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Sampling with Niskin bottles and microfiltration reveals a high prevalence of microfibers

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ABSTRACT

Microfibers were identified in filtered water from Lake Superior, Lake Michigan and northern Lake Huron in the United States. Sampling with a Niskin bottle followed by microfiltration (0.45 µm filter), a sampling methodology rarely used in microplastics sampling, revealed an average microfiber concentration of 0.119 +/- 0.04 microfibers/mL. Microfibers may be under-represented in estimates of micro- and nanoplastic pollution if microfiltering is not included in the sampling scheme.

1. Introduction

Due to the ubiquitous nature of micro- and nanoplastic pollution and increasing concerns about the associated hazards, characterizing and qunatifying these plastics in aquatic ecosystems has become a priority (Arthur et al., 2009). Ingestion of plastic of all sizes has been documented in a variety of taxa (e.g. Cole et al., 2014). Microplastics (< 5 µm) and nanoplastics (< 1 µm), are now being identified as a concern to zooplankton and filter feeders, and include artificial fibers (Cole and Galloway, 2015). Ingested plastics may leach chemical constituents, or contain concentrated persistent organic pollutants (Andrady, 2011). The miniscule size of micro- and nanoplastics makes them available to filter feeders, detritivores and planktivores at the base of an aquatic food web (e.g. Cole et al., 2011).

Sampling of microplastics often includes sampling with neuston nets and/or manta trawls (Baldwin et al., 2016; Eriksen et al., 2013), or filtering directly from the water (La Daana et al., 2018; Setälä et al., 2016). Some studies that use filtering have reported higher counts of fibers (e.g. Hendrickson et al., 2018; Wang et al., 2018), but the use of filters with very small pore sizes is rare. Microfiltering may be useful for sampling small plastic particles, especially fibers, for studies that are focusing on nano-/microplastics (Dai et al., 2018; Di Mauro et al., 2017; Green et al., 2018; Wang et al., 2018). Setälä et al. (2016) recovered higher concentrations of microplastics from the Baltic Sea using a pump and filter system than with nets, and further, a 100 µm filter recovered more microplastics than a 300 µm filter. Likewise, Anderson et al. (2018) identified more fibers in the sea surface microlayer of UK estuaries than previously reported studies by sampling with a dipped glass plate, a new methodology that may be well suited for identifying microfibers. Therefore, alternative methods may be preferable to net sampling when recovering plastics that would be available for ingestion by neuston and zooplankton feeding on micro-sized food items.

The goal of this project was to sample near-surface water from Lake Superior, Lake Michigan and northern Lake Huron with Niskin bottles and filter the water samples using a 0.45 µm filter to determine the presence of particles that could be bioavailable to zooplankton. Niskin sampling, which may reduce aerial contamination, has rarely been used in microplastic sampling (Dai et al., 2018; La Daana et al., 2018) and to date, only one published study used a 0.45 µm filter (Green et al., 2018). We report new data regarding microfibers sampled from the open waters of the Great Lakes.

2. Methods

2.1. Sampling

Sampling was conducted in the R/V Blue Heron along a transect through Lake Superior (n = 3), St. Mary's River (n = 1), Lake Michigan (n = 1), and the northern region of Lake Huron (n = 2) in the United States from June 8–10, 2017 (Fig. 1). Sampling in Lake Michigan was interrupted due to inclement weather, resulting in only one sampling station. All water samples were taken using stainless steel Niskin bottles in a CTD rosette sitting at the surface, providing a near-surface sample of water. At each station, one liter of water was collected from the

