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Article

Microplastics in subsurface water and zooplankton from eight lakes in British Columbia

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Abstract

Microplastics are a global contaminant of concern, but we have little information on the characteristics and bioavailability of these pollutants in western Canadian lakes. Here, we quantify and characterize microplastics in subsurface water and zooplankton from eight lakes in BC, Canada. By sampling water and zooplankton, we provide insight into the fraction of microplastics entering the food web. We found 0.607 ± 0.153 microplastics per litre in subsurface water, 0.01 ± 0.011 microplastics per copepod, and 0.02 ± 0.014 microplastics per *Daphnia*. Microplastic pollution was similar in all lakes sampled and showed no relationship with local population density. Fibers were the dominant morphology observed in all lakes, and Raman spectroscopy identified polyester as the dominant polymer found both in lakes and within zooplankton. Zooplankton generally ingested microplastics that were shorter than their body length and that fell on the smaller end of the range of available microplastics. The prominence of polyester fibers and PET films and fragments suggests that the likely sources of microplastics to these lakes are recreational activities and atmospheric deposition.

Key words: microplastic, freshwater, lake, zooplankton, *Daphnia*, copepod, fibers, Canada

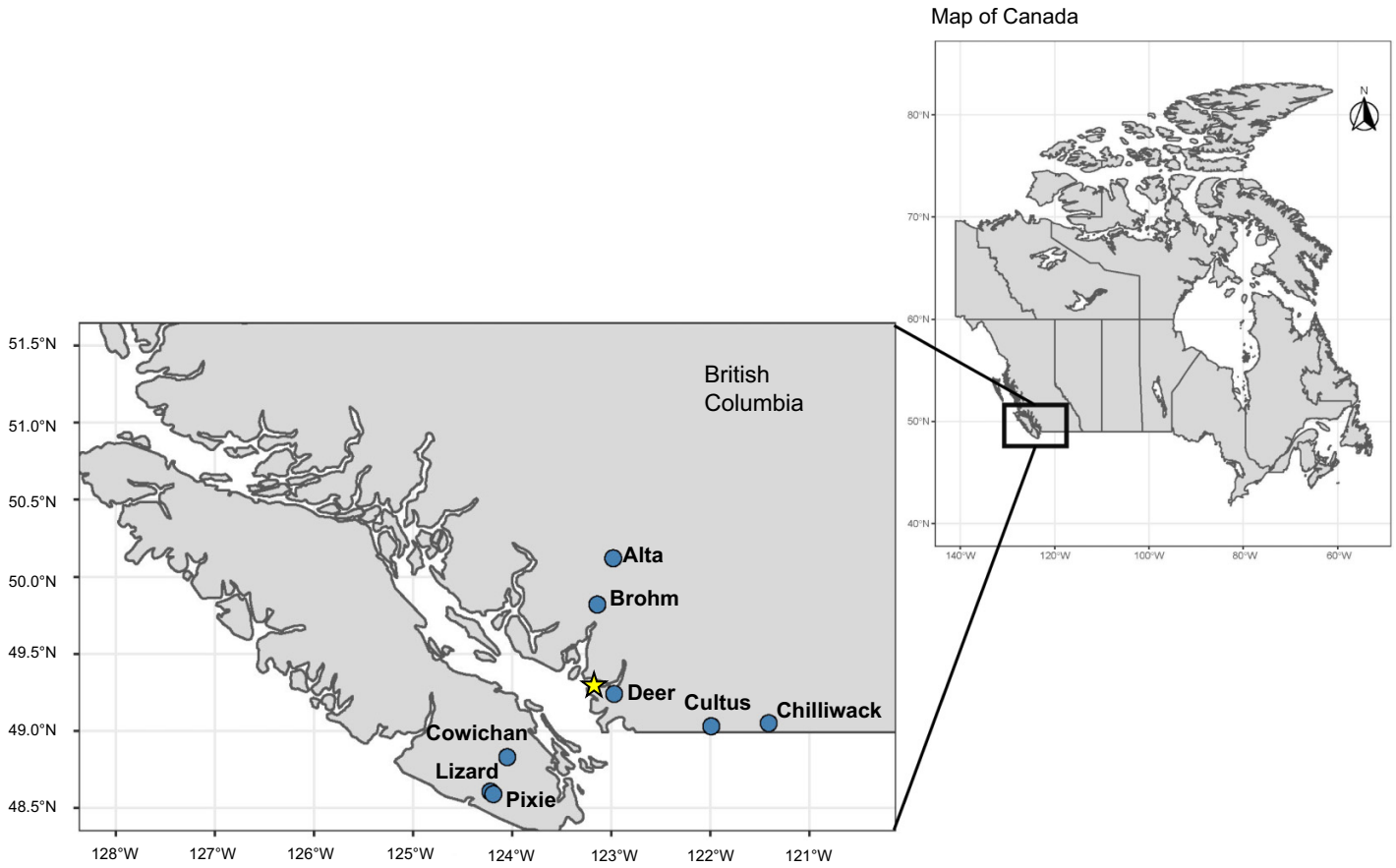
1. Introduction

Microplastics have emerged over the past decade as a global contaminant of concern because of their widespread presence, persistent nature, and demonstrated potential to cause adverse effects when ingested (Thompson et al. 2004; Hamid et al. 2018; Bucci et al. 2020). Often defined as synthetic particles smaller than 5 mm in size, microplastics are classified into primary (particles manufactured to be smaller than 5 mm) or secondary microplastics (particles that have formed because of the fragmentation of larger plastic items) (GESAMP 2016). The term microplastic is a catch-all term for a highly diverse suite of contaminants, which include particles with different shapes, sizes, colours, and polymers, with complex mixtures of associated chemicals and sorbed pollutants (Rochman et al. 2019).

There has been a push in recent years to increase knowledge on microplastic pollution in freshwater ecosystems because historically microplastic research has primarily focused on marine environments. Although research efforts have increased, studies in marine ecosystems still accounted for over 60% of published studies in 2020 (D'Avignon et al. 2022). In Canada, microplastic studies have been conducted in the three surrounding oceans (Desforges et al. 2014; Hamilton et al. 2021; Mathalon and Hill 2014), while freshwater studies have been concentrated in the Great Lakes—St. Lawrence River system (Corcoran et al. 2015; Vermaire et al. 2017; Crew et al. 2020; Earn et al. 2021), with few studies occurring in other freshwater ecosystems (D'Avignon et al. 2022). In western Canada, microplastic research has been marine dominated (Collicutt et al. 2018; Covernton et al. 2019; Mahara et al. 2022), with only two published studies investigating freshwater ecosystems, both riverine (Campbell et al. 2017; Bujaczek et al. 2021).

In this study, we investigated microplastic pollution in freshwater lakes in British Columbia (BC), Canada, by quantifying and characterizing microplastics in subsurface water and investigating the uptake of microplastics by zooplankton. Increasing our understanding of the current state of microplastic pollution in lakes is critical for pinpointing sources of microplastics, assessing overall ecological risk, and developing potential management options for these ecosystems. By sampling both subsurface water and zooplankton, we provide unique insight into the fraction of microplastics entering the base of the food web. Zooplankton have been documented to ingest microplastics in marine and estuarine systems (Desforges et al. 2015; Sipps et al. 2022), and adverse effects of ingestion have been demonstrated for various species in laboratory experiments (Sorrentino and Senna 2021), but there is limited data on microplastic ingestion by freshwater zooplankton in nature. Zooplankton play a key role in lake ecosystems for energy transfer as they are dominant grazers of phytoplankton and important food sources for higher trophic levels (Lampert et al. 1986; Forró et al. 2008). Understanding the magnitude and types of microplas-

Fig. 1. Map of lakes sampled for microplastics. Star denotes Vancouver, BC.



tics that are ingested will provide useful information to assess the threat of microplastics to zooplankton and to guide future experiments looking to simulate environmentally realistic exposure scenarios. The aims of our study were to (1) provide baseline quantification and characterization of microplastics in these selected lakes, (2) compare microplastics found in zooplankton to those found in water samples, and (3) investigate whether concentration and characterization differed among lakes for both sample matrices.

2. Methods

2.1. Study area

We sampled microplastics from eight lakes located in southwest BC, Canada (Fig. 1). Six of eight lakes were selected because they are a part of the British Columbia Lake Monitoring Network (Ministry of Environment and Climate Change Strategy, *n.d.*). This program collects long-term abiotic and biotic data from lakes throughout the province. The Lake Monitoring Network is currently not collecting data on microplastics. Although we selected a small subset of the available lakes in the network, future studies can build on the protocols and data reported here. The two lakes in our study that are not part of the Monitoring Network were sampled opportunistically due to their location (Pixie Lake was sampled because of its proximity to Lizard Lake, and Deer Lake was sampled because of its proximity to Vancouver and the University of British Columbia) and to increase our overall sample size. In an attempt to obtain a representative baseline for lakes in our study region, we selected lakes located in both urban and rural areas. Information on each lake can be found in Table 1.

2.2. Qa/qc

To limit field and laboratory procedural contamination, we followed protocols suggested by Brander et al. (2020) and Hung et al. (2021). Prior to field sampling, all materials that were to be used in the field were washed with soap and water and then triple rinsed with reverse osmosis (RO) water and covered with aluminum foil until use in the field. To check for procedural contamination from our Kemmerer water sampler, we ran a blank consisting of RO water through the sampler. We did not find evidence in our blank that the sampler itself would contaminate our environmental samples. In the field, participants wore non-synthetic clothing and potential sources of contamination were noted. Each lake had a paired background contamination control (field blank). Before sampling began, we opened a clean glass Petri dish that contained a single 14 μm polycarbonate track etch (PCTE) filter membrane (Sterlitech Corporation). The filter was wetted with a few drops of RO water and left exposed adjacent to the sample collection area on the vessel until sampling ended.

