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DOUTORAMENTO EM CIÊNCIA ANIMAL

ESPECIALIZAÇÃO EM MORFOLOGIA E FISIOLOGIA

Microplastics in aquaculture - the impacts on fish welfare and food safety

Ricardo Silva Matias

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INSTITUTO DE CIÊNCIAS BIOMÉDICAS ABEL SALAZAR

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Tese de Candidatura ao grau de Doutor em Ciência Animal, Especialidade em Morfologia e Fisiologia, submetida ao Instituto de Ciências Biomédicas Abel Salazar (ICBAS), enquanto instituição pertencente à Universidade do Porto.

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Microplastics in aquaculture – the impacts on fish welfare and food safety

Thesis for applying to a Doctoral degree in Animal Science, Specialization in Morphology and Physiology, submitted to the School of Medicine and Biomedical Sciences (ICBAS), as an institution of the University of Porto.

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"*Difficulties are just things to overcome, after all*"

- Sir Ernest Shackleton (1874-1922)

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Matias R.S., Monteiro M., Sousa V., Pinho B., Guilhermino L., Valente L.M.P., & Gomes S. (2024). A multiple biomarker approach to understand the effects of microplastics on the health status of European seabass farmed in earthen ponds on the NE Atlantic coast. *Environmental Research* 263(Part 3), 120208 https://doi.org/10.1016/j.envres.2024.120208

Matias R.S., Holhorea P., Gomes S., Calduch-Giner J., Guilhermino L., Naya-Català F., Pérez-Sánchez, J., & Valente, L.M.P. It's not just synthetic microplastics: Multi-tissue approach reveals a wide range of transcriptional changes in European seabass exposed to viscose-rayon microfibres. Submitted to a scientific peer-reviewed journal in March 2025.

Abstract

Plastics have drawn attention worldwide over recent decades due to their environmental persistence in aquatic environments. There, plastic gradually fragments and changes through a number of processes, such as mechanical abrasion and UV radiation exposure, among others. Microplastics (MPs, *plural* of MP), with sizes <5 mm, are capable of travelling long distances thus being widespread and available to be ingested by biota. Upon ingestion or uptake by other routes, MPs can negatively impact animal health in several ways, such as damaging and impairing intestinal and branchial functions, and inducing other toxic effects on tissues. Therefore, MP pollution has become a growing concern due to its potential risks for the biota, ecosystem functioning and human health. Despite MP pollution concerns, the knowledge regarding its occurrence and impacts in aquaculture is still limited. Aquaculture is susceptible to endogenous and exogenous contamination, through water, aquafeeds, plastic gear, and other materials. MP contamination in farmed seafood has been increasingly documented in the last few years. The presence of MPs in edible tissues has been reported both in wild and farmed fish, as well as in fish exposed to MPs under laboratorial conditions. Still, the consumption of farmed seafood is an understudied route for human exposure. Therefore, future research addressing MP impacts on food safety is essential for developing cleaner, safer, and more sustainable practices for the aquaculture industry.

In line with the 'One Health' concept, which recognises the interconnectedness of environmental, animal, and human health, this Thesis aimed to help fill critical gaps of knowledge regarding MP contamination and its effects within the aquaculture sector. Specifically, the research focused on European seabass (*Dicentrarchus labrax*), a species of high economic relevance to Mediterranean aquaculture. The main research questions addressed were:

1. Does MP contamination affect different aquaculture production systems of seabass (cages, ponds, and recirculating aquaculture systems) in distinct ways?
2. Are higher levels of MPs in farmed fish tissues associated with changes in fish health biomarkers?
3. Is there a relationship between MP levels in seabass muscle and the accumulation of trace and non-essential metals, and do these contaminants represent a significant exposure route for human consumers?
4. Does the most predominant MP type observed in aquaculture production systems of seabass affect fish performance and health status under aquaculture-relevant experimental conditions?

To address the first question, the presence of MPs and other anthropogenic particles were evaluated in the water, aquafeed, and fish tissues (*i.e.*, gastrointestinal tract (GIT), gills, liver, dorsal muscle) of seabass collected from three different aquaculture production systems: a cage farm, an earthen-pond farm, and a pilot recirculating aquaculture system (RAS). Particles were visually characterised using stereomicroscopy and chemically identified through micro-Fourier Transformed Infrared Spectroscopy (μ-FTIR). The RAS exhibited the highest mean (± standard deviation) MP concentrations in water (37.2 ± 1.9 MP/L), aquafeed (3.9 ± 1.4 MP/g), and fish tissues (0.18 ± 0.13 MP/g). Black, blue, and transparent microfibres, mainly composed of man-made cellulose/rayon and polyethylene terephthalate, predominated over the other types of MPs. In RAS, the most common MPs were not linked to any plastic gear present. Our findings indicate differences of MP contamination among the three production systems, with observable impacts on MP levels in fish tissues.

To address the second question, for both RAS and pond farms, two groups of fish were selected for each production system based on the lowest and highest MP levels in their tissues. Oxidative stress biomarkers were evaluated in the liver, including the enzymatic activities of superoxide dismutase, catalase, glutathione reductase, glutathione S-transferase, and glutathione peroxidase, along with lipid peroxidation levels in both liver and dorsal muscle. No significant relationship was found between MP levels and oxidative stress biomarkers in either aquaculture production system. The rectum histomorphology was also evaluated through microscopy and Alcian-Blue/Periodic Acid Schiff (AB/PAS) colouration. Pond-farmed seabass with higher MP levels displayed a greater number of acid goblet cells (GCs) in the intestinal epithelium, suggesting increased mucus production that may act as a protective response to foreign particles. This may indicate that, while different MP levels were insufficient to trigger oxidative stress, they were high enough in pond-farmed seabass with higher MP levels to stimulate localised mucosal defence mechanisms.

To address the third question, the co-occurrence of MPs with trace and non-essential metals was evaluated in the dorsal muscle of seabass collected from the three aquaculture production systems. In each production system, two groups of fish were selected based on the lowest and highest MP levels in their tissues. Metal concentration, including cadmium, copper, chromium, led, mercury, nickel, and zinc, were determined using Atomic Absorption Spectrometry (AAS). No significant relationship was found between MP and metal levels in the aquaculture production systems. Importantly, all metal levels were below the maximum allowable concentrations for this species and represented no toxicological risk to human consumers, based on the health-based guidance values provided by the European Food Safety Authority (EFSA) and the seafood consumption data from the European Market Observatory for Fisheries and Aquaculture Product (EUMOFA). It was estimated that human

consumers are exposed to a total of 27-54 MPs in 150 g of fillet of farmed seabass. Further research applied to different fish species is advised to better estimate human exposure to MPs and associated metals through the consumption of farmed seafood.

To address the fourth question, a dedicated *in vivo* trial was conducted to evaluate the effects of cellulosic microfibres, more specifically viscose-rayon (RFs), on the growth performance, feed efficiency, and gene expression patterns in various tissues of seabass. This experiment was designed under aquaculture-relevant conditions, based on *in situ* data. Over 68 days, four groups of fish were separately exposed to a control diet with no added RFs (CTRL), or to three diets containing increasing RF doses: RF1 (0.001 g/kg ≈ 1.8 RF/g), RF2 (0.01 g/kg ≈ 14.8 RF/g), and RF3 (0.1 g/kg ≈ 195.0 RF/g). RF exposure did not affect growth performance and feed efficiency, with fish showing high and efficient growth (Specific Growth Rate, 2.40–2.45%; Feed Conversion Ratio, 0.95–0.96). However, the hepatosomatic index increased progressively with RF concentrations, from 1.49 to 1.98%. Gene expression analyses in the liver, white skeletal muscle (WSM), anterior intestine, and head kidney (HK) revealed consistent transcriptional changes across tissues. These included the upregulation of genes involved in lipogenesis and mitochondrial functions in the liver, and increased expression of genes linked to opposing regulatory mechanisms governing both WSM growth and immune response either at systemic (HK) and local (anterior intestine) levels. These findings show that dietary exposure to RFs can induce sublethal physiological changes in seabass, highlighting that cellulosic microfibres, whether with a natural or semi-synthetic origin, can impact fish health.

This Thesis significantly contributes to understanding MP contamination in aquaculture, highlighting its occurrence, potential effects on fish health, and implications for food safety. By addressing key gaps of knowledge, it emphasises the need for continued monitoring and adoption of sustainable practices to mitigate plastic pollution. Under the ‘One Health’ approach, this work contributes for future research and strategy development to ensure the safety and sustainability of farmed seafood.

Resumo

Os plásticos têm atraído atenção mundial nas últimas décadas devido à sua persistência elevada nos ambientes aquáticos. Nestes, os plásticos fragmentam-se gradualmente e sofrem alterações através de vários processos, como a abrasão mecânica e a exposição à radiação UV, entre outros. Os microplásticos (MPs, *plural* de MP), com tamanhos <5 mm, são capazes de percorrer longas distâncias, tornando-se amplamente distribuídos e acessíveis para serem ingeridos por organismos aquáticos. Após ingestão ou absorção por outras vias, os MPs podem impactar negativamente a saúde animal de várias formas, causando danos e comprometendo funções intestinais e branquiais; podem ainda ter efeitos tóxicos em tecidos. Assim, a poluição por MPs tornou-se uma preocupação devido aos riscos para a biota, o funcionamento dos ecossistemas e a saúde humana. Apesar da preocupação relacionada com a poluição por MPs, o conhecimento sobre a sua ocorrência e impactos na aquacultura ainda é limitado. A aquacultura é suscetível à contaminação endógena e exógena por MPs, através da água, rações, equipamentos plásticos, e outros materiais. A documentação sobre produtos aquícolas contaminados por MPs tem vindo a aumentar nos últimos anos. Além disso, a presença de MPs em tecidos comestíveis é relatada tanto em peixes selvagens como de aquacultura, bem como em peixes expostos a MPs em condições laboratoriais. Estes relatos destacam o consumo de peixe de aquacultura como uma via relevante de exposição para os consumidores ainda pouco estudada. Assim, é essencial que estudos futuros tenham como prioridade avaliar os impactos dos MPs na segurança alimentar, a fim de desenvolver práticas mais limpas, seguras e sustentáveis para o sector da aquacultura.

Em conformidade com o conceito de 'One Health', que reconhece a interligação entre a saúde ambiental, animal e humana, a presente Tese procurou contribuir com o preenchimento de lacunas significativas do conhecimento relativamente à contaminação por MPs e aos seus efeitos no setor da aquacultura. Especificamente, a investigação focou-se no robalo europeu (*Dicentrarchus labrax*), uma espécie de elevada importância económica para a aquacultura nos países mediterrânicos. As principais questões de investigação abordadas foram:

1) A contaminação por MPs afeta de forma distinta os diferentes sistemas de produção aquícola (jangadas, tanques de terra e sistemas de recirculação) de robalo?
2) Níveis de MPs mais elevados nos tecidos dos peixes de aquacultura estão associados a alterações nos biomarcadores de saúde?
3) Existe uma relação entre os níveis de MPs no músculo de robalo e os níveis de oligometais e metais não-essenciais? E poderão estes representar uma via relevante de exposição para os consumidores?

4) O tipo de MPs mais predominantemente observado tem efeitos negativos na saúde do robalo produzido em condições experimentais relevantes para a aquacultura?

Para responder à primeira questão, avaliou-se a presença de MPs e outras partículas antropogénicas na água, ração e tecidos (*i.e.*, trato gastrointestinal (GIT), brânquias, fígado e músculo dorsal) de robalos provenientes de três sistemas de produção aquícola: uma aquacultura em jangadas, uma aquacultura em tanques de terra, e um sistema de aquacultura em recirculação (RAS) piloto. As partículas foram caracterizadas visualmente por estereomicroscopia e quimicamente identificadas por microespectroscopia de Infravermelho por Transformada de Fourier (μ-FTIR). O RAS apresentou as maiores concentrações médias (± desvio-padrão) de MPs na água (37,2 ± 1,9 MP/L), na ração (3,9 ± 1,4 MP/g) e tecidos de peixe (0,18 ± 0,13 MP/g). As microfibras pretas, azuis e transparentes, compostas maioritariamente por celulose artificial/rayon e tereftalato de polietileno, predominaram em relação a outros tipos de MPs. No RAS, os MPs mais comuns não se relacionaram com qualquer equipamento plástico presente. Os nossos resultados indicam diferenças na contaminação por MPs entre os sistemas de produção, com impactos observáveis nos níveis de MP nos tecidos dos peixes.

Para responder à segunda questão, foram selecionados dois grupos, para o RAS e para a aquacultura em tanques de terra, com os níveis mais baixos e mais elevados de MPs nos tecidos. Foram avaliados biomarcadores de stress oxidativo no fígado, incluindo as atividades enzimáticas da superóxido dismutase, catalase, glutationa redutase, glutationa S-transferase e glutationa peroxidase, bem como os níveis de peroxidação lipídica no fígado e no músculo dorsal. Não foi encontrada nenhuma relação entre os níveis de MPs nos tecidos e os biomarcadores de stress oxidativo em ambos sistemas de produção. A histomorfologia do reto também foi avaliada através de microscopia e com recurso à coloração azul alciano/ácido periódico Schiff (AB/PAS). Verificou-se que os peixes produzidos em tanques de terra e com níveis mais elevados de MPs apresentaram um maior número de células caliciformes (GCs) ácidas no epitélio retal, sugerindo uma maior produção de muco que poderá atuar como resposta protetora a partículas estranhas. Isto pode indicar que, embora os diferentes níveis de MPs não tenham sido suficientes para induzir stress oxidativo, foram elevados o suficiente para estimular mecanismos de defesa da mucosa em peixes produzidos em viveiros de terra.

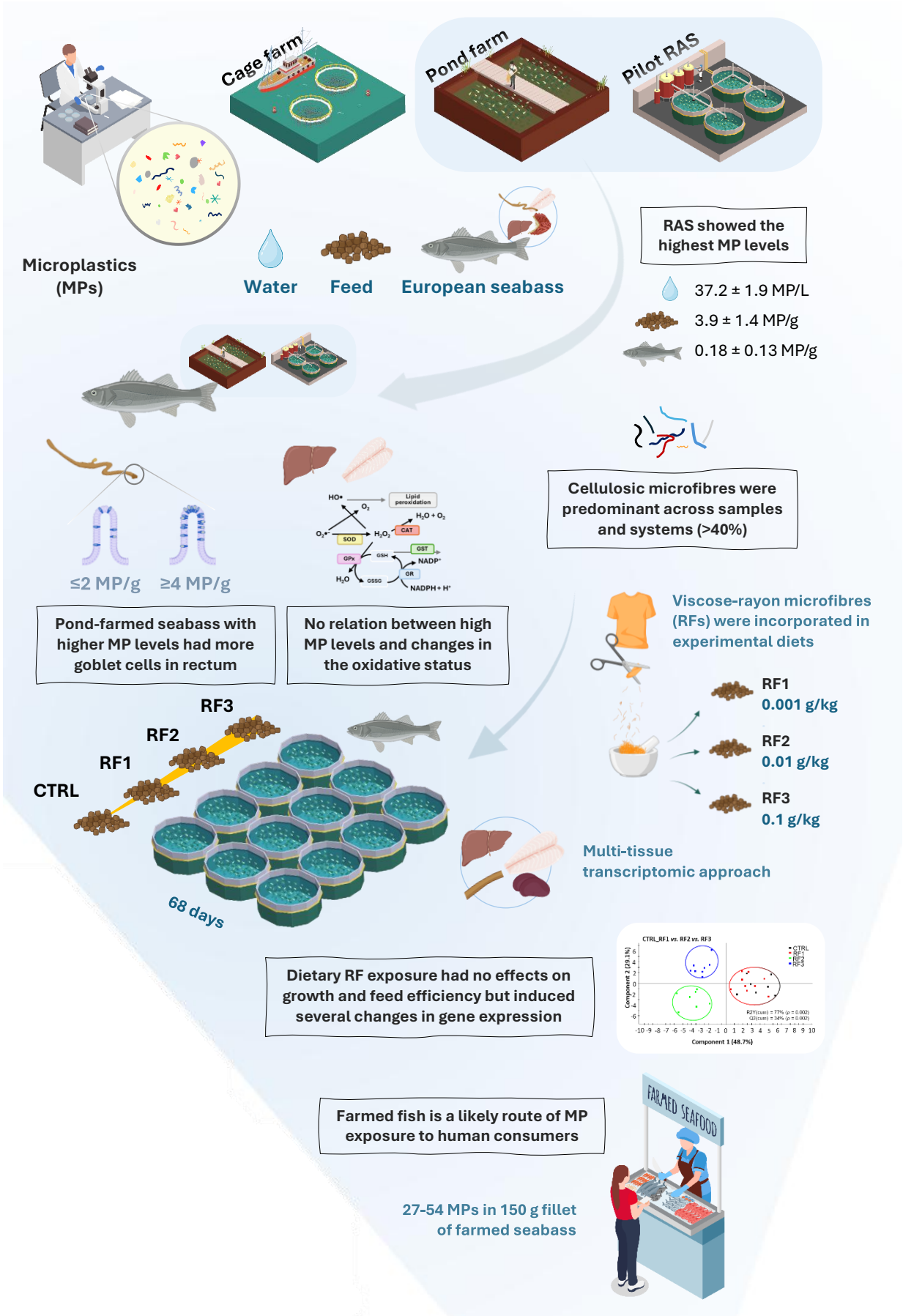
Para responder à terceira questão, foi avaliada a coocorrência de MPs com oligometais e metais não-essenciais no músculo dorsal dos robalos recolhidos nos três sistemas de produção aquícola. Em cada sistema, foram também considerados dois grupos de peixes com base nos níveis mais baixos e mais elevados de MPs nos tecidos. As concentrações de metais, incluindo cádmio, cobre, crómio, chumbo, mercúrio, níquel e

zinco, foram determinados por Espectrometria de Absorção Atómica (AAS). Não foi encontrada qualquer relação significativa entre os níveis de MPs e metais nos sistemas de produção. Importa destacar que todos os níveis de metais estavam abaixo das concentrações máximas permitidas para a espécie e não representaram risco toxicológico para o consumidor humano com base nos valores de referência da Autoridade Europeia para a Segurança dos Alimentos (EFSA) e nos dados de consumo de pescado do Observatório Europeu do Mercado de Produtos da Pesca e da Aquacultura (EUMOFA). Foi estimado que os consumidores são expostos entre 27-54 MPs por cada 150 g de fillet de robalo de aquacultura. Uma vez que a dieta humana é diversificada, recomendam-se que estudos futuros avaliem diferentes espécies de peixes para melhor estimar a exposição humana a MPs e metais associados através do consumo de produtos de aquacultura.

Para responder à quarta questão, foi realizado um ensaio *in vivo* para avaliar os efeitos de microfibras celulósicas, especificamente viscose-rayon (RFs), no crescimento, eficiência alimentar e padrões de expressão génica em vários tecidos de robalo. Esta experiência foi realizada sob condições relevantes para a aquacultura, com base nos dados *in situ*. Durante 68 dias, quatro grupos de peixes foram expostos separadamente a uma dieta controlo sem RFs (CTRL), ou a três dietas com doses crescentes de RFs: RF1 (0,001 g/kg ≈ 1,8 RF/g), RF2 (0,01 g/kg ≈ 14,8 RF/g), RF3 (0,1 g/kg ≈ 195,0 RF/g). A exposição às RFs não afetou o crescimento nem a eficiência alimentar, com os peixes apresentando um crescimento rápido e eficiente (taxa de crescimento específica, 2,40-2,45; índice de conversão alimentar, 0,95-0,96). O índice hepatossomático aumentou progressivamente com as doses de RFs, de 1,49 para 1,98%. As análises da expressão génica no fígado, músculo esquelético branco (WSM), intestino anterior e rim cefálico (HK) revelaram alterações consistentes nos padrões de expressão de genes dos tecidos. Estas incluíram a regulação positiva de genes envolvidos na lipogénese e funções mitocondriais no fígado, regulação positiva e negativa de genes envolvidos no crescimento muscular (WSM), bem como na resposta imune, tanto a nível sistémico (HK) como local (intestino anterior). Estes resultados demonstram que as RFs induzem alterações fisiológicas subletais nos robalos, evidenciando que microfibras celulósicas, quer de origem natural quer semissintética, poderão afetar a saúde do peixe.

Esta Tese contribui significativamente para a compreensão da contaminação por MPs na aquacultura, destacando a sua ocorrência, os seus potenciais efeitos na saúde dos peixes e implicações para a segurança alimentar. Ao abordar lacunas de conhecimento, sublinha a necessidade da monitorização contínua e da implementação de práticas sustentáveis para mitigar a poluição plástica. Alinhado com a abordagem “Uma Saúde”, esta Tese contribui para investigação e desenvolvimento de estratégias no futuro, visando a segurança e sustentabilidade de produtos aquícolas.

Graphical Abstract



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List of Abbreviations

A
AAS – Atomic Absorption Spectrometry
ABW – Average Body Weight
AB/PAS – Alcian-blue/periodic acid Schiff
AI – Anterior intestine
AK – Alkyd resin
ANCOVA – Analysis of covariance
ANOVA – Analysis of variance
ASC – Aquaculture Stewardship Council
ATR – Attenuated total reflectance

B
BAF – Bioaccumulation factor
BCF – Bioconcentration factor
BHT - Butylhydroxytoluene
$BMDL_{01}$ – Benchmark Dose Lower Limit with 1% of extra risks

C
CAT – Catalase enzyme
Cd – Cadmium
cDNA – Complementary desoxyribonucleic acid
CNDB – 1-chloro-2,4-dinitrobenzene
Cr – Chromium
CTRL – Control
Cu – Copper

D
DM – Dry matter
DTNB – 5,5'-dithiobis-2-nitrobenzoic acid

E
EFSA – European Food Safety Authority
EPDM – Ethylene-propylene diene
EPS – Expanded polystyrene
EU – Enzyme unit
EU REACH – European Union's regulation for the 'Registration, Evaluation and Authorization of Chemicals"
EUMOFA – European Market Observatory for Fisheries and Aquaculture Products
EVA – Ethylene-vinyl acetate

F
FA – Fatty acid (*plural*, FAs)
FAO – Food and Agriculture Organization of the United Nations
FBL – Final body length
FBW – Final body weight
FCR – Feed Conversion Ratio

G
GC – goblet cell
Ge – Germanium
GIT – Gastrointestinal tract
GPx – Glutathione peroxidase enzyme
GR – Glutathione reductase enzyme
GSH – Reduced glutathione
GSSG – Oxidised glutathione
GST – Glutathione S-transferase enzyme

H
HBGV – Health-based guidance value
HDPE – High-density polyethylene
Hg – Mercury
HK – Head kidney
HNO_3 – Nitric acid
HSI – Hepatosomatic index
hsl – Hormone-sensitive lipase
H_2O_2 – Hydrogen peroxide

I
IBW – Initial body weight
IgM – Total immunoglobulin M

K
K – Fulton's condition factor
KOH – Potassium hydroxide

L
LC-HRMS – Liquid Chromatography with High-Resolution Mass Spectrometry
LDPE – Low-density polyethylene
LoD – Limit of detection
LPO – Lipid peroxidation

M
MCT – Mercury cadmium telluride
meso-PL - Mesoplastic
MDA - Malondialdehyde
MGG – May-Grunwald Giemsa
MMT – Million metric tonnes
MP – Microplastic (*plural*, MPs)
MPC – Maximum Permissible Concentration
MS-222 – tricaine methanesulfonate

N
NADPH – Nicotinamine adenine dinucleotide phosphate
NAFLD – Non-alcoholic Fatty Liver Disease
Ni – Nickel
NP – Nanoplastic (*plural*, NPs)

O
OD – Optical density

P
PA – Polyamide
PAHs – Polycyclic aromatic hydrocarbons (PAHs)
PAM – Polyacrylamide
PAN – Polyacrylonitrile
PAR – Polyamide resin
Pb – Lead
PCBs – Polychlorinated biphenyls
PCR – Polymerase Chain Reaction
PE – Polyethylene
PER – Protein Efficiency Ratio
PES – Polyester
PET – Polyethylene terephthalate
PFAS – Perfluoroalkyl substances
PLS-DA – Partial Least Square-Discriminant Analysis
PL – Plastic particle (*plural*, PLs)
PMMA – Polymethyl methacrylate
PMS – Post-mitochondrial supernatant
PP – Polypropylene
PS – Polystyrene
PTFE – Polytetrafluoroethylene
PUFA – Polyunsaturated fatty acid
PUR – Polyurethane
PVC – Polyvinyl chloride

R
RAS – Recirculating Aquaculture System
RF – Viscose-rayon microfibre (*plural*, RFs)
ROS – Reactive oxygen species

S
SD – Standard deviation
SGR – Specific Growth Rate
SEM – Standard error of the mean
SOD – Superoxide dismutase

T
TAC – Total antioxidant capacity
TBA – Thiobarbituric acid
TBARS – Thiobarbituric acid reactive substances
TDI – Tolerable daily intake
TG – Triglyceride (*plural*, TGs)
TL – Total body length
TW – Total body weight
TWI – Tolerable weekly intake

U
UL – tolerable upper intake level

V
VFI – Voluntary Feed Intake
VIP – Variable Importance in the Projection
VSI – Viscerosomatic index

W
WSM – White skeletal muscle
ww – Wet weight

Z
Zn – zinc

Others
µFTIR – micro-Fourier Transformed Infrared Imaging Microscopy

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Chapter I

General introduction

Driven by the search for alternatives to expensive rare materials, such as tortoise shell and whalebone, material innovations date back mainly to the early 19th century. Early attempts with cellulose-based materials led to the creation of the first semi-synthetic polymers with significant advances in the production of heat-mouldable plastics (*i.e.*, thermoplastics) such as *Parkesine* by Alexander Parks in 1856 and celluloid by John Wesley Hyatt in 1869 (Rasmussen, 2021). This period led to the creation of *Bakelite* in 1909 by Leo Baekeland, which was the first durable and fully synthetic polymer capable of retaining its shape under heat conditions (*i.e.*, thermosetting material) (Baekeland, 1909). Marketed as "the material of a thousand uses", *Bakelite* became a staple polymer in the manufacturing of a wide range of products, such as electrical insulators, telephone and radio casings, kitchenware, jewellery and children's toys (Meikle, 1995). Baekeland's discovery is often considered the beginning of a new era in polymer science known as the "Age of Plastics" (Rasmussen, 2021; Thompson *et al.*, 2009).

In the first half of the 20th century, the global production of plastics was less than one million metric tonnes (MMT) per year and plastics were mainly used for military purposes until after World War II, 1939-1945 (Madden *et al.*, 2019; Meikle, 1995). During that period, significant advances were made in the development of modern plastics as these were durable, lightweight, cheap, and good alternatives to natural resources (Smith Jr., 1988). Some of the advances include the development of rayon, neoprene, nylon, and polymethyl methacrylate that were rapidly implemented in the manufacturing of several military gear in high demand, such as garments, tires, parachutes, and even aircraft parts (Smith Jr., 1988). This period of innovation set the stage for a post-war explosion in plastic use across sectors, due to new consumer habits (Madden *et al.*, 2019; Thompson *et al.*, 2009). By the end of the 20th century, plastic production had surpassed other man-made materials, driven by products made of low- and high-density polyethylene (LDPE and HDPE, respectively), polypropylene (PP), polyvinyl chloride (PVC), and polystyrene (PS) (Geyer, 2020).

Global plastic production continued to rise, reaching an annual production of 413.8 MMT in 2023 (Plastics Europe, 2024) (Fig. 1.1). In 2023, China had the highest share of annual global plastic production accounting for 33.3%, followed by North America with 17.1%, and Europe with 12.3% (Plastics Europe, 2024). In total weight, plastic production in Europe has remained relatively constant and lead to a decrease of its share in global production from 22% in 2014 to 12.3% in 2023 (Plastics Europe, 2024) (Fig. 1.1). Estimates point to a cumulative total of 8300 MMT of plastic produced worldwide between 1950 and 2015 (Geyer *et al.*, 2017). Currently, most produced polymers include LDPE, HDPE, PP, PVC, polyethylene terephthalate (PET), PS, polyurethane (PUR), and polyamide (PA) (Geyer *et al.*, 2017; Plastics Europe, 2024). In addition to polymers, plastic industry involves a large number of additives (*e.g.*, plasticisers, flame retardants, stabilisers) which confer to

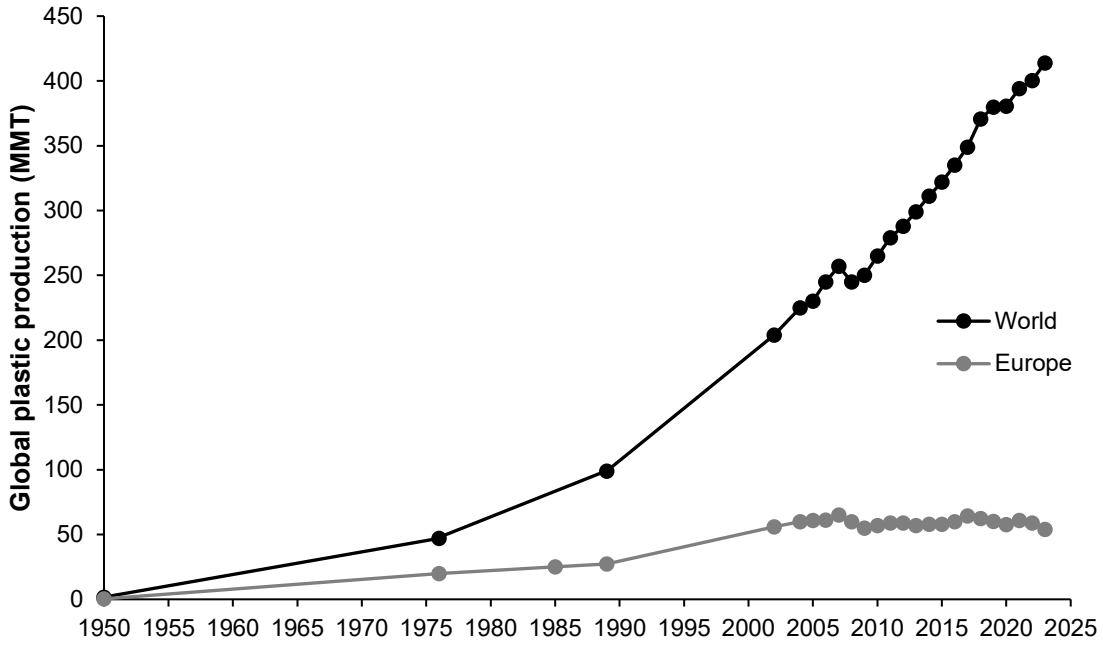

Figure 1.1. Plastic production in million metric tonnes (MMT) from 1950 to 2022. Data accounts for plastic production from polymerisation and mechanical recycling; and does not include plastic used for the production of textiles, coatings, among others. Adapted from data of Plastics Europe.

plastics the desired physicochemical properties for different applications, such as the high strength-to-weight ratio, water and shock resistance, thermal and electrical insulation, among others (Hahladakis *et al.*, 2018). These properties helped plastics to become key to reduce material and energy expenditure (Andrady & Neal, 2009). For instance, plastic packaging and replacing metal parts by plastics in automobiles reduces vehicle weight and their fuel consumption (Andrady & Neal, 2009).Plastics are now present in agriculture, construction, electronics, textiles and other household goods (Geyer, 2020). Notably, in the fishery, plastic gear has been extensively used (*e.g.*, nets, ropes, floats) and enhance the efficiency of fishery operations due to their higher resistance (Geyer, 2020). Beyond the societal benefits, plastics have proven to be crucial in the health sector, such as personal protective equipment to avoid the transmission and spread of infectious diseases, as seen during the SARS-CoV-2 pandemic (de Sousa, 2021). Undoubtedly, plastics are one of the most transformative technological innovations, not only revolutionising the human life but also continuing to drive progress in the 21st century. However, has every rose has its thorn, the high production of plastics has soon begun to raise serious concerns.

1.1. Marine plastic debris and pervasive occurrence of microplastics

In 1969, Kenyon & Kridler (1969) found plastic debris in the stomach content of Layson albatross chicks (*Phoebastria immutabilis*) from North West Hawaiian islands. Among other indigestible items, an average of 2 g of plastic per bird was found in 74 of the 100 chicks that died before fledging in 1966. Parslow & Jefferies (1972) reported the occurrence of elastic rubber threads in the stomach of non-breeding Atlantic puffins (*Fratercula arctica*) collected between 1969 and 1971. Other studies have also reported plastic ingestion in green sea turtle (*Chelonia mydas*) and seabird samples collected before 1966 but only appeared later than 1972 (Balazs, 1985; Harper & Fowler, 1987; Rothstein, 1973). However, earlier reports of the impact of marine plastic debris in biota only gained notoriety with the works of Carpenter & Smith (1972) and Carpenter *et al.* (1972) reporting their occurrence in the Sargasso Sea, and coastal waters and fish of southern New England, respectively. These studies were particularly important because as not only observed high abundances of plastic particles but also warned about threats to organisms, namely the ingestion of plastic debris leading to intestinal obstruction, malnutrition, and starvation, as well as the presence of bacteria and polychlorinated biphenyls (PCBs) adhered to the MP surface.

The number of reports alarming to the high occurrence of plastic debris in open waters of the Atlantic and North Pacific Ocean grew during the 1970s (Colton *et al.*, 1974; Venrick *et al.*, 1973; Wong *et al.*, 1974). High plastic occurrence was also reported nearby highly populated or industrialised regions of North America (Austin & Stoops-Glas, 1977; Hays & Cormons, 1974), United Kingdom (Kartar *et al.*, 1973; Morris & Hamilton, 1974) and New Zealand (Gregory, 1977). These studies clarified that plastic pollution was not confined to isolated areas of high anthropogenic presence and extends to open waters and remote areas. The number of reports of deleterious interactions between plastic and biota also grew in the Northern and Southern Hemispheres, including seabirds and mammals (Crockett & Reed, 1976; Forrester *et al.*, 1975; Gochfeld, 1973; Ohlendorf *et al.*, 1978). Beyond ingestion, entanglement in marine debris was also identified as a threat to biota. “Ghost fishing” by abandoned, lost, or discarded fishing gear raised concerns after reports of the death of aquatic organisms, from fish to marine mammals (Bourne, 1977). This outcome became more evident with the growing use of synthetic materials (*e.g.*, nylon, polyethylene (PE), PP) in fisheries, which can persist in the environment for decades and trap organisms long after their use.

These findings led to the first dedicated scientific event in 1984: the Workshop on the Fate and Impact of Marine Debris, held in Honolulu, Hawaii. This event brought together the scientific community to discuss the growing evidence and harmful impacts of plastic

debris on the marine environment. A technical memorandum by the National Oceanic and Atmospheric Administration (NOAA) of the United States of America (US) that identified research priorities and recommendations of mitigation strategies to combat marine pollution by plastic debris (Shomura & Yoshida, 1985). Since then, one of the most significant measures was the implementation of Annex V by the International Convention for the Prevention of Pollution from Ships (MARPOL) in 1988 that specifically prohibits plastic disposal at sea. Studies commonly reported high abundances of marine plastic debris and associated their occurrence to ship-based sources (Horsman, 1982; Scott, 1972). Plastic pollution was also incorporated as part of the United Nations (UN) 2030 Agenda for Sustainable Development in 2015, specifically under the Goal 14 "*Life Below Water*" which calls for the prevention and reduction of marine pollution by plastics and other harmful contaminants (UN, 2015). Since then, international and national efforts have been made to mitigate this issue, for example through the enforcement of single-use plastic ban laws for several items, such as disposable kitchenware, cotton bud sticks, and shopping bags (EU, 2019; Knoblauch & Mederake, 2021; Muposhi *et al.*, 2022). Beach cleanups and other initiatives involving citizens became popular (Lawen *et al.*, 2024; Mishra *et al.*, 2023).

At the beginning of the 21st century, the presence of marine plastic debris defined as "any persistent, manufactured or processed solid material discarded, disposed or abandoned in marine and coastal environments" (UNEP, 1995), had already been widely described. Estimates indicate that ~11 MMT of plastic waste enters in oceans every year (Jambeck *et al.*, 2015). This influx of plastic debris is expected to reach ~21.5 and ~29.0 MMT by 2030 and 2040, respectively, if no effective mitigation measures are implemented (Lau *et al.*, 2020). Some of the pathways for plastics into aquatic environments include improper disposal of waste and sewage management, transportation by wind, urban run-off waters, rivers, and tidal influence (Barnes *et al.*, 2009). It should be noted that extreme weather events, such as floods and hurricanes, are likely to increase the transfer of land-based waste into marine ecosystems (Lincoln *et al.*, 2022).

Marine plastic debris have been found to accumulate in subtropical oceanic gyres located in the Atlantic, Pacific, and Indian Oceans (Connan *et al.*, 2021; Law *et al.*, 2010; Moore *et al.*, 2001), and other basins such as the Mediterranean Sea (Suaria *et al.*, 2016). Their widespread presence extends to remote oceanic islands (Lavers & Bond, 2017) and polar regions (Bergmann *et al.*, 2022; De-la-Torre *et al.*, 2024). This became more evident following the findings of Moore *et al.* (2001), who reported plastic densities in the North Pacific gyre exceeding 300,000 particles/km^2, an amount by total weight six-fold higher than zooplankton. Another important study conducted by Thompson *et al.* (2004) showed an increasing plastic concentration in sediments and seawater from the North Atlantic between the 1960s and 1990s, particularly of particles within microscopic sizes. These

findings were in line with the first studies on marine plastic debris (Carpenter *et al.*, 1972; Colton *et al.*, 1974; Wong *et al.*, 1974), but challenged the existing assumption that plastic debris would fully degrade and disperse as dust over time. Further studies consolidated these findings and raised concerns regarding these microscopic plastic particles.

In the environment, plastic debris fragments into smaller pieces due to several physicochemical processes, such as mechanical abrasion and UV radiation exposure, among others (Shi *et al.*, 2023). Plastic debris fragmentation can also occur in the digestive tract of biota after ingestion (Cau *et al.*, 2020; Dawson *et al.*, 2018) or due to biofouling (Galloway *et al.*, 2017; Póvoa *et al.*, 2021). These processes can lead to microplastics (MPs), which are generally defined as "any particle <5 mm in diameter, which includes particles in the nano-size range" (GESAMP, 2016). More recently, plastic particle with size equal or <100 nm are commonly indicated as nanoplastics (NPs) however some studies consider NPs <1000 nm (GESAMP, 2019; Gigault *et al.*, 2018; Mitrano *et al.*, 2021). However, the detection and quantification of NPs environmental samples is in its infancy and remains a caveat to determine if NPs are as widespread as other forms of plastic pollution (Mitrano *et al.*, 2021; Zhang *et al.*, 2022e).

Due to their small size and weight, MPs are very susceptible to travel long distances and were already found in a wide range of ecosystems worldwide (Thompson *et al.*, 2024). Recent estimates indicate an average of 172 trillion particles floating in world's oceans in 2019, ranging from 82 to 358 trillion particles afloat (Eriksen *et al.*, 2023). Some MPs are deposited in low-energy locations such as the deep sea (Woodall *et al.*, 2014). However, understanding the environmental dynamics and fate of MPs is complex due to the different MP characteristics (*e.g.*, shape, size, and density) which affect their transport, dispersion range and settling (Qian *et al.*, 2024; Stride *et al.*, 2024). Current literature shows that the polymers most produced are similar to those commonly found in aquatic environments (Thompson *et al.*, 2024). Kannankai *et al.* (2022) conducted a meta-analysis and found that most common MPs were PE (79.9%), PP (77.2%), PA (52.3%), PS (47.7%), PET (43%), PVC (42.3%), and polyester (PES, 38.3%). It should be mentioned that these polymers fall under the category of polymers of concern due to their monomer toxicity and high persistence (Yuan *et al.*, 2022). Estimates indicate that even if plastic emissions stop now, the fragmentation of plastic debris already available would be sufficient to increase of MP environmental contamination (Thompson *et al.*, 2024).

1.2. Interactions between microplastics and fish

So far, it has been recognised that aquatic and terrestrial organisms face great risks from plastic pollution as these can easily come across with plastic particles occurring in the environment. Until now, a total of 4076 species have been found to interact with plastic debris in aquatic environments, ranging from bacteria, algae and plants, to zooplankton and higher-order animals, such as fish, seabirds, and mammals (LITTERBASE/AWI). According to this database, from a total of 1956 publications, ingestion is the most common interaction observed between biota and plastic debris, involving 40.4% of the species. As larger plastic items fragment into smaller pieces, reaching MP and NP sizes, their widespread distribution in aquatic environments increases their availability to organisms across the food chain.

Ingestion is one of the major routes of fish exposure to MPs. In fact, the presence of MPs in the gastrointestinal tract (GIT) of fish has been detected in global aquatic environments, and in a wide range of freshwater and marine species, namely over 522 species, 141 families and 42 orders (Sacco *et al.*, 2024). When in the environment, fish can ingest MPs by confounding them as prey, directly from water, and through MP-contaminated prey (de Sá *et al.*, 2015; Li *et al.*, 2021a; Setälä *et al.*, 2014). MP ingestion is influenced by the number of MPs locally bioavailable to fish and their preys, properties of the MPs, such as shapes, colours, sizes, odour (Horie *et al.*, 2024; Lehtiniemi *et al.*, 2018; Procter *et al.*, 2019; Savoca *et al.*, 2017; Xiong *et al.*, 2019), biological traits of fish, such as mouth-to-body size ratio (Siddique *et al.*, 2024), among other factors. Once ingested, MPs can accumulate in the GIT and remain there for extended periods of time, potentially leading to a false food satiation sense and lower food intake (Zhang *et al.*, 2024b). In high exposure scenarios, the absence of an adequate nutrition from MPs can lead to decreased energy reserves and compromise the overall fish health (Chen *et al.*, 2020b; Yin *et al.*, 2018).

Branchial and dermal exposure are also important routes for waterborne MP in fish. During respiration, MPs can be retained with the mucus in gills as the water flows out. Indeed, several studies have previously observed the retention of MPs in the mucosal layer within gill filaments (Abarghouei *et al.*, 2021; Batel *et al.*, 2018; Zitouni *et al.*, 2021). As fish gills is a sophisticated and delicate organ where multiple physiological functions occur in addition to gas exchange, it is likely that MPs negatively impact the well-functioning of the tissue (Cao *et al.*, 2023; Hamed *et al.*, 2021). Nonetheless, ingestion and branchial uptake may not be entirely separated since waterborne MPs retained in gills may form clumps and get dragged into the GIT alongside food (Li *et al.*, 2021a; Liang *et al.*, 2023). These studies also found increased coughing rates in fish exposed to waterborne MP fibres and the successful elimination of most fibres gulped in, suggesting that the branchial uptake of MPs is an important pathway but possibly minor in comparison to MP

ingestion. MPs also interact with fish through the skin, which represents the largest surface area of fish. It has been reported significantly higher MP levels in the skin of scaleless fish species in comparison to scaled fish (Feng *et al.*, 2019). Recently, Thiyagarajan *et al.* (2024) has found that keratocytes, scavenger cells located in the skin epithelial layer, of Atlantic salmon (*Salmo salar*) could fully internalise PS particles measuring up to 1 µm. Nevertheless, dermal exposure remains an overlooked uptake pathway.

The presence of MPs in the GIT has been the main focus of available literature regarding the occurrence of MPs in fish (Sacco *et al.*, 2024). The presence of MPs in fish GIT was found in museum specimens collected from 1950 onwards (Ilechukwu *et al.*, 2023). Currently, Lopes *et al.* (2023) recently reported MP occurrence in the GIT of fish ranging from 0.93 to 3.45 MP/g, namely the European anchovy (*Engraulis encrasicolus*), European sardine (*Sardina pilchardus*), and Atlantic horse mackerel (*Trachurus trachurus*), collected along the coast of Portugal, where the present thesis was conducted. Most MPs identified in these fish species were fibres, of blue colouration, measured <500 µm, and the most common polymers were fibre-related including cellulose, polyacrylate, and PES. Previous studies have also reported MP ingestion in fish species of commercial interest from Portuguese waters (Barboza *et al.*, 2020b; Bessa *et al.*, 2018; Guilhermino *et al.*, 2021; Lopes *et al.*, 2020; Prata *et al.*, 2022). In the eastern Mediterranean, Eryaşar *et al.* (2024) recently reported the ingestion of MPs in 11 demersal, benthopelagic, and pelagic fish species, ranging from 0.17 ± 0.1 MP/indv in striped seabream (*Lithognathus mormyrus*) and 1.08 ± 0.1 MP/indv in Atlantic mackerel (*Scomber scomber*). Both fibres and fragments represented around 50% of the MPs and these were mostly black (65%) and composed of PE (45%) and PET (31%). Reports of MP ingestion in Mediterranean fish also extends to other commercial species (Capone *et al.*, 2020; Chenet *et al.*, 2021; Rios-Fuster *et al.*, 2022; Solomando *et al.*, 2022). Alongside the review made by Sacco *et al.* (2024), literature's findings indicate that fibres are the most prevalent MP shape; blue, black, and red are the most prevalent MP colours; and PE and PP are the most prevalent polymers in fish populations in the natural environment.

Additionally, MPs may cross biological barriers and travel within the organism through the circulatory system and lymphatic systems, becoming retained in internal organs. It is currently hypothesised that only MPs <150 µm can cross the epithelia and lead to systemic exposure, and that only MPs measuring <1.5 µm penetrate deeper in tissues (EFSA, 2016). Vagner *et al.* (2022) reported that PS-NPs (Ø 50 nm) successfully crossed the intestinal barrier of European seabass (*Dicentrarchus labrax*) in an *ex vivo* trial. Also, it has been experimentally demonstrated that MPs could reach the liver, brain, and muscle (Ding *et al.*, 2018; Zeytin *et al.*, 2020; Zhang *et al.*, 2019a; Zitouni *et al.*, 2021). So far, MP translocation mechanisms remain poorly understood. Fifty years ago, it was hypothesised

that particles measuring up to 110 µm, including PVC-MPs, could cross between intestine's enterocytes through a mechanism referred to as persorption, reaching the mucosal layer and travel in the organism through lymph vessels and mesenteric veins (Volkheimer, 1974, 1975). Since then, other studies have identified endocytosis as an important pathway for cellular uptake of MPs, but likely hindered for MPs measuring >15 µm (Hua & Wang, 2022). Additionally, cellular internalisation by macrophages has been documented and seems to vary due to several factors, such as the presence of an eco-corona (*i.e.*, biomolecules adhered to MP surface), the MP polymer type, and tissue (Abihssira-García *et al.*, 2020; Ramsperger *et al.*, 2020). Nevertheless, the understanding of translocation mechanisms for MPs still remains limited and estimates indicate that only ≤0.3% of particles <150 µm are capable of being absorbed and reach deeper tissue (EFSA, 2016).

Several studies have detected the occurrence of MPs in the internal tissues of wild fish, and there Is a growing concern about their potential health effects, which are further detailed in this chapter. Collard *et al.* (2017) detected for the first time the presence of MPs (Ø 124-438 µm) in 79% of the analysed liver samples from European anchovies, European sardines, and Atlantic herring (*Clupea harengus*) collected in the Mediterranean Sea. Since then, other studies have also reported the presence of MPs in liver samples of commercial fish in other world regions (Abbasi *et al.*, 2018; McIlwraith *et al.*, 2021; Rahman *et al.*, 2024), including Guilhermino et al. (2021) who reported an average MP presence equal to 0.7 ± 0.2 MP/g in the liver of common carp (*Cyprinus carpio*), European flounder (*Platichthys flesus*), and flathead grey mullet (*Mugil cephalus*) collected in Portuguese waters. It has been hypothesised that MPs are retained in liver through the portal vein after which are excreted by exocytosis with the bile (Handy *et al.*, 2008). Furthermore, MPs have also been detected in the brain of red mullet (*Mullus barbatus*) and Pontic shad (*Alosa immaculata*) collected from the Black Sea (Atamanalp *et al.*, 2021), and European seabass (0.3 ± 0.8 MP/indv; 2 ± 6 MP/g) collected from the Douro estuary in Portugal (Barboza *et al.*, 2023). Altogether, current findings suggest that MPs can be found in a wide range of tissues that may potentially lead to systemic effects.

In addition, the presence of MPs had been detected in the muscle of wild specimens of commercial species, many of them already aforementioned, collected from global aquatic environments. For instance, previous studies have reported MP levels in muscle reaching up to 2.9 MP/g in fish from NE Atlantic waters (Akoueson *et al.*, 2020; Barboza *et al.*, 2020b; Guilhermino *et al.*, 2021; Lopes *et al.*, 2023). Similar findings were reported in fish from the Mediterranean Sea, being worthwhile mentioning the bluefin tuna (*Thunnus thynnus*), common sole (*Solea solea*), gilthead seabream (*Sparus aurata*), and swordfish (*Xiphias gladius*) due to their importance for the human consumption (Di Giacinto *et al.*, 2023; Djekoun *et al.*, 2024; Ferrante *et al.*, 2022; Zitouni *et al.*, 2020). It should be noted that

Ferrante *et al.* (2022) found considerably higher MP levels in fish muscle, 95,000 ± 66,400 MP/g, ranging within Ø 1.7-2.8 µm through Scanning Electron Microscopy coupled with Energy Dispersive X-ray Spectroscopy (SEM-EDX), indicating that MP quantification can greatly vary, particularly for smaller particles, with the methodology employed. Furthermore, MP presence in fish muscle has been reported in fish from the Black Sea and Persian Gulf (Abbasi *et al.*, 2018; Ahmadi *et al.*, 2022; Akhbarizadeh *et al.*, 2018; 2019; Atamanalp *et al.*, 2021), and other regions of the world (Dawson *et al.*, 2022; Li *et al.*, 2022b; Selvam *et al.*, 2021). While predators consume the whole fish and ingest all MPs retained in prey, MP presence in muscle is particularly relevant for the human consumer which typically consumes muscle.

Another significant concern regarding MPs is their role as vectors of several threats that disperse in the environment and directly harm living organisms. MPs have chemical additives in their composition that are released as plastics degrade (Costa *et al.*, 2023). Bisphenol A and analogs, and phthalate esters (PAEs) are used in plastic production and classify as xenoestrogens since their properties allow them to bind to estrogen receptors and disrupt the endocrine system (Cao *et al.*, 2022; Czarny-Krzymińska *et al.*, 2023). Higher MP intake has been linked with high levels of bisphenols and PAEs in internal tissues of biota (Barboza *et al.*, 2020a; Rios-Fuster *et al.*, 2022). The high surface area-to-volume ratio and hydrophobic properties of MPs increase their adsorption ability of environmentally available contaminants, such as persistent organic pollutants (POPs), drugs, and non-essential elements (Zambrano-Pinto *et al.*, 2024). For instance, it has been observed the bioaccumulation of POPs in the dorsal muscle of Atlantic salmon and European seabass exposed to spiked LDPE-MPs included in diets (Granby *et al.*, 2024; 2018). Interestingly, both cited studies reported lower bioaccumulation of POPs when exposing fish to diets with virgin MPs and pollutants in the interior and coat of the feed pellets, respectively (*i.e.*, MPs only encountered POPs in GIT). Also, MPs can carry pathogens with potential deleterious effects to heath, such as antibiotic resistance genes, viruses, and harmful bacteria (Seeley *et al.*, 2023; Viršek *et al.*, 2017; Zhang *et al.*, 2022d). For these reasons, MPs pose a persistent and complex environmental challenge with significant risks to the health.

MP effects have been extensively documented in dedicated experimental studies. This is a growing field of study, with approximately 20 experimental studies published by 2018 and reaching over 100 only in 2022, with the most representative fish species being zebrafish (*Danio rerio*) and the most commonly tested MP polymer type being PS (Sacco *et al.*, 2024). Most studies primarily focus on assessing the health effects of different MP concentrations, including pharmacological doses to effectively produce a biological response and evaluate the underlying toxicity mechanisms but may not reflect environmental MP concentrations (Cunningham & Sigwart, 2019). This highlights the need

for further studies to incorporate environmentally relevant conditions in their experimental design to better assess if MP contamination in ecosystems is sufficient to impact the health of aquatic organisms. Nonetheless, considerable knowledge has already been gained on how MPs interact with fish and their potential effects.

Focusing on ingestion, it is essential to understand for how long MPs will remain in the GIT for further determining the average egestion rates and cumulative exposure to which fish are exposed to over a given period or lifetime. This may be also relevant to study MP dynamics at the ecosystem-scale, since, for instance, lower egestion rates in migratory fish may enhance the role of fish as MP vectors across ecosystems (Justino *et al.*, 2022). Previous experimental studies have identified factors influencing MP egestion rates. Different GIT morphology among species seems to interfere with ingestion rates, as observed in previous studies describing lower MP egestion rates in fish with true stomach in comparison to stomachless species (Ohkubo *et al.*, 2020; Roch *et al.*, 2021). Particle characteristics also seem to have an impact on egestion rates although controversial results can be found. Previous studies found no differences in egestion rates of particles with different shapes and sizes, being >90% of MPs passively excreted along with food within 48 h (Grigorakis *et al.*, 2017; Ohkubo *et al.*, 2020). It should be noted that Roch *et al.* (2021) found that smaller MPs (Ø 42.7 µm) had higher egestion times (7.3-12.1 h) in relation with larger particles (Ø 1086 µm) (4.0-4.6 h) in both rainbow trout (*Onchorhynchus mykiss*), and common carp. Moreover, Hoang & Felix-Kim (2020) found similar results with fathead minnows (*Pimephales promelas*) successfully egesting larger MPs within 12 h post exposure (Ø 125-155 µm) but still retaining a significant number of smaller MPs after 24 h (Ø 63-75 µm). This is particularly relevant since small-sized MPs can be retained longer in GIT thus increasing the likelihood of MP exposure-associated health effects.

When in the GIT and gills, MP exposure can lead to several alterations in the tissue and cellular morphology (Banaee *et al.*, 2024). Particularly in the intestine, some of the damages commonly reported include altered *villi* structure (*i.e.*, shortening and widening, disepithelisation, and even beheading), oedematous tissue, vacuolisation of enterocytes, goblet cell hyperplasia, and leukocyte infiltration (Del Piano *et al.*, 2023b; Hao *et al.*, 2023). Histomorphology alterations are closely linked to the duration and degree of exposure, as seen in previous studies reporting potential irreversible damages due to longer and higher exposure concentrations (Espinosa *et al.*, 2019; Pedà *et al.*, 2016). The severity of tissue damage can also be influenced by other factors, such as MP characteristics. For instance, Jabeen *et al.* (2018) and Qiao *et al.* (2019) reported that goldfish (*Carassius auratus*) and zebrafish exposed to MP fibres showed severer intestinal damage than those exposed to MP fragments and spheres. Different polymer types seem also to differ in the degree of intestinal alterations, as Espinosa *et al.* (2019) observed that the intestinal alterations of

European seabass exposed to PE-MPs were likely more related to mechanical abrasion while those exposed to PVC-MPs were more related to chemical injury. Moreover, the severity of histomorphology alterations increases when fish are exposed to MPs spiked with other chemical contaminants, such as PCBs and polybrominated diphenyl ethers (PBDEs) (Espinosa-Ruiz *et al.*, 2023; Montero *et al.*, 2022; Pedà *et al.*, 2016). These structural effects compromise tissue integrity and functionality, ultimately increasing the risk of systemic effects in the fish health.

Studies indicate that MPs can significantly disrupt the normal fish feeding behaviour and foraging efficiency, potentially leading to long-term impacts on the individual fish or the entire population. For instance, Zhang *et al.* (2024b) observed that the exposure of goldfish to MPs significantly led to reduced food-searching behaviour and food intake, and longer feeding period. It was also found the reduction of orexigenic and increase of anorexigenic factors in blood serum, indicating appetite dysregulation. Previous studies also point to alterations in the overall fish behaviour, with MP-exposed fish showing more assertive social interactions (Hollerova *et al.*, 2023; Rios-Fuster *et al.*, 2021b). The presence of chemical contaminants adsorbed to MPs can significantly increase the effects on behaviour, like it was found by Barboza *et al.* (2018d), who reported decreased swimming velocity and resistance to water flow in European seabass exposed solely, or in combination, to benzoguanamine-MPs (0.26-0.69 mg/L) and mercury (0.010-0.016 mg/L). These behaviour alterations may be a result from underlying physiological and neurological issues, leading to reduced food intake and, ultimately, the depletion of energy reserves (Chen *et al.*, 2020b; Yin *et al.*, 2018). For that reason, several studies have already reported MP effects on decreased feed efficiency and growth performance in several commercial fish species, including the Nile tilapia (*Oreochromis niloticus*) (Lu *et al.*, 2022b; Nair & Perumal, 2024), rainbow trout (Roch *et al.*, 2022), marine jacopever (*Sebastes schlegelii*) (Sun *et al.*, 2023b; Yin *et al.*, 2018), and adults of walking catfish (*Clarias batrachus*) (Fatema *et al.*, 2023). Nonetheless, current literature shows controversial results regarding MP effects on growth performance, with several studies reporting no differences between MP-exposed fish and control groups (Del Piano *et al.*, 2023b; Granby *et al.*, 2024; 2018; Hollerova *et al.*, 2023). Given that various factors can influence the degree of the effects, such as exposure duration and concentration, and fish developmental stage, it still remains poorly understood to which MP levels can fish be exposed to without detrimental effects on growth.

The toxic effects of MP exposure have been already extensively investigated, with particular focus on oxidative stress. Previous studies have found a significant relationship between higher MP levels and altered oxidative stress status in wild fish populations (Barboza *et al.*, 2020b; Cohen-Sánchez *et al.*, 2023; Solomando *et al.*, 2022). Upon exposure, MPs disrupt the normal functioning of mitochondria, which is an important part of

cellular energy metabolism, by increasing the free radical formation (Hua & Wang, 2022). This may lead to increased production of reactive oxygen species (ROS) if these surpass the antioxidant scavenging capacity of tissues, ultimately could result in damage to cellular components such as proteins, lipids, and DNA (Das, 2023). Due to their larger surface area and likelihood of cellular internalisation, smaller MPs seem to have an enhanced ability to induce oxidative damage and apoptosis, as demonstrated in previous studies with fish (Abarghouei *et al.*, 2021; Bobori *et al.*, 2022a). Degraded MPs may also lead to higher oxidative stress than virgin MPs because of free radicals present in the MP surface resultant from degradation processes (Ge *et al.*, 2023), as demonstrated in previous studies showing that aged MPs induced higher impact on the activities of antioxidant and detoxification enzymes of fish in comparison to virgin MPs (Michailidou *et al.*, 2024; Zhao *et al.*, 2024). Indeed, degraded MPs often bind to other chemical contaminants which synergistically enhance the adverse effects on the antioxidant and detoxification pathways. For instance, Wei *et al.* (2023) observed that the co-exposure to MPs and cadmium led to enhanced lipid peroxidation levels and the reduced activity of antioxidant enzymes, such as superoxide dismutase and catalase (CAT), in the liver of crucian carp (*Carassius carassius*). This reduction may be explained by the depletion of antioxidant enzymes after excessive ROS production. Similarly, Hoyo-Alvarez *et al.* (2022) observed that the exposure to environmentally spiked MPs enhanced the oxidative stress status, indicated by the higher activity of CAT and glutathione-S-transferase in the brain of gilthead seabream. The resultant imbalance of oxidative stress further contributes to metabolic irregularities leading to a cascade of effects on fish health.

MP exposure also leads to other systemic effects, including disruption of energy and lipid metabolism, immune response modulation, neuromuscular toxicity, and even reproductive and transgenerational effects. For instance, Del Piano *et al.* (2024) reported significantly higher hepatosomatic index (HSI) in gilthead seabream exposed to 25-250 mg PS-MPs per kg of feed (Ø 1-20 μm). This phenotypical biomarker resulted due to excessive lipid accumulation (*i.e.*, hepatic steatosis) and higher inflammatory response, leading to the onset and progression of hepatic metabolic disorders. Other studies have reported similar effects in the hepatic metabolism of MP exposed fish (Du *et al.*, 2023; Lai *et al.*, 2021). Regarding immunotoxicity, current literature indicates the up- and downregulation of the immune activity depending on the exposure concentration and duration (Li *et al.*, 2024a). Recently, Espinosa-Ruiz *et al.* (2023) reported the overall suppression of the immune response in the head kidney of European seabass exposed to 100 mg of PE-MPs per kg of feed (Ø 150-500 μm). Interestingly, it was observed an upregulation of the immune response in the gut, possibly indicating a compensatory response through the mobilisation of immune cells to tissues with higher inflammation. However, chronic inflammation may

decrease the immune system response to pathogens, becoming susceptible to diseases (González-Fernández & Cuesta, 2022; Seeley *et al.*, 2023; Yang *et al.*, 2024). Several studies have also documented the loss of cholinergic signalling in the neuromuscular system (*i.e.*, inhibition of AChE activity), possibly leading to the disruption of motor function and behavioural patterns (Barboza *et al.*, 2018c; Choi *et al.*, 2023; Iheanacho & Odo, 2020). Neurotoxic effects associated with higher MP levels have also been documented in wild fish populations (Barboza *et al.*, 2020b; 2023; Zitouni *et al.*, 2022). Furthermore, fish exposed to MPs have shown reduced fecundity and developmental effects, possibly due to MP transfer to offspring, reduced gamete quality, and epigenetic changes (Yi *et al.*, 2024). These combined effects highlight the multiple fish health risks associated to MP exposure, underscoring the need for further research and environmental management strategies to mitigate the impact of MPs on aquatic ecosystems.

1.3. Microplastics in aquaculture: an environmental threat to food safety?

Fish constitutes a crucial component of a healthy human diet, providing high-quality protein, vitamins (*e.g.*, A, B-complex, D) and minerals (*e.g.*, iodine, iron, selenium, zinc) (FAO, 2023; Tacon *et al.*, 2020). Another nutritional benefit associated with consuming fish is their high levels of omega-3 polyunsaturated fatty acids (*n*-3 PUFAs), such as the eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Virtually absent from terrestrial-based foods, both EPA and DHA play important roles in regulating cardiovascular function and stimulating cognitive development (Zhang *et al.*, 2019b). The awareness of benefits associated with EPA and DHA intake has contributed to an increase in the global demand for seafood products rich in these *n*-3 PUFAs (FAO, 2023; Tacon *et al.*, 2020). However, wild fish stocks are currently threatened by multiple environmental factors, such as overexploitation, habitat degradation, and climate change, making aquaculture a more stable alternative to support food security as wild populations decline (FAO, 2024). For the first time, global aquaculture production reached a new record of 94.4 MMT, surpassing capture fisheries (91.0 MMT) in live-weight volume (FAO, 2024).

Global aquaculture production has steadily increased over the past decades (Figure 1.2), currently supplying over 57% of the aquatic organisms, namely fish, shellfish, and molluscs, destined for human consumption (FAO, 2024). Within global aquaculture, the marine and inland aquaculture represented 35.6 and 58.8% of production, respectively. Asia remains the leading producer, with China contributing to the majority of this output, harvesting a total of 83.4 MMT of aquatic organisms. Europe is the second-largest producer, harvesting 3.6 MMT of aquatic organisms. Particularly in Europe, Norway, UK, Spain, France, and Greece are the major contributors with the farming of marine fish such as

salmonids (*e.g.*, Atlantic salmon and rainbow trout), as well as the European seabass and gilthead seabream (EUMOFA, 2023; FEAP, 2023).

Particularly in Mediterranean countries, the economic importance of aquaculture has accompanied the increasing trend in production (FEAP, 2023). The European seabass and gilthead seabream remain the dominant marine fish species, representing 46.2 and 44.7% of the total production in 2022 (EUMOFA, 2023; FEAP, 2023). The production systems in Mediterranean are highly diversified due to a wide range of geographical, historical, and socio-economic factors (Massa *et al.*, 2017). For instance, European seabass and gilthead seabream are commonly reared in cages located along the coast but also in earthen ponds usually located in salt marshes (Briones-Hidrovo *et al.*, 2023; Papageorgiou *et al.*, 2021). Nonetheless, innovative technologies and more sustainable practices are being integrated to prepare aquaculture for environmental challenges. For instance, the adoption of complex production systems such as recirculating aquaculture systems (RAS), which are land-based intensive facilities with minimal water use and good water quality, is increasing globally (Cooney *et al.*, 2023), enabling the production of high quantities of fish closer to end consumers (Lindholm-Lehto, 2023).

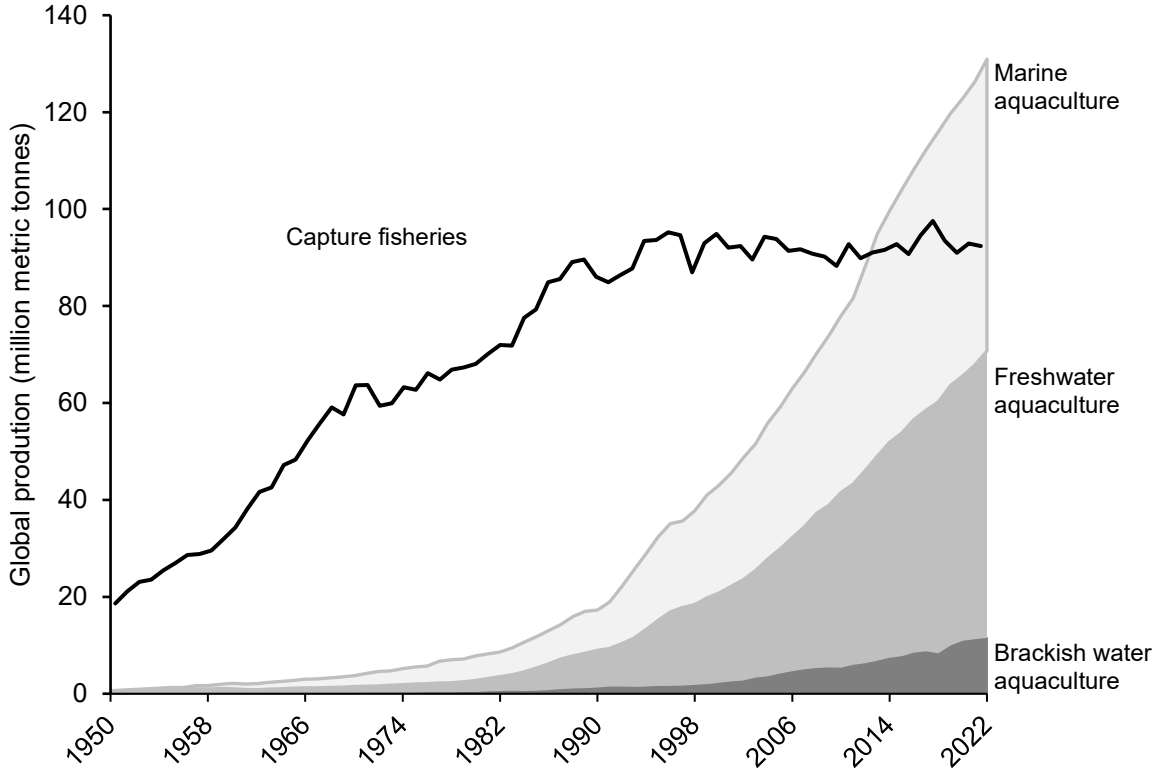

Figure 1.2. Global production from capture fisheries (black) and aquaculture (grey) from 1950 to 2022. The aquaculture production in marine, brackish, and freshwater environments is presented cumulatively by different shades of grey. Data obtained from FishStatJ, includes production of algae and seaweeds, crustaceans and molluscs, and fish and reptiles.

Under the UN's 2030 Agenda for Sustainable Development (UN, 2015), aquaculture advancements focus on both preparing seafood production to climate-related uncertainties and mitigation of aquaculture-associated environmental impacts. For instance, alternative feed ingredients, such as plant- and insect-based meals, have been adopted to reduce the dependence on wild fish-based fishmeal and fish oil (Jia *et al.*, 2022a; Monteiro *et al.*, 2024; Napier & Betancor, 2023). Fishmeal and fish oil derived from by-products is also increasing, representing 34 and 53% of total production in 2022 (FAO, 2024). Moreover, research on vaccination and functional feed additives has significantly improved disease management by reducing the use of antibiotics and boosting fish immunity with the inclusion of pre- and probiotics in aquafeeds (Hoseinifar *et al.*, 2024). The ongoing commitment of aquaculture to reduce the ecological footprints reflects the need to ensure stable and nutritious food sources for a growing global population (FAO, 2024).

1.3.1. Microplastic contamination sources in aquaculture

Aquaculture production systems often rely on plastic equipment, such as nets, cages, buoys, and ropes, which degrade over time and release MPs into aquatic environments (Miao *et al.*, 2023). Despite the efforts to minimise environmental impacts, plastic pollution is a growing concern for the aquaculture industry. As detailed in Table 1.1, aquaculture plastic gear is made from a wide range of polymers (*e.g.*, nylon, PE, PP), and different production systems also vary in gear requirements (Huntington, 2019). Aquaculture constitutes a challenging environment for plastic gear, due to its chemical and physical characteristics, such as high salinity, mechanical abrasion, UV radiation exposure, interaction with biota, among others. These conditions seem to increase fragmentation, leading to higher MP release to the surrounding environment (Mackay & Ridge, 2022; Napper *et al.*, 2022). Previous studies have identified aquaculture-derived MPs in surface water and sediments in areas with high aquaculture activity, and linked the source to aquaculture equipment based on the similarity between MPs and plastic materials used in the facilities (Chen *et al.*, 2020a; 2018; Lee *et al.*, 2013; Sui *et al.*, 2020b).

Aquafeeds have also been identified as an important source of MP contamination (Su *et al.*, 2024). During 2021 and 2022, the beginning of this thesis, many studies reported the presence of MPs in fishmeal products (from 0.03 to 17.3 MP/g) (Gündoğdu *et al.*, 2021; Karbalaei *et al.*, 2020; Thiele *et al.*, 2021; Wang *et al.*, 2022c; Yao *et al.*, 2021). Fishmeal is a key protein ingredient in aquafeeds, particularly for carnivorous fish species. It can be produced from whole fish or fish by-products, such as heads, viscera, gills or bones. These tissues, particularly GIT and gills, are likely to contain higher MP levels (Lopes *et al.*, 2023; Vazirzadeh *et al.*, 2025). Only more recently, MP contamination has also been reported in aquafeeds, ranging from 0.03 to 11.6 MP/g (Devi *et al.*, 2024; Egea-Corbacho *et al.*, 2023;

Mohsen *et al.*, 2024a; Muhib & Rahman, 2023; Siddique *et al.*, 2023; Zhou *et al.*, 2024). Also, MP contamination has been found not only in other animal-based ingredients, such as poultry by-products (2.85 MP/g) (Rimoldi *et al.*, 2024), but also in plant-based products, such as soybean meal (0.69-1.23 MP/g), rice bran (0.55 MP/g), wheat bran (0.07 MP/g), and wheat flour (0.17 MP/g) (Mohsen *et al.*, 2024b; Walkinshaw *et al.*, 2022). Notably, MP contamination in aquafeeds produced across countries, mainly depends on the background levels of contamination of raw materials and on the manufacturing conditions. For instance, fishmeal and aquafeeds produced using fish collected from polluted fishing grounds, such as southern Asia, tends to have high MP levels (Devi *et al.*, 2024; Gündoğdu *et al.*, 2021; Mohsen *et al.*, 2024b; Wang *et al.*, 2022c; Yao *et al.*, 2021). Furthermore, MPs can be introduced during several stages of aquafeed manufacturing and packaging, highlighting the importance of monitoring MP contamination throughout the process for ensuring the production of safer fish products (Gündoğdu *et al.*, 2021; Thiele *et al.*, 2021).

Table 1.1. Plastic components and most common polymer composition of microplastics (MPs) collected from different fish aquaculture production systems. Table content adapted from Huntington (2019).

Plastic components in aquaculture	Most common polymers
Open water cages	
Floating collars (including handrails)	HDPE, PVC
Collar floatation	EPS
Buoys (in mooring systems)	HDPE, LDPE, PE
Ropes (in mooring systems)	Nylon, PE, PET, PP
Net enclosures	HDPE, Nylon, PP, UHMWPE
Predator and other nets	HDPE, Nylon, PE
Feeding systems (pipes and hoppers)	FRP, HDPE, PVC
Coastal and inland ponds	
Pond liners	HDPE, LLDPE, LDPE
Harvesting nets	HDPE, Nylon, PP, UHMWPE
Plastic greenhouse	LDPE
Aerators/pumps	HDPE, PVC
Feeding systems (pipes, feeders, and trays)	FRP, HDPE, PVC
Tank-based systems (including RAS)	
Stock holding tanks	FRP, HDPE
Pipework (connectors and valves)	FRP, HDPE, PVC

EPS, expanded polystyrene; FRP, fibre-reinforced plastic; HDPE, high-density polyethylene; LDPE, low-density polyethylene; LLDPE, linear low-density polyethylene; PE, polyethylene; PP, polypropylene; PVC, polyvinyl chloride; UHMWPE, ultra-high molecular weight polyethylene

Aquaculture environments are also susceptible to external contamination sources, such as the inflow of MP contaminated water. Several studies have already identified the occurrence of MPs in water and sediment samples from regions with high aquaculture activity worldwide (Table 1.2). To date, the highest MP levels in aquaculture have been recorded in farms located in the southern coast of Asia, particularly in farming ponds where level can range from 6.6-263.6 MP/L and 3.3-2500 MP/kg in water and sediment, respectively (Table 1.2) (Hossain *et al.*, 2023a; Li *et al.*, 2021e; Ma *et al.*, 2020; Yu *et al.*, 2023a). Moreover, MP contamination may differ between production systems. Huang *et al.* (2023) observed that water in cement and earthen ponds contained significantly higher levels of MPs (10.09 and 13.81 MP/L, respectively) compared to RAS and indoor aquarium water (1.67 and 2.47 MP/L, respectively). The study hypothesised that this difference was due to the more complete and efficient filtration equipment in RAS. Other studies have also documented differences between aquaculture production systems (Lv *et al.*, 2020; Song *et al.*, 2024). However, the relationship between MPs and aquaculture facilities is extremely complex due to the variability of the surrounding environment, and leads to interactions between MPs and farmed organisms which may be potentially harmful (Miao *et al.*, 2023; Wu *et al.*, 2023a).

Table 1.2. Studies reporting microplastic (MP) occurrence in marine aquaculture environments, namely water and sediment (in dry weight) samples. Data included indicates MP concentration and the most predominant MP types (shape, colour, size, and polymer).

Location	Sample type	Concentration	Predominant MP types	References
Brazil				
Espirito Santo (bivalve mariculture)	surface water	53.7 ± 14.8 MP/L	fibres (74%), black (56%), 0.1-0.35 mm (~32%)	Bom *et al.* (2022)
Bulgary				
Black Sea coast	surface water	7.3 ± 4.9 MP/L (1.3-4.9 MP/L)	fibres (73.3%), transparent (38%)	Georgieva *et al.* (2023)
Canada				
Halifax Harbour	beach and tide sediments	2.0-8.0 MP/g	fibres (*n.s.*)	Mathalon & Hill (2014)
Chile				
Inner Sea of Chiloé (salmon farming)	sediment	72.2 ± 32.4 MP/kg	fibres (88%), white (40%), cellulose (45%)	Jorquera *et al.* (2022)
China				
Shandong province Laizhou Bay	surface water	858.3 ± 573.2 MP/m^3 (30.2320 MP/m^3)	fibres (99.3%), transparent (66.7%), 1-2 mm (28.5%), rayon (77.5%)	Chen *et al.* (2024)
	sediment	151.0 ± 77.4 MP/kg (50-300 MP/kg)	fibres (91.7%), transparent (32.2%), 0.5-1 mm (27.9%), rayon (73.7%)	
Yantai (mariculture area)	surface water	144.3 ± 42.5 MP/L	fragments (*n.s.*), <50 µm (85.2%), PA (74.3%)	Li *et al.* (2024b)
	offshore sediment	100.0 ± 56.8 MP/g	fragments (*n.s.*), <50 µm (87%), PVC (19.0%)	
Weihai (mariculture area)	surface water	11.49 MP/m^3	fragments (~45%), transparent (~42%), <300 µm (36.8%), PE (~40%)	Zhang *et al.* (2021e)
Yantai & Weihai coast	surface water	*n.s.*	fibres (*n.s.*), transparent (30-60%)	Sui *et al.* (2020a)
Sanggou Bay (mariculture area)	sediment	1674 ± 526 MP/kg (699-2824 MP/kg)	pellets (32.3-86.7%), transparent (64.7-95.2%), <0.5 mm (75.0-97.1%), PE (43.3-60.6%)	Sui *et al.* (2020b)
Laizhou Bay	surface water	1.7 ± 1.5 MP/m^3 (0.1-6.7 MP/m^3)	fibres (96.1%), 1660.0 ± 1310.4 µm, PET (32.8%)	Teng *et al.* (2020)
	sediment	461.6 ± 167.0 MP/kg (193-1053 MP/kg)	fibres (94.1%), 876.8 ± 1027.5 µm, cellophane (85.4%)	

Table 1.2. Continuation (part 2/4).

Location	Sample type	Concentration	Predominant MP types	References
China				
Shandong province Yellow & Bohai Sea	sediment	20-1040 MP/kg	fibres (97%), blue (48%), ≤1 mm (82%), cellophane (*n.s.*)	Mohsen *et al.* (2019)
Shanghai area (fish, prawn, crab farms)	pond water	36.3 ± 6.8 MP/L	fibres (40.9%), black (*n.s.*), <1 mm (79.4%), PE (55%)	Huang *et al.* (2024)
	sediment	271.7 ± 164.8 MP/kg	fibres (58.6%), transparent (*n.s.*), 0.3-3 mm (*n.s.*), PE (34%)	
(SHOU aquaculture station)	RAS water	1.67 MP/L	fibres (77.6%), black (56.9%), 300-1000 µm (*n.s.*), rayon (60.9%)	Huang *et al.* (2023)
	aquarium water	2.47 MPL	fibres (85.9%), black (47.5%), 50-300 µm (*n.s.*), PP (47.8%)	
	cement pond water	10.09 MP/L	fibres (43.6%), blue (37.7%), 300-1000 µm (*n.s.*), PP (41.5%)	
	earthen pond water	13.81 MP/L	fibres (68.1%), blue (40%), 3000-5000 µm (*n.s.*), PP (38.2%)	
Yangtze estuary	pond water	4.4-10.8 MP/L	fibres (66.8%), blue (32.0%), 1-3 mm (33.5%), PE & PET (45.7% each)	Yu *et al.* (2023a)
	sediment	286-543 MP/kg	fibres (54.8%), blue (44.4%), 0.1-0.3 mm (33.6%), PE (43.6%)	
Guangdong province Dapeng Cove	surface water	1333 ± 773 MP/m^3	fibres (65.4%), <1 mm (69.6%), PE (40.6%)	Xu *et al.* (2024b)
	sediment	1381 ± 945 MP/kg	fibres (52.1%), <1 mm (65.2%), PP (39.1%)	
Zhanjiang Bay	surface water	0.37 ± 0.57 MP/m^3 (0-2.65 MP/m^3)	fragments (51%), white (51%), <3 mm (81%), PE (20%)	Chen *et al.* (2022a)
Xiangshan Bay	surface water	33.3-240.0 MP/kg	fibres (64.9%), colour (*n.s.*), ≤2 mm (84.4%), rayon (42.5-78.4%)	Yu *et al.* (2022a)
	sediment	0.03-0.70 MP/m^3	foam (64.4%), ≤2 mm (75.6%), PS (66.9%)	
Pearl River estuary (fish & shrimp farms)	pond water	6.6-263.6 MP/L	fibres (98.04%), transparent (90.7%), <0.5 mm (42.8%), celulose	Li *et al.* (2021e)
	sediment	566.7-2500.0 MP/kg	fibres (81.7%), transparent (62.7%), <0.5 mm (*n.s.*), PP (25%)	
Pearl River estuary (pond culture area)	pond water	10.3-87.5 MP/L	fibres (68.1-78.9%), blue (*n.s.*), <1 mm (56.3-87.7%), PP (*n.s.*)	Ma *et al.* (2020)
Xiangshan Bay	sediment	73.9 ± 30.4 MP/kg (33-113 MP/kg)	fibres (97.7%), 500-2500 µm (72.5%), cellulose (60-88%)	Wu *et al.* (2020)
Guangxi province Beibu Gulf	sediment	4765 ± 116 MP/kg	fibres (48.4%), black (39.8%), 20-50 µm (51.2%), PA (31.5%)	Liu *et al.* (2023a)
Maowei Sea (oyster farms)	surface water	1.47-7.61 MP/L	fibres (~82%), blue (~57%), 1-5mm (~56%), PET (~60%)	Zhu *et al.* (2021)
Zheijang province Lianyungang (shrimp farms)	pond water	0.57-6.47 MP/L	fibres (74.0%), black-grey (~51.6%), <1 mm (~62.8%), cellophane (39.1%)	Song *et al.* (2024)
	tank water	0.35-2.06 MP/L	fibres (74.3%), black-grey (~56.7%), <1 mm (~69.3%), PE (36.9%)	
Lianyungang (bivalve farms)	inland water	6.03 ± 4.95 MP/L	fibres (66.8%), black-grey (61%), <1 mm (68.9%), cellophane (33.1%)	Song *et al.* (2023)
	coastal water	4.76 ± 2.99 MP/L	fibres (50.4%), black-grey (74.1%), <1 mm (64.6%), cellophane (33.5%)	
	marine water	2.37 ± 1.83 MP/L	fibres (87.5%), blue-green (51.4%), <1 mm (73.9%), PET (48.3%)	
Hangzhou Bay	surface water	1.8 ± 1.0 MP/m^3 (0.8-9.6 MP/m^3)	fibres (42%), PE (47%)	Qu *et al.* (2022)
	sediment	106 ± 55 MP/kg (44-208 MP/kg)	fibres (57%), cellulose (60%)	
Fujian province Longjiao Bay	surface water	250-5150 MP/m^3	fibres (41.4%), white (25-75%), 0.3-5 mm (*n.s.*), PET (30.2%)	Chen *et al.* (2020a)
	RAS water	58-72 MP/m^3	fibres (*n.s.*), 0.2-2 mm (*n.s.*), PET (*n.s.*)	Lu *et al.* (2019)
n.s.	RAS water	1.53 ± 0.21 MP/L	fibres (77.2%), blue (*n.s.*), 20-500 µm (*n.s.*), PA (38.8%)	Zhou *et al.* (2024)
Colombia				
Caribbean coastal lagoon	surface water	0-0.3 MP/L	fibres (*n.s.*), blue (*n.s.*), PE (~25%)	Garcés-Ordóñez *et al.* (2022)
	sediment	0-3.1 MP/kg	fibres (*n.s.*), colourless (*n.s.*), HDPE (~50%)	

Table 1.2. Continuation (part 3/4).

Location	Sample type	Concentration	Predominant MP types	References
Fiji				
Laucala Bay	surface water	0.8 ± 0.2 MP/L	fibres (73%*), black (42%*), 1.6-3 mm (36%*), nylon (31%*)	Vanukon *et al.* (2024)
French Polynesia				
Tuamotu Archipelago lagoons	surface water	0.2-8.4 MP/m^3	fibres (~84.5%), black/grey (40%), 20-200 µm (70%), PE (36.8%)	Gardon *et al.* (2021)
	water column	14.0-716.2 MP/m^3	fragments (~90%), black/grey (45.4%), 20-200 µm (93.2%), PE (29.2%)	
Greece				
North Aegean Sea Thermaic Gulf	surface water	1.9 ± 2.0 MP/m^3 (750.8 ± 838.0 MP/km^2)	fragments (67%), white (45%), 1-1.5 mm (~22.1%), PP (27.5%)	Kermenidou *et al.* (2023)
	sediment	733.6 ± 136.6 MP/m^2	fragments (83%), white (33%), <0.3 mm (~59.5%), PP (42%)	
NW Ionian Sea Igoumenitsa	surface water	*n.s.*	fragments & films (30% each), PE (40%)	Miserli *et al.* (2023)
India				
Chilika lake	sediment	440 ± 3.53 MP/kg wet (1-70 MP/kg wet)	fibres (48%size), black (38.8%), 50-500 µm (*n.s.*), HDPE (37%)	Kumar *et al.* (2024)
	surface water	0.6 ± 0.3 MP/L (0.26-1.10 MP/L)	foams (70%), white/pale (83.4%), <1 mm (53.9%), PP (28.8%)	Singh *et al.* (2023)
	sediment	14.9 ± 6.5 MP/kg (2.2-25.2 MP/kg)	fragments (35.1%), coloured (44.2%), 2-5 mm (33.2%), PS (61.2%)	
Gulf of Mannar	surface water	54-619 MP/L	fragments (40.9%), blue (41.9%), 0.5-1mm (31.1%), PE (49.8%)	Keerthika *et al.* (2022)
	sediment	32-232 MP/kg	fragments (52.7%), blue (52.3%), 0.5-1mm (44.0%), PE (47.6%)	
Italy				
Lagoon of Venice	sediments	672-2175 MP/kg	fragments (86&), <100 µm (55.3%), PE (48.4%)	Vianello *et al.* (2013)
Indonesia				
Gresik, East Java	pond water	7.13-10.40 MP/L	fragments (34%), yellow (20%), 100-500 µm (*n.s.*)	Anjeli *et al.* (2024)
	pond sediment	0.91-1.15 MP/g	fragments (47%), black (21%), <100 µm (*n.s.*)	
Jakarta Bay	pond water	90.7 ± 17.4 MP/L	fibres (*n.s.*)	Priscilla & Patria (2019)
	pond sediment	82.48 ± 11.23 MP/g	fibres (*n.s.*)	
Iran				
Persian Gulf	farm water	955 ± 114 MP/L	fibres (50%), grey-black (45.8%), ≥1000 µm (33.3%), PUR (18.6%*)	Vazirzadeh *et al.* (2025)
	sediment	34 ± 17 MP/kg	fibres (73%), grey-black (43.2%), ≥1000 µm (41.3%), PUR (18.6%*)	
Malaysia				
Kerteh Estuary	pond water	127.9 ± 15.0 MP/L (860.4-196.0 MP/L)	fibres (66.7%), transparent (60.4%), 50-500 µm (47.7%)	Hossain *et al.* (2023b)
	pond sediment	47.5 ± 11.9 MP/g (37.1-57.8 MP/g)	fibres (77.7%), transparent (57.4%), >1-5mm (47.8%)	
Johor Strait Estuary	sediments	29-60 MP/kg	black & red (32% each), fragments (79%), 0.1-0.5 mm (63%), PP (*n.s.*)	Zin *et al.* (2023)
Philippines				
Butuan Bay	surface water	0.2-1.0 MP/L	fibres (36%*), blue (43%*), EVA (54%*)	Similatan *et al.* (2023)
South Korea				
Jinhae Bay (aquaculture site)	sediment	5121 ± 2428 MP/kg (2916-8796 MP/kg)	fragments (86.6%), PP (47.6%)	Eo *et al.* (2023)
Spain				
n.s.	RAS water	>257.7 ng/L	PI (66.7%)	Blonç *et al.* (2023)
Cadiz	RAS water	0-32.5 MP/L	63-100 µm (*n.s.*), PE (38.6%)	Egea-Corbacho *et al.* (2023)
Balearic Islands	surface water	0.27 ± 0.14 MP/m^2	n.a.	Capó *et al.* (2021b)
Thailand				
Phetchaburi coast	surface water	16.8 ± 7.5 MP/L	fragments (71%), 0.3-0.5 mm (38.0%), PP & HDPE (41% each)	Imasha & Babel (2023)

Table 1.2. Continuation (part 4/4).

Location	Sample type	Concentration	Predominant MP types	References
Thailand				
Sriracha Bay	surface water	41.5 ± 6.0 MP/L	fragments (79%), 50-300 µm (32.5%), HDPE (30%)	Imasha & Babel (2023)
	sediment	474.6 ± 102.6 MP/kg	fragments (59%), 0.05-0.3 mm (38.0%), HDPE (40%)	
Bandon Bay	surface water	0-2.8 MP/L	fibres (98.4%), <1 mm (68.3%)	Chinfak *et al.* (2021)
	sediment	5-160 MP/kg	fibres (92.9%), <1 mm (59.3%)	
Chao Phraya estuary	surface water	48 ± 8 MP/m^3	fragments (~70%), white (~49%*), 50-300 µm (~41%*), PP (~65%)	Ta & Babel (2023)
	sediment	37 ± 14 MP/kg	fragments (70%), white (49%*), 50-300 µm (~41%*), PVC (40%)	
Chao Phraya estuary	surface water	48 ± 8 MP/m^3	fragments (65-72%), white (*n.s.*), 50-300 µm (*n.s.*), PP (~70%)	Ta & Babel (2020)
	sediment	39 ± 14 MP/kg	fragments (60-70%), white (*n.s.*), 50-300 µm (100%), PE (50%)	
USA				
Niantic Bay, CT	surface water	0.18 ± 0.10 MP/L	fibres (54.5%), ~100-150 µm (*n.s.*), PES (27.3%)	Mladinich *et al.* (2024)
Vietnam				
Cha Va estuary	surface water	9-27 MP/m^3	fibres (77-92%), blue (~45-90%), PP (21.8%*)	Dao *et al.* (2023)
	sediment	3300-8000 MP/kg	fibres (97-100%), blue (80-90%), PP (21.8%*)	
Mekong river delta	surface water	53.8 ± 140.7 MP/m^3	fibres (85%), blue (59.5%), PE & PP (67%)	Kieu-Le *et al.* (2023)

Abbrv. EVA, ethylene vinyl-acetate; HDPE, high-density polyethylene; PA, polyamide; PE, polyethylene; PES, polyester; PET, polyethylene terephthalate; PI, polyisoprene; PP, polypropylene; PVC, polyvinyl chloride; PUR, polyurethane.
*Value encompasses data from other sample types

1.3.2. Microplastic contamination in aquaculture organisms

MP ingestion has been also documented in farmed aquatic organisms. For instance, significantly higher MP levels (~375 MP/5 indv) were found in the edible tissues of farmed blue mussel (*Mytilus edulis*) than wild specimens (~170 MP/5 indv) (Mathalon & Hill, 2014). Similar results were found by Davidson & Dudas (2016) reporting that farmed Manila clams (*Venerupis philippinarum*) had higher MP levels (11.3 ± 6.6 MP/indv, 1.7 ± 1.2 MP/g) than wild clams (8.4 ± 8.5 MP/indv, 0.9 ± 0.9 MP/g). Contrarily, Tunçelli & Erkan (2024) reported lower MP levels (~3 ± 1 MP/indv, ~4.5 ± 2 MP/g) in farmed Mediterranean mussels (*Mytilus galloprovincialis*) than in wild mussels (~8 ± 3 MP/indv; ~6.5 ± 2.5 MP/g). Other studies found no difference in MP levels between farmed and wild bivalves (Covernton *et al.*, 2019; Renzi *et al.*, 2018). Additionally, My *et al.* (2023) reported higher MP levels (7.7-8.6 MP/indv) in farmed shrimp (*vs.* wild shrimp; 2.3 ± 0.7 to 2.5 ± 0.5 MP/indv). Also, Oliveira *et al.* (2020) reported higher MP levels in the GIT and digestive gland (0.14 ± 0.04 MP/g) of wild cuttlefish (*Sepia officinalis*) in relation to farmed specimens (0.06 ± 0.02 MP/g). More specifically in fish, similar controversial results are also present in the available literature. For instance, Song *et al.* (2022) found high levels of MPs in wild and farmed fugu (*Takifugu bimaculatus*) (98 and 100%, respectively), with MPs being more prevalent in the gills of wild fugu and in the GIT of farmed fugu (Song *et al.*, 2022). Cheung *et al.* (2018a) observed that only 16.7% of farmed flathead grey mullets contained MPs in GIT (0.2 ± 0.5 MP/indv), in comparison to

60% wild specimens (4.3 ± 14.5 MP/indv). Similarly, Garcia *et al.* (2021) reported that only 44% of the farmed Nile tilapia contained MPs in relation to 75% wild individuals of other species. Sultana *et al.* (2023) observed in several freshwater fish species from Bangladesh, detailed in Table 1.3, that 66.7% of farmed fish contained MPs in GIT (0.9 ± 0.8 MP/indv), in comparison to 88.4% of wild fish (1.64 ± 1.15 MP/indv). Moreover, Chan *et al.* (2019) found no significant differences in MP occurrence in the GIT between farmed star snapper (*Lutjanus stellatus*) (58%; 2.0 ± 1.1 MP/indv) in relation to wild fish species collected in the surrounding environment (54-67%; 1.9 ± 1.2 MP/indv to 3.2 ± 4.1 MP/indv).

In addition, since MP contamination varies across aquaculture production systems in geographic proximity (Table 1.3), different MP levels are expected to be found in farmed aquatic organisms. For instance, Lv *et al.* (2020) reported no significant difference on the MP levels found in tissues of Asian swamp eel (*Monopterus albus*) produced either in recycled water, cement pond, or net-cage systems in China (2.4 ± 0.8 MP/indv; 0.02 ± 0.01 MP/g). Although no differences were found in sediment samples among aquaculture production systems (from 16.1 ± 5.4 to 27.1 ± 4.7 MP/kg), the cited study found significantly high MP levels in wastewater samples of net-cage and rice-field systems (Lv *et al.*, 2020). Also, Song *et al.* (2024) reported higher MP levels in 2-month-old whiteleg shrimp (*Penaeus vannamei*) produced in a pond aquaculture in comparison to specimens from a RAS facility. This difference was not found in 4-month-old specimens, corresponding to the harvest stage (Song *et al.*, 2024). It was hypothesised that MP levels in the pond aquaculture system were highly influenced by the contamination levels from supplying water bodies since water is regularly added to the pond to compensate for evaporation and result in MP build-up.

The occurrence of MPs in aquaculture organisms, particularly fish, was a significant gap of knowledge during the first half of the present thesis until 2022 (Chen *et al.*, 2021; Zhou *et al.*, 2021a). As detailed in Table 1.3, available studies focused on MP occurrence in the GIT of marine and freshwater fish produced in Asian farms. There were also studies reporting MP ingestion by wild fish specimens collected at locations with high aquaculture activity, highlighting this sector as a potential source of MP contamination for surrounding environments (Feng *et al.*, 2019; Zhu *et al.*, 2019). In South America, one study was published reporting MP ingestion in Nile tilapia produced in freshwater ponds in Colombia (Garcia *et al.*, 2021). In Europe, three studies were already available regarding MP ingestion in farmed specimens of commercially important fish, namely the European seabass, gilthead seabream, and common carp (Reinold *et al.*, 2021; Sánchez-Almeida *et al.*, 2022; Savoca *et al.*, 2021). Meanwhile other studies have contributed to this important topic, highlighting the high frequency (>80%) of individuals with at least one MP recovered from GIT and reaching up to 546 ± 52 MP/g as reported by Liu *et al.* (2023a) (Table 1.3).

Table 1.3. Studies reporting microplastic (MP) occurrence in farmed fish. The values presented indicate the frequency of positive samples from where MPs were recovered (%), the MP concentration, and the most predominant MP type (shape, colour, and polymer).

Species/Location and production system	*n*	Tissue	%	MP concentration	Predominant MP type	Reference
Chanos chanos (milkfish)						
Butuan Bay, Philippines (fish cages)	30	GIT	96.7	5.40-10.3 MP/indv *0.17-0.34 MP/g*	fibres (54%), blue (43%), EVA (54%)	Similatan *et al.* (2023)
South Sulawesi, Indonesia (fishponds)	50	GIT	92	3.5 ± 2.9 MP/indv	fibres (92.6%), blue (40%), <1 mm (51%)	Amelinda *et al.* (2021)
Jakarta Bay, Indonesia (fishponds)	12	GIT	*n.s.*	*2.09-3.01 MP/indv* *8.80-9.8 MP/g*	fibres (*n.s.*)	Priscilla & Patria (2019)
Cirrhinus molitorella (mud carp)						
Guangzhou, China (fishponds)	16	GIT	87.5	0.89 ± 0.93 MP/g	fibres (88-98.9%*), blue (87%*), 1-2 mm (23.2-44.8%*), PP & PE (18.8% each*)	Aiguo *et al.* (2022)
	16	gills	87.5	2.72 ± 4.07 MP/g		
	16	sum	*n.s.*	11.0 ± 11.0 MP/indv *1-40 MP/indv* 0.10 ± 0.11 MP/g *0.01-0.46 MP/g*		
Ctenopharyngodon Idella (grass carp)						
Guangzhou, China (fishponds)	15	GIT	93.3	19.33 ± 8.98 MP/g	fibres (88-98.9%*), blue (87%*), 1-2 mm (23.2-44.8%*), PP & PE (18.8% each*)	Aiguo *et al.* (2022)
	15	gills	86.7	0.42 ± 0.47 MP/g		
	15	sum	*n.s.*	13.0 ± 8.6 MP/indv *3-29 MP/indv* 0.04 ± 0.03 MP/g *0.02-0.12 MP/g*		
Cyprinus carpio (common carp)						
Croatia (fishponds)	20	GIT	55.5	0.25 MP/indv	fibres (55.5%), azure (55.5%), ≤1 mm (*n.s.*), cellulosic (25%)	Savoca *et al.* (2021)
Dicentrarchus labrax (European seabass)						
Cadiz, Spain (RAS facility)	45	head	27	*n.s.*	PE (55.6%)	Egea-Corbacho *et al.* (2023)
	45	muscle (+ skin)	33	*n.s.*		
Greee (fish cage)	*n.s.*	GIT	*n.s.*	2.2 ± 2.1 MP/indv	~7.5 µm (*n.s.*), PE (47.4%)	Miserli *et al.* (2023)
Greece, Italy, Turkey (offshore aquacultures)	17	GIT	29.4*	<3 MP/indv*	fibres (68%*), black (40%*), <5 mm (50%*), PES (50%*)	Mosconi *et al.* (2023)
	17	liver	14*	<1 MP/indv*		
	17	muscle	15*	<1 MP/indv*		
	17	sum	35	1.39 ± 0.65 MP/indv*		
Hatay, Turkey (local aquacultures)	21	GIT	52	1.0 ± 1.1 MP/indv	fibres (75%), black (54%), 0.3-0.5 mm (~29%), PE (25%)	Kılıç (2022)
Tenerife, Canary Island (fish cages)	45	GIT	97.8	5.4 ± 4.2 MP/indv	fibres (100%), colourless (47.7%), 0.8-1.2 mm (*n.s.*), cellulosic (60.7%)	Sánchez-Almeida *et al.* (2022)
Tenerife, Canary Island (fish cages)	83	GIT	65	1.4 ± 1.8 MP/indv	fibres (81%), blue (26.3%), cellulose (27.5%)	Reinold *et al.* (2021)
Epinephelus cyanopodus (speckled blue grouper)						
Shandong Peninsula, China (RAS facility)	24	GIT	*n.s.*	*0.07-0.08 MP/g*	fibres (88.7%*), blue (50.7%*), 20-500 µm (37.7-42.4%*), PET (30.2%*)	Zhou *et al.* (2024)
	24	gills	*n.s.*	*0.21-0.27 MP/g*		
	24	sum	*n.s.*	*2.25-2.75 MP/indv*		
Epinephelus fuscoguttatus x E. lanceolatus (hybrid grouper)						
Guangdong province, China (fish cages)	90	stomach	100	12.8 MP/indv 0.37 MP/g	fibres (70.1%), transparent (51.1%), 0.11-0.50 mm (32.5%), HDPE & PS (44.4% each)	Lam *et al.* (2022)
	90	intestine	100	23.9 MP/indv 1.10 MP/g		
	90	sum	100	35.4 MP/indv 0.62 MP/g		
Heteropneustes fossilis (Asian stinging catfish)						
Bangladesh (aquaculture)	7	GIT	71.4	1.0 ± 0.8 MP/indv 1.43 ± 1.73 MP/g	fibres (56.3%), transparent (~46%), 500-1000 µm (68.8%), PUR (*n.s.*)	Sultana *et al.* (2023)
Hypophthalmichthys molitrix (silver carp)						
Guangzhou, China (fishponds)	12	GIT	91.7	0.56 ± 0.54 MP/g	fibres (88-98.9%*), blue (87%*), 1-2 mm (23.2- 44.8%*), PP & PE (18.8% each*)	Aiguo *et al.* (2022)
	12	gills	100	0.27 ± 0.26 MP/g		

Table 1.3. Continuation (part 2/3).

