Marine Pollution Bulletin 130 (2018) 123–131

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

Virgin microplastics are not causing imminent harm to fish after dietary exposure

Boris Jovanović[a,*], Kerem Gökdağ[b], Olgaç Güven[b], Yilmaz Emre[c], Elizabeth M. Whitley[d], Ahmet Erkan Kideys[b]

[a] Department of Natural Resource Ecology and Management, Iowa State University, Ames, IA, USA
[b] Institute of Marine Sciences, Middle East Technical University, Erdemli, Mersin, Turkey
[c] Faculty of Science, Akdeniz University, Antalya, Turkey
[d] Pathogenesis, LLC, Gainesville, FL, USA

ARTICLE INFO

Keywords:
Microplastics
Marine litter
Fish
Histopathology
Diet
Toxicity

ABSTRACT

Among aquatic organisms, fish are particularly susceptible to ingesting microplastic particles due to their attractive coloration, buoyancy, and resemblance to food. However, in previous experimental setups, fish were usually exposed to unrealistically high concentrations of microplastics, or the microplastics were deliberately contaminated with persistent organic chemicals; also, in many experiments, the fish were exposed only during the larval stages. The present study investigated the effects of virgin microplastics in gilt-head seabream (*Sparus aurata*) after 45 days' exposure at 0.1 g kg^{-1} bodyweight day^{-1} to 6 common types of microplastics. The overall growth, biochemical analyses of the blood, histopathology, and the potential of the microplastics to accumulate in gastrointestinal organs or translocate to the liver and muscles were monitored and recorded. The results revealed that ingestion of virgin microplastics does not cause imminent harm to the adult gilt-head seabream during 45 days of exposure and an additional 30 days of depuration. The retention of virgin microplastics in the gastrointestinal tract was fairly low, indicating effective elimination of microplastics from the body of the fish and no significant accumulation after successive meals. Therefore, both the short- and the long-term retention potential of microplastics in the gastrointestinal tract of fish is close to zero. However, some large particles remained trapped in the liver, and 5.3% of all the livers analyzed contained at least one microplastic particle. In conclusion, the dietary exposure of *S. aurata* to 6 common types of virgin microplastics did not induce stress, alter the growth rate, cause pathology, or cause the microplastics to accumulate in the gastrointestinal tract of the fish.

1. Introduction

Every year, between 4.8 and 12.7 million metric tons (MT) of plastic waste enters the ocean (Jambeck et al., 2015). In the last two decades plastic already outweighs plankton in certain parts of the ocean (Moore et al., 2001), and by 2050 it is expected that plastic will surpass fish stocks in the ocean by weight. In 2014, the estimated number of floating plastic particles in the world's oceans was 5.25 trillion (269,000 MT), out of which 4.85 trillion particles were microplastics of < 4.75 mm in size (Eriksen et al., 2014). The difference between the yearly plastic waste discharge into the ocean and the amount of floating plastic estimated by Eriksen and colleagues is perhaps because it has sunk below the surface, washed ashore onto beaches, or been ingested by marine animals. The average concentration of plastic for the whole ocean is estimated to be 2 ng L^{-1} (Koelmans et al., 2016), which may not look so significant. However, microplastics can reach a high concentration in specific areas. For example, the Swedish west coast harbor adjacent to a polyethylene factory has a microplastics concentration of 102,000 particles m^{-3} (Lozano and Mouat, 2009). With most of the microplastics particles weighing < 0.01 g (Morét-Ferguson et al., 2010), or more specifically around 0.02 mg (Gökdağ, 2017), in this extreme case, their concentration would be around 0.02–1 g L^{-1}. Therefore, it is of no surprise that scientific literature on the topic of the potential toxic effects of microplastics on aquatic organisms is steadily growing. Microplastic exposure has been identified as having a negative effect on: growth, development, behavior, reproduction, intestinal blockage, physical damage, and the mortality of aquatic organisms (Chae and An, 2017; Jovanović, 2017). However, in past experimental setups, organisms were usually exposed to microplastic concentrations which are unrealistically high and not environmentally relevant

