

# Microplastics Flowing into Lake Winnipeg: Densities, Sources, Flux, and Fish Exposures

Sarah Warrack<sup>1</sup>, Jonathan K. Challis<sup>2</sup>, Mark L. Hanson<sup>1</sup>, Michael D. Rennie<sup>3</sup>

<sup>1</sup>Department of Environment and Geography, University of Manitoba, Winnipeg, Canada, R3T 2N2

<sup>2</sup>Department of Chemistry, University of Manitoba, Winnipeg, Canada, R3T 2N2

<sup>3</sup>Department of Biology, Lakehead University, Ontario, Canada, P7B 5E1

Corresponding Author: Sarah Warrack (umwarras@myumanitoba.ca)

## Abstract

*Microplastics (plastic particles < 5.0 mm in diameter) have been detected in freshwater ecosystems worldwide. Recently, surface concentrations of microplastics in Lake Winnipeg, Manitoba were shown to be comparable to those observed in Lake Erie, Ontario despite large differences between the lakes in terms of population density and industrial activity. To better understand potential sources of microplastics into Lake Winnipeg, two inflowing tributaries (the Red and Assiniboine rivers) and the lake outflow (the Nelson River) were sampled for microplastics. To determine the role of wastewater treatment plants in contributing to microplastic pollution, microplastic densities upstream and downstream of wastewater treatment plants in the city of Winnipeg were compared. Finally, to determine the bioavailability of microplastics to fishes, we evaluated the presence of microplastics in the gastrointestinal tracts of two fish species, common carp (Cyprinus carpio), and sauger (Sander canadensis), collected from the Red River. Microplastics in the Red and Assiniboine rivers were comparable to those from Great Lake tributaries, but were elevated four to six times relative to concentrations observed in the Nelson River, suggesting significant losses to settling in Lake Winnipeg. On average, densities of microplastics downstream of wastewater treatment plants were elevated and a significant correlation was observed between standardized daily effluent discharge from Winnipeg and river flux of microplastics/m<sup>2</sup>/s. On average, sauger were found to contain one microplastic particle and carp were found to contain seven microplastics within their gastrointestinal tracts. The number of particles ingested did not appear to affect body condition of fish collected in this study.*

**Keywords:** microplastics, plastic debris, rivers, freshwater contamination

## 1 INTRODUCTION

Globally, there is heavy reliance on the use of plastics in the manufacturing of consumer products (Romeo et al., 2015) and many plastics eventually reach our waterways (Klein et al., 2015). Microplastics are defined as small plastic particles less than 5.0 mm in diameter (Eerkes-Merando et al., 2015) which can enter the environment in one of two ways, either directly as microscopic-sized plastics such as microbeads in cosmetic products or scrubbers in cleaning products, or indirectly as larger plastic debris that continuously fragment and degrade into smaller particles (Browne et al., 2011; Arthur et al., 2010; Cole et al., 2014). These larger plastics fragment in the environment due to photolytic, mechanical, or biological degradation (Browne et al., 2007). Current global plastic production is estimated to be 300 million metric tonnes annually and is increasing by 20 million metric tonnes each year (Europe, 2015). By comparison to other forms of anthropogenic pollution (i.e. non-plastics), the degradation time of plastic is very slow, potentially hundreds of years (Europe, 2015).

Microplastic sources include consumer products (microbeads), manufacturing products (larger pellets), textiles (fibres), atmospheric fallout (dust particles), and larger plastics, which break down over time into smaller particles (Browne et al., 2011; Driedger et al., 2015; Dris et al., 2016). When clothing is washed, synthetic microplastic fibres shed, making their way into the sewage (Browne et al., 2011) that is then treated by wastewater treatment plants. Plastic particles not captured by wastewater treatment plants are eventually released, via effluent, into freshwater systems (Browne et al., 2011). Wastewater treatment plants are a point source of microplastics (Eerkes-Merando et al., 2015) as plastic particles are small enough to pass through the filtration process and thus have the potential to enter lakes, rivers, and streams (Browne et al., 2007).

The transportation and accumulation of microplastics are unique in river systems, as the unidirectional current is flowing downstream. Densities of microplastics in the Chicago River were greater in riparian zones than in bottom sediments (Driedger et al., 2015), as the high velocities of the river prevents plastic particles from settling. Proximity to wastewater treatment plants, water body size, depth, ship



