

Original article

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## Microplastic ingestion by fish with different feeding habits in the Ob and Yenisei rivers

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**Abstract.** Microplastic particles in the size range 0.15 to 5.00 mm were quantified and characterised in the gastrointestinal tracts of three wild freshwater fish (n = 141) from the Ob and Yenisei rivers, including common ruffe (*Gymnocephalus cernua* L.), Siberian dace (*Leuciscus baicalensis* Dybowski) and European perch (*Perca fluviatilis* L.). Microplastics were found in the gastrointestinal tract of 62% of the fish examined, ranging from 18.5% in the Yenisei perch sample to 92.6% in the Ob ruffe sample. The vast majority of microplastics in all fish species were fibres (up to 99.7% of all particles detected in perch), followed by irregularly shaped fragments (up to 22.7% in ruffe), with no preference between the three species. Spheres and films were found exclusively in dace and ruffe, with proportions of 3.7% and 1.2% respectively. Particles consisted of polyethylene terephthalate, polypropylene, polyamide and other synthetic polymers with a significant proportion of highly degraded polymers. The mean MP content in fish GITs ranged from 0.44 ± 0.25 items per individual in Yenisei perch to 3.81 ± 0.55 items per individual in Ob ruffe. Particle burdens in fish were significantly higher in the Ob than in the Yenisei (p < 0.05), which may reflect the common level of plastic contamination in these two rivers. MP ingestion varied in species with different feeding habits as ruffe (benthivorous) > dace (omnivorous) > perch (hunter) in both rivers (p < 0.01). This study was the first to quantify MP consumption by freshwater fish of different species in the Ob and Yenisei rivers and to identify patterns associated with different feeding habits.

*The paper contains 4 Figures, 2 Tables and 37 References.*

**Keywords:** microplastics, freshwater fish, Ob River, Yenisei River, bioindication, particle ingestion patterns, feeding habits

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Научная статья

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## Потребление микропластика рыбами с различным пищевым поведением в реках Обь и Енисей

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**Аннотация.** Проведен количественный учет и дана характеристика микропластика размерного диапазона от 0,15 до 5,00 мм в желудочно-кишечном тракте трех видов диких пресноводных рыб ( $n = 141$ ) из рек Обь и Енисей, включая обыкновенного ерша (*Gymnocephalus cernua* L.), сибирского ельца (*Leuciscus baicalensis* Dybowski) и речного окуня (*Perca fluviatilis* L.). Микропластик был обнаружен в желудочно-кишечном тракте 62% исследованных рыб, доля рыб с пластиком варьировала от 18,5% в выборке енисейского окуня до 92,6% в выборке обского ерша. Подавляющее большинство микропластика во всех видах рыб составляли волокна (до 99,7% всех частиц, обнаруженных в желудочно-кишечном тракте окуней), затем следовали фрагменты неправильной формы (до 22,7% у ерша). Достоверные отличия в поглощении частиц той или иной формы между тремя видами рыб отсутствовали. Сферы и пленки были обнаружены исключительно в выборках ельца и ерша, их доля составляла лишь 3,7 и 1,2%, соответственно. Найденные в рыбах частицы состояли из полиэтилентерефталата, полипропилена, полиамида и других синтетических полимеров со значительной долей деградированных полимеров. Среднее содержание микропластика в желудочно-кишечном тракте рыб варьировало от  $0,44 \pm 0,25$  шт. на особь у окуней из Енисея до  $3,81 \pm 0,55$  шт. на особь у ершей из Оби. Содержание частиц в рыбах было значимо выше для выборок из Оби, чем из Енисея ( $p < 0,05$ ), что может отражать общий уровень пластикового загрязнения в этих двух реках. Поглощение микропластика у видов с разным пищевым поведением распределялось в последовательности: ерш (бенгофаг) > елец (эврифаг) > окунь (хищник) в обеих реках ( $p < 0,01$ ). В данном исследовании впервые проведена количественная оценка потребления микропластика пресноводными рыбами разных видов в реках Обь и Енисей и выявлены закономерности, связанные с различным пищевым поведением.

