

## ЭКОЛОГИЯ

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### **Microplastics in fish gut, first records from the Tom River in West Siberia, Russia**

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*This preliminary study investigated the abundance of microplastic particles in gastrointestinal tracts of the dace (*Leuciscus leuciscus* L.) from the Tom River, a large tributary of the Ob River in West Siberia. A total of 13 dace specimens of 2+ to 4+ years of age were studied. Microplastic particles extracted from fish guts were counted and classified by shapes and sizes. In average  $204 \pm 28.7$  items of microplastics were detected for one dace specimen. Microplastic particles were categorized as fragments of irregular shape (70%), spheres (16%), films (7%) and fibers (7%), with size ranging from  $<0.15$  to  $2.00$  mm. The vast majority of detected microplastic particles (almost 80%) were less than  $0.15$  mm by their largest dimension. These data provide the first evidence of microplastics in fish from the Ob River system.*

**Key words:** microplastics; aquatic pollution; rivers; hydrobionts; fish; food chains.

#### **Introduction**

In recent decades, plastic debris have been found in aquatic ecosystems around the world as a direct consequence of industrial, consumer waste and wastewater emissions [1]. Pollution of the marine environment with microplastics (particles  $< 5$  mm) is especially intensively studied. The number of studies analyzing the abundance of microplastics in the marine environment began to grow rapidly after 2004, when the seminal paper by Thompson et al. [2] had been published. Microplastics are currently defined as polymer particles smaller than 5 mm [3], or 1 mm [4] in the largest axis. Some authors also use the terms “large” and “small” for microplastics (2-5 mm and 0.5-2 mm, respectively) [5].

The adverse effects of plastics when swallowed by hydrobionts, suffocating or entangling them, have been documented for a variety of marine species, so these

materials were found hazardous to marine fauna [6]. Since plastic breaks down into smaller pieces in an aquatic environment to form microplastics, it is believed that it can enter food chains [6-7]. Both field and laboratory studies suggest that fish absorb micro-sized plastic particles, e.g. originating from synthetic clothing and cleaning products containing plastic granules [7-8]. Ingestion of microplastics by hydrobionts and their accumulation in food chains provides a potential pathway for the transfer of other pollutants and potentially toxic additives to living organisms up to humans with uncertain consequences for their health [9-12].

Most studies on microplastics abundance have focused on marine organisms. Microscopic plastic particles have been detected in marine benthic organisms, especially in bivalves [7, 13-15]. Several reports describe microplastics in the gut of marine fish. Microplastics were detected in semipelagic fish bogue (*Boops boops* L.) around the Balearic Islands [16]. Microplastic ingestion is documented in commercially relevant fish species from the Spanish Atlantic coast and Mediterranean Sea - *Scyliorhinus canicula* L., *Merluccius merluccius* L. and the *Mullus barbatus* L. [17-18]. Ingestion of anthropogenic microfibrils and microfragments by the European anchovy (*Engraulis encrasicolus* L.) of the Mediterranean Sea has been recently studied [19].

Much less attention is paid to riverine fish. First evidence of microplastics ingestion by fish from the Amazon River was received not so long ago [20]. McNeish et al. [21] measured microplastic abundance in fish from three major tributaries of Lake Michigan, the Muskegon River, the Milwaukee River, and the St. Joseph River. The results obtained from these two and several other [22] studies suggested microplastic pollution is common in river food webs.

The aim of this research is to assess the ingestion of microplastics by fish from the Tom River, a large tributary of the Ob River in West Siberia. It should be noted that the abundance of microplastics in fish of the Ob River system has not been studied to date, as well as in fish in other rivers of Russia.

### Materials and Methods

The object of the study was common dace, (*Leuciscus leuciscus* L.) from the Tom River in Western Siberia, Russia. *L. leuciscus* is a widespread freshwater fish of Cyprinidae family [23]. Thirteen specimens of common dace were caught using a fishing rod on the right bank of the Tom River within the city of Tomsk (56°27'33"N, 84°56'056"E) on April 01, 2020. Fish were frozen, transported to the laboratory and stored at -20 °C before the laboratory analysis. Subsequently, each fish was defrosted and examined.