* Corresponding author at: Iowa State University, Department of Natural Resource Ecology and Management, 107 Science II, 2310 Pammel Drive, Ames, IA 50011, USA.
E-mail address: nanoaquatox@gmail.com (B. Jovanović).

https://doi.org/10.1016/j.marpolbul.2018.03.016
Received 13 December 2017; Received in revised form 8 March 2018; Accepted 9 March 2018

(Phuong et al., 2016). Furthermore, in dietary exposure studies microplastics are often deliberately contaminated with persistent organic chemicals in order to simulate their adsorption to microplastics in the aquatic environment (Batel et al., 2016; Rochman et al., 2013). Therefore, due to a high microplastic concentration, not only have such studies often been associated with great contaminant stress that does not necessarily occur in the natural environment (Phuong et al., 2016), but also the intrinsic toxicity information (if any) of virgin microplastics is lost. At least in the case of hydrophobic organic toxicants associated with microplastics, the ingestion of an environmentally relevant concentration of microplastics is not likely to increase exposure (and thus risk) to marine organisms (Koelmans et al., 2016). Among aquatic organisms, fish are particularly susceptible to the ingestion of microplastic particles due to their attractive coloration, buoyancy, and resemblance to food (Güven et al., 2017; Jovanović, 2017). In summary, although intestinal blockage, physical damage, histopathological alterations in the intestines, changes in behavior, changes in the lipid metabolism, and transfer to the liver are the observed effects of microplastic ingestion by fish, these effects are frequently observed in larval fish or in studies with high concentration of microplastics and/or contaminant laden microplastics (Jovanović, 2017). Therefore, the aim of the present study was to evaluate the effects of virgin microplastics in adult fish, *Sparus aurata*, Linnaeus, 1758, after 45 days of dietary exposure to environmentally relevant concentrations of 6 common types of microplastics. *S. aurata* was used in the present research, as it is one of the well studied model species in aquaculture (Grigorakis, 2007; Koven et al., 2001).

2. Methods

2.1. Microplastics

Six different types of microplastic particles were purchased from Sigma-Aldrich: 1) polyvinyl chloride high molecular weight (PVCHMW) - catalog number 81387; 2) polyamide (PA) - catalog number 02395; 3) ultra-high molecular weight polyethylene (UHMWPE) - catalog number 434272; 4) polystyrene (PS) - catalog number 430102; 5) average molecular weight medium density polyethylene (MDPE) - catalog number 427772; and 6) polyvinyl chloride low molecular weight (PWCLMW) - catalog number 81388. With the exception of PS all other products were used in the form in which they were received. PS microplastic spherical pellets were too big (approximately 2 mm in diameter) compared to the other products and were thus ground using a coffee grinder. In order to estimate the average size of each product, 50–100 particles were placed under a binocular scope and photos were taken. The Lapazz TWMM853 Graphic Tablet with ImageJ software was used to calculate the size of each particle.

2.2. Fish and dietary exposure to microplastics

500 L tanks with a single pass water flow were used to house juvenile gilt-head seabream - *S. aurata*. Each of the 7 tanks had 50 fish to start with, which were acclimated for a week to the new housing environment before the start of the experiments. The *S. aurata* were bred in house at the Mediterranean Fisheries Research Production and Training Institute, Demre-Antalya-Turkey. Before placement in the tanks, each fish was weighed. The total biomass per tank ranged between 375.1 g and 377.4 g. There was no statistical difference in the fish mass between any of the tanks. The mean mass of the fish ± standard deviation (SD) in the 7 tanks was: 7.54 ± 0.32; 7.55 ± 0.31; 7.53 ± 0.31; 7.52 ± 0.31; 7.53 ± 0.32; 7.50 ± 0.30; and 7.50 ± 0.29 g in no particular order.