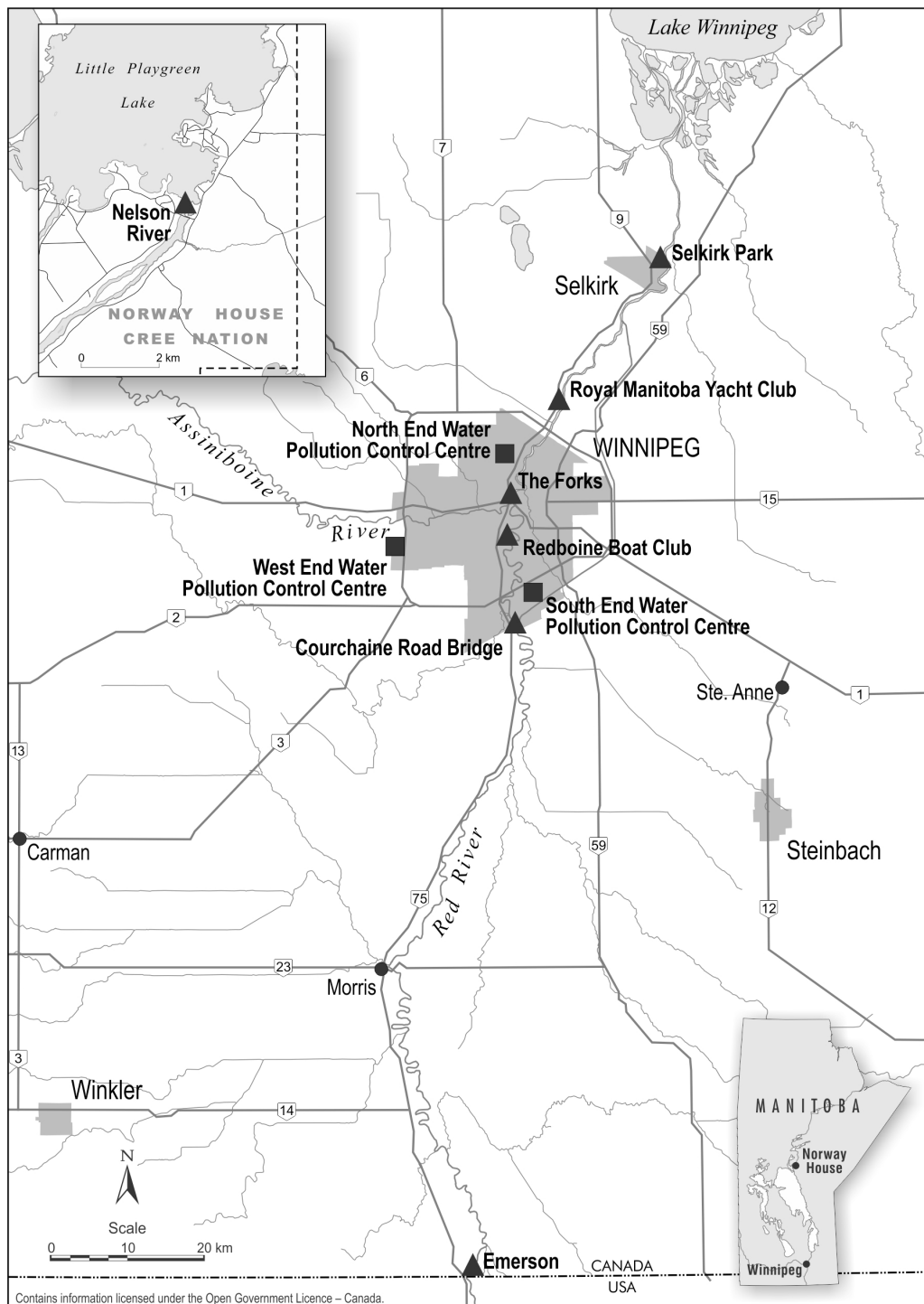


Figure 1: Map of surface water and fish (Selkirk Park) sample collection sites (triangles) and Winnipeg's three wastewater treatment plants (squares): South End Water Pollution Control Centre (SEWPCC), North End Water Pollution Control Centre (NEWPCC), and West End Water Pollution Control Centre (WEWPC). Emerson and Courchaine Road Bridge were upstream of SEWPCC. Redboine Boat Club downstream of SEWPCC. Royal Manitoba Yacht Club (RMYC) downstream of NEWPCC. The Forks site was downstream of WEWPC.

traffic, water turbulence events, seasonal events, water temperature, weather events, and geomorphological characteristics may all influence microplastic transport and possi-

ble accumulation of microplastics within a freshwater system (Mani et al., 2015; Fischer et al., 2016). Freshwater systems are likely a potential contributor to microplastic



loading in oceans (Eerkes-Merando et al., 2015). It is estimated that there are a minimum of five trillion plastic particles currently in the world's oceans, weighing approximately 250,000 tonnes (Eriksen et al., 2014).

Microplastics have been found inside the bodies of a wide variety of marine and freshwater organisms including invertebrates, fish, birds, and mammals (GESAMP, 2015), but the long-term impacts of microplastics on aquatic wildlife are not well understood (Masura et al., 2015). Microplastics may pose a possible hazard to human health through human consumption of aquatic species that contain plastic particles (Romeo et al., 2015), but there is no evidence of any human health impacts at this time.

Lake Winnipeg is the fifth largest Canadian lake and has the second largest watershed in Canada, over 982,000 km<sup>2</sup> (Anderson et al., 2017). The watershed is home to seven million people (20% of the Canadian population) and spans four Canadian provinces and four US states (Schindler, 2009). Lake Winnipeg has a greater density of microplastics per km<sup>2</sup> (193,420 ± 115,567 microplastics/km<sup>2</sup>) compared to Lake Superior (5,391 ± 4,552 microplastics/km<sup>2</sup>) and Lake Huron (2,779 ± 2,440 microplastics/km<sup>2</sup>) (Anderson et al., 2017). By contrast, Lake Erie supports 12 million people in a watershed 1/10th the size of Lake Winnipeg (Anderson et al., 2017). The comparable densities between Lake Erie (105,503 ± 173,587 microplastics/km<sup>2</sup>) and Lake Winnipeg (193,420 ± 115,567 microplastics/km<sup>2</sup>) suggest that either long-range transport of microplastics from rivers is a major contributing source, or a potential source in the watershed may be missed with existing sampling campaigns (Anderson et al., 2017). The Red and Assiniboine rivers flow into Lake Winnipeg, and given their drainage through the city of Winnipeg likely contribute microplastics into the lake. The Nelson River drains Lake Winnipeg, and may be taking microplastics out of the lake. Understanding the potential inflow and outflow of microplastics in Lake Winnipeg by these three rivers provides important context for understanding the high densities observed in the lake (Anderson et al., 2017).

The purpose of this study was to collect and quantify microplastic densities in the surface waters of three Manitoban rivers, as well as to quantify fish ingestion of microplastics to establish a baseline for future monitoring. The data collected on the densities of microplastics in the inflow (Red and Assiniboine rivers) and outflow (Nelson River) of Lake Winnipeg will help to calculate microplastic loading in the lake. The study was also designed to investigate the potential influence that wastewater treatment plants may have on plastic densities in the rivers, as well as spatial and temporal trends. Specifically, we hypothesized that: (1) microplas-

tic densities would be greatest downstream of wastewater treatment plants, and (2) microplastics would be ingested at higher numbers in benthic feeding species, where plastics are likely to be densest. The Assiniboine, Red, and Nelson rivers provide habitat for many fish and waterfowl species, and are culturally and economically important to Manitobans. Characterizing sources, ingestion by fish, and potential impacts of microplastics within the Assiniboine, Red, and Nelson rivers are important steps to further our understanding of this emerging environmental contaminant in freshwater systems.

