environments

MDPI

Article

Baseline Assessment of Anthropogenic Particles in Ogaa (*Sander vitreus*) and Asaawe (*Perca flavescens*) from the Mississippi Headwaters

Colin Eagle [1,*], Carl Isaacson [2], Melinda Neville [1], Michelle Anderson [1] and Muhammad Hasnain [1]

[1] Indigenous Science Department, Leech Lake Tribal College, Cass Lake, MN 56633, USA; mianderson201@gmail.com (M.A.); muhammad.hasnain@lltc.edu (M.H.)
[2] Department of Environmental Studies, Center for Sustainability Studies, Bemidji State University, Bemidji, MN 56601, USA; carl.isaacson@bemidjistate.edu
* Correspondence: colin.eagle@lltc.edu

Abstract

Anthropogenic particles (APs), including suspected microplastics, are increasingly reported in freshwater ecosystems; however, baseline data remain limited for culturally important freshwater fish in the Mississippi River Headwaters. This study assessed the occurrence and morphology of APs in Ogaa (walleye; *Sander vitreus*) and Asaawe (yellow perch; *Perca flavescens*) collected from five northern Minnesota lakes. Gastrointestinal tract, liver, and fillet tissues from 49 fish were processed using a modified hydrogen peroxide digestion protocol and examined by stereomicroscopy. Because polymer confirmation was not conducted, visually identified particles are conservatively reported as anthropogenic particles rather than confirmed microplastics. Forty-one APs were identified across all tissues, with 43% of fish containing at least one particle ($0.83 \pm 1.$ particles per fish). Fibers were the predominant morphology, followed by fragments, and walleye exhibited greater AP prevalence and abundance than yellow perch. Although descriptive differences were observed among lakes and tissues, statistical analyses did not detect significant relationships among the variables examined. These findings establish baseline information on AP occurrence in culturally important freshwater fish from the Mississippi River Headwaters and provide a foundation for future investigations incorporating polymer confirmation and expanded spatial and temporal sampling.

Keywords: Ogaa; Asaawe; Anishinaabe; Bemidjigamaag; anthropogenic particles; freshwater fish; Mississippi River Headwaters; Indigenous land stewardship

Academic Editor: Claudio Agnisola

Received: 18 June 2026
Revised: 23 July 2026
Accepted: 25 July 2026
Published: 4 August 2026

1. Introduction

The production of plastic in the world has skyrocketed since its first use in the early 20th century, proving beneficial to the global economy. Between 1950 and 2015, humans produced about 8300 million metric tons of plastic, generating roughly 6300 million metric tons of plastic waste. Of this waste, only about 9% has been recycled, 12% incinerated, and the vast majority around 79% has accumulated in landfills or the natural environment [1] . The lifespan of plastic in ecosystems ranges from hundreds to thousands of years, depending on its molecular structure and weight. Furthermore, adding antioxidants and stabilizers to its production can increase its lifespan [2]. The durability and moldability of plastic, which made it applicable to society, have made it consequential in the environment [3]. Plastic pollution in rural and urban settings can accumulate as suspended floating articles

in aquatic ecosystems [4,5]. UV radiation and other environmental factors, such as ocean waves, can fragment the plastic into smaller particles (<5 mm) called microplastics [6], which we define as anthropogenic particles (APs).

There are two types of AP: primary and secondary. Primary AP is manufactured in its current microsize, while secondary AP is created through the fragmentation of larger plastic litter. Examples of primary AP include cosmetic products, industrial cleaning powders, and nurdles. Secondary microplastics are often formed from fragmented trash and fibrous materials from clothing [7]. Furthermore, AP is categorized by shape: spongy foams, spindly fibers, fragments, round pellets, thin films, and nurdles [8].

The presence of AP contamination in freshwater systems arises from multiple sources, including sewage discharge and fishing-related debris, highlighting the need for broader pollution mitigation strategies [9]. Surprisingly, seemingly innocuous activities like domestic laundry, mediated through washing machines, have been identified as sources of AP fibers that find their way into wastewater [10]. Anthropogenic contributions to microplastic pollution in freshwater ecosystems are exemplified by tire wear particles from urban runoff and synthetic bait used in recreational fishing, both of which are significant sources of anthropogenic particles [11,12].

The introduction of AP in freshwater systems has resulted in it being recognized as a significant pollutant in freshwater fish [13,14], but the severity of the effects of MP pollution is still undetermined. For instance, in some studies, the average number of particles within fish varied by over an order of magnitude [14,15], suggesting that species and the environment drive particle ingestion. Particle ingestion can reduce prey consumption and be further compounded by negative effects on growth, reproduction, and survival for some fish [16].

Furthermore, some studies suggest that MP concentrations within fish are directly related to anthropogenic factors such as the density of urban populations. For example, researchers have found that in the Thames River, fish from urban areas had higher amounts of MP than fish from rural areas [17]. Another study found that urbanization played a significant role in introducing MP into the system, contributing to the concentration of MP within the studied fish [13]. Furthermore, a study conducted in Texas found that MP ingestion levels were significantly higher at certain sites compared to both upstream and downstream locations [18]. Conversely, Roch et al. executed a substantial study done on rivers in Germany and found no relationship between urbanization and the amount of MP found within their fish [19]. These conflicting results suggest that more research is needed to investigate the relationship between urbanization and the amount of MP found within freshwater fish.

In this study, we aim to (1) quantify anthropogenic particle (AP) concentrations in the gastrointestinal tract (GIT), liver, and fillet tissues of wild-caught fish from the Mississippi River Headwaters watershed; (2) characterize the morphological characteristics (e.g., shape and color) of AP recovered from the GIT, liver, and fillet tissues of wild-caught fish and (3) explore potential relationships between AP occurrence in fish tissues and lake-level environmental characteristics, including urbanization, within the Mississippi River Headwaters watershed.

This research is especially important in the Mississippi River Headwaters, where Anishinaabe communities continue to exercise treaty-protected rights to fish, hunt, and gather while maintaining enduring cultural relationships with the land and water. According to Anishinaabe oral history, the people migrated to this region in search of Manoomin (wild rice), a sacred food that grows on water [20]. These relationships were recognized through the 1855 Treaty and have been reaffirmed by the U.S. Supreme Court in Minnesota v. Mille Lacs Band of Chippewa Indians [20,21]. The species examined in this study, Ogaa

(walleye) and Asaawe (yellow perch), remain culturally significant and central to subsistence practices. Ogaa are common top predators in north-temperate lakes and forage across both inshore and offshore habitats, with juvenile Asaawe serving as an important prey species [22]. Asaawe are visual feeders that undergo ontogenetic dietary shifts from zooplankton to macroinvertebrates and small fish, often accompanied by shifts between littoral and pelagic habitats [15]. These ecological differences provide context for potential AP exposure pathways; however, diet and habitat use were not directly measured in this study. Consequently, understanding anthropogenic particle occurrence in these species not only addresses an important scientific knowledge gap but also contributes to the long-term stewardship of culturally significant freshwater resources.

