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A watershed-scale, citizen science approach to quantifying microplastic concentration in a mixed land-use river

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ABSTRACT

Microplastic (particles < 5 mm) pollution dynamics are well documented in oceans and increasingly studied in freshwater. We used a watershed-scale approach to examine spatial and temporal patterns in microplastic concentrations in the Gallatin River watershed (Montana, USA). At 72 sites, trained volunteers collected ~1-L grab samples at 4 seasons per year over 2 years (n = 714 samples). Microplastics were found in 57% of the samples (mean = 1.2 particles L^{-1}). The majority of particles were fibers (80%), 0.1–1.5 mm long. Chemical identification determined 93% of particles measured by μFT-IR were synthetic or semi-synthetic materials. Microplastic concentration differed significantly among dates, but showed no longitudinal pattern or relationship to land-use among subwatersheds. At two sites with gaging stations, microplastic was negatively related to discharge when compared across dates. This suggests stormwater is not a source of microplastic in this watershed, but instead dilutes microplastic inputs from other sources. We conclude that microplastic sources are diverse, and measurements of microplastic deposition, resuspension, and transport may be needed to clarify the role of land-use patterns on microplastic pollution. This large scale, citizen science based approach provides a model for future analysis which can further expand microplastic collection at the watershed scale.

1. Introduction

Plastic pollution is a growing problem occurring at a global scale. Large scale industrial production of plastics has accelerated since the beginning of the plastics industry in the mid-1900s (Geyer et al., 2017). It is a preferred material due to its low cost, durability, low density, and the perception of its expendability. The development of single-use plastic, most notably in packaging, has increased its popularity and share of global municipal solid waste (Geyer et al., 2017; Jambeck et al., 2015). Plastic entering the environment accumulates and persists in near permanence (Barnes et al., 2009). Accordingly, plastic pollution has been documented in all major ocean basins and a growing number of freshwater and terrestrial environments (Rillig, 2012; Wagner et al., 2014).

Microplastics (i.e., particles < 5 mm) are an important component of global plastic pollution (Arthur et al., 2009; Cole et al., 2011; Dris et al., 2017). Primary microplastics are manufactured to this size class, such as microbeads (e.g., personal care products and industrial cleaners) and pre-production plastic pellets. In contrast, secondary microplastics originate as larger items and fragment or degrade into microplastic (Andrady, 2011; Browne et al., 2011). Secondary microplastics include uneven fragments, pellets, rounded granules, or filaments known as microfibers.

Microfibers are among the most common microplastic encountered on shorelines, lakes, and rivers worldwide (Browne et al., 2011; McCormick et al., 2016; Miller et al., 2017; Wang et al., 2017). Microfibers are threads, approximately 0.1–5 mm long and ~0.04 mm wide (Barrows et al., 2017b). Not all microfibers are plastic, as some are derived from non-synthetic textiles such as cotton, wool, silk or cellulose, the latter frequently made into a semi-synthetic material via further processing (e.g. rayon, viscose). A primary source of microfiber pollution is effluent from

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wastewater treatment plants which contains fibers fragmented from clothes during washing (Browne et al., 2011; Hoellein et al., 2017; McCormick et al., 2016). Atmospheric deposition is another potentially significant microfiber source into the aquatic environment (Dris et al. 2016, 2017).

While considered a major transporter of plastic pollution to global oceans (Lebreton et al., 2017), initial studies of microplastic in rivers show high spatial and temporal variation in plastic concentration (Miller et al., 2017; Vermaire et al., 2017; Yonkos et al., 2014). Like naturally occurring allochthonous particles (e.g., wood, leaf litter, and sediments), plastic particles that enter rivers can be transported, retained, buried, or degraded (Webster et al., 1999). Biological and physical degradation of plastic is very slow, so most research has focused on measuring abundance and movement of plastic within and across riverine habitats (McCormick and Hoellein, 2016). For example, longitudinal assessments of microplastic have been attempted to infer major sources and sinks in rivers. Some studies showed higher concentrations of microplastics near urbanized regions (Mani et al., 2015, McNeish et al., 2018) or downstream of wastewater treatment plant outfalls (McCormick et al. 2014, 2016), while others show high longitudinal variation and no clear correlation with watershed land-use or landscape features (Hoellein et al., 2017; Klein et al., 2015; Miller et al., 2017). High variation of microplastic concentration appears to reflect the reality of *in situ* microplastic heterogeneity in river ecosystems. However, no previous research has used a watershed-scale approach to examine spatial and temporal patterns in microplastic concentrations. Quantifying microplastic throughout an individual watershed that contains contrasting land-use, and completed over different seasons, could help reveal factors driving microplastic abundance in lotic ecosystems.

The study was conducted within the Gallatin River Watershed (area = 485,622 ha) using a citizen science approach. The watershed contains a diversity of land-use types. The upper reaches include protected areas in Yellowstone National Park, National Forest lands, and a wilderness area. Much of the watershed is situated in Gallatin County, MT, one of the fastest growing regions in the United States (pop. 100,000). The Gallatin River, at the headwaters of the Missouri River watershed, is categorized as a 'Blue Ribbon Trout stream' (i.e., exceptionally high-quality fishing). The Gallatin watershed is popular for outdoor recreation, including kayaking, rafting, fishing, mountain biking, hiking, climbing, skiing, snowshoeing, ice climbing, and camping. An estimated 2.8 million non-Montana residents visited Gallatin County in 2017 with visitor surveys showing that 41% participated in hiking, and four of the top five sites visited were public lands. Because of the large study area, narrow time frames for seasonal sampling used in this analysis, and financial constraints, we used trained volunteers (i.e. citizen scientists) for data collection. Citizen science is growing in use in studies of large scale data collection on research for plastic pollution in general (Hoellein et al., 2015; McKinley et al., 2016) and microplastic in particular (Eriksen et al., 2014; Hidalgo-Ruz and Thiel, 2013; Law et al., 2014; Ogata et al., 2009; Zettler et al., 2017). We placed emphasis on data quality as opposed to providing an environmental education experience for volunteers, which should be mirrored in future citizen science efforts on microplastic research.

This study provides the first temporally and spatially explicit measurement of microplastic throughout an individual watershed. We hypothesized that (1) water samples near populated areas and in more developed sub-watersheds will show higher microplastic concentrations, (2) microfibers will be the predominant microplastic shape found at all sites and dates, and (3) microplastic will be highest at periods of peak discharge.

