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Evaluation of the impact of polyethylene microbeads ingestion in European sea bass (*Dicentrarchus labrax*) larvae

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ABSTRACT

Microplastics are present in marine habitats worldwide and may be ingested by low trophic organisms such as fish larvae, with uncertain physiological consequences. The present study aims at assessing the impact of polyethylene (PE 10–45 μM) microbeads ingestion in European sea bass (*Dicentrarchus labrax*) larvae. Fish were fed an inert diet including 0, 10^4 and 10^5 fluorescent microbeads per gram from 7 until 43 days post-hatching (dph). Microbeads were detected in the gastrointestinal tract in all fish fed diet incorporating PE. Our data revealed an efficient elimination of PE beads from the gut since no fluorescent was observed in the larvae after 48 h depuration. While the mortality rate increased significantly with the amount of microbeads scored per larvae at 14 and 20 dph, only ingestion of the highest concentration slightly impacted mortality rates. Larval growth and inflammatory response through Interleukine-1-beta (*IL-1β*) gene expression were not found to be affected while cytochrome-P450-1A1 (*cyp1a1*) expression level was significantly positively correlated with the number of microbeads scored per larva at 20 dph. Overall, these results suggest that ingestion of PE microbeads had limited impact on sea bass larvae possibly due to their high potential of egestion.

1. Introduction

Microplastics, tiny plastic fragments with diameters of <5 mm, are widespread and ubiquitous within the marine environment (Lusher, 2015; Thompson, 2015). It is suggested that they are now the most abundant form of solid-waste pollution on Earth (Derraik, 2002; Galgani et al., 2015). Microplastics are originated from the industry, from the domestic use of a wide panel of personal care products which contain microparticles (e.g facial cleansers and toothpaste) (Ghelardini et al., 1996; Zitko and Hanlon, 1991) and from the wastewater of washing machines (Browne, 2015). Waste microplastics also result from the breakdown of larger plastic debris (Andrady, 2011). Polyethylene (PE), polypropylene (PP), polyvinyl chloride (PVC), polystyrene (PS) and polyethylene terephthalate (PET) are among the most widely used polymers in the industry and thereby ultimately found in the ocean (Andrady and Neal, 2009). Presence of microplastics is documented in several species at the base of the food chain such as plankton or filter and deposit feeders because of their microscopic size and their ubiquitous presence (Avio et al., 2015a; Cole et al., 2013; Collignon et al., 2012; Frias et al., 2014; Thompson et al., 2004; Van Cauwenberghe and Janssen, 2014). The plankton, which is a source of food for other animals, could pass microplastics up the food web to top predator species (fish, birds, marine and terrestrial mammals) (Ivar do Sul and Costa, 2014; Wright et al., 2013a).

Several species of fish have been recorded to ingest plastic debris including microplastics (Boerger et al., 2010; Carpenter et al., 1972; Foekema et al., 2013; Lusher et al., 2013). Recent papers reported that microplastics ingestion appears to be common across a range of fish species (pelagic and demersal) from the English Channel (Lusher et al., 2013; Foekema et al., 2013) and Mediterranean sea (Avio et al., 2015b; Deudero and Alomar, 2015; Romeo et al., 2015). It is also documented that all ontogenic phases including early life stages of fish can be concerned by plastic debris

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ingestion (Carpenter et al., 1972; Hoss and Settle, 1990; Possatto et al., 2011). Carpenter et al. (1972), working on fish larvae, reported that of 14 sampled species, 8 contained plastic in their guts. Kartar et al. (1973) found as many as 30 PS particles in the stomachs of flounder, *Platichthys flesus*, sampled in the Severn Estuary in the United Kingdom. Since fish larval ontogenesis is particularly sensitive to environmental stressors (Houde, 1997), ingestion of plastic by larvae could compromise their survival and may have detrimental consequences on recruitment into the adult population.

Until now, however, there is little information available relative to the biological impacts of microplastic ingestion on fish larval stages. In addition to the chemical effects attributed to organic pollutants that can be adsorbed on the plastic debris, some specific effects of plastic ingestion on marine organisms have been described in the literature. Ingestion of microplastics has been shown to impair feeding, leading to reductions in ingested carbon biomass and energy depletion which result in decreased hatching success in zooplankton (Cole et al., 2015; Lee et al., 2013). It is also suggested that depending on the size of the debris, plastic particles may be retained in the intestine, induce internal injury and clog the digestive system in various marine species including fish (Carpenter et al., 1972; Derraik, 2002). More recently, studies in mussels (*Mytilus edulis*) indicated that ingested microplastics can also pass through the gut and translocate to the circulatory system (Browne et al., 2008). Their potential presence in tissues allows a glimpse of the effects on essential physiological functions other than the digestive one. In mussels, ingestion of non-contaminated microplastics has been shown to induce immunological effects and inflammatory response (Avio et al., 2015a; Von Moos et al., 2012; Wright et al., 2013b). Concerning fish species, studies from Oliveira et al. (2013) suggested adverse effects of virgin microplastics in neurofunction of the common goby *Pomatoschistus microps*. Rochman et al. (2013, 2014) demonstrated early signs of endocrine disruption as well as hepatic stress in adult medaka *Oryzias latipes* after ingestion of virgin polyethylene. In contrast, recent work performed on larvae of invertebrates (sea urchin, *Tripneustes gratilla*) indicated very limited biological impact of microplastics ingestion suggesting that effect of plastic ingestion could be species and/or stage specific and can depend on the nature of the ingested plastic (Kaposi et al., 2014).