2. Study Area

This study is in the north-central region of Minnesota within the Mississippi Headwaters watershed, characterized by its coniferous forests, secluded lakes, and relatively low population density along the Upper Mississippi River (see Figure 1). Fish were collected from Elk, Marquette, Irving, Bemidji, and Stump Lakes all part of Minnesota's Northern Lakes and Forests Ecoregion. The lakes along this portion of the watershed range from minimally to highly developed.

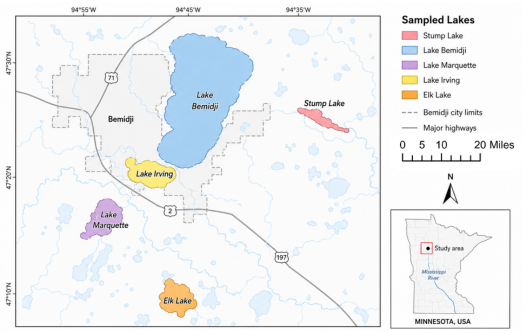

Figure 1. Locations of the five sampled lakes in northern Minnesota. Dashed gray boundaries indicate the Bemidji city limits, and solid gray lines indicate major highways. The inset shows the location of the study area within Minnesota and the Mississippi River.

The upstream lakes of the City of Bemidji include Elk, Marquette, and Irving. Elk Lake, located in Itasca State Park, is a small, deep, rural lake with minimal shoreline development, though it experiences seasonal group camping (MN DNR). Its shores are primarily forested and bordered by wetlands, supporting a healthy fish population (see Table 1). Marquette Lake is shallow and surrounded by moderate shoreline development and varying agricultural land use within its watershed. It is classified as mildly eutrophic, with a Trophic State Index (TSI) of 51, placing it within the Northern Lakes and Forests Ecoregion (RMB Environmental Laboratories, Inc., Detroit Lakes, MN, USA). Lake Irving, a highly developed eutrophic lake with multiple inlets, serves as the final upstream basin before the Mississippi River flows into Lake Bemidji.(Note: Water samples were analyzed by RMB Environmental Laboratories, Inc. (Detroit Lakes, MN, USA); trophic state indices and ecoregion assignments follow Minnesota Pollution Control Agency (MPCA) guidelines.)

The city of Bemidji (population 15,000) surrounds Bemidjigamaag (Lake Bemidji), a large and heavily developed recreational lake. The Mississippi River flows directly through Bemidjigamaag and exits via the Bemidji Dam, which regulates outflow of Stump Lake. Stump Lake, a small, shallow impoundment, lies immediately above the dam before the Mississippi River flows into the Leech Lake Reservation, which spans parts of Beltrami, Cass, and Hubbard counties. This geographic intersection is especially significant, as it highlights Indigenous land stewardship in an area affected by anthropogenic particle (AP)

pollution. Bemidjigamaag is classified as moderately eutrophic, with frequent summer algal blooms resulting from nutrient loading [23]. The lake supports numerous recreational activities, including boating, swimming, netting, and fishing, making it both a cultural and ecological focal point for local communities.

Table 1. Land Cover Composition Around Selected Lakes.

Lake Name	Developed Land (%)	Barren (%)	Forest (%)	Shrubland (%)	Herbaceous (%)	Planted (%)	Wetlands (%)
Elk	5.8	0.1	76.5	0.0	0.5	0.0	17.1
Marquette	18.0	0.0	32.0	1.7	2.9	5.4	40.0
Irving	39.8	0.0	11.7	0.0	0.7	0.4	47.3
Lake Bemidji(BEMIDJIGAMAAG)	34.3	0.1	38.0	1.0	1.5	2.2	23.0
Stump	34.3	0.1	38.0	1.0	1.5	2.2	23.0

3. Materials and Methods

3.1. Fish Collection, Sample Preparation, and Particle Identification

Fish (N = 49) were obtained through the Minnesota Department of Natural Resources (MN DNR) annual fisheries assessment program. Specimens were collected using standardized gill-net surveys from sampling locations within the Mississippi River Headwaters watershed. Fish were measured (total length), weighed, and dissected using sterilized stainless-steel instruments inside a clean fume hood. Gastrointestinal tract (GIT), liver, and fillet tissues from Sander vitreus (Ogaa) and Perca flavescens (Asaawe) were transferred to pre-labeled Erlenmeyer flasks covered with aluminium foil to minimize airborne contamination.

The tissue digestion followed a modified version of the protocol described by [24] (Appendix A). Tissue samples were digested in 100 mL of 30% filtered hydrogen peroxide (H_2O_2) and incubated at 50 °C for 48–72 h until the tissues were completely digested. Larger tissue samples were homogenized as necessary to facilitate complete digestion. Digests were vacuum-filtered, and the filters were transferred to covered glass Petri dishes and stored until microscopic examination.

Filters were examined using an AmScope trinocular stereomicroscope (United Scope LLC, Irvine, CA, USA) (2×–225× magnification). Suspected anthropogenic particles (APs) were visually identified and classified according to particle morphology (e.g., fibers, fragments, films, foam, and beads) following [25]. Candidate particles were evaluated using multiple morphological characteristics, including uniform thickness, consistent coloration, absence of visible cellular or biological structures, and overall appearance consistent with anthropogenic materials. Particles were examined under multiple focal planes and lighting angles to distinguish suspected APs from residual biological material and other non-target debris. Because polymer composition was not confirmed using spectroscopic techniques (e.g., FTIR or Raman spectroscopy), all visually identified particles are conservatively reported as anthropogenic particles (APs) rather than confirmed microplastics.

3.2. Quality Assurance and Quality Control (QA/QC)

To minimize laboratory contamination, all laboratory surfaces and equipment were cleaned before sample processing. Glassware was rinsed three times with filtered deionized water before use and covered with aluminum foil when not actively in use. Laboratory glassware and equipment were cleaned using plant-based cellulose sponges to reduce the introduction of synthetic fibers during sample preparation. All digestion reagents and deionized water were filtered prior to use. Personnel wore non-synthetic laboratory coats,

nitrile gloves, and safety glasses throughout sample preparation and analysis. Sample preparation and dissections were conducted inside a clean fume hood whenever possible.

Eight laboratory blanks were processed throughout the study to assess background contamination. Each blank consisted of 150 mL of filtered deionized water exposed to the laboratory environment during the same periods that biological samples were uncovered and processed. Blank samples were vacuum filtered using the same filtration apparatus and filter paper as biological samples, transferred to covered glass Petri dishes, stored, and examined microscopically using the same visual identification criteria applied to biological samples.