The 6 treatments and the control group were assigned randomly to the tanks. The treatments were: 1. PVCHMW; 2. PA; 3. UHMWPE; 4. PS; 5. MDPE; 6. PWCLMW; and 7. Control.

Table 1
Semi-quantitative histopathology severity scale score.

Score	Severity	Proportion of affected parenchyma
0	No change	None
1	Minimal change	Very small amount
2	Mild change	Small amount
3	Moderate change	Medium amount
4	Severe change	Large amount
5	Markedly severe	All

It is hard to say what the daily microplastic ingestion load of a fish is in its natural environment, as such studies do not exist (Jovanović, 2017). We assumed that the ingested microplastic content by fish per day would not exceed 0.3% of the total ingested daily feed, even in marine environments with a high microplastic concentration. The microplastics were mixed into the fish feed, and feed pellets were made at a concentration of 3.33 g kg^{-1} of feed. The pellets were 3.0 mm in size and were made with a cold extrusion machine. The pellets were dried in an oven at 40 °C for 24 h and stored in airtight bags until use. The approximate composition of the feed was: crude protein 48.66%, crude lipid 18.54%, crude ash 7.77%, crude cellulose 1.27%, total phosphorous 2.71% and crude starch 8.50%. The fish were fed 3% of their body mass daily and were therefore exposed to the microplastics at approximately 0.1 g per kg^{-1} body mass. A control group of fish was fed with the same feed, only without the addition of microplastics. Since, initially, the fish weighed approximately 7.5 g and the microplastic particles in general were around 75 μm in size, each fish at the start of the experiment could potentially ingest a maximum of 0.75 mg of plastic or around 2800 particles per day. For this approximation, the particles were considered as a perfect sphere and the mass of a single microplastic particle was calculated accordingly as the mass of a sphere ($M = 4/3\pi r^3\rho$, where r is assumed to be 0.0375 mm and ρ is 1.2 mg mm^{-3}). This is, however, only a rough approximation of the potential number of particles. In reality, the fish ingested a smaller number of particles per day as fish do have numerous adaptations for the exclusion of sediment from the buccal cavity and microplastic is likely not an exception. Therefore, in terms of particle concentration, mass, and number we believe that the present exposure scenario is environmentally relevant, and not an exaggeration.

The fish were fed for 45 days, starting June 18, 2015. The water temperature was recorded daily in each tank. There was no difference in the average daily temperature between the tanks and it was typically in the range of 25.7 °C to 25.8 °C. The maximum difference in the water temperature between any of the 2 tanks on the same day was no bigger than 0.2 °C. Every two weeks, 10 random fish from each tank were netted and weighed in order to further adjust the daily amount of feed given (3% of body mass) if necessary.

At the end of the feeding trial 3 random fish from each tank were euthanized, their blood was collected from the puncture of caudal vein using a syringe and collected into micro tubes (0.5 mL). Levels of glucose, AST, ALT, LDH, and GGT were measured in serum of each fish using automated chemical analyzer.

24 h after the last feeding, 15 random fish per tank were euthanized. First, a sample of the caudal muscles was taken, followed by a liver sample. In order to avoid contamination, the gastrointestinal tract was dissected only after the samples of muscles and liver were collected. The stomach, intestines, liver, and muscles samples were placed in 50 mL centrifuge tubes and treated with 30 mL of 4 M KOH for one hour at 60 °C in a water bath. After one hour, the samples were washed with distilled water and filtered through a 10 μm zooplankton mesh. The microplastic particles were counted using an Olympus SZX16 Stereomicroscope (max magnification 30×) equipped with a DP26 - Olympus 5.0 MP High Color Fidelity Microscope Digital Camera. The photos were taken and processed using the Olympus cellSens platform (Image Analysis software) in order to determine the diameter/length of