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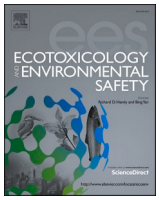

High microplastics concentration in liver is negatively associated with condition factor in the Benguela hake *Merluccius polli*

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ABSTRACT

Microplastics (MPs) affect both marine and terrestrial biota worldwide for their harmful effects, which range from physical cell damage to physiological deterioration. In this research, microplastics were quantified from gills, liver and muscle of demersal Benguela hakes *Merluccius polli* (n = 94), caught by commercial trawling from northwest African waters. Plastic polymers were identified using Fourier Transformed-infraRed spectroscopy (FT-iR). Fulton's *k* condition factor and the degree of DNA degradation in liver were measured. None of the individuals were free of MPs, whose concentration ranged from 0.18 particles/g in muscle to 0.6 in liver. Four hazardous polymers were identified: 2-ethoxyethylmethacrylate, polyester, polyethylene terephthalate, and poly-acrylics. MP concentration in liver was correlated negatively with the condition factor, suggesting physiological damage. Positive association of MP concentration and liver DNA degradation was explained from cell breakage during trawl hauls during decompression, suggesting an additional way of MPs harm in organisms inhabiting at great depth. This is the first report of potential MPs-driven damage in this species; more studies are recommended to understand the impact of MP pollution on demersal species.

1. Introduction

Plastic polymers have been used worldwide for the last century, for their cheap manufacture, lightness, malleability, reusability, and resistance (Andrady and Neal, 2009). The uncontrolled use and disposal of plastic has positioned it as a global problem of huge environmental impact, for the ubiquity of plastic polymers in all known ecosystems has been proven (Rochman, 2018). As it has been widely described (Zhang et al., 2021; Li et al., 2022), plastics suffer from physical-chemical degradations leading to the appearance of microplastics (MPs thereafter). Larger pieces of plastic are actively broken by many physic-chemical factors in the marine environment such as waves (Zhang et al., 2021), sunlight (Bao et al., 2022), or even the biotic pressure (Gallitelli et al., 2022). The resulting particles of this degradation are called secondary MPs when < 5 mm length (Arthur et al., 2008). MPs that are directly manufactured of this size or smaller are called primary MPs and are generally employed in personal care products and cleansers. MPs are found in different shapes, such as fibers, fragments, films, microbeads, or pellets (Ngo et al., 2019; Lorenzo-Navarro et al., 2021). Although the variety is enormous in different marine fish, blue and black fibers are the most abundant type (Hossain et al., 2019; Abidli et al., 2021; Menéndez et al., 2022).

According to Ryan et al. (2019), Carpenter and Smith (1972) reported the first evidence of MPs in an aquatic system in 1972. The occurrence of MPs in the marine environment as well as in hundreds of marine species has been widely studied all along the coasts and seas of Africa, Eurasia, Australia, and America (Kroon et al., 2018; Ita-Nagy et al., 2022; Masiá et al., 2022a and 2022b; Piyawardhana et al., 2022; Bilbao-Kareaga et al., 2023), and even in the polar regions (Morgana et al., 2018; Kögel et al., 2022). For the last 50 years, thousands of studies have reported potential effects of plastics in the marine environment, many focusing on marine species ranging from plankton (Lima et al., 2015; Rodrigues et al., 2021) to big cetaceans (Fossi et al., 2012; Zhu et al., 2019a), including filter feeders (Naji et al., 2018; Expósito et al., 2022), fishes (Neves et al., 2015; Menéndez et al., 2022), or algae (Wu et al., 2019; Menendez et al., 2021), among others. MPs can be ingested by many species (Boerger et al., 2010; Nicastro et al., 2018; Markic et al., 2020; Collard and Ask, 2021). One of the dangers derived

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from microplastic ingestion is chemical damage. Plastics categorised as "Group 7 plastics" such as epoxy resin or polycarbonates are manufactured with Bisphenol-A (BPA) (Yang et al., 2011) which is an endocrine disruptor. Its consumption can lead to a wide range of diseases such as neonate malformations, infertility or even cancer (Vandenberg et al., 2007). Also "Group 3 plastics" (PolyVinyl Chloride – PVC) are toxic, and their intake has been reported to induce cancer and to reduce the hepatic functioning (Wagoner, 1983) as well as DNA damage (Lei et al., 2004), among other effects. Harmful effects on both animals and environment have been also documented not only for the plastics themselves but also for the compounds attached to their surfaces (Brennecke et al., 2016; Ribeiro et al., 2017; Rehse et al., 2018; Yuan et al., 2020). Plastics can retain hydrophobic compounds such as heavy metals that are frequent pollutants of aquatic ecosystems (Rainbow, 1985; Copat et al., 2013; Makedonski et al., 2017; Tian et al., 2020; Javanshir Khoei, 2022). The exposure to these elements leads to the development of different diseases and medical conditions such as mitochondrial dysfunction (Sun et al., 2022), kidney damage (Achparaki et al., 2012) or cancer (Jaishankar et al., 2014), among others. Some heavy metals can actively cause DNA damage (Hengstler et al., 2003; Zocche et al., 2010; Ngo et al., 2021). For the elements attached to MPs, or alone, Reactive Oxygen Species (ROS) metabolism modifications and even cell apoptosis (Xia et al., 2008; Thubagere and Reinhard, 2010; Chiu et al., 2015) have been reported to occur due to the presence of MPs (Avio et al., 2015; Ribeiro et al., 2017).

Besides toxicity, another risk of the ingestion of MPs is physical damage caused mechanically. To give a few examples, the obstruction of cavities and ducts by MPs can be lethal (Roman et al., 2021). At a cellular level, MPs can induce cellular breakage (Espinosa et al., 2019), and cell injury and destruction have been reported (Wang et al., 2022a, 2022b, 2022c; Manu et al., 2023). This can be due to modifications in the cellular membrane permeability and the induction of an inflammatory reaction (Deng et al., 2017). Mechanical cell destruction due to MPs has been also reported (Fleury and Baulin, 2021; Wang et al., 2022a, 2022b, 2022c). In addition, DNA damage can be induced when MPs are abundant in the tissue (Prokić et al., 2019; Masiá et al., 2021).

Although the presence of MPs in marine organisms has been described from the Artic (Herzke et al., 2021) to tropical and subtropical marine ecosystems (Costa and Barletta, 2015), only a few studies have focused on MP pollution in the north-western coast of Africa (Kim et al., 20188; Maaghloud et al., 2020, 2021; Wang et al., 2022a, 2022b, 2022c). Masiá et al. (2022a, 2022b) alerted of the potential risk of MP pollution for African fishing resources. One of the important resources in Atlantic African waters is the Benguela hake (*Merluccius polli* Cadenat, 1950), that is abundant in the north-west coast due to the accused seasonal upwelling (Mbaye et al., 2015). Its fisheries are also of great importance for the European fleet (Rey et al., 2012). Spanish trawlers have been fishing from Morocco, Senegal and Mauritania waters for the last 40 years (FAO, 2020; Soto et al., 2022). Hakes inhabiting Atlantic (Neves et al., 2015; Cabanilles et al., 2022) and Mediterranean (Mistri et al., 2022) waters may carry a considerable MP charge. However, to our knowledge, and despite the risk of MP pollution in west African coasts (Masiá et al., 2022a, 2022b), the content of MPs in Benguela hake has not been investigated yet.

In this study, we have quantified and analysed the abundance of MPs in different tissues of Benguela hake adults: muscle, which is the edible tissue in hakes; gills, that are the first contact with MPs from the water column (Guilhermino et al., 2021), and liver. MP accumulation in liver may negatively affect the health of the organisms (Yu et al., 2018), something that in fish can be reflected in a worse condition factor (Amorim et al., 2020). The status of the sampled hakes was evaluated from Fulton's *k* condition factor, and the possible damage to liver from the degree of DNA degradation (Masiá et al., 2021). From the negative association between MP charge and fish status, the starting hypothesis will be that the individuals with more MPs in liver would exhibit a worse condition factor and a higher level of DNA degradation.

2. Materials and methods

2.1. Species in study and samples analysed

Merluccius polli is an Actinopterygii from the Merlucciidae family. Its natural distribution ranges from southern Morocco (NW Africa, 28°N) (Manchih et al., 2018) to the Northern coast of Namibia (SW Africa, 18.30°S)(Lloris et al., 2005); according to the International Union for the Conservation of Nature (IUCN), it is catalogued as Least Concern (https://www.iucnredlist.org/es/species/15522226/15603610). It is sympatric to Senegalese hake (*Merluccius senegalensis*), although the Benguela hake can occupy a greater range of pressures and temperatures (Fernández-Peralta et al., 2011). Benguela hake preys upon small fishes, little squids, and shrimps (FAO, 1990), with some cannibalistic behaviour in adults, depending on the availability of food and resources (Kilongo and Mehl, 1997). Its exploitation has increased exponentially for the last 20 years. In 2006 approximately 9000 tonnes of Benguela hakes were caught, while in 2018 the amount rose to 20,000 tonnes according to FAO (2019).

A total of 94 commercial individuals, in whole and fresh (kept in ice), were kindly provided by the Cádiz Fish Market (Lonja de Cádiz). The samples had been fished by trawling in the 34.1.3 FAO Fishing Area (Fig. 1), in the Western coast of Africa. Since they were caught by commercial fleet for selling, not sampled in purpose for this study, an ethic statement is not needed for this research.

Individuals were measured (standard length was taken), weighted, and dissected for the recovery of muscle, liver, and gills. Samples were labelled and stored in the freezer until processing. For all the samples, approximately 20 g of dorsolateral muscle, 4 gill arches (the same side as the muscle) and 5 g of liver were processed.

