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The determination of microplastic contamination in freshwater environments using sampling methods – A case study

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Abstract: We compared different net sampling methods for microplastic quantitative collection by sampling different water volumes with nets of different mesh sizes. Sampling covered freshwater lake and reservoir with a significant degree of eutrophication located in Central Poland. The fibres were the main type of plastic collected from sampling sites and constituted 83% of all microplastic particles. Fibres of 700–1900 µm dominated in the samples. The size of mesh affected the amount of fibres collected. Small fibres of 10–200 µm in length were collected using only a fine net of 20 µm mesh size. The total amount of fibres depended on sample volumes; concentrations of microplastics were higher for smaller water volumes. It is likely that clogging with phytoplankton and suspended particles reduced the filtration capacity of the finest nets when large volumes were sampled, which led to an underestimation of microplastic. To our knowledge, this is the first study to provide evidence that the amount of small microfibres depends on mesh size and that the total microplastic abundance in freshwaters in Poland depends on the sample volume. We suggest sampling rather larger than smaller water volumes to assess the level of microplastic contamination more accurately, but clogging, which reduces the filtration capacity of finest nets, should be taken into account when eutrophic freshwater environments are studied.

Keywords: fraction of microfibres, mesoplastic, microplastic contamination, water pollution

INTRODUCTION

Plastic has become a significant global problem, threatening both the environment and people. Nowadays, the demand for plastic has become so high that it has outpaced its manageability, resulting in the continuous accumulation of microplastic (MP) particles that originate from the decomposition of plastics. MPs are defined as plastic particles (e.g. fibres, granules, foams, foils) less than 5 mm in size (Arthur *et al.*, 2009; Barnes *et al.*, 2009; Andrady, 2011). MPs are present even in sites that are not easily accessible to humans, such as polar waters and deep seas (Woodall *et al.*, 2014; Lusher *et al.*, 2015; Covernton *et al.*, 2019). The accumulation of MP particles in the natural environment has been documented mainly in marine ecosystems, freshwater lakes, and rivers. Examples of freshwater environments include St. Lawrence River, flowing through the US and Canada (Castañeda *et al.*, 2014); the Beijiang River in Southern China (Wang *et al.*, 2017); the Danube River (Lechner *et al.*, 2014); Hovsgol Mountain Lake, which is a national park in Mongolia (Free *et al.*, 2014); the Great Lakes of North America (Zbyszewski *et al.*, 2014); Geneva Lake (Faure *et al.*, 2012); freshwaters in Hungary (Bordós *et al.*, 2019); and lakes and rivers in Poland (Nocoń *et al.*, 2018; Kaliszewicz *et al.*, 2020; Dacewicz *et al.*, 2022). According to many studies, MP is a real threat to aquatic organisms and ecosystems worldwide (Lusher, 2015; GESAMP, 2015; Rochman *et al.*, 2016; Tavşanoğlu *et al.*, 2020). MPs are ingested by aquatic organisms, and some of these animals are then consumed by humans (Chae and An, 2017; Lusher *et al.*, 2017). Studies on the health effects of MPs on living organisms indicate that MP consumption can have negative effects on the growth rate, fertility, and survival of animals (Wegner *et al.*, 2012; Besseling *et al.*, 2013; Wright *et al.*, 2013; Cole *et al.*, 2015; Cole *et al.*, 2016; Sussarellu *et al.*, 2016; Welden and Cowie, 2016; Cole *et al.*, 2019).

