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Microplastics cause neurotoxicity, oxidative damage and energy-related changes and interact with the bioaccumulation of mercury in the European seabass, *Dicentrarchus labrax* (Linnaeus, 1758)

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ABSTRACT

Microplastics pollution is a global paradigm that raises concern in relation to environmental and human health. This study investigated toxic effects of microplastics and mercury in the European seabass (*Dicentrarchus labrax)*, a marine fish widely used as food for humans. A short-term (96 h) laboratory bioassay was done by exposing juvenile fish to microplastics (0.26 and 0.69 mg/L), mercury (0.010 and 0.016 mg/L) and binary mixtures of the two substances using the same concentrations, through test media. Microplastics alone and mercury alone caused neurotoxicity through acetylcholinesterase (AChE) inhibition, increased lipid oxidation (LPO) in brain and muscle, and changed the activities of the energy-related enzymes lactate dehydrogenase (LDH) and isocitrate dehydrogenase (IDH). All the mixtures caused significant inhibition of brain AChE activity (64–76%), and significant increase of LPO levels in brain (2.9–3.4 fold) and muscle (2.2–2.9 fold) but not in a concentration-dependent manner; mixtures containing low and high concentrations of microplastics caused different effects on IDH and LDH activity. Mercury was found to accumulate in the brain and muscle, with bioaccumulation factors of 4–7 and 25–40, respectively. Moreover, in the analysis of mercury concentrations in both tissues, a significant interaction between mercury and microplastics was found. The decay of mercury in the water increased with microplastics concentration, and was higher in the presence of fish than in their absence. Overall, these results indicate that: microplastics influence the bioaccumulation of mercury by *D. labrax* juveniles; microplastics, mercury and their mixtures (ppb range concentrations) cause neurotoxicity, oxidative stress and damage, and changes in the activities of energy-related enzymes in juveniles of this species; mixtures with the lowest and highest concentrations of their components induced different effects on some biomarkers. These findings and other published in the literature raise concern regarding high level predators and humans consuming fish being exposed to microplastics and heavy metals, and highlight the need of more research on the topic.

1. Introduction

The presence of microplastics in the marine environment due to primary and secondary sources (e.g. pre-production pellets, synthetic textiles, cosmetics, fragmentation of plastic debris) has been reported worldwide (Barboza and Gimenez, 2015; Cózar et al., 2014; van Sebille et al., 2015). Since these particles have been found to induce adverse effects in a considerable variety of organisms (e.g. Avio et al., 2015; Ferreira et al., 2016; Gall and Thompson, 2015; Ribeiro et al., 2017), concerns regarding environmental, animal and human health exist (Thompson, 2015). Thus, regulations to monitor and investigate the problem to minimize its impacts have been implemented (e.g. European

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Marine Strategy Framework Directive).

Microplastics present in the environment can be ingested by different types of organisms (Besseling et al., 2013; Fossi et al., 2012; Frias et al., 2014; Goldstein and Goodwin, 2013; Güven et al., 2017; Romeo et al., 2015; Rummel et al., 2016; de Sá et al., 2015) including species widely used in the human diet (Battaglia et al., 2016; Neves et al., 2015; Rochman et al., 2015; Silva-Cavalcanti et al., 2017). Microplastics can induce toxic effects *per se* (Ferreira et al., 2016; Oliveira et al., 2013). They may also contain very hazardous chemicals that are introduced in organisms when microplastics are taken up potentially leading to increased accumulation of these substances in food webs (Batel et al., 2016; Setälä et al., 2014; Teuten et al., 2009). Thus, special concerns regarding top predators exist, especially because some of them are consumed by humans. In fish, microplastics have been found to cause several adverse effects, including decreased predatory performance, endocrine disruption, hepatic stress, intestinal alterations, oxidative stress, among others (de Sá et al., 2015; Ferreira et al., 2016; Oliveira et al., 2013; Pedà et al., 2016; Rochman et al., 2013).

A complex problem associated to microplastics is their capability to sorb and interact in other ways with other common environmental contaminants, such as metals (Ashton et al., 2010; Holmes et al., 2012; Rochman et al., 2014a,2014b), pharmaceuticals (Wu et al., 2016), and other contaminants (Rochman et al., 2013; Tosetto et al., 2016; Wang et al., 2015). Therefore, microplastics can influence the fate of these substances in the environment and in organisms, as well as their toxicity. For example, microplastics have been found to influence the localization, biotransformation and/or toxicity of polycyclic aromatic hydrocarbons (PAHs) and polybrominated diphenyl ethers (PBDEs) in fish (Oliveira et al., 2013; Rochman et al., 2013) and in other organisms (Avio et al., 2015; Chua et al., 2014; Paul-Pont et al., 2016), of pharmaceuticals and personal care products in fish (Fonte et al., 2016; Wardrop et al., 2016), and of metals in fish (Khan et al., 2015; Luís et al., 2015). However, more knowledge on such interactions in needed to assess the risks and increase the safety in the use and management of microplastics and other common environmental contaminants.