2. Materials & methods

2.1. Description of study area

The Gallatin Watershed is located in Southwest Montana and Northwest Wyoming (Fig. 1). The mainstem of the Gallatin River originates in Yellowstone National Park's high plateau and descends north along Montana highway 191, flanked by the Gallatin and Madison mountain ranges. The mainstem receives tributary input from protected wilderness areas and developed communities of Big Sky, Gallatin Gateway, and Four Corners. The East Gallatin River is east of the city of Bozeman, the watershed's largest city, and crosses mostly private lands in Bozeman, Belgrade and Manhattan. Tributaries to the East Gallatin descend from the Bridger and Gallatin Mountains. The Gallatin River and East Gallatin River meet near the town of Manhattan. Land ownership in the watershed is 49% public and 51% private, and includes 634 river kilometers (MSDI, 2017).

2.2. Experimental design

All sampling was conducted with trained volunteers (n = 120) acting as citizen scientists. Adventure Scientists, a Bozeman, Montana-based 501(c)3 conservation organization, recruited, trained, and managed volunteers for this study. Rigorous training methods prepared citizen scientists for data collection, while quality assurance measures built into protocols ensured proper collection methods were used in the field (see Text S1). The citizen scientists had a wide range of both scientific expertise (from no previous training to professional scientists), and field experience (strong outdoor competency to professional outdoor athletes). For a volunteer to participate in the project, they were required to participate in a day-long field protocol training event, completed by a successful sampling, witnessed and approved by Adventure Scientists' staff. Each subsequent sampling event commenced with in-person training to review and confirm volunteers' understanding of sampling procedures.

We collected water samples throughout the study watershed (Fig. 1). The 72 sample sites were distributed at 10 km intervals along the length of the Gallatin River and tributaries from the headwaters to the most downstream site at Logan (Fig. 1). Sample sites were along the mainstem river (n = 22) and tributaries (n = 50). We used a grab sample approach to collect surface water for microplastic using ~1 L of surface water (average of 1.3 L) (Barrows et al., 2017b; McNeish et al., 2018; Miller et al., 2017). Stainless steel sample bottles were triple-rinsed with tap water, sealed, and then triple-rinsed *in situ* with stream water. Samples were collected upstream of the volunteer and sample bottles were immediately capped underwater to reduce air contamination. Citizen scientists collected samples by wading, standing on ice, using a sampling pole from the streambank, or from a kayak. Sampling locations included remote, rural, and urban environments.

Microplastic samples and environmental data were collected by volunteers at mainstem and tributary sites using slightly different approaches. Citizen scientists were given GPS coordinates to direct sampling locations. During the first sampling year, mainstem river samples were taken along a perpendicular transect from river left, center, and right at a sampling site. In the second year, mainstem samples were reduced to one per site as no difference was detected in transect samples from year 1. For tributaries, citizen scientists were directed to collect samples at 2 locations. The first site was approximately 15 m upstream from the confluence with the mainstem. The second site was a minimum of 5 km upstream on the tributary. In the winter, volunteers collected samples in as close proximity to the 5 km mark as possible where water was flowing

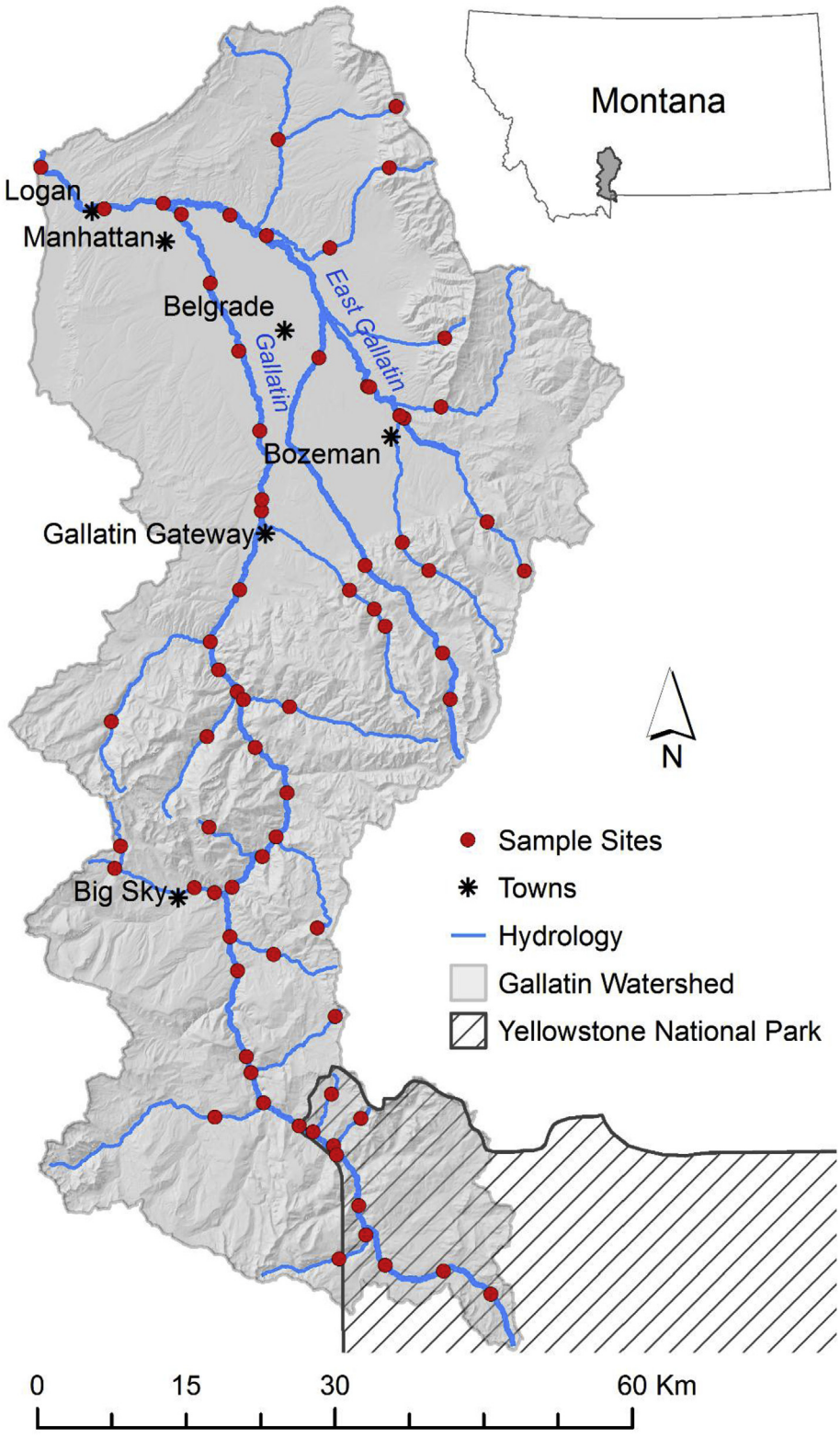

Fig. 1. Gallatin watershed study area (Agriculture 1983; USGS, 2017).

and unfrozen (at some site ice removal was necessary for water access). At all sites, citizen scientists were asked to record the time, GPS coordinates, and field site data (i.e., water temperature, depth, substrate type, presence/absence of exposed rocks, site notes). Citizen scientists used a smartphone application and field data sheet to record information. For quality assurance, citizen scientists answered self-assessment questions regarding their sampling technique (e.g. "Did you remember to cap your bottle underwater?"). They were also asked to submit photos of the clothing worn while sampling, which were later used to determine potential

contamination during sample particle analysis.

