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Trophic transfer of DDE, BP-3 and chlorpyrifos from microplastics to tissues in *Dicentrarchus labrax*

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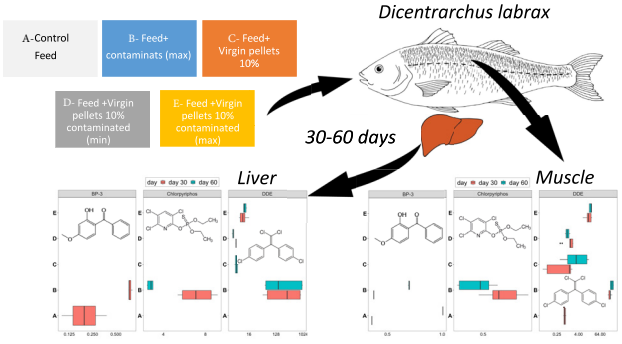
HIGHLIGHTS

- Trophic transfer of chemical pollutants to liver and muscle was observed in treatment consist in feed plus contaminants.
- In treatment consist in feed plus contaminants, BP-3 was present in liver at day 30 and was not detected at day 60.
- DDE-p,p' transfer was also observed in liver and muscle in treatments consisting of feed plus MPs with contaminants.
- Highest biomagnification factor in muscle was for DDE-p,p' in treatment with feed plus virgin MPs.
- No significant differences between treatments were observed in condition indexes in fish.

GRAPHICAL ABSTRACT



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ABSTRACT

Microplastic pollution and associated chemical contaminants is a topic of growing interest. In recent years, the number of publications reporting the presence of microplastics (MPs) in marine organisms has increased exponentially. However, there is a gap in knowledge about the trophic transfer of contaminants from microplastics to animal tissues, as well as possible health effects. In this study we analyzed the trophic transfer and biomagnification of three chemical pollutants present in microplastics: dichlorodiphenyldichloroethylene (DDE-p,p'), benzophenone 3 (BP-3) and chlorpyrifos (CPS). The reference values used were concentrations found in environmental microplastics in the Canary Islands (minimum and maximum). European seabass (Dicentrarchus labrax) were fed for 60 days with 5 different treatments: A) feed; B) feed with chemical pollutants at maximum concentration; C) feed + 10 % virgin MPs; D) feed + 10 % MPs with chemical pollutants at minimum concentration; E) feed + 10 % MPs with chemical pollutants at maximum concentration. We detected trophic transfer of DDE-p,p', CPS and BP-3 from the feed (treatment B) to the muscle and liver of fish. In the case of DDE-p,p', transfer to liver and muscle was also observed in the treatments consisting of feed plus plastics with different levels of contamination (C, D and E). No effect of the experimental treatments on fish condition indices was observed.

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1. Introduction

Plastic pollution is a global problem affecting marine ecosystems (Borrelle et al., 2020; Geyer et al., 2017; Jambeck et al., 2015). One of the main problems associated with plastic pollution is the presence of microplastics in the ocean (MPs), which, due to their size, between 1 μm and 5 mm (Hartmann et al., 2019), can be ingested by marine organisms. There are numerous studies reporting ingestion of MPs in different species (Ugwu et al., 2021), and especially in fish (Compa et al., 2018; Herrera et al., 2019; Nadal et al., 2016; Reinold et al., 2021; Wootton et al., 2021). Once ingested, MPs can cause different effects, ranging from physical damage (Wright et al., 2013), to toxicological effects due to chemical contaminants associated with plastics (Barboza et al., 2020; Barboza et al., 2018a, b, c; Choi et al., 2018; Ding et al., 2020; Rios-Fuster et al., 2021a). These contaminants can be additives that are deliberately added to plastics during their manufacture such as flame retardants, plasticizers, UV filters (Campanale et al., 2020); or persistent chemical contaminants present in low concentrations in the ocean that adsorb and accumulate on the MPs surface (Camacho et al., 2019; Hirai et al., 2011; Ogata et al., 2009). These compounds include organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), UV filters, and others; once microplastics are ingested, these chemical contaminants can release and bioaccumulate in tissues (Teuten et al., 2009; Teuten et al., 2007).

However, to date there is no scientific consensus on whether microplastics act as vectors for the transfer of chemical contaminants to organisms (Hartmann et al., 2017; Koelmans et al., 2022; Koelmans et al., 2016). There are very few studies conducted under environmentally relevant conditions (Rochman et al., 2013), or conducted with "environmental" microplastics. Previous studies conducted in European seabass (*Dicentrarchus labrax*) show that under realistic conditions, in the worst-case scenario, ingestion of environmental microplastics causes accumulation of flame retardants (PBDEs) in the liver (Herrera et al., 2022). Another gap in knowledge about microplastic pollution is the effect on the health of organisms due to ingestion of microplastics. While several studies indicate effects such as neurotoxicity and higher oxidative stress activity in fish tissues (Barboza et al., 2020; Barboza et al., 2018a, b, c; Rochman et al., 2013), or intestinal and microbiota alterations (Montero et al., 2022; Pedà et al., 2016); the effects on fish health due to ingestion of MPs, and the implications to human health, are still unknown.

In the study conducted by Herrera et al. (2020) on microplastic abundance and neustonic zooplankton in Macaronesian waters, areas with very high concentrations of microplastics were found. In these accumulation zones, the ratio of microplastics/zooplankton in wet weight was between 0.08 and 0.14, meaning that a filter-feeding organism could be eating >10 % microplastics with their food (Herrera et al., 2020). On the other hand, Camacho et al. (2019) studied the chemical pollutants associated with microplastics in the same region, shown high levels of chemical contaminants associated with microplastics, in particular pesticides and UV filters.

In the environment, a planktivorous fish could be ingesting up to 10 % of microplastics with different levels of chemical contaminants in these areas of MPs accumulation. Therefore, the present study aims to determine whether there is a trophic transfer of chemical contaminants due to the ingestion of MPs in fish, with environmentally relevant values. In addition, it aims to evaluate the effect on fish health by using different condition indices.

We chose three different pollutants for this experiment, dichlorodiphenyldichloroethylene (DDE-p,p') within the organochlorine pesticides (OCPs), benzophenone 3 (BP-3) and chlorpyrifos (CPS) within the emerging pollutants (EPs), because of their importance, especially in the Canary Islands. In the MPs studied on the coasts of the Canary Islands, the sum of DDTs reached very high values in some samples (ranged from 0.4 to 13,488 ng/g), among the highest found in the literature (Camacho et al., 2019). On the other hand, both BP-3 (UV filter) and chlorpyrifos are considered EPs, these are chemical compounds that are not commonly monitored but have the potential to enter the environment and cause adverse ecological and human health effects (Geissen et al., 2015). Therefore, is necessary to carry out studies to investigate both their bioaccumulation and biomagnification, as well as the possible effects on the health of organisms. In the Canary Islands, high concentrations of UV filters associated with MPs were obtained (ranged from 0 to 3740 ng/g), particularly in beaches with high anthropogenic pressure, which may represent a risk for organisms that ingest them (Camacho et al., 2019). Chlorpyrifos was the most widely used insecticide in the European Union (EU) until 2020 (Saunders et al., 2012; Wołejko et al., 2022), in the Canary Islands was a highly employed in the agriculture (i.e. banana production). Chlorpyrifos was also found in microplastic samples collected on the Canarian beaches (ranged from 0 to 1508 ng/g), for which reason it is necessary to study its possible transfer through MPs (Camacho et al., 2019).