To limit procedural contamination in the laboratory, white cotton lab coats were worn at all times, all glassware and

Table 1. Characteristics of lakes sampled for microplastics.

Lake	Surface area (km^2)[a]	Maximum depth (m)[a]	Elevation (m)[a]	Inflow[b]	Outflow[b]	Trophic status[a]	Population density (per km^2)[c]	Activities on lake[d,e]
Alta	0.97	18	641	Yes	Yes	Oligotrophic	58.3	Recreational area (public beaches and parks with picnic sites), fishing, walking trails, motorized boating and nonmotorized water activities, and housing development on lake
Brohm	0.12	16	274	Yes	Yes	Oligotrophic	227.5	Recreational area (picnic site), fishing, walking trail, and nonmotorized water activities
Chilliwack	11.82	114	621	Yes	Yes	Oligotrophic	2.4	Recreational area (public beach, campground), fishing, walking/hiking trails, motorized boating and nonmotorized water activities, and small housing development on lake
Cowichan	62.14	150	164	Yes	Yes	Oligotrophic	403	Recreational area (public beaches and parks with picnic sites, multiple campgrounds), fishing, walking/hiking trails, motorized boating and nonmotorized water activities, housing developments on lake, and other accommodations for tourists
Cultus	6.31	42	46	Yes	Yes	Oligotrophic	22.6	Recreational area (public beaches and parks with picnic sites, multiple campgrounds), fishing, walking/hiking trails, motorized boating and nonmotorized water activities, and housing developments on lake
Deer	0.30[f]	6[g]	18[g]	Yes	Yes	n/a	2750	Recreational area (public beach and park with picnic sites), fishing, walking trails, nonmotorized water activities, heritage and cultural facilities on lake including an art gallery, heritage homes, a museum, and a festival lawn[h]
Lizard	0.08	16	65	No	Yes	Oligotrophic	0.3	Recreational area (public beach and park with picnic sites, one small campsite), fishing, nonmotorized water activities
Pixie	0.05[i]	4[i]	84[i]	Yes	Yes	n/a	0.3	Recreational area (small dock) and fishing

Note: Alphabetical superscripts indicate the references used to obtain characteristic values and information. The values and information in the table for each column correspond to the alphabetical superscript in the first row of each column, unless otherwise specified in the table.

[a]Ministry of Environment and Climate Change Strategy (no date) *B.C. Lake Monitoring Network, Province of British Columbia*. Province of British Columbia. Available from https://www2.gov.bc.ca/gov/content/environment/research-monitoring-reporting/monitoring/lake-monitoring/bc-lake-monitoring-network.

[b]National Hydrographic Network (2019) Natural Resources Canada. Government of Canada. Available from https://www.nrcan.gc.ca/science-and-data/science and-research/earth-sciences/geography/topographic-information/geobase-surface-water-program-geeau/national-hydrographic-network/21361.

[c]*Population and dwelling counts: Canada and census subdivisions (municipalities)* (2022) *Statistics Canada*. Government of Canada. Available from https://www150.statcan.gc.ca/t1/tbl1/en/tv.action?pid=9810000201.

[d]Personal observations while collecting field samples.

[e]Ministry of Environment (no date) *Discover BC Parks, BC Parks*. Province of British Columbia. Available from https://bcparks.ca/discover/.

[f]*Deer Lake, British Columbia* (no date) *Angler's atlas*. Available from https://www.anglersatlas.com/place/99960/deer-lake.

[g]*Deer Lake—Vancouver* (no date) *Sharp Hooks*. Available from https://www.sharphooks.com/tripplanner.aspx?subpage=lakeinfo&lake=deer%2Blake%2B-%2Bvancouver&lakeid=26 (accessed 15 November 2022).

[h]*Deer Lake Park* (no date). City of Burnaby. Available from https://www.burnaby.ca/explore-outdoors/parks/deer-lake-park.

[i]*Pixie Lake Fishing Map* (no date) *Nautical Charts App*. Available from https://www.gpsnauticalcharts.com/main/ca_bc_pixie_lake__bc-pixie-lake-nautical-chart.html.

materials were washed with soap and water and then triple rinsed with RO water, all glassware and materials were covered with aluminum foil when not in use, all reagents used (with the exception of RO water) were vacuum-filtered through 1.5 μm glass fiber filters (VWR International) before use, all samples were processed in either a designated clean room or under a laminar flow hood, and a laboratory blank (RO water) was concurrently taken with each set of lake samples. All samples (water and zooplankton) from a lake were processed in full before a new set of samples from another lake was processed, and concurrent laboratory and associated field blanks were treated as normal samples.

Suspected microplastics found in field and laboratory blanks were analyzed for chemical identity using Raman spectroscopy. Methodological details for the Raman spectral analysis is included in Section 2.4.4. To remove contamination introduced from field or laboratory contamination, lake samples were blank-corrected by colour-polymer-morphology combination using the paired field and laboratory blanks for each lake. For example, one green polyvinyl chloride fiber and one black polyester fiber were found in the laboratory blank for Pixie Lake, and zero microplastics were present in Pixie's field blank. Therefore, we subtracted one green polyvinyl chloride fiber and one black polyester fiber from our Pixie Lake water and zooplankton counts. Particle identities and characteristics from blanks can be found in Supplementary data (Table S1), and information on the results of our blanks can be found in Appendix A.

2.3. Field collection of samples

Each lake was sampled once in August or September 2021. At each lake, we collected grab subsurface water samples at four locations and vertical zooplankton tows at two of these four locations. We used depth and contour maps to estimate the approximate deepest areas for each lake and all samples were collected at these locations. Subsurface water samples were collected by deploying a 4.2 L Kemmerer water sampler approximately 2 m below the surface at predetermined deep spots in each lake. We chose to sample approximately 2 m below the surface because we were interested in the concentration of microplastics present in the area of the water column where zooplankton often feed. The Kemmerer was emptied into a 16 L glass carboy. The carboy's mouth was covered with aluminum foil, and only the nozzle of the Kemmerer was inserted into the mouth to limit airborne contamination. At each sampling site within a lake, the Kemmerer was deployed three times in succession to collect 12–13 L total of bulk water. Carboys were capped with a cork plug that was further covered in aluminum foil for transportation to the laboratory at the University of British Columbia.

Zooplankton samples were obtained by lowering a 30 cm diameter, 50 μm mesh, 90 cm length vertical tow net 10 m below the surface at approximately the same locations as two of the water samples. The maximum depth of two of the lakes was less than 10 m (Deer, Pixie); thus, we used 4 m depth tows in these lakes. Because we were not estimating overall zooplankton density per lake, we do not believe that the decreased volume of water that was sampled for zooplankton for Deer and Pixie Lakes affected the overall results. The net was lowered using a measured and marked nylon rope and then pulled up at a speed of 1 m/s. The cod end of the net was rinsed into a clean 200 mL glass jar using RO water, and at least 20 mL of ethanol was added to top up the sample for preservation. Two zooplankton samples (one tow equals one sample) were taken per lake. Once in the laboratory at the University of British Columbia, zooplankton samples were sieved using a 63 μm sieve, and the sieve was rinsed with ethanol back into the glass jar until further analysis.

2.4. Laboratory analysis

2.4.1. Bulk water reduction and filtration

Bulk water samples were volume-reduced via vacuum filtration in a laboratory clean room within one week of collection. For each water sample, 12 L was filtered onto 14 μm pore, 47 mm diameter PCTE filter membranes (Sterlitech Corporation). To maximize the transfer of microparticles onto the PCTE filter membrane, all parts of the vacuum filtration apparatus were triple-rinsed with RO water. Filters were transferred from the filtration device using stainless steel tweezers and placed in a clean Petri dish, which was stored at −18 °C until chemical digestion. In some cases, large amounts of biotic material present in the water sample necessitated the use of multiple PCTE filters for a single water sample.

2.4.2. Zooplankton selection

From each zooplankton tow sample ($n = 2$ per lake), *Daphnia* and copepods were analyzed for microplastic uptake. These groups were selected because they are commonly found in temperate freshwater lakes in BC and often make up the bulk of the crustacean zooplankton in these ecosystems. Zooplankton were not identified to the species level. Each zooplankton sample was transferred into a Bogorov Chamber and following the protocols of previously published zooplankton studies, 30–100 individuals of each taxonomic group were selected (Sun et al. 2018; Md Amin et al. 2020; Zheng et al. 2020). The selected individuals were placed in filtered RO water in a clean, square Petri dish. The exact number of individuals sampled depended on the abundance of each group found in the sample (Supplementary data, Table S2). To ensure we only reported ingested microplastics, each individual zooplankton was inspected for external microplastics under a dissecting microscope (Zeiss Stemi 305 Stereo Microscope, Oberkochen Germany). Every 10th individual was imaged (Zeiss Axiocam 105, Zen 2 software), and the body size (length and width) was measured using Image J (Fiji project: Schindelinet al. 2012) (Supplementary data, Table S3). After inspection, the individuals of each zooplankton group were transferred into clean, glass beakers for immediate chemical digestion.

2.4.3. Chemical digestion of water and zooplankton samples

Following the protocols of López-Rosales et al. (2021) and Mahara et al. (2022), we used chemical digestion to break down biological material present in our water sample filters and the grouped zooplankton individuals. To digest both the water and zooplankton samples, a 10% KOH solution was added to the sample (3× the sample volume, minimum 30 mL) and left for 48 h in a 40 °C incubator on a rotating plate (Corning Low Speed Orbital Shaker, RPM = 20). Glass watch glasses were placed on top of the sample beakers and sealed with tape to prevent airborne contamination. After 48 h, a 10% H_2O_2 solution was added to our water samples only (5% of the sample volume, minimum 5 mL) to digest any remaining biological material. The water samples remained on the rotating plate in the 40 °C incubator for an additional 24 h. The zooplankton samples were removed after the 48 h KOH digestion. It has been demonstrated that 40 °C is a safe temperature for digestion because it does not result in degradation of microplastics (Munno et al. 2018). After digestion was completed, samples were filtered onto 14 μm pore, 47 mm PCTE filter membranes using vacuum filtration in a laminar flow hood. All parts of the vacuum filtration apparatus were triple-rinsed with RO water to ensure all microparticles transferred to the filter. Stainless steel tweezers were used to remove the filter and place it in a clean, glass Petri dish.