**Ключевые слова:** микропластик, пресноводные рыбы, Обь, Енисей, биоиндикация, особенности поглощения частиц, пищевое поведение

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## Introduction

Freshwater fish, which are considered to be the main recipients and bioindicators of microplastic < 5 mm (MPs) pollution in rivers and lakes, are a valuable model for developing knowledge on the effects of plastics on biota [1]. Published data show that MPs and smaller particles (nanoplastics) can have adverse effects on freshwater fish at almost all biological levels, including the cellular, tissue and organ, individual, population, aquatic community and ecosystem levels, causing metabolic disorders, altered feeding behaviour, mortality and changes in community structure [2-3]. The uptake and effects of MP particles in freshwater fish were first documented in 2013 [4], and the number of publications has steadily increased since then. More recently, evidence of plastic particles in commercial wild fish and fish products has emerged [5], highlighting the potential impact of MP contamination of the hydrosphere on human health [6-7]. Freshwater fish are important contributors to both aquatic and terrestrial food chains; in aquatic ecosystems, fish are among the most important consumers or top predators, occupying an obvious position in the food web [8]. Many of Siberia's river fish are commercially important and serve as a source of protein for local populations, highlighting the importance of research in this area.

Research on the uptake of MPs by wild fish around the world is mainly based on examination of the gastrointestinal tract (GIT), as ingestion is considered to be the main external route for particles [9]. The trophic guild can be an important factor in explaining the presence and abundance of MPs in the GIT of a fish [10]. MPs can be ingested by fish with different feeding habits in several ways [11]: (1) predators ingest particles as prey; (2) planktophages ingest MPs passively when filtering nutrients; (3) organisms ingest MPs accidentally from the environment during non-filtering feeding; (4) organisms obtain MPs through food chains; and (5) organisms ingest MPs passively from the surrounding aquatic environment during respiration.

MP ingestion has previously been demonstrated for omnivorous dace in tributaries of the Ob and Yenisei [12-13], but not in the main rivers. The consumption of MP by perch and ruffe in Siberian rivers and its patterns for fish with different feeding habits are of interest. This study aimed to quantitatively evaluate the consumption of MP by three freshwater fish species with different feeding strategies,

including Siberian dace, European perch and common ruffe in the main rivers of the Ob and Yenisei. The Siberian dace is a freshwater benthopelagic omnivorous fish of the Cyprinidae family that feeds on insect larvae such as caddis and mayflies, terrestrial insects, cladocerans, copepods, algae and higher aquatic plants [14]. The European perch and the common ruffe are two demersal carnivorous freshwater fish of Percidae family [15]. The larvae and small juveniles of perch usually feed on planktonic invertebrates; the adult is a typical hunter, while the main food of adult ruffe is zoobenthic and nektobenthic organisms [15-16]. It was shown that the niche widths of perch and ruffe differ in terms of temperature, light intensity and distance from the bottom, providing evidence for complementary niche widths [17]. For the purposes of this study, we have classified dace, perch and ruffe as 'omnivorous', 'hunters' and 'benthivorous' respectively, based on their feeding habits.

## Materials and Methods

*Fish Sampling and Biological Analysis.* The objects of the study were freshwater fish commonly found in Siberian rivers, namely European perch (*Perca fluviatilis* L., 1758), common ruffe (*Gymnocephalus cernua* L., 1758) and Siberian dace (*Leuciscus baicalensis* Dybowski, 1874). A total of 141 fish individuals of these species were caught using a fishing rod and analysed from the Ob (n = 74) and Yenisei (n = 67) rivers. The Yenisei fish collection was carried out in the Krasnoyarsk region from 20 to 29 June 2022. Dace (n = 20) and perch (n = 20) were collected from the Yenisei River within Krasnoyarsk (55°59' N 92°50' E), another 7 perch individuals were caught near Bor settlement (61°35' N 90°02' E), ruffe (n = 20) were collected in Dudinka (69°24' N 86°09' E). All Ob samples were caught in the Nizhnyaya Fedorovka settlement, Molchanovo district, Tomsk Oblast (57°42'24' N 83°51'32' E). Perch (n = 27) and ruffe (n = 24) were caught in June 2022; dace (n = 23) were caught in August 2023 at the same site.

Total fish length (L) and standard body length (l) were measured to the nearest 1 mm. Total body weight (Q) and body weight without viscera (q) ( $\pm 0.1$ – $0.01$  g) were determined using an electronic balance. Fish GIT fullness was determined visually and scored from 1 to 5 using the Lebedev scale [18]. Fish age was determined by the number of annual rings on the fish scales under a dissecting microscope. The sex of the fish was determined visually from the gonads as described by Pravdin [19].