There are two sources of MPs: primary, or factory-made (e.g., from cosmetics), and secondary, resulting from mechanical fragmentation of plastics into smaller particles (e.g. from fragmentation of plastic elements or clothing) (Andrady, 2011; Cole *et al.*, 2011; Dris *et al.*, 2016; Sutton *et al.*, 2016; Gies *et al.*, 2018). Sizes of primary MPs are determined during their production. Secondary MPs are created as a result of size reduction by physicochemical processes and photooxidation. In the literature, the most common shape of MPs reported is fibre (Lusher *et al.*, 2015; Zhang *et al.*, 2016; Fang *et al.*, 2018; Covernton *et al.*, 2019; Gonzalez-Pleiter *et al.*, 2020). The most common types of polymers that constitute MPs are polyethylene (PE), polypropylene (PP), polyethylene terephthalate (PET), polystyrene, polyvinyl chloride, polylactic acid, and polyamide (Carr *et al.*, 2016; Courtene-Jones *et al.*, 2017; Miller *et al.*, 2017; Tavşanoğlu, 2020). Appropriate methods of collecting MPs from the environment are extremely important, both in quantitative research and in studies of the proportion of contamination by certain polymers. Nets with mesh sizes of 333–350 µm are the most commonly used for sampling MPs from water columns and were recommended by the Marine Strategy Framework Directive for Monitoring (Hidalgo-Ruzi *et al.*, 2012; Gago *et al.*, 2016; Lusher *et al.*, 2016; Michida *et al.*, 2019). Plastic fragments larger than 300 µm were recorded in most field studies, most likely because smaller fractions can pass through the mesh (Conkle *et al.*, 2018; Covernton *et al.*, 2019). As a result of studies with two nets of different mesh sizes, the concentration of MPs using the 100 µm mesh is 10 times higher than in the case of the 500 µm mesh, and there is a 2.5-fold increase in plastic fragments using the 100 µm mesh compared to the 333 µm mesh (Lindeque *et al.*, 2020). Covernton *et al.* (2019) reported that using the 300–350 µm mesh can underestimate total MP concentrations by one to four orders of magnitude compared to samples that are filtered through much smaller mesh sizes (e.g., <100 µm). On the other hand, when using nets with too small mesh sizes, there is a risk of underestimating results due to clogging of meshes with filtered suspension – especially in the case of a higher degree of eutrophication (Kang *et al.*, 2015; Barrows *et al.*, 2017; Lindeque *et al.*, 2020; Tadashi *et al.*, 2021).

In this study, we focused on the comparison between different methods of MP quantitative collection by sampling different water volumes with nets of different mesh sizes. We collected MP samples from freshwater lakes in Central Poland with a significant degree of eutrophication. To the best of our knowledge, this is the first study to show the possible effect of mesh size and sample volume on the assessed level of MP contamination in freshwaters in Poland.

MATERIALS AND METHODS

THE STUDY SITES AND MICROPLASTIC SAMPLING

The samples were taken from two freshwater sites with a significant degree of eutrophication. The sites were located in Central Poland near large urban agglomerations (Fig. 1). Lake Dziekanowskie (52°37' N, 20°84' E) is located in the Vistula River Basin and is connected to the river by Struga Dziekanowska (Romanowski *et al.*, 2013). The lake is located north of the town of Łomianki, between the Vistula River and the village of Dziekanów Polski. The lake has an area of 27.6 ha and a maximum depth of 10 m.

The reservoir Ruda (52°04' N, 20°44' E) has been created artificially on the Pisia-Gągolina River. It is located in the centre of the city of Żyrardów. It has an area of 1.3 ha.

The net sampling method was used to collect MPs along the shore of the abovementioned water reservoirs. Three plankton nets with a 23 cm diameter grid inlet and mesh size of 20, 200, and 500 µm were used in the study. The net was trawled just below the water surface on a transect of 2, 8, and 14 m at a distance of about 5 m from the shore. This translates into 14.4 dm^3, 57.6 dm^3, and 100.8 dm^3 of water passed through the net, respectively. The samples were concentrated to approx. 50 cm^3 each. For each mesh/transect combination, 27 samples were collected from the site. Each sample was placed in 100 cm^3 container with a screw cap and transported to the laboratory.