Species/Location and production system	*n*	Tissue	%	MP concentration	Predominant MP type	Reference
Hypophthalmichthys molitrix (silver carp)						
	12	sum	*n.s.*	9.1 ± 4.7 MP/indv *2-20 MP/indv* 0.04 ± 0.03 MP/g *0.01-0.09 MP/g*		Aiguo *et al.* (2022)
Konosirus punctatus (dotted gizzard shad)						
Xiangshan Bay, China (mariculture area)	*n.s.*	GIT & muscle	90	2.1 ± 0.4 MP/indv 0.04 ± 0.03 MP/g	fibres (~97.3%), <500 µm (38%), cellulose (~72%)	Wu *et al.* (2020)
Labeo catla (catla carp)						
Bangladesh (aquaculture)	3	GIT	66.7	1.7 ± 1.5 MP/indv 0.03 ± 0.03 MP/g	fibres (62.5%), transparent (~46%), 500-1000 µm (62.5%), PA-6 (*n.s.*)	Sultana *et al.* (2023)
Labeo rohita (rohu carp)						
Bangladesh (aquaculture)	7	GIT	83.3	1.0 ± 0.8 MP/indv 0.07 ± 0.07 MP/g	fibres (46.7%), transparent (~46%), 500-1000 µm (53.3%), PES (*n.s.*)	Sultana *et al.* (2023)
Larimichthys crocea (large yellow croaker)						
Xiangshan Bay, China (mariculture area)	*n.s.*	GIT & muscle	80	1.8 ± 0.4 MP/indv 0.01 ± 0.01 MP/g	fibres (~97.3%), 500-1000 µm (~45%), cellulose (~65%)	Wu *et al.* (2020)
Lates calcarifer (Asian seabass)						
Persian Gulf, Iran (fish cage)	144	GIT	*n.s.*	0.05 ± 0.07 MP/g	fibres (49.3%), black-grey (36.2%), <100 µm (30.3%)	Vazirzadeh *et al.* (2025)
	144	gills	*n.s.*	0.19 ± 0.22 MP/g	fibres (87.5%), black-grey & red-pink (29.1% each), ≥1 mm (55.2%)	
	144	muscle	*n.s.*	0.02 ± 0.06 MP/g	fibres (50.0%), white-transparent (41.7%), <100 & 100-250 (33.3% each)	
	144	sum	*n.s.*	0.05 ± 0.06 MP/g	PUR (*n.s.*)	
Lutjanus stellatus (star snapper)						
Hong Kong, China (fish farms)	26	stomach	58	2.0 ± 1.1 MP/indv	Fragments (*n.s.*), PE (*n.s.*)	Chan *et al.* (2019)
Misgurnus anguillicaudatus (pond loach)						
Shanghai, China (rice-fish co-culture)	16	GIT	*n.s.*	1.8-2.2 MP/indv	fibres (~60-95%), white (~55-65%), <1 mm (~70%), PE (90.9%)	Lv *et al.* (2019)
Monopterus albus (Asian swamp eel)						
Shanghai, China (experimental base)	*n.s.*	GIT	*n.s.*	0.5-3.8 MP/indv	films (n.s.), 0.1-1 mm (*n.s.*), translucent (*n.s.*)	Lv *et al.* (2020)
	n.s.	head, muscle & bones	*n.s.*	0.5 ± 0.2 MP/indv		
Shanghai, China (rice-fish co-culture)	7	GIT	*n.s.*	3.3-4.2 MP/indv	fibres (~60-95%), white (~55-65%), <1 mm (~70%), PE (90.9%)	Lv *et al.* (2019)
Mugil cephalus (flathead grey mullet)						
Hong Kong, China (fishponds)	30	GIT	16.7	0.2 ± 0.5 MP/indv	fibres (100%), blue & purple (33% each), <2 mm (83%), PP (42%)	Cheung *et al.* (2018a)
Mystus tengara (stripped dwarf catfish)						
Bangladesh (aquaculture)	7	GIT	57.4	1.9 ± 3.6 MP/indv 0.57 ± 0.53 MP/g	fragment (42.9%), transparent (~46%), 0.5-1 mm (85.7%), PUR (*n.s.*)	Sultana *et al.* (2023)
Oncorhynchus mykiss (rainbow trout)						
Hatay, Turkey (local aquacultures)	30	GIT	63	1.2 ± 1.3 MP/indv	fibres (89%), black (60%), 1-2.5 mm (32%), PE (25%)	Kılıç (2022)
Ompok pabda (butter catfish)						
Bangladesh (aquaculture)	6	GIT	57.1	0.7 ± 0.5 MP/indv 0.86 ± 0.69 MP/g	fragments (66.7%), transparent (~46%), <0.5-1 mm (50%), PE (*n.s.*)	Sultana *et al.* (2023)
Oplegnathus punctatus (spotted knifejaw)						
Shandong Peninsula, China (RAS facility)	24	GIT	n.s.	0.21-0.48 MP/g	fibres (88.7%*), blue (50.7%*), 20-500 µm (37.7-42.4%*), PET (30.2%*)	Zhou *et al.* (2024)
	24	gills	n.s.	0.46-0.66 MP/g		
	24	sum	n.s.	1.50-2.25 MP/indv		
Oreochromis niloticus (Nile tilapia)						
Spain (Aquaponic RAS facility)	4	stomach	25	*n.s.*	PE (38.2%)	Blonç *et al.* (2023)
	4	gut	100	n.s.		

Table 1.3. Continuation (part 3/3).

Species/Location and production system	*n*	Tissue	%	MP concentration	Predominant MP type	Reference
Oreochromis niloticus (Nile tilapia)						
	8	liver	0	/		Blonç *et al.* (2023)
	8	muscle	100	*n.s.*		
	4	gonads	0	/		
	4	brain	100	*n.s.*		
Guangzhou, China (fishponds)	14	GIT	92.9	1.44 ± 2.22 MP/g	fibres (88-98.9%), blue (87%), 1-2 mm (23.2- 44.8%), PP & PE (18.8% each)	Aiguo *et al.* (2022)
	14	gills	100	2.63 ± 3.67 MP/g		
	14	sum	*n.s.*	9.4 ± 7.1 MP/indv 0.13 ± 0.16 MP/g		
Huila region, Colombia (fishponds)	18	gut	44	~3.4 MP/g	fragments (*n.s.*), PET (36.4%)	Garcia *et al.* (2021)
	18	gill	44	~2.8 MP/g		
	18	muscle	44	~3.0 MP/g		
Plectropomus leopardus (leopard coral grouper)						
Shandong Peninsula, China (RAS facility)	24	GIT	*n.s.*	0.17-0.19 MP/g	fibres (88.7%*), blue (50.7%*), 20-500 µm (37.7-42.4%*), PET (30.2%*)	Zhou *et al.* (2024)
	24	gill	*n.s.*	0.27-0.59 MP/g		
	24	sum	*n.s.*	1.50-3.00 MP/indv		
Sparus aurata (gilthead seabream)						
Thesprotia, Greee (fish cage)	*n.s.*	GIT	*n.s.*	40.0 ± 3.9 MP/indv	~7.5 µm (*n.s.*), PE (70%)	Miserli *et al.* (2023)
Greece, Italy, Turkey (offshore aquacultures)	17	GIT	29.4*	<3 MP/indv*	fibres (68%*), black (40%*), <5 mm (50%*), PES (50%*)	Mosconi *et al.* (2023)
	17	liver	14*	<1 MP/indv*		
	17	muscle	15*	<1 MP/indv*		
	17	sum	41	1.39 ± 0.65 MP/indv*		
Tunisian coast (farmed)	10	muscle	100	86600 ± 24300 MP/g *63700-112500 MP/g*	1.8-2.6 µm	Ferrante *et al.* (2022)
Hatay, Turkey (local aquacultures)	22	GIT	50	0.8 ± 1.1 MP/indv	fibres (83%), black (65%), 1-2.5 mm (40%), PE (25%)	Kılıç (2022)
Tenerife, Canary Island (fish cages)	41	GIT	78.9	5.1 ± 5.1 MP/indv	fibres (96.1%), colourless (60.9%), 0.8-1.2 mm (*n.s.*), cellulosic (56.1%)	Sánchez-Almeida *et al.* (2022)
Italy (offshore cage farm)	20	GIT	66.6	1.3 MP/indv	fibres (100%), black (46.2%), 1-2mm (*n.s.*), cellulosic (43.8%)	Savoca *et al.* (2021)
Tachysurus fulvidraco (yellow catfish)						
Guangzhou, China (fishponds)	11	GIT	90.9	3.35 ± 2.38 MP/g	fibres (88-98.9%), blue (87%), 1-2 mm (23.2- 44.8%), PP & PE (18.8% each)	Aiguo *et al.* (2022)
	11	gills	81.8	1.97 ± 1.51 MP/g		
	11	sum	*n.s.*	6.9 ± 4.2 MP/indv 0.21 ± 0.12 MP/g		
Takifugu bimaculatus (fugu fish)						
Jiangsu, China (aquacultures)	175	GIT	~95	~2.9 MP/indv ~0.9 MP/g	fibres (96.3%), black (72.3%), <1 mm (53%), PE (35.3%)	Song *et al.* (2022)
	175	gills	~85	~1.8 MP/indv ~1.0 MP/g		
	175	liver	~78	~1.8 MP/indv ~0.1 MP/g		
	175	skin	~96	~2.0 MP/indv ~0.1 MP/g		
	175	sum	100	7.9 ± 2.2 MP/indv 0.06 ± 0.02 MP/g		
Trachinotus ovate (golden pompano)						
Guangxi, China (fish cages)	81	GIT	*n.s.*	54-546 MP/g	fragments (35.2%), blue (28.6%), 20-50 µm (92.8%), PE (52.6%)	Liu *et al.* (2023a)

Abbrv: EVA, ethylene vinyl-acetate; HDPE, high-density polyethylene; PA-6, nylon 6; PE, polyethylene; PET, polyethylene terephthalate; PP, polypropylene; PS, polystyrene; PUR, polyurethane.
*Data presented encompasses other sample types.

Although most studies have focused on MP ingestion, these particles have also been detected in gills and skin of farmed fish since these are important first-barrier tissues (Aiguo *et al.*, 2022; Garcia *et al.*, 2021; Song *et al.*, 2022; Vazirzadeh *et al.*, 2025; Zhou *et al.*, 2024). In the gills, MP uptake occurs when water passes through the gill filaments, where MPs become trapped. Current literature suggests that waterborne MP contamination is an important MP exposure pathway, reporting MP levels in gills (0.19-2.8 MP/g) ranging within those observed in GIT (Table 1.3). Song *et al.* (2022) have also evaluated the MP occurrence in skin of farmed fugu fish, reporting ~2.0 MP/indv (~0.1 MP/g). Moreover, in wild fish collected from a mariculture area in a Haizhou Bay (China), Feng *et al.* (2019) evaluated MP occurrence in the skin of several scaly and scaleless fish species. In the cited study it was observed significantly higher MP levels in the skin of scaleless species, possibly facilitated by the higher skin mucus production.

MPs may translocate from GIT, gills, and skin, into internal tissues. Indeed, several studies have already reported the occurrence of MPs, although at lower concentrations, in internal organs, such as liver, brain, and muscle (Blonç *et al.*, 2023; Mosconi *et al.*, 2023; Song *et al.*, 2022; Vazirzadeh *et al.*, 2025). This capacity for crossing biological tissue barriers seems to increase for smaller particles, as seen by Song *et al.* (2022) and Vazirzadeh *et al.* (2025) reporting higher abundance of MPs of lower size classes in comparison to first-barrier tissues. The presence of MPs also extends to edible tissues, such as farmed fish dorsal muscle. Egea-Corbacho *et al.* (2023) found MPs in 35.7% of muscle samples of gilthead seabream produced in a RAS farm in Northern Spain, however these samples also contained skin which can contribute to the MP levels detected. Also, Mosconi *et al.* (2023) found MPs in ~15% of muscle samples of European seabass and gilthead seabream produced in aquacultures from Italy, Greece, and Turkey. Recently, Vazirzadeh *et al.* (2025) reported 0.015 ± 0.055 MP/g in the muscle of Asian seabass (*Lates calcarifer*) produced in a cage farm in the northern Persian Gulf. It should be noted that the aforementioned studies relied on microscopy techniques to quantify MPs, therefore being limited on the detection of smaller particles. For instance, Ferrante *et al.* (2022) found high levels of MPs (86,600 ± 24,300 MP/g), ranging within Ø 1.8-2.8 µm, in the muscle of farmed gilthead seabream using SEM-EDX. Blonç *et al.* (2023) also reported the presence of PE-MPs ranging from 0.23-79.57 ng/g of muscle of Nile tilapia produced in a RAS facility in Spain, being the MPs quantified and characterised through size exclusion chromatography. As there still is lack of knowledge about the influence of aquaculture production systems on MP levels in farmed organisms, more specifically in edible tissues such as muscle, further studies are required to provide more data.

1.3.3. Microplastic contamination in seafood products: risk to human consumers

The detection of MPs in fish muscle is a growing concern not only due to the potential health impacts on fish itself, but also because muscle comprises the majority of fish's body and is the primary tissue consumed by humans. In fact, Cox *et al.* (2019) estimated human consumption of microplastics and ranked seafood (*i.e.*, fish, shellfish, and molluscs) among the leading three sources of human exposure alongside air and bottled water. It is important to mention that, at that time, most MP monitoring studies in food items focused on seafood. So far, other studies have identified foodborne MP sources, such as drinking water and beverages, salt, honey, sugar, fruit and vegetables, and other animal products such as dairy and poultry (Garrido Gamarro & Constanzo, 2022; Vitali *et al.*, 2023). Moreover, plastic packaging materials often made up by thermoplastics (*e.g.*, LD- and HDPE, PET, PP) can release MPs and contribute to the contamination levels in food items (Jadhav *et al.*, 2021). Consequently, MP occurrence was already found in human stool and urine, demonstrating that MP contamination poses a direct risk to human health as well (Hartmann *et al.*, 2024; Rotchell *et al.*, 2024; Zhu *et al.*, 2024). Interestingly, based on dietary questionnaire data, it has been observed that food habits including processed items, and use of plastic containers contribute to higher MP intake (Hartmann *et al.*, 2024; Zhu *et al.*, 2024).

When discussing seafood consumption, particularly fish, it is important to recognize that not only muscle or fillet are typically part of the menu, with various parts of the fish also being regularly consumed depending on cultural backgrounds or culinary preferences. The consumption of these may contribute to higher intakes of MPs. For instance, in farmed fish, Lv *et al.* (2020) found 0.5 ± 0.2 MP/indv in whole gutted Asian swamp eel (*i.e.*, head, bones, and muscle), which is commonly eaten in this manner in Asian countries. Fish muscle with skin attached is often consumed, which may increase the overall MP contamination of the meal, as some studies found that in wild fish collected in an important mariculture area have MP levels in skin exceeding those found in GIT and gills, and are not easily removed by washing (Feng *et al.*, 2019). Various internal organs are consumed worldwide, such as the whole viscera (*e.g.*, fish guts soup in Asian countries), liver (*e.g., foie de lotte* in French cuisine), swim bladder (*e.g.*, dried fish maw in Chinese cuisine), and gonads (*e.g.*, caviar). Other studies have also highlighted MP contamination in processed seafood products such as salt cured and dried fish (Kutralam-Muniasamy *et al.*, 2023; Rukmangada *et al.*, 2023) and canned seafood (Akhbarizadeh *et al.*, 2020; Silva *et al.*, 2024). However, studies comparing fresh and processed seafood are still scarce, with some mentioning no significant differences between these (Nalbone *et al.*, 2024).

Furthermore, meal preparation practices can influence human MP consumption. One of the best studied MP sources in the kitchen are plastic cutting boards. For instance,

Yadav *et al.* (2023) reported the release of 1.23 ± 0.49 mg MP after one chopping cycle and reaching up to 5.00 ± 3.99 mg MP by six chopping cycles (*i.e.*, 500 knife cuts each cycle). Other types of cookware have been identified as potential MP source, such as non-stick pans may release up to 4968 particles of polytetrafluoroethylene (PTFE, also known by Teflon) per year into homecooked food (Cole *et al.*, 2024). Besides cookware, the exposure of food to household dust through air, mainly composed by fibres, during meal preparation can significantly increase MP contamination (Catarino *et al.*, 2018). Also, different cooking methods can affect the quantity of MPs although current literature points out to controversial results. For instance, Eshun & Pobee (2022) reports that frying fish lead to lower MP levels in the final food item, but MP leached into the oil, raising concerns about the potential health implications of meals prepared with MP-contaminated oil. Contrarily, Li *et al.* (2022a) found that frying significantly increased the number of MPs in some seafood samples, while boiling and steaming resulted in lower MP contaminated seafood. Altogether, current literature indicates that selecting non-plastic cookware and appropriate cooking methods can mitigate additional contamination of items during meal preparation.

Indeed, humans are daily exposed to MPs through consumption of MP contaminated food. Besides bodily fluids, MPs have already been identified in other tissues, such as blood (Leonard *et al.*, 2024), liver (Horvatits *et al.*, 2022), and brain (Amato-Lourenço *et al.*, 2024). The presence of MPs has also been found in placenta and meconium (*i.e.*, first stool of a newborn baby), as well as in breast milk and infant stools, indicating maternal transfer of MPs during and post-pregnancy (Liu *et al.*, 2023b). Moreover, current literature already raises some concerns regarding MP impacts on human health, although it is still not entirely understood if MPs are linked to the origin of the disease or that are easily retained in diseased tissues. For instance, Yan *et al.* (2022) found that patients with inflammatory bowel disease had significantly higher mean faecal MP levels in comparison to healthy patients (41.8 and 28.0 MPs/g dw, respectively). Also, high MP levels have been found in cirrhotic liver samples (1.4 MP/g; 4.6-9.9 MP/g) of patients with chronic liver disease in relation to those found in liver of deceased individuals with no underlying hepatic condition (0.0-1.5 MP/g) (Horvatits *et al.*, 2022). For that reason, MP research on food items and in particular farmed seafood, is crucial to understand how the aquaculture production system and surrounding environment affect the MP contamination levels in fish, with the ultimate goal of providing MP-safer products to the human consumer.

1.4. The main goals of this thesis

The main goals of the present Thesis were to investigate the MP contamination and its potential adverse effects in the European seabass (*Dicentrarchus labrax*) produced in different aquaculture production systems, while simultaneously evaluating the potential exposure for human consumers. European seabass was selected as a model of farmed fish because it is a valuable aquaculture species in Mediterranean countries, as exemplified by a production volume of 92,081 tonnes corresponding to a total value of €680 million in 2022 (EUMOFA, 2024). Considering the gaps of knowledge identified in the introductory chapter, the specific questions this Thesis aimed to address were:

1. Does MP contamination affect different aquaculture production systems (cages, ponds, and recirculation) of seabass in distinct ways?
2. Are higher levels of MPs in farmed fish tissues associated with changes in fish health biomarkers?
3. Is there a relationship between MP levels in seabass muscle and the accumulation of trace and non-essential metals, and do these contaminants represent a significant exposure route for human consumers?
4. Does the most predominant MP type observed in different aquaculture production systems impact the health of farmed European seabass under aquaculture-relevant experimental conditions?

The following chapters aimed to address the questions accordingly. 1) to determine the occurrence of MPs and other anthropogenic particles in the water, aquafeed, and tissues of seabass collected from three different aquaculture production systems (*chapters II to IV*). This objective aimed to determine MP contamination and highlight aquaculture environments not only as sources but also as recipients of MP pollution. 2) to evaluate, under field conditions, the potential relation between MP occurrence and altered biomarkers of fish health status (*chapters II and IV*). This objective aimed to link MP occurrence and physiological changes in farmed fish, contributing to the better understanding of their sublethal effects. 3) to evaluate potential relation between MPs and other contaminants while determining their risk for human consumers (*chapter III*). This objective aimed to evaluate the co-occurrence of MPs and metals in the dorsal muscle of seabass, while estimating human exposure through consumption data. 4) to evaluate the exposure effects of the most predominant type of MPs found, namely cellulosic microfibres, through a dedicated *in vivo* trial under aquaculture-relevant conditions based on the previously gathered data (*chapter V*). This objective aimed to understand physiological responses to

dietary exposure of viscose-rayon, highlighting the potential effects of natural and semi-synthetic microfibres in biota.

Through the knowledge gathered in field and experimental studies contained in this thesis, it is hoped that this project will enable to a better understanding of the potential impacts of MPs at a multiscale approach and relevant aquaculture exposure conditions. Moreover, the Thesis will contribute to the advancement of the knowledge on MP pollution in aquaculture, under the ‘One Health’ approach, which recognises the interconnectedness of environmental, animal, and human health.

Chapter II

Microplastics in water, feed and tissues of European seabass reared in a recirculation aquaculture system (RAS)

by

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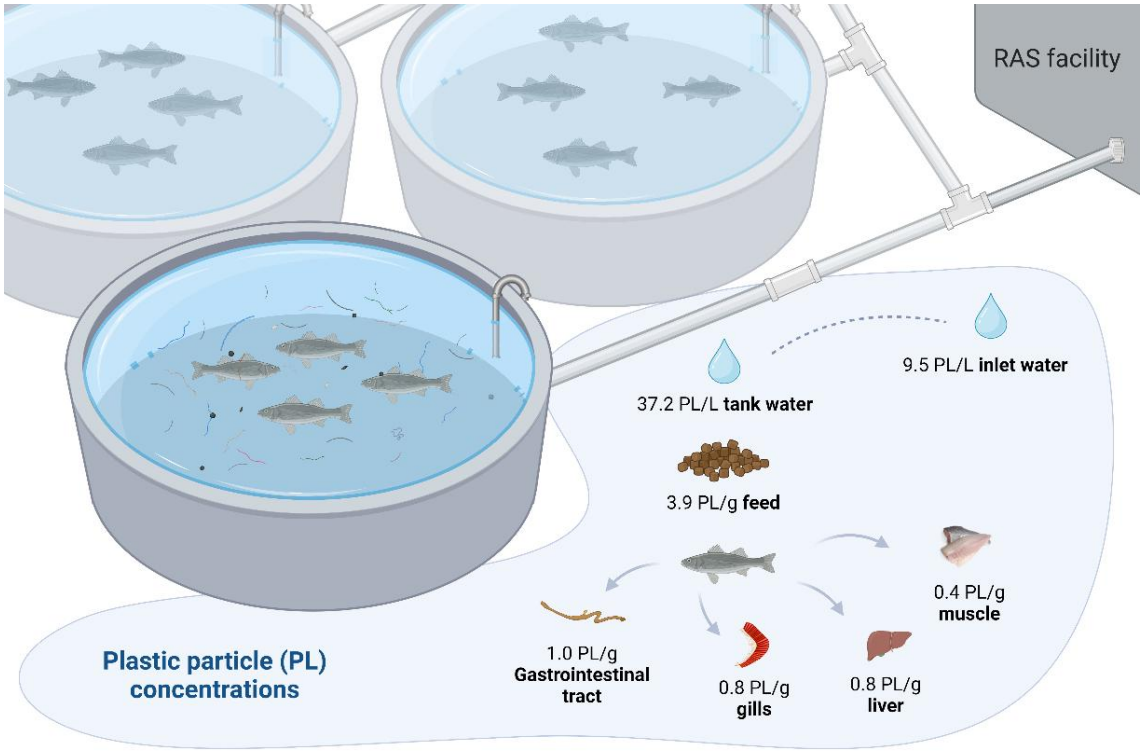

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Abstract

Plastic particles (PLs) are ubiquitous in aquatic ecosystems, and aquaculture production is susceptible to contamination from external or endogenous sources. This study investigated PL presence in water, fish feed and body sites of 55 European seabass produced in a recirculating aquaculture system (RAS). Fish morphometric parameters and health status biomarkers were determined. A total of 372 PLs were recovered from water (37.2 PL/L), 118 PLs from feed (3.9 PL/g), and 422 from seabass (0.7 PL/g fish; all body sites analysed). All 55 specimens had PLs in at least two of the four body sites analysed. Concentrations were higher in the gastrointestinal tract (GIT; 1.0 PL/g) and gills (0.8 PL/g) than in the liver (0.8 PL/g) and muscle (0.4 PL/g). PL concentration in GIT was significantly higher than in muscle. Black, blue, and transparent fibres made of man-made cellulose/rayon and polyethylene terephthalate were the most common PLs in water and seabass, while black fragments of phenoxy resin were the most common in feed. The levels of polymers linked to RAS components (polyethylene, polypropylene, and polyvinyl chloride) were low, suggesting a limited contribution to the overall PL levels found in water and/or fish. The mean PL size recovered from GIT (930 μm) and gills (1047 μm) was significantly larger than those found in the liver (647 μm) and dorsal muscle (425 μm). Considering all body sites, PLs bioconcentrated in seabass (BCF_{Fish} >1), but their bioaccumulation did not occur (BAF_{Fish} <1). No significant differences were observed in oxidative stress biomarkers between fish with low (<7) and high (≥7) PL numbers. These findings suggest that fish produced in RAS are mainly exposed to PLs through water and feed. Further monitoring under commercial conditions and risk assessment are warranted to identify potential threats to fish and human health and define mitigating measures.

Keywords

Bioconcentration; Microplastics; ‘One health’ concept; Recirculating aquaculture systems (RAS); Seafood contamination

2.1. Introduction

As emerging contaminants, microplastics (MPs) have raised concerns within the aquaculture sector due to plastic pollution levels in aquatic environments and their presence in various stages of seafood production (FAO, 2022). Indeed, the aquaculture sector is the fastest-growing food industry, becoming a vital contributor to human nutrition through the production of over 50% of fish in markets (Béné *et al.*, 2016; FAO, 2022). MPs may also carry other contaminants incorporated during plastic manufacturing or adsorbed through environmental exposure, which may further compromise human health (Campanale *et al.*, 2020; Liu *et al.*, 2022a). For that reason, MP presence in food commodities, including seafood, has become a global food safety concern (Garrido Gamarro & Constanzo, 2022).

MPs are plastic particles smaller than 5000 µm (GESAMP, 2016) and can reach aquaculture systems from several exogenous and endogenous sources. Exogenous MPs may originate from human activities and reservoir environments, such as the surrounding atmosphere and water bodies (Chen *et al.*, 2021; Zhou *et al.*, 2021a). The occurrence of MPs has been widely reported in many aquatic ecosystems (Ajith *et al.*, 2020; Stenger *et al.*, 2021), including in regions with elevated aquaculture production (Chen *et al.*, 2020a; Feng *et al.*, 2019; Lin *et al.*, 2022; Ma *et al.*, 2020; Wu *et al.*, 2020; Xiong *et al.*, 2022). Farmed fish can also be exposed to other external MP sources, like fish feed, that may be contaminated by the ingredients, such as fishmeal, or during the different stages of the feed production process (Gündoğdu *et al.*, 2021; Karbalaei *et al.*, 2020; Rahman *et al.*, 2022; Thiele *et al.*, 2021; Wang *et al.*, 2022c). Endogenous MPs may originate from the weathering of plastic components used in aquacultures. Fish exposure to these MPs may depend on the reliance of aquaculture systems on plastic components (*e.g.* fishing gear, tanks, and filters), and their potential release into the water system (Lu *et al.*, 2019; Wu *et al.*, 2020). The uptake of MPs has been reported in several commercial fish species, including Nile tilapia (*Oreochromis niloticus*), Atlantic salmon (*Salmo salar*), common carp (*Cyprinus carpio*), European seabass (*Dicentrarchus labrax*) and gilthead seabream (*Sparus aurata*), either collected from the wild or farmed in cages and ponds (Aiguo *et al.*, 2022; Alomar *et al.*, 2022; Barboza *et al.*, 2020b; Corami *et al.*, 2022; Dehm *et al.*, 2022; Ferrante *et al.*, 2022; Garcia *et al.*, 2021; Guilhermino *et al.*, 2021; Kılıç, 2022; Liboiron *et al.*, 2019; Reinold *et al.*, 2021; Sánchez-Almeida *et al.*, 2022; Savoca *et al.*, 2021).

Recirculation aquaculture systems (RAS) provide an opportunity for seafood production to address environmental sustainability since it is based on the principles of nutrient recycling, reduced water usage and improvement of waste management (Belton *et al.*, 2020; Naylor *et al.*, 2021). Current technology in RAS guarantees optimal water quality and all-year-round controlled rearing conditions (d'Orbcastel *et al.*, 2009). However, RAS

might face some challenges due to the potential accumulation of contaminants, such as heavy metals, drug residues, and metabolites (Deviller *et al.*, 2005; Martins *et al.*, 2009). The occurrence of MPs in fish reared in this production system type remains poorly documented (Lv *et al.*, 2020). But, as RAS heavily relies on plastic components, MP impacts on seafood health and quality have raised concerns within the aquaculture sector, and they have already stimulated the development of non-plastic alternative components, such as natural biofiltration media (Lu *et al.*, 2019; Mnyoro *et al.*, 2022).

Based on experimental studies exposing fish to MPs, either through the uptake of contaminated water or food, lesions associated with immune responses in first-barrier tissues, such as the intestine, gills, and skin, were often observed (Abarghouei *et al.*, 2021; Espinosa *et al.*, 2019; Karbalaei *et al.*, 2021; Montero *et al.*, 2022). Other effects associated with MP exposure include tissue oxidative damage (Alomar *et al.*, 2017; Barboza *et al.*, 2018b; Espinosa *et al.*, 2019; Lu *et al.*, 2016), neurotoxicity and behavioural changes (Barboza *et al.*, 2018c; 2018d; Mattsson *et al.*, 2017; Shi *et al.*, 2021), and growth impairment (Jabeen *et al.*, 2018; Naidoo & Glassom, 2019). Moreover, MPs can cross biological barriers and enter into the bloodstream, potentially being retained in edible tissues, such as muscle, as already reported in some farmed fish species (Ferrante *et al.*, 2022; Garcia *et al.*, 2021; Lv *et al.*, 2020; Rahman *et al.*, 2022; Wu *et al.*, 2020). Such physiological responses may reduce the nutritional quality of fish to their predators and humane consumers (Hanachi *et al.*, 2021; Lai *et al.*, 2021; Yin *et al.*, 2018).

The European seabass (*Dicentrarchus labrax*, Linnaeus 1758) is a marine species with high commercial value in European countries, representing €262 million in economic weight (EUMOFA, 2021). Studies have reported MP uptake in wild seabass (Akoueson *et al.*, 2020; Barboza *et al.*, 2020b) and specimens produced in cage and pond aquacultures in Atlantic and Mediterranean waters (Kılıç, 2022; Reinold *et al.*, 2021; Sánchez-Almeida *et al.*, 2022). But so far, MP presence has only been reported in the edible tissues, specifically muscle, of wild seabass (Akoueson *et al.*, 2020; Barboza *et al.*, 2020b). The main goal of the present study was to investigate the direct exposure to plastic particles (PLs) from water and feed of European seabass specimens produced in a RAS facility and determine their presence in fish body sites (*i.e.,* gastrointestinal tract, gills, liver, and dorsal muscle). Antioxidant and detoxification biomarkers in the liver were evaluated to determine the potential effects of PL exposure on fish health status.

2.2. Materials & methods

2.2.1. Fish collection and sample preparation

European seabass juveniles were reared in a recirculation aquaculture system (RAS) pilot facility located in Portugal, with 12 tanks of 350 L, supplied with underground filtered and heated saltwater at 20 ± 1 °C and a salinity of 18 ppm, equivalent to a density of 1.01 g/cm^3. During the trial, a total of 2000 L of water entered the system daily, replacing 5% of the RAS total volume (40,000 L). The RAS facility integrates several plastic components, such as: the tanks and tubing made of polyvinyl chloride (PVC); mechanical biofilters, skimmer, and water deposits made of polypropylene (PP) and polyethylene (PE); and mesh filters made of PP and polyester, also referred to as polyethylene terephthalate (PET).

Alive fish handling was performed according to the ethical principles and regulations given by the European Union (Directive, 2010/63/EU) and the Portuguese legislation (Decree-Law nº 113/2013) on the protection of animals used for scientific purposes.

For eight months, fish were fed the same commercial feed for the species containing 50% crude protein and 20% crude fat. After this period, 55 fish were sampled following practical conditions used in commercial seabass aquaculture production: after a 24 h fasting period, fish were harvested with a net and euthanized by immersion in an ice slurry (<4 °C). Fish were then put into ice-filled boxes and transported to the Interdisciplinary Centre of Marine and Environmental Research (CIIMAR) facilities in Porto, Portugal. Using glass flasks, 10 L of water were collected using glass flasks from the tanks where fish were maintained and from the water inlet reservoir. Also, a sample of the feed (~50 g) used was collected.

Prior to dissection, all fish were individually measured (21.6 ± 1.9 cm) and weighed (122.0 ± 35.4 g). Fish were externally rinsed with distilled water to remove skin impurities, and body sites were collected from each fish, namely the gastrointestinal tract (GIT; from the oesophagus to the rectum), gills, half portion of the liver, the dorsal muscle and stored at –20 °C for later assessment of the PL presence. The mean sample weight (± SD) can be consulted in Table S2.1. All the fish collected presented empty GIT at the time of sample collection. From each fish, muscle and the remaining half portion of the liver were also isolated, quickly deep-frozen in liquid nitrogen, and stored at –80 °C to evaluate antioxidant and detoxification biomarkers.

2.2.2. Plastic particle isolation, visual identification, and chemical characterisation

Water, feed, and seabass tissue samples were processed for PL isolation and characterisation. In brief, water samples were collected through the grab sampling method as described by Barrows *et al.* (2017), enabling the capture of nano- and MPs of smaller sizes in relation to net-based methods. Water samples were stirred and filtered under vacuum conditions (pump Millipore WP6122050, Merck, KGaA, Darmstadt, Germany) through glass-microfibre filter membranes with 1.2 µm pore size (Munktell & Filtrak, GmbH, Germany). Regarding feed, six replicates of 5 g (30 g in total) were digested in glass flasks filled with 30% hydrogen peroxide (H_2O_2) solution. To each flask, the added solution volume corresponded to three folds of the sample weight. Then, samples were incubated at 60 °C for 24 h, vacuum-filtered, and oven-dried at 40 °C for 24 h (Binder BD 53, Tuttlingen, GmbH, Germany). Digested feed was vacuum-filtered using the 1.2 µm filter membranes (Wang *et al.*, 2022c). Fish body sites were also individually digested inside glass flasks, covered with 10% potassium hydroxide (KOH) solution prepared at room temperature with ultrapure water (Milli-Q®, Millipore Corporation, MA, USA) in a volume corresponding to three-folds of the sample weight. Each tissue sample was incubated at 60 °C for 24 h, vacuum-filtered using 1.2 µm filter membranes, and dried at 40 °C for 24 h (Barboza *et al.*, 2020b; Dehaut *et al.*, 2016; Guilhermino *et al.*, 2021).

The particles present in filters suspect of being composed of plastic or man-made were quantified using a stereomicroscope coupled with an integrated camera (Nikon SMZ1000 with the DS-Fi1, Japan). The particles were catalogued according to the guidelines provided for plastic pollution monitoring studies (GESAMP, 2019), and sorted into different categories according to their shape (fibres, fragments, films, pellets, and foams), colour (black, blue, transparent, multicolour, among others), and size (microplastics, <100-4999 µm; mesoparticles, 5000-25,000 µm). PLs, refer to all MPs and mesoplastics (meso-PLs) recovered. Particle size was defined based on the largest cross section and measured using imaging analysis software (Olympus Cell^B, GmbH, Hamburg, Germany). For the analysis, particles were included in seven size classes: <100 µm, 100-149 µm, 150-499 µm, 500-1499 µm, 1500-2999 µm, 3000-4999 µm, and ≥5000 µm.

Approximately 50% of all particles recovered were selected considering the diversity of their visual characteristics to be chemically identified through micro-Fourier Transformed Infrared Imaging Microscopy (µFTIR). The analysis was carried out in a Nicolet™ iN10 MX Infrared Imaging Microscope equipped with a mercury cadmium telluride (MCT) array cooled by liquid nitrogen (Thermo Fischer Scientific, Waltham, MA, USA). Several spectra per particle were collected in reflectance mode with a resolution set at 8 cm^{-1} ranging from 4000 to 600 cm^{-1} with 16 scans and further compared with available polymer and

additive (coatings and paint) databases using OMNIC software (Thermo Fischer Scientific, Waltham, MA, USA). The chemical identification of the particles was only accepted if the spectrum under analysis matched 70% with reference spectra and, whenever possible, inferred for the non-analysed remaining particles through their visual appearance relative to μFTIR-confirmed particles (Fig. S2.1).

The concentration of PLs (sum of MPs and meso-PLs) was expressed as the number of particles per individual fish (PL/fish) and per gram of wet sample weight (PL/g). For water and feed, PL concentration was expressed as the number of particles per litre (PL/L) and the number of particles per gram of feed (PL/g).

2.2.3. Contamination control

To avoid external PL contamination during sample manipulation, several recommended procedures followed Bessa *et al.* (2019) and Barboza *et al.* (2020b). Specifically, samples were collected and processed in a room without potential airborne PL contamination sources. During all the procedure, white 100% cotton lab coats and powder-free nitrile gloves were worn, and, whenever possible, plastic equipment was replaced by glassware. All surfaces and dissection utensils were cleaned with ethanol 70% and ultrapure water in order to avoid cross-contamination between and in-between samples. Moreover, during all procedures, three glass Petri dishes with clean filters (previously inspected under the stereomicroscope for PL presence) were placed nearby each working station for samples' contamination control. Aliquots of the solutions prepared (*e.g.*, 10% KOH) were collected and filtered through 1.2 μm filter membranes to determine potential PL contamination. A total of 128 particles were found in procedural blanks. In order to compensate for the contamination during sample handling, particles with similar characteristics (*i.e.,* shape, colour, size, and polymer type) to those found in procedural blanks were subtracted from each sample.

2.2.4. Calculation of plastic particles' bioconcentration and bioaccumulation factors

The bioconcentration (BCF) and bioaccumulation (BAF) factors of PLs were determined for seabass, as described by Miller *et al.* (2023). The PL concentrations in tank water, feed, and seabass were transformed into comparable units, namely in tank water (PL/L), feed (PL/kg), and seabass (PL/kg). PL concentrations in tank water were adjusted considering the estimated water density of 1.01 g/cm^3. The equations used were:

1) $\mathrm{BCF} = \mathrm{C_{Fish}}/\mathrm{C_{Water}}$
2) $\mathrm{BAF} = \mathrm{C_{Fish}}/(\mathrm{C_{Water}} + \mathrm{C_{Feed}})$

BCF and BAF in seabass were calculated considering the mean PL concentration in the body sites analysed (PL/kg). Due to the direct exposure to PLs, BCF and BAF were calculated for GIT and gills as well (PL/kg). In gills, only the BCF was calculated since plastic exposure mainly occurs through tank water and not feed. BCF and BAF values >1 indicate bioconcentration and bioaccumulation in the organism/tissue, respectively.

2.2.5. Determination of fish health indexes and oxidative stress biomarkers

General fish health indexes were determined in all sampled fish based on biometric values, namely the total body length (TL) and weight (TW) as well as the weight of liver and viscera. The Fulton's body condition factor (K) was estimated as $K = 100 \times (\mathrm{TW}/\mathrm{TL}^3)$, where TW is in g and TL in cm. The hepatosomatic index (HSI) and viscerosomatic index (VSI) were estimated using the formulas, $\mathrm{HSI}\ (\%) = 100 \times (liver\ weight/\mathrm{TW})$ and $\mathrm{VSI}\ (\%) = 100 \times (viscera\ weight/\mathrm{TW})$.

After the quantification of PLs in all fish body sites, 23 of the 55 sampled fish were selected for the determination of biomarkers involved in the antioxidant and detoxification processes, namely in liver: superoxide dismutase (SOD) activity, catalase (CAT) activity, glutathione-S-transferase (GST) activity, glutathione peroxidase (GPx) activity, glutathione reductase (GR) activity, total antioxidant capacity (TAC), and lipid peroxidation (LPO) levels. The LPO levels were also determined in dorsal muscle. As PLs were identified in all sampled fish, being recovered from at least two of the analysed body sites, and as the non-identification of PLs in a tissue sample may not imply its complete absence, two groups of fish were established (<7 and ≥7 PL items/fish) following the approach suggested by Solomando *et al.* (2022).

Liver and muscle samples were homogenised in ice-cold K-phosphate buffer (0.1 M, pH 7.4) in a proportion of 1:10 (*w/m)* using a UP200Ht sonicator (Hielscher Ultrasonics, GmbH, Germany), and centrifuged (10,000, 4 °C, 20 min) to obtain a homogenate supernatant. Aliquots were made from the supernatants and stored at –80 °C until further biomarker determination. To prevent lipid peroxidation in liver and muscle, butylhydroxytoluene (BHT) was added aliquots destined to LPO determination. Biomarkers were determined using well established methods previously described in Pereira *et al.* (2022). All determinations were performed in triplicate in a Synergy HTX Multi-Mode Microplate Reader (BioTek Instruments Inc., Foster City, CA, USA).

2.2.6. Statistical analyses

The normal distribution of each variable was assessed using Shapiro-Wilk test, and the homogeneity of variances was using Levene's test. The data regarding the presence of PLs based on characteristics such as shape, colour, size, and polymer type were evaluated

using Kruskal-Wallis test. In cases where significant treatment effects were observed, post hoc Dunn's pairwise tests were conducted. The biometric values and oxidative stress parameters of two groups, categorised by the number of PLs recovered (<7 and ≥7 PL/fish), were compared using Student's T-tests. As data normality could not be achieved in the distribution of PLs, Spearman's correlation coefficient was employed for the following correlation analyses: seabass sample weight *vs*. the number of PLs recovered; predominant shapes *vs*. predominant colours, size classes, and polymer types; predominant colours *vs*. predominant size classes and polymer types; predominant size classes *vs*. predominant polymer types; and total PL count *vs*. biometric values and oxidative stress biomarkers.

All statistical analyses were performed using the SPSS statistical analysis package (version 27) and a significance level of 0.05 was considered in all tests.

2.3. Results

2.3.1. Characteristics of the plastic particles recovered from water and feed

In the water inlet reservoir, 95 particles (9.5 ± 5.9 PL/L) were recovered, indicating a lower PL occurrence relative to tank water ($p < 0.001$). A total of 570 particles were recovered from tank water and 188 from feed. All particles with visual and chemical similarities to those found in procedural blanks were excluded, specifically cellulose-based fibres, as well as natural organic and inorganic particles. However, several cellulose-based fibres displayed visible signs of degradation and decolouration, and/or exhibited at least one spectrum that significantly matched with plasticizers, which was not verified in particles recovered from procedural blanks, so these fibres were accepted. Thereafter, only 372 particles (37.2 ± 1.9 PL/L) recovered from water and 118 (3.9 ± 1.4 PL/g) recovered from feed were considered (Table 2.1). Examples of the visual diversity of PLs recovered in tank water and feed are depicted in Figure 2.1.

In tank water, fibres accounted for 83.1% of the recovered PLs, followed by fragments (9.4%), films (4.6%), and pellets (2.9%), as shown in Figure 2.2A. Fibre occurrence was significantly higher ($p = 0.04$) in relation to the other shapes. PLs were sorted into twelve different colour categories that significantly differed ($p < 0.001$) in their occurrence level (Fig. 2.2B). Specifically, the most common colours were blue (39.8%), black (27.4%), transparent (11.6%); other colours represented less than 7%. The most representative size class was 150–499 µm (30.4%), followed by 500–1499 µm (25.0%), and <100 µm (21.0%), as shown in Fig. 2.2C. The sizes of particles found in water ranged from 16 to 11 264 µm (Table 2.1). The fibres and films were significantly larger ($p < 0.01$) than fragments and pellets (59 ± 26 µm); only 5 meso-PLs with a fibre shape could be found in water (Table 2.2). Concerning polymer type, man-made cellulose/rayon particles were significantly more abundant (58.6%) than other polymers identified in water ($p < 0.01$). The occurrence levels of the remaining thirteen polymers, including PET, PP, and polyacrylonitrile (PAN), are detailed in Fig. 2.3A.

Regarding feed, fragments (53.4%) and fibres (30.9%) were significantly more abundant ($p < 0.001$) than pellets (8.5%) and films (4.0%), as shown in Figure 2.2A. In total, 10 different colour categories were observed in the MPs found in the feed (Fig. 2.2B). The occurrence of black (45.8%) and blue (22.9%) PLs was significantly higher compared to other colours ($p < 0.001$). The most common size classes were 150–499 µm (33.9%), and <100 µm (32.2%) (Fig. 2.2C). The size of PLs in feed ranged from 37 to 4526 µm (Table 2.1), with fibres being significantly larger ($p < 0.01$) than fragments, films, and pellets (178 ± 161 µm) (Table 2.2). No meso-PLs were found. In total, 33.1% of the particles were identified as phenoxy resin (*i.e.*, epoxy resin derived by bisphenol A

and epichlorohydrin), followed by man-made cellulose/rayon (25.4%), and PET (11.0%), among others (Fig. 2.3B).

2.3.2. Characteristics of the plastic particles recovered from seabass

From the examined fish samples, a total number of 780 particles were recovered, from which 33% were subtracted due to the presence of similar particles in procedural blanks, specifically cellulose-based fibres. As described in water and feed analyses, only fibres displaying visible signs of degradation and decolouration, and/or exhibiting at least one spectrum that significantly matched with plasticizers, were accepted. Hence, 422 particles recovered from the seabass body sites were considered for the analysis. PLs could be identified in all fish, being recovered from at least two of the analysed body sites (Table 2.1). Total PL count ranged from 2 to 14 particles per individual, with a mean of 7.7 ± 3.2 PL/fish. In Figure 2.1, some examples of the visual diversity of the PLs found in seabass body sites are shown. No correlation ($p > 0.100$) was found between sample weight and the number of PL items recovered from each seabass body site (Table S2.1).

Considering all tissues analysed, fibres (66.8%) were the most abundant particle shape recovered from seabass ($p < 0.001$), followed by fragments (20.9%), films (6.6%), and pellets (5.7%), as depicted in Figure 2.2A. Similarly, a higher abundance of fibres ($p < 0.001$) was observed in GIT (61.8%), gills (82.5%), liver (58.9%), and dorsal muscle (67.2%) (Fig. 2.2A). Eleven colour categories were observed in the PLs recovered from all seabass body sites (Fig. 2.2B), of which black (24.2%), blue (32.9%), and transparent (20.9%) were the most abundant ($p < 0.001$). Black, blue, and transparent PLs were also the three most abundant ($p < 0.001$) in all body sites (Table 2.2). Also, the PL size classes 500–1499 µm (30.6%) and 150–499 µm (30.1%) were significantly more abundant ($p < 0.001$) than the others (Fig. 2.2C). Correlation values were established between particle shape and the most common, colours and size classes recovered from European seabass body sites (Table S2.2).

The six most common polymers in seabass' body sites were man-made cellulose/rayon (53.1%), PET (7.6%), PVC (7.1%), PAN (3.3%), ethylene-propylene-diene copolymer (EPDM) (3.3%), and an organoclay-based coating (3.1%) (Fig. 2.3C). Other recovered polymers represented less than 3% and include phenoxy resin, PE, PP, polyacrylamide (PAM), and polystyrene (PS) (Fig. 2.3C). Fibres were mostly made of man-made cellulose/rayon, PET, and PAN. Correlation values between particle shapes and polymer types are detailed in Table S2.2. As depicted in Fig. S2.2, man-made cellulose/rayon particles were mostly blue (39.7%) and transparent (29.9%), measuring

Table 2.1. Total number (*n*) and tissue distribution of plastic particles (PLs) in the water (10 L), fish feed (30 g) and 55 European seabass specimens produced in a recirculation aquaculture system (RAS) for 8 months.

			Seabass				
	Tank water	**Feed**	**All**	**GIT**	**Gills**	**Liver**	**Muscle**
Fish with PLs							
N (%)	-	-	55	50 (91.0)^{a}	37 (67.3)^{b}	42 (74.6)^{b}	45 (81.8)^{a,b}
Number of PLs							
n (%)	372	118	422	144 (34.1)	80 (19.0)	73 (17.3)	125 (29.6)
Mean PL/fish	-	-	7.67 ± 3.18	2.62 ± 1.90^{a,b}	1.45 ± 1.60^{c}	1.33 ± 1.08^{c}	2.27 ± 1.86^{b}
Mean PL/g or L	37.20 ± 1.94	3.93 ± 1.38	0.73 ± 0.27	1.01 ± 0.78^{a}	0.75 ± 0.94^{b}	0.80 ± 0.86^{a,b}	0.36 ± 0.32^{c}
Mean PL size (µm)	887 ± 871	410 ± 713	748 ± 893	930 ± 1022^{a}	1047 ± 1117^{a}	647 ± 713^{b}	425 ± 445^{b}
PL size range	16-11,261	37-4526	30-6672	42-5194	57-6672	30-3325	30-2181
MPs (%)	98.7	100	99.3	98.6	98.7	100	100
Meso-PLs (%)	1.3	0	0.7	1.4	1.3	0	0

The number of PLs is represented by the mean and standard deviation (mean ± SD). Different superscript letters indicate significant differences between tissues (Kruskal Wallis test, $p < 0.05$). PL – sum of all microplastics (MPs) and mesoplastics (Meso-PLs). All – sum of the PL occurrence in the gastrointestinal tract (GIT), gills, liver, and dorsal muscle.

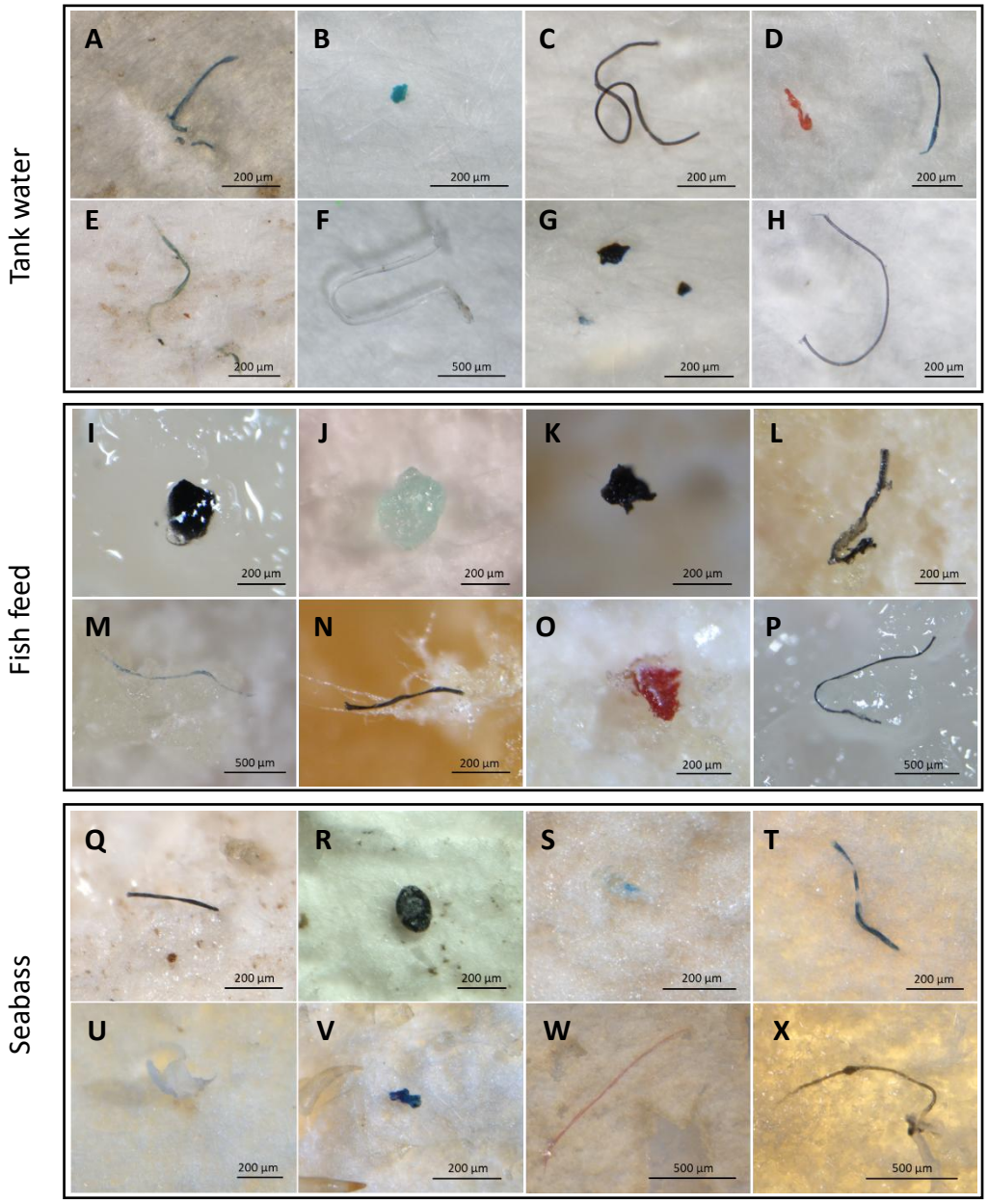

Figure 2.1. Visual diversity of the plastic particles recovered from water, feed, and European seabass body sites produced in a recirculating aquaculture system (RAS). A, D (blue item), E, M, N, and T – man-made cellulose/rayon; B and U – polyvinyl chloride (PVC); C, D (red item), H, L, P, and X – polyethylene terephthalate (PET); F – polypropylene (PP); G, I and K – phenoxy resin; J and R – polyethylene (PE); V – polystyrene (PS); W – polyacrylonitrile (PAN); and O, Q, and S – unidentified anthropogenic particles.

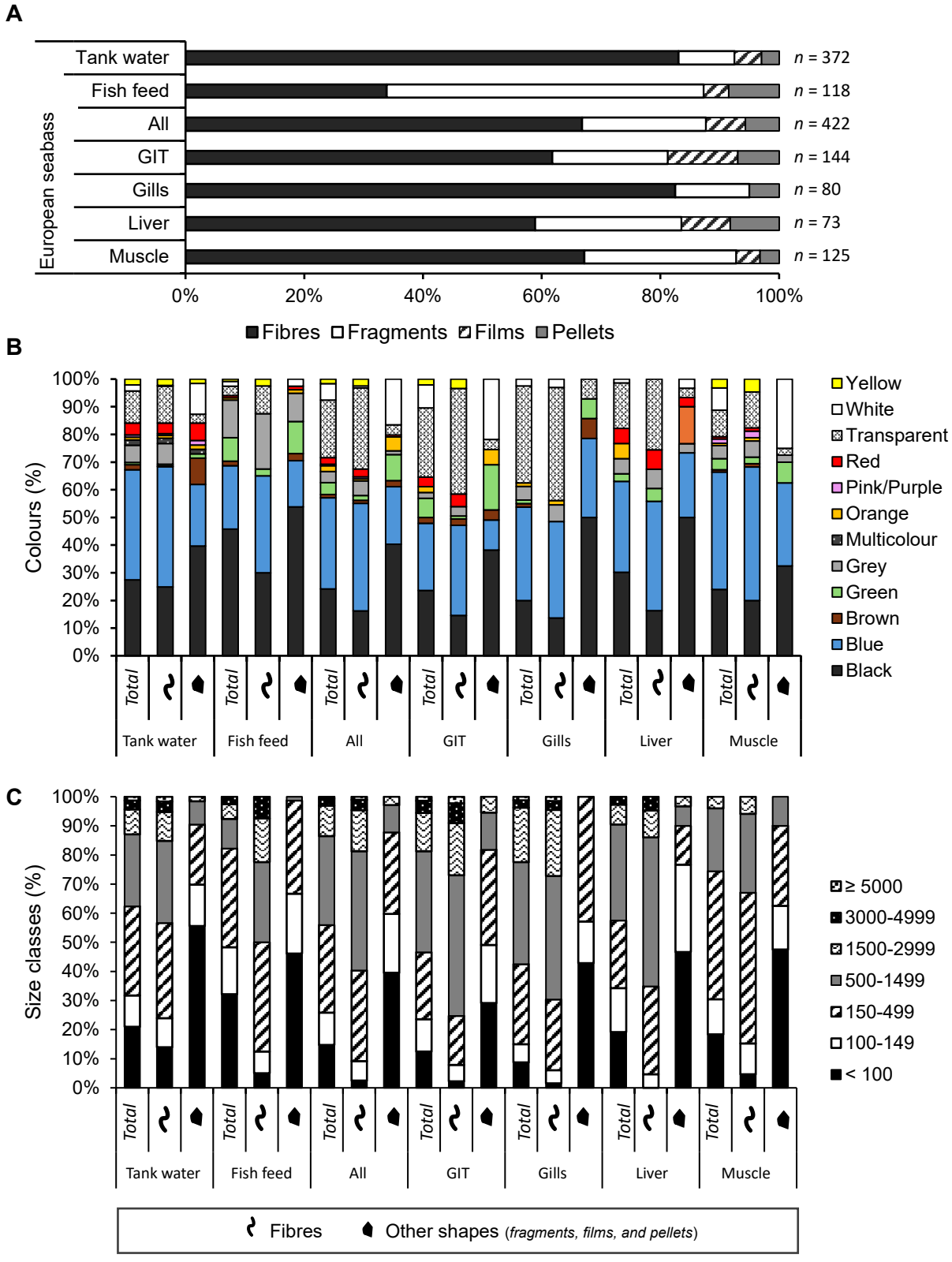

Figure 2.2. Percentage of the shape (A), colour (B), and size classes (C; in µm) of the plastic particles (PLs) recovered from 10 L of tank water collected from recirculating aquaculture system (RAS), 30 g of fish feed, and 55 European seabass. "*All*" refers to the sum of PLs found in all seabass body sites analysed, namely the gastrointestinal tract (GIT), gills, liver, and muscle. In A, *n* values represent the total PL number found in each sample type.

within 150–499 µm (29.0%) and 500–1499 µm (42.4%); PET particles were mostly blue (50.0%) and measured within 150–499 µm (65.6%); PVC particles were mostly white (63.3%); EPDM particles were mostly black (85.7%) and measured <100 µm (85.7%); particles made of organoclay-based coating and phenoxy resin were mostly black (76.9 and 100%, respectively).

2.3.3. Presence of plastic particles in European seabass body sites

All 55 sampled fish contained PLs in at least two of the body sites examined. More fish contained MPs in GIT, followed by muscle, liver, and gills ($p = 0.019$) (Table 2.1). For each body site, the total number of PLs per gram was higher in the GIT ($p < 0.001$) (Table 2.1).

Regarding PL shape, the number of fibres per gram of tissue was significantly higher in GIT than in the liver and muscle ($p = 0.003$) (Table 2.2). The fibres recovered from muscle were significantly smaller than those found in other body sites ($p < 0.001$) (Table 2.2). Also, PL films were significantly more abundant in GIT than in gills, liver, and muscle ($p < 0.001$). Between body sites, no significant differences in PL colours were found, except for white particles which were more abundant ($p = 0.008$) in GIT than in the other tissues (Table 2.2). The mean PL size found in GIT and gills was significantly higher than that of particles recovered from the liver and muscle ($p < 0.01$), as detailed in Table 2.1. The presence of particles measuring >1500 µm was mainly found in GIT and gills (Fig. 2.2C). The largest non-fibre-shaped PL found measured 2180 µm and overall PLs measuring >3000 µm represented less than 3% of the total particles found in seabass body sites. Only three meso-PL with fibre shape were recovered, two from the GIT and one from the gills. Concerning polymer type, man-made cellulose/rayon was the most abundant among body sites ($p < 0.001$) (Fig. 2.4). Man-made cellulose/rayon, organoclay-based coating, and PAM prevailed in GIT ($p < 0.05$); phenoxy resin only occurred in GIT ($p < 0.01$); PVC particles were more abundant in GIT and muscle; and PET particles were significantly more abundant ($p < 0.001$) in muscle (Table 2.2). Also, MPs made of man-made cellulose/rayon ($p < 0.001$) and PVC ($p = 0.01$) were significantly smaller in muscle in relation to GIT (Table 2.2).

Considering all body sites analysed a BCF_{Fish} equal to 19.9 indicates bioconcentration (*i.e.*, >1) of PLs in seabass, and a similar trend was observed in both GIT and gills ($BCF_{GIT} = 27.3$ and $BCF_{Gills} = 20.2$). On the other hand, when considering only PL exposure through feed, a total BAF_{Fish} equal to 0.2 and, in GIT, a BAF_{GIT} equal to 0.3 indicate that PL bioaccumulation did not occur in the fish sampled from this RAS facility.

Table 2.2. Occurrence of micro- and mesoplastic particles (PL) in water (10 L), fish feed (30 g), and body sites of 55 European seabass according to their most relevant shape, colour, and polymer type. Mean PL occurrence in water is expressed in PL/L, mean PL occurrence in seabass (sum of all body sites) is expressed in mean PL/fish, and mean PL occurrence in feed and tissues is expressed in PL/g feed and PL/g tissue, respectively.

PL characteristics	Tank water	Feed	Seabass				
			All	GIT	Gills	Liver	Muscle
Shapes							
Fibres							
n (%)	309 (83.1)	40 (30.9)	282 (66.8)	89 (61.8)	66 (82.5)	43 (58.9)	84 (67.2)
Mean PL occurrence	30.90 ± 3.94	1.30 ± 0.67	5.13 ± 2.82	0.63 ± 0.58^{a}	0.64 ± 0.94^{a,b}	0.47 ± 0.57^{b,c}	0.25 ± 0.27^{c}
Mean PL size (µm)	830 ± 1286	935 ± 1038	887 ± 871	1307 ± 1109^{a}	1232 ± 1147^{a}	891 ± 755^{a}	528 ± 484^{b}
Fragments							
n (%)	35 (9.4)	63 (53.4)	88 (20.9)	28 (19.4)	10 (12.5)	18 (24.7)	32 (25.6)
Mean PL occurrence	3.50 ± 1.81	2.10 ± 1.18	1.60 ± 1.34	0.19 ± 0.35	0.07 ± 0.18	0.21 ± 0.41	0.09 ± 0.18
Mean PL size (µm)	119 ± 136	133 ± 73	210 ± 190	221 ± 174	213 ± 142	210 ± 248	201 ± 186
Films							
n (%)	17 (4.6)	5 (4.02)	28 (6.6)	17 (11.8)	0	6 (8.2)	5 (4.0)
Mean PL occurrence	1.7 ± 0.9	0.17 ± 0.20	0.51 ± 0.72	0.12 ± 0.23^{a}	0^{b}	0.08 ± 0.25^{b}	0.01 ± 0.04^{b}
Mean PL size (µm)	462 ± 489	148 ± 78	533 ± 613	602 ± 564	n.a.	463 ± 842	346 ± 543
Colours							
Black							
n (%)	102 (27.4)	54 (45.8)	102 (24.2)	34 (23.6)	16 (20.0)	22 (30.1)	30 (24.0)
Mean PL occurrence	10.20 ± 1.82	1.77 ± 0.83	1.85 ± 1.33	0.26 ± 0.44	0.13 ± 0.28	0.25 ± 0.35	0.09 ± 0.15
Mean PL size (µm)	615 ± 1439	305 ± 667	358 ± 516	387 ± 581	615 ± 797	274 ± 354	249 ± 262
Blue							
n (%)	148 (39.8)	27 (22.9)	139 (32.9)	35 (24.3)	27 (33.8)	24 (32.9)	53 (42.4)
Mean PL occurrence	14.80 ± 1.76	0.90 ± 0.83	2.53 ± 1.76	0.24 ± 0.33	0.26 ± 0.52	0.25 ± 0.38	0.15 ± 0.21
Mean PL size (µm)	456 ± 597	491 ± 659	648 ± 867	1176 ± 1275^{a}	737 ± 836^{a,b}	453 ± 561^{a,b}	342 ± 370^{b}
Transparent							
n (%)	43 (11.6)	4 (3.4)	88 (20.9)	36 (25.0)	28 (35.0)	12 (16.4)	12 (9.6)
Mean PL occurrence	4.30 ± 0.17	0.13 ± 0.24	1.60 ± 1.66	0.25 ± 0.31^{a}	0.25 ± 0.47^{a,b}	0.11 ± 0.25^{b}	0.04 ± 0.10^{b}
Mean PL size (µm)	1483 ± 1224	1165 ± 1276	1428 ± 995	1353 ± 817	1713 ± 1357	1400 ± 77	1018 ± 405
White							
n (%)	8 (2.1)	2 (1.7)	25 (5.9)	12 (8.3)	2 (2.5)	1 (1.4)	10 (8.0)
Mean PL occurrence	0.80 ± 0.75	0.07 ± 0.10	0.45 ± 0.37	0.09 ± 0.21^{a}	0.02 ± 0.12^{b}	0.01 ± 0.10^{b}	0.03 ± 0.09^{a,b}
Mean PL size (µm)	677 ± 695	677 ± 695	650 ± 500	821 ± 547^{a}	1275 ± 37^{a}	703^{a,b}	316 ± 405^{b}
Polymer							
Cellulose/Rayon							
n (%)	218 (58.6)	30 (25.4)	224 (53.1)	80 (55.6)	52 (65.0)	37 (50.7)	55 (44.0)
Mean PL occurrence	21.80 ± 1.51	1.00 ± 0.47	4.07 ± 2.44	0.55 ± 0.47^{a}	0.51 ± 0.76^{b}	0.40 ± 0.45^{b}	0.16 ± 0.18^{c}
Mean PL size (µm)	607 ± 1042	622 ± 682	958 ± 866	1170 ± 1016^{a}	1133 ± 816^{b}	874 ± 812^{b,c}	542 ± 494^{c}
Phenoxy resin							
n (%)	10 (2.7)	39 (33.1)	9 (2.1)	9 (6.3)	0	0	0
Mean PL occurrence	1.00 ± 0.35	1.27 ± 0.62	0.16 ± 0.46	0.07 ± 0.23^{a}	0^{b}	0^{b}	0^{b}
Mean PL size (µm)	100 ± 41	124 ± 59	222 ± 196	222 ± 196	n.a.	n.a.	n.a.
Organoclay-based coating							
n (%)	1 (0.27)	1 (0.85)	13 (3.08)	8 (5.56)	1 (1.25)	3 (4.11)	1 (0.80
Mean PL occurrence	0.10 ± 0.17	0.03 ± 0.08	0.24 ± 0.47	0.05 ± 0.15^{a}	0.01 ± 0.07^{b}	0.04 ± 0.19^{a,b}	0.002 ± 0.01^{b}
Mean PL size (µm)	83	140	123 ± 69	123 ± 79^{a}	196^{b}	123 ± 35^{b}	53^{a,b}
PAM							
n (%)	12 (3.23)	0	7 (1.66)	6 (4.17)	0	1 (1.37)	0
Mean PL occurrence	1.20 ± 0.90	0	0.13 ± 0.39	0.05 ± 0.16^{a}	0^{b}	0.01 ± 0.06^{b}	0^{b}
Mean PL size (µm)	561 ± 248	n.a.	715 ± 737	829 ± 736	n.a.	30	n.a.
PET							
n (%)	58 (15.6)	13 (11.0)	32 (7.6)	3 (2.1)	5 (6.3)	4 (5.5)	20 (16.0)
Mean PL occurrence	5.80 ± 3.02	0.43 ± 0.23	0.58 ± 1.13	0.02 ± 0.08^{a}	0.04 ± 0.23^{a}	0.05 ± 0.19^{a}	0.06 ± 0.17^{b}
Mean PL size (µm)	1116 ± 1780	1437 ± 1441	702 ± 1203	570 ± 631	1954 ± 2709	772 ± 577	395 ± 453
PVC							
n (%)	9 (2.4)	0	30 (7.1)	12 (8.3)	0	1 (1.37)	17 (13.6)
Mean PL occurrence	0.90 ± 0.30	0	0.55 ± 0.90	0.09 ± 0.21^{a}	0^{b}	0.01 ± 0.10^{b}	0.05 ± 0.12^{a}
Mean PL size (µm)	86 ± 87	n.a	472 ± 495	767 ± 591^{a}	n.a.	384^{a,b}	270 ± 174^{b}

The mean PLs occurrence and size is presented by the mean and standard deviation (mean ± SD). Different superscript letters indicate significant differences among seabass tissues (Kruskal Wallis test, $p < 0.05$). GIT – gastrointestinal tract, PAM – polyacrylamide, PET – polyethene terephthalate, PVC – polyvinyl chloride.

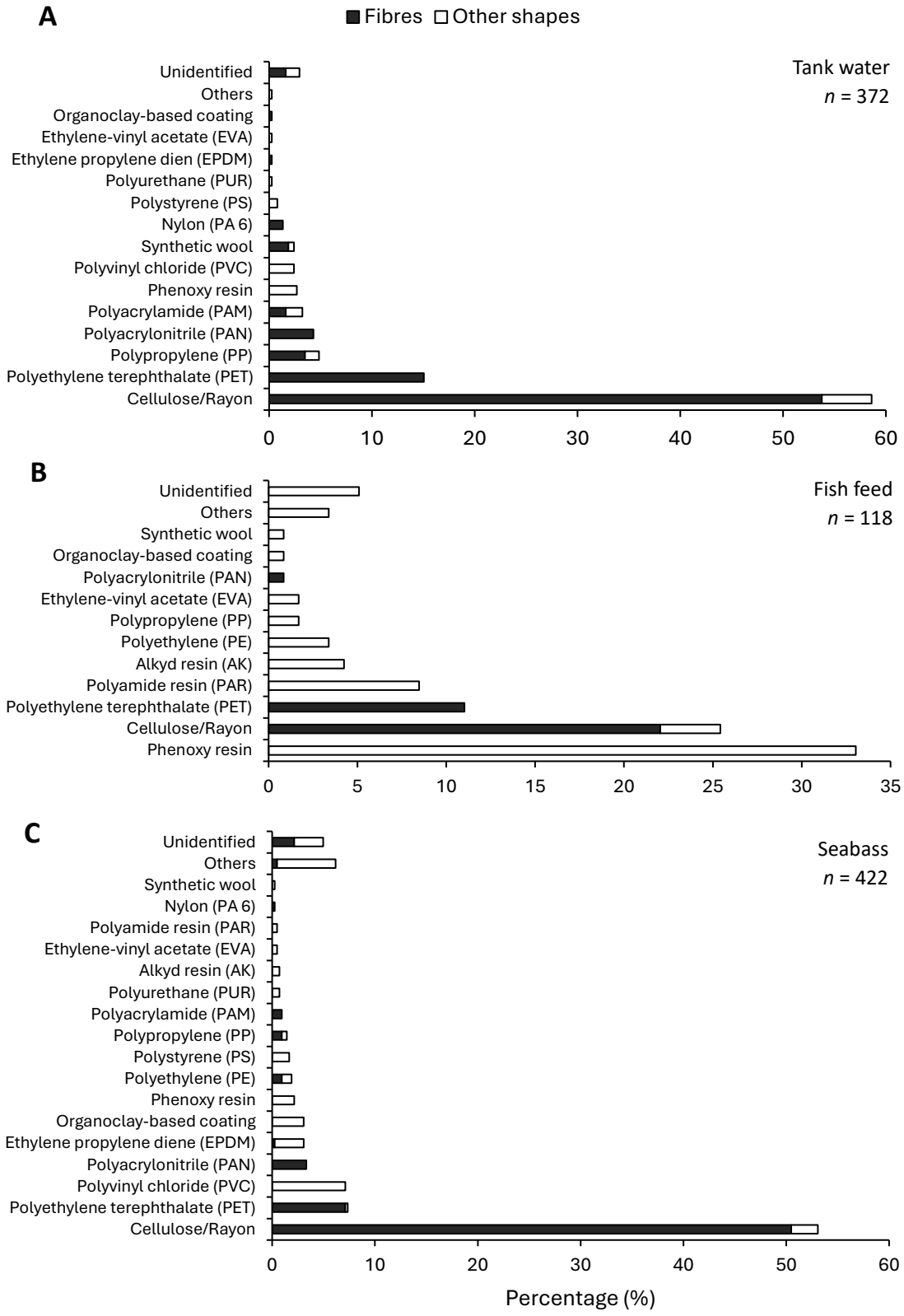

Fig. 2.3. Percentage of plastic polymers according to their shape, namely fibres (black), and fragments, films, and pellets (white), identified μFTIR, among the PLs recovered from (A) water, (B) fish feed, and (C) European seabass. Fragments, films, and pellets were included in "other shape" group due to their significantly lower abundance in general relative to fibres. The *n* value represents the PL number found in each sample type.

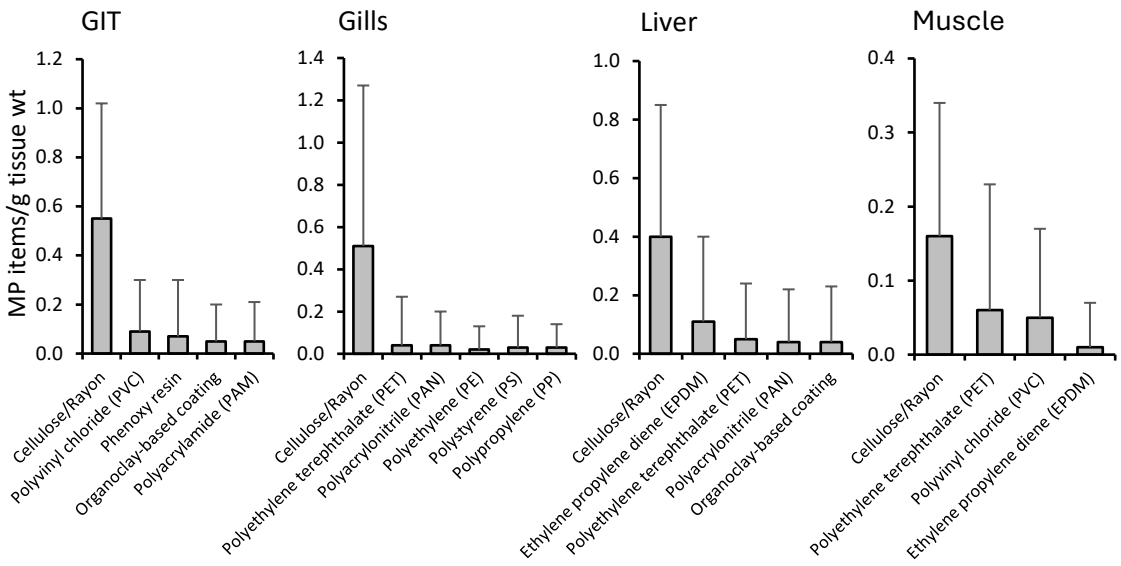

Fig. 2.4. Percentage of the most common polymers (>3% of occurrence) recovered from European seabass body sites, namely the gastrointestinal tract (GIT), gills, liver, and muscle.

2.3.4. Fish health and biomarkers of oxidative stress

The Fulton's body condition factor (K) for the examined fish (*N* = 55) was 1.18 ± 0.12 based on the TL and TW values (21.61 ± 1.86 cm and 122.04 ± 35.37 g) above-mentioned. The HSI and the VSI determined were 1.6 ± 0.5% and 12.1 ± 5.9%, respectively. PLs were found in all tissues of all sampled fish. So, biomarkers were evaluated in two groups of fish taking into consideration the mean PL per fish, specifically a group of fish with less than 7 MP and a group of fish with 7 or more MPs per fish. For all fish analysed (*N* = 23), the mean values for each biometric parameter and biomarkers in the liver (*i.e.*, CAT, GPx, GR, GST, SOD, TAC, LPO) and muscle (*i.e.*, LPO) are presented in Table 2.3, without significant differences between groups. No significant correlations ($p > 0.05$) could be found between total PL count vs. biometric values and oxidative stress biomarkers (Table S2.3).

Table 2.3. Biometric data, antioxidant and detoxification biomarkers determined in the liver and muscle of European seabass produced in a recirculating aquaculture system (RAS) for 8 months. "*All*" refers to the overall values for the analysed subpopulation.

	All	**Fish with < 7 PLs**	**Fish with ≥ 7 PLs**
N	23	11	12
Fish length (cm)	21.5 ± 1.7	21.3 ± 1.7	21.7 ± 5.5
Fish weight (g)	118.3 ± 32.9	111.3 ± 35.2	124.8 ± 44.1
K	1.2 ± 0.1	1.1 ± 0.1	1.2 ± 0.3
HSI (%)	1.8 ± 0.6	1.8 ± 0.7	1.7 ± 0.8
VSI (%)	11.6 ± 4.2	12.0 ± 6.0	11.2 ± 5.4
Liver			
CAT (µmol/min mg protein)	730.89 ± 50.96	736.79 ± 54.21	725.49 ± 51.88
SOD (µmol/min mL)	62.12 ± 65.73	81.66 ± 88.66	44.04 ± 33.82
GPx (nmol/min mg protein)	356.22 ± 116.92	350.87 ± 125.03	361.12 ± 119.66
GR (nmol/min mg protein)	56.22 ± 12.64	58.58 ± 10.77	54.06 ± 14.76
GST (nmol/min mg protein)	241.96 ± 74.75	257.36 ± 71.98	227.85 ± 80.72
TAC (Trolox eq. mM)	13.18 ± 2.62	13.22 ± 3.66	13.14 ± 1.45
LPO (nmol TBARS/g)	42.94 ± 19.52	38.73 ± 25.10	46.79 ± 13.80
Muscle			
LPO (nmol TBARS/g)	12.06 ± 6.23	13.66 ± 7.74	10.59 ± 4.68

K - Fulton's body condition factor; HSI - Hepatosomatic index; VSI - Viscerosomatic index. Values are presented by the mean and standard deviation (mean ± SD). No significant differences between groups were found (Student's T-test, $p > 0.05$).

2.4. Discussion

This study provides the first contribution to the existent knowledge on the presence of PLs in body sites of European seabass produced in a RAS pilot facility, while also investigating PLs in tank water and feed, which are two direct exposure pathways for RAS-farmed fish. Also, our results confirm the existent data on PL occurrence in wild-caught specimens and those reared in open aquaculture systems (summarised in Table S2.4). The present findings demonstrated that most PLs recovered from the tank water, feed and seabass body sites were MPs (<5000 µm), and their heterogeneity illustrates well the particle diversity found in the analysed RAS. Results also showed that fish produced in RAS are mainly exposed to MP through water and feed, with a limited contribution from polymers linked to RAS components.

2.4.1. Plastic particles in water

Several PLs were detected in tank water, mostly MPs, with a mean availability of 37.2 ± 1.9 PL/L that might be further uptaken by fish. Our results showed a higher abundance of particles than previous observations by Lu *et al.* (2019), reporting a presence of 0.58–0.72 PL/L in RAS water. These results are difficult to be directly compared with the present data, as relied on a different method for PL collection (*i.e.*, net with 300 µm mesh size *vs.* glass jar), which might compromise the collection of nano- and microplastics (<300 µm). Indeed, Lu *et al.* (2019) have only reported the presence of fibres which ranged from 200 to 2000 µm, whilst in the present study the PL size ranged from 16 to 11 261 µm. If a similar methodology had been applied for PL collection in the present study (*i.e.*, 300 µm nets), almost 50% of the MPs would have not been recovered (<300 µm), but MP levels in the analysed tank water would still be considerably higher than those reported by Lu *et al.* (2019). High PL occurrence level, ranging from 10.3 to 87.5 PL/L, was also observed in fishponds of the Pearl River estuary in China, after collecting water with glass jars (Ma *et al.*, 2020). Such PL concentrations are considerably higher than those reported in sea surface waters collected with ~300 µm nets: 0.001–0.004 PL/L in North-East Atlantic water (Kanhai *et al.*, 2017; Maes *et al.*, 2017) and 0.005–0.020 PL/L in more polluted locations such as the Maowei Sea and the Eastern China Sea (Chen *et al.*, 2018; Zhu *et al.*, 2019). Besides the lack of a standard methodology for water collection, other factors may difficult the direct comparisons between studies, such as proximity to urban or industrialised areas, climate, and hydrology context (Chen *et al.*, 2018; Ma *et al.*, 2020; Zhu *et al.*, 2019).

Fibres were the most common PL shape in tank water, confirming Lu *et al.* (2019) observations. PLs had highly diverse colours and the most abundant (*i.e.*, blue, black, and transparent) were mainly associated with fibre-shaped particles. Although PL

concentration in the water inlet reservoir that supplies the system is much lower compared to that in fish tanks, their presence indicates a potential contribution of PLs to the system. Indeed, RAS-farmed fish seem to be mainly exposed to MPs with similar shapes and colours to those reported in natural aquatic environments (Stenger *et al.*, 2021; Suaria *et al.*, 2020), suggesting a potential link with textiles-derived pollution (Browne *et al.*, 2011; Cesa *et al.*, 2017). But PLs occurrence does not seem to be exclusively linked to RAS components (*e.g.*, PET fibres from filtration systems) or water inlet contamination; the deposition of airborne fibres may also contribute to the high presence of fibres in tank water (Dris *et al.*, 2017; Kacprzak & Tijing, 2022). The low occurrence of large-sized PLs >1500 µm (13%) in tank water might be a direct result of the filtration methods capable of removing or immobilizing such particles, as previously observed by Lu *et al.* (2019) and by Lv *et al.* (2019) in the wastewater of a RAS facility. In addition, according to Dris *et al.* (2017), PLs available in indoor and outdoor air, as well as dust fall, usually fall within the 100-5000 µm size range, with PLs of bigger sizes being less abundant, reinforcing the possible contribution of airborne fibre deposition to the observed PL values in RAS water.

Man-made cellulose/rayon and PET were the most common polymer type and were often associated with fibre-shape particles. The high abundance of fibres made of these polymers suggests that RAS is susceptible to PL contamination similar to natural aquatic environments (Lu *et al.*, 2019; Stenger *et al.*, 2021; Suaria *et al.*, 2020). The low occurrence of polymers linked to RAS components (*e.g.*, PE and PP) suggests a lower contribution of the system to the overall PL levels found in water. The availability of certain polymers may be affected by their specific density (*e.g.*, PE, 0.86–0.97 g/cm^3; PP, 0.90–0.92 g/cm^3; PET, 1.37–1.46 g/cm^3; cellulose, 1.54–1.63 g/cm^3), potentially leading to a misrepresentation of polymers positive- or negative-buoyant (Coyle *et al.*, 2020). In fact, considering the low abundance of polymers (*e.g.*, PE and PP) with lower densities than the collected tank water (1.01 g/cm^3), and the reduced water usage in RAS (5% daily renewal), it is possible to suggest that airborne and waterborne contamination of the inlet reservoir may have accounted to the high levels of fibres found in this system. Overall, these results point to a need to carefully monitor PL occurrence in air and water, particularly in indoor RAS facilities, in order to implement adequate mitigating measures if required.

2.4.2. Plastic particles in feed

The mean number of PLs detected in the analysed feed (3.9 ± 1.4 PL/g) correspond well to values previously reported in commercial diets for catfish, *Heteropneustes fossilis*, 2.0 to 5.7 PL/g (Rahman *et al.*, 2022). To the best of our knowledge, the majority of studies have primarily focused on the occurrence of MPs in feed ingredients, particularly fishmeal. Fishmeal is a key ingredient used in the diets of carnivorous marine species,

and its MP content can vary from 0 to 17.3 PL/g, depending on the fishing grounds (Gündoğdu *et al.*, 2021; Hanachi *et al.*, 2019; Walkinshaw *et al.*, 2022; Wang *et al.*, 2022c). It is worth noting that the highest levels of MPs were detected in fishmeal derived from fish caught in polluted fishing areas, underscoring the influence of marine pollution on the safety of feed ingredients (Gündoğdu *et al.*, 2021; Wang *et al.*, 2022c). Furthermore, plant-based meals commonly used as alternatives to fishmeal in feed production, such as soybean meal, exhibited levels of MPs (from 0.8 to 1.7 PL/g) comparable to certain fishmeal sources (Walkinshaw *et al.*, 2022). Apart from the nature and quality of the ingredients, the processing technology employed could potentially contribute to the observed MPs in feed, but due to the limited number of studies available, further research is necessary to investigate this matter more extensively.

Fragments were the most common particle shape, in accordance with previous reports on fishmeal (Gündoğdu *et al.*, 2021; Hanachi *et al.*, 2019; Karbalaei *et al.*, 2020). However, in a recent study by Wang *et al.* (2022c), it was fibres, and not fragments, that accounted for the majority (96.1%) of all MPs recovered from fishmeal produced in ten of the main producing countries. Also in feed, Rahman *et al.* (2022) reported a higher percentage of fibres in relation to fragments. Blue and black were the most common colours found in MPs recovered from European seabass feed, corresponding to previous descriptions in fishmeal (Gündoğdu *et al.*, 2021; Thiele *et al.*, 2021). Also, feed particle size was generally smaller than 500 µm although previous studies have reported larger particles in fishmeal (Gündoğdu *et al.*, 2021; Karbalaei *et al.*, 2020; Wang *et al.*, 2022c). MPs size in feeds may depend on the fish species and fish size used for fishmeal production (Thiele *et al.*, 2021), and/or on the application of physical forces in several stages of the feed production process (*e.g.*, pressing and grinding). It is important to note that MPs can also be introduced during further production stages, such as packaging (Gündoğdu *et al.*, 2021; Wang *et al.*, 2022c).

Man-made cellulose/rayon and PET were also found in feed, although phenoxy resin was the most common polymer. The occurrence of this resin has not been verified in previous studies monitoring MP contamination in feed ingredients. On the other hand, the presence of cellulose-based polymers and PET has already been reported in fishmeal (Hanachi *et al.*, 2019; Karbalaei *et al.*, 2020; Wang *et al.*, 2022c). However, it is worth mentioning that the processing conditions applied during feed manufacture may interfere with the identification of MPs. Specifically, during extrusion, ingredients are submitted to high temperatures (>100 °C) which can further degrade certain polymers, as hypothesised by Gündoğdu *et al.* (2021). Low-density polyethylene, for instance, has a lower melting point of 110 °C than other polymers, such as PP, PS and PET are 160 °C, 240 °C and 260 °C, respectively. Further studies are needed to monitor ingredient quality and the impact of the

processing conditions on MP contamination levels in feed. This information is crucial for selecting more suitable dietary formulations for each species' life stages and identifying novel methods to mitigate MP presence in aquafeeds.

2.4.3. Plastic particles in fish body sites

Our results show that from all the body sites analysed from RAS-farmed seabass, GIT had the highest MP levels. Such findings are in line with previous studies monitoring MP levels in wild-caught and farmed seabass produced in open systems, indicating a natural trend for this species to ingest plastics (Akoueson *et al.*, 2020; Barboza *et al.*, 2020b; Bessa *et al.*, 2018; Kılıç, 2022; Reinold *et al.*, 2021; Sánchez-Almeida *et al.*, 2022) (Table S4). Within the 144 PLs recovered from GIT, fibres and transparent, blue, and black PLs were the most common shape and colours. Regarding size, PLs found in GIT ranged from 42 to 5194 µm and had an overall particle size higher than other body sites. Man-made cellulose/rayon accounted for more than half of the PLs found in GIT. The predominance of these PLs can be explained by their abundance in the RAS water and feed, evidencing the importance of this pathway for PL uptake by fish. Indeed, seabass is a visual predator and PL ingestion directly from water either inadvertently (Li *et al.*, 2021a) or due to confusion between MPs and prey (de Sá *et al.*, 2015; Lehtiniemi *et al.*, 2018), may influence the type of particles found in seabass tissues. Similar levels of PLs in GIT have been reported in larger European seabass specimens produced in cage-aquacultures off the coast of the Canary Islands, ranging from 2.7 ± 1.9 PL/fish up to 5.4 ± 4.2 PL/fish (Reinold *et al.*, 2021; Sánchez-Almeida *et al.*, 2022) (Table S2.4). In the present study, fish were starved for 24 h before sampling, and the majority of ingested MPs are efficiently excreted within this period (Grigorakis *et al.*, 2017; Ohkubo *et al.*, 2020; Roch *et al.*, 2021). Indeed, at the time of sample collection, all fish presented an empty GIT, so a large number of PLs might have already been passively excreted along with faeces. Caution should be taken when considering fish with GIT containing food as these may overestimate PL levels in GIT.

Similar to GIT, gills are also a first barrier to stressor exposure thus likely to retain more particles via passive filtration during the water passage through gill filaments (Bour *et al.*, 2020; Lu *et al.*, 2016). Regarding PL characteristics, gills had the highest percentage of fibres in a total of 80 MPs per body site, which may be explained by the predominance of this shape in the water, and by the retention potential of fibres in this body site during respiration (Bour *et al.*, 2020). The colours and size range (*i.e.*, 57–6672 µm) of the PLs found in the gills were similar to those found in the GIT. Several studies have also reported fibre occurrence in the gills of several species, including European seabass (Abbasi *et al.*, 2018; Akoueson *et al.*, 2020; Barboza *et al.*, 2020b; Guilhermino *et al.*, 2021). Also, despite the presence of polymers which may have been released by some RAS

components into the water, the majority of recovered particles were man-made cellulose/rayon fibres, highlighting the potential contribution of water inlet and airborne contamination in the RAS facility (Dris *et al.*, 2017). The results of the present study support the importance of GIT and gills as sentinel organs to monitor MP contamination levels in both natural and artificial aquatic environments (GESAMP, 2019).

The presence of PLs in the liver and dorsal muscle, with similar characteristics to those recovered from tank water, feed, GIT, and gills suggests the retention of MPs in internal organs (Abbasi *et al.*, 2018; Barboza *et al.*, 2020b; Guilhermino *et al.*, 2021; McIlwraith *et al.*, 2021). The presence of PLs in RAS-farmed seabass muscle corresponded well to values reported in wild seabass, specifically 0.04 ± 0.07 PL/g (Barboza *et al.*, 2020b) and 1.04 ± 0.07 PL/g (Akoueson *et al.*, 2020). Similar to GIT and gills, fibres accounted for over 50% of all PLs recovered from the liver and muscle. Moreover, the colours blue, black, and transparent prevailed. Man-made cellulose/rayon fibres were again the most common in the liver and muscle, but the presence of PLs made of polymers possibly present in RAS has also been noted in all tissues, such as white PVC fragments in GIT and muscle. PLs found in the liver and muscle were significantly smaller than those recovered from GIT and gills, with over 55% of the particles measuring less than 500 µm. These results may be explained by the lack of limitation regarding the PL size that GIT and gills are exposed to, and to the apparent size-dependent translocation of smaller particles into internal organs (Abarghouei *et al.*, 2021; Zitouni *et al.*, 2021). Moreover, MP absorption may be also limited to particles inferior to 150 µm (EFSA, 2016), suggesting that only 34.3 and 30.4% of the MPs found in the liver and muscle, respectively, might have been absorbed.

The estimated values of BCF (19.9) and BAF (0.2) in European seabass are in line with previous studies reporting PL bioconcentration, but no PL bioaccumulation in fish tissues (Elizalde-Velázquez *et al.*, 2020; Miller *et al.*, 2023). According to the EU REACH guidelines, only substances with a BCF >2000 are regarded as bio-accumulative, indicating a low bio-accumulative potential of PLs in European seabass. It was also observed that PLs are mostly concentrated in GIT and gills, albeit according to literature the majority of particles seem to be efficiently excreted with faeces or released through gills (Grigorakis *et al.*, 2017; Ohkubo *et al.*, 2020; Roch *et al.*, 2021). However, the mechanisms underlying the translocation of MPs into internal organs still remain unclear. Endocytosis has been identified as one of the possible pathways for MP absorption in tissues, but likely hindered by the limited capability of cells to phagocyte particles larger than 10 µm (Abihssira-García *et al.*, 2020; Sendra *et al.*, 2020; von Moos *et al.*, 2012). Granulocytoma formation has been proposed by von Moos *et al.* (2012) as a potential pathway for MP translocation in blue mussel *Mytilus edulis*, after observing the phagocytosis of MPs by eosinophilic granulocytes and their migration via enterocytes into the tissues. Previous

studies have also reported the phagocytic uptake of MPs from fish and mice macrophages (Abihssira-García *et al.*, 2020; Ramsperger *et al.*, 2020). As these immune cell types are present in the seabass innate immune system, it is therefore likely that this pathway for MP retention also occurs in seabass gills and intestinal epithelia. Nevertheless, more research on this topic is required since the uptake and absorption of MPs seem to be both tissue- and polymer-dependent (Abihssira-García *et al.*, 2020). Also, the direct contact between MPs and the aquatic environment may lead to the adsorption of biomolecules (*e.g.*, carbohydrates and lipids), which might enhance cellular internalization (Ramsperger *et al.*, 2020). For particles <150 µm, MPs might also penetrate deeper tissue layers by paracellular resorption, also known as persorption, and be transported via lymph vessels into circulation (Hussain *et al.*, 2001; Volkheimer, 1974). This pathway has been investigated mostly in bird and mammal intestine and seems to be affected by particle size and rigidity, which may limit MP persorption ability (Volkheimer, 1974). In fish, this uptake pathway remains however yet to be clarified.

2.4.4. Potential effects of plastic exposure on fish oxidative stress

All fish used in the present study contained PLs in at least two of the sampled body sites. It is important to mention that the total number of PLs identified in fish may still be underestimated as results from the sum of PL determined in individual tissues, and not from the analysis of the whole fish. All sampled fish had similar body sizes and were produced under controlled conditions in a RAS, for 8 months, being hence exposed to the same PL levels. Presumably, this might explain the absence of fish where MP presence was not observed and the lack of significant differences for any of the tested biomarkers between the two established groups of fish (<7 MPs and ≥7 MPs per fish). Previous studies reported a significant increase in the activity of SOD, CAT, and GST in the liver of fish with increased MP levels (Alomar *et al.*, 2017; Solomando *et al.*, 2022; Zitouni *et al.*, 2021). Increased LPO levels have been also reported in the brain, gills, and dorsal muscle of fish from the North-East Atlantic Ocean with high PL count (Barboza *et al.*, 2020b), but this could not be confirmed in the present study. This may suggest that either seabass individuals were not exposed to PL levels sufficiently high or long enough to trigger a physiological response, and/or that MP exposure in a RAS is constant and thus likely to be equally reflected in all specimens. For this reason, the values of each biomarker are provided as a baseline for future studies monitoring the effects of MP exposure in RAS.

2.5. Conclusion

Now, more than ever, the aquaculture sector aims towards sustainable production systems capable of rearing seafood products with high quality and safety. Within the different production systems, RAS are equipped with state-of-the-art technology that guarantee stable rearing conditions for growth. The present study reveals for the first time the presence of several PLs in tank water, feed, and European seabass produced in a RAS facility. All sampled fish contained PLs, with the highest mean PL/g in GIT. The mean PL size in GIT and gills was significantly higher than those found in the liver and muscle. The most abundant MPs in RAS were black, blue, and transparent man-made cellulose/rayon and PET fibres measuring within 150–1499 µm. PLs seem to bioconcentrate in fish, but not bioaccumulate in tissues, indicating the PL low bio-accumulative potential in European seabass. No significant differences were observed in oxidative stress biomarkers between fish with low (<7) and high (≥7) total PL numbers. The low occurrence of polymers linked to the analysed RAS components (*e.g.*, PE and PP) suggests a limited contribution of the system to the PL levels found in tank water. However, the implementation of alternative components (*i.e.*, either non-plastic or more resistant to weathering) and the continuous monitoring of plastic components' lifetime are advised to mitigate possible leakage of MPs and plastic additives. These findings suggest that fish produced in RAS are mainly exposed to MP through water and feed. Further studies are needed to monitor the quality of water and feed ingredients in order to minimise the potential hazards of PL pollution in the aquaculture production systems. Risk assessment of MPs should be also conducted to determine the extent to which they may pose a risk for both fish and human health. This information can be valuable for policymakers and entities in defining effective mitigating measures.