Fish individuals were dissected and the entire gastrointestinal tract (GIT), including oesophagus, stomach and intestines, was removed for further processing as previously described [20]. GITs were fixed individually in 70% ethyl alcohol [21] prior to MP extraction.

*Extraction, Quantification and Identification of Microplastics.* To extract MPs from fish GITs, we used a protocol based on alkaline digestion of soft tissues followed by density separation [22]. The digestion procedure involved the destruction of fish organs for 48 h in 100 mL of 10% KOH at 55°C. After tissue destruction, MPs were collected by density separation in a saturated NaCl solution (1.19 g/cm<sup>3</sup>) overnight to avoid mineral particles. To remove products of fat saponification, the upper phase from the separation funnel was additionally treated

with 96% ethyl alcohol (10% of sample volume). After treatment, each sample was individually vacuum filtered through a glass fiber filter with a pore size of 1  $\mu\text{m}$  (Membrane Solutions, China).

Particles collected on the membrane filters were first examined by light microscopy (Micromed MC2 stereomicroscope) using a digital camera and ToupView 3.7.6273 software, and probed using a 'hot needle test' [23]. The polymer composition of the particles was determined using microscopy coupled with Raman spectroscopy ( $\mu\text{Raman}$ ) as described previously [24]. Spectra were obtained using an InVia Basic (Renishaw, UK) confocal Raman dispersion spectrometer fitted with a DM 2500 M microscope (Leica, Germany). Excitation was performed with a continuous wave semiconductor laser (wavelength 785 nm, 100 mW). The maximum laser intensity did not exceed 10% to avoid heating and destruction of the sample. The signal spectrum was accumulated during 1 s and the number of scans reached 200. The spectra were measured in the range of 100 to 1800  $\text{cm}^{-1}$  with a spectral resolution of 1  $\text{cm}^{-1}$ . The spectra obtained were then compared with those of known plastic materials available in the PublicSpectra database.

*Quality Assurance and Control.* Biological analysis was performed directly after sampling to avoid particle loss and underestimation, as recommended [25]. Each individual fish was rinsed with distilled water prior to dissection to remove any contamination from the fish body, and final dissection was performed in a filtered air laminar flow box. Blanks containing no biological material were analysed to control for air and reagent contamination as previously described [21] ( $n = 5$  per 10 fish). Quantitative data were then normalised to account for blank results (0 to 2 fibers per filter).

*Analysis and Interpretation of the Data.* MP abundance in GITs was assessed as the number of particles per individual fish (items/ind.) and interpreted in the paper as the arithmetic mean  $\pm$  standard error of the mean for each sample. Standard deviation and variation of MP content values per individual are also shown. MP particles extracted from fish GITs were classified by shape into four groups: spheres, films, fibers and irregularly shaped fragments, as previously done for Siberian dace in the Yenisei tributary [13]. The particles were also classified into groups according to their largest dimension: 0.15-0.30 mm, 0.31-1.00 mm, 1.01-5.00 mm. The proportion (%) of each shape and size group of MPs was calculated for each of the six fish samples examined.

Differences in MP uptake by dace, perch and ruffe between the Ob and the Yenisei were assessed using the Mann-Whitney U test, as were differences in MP abundance between males and females, and adults and juveniles. The Kruskal-Wallis H test was used to determine the significance of differences in MP uptake by species within each river and in particle shape and size distribution in the GITs of three fish species. Differences were considered statistically significant at  $p < 0.05$ .

## Results and Discussion

*Abundance of Microplastics in Fish GITs and Associations with Biological Characteristics.* The biological characteristics of the investigated samples from

the Ob and Yenisei rivers, as well as the content of MPs in the GIT of fish, are presented in Table 1.