The total length of the body (L) and the standard length (from the tip of the snout to the posterior end of the midlateral portion of the hypural plate, l) were measured using a caliper to the nearest 1 mm. Total weight (Q) and body weight without viscera (q) (wet weight, ± 1 g) were determined using an electronic balance. Scales were taken in the region of the dorsal fin (10-15 pcs. in each

specimen). The fish age was determined by the number of annual rings on the fish scales using a dissecting microscope. The sex of the fish was determined visually by gonads as described by Pravdin [24].

The fish was dissected, the gastrointestinal tract (oesophagus, stomach, and intestine) was removed for further processing and stored at  $-20\text{ }^{\circ}\text{C}$  until analysis according to the method published by Bellas et al. [17]. To extract microplastics from the gut, we used modified protocol developed by Claessens et al. [25] based on acid digestion of the soft tissues. The digestion procedure consisted of 12 h destruction of the fish guts in 25 mL of  $\text{HNO}_3$  (22.5 M) at room temperature, followed by 2 h of boiling in a water bath. Then the mixture was diluted to 100 mL with 26% NaCl solution for total salt concentration of 20% and left for additional 12 h for the density separation. After separation, the upper fraction was vacuum filtered using  $0.45\text{ }\mu\text{m}$  mixed cellulose ester membrane filter (MF-Millipore). Filters were rinsed with 2% KOH solution for saponification of fats and inspected by light microscopy (stereomicroscope Micromed MC2) using digital camera and ToupView 3.7.6273 software.

The abundance of microplastics of different shape and sizes was evaluated as the number of particles per fish specimen. The microplastics particles extracted from fish guts were classified into four groups by their shape [26]: spheres, films, fibers/lines, and fragments of irregular shape (including foams). The particles of microplastics were also classified by their major dimension into seven groups:  $<0.15\text{ mm}$ ,  $0.15\text{-}0.30\text{ mm}$ ,  $0.30\text{-}1.00\text{ mm}$ ,  $1.00\text{-}2.00\text{ mm}$ ,  $2.00\text{-}3.00\text{ mm}$ ,  $3.00\text{-}4.00\text{ mm}$  and  $4.00\text{-}5.00\text{ mm}$ .

The Mann-Whitney U test [27] was used to compare differences in biological parameters and microplastics abundance. Statistically significant differences were considered at  $p \leq 0.05$

## Results and Discussion

This research analyzed the anthropogenic microparticles in the guts of the dace (*Leuciscus leuciscus* L.) from the Tom River, the right tributary of the Ob River. The daces caught in the Tom River on April 01, 2020 were represented by three age groups: 2+ years (4 specimens), 3+ years (7 specimens), and 4+ years (2 specimens). The sex ratio in the group was: females - 3 specimens (23%), males - 10 specimens (77%). The dimensional features of the fish specimens are presented in Table 1. Statistical comparison of microplastics abundance in the gastrointestinal tract of fish using the nonparametric Mann-Whitney test did not reveal significant differences between groups of males and females, as well as between groups of different ages (2+ and 3+).

According to the analysis results,  $204 \pm 28.7$  items of microplastics were detected in each dace specimen (Table 2). The found value for total microplastic particles per fish (items fish<sup>-1</sup>) is quite high. The average of microparticle content in the gut of the Mediterranean Sea fish were  $< 1.00$  items per fish for anchovies [19]

and  $1.56 \pm 0.50$  items per fish for the red mullet [17]. The number of microplastics found in the gastrointestinal tract of *Dicentrarchus labrax* L., *Trachurus trachurus* L. and *Scomber colias* Gmelin from North East Atlantic Ocean was  $1.3 \pm 2.5$  items per individual [28]. Microplastics abundance in riverine fish in tributaries of Lake Michigan, USA ranged from  $10.0 \pm 2.30$  to  $13.0 \pm 1.60$  items fish<sup>-1</sup> and was not significantly different among the three rivers, the Muskegon River, the Milwaukee River, and the St. Joseph River [20].