## 2 METHODS

### 2.1 Sampling Sites

Surface water from six study sites along the Assiniboine, Red, and Nelson rivers in Manitoba, Canada were sampled for microplastic densities (Figure 1). The six sites were selected based on accessibility when using the manta trawl, and their location along the rivers (upstream and downstream of Winnipeg's three wastewater treatment plants: North End Water Pollution Control Centre (NEWPCC) and South End Water Pollution Control Centre (SEWPCC) on the Red River and the West End Water Pollution Control Centre (WEPCC) on the Assiniboine River). The five inflow study sites were: Emerson (to assess contributions from the United States), Courchaine Road Bridge, Redboine Boat Club, the Forks Historic Rail Bridge (Forks), the Royal Manitoba Yacht Club (RMYC), and the outflow study site was the Nelson River in Norway House Cree Nation, Manitoba (Figure 1).

### 2.2 Surface Water Sample Collection, Processing, and Quantification

Surface water was sampled for microplastics using a manta trawl. The manta trawl has a net with a mesh size of 333 μm, is 295 cm long, has an aperture width of 61 cm, and a height of 18 cm. The trawl was deployed facing the rivers current off of bridges or docks. A total of 14 samples were collected from the six study sites in June (Redboine Boat Club, Forks and RMYC), July (Courchaine Road Bridge, Redboine Boat Club, Forks, and RMYC), October (Emerson, Redboine Boat Club, Forks, RMYC) (2016) and May (Nelson River) (2017). The variation in numbers of samples per site was due to logistical issues such as weather and time constraints. The four months were chosen opportunistically and sampled based on field season availability. Seasonality effects may play a role in the densities of microplastics across sites. Variation in



sampling sites was accounted for by grouping sites into categories (upstream versus downstream) and using a flow meter to correct for differences in water flow across sampling sites and times. The shorter sampling time in June was a result of high river flows, making longer deployments of the manta trawl very challenging. Materials captured by the trawl were passed through a 355  $\mu\text{m}$  sieve using MilliQ water. Debris retained in the sieve was placed into labelled mason jars and preserved with 70% ethanol for future processing and analysis.

At time of analysis, samples were filtered through a 355  $\mu\text{m}$  mesh brass sieve and rinsed with deionized (DI) water to remove the ethanol. DI water was added to the sample to reconstitute it to 1,250 mL and a subsample of 250 mL was collected and processed using a wet peroxide oxidation (WPO) treatment (Masura et al., 2015; Mason et al., 2016). The 250 mL subsample was stirred and heated to 75°C, followed by 20 mL additions of a 0.05 M Fe (II) solution and 30% hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) to facilitate chemical digestion of organic material. All digestions were conducted in a laminar flow hood. At approximately 10 minute intervals, samples were re-examined and additional  $\text{H}_2\text{O}_2$  was added. The process was repeated until all organic material was digested. Samples were covered and left for 24 hours to digest fully. Blanks were deployed to account for possible airborne and waterborne (in our DI water) microplastic contamination from the lab while samples were digested and enumerated. The WPO treatment has the potential to digest some of the microplastics within our samples as temperatures are elevated to (>80°C) immediately after peroxide additions.

After filtering the sample through the sieve and rinsing with DI water to remove  $\text{H}_2\text{O}_2$ , the contents were placed into glass Petri dishes. The number and type of microplastic particles were visually examined in the Petri dish using a dissecting microscope. Microplastics were counted and types were recorded. Our microplastic particles were categorized into five shape categories: fragments (hard with jagged edges), foams (sponge-like and light weight), fibres (thin lines), pellets (hard and spherical in shape), and films (thin and flimsy) (Figure 2). These types were part of our primary search pattern based on their presence in other marine and freshwater samples reported in the literature (e.g., Eriksen et al. (2013), Anderson et al. (2017), Baldwin et al. (2016)). Microplastic particles were transferred using a fine-tipped probe to a glass vial containing ethanol and sealed with a rubber stopper for long-term storage. The number of microplastic particles collected was calculated for each trawl and used to calculate the densities of microplastics/ $\text{km}^2$ .

The same methods, lab, and personnel were used to visually sort and identify the microplastics as Anderson et al.

(2017). That study found a 78% success rate at identifying plastic visually compared with the examination of samples using scanning electron microscopy and energy dispersive X-ray spectroscopy (Anderson et al., 2017). The densities of microplastics reported here were corrected for this identification bias (microplastic densities multiplied by 0.78).

### 2.3 Fish Collection, Processing, and Quantification

Fish were collected at Selkirk Park in Selkirk, Manitoba (Figure 1) following approved collection protocols (F16-029). Two fish species, *Cyprinus carpio* (common carp) and *Sander canadensis* (sauger) were obtained with the help of the Department of Fisheries and Oceans Canada's electrofishing boat in October 2016. The two fish species were selected as they occupy different ecological niches. Carp are a benthic filter feeder and sauger are a pelagic predatory species. Sampling took place near the shoreline and the fish were captured using long dip nets after being electroshocked and having floated to the surface of the water. The fish were then placed in a water bath and euthanized on site using an overdose of tricaine methylsulfate (TMS-MS-222). The fish were placed into freezer bags, placed on ice, and transported back to the University of Manitoba. The fish were placed in a freezer at -20°C for later processing. After thawing, nine sauger and eight carp were weighed and fork and total lengths were measured.

The fish were then dissected and the whole gastrointestinal tract removed from the esophagus to the anus. The entire gastrointestinal tract for each fish, fully intact, was processed using the same WPO method to digest organic material (Masura et al., 2015), with a small adjustment to deal with the high fat content of the fish. Right after the sample was processed for the first time, the sample was sieved and processed again. The samples were also rinsed with a solvent (ethanol)

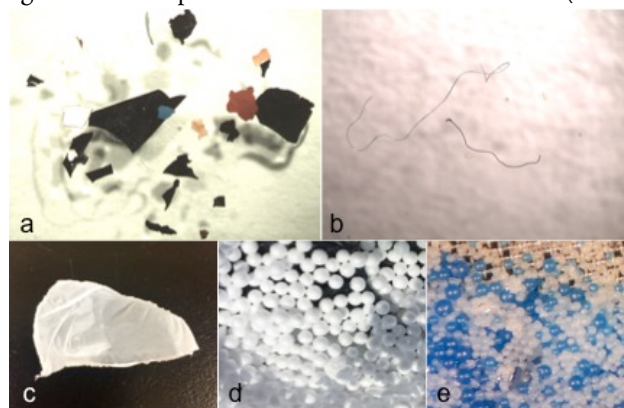


Figure 2: The five shapes of microplastics: (a) fragments, (b) fibres, (c) films, (d) foams, (e) pellets. Photo credit: Sarah Warrack, University of Manitoba.