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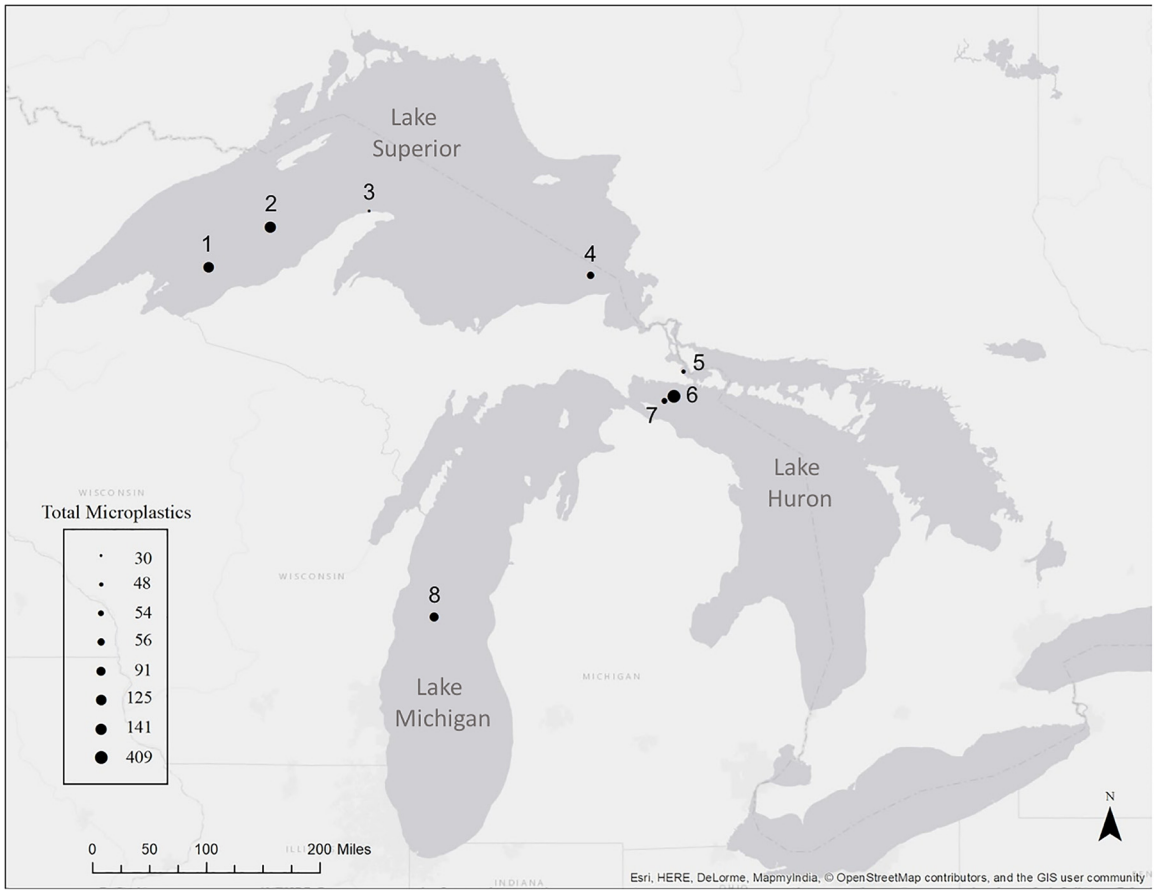

Fig. 1. Stations (1–8) occur along a transect through Lakes Superior, Huron and Michigan. One-liter water samples were collected from each station and filtered immediately.

Niskin bottle and immediately filtered. Filtering was performed using 0.45 µm gridded cellulose nitrate filters (Cole-Parmer, Vernon Hills, Illinois) and a hand-operated pump.

To reduce contamination in the field and lab, all filtering equipment was rinsed with deionized water before and after use. Whenever possible, metal and glass were used in place of plasticware. Water samples were covered to prevent contamination from airborne microplastics. Deionized water was pumped through the filtering equipment to remove any microplastics between sampling stations. To identify the level of airborne contamination, blanks (n = 8), consisting of filtered deionized water (1 L), were performed between filtration of samples. Filters were stored in sealed petri dishes and not opened until identification and quantification. Once opened, the filters were processed immediately.

2.2. Microplastic identification

Microplastics were identified and quantified by direct examination of each square of gridded filter samples using a compound microscope (4x and 10x magnification). Visual identification was performed to accurately differentiate plastics from other natural organic debris such as algae, sediment, invertebrates, and plant material.

