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# Microplastic Monitoring in Richardsonius balteatus from Ross Lake, WA

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## Abstract

Recent work has shown that microplastics are present in glaciers. This is a concern for water bodies such as Ross Lake (WA) where glacier runoff may transport the microplastics into the watershed and be available to aquatic organisms. Currently there is no evaluation of how organism storage methods may impact microplastic recovery. In this study microplastic type and color in whole body *Richardsonius balteatus* (redside shiners) from Ross Lake were counted. Fish were collected from Ross Lake on July 6th, 2019. Approximately half of the samples were stored in ethanol and the remainder on ice. Characteristics including color and type (fragment, pellet, fiber, film, and foam) of recovered microplastics were compared between the two groups. To date, 43 fish stored in ethanol and 53 stored on ice have been analyzed. The average wet mass of ethanol-stored and ice-stored was 0.4329 and 0.3590g. A total of 478 total microplastics were identified in fish stored in ethanol (11.4 per fish). All the samples in ethanol had fibers; black fibers were the most prevalent at 39.11% of all fibers. Fragments, films, and foams were in 1% of the samples, and no pellets were observed. A total of 283 total microplastics were found in the samples stored on ice (5.3 per fish). Fibers were found in 97% of the fish; black fibers account for 29.93% of all fibers. Fragments and films were in 3.2% of plastics in all samples stored on-ice, with no pellets or foams observed.

## Introduction

Ross Lake is a large reservoir, 35.5 km long and 1.6 average km wide, in the Skagit River watershed. This reservoir is in the North Cascades National Park and is commonly used for fishing and boating activities. The Ross Lake Resort, a small resort accessible by boat, is located on the southern tip and campsites around the lake are only accessible by hiking trails and boat. The limited access means local sources of plastics are primarily fishing line, nets, and synthetic clothing. In addition to local sources, microplastics have also been shown to travel large distances to remote mountains where they can enter freshwater systems (Allen et al., 2019). Microplastics are an emerging contaminant that has become a concern among the public and government authorities (Li et al. 2018). They have been studied mainly in marine systems and only recently in freshwater systems; data on microplastics in freshwater fish is very limited (Wagner et al., 2014). The redside shiner is an introduced species to Ross Lake with previously measured length range from 16 to 111 mm (Welsh 2012). Our work describes the extent of microplastic contamination in redside shiners in a high alpine lake in a National Park (Figure 1).


Figure 1- Ross Lake reservoir in the North Cascades National Park.

## Methods

- Samples were collected from the same drainage in 2 separate batches with a beach seine.
- Ethanol samples were stored in Whirl-pak bags with 95% ethanol
- Frozen samples were wrapped in aluminum foil and immediately put on ice
- Samples were rinsed, weighed, and measured prior to digestions (Thiele at al., 2019)
- Samples were digested in 10% potassium hydroxide (KOH) and dried at 40°C for 48 hrs, then neutralized with citric acid to a pH of 6.8-7.5 and vacuum filtered through a 1.2 µm glass filter pad
- Microplastics were identified at 40X magnification with a dissecting microscope and archived into individual vials
- Microplastics were categorized according to the MERI (2017) identification guidelines as fibers, fragments, films, foam, pellets. Color was also recorded.
- For each sample a microscope blank was used to account for contamination in the lab, the filter pad was uncovered every time the sample was out. A process blank was also produced for every 10th sample.

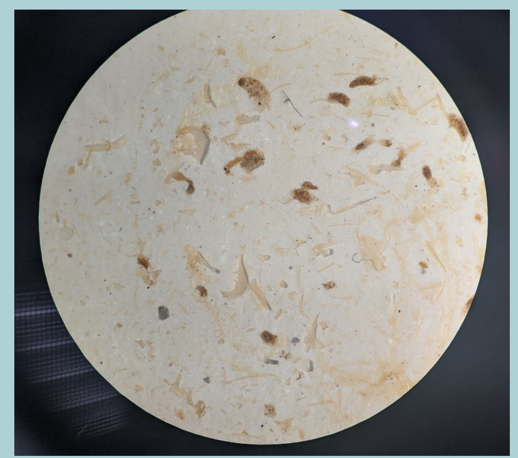

Figure 2- Microplastics retrieved from Redside shiners.

## Results & Discussion

Microplastics were found in all but one of our samples. Plastics found consisted of fibers, films and fragments. For the on-ice samples, fibers made up 96.82 percent of all plastics found, while fragments made up 1.76 percent, and films 1.41 percent. For the ethanol samples, fibers made up 99.16 percent of all plastics found, while fragments made up 0.63 percent, and films were 0 percent.

For both the samples stored on ice and in ethanol the three main fiber colors were black, transparent, and blue. In the ice samples these comprised 83.58 percent of all the fibers. In the ethanol samples these comprised 87.31 percent of all the fibers (Figures 3 and 4).

The fish stored on ice have more fibers per kilogram dry weight fish than those stored in ethanol. The majority of ice stored fish have an average of 0.12 and a median of 0.11 fibers per milligram while those stored in ethanol have an average of 0.058 and a median of 0.030 fibers per milligram (Figure 4).

There was a non-significant trend between size (based on dry weight) and number of fibers per individual for both the ethanol stored fish and the on-ice fish. (Figures 5 and 6). The $R^2$ value for both the ethanol and on-ice fish is significantly lower than 1, showing there is not a correlation between fibers/individual and dry weight.

