Chemosphere 213 (2018) 323–332

Contents lists available at ScienceDirect

Chemosphere

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

Effects of virgin microplastics on goldfish (*Carassius auratus*)

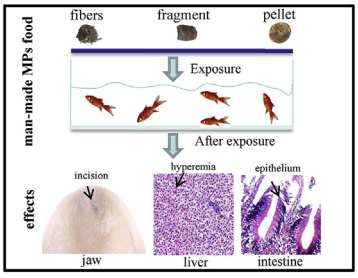
Khalida Jabeen [a], Bowen Li [a], Qiqing Chen [a], Lei Su [a], Chenxi Wu [b], Henner Hollert [c], Huahong Shi [a, d, *]

[a] State Key Laboratory of Estuarine and Coastal Research, East China Normal University, Shanghai, 200062, China
[b] State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, 430072, China
[c] Department of Ecosystem Analysis, Institute for Environmental Research, ABBt – Aachen Biology and Biotechnology, RWTH Aachen University, 1 Worringerweg, 52074, Aachen, Germany
[d] Institute of Eco-Chongming, East China Normal University, Shanghai, 200062, China

HIGHLIGHTS

- Ingestion and effects of MPs in fish are largely dependent on their shape and size.
- Only fiber-based food was ingested but fragment and pellet-based were expelled.
- Fibers were found in the gills, gastrointestinal tract, and feces.
- Pronounced and severe damages were found in the liver of the fiber-based group.
- Severe damage in the jaws was observed in fragment-based group due to chewing.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 27 April 2018
Received in revised form
12 August 2018
Accepted 4 September 2018
Available online 11 September 2018

Handling Editor: Tamara S. Galloway

Keywords:
Microplastics
Goldfish
Exposure
Chewing
Ingestion
Histopathology

ABSTRACT

Microplastics (MPs) are abundant in freshwater and marine environments. They are diverse shape and size and are ingested by organisms. In this study, goldfish (*Carassius auratus*) were exposed via diet to three types of virgin MPs material types and shapes including fibers, fragments, and pellets. After six weeks of exposure, various sub-lethal effects, but no mortality, was observed. Fish exposed to plastic showed significant weight loss compared with the control. Fibers were found in the gills, gastrointestinal tract (GIT), and feces were not likely to accumulate in the GIT. Pronounced and severe alterations were found in the livers of fish exposed to fibers. The distal intestine showed more pronounced and severe changes compared to the proximal intestine, likely due to an intake of fibers. The ingestion of fibers caused the highest frequencies of progressive and inflammatory changes in the livers and intestines. This is in accordance with the higher organ index in these organs compared to other texa. Conversely, fragments and pellets were not ingested but chewed and expelled. Chewing process resulted in damages to the jaws as ranging from slight exfoliation to deep incisions. The highest frequency of regressive and circulatory (e.g., dilated sinusoids) changes was found in fish exposed to fragments, specifically in the upper and lower jaw, and in lower jaw and liver, respectively. Together, these results demonstrate that ingestion and chewing of MPs lead to damages in various organs and tissues of the gastrointestinal system, and suggest that different materials can have drastically different impacts on fish.

* Corresponding author. State Key Laboratory of Estuarine and Coastal Research, East China Normal University, Shanghai 200062, China.
E-mail address: hhshi@des.ecnu.edu.cn (H. Shi).

https://doi.org/10.1016/j.chemosphere.2018.09.031
0045-6535/

1. Introduction

Microplastics are ubiquitous within marine and freshwater environments and are bioavailable to a wide range of organisms, particularly due to their small size (<5 mm) (Eerkes-Medrano et al., 2015; Lusher et al., 2015; Thompson, 2015). Different materials and shapes, especially irregular shapes, of particles may pose a physical risk to organisms (Mazurais et al., 2015; Rainieri et al., 2018). The impacts of microplastics have been reported in marine invertebrates, but little work has been done on freshwater species (von Moos et al., 2012; Browne et al., 2013; Wright et al., 2013; Au et al., 2015).

MPs can impact animal physiology, cause intestinal blockage, alter development, increase mortality, and change ecologically relevant behavior such as foraging, social interactions, swimming, anti-predatory behavior, and reproduction (Clotfelter et al., 2004; Soffker and Tyler, 2012; Jovanovic, 2017; Choi et al., 2018). Changes in behavior may also occur following exposure to small quantities of related pollutants, which may affect the biological fitness of an animal in an ecological context (Scott and Sloman, 2004; Bae and Park, 2014). Such behavioral responses are indicators of the effects of these pollutants on individuals (Weis, 2014), and the use of these responses to detect contaminants is a growing tool in ecotoxicology (Oulton et al., 2014).

Several studies have reported ingestion plastic debris by a wide range of fish species worldwide (Foekema et al., 2013; Lusher et al., 2013; Sanchez et al., 2014; Romeo et al., 2015; Biginagwa et al., 2016; Jabeen et al., 2017). Ingested microplastics may cause physical and/or chemical harm, e.g. blocking the GIT and toxicity from leaching of chemical additives (Wright et al., 2013). Such interactions within the digestive system can lead to the reduction of food uptake and energy assimilation, and may also result in the alterations at the tissue and cell level (Besseling et al., 2012; Rochman et al., 2013). For example in sea bass (*Dicentrarchus labrax* (Linnaeus, 1758)) MPs ingestion was shown to lower the hatching success of embryos (Lu et al., 2016; Peda et al., 2016).

The adverse effects of virgin microplastics have been reported in other fish species. This includes alterations in the neurofunction of the common goby (*Pomatoschistus microps* (Oliveira et al., 2013)) as well as hepatic stress and early signs of endocrine disruption in adult medaka (*Oryzias latipes*) (Rochman et al., 2013). In comparison, ingestion of MPs by invertebrate larvae (e.g., sea urchin, *Tripneustes gratilla*) indicates that effects depend on the nature of the ingested plastic items (Kaposi et al., 2014).

Although MPs are commonly detected in the intestinal tracts of fish, there is limited information characterizing their retention. Particle size and shape are the most influential factors affecting the changes in physiology of within the GIT of exposed fish, specifically the retention of MPs items (Grigorakis et al., 2017), but comparisons between different types of MPs is very limited. Recent studies have observed a higher abundance of fibers in the gut contents of fish compared to fragments (Neves et al., 2015; Jabeen et al., 2017). Some studies reported the negative impacts of microplastic fragments on early life stages of fish (Mazurais et al., 2015; van Pomeren et al., 2017). For example, Chen et al. (2017a,b) observed neurotoxic effects and changes in the behavior Zebrafish (*Danio rerio*) larvae. MPs have also been described as inflammatory agents and stressors in fish (Ferreira et al., 2016; Greven et al., 2016) as evidenced by histological alterations in fish exposed to different types of MP polymers (Wang et al., 2013; Peda et al., 2016; Karami et al., 2016b).

Polyethylene and polystyrene are among the most abundant polymers, constituting more than half of the plastic production in the world (Plastic Europe, 2015) and are the primary components of plastic debris observed in the environment (Rochman et al., 2013; Sadri and Thompson, 2014). Many organisms including fish have been reported to ingest and egest these plastic items, but the adverse effects of this uptake are limited (Peda et al., 2016). Goldfish (*Carassius auratus*) were selected as a model organism for the present study because they are easy to culture under laboratory conditions, have a higher tolerance to a range of conditions, and accept different diets. Additionally, these fish are routinely used to investigate adverse toxicological effects (Atamaniuk et al., 2013; Ghosha et al., 2017; Grigorakis et al., 2017).

The aims of the current study are 1) to observe the ingestion and egestion of MP particles in goldfish; 2) to examine the morphological and histopathological impacts after dietary exposure to the MPs; and 3) to obtain insight into the effects of MPs of different shapes and sizes.