The study was carried out with reference values obtained in the study by Camacho et al. (2019) for UV filters and pesticides, and diets were prepared based on the MPs/zooplankton ratio in wet weight 0.1, which represents 10 % of the zooplankton samples collected in accumulation zones of MPs (Herrera et al., 2020).

2. Material and methods

2.1. Ethics statement

All procedures involving fish complied with the guidelines of the European Union Council (86/609/EU) and Spanish legislation (RD 53/2013) and were approved by Bioethical Committee of the University of Las Palmas de Gran Canaria (Ref. 06/2021 CEBA ULPGC).

2.2. Experimental conditions

The experiment was carried out during two months, in September and October 2019. The experimental conditions and diet production has been previously published (Montero et al., 2022). In brief, 300 fish (mean weight 80.91 ± 13.28 g and mean length 17.98 ± 1.06 cm) from the experimental facilities of the ECOAQUA Institute, ULPGC (Canary Islands, Spain) were subjected to the experimental conditions. Those fish are naïve with respect to the variables measured in the present experiment, and their life-story conditions (feeding regime and environmental conditions) were equal for all the fish as they grew under standardized conditions in the facilities. The fish were distributed in fifteen cylindroconical 500 L tanks (triplicate tanks for each dietary group, 20 fish per tank) provided with a flow-through system. The water conditions were monitored daily, maintaining salinity at 37 mg L^{-1}, oxygen values at 6.0 ± 0.5 ppm O_2 and temperature at 22 ± 1 °C. Fish were fed by hand 3 times per day to apparent satiety. All diets were well accepted during the whole trial, and fish grew properly, doubling their body weight at the end of the experimental period. No significant differences were found among dietary groups in terms of growth performance and feeding efficiency, as previously reported (Montero et al., 2022).

At day 30 and 60 of the experiment, 12 fish per dietary treatment (4 fish/tank) were euthanized with an overdose of clove oil anesthesia by immersion to obtain samples of different tissues after dissection. Liver and muscle samples were obtained for analysis of chemical contaminants. Pooling of 4 fish livers per tank was performed.

2.3. Experimental diets

Commercial feed was used (D-2 Optibream AE 1P, Skretting Spain Spa crude protein 48 %, fat 18 %, ash 6.3 %, and fiber 3.6 %), to produce all diets. Once artificially contaminated, MPs were shredded using a cutting mil (Retsch-SM100, Haan, Germany), to obtain a size fraction between 0.7- and 1-mm. Feed was shredding too following the same process. Once the diets were mixed were put into the granulator with a 3 mm output diameter, obtaining pellets. In order to no affect the stability of the pollutants, the granulator was kept cold with ice to avoid the temperature rising up 50 °C, and the process was done under stable pressure of 2–3 atm. The

feed pellets were dried in an oven at 37–39 °C. The humidity of the diets at the end of the process was between 6.75 ± 0.75 %.

As shown in the Fig. 1, the experimental design included a control (Treatment A) consisting of a commercial feed, and microplastic control (Treatment C) consisting of feed plus 10 % virgin MPs. The virgin microplastics were resins pellets, low density polypropylene (LDPP), (Sigma-Aldrich, ref. 428,116), listed as free of bioaccumulative additives that are toxic and persistent or very bioaccumulative and very persistent at levels of 0.1 % or higher. Polypropylene is one of the most widely produced plastic polymers globally (PlasticsEurope, 2021) and one of the most frequently found in the environment and in ingestion studies in marine organisms (Ugwu et al., 2021).

In order to analyze the trophic transfer of contaminants; DDE-p,p', BP-3 and CPS were included at maximum concentration (Treatment B). In addition, to study the effect of combined plastic and chemical pollutants, two diets were performed adding 10 % MPs with two different DDE-p,p', BP-3 and CPS concentrations: minimum and maximum concentration (Treatments D and E respectively). The proposed reference minimum values to artificially contaminate the virgin MPs were obtained from Camacho et al. (2019) in which the pollutants associated with resin pellets accumulated on Canary Islands beaches were analyzed. The maximum value is proposed as the worst case scenario, calculated as an order of magnitude higher than the minimum value. The minimum and maximum reference values for BP-3, CPS and DDE-p,p', were 600 and 6000 ng/g; 200 and 2000 ng/g; and 1500 and 15,000 ng/g respectively. The purity of the used BP-3, CPS and DDE-p,p' (Sigma-Aldrich analytical standards) was 98.5 %, 97.5 % and 99.9 % respectively.

Once the proportional amount of contaminants was calculated and weighed according to the required concentrations, the first step was to dissolve the compounds in organic solvents: 1 L ethanol (100 %) and 10 mL pure acetone per 500 g of solute (MPs or feed). The feed and the MPs were distributed in glass bins (20 × 20 cm) in a homogeneous layer. The solutions were spread on the bin and removed with a metal spoon. Finally, the ethanol was left to evaporate inside the hood for 5 days. The analysis of levels BP-3, CPS and DDE-p,p' in the MPs was performed following the analysis methodology presented in Camacho et al. (2019). The limits of quantification (LOQ) of the analytical method for the determination of target compounds in MPs have been added as supplementary data in Table S1. The final values in ng/g in MPs are presented in Table 1 and Fig. 2.

Feed used in all diets was D-2 Optibream AE 1P, Skretting Spain Spa (crude protein 48 %, fat 18 %, ash 6.3 %, and fiber 3.6 %). A total of 5 kg of experimental feed were produced for each diet.

Table 1
Concentration in ng/g of BP-3, CPS and DDE-p,p' in MPs at minimum and maximum contamination level.

Pollutant	Concentration in ng/g of plastic (mean ± SD)	
	Contaminated Min	Contaminated Max
BP-3	873.6 ± 42.3	6525.1 ± 325.4
CPS	287.8 ± 22.08	1733.4 ± 126.1
DDE-p,p'	1440.9 ± 36.17	14,206.1 ± 1726.9

The biomagnification factor (BMF) was calculated as defined by Gobas et al. (2009), for laboratory organisms in controlled experiments in which the test organisms are exposed to chemical in the diet, being the ratio between the concentration in the organisms (C_B, ng/g) and the concentration in the diet (C_D, ng/g).

$$BMF = C_B/C_D$$

2.4. Fish condition indices

As indices of fish condition, the hepatosomatic index (HPS), Fulton's condition factor (K) and cellular energy allocation (CEA) index were analyzed.

Hepatosomatic index (HSI) was determined according to Wootton et al. (1978) and calculated as:

$$HSI = \frac{Liver\ weight\ (g)}{Fish\ weight\ (g)} \times 100.$$

Fulton's condition factor (K) (Frias and Nash, 2019; Ricker, 1975) was calculated as follow:

$$K = 100 \times \frac{Weight\ (g)}{Lenght(cm)^3}$$

We calculated the CEA index defined as the relationship between the energy available (Ea) and energy consumption (Ec) (De Coen and Janssen, 1997; Verslycke and Janssen, 2002).

$$CEA\ index = Ea/Ec$$

Methodology of biochemical analysis of lipids, carbohydrates, proteins, and electron transport system activity (ETS activity) to analyze CEA index is specified in Supplementary Material.