2.2. Molecular identification by PCR-RFLPs

All individuals came labelled as *Merluccius polli* from the supplier. Additionally, the species assignation was checked with PCR-RFLPs, as this species is captured in mixed fisheries with its sympatric species (*Merluccius senegalensis)* and both species are often mislabelled (Blanco-Fernandez et al., 2022). DNA was extracted using Chelex®, following the protocol developed by Estoup et al. (1996). The mitochondrial control region was selected as target to discriminate between both species, since it is known to be a variable region with polymorphisms between the different species of the *Merluccius* genus (Machado-Schiaffino et al., 2008). A fragment of control region (450pb) was amplified using the primers MmerHk01 and MmerHk02 developed by Lundy et al. (2000). Amplifications were performed in a final volume of 40 μL using each primer in a final concentration of 0.5 μM, dNTPs at 0.25 mM, $MgCl_2$ at 1.5 mM, Green GoTaq® G2 Flexi Buffer 1x, and DNApol GoTaq® G2 Flexi DNA Polymerase at 0.0375 U/μL. PCR conditions were set to an initial denaturing step of 5' at 95 °C, followed by 35 cycles consisting on denaturing for 30'' at 95 °C, annealing for 30'' at 55 °C and extension for 30'' at 72 °C, and then a final extension step at 72 °C for 15'.

The restriction enzyme BseGI (BTSCI) was selected to generate RFLPs after validation. The enzyme was choosen after searching for a polymorphism that would allow for a differential cut between *M. polli* and *M. senegalensis*. This search was carried out using *in silico* simulator NEBcutter v3 (https://nc3.neb.com/NEBcutter/) to locate the target sequences for commercial restriction enzymes. The enzyme BtsCI would digest the amplicon in two fragments of 216 bp and 226 bp respectively for *M. polli*, while amplicons from *M. senegalensis* remained undigested (442 bp fragment). For its *in silico* validation, 93 sequences corresponding to haplotypes of 806 individuals of both species (60 sequences belonging to *M. polli* and 33 to *M. senegalensis*) were taken from Blanco-Fernandez et al. (2022) (GenBank accession numbers from MZ703314 to MZ703406). All sequences were aligned using MUSCLE in BioEdit and the target position was checked to see whether the

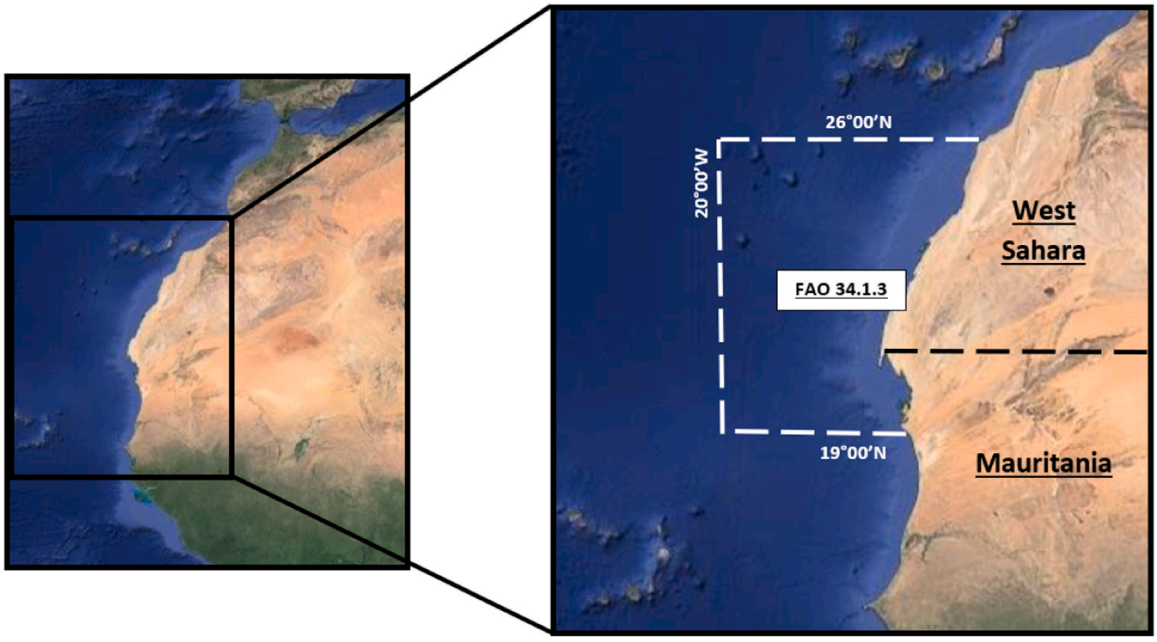

Fig. 1. Fishing area where the individuals analysed in this study were caught from commercial trawling.

polymorphism was maintained along all samples of both species. Out of the 806, only one specimen of *M. polli* did not present the polymorphism that would allow for the digestion, setting an overall error of 0.12 %. After the *in silico* validation, a digestion was performed with known samples from *M. polli* and *M. senegalensis*. For the digestion, we added 10 μL of the previous PCR as template, BseGI(BTSCI) at 0.67 U/μL, and Thermo Scientific™ 1x Buffer Tango in a final volume of 30 μL. The reaction was left incubating at 55 °C for 3 h. Then, results were visualised in agarose gel 2 % stained with 2.5 μL SimpleSafe (Eurx) and run at 120 V for 30 min. Once this methodology was established, the same procedure was applied to the samples of this study. Additionally, 30 randomly chosen from the 94 samples were Sanger sequenced for further verification.

2.3. Microplastic extraction and quantification

First, tissues were digested using 10 % KOH (Thermo Fisher Scientific®). The proportion of reagent per gram of tissue was 1:5 (w/v) for muscle, proportion 1:10 for gills, and 1:50 for liver. Tissue digestion was carried out at 40 °C for 48 h in glass jars covered with aluminium foil to avoid contamination. Blanks consisting of 100 mL of KOH were placed in every oven together with the samples to control for possible contamination during the lab work.

After digestion, two phases were obtained from liver samples: the lower, which is the digested material, and the top one, which is non-digested fat. Jars were refilled with 100 mL of filtered neutral laboratory soap (Labbox, Spain) per 100 mL of digestion to disaggregate and dissolve the fat, for further filtration. This process takes between one and two hours with periodical manual shaking to detach the fat from jar walls. For gill and muscle samples, and blanks, 100 mL of filtered distilled water were added and the same process was followed (1–2 h, manual shaking) to homogenise the process across samples for the control of procedural contaminations.

Finally, all digestions were filtered through 1.2 μm pore size glass microfiber filters (Whatman GF/C, 47 mm diameter) that were allowed to dry in glass petri dishes for 48 h before MP counting. Once dried, filters' surfaces were observed individually under the microscope and all potential plastic particles were counted following Hidalgo-Ruz et al. (2012). Due to the colour and morphology of the glass microfiber filters, special attention was paid to white and transparent particles. A heated needle was approached to potential particles to confirm they were of plastic (plastic bends when heated while organic matter and glass do not). Counting and visual identification were carried out under a Leica 2000 stereomicroscope at 40x magnification (Masiá et al., 2019; Menéndez et al., 2022). Only < 5 mm particles were considered for further analysis.

MPs were first classed by shape as in previous studies (Kumar et al., 2018; Hossain et al., 2019; Wang et al., 2022a, 2022b, 2022c). Three main groups were identified: fibers (elongated, uniform colouration and mostly cylindrical), fragments (irregular shapes, generally with sharp angles), and microbeads (plastic spheres, after checking for possible misidentification with small eggs) (Neves et al., 2015; Güven et al., 2017; Yin et al., 2022). Colour was also recorded (Zhu et al., 2019b; Guilhermino et al., 2021).

A 16 % of putative plastic particles ($n = 120$), roughly representative of all the shapes and colours found in the samples, were analysed by Fourier Transformed infrared spectroscopy (FT-iR) (Uurasjärvi et al., 2021) in the Autonomous University of Madrid. They were picked from the petri dishes under laminar flow cabin to prevent airborne contamination. The analyses were performed using a wavelength between 4000 and 500 cm^{-1} and a germanium glass, Varian 620-IR and Varian 670-IR. Results with a bibliographic search score over 60 % were used. The potential toxicity of the compounds for the aquatic life and/or for humans was checked in the European Chemicals Agency (ECHA; https://echa.europa.eu/es/home, accessed March 2023).

2.4. Contamination control

To control for potential contamination from airborne particles, all procedures were performed into a semi-closed laminar flow cabinet. A cotton white lab coat was constantly worn by the researchers as well as nitrile gloves. All materials in contact with the samples (scissors, tweezers, glass jars…) and implicated in the filtering (vacuum pump) were previously rinsed with filtered distilled water. Distilled water was filtered through 0.22 μm pore size PES filters (PALL Corporation®, 47 mm diameter). Also, the laboratory soap was filtered in the same conditions, while KOH was filtered through 1.2 μm pore size glass microfiber filters (Whatman GF/C, 47 mm diameter). Filters were stored in clean petri dishes which remained closed until the particle's selection for the chemical analysis.

2.5. Genomic DNA extraction and DNA degradation analyses

DNA was extracted from the liver of 46 individuals (48.9 % of the total sample) representing all the range of MPs concentrations found in this study. A small piece of tissue (≤ 25 mg) was disaggregated mechanically, and the genomic DNA was extracted with a commercial kit (DNeasy Blood & Tissue kit, Qiagen, Hilden, Germany), following the manufacturer's instructions. Extractions were quantified using a Qubit 4 Fluorimeter (Thermo Fisher Scientific, Inc). The electrophoresis was performed based on Mičić et al. (2002) and Masiá et al. (2021): 30 ng of DNA (variable μL of each extraction) was run in a 1.3 % agarose gel for 2 h at 90 mV. The level of DNA degradation was categorised in four groups (G1-G4) following Masiá et al. (2021) (Supplementary Figure 1).

2.6. Condition factor

Fulton's *k* condition factor of each individual (Fulton, 1904) was calculated following the equation:

$$K = 100x\frac{W}{L^3}$$

Being W the full body weight in grams and L the standard length in cm (Froese, 2006).

2.7. Statistical analysis

Differences between groups of samples for variables distributed in discrete categories (for example proportion of MPs of different colours or shapes) were tested using contingency chi-square analysis.