The average blank particle count was subtracted from biological sample counts prior to calculating particle concentrations and conducting statistical analyses.

3.3. Statistical Analysis

The shoreline development metric for each lake was calculated by dividing the total number of shoreline developments (e.g., homes, cabins, and other structures) by shoreline length (m). Descriptive statistics, including means, standard deviations, ranges, and frequency distributions, were calculated for anthropogenic particle abundance and concentration by lake, species, and tissue type. Pearson correlation coefficients were used to evaluate relationships between fish morphological characteristics (total length and weight) and AP abundance or concentration. Because AP count data were non-normally distributed and characterized by numerous zero observations following blank correction, differences among species–tissue groups were evaluated using the Kruskal–Wallis rank-sum test. Statistical analyses were performed using RStudio, R 4.4.2 with RStudio 2024 (R Foundation for Statistical Computing, Vienna, Austria), and statistical significance was assessed at $\alpha = 0.05$.

4. Results

4.1. Anthropogenic Particle Occurrence by Species

A total of 41 anthropogenic particles (APs) were detected across liver, gastrointestinal tract (GIT), and fillet tissues from 49 wild-caught *giigoo* (fish), yielding a mean (±SD) of 0.83 ± 1.50 particles per fish. Overall AP prevalence was 43%, with individual fish containing between 0 and 6 particles (Table 2).

Table 2. Summary statistics for anthropogenic particles (APs) found within fish and their respective tissues.

Species/Tissue	AP Total	Samples	Fish with APs	Prevalence (%)	Mean	Min	Max
Ogaa	25	24	12	50%	1.04 ± 1.67	0	6
Asaawe	16	25	9	36%	0.64 ± 1.29	0	6
Ogaa GIT	11	24	4	17%	2.75 ± 1.35	0	6
Ogaa Liver	6	23	4	17%	1.50 ± 0.689	0	3
Ogaa Fillet	8	23	6	26%	1.14 ± 0.656	0	2
Asaawe GIT	10	25	4	16%	2.50 ± 1.35	0	6
Asaawe Liver	4	25	3	12%	1.30 ± 0.473	0	2
Asaawe Fillet	2	25	2	8%	1.00 ± 0.277	0	1
Total	41	49	21	43%	—	0	6

Two species were examined: *Ogaa* (*Sander vitreus*) and *Asaawe* (*Perca flavescens*). Among 24 *Ogaa*, 25 APs were detected, with a mean of 1.04 ± 1.67 particles per fish and a prevalence of 50%. Individual *Ogaa* contained between 0 and 6 particles, with blue fibers representing the most frequently observed AP morphology, followed by fragments.

Among 25 *Asaawe*, 16 APs were detected, with a mean of 0.64 ± 1.29 particles per fish and a prevalence of 36%. Individual *Asaawe* also contained between 0 and 6 particles.

As with *Ogaa*, blue fibers were the predominant morphology, with blue fibers occurring most frequently.

4.2. Particle Counts by Tissue Type

Across both species, the gastrointestinal tract (GIT) contained the highest observed total AP counts, with 11 APs detected in *Ogaa* and 10 APs in *Asaawe*. In *Ogaa*, the GIT had a mean tissue particle count of 2.8 ± 1.4 particles and a prevalence of 17%, with counts ranging from 0–6 particles. Similarly, the GIT of *Asaawe* had a mean tissue particle count of 2.5 ± 1.4 particles, a prevalence of 16%, and counts ranging from 0–6 particles.

Liver tissues contained fewer APs than the GIT. *Ogaa* livers contained 6 APs, with a mean tissue particle count of 1.5 ± 0.7 particles, a prevalence of 17%, and counts ranging from 0–3 particles. *Asaawe* livers contained 4 APs, with a mean tissue particle count of 1.3 ± 0.5 particles, a prevalence of 12%, and counts ranging from 0–2 particles.

Fillet tissues also contained relatively few APs. *Ogaa* fillets contained 8 APs, with a mean tissue particle count of 1.1 ± 0.66 particles, a prevalence of 26%, and counts ranging from 0–2 particles. In comparison, *Asaawe* fillets contained 2 APs, with a mean tissue particle count of 1.0 ± 0.28 particles, a prevalence of 8%, and counts ranging from 0–1 particle.

4.3. AP Concentrations in Tissues (p/g)

The overall concentration of anthropogenic particles (APs) across all tissues was 0.09 ± 0.32 particles per gram wet weight (p/g), with concentrations ranging from 0–2.8 p/g. *Ogaa* tissues ($n = 70$) had a mean concentration of 0.08 ± 0.23 p/g (range: 0–1.6 p/g), whereas *Asaawe* tissues ($n = 75$) had a mean concentration of 0.09 ± 0.23 p/g (range: 0–2.8 p/g).

Descriptively, liver tissues exhibited the highest AP concentrations (0.14 ± 0.49 p/g; range: 0–2.8 p/g), followed by the gastrointestinal tract (GIT) (0.08 ± 0.25 p/g; range: 0–1.4 p/g) and fillet tissues (0.04 ± 0.10 p/g; range: 0–0.52 p/g).

4.3.1. AP Concentrations by Species and Tissue

In *Ogaa*, mean AP concentrations were highest in the liver (0.10 ± 0.36 p/g), followed by the fillet (0.07 ± 0.13 p/g) and the gastrointestinal tract (GIT) (0.05 ± 0.15 p/g). Concentrations ranged from 0–1.6 p/g in the liver, 0–0.52 p/g in the fillet, and 0–0.61 p/g in the GIT.

In *Asaawe*, the liver also exhibited the highest mean AP concentration (0.17 ± 0.60 p/g) (see Figure 2), followed by the GIT (0.10 ± 0.32 p/g) and the fillet (0.02 ± 0.06 p/g). Concentration ranges were 0–2.8 p/g for the liver, 0–1.4 p/g for the GIT, and 0–0.22 p/g for the fillet.

4.3.2. AP Distribution in Fish Across Multiple Lakes

Anthropogenic particle (AP) occurrence varied among the five sampled lakes. The following sections summarize total particle abundance, mean AP concentrations, and species- and tissue-level variability observed across the study lakes. These comparisons are descriptive and are intended to illustrate observed patterns among sampling locations.

Total Particles by Lake

A total of 49 fish were collected across five lakes in the Mississippi River Headwaters watershed. Fish sample sizes and the total number of anthropogenic particles (APs) recovered from each lake are summarized in Table 3. Descriptively, AP abundance varied among the five sampled lakes. Stump Lake had the highest observed particle count ($n = 16$), followed by Marquette Lake ($n = 11$), Elk Lake ($n = 8$), Irvine Lake ($n = 5$), and Lake Bemidji ($n = 1$).