Table 1

**Biological characteristics of fish samples and MP content in fish gastrointestinal tracts**

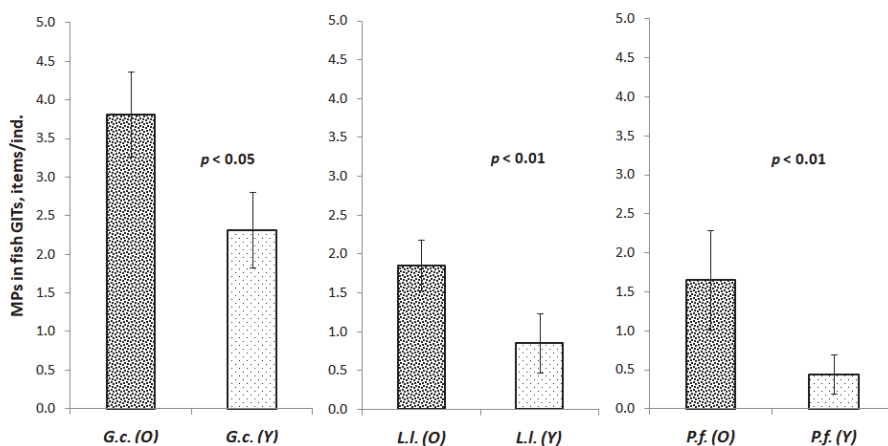
Parameter	L, mm	l, mm	Q, g	q, g	Age, years (n)	$\frac{n}{\text{♀/♂/juv}}$	GIT fullness, score	% of fish with MPs	MPs, items/ind.
<i>G. cernua</i> , the Ob River, 2023									
$\bar{x}$	93.41	84.96	13.97	13.02	1+ (2) 2+ (13) 3+ (11) 4+ (1)	27 12/12/3	1.65	92.59	3.81
SD	19.07	14.93	8.05	7.81			0.65		2.88
$m_{\bar{x}}$	3.7	2.87	1.55	1.5			0.13		0.55
lim	70-135	67-120	5.57-33.08	4.77-31.73			1-3		0-11
<i>L. baicalensis</i> , the Ob River, 2023									
$\bar{x}$	96.78	88.26	9.95	8.55	1+ (16) 2+ (7)	23 3/0/20	2	91.3	1.85
SD	14.16	12.77	5.17	4.96			1		1.58
$m_{\bar{x}}$	2.95	2.66	1.08	1.04			0.21		0.33
lim	71-128	63-118	2.95-21.5	1.4-19			1-4		0-6
<i>P. fluviatilis</i> , the Ob River, 2023									
$\bar{x}$	200.88	181.75	131.6	122.56	1+ (1) 3+ (12) 4+ (9) 5+ (2)	24 14/10/0	1.83	66.66	1.65
SD	17.38	17.72	30.96	29.33			0.98		3.11
$m_{\bar{x}}$	3.55	3.62	6.32	5.99			0.21		0.63
lim	158-230	142-215	61.3-199.02	57.45-188.01			1-4		0-15
<i>G. cernua</i> , the Yenisei River, 2022									
$\bar{x}$	126.5	115.2	18.57	16.25	1+ (2) 2+ (10) 3+ (8)	20 13/7/0	2.68	75	2.32
SD	12.54	11.74	6.41	5.53			1.12		2.2
$m_{\bar{x}}$	2.8	2.63	1.43	1.24			0.25		0.49
lim	109-157	100-145	11.10-37.9	10.20-32.3			1-5		0-9
<i>L. baicalensis</i> , the Yenisei River, 2022									
$\bar{x}$	208.15	188.25	110.85	95.18	6+ (6) 7+ (5) 8+ (8) 9+ (1)	20 12/8/0	3.33	30	0.86
SD	16.41	15.25	30.00	22.98			1.08		1.71
$m_{\bar{x}}$	3.67	3.41	6.71	5.14			0.24		0.38
lim	180-232	159-212	60.20-155.7	56.10-138.7			2-5		0-6
<i>P. fluviatilis</i> , the Yenisei River, 2022									
$\bar{x}$	213.89	189.93	154.79	141.25	1+ (1) 3+ (8) 4+ (9) 5+ (7) 6+ (1) 8+ (1)	27 9/18/0	3.37	18.52	0.44
SD	48.3	43.8	128.83	120.62			1.44		1.28
$m_{\bar{x}}$	9.3	8.43	24.79	23.21			0.28		0.25
lim	166-335	147-314	60.20-555.4	53.30-519.8			1-6		0-6

Note. n - sample size;  $\bar{x}$  - arithmetic mean; SD - standard deviation;  $m_{\bar{x}}$  - standard error of the mean; lim - variation in values; ♀ - female; ♂ - male; juv. - immature; ind. - fish individual.