Quantitative data like MPs/g were compared between groups of samples (e.g., tissues) using one-way ANOVA, after checking normality from Shapiro-Wilk test and homoscedasticity from Breusch-Pagan test. Post-hoc Tukey's pairwise tests were performed after significant ANOVAs.

Multiple linear regression analysis was applied for condition factor as dependent variable and MP concentration in the different tissues as independent variables, to infer if any tissue pollution could be a predictor of the hake condition. Pairwise Pearson's correlation tests were run to check for associations between variables, e.g., MP concentration and DNA degradation.

Statistical results were interpreted under a 95 % confidence interval (standard significance threshold $p < 0.05$), applying Bonferroni correction for multiple comparison when needed. Statistical analysis was done in PAST free Software V.2.17 (Hammer et al., 2001).

3. Results

3.1. Microplastics content in Benguela hake tissues

BseGI PCR-RFLPs successfully assigned all individuals as *Merluccius polli*, as had been reported. A total of 747 particles identified visually as putative MPs were obtained from the 94 Benguela hakes analysed (Supplementary Table 1). All the individuals had at least one tissue with MPs, from 80 % of gill to 94 % of muscle samples; for the shape, the majority of particles were fibers, a few fragments, and only one microbead in gills (Table 1).

Blanks run all over the experimental work showed a concentration of 0.003 MPs/g, which is two orders of magnitude lower than the tissue samples (see Table 1). Thus, significant procedural contamination could be discarded.

The relative MP load was higher in livers and gills than in muscle (Table 1). The difference among tissues was highly significant (ANOVA with $F_{(2279)} = 25.81$, $p < 0.0001$). Post-hoc Tukey's test showed significant difference between muscle and liver ($t = 6.68$, $p < 0.0001$) as well as between muscle and gills ($t = 7.67$, $p < 0.0001$).

From the relative frequency of different types of particles, MPs were classed for analysis in six groups: blue, black, transparent, and other (including reddish, green, orange, and purple) fibers, blue fragments, and transparent microbeads. Fig. 2 shows the profile of the particles recovered from the three tissues. The majority of MPs were black, followed by blue, transparent fibers, fibers of other colours, blue fragments and one transparent microbead in a gill. The global contingency chi-square was statistically significant (χ^2=28.87, d.f.=10, $p = 0.016$; Cramer's V = 0.12) due to the difference between muscle and liver (χ^2=14.01, d.f.=4, $p = 0.007$; Cramer's V = 0.16); the rest of comparisons between tissues were not significant (data not shown). Livers contained more black and fewer blue fibers than muscle samples (Fig. 2).

FT-iR analysis was done on 53 particles recovered from muscle, 39 from liver and 28 from gill tissues. Seven different compounds were identified (Fig. 3): Rayon (55 %), PEI – Polyethyleneimine cellulose (9.17 %), PET-Polyethylene terephthalate (7.5 %), Polyester (5.83 %), PAN/PAA – Polyacrylenitrile/Polyacrylic acid (2.5%) and 2-ethoxyethyl methacrylate (0.83 %). The remaining 19.18 % particles were of natural compounds such as cellulose. Excluding cellulose, contingency chi-square analysis did not show significant differences between tissues (χ^2=12.461, d.f.=10, $p = 0.255$).

From the ECHA, four of those compounds are harmful for living beings (Table 2); therefore, Benguela hake is expected to be affected by those MPs.

3.2. Condition factor

Fulton's '*k*' condition factor varied widely in the samples analysed, ranging between 0.401 and 1.114 g/cm^3 with an average of 0.886 (SD 0.094). The modal class was the group with *k* [0.839–0.912] (Fig. 4).

From multiple linear regression, with the condition factor as dependent and the concentration of MPs/g in different tissues as independent variables, only the MPs concentration in liver significantly predicted the condition factor (Table 3). The regression slope was significantly negative ($r = -0.234$, $p = 0.024$).

3.3. DNA degradation in liver

The individual results obtained for liver DNA quantification and the assigned degree of degradation (Masiá et al., 2021) are in the Supplementary Table 2. Mean DNA concentration of the 46 liver samples analysed was 18.7 (SD 12.6) ng/μL. The average level of DNA degradation was 2.65 (SD 0.90) over a maximum of four, implying that quite fragmented DNA was found for many individuals (Fig. 5). Only six individuals exhibited a clear band of large genomic DNA (group G1, not degraded), while seven individuals yielded very small DNA fragments (group G4) (Fig. 5).

As expected, a positive significant correlation was found between the estimated DNA degradation and the concentration of MPs/g in liver ($r = 0.35$, $p = 0.015$; see blue trend line of MPs/g in Fig. 5), suggesting that MPs may contribute to degrade DNA. To explore this effect further,

Table 1
Overview of MPs identified from each tissue. N: number of particles identified.

Tissue	% individuals affected	Mean MPs/g (variance)	Fibers	Fragments	Microbeads	N
Gills	80 %	0.517 (0.168)	95.4 %	4 %	0.6 %	175
Liver	83 %	0.599 (0.355)	98.2 %	1.8 %	0 %	224
Muscle	94 %	0.181 (0.012)	96.55 %	3.45 %	0 %	348

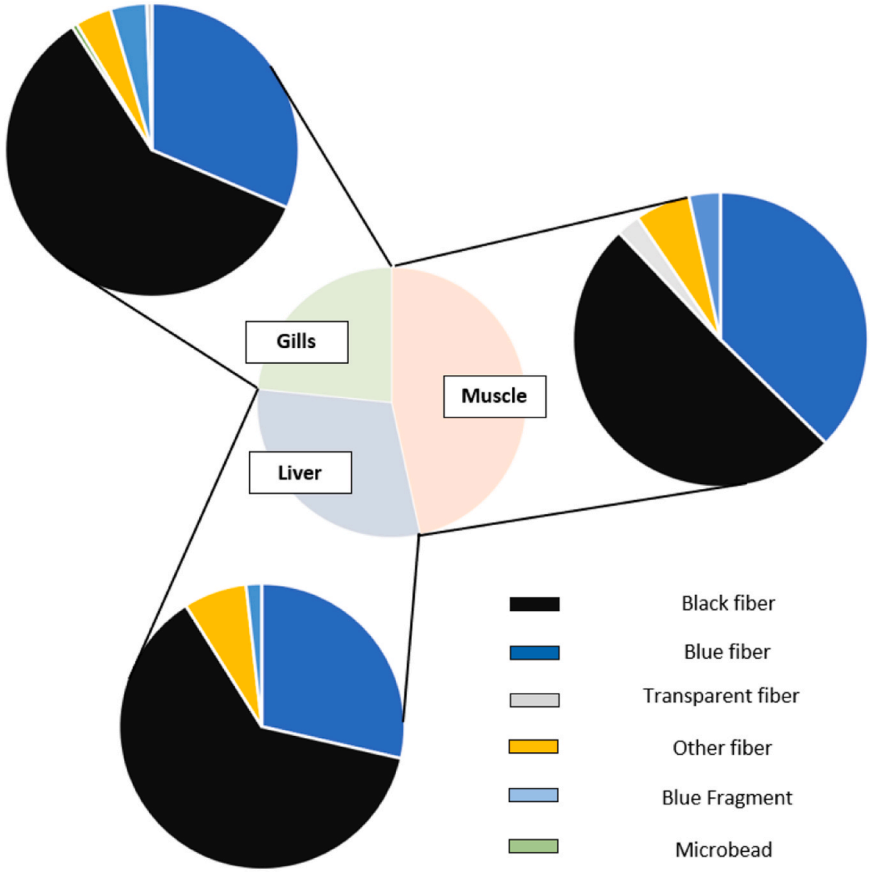

Fig. 2. Colour and shape of the microparticles analysed from muscle, gills and liver. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

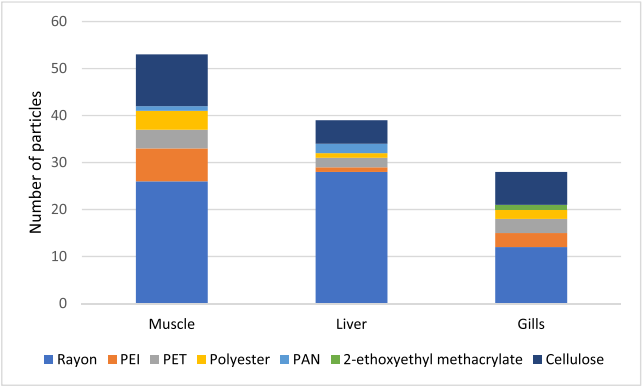

Fig. 3. Proportion of particles of different compounds in the Benguela hake tissues analysed.

we divided the samples in three groups: one with no MPs found in liver ($n = 13$; mean DNA degradation 2.0, SD=0.82), another with MP concentrations up to 1 MPs/g ($n = 20$; mean DNA degradation 2.95, SD = 0.67), and another with ≥ 1 MPs/g ($n = 13$; mean DNA degradation 2.85, SD =0.99). One-way ANOVA for DNA degradation was highly significant ($F_{2,45} = 5.85$, $p = 0.005$), and the post-hoc Tukey's test showed that the group of hakes without MPs in liver had significantly less degraded DNA than the group with between 0 and 1 MPs/g ($t = 4.47$, $p = 0.01$), and also less than the group with more than 1 MPs/g ($t = 3.98$, $p = 0.02$). The difference between the two groups with MPs in liver was not significant ($t = 0.49$, $p = 0.9$ n.s.).

While the correlation between the condition factor and the concentration of MPs in liver in this subsample was significantly negative ($r = -0.32$, $p = 0.03$), as it was in the whole sample of 94 hakes (see results in 3.2 above), the correlation between the condition factor and liver DNA degradation was not significant ($r = -0.23$, $p = 0.118$); a quite flat brown trend line can be observed in Fig. 5. This suggests that, unlike liver MPs content, the level of DNA degradation found in this assay is not related with the physiological condition of hakes (see below).