Chapter III

Occurrence of microplastics and metals in European seabass produced in different aquaculture systems: Implications for human exposure, risk, and food safety

by

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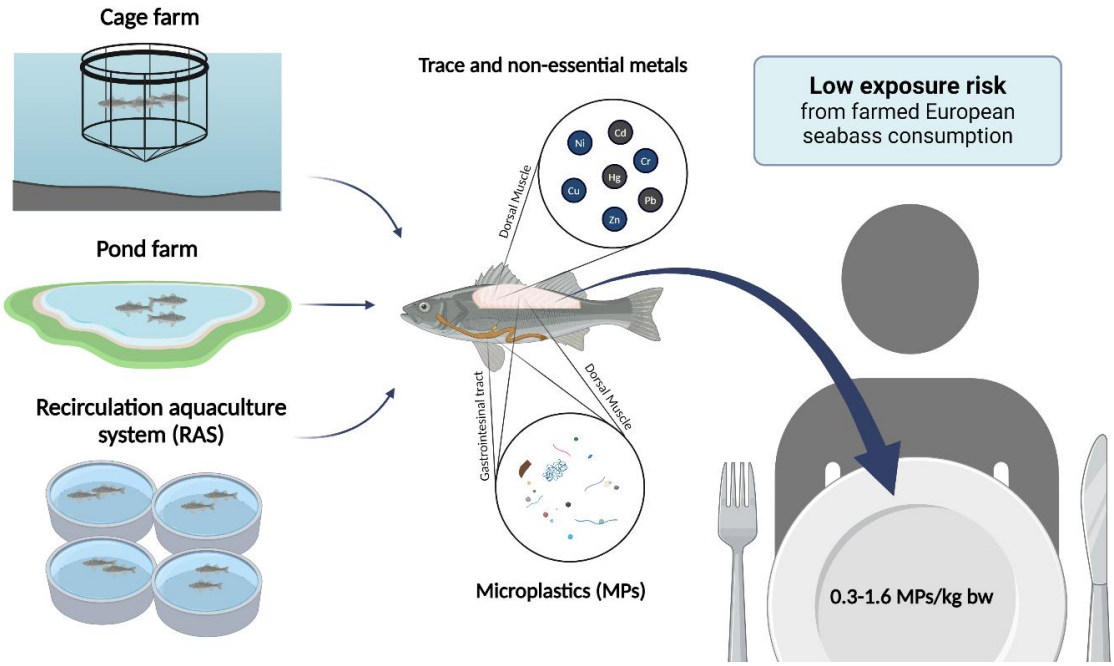

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Abstract

Microplastics (MPs) are emerging contaminants of increasing concern as they may cause adverse effects and carry other contaminants, which may potentially compromise human health. Despite occurring in aquatic ecosystems worldwide, the knowledge about MP presence in different aquaculture systems and their potential impact on seafood products is still limited. This study aimed to determine the levels of MPs in water, feed, and European seabass (*Dicentrarchus labrax*) from three relevant aquaculture systems and estimate human exposure to MPs and metals through seabass consumption. The recirculating aquaculture system (RAS) had the highest MP occurrence in water and feed. MP levels in seabass followed the aquaculture system's levels in water and feed, with RAS-farmed fish presenting the highest MP load, both in the fish gastrointestinal tract (GIT) and muscle, followed by pond-, and cage-farmed fish. MPs' characteristics across aquaculture systems and fish samples remained consistent, with the predominant recovered particles falling within the MP size range. The particles were visually characterized and chemically identified by micro-Fourier Transform Infrared Spectroscopy (μFTIR). Most of these particles were fibres composed of man-made cellulose and PET. MP levels in GIT were significantly higher than in muscle for pond- and RAS-farmed fish, MPs' bioconcentration factors >1 indicated bioconcentration in farmed seabass. Metal concentrations in fish muscle were below permissible limits, posing low intake risks for consumers according to the available health-based guidance values and estimated dietary scenarios.

Keywords

Anthropogenic contaminants; Bioconcentration; Seafood contamination Food safety; ‘One health’ concept

3.1. Introduction

The presence of microplastics (MPs), i.e., plastic particles <5 mm, in several food items raises global concerns regarding the impacts of this emerging pollutant on food safety (Garrido Gamarro & Constanzo, 2022). Human ingestion of MPs and their excretion has been documented through their presence in stools (Schwabl *et al.*, 2019; Yan *et al.*, 2022). Moreover, MPs have been detected in the human bloodstream, indicating their potential to reach other tissues (Leslie *et al.*, 2022). Although exposure may vary between individuals (e.g., location, diet, occupation), estimates predict a median daily intake rate between 553 and 883 MPs per capita, including ingestion and inhalation (Mohamed Nor *et al.*, 2021). MPs induce toxic effects in animals, such as fish (Barboza *et al.*, 2018a), and may also carry other contaminants either used during plastic manufacturing or adsorbed from the environment – including non-essential metals, polychlorinated biphenyls (PCBs), among others – which may further compromise human health (Barboza *et al.*, 2018a; Campanale *et al.*, 2020; Liu *et al.*, 2022a). Among food items, seafood products such as fish and shellfish have been identified as potential human dietary exposure pathways to MPs and associated contaminants (Cunha *et al.*, 2022; Garrido Gamarro & Constanzo, 2022; Li *et al.*, 2021d; Sequeira *et al.*, 2020). The aquaculture sector plays a pivotal role in meeting the demands of consumers, globally producing >50 % of the fish available in markets. It serves as a sustainable alternative to conventional fisheries that heavily rely on currently threatened natural stocks, contributing to global food security (FAO, 2022). Aquaculture is practised in diverse environments, including coastal and estuarine regions, utilizing various production systems to ensure a year-round supply of seafood products (FAO, 2022; Naylor *et al.*, 2021). However, previous studies have reported the presence of MPs in aquatic environments near aquaculture production sites, ranging from 0.004 to 87.5 MPs/L (Chen *et al.*, 2018; Huang *et al.*, 2023; Krüger *et al.*, 2020; Lin *et al.*, 2022; Ma *et al.*, 2020; Wu *et al.*, 2020; Xiong *et al.*, 2022; Zhu *et al.*, 2019). Many aquaculture facilities are located near urbanized and industrialized areas, which are known hotspots for plastic pollution and are particularly vulnerable to MP contamination (Chen *et al.*, 2021; Zhou *et al.*, 2021a). Additionally, fish farms employ plastic equipment such as ropes, floats, and impermeable liners, as well as use feed ingredients that may contain MPs, potentially contributing to their release into the environment (Lusher *et al.*, 2017).

Most fish and shellfish species commonly consumed by humans have been found to ingest MPs (Sequeira *et al.*, 2020; Walkinshaw *et al.*, 2020). In the context of aquaculture, recent studies reported the uptake of MPs in several economically relevant species (Alomar *et al.*, 2022; Garcia *et al.*, 2021; Kılıç, 2022; Sánchez-Almeida *et al.*, 2022; Savoca *et al.*, 2021; Wu *et al.*, 2020). The contamination of organisms with MPs is

influenced by numerous site-specific factors that determine the levels of MP exposure. In particular, the equipment used in different aquaculture production systems may impact MP availability within systems (Huang *et al.*, 2023). To the best of our knowledge, Lv *et al.* (2020) is the only study that has examined the role of different aquaculture production systems (***e.g.***, net cages, cement- and earthen-pond farms) in MP occurrence in Asian swamp eel, *Monopterus albus*. While no significant differences were observed among fish from different systems (2.4 MP/indv), significantly higher MP levels were found in wastewater samples collected during post-rearing stages from the net-cage and earthen-pond systems (1.6 and 1.8 MP/L) compared to the recycled-water system (0.1 MP/L) (Lv *et al.*, 2020). So, more research on the topic of MP presence in fish from different aquaculture systems is needed.

Exposure to MPs can have a substantial impact on fish health, which is influenced by particle concentration and their characteristics including shape, size, and polymer type, among other factors (Abarghouei *et al.*, 2021; Ding *et al.*, 2020; Lehtiniemi *et al.*, 2018; Liu *et al.*, 2021). MP exposure levels in experimental studies, ranging between 0.05 and 300 mg/L (ca. 250–10^6 MP/L), resulted in structural alterations in tissues and triggered immune response mechanisms, potentially leading to reduced fitness in affected organisms (Abarghouei *et al.*, 2021; Ding *et al.*, 2020; Lehtiniemi *et al.*, 2018; Liu *et al.*, 2021). Moreover, organisms can be simultaneously exposed to other contaminants, such as metals, present in water, food, and adsorbed to MPs, among other sources. In organisms, metals can accumulate in tissues and enhance the toxicological risk to organisms (Chen *et al.*, 2023b). Only a few studies have simultaneously evaluated MPs and metal levels in the tissues of wild fish and shellfish, and they have generally not found significant relationships (Akhbarizadeh *et al.*, 2018; da Silva *et al.*, 2022; Esmaeilbeigi *et al.*, 2023; Pradit *et al.*, 2021; Selvam *et al.*, 2021; Vieira *et al.*, 2021). This could be due to both low exposure levels and the influence of abiotic and biotic factors, such as water conditions or species' ecology. The interaction between MPs and metals in farmed organisms has been poorly evaluated but deserves particular attention due to the potential MP retention and metal bioaccumulation in species within aquaculture environments. It is crucial to comprehensively understand the interdependence among environments, aquaculture practices, and consumption concerning these contaminants to effectively plan and adopt mitigation strategies with the ultimate goal of enhancing seafood safety (Chen *et al.*, 2021; Zhou *et al.*, 2021a).

This study focused on the European seabass *Dicentrarchus labrax*, a highly valued fish species for European consumers. European seabass production predominantly occurs in Mediterranean countries, utilizing cage and pond aquaculture systems (EUMOFA, 2021). Previous research has reported the occurrence of MPs in both wild and farmed seabass

(Akoueson *et al.*, 2020; Barboza *et al.*, 2020b; Kılıç, 2022; Reinold *et al.*, 2021; Sánchez-Almeida *et al.*, 2022) as well as the presence of metals (Bat *et al.*, 2022; Cammilleri *et al.*, 2022; Milenkovic *et al.*, 2019; Renieri *et al.*, 2019; Ulusoy & Mol, 2022; Varol *et al.*, 2019). To the best of our knowledge, no study has yet evaluated simultaneously MPs and metals in farmed fish. Moreover, our previous study described the presence of MPs in various tissues of European seabass specimens produced in a recirculation aquaculture system (RAS) facility (Matias *et al.*, 2023), highlighting the relevance of water and feed as the primary pathways of MP exposure, a topic that needs more research. The main goal of this study was to compare the levels of MPs and other anthropogenic particles in water and fish feed, *i.e.*, the direct exposure pathways, and in seabass specimens produced in three different production systems (cage, pond, and RAS). Additionally, the levels of trace (Cr, Ni, Cu, and Zn) and non-essential metals (Pb, Cd, and Hg) in the dorsal muscle of fish were analysed. Estimations of human exposure through the consumption of farmed seabass fillets were conducted based on data provided by EUMOFA (2021). The human exposure scenarios to contaminants from European seabass fillet were further estimated. This study adopted the 'One Health' concept and focused on the interconnection between environmental, animal, and human health, by investigating the occurrence of MPs in the water, feed, and European seabass across three relevant aquaculture systems, and estimating seabass intake risk to consumer.

3.2. Materials & methods

3.2.1. Sample collection and aquaculture description

A total of 151 European seabass specimens were collected from three aquaculture production systems: 46 fish from cage aquaculture, 50 fish from a semi-intensive pond aquaculture, and 55 fish from a RAS pilot facility. The selected fish farms represent the main production systems for European seabass in countries involved in the commercial production of this species. Cage-farmed fish were provided by a large European retailer that was supplied by a facility located in the coastal Turkish waters of the Aegean Sea. Pond-farmed fish were directly obtained from an aquaculture facility on the NE Atlantic coast of Portugal, supplied with brackish water from an adjacent estuary. RAS-farmed fish were obtained from a Portuguese pilot facility, where the fish were maintained in plastic tanks with a daily renewal of 5 % of the water (Matias *et al.*, 2023). In order to protect the identity of the aquacultures, the exact location of each farm remains undisclosed. All fish were collected at the same season and were representative of a certain production batch within each system. All fish were collected using nets after a 24-h fasting period and euthanized in an ice slurry (<4 °C), following current aquaculture practices. Then, fish were transported to the laboratory in ice-filled boxes and processed for the study upon arrival.

Regarding pond and RAS water, glass jars were used to collect approximately 10 L of tank water where fish were produced (Barrows *et al.*, 2017; Matias *et al.*, 2023). No water samples could be obtained from the cage aquaculture, so MP concentration in water was estimated based on available data for that geographical area (Adamopoulou *et al.*, 2021; Yozukmaz, 2021). The fish feed used in the study was directly provided by each aquaculture facility under study.

At the laboratory, fish biometric parameters, including total body length (cm) and weight (g), were recorded (Table 3.1). Prior to further procedures, all fish were externally rinsed with distilled water to remove skin impurities, such as particles or debris. Subsequently, the whole gastrointestinal tract (GIT, from the oesophagus to the anus) and approximately 10 g of dorsal muscle sample were collected from each fish. These samples were wrapped in aluminium foil and preserved at a temperature of −20 °C until later determination of MP occurrence. An additional muscle sample was collected from each fish using a similar procedure and stored at −20 °C until further lyophilisation for the quantification of metal levels.

Table 3.1. Biometric and health index values, namely hepatosomatic (HSI) and viscerosomatic (VSI) indexes, for European seabass (*Dicentrarchus labrax*) collected from three different aquaculture production systems.

	Cage	Pond	RAS
Number of fish	46	50	55
Total length (cm)	30.1 ± 1.1[a]	23.3 ± 0.7[b]	21.6 ± 1.9[c]
Total weight (g)	269.0 ± 21.8[a]	138.4 ± 12.3[b]	122.0 ± 35.4[c]
Fulton's condition factor K	1.0 ± 0.1[c]	1.1 ± 0.1[b]	1.2 ± 0.1[a]
HSI (%)	1.5 ± 0.3[a]	1.3 ± 0.4[b]	1.6 ± 0.5[a]
VSI (%)	9.2 ± 1.6[b]	10.0 ± 2.3[a]	12.1 ± 5.9[a]

Different superscript letters indicate significant differences between aquaculture systems ($p < 0.05$).

3.2.2. Microplastic isolation, quantification, and characterization

Particle isolation, quantification, and characterization were conducted as previously described in Barboza *et al.* (2020b) and Matias *et al.* (2023). Briefly, the water samples were directly filtered through glass-microfibre filter membranes with a 1.2 µm pore size (Munktell & Filtrak, Germany) under vacuum conditions (pump Millipore WP6122050, Merck, Germany). For each feed sample, 30 g (six replicates of 5 g) was digested in glass flasks filled with 30 % hydrogen peroxide solution (H_2O_2; Merck, Germany) in a volume corresponding to three folds of the sample weight. In the case of seabass' GIT and muscle samples, digestion was performed in glass flasks filled with 10 % potassium hydroxide (KOH; Merck, Germany) solution prepared with ultrapure Milli-Q water (Millipore Corporation, USA) in a volume corresponding to three folds of the sample weight. Both fish feed and seabass tissue samples were then incubated at 60 °C for 24-h (Binder 53 BD, Germany), and vacuum-filtered through glass-microfibre filter membranes. The filters were subsequently dried at 40 °C for 24-h, before further analysis.

From each filter, particles were quantified and characterized under a stereomicroscope with an integrated camera (Nikon SMZ1000 with DS-Fi1, Japan), according to current guidelines provided for MP monitoring studies (GESAMP, 2019). Particle size was determined based on the largest cross section and measured using adequate imaging software (Olympus Cell^B, Germany). Then, particles were sorted by shape (fibres, fragments, films, foams, pellets), colour (black, blue, brown, green, grey, multicoloured, orange, pink/purple, red, transparent, white, yellow), and size classes (microparticles, <100 µm, 100–149 µm, 150–499 µm, 500–1499 µm, 1500–2999 µm, 3000–4999 µm; and mesoparticles, 5000–25,000 µm). However, due to the low number of mesoplastics (meso-PLs) recovered from samples, all plastic particles will be referred to as microplastics (MPs) for simplification.

Based on particle's visual characterization and heterogeneity, approximately 50 % of all particles recovered were selected to be chemically characterized through micro-Fourier Transformed Infrared Imaging Microscopy (μFTIR). The polymer identification was performed using a Nicolet™ iN10 MX Infrared Imaging Microscope (Thermo Fischer Scientific, USA). The selected particles were directly transferred from the filter to the equipment using a stereomicroscope. Several spectra per particle were collected in reflectance and attenuate total reflectance (ATR) modes with a resolution set at 8 cm^{-1} ranging from 4000 to 600 cm^{-1} with 16 scans. The resulting spectra were compared with available reference libraries with plastic polymers and additives spectra using OMNIC software (Thermo Fischer Scientific, USA), and only accepted for >70 % match. For non-analysed particles, chemical composition was inferred based on their visual similarity to already-confirmed particles through μFTIR (Matias *et al.*, 2023). All MP data presented encompasses fully synthetic MPs and other anthropogenic particles, such as cellulose-based polymers.

3.2.3. Quality control and quality assurance (QA/QC) procedures for microplastics

To prevent external contamination from MPs, strict protocols were followed during the handling of the samples, as recommended by GESAMP (2019). Dissections, samples preparation, and filtration procedures were performed in a clean room with restricted access to reduce external contamination. To avoid cross-contamination between fish and each body tissue sampled, all materials used in procedures were cleaned using 70 % ethanol and ultrapure water. Dissection materials were made of stainless steel and both white lab coats made of 100 % cotton and nitrile gloves were worn during procedures. Procedural blank samples (i.e., Petri dishes with clean filters) were incorporated at each stage of the process to monitor airborne contamination, and all filter membranes were carefully inspected under a stereomicroscope before use. Additionally, control samples containing KOH solution in ultra-pure water (10 % v/v) were employed as an additional precautionary measure (Barboza *et al.*, 2020b; Matias *et al.*, 2023). A total of 92 particles were found in procedural blanks related to the manipulation of cage aquaculture samples, 138 particles related to the pond aquaculture, and 128 particles related to the RAS. To compensate for contaminations during sample manipulation, all particles with similar characteristics (*i.e.*, shape, colour, size, and polymer type) to those found in procedural blanks were excluded from each sample.

3.2.4. Microplastic bioconcentration and bioaccumulation factors

The calculations of the bioconcentration (BCF) and bioaccumulation (BAF) factors were performed as previously described in Miller *et al.* (2023) and Matias *et al.* (2023).

To ensure compatibility, MP concentrations in water, feed, and seabass were converted into comparable units (MPs/L and MPs/kg). Considering the variability in water parameters among different aquacultures, it was assumed that 1 L of water, whether brackish or saltwater, was equivalent to 1 kg of water. The equations used for calculation were: 1) $BCF = C_{Fish} / C_{Water}$ and 2) $BAF = C_{Fish} / (C_{Water} + C_{Feed})$. BCF (L/kg) and BAF (unitless) values serve as predictors of the bioaccumulative potential of contaminants, with values >1 indicating bioconcentration and bioaccumulation in the organism/tissue, respectively. Only substances with a BCF > 2000 are considered bio-accumulative according to EU REACH guidelines (ECHA, 2017).

3.2.5. Metals in the muscle of European seabass

For each aquaculture, approximately 0.5 g of lyophilized muscle sample ($N = 6$) were digested in a polytetrafluoroethylene (PTFE) reactor using 1 mL of concentrated nitric acid (HNO_3) and 5 mL of 30 % H_2O_2 solution (Sigma-Aldrich, Germany). The digestion process was carried out in a high-pressure microwave system (ETHOS 1, Milestone S.r.l., Italy), using a previously optimised temperature programme: 5 min at 250 W, 5 min at 400 W, 5 min at 500 W, and 10 min at 0 W (Rocha *et al.*, 2019). After digestion, the flasks were cooled down at room temperature and each solution was subsequently transferred to separate tubes, followed by a final volume adjustment to 10 mL with deionized water.

The levels of several metals, namely cadmium (Cd), chromium (Cr), copper (Cu), led (Pb), mercury (Hg), nickel (Ni), and zinc (Zn), were determined through Atomic Absorption Spectrometry (AAS). Flame atomisation AAS was employed for Cu and Zn (Thermo Scientific iCE 3300, USA). Electrothermal atomisation AAS was employed for Cd, Cr, Ni, and Pb (Perkin-Elmer PinAAcle 900, USA). For Hg, cold vapour AAS was used (Thermo Scientific iCE 3000 series coupled with VP100 accessory, USA). Aqueous matched metal standard solutions were used for aqueous external calibrations curves and the detection limits (LoD) were determined in function of sample dry weight: <0.01 µg/g (Cd), <0.02 µg/g (Hg), <0.05 µg/g (Ni), <0.10 µg/g (Cr and Pb), <0.25 µg/g (Zn), and <1.0 µg/g (Cu). Then, metal concentrations were corrected and expressed as a function of wet weight.

All procedures were previously optimized and validated with the certified materials DORM-2 (dogfish muscle) and TORT-2 (lobster hepatopancreas), with satisfying values (between 90 and 113 % recovery) for the triplicate analysis of reference materials being obtained (Rocha *et al.*, 2019). Blank solutions were prepared using all the microwave digestion and analysis procedures with no significant metal contents being detected. All material used was previously washed with deionized water, soaked in a 20 % (v/v) nitric acid solution for 12 h and washed again with deionized water to avoid metal contaminations.

3.2.6. Human exposure scenarios to contaminants from European seabass fillet

Human exposure scenarios to MPs and metals from the consumption of farmed seabass fillets were evaluated through a deterministic approach, following the recommendations provided by EFSA (2012b). Dietary exposures were calculated for two specific age groups: toddlers (1 to <3 years old, with an average body weight of 12 kg) and adults (18 to <65 years old, with an average body weight of 70 kg). These age groups are likely to encompass exposure levels of older children and adolescents (EFSA, 2012b).

For the assessment of chronic human exposure, the dietary scenarios were developed based on data from the European Market Observatory for Fisheries and Aquaculture Products regarding the monthly household consumption of seafood and seabass (EUMOFA, 2021). The scenarios were also calculated for toddlers and adults from the countries where EUMOFA data on seabass consumption were available, namely Italy, Portugal, Spain, and the United Kingdom. To determine consumption scenarios, 2019 values for annual seafood consumption per capita were considered: Italy (31.21 kg/capita/year); Portugal (59.91 kg/capita/year); Spain (46.02 kg/capita/year); and the UK (20.13 kg/capita/year). The apparent seabass per capita consumption was obtained by the ratio between the mean volume-weight monthly consumption of seafood and that of seabass (EUMOFA, 2021). The mean monthly exposure to MPs, as well as trace and non-essential metals, was expressed, respectively, in terms of MPs and µg per unit of body weight.

To assess the toxicological risks associated with acute exposure, a meal portion of 150 g of seabass fillet was considered. The assessment relied on available health-based guidance values (HBGVs) as follows: a tolerable weekly intake (TWI) of 2.5 µg per kg of body weight per week (µg/kg bw/week) for Cd (EFSA, 2011); a tolerable daily intake (TDI) of 300 µg per kg of body weight per/day (µg/kg bw/day) for Cr(III) (EFSA, 2014); a tolerable upper intake level (UL) of 1000–4000 µg/day for Cu (EFSA, 2015); a TWI of 4 µg/kg bw/week for total Hg (EFSA, 2012c); a TDI of 13 µg/kg bw/day for Ni (EFSA, 2020b); a benchmark dose lower limit ($BMDL_{01}$) of 0.50 µg/kg bw/day for Pb (EFSA, 2010); and a UL of 7000–25,000 µg/day for Zn (EFSA, 2006). No HBGVs are currently available specifically for MP exposure from food (EFSA, 2016). The dietary exposure estimates were calculated using the formula, intake (%) = $([metal]_{muscle}/HBGV) \times 100$. For metals not detected in the muscle samples, concentrations were assumed to be as high as half the LoD (corrected for ww) to calculate the maximum detectable exposure (USEPA, 1991).

3.2.7. Statistical analyses

For each aquaculture system, the mean concentration of MPs found in water was expressed as MPs/L, while the number of particles per gram of feed and wet seabass tissue was expressed as MPs/g. Mean MP concentrations in fish considered all fish analysed. It is worth mentioning that the comparison of fish based on the presence or absence of MPs in the analysed body tissues is a simplification. As the whole fish body was not analysed for MP occurrence, the presence of MPs in fish tissues where no MPs were found cannot be excluded, although they were likely at lower levels.

For each variable, the data normality (Shapiro-Wilk test) and homogeneity of variances (Levene's test) were verified. The fish biometrics among aquaculture systems were evaluated using a one-way ANOVA, followed by a post hoc Tukey test. Due to the significant differences found among rearing systems, fish weight was included as a covariate in the subsequent contaminant analysis. A non-parametric ANCOVA with production systems as the main factor and fish weight as covariate was employed for MPs, followed by the post hoc Fisher's Least Significant Square (LSD) test. For metal concentrations, a parametric ANCOVA was used, followed by a Pairwise Comparison test. Within systems, the Mann-Whitney test was used to compare MP concentrations between GIT and muscle. The data regarding MP occurrence in water and feed, as well as MP characteristics, were compared among systems using a Kruskal Wallis test followed by the post-hoc Dunn's pairwise test. All statistical analyses were performed using the SPSS statistical analysis package (version 27) and a significance level < 0.05 was considered as the threshold for the tests used.

3.3. Results

3.3.1. Microplastics in water

In the pond aquaculture, a total of 63 particles were recovered from water samples and only 2 were discounted based on their similarity to particles found in procedural blanks. Thus, 61 particles were considered with a mean concentration of 6.10 ± 2.33 MPs/L water (Fig. 3.1A). Among them, the most prevalent shape was fibres (67.2 %), followed by fragments (16.4 %), pellets (8.2 %), films (4.9 %), and foams (3.3 %) (Fig. 3.2A). The different MP colour categories observed are depicted in Fig. 3.2B, with black and blue being the most commonly found. The mean MPs size was 654 ± 1263 µm, ranging from 68 to 9567 µm. MPs within the size range of 150–499 µm and 500–1499 µm accounted for 54.1 and 21.3 %, respectively (Fig. 3.2C). It is worth noting that only one fibre-shaped meso-PL was recovered from the pond water. Fibres were significantly larger (811 ± 1506 µm) than pellets (140 ± 74 µm) ($p = 0.041$). Among the identified polymers, the most common polymers were man-made cellulose/rayon (52.2 %), followed by phenoxy resins (9.8 %), polyacrylamide (PAM; 6.6 %), nylon (4.9 %), and polyethylene terephthalate (PET; 4.9 %). Other polymers, such as polyacrylonitrile (PAN) and polytetrafluoroethylene (PTFE), represented <4 % (Fig. 3.3A).

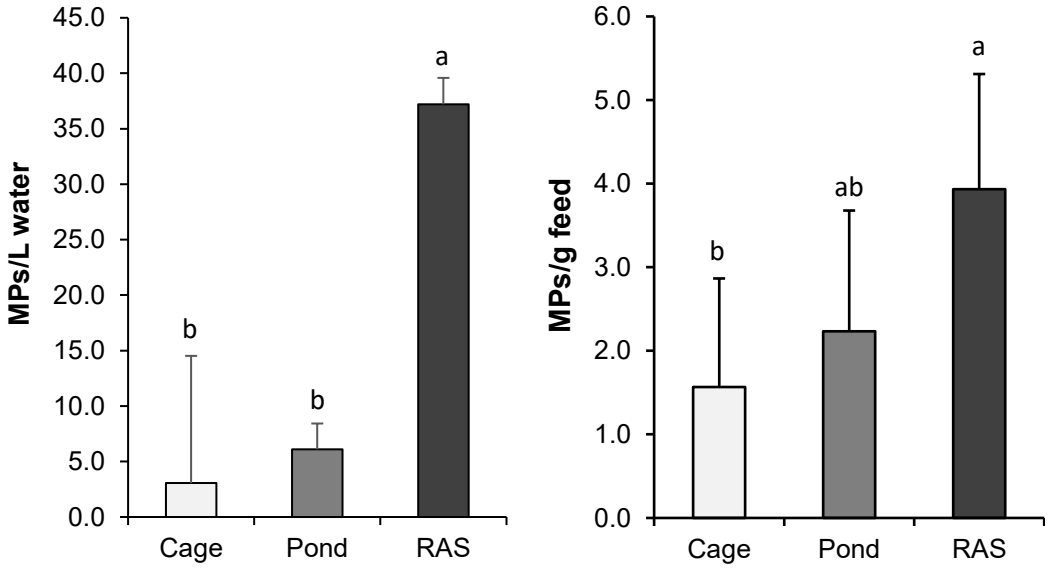

Figure 3.1. Mean concentration of microplastics (MPs) in water and feed (MPs/L and MPs/g, respectively) from three different production systems of European seabass, namely cage, pond, and recirculation aquacultures. Different letters indicate significance groups in water and feed. MPs values in water and feed from the recirculating aquaculture system (RAS) are adapted from Matias *et al.* (2023). MPs concentration in Eastern Mediterranean surface waters is an estimation of the values registered (i.e., using 333 µm nets) in locations close to where the cage aquaculture is placed (Adamopoulou *et al.*, 2021; Yozukmaz, 2021).

As described in more detail in Matias et al. (2023), 570 particles were recovered from the RAS tank water, but only 372 were considered with a mean concentration of 37.20 ± 1.94 MPs/L water (Fig. 3.1A). Fibres were significantly more abundant than other particle shapes ($p < 0.001$) accounting for 83.1 % (Fig. 3.2A). The most common colours observed were blue (39.8 %), black (27.4 %), and transparent (11.6 %) (Fig. 3.2B). MPs in RAS water ranged in size from 16 to 11,264 µm, with 30.6 and 24.7 % of the MPs measuring within 150–499 µm and 500–1499 µm, respectively (Fig. 3.2C). The predominant polymers were man-made cellulose/rayon (58.6 %) and PET (15.6 %). Other polymers, such as polypropylene (PP), PAN, PAM, and polyvinyl chloride (PVC), represented <5 % (Fig. 3.3A).

Inferred by the available data, a mean MP concentration equal to 4.47 ± 11.14 MPs/L was considered for Turkish waters surrounding the analysed cage aquaculture (Adamopoulou et al., 2021; Yozukmaz, 2021). Among the three aquaculture systems, water samples from RAS showed significantly higher concentrations of MPs relative to the cage and pond systems ($p = 0.003$) (Fig. 3.1A). For the water samples, fibres and fragment concentrations were significantly higher ($p < 0.05$) in RAS (30.90 ± 4.83 fibres/L and 3.50 ± 2.21 fragments/L) than pond water (4.10 ± 1.43 fibres/L and 0.50 ± 0.61 fragments/L). The concentrations of blue, black, red, transparent, and red MPs were significantly higher ($p < 0.05$) in RAS water (14.80 ± 1.76 blue MPs/L, 10.20 ± 1.82 black MPs/L, 4.30 ± 0.17 transparent MPs/L, and 1.60 ± 0.62 red MPs/L). With the exception of meso-PLs, the concentrations of all size classes below 5 mm were significantly higher in RAS water ($p < 0.05$). Within polymers, man-made cellulose/rayon was the most abundant followed by, respectively, PET in RAS water, and both phenoxy resin and PAM in pond water samples (Fig. 3.3A). However, the concentrations of man-made cellulose/rayon, PAN, PET, and phenoxy resin MPs were significantly higher in RAS water ($p = 0.036$).

3.3.2. Microplastics in seabass feeds

From the feed provided by the cage aquaculture, a total of 47 MPs were obtained and considered with a mean concentration of 1.57 ± 1.30 MPs/g feed (Fig. 3.1B). Fragments accounted for 51.1 %, followed by fibres representing 36.1 %, and films and pellets each representing 6.4 % (Fig. 3.2D). Eight different colour categories in MPs were observed (Fig. 3.2E). The size of the MPs ranged from 46 to 3543 µm, with a mean MP size of 462 ± 729 µm. Approximately 36.2 % of the MPs measured were within 150–499 µm and the relative occurrence of the different size classes is depicted in Figure 3.2F. The most common polymers identified in the feed were man-made cellulose (21.3 %) and phenoxy resin (12.8 %). Other polymers occurring <10 % are detailed in Figure 3.3B.

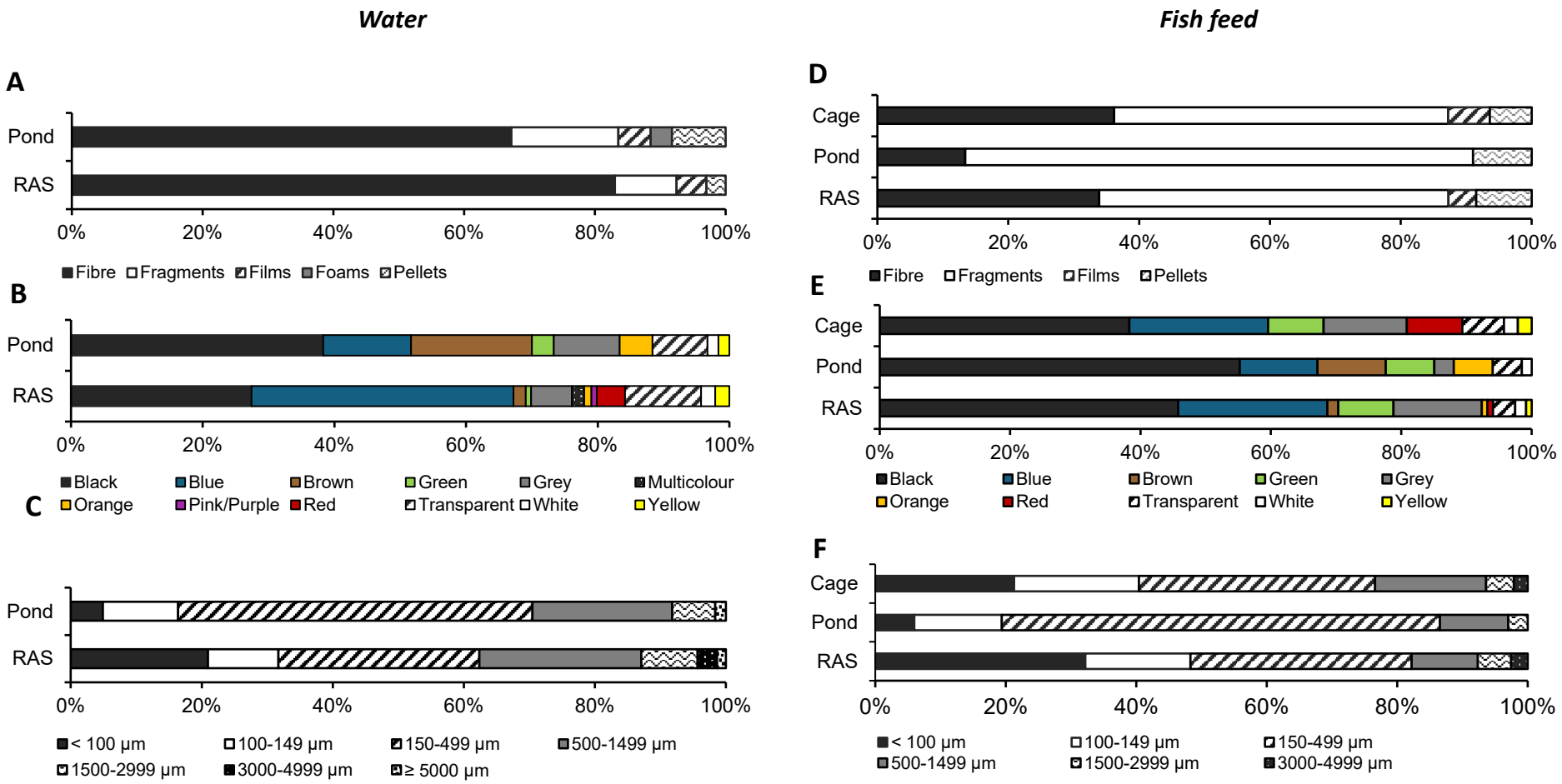

Figure 3.2. Percentage of the microplastics (MPs) per shape, colour, and size class, recovered from the water (A–C) and feeds (D–F) from three production systems of European seabass.

In the feed used at the pond aquaculture, a total of 67 MPs were recovered and considered with a mean concentration of 2.33 ± 1.44 MPs/g feed (Fig. 3.1B). Fragments were the most common shape, accounting for 76.1 %, followed by fibres (14.9 %), and pellets (9.0 %) (Fig. 3.2D). A total of 8 different MP colour categories were observed (Fig. 3.2E). The mean size of the MPs ranged from 32 to 2751 µm, with 67.2 % of the MPs measuring within 150–499 µm (Fig. 3.2F). Fibres were found to be significantly larger (1021 ± 846 µm) compared to fragments (232 ± 139 µm) and pellets (160 ± 73 µm) ($p < 0.001$). Within polymers, phenoxy resin and man-made cellulose/rayon were the most common polymers, accounting for 35.8 and 14.9 % of MPs, respectively. Other polymers representing <8 % of MPs are detailed in Figure 3.3B.

Moreover, as detailed in Matias *et al.* (2023), the RAS feed samples contained 188 particles but only 118 were considered, with a mean concentration of 3.93 ± 1.38 MP/g (Fig. 3.1B). Fragments and fibres were the most abundant shapes, accounting for 53.4 and 33.9 %, respectively (Fig. 3.2D). Regarding colour, the most common were black (45.8 %) blue (22.9 %), grey (13.6 %) (Fig. 3.2F). MP size ranged from 37 to 4526 µm, with a mean size of 410 ± 713 µm. The majority of particles were smaller than 100 µm (32.2 %) or within 150–499 µm (33.9 %) (Fig. 3.2E). The most common polymers were phenoxy resin (33.1 %), man-made cellulose (25.4 %), and PET (11.0 %). Additional polymers identified in MPs are depicted in Fig. 3.3B.

Significant differences were found between the analysed feeds ($p = 0.046$), with the RAS feed showing a significantly higher mean of MP concentration relative to the cage feed but not to the pond feed (Fig. 3.1B). Among shapes, only fibre concentration was significantly higher ($p = 0.040$) in the RAS feed (1.33 ± 0.67 fibres/g) relative to pond feed (0.33 ± 0.24 fibres/g), but not to cage feed (0.53 ± 0.47 fibres/g). No significant differences were found in the overall mean MP size among feeds, however, when considering shape and size, fragments in the pond feed (232 ± 139 µm) were significantly larger ($p < 0.001$) than fragments observed in RAS and cage feeds (133 ± 73 µm and 207 ± 160 µm, respectively). No meso-PLs were found in any feed samples. In the feed of the three systems, the most abundant polymers were man-made cellulose/rayon and phenoxy resins (Fig. 3.3B). Regarding polymer type, man-made cellulose concentration was significantly higher ($p = 0.030$) in the RAS feed (1.00 ± 0.47 cellulose MPs/g) than in both cage (0.40 ± 0.32 cellulose MPs/g) and pond (0.40 ± 0.20 cellulose MPs/g) feeds; PET concentration was significantly higher ($p = 0.004$) in RAS feed (0.43 ± 0.23 PET-MPs/g) relatively to pond, where PET-MPs were not observed.

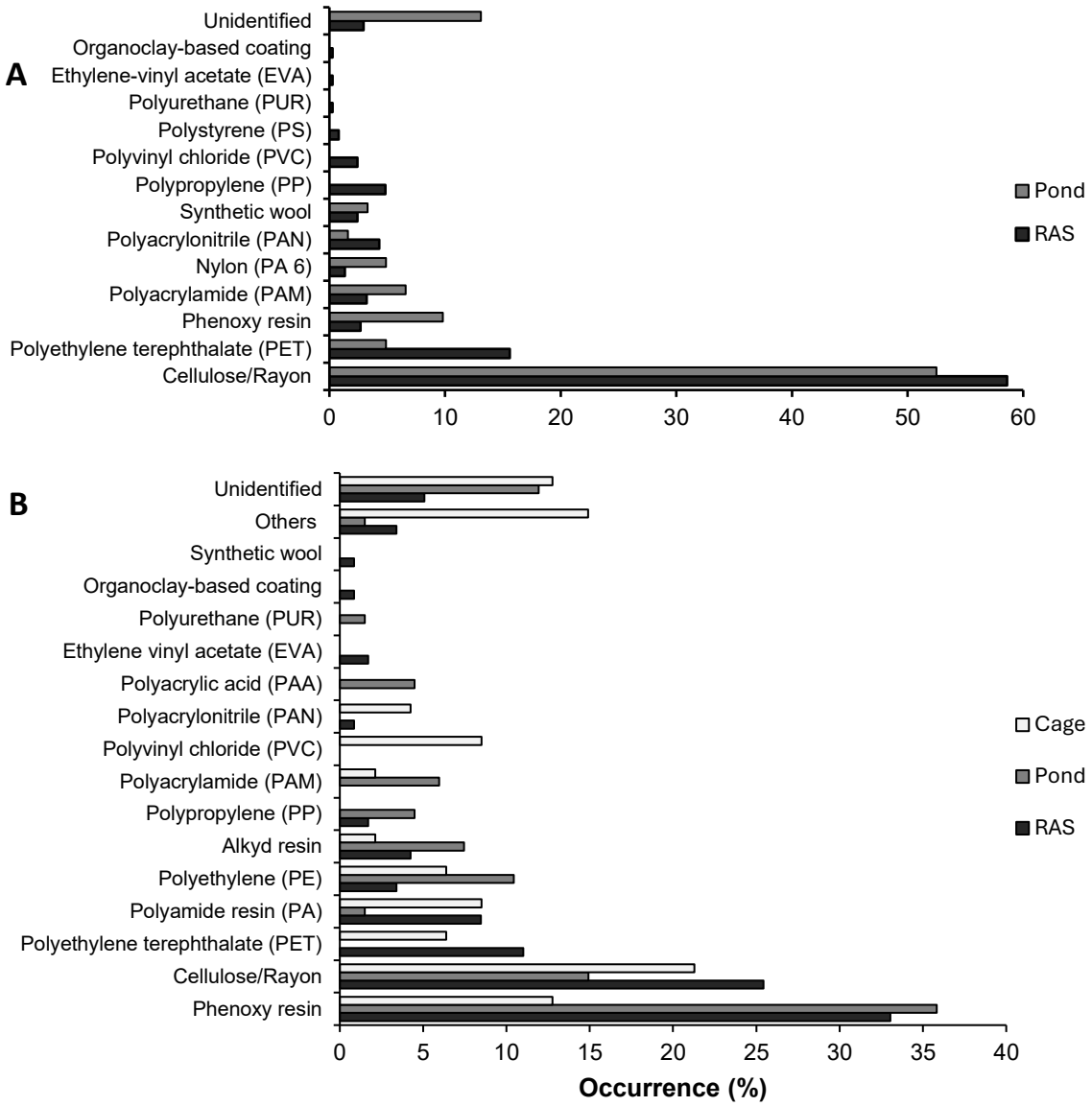

Figure 3.3. Percentage of the different polymers recovered from water (A) and feeds (B) from three production systems of European seabass.

3.3.3. Retention of microplastics in European seabass

Significant differences ($p < 0.001$) were found in the total body length and weight among the fish produced in the different aquaculture systems. The cage-farmed fish were found to be the largest, followed by the pond- and RAS-farmed fish (Table 3.1).

In cage-farmed seabass, a total of 197 particles were found but, after discounting particles similar to those found in procedural blanks, only 148 were considered. Out of the 46 analysed fish, 41 specimens contained particles in the GIT and dorsal muscle (3.2 ± 2.3 MPs/indv or 0.18 ± 0.13 MPs/g) (Fig. 3.4). There was no significant difference ($p = 0.912$) between MP concentrations in GIT and muscle (Table 3.2). The visual

characteristics of MPs in GIT and muscle are depicted in Fig. 3.5. Fibres and fragments accounted for 58.8 and 22.3 %, respectively (Fig. 3.5A). Blue (37.2 %), black (36.5 %), and grey (10.8 %) were the most common colours observed among MPs (Fig. 3.5B). Furthermore, MPs detected in cage-farmed seabass measured mostly within the three most common size classes, namely 150–499 µm (30.4 %), <100 µm (25.0 %), and 500–1499 µm (20.3 %) (Fig. 3.5C). There were no significant differences between MP sizes in GIT and muscle (p = 0.082), although meso-PLs were only found in the GIT samples (Table 3.2). Among polymers, man-made cellulose/rayon (40.5 %), PET (13.5 %), and phenoxy resin (7.4 %) were the most common. Other polymers found in cage-farmed fish are depicted in Fig. 3.6.

From pond-farm seabass, a total of 389 particles were recovered from filters and only 212 were considered for the study. The particles were found in 47 out of the 50 analysed fish with a mean concentration of 4.2 ± 2.8 MPs/fish or 0.53 ± 0.40 MPs/g (Fig. 3.4). GIT showed a significantly higher MP concentration than in muscle (p < 0.001) (Table 3.2). Fibres (47.6 %) and fragments (36.3 %) were the most common (Fig. 3.5A). Within 12 colour categories, 54.7 % were black and 21.2 % were blue (Fig. 3.5B). The most common MP size classes were 150–499 µm (30.2 %), 500–1499 µm (21.7 %), 100–149 µm (17.5 %), and <100 µm (17.0 %) (Fig. 3.5C). The mean MP size in GIT was significantly larger than in the muscle (p = 0.003) (Table 3.2). Phenoxy resin (35.4 %) and man-made cellulose/rayon (30.2 %) were the most common polymers, and the additional are depicted in Figure 3.6.

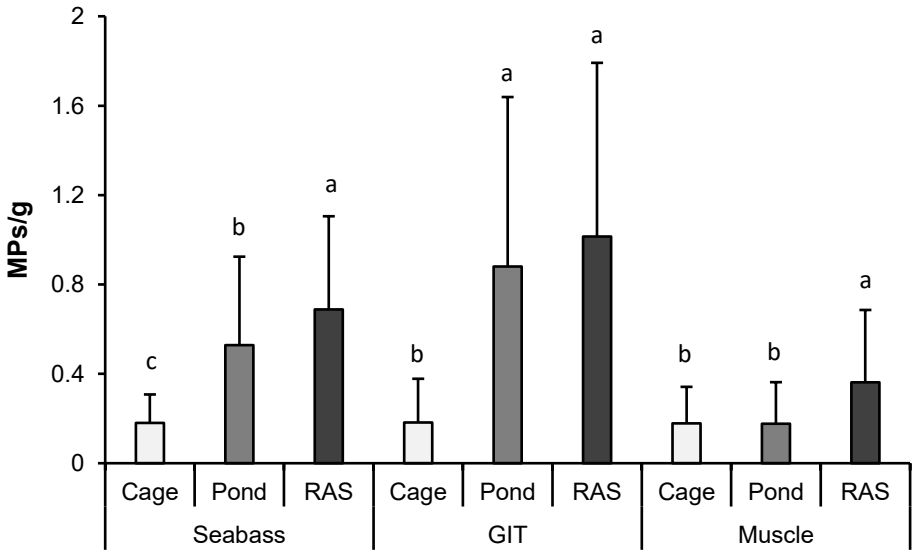

Figure 3.4. Microplastics (MPs) per gram of European sea bass body tissues, as well as per gram of gastrointestinal tract (GIT) and muscle, obtained from three different production systems. Different letters indicate significant differences among production systems.

Table 3.2. Number of microplastics (MPs), mean concentration (± standard deviation), and mean size and size range of the MPs recovered from European seabass (*Dicentrarchus labrax*) obtained from different aquaculture production systems.

	Cage	Pond	RAS
Fish with MPs			
N (%)	41 (89.1)	47 (94.0)	53 (96.4)
GIT			
Number MPs	58	140	144
Mean MPs/g	0.18 ± 0.20^{b}	$0.88 \pm 0.76^{a*}$	$1.01 \pm 0.78^{a*}$
Mean MPs size	680 ± 1123^{b}	$641 \pm 1027^{b*}$	$930 \pm 1022^{a*}$
MPs size range	19-6476	39-7042	42-5194
Muscle			
Number MPs	90	72	125
Mean MPs/g	0.18 ± 0.16^{b}	0.18 ± 0.19^{b}	0.36 ± 0.32^{a}
Mean MPs size	$587 \pm 708^{a,b}$	682 ± 752^{a}	425 ± 445^{b}
MPs size range	23-3988	92-4362	30-2181

Different superscript letters indicate significant differences between aquaculture systems, and "*" indicates significant differences between tissues ($p < 0.05$).

In RAS-farmed seabass, a total of 507 particles were recovered from filters. Only 269 particles were recovered from the GIT (n = 144) and muscle (n = 125) of 53 out of the 55 analysed fish, with a mean concentration of 4.89 ± 2.50 MPs/fish or 0.69 ± 0.42 MPs/g (adapted from Matias *et al.* (2023)) (Fig. 3.4). MP concentrations in GIT were significantly higher ($p < 0.001$) than those in muscle (Table 3.2). Visual characteristics (*i.e.*, shape, colour, and size classes) are depicted in Figure 3.5 and can be consulted in detail in Matias *et al.* (2023). Man-made cellulose/rayon (50.19 %), PVC (10.78 %), and PET (8.18 %) were the most common polymers in RAS-farmed seabass' GIT and muscle (Fig. 3.6).

Among aquaculture systems, the MP concentrations observed in the GIT of cage-farmed seabass were significantly lower (ANCOVA, p = 0.044) than in pond- and RAS-farmed seabass (Fig. 3.4). Conversely, the MP concentrations in muscle of RAS-farmed seabass were significantly higher (ANCOVA, p = 0.015) than those observed in cage- and pond-farmed seabass (Fig. 3.4). Man-made cellulose/rayon was the most abundant polymer in the tissues of the three analysed systems, followed by a high occurrence of PET in cage seabass, phenoxy resin in pond seabass, and PVC in RAS seabass (Fig. 3.6).

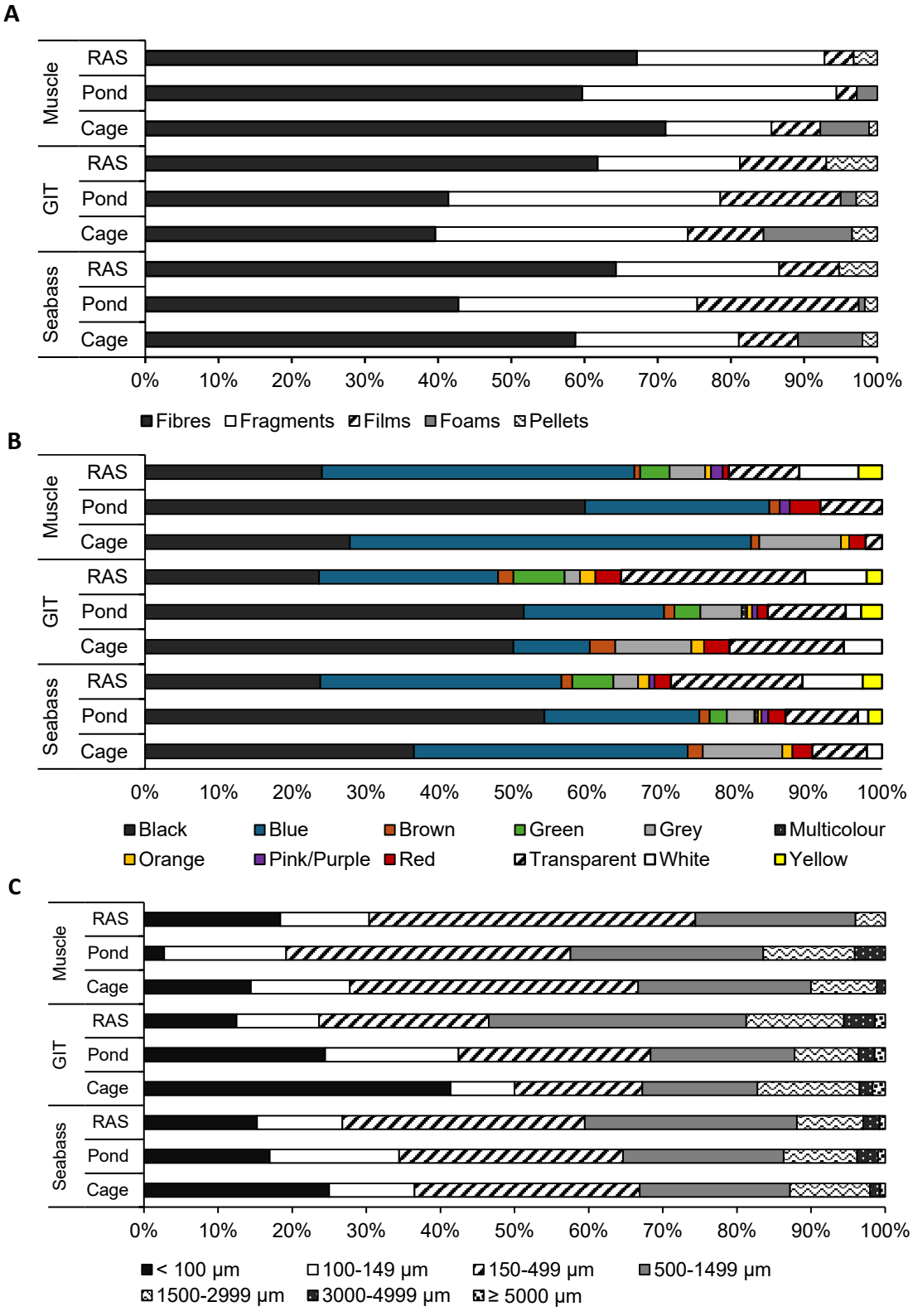

Figure 3.5. Percentage of the microplastics (MPs) per shape, colour, and size class, recovered from the gastrointestinal tract (GIT) and dorsal muscle of European seabass obtained from three production systems.

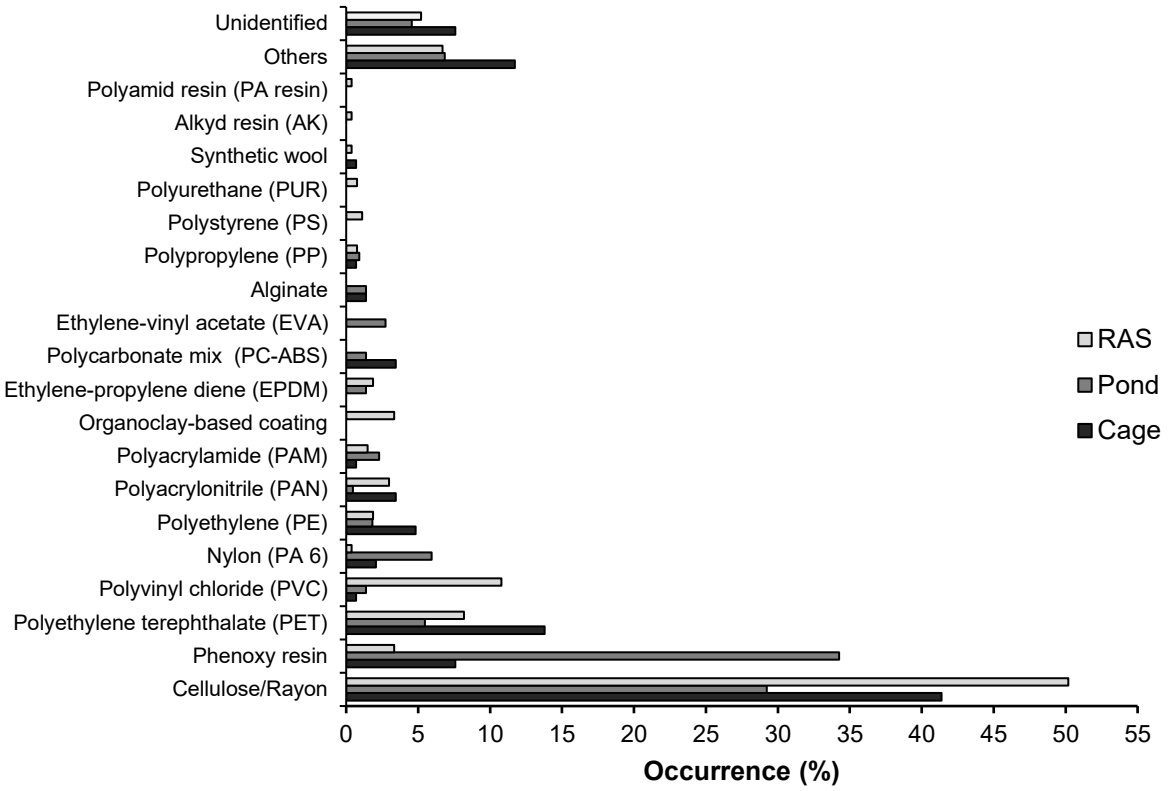

Figure 3.6. Percentage of polymers recovered from the gastrointestinal tract (GIT), and dorsal muscle of European seabass (*Dicentrarchus labrax*) specimens obtained from three production systems, namely cage, pond, and recirculation aquacultures.

When only considering the GIT and muscle, the BCF_{Fish} values calculated for cage-, pond- and RAS-farmed seabass were 40.2, 86.61, and 18.5 L/kg, respectively. BCF values >1 indicate bioconcentration of MPs relative to the surrounding environment, particularly from water. The BAF_{Fish} values for cage-, pond- and RAS-farmed seabass equalled to 0.11, 0.23, and 0.17, respectively. BAF values <1 indicate that no MP bioaccumulation occurred in farmed seabass.

3.3.4. Trace and non-essential metal levels in seabass muscle

Only Cr, Ni, Zn, Cd, and Hg were detected in fish muscle samples above their respective detection limits (Table 3.3). Among the three aquaculture systems, no significant differences were found in the concentrations of Cr, Ni, Cd, and Hg in the muscle samples (ANCOVA, $p = 0.417$, $p = 0.676$, $p = 0.944$, and $p = 0.908$, respectively). However, the Zn concentrations in muscle samples from pond-farmed seabass (5.542 ± 0.724 µg/g ww) were significantly higher (ANCOVA, $p < 0.001$) compared to those from RAS-farmed fish (3.657 ± 0.045 µg/g ww). The concentrations of all analysed metals were below the maximum permissible concentrations (MPCs) currently established for fresh fish, particularly for seabass: Cd, 0.05 µg/g; Cr, 0.05 µg/g; Cu, <20–70 µg/g; total Hg, 0.5 µg/g;

Ni, 0.5 µg/g; Pb, 0.2 µg/g; Zn, 30 µg/g (EC, 2005; FAO, 2003; Nauen, 1893; WHO, 1991). No significant differences were found ($p > 0.05$) between the established groups within aquaculture systems (i.e., fish with no MPs and fish with the highest MP level).

3.3.5. Human exposure scenarios to contaminants from European seabass fillet

Based on the EUMOFA data, significant differences ($p < 0.001$) were observed in the apparent seabass consumption per capita between countries (Fig. S3.1). Portugal showed the highest consumption (308 ± 23 g/capita/month), followed by Spain (146 ± 21 g/capita/month), Italy (144 ± 23 g/capita/month), and the UK (59 ± 9 g/capita/month). For each contaminant, the estimated exposure per month was calculated and detailed in Table 3.4. Among population groups, our results suggest that Portuguese toddlers are the most exposed group due to their lower body weight and high seabass consumption.

The human exposure scenarios to the analysed contaminants through the consumption of a meal with 150 g of seabass fillet did not exceed the available HBGVs. For MPs, the estimated intake for toddlers and adults (12 and 70 kg, respectively) were: 2.23 and 0.38 MPs per body weight (MPs/bw) from RAS-farmed seabass, 2.21 and 0.38 MPs/bw from pond-farmed seabass, and 4.53 and 0.78 MPs/bw from cage-farmed seabass. About metals and the available HBGVs, the toxicological risk for humans is considerably low (Table 3.5). For metals whose levels obtained were lower than the LoD, exposure estimations indicate that the seabass fillet from farmed sources provided for toddlers and

Table 3.3. Total metal concentrations (mean ± SD) found in the muscle of European seabass (*Dicentrarchus labrax*) produced in three different aquaculture systems. *All* refers to the mean concentrations for all fish; MPC refers to the maximum permissible concentrations set for each contaminant in seabass muscle (EC, 2005; FAO, 2003; Nauen, 1893; WHO, 1991); and < LoD refers to contaminants whose detection was below the analytical limits (Pb, < 0.10 µg/g; Cu, < 1.0 µg/g). All concentrations are expressed in µg/g ww.

Metals	All	Cage	Pond	RAS	MPC
Trace metals					
Chromium (Cr)	0.007 ± 0.007	0.008 ± 0.007	0.005 ± 0.003	0.010 ± 0.009	0.05
Nickel (Ni)	0.018 ± 0.008	0.019 ± 0.013	0.014 ± 0.003	0.020 ± 0.004	0.5
Zinc (Zn)	4.672 ± 0.971	4.647 ± 0.745[a]	5.542 ± 0.724[a]	3.657 ± 0.045[b]	30
Copper (Cu)	< LoD	< LoD	< LoD	< LoD	< 20-70
Non-essential metals					
Cadmium (Cd)	0.004 ± 0.002	0.005 ± 0.003	0.003 ± 0.002	0.003 ± 0.001	0.05
Lead (Pb)	< LoD	< LoD	< LoD	< LoD	0.2
Mercury (Hg)	0.028 ± 0.007	0.031 ± 0.009	0.027 ± 0.007	0.027 ± 0.005	0.5

adults, respectively: 0.31 and 0.05 % of the UL of Cu; and 31.25 and 5.36 % of the $BMDL_{01}$ value set for Pb (Table 3.5). The human exposure scenarios reveal that toddlers are more vulnerable to contaminants due to their lower body weight, even though metal concentrations in the fillet from all three aquaculture systems were considerably low to exert associated toxicological impacts.

Table 3.4. Mean monthly exposure to different contaminants, i.e., plastic particles (PLs), and trace and non-essential metals, observed through the consumption of farmed seabass fillet in four different countries using EUMOFA data. All values are presented for toddlers and adults (inside brackets) and expressed as PL/kg and µg/kg in function of mean body weight, 12 kg bw (70 kg bw), respectively.

	Portugal	**Spain**	**Italy**	**UK**
Plastic particles (PL)	9.29 (1.59)	3.25 (0.70)	4.33 (0.74)	1.78 (0.30)
Trace metals				
Chromium (Cr)	2.57 (0.44)	1.22 (0.19)	1.20 (0.21)	0.49 (0.08)
Nickel (Ni)	0.51 (0.09)	0.24 (0.04)	0.24 (0.04)	0.10 (0.02)
Zinc (Zn)	142.30 (24.30)	67.42 (10.77)	66.37 (11.38)	27.22 (4.67)
Copper (Cu)	25.68 (4.40)	12.17 (1.94)	11.97 (2.05)	4.91 (0.84)
Non-essential metals				
Cadmium (Cd)	0.13 (0.02)	0.06 (0.01)	0.06 (0.01)	0.02 (0.004)
Lead (Pb)	1.28 (0.22)	0.61 (0.10)	0.60 (0.10)	0.25 (0.04)
Mercury (Hg)	0.80 (0.14)	0.38 (0.06)	0.37 (0.06)	0.15 (0.03)

Table 3.5. Acute dietary intake (%) of the different contaminants in function of different body weights (toddler, 12 kg; adults, 70 kg (inside brackets) and relative to health-based guidance values (HBGV), for one 150 g meal of muscle seabass from different aquaculture systems. TDI and TWI, tolerable daily and weekly intakes, respectively; UL, tolerable upper intake level; $BMDL_{01}$, lower benchmark dose level.

	Cage	**Pond**	**RAS**	**HBGV**			
Trace metals							
Chromium (Cr)	0.033 (0.006)	0.021 (0.004)	0.042 (0.007)	TDI	300	µg/kg bw/day	EFSA (2014)
Nickel (Ni)	1.83 (0.31)	1.35 (0.23)	1.92 (0.33)	TDI	13	µg/kg bw/day	EFSA (2020b)
Zinc (Zn)	9.96 (2.79)	11.88 (3.33)	7.84 (2.19)	UL	7000-25,000	µg/kg	EFSA (2006)
Copper (Cu)	0.16 (0.03)	0.16 (0.03)	0.16 (0.03)	UL	1000-4000	µg/kg	EFSA (2015)
Non-essential metals							
Cadmium (Cd)	2.50 (0.43)	1.50 (0.26)	1.50 (0.26)	TWI	2.5	µg/kg bw/week	EFSA (2011)
Lead (Pb)	31.25 (5.36)	31.25 (5.36)	31.25 (5.36)	$BMDL_{01}$	0.5	µg/kg bw/day	EFSA (2010)
Mercury (Hg)	9.69 (0.43)	7.19 (0.26)	9.06 (0.26)	TWI	4	µg/kg bw/week	EFSA (2012c)

3.4. Discussion

Following a holistic approach under the 'One Health' concept, this study provides a first comparison of the occurrence of MP in the water, feed, and European seabass across three relevant aquaculture systems, and estimates human exposure to MPs and metals through seabass consumption. It was concluded that the levels of microplastics (MPs) in seabass were aligned with the concentrations of MPs found in both water and feed within each aquaculture system. The selected RAS-farmed fish presented the highest MP load, both in the fish GIT and muscle, followed by pond-, and cage-farmed fish. Nevertheless, it is important to mention that MP occurrence in aquatic environments is affected by multiple factors, including proximity to pollution sources, tidal cycles, river flow, wind intensity and/or direction, and rainfall (Malli *et al.*, 2022). Metal levels observed in farmed seabass muscle across aquaculture production systems were below MPC. Based on the estimated scenarios, human exposure to MPs and metals through seabass consumption is considered low. However, it is highlighted the complexity of environmental dynamics, underscoring the importance of gathering additional evidence from diverse farms under each production system to better understand MP and metal occurrence in farmed fish species.

3.4.1. Diversity of microplastics in water and fish feed

The predominance of fibres in water samples, as well as the presence of certain colours (e.g., black and blue) and polymers (e.g., man-made cellulose, PET, PAM), found in the pond and RAS aquaculture align with the most common characteristics of MPs observed in fish collected from Atlantic coastal and estuarine waters in Portugal (Bessa *et al.*, 2018; Guilhermino *et al.*, 2021; Lopes *et al.*, 2020). The occurrence of other MP types in the form of fragments and pellets made of PE, PP and polystyrene (PS) have been also found in Portuguese water and sediments (Antunes *et al.*, 2018; 2013; Frias *et al.*, 2014; 2010; Morgado *et al.*, 2022). Likewise, MPs with similar characteristics were found in coastal water and farmed fish in Turkey (Kılıç, 2022; Yozukmaz, 2021). It is worth noting that Adamopoulou *et al.* (2021) reported a higher abundance of fragments in the Aegean Sea, although different site-specific conditions and sampling methodology (*i.e.*, water currents, neuston nets at open sea) might influence the abundance of the different MP shapes. In the present study, the heterogeneity of MPs was mainly related to colour, size class, and polymer types; however, MP diversity in aquatic environments might vary greatly due to numerous site-specific and temporal factors, such as proximity to cities or precipitation level (Jambeck *et al.*, 2015). Regarding the feeds used in cage, pond and RAS facilities, the predominance of fragments, some of the colours (*e.g.*, black and blue) and polymers (*e.g.*, phenoxy resin, man-made cellulose, PE) found was in line with previous

studies monitoring MPs in fishmeal (Gündoğdu *et al.*, 2021; Hanachi *et al.*, 2019; Karbalaei *et al.*, 2020; Thiele *et al.*, 2021; Wang *et al.*, 2022c) and feed (Walkinshaw *et al.*, 2022).

Fibres constituted the prevalent shape of MPs found in the aquaculture environments where the analysed European seabass was farmed. This high fibre occurrence in the water has been previously observed in pond and RAS facilities (Huang *et al.*, 2023; Lu *et al.*, 2019; Lv *et al.*, 2020; 2019; Ma *et al.*, 2020), which is consistent with the findings in aquatic environments worldwide (Stenger *et al.*, 2021; Suaria *et al.*, 2020). Marine pollution from fibres is often linked to abandoned, lost, and discarded fishing gear used in aquaculture and fisheries, including ropes, lines, and nets. These materials are predominately composed of fibres which undergo weathering processes over time (Napper & Thompson, 2020). Moreover, wastewater from domestic washing machines contain thousands of natural and synthetic textile fibres which might reach aquatic environments due to the difficulties in retaining this particle type (*i.e.*, high length-to-diameter ratio) in wastewater treatment plants (Browne *et al.*, 2011; Cesa *et al.*, 2017). Indeed, man-made cellulose and PET are polymers commonly used in the production of textiles, being primarily associated with fibre-shaped particles in this study. Fibre contamination can also occur through airborne sources since fibres are common in both indoor and outdoor air. This has been suggested as a significant pathway to environmental contamination (Dris *et al.*, 2017; Napper *et al.*, 2023). As a result, fibres are frequently the most prevalent MP shape detected in aquatic organisms (Rebelein *et al.*, 2021; Sequeira *et al.*, 2020) and in fishery by-products such as fishmeal (Wang *et al.*, 2022c).

The higher abundance of MPs (*i.e.*, <5000 µm) may be a result of MP release and fragmentation of larger plastic debris in surrounding environments, which can explain the lower occurrence of particles >5000 µm that reach aquaculture environments (Barnes *et al.*, 2009). However, MP size distribution seems to differ between selected aquaculture systems. For instance, RAS facilities have filtration systems capable of retaining larger MPs (Huang *et al.*, 2023). In aquatic environments, MP size seems to control their dispersion and concentration, with meso- and larger MPs selectively drifting closer to the shoreline (Isobe *et al.*, 2014). For these reasons, it is worth mentioning that while MP levels in RAS water were significantly higher compared to other systems, the artificial configuration and effective water treatment capabilities of RAS are likely to significantly minimize MP contamination. In a previous study, it was observed that MP levels in a RAS were four times lower than the levels found in inlet water (Matias *et al.*, 2023). This indicates a potential build-up of MP and other anthropogenic particles within the tanks where fish are kept, possibly through airborne contamination or the fragmentation of plastic components. As safer system components are developed for RAS, it is expected that the potential for MP contamination can be further mitigated.

In feed, most MPs were identified as fragments and generally measured <500 µm, but particle size can vary depending on the source and type of ingredients used, namely the amount and fish species used to produce of fishmeal (Walkinshaw *et al.*, 2022). Moreover, various fishmeal and feed production stages, such as grinding or pressing, can promote plastic fragmentation (Gündoğdu *et al.*, 2021). To minimize fish exposure to contaminants, it is crucial to opt for ingredients with low MP contamination and implement a consistent monitoring system for MPs in aquafeeds.

3.4.2. Seabass contamination by microplastics

In the present study, the MP ingestion was observed in seabass from all three production systems analysed. The mean MP concentration in GIT ranged from 1.26 to 2.80 MPs per fish in cage and pond farms, respectively. Seabass from the pond and RAS facility exhibited higher MP levels per g of GIT, which might have been related to the significantly higher MP availability in the systems. A wider range of levels of MP ingestion were documented in seabass farmed in several cage farms in the Canary Islands, ranging from 0.6 to 5.4 ± 4.2 MPs/fish (Reinold *et al.*, 2021; Sánchez-Almeida *et al.*, 2022). In Turkish farms, smaller-sized seabass were found to have an average of 0.95 ± 1.1 MPs/fish, though no information was available regarding the specific type of production system (Kılıç, 2022). These results are comparable to those observed in wild specimens, ranging from 0.30 to almost 2.0 MPs/fish (Akoueson *et al.*, 2020; Barboza *et al.*, 2020b; Bessa *et al.*, 2018). The variability in MP ingestion levels may be explained by different fish sizes and site-specific environmental factors influencing MP availability and distribution in the water column of the aquatic environments where these specimens were collected. Nevertheless, according to Covernton *et al.* (2021), MP occurrence in wild fish does not seem to increase with trophic level or body size. In the present study, within each system, fish biometrics could not be associated with MP concentrations in seabass tissues (non-significant correlation was found, data not included), suggesting that the observed differences are mainly related to the aquaculture environment. Another contributing factor to MP ingestion by farmed fish might be their interaction with cages and fishing gear during harvesting. Reinold *et al.* (2021) reported that particles with colours similar to those of equipment showed bite marks, suggesting a nibbling, or attacking behaviour towards gear. The similarity between MPs recovered from water, feed, and fish, indicates that water and feed are two important exposure pathways for farmed seabass.

The presence of MPs in the muscle tissue of seabass produced in all three aquaculture systems indicates that MPs were uptaken by fish and reached the dorsal muscle where they were retained, in line with other studies. Muscle's MP concentrations in the present study were lower compared to those reported in wild seabass specimens by

Akoueson *et al.* (2020), ~1.0 MPs/g tissue, but considerably higher than those reported by Barboza *et al.* (2020b), 0.04 MPs/g tissue. Previous studies have also documented the occurrence of MPs, ranging from 0.1 to 6.0 MPs/g tissue, in the muscle tissue of other species captured in Portuguese (Guilhermino *et al.*, 2021) and Turkish waters (Atamanalp *et al.*, 2021), as well as in other regions worldwide (Abbasi *et al.*, 2018; Akhbarizadeh *et al.*, 2018; Selvam *et al.*, 2021; Zitouni *et al.*, 2020). Other studies did not find MPs in fish muscle samples (Hosseinpour *et al.*, 2021; Wu *et al.*, 2020). While the exact mechanisms allowing MPs to end up in the dorsal muscle are not completely understood, studies have demonstrated that particles smaller than 10 µm can be phagocyted by immune cells of fish and murine (Abihssira-García *et al.*, 2020; Ramsperger *et al.*, 2020). It is also hypothesized that larger MPs may be able to penetrate deep tissues through processes such as tissue persorption and transportation via the lymphatic system (Hussain *et al.*, 2001; Volkheimer, 1974). Therefore, despite further studies are required to fully understand MP translocation, evidence of MP internalization in fish muscle has been documented through in vivo studies (Zeytin *et al.*, 2020). It is currently accepted that MPs measuring <150 µm are likely to translocate across the gut epithelium and cause systemic exposure (EFSA, 2016). The high fibre occurrence suggests that this type of particle is more likely to be ingested and internalized by seabass compared to other particles, possibly due to their smaller diameter usually measuring a few micrometres (*i.e.*, ~10 µm) (Rebelein *et al.*, 2021). However, considering the current EFSA opinion on the absorption limit of particles smaller than 150 µm, only approximately 25 % of the MPs recovered from the seabass muscle of the three aquaculture systems would have resulted of internalization.

3.4.3. Trace and non-essential metals in farmed seabass muscle

Our results revealed that trace metal levels in seabass were below the maximum permissible concentration values (MPCs), and there were no significant differences observed among the selected aquaculture systems. Cr, Ni, Cu, and Zn are trace metals that are associated with specific proteins in metalloenzymes and play important roles in various catalytic functions (Wood *et al.*, 2011). However, dietary overexposure to these trace elements can have detrimental effects on human life. For example, the hexavalent forms of Cr have been associated with hepatic toxicity and an increased risk of cancer (EFSA, 2014).

These results are consistent with previous observations made in seabass produced in Portuguese (Custódio *et al.*, 2011; Fernandes *et al.*, 2008; Lourenço *et al.*, 2012), Turkish (Erkan & Özden, 2007; Özden & Erkan, 2008; Türkmen *et al.*, 2010; Varol *et al.*, 2019; Yildiz, 2008), Greek (Kalantzi *et al.*, 2016a; 2016b), and Italian aquacultures (Lomolino *et al.*, 2016) (Table S3.1). In previous studies, higher total concentrations of Cr exceeding the MPC have been reported in farmed seabass from Portugal and Greece, with

mean values ranging from 0.31 to 1.06 µg/g ww, respectively (Lomolino *et al.*, 2016; Lourenço *et al.*, 2012). Moreover, compared to wild specimens collected in Portuguese waters, Ferreira *et al.* (2010) reported higher Cu levels in farmed seabass, despite no significantly differing levels being detected between wild and farmed fish in a subsequent study (Custódio *et al.*, 2011). In other studies, in cage-farmed seabass, Cu levels were generally higher compared to pond-farmed seabass, possibly due to unknown additional sources of exposure (Fernandes *et al.*, 2008). The use of nets with antifouling coatings has been suggested as a potential contributor to the unpredictable release of trace metals, but no subsequent increase in metal levels in farmed fish was observed (Kalantzi *et al.*, 2016b). When compared with other studies conducted in the Mediterranean Sea, the metal values observed in seabass were either comparable or within the lower range (Alasalvar *et al.*, 2002; Ersoy *et al.*, 2006; Marengo *et al.*, 2018; Squadrone *et al.*, 2016).

Regarding non-essential metals, it is important to note that Pb, Cd, and Hg are considered harmful to human health, and do not have any essential function in the human body (Koller & Saleh, 2018). These metals are found in food in trace concentrations, both in inorganic and organic forms, with the latter generally being more toxic. Specifically, dietary exposure to Pb mostly affects the circulatory and central nervous system (EFSA, 2010), while Cd is known to cause kidney failure and increase the risk of cancer (EFSA, 2012a). Moreover, Hg, particularly its organic forms, is highly neurotoxic and potentially linked to the development of coronary heart diseases (EFSA, 2012c). These metals occur naturally in the environment and are capable of bioaccumulating throughout trophic levels. Therefore, in the case of farmed fish, feed is supposed to be the main exposure pathway.

In the muscle tissue of seabass from the three aquaculture systems analysed, Pb concentrations were lower than the LoD (*i.e.*, <1.0 µg/g), similar to the findings of Kalantzi *et al.* (2016a) and Varol *et al.* (2019). In general, Pb concentrations reported in the literature for seabass are below MPCs. However, Ersoy *et al.* (2006) reported a higher mean Pb level in raw fillets from Turkish farmed seabass (0.28 µg/g). Moreover, no significant differences were found in Cd and Hg levels between the different aquaculture systems, and these values were within the same order of magnitude, or the lower range of metal levels reported in wild and farmed seabass from the Mediterranean Sea (Cammilleri *et al.*, 2022; Ferreira *et al.*, 2010; Kalantzi *et al.*, 2016a; Lourenço *et al.*, 2012; Marengo *et al.*, 2018; Renieri *et al.*, 2019; Squadrone *et al.*, 2016; Trocino *et al.*, 2012; Varol *et al.*, 2019; Yabanli *et al.*, 2012) (Table S3.1). It is important to mention that metal concentrations in fish can vary across different regions and/or environmental conditions. For instance, previous studies have reported Cd levels (0.05 to 0.17 µg/g) exceeding the MPCs in seabass produced in Turkish farms (Türkmen *et al.*, 2010; Ulusoy & Mol, 2022). It has been hypothesized that these elevated Cd levels may be associated with seasonal harmful algal

blooms in the Aegean Sea, which have become more and more frequent and intense (Ulusoy & Mol, 2022). On the other hand, no studies have reported Hg levels above the MPCs in seabass farmed in European countries, indicating that, arguably, this metal is not a major concern in European seabass farming.

3.4.4. Interaction of microplastics and metals on fish

In the present study, the metal levels in fish with low and higher MP levels did not differ. Observational studies with wild fish have found positive relationships between MPs and metals however, this may be explained by differential background pollution levels in sampling points as well as different habitat and feeding ecology, and organism condition, among others (Akhbarizadeh *et al.*, 2018; Selvam *et al.*, 2021). This is supported by previous studies that demonstrated experimentally that MP exposure can influence metal assimilation in fish tissues. For instance, Barboza *et al.* (2018b) observed that seabass exposed to MPs (0.26, 0.69 mg/L) and Hg (0.010, 0.016 mg/L) in combination exhibited higher Hg levels in the gills and liver. Zuo *et al.* (2022) observed that grass carp (*Ctenopharyngodon idella*) exposed to 700 µg of MPs/L and 100 µg of Cd/L had higher Cd levels in the intestine. Hoseini *et al.* (2022) observed that common carp (*Cyprinus carpio*) exposed to 0.5 mg of MPs/L and 0.25 mg of Cu/L had higher Cd levels in the liver. In African fish (*Clarias gariepinus*), Jang *et al.* (2022) observed that fish exposed to 100 g of MPs/kg feed in combination with Cu (0.050 mg/L) or Pb (0.060 mg/L) lead to higher assimilation of these metals in several tissues. Conversely, also in African catfish, Soliman *et al.* (2023) observed no significant differences in the blood levels of Pb between fish solely exposed to 1 mg of Pb/L or in combination with 100 mg of MPs/L. It is important however to mention that MP levels used in exposure studies are usually high and/or expressed in units incomparable to those reported in environmental conditions (Cunningham & Sigwart, 2019). Further studies monitoring MP and metal concentration levels in aquaculture systems are required to better assess fish exposure to these contaminants and investigate the interactions between them.

3.4.5. Human exposure to MPs via contaminated seabass fillet and health implications

As farmed fish is becoming a significant source of proteins and omega-3 fatty acids, particularly in South European countries, the control of contaminants is of utmost importance. However, it is important to remember that seafood constitutes only a fraction of the overall human diet, and thus human exposure to MPs and metals daily is expected to be considerably higher due to their presence in various food items (Garrido Gamarro & Constanzo, 2022). Indeed, estimations based on EUMOFA (2021) data indicated that European seabass only represented on average 3.5 to 6.2 % of the per capita seafood

consumption in the United Kingdom and Portugal, respectively. Furthermore, it should be considered that food preparation and consumption practices influence contaminant levels. Previous studies have reported increased MP levels in food items following food preparation (Catarino *et al.*, 2018; Habib *et al.*, 2022; Zhang *et al.*, 2022c). The effects of different cooking methods on metal levels in seabass fillets have also been reported (Ersoy *et al.*, 2006; Lomolino *et al.*, 2016).

Intuitively, exposure to contaminants from seabass fillets is dependent on seabass consumption. Based solely on the observed concentrations of contaminants in fish muscle, it can be concluded that Portuguese toddlers and British adults had the highest and lowest exposure, respectively, due to differences in apparent per capita seabass consumption and body weights between countries. Regarding MPs, monthly per capita exposure ranged from 1.8 to 9.3 MPs per unit of body weight for British and Portuguese seabass consumers, respectively. Since there are no available HBGVs for MPs in food, estimating the toxicological risk for consumers is challenging. Therefore, further studies are needed to monitor MP levels in the muscle of farmed fish to estimate human consumer exposure more accurately. In a study involving human subjects, Yan *et al.* (2022) found higher faecal MP levels in patients with inflammatory bowel disease compared to healthy patients (41.8 and 28.0 MPs/g dw). However, it could not be determined whether MP exposure could be related to the aetiology of the disease or if this condition could potentiate MP retention.

Regarding trace and non-essential metals, since all concentrations in muscle tissue of seabass were below MPCs, the associated human risk associated with their ingestion is negligible. In fact, one person would need to ingest an unrealistic amount of seabass to reach the daily or weekly HGBVs. Based on a mean portion size of 150 g and data from EUMOFA (2021), the number of seabass meals per month per capita was roughly estimated to be 2 for Portuguese, 1 for Spanish and Italian, and 0.5 for British consumers. Considering the acute exposure through the consumption of one meal per week, only the levels of Zn in pond-farmed seabass were significantly higher compared to the other aquaculture systems' seabass. Considering toddlers and the higher metal levels within systems, the dietary intake of trace metals ranged from 0.02 % of the TDI set for Cr and 11.88 % of the UL set for Zn. For non-essential metals, the dietary intake ranged from 2.50 % of the TWI set for Cd and to <31.25 % of the $BMDL_{01}$ set for Pb. However, it is important to stress that seafood consumption varies regionally and within different population groups (Cardoso *et al.*, 2013; Esteve-Llorens *et al.*, 2021; Pulcini *et al.*, 2020). Therefore, the potential acute toxicological effects related to trace and non-essential metals should not be discarded for individuals who are high consumers of seabass. Moreover, other contaminants, such as dioxin-like polychlorinated biphenyls (PCBs) and perfluoroalkyl substances (PFAs), should be considered to properly assess the toxicological risk as they have been reported even at

low levels, in both wild-caught and farmed seabass specimens (Barbarossa *et al.*, 2016; Trocino *et al.*, 2012). Therefore, monitoring the levels of MPs and other contaminant levels both in wild and farmed seafood products, including their different sources and production systems, is essential for ensuring the provision of safer and secure products with high quality to consumers.

3.5. Conclusion

This work aligns with the holistic One Health concept, recognizing the interdependence of human, animal, and environmental health. The diversity of MPs found in water, feed, and fish tissues, indicates that European seabass uptakes MPs from water and feed. The majority of recovered particles were within the MP size range and were primarily fibres composed of man-made cellulose and PET. Among the selected production systems, cage-farmed seabass exhibited the lowest MP occurrence, with 89 % of the specimens having at least one particle detected in the tissues. The GIT had the highest MP concentration compared to the muscle tissue, except for cage-farmed fish where identical values were registered. Based on the BCF and BAF values, it can be inferred that MPs are only bioconcentrated in seabass, suggesting a low potential for bioaccumulation in fish. The trace and non-essential metal concentrations in the muscle tissue of farmed seabass were below the respective MPCs. Moreover, the human dietary exposure scenarios indicated a relatively low toxicological risk for consumers based on the HGBVs set by EFSA for metals. Although the selected RAS showed the highest MP levels, this system type is equipped with filtration systems able to minimize MP contamination due to its controlled conditions and the ability to implement mitigation strategies, such as using system components made of natural materials. Due to the multiple factors influencing MP occurrence, further studies monitoring MP contamination in other aquacultures and surrounding environments are warranted. The comparison between farmed fish and their wild counterparts is also crucial for better understanding the impacts of MPs on fish health and ultimately, assessing human exposure levels through seafood consumption.

Chapter IV

A multiple biomarker approach to understand the effects of microplastics on the health status of European seabass farmed in earthen-ponds on the NE Atlantic coast

by

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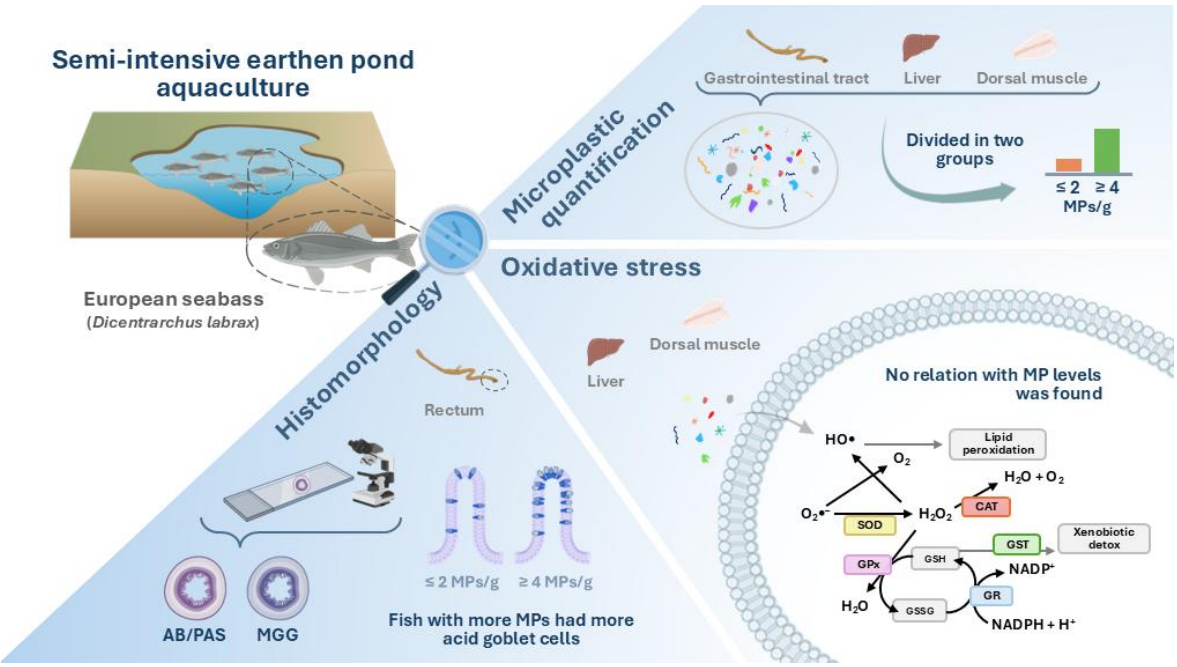

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Abstract

The occurrence of microplastics (MPs) in aquaculture environments is a growing concern to due to their potential negative effects on fish health and, ultimately, on seafood safety. Earthen pond aquaculture, a prevalent aquaculture system worldwide, is typically located in coastal and estuarine areas thus vulnerable to MP contamination. The present study investigated the possible relation between MP levels of European seabass (*Dicentrarchus labrax*) farmed in an earthen pond, and its health status. More precisely, two groups of fish established based on the lowest and highest number of MPs found collectively in their gastrointestinal tract (GIT), liver, and dorsal muscle: fish with ≤ 2 MP/g and fish with ≥ 4 MP/g. The intestinal integrity and oxidative stress biomarkers in liver and dorsal muscle were evaluated in the established groups. No significant differences in the biometric and organosomatic parameters between groups were observed. The results indicated a significant increase in the number of acid goblet cells (GC) in the rectum of fish with higher MP levels (p = 0.016). Increased acid GC number may constitute a first defence strategy against foreign particles to protect the intestinal epithelium. No significant differences in oxidative stress biomarkers between the two fish groups were observed, namely in the activity of superoxide dismutase, catalase, glutathione reductase, and glutathione S-transferase in the liver, or in lipid peroxidation levels in the liver and dorsal muscle. The overall results suggest that MP levels were possibly related to an intestinal response but its potential implications on the health status of pond-farmed seabass warrant further investigation. Monitoring MP occurrence across stages of aquaculture production could help to elucidate the potential threats of MPs to fish health.

Keywords

Anthropogenic particles; Farmed fish; Histology; Oxidative stress; ‘One Health’

4.1. Introduction

Fish occupy key ecological roles and are a valuable human food resource. For several decades, aquaculture production has grown as an alternative to overexploited wild fish stocks and to meet the nutritional needs of a growing population (FAO, 2024). Indeed, human ingestion of MPs has been reported in previous studies confirming their occurrence in stools (Barceló *et al.*, 2023; Hartmann *et al.*, 2024).

Within aquaculture, constructed earthen ponds are the most globally adopted aquaculture production system for fish species, accounting for more than half of the fish produced in aquaculture (FAO, 2024). Particularly in European countries, these are usually located in salt marshes adjacent to estuarine and coastal waters due to the high productivity of these ecosystems, resorting to semi-intensive techniques (Gutiérrez-Estrada *et al.*, 2022; Rocha *et al.*, 2022). Briefly, semi-intensive techniques include fish stocking in earthen ponds, frequent water exchanges from supplying water bodies, and fish feeding relying on natural sources and supplementary feed (Oddsson, 2020). Rather than active mechanical filtration systems, these facilities often rely on natural processes, such as sedimentation, biological activity of plants/microorganisms, or the physical structure of the pond, to filter the inlet water and maintain its quality (Henares *et al.*, 2020). Nonetheless, these aquacultures are often located close to urban or industrial areas, meaning that their seafood products are susceptible to contaminations from exogenous sources typically associated with anthropogenic activity (Häder *et al.*, 2020; Lu *et al.*, 2022c).

The presence of microplastics (MPs; plastic particles with dimensions inferior to 5 mm) in aquatic ecosystems has sparked concerns about the potential consequences for aquaculture systems and the need to ensure the safety of seafood products (Garrido Gamarro & Constanzo, 2022). These particles can reach aquaculture environments from various sources, such as water bodies and air deposition, as well as from the products or materials used, such as feeds and fishing gear (Miao *et al.*, 2023; Nabi *et al.*, 2024). Indeed, the abundance of MPs and other anthropogenic particles has been previously reported in aquaculture environments (Miao *et al.*, 2023) with values registered in aquaculture ponds ranging from 0.25 MPs/L (Chen *et al.*, 2020a) up to 87.5 and 103.8 MP/L (Ma *et al.*, 2020; Priscilla & Patria, 2019). The MP occurrence in NE Atlantic salt marshes has been reported to range from 39.9 to 469 MP/kg of sediments collected from lower to upper estuarine locations (Morgado *et al.*, 2022). The same study reports similar MP occurrence in sediments in Portuguese estuaries, located in NE Atlantic coasts. Alongside geographical variations, different sampling techniques (e.g., glass jars vs. nets) may explain the variation in the number of particles found in water (Barrows *et al.*, 2017; Hung *et al.*, 2021). Moreover, MP levels in the sediment of aquaculture ponds were observed to range

from 3.3-543 MPs/kg dry weight (Hossain *et al.*, 2023a; Le *et al.*, 2022; Xiong *et al.*, 2021; Yu *et al.*, 2023a). In water, several studies have documented a 1.4 to 8.3-fold variation in MP occurrence between different production systems (Lv *et al.*, 2020; Matias *et al.*, 2024) with some reporting pond systems as the most contaminated (Huang *et al.*, 2023; Song *et al.*, 2023). For that reason, monitoring MP contamination in aquaculture environments is essential to better understand its implications to farmed organisms.

MP-associated hazards have been observed in several taxa, including livestock and poultry (Dong *et al.*, 2023). The interactions between MPs and fish can occur through several pathways, including the intentional or unintentional ingestion of MPs and contaminated food items, water passage through gills, and adsorption through the skin epithelium (Wang *et al.*, 2020a). Several studies have already reported that MPs can trigger several physiological responses and potentially compromise fish health and performance (Gündogdu *et al.*, 2022; Wang *et al.*, 2020a). These effects can be enhanced when MPs occur in combination with other environmental pollutants adsorbed to their surface, such as heavy metals and polycyclic aromatic hydrocarbons (PAHs) (Sun *et al.*, 2023a). Regarding ingestion, some of the commonly reported effects of MP exposure in commercially relevant fish species include the induction of physical damage in the gastrointestinal tract (GIT) during MP retention. Specifically, these alterations include goblet cell (GC) hyperplasia, shortening and swelling of *villi*, leucocyte infiltration in the *mucosa*, and high enterocyte vacuolisation, observed in species such as European seabass (*Dicentrarchus labrax*) (Espinosa *et al.*, 2019; Montero *et al.*, 2022; Pedà *et al.*, 2016), gilthead seabream (*Sparus aurata*) (Del Piano *et al.*, 2023a; 2023b; Jacob *et al.*, 2021; Varó *et al.*, 2021), Nile tilapia (*Oreochromis niloticus*) (Hasan *et al.*, 2023; Wu *et al.*, 2024; Zhang *et al.*, 2022b), and rainbow trout (*Oncorhynchus mykiss*) (Karbalaei *et al.*, 2021). The cited studies observed significant effects, particularly in the posterior intestine and rectum, after exposing fish to MPs of varying characteristics (i.e., shape, size, polymer type) through food (5-500 mg/kg diet) and water (1-300 mg/L water). Depending on the extent of MP effects on the GIT, these alterations can lead to abnormal intestinal functioning and compromise nutrient absorption and overall physiological functions (Lu *et al.*, 2022a; Yin *et al.*, 2018).

Oxidative stress is a common biological response to various environmental stressors, including MPs (Hu & Palić, 2020). These may lead to increased production of reactive oxygen species (ROS), generating a redox imbalance as ROS production surpasses the antioxidant scavenging capacity of tissues (Das, 2023). This redox imbalance can ultimately result in damage to cellular components such as proteins, lipids, and DNA (Bergamini *et al.*, 2004). Previous studies have documented an increase in the lipid peroxidation (LPO) levels in several tissues of European seabass exposed to MPs (0.26-0.69 mg/L water and 750 g/kg diet) (Barboza *et al.*, 2018b; 2018c; Zitouni *et al.*, 2021).

Alongside an increase in LPO levels, increased protein carbonyl compounds have been reported in the gut of common carp (*Cyprinus carpio*) and the liver of gilthead seabream exposed to MP concentrations of 175-1400 µg/L water and 100 g/kg feed, respectively (Banaei *et al.*, 2022; Solomando *et al.*, 2020). To counteract the negative effects of ROS, organisms are equipped with defence mechanisms capable of neutralizing these highly reactive molecules such as the action of antioxidant and detoxification enzymes: superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), and glutathione S-transferase (GST). Under controlled conditions, the modulatory effects of MP exposure in oxidative stress biomarkers have been extensively documented experimentally in fish species (Capó *et al.*, 2021a; Hollerova *et al.*, 2023; Jawdhari *et al.*, 2023) like the European seabass (*Dicentrarchus labrax*) (Barboza *et al.*, 2018b; Espinosa *et al.*, 2019; Pedà *et al.*, 2022). Similarly, other studies with wild fish populations have reported significant changes in oxidative stress biomarkers of fish with higher MP levels in tissues (Alomar *et al.*, 2017; Barboza *et al.*, 2020b; Zitouni *et al.*, 2020).

Focusing on the Mediterranean countries, the European seabass (*Dicentrarchus labrax*) is an economically important species with a production volume of 97,090 tonnes representing a total value of €590 million in 2021 and often produced in semi-intensive pond aquacultures (EUMOFA, 2023; Rocha *et al.*, 2022). Also, the occurrence of MPs in European seabass aquacultures was previously identified but did not related MPs levels in fish with their health status (Kılıç, 2022; Matias *et al.*, 2024; Reinold *et al.*, 2021). Therefore, the present study aimed to explore the potential relationship between MP levels in several European seabass tissues (GIT, liver, and dorsal muscle) with gut integrity and oxidative stress response in liver and dorsal muscle.

4.2. Materials & methods

4.2.1. Fish and sample collection

European seabass specimens were reared for 8 months in a semi-intensive commercial farm with earthen ponds supplied with brackish water, located in the salt marshes of the Mondego estuary in the central region of Portugal (40°08' N, 8°50' W). All specimens were sourced from the same hatching facility and maintained under the same conditions. In total, 50 specimens were collected following the procedures indicated in Matias *et al.* (2024). Briefly, before collection, all specimens underwent a fasting period of 24 hours. Fish were euthanized using an ice slurry bath and transported within the shortest time possible to the Interdisciplinary Centre of Marine and Environmental Research (CIIMAR) facilities in Matosinhos, Portugal. Additionally, samples of the pond water (*i.e.* five 2L replicates) where the fish were housed were collected using glass flasks, along with the remaining commercial fish feed, following the procedures described in Matias *et al.* (2024).

Before tissue dissection, all fish were externally rinsed with distilled water to remove most particles adhered to the skin. The biometric parameters were registered, namely the total length (TL) and the total body wet weight (TW). Fulton's condition factor (K) was calculated as an indicator of the physical condition using the formula: K = [total body weight/(total body length)^3]. The wet weight of the liver and viscera were registered for the calculation of organosomatic indices, namely the hepato- and viscerosomatic indices (HSI and VSI, respectively). From each fish, a small cross-sectional (0.5 cm) rectum segment was carefully washed and fixed in 4% formaldehyde (pH 7.0 ± 0.1) for histological analysis. The remaining gastrointestinal tract (GIT, from the oesophagus to the rectum), half of the liver, and a dorsal muscle sample (~ 5 g) were collected, and the wet weight was registered, to quantify their contamination with MPs. Additionally, half of the liver and ~ 10 g of dorsal muscle were promptly collected and deep-frozen in liquid nitrogen and stored at –80 °C for the determination of oxidative stress biomarkers.

