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THE FIRST OBSERVATION OF THE PRESENCE OF MICROPLASTICS IN WILD COMMON BLEAK (*Alburnus alburnus* L.) AND STANDARDIZATION OF EXTRACTION PROTOCOLS

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ABSTRACT. The presence of microplastics (MPs) in the gastrointestinal tract, muscle, and whole-body samples of common bleak *Alburnus alburnus* L. from Gruža Reservoir (Central Serbia) was studied for the first time. Different protocols for MPs extraction were applied to determine the most efficient one. The study aimed to modify existing protocols to be cost-effective, efficient in digestion, and with no detrimental effect on potentially present MPs polymers. In this study, the digestion with 10% KOH during 48 h at 40°C was efficient for the gastrointestinal tract and muscle. Digestion with 10% KOH during 72 h at 40°C was the most efficient for whole-body samples. The usage of NaClO proved successful in digestion of the gastrointestinal tract overnight at room temperature. Fibers detected in the samples are assumed to be of plastic origin. The general goal was to establish a protocol for extracting MPs from fish tissue in wild populations to obtain results and determine the degree of pollution.

Key words: bioindicators, digestion protocol, fibers, freshwater, Gruža Reservoir, Serbia.

INTRODUCTION

The enormous expansion of plastics has increased convenience but simultaneously created enormous quantities of plastic waste (PROKIĆ *et al.*, 2021). Plastic production reached nearly 58 million tonnes in Europe, about 16% of the world's plastics production (ANONYMOUS, 2019). Plastic is widely present in agriculture, electronic equipment, pharmaceutical, cosmetic, car, and footwear industries (PROKIĆ *et al.*, 2019). This raised numerous environmental concerns (PROKIĆ *et al.*, 2021), especially regarding the rapid increase in the production and distribution of plastic materials resistant to degradation (LUSHER, 2015). Concerns related to interactions between plastics and their derivates with

different organisms have progressively increased in the last few decades (HARRISON *et al.*, 2011; DE SOUZA MACHADO *et al.*, 2017; BERLINO *et al.*, 2021; CAO *et al.*, 2021).

THOMPSON *et al*. (2004) firstly introduced the concept of microplastics. Based on their size, small plastics particles are divided into microplastics (100 nm – 5 mm) and nanoplastics (< 100 nm) (NG *et al.*, 2018). Furthermore, by their source, microplastics (MPs) are categorized as primary and secondary (YAO *et al.*, 2020). Primary MPs are small fragments that originate from industrial and domestic use. They include various plastic fragments, fibers, pellets, seeds, and spheres used in agriculture, cosmetics, and pharmaceutical industries, residues of the ship-breaking process, and materials applied in air blasting technology (FENDALL and SEWELL, 2009; GUZZETTI *et al.*, 2018). Secondary MPs result from extended physical, chemical, or biological breakdown of the larger plastic parts (GUZZETTI *et al.*, 2018).

MPs debris are not localized in one habitat and do not have just one primary source. For a large number of MPs, the land is considered one of the principal sources, and the ocean an aggregate space (GONG and XIE, 2020). Freshwater ecosystems are the link between terrestrial and marine MPs polluted habitats (PROKIĆ *et al.*, 2021). MPs enter the ocean primarily through water flows (PROKIĆ *et al.*, 2021), as rivers continue to be the main source of MPs (< 5 mm) (ARTHUR *et al.*, 2009; LIMA *et al.*, 2014; LEBRETON *et al.*, 2017). MPs trapped within sediments may eventually permeate into groundwaters or aquifers before re-joining the water cycle (O'CONNOR *et al.*, 2019; RE, 2019), or may be released by storm and rain events that may resuspend trapped MPs and introduce them back into aquatic systems (BONDELIND *et al.*, 2020; DE JESUS PIÑON-COLIN *et al.*, 2020; OCKELFORD *et al.*, 2020). Fishes could easily ingest MPs due to their small size, which is similar to the size of zooplankton (FERNANDEZ *et al.*, 2020). Consequently, MPs have been discovered in a broad spectrum of living organisms (CHANG *et al.*, 2019).

Fish species are exposed to MPs in water through gills and food ingestion, which produces adverse effects on various biological processes (GAMARRO *et al.*, 2020; WANG *et al.*, 2020). Some authors suggest that ingestion of MPs can occur accidentally during fish feeding (BESSA *et al.*, 2018), or by the transfer of MPs within the food chain from prey to predator (SANTANA *et al.*, 2017). According to WANG *et al.* (2020), MPs can have adverse effects on the food chain. Fish may unintentionally ingest MPs that may resemble the color or shape of their usual food (ORY *et al.*, 2017). This will depend on the feeding behavior of the fish and the properties of MPs (ROCH *et al.*, 2020).

Freshwater fish populations can be continuously exposed to a range of MPs (GAMARRO *et al.*, 2020, WANG *et al.*, 2020), so they have to adapt or tolerate any changes in their local environment, especially in cases where their movement is limited due to adaptation to changes in environmental factors along with the river flow (GRILL *et al.*, 2019). Therefore, freshwater fishes are considered bioindicators of MPs pollution. They represent a good model for analyzing MPs effects on the ecology and behavior of animals, from individual to population, community and ecosystem levels (PARKER *et al.*, 2021). According to BESSA *et al.* (2019), it is necessary to focus on a relatively small set of indicator species that would serve as candidates for MPs monitoring purposes.

For the first time in the territory of Serbia, a wild fish species common bleak *(Alburnus alburnus* Linnaeus, 1758) was used to modify protocols for MPs extraction (KARAMI *et al.*, 2016a; COLLARD *et al.*, 2018) and determination of its presence. Common bleak is planktivorous fish species, widespread from medium to large rivers and lakes across Europe and Asia (KOTTELAT, 1997; KOTTELAT and FREYHOF, 2007). In addition, it is easy to collect, gregarious, important food for predatory fish species, and is categorized as Least Concern by the International Union for the Conservation of Nature (FREYHOF and KOTTELAT, 2008). All these characteristics make common bleak a favorable candidate for a bioindicator species of MPs detection.

This study aimed to modify the existing nondestructive MPs protocols (KARAMI *et al.*, 2016a; LUSHER *et al.*, 2017; COLLARD *et al.*, 2018; BESSA *et al.*, 2019) to develop a new cost-effective one. The proposed protocol should efficiently digest the fish tissues (gastrointestinal tract, muscles, and whole-body samples) and have no detrimental effect on potentially present MPs polymers. Also, in order to conduct future detailed analyzes, we wanted to make a visual confirmation of the present microplastics polymers.

MATERIALS AND METHODS

Study area and sampling

Gruža Reservoir (Fig. 1) is an artificial lake, primarily used for water supply, situated in Central Serbia (ČOMIĆ and OSTOJIĆ, 2005). Additionally, fish from this lake are commonly used in the human diet in this part of the country (SIMIĆ *et al.*, 2016).