Table 1  
Dimensional features of the dace, the Tom River, Tomsk, April 01, 2020

Parameters	L, cm	l, cm	Q, g	q, g
$M \pm m_M$	162 $\pm$ 2.10	133 $\pm$ 1.68	36.2 $\pm$ 1.40	31.7 $\pm$ 1.46
Min-Max	148-175	123-145	27.7-43.9	23.7-40.3
Sample variance	57.1	36.9	25.7	27.6
Standard deviation	7.55	6.07	5.06	5.25
Coefficient of variation	4.67	4.57	14.0	16.6

Note: *L* - Total length, *l* - Standard length, *Q* - Total weight, *q* - Body weight without viscera.

Table 2  
The content of microplastic particles of different shapes and sizes in the fish gut (items fish<sup>-1</sup>)

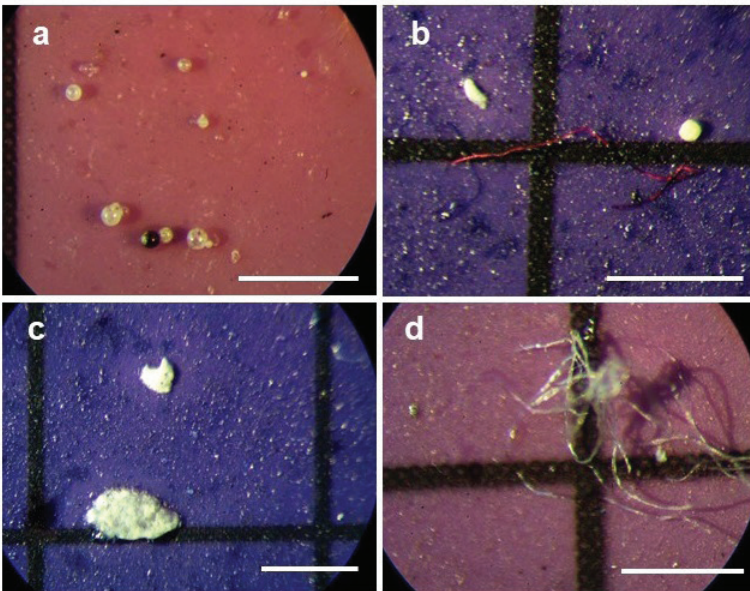
Parameters	Spheres	Films	Fibers	Fragments	<0.15	0.15-0.30	0.30-1.00	1.00-2.00	Total items fish <sup>-1</sup>
$M \pm m_M$	31.5 $\pm$ 24.4	14.9 $\pm$ 2.10	13.9 $\pm$ 2.90	144 $\pm$ 10.2	162 $\pm$ 27.9	24.2 $\pm$ 2.50	16.2 $\pm$ 4.00	1.20 $\pm$ 0.60	204 $\pm$ 28.7
Min-Max	0.00-323	5.00-26.0	2.00-35.0	96.0-225	74.0-468	11.0-39.0	1.00-37.0	0.00-7.00	114-512
Standard deviation	88.0	7.60	10.4	36.8	101	9.00	14.5	2.00	104
Coefficient of variation	279	50.9	74.8	25.7	62.0	37.1	89.1	173	50.7

Thus, river fish are in some cases characterized by a relatively higher content of microplastics in the gastrointestinal tract as compared to sea fish. Riverine fish are more susceptible to ingesting microplastics because watercourses flow through many settlements which are anthropogenic sources of primary microplastics and secondary microplastics derived from plastic waste. Accumulation of microplastics in water systems in proximity to cities with a relatively high plastic use was documented in several studies reviewed by Wong et al. [22]. According to the review, microplastics were more common in areas with a high population density or proximity to urban centers. A study on microplastics in the Laurentian Great Lakes attributed the large spatial variability of pollution to population density by Eriksen et al. [29]. The urban sources of microplastics were also reported by Sanchez et al. [30] who detected the presence of microplastics in the