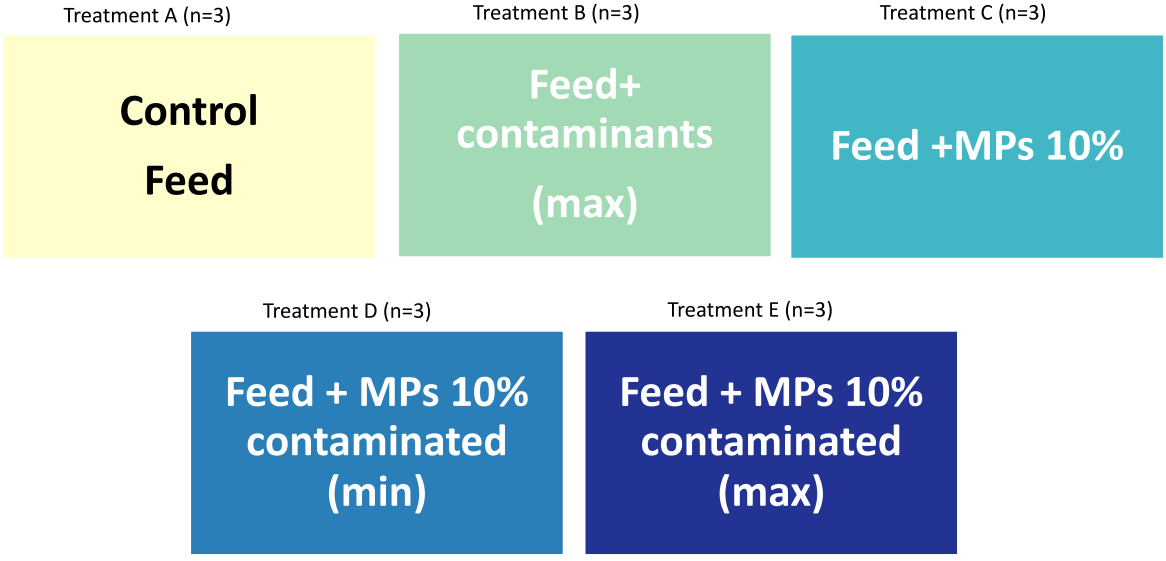

Fig. 1. Composition of each diet A) Feed; B) Feed plus BP-3, CPS and DDE-p,p' at maximum; C) Feed plus 10 % virgin MPs; D) Feed plus 10 % MPs contaminated at minimum; E) Feed plus 10 % MPs contaminated at maximum.

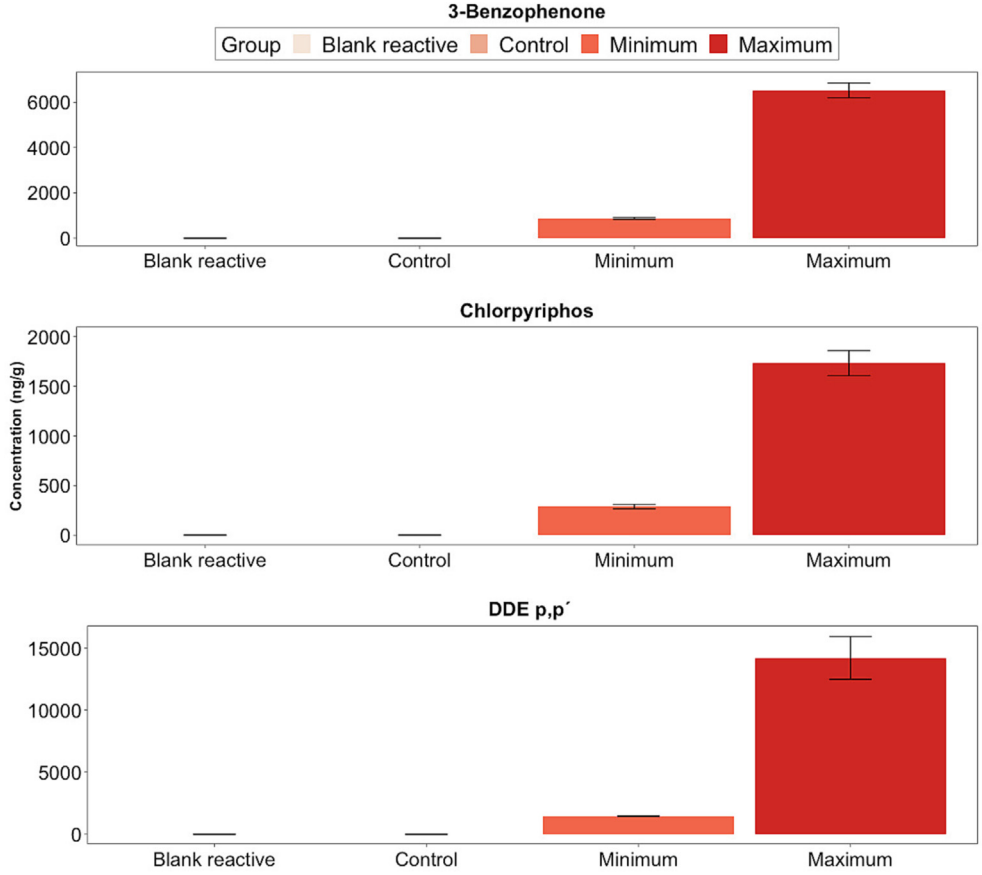

Fig. 2. Concentration in ng/g of BP-3, CPS and DDE-p,p' in MPs in blank reactive, control, minimum contamination, and maximum contamination.

2.5. Analysis of chemical contaminants in liver, feed, muscle, and MPs

The limits of quantification (LOQ) and validation results for the compounds analyzed in this study by this method have been added as supplementary data in Table S1.

2.5.1. Reagents and chemicals for the extractions and analysis

Acetonitrile (ACN), methanol (MeOH), acetone (Ac) and formic acid (FA, HCOOH) of analytical grade were obtained from Honeywell (Morristown, NJ, USA) while cyclohexane (CHX) and ethyl acetate (AE) were purchased from Merck (Darmstadt, Germany). AOAC QuEChERS method salts were purchased from Agilent Technologies (Palo Alto, CA, USA).

We prepared working solutions at 1 μg/mL in MeOH, ACN and Ac of BP-3, CPS and DDE-p,p', respectively. PCB 200 and chlorpyrifos-d10 were used as procedural internal standard (P-IS) and were both acquired from Dr. Ehrenstorfer (Augsburg, Germany) at 10 μg/mL in Ac and solid (98,9 % purity), respectively. From them, a working solution was prepared at 1 μg/m in Ac. These solutions were stored in glass amber vials at −20 °C and checked periodically for stability.