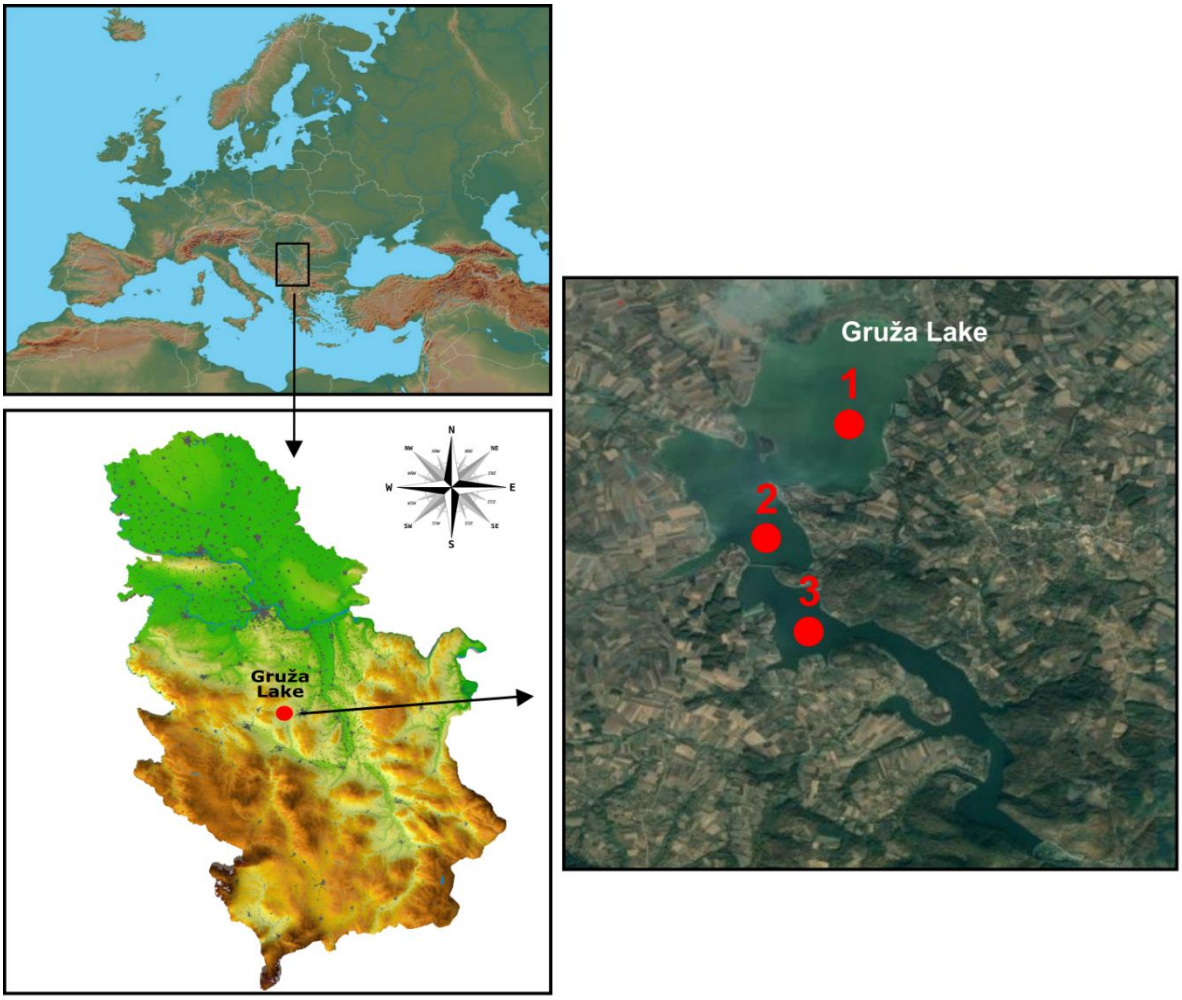

Figure 1. Gruža Reservoir in Central Serbia; sampling localities.

Potential pollutants and sources of MPs can be found in many cottages, houses, and several restaurants that surround the lake. The 32 common bleak specimens were collected in three localities on Gruža Reservoir in August 2020 (Table 1). Fish were collected with gill nets (mesh size 10 mm), dimensions 120 cm in length and 80 cm in width. The total length (TL) of each analyzed fish was measured to the nearest mm and weighted (W) to the nearest g. Specimens were stored at -20°C prior to the laboratory experiments.

Table 1. Description of the sampling localities in the Gruža Reservoir and collected material.

GPS coordinates	Number of specimens	Mean length (cm)	Mean weight (g)
1. locality N43°55'062″ E20°40'645″ 2. locality N43°55'331″ E20°40'724″ 3. locality N43°55'906″ E20°40'779″	32	10.22	10.9

Preventing contamination and procedural blanks

Throughout the entire isolation process, white cotton lab coats, masks, and latex gloves were worn to minimize sample contamination. All dissection tools and glassware were rinsed with distilled water before each use, and the work surface was thoroughly cleaned with 99% ethanol. All plates, stainless steel tackles, and equipment were left under the aluminum foil to prevent airborne contamination. Three procedural blanks were performed to evaluate whether materials, solutions, workspace or equipment were contaminated by MPs. The first one is during the filtration of chemicals and the process of dissection, the second is during the digestion process, and the third one is during microscopy. Wet filter papers were placed in the glass Petri dishes that were left open for the whole duration of the experiment.

Modyfied protocols for digestion of biogenic material and isolation of MPs

For the experiments, we analyzed whole-body samples, the gastrointestinal tracts, and muscle tissue samples of 32 common bleak specimens. Experiments were conducted using 4% NaClO and 10% KOH. Table 2 contains the number of specimens used for each of the modifications of protocols performed in this study. Fish were dissected and the gastrointestinal tract and muscle tissue samples were taken from each specimen (6 in total per each protocol). Also, whole-body samples were used in the experiment (5 per protocol).

Table 2. Number of specimens per modified protocols.

Tissue	4% NaClO		10% KOH	
	Room temperature overnight	Room temperature 24h	40°C/48h	40°C/72h
Gastrointestinal tract and muscle tissue	6	/	6	/
Whole body sample	5	5	5	5
In total	16		16	

The NaClO and KOH solutions were made with distilled water previously filtered through a cellulose membrane, pore diameter of 11 µm (Whatman Inc., No. 1). The prepared solutions were filtered once more and stored in glass bottles before the experiment.

The first protocol was the modification of the protocol described by COLLARD *et al.* (2018). Modification considered the usage of a less concentrated NaClO solution. The first three samples were digested using 4% sodium hypochlorite (NaClO, Centrohem, Stara Pazova, Serbia) in a ratio of approximately 1:100 (v/v) at room temperature overnight. The fourth sample was digested longer, 24h, until the complete digestion of the tissue. After the

digestion, tissues were filtered with 4% NaClO solution through a cellulose filter membrane. The filtered membranes, which potentially contained particles of MPs, were stirred with 99% ethanol and filtered with a vacuum system. Ethanol is commonly used to conserve samples containing MPs (SÁNCHEZ *et al.*, 2014; PHILLIPS and BONNER, 2015; ORY *et al.*, 2017; COLLARD *et al.*, 2018). Finally, the filter membranes were left at room temperature for 2 h to evaporate the ethanol before microscopic analyses.

For the second protocol, samples were kept in four glass bottles that contained 10% KOH (Moss & Hemoss, Belgrade, Serbia) in a ratio of 1:10 (v/v) and prepared for the digestion process. Tissues digestion with 10% KOH at 40°C/48 h was performed, similarly to the protocol described in the study by KARAMI *et al.* (2016a). The first, second, and third sample bottles were placed in a water bath at 40°C (BIANCHI *et al.*, 2020) for 48 hours. The fourth bottle with the whole body sample was left in a water bath at 40°C for 72 h to compare the effect of incubation time on the digestion of the whole body sample. Digested tissues were filtered through an 11 µm cellulose membrane using a vacuum system. In the case of undigested biogenic material occurrence on filter membranes, 30 ml NaI (Centrohem, Stara Pazova, Serbia) solution was added (NUELLE *et al.*, 2014), manually stirred, and the mixture was left for a few minutes. The top layer of the sample was collected with a pipette (volume 5 ml), filtered again, and prepared for microscopy.