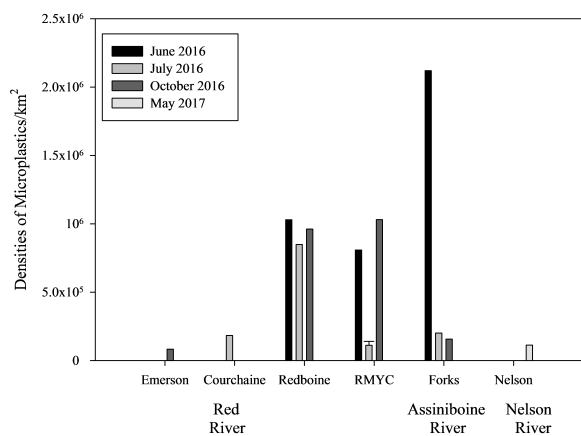


Figure 3: Densities of microplastics/ $\text{km}^2$  calculated for each sampling date at four Red River sites arranged South to North in direction of flow: Emerson, Courchaine Road Bridge, Redboine Boat Club, and the Royal Manitoba Yacht Club (RMYC), the Assiniboine River at the Forks, and the Nelson River. Density calculations were determined based on the number of microplastics counted in a given sample and the approximate surface area sampled by the manta trawl over a given deployment time. A triplicate sample was collected in July at the RMYC, the average and standard error calculated was  $112,002 \pm 29,339$  microplastics/ $\text{km}^2$ .

when sieving to get rid of any excess fat that the WPO did not digest. Once all organic material was digested, the contents were sieved, placed into glass Petri dish, and the number and type of microplastic particles were visually examined using a dissecting microscope. The number of microplastics found within each individual fish's digestive tract was counted and transferred to ethanol in a glass vial with a rubber stopper for long-term storage.

## 2.4 Blanks

Lab blanks were used to determine possible contamination from either air or DI water. DI water blanks were run under the DI water tap at a rate of eight L/min (480 L total) at the University of Manitoba for 60 minutes using a clean  $355 \mu\text{m}$  brass sieve. Air blanks were employed by leaving one L mason jars of Milli-Q water out on the lab counter for 24 hours.

## 2.5 Data Analysis

Microplastic densities (microplastics/ $\text{km}^2$ ) were analyzed by a Student's *t*-test using densities and site location (upstream versus downstream of wastewater treatment plants) as variables. A Pearson linear correlation was used to evaluate association between river velocity and the density of microplastics sampled, as well as association between sewage discharge and flux of microplastics in the river. The latter was analyzed using Z-scores, calculated for both flux and discharge (value

– mean / standard deviation). Standardizing the data using the Z-score allowed for comparisons across sites. The flux calculation was used to understand the amount of microplastics in a cross-sectional area in the rivers at a given time, accounting for differences in discharge volume at different sampling sites. Discharge ( $\text{m}^3/\text{sec}$ ) data was obtained from the Government of Canada's hydrometric monitoring data (Government of Canada: Water Office, 2017). A station (closest to our site) was selected and an average of the daily maximum and minimum discharge values were taken. Linear regression was used to compare counts of ingested microplastics with fish weight. A Student's *t*-test was also used to compare the number of microplastics ingested by the two fish species to help determine if fish species had different rates of microplastic ingestion. All statistical analyses were conducted using Sigma Plot with statistical significance considered for  $p < 0.05$ .

## 3 RESULTS

### 3.1 Quality Assurance and Quality Control

The DI water blanks contained 13, 5, 16, and 9 particles (all fibres). These data suggest that on average, in our laboratory, one microplastic fibre is introduced for every 48 L of DI water used when processing the samples. The average rinse time of a sample is five minutes with DI water (at eight L/minute), with reconstitution to 1.25 L prior to subsampling, resulting in, on average, an estimated 0.85 fibres introduced to our samples from DI water alone. Two air blanks recorded eight and seven fibres over the 24 hour time period, or 0.3 fibres/hour. With an average microscope analysis time of four hours, we estimate that on average 1.25 microplastic particles were introduced from the lab air. In total, while processing samples, about two fibres/sample were likely introduced due to DI water and air. All water and fish sample counts were corrected by this blank contamination factor (two subtracted from all fibre counts). This contamination of microplastics likely affected the overall counts of microplastics in the gastrointestinal tract contents of fish more significantly than surface water samples, since the counts in fish were much lower than in samples (e.g.,  $\leq$  six fibres per fish).

Triplicate samples were taken at the Red River site, RMYC (downstream of NEWPCC) in July, where the manta trawl was deployed three times to evaluate within-site variability. The three densities of microplastics in the triplicates were: 161,275; 59,771; and 114,960 microplastics/ $\text{km}^2$ . The average and standard error calculated for RMYC in July was  $112,002 \pm 29,339$  microplastics/ $\text{km}^2$ .