2.5.2. Extraction of chemical pollutants in environmental and virgin microplastics

Into an amber glass vial containing 1 ± 0.05 g of pulverized MPs, 10 μL of P-IS solution was added, shaken and left for one hour. Next, 5 mL of a CHX:AE mixture (1:1, *v*/v) was added. The vials were shaken vigorously and subjected to ultrasound for 20 min (Selecta, Barcelona, Spain). The samples were then shaken for 24 h on a rotary shaker (Ovan, Barcelona, Spain). Next, the 5 mL of solvent was transferred to a clean vial and the process was repeated two more times until a final volume of 15 mL of the extraction mixture was reached after 72 h. Finally, 1 mL of this mixture was filtered through a 0.20 μm Chromafil® PET filters (Macherey-Nagel, Düren, Germany) to an amber glass chromatography vial and evaporated in a vacuum concentrator RVC 2–25 CD plus (Christ, Germany) to dryness. The samples were resuspended in 100 μL CHX and analyzed by GC–MS/MS.

2.5.3. Extraction of chemical pollutants in feed

First, 1 ± 0.05 g of feed diet was weighted into a 7 mL plastic hermetic tube, 4 mL of ultrapure water was added along with a few stainless-steel beads and samples were subjected to homogenization for 2 min in a sample preparation equipment (Precellys Evolution, Bertin Technologies, Montigny-le-Bretonneux, France). Then, 1 g of the homogenate was taken into a 5 mL Eppendorf tube and 2 mL of extraction solvent (ACN 1%FA) was added and samples were shaken vigorously for 1 min. Then, they were subjected to ultrasound for 20 min. After that, 480 mg of $MgSO_4$ and 120 mg of CH_3COONa were added to each tube and they were shaken for another minute. Finally, samples were centrifuged for 10 min at 4200 rpm (3175.16 × g) (5804 R, Eppendorf, Hamburg, Germany) and 1 mL of supernatant was filtered into chromatography glass amber vials through a 0.20 μm Chromafil® PET filters.

All samples, calibration curves and blanks were spiked with 10 μL of P-IS solution to the 1 g homogenate and were left in darkness for 1 h prior to extraction.

2.5.4. Extraction of pollutants in liver and muscle

The samples were freeze-dried and sifted to a particle size smaller than 300 μm to have a powder, which allowed to obtain a uniform sample. The freeze-dried of the samples was carried out by a LyoQuest instrument (Telstar, Barcelona, Spain). The extraction of target analytes from the matrix was carried out by triplicate employing ultrasound assisted extraction (UAE). First, 100 g of sample was spiked with 10 μL of P-IS, left for one hour and then extracted with 5 mL of hexane for 5 min. Next, the obtained extract was centrifugated at 4000 rpm for 10 min, evaporated and reconstituted in 2 mL of ACN. Finally, the solution was passed through a 0.20 μm Chromafil® PET filters before to be introduced in the determination system.

2.5.5. Instrumental analyses

The analyses were performed with a gas chromatograph coupled to a mass spectrometer GC System 7890B and Triple Quad 7010 (Agilent Technologies, Palo Alto, CA, USA) using two 15 m columns (Agilent J&WHP-5MS, 0.25 mm inner diameter and 0.25 μm film thickness each) joined together using a purged junction to perform the chromatographic separations. The flow rate of the carrier gas (helium, 99.999 %) was adjusted with the retention time locking function (RTL), using chlorpyrifos methyl (tR = 9.143 min) as a reference. The temperature ramp was programmed as follows: (a) 80 °C—1.8 min; (b) 80 °C to 170 °C at a rate of 40 °C min^{-1}; (c) 170 °C to 310 °C at a rate of 10 °C min^{-1}; (d) 310 °C for 3 min. The total time for each analysis was 20 min and 45 s. For each analysis, 1.5 μL of the extract were injected in splitless mode. A 4 mm ultra-inert liner with glass wool was used. The injector temperature was programmed at 250 °C. Helium backflushing at 5.8 mL/min at a temperature of 315 °C for 5 min was used to clean the injector after each analysis.

The MS/MS analyses were performed in multiple reaction monitoring (MRM). The ionization source (electron impact, 70 eV) was maintained at a temperature of 280 °C. Nitrogen gas of the highest purity available (99.9999 %, Linde, Dublin, Ireland) was used for Q2 fragmentation of the parent ions at a flow rate of 1.5 mL/min. The transfer line temperature was 280 °C. For data acquisition, a delay of 3.7 min was programmed to allow the solvent front to pass. The retention times, transitions, and collision energies of each compound and P-IS can be found in Table S2 of the Supplementary Material.

2.5.6. QC/QA

The quantification was based on peak areas, using matrix-matched calibration for the liver, muscle, and diet samples. Quality Control samples (QCs), blanks and calibration curve were prepared in chicken liver matrix free of the target analytes. A ten-point calibration curve covering the range 100–0.195 ng/g and was prepared by spiking with the appropriate volume of working solutions at 1 μg/mL 1 g of chicken liver homogenate and extracting it using the same procedure as in the samples. Similarly, QCs were prepared at a single concentration of 2.5 ng/g in the same matrix. All samples, QCs, calibration points, and blanks were added 10 μL of P-IS solution at 1 μg/mL and were left to stand for 1 h in dark prior to extraction. For the MPs, calibration curves were prepared in CHX with 1 % olive oil to emulate the matrix load in the samples. Blank reactive were prepared with the same methodology to avoid material and reactives interferences. Similarly, 10 μL of P-IS solution at 1 μg/mL was added to all samples and blanks before each extraction repetition previous to the solvent addition step.

2.6. Statistical analysis

Statistical analysis was performed using the R Version 4.0.5 with RStudio Version 2022.07.2. Normality and homoscedasticity of data were checked by Shapiro–Wilks and Levene's test, respectively. *Kruskal-Wallis Test* was applied to determine if there were significant differences (p-value <0.05), and *Tukey's* post hoc for multiple comparisons when *Kruskal-Wallis Test* indicated significant differences among treatments. Graphics were performed in RStudio using ggplot2 package. For statistical analyses, concentrations below the LOQ but above the LOD were assigned a random value between these two limits.

3. Results

3.1. Pollutants in feed

In control diet (treatments A) the mean concentration of BP-3, CPS and DDE-p,p' were 24.8 ± 9.9 ng/g, 0 ± 0 ng/g and 3.0 ± 0.2 ng/g respectively (Table 2, Fig. 3). In treatment C mean values of BP-3, CPS and DDE-p,p' were 16.8 ± 6.2 ng/g, 0 ± 0 ng/g and 20.8 ± 0.9 ng/g respectively (Table 2, Fig. 3).

Table 2
Mean, SD and median concentration of chemical pollutants (ng/g) in each feed treatment.