2.4.4. Microplastic quantification and characterization

Each filter was analyzed for suspected microplastic particles under a dissecting microscope (Zeiss Stemi 305). The microscope itself was encased in a transparent polyethylene garbage bag to minimize background contamination. The filter was visually scanned square by square following a 100-square grid sticker placed underneath the Petri dish holding the filter. Suspected microplastics, i.e., particles that appeared anthropogenic (e.g., presence of colour, smooth surface, and even width for fibers) and withstood prodding with tweezers (to ensure it was anthropogenic and not glass, mineral, or sediment), were transferred from the filter using fine point tweezers and placed onto double-sided sticky tape that was pre-mounted on transparent paper. Examples of microplastics found in samples can be found in Appendix A (Fig. A1). Particles were characterized by morphology and by colour (Helm 2017), imaged under the microscope (Axiocam 105, ZEN 2 software), and measured (longest dimension and width) in ImageJ (Fiji project: Schindelin et al. 2012). The minimum detectable size of microplastics using these standardized methods is approximately 75 μm.

We analyzed all extracted suspected microplastics ($n = 400$) with Raman spectroscopy and thus we are able to report with certainty and without any extrapolation on the concentration and characterization of microplastics in our samples. Raman spectroscopy was completed by our analytical laboratory partners, Ocean Diagnostics (Victoria, BC, Canada). Suspected microplastic particles were personally driven or shipped to Ocean Diagnostics for analysis. Ocean Diagnostics received suspected microplastics that were isolated on double-sided tape mounted on transparent paper in round, plastic Petri dishes, and each particle was circled and labelled with a number, which corresponded to a datasheet that held information for each specific particle.

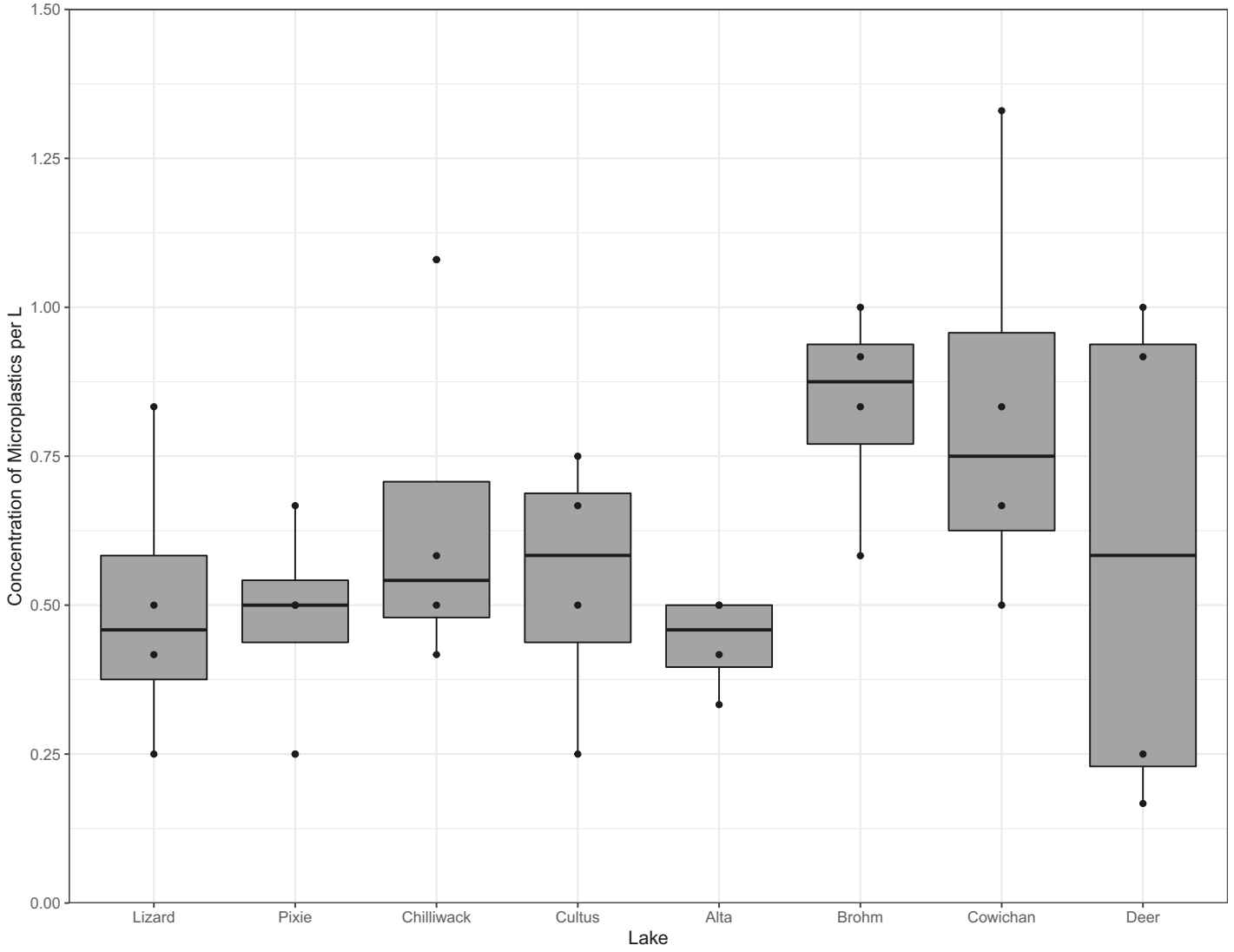
At Ocean Diagnostics, suspected microplastic particles were first identified using a light microscope under bright field mode and focused using a 20×/0.40 magnification objective lens (Leica, N Plan EPI). Particles were measured by single-point analysis using an InVia™ confocal Raman microscope (Renishaw, UK), using a 532 nm excitation laser (RL532C50 model). The spectrometer is equipped with a diffraction grating with a density of 2400 l/mm and the detector is a Centrus 08HQ36 sensor (Renishaw). A piece of a silicon wafer was used to calibrate the instrument before isolated particles were measured. All spectra collected as a function of the Stokes shift had a range of 900–2000 cm^2 and were centered at 1500 cm^2. Open-source libraries were used to compare collected spectra to reference spectra (OpenSpecy: Cowger et al. 2021, SLoPP and SLoPP-E: Munno et al. 2020) using Ocean Diagnostics Software and first derivative Pearson correlation approach, which was used directly as the Hit Quality Index (HQI). HQIs > 0.80 were deemed successful matches, while particles with HQI between 0.65 and 0.85 had their spectra manually examined to confirm the match. HQI below 0.65 resulted in re-examination of the spectrum and further action, including re-measurement and re-matching, physical examination for polymer identification class, or calculating the Pearson correlation coefficient instead of the aforementioned first derivative for HQI. Particles that yielded spectra HQI < 0.5 were deemed as "Not Identified."

Particles were categorized into four different groups based on their chemical identification. Categories included plastic (Raman spectra matched to a specific polymer type), dyed cellulose (cellulose spectra detected and presence of a dye), natural (non-dyed cellulose, minerals), and unknown (when there was no spectra match or HQI < 0.5). Raman spectroscopy confirmed that 65% of particles from water and zooplankton samples were plastic, 17% of particles were dyed cellulose, 16% were unknown, and 2% were natural. In this study, we only report confirmed microplastic counts. Information on all particles, including those identified to the other categories in this study, can be found in Supplementary data (Table S4).

2.5. Data analysis

We calculated the concentration of microplastics per litre by dividing the number of confirmed microplastics per water sample by 12 (the volume of water processed per sample). Similarly, we calculated the number of microplastic particles per individual zooplankton by dividing the number of confirmed microplastics in a sample by the number of individuals processed for that sample ($n = 30$–100). We used analysis of variance (ANOVA) to assess whether there were significant differences in microplastic concentration in subsurface water samples among lakes. A Shapiro–Wilk test indicated that copepod ingestion values were not normally distributed even when the data were log-transformed. Thus, a non-parametric two sample Wilcoxon rank test was used to assess whether

Fig. 2. Concentration of microplastics per litre found in subsurface water samples from each lake. Lakes are arranged in ascending order from lowest to highest population density per km^2. ANOVA found no significant differences among lakes for microplastic concentration ($F_{[7,24]} = 1.29$, $p = 0.297$).



there was a difference in the number of ingested microplastics between *Daphnia* and copepods, averaged across all lakes. We applied separate Kruskal–Wallis H tests to assess whether there were significant differences in microplastic ingestion among lakes for both *Daphnia* and copepod groups as Levene's test indicated the data for both violated homogeneity of variance. We used linear regression to assess whether the average concentration of microplastics per litre predicted the average number of microplastics ingested by *Daphnia* and copepod zooplankton per lake. We used separate linear regressions to assess whether local human population density was a significant predictor of (a) the concentration of microplastics in subsurface water, (b) the ingestion of microplastics by *Daphnia*, or (c) the ingestion of microplastics by copepods. Data for local population density (per km^2) were obtained from Statistics Canada (*Population and dwelling counts: Canada and census subdivisions (municipalities)*) (Government of Canada, S.C. 2022). Population density was log-transformed. All statistical tests were performed in R version 4.1.2.