Table 2
Potential hazard of the compounds identified in this study, according to the European Chemical Agency. Rayon and PET are under research.

Compound	Harmful to aquatic life	Harmful if swallowed	Irritative	Affects fertility and unborn child
Rayon	Pre-registered, no information available			
PEI	X	X		
PET	Pre-registered, no information available			
Polyester	X			
PAN/PAA	X		X	
2- Ethoxyethyl methacrylate			X	X

4. Discussion

To the best of our knowledge, this is the first study that evidences the occurrence of MPs in Benguela Hake (100 % prevalence), some of compounds identified as harmful to aquatic life. From these results, we could expect the most MP-polluted hakes exhibit a poorer physiological condition, as found in other fish (Amorim et al., 2020), especially if MPs

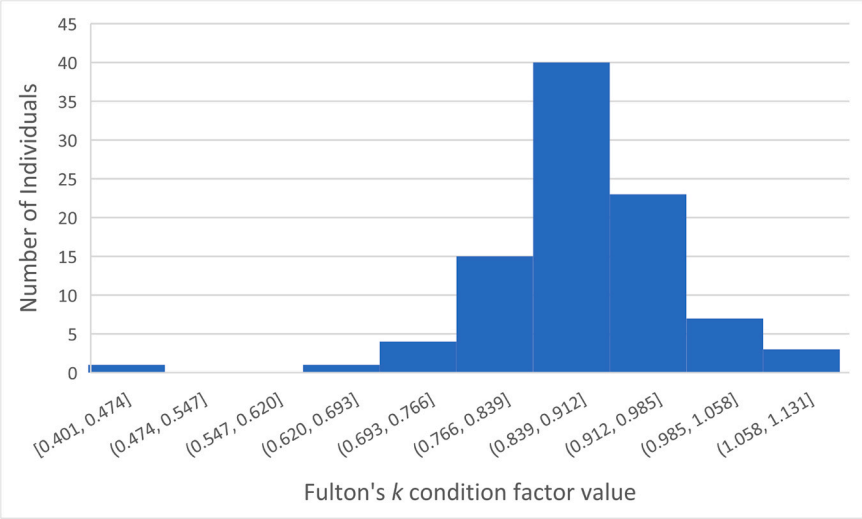

Fig. 4. Distribution of the analysed Benguela hakes by Fulton's k condition factor.

Table 3
Linear regression results with Fulton's k Condition Factor as dependent variable and the concentration of MPs in different tissues as independent variables. SE, standard error.

	Coefficient	SE	t	*p*	r^2
Constant	0.92	0.023	39.767	0.000	
Muscle MP	-0.045	0.088	0.515	0.608	0.003
Liver MP	-0.037	0.016	22.926	0.024	0.055
Gills MP	-0.006	0.021	0.244	0.807	0.001

are in liver (Yu et al., 2018). This was confirmed from a significant negative association between MP content in liver and hake condition factor and would suggest that MP pollution is endangering this important fishing resource. In our study, the MPs in muscle were statistically different from those found in liver and gills. This may suggest that the MPs that reach the liver and the gills are similar; more studies should be carried out to confirm this relationship. As in other fish (Guilhermino et al., 2021), liver exhibited the highest MP concentration, supporting its suggested role of bioaccumulation of these pollutants (Lu et al., 2016; Collard et al., 2017; Yu et al., 2018). The accumulation of plastic particles in the liver may affect the health status of the organism, which is suggested in our study by negative correlation between MPs content and condition factor. Table 3

The mechanisms linking MPs in liver and worse physiological conditions are probably a combination of chemical and mechanical damage. Harmful polymers (Table 2) surely interfere with tissue functioning. DNA damage is known as an effective indicator of environmental ecotoxicity (Dimitriadi et al., 2021), and a higher amount of MPs may lead to higher DNA damage (Shen et al., 2022). Moreover, the association between MP content and DNA degradation, found here for the first time in fish, would suggest a higher level of cell breakage in liver in the individuals with MPs. It can be interpreted as a signal of broken cells where DNA is no longer protected inside the nucleus wall, because the presence of MPs in soft tissues might lead to the cell and/or DNA breakdown (Wright et al., 2013; Espinosa et al., 2019; Masiá et al., 2021; Sobhani et al., 2021). Indeed, highly degraded liver DNA in our study does not mean that there were no integer cells when the hakes were alive (they could not survive without functional liver cells); it could be attributed to a higher cell breakage in livers with MPs during the fishing process instead. For that, the level of DNA degradation found in this

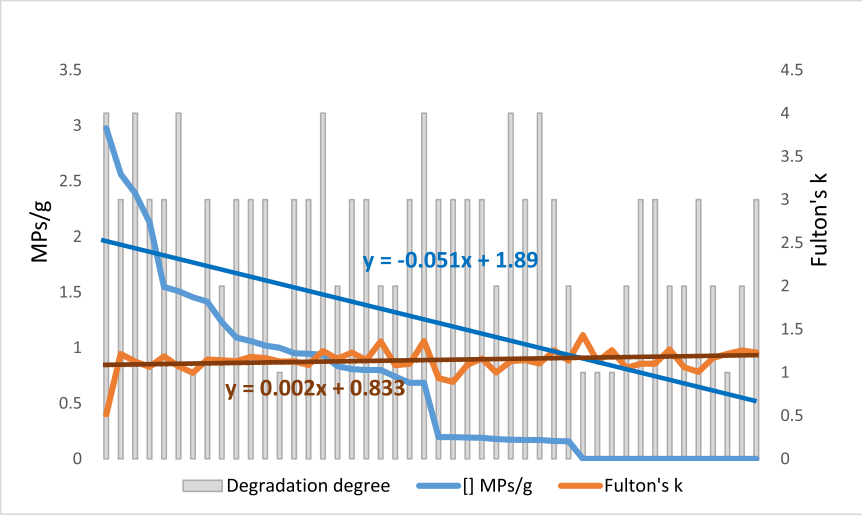

Fig. 5. Relation between the concentration of MPs/g (blue irregular line and blue trend line), Fulton's k Condition Factor (orange irregular line and brown trend line) and DNA Degradation Degree (grey columns, scores 1–4). The equation of trend lines is shown. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

assay is not related with the physiological condition of hakes. *Merluccius polli* is a demersal/bathydemersal fish, so its body is naturally subjected to high pressures (depth down to 1000 m), and the samples here analysed were obtained from commercial trawling. Fish internal organs are often damaged during haul backs for rapid decompression (Suuronen, 2005); likely the presence of MPs contributed to mechanical damage of the organs under decompression, facilitating cell breakage (Espinosa et al., 2019; Wang et al., 2022a, 2022b, 2022c) and subsequent DNA degradation (Masiá et al., 2021). Therefore, as it was showed in the results section, the level of DNA degradation found in this assay is not related to the physiological condition of the hake.

Regarding the prevalence and quantity of MPs, the results showed that all the hakes analysed contained MPs at least in one of the three tissues; which is relatively high if compared with other *Merluccius* hakes from Gulf of Cadiz (Bellas et al., 2016) or the South Pacific (Pozo et al., 2019), with the exception of a few *M. merluccius* individuals from the Cantabrian Sea that were highly contaminated with MPs (Cabanilles et al., 2022). MPs concentrations were also higher than those reported for hakes in Portugal (Neves et al., 2015), in the Mediterranean (Anastasopoulou et al., 2013; Giani et al., 2019; Mancuso et al., 2019; Bošković et al., 2022; Mistri et al., 2022) or in Newfoundland (Liboiron et al., 2018); similar or slightly higher than those found in South African hakes (Sparks and Immelman, 2020), and lower than MP content in Pacific *M. productus* from Monterey Bay (Hamilton et al., 2021). In general we could say that these hakes are relatively very polluted, at least in comparison with hakes from other regions; however, it should be noted that the comparison is not straightforward because the majority of studies on hakes have analysed MPs in the gastrointestinal tract, while in our study we analysed individual's tissues.

Compared with other marine fish of Northwest African waters, our data on hake would be also relatively higher than those reported for pelagic species (Maaghloud et al., 2020, 2021; Sánchez-Almeida et al., 2022), except for chub mackerel *Scomber colias* from Canary Islands (Herrera et al., 2019); to be noted again that these data are from the gastrointestinal tract. Murphy et al. (2017) found more MPs in demersal than in pelagic fish; perhaps pelagic fish are less exposed to plastic debris that accumulates near shores, flocculates and deposits over the seafloor. According with the type of MPs, our results were in concordance with previous data of MPs in marine species that are principally black and blue fibers (Hossain et al., 2019; Abidli et al., 2021; Menéndez et al., 2022). In this study, we found a majority of the same MP types. Fibers are, due to their morphology and typology, the least retained in wastewater treatment plants WWTPs (Ngo et al., 2019; Masiá et al., 2020) and are the most likely type of particle obtained from the degradation of fishing gears and nets (Montarsolo et al., 2018; Wright et al., 2021) or released from clothes (De Falco et al., 2019). Also, dark particles tend to be more consumed by fishes for their confusion with plankton (Ma et al., 2020). It is worth noting that hakes are carnivorous so many MPs might have reached the animal by trophic transfer (Au et al., 2017; Carbery et al., 2018).

Masiá et al. (2022, 2022b) found in their meta-analysis that fish from Northwest Africa were relatively little polluted with MPs, but these new data on hakes would reveal more contamination than expected. As explained above, the majority of studies from this area were on pelagic fish, while Benguela hake is demersal and would be more exposed to MPs (Murphy et al., 2017). Another explanation could be a recent accumulation of MPs transported by currents, as it happens in Japanese waters (Iwasaki et al., 2017). According to NOAA (https://nowcoast.noaa.gov/ Last accessed February, 2023) the main currents that run through the sampling area have their origin in the Strait of Gibraltar and have North-south directionality. High MP pollution in fish has been reported from different areas of the Mediterranean Sea (Akhbarizadeh et al., 2019; Masiá et al., 2022a, 2022b). Recent studies (Akarsu et al., 2020; Fytianos et al., 2021; Pedrotti et al., 2021) have revealed a large amount of MPs reaching the Mediterranean Sea after escaping the retention systems of WWTPs that are sources of MPs in the aquatic environment (Sun et al., 2019; Liu et al., 2021). Therefore, it is not unreasonable to consider the Mediterranean Sea as a potential source of pollution for the waters of northwest Africa. This does not exclude other MP sources like rivers (Jiang et al., 2019; Kataoka et al., 2019), or airborne plastic particles (Allen et al., 2021).