2. Materials and methods

2.1. Experimental design

Fibers, fragments, or pellets were used for exposure experiment. All plastic particles were less than 5 mm in size. Fibers ranged from 0.7 mm to 5.0 mm while fragments and pellets ranged between 2.5-3.0 mm and 4.9–5.0 mm, respectively. Polymer type of plastic items was identified via Raman spectroscopy (DXR2, Thermo Fisher Scientific Co.) to be ethylene vinyl acetate (EVA) fibers, polystyrene (PS) fragments, and polyethylene acrylate (PA) pellets (Table 1). Fibers were collected using forceps from the football field on the East China Normal University campus in Shanghai, China. Fragments and pellets were purchased from production sites. Goldfish were introduced into twelve glass tanks ($30 \times 22 \times 25$ cm) with continuous aeration, with five fish per tank, and three replicated per group. Fish were exposed to different types of plastic-amended food (see section 2.2 and Fig. 1) for duration of six weeks. Tank water was renewed daily, average temperature was of 24 °C ± 0.6, and there was a 14 h:10 h light/dark cycle throughout the experiment.

2.2. Microplastic food preparation

Commercial fish food (Jin Yue, Koi food in the form of pellets), completely free from MPs contamination, was soaked in tap water to soften. Fibers, fragments or pellets were mixed with the softened food and then air dried under sunlight. Fiber-containing food was prepared by weight (amending 0.03 g of fibers in 15 pellets of commercial food pellets), with each fiber-based pellet containing 55 to 76 fibers. Fragment- and pellet-containing food was prepared by using particles numbers; each food pellet contained either one

Table 1
Basic parameters of fish exposed to different food types.

Food type	Polymer type	No. of fish	Exposure time	Average weight (g)	Average length (cm)	Condition factor ($W/L^3 x100$)
Control	-	15	6 weeks	17.7 ± 2.3 (11.5–20.0)	11.8 ± 0.7 (10.4–12.5)	1.06 ± 0.09
Fiber	EVA	15	6 weeks	14.6 ± 2.6 (11.4–17.1)	12.0 ± 0.6 (11.0–12.8)	0.84 ± 0.18
Fragment	PS	15	6 weeks	13.9 ± 2.8 (10.2–20.4)	12.0 ± 1.4 (9.7–15.4)	0.83 ± 0.27
Pellet	PA	15	6 weeks	13.9 ± 1.9 (10.5–17.2)	11.7 ± 0.9 (10.0–13.7)	0.9 ± 0.25

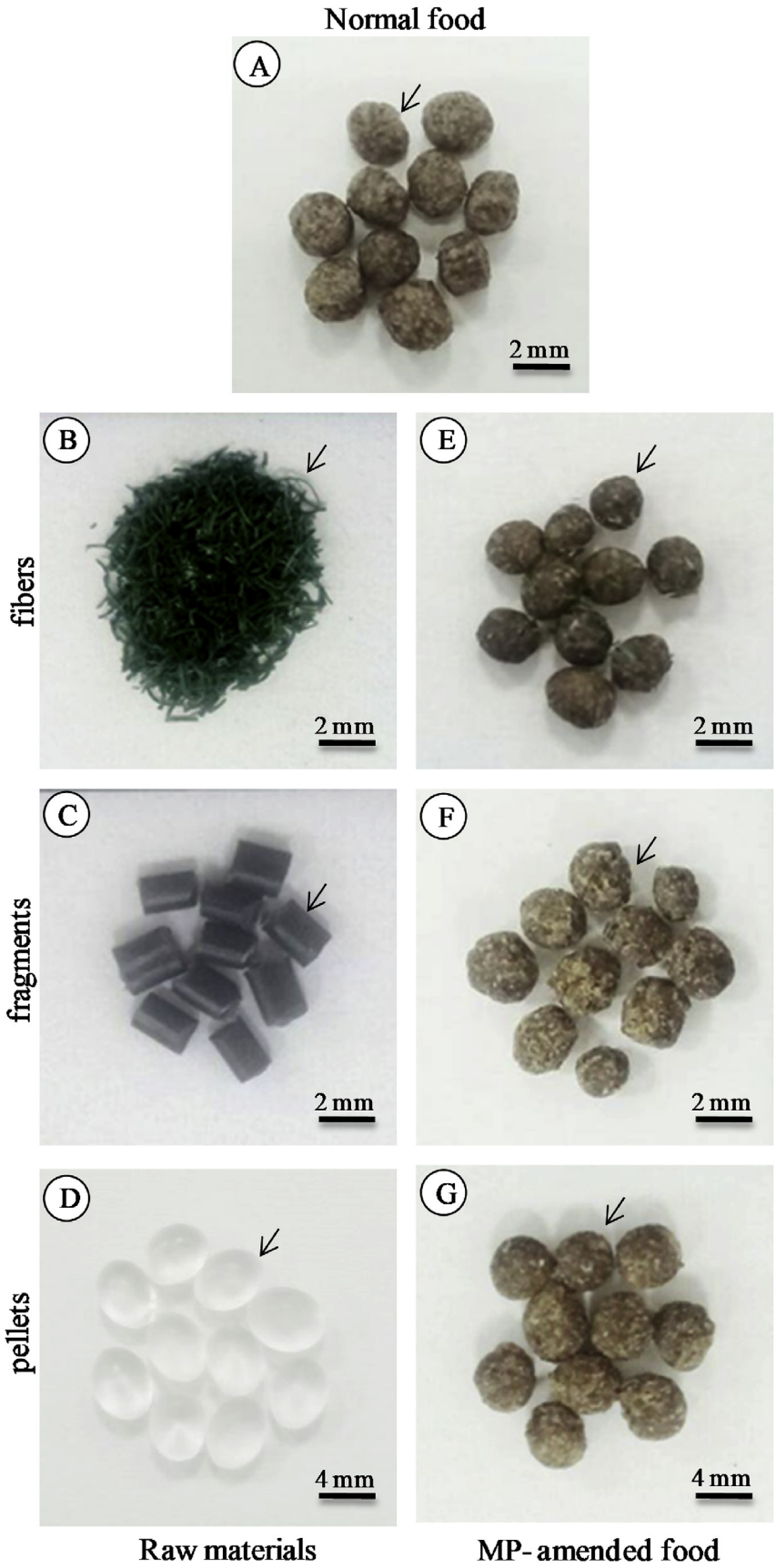


Fig. 1. Different types of food. Normal food (**A**), virgin MPs (**B:** fibers, **C:** fragments, **D:** pellets) and MP-amended food (**E:** fiber, **F:** fragment, **G:** pellet). Arrows point toward the food particles (scale bar are 2 mm and 4 mm).

fragment or one pellet (Fig. 1). There may have be small variations, but on average, fish were dosed with a concentration of 0.96%, 1.36%, 1.94% and 3.81% (g (food + MPs)/g ww fish), which was equal to 0, 55–76, 15, 15 and 15 MP particles fed to each fish for control, fibers, fragments and pellets groups respectively. All types of food were prepared in the same way, and control food pellets contained no plastic items.

2.3. Feeding strategy and observation

Fish were fasted for 48 h before the start of the experiment. Then, fish were fed three days a week, once each day at 9 a.m., for six weeks. During, each feeding, three food pellets per fish (e.g., fifteen pellets per tank) were provided to fish in all groups (n = 4). Each group was observed twice a day. During the first observation, feeding response of fish to food particles was examined during the first 5 min. Ingestion or rejection of food was recorded after 1 h by counting the number of food items left in the tank. The leftover food was then removed in order to avoid contamination.