The watershed sampling regime was organized into 10-day windows in each season, including September (after peak flow), December (at low flow), March (winter), and June (during peak flow). The study was conducted from September 2015 to June 2017. A total of 774 samples were collected and analyzed for the project over two years, representing the efforts of 117 volunteers. Finally, a minimum of 10 randomly assigned duplicate samples were taken each sampling regime (n = 82) to use as a measure of quality assurance (Table S2.). Duplicate samples were representative of the field samples and were included in site analysis.

2.3. Laboratory analysis

Trained scientists processed the samples by vacuum filtration and particles were counted under a stereo microscope (Barrows et al., 2017b; Hidalgo-Ruz et al., 2012; Miller et al., 2017). The samples were vacuumed pumped over a gridded 0.45 μm filter (Whatman mixed cellulose nitrate, 47 mm diameter, GE Life Sciences). Water volume was measured and recorded at time of filtration. Before filtration, lab surfaces were wiped with a bright colored sponge to reduce and recognize potential contamination. All glassware and tools were triple-rinsed, as were forearms and hands. White 100% cotton lab coats were worn. After filtration, the filters were stored in a triple-rinsed glass petri dish, closed immediately and left to dry for at least 24 h.

Particles were subject first to visual inspection, and then a representative subset of particles identified for polymer type. Filters were inspected under a stereo microscope at 45 × magnification. Particles were identified based on a lack of cellular structure and a uniformity of shape (Hidalgo-Ruz et al., 2012). If the piece could not be categorized as microplastic under a stereo microscope, it was examined under a compound microscope and subjected to the hot needle test (De Witte et al., 2014; Devriese et al., 2015). The hot needle test was used as a supplement our visual identification before subsampling for material characterization using micro-FTIR. If there was still doubt after inspection under the compound microscope, it was not counted as microplastic but noted as a potential non-synthetic. Particles were categorized by shape (round, fiber, fragment), color (blue, red, transparent, black, other), and size (except September 2015). Since initial studies suggest organisms differently ingest microplastic in different size classes (Bellas et al., 2016; Desforges et al., 2015; Gusmao et al., 2016), we further defined categories of 0.1–1.5 mm, 1.6–3.1 mm, 3.2–5 mm, and 5.1–9.6 mm. Size classes were delineated based on the filter grid and the lower limit of 0.1 mm based on limitations of 45 × magnification. We acknowledge that particles in the 9.6 mm size class are not technically microplastic, but likely have similar sources, particle transport mechanisms, and biological interactions as smaller particles so were included here. Sample volume varied from 0.7 to 1.75 L (median = 1.28 L). Concentration (No/L) was generated by dividing the total number microplastics by the sample volume.

A representative subsample of particles was used to determine chemical composition via micro Fourier Transform-Infrared Spectroscopy (μFT-IR) (n = 56). We used a random number generator to choose samples for analysis. In each sample, particles were picked randomly by removing the first microplastic encountered within the filter grid, regardless of color (including transparent particles). The relative abundance of color categories in the subset of fibers selected for FTIR analysis was well matched to the relative colors for all particles in the dataset (Table S4). The number of particles for polymer analysis was approximately 5% of total number of particles counted. This value was determined by financial limits, and we recommend future studies report and maximize this value. MicroVision Laboratories analyzed each particle using μFT-IR analysis (a Bruker LUMOS FT-IR operated in reflectance mode). The material spectra were compared to known standards within the Bruker reference library to determine material type for the particle. The LUMOS has a spectral range from 7000 to 600 cm^{-1}, with 32 scans at a spectral resolution of 4 cm^{-1}. The instrument was operated using OPUS software.

2.4. Laboratory controls

To reduce airborne contamination, the laboratory floor and surfaces were vacuumed a minimum of once a week. Lab water and air blanks were run during sample handling to determine any possible lab contamination. Before filtering samples, we filtered tap water as a control (i.e., blank). A blank was also run of the filtrate used to rinse the sample bottle and filtration apparatus. The volume of filtrate blank was 0.25 L–1.2 L. During filtering, a filter was exposed to air for 30 s to mimic the maximum amount of time the sample could have been exposed when transferred from the sample bottle to the filtration apparatus. When filters were open under the stereo microscope for counting, an air exposure blank was placed next to the microscope. We conducted 74 water blanks and 53 air blanks during laboratory processing. There was an average combined (air and water) contamination of 0.17 particles per sample (see Text S2). This level of contamination is identical or lower than previous research in this laboratory and elsewhere (Barrows et al., 2017a; McCormick et al., 2016; Miller et al., 2017). Results were administered to a quality assurance process before completing the dataset (Table S1). Of the 774 samples collected by citizen scientists, 8% (n = 60) were excluded from final analysis due to incomplete field data, potential contamination (as determined from sample clothing photographs or other events), or outlying results (n = 2). All final calculations completed these accounting parameters and represent a conservative assessment of concentration (n = 714 samples).

2.5. Watershed land-use

Land-use data labels were determined by the USGS Gap Analysis Project (GAP) (USGS, 2016). The Gallatin Watershed sites fell into five categories: forested or natural, agriculture, extraction, rural, or urban. Natural sites included areas that were recently disturbed or modified (introduced vegetation, recently burned), all of the other land-use labels encompass land that was used or impacted by humans. We used “undeveloped” as a category that summed natural and forested land-use together. We calculated relative land-use (%) for each category as the land-use area for each type divided by watershed area. All but three of the study sites contained more than 50% undeveloped land.

2.6. Statistical analysis

We used non-parametric Mann-Whitney and Kruskal-Wallis tests to compare microplastic concentration among locations and sampling times. Simple linear regression was used to compare microplastic concentration to sub-watershed land-use, conducted with all data combined, comparing each collection date individually, and comparing among dates with each site individually. We also used simple linear regression to compare microplastic concentration to mean daily discharge at two sites with USGS gaging stations.