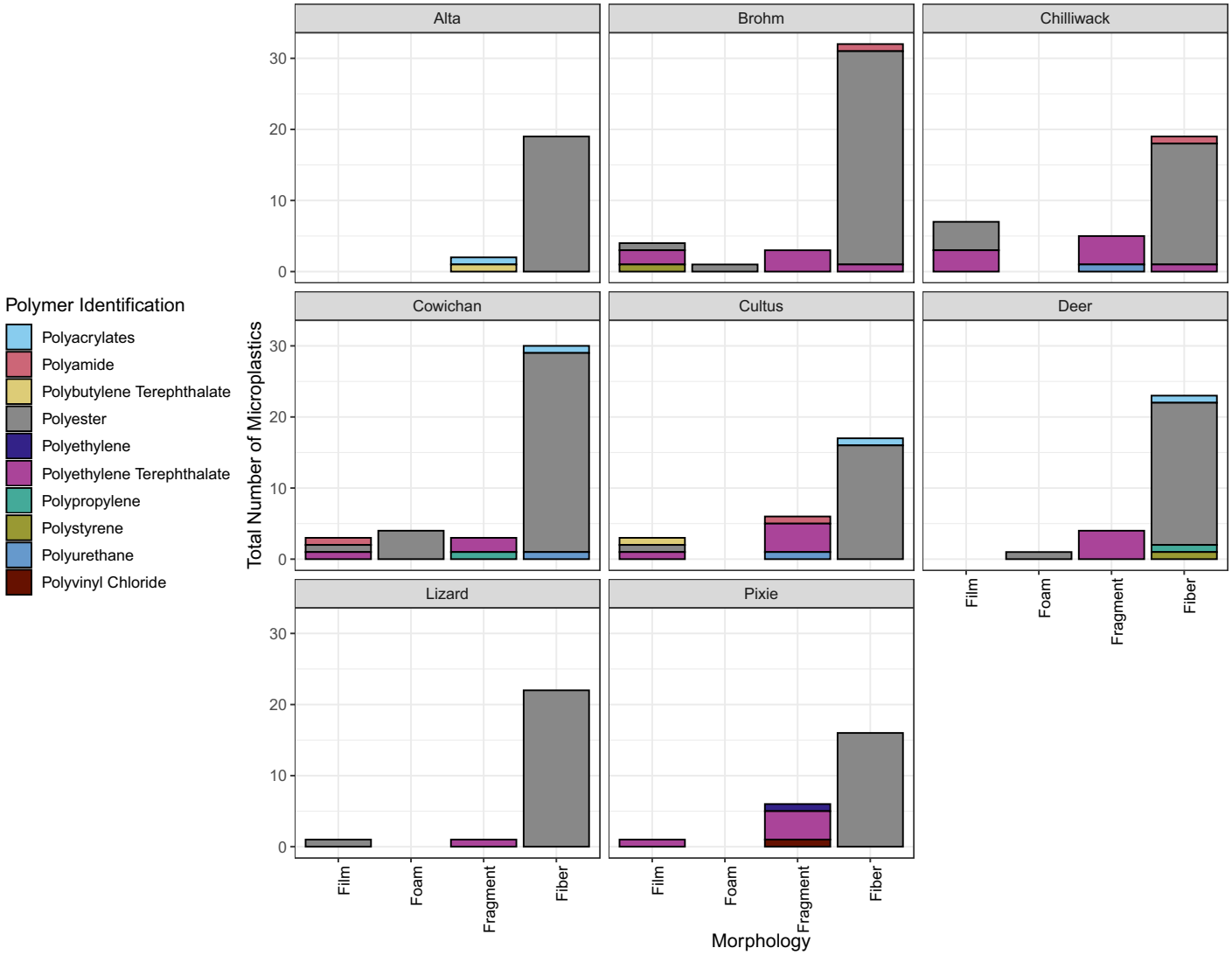
3. Results

3.1. Quantification and characterization of microplastics in subsurface lake water

Microplastics were found in all subsurface water samples from every lake (Fig. 2, Table A1). Across all lakes, the mean (±SD) microplastic concentration was 0.607 ± 0.153 particles per litre (range: 0.167–1.330). There was clear among-sample variation in microplastic concentration within lakes (Fig. 2), and although some lakes contained double the concentration of microplastics than others (e.g., Brohm Lake: 0.833 ± 0.180 vs. Alta Lake: 0.438 ± 0.079), among-lake differences were not statistically significant ($F_{[7,24]} = 1.29$, $p = 0.297$).

In every lake, fibers were the most dominant microplastic morphology observed (Fig. 3), and across lakes they accounted for 76% of all particles. Fragments (13%), film (8%), and foam (3%) morphologies were also found in water

Fig. 3. Morphological characterizations and polymer identities for microplastic particles found in each lake for subsurface water samples.



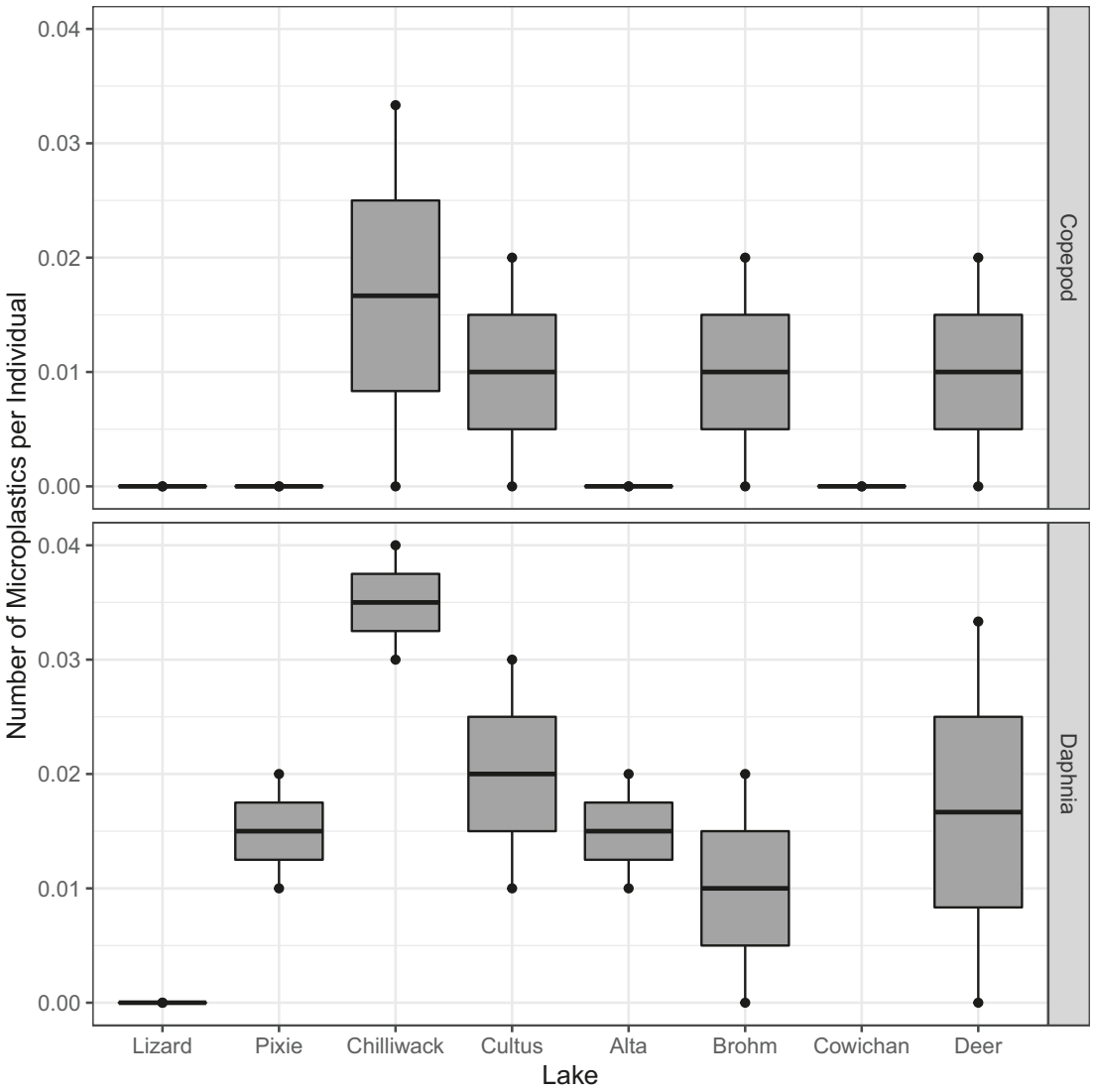
samples. In total, 10 different polymers were identified via Raman spectroscopy: polyacrylates (2%), polyamide (2%), polybutylene terephthalate (<1%), polyester (78%) polyethylene (<1%), polyethylene terephthalate (14%), polypropylene (<1%), polystyrene (<1%), polyurethane (1%), and polyvinyl chloride (<1%) (Fig. 3). In every lake, polyester was the dominant polymer type observed. With respect to microplastic colour, clear, blue, and black were the most common colours observed (Fig. A2).

3.2. Quantification and characterization of microplastics in zooplankton samples

Daphnia and copepods were found in all lakes except Cowichan Lake, where we did not collect any *Daphnia* in our tows. Thus, for Cowichan Lake, we only report the number of microplastics found in copepods. The mean (±SD) number of microplastics per copepod was 0.01 ± 0.011 (range: 0–0.033) (Fig. 4, Table A1). No microplastics were found in copepods sampled from Alta, Lizard, Cowichan, and Pixie Lakes. The mean (±SD) number of microplastics per *Daphnia* was 0.02 ± 0.014 (range: 0–0.067) (Fig. 4, Table A1). Averaged across all lakes, the mean number of microplastics per individual was significantly different between *Daphnia* and copepods (Wilcoxon Rank test $p = 0.025$). There were no significant differences among lakes in microplastics per copepod (Kruskal–Wallis $H_7 = 5.04$, $p = 0.655$) or per *Daphnia* ($H_6 = 6.83$, $p = 0.337$). The concentration of microplastics in water did not predict the number of microplastics observed in either taxonomic group ($F_{[1,6]} = 0.531$, $p = 0.494$ (copepods); $F_{[1,5]} = 0.085$, $p = 0.782$ (*Daphnia*)).