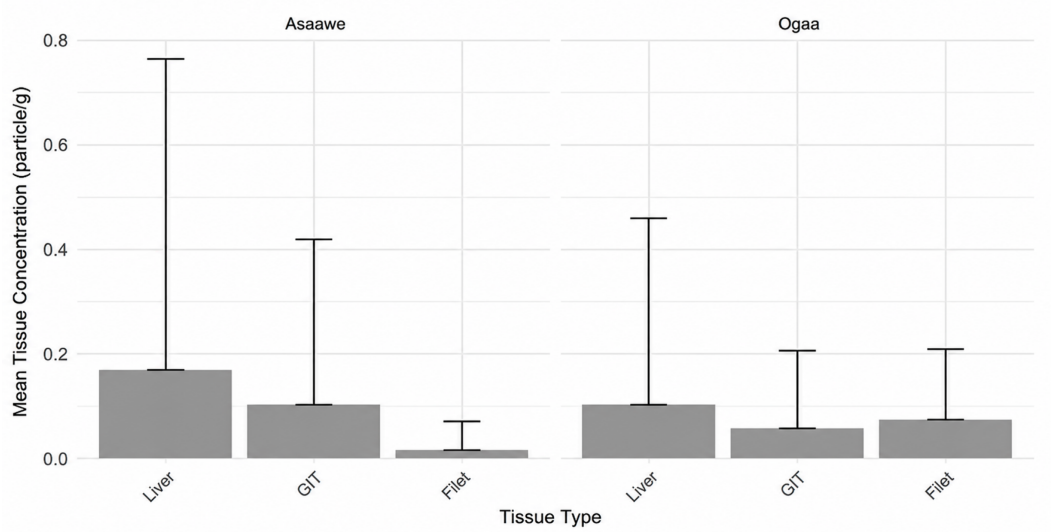

Figure 2. Mean anthropogenic particle (AP) concentrations in liver, gastrointestinal tract (GIT), and fillet tissues of *Ogaa* and *Asaawe*. Bars represent mean concentrations (particles g^{-1} wet weight), and error bars represent ±1 standard deviation (SD). Tissue sample sizes were: *Ogaa*, GIT ($n = 24$), liver ($n = 23$), and fillet ($n = 23$); and *Asaawe*, GIT ($n = 25$), liver ($n = 25$), and fillet ($n = 25$). Portions of error bars extending below zero represent statistical dispersion around the mean and do not indicate observed negative AP concentrations.

Table 3. Number of fish analyzed (*n*) and total anthropogenic particles (APs) recovered from each sampled lake.

Lake	Fish (*n*)	APs (*n*)
Elk	10	8
Marquette	10	11
Irving	10	5
Bemidji	10	1
Stump	9	16
Total	49	41

Mean Concentrations by Lake

Mean AP concentrations (p/g wet weight) followed a similar pattern, with Stump Lake exhibiting the highest mean concentration (0.203 ± 0.575 p/g). Marquette, Irvine, and Elk exhibited intermediate mean concentrations ranging from 0.049 to 0.086 p/g, whereas Bemidji had the lowest mean concentration (0.030 ± 0.166 p/g).

Species- and Tissue-Level Variability

Among *Asaawe*, liver tissues exhibited the highest mean AP concentration at Stump Lake (0.666 ± 1.24 p/g), whereas liver APs were not detected in several other lakes. GIT concentrations were also elevated in Marquette and Stump but were not detected in Bemidji or Irvine. Fillet concentrations were generally low, with the highest observed mean concentration occurring in Elk (0.444 ± 0.099 p/g).

Among *Ogaa*, GIT concentrations were modestly elevated in Marquette, Irvine, and Stump but were not detected in Elk or Bemidji. Fillet tissues exhibited the highest mean concentration in Elk (0.215 ± 0.406 p/g), whereas liver concentrations were generally low across lakes, with the highest observed mean concentration occurring in Stump (0.14 ± 0.23 p/g) (see Figure 3).

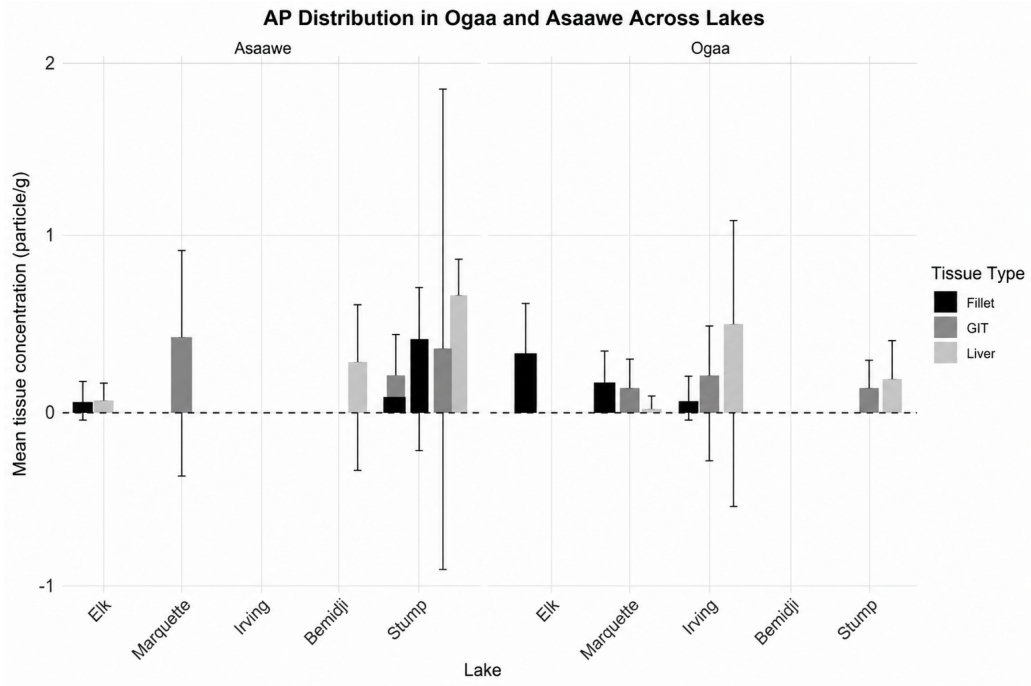

Figure 3. Mean anthropogenic particle (AP) concentrations across sampled lakes by species and tissue type. Bars represent mean tissue concentrations (particles g^{-1} wet weight), and error bars represent ± 1 standard deviation (SD). Fish sample sizes by lake were Elk ($n = 10$), Marquette ($n = 10$), Irvine ($n = 10$), Bemidji ($n = 10$), and Stump ($n = 9$). Portions of error bars extending below zero represent statistical dispersion around the mean and do not indicate observed negative AP concentrations.