3. Results

Microplastics were found in 57% of the surface water samples

collected in the Gallatin watershed. Samples contained an average of 1.2 microplastics L^{-1}, ranging from 0 to 67.5 microplastics L^{-1}. There was a significant difference in the average concentration between mainstem (1.6 L^{-1}) and tributary sites (0.97 L^{-1}) based on a Mann-Whitney *U* Test ($p < 0.001$) (Fig. 2).

The distribution of particle shapes and colors were relatively uniform among samples. Microfibers comprised 80% of the microplastic particles collected. Fragments comprised 19.7%, and microbeads 0.3%. Blue and clear/transparent were the dominant colors, at 33% and 30%, respectively (Fig. 3). The remaining colors were red (13%), other (13%), or black (11%). The majority of particles (71%) were 0.1–1.5 mm long, with decreasing numbers of in each of the larger size classes: 20% 1.6–3.1 mm, 6% 3.2–5 mm, and 2% 5.1–9.6 mm (Fig. 3). Although not technically 'micro' plastics, the 2% of particles in the > 5 mm size class were included in our calculations.

We found a significant annual and seasonal difference when comparing the sampling events over 2 years. A Mann-Whitney *U* test showed a difference between year 1 and year 2 ($p < 0.001$). The monthly sampling data displayed a significant difference ($p < 0.05$) across all months except December and March (Fig. 2). Kruskal-Wallis analysis showed a significant seasonal difference across months in microplastic average concentration ($p = 3.15 \times 10^{-12}$). March had the highest microplastic concentration and June had the lowest (Fig. 2). Using a regression analysis, we found a significant correlation between original samples and their corresponding duplicates over the two-year study period (95% confidence level, $r^2 = 0.15$, $p < 0.001$).

Microplastic concentration showed high variation among all data collection sites, when considered on each date individually (Fig. 2) or when data from all dates were combined (Fig. 4). There were no significant relationships between microplastic concentration and any watershed land-use category (simple linear regression, $p > 0.05$) (Fig. S1). No longitudinal patterns were identified in microplastic concentration according to elevation and distance from the mouth of the Gallatin River (Fig. 5).

Through μFT-IR spectral analysis we characterized material type for 56 particles visually identified as plastic. Fibers comprised 86% of the characterized microplastic shapes with the remaining 14% categorized as fragments. Of the 56 particles, over 90% were identified as semi-synthetic or synthetic and the remaining 7% were categorized as non-synthetic (cotton, n = 3, wool n = 1). Semi-synthetic cellulose (commonly known as rayon or viscose) and polyester were the two most common materials (Fig. 6).

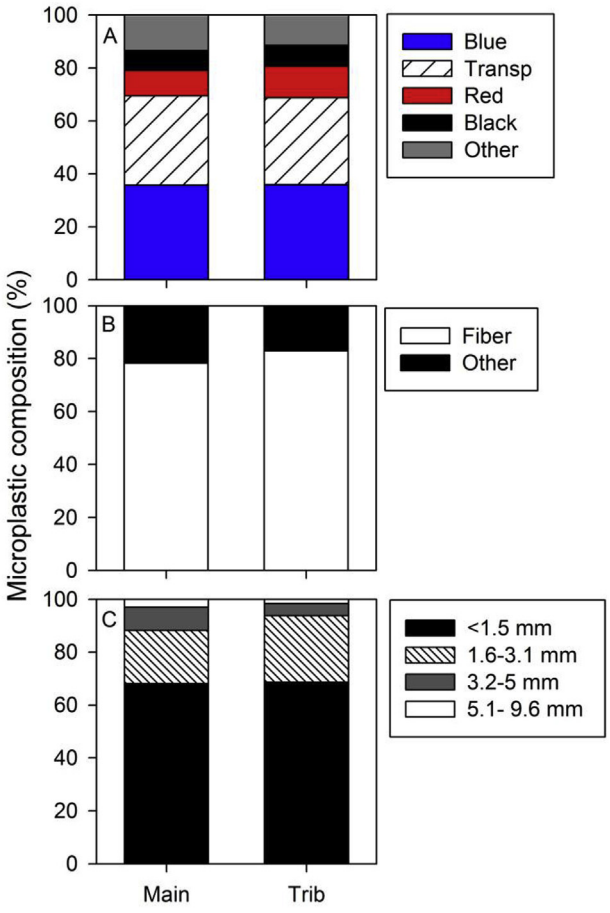

Fig. 3. Microplastic composition in mainstem (main) and tributaries (trib) of the Gallatin River Watershed according to relative (A) color, (B) shape, and (C) size. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

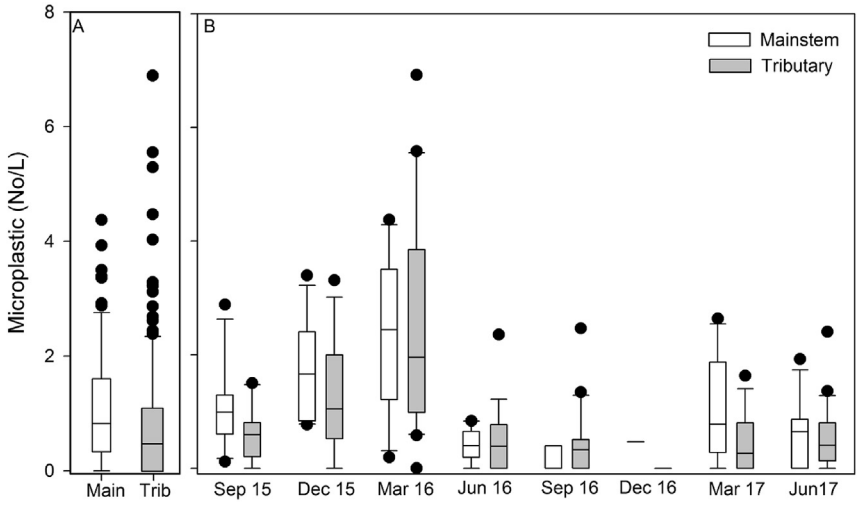

Fig. 2. Microplastic concentration as number (No) of pieces per liter in the mainstem (main) and tributaries (trib) of the Gallatin River Watershed for (A) all dates combined, and (B) for each collection date separately. The central line indicates median, the bottom and top of the box show 25% and 75% percentile, error bars show 5% and 95% percentile, and dots represent data points outside 5–95%. A Mann-Whitney *U* Test ($p < 0.001$) indicates a significant difference in the average microplastics L^{-1} between mainstem (1.6 L^{-1}) and tributary sites (0.97 L^{-1}).