Fibers were the most dominant microplastic morphology observed in zooplankton, accounting for 92% of the total particles (Fig. 5). Fragments accounted for the remaining 8% of microplastics; however, they were only observed in copepods, not in *Daphnia* (Fig. 5). No other particle morphologies (besides fibers and fragments) were observed. Four different polymers were identified via Raman spectroscopy: polyamide (4%), polyester (88%), polyethylene (4%), and polyisoprene

Fig. 4. Number of microplastics per individual for copepods and *Daphnia*. Lakes are arranged in ascending order from lowest to highest population density per km^2. Separate Kruskal–Wallis H tests found the differences among the lakes for both zooplankton groups to be insignificant ($H_7 = 5.04$, $p = 0.655$ (copepods), $H_6 = 6.83$, $p = 0.337$ (*Daphnia*)).



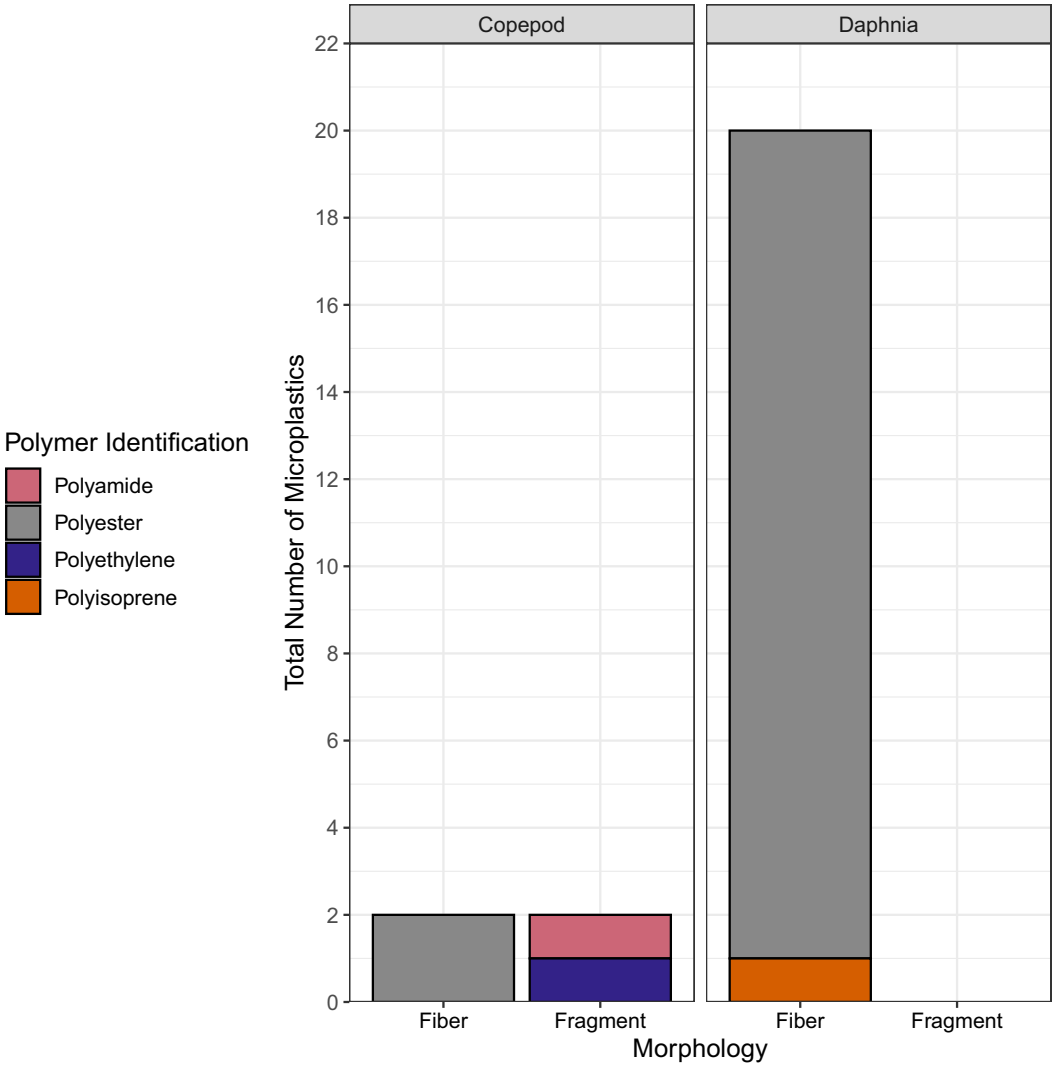
(4%), and similar to the water samples, polyester was by far the most dominant polymer type detected (Fig. 5). The distribution of colours for microplastics found in zooplankton is plotted in Fig. A3.

3.3. Comparison of the sizes of microplastics found in zooplankton to those found in subsurface water and to zooplankton body size

The sizes (longest dimension) of microplastics found in our water samples ranged from 60 to 3295 μm with a mean (±SD) size of 753 ± 563 μm and a median value of 583 μm (Fig. 6). The sizes of microplastics found in copepods ranged from 54 to 152 μm, with a mean (±SD) size of 94 ± 44.6 μm and a median value of 84 μm, and the sizes of microplastics found in *Daphnia* ranged from 182 to 2200 μm, with a mean (±SD) size of 523 ± 466 μm and a median value of 356 μm (Fig. 6). Fragments, which were only observed in copepods, ranged from 55 to 105 μm, with a mean (±SD) size of 80 ± 36 μm and median value of 80 μm, while fibers, which were found in both zooplankton groups, ranged from 63 to 2200 μm, with a mean (±SD) size of 493 ± 460 μm and a median value of 329 μm. The mean (±SD) width of fibers found in zooplankton was 16 ± 8 μm.

The sizes of all the microplastics ingested by zooplankton were within the broader distribution of microplastics found in the water samples, and the majority fell on the left side of the distribution and were smaller than 500 μm (Fig. 6). The average body size (length) (±SD) of copepods was 921 ± 275 μm and the average size (±SD) of *Daphnia* was 1344 ± 366 μm. The majority of microplastics found in copepods and *Daphnia* were smaller in their longest dimension than the smallest zooplankton measured for each group, and both the mean (94 μm (copepods) and 523 μm (*Daphnia*)) and median sizes (84 μm (copepods) and 356 μm (*Daphnia*)) of

Fig. 5. Morphological characterizations and polymer identifies for microplastic particles found in each zooplankton group.



ingested microplastics observed were smaller than the average size for both zooplankton groups.

3.4. Relationship between local population density and quantities of microplastics in subsurface water and zooplankton

Local human population density (per km^2) around the sampled lakes did not predict the mean concentrations of microplastics in subsurface water ($F_{[1,6]} = 2.05$, $p = 0.202$) or the mean number of microplastics ingested by zooplankton (*Daphnia*: $F_{[1,5]} = 0.04$, $p = 0.851$; copepods: $F_{[1,6]} = 0.29$, $p = 0.611$) (Fig. 7).