To avoid misidentification and inaccurate estimation of microplastics, visual criteria, including examination for cellular and organic structures, equal thickness throughout the length of fibers, and homogeneous particle color, similar to Hidalgo-Ruz et al. (2012) were used. To distinguish between plastic and natural organic material, fibers were tested with two procedures. A metal probe was heated and placed next to the putative microplastic. Hendrickson et al. (2018) found that using the "melt test," all plastic fibers melted, but cotton and wool fibers burned. Therefore, in this study, if the item melted it was considered a microplastic (Hidalgo-Ruz et al., 2012; Desforges et al., 2013). A small subset (21 fibers) were also investigated using micro-fourier transform infrared spectroscopy (micro-FTIR) of 21 microfibers was performed using a Thermo® Nicolet iN10 (liquid nitrogen cooled MCT-A detector with a wavenumber range of 11,700–600 cm^{-1}). Scans were repeated in triplicate. Spectra were compared with reference databases to determine matches.

Approximately, 75% of the detected fibers were measured and color was recorded. Measurements were collected using ImageJ v 1.52a bundled with Java 1.8.0_172 for Windows. Photographs of each filter were taken with a Nikon DS-Fi2 microscope and corresponding software, NIS-Elements, was used to burn the set scale of 100 µm to every photo when capturing each image. The process of measuring required the scale to be set on each individual photo as each filter had multiple photos to capture all plastic debris. Using the straight line tool in ImageJ, the scale line was traced and set before beginning measurements. All fibers were traced using the freehand tool to accommodate twisting and bending of fibers.

2.3. Analyses

A one-way Analysis of Variance (ANOVA) and Tukey's multiple comparison adjustment was performed to determine if there were any differences between lakes and stations by total, fiber length or color. Further, an ANOVA was used to validate that samples were statistically different than filtration blanks. Analyses were performed with R software (Package: stats, cran mirror: USA PA1, Chambers and Hastie, 1992).

Table 1
Microplastics identified for each station and filtration blanks. Abundance, expressed as microplastics/liter of fibers, fragments and beads for each station.

Station No.	Date/Time	Latitude	Longitude	Fibers	Fragments	Beads	Total (n)
1	8June 12:30	46º 59.840	−91º 05.160	125	0	0	125
2	8June 22:30	47º 20.609	−89º 17.776	141	0	0	141
3	9June 07:15	47º 28.871	−88º 02.079	30	0	0	30
4	9June 23:00	46º 55.461	−85º 12.965	54	1	1	56
5	10June 10:30	46º 04.866	−84º 01.907	48	0	0	48
6	10June 15:30	45º 51.693	−84º 09.276	407	2	0	409
7	10June 18:30	45º 49.632	−84º 16.421	54	0	0	54
8	11June 03:20	43º 52.527	−87º 12.484	91	0	0	91
Total				**950**	**3**	**1**	**954**

3. Results

Sampling of neustonic waters of Lake Superior, Lake Michigan and northern Lake Huron using a Niskin bottle followed by filtration (0.45 µm filter) for microplastics revealed a total microplastic concentration of 0.119 microplastics/mL, of which 99% were microfibers (Table 1). Specifically, a total of 950 microfibers were counted from eight liters of water that were filtered (Table 1). Only two microfragments were found (Station 6) and one microbead was identified (Station 4).

Fibers were of primarily red, black, blue and varied in length from, on average, 95–274 µm. Representative fibers are pictured in Fig. 2. All fibers that were examined with micro-FTIR were identified as plastic, specifically styrene and polystyrene. Filtration blanks had significantly fewer and shorter microfibers than water samples ($p < 0.001$, Fig. 3).

There was no statistical difference between lakes for the number of fibers identified ($p = 0.64$), despite differences in human populations of neighboring cities. For instance, Stations 1 and 2 ($n = 125$, $n = 141$, respectively) are close to Duluth, Minnesota, while Station 3 ($n = 30$) is near the Keweenaw Peninsula, Michigan, which has roughly half the population of Duluth. Likewise, there was also no difference between lakes for length ($p = 0.66$) or color ($p = 0.74$) of fibers.