The concentration of 0.15-5.00 mm plastic particles in the investigated fish varied between samples from  $0.44 \pm 0.25$  items/ind. in perch from the Yenisei to  $3.81 \pm 0.55$  in ruffe from the Ob. In our study, 62% of fish (n = 141) contained MPs in their GIT, ranging from 18.5% of the Yenisei perch

sample to 92.6% of the Ob ruffe sample (Table 1). In addition to more individuals from the Ob containing MPs in their GIT compared to fish from the Yenisei, there were statistically significant differences in MP uptake by fish of the same species in the two rivers (Fig. 1). Ruffe caught in the Ob River ingested more particles ( $p < 0.05$ ) than ruffe caught in the Yenisei River. The GITs of the Ob samples of dace and perch also showed a higher content of MPs ( $p < 0.01$ ) than those of the Yenisei samples. This may be a bioindication of a higher MP load in the Ob system compared to the Yenisei, as estimated in preliminary surface water and sediment pollution studies [26-27].

The uptake and accumulation of MPs by freshwater fish has been studied worldwide, with the two most intensively studied species being common carp (*Cyprinus carpio* L., 1758) and Nile tilapia (*Oreochromis niloticus* L., 1758) [9]. Much less attention is paid to the fish species analysed in our study. MPs in GIT were counted in perch from four Italian southern alpine lakes, showing that plastic particles  $< 0.4$  mm were present in 86% of the samples analysed ( $n = 80$ ), with mean values ranging from  $1.24 \pm 1.04$  items/ind. in Lake Como to  $5.59 \pm 2.61$  items/ind. in Lake Garda [28].



**Fig. 1.** Content of the MPs in GIT and differences in particle ingestion by fish between the rivers, based on the Mann-Whitney U-test, significant at  $p < 0.05$  and  $p < 0.01$ . Designations (hereinafter): *G.c.* (O) and *G.c.* (Y) - *G. cernua* from the Ob and Yenisei rivers; *L.I.* (O) and *L.I.* (Y) - *L. baicalensis* from the Ob and Yenisei rivers; *P.f.* (O) and *P.f.* (Y) - *P. fluviatilis* from the Ob and Yenisei rivers

Individuals of common dace (*Leuciscus leuciscus* L., 1758) from rivers and lakes in Baden-Württemberg, south-west Germany, contained no MPs in the GITs, whereas perch and ruffe, among other freshwater fish studied, ingested particles of  $899 \pm 1050$   $\mu\text{m}$  [29]. For fish from Siberian rivers, previous studies have only been carried out on samples of dace from tributaries of the Ob and Yenisei. In the GIT of dace from a tributary of the Ob, the Tom River ( $n = 13$ ), the content of particles in the size range 0.15-5.00 mm was high, averaging 41.7 items/ind. [12]. However, the quantitative analysis was preliminary and without verification of the polymeric nature of the particles, which may account for the overestimated

content. Another possible explanation is the use of strong acid hydrolysis of fish GITs, which may lead to additional fragmentation of MPs and their overestimation. Data on the content of MPs with confirmed polymer composition in the GIT of Siberian dace sampled from a tributary of the Yenisei, the Nizhnyaya Tunguska River (n = 40), have been published [13], which averaged 1.76 items/ind. and was significantly lower ( $p < 0.05$ ) than that obtained in the present study for daces from the main river.

Patterns in the ingestion of MPs by freshwater fish are often related to individual biological traits such as body size [1]. For example, a significant correlation between MP uptake and body length/weight ( $p < 0.01$ ) was observed in fish from the Han River, South Korea [30]. In our study, MP content in fish GIT was moderately correlated with linear size (L, l) and body weight (Q, q) in ruffe and perch from the Ob River samples (Table 2). No significant correlation was found between fish GIT fullness and the number of MPs in the fish.