2.4. Sample processing

After six weeks of exposure, all fish were anesthetized with MS-222 (100 mg L^{-1}) and sacrificed for immediate analysis. Total length (cm), wet weight (g), and condition factor (CF=W/L^3x100, where CF = condition factor, W = weight, L = standard length) of each fish was recorded (Table 1). Each fish was decapitated and the GIT and liver were removed through a ventral incision. Heads of all fish and the GITs and livers of 24 out of 60 fish (6 fish from each group) were preserved in 6% formalin (Sinopharm Co., Shanghai, China) for further investigation. The GITs of 36 out of 60 fish (9 fish from each group) were frozen at −20 °C for later investigation under a microscope.

2.5. Microscopic observations

Each of the preserved heads was cut horizontally on each lateral side of the buccal cavity to the branchial cavity so that the gills could be removed. Gills, upper jaw, and lower jaw were observed under a stereomicroscope (Carl Zeiss Discovery V8, MicroImaging160 GmbH, Göttingen, Germany) for any apparent change. The images of plastic items were taken with an AxioCam digital camera. Gills and GIT of 36 fish (9 fish from each treatment group) were investigated under the microscope for the presence or absence of MPs. The numbers of fish containing plastic items in gills and GITs as well as the amount/number of recovered plastic items were recorded.

2.6. Histopathological examination of jaws, liver and intestine

Based on microscopic examination, 24 fish (6 fish from each group) were selected for histological evaluation of jaws, liver, and GIT. Upper and lower jaws were divided into three parts (Supplementary Fig. 1). In case of jaws, a small tissue sample was excised using a sharp surgical blade. Two subsamples were excised from each liver, with the placement of sample within the liver chosen at random. The GIT was divided into the proximal and distal portions, with three samples were taken from the proximal and distal intestine, specifically selecting those areas where more food was present. Histological protocol with slight modifications was followed according to Walker et al. (2004) and Peda et al. (2016). Samples were fixed in 6% buffered formalin, dehydrated with an ethanol series, cleared with xylene, embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin.

Tissue sections from all sampled organs were observed using the brightfield function on an Olympus BX53 fluorescent microscope (Olympus Optical Co., Ltd, Tokyo, Japan), and images were taken with an Olympus DP 80 camera (Olympus Optical Co., Ltd). Randomly selected images ($n = 60$) from each organ were analyzed for the presence of or absence of any change. Histological alterations were evaluated according to Bernet et al. (1999). Alterations were divided into circulatory, regressive, and progressive and inflammatory changes. A pathological importance factor (importance factor from 1 to 3) was assigned to each change (Supplementary Table 3). The extent of alterations was assessed by using the scoring values described by Zimmerli et al. (2007) and Peda et al. (2016): normal (0), slight damage (1), medium damage (2), pronounced damage (3), and severe damage (4).

An organ index was calculated by multiplying the sum of the importance factors and the sum of the score values of all changes found within an organ. This organ index was used to represent the degree of damage. The finding of microgranuloma was included in the inflammatory category while portal fibrosis and lipid droplets were placed in the category of regressive changes. Jaws were evaluated under the reaction pattern of skin. The frequency of occurrence of changes was calculated from randomly selected images and recorded for each alteration.

2.7. Data analysis

Data were analyzed using SPSS 16.0 software. Differences in weight and length of fish were analyzed using a one-way analysis of variance (ANOVA) followed by Tukey's HSD test. The ANOVA comparisons were also conducted within each organ among different treatments and control group. Asterisks (*) indicate statistically significant differences between treatments within the same organ. Significant differences were recorded at $^{*} = p < 0.05$ and $^{**} = p < 0.01$.

3. Results

3.1. Behavior, ingestion and accumulation

External appearance did not differ in fish from different groups, and no mortality or acute toxicity was observe. All fish were observed to consume the MP amended food. The behavior of fish towards food was the same in all groups during the first day. From the second day on, fish consumed all food quickly after its introduction into the tanks. The condition factor of MP fed fish from fiber and fragment groups (0.84 ± 0.18 and 0.83 ± 0.27, respectively) was significantly lower compared to fish from the control group (1.06 ± 0.09) (p=0.015) (Table 1). Fish exposed to different MP-amended food groups (fiber (14.6 ± 2.6), fragment (13.9 ± 2.8), and pellet (13.9 ± 1.9)) showed significantly lower weight compared with the control group (17.7 ± 2.3) ($p = 0.00$).

Only fibers were found in GITs and gills of exposed fish. 367 fibers out starting number of 458 fibers (80.1%) were found in the GITs, and fibers were found in the feces (Fig. 2F, I). Fragments and pellets were not ingested but, rather, fish chewed them and then expelled them. The duration of chewing and expelling lasted for approximately 30 s for each of the fragments or pellets. Within gills, most of the fibers were observed on the filaments part, 38 fibers of 458 fibers (8.3%), and broken filaments were also observed (Fig. 2G; Table 2).

3.2. Structural observations of buccal cavity of fish

Observation of jaws revealed that chewing of fragments resulted in damage to the buccal cavity in 12 out 15 fish, (80.0%). The severity of damage varied from slight exfoliation of the buccal

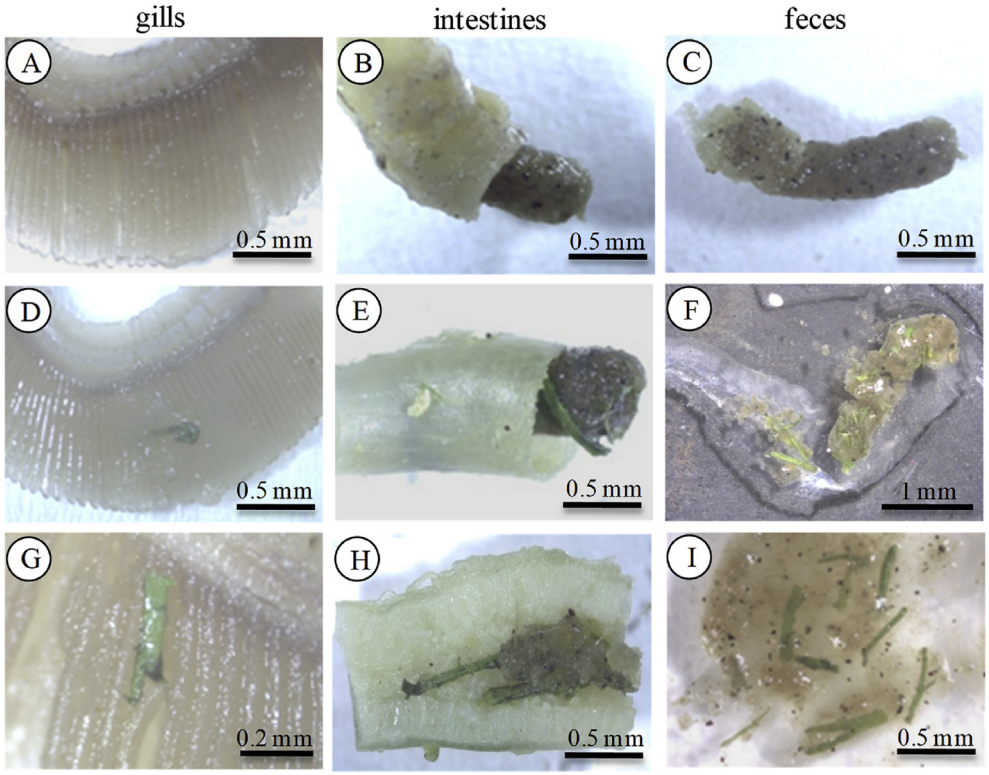


Fig. 2. Control group (**A-C**), fibers in gills **(A,D,G),** intestines **(B,E,H)** and feces (**C,F,I**) of gold fish. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

epithelium to marked abrasion/roughness to deep incisions at the peripheral and medial region of both the upper and lower jaws of fish from different treatment groups (Fig. 3).