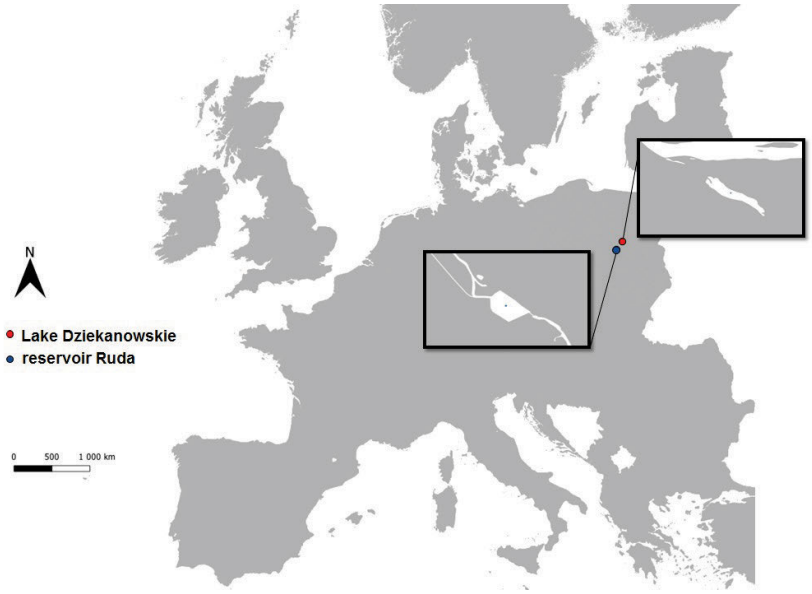

Fig. 1. Map of the sampling region in Central Poland; source: own elaboration

METHODS TO VISUALISE MICROPLASTIC PRESENT IN THE SAMPLE

In order to evaporate water, the samples were placed in a laboratory drying oven at 60°C for 72 h. Then, to remove organic matter from the samples, 69% nitric acid and 5 cm^3 of 30% hydrogen peroxide were added at a 3:1 ratio to each flask. The flasks were covered with glass plates and left for 72 h for organic matter to degrade. The samples were then filtered using a Labor s. c. PL2/1 SN 1309 vacuum pump kit with glass microfibre filters. The filters had 47 mm in diameter and pore sizes of 1.2 µm (Whatman, GF/C^{TM}). The filters were placed individually in glass Petri dishes with a lid and left to dry for 24 h. Each filter was photographed at high resolution using a Keyence VHX-7000 digital microscope at 500–1000× magnification to avoid overlooking smaller and transparent particles and fibres during visual examination. The fibres were identified on the basis of known physical characteristics (Kaliszewicz *et al.*, 2020) and counted and measured individually from the images using Keyence VHX-7000 digital microscope software.

To check whether there was any MP contamination resulting from the sampling method, we used our standardised control procedure (Kaliszewicz *et al.*, 2020). We poured 14.4 dm^3 of deionised water through the plankton net and treated it with the same procedure used for the samples from the sites. We also checked whether there were any MP fibres in the laboratory air. We used clean glass microfibre filters that were placed in open Petri dishes for 4 h in the working area of the laboratory.

STATISTICAL ANALYSES

The number of MP particles for each transect length and mesh size was converted into a volume of 1 m^3 of water. Calculations were performed using one-way ANOVA, and if the data distribution was not close to normal, the nonparametric Kruskal–Wallis test was applied. The data were analysed for normality using the Shapiro–Wilk test. Tukey's post hoc test was used to establish differences between variants for ANOVA. The nonparametric Kruskal–Wallis test was followed by Mann–Whitney U test. A significance level of $\alpha = 0.05$ was used for the statistical analysis. All statistical analyses were performed using Statistica (StatSoft Inc.).

RESULTS

SAMPLING BY NETS OF DIFFERENT MESH SIZES

Fibres were the main type of plastic collected from the sampling sites and constituted 83% of all particles. The distribution of MPs is not uniform in water. Fibres were detected in almost every sample but their amount differ between samples and sites. The total amount of fibres was 174–3125 items per m^3 in reservoir Ruda, and 10–1250 items per m^3 in Lake Dziekanowskie. The amount of fibres collected varied depending on the size of mesh. We found a maximum of 3125 fibres per m^3 by using mesh size of 20 µm, 2917 fibres per m^3 by using mesh size of 200 µm, and 694 fibres per m^3 by using mesh size of 500 µm. The fibres were divided into four size classes: (a) small (10–200 µm), (b) medium (201–1000 µm), (c) large (1001–5000 µm) microfibres, and (d) mesofibres (5001–25,000 µm). Medium and large fibres dominated in both study sites (maximum of 2153 items per m^3 and 1736 items per m^3, respectively). Fibres less than 200 µm in length (Fig. 2) were collected using only a 20 µm mesh size (Kruskal–Wallis test, $p = 0.046$). In the case of larger mesh sizes, these fibres were not present (Fig. 3). As regards mesofibres (5001–25,000 µm, Fig. 3) in Lake Dziekanowskie, an opposite trend was discovered: the larger the mesh size, the more fibres were collected (Kruskal–Wallis test, $p = 0.05$).