As a final remark, the results found in this study suggest MPs pose an additional threat to demersal fish. According to the OECD, about 6.1 Mega Tonnes of MPs were released to the aquatic environments in 2019, leading to an historical accumulation of 30 Mt in the oceans (OECD, https://www.oecd-ilibrary.org/sites/de747aef-en/index.html?itemId=/content/publication/de747aef-en). The only way to stop the deterioration of these valued fishing resources is to reduce the human dependence on plastic while improving plastic management.

5. Conclusions

The Benguela Hake was used here as a representative of the marine top-predators from the North-West coast of Africa. Multiple plastic polymers were found from all the individuals studied in, at least, one tissue analysed (showing a high prevalence of black and blue fibers). While most of the particles have not been reported as dangerous for the aquatic life or the human consumer (rayon was the mostly found polymer), a few compounds (in minor quantities) recognised as hazardous, were also identified.

The gills and the liver, as a direct entrance for MPs in the organisms, showed a high and similar number of particles per gram of tissue. Otherwise, the muscle, studied as an edible tissue by the potential human consumer, showed a lower but still warning presence of plastics. Once the presence and abundance of MPs was studied, their biological harmful effect has been proven in the liver, which acts as an active bioaccumulator of MPs. In the liver, the increase on the number of MPs per gram of tissue is translated into a negative effect in both the physiological condition and the DNA integrity (the higher MP concentration, the lower Condition Factor, and the higher DNA degradation, respectively).

The Benguela hake is now included in a long list of species affected by microplastics in the African coast, but a short list when we focus on the northwest coast, where not enough studies have been carried out yet. Their demersal distribution as well as their environmental importance might be taken as a wake-up call for further analyses. The identification of the preys, the potential trophic transfer as well as the study of the waters where the Benguela hake inhabits might be enough for the correct implementation of management measurements that avoid or, at least, limit the arrival of a large number of pollutants in the area.

New policies aimed at responsible waste management (land and water waste) as well as the exhaustive limitation of the distribution of single-use plastics should be a priority to preserve a balanced health status of the seas. These actions together with a proper public warning and population education must be carried out in an efficient manner to protect marine ecosystems and, therefore, human health.

CRediT authorship contribution statement

Daniel Menéndez: Methodology, Investigation, Software, Writing–original draft. **Carmen Blanco-Fernandez:** Investigation, Writing – review & editing. **Gonzalo Machado-Schiaffino:** Visualization, Writing – review & editing. **Alba Ardura:** Supervision, Visualization, Writing – review & editing. **Eva Garcia-Vazquez**: Conceptualization, Data curation, Visualization, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2023.115135.

References

Abidli, S., Akkari, N., Lahbib, Y., Trigui El Menif, N., 2021. First evaluation of microplastics in two commercial fish species from the lagoons of Bizerte and Ghar El Melh (Northern Tunisia). Reg. Stud. Mar. Sci. 41, 101581 https://doi.org/10.1016/j.rsma.2020.101581.

Achparaki, M., Thessalonikeos, E., Tsoukali, H., Mastrogianni, O., Zaggelidou, E., Chatzinikolaou, F., Raikos, N., 2012. Heavy metals toxicity. Aristotle Univ. Med. J. 39 (1), 29–34.

Akarsu, C., Kumbur, H., Gökdağ, K., Kıdeyş, A.E., Sanchez-Vidal, A., 2020. Microplastics composition and load from three wastewater treatment plants discharging into Mersin Bay, north eastern Mediterranean Sea. Mar. Pollut. Bull. 150, 110776 https://doi.org/10.1016/j.marpolbul.2019.110776.

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.

Allen, S., Allen, D., Baladima, F., Phoenix, V.R., Thomas, J.L., Le Roux, G., Sonke, J.E., 2021. Evidence of free tropospheric and long-range transport of microplastic at Pic du Midi Observatory. Nat. Commun. 12 (1), 7242. https://doi.org/10.1038/s41467-021-27454-7.

Amorim, A.L.A. de, Ramos, J.A.A., Nogueira Júnior, M., 2020. Ingestion of microplastic by ontogenetic phases of *Stellifer brasiliensis* (Perciformes, Sciaenidae) from the surf zone of tropical beaches. Mar. Pollut. Bull. 158, 111214 https://doi.org/10.1016/j.marpolbul.2020.111214.

Anastasopoulou, A., Mytilineou, C., Smith, C.J., Papadopoulou, K.N., 2013. Plastic debris ingested by deep-water fish of the Ionian Sea (Eastern Mediterranean. Deep Sea Res. Part I Oceanogr. Res. Pap. 74, 11–13. https://doi.org/10.1016/j.dsr.2012.12.008.

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.

Arthur, C., Baker, J., Bamford, H. , (2008, September). International research workshop on the occurrence, effects, and fate of microplastic marine debris. In: Conference Proceedings. Sep, 9–11.

Au, S.Y., Lee, C.M., Weinstein, J.E., van den Hurk, P., Klaine, S.J., 2017. Trophic transfer of microplastics in aquatic ecosystems: identifying critical research needs: factors influencing microplastic trophic transfer. Integr. Environ. Assess. Manag. 13 (3), 505–509. https://doi.org/10.1002/ieam.1907.

Avio, C.G., Gorbi, S., Milan, M., Benedetti, M., Fattorini, D., d'Errico, G., Regoli, F., 2015. Pollutants bioavailability and toxicological risk from microplastics to marine mussels. Environ. Pollut. 198, 211–222.

Bao, R., Cheng, Z., Hou, Y., Xie, C., Pu, J., Peng, L., Gao, L., Chen, W., Su, Y., 2022. Secondary microplastics formation and colonized microorganisms on the surface of conventional and degradable plastic granules during long-term UV aging in various environmental media. J. Hazard. Mater. 439, 129686 https://doi.org/10.1016/j.jhazmat.2022.129686.

Bellas, J., Martínez-Armental, J., Martínez-Cámara, A., Besada, V., Martínez-Gómez, C., 2016. Ingestion of microplastics by demersal fish from the Spanish Atlantic and Mediterranean coasts. Mar. Pollut. Bull. 109 (1), 55–60. https://doi.org/10.1016/j.marpolbul.2016.06.026.

Bilbao-Kareaga, A., Menendez, D., Peón, P., Ardura, A., Garcia-Vazquez, E., 2023. Microplastics in jellifying algae in the Bay of Biscay. Implications for consumers' health. Algal Res., 103080

Blanco-Fernandez, C., Erzini, K., Rodriguez-Diego, S., Alba-Gonzalez, P., Thiam, N., Sow, F.N., et al., 2022. Two fish in a pod. mislabelling on board threatens sustainability in mixed fisheries. Front. Mar. Sci. 9, 1–10. https://doi.org/10.3389/fmars.2022.841667.

Boerger, C.M., Lattin, G.L., Moore, S.L., Moore, C.J., 2010. Plastic ingestion by planktivorous fishes in the North Pacific Central Gyre. Mar. Pollut. Bull. 60 (12), 2275–2278. https://doi.org/10.1016/j.marpolbul.2010.08.007.

Bošković, N., Joksimović, D., Bajt, O., 2022. Microplastics in fish and sediments from the Montenegrin coast (Adriatic Sea): similarities in accumulation. Sci. Total Environ. 850, 158074 https://doi.org/10.1016/j.scitotenv.2022.158074.

Brennecke, D., Duarte, B., Paiva, F., Caçador, I., Canning-Clode, J., 2016. Microplastics as vector for heavy metal contamination from the marine environment. Estuar., Coast. Shelf Sci. 178, 189–195.

Cabanilles, P., Acle, S., Arias, A., Masiá, P., Ardura, A., Garcia-Vazquez, E., 2022. Microplastics risk into a three-link food chain inside european hake. Diversity 14 (5), 308. https://doi.org/10.3390/d14050308.

Carbery, M., O'Connor, W., Palanisami, T., 2018. Trophic transfer of microplastics and mixed contaminants in the marine food web and implications for human health. Environ. Int. 115, 400–409. https://doi.org/10.1016/j.envint.2018.03.007.

Carpenter, E.J., Smith Jr, K.L., 1972. Plastics on the sargasso sea surface. Science 175 (4027), 1240–1241.

Chiu, H.W., Xia, T., Lee, Y.H., Chen, C.W., Tsai, J.C., Wang, Y.J., 2015. Cationic polystyrene nanospheres induce autophagic cell death through the induction of endoplasmic reticulum stress. Nanoscale 7 (2), 736–746.

Collard, F., Ask, A., 2021. Plastic ingestion by Arctic fauna: a review. Sci. Total Environ. 786, 147462 https://doi.org/10.1016/j.scitotenv.2021.147462.

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.

Copat, C., Arena, G., Fiore, M., Ledda, C., Fallico, R., Sciacca, S., Ferrante, M., 2013. Heavy metals concentrations in fish and shellfish from eastern Mediterranean Sea: consumption advisories. Food Chem. Toxicol. 53, 33–37. https://doi.org/10.1016/j.fct.2012.11.038.

Costa, M.F., Barletta, M., 2015. Microplastics in coastal and marine environments of the western tropical and sub-tropical Atlantic Ocean. Environ. Sci. Process. Impacts 17 (11), 1868–1879.

De Falco, F., Di Pace, E., Cocca, M., Avella, M., 2019. The contribution of washing processes of synthetic clothes to microplastic pollution. Sci. Rep. 9 (1), 6633. https://doi.org/10.1038/s41598-019-43023-x.