4.2.2. Microplastic isolation and characterisation in the liver of pond-farmed seabass

Each liver portion was individually digested in glass flasks with a 10% potassium hydroxide (KOH) solution prepared with ultrapure water (Millipore, Corporation, USA) as previously described in Matias *et al.* (2023). After digestion, the samples were filtered under vacuum conditions through 1.2 µm glass-microfibre filter membranes. The particles retained in filters were quantified and their visual characteristics were analysed under the stereomicroscope (Nikon SMZ100 with Fi1, Japan) using imaging software (Olympus CellˆB, Hamburg, Germany). The particles were also chemically characterised through micro-Fourier Transformed Infrared Imaging Microscopy (µFTIR) (Nicolet™ iN10 MX,

Thermo Fischer Scientific, Waltham, MA, USA) and the obtained spectra were analysed using the OMNIC software (Thermo Fischer Scientific, Waltham, MA, USA). The MP data presented in this study encompasses synthetic polymers and other anthropogenic particles, such as cellulosic microfibres. MP concentrations in feed and seabass tissues are expressed per gram of wet tissue (MP/g).

During all procedures, the samples were handled following strict procedures to avoid external and cross-contamination of samples (GESAMP, 2019). Samples were manipulated in a previously clean room with restricted access. The researchers used 100% cotton laboratory coats and nitrile gloves, and all the plastic equipment was substituted whenever glassware and other feasible alternatives were available. During dissections, all equipment (*e.g.,* washing materials, dissection instruments) used was sterilised and thoroughly cleaned with ultrapure water between tissues and specimens. Also, three procedural blanks (i.e., clean filters in Petri dishes) were run alongside samples by air-exposing them in the laboratory at each methodological stage to monitor airborne contamination. Three replicates of KOH solutions were filtered to monitor potential contamination. All particles recovered from samples exhibiting similar characteristics (*e.g.,* shape, colour, and polymer type) to those found in procedural blanks and solutions were discounted from the analysis. As size can be highly variable within certain particle types, this characteristic was not used as a criterion for exclusion from analysis.

Based on the sum of the mean MP levels found in GIT, liver, and dorsal muscle of the 50 fish, two groups were established representing the least (≤2 MP/g) and most (≥4 MP/g) contaminated specimens. Since all the fish had at least one particle recovered from one of the analysed tissues, and to ensure a good separation between groups, only 8 fish per group were considered for further analyses, namely for histological evaluation of the rectum and determination of oxidative stress biomarkers.

4.2.3. Histomorphology evaluation of the rectum

After a 24 h fixation period, the rectum samples were preserved in 70% ethanol. Thereafter, the samples of the 16 selected fish (*i.e.,* 8 fish with ≤ 2 MP/g and 8 fish with ≥ 4 MP/g) were processed according to histological procedures and were embedded in paraffin (Ferreira *et al.*, 2023). Rectum cross-sections with 3 µm thickness were obtained using a semi-automated rotary microtome (Leica RM 2245, Leica Biosystems, Germany). These sections were stained using Alcian Blue/Periodic Acid Schiff (AB/PAS, pH 2.5) and May Grünwald-Giemsa (MGG) techniques. Digital images of the stained sections were obtained at a 20x ampliation using a light microscope equipped with an integrated camera (Olympus BX51 with DP50, Germany). These were then used for the quantitative analysis using

adequate software (Olympus cellSens Standard 2.2), as depicted in Figure 4.1, and described in Ferreira *et al.* (2023).

In the AB/PAS-stained digital images, both the cross-sectional perimeter (mm) and absorption area (area occupied by the *villi* and lamina propria) were semi-automatically determined (Fig. 4.1) as described by Ferreira *et al.* (2023). The *muscularis* and *submucosa* thickness were calculated as the average of eight thickness measurements representative of each layer. Additionally, the mean *villi* length was also measured in eight complete and representative *villi,* and the *lamina propria* thickness was measured considering the average of three points (basal, intermediate, and apical) of the *villi* selected (Fig. 4.1A). Based on the pixel colour range, the total neutral and acid GC (magenta and blue-stained cells, respectively) numbers and mean GC size were automatically quantified across the entire cross-section (Fig. 4.1B).

The mean *microvilli* length was determined in the MGG-stained digital images, through eight representative measurements on each of the eight *villi* previously measured. The lymphoid cells (LC) (small, blue-stained cells) and the granulocytes (GN) (large, oval-shaped cells with pink granules in the cytoplasm and blue-stained nucleus at the periphery) were identified and counted across the full extent of the *lamina propria* and were expressed as total number of cells per *villus* (Fig. 4.1). The number of LC and GN were counted in the *submucosa* area at the base of the selected *villi*, and were expressed as number of cells per μm^2, as shown in Figure 4.1D.

4.2.4. Determination of oxidative stress biomarkers

The activities of CAT, SOD, GPx, GR, and GST were determined in the liver, and LPO levels in both liver and dorsal muscle. Both tissues were homogenised in K-phosphate buffer (0.1 M, pH 7.4) in a proportion of 1:10 (m/v) using an ultrasonic homogenizer (UP200Ht, Hielsch Ultrasonics, Germany). For LPO assays, the aliquots of the homogenates were prepared with the addition of 2.5 µL of butylhydroxytoluene (BHT) to 150 µL of sample. For enzymatic activity assays, the liver homogenates, without BHT, were centrifuged (Eppendorf Centrifuge 5430 R, USA) at 10,000 *g* and 4° C for 20 min, and the post-mitochondrial supernatant (PMS) was aliquoted (Resende *et al.*, 2023). All aliquots were preserved at –80 °C until further analyses. Except for LPO analyses, all the conducted assays were normalised considering the soluble protein concentration determined in the PMS through the Bradford (1976) method adapted to a microplate (Resende *et al.*, 2023). All the assays were adapted to a microplate and the measurements were performed at room temperature (25°C) in triplicate in a Synergy HTX Multi-Mode Microplate Reader (BioTek Instruments Inc., USA).

CAT activity was determined according to Clairborne (1985), using 30% H_2O_2 as substrate. The absorbance variation resulting from H_2O_2 consumption was recorded at 240 nm (ε = 40 M^{-1} cm^{-1}) and CAT activity was expressed as µmol of H_2O_2 consumed per min per mg of protein (µmol/min/mg protein). SOD activity was determined using a SOD Determination Kit (Sigma-Aldrich 19160-1KT-F), according to the manufacturer's instructions. Results were expressed in units of enzyme catalytic activity per mg of protein homogenate (U mg $protein^{-1}$). One unit was considered the amount required for 50 % inhibition of the formation of WST-1 formazan. GPx activity was determined indirectly according to Mohandas *et al.* (1984), based on the oxidation of reduced glutathione (GSH) to oxidized glutathione (GSSG). The oxidation of nicotinamide adenine dinucleotide phosphate (NADPH) was recorded at 340 nm (ε = 6.22 x 10^3 M^{-1} cm^{-1}) and expressed as nmol of oxidized NADPH per min per mg of protein (nmol/min/mg protein). GR activity was determined according to Smith *et al.* (1988), by assessing the reduction of 5.5'dithiobis(2-nitrobenzoic acid) (DTNB) by GSH at 412 nm (ε = 6.22 x 10^3 M^{-1} cm^{-1}). The result was expressed in nmol of DTNB per min per mg of protein (nmol/min/mg protein). GST activity was determined according to Habig *et al.* (1974), by monitoring 1-chloro-2,4-dinitrobenzene (CNBD) conjugation with reduced glutathione (GSH), at 340 nm (ε = 9.6 x 10^3 M^{-1} cm^{-1}), and values were expressed as nmol per min per mg of protein (nmol/min/mg protein). The LPO levels in the liver and dorsal muscle were determined by the quantification of the thiobarbituric acid (TBA) reactive substances (TBARS), according to Ohkawa *et al.* (1979). Briefly, the decomposition of unstable peroxides resulting from polyunsaturated fatty acid (PUFA) oxidation forms TBARS, namely malondialdehyde (MDA), which reacts with thiobarbituric acid (ε = 1.56 x 10^5 M^{-1} cm^{-1}). MDA can be quantified at 535 nm and LPO values were expressed as nmol TBARS per g of wet tissue (nmol TBARS/g).

4.2.5. Statistical analysis

For each variable, the data normality and homogeneity of variances were checked through the Shapiro-Wilk and Levene's tests, respectively. Student's *t*-test was used to check for significant differences between the two established groups (≤2 MP/g and ≥4 MP/g). Whenever data normality or homogeneity could not be achieved, data transformation of equivalent nonparametric test (Mann-Whitney test) was employed. Relationships between the different parameters analysed were checked using the Pearson correlation test. All statistical analyses were performed using the SPSS statistical analysis package (version 27) and a threshold < 0.05 was considered for statistical significance for the tests used.

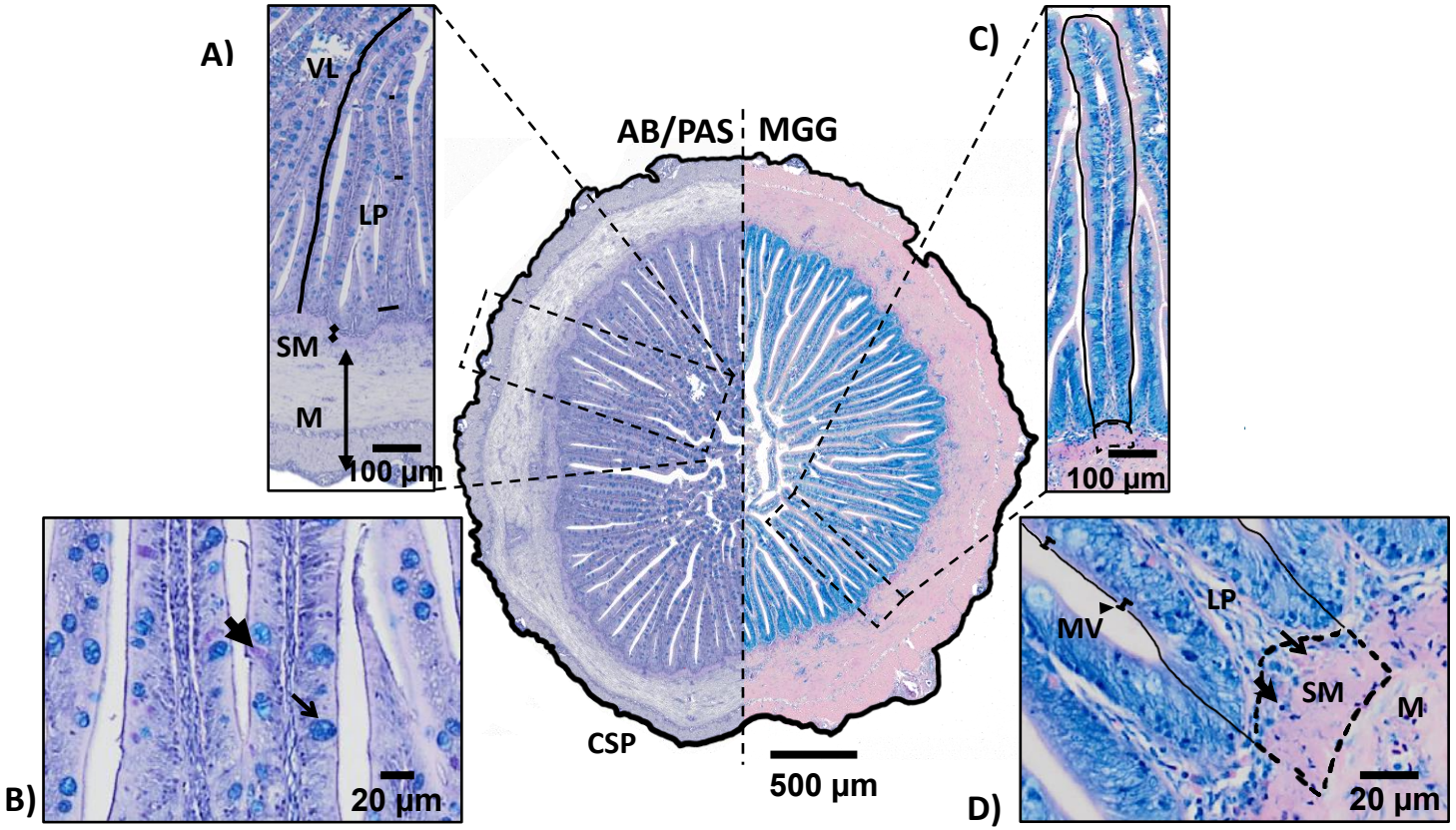

Figure 4.1. Schematic representation of the quantitative analysis of the histological sections of the rectum of European seabass. All histological measurements were performed in Alcian-Blue/Periodic Acid-Schiff (AB/PAS, pH 2.5) (A, B) and May-Grünwald Giemsa (MGG) (C, D) stained digital images, including the cross-section perimeter (CSP), muscularis thickness (M), submucosa thickness (SM), villi length (VL), lamina propria thickness (LP) and microvilli length (MV). (B) The number of acid (blue-stained, thick arrow) and neutral (magenta-stained, thin arrow) goblet cells was quantified in the entire cross-section. (C) The number of lymphoid cells (LC) and granulocytes (GN) were quantified in the same villi (full line) and in the submucosa region directly at the base (dashed line). (D) Visual representation of the submucosa area (dashed line) where both LC (thick arrow) and GN (thin arrow) were counted. All measurements were performed using the imaging software Olympus cellSens Standard 2.2.

4.3. Results

4.3.1. Microplastic quantification and characterisation in seabass body sites

A total of 91 particles were recovered from the liver of the pond-farmed European seabass. Only 70 particles were considered because of the similarity between the excluded ones and those found in procedural blanks. Out of the 50 fish collected, particles were recovered from 34 individuals and only 16 fish had no particles recovered from the liver analysed. The mean MP concentration equalled to 1.81 ± 2.04 MP/g of tissue (1.40 ± 1.31 MPs per individual). Regarding their characteristics, fibres were the most predominant particle shape (82.9%), followed by fragments (15.7%) and pellets (1.4%) (Fig. 4.2). Blue (41.4%) and black (18.6%) colours were the most common, followed by grey (15.7%) and transparent (15.7%). Other colours represented less than 5% as detailed in Figure 4.2. The mean particle size equalled to 741.0 ± 753.5 µm, with the most predominant size classes being 500-1499 µm (37.3%) and 150-499 µm (33.3%) (Fig. 4.2). Regarding their chemical composition, 65.7% of the particles were man-made cellulose/rayon, followed by polyethylene terephthalate (PET; 8.6%), and phenoxy resin (5.7%). Other polymers representing less than 5% including polyethylene (PE), polypropylene (PP), and polystyrene (PS), are detailed in Figure 4.2.

A total of 282 particles were recovered from three body sites (GIT, dorsal muscle and liver) of all 50 sampled seabass. All of the fish had at least one particle recovered from one of the analysed tissues. In our previous study, it is reported a total of 140 and 72 particles recovered from GIT and dorsal muscle, respectively (Matias *et al.*, 2024). Regarding their characteristics, fibres (56.4%) and fragments (31.2%) were the most common particle shapes, followed by films (8.9%), foams (1.8%) and pellets (1.8%) (Fig. 4.2). From 12 different colour categories, black and blue were also the most common (45.4 and 26.1%, respectively) followed by transparent (11.3%) and grey (6.7%). Other colours represented less than 3% (Fig. 4.2). The most predominant size classes were 150-499 µm (31.7%) and 500-1499 µm (26.3%) (Fig. 4.2). Within polymers, man-made cellulose/rayon (38.1%) and phenoxy resin (27.3%) were the most predominantly found, followed by PET (6.2%) and nylon (5.2%). Other polymers representing less than 5% are depicted in Figure 4.2. Example images of the MPs recovered from the three tissues are depicted in Figure 4.3.

Based on the sum of the mean MP levels in all body sites (2.87 MPs/g tissues), two groups of fish were established for further analyses: 8 fish with ≤ 2 MP/g (least contaminated) and another 8 fish with ≥ 4 MP/g (most contaminated) (Table 4.1). The 16 selected fish were homogeneous in total length (TL) (23.3 ± 0.7 cm), weight (TW) (138.18 ± 12.6 g), and Fulton's condition factor (K) (1.10 ± 0.06 g/dm^3). Similarly, the

organosomatic indices were homogeneous (HSI, 1.36 ± 0.30%; VSI, 10.18 ± 1.13%). No significant differences were found in biometric parameters and organosomatic indices between groups. The values for each group are detailed in Table 4.1.

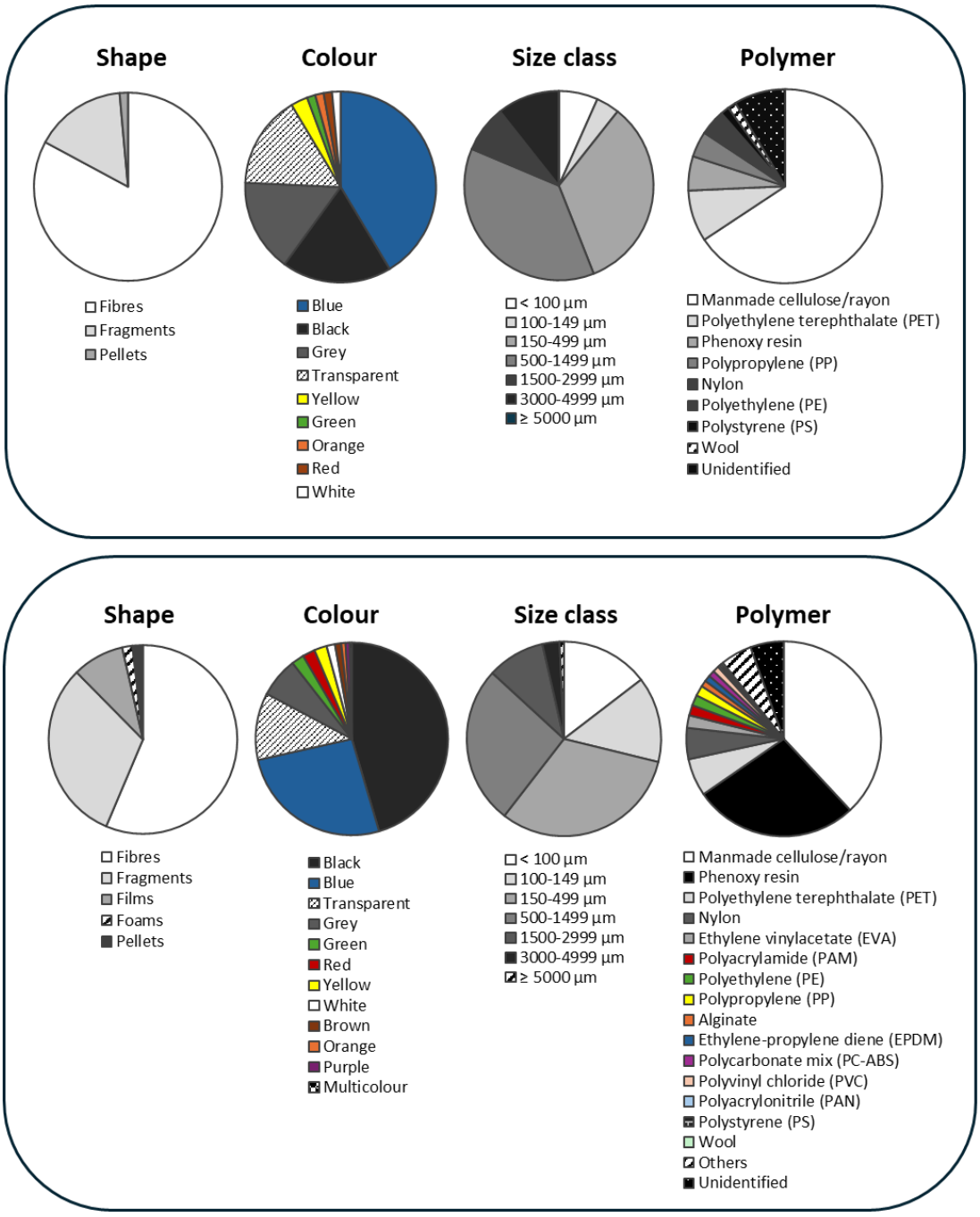

Figure 4.2. Shape, colour, size classes, and polymers of the microplastics found in the liver (top), and in three tissues (bottom) (gastrointestinal tract (GIT), liver, dorsal muscle) of pond-farmed European seabass (n = 50). Data for GIT and muscle is available in Matias *et al.* (2024).

Figure 4.3. Example of the diversity of microplastics (MPs) found in the gastrointestinal tract, liver, and dorsal muscle of European seabass (*Dicentrarchus labrax*) produced in a semi-intensive earthen pond aquaculture.

Table 4.1. Biometric parameters, organosomatic indices, and the number of microplastics (MPs) recovered per gram of gastrointestinal tract (GIT), liver, and muscle of pond-farmed European seabass based on the microplastic (MPs) levels (≤ 2 MP/g and ≥ 4 MP/g).

	≤2 MPs/g	**≥4 MPs/g**	***p*-value**
Number of MPs per g of tissue			
Gastrointestinal tract (*GIT*)	0.16 ± 0.25[b]	1.63 ± 0.96[a]	0.003
Liver	1.67 ± 1.03	2.42 ± 1.15	0.188
Muscle	0.04 ± 0.08[b]	0.40 ± 0.27[a]	<0.001
GIT + Liver + Muscle	1.86 ± 1.03[b]	4.46 ± 1.07[a]	<0.001
Biometric parameters			
Total length (cm)	23.3 ± 0.8	23.2 ± 0.8	0.820
Total weight (g)	138.56 ± 11.90	137.80 ± 13.99	0.908
Fulton's condition factor (*K*)	1.09 ± 0.07	1.10 ± 0.06	0.895
Hepatosomatic index (*HSI*) (%)	1.37 ± 0.38	1.34 ± 0.22	0.828
Viscerosomatic index (*VSI*) (%)	9.94 ± 1.01	10.42 ± 1.26	0.408

Values are expressed as mean ± SD (standard deviation). Statistical *p*-value (Student's test, $p<0.05$) refers to differences between the two groups (≤2 MP/g and ≥4 MP/g).

4.3.2. Histomorphology evaluation of rectum based on MP levels

Potential histomorphological changes produced in the intestine of pond-seabass between fish with distinct MP levels were evaluated by AB/PAS and MGG-stained sections of the rectum. As detailed in Table 4.2, no significant differences were found in the histomorphological parameters evaluated between the two established groups (with ≤2 MP/g and ≥4 MP/g). Nevertheless, the total number of goblet cells (GC) was significantly higher (p = 0.014) in fish with ≥4 MP/g (1886.0 ± 639.0 GCs) than fish with ≤2 MP/g (1148.9 ± 381.2 GCs). This increase is associated with a higher number of acid GC (p = 0.016), but not with a higher number of neutral GC (Fig. 4.4, Table 4.2). No differences were found in the mean GC size, ranging approximately from 70 to 90 μm^2 between groups (Table 4.2). Furthermore, no significant differences were observed in the number of lymphoid cells and infiltrated granulocytes present in the *submucosa* (SM) and *lamina propria* (LP) across the two groups (Table 4.2).

Table 4.2. Histomorphological parameters of the rectum of pond-farmed European seabass. Values are presented for *all* fish analysed (n = 16) and per group (≤ 2 MP/g and ≥ 4 MP/g, 8 fish per group) established based on the number of microplastics recovered from seabass tissues (MP/g tissue).

	All	**≤ 2 MPs/g**	**≥ 4 MPs/g**	***p*-value**
Cross-section perimeter (mm)	11.3 ± 1.7	11.1 ± 1.3	11.6 ± 2.1	0.543
Absorption area (mm^2)	3.3 ± 1.0	3.3 ± 1.0	3.3 ± 1.1	0.903
Muscularis thickness (μm)	298.5 ± 75.61	300.5 ± 71.5	296.6 ± 84.4	0.922
Submucosa thickness (μm)	75.8 ± 16.6	78.8 ± 19.8	72.8 ± 13.3	0.493
Villi length (μm)	737.7 ± 175.2	698.4 ± 207.3	777.1 ± 138.9	0.388
Lamina propria thickness (μm)	17.8 ± 4.4	16.4 ± 3.9	19.3 ± 4.6	0.196
Microvilli length (μm)	1.8 ± 0.4	1.8 ± 0.4	1.9 ± 0.6	0.897
Total goblet cells	1517.4 ± 635.0	1148.9 ± 381.2[b]	1886.0 ± 639.0[a]	0.014
Acid goblet cells	1419.4 ± 643.9	1051.6 ± 430.7[b]	1787.1 ± 627.6[a]	0.016
Neutral goblet cells	99.5 ± 59.2	97.3 ± 71.2	101.8 ± 49.3	0.395
Mean goblet cells size (μm^2)	80.0 ± 26.7	70.3 ± 21.8	89.8 ± 29.0	0.151
Total lymphoid cells	1969.8 ± 85.8	1745.1 ± 406.7	1648.5 ± 285.3	0.591
Lamina propria lymphoid cells (cell/villus)	194.6 ± 39.7	199.7 ± 47.4	189.5 ± 32.7	0.622
Submucosa lymphoid cells (cell/μm^2)	0.0041 ± 0.0019	0.0046± 0.0018	0.0036 ± 0.0019	0.307
Total granulocytes	109.2 ± 8.0	116.4 ± 32.9	102.0 ± 31.2	0.385
Lamina propria granulocytes (cell/villus)	7.1 ± 3.5	7.6 ± 3.6	6.6 ± 3.6	0.579
Submucosa granulocytes (cell/μm^2)	0.0015 ± 0.0007	0.0018 ± 0.0006	0.0013 ± 0.0007	0.137

Statistical p-value (Student's test, p<0.05) refers to differences between the two groups (≤2 MP/g and ≥4 MP/g).

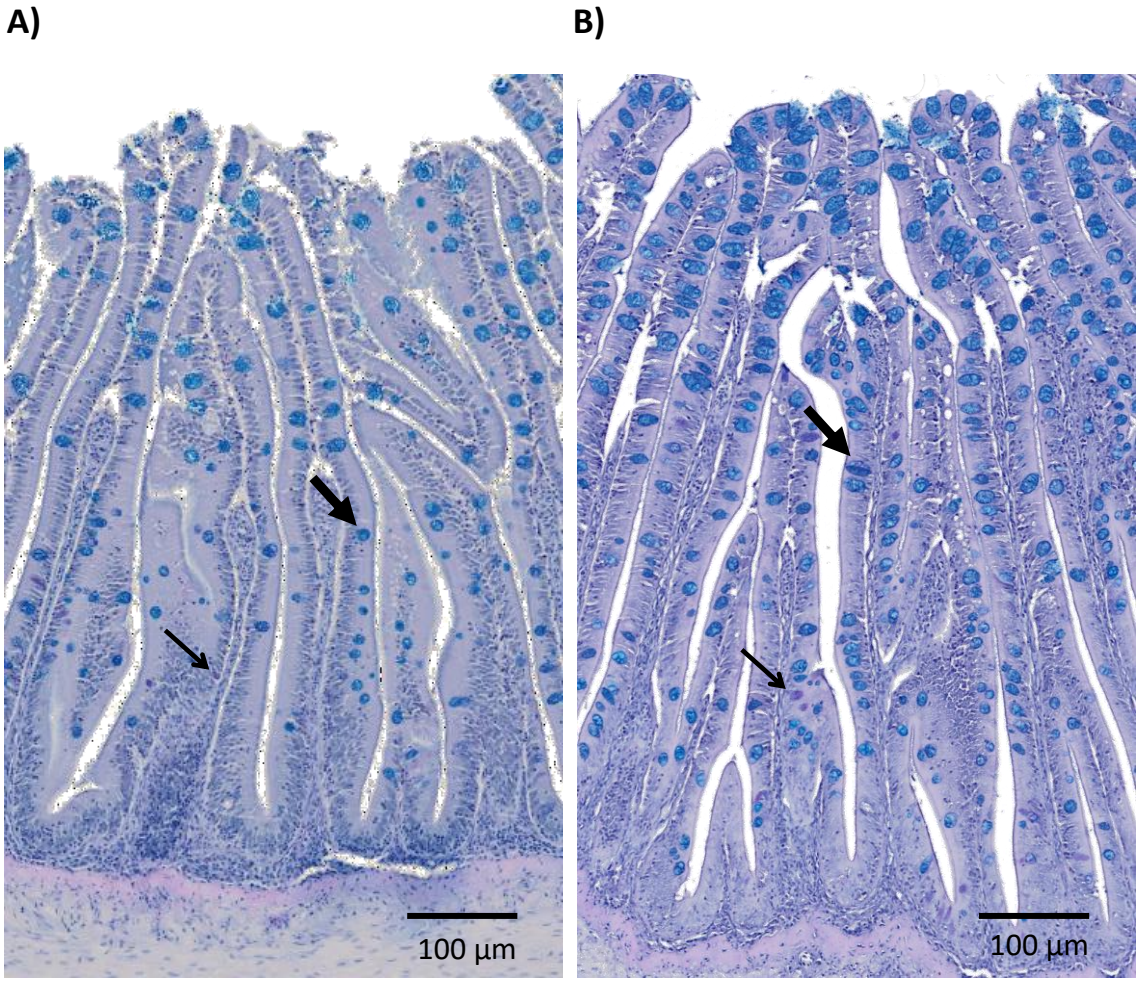

Figure 4.4. Representative micrographs of the distribution of goblet cells (GCs), acid GC (coloured blue; thick arrow) and neutral GC (coloured magenta; thin arrow) across transverse sections of rectum of pond-farmed European seabass with the (**A**) lowest (≤ 2 MP/g) and (**B**) highest (≥ 4 MP/g) microplastic number recovered from tissues. Cross sections were stained with Alcian-Blue/Periodic Acid-Schiff (AB/PAS, pH 2.5). Scale bar 100 µm.

4.3.3. Oxidative stress biomarkers changes induced by MP levels

No significant differences were observed between the two groups concerning oxidative stress biomarkers (Fig. 4.5): the enzymatic activities in liver of CAT (average of 65.97 ± 10.92 µmol/min/mg protein), SOD (average of 180.69 ± 31.31 U/mg protein), GR (average of 7.26 ± 1.22 nmol/min/mg protein), and GST (average of 16.26 ± 4.79 nmol/min/mg protein) remained similar between the two groups of fish. Likewise, the LPO levels in liver (average of 84.01 ± 28.51 nmol TBARS/g tissue) and muscle (average of 14.78 ± 6.14 nmol TBARS/g tissue) also remained similar between groups. No GPx activity in the liver was detected using the employed methodology. No correlations were observed between MP levels in each tissue or collectively with the evaluated biomarkers (p >0.05).

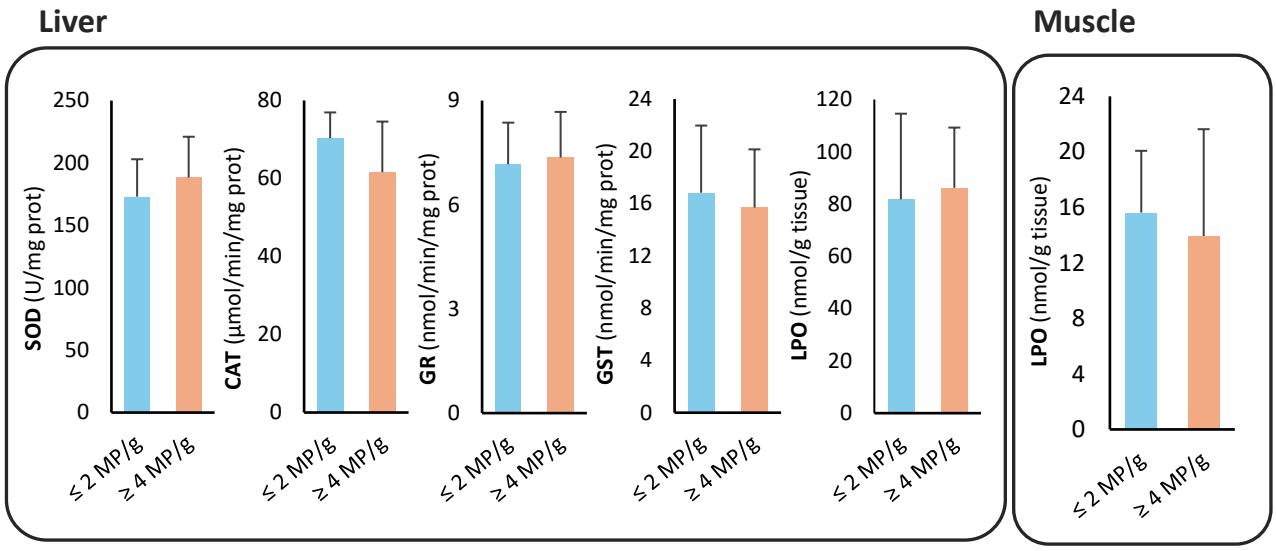

Figure 4.5. Oxidative stress biomarkers determined in the liver and dorsal muscle of two established groups based on the number of microplastics per gram (MP/g tissue) found in tissues of pond-farmed European seabass (≤2 MP/g and ≥4 MP/g). The error bars represent the standard deviation to the mean. The lack of different letters/symbols indicate no significant differences between groups (p >0.05).

4.3.4. Relationship between intestinal histomorphology and oxidative stress biomarkers

Several correlations were identified between the parameters quantified in the rectum and the oxidative stress biomarkers in the liver and dorsal muscle. The cross-section perimeter correlated negatively with CAT activity in liver (r = -0.713, p = 0.002) and LPO levels in muscle (r = -0.512, p = 0.043). The absorption area correlated positively with the GST (r = 0.729, p = 0.001), and negatively with the CAT (r = -0.649, p = 0.007) and GR (r = -0.576, p = 0.019). The *villus* length correlated positively with the GST (r = 0.555, p = 0.026) and negatively with the CAT (r = -0.667, p = 0.005). Mean microvillus length correlated positively with the GST (r = 0.582, p = 0.018), and negatively correlated with the CAT (r = -0.500, p = 0.049) and GST (r = -0.865, p < 0.001). The *lamina propria* thickness correlated negatively with CAT (r = -0.029, p = 0.009). CAT correlated negatively with the total number of acid GCs (r = -0.738, p = 0.001) but not neutral GCs (r = 0.281, p = 0.292). CAT was also negatively correlated with the mean GC size (r = -0.517, p = 0.021). Also, the granulocyte number in the *submucosa* correlated positively with the CAT (r = 0.618, p = 0.011) and GST (r = 0.577, p = 0.019), whereas the number of granulocytes in the *lamina propria* correlated positively with the GST (r = 0.582, p = 0.018).

4.4. Discussion

The present study focused on evaluating the potential impact of MPs on the gut morphology and oxidative stress biomarkers in European seabass reared in a semi-intensive pond aquaculture system located in the Mondego estuary, Portugal. Two groups of fish were established concerning the presence of MP: ≤2 MP/g and ≥4 MP/g. There were no significant differences found in the biometric and organosomatic parameters or oxidative stress biomarkers between groups, but a significant increase in acid goblet cells was observed in the rectum of fish with higher MP levels.

In the Mondego region, MP occurrence levels have been reported to range from 39.9 to 469 MPs/kg of sediments collected across lower and upper estuarine locations (Morgado *et al.*, 2022). The same study reports similar MP occurrence levels in sediments between the estuaries of Mondego and other rivers of Portugal. Nevertheless, coastal and estuarine environments are highly dynamic and affected by multiple environmental factors, which may also explain such variability on MP occurrence. For instance, Leads *et al.* (2023) found a 2.5-fold change in MP abundance over 24 h and a 20-fold change throughout a 17-day sampling (6.0 to 23.5 MP/kg dry weight of sediment) in a U. S. estuary. This study attributed precipitation levels and wind direction as important environmental factors contributing to MP occurrence. On larger temporal scales, other studies have also observed the contributing effect of precipitation levels and wind direction on MP occurrence (Bermúdez *et al.*, 2021; Cheung *et al.*, 2018b; Pazos *et al.*, 2021). Ultimately, the temporal variability of MP occurrence in estuarine environments has been shown to influence MP levels in aquatic organisms (Guilhermino *et al.*, 2021; Lima *et al.*, 2015; Rodrigues *et al.*, 2019). For that reason, further monitoring studies in aquaculture environments are likely to require a finer temporal scale, to better understand MP dynamics and assess their risks for the fish health and consequently farmed seafood safety.

As expected, the two groups significantly differed in the MP levels observed in the GIT and dorsal muscle and, despite no differences observed in the liver, the two groups still significantly differed in the MP mean levels across all three tissues combined. It is noteworthy that since not all fish tissues or the whole fish were analysed it is likely that the occurrence of MPs is underestimated. Nevertheless, the apparent absence or lower occurrence of MPs in the GIT and dorsal muscle suggests lower MP contamination. The results of the present study align with those reported for RAS-farmed seabass, which had similar MP levels in the GIT (1.0 ± 0.7 MP/g), liver (0.8 ± 0.9 MP/g), and dorsal muscle (0.4 ± 0.3 MP/g) (Matias *et al.*, 2023). Other studies monitoring MPs in wild and farmed seabass have found MP levels in GIT ranging from 0.04 to 5.4 MPs per individual (Akoueson *et al.*, 2020; Barboza *et al.*, 2020b; Bessa *et al.*, 2018; Kılıç, 2022; Reinold *et al.*, 2021;

Sánchez-Almeida *et al.*, 2022). Particularly in the Mondego estuary, Bessa *et al.* (2018) reported 0.3 ± 0.6 MP/fish in the GIT of juvenile wild seabass (total length, 14.3 ± 0.1 cm; total weight, 49.5 ± 15.4 g) but are lower than the 2.8 ± 2.3 MP per GIT reported in pond-farmed seabass specimens (Matias *et al.*, 2024). In addition, MP occurrence level in internal organs has been previously reported, particularly in European seabass dorsal muscle, ranging from 0.04 to 1.04 MP per gram of tissue (Akoueson *et al.*, 2020; Barboza *et al.*, 2020b). The most common particle categories found in liver were similar to those found in the seabass GIT, dorsal muscle, water and feed as detailed in Matias *et al.* (2024). More precisely, particles with a fibre shape, with black or blue colouration, and constituted of man-made cellulose/rayon were the most common, and align with MPs previously observed in fish collected from Portuguese coastal and estuarine waters (Barboza *et al.*, 2020b; Guilhermino *et al.*, 2021; Lopes *et al.*, 2020), including wild seabass from the Mondego estuary (Bessa *et al.*, 2018). The characteristics of the MPs found in sediments from the Mondego estuary Morgado *et al.* (2022) were in general much different, mostly with an irregular shape (*i.e.*, fragment) and with white or translucent colouration, as well as constituted by polypropylene (PP) and high-density polyethylene (HDPE). Although no sediments were collected from the earthen ponds, studies report different MP characteristics between sediments and water (Rodrigues *et al.*, 2018; Scherer *et al.*, 2020). This can be justified by different MP characteristics (*e.g.*, shape, size, polymer density) as well as environmental conditions, such as water parameters like as salinity and flow strength, which interfere with the settling velocities of MPs (Laursen *et al.*, 2023; Yu *et al.*, 2022b). As alterations in these environmental conditions may be enhanced under a climate change context (Haque & Fan, 2023), further dedicated studies are required to better understand MP dynamics within aquacultures.

No significant differences were observed in fish biometric parameters (TL, TW, and K) and organosomatic indices (HSI and VSI) between the two established groups. Previous field studies have also not observed a significant relation between MP levels and biometric parameters in wild fish populations, including European seabass, from the NE Atlantic Ocean (Barboza *et al.*, 2020b), Mediterranean Sea (Bottari *et al.*, 2022; Capó *et al.*, 2022; Garcia-Garin *et al.*, 2019; Mancia *et al.*, 2020; Mancuso *et al.*, 2022; Rodríguez-Romeu *et al.*, 2020; Sbrana *et al.*, 2020), North and Baltic Seas (Rummel *et al.*, 2016), Greenland Sea (Morgana *et al.*, 2018). So far, Compa *et al.* (2018) reported a significant decrease in the K of European pilchard (*Sardina pilchardus*) with higher MP levels in GIT (K = 0.69 ± 0.01 and 0.53 MP/fish) relative to less contaminated fish (K = 0.85 ± 0.03 and no MP/fish) collected along the Spanish Mediterranean coastal waters. Lower K values in fish with higher MP levels may imply a negative effect on the overall fitness levels of the individuals. Nonetheless, most of these studies analysed fish of different sizes and ages which

influence MP levels found, although studies report controversial results (Chen *et al.*, 2022e; Ding *et al.*, 2023). Under controlled conditions, several experimental studies exposing European seabass to MPs have no effects on growth performance and organosomatic indices (Mazurais *et al.*, 2015; Montero *et al.*, 2022; Zeytin *et al.*, 2020). Likewise, no significant changes in these metrics were observed for gilthead seabream (*Sparus aurata*) (Del Piano *et al.*, 2023b; Solomando *et al.*, 2020) and rainbow trout (*Oncorhynchus mykiss*) (Hodkovicova *et al.*, 2021; Roch *et al.*, 2021). Contrarily, Pedà *et al.* (2022) reported lower CF (0.98 *vs.*1.12 g/cm^3) and HSI (1.26 *vs.* 1.67%) values in European seabass exposed to MPs through diet after 90 days (10 g/kg of diet), indicating decreased growth and changes in liver metabolism Roch *et al.* (2022) reported a dose-dependent decrease in specific growth rate (1.40 *vs.* 1.44%/day) and an increase in the feed conversion rate (0.89 *vs.* 0.83) in rainbow trout, resulting in a lower final weight (115 *vs.* 123 g) of the fish exposed to MPs through diet for 120 days (13 to 73 MP/fish every 2 days). In addition, Lu *et al.* (2022b) reported dose-dependent effects on Nile tilapia (*Oreochromis niloticus*), with fish exposed to MPs through diets (40 and 80 g/kg of diet) for 9 weeks showing higher HSI values (1.02 *vs.* 0.86%). As these latter studies have in common longer trial durations (>60 days), their results may be an indication that the exposure period to MPs is important to manifest on these metrics. Nevertheless, in the context of our study, no relationship between MP levels and fish biometric parameters and organosomatic indices of pond-farmed seabass was observable.

The histomorphological parameters evaluated between the two established groups (with ≤2 MP/g and ≥4 MP/g) suggests no relationship between MP levels and the integrity of the gastrointestinal tract. Nevertheless, the higher goblet cells (GC) (1886.0 ± 639.0) in the rectums of pond-farmed seabass with ≥4 MP/g suggests an increased mucus production and secretion as this is the first defence strategy against foreign particles to protect the intestinal epithelium. Previous experimental studies have also observed increased GC number after exposing fish to MP-contaminated diets (10 to 500 mg of MPs/kg) (Ašmonaitė *et al.*, 2018b; Espinosa *et al.*, 2019; Pedà *et al.*, 2016) and water (0.001 to 100 mg of MPs/L) (Hamed *et al.*, 2021; Xu *et al.*, 2023; Zheng *et al.*, 2024). In these studies, moderate to severe changes in the intestinal structure, such as the loss of epithelial integrity, high enterocyte vacuolisation, and shortening and thickening of *villi*, were reported in those fish. Montero *et al.* (2022) reported a higher number of lymphocyte cells in the *submucosa* of the anterior intestine and rectum in European seabass (fish with ~134 g) fed with a MP contaminated diet for 60 days (10% w/w). However, most of these studies tested high MP concentrations over time, which may not accurately reflect the MP levels that wild and farmed fish are exposed to (Cunningham & Sigwart, 2019). In the present study, the lack of significant effects in other parameters may indicate that pond-

farmed seabass may be exposed to lower MP levels, but high enough to manifest in an increased acid GC number in the rectum. In addition, it is worth noting that these MPs were environmentally enriched, possibly with other contaminants adsorbed to their surface, which may enhance MP exposure effects in the histomorphology of the rectum (Montero *et al.*, 2022; Pedà *et al.*, 2016). Further research is needed to conduct a more robust evaluation of aquacultures in different geographical locations subject to varying levels of MPs, to better determine if such levels to which farmed fish are exposed may result in histological responses.

There was no relationship between MP levels in the analysed tissues and oxidative stress biomarkers in the liver and muscle. This result may suggest that the higher MP levels observed were not sufficiently high to induce oxidative stress and cellular damage in the liver and dorsal muscle of fish. Moreover, since all fish were maintained in the same earthen pond under similar conditions thus it is possible that MP effects are equally reflected in all individuals. Other possible explanation is that fish with higher MP levels have compensatory mechanisms to maintain homeostasis, as an adaptive response to MP exposure (Rangasamy *et al.*, 2022). Nevertheless, previous studies exposing seabass to MPs under controlled conditions have reported a significant oxidative stress response. For instance, exposure from 0.26 to 0.69 mg of MP/L water or 750 g of MP/kg diet increased the activity of SOD, CAT, and GST activities, and LPO levels in several European seabass tissues after 3 days (Barboza *et al.*, 2018b; 2018c; Zitouni *et al.*, 2021). With higher exposure periods, Espinosa *et al.* (2019) reported a significant decrease in SOD and CAT activities in the liver after 21 days of MP exposure (100 and 500 mg/kg of diet). Pedà *et al.* (2022) also reported significant changes in CAT activity and LPO levels in the liver of European seabass after 30 and 90 days of exposure to 100 g of MP/kg of diet. Indeed, MP effects on the oxidative stress biomarkers of fish have been extensively documented (Compa *et al.*, 2024; Sun *et al.*, 2024) but many of these exposure studies tested MP concentrations that do not reflect aquaculture-relevant conditions, highlighting the need for further studies.

Other field studies have reported significant differences in oxidative stress biomarkers concerning to the number of MPs found in the tissues of wild fish. In the Balearic Islands coastal region, Solomando *et al.* (2022) reported higher SOD, CAT, and GST activities in great amberjack (*Seriola dumerili*) with >8 MPs recovered from GIT compared to fish with <8 MPs. Other studies establishing fish groups based on the presence and absence of MPs recovered from dietary content or tract also observed significant increases in the oxidative stress biomarkers in wild specimens of commercial fish species caught off the Balearic Islands (Alomar *et al.*, 2017; Capó *et al.*, 2022; Cohen-Sánchez *et al.*, 2023). Barboza *et al.* (2020b) reported increased LPO levels in the dorsal muscle of European seabass with MPs recovered from the GIT of fish captured along the Northeastern

Portuguese coast. Although other factors may influence the degree of MP effects, such as particle size (Ašmonaitė *et al.*, 2018a), the evidence of oxidative stress resulting from MP occurrence in wild fish suggests that MP levels in aquatic environments may be high enough to have a significant impact. It is important to mention that the above-cited studies, as well as ours, used different criteria to establish groups that can influence results. Therefore, a better understanding of the most adequate approaches to evaluate MP effects on field studies is advised. We hypothesize that the MP occurrence in the aquaculture facility analysed in the present study was not sufficient to affect the oxidative status in pond-farmed seabass. However, as numerous site-specific factors can influence MP occurrence in different aquaculture systems further studies are required to monitor MP occurrence in this sector and its potential effects on fish oxidative stress status.

As the present study constitutes a first approach to evaluate the potential relation between MP contamination and health status of seabass produced in an earthen pond aquaculture, it is highlighted the need to further field research monitoring MP levels to include the determination of biomarkers in their experimental designs. This is supported not only by our findings (*i.e.,* higher number of acid goblet cells) but also by the above-mentioned studies reporting higher oxidative status and neuromuscular toxicity in wild fish with higher MP levels (Barboza *et al.*, 2020b; 2023). Since aquaculture environments are inherently richer in plastic sources, it is important to monitor MP contamination and use the collected data to further experimental studies exposing fish to more aquaculture-relevant conditions. Regarding semi-intensive earthen pond farms, several practices should be implemented to reduce MP contamination, such as enhancing both natural (*e.g.*, sedimentation tanks) and mechanical filtration, reduce plastic gear usage, and choose aquafeeds with lower MP contamination (Jeyasanta *et al.*, 2024; Wu *et al.*, 2023a). Although at the present study no detrimental effects were observed, MP effects on fish health have been extensively documented (*e.g.,* inflammatory responses, behavioural changes) (Banaee *et al.*, 2024). Therefore, it is important to safeguard fish health and, ultimately, the consumers' health by providing safer and better seafood products.

4.5. Conclusion

Under the ‘One Health’ approach, this study first highlights the need to monitor MP occurrence in aquacultures to better understand their potential effects on fish health and seafood quality. Our findings point to higher acid goblet cell number in the rectum of pond-farmed European seabass with higher MP levels (≥4 MP/g). This may be an indication of a defensive response to protect the intestinal epithelium from ingested particles. Nonetheless, no detrimental effects related to higher MP levels are apparent as observed by the lack of significant results regarding biometric parameters and oxidative stress biomarkers. Further experimental studies should base the experimental design on accurate conditions. Moreover, aquaculture location should consider water quality thus requiring dedicated studies to better determine spatial and temporal variability (*e.g.,* production stages) of MP contamination. Moreover, aquaculture practices should search and implement mitigation strategies to minimize MP contamination, such as enhancing water filtration, reducing plastic gear usage, and aquafeeds with lower MP contamination.

Chapter V

It's not just synthetic microplastics: Multi-tissue approach reveals a wide range of transcriptional changes in European seabass exposed to viscose-rayon microfibres

by

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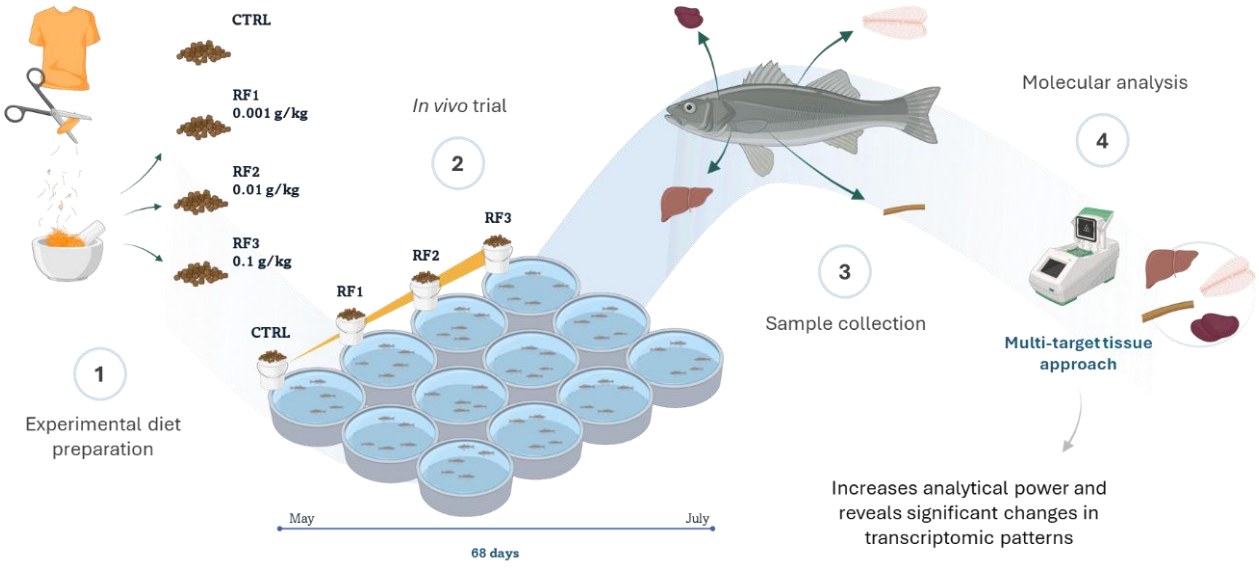

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Abstract

The impacts of dietary viscose-rayon microfibres (RFs) on European seabass were evaluated on the growth performance and feed efficiency, as well as on gene expression patterns using tissue-specific customised PCR-arrays. Juveniles (5.8 g) were fed diets either without RFs (CTRL) or with increasing RF levels: RF1 (0.001 g/kg), RF2 (0.01 g/kg), RF3 (0.1 g/kg), to visual satiety for 68 days. While growth performance remained unaffected by RF exposure, the hepatosomatic index (HSI) increased from ~1.5 in CTRL and RF1 to 1.98 in RF3 fish. HSI was related to hepatic transcriptional changes, such as activation of lipogenesis and mitochondrial respiration uncoupling, combined with overexpression of markers of cholesterol metabolism, and triacylglycerol- and fatty acid catabolism. RFs also affected the transcriptional profile of muscle, revealing enhanced regulation of positive and negative modulators of muscle growth. Several pro-inflammatory markers were altered by RF exposure, denoting an opposite regulation of immune response at systemic and local level, that contributed to discriminate CTRL_RF1 from RF2_RF3. The multi-tissue approach identified three distinct transcriptional signatures driven by 43 genes (CTRL_RF1, RF2, RF3). Intestine and muscle made a major contribution to this group separation. These findings indicate that RF exposure induces transcriptional changes with potential impacts on fish health.

Keywords

Transcriptional signatures; Lipid metabolism; Mitochondrial respiration; Muscle growth; Pro-inflammatory markers

5.1. Introduction

Microplastics are persistent contaminants in aquatic environments worldwide (Barrows *et al.*, 2018). This growing environmental concern also applies to microfibres, defined as any natural, semi-synthetic or synthetic particle of a fibrous shape with less than 5 mm in length and 50 µm in diameter (Liu *et al.*, 2019), that are specially abundant in the aquatic media (Suaria *et al.*, 2020), including aquaculture systems (Matias *et al.*, 2024; 2023). Although fishing nets and ropes are potential sources of microfibres, household laundry is a major annual contributor, releasing an estimated 0.28 million metric tons (MMT) (Belzagui *et al.*, 2020). However, previous studies have likely underestimated microfibre occurrence by excluding cellulosic microfibres, either due to concerns about airborne contamination or the assumption that these fibres are easily degradable and harmless in the environment (Athey & Erdle, 2022). Microfibres of natural and semi-synthetic origin, such as cellulosic microfibres, are degraded faster than synthetic polymers, but can still persist for months to years (Allison *et al.*, 2023; Belzagui *et al.*, 2021; Royer *et al.*, 2023). For instance, their half-life can be extended by some modifications, such as the addition of some chemical additives, including dyes, softeners, water repellents, and antimicrobial agents (Lykaki *et al.*, 2021; Zambrano *et al.*, 2021). Most of these chemicals (*e.g.*, bisphenols, phthalate esters, and per- and polyfluorinated alkyl substances) are highly toxic for aquatic biota (Cao *et al.*, 2022; Czarny-Krzymińska *et al.*, 2023; Ma *et al.*, 2023). Spheroidal particles composed by polystyrene (PS), polyethylene (PE), and polypropylene (PP), continue to be the most common form of microplastic in toxicological studies, while microfibres have been overlooked despite their larger predominance in aquatic environments (Rebelein *et al.*, 2021; Sacco *et al.*, 2024). Toxicological studies have reported changes in behaviour, epithelial mucosal histomorphology, and pro-inflammatory responses in fish exposed to fully synthetic microfibres (*e.g.*, polyester (PES), PP, nylon) (Hu *et al.*, 2020; Liang *et al.*, 2023; Qiao *et al.*, 2019). Moreover, there is now evidence that both types of microfibres can compromise host tissues, allowing pathogens to bypass the host fish defences (MacAulay *et al.*, 2023; Seeley *et al.*, 2023).

Cellulosic microfibres have been found at high levels in feeds and feedstuffs of farmed fish (Thiele *et al.*, 2021; Walkinshaw *et al.*, 2022; Wang *et al.*, 2022c). In addition, the predominance of cellulosic microfibres over other types of microplastics has been documented in the gastrointestinal tract of farmed fish, such as European seabass (*Dicentrarchus labrax*) (Matias *et al.*, 2024; Reinold *et al.*, 2021; Sánchez-Almeida *et al.*, 2022), gilthead seabream (*Sparus aurata*) (Sánchez-Almeida *et al.*, 2022; Savoca *et al.*, 2021), common carp (*Cyprinus carpio*) (Savoca *et al.*, 2021) and Nile tilapia (*Oreochromis niloticus*) (Garcia *et al.*, 2021), among other freshwater and marine species (Aiguo *et al.*,

2022; Feng *et al.*, 2019; Wu *et al.*, 2020). Siddiqui *et al.* (2023) investigated the dose-dependent effects of cotton microfibres in water (3, 10, 30 microfibres/mL) in mysid shrimp (*Americamysis bahia*) and inland silversides fish (*Menidia beryllina*). Their findings evidenced some detrimental effects on growth and/or behaviour in association with the highest salinity. Similarly, it has been observed that short-term exposure to lyocell-rayon microfibres at concentrations ranging from 500 to 2000 mg/L damaged the gut of both water flea (*Daphnia magna*) and brine shrimp (*Artemia franciscana*) (Kim *et al.*, 2021a; 2021b). These *in vivo* exposure studies with aquatic biota suggest that cellulosic microfibres are generally less harmful compared to fully synthetic microfibres. Conversely, Mateos-Cárdenas *et al.* (2021) observed that the ingestion of cellulosic microfibres had no acute effects in freshwater amphipods (*Gammarus duebeni*) after exposure to 600 particles/L for 96 h. Also, Bunge *et al.* (2022) reported no significant effects on growth, development, and immunological parameters in three-spined sticklebacks (*Gasterosteus aculeatus*) following dietary exposure to polyester (PES) and cotton microfibres (0.2-2 mg/g feed).

Taking altogether the above findings, current literature raises concerns that both natural and semi-synthetic cellulosic microfibres may pose toxicological risks, especially in the context of increasing global textile fibre production that has reached 124 MMT in 2023 and is projected to grow to 160 MMT by 2030 (Textile Exchange, 2024). Accordingly, the present study aimed to address current gaps of knowledge by evaluating the impact of dietary exposure to a semi-synthetic cellulosic fibre, viscose-rayon, on a commercially relevant farmed fish, the European seabass. Such approach considered up to three different exposure levels to comprehensively evaluate the effects of viscose-rayon microfibers (RFs) on growth performance, feed efficiency, and the gene expression profiles of selected biomarkers across key biological processes in essential tissues (liver, white skeletal muscle, anterior intestine, and head kidney). Gene expression data were considered separately for each target tissue and collectively across all four tissues, enabling a holistic, integrated approach to uncover multi-tissue effects of RF dietary exposure that might otherwise remain undetected.

5.2. Materials and methods

5.2.1. Ethical statement

The trial was carried out in the Institute of Aquaculture Torre la Sal (IATS), and all procedures were approved by the IATS and CSIC Ethics Committee (permission 1135/2021) and Generalitat Valenciana (permission 2021-VSC-PEA-0192). They complied with the guidelines provided by the European Union Council (2010/63/EU) and Spanish legislation (Royal Decree RD53/2013) on the protection of animals used for scientific purposes.

5.2.2. Viscose-rayon microfibres characterisation

The RF particles used in the study were isolated from a bright orange 100% viscose-rayon blouse. The deliberate choice of bright orange colour aimed to facilitate the identification of these fibres in various samples, given the apparently limited occurrence of orange particles in previous environmental studies (Ugwu *et al.*, 2021). Briefly, small pieces of fabric were finely cut, avoiding stich lines, and separated using a mortar with the addition of small volumes of ultrapure water. The RFs passing through a mesh sieve of 150 µm were collected in a glass flask and allowed to settle ($\rho \sim 1.53$ g/cm^3). Most of the water was removed without disturbing the settled RFs, and the remaining material was dried at 60 °C for 24 h (Binder BD53, GmbH, Germany). From here on, “RFs” refers specifically to the isolated bright orange viscose-rayon microfibres.

The cellulosic origin of the isolated RFs was confirmed by micro-Fourier Transformed Infrared Spectroscopy (µ-FTIR) with ATR sampling. The analysis was performed in an iN10 Infrared microscope with a Ge ATR tip (Thermo Scientific, WI, USA). The spectra were collected with an MCT-A detector using 256 scans from 4000 to 675 cm^{-1} (8 cm^{-1} resolution). The spectra were further compared to available spectral databases for plastic polymers and additives using the OMNIC software as presented in Figure S5.1. Automated measurements of RF dimensions, including length and diameter, were performed in the same equipment. Based on their length, RFs were categorized into different size classes: < 100 µm (19.50%), 100-149 µm (15.40%), 150-499 µm (58.61%), 500-1499 µm (6.44%), and 1500-2999 µm (0.05%). The mean RF length was 235.2 ± 165.4 µm, whereas the RF diameter averaged approximately 12.0 ± 1.0 µm (mean ± SD).

Chemical additives from RFs were extracted with methanol and analysed by Liquid Chromatography with High-Resolution Mass Spectrometry (LC-HRMS). The analysis was performed in a Dionex UltiMate 3000 UHPLC (Thermo Scientific, Germering, Germany) coupled with an Accucore RP-18 (2.1 x 100 mm, 2.6 µm) column with a mobile phase water (A)/methanol (B) for Atmospheric Pressure Chemical Ionization (APCI) as described by Costa *et al.* (2024). LC-HRMS profiles annotated three different compounds: decyl hexyl

phthalate, benzothiazole, and N-octyl-2-pyrrolidone. Data analysis was performed using Xcalibur 4.1 (Thermo Fischer Scientific), and the compound annotation and untargeted analysis were performed using Compound Discoverer 3.3 (Thermo Fischer Scientific).

5.2.3. Experimental diets preparation

Prior to diet formulation, microplastic occurrence was evaluated in several fishmeal products, as main protein source in aquafeeds, following the methodology described in Matias *et al.* (2024). The fishmeal with the lowest microplastic loads (0.9 ± 0.1 particles/g) and without orange particles was selected as the main dietary protein source in the study. A commercial-based diet (CTRL) was formulated without the addition of RFs, while three diets with RFs (from a 100% viscose-rayon blouse) were prepared using the same basal formulation, but with increasing levels of RFs: RF1 (0.001 g/kg), RF2 (0.01 g/kg), RF3 (0.1 g/kg) (Table 5.1). All diets, manufactured by Sparos Lda (Olhão, Portugal), were cold extruded to prevent the damage of the RFs, which were incorporated alongside oils.

The proximate diet composition was analysed following standardized methods (AOAC, 2006). The dry matter (DM) was determined by the weight difference after sample drying in a lab oven (24 h at 100 ± 1 °C); the ash content was determined by the weight difference after combustion in a muffle furnace (15 h at 600 ± 1 °C), crude protein was calculated after determining total nitrogen (N x 6.25) in a nitrogen analyser (Model FP 528; Leco Corporation, St. Joseph, USA); crude fat was determined after extraction with petroleum ether (Model ST 2055 Soxtec™; FOSS, Hillerod, Denmark); gross energy was measured in an adiabatic bomb calorimeter; and phosphorus content by digestion at 230 °C and absorbance determination at 820 nm (adapted from AFNOR V 04-406) as detailed in Basto *et al.* (2020).

The RFs distribution in experimental diets was assessed as described in Matias *et al.* (2024). Briefly, five replicates of 5 g were digested in glass flasks through the addition of 30% hydrogen peroxide (H_2O_2) solution at 60 °C for 24 h. Digested feed samples were vacuum-filtered through glass-microfibre filter membranes with 1.2 µm of pore size (Munktell & Filtrak, Germany). The absence of any orange particles was confirmed in the CTRL diet. The occurrence of RFs in each experimental diet proportionally matched the mass added in the feed mixture: RF1, 1.8 ± 0.5 RFs/g; RF2, 14.8 ± 3.0 RFs/g; and RF3, 195.0 ± 6.8 RFs/g (Figure 5.1A). After this validation procedure, CTRL and experimental diets were considered suitable for the feeding trial.

Table 5.1. Ingredients and proximate composition of experimental diets.

	CTRL	RF1	RF2	RF3
Ingredients				
Fishmeal LT70 (%)[1]	25.00	25.00	25.00	25.00
Soy protein concentrate (%)[2]	12.00	12.00	12.00	12.00
Wheat gluten (%)[3]	10.00	10.00	10.00	10.00
Corn gluten meal (%)[4]	8.00	8.00	8.00	8.00
Guar korma (%)[5]	5.00	5.00	5.00	5.00
Soybean meal (%)[6]	8.00	8.00	8.00	8.00
Rapeseed meal (%)[7]	3.00	3.00	3.00	3.00
Wheat meal (%)[8]	7.80	7.80	7.80	7.80
Whole peas (%)[9]	4.00	4.00	4.00	4.00
Vitamin and mineral premix (%)[10]	1.00	1.00	1.00	1.00
Choline chloride (%)	0.20	0.20	0.20	0.20
Monoammonium phosphate (%)	1.20	1.20	1.20	1.20
Fish oil (%)[11]	5.90	5.90	5.90	5.90
Rapeseed oil (%)[12]	8.90	8.90	8.90	8.90
Viscose rayon microfibres (g/kg)	0.0	0.0008	0.008	0.08
Proximate composition				
Dry matter (DM) (%)	97.42	96.43	97.00	95.89
Protein (% DM)	52.94	53.12	53.22	53.66
Energy (kJ g^{-1} DM)	22.47	23.03	23.16	23.18
Lipids (% DM)	18.22	18.58	18.32	18.09
Ash (% DM)	7.19	7.30	7.18	7.19
Phosphorous (% ash)	0.92	0.95	0.98	1.01

[1] Fishmeal NORVIK LT. Sopropêche, France (72% crude protein (CP), 7% crude fat (CF)); [2] Soy protein concentrate Soycomil®-P, ADM, Animal Nutrition™, Netherlands (62% CP, 0.7% CF); [3] Wheat gluten, Roquette Frères, France (80% CP, 7% CF); [4] Corn gluten meal COPAM, Portugal (61% CP, 6% CF); [5] Guar korma, Sopropêche, France (55.3% CP, 7.8% CF); [6] Dehulled solvent extracted soybean meal, Cargill, Spain (48% CP, 2% CF); [7] Rapeseed meal. Ribeiro & Sousa Lda, Portugal (34% CP, 2.1% CF); [8] Wheat meal Casa Lanchinha Lda, Portugal (11% CP, 2% CF); [9] Whole peas, Ribeira & Sousa Lda., Portugal (19.6% CP, 2.2% CF); [10] Vitamin and mineral premix WISIUM MIX AQUA 1.5%, ADM Portugal S.A., Portugal; [11] Sardine oil, Sopropêche, France; [12] Rapeseed oil, Henry Lamotte Oils GmbH, Germany

5.2.4. Experimental setup and sample collection

European seabass fingerlings of Mediterranean origin were obtained from a commercial fish hatchery (Avramar, Burriana, Spain). Upon arrival at the indoor marine IATS infrastructure, fish were acclimatized for 2 weeks in a 3,000 L tank, connected to a flow-through water system. At this time, fish were fed with a commercial feed (BioMar, Palencia, Spain), after which 720 fish of 5.8 g in average were equally distributed in 12 tanks of 500 L capacity each one. Three replicate tanks were used per dietary treatment with a complete water renewal every 1.5 h. Fish were fed three times per day at 9:00 h, 12:00 h, and 14:30 h with automatic feeders near to visual satiation. This was achieved by augmenting the feed supply to each tank by 2.5-3.5% daily, if no feed losses were observed. The amount of feed

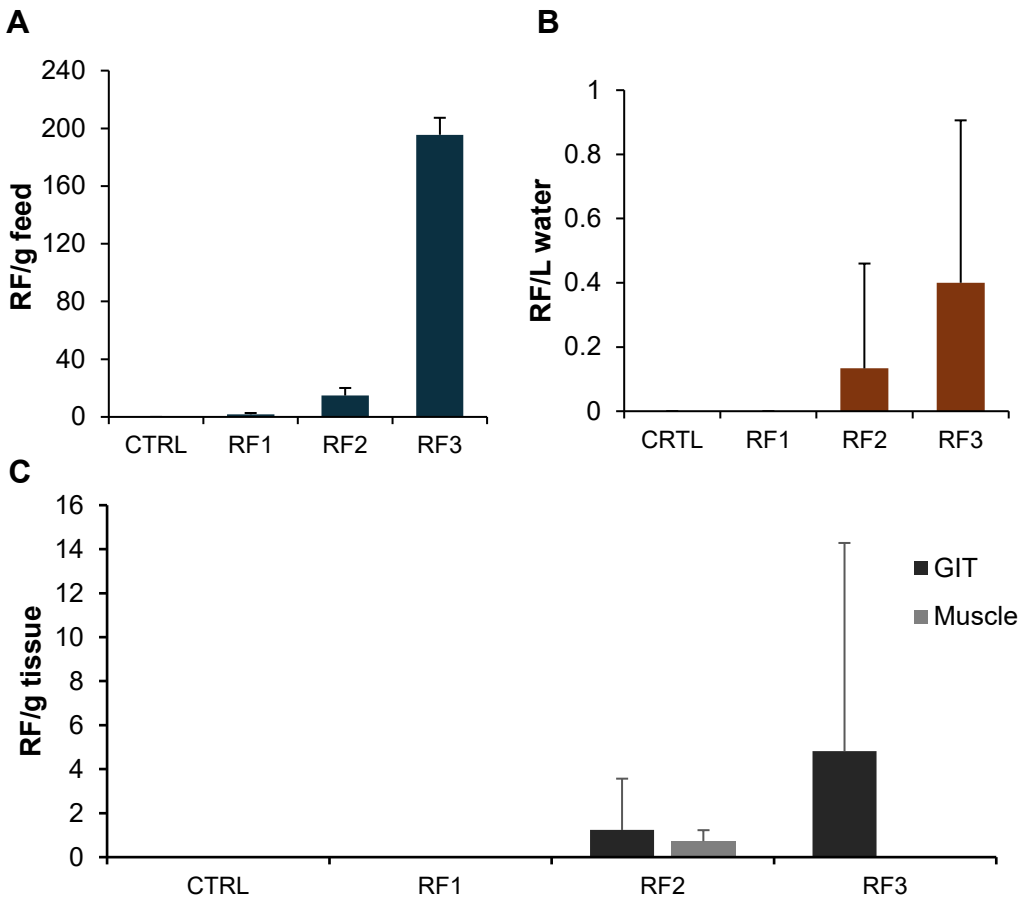

Figure 5.1. Quantification of orange viscose-rayon microfibres (RFs) in the experimental diets (A), water samples from the tanks (B), and from both seabass body sites (C), the gastrointestinal tract (GIT) and muscle.

distributed per tank was closely monitored throughout the trial. The trial lasted 68 days (May-July 2023) following the natural spring/summer variations at IATS latitude (40° 5'N, 0° 10'E), increasing the daily average water temperature from 20 °C to 27 °C over the course of the trial. Water oxygen (O_2) levels were monitored continuously, remaining above 75% air saturation (5-6.5 ppm) over the course of the trial. For growth recording, fish were collectively (at the beginning of trial) and individually (at the end of trial) weighed and measured (fork length) using an FR-200 FishReader W (Trovan, Madrid, Spain). The amount of feed distributed per tank was closely monitored throughout the trial and used for determining the voluntary feed intake (VFI) and feed conversion ratio (FCR).

At the end of trial, water samples (2.5 L each) were taken from the inlet water of each tank, just before the usual first feeding of the day. Such water samples were immediately filtered through glass-microfibre membranes with a pore size of 1.2 µm (Munktell & Filtrak, Germany) under vacuum conditions. At the same time (in three consecutive days) and following overnight fasting, ten fish per tank (*i.e.,* 30 per dietary treatment) were randomly selected and anaesthetized with 0.1 g/L of MS-222 (Sigma, Saint

Louis, MO, USA). Blood samples from 5 fish were taken from caudal vessels using heparinized syringes and tubes, centrifuged at 3,000 x *g* for 20 min at 4 °C, and stored until determination of plasma immune parameters. These fish were then sacrificed by decapitation, and the liver and viscera weights were registered for calculating the hepatosomatic (HSI) and viscerosomatic (VSI) indices. Portions of liver, white skeletal muscle (WSM; left side), anterior intestine (AI), and head kidney (HK) were sampled and preserved in RNA later for gene expression analyses. The remaining gastrointestinal tract (GIT) and approximately 1 g of dorsal WSM (right side) were sampled for RF quantification. The other five fish per tank were pooled for whole-body composition analyses. All samples were preserved at –80 °C until analytical procedures.

To avoid sources of contamination other than diet by itself, no orange clothing was worn by operators in the culture and experimental area, and the equipment used for daily maintenance operations was not shared among experimental tanks. Additionally, all dissection materials used during sample collection were made of stainless steel and thoroughly cleaned between samples. Procedural blank samples (*i.e.,* Petri dishes with clean filters) were also incorporated at each sampling stage to confirm the absence of any airborne contamination during sample manipulation.

5.2.5. Whole-body composition

The five fish per tank selected for whole-body composition analyses were immediately pooled and grinded using a food mincer to obtain a homogeneous sample. One aliquot was used to evaluate the dry matter (DM) content, while the remaining sample was immediately freeze-dried for further determination of ash, energy, crude protein (N x 6.25), crude lipids, and phosphorous as previously described for diets.

5.2.6. Growth performance and biometric indices

Calculations of growth performance and biometric indices were done as follows: Average body weight (ABW) (g) = (final body weight (g) + initial body weight (g)) / 2; Fulton's condition factor (K) = [final body weight (g) / (final fork length (cm))^3]; Feed intake (g/fish) = dry feed intake (g) / number of fish; Voluntary feed intake (VFI) (% body weight/day) = 100 x (dry feed intake (g) /ABW (g) / number of days); Specific growth rate (SGR) (%) = 100 x [(ln final body weight (g)) – (ln initial body weight (g))] / number of days; Feed conversion ratio (FCR) = dry feed intake (g) / weight gain (g); Hepatosomatic index (HSI) (%) = 100 x (liver weight (g) / body weight (g)); and Viscerosomatic index (VSI) (%) = 100 x (viscera weight (g) / body weight (g)).

5.2.7. *Microfibre identification and quantification in fish tissues*

GIT and WSM samples were digested in clean glass flasks with 30% H_2O_2 solution at a volume corresponding to three folds of the sample weight, following an incubation at 60 °C for 24 h, as described for the identification of RFs in the diets. The digested samples were filtered under vacuum conditions (Millipore Corporation, USA), and filtered through glass filter membranes with 1.2 µm pore size (Munktell & Filtrak, Germany). Filters were subsequently dried at 40 °C for 24 h, being orange RFs quantified under a stereomicroscope with an integrated camera (Nikon SMZ1000 with DS-Fi1, Japan) and ImageJ software (Schneider *et al.*, 2012). All orange RFs found analysed by µFTIR to confirm their cellulosic origin.

5.2.8. *Gene expression analyses*

The gene expression analysis followed the methodology described by Holhorea *et al.* (2023). Briefly, the liver, WSM, AI, and HK samples were homogenized in TRI reagent and the total RNA was extracted using the MagMAX-96 total RNA isolation kit (Life Technologies, USA). The RNA quantity and purity were determined with absorbance ratios at 260 nm/280 nm of 1.9-2.1 by Nanodrop (Thermo Scientific, USA). Using the High-Capacity cDNA Archive Kit, random decamers were used to perform the reverse transcription (RT) of 500 ng of total RNA (Applied Biosystems, USA). The RT reactions, including a negative control without reverse transcriptase, were incubated for 10 min at 25 °C and 2 h at 37 °C. The synthesized cDNA was used for PCR quantification with SYBR Green Master Mix (Bio-Rad, USA), and specific primers at a final concentration of 0.9 µM (Table S5.1). For each tissue, one customized PCR-array layout was assembled for the simultaneous gene expression profiling of 29 genes. In total, 91 genes were analysed with some overlapping between tissues. In liver and WSM, the selected biomarkers (49 genes in total) focused on the GH/IGF system (10), lipid metabolism (12), energy metabolism (11), muscle cell proliferation and differentiation (12), and protein turnover (4) (Table 5.2). Likewise in the AI and HK, the selected biomarkers (42 genes in total) covered different immunological functions: antibacterial peptides (5), complement system and acute phase proteins (5), mucins and pathogen recognition system (2), cytokines and transcription factors (19), and antigen-specific immune response (11) (Table 5.3).

The qPCR reactions were performed with an initial denaturation step at 95 °C for 3 min, followed by 40 cycles of denaturation at 95 °C for 15 s, and annealing/extension at 60 °C for 45 s. The efficiency and specificity of qPCRs reactions was assessed by analyses of melting curves and linearity of serial dilutions of RT reactions, respectively. All pipetting operations were done by an EpMotion 5070 Liquid Handling Robot (Eppendorf, Germany) to improve data reproducibility. Fluorescence data acquired during the extension phase

were normalized by the delta-delta Ct method (Livak & Schmittgen, 2001). Due to the β-actin gene (*actb)* stability (GeNorm software, M score = 0.21), it was used as housekeeping gene in the normalization procedure. For multigene expression analysis, all values in each tissue were referenced to the expression of a given gene in CTRL fish with an arbitrary assigned value of one, namely the fatty acid desaturase 2 (*fads2*) in liver, the growth hormone receptor 1 (*ghr1*) in WSM, the nuclear factor NF-κ-B p1000 subunit (*nfkb2*) in AI, and the monocyte to macrophage differentiation factor (*mmd*) in HK.

5.2.9. Humoral immune parameters

Plasma lysozyme, peroxidase, and total immunoglobulin M (IgM) content were measured in a microplate spectrophotometer (BioTek Synergy HT, Vermont, USA) in triplicate. Briefly, the activity of lysozyme (μg/mL of plasma) was determined using a turbidimetric assay (Hutchinson & Manning, 1996) adapted to a microtiter (Resende *et al.*, 2023). Serial dilutions of lysozyme from lyophilized hen egg white (Sigma) were used to obtain a calibration curve. The peroxidase activity was determined following Quade & Roth (1997), by defining that one unit of peroxidase (EU) produces an absorbance change of one OD (EU/mL of plasma). Total immunoglobulin (Ig) content was determined following Magalhães *et al.* (2023), based on the difference of plasma total protein content before and after the precipitation of Ig molecules by using a 12% solution of polyethylene glycol (PEG) (Pierce™ BCA Protein Assay Kit, Thermo Scientific, ref: 23225, Illinois, USA).

5.2.10. Statistical analysis

The data normality and homogeneity of variances were checked through the Shapiro-Wilk and Levene's tests, respectively. In a normal and homoscedastic scenario, One-way ANOVA followed by a Tukey *post hoc* test was used to assess statistical differences. The possible relation between humoral immune parameters in plasma and gene expression in AI and HK was checked using Pearson correlation ($p < 0.05$). Whenever data normality or homogeneity could not be achieved, data transformation (*i.e.,* logarithmic) or nonparametric statistical analysis (*i.e.,* Kruskal-Wallis for gene expression, Spearman for correlation; $p < 0.05$) was employed. All statistical analyses were performed using the SPSS statistical analysis package (version 27) or SigmaPlot (version 13) and a significance level of $p < 0.05$ was considered as the threshold for the tests used.

A partial least-squares discriminant analysis (PLS-DA) was used to analyse the gene expression patterns using EZinfo v3.0 (Umetrics, Sweden). Four separate PLS-DA analyses were conducted, one for each of the analysed tissues, to assess tissue-specific variations. For a more comprehensive evaluation across tissues, an integrated multi-tissue PLS-DA was subsequently performed. To avoid group separation due to differences in the

gene expression level between tissues, the multi-tissue PLS-DA was done using the fold-change (FC) value of each gene, calculated as the relation between a gene expression value and the average expression value of their respective experimental group. The explained variance and the predictive capability, given by the R^2Y (cum) and Q^2 (cum) values respectively, were used as quality indicators of the PLS-DA models. Hotelling's T^2 statistic was calculated by the multivariate software package EZinfo v3.0. All points in the current study that were above the 95% confidence limit for T^2 were detected and discarded. Loadings plot was used to identify the top genes with higher or lower values in the x-axis, and the top genes with higher or lower values in y-axis. The obtained models were validated through 500 random permutational tests (Ojala & Garriga, 2010) implemented in the Bioconductor R package *ropls* (Thévenot *et al.*, 2015). The genes significantly contributing to group separation were selected according to their Variable Importance in the Projection (VIP) values, with discriminant genes considered with a VIP threshold ≥1.0 (Li *et al.*, 2012; Wold *et al.*, 2001).

Table 5.2. PCR-array layout for hepatic (*) and white skeletal muscle (†) gene expression profiling.

Function	Gene	Symbol	GenBank
PERFORMANCE GH/IGF system	Growth hormone receptor-type 1	*ghr1* *†	AF438177
	Growth hormone receptor-type 2	*ghr2* *†	AY642116
	Insulin-like growth factor 1	*igf1* *†	AY800248
	Insulin-like growth factor 2	*igf2* *†	AY839105
	Insulin-like binding-protein 1b	*igfbp1b* *	GCA_000689215
	Insulin-like binding-protein 2b	*igfbp2b* *	EU526670
	Insulin-like binding-protein 3a	*igfbp3a* †	GCA_000689215
	Insulin-like binding-protein 4	*igfbp4* *	MN045298
	Insulin-like binding-protein 5b	*igfbp5b* †	GCA_000689215
	Insulin-like binding-protein 6b	*igfbp6b* †	GCA_000689215
LIPID METABOLISM FA elongases, FA desaturases, Lipases	Adipose triglyceride lipase	*atgl* *	KF857294
	Elongation of very long chain fatty acids 1	*elovl1* *	KF857295
	Elongation of very long chain fatty acids 4	*elovl4* *	KF857296
	Elongation of very long chain fatty acids 5	*elovl5* *	FR717358
	Elongation of very long chain fatty acids 6	*elovl6* *	KF857297
	Fatty acid desaturase 2	*fads2* *	EU647692
	Hormone-sensitive lipase	*hsl* *	KF857293
	Stearoyl-CoA desaturase 1b	*scd1b* *†	FN868643
	Lipoprotein lipase	*lpl* *	AM411614
	Hepatic lipase	*hl* *	KF857289
	Peroxisome proliferator-activated receptor α	*pparα* *	AY590300
	Peroxisome proliferator-activated receptor γ	*pparγ* *	AY590303
ENERGY METABOLISM	Carnitine palmitoyltransferase 1a	*cpt1a* *†	KF857302
	Citrate synthase	*cs* *†	KF857304
	Cholesterol 7-α-monooxygenase	*cyp7a1* *	KF857306
	Cytochrome b	*cyb* *	EF427553
	Cytochrome c oxidase subunit I	*cox1* *	KF857308
	Mitochondrial uncoupling protein 1	*ucp1* *	MH138003
	Mitochondrial uncoupling protein 3	*ucp3* †	GCA_000689215
	NADH dehydrogenase subunit 5	*nd5* *	KF857307
	Sirtuin 1	*sirt1* *†	MH138004
	Sirtuin 2	*sirt2* *†	MK983171
	Succinate dehydrogenase cytochrome b560 subunit	*sdhc* *	KF857305
MUSCLE CELL PROLIFERATION & DIFFERENTIATION	Fibroblast growth factor 4	*fgf4* †	GCA_000689215
	Fibroblast growth factor 6	*fgf6* †	AY831723
	Follistatin	*fst* †	MK983166
	Myoblast determination protein 1	*myod1* †	GCA_000689215
	Myoblast determination protein 2	*myod2* †	GCA_000689215
	Myogenic regulatory factor 4	*mrf4* †	GCA_000689215
	Myogenic factor 5	*myf5* †	GCA_000689215
	Myogenin	*myog* †	GCA_000689215
	Myomaker	*mymk* †	GCA_000689215
	Myostatin	*mstn* †	AY839106
	Muscle atrophy F-box	*mafbx/atrogin 1* †	MK983167
	Muscle RING-finger protein 1	*murf1* †	GCA_000689215
PROTEIN TURNOVER	Calpain 1	*capn1* †	FJ821591
	Calpain 2	*capn2* †	MK983168
	Calpain 3	*capn3* †	MK983169
	Calpastatin	*cpst* †	MK983170

Table 5.3. PCR-array layout for anterior intestine (*) and head kidney (†) gene expression profiling.

Function	Gene	Symbol	GenBank
ANTIBACTERIAL PEPTIDES & ENZYMES	β-defensin	*defb* *†	MG596340
	Dicentracine	*dic* *	AY303949
	Hepcidin precursor	*hepc* *	DQ131605
	Lysozyme C	*lyz* †	MG596338
	Liver-expressed antimicrobial peptide 2	*leap2* *†	MG596338
COMPLEMENT SYSTEM & ACUTE PHASE PROTEINS	α-2 macroglobulin	*a2m* †	MG596342
	Complement C3	*c3* †	HM563078
	C-reactive protein	*crp* †	EU660933
	Serum amyloid P component	*sap* †	KP642765
	Transferrin	*trf* †	FJ197144
MUCINS & PATHOGEN RECOGNITION	Galectin-8-like isoform X1	*lgals8x1* †	MG596345
	Mucin 2	*muc2* *	MK956789
CYTOKINES & TRANSCRIPTION FACTORS	Atypical chemokine receptor 4/C-C chemokine receptor type 11	*ackr4* †	KM225782
	CC chemokine ligand 2	*ccl2* *	AM490066
	CC chemokine ligand 4	*ccl4* *	AM490064
	CC chemokine receptor type 3	*ccr3* *†	KM225781
	CC chemokine receptor type 9	*ccr9* *†	FN665390
	Interferon regulatory factor 8	*irf8* †	KM225789
	Interleukin 1β	*il1b* *†	AJ311925
	Interleukin 6	*il6* *	AM490062
	Interleukin 8	*il8* *†	KM225777
	Interleukin 10	*il10* *†	DQ821114
	Interleukin 17A/F	*il17a/f* *	KJ818335
	Interleukin 20	*il20* *†	KM225779
	Interleukin 22	*il22* *	KJ818327
	Interleukin 34	*Il34* †	KM225780
	Macrophage colony-stimulating factor 1 receptor	*csf1r* *†	KM225787
	Macrophage migration inhibitory factor	*mif* *†	FN582353
	Monocyte to macrophage differentiation factor	*mmd* *†	KM225788
	Nuclear factor NF-κ-B p100 subunit	*nfkb2* *†	KM225790
	Tumour necrosis factor α	*tnfα* *†	DQ070246
AG-SPECIFIC IMMUNE RESPONSE	Immunoglobulin M membrane-bound	*igmb* †	MG596341
	Immunoglobulin M secreted	*igms* *†	KY173353
	Immunoglobulin T	*igt* *	KP096356
	Major Histocompatibility Complex Class I	*mhc1* *	AM943118
	Major Histocompatibility Complex Class II	*mhc2* *	AM113466
	Myeloid cell surface antigen CD33	*cd33* †	KM225786
	Myeloid differentiation primary response protein MyD88	*myd88* †	KM225785
	T-cell surface glycoprotein cd3 ζ chain	*cd247* *†	KM225783
	T-cell surface glycoprotein cd4	*cd4* *	AM849812
	T-cell surface glycoprotein cd8 α	*cd8a* *	AJ846849
	T-cell surface glycoprotein cd8 β	*cd8b* *†	KM225784

5.3. Results

5.3.1. Growth performance and whole-body composition

As shown in Table 5.4, all fish accepted the experimental diets equally well, resulting in similar voluntary feed intake (VFI, 1.9%), feed conversion ratio (FCR, 0.95-0.96), and growth performance (SGR, 2.40-2.45%) across dietary groups. Likewise, there were no significant differences in fish weight and length, resulting in an unaltered Fulton's condition factor (K). A trend of increasing hepatosomatic index (HSI) with RF exposure was observed, ranging from 1.48-1.55 in CTRL and RF1 fish to 1.82 in RF2 and 1.98 in RF3 fish. This increase was statistically significant when comparing RF1 and RF3 (p = 0.037). The increase of VSI with the highest RF exposure was less pronounced, with no significant differences found among dietary groups. Similarly, whole-body composition showed minimal variation, with protein (17.22-17.46% ww) and lipid (13.37-14.66% ww) contents remaining consistent across experimental groups.

Table 5.4. Data on growth performance of European seabass fed the CTRL and experimental diets (RF1, RF2 and RF3) for 68 days. Values are the mean ± SEM of triplicated tanks. Different superscript letters indicate statistically significant differences among groups (one-way ANOVA followed by Tukey post hoc test, $p < 0.05$).

	CTRL	RF1	RF2	RF3	*p*-value
Growth performance					
IBW (g)	5.86 ± 0.02	5.85 ± 0.03	5.91 ± 0.04	5.89 ± 0.04	0.557
FBW (g)	30.84 ± 0.50	30.91 ± 0.34	30.62 ± 0.67	30.34 ± 0.53	0.863
FBL (mm)	113.12 ± 1.00	113.50 ± 0.50	113.10 ± 1.11	112.28 ± 0.62	0.778
K	2.11 ± 0.03	2.09 ± 0.01	2.09 ± 0.01	2.12 ± 0.002	0.557
Feed intake (g/fish)	23.66 ± 0.23	23.77 ± 0.28	22.87 ± 0.34	23.36 ± 0.48	0.329
VFI (%/day)	1.91 ± 0.02	1.90 ± 0.01	1.89 ± 0.02	1.90 ± 0.02	0.880
Liver weight (g)	0.51 ± 0.03	0.51 ± 0.03	0.56 ± 0.03	0.62 ± 0.05	0.161
Viscera weight (g)	3.53 ± 0.19	3.40 ± 0.12	3.47 ± 0.19	3.42 ± 0.19	0.954
HSI (%)	1.55 ± 0.15^{ab}	1.48 ± 0.11^{b}	1.82 ± 0.08^{ab}	1.98 ± 0.08^{a}	**0.037**
VSI (%)	10.57 ± 0.67	9.82 ± 0.32	11.18 ± 0.38	11.08 ± 0.34	0.206
SGR (%)	2.43 ± 0.02	2.45 ± 0.03	2.40 ± 0.02	2.41 ± 0.01	0.274
FCR	0.96 ± 0.01	0.95 ± 0.01	0.95 ± 0.01	0.96 ± 0.01	0.968
Whole body composition					
Moisture (% ww)	65.28 ± 0.29	66.21 ± 0.85	65.41 ± 0.93	65.51 ± 0.50	0.789
Protein (% ww)	17.22 ± 0.26	17.26 ± 0.60	17.46 ± 0.35	17.26 ± 0.06	0.966
Lipids (% ww)	14.66 ± 0.32	13.37 ± 0.45	13.64 ± 0.84	13.75 ± 0.78	0.541
Energy (kJ/g ww)	8.54 ± 0.11	8.25 ± 0.16	8.59 ± 0.34	8.44 ± 0.30	0.769
Phosphorous (% ww)	0.55 ± 0.02	0.56 ± 0.03	0.57 ± 0.02	0.53 ± 0.01	0.420
Ash (% ww)	4.07 ± 0.11	3.98 ± 0.15	4.26 ± 0.10	3.80 ± 0.02	0.073

IBW, initial body weight; FBW, final body weight; FBL, final body (fork) length; K, Fulton's body condition factor; VFI, voluntary feed intake; VSI, viscerosomatic index; HSI, hepatosomatic index; FCR, feed conversion rate; SGR, specific growth rate.

5.3.2. Quantification of viscose-rayon microfibres

The number of RFs in feed, water and fish samples was established by μFTIR, increasing exponentially the number of RFs in feeds from zero (CTRL) to 195.0 ± 6.8 RFs/g in the extreme RF3 diet (Figure 5.1A). No RFs could be detected in inlet water samples. Likewise, RFs were not detectable in the water samples from tanks of CTRL and RF1 fish, but their occurrence increased up to 0.13 ± 0.13 RFs/L and 0.40 ± 0.21 RFs/L in tanks of RF2 and RF3 fed fish, respectively (Figure 5.1B). The presence of RFs in the GIT of fish also reflected the dietary challenge. RFs were not detected in CTRL and RF1 fish, while their presence increased exponentially in RF2 and RF3 fish, rising from 9 to 23 RFs. This corresponds to concentrations of 1.24 ± 0.60 RFs/g and 4.82 ± 2.44 RFs/g, respectively (Figure 5.1C). The presence of RFs in WSM was highly variable and did not show a direct relation with dietary RF exposure, being limited to 13 detectable RFs in RF2 fish, the equivalent to 0.78 ± 0.50 RFs/g (Figure 5.1C) with an average length of 138.1 ± 84.3 μm.

5.3.3. Effects of RFs on tissue gene expression profiles

The expression pattern of 18 genes was significantly ($p < 0.05$; one-way ANOVA) affected by RF exposure, more specifically 7 in liver (Table S5.2), 4 in WSM (Table S5.3), 2 in AI (Table S5.4), and 4 in HK (Table S5.5). In the liver tissue, the expression level of lipogenic enzymes, and energy-related genes such as elongation of very long chain fatty acids 5 (*elovl5*), stearoyl-CoA desaturase 1b (*scd1b*), cytochrome b (*cyb*), cytochrome c oxidase subunit 1 (*cox1*), cholesterol 7-α-monooxygenase (*cyp7a1*) and the mitochondrial respiratory uncoupling protein 1 (*ucp1*) were upregulated in either RF2 or RF3 fish in relation to CTRL or RF1 fish. Conversely, the lowest expression level of the catabolic marker, carnitine palmitoyltransferase 1a (*cpt1a*), was achieved in fish fed RF2 diet, followed by those fed RF3 (Table S5.2). In the WSM, the expression patterns did not clearly reflect the increasing dietary RF content. Fish fed the RF3 diet showed the highest expression levels of mitochondrial respiratory uncoupling protein 3 (*ucp3*), but without differing significantly from the control. The same expression pattern was found for the muscle atrophy F-box (*mafbx/atrogin 1*) which was highest in fish fed RF3. The muscle RING-finger protein 1 (*murf1*) and the myogenic regulatory factor 4 (*mrf4*) also varied significantly among groups, though their expression pattern did not reflect the increasing dietary RF content (Table S5.3). In the case of AI, the significant changes in gene expression were almost reduced to both tumour necrosis factor α (*tnfα*) and interleukin 1β (*il1b*), which achieved the highest expression level in RF2 fish (Table S5.4). In the HK, changes in immunoregulatory gene expression were more evident than in other tissues, specifically the gut. The trend for differentially expressed genes in HK, including immunoglobulin M secreted (*igms*), interferon regulatory factor 8 (*irf8)*, and myeloid cell surface antigen CD33

(*cd33*), indicated downregulation in RF2 or RF3 fish in relation to CTRL fish. Also, interleukin 20 (*il20*) was significantly downregulated in RF3 fish in relation to RF1 fish but not to the CTRL (Table S5.5).

When a partial least-squares discriminant analysis (PLS-DA) was used to analyse the gene expression patterns within a tissue, two clear groups could be established in most tissues, corresponding to CTRL_RF1 and RF2_RF3, with a number of discriminant genes with a VIP score ≥ 1.0. In the case of liver, such approach allows us to explain the 73% of variance observed and the 45 % of the predicted variance (R^2Y (cum) = 73 %, p = 0.006; Q^2 (cum) = 45%, p = 0.012), which was driven by 12 discriminant genes that were up- (8) or downregulated (4) in response to RF exposure (Figure 5.2A). This set of genes, ordered by its discriminant value, include a number of markers related to lipid/energy metabolism and growth regulation (s*cd1b*; *elovl5*; *cyp7a1; cpt1α; cox1; ucp1;* adipose triglyceride lipase (*atgl*); fatty acid desaturase 2 (*fads2*); *cox1*; growth hormone receptor-type 1 (*ghr1*); lipoprotein lipase (*lpl*); and hormone-sensitive lipase (*hsl*) (Figure 5.2A). Likewise, the muscle PLS-DA model disclosed the separation of CTRL_RF1 and RF2_RF3 (R^2Y (cum) = 68%, p = 0.012; Q^2 (cum) = 21%, p = 0.006) (Figure 5.2B), which was driven by 13 differentially expressed genes that were upregulated (10) rather than downregulated (3) by RF exposure. This expanded list of discriminant genes is ordered by VIP values as follows: myomaker (*mymk*); calpain 3 (*capn3*); fibroblast growth factor *6* (*fgf6*); calpain 1 (*capn1*); sirtuin 2 (*sirt2*); fibroblast growth factor 4 (*fgf4*); citrate synthase (*cs*); growth hormone receptor-type 2 (*ghr2*); myoblast determination protein 2 (*myod2*); insulin-like binding-protein 6b (*igfbp6b*); myogenic regulatory factor 4 (*mrf4*); *cpt1α*; and myoblast determination protein 1 (*myod1*) (Figure 5.2B). In the case of AI, the multivariate analysis was not able to discriminate the combined groups of CTRL_RF1 and RF2_RF3 (R^2Y (cum) = 58%, p = 0.216; Q^2 (cum) = 14%, p = 0.252) (Figure 5.3A). Conversely, in the HK, the separation was driven by 13 immunoregulatory genes (R^2Y (cum) = 66%, p = 0.010; Q^2 (cum) = 33%, p = 0.004) that were downregulated rather than upregulated by RFs (Figure 5.3B). At a closer look, these genes are components of the immune acute phase response (C-reactive protein, *crp*), cytokines & transcription factors (*il20*; interleukin 10, *il10*; *tnfα*, macrophage-colony-stimulating factor 1 receptor, *csf1r;* CC chemokine receptor type 9, *ccr9;* macrophage migration inhibitory factor, *mif*), and markers related to the antigen-specific immune response (*cd33*; immunoglobulin M membrane-bound, *igmb*, β-defensin, *defb*; T-cell surface glycoprotein cd8β, *cd8b*; *irf8*; *igms*). The separation obtained by the three PLS-DA models and the validity of the discriminant gene (VIP ≥ 1.0) were further confirmed by the statistical differences obtained through the One-Way ANOVA test using the same grouping applied for the multivariate analysis, which considered CTRL_RF1

and RF2_RF3 as experimental super-groups. The results of the comparison can be accessed at Table S5.6.