4.4. AP Morphology

The recovered anthropogenic particles were classified into morphological categories, including fibers and fragments (see Figure 4 for visual comparison). Across all tissues and lakes, fibers were the predominant particle type, followed by fragments. Fibers were observed most frequently in gastrointestinal tract (GIT) samples, although they were also detected in liver and fillet tissues.

Morphological composition was generally similar among the sampled lakes. Stump Lake contained the greatest number of recovered fibers, reflecting its higher overall AP abundance, whereas particles recovered from Marquette, Elk, Bemidji, and Irvine included a mixture of fibers and fragments. Because particle morphology was determined following chemical digestion and visual microscopy, these observations are presented as descriptive classifications.

4.5. Statistical Analyses

Pearson correlation analyses were conducted to evaluate relationships between fish morphological characteristics (length and weight) and anthropogenic particle (AP) occurrence. Correlation coefficients were negligible, indicating little evidence of linear relationships between total length and AP concentration ($r = 0.0019$) or between fish weight and tissue particle totals ($r = 0.0296$).

Differences in AP counts among species–tissue combinations were evaluated using a Kruskal–Wallis rank-sum test. No statistically significant differences were detected among groups ($\chi^2 = 4.80$, $df = 5$, $p = 0.441$).

4.6. QA/QC

Eight laboratory blanks yielded a mean of 2.9 anthropogenic particles per blank. Fibers were the predominant morphology, with blue fibers occurring most frequently.

Blank-corrected data remained non-normally distributed and were therefore analyzed using nonparametric statistical approaches.

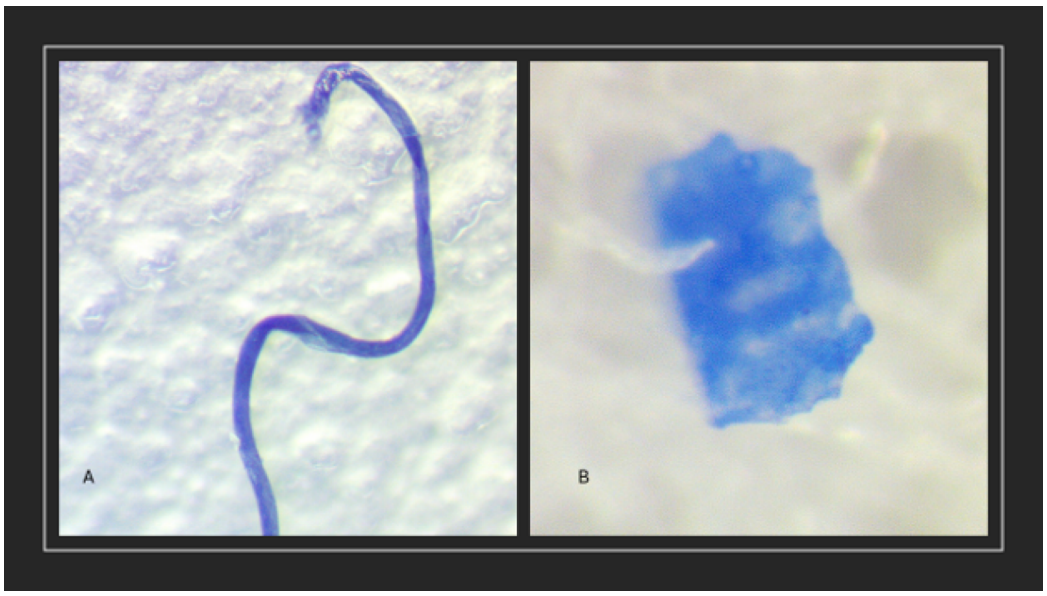

Figure 4. Representative anthropogenic particles (APs) recovered from digested fish tissues. (**A**) Fiber and (**B**) fragment identified by stereomicroscopy following chemical digestion. Particle classifications were based on visual morphology.

5. Discussion

5.1. Anthropogenic Particle Occurrence Across Lakes

Anthropogenic particle (AP) abundance varied among the five sampled lakes but showed no clear relationship with shoreline development or the hypothesized urbanization gradient. Stump Lake, the most downstream lake sampled, exhibited the highest AP abundance, whereas Bemidji, despite having the greatest surrounding development and receiving wastewater treatment plant (WWTP) effluent upstream, exhibited the lowest AP abundance. Similar variability among lakes has been reported in other Minnesota freshwater studies that likewise found no significant differences in AP occurrence among sampled lakes [26].

The elevated AP abundance observed in Stump Lake may reflect its downstream position within the watershed and the influence of impoundment by a dam. Reduced water velocity associated with reservoirs can promote the settling and accumulation of suspended anthropogenic particles, potentially increasing exposure to aquatic organisms [26]. Multiple studies have similarly reported greater AP accumulation in downstream water bodies where particles are transported and retained within connected freshwater systems [27,28] . Conversely, the relatively low AP abundance observed in Bemidji suggests that shoreline development alone may not adequately predict AP occurrence in freshwater fish, despite the presence of urban development and upstream WWTP discharge. Although WWTPs are recognized sources of APs to freshwater environments [29–31], our findings indicate that additional watershed processes likely influence particle exposure.

The lack of a consistent relationship among lakes emphasizes the complexity of AP transport within interconnected freshwater systems. Differences in hydrology, watershed connectivity, lake morphology, particle retention, species ecology, and local environmental conditions may collectively influence AP availability. Similar conclusions have been reported for Minnesota lakes, where substantial variability occurred despite comparable regional settings [15]. Because these factors were not directly measured in the present study,

they should be considered plausible explanations requiring further investigation rather than direct causal mechanisms.

5.2. AP Occurrence in Wild-Caught Fish

Anthropogenic particles were detected in all tissue types examined, with 41 total APs identified among 49 fish and an overall mean of 0.83 particles per fish. Approximately 43% of sampled fish contained at least one observed AP. These findings are comparable to recent studies conducted in Minnesota freshwater systems that reported similar prevalence and particle abundance, including 48% prevalence and a mean of 0.86 particles per fish [26]. Differences among studies likely reflect variation in sample size, digestion protocols, fish species, analytical procedures, and reporting metrics.

Reports of AP occurrence in freshwater fish remain highly variable. For example, one investigation reported approximately 2.1 ± 1.26 particles per fish [26] , whereas [32] observed substantially greater particle burdens (17 ± 15.5 particles per fish) and [14] detected only 18 particles among 60 freshwater fish. Such discrepancies likely reflect methodological differences, including digestion techniques, particle identification criteria, and tissue sampling strategies, as well as biological differences among fish species and environmental variation among freshwater systems. Collectively, these comparisons highlight the continuing need for standardized methodologies to improve comparisons among studies.