4. Discussion

The high level of microfibers recovered in this study is not a surprising result. Discrepancies of microfiber counts between this study and a previous study using nets to sample plastics in the Great Lakes (Eriksen et al., 2013) are likely due to differences in sampling methodologies. Eriksen et al. (2013) did not report the presence of microfibers from sampling with manta trawls towed for 60 min, which is a larger sample size than what was used in the methods presented in this short communication. In corroboration with our findings, Hendrickson et al. (2018) and Baldwin et al. (2016) reported that microfibers were a predominant microplastic in water samples from Lake Superior and tributaries of the Great Lakes. Additionally, a study using filtration (50 µm filter) of 20 liters from each site also identified a high abundance of fibers, which the authors attributed to the use of filtration

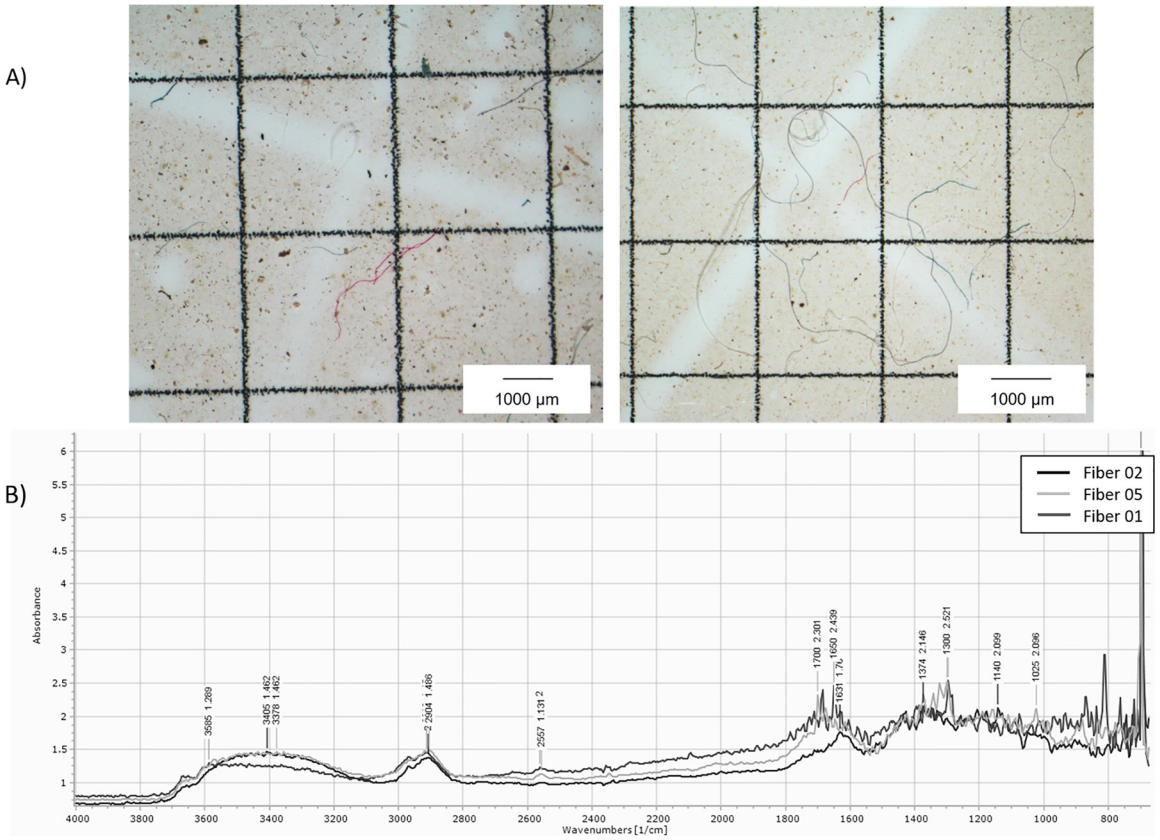

Fig. 2. Representative microfibers photographed on 0.45 µm gridded cellulose nitrate filter from Lake Michigan. Each filter has an abundance of algae, which appears as shaded area in the photograph. The size of fibers varied widely.