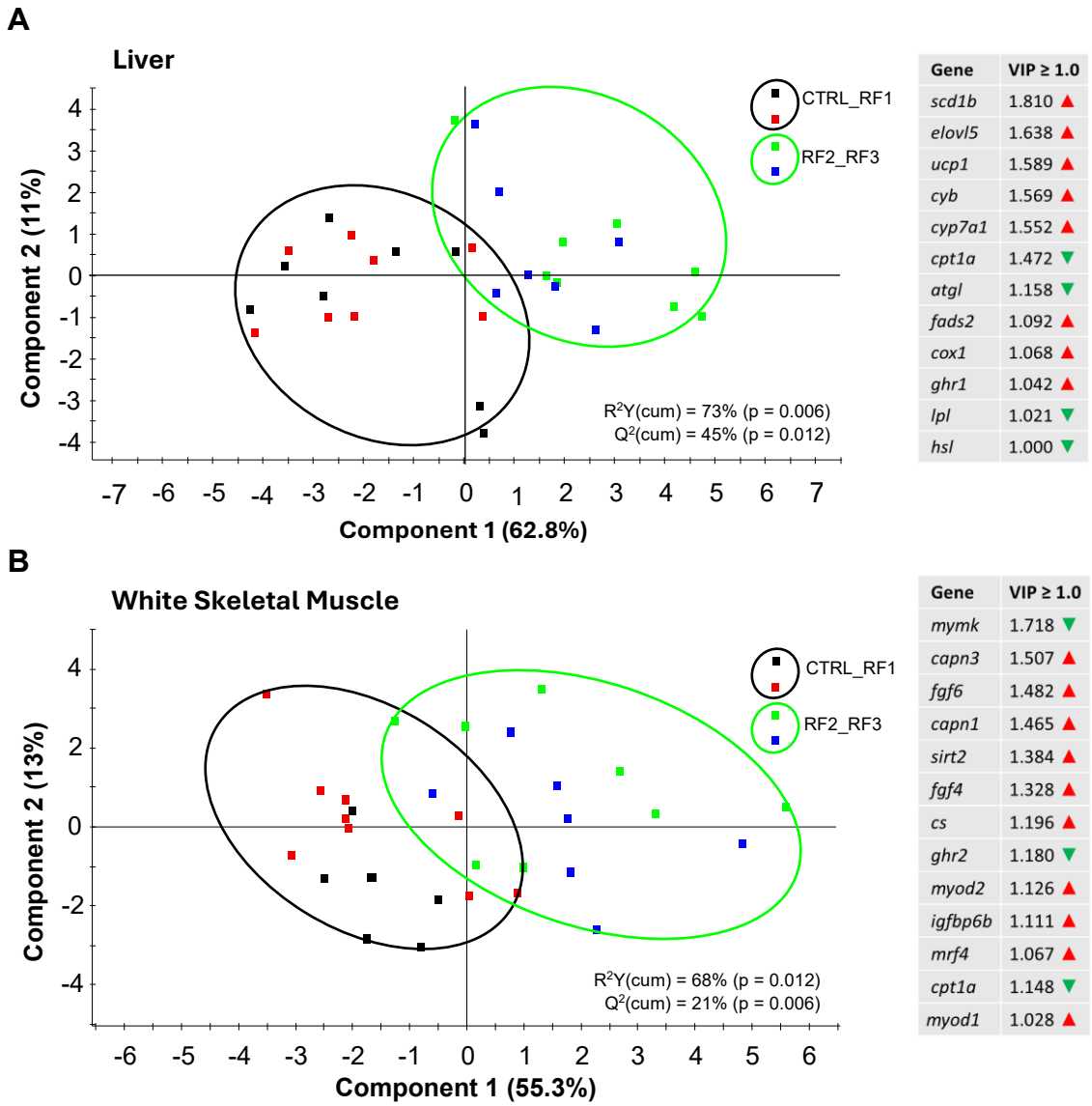

Figure 5.2. Two-dimensional PLS-DA score plots of (A) liver and (B) white skeletal muscle gene expression, representing the distribution of the samples between the first two components in the model. The cumulative explained $R^2Y(cum)$ and predicted $Q^2(cum)$ variance, as well as the *p*-values of the permutation plot can be found at the bottom-right side of the figure. Discriminant genes are ordered by variable importance in the projection (VIP). Up-regulated genes in RF2_RF3 group are indicated by a red upward arrow, and down-regulated genes by a green downward arrow.

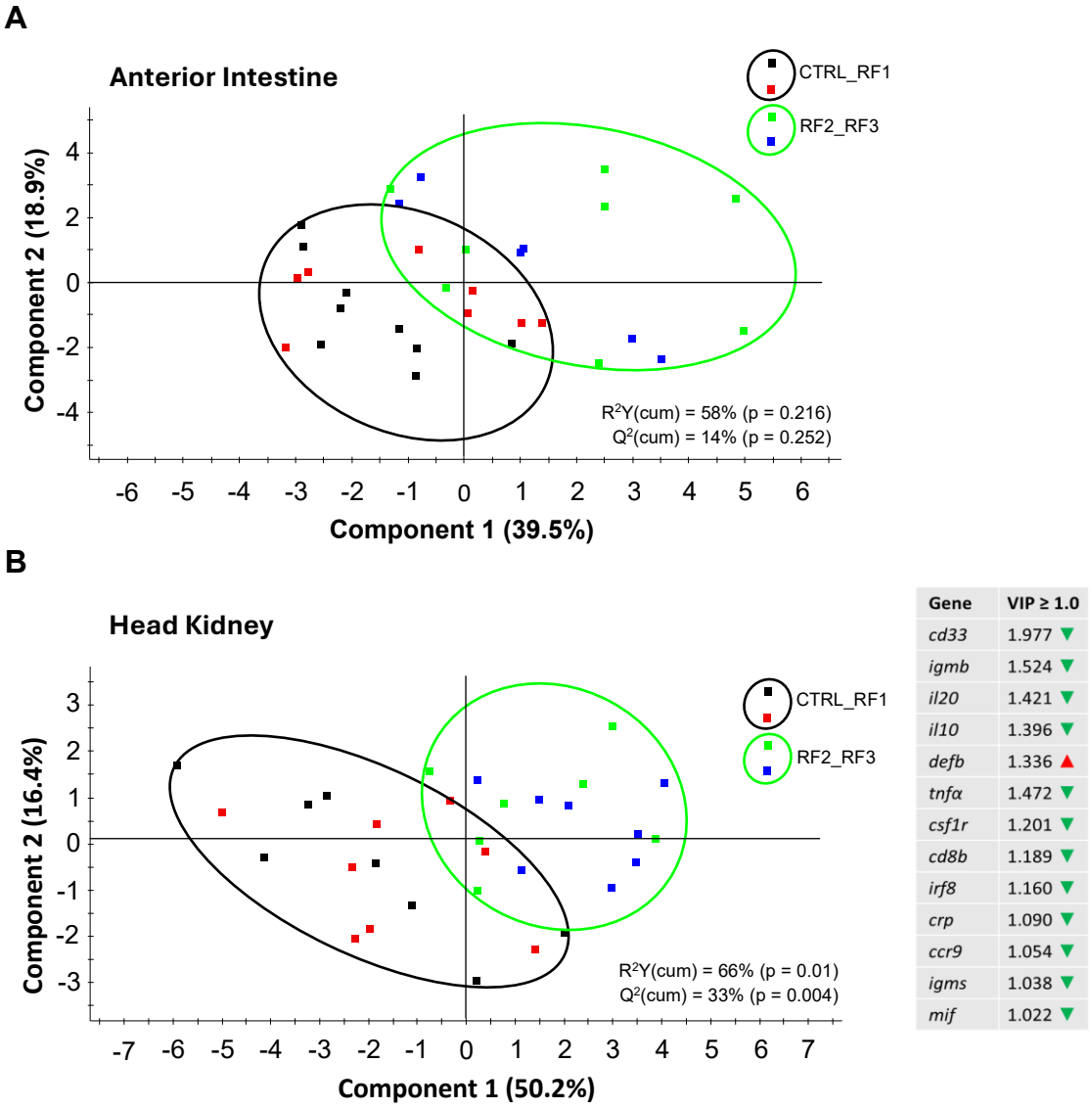

Figure 5.3. Two-dimensional PLS-DA score plots of (A) anterior intestine and (B) head kidney gene expression, representing the distribution of the samples between the first two components in the model. The cumulative explained $R^2Y(cum)$ and predicted $Q^2(cum)$ variance, as well as the *p*-values of the permutation plot can be found at the bottom-right side of the figure. Discriminant genes are ordered by variable importance in the projection (VIP). Upregulated genes in RF2_RF3 group are indicated by a red upward arrow, and downregulated genes by a green downward arrow.

When considered together all the tissue-gene expression patterns, the resulting PLS-DA model was able to clearly differentiate up to three main groups, corresponding to CTRL_RF1, RF2 and RF3 fish (Figure 5.4A). This improved group separation was driven by an increased number of discriminant genes (43 *vs.* 38) that were clustered in a heat map as down- (25) and upregulated genes (18) by RF exposure (Figure 5.4B). The downregulated group was enriched in immunoregulatory HK genes that mostly co-regulated

together in three main sub-nodes that embraced up to 2 (nuclear factor NF-κ-B p100 subunit, *nfkb2*; *cd33*); 8 *(defb*; *il10*; *il20*; *mif*; *crp*; *cd8b*; *irf8*; *csf1r*; *tnfα)* and 5 (atypical chemokine receptor 4/C-C chemokine receptor type 11, *ackr4*; *igmb*; *ccr9*; *igms*; lysozyme C, *lyz*) genes. The other subset of downregulated genes clustered separately liver (*igfbp4*, *lpl*, *atgl*, *cpt1a*, *hsl*) and WSM (*mymk, ghr2, mafbx/atrogin 1*) markers. The cluster of upregulated genes included up to 7 genes (*cox1*; *cyb*; *cyp7a1*; NADH dehydrogenase subunit 5, *nd5*; *ucp1*, *scd1b*, *elovl5*) from liver; 4 (myostatin (*mstn*); *ucp3*; *myod2*; insulin growth factor binding-protein 5b, *igfbp5b*) from WSM; 6 (*il1b*; C-C chemokine ligand 4, *ccl4*; *nkfb2*; *il10*; hepcidin precursor, *hepc*; *il20*) from AI; and 1 (*defb*) from HK without a clear tissue-specific co-regulated expression pattern. After loading plot re-ordering of data, 8 x-axis genes corresponded to liver (*elovl5*, *ucp1*, *cyb*, *scd1b*, *cyp71a*, *lpl*, *atgl*, *cpt1a*), and 4 corresponded to HK (*il10*,*il20*, *cd33*, *defb*) were identified; whereas the y-axis genes were subdivided in 5 WSM genes (*ucp3*, *mstn*, *mafbx/atrogin 1*, *igfbp5b*, *myod2*) and 2 AI genes (*il1b*, *il20*) (Figure 5.4C).

5.3.4. Correlation analyses between humoral and immune-related markers

As depicted in Figure 5.5, no significant differences were found in humoral immune parameters among groups: lysozyme activity (p = 0.860), peroxidase activity (p = 0.259), and total immunoglobulin content (p = 0.466). Considering all data together, correlation analysis depicted a close association between lysozyme and peroxidase activities (r = -0.477, p = 0.005). Likewise, significant correlations between humoral parameters and gene expression levels of AI and HK markers were also found. Thus, plasma lysozyme activity disclosed a negative association with the expression level of mucin *2* (*muc2*) (r = -0.427, p = 0.018) and interleukin 6 (*il6*) (r = -0.376, p = 0.031) in the AI. The plasma peroxidase activity was positively correlated with the HK expression level of *ccr9* (r = 0.454, p = 0.008), *igmb* (r = 0.485, p = 0.004), and *csf1r* (r = 0.358, p = 0.041). The total amount of plasma immunoglobulins was positively correlated with the expression level of *ccr3* (r = 0.409, p = 0.031) and *il10* (r = 0.413, p = 0.029) in the AI, as well as with *csf1r* (r = 0.458, p = 0.009), *crp* (r = 0.421, p = 0.029), *igms* (r = 0.383, p = 0.044), *cd8b* (r = 0.435, p = 0.021) and *tnfα* (r = 0.506, p = 0.006) at the level of HK. The values of correlation analysis are available in Table S5.7.

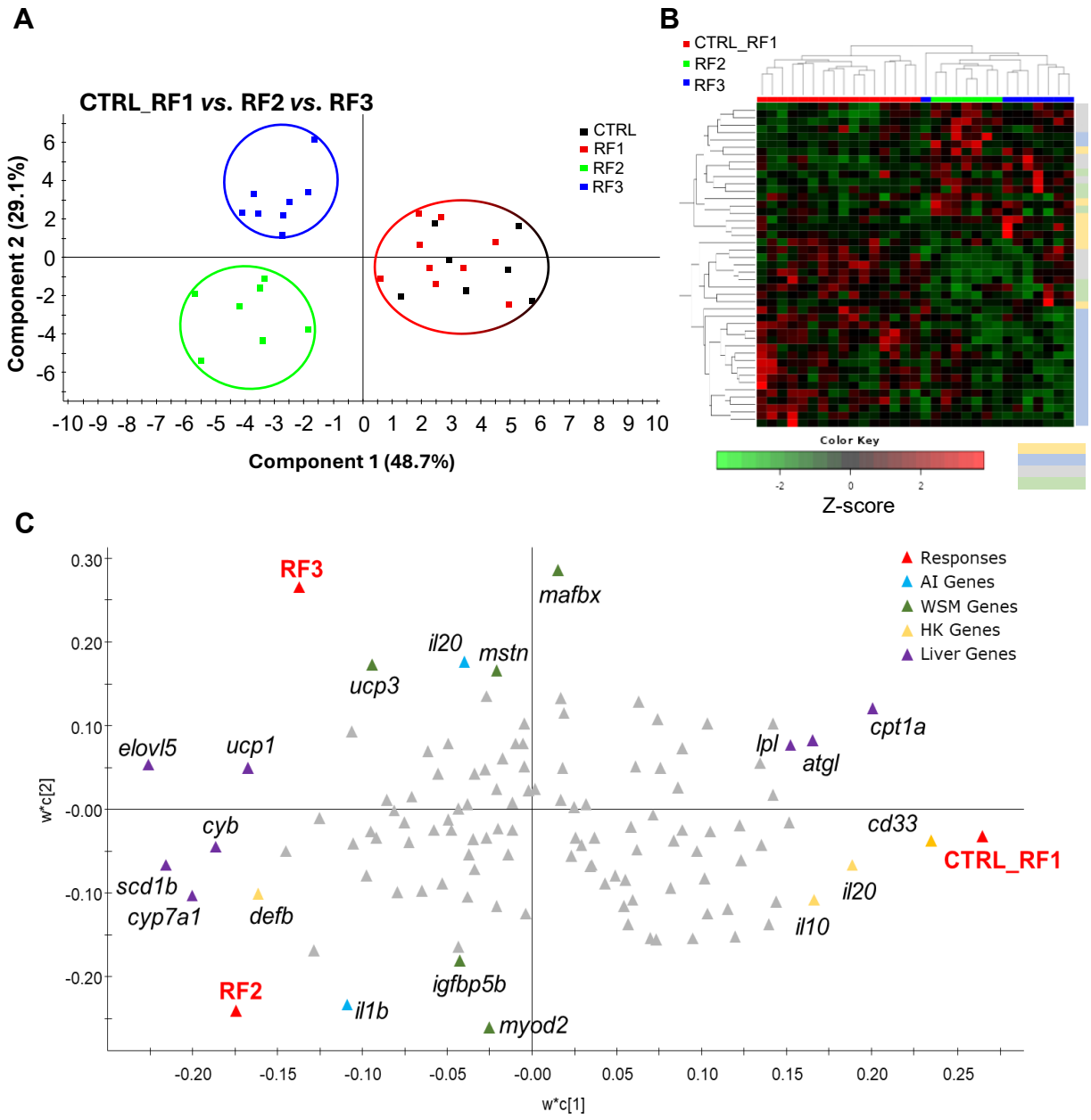

Figure 5.4. (A) Two-dimensional PLS-DA score plot of multi-target (anterior intestine, head kidney, liver and muscle) gene expression, representing the distribution of the samples between the first two components in the model. The cumulative explained $R^2Y(cum)$ and predicted $Q^2(cum)$ variance, as well as the p-values of the permutation plot can be found at the bottom-right side of the figure. (B) Heatmap representing the abundance distribution (Z-score) of the genes identified to be driving the separation by diet (VIP ≥ 1). (C) Loading plot representing the top genes contributing to the separation between groups (VIP ≥ 1.0; coloured triangles). Red triangles represent the experimental conditions used to represent multi-tissue PLS-DA; grey triangles indicate other genes in the model.

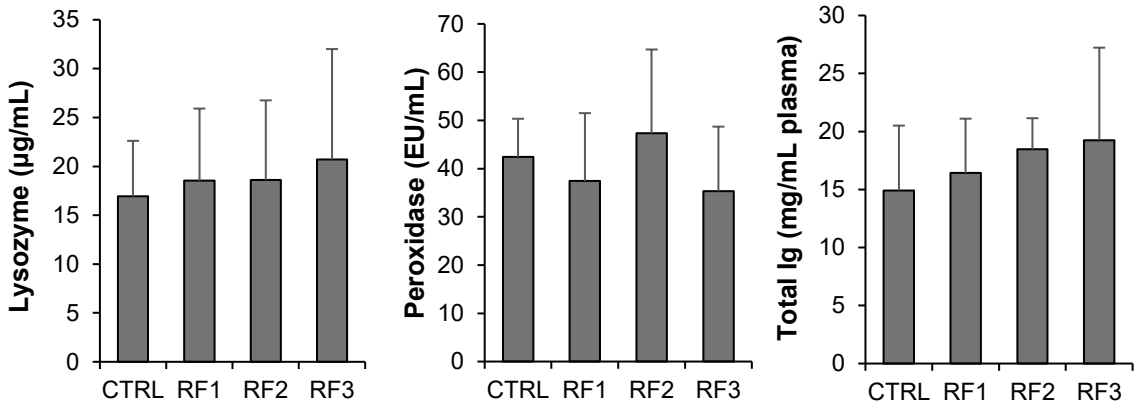

Figure 5.5. Humoral immune parameters in European seabass exposed to increasing concentrations of viscose-rayon microfibres through diets (RF1, RF2, and RF3). Total Ig refers to the total immunoglobulin content.

5.4. Discussion

The exposure of European sea bass to increasing levels of viscose-rayon microfibres (RFs) did not affect growth performance or feed efficiency. However, the gene expression analyses in liver, WSM, AI, and HK revealed tissue-specific physiological adaptations in response to RF exposure. The 1.8 RFs/g feed in diet RF1 falls within the previously reported range of microplastics and other anthropogenic particles in aquafeeds (from 1.57 to 3.93 particles per gram) (Matias *et al.*, 2024; 2023). The RF2 diet reaches up to 14.8 RFs/g, which is in line with the highest reported concentrations in aquafeeds of 11.6 MP/g (Muhib & Rahman, 2023). In contrast, the RF3 diet contains a microfibre concentration 10 times higher than any previously reported in aquafeeds. This aspect is particularly important since most studies use high doses to intentionally induce a biological response and/or adopt quantification units that make it difficult to ascertain their environmental relevance and the comparison across the literature studies (Cunningham & Sigwart, 2019). On the other hand, it must be noted that only a small quantity of RFs (< 0.5 RFs/L) was found in the water samples from fish reared with the RF2 and RF3 diets. This suggests that RFs did not accumulate within the rearing system and that waterborne exposure pathway likely had a minimal contribution to the observed effects.

After the 68-day feeding trial, all the fish grew fast (SGR, 2.40-2.43) and efficiently (FCR, 0.95-0.96) regardless of the experimental group. This suggests that exposure to increasing dietary levels of RFs had a negligible impact on growth performance and feed efficiency under the tested conditions. In fact, fish performance was comparable to studies conducted under optimal feeding conditions (Kousoulaki *et al.*, 2015). These findings align with previous studies exposing European seabass of similar size (5-8 g) to diets having relatively high levels of PE, polyvinyl chloride (PVC), or benzoguanamine resin microplastics (0.05 to 0.50 g/kg) which resulted in no significant effects on growth performance (Espinosa *et al.*, 2019; Zeytin *et al.*, 2020). Likewise, other studies in European seabass observed no changes in growth performance after exposure of 70-140 g fish to diets containing 0.1-10% (*w*/*w*) of PE, PP, PVC, or amino formaldehyde microplastics (Granby *et al.*, 2018; Montero *et al.*, 2022; Pedà *et al.*, 2022; Zarantoniello *et al.*, 2024). The same trend has been described in other important farmed fish for the European aquaculture, such as gilthead seabream (Brandts *et al.*, 2021b; Capó *et al.*, 2021a; Del Piano *et al.*, 2023b; Espinosa *et al.*, 2017; Jovanović *et al.*, 2018; Solomando *et al.*, 2020; Varó *et al.*, 2021) and rainbow trout (Hodkovicova *et al.*, 2021; Hollerova *et al.*, 2023). Conversely, Lu *et al.* (2022b) reported decreased growth and survival rates after exposure of 5 g juveniles of Nile tilapia for 9 weeks to diets containing 4-8 % (*w*/*w*) of PE microplastics. Similarly, with fish of the same class of size, Nair & Perumal (2024) reported an impaired

growth following PP microplastic (1.5-4.5 g/kg) exposure for 7 weeks. Roch *et al.* (2022) also reported decreased growth and feed efficiency after exposing juvenile (21 g) rainbow trout for 120 days to diets containing polymethyl methacrylate (PMMA) microplastics (~2729 to 3526 particles/kg). Detrimental growth effect were observed by Fatema *et al.* (2023) in adult (100 g) walking catfish (*Clarias batrachus*) after 60 days of exposure to diets containing 5 % (*w*/*w*) of PE or polyethylene terephthalate (PET) microplastics. Contrarily to the present data, previous studies reported lower feed intake in the marine jacopever (*Sebastes schlegelii*) after a 14-day exposure to 0.23 mg PS/L and 1×10^6 PS particles/L, ultimately compromising fish energy reserves and growth rates (Sun *et al.*, 2023b; Yin *et al.*, 2018). Detrimental effects on growth and survival rates have also been described after plastic pollutant exposure in model fish species, such as zebrafish (*Danio rerio*) and medaka (*Oryzias melastigma*) (Cormier *et al.*, 2022; Teng *et al.*, 2022; Zhao *et al.*, 2020). Current literature reports varying results, likely due to differences in study conditions. Life stages and exposure duration appear to be key factors, as the susceptibility to microplastic exposure is significantly higher during early life stages, such as larvae fish (Uy & Johnson, 2022; Xia *et al.*, 2020; Xu *et al.*, 2024a; Yang *et al.*, 2020). Additionally, in most studies, the observed effects tend to be amplified after long-term exposure throughout the life or full production cycle.

The HSI, an indicator of hepatoxicity, showed a RFs-associated pattern which resulted in a progressive rise from 1.4-1.6 % in CTRL and RF1 fish to 1.8-2 % in RF2 and RF3 fish (Table 4). Likewise, several authors have related the fish microplastic exposure to the increase of HSI in concurrence with hepatocyte hyperplasia and impaired oxidative status (Hu *et al.*, 2023; Lu *et al.*, 2022a; 2022b). Conversely, other authors have reported a significant HSI reduction, but only as a result of an evident histopathological damage. In particular, Granby *et al.* (2018) reported a lower HSI in European seabass exposed during 80-days to PE, added to experimental diets at 2-4 % (*w*/*w*). In the same fish species, Pedà *et al.* (2022) showed a reduction of HSI after 90 days of dietary exposure to 0.1 % (*w*/*w*) PVC diet. Similar results have been reported in other fish species, but again the decrease of liver size after microplastic exposure is associated with signs of histopathological damage, abnormal cell morphology, and serious lipid accumulation in hepatocytes, which highlighted a severe inflammatory and oxidative status (Chen et al., 2022; Espinosa et al., 2019; Hollerova et al., 2023; Liu et al., 2023).

Although liver morphology was not evaluated in the present study, data related to HSI are corroborated by RF-mediated transcriptional changes in the liver, which become especially evident, with up 12 discriminant genes identified when comparing CTRL_RF1 and RF2_RF3 fish (Table S2, Fig. 2A). This is not surprising because the connection of environmental pollutants with the detoxifying effects of liver have been

extensively documented in rodents and fish, among other animal models (Das, 2023; Solomando *et al.*, 2022; Sun *et al.*, 2024). Thus, several studies have indicated that MPs or NPs can affect the mitochondria by disrupting the electron transport chain and damaging the inner mitochondrial membrane (Bhuyan, 2022; Subaramaniyam *et al.*, 2023). This resulted in decreased efficiency of aerobic ATP production that is counteracted by enhancing the oxidative phosphorylation and proton movement across the mitochondrial respiratory chain (Song & Villeneuve, 2021). In the present study, this would be exemplified by the upregulation of *cyb* (respiratory chain complex III) and *cox1* (respiratory chain complex IV*)* in RF2_RF3 fish, which ultimately would increase the production of reactive oxygen species (ROS) with the subsequent risk of overcoming the antioxidant defence system (Lushchak, 2016; Selivanov *et al.*, 2011). In agreement with this, Brandts *et al.* (2018) reported in European seabass the upregulated expression of *nd5* (respiratory chain complex I) after waterborne exposure to PMMA nanoplastics (0.02 to 2 mg/L). Hence, some protective actions should be required to minimise any cellular damage due to the concomitant increase of mitochondrial activity and ROS production. Accordingly, the changes in mitochondrial respiration uncoupling via the upregulated expression of hepatic *ucp1* in RF2_RF3 fish can be viewed as an adaptive mechanism, acting as a redox-sensor to minimise the risk of oxidative stress with the increase of mitochondrial activity (Bermejo-Nogales *et al.*, 2010; Ledesma *et al.*, 2002). Such notion was also supported by observations made by other authors, who stated a mitochondrial respiration uncoupling in gilthead seabream (Espinosa *et al.*, 2017) and sea cucumber (*Apostichopus japonicus*) (Li *et al.*, 2024c) following exposure to PE microplastics.

Many aspects of hepatic lipid metabolism are also modified by a number of environmental pollutants, increasing or limiting the provision of fatty acids (FA) for oxidative purposes, which ultimately alters the ROS production and the risk of oxidative stress (Juan *et al.*, 2021; Mustafa *et al.*, 2024). Indeed, changes in circulating lipoprotein clearance and lipogenic/lipolytic pathways are shaped in fish and other animal models to cope with different stressogenic agents such as thermal stress, high stocking density, and hypoxia (Domingo-Bréton *et al.*, 2025; Holhorea *et al.*, 2023; Martos-Sitcha *et al.*, 2019; Naya-Català *et al.*, 2021; Toxqui-Rodríguez *et al.*, 2024). Likewise, several authors have documented the effects of microplastics on lipid metabolism, highlighting the potential risk to develop hepatic steatosis and, ultimately the onset and progression of non-alcoholic fatty liver disease (NAFLD) (Del Piano *et al.*, 2024; Zhou *et al.*, 2023). In the present study, the progressive increase of HSI in response to RF exposure may be an indication of abnormal lipid deposition rates, first driven by the upregulation of *scd1b and fads2* desaturases, two key lipogenic enzymes that are known to be nutritionally and/or epigenetically regulated in gilthead sea bream (Perera *et al.*, 2020). This was concurrent with the overexpression of

elovl5 that alters in humans (Shikama *et al.*, 2015) and rodents (Tripathy *et al.*, 2014) the expression of FA desaturases- and triglyceride (TG)-related genes. The products of ELOV5 participate in activating TG catabolism and the negative feedback regulation of *de novo* FA synthesis that might serve to maintain regulated at a low level the lipogenic activity. Certainly, in gilthead seabream, changes in the expression pattern of *elovl5* have been documented with the use of new fish feed formulations that can compromise at certain degree the overall fish performance (Fernandes *et al.*, 2024). However, herein the diets were formulated to clearly fulfil fish nutritional requirements and the observed alterations in the expression pattern would be entirely mediated by the dietary RF exposure. At the same time, the PLS-DA denoted a downregulated expression of key markers of tissue FA uptake (*lpl*) and TG catabolism (*atgl, hl*) in the liver of RF2_RF3 fish, which highlighted a clear dominance of lipid storage upon lipid catabolism with the subsequent risk to lead to hepatic steatosis (Bai & Li, 2019; Zhou *et al.*, 2021b). Such observation is reinforced by the downregulated expression of hepatic *cpt1a* in RF2_RF3 fish. The *cpt1a* is in fact a rate-limiting enzyme of FA oxidation that catalyses the transfer of the long-chain acyl group in acyl-CoA ester to carnitine, allowing FAs to enter the mitochondrial matrix for oxidation. Therefore, the downregulation of this enzyme would limit the catabolism of FAs, which in turn minimises the production of ROS and the risk of oxidative stress (Jiang *et al.*, 2022; Luo *et al.*, 2020). Otherwise, RF exposure resulted in the upregulation of the hepatic expression level of *cyp7a1*, the first and rate limiting step on the bile acid synthesis (Chiang & Ferrell, 2020), which have diverse effects on physiological processes, including oxidative stress and inflammation (Jin *et al.*, 2019; Lv *et al.*, 2023; Zhao *et al.*, 2021). Certainly, bile acids shape the gut microbiota composition through their antimicrobial activity and the activation of different signalling pathways, which can contribute for instance to mitigate heat stress, maintaining regulated hepatic lipogenesis and adiposity in chicken broilers and fish, including gilthead seabream (Domingo-Bréton *et al.*, 2025; Ruiz *et al.*, 2023). Altogether, results highlight a strong alteration of hepatic lipid and oxidative metabolism by RF exposure, as already reported for PS in a number of fish species, including gilthead seabream (Del Piano *et al.*, 2024), zebrafish (Du *et al.*, 2023) and large yellow croaker (*Larimichthys crocea*) (Lai *et al.*, 2021).

Exposure to increasing dietary levels of RFs had a negligible effect on growth performance, but the PLS-DA was able to discriminate two main groups: CTRL_RF1 and RF2_RF3, based on a discriminant gene expression pattern enriched on muscle development- and growth-related genes. In RF2_RF3, this gene expression pattern was first underlined by a downregulation of the muscle *mymk*, a highly conserved transmembrane protein that is required for myoblast fusion during myogenesis (Perelló-Amorós *et al.*, 2022). The critical role of *mymk* in muscle growth has been

demonstrated in zebrafish, where *mymk* knockout resulted in defective myoblast fusion, reduced growth, and increased adiposity infiltration (Shi *et al.*, 2018). The expression pattern observed in RF2_RF3 also revealed an upregulation of calcium-regulated non-lysosomal thiol-proteases (*capn1* and *capn3*), which catalyse the limited proteolysis involved in cytoskeletal remodelling and signal transduction (Preziosa *et al.*, 2013). Despite these transcriptomic changes, RF2_RF3 also showed increased expression of fibroblast growth factors (*fgf4* and *fgf6*) and myogenic cell differentiation factors (*myod1* and *myod2*), which are essential to myoblast proliferation and differentiation (Jin *et al.*, 2023). These findings suggest a compensatory mechanism in which the reinforced regulation of genes associated with muscle growth and differentiation mitigates the negative effects of RF exposure, resulting in similar growth performance across groups. Further studies are required to evaluate whether prolonged RF exposure could exceed the organism's adaptative capability potentially leading to adverse effects on fish health and growth.

The dietary RF exposure also altered the gene expression profile of the AI and HK, two important lymphoid and endocrine organs highly involved in the organization of immune and stress responses (Geven & Klaren, 2017). In the case of AI, the observed transcriptional changes were commanded by the upregulation of two well-known pro-inflammatory cytokines (*il1b* and *tnfα)* that play a key immunoregulatory role, promoting the migration of immune cells to the inflammation site (Zou & Secombes, 2016). Interestingly, such transcriptional pattern becomes especially evident in RF2 fish instead of RF3 fish (Table S5.3), which suggests some adaptive attenuation of the inflammatory response at the local site with the highest tested RF dose. Indeed, discriminant analysis failed to differentiate significantly CTRL_RF1 and RF2_RF3 fish groups due, at least in part, to a certain inactivation of the pro-inflammatory response in RF3 fish (Fig. 5.3). Otherwise, Montero *et al.* (2022) reported a downregulation of *il1b* and *tnfα* in the AI of European seabass exposed during 60 days to PP microplastics included in the diet at the 10 % (*w*/*w*); while a pro-inflammatory response mediated by these and other cytokines is a common response in mice (Jia *et al.*, 2023; Liu *et al.*, 2022b) and several fish models (Del Piano *et al.*, 2023b; Wang *et al.*, 2023; Zheng *et al.*, 2023) after microplastic exposure. Certainly, Kim *et al.* (2021a) and Kim *et al.* (2021b) have specifically reported signs of gut damage on water flea and brine shrimp after exposure to lyocell-rayon microfibres, respectively. In contraposition to the AI, the HK disclosed in our experimental model a downregulated expression pattern for a wide representation of immune-related genes (*e.g.*, *il20*, *csf1r, tnfα*, *cd33*, *igms, igmb*, *irf8*, *ccr9*), which led again to discriminate significantly the group of RF2_RF3 fish (Fig. 5.4). Such transcriptional signature can compromise a number of processes, including immune cell trafficking and activation as well as pathogen recognition processes. However, this opposite AI and HK trade-off is not new,

as it has been also reported in European seabass exposed for 21 days to PE microplastics (100 mg/kg diet) (Espinosa-Ruiz *et al.*, 2023). Therefore, it is tempting to suggest that, in our experimental model, the anti-inflammatory HK response at the systemic level represents an adaptive feature to maintain the local defence system at a relatively low activation state, preventing an excessive inflammatory response and the associated tissue damage. Such assumption is supported by the absence of significant differences in the humoral immune parameters across experimental groups. However, the correlation analyses highlighted some associations between humoral parameters and gene expression patterns, which would reflect the individual variability as a whole rather than any dietary mediated response (Table S5.7). In any case, to the best of our knowledge, the increased susceptibility to diseases in response to environmental pollutants, such as microplastics and other anthropogenic particles, remain understudied in both wild and farmed animals, which will likely become aggravated in a context of climate change (González-Fernández & Cuesta, 2022; Seeley *et al.*, 2023; Yang *et al.*, 2024).

In summary, the observed gene expression results seem to indicate several tissue-specific physiological adaptations in response to RF exposure depending on the metabolic/physiological capabilities of each organ and tissue. However, studying each tissue independently may overlook inter-tissue similarities and dependencies that might lead to an underestimation of the overall transcriptional outcomes (Erola *et al.*, 2020). Accordingly, a multi-tissue approach combining the results of the four analysed tissues represented a step beyond the group separation with 43 discriminant genes that served to differentiate the gene expression profile of RF3 fish from RF2 fish and from the CTRL_RF1 super-group. Indeed, the multi-tissue approach disclosed up to 14 new driving genes. Moreover, once re-ordered by loading values, a major contribution of liver and HK genes was disclosed separating CTRL_RF1 from the other two groups; while the RF2 and RF3 separation was mainly commanded by the differentially regulated expression of AI and WSM biomarkers. The precise physiological significance of this finding remains uncertain, but it must be noted that the pro-inflammatory response of AI in RF2 and RF3 was driven by cytokines that contribute differentially to a low/acute inflammatory condition. Certainly, *il1b* is associated to the start of the inflammatory response, and consequently its activation is very often ligated to a wide range of epithelial injuries (Sakai *et al.*, 2021; Soliman & Barreda, 2023). Conversely, *il20* belongs to the IL-10 family and its overexpression has been associated to a chronically low inflammatory condition thanks to a dual action that dictates a pro-inflammatory response (Wang *et al.*, 2022a), but also some positive effects on gut health promoting intestinal cell proliferation and mucosal healing (Chiriac *et al.*, 2024). Thus, it is likely to consider that *il1b* primes the first stages of the inflammatory response, whereas the upregulated expression of *il20* instead of *il1b* served to disclose in

RF3 fish an attenuated inflammatory response with the exposure to the RF3 diet. On the other hand, regarding WSM, the multi-tissue approach indicated an increased mitochondrial respiration uncoupling with the upregulated expression of muscle *ucp3*. Although few information is available on the modulation of muscle uncoupling proteins by RFs in fish, their upregulation has been observed in glycolytic and oxidative muscle tissues in response to other stressors, such as hypoxia and limited feed supply (Bermejo-Nogales *et al.*, 2014; Martos-Sitcha *et al.*, 2019). This enhanced regulation of gene expression in muscle was further evidenced herein by the simultaneous up- and downregulation of key negative and positive modulators of growth. This includes the upregulation of *mstn*, a negative regulator of growth that inhibits Akt/TORC1 signalling (McFarland et al., 2006), and *mafbx/atrogin 1*, which promotes protein degradation and muscle atrophy by targeting muscle proteins for proteasomal breakdown (Bodine & Baehr, 2014). At the same time, RF3 fish showed a downregulated expression of *igfbp5b* and *myod2,* which act as myogenic factors (positive regulators of muscle growth and development) in fish and other animal models (Pérez-Sánchez *et al.*, 2018; Tan & Du, 2002). Although no effects were observed on growth under the tested conditions, a multi-tissue approach proved to be a valuable tool for future ecotoxicology studies. It enabled the detection of RF-mediated effects, highlighting tissue-specific trends, which otherwise would remain subtle or diluted.

5.5. Conclusion

The growth performance of European seabass growing at high rates during the juvenile stage remains mostly unaffected at short-term by dietary RF exposure. However, consistent changes in the gene expression pattern were found in liver, white skeletal muscle, anterior intestine and head kidney following the exponential increase of the dietary inclusion of RFs from zero (CTRL) to approximately 0.1 g/kg (RF3). Such transcriptional profile, associated to the increase of the hepatosomatic index, served to differentiate two main groups corresponding to the extreme RF dietary concentrations. However, it is noteworthy that the discriminatory capacity was really improved by the use of a multi-tissue approach, which considered together all the analysed tissues. This holistic methodology allowed us to shape new discriminant genes, highlighting the different tissue trends that could potentially lead to significant health effects with longer exposures. These effects, which would otherwise remain barely visible and diluted when considering each tissue-specific response separately, become more evident through this comprehensive approach. Although requiring further validation through the analysis of molecular and histopathological markers, this integrated approach offers the possibility to more precisely survey the negative effects of a wide range of environmental pollutants in wild and farmed aquatic organisms, giving attention not only to microplastics but also to other anthropogenic particles such as cellulosic microfibres.

Chapter VI

General discussion

The present Thesis set out to investigate the occurrence and potential impacts of microplastic (MP) contamination in European seabass (*Dicentrarchus labrax*) farmed under different aquaculture production systems. The findings presented across the preceding chapters contribute to a growing body of literature highlighting aquaculture environments as both sources and recipients of MP pollution. Additionally, this work also provides new insights into the relationship between MP occurrence, and potential fish health effects and human exposure through consumption. The following section provides a general discussion of this Thesis' findings, highlighting their implications for aquaculture sustainability and food safety within the *One Health* framework.

6.1. Microplastic occurrence in water and feed

The first step to better understand the impacts of MP contamination in aquaculture environments was to evaluate the MP occurrence in water collected from the three different production systems. Our findings point to RAS as the analysed production system with the highest MP levels in water (37.20 ± 1.94 MP/L) in comparison to the cage and earthen-pond systems and the surrounding environment. Regarding water, this difference seems to be influenced by the relation between production systems and the surrounding environment. For instance, the water in both cage and earthen-pond systems is renewed more often, either passively or actively. The same stands for the MPs present in those systems. In RAS, the number of MPs recovered from the water of the fish tanks where fish were housed was higher than those from the inlet reservoir tank (9.5 ± 5.9 MP/L). This may be related to the low daily renewal of 5% of the total water volume recirculating within the system that contributed to MP accumulation. Recent findings by Huang *et al.* (2023), reported that intensive production systems equipped with filtration systems, such as RAS (1.7 MP/L) and glass aquariums (2.5 M/L), had lower MP levels in water in comparison to cement- (10.1 MP/L) and earthen-ponds (13.8 MP/L). The authors attributed these results to an effective filtering equipment as values were even lower than tap water. Other studies similarly reported differences between aquaculture production systems (Lv *et al.*, 2020; Song *et al.*, 2024). As several site-specific factors may influence MP contamination in aquaculture environments, further research is required to understand the MP dynamics and impacts in each aquaculture facilities under a broader perspective.

It should be noted that the findings presented in this Thesis require careful consideration. For instance, in the cage system, the MP levels considered from water of the farm in Turkey (4.5 ± 11.1 MP/L) were retrieved from data previously generated in the same geographic region, although within different hydrological contexts, and collect using different methods and periods (Adamopoulou *et al.*, 2021; Yozukmaz, 2021). Moreover, the different methodologies employed to collect water can influence MP levels found. For instance,

Yozukmaz (2021) reported approximately 18.2 MP/L in the surface water of the Izmir Bay after sampling it in glass flasks and later filtered this through micromembrane filters with 0.7 µm pore size. Contrarily, Adamopoulou *et al.* (2021) found 1.7 ± 1.8 MP/L after sampling open waters of the Aegean Sea using a manta trawl with a 330 µm mesh size. Collecting water in glass flasks may not adequately represent the overall environment due to the limited volumes sampled. Similarly, manta trawls are unsuitable for use in smaller spaces (*e.g.*, RAS tanks and earthen-ponds) but ineffective at capturing MPs smaller than the mesh size. Therefore, pump filtration is an alternative approach for collecting MPs in larger volumes of water and more comparable across aquaculture production systems.

The identification of MPs with a variety of characteristics (*i.e.*, shape, colour, size, polymer type) highlighted the vulnerability of aquaculture production systems to several endogenous and exogenous sources of MP pollution. Man-made cellulose/rayon particles with a fibrous shape were the most abundant type of particle across all production systems. This finding aligns with the growing awareness of the dominance of natural fibres, such as cellulosic (79.5%) and proteinaceous (12.3%) in surface oceanic waters, in relation to those of synthetic origin (8.6%) (Suaria *et al.*, 2020). Particularly in RAS, the detection of polymers on MPs corresponding to those of RAS components was limited, such as polyvinyl chloride (PVC, 0.9 ± 0.3 MP/L), whereas man-made cellulose/rayon particles were significantly more abundant (21.8 ± 1.5 MP/L). Interestingly, Huang *et al.* (2023) similarly reported that rayon microfibres were the most predominant MP type in the analysed RAS (60.87%) and due to the absence of RAS components made of this polymer, it was hypothesised that these may have arrived the facility through tap water. In any case, particularly in RAS, it is likely that the external environment highly contributed to the overall MP contamination of the analysed facility not only from the water inlet reservoir, but also through the deposition of suspended particles and fibre shedding from the clothing (*i.e.*, cellulose is commonly used in textiles) of workers during daily maintenance tasks. For that reason, the used of advanced filtration mechanisms are essential to reduce MP contamination levels. Nevertheless, further research is required to better understand MP dynamics in aquaculture environments, while considering the numerous site-specific factors that can influence MP contamination.

Considering these findings, one of the contributions of the Thesis is highlighting the importance of air quality in aquaculture environments, particularly indoors such as RAS. An approach to mitigate MP contamination by cellulosic microfibres would be to reduce the presence of contamination sources and, if possible, to implement strategies that minimise interactions between operators and the system. Additionally, another possible MP sources can come from air conditioning and ventilation equipment, which are known to be sinks and sources of airborne MPs (*i.e.*, mostly fibres) in indoor environments (Chen *et al.*, 2022d). Indeed, most RAS facilities rely on these types of equipment to control the thermal

environment through air-to-water heat transfer (Gehlert *et al.*, 2018). Following this, an additional and indirect approach would be the incorporation and periodic maintenance of appropriate filtration in air conditioning and ventilation equipment, which can capture and retain airborne MPs more efficiently. It should be noted that upgrading existing RAS facilities or designing new ones to employ such strategies can become complex and very costly, thus requiring further evaluation to more economically mitigate MP contamination.

Another significant contribution from the findings of the present Thesis concern to MPs contamination in aquafeeds. Indeed, MPs were detected in all of the three analysed aquafeed products with concentrations ranging from 1.6 ± 1.3 to 3.9 ± 1.4 MP/g. The levels registered align with those reported in available literature ranging from 0.03 to 11.6 MP/g (Devi *et al.*, 2024; Egea-Corbacho *et al.*, 2023; Jeyasanta *et al.*, 2024; Mohsen *et al.*, 2024a; Muhib & Rahman, 2023; Siddique *et al.*, 2023; Zhou *et al.*, 2024). This finding highlights the role of aquafeeds as important sources of MP contamination in aquaculture environments. This is particularly relevant in semi- and intensive aquacultures where the nutritional needs of aquatic organisms are partially or entirely sustained by aquafeeds. Moreover, recent studies have shown comparable levels of MP contamination in feeds for terrestrial animals, such as poultry (0.1-0.3 MP/g) and ruminants (8.4-39.3 MP/g) (Jeyasanta *et al.*, 2024; Patrucco *et al.*, 2024). Altogether the increasing body of literature regarding this topic highlights that MP contamination in feeds is a growing concern in the animal production industry, warranting further studies to evaluate MP levels in commercially available feeds to ultimately estimate exposure and potential effects on animal health.

The reported MP levels in European seabass aquafeeds are in line with those documented in fishmeal and other common ingredients currently used in aquafeed formulations, such as poultry by-products and plant-based products (Mohsen *et al.*, 2024b; Rimoldi *et al.*, 2024; Walkinshaw *et al.*, 2022). Due to its direct connection to the aquatic environment and role as main protein source in aquafeeds, fishmeal has received more attention from studies monitoring MP contamination, with reported levels matching those found in aquafeeds (Jeyasanta *et al.*, 2024). Nevertheless, the inclusion of fishmeal in aquafeeds currently corresponds to 5-25% and has been steadily decreasing due to the cost and sustainability concerns, promoting the reliance on plant-based ingredients and alternative protein sources (Glencross *et al.*, 2024). This critical nuance highlights a potential underestimated contribution of other ingredients to the overall MP levels observed in aquafeeds. For that reason, further studies should monitor MP contamination in a wider range of ingredients other than fishmeal, ultimately contributing to inform and select safe feed formulations. Also, it is recommended that such studies are conducted in an industrial context to account for the contribution of manufacturing processes to MP contamination, from the raw materials to the end-product.

Several challenges arose while isolating MPs from complex matrices such as aquafeeds due to the presence of ingredients from different sources (*i.e.*, mostly animal and vegetable). Although not detailed in previous chapters, MPs were extracted from European seabass tissues and feeds using different digestion methods, namely an alkaline (10% KOH) and oxidative (30% H_2O_2) digestions, respectively. Both approaches are used for extracting MPs from environmental samples, such as fish tissues and organic-rich sediments, while preserving the particle characteristics (GESAMP, 2019). Different methods were used since 10% KOH was inefficient for digesting aquafeeds. So far, it has been found that digesting lipid-rich organic matter with KOH is not very effective due to the production of a suspension of glycerol and fatty acids, also known as saponification. These products trap MPs and clog micromembrane filters, hindering the digestion and filtration efficiency, thus the recovery of particles (Dawson *et al.*, 2020). Likewise, it is also probable that the presence of other constituents, such as lignocellulose from plant-based ingredients, may require protocol adjustments to more efficiently recover MPs (Patrucco *et al.*, 2024). The extraction of MPs from aquafeeds was achieved with 30% H_2O_2 but still a higher number of micromembrane filters were required to be used per sample to avoid clogging and make it easier to facilitate the detection of MPs through stereomicroscopy. Recently, Ge *et al.* (2024) reported that 30% H_2O_2 was the most efficient MP extraction protocol evaluated without affecting their characteristics, but additional steps of density separation were required for better efficiency. As the number of studies monitoring MPs in aquafeeds increases, as well as the adoption of alternative ingredients in aquafeed formulations, it is highlighted the need for further optimising and standardising protocols for MP extraction.

6.2. Mitigation strategies in fish farms

As previously stated, aquaculture environments are vulnerable to MP contamination from system components or from external sources, such as air, water, and aquafeeds, thus requiring several mitigation strategies. Alongside those already provided in this Thesis, several recommendations have been provided by the Aquaculture Stewardship Council (ASC) for mitigating the contribution of this industrial sector to plastic pollution of aquatic environments, and consequently, in aquacultures (Huntington, 2019). Briefly, it is advised the use of plastic equipment in the appropriate physical and chemical environments for their characteristics and ensuring their overall lifetime by minimising the impact of weathering agents. Plastic equipment should be replaced within their expected lifetime, since aged equipment is more prone to failure and releases more MPs. For instance, Song *et al.* (2017) found that expanded polystyrene (EPS) produced significantly more MPs in relation to polyethylene (PE) and polypropylene (PP) when applied mechanical abrasion. The same study also found that UV exposure increases MP release after applying mechanical

abrasion due to the embrittlement at the surface of polymers. This occurs as the energy of UV radiation is enough to produce free radicals and dissociate C-C and C-H bonds from the polymer backbone (Feldman, 2002). Over time, the repetitive sequence of degradation, embrittlement, and fragmentation increases MP release, as reported by Napper *et al.* (2022) in older PP maritime ropes (≥2 years). Moreover, the ASC recommends the reduction of the use of single-use and low-recyclability plastics, as well as equipment made from mixed polymers or with coating treatments that complicate or impair their recycling process. It is also recommended the implementation of standard operation procedures which maximise the use of recyclable plastics or other-material alternatives, within the aquaculture and ensure the responsible fate of decommissioning plastic equipment.

Nevertheless, the available literature regarding the applicability of the mitigation strategies above mentioned remain limited under an aquaculture context. For instance, several studies have highlighted the adoption of Integrated Multi-Trophic Aquaculture systems since the co-culture of filter-feeders and seaweeds as bioremediatory agents can trap MPs and increase the overall water quality (Falkenberg *et al.*, 2024; Stabili *et al.*, 2024). Moreover, particularly in filtered systems such as RAS, Mnyoro *et al.* (2022) found that plastic biofilter media, which fragment and release MPs, could be replaced by local-sourced biofilter media, such as coconut shells, with similar performance in water quality maintenance. However, it is crucial to better understand the advantages and disadvantages of certain strategies for mitigating MP pollution. Comas *et al.* (2021) reported that copper-coated nets are a good alternative for polyurethane-coated nets, since the former performed better structurally and did not result in Cu bioaccumulation in the tissues of gilthead seabream farmed in fishponds in Spain. Contrarily, Canada *et al.* (2020) found that copper nets from cage aquacultures in Madeira Island in Portugal had a less diverse microbiome with higher abundance of *Tenacibaculum* genus, which is known to harbour pathogenic bacteria, in relation to nylon nets from aquacultures in the same region. In addition, similar to other industrial sectors, efforts are being made by aquacultures to substitute conventional plastic gear by lower-impact biopolymers despite the limited availability of data regarding their functionality and durability in aquatic environments (Arantzamendi *et al.*, 2023).Therefore, further studies are warranted since the implementation of certain mitigation strategies, such as alternative materials, can represent increased threats for the health of farmed fish

6.3 Microplastics in European seabass tissues and their characteristics

The presence of MPs in different tissues of European seabass was confirmed in fish from the three different production systems, in chapters II to IV. In chapter V, and although at lower levels, it was also documented the presence of viscose-rayon microfibres (RFs) in

the muscle of European seabass after 68 days exposed to 0.01 g/kg of feed (RF2) under experimental conditions, suggesting the successful translocation into internal tissues. From the three production systems, RAS-farmed seabass stood out as the most contaminated (1.01 ± 0.78 MP/g). Among fish tissues, the MP levels in GIT were consistently higher across production systems (2.3 MP/indv), which is in line with values reported for seabass (0.6-22 MP/indv) and other species relevant to the European context like gilthead seabream (0.8-40 MP/indv) (Kılıç, 2022; Miserli *et al.*, 2023; Mosconi *et al.*, 2023; Reinold *et al.*, 2021; Sánchez-Almeida *et al.*, 2022). Our findings highlight the role of the environment in determining the MP levels in seabass tissues.

The MP levels found in liver (0.8-1.4 MP/g) and dorsal muscle (0.18-0.36 MP/g) of European seabass were also similar to observations in gilthead seabream, and Asian seabass (*Lates calcarifer*) reared in cage and pond systems (Mosconi *et al.*, 2023; Vazirzadeh *et al.*, 2025). Likewise, our findings and both cited studies found that MPs recovered from internal tissues are less abundant and generally smaller than those from GIT and gills. Lower levels in internal tissues may be explained by the size-dependent MP translocation across tissues (Ding *et al.*, 2018; Vagner *et al.*, 2022; Zeytin *et al.*, 2020; Zhang *et al.*, 2019a). As aforementioned, it should be noted the limitations of the present thesis and both studies, Mosconi *et al.* (2023) and Vazirzadeh *et al.* (2025), which relied on stereomicroscopy for MP quantification which limits the detection of smaller MPs belonging to the <100 µm size class. Some of the factors influencing MP detection are the lighting and magnification power of the equipment, or the experience of the researcher in visually detecting particles suspect of having anthropogenic origin. Indeed, the anecdotal detection of MPs < 30 µm contrasts with the MP levels reported by Ferrante *et al.* (2022) in the dorsal muscle of farmed gilthead seabream (86,600 ± 24,300 MP/g measuring Ø 1.8-2.8 µm) through Scanning Electron Microscopy coupled with Energy Dispersive X-ray Spectroscopy (SEM-EDX). The order of magnitude's difference on MPs reported reinforces the need for new approaches to detect and characterise smaller particles, which are often missed by the methods generally adopted by MP monitoring studies. Altogether, this highlights that MP occurrence in internal tissues is still a significant gap of knowledge and supports the growing concern for the safety of farmed seafood products.

Regarding MP characteristics, those found in the analysed seabass tissues were similar to the ones recovered from water and feed samples. They were predominantly fibres, were mostly blue, black, and transparent, and were also made of man-made cellulose/rayon and polyethylene terephthalate (PET). These findings are in line with those commonly found to be ingested by fish (Sacco *et al.*, 2024). MPs made of semisynthetic and natural polymers, such as cellulosic fibres, have been reported to dominate aquatic environments, but are often underestimated. Many studies exclude cellulosic fibres as these can result

from potential sample contamination or it is assumed that they easily degrade in the environment (Athey & Erdle, 2022; Stanton *et al.*, 2024). It should be noted that aquaculture environments are susceptible to similar MP pollution sources to those often reported in other field observations worldwide. Beyond literature reports in several environmental compartments, the wide applications of certain polymers and their predominance may limit conclusions regarding potential pollution sources and consequently hinder the implementation of targeted measures for mitigating MP contamination. In any case, monitoring MP characteristics seems useful in aquaculture production systems, such as RAS and earthen-pond facilities, due to the close proximity to known MP sources.

6.4. Impact of microplastics in fish physiological traits

For RAS and pond-farmed seabass, no significant differences were found in the oxidative stress biomarkers between fish with lower and higher MP levels in tissues. The lack of differences suggests that MP levels in more contaminated fish were not high enough to alter oxidative stress status or that the effects of MP exposure were equally reflected in all fish of each production system. Indeed, fish oxidative stress modulation by MP exposure has been demonstrated not only experimentally (Das, 2023), but also in wild fish (Table 6.1). It is also possible that MP exposure led to an adaptive response in farmed fish. However, high MP levels seemed to have slight effects in the histomorphology of the rectum of pond-farmed seabass, with a significant higher number of acid goblet cells (GCs). This MP-induced increase of acid GCs has been experimentally reported in European seabass and it was associated with increased mucus production to protect the intestinal epithelia from foreign particles (Espinosa *et al.*, 2019; Pedà *et al.*, 2016). Recently, Vazirzadeh *et al.* (2025) found that cage-farmed Asian seabass (*Lates calcarifer*) with higher MP levels in tissues (*i.e.*, 51-100 and >100 MP/kg) showed higher hepatic expression of detoxification biomarkers and substantial changes in the serum biochemistry that indicated impaired hepatic functions and immune response. Another potential factor influencing the lack of significant relationships between MP levels and fish health biomarkers is that the latter were not enough to discriminate the established groups. Therefore, further studies with farmed fish are advised to incorporate multi-tissue approaches to better understand under a holistic perspective the potential relationship between MP occurrence in fish tissues and effects on overall fish health.

The placement of the exposure trial in the timeline of the Thesis was purposeful to ensure that the experiment was conducted under aquaculture-relevant conditions. This was achieved by incorporating the findings of the previous chapters in the experimental design, namely the most common particle type (*i.e.*, cellulosic microfibres) and the levels of MPs found in aquafeeds. The general overlook regarding cellulosic microfibres is associated with

the misconception that natural and semisynthetic polymers biodegrade rapidly and have reduced impacts on environment (Stanton *et al.*, 2024). Several modifications are often made to cellulose-based textile fibres, such as mercerisation or acetylation, which alters cellulose's polymeric structure and enhances their durability and persistence in the environment (Allison *et al.*, 2023; Belzagui *et al.*, 2021; Royer *et al.*, 2023). Nevertheless, the impacts of cellulosic microfibres in the health of organisms remains a significant gap of knowledge in aquatic pollution.

A dedicated exposure trial was performed to evaluate the impacts of dietary exposure to viscose-rayon microfibres (RFs) (*i.e.*, semisynthetic cellulose-based polymer) on the growth performance and transcriptomic traits of several tissues of European seabass. Based on the findings of chapter V, it was found that none of the experimental diets, including the one with the highest dose of RFs (RF3, 0.1 g/kg), affected on growth performance and feed efficiency. This is supported by the good zootechnical results achieved across the four dietary treatments (SGR, 2.40-2.45; FCR, 0.95-0.96), with fish exhibiting fast and efficient growth (*i.e.*, from 5.8 to 30.3-30.9 g in 68 days). However, a multi-target tissue approach – liver, white skeletal muscle, anterior intestine, and head kidney – revealed consistent changes in fish fed with the RF2 and RF3 diets, namely altered expression of genes related to energy and lipid metabolism, muscle growth and differentiation, and immune response. These transcriptomic changes raise concerns about potential long-term effects of dietary RF exposure on fish health, that ultimately may impact fish growth performance. Notably, RF levels in the RF2 diet (14.8 ± 3.0 RF/g) are close to the highest MP levels reported in aquafeeds, namely 11.3 MP/g (Muhib & Rahman, 2023). This emphasises the importance of monitoring MP levels in feeds for mitigating exposure. Moreover, the use of a Partial Least Squares-Discriminant Analysis (PLS-DA) and the integration of the transcriptomic responses of all analysed tissues combined enhanced the discriminatory capacity of the model to uncovered systemic changes that would otherwise remain undetected with single-tissue or univariate approaches. Furthermore, either under field or experimental conditions, future studies may benefit from building on similar approaches to evaluate the sublethal effects of extended exposure periods to a broader range of MPs, including natural and semisynthetic particles of anthropogenic origin.

Table 6.1. Example of studies reporting a relation between MP levels and alterations in biomarkers of fish health status in wild populations.

Species / Region	*n*	% fish with MPs	MP levels	Biomarker response	Reference
Boops boops (bogue)					
Balearic Islands, Mediterranean Sea	51	*n.s.*	**GIT,** 4.74 ± 1.04 MP/indv	**Liver** ↔ CAT, SOD, GST, LPO, MDA **Brain** ↑ CAT, SOD, GST / ↔ AChE, LPO, MDA	Capó *et al.* (2022)
Chrysichthys nigrodigitatus (silver catfish)					
Lagos lagoon, Nigeria	8	*n.s.*	**Liver,** ~7.5 ± 2.0 MP/indv	**Liver** ↓ *cyp1a* / ↔ *hsp70*	Akinhanmi *et al.* (2024)
Clarias garipenus (African catfish)					
Lagos lagoon, Nigeria	8	*n.s.*	**Liver,** ~8.5 ± 5.0 MP/indv	**Liver** ↓ *cyp1a* / ↔ *hsp70*	Akinhanmi *et al.* (2024)
Cyprinus carpio (common carp)					
Minho River estuary, Portugal	41	98	**GIT,** 6 ± 6 MP/indv, 0.3 ± 0.3 MP/g **Gills,** 0.6 ± 1.5 MP/indv, 0.6 ± 1.4 MP/g **Liver,** 0.2 ± 0.5 MP/indv, 0.5 ± 1.7 MP/g **Muscle,** 0.8 ± 1.6 MP/indv, 0.1 ± 0.2 MP/g	**Liver** ↑ GR / ↔ GST, GPx, CAT, LPO **Gills** ↑ CAT / ↔ GST, GR, GPx, LPO **Brain** ↔ AChE, LPO **Muscle** ↔ ChE, LPO	Martins *et al.* (2025)
Dicentrarchus labrax (European seabass)					
Douro River estuary, Portugal	180	5	**Brain,** 0.3 ± 0.8 MP/indv, 2 ± 6 MP/g	**Brain** ↓ AchE	Barboza *et al.* (2023)
NE Atlantic Ocean, Portuguese coast	50	42	**GIT,** 1.3 ± 2.5 MP/indv **Gills,** 0.8 ± 1.4 MP/indv **Muscle,** 0.04 ± 0.07 MP/g	**Gills** ↑ LPO **Brain** ↑ AChE, LPO **Muscle** ↑ LPO / ↔ ChE	Barboza *et al.* (2020b)
Engraulis encrasicolus (European anchovy)					
Balearic Islands, Mediterranean Sea	34	*n.s.*	**GIT,** 3.74 ± 0.41 MP/indv	**Liver** ↑ CAT, SOD, MDA / ↔ GST, LPO **Brain** ↑ SOD, LPO, MDA / ↔ CAT, GST, AChE	Capó *et al.* (2022)
Galeus melastomus (blackmouth catshark)					
Balearic Islands, Mediterranean Sea	16	82	**GIT,** 8.31 ± 2.46 MP/indv	**Gut** ↑ SOD, GSH / ↔ CAT, MDA	Torres *et al.* (2024)
Gymnarchus niloticus (African knifefish)					
Lagos lagoon, Nigeria	8	*n.s.*	**Liver,** ~9.5 ± 4.4 MP/indv	**Liver** ↓ *cyp1a* / ↔ *hsp70*	Akinhanmi *et al.* (2024)
Merluccius merluccius (European hake)					
Central Adriatic Sea	16	*n.s.*	**GIT,** 9.69 ± 4.09 MP/indv	**Gut** ↑ *il1b*, *il10*, *ifn*, *cat*, *sod* / ↔ *il8*	Cocci *et al.* (2022)
Mugil cephalus (mullet)					
Minho River estuary, Portugal	43	100	**GIT,** 9 ± 9 MP/indv, 0.3 ± 0.2 MP/g **Gills,** 0.4 ± 0.8 MP/indv, 0.3 ± 0.7 MP/g **Liver,** 0.4 ± 0.8 MP/indv, 0.4 ± 0.8 MP/g **Muscle,** 0.7 ± 1.0 MP/indv, 0.1 ± 0.2 MP/g	**Liver** ↑ GST, GR / ↔ GPx, CAT, LPO **Gills** ↑ GR, GPx / ↓ LPO / ↔ GST, CAT **Brain** ↑ LPO / ↓ AChE **Muscle** ↔ ChE, LPO	Martins *et al.* (2025)
Mullus barbatus (red mullet)					
Balearic Islands, Mediterranean Sea	16	*n.s.*	**GIT,** 8.62 ± 5.69 MP/indv	**Gut** ↑ *il1b*, *il8*, *il10*, *ifn*, *cat*, *sod*	Cocci *et al.* (2022)

Table 6.1. Continuation (part 1/2).

Species / Region	*n*	% fish with MPs	MP levels	Biomarker response	Reference
Mullus surmuletus (striped mullet)					
Balearic Islands, Mediterranean Sea	44	*n.s.*	**GIT,** 2.05 ± 0.36 MP/indv	**Liver** ↑ GST / ↔ CAT, SOD, LPO, MDA **Brain** ↑ CAT / ↔ SOD, GST, AChE, LPO, MDA	Capó *et al.* (2022)
Balearic Islands, Mediterranean Sea	417	27.3	**GIT,** 0.42 ± 0.04 MP/indv	**Liver** ↑ GST / ↔ CAT, SOD, MDA	Alomar *et al.* (2017)
Oreochromis niloticus (Nile tilapia)					
Lagos lagoon, Nigeria	8	*n.s.*	**Liver,** 7.2 ± 1.9 MP/indv	**Liver** ↓ *cyp1a* / ↔ *hsp70*	Akinhanmi *et al.* (2024)
Platichthys flesus (European flounder)					
Minho River estuary, Portugal	44	79	**GIT,** 1 ± 1 MP/indv, 0.2 ± 0.2 MP/g **Gills,** 0.5 ± 0.8 MP/indv, 0.8 ± 2.1 MP/g **Liver,** 0.4 ± 0.8 MP/indv, 1 ± 3 MP/g **Muscle,** 0.5 ± 0.9 MP/indv, 0.1 ± 0.2 MP/g	**Liver** ↑ GST / ↔ GR, GPx, CAT **Gills** ↔ GST, GR, GPx, CAT, LPO **Brain** ↔ AChE, LPO **Muscle** ↑ ChE / ↔ LPO	Martins *et al.* (2025)
Scomber colias (Atlantic chub mackerel)					
NE Atlantic Ocean, Portuguese coast	50	62	**GIT,** 1.2 ± 1.6 MP/indv **Gills,** 0.7 ± 1.0 MP/indv **Muscle,** 0.06 ± 0.08 MP/g	**Gills** ↑ LPO **Brain** ↑ AChE, LPO **Muscle** ↑ LPO / ↔ ChE	Barboza *et al.* (2020b)
Seriola dumerili (greater amberjack)					
Balearic Islands, Mediterranean Sea	52	98	**GIT,** 12.2 ± 1.3 MP/indv (± SEM)	**Liver** ↑ SOD, CAT, GST / ↔ MDA, EROD	Solomando *et al.* (2022)
Serranus scriba (painted comber)					
Strait of Sicily, Italy	120	22-43	**Liver,** 8.88 ± 1.03 MP/g	**Liver** ↑ CAT, GST, MDA / ↔ AChE	Zitouni *et al.* (2022)
Scyliorhinus canicula (small-spotted catshark)					
Balearic Islands, Mediterranean Sea	16	63	**GIT,** 4.38 ± 1.77 MP/indv	**Gut** ↑ SOD, GSH / ↔ CAT, MDA	Torres *et al.* (2024)
Trachurus trachurus (Atlantic horse mackerel)					
NE Atlantic Ocean, Portuguese coast	50	62	**GIT,** 1.0 ± 1.9 MP/indv **Gills,** 0.7 ± 1.4 MP/indv **Muscle,** 0.07 ± 0.13 MP/g	**Gills** ↑ LPO **Brain** ↑ AChE, LPO **Muscle** ↑ LPO / ↔ ChE	Barboza *et al.* (2020b)

AChE – acetylcholinesterase; CAT, *cat* – catalase; ChE – cholinesterase; *cyp1a* – cytochrome P450 1a; EROD – ethoxyresorufin-O-deethylase; GSH – reduced glutathione; GST – glutathione S-transferase; *hsp70* – heat shock protein 70; *ifn* – interferon; *il1b* – interleukin-1β; *il8* – interleukin-8; *il10* – interleukin-10; LPO – lipid peroxidation, MDA – malondialdehyde, SOD – superoxide dismutase

Previous studies have reported gut damage and behaviour alterations in aquatic invertebrates and small fish species exposed to cotton and lyocell-rayon microfibres (Kim *et al.*, 2021a; 2021b; Siddiqui *et al.*, 2023). Particularly in aquaculture-relevant fish species, the evaluation of the impacts of cellulose-based MPs were virtually absent from a total of 155 ecotoxicological studies collected (Table S6.1). In fact, most of these studies evaluated the health effects of synthetic polymers, such as PS (44.7%), PE (11.2%), low-density polyethylene (LDPE; 8.8%), PVC (5.9%), and PP (5.3%). On a broader look, most studies also evaluated the impacts of spherical and irregular-shaped MPs (47.6 and 39.0%, respectively). Altogether, current literature indicates that ecotoxicological studies with fish, although necessary, have disproportionally focused on the effects of MPs within a narrow set of characteristics, overlooking the environmental-relevance of cellulosic microfibres in MP pollution research (Rebelein *et al.*, 2021).

6.5. Risks for human consumption

Considering the MP levels found in dorsal muscle of farmed European seabass (0.18-0.36 MP/g), it was estimated that human consumers would be exposed on average to 27-54 MPs from a 150 g fillet. Such MP levels translate into an exposure ranging from 0.38-2.23 MP/kg of body weight for adults (70 kg bw) and toddlers (12 kg bw). It should be noted that these estimates can vary regionally and nationally depending on the averaged seafood consumption per capita, as detailed for the United Kingdom (20.13 kg/capita/year) and Portugal (59.91 kg/capita/year). Additionally, the levels of trace (Cu, Cr, Ni, Zn) and non-essential (Cd, Hg, Pb) metals quantified in the dorsal muscle of farmed European seabass were inferior to the maximum allowable concentrations for this species. No differences in risks for human consumption were found among aquaculture production systems concerning metals, and no relation was found between metal and MP levels. Due to gaps of knowledge on MP research that hinder proper risk assessment, the toxicological risk associated to the consumption of farmed seabass fillet consumption was not possible to determine since no health-based guidance values (HBGVs) are currently available for this type of contaminants (EFSA, 2020a). But regarding metals, it was concluded that farmed seabass represented a low toxicological risk considering the estimated data of seabass consumption per capita and available HBGVs provided by the European Food Safety Authority for trace (EFSA, 2006, 2014, 2015, 2020b) and non-essential metals (EFSA, 2010, 2011, 2012c).

MPs have been found in several food items (Garrido Gamarro & Constanzo, 2022) with seafood being the most studied. Notably, seafood has been identified among the top three sources of human MP ingestion (Cox *et al.*, 2019). For that reason, further studies

monitoring MP levels in food products available in markets are warranted in order to better determine the overall human MP ingestion on a daily basis, compare MP contamination between seafood and other food items, and evaluate the impact of food origin (*i.e.*, wild *vs.* farmed seafood products) on MP exposure. It is also important to consider that human diet comprises a wider range of seafood products, including those more processed such as canned fish, which may significantly contribute to the overall MP ingestion. Moreover, although the present Thesis may indicate that farmed seabass is not a significant vector of metals to humans, it should be reinforced the need for monitoring potential synergisms between MPs and metals that can lead to higher levels in wild and farmed seafood.

Chapter VII

Concluding remarks and future perspectives

So far, numerous studies have extensively documented the widespread presence of microplastics (MPs) in aquatic environments. Nevertheless, the occurrence and impacts of MPs in aquacultures represented significant gaps of knowledge at the beginning of this Thesis. Indeed, aquaculture environments are not only exposed to various MP sources, such as aquafeed or plastic gear, but are also susceptible to MP contamination from the surrounding environment. These particles interact with farmed organisms and can be retained in internal organs and negatively affect fish health. Ultimately, MP occurrence in aquacultures may compromise the quality and safety of seafood products. Considering this, the aim of the present Thesis was to determine the MP levels in water, fish feed, and tissues of European seabass (*Dicentrarchus labrax*) produced in different aquaculture production systems, while evaluating the potential relation between MP levels and fish health status. Additionally, trace and non-essential metals were quantified in the muscle of farmed seabass muscle and the potential exposure and risk to human consumers was estimated through consumption data.

The main conclusions of this Thesis are:

1. MPs were detected in the water, aquafeed, and European seabass (*Dicentrarchus labrax*) specimens obtained from cage, earthen-pond, and RAS facilities. Among the production systems investigated, RAS was the most contaminated with MPs, with cellulosic microfibres being the predominant type of particles in all systems. Findings indicate that aquaculture systems are both a source and a recipient of MP contamination.
2. Under field conditions, there were no significant relationships between the occurrence of MPs in seabass tissues and the selected oxidative stress biomarkers. In pond-farmed seabass, fish with higher MP levels (≥4 MP/g) showed a higher number of acid goblet cells in the rectum epithelia suggesting an increased protection through mucus production.
3. In the three production systems, the levels of both trace and non-essential metals analysed in fish dorsal muscle were below the maximum allowable concentrations. No significant relationships between MP and metal concentrations were observed. Estimates of human exposure to MPs through the consumption of fillet from farmed seabass range between 27-54 MPs in a 150 g fillet, although no health-based guideline values are currently available for MPs.
4. For 68 days, dietary exposure to viscose-rayon microfibres (RFs) at concentrations that occur in aquafeeds did not affect growth performance and feed efficiency. However, RF exposure induced transcriptional changes related with energy and lipid metabolism, muscle growth and differentiation, and immune signalling. Most of the changes could

only be detected through a multiple tissue approach. At longer-term, several of the observed alterations may negatively affect fish growth, health and welfare.

In the continuation of the studies conducted within the scope of the present Thesis, further investigation is needed to improve our understanding and manage the risks inherent to the environmental contamination by MPs in aquacultures. For instance, the importance of addressing several methodological challenges faced while quantifying MPs in water and aquafeeds, and to standardise them. Monitoring MP contamination in ingredients and throughout the aquafeed production is also crucial to reduce this potential MP source to aquacultures. Another priority topic is the determining potential relationships between MP occurrence and physiological responses in farmed fish throughout their whole production life cycle. Particularly in hatcheries, MPs represent an increased threat due to more approximate dimensions of particles to the gastrointestinal tract of larvae and juveniles, potentially leading to malnutrition and increased mortality rates (Campos *et al.*, 2021). Moreover, further research is required to better understand the toxicological interactions between MPs and other environmental contaminants under an aquaculture context. Cage and earthen-pond aquacultures which are often located in coastal or estuarine areas are also likely exposed to a high number of emerging contaminants, not only chemical additives present in plastics, but also other compounds related to anthropogenic activity, such as drugs and pesticides. On the other hand, the high reliance of RAS on plastic components raises the concern of potential leakage of certain chemical additives, such as phthalate esters from PVC tanks and pipes. Dedicated research is required to monitor the physical state of aquaculture gear and find safer alternatives. Also, more data should be generated in aquaculture environments to determine if MP occurrence is compromising fish health and consequently the safety and quality of farmed seafood products. In addition to that, the detection of the effects of MPs and other environmental pollutants, particularly in subacute exposure conditions, may benefit from an integrated approach which evaluates the potential cascade of effects on several tissues.

Finally, based on the findings of the present thesis, it is evident that understanding MP contamination in aquaculture environments requires an integrated and multidisciplinary approach – one that the *'One Health'* perspective is capable of providing. Indeed, the recognition of the intricate connections between environmental integrity, animal and human heath enabled a holistic perspective on the issue and it is strongly recommended for future research. This perspective not only constitutes a valuable tool to address the effects of many environmental stressors (*e.g.*, MPs and other pollutants, climate change) in biological systems, but also to inform more effective monitoring and mitigation strategies.

References

Abarghouei, S., Hedayati, A., Raeisi, M., Hadavand, B.S., Rezaei, H., Abed-Elmdoust, A., 2021. Size-dependent effects of microplastic on uptake, immune system, related gene expression and histopathology of goldfish (*Carassius auratus*). *Chemosphere* 276, 129977. https://doi.org/10.1016/j.chemosphere.2021.129977

Abbasi, S., Soltani, N., Keshavarzi, B., Moore, F., Turner, A., Hassanaghaei, M., 2018. Microplastics in different tissues of fish and prawn from the Musa Estuary, Persian Gulf. *Chemosphere* 205, 80-87. https://doi.org/10.1016/j.chemosphere.2018.04.076

Abihssira-García, I.S., Park, Y., Kiron, V., Olsvik, P.A., 2020. Fluorescent microplastic uptake by immune cells of Atlantic salmon (*Salmo salar* L.). *Front. Environ. Sci.* 8. https://doi.org/10.3389/fenvs.2020.560206

Adamopoulou, A., Zeri, C., Garaventa, F., Gambardella, C., Ioakeimidis, C., Pitta, E., 2021. Distribution patterns of floating microplastics in open and coastal waters of the Eastern Mediterranean Sea (Ionian, Aegean, and Levantine Seas). *Front. Mar. Sci.* 8. https://doi.org/10.3389/fmars.2021.699000

Ahmadi, A., Moore, F., Keshavarzi, B., Soltani, N., Sorooshian, A., 2022. Potentially toxic elements and microplastics in muscle tissues of different marine species from the Persian Gulf: Levels, associated risks, and trophic transfer. *Mar. Pollut. Bull.* 175, 113283. https://doi.org/10.1016/j.marpolbul.2021.113283

Ahmadifar, E., Kalhor, N., Dawood, M.A.O., Ahmadifar, M., Moghadam, M.S., Abarghouei, S., Hedayati, A., 2021. Effects of polystyrene microparticles on inflammation, antioxidant enzyme activities, and related gene expression in Nile tilapia (*Oreochromis niloticus*). *Environ. Sci. Pollut. Res.* 28(12), 14909-14916. https://doi.org/10.1007/s11356-020-11731-x

Aiguo, Z., Di, S., Chong, W., Yuliang, C., Shaolin, X., Peiqin, L., Guohuan, X., Huijuan, T., Jixing, Z., 2022. Characteristics and differences of microplastics ingestion for farmed fish with different water depths, feeding habits and diets. *J. Environ. Chem. Eng.* 10(2), 107189. https://doi.org/10.1016/j.jece.2022.107189

Ajith, N., Arumugam, S., Parthasarathy, S., Manupoori, S., Janakiraman, S., 2020. Global distribution of microplastics and its impact on marine environment - a review. *Environ. Sci. Pollut. Res.* 27(21), 25970-25986. https://doi.org/10.1007/s11356-020-09015-5

Akhbarizadeh, R., Dobaradaran, S., Nabipour, I., Tajbakhsh, S., Darabi, A.H., Spitz, J., 2020. Abundance, composition, and potential intake of microplastics in canned fish. *Mar. Pollut. Bull.* 160, 111633. https://doi.org/10.1016/j.marpolbul.2020.111633

Akhbarizadeh, R., Moore, F., Keshavarzi, B., 2018. Investigating a probable relationship between microplastics and potentially toxic elements in fish muscles from northeast of Persian Gulf. *Environ. Pollut.* 232, 154-163. https://doi.org/10.1016/j.envpol.2017.09.028

Akhbarizadeh, R., Moore, F., Keshavarzi, B., 2019. Investigating microplastics bioaccumulation and biomagnification in seafood from the Persian Gulf: a threat to human health? *Food Addit. Contam. Part A* 36(11), 1696-1708. https://doi.org/10.1080/19440049.2019.1649473

Akinhanmi, F.O., Ayanda, O.I., Dedeke, G.A., 2024. Assessment of microplastic-contaminated liver through gene expression profiling of four commercial fish species in the Lagos Lagoon, Nigeria. *Sci. Afr.* 24, e02242. https://doi.org/10.1016/j.sciaf.2024.e02242

Akoueson, F., Sheldon, L.M., Danopoulos, E., Morris, S., Hotten, J., Chapman, E., Li, J., Rotchell, J.M., 2020. A preliminary analysis of microplastics in edible versus non-edible tissues from seafood samples. *Environ. Pollut.* 263(Pt A), 114452. https://doi.org/10.1016/j.envpol.2020.114452

Alasalvar, C., Taylor, K.D.A., Zubcov, E., Shahidi, F., Alexis, M., 2002. Differentiation of cultured and wild sea bass (*Dicentrarchus labrax*): total lipid content, fatty acid and trace mineral composition. *Food Chem.* 79(2), 145-150. https://doi.org/10.1016/S0308-8146(02)00122-X

Ali, M.H., Huang, Y.-p., Johnson, D., Tu, Z.-y., Yuan, X., 2024. Effects of polystyrene microspheres on the swimming behavior and metabolism of grass carp (*Ctenopharyngodon idella*). *Aquat. Toxicol.* 273, 107009. https://doi.org/10.1016/j.aquatox.2024.107009

Allison, T., Ward, B.D., Harbottle, M., Durance, I., 2023. Do flushed biodegradable wet wipes really degrade? *Sci. Total Environ.* 894, 164912. https://doi.org/10.1016/j.scitotenv.2023.164912

Alomar, C., Compa, M., Sanz-Martín, M., Fagiano, V., Álvarez, E., Valencia, J.M., Deudero, S., 2022. A holistic approach to plastic pollution in integrated multi-trophic aquaculture facilities: Plastic ingestion in *Sparus aurata* and *Mytilus galloprovincialis*. *Aquaculture* 561, 738666. https://doi.org/10.1016/j.aquaculture.2022.738666

Alomar, C., Sureda, A., Capó, X., Guijarro, B., Tejada, S., Deudero, S., 2017. Microplastic ingestion by *Mullus surmuletus* Linnaeus, 1758 fish and its potential for causing oxidative stress. *Environ. Res.* 159, 135-142. https://doi.org/10.1016/j.envres.2017.07.043

Amato-Lourenço, L.F., Dantas, K.C., Júnior, G.R., Paes, V.R., Ando, R.A., de Oliveira Freitas, R., da Costa, O.M.M.M., Rabelo, R.S., Soares Bispo, K.C., Carvalho-Oliveira, R., Mauad, T., 2024. Microplastics in the olfactory bulb of the human brain. *JAMA Netw. Open* 7(9), e2440018-e2440018. https://doi.org/10.1001/jamanetworkopen.2024.40018

Amelinda, C., Werorilangi, S., Burhanuddin, A.I., Tahir, A., 2021. Occurrence of microplastic particles in milkfish (*Chanos chanos*) from brackishwater ponds in Bonto Manai village, Pangkep Regency, South Sulawesi, Indonesia. *IOP Conf. Ser.: Earth Environ. Sci.* 763(1), 012058. https://doi.org/10.1088/1755-1315/763/1/012058

Ammar, E., Mohamed, M.S., Sayed, A.E.-D.H., 2022. Polyethylene microplastics increases the tissue damage caused by 4-nonylphenol in the common carp (*Cyprinus carpio*) juvenile. *Front. Mar. Sci.* 9. https://doi.org/10.3389/fmars.2022.1041003

Andrady, A.L., Neal, M.A., 2009. Applications and societal benefits of plastics. *Philos. Trans. R. Soc. B: Biol. Sci.* 364(1526), 1977-1984. https://doi.org/10.1098/rstb.2008.0304

Anjeli, U.G., Sartimbul, A., Sulistiyati, T.D., Yona, D., Iranawati, F., Seftiyawan, F.O., Aliviyanti, D., Lauro, F.M., Matallana-Surget, S., Fanda, A.M., Winata, V.A., 2024. Microplastics contamination in aquaculture-rich regions: A case study in Gresik, East Java, Indonesia. *Sci. Total Environ.* 927, 171992. https://doi.org/10.1016/j.scitotenv.2024.171992

Antunes, J., Frias, J., Sobral, P., 2018. Microplastics on the Portuguese coast. *Mar. Pollut. Bull.* 131, 294-302. https://doi.org/10.1016/j.marpolbul.2018.04.025

Antunes, J.C., Frias, J.G.L., Micaelo, A.C., Sobral, P., 2013. Resin pellets from beaches of the Portuguese coast and adsorbed persistent organic pollutants. *Estuar. Coast. Shelf Sci.* 130, 62-69. https://doi.org/10.1016/j.ecss.2013.06.016

AOAC, 2006. Official methods of analysis of AOAC International, 18th ed. AOAC International, Gaithersburg, Maryland.

Arantzamendi, L., Andrés, M., Basurko, O.C., Suárez, M.J., 2023. Circular and lower impact mussel and seaweed aquaculture by a shift towards bio-based ropes. *Rev. Aquac.* 15(3), 1010-1019. https://doi.org/10.1111/raq.12816

Ašmonaitė, G., Larsson, K., Undeland, I., Sturve, J., Almroth, B.C., 2018a. Size matters: Ingestion of relatively large microplastics contaminated with environmental pollutants posed little risk for fish health and fillet quality. *Environ. Sci. Technol.* 52(24), 14381-14391. https://doi.org/10.1021/acs.est.8b04849

Ašmonaitė, G., Sundh, H., Asker, N., Almroth, B.C., 2018b. Rainbow trout maintain intestinal transport and barrier functions following exposure to polystyrene microplastics. *Environ. Sci. Technol.* 52(24), 14392-14401. https://doi.org/10.1021/acs.est.8b04848

Atamanalp, M., Köktürk, M., Uçar, A., Duyar, H.A., Özdemir, S., Parlak, V., Esenbuğa, N., Alak, G., 2021. Microplastics in tissues (brain, gill, muscle and gastrointestinal) of *Mullus barbatus* and *Alosa immaculata*. *Arch. Environ. Contam. Toxicol.* 81(3), 460-469. https://doi.org/10.1007/s00244-021-00885-5

Athey, S.N., Erdle, L.M., 2022. Are we underestimating anthropogenic microfiber pollution? A critical review of occurrence, methods, and reporting. *Environ. Toxicol. Chem.* 41(4), 822-837. https://doi.org/10.1002/etc.5173

Austin, H.M., Stoops-Glas, P.M., 1977. The distribution of polystyrene spheres and nibs in Block Island Sound during 1972–1973. *Chesap. Sci.* 18(1), 89-92. https://doi.org/10.2307/1350372

Baekeland, L.H., 1909. The synthesis, constitution, and uses of Bakelite. *Ind. Eng. Chem.* 1, 149-161.

Bai, L., Li, H., 2019. Innate immune regulatory networks in hepatic lipid metabolism. *J. Mol. Med.* 97(5), 593-604. https://doi.org/10.1007/s00109-019-01765-1

Balazs, G.H., 1985. Impact of ocean debris on marine turtles: entanglement and ingestion, Proceedings of the Workshop on the Fate and Impact of Marine Debris. NOAA Technical Memorandum, NMFS, SWFC54, Honolulu, Hawaii, pp. 387-429.

Banaee, M., Multisanti, C.R., Impellitteri, F., Piccione, G., Faggio, C., 2024. Environmental toxicology of microplastic particles on fish: A review. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, 110042. https://doi.org/10.1016/j.cbpc.2024.110042

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*). *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology* 261, 109423. https://doi.org/10.1016/j.cbpc.2022.109423

Banihashemi, E.A., Soltanian, S., Gholamhosseini, A., Banaee, M., 2022. Effect of microplastics on *Yersinia ruckeri* infection in rainbow trout (*Oncorhynchus mykiss*). *Environ. Sci. Pollut. Res.* 29(8), 11939-11950. https://doi.org/10.1007/s11356-021-16517-3

Barbarossa, A., Gazzotti, T., Farabegoli, F., Mancini, F.R., Zironi, E., Badiani, A., Busani, L., Pagliuca, G., 2016. Comparison of perfluoroalkyl substances contamination in farmed and wild-caught European sea bass (*Dicentrarchus labrax*). *Food Control* 63, 224-229. https://doi.org/https://doi.org/10.1016/j.foodcont.2015.12.011

Barboza, L.G.A., Cunha, S.C., Monteiro, C., Fernandes, J.O., Guilhermino, L., 2020a. Bisphenol A and its analogs in muscle and liver of fish from the North East Atlantic Ocean in relation to microplastic contamination. Exposure and risk to human consumers. *J. Hazard. Mater.* 393, 122419. https://doi.org/10.1016/j.jhazmat.2020.122419

Barboza, L.G.A., Dick Vethaak, A., Lavorante, B.R.B.O., Lundebye, A.-K., Guilhermino, L., 2018a. Marine microplastic debris: An emerging issue for food security, food safety and human health. *Mar. Pollut. Bull.* 133, 336-348. https://doi.org/10.1016/j.marpolbul.2018.05.047

Barboza, L.G.A., Lopes, C., Oliveira, P., Bessa, F., Otero, V., Henriques, B., Raimundo, J., Caetano, M., Vale, C., Guilhermino, L., 2020b. 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. https://doi.org/10.1016/j.scitotenv.2019.134625

Barboza, L.G.A., Otero, X.L., Fernández, E.V., Vieira, L.R., Fernandes, J.O., Cunha, S.C., Guilhermino, L., 2023. Are microplastics contributing to pollution-induced neurotoxicity? A pilot study with wild fish in a real scenario. *Heliyon* 9(1), e13070. https://doi.org/10.1016/j.heliyon.2023.e13070

Barboza, L.G.A., Vieira, L.R., Branco, V., Carvalho, C., Guilhermino, L., 2018b. Microplastics increase mercury bioconcentration in gills and bioaccumulation in the liver, and cause oxidative stress and damage in *Dicentrarchus labrax* juveniles. *Sci. Rep.* 8(1), 15655. https://doi.org/10.1038/s41598-018-34125-z

Barboza, L.G.A., Vieira, L.R., Branco, V., Figueiredo, N., Carvalho, F., Carvalho, C., Guilhermino, L., 2018c. Microplastics cause neurotoxicity, oxidative damage and energy-related changes and interact with the bioaccumulation of mercury in the European seabass, *Dicentrarchus labrax* (Linnaeus, 1758). *Aquat. Toxicol.* 195, 49-57. https://doi.org/10.1016/j.aquatox.2017.12.008

Barboza, L.G.A., Vieira, L.R., Guilhermino, L., 2018d. Single and combined effects of microplastics and mercury on juveniles of the European seabass (*Dicentrarchus labrax*): Changes in behavioural responses and reduction of swimming velocity and resistance time. *Environ. Pollut.* 236, 1014-1019. https://doi.org/10.1016/j.envpol.2017.12.082

Barceló, D., Picó, Y., Alfarhan, A.H., 2023. Microplastics: Detection in human samples, cell line studies, and health impacts. *Environ. Toxicol. Pharmacol.* 101, 104204. https://doi.org/10.1016/j.etap.2023.104204

Barnes, D.K.A., Galgani, F., Thompson, R.C., Barlaz, M., 2009. Accumulation and fragmentation of plastic debris in global environments. *Philos. Trans. R. Soc. B: Biol. Sci.* 364(1526), 1985-1998. https://doi.org/10.1098/rstb.2008.0205

Barrows, A.P.W., Cathey, S.E., Petersen, C.W., 2018. Marine environment microfiber contamination: Global patterns and the diversity of microparticle origins. *Environ. Pollut.* 237, 275-284. https://doi.org/10.1016/j.envpol.2018.02.062

Barrows, A.P.W., Neumann, C.A., Berger, M.L., Shaw, S.D., 2017. Grab *vs.* neuston tow net: a microplastic sampling performance comparison and possible advances in the field. *Anal. Methods* 9(9), 1446-1453. https://doi.org/10.1039/C6AY02387H

Basto, A., Matos, E., Valente, L.M.P., 2020. Nutritional value of different insect larvae meals as protein sources for European sea bass (*Dicentrarchus labrax*) juveniles. *Aquaculture* 521, 735085. https://doi.org/10.1016/j.aquaculture.2020.735085

Bat, L., Şahin, F., Bhuyan, M.S., Arici, E., Öztekin, A., 2022. Metals in wild and cultured *Dicentrarchus labrax* (Linnaeus, 1758) from fish markets in Sinop: Consumer's health risk assessment. *Biol. Trace Elem. Res.* 200(11), 4846-4854. https://doi.org/10.1007/s12011-021-03064-8

Batel, A., Borchert, F., Reinwald, H., Erdinger, L., Braunbeck, T., 2018. Microplastic accumulation patterns and transfer of benzo[a]pyrene to adult zebrafish (*Danio rerio*) gills and zebrafish embryos. *Environ. Pollut.* 235, 918-930. https://doi.org/10.1016/j.envpol.2018.01.028

Belton, B., Little, D.C., Zhang, W., Edwards, P., Skladany, M., Thilsted, S.H., 2020. Farming fish in the sea will not nourish the world. *Nat. Commun.* 11(1), 5804. https://doi.org/10.1038/s41467-020-19679-9

Belzagui, F., Buscio, V., Gutiérrez-Bouzán, C., Vilaseca, M., 2021. Cigarette butts as a microfiber source with a microplastic level of concern. *Sci. Total Environ.* 762, 144165. https://doi.org/10.1016/j.scitotenv.2020.144165

Belzagui, F., Gutiérrez-Bouzán, C., Álvarez-Sánchez, A., Vilaseca, M., 2020. Textile microfibers reaching aquatic environments: A new estimation approach. *Environ. Pollut.* 265, 114889. https://doi.org/10.1016/j.envpol.2020.114889

Béné, C., Arthur, R., Norbury, H., Allison, E.H., Beveridge, M., Bush, S., Campling, L., Leschen, W., Little, D., Squires, D., Thilsted, S.H., Troell, M., Williams, M., 2016. Contribution of fisheries and aquaculture to food security and poverty reduction: Assessing the current evidence. *World Dev.* 79, 177-196. https://doi.org/10.1016/j.worlddev.2015.11.007

Bergamini, C.M., Gambetti, S., Dondi, A., Cervellati, C., 2004. Oxygen, reactive oxygen species and tissue damage. *Curr. Pharm. Des.* 10(14), 1611-1626. https://doi.org/10.2174/1381612043384664

Bergmann, M., Collard, F., Fabres, J., Gabrielsen, G.W., Provencher, J.F., Rochman, C.M., van Sebille, E., Tekman, M.B., 2022. Plastic pollution in the Arctic. *Nat. Rev. Earth Environ.* 3(5), 323-337. https://doi.org/10.1038/s43017-022-00279-8

Bermejo-Nogales, A., Calduch-Giner, J.A., Pérez-Sánchez, J., 2010. Gene expression survey of mitochondrial uncoupling proteins (UCP1/UCP3) in gilthead sea bream (*Sparus aurata* L.). *J. Comp. Physiol. B* 180(5), 685-694. https://doi.org/10.1007/s00360-009-0441-6

Bermejo-Nogales, A., Calduch-Giner, J.A., Pérez-Sánchez, J., 2014. Tissue-specific gene expression and functional regulation of uncoupling protein 2 (UCP2) by hypoxia and nutrient availability in gilthead sea bream (*Sparus aurata*): implications on the physiological significance of UCP1–3 variants. *Fish Physiol. Biochem.* 40(3), 751-762. https://doi.org/10.1007/s10695-013-9882-7

Bermúdez, M., Vilas, C., Quintana, R., González-Fernández, D., Cózar, A., Díez-Minguito, M., 2021. Unravelling spatio-temporal patterns of suspended microplastic concentration in the Natura 2000 Guadalquivir estuary (SW Spain): Observations and model simulations. *Mar. Pollut. Bull.* 170, 112622. https://doi.org/10.1016/j.marpolbul.2021.112622

Bessa, F., Barría, P., Neto, J.M., Frias, J.P.G.L., Otero, V., Sobral, P., Marques, J.C., 2018. Occurrence of microplastics in commercial fish from a natural estuarine environment. *Mar. Pollut. Bull.* 128, 575-584. https://doi.org/10.1016/j.marpolbul.2018.01.044

Bessa, F., Frias, J., Kögel, T., Lusher, A., Andrade, J.M., Antunes, J., Sobral, P., Pagter, E., Nash, R., O'Connor, I., Pedrotti, M.L., Kerros, M.E., León, V., Tirelli, V., Suaria, G., Lopes, C., Raimundo, J., Caetano, M., Gago, J., Viñas, L., Carretero, O., Magnusson, K., Granberg, M., Dris, R., Fischer, M., Scholz-Böttcher, B., Muniategui, S., Grueiro, G., Fernández, V., Palazzo, L., de Lucia, A., Camedda, A., Avio, C.G., Gorbi, S., Pittura, L., Regoli, F., Gerdts, G., 2019. Harmonized protocol for monitoring microplastics in biota, Deliverable D4.3. JPI-Oceans BASEMAN Project. p. 28.