Although APs were observed in liver and fillet tissues, concentrations remained relatively low. Detection of APs outside the gastrointestinal tract is consistent with previous reports suggesting that particles may move beyond the digestive tract under some conditions [14,32]. However, the mechanisms governing particle occurrence within internal tissues remain poorly understood, and conflicting findings among freshwater studies emphasize the importance of future investigations incorporating spectroscopic polymer confirmation and standardized analytical approaches.

5.3. Species and Tissue Differences

Ogaa contained more APs overall than Asaawe; however, no statistically significant differences were identified between species. Similar AP abundances within gastrointestinal tract samples suggest that both species experience comparable exposure within the study area despite differences in feeding ecology. Previous studies have reported inconsistent relationships between trophic position and AP burden, with some investigations reporting greater accumulation in piscivorous species while others observed no consistent trophic patterns [32–36]. Together with our findings, these studies suggest that species ecology alone may not fully explain AP occurrence in freshwater fish.

Across all samples, APs were most frequently detected within gastrointestinal tract tissues, followed by liver and fillet tissues. This distribution is consistent with ingestion representing a significant pathway of AP exposure. APs were observed in liver and fillet samples; however, the low particle counts, absence of polymer confirmation, and possibility of procedural or dissection-related contamination prevent conclusions regarding translocation into internal tissues. Variation among published studies further illustrates the uncertainty surrounding particle occurrence within fish tissues. Differences in reported AP occurrence likely reflect methodological variation, including tissue mass analyzed, particle detection limits, digestion procedures, and analytical confirmation. Consequently, standardized methodologies and polymer-confirmed analyses remain essential for improving comparisons among studies and clarifying the biological processes governing AP occurrence within freshwater fishes.

5.4. Particle Morphology

Fibers were the predominant AP morphology observed across species, tissues, and lakes, followed by fragments, with blue particles representing the most frequently observed color. Similar dominance of fibers has been reported in numerous freshwater fish studies and may reflect the widespread occurrence of synthetic textile fibers, fishing-related materials, and other diffuse anthropogenic sources. Although fragments have been reported as the dominant morphology in some freshwater investigations, differences in particle composition among studies likely reflect regional environmental conditions, local sources, and methodological variation.

The predominance of fibers observed in gastrointestinal tissues supports previous freshwater studies reporting similar morphological distributions [32]. Variation in particle morphology among liver and fillet tissues should be interpreted cautiously because relatively few particles were detected outside the gastrointestinal tract. Future investigations incorporating spectroscopic identification will improve understanding of particle composition and potential environmental sources.

5.5. Study Limitations and Future Research

Several limitations should be considered when interpreting these findings. Sample size limited statistical power to detect subtle biological or environmental relationships. Particle identification relied on visual microscopy without polymer confirmation, preventing definitive identification of plastics and supporting the use of the broader term anthropogenic particles. In addition, only portions of some tissues were analyzed, and particles below the visual detection limit may have been missed.

Despite these limitations, this study establishes baseline information on AP occurrence in culturally important freshwater fish from the Mississippi Headwaters watershed. Future investigations should incorporate larger sample sizes, standardized methodologies, seasonal sampling, environmental matrices (e.g., water, sediment, and prey organisms), and spectroscopic confirmation using techniques such as FTIR or Raman spectroscopy. Continued long-term monitoring will improve understanding of AP occurrence, transport, and ecological significance within northern freshwater ecosystems.

6. Conclusions

This study provides a baseline assessment of anthropogenic particle (AP) occurrence in Ogaa (*Sander vitreus*) and Asaawe (*Perca flavescens*) collected from the Mississippi Headwaters watershed of northern Minnesota. Anthropogenic particles were detected in gastrointestinal tract, liver, and fillet tissues, with fibers representing the predominant particle morphology. Although AP occurrence varied among lakes, species, and tissue types, no statistically significant relationships were identified among the biological or environmental variables examined, suggesting that AP exposure within these freshwater systems is influenced by factors more complex than shoreline development alone.

The detection of APs in culturally important fish species contributes baseline information for a region where data remain limited and establishes a foundation for long-term monitoring of freshwater anthropogenic particle contamination. While visual microscopy provided a practical approach for baseline assessment, future investigations should incorporate larger sample sizes, standardized methodologies, and spectroscopic confirmation using techniques such as FTIR or Raman spectroscopy to improve particle identification and strengthen comparisons among freshwater studies. Together, these findings provide an important reference for future ecological monitoring and support continued investigation of anthropogenic particles within the Mississippi Headwaters watershed. These

findings should be interpreted in light of the limited sample size and the reliance on visual identification without spectroscopic polymer confirmation.

Author Contributions: Conceptualization, C.E.; methodology, C.E. and M.A.; formal analysis, C.E.; investigation, C.E.; data curation, C.E.; writing-original draft preparation, C.E.; writing-review and editing, C.E. and M.H.; visualization, C.E. and M.H.; supervision, C.I. and M.N. M.H. additionally contributed to manuscript development, reference and citation management, journal selection, formatting, and preparation of the manuscript for submission. All authors have read and agreed to the published version of the manuscript.

Funding: This program was funded by the Bemmidji State University Graduate Program, Niizhoo-gwayakochigewin program and USDA Mcintire Stennis (NIFA).

Data Availability Statement: The data supporting the findings of this study are available from the corresponding author upon reasonable request. The data are not currently available in a publicly accessible repository.

Acknowledgments: I want to express my profound gratitude to Gitchii-manitou for the gift of creation and the wisdom and foresight to complete this project. I am incredibly thankful to the fish whose lives were given to enable this study. My sincere appreciation extends to the Minnesota Department of Natural Resources Fisheries for allowing me to participate in their fish surveys, which were essential to my research. I also want to express my deep gratitude to my committee members, including Carl Isaacson and Miriam Rios-Sanchez from Bemidji State University, and Melinda Neville from Leech Lake Tribal College, for their steadfast support during the planning and implementation of this project. Your resources, guidance, and encouragement have been incredibly valuable. Furthermore, I am profoundly grateful to Michelle Anderson. Her exceptional work on this project has far exceeded my expectations, and her contributions have been invaluable. I am also grateful to Muhammad Hasnain for his assistance in reviewing and preparing this work for journal publication, including manuscript preparation, citation and reference management, formatting, and journal selection. Finally, I want to express my heartfelt thanks to my kids, Colin Eagle Jr and Jazlynn Rice. Your patience, support, and understanding while I worked on this thesis have been immeasurable. Dad loves you dearly and could not have completed this journey without your help and encouragement. Chii-miigwech.