### 3.2 Surface Water

Microplastics were found in samples from all sites. Within the Red River inflow there was an average surface density of 632,489 microplastics/km<sup>2</sup> (n=8), and within the Assiniboine River inflow there was an average surface density of 812,672 microplastics/km<sup>2</sup> (n=3) across all sampling sites and dates (Figure 3). Density calculations were determined based on the number of microplastics counted in a given sample and the approximate surface area sampled by the manta trawl over a given deployment time. Surface area was calculated from the distance trawled, determined using a General Oceanics 2030R mechanical flow meter, and the width of the manta net opening (61 cm). A fundamental assumption to these calculations is the fact that while we report surface densities, in fact, there is a volume of water being sampled. One third of the net was deployed below the surface of the water during deployment, which was used to calculate the volume of water sampled. To remain consistent with the current literature, we report both microplastic surface (Eriksen et al., 2013; Anderson et al., 2017) and volume densities (Baldwin et al., 2016) (Table 1). The greatest inflow densities of microplastics/km<sup>2</sup> in the Red River were observed in June, downstream of the SEWPCC (1,030,091 microplastics/km<sup>2</sup>) and October downstream of the NEWPCC (1,030,922 microplastics/km<sup>2</sup>). In the Red River, the density of microplastics collected in June and October increased with the direction of flow from south

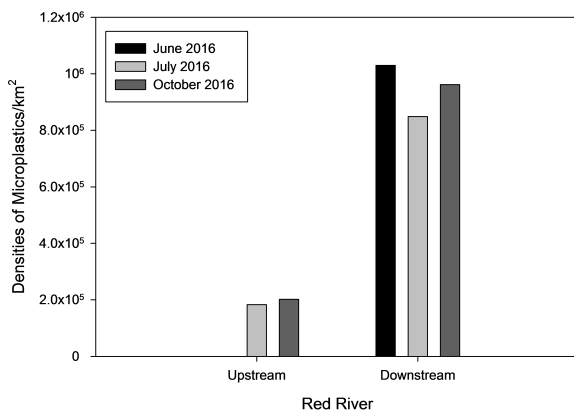


Figure 4: Mean densities of microplastics/km<sup>2</sup> in the Red River upstream and downstream of the South End Water Pollution Control Centre (SEWPCC). No upstream site was sampled in June 2016 (preliminary sampling). Upstream SEWPCC sites: Emerson and Courchaine Road Bridge, and downstream SEWPCC site: Redboine Boat Club.

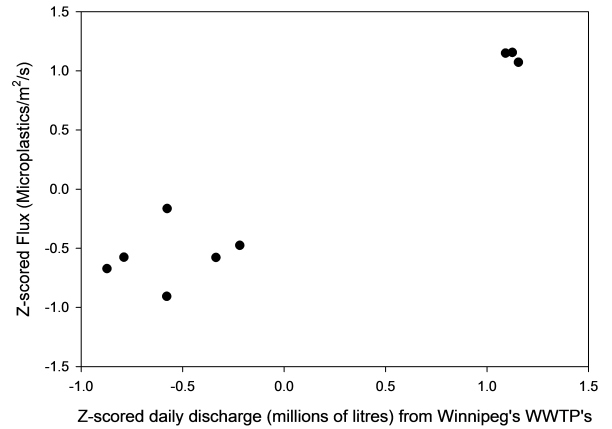


Figure 5: Z-scored river flux of microplastics from the Red and Assiniboine rivers associated with Z-scored Waste Water Treatment Plant (WWTP) flux confirmed significant relationship (Pearson Linear Correlation:  $r=0.96$ ,  $n=9$ ,  $p\text{-value}=0.00005$ ). Redboine was sampled downstream of the South End Water Pollution Control Centre, the Forks was sampled downstream of the West End Water Pollution Control Centre and Royal Manitoba Yacht Club (RMYC) was sampled downstream of North End Water Pollution Control Centre.

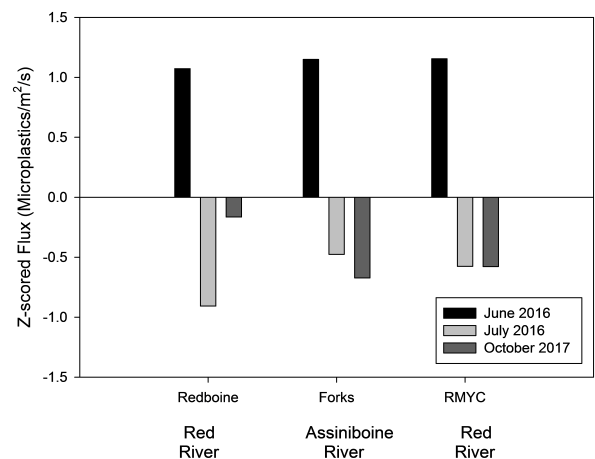


Figure 6: Flux of microplastics/m<sup>2</sup>/s (Z-scored) within the Red and Assiniboine rivers as a function of season (summer/ fall) where flux of microplastics/m<sup>2</sup>/s was higher in early summer (June 27), and lower in both mid-summer and fall (July and October). Redboine was sampled downstream of the South End Water Pollution Control Centre (SEWPCC), the Forks was sampled downstream of the West End Water Pollution Control Centre (WEWPCC), and Royal Manitoba Yacht Club (RMYC) was sampled downstream of North End Water Pollution Control Centre (NEWPCC).



Table 1: *Microplastic densities in surface areas (microplastics/km<sup>2</sup>) and volume (microplastics/m<sup>3</sup>) for Emerson, Courchaine, Redboine, Royal Manitoba Yacht Club (RMYC) and the Forks. The sites are arranged from south to north on the Red River.*

Date	Site	River	Surface Area Densities	Volume Densities
06-Oct-16	Emerson	Red	65,626.93	0.70
07-Jul-16	Courchaine	Red	143,201.90	1.53
27-Jun-16	Redboine	Red	806,763.83	8.62
07-Jul-16	Redboine	Red	667,045.81	7.13
06-Oct-16	Redboine	Red	751,743.85	8.03
27-Jun-16	RMYC	Red	631,540.63	6.75
07-Jul-16	RMYC	Red	126,142.26	0.94
06-Oct-16	RMYC	Red	808,549.79	8.64
27-Jun-16	Forks	Assiniboine	1,655,485.65	17.69
07-Jul-16	Forks	Assiniboine	157,686.44	1.69
06-Oct-16	Forks	Assiniboine	122,920.24	1.31
04-May-17	Nelson	Nelson	88,832.44	0.95

to north (Figure 3). In October, the density of microplastics along the southern part of the Red River was lower (Emerson site) and plateaued as sites moved north (Redboine Boat Club, RMYC). The densities of microplastics in the Assiniboine River sampled at the Forks in June 2016 had the greatest density of microplastics of all the sites (2,120,066 microplastics/km<sup>2</sup>). In the Assiniboine River, the densities of microplastics decreased from June to October. The Nelson River had 88,832 microplastics/km<sup>2</sup>, when sampled in May. The estimated daily inflow of microplastics from the Red River into Lake Winnipeg is 1,100,000 microplastics, so the Red River is contributing 401,500,000 microplastics annually to Lake Winnipeg, which contains a total of about 4,800,000,000 microplastics (Anderson et al., 2017). The estimated daily outflow of microplastics from Lake Winnipeg to the Nelson River is 10,800 microplastics, therefore the Nelson River is taking out 3,942,039 microplastics from Lake Winnipeg annually.