Deng, Y., Zhang, Y., Lemos, B., Ren, H., 2017. Tissue accumulation of microplastics in mice and biomarker responses suggest widespread health risks of exposure. Sci. Rep. 7 (1), 46687.

Dimitriadi, A., Papaefthimiou, C., Genizegkini, E., Sampsonidis, I., Kalogiannis, S., Feidantsis, K., Bikiaris, D.N., 2021. Adverse effects polystyrene microplastics exert on zebrafish heart–Molecular to individual level. J. Hazard. Mater. 416, 125969.

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.

Estoup, A., Largiader, C., Perrot, E., Chourrout, D., 1996. Rapid one-tube DNA extraction for reliable PCR detection of fish polymorphic markers and transgenes. Mol. Mar. Biol. Biotechnol. 5 (4), 295–298.

Expósito, N., Rovira, J., Sierra, J., Gimenez, G., Domingo, J.L., Schuhmacher, M., 2022. Levels of microplastics and their characteristics in molluscs from North-West Mediterranean sea: human intake. Mar. Pollut. Bull. 181, 113843 https://doi.org/10.1016/j.marpolbul.2022.113843.

FAO , Species catalogue—vol. 10 Gadiform Fishes of the World. 1990.

Fernández-Peralta, L., Salmerón, F., Rey, J., Puerto, M., García-Cancela, R., 2011. Reproductive biology of black hakes (Merluccius polli and M. senegalensis) off Mauritania. Cienc. Mar. 37 (4B), 527–546. https://doi.org/10.7773/cm.v37i4B.1841.

Fleury, J.B., Baulin, V.A., 2021. Microplastics destabilize lipid membranes by mechanical stretching. Proc. Natl. Acad. Sci. USA 118 (31), e2104610118.

Fossi, M.C., Panti, C., Guerranti, C., Coppola, D., Giannetti, M., Marsili, L., Minutoli, R., 2012. Are baleen whales exposed to the threat of microplastics? A case study of the Mediterranean fin whale (Balaenoptera physalus. Mar. Pollut. Bull. 64 (11), 2374–2379. https://doi.org/10.1016/j.marpolbul.2012.08.013.

Froese, R., 2006. Cube law, condition factor and weight-length relationships: history, meta-analysis and recommendations. J. Appl. Ichthyol. 22 (4), 241–253. https://doi.org/10.1111/j.1439-0426.2006.00805.x.

Fulton, T.W., 1904. Twenty-Second Annual Report. Fisheries Board of Scotland, pp. 41–241.

Fytianos, G., Ioannidou, E., Thysiadou, A., Mitropoulos, A.C., Kyzas, G.Z., 2021. Microplastics in Mediterranean coastal countries: a recent overview. J. Mar. Sci. Eng. 9 (1), 98. https://doi.org/10.3390/jmse9010098.

Gallitelli, L., Zauli, A., Scalici, M., 2022. Another one bites the plastics. Ecol. Evol. 12 (9) https://doi.org/10.1002/ece3.9332.

Giani, D., Baini, M., Galli, M., Casini, S., Fossi, M.C., 2019. Microplastics occurrence in edible fish species (Mullus barbatus and Merluccius merluccius) collected in three different geographical sub-areas of the Mediterranean Sea. Mar. Pollut. Bull. 140, 129–137. https://doi.org/10.1016/j.marpolbul.2019.01.005.

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.

Güven, O., Gökdağ, K., Jovanović, B., Kıdeyş, A.E., 2017. Microplastic litter composition of the Turkish territorial waters of the Mediterranean Sea, and its occurrence in the gastrointestinal tract of fish. Environ. Pollut. 223, 286–294. https://doi.org/10.1016/j.envpol.2017.01.025.

Hamilton, B., Rochman, C., Hoellein, T., Robison, B., Van Houtan, K., Choy, C., 2021. Prevalence of microplastics and anthropogenic debris within a deep-sea food web. Mar. Ecol. Prog. Ser. 675, 23–33. https://doi.org/10.3354/meps13846.

