

Article

Presence of Microplastics in the Food Web of the Largest High-Elevation Lake in North America

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Abstract: Microplastics have been documented in aquatic and terrestrial ecosystems throughout the world. However, few studies have investigated microplastics in freshwater fish diets. In this study, water samples and three trophic levels of a freshwater food web were investigated for microplastic presence: amphipods (*Gammarus lacustris*), Yellowstone cutthroat trout (*Oncorhynchus clarkii bouvieri*), and lake trout (*Salvelinus namaycush*). Microplastics and other anthropogenic materials were documented in water samples, amphipods, and fish, then confirmed using FTIR (Fourier-transform infrared) and Raman spectroscopy. Our findings confirmed the presence of microplastics and other anthropogenic materials in three trophic levels of a freshwater food web in a high-elevation lake in a national park, which corroborates recent studies implicating the global distribution of microplastics. This study further illustrates the need for global action regarding the appropriate manufacturing, use, and disposal of plastics to minimize the effects of plastics on the environment.

Keywords: Yellowstone lake; microplastics; Yellowstone cutthroat trout; food web; freshwater; protected area



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1. Introduction

Anthropogenic materials infiltrate our environment at an alarming rate, including in some of the most remote locations on earth; from diets of freshwater fish in the Amazon [1] to waters of the Arctic region [2]. Microplastics, which are particles less than 5 mm in size, have been a focus in recent environmental pollution research. Microplastics come in many colors and shapes, sometimes originating from degrading plastic debris [3]. Concerns of rapid accumulation, atmospheric transportation, and harmful health implications associated with microplastics have led to an increase in research of this phenomenon. Microplastics pose a major risk to the environment, with bioaccumulation in aquatic ecosystems [4] that has been shown to transfer among trophic levels [5]. Exposure to microplastics has been shown to negatively affect physiology [6,7], feeding behavior [8], and fitness [9] in aquatic organisms.

Research on microplastic distribution and threats to freshwater environments have increased in recent years [10,11]. The abundance and prevalence of microplastics can vary based on land use and population density [12] and have been documented even in remote catchments [13,14]. The small size of microplastics allows for ingestion by organisms of many different trophic levels [15], from invertebrates to piscivores. Few studies have looked at remote freshwater systems; even fewer studies have investigated microplastic presence in freshwater fish or invertebrate diets [16].

Located in Yellowstone National Park, Wyoming, USA, Yellowstone Lake is considered to be a pristine environment situated within the most remote watershed in the

contiguous United States [17]. Yellowstone Lake contains the largest nonhybridized population of Yellowstone cutthroat trout (*Oncorhynchus clarkii bouvieri*), a keystone species for the Greater Yellowstone Ecosystem [18]. Identifying and understanding potential threats to Yellowstone Lake, including climate change, non-native species, and other anthropogenic impacts, is critical to the long-term functioning and management of this notable ecosystem.

With the prevalence of microplastics in freshwater systems elsewhere and documented threats to organism health, we aimed to determine whether microplastics were present in the Yellowstone Lake food web. This study addressed the following questions in Yellowstone Lake: (1) are microplastics present in the water, (2) are microplastics present in diets of amphipods and fishes, and (3) if microplastics are present, what are the material types?

2. Materials and Methods

Yellowstone National Park is one of the most popular national parks in the United States and has an average of over 4 million visitors annually [19]—most of which experience the park by motor vehicle. The largest body of water in Yellowstone National Park is Yellowstone Lake, with a surface area of 341 km², an average depth of 43 m and maximum depth of 199 m [20]; strong prevailing winds from the southwest (247°) cause surface currents and upwelling throughout the lake [21] (Figure 1). Yellowstone Lake is also the largest freshwater lake above 2000 m in North America [22]. Areas of relatively high public use are congregated along the western shores, and 53 km of road borders the north and west shorelines of the lake. Yellowstone Lake also has recreational fisheries for lake trout (*Salvelinus namaycush*) and Yellowstone cutthroat trout, an annual gillnetting assessment program, and a suppression program that uses 10,000 km of gillnet per year to suppress non-native lake trout [23]. Lake trout occupy the highest trophic position in Yellowstone Lake and consume mainly Yellowstone cutthroat trout and amphipods. Yellowstone cutthroat trout consume mainly amphipods and zooplankton [24,25].