Estuaries and other coastal areas of industrial and urbanized impacted regions are considered microplastics hotspots (Gallagher et al., 2016; Isobe et al., 2015; Peters and Bratton, 2016). Such ecosystems are also contaminated with a high number of other chemicals, including several ubiquitous pollutants. Among these, mercury raises special concern mainly because is very toxic at low concentrations and its organic forms, methylmercury in particular, are biomagnified in trophic webs, increasing the risk of exposure and toxic effects on top predators and humans consuming them (Atchison et al., 1987; Branco et al., 2004; Carvalho et al., 2008; Selin, 2009). In addition, because of its high degree of toxicity, mercury is among the priority pollutants considered under the United Nations Environment Programme (UNEP), United States Environmental Protection Agency (US EPA) and European Commission (EU).

To the best of our knowledge, the toxic effects resulting from the simultaneous exposure to microplastics and mercury through the water were not investigated before in fish. Thus, the goals of the present study were to investigate the short-term toxic effects of microplastics and mercury exposures, individually and in binary mixtures, on juveniles of the European seabass *Dicentrarchus labrax* (Linnaeus, 1758). *D. labrax* was selected as model species for this study mainly because it is a key species in several European estuaries and in other marine ecosystems, is used as food for humans being a very appreciated marine fish and therefore having a high commercial value, and recent studies have investigated the effects of microplastics in this species (e.g survival, growth and intestinal alterations) (Mazurais et al., 2015; Pedà et al., 2016).

2. Material and methods

2.1. Chemicals

Fluorescence red polymer microspheres, 1–5 μm diameter (lot number: 4-0906-0661), purchased from Cospheric − Innovations in Microtechnology (USA), were used as microplastics model. According to the manufacturer, the particles are spherical, red opaque, 1.3 g/cc density, and can be detected by spectrofluorimetry (excitation wavelength of 575 nm and emission wavelength of 607 nm). Mercury chloride (≥ 99.5% pure, lot number: 031M0173 V) was purchased from Sigma-Aldrich (USA). The other chemicals used were all of the highest purity available and purchased from Sigma-Aldrich (USA) or Merck (Germany). The Bradford reagent used for protein determinations was from BIORAD (Germany).

2.2. Ethical issues

Experiments were conducted in accordance with ethical principles and other requirements of the Portuguese and European regulations for the protection of animals used for scientific purposes, including authorization of the Portuguese National Authority: “Direção Geral de Alimentação e Veterinária” (DGAV): 0421/000/000/2017, 014227, 31st May 2017. L. Guilhermino, L. R. Vieira and F. Carvalho are accredited by the DGAV as investigator/coordinator (equivalent to FELASA category C) to carry animal experimentation. The experiments were carried out in the CIIMAR bioterium that is accredited by DGAV for studies with aquatic animals.

2.3. Fish maintenance and acclimatization

Seabass juveniles were purchased from a saltwater fish aquaculture (Vigo, Spain) and acclimatized to laboratory conditions for 4 months. During this period, they were maintained in 2000 L tanks with aerated, biologically and UV-filtered seawater (salinity: 34 ± 1 g L^{-1}), hereafter indicated as water. Partial water renewal was made every week and water abiotic parameters (temperature, conductivity, salinity, dissolved oxygen, pH, ammonia, nitrates, and nitrites) were periodically monitored. During this period, fish were fed with commercial fish food (Tetramin®). Fifteen days before the bioassay, fish were put in a room with control of temperature (19 ± 1 °C) and photoperiod (14 h light: 10 h dark), with water temperature maintained at 18 °C ± 1 °C. Here, they were maintained in 5 L glass beakers (with 4 L of water), 1 animal per beaker, with continuous air supply. The water was changed every other day, the water parameters above mentioned were determined every day. Fish were fed *ad libitum* with commercial fish food (Tetramin®) and observed two times per day. No mortality was observed during the acclimatization period. Forty-eight hours before the start of the bioassay, fish were transferred to beakers with clean water and feeding was stopped.

2.4. Preliminary assay without fish

Prior to the bioassay, a preliminary assay without fish was carried out to investigate the behavior of mercury and microplastics in the water. Briefly, the assay was carried out for 96 h; photoperiod, water temperature and salinity were as indicated in Section 2.3. Treatments were: 1 control (water only), 1 treatment containing a low microplastics concentration (MPs-L: 0.25 mg/L); 1 treatment containing a high microplastics concentration (MPs-H: 0.69 mg/L); 1 treatment containing a low mercury concentration (Hg-L: 0.009 mg/L); 1 treatment containing a high mercury concentration (Hg-H: 0.016 mg/L); and 4 binary mixtures containing microplastics and mercury simultaneously (MPs-L + Hg-L; MPs-L + Hg-H; MPs-H + Hg-L; MPs-H + Hg-H). The concentrations of microplastics and mercury above indicated are the mean of mid-point actual concentrations in treatments containing the lowest