3.3. Histological changes and evaluation of organ indices

The occurrence of regressive changes was higher in the lower jaws (30.4%) and upper jaws (27.0%) of fish exposed to fragments, followed by the jaws in fish exposed to pellet-amended food. Progressive changes were found in the upper jaws (15.2%) and lower jaws (10.7%) of fish from fiber and pellet groups, respectively (Supplementary Table 3). Examination under the microscope revealed severe breakage of the dermal layer with hemorrhages in the lower jaws of fish exposed to fragments (Fig. 4D). Circulatory changes were most prevalent in livers from the fragment exposure group, with 13.1% of fish exhibiting dilated sinusoids. The livers from fish in fiber group had fewer occurrences of sinusoid dilation (7.5%), but 3.0% of these fish showed passive hyperemia (Fig. 4E) (Supplementary Table 3).

Fish exposed to fibers also showed an inflammatory response (9.0%), with infiltration and microgranuloma in the livers (Supplementary Table 3). These fish also had the highest frequency of regressive changes in the GIT. In particular, the proximal intestine had the most regressive (67.5%) and no progressive changes (Fig. 4F). Inflammation was observed in 12.5% of the fish in this group, specifically in the distal intestine; hypertrophic goblet cells were also observed here (Supplementary Table 3).

Jaws, liver, and intestines from control fish had normal (level 0) and slight change (level 1) scores for these structures. The frequency of pronounced changes (level 3) was higher in the upper jaw (19.4%) for fish from the fiber group. The greatest number of fish exhibiting some level of change (1–4) was recorded in the lower jaw from fish exposed to fragment-amended food (51.9%). Severe changes were only found in the jaws (3.3% upper jaw; 7.5% lower jaw) from fragment group. Medium changes (level 2) were observed only in the jaws of pellet-fed fish. The only liver changes were in the fiber group, which were pronounced (level 3) and severe changes (level 4). Proximal intestine consisted of slight (level 1) and moderate changes (level 2). The distal intestine had higher proportion of fish with pronounced (12.9%) and severe (13.2%) changes. The fiber-fed fish also had the lowest frequency of normal scores for GIT (22.0% proximal intestine; 45.2% distal intestine) (Fig. 5A).

Higher organ index values were observed in MPs-fed fish compared to control. Higher organ indices were observed for the upper jaw, liver, and intestines of fish exposed to fibers compared

Table 2
Number and percentage of plastic items found in gills and gastrointestinal tracts of fish (9 fish out of 15 fish).

Food type	Gill arches		Gill rakers		Filaments		GIT	
	no. of fish	no. of items (%)	no. of fish	no. of items (%)	no. of fish	no. of items (%)	no. of fish	no. of items (%)
Fiber	8	4.0 ± 2.8 (7.9)	8	1.9 ± 1.5 (3.7)	9	4.2 ± 3.1 (8.3)	9	40.8 ± 16.5 (80.1)
Fragment	9	0	9	0	9	0	9	0
Pellet	9	0	9	0	9	0	9	0

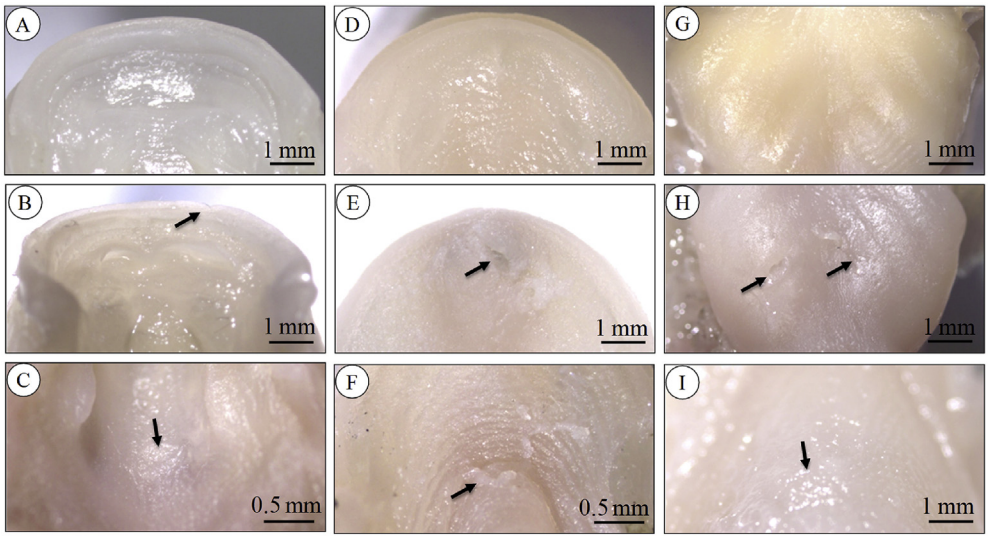


Fig. 3. Upper and lower jaws from control and treatment groups. Arrows indicate damage in treatment groups exposed to MPs-amended food. **Upper jaw** (**A:** upper lip from control; **B:** slight exfoliation in the upper lip from fiber group; **C:** slight incision in the pharyngeal cavity from pellet group); **Lower jaw** (**D:** lower lip from control; **E:** deep incision in the lower lip from fragment group; **F:** abrasion of rudimentary tongue from fragment group); **G:** pharyngeal pad from control; **H:** deep incision in the pharyngeal pad from fragment group; **I:** abrasion of ventral pharyngeal floor from pellet group.

to other treatment groups. The upper jaws of fish fed fibers and lower jaws of those fed fragments showed the highest organ indices, 1.2 ± 1.6 and 1.1 ± 1.5, respectively. The organ index value of livers from the fiber group was significantly higher (0.88 ± 0.8) than the livers from the control group (0.08 ± 0.1) ($p = 0.043$). Proximal and distal intestines of fish from fiber group had higher organ index values compared to control, fragment, and pellet groups. The value of organ index for proximal intestine was significantly higher in fiber group than the control group ($p = 0.002$) (Fig. 5B).

4. Discussions

4.1. Ingestion and egestion of microplastics

We investigated the ingestion MPs via diet using goldfish. Condition factor is an important indicator of energy status in fish and is used to evaluate the impacts of contaminants (Wijeyaratne and Pathiratne, 2006; Karami et al., 2016b). The reduction in weight and low condition factors could be related to a low energy status resulting from the stress of chewing and ingestion of plastic items. Ingestion of MPs was shown to reduce the energy status in marine worms (*Arnicola marina*) and marine copepods (*Tigriopus japonicus*) (Lee et al., 2013; Wright et al., 2013; Cole and Galloway, 2015). Changes in the feeding behavior with MPs have also been reported in marine isopods (*Idotea emarginata*) and Pacific oyster exposed to polystyrene (Hamer et al., 2014; Jambeck et al., 2015; Sussarellu et al., 2016). However, fish in our study responded to MPs in their food without changes in their feeding behavior.

Different shapes and size of plastics have been reported in the environment (Jabeen et al., 2017). However, there are no studies on the environmental concentration of MPs in fish food nor is there an exact knowledge on the daily ingestion of MPs by fish in the environment. Therefore, in this study, fish were exposed to different types of MPs from 1 to 76 particles in one food pellet to verify the possible impact when they encounter MPs in their diet. Fibers are the dominant composition pattern in most field studies (Nadal et al., 2016; Jabeen et al., 2017; Halstead et al., 2018) and were the only ingested food by fish in this study. Fragment and pellet amended food were not rejected immediately but, rather, there was a trend towards chewing and expelling both fragments and pellets. Ingestion of fiber-amended food seems to be a passive. This means that fish select of food based on the morphology of plastic particles. It seems that smaller particles are passively ingested by fish and can be transferred to other organs while larger particles with hard and sharp edges are not ingested. For example, polystyrene particles with 5 μm diameter were reported in the gills, liver, and gut of zebrafish while the larger size particles (20 μm) were found only in the gills and gut but not accumulated (Lu et al., 2016). Several studies also observed that the ingested fibers can be egested by goldfish and other species such as sea bass and marine isopods (*Idotea emarginat* (Hamer et al., 2014; Mazurais et al., 2015; Grigorakis et al., 2017). Therefore, not only shape but also the size and polymer composition of MP particles may contribute to morphological and toxicological impacts.