Concentration in ng/g feed
Mean, sd and median

pollutant	treatment	mean	sd	median
3-Benzophenone	FEED A	24.8	9.9	26.9
3-Benzophenone	FEED B	5424.4	90.3	5468.1
3-Benzophenone	FEED C	16.8	10.2	11.9
3-Benzophenone	FEED D	156.4	4.8	154.9
3-Benzophenone	FEED E	1064.9	15.2	1062.0
Chlorpyrifos	FEED A	0.0	0.0	0.0
Chlorpyrifos	FEED B	1224.5	12.4	1229.0
Chlorpyrifos	FEED C	0.0	0.0	0.0
Chlorpyrifos	FEED D	42.0	5.5	39.2
Chlorpyrifos	FEED E	204.3	8.3	203.6
DDE	FEED A	3.0	0.2	3.0
DDE	FEED B	12245.0	221.6	12331.7
DDE	FEED C	20.8	0.9	20.8
DDE	FEED D	297.6	10.6	296.4
DDE	FEED E	2630.7	77.9	2590.2

Regarding the diets that were artificially contaminated, for BP-3 in diet B the mean concentration was 5424.4 ± 90.3 ng/g, while in diets D and E the mean concentrations was 156.4 ± 4.8 ng/g and 1064.9 ± 15.2 ng/g respectively. For CPS in diet B the mean concentration was 1224.5 ± 12.4 ng/g, while in diets D and E, the mean concentrations was 42.0 ± 5.5 ng/g and 204.3 ± 8.3 ng/g respectively. Finally mean values for DDE-p-p' were: in diet B 12245.0 ± 221.6 ng/g, while in diets D and E 297.6 ± 10.6 ng/g and 2630.7 ± 77.9 ng/g respectively (Table 2, Fig. 3).

3.2. Pollutants in Dicentrarchus labrax liver and muscle

Trophic transfer of BP-3, CPS and DDE-p,p' to both liver and muscle was confirmed in treatment B (feed + chemical pollutants) (Figs. 4 and 5, Tables 3 and 4).

In the liver, significant differences were found in the accumulation of DDE-p,p' for treatment B at both day 30 and day 60, being higher than in the rest of the treatments ($p < 0.05$), and for treatment E, which was higher than in the control treatment (A) ($p < 0.05$) (Figs. 4 and 5). In the case of BP-3 and CPS the accumulation was significantly higher in the liver in treatment B with respect to the rest of the treatments, both for day 30 and day 60 ($p < 0.05$) (Figs. 4 and 5).

In the muscle, trophic transfer of DDE-p,p' was observed in all treatments, being significantly higher in treatment B than the rest of the treatments, and in treatment E than control treatment (A), both for day 30 and 60 ($p < 0.05$) (Figs. 4 and 5). While for BP-3 and for CPS in muscle significant differences were found in treatment B with all other treatments ($p < 0.05$) (Figs. 4 and 5) at day 30 and day 60 of the experiment.

Biomagnification factors were highest in DDE-p,p' for treatments B and C in both liver and muscle (Tables 3 and 4).

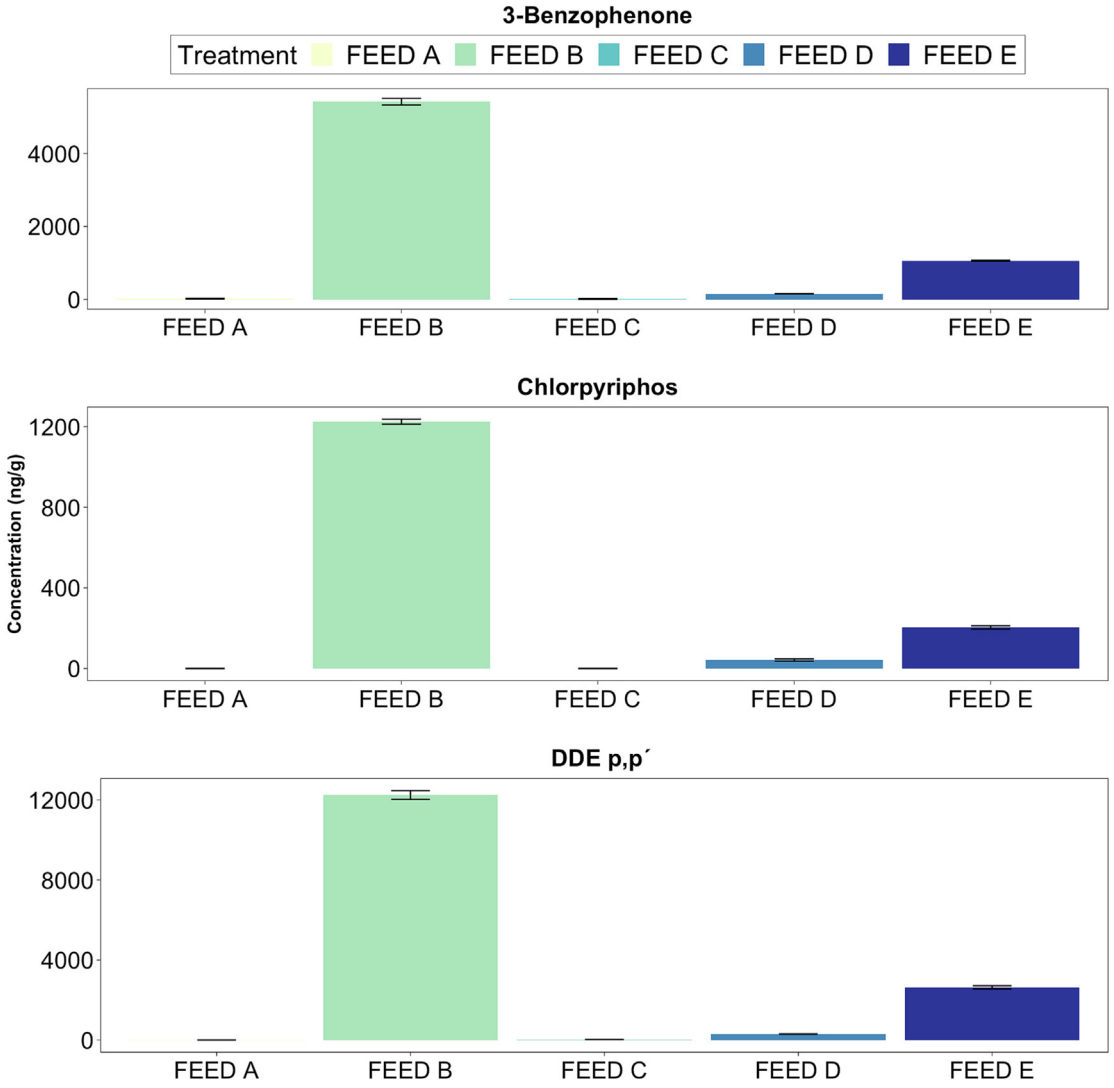

Fig. 3. Concentration of chemical pollutants (ng/g) (mean ± SD) in each treatment: Feed A (Control); Feed B (feed plus BP-3, CPS and DDE-p,p' at maximum concentration); Feed C (feed plus 10 % virgin MPs); Feed D (feed plus 10 % MPs contaminated with BP-3, CPS and DDE-p,p' at minimum concentration); Feed E (feed plus 10 % MPs contaminated with BP-3, CPS and DDE-p,p' at maximum concentration).

3.3. Fish condition indexes

No significant differences were observed between treatments in the Fulton's condition factor (K), nor in the HPS and CEA indexes ($p > 0.05$), both for day 30 and 60 of the experiment (Supplementary Material, Figs. S1, S2 and S3).