Microscopic analyses

The samples were analyzed under the light microscope Motic BA310. The microphotographs of potential MPs particles were taken by digital camera BRESSER (9MP) using the software package MicroCamLab® (BRESSER GmbH, Rhede, Germany).

RESULTS AND DISCUSSION

Information regarding mean values of length and weight of analyzed specimens are given in Table 1, where the measured mean length is 10.22 cm and the mean weight is 10.9 g. A comparative presentation of the results regarding the efficiency of digestion protocols of biogenic material is presented in Table 3. The most effective protocol for digestion of the gastrointestinal tract was with 4% NaClO at room temperature, overnight. The 10% KOH at 40 °C for 72 h was the most effective digestion protocol for the whole-body sample.

Table 3. The efficiency of different digestion protocols of tissues of common bleak for MPs detection.

	4% NaClO		**10% KOH (48h)**		**10% KOH (72h)**	
Tissue	**Before treatment (g)**	**After treatment (g)**	**Before treatment (g)**	**After treatment (g)**	**Before treatment (g)**	**After treatment (g)**
Gastrointestinal tract	2.6	0.1	3.4	0.2	/	/
Muscle tissue	6.3	/	6.7	0.2	/	/
Whole fish sample	56.1	/	33.5	1.2	34	1.1

The number of species exposed to plastic pollution is alarming (GALLOWAY, 2015). HOU *et al.* (2021) measured MPs in digestive tissues of specimens of four fish species collected from 1900 to 2018, preserved in museum collections. Their results showed that no MPs were detected in any fish before 1950, implying that from the mid-20th century to 2018,

concentrations of MPs showed a significant increase. All detected particles were fibers and represented plastic polymers (e.g., polyester), along with mixtures of natural and synthetic textiles. Since wild aquatic organisms can contain MPs (BOERGER *et al.*, 2010; DAVISON and ASCH, 2011; SÁNCHEZ *et al.*, 2019), there is a need for an effective digesting protocol that could fully extract all the types of plastic polymers without their degradation (KARAMI *et al.*, 2016a). The number of studies regarding MPs pollution and its impact is growing; however, only a few examine freshwater organisms (COLLARD *et al.*, 2019). Moreover, the collection and analysis of MPs from freshwater field data are currently conducted globally by diverse and not standardized methods (O'CONNOR *et al.*, 2019), making the comparison of the results difficult. Methods used to detect microplastics in biota have been in progress in recent years, but there is still no harmonization of methods for MPs extraction. There are different established protocols for extraction and/or quantification of MPs in biota, categorized into two main classes – destructive and nondestructive methods (BESSA *et al.*, 2019).

In this study, for the first time, protocols for MPs extraction were conducted on common bleak with the main goal to determine the potential usage of planktonic freshwater species as bioindicators. Since the whole body of the common bleak is used in the human diet, the obtained results prove that the consumption of this fish does not necessarily eliminate the risk of MPs intake.

Gastrointestinal tract

Gastrointestinal tracts were successfully digested with both chemicals (Table 3), and there was no need for additional sample purification. In the protocol with 10% KOH at 40°C for 48 h, traces of fat particles were detected on the filter paper. The digestion protocol with 4% NaClO at room temperature overnight obtained better results, with no biological material or undigested fat detected. Similar results to the ones provided in this study were obtained by COLLARD *et al.* (2015), wherein stomach contents of three planktivorous fish species are almost completely degraded by 3% NaClO solution at room temperature overnight. However, the same authors also applied a mixture of NaClO/HNO_3 for 5 minutes, resulting in total digestion. They reported the formation of secondary particles (probably $NaNO_3$), which could resemble MPs. The digestion protocol for MPs extraction from species *Engraulis encrasicolus* liver was successful with an increased concentration of NaClO (9%) (COLLARD *et al.*, 2017). By reducing the acidity of the solution, we wanted to minimize the possibility of damaging plastic polymers, but also digest the tissue successfully, since BIANCHI *et al.* (2020) proved that increasing the acidity of solution leads to significant loss of polymer, especially nylon that melts with acid.

KOH was the first time used to digest biological materials for MPs extraction by FOEKEMA *et al.* (2013), followed by several more studies (KARAMI *et al.*, 2016a, 2016b, 2017; THIELE *et al.*, 2019; PRATA *et al.*, 2021). Our results proved better digestion of the gastrointestinal tract in a 4% NaClO solution compared to 10% KOH solution, after which traces of undigested fat stains remained. According to JAYA-RAM *et al.* (2018), the tissue of cyprinid fish contains a large amount of Omega-3 long-chain polyunsaturated fatty acids, which is harder to digest compared to fish from the Cobitidae family. HERMSEN *et al.* (2018) emphasized that digestion in 10% KOH is not particularly suitable for tissues with a considerable amount of fat because the digestion may take several days and may partially damage some polymers. According to BESSA *et al.* (2019), a protocol with 10% KOH has a fast approach, but the protocol is not appropriate for fat tissues. Establishing a 4% acidity of the NaClO solution in our study proved effective for the gastrointestinal tract. Differences in the digestion of biological material by similar solutions are probably present due to different sources of biological material, soft fish tissue, or stomach contents (KARAMI *et al.*, 2016a).

Muscle tissue

Muscle tissue was successfully digested in 10% KOH solution at 40°C for 48 h. On the other hand, this tissue was not digested in a 4% NaClO solution (Table 3.). NaClO was not sufficiently concentrated for the tissue, and the sample contained skin particles which are difficult to digest. The same results are obtained by KARAMI *et al.* (2016a), where biological material was incubated (muscle and skin) with NaClO solution at different temperatures, which did not lead to an optimum digestion efficiency within 96 h.

ZITOUNI *et al.* (2020) conducted the digestion protocol with 10% KOH to extract MPs from the gastrointestinal tract and muscle. They highlighted the presence of MPs (≤ 3 μm) in all samples of adult benthopelagic fish *Serranus scriba*. Thus, their protocol was successful as the one provided in this work. Also, RASTA *et al.* (2021) reported that MPs were found in all treated tissues (muscle samples, gastrointestinal tract, and gonads) after digestion in 10% KOH for 24 h at 60°C. In contrast, PATIL and SHARMA (2011) proved that the KOH solution is more aggressive at higher temperatures. Based on this, HERMSEN *et al.* (2018) consider that such results might be attributed to differences in ecological parameters, or the use of variable extraction and characterization methodologies.

Whole-body sample

In our study, the whole body sample was digested in 10% KOH solution after 48 h at 40°C, but undigested biological material during filtration was observed. Consequently, the incubation time was extended to 72 h, and digestion was successful. The protocol conducted by DEHAUT *et al.* (2016) with 10% KOH solution and incubation at 60°C for 24 h was proposed as a good compromise for extraction and characterization of MPs from seafood. KÜHN *et al.* (2016) suggested that the concentration of KOH solution and duration of digestion should be increased depending on the tissue that needs to be dissolved. According to the results of KARAMI *et al.* (2016a), the whole body sample was fully digested in 10% KOH solution at 40°C for 48 h, and the bone fragments were successfully separated with NaI. Protocol with 4% NaClO in our study did not give digestion results, as in the study of STOJIČIĆ *et al*. (2010), where the authors experimented with different solution concentrations and different temperatures.