4.2. Structural damage and histological changes of fish

MPs caused structural damage to different organs and tissues of goldfish. This damage also depended on the size and shape of plastic particles. Fibers were detected in the gills and resulted in the breakage of filaments, likely due to direct contact. Similar findings have also been reported by Karami et al. (2016a) and Erkmen et al. (2017). We also have observed that fish exposed to different types of MPs-amended food had different impacts on jaws. Fish exposed to fragments had the fewest normal structures and the highest frequency of severe changes. Fish exposed to pellets more frequently exhibited medium level changes. These differences may be largely due to the sharp edges of fragments compared with smoother pellets. Indeed pellets were generally not associated with as much damage compared to fragments. It has been reported that exposure to plastic items has interfered with the normal functioning of the digestive systems of fish (Besseling et al., 2012). In the current study, ingested fibers impacted the intestinal lining and were found in the feces, indicating that they likely do not accumulate in the gut. Grigorakis et al. (2017) found similar result in that microfibers and microbeads in the diet were egested by goldfish. Although, fragments and pellets were not ingested in our study, Peda et al. (2016) showed that smaller size polyvinyl chloride

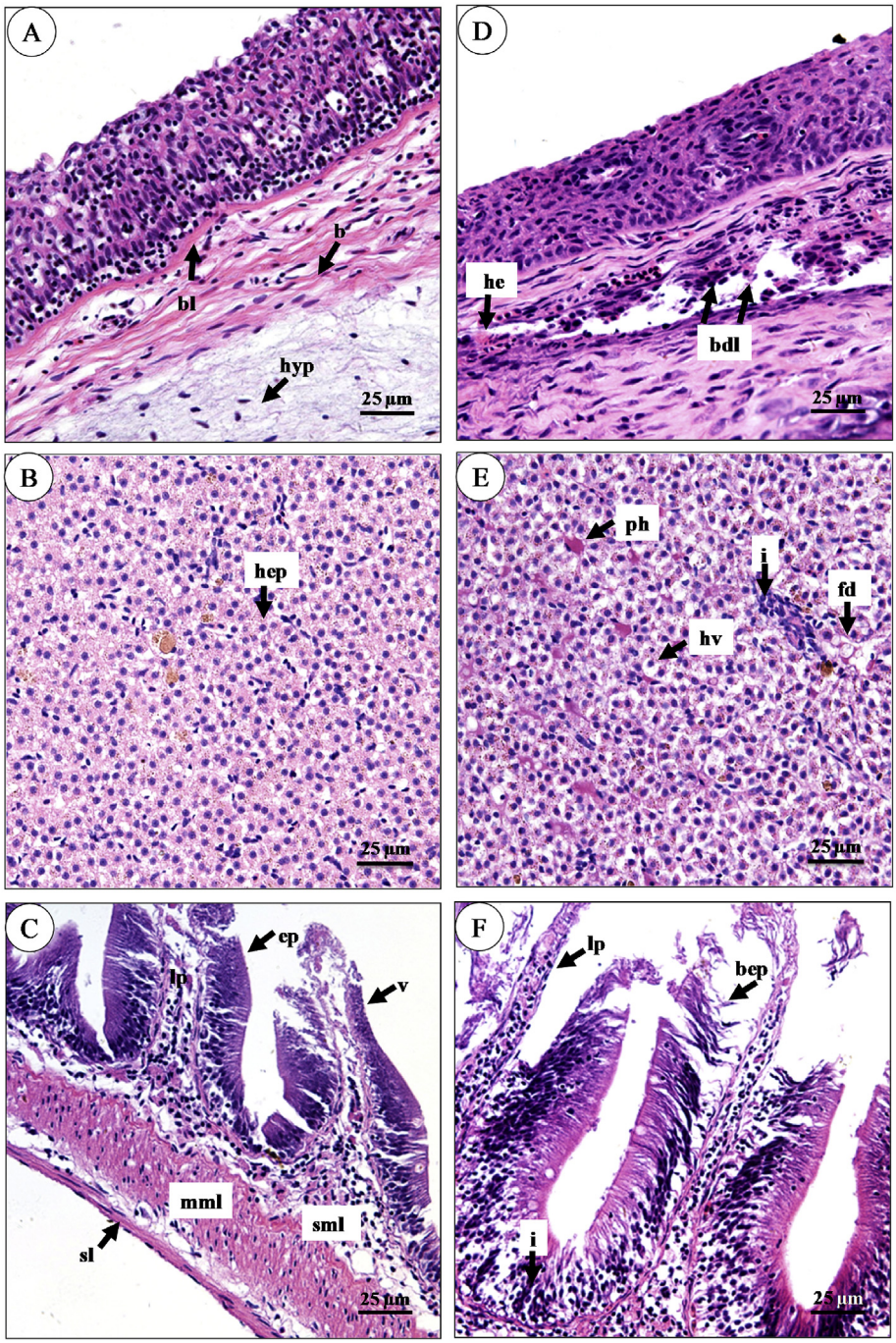


Fig. 4. Microphotographs show the normal structure lower jaw (**A**), liver (**B**), and proximal intestine (**C**) from control group (**bl:** basal layer, **d:** dermis, **hyp:** hypodermis, **hep:** hepatocytes, **v:** villus, **ep:** epithelium, **lp:** lamina propria, **sl:** serous layer, **mml:** muscularis mucosa layer, **sml:** sub-muscular layer), **D:** lower jaw from fragment group (**he:** hemorrhage, **bdl:** breakage of dermal layer), **E:** liver from fiber group (**ph:** passive hyperemia, **hv:** hydrophic vacuolization, **i:** infiltration, **fd:** lipid droplet), **F:** proximal intestine from fiber group (**lp:** lamina propria, **bep:** breakage of epithelium).

(PVC) fragments ingested by sea bass impacted their intestines. The size and shape of plastic particles has been shown to be important factors in their uptake (Wright et al., 2013; Grigorakis et al., 2017; Romano et al., 2018).

Some field studies have revealed that ingestion of plastic may lead to internal blockages and injury to the digestive tract of fish (Jackson et al., 2000; Cannon et al., 2016; Nadal et al., 2016). Karami et al. (2016b) and Peda et al. (2016) demonstrated how MPs can cause abrasion and organ damage, but particles with smooth spherical surfaces likely less of this abrasion (Mazurais et al., 2015; Karami et al., 2016b). Within the GIT of silver barb (*Barbodes gonionotus*), MPs also enhanced digestive enzyme activity in an attempt to digest pristine PVC fragments, resulting in localized thickening of mucosal epithelium (Romano et al., 2018).