Conflicts of Interest: The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

Abbreviations

The following abbreviations are used in this manuscript:

AP	Anthropogenic particle(s)
FTIR	Fourier-transform infrared spectroscopy
GIT	Gastrointestinal tract
H_2O_2	Hydrogen peroxide
MN DNR	Minnesota Department of Natural Resources
MP	Microplastic(s)
p/g	Particles per gram
QA/QC	Quality assurance/quality control
R	R statistical computing environment
RMB	RMB Environmental Laboratories, Inc.
SD	Standard deviation
TSI	Trophic State Index
U.S.	United States
UV	Ultraviolet
WWTP	Wastewater treatment plant

Appendix A. Detailed Laboratory Protocol for Sample Digestion, Filtration, and Visual Identification of Anthropogenic Particles

The protocol includes sample preparation, dissection, oxidation with H_2O_2, filtration steps, and contamination-control measures used throughout the study.

Appendix A.1. Preparation and Cleaning

- Thaw fish samples before processing.
- Wash and label all necessary Erlenmeyer flasks ($\geq$60) using filtered deionized (FDI) water.
- Clean laboratory surfaces with plant-based sponges.
- Dry-mop floors with a Swiffer prior to use.
- Wear non-synthetic laboratory coats, gloves, and safety glasses during all procedures.
- Rinse flasks three times with filtered deionized water and cover them with aluminum foil to prevent contamination.

Appendix A.2. Dissection

- All chemicals were filtered before use.
- All dissection steps were performed inside a clean fume hood using sterilized dissection kits.
- Fish were measured (total length from caudal fin to mouth) and weighed before dissection.
- The liver, gastrointestinal tract (GIT), and fillet were removed using sterile tools and placed into pre-labeled, foil-covered Erlenmeyer flasks.
- Tissue samples weighing more than approximately 5–7 g were chopped into smaller pieces to match digestion flask volume and optimize processing.

Appendix A.3. Digestion and Filtration

- Each tissue sample was combined with 100 mL of 30% H_2O_2 in a pre-rinsed flask.
- Flasks were incubated in a convection oven at 50 °C for 48–72 h.
- After digestion, samples were filtered using a vacuum filtration system with 20 µm filter paper to avoid clogging.
- If clogging occurred, undigested portions were transferred into smaller beakers and re-filtered as needed.
- Filters were stored in labeled Petri dishes for inspection.

Appendix A.4. Visualization and Identification

- Particles retained on filters were first examined using an AmScope 2×–225× trinocular boom microscope.
- Each particle was classified by shape (fragment, fiber, bead, or foam) following Egessa et al. (2020) [26].
- Particles were evaluated based on:
  - size (<5 mm);
  - thickness uniformity;
  - color consistency, especially for transparent or white particles;
  - absence of cellular or biological structures, following Laju et al. (2022) [11].

References

1. Geyer, R.; Jambeck, J.R.; Law, K.L. Production, use, and fate of all plastics ever made. *Sci. Adv.* **2017**, *3*, e1700782. [CrossRef]
2. Chamas, A.; Moon, H.; Zheng, J.; Qiu, Y.; Tabassum, T.; Jang, J.H.; Abu-Omar, M.; Scott, S.L.; Suh, S. Degradation rates of plastics in the environment. *ACS Sustain. Chem. Eng.* **2020**, *8*, 3494–3511. [CrossRef]