Table 2

**Spearman correlation between MP ingestion and biological parameters of the fish studied**

Sample	MPs - L	MPs - l	MPs - Q	MPs - q	MPs - GIT fullness	MPs - Age
<i>G. cernua</i> , the Ob River, 2023	$r_s = 0.59$ ( $p < 0.01$ )	$r_s = 0.66$ ( $p < 0.01$ )	$r_s = 0.55$ ( $p < 0.05$ )	$r_s = 0.53$ ( $p < 0.05$ )	no	no
<i>L. baicalensis</i> , the Ob River, 2023	no	no	no	no	no	no
<i>P. fluviatilis</i> , the Ob River, 2023	$r_s = 0.42$ ( $p < 0.05$ )	$r_s = 0.40$ ( $p < 0.05$ )	$r_s = 0.41$ ( $p < 0.05$ )	$r_s = 0.41$ ( $p < 0.05$ )	no	$r_s = 0.39$ ( $p < 0.05$ )
<i>G. cernua</i> , the Yenisei River, 2022	no	no	no	no	no	no
<i>L. baicalensis</i> , the Yenisei River, 2022	no	no	no	no	no	no
<i>P. fluviatilis</i> , the Yenisei River, 2022	no	no	no	no	no	$r_s = 0.46$ ( $p < 0.05$ )

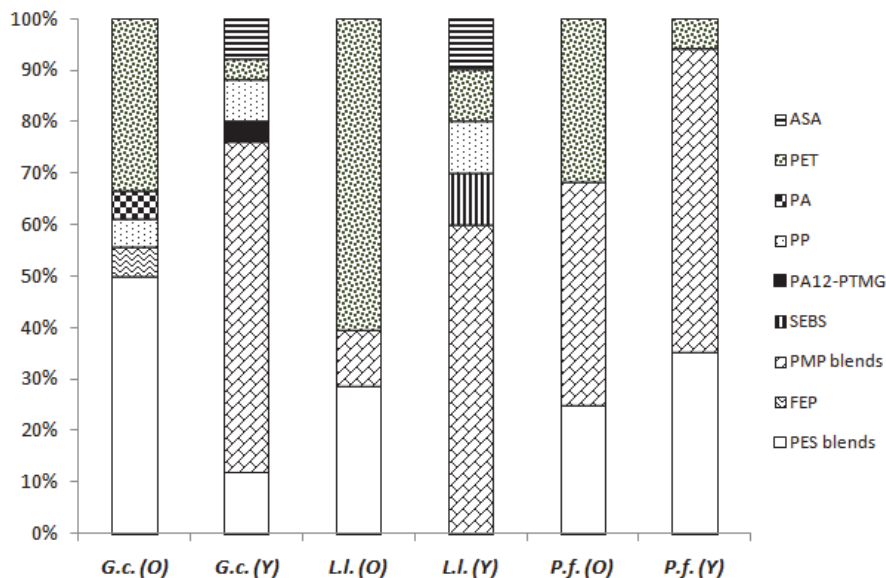
Note. MPs - number of MP particles in GITs, items/individual;  $r_s$  - Spearman's rank correlation coefficient, no - no significant correlation.

No significant association was observed between fish age and MP intake for dace and ruffe samples. However, a moderate positive Spearman correlation ( $p < 0.05$ ) was found between age and MPs in the GIT for perch in both the Ob and Yenisei samples (Table 2). Statistical comparison of MP abundance in the GIT of fish using the non-parametric Mann-Whitney test showed no differences between groups of males and females and between adults and juveniles. Sex has been suggested as a possible explanation for differences in MP patterns in freshwater fish in several studies [31-32]. However, this is probably an artefact of body size associated with sexual dimorphism in fish [1, 33].

*Characteristics of Microplastic Found in the Gastrointestinal Tract of Fish.* The MPs found in fish GIT were diverse in their chemical structure and represented both single polymers and mixtures (Fig. 2). Of the individual polymers,