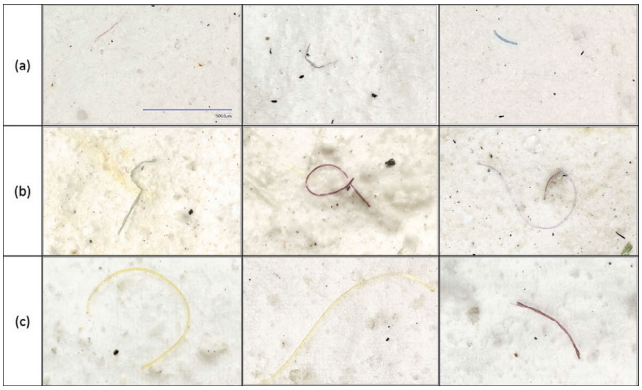

Fig. 2. Examples of microplastic fibres collected with nets of different mesh sizes: a) 20 µm b) 200 µm c) 500 µm; all images of the fibres are of the same scale; source: own study

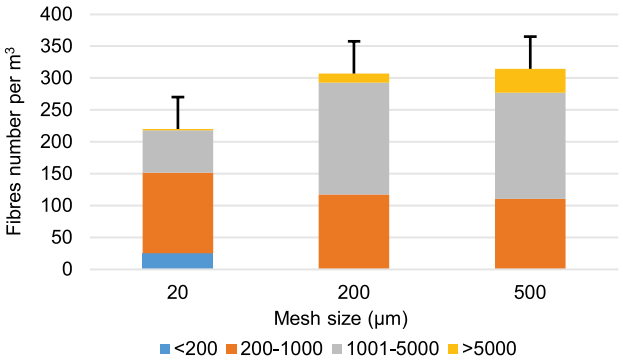

Fig. 3. Total number of fibres (mean ± 1*SE*) collected from the study sites by sampling different water volumes (dm^3) with nets of different mesh sizes (µm); source: own study

SAMPLING OF DIFFERENT WATER VOLUMES

The number of fibres collected depended not only on the size of mesh but also on water volumes (ANOVA, $F_{2, 38} = 4.67$, $p = 0.015$). It appeared that there were significant differences in the number of fibres between samples of 14.4 dm^3 (139–3125 items per m^3), and 100.8 dm^3 (10–268 items per m^3) of water passed through the plankton net (Tukey's post hoc test, $p = 0.02$). In the latter case, a lower total fibre amount was observed when samples were collected with mesh sizes of 20 µm and 200 µm (Fig. 4). In the case of mesh size 500 µm no differences were observed ($p > 0.05$, Fig. 4). For medium microfibres (201–1000 µm) analysed, significant differences in the amount of fibre appeared (ANOVA, $F_{2, 38} = 4.23$, $p = 0.022$). There were differences between the samples of 14.4 dm^3 (69–2153 items per m^3), and 100.8 dm^3 (10–69 items per m^3; Tukey's post hoc test, $p = 0.03$). A smaller amount of medium microfibres was observed at a volume of 100.8 dm^3 (Fig. 5a).

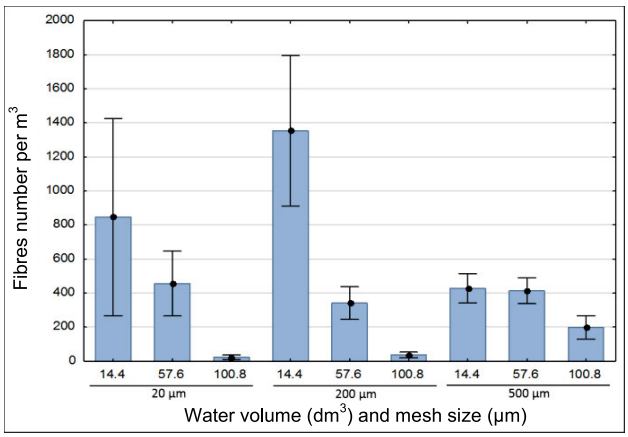

Fig. 4. The mean number of fibres divided into four size classes: small (10–200 µm), medium (201–1000 µm), large (1001–5000 µm) microfibres, and mesofibres (5001–25000 µm) collected with nets of different mesh sizes (20, 200, and 500 µm) from Lake Dziekanowskie; error bars represent ± 1*SE*; source: own study

Similar results were obtained for large (1001–5000 µm) microfibres (ANOVA, $F_{2,\ 37} = 3.71$, $p = 0.035$). There were also differences between the samples of 14.4 dm^3 (69–1736 items per m^3), and 100.8 dm^3 (10–179 items per m^3; Tukey's post hoc test, $p = 0.034$). In the latter, fewer microfibres were observed (Fig. 5b).