All samples (water, amphipods, and fish) were collected during August 2019. Water samples were collected from four historical zooplankton sampling locations: West Thumb, South Arm, Southeast Arm, and the water southeast of Stevenson Island [26] (Figure 1). Sampling locations were selected for standardization in anticipation of future sampling occasions regarding the monitoring of microplastics. Additionally, the sites have unique environmental characteristics that could influence microplastic presence or concentration: e.g., proximity to roads (West Thumb and Stevenson Island), proximity to the main tributary (South Arm), and remote or wilderness-adjacent (South Arm and Southeast Arm). Water was filtered using a plankton net (Wildco® mesh size 80 µm, diameter 20.32 cm, net material Nitex® nylon), with one 5-meter vertical (“water vertical”) and one 5-meter horizontal tow (“water surface”) conducted at each location. Each sampling event filtered 162 liters of water for a total of 4 vertical samples and 4 surface samples. Horizontal tows were sampled at 5-meter lengths as the distance allowed for >100 liters of water to be sampled while also providing a short enough distance to safely sample from the boat while anchored. To standardize the volume of water sampled, vertical tows were sampled at the same increment of 5 meters. Samples were stored in glass jars and frozen until analyzed for microplastics.

Amphipods (*Gammarus lacustris*) were sampled from Carrington Island (Figure 1) using a fry trap (made of steel cone and PVC capture tube); 32 were sacrificed and frozen in glass jars until dissection. Five lake trout and five Yellowstone cutthroat trout were collected by gillnetting (gillnets made of monofilament line), adjacent to Stevenson Island, as part of the lake-wide lake trout suppression program (see [23] for detailed methods on the suppression program). Whole stomachs were extracted from fish, immediately transferred to glass jars, and frozen until dissection. The methods used for storage of stomach samples were selected to prevent contamination in the field; whole stomachs were “sealed” from outside contamination until lanced in the laboratory, no plastic tools or jars were used in the field, and no ethanol was used to prevent possible contamination from a foreign liquid.