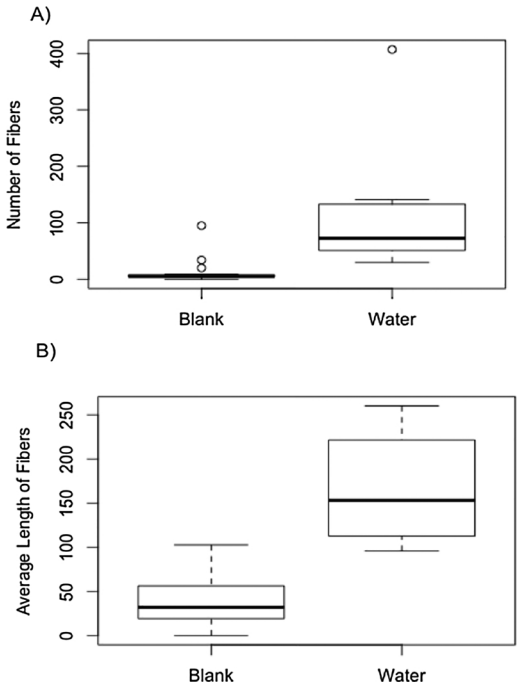

Fig. 3. Filtration blanks (n = 8) were taken during sampling to account for contamination. An analysis of variance showed that there is a statistical difference between blank and sample measurements ($p < 0.001$).

(Wang et al., 2018). Further, the results of this study are similar to findings of Green et al. (2018), a recent study that showed bongo nets and manta trawls caused underestimation (by an order of 3 magnitudes) of microplastics and that sampling with Niskin bottles is superior for collecting very small plastics (Green et al., 2018).

Fibers in environmental samples come from a variety of sources, including clothing, cigarette butts (Wright et al., 2013), ropes, nets, fishing activity (Wang et al., 2018), and atmospheric deposition (Dris et al., 2016). Sampling from wastewater produced by washing machines showed that washing one article of clothing can result in the release of more than 1900 microfibers (Browne et al., 2011). Fibers may be nylon, polyester, or polypropylene, depending on the source of the microfiber (Andrady, 2011; Hidalgo-Ruz et al., 2012). While most fibers from clothing consist of polyester, plasticizers found in waterproof boating ropes, which may be styrene and polystyrene, could be the source of the fibers identified here. However, only a small subset of fibers was investigated using micro-FTIR, and a previous study reported that approximately half of fibers collected may be plastic (Cincinelli et al., 2017), therefore it is not likely that all fibers in this study are plastic. Microfibers collected in a previous study from Lake Superior were most often found to be polyethylene terephthalate (Hendrickson et al., 2018). The chemical constituents in microfibers can be difficult to discern due to their low mass, small size, and the twisting or folding that makes focusing of the micro-FTIR difficult.

The ubiquitous and abundant nature of microplastics recovered from this study is a cause for concern for zooplankton. A number of marine organisms are known to ingest fibers, including *Mytilus edulis* (blue mussel, Mathalon and Hill, 2014), *Euphausia pacifica* (Northern Pacific krill), and *Neocalanus cristatus* (subarctic copepod; Desforges et al., 2015). Ingested microfibers, natural or plastic, can cause blockage within the digestive tract of aquatic organisms. Plastic fibers are especially concerning because they cannot be broken down and may translocate within the organism (Cole, 2016; Wright et al., 2013). The effects of styrene and polystyrene on zooplankton are unclear, but there is evidence that styrene and polystyrene are potentially carcinogenic and toxic (Saido et al., 2014) and could bioaccumulate once in the food web.

5. Conclusions

This study is of note because the sampling methodology (Niskin bottle collection followed by 0.45 μm gridded cellulose filtration) has not been previously used in the Great Lakes and rarely used globally. Using Niskin bottles for sampling, followed by covered filtration, reduces the likelihood of contamination (atmospheric deposition) during sampling and may provide improved estimates of micro- and nanoplastics.

Future work that incorporates microfiltering should include an increased water volume to improve explanatory power. As larger fibers break down, they become bioavailable to smaller organisms, potentially affecting the underpinnings of the whole food web. Understanding the types and abundance of microplastics will lead to future research on bioaccumulation of microplastic fibers and associated pollutants, furthering insight on the movement of plastics through food webs. This study provides evidence that microfiltration should be used in a complete nano-/microplastic sampling scheme, especially when research questions address the potential effects on neuston and zooplankton.

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