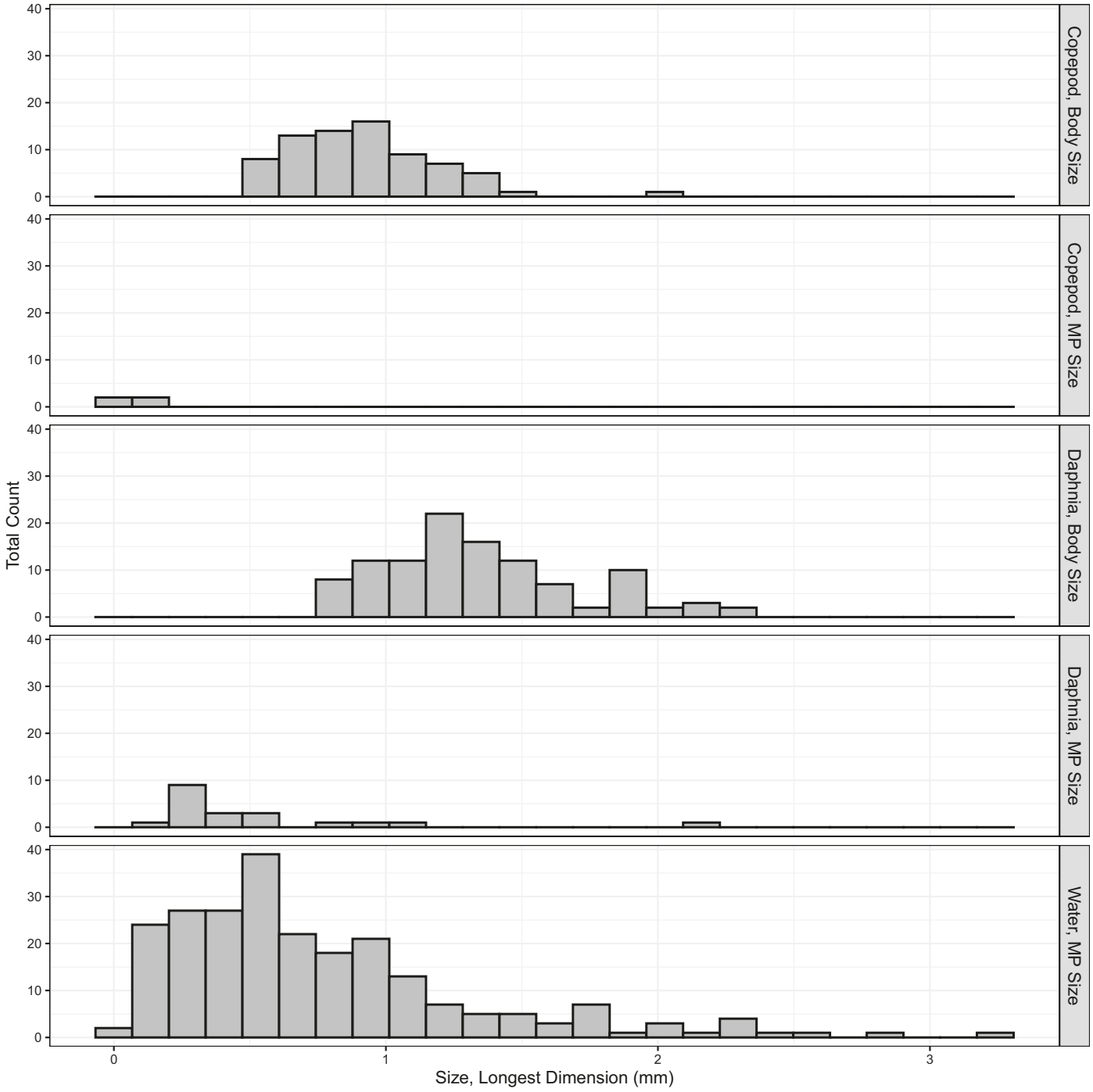
4. Discussion

Overall, we found that microplastics were present in all lakes sampled in this study suggesting that microplastic pollution is likely widespread throughout freshwater lakes in southwest BC. We also report that approximately 1 in 100 copepods and 2 in 100 *Daphnia* ingested microplastics. Because zooplankton are a key prey item for larger invertebrates and fish, these results provide evidence that microplastics are entering the aquatic food web via zooplankton uptake. Below, we discuss in depth the potential sources of variation in the microplastic concentrations observed in these lakes and in zooplankton, and we compare our results to similar studies in other geographic locations.

4.1. Quantification and characterization of microplastics in subsurface water samples

For subsurface water samples, we observed more variation in microplastic concentrations within lakes than among lakes. Intra-lake variation has been reported previously and has been attributed to the spatial and temporal distributional heterogeneity of microplastic pollution (Wang et al. 2018; Yuan et al. 2019; Tamminga and Fischer 2020). How microplastics are distributed throughout lake ecosystems is influenced by a variety of factors relating to the specific particle itself (density, shape, and extent of biofouling) and processes within the lake (degree of biological productivity, organism

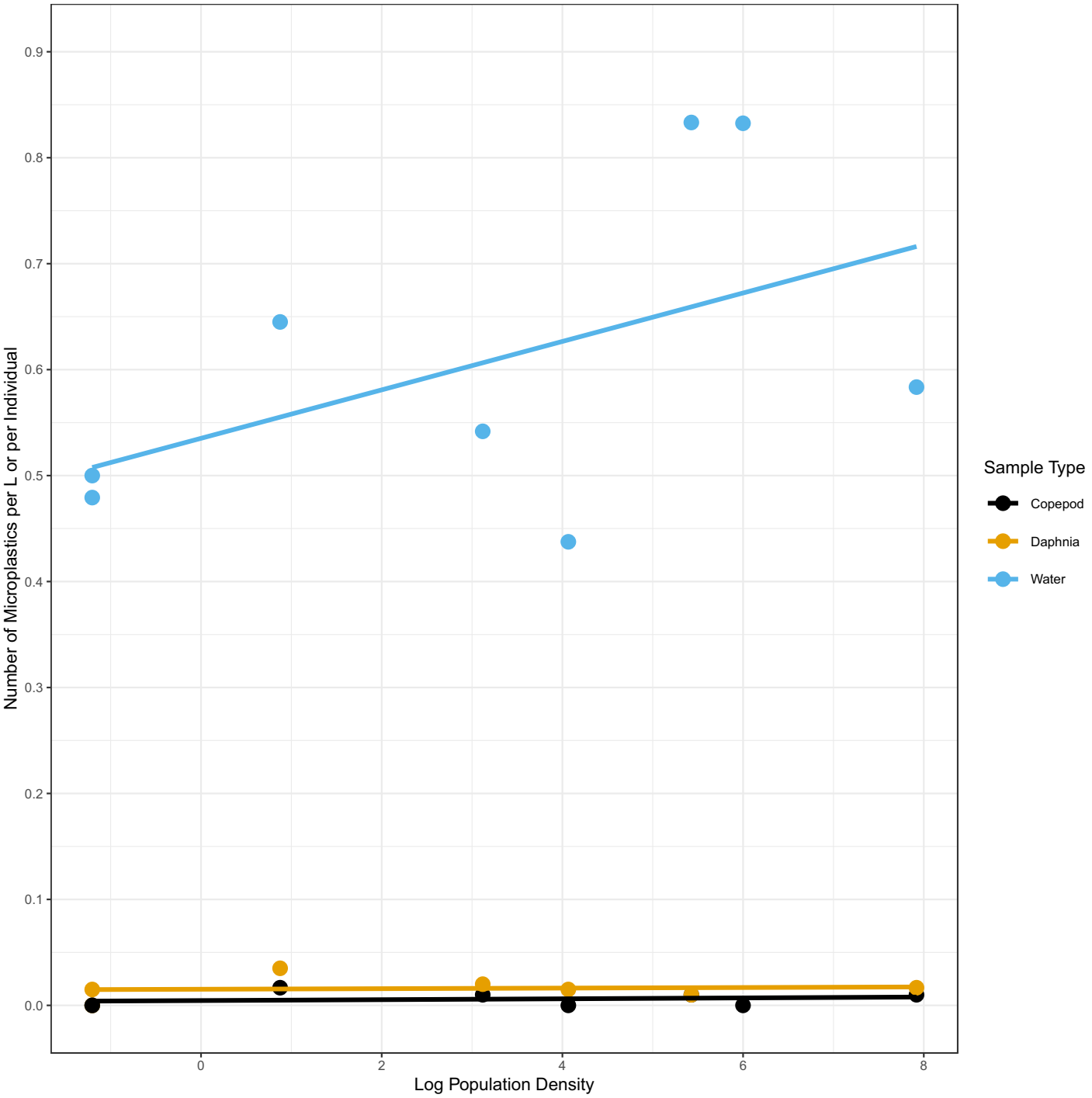
Fig. 6. Size distribution of microplastics found in copepods, *Daphnia*, and subsurface water and the body size (length) distribution of copepods and *Daphnia* (MP, microplastic).



ingestion and transfer, resuspension, local wind patterns, water currents, and flow and circulation patterns) (Cole et al. 2011; Lenaker et al. 2019; Tamminga and Fischer 2020). Because of these complex and interacting processes, the distribution and thus concentration of microplastics is constantly in flux in the water column, causing the high variation observed within all the lakes.

We also observed among-lake variation in microplastic concentrations, which could be due to differences in physical characteristics (size and shape of lakes) or due to inter-lake variation in the sources of microplastics entering each lake. The lakes sampled here ranged in size, with surface areas <1–62 km^2. The size of a lake can affect the concentration of microplastics observed as larger lakes with more area and volume can dilute microplastics (Free et al. 2014). Previous studies have shown that major pathways and sources of microplastic pollution to inland waters include wastewater effluent, agricultural runoff, surface runoff, atmospheric

Fig. 7. Separate linear regressions of population density (per km^2) and the mean concentration of microplastics in subsurface water, the number of microplastics per individual copepod, and the number of microplastics per individual *Daphnia*. All sample types: $p > 0.05$.



deposition, recreational activities and tourism, and improperly disposed plastics (Fischer et al. 2016; Mason et al. 2016; Dris et al. 2017; Li et al. 2018; Stanton et al. 2019). To our knowledge, none of our study lakes received effluent. Two of the lakes (Cowichan and Cultus) were located near but upstream of agricultural areas and thus were unlikely to receive agricultural runoff. Therefore, microplastics found in our lakes likely came from atmospheric deposition, recreational activities and tourism, surface runoff, and improperly disposed plastics.

We can further narrow the sources of microplastics by examining the most commonly observed microplastic morphologies. In this case, microplastic fibers were the dominant type of microplastic recorded. Fibers, which shed from clothing and textiles, are often the most commonly reported form of microplastics in the environment and could have entered

these BC lakes through atmospheric deposition, runoff, and recreational activities (De Falco et al. 2019; Henry et al. 2019; Napper et al. 2023). All lakes in our study could be considered recreational areas, and they attract visitors year-round but particularly in the summer (when we conducted our sampling). Most of the fibers were found to be polyester, which is a dominant polymer used in textiles (Henry et al. 2019). We also found polyethylene terephthalate (PET, another form of polyester) films and fragments, and PET is commonly used to produce plastic bottles, food wrappers, and bags, which are common waste products associated with recreational areas (Thushari et al. 2017). Other polymer types observed in our study can be linked to boating, as boats and vessels have been shown to be sources for paint-derived microplastic fragments (Hamilton et al. 2021). Both polyurethane and polyacrylates are employed primarily in paints, and we observed them in lakes with boating activities. Thus overall, we believe that the inter-lake variation in microplastics concentrations observed here was likely due to a combination of among-lake variation in lake size and variation in levels of recreational activities.

Much of the research in Canadian freshwaters has focused on the Great Lakes. We refrain from comparing our results with those of the Great Lakes because of the significant size difference between our study lakes and the Great Lakes, in addition to differences in sampling methodologies (e.g., we used grab samples, while many of the Great Lake's studies have used manta trawls (Eriksen et al. 2013; Hendrickson et al. 2018; Minor et al. 2020)). The use of manta trawls allows for greater volumes of water to be sampled compared to grab samples, and microplastic concentrations are typically reported in particles per m^2, while we report our concentrations in microplastic particles per litre. A comparison of the use of grab vs. manta trawls has shown that these two methods also have different particle capture capabilities (Barrows et al. 2017). Therefore, within Canada, our results are most comparable with Felissimo et al. (2021), who also analyzed grab samples from Lake Simcoe (ON, Canada). We found higher quantities in our samples (0.6 particles per litre) compared to this study, which found on average 0.04 microplastics per litre. Lake Simcoe is much larger and deeper than all of our sampled lakes, which could have a volume dilution effect on microplastic concentration. Furthermore, the differences in concentrations observed between our findings and the Lake Simcoe study could be because we collected grab samples of larger volumes and took samples from a different depth. The types of polymers observed also differed between our studies, which was also likely influenced by sampling depth. By sampling at ~2 m below the surface, we captured microplastics that were denser than those often reported when sampling surface water: in our study, we found mostly polyester, while greater proportions of less dense polymers have been reported in studies that sampled near or at the surface, such as polyurethane (reported in Felissimo et al.'s 2021 grab samples) or polyethylene (most commonly reported polymer found in freshwater surface waters (Kooi et al. 2021)).