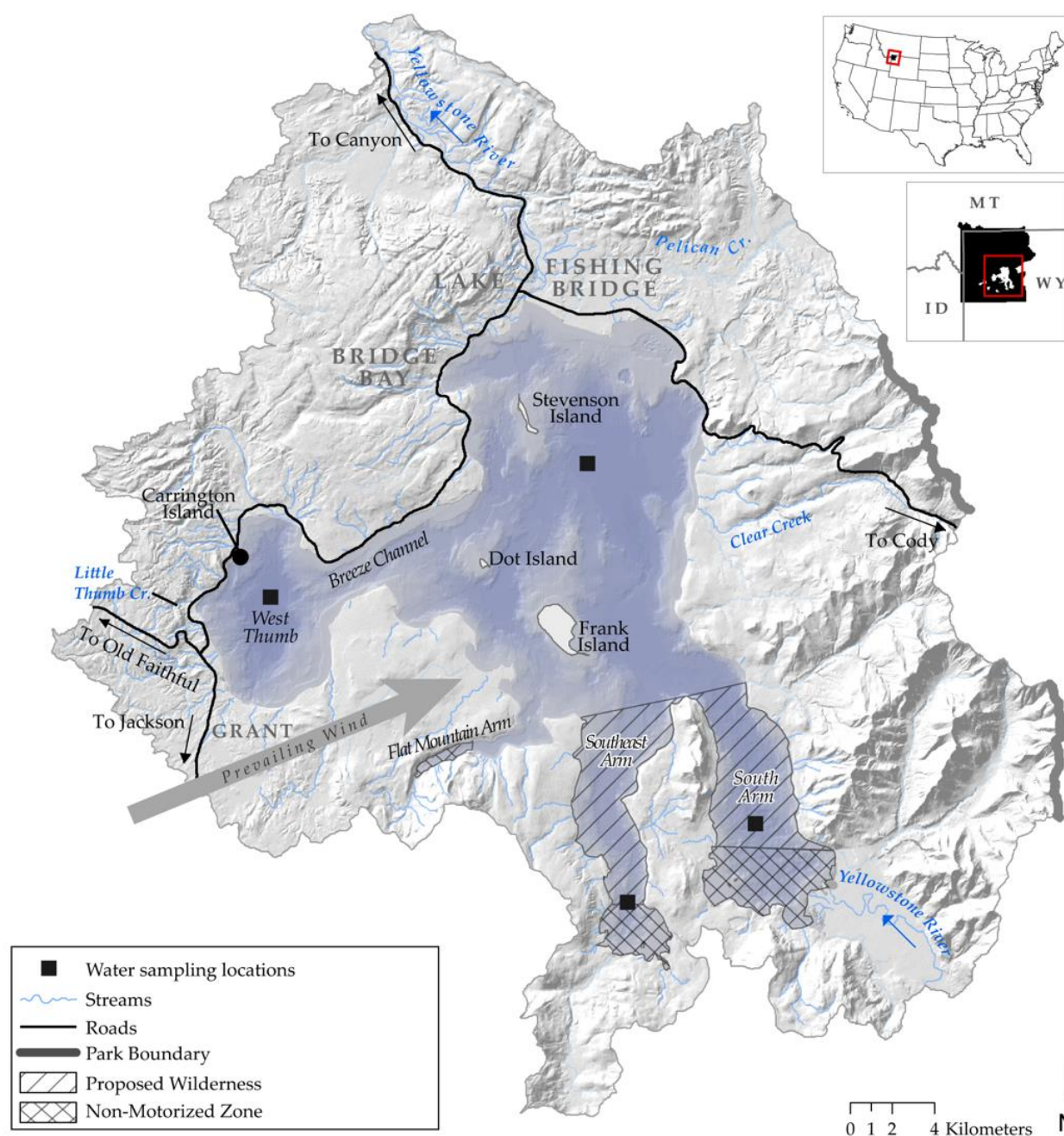


Figure 1. Yellowstone Lake within Yellowstone National Park in northwestern Wyoming, USA, with four water sampling locations indicated (West Thumb, southeast of Stevenson Island, South Arm, and Southeast Arm) and Carrington Island, where amphipod samples were collected.

All non-organic materials were removed manually from water, amphipod, and fish samples using a microscope (LEICA S8APO) and dissection tools. Water samples were visually inspected and non-organic materials manually extracted, while amphipod digestive tracts and fish stomachs were individually dissected and visually inspected to identify non-organic materials. Dissection, visual inspection, and isolation of non-organic materials from samples were completed in a clean space created by covering a wood frame with cotton material and filtering the air. The clean space air was filtered using a 3M Filtrete Elite Room purifier with true HEPA filters. The air purifier was operated for a minimum of 14 days prior to use of the clean space and during all identification and dissection events. Only glass or metal tools were used within the clean space, and only cotton clothing was

worn to prevent plastic contamination. Furthermore, any tool used in the clean space was rinsed three times with ultrapure water prior to use and in between samples. A control petri dish was placed in the clean space during dissection or identification events; if a particle was found in the petri dish during the event, the color of the particle was noted, and particles found in the sample of the same color were excluded from results and further analysis. All white fibers were rejected from identification due to possible cross-contamination of white cotton materials used in the construction of the clean space and the white PVC present in the fry traps; all clear fibers were rejected from identification due to possible cross-contamination from the zooplankton net.

Fourier-transform infrared (FTIR) and Raman spectroscopy have been used in numerous studies (e.g., [27,28]) to identify anthropogenic materials. We selected 10 particles from water, amphipods, and fish samples to be identified using FTIR spectroscopy at Materials Research Laboratories in Struthers, Ohio. We selected particles based on color and sample type; that is, we wanted to investigate if different particle colors represented different materials, and also chose to submit to the FTIR laboratory at least one particle from each sample type. Raman spectroscopy was used for samples that were too small to be identified using FTIR. We did not analyze all particles found due to monetary constraints.