polyethylene terephthalate (PET) was detected in all six fish samples and accounted for up to 60% of all particles in Siberian dace from the Ob River. Only ruffe and dace ingested polypropylene (PP) and acrylonitrile styrene acrylate (ASA) microparticles in proportions of < 10%; polyacrylamide (PA) fibres and styrene-ethylene-butylene-styrene (SEBS) MPs were detected exclusively in the Ob ruffe and Yenisei dace, respectively (Fig. 2). Approximately 4% of the MPs in the Yenisei ruffe were represented by a PA12-PTMG copolymer consisting of polyamide blocks and polyether blocks, and 5.6% of the MPs in the Ob ruffe were particles of high-density fluorinated ethylene-propylene copolymer (FEP). A comparatively large proportion of MPs in the GIT of Yenisei ruffes, daces and perches were identified as thermoplastic polyolefin polymethylpentene (PMP) with the low spectral similarity of < 35%. A similar situation was observed with particles of polymer based on industrially used polyethersulphone (PES) (Fig. 2). The problem of identifying MPs was previously highlighted by Galafassi et al. [28], who observed that 43% of plastic particles from perch GITs were highly degraded, making it impossible to identify them down to the polymer type. The authors characterised the polymer composition of such MPs using the terms ‘aliphatic polymers’ and ‘aromatic polymers’ based on narrow and intense infrared peaks in the aromatic and aliphatic C-H stretching region (2800-3150 cm<sup>-1</sup>).



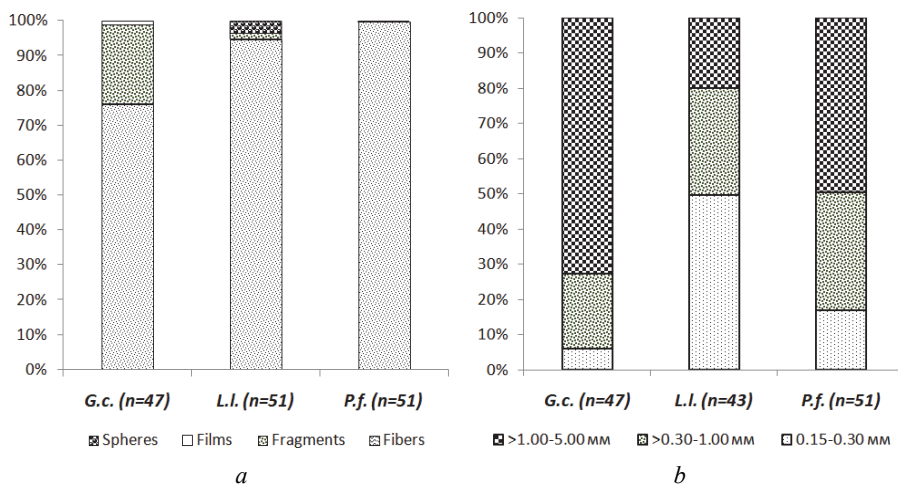
**Fig. 2.** Polymer structure of the MPs detected in the GITs of fish from the Ob and Yenisei rivers based on the results of the  $\mu$ Raman identification

In terms of morphology, the majority of the MPs from GITs were fibers, which accounted for up to 99.7% in 51 perch individuals from two rivers (Fig. 3a). The proportion of fragments was higher in ruffe, reaching 22.7% of the total number of MPs; spheres and films were found exclusively in dace and ruffe, with a proportion of 3.7% and 1.2% respectively. Based on the meta-analysis of plastics

ingested by fish worldwide, fibers were the most common MPs ingested by fish, with a relative abundance of about 72% [34]. Otherwise, more than half of all MPs detected in the GITs of freshwater fish in rivers and lakes of south-west Germany were irregularly shaped fragments, followed by fibers, which accounted for almost 40% [29]. Plastic films and spheres were rare, similar to the results obtained for Siberian fish in the current study.

In the literature, MPs have been divided into two groups called ‘small microplastics’ (< 1 mm) and ‘large microplastics’ (1-5 mm) [35]. Of the studies that reported the size of MP ingested by fish globally, 74% found small particles to be the as the predominant size class [34]. The maximum content of the smallest particles of 0.15-0.30 mm (37%) was found in the GITs of omnivorous dace from Siberian rivers; ‘large’ MPs of 1.00-5.00 mm were relatively more abundant in benthivorous ruffe, accounting for up to 67% of the MP number (fig. 3b). However, the Kruskal-Wallis test showed no preference for MPs of different shapes and sizes among the three fish species ( $p > 0.05$ ).

*Microplastic Ingestion by Fish with Different Feeding Habits.* The level of MP ingestion was dependent on the feeding habits of the fish species, with a significant decrease in the raw benthivorous ruffe – omnivorous dace – hunting perch in two Siberian rivers ( $p < 0.01$ ) (Fig. 4). It is thought that predatory or fish-eating fish are more vulnerable to MP consumption than fish with a different feeding strategy [36], but published data are inconsistent. Other studies support our findings on the minimum MP consumption by perch, the only fish analysed with the smaller fish found in GIT. For example, a study of MP uptake by freshwater fish in southwest Germany shows a significantly lower particle loads in piscivorous fish than in lower trophic groups [29].