Microphotograph of MPs fibers found in samples

In five whole-body samples digested with 10% KOH solution at 40°C for 72 h, 14 fibers were observed (13 blue, and one red). Six fibers were observed in six digested gastrointestinal tracts treated with 4% NaClO solution at room temperature overnight (five blue, and one red). On the other hand, in the digested muscle tissue samples with both chemicals, no fibers were observed.

The longest measured fiber was 520 μm, and the smallest one 110 μm. We assume that all found fibers are of plastic origin (Fig. 2).

KHAN *et al.* (2020) found that over 75% of the fish sampled from local sellers in Cairo contained MPs in the digestive tracts. The most abundant MPs type were fibers (65%), followed by films (26.5%), and the remaining MPs were fragments. In our study, fibers suspected to be of plastic origin were found in the whole body samples (digested in 10% KOH solution for 72 h at 40°C) and in the gastrointestinal tract (digested in 4% NaClO solution at room temperature overnight) (Fig 2b). Results published by COLLARD *et al.* (2017) showed that 80% of *E. encrasicolus* livers contained relatively large MPs. UURASJÄRVI *et al*. (2020) showed that 17% of *Perca fluviatilis* and 25% *Coregonus albula* specimens from Lake Kallavesi had ingested MPs.

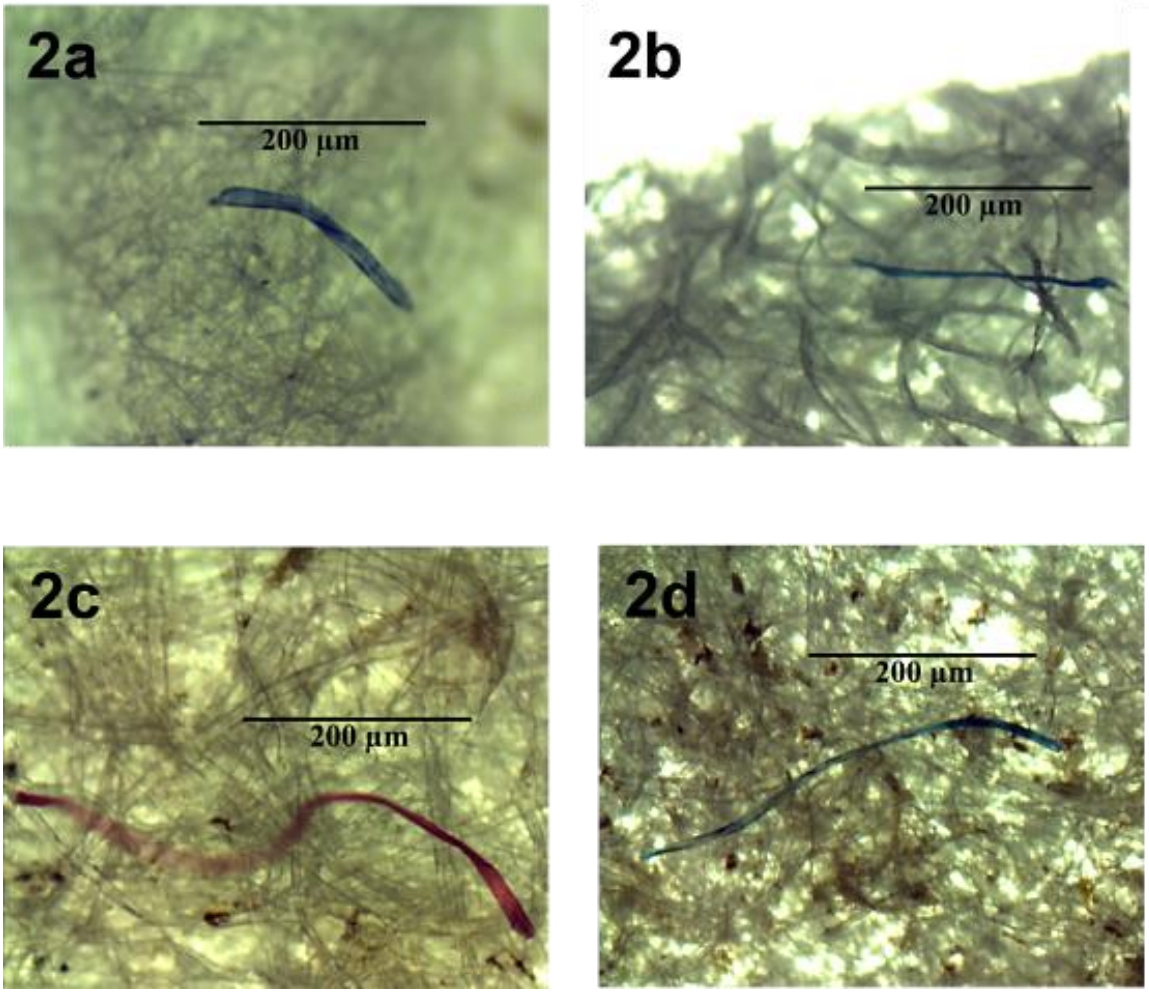

Figure 2. MPs in gastrointestinal tract digested with NaClO (2a) and whole body sample of common bleak digested in 10% KOH (2b, 2c, 2d).

According to UURASJÄRVI *et al*. (2020) the particle size was categorized into fibers or fragments by dividing the major measured dimension with the minor measured dimension. If the resulting ratio was > 5, particles were categorized as fiber, otherwise as fragments. In this study, the smallest detected fiber was 110 µm. COLLARD *et al*. (2018) found dominant fibers in *Squalius cephalus* stomach contents, not less than 390 µm. According to QIAO *et al*. (2019), fibers have a higher tendency to be accumulated compared to fragments and pellets since they are harder to digest. The first evidence of MPs ingestion by freshwater fish was published by SÁNCHEZ *et al.* (2014). Before them, FAURE *et al.* (2012) analyzed the gut content of 41 fish samples from Geneva Lake, but no MPs were observed. NEMATOLLAHI *et al.* (2021) recorded only MPs fibers in the gut of highly consumed fish species *Chelon saliens*, *Cyprinus carpio* and *Rutilus caspicus* in the southern Caspian Sea. In the same study, the estimated condition index reflected a significant difference between the species, implying that MPs may pose adverse health impacts on *C. saliens* and *C. carpio*, with no effect on *R. caspicus*. No significant relationship exists between biological parameters and the MPs frequency in the fish gut (NEMATOLLAHI *et al.*, 2021). MCNEISH *et al.* (2018) concluded that the feeding characteristics had a notable effect on MPs uptake. According to some authors, higher numbers of MPs have been detected in the digestive tracts of fishes in urban environments compared to more pristine areas (SÁNCHEZ *et al.*, 2014; PETERS and BRATTON, 2016; FERREIRA *et al.*, 2020).