3. Results

Each sample type had at least one non-organic particle present. All surface tows had at least three non-organic particles, and three of four vertical tows had at least two non-organic particles present (Tables 1 and 2). The number of particles in water samples was greater than the number of particles in biota samples; surface water samples had a higher number of particles than the vertical water samples. Of the 32 amphipods sampled, only one contained an identifiable non-organic particle (Tables 1 and 2). Two lake trout diets had three particles in total, and one Yellowstone cutthroat trout diet contained three particles. Of the 50 samples among sample type and locations, 22% of samples contained non-organic particles.

Table 1. Number of samples, and samples with extracted particles by sample type and location, collected during 2019 in Yellowstone Lake, Yellowstone National Park, Wyoming, USA. For water and amphipod sampling locations refer to Figure 1.

Sample Type	Locations	Number of Samples	Number of Samples with Extracted Particles
Water surface	Stevenson Island, West Thumb, South Arm, Southeast Arm	4	4
Water vertical	Stevenson Island, West Thumb, South Arm, Southeast Arm	4	3
Amphipod	Carrington Island	32	1
Lake trout	Adjacent to Stevenson Island	5	2
Yellowstone cutthroat trout	Adjacent to Stevenson Island	5	1

Table 2. Color and number of particles sampled by sample type and location collected during 2019 in Yellowstone Lake, Yellowstone National Park, Wyoming, USA. Colors with an asterisk (*) denote the microplastic was sent for Fourier-transform infrared (FTIR) or Raman spectroscopy identification.

Sample Type	Location	Number of Particles in Sample	Color of Particles (Count)
Water surface	Stevenson Island	11	Black (2), blue (2), red (3), light blue (1), green/blue * (1), brown (2)
Water surface	West Thumb	7	White (2), black (1), green (2), blue * (2)
Water surface	South Arm	11	Red * (2), blue * (2), black (2), dark blue (1), black/grey (1), white/grey (1), red (1), green (1)
Water surface	Southeast Arm	3	Black/grey (1), tan (1), light blue (1)
Water vertical	West Thumb	5	Black (2), blue (3), green (1)
Water vertical	South Arm	3	Light blue (1), red (1), green (1)
Water vertical	Southeast Arm	2	Blue * (1), grey (1)
Amphipod	Carrington Island	1	Green/blue * (1)
Lake trout	Adjacent to Stevenson Island	2	Black * (1), blue (1)
Lake trout	Adjacent to Stevenson Island	1	Blue (1)
Yellowstone cutthroat trout	Adjacent to Stevenson Island	3	Blue (3)

The color of each particle found was noted during inspection to determine if a specific color was prevalent in Yellowstone Lake water, if organisms appeared to consume a specific color, or if specific colors were identified as the same materials during FTIR or Raman spectroscopy. Thirteen different colors were present in water samples (Table 2). Fish and amphipod diets contained only three particle colors; blue, blue/green, and black (Table 2).

Ten samples were identified using either FTIR or Raman spectroscopy to determine whether the non-organic particle was plastic-based. Four samples were identified as a plastic or rubber material, two in water samples, one in a fish diet, and another in the amphipod diet (Table 3). The other particles were either a glass fiber or cellulosic material (Table 3). The width of particles averaged 0.03 mm, lengths varied from 0.62 mm to 2.46 mm; one sample was more spherical than the other particles and had a diameter of 0.51 mm (Table 3).

Table 3. Material identification and measurements of 10 particles from Yellowstone Lake, Yellowstone National Park, Wyoming, USA sampled in 2019. Particles were analyzed using either Fourier-transform infrared (FTIR) or Raman spectroscopy. An asterisk (*) denotes a plastic- or rubber-similar material.