Bhuyan, M.S., 2022. Effects of microplastics on fish and in human health. *Front. Environ. Sci.* 10. https://doi.org/10.3389/fenvs.2022.827289

Blonç, M., Husson, F., Llorca, M., Farré, M.L., Tort, L., Brandts, I., Teles, M., 2023. Occurrence of micro- nanoplastics in a commercial recirculated aquaculture system and their translocation to cultured fish organs: A baseline study. *J. Hazard. Mater. Adv.* 12, 100381. https://doi.org/10.1016/j.hazadv.2023.100381

Bobori, D.C., Dimitriadi, A., Feidantsis, K., Samiotaki, A., Fafouti, D., Sampsonidis, I., Kalogiannis, S., Kastrinaki, G., Lambropoulou, D.A., Kyzas, G.Z., Koumoundouros, G., Bikiaris, D.N., Kaloyianni, M., 2022a. Differentiation in the expression of toxic effects of polyethylene-microplastics on two freshwater fish species: Size matters. *Sci. Total Environ.* 830, 154603. https://doi.org/10.1016/j.scitotenv.2022.154603

Bobori, D.C., Feidantsis, K., Dimitriadi, A., Datsi, N., Ripis, P., Kalogiannis, S., Sampsonidis, I., Kastrinaki, G., Ainali, N.M., Lambropoulou, D.A., Kyzas, G.Z., Koumoundouros, G., Bikiaris, D.N., Kaloyianni, M., 2022b. Dose-dependent cytotoxicity of polypropylene microplastics (PP-MPs) in two freshwater fishes. Int. J. Mol. Sci. 23(22). https://doi.org/10.3390/ijms232213878

Bodine, S.C., Baehr, L.M., 2014. Skeletal muscle atrophy and the E3 ubiquitin ligases MuRF1 and MAFbx/atrogin-1. *Am. J. Physiol. Endocrinol. Metab.* 307(6), E469-484. https://doi.org/10.1152/ajpendo.00204.2014

Bom, F.C., de Brito, W.V.F., Sá, F., 2022. Microplastics concentration in bivalve of economic importance, a case study on the southeastern Brazilian coast. *Reg. Stud. Mar. Sci.* 52, 102346. https://doi.org/10.1016/j.rsma.2022.102346

Bottari, T., Mancuso, M., Pedà, C., De Domenico, F., Laface, F., Schirinzi, G.F., Battaglia, P., Consoli, P., Spanò, N., Greco, S., Romeo, T., 2022. Microplastics in the bogue, *Boops boops*: A snapshot of the past from the southern Tyrrhenian Sea. *J. Hazard. Mater.* 424, 127669. https://doi.org/10.1016/j.jhazmat.2021.127669

Bour, A., Hossain, S., Taylor, M., Sumner, M., Carney Almroth, B., 2020. Synthetic microfiber and microbead exposure and retention time in model aquatic species under different exposure scenarios. *Front. Environ. Sci.* 8. https://doi.org/10.3389/fenvs.2020.00083

Bourne, W.R.P., 1977. Nylon netting as a hazard to birds. *Mar. Pollut. Bull.* 8(4), 75-76.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72(1), 248-254. https://doi.org/10.1016/0003-2697(76)90527-3

Brandts, I., Balasch, J.C., Gonçalves, A.P., Martins, M.A., Pereira, M.d.L., Tvarijonaviciute, A., Teles, M., Oliveira, M., 2021a. Immuno-modulatory effects of nanoplastics and humic acids in the European seabass (*Dicentrarchus labrax*). *J. Hazard. Mater.* 414, 125562. https://doi.org/10.1016/j.jhazmat.2021.125562

Brandts, I., Barría, C., Martins, M.A., Franco-Martínez, L., Barreto, Â., Tvarijonaviciute, A., Tort, L., Oliveira, M., Teles, M., 2021b. Waterborne exposure of gilthead seabream (*Sparus aurata*) to polymethylmethacrylate nanoplastics causes effects at cellular and molecular levels. *J. Hazard. Mater.* 403, 123590. https://doi.org/10.1016/j.jhazmat.2020.123590

Brandts, I., Teles, M., Tvarijonaviciute, A., Pereira, M.d.L., Martins, M.A., Tort, L., Oliveira, M., 2018. Effects of polymethylmethacrylate nanoplastics on *Dicentrarchus labrax*. *Genomics* 110(6), 435-441. https://doi.org/10.1016/j.ygeno.2018.10.006

Briones-Hidrovo, A., Quinteiro, P., Dias, A.C., 2023. Investigating the environmental sustainability of a seabass and seabream aquaculture system in Portugal based on life cycle and nexus approaches. *Sci. Total Environ.* 890, 164195. https://doi.org/10.1016/j.scitotenv.2023.164195

Browne, M.A., Crump, P., Niven, S.J., Teuten, E., Tonkin, A., Galloway, T., Thompson, R., 2011. Accumulation of microplastic on shorelines woldwide: Sources and sinks. *Environ. Sci. Technol.* 45(21), 9175-9179. https://doi.org/10.1021/es201811s

Bunge, A., Lugert, V., McClure, M., Kammann, U., Hanel, R., Scharsack, J.P., 2022. Less impact than suspected: Dietary exposure of three-spined sticklebacks to microplastic fibers does not affect their body condition and immune parameters. *Sci. Total Environ.* 819, 153077. https://doi.org/10.1016/j.scitotenv.2022.153077

Cammilleri, G., Calabrese, V., Accordino, L., Pantano, L., Migliazzo, A., Galluzzo, F.G., Parrino, V., Brunone, A., Lo Dico, G.M., Bacchi, E., Giangrosso, G., Calvacca, P.F., Ferrantelli, V., 2022. Toxic metals and total lipids comparison between wild and farmed fish of South mediterranean. *Nat. Prod. Res.*, 1-11. https://doi.org/10.1080/14786419.2022.2037588

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. Gastron. Food Sci. 17(4). https://doi.org/10.3390/ijerph17041212

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., Silva, A.L.P., 2021. Are microplastics impairing marine fish larviculture?—Preliminary results with *Argyrosomus regius*. *Water* 13(1), 104. https://doi.org/10.3390/w13010104

Canada, P., Pereira, A., Nogueira, N., Png-Gonzalez, L., Andrade, C., Xavier, R., 2020. Analysis of bacterial microbiome associated with nylon and copper nets in an aquaculture context. *Aquaculture* 516, 734540. https://doi.org/10.1016/j.aquaculture.2019.734540

Cao, J., Xu, R., Wang, F., Geng, Y., Xu, T., Zhu, M., Lv, H., Xu, S., Guo, M.-y., 2023. Polyethylene microplastics trigger cell apoptosis and inflammation via inducing oxidative stress and activation of the NLRP3 inflammasome in carp gills. *Fish Shellfish Immunol.* 132, 108470. https://doi.org/10.1016/j.fsi.2022.108470

Cao, Y., Lin, H., Zhang, K., Xu, S., Yan, M., Leung, K.M.Y., Lam, P.K.S., 2022. Microplastics: A major source of phthalate esters in aquatic environments. *J. Hazard. Mater.* 432, 128731. https://doi.org/10.1016/j.jhazmat.2022.128731

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., 2021a. Long-term exposure to virgin and seawater exposed microplastic enriched-diet causes liver oxidative stress and inflammation in gilthead seabream *Sparus aurata*, Linnaeus 1758. *Sci. Total Environ.* 767, 144976. https://doi.org/10.1016/j.scitotenv.2021.144976

Capó, X., Morató, M., Alomar, C., Rios-Fuster, B., Valls, M., Compa, M., Deudero, S., 2022. A biomarker approach as responses of bioindicator commercial fish species to microplastic ingestion: Assessing tissue and biochemical relationships. Biol. 11(11). https://doi.org/10.3390/biology11111634

Capó, X., Rubio, M., Solomando, A., Alomar, C., Compa, M., Sureda, A., Deudero, S., 2021b. Microplastic intake and enzymatic responses in *Mytilus galloprovincialis* reared at the vicinities

of an aquaculture station. *Chemosphere* 280, 130575. https://doi.org/10.1016/j.chemosphere.2021.130575
Capone, A., Petrillo, M., Misic, C., 2020. Ingestion and elimination of anthropogenic fibres and microplastic fragments by the European anchovy (*Engraulis encrasicolus*) of the NW Mediterranean Sea. *Mar. Biol.* 167(11), 166. https://doi.org/10.1007/s00227-020-03779-7
Cardoso, C., Lourenço, H., Costa, S., Gonçalves, S., Nunes, M.L., 2013. Survey into the seafood consumption preferences and patterns in the portuguese population. Gender and regional variability. *Appetite* 64, 20-31. https://doi.org/10.1016/j.appet.2012.12.022
Carpenter, E.J., Anderson, S.J., Harvey, G.R., Miklas, H.P., Peck, B.B., 1972. Polystyrene spherules in coastal waters. *Science* 178(4062), 749-750. https://doi.org/10.1126/science.178.4062.749
Carpenter, E.J., Smith, K.L., 1972. Plastics on the Sargasso Sea surface. *Science* 175(4027), 1240-1241. https://doi.org/10.1126/science.175.4027.1240
Castritsi-Catharios, J., Neofitou, N., Vorloou, A.A., 2015. Comparison of heavy metal concentrations in fish samples from three fish farms (Eastern Mediterranean) utilizing antifouling paints. *Toxicol. Environ. Chem.* 97(1), 116-123. https://doi.org/10.1080/02772248.2014.943226
Catarino, A.I., Macchia, V., Sanderson, W.G., Thompson, R.C., Henry, T.B., 2018. Low levels of microplastics (MP) in wild mussels indicate that MP ingestion by humans is minimal compared to exposure via household fibres fallout during a meal. *Environ. Pollut.* 237, 675-684. https://doi.org/10.1016/j.envpol.2018.02.069
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. *Environ. Sci. Technol.* 54(8), 4886-4892. https://doi.org/10.1021/acs.est.9b07705
Cesa, F.S., Turra, A., Baruque-Ramos, J., 2017. Synthetic fibers as microplastics in the marine environment: A review from textile perspective with a focus on domestic washings. *Sci. Total Environ.* 598, 1116-1129. https://doi.org/10.1016/j.scitotenv.2017.04.172
Chan, H.S.H., Dingle, C., Not, C.e., 2019. Evidence for non-selective ingestion of microplastic in demersal fish. *Mar. Pollut. Bull.* 149, 110523. https://doi.org/10.1016/j.marpolbul.2019.110523
Chen, B., Fan, Y., Huang, W., Rayhan, A.B.M.S., Chen, K., Cai, M., 2020a. Observation of microplastics in mariculture water of Longjiao Bay, southeast China: Influence by human activities. *Mar. Pollut. Bull.* 160, 111655. https://doi.org/10.1016/j.marpolbul.2020.111655
Chen, F., Lao, Q., Liu, M., Huang, P., Chen, B., Zhou, X., Chen, P., Chen, K., Song, Z., Cai, M., 2022a. Impact of intensive mariculture activities on microplastic pollution in a typical semi-enclosed bay: Zhanjiang Bay. *Mar. Pollut. Bull.* 176, 113402. https://doi.org/10.1016/j.marpolbul.2022.113402
Chen, G., Li, Y., Wang, J., 2021. Occurrence and ecological impact of microplastics in aquaculture ecosystems. *Chemosphere* 274, 129989. https://doi.org/10.1016/j.chemosphere.2021.129989
Chen, H., Jiang, F., Li, J., Cao, W., Zhang, D., Zhang, F., Wang, S., Sun, C., 2024. Interlinked water and sediment microplastics in the Laizhou Bay of China. *J. Oceanol. Limnol.* https://doi.org/10.1007/s00343-024-4032-x
Chen, J., Rao, C., Yuan, R., Sun, D., Guo, S., Li, L., Yang, S., Qian, D., Lu, R., Cao, X., 2022b. Long-term exposure to polyethylene microplastics and glyphosate interferes with the behavior, intestinal microbial homeostasis, and metabolites of the common carp (*Cyprinus carpio* L.). *Sci. Total Environ.* 814, 152681. https://doi.org/10.1016/j.scitotenv.2021.152681
Chen, M., Bao, X., Yue, Y., Yang, K., Liu, H., Yang, Y., Yu, H., Yu, Y., Duan, N., 2023a. Combined effects of cadmium and nanoplastics on oxidative stress, histopathology, and intestinal microbiota in largemouth bass (*Micropterus salmoides*). *Aquaculture* 569, 739363. https://doi.org/10.1016/j.aquaculture.2023.739363
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. *Environ. Pollut.* 242, 1146-1156. https://doi.org/10.1016/j.envpol.2018.07.133
Chen, M., Yue, Y., Bao, X., Feng, X., Ou, Z., Qiu, Y., Yang, K., Yang, Y., Yu, Y., Yu, H., 2022c. Effects of polystyrene nanoplastics on oxidative stress, histopathology and intestinal microbiota in largemouth bass (*Micropterus salmoides*). *Aquac. Res.* 27, 101423. https://doi.org/10.1016/j.aqrep.2022.101423
Chen, Q., Lackmann, C., Wang, W., Seiler, T.-B., Hollert, H., Shi, H., 2020b. Microplastics lead to hyperactive swimming behaviour in adult zebrafish. *Aquat. Toxicol.* 224, 105521. https://doi.org/10.1016/j.aquatox.2020.105521

Chen, Q., Zhao, H., Liu, Y., Jin, L., Peng, R., 2023b. Factors affecting the adsorption of heavy metals by microplastics and their toxic effects on fish. Toxics 11(6). https://doi.org/10.3390/toxics11060490
Chen, Y., Li, X., Zhang, X., Zhang, Y., Gao, W., Wang, R., He, D., 2022d. Air conditioner filters become sinks and sources of indoor microplastics fibers. *Environ. Pollut.* 292, 118465. https://doi.org/10.1016/j.envpol.2021.118465
Chen, Y., Shen, Z., Li, G., Wang, K., Cai, X., Xiong, X., Wu, C., 2022e. Factors affecting microplastic accumulation by wild fish: A case study in the Nandu River, South China. *Sci. Total Environ.* 847, 157486. https://doi.org/10.1016/j.scitotenv.2022.157486
Chenet, T., Mancia, A., Bono, G., Falsone, F., Scannella, D., Vaccaro, C., Baldi, A., Catani, M., Cavazzini, A., Pasti, L., 2021. Plastic ingestion by Atlantic horse mackerel (*Trachurus trachurus*) from central Mediterranean Sea: A potential cause for endocrine disruption. *Environ. Pollut.* 284, 117449. https://doi.org/10.1016/j.envpol.2021.117449
Cheung, L.T.O., Lui, C.Y., Fok, L., 2018a. Microplastic contamination of wild and captive flathead grey mullet (*Mugil cephalus*). *Int. J. Environ. Res. Public Health* 15(4). https://doi.org/10.3390/ijerph15040597
Cheung, P.K., Fok, L., Hung, P.L., Cheung, L.T.O., 2018b. Spatio-temporal comparison of neustonic microplastic density in Hong Kong waters under the influence of the Pearl River Estuary. *Sci. Total Environ.* 628-629, 731-739. https://doi.org/10.1016/j.scitotenv.2018.01.338
Chiang, J.Y.L., Ferrell, J.M., 2020. Up to date on cholesterol 7 alpha-hydroxylase (CYP7A1) in bile acid synthesis. *Liver Res.* 4(2), 47-63. https://doi.org/https://doi.org/10.1016/j.livres.2020.05.001
Chinfak, N., Sompongchaiyakul, P., Charoenpong, C., Shi, H., Yeemin, T., Zhang, J., 2021. Abundance, composition, and fate of microplastics in water, sediment, and shellfish in the Tapi-Phumduang River system and Bandon Bay, Thailand. *Sci. Total Environ.* 781, 146700. https://doi.org/10.1016/j.scitotenv.2021.146700
Chiriac, M.T., Hracsko, Z., Günther, C., Gonzalez-Acera, M., Atreya, R., Stolzer, I., Wittner, L., Dressel, A., Schickedanz, L., Gamez-Belmonte, R., Erkert, L., Hundorfean, G., Zundler, S., Rath, T., Vetrano, S., Danese, S., Sturm, G., Trajanoski, Z., Kühl, A.A., Siegmund, B., Hartmann, A., Wirtz, S., Siebler, J., Finotto, S., Becker, C., Neurath, M.F., 2024. IL-20 controls resolution of experimental colitis by regulating epithelial IFN/STAT2 signalling. *Gut* 73(2), 282. https://doi.org/10.1136/gutjnl-2023-329628
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. https://doi.org/10.1016/j.etap.2023.104199
Choi, J.-H., Lee, J.-H., Jo, A.H., Choi, Y.J., Choi, C.Y., Kang, J.-C., Kim, J.-H., 2023. Microplastic polyamide toxicity: Neurotoxicity, stress indicators and immune responses in crucian carp, *Carassius carassius*. *Ecotoxicol. Environ. Saf.* 265, 115469. https://doi.org/10.1016/j.ecoenv.2023.115469
Clairborne, A., 1985. Catalase activity, in: Greenwald, R.A. (Ed.), Handbook of Methods for Oxygen Radical Research. CRC Press, Boca Raton, pp. 283-284.
Cocci, P., Gabrielli, S., Pastore, G., Minicucci, M., Mosconi, G., Palermo, F.A., 2022. Microplastics accumulation in gastrointestinal tracts of *Mullus barbatus* and *Merluccius merluccius* is associated with increased cytokine production and signaling. *Chemosphere* 307, 135813. https://doi.org/10.1016/j.chemosphere.2022.135813
Cohen-Sánchez, 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(4). https://doi.org/10.3390/toxics11040365
Cole, M., Gomiero, A., Jaén-Gil, A., Haave, M., Lusher, A., 2024. Microplastic and PTFE contamination of food from cookware. *Sci. Total Environ.* 929, 172577. https://doi.org/10.1016/j.scitotenv.2024.172577
Collard, F., Gilbert, B., Compère, P., Eppe, G., Das, K., Jauniaux, T., Parmentier, E., 2017. Microplastics in livers of European anchovies (*Engraulis encrasicolus*, L.). *Environ. Pollut.* 229, 1000-1005. https://doi.org/10.1016/j.envpol.2017.07.089
Colton, J.B., Burns, B.R., Knapp, F.D., 1974. Plastic particles in surface waters of the Northwestern Atlantic. *Science* 185(5260), 491-497. https://doi.org/10.1126/science.185.4150.491
Comas, J., Parra, D., Balasch, J.C., Tort, L., 2021. Effects of fouling management and net coating strategies on reared gilthead sea bream juveniles. Animals 11(3). https://doi.org/10.3390/ani11030734

Compa, M., Capó, X., Alomar, C., Deudero, S., Sureda, A., 2024. A meta-analysis of potential biomarkers associated with microplastic ingestion in marine fish. *Environ. Toxicol. Pharmacol.* 107, 104414. https://doi.org/10.1016/j.etap.2024.104414

Compa, M., Ventero, A., Iglesias, M., Deudero, S., 2018. Ingestion of microplastics and natural fibres in *Sardina pilchardus* (Walbaum, 1792) and *Engraulis encrasicolus* (Linnaeus, 1758) along the Spanish Mediterranean coast. *Mar. Pollut. Bull.* 128, 89-96. https://doi.org/10.1016/j.marpolbul.2018.01.009

Connan, M., Perold, V., Dilley, B.J., Barbraud, C., Cherel, Y., Ryan, P.G., 2021. The Indian Ocean 'garbage patch': Empirical evidence from floating macro-litter. *Mar. Pollut. Bull.* 169, 112559. https://doi.org/https://doi.org/10.1016/j.marpolbul.2021.112559

Cooney, R., de Sousa, D.B., Fernández-Ríos, A., Mellett, S., Rowan, N., Morse, A.P., Hayes, M., Laso, J., Regueiro, L., Wan, A.H.L., Clifford, E., 2023. A circular economy framework for seafood waste valorisation to meet challenges and opportunities for intensive production and sustainability. *J. Clean. Prod.* 392, 136283. https://doi.org/https://doi.org/10.1016/j.jclepro.2023.136283

Corami, F., Rosso, B., Sfriso, A.A., Gambaro, A., Mistri, M., Munari, C., Barbante, C., 2022. Additives, plasticizers, small microplastics (<100 μm), and other microlitter components in the gastrointestinal tract of commercial teleost fish: Method of extraction, purification, quantification, and characterization using Micro-FTIR. *Mar. Pollut. Bull.* 177, 113477. https://doi.org/10.1016/j.marpolbul.2022.113477

Cormier, B., Cachot, J., Blanc, M., Cabar, M., Clérandeau, C., Dubocq, F., Le Bihanic, F., Morin, B., Zapata, S., Bégout, M.-L., Cousin, X., 2022. Environmental microplastics disrupt swimming activity in acute exposure in *Danio rerio* larvae and reduce growth and reproduction success in chronic exposure in *D. rerio* and *Oryzias melastigma*. *Environ. Pollut.* 308, 119721. https://doi.org/10.1016/j.envpol.2022.119721

Costa, C.Q.V., Afonso, I.I., Cruz, J., Teodósio, M.A.A., Jockusch, S., Ramamurthy, V., Power, D.M., Da Silva, J.P., 2024. Environmental markers of plastics and microplastics. *Environ. Sci. Technol.* 58(20), 8889-8898. https://doi.org/10.1021/acs.est.3c09662

Costa, J.P.d., Avellan, A., Mouneyrac, C., Duarte, A., Rocha-Santos, T., 2023. Plastic additives and microplastics as emerging contaminants: Mechanisms and analytical assessment. *Trends Analyt. Chem.* 158, 116898. https://doi.org/https://doi.org/10.1016/j.trac.2022.116898

Covernton, G.A., Collicutt, B., Gurney-Smith, H.J., Pearce, C.M., Dower, J.F., Ross, P.S., Dudas, S.E., 2019. Microplastics in bivalves and their habitat in relation to shellfish aquaculture proximity in coastal British Columbia, Canada. *Aquac. Environ. Interact.* 11, 357-374. https://doi.org/10.3354/aei00316

Covernton, G.A., Davies, H.L., Cox, K.D., El-Sabaawi, R., Juanes, F., Dudas, S.E., Dower, J.F., 2021. A Bayesian analysis of the factors determining microplastics ingestion in fishes. *J. Hazard. Mater.* 413, 125405. https://doi.org/10.1016/j.jhazmat.2021.125405

Cox, K.D., Covernton, G.A., Davies, H.L., Dower, J.F., Juanes, F., Dudas, S.E., 2019. Human consumption of microplastics. *Environ. Sci. Technol.* 53(12), 7068-7074. https://doi.org/10.1021/acs.est.9b01517

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 Stud. Chem. Environ. Eng.* 2, 100010. https://doi.org/10.1016/j.cscee.2020.100010

Crockett, D.E., Reed, S.M., 1976. Phenomenal Antarctic fulmar wreck. 23, 250-252.

Cui, J., Zhang, Y., Liu, L., Zhang, Q., Xu, S., Guo, M.-y., 2023. Polystyrene microplastics induced inflammation with activating the TLR2 signal by excessive accumulation of ROS in hepatopancreas of carp (*Cyprinus carpio*). *Ecotoxicol. Environ. Saf.* 251, 114539. https://doi.org/10.1016/j.ecoenv.2023.114539

Cunha, S.C., Menezes-Sousa, D., Mello, F.V., Miranda, J.A.T., Fogaca, F.H.S., Alonso, M.B., Torres, J.P.M., Fernandes, J.O., 2022. Survey on endocrine-disrupting chemicals in seafood: Occurrence and distribution. *Environ. Res.* 210, 112886. https://doi.org/10.1016/j.envres.2022.112886

Cunningham, E.M., Sigwart, J.D., 2019. Environmentally accurate microplastic levels and their absence from exposure studies. *Integr. Comp. Biol.* 59(6), 1485-1496. https://doi.org/10.1093/icb/icz068

Custódio, P.J., Pessanha, S., Pereira, C., Carvalho, M.L., Nunes, M.L., 2011. Comparative study of elemental content in farmed and wild life sea bass and gilthead bream from four different sites by FAAS and EDXRF. *Food Chem.* 124(1), 367-372. https://doi.org/10.1016/j.foodchem.2010.06.020

Czarny-Krzymińska, K., Krawczyk, B., Szczukocki, D., 2023. Bisphenol A and its substitutes in the aquatic environment: Occurrence and toxicity assessment. *Chemosphere* 315, 137763. https://doi.org/10.1016/j.chemosphere.2023.137763

d'Orbcastel, E.R., Blancheton, J.-P., Aubin, J., 2009. Towards environmentally sustainable aquaculture: Comparison between two trout farming systems using Life Cycle Assessment. *Aquac. Eng.* 40(3), 113-119. https://doi.org/10.1016/j.aquaeng.2008.12.002

da Silva, J.M., Alves, L.M.F., Laranjeiro, M.I., Bessa, F., Silva, A.V., Norte, A.C., Lemos, M.F.L., Ramos, J.A., Novais, S.C., Ceia, F.R., 2022. Accumulation of chemical elements and occurrence of microplastics in small pelagic fish from a neritic environment. *Environ. Pollut.* 292, 118451. https://doi.org/10.1016/j.envpol.2021.118451

Dao, T.-S., Kieu-Le, T.-C., La, X.-T., Truong, N.-T.-S., Thuong, Q.-T., Nguyen, V.-T., Nguyen, Q.-H., Strady, E., 2023. Microplastic accumulation in oysters: Insights from aquaculture and laboratory conditions. *Reg. Stud. Mar. Sci.* 68, 103251. https://doi.org/https://doi.org/10.1016/j.rsma.2023.103251

Das, A., 2023. The emerging role of microplastics in systemic toxicity: Involvement of reactive oxygen species (ROS). *Sci. Total Environ.* 895, 165076. https://doi.org/10.1016/j.scitotenv.2023.165076

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. https://doi.org/10.1016/j.jhazmat.2023.131916

Davidson, K., Dudas, S.E., 2016. Microplastic ingestion by wild and cultured manila clams (*Venerupis philippinarum*) from Baynes Sound, British Columbia. *Arch. Environ. Contam. Toxicol.* 71(2), 147-156. https://doi.org/10.1007/s00244-016-0286-4

Dawson, A.L., Kawaguchi, S., King, C.K., Townsend, K.A., King, R., Huston, W.M., Bengtson Nash, S.M., 2018. Turning microplastics into nanoplastics through digestive fragmentation by Antarctic krill. *Nat. Commun.* 9(1), 1001. https://doi.org/10.1038/s41467-018-03465-9

Dawson, A.L., Li, J.Y.Q., Kroon, F.J., 2022. Plastics for dinner: Store-bought seafood, but not wild-caught from the Great Barrier Reef, as a source of microplastics to human consumers. *Environ. Adv.* 8, 100249. https://doi.org/10.1016/j.envadv.2022.100249

Dawson, A.L., Motti, C.A., Kroon, F.J., 2020. Solving a sticky situation: Microplastic analysis of lipid-rich tissue. *Front. Environ. Sci.* 8. https://doi.org/10.3389/fenvs.2020.563565

De-la-Torre, G.E., Santillán, L., Dioses-Salinas, D.C., Yenney, E., Toapanta, T., Okoffo, E.D., Kannan, G., Madadi, R., Dobaradaran, S., 2024. Assessing the current state of plastic pollution research in Antarctica: Knowledge gaps and recommendations. *Chemosphere* 355, 141870. https://doi.org/10.1016/j.chemosphere.2024.141870

de Sá, L.C., Luís, L.G., Guilhermino, L., 2015. Effects of microplastics on juveniles of the common goby (*Pomatoschistus microps*): Confusion with prey, reduction of the predatory performance and efficiency, and possible influence of developmental conditions. *Environ. Pollut.* 196, 359-362. https://doi.org/10.1016/j.envpol.2014.10.026

de Sousa, F.D.B., 2021. Plastic and its consequences during the COVID-19 pandemic. *Environ. Sci. Pollut. Res.* 28(33), 46067-46078. https://doi.org/10.1007/s11356-021-15425-w

Dehaut, A., Cassone, A.-L., Frère, L., Hermabessiere, L., Himber, C., Rinnert, E., Rivière, G., Lambert, C., Soudant, P., Huvet, A., Duflos, G., Paul-Pont, I., 2016. Microplastics in seafood: Benchmark protocol for their extraction and characterization. *Environ. Pollut.* 215, 223-233. https://doi.org/10.1016/j.envpol.2016.05.018

Dehm, J., Volau, M., Ledua, E., Hewavitharane, C., 2022. Occurrence of microplastics within a freshwater aquaculture system in the Pacific Islands, Viti Levu, Fiji. *Environ. Monit. Assess.* 194(9), 624. https://doi.org/10.1007/s10661-022-10305-3

Del Piano, F., Almroth, B.C., Lama, A., Piccolo, G., Addeo, N.F., Paciello, O., Martino, G., Esposito, S., Mercogliano, R., Pirozzi, C., Meli, R., Ferrante, M.C., 2024. Subchronic oral exposure to polystyrene microplastics affects hepatic lipid metabolism, inflammation, and oxidative balance in gilthead seabream (*Sparus aurata*). *Ecotoxicol. Environ. Saf.* 279, 116455. https://doi.org/10.1016/j.ecoenv.2024.116455

Del Piano, F., Lama, A., Monnolo, A., Pirozzi, C., Piccolo, G., Vozzo, S., De Biase, D., Riccio, L., Fusco, G., Mercogliano, R., Meli, R., Ferrante, M.C., 2023a. Subchronic exposure to polystyrene microplastic differently affects redox balance in the anterior and posterior intestine of *Sparus aurata*. Animals 13(4). https://doi.org/10.3390/ani13040606

Del Piano, F., Lama, A., Piccolo, G., Addeo, N.F., Iaccarino, D., Fusco, G., Riccio, L., De Biase, D., Mattace Raso, G., Meli, R., Ferrante, M.C., 2023b. Impact of polystyrene microplastic

exposure on gilthead seabream (*Sparus aurata* Linnaeus, 1758): Differential inflammatory and immune response between anterior and posterior intestine. *Sci. Total Environ.* 879, 163201. https://doi.org/10.1016/j.scitotenv.2023.163201

Devi, S.S., Jayan, S., Kumar, A.B., 2024. Microplastic assessment in aquaculture feeds: Analyzing polymer variability across commercial fishfeeds from three continents. *J. Hazard. Mater.* 479, 135621. https://doi.org/10.1016/j.jhazmat.2024.135621

Deviller, G., Palluel, O., Aliaume, C., Asanthi, H., Sanchez, W., Franco Nava, M.A., Blancheton, J.P., Casellas, C., 2005. Impact assessment of various rearing systems on fish health using multibiomarker response and metal accumulation. *Ecotoxicol. Environ. Saf.* 61(1), 89-97. https://doi.org/10.1016/j.ecoenv.2004.07.011

Di Giacinto, F., Di Renzo, L., Mascilongo, G., Notarstefano, V., Gioacchini, G., Giorgini, E., Bogdanović, T., Petričević, S., Listeš, E., Brkljača, M., Conti, F., Profico, C., Zambuchini, B., Di Francesco, G., Giansante, C., Diletti, G., Ferri, N., Berti, M., 2023. Detection of microplastics, polymers and additives in edible muscle of swordfish (*Xiphias gladius*) and bluefin tuna (*Thunnus thynnus*) caught in the Mediterranean Sea. *J. Sea Res.* 192, 102359. https://doi.org/10.1016/j.seares.2023.102359

Ding, J., Huang, Y., Liu, S., Zhang, S., Zou, H., Wang, Z., Zhu, W., Geng, J., 2020. Toxicological effects of nano- and micro-polystyrene plastics on red tilapia: Are larger plastic particles more harmless? *J. Hazard. Mater.* 396, 122693. https://doi.org/10.1016/j.jhazmat.2020.122693

Ding, J., Ju, P., Ran, Q., Li, J., Jiang, F., Cao, W., Zhang, J., Sun, C., 2023. Elder fish means more microplastics? Alaska pollock microplastic story in the Bering Sea. *Sci. Adv.* 9(27), eadf5897. https://doi.org/10.1126/sciadv.adf5897

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*). *Environ. Pollut.* 238, 1-9. https://doi.org/10.1016/j.envpol.2018.03.001

Djekoun, M., Gaaied, S., Romdhani, I., Rida, A.M., Missaoui, Y., Boebekeur, M.S., Trea, F., Lakbar, C., Ouali, K., 2024. Abundance and distribution of environmental microplastic in edible fish and muscle from the south Mediterranean coasts. *Mar. Pollut. Bull.* 206, 116705. https://doi.org/10.1016/j.marpolbul.2024.116705

Domingo-Bréton, R., Cools, S., Moroni, F., Belenguer, Á., Calduch-Giner, J.À., 2025. Intestinal microbiota shifts as a marker of thermal stress during extreme heat summer episodes in farmed gilthead sea bream (*Sparus aurata*). *Aquac. Res.*

Dong, R., Zhou, C., Wang, S., Yan, Y., Jiang, Q., 2022. Probiotics ameliorate polyethylene microplastics-induced liver injury by inhibition of oxidative stress in Nile tilapia (*Oreochromis niloticus*). *Fish Shellfish Immunol.* 130, 261-272. https://doi.org/10.1016/j.fsi.2022.09.022

Dong, X., Liu, X., Hou, Q., Wang, Z., 2023. From natural environment to animal tissues: A review of microplastics(nanoplastics) translocation and hazards studies. *Sci. Total Environ.* 855, 158686. https://doi.org/https://doi.org/10.1016/j.scitotenv.2022.158686

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. https://doi.org/10.1016/j.envpol.2016.12.013

Du, J., Hu, Y., Hou, M., Zhou, J., Xiang, F., Zheng, H., Zhang, X., He, X., Xiao, H., 2023. Combined effects of high-fat diet and polystyrene microplastic exposure on microplastic bioaccumulation and lipid metabolism in zebrafish. *Fish Shellfish Immunol.* 137, 108803. https://doi.org/10.1016/j.fsi.2023.108803

Dugo, G., La Pera, L., Bruzzese, A., Pellicanò, T.M., Turco, V.L., 2006. Concentration of Cd (II), Cu (II), Pb (II), Se (IV) and Zn (II) in cultured sea bass (*Dicentrarchus labrax*) tissues from Tyrrhenian Sea and Sicilian Sea by derivative stripping potentiometry. *Food Control* 17(2), 146-152. https://doi.org/10.1016/j.foodcont.2004.09.014

Dural, M., Göksu, M.Z.L., Özak, A.A., 2007. Investigation of heavy metal levels in economically important fish species captured from the Tuzla lagoon. *Food Chem.* 102(1), 415-421. https://doi.org/10.1016/j.foodchem.2006.03.001

Dural, M., Lugal Göksu, M.Z., Özak, A.A., Derici, B., 2006. Bioaccumulation of some heavy metals in different tissues of *Dicentrarchus labrax* L, 1758, *Sparus aurata* L, 1758 and *Mugil cephalus* L, 1758 from the ÇamlIk Lagoon of the Eastern cost of Mediterranean (Turkey). *Environ. Monit. Assess.* 118(1), 65-74. https://doi.org/10.1007/s10661-006-0987-7

EC, 2005. Commission Regulation (EC) No 78/2005 of 19 January 2005 amending Regulation (EC) No 466/2001 as regards heavy metals. *OJEU*, L16/43-L13/45.

ECHA, 2017. Guidance on information requirements and chemical safety assessment, in: Chapter R.7c: Endpoint specific guidance. Helsinki, Finland.
EFSA, 2006. Tolerable upper intake levels for vitamins and minerals.
EFSA, 2010. Scientific Opinion on lead in food. *EFSA J.* 8(4), 1570. https://doi.org/10.2903/j.efsa.2010.1570
EFSA, 2011. Statement on tolerable weekly intake for cadmium. *EFSA J.* 9(2), 1975. https://doi.org/10.2903/j.efsa.2011.1975
EFSA, 2012a. Cadmium dietary exposure in the European population. *EFSA J.* 10(1), 2551. https://doi.org/10.2903/j.efsa.2012.2551
EFSA, 2012b. Guidance on selected default values to be used by the EFSA Scientific Committee, Scientific Panels and Units in the absence of actual measured data. *EFSA J.* 10(3), 2579. https://doi.org/10.2903/j.efsa.2012.2579
EFSA, 2012c. Scientific Opinion on the risk for public health related to the presence of mercury and methylmercury in food. *EFSA J.* 10(12), 2985. https://doi.org/10.2903/j.efsa.2012.2985
EFSA, 2014. Scientific Opinion on the risks to public health related to the presence of chromium in food and drinking water. *EFSA J.* 12(3), 3595. https://doi.org/https://doi.org/10.2903/j.efsa.2014.3595
EFSA, 2015. Scientific Opinion on dietary reference values for copper. *EFSA J.* 13(10), 4253. https://doi.org/10.2903/j.efsa.2015.4253
EFSA, 2016. Presence of microplastics and nanoplastics in food, with particular focus on seafood. *EFSA J.* 14(6), e04501. https://doi.org/https://doi.org/10.2903/j.efsa.2016.4501
EFSA, 2020a. Risk assessment and toxicological research on micro- and nanoplastics after oral exposure via food products. *EFSA J.* 18(S1), e181102. https://doi.org/10.2903/j.efsa.2020.e181102
EFSA, 2020b. Update of the risk assessment of nickel in food and drinking water. *EFSA J.* 18(11), e06268. https://doi.org/10.2903/j.efsa.2020.6268
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.-j., 2023. Microplastic in industrial aquaculture: Occurrence in the aquatic environment, feed and organisms (*Dicentrarchus labrax*). *Sci. Total Environ.* 904, 166774. https://doi.org/10.1016/j.scitotenv.2023.166774
Elizalde-Velázquez, A., Carcano, A.M., Crago, J., Green, M.J., Shah, S.A., Cañas-Carrell, J.E., 2020. Translocation, trophic transfer, accumulation and depuration of polystyrene microplastics in *Daphnia magna* and *Pimephales promelas*. *Environ. Pollut.* 259, 113937. https://doi.org/10.1016/j.envpol.2020.113937
Eo, S., Hong, S.H., Cho, Y., Song, Y.K., Han, G.M., Shim, W.J., 2023. Spatial distribution and historical trend of microplastic pollution in sediments from enclosed bays of South Korea. *Mar. Pollut. Bull.* 193, 115121. https://doi.org/10.1016/j.marpolbul.2023.115121
Eriksen, M., Cowger, W., Erdle, L.M., Coffin, S., Villarrubia-Gómez, P., Moore, C.J., Carpenter, E.J., Day, R.H., Thiel, M., Wilcox, C., 2023. A growing plastic smog, now estimated to be over 170 trillion plastic particles afloat in the world's oceans—Urgent solutions required *PLOS ONE* 18(3), e0281596. https://doi.org/10.1371/journal.pone.0281596
Erkan, N., Özden, Ö., 2007. Proximate composition and mineral contents in aqua cultured sea bass (*Dicentrarchus labrax*), sea bream (*Sparus aurata*) analyzed by ICP-MS. *Food Chem.* 102(3), 721-725. https://doi.org/10.1016/j.foodchem.2006.06.004
Erola, P., Björkegren, J.L.M., Michoel, T., 2020. Model-based clustering of multi-tissue gene expression data. *Bioinformatics* 36(6), 1807-1813. https://doi.org/10.1093/bioinformatics/btz805
Ersoy, B., Yanar, Y., Küçükgülmez, A., Çelik, M., 2006. Effects of four cooking methods on the heavy metal concentrations of sea bass fillets (*Dicentrarchus labrax* Linne, 1785). *Food Chem.* 99(4), 748-751. https://doi.org/10.1016/j.foodchem.2005.08.055
Eryaşar, A.R., Mutlu, T., Karaoğlu, K., Veske, E., Gedik, K., 2024. Assessment of microplastic pollution in eleven commercial fish species in the Gulf of İzmir (Aegean Sea, eastern Mediterranean). *Mar. Pollut. Bull.* 208, 116932. https://doi.org/10.1016/j.marpolbul.2024.116932
Eshun, F., Pobee, A.N.A., 2022. Effects of frying on microplastics load in fish and implications on health. *Food Front.* 3(4), 543-549. https://doi.org/10.1002/fft2.164
Esmaeilbeigi, M., Kazemi, A., Gholizadeh, M., Rezaeiyeh, R.D., 2023. Microplastics and heavy metals contamination in *Atropus atropos* and associated health risk assessment in the

northwest of the Persian Gulf, Iran. *Reg. Stud. Mar. Sci.* 57, 102750. https://doi.org/10.1016/j.rsma.2022.102750
Espinosa-Ruiz, C., González-Fernández, C., Cormier, B., Keiter, S.H., Vieira, L.R., Guilhermino, L., Clérandeau, 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. https://doi.org/10.1016/j.fsi.2023.108793
Espinosa, C., Cuesta, A., Esteban, M.Á., 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 Immunol.* 68, 251-259. https://doi.org/10.1016/j.fsi.2017.07.006
Espinosa, C., Esteban, M.Á., 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. https://doi.org/10.1016/j.fsi.2019.10.072
Esteve-Llorens, X., Van Dooren, C., Álvarez, M., Moreira, M.T., Feijoo, G., González-García, S., 2021. Environmental and nutritional profile of food consumption patterns in the different climatic zones of Spain. *J. Clean. Prod.* 279, 123580. https://doi.org/10.1016/j.jclepro.2020.123580
Estrela, F.N., Batista Guimarães, A.T., Silva, F.G., Marinho da Luz, T., Silva, A.M., Pereira, P.S., Malafaia, G., 2021. Effects of polystyrene nanoplastics on *Ctenopharyngodon idella* (grass carp) after individual and combined exposure with zinc oxide nanoparticles. *J. Hazard. Mater.* 403, 123879. https://doi.org/10.1016/j.jhazmat.2020.123879
EU, 2019. Directive (EU) 2019/904 of the European parliament and of the council of 5 June 2019 on the reduction of the impact of certain plastic products on the environment.
EUMOFA, 2021. The EU fish market: 2021 edition. Publications Office of the European Union.
EUMOFA, 2023. The EU fish market: 2023 edition. Publications Office of the European Union.
EUMOFA, 2024. The EU fish market - 2024 edition. Publications Office of the European Union.
Falkenberg, L.J., Cornet, J.E., Joyce, P.W.S., 2024. Nature-based solutions to the management of legacy plastic pollution: Filter-feeders as bioremediation tools for coastal microplastics. *Sci. Total Environ.* 956, 177237. https://doi.org/10.1016/j.scitotenv.2024.177237
FAO, 2003. Heavy metal regulations, Legal notice nº 66/2003. p. 8.
FAO, 2022. The state of world fisheries and aquaculture 2022: Towards blue transformation. FAO, Rome, Italy, p. 266.
FAO, 2023. Summary report of the Joint FAO/WHO Expert Consultation on risks and benefits of fish consumption. Rome, Italy, p. 9.
FAO, 2024. The state of world fisheries and aquaculture 2024: Blue transformation in action. Rome, Italy.
Fatema, K., Auditi, T.I., Biswas, S., Ayesha, S.B., Helal Uddin, M., Sumon, K.A., Goswami, C., Bhandari, R.K., Rashid, H., 2023. Investigations of hemato-biochemical and histopathological parameters, and growth performance of walking catfish (*Clarias batrachus*) exposed to PET and LDPE microplastics. *Environ. Toxicol. Pharmacol.* 102, 104250. https://doi.org/10.1016/j.etap.2023.104250
FEAP, 2023. European aquaculture production report 2016-2022.
Feldman, D., 2002. Polymer weathering: Photo-oxidation. *J. Polym. Environ.* 10(4), 163-173. https://doi.org/10.1023/A:1021148205366
Feng, Z., Zhang, T., Li, Y., He, X., Wang, R., Xu, J., Gao, G., 2019. The accumulation of microplastics in fish from an important fish farm and mariculture area, Haizhou Bay, China. *Sci. Total Environ.* 696, 133948. https://doi.org/10.1016/j.scitotenv.2019.133948
Fernandes, A.M., Calduch-Giner, J.À., Pereira, G.V., Gonçalves, A.T., Dias, J., Johansen, J., Silva, T., Naya-Català, F., Piazzon, C., Sitjà-Bobadilla, A., Costas, B., Conceição, L.E.C., Fernandes, J.M.O., Pérez-Sánchez, J., 2024. Sustainable fish meal-free diets for gilthead sea bream (*Sparus aurata*): integrated biomarker response to assess the effects on growth performance, lipid metabolism, antioxidant defense and immunological status. Animals 14(15). https://doi.org/10.3390/ani14152166
Fernandes, D., Zanuy, S., Bebianno, M.J., Porte, C., 2008. Chemical and biochemical tools to assess pollution exposure in cultured fish. *Environ. Pollut.* 152(1), 138-146. https://doi.org/10.1016/j.envpol.2007.05.012
Ferrante, M., Pietro, Z., Allegui, C., Maria, F., Antonio, C., Pulvirenti, E., Favara, C., Chiara, C., Grasso, A., Omayma, M., Gea, O.C., Banni, M., 2022. Microplastics in fillets of Mediterranean

seafood. A risk assessment study. *Environ. Res.* 204, 112247. https://doi.org/10.1016/j.envres.2021.112247
Ferreira, M., Caetano, M., Antunes, P., Costa, J., Gil, O., Bandarra, N., Pousão-Ferreira, P., Vale, C., Reis-Henriques, M.A., 2010. Assessment of contaminants and biomarkers of exposure in wild and farmed seabass. *Ecotoxicol. Environ. Saf.* 73(4), 579-588. https://doi.org/10.1016/j.ecoenv.2010.01.019
Ferreira, M., Sousa, V., Oliveira, B., Canadas-Sousa, A., Abreu, H., Dias, J., Kiron, V., Valente, L.M.P., 2023. An in-depth characterisation of European seabass intestinal segments for assessing the impact of an algae-based functional diet on intestinal health. *Sci. Rep.* 13(1), 11686. https://doi.org/10.1038/s41598-023-38826-y
Forrester, D.J., White, F.H., Woodard, J.C., Thompson, N.P., 1975. Intussusception in a Florida manatee. *J. Wildl. Dis.* 11(4), 566-568. https://doi.org/10.7589/0090-3558-11.4.566
Frias, J.P.G.L., Otero, V., Sobral, P., 2014. Evidence of microplastics in samples of zooplankton from Portuguese coastal waters. *Mar. Environ. Res.* 95, 89-95. https://doi.org/10.1016/j.marenvres.2014.01.001
Frias, J.P.G.L., Sobral, P., Ferreira, A.M., 2010. Organic pollutants in microplastics from two beaches of the Portuguese coast. *Mar. Pollut. Bull.* 60(11), 1988-1992. https://doi.org/10.1016/j.marpolbul.2010.07.030
Fuentes, A., Fernández-Segovia, I., Serra, J.A., Barat, J.M., 2010. Comparison of wild and cultured sea bass (*Dicentrarchus labrax*) quality. *Food Chem.* 119(4), 1514-1518. https://doi.org/10.1016/j.foodchem.2009.09.036
Galloway, T.S., Cole, M., Lewis, C., 2017. Interactions of microplastic debris throughout the marine ecosystem. *Nat. Ecol. Evol.* 1(5), 0116. https://doi.org/10.1038/s41559-017-0116
Garcés-Ordóñez, O., Saldarriaga-Vélez, J.F., Espinosa-Díaz, L.F., Patiño, A.D., Cusba, J., Canals, M., Mejía-Esquivia, K., Fragozo-Velásquez, L., Sáenz-Arias, S., Córdoba-Meza, T., Thiel, M., 2022. Microplastic pollution in water, sediments and commercial fish species from Ciénaga Grande de Santa Marta lagoon complex, Colombian Caribbean. *Sci. Total Environ.* 829, 154643. https://doi.org/10.1016/j.scitotenv.2022.154643
Garcia-Garin, O., Vighi, M., Aguilar, A., Tsangaris, C., Digka, N., Kaberi, H., Borrell, A., 2019. *Boops boops* as a bioindicator of microplastic pollution along the Spanish Catalan coast. *Mar. Pollut. Bull.* 149, 110648. https://doi.org/10.1016/j.marpolbul.2019.110648
Garcia, A.G., Suárez, D.C., Li, J., Rotchell, J.M., 2021. A comparison of microplastic contamination in freshwater fish from natural and farmed sources. *Environ. Sci. Pollut. Res.* 28(12), 14488-14497. https://doi.org/10.1007/s11356-020-11605-2
Gardon, T., El Rakwe, M., Paul-Pont, I., Le Luyer, J., Thomas, L., Prado, E., Boukerma, K., Cassone, A.-L., Quillien, V., Soyez, C., Costes, L., Crusot, M., Dreanno, C., Le Moullac, G., Huvet, A., 2021. Microplastics contamination in pearl-farming lagoons of French Polynesia. *J. Hazard. Mater.* 419, 126396. https://doi.org/10.1016/j.jhazmat.2021.126396
Garrido Gamarro, E., Constanzo, V., 2022. Microplastics in food commodities - A food safety review on human exposure through dietary sources, Food Safety and Quality Series. FAO, Rome, Italy
Ge, A., Zhao, S., Sun, C., Yuan, Z., Liu, L., Chen, L., Li, F., 2024. Comparison of three digestion methods for microplastic extraction from aquaculture feeds. *Sci. Total Environ.* 912, 168919. https://doi.org/10.1016/j.scitotenv.2023.168919
Ge, J., Wang, M., Liu, P., Zhang, Z., Peng, J., Guo, X., 2023. A systematic review on the aging of microplastics and the effects of typical factors in various environmental media. *Trends Analyt. Chem.* 162, 117025. https://doi.org/https://doi.org/10.1016/j.trac.2023.117025
Gehlert, G., Griese, M., Schlachter, M., Schulz, C., 2018. Analysis and optimisation of dynamic facility ventilation in recirculation aquacultural systems. *Aquac. Eng.* 80, 1-10. https://doi.org/10.1016/j.aquaeng.2017.11.003
Georgieva, S.K., Peteva, Z.V., Stancheva, M.D., 2023. Evaluation of abundance of microplastics in the Bulgarian coastal waters. *BioRisk* 20, 59-69. https://doi.org/10.3897/biorisk.20.97555
GESAMP, 2016. Sources, fate and effects of microplastics in the marine environment: part two of a global assessment, in: Kershaw, P.J., Rochman, C.M. (Eds.), Rep. Stud.-IMO/FAO/UNESCO/WMO/WHO/IAEA/UN/UNEP Jt. Group Experts Sci. Asp. Mar. Pollut.-GESAMP. IMO/FAO/UNESCO-IOC/UNIDO/WMO/IAEA/UN/UNEP/UNDP/ISA Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection, p. 123.
GESAMP, 2019. Guidelines for the monitoring and assessment of plastic litter in the ocean, in: Kershaw, P.J., Turra, A., Galgani, F. (Eds.), Rep. Stud.-IMO/FAO/UNESCO/WMO/WHO/IAEA/UN/UNEP Jt. Group Experts Sci. Asp. Mar. Pollut.-

GESAMP. IMO/FAO/UNESCO-IOC/UNIDO/WMO/IAEA/UN/UNEP/UNDP/ISA Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection, p. 123.
Geven, E.J.W., Klaren, P.H.M., 2017. The teleost head kidney: Integrating thyroid and immune signalling. *Dev. Comp. Immunol.* 66, 73-83. https://doi.org/10.1016/j.dci.2016.06.025
Geyer, R., 2020. A brief history of plastics, in: Streit-Bianchi, M., Cimadevila, M., Trettnak, W. (Eds.), *Mare Plasticum* - The Plastic Sea: Combatting plastic pollution through science and art. Springer International Publishing, Cham, pp. 31-47.
Geyer, R., Jambeck, J.R., Law, K.L., 2017. Production, use, and fate of all plastics ever made. *Sci. Adv.* 3(7), e1700782. https://doi.org/10.1126/sciadv.1700782
Ghasemi, A., Shadi, A., 2024. Combined effects of microplastics and benzo[a]pyrene on Asian sea bass *Lates calcarifer* growth and expression of functional genes. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology* 283, 109966. https://doi.org/10.1016/j.cbpc.2024.109966
Gigault, J., Halle, A.t., Baudrimont, M., Pascal, P.-Y., Gauffre, F., Phi, T.-L., El Hadri, H., Grassl, B., Reynaud, S., 2018. Current opinion: What is a nanoplastic? *Environ. Pollut.* 235, 1030-1034. https://doi.org/10.1016/j.envpol.2018.01.024
Glencross, B., Ling, X., Gatlin, D., Kaushik, S., Øverland, M., Newton, R., Valente, L.M.P., 2024. A SWOT analysis of the use of marine, grain, terrestrial-animal and novel protein ingredients in aquaculture feeds. *Rev. Fish. Sci. Aquac.* 32(3), 396-434. https://doi.org/10.1080/23308249.2024.2315049
Gochfeld, M., 1973. Effect of artefact pollution on the viability of seabird colonies on Long Island, New York. *Environ. Pollut.* 4(1), 1-6. https://doi.org/10.1016/0013-9327(73)90025-6
González-Fernández, C., Cuesta, A., 2022. Nanoplastics increase fish susceptibility to nodavirus infection and reduce antiviral immune responses. Int. J. Mol. Sci. 23(3). https://doi.org/10.3390/ijms23031483
Granby, K., Bhattarai, B., Johannsen, N., Kotterman, M.J.J., Sloth, J.J., Cederberg, T.L., Marques, A., Larsen, B.K., 2024. Microplastics in feed affect the toxicokinetics of persistent halogenated pollutants in Atlantic salmon. *Environ. Pollut.* 357, 124421. https://doi.org/10.1016/j.envpol.2024.124421
Granby, K., Rainieri, S., Rasmussen, R.R., Kotterman, M.J.J., Sloth, J.J., Cederberg, T.L., Barranco, A., Marques, A., Larsen, B.K., 2018. The influence of microplastics and halogenated contaminants in feed on toxicokinetics and gene expression in European seabass (*Dicentrarchus labrax*). *Environ. Res.* 164, 430-443. https://doi.org/10.1016/j.envres.2018.02.035
Gregory, M.R., 1977. Plastic pellets on New Zealand beaches. *Mar. Pollut. Bull.* 8(4), 82-84. https://doi.org/10.1016/0025-326X(77)90193-X
Grigorakis, S., Mason, S.A., Drouillard, K.G., 2017. Determination of the gut retention of plastic microbeads and microfibers in goldfish (*Carassius auratus*). *Chemosphere* 169, 233-238. https://doi.org/10.1016/j.chemosphere.2016.11.055
Gu, H., Wang, S., Wang, X., Yu, X., Hu, M., Huang, W., Wang, Y., 2020. Nanoplastics impair the intestinal health of the juvenile large yellow croaker *Larimichthys crocea*. *J. Hazard. Mater.* 397, 122773. https://doi.org/10.1016/j.jhazmat.2020.122773
Guilhermino, L., Martins, A., Lopes, C., Raimundo, J., Vieira, L.R., Barboza, L.G.A., Costa, J., Antunes, C., Caetano, M., Vale, C., 2021. Microplastics in fishes from an estuary (Minho River) ending into the NE Atlantic Ocean. *Mar. Pollut. Bull.* 173, 113008. https://doi.org/10.1016/j.marpolbul.2021.113008
Guimarães, A.T.B., Estrela, F.N., Rodrigues, A.S.d.L., Chagas, T.Q., Pereira, P.S., Silva, F.G., Malafaia, G., 2021. Nanopolystyrene particles at environmentally relevant concentrations causes behavioral and biochemical changes in juvenile grass carp (*Ctenopharyngodon idella*). *J. Hazard. Mater.* 403, 123864. https://doi.org/10.1016/j.jhazmat.2020.123864
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, 736316. https://doi.org/10.1016/j.aquaculture.2020.736316
Gündogdu, S., Rathod, N., Hassoun, A., Jamroz, E., Kulawik, P., Gokbulut, C., Aït-Kaddour, A., Özogul, F., 2022. The impact of nano/micro-plastics toxicity on seafood quality and human health: facts and gaps. *Crit. Rev. Food Sci. Nutr.*, 1-19. https://doi.org/10.1080/10408398.2022.2033684
Gutiérrez-Estrada, J.C., Pulido-Calvo, I., Castro-Gutiérrez, J., Peregrín, A., López-Domínguez, S., Gómez-Bravo, F., Garrocho-Cruz, A., de la Rosa-Lucas, I., 2022. Fish abundance estimation

with imaging sonar in semi-intensive aquaculture ponds. *Aquac. Eng.* 97, 102235. https://doi.org/10.1016/j.aquaeng.2022.102235
Guven, O., Bach, L., Munk, P., Dinh, K.V., Mariani, P., Nielsen, T.G., 2018. Microplastic does not magnify the acute effect of PAH pyrene on predatory performance of a tropical fish (*Lates calcarifer*). *Aquat. Toxicol.* 198, 287-293. https://doi.org/10.1016/j.aquatox.2018.03.011
Habib, R.Z., Poulose, V., Alsaidi, R., al Kendi, R., Iftikhar, S.H., Mourad, A.-H.I., Kittaneh, W.F., Thiemann, T., 2022. Plastic cutting boards as a source of microplastics in meat. *Food Addit. Contam. Part A* 39(3), 609-619. https://doi.org/10.1080/19440049.2021.2017002
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(22), 7130-7139. https://doi.org/10.1016/S0021-9258(19)42083-8
Häder, D.-P., Banaszak, A.T., Villafañe, V.E., Narvarte, M.A., González, R.A., Helbling, E.W., 2020. Anthropogenic pollution of aquatic ecosystems: Emerging problems with global implications. *Sci. Total Environ.* 713, 136586. https://doi.org/10.1016/j.scitotenv.2020.136586
Hahladakis, J.N., Velis, C.A., Weber, R., Iacovidou, E., Purnell, P., 2018. An overview of chemical additives present in plastics: Migration, release, fate and environmental impact during their use, disposal and recycling. *J. Hazard. Mater.* 344, 179-199. https://doi.org/10.1016/j.jhazmat.2017.10.014
Hamed, M., Soliman, H.A.M., Badrey, A.E.A., Osman, A.G.M., 2021. Microplastics induced histopathological lesions in some tissues of tilapia (*Oreochromis niloticus*) early juveniles. *Tissue and Cell* 71, 101512. https://doi.org/10.1016/j.tice.2021.101512
Hamed, M., Soliman, H.A.M., Eid, Z., Al Naggar, Y., Sayed, A.E.-D.H., 2022. Dietary feeding lycopene, citric acid, and *Chlorella* alleviated the neurotoxicity of polyethylene microplastics in African catfish (*Clarias gariepinus*). *Front. Environ. Sci.* 10. https://doi.org/10.3389/fenvs.2022.869727
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. https://doi.org/10.1016/j.chemosphere.2019.04.153
Hamed, M., Soliman, H.A.M., Osman, A.G.M., Sayed, A.E.-D.H., 2020. Antioxidants and molecular damage in Nile Tilapia (*Oreochromis niloticus*) after exposure to microplastics. *Environ. Sci. Pollut. Res.* 27(13), 14581-14588. https://doi.org/10.1007/s11356-020-07898-y
Hanachi, P., Karbalaei, S., Walker, T.R., Cole, M., Hosseini, S.V., 2019. Abundance and properties of microplastics found in commercial fish meal and cultured common carp (*Cyprinus carpio*). *Environ. Sci. Pollut. Res.* 26(23), 23777-23787. https://doi.org/10.1007/s11356-019-05637-6
Hanachi, P., Karbalaei, S., Yu, S., 2021. Combined polystyrene microplastics and chlorpyrifos decrease levels of nutritional parameters in muscle of rainbow trout (*Oncorhynchus mykiss*). *Environ. Sci. Pollut. Res.* 28(45), 64908-64920. https://doi.org/10.1007/s11356-021-15536-4
Handy, R.D., Henry, T.B., Scown, T.M., Johnston, B.D., Tyler, C.R., 2008. Manufactured nanoparticles: their uptake and effectson fish—a mechanistic analysis. *Ecotoxicology* 17, 396-409. https://doi.org/10.1007/s10646-008-0205-1
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. https://doi.org/10.1016/j.scitotenv.2022.159270
Haque, F., Fan, C., 2023. Fate of microplastics under the influence of climate change. *iScience* 26(9), 107649. https://doi.org/10.1016/j.isci.2023.107649
Harper, P.C., Fowler, J.A., 1987. Plastic pellets in New Zealand storm-killed prions (*Pachyptila* spp.) 1958-1977. *Notornis* 34, 65-70.
Hartmann, C., Lomako, I., Schachner, C., El Said, E., Abert, J., Satrapa, V., Kaiser, A.-M., Walch, H., Köppel, S., 2024. Assessment of microplastics in human stool: A pilot study investigating the potential impact of diet-associated scenarios on oral microplastics exposure. *Sci. Total Environ.* 951, 175825. https://doi.org/10.1016/j.scitotenv.2024.175825
Hasan, J., Siddik, M.A., Ghosh, A.K., Mesbah, S.B., Sadat, M.A., Shahjahan, M., 2023. Increase in temperature increases ingestion and toxicity of polyamide microplastics in Nile tilapia. *Chemosphere* 327, 138502. https://doi.org/10.1016/j.chemosphere.2023.138502
Hays, H., Cormons, G., 1974. Plastic particles found in tern pellets, on coastal beaches and at factory sites. *Mar. Pollut. Bull.* 5(3), 44-46. https://doi.org/10.1016/0025-326X(74)90234-3
Henares, M.N.P., Medeiros, M.V., Camargo, A.F.M., 2020. Overview of strategies that contribute to the environmental sustainability of pond aquaculture: rearing systems, residue treatment, and

environmental assessment tools. *Rev. Aquac.* 12(1), 453-470. https://doi.org/10.1111/raq.12327

Herrera, A., Acosta-Dacal, A., Pérez-Luzardo, O., Martínez, I., Rapp, J., Reinold, S., Montesdeoca-Esponda, S., Montero, D., Gómez, M., 2023. Trophic transfer of DDE, BP-3 and chlorpyrifos from microplastics to tissues in *Dicentrarchus labrax*. *Sci. Total Environ.* 882, 163295. https://doi.org/10.1016/j.scitotenv.2023.163295

Herrera, A., Acosta-Dacal, A., Pérez Luzardo, O., Martínez, I., Rapp, J., Reinold, S., Montesdeoca-Esponda, S., Montero, D., Gómez, M., 2022. Bioaccumulation of additives and chemical contaminants from environmental microplastics in European seabass (*Dicentrarchus labrax*). *Sci. Total Environ.* 822, 153396. https://doi.org/10.1016/j.scitotenv.2022.153396

Hoang, T.C., Felix-Kim, M., 2020. Microplastic consumption and excretion by fathead minnows (Pimephales promelas): Influence of particles size and body shape of fish. *Sci. Total Environ.* 704, 135433. https://doi.org/https://doi.org/10.1016/j.scitotenv.2019.135433

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*)? *Sci. Total Environ.* 793, 148490. https://doi.org/https://doi.org/10.1016/j.scitotenv.2021.148490

Holhorea, P.G., Naya-Català, F., Belenguer, Á., Calduch-Giner, J.A., Pérez-Sánchez, J., 2023. Understanding how high stocking densities and concurrent limited oxygen availability drive social cohesion and adaptive features in regulatory growth, antioxidant defense and lipid metabolism in farmed gilthead sea bream (*Sparus aurata*). *Front. Physiol.* 14, 1272267. https://doi.org/10.3389/fphys.2023.1272267

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. *Sci. Total Environ.* 858, 159976. https://doi.org/10.1016/j.scitotenv.2022.159976

Horie, Y., Mitsunaga, K., Yamaji, K., Hirokawa, S., Uaciquete, D., Ríos, J.M., Yap, C.K., Okamura, H., 2024. Variability in microplastic color preference and intake among selected marine and freshwater fish and crustaceans. *Discover Oceans* 1(1), 5. https://doi.org/10.1007/s44289-024-00005-w

Horsman, P.V., 1982. The amount of garbage pollution from merchant ships. *Mar. Pollut. Bull.* 13, 167–169.

Horvatits, T., Tamminga, M., Liu, B., Sebode, M., Carambia, A., Fischer, L., Püschel, K., Huber, S., Fischer, E.K., 2022. Microplastics detected in cirrhotic liver tissue. *eBioMedicine* 82. https://doi.org/10.1016/j.ebiom.2022.104147

Hoseini, S.M., Khosraviani, K., Hosseinpour Delavar, F., Arghideh, M., Zavvar, F., Hoseinifar, S.H., Van Doan, H., Zabihi, E., Reverter, M., 2022. Hepatic transcriptomic and histopathological responses of common carp, *Cyprinus carpio*, to copper and microplastic exposure. *Mar. Pollut. Bull.* 175, 113401. https://doi.org/10.1016/j.marpolbul.2022.113401

Hoseinifar, S.H., Ashouri, G., Marisaldi, L., Candelma, M., Basili, D., Zimbelli, A., Notarstefano, V., Salvini, L., Randazzo, B., Zarantoniello, M., Pessina, A., Sojan, J.M., Vargas, A., Carnevali, O., 2024. Reducing the use of antibiotics in European aquaculture with vaccines, functional feed additives and optimization of the gut microbiota. J. Mar. Sci. Eng. 12(2). https://doi.org/10.3390/jmse12020204

Hossain, M.B., Banik, P., Nur, A.-A., Choudhury, T.R., Liba, S.I., Albeshr, M.F., Yu, J., Arai, T., 2023a. Microplastics in fish culture ponds: abundance, characterization, and contamination risk assessment. *Front. Environ. Sci.* 11. https://doi.org/10.3389/fenvs.2023.1251158

Hossain, S., Ahmad Shukri, Z.N., Waiho, K., Ibrahim, Y.S., Minhaz, T.M., Kamaruzzan, A.S., Abdul Rahim, A.I., Draman, A.S., Khatoon, H., Islam, Z., Kasan, N.A., 2023b. Microplastics pollution in mud crab (*Scylla* sp.) aquaculture system: First investigation and evidence. *Environ. Pollut.* 329, 121697. https://doi.org/10.1016/j.envpol.2023.121697

Hosseinpour, A., Chamani, A., Mirzaei, R., Mohebbi-Nozar, S., L., 2021. Occurrence, abundance and characteristics of microplastics in some commercial fish of northern coasts of the Persian Gulf. *Mar. Pollut. Bull.* 171, 112693. https://doi.org/10.1016/j.marpolbul.2021.112693

Hoyo-Alvarez, E., Arechavala-Lopez, P., Jiménez-García, M., Solomando, A., Alomar, C., Sureda, A., Moranta, D., Deudero, S., 2022. Effects of pollutants and microplastics ingestion on oxidative stress and monoaminergic activity of seabream brains. *Aquat. Toxicol.* 242, 106048. https://doi.org/10.1016/j.aquatox.2021.106048

Hu, J., Zuo, J., Li, J., Zhang, Y., Ai, X., Zhang, J., Gong, D., Sun, D., 2022. Effects of secondary polyethylene microplastic exposure on crucian (*Carassius carassius*) growth, liver damage, and gut microbiome composition. *Sci. Total Environ.* 802, 149736. https://doi.org/10.1016/j.scitotenv.2021.149736

Hu, L., Chernick, M., Lewis, A.M., Ferguson, P.L., Hinton, D.E., 2020. Chronic microfiber exposure in adult Japanese medaka (*Oryzias latipes*). *PLOS ONE* 15(3), e0229962. https://doi.org/10.1371/journal.pone.0229962

Hu, M., Palić, D., 2020. Micro- and nano-plastics activation of oxidative and inflammatory adverse outcome pathways. *Redox Biol.* 37, 101620. https://doi.org/10.1016/j.redox.2020.101620

Hu, X., Meng, L.-J., Liu, H.-D., Guo, Y.-S., Liu, W.-C., Tan, H.-X., Luo, G.-Z., 2023. Impacts of Nile tilapia (*Oreochromis niloticus*) exposed to microplastics in bioflocs system. *Sci. Total Environ.* 901, 165921. https://doi.org/10.1016/j.scitotenv.2023.165921

Hua, X., Wang, D., 2022. Cellular uptake, transport, and organelle response after exposure to microplastics and nanoplastics: Current knowledge and perspectives for environmental and health risks. *Rev. Environ. Contam. Toxicol.* 260(1), 12. https://doi.org/10.1007/s44169-022-00013-x

Huang, J.-N., Xu, L., Wen, B., Gao, J.-Z., Chen, Z.-Z., 2024. Characteristics and risks of microplastic contamination in aquaculture ponds near the Yangtze Estuary, China. *Environ. Pollut.* 343, 123288. https://doi.org/10.1016/j.envpol.2024.123288

Huang, J.-N., Yang, B.-T., Wen, B., Gao, J.-Z., Chen, Z.-Z., 2023. Occurrence and characteristics of microplastics contamination in different intensive aquaculture systems nearby the Yangtze Estuary, China. *Bull. Environ. Contam. Toxicol.* 110(1), 1. https://doi.org/10.1007/s00128-022-03643-y

Huang, Y., Ding, J., Zhang, G., Liu, S., Zou, H., Wang, Z., Zhu, W., Geng, J., 2021. Interactive effects of microplastics and selected pharmaceuticals on red tilapia: Role of microplastic aging. *Sci. Total Environ.* 752, 142256. https://doi.org/10.1016/j.scitotenv.2020.142256

Hung, C., Klasios, N., Zhu, X., Sedlak, M., Sutton, R., Rochman, C.M., 2021. Methods matter: Methods for sampling microplastic and other anthropogenic particles and their implications for monitoring and ecological risk assessment. *Integr. Environ. Assess. Manag.* 17(1), 282-291. https://doi.org/10.1002/ieam.4325

Huntington, T., 2019. Marine litter and aquaculture gear - white paper. Report produced by Poseidon Aquatic Resources Management Ltd for the Aquaculture Stewardship Council. p. 20.

Hussain, N., Jaitley, V., Florence, A.T., 2001. Recent advances in the understanding of uptake of microparticulates across the gastrointestinal lymphatics. *Adv. Drug Deliv. Rev.* 50(1), 107-142. https://doi.org/10.1016/S0169-409X(01)00152-1

Hutchinson, T.H., Manning, M.J., 1996. Seasonal trends in serum lysozyme activity and total protein concentration in dab (*Limanda limanda* L.) sampled from Lyme Bay, U.K. *Fish Shellfish Immunol.* 6(7), 473-482. https://doi.org/10.1006/fsim.1996.0045

Iheanacho, S.C., Odo, G.E., 2020. Neurotoxicity, oxidative stress biomarkers and haematological responses in African catfish (*Clarias gariepinus*) exposed to polyvinyl chloride microparticles. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology* 232, 108741. https://doi.org/10.1016/j.cbpc.2020.108741

Ilechukwu, I., Das, R.R., Reimer, J.D., 2023. Review of microplastics in museum specimens: An under-utilized tool to better understand the Plasticence. *Mar. Pollut. Bull.* 191, 114922. https://doi.org/10.1016/j.marpolbul.2023.114922

Imasha, H.U.E., Babel, S., 2023. Microplastics contamination in the green mussels (*Perna viridis*) cultured for human consumption in Thailand. *Reg. Stud. Mar. Sci.* 67, 103203. https://doi.org/10.1016/j.rsma.2023.103203

Ismail, R.F., Saleh, N.E., Sayed, A.E.-D.H., 2021. Impacts of microplastics on reproductive performance of male tilapia (*Oreochromis niloticus*) pre-fed on Amphora coffeaeformis. *Environ. Sci. Pollut. Res.* 28(48), 68732-68744. https://doi.org/10.1007/s11356-021-14984-2

Isobe, A., Kubo, K., Tamura, Y., Kako, S.i., Nakashima, E., Fujii, N., 2014. Selective transport of microplastics and mesoplastics by drifting in coastal waters. *Mar. Pollut. Bull.* 89(1), 324-330. https://doi.org/10.1016/j.marpolbul.2014.09.041

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. https://doi.org/10.1016/j.chemosphere.2018.09.031

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. https://doi.org/10.1016/j.aquatox.2021.106004
Jadhav, E.B., Sankhla, M.S., Bhat, R.A., Bhagat, D.S., 2021. Microplastics from food packaging: An overview of human consumption, health threats, and alternative solutions. *Environ. Nanotechnol. Monit. Manag.* 16, 100608. https://doi.org/10.1016/j.enmm.2021.100608
Jakubowska, M., Białowąs, M., Stankevičiūtė, M., Chomiczewska, A., Pažusienė, J., Jonko-Sobuś, K., Hallmann, A., Urban-Malinga, B., 2020. Effects of chronic exposure to microplastics of different polymer types on early life stages of sea trout *Salmo trutta*. *Sci. Total Environ.* 740, 139922. https://doi.org/10.1016/j.scitotenv.2020.139922
Jambeck, J.R., Geyer, R., Wilcox, C., Siegler, T.R., Perryman, M., Andrady, A., Narayan, R., Law, K.L., 2015. Plastic waste inputs from land into the ocean. *Science* 347(6223), 768-771. https://doi.org/10.1126/science.1260352
Jang, F.H., Wong, C., Choo, J., Aun Sia, E.S., Mujahid, A., Müller, M., 2022. Increased transfer of trace metals and *Vibrio* sp. from biodegradable microplastics to catfish *Clarias gariepinus*. *Environ. Pollut.* 298, 118850. https://doi.org/10.1016/j.envpol.2022.118850
Jawdhari, A., Mihăilescu, D.F., Stan, M.S., Bălănescu, M.-V., Vlăsceanu, R.-I., Staicu, C.A., Crăciun, N., Deák, G., 2023. Ingestion of polyvinylchloride powder particles induces oxidative stress and hepatic histopathological changes in *Oreochromis niloticus* (Nile tilapia) - A preliminary study. Sustainability 15(8). https://doi.org/10.3390/su15086494
Jeyasanta, I., Sathish, M.N., Patterson, J., Esmeralda, V.G., R.L, L., 2024. Microplastics contamination in commercial fish meal and feed: a major concern in the cultured organisms. *Chemosphere* 363, 142832. https://doi.org/10.1016/j.chemosphere.2024.142832
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(3). https://doi.org/10.3390/toxics11030282
Jia, R., Han, J., Liu, X., Li, K., Lai, W., Bian, L., Yan, J., Xi, Z., 2023. Exposure to polypropylene microplastics via oral ingestion induces colonic apoptosis and intestinal barrier damage through oxidative stress and inflammation in mice. Toxics 11(2). https://doi.org/10.3390/toxics11020127
Jia, S., Li, X., He, W., Wu, G., 2022a. Protein-sourced feedstuffs for aquatic animals in nutrition research and aquaculture, in: Wu, G. (Ed.), Recent advances in animal nutrition and metabolism. Springer International Publishing, Cham, pp. 237-261.
Jia, X., Liu, Y., He, Y., Yu, H., Liu, Y., Shen, Y., Xu, X., Li, J., 2022b. Exposure to microplastics induces lower survival, oxidative stress, disordered microbiota and altered metabolism in the intestines of grass carp (*Ctenopharyngodon idella*). *Aquac. Fish.* https://doi.org/10.1016/j.aaf.2022.09.004
Jiang, N., Xing, B., Peng, R., Shang, J., Wu, B., Xiao, P., Lin, S., Xu, X., Lu, H., 2022. Inhibition of Cpt1a alleviates oxidative stress-induced chondrocyte senescence via regulating mitochondrial dysfunction and activating mitophagy. *Mech. Ageing Dev.* 205, 111688. https://doi.org/https://doi.org/10.1016/j.mad.2022.111688
Jin, C., Yan, K., Wang, M., Song, W., Kong, X., Zhang, Z., 2023. Identification, characterization and functional analysis of fibroblast growth factors in black rockfish (*Sebastes schlegelii*). *Int. J. Mol. Sci.* 24(4). https://doi.org/10.3390/ijms24043626
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. https://doi.org/10.1016/j.scitotenv.2018.08.353
Jorquera, A., Castillo, C., Murillo, V., Araya, J., Pinochet, J., Narváez, D., Pantoja-Gutiérrez, S., Urbina, M.A., 2022. Physical and anthropogenic drivers shaping the spatial distribution of microplastics in the marine sediments of Chilean fjords. *Sci. Total Environ.* 814, 152506. https://doi.org/10.1016/j.scitotenv.2021.152506
Jovanović, B., Gökdağ, K., Güven, 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. https://doi.org/10.1016/j.marpolbul.2018.03.016
Juan, C.A., Pérez de la Lastra, J.M., Plou, F.J., Pérez-Lebeña, E., 2021. The chemistry of reactive oxygen species (ROS) revisited: Outlining their role in biological macromolecules (DNA, lipids and proteins) and induced pathologies. Int. J. Mol. Sci. 22(9). https://doi.org/10.3390/ijms22094642
Justino, A.K.S., Ferreira, G.V.B., Schmidt, N., Eduardo, L.N., Fauvelle, V., Lenoble, V., Sempéré, R., Panagiotopoulos, C., Mincarone, M.M., Frédou, T., Lucena-Frédou, F., 2022. The role of

mesopelagic fishes as microplastics vectors across the deep-sea layers from the Southwestern Tropical Atlantic. *Environ. Pollut.* 300, 118988. https://doi.org/10.1016/j.envpol.2022.118988
Kacprzak, S., Tijing, L.D., 2022. Microplastics in indoor environment: Sources, mitigation and fate. *J. Environ. Chem. Eng.* 10(2), 107359. https://doi.org/10.1016/j.jece.2022.107359
Kalantzi, I., Pergantis, S.A., Black, K.D., Shimmield, T.M., Papageorgiou, N., Tsapakis, M., Karakassis, I., 2016a. Metals in tissues of seabass and seabream reared in sites with oxic and anoxic substrata and risk assessment for consumers. *Food Chem.* 194, 659-670. https://doi.org/10.1016/j.foodchem.2015.08.072
Kalantzi, I., Zeri, C., Catsiki, V.-A., Tsangaris, C., Strogyloudi, E., Kaberi, H., Vergopoulos, N., Tsapakis, M., 2016b. Assessment of the use of copper alloy aquaculture nets: Potential impacts on the marine environment and on the farmed fish. *Aquaculture* 465, 209-222. https://doi.org/10.1016/j.aquaculture.2016.09.016
Kaloyianni, M., Bobori, D.C., Xanthopoulou, D., Malioufa, G., Sampsonidis, I., Kalogiannis, S., Feidantsis, K., Kastrinaki, G., Dimitriadi, A., Koumoundouros, G., Lambropoulou, D.A., Kyzas, G.Z., Bikiaris, D.N., 2021. Toxicity and functional tissue responses of two freshwater fish after exposure to polystyrene microplastics. Toxics 9(11). https://doi.org/10.3390/toxics9110289
Kanhai, L.D.K., Officer, R., Lyashevska, O., Thompson, R.C., O'Connor, I., 2017. Microplastic abundance, distribution and composition along a latitudinal gradient in the Atlantic Ocean. *Mar. Pollut. Bull.* 115(1), 307-314. https://doi.org/10.1016/j.marpolbul.2016.12.025
Kannankai, M.P., Babu, A.J., Radhakrishnan, A., Alex, R.K., Borah, A., Devipriya, S.P., 2022. Machine learning aided meta-analysis of microplastic polymer composition in global marine environment. *J. Hazard. Mater.* 440, 129801. https://doi.org/10.1016/j.jhazmat.2022.129801
Karami, A., Romano, N., Galloway, T., Hamzah, H., 2016. Virgin microplastics cause toxicity and modulate the impacts of phenanthrene on biomarker responses in African catfish (*Clarias gariepinus*). *Environ. Res.* 151, 58-70. https://doi.org/10.1016/j.envres.2016.07.024
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. *Mar. Pollut. Bull.* 150, 110687. https://doi.org/10.1016/j.marpolbul.2019.110687
Karbalaei, S., Hanachi, P., Rafiee, G., Seifori, P., Walker, T.R., 2021. Toxicity of polystyrene microplastics on juvenile *Oncorhynchus mykiss* (rainbow trout) after individual and combined exposure with chlorpyrifos. *J. Hazard. Mater.* 403, 123980. https://doi.org/10.1016/j.jhazmat.2020.123980
Kardgar, A.K., 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. https://doi.org/10.1016/j.scitotenv.2023.163425
Kartar, S., Milne, R.A., Sainsbury, M., 1973. Polystyrene waste in the Severn Estuary. *Mar. Pollut. Bull.* 4(9), 144. https://doi.org/10.1016/0025-326X(73)90010-6
Keerthika, K., Padmavathy, P., Rani, V., Jeyashakila, R., Aanand, S., Kutty, R., 2022. Spatial, seasonal and ecological risk assessment of microplastics in sediment and surface water along the Thoothukudi, south Tamil Nadu, south east India. *Environ. Monit. Assess.* 194(11), 820. https://doi.org/10.1007/s10661-022-10468-z
Kenyon, K.W., Kridler, E., 1969. Laysan albatrosses swallow indigestible matter *Auk* 86(2), 339-343. https://doi.org/10.2307/4083505
Kermenidou, M.V., Frydas, I.S., Moschoula, E., Kousis, D., Christofilos, D., Karakitsios, S.P., Sarigiannis, D.A.A., 2023. Quantification and characterization of microplastics in the Thermaic Gulf, in the North Aegean Sea. *Sci. Total Environ.* 892, 164299. https://doi.org/10.1016/j.scitotenv.2023.164299
Kieu-Le, T.-C., Thuong, Q.-T., Truong, T.-N.-S., Le, T.-M.-T., Tran, Q.-V., Strady, E., 2023. Baseline concentration of microplastics in surface water and sediment of the northern branches of the Mekong River Delta, Vietnam. *Mar. Pollut. Bull.* 187, 114605. https://doi.org/10.1016/j.marpolbul.2023.114605
Kılıç, E., 2022. Microplastic ingestion evidence by economically important farmed fish species from Turkey. *Mar. Pollut. Bull.* 183, 114097. https://doi.org/10.1016/j.marpolbul.2022.114097
Kim, D., Kim, H., An, Y.-J., 2021a. Effects of synthetic and natural microfibers on Daphnia magna–Are they dependent on microfiber type? *Aquat. Toxicol.* 240, 105968. https://doi.org/10.1016/j.aquatox.2021.105968
Kim, J., Poirier, D.G., Helm, P.A., Bayoumi, M., Rochman, C.M., 2020. No evidence of spherical microplastics (10–300 μm) translocation in adult rainbow trout (*Oncorhynchus mykiss*) after a

two-week dietary exposure. *PLOS ONE* 15(9), e0239128. https://doi.org/10.1371/journal.pone.0239128
Kim, L., Kim, S.A., Kim, T.H., Kim, J., An, Y.-J., 2021b. Synthetic and natural microfibers induce gut damage in the brine shrimp *Artemia franciscana*. *Aquat. Toxicol.* 232, 105748. https://doi.org/10.1016/j.aquatox.2021.105748
Knoblauch, D., Mederake, L., 2021. Government policies combatting plastic pollution. *Curr. Opin. Toxicol.* 28, 87-96. https://doi.org/10.1016/j.cotox.2021.10.003
Koller, M., Saleh, H.M., 2018. Introductory chapter: Introducing heavy metals, in: Saleh, H.E.-D.M., Aglan, R.F. (Eds.), Heavy metals.
Kousoulaki, K., Sæther, B.-S., Albrektsen, S., Noble, C., 2015. Review on European sea bass (*Dicentrarchus labrax*, Linnaeus, 1758) nutrition and feed management: a practical guide for optimizing feed formulation and farming protocols. *Aquac. Nutr.* 21(2), 129-151. https://doi.org/10.1111/anu.12233
Krüger, L., Casado-Coy, N., Valle, C., Ramos, M., Sánchez-Jerez, P., Gago, J., Carretero, O., Beltran-Sanahuja, A., Sanz-Lazaro, C., 2020. Plastic debris accumulation in the seabed derived from coastal fish farming. *Environ. Pollut.* 257, 113336. https://doi.org/10.1016/j.envpol.2019.113336
Kumar, M., Naik, D.K., Maharana, D., Das, M., Jaiswal, E., Naik, A.S., Kumari, N., 2024. Sediment-associated microplastics in Chilika lake, India: Highlighting their prevalence, polymer types, possible sources, and ecological risks. *Sci. Total Environ.* 914, 169707. https://doi.org/10.1016/j.scitotenv.2023.169707
Kutralam-Muniasamy, G., Shruti, V.C., Pérez-Guevara, F., Roy, P.D., Martínez, I.E., 2023. Consumption of commercially sold dried fish snack “Charales” contaminated with microplastics in Mexico. *Environ. Pollut.* 332, 121961. https://doi.org/10.1016/j.envpol.2023.121961
Lai, W., Xu, D., Li, J., Wang, Z., Ding, Y., Wang, X., Li, X., Xu, N., Mai, K., Ai, Q., 2021. Dietary polystyrene nanoplastics exposure alters liver lipid metabolism and muscle nutritional quality in carnivorous marine fish large yellow croaker (*Larimichthys crocea*). *J. Hazard. Mater.* 419, 126454. https://doi.org/10.1016/j.jhazmat.2021.126454
Lam, T.W.L., Fok, L., Ma, A.T.H., Li, H.-X., Xu, X.-R., Cheung, L.T.O., Wong, M.H., 2022. Microplastic contamination in marine-cultured fish from the Pearl River Estuary, South China. *Sci. Total Environ.* 827, 154281. https://doi.org/10.1016/j.scitotenv.2022.154281
Lau, W.W.Y., Shiran, Y., Bailey, R.M., Cook, E., Stuchtey, M.R., Koskella, J., Velis, C.A., Godfrey, L., Boucher, J., Murphy, M.B., Thompson, R.C., Jankowska, E., Castillo Castillo, A., Pilditch, T.D., Dixon, B., Koerselman, L., Kosior, E., Favoino, E., Gutberlet, J., Baulch, S., Atreya, M.E., Fischer, D., He, K.K., Petit, M.M., Sumaila, U.R., Neil, E., Bernhofen, M.V., Lawrence, K., Palardy, J.E., 2020. Evaluating scenarios toward zero plastic pollution. *Science* 369(6510), 1455-1461. https://doi.org/10.1126/science.aba9475
Laursen, S.N., Fruergaard, M., Dodhia, M.S., Posth, N.R., Rasmussen, M.B., Larsen, M.N., Shilla, D., Shilla, D., Kilawe, J.J., Kizenga, H.J., Andersen, T.J., 2023. Settling of buoyant microplastic in estuaries: The importance of flocculation. *Sci. Total Environ.* 886, 163976. https://doi.org/10.1016/j.scitotenv.2023.163976
Lavers, J.L., Bond, A.L., 2017. Exceptional and rapid accumulation of anthropogenic debris on one of the world's most remote and pristine islands. *Proc. Natl. Acad. Sci.: Biol. Sci.* 114(23), 6052-6055. https://doi.org/10.1073/pnas.1619818114
Law, K.L., Morét-Ferguson, S., Maximenko, N.A., Proskurowski, G., Peacock, E.E., Hafner, J., Reddy, C.M., 2010. Plastic accumulation in the North Atlantic subtropical gyre. *Science* 329(5996), 1185-1188. https://doi.org/doi:10.1126/science.1192321
Lawen, A., Lawen, J., Turner, A., 2024. Beached plastic and other anthropogenic debris in the inner Seychelles islands: Results of a citizen science approach. *Mar. Pollut. Bull.* 201, 116176. https://doi.org/10.1016/j.marpolbul.2024.116176
Le, N.D., Hoang, T.T.H., Duong, T.T., Lu, X., Pham, T.M.H., Phung, T.X.B., Le, T.M.H., Duong, T.H., Nguyen, T.D., Le, T.P.Q., 2022. First observation of microplastics in surface sediment of some aquaculture ponds in Hanoi city, Vietnam. *J. Hazard. Mater. Adv.* 6, 100061. https://doi.org/10.1016/j.hazadv.2022.100061
Leads, R.R., Weinstein, J.E., Kell, S.E., Overcash, J.M., Ertel, B.M., Gray, A.D., 2023. Spatial and temporal variability of microplastic abundance in estuarine intertidal sediments: Implications for sampling frequency. *Sci. Total Environ.* 859, 160308. https://doi.org/10.1016/j.scitotenv.2022.160308
Ledesma, A., de Lacoba, M.G., Rial, E., 2002. The mitochondrial uncoupling proteins. *Genome Biology* 3(12). https://doi.org/10.1186/gb-2002-3-12-reviews3015

Lee, J., Hong, S., Song, Y.K., Hong, S.H., Jang, Y.C., Jang, M., Heo, N.W., Han, G.M., Lee, M.J., Kang, D., Shim, W.J., 2013. Relationships among the abundances of plastic debris in different size classes on beaches in South Korea. *Mar. Pollut. Bull.* 77(1), 349-354. https://doi.org/10.1016/j.marpolbul.2013.08.013

Lehtiniemi, M., Hartikainen, S., Näkki, P., Engström-Öst, J., Koistinen, A., Setälä, O., 2018. Size matters more than shape: Ingestion of primary and secondary microplastics by small predators. *Food Webs* 17, e00097. https://doi.org/10.1016/j.fooweb.2018.e00097

Leonard, S.V.L., Liddle, C.R., Atherall, C.A., Chapman, E., Watkins, M., Calaminus, S.D.J., Rotchell, J.M., 2024. Microplastics in human blood: Polymer types, concentrations and characterisation using μFTIR. *Environ. Int.* 188, 108751. https://doi.org/10.1016/j.envint.2024.108751

Leslie, H.A., van Velzen, M.J.M., Brandsma, S.H., Vethaak, A.D., Garcia-Vallejo, J.J., Lamoree, M.H., 2022. Discovery and quantification of plastic particle pollution in human blood. *Environ. Int.* 163, 107199. https://doi.org/https://doi.org/10.1016/j.envint.2022.107199

Li, B., Liang, W., Liu, Q.-X., Fu, S., Ma, C., Chen, Q., Su, L., Craig, N.J., Shi, H., 2021a. Fish ingest microplastics unintentionally. *Environ. Sci. Technol.* 55(15), 10471-10479. https://doi.org/10.1021/acs.est.1c01753

Li, H., Liu, H., Bi, L., Liu, Y., Jin, L., Peng, R., 2024a. Immunotoxicity of microplastics in fish. *Fish Shellfish Immunol.* 150, 109619. https://doi.org/10.1016/j.fsi.2024.109619

Li, H., Ma, M.-L., Luo, S., Zhang, R.-M., Han, P., Hu, W., 2012. Metabolic responses to ethanol in *Saccharomyces cerevisiae* using a gas chromatography tandem mass spectrometry-based metabolomics approach. *Int. J. Biochem. Cell Biol.* 44(7), 1087-1096. https://doi.org/10.1016/j.biocel.2012.03.017

Li, J., Liu, J., Wang, X., Zhang, T., Wang, D., Shan, E., Teng, J., Zhao, J., Wang, Q., 2024b. Vertical transfer of microplastics in nearshore water by cultured filter-feeding oysters. *J. Hazard. Mater.* 475, 134769. https://doi.org/10.1016/j.jhazmat.2024.134769

Li, J., Zhang, L., Dang, X., Su, L., Jabeen, K., Wang, H., Wang, Z., 2022a. Effects of cooking methods on microplastics in dried shellfish. *Sci. Total Environ.* 837, 155787. https://doi.org/10.1016/j.scitotenv.2022.155787

Li, K., Wang, F., Liu, S., Cheng, X., Xu, J., Liu, X., Zhang, L., 2024c. Response and adaptation mechanisms of *Apostichopus japonicus* to single and combined anthropogenic stresses of polystyrene microplastics or cadmium. *Mar. Pollut. Bull.* 204, 116519. https://doi.org/10.1016/j.marpolbul.2024.116519

Li, L.a., Gu, H., Chang, X., Huang, W., Sokolova, I.M., Wei, S., Sun, L., Li, S., Wang, X., Hu, M., Zeng, J., Wang, Y., 2021b. Oxidative stress induced by nanoplastics in the liver of juvenile large yellow croaker *Larimichthys crocea*. *Mar. Pollut. Bull.* 170, 112661. https://doi.org/10.1016/j.marpolbul.2021.112661

Li, L.a., Xu, R., Jiang, L., Xu, E.G., Wang, M., Wang, J., Li, B., Hu, M., Zhang, L., Wang, Y., 2021c. Effects of microplastics on immune responses of the yellow catfish *Pelteobagrus fulvidraco* under hypoxia. *Front. Physiol.* 12. https://doi.org/10.3389/fphys.2021.753999

Li, Q., Ma, C., Zhang, Q., Shi, H., 2021d. Microplastics in shellfish and implications for food safety. *Curr. Opin. Food Sci.* 40, 192-197. https://doi.org/10.1016/j.cofs.2021.04.017

Li, W., Pan, Z., Xu, J., Liu, Q., Zou, Q., Lin, H., Wu, L., Huang, H., 2022b. Microplastics in a pelagic dolphinfish (*Coryphaena hippurus*) from the Eastern Pacific Ocean and the implications for fish health. *Sci. Total Environ.* 809, 151126. https://doi.org/10.1016/j.scitotenv.2021.151126

Li, Y., Chen, G., Xu, K., Huang, K., Wang, J., 2021e. Microplastics environmental effect and risk assessment on the aquaculture systems from South China. Int. J. Gastron. Food Sci. 18(4). https://doi.org/10.3390/ijerph18041869

Li, Z., Chen, F., Wei, M., Zhi, L., Su, Z., Chong, Y., Xiao, Z., Wang, J., 2024d. Concurrent impacts of polystyrene nanoplastic exposure and *Aeromonas hydrophila* infection on oxidative stress, immune response and intestinal microbiota of grass carp (*Ctenopharyngodon idella*). *Sci. Total Environ.* 912, 169225. https://doi.org/10.1016/j.scitotenv.2023.169225

Li, Z., Yan, L., Junaid, M., Chen, X., Liao, H., Gao, D., Wang, Q., Zhang, Y., Wang, J., 2023. Impacts of polystyrene nanoplastics at the environmentally relevant and sub-lethal concentrations on the oxidative stress, immune responses, and gut microbiota to grass carp (*Ctenopharyngodon idella*). *J. Hazard. Mater.* 441, 129995. https://doi.org/10.1016/j.jhazmat.2022.129995

Liang, W., Li, B., Jong, M.-C., Ma, C., Zuo, C., Chen, Q., Shi, H., 2023. Process-oriented impacts of microplastic fibers on behavior and histology of fish. *J. Hazard. Mater.* 448, 130856. https://doi.org/10.1016/j.jhazmat.2023.130856

Liao, H., Liu, S., Junaid, M., Gao, D., Ai, W., Chen, G., Wang, J., 2022. Di-(2-ethylhexyl) phthalate exacerbated the toxicity of polystyrene nanoplastics through histological damage and

intestinal microbiota dysbiosis in freshwater *Micropterus salmoides*. *Water Res.* 219, 118608. https://doi.org/https://doi.org/10.1016/j.watres.2022.118608
Liboiron, M., Melvin, J., Richárd, N., Saturno, J., Ammendolia, J., Liboiron, F., Charron, L., Mather, C., 2019. Low incidence of plastic ingestion among three fish species significant for human consumption on the island of Newfoundland, Canada. *Mar. Pollut. Bull.* 141, 244-248. https://doi.org/10.1016/j.marpolbul.2019.02.057
Lima, A.R.A., Barletta, M., Costa, M.F., 2015. Seasonal distribution and interactions between plankton and microplastics in a tropical estuary. *Estuar. Coast. Shelf Sci.* 165, 213-225. https://doi.org/10.1016/j.ecss.2015.05.018
Lin, F., Zhang, Q., Xie, J., Lin, Y., Chen, Y., Mao, K., Qin, Y., Diao, X., 2022. Microplastics in biota and surface seawater from tropical aquaculture area in Hainan, China. *Gondwana Res.* 108, 41-48. https://doi.org/10.1016/j.gr.2021.11.008
Lincoln, S., Andrews, B., Birchenough, S.N.R., Chowdhury, P., Engelhard, G.H., Harrod, O., Pinnegar, J.K., Townhill, B.L., 2022. Marine litter and climate change: Inextricably connected threats to the world's oceans. *Sci. Total Environ.* 837, 155709. https://doi.org/10.1016/j.scitotenv.2022.155709
Lindholm-Lehto, P., 2023. Water quality monitoring in recirculating aquaculture systems. *Aquac. Fish Fish.* 3(2), 113-131. https://doi.org/10.1002/aff2.102
LITTERBASE/AWI. Tekman, M.B., Gutow, L., Macario, A., Haas, A., Walter, A., Bergmann, Melanie, Alfred-Wegener-Institut Helmholtz-Zentrum für Polar- und Meeresforschung.
Liu, J., Yang, Y., Ding, J., Zhu, B., Gao, W., 2019. Microfibers: a preliminary discussion on their definition and sources. *Environ. Sci. Pollut. Res.* 26(28), 29497-29501. https://doi.org/10.1007/s11356-019-06265-w
Liu, M.-J., Guo, H.-Y., Gao, J., Zhu, K.-C., Guo, L., Liu, B.-S., Zhang, N., Jiang, S.-G., Zhang, D.-C., 2023a. Characteristics of microplastic pollution in golden pompano (*Trachinotus ovatus*) aquaculture areas and the relationship between colonized-microbiota on microplastics and intestinal microflora. *Sci. Total Environ.* 856, 159180. https://doi.org/10.1016/j.scitotenv.2022.159180
Liu, M., Liu, X., Xu, J., 2024. Influence of microplastic pollution on the toxicity of potamodromous fish grass carp (*Ctenopharyngodon idellus*) and its swimming capacity. *J. Environ. Chem. Eng.* 12(5), 113520. https://doi.org/10.1016/j.jece.2024.113520
Liu, S., Guo, J., Liu, X., Yang, R., Wang, H., Sun, Y., Chen, B., Dong, R., 2023b. Detection of various microplastics in placentas, meconium, infant feces, breastmilk and infant formula: A pilot prospective study. *Sci. Total Environ.* 854, 158699. https://doi.org/https://doi.org/10.1016/j.scitotenv.2022.158699
Liu, S., Huang, J., Zhang, W., Shi, L., Yi, K., Yu, H., Zhang, C., Li, S., Li, J., 2022a. Microplastics as a vehicle of heavy metals in aquatic environments: A review of adsorption factors, mechanisms, and biological effects. *J. Environ. Manage.* 302, 113995. https://doi.org/10.1016/j.jenvman.2021.113995
Liu, S., Li, H., Wang, J., Wu, B., Guo, X., 2022b. Polystyrene microplastics aggravate inflammatory damage in mice with intestinal immune imbalance. *Sci. Total Environ.* 833, 155198. https://doi.org/10.1016/j.scitotenv.2022.155198
Liu, S., Yan, L., Zhang, Y., Junaid, M., Wang, J., 2022c. Polystyrene nanoplastics exacerbated the ecotoxicological and potential carcinogenic effects of tetracycline in juvenile grass carp (*Ctenopharyngodon idella*). *Sci. Total Environ.* 803, 150027. https://doi.org/10.1016/j.scitotenv.2021.150027
Liu, Y., Jia, X., Zhu, H., Zhang, Q., He, Y., Shen, Y., Xu, X., Li, J., 2022d. The effects of exposure to microplastics on grass carp (*Ctenopharyngodon idella*) at the physiological, biochemical, and transcriptomic levels. *Chemosphere* 286, 131831. https://doi.org/10.1016/j.chemosphere.2021.131831
Liu, Y., Qiu, X., Xu, X., Takai, Y., Ogawa, H., Shimasaki, Y., Oshima, Y., 2021. Uptake and depuration kinetics of microplastics with different polymer types and particle sizes in Japanese medaka (*Oryzias latipes*). *Ecotoxicol. Environ. Saf.* 212, 112007. https://doi.org/10.1016/j.ecoenv.2021.112007
Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2−ΔΔCT method. *Methods* 25(4), 402-408. https://doi.org/10.1006/meth.2001.1262
Lomolino, G., Crapisi, A., Cagnin, M., 2016. Study of elements concentrations of European seabass (*Dicentrarchus labrax*) fillets after cooking on steel, cast iron, teflon, aluminum and ceramic pots. *Int. J. Gastron. Food Sci.* 5-6, 1-9. https://doi.org/10.1016/j.ijgfs.2016.06.001

Lopes, C., Ambrosino, A.C., Figueiredo, C., Caetano, M., Santos, M.M., Garrido, S., Raimundo, J., 2023. Microplastic distribution in different tissues of small pelagic fish of the Northeast Atlantic Ocean. *Sci. Total Environ.* 901, 166050. https://doi.org/10.1016/j.scitotenv.2023.166050

Lopes, C., Raimundo, J., Caetano, M., Garrido, S., 2020. Microplastic ingestion and diet composition of planktivorous fish. *Limnol. Oceanogr. Bull.* 5(1), 103-112. https://doi.org/10.1002/lol2.10144

Lourenço, H.M., Afonso, C., Anacleto, P., Martins, M.F., Nunes, M.L., Lino, A.R., 2012. Elemental composition of four farmed fish produced in Portugal. *Int. J. Food Sci. Nutr.* 63(7), 853-859. https://doi.org/10.3109/09637486.2012.681632

Lu, J., Zhang, Y., Wu, J., Luo, Y., 2019. Effects of microplastics on distribution of antibiotic resistance genes in recirculating aquaculture system. *Ecotoxicol. Environ. Saf.* 184, 109631. https://doi.org/10.1016/j.ecoenv.2019.109631

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., Rios Mendoza, L.M., 2022a. Chronic exposure to high-density polyethylene microplastic through feeding alters the nutrient metabolism of juvenile yellow perch (*Perca flavescens*). *Anim. Nutr.* 9, 143-158. https://doi.org/10.1016/j.aninu.2022.01.007

Lu, X., Zhang, J.-X., Zhang, L., Wu, D., Tian, J., Yu, L.-J., He, L., Zhong, S., Du, H., Deng, D.-F., Ding, Y.-Z., Wen, H., Jiang, M., 2022b. Comprehensive understanding the impacts of dietary exposure to polyethylene microplastics on genetically improved farmed tilapia (*Oreochromis niloticus*): tracking from growth, microbiota, metabolism to gene expressions. *Sci. Total Environ.* 841, 156571. https://doi.org/10.1016/j.scitotenv.2022.156571

Lu, Y., Wang, P., Wang, C., Zhang, M., Cao, X., Chen, C., Wang, C., Xiu, C., Du, D., Cui, H., Li, X., Qin, W., Zhang, Y., Wang, Y., Zhang, A., Yu, M., Mao, R., Song, S., Johnson, A.C., Shao, X., Zhou, X., Wang, T., Liang, R., Su, C., Zheng, X., Zhang, S., Lu, X., Chen, Y., Zhang, Y., Li, Q., Ono, K., Stenseth, N.C., Visbeck, M., Ittekkot, V., 2022c. Multiple pollutants stress the coastal ecosystem with climate and anthropogenic drivers. *J. Hazard. Mater.* 424, 127570. https://doi.org/10.1016/j.jhazmat.2021.127570

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. *Environ. Sci. Technol.* 50(7), 4054-4060. https://doi.org/10.1021/acs.est.6b00183

Luo, X., Sun, D., Wang, Y., Zhang, F., Wang, Y., 2020. Cpt1a promoted ROS-induced oxidative stress and inflammation in liver injury via the Nrf2/HO-1 and NLRP3 inflammasome signaling pathway. *Can. J. Physiol. Pharmacol.* 99(5), 468-477. https://doi.org/10.1139/cjpp-2020-0165

Lushchak, V.I., 2016. Contaminant-induced oxidative stress in fish: a mechanistic approach. *Fish Physiol. Biochem.* 42(2), 711-747. https://doi.org/10.1007/s10695-015-0171-5

Lusher, A.L., Hollman, P.C.H., Mendoza-Hill, J.J., 2017. Microplastics in fisheries and aquaculture: Status of knowledge on their occurrence and implications for aquatic organisms and food safety. FAO, Rome, Italy, p. 147.