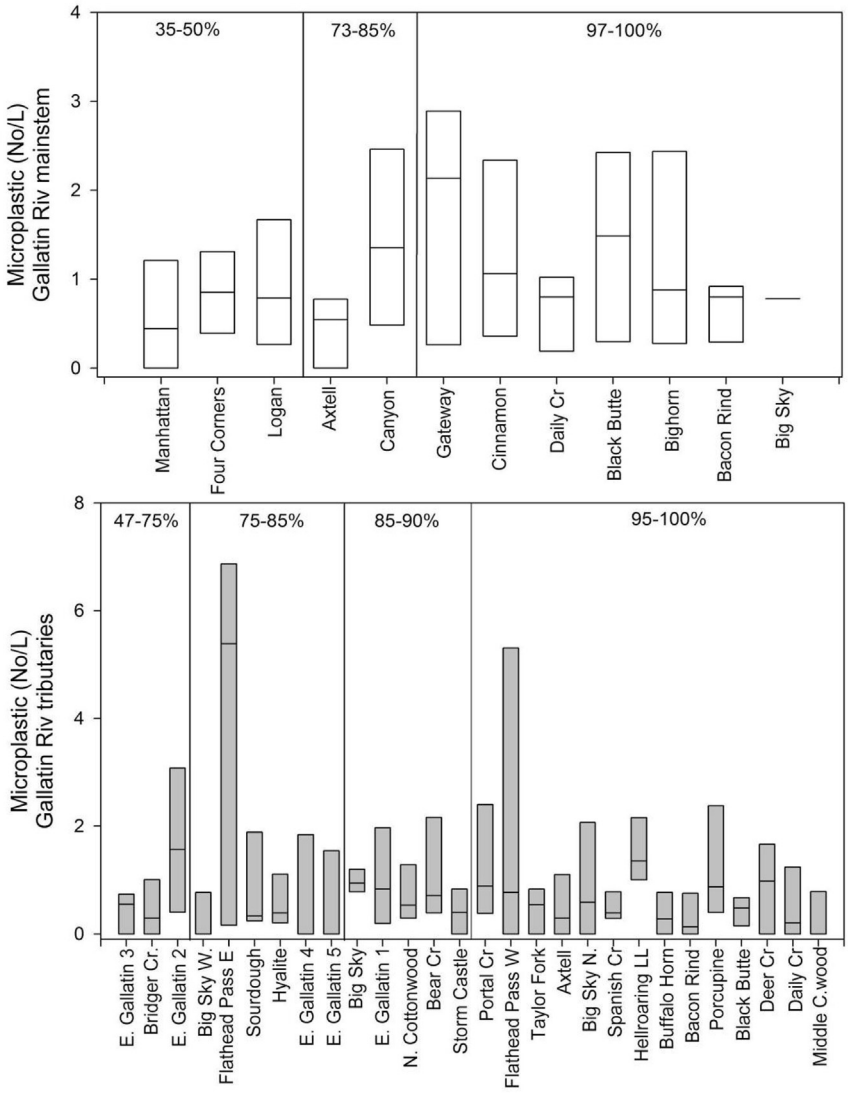

Fig. 4. Microplastic concentration at each site in (A) the mainstem and (B) tributaries of Gallatin River. The central line is the median and box represents 25–75% percentile. Sites are grouped according to proportion of watershed land-use that is undeveloped (%), indicated at the top of each panel.

We calculated the daily mean discharge at two sites with USGS gaging stations: Gallatin Gateway and Logan (Fig. 7) (USGS, 2017). Linear regression showed a significant negative relationship between microplastic concentration and discharge ($r^2 = 0.571$, $p = 0.002$) when compared across sampling months at the two sites.

4. Discussion

This study documents microplastic pollution throughout the Gallatin River watershed, spanning gradients of land-use from undeveloped catchments in protected conservation areas, to more developed areas in the town of Bozeman. This demonstrates the pervasiveness of plastics in the environment and the need for research which clarifies its sources, retention, and transport in rivers.

4.1. Assessing spatial and temporal variation to infer microplastic sources

Microplastic in the Gallatin River watershed was relatively ubiquitous across the mainstem and tributaries, which makes isolation of microplastic sources challenging. Despite the variation in topography and proximity to potential pollution sources, microplastic pollution was common throughout the river. Differences in magnitude among sites were relatively minor, even though most of the tributaries flowed through undeveloped wilderness areas with seemingly scarce pollution sources. Thus, we are left to speculate on some possible sources of microplastic such as recreation activities and aerial deposition. Most of the tributaries are undeveloped, but are visited by people enjoying outdoor activities such as hiking and camping, which may be a source of fibers. In addition, mountain biking is popular on trails adjacent to the river, which may be source pollution as tire rubber particles (i.e., butadiene/butyl rubber; Fig. 6), shoes, or other elastomeric products. To date, only tire rubber from cars has been studied as a microplastic pollution source (Boucher and Friot, 2017), but bike tires also shed during use. Last, aerial deposition is another potential source of microplastic in uninhabited areas. For example, Free et al. (2014) found higher than expected microplastic pollution in a remote lake in Mongolia. The authors determined the source of microplastic was in part due to prevailing winds dispersing microplastic

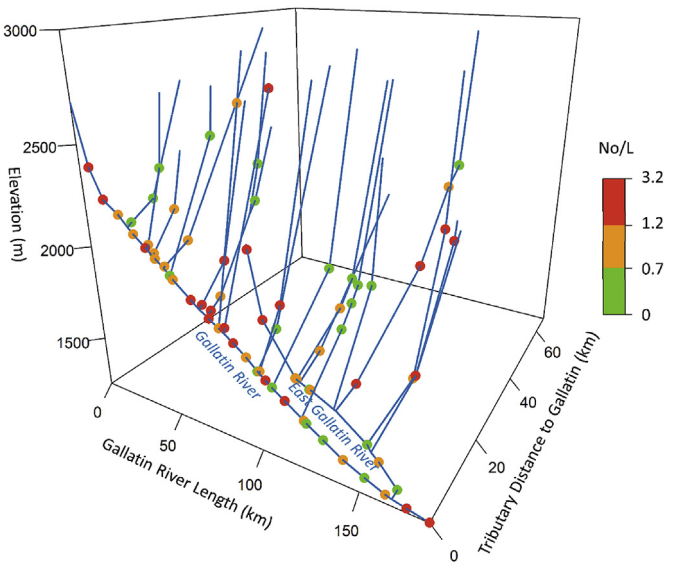

Fig. 5. Mean microplastic concentration at each sample site in the Gallatin River (blue) according to elevation above sea level (m), distance from the most downstream point in the Gallatin River (km), and distance along each sampled tributary. Hydrology to scale in length and order but not in orientation to allow for condensed viewing (Agriculture 1983; USGS, 2017). Samples within each tributary were considered independent of samples within other tributaries. Samples along the same tributary and on the Gallatin and East Gallatin River were partially comprised of the same water, and were thus not considered independent of one another. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

from more populated areas. Few measurements of aerial concentrations and movement have been completed (Dris et al., 2016, 2017), but these studies suggest aerial deposition may contribute to the spatial patterns found in this watershed.