Still, the total number of field studies is relatively low (n = 76) compared to laboratory studies (KUKKOLA *et al.*, 2021). Therefore, this study is of great importance, because protocols for the extraction of microplastics were conducted on the tissues and whole-body samples of wild fish specimens. Few studies investigated MPs ingestion by wild fish species (SÁNCHEZ *et al.*, 2014; PAZOS *et al.*, 2017; COLLARD *et al.*, 2019; BARBOZA *et al.*, 2020), while some of those have focused only on stomach contents (SÁNCHEZ *et al.*, 2014; PAZOS *et al.*, 2017). Moreover, several studies have identified MPs bioaccumulation within the liver, brain, and muscle of freshwater fish (BATEL *et al.*, 2016; ABBASI *et al.*, 2018; SU *et al.*, 2019; DE SALES-RIBEIRO *et al.*, 2020; DING *et al.*, 2020). However, the possibility of MPs detection in the edible parts of fish is the greatest concern (KARAMI *et al.*, 2017) since posing a risk to human health (AKOUESON *et al.*, 2020). So, all plastic debris can be traced back to human activities, either on land or at sea (THIELE *et al.*, 2021).

In our study, fibers suspected to be of plastic origin were found in the gastrointestinal tract of six (from 32 analyzed) *A. alburnus* individuals digested with 4% NaClO solution overnight at room temperature, and five whole-body samples digested with 10% KOH for 72 h at 40°C. Therefore, fibers were detected in 11 samples out of 32, which is a significant number since it represents one-third of the total sample. For further analyses, it is crucial to determine the type of found MPs. Additionally, it is necessary to establish a protocol for the extraction of MPs from the tissue of wild fish specimens. Based on our study results, for the common bleak, we suggest the usage of the protocol with 4% NaClO overnight at room temperature for the gastrointestinal tract, 10% KOH at 40°C during 48 h for muscle tissue, and 10% KOH at 40°C during 72 h for whole body sample.

Acknowledgements

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References

[1] ABBASI, S., SOLTANI, N., KESHAVARZI, B., MOORE, F., TURNER, A., HASSANAGHAEI, M. (2018): Microplastics in different tissues of fish and prawn from the Musa Estuary, Persian Gulf. *Chemosphere* **205**: 80–87. doi: 10.1016/j.chemosphere.2018.04.076

[2] AKOUESON, F., SHELDON, L., M., DANOPOULOS, E., MORRIS, S., HOTTEN, J., CHAPMAN, E., LI, J., ROTCHELL, J.M. (2020): A preliminary analysis of microplastics in edible versus non-edible tissues from seafood samples. *Environmental Pollution* **263**: 14452. doi: 10.1016/j.envpol.2020.114452

[3] ANONYMOUS (2019): Plastics the facts 2019, An Analysis of European Latest Plastics Production. https://plasticseurope.org/wp-content/uploads/2021/10/2019-Plastics-the-facts.pdf

[4] ARTHUR, C., BAKER, J., BAMFORD, A. (eds.) (2009): *Proceedings of the International Research Workshop on the Occurrence, Effects and Fate of Microplastics Marine Debris*. Sept 9-11, 2008. NOAA Technical Memorandum NOS-OR&R-30.

[5] BARBOZA, A.L.G., LOPES, C., OLIVEIRA, P., BESSA, F., OTERO, V., HENRIQUES, B., RAIMUNDO, J., CAETANO, M., VALE, C., GUILHERMNO, L. (2020): Microplastics in wild

fish from North East Atlantic Ocean and its potential for causing neurotoxic effects, lipid oxidative damage, and human health risks associated with ingestion exposure. *Science of the Total Environment* **717**: 134625. doi: 10.1016/j.scitotenv.2019.134625

[6] BATEL, A., LINTI, F., SCHERER, M., ERDINGER, L., BRAUNBECK, T. (2016): Transfer of benzo[a]pyrene from microplastics to *Artemia nauplii* and further to zebrafish via a trophic food web experiment: CYP1A induction and visual tracking of persistent organic pollutants. *Environmental Toxicology and Chemistry* **35**: 1656–1666. doi: 10.1002/etc.3361

[7] BERLINO, M., MANGANO, M.C., DE VITTOR, C., SARCI, G. (2021): Effects of microplastics on the functional traits of aquatic benthic organisms: A global-scale meta-analysis. *Environmental Pollution* **285**: 117174. doi: 10.1016/j.envpol.2021.117174

[8] BESSA, F., FRIAS, J., KOGEL, T., LUSHER, A., ANDRADE, J.M., ANTUNES, J., SOBRAL, P., PAGTER, E., NASH, R., O'CONNOR, I., PEDROTTI, M.L., KERROS, M.E., LEON, V., TIRELLI, V., SUARIA, G., LOPES, C., RAIMUNDO, J., CAETANO, M., GAGO, J., VIÑAS, L., ... GERDTS, G. (2019): Harmonized protocol for monitoring microplastics in biota. *Techical Report*, JPI-Oceans BASEMAN project. doi: 10.13140/RG.2.2.28588.72321/1

[9] BESSA, F., BARRÍA, P., NETO, J.N., FRIAS, J.P.G.L., OTERO, V., SOBRAL, P., MARQUES, J.C. (2018): Occurrence of microplastics in commercial fish from a natural estuarine environment. *Marine Pollution Bulletine* **128**: 575–584. doi: 10.1016/j.marpolbul.2018.01.044

[10] BIANCHI, J., VALENTE, T., SCACCO, U., CIMMARUTA, R., SBRANA, A., SILVESTRI, C., MATIDDI, M. (2020): Food preference determines the best suitable digestion protocol for analysing microplastic ingestion by fish. *Marine Pollution Bulletine* **154**: 11050. doi: 10.1016/j.marpolbul.2020.111050

[11] BOERGER, C.M., LATTIN, G.L., MOORE, S.L., MOORE, C.J. (2010): Plastic ingestion by planctivorous fishes in the North Pacific Central Gyre. *Marine Pollution Bulletine* **60**: 2275–2278. doi: 10.1016/j.marpolbul.2010.08.007

[12] BONDELIND, M., SOKOLOVA, E., NGUYEN, A., KARLSSON, D., KARLSSON, A., BJÖRKLUND, K. (2020): Hydrodynamic modelling of traffic-related microplastics discharged with stormwater into the Göta River in Sweden. *Environmental Science and Pollution Research* 27: 24218–24230. doi: 10.1007/s11356-020-08637-z

[13] CAO, Y., ZHAO, M., MA, X., SONG, Y., ZUO, S., LI, H., DENG, W. (2021): A crtical review on the interactions of microplastics with heavy metals: Mechanism and their conbined effect on organosms and humans. *Science of the Total Environment* **788**: 147620. doi: 10.1007/s11356-020-08637-z

[14] CHANG, X., XUE, Y., LI, J., ZOU, I., TANG, M. (2019): Potential health impact of environmental micro and nanoplastics pollution. *Journal of Appllied Toxicology* 40: 4–15. doi: 10.1002/jat.3915

[15] COLLARD, F., GASPERI, J., GABRIELSEN, W.G., TASSIN, B. (2019): Plastic particle ingestion by wild freshwater fish: a critical review. *Environmental Science and Pollution Research* **53** (22): 12974–12988. doi: 10.1021/acs.est.9b03083

[16] COLLARD, F., GASPERI, J., GILBERT, B., EPPE, G., AZMI, S., ROCHER, V., TASSIN, B. (2018): Anthropogenic particles in the stomach contents and liver of freshwater fish *Squalius cephalus. Science of The Total Environment* **643**: 1257–1264. doi: 10.1016/j.scitotenv.2018.06.313