Sample Type	Location	Material Identification	Particle Measurements (mm)
Water surface	Stevenson Island	Glass fiber	0.62 (length), 0.03 (width)
Water surface	Stevenson Island	Polyvinyl alcohol material (PVA) *	NA
Water surface	West Thumb	Isotactic polypropylene *	0.51 (diameter)
Water surface	West Thumb	Glass fiber	0.48 (length), 0.03 (width)
Water surface	South Arm	Glass fiber	2.46 (length)
Water surface	South Arm	Cellulosic material	0.974 (length)
Water vertical	Southeast Arm	Cellulosic material	1.52 (length), 0.03 (width)

Table 3. Cont.

Amphipod	Carrington Island	Tyrin 4211 (chlorinated polyethylene) *	0.68 (length), 0.03 (width)
Lake trout	Adjacent to Stevenson Island	Polysulfide rubber *	1.95 (length), 0.03 (width)
Lake trout	Adjacent to Stevenson Island	Cellulose fiber	1.33 (length), 0.02 (width)

4. Discussion

We found microplastics in the water and organisms of Yellowstone Lake, including the Yellowstone cutthroat trout, a species of conservation concern. Little research has investigated the presence of microplastics in remote freshwater systems, and even fewer studies have investigated microplastic presence in freshwater fish or invertebrate diets [16]. Surface water samples had the greatest concentrations of microplastics in our study; surface water has been noted in reviews of microplastics pollution in freshwater environments to contain higher concentrations of microplastics than water column samples [29]. Additionally, we found microplastics in three trophic levels of the Yellowstone Lake food web, including amphipods, Yellowstone cutthroat trout, and lake trout, indicating that particles may be passed through trophic levels by consumption. One-third of our fish samples had microplastic particles in the stomach, which is a similar ratio of fish with microplastics for studies conducted on freshwater fishes [30]. Our study is unique in that investigations into microplastic pollution in trophic webs of other remote lake ecosystems did not detect microplastics in the food web [13].

Our field and laboratory sampling events were extremely conservative, thus, we are confident that the microplastics identified in our samples are not a relic of contamination, given the clean space and the strict air and tool-cleaning protocol. The microplastics identified in our study to be plastic- or rubber-like particles included polyvinyl alcohol material (PVA), isotactic polypropylene, polysulfide rubber, and chlorinated polyethylene (Tyrin 4211). A point source of microplastic deposition is likely not identifiable in Yellowstone National Park, as atmospheric transport and deposition can occur, contributing to microplastic accumulation at remote locations [14,31]. However, it is highly plausible that some of the plastic observed in this study is a byproduct from the 4 million people who visit Yellowstone National Park annually.

Though we identified microplastics in Yellowstone Lake and within the food web, our sample size was limited, and we cannot determine the effect of microplastics on individual organisms or the ecosystem. Research has shown amphipods (*Gammarus duebeni*) breakdown microplastics into smaller particles leading to rapid fragmentation [32], and that microplastics may compromise growth and reproduction [33]. Some fishes have been shown to successfully pass spherical microplastics within 24 hours of ingestion and have no signs of microplastic translocation after a two-week exposure period [11]. However, variation in response to microplastic exposure should be considered for each species [34]. Microplastics can contain surface chemicals or accumulate heavy metals [35] that can result in acute mortality [36], endocrine disruption, or reduced predatory behavior [37]. Research conducted to further understand how microplastics in Yellowstone Lake may affect lake organisms, and Yellowstone cutthroat trout, in particular, would be valuable.

This study provides the first report of microplastics in Yellowstone National Park. This initial evidence of microplastic prevalence in the water and organisms of Yellowstone Lake will assist the US National Park Service with the development of further research or potential mitigation plans. Microplastics can become concentrated in our waterways [12,38,39] and transcend trophic levels in aquatic environments [5], as demonstrated in our study. As global plastics production continues to rise, so does the need to identify and quantify the prevalence of microplastics, especially in freshwater ecosystems and freshwater fishes in highly protected, otherwise pristine environments.

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