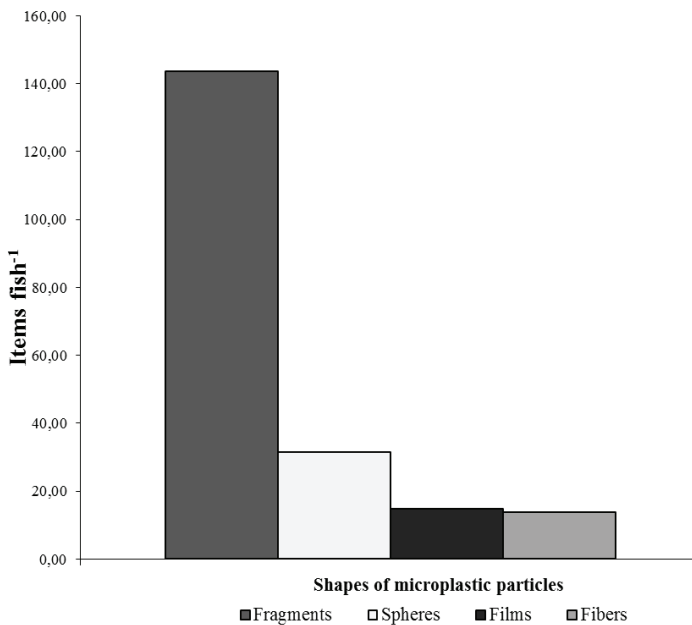
guts of gudgeon (*Gobio gobio* L.) sampled from French urban rivers whereas none were found in gudgeons from sparsely populated areas.

In our study, 100% of the fish contained microplastics in their gut. As previously shown, 28% of 76 sea fish in Indonesia contained microparticles in the gut [31]; 67% of fish caught off the coast of Portugal contained at least one plastic particle [32]; in recent study microplastics were found in 42-62% of fish specimens from Portuguese coastal waters, depending on the species and their feeding type [26]. The frequency of occurrence of plastic debris per species from the Amazon River estuary varied between 18.7% (*Cynoscion microlepidotus* Cuvier) and 100% (*Bagre marinus* Mitchell, *Caranx hippos* L.), a positive correlation was found between fish standard length and number of microplastic particles in the gut [20]. 85% of fish individuals from Lake Michigan tributaries contained microplastic in their digestive tracts, plastic debris content in fish digestive tracts was different among species and feeding groups [21].

Microplastics found in dace from the Tom River were diverse in shape (Fig. 1) and included fragments, spheres, fibers and films ranging from <0.15 to 2.00 mm. Microfragments were the most abundant. In average  $144 \pm 10.2$  fragments fish<sup>-1</sup> were detected, that comprised 70% of the total particles (Table 2 and Fig. 1). The remaining 30% of microplastic particles were spheres (16%), films (7%) and fibers (7%) as shown in Fig. 2. The content of fragments of microplastics in the fish gut was significantly higher ( $p \leq 0.01$ ) than other types of particles (Fig. 2).



**Fig. 1.** Diversity of microplastics from the guts of the Tom River dace: spheres (a), sphere, fibers and fragment (b), fragments (c), fibers (d). Scale bar is 1 mm. Microphotographs by Dmitry V. Antsiferov