Previous studies have attempted to use spatial variation to quantify the sources and movement of microplastic in rivers, with equivocal results. For example, longitudinal measurements of microplastic in sediments and surface waters of the Rhine River showed high variation among sites (Klein et al., 2015; Mani et al., 2015). Hoellein et al. (2017) also found no clear longitudinal pattern for microplastic in sediment and surface water over several kilometers of an urban river, downstream of a wastewater treatment plant. Studies which attempt to isolate particular point sources cover much smaller geographic distance, but have been more effective at determining microplastic sources. McCormick et al. (2016) compared microplastic concentration upstream and downstream of wastewater treatment plants to show point sources of microplastic in effluent. Klein et al. (2015) suggested the Main River tributary to the Rhine was an important source of microplastic by measuring concentration just upstream and downstream of the confluence. Finally, our sampling regime offered insight into the scale of *in situ* heterogeneity in microplastic concentrations at individual sampling sites by conducting 'duplicate' samples (i.e., water samples collected in quick succession). Regression showed a significant positive relationship between duplicates collected across the sampling regime, but explained a relatively low amount of variability ($r^2 = 0.15$, $p < 0.001$) (see Table S3., Figure S2). These data suggest higher replication both within and among sites may be needed for resolution of microplastic dynamics in flowing waters. Overall, results from this study are consistent with previous research by suggesting widespread but diverse sources of microplastic which are difficult to isolate at the watershed scale, despite the high coverage of sampling sites used for this analytical approach. We suggest that future studies may benefit from measuring the influence of individual suspected sources or retention sites to better inform microplastic budgets in rivers. In addition, measuring discharge and temporal variability at sampling sites could further reveal the influence of hydrology and seasonality of sources or transport patterns.

No previous studies have measured seasonality of microplastic in rivers, and the high variation of microplastic concentration in the Gallatin Watershed among sample dates allows for some inference regarding microplastic sources and movement. Our results show high temporal variation for all study sites. However, the relationship between microplastic concentration and discharge suggests

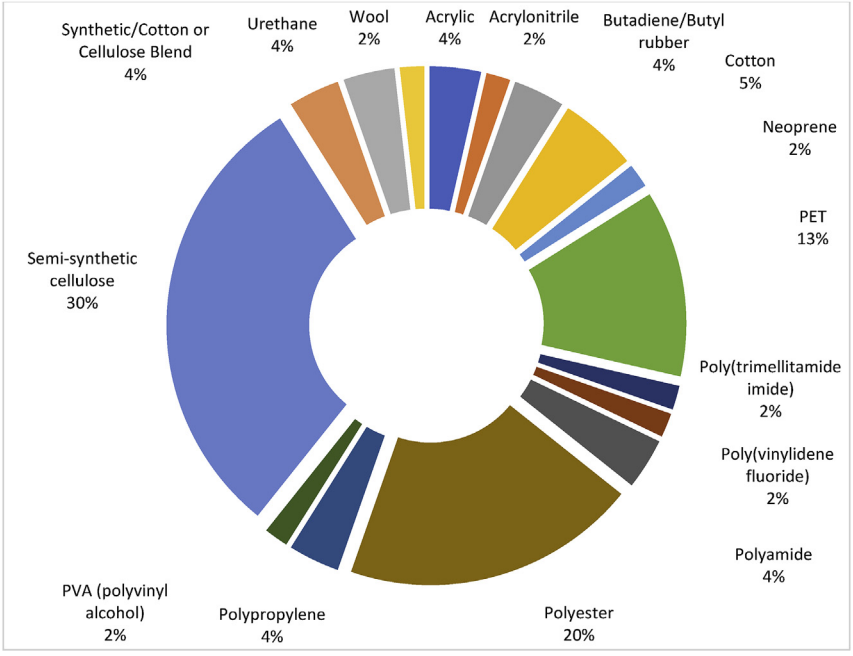

Fig. 6. Microplastic particle type and percentage as characterized by μFT-IR.