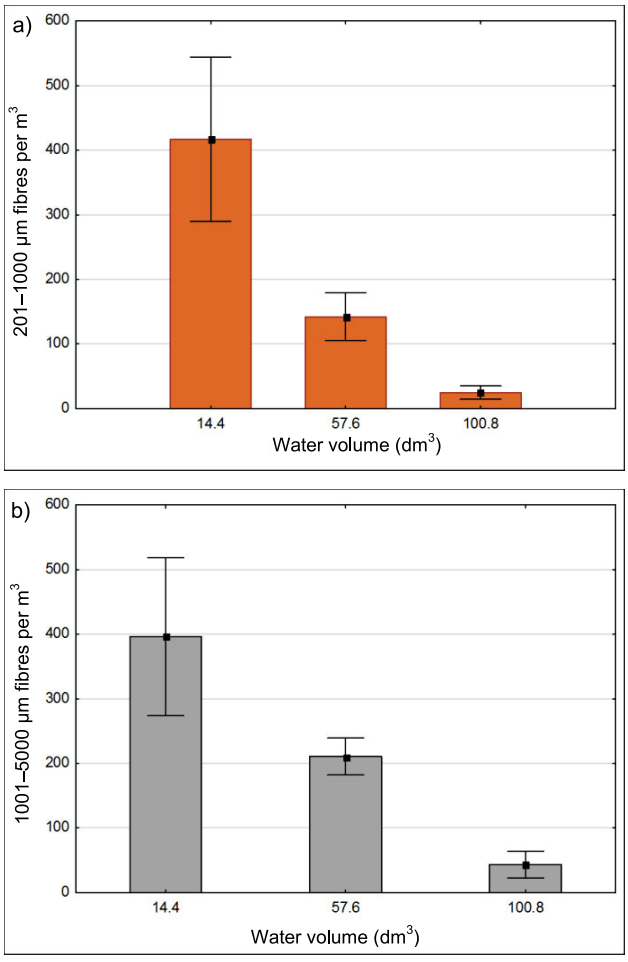

Fig. 5. The mean number of microfibres of different sizes (a) medium – 201–1000 µm, (b) large – 1001–5000 µm) collected by sampling different water volumes; error bars represent ±1*SE*; source: own study

DISCUSSION

Our results indicate that both the mesh size and sampled water volumes affected the amount of MPs collected. Fibres were the main types of plastic collected from the sampling sites. Interestingly, only the smallest fraction of fibres (10–200 µm) depended on the mesh size. Such microfibres were the most abundant in samples collected using 20 µm nets and these were not present when 200 µm and 500 µm nets were used. Our results clearly indicated that the size of the mesh determined the size of the MP captured. Small fibres can escape from larger mesh sizes and are unaccounted for (Koelmans *et al.*, 2019; Tokai *et al.*, 2021). We clearly indicated underestimation of small MPs, especially microfibres, for which width-to-length ratios were usually <0.1. Small MPs (<300 µm) consist of the most common microbeads found in cosmetic products and are predominant in aquatic studies (Covernton *et al.*, 2019; Bujaczek *et al.*, 2021). This small fraction of MPs can usually be ingested by a range of aquatic organisms (e.g. zooplankton, benthic invertebrates, and fish) (Ziajahromi *et al.*, 2017; Domogalla-Urbansky *et al.*, 2019; Sarijan *et al.*, 2021). MP fragments smaller than the mesh size may escape or can only be collected partially (Conkle *et al.*, 2018). When we analysed fibres, our results indicated that those of 200 µm in length could not be collected by the mesh of the same width of the sieve. The collection efficiency in the case of MP fragments close in size to the mesh has also been described in other studies as limited compared to larger particles (Stanton *et al.*, 2020; Tamminga *et al.*, 2022). The width of a fibre should be used to indicate whether it can be captured by a sieve of a certain size. However, the longer the fibre, the more likely it is to be captured by the mesh. In our study, the coarse net (500 µm) increased the probability that long fibres (>5 mm) were collected.