In the present study, we investigated the impact of *per os* administration of PE microbeads on European sea bass (*Dicentrarchus labrax*) larvae. Sea bass, with most marine fish species, exhibits an extended pelagic planktotrophic larval period and thereby potentially encounters and ingests microplastic particles during its development. Using an inert diet incorporating PE microbeads, the main objectives of our study were to assess (i) the effective retention of microplastics in the gut of sea bass larvae and (ii) the potential impact of microplastic ingestion on their survival, growth and some physiological parameters. The inflammatory response, suggested to be impacted in other species (Von Moos et al., 2012; Wright et al., 2013b), was addressed through investigation of a proxy of this pathway, the Interleukin-1 beta (*IL-1β*) gene expression (Ogryzko et al., 2014). Potential chemotoxic effect of fluorescent PE microbeads possibly due to hazardous decomposition by-products induced in digestive tract was tackled through the analysis of the expression of cytochrome-P450-1A1 (*cyp1a1*) involved in the biotransformation of toxicants.

2. Materials and methods

2.1. Animals and experimental diets

European sea bass larvae were provided by the marine farm Aquastream (Ploemeur, France) and reared from 2 days after hatching (dph) to 45 dph at IFREMER, Centre de Brest (France). Larvae were distributed into 18 conical fiberglass tanks (35 L) at 3 dph, with initial stocking density of 60 larvae·L^{-1} and were reared according to Darias et al. (2010) until 45 dph. Briefly, the tanks were supplied with running seawater at 20 °C, which had been filtered through a sand filter and then passed successively through a tungsten heater and degassing column packed with plastic rings. To prevent any dumping of PE microbeads to waste water and subsequently at sea, outflow of seawater was filtered on a 1 μm filter renewed every week and then burned by a waste management company.

From 7 to 43 dph, larvae were fed on microparticulate diets (WO 0064273) prepared in our laboratory as described by Cahu et al. (2003), 6 replicate tanks each, including 0 (control, C), 10^4 (i.e. 1.2 mg; 1X) or 10^5 (i.e. 12 mg; 10X) fluorescent microbeads of polyethylene (#UVPMS-BR, mix of 10–45 μm, 1.050 g/cc, Cospheric, Santa Barbara, CA, USA) per gram of diet. The dietary ingredients, including microbeads, were mixed with water, pelletized, and dried at 50 °C for 60 min. The pellets were sieved to obtain particles with size lower than 400 μm. The concentration of microbeads in the three diets was confirmed by counting fluorescent beads under microscope. The larvae were fed in excess with belt feeders 16 h per day (10:00 AM–02:00 AM). The fluorescent PE microbead concentrations incorporated in the feed were used to correspond to high environmentally relevant concentration of microplastics that larvae could ingest in the wild environment (see discussion part).

Non-ingested food and faeces were collected using a filter to avoid dissemination of microbeads in effluent. From 43 to 45 dph, all groups were fed control diet.

Experiments were conducted within IFREMER facilities having authorization for animal experimentation. Present work was performed in accordance with French and European policies and guidelines of the IFREMER institute (Agreement number: 01964.01).

2.2. Monitoring of microbeads ingestion and retention

The presence of fluorescent microbeads in European sea bass larvae was followed by microscopic analysis. At 14, 20, 34 (exposure phase) and 45 (depuration phase) dph, 20 larvae per tank (120 per group) were randomly sampled, fixed in ethanol-formalin-acetic acid (ethanol 95% 6 V; formaldehyde 40% 3 V, glacial acetic acid, 1 V) for 48 h then immersed in ethanol (100%) for microscope examination. The number of larvae containing microbeads was scored and the number of beads detected per larvae was counted.

2.3. Survival and growth

Survival in each experimental group (mean of the 6 replicates) was assessed by scoring the number of alive larvae at the end of the experiment (45 dph) and by considering the initial number of larvae as well as amount of larvae sampled in each tank for analysis. 50–60 larvae randomly sampled at 20, 27, 34 and 43 dph in each of the 6 replicated tanks were weighed to determine larval growth for each experimental group.

2.4. RNA extraction, cDNA synthesis and real time PCR

Total RNA was extracted from pools of whole larvae (1 pool per tank) at 27 and 43 dph with Extract-All (Eurobio, France), following manufacturer's instructions. Thirty larvae were sampled per pool at 27 dph to get around 100 mg of fresh tissue while 7 to 10 larvae were necessary at 43 dph. Potential DNA contaminants were removed from extracted RNA by using RTS DNase ™ kit (Mo bio laboratories, Carlsbad, USA) following manufacturer's instructions.