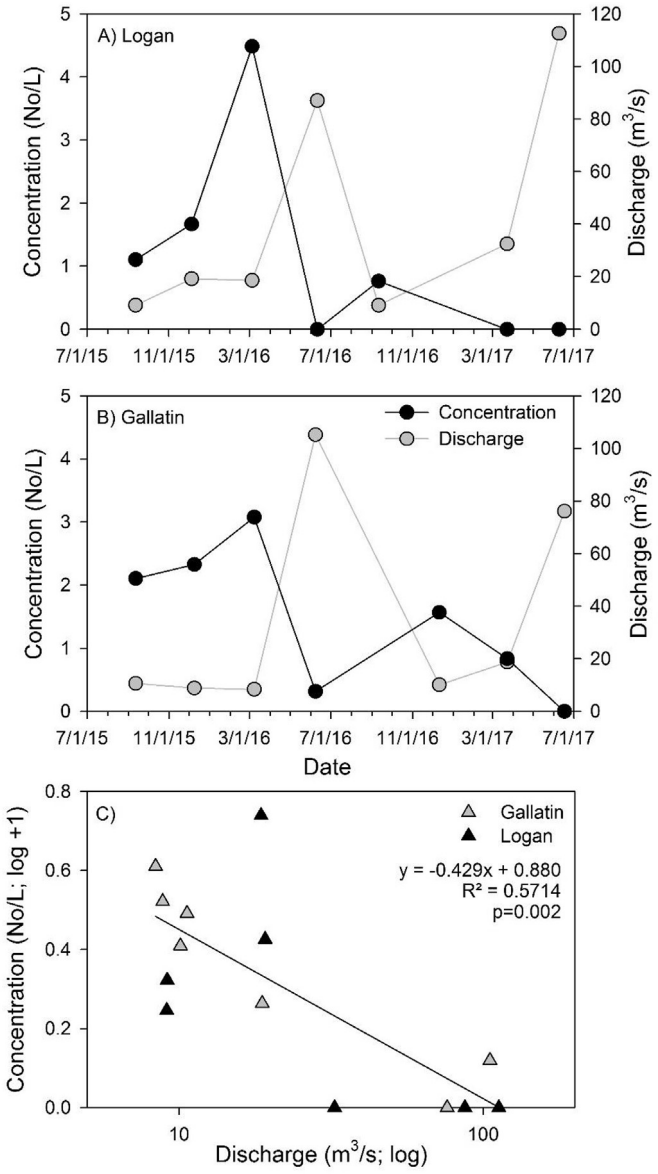

Fig. 7. Mean daily discharge (m^3/s) and mean microplastic concentration (No/L) at (A) Gallatin Gateway and (B) Logan over the two-year sampling period. (C) Simple linear regression between microplastic concentrations and discharge across sampling dates.

meaningful ecological dynamics are ongoing. We hypothesized that the peak water flow month of June would have the highest concentration of microplastics. We predicted this would occur as the relatively constant source of particles from point sources (i.e., wastewater treatment plants) would be combined with potential microplastic inputs that could occur during high flows (i.e., snowmelt, and runoff from impervious surfaces and overland flow). This prediction was not supported, as microplastic concentrations in June were low, and discharge was negatively correlated with microplastic concentration at Logan and Gallatin Gateway collection sites. Results suggest that stormwater and high flows that occur in summer are not sources of microplastic to the river, but instead appear to dilute microplastic that may come from other sources, such as wastewater effluent or atmospheric deposition, which are not affected by flooding. The Bozeman metropolitan area does not have combined sewers, and it appears high flows reduce plastic concentration, rather than add to it. In rivers elsewhere, stormwater may be a source of microplastic if it contributes plastic pollution to the river combined sewer outfalls. In addition, much previous research has documented that stormwater-derived contaminants in rivers occurs at the 'first flush' of water that occurs at the beginning of storms, and input is reduced as storms and floodwaters proceed (Lee et al., 2002). Given the seasonal approach and 2-year duration, our study was not designed to capture microplastic concentrations during the hydrograph of individual storm events. To our knowledge, microplastic inputs during the first flush of stormwater is an important and potentially fruitful area for future research that has not yet been examined. Overall, we conclude that high flow time periods are not associated with increased microplastic concentrations, which contributes to our understanding of microplastic ecology at this site.

4.2. Material identification can suggest microplastic sources and ecological impacts

Polymer identification of microplastic particles showed a mixture of commonly used products (i.e., semi-synthetic cellulose, PET and polyester), as well as a few material types closely associated with recreation (i.e., polyvinyl alcohol (PVA) and neoprene). Semi-synthetic cellulose has been reported in microplastic collected from marine, freshwater, and terrestrial environments (Dris et al., 2015, 2016, 2018; Lusher et al., 2014; Miller et al., 2017; Remy et al., 2015; Zhao et al., 2016). Semi-synthetic cellulose and polyester are among the most common synthetic textiles produced, and production of apparel accounts for approximately half of the end use of PET fibers (Carr, 2017; Comnea-Stancu et al., 2017). Neither semi-synthetic cellulose, polyester, nor PET help identify sources of particles in this watershed because of their widespread applications. However, PVA is commonly used for temporary attachments for lures, bags, and fishing line by freshwater anglers, and can eventually 'dissolve' when discarded in the environment (DeMerlis and Schoneker, 2003). Neoprene has a wide array of applications because it maintains flexibility over a large temperature range (Martins et al., 2004) and it is used for waders and wetsuits in the Gallatin River area. Both PVA and neoprene support the hypothesis that recreation may contribute to microplastic at even remote locations. Overall, the mixture of polymers in microplastic across the Gallatin River watershed suggest both diffuse sources (widespread synthetics in textiles) as well as the potential for local point sources via outdoor recreation.

Even though semi-synthetic microfibers have less plastic and are composed of some fraction of naturally occurring fibers, synthetic and semi-synthetic microfibers can cause similar physical and chemical effects when ingested by aquatic animals (Remy et al., 2015). Some dyes and chemicals used in production of natural, semi-synthetic, and synthetic textile production are carcinogenic in animals (Remy et al., 2015). Degradation of non-synthetic particles can disperse additives in the environment (Remy et al., 2015) and may be completely digested following ingestion (Zhao et al., 2016). We categorized 30% of the microplastics in the study as clear/transparent, indicating that they either entered the environment this way or the original dye was degraded. Animals may select colored particles over clear (Shaw and Day, 1994), which could sustain a higher relative abundance of clear microplastics in the environment, although this has not been measured.

4.3. Comparison to literature values and methodological considerations

We compared our results to the other publications of river microplastic concentrations using the same method (Fig. 8A). Results from the Gallatin River were in the same range as the Hudson River ($1.6\,L^{-1}$; New York) and the Muskegon River ($2.9\,L^{-1}$;

Michigan). Sites from McNeish et al. (2018) are large river tributaries of Lake Michigan that span land-use gradients that exceed the Gallatin River watershed for human impacts (McNeish et al., 2018). Watershed land-use in the Muskegon River is 39% forested, 20% agriculture, and 9% urban. Land-use in the Milwaukee River watershed is 12% forested, 43% agriculture, and 30% urban, while the St. Joseph River watershed is 11% forested, 58% agriculture, and 14% urban (Horton et al., 2017, 2018, McNeish et al., 2018). Combining results across published studies suggests that the higher intensity of human development could enhance microplastic concentrations. Potential sources for microplastic in more developed locations include sewage outfalls, wastewater treatment, stormwater runoff, industry, agriculture, and atmospheric deposition (Dris et al., 2016; Horton et al., 2017, 2018; Mahon et al., 2017).

The high variation shown in plastic concentration in this study and others may be affected by the 1-L volume of the grab samples. This method assumes microplastic concentration taken closer to the surface of the water column is representative of the concentration throughout the water column. In the Gallatin River, sample sites were 0.7 m deep on average, so we suggest sample collection depths were representative of the water column. However, no assessments of vertical distribution of microplastic in the water column have been attempted. These measurements would be needed for calculation of depth-integrated values in rivers. We acknowledge this potential limitation, but also note that consistency in the results among widely distributed locations and researcher groups (Fig. 8A) provides support for values generated from 1-L grab samples.