Laboratory studies have also utilized histology to show how exposure to plastic has negative impacts on fishes (Rochman et al., 2013; Peda et al., 2016). In the present study, histological examinations revealed the upper and lower jaws to severely impacted, with regressive changes caused by fragments and pellets, e.g., the erosion of mucous cells in the upper jaw and detachment of

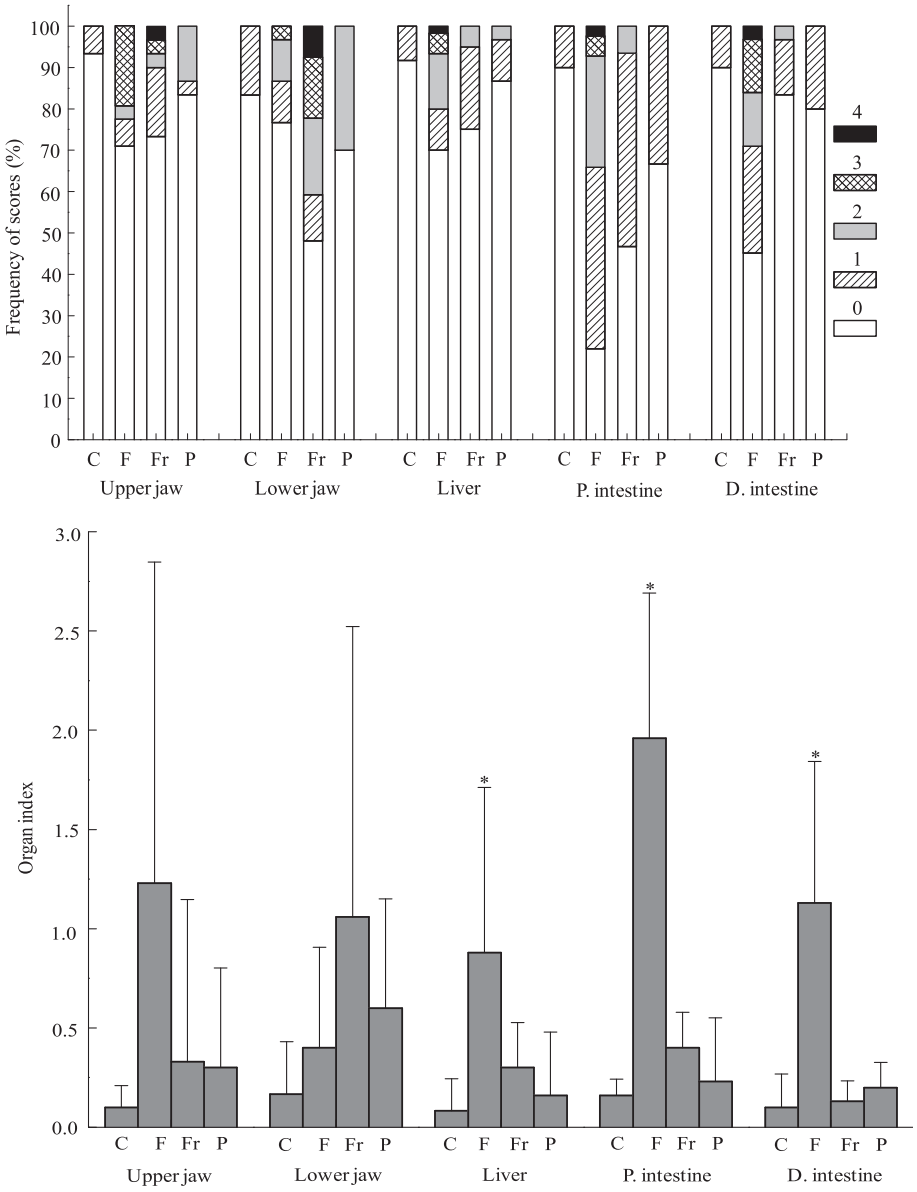


Fig. 5. 0020(A) Frequency of scores (%) (0–4) assigned to the histological changes in different organs of fish exposed to different types of food. **(B)** Organ indices of the jaws, liver and intestine of goldfish exposed to different types of food (C: control food, F: fiber-amended food, Fr: fragment-amended food, P: pellet-amended food). * represents significant differences with other groups within the same organ section ($p < 0.05$).

superficial layer and breakage of the dermal and hypodermal layer in the lower jaw. Hypertrophy of mucous cells was only observed in the lower jaw in fish exposed to pellets. These alterations were most likely due to chewing of fragments and pellets. Similarly, the hyperplasia of the dermal layer, epithelial hyperplasia, and infiltration of leukocytes showed inflammation in the upper jaw from fish exposed to fibers. The hypertrophy and hyperplasia are the basic responses to protect the organism from toxicants (Nowak, 1992; Xu et al., 2014).

The liver is the vital organ for the detoxification processes (Van der Oost et al., 2003). Pronounced and sever changes were found only in the livers from fish exposed to fiber-amended food, as well as higher organ index value. Both may indicate the stress due to the ingestion of fibers. Passive hyperaemia, dilated sinusoids, and hydrophic vacuolization were found in livers of fish exposed to MPs-amended food compared with the control group. Similar findings have been demonstrated in the livers of Nile tilapia (*Oreochromis niloticus*) exposed to the plasticizer di-*n*-butyl phthalate (10 mg L^{-1}) (Erkmen et al., 2017). Vacuolization has been related to of energy depletion and inhibition of protein synthesis in response to chemical stress (Liao et al., 2006). Although no MPs were found in the liver, the chemical stress might be due to toxicants leaching during chewing and ingestion. But, further validation for this is needed. After 45 days exposure to 100 or 500 μgL^{-1}dibutylphthalate (DBP), vacuolation and accumulation of lipid droplets was observed in the livers of male zebrafish (Xu et al., 2014). Inflammatory responses and lipid droplets have also been reported in the livers of zebrafish exposed to 5 μm, 20 μm, or 70 nm sized fluorescent-labeled polystyrene particles (Lu et al., 2016). An elevated degree of tissue change has also been reported in the livers of juvenile African catfish (*Clarias gariepinus*) exposed to virgin low-density polyethylene fragments (Karami et al., 2016b).

Ingestion of plastic items greatly affects the gastrointestinal tract of fish. We observed structural alterations in both the

proximal and distal intestine, likely due to ingestion of food containing fibers. These alterations included the breakage of epithelium, complete detachment of epithelium, erosion of villi, and detachment of the lamina propria. Infiltration of leukocytes showed the immune response to these structural changes. Similar, leukocyte infiltration has been reported in response to stress condition, physical and chemical injury (Manera and Dezfuli, 2004; Peda et al., 2016). If a comparison is made between the width of the proximal and distal intestine, the latter is narrower. This may be the reason for these finding more pronounced and severe changes, specifically the breakage of epithelium and hypertrophy in the distal intestine. In sea bass exposed to native and polluted polyvinylchloride- PVC plastics, histopathological changes in the distal intestine (e.g., breakage of epithelium and leukocyte infiltration) were also reported (Peda et al., 2016).

5. Conclusion

We reported the effects of dietary exposure to virgin microplastics on goldfish (*Carassius auratus*) under laboratory conditions. We found that uptake and ingestion depended on the size and shape of plastic items. Both chewing and ingestion caused severe impacts, with chewing plastic items resulting in damage the surface morphology of the buccal cavity, from slight abrasion to severe incision. Fibers were shown to be passively ingested by fish. We observed histological changes in the jaws, liver, proximal and distal intestine of exposed fish. The changes in the distal intestine were more pronounced and severe compared to the proximal intestine. The lower conditioning factors and changes in organs observed can lead to adverse effect on fish in the environment. This study provides valuable data on ingestion, behavior and histopathological effects of MPs using a laboratory model organism for aquatic vertebrates. However, this study clearly shows that additional research is needed to get a more detailed insight into the impacts of MP exposure in the aquatic environment and to evaluate the combined adverse effects of MPs together with chemicals. Future studies of biomarkers would provide needed assessments of the energy status of fish. Additionally, a comparison between laboratory experiments and wild-caught fish exposed to plastics is needed. This study also demonstrates that fish should be exposed to diverse plastic items as they can have differing impacts, and various levels of biological organization should be evaluated to detect change.

Acknowledgements

This work was supported by grant from the National Key Research and Development (2016YFC1402204). We thank Miss Melissa Chernick (Nicholas School of the Environment, Duke University, Durham, North Carolina, United States) for her editing the manuscript.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.chemosphere.2018.09.031.