Our findings are only partially consistent with other studies in which a smaller mesh size allowed to collect more MPs in general (Tavşanoğlu *et al.*, 2020; Ben-David *et al.*, 2021), and rarely negligible differences were observed (100 µm vs 333 µm mesh size) (Lindeque *et al.*, 2020). Discrepancy between our results and the literature data indicating higher amount of MPs collected when the finer mesh is used (20–100 µm instead of >300 µm) could arise from clogging of the nets. This mainly applies to small mesh sizes (20 µm), which can easily be clogged with organic material (e.g. phytoplankton blooms). The sites represented eutrophic freshwater ecosystems with phytoplankton biomass (usually diatoms and blue-green algae) of 7–10 mg·dm^{-3} on average. Clogging with phytoplankton and suspended particles reduces the filtration capacity of the finest nets and leads to an underestimation of the MP collected. This seems to be especially true for mesotrophic and eutrophic freshwater environments (Tavşanoğlu *et al.*, 2020).

Fibres of 700–1900 µm dominated our samples independently of the method used. The predominant fibrous fraction of MPs has been described in many studies (Desforges *et al.*, 2014; Lindeque *et al.*, 2020; Tavşanoğlu *et al.*, 2020). However, the dominant size of the fibres varies between studies. The airborne fibres were mainly of 50–450 µm (Dris *et al.*, 2017). Vianello *et al.* (2013) found that small fibres of 30–500 µm dominated sediments of the Lagoon of Venice. The sludge sample of a secondary wastewater treatment plant contained the most fibres of 1,000–2,000 µm (Vardar *et al.*, 2021). The MPs in the water column and sediments of freshwaters in China were dominated by fibres of 500–5000 µm (Zhao *et al.*, 2022). The size of the predominant

fraction of MPs seems to be more dependent on the source of contamination and the environment than on the method used.

The results of a previous study indicated the dominance of conventional plastic polymers in freshwater environments in Poland (Kaliszewicz *et al*., 2020). Fibres are composed mainly of PET, a polyester family, and PP. Polyester is the fourth most commonly used and accounts for around 18% of the world polymer production; additionally, it is the most important textile fibre (Shamsi and Sadeghi, 2016). The results are in line with the literature data and most of fibres found in ecosystems are composed of PE, PET, or polyurethane (PU) (Yu *et al*., 2018; Covernton *et al*., 2019; Parolini *et al*., 2021).

Contrary to different mesh sizes used in many studies of aquatic MP, different sampling volumes have been rarely analysed (Lusher *et al*., 2014; Tamminga *et al*., 2019). Lusher *et al*. (2014) indicated that the concentration of MPs was higher in smaller water volumes. Tamminga *et al*. (2019) did not find differences in MP particle concentration between sample volumes, but the median concentration was higher in the case of smaller water volumes. The results presented in this study are partially consistent with those described in the literature. A small sample volume yielded a larger number of fibres, but the net with the largest mesh size used (500 µm) was excluded. The variance of microfibre abundance in low-volume samples was higher than in large-volume samples. Similar observations have already been reported in the literature (Tamminga *et al*., 2019). A larger sample volume can improve the accuracy of estimates, especially when small mesh sizes are used. This allows to collect smaller MP particles.

CONCLUSIONS

Nets with mesh sizes of 333–350 µm are the most commonly used in microplastic sampling methods. MP fragments smaller than the mesh size may escape or can only be collected partially. The comparison of three mesh sizes and three water volumes from eutrophic freshwater environments in Poland indicated that the smallest fraction of microfibres (10–200 µm) were collected by 20 µm nets and were not present when 200 µm and 500 µm nets were used. A smaller mesh size did not allow to collect more MPs in general. Contrary to different mesh sizes, the sample volume affected the amount of MPs collected. A small sample volume yielded a larger number of fibres, which prevailed in samples, with the exception of the largest 500 µm net. Moreover, the variance of microfibre abundance in low-volume samples was higher than in large-volume samples. The reason may be that small mesh sizes can be easily clogged with organic material (e.g. phytoplankton blooms). It reduces the filtration capacity of the finest nets when a large volume of water is sampled and leads to an underestimation of the MP amount in eutrophic freshwater environments. Despite clogging, our results suggest to use plankton nets with mesh sizes of 20 µm and a minimum volume of 50 dm^3. The use of larger net sizes and smaller water volumes may lead to underestimation of the MP amount, especially its small fraction.

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