Mann–Whitney U-test

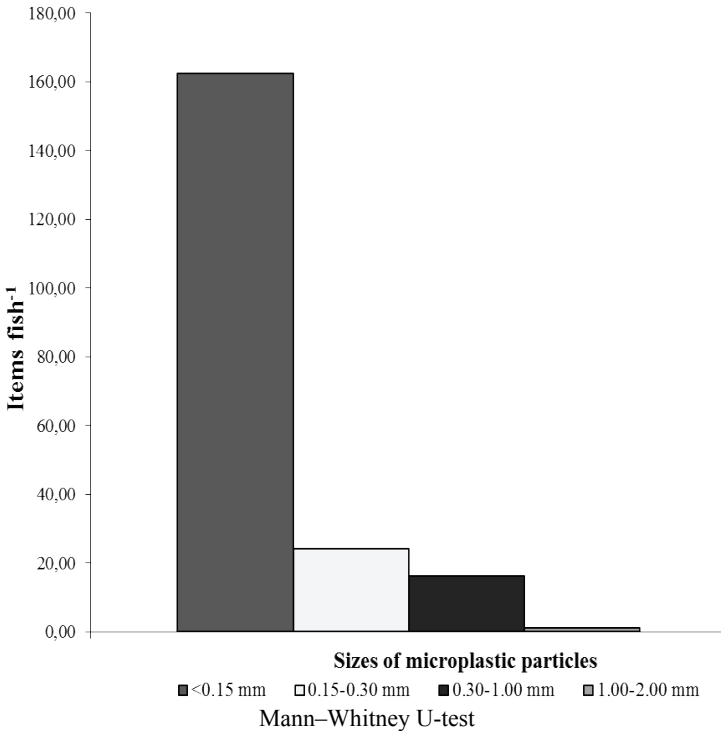
	Fr	Sp	Ff
Sp	$p \leq 0.01$		
Ff	$p \leq 0.01$	no	
Fb	$p \leq 0.01$	no	no

Fr - Fragments, Sp - Spheres, Ff - Films, Fb - Fibers

**Fig. 2.** Abundance of microplastic shapes in the guts of the Tom River dace (a), differences in microplastic shapes abundance (b)

The prevalence of one type of particle or another may differ from site to site. In contrast to our data, fragments were rare in fish from Lake Michigan tributaries and accounted for approximately 2.5-3% while fibers comprised over 90% of the total microplastics [21]. Microspheres, namely pellets, were the most abundant (97.4%) among plastic particles ingested by fish from the Amazon River estuary [20].

The majority of detected microplastic particles ( $162 \pm 27.9$  items fish<sup>-1</sup> or almost 80%) were less than 0.15 mm by their largest dimension (Table 2 and Figure 3). The content of the smallest (<0.15 mm) microplastics in the fish gut was significantly higher ( $p \leq 0.01$ ) than other sizes of particles (Fig. 3). 20% of the particles detected in the fish gut were in the 0.15-2.00 mm size range. The largest fraction of the plastic particles between 0.15 and 2.00 mm were in the 0.15-0.30 mm size range followed by 0.30-1.00 mm sized particles (Table 2 and Fig. 3). Only one fragment of 3.00-4.00 mm size range was found in the studied dace digestive tracts. No particles of 2.00-3.00 or 4.00-5.00 mm were detected.



Mann–Whitney U-test

	<0.15	0.15-0.30	0.30-1.00
0.15-0.30	$p \leq 0.01$		
0.30-1.00	$p \leq 0.01$	no	
1.00-2.00	$p \leq 0.01$	$p \leq 0.01$	$p \leq 0.01$

**Fig. 3.** Abundance of microplastic sizes in the guts of the Tom River dace (a), differences in microplastic sizes abundance (b)

The size of microparticles of plastic ingested by aquatic organisms can affect their behavior in the body. It is accepted that microplastics of size less than 0.150 mm are absorbed by the intestine [10]. Micro- and nanoplastics can translocate from the intestine to the circulatory system or surrounding tissue and persist in the animal’s body [11], promoting uptake of plastic debris in food chains.

### Conclusions

Preliminary assessment conducted in this study suggests that the abundance of microplastics in fish of the Tom River is very high. 100% of thirteen dace (*Leuciscus leuciscus* L.) individuals from the Tom River caught in April, 2020 contained plastic debris in their gastrointestinal tract. In average  $204 \pm 28.7$  items of microplastics were detected for one dace specimen. Fragments were the most



abundant comprising 70% of the total particles, the remaining 30% of microplastic particles were spheres (16%), films (7%) and fibers (7%). The vast majority of the detected particles (almost 80%) were less than 0.15 mm by their largest dimension. 20% of the particles detected in the fish gut were in the 0.15-2.00 mm size range.

There were no significant differences in the abundance of microplastics in the gastrointestinal tract of fish between groups of males and females, as well as between groups of different ages. To identify such relationships, a long-term and large-scale study should be carried out.

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