In the Red River, upstream of the SEWPCC (July 2016 and October 2016) the microplastic densities were significantly different from downstream of the SEWPCC, potentially due to wastewater inputs (Figure 4; one-tailed t-test, d.f= 3, p=0.00093). The Assiniboine River enters the Red River at the Forks, which is upstream of the RMYC.

A linear regression indicated no significant relationship between microplastic densities (microplastics/km<sup>2</sup>) and river velocity ( $R^2=0.00003$ ,  $n=14$ ,  $p\text{-value}=0.98$ ). In contrast, there was a significant correlation between the standardized (Z-scored) volume of wastewater treatment plant discharge and standardized (Z-scored) microplastic flux in the Red and Assiniboine rivers at regions near wastewater treatment plants (Pearson Linear Correlation:  $R=0.96$ ,  $n=9$ ,

$p\text{-value}=0.00005$ ) (Figure 5). Flux of microplastics in the Red and Assiniboine followed a seasonal pattern of highest in spring (June) and lower in both summer and fall (July and October) (Figure 6), as was discharge from wastewater treatment plants.

The most common type of microplastics across all sites was identified as fibres (89%) (Figure 7). Pellets (0.2%), foams (0.32%), and films (0.2%) were the least common microplastic types detected (Figure 7).

### 3.3 Fish

Plastics were detected in seven of nine sauger; however, when corrected for possible contamination during processing, only four of nine sauger contained plastic. The average (corrected) count of microplastics within the nine sauger were one microplastic particle per fish. The average weight of sauger was  $232.7 \pm 17.2$  grams and average fork length was  $23.5 \pm 2.9$  cm (total length was  $29.3 \pm 0.9$  cm). Linear regression indicated no significant relationship between the counts of microplastics and sauger size ( $R^2=0.06$ ,  $n=9$ ,  $p\text{-value}=0.53$ ).

Plastics were detected in eight of eight carp, and when corrected for possible contamination during processing, only seven of eight carp contained plastic. The average (corrected) count of microplastics within the eight carp were seven microplastic particles. The average weight of the carp was  $3849.5 \pm 17.2$  grams and average fork length was  $55.2 \pm 0.97$  cm (total length was  $60.5 \pm 0.93$  cm). Linear regression revealed that there was no significant relationship between counts of microplastics and carp size ( $R^2=0.04$ ,  $n=8$ ,  $p\text{-value}=0.64$ ).



Significant differences in the number of ingested microplastics were observed for sauger ( $1 \pm 1.5$ ,  $n=9$ ) and carp ( $7.1 \pm 7$ ,  $n=8$ ) collected within the Red River, Manitoba, Canada in October 2016 (Student's *t*-test two-tailed  $\alpha=0.05$ ,  $d.f=15$ ,  $p=0.01$ ). Both carp and sauger had ingested fibres and fragments. Only one carp had ingested a film. Of the 17 fish processed, 65% contained plastic (44% of sauger and 88% of carp).

## 4 DISCUSSION

### 4.1 Surface water

Microplastics were present in the surface waters of the 14 samples examined and contained on average 806,352 microplastics/km<sup>2</sup> (Red River), 1,241,085 microplastics/km<sup>2</sup> (Assiniboine River), and 113,888 microplastics/km<sup>2</sup> (Nelson River) (Table 1).

The densities of microplastics in these rivers (Table 1) are comparable to those in other rivers reported elsewhere. In the Rhine River in Germany, 892,277 microplastics/km<sup>2</sup> were reported (Mani et al., 2015). Four estuarine rivers in Chesapeake Bay, USA found average densities of microplastics to range between 40,852 and 155,374 microplastics/km<sup>2</sup> (Yonkos et al., 2014). Volumetric estimates of microplastics within the Red, Assiniboine and Nelson rivers ranged from 0.7 microplastics/m<sup>3</sup> to 18 microplastics/m<sup>3</sup>, with an average of 5.3 microplastics/m<sup>3</sup> (Table 1). These estimates are also comparable to those reported for Great Lakes tributaries which ranged from 0.5 to 32 microplastics/m<sup>3</sup> with an average of 4.3 microplastics/m<sup>3</sup> (Baldwin et al., 2016). Some evidence exists to suggest that microplastic densities are higher in rivers with greater population density (Yonkos et al., 2014). We also found this to be the case in our study, where the density of microplastics was lower in the Nelson River (downstream of Norway House, population approximately 5,000), compared to the Red River (Winnipeg, population approximately 700,000). In addition, microplastics appear to be at lowest densities at higher latitudes (Lusher et al., 2015). Plastics have an anthropogenic origin, and as latitude increases, human population densities decrease (Browne et al., 2011; Eriksen et al., 2013).

Of note is that the inflow densities in the Red and Assiniboine rivers are four to six times greater than densities observed in Lake Winnipeg (200,000 microplastics/km<sup>2</sup>) (Anderson et al., 2017), whereas the outflow densities are only 50% of the mean lake wide density. This large negative gradient in surface densities from the Red River, through Lake Winnipeg and into the Nelson is highly suggestive of significant losses due to settling within the lake. While inputs from

the Saskatchewan River and Winnipeg River are not quantified here, they together contribute nearly 75% of the water input to Lake Winnipeg (Manitoba Water Stewardship, 2011). Thus, water inputs from these other tributaries may act to dilute inputs from the Red, or, if microplastic densities in these other tributaries are comparable, it suggests that Lake Winnipeg could be an even greater sink for microplastics in this system than suggested by the current study.