4. Discussion

For treatments with MPs (diets C, D and E), no trophic transfer of BP-3 and CPS to muscle or liver was observed. This could be due to the fact that the concentrations of contaminants in the plastics were not very high, added to the fact that the n-octanol-water partition coefficients (K_{OW}) (defined as the ratio of the concentration of a solute in a water-saturated octanolic phase to its concentration in an octanol-saturated aqueous phase) of both BP-3 (Log K_{OW} = 3.79) and CPS (Log K_{OW} = 4.96) (USA National Institutes of Health, 2022) are relatively low.

Other studies on the effect of MPs and CPS on crustaceans and earthworms suggest that microplastics can significantly modulate the effects of co-exposed chemical contaminants (Dolar et al., 2021), and that the presence of MPs significantly reduces CPS bioaccumulation (Ju et al., 2023). A similar effect has been reported for microalgae, in general, the presence of MPs modulates the toxicity of CPF, decreasing the toxic effects, probably due to a lower bioavailability of the pollutants (Garrido et al., 2019; Pinto et al., 2023). However, studies carried out in the marine copepod *Acartia tonsa* show that MPs do act as vectors of CPS, increasing its toxicity (Bellas and Gil, 2020). Therefore, the effect of MPs in the presence of chemical contaminants is not clear; it will probably depend on the organism, the pollutant and their affinity for plastics, the concentration of pollutant and the type of polymer.

Adverse effects due to ingestion of MPs and BP-3 were found in *Dapnia magna* (Na et al., 2021; Song et al., 2021), including transgenerational effects (Song et al., 2022). In addition, studies in the clam *Scrobicularia plana* indicate that ingestion of MPs with adsorbed BP-3 induce higher oxidative stress and damage, and neurotoxic effects, compared to ingestion of MPs without pollutants (O'Donovan et al., 2020).

In the present study, BP-3 in treatment B was present in the liver at day 30, although in very low concentrations, and at day 60 its presence was not detected, which could indicate that *Dicentrarchus labrax* metabolizes this contaminant. It has been observed that other contaminants such as BDE-99 are metabolized in the liver (Granby et al., 2018; Roberts et al., 2011; Yokota et al., 2022). Other studies carried out on wild marine organisms in Gran Canaria, also detected very low values of BP-3 in relation to other UV filters, due to its lowest octanol–water coefficient (Log Kow) value

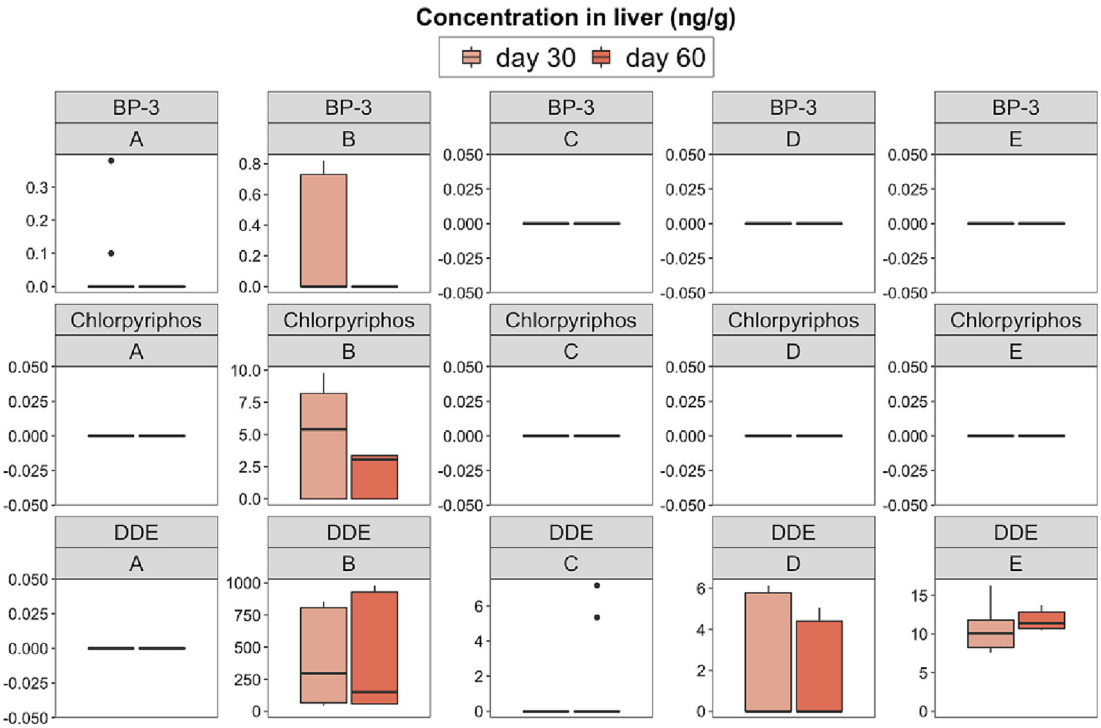

Fig. 4. Concentration of chemical pollutants (ng/g) in *Dicentrarchus labrax* liver after 30 and 60 days feeding each treatment.