A few other studies have shared similar aims to ours and have sampled multiple lakes and compared the quantities and characterizations observed among lakes (Wang et al. 2017; Alfonso et al. 2020; Malygina et al. 2021). In terms of average concentration of microplastics across lakes, our set of eight BC lakes contained more microplastics compared to nine rural lakes sampled in Patagonia (Alfonso et al. 2020) but lower concentrations when compared to 20 urban lakes in China (Wang et al. 2017). One possible reason why our results were intermediate to those lakes in Patagonia and China is that we attempted to sample lakes from both rural and urban areas. Congruent with our findings, polyester fibers were the most common microplastic observed in lakes in Patagonia and China. This finding suggests that atmospheric deposition of fibrous particles affects all lakes regardless of proximity to urbanization. Finally, quantities of microplastics found in these BC lakes is lower than the global mean concentration reported for inland surface waters and for lentic systems, which was found to be approximately 1.9 and 1 microplastics per litre, respectively (D'Avignon et al. 2022).

4.2. Quantification and characterization of microplastics in zooplankton samples

The ingestion of microplastics by zooplankton is known to occur and has been documented by various marine species in situ; however, the ingestion by freshwater zooplankton has rarely been documented in natural ecosystems (Sorrentino and Senna 2021). The findings of this study illustrate that freshwater zooplankton ingest microplastics that are representative of those present in their surrounding environments, with respect to morphology and polymer identity. Polyester fibers were the most common microplastic detected in water samples and were also the most common microplastic found to be ingested by both zooplankton groups. Microplastic concentrations present in water did not predict the number of microplastics ingested by either zooplankton group.

We found more *Daphnia* than copepods ingested microplastics, and the difference in uptake was statistically significant. However, there were differences in the number of *Daphnia* and copepods analyzed for each sample in a lake and across lakes due to the differences in abundance of these zooplankton groups present in each lake and within each tow. Because of this sample size variation, and the low number of microplastics found in zooplankton, we refrain from suggesting there are taxon-specific differences in microplastic uptake between *Daphnia* and copepods. Also, because we pooled different species into broader taxonomic groups, it is unclear whether different feeding strategies affected microplastic uptake between the groups. While *Daphnia* are widely recognized as filter feeders (Dodds and Whiles 2010; Scherer et al. 2017), the feeding mechanism for copepods depends on the species (Sandercock and Scudder 1994). It would be advisable for future studies looking to investigate microplastic uptake in zooplankton samples from the field to first quantify the abundance of different groups/species of zooplankton in a tow sample and then adjust the number of replicate tows taken to ensure a robust sample size. Additionally, laboratory experiments could answer whether different taxonomic groups and(or) different feeding strategies affect microplastic ingestion. Although our results are limited by

the low number of microplastics recovered from zooplankton, they do align with laboratory studies that show filter-feeding zooplankton are more likely to ingest microplastics due to their indiscriminate feeding strategy (Scherer et al. 2017). Interestingly, fragments were only found in copepods in our study. This finding could indicate that there are group-specific differences in particle selection or retention of certain morphologies; however, once again because of the low total number of microplastics recovered from zooplankton, we are reluctant to speculate further on why we observed different microplastic morphologies in *Daphnia* vs. copepods.

We could not find any published studies documenting in situ microplastic ingestion by freshwater zooplankton; therefore, here we compare our findings to those of marine studies. Our results align with the available data from natural environments that illustrate overall low ingestion and retention of microplastics by zooplankton. We found the mean ingestion of microplastics for copepods and *Daphnia* to be 0.01 and 0.02 microplastics per individual. Other studies quantifying microplastics in marine copepods have found between 0.005 and 0.82 microplastics per individual (Sun et al. 2017, 2018; Md Amin et al. 2020; Zheng et al. 2020; Botterell et al. 2022; Sipps et al. 2022). Similar to Zheng et al. (2020) and Sun et al. (2018), we found majority fibrous particles, but different microplastic morphologies have been documented to be ingested by marine zooplankton, for example, Botterell et al. (2022) and Md Amin et al. (2020) found zooplankton ingested mostly fragments.

It is unclear at this point how the ingestion of 0.01 or 0.02 microplastics per individual zooplankton will affect longer-term population dynamics. Laboratory toxicity studies on freshwater zooplankton have typically used unrealistically high doses and uncommon morphologies to demonstrate negative effects of microplastic ingestion on zooplankton fitness. Studies that have evaluated the effects of fibers on freshwater zooplankton have also exposed organisms to concentrations unrepresentative of what has been documented in nature (Jemec et al. 2016; Ogonowski et al. 2016; Ziajahromi et al. 2017; Kim et al. 2021). To better evaluate the effects of microplastic ingestion, laboratory experiments should utilize at least one treatment that represents an environmentally relevant situation for their chosen study organism. To truly understand ecological implications of microplastics, treatments must be created by selecting relevant morphologies, polymer types and sizes, and by combining in situ water concentration values with ingestion data (Connors et al. 2017).

4.3. Comparison of the sizes of microplastics found in zooplankton to those found in subsurface water and to zooplankton body size

In an effort to improve the understanding of what microplastic sizes are more bioavailable to freshwater zooplankton, we compared the sizes of microplastics found in water to what was observed in zooplankton. We further compared the sizes of the particles found in each zooplankton group to the body size of *Daphnia* and copepods to understand how individual size may affect what particles are ultimately ingested. A caveat of using microscopy for initial detection of microplastics in environmental samples is that it is very difficult to detect particles <50 μm in size (Kotar et al. 2022). Conservatively, we estimate that we missed particles <75 μm from our water and zooplankton samples. We suggest keeping this caveat in mind while interpreting the results and our conclusions regarding size.

The size of microplastics found in zooplankton fell within the range of the sizes of microplastic particles present in water; however, they were on the smaller end of the range found, illustrating that smaller particles are generally more bioavailable to zooplankton. Botterell et al. (2022) also found microplastics ingested by zooplankton were smaller in comparison to particles found in water samples, although they reported only fragments were ingested by zooplankton. *Daphnia* on average were larger than copepods, and they ingested particles that were larger than those ingested by copepods, suggesting that taxonomic size plays a role in the size of ingested particles. The finding that smaller-sized particles are generally more bioavailable and that taxonomic size influences microplastic bioavailability has been previously described for marine zooplankton (Botterell et al. 2019). Most of the particles found in zooplankton were smaller than the mean size of the zooplankton, with the exception of one very long fiber measured at 2.2 mm. Over half of the particles ingested were <300 μm, which falls within the size range of phytoplankton and the prey size of most zooplankton (Sun et al. 2018). All the particles >300 μm were fibers, which can be twisted or folded to smaller, more ingestible sizes in the natural environment (Desforges et al. 2015). Furthermore, the small width of fibers (typically 10–20 μm) increases their bioavailability as this size allows them to easily pass through the mouths of different zooplankton (Zheng et al. 2020).

The range of sizes we found to be ingested by zooplankton are comparable to sizes of ingested microplastics found in zooplankton from the Yellow Sea (mean 155 ± 153 μm) (Sun et al. 2018), the northeast Pacific (461–1778 μm (fibers) and 168–299 μm (fragments)) (Desforges et al. 2015); the Fram Strait (8–236 μm (fragments)) (Botterell et al. 2022); and the southern South China Sea (mean 534 ± 372 μm (fibers) and 61 ± 12 μm (fragments)) (Md Amin et al. 2020). Overall, these findings demonstrate microplastic size and taxonomic size influence what microplastics can be ingested by zooplankton, but the range of particle sizes that has been shown to be ingested is broad.

4.4. Relationship between local population density and quantities of microplastics in subsurface water and zooplankton

The abundance and characterizations of microplastics found in aquatic ecosystems have previously been demonstrated to be correlated to metrics relating to urbanization, such as distance to city centers (Wang et al. 2017), total population (Rochman et al. 2022), and population density (Yonkos et al. 2014). As urban areas have intense human activity, they typically have more pollution sources and greater overall emissions. Thus, it has been postulated that aquatic ecosystems in urban areas are at higher risk of microplastic

pollution compared to rural areas (Eckert et al. 2018). However, urban areas are often associated with superior wastewater and solid waste facilities that would mitigate some microplastic pollution. There could also be differences in environmental protection measures and eco-awareness between urban and rural areas, which could also lead to higher pollution in rural areas compared to urban areas (Yin et al. 2020). In our study, we found no relationship between population density and quantities of microplastics in water or zooplankton. The lack of a significant relationship could be due to low sample sizes or to minimal variation in recreational activity among lakes despite their different proximities to urban areas.

5. Conclusion

Our findings provide a first look into microplastic pollution in freshwater lakes in BC, demonstrating the pervasive occurrence of microplastics, particularly polyester fibers, in subsurface waters. We provide evidence that key freshwater zooplankton groups ingest microplastics that reflect the microplastics present in their surrounding environment, albeit smaller in size. Microplastic particles were similar in terms of quantities and characterizations for both subsurface water and zooplankton among lakes. Our study suggests recreation and atmospheric deposition as important contributors of microplastic pollution to these ecosystems. Ultimately, this study should be regarded as a snapshot into microplastic pollution in BC. We encourage further sampling of these lakes in different seasons, for greater volumes of water, and for more matrices, in addition to sampling potential point sources (i.e., atmospheric deposition) to better characterize microplastic pollution in these ecosystems.