Lv, W., Shen, Y., Xu, S., Wu, B., Zhang, Z., Liu, S., 2023. Underestimated health risks: Dietary restriction magnify the intestinal barrier dysfunction and liver injury in mice induced by polystyrene microplastics. *Sci. Total Environ.* 898, 165502. https://doi.org/10.1016/j.scitotenv.2023.165502

Lv, W., Yuan, Q., He, D., Lv, W., Zhou, W., 2020. Microplastic contamination caused by different rearing modes of Asian swamp eel (*Monopterus albus*). *Aquac. Res.* 51(12), 5084-5095. https://doi.org/10.1111/are.14847

Lv, W., Zhou, W., Lu, S., Huang, W., Yuan, Q., Tian, M., Lv, W., He, D., 2019. Microplastic pollution in rice-fish co-culture system: A report of three farmland stations in Shanghai, China. *Sci. Total Environ.* 652, 1209-1218. https://doi.org/10.1016/j.scitotenv.2018.10.321

Lykaki, M., Zhang, Y.-Q., Markiewicz, M., Brandt, S., Kolbe, S., Schrick, J., Rabe, M., Stolte, S., 2021. The influence of textile finishing agents on the biodegradability of shed fibres. *Green Chem.* 23, 5212-5221. https://doi.org/10.1039/D1GC00883H

Ma, J., Niu, X., Zhang, D., Lu, L., Ye, X., Deng, W., Li, Y., Lin, Z., 2020. High levels of microplastic pollution in aquaculture water of fish ponds in the Pearl River Estuary of Guangzhou, China. *Sci. Total Environ.* 744, 140679. https://doi.org/10.1016/j.scitotenv.2020.140679

Ma, T., Wu, P., Wang, L., Li, Q., Li, X., Luo, Y., 2023. Toxicity of per- and polyfluoroalkyl substances to aquatic vertebrates. *Front. Environ. Sci.* 11. https://doi.org/10.3389/fenvs.2023.1101100

MacAulay, S., Masud, N., Davies-Jones, J., Ward, B.D., Cable, J., 2023. The impacts of synthetic and cellulose-based fibres and their associated dyes on fish hosts and parasite health. *Environ. Sci. Pollut. Res.* 30(58), 121558-121568. https://doi.org/10.1007/s11356-023-30794-0

Mackay, T., Ridge, I.M.L., 2022. Abrasion testing of fibre rope on impermeable fabrics for wave energy converter systems. *Renew. Energy* 201, 993-1009. https://doi.org/10.1016/j.renene.2022.11.052
Madden, O., Charola, A.E., Cobb, K.C., DePriest, P.T., Koestler, R.J., 2019. The Age of Plastic: Ingenuity and responsibility (Proceedings of the 2012 MCI Symposium). Smithsonian Institution Scholarly Press.
Maes, T., Van der Meulen, M.D., Devriese, L.I., Leslie, H.A., Huvet, A., Frère, L., Robbens, J., Vethaak, A.D., 2017. Microplastics baseline surveys at the water surface and in sediments of the North-east Atlantic. *Front. Mar. Sci.* 4. https://doi.org/10.3389/fmars.2017.00135
Magalhães, R., Martins, N., Fontinha, F., Olsen, R.E., Serra, C.R., Peres, H., Oliva-Teles, A., 2023. Dietary ARA, DHA, and carbohydrate ratios affect the immune status of gilthead sea bream juveniles upon bacterial challenge. Animals 13(11). https://doi.org/10.3390/ani13111770
Malli, A., Corella-Puertas, E., Hajjar, C., Boulay, A.-M., 2022. Transport mechanisms and fate of microplastics in estuarine compartments: A review. *Mar. Pollut. Bull.* 177, 113553. https://doi.org/10.1016/j.marpolbul.2022.113553
Mancia, A., Chenet, T., Bono, G., Geraci, M.L., Vaccaro, C., Munari, C., Mistri, M., Cavazzini, A., Pasti, L., 2020. Adverse effects of plastic ingestion on the Mediterranean small-spotted catshark (*Scyliorhinus canicula*). *Mar. Environ. Res.* 155, 104876. https://doi.org/10.1016/j.marenvres.2020.104876
Mancuso, M., Panarello, G., Falco, F., Di Paola, D., Serena, S., Capillo, G., Romeo, T., Presti, G., Gullotta, E., Spanò, N., Bono, G., Salvatore, G., Bottari, T., 2022. Investigating the effects of microplastic ingestion in *Scyliorhinus canicula* from the South of Sicily. *Sci. Total Environ.* 850, 157875. https://doi.org/10.1016/j.scitotenv.2022.157875
Marengo, M., Durieux, E.D.H., Ternengo, S., Lejeune, P., Degrange, E., Pasqualini, V., Gobert, S., 2018. Comparison of elemental composition in two wild and cultured marine fish and potential risks to human health. *Ecotoxicol. Environ. Saf.* 158, 204-212. https://doi.org/10.1016/j.ecoenv.2018.04.034
Martins, A., Barboza, L.G., Vieira, L.R., Botelho, M.J., Vale, C., Guilhermino, L., 2025. Relations between microplastic contamination and stress biomarkers under two seasonal conditions in wild carps, mullets and flounders. *Mar. Environ. Res.* 204, 106925. https://doi.org/10.1016/j.marenvres.2024.106925
Martins, C.I.M., Pistrin, M.G., Ende, S.S.W., Eding, E.H., Verreth, J.A.J., 2009. The accumulation of substances in Recirculating Aquaculture Systems (RAS) affects embryonic and larval development in common carp *Cyprinus carpio*. *Aquaculture* 291(1), 65-73. https://doi.org/10.1016/j.aquaculture.2009.03.001
Martos-Sitcha, J.A., Simó-Mirabet, P., de las Heras, V., Calduch-Giner, J.À., Pérez-Sánchez, J., 2019. Tissue-specific orchestration of gilthead sea bream resilience to hypoxia and high stocking density. *Front. Physiol.* 10. https://doi.org/10.3389/fphys.2019.00840
Massa, F., Onofri, L., Fezzardi, D., Nunes, P.A.L.D., Svensson, L.E., Markandya, A., 2017. Handbook on the Economics and Management of Sustainable Oceans, in: Chapter 4: Aquaculture in the Mediterranean and the Black Sea: a Blue Growth perspective. Edward Elgar Publishing.
Mateos-Cárdenas, A., O'Halloran, J., van Pelt, F.N.A.M., Jansen, M.A.K., 2021. Beyond plastic microbeads – Short-term feeding of cellulose and polyester microfibers to the freshwater amphipod *Gammarus duebeni*. *Sci. Total Environ.* 753, 141859. https://doi.org/10.1016/j.scitotenv.2020.141859
Mathalon, A., Hill, P., 2014. Microplastic fibers in the intertidal ecosystem surrounding Halifax Harbor, Nova Scotia. *Mar. Pollut. Bull.* 81(1), 69-79. https://doi.org/10.1016/j.marpolbul.2014.02.018
Matias, R.S., Gomes, S., Barboza, L.G.A., Almeida, C.M.R., Marques, A., Guilhermino, L., Valente, L.M.P., 2024. Occurrence of microplastics and metals in European seabass produced in different aquaculture systems: Implications for human exposure, risk, and food safety. *Sci. Total Environ.* https://doi.org/10.1016/j.scitotenv.2024.172535
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/10.1016/j.chemosphere.2023.139055
Mattsson, K., Johnson, E.V., Malmendal, A., Linse, S., Hansson, L.-A., Cedervall, T., 2017. Brain damage and behavioural disorders in fish induced by plastic nanoparticles delivered through the food chain. *Sci. Rep.* 7(1), 11452. https://doi.org/10.1038/s41598-017-10813-0
Mazurais, D., Ernande, B., Quazuguel, P., Sévère, A., Huelvan, C., Madec, L., Mouchel, O., Soudant, P., Robbens, J., Huvet, A., Zambonino-Infante, J.-L., 2015. Evaluation of the impact of

polyethylene microbeads ingestion in European sea bass (*Dicentrarchus labrax*) larvae. *Mar. Environ. Res.* 112, 78-85. https://doi.org/10.1016/j.marenvres.2015.09.009
McFarland, D.C., Velleman, S.G., Pesall, J.E., Liu, C., 2006. Effect of myostatin on turkey myogenic satellite cells and embryonic myoblasts. *Comp. Biochem. Physiol. Part A: Mol. Integr. Physiol.* 144(4), 501-508. https://doi.org/10.1016/j.cbpa.2006.04.020
McIlwraith, H.K., Kim, J., Helm, P., Bhavsar, S.P., Metzger, J.S., Rochman, C.M., 2021. Evidence of microplastic translocation in wild-caught fish and implications for microplastic accumulation dynamics in food webs. *Environ. Sci. Technol.* 55(18), 12372-12382. https://doi.org/10.1021/acs.est.1c02922
Meikle, J.L., 1995. American plastic: A cultural history. Rutgers University Press, New Jersey, USA.
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/10.1016/j.chemosphere.2023.139924
Michailidou, K., Palisidou, C., Feidantsis, K., Ainali, N.M., Kastrinaki, G., Lambropoulou, D.A., Kyzas, G.Z., Bikiaris, D.N., Kaloyianni, M., Bobori, D.C., 2024. Impact of aged and virgin polyethylene microplastics on multi end-points effects of freshwater fish tissues. *Sci. Total Environ.* 948, 174704. https://doi.org/10.1016/j.scitotenv.2024.174704
Milenkovic, B., Stajic, J.M., Stojic, N., Pucarevic, M., Strbac, S., 2019. Evaluation of heavy metals and radionuclides in fish and seafood products. *Chemosphere* 229, 324-331. https://doi.org/10.1016/j.chemosphere.2019.04.189
Miller, M.E., Motti, C.A., Hamann, M., Kroon, F.J., 2023. Assessment of microplastic bioconcentration, bioaccumulation and biomagnification in a simple coral reef food web. *Sci. Total Environ.* 858, 159615. https://doi.org/10.1016/j.scitotenv.2022.159615
Miserli, K., Lykos, C., Kalampounias, A.G., Konstantinou, I., 2023. Screening of microplastics in aquaculture systems (fish, mussel, and water samples) by FTIR, Scanning Electron Microscopy–Energy Dispersive Spectroscopy and Micro-Raman Spectroscopies. Appl. Sci. 13(17). https://doi.org/10.3390/app13179705
Mishra, P., Kaviarasan, T., Sambandam, M., Dhineka, K., Murthy, M.V.R., Iyengar, G., Singh, J., Ravichandran, M., 2023. Assessment of national beach litter composition, sources, and management along the Indian coast - a citizen science approach. *Mar. Pollut. Bull.* 186, 114405. https://doi.org/10.1016/j.marpolbul.2022.114405
Mitrano, D.M., Wick, P., Nowack, B., 2021. Placing nanoplastics in the context of global plastic pollution. *Nat. Nanotechnol.* 16(5), 491-500. https://doi.org/10.1038/s41565-021-00888-2
Mladinich, K., Holohan, B.A., Shumway, S.E., Ward, J.E., 2024. Abundance of microplastics at and near a shellfish aquaculture farm: An eastern oyster (*Crassostrea virginica*) transplant study. *Mar. Environ. Res.* 199, 106606. https://doi.org/10.1016/j.marenvres.2024.106606
Mnyoro, M.e.S., Munubi, R.N., Pedersen, L.-F., Chenyambuga, S.W., 2022. Evaluation of biofilter performance with alternative local biomedia in pilot scale recirculating aquaculture systems. *J. Clean. Prod.* 366, 132929. https://doi.org/10.1016/j.jclepro.2022.132929
Mohamed Nor, N.H., Kooi, M., Diepens, N.J., Koelmans, A.A., 2021. Lifetime accumulation of microplastic in children and adults. *Environ. Sci. Technol.* 55(8), 5084-5096. https://doi.org/10.1021/acs.est.0c07384
Mohandas, J., Marshall, J.J., Duggin, G.G., Horvath, J.S., Tiller, D.J., 1984. Differential distribution of glutathione and glutathione-related enzymes in rabbit kidney: Possible implications in analgesic nephropathy. *Biochem. Pharmacol.* 33(11), 1801-1807. https://doi.org/10.1016/0006-2952(84)90353-8
Mohsen, M., Lin, J., Lu, K., Wang, L., Zhang, C., 2024a. Microplastic pollution in aquafeed of diverse aquaculture animals. *Heliyon* 10(17). https://doi.org/10.1016/j.heliyon.2024.e37370
Mohsen, M., Su, F., Lin, J., Li, X., Lu, K., Zhang, C., 2024b. Microplastic contamination in aquafeed ingredients used as protein and carbohydrate sources. *Bull. Environ. Contam. Toxicol.* 113(4), 41. https://doi.org/10.1007/s00128-024-03952-4
Mohsen, M., Wang, Q., Zhang, L., Sun, L., Lin, C., Yang, H., 2019. Microplastic ingestion by the farmed sea cucumber *Apostichopus japonicus* in China. *Environ. Pollut.* 245, 1071-1078. https://doi.org/10.1016/j.envpol.2018.11.083
Monteiro, M., Costa, R.S., Sousa, V., Marques, A., Sá, T., Thoresen, L., Aldaghi, S.A., Costamagna, M., Perucca, M., Kousoulaki, K., Valente, L.M.P., 2024. Towards sustainable aquaculture: Assessing polychaete meal (Alitta virens) as an effective fishmeal alternative in European seabass (Dicentrarchus labrax) diets. *Aquaculture* 579, 740257. https://doi.org/https://doi.org/10.1016/j.aquaculture.2023.740257

Montero, D., Rimoldi, S., Torrecillas, S., Rapp, J., Moroni, F., Herrera, A., Gómez, M., Fernández-Montero, Á., 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. https://doi.org/10.1016/j.scitotenv.2021.150402

Moore, C.J., Moore, S.L., Leecaster, M.K., Weisberg, S.B., 2001. A comparison of plastic and plankton in the North Pacific central gyre. *Mar. Pollut. Bull.* 42(12), 1297-1300. https://doi.org/https://doi.org/10.1016/S0025-326X(01)00114-X

Morgado, V., Gomes, L., Bettencourt da Silva, R.J.N., Palma, C., 2022. Microplastics contamination in sediments from Portuguese inland waters: Physical-chemical characterisation and distribution. *Sci. Total Environ.* 832, 155053. https://doi.org/10.1016/j.scitotenv.2022.155053

Morgana, S., Ghigliotti, L., Estévez-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. https://doi.org/10.1016/j.envpol.2018.08.001

Morris, A.W., Hamilton, E.I., 1974. Polystyrene spherules in the Bristol Channel. *Mar. Pollut. Bull.* 5(2), 26-27. https://doi.org/10.1016/0025-326X(74)90311-7

Mosconi, G., Panseri, S., Magni, S., Malandra, R., D'Amato, A., Carini, M., Chiesa, L., Della Torre, C., 2023. Plastic contamination in seabass and seabream from off-shore aquaculture facilities from the Mediterranean Sea. J. Xenobiotics 13(4), 625-640. https://doi.org/10.3390/jox13040040

Muhib, M.I., Rahman, M.M., 2023. Microplastics contamination in fish feeds: Characterization and potential exposure risk assessment for cultivated fish of Bangladesh. *Heliyon* 9(9), e19789. https://doi.org/10.1016/j.heliyon.2023.e19789

Muposhi, A., Mpinganjira, M., Wait, M., 2022. Considerations, benefits and unintended consequences of banning plastic shopping bags for environmental sustainability: A systematic literature review. *Waste Manag. Res.* 40(3), 248-261. https://doi.org/10.1177/0734242x211003965

Mustafa, S.A., Al-Rudainy, A.J., Salman, N.M., 2024. Effect of environmental pollutants on fish health: An overview. *Egypt. J. Aquat. Res.* 50(2), 225-233. https://doi.org/10.1016/j.ejar.2024.02.006

My, T.T., Dat, N.D., Hung, N.Q., 2023. Occurrence and characteristics of microplastics in wild and farmed shrimps collected from Cau Hai Lagoon, Central Vietnam. Molecules 28(12). https://doi.org/10.3390/molecules28124634

Nabi, N., Ahmad, I., Amin, A., Rather, M.A., Ahmed, I., Hajam, Y.A., Khursheed, S., Malik, M.M., Abubakr, A., 2024. Understanding the sources, fate and effects of microplastics in aquatic environments with a focus on risk profiling in aquaculture systems. *Rev. Aquac.* n/a(n/a). https://doi.org/10.1111/raq.12941

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. https://doi.org/10.1016/j.marpolbul.2019.02.037

Nair, H.T., Perumal, S., 2024. Growth performance, hematological and oxidative stress responses in Nile tilapia (*Oreochromis niloticus*) exposed to polypropylene microplastics. *Environ. Qual. Manag.* 34(1), e22179. https://doi.org/10.1002/tqem.22179

Nalbone, L., Giarratana, F., Genovese, M., Panebianco, A., 2024. Occurrence of microplastics in store-bought fresh and processed clams in Italy. *Mar. Pollut. Bull.* 206, 116739. https://doi.org/10.1016/j.marpolbul.2024.116739

Napier, J.A., Betancor, M.B., 2023. Engineering plant-based feedstocks for sustainable aquaculture. *Curr. Opin. Plant Biol.* 71, 102323. https://doi.org/10.1016/j.pbi.2022.102323

Napper, I.E., Parker-Jurd, F.N.F., Wright, S.L., Thompson, R.C., 2023. Examining the release of synthetic microfibres to the environment via two major pathways: Atmospheric deposition and treated wastewater effluent. *Sci. Total Environ.* 857, 159317. https://doi.org/10.1016/j.scitotenv.2022.159317

Napper, I.E., Thompson, R.C., 2020. Plastic debris in the marine environment: History and future challenges. *Global Chall.* 4(6), 1900081. https://doi.org/10.1002/gch2.201900081

Napper, I.E., Wright, L.S., Barrett, A.C., Parker-Jurd, F.N.F., Thompson, R.C., 2022. Potential microplastic release from the maritime industry: Abrasion of rope. *Sci. Total Environ.* 804, 150155. https://doi.org/10.1016/j.scitotenv.2021.150155

Nauen, C.E., 1893. Compilation of legal limits for hazardous substances in fish and fishery products. *FAO Fisheries Circular* 764, 102.

Naya-Català, F., Martos-Sitcha, J.A., de las Heras, V., Simó-Mirabet, P., Calduch-Giner, J.À., Pérez-Sánchez, J., 2021. Targeting the mild-hypoxia driving force for metabolic and muscle

transcriptional reprogramming of gilthead sea bream (*Sparus aurata*) juveniles. Biol. 10(5). https://doi.org/10.3390/biology10050416
Naylor, R.L., Hardy, R.W., Buschmann, A.H., Bush, S.R., Cao, L., Klinger, D.H., Little, D.C., Lubchenco, J., Shumway, S.E., Troell, M., 2021. A 20-year retrospective review of global aquaculture. *Nature* 591(7851), 551-563. https://doi.org/10.1038/s41586-021-03308-6
Oddsson, G.V., 2020. A definition of aquaculture intensity based on production functions—The aquaculture production intensity scale (APIS). Water 12(3). https://doi.org/10.3390/w12030765
Ohkawa, H., Ohishi, N., Yagi, K., 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal. Biochem.* 95(2), 351-358. https://doi.org/10.1016/0003-2697(79)90738-3
Ohkubo, N., Ito, M., Hano, T., Kono, K., Mochida, K., 2020. Estimation of the uptake and gut retention of microplastics in juvenile marine fish: Mummichogs (*Fundulus heteroclitus*) and red seabreams (*Pagrus major*). *Mar. Pollut. Bull.* 160, 111630. https://doi.org/10.1016/j.marpolbul.2020.111630
Ohlendorf, H.M., Risebrough, R.W., Vermeer, K., 1978. Exposure of marine birds to environmental pollutants. U.S. Fish and Wildlife Service Research Report 9, Washington D. C.
Ojala, M., Garriga, G.C., 2010. Permutation tests for studying classifier performance. *J. Mach. Learn. Res.* 11(62), 1833-1863. https://doi.org/10.5555/1756006.1859913
Oliveira, A.R., Sardinha-Silva, A., Andrews, P.L.R., Green, D., Cooke, G.M., Hall, S., Blackburn, K., Sykes, A.V., 2020. Microplastics presence in cultured and wild-caught cuttlefish, *Sepia officinalis*. *Mar. Pollut. Bull.* 160, 111553. https://doi.org/10.1016/j.marpolbul.2020.111553
Ou, M., Zhang, C., Wang, X., Zheng, C., Zhang, H., Xue, Y., 2024. Automated video analysis of the specific effects of the size and concentration of polystyrene microplastics on the swimming behavior of the crucian carp (*Carassius carassius*). *IEEE Access* 12, 125032-125042. https://doi.org/10.1109/ACCESS.2024.3452774
Özden, Ö., Erkan, N., 2008. Comparison of biochemical composition of three aqua cultured fishes (*Dicentrarchus labrax*, *Sparus aurata*, Dentex dentex). *Int. J. Food Sci. Nutr.* 59(7-8), 545-557. https://doi.org/10.1080/09637480701400729
Papageorgiou, N., Dimitriou, P.D., Moraitis, M.L., Massa, F., Fezzardi, D., Karakassis, I., 2021. Changes of the Mediterranean fish farm sector towards a more sustainable approach: A closer look at temporal, spatial and technical shifts. *Ocean Coast. Manag.* 214, 105903. https://doi.org/10.1016/j.ocecoaman.2021.105903
Parslow, J.L.F., Jefferies, D.J., 1972. Elastic thread pollution of puffins. *Mar. Pollut. Bull.* 3(3), 43-45. https://doi.org/10.1016/0025-326X(72)90142-7
Patrucco, S.G., Rivoira, L., Bruzzoniti, M.C., Barbera, S., Tassone, S., 2024. Development and application of a novel extraction protocol for the monitoring of microplastic contamination in widely consumed ruminant feeds. *Sci. Total Environ.* 947, 174493. https://doi.org/10.1016/j.scitotenv.2024.174493
Pazos, R.S., Amalvy, J., Cochero, J., Pecile, A., Gómez, N., 2021. Temporal patterns in the abundance, type and composition of microplastics on the coast of the Río de la Plata estuary. *Mar. Pollut. Bull.* 168(112382). https://doi.org/10.1016/j.marpolbul.2021.112382
Pedà, 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. https://doi.org/10.1016/j.envpol.2016.01.083
Pedà, 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., Maricchiolo, G., Consoli, P., Fossi, M.C., 2022. Integrated biomarker responses in European seabass *Dicentrarchus labrax* (Linnaeus, 1758) chronically exposed to PVC microplastics. *J. Hazard. Mater.* 438, 129488. https://doi.org/10.1016/j.jhazmat.2022.129488
Pereira, R., Costa, M., Velasco, C., Cunha, L.M., Lima, R.C., Baião, L.F., Batista, S., Marques, A., Sá, T., Campos, D.A., Pereira, M., Jesus, D., Fernández-Boo, S., Costas, B., Pintado, M., Valente, L.M.P., 2022. Comparative analysis between synthetic vitamin E and natural antioxidant sources from tomato, carrot and coriander in diets for market-sized *Dicentrarchus labrax*. Antioxidants 11(4). https://doi.org/10.3390/antiox11040636
Perelló-Amorós, M., Otero-Tarrazón, A., Jorge-Pedraza, V., García-Pérez, I., Sánchez-Moya, A., Gabillard, J.-C., Moshayedi, F., Navarro, I., Capilla, E., Fernández-Borràs, J., Blasco, J., Chillarón, J., García de la serrana, D., Gutiérrez, J., 2022. Myomaker and myomixer characterization in gilthead sea bream under different myogenesis conditions. Int. J. Mol. Sci. 23(23). https://doi.org/10.3390/ijms232314639

Perera, E., Turkmen, S., Simó-Mirabet, P., Zamorano, M.J., Xu, H., Naya-Català, F., Izquierdo, M., Pérez-Sánchez, J., 2020. Stearoyl-CoA desaturase (*scd1a*) is epigenetically regulated by broodstock nutrition in gilthead sea bream (*Sparus aurata*). *Epigenetics* 15(5), 536-553. https://doi.org/10.1080/15592294.2019.1699982

Pérez-Sánchez, J., Simó-Mirabet, P., Naya-Català, F., Martos-Sitcha, J.A., Perera, E., Bermejo-Nogales, A., Benedito-Palos, L., Calduch-Giner, J.À., 2018. Somatotropic axis regulation unravels the differential effects of nutritional and environmental factors in growth performance of marine farmed fishes. *Frontiers in Endocrinology* 9. https://doi.org/10.3389/fendo.2018.00687

Permatasari, D., Fadjar, M., Yuniarti, A., 2023. Survival rate and growth length of catfish (*Clarias gariepinus*) exposed to microplastics. *J. Aquac. Fish Health* 12(1). https://doi.org/10.20473/jafh.v12i1.36844

Plastics Europe, 2024. Plastics - the *fast* facts 2024.

Póvoa, A.A., Skinner, L.F., de Araújo, F.V., 2021. Fouling organisms in marine litter (rafting on abiogenic substrates): A global review of literature. *Mar. Pollut. Bull.* 166, 112189. https://doi.org/10.1016/j.marpolbul.2021.112189

Pradit, S., Noppradit, P., Goh, B.P., Sornplang, K., Ong, M.C., Towatana, P., 2021. Occurrence of microplastics and trace metals in fish and shrimp from Songkhla Lake, Thailand during the COVID-19 pandemic. *Appl. Ecol. Environ. Res.* https://doi.org/10.15666/aeer/1902_10851106

Prata, J.C., da Costa, J.P., Duarte, A.C., Rocha-Santos, T., 2022. Suspected microplastics in Atlantic horse mackerel fish (Trachurus trachurus) captured in Portugal. *Mar. Pollut. Bull.* 174, 113249. https://doi.org/https://doi.org/10.1016/j.marpolbul.2021.113249

Preziosa, E., Liu, S., Terova, G., Gao, X., Liu, H., Kucuktas, H., Terhune, J., Liu, Z., 2013. Effect of nutrient restriction and re-feeding on Calpain family genes in skeletal muscle of channel catfish (*Ictalurus punctatus*). *PLOS ONE* 8(3), e59404. https://doi.org/10.1371/journal.pone.0059404

Priscilla, V., Patria, M.P., 2019. Comparison of microplastic abundance in aquaculture ponds of milkfish *Chanos chanos* (Forsskål, 1775) at Muara Kamal and Marunda, Jakarta Bay. *IOP Conf. Ser.: Earth Environ. Sci.* 404. https://doi.org/10.1088/1755-1315/404/1/012027

Procter, J., Hopkins, F.E., Fileman, E.S., Lindeque, P.K., 2019. Smells good enough to eat: Dimethyl sulfide (DMS) enhances copepod ingestion of microplastics. *Mar. Pollut. Bull.* 138, 1-6. https://doi.org/10.1016/j.marpolbul.2018.11.014

Pulcini, D., Franceschini, S., Buttazzoni, L., Giannetti, C., Capoccioni, F., 2020. Consumer preferences for farmed seafood: An Italian case study. *J. Aquat. Food Prod. Technol.* 29(5), 445-460. https://doi.org/10.1080/10498850.2020.1749201

Qian, S., Qiao, X., Zhang, W., Yu, Z., Dong, S., Feng, J., 2024. Machine learning-based prediction for settling velocity of microplastics with various shapes. *Water Res.* 249, 121001. https://doi.org/https://doi.org/10.1016/j.watres.2023.121001

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/10.1016/j.chemosphere.2019.07.065

Qu, J., Wu, P., Pan, G., Li, J., Jin, H., 2022. Microplastics in seawater, sediment, and organisms from Hangzhou Bay. *Mar. Pollut. Bull.* 181, 113940. https://doi.org/10.1016/j.marpolbul.2022.113940

Quade, M.J., Roth, J.A., 1997. A rapid, direct assay to measure degranulation of bovine neutrophil primary granules. *Vet. Immunol. Immunopathol.* 58(3), 239-248. https://doi.org/10.1016/S0165-2427(97)00048-2

Rahman, R.R., Baqee, A., Alam, M., Khan, M.W., Muhib, M.I., Kabir, A., 2024. Organ-specific bioaccumulation of microplastics in market fish of Dhaka and size-dependent impacts of PVC microplastics on growth of *Anabus testudineus*. *Environ. Pollut.* 361, 124807. https://doi.org/10.1016/j.envpol.2024.124807

Rahman, T., Mustakima, S., Ferdous, Z., Tabassum, T., Mahamud, A.G.M.S.U., Siddika, M., Akter, M., Alam, M.S., Haque, M.N., 2022. Properties and abundance of microplastics found in fish feed, tissues, and culture water of catfish (*Heteropneustes fossilis*). *Int. J. Aquat. Biol.* 10(1), 1-10. https://doi.org/10.22034/ijab.v10i1

Ramsperger, A.F.R.M., Narayana, V.K.B., Gross, W., Mohanraj, J., Thelakkat, M., Greiner, A., Schmalz, H., Kress, H., Laforsch, C., 2020. Environmental exposure enhances the internalization of microplastic particles into cells. *Sci. Adv.* 6(50), eabd1211. https://doi.org/10.1126/sciadv.abd1211

Rangasamy, B., Malafaia, G., Maheswaran, R., 2022. Evaluation of antioxidant response and Na+-K+-ATPase activity in zebrafish exposed to polyethylene microplastics: Shedding light on a physiological adaptation. *J. Hazard. Mater.* 426, 127789. https://doi.org/10.1016/j.jhazmat.2021.127789

Rasmussen, S.C., 2021. From parkesine to celluloid: the birth of organic plastics. *Angew. Chem.* 133(15), 8090-8094. https://doi.org/10.1002/ange.202015095

Raza, T., Rasool, B., Asrar, M., Manzoor, M., Javed, Z., Jabeen, F., Younis, T., 2023. Exploration of polyacrylamide microplastics and evaluation of their toxicity on multiple parameters of *Oreochromis niloticus*. *Saudi J. Biol. Sci.* 30(2), 103518. https://doi.org/10.1016/j.sjbs.2022.103518

Rebelein, A., Int-Veen, I., Kammann, U., Scharsack, J.P., 2021. Microplastic fibers - Underestimated threat to aquatic organisms? *Sci. Total Environ.* 777, 146045. https://doi.org/10.1016/j.scitotenv.2021.146045

Reinold, S., Herrera, A., Saliu, F., Hernández-González, C., Martinez, I., Lasagni, M., Gómez, M., 2021. Evidence of microplastic ingestion by cultured European sea bass (*Dicentrarchus labrax*). *Mar. Pollut. Bull.* 168, 112450. https://doi.org/10.1016/j.marpolbul.2021.112450

Renieri, E.A., Safenkova, I.V., Alegakis, A.K., Slutskaya, E.S., Kokaraki, V., Kentouri, M., Dzantiev, B.B., Tsatsakis, A.M., 2019. Cadmium, lead and mercury in muscle tissue of gilthead seabream and seabass: Risk evaluation for consumers. *Food Chem. Toxicol.* 124, 439-449. https://doi.org/10.1016/j.fct.2018.12.020

Renzi, M., Guerranti, C., Blašković, A., 2018. Microplastic contents from maricultured and natural mussels. *Mar. Pollut. Bull.* 131, 248-251. https://doi.org/10.1016/j.marpolbul.2018.04.035

Resende, D., Pereira, R., Domínguez, D., Pereira, M., Pereira, C., Pintado, M., Valente, L.M.P., Velasco, C., 2023. Stress response of European seabass (*Dicentrarchus labrax*) fed plant-based diets supplemented with swine blood hydrolysates. *Aquac. Res.* 30, 101600. https://doi.org/10.1016/j.aqrep.2023.101600

Rimoldi, S., Ponti, J., Valsesia, A., Saroglia, G., La Spina, R., Fumagalli, F., Terova, G., 2024. Detection of microplastic contamination in commercial insect meals. Environments 11(6). https://doi.org/10.3390/environments11060112

Rios-Fuster, B., Alomar, C., Paniagua González, G., Garcinuño Martínez, R.M., Soliz Rojas, D.L., Fernández 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. https://doi.org/10.1016/j.envres.2022.114034

Rios-Fuster, B., Alomar, C., Viñas, L., Campillo, J.A., Pérez-Fernández, B., Álvarez, E., Compa, M., Deudero, S., 2021a. Organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) occurrence in *Sparus aurata* exposed to microplastic enriched diets in aquaculture facilities. *Mar. Pollut. Bull.* 173, 113030. https://doi.org/10.1016/j.marpolbul.2021.113030

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

Roch, S., Rebl, A., Wolski, W., Brinker, A., 2022. Combined proteomic and gene expression analysis to investigate reduced performance in rainbow trout (*Oncorhynchus mykiss*) caused by environmentally relevant microplastic exposure. *Microplast. Nanoplast.* 2(1), 14. https://doi.org/10.1186/s43591-022-00034-2

Roch, S., Ros, A.F.H., Friedrich, C., Brinker, A., 2021. Microplastic evacuation in fish is particle size-dependent. *Freshw. Biol.* 66(5), 926-935. https://doi.org/10.1111/fwb.13687

Rocha, C.P., Cabral, H.N., Marques, J.C., Gonçalves, A.M.M., 2022. A global overview of aquaculture food production with a focus on the activity's development in transitional systems - The case study of a South European country (Portugal). *J. Mar. Sci. Eng.* 10(3), 417. https://doi.org/10.3390/jmse10030417

Rocha, F., Rocha, A.C., Baião, L.F., Gadelha, J., Camacho, C., Carvalho, M.L., Arenas, F., Oliveira, A., Maia, M.R.G., Cabrita, A.R., Pintado, M., Nunes, M.L., Almeida, C.M.R., Valente, L.M.P., 2019. Seasonal effect in nutritional quality and safety of the wild sea urchin Paracentrotus lividus harvested in the European Atlantic shores. *Food Chem.* 282, 84-94. https://doi.org/10.1016/j.foodchem.2018.12.097

Rodrigues, M.O., Abrantes, N., Gonçalves, F.J.M., Nogueira, H., Marques, J.C., Gonçalves, A.M.M., 2018. Spatial and temporal distribution of microplastics in water and sediments of a freshwater system (Antuã River, Portugal). *Sci. Total Environ.* 633, 1549-1559. https://doi.org/10.1016/j.scitotenv.2018.03.233

Rodrigues, S.M., Almeida, C.M.R., Silva, D., Cunha, J., Antunes, C., Freitas, V., Ramos, S., 2019. Microplastic contamination in an urban estuary: Abundance and distribution of microplastics and fish larvae in the Douro estuary. *Sci. Total Environ.* 659, 1071-1081. https://doi.org/10.1016/j.scitotenv.2018.12.273

Rodríguez-Romeu, O., Constenla, M., Carrassón, M., Campoy-Quiles, M., Soler-Membrives, A., 2020. Are anthropogenic fibres a real problem for red mullets (*Mullus barbatus*) from the NW Mediterranean? *Sci. Total Environ.* 733, 139336. https://doi.org/10.1016/j.scitotenv.2020.139336

Rotchell, J.M., Austin, C., Chapman, E., Atherall, C.A., Liddle, C.R., Dunstan, T.S., Blackburn, B., Mead, A., Filart, K., Beeby, E., Cunningham, K., Allen, J., Draper, H., Guinn, B.-a., 2024. Microplastics in human urine: Characterisation using μFTIR and sampling challenges using healthy donors and endometriosis participants. *Ecotoxicol. Environ. Saf.* 274, 116208. https://doi.org/10.1016/j.ecoenv.2024.116208

Rothstein, S.I., 1973. Plastic particle pollution of the surface of the Atlantic Ocean: Evidence from a seabird. *Condor* 75, 344-366.

Royer, S.-J., Greco, F., Kogler, M., Deheyn, D.D., 2023. Not so biodegradable: Polylactic acid and cellulose/plastic blend textiles lack fast biodegradation in marine waters. *PLOS ONE* 18(5), e0284681. https://doi.org/10.1371/journal.pone.0284681

Ruiz, A., Andree, K.B., Furones, D., Holhorea, P.G., Calduch-Giner, J.À., Viñas, M., Pérez-Sánchez, J., Gisbert, E., 2023. Modulation of gut microbiota and intestinal immune response in gilthead seabream (*Sparus aurata*) by dietary bile salt supplementation. *Frontiers in Microbiology* 14. https://doi.org/10.3389/fmicb.2023.1123716

Rukmangada, R., Naidu, B.C., Nayak, B.B., Balange, A., Chouksey, M.K., Xavier, K.A.M., 2023. Microplastic contamination in salted and sun dried fish and implications for food security – A study on the effect of location, style and constituents of dried fish on microplastics load. *Mar. Pollut. Bull.* 191, 114909. https://doi.org/10.1016/j.marpolbul.2023.114909

Rummel, C.D., Löder, M.G.J., Fricke, N.F., Lang, T., Griebeler, E.-M., Janke, M., Gerdts, G., 2016. Plastic ingestion by pelagic and demersal fish from the North Sea and Baltic Sea. *Mar. Pollut. Bull.* 102(1), 134-141. https://doi.org/10.1016/j.marpolbul.2015.11.043

Sacco, V.A., Zuanazzi, N.R., Selinger, A., Alliprandini da Costa, J.H., Spanhol Lemunie, É., Comelli, C.L., Abilhoa, V., Sousa, F.C.d., Fávaro, L.F., Rios Mendoza, L.M., de Castilhos Ghisi, N., Delariva, R.L., 2024. What are the global patterns of microplastic ingestion by fish? A scientometric review. *Environ. Pollut.* 350, 123972. https://doi.org/10.1016/j.envpol.2024.123972

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. https://doi.org/10.1016/j.etap.2022.104059

Sai, S., Mani, R., Vijayakumar, P., Ganesan, M., Velu, K., Ayyamperumal, R., Rajagopal, R., Chang, S.W., Alfarhan, A., Ravindran, B., 2022. Risk assessment of potential toxicity induced by bio and synthetic plastic microspheres in *Lates calcarifer*. *Chemosphere* 298, 134269. https://doi.org/10.1016/j.chemosphere.2022.134269

Sakai, M., Hikima, J.-i., Kono, T., 2021. Fish cytokines: current research and applications. *Fisheries Science* 87(1), 1-9. https://doi.org/10.1007/s12562-020-01476-4

Sánchez-Almeida, R., Hernández-Sánchez, C., Villanova-Solano, C., Díaz-Peña, F.J., Clemente, S., González-Sálamo, J., González-Pleiter, M., Hernández-Borges, J., 2022. Microplastics determination in gastrointestinal tracts of European sea bass (*Dicentrarchus labrax*) and gilt-head sea bream (*Sparus aurata*) from Tenerife (Canary Islands, Spain). *Polymers* 14(10). https://doi.org/10.3390/polym14101931

Santana, L.M.B.M., Rodrigues, A.C.M., Campos, D., Kaczerewska, O., Figueiredo, J., Silva, S., Sousa, I., Maia, F., Tedim, J., Abessa, D.M.S., Pousão-Ferreira, P., Candeias-Mendes, A., Soares, F., Castanho, S., Soares, A.M.V.M., Rocha, R.J.M., Gravato, C., Patrício Silva, A.L., Martins, R., 2022. Can the toxicity of polyethylene microplastics and engineered nanoclays on flatfish (*Solea senegalensis*) be influenced by the presence of each other? *Sci. Total Environ.* 804, 150188. https://doi.org/10.1016/j.scitotenv.2021.150188

Savoca, M.S., Tyson, C.W., McGill, M., Slager, C.J., 2017. Odours from marine plastic debris induce food search behaviours in a forage fish. *Proc. R. Soc. B.: Biol. Sci.* 284(1860), 20171000. https://doi.org/10.1098/rspb.2017.1000

Savoca, S., Matanović, K., D'Angelo, G., Vetri, V., Anselmo, S., Bottari, T., Mancuso, M., Kužir, S., Spanò, N., Capillo, G., Di Paola, D., Valić, D., Gjurčević, E., 2021. Ingestion of plastic and non-plastic microfibers by farmed gilthead sea bream (*Sparus aurata*) and common carp (*Cyprinus carpio*) at different life stages. *Sci. Total Environ.* 782, 146851. https://doi.org/10.1016/j.scitotenv.2021.146851

Sayed, A.E.-D.H., Hamed, M., Badrey, A.E.A., Soliman, H.A.M., 2021. Bioremediation of hemotoxic and oxidative stress induced by polyethylene microplastic in *Clarias gariepinus* using lycopene, citric acid, and chlorella. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology* 250, 109189. https://doi.org/10.1016/j.cbpc.2021.109189

Sayed, A.E.-D.H., Hamed, M., Ismail, R.F., 2022. Natural antioxidants can improve microplastics-induced male reproductive impairment in the African catfish (*Clarias gariepinus*). *Front. Environ. Sci.* 9. https://doi.org/10.3389/fenvs.2021.811466

Sayed, A.E.-D.H., Hana, M.N., Hamed, M., Abdel-Latif, H.M.R., Lee, J.-S., Soliman, H.A.M., 2023. Protective efficacy of dietary natural antioxidants on microplastic particles-induced histopathological lesions in African catfish (*Clarias gariepinus*). *Environ. Sci. Pollut. Res.* 30(9), 24424-24440. https://doi.org/10.1007/s11356-022-23789-w

Sbrana, A., Valente, T., Scacco, U., Bianchi, J., Silvestri, C., Palazzo, L., de Lucia, G.A., Valerani, C., Ardizzone, G., Matiddi, M., 2020. Spatial variability and influence of biological parameters on microplastic ingestion by *Boops boops* (L.) along the Italian coasts (Western Mediterranean Sea). *Environ. Pollut.* 263, 114429. https://doi.org/10.1016/j.envpol.2020.114429

Scherer, C., Weber, A., Stock, F., Vurusic, S., Egerci, H., Kochleus, C., Arendt, N., Foeldi, C., Dierkes, G., Wagner, M., Brennholt, N., Reifferscheid, G., 2020. Comparative assessment of microplastics in water and sediment of a large European river. *Sci. Total Environ.* 738, 139866. https://doi.org/10.1016/j.scitotenv.2020.139866

Schmieg, H., Burmester, J.K.Y., Krais, S., Ruhl, A.S., Tisler, S., Zwiener, C., Köhler, H.-R., Triebskorn, R., 2020a. Interacting effects of polystyrene microplastics and the antidepressant amitriptyline on early life stages of brown trout (*Salmo trutta* f. *fario*). Water 12(9). https://doi.org/10.3390/w12092361

Schmieg, H., Huppertsberg, S., Knepper, T.P., Krais, S., Reitter, K., Rezbach, F., Ruhl, A.S., Köhler, H.-R., Triebskorn, R., 2020b. Polystyrene microplastics do not affect juvenile brown trout (*Salmo trutta* f. *fario*) or modulate effects of the pesticide methiocarb. *Environ. Sci. Eur.* 32(1), 49. https://doi.org/10.1186/s12302-020-00327-4

Schmieg, H., Krais, S., Kübler, K., Ruhl, A.S., Schmidgall, I.M., Zwiener, C., Köhler, H.-R., Triebskorn, R., 2022. Effects of the antidepressant amitriptyline on juvenile brown trout and their modulation by microplastics. Toxics 10(12). https://doi.org/10.3390/toxics10120763

Schneider, C.A., Rasband, W.S., Eliceiri, K.W., 2012. NIH Image to ImageJ: 25 years of image analysis. *Nat. Methods* 9, 671-675. https://doi.org/10.1038/nmeth.2089

Schwabl, P., Köppel, S., Königshofer, P., Bucsics, T., Trauner, M., Reiberger, T., Liebmann, B., 2019. Detection of various microplastics in human stool. *Ann. Intern. Med.* 171(7), 453-457. https://doi.org/10.7326/M19-0618

Scott, G., 1972. Plastics packaging and coastal pollution. *Int. J. Environ. Stud.* 3, 35–36.

Seeley, M.E., Hale, R.C., Zwollo, P., Vogelbein, W., Verry, G., Wargo, A.R., 2023. Microplastics exacerbate virus-mediated mortality in fish. *Sci. Total Environ.* 866, 161191. https://doi.org/10.1016/j.scitotenv.2022.161191

Selivanov, V.A., Votyakova, T.V., Pivtoraiko, V.N., Zeak, J., Sukhomlin, T., Trucco, M., Roca, J., Cascante, M., 2011. Reactive oxygen species production by forward and reverse electron fluxes in the mitochondrial respiratory chain. *PLOS Comput, Biol.* 7(3), e1001115. https://doi.org/10.1371/journal.pcbi.1001115

Selvam, S., Manisha, A., Roy, P.D., Venkatramanan, S., Chung, S.-Y., Muthukumar, P., Jesuraja, K., Elgorban, A.M., Ahmed, B., Elzain, H.E., 2021. Microplastics and trace metals in fish species of the Gulf of Mannar (Indian Ocean) and evaluation of human health. *Environ. Pollut.* 291, 118089. https://doi.org/10.1016/j.envpol.2021.118089

Sendra, M., Saco, A., Yeste, M.P., Romero, A., Novoa, B., Figueras, A., 2020. Nanoplastics: From tissue accumulation to cell translocation into *Mytilus galloprovincialis* hemocytes. resilience of immune cells exposed to nanoplastics and nanoplastics plus *Vibrio splendidus* combination. *J. Hazard. Mater.* 388, 121788. https://doi.org/10.1016/j.jhazmat.2019.121788

Sequeira, I.F., Prata, J.C., da Costa, J.P., Duarte, A.C., Rocha-Santos, T., 2020. Worldwide contamination of fish with microplastics: A brief global overview. *Mar. Pollut. Bull.* 160, 111681. https://doi.org/10.1016/j.marpolbul.2020.111681

Setälä, O., Fleming-Lehtinen, V., Lehtiniemi, M., 2014. Ingestion and transfer of microplastics in the planktonic food web. *Environ. Pollut.* 185, 77-83. https://doi.org/10.1016/j.envpol.2013.10.013
Shi, H., Frias, J., El-Din H. Sayed, A., De-la-Torre, G.E., Jong, M.-C., Uddin, S.A., Rajaram, R., Chavanich, S., Najii, A., Fernández-Severini, M.D., Ibrahim, Y.S., Su, L., 2023. Small plastic fragments: A bridge between large plastic debris and micro- & nano-plastics. *Trends Analyt. Chem.* 168, 117308. https://doi.org/10.1016/j.trac.2023.117308
Shi, J., Cai, M., Si, Y., Zhang, J., Du, S., 2018. Knockout of myomaker results in defective myoblast fusion, reduced muscle growth and increased adipocyte infiltration in zebrafish skeletal muscle. *Hum. Mol. Genet.* 27(20), 3542-3554. https://doi.org/10.1093/hmg/ddy268
Shi, W., Sun, S., Han, Y., Tang, Y., Zhou, W., Du, X., Liu, G., 2021. Microplastics impair olfactory-mediated behaviors of goldfish *Carassius auratus*. *J. Hazard. Mater.* 409, 125016. https://doi.org/10.1016/j.jhazmat.2020.125016
Shikama, A., Shinozaki, H., Takeuchi, Y., Matsuzaka, T., Aita, Y., Murayama, T., Sawada, Y., Piao, X., Toya, N., Oya, Y., Takarada, A., Masuda, Y., Nishi, M., Kubota, M., Izumida, Y., Nakagawa, Y., Iwasaki, H., Kobayashi, K., Yatoh, S., Suzuki, H., Yagyu, H., Kawakami, Y., Yamada, N., Shimano, H., Yahagi, N., 2015. Identification of human *ELOVL5* enhancer regions controlled by SREBP. *Biochem. Biophys. Res. Commun.* 465(4), 857-863. https://doi.org/10.1016/j.bbrc.2015.08.101
Shomura, R.S., Yoshida, H., O., 1985. Proceedings of the Workshop on the Fate and Impact of Marine Debris.
Siddique, M.A.M., Shazada, N.E., Ritu, J.A., Turjo, K.E.Z., Das, K., 2024. Does the mouth size influence microplastic ingestion in fishes? *Mar. Pollut. Bull.* 198, 115861. https://doi.org/10.1016/j.marpolbul.2023.115861
Siddique, M.A.M., Tahsin, T., Hossain, I., Hossain, M.S., Shazada, N.E., 2023. Microplastic contamination in commercial fish feeds: A major concern for sustainable aquaculture from a developing country. *Ecotoxicol. Environ. Saf.* 267, 115659. https://doi.org/10.1016/j.ecoenv.2023.115659
Siddiqui, S., Hutton, S.J., Dickens, J.M., Pedersen, E.I., Harper, S.L., Brander, S.M., 2023. Natural and synthetic microfibers alter growth and behavior in early life stages of estuarine organisms. *Front. Mar. Sci.* 9. https://doi.org/10.3389/fmars.2022.991650
Silva, D.M., Almeida, C.M.R., Guardiola, F.A., Pereira, R., Rodrigues, S.M., Ramos, S., 2024. Uncovering microplastics contamination in canned seafood. *Food Chem.* 448, 139049. https://doi.org/10.1016/j.foodchem.2024.139049
Similatan, K.M., Arcadio, C.G.L.A., Navarro, C.K.P., Capangpangan, R.Y., Bacosa, H.P., 2023. Microplastic ingestion by adult milkfish *Chanos chanos* (Forsskål, 1775) in aquaculture system: The case of Butuan Bay, Philippines. *Mar. Pollut. Bull.* 194, 115409. https://doi.org/10.1016/j.marpolbul.2023.115409
Singh, N., Mondal, A., Abhinav, G., Nagamani, P.V., Darbha, G.K., 2023. Microplastics in Indian brackish water lagoon: Occurrence and distribution in the Chilika Lake. *Water Air Soil Pollut.* 234(10), 614. https://doi.org/10.1007/s11270-023-06627-8
Smith, I.K., Vierheller, T.L., Thorne, C.A., 1988. Assay of glutathione reductase in crude tissue homogenates using 5,5′-dithiobis(2-nitrobenzoic acid). *Anal. Biochem.* 175(2), 408-413. https://doi.org/10.1016/0003-2697(88)90564-7
Smith Jr., J.K., 1988. World War II and the transformation of the American chemical industry, in: Mendelsohn, E., Smith, M.R., Weingart, P. (Eds.), Science, Technology and the Military. Springer Netherlands, Dordrecht, pp. 307-322.
Soliman, A.M., Barreda, D.R., 2023. The acute inflammatory response of teleost fish. *Dev. Comp. Immunol.* 146, 104731. https://doi.org/10.1016/j.dci.2023.104731
Soliman, H.A.M., Salaah, S.M., Hamed, M., Sayed, A.E.-D.H., 2023. Toxicity of co-exposure of microplastics and lead in African catfish (*Clarias gariepinus*). *Front. Vet. Sci.* 10, 1279382. https://doi.org/10.3389/fvets.2023.1279382
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. *Environ. Pollut.* 266, 115295. https://doi.org/10.1016/j.envpol.2020.115295
Solomando, A., Capó, 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. https://doi.org/1016/j.scitotenv.2021.147479

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, 113369. https://doi.org/10.1016/j.envres.2022.113369

Song, K., Du, W., Ma, X., Chen, Y., Sun, Y., Zhang, T., Huang, W., Feng, Z., 2022. Accumulation of microplastics in fugu (*Takifugu bimaculatus*): A comparative study between fishing grounds and aquafarms. *Mar. Pollut. Bull.* 185, 114200. https://doi.org/10.1016/j.marpolbul.2022.114200

Song, K., Wang, R., Yang, G., Xie, S., Chen, Y., Yang, F., Huang, W., Zhang, T., Feng, Z., 2023. Pollution concerns in mariculture water and cultured economical bivalves: Occurrence of microplastics under different aquaculture modes. *J. Clean. Prod.* 406, 136913. https://doi.org/10.1016/j.jclepro.2023.136913

Song, K., Xie, S., Li, C., He, J., Jin, W., Wang, R., Huang, W., Li, P., Feng, Z., 2024. The occurrence of pollutants in organisms and water of inland mariculture systems: Shrimp aquaculture is a procession of microplastics accumulation. *Environ. Pollut.* 342, 123072. https://doi.org/10.1016/j.envpol.2023.123072

Song, Y., Villeneuve, D.L., 2021. AOP report: Uncoupling of oxidative phosphorylation leading to growth inhibition via decreased cell proliferation. *Environ. Toxicol. Chem.* 40(11), 2959-2967. https://doi.org/10.1002/etc.5197

Song, Y.K., Hong, S.H., Jang, M., Han, G.M., Jung, S.W., Shim, W.J., 2017. Combined effects of UV exposure duration and mechanical abrasion on microplastic fragmentation by polymer type. *Environ. Sci. Technol.* 51(8), 4368-4376. https://doi.org/10.1021/acs.est.6b06155

Spanjer, A.R., Liedtke, T.L., Conn, K.E., Weiland, L.K., Black, R.W., Godfrey, N., 2020. Evidence for rapid gut clearance of microplastic polyester fibers fed to Chinook salmon: A tank study. *Environ. Pollut.* 265, 115083. https://doi.org/10.1016/j.envpol.2020.115083

Squadrone, S., Brizio, P., Stella, C., Prearo, M., Pastorino, P., Serracca, L., Ercolini, C., Abete, M.C., 2016. Presence of trace metals in aquaculture marine ecosystems of the northwestern Mediterranean Sea (Italy). *Environ. Pollut.* 215, 77-83. https://doi.org/10.1016/j.envpol.2016.04.096

Stabili, L., Quarta, E., Giotta, L., 2024. The seaweed *Chaetomorpha linum* cultivated in an integrated multitrophic aquaculture system: A new tool for microplastic bioremediation? *Sci. Total Environ.* 954, 176262. https://doi.org/10.1016/j.scitotenv.2024.176262

Stanton, T., James, A., Prendergast-Miller, M.T., Peirson-Smith, A., KeChi-Okafor, C., Gallidabino, M.D., Namdeo, A., Sheridan, K.J., 2024. Natural fibers: Why are they still the missing thread in the textile fiber pollution story? *Environ. Sci. Technol.* 58(29), 12763-12766. https://doi.org/10.1021/acs.est.4c05126

Stenger, K.S., Wikmark, O.-G., Bezuidenhout, C.C., Molale-Tom, L.G., 2021. Microplastics pollution in the ocean: Potential carrier of resistant bacteria and resistance genes. *Environ. Pollut.* 291, 118130. https://doi.org/10.1016/j.envpol.2021.118130

Stride, B., Abolfathi, S., Bending, G.D., Pearson, J., 2024. Quantifying microplastic dispersion due to density effects. *J. Hazard. Mater.* 466, 133440. https://doi.org/10.1016/j.jhazmat.2024.133440

Su, Z., Wei, L., Zhi, L., Huang, X., Wang, X., Wang, J., 2024. Microplastics in aquafeeds: Occurrence, sources, effects and considerations for aquatic food production. *Trends Analyt. Chem.* 176, 117760. https://doi.org/10.1016/j.trac.2024.117760

Suaria, G., Achtypi, A., Perold, V., Lee, J.R., Pierucci, A., Bornman, T.G., Aliani, S., Ryan, P.G., 2020. Microfibers in oceanic surface waters: A global characterization. *Sci. Adv.* 6(23), 8493. https://doi.org/10.1126/sciadv.aay8493

Suaria, G., Avio, C.G., Mineo, A., Lattin, G.L., Magaldi, M.G., Belmonte, G., Moore, C.J., Regoli, F., Aliani, S., 2016. The Mediterranean Plastic Soup: synthetic polymers in Mediterranean surface waters. *Sci. Rep.* 6(1), 37551. https://doi.org/10.1038/srep37551

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. https://doi.org/10.3389/fphys.2023.1217666

Sui, M., Lu, Y., Wang, Q., Hu, L., Huang, X., Liu, X., 2020a. Distribution patterns of microplastics in various tissues of the Zhikong scallop (*Chlamys farreri*) and in the surrounding culture seawater. *Mar. Pollut. Bull.* 160, 111595. https://doi.org/10.1016/j.marpolbul.2020.111595

Sui, Q., Zhang, L., Xia, B., Chen, B., Sun, X., Zhu, L., Wang, R., Qu, K., 2020b. Spatiotemporal distribution, source identification and inventory of microplastics in surface sediments from

Sanggou Bay, China. *Sci. Total Environ.* 723, 138064. https://doi.org/10.1016/j.scitotenv.2020.138064
Sultana, N., Tista, R.R., Islam, M.S., Begum, M., Islam, S., Naser, M.N., 2023. Microplastics in freshwater wild and farmed fish species of Bangladesh. *Environ. Sci. Pollut. Res.* 30(28), 72009-72025. https://doi.org/10.1007/s11356-023-26512-5
Sun, N., Shi, H., Li, X., Gao, C., Liu, R., 2023a. Combined toxicity of micro/nanoplastics loaded with environmental pollutants to organisms and cells: Role, effects, and mechanism. *Environ. Int.* 171, 107711. https://doi.org/10.1016/j.envint.2022.107711
Sun, X., Wang, X., Booth, A.M., Zhu, L., Sui, Q., Chen, B., Qu, K., Xia, B., 2023b. New insights into the impact of polystyrene micro/nanoplastics on the nutritional quality of marine jacopever (*Sebastes schlegelii*). *Sci. Total Environ.* 903, 166560. https://doi.org/10.1016/j.scitotenv.2023.166560
Sun, Y., Deng, Q., Zhang, Q., Zhou, X., Chen, R., Li, S., Wu, Q., Chen, H., 2024. Hazards of microplastics exposure to liver function in fishes: A systematic review and meta-analysis. *Mar. Environ. Res.* 196, 106423. https://doi.org/https://doi.org/10.1016/j.marenvres.2024.106423
Ta, A.T., Babel, S., 2020. Microplastics pollution with heavy metals in the aquaculture zone of the Chao Phraya River Estuary, Thailand. *Mar. Pollut. Bull.* 161, 111747. https://doi.org/10.1016/j.marpolbul.2020.111747
Ta, A.T., Babel, S., 2023. Occurrence and spatial distribution of microplastic contaminated with heavy metals in a tropical river: Effect of land use and population density. *Mar. Pollut. Bull.* 191, 114919. https://doi.org/10.1016/j.marpolbul.2023.114919
Tacon, A.G.J., Lemos, D., Metian, M., 2020. Fish for health: Improved nutritional quality of cultured fish for human consumption. *Rev. Fish. Sci. Aquac.* 28(4), 449-458. https://doi.org/10.1080/23308249.2020.1762163
Tan, X., Du, S., 2002. Differential expression of two *MyoD* genes in fast and slow muscles of gilthead seabream (*Sparus aurata*). *Dev. Genes Evol.* 212(5), 207-217. https://doi.org/10.1007/s00427-002-0224-5
Teng, J., Zhao, J., Zhang, C., Cheng, B., Koelmans, A.A., Wu, D., Gao, M., Sun, X., Liu, Y., Wang, Q., 2020. A systems analysis of microplastic pollution in Laizhou Bay, China. *Sci. Total Environ.* 745, 140815. https://doi.org/10.1016/j.scitotenv.2020.140815
Teng, M., Zhao, X., Wang, C., Wang, C., White, J.C., Zhao, W., Zhou, L., Duan, M., Wu, F., 2022. Polystyrene nanoplastics toxicity to zebrafish: Dysregulation of the brain–intestine–microbiota axis. *ACS Nano* 16(5), 8190-8204. https://doi.org/10.1021/acsnano.2c01872
Textile Exchange, 2024. Materials market report. pp. 1-76.
Thévenot, E.A., Roux, A., Xu, Y., Ezan, E., Junot, C., 2015. Analysis of the human adult urinary metabolome variations with age, body mass index, and gender by implementing a comprehensive workflow for univariate and OPLS statistical analyses. *J. Proteome Res.* 14(8), 3322-3335. https://doi.org/10.1021/acs.jproteome.5b00354
Thiele, C.J., Hudson, M.D., Russell, A.E., Saluveer, M., Sidaoui-Haddad, G., 2021. Microplastics in fish and fishmeal: an emerging environmental challenge? *Sci. Rep.* 11(1), 2045. https://doi.org/10.1038/s41598-021-81499-8
Thiyagarajan, D.B., Stette, M.H.S., Afzal, B.M., Ahluwalia, B.S., Agarwal, K., Dalmo, R.A., Wolfson, D.L., 2024. 3D imaging shows nano- and microparticles are internalized by salmon skin and corneal epithelial cells. *Front. Mar. Sci.* 11. https://doi.org/10.3389/fmars.2024.1422748
Thompson, R.C., Courtene-Jones, W., Boucher, J., Pahl, S., Raubenheimer, K., Koelmans, A.A., 2024. Twenty years of microplastics pollution research—what have we learned? *Science*, eadl2746. https://doi.org/10.1126/science.adl2746
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), 838-838. https://doi.org/10.1126/science.1094559
Thompson, R.C., Swan, S.H., Moore, C.J., vom Saal, F.S., 2009. Our plastic age. *Philos. Trans. R. Soc. B: Biol. Sci.* 364(1526), 1973-1976. https://doi.org/10.1098/rstb.2009.0054
Tongo, I., Erhunmwunse, N.O., 2022. Effects of ingestion of polyethylene microplastics on survival rate, opercular respiration rate and swimming performance of African catfish (*Clarias gariepinus*). *J. Hazard. Mater.* 423, 127237. https://doi.org/10.1016/j.jhazmat.2021.127237
Torres, S., Compa, M., Box, A., Pinya, S., Sureda, A., 2024. Presence and potential effects of microplastics in the digestive tract of two small species of shark from the Balearic Islands. Fishes 9(2). https://doi.org/10.3390/fishes9020055
Toxqui-Rodríguez, S., Holhorea, P.G., Naya-Català, F., Calduch-Giner, J.À., Sitjà-Bobadilla, A., Piazzon, C., Pérez-Sánchez, J., 2024. Differential reshaping of skin and intestinal microbiota

by stocking density and oxygen availability in farmed gilthead sea bream (*Sparus aurata*): A behavioral and network-based integrative approach. Microorganisms 12(7). https://doi.org/10.3390/microorganisms12071360
Tripathy, S., Lytle, K.A., Stevens, R.D., Bain, J.R., Newgard, C.B., Greenberg, A.S., Huang, L.-S., Jump, D.B., 2014. Fatty acid elongase-5 (Elovl5) regulates hepatic triglyceride catabolism in obese C57BL/6J mice [S]. *J. Lipid Res.* 55(7), 1448-1464. https://doi.org/10.1194/jlr.M050062
Trocino, A., Xiccato, G., Majolini, D., Tazzoli, M., Tulli, F., Tibaldi, E., Messina, C.M., Santulli, A., 2012. Levels of dioxin-like polychlorinated biphenyls (DL-PCBs) and metals in European sea bass from fish farms in Italy. *Food Chem.* 134(1), 333-338. https://doi.org/10.1016/j.foodchem.2012.02.153
Tunçelli, İ.C., Erkan, N., 2024. Microplastic pollution in wild and aquacultured Mediterranean mussels from the Sea of Marmara: Abundance, characteristics, and health risk estimations. *Environ. Res.* 242, 117787. https://doi.org/10.1016/j.envres.2023.117787
Türkmen, A., Türkmen, M., Tepe, Y., Çekiç, M., 2010. Metals in tissues of fish from Yelkoma Lagoon, northeastern Mediterranean. *Environ. Monit. Assess.* 168(1), 223-230. https://doi.org/10.1007/s10661-009-1106-3
Ugwu, K., Herrera, A., Gómez, M., 2021. Microplastics in marine biota: A review. *Mar. Pollut. Bull.* 169, 112540. https://doi.org/10.1016/j.marpolbul.2021.112540
Ulusoy, Ş., Mol, S., 2022. Trace elements in seabass, farmed by Turkey, and health risks to the main consumers: Turkish and Dutch populations. *Environ. Monit. Assess.* 194(3), 224. https://doi.org/10.1007/s10661-022-09806-y
UN, 2015. Transforming our world: The 2030 agenda for sustainable development.
UNEP, 1995. Global Programme of Action for the Protection of the Marine Environment from Land-Based Activities (GPA).
USEPA, 1991. Regional guidance on handling chemical concentration data near the detection limit in risk assessments. https://www.epa.gov/risk/regional-guidance-handling-chemical-concentration-data-near-detection-limit-risk-assessments.
Uy, C.A., Johnson, D.W., 2022. Effects of microplastics on the feeding rates of larvae of a coastal fish: direct consumption, trophic transfer, and effects on growth and survival. *Mar. Biol.* 169(2), 27. https://doi.org/10.1007/s00227-021-04010-x
Vagner, M., Boudry, G., Courcot, L., Vincent, D., Dehaut, A., Duflos, G., Huvet, A., Tallec, K., Zambonino-Infante, J.L., 2022. Experimental evidence that polystyrene nanoplastics cross the intestinal barrier of European seabass. *Environ. Int.* 166, 107340. https://doi.org/10.1016/j.envint.2022.107340
Vanukon, M.S., Dehm, J., Pickering, T., Yabakiva, M., Rico, C., Hewavitharane, C., 2024. First assessment of microplastic concentrations in oysters, water and sediment in Laucala Bay, Fiji Islands. *Int. J. Environ. Sci. Technol.* https://doi.org/10.1007/s13762-024-05739-w
Varó, I., Osorio, K., Estensoro, I., Naya-Català, F., Sitjà-Bobadilla, A., Navarro, J.C., Pérez-Sánchez, J., Torreblanca, A., Piazzon, M.C., 2021. Effect of virgin low density polyethylene microplastic ingestion on intestinal histopathology and microbiota of gilthead sea bream. *Aquaculture* 545, 737245. https://doi.org/10.1016/j.aquaculture.2021.737245
Varol, M., Kaya, G.K., Sünbül, M.R., 2019. Evaluation of health risks from exposure to arsenic and heavy metals through consumption of ten fish species. *Environ. Sci. Pollut. Res.* 26(32), 33311-33320. https://doi.org/10.1007/s11356-019-06450-x
Vazirzadeh, A., Ergun, S., Mossafa, H., Farhadi, A., Keshavarzifard, M., Yigit, M., Yilmaz, S., 2025. Microplastics contamination suppressed immune and health status in cage cultured Barramundi: An investigation on pollution sources, ecotoxicological impacts, and transcription of genes involved in detoxification. *Aquaculture* 594, 741370. https://doi.org/10.1016/j.aquaculture.2024.741370
Venrick, E.L., Backman, T.W., Bartram, W.C., Platt, C.J., Thornhill, M.S., Yates, R.E., 1973. Man-made objects on the surface of the Central North Pacific Ocean. *Nature* 241(5387), 271-271. https://doi.org/10.1038/241271a0
Vianello, A., Boldrin, A., Guerriero, P., Moschino, V., Rella, R., Sturaro, A., da Ros, L., 2013. Microplastic particles in sediments of Lagoon of Venice, Italy: First observations on occurrence, spatial patterns and identification. *Estuar. Coast. Shelf Sci.* 130, 54-61. https://doi.org/10.1016/j.ecss.2013.03.022
Vieira, K.S., Baptista Neto, J.A., Crapez, M.A.C., Gaylarde, C., Pierri, B.d.S., Saldaña-Serrano, M., Bainy, A.C.D., Nogueira, D.J., Fonseca, E.M., 2021. Occurrence of microplastics and heavy metals accumulation in native oysters *Crassostrea gasar* in the Paranaguá estuarine system, Brazil. *Mar. Pollut. Bull.* 166, 112225. https://doi.org/10.1016/j.marpolbul.2021.112225

Viršek, M.K., Lovšin, M.N., Koren, Š., Kržan, A., Peterlin, M., 2017. Microplastics as a vector for the transport of the bacterial fish pathogen species *Aeromonas salmonicida*. *Mar. Pollut. Bull.* 125(1), 301-309. https://doi.org/10.1016/j.marpolbul.2017.08.024

Vitali, C., Peters, R.J.B., Janssen, H.-G., Nielen, M.W.F., 2023. Microplastics and nanoplastics in food, water, and beverages; part I. occurrence. *Trends Analyt. Chem.* 159, 116670. https://doi.org/https://doi.org/10.1016/j.trac.2022.116670

Volkheimer, G., 1974. Passage of particles through the wall of the gastrointestinal tract. *Environ. Health Perspect.* 9, 215-225. https://doi.org/10.1289/ehp.749215

Volkheimer, G., 1975. Hematogenous dissemination of ingested polyvinyl chloride particles. *Ann. N. Y. Acad. Sci.* 246(1), 164-171. https://doi.org/10.1111/j.1749-6632.1975.tb51092.x

von Moos, N., Burkhardt-Holm, P., Köhler, A., 2012. Uptake and effects of microplastics on cells and tissue of the blue mussel *Mytilus edulis* L. after an experimental exposure. *Environ. Sci. Technol.* 46(20), 11327-11335. https://doi.org/10.1021/es302332w

Walkinshaw, C., Lindeque, P.K., Thompson, R., Tolhurst, T., Cole, M., 2020. Microplastics and seafood: lower trophic organisms at highest risk of contamination. *Ecotoxicol. Environ. Saf.* 190, 110066. https://doi.org/10.1016/j.ecoenv.2019.110066

Walkinshaw, C., Tolhurst, T.J., Lindeque, P.K., Thompson, R., Cole, M., 2022. Detection and characterisation of microplastics and microfibres in fishmeal and soybean meal. *Mar. Pollut. Bull.* 185, 114189. https://doi.org/10.1016/j.marpolbul.2022.114189

Wang, F., Zhang, Q., Cui, J., Bao, B., Deng, X., Liu, L., Guo, M.-y., 2023. Polystyrene microplastics induce endoplasmic reticulum stress, apoptosis and inflammation by disrupting the gut microbiota in carp intestines. *Environ. Pollut.* 323, 121233. https://doi.org/10.1016/j.envpol.2023.121233

Wang, H.-H., Chen, W.-Y., Huang, Y.-H., Hsu, S.-M., Tsao, Y.-P., Hsu, Y.-H., Chang, M.-S., 2022a. Interleukin-20 is involved in dry eye disease and is a potential therapeutic target. *J. Biomed. Sci.* 29(1), 36. https://doi.org/10.1186/s12929-022-00821-2

Wang, J., Li, X., Li, P., Li, L., Zhao, L., Ru, S., Zhang, D., 2022b. Porous microplastics enhance polychlorinated biphenyls-induced thyroid disruption in juvenile Japanese flounder (*Paralichthys olivaceus*). *Mar. Pollut. Bull.* 174, 113289. https://doi.org/10.1016/j.marpolbul.2021.113289

Wang, Q., Duan, X., Huang, F., Cheng, H., Zhang, C., Li, L., Ruan, X., He, Q., Yang, H., Niu, W., Qin, Q., Zhao, H., 2021. Polystyrene nanoplastics alter virus replication in orange-spotted grouper (*Epinephelus coioides*) spleen and brain tissues and spleen cells. *J. Hazard. Mater.* 416, 125918. https://doi.org/10.1016/j.jhazmat.2021.125918

Wang, Q., Li, J., Zhu, X., Sun, C., Teng, J., Chen, L., Shan, E., Zhao, J., 2022c. Microplastics in fish meals: An exposure route for aquaculture animals. *Sci. Total Environ.* 807, 151049. https://doi.org/10.1016/j.scitotenv.2021.151049

Wang, S., Xie, S., Wang, Z., Zhang, C., Pan, Z., Sun, D., Xu, G., Zou, J., 2022d. Single and combined effects of microplastics and cadmium on the cadmium accumulation and biochemical and immunity of *Channa argus*. *Biol. Trace Elem. Res.* 200(7), 3377-3387. https://doi.org/10.1007/s12011-021-02917-6

Wang, S., Xie, S., Zhang, C., Pan, Z., Sun, D., Zhou, A., Xu, G., Zou, J., 2022e. Interactions effects of nano-microplastics and heavy metals in hybrid snakehead (Channa maculata ♀ × Channa argus ♂). *Fish Shellfish Immunol.* 124, 74-81. https://doi.org/https://doi.org/10.1016/j.fsi.2022.03.045

Wang, W., Ge, J., Yu, X., 2020a. Bioavailability and toxicity of microplastics to fish species: A review. *Ecotoxicol. Environ. Saf.* 189, 109913. https://doi.org/10.1016/j.ecoenv.2019.109913

Wang, X., Zheng, H., Zhao, J., Luo, X., Wang, Z., Xing, B., 2020b. Photodegradation elevated the toxicity of polystyrene microplastics to grouper (*Epinephelus moara*) through disrupting hepatic lipid homeostasis. *Environ. Sci. Technol.* 54(10), 6202-6212. https://doi.org/10.1021/acs.est.9b07016

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. https://doi.org/10.1016/j.ecoenv.2023.115104

WHO, 1991. Nickel, nickel carbonyl, and some nickel compounds: Health and safety guide. Geneva, Switzerland.

Wold, S., Sjöström, M., Eriksson, L., 2001. PLS-regression: a basic tool of chemometrics. *Chemometr. Intell. Lab. Syst.* 58(2), 109-130. https://doi.org/https://doi.org/10.1016/S0169-7439(01)00155-1

Wong, C.S., Green, D.R., Cretney, W.J., 1974. Quantitative tar and plastic waste distributions in the Pacific Ocean. *Nature* 247(5435), 30-32. https://doi.org/10.1038/247030a0
Wood, C.M., Farrell, A.P., Brauner, C.J., 2011. Homeostasis and toxicology of essential metals, in: Fish Physiology. Academic Press, p. i.
Woodall, L.C., Sanchez-Vidal, A., Canals, M., Paterson, G.L.J., Coppock, R., Sleight, V., Calafat, A., Rogers, A.D., Narayanaswamy, B.E., Thompson, R.C., 2014. The deep sea is a major sink for microplastic debris. *Royal Soc. Open Sci.* 1(4), 140317. https://doi.org/10.1098/rsos.140317
Wu, D., Lu, X., Dong, L.-X., Tian, J., Deng, J., Wei, L., Wen, H., Zhong, S., Jiang, M., 2024. Nano polystyrene microplastics could accumulate in Nile tilapia (*Oreochromis niloticus*): Negatively impacts on the liver and intestinal health through water exposure. *J. Environ. Sci.* 137, 604-614. https://doi.org/10.1016/j.jes.2023.02.018
Wu, F., Wang, Y., Leung, J.Y.S., Huang, W., Zeng, J., Tang, Y., Chen, J., Shi, A., Yu, X., Xu, X., Zhang, H., Cao, L., 2020. Accumulation of microplastics in typical commercial aquatic species: A case study at a productive aquaculture site in China. *Sci. Total Environ.* 708, 135432. https://doi.org/10.1016/j.scitotenv.2019.135432
Wu, H., Hou, J., Wang, X., 2023a. A review of microplastic pollution in aquaculture: Sources, effects, removal strategies and prospects. *Ecotoxicol. Environ. Saf.* 252, 114567. https://doi.org/10.1016/j.ecoenv.2023.114567
Wu, X., Li, M., Yang, S., Dong, J., Pan, W., Ning, Y., Hua, X., Dong, D., Liang, D., 2023b. Liver metabolic dysregulation induced by polypropylene nano- and microplastics in Nile tilapia using Internal Extractive Electrospray Ionization Mass Spectrometry. *Anal. Chem.* 95(20), 7863-7871. https://doi.org/10.1021/acs.analchem.2c05672
Xia, X., Sun, M., Zhou, M., Chang, Z., Li, L., 2020. Polyvinyl chloride microplastics induce growth inhibition and oxidative stress in *Cyprinus carpio* var. larvae. *Sci. Total Environ.* 716, 136479. https://doi.org/10.1016/j.scitotenv.2019.136479
Xie, M., Lin, L., Xu, P., Zhou, W., Zhao, C., Ding, D., Suo, A., 2021. Effects of microplastic fibers on *Lates calcarifer* juveniles: Accumulation, oxidative stress, intestine microbiome dysbiosis and histological damage. *Ecol. Indic.* 133, 108370. https://doi.org/10.1016/j.ecolind.2021.108370
Xie, M., Xu, P., Zhou, W., Xu, X., Li, H., He, W., Yue, W., Zhang, L., Ding, D., Suo, A., 2022. Impacts of conventional and biodegradable microplastics on juvenile *Lates calcarifer*: Bioaccumulation, antioxidant response, microbiome, and proteome alteration. *Mar. Pollut. Bull.* 179, 113744. https://doi.org/10.1016/j.marpolbul.2022.113744
Xiong, X., Liu, Q., Chen, X., Wang, R., Duan, M., Wu, C., 2021. Occurrence of microplastic in the water of different types of aquaculture ponds in an important lakeside freshwater aquaculture area of China. *Chemosphere* 282, 131126. https://doi.org/10.1016/j.chemosphere.2021.131126
Xiong, X., Tu, Y., Chen, X., Jiang, X., Shi, H., Wu, C., Elser, J.J., 2019. Ingestion and egestion of polyethylene microplastics by goldfish (*Carassius auratus*): influence of color and morphological features. *Heliyon* 5(12), e03063. https://doi.org/10.1016/j.heliyon.2019.e03063
Xiong, X., Xie, S., Feng, K., Wang, Q., 2022. Occurrence of microplastics in a pond-river-lake connection water system: How does the aquaculture process affect microplastics in natural water bodies. *J. Clean. Prod.* 352, 131632. https://doi.org/10.1016/j.jclepro.2022.131632
Xu, J., Li, D., 2021. Feeding behavior responses of a juvenile hybrid grouper, *Epinephelus fuscoguttatus*♀ × *E. lanceolatus*♂, to microplastics. *Environ. Pollut.* 268, 115648. https://doi.org/10.1016/j.envpol.2020.115648
Xu, M., Fang, W., Lin, G., Zhu, X., Lu, J., 2024a. Transcriptomic responses and larval-stage growth of protandrous yellowfin seabream (*Acanthopagrus latus*) to different polyethylene microplastics exposure. *Mar. Biotechnol.* https://doi.org/10.1007/s10126-024-10334-8
Xu, T., Cui, J., Xu, R., Cao, J., Guo, M.-y., 2023. Microplastics induced inflammation and apoptosis via ferroptosis and the NF-κB pathway in carp. *Aquat. Toxicol.* 262, 106659. https://doi.org/10.1016/j.aquatox.2023.106659
Xu, Y., Li, S., Chen, J., Cui, Z., 2024b. Assessment of mariculture-derived microplastic pollution in Dapeng Cove, China. *Front. Mar. Sci.* 11. https://doi.org/10.3389/fmars.2024.1382249
Yabanli, M., Arlparslan, Y., Baygar, T., 2012. Assessment of cadmium, mercury and lead contents of frozen European sea bass (*Dicentrarchus labrax* L., 1758) and Gilthead sea bream (*Sparus aurata* L., 1758) fillet from Turkey *Agric. Sci.* 3, 669-673. https://doi.org/10.4236/as.2012.35081
Yadav, H., Khan, M.R.H., Quadir, M., Rusch, K.A., Mondal, P.P., Orr, M., Xu, E.G., Iskander, S.M., 2023. Cutting boards: An overlooked source of microplastics in human food? *Environ. Sci. Technol.* 57(22), 8225-8235. https://doi.org/10.1021/acs.est.3c00924

Yan, L., Yao, X., Wang, P., Zhao, C., Zhang, B., Qiu, L., 2024. Effect of polypropylene microplastics on virus resistance in spotted sea bass (*Lateolabrax maculatus*). *Environ. Pollut.* 342, 123054. https://doi.org/10.1016/j.envpol.2023.123054

Yan, Z., Liu, Y., Zhang, T., Zhang, F., Ren, H., Zhang, Y., 2022. Analysis of microplastics in human feces reveals a correlation between fecal microplastics and inflammatory bowel disease status. *Environ. Sci. Technol.* 56(1), 414-421. https://doi.org/10.1021/acs.est.1c03924

Yang, H., Ju, J., Wang, Y., Zhu, Z., Lu, W., Zhang, Y., 2024. Micro-and nano-plastics induce kidney damage and suppression of innate immune function in zebrafish (*Danio rerio*) larvae. *Sci. Total Environ.* 931, 172952. https://doi.org/10.1016/j.scitotenv.2024.172952

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. *J. Hazard. Mater.* 388, 122058. https://doi.org/10.1016/j.jhazmat.2020.122058

Yang, J., Karbalaei, S., Hussein, S.M., Ahmad, A.F., Walker, T.R., Salimi, K., 2023. Biochemical effects of polypropylene microplastics on red tilapia (*Oreochromis niloticus*) after individual and combined exposure with boron. *Environ. Sci. Eur.* 35(1), 71. https://doi.org/10.1186/s12302-023-00771-y

Yao, C., Liu, X., Wang, H., Sun, X., Qian, Q., Zhou, J., 2021. Occurrence of microplastics in fish and shrimp feeds. *Bull. Environ. Contam. Toxicol.* 107(4), 684-692. https://doi.org/10.1007/s00128-021-03328-y

Yedier, S., Yalçınkaya, S.K., Bostancı, D., 2023. Exposure to polypropylene microplastics via diet and water induces oxidative stress in *Cyprinus carpio*. *Aquat. Toxicol.* 259, 106540. https://doi.org/10.1016/j.aquatox.2023.106540

Yi, J., Ma, Y., Ruan, J., You, S., Ma, J., Yu, H., Zhao, J., Zhang, K., Yang, Q., Jin, L., Zeng, G., Sun, D., 2024. The invisible threat: Assessing the reproductive and transgenerational impacts of micro- and nanoplastics on fish. *Environ. Int.* 183, 108432. https://doi.org/10.1016/j.envint.2024.108432

Yildiz, M., 2008. Mineral composition in fillets of sea bass (*Dicentrarchus labrax*) and sea bream (*Sparus aurata*): a comparison of cultured and wild fish. *J. Appl. Ichthyol.* 24(5), 589-594. https://doi.org/10.1111/j.1439-0426.2008.01097.x

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. https://doi.org/10.1016/j.jhazmat.2018.07.110

Yin, L., Liu, H., Cui, H., Chen, B., Li, L., Wu, F., 2019. Impacts of polystyrene microplastics on the behavior and metabolism in a marine demersal teleost, black rockfish (*Sebastes schlegelii*). *J. Hazard. Mater.* 380, 120861. https://doi.org/10.1016/j.jhazmat.2019.120861

Yozukmaz, A., 2021. Investigation of microplastics in edible wild mussels from İzmir Bay (Aegean Sea, Western Turkey): A risk assessment for the consumers. *Mar. Pollut. Bull.* 171, 112733. https://doi.org/10.1016/j.marpolbul.2021.112733

Yu, F., Pei, Y., Zhang, X., Wu, X., Zhang, G., Ma, J., 2023a. Occurrence and distribution characteristics of aged microplastics in the surface water, sediment, and crabs of the aquaculture pond in the Yangtze River Delta of China. *Sci. Total Environ.* 871, 162039. https://doi.org/10.1016/j.scitotenv.2023.162039

Yu, X., Huang, W., Wang, Y., Wang, Y., Cao, L., Yang, Z., Dou, S., 2022a. Microplastic pollution in the environment and organisms of Xiangshan Bay, East China Sea: An area of intensive mariculture. *Water Res.* 212, 118117. https://doi.org/10.1016/j.watres.2022.118117

Yu, Y.-B., Choi, J.-H., Choi, C.Y., Kang, J.-C., Kim, J.-H., 2023b. Toxic effects of microplastic (polyethylene) exposure: Bioaccumulation, hematological parameters and antioxidant responses in crucian carp, *Carassius carassius*. *Chemosphere* 332, 138801. https://doi.org/10.1016/j.chemosphere.2023.138801

Yu, Z., Yang, G., Zhang, W., 2022b. A new model for the terminal settling velocity of microplastics. *Mar. Pollut. Bull.* 176, 113449. https://doi.org/10.1016/j.marpolbul.2022.113449

Yuan, Z., Nag, R., Cummins, E., 2022. Ranking of potential hazards from microplastics polymers in the marine environment. *J. Hazard. Mater.* 429, 128399. https://doi.org/10.1016/j.jhazmat.2022.128399

Yue, Y., Guo, X., Wang, Z., Gan, L., Dong, X., Zhang, M., Jiang, H., An, M., Shao, J., 2024. Alleviating effect of EMs on oxidative stress and inflammation of *Micropterus salmoides* after microplastics exposure. *Aquac. Int.* 32(3), 3719-3732. https://doi.org/10.1007/s10499-023-01347-6

Zambrano-Pinto, M.V., Tinizaray-Castillo, R., Riera, M.A., Maddela, N.R., Luque, R., Díaz, J.M.R., 2024. Microplastics as vectors of other contaminants: Analytical determination techniques and

remediation methods. *Sci. Total Environ.* 908, 168244. https://doi.org/https://doi.org/10.1016/j.scitotenv.2023.168244
Zambrano, M.C., Pawlak, J.J., Daystar, J., Ankeny, M., Venditti, R.A., 2021. Impact of dyes and finishes on the aquatic biodegradability of cotton textile fibers and microfibers released on laundering clothes: Correlations between enzyme adsorption and activity and biodegradation rates. *Mar. Pollut. Bull.* 165, 112030. https://doi.org/10.1016/j.marpolbul.2021.112030
Zarantoniello, M., Cattaneo, N., Conti, F., Carrino, M., Cardinaletti, G., Şener, İ., Olivotto, I., 2024. Mitigating dietary microplastic accumulation and oxidative stress response in European seabass (*Dicentrarchus labrax*) juveniles using a natural microencapsulated antioxidant. Antioxidants 13(7). https://doi.org/10.3390/antiox13070812
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*. *Mar. Pollut. Bull.* 156, 111210. https://doi.org/10.1016/j.marpolbul.2020.111210
Zhang, C., Wang, F., Wang, Q., Zou, J., Zhu, J., 2023. Species-specific effects of microplastics on juvenile fishes. *Front. Physiol.* 14. https://doi.org/10.3389/fphys.2023.1256005
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. https://doi.org/10.1016/j.fsi.2021.04.010
Zhang, C., Wang, J., Zhou, A., Ye, Q., Feng, Y., Wang, Z., Wang, S., Xu, G., Zou, J., 2021b. Species-specific effect of microplastics on fish embryos and observation of toxicity kinetics in larvae. *J. Hazard. Mater.* 403, 123948. https://doi.org/10.1016/j.jhazmat.2020.123948
Zhang, C., Wang, Q., Wang, S., Pan, Z., Sun, D., Cheng, Y., Zou, J., Xu, G., 2021c. Effects of virgin microplastics on growth, intestinal morphology and microbiota on largemouth bass (*Micropterus salmoides*). Appl. Sci. 11(24). https://doi.org/10.3390/app112411921
Zhang, C., Wang, Q., Zuo, Z., Ding, J., Xu, G., Zou, J., 2021d. Interactive effects of microplastics and tetracycline on bioaccumulation and biochemical status in Jian carp (*Cyprinus carpio* var. Jian). *Front. Environ. Sci.* 9. https://doi.org/10.3389/fenvs.2021.764344
Zhang, C., Zuo, Z., Wang, Q., Wang, S., Lv, L., Zou, J., 2022a. Size effects of microplastics on embryos and observation of toxicity cinetics in larvae of grass carp (*Ctenopharyngodon idella*). Toxics 10(2). https://doi.org/10.3390/toxics10020076
Zhang, F., Li, D., Yang, Y., Zhang, H., Zhu, J., Liu, J., Bu, X., Li, E., Qin, J., Yu, N., Chen, L., Wang, X., 2022b. Combined effects of polystyrene microplastics and copper on antioxidant capacity, immune response and intestinal microbiota of Nile tilapia (*Oreochromis niloticus*). *Sci. Total Environ.* 808, 152099. https://doi.org/10.1016/j.scitotenv.2021.152099
Zhang, K., Chen, J., Wang, Y., Chen, M., Bao, X., Chen, X., Xie, S., Lin, Z., Yu, Y., 2024a. Investigating polystyrene nano-plastic effects on largemouth bass (*Micropterus salmoides*) focusing on mRNA expression: Endoplasmic reticulum stress and lipid metabolism dynamics. Fishes 9(9). https://doi.org/10.3390/fishes9090342
Zhang, K., Liang, J., Liu, T., Li, Q., Zhu, M., Zheng, S., Sun, X., 2022c. Abundance and characteristics of microplastics in shellfish from Jiaozhou Bay, China. *J. Oceanol. Limnol.* 40(1), 163-172. https://doi.org/10.1007/s00343-021-0465-7
Zhang, P., Lu, G., Sun, Y., Yan, Z., Dang, T., Liu, J., 2022d. Metagenomic analysis explores the interaction of aged microplastics and roxithromycin on gut microbiota and antibiotic resistance genes of *Carassius auratus*. *J. Hazard. Mater.* 425, 127773. https://doi.org/10.1016/j.jhazmat.2021.127773
Zhang, S., Ding, J., Razanajatovo, R.M., Jiang, H., Zou, H., Zhu, W., 2019a. Interactive effects of polystyrene microplastics and roxithromycin on bioaccumulation and biochemical status in the freshwater fish red tilapia (*Oreochromis niloticus*). *Sci. Total Environ.* 648, 1431-1439. https://doi.org/10.1016/j.scitotenv.2018.08.266
Zhang, T.-T., Xu, J., Wang, Y.-M., Xue, C.-H., 2019b. Health benefits of dietary marine DHA/EPA-enriched glycerophospholipids. *Prog. Lipid Res.* 75, 100997. https://doi.org/10.1016/j.plipres.2019.100997
Zhang, W., Tian, D., Yu, Y., Tong, D., Zhou, W., Yu, Y., Lu, L., Li, W., Liu, G., Shi, W., 2024b. Micro/nanoplastics impair the feeding of goldfish by disrupting the complicated peripheral and central regulation of appetite. *Sci. Total Environ.* 946, 174112. https://doi.org/10.1016/j.scitotenv.2024.174112
Zhang, W., Wang, Q., Chen, H., 2022e. Challenges in characterization of nanoplastics in the environment. *Front. Environ. Sci. Eng.* 16(11). https://doi.org/10.1007/s11783-021-1445-z

Zhang, X., Li, S., Liu, Y., Yu, K., Zhang, H., Yu, H., Jiang, J., 2021e. Neglected microplastics pollution in the nearshore surface waters derived from coastal fishery activities in Weihai, China. *Sci. Total Environ.* 768, 144484. https://doi.org/10.1016/j.scitotenv.2020.144484

Zhao, Y., Bao, Z., Wan, Z., Fu, Z., Jin, Y., 2020. Polystyrene microplastic exposure disturbs hepatic glycolipid metabolism at the physiological, biochemical, and transcriptomic levels in adult zebrafish. *Sci. Total Environ.* 710, 136279. https://doi.org/10.1016/j.scitotenv.2019.136279

Zhao, Y., Ma, C., Wei, W., Wang, Y., Cao, H., Cui, N., Liu, Y., Liang, H., 2024. Effects of single and combined exposure of virgin or aged polyethylene microplastics and penthiopyrad on zebrafish (*Danio rerio*). *Sci. Total Environ.* 921, 171160. https://doi.org/10.1016/j.scitotenv.2024.171160

Zhao, Y., Qin, Z., Huang, Z., Bao, Z., Luo, T., Jin, Y., 2021. Effects of polyethylene microplastics on the microbiome and metabolism in larval zebrafish. *Environ. Pollut.* 282, 117039. https://doi.org/10.1016/j.envpol.2021.117039

Zheng, Q., Cui, L., Liao, H., Junaid, M., Li, Z., Liu, S., Gao, D., Zheng, Y., Lu, S., Qiu, J., Wang, J., 2023. Combined exposure to polystyrene nanoplastics and bisphenol A induces hepato- and intestinal-toxicity and disturbs gut microbiota in channel catfish (*Ictalurus punctatus*). *Sci. Total Environ.* 891, 164319. https://doi.org/10.1016/j.scitotenv.2023.164319

Zheng, S., Tang, B.Z., Wang, W.-X., 2024. Microplastics and nanoplastics induced differential respiratory damages in tilapia fish *Oreochromis niloticus*. *J. Hazard. Mater.* 465, 133181. https://doi.org/10.1016/j.jhazmat.2023.133181

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

Zhou, B., Liu, C., Xu, L., Yuan, Y., Zhao, J., Zhao, W., Chen, Y., Qiu, J., Meng, M., Zheng, Y., Wang, D., Gao, X., Li, X., Zhao, Q., Wei, X., Wu, D., Zhang, H., Hu, C., Zhuo, X., Zheng, M., Wang, H., Lu, Y., Ma, X., 2021b. N^6-methyladenosine reader protein YT521-B homology domain-containing 2 suppresses liver steatosis by regulation of mRNA stability of lipogenic genes. *Hepatol.* 73(1). https://doi.org/10.1002/hep.31220

Zhou, W., Shi, W., Du, X., Han, Y., Tang, Y., Ri, S., Ju, K., Kim, T., Huang, L., Zhang, W., Yu, Y., Tian, D., Yu, Y., Chen, L., Wu, Z., Liu, G., 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 Perspect.* 131(4), 047006. https://doi.org/10.1289/EHP11600

Zhou, Z., Song, X., Dong, D., Li, X., Sun, Y., Wang, L., Huang, Z., Li, M., 2024. Occurrence, distribution and sources of microplastics in typical marine recirculating aquaculture system (RAS) in China: The critical role of RAS operating time and microfilter. *Water Res.* 255, 121476. https://doi.org/10.1016/j.watres.2024.121476

Zhu, J., Zhang, Q., Huang, Y., Jiang, Y., Li, J., Michal, J.J., Jiang, Z., Xu, Y., Lan, W., 2021. Long-term trends of microplastics in seawater and farmed oysters in the Maowei Sea, China. *Environ. Pollut.* 273, 116450. https://doi.org/10.1016/j.envpol.2021.116450

Zhu, J., Zhang, Q., Li, Y., Tan, S., Kang, Z., Yu, X., Lan, W., Cai, L., Wang, J., Shi, H., 2019. Microplastic pollution in the Maowei Sea, a typical mariculture bay of China. *Sci. Total Environ.* 658, 62-68. https://doi.org/10.1016/j.scitotenv.2018.12.192

Zhu, L., Wu, Z., Dong, J., Zhao, S., Zhu, J., Wang, W., Ma, F., An, L., 2024. Unveiling small-sized plastic particles hidden behind large-sized ones in human excretion and their potential sources. *Environ. Sci. Technol.* 58(27), 11901-11911. https://doi.org/10.1021/acs.est.3c11054

Zin, M.M., Azman, S., Anaziah, S.H., Khalid, N., Jumali, S., Yusof, N.A.U., 2023. Microplastic contamination in the sediment of the Johor Strait Estuary, Malaysia. *IOP Conf. Ser.: Earth Environ. Sci.* 1263(1), 012039. https://doi.org/10.1088/1755-1315/1263/1/012039

Zitouni, N., Bousserrhine, N., Belbekhouche, S., Missawi, O., Alphonse, V., Boughatass, I., Banni, M., 2020. First report on the presence of small microplastics (≤ 3 µm) in tissue of the commercial fish *Serranus scriba* (Linnaeus. 1758) from Tunisian coasts and associated cellular alterations. *Environ. Pollut.* 263, 114576. https://doi.org/10.1016/j.envpol.2020.114576

Zitouni, N., Bousserrhine, N., Missawi, O., Boughattas, I., Chèvre, N., Santos, R., Belbekhouche, S., Alphonse, V., Tisserand, F., Balmassiere, L., Dos Santos, S.P., Mokni, M., Guerbej, H., Banni, M., 2021. Uptake, tissue distribution and toxicological effects of environmental microplastics in early juvenile fish *Dicentrarchus labrax*. *J. Hazard. Mater.* 403, 124055. https://doi.org/10.1016/j.jhazmat.2020.124055

Zitouni, N., Cappello, T., Missawi, O., Boughattas, I., De Marco, G., Belbekhouche, S., Mokni, M., Alphonse, V., Guerbej, H., Bousserrhine, N., Banni, M., 2022. Metabolomic disorders unveil

hepatotoxicity of environmental microplastics in wild fish *Serranus scriba* (Linnaeus 1758). *Sci. Total Environ.* 838, 155872. https://doi.org/10.1016/j.scitotenv.2022.155872

Zou, J., Secombes, C.J., 2016. The function of fish cytokines. Biol. 5(2). https://doi.org/10.3390/biology5020023

Zu, B., Li, W., Yang, Q., Guo, J., An, J., Li, J., Mei, X., 2023. Ingestion of microplastics by silver carp (*Hypophthalmichthys molitrix*) larvae: Quantification of ingestion and assessment of microbiota dysbiosis. *Aquat. Toxicol.* 257, 106475. https://doi.org/10.1016/j.aquatox.2023.106475

Zuo, Z., Wang, Q., Zhang, C., Zou, J., 2022. Single and combined effects of microplastics and cadmium on juvenile grass carp (*Ctenopharyngodon idellus*). *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology* 261, 109424. https://doi.org/10.1016/j.cbpc.2022.109424

Appendices

Appendix A - Supplementary materials of the Chapter II

Table S2.1. Results of correlation analyses between the sample weight and the number of plastic particles recovered from European seabass (*Dicentrarchus labrax*) samples from all body sites together and separately. GIT – Gastrointestinal tract. *N* – number of fish analysed. *r* – Spearman's correlation coefficient.