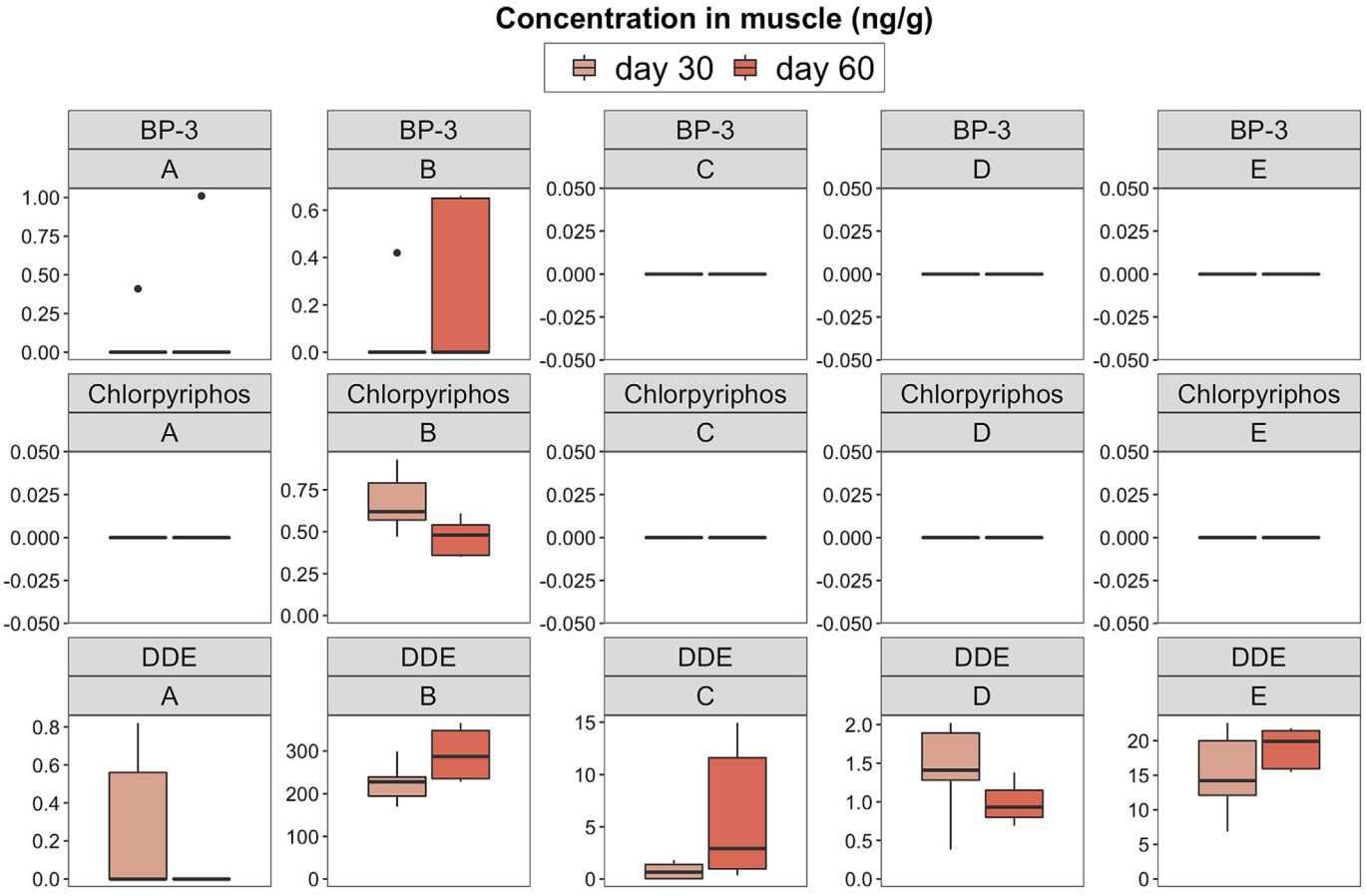

Fig. 5. Concentration of chemical pollutants (ng/g) in *Dicentrarchus labrax* muscle after 30 and 60 days feeding each treatment.

Table 3
Concentration of chemical pollutants in ng/g (mean, standard deviation (sd), and median) and biomagnification factor (BMF) in *Dicentrarchus labrax* liver after 30 and 60 days feeding each treatment: A-control, B, C, D and E. NaN (Not a Number): It has not been possible to calculate, because the values in the feed are zero.

Concentration in liver ng/g
Mean, sd and median

pollutant	treatment	day	mean	sd	median	BMF
BP-3	A	day 30	0.05	0.13	0.00	0.00
BP-3	A	day 60	0.00	0.00	0.00	0.00
BP-3	B	day 30	0.25	0.38	0.00	0.00
BP-3	B	day 60	0.00	0.00	0.00	0.00
BP-3	C	day 30	0.00	0.00	0.00	0.00
BP-3	C	day 60	0.00	0.00	0.00	0.00
BP-3	D	day 30	0.00	0.00	0.00	0.00
BP-3	D	day 60	0.00	0.00	0.00	0.00
BP-3	E	day 30	0.00	0.00	0.00	0.00
BP-3	E	day 60	0.00	0.00	0.00	0.00
Chlorpyriphos	A	day 30	0.00	0.00	0.00	NaN
Chlorpyriphos	A	day 60	0.00	0.00	0.00	NaN
Chlorpyriphos	B	day 30	4.79	3.94	5.40	0.00
Chlorpyriphos	B	day 60	2.15	1.62	3.06	0.00
Chlorpyriphos	C	day 30	0.00	0.00	0.00	NaN
Chlorpyriphos	C	day 60	0.00	0.00	0.00	NaN
Chlorpyriphos	D	day 30	0.00	0.00	0.00	0.00
Chlorpyriphos	D	day 60	0.00	0.00	0.00	0.00
Chlorpyriphos	E	day 30	0.00	0.00	0.00	0.00
Chlorpyriphos	E	day 60	0.00	0.00	0.00	0.00
DDE	A	day 30	0.00	0.00	0.00	0.00
DDE	A	day 60	0.00	0.00	0.00	0.00
DDE	B	day 30	394.91	346.77	296.31	0.03
DDE	B	day 60	389.52	426.72	151.13	0.03
DDE	C	day 30	0.00	0.00	0.00	0.00
DDE	C	day 60	1.39	2.79	0.00	0.07
DDE	D	day 30	1.99	2.99	0.00	0.01
DDE	D	day 60	1.60	2.40	0.00	0.01
DDE	E	day 30	10.75	2.88	10.05	0.00
DDE	E	day 60	11.71	1.24	11.38	0.00

Table 4
Concentration of chemical pollutants in ng/g (mean, standard deviation (sd), and median) and biomagnification factor (BMF) in *Dicentrarchus labrax* muscle after 30 and 60 days feeding each treatment: A-control, B, C, D and E. NaN (Not a Number): It has not been possible to calculate, because the values in the feed are zero.

Concentration in muscle ng/g
Mean, sd and median

pollutant	treatment	day	mean	sd	median	BMF
BP-3	A	day 30	0.05	0.14	0.00	0.00
BP-3	A	day 60	0.17	0.41	0.00	0.01
BP-3	B	day 30	0.05	0.14	0.00	0.00
BP-3	B	day 60	0.22	0.33	0.00	0.00
BP-3	C	day 30	0.00	0.00	0.00	0.00
BP-3	C	day 60	0.00	0.00	0.00	0.00
BP-3	D	day 30	0.00	0.00	0.00	0.00
BP-3	D	day 60	0.00	0.00	0.00	0.00
BP-3	E	day 30	0.00	0.00	0.00	0.00
BP-3	E	day 60	0.00	0.00	0.00	0.00
Chlorpyriphos	A	day 30	0.00	0.00	0.00	NaN
Chlorpyriphos	A	day 60	0.00	0.00	0.00	NaN
Chlorpyriphos	B	day 30	0.68	0.16	0.62	0.00
Chlorpyriphos	B	day 60	0.48	0.10	0.48	0.00
Chlorpyriphos	C	day 30	0.00	0.00	0.00	NaN
Chlorpyriphos	C	day 60	0.00	0.00	0.00	NaN
Chlorpyriphos	D	day 30	0.00	0.00	0.00	0.00
Chlorpyriphos	D	day 60	0.00	0.00	0.00	0.00
Chlorpyriphos	E	day 30	0.00	0.00	0.00	0.00
Chlorpyriphos	E	day 60	0.00	0.00	0.00	0.00
DDE	A	day 30	0.23	0.35	0.00	0.08
DDE	A	day 60	0.00	0.00	0.00	0.00
DDE	B	day 30	222.50	39.30	227.82	0.02
DDE	B	day 60	291.53	53.32	287.34	0.02
DDE	C	day 30	0.77	0.82	0.66	0.04
DDE	C	day 60	5.63	5.93	2.92	0.27
DDE	D	day 30	1.36	0.60	1.41	0.00
DDE	D	day 60	1.00	0.26	0.93	0.00
DDE	E	day 30	15.35	5.27	14.21	0.01
DDE	E	day 60	19.05	2.62	19.92	0.01

and the greatest water solubility among the target compounds studied (Cadena-Aizaga et al., 2022).