[17] COLLARD, F., GILBERT, B., COMPÈRE, P., EPPE, G., DAS, K., JAUNIAUX, T., PARMENTIER E. (2017): Microplastics in livers of European anchovies (*Engraulis encrasicolus*, L.). *Environmental Pollution* **229**: 1000–1005. doi: 10.1016/j.envpol.2017.07.089

[18] COLLARD, F., GILBERT, B., EPPE, G., PARMENTIER, E., DAS, K. (2015): Detection of anthroppogenic particles in fish stomachs: an isolation method adapted to identification by Raman spectroscopy. *Archives of Environmental Contamination and Toxicology* **69**: 331–339. doi: 10.1007/s00244-015-0221-0

[19] ČOMIĆ, LJ., OSTOJIĆ, A. (2005): River and Reservoir Gruža. *In:* Čomić, LJ., Ostojić, A. (eds), The Reservoir Gruža – monography. pp. 21–28. Faculty of Science, Kragujevac. [in Serbian with English summary]

[20] DAVISON, P., ASCH, R.G. (2011): Plastic ingestion by mesopelagic fishes in the North Pacific Subtropical Gyre. *Marine Ecology Progress Series* **432**: 173–180. doi:10.3354/meps09142

[21] DE JESUS PIÑON-COLIN, T., RODRIGUEZ-JIMENEZ, R., ROGEL-HERNANDEZ, E., ALVAREZ-ANDRADE, A., TOYOHIKO WAKIDA, F. (2020): Microplastics in stormwater runoff in a semiarid region, Tijuana, Mexico. *Science of the Total Environment* 704: 135411. doi: 10.1016/j.scitotenv.2019.135411

[22] DE SALES-RIBEIRO, C., BRITO-CASILLAS, Y., FERNANDEZ, A., CABALLERO, M.J. (2020): An end to the controversy over the microscopic detection and effects of pristine microplastics in fish organs. *Scientific Reports* **10** (1): 1–19. doi: 10.1038/s41598-020-69062-3

[23] DE SOUZA MACHADO, A.A., KLOAS, W., ZARFI, C., HAMPEL, S., RILLING, M.C. (2017): Microplastics as an emerging threat to terrestrial ecosystems. *Global Change Biology* **24** (4): 1405–1416. doi: 10.1111/gcb.14020

[24] DEHAUT, A., CASSONE, A.L., FRÈRE, L., HERMABESSIERE, L., HIMBER, C., RINNERT, E., RIVIÈRE, G., LAMBERT, C., SOUDANT, P., HUVET, A., DUFLOS, G., PAUL-PONT, I. (2016): Microplastics in seafood: Benchmark protocol for their extraction and characterization. *Environment Pollution* **215**: 223–233. doi: 10.1016/j.envpol.2016.05.018

[25] DING, J., HUANG, Y., LIU, S., ZHANG, S., ZOU, H., WANG, Z., ZHU, W., GENG, J. (2020): Toxicological effects of nano- and micro-polystyrene plastics on red tilapia: Are larger plastic particles more harmless? *Journal of Hazardous Materials* **396**: 122693. doi:10.1016/j.jhazmat.2020.122693

[26] FAURE, F., CORBAZ, M., BAECHER, H., DE ALENCASTRO, L.F. (2012): Pollution due to plastics and microplastics in Lake Geneva and in the Mediterranean Sea. *Archives des Sciences* **65**: 157–164.

[27] FENDALL, I., SEWELL, M. (2009): Contributing to marine pollution by washing your face: Microplastics in facial cleansers. *Marine Pollution Bulletine* **58**: 1225–1228. doi: 10.1016/j.marpolbul.2009.04.025

[28] FERNANDEZ, B., SANTOS-ECHEANDIA, J., RIVERA-HERNANDEZ, J.R., GARRIDO, S., ALBENTOSA, M. (2020): Mercury interactions with algal and plastic microparticules: comparative role as vectors of metals for the mussel, Mytilus galloprovincialis. *Jornal of Hazardous Materials* **396**: 122739. doi: 10.1016/j.jhazmat.2020.122739

[29] FERREIRA, M., THOMPSON, J., PARIS, A., ROHINDRA, D., RICO, C. (2020): Presence of microplastics in water, sediments and fish species in a urban coastal environment of Fiji, a Pacific small island developing state. *Marine Pollution Bulletin* **153**: 110991. doi: 10.1016/j.marpolbul.2020.110991

[30] FOEKEMA, E.M., DE GRUIJTER, C., MERGIA, M.T., VAN FRANEKER, J.A., MURK, A.J., KOELMANS, A.A. (2013). Plastic in North Sea fish. *Environmental Science of Technology* **47**: 8818–8824. doi: 10.1021/es400931b

[31] FREYHOF, J., KOTTELAT, M. (2008). *Alburnus alburnus* (errata version published in 2020). *The IUCN Red List of Threatened Species* 2008: e.T789A174775859. https://dx.doi.org/10.2305/IUCN.UK.2008.RLTS.T789A174775859.en. Accessed on 17 February 2022.

[32] GALLOWAY, T.S. (2015): Micro and Nano-plastics and Human Health. *In:* Bergmann, M., Gutaw, L., Klages, M. (eds) Marine Anthropogenic Litter. *Springer International Publishing*. p. 13. doi: 10.1007/978-3-319-16510-3

[33] GAMARRO, E., RYDER, J., EDELL. E., OLSEN, R. L. (2020): Microplastics in fish and shellfish- a threat to seafood safaty? *Journal of Aquatic Food Product Technology* **29**: 417 –425. doi: 10.1080/10498850.2020.1739793

[34] GONG, J., XIE, P. (2020): Research progress in sources, analytical methods, eco-environmental effects, and control easures of microplastic. *Chemosphere* **254**: 126790. doi: 10.1016/j.chemosphere.2020.126790

[35] GRILL, G., LEHNER, B., THIEME, M., GEENEN, B., TICKNER, D., ANTONELLI, F., BABU, S., BORRELLI, P., CHENG, L., CROCHETIERE, H., EHALT MACEDO, H., FILGUEIRAS, R., GOICHOT, M., HIGGINS, J., HOGAN, Z., LIP, B., MCCLAIN, M.E., MENG, J., MULLIGAN, M., NILSSON, C., ET AL. (2019): Mapping the world's free-flowing rivers. *Nature* **569**: 215–221. doi: 10.1038/s41586-019-1111-9

[36] GUZZETTI, E., SUREDA, A., TEJADA, S., FAGGIO, C. (2018). Microplastic in marine organism: environmental and toxicological effects. *Environmental Toxicology and Pharmacology* **64**: 164–171. doi: 10.1016/j.etap.2018.10.009

[37] HARRISON, J.P., SAPP, M., SCHRATZBERGER, M., OSBOR, A.M. (2011). Interactions between microorganisms and Marine microplastics: A call for research. *Marine Technology Society Journal* **45** (2): 12–20. doi: 10.4031/MTSJ.45.2.2

[38] HERMSEN, E., MINTEINIG, S.M., BESSELING, E., KOELMANS, A.A. (2018). Quality criteria for the analysis of microplastic in biota samples: a critical review. *Environmental Science & Technology* **52** (18): 10230–10240.
doi: 10.1021/acs.est.8b01611