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Data availability

Data generated and analyzed during this study are provided in full within the published article and its supplementary materials.

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Competing interests

The authors declare there are no competing interests.

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Supplementary material

Supplementary data are available with the article at https://doi.org/10.1139/cjfas-2022-0293.

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Appendix A

Blank subtraction results

In the blanks taken for each set of lake samples (one field blank and one laboratory blank per set of lake samples), 0–3 microplastics were identified and subsequently subtracted from the corresponding lake samples (water and zooplankton). By taking a field blank at each lake site and then one laboratory blank during the processing of each set of lake samples, we were able to account for contamination specific to each set of lake samples. In total, 16 blanks were taken (8 laboratory, 8 field blanks) and 14 microplastics in total were found. Of these 14 microplastics, 12 were found in laboratory blanks and 2 were found in field blanks. In terms of morphologies, 12 microplastics were fibers and 2 were fragments. The two microplastics found in field blanks were identified as polyester fibers, while polymers identified in laboratory blanks included polyester, polypropylene, polyethylene, and polyvinyl chloride. Particle identities and characteristics from blanks can be found in the Supplementary data (Table S1). We found no microplastics in both blanks taken for Cultus Lake.

Table A1. Mean concentration of microplastics per litre and mean number of microplastics per individual zooplankton (±SD) for each lake.

Lake	Mean concentration of microplastics per litre in subsurface water	Mean number of microplastics per individual *Daphnia*	Mean number of microplastics per individual copepod
Alta	0.438 ± 0.079	0.015 ± 0.007	0 ± 0
Brohm	0.833 ± 0.180	0.010 ± 0.014	0.010 ± 0.014
Chilliwack	0.645 ± 0.298	0.035 ± 0.007	0.017 ± 0.024
Cowichan	0.832 ± 0.358	N/A	0 ± 0
Cultus	0.542 ± 0.221	0.020 ± 0.014	0.010 ± 0.014
Deer	0.584 ± 0.436	0.017 ± 0.024	0.010 ± 0.014
Lizard	0.500 ± 0.245	0 ± 0	0 ± 0
Pixie	0.479 ± 0.172	0.015 ± 0.007	0 ± 0

Fig. A1. Microplastics found in lake samples: (A, B) polyester fibers, (C) polyurethane fiber, (D) polyethylene terephthalate film, (E) polyethylene terephthalate fragment, and (F) polyester foam.

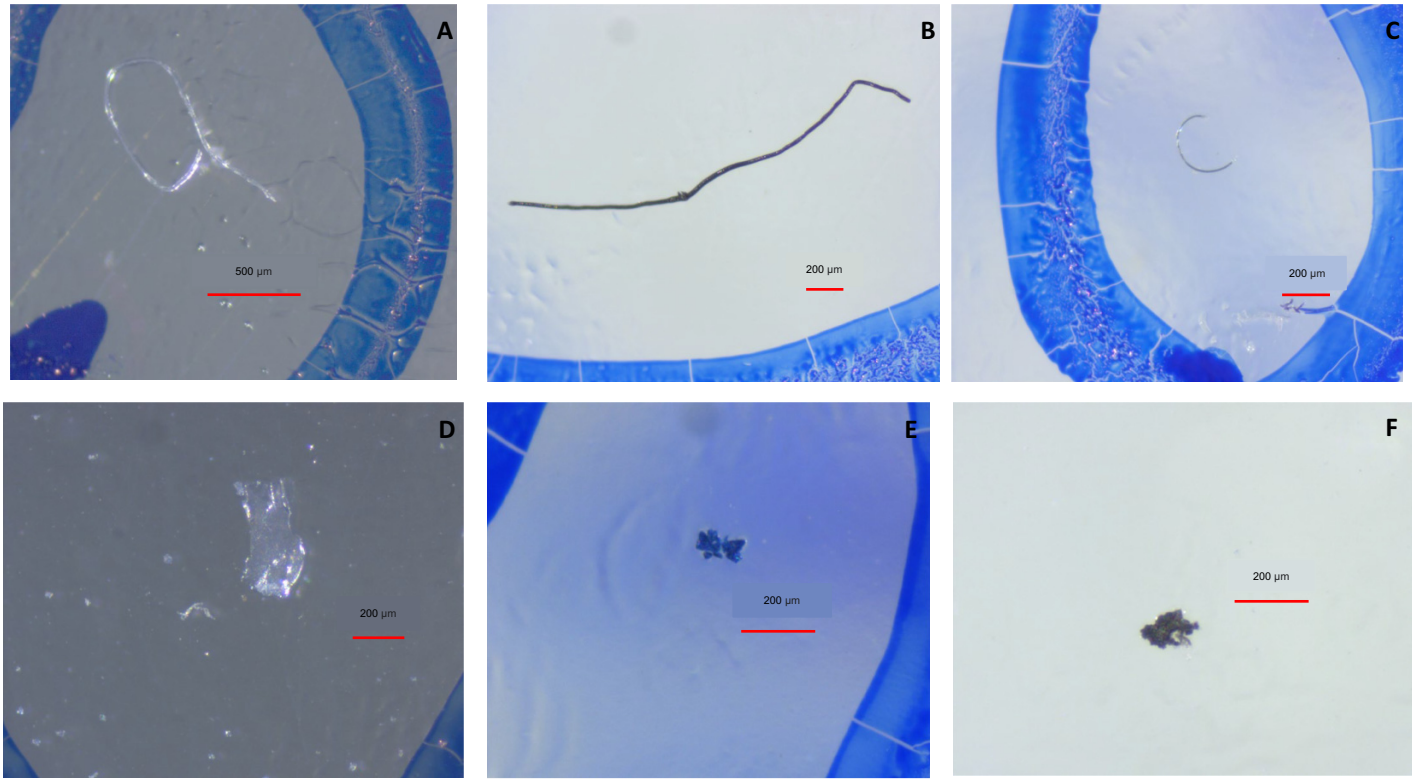

Fig. A2. Colours of microplastics found in subsurface water samples.

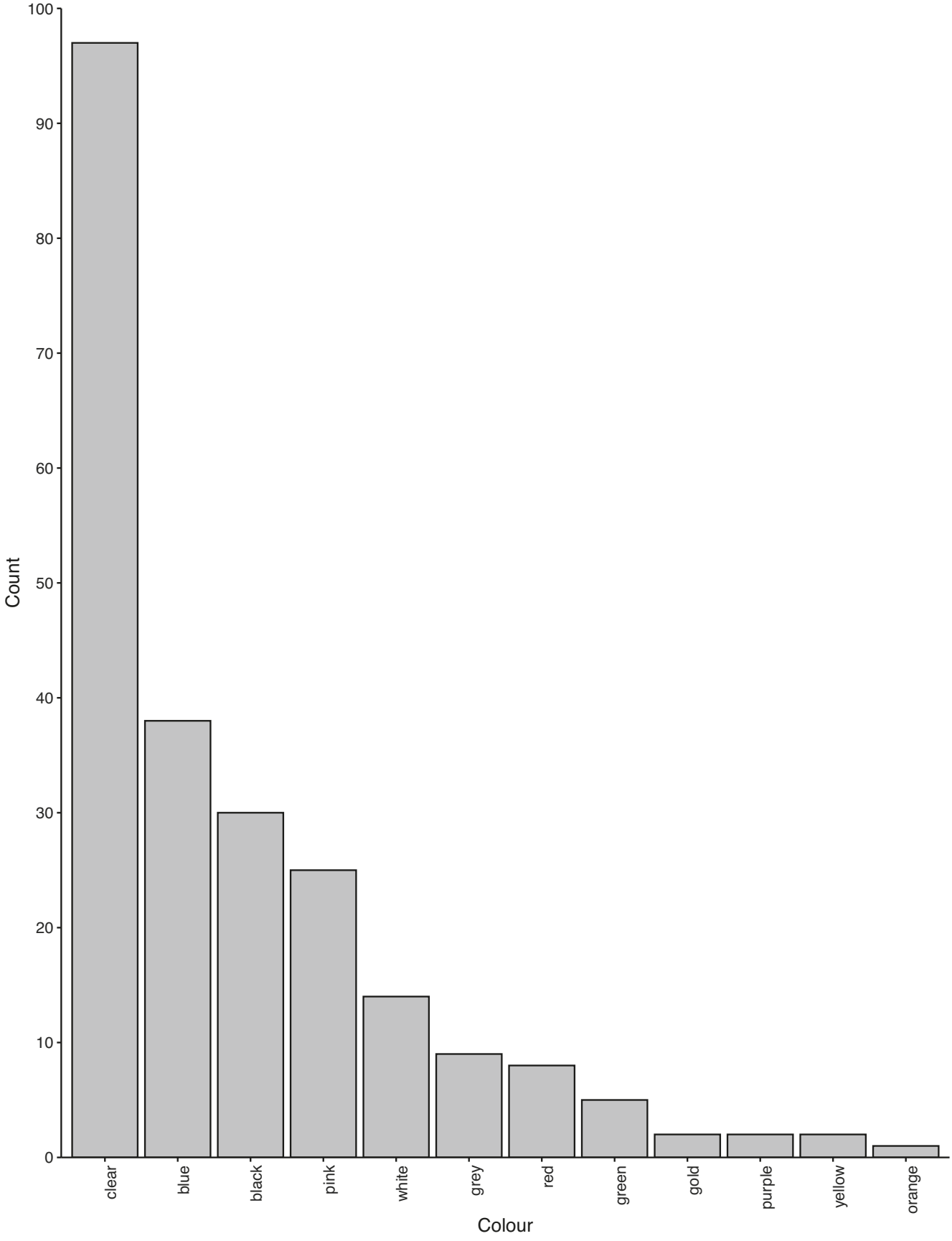

Fig. A3. Colours of microplastics found in zooplankton samples.