**Fig. 3.** Distribution of MP shapes (a) and sizes (b) in the GITs of fish from the Ob and Yenisei rivers

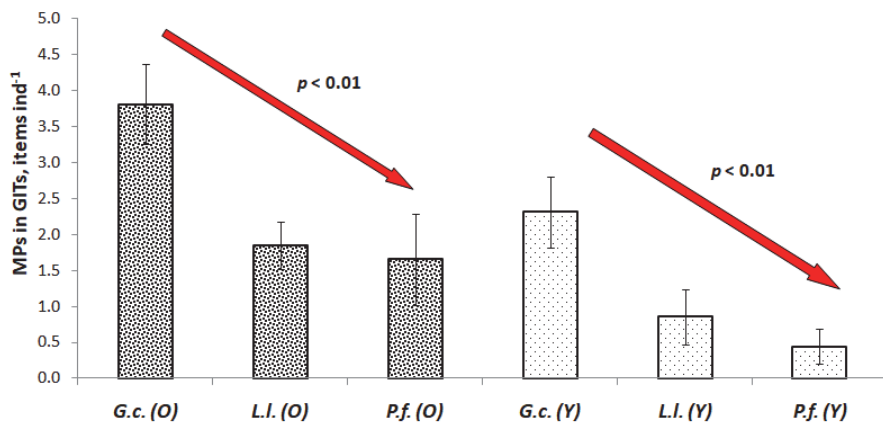


Fig. 4. MP ingestion by fish with different feeding habits, significant at  $p < 0.01$  based on the Kruskal-Wallis H-test

Previously, omnivorous fish were shown to have higher levels of MPs in their GIT (namely, fibers) than herbivores and carnivores [37]. Other research has shown that omnivorous and insectivorous fish contain more MPs than carnivorous and herbivorous fish [30]. In the current study, the MP content in the GIT of ruffe and perch, both carnivorous fish, was significantly different ( $p < 0.01$ ), showing that not only the feeding type influences particle uptake but also the feeding behaviour and living habitat. As bottom sediments are a sink for MPs, demersal and benthic fish are likely to be more exposed to MPs than pelagic species, which has been shown in many studies for freshwater fish as reviewed by Collard et al. [36]. We detected the highest particle content in the GIT of benthivorous ruffe in the Ob and Yenisei rivers compared to two other fish species with different feeding habits ( $p < 0.01$ ). This may be related to the release of MP deposited on the surface and inside sediments during ruffe feeding, supporting previous findings.

## Conclusions

It has been shown that wild freshwater fish in the Ob and Yenisei rivers ingest MPs. Quantitative analysis coupled with  $\mu$ Raman spectroscopy revealed that 62% of the fish studied ( $n = 141$ ) in two Siberian rivers contained MPs of 0.15-5.00 mm in their GIT, ranging from 18.5% of Yenisei perch to 92.6% of Ob ruffe. MP content in fish GIT varied between samples from  $0.44 \pm 0.25$  items/individual in perch from the Yenisei to  $3.81 \pm 0.55$  in ruffe from the Ob. The ingestion of MPs depended on the feeding habits of the fish species ( $p < 0.01$ ), e.g. benthivorous ruffe > omnivorous dace > hunting perch, which was true for both rivers. Most of the ingested particles were fibers (up to 99.7% in the GITs of perch). The maximum content of the smallest particles of 0.15-0.30 mm was found in the GITs of omnivorous dace from Siberian rivers, but 'large' MPs of 1.00-5.00 mm were relatively more abundant in ruffe. However, there was no significant preference for MPs of different shapes and sizes among the three fish species.

All three fish species samples caught in the Ob River ingested significantly more particles ( $p < 0.05$ ) compared to the Yenisei River, which may indicate a higher MP load in the Ob system compared to the Yenisei. At the same time, the consumption of MPs by different fish species differed significantly in each of the rivers. The data may be useful for the selection of fish species for bioindication of plastic pollution in Siberian rivers in the future.

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