Densities of microplastics in the surface waters of the Red and Assiniboine rivers appear to be influenced by daily discharge of effluent from Winnipeg's three wastewater treatment plants (Figure 5). If so, our findings are consistent with other studies that have found significant correlation between wastewater treatment plant discharge and densities of microplastics (A. R. McCormick et al., 2016). Densities of microplastics were greater downstream of the SEW-PCC (Figure 4) which is consistent with a study conducted in Illinois, United States which found greater microplastic densities downstream of wastewater treatment plants compared to upstream in seven of nine rivers ( $p \leq 0.001$ ) (A. R. McCormick et al., 2016). Seasonality may be driving the correlation between wastewater treatment plant and density of microplastics in the surface waters of the rivers (Yonkos et al., 2014) as June had the highest wastewater treatment plant daily discharge of raw effluent for each of Winnipeg's three wastewater treatment plants' and June also had the highest flux of microplastics within the Red and Assiniboine rivers. Further investigation is required to better understand seasonal trends in microplastic densities in these rivers.

Microplastics in surface waters are influenced by wind (Browne et al., 2011) and rain events (Moore et al., 2011) as they can transfer terrestrial debris into the waterway, increasing the amount of microplastics in the system (Yonkos et al., 2014). Microplastic densities were elevated in June for the Red and Assiniboine rivers (Figure 3), which may be attributed to rain that occurred a few days before sampling. For the June 27, 2016 sampling, it rained June 24-26 and rainfall ranged from a low of 4.3 mm to a high of 39.4 mm (City of Winnipeg, 2017). July 7, 2016 rainfall ranged from a low of 0.3 mm to a high of 30.4 mm (City of Winnipeg, 2017). For the October 6, 2016 sampling it rained October 4th and 5th, ranging from a low of 7.8 mm to a high of 23.3 mm (City of Winnipeg, 2017). Therefore it rained more in June than July and October, which may have added to the higher densities of microplastics in June.

Fibres were the predominant type of microplastic particles found in the surface waters of the rivers sampled here (89%) (Figure 7). Other studies in freshwater (both rivers and lakes) have also found the majority of particles in their samples to be fibres (71% to 86%) (Baldwin et al., 2016; An-





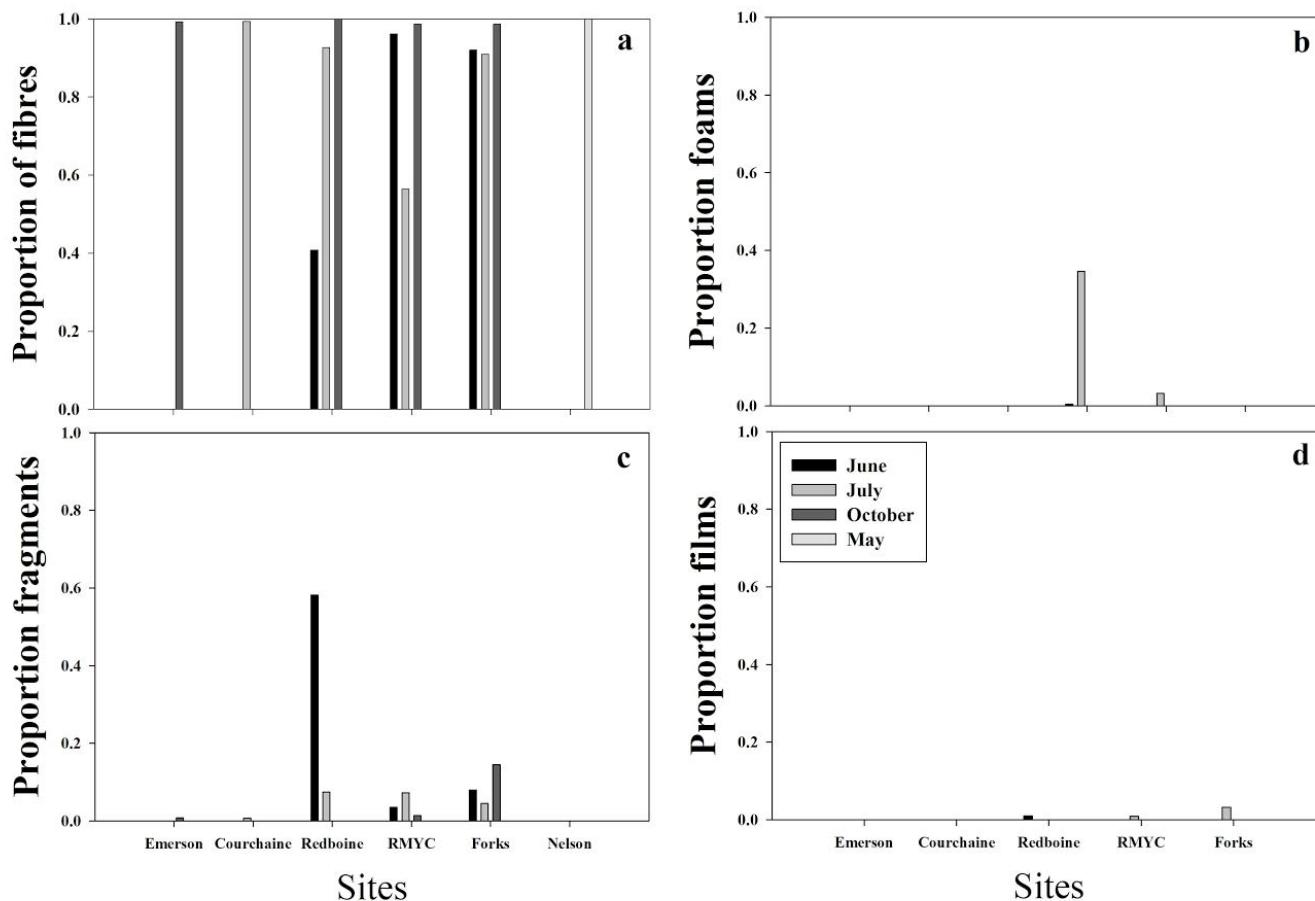


Figure 7: The proportion of the four types of microplastic particles a) fibres, b) foams, c) fragments, and d) films, found at the 6 sites. Red River sites were Emerson, Courchaine, Redboine, and Royal Manitoba Yacht Club (RMYC), the Assiniboine River site was the Forks, and the Nelson River site. The Nelson River site only contained fibres.

derson et al., 2017; Campbell et al., 2017). It remains unclear whether wastewater effluent is a primary source of microplastic fibres. Some studies have found that wastewater effluent is a significant source of fibres (Browne et al., 2011; A. McCormick et al., 2014; Mason et al., 2016), while other studies have found that wastewater treatment plants are effective at removing plastic particles (primarily fibres) as most if not all particles settle out in the sludge (Carr et al., 2016; Dris et al., 2016). However, the remaining 1.5% of plastic particles in wastewater treatment plant effluent still results in a major source of millions of microplastics discharged to rivers daily (Mason et al., 2016).