In our study, no significant differences were observed in any of the fish condition factors analyzed (Fulton condition factor (K), HSI index and CEA index), indicating that at the bioaccumulation levels reached there is no effect on fish health. Similar results were obtained in HSI and condition factor (K) by Rios-Fuster et al. (2021a). However, other studies have shown that ingestion of microplastics and chemical pollutants produces oxidative stress in European seabass (Barboza et al., 2018a, 2018b) and *Sparus aurata* (Rios-Fuster et al., 2021b; Solomando et al., 2020).

In the case of DDE-p,p', transfer to both liver and muscle was observed in the fish subjected to treatments with MPs (diets C, D and E). In the case of DDE-p,p' the concentrations in food were high (although in the range of the values obtained in the environment) and bioaccumulation in muscle tissue was observed. Other studies have demonstrated trophic transfer of contaminants to tissue by ingestion of MPs contaminated with environmentally relevant values (Herrera et al., 2022; Rochman et al., 2013; van der Hal et al., 2020).

DDE-p,p' n-octanol-water partition coefficients (Log K_{OW} = 6.51) (USA National Institutes of Health, 2022) is higher than BP-3 and CPS, and therefore presents higher biomagnification factors. The octanol-water partition coefficient, K_{OW}, can be a good indicator of adsorption capacity for some types of polymers and organic pollutants; however, the hydrophobicity of the pollutants alone may not necessarily indicate adsorption affinity. Other factors such as polymer type, MPs size, surface area, porosity, water type, and adsorption isotherm parameters of the respective MPs influence their organic pollutants adsorption and their predictability with K_{OW} (Costigan et al., 2022).

The fact that the biomagnification factors were highest in the DDE-p,p' for treatment C (food + virgin plastic) (Table 4), both in liver and muscle, could mean that when the food does not have high levels of contaminants (Fig. 3), the presence of plastics without contaminants could increase the transfer of contaminants to the tissues, and therefore increase biomagnification. One possible hypothesis is that in such cases plastics adsorb chemical contaminants from feed and concentrate them, making them more easily leached.

The mechanism through which contaminants can be transferred from MPs to tissues and bioaccumulate is highly complex and has been extensively discussed by several authors (Bakir et al., 2016; Koelmans, 2015; Koelmans et al., 2022; Mohamed Nor and Koelmans, 2019). Other studies in juvenile gilthead seabream (*Sparus aurata*) show that DDE-p,p' levels decreased during the second month of the experiment (T60) in the MPs treatments, the authors attribute this to the fact that the levels in the tissues became higher than in the diets, and therefore, adsorption of contaminants to the MPs occurred (Rios-Fuster et al., 2021a). The authors conclude that MPs could, therefore, modulate the accumulation of chemical pollutants in tissues and prevent unlimited bioaccumulation (Rios-Fuster et al., 2021a). Studying the bioaccumulation of contaminants associated with MPs in fish is complex, as it depends on many factors, not only adsorption/desorption from MPs, but there are also movements of lipid reserves that can transport pollutants to other tissues, and there are also different processes that allow the metabolization of contaminants. In the study by Herrera et al. (2022) feeding European seabass with food and 10 % environmental microplastics, it was observed that seabass metabolized BDE-99 to BDE-47 by a reductive debromination (Granby et al., 2018; Herrera et al., 2022). All these factors could mask bioaccumulation and biomagnification of chemical contaminants in tissues, therefore long-term studies analyzing chemical contaminants and their metabolic products are recommended.

The highest biomagnification factor was for DDE-p,p' in treatment C, with values of 0.27, which although they do not reach risk values (<1) (Arnot and Gobas, 2006; Gobas et al., 2009) could be considered high, since the organisms were only exposed to this treatment for 60 days. However, the results obtained by Gouin et al. (2011) showed that chemicals with log K_{OW} > 5 have the potential to partition >1 % to polyethylene and from food web models estimated that the tissue concentration of non-polar organic chemicals is reduced in substances with log K_{OW} between 5.5 and 6.5. Therefore, MPs have relative importance as a vector of chemical pollutants to organisms compared to other exposure routes (Gouin et al., 2011).

Further studies are needed to determine the trophic transfer of contaminants to fish tissues in the presence and absence of microplastics (Fauser et al., 2022). There are many factors involved in bioaccumulation, the n-octanol-water partition coefficients of each pollutant, the digestive fluids of the organism, and the capacity of the organisms to metabolize each pollutant. According to Koelmans (2015), the ingestion of plastic can clean or contaminate, depending on the gradient of chemical fugacity between the ingested plastic and the tissue of the organism. Many studies are based on model simulation (Bakir et al., 2016; Bakir et al., 2014; Diepens and Koelmans, 2018; Gouin et al., 2011; Koelmans et al., 2016; Mohamed Nor and Koelmans, 2019), and more laboratory studies are needed to test trophic transfer and bioaccumulation and biomagnification in different possible scenarios (Carbery et al., 2018; Hermsen et al., 2018; Koelmans et al., 2022; Miller et al., 2020).

5. Conclusions

Trophic transfer of contaminants to tissues occurred both in the absence and presence of microplastics when contaminant levels are high, as is the case for DDE-p,p'. When contamination levels in the food are low, e.g. DDE-p,p' in treatment C, the presence of microplastics seems to favor the trophic transfer of contaminants, resulting in a higher biomagnification factor than in the rest of the treatments. However, with low concentrations of BP-3 and CPS, no trophic transfer to tissues was observed in the treatments with MPs.

On the other hand, no significant differences were observed between treatments in the fish condition indices analyzed, this seems to indicate that, at least in 60 days of treatment, there is no effect on the fish health.

Given the worrying increase in the amount of microplastics present in the ocean, and the amount and diversity of contaminants they accumulate, more studies are needed at "realistic" concentrations to determine the risk to the health of marine organisms, and also to humans through the consumption of fish and seafood. The processes of bioaccumulation and biomagnification are complex, since many factors are involved, not only the adsorption/desorption capacity of the MPs, the type of polymer, the degradation processes that the MPs have experienced, it also depends on the exposure time of the organisms to the contaminated MPs, the retention time in the digestive system, the digestive processes and fluids, the movement of fats in the tissues, the ability of the species to metabolize chemical pollutants, etc., which makes it so difficult to obtain conclusive results in this regard.

Therefore, it is necessary to carry out long-term experiments in different species, with concentrations of MPs and chemical pollutants similar to those in the environment and in conditions as realistic as possible, as well as the use of biomarkers, in order to understand how this problem may be affecting marine organisms and how it will affect them in the future.

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CRediT authorship contribution statement

Alicia Herrera: conceptualization, experimental investigation, data curation and writing. **Andrea Acosta-Dacal**, **Octavio Pérez-Luzardo** and **Sarah Montesdeoca-Esponda**: analysis of chemical pollutants, experimental investigation, methodology. **Jorge Rapp** and **Stefanie Reinold**: experimental investigation, methodology. **Ico Martínez**, **Daniel Montero** and **May Gómez**: conceptualization, experimental investigation. All authors contributed to the acquisition of the data, review the manuscript, and approved the final version.

Data availability

Data will be made available on request.

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.

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