	All body sites	**GIT**	**Gills**	**Liver**	**Muscle**
Number of PLs					
n	422	144	80	73	125
PL/g	0.58 ± 0.32	1.01 ± 0.78	0.75 ± 0.94	0.80 ± 0.86	0.36 ± 0.32
Sample weight (g)					
Mean ± SD	14.05 ± 3.55	2.84 ± 0.98	2.20 ± 0.95	1.98 ± 0.76	7.04 ± 2.22
	Number of plastics *versus* sample weight				
	All body sites	**GIT**	**Gills**	**Liver**	**Muscle**
Seabass (*N* = 55)	*r* = 0.103 *p* = 0.453	*r* = 0.078 *p* = 0.573	*r* = 0.207 *p* = 0.129	*r* = 0.060 *p* = 0.662	*r* = 108 *p* = 0.431

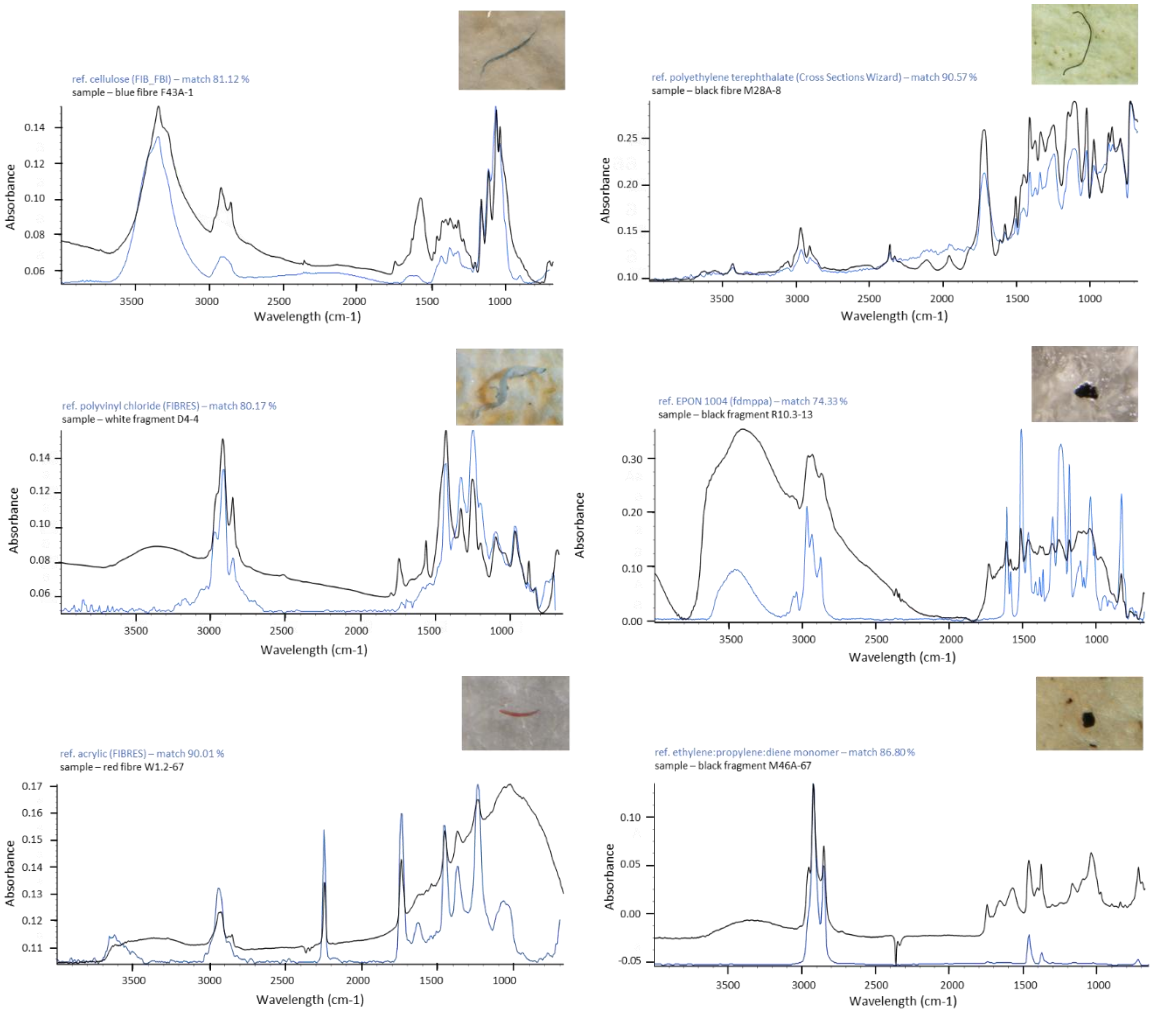

Figure S2.1. Representative spectra of plastic polymers recovered from recirculating aquaculture system (RAS), namely water from tanks, fish feed, and several body sites of European seabass (*Dicentrarchus labrax*): A – cellulose, B – polyethylene terephthalate (PET), C – polyvinyl chloride (PVC), D – phenoxy resin, E – polyacrylonitrile (PAN) and F – ethylene-propylene diene (F). Reference spectra of the plastic polymers identified by μFTIR analyses are presented in blue.

Table S2.2. Results of the correlation (Spearman's correlation coefficient – r) analyses between the shape and other characteristics (colours, size classes in µm, and polymer type) observed in the plastic particles recovered from European seabass (*Dicentrarchus labrax*) produced in a recirculation aquaculture system (RAS) for 8 months. *n* = 422 for all the data. *n* = 282 for fibres, *n* = 88 for fragments, *n* = 28 for films, *n* = 24 for pellets.

Variable 1	Variable 2	*r*	*p*	Variable 1	Variable 2	*r*	*p*	Variable 1	Variable 2	*r*	*p*	Variable 1	Variable 2	*r*	*p*
Fibre	Black	-0.264	< 0.001	**Fragment**	Black	0.173	< 0.001	**Film**	Black	0.146	0.003	**Pellet**	Black	0.076	0.117
	Blue	0.180	< 0.001		Blue	-0.099	0.042		Blue	-0.183	< 0.001		Blue	0.002	0.966
	Brown	0.063	0.196		Brown	0.106	0.030		Brown	-0.029	0.558		Brown	-0.027	0.582
	Green	-0.176	< 0.001		Green	0.151	0.002		Green	-0.007	0.882		Green	0.100	0.040
	Grey	0.092	0.058		Grey	-0.046	0.348		Grey	-0.054	0.272		Grey	-0.050	0.302
	Orange	-0.141	0.004		Orange	0.086	0.079		Orange	-0.039	0.429		Orange	0.176	< 0.001
	Pink/Purple	0.048	0.322		Pink/Purple	-0.035	0.468		Pink/Purple	-0.018	0.712		Pink/Purple	-0.017	0.729
	Red	0.076	0.119		Red	-0.080	0.101		Red	-0.041	0.404		Red	0.029	0.552
	Transparent	0.298	< 0.001		Transparent	-0.235	< 0.001		Transparent	-0.063	0.199		Transparent	-0.126	0.010
	White	-0.315	< 0.001		White	0.192	< 0.001		White	0.345	< 0.001		White	-0.062	0.206
	Yellow	0.091	0.062		Yellow	-0.067	0.172		Yellow	-0.034	0.487		Yellow	-0.032	0.514
Fibre	< 100 µm	-0.492	< 0.001	**Fragment**	< 100 µm	0.298	< 0.001	**Film**	< 100 µm	0.083	0.089	**Pellet**	< 100 µm	0.389	< 0.001
	100-149 µm	-0.201	< 0.001		100-149 µm	0.189	< 0.001		100-149 µm	0.031	0.531		100-149 µm	0.043	0.376
	150-499 µm	0.038	0.434		150-499 µm	0.027	0.586		150-499 µm	-0.026	0.589		150-499 µm	-0.096	0.048
	500-1499 µm	0.317	< 0.001		500-1499 µm	-0.235	< 0.001		500-1499 µm	-0.066	0.176		500-1499 µm	-0.161	< 0.001
	1500-2999 µm	0.177	< 0.001		1500-2999 µm	-0.177	< 0.001		1500-2999 µm	0.035	0.471		1500-2999 µm	-0.085	0.082
	3000-4999 µm	0.103	0.034		3000-4999 µm	-0.076	0.120		3000-4999 µm	-0.039	0.429		3000-4999 µm	-0.036	0.458
	≥ 5000 µm	0.059	0.224		≥ 5000 µm	-0.043	0.373		≥ 5000 µm	-0.022	0.650		≥ 5000 µm	-0.021	0.670
Fibre	AK	-0.140	0.004	**Fragment**	AK	0.070	0.150	**Film**	AK	-0.026	0.600	**Pellet**	AK	0.187	< 0.001
	Cellulose/Rayon	0.634	< 0.001		Cellulose/Rayon	-0.429	< 0.001		Cellulose/Rayon	-0.278	< 0.001		Cellulose/Rayon	-0.241	< 0.001
	EPDM	-0.208	< 0.001		EPDM	0.133	0.006		EPDM	0.114	0.019		EPDM	0.069	0.158
	EPON	-0.211	< 0.001		EPON	0.086	0.079		EPON	0.095	0.050		EPON	0.176	< 0.001
	EVA	-0.098	0.043		EVA	0.050	0.310		EVA	-0.018	0.712		EVA	0.132	0.007
	Nylon	0.034	0.484		Nylon	-0.025	0.608		Nylon	-0.013	0.794		Nylon	-0.012	0.806
	Organoclay-based coating	-0.254	< 0.001		Organoclay-based coating	0.044	0.373		Organoclay-based coating	0.346	< 0.001		Organoclay-based coating	0.075	0.126
	PAM	-0.027	0.574		PAM	0.025	0.613		PAM	-0.034	0.487		PAM	0.048	0.323
	PAN	0.130	0.008		PAN	-0.095	0.051		PAN	-0.048	0.321		PAN	-0.045	0.351
	PA resin	-0.098	0.043		PA resin	0.050	0.310		PA resin	-0.018	0.712		PA resin	0.132	0.007
	PE	-0.067	0.170		PE	0.070	0.149		PE	-0.034	0.487		PE	0.048	0.323
	PET	0.182	< 0.001		PET	-0.147	0.002		PET	-0.038	0.432		PET	-0.070	0.149
	PP	-0.001	0.984		PP	-0.012	0.800		PP	0.050	0.302		PP	-0.029	0.546
	PS	-0.185	< 0.001		PS	0.070	0.149		PS	0.050	0.302		PS	0.128	0.008
	PUR	-0.098	0.043		PUR	0.134	0.006		PUR	0.118	0.016		PUR	-0.017	0.729
	PVC	-0.395	< 0.001		PVC	0.267	< 0.001		PVC	-0.018	0.712		PVC	0.012	0.811
	Wool	-0.070	0.154		Wool	-0.025	0.608		Wool	-0.013	0.794		Wool	0.198	< 0.001
	Others	-0.315	< 0.001		Others	0.341	< 0.001		Others	-0.066	0.179		Others	0.112	0.022
	Unidentified	-0.130	0.007		Unidentified	0.168	< 0.001		Unidentified	-0.018	0.716		Unidentified	-0.012	0.813

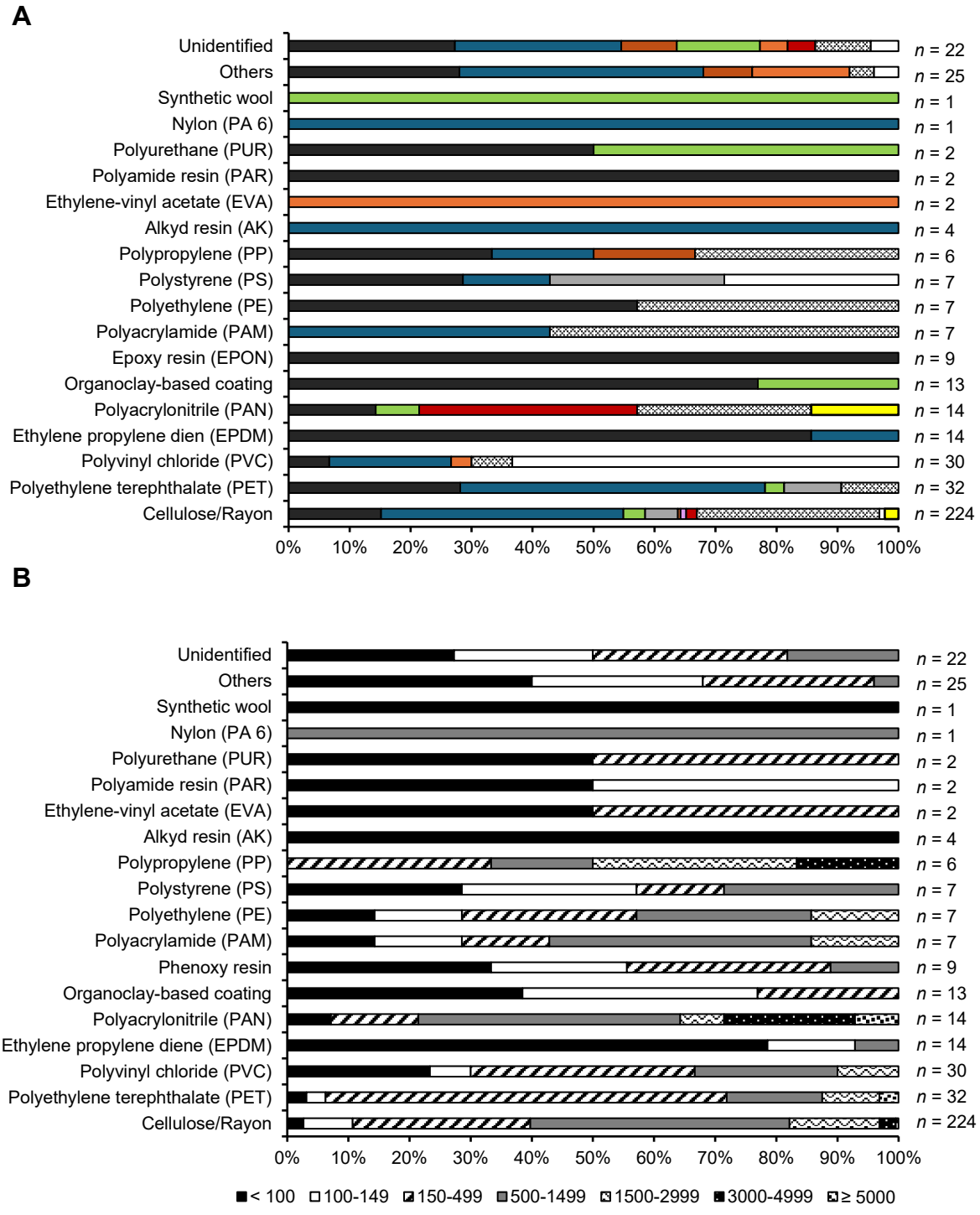

Figure S2.2. Percentage of the different colours (A) and size classes in µm (B) per polymer type observed in the 422 plastic particles (PLs) recovered from European seabass body sites, namely the gastrointestinal tract (GIT), gills, liver, and dorsal muscle. The total number of PLs found is given by the *n* value presented.

Table S2.3. Spearman correlation values were evaluated between total plastic particles (PLs) recovered from European seabass (*Dicentrarchus labrax*) and fish biometric and oxidative stress biomarkers.

	PL number	
	r	***p-value***
Fish length (cm)	-0.092	0.718
Fish weight (g)	0.249	0.252
K	0.364	0.087
HSI (%)	-0.074	0.739
VSI (%)	0.017	0.939
Liver		
CAT (µmol/min mg protein)	-0.072	0.745
SOD (µmol/min mL)	-0.098	0.657
GPx (nmol/min mg protein)	-0.025	0.908
GR (nmol/min mg protein)	-0.206	0.346
GST (nmol/min mg protein)	-0.307	0.154
TAC (Trolox eq. mM)	0.029	0.897
LPO (nmol TBARS/g)	0.318	0.091
Muscle		
LPO (nmol TBARS/g)	-0.071	0.747

K - Fulton's body condition factor; HSI - Hepatosomatic index; VSI - Viscerosomatic index.

Table S2.4. Summary of studies reporting the occurrence of micro- and mesoplastic particles (PL) in several body sites of European seabass (*Dicentrarchus labrax*) both captured in the wild and produced in aquaculture, including results obtained in the present study in a recirculating aquaculture system (RAS). Examples of the most common particle shapes, colours, sizes, and polymers are listed.

Body sites	Occurrence levels	Shape	Colour	Size range	Polymers	Location	Reference
Gastrointestinal tract (GIT)	0.30 ± 0.61 PL/indv	Fibre, fragments	Blue, white, red	≤1000 – 5000 µm	PET, PP, PAN, PE, Nylon, Rayon*	Mondego Estuary, Portugal *Wild-caught fish*	Bessa *et al.* (2018)
	~1.7 PL/g tissue	Fibres, fragments	not specified	5 – 5000 µm	Cellulose, PET, PE *	Mediterranean Sea *Wild-caught fish*	Akoueson *et al.* (2020)
	0.6 ± 0.8 PL/indv 2.7 ± 1.9 PL/indv	Fibres, fragments, films, lines	Blue, black, transparent, yellow	≤1000 – 5000 µm	Cellulose, Nylon, PE, PP	Canary Islands *Fish farmed in cages*	Reinold *et al.* (2021)
	1.3 ± 2.5 PL/indv	Fibres, fragments	Blue, white, black, red, yellow*	<100 – 5000 µm	PE, PET, rayon*	North-East Atlantic *Wild-caught fish*	Barboza *et al.* (2020b)
	5.4 ± 4.2 PL/indv	Fibres, fragments, films	Transparent, blue, black	50 – 1200 µm	Rayon, PAN, PET, PUR	Canary Islands *Fish farmed in cages*	Sánchez-Almeida *et al.* (2022)
	2.6 ± 1.9 PL/indv	Fibres, fragments, films, pellets	Blue, black, transparent, white	42 – 5194 µm	Cellulose/rayon, PET, PVC	Portugal *Fish farmed in RAS*	***This study***
	0.95 ± 1.1 PL/indv	Fibres, fragments	Black, blue, green	3 - ≥5000 µm	PET, Nylon, PE	Turkey *Farmed fish from local market*	Kılıç (2022)
Gills	~0.9 PL/g tissue	Fibres, fragments	n.d.	5 – 5000 µm	Cellulose, PET, PE*	Mediterranean Sea *Wild-caught fish*	Akoueson *et al.* (2020)
	0.8 ± 1.4 PL/indv	Fibres, fragments	Blue, white, black, red, yellow*	<100 – 3000 µm	PE, PET, rayon*	North-East Atlantic *Wild-caught fish*	Barboza *et al.* (2020b)
	1.5 ± 1.6 PL/indv	Fibres, fragments, films, pellets	Blue, black, transparent	57 – 6672 µm	Cellulose/rayon, PET, acrylic	Portugal *Fish farmed in RAS*	***This study***
Liver	0.8 ± 0.9 PL/g tissue	Fibres, fragments, films, pellets	Blue, black, transparent	30 – 3325 µm	Cellulose/rayon, EPDM, PET, acrylic	Portugal *Fish farmed in RAS*	***This study***
Dorsal muscle	1.04 ± 0.07 PL/g tissue	Fibres, fragments	n.d.	5 – 5000 µm	Cellulose, PET, PE*	Mediterranean Sea *Wild-caught fish*	Akoueson *et al.* (2020)
	0.04 ± 0.07 PL/g tissue	Fibres, fragments	Blue, white, black, red, yellow*	<100 – 3000 µm	PE, PET, rayon*	North-East Atlantic *Wild-caught fish*	Barboza *et al.* (2020b)
	0.36 ± 0.32 PL/g tissue	Fibres, fragments, films, pellets	Blue, black, transparent, white	30 – 2181 µm	Cellulose/rayon, PET, PVC	Portugal *Fish farmed in RAS*	***This study***

*Not specified per tissue, results are shown in relation to the total number of particles found per species or in the study.

Appendix B - Supplementary materials of the Chapter III

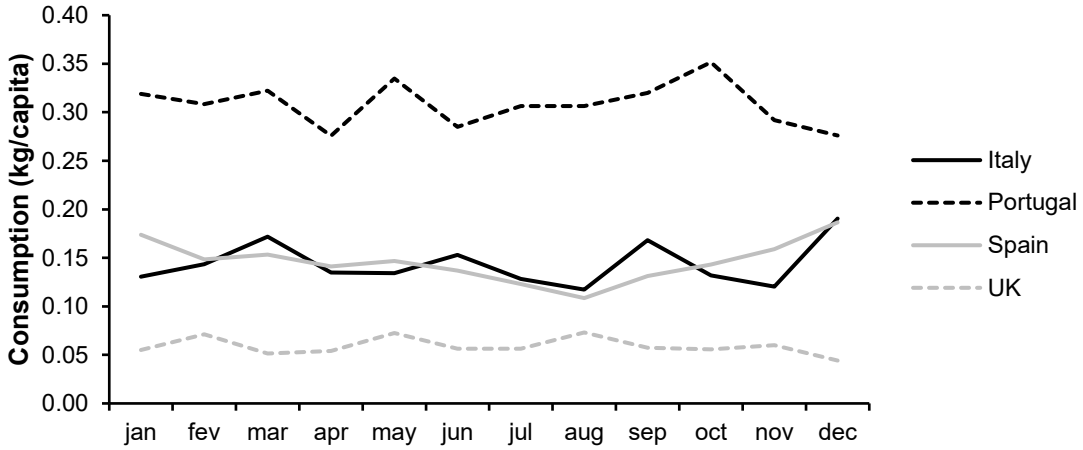

Figure S3.1. Estimated per capita seabass consumption derived from EUMOFA data on monthly household seafood and seabass consumption in volume weight – Italy (2015-2022), Portugal (2012-2022), Spain (2013-2022), United Kingdom (2014-2020) –, and seafood consumption per capita from 2019.

Table S3.1. Mean (± SD) metal concentrations reported in the muscle of European seabass (*Dicentrarchus labrax*) collected from aquaculture facilities or in the wild in the Mediterranean Sea. Bold values indicate observations over the maximum permitted concentrations of metals (µg/kg) in function of wet weight.

Country	Source	Trace metals				Non-essential metals			Reference
		Cr	Ni	Cu	Zn	Pb	Cd	Hg	
Turkey	**Cage Farm**	0.008	0.019	< 1.0	4.647	< 0.1	0.005	-	***The present study***
Portugal	**Pond Farm**	0.005	0.014	< 1.0	5.542	< 0.1	0.003	-	
	RAS Farm	0.010	0.020	< 1.0	3.657	< 0.1	0.003	-	
Turkey	**Farmed**	-	-	0.27	17.0	0.038	0.012	0.02	Bat *et al.* (2022)
	Wild	-	-	0.22	16.7	0.047	0.017	0.03	
Italy	**Farm & Wild**	-	-	-	-	0.008	< 0.05	< LoD	Cammilleri *et al.* (2022)
Turkey	**Farm**	-	-	-	-	0.03	**0.06**	0.03	Ulusoy & Mol (2022)
Croatia	**Processed**	-	-	-	-	0.01	**0.63**	0.14	Milenkovic *et al.* (2019)
Greek	**Processed**	-	-	-	-	0.01	**0.13**	0.11	
Crete	**Farm & Wild**	-	-	-	-	0.044	0.002	0.034	Renieri *et al.* (2019)
Turkey	**Cage farm**	0.040	0.016	0.20	4.25	< LoD	0.0027	-	Varol *et al.* (2019)
Corsica	**Farmed**	0.011	-	0.440	3.819	0.003	0.002	-	Marengo *et al.* (2018)
	Wild	0.020	-	0.330	4.221	0.003	0.001	-	
Aegean Sea	**Farm**	0.055	< LoD	< LoD	5.885	< LoD	< LoD	0.065	Kalantzi *et al.* (2016a)
Ionian Sea	**Farm**	0.05	< LoD	< LoD	5.91	< LoD	< LoD	0.06	
Greece	**Farm**	-	-	0.725	-	-	-	-	Kalantzi *et al.* (2016b)
Greece	**Farmed**	**1.06**	0.14	0.47	5.23	0.11	-	-	Lomolino *et al.* (2016)
Italy	**Cage Farm**	0.033	0.039	0.992	4.920	< 0.010	< 0.010	0.036	Squadrone *et al.* (2016)
Portugal		**0.31**	0.04	0.46	5.2	0.02	< 0.01	0.14	Lourenço *et al.* (2012)
Italy		0.08	-	0.99	4.97	0.11	< LoD	0.006	Trocino *et al.* (2012)
Turkey	**Processed**	-	-	-	-	0.0577	0.0073	0.0977	Yabanli *et al.* (2012)
Spain		-	-	0.27	1.68	-	-	-	Fuentes *et al.* (2010)
Greece		-	-	0.29	2.34	-	-	-	
Turkey	**Farm**	0.**17**	0.11	0.71	6.01	0.19	**0.10**	-	Türkmen *et al.* (2010)
Turkey	**Cage Farm**	-	-	-	2.89	-	**-**	-	Özden & Erkan (2008)
Turkey		-	-	-	2.8	-	-	-	Erkan & Özden (2007)
Italy	**Cage Farm**	-	-	1.17	3.35	0.18	**0.09**	-	Dugo *et al.* (2006)
	Cage Farm	-	-	0.88	2.63	0.12	< LoD	-	
Turkey	**Farmed**	**0.112**	< 0.06	-	-	**0.278**	< 0.02	-	Ersoy *et al.* (2006)
Expressed in µg/g dw									
Aegean Sea	**Wild**	0.91	-	0.86	8.40	-	-	-	Castritsi-Catharios *et al.* (2015)
Portugal	**Pond-farmed**	-	-	1.88	-	0.040	0.0024	-	Ferreira *et al.* (2010)
	Wild	-	-	1.22	-	0.034	0.0045	-	
Turkey	**Farm**	1.2	0.6	0.25	4.3	0.9	0.25	-	Yildiz (2008)
	Wild	0.4	0.2	0.1	3.1	0.6	0.2	-	
Turkey	**Wild**	-	-	0.34	52.22	0.82	0.06	-	Dural *et al.* (2007)
Turkey	**Wild**	-	-	-	82.65	-	0.075	-	Dural *et al.* (2006)
Greece	**Farmed**	0.17	4.89	3.87	45.1	1.03	0.27	-	Alasalvar *et al.* (2002)
	Wild	0.15	3.43	2.96	43.6	0.84	0.17	-	

Appendix C - Supplementary materials of the Chapter V

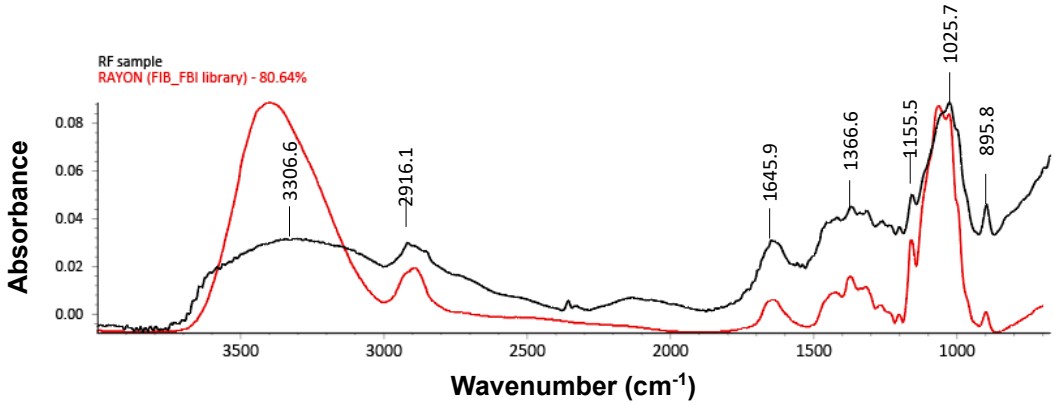

Figure S5.1. Averaged ATR-μFTIR spectra plus identified peaks obtained from the isolated microfibres (black line) in comparison with library reference (red line).

Table S5.1. Forward and reverse primers for the pathway-focused PCR-array.

Gene name	Symbol		Primer sequence (5'-3')
Adipose triglyceride lipase	*atgl*	**F**	GGA GCC CTC ACT GCC ACT
		R	ATT CGC ACC AGT CTC TCC AAG A
α-2 macroglobulin	*a2m*	**F**	GTG GTC ACA CGG TCT CTC ATA GTA AAG GC
		R	GCA GCC AGT TGT ACG TCT TAG TCA TGT CAG
Atypical chemokine receptor 4/C-C chemokine receptor type 11	*ackr4*	**F**	TAC TTC TCT TCA CCC TGC CTT TCT G
		R	GCT GCC GAA CCC AAC TTC CA
β-actin	*actb*	**F**	TCC TGC GGA ATC CAC GAG A
		R	AAC GTC GCA CTT CAT GAT GCT
β-defensin	*defb*	**F**	GGG CTG AGC TTG GTT CTC CTT GT
		R	CCT CCC CAA CTG CGA GCA TCA
Carnitine palmitoyltransferase 1a	*cpt1a*	**F**	TGC CAA GAG GTC ATC CAG AGT TCT
		R	AGT CCA CAT CAT CCG CCA GAG A
Calpain 1	*capn1*	**F**	CTA CAG AGG AAA TCC GAC TAA GC
		R	CGG TCC ATT CCA CTT CCC
Calpain 2	*capn2*	**F**	AAC GAA CTG ACA TCC GAA CTG A
		R	ATT GCC GCT GTC ATC CAT CA
Calpain 3	*capn3*	**F**	ATA CCG ACG GGA CAG GGA AG
		R	GCT GCC ACG CCT TGA TCT T
Calpastatin	*cpst*	**F**	AGA CGA CAC GCT GCC TCC A
		R	CTC AGT GGT TTA GGG ACA TCC TTG GGT TT
Cholesterol 7-α monooxygenase	*cyp7a1*	**F**	TGC CAT CAA AGT CCC ACC TCT T
		R	CAC ATC ATA GGT AGG CTG GAG GAT TC
Citrate synthase	*cs*	**F**	GTG TAT GAG ACC TCC GTG TTG G
		R	AGC AAC TTC TGA CAC TCT GGA ATG
Complement C3	*c3*	**F**	ACA GAC GGT GCA GCT TGT GT
		R	CTC GCT GGC TTT CCA TCT CCT TAC
Cytochrome b	*cyb*	**F**	TGC CTA CGC TAT CCT TCG CTC GAT CC
		R	TAA CGC CAA CAC CCC GCC CAA T
Cytochrome c oxidase subunit I	*coxi*	**F**	ATA CTT CAC ATC CGC AAC CAT AA
		R	AAG CCT CCG ACT GTA AAT AAG AAA
C-C chemokine ligand 2	*ccl2*	**F**	GCA TAA AGC CTG GTG TGT GGA T
		R	CCT CTT CTC TGT AAT CTC TGG AGT CTG
C-C chemokine ligand 4	*ccl4*	**F**	TGG CTG CTC CTC GTC TCA
		R	GAC CAC GCA CAC CTT CAG T
C-C chemokine receptor type 3	*ccr3*	**F**	TGA CCT TCG ACC GAC ACC TA
		R	ACA ATA CAG GAG ACT ACC GCA TAG C
C-C chemokine receptor type 9	*ccr9*	**F**	CCT GTG TGT CTG GCT TGT TTC TAC TCT C
		R	TCG CTC TTC ACC TGG GCA AAG ATA AAC TC
Dicentracine	*dic*	**F**	GTG CTG TCG ATG GTC GTC CTC
		R	TGT GGT GAA AGA AGG CGT CCC
Elongation of very long chain fatty acids 1	*elovl1*	**F**	TAC ACA TCT TCC ACC ACT CCT TCA T
		R	CCA TTC CAC CAG GAG CAT AGG
Elongation of very long chain fatty acids 4	*elovl4*	**F**	ACC ATG CTT ACC GAC GCA AAC CTT
		R	CGA CGT GCT TGC CTC CCT TCT G
Elongation of very long chain fatty acids 5	*elovl5*	**F**	CAG TCA TGT ACC TTC TGA TCG TGT GGA TGG
		R	GGA GTA CGG CTG CCT GTG TTT CAT
Elongation of very long chain fatty acids 6	*elovl6*	**F**	ACA TCA CCG TGC TGC TCT ACT CCT G
		R	CCG CCA CCT GGT CCT TGT AGC A
Fatty acid desaturase 2	*fads2*	**F**	CCG CCG TGA CTG GGT GGA T
		R	GCA CAG GTA GCG AAG GTA GTA AGA CAT AGA
Fibroblast growth factor 4	*fgf4*	**F**	GGC TTT GTG ACC GGA ATG G
		R	GTC CGC TGT CCC GTT CAG
Fibroblast growth factor 6	*fgf6*	**F**	CAA CGC CTA CGA GTC TCT GGT CTA C
		R	GCC ATG CTT GCT GAG TGC TAT GT
Follistatin	*fst*	**F**	GTG CCA GTG ACA ACA CCA CAT ATC C
		R	ATC CCG AGT GCT TGA CTT CCA
Galectin-8-like isoform X1	*lgals8x1*	**F**	GTC TTC GTC AGA AAC TCC TTC CT
		R	CCA GCT TCG TCT CCT CGG
Growth hormone receptor-type 1	*ghr1*	**F**	GGT GGA TGC TGA GGA TGC
		R	GGT GTC TGA GCC CTG GTT
Growth hormone receptor-type 2	*ghr2*	**F**	TCC AGT CCA GAG CCC TAC
		R	ACG ACC TCA CCT CAC TCA
Hepatic lipase	*hl*	**F**	CGC AGT GGC ACC AGC AAG A
		R	CGG CAT CCG AGA CCG TGT T
Hepcidin precursor	*hepc*	**F**	GCT GGA GGA GCC AAT GAG CAA TGA G
		R	CGCTTGTGTCTGTTGTTATACGGCATCTT
Hormone-sensitive lipase	*hsl*	**F**	GCC CTG TCT CCA GAC TAT TGC TAT C
		R	GCT GCT ACA CCT ATT CCT GAC TGA T
Immunoglobulin M membrane-bound	*lgmb*	**F**	ACA GAG GAA GAT AAC ATG GCG GTG G
		R	TGG TTA CAA TGG TGA ACA GCA GAG TGAT
Immunoglobulin M secreted	*lgms*	**F**	GTG TCT TGG CTT GTT GAT GAC GAG GAA
		R	TCT ACA GGC TTT GTG GTA TGG AAC TCA

Table S5.1. Continuation (part 2/3).

Gene name	Symbol		Primer sequence (5'-3')
Immunoglobulin T	*igt*	**F**	TGA GCT GCA CAG ATG AAG AGG ATG AGT T
		R	GGA ATA TGA AGG AGG AGG CTG TGG ACC
Insulin-like growth factor 1	*igf1*	**F**	TAG CCA CAC CCT CTC ACT ACT G
		R	CCT GTT GCC GTC GGA GTC
Insulin-like growth factor 2	*igf2*	**F**	AGA CAC GGA CAC CAC ACA CTT TG
		R	CTC TTG ACC TTC ATT CTG CTG CTC TC
Insulin-like binding-protein 1b	*igfbp1b*	**F**	AGT GTG AAT CAT CTC TGG TTG GA
		R	CCC ATT CCA GGA AGA GAC ACA
Insulin-like binding-protein 2b	*igfbp2b*	**F**	GCA CGG AGG CTG ACT TAC C
		R	CTT GGT CCA GAG TTG TTG TGA GAT
Insulin-like binding-protein 3a	*igfbp3a*	**F**	GCG TGG CAA CCG TGA AGG
		R	GCC TGG TGT CCA CAG ATC C
Insulin-like binding-protein 4	*igfbp4*	**F**	ACT CAG CGA TGG ACA GGC AGG AT
		R	CGG ATG TTG CTG TTG TTG GGA TGC T
Insulin-like binding-protein 5b	*igfbp5b*	**F**	GTG CCA CTC CTT CCC AAA GAC AT
		R	CTG CTT GCC CAG CTT CCT CT
Insulin-like binding-protein 6b	*igfbp6b*	**F**	CCA GGG ACC ATA ATG TTG CC
		R	TAC ACA CCA CAG GGC TCT C
Interferon regulatory factor 8	*irf8*	**F**	TCT GAA GGC TGC CGA ATC TCC
		R	CTG TCT GAA CTG TAT AGG GCA CCA C
Interleukin-1β	*il1b*	**F**	CAT GAG CGA GAT GTG GAG ATC CAA GAT
		R	CAT TGT CAG TGG GTG GTG GGT AAT C
Interleukin-6	*il6*	**F**	CAT GCC CTG AGA AGT CCA
		R	TTG AGA AGA GCT GTG TAA GTG A
Interleukin-8	*il8*	**F**	CAA TCA GCA GGG ACT ACA ACA CAC A
		R	CTG TCT GGA GGG ATG ATC CTT GAC T
Interleukin-10	*il10*	**F**	CAG TGC TGT CGT TTT GTG GAG GGT TTC
		R	TCT CTG TGA AGT CTG CTC TGA GTT GCC TTA
Interleukin-17A/F	*Il17a/f*	**F**	GTT GTT TGC TGA ACG GCT GTC TC
		R	CTC CAG GTT CGG GTC CTC TCT
Interleukin-20	*il20*	**F**	GCT AGA AAT AAA GGA GGC GGC ACA GAA GG
		R	CAG TCC AGC ACA GTG TCC AGT TCT C
Interleukin-22	*il22*	**F**	AGC AAG CCA CTG CGA GAC
		R	CGT CTG CTG AGC GTG TTG
Interleukin-34	*il34*	**F**	AGA ACC CGA CAG AGT GCC AGA GT
		R	CAG GAG GGA TTT TGG GGA CGC ATA TC
Lipoprotein lipase	*lpl*	**F**	CAA TGT GAT CGT GGT GGA CTG
		R	CGT CGG GTA GTG CTG GTT
Liver-expressed antimicrobial peptide 2	*leap2*	**F**	GGT TTG CTC CAA CGG ACC AA
		R	CAC AGG CTT CAT GCT GTT CCA
Lysozyme C	*lyz*	**F**	CGG AGC CAT CAA CCA CAA CAC TG
		R	GCC ATT ATT ACA CCA CCA GCG ACT GT
Macrophage colony-stimulating factor 1 receptor	*csf1r*	**F**	CGG GCA GGA ACA GCT AAT CTA CCA
		R	ACT TGG GCT CAT CAC ACA CTT CAC
Macrophage migration inhibitory factor	*mif*	**F**	GCT CCC TCC ACA GTA TTG GCA AGA T
		R	TTG AGC AGT CCA CAC AGG AGT TTA GAG T
Major Histocompatibility Complex Class I	*mhci*	**F**	ACA TAG CAT TTG ACC TGA GGA CAG AGA CAT
		R	CCT GTG GCA CTG GAG CGA CC
Major Histocompatibility Complex Class II	*mhcii*	**F**	GCC AGC CTG AGA GAA CCT
		R	GCT CCG ATG GCG ATC TTG T
Mitochondrial respiratory uncoupling protein 1	*ucp1*	**F**	CGA TTC CAA GCC CAG ACG AAC CT
		R	TGC CAG TGT AGC GAC GAG CC
Mitochondrial respiratory uncoupling protein 3	*ucp3*	**F**	CCA TGC TGA GAC AGG AAG GAC CCA CAT
		R	CCA TGC TGA GAC AGG AAG GAC CCA CAT
Monocyte to macrophage differentiation factor	*mmd*	**F**	GGT CAT CTA CTT CTT CAT CGC TGC CTC CTA
		R	CCA ACT CTC GCA GGT TCA ACC AAG GT
Mucin 2	*muc2*	**F**	GAC TGA AGA TTG TTG GTC CAC ACC TGC C
		R	AGG GCA AGT AGG TAC AGA AGG TTT CGT TGT
Muscle atrophy F-box	*mafbx/atrogin 1*	**F**	ACT GAG GAC CGA CTG CTG TGG AAG A
		R	TGT CTG TCT GTG AAG TGG TAC TGG CAA AGT
Muscle RING-finger protein 1	*murf1*	**F**	TGG TGC GTC CTG TCA GTG
		R	TGG TGC GTC CTG TCA GTG
Myeloid cell surface antigen CD33	*cd33*	**F**	CTG TTC ATT CAC CCA TCC TAG AG
		R	GGT CGA ACG ATG CCA GAT T
Myeloid differentiation primary response protein MyD88	*myd88*	**F**	CCA ATT CAG GTT GAT GAG GTT GAC A
		R	TCC TCC AGG GTG ATA CCA ATC C
Myoblast determination protein 1	*myod1*	**F**	GAC CGA CCT GTC AGT CCA ACC G
		R	TGG AGT CTC GGA GAA ATA AGA GCT GTT GT
Myoblast determination protein 2	*myod2*	**F**	CTG CTG ATG ACC TCT ACG ATG AC
		R	GGC GTC CAG GTC GTC AAA
Myogenic factor 5	*myf5*	**F**	CGC AAC GCC ATC CAG TAC ATC G
		R	GCC GTA GTA GTT TTC CAC CTG CTC AT

Table S5.1. Continuation (part 3/3).

Gene name	Symbol		Primer sequence (5'-3')
Myogenic regulatory factor 4	*mrf4*	**F**	GTC TCC TCT ATA CAA CGG CAA T
		R	CTG TCT CGG ACG GAA CAT TAT C
Myogenin	*myog*	**F**	GAC CAA CCC TTA TTT CTT
		R	CAT CAT GGA GTT CCT ATC
Myomaker	*mymk*	**F**	ATC TGT CTC TGG CTG TGT CCT TCA T
		R	CAG CAT TTC GTC CCG TCC CT
Myostatin	*mstn*	**F**	GCA GCA GCT TCT CGA CCA GTA
		R	ATC GTC GTC CTC CAT AAC CAC ATC
NADH dehydrogenase subunit 5	*nd5*	**F**	CCC GAT TTC TGT GCC CTA CTA
		R	AGG AAA GGA GTG CCT GTG A
Nuclear factor NF-κ-B p100 subunit	*nfkb2*	**F**	CTG GAG GAA ACT GGC GGA GAA GC
		R	CAG GTA CAG GTG AGT CAG CGT CAT C
Pentraxin (C-reactive protein)	*crp*	**F**	CAG ACC TCA AAA GAG ACC ACG TCC TT
		R	CAT CCC AGA AAA GCA GAA AAG CAT TGG
Pentraxin (serum amyloid P component 3)	*sap*	**F**	GGC TGA CTA CAA CAA GAC AGA ATT TGA G
		R	GGC TAT GTG TTC TAC GGA GGT CT
Peroxisome proliferator-activated receptor α	*pparα*	**F**	CGT GCC TCT AGT GGA ACA GC
		R	AGC AGG TGG AGC CGT AGT
Peroxisome proliferator-activated receptor γ	*pparγ*	**F**	CAG GAC ACG CAC AAC TCA ATC A
		R	GGA GAA CAC GGG ACA GTC AGA A
Stearoyl-CoA desaturase 1b	*scd1b*	**F**	GCT TGT GGC ATA CTT CAT CCC TGG ACT C
		R	GGT GGC GTT GAG CAT CAC GGT GTA
Sirtuin 1	*sirt1*	**F**	GGT GGA CCT CTT GAT TGT CAT TGG CTC TTC
		R	GGG ATG AGG GCA ACT GGT CGG ACT TTA
Sirtuin 2	*sirt2*	**F**	TCT AAT TGA GGC TCA CGG AAC
		R	GAC GGG TAG ATT CTC TCC AAA G
Succinate dehydrogenase cytochrome b560 subunit	*sdhc*	**F**	ACA TGG GCA AGG GCT TCA AA
		R	CGA TGA TGG ACA GAC CGA TAA CG
Transferrin	*trf*	**F**	ACC GTT GAT TGC CGG AAT GCC
		R	ACT GCC ATT GCG TCA GCC TCT T
Tumour necrosis factor α	*tnfα*	**F**	CAG GTA CAG GTG AGT CAG CGT CAT C
		R	CCG CAC TTT CCT CTT CAC CAT CGT
T-cell surface glycoprotein cd3 ζ chain	*cd247*	**F**	CTG ATG CGT CTG AAG AGA ATG GAG GC
		R	GTT CAA GCA CCT GGT AAG GAT CAG CAT C
T-cell surface glycoprotein cd4	*cd4*	**F**	GGG ACA TTG AGG GAG GAA AGT GGG AAT
		R	AGA GGG AGA AGA GCA TCT GTG GAG CAT TA
T-cell surface glycoprotein cd8 α	*cd8a*	**F**	AGT GCC CAC CAT CAA ACC AAC TCT ATG C
		R	CCT TCT TGT TAC ACA CAC ATG GCG TGG TAG
T-cell surface glycoprotein cd8 β	*cd8b*	**F**	AGT GAT CCC GCC AAC ATT ACC TCC TA
		R	TCT TCT TAG GGC AGC GAC AGA CT

Table S5.2. Relative gene expression of liver mRNA transcripts of fish fed the experimental diets. Values are the mean ± SEM of 9 fish per experimental condition. All data are in reference to the expression level of *fads2* of CTRL fish with an arbitrarily assigned value of 1.

Gene	CTRL	RF1	RF2	RF3	*p*-value
ghr1	2.97 ± 0.35	3.64 ± 0.40	4.18 ± 0.42	4.06 ± 0.77	0.306
ghr2	1.75 ± 0.32	1.68 ± 0.16	1.72 ± 0.28	1.34 ± 0.23	0.519
igf1	9.71 ± 1.11	9.61 ± 1.07	8.21 ± 0.88	9.12 ± 2.03	0.858
igf2	1.61 ± 0.27	1.95 ± 0.25	1.77 ± 0.24	1.59 ± 0.37	0.784
igfbp1b	2.52 ± 0.47	2.58 ± 0.41	2.54 ± 0.88	2.24 ± 0.63	0.784
igfbp2b	17.24 ± 2.11	16.36 ± 1.86	19.53 ± 1.39	16.93 ± 2.55	0.702
igfbp4	1.18 ± 0.16	1.58 ± 0.14	1.11 ± 0.13	1.20 ± 0.22	0.203
atgl	0.37 ± 0.07	0.31 ± 0.04	0.25 ± 0.06	0.25 ± 0.04	0.271
elovl1	0.17 ± 0.02	0.23 ± 0.04	0.19 ± 0.02	0.17 ± 0.03	0.349
elovl5	0.007 ± 0.001^{a}	0.006 ± 0.001^{a}	0.013 ± 0.003^{ab}	0.016 ± 0.003^{b}	**0.004**
elovl6	1.14 ± 0.23	1.33 ± 0.20	1.13 ± 0.14	1.59 ± 0.53	0.720
fads2	1.03 ± 0.08	1.24 ± 0.24	1.52 ± 0.16	1.38 ± 0.12	0.148
hsl	0.58 ± 0.08	0.58 ± 0.05	0.46 ± 0.06	0.51 ± 0.06	0.505
scd1b	1.58 ± 0.46^{a}	2.85 ± 0.96^{a}	7.94 ± 1.95^{b}	7.36 ± 2.03^{b}	**0.007**
lpl	6.74 ± 0.74	7.48 ± 0.94	5.52 ± 0.39	5.58 ± 0.54	0.303
hl	5.20 ± 0.33	5.45 ± 0.44	5.52 ± 0.39	5.58 ± 0.54	0.928
pparα	0.05 ± 0.01	0.05 ± 0.01	0.06 ± 0.01	0.05 ± 0.01	0.934
pparγ	0.25 ± 0.02	0.28 ± 0.03	0.031 ± 0.03	0.27 ± 0.01	0.330
cpt1a	0.21 ± 0.03^{ab}	0.22 ± 0.02^{a}	0.14 ± 0.02^{b}	0.17 ± 0.02^{ab}	**0.032**
cs	0.86 ± 0.06	1.06 ± 0.04	0.96 ± 0.05	0.98 ± 0.07	0.170
cyp7a1	6.58 ± 0.69^{ab}	5.80 ± 0.46^{a}	11.25 ± 1.40^{c}	9.58 ± 1.07^{bc}	**0.001**
cyb	0.28 ± 0.03^{ab}	0.25 ± 0.03^{a}	0.40 ± 0.03^{c}	0.37 ± 0.03^{bc}	**0.003**
cox1	250.38 ± 17.86^{a}	233.00 ± 13.49^{a}	306.78 ± 11.33^{b}	246.48 ± 13.43^{a}	**0.007**
ucp1	6.57 ± 0.59^{a}	6.46 ± 0.71^{a}	8.31 ± 0.49^{b}	8.89 ± 0.84^{b}	**0.034**
nd5	83.02 ± 5.37	80.57 ± 6.69	96.31 ± 6.14	75.41 ± 4.73	0.089
sirt1	0.08 ± 0.01	0.09 ± 0.01	0.09 ± 0.01	0.08 ± 0.01	0.893
sirt2	0.77 ± 0.05	0.73 ± 0.09	0.86 ± 0.05	0.78 ± 0.06	0.529
sdhc	2.25 ± 0.13	2.13 ± 0.12	2.26 ± 0.09	2.00 ± 0.20	0.536

Table S5.3. Relative gene expression of white skeletal muscle mRNA transcripts of fish fed the experimental diets. Values are the mean ± SEM of 9 fish per experimental condition. All data are in reference to the expression level of *ghr1* of CTRL fish with an arbitrarily assigned value of 1.

Gene	CTRL	RF1	RF2	RF3	*p*-value
ghr1	1.01 ± 0.06	0.97 ± 0.11	1.00 ± 0.13	1.00 ± 0.14	0.994
ghr2	0.74 ± 0.12	0.69 ± 0.07	0.51 ± 0.07	0.72 ± 0.12	0.332
igf1	0.05 ± 0.01	0.04 ± 0.01	0.05 ± 0.01	0.04 ± 0.01	0.495
igf2	0.90 ± 0.11	0.81 ± 0.08	0.72 ± 0.07	0.89 ± 0.11	0.488
igfbp3a	1.24 ± 0.07	1.12 ± 0.10	1.24 ± 0.14	1.26 ± 0.13	0.809
igfbp5b	1.95 ± 0.15	1.96 ± 0.19	2.43 ± 0.31	1.93 ± 0.28	0.407
igfbp6b	0.29 ± 0.02	0.26 ± 0.04	0.31 ± 0.06	0.29 ± 0.04	0.882
scd1b	0.39 ± 0.03	0.47 ± 0.11	0.49 ± 0.11	0.51 ± 0.08	0.893
cpt1a	0.95 ± 0.09	0.73 ± 0.09	0.67 ± 0.05	0.82 ± 0.11	0.148
cs	17.31 ± 0.83	15.51 ± 0.85	17.83 ± 0.81	17.58 ± 1.09	0.273
ucp3	4.48 ± 1.07^{ab}	3.62 ± 0.55^{ab}	3.71 ± 0.90^{a}	6.69 ± 1.00^{b}	**0.049**
sirt1	0.26 ± 0.01	0.26 ± 0.02	0.24 ± 0.01	0.27 ± 0.02	0.603
sirt2	1.62 ± 0.06	1.53 ± 0.08	1.66 ± 0.09	1.77 ± 0.09	0.246
fgf4	0.34 ± 0.02	0.29 ± 0.01	0.35 ± 0.01	0.33 ± 0.02	0.200
fgf6	0.46 ± 0.04	0.40 ± 0.02	0.51 ± 0.05	0.50 ± 0.05	0.379
fst	0.50 ± 0.06	0.44 ± 0.08	0.46 ± 0.07	0.50 ± 0.05	0.882
myod1	9.41 ± 0.58	8.40 ± 0.62	9.55 ± 0.54	9.11 ± 0.82	0.607
myod2	4.61 ± 0.39	4.21 ± 0.31	5.30 ± 0.35	4.10 ± 0.35	0.093
mrf4	0.75 ± 0.04^{ab}	0.62 ± 0.04^{a}	0.79 ± 0.04^{b}	0.73 ± 0.04^{ab}	**0.031**
myf5	0.55 ± 0.03	0.54 ± 0.04	0.54 ± 0.03	0.52 ± 0.03	0.922
myog	2.58 ± 0.24	2.34 ± 0.14	2.47 ± 0.21	2.42 ± 0.25	0.887
mymk	0.10 ± 0.02	0.12 ± 0.01	0.07 ± 0.01	0.07 ± 0.02	0.089
mstn	0.81 ± 0.20	0.79 ± 0.29	0.85 ± 0.24	1.33 ± 0.35	0.362
mafbx/atrogin 1	0.61 ± 0.16^{ab}	0.48 ± 0.10^{a}	0.40 ± 0.06^{a}	1.24 ± 0.44^{b}	**0.047**
murf1	11.87 ± 0.96^{a}	8.62 ± 0.49^{b}	9.81 ± 0.59^{ab}	11.10 ± 0.76^{ab}	**0.017**
capn1	1.95 ± 0.05	1.92 ± 0.09	2.11 ± 0.10	2.14 ± 0.10	0.251
capn2	0.94 ± 0.05	0.90 ± 0.03	0.97 ± 0.05	0.87 ± 0.03	0.337
capn3	4.77 ± 0.60	4.33 ± 0.38	5.67 ± 0.81	4.86 ± 0.49	0.450
cpst	0.79 ± 0.06	0.86 ± 0.06	0.90 ± 0.05	0.78 ± 0.04	0.405

Table S5.4. Relative gene expression of anterior intestine mRNA transcripts of fish fed the experimental diets. Values are the mean ± SEM of 9 fish per experimental condition. All data are in reference to the expression values of *nfkb2* of CTRL fish with an arbitrarily assigned value of 1.

Gene	CTRL	RF1	RF2	RF3	*p*-value
defb	0.002 ± 0.001	0.002 ± 0.001	0.002 ± 0.001	0.004 ± 0.001	0.556
dic	31.81 ± 9.32	33.35 ± 6.17	31.51 ± 5.74	26.36 ± 7.07	0.913
hepc	0.012 ± 0.03	0.021 ± 0.007	0.007 ± 0.001	0.048 ± 0.027	0.335
leap2	55.19 ± 10.41	60.73 ± 9.55	67.45 ± 18.02	75.00 ± 15.99	0.771
muc2	22.29 ± 1.51	24.89 ± 2.67	25.06 ± 4.78	24.11 ± 2.30	0.904
ccl2	0.038 ± 0.013	0.029 ± 0.008	0.013 ± 0.003	0.018 ± 0.006	0.352
ccl4	0.46 ± 0.04	0.51 ± 0.08	0.80 ± 0.18	0.69 ± 0.13	0.182
ccr3	0.67 ± 0.07	0.61 ± 0.09	0.68 ± 0.11	0.56 ± 0.08	0.776
ccr9	0.35 ± 0.04	0.35 ± 0.04	0.40 ± 0.04	0.39 ± 0.06	0.822
il1b	0.007 ± 0.001^{a}	0.006 ± 0.001^{a}	0.015 ± 0.003^{b}	0.009 ± 0.001^{ab}	**0.001**
il6	0.058 ± 0.007	0.058 ± 0.005	0.069 ± 0.016	0.056 ± 0.010	0.874
il8	0.032 ± 0.005	0.034 ± 0.009	0.031 ± 0.007	0.044 ± 0.009	0.587
il10	0.012 ± 0.001	0.011 ± 0.001	0.014 ± 0.001	0.016 ± 0.003	0.179
il17a/F	0.003 ± 0.001	0.005 ± 0.001	0.007 ± 0.002	0.005 ± 0.001	0.152
il20	0.028 ± 0.003	0.034 ± 0.002	0.030 ± 0.003	0.041 ± 0.009	0.284
il22	0.016 ± 0.004	0.014 ± 0.004	0.015 ± 0.001	0.016 ± 0.003	0.837
csf1r	0.41 ± 0.03	0.48 ± 0.06	0.47 ± 0.09	0.52 ± 0.09	0.738
mif	4.84 ± 0.49	5.58 ± 0.27	5.67 ± 0.70	5.83 ± 0.40	0.398
mmd	3.39 ± 0.24	3.50 ± 0.21	3.66 ± 0.42	3.80 ± 0.32	0.807
nfkb2	1.04 ± 0.10	1.26 ± 0.11	1.46 ± 0.22	1.44 ± 0.20	0.258
tnfα	0.056 ± 0.005^{a}	0.063 ± 0.004^{ab}	0.078 ± 0.008^{b}	0.075 ± 0.007^{ab}	**0.027**
igms	3.30 ± 0.50	3.02 ± 0.35	3.05 ± 0.44	4.25 ± 1.05	0.953
igt	1.21 ± 0.30	0.98 ± 0.25	0.69 ± 0.26	0.54 ± 0.18	0.279
mhc1	5.85 ± 0.53	6.80 ± 0.63	7.34 ± 1.00	6.93 ± 0.73	0.549
mhc2	37.06 ± 1.79	44.49 ± 5.33	46.19 ± 4.83	44.33 ± 5.55	0.519
cd247	1.12 ± 0.09	1.00 ± 0.06	1.09 ± 0.08	0.96 ± 0.08	0.499
cd4	0.023 ± 0.002	0.019 ± 0.001	0.021 ± 0.002	0.018 ± 0.003	0.344
cd8a	0.32 ± 0.03	0.38 ± 0.03	0.36 ± 0.04	0.37 ± 0.04	0.671
cd8b	0.032 ± 0.003	0.038 ± 0.004	0.040 ± 0.004	0.036 ± 0.006	0.751

Table S5.5. Relative gene expression of head kidney mRNA transcripts of fish fed the experimental diets. Values are the mean ± SEM of 9 fish per experimental condition. All data are in reference to the expression level of *mmd* of CTRL fish with an arbitrarily assigned value of 1.

Gene	CTRL	RF1	RF2	RF3	*p*-value
defb	0.007 ± 0.003	0.003 ± 0.001	0.013 ± 0.004	0.008 ± 0.003	0.138
lyz	0.040 ± 0.019	0.029 ± 0.003	0.025 ± 0.002	0.020 ± 0.003	0.264
leap2	0.038 ± 0.016	0.018 ± 0.007	0.027 ± 0.010	0.029 ± 0.009	0.321
a2m	0.009 ± 0.004	0.005 ± 0.003	0.003 ± 0.001	0.011 ± 0.008	0.806
c3	0.009 ± 0.002	0.005 ± 0.001	0.004 ± 0.001	0.012 ± 0.007	0.910
crp	43.23 ± 4.62	44.83 ± 4.79	41.60 ± 3.39	35.98 ± 3.73	0.470
sap	0.046 ± 0.008	0.042 ± 0.011	0.037 ± 0.006	0.041 ± 0.010	0.908
trf	0.116 ± 0.055	0.028 ± 0.012	0.013 ± 0.005	0.027 ± 0.012	0.065
lgals8x1	2.72 ± 0.29	3.08 ± 0.31	3.10 ± 0.33	3.20 ± 0.32	0.709
ackr4	0.34 ± 0.02	0.31 ± 0.02	0.31 ± 0.02	0.28 ± 0.02	0.293
ccr3	4.26 ± 0.68	4.42 ± 0.91	4.16 ± 0.74	3.56 ± 0.62	0.858
ccr9	2.79 ± 0.35	2.49 ± 0.25	2.27 ± 0.17	2.03 ± 0.28	0.250
irf8	8.09 ± 0.63^{a}	7.79 ± 0.72^{ab}	6.72 ± 0.33^{ab}	6.08 ± 0.40^{b}	**0.037**
il1b	0.13 ± 0.03	0.16 ± 0.05	0.10 ± 0.02	0.09 ± 0.02	0.860
il8	1.21 ± 0.15	1.14 ± 0.20	1.23 ± 0.23	1.30 ± 0.19	0.951
il10	0.23 ± 0.03	0.22 ± 0.02	0.18 ± 0.02	0.15 ± 0.01	0.086
il20	0.15 ± 0.01^{ab}	0.17 ± 0.01^{a}	0.13 ± 0.01^{ab}	0.12 ± 0.01^{b}	**0.021**
il34	1.19 ± 0.12	1.19 ± 0.12	1.04 ± 0.06	1.10 ± 0.08	0.646
csf1r	4.30 ± 0.39	4.81 ± 0.37	4.40 ± 0.27	4.09 ± 0.26	0.484
mif	16.78 ± 1.68	19.69 ± 1.87	16.41 ± 1.31	16.35 ± 1.09	0.359
mmd	1.01 ± 0.04	1.26 ± 0.09	1.11 ± 0.08	1.09 ± 0.08	0.109
nfkb2	8.64 ± 0.52	9.61 ± 0.40	8.55 ± 0.31	8.30 ± 0.38	0.154
tnfα	0.37 ± 0.03	0.41 ± 0.06	0.33 ± 0.02	0.31 ± 0.03	0.286
igmb	49.66 ± 5.97	55.69 ± 5.34	45.16 ± 5.81	40.16 ± 6.04	0.291
igms	44.15 ± 9.82^{a}	43.43 ± 4.11^{ab}	35.61 ± 4.03^{ab}	23.85 ± 2.05^{b}	**0.037**
cd33	13.67 ± 1.21^{a}	15.33 ± 1.37^{a}	8.56 ± 0.65^{b}	8.46 ± 0.69^{b}	**<0.001**
myd88	4.40 ± 0.26	4.34 ± 0.18	4.15 ± 0.20	4.25 ± 0.25	0.875
cd247	1.33 ± 0.07	1.38 ± 0.14	1.21 ± 0.09	1.16 ± 0.12	0.382
cd8b	1.29 ± 0.40	1.32 ± 0.36	1.02 ± 0.13	0.68 ± 0.15	0.414

Table S5.6. Relative gene expression in three tissues (Liver, WSM, HK), grouping together CTRL & RF1 and RF2 & RF3 experimental diets. Values are the mean ± SEM of 15-18 fish per experimental super-group. All data are in reference to the expression level of a CTRL fish gene as described in the Supplementary tables of each tissue.

Gene	CTRL_RF1	RF2_RF3	*p*-value
Liver			
ghr1	3.1 ± 0.26	4.11 ± 0.38	**0.035**
elovl5	0.006 ± 0.001	0.02 ± 0	**<0.001**
fads2	1.05 ± 0.1	1.44 ± 0.11	**0.013**
scd1b	1.89 ± 0.57	8.98 ± 1.45	**<0.001**
lpl	7.11 ± 0.66	5.11 ± 0.57	**0.031**
atgl	0.35 ± 0.04	0.21 ± 0.03	**0.016**
hsl	0.59 ± 0.05	0.44 ± 0.04	**0.029**
cpt1a	0.22 ± 0.02	0.14 ± 0.01	**<0.001**
cyb	0.26 ± 0.02	0.4 ± 0.02	**<0.001**
cox1	235.93 ± 10.92	277.61 ± 12.37	**0.028**
cyp7a1	6.18 ± 0.47	10.62 ± 0.89	**<0.001**
ucp1	6.11 ± 0.4	8.98 ± 0.53	**<0.001**
WSM			
ghr2	0.76 ± 0.07	0.59 ± 0.06	0.072
igfbp6b	0.28 ± 0.02	0.32 ± 0.04	0.27
cpt1a	0.84 ± 0.07	0.71 ± 0.04	0.13
cs	15.99 ± 0.59	18.13 ± 0.66	**0.022**
myod1	8.68 ± 0.45	9.9 ± 0.44	0.061
myod2	4.17 ± 0.21	4.97 ± 0.29	0.058
mrf4	0.68 ± 0.03	0.78 ± 0.03	**0.047**
fgf4	0.31 ± 0.01	0.35 ± 0.01	**0.010**
fgf6	0.41 ± 0.02	0.54 ± 0.03	**0.008**
mymk	0.12 ± 0.01	0.06 ± 0.01	**0.001**
capn1	1.9 ± 0.06	2.2 ± 0.07	**0.004**
capn3	4.14 ± 0.22	5.75 ± 0.46	**0.001**
sirt2	1.55 ± 0.05	1.78 ± 0.06	**0.007**
HK			
defb	0.004 ± 0.001	0.009 ± 0.002	**0.034**
ccr9	2.68 ± 0.21	2.01 ± 0.16	**0.018**
igmb	54.21 ± 3.45	37.39 ± 3.19	**0.001**
igms	44.33 ± 5.48	28.53 ± 3.02	**0.004**
irf8	7.66 ± 0.44	6.19 ± 0.28	**0.010**
il10	0.23 ± 0.02	0.15 ± 0.01	**0.001**
il20	0.17 ± 0.01	0.12 ± 0.01	**0.003**
csf1r	4.55 ± 0.19	4.02 ± 0.17	0.050
mif	18.18 ± 1.16	15.13 ± 0.42	**0.044**
cd33	14.14 ± 0.88	8.44 ± 0.53	**<0.001**
crp	43.37 ± 2.76	36.08 ± 2.35	0.054
cd8b	1.12 ± 0.24	0.81 ± 0.1	0.32
tnfα	0.36 ± 0.02	0.3 ± 0.01	0.063

Table S5.7. Pearson **c**orrelation values between humoral immune parameters in plasma and the gene expression of immune-related genes in the anterior intestine and head kidney of European seabass (*Dicentrarchus labrax*) exposed to viscose-rayon microfibres through experimental diets. Genes marked with "*" were non-normally distributed and evaluated by Spearman correlation.

	Lysozyme activity	**Peroxidase activity**	**Total Ig content**
Plasma			
Lysozyme activity	-	-	-
Peroxidase activity	**r = -0.477, *p* = 0.005**	-	-
Total Igl content	r = 0.277, *p* = 0.154	r = 0.014, *p* = 0.945	-
Anterior intestine			
defb	r = -0.081, *p* = 0.655	r = 0.149, *p* = 0.409	r = -0.228, *p* = 0.244
dic	r = -0.190, *p* = 0.298	r = 0.234, *p* = 0.198	r = 0.245, *p* = 0.219
*hepc**	r = -0.094, *p* = 0.621	r = -0.099, *p* = 0.601	r = 0.092, *p* = 0.654
leap2	r = -0.203, *p* = 0.258	r = 0.098, *p* = 0.589	r = 0.041, *p* = 0.838
muc2	**r = -0.427, *p* = 0.018**	r = -0.249, *p* = 0.184	r = 0.014, *p* = 0.946
ccl2	r = 0.066, *p* = 0.726	r = -0.072, *p* = 0.700	r = 0.307, *p* = 0.128
ccl4	r = 0.062, *p* = 0.479	r = -0.023, *p* = 0.905	r = 0.224, *p* = 0.292
ccr3	r = -0.026, *p* = 0.884	r = -0.025, *p* = 0.892	**r = 0.409, *p* = 0.031**
ccr9	r = -0.055, *p* = 0.761	r = -0.062, *p* = 0.732	r = 0.205, *p* = 0.296
*il1b**	r = -0.203, *p* = 0.266	r = -0.083, *p* = 0.652	r = 0.234, *p* = 0.231
il6	**r = -0.376, *p* = 0.031**	r = 0.139, *p* = 0.442	r = 0.301, *p* = 0.120
il8	r =-0.082, *p* = 0.655	r = 0.043, *p* = 0.817	r = 0.033, *p* = 0.869
il10	r = 0.074, *p* = 0.684	r = -0.014, *p* = 0.939	**r = 0.413, *p* = 0.029**
il17a/F	r = 0.164, *p* = 0.368	r = -0.117, *p* = 0.523	r = 0.244, *p* = 0.221
il20	r = 0.015, *p* = 0.936	r = -0.143, *p* = 0.426	r = 0.041, *p* = 0.835
il22	r = -0.327, *p* = 0.078	r = 0.190, *p* = 0.315	r = 0.108, *p* = 0.608
csf1r	r = 0.008, *p* = 0.966	r = -0.005, *p* = 0.977	r = 0.212, *p* = 0.279
mif	r = 0.008, *p* = 0.967	r = -0.194, *p* = 0.280	r = -0.051, *p* = 0.795
mmd	r = 0.032, *p* = 0.860	r = 0.179, *p* = 0.318	r = 0.050, *p* = 0.800
nfkb2	r = 0.101, *p* = 0.584	r = 0.268, *p* = 0.138	r = 0.054, *p* = 0.788
tnfα	r = 0.133, *p* = 0.459	r = -0.065, *p* = 0.719	r = 0.254, *p* = 0.192
igms	r = 0.085, *p* = 0.638	r = 0.167, *p* = 0.352	r = -0.070, *p* = 0.724
igt	r = -0.079, *p* = 0.677	r = -0.168, *p* = 0.374	r = -0.031, *p* = 0.883
mhc1	r = -0.004, *p* = 0.984	r = -0.027, *p* = 0.880	r = 0.124, *p* = 0.530
mhc2	r = 0.063, *p* = 0.727	r = -0.037, *p* = 0.839	r = 0.216, *p* = 0.270
cd247	r = 0.040, *p* = 0.826	r = -0.188, *p* = 0.296	r = 0.323, *p* = 0.093
cd4	r = -0.185, *p* = 0.303	r = 0.120, *p* = 0.505	r = 0.132, *p* = 0.503
cd8a	r = -0.042, *p* = 0.817	r = -0.017, *p* = 0.926	r = 0.108, *p* = 0.586
cd8b	r = -0.012, *p* = 0.949	r = -0.055, *p* = 0.760	r = 0.341, *p* = 0.076
Head kidney			
*defb**	r = -0.028, *p* = 0.879	r = 0.073, *p* = 0.689	r = 0.110, *p* = 0.585
lyz	r = 0.001, *p* = 0.995	r = -0.053, *p* = 0.783	r = 0.262, *p* = 0.186
leap2	r = 0.121, *p* = 0.504	r = 0.023, *p* = 0.901	r = -0.034, *p* = 0.862
a2m	r = 0.572, *p* = 0.084	r = -0.576, *p* = 0.081	r = 0.198, *p* = 0.638
c3	r = 0.217, *p* = 0.403	r = -0.332, *p* = 0.192	r = 0.246, *p* = 0.418
crp	r = -0.026, *p* = 0.886	r = 0.180, *p* = 0.324	**r = 0.421, *p* = 0.029**
sap	r = -0.006, *p* = 0.975	r = 0.325, *p* = 0.069	r = 0.285, *p* = 0.150

Table S5.6. Continuation (part 2/2).

	Lysozyme activity	Peroxidase activity	Total Ig content
Head kidney			
trf	r = 0.028, *p* = 0.900	r = 0.021, *p* = 0.924	r = -0.110, *p* = 0.653
lgals8x1	r = 0.034, *p* = 0.851	r = 0.246, *p* = 0.167	r = 0.232, *p* = 0.234
ackr4	r = -0.075, *p* = 0.679	r = 0.272, *p* = 0.126	r = 0.105, *p* = 0.595
ccr3	r = -0.078, *p* = 0.666	r = 0.243, *p* = 0.173	r = 0.211, *p* = 0.282
ccr9	r = -0.274, *p* = 0.123	**r = 0.454, *p* = 0.008**	r = 0.117, *p* = 0.553
irf8	r = -0.266, *p* = 0.135	r = 0.295, *p* = 0.095	r = 0.340, *p* = 0.077
il1b	r = -0.061, *p* = 0.741	r = 0.199, *p* = 0.274	r = 0.178, *p* = 0.376
il8	r = 0.034, *p* = 0.850	r = 0.224, *p* = 0.211	r = 0.159, *p* = 0.419
il10	r = -0.187, *p* = 0.298	r = 0.226, *p* = 0.207	r = 0.278, *p* = 0.151
il20	r = -0.159, *p* = 0.378	r = 0.095, *p* = 0.598	r = 0.126, *p* = 0.521
il34	r = -0.073, *p* = 0.688	r = 0.120, *p* = 0.504	r = 0.283, *p* = 0.144
csf1r	r = -0.319, *p* = 0.070	**r = 0.358, *p* = 0.041**	**r = 0.458, *p* = 0.009**
mif	r = -0.001, *p* = 0.997	r = 0.141, *p* = 0.435	r = 0.351, *p* = 0.067
mmd	r = 0.044, *p* = 0.809	r = 0.188, *p* = 0.296	r = 0.349, *p* = 0.069
nfkb2	r = -0.062, *p* = 0.737	r = -0.069, *p* = 0.707	r = -0.005, *p* = 0.981
tnfα	r = 0.002, *p* = 0.993	r = 0.265, *p* = 0.137	**r = 0.506, *p* = 0.006**
imgb	r = -0.330, *p* = 0.061	**r = 0.485, *p* = 0.004**	r = -0.028, *p* = 0.888
igms	r = -0.026, *p* = 0.888	r = 0.066, *p* = 0.716	**r = 0.383, *p* = 0.044**
cd33	r = -0.113, *p* = 0.540	r = 0.021, *p* = 0.907	r = 0.246, *p* = 0.206
myd88	r = -0.181, *p* = 0.312	r = 0.112, *p* = 0.535	r = 0.339, *p* = 0.077
cd247	r = -0.014, *p* = 0.940	r = 0.115, *p* = 0.523	r = 0.269, *p* = 0.167
cd8b	r = 0.061, *p* = 0.737	r = -0.164, *p* = 0.362	**r = 0.435, *p* = 0.021**

Appendix D - Supplementary materials of the Chapter VI

TableS6.1. List of *in vivo* trials exposing fish to microplastics (MPs) and other anthropogenic particles. Only studies focusing on species with commercial interest for human consumption were considered.

Species	Particle size	Exposure concentration	Exposure period	Experimental variable	Reference
Anabus testunideus (climbing perch)					
Dietary exposure					
PVC fragments	75-150 µm 150-300 µm 300-1180 µm	100, 300 µg/day	15-90 d	Particle type (*size*) MP concentration	Rahman *et al.* (2024)
Carassius carassius (crucian carp)					
Dietary exposure					
PE fragments	0-2000 µm (90%, <848.4 µm)	6.4, 12.2, 22.3 MP/day/fish	30 d	MP concentration	Hu *et al.* (2022)
Waterborne exposure					
PS spheres	0.08, 8 µm	0.3, 1.2, 4.8, 19.2 mg/L	20 h (+20 h *depur.*)	Particle type (*size*) MP concentration	Ou *et al.* (2024)
PA fragments	0.43-255 µm	4, 8, 16, 32, 64 mg/L	1, 2 w	MP concentration	Choi & Kim (2023) Choi *et al.* (2023)
PS spheres	100 ± 0.4 nm	1 mg/L	96 h, 21 d	MP & Cd interaction	Wei *et al.* (2023)
PE spheres	22-71 µm (~90%)	4-64 mg/L	1, 2 w	MP concentration	Yu *et al.* (2023b)
Channa argus (northern snakehead)					
Waterborne exposure					
PS spheres	80 nm	50, 500 µg/L	24-96h (*+48 h depur.*)	MP & Cd interaction MP concentration Exposure period	Wang *et al.* (2022d)
Channa maculata x Channa argus (hybrid snakehead)					
Waterborne exposure					
PS spheres	80 nm	50, 500 µg/L	24-96h (*+48 h depur.*)	MP & Cd interaction MP concentration	Wang *et al.* (2022e)
PS spheres	5 µm	10 mg/L	6-72h	MP presence Species	Zhang *et al.* (2021b)
Clarias batrachus (walking catfish)					
Dietary exposure					
LDPE spheres PET fragments	LDPE, 2.9-3.0 mm PET, 1.0-2.0 mm	LDPE, 1-2 MP/L PET, 3-6 MP/L	30, 60 d	Particle type (*shape, size, polymer*)	Fatema *et al.* (2023)
Clarias gariepinus (African catfish)					
Dietary exposure					
PS fragments	197.8-819.1 µm	5, 10, 15% (w/*w*)	30 d	MP concentration	Permatasari *et al.* (2023)
PE fragments	>100 nm (~90%)	500 mg/kg	15 d	MP & antioxidants interactions	Sayed *et al.* (2023) Hamed *et al.* (2022) Sayed *et al.* (2022) Sayed *et al.* (2021)
PA, PLA (*shape n.s.*)	*n.s.*	10% (w/w) (*~99.7 g/kg*)	30-90 d	Particle type (*polymer*) Exposure period	Jang *et al.* (2022)
PVC (*shape n.s.*)	95.4 ± 4.2 µm	0.5, 1.5, 3.0% (w/w)	15-45 d (*+30 d depur.*)	MP concentration	Iheanacho & Odo (2020)
Waterborne exposure					
n.s.	*n.s.*	100 mg/L	15 d	MP & Pb interaction	Soliman *et al.* (2023)
LDPE fragments	50-500 µm (*>98%*)	0.5, 1, 1.5, 2 g/L	4 d	MP concentration	Tongo & Erhunmwunse (2022)
LDPE fragments	<60 µm (*~95%*)	50, 500 µg/L (*1400, 14000 MP/L*)	96 h	MP & Phe interaction MP concentration	Karami *et al.* (2016)
Cirrhinus mrigala (mrigal carp)					
Waterborne exposure					
PS spheres	5 µm	10 mg/L	6-72h	MP presence Species	Zhang *et al.* (2021b)
Ctenopharyngodon idella (grass carp)					
Waterborne exposure					
PS spheres	0.07, 0.5, 5 µm	200 µg/L	2-15 d	Particle type (*size*)	Ali *et al.* (2024)

Figure S6.1. Continuation (part 2/6)

Species	Particle size	Exposure concentration	Exposure period	Experimental variable	Reference
Ctenopharyngodon idella (grass carp)					
PS spheres	80 nm	10-1000 µg/L (*10-fold incr.*)	24-72 h	MP & pathogen interaction	Li *et al.* (2024d)
PS spheres	5 µm	0.1, 1, 10 mg/L	1-6 d	MP concentration	Liu *et al.* (2024)
PS spheres	0.5, 15 µm	100, 500 µg/L	7, 14 d (*+7 d depur.*)	Particle type (*size*) MP concentration	Hao *et al.* (2023)
PS spheres	80 nm	300, 3000 ng/L	24-120 h	MP concentration	Li *et al.* (2023)
PS spheres	0.08, 8 µm	0.02, 0.2, 2 mg/L	24, 48 h	Particle type (*size*) MP concentration	Zhang *et al.* (2023)
PS spheres	32-40 µm	100, 1000 µg/L	21 d	MP concentration	Jia *et al.* (2022b) Liu *et al.* (2022d)
PS spheres	80 nm	20, 200, 2000 µg/L	1-7 d	MP & TC interaction	Liu *et al.* (2022c)
PS spheres	0.08, 8 µm	5, 15, 45 mg/L	2, 4, 6, 8 h	Particle type (*size*) MP concentration	Zhang *et al.* (2022a)
PS spheres	5 µm	700 µg/L	24, 48h	MP & Cd interaction	Zuo *et al.* (2022)
PS spheres	20-26 nm	760 µg/L	3 d	MP & ZnO particles interaction	Estrela *et al.* (2021)
PS spheres	23.03 ± 0.27 nm	0.04, 34 ng/L 34 µg/L	20 d	MP concentration	Guimarães *et al.* (2021)
Cyprinus carpio (common carp)					
Dietary exposure					
PMMA fragments	20-1086 µm	85 ± 39 MP/pellet	8-64 h (*every 24 h*)	Depuration period Species	Roch *et al.* (2021)
Waterborne exposure					
PS spheres	8 µm	1000 ng/L	21 d	MP & feed interaction	Cui *et al.* (2023)
PS spheres	0.08, 8 µm	0.02, 0.2, 2 mg/L	24, 48 h	Particle type (*size*) MP concentration	Zhang *et al.* (2023)
HDPE (*shape n.s.*)	<200 µm	175, 350, 700, 1400 µg/L	30 d	MP concentration	Banaei *et al.* (2022)
PE (*shape n.s.*)	*n.s.*	10 mg/L	2 w	MP & 4-NP interaction	Ammar *et al.* (2022)
PE fragments	~8-12 µm	1.5, 4.5 mg/L	60 d	MP & GLY interaction	Chen *et al.* (2022b)
PVC spheres	~140.7 µm	0.5 mg/L	14 d	MP & Cu interaction MP concentration	Hoseini *et al.* (2022)
PS spheres	5 µm	700 µg/L	48, 96 h	MP & TC interaction	Zhang *et al.* (2021d)
PVC fragments	100-200 µm	45.6, 91.1, 136.7 µg/L	30, 60 d	MP concentration	Xia *et al.* (2020)
Dietary & waterborne exposure					
PP fragments	~ 900 µm	100, 250 mg/g 1.0, 2.5 g/L	7 d (*every 24 h*)	Exposure pathway MP concentration	Yedier *et al.* (2023)
Dicentrarchus labrax (European seabass)					
Dietary exposure					
PE (*shape n.s.*)	150-500 µm (267 ± 90 µm)	100 mg/kg	21 d	MP & PFOS interaction	Espinosa-Ruiz *et al.* (2023)
LDPP fragments	700-1000 µm	10% (w/w)	60 d	MP & PBDE/p.p'-DDE/PCB interaction	Herrera *et al.* (2023) Herrera *et al.* (2022)
LDPP fragments	700-1000 µm	10% (w/w)	60 d	MP & CPF/DDE/BP-3 interaction	Montero *et al.* (2022)
PVC fragments	< 300 µm	0.1% (w/w)	30-90 d	MP & PCB/PAE interaction	Pedà *et al.* (2022), Pedà *et al.* (2016)
BR spheres	1-5 µm	0.05 g/kg	16 w	MP presence	Zeytin *et al.* (2020)
PE & PVC fragments	40-150 µm PE (78±18 µm) PVC (104±36 µm)	100, 500 MP/kg	3 w	Particle type (*polymer*) MP concentration	Espinosa *et al.* (2019)
LDPE fragments	125-250 µm	2, 4% (w/w)	20-80 d (*+51 d depur.*)	MP & PCB/BFR & feed interaction	Granby *et al.* (2018)
PE spheres	10-45 µm	1.2, 2 mg/g (10^4, 10^5 *MP/g*)	14, 20, 34 d	MP concentration	Mazurais *et al.* (2015)

Figure S6.1. Continuation (part 3/6)

Species	Particle size	Exposure concentration	Exposure period	Experimental variable	Reference
Dicentrarchus labrax (European seabass)					
Waterborne exposure					
PS fragments	~53 nm	0.02, 20 mg/L	96 h	MP & HA interaction	Brandts *et al.* (2021a)
PMMA spheres	~45 nm	0.02-20 mg/L (*10-fold incr.*)	96 h	MP concentration	Brandts *et al.* (2018)
BR spheres	1-5 µm	0.26, 0.69 mg/L	96 h	MP & Hg interaction MP concentration	Barboza *et al.* (2018b) Barboza *et al.* (2018c) Barboza *et al.* (2018d)
Epinephelus coioides (orange-spotted grouper)					
Waterborne exposure					
PS	100.5 ± 8.0 nm	300 µg/mL	7 d (*+10 d depur.*)	MP & virus interaction	Wang *et al.* (2021)
Epinephelus fuscoguttatus x Epinephelus lanceolatus (hybrid grouper)					
Waterborne exposure					
PA6 & PVC (*shape n.s.*)	*n.s.*	1 MP/fish after feed	30 min	Particle type (*colour, polymer*)	Xu & Li (2021)
Epinephelus moara (kelp grouper)					
Dietary exposure					
PS spheres & fragments	spheres, ~22.3 µm fragments, 20-100µm	2.0-20 mg/g	1-26 d (*every ~6 d*)	Particle type (*shape*) MP concentration	Wang *et al.* (2020b)
Hypophthalmichthys molitrix (silver carp)					
Dietary exposure					
PS spheres	50, 150, 300, 500 µm	10^4 MP/L	1, 4, 7 d	Particle type (*size*)	Zu *et al.* (2023)
Waterborne exposure					
PS spheres	5 µm	80, 800 µg/L	48 h (*+48 h depur.*)	MP concentration	Zhang *et al.* (2021a)
Hypophthalmichthys nobilis (bighead carp)					
Waterborne exposure					
PES fibres	0.29-2.77 mm	100 MP/L	24 h	MP presence Species	Li *et al.* (2021a)
PS spheres	5 µm	10 mg/L	6-72h	MP presence Species	Zhang *et al.* (2021b)
Larimichthys crocea (large yellow croaker)					
Dietary exposure					
PS spheres	8 nm	1, 10, 100 mg/kg	21 d	MP concentration	Lai *et al.* (2021)
Waterborne exposure					
PS spheres	100 nm	10, 10^4, 10^6 MP/L	14 d (*+7 d depur.*)	MP concentration	Gu *et al.* (2020) Li *et al.* (2021b)
Lateolabrax maculatus (spotted seabass)					
Waterborne exposure					
PP fragments	1-6, 10-30 µm	50-5000 µg/L (*10-fold incr.*)	7, 14 d	Particle type (*size*) MP concentration	Yan *et al.* (2024)
Lates calcarifer (Asian seabass)					
Dietary exposure					
PLA-PBAT & PE films	PLA-PBAT, ~3.1 mm PE, ~3.0 mm	1 MP/pellet	21 d	Particle type (*polymer*)	Xie *et al.* (2022)
PE fibres	2-3 mm	1% (w/w)	56 d	MP presence	Xie *et al.* (2021)
Waterborne exposure					
PE spheres	30-50 µm	0.1, 1 mg/L	1-56 d	MP & BaP interaction MP concentrations	Ghasemi & Shadi (2024)
PS spheres	8 µm	0.05, 0.5, 5 mg/L	15 d	MP concentration MP & oil pollutant interaction	Sahabuddin *et al.* (2023)
PHB spheres PS spheres	1 µm	10^{10} MP/L	48 h	Particle type (*polymer*)	Sai *et al.* (2022)
PS spheres	97 µm	100 MP/L	24 h	MP & PAH interaction	Guven *et al.* (2018)

Table S6.1. Continuation (part 4/6).

Species	Particle size	Exposure concentration	Exposure period	Experimental variable	Reference
Micropterus salmoides (largemouth bass)					
Waterborne exposure					
PS spheres	5 µm	5 mg/L	96 h (*+96 h depur.*)	MP presence	Yue *et al.* (2024)
PS spheres	100 nm	100 µg/L	7, 19 d	MP presence	Zhang *et al.* (2024a)
PS spheres	100 nm	100 µg/L	7, 19 d	MP & Cd interaction	Chen *et al.* (2023a)
PS spheres	0.08, 8 µm	0.02, 0.2, 2 mg/L	24, 48 h	Particle type (*size*) MP concentration	Zhang *et al.* (2023)
PS spheres	100 nm	10, 100 µg/L	7, 19 d	MP concentration	Chen *et al.* (2022c)
PS spheres	500 nm	2, 10, 40 mg/g	21 d	MP & DEHP interaction MP concentration	Liao *et al.* (2022)
Dietary & waterborne exposure					
PP fibres & fragments	3-4 mm	0.3% (w/w)	28 d	Exposure pathway	Zhang *et al.* (2021c)
Oncorhynchus mykiss (rainbow trout)					
Dietary exposure					
PS spheres	39.7-78.1 µm (*52.5 ± 11.5 µm*)	0.5, 2, 5% (w/w)	6 w	MP concentration	Hollerova *et al.* (2023)
HDPE fragments	<20 µm	500, 1000 mg/kg	30 d	MP & bacteria interaction MP concentration	Banihashemi *et al.* (2022)
PMMA fragments	27-778 µm (*149 ± 128 µm*)	13, 73 MP/fish/2 days	120 d (*~every 7 d*)	MP concentration	Roch *et al.* (2022)
PS fragments	21.9-466.7 µm (*<100 µm, 60%*)	30, 300 µg/L	96 h	MP & CPF interaction MP concentration	Hanachi *et al.* (2021) Karbalaei *et al.* (2021)
PE fragments	10-80 µm	0.5, 2, 5% (w/w)	6 w	MP concentration	Hodkovicova *et al.* (2021)
PMMA fragments	20-1086 µm	81 ± 24 MP/pellet	0, 8, 24, 48, 56, 72 h	Depuration period Species	Roch *et al.* (2021)
PE spheres	10-20, 27-32, 63-75, 125-150, 250-300 µm	1400, 980 MP/g	14 d	Particle type (*size*) MP concentration	Kim *et al.* (2020)
PS fragments	100-400 µm	500-700, 2226-2411 MP/fish/day	28 d	MP & pollutant mix interaction	Ašmonaitė *et al.* (2018a) Ašmonaitė *et al.* (2018b)
Waterborne exposure					
PS fragment PA fibres	PS, ~26.8 µm PA, ~500 µm	0.1, 1, 10 mg/L	31, 35, 42, 56 d	MP & virus interaction Particle type (*shape, size, polymer*) MP concentration	Seeley *et al.* (2023)
Oncorhynchus tshawytscha (Chinook salmon)					
Dietary exposure					
PES fibres	~ 5000 µm	~240 MP/fish/day	3, 5, 7, 10 d	Exposure period	Spanjer *et al.* (2020)
Oreochromis mossambicus (Mozambique tilapia)					
Dietary exposure					
PP films	11.9-44.6 µm	100, 500, 1000 mg/kg	4, 14 d	MP concentration	Jeyavani *et al.* (2023)
Oreochromis niloticus (Nile tilapia)					
Dietary exposure					
PVC fragments	50 µm	500, 1000 mg/kg	40 d	MP concentration	Jawdhari *et al.* (2023)
PP spheres	0.1, 100 µm	1, 10, 100 mg/g	21 d	Particle type (*size*) MP concentration	Wu *et al.* (2023b)
HDPE fragments	27, 63 µm	4, 8% (w/w)	9 w	Particle type (*size*) MP concentration	Lu *et al.* (2022b)
Waterborne exposure					
PS (*shape n.s.*)	0.08, 0.8, 8, 80 µm	1 mg/L	14 d	Particle type (*size*)	Wu *et al.* (2024)
PS spheres	0.08, 2, 20 µm	100 µg/L	28 d	Particle type (*size*)	Zheng *et al.* (2024)
PS spheres	1 µm	0.01, 0.1, 1 mg/L	14 d	MP concentration	Das *et al.* (2023)
PA fibres	500-4000 µm	10 mg/L	15 d	MP & temperature interaction	Hasan *et al.* (2023)

Table S6.1. Continuation (part 5/6).

Species	Particle size	Exposure concentration	Exposure period	Experimental variable	Reference
Oreochromis niloticus (Nile tilapia)					
PMMA spheres	0.1-0.4 mm	0.018, 0.03, 0.09 g/L	28 d	MP concentration	Raza *et al.* (2023)
PP fragments	< 100 µm (~ *82%*)	10, 100 mg/L	21 d	MP & B interaction MP concentration	Yang *et al.* (2023)
PS spheres	0.5 µm	1 mg/L	8 w	MP & probiotics interaction	Dong *et al.* (2022)
PS spheres	0.1 µm	1 mg/L	4, 14 d	MP & Cu interaction	Zhang *et al.* (2022b)
n.s. fragments	<100 nm (> *90%*)	1, 10, 100 mg/L	15 d (+ *15 d depur.*)	MP concentration	Hamed *et al.* (2021) Hamed *et al.* (2020) Hamed *et al.* (2019)
PS spheres	5 µm	10 µg/L	14 d (+ *14 d depur.*)	MP & PRP/SMX interaction	Huang *et al.* (2021)
PS spheres	0.35, 9 µm	5 mg/L	28 d	Particle type (*size*)	Ahmadifar *et al.* (2021)
n.s. fragments	< 100 nm (> *90%*)	10 mg/L	15 d	MP & supplement interaction	Ismail *et al.* (2021)
PS spheres	0.1 µm	1, 10, 100 µg/L	1, 3, 6, 10, 14 d	MP & ROX interaction MP concentration	Zhang *et al.* (2019a)
PS spheres	0.1 µm	1, 10, 100 µg/L	14 d	MP concentration	Ding *et al.* (2018)
Paralichthys olivaceus (olive flounder)					
Waterborne exposure					
PS spheres	10 µm	2, 20, 200 µg/L	21 d	MP concentration	Wang *et al.* (2022b)
Perca flavescens (yellow perch)					
Dietary exposure					
HDPE fragments	100-125 µm	1, 2, 4, 8 g/100g	9 w	MP concentration	Lu *et al.* (2022a)
Perca fluviatilis (European perch)					
Dietary exposure					
PLA fragments	90-150 µm	2% (w/w)	6 w	MP presence Plastic *vs.* kaolin particles	Kardgar *et al.* (2023)
LDPE spheres	10-45 µm 106-125 µm	10 mg/g	21 d	Particle type (*size*)	Bobori *et al.* (2022a)
PP spheres	8-10 µm	1, 10 mg/g	21 d	MP concentration	Bobori *et al.* (2022b)
PS spheres	5-12 µm	1, 50, 100 mg/g 134 mg/g	21 d	MP concentration Species	Kaloyianni *et al.* (2021)
Salmo salar (Atlantic salmon)					
Dietary exposure					
LDPE fragments	125-250 µm	2% (w/w)	19-98 d (+*56 d depur.*)	MP & PCB/BFR interaction	Granby *et al.* (2024)
Salmo trutta (sea trout)					
Waterborne exposure					
PE, PET, PS spheres	~3000 µm	0.1% (w/v)	113 d	Particle type (*polymer*)	Jakubowska *et al.* (2020)
PS fragments	30-60 µm (~*92%*)	10^4 MP/L	96 h	MP & Methiocarb interaction	Schmieg *et al.* (2020b)
PS fragments	2-60 µm (~*98%*)	10^4, 10^5 MP/L	3 w	MP & Amitriptyline interaction	Schmieg *et al.* (2020a) Schmieg *et al.* (2022)
Sebastes schlegelii (marine jacopever)					
Waterborne exposure					
PS spheres	0.1, 5 µm	0.23 mg/L	15 d	MP presence	Sun *et al.* (2023b)
PS spheres	0.5, 15 µm	190 µg/L	14 d (+*7 d depur.*)	MP concentration	Yin *et al.* (2019)
PS spheres	15 µm	10^6 MP/L	14 d (+*7 d depur.*)	MP presence	Yin *et al.* (2018)
Solea senegalensis (Senegalese sole)					
Waterborne exposure					
LDPE fragments Nanoclay coating	LDPE, 64-125 µm Nanoclay, *n.s.*	LDPE, 0.1, 1, 10 mg/L Nanoclay, 0.33, 1, 3.33 mg/L	3 h	MP & Nanoclay interaction	Santana *et al.* (2022)

Table S6.1. Continuation (part 6/6).

Species	Particle size	Exposure concentration	Exposure period	Experimental variable	Reference
Sparus aurata (gilthead seabream)					
Dietary exposure					
PS spheres	1-20 µm	0.5, 5% (w/w)	21 d	MP concentration	Del Piano *et al.* (2024) Del Piano *et al.* (2023b) Del Piano *et al.* (2023a)
LDPE fragments	100-500 µm	10% (w/w)	90 d (*+ 30 d depur.*)	MP & pollutant mix interaction	Hoyo-Alvarez *et al.* (2022) Capó *et al.* (2021a)
PE spheres	10-20 µm	87 ± 18 µg/day	14, 35 d	MP presence	Jacob *et al.* (2021)
LDPE fragments	n. s.	10% (w/w)	90 d (*+ 30 d depur.*)	MP & OC/PCB interaction	Rios-Fuster *et al.* (2021a)
LDPE fragments	100-500 µm	10% (w/w)	21 d	MP & pollutant interaction	Rios-Fuster *et al.* (2021b)
LDPE fragments	200-500 µm	10% (w/w)	90 d (*+ 30 d depur.*)	MP presence	Solomando *et al.* (2021) Solomando *et al.* (2020)
LDPE films LDPE fragments	200-500 µm 501-1000 µm	0.12% (w/w)	30 d	Particle type (*shape, size*)	Varó *et al.* (2021)
MDPE, PA, PVCLMW, UHMWPE fragments	~75 µm	0.1 g/kg	45 d	Particle type (*polymer*)	Jovanović *et al.* (2018)
PVC fragments	40-150 µm	100, 500 mg/kg	15, 30 d	MP concentration	Espinosa *et al.* (2017)
Waterborne exposure					
PMMA spheres	45 nm	0.001-10 mg/L (*10-fold incr.*)	24, 96 h	MP concentration	Brandts *et al.* (2021b)
Tachysurus fulvidraco (Yellowhead catfish)					
Waterborne exposure					
PES fibres	<5 mm	100 MP/L	24 h	MP presence Species	Li *et al.* (2021a)
PS spheres	20 µm	0.115, 11.5 µg/L (*25.8, 2580 MP/L*)	15 d	MP concentration	Li *et al.* (2021c)

B, boron; BaP, benzo(a)pyrene; BFR, brominated flame retardants; BP-3, benzophenone; Br, Cd, cadmium; CPF, chlorpyrifos; Cu, copper; DEHP, di(2-ethylhexyl) phthalate; GLY, glyphosate; HA, humic acid; Hg, mercury; LDPE, low-density polyethylene; LDPP, low-density polypropylene; MDPE, medium-density polyethylene; PA, polyamide; PAE, phthalate ester; PAH, polyaromatic hydrocarbons; PAN, polyacrylonitrile; Pb, lead; PBDE, polybrominated diphenyl ethers; PCB, polychlorinated biphenyl; PE, polyethylene; PES, polyester; PET, polyethylene terephthalate; PLA, polylactic acid; PMMA, polymethyl methacrylate; PP, polypropylene; PRP, propranolol; PS, polystyrene; PVC, polyvinyl chloride; PVCLMW, polyvinyl chloride low molecular weight; p.p'-DDE, dichlorodiphenyldichloroethylene; OC, organochlorine compound; OCP, organochlorine pesticide; ROX, roxithromycin; SMX, sulfamethoxazole; TC, tetracycline; UHMWPE, ultra-high molecular weight polyethylene; ZnO, zinc oxide; 4-NP, 4-nonyphenol