Our analysis suggests that large amounts of microplastics are being deposited in Lake Winnipeg sediments every year. The estimated total surface density of microplastics in Lake Winnipeg appears to be constant at approximately 4.8 billion from 2014 to 2016 (average density of mi-

croplastics over three years of study approximately 200,000 microplastics/km<sup>2</sup> (Anderson et al., 2017) with a surface area of the lake approximately 24,000 km<sup>2</sup>). Based on an estimate of daily flux from the RMYC (site closest to inflow of Lake Winnipeg) of 20,000 microplastics per day, and an estimated 55 m<sup>2</sup> cross sectional area of the Red River at RMYC, we estimate the annual input of microplastics from the Red and Assiniboine rivers is roughly 0.4 billion particles. By comparison, flux estimates from the Nelson River indicate that only 0.004 billion microplastics (1.0% of the input from the Red and Assiniboine rivers) are lost annually through the Nelson River (outflow of Lake Winnipeg). If we assumed that the contributions of other tributaries to Lake Winnipeg provided negligible amounts of microplastics (a highly conservative assumption), then the riverine input from the Red and Assiniboine rivers of 0.4 billion microplastics annually is three orders of magnitude greater than the measured loss



from the Nelson River, leaving 99% of the microplastics entering the lake through the Red River unaccounted for. The inclusion of any potential additional inputs (e.g., from the South Saskatchewan River) would only increase this loss rate even higher. One possible route for losses of microplastics is settling or sedimentation. Very little is currently known about sedimentation rates of microplastics, and our results strongly suggest value in pursuing investigations into quantifying microplastic settling rates and determining what mechanisms influence settling.

## 4.2 Fish

Microplastic ingestion by fish in the Red River was species-dependent. Carp had an average of seven microplastic particles whereas sauger had an average of one microplastic particle in their gastrointestinal tracts at the time of sampling. This difference between species may be due to their feeding strategy. Microplastic ingestion may be occurring accidentally when the fish are feeding (Lusher et al., 2013) or breathing. Carp are benthic feeders, and ingest all particles from the bottom layer of the river; non-food particles (sediments and plastics) are released through their gills (Food and Agriculture Organization of the United Nations, 2017). As carp do this, they stir up the sediments, possibly re-suspending microplastics that have settled (Food and Agriculture Organization of the United Nations, 2017). Sauger are predators, and may be less likely to encounter plastics compared to filter-feeding carp, and therefore ingest lower densities of microplastics.

Our findings differed from Lusher et al. (2013) who found no significant difference between the amount of microplastics in benthic versus pelagic fish in the English Channel (saltwater). Lusher et al. (2013) found that both pelagic and benthic fish species ingested 1-2 microplastics on average and total ingestion ranged from 1-15 microplastics. Our study found ingested microplastic particles ranging from 1-24. About 65% of all fish had ingested microplastics in our study (after blank corrections), compared to Lusher et al. (2013) where only 37% of all fish had ingested microplastics. This also suggests that there may be more microplastics in the sediments of the rivers, and future sampling should focus on sediment sampling of microplastics. No studies have been conducted on excretion of microplastic particles, so we are unsure of how many particles fish are ingesting throughout their life. The presence of plastics within the fish at the moment of sampling only indicates that the fish have recently ingested plastic (Foekema et al., 2013). The microplastic particles are as small as, or even smaller than, what fish typically eat. Therefore, it is not likely that fish are retaining these particles and they are most likely being excreted with the other

waste products (Foekema et al., 2013). The particles are too small for the fish sampled in this study to likely feel satiated, cause intestinal blockage, or be in the fish long enough to be a vector of harmful contamination (Foekema et al., 2013). Due to the low numbers of microplastics fish in the Red River ingested, it does not appear likely that plastics affect body condition in this study.

In this study, the amount of microplastics ingested by two fish species (carp and sauger) differed ( $7.1 \pm 7$  and  $1 \pm 1.5$ ) and feeding strategy may explain the difference. Campbell et al. (2017) did not find significant differences in the number of microplastics in the gastrointestinal tracts of different benthic and planktivorous fish species, yet northern pike, an apex predator, ingested the most microplastics. This higher density of microplastics may be due to trophic transfer of microplastics from ingested prey species (Campbell et al., 2017).

## 5 CONCLUSIONS

Microplastics were found in the Red, Assiniboine, and Nelson rivers. The majority of microplastics found in the rivers were fibres (89%). Other studies have also found that the majority of plastic particles are fibres (Baldwin et al., 2016; Anderson et al., 2017; Campbell et al., 2017). There were significant differences in the densities of microplastics found upstream versus downstream of wastewater treatment plants in the Red River, suggesting that wastewater treatment plants are a point source of microplastics in surface waters (Eerkes-Merando et al., 2015). The long-range transport of microplastics in rivers is a major contributor to the input and output of microplastics within their watersheds (Anderson et al., 2017). The Red and Assiniboine rivers are contributing 0.4 billion microplastics annually to Lake Winnipeg, and appear to be the major contributors of microplastics to Lake Winnipeg.

Future research efforts should focus on quantifying settling rates and sedimentation processes of microplastics in freshwater systems (both rivers and lakes). Quantifying settling rates of microplastics will help to understand how long microplastics persist in both lentic and lotic environments, and the processes that increase their settling rate.

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