References

Atamaniuk, T.M., Kubrak, O.I., Storey, K.B., Lushchak, V.I., 2013. Oxidative stress as mechanism for toxicity of 2,4-dichlorophenoxyacetic acid (2,4-D): studies with goldfish gills. Ecotoxicol 22, 1498–1508.

Au, S.Y., Bruce, T.F., Bridges, W.C., Klaine, S.J., 2015. Responses of *Hyalella azteca* to acute and chronic microplastic exposures. Environ. Toxicol. Chem. 34, 2564–2572.

Bae, M.J., Park, Y.S., 2014. Biological early warning system based on the responses of aquatic organisms to disturbances: a review. Sci. Total Environ. 466–467, 635–649.

Bernet, D., Schmidt, H., Meier, W., Burkhardt-Holm, P., Wahli, T., 1999. Histopathology in fish: proposal for a protocol to assess aquatic pollution. J. Fish. Dis. 22, 25–34.

Besseling, E., Wegner, A., Foekema, E.M., van den Heuvel-Greve, M.J., Koelmans, A.A., 2012. Effects of microplastic on fitness and PCB bioaccumulation by the lugworm *Arenicola marina* (L.). Environ. Sci. Technol. 47, 593–600.

Biginagwa, F.J., Mayoma, B.S., Shashoua, Y., Syberg, K., Khan, F.R., 2016. First evidence of microplastics in the african great lakes: recovery from lake victoria nile perch and nile tilapia. J. Great Lake. Res. 42, 146–149.

Browne, M.A., Niven, S.J., Galloway, T.S., Rowland, S.J., Thompson, R.C., 2013. Microplastic moves pollutants and additives to worms, reducing functions linked to health and biodiversity. Curr. Biol. 23, 2388–2392.

Cannon, S.M.E., Lavers, J.L., Figueireda, B., 2016. Plastic ingestion by fish in the Southern Hemisphere: a baseline study and review of methods. Mar. Pollut. Bull. 107, 286–291.

Chen, Q.Q., Gundlach, M., Yang, S.Y., Jiang, J., Velki, M., Yin, D.Q., Hollert, H., 2017a. Quantitative investigation of the mechanisms of microplastics and nanoplastics toward zebrafish larvae locomotor activity. Sci. Total Environ. 584, 1022–1031.

Chen, Q.Q., Yin, D., Jia, Y., Schiwy, S., Legradi, J., Yang, S., Hollert, H., 2017b. Enhanced uptake of BPA in the presence of nanoplastics can lead to neurotoxic effects in adult zebrafish. Sci. Total Environ. 609, 1312–1321.

Choi, J.S., Jung, U.J., Hong, N.H., Hong, S.H., Park, J.W., 2018. Toxicological effects of irregularly shaped and spherical microplastics in a marine teleost, the sheepshead minnow (*Cyprinodon variegatus*). Mar. Pollut. Bull. 129, 231–240.

Clotfelter, E.D., Bell, A.M., Levering, K.R., 2004. The role of animal behaviour in the study of endocrine-disrupting chemicals. Anim. Behav. 68, 665–676.

Cole, M., Galloway, T.S., 2015. Ingestion of nanoplastics and microplastics by pacific oysterlarvae. Environ. Sci. Technol. 49, 14625–14632.

Eerkes-Medrano, D., Thompson, R.C., Aldridge, D.C., 2015. Microplastics in freshwater systems: a review of the emerging threats, identification of knowledge gaps and prioritisation of research needs. Water Res. 75, 63–82.

Erkmen, B., Benli, A.C.K., Agus, H.H., Yildirim, Z., Mert, R., Erkoc, F., 2017. Impact of sublethal di-n-butyl phthalate on the aquaculture fish species Nile tilapia (*Oreochromis niloticus*): histopathology and oxidative stress assessment. Aquat. Res. 48, 675–685.

Ferreira, P., Fonte, E., Soares, M.E., Carvalho, F., Guilhermino, L., 2016. Effects of multi stressors on juveniles of the marine fish *Pomatoschistus microps*: gold nanoparticles, microplastics and temperature, Aquat. Toxicology 170, 89–103.

Foekema, E.M., Gruijter, C.D., Mergia, M.T., van Franeker, J.A., Murk, A.J., Koelmans, A.A., 2013. Plastic in North sea fish. Environ. Sci. Technol. 47, 8818–8824.

Ghosha, P., Thakurb, I.S., Kaushik, A., 2017. Bioassays for toxicological risk assessment of landfill leachate: a review. Ecotoxicol. Environ. Saf. 141, 259–270.

Greven, A.C., Merk, T., Karagoz, F., Mohr, K., Klapper, M., Jovanovic, B., Palic, D., 2016. Polycarbonate and polystyrene nanoplastic particles act as stressors to the innate immune system of fathead minnow (*Pimephales promelas*). Environ. Toxicol. Chem. 35, 3093–3100.

Grigorakis, S., Mason, S.A., Drouillard, K.G., 2017. Determination of gut retention of plastic microbeads and microfibers in goldfish (*Carrasius auratus*). Chemosphere 169, 233–238.

Halstead, J.E., James, A.S., Elizabeth, A.C., Peter, A.L., Emma, L.J., 2018. Assessment tools for microplastics and natural fibers ingested by fish in an urbanised estuary. Environ. Pollut. 234, 552–561.

Hamer, J., Gutow, L., Kohler, A., Saborowski, R., 2014. Fate of microplastics in the marine isopod *Idotea emarginata*. Environ. Sci. Technol. 48, 13451–13458.

Jabeen, K., Su, L., Li, J.J., Yang, D., Tong, C., Mu, J., Shi, H., 2017. Microplastics and mesoplastics in fish from coastal and fresh waters of China. Environ. Pollut. 221, 141–149.

Jackson, G.D., Buxton, N.G., George, M.J., 2000. Diet of the southern opah *Lamprisim maculates* on the Patagonian Shelf; the significance of the squid *Moroteuthis ingens* and anthropogenic plastic. Mar. Ecol. Prog. Ser. 206, 261–271.

Jambeck, J.R., Geyer, R., Wilcox, C., Siegler, T.R., Perryman, M., Andrady, A., Narayan, R., Law, K.L., 2015. Plastic waste inputs from land into the ocean. Science 347, 768–771.

Jovanovic, B., 2017. Ingestion of microplastics by fish and its potential consequences from a physical perspective. Integrated Environ. Assess. Manag. 13, 510–515.

Kaposi, K.L., Mos, B., Kelaher, B.P., Dworjanyn, S.A., 2014. Ingestion of microplastic has limited impact on a marine larva. Environ. Sci. Technol. 48, 1638–1645.

Karami, A., Goh, Y.M., Jahromi, M.F., Lazorchak, J.M., Abdullah, M., Courtenay, S.C., 2016a. Diploid and triploid African catfish (*Clarias gariepinus*) differ in biomarker responses to the pesticide chlorpyrifos. Sci. Total Environ. 557–558, 204–211.

Karami, A., Romano, N., Galloway, T., Hamzah, H., 2016b. Virgin microplastics cause toxicity and modulate the impacts of phenanthrene on biomarker responses in African catfish (*Clarias gariepinus*). Environ. Res. 151, 58–70.

Lee, K.W., Shim, W.J., Kwon, O.Y., Kang, J.H., 2013. Size-dependent effects of micro polystyrene particles in the marine copepod *Tigriopus japonicus*. Environ. Sci. Technol. 47, 11278–11283.

Liao, C.Y., Fu, J.J., Shi, J.B., Zhou, Q.F., Yuan, C.G., Jiang, G.B., 2006. Methylmercury accumulation, histopathology effects, and cholinesterase activity alterations in medaka (*Oryzias latipes*) following sublethal exposure to methylmercury chloride. Environ. Toxicol. Pharmacol. 22, 225–233.