[39] HOU, L., MCMAHAN, C.D., MCNEISH, R.E., MUNNO, K., ROCHMAN, C.M., HOELLEIN, T.J. (2021): A fish tale: a century of museum specimens reveals increasing microplastic concentrations in freshwater fish. *Ecological Applications* **31** (5): 1553835. doi: 10.1002/eap.2320

[40] JAYA-RAM, A., FUAD, F., ZAKEYUDDIN, M.S., SAH, A. (2018): Muscle fatty acide content in selected freshwater fish from Bukit Merah Reservoir, Perak, Malaysia. *Tropical life sciences research* **29** (2): 103–117. doi: 10.21315/tlsr2018.29.2.8

[41] KARAMI, A., GOLIESKARDI, A., CHOO, C.K., ROMANO, N., HO, Y.B., SALAMATINIA, B. (2016a): A high-performance protocol for extraction of microplastics in fish. *Science of the Total Environment* **578**: 485–494. doi:10.1016/j.scitotenv.2016.10.213

[42] KARAMI, A., GOLIESKARDI, A., HO, Y. B., LARAT, V., SALAMATINIA, B. (2017): Microplastics in eviscerated flesh and excised organs of dried fish. *Scientific Reports* **7** (1): 1-9. doi:10.1038/s41598-017-05828-6

[43] KARAMI, A., ROMANO, N., GALLOWAY, T.S., HAZILAWATI, H. (2016b): Virgin microplastics cause toxicity and modulate the impacts of phenanthrene on biomarker responses in African catfish (*Clarias gariepinus*). *Environmental Research* **151**: 58–70. doi:10.1016/j.envres.2016.07.024

[44] KHAN, F., SHASHOUA, Y., CRAWFORD, A., DRURY, A., SHEPPARD, K., STEWART, K., SCULTHORP, T. (2020): 'The Plastic Nile': First evidence of microplastic contamination in fish from the Nile River (Cairo, Egypt). *Toxics* **8** (2): p. 22. doi: 10.3390/toxics8020022

[45] KOTTELAT, M., FREYHOF, J. (2007): Handbook of European freshwater fishes. *Kottelat, Cornol, Switzerland and Freyhof*: Berlin, Germany. ISBN 978-2-8399-0298-4

[46] KOTTELAT, M. (1997): European freshwater fishes of Europe (exclusive of former USSR), with an introduction for non-systematists and comments on nomenclature and conservation. *Biologia*, Bratislava 52/ Suppl. 5: 1–271.

[47] KÜHN, S., VAN WERVEN, B., VAN OYEN, A., MEIJBOOM, A., BRAVO REBOLLEDO, E.L., VAN FRANEKER, J.A. (2016): The use of potassium hydroxide (KOH) solution as a suitable approach to isolate plastics ingested by marine organisms. *Marine Pollution Bulletin* 115: 86–90. doi: 10.1016/j.marpolbul.2016.11.034

[48] KUKKOLA, A., KRAUSE, S., LYNCH, I., SAMBROOK SMITH, G.H., NEL, H. (2021): Nano and microplastic interactions with freshwater biota – Current knowledge, challenge and future solutions. *Environment International* 152: 106504. doi: 10.1016/j.envint.2021. 106504

[49] LEBRETON, L.C.M., VAN DER ZWET, J., DAMSTEEG, J.W., SLAT, B., ANDRADY, A., REISSER, J. (2017): River plastic emissions to the world's oceans. *Nature Communications* **8**: 15611. doi: 10.1038/ncomms15611

[50] LIMA, A.R.A., COSTA, M.F., BARLETTA, M. (2014): Distribution patterns of microplastics within the plankton of a tropical estuary. *Environmental Research* **132**: 146–155. doi: 10.1016/j.envres.2014.03.031

[51] LUSHER, A.L., WELDEN, N.A., SOBRAL, P., COLE, M. (2017): Sampling, isolating and identifying microplastics ingested by fish and invertebrates. *Analytical Methods* **9** (9): 1346–1360. doi: 10.1039/c6ay02415g

[52] LUSHER, A. (2015): Microplastics in the Marine environment distribution, interactions and effects. *Marine Anthropogenic Litter* 245–307: doi: 10.1007/978-3-319-16510-3_10

[53] MCNEISH, R.E., KIM, L.H., BARRETT, H.A., MASON, S.A., KELLY, J.J., HOELLEIN, T.J. (2018): Microplastics in riverine fish is connected to species traits. *Scientific Reports* **8**: 1–12. doi: 10.1038/s41598-018-29980-9

[54] NEMATOLLAHI, M.J., KESHAVARZI, B., MOORE, F., ESMAEILI, H.R., NASROLLAHZADEH SARAVI, H., SOROOSHIAN, A. (2021): Microplastic fibers in the gut of highly consumed fish species from the southern Caspian Sea. *Marine Pollution Bulletin* **168**: 112461. doi: 10.1016/j.marpolbul.2021.112461

[55] NG, E.I., HUERTA LWANGA, E., ELDRIDGE, S.M., JOHNSTON, P., HU, H.W., GEISSEN, V., CHEN, D. (2018): An overview of microplastic and nanoplastic pollution in agroecosystems. *Science of the Total Environment* **627**: 1377–1388. doi: 10.1016/j.scitot env.2018.01.341

[56] NUELLE, M.T., DEKIFF, J.H., REMY, D., FRIES, E. (2014): A new analytical approach for monitoring microplastic in marine sediments. *Environmental Pollution* **184**: 161–169. doi: 10.1016/j.envpol.2013.07.027

[57] OCKELFORD, A., CUNDY, A. EBDON, J.E. (2020): Storm response of fluvial sedimentary microplastics. *Scientific Reports* **10**: 1865. doi: 10.1038/s41598-020-58765-2

[58] O'CONNOR, D., PAN, S., SHEN, Z., YINAN SONG, Y., JIN, Y., WU, W.M., HOU, D. (2019): Microplastics undergo accelerated vertical migration in sand soil due to small size and wet-dry cycles. *Environment Pollution* **247**: 527–53. doi: 10.1016/j.envpol.2019.03.092

[59] ORY, N.C., SOBRAL, P., FERREIRA, J.L., THIELE, M. (2017): Amberstripe scad *Decapterus muroadsi* (Carangidae) fish ingest blue microplastics resembling their copepod prey along the coast of Rapa Nui (Easter Island) in the South Pacific subtropical gyre. *Science of the Total Environment* 586: 430–137. doi: 10.1016/j.scitot env.2017.01.175

[60] PARKER, B., ANDREOU, D., GREEN, I.D., BRITTON, J.R. (2021): Microplastics in freshwater fishes: Occurrence, impacts and future perspectives. *Fish and Fisheries* **22** (3): 467–488. doi: 10.1111/faf.12528

[61] PATIL, D.B., SHARMA, A.R. (2011): Study on the corrosion kinetics of iron in acid and base medium. *Journal of Chemistry* **8**: S358-S362. doi: 10.1155/2011/294792

[62] PAZOS, R.S., MAIZTEGUI, T., COLAUTTI, D.C., PARACAMPO, A.H., GOMEZ, N. (2017): Microplastics in gut contents of coastal freshwater fish from Riodela Plata estauary. *Marine Pollution Bulletin* **122**: 85–90. doi: 10.1016/j.marpolbul.2017.06.007