The size distribution of fish was different between the two fish populations despite being collected from the same population of fish within about 10 minutes of each other. When the total number of fibers per individual were compared, there were more fibers in the ethanol stored fish than in the fish stored on-ice. We are considering how comparable the two dry weights of fish are and whether there is evidence of regurgitation in the ethanol stored fish.

Microscope blanks (MB) were used in order to account for contamination during the counting process. For the on-ice samples, the maximum amount of fibers found on the MBs was 5, while the minimum was 0. The average amount of fibers found on the MBs for the on-ice samples was 0.5 per sample. For the ethanol samples, the maximum number of fibers found on the MBs was 4, while the minimum was 0. On average 0.30 fibers were found on the MBs for the ethanol samples.

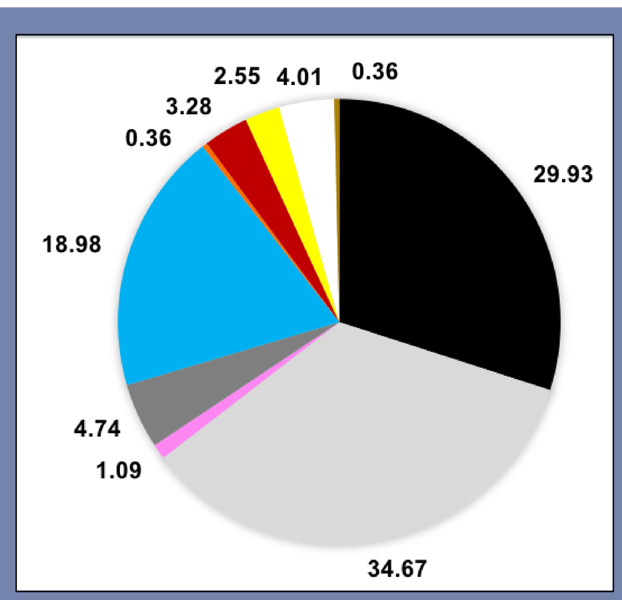


Figure 3- Distribution of the color of all fibers identified in Redside shiners stored on ice.

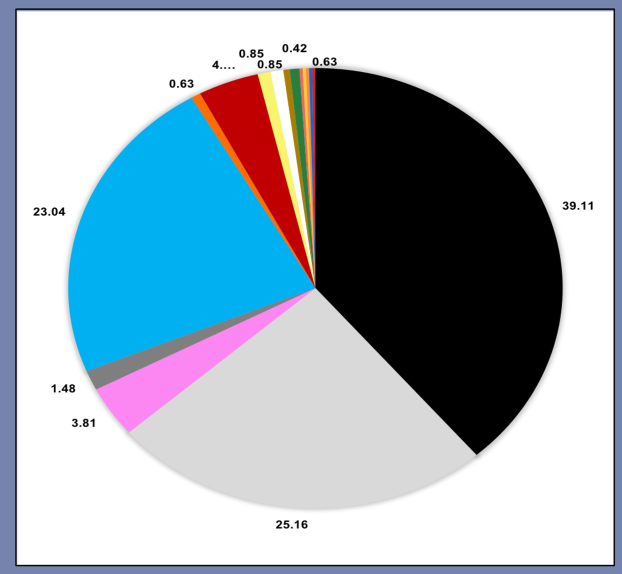


Figure 4- Distribution of the color of all fibers identified in Redside shiners stored in ethanol.

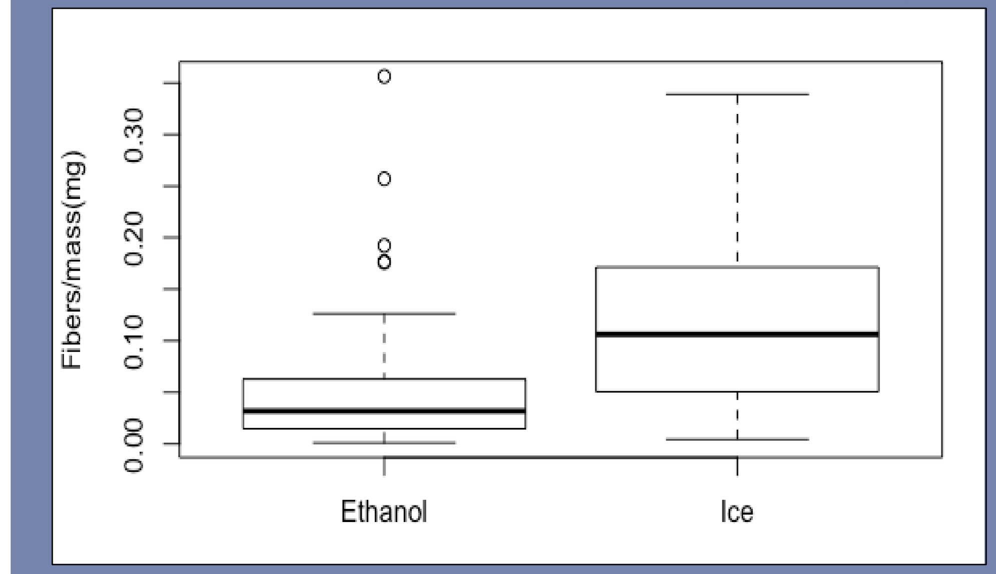


Figure 4- This figure shows that the amount of fibers/mass (mg) is significantly different between the ethanol and ice samples. The ice samples have a higher median fibers/mass(mg) then the ethanol samples.