Lu, Y., Zhang, Y., Deng, Y., Jiang, W., Zhao, Y., Geng, J., Ding, L., Ren, H.Q., 2016. Uptake and accumulation of polystyrene microplastics in zebrafish (*Danio rerio*) and

toxic effects in liver. Environ. Sci. Technol. 50, 4054–4060.
Lusher, A.L., Hernandez-Milian, G., O'Brien, J., Berrow, S., O'Connor, I., Officer, R., 2015. Microplastic and macroplastic ingestion by a deep diving, oceanic cetacean: the True's beaked whale *Mesoplodon mirus*. Environ. Pollut. 199, 185–191.
Lusher, A.L., McHugh, M., Thompson, R.C., 2013. Occurrence of microplastics in the gastrointestinal tract of pelagic and demersal fish from the English Channel. Mar. Pollut. Bull. 67, 94–99.
Manera, M., Dezfuli, B.S., 2004. Rodlet cells in teleosts: a new insight into their nature and function. Review paper. J. Fish. Biol. 65, 597–619.
Mazurais, D., Ernande, B., Quazuguel, P., Severe, A., Huelvan, C., Madec, L., Mouchel, O., Soudant, P., Robbens, J., Huvet, A., Zamonino-Infante, J., 2015. Evaluation of the impact of polyethylene microbeads ingestion in European sea bass (*Dicentrarchus labrax*) larvae. Mar. Environ. Res. 112, 78–85.
Nadal, M.A., Alomar, C., Deudero, S., 2016. High levels of microplastic ingestion by the semi-pelagic fish bogue *Boops boops* (L.) around the Balearic Islands. Environ. Pollut. 214, 517–523.
Neves, D., Sobral, P., Ferreira, J.L., Pereira, T., 2015. Ingestion of microplastics by commercial fish off the Portuguese coast. Mar. Pollut. Bull. 101, 119–126.
Nowak, B., 1992. Histological changes in gills induced by residues of endosulfan. Aquat. Toxicol. 23, 65–84.
Oliveira, M., Ribeiro, A., Hylland, K., Guilhermino, L., 2013. Single and combined effects of microplastics and pyrene on juveniles (0+group) of the common goby *Pomatoschistus microps* (Teleostei, Gobiidae). Ecol. Indicat. 34, 641–647.
Oulton, L.J., Taylor, M.P., Hose, G.C., Brown, C., 2014. Sublethal toxicity of untreated and treated storm water Zn concentrations on the foraging behaviour of *Paratya australiensis* (Decapoda: Atyidae). Ecotoxicology 23, 1022–1029.
Pedà, C., Caccamo, L., Fossi, M.C., Gai, F., Andaloro, F., Genovese, L., Perdichizzi, A., Romeo, T., Maricchiolo, G., 2016. Intestinal alterations in European sea bass *Dicentrarchus labrax* (Linnaeus, 1758) exposed to microplastics: preliminary results. Environ. Pollut. 212, 251–256.
Plastics Europe, 2015. Plastics-the Facts 2015. http://www.plasticseurope.org/document/plastics-thefacts-2015.aspx.
Rainieri, S., Conlledo, N., Larsen, B.K., Granby, K., Barranco, A., 2018. Combined effects of microplastics and chemical contaminants on the organ toxicity of zebrafish (*Danio rerio*). Environ. Res. 162, 135–143.
Rochman, C., Hoh, E., Kurobe, T., Teh, S., 2013. Ingested plastic transfers hazardous chemicals to fish and induces hepatic stress. Sci. Rep. 3, 3263.
Romano, N., Ashikin, M., TheM, J.C., Syukri, F., Karami, A., 2018. Effects of pristine polyvinyl chloride fragments on whole body histology and protease activity in silver barb *Barbodes gonionotus* fry. Environ. Pollut. 223, 154–163.
Romeo, T., Pietro, B., Peda, C., Consoli, P., Andaloro, F., Fossi, M.C., 2015. First evidence of presence of plastic debris in stomach of large pelagic fish in the Mediterranean Sea. Mar. Pollut. Bull. 95, 358–361.
Sadri, S.S., Thompson, R.C., 2014. On the quantity and composition of floating plastic debris entering and leaving the Tamar Estuary, Southwest England. Mar. Pollut. Bull. 81, 55–60.
Scott, G.R., Sloman, K.A., 2004. The effects of environmental pollutants on complex fish behaviour: integrating behavioral and physiological indicators of toxicity. Aquat. Toxicol. 68, 369–392.
Sanchez, W., Bender, C., Porcher, J.M., 2014. Wild gudgeons (*Gobio gobio*) from French rivers are contaminated by microplastics: preliminary study and first evidence. Environ. Res. 128, 98–100.
Soffker, M., Tyler, C.R., 2012. Endocrine disrupting chemicals and sexual behaviors in fish-a critical review on effects and possible consequences. Crit. Rev. Toxicol. 42, 653–668.
Sussarellu, R., Suquet, M., Thomas, Y., Lambert, C., Fabioux, C., Pernet, M.E.J., Goic, N.L., Quillien, V., Mingant, C., Epelboin, Y., Corporeau, C., Guyomarch, J., Robbens, J., Paul-Pont, I., Soudant, P., Huvet, A., 2016. Oyster reproduction is affected by exposure to polystyrene microplastics. P. Nat. Acad. Sci. 113, 2430–2435.
Thompson, R.C., 2015. Microplastics in the Marine Environment: Sources, Consequences and Solutions. Marine Anthropogenic Litter. Springer, pp. 185–200.
Van der Oost, R., Beyer, J., Vermeulen, N.P., 2003. Fish bioaccumulation and biomarkers in environmental risk assessment: a review. Environ. Toxicol. Pharmacol. 13, 57–149.
van Pomeren, M., Brun, N.R., Peijnenburg, W.J.G.M., Vijver, M.G., 2017. Exploring uptake and biodistribution of polystyrene (nano) particles in zebrafish embryos at different development-tal stages. Aquat. Toxicol. 190, 40–45.
von Moos, N., Burkhardt-Holm, P., Kohler, A., 2012. Uptake and effects of microplastics on cells and tissue of the blue mussel *Mytilus edulis* L. after an experimental exposure. Environ. Sci. Technol. 46, 11327–11335.
Walker, R.L., Buret, A.G., Jackson, C.L., Scott, K.G.F., Bajwa, R., Habibi, H.R., 2004. Effects of growth hormone on leucine absorption, intestinal morphological, and ultrastructure of the goldfish intestine. Can. J. Physiol. Pharmacol. 82, 951–959.
Wang, F., Bexiga, M.G., Anguissola, S., Boya, P., Simpson, J.C., Salvati, A., Dawson, K.A., 2013. Time resolved study of cell death mechanisms induced by amine modified polystyrene nanoparticles. Nanoscale 5, 10868–10876.
Weis, J., 2014. Physiological, Developmental and Behavioral Effects of Marine Pollution. Springer, Dordre-cht, Netherlands.
Wijeyaratne, W.M.D.N., Pathiratne, A., 2006. Acetyl cholinesterase inhibition and gill lesions in *Rasbora caverii*, an indigenous fish inhabiting rice field associated water bodies in Sri Lanka. Ecotoxicology 15, 609–619.
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.
Xu, N., Pengyu, C., Lei, L., Yaqiong, Z., Haixia, Zhou, Song, L., 2014. Effects of combined exposure to 17α-ethynylestradiol and dibutyl phthalate on the growth and reproduction of adult male zebrafish (*Danio rerio*). Ecotoxicol. Enviorn. saf. 107, 61–70.
Zimmerli, S., Bernet, D., Burkhardt-Holm, P., Schmidt-Posthaus, H., Vonlanthen, P., Wahli, T., Segner, H., 2007. Assessment of fish health status in four Swiss rivers showing a decline of brown trout catches. Aquat. Sci. 69, 11–25.