[63] PETERS, C.A., BRATTON, S.P. (2016): Urbanization is a major influence on microplastic ingestion by fishes in watersheds of the Gulf of Mexico. *Marine Pollution Bulletin* **210**: 380-387. doi: 10.1016/j.envpol.2016.01.018

[64] PHILLIPS, M.B., BONNER, T.H. (2015): Occurrance and amount of microplastic ingested by fishes in watersheds of the Gulf of Mexico. *Marine Pollution Bulletin* **100** (1): 264–269. doi: 10.1016/j.marpolbul.2015.08.041

[65] PRATA, J.C., SEQUEIRA, I.F., MONTEIRO, S.S., PATRÍCIO SILVA, A.L., DA COSTA, J.P., DIAS-PEREIRA, P., SILVA FERNANDES, A.J., COSTA, F.M., DUARTE, A.C., ROCHA-SANTOS, T. (2021): Preparation of biological samples for microplastic identification by Nile Red. *Science of the Total Environmental* 783: 147065. doi: 10.1016/j.scitotenv. 2021.147065

[66] PROKIĆ, M.D., GAVRILOVIĆ, B.R., RADOVANOVIĆ, T.B, GAVRIĆ, J.P., PETROVIĆ, T.G., DESPOTOVIĆ, S.G., FAGGIO, C. (2021): Studying microplastics lessons from evaluated literature on animal model organisms and experimental approaches. *Journal of Hazardous Materials* **414**: 125476. doi: 10.1016/j.jhazmat.2021.125476

[67] PROKIĆ, M.D., RADOVANOVIĆ, T.B., GAVRIĆ, J.P, FAGGIO, C. (2019): Exotoxicological effects of microplastics: exanimation of biomarkers, current, state and future perspectives. *Trends in Analytical Chemistry* **111**: 37–46. doi: 10.1016/j.trac.2018. 12.001

[68] QIAO, R., LU, K., DENG, Y., REN, H., ZHANG, Y. (2019): Combined effects of polystyrene microplastics and natural organic matter on the accumulation and toxicity of copper in zebrafish. *Science of the Total Environment* **682**: 128–137. doi: 10.1016/j.scitotenv.2019.05.163

[69] RASTA, M., SATTARI, M., TALESHI, M.S., NAMIN, J.I. (2021): Microplastics in different tissues of some commercially important fish species from Anzali Wetland in the Southwest Caspian Sea, Northern Iran. *Marine Pollution Bulletin* **169**: 112479. doi: 10.1016/ j.marpolbul.2021.112479

[70] RE, V. (2019): Shedding light on the invisible: addressing the potential for groundwater contamination by plastic microfibers. *Hydrogeology Journal* **27**: 2719–2727. doi:10.1007/s10040-019-01998-x

[71] ROCH, S., FRIEDRICH, C. BRINKER, A. (2020): Uptake routes of microplastics in fishes: practical and theoretical approaches to test existing theories. *Scientific Reports* **10**: 3896. doi: 10.1038/s41598-020-60630-1

[72] SÁNCHEZ, L.S., GRELAUD, M., ORELLANA, J.G., ZIVERI, P. (2019): River deltas as hotspots of microplastic accumulation: The case study of the Ebro River (NW Mediterranean). *Science of the Total Environment* **687**: 1186–1196. https://doi.org/10.1016/j.scitotenv.2019.06.168

[73] SÁNCHEZ, W., BENDER, C., PORCHER, J.M. (2014). Wild gudgeons (*Gobio gobio*) from French rivers are contaminated by microplastics: preliminary study and first evidence. *Environmental Research* **128**: 98–100. doi: 10.1016/j.envres.2013.11.004

[74] SANTANA, M.F.M., MOREIRA, F.T., TURRA, A. (2017): Trophic transference of microplastics under a low exposure scenario: Insights on the likelihood of particle cascading along marine food-webs. *Marine Pollution Bulletin* **121**: 154–159. doi: 10.1016/j.marpolbul.2017.05.061

[75] SIMIĆ, V., SIMIĆ, S., PAUNOVIĆ, M., RADOJKOVIĆ, N., PETROVIĆ, A., TALEUSI, T., MILOŠEVIĆ, Đ. (2016): The *Alburnus* benthopelagic fish species of the Western Balkan Peninsula: An Assesment of their sustainable use. *Science of The Total Environment* **540**: 410–417. doi: 10.1016/j.scitotenv.2015.07.030

[76] STOJIČIĆ, S., ŽIVKOVIĆ, S., QIAN, W., ZBANG, H., HAAPASALO, M. (2010): Tissue dissolution by sodium hypochlorite: effect of concentration, temperature, agitation, and surfactant. *Journal of Endodontics* **36**: 1558–1562. doi: 10.1016/j.joen.2010.06.021

[77] SU, L., NAN, B., HASSELL, K.L., CRAIG, N.J., PETTIGROVE, V. (2019): Microplastics biomonitoring in Australian urban wetlands using a common noxious fish (*Gambusia holbrooki*). *Chemosphere* **228**: 65–74. doi: 10.1016/j.chemosphere.2019.04.114

[78] THIELE, C.J., HUDSON, M.D., RUSSELL, A.E., SALUVEER, M., SIDAAN-HADDAD G. (2021): Microplastics in fish and fishmeal: an emerging environmental challenge? *Scientific Reports* **11**: 2045. doi: 10.1038/s41598-021-81499-8

[79] THIELE, C.J., HUDSON, M.D., RUSSELL, A.E. (2019): Evaluation of existing methods to extract microplastics from bivalve tissue: Adapted KOH digestion protocol improves filtration at single digit pore size. *Marine Pollution Bulletin* **142**: 384–393. doi: 10.1016 /j.marpolbul.2019.03.003

[80] THOMPSON, R., OLSEN, Y., MITCHELL, R., DAVIS, A., ROWLAND, S., JOHN, A., MCGONIGLE, D., RUSSELL, A. (2004): Lost at sea: where is all the plastic? *Science* **304**: 838–838. doi: 10.1126/science.1094559

[81] UURASJÄRVI, E., SAINIO, E., SETÄLÄ, O., LEHTINIEMI, M., KOISTINEN, A. (2020): Validation of an imaging FTIR spectroscopic method for analyzing microplastics ingestion by Finish lake fish (*Perca fluviatilis* and *Coregonus albula*). *Environmental Pollution* **288**: 117780. doi: 10.1016/j.envpol.2021.117780

[82] WANG, W., GE, J., YU, X. (2020): Bioavailability and toxicity of microplastics to fish species: A review. *Ecotoxicology and Environmental Safety* **189**: 109913. doi: 10.1016/j.ecoenv.2019.109913

[83] YAO, L., HUI, Z., YANG, Z., CHEN, X., XIAO, A. (2020): Freshwater microplastics pollution: detecting and visualizing emerging trends based on Citespace II. *Chemosphere* **245**: 125627. doi: 10.1016/j.chemosphere.2019.125627

[84] ZITOUNI, N., BOUSSERRHINE, N., BELBEKHOUCHE, S., MISSAWI, O., ALPHONSE, V., BOUGHATASS, I., BANNI, M. (2020): First report on the presence of small microplastics (≤ 3µm) in tissue of the commercial fish *Serranus scriba* (Linnaeus. 1758) from Tunisian coasts and associated cellular alterations. *Environmental Pollution* **263**A: 114576. doi: 10.1016/j.envpol.2020.114576