To further understand how the 1-L samples may affect our conclusions we quantified variability in microplastic measurements among sites and sample locations with different levels of microplastic. At each Gallatin River mainstem location in year 1, volunteers collected samples at the left, center, and right side of the river. We considered the three measurements as replicates (i.e., assumed microplastic was well mixed across the river), calculated the coefficient of variation (CV; %) as the standard deviation/mean, and completed a regression of the CV relative to concentration. We added results from this study to 3 data points from McNeish et al. (2018) which collected replicates using identical methods. The comparison showed a negative relationship between CV and microplastic concentration (Fig. 8B). That is, sites with higher mean microplastic concentrations showed lower variability among replicate measurements in 1-L samples. The exception to this pattern was 8 times where all three replicates showed zero microplastic, in which case CV cannot be calculated (i.e., dividing by zero is not possible). These data suggest that confidence in microplastic measurements using this method increases with concentration. More variable results at low concentrations suggest we were less likely to capture the central tendency in microplastic concentration using 1-L samples. Alternatively, results could indicate the plastic was not well mixed and the high variation in the replicates accurately represents the variability in the stream. In either case, we conclude that at sites with low concentrations, researchers would benefit from using higher volume in samples as well as greater replication to reduce variability and increase confidence. Thus, this approach could be tailored to conditions at specific locations, and generate more stable, explicable assessment of *in situ* microplastic patterns at varying levels of concentration.

Other studies using nets to capture microplastic in rivers and marine environments have shown lower concentrations than grab samples (McCormick et al., 2016; McNeish et al., 2018; Miller et al., 2017). However, net samples have a minimum collection size based on the net pore size, and may let some fibers escape given their very narrow width (0.04 mm). For grab samples, there is no minimum detection size (filter pore size = 0.45 μm) and thin fibers do not escape collection in bottles. Thus, grab samples capture smaller size classes than nets. In contrast, nets are critical for capturing particles that float at the water surface, which may represent different polymer classes (i.e., polyethene, foamed polystrene) and would not be captured by grab samples collected under the water surface and surface microlayer (Barrows et al., 2017b; Song et al., 2014). Overall, we suggest the grab sample method captures an accurate microplastic particle concentration representative of the river water column. We acknowledge the field of research in microplastic ecology is quickly developing, and development of methods is in progress. For example, we recommend future studies consider comparisons of microplastic concentration from grab samples collected across volume gradients for a greater understanding of methodological approaches. This study will make an important contribution to ongoing syntheses.

The final methodological consideration for this dataset was the collaboration with citizen scientists. We determined the citizen science approach was the best method for data collection due to the large study area and constrained time periods. This method has important advantages for ecological research, as well as critical

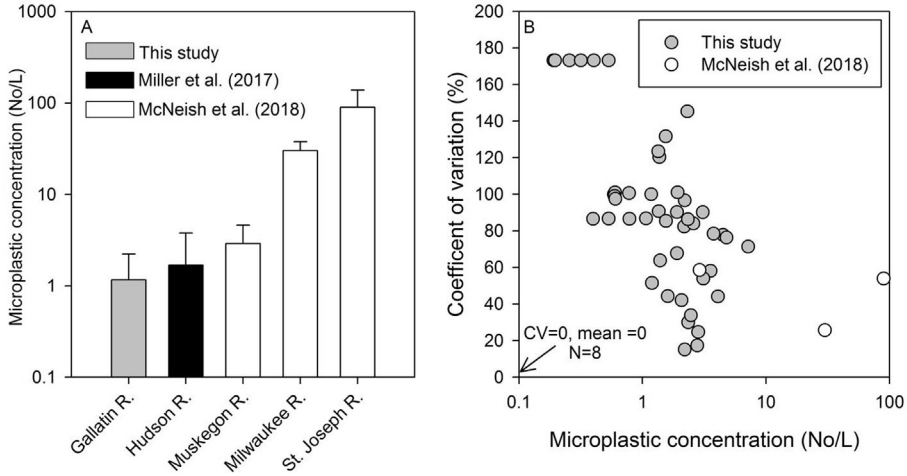

Fig. 8. (A) Mean (±standard deviation) microplastic concentration from 5 rivers, using the same sampling and counting methods. (B) The coefficient of variation (CV) relative to mean microplastic concentration for data collected in the Gallatin River in year 1 of this study, as well as results from 3 rivers assessed by McNeish et al. (2018) using identical methods. In 8 instances, the CV could not be calculated where the mean microplastic value was zero.

considerations for data interpretation. Adventure Scientists recruited volunteers from local communities within the watershed, targeting outdoor recreationists due to their competency for safely accessing remote sample locations. Volunteers underwent a competitive application process and rigorous training (detailed in Text S1), resulting in a reliable group of volunteers and high volunteer retention. These processes also fostered personal relationships between volunteers and project management staff, establishing pathways for communication and trust that contributed to data quality. For example, 92% of volunteer-collected samples passed strict quality assurance measures. Volunteer connection to the issue of microplastic was high, as an informal, project-end volunteer survey revealed that 80% had taken steps to address microplastic or plastic pollution in their communities. Some challenges arose, including the use of data collection technology in the field. We attribute problems to programming errors in the computer application as well as volunteer user error. This was overcome by guiding volunteers through application use and requiring paper data sheets as back-ups. We also acknowledge that volunteer-based data collection in any scientific research may have some limitations (e.g., consistency among measurements (Hoellein et al., 2015)) and the numerous quality-assurance protocols we employed were designed to overcome this limitation. Moreover, the vast scope of data collection for this project could not be accomplished by any other means for data collection.

5. Conclusions

This study shows that volunteer-based research on microplastic concentrations is a valuable approach for quantifying microplastic abundance in a large, mixed land-use watershed. Microfibers were the dominant type of microplastic discovered, reflecting similar findings from studies worldwide. The presence of microplastics in isolated tributaries raises further questions about atmospheric transport and other pollution sources that are not yet documented. Material type of microplastics may indicate recreational use of the Gallatin Watershed as a contributor to aquatic pollution, suggesting that remote watersheds are impacted by a previously unstudied source. Periods of high discharge were associated with diluted microplastic concentrations in the watershed, but patterns may vary in watersheds with different stormwater management infrastructure, and during the course of an individual flood event. Finally, initial comparisons of microplastic contamination from this watershed and other more developed locations generated a wider gradient of human impacts which was helpful to suggest watershed development as source of microplastic among rivers at a continental scale. Additional analyses are needed to more fully understand source of microplastic in rivers, to inform the position of rivers in global budgets of microplastic, and to measure the effects of microplastic on biological processes in freshwater environments.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.watres.2018.10.013.

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