3. Thompson, R.C.; Moore, C.J.; Saal, F.S.V.; Swan, S.H. Plastics, the environment and human health: Current consensus and future trends. *Philos. Trans. R. Soc. B Biol. Sci.* **2009**, *364*, 2153–2166. [CrossRef] [PubMed]
4. Lebreton, L.; Slat, B.; Ferrari, F.; Sainte-Rose, B.; Aitken, J.; Marthouse, R.; Hajbane, S.; Cunsolo, S.; Schwarz, A.; Levivier, A.; et al. Evidence that the Great Pacific Garbage Patch is rapidly accumulating plastic. *Sci. Rep.* **2018**, *8*, 4666. [CrossRef] [PubMed]
5. Anderson, P.J.; Warrack, S.; Langen, V.; Challis, J.K.; Hanson, M.L.; Rennie, M.D. Microplastic contamination in Lake Winnipeg, Canada. *Environ. Pollut.* **2017**, *225*, 223–231. [CrossRef] [PubMed]
6. Barnes, D.K.A.; Galgani, F.; Thompson, R.C.; Barlaz, M. Accumulation and fragmentation of plastic debris in global environments. *Philos. Trans. R. Soc. B Biol. Sci.* **2009**, *364*, 1985–1998. [CrossRef] [PubMed]
7. Atugoda, T.; Vithanage, M.; Wijesekara, H.; Bolan, N.; Sarmah, A.K.; Bank, M.S.; You, S.; Ok, Y.S. Interactions between microplastics, pharmaceuticals and personal care products: Implications for vector transport. *Environ. Int.* **2021**, *149*, 106367. [CrossRef] [PubMed]
8. Baldwin, A.K.; Spanjer, A.R.; Rosen, M.R.; Thom, T. Microplastics in Lake Mead National Recreation Area, USA: Occurrence and biological uptake. *PLoS ONE* **2020**, *15*, e0228896. [CrossRef] [PubMed]
9. Yuan, W.; Liu, X.; Wang, W.; Di, M.; Wang, J. Microplastic abundance, distribution and composition in water, sediments, and wild fish from Poyang Lake, China. *Ecotoxicol. Environ. Saf.* **2019**, *170*, 180–187. [CrossRef] [PubMed]
10. Cai, Y.; Yang, T.; Mitrano, D.M.; Heuberger, M.; Hufenus, R.; Nowack, B. Systematic study of microplastic fiber release from 12 different polyester textiles during washing. *Environ. Sci. Technol.* **2020**, *54*, 4847–4855. [CrossRef] [PubMed]
11. Laju, R.L.; Jayanthi, M.; Jeyasanta, K.I.; Patterson, J.; Asir, N.G.G.; Sathish, M.N.; Edward, J.P. Spatial and vertical distribution of microplastics and their ecological risk in an Indian freshwater lake ecosystem. *Sci. Total Environ.* **2022**, *820*, 153337. [CrossRef] [PubMed]
12. de Carvalho, A.R.; Imbert, A.; Parker, B.; Euphrasie, A.; Boulêtreau, S.; Britton, J.R.; Cucherousset, J. Microplastic in angling baits as a cryptic source of contamination in European freshwaters. *Sci. Rep.* **2021**, *11*, 11255. [CrossRef] [PubMed]
13. Silva-Cavalcanti, J.S.; Silva, J.D.B.; de França, E.J.; de Araújo, M.C.B.; Gusmão, F. Microplastics ingestion by a common tropical freshwater fishing resource. *Environ. Pollut.* **2017**, *221*, 218–226. [CrossRef] [PubMed]
14. McIlwraith, H.K.; Kim, J.; Helm, P.; Bhavsar, S.P.; Metzger, J.S.; Rochman, C.M. Evidence of microplastic translocation in wild-caught fish and implications for microplastic accumulation dynamics in food webs. *Environ. Sci. Technol.* **2021**, *55*, 12372–12382. [CrossRef] [PubMed]
15. Conowall, P.; Schreiner, K.M.; Minor, E.C.; Hrabik, T.; Schoenebeck, C.W. Variability of microplastic loading and retention in four inland lakes in Minnesota, USA. *Environ. Pollut.* **2023**, *328*, 121573. [CrossRef] [PubMed]
16. Foley, C.J.; Feiner, Z.S.; Malinich, T.D.; Höök, T.O. A meta-analysis of the effects of exposure to microplastics on fish and aquatic invertebrates. *Sci. Total Environ.* **2018**, *631–632*, 550–559. [CrossRef] [PubMed]
17. Wardlaw, C.M.; Corcoran, P.L.; Neff, B.D. Factors influencing the variation of microplastic uptake in demersal fishes from the upper Thames River Ontario. *Environ. Pollut.* **2022**, *313*, 120095. [CrossRef] [PubMed]
18. Peters, C.A.; Bratton, S.P. Urbanization is a major influence on microplastic ingestion by sunfish in the Brazos River Basin, Central Texas, USA. *Environ. Pollut.* **2016**, *210*, 380–387. [CrossRef] [PubMed]
19. Roch, S.; Walter, T.; Ittner, L.D.; Friedrich, C.; Brinker, A. A systematic study of the microplastic burden in freshwater fishes of south-western Germany—Are we searching at the right scale? *Sci. Total Environ.* **2019**, *689*, 1001–1011. [CrossRef] [PubMed]
20. Bouayad, A. Wild rice protectors: An Ojibwe odyssey. *Environ. Law Rev.* **2020**, *22*, 25–42. [CrossRef]
21. Earle, B.H.; Madek, G.A. Minnesota v. Mille Lacs Band of Chippewa: 19th Century U.S. Treaty-Guaranteed Usufructuary Property Rights, the Foundation for 21st Century Indigenous Sovereignty. *Minn. J. Law Inequal.* **2015**, *33*, 143. [CrossRef]
22. Fetzer, W.W.; Farrell, C.J.; Jackson, J.R.; Rudstam, L.G. Year-class variation drives interactions between warm-water predators and yellow perch. *Can. J. Fish. Aquat. Sci.* **2016**, *73*, 1330–1341. [CrossRef]
23. Lake Bemidji—04-0130-02 Lake Water Quality. WRL Digital Asset Management. Available online: https://wrl.mnpals.net/islandora/object/WRLrepository%3A3570 (accessed on 20 July 2026).
24. Karami, A.; Golieskardi, A.; Choo, C.K.; Romano, N.; Ho, Y.B.; Salamatinia, B. A high-performance protocol for extraction of microplastics in fish. *Sci. Total Environ.* **2017**, *578*, 485–494. [CrossRef] [PubMed]
25. Egessa, R.; Nankabirwa, A.; Ocaya, H.; Pabire, W.G. Microplastic pollution in surface water of Lake Victoria. *Sci. Total Environ.* **2020**, *741*, 140201. [CrossRef] [PubMed]
26. Conowall, P.; Schreiner, K.M.; Marchand, J.; Minor, E.C.; Schoenebeck, C.W.; Maurer-Jones, M.A.; Hrabik, T.R. Variability in the drivers of microplastic consumption by fish across four lake ecosystems. *Front. Earth Sci.* **2024**, *12*, 1339822. [CrossRef]
27. Gad, A.K.; Toner, K.; Benfield, M.C.; Midway, S.R. Microplastics in mainstem Mississippi River fishes. *Front. Environ. Sci.* **2023**, *10*, 1065583. [CrossRef]
28. Watkins, L.; McGrattan, S.; Sullivan, P.J.; Walter, M.T. The effect of dams on river transport of microplastic pollution. *Sci. Total Environ.* **2019**, *664*, 834–840. [CrossRef] [PubMed]
29. Mason, S.A.; Garneau, D.; Sutton, R.; Chu, Y.; Ehmann, K.; Barnes, J.; Fink, P.; Papazissimos, D.; Rogers, D.L. Microplastic pollution is widely detected in US municipal wastewater treatment plant effluent. *Environ. Pollut.* **2016**, *218*, 1045–1054. [CrossRef] [PubMed]

30. Murphy, F.; Ewins, C.; Carbonnier, F.; Quinn, B. Wastewater treatment works (WwTW) as a source of microplastics in the aquatic environment. *Environ. Sci. Technol.* **2016**, *50*, 5800–5808. [CrossRef] [PubMed]
31. Horton, A.A.; Jürgens, M.D.; Lahive, E.; van Bodegom, P.M.; Vijver, M.G. The influence of exposure and physiology on microplastic ingestion by the freshwater fish *Rutilus rutilus* (roach) in the River Thames, UK. *Environ. Pollut.* **2018**, *236*, 188–194. [CrossRef] [PubMed]
32. Garcia, A.G.; Suárez, D.C.; Li, J.; Rotchell, J.M. A comparison of microplastic contamination in freshwater fish from natural and farmed sources. *Environ. Sci. Pollut. Res.* **2021**, *28*, 14488–14497. [CrossRef] [PubMed]
33. Collard, F.; Gasperi, J.; Gilbert, B.; Eppe, G.; Azimi, S.; Rocher, V.; Tassin, B. Anthropogenic particles in the stomach contents and liver of the freshwater fish *Squalius cephalus*. *Sci. Total Environ.* **2018**, *643*, 1257–1264. [CrossRef] [PubMed]
34. Galafassi, S.; Sabatino, R.; Sathicq, M.B.; Eckert, E.M.; Fontaneto, D.; Fontana, G.D.; Mossotti, R.; Corno, G.; Volta, P.; Di Cesare, A. Contribution of microplastic particles to the spread of resistances and pathogenic bacteria in treated wastewaters. *Water Res.* **2021**, *201*, 117368. [CrossRef] [PubMed]
35. Uurasjärvi, E.; Pääkkönen, M.; Setälä, O.; Koistinen, A.; Lehtiniemi, M. Microplastics accumulate to thin layers in the stratified Baltic Sea. *Environ. Pollut.* **2021**, *268*, 115700. [CrossRef] [PubMed]
36. Driscoll, S.C.; Glassic, H.C.; Guy, C.S.; Koel, T.M. Presence of microplastics in the food web of the largest high-elevation lake in North America. *Water* **2021**, *13*, 264. [CrossRef]