Hammer, Ø., Harper, D.A., Ryan, P.D., 2001. PAST: Paleontological statistics software package for education and data analysis. Palaeontol. Electron. 4 (1), 9.
Hengstler, J.G., Bolm-Audorff, U., Faldum, A., Janssen, K., Reifenrath, M., Götte, W., Oesch, F., 2003. Occupational exposure to heavy metals: DNA damage induction and DNA repair inhibition prove co-exposures to cadmium, cobalt and lead as more dangerous than hitherto expected. Carcinogenesis 24 (1), 63–73.
Herrera, A., Ŝtindlová, A., Martínez, I., Rapp, J., Romero-Kutzner, V., Samper, M.D., Montoto, T., Aguiar-González, B., Packard, T., Gómez, M., 2019. Microplastic ingestion by Atlantic chub mackerel (Scomber colias) in the Canary Islands coast. Mar. Pollut. Bull. 139, 127–135.
Herzke, D., Ghaffari, P., Sundet, J.H., Tranang, C.A., Halsband, C., 2021. Microplastic fiber emissions from wastewater effluents: abundance, transport behavior and exposure risk for biota in an Arctic Fjord. Front. Environ. Sci. 9, 662168 https://doi.org/10.3389/fenvs.2021.662168.
Hidalgo-Ruz, V., Gutow, L., Thompson, R.C., Thiel, M., 2012. Microplastics in the marine environment: a review of the methods used for identification and quantification. Environ. Sci. Technol. 46 (6), 3060–3075. https://doi.org/10.1021/es2031505.
Hossain, M.S., Sobhan, F., Uddin, M.N., Sharifuzzaman, S.M., Chowdhury, S.R., Sarker, S., Chowdhury, M.S.N., 2019. Microplastics in fishes from the Northern Bay of Bengal. Sci. Total Environ. 690, 821–830. https://doi.org/10.1016/j.scitotenv.2019.07.065.
Ita-Nagy, D., Vázquez-Rowe, I., Kahhat, R., 2022. Prevalence of microplastics in the ocean in Latin America and the Caribbean. J. Hazard. Mater. Adv. 5, 100037 https://doi.org/10.1016/j.hazadv.2021.100037.
Iwasaki, S., Isobe, A., Kako, S.I., Uchida, K., Tokai, T., 2017. Fate of microplastics and mesoplastics carried by surface currents and wind waves: a numerical model approach in the Sea of Japan. Mar. Pollut. Bull. 121 (1–2), 85–96.
Jaishankar, M., Tseten, T., Anbalagan, N., Mathew, B.B., Beeregowda, K.N., 2014. Toxicity, mechanism and health effects of some heavy metals. Interdiscip. Toxicol. 7 (2), 60–72. https://doi.org/10.2478/intox-2014-0009.
Javanshir Khoei, A., 2022. A comparative study on the accumulation of toxic heavy metals in fish of the Oman Sea: effects of fish size, spatial distribution and trophic level. Toxin Rev. 1–8. https://doi.org/10.1080/15569543.2022.2040033.
Jiang, C., Yin, L., Li, Z., Wen, X., Luo, X., Hu, S., Yang, H., Long, Y., Deng, B., Huang, L., Liu, Y., 2019. Microplastic pollution in the rivers of the Tibet Plateau. Environ. Pollut. 249, 91–98. https://doi.org/10.1016/j.envpol.2019.03.022.
Kataoka, T., Nihei, Y., Kudou, K., Hinata, H., 2019. Assessment of the sources and inflow processes of microplastics in the river environments of Japan. Environ. Pollut. 244, 958–965. https://doi.org/10.1016/j.envpol.2018.10.111.
Kilongo, K., Mehl, S. , 1997. Predation by Benguela hake (Merluccius polli) on cOmmercially Exploited Shrimps (Parapenaeus longirostris and Aristeus varidens) in Angolan Waters.
Kim, J.-S., Lee, H.-J., Kim, S.-K., Kim, H.-J., 2018. Global pattern of microplastics (MPs) in commercial food-grade salts: sea salt as an indicator of seawater MP pollution. Environ. Sci. Technol. 52 (21), 12819–12828. https://doi.org/10.1021/acs.est.8b04180.
Kögel, T., Hamilton, B.M., Granberg, M.E., Provencher, J., Hammer, S., Gomiero, A.,. Lusher, A.L. , 2022. Current efforts on microplastic monitoring in Arctic fish and how to proceed. Arctic Sci.
Kroon, F.J., Motti, C.E., Jensen, L.H., Berry, K.L.E., 2018. Classification of marine microdebris: a review and case study on fish from the Great Barrier Reef, Australia. Sci. Rep. 8 (1), 16422. https://doi.org/10.1038/s41598-018-34590-6.
Kumar, V.E., Ravikumar, G., Jeyasanta, K.I., 2018. Occurrence of microplastics in fishes from two landing sites in Tuticorin, South east coast of India. Mar. Pollut. Bull. 135, 889–894. https://doi.org/10.1016/j.marpolbul.2018.08.023.
Lei, Y.-C., Yang, H.-T., Ma, Y.-C., Huang, M.-F., Chang, W.P., Cheng, T.-J., 2004. DNA single strand breaks in peripheral lymphocytes associated with urinary thiodiglycolic acid levels in polyvinyl chloride workers. Mutat. Res. /Genet. Toxicol. Environ. Mutagen. 561 (1–2), 119–126. https://doi.org/10.1016/j.mrgentox.2004.04.002.
Li, J., Peng, D., Ouyang, Z., Liu, P., Fang, L., Guo, X., 2022. Occurrence status of microplastics in main agricultural areas of Xinjiang Uygur Autonomous Region, China. Sci. Total Environ. 828, 154259.
Liboiron, F., Ammendolia, J., Saturno, J., Melvin, J., Zahara, A., Richárd, N., Liboiron, M., 2018. A zero percent plastic ingestion rate by silver hake (Merluccius bilinearis) from the south coast of Newfoundland, Canada. Mar. Pollut. Bull. 131, 267–275. https://doi.org/10.1016/j.marpolbul.2018.04.007.
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.
Liu, W., Zhang, J., Liu, H., Guo, X., Zhang, X., Yao, X., Cao, Z., Zhang, T., 2021. A review of the removal of microplastics in global wastewater treatment plants: Characteristics and mechanisms. Environ. Int. 146, 106277 https://doi.org/10.1016/j.envint.2020.106277.
Lloris D., Matallanas J., Oliver P., 2005 Hakes of the world (family Merlucciidae): an annotated and illustrated catalogue of hake species known to date. FAO Species Catalogue for Fishery Purposes, No. 2, FAO, Rome, 57.
Lorenzo-Navarro, J., Castrillón-Santana, M., Sánchez-Nielsen, E., Zarco, B., Herrera, A., Martínez, I., Gómez, M., 2021. Deep learning approach for automatic microplastics counting and classification. Sci. Total Environ. 765, 142728 https://doi.org/10.1016/j.scitotenv.2020.142728.
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.
Lundy, C.J., Rico, C., Hewitt, G.M., 2000. Temporal and spatial genetic variation in spawning grounds of European hake (*Merluccius merluccius*) in the Bay of Biscay. Mol. Ecol. 9, 2067–2079. https://doi.org/10.1046/j.1365-294X.2000.01120.x.
Ma, H., Pu, S., Liu, S., Bai, Y., Mandal, S., Xing, B., 2020. Microplastics in aquatic environments: toxicity to trigger ecological consequences. Environ. Pollut. 261, 114089 https://doi.org/10.1016/j.envpol.2020.114089.
Maaghloud, H., Houssa, R., Bellali, F., El Bouqdaoui, K., Ouansafi, S., Loulad, S., Fahde, A., 2021. Microplastic ingestion by Atlantic horse mackerel (Trachurus trachurus) in the North and central Moroccan Atlantic coast between Larache (35° 30′ N) and Boujdour (26° 30′ N). Environ. Pollut. 288, 117781.
Maaghloud, H., Houssa, R., Ouansafi, S., Bellali, F., El Bouqdaoui, K., Charouki, N., Fahde, A., 2020. Ingestion of microplastics by pelagic fish from the Moroccan Central Atlantic coast. Environ. Pollut. 261, 114194.
Machado-Schiaffino, G., Martinez, J.L., Garcia-Vazquez, E., 2008. Detection of mislabeling in hake seafood employing mtSNPs-based methodology with identification of eleven hake species of the genus Merluccius. J. Agric. Food Chem. 56, 5091–5095. https://doi.org/10.1021/jf800207t.
Makedonski, L., Peycheva, K., Stancheva, M., 2017. Determination of heavy metals in selected black sea fish species. Food Control 72, 313–318. https://doi.org/10.1016/j.foodcont.2015.08.024.
Manchih, K., Peralta, L.F., Bensbai, J., Najd, A., Bekkali, M., 2018. Distribution of black hakes *Merluccius senegalensis* and *Merluccius polli* along the Moroccan Atlantic coast. AACL Bioflux 11, 245–258.
Mancuso, M., Savoca, S., Bottari, T., 2019. First record of microplastics ingestion by European hake *Merluccius merluccius* from the Tyrrhenian Sicilian coast (Central Mediterranean Sea). J. Fish. Biol. 94 (3), 517–519. https://doi.org/10.1111/jfb.13920.
Manu, M.K., Luo, L., Kumar, R., Johnravindar, D., Li, D., Varjani, S., Wong, J., 2023. A review on mechanistic understanding of microplastic pollution on the performance of anaerobic digestion. Environ. Pollut., 121426
Markic, A., Gaertner, J.-C., Gaertner-Mazouni, N., Koelmans, A.A., 2020. Plastic ingestion by marine fish in the wild. Crit. Rev. Environ. Sci. Technol. 50 (7), 657–697. https://doi.org/10.1080/10643389.2019.1631990.
Masiá, P., Ardura, A., Garcia-Vazquez, E., 2019. Microplastics in special protected areas for migratory birds in the Bay of Biscay. Mar. Pollut. Bull. 146, 993–1001. https://doi.org/10.1016/j.marpolbul.2019.07.065.
Masiá, P., Ardura, A., García-Vázquez, E., 2021. Virgin polystyrene microparticles exposure leads to changes in gills DNA and physical condition in the Mediterranean mussel Mytilus galloprovincialis. Animals 11 (8), 2317.
Masiá, P., Ardura, A., Garcia-Vazquez, E., 2022a. Microplastics in seafood: relative input of Mytilus galloprovincialis and table salt in mussel dishes. Food Res. Int. 153, 110973.
Masiá, P., Mateo, J.L., Arias, A., Bartolomé, M., Blanco, C., Erzini, K., Garcia-Vazquez, E., 2022b. Potential microplastics impacts on African fishing resources. Sci. Total Environ. 806, 150671.
Masiá, P., Sol, D., Ardura, A., Laca, A., Borrell, Y.J., Dopico, E., Garcia-Vazquez, E., 2020. Bioremediation as a promising strategy for microplastics removal in wastewater treatment plants. Mar. Pollut. Bull. 156, 111252.
Mbaye, B.C., Brochier, T., Echevin, V., Lazar, A., Lévy, M., Mason, E., Gaye, A.T., Machu, E., 2015. Do Sardinella aurita spawning seasons match local retention patterns in the Senegalese–Mauritanian upwelling region? Fish. Oceanogr. 24 (1), 69–89.
Menéndez, D., Álvarez, A., Acle, S., Peón, P., Ardura, A., Garcia-Vazquez, E., 2022. Microplastics across biomes in diadromous species. Insights from the critically endangered Anguilla anguilla. Environ. Pollut. 305, 119277.
Menendez, D., Alvarez, A., Peon, P., Ardura, A., Garcia-Vazquez, E., 2021. From the ocean to jellies forth and back? Microplastics along the commercial life cycle of red algae. Mar. Pollut. Bull. 168, 112402.
Mičić, M., Bihari, N., Jakšić, Ž., Müller, W.E., Batel, R., 2002. DNA damage and apoptosis in the mussel Mytilus galloprovincialis. Mar. Environ. Res. 53 (3), 243–262.
Mistri, M., Sfriso, A.A., Casoni, E., Nicoli, M., Vaccaro, C., Munari, C., 2022. Microplastic accumulation in commercial fish from the Adriatic Sea. Mar. Pollut. Bull. 174, 113279 https://doi.org/10.1016/j.marpolbul.2021.113279.
Montarsolo, A., Mossotti, R., Patrucco, A., Caringella, R., Zoccola, M., Pozzo, P.D., Tonin, C., 2018. Study on the microplastics release from fishing nets. Eur. Phys. J. 133 (11), 494. https://doi.org/10.1140/epjp/i2018-12415-1.
Morgana, S., Ghigliotti, L., Estévez-Calvar, N., Stifanese, R., Wieckzorek, A., Doyle, T., 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.
Murphy, F., Russell, M., Ewins, C., Quinn, B., 2017. The uptake of macroplastic & microplastic by demersal & pelagic fish in the Northeast Atlantic around Scotland. Mar. Pollut. Bull. 122 (1–2), 353–359. https://doi.org/10.1016/j.marpolbul.2017.06.073.
Naji, A., Nuri, M., Vethaak, A.D., 2018. Microplastics contamination in molluscs from the northern part of the Persian Gulf. Environ. Pollut. 235, 113–120. https://doi.org/10.1016/j.envpol.2017.12.046.
Neves, D., Sobral, P., Ferreira, J.L., Pereira, T., 2015. Ingestion of microplastics by commercial fish off the Portuguese coast. Mar. Pollut. Bull. 101 (1), 119–126. https://doi.org/10.1016/j.marpolbul.2015.11.008.
Ngo, H.T.T., Liang, L., Nguyen, D.B., Doan, H.N., Watchalayann, P., 2021. Blood heavy metals and DNA damage among children living in an informal E-waste processing area in Vietnam. Hum. Ecol. Risk Assess. Int. J. 27 (2), 541–559.
Ngo, P.L., Pramanik, B.K., Shah, K., Roychand, R., 2019. Pathway, classification and removal efficiency of microplastics in wastewater treatment plants. Environ. Pollut. 255, 113326 https://doi.org/10.1016/j.envpol.2019.113326.

Nicastro, K.R., Lo Savio, R., McQuaid, C.D., Madeira, P., Valbusa, U., Azevedo, F., Casero, M., Lourenço, C., Zardi, G.I., 2018. Plastic ingestion in aquatic-associated bird species in southern Portugal. Mar. Pollut. Bull. 126, 413–418. https://doi.org/10.1016/j.marpolbul.2017.11.050.

Pedrotti, M.L., Petit, S., Eyheraguibel, B., Kerros, M.E., Elineau, A., Ghiglione, J.F., Loret, J.F., Rostan, A., Gorsky, G., 2021. Pollution by anthropogenic microfibers in North-West Mediterranean Sea and efficiency of microfiber removal by a wastewater treatment plant. Sci. Total Environ. 758, 144195 https://doi.org/10.1016/j.scitotenv.2020.144195.

Piyawardhana, N., Weerathunga, V., Chen, H.-S., Guo, L., Huang, P.-J., Ranatunga, R.R. M.K.P., Hung, C.-C., 2022. Occurrence of microplastics in commercial marine dried fish in Asian countries. J. Hazard. Mater. 423, 127093 https://doi.org/10.1016/j.jhazmat.2021.127093.

Pozo, K., Gomez, V., Torres, M., Vera, L., Nuñez, D., Oyarzún, P., Mendoza, G., Clarke, B., Fossi, M.C., Baini, M., Přibylová, P., Klánová, J., 2019. Presence and characterization of microplastics in fish of commercial importance from the Biobío region in central Chile. Mar. Pollut. Bull. 140, 315–319.

Prokić, M.D., Radovanović, T.B., Gavrić, J.P., Faggio, C., 2019. Ecotoxicological effects of microplastics: examination of biomarkers, current state and future perspectives. TrAC Trends Anal. Chem. 111, 37–46.

Rainbow, P.S., 1985. The biology of heavy metals in the sea. Int. J. Environ. Stud. 25 (3), 195–211. https://doi.org/10.1080/00207238508710225.

Rehse, S., Kloas, W., Zarfl, C., 2018. Microplastics reduce short-term effects of environmental contaminants. Part I: effects of bisphenol A on freshwater zooplankton are lower in presence of polyamide particles. Int. J. Environ. Res. Public Health 15 (2), 280.

, 2019Report of the FAO/CECAF Working Group on the Assessment of Demersal Resources – Subgroup North Nouakchott, Mauritania, 2–10 December 2019 / Rapport du Groupe de travail FAO/COPACE sur l'évaluation des ressources démersales – Sous-groupe Nord Nouakchott, Mauritanie, 2–10 decembre 2019. (2020). FAO. https://doi.org/10.4060/cb1539b.

Rey, J., Fernández-Peralta, L., Esteban, A., García-Cancela, R., Salmerón, F., Puerto, M.Á., Piñeiro, C., 2012. Does otolith macrostructure record environmental or biological events? The case of black hake (Merluccius polli and Merluccius senegalensis). Fish. Res. 113 (1), 159–172. https://doi.org/10.1016/j.fishres.2011.10.010.

Ribeiro, F., Garcia, A.R., Pereira, B.P., Fonseca, M., Mestre, N.C., Fonseca, T.G.,. & Bebianno, M.J. (2017). Microplastics effects in Scrobicularia plana. Mar. Pollut. Bull., 122(1–2), 379–391.

Rochman, C.M., 2018. Microplastics research—from sink to source. Science 360 (6384), 28–29.

Rodrigues, S.M., Elliott, M., Almeida, C.M.R., Ramos, S., 2021. Microplastics and plankton: knowledge from laboratory and field studies to distinguish contamination from pollution. J. Hazard. Mater. 417, 126057 https://doi.org/10.1016/j.jhazmat.2021.126057.

Roman, L., Schuyler, Q., Wilcox, C., Hardesty, B.D., 2021. Plastic pollution is killing marine megafauna, but how do we prioritize policies to reduce mortality. Conserv. Lett. 14 (2) https://doi.org/10.1111/conl.12781.

Ryan, M.G., Watkins, L., Walter, M.T., 2019. Hudson River juvenile Blueback herring avoid ingesting microplastics. Mar. Pollut. Bull. 146, 935–939. https://doi.org/10.1016/j.marpolbul.2019.07.004.

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), 1931.

Shen, R., Yang, K., Cheng, X., Guo, C., Xing, X., Sun, H., Wang, D., 2022. Accumulation of polystyrene microplastics induces liver fibrosis by activating cGAS/STING pathway. Environ. Pollut. 300, 118986.

Sobhani, Z., Panneerselvan, L., Fang, C., Naidu, R., Megharaj, M., 2021. Chronic and transgenerational effects of polystyrene microplastics at environmentally relevant concentrations in earthworms (Eisenia fetida). Environ. Toxicol. Chem. 40 (8), 2240–2246.

Soto, M., Fernández-Peralta, L., Pennino, M.G., Kokkalis, A., Rey, J., Salmerón, F., Liébana, M., Meissa, B., Kell, L., 2022. Effects of misreporting landings, discards, and Catch Per Unit of Effort index in state-space production models: The case of black hake in northwest Africa. ICES J. Mar. Sci., fsac188. https://doi.org/10.1093/icesjms/fsac188.

Sparks, C., Immelman, S., 2020. Microplastics in offshore fish from the Agulhas Bank, South Africa. Mar. Pollut. Bull. 156, 111216 https://doi.org/10.1016/j.marpolbul.2020.111216.

Summary Report—FAO Working Group on the Assessment of Demersal Resources off Northwest Africa. 2019.

Sun, J., Dai, X., Wang, Q., van Loosdrecht, M.C.M., Ni, B.-J., 2019. Microplastics in wastewater treatment plants: detection, occurrence and removal. Water Res. 152, 21–37. https://doi.org/10.1016/j.watres.2018.12.050.

Sun, Q., Li, Y., Shi, L., Hussain, R., Mehmood, K., Tang, Z., Zhang, H., 2022. Heavy metals induced mitochondrial dysfunction in animals: Molecular mechanism of toxicity. Toxicology 469, 153136. https://doi.org/10.1016/j.tox.2022.153136.

Suuronen, P. (2005). Mortality of fish escaping trawl gears. FAO Fisheries Technical Paper, 478, ISSN 0429–9345. FAO, Rome. ⟨https://www.fao.org/3/y6981e/y6981e00.htm#Contents⟩. (Accessed April 2023).

Thubagere, A., Reinhard, B.M., 2010. Nanoparticle-induced apoptosis propagates through hydrogen-peroxide-mediated bystander killing: insights from a human intestinal epithelium in vitro model. ACS Nano 4 (7), 3611–3622.

Tian, K., Wu, Q., Liu, P., Hu, W., Huang, B., Shi, B., Zhou, Y., Kwon, B.-O., Choi, K., Ryu, J., Seong Khim, J., Wang, T., 2020. Ecological risk assessment of heavy metals in sediments and water from the coastal areas of the Bohai Sea and the Yellow Sea. Environ. Int. 136, 105512 https://doi.org/10.1016/j.envint.2020.105512.

Uurasjärvi, E., Sainio, E., Setälä, O., Lehtiniemi, M., Koistinen, A., 2021. Validation of an imaging FTIR spectroscopic method for analyzing microplastics ingestion by Finnish lake fish (Perca fluviatilis and Coregonus albula). Environ. Pollut., 117780 https://doi.org/10.1016/j.envpol.2021.117780.

Vandenberg, L.N., Hauser, R., Marcus, M., Olea, N., Welshons, W.V., 2007. Human exposure to bisphenol A (BPA). Reprod. Toxicol. 24 (2), 139–177. https://doi.org/10.1016/j.reprotox.2007.07.010.

Wagoner, J.K., 1983. Toxicity of vinyl chloride and poly (vinyl chloride): a critical review. Environ. Health Perspect. 52, 61–66.

Wang, Q., Li, J., Zhu, X., Sun, C., Teng, J., Chen, L., Shan, E., Zhao, J., 2022a. 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, Y., Wang, S., Xu, T., Cui, W., Shi, X., Xu, S., 2022b. A new discovery of polystyrene microplastics toxicity: the injury difference on bladder epithelium of mice is correlated with the size of exposed particles. Sci. Total Environ. 821, 153413.

Wang, W., Zhang, J., Qiu, Z., Cui, Z., Li, N., Li, X., Zhao, C., 2022c. Effects of polyethylene microplastics on cell membranes: a combined study of experiments and molecular dynamics simulations. J. Hazard. Mater. 429, 128323.

Wright, L.S., Napper, I.E., Thompson, R.C., 2021. Potential microplastic release from beached fishing gear in Great Britain's region of highest fishing litter density. Mar. Pollut. Bull. 173, 113115 https://doi.org/10.1016/j.marpolbul.2021.113115.

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

Wu, Y., Guo, P., Zhang, X., Zhang, Y., Xie, S., Deng, J., 2019. Effect of microplastics exposure on the photosynthesis system of freshwater algae. J. Hazard. Mater. 374, 219–227. https://doi.org/10.1016/j.jhazmat.2019.04.039.

Xia, T., Kovochich, M., Liong, M., Zink, J.I., Nel, A.E., 2008. Cationic polystyrene nanosphere toxicity depends on cell-specific endocytic and mitochondrial injury pathways. ACS Nano 2 (1), 85–96.

Yang, C.Z., Yaniger, S.I., Jordan, V.C., Klein, D.J., Bittner, G.D., 2011. Most plastic products release estrogenic chemicals: a potential health problem that can Be solved. Environ. Health Perspect. 119 (7), 989–996. https://doi.org/10.1289/ehp.1003220.

Yin, X., Wu, J., Liu, Y., Chen, X., Xie, C., Liang, Y., Li, J., Jiang, Z., 2022. Accumulation of microplastics in fish guts and gills from a large natural lake: selective or non-selective. Environ. Pollut. 309, 119785 https://doi.org/10.1016/j.envpol.2022.119785.

Yu, P., Liu, Z., Wu, D., Chen, M., Lv, W., Zhao, Y., 2018. Accumulation of polystyrene microplastics in juvenile *Eriocheir sinensis* and oxidative stress effects in the liver. Aquat. Toxicol. 200, 28–36. https://doi.org/10.1016/j.aquatox.2018.04.015.

Yuan, W., Zhou, Y., Chen, Y., Liu, X., Wang, J., 2020. Toxicological effects of microplastics and heavy metals on the Daphnia magna. Sci. Total Environ. 746, 141254.

Zhang, K., Hamidian, A.H., Tubić, A., Zhang, Y., Fang, J.K., Wu, C., Lam, P.K., 2021. Understanding plastic degradation and microplastic formation in the environment: a review. Environ. Pollut. 274, 116554.

Zhu, J., Yu, X., Zhang, Q., Li, Y., Tan, S., Li, D., Yang, Z., Wang, J., 2019a. Cetaceans and microplastics: first report of microplastic ingestion by a coastal delphinid, Sousa chinensis. Sci. Total Environ. 659, 649–654. https://doi.org/10.1016/j.scitotenv.2018.12.389.

Zhu, J., Yu, X., Zhang, Q., Li, Y., Tan, S., Li, D., Yang, Z., Wang, J., 2019b. Cetaceans and microplastics: first report of microplastic ingestion by a coastal delphinid, Sousa chinensis. Sci. Total Environ. 659, 649–654. https://doi.org/10.1016/j.scitotenv.2018.12.389.

Zocche, J.J., Leffa, D.D., Damiani, A.P., Carvalho, F., Mendonça, R.Á., Dos Santos, C.E.I., de Andrade, V.M., 2010. Heavy metals and DNA damage in blood cells of insectivore bats in coal mining areas of Catarinense coal basin, Brazil. Environ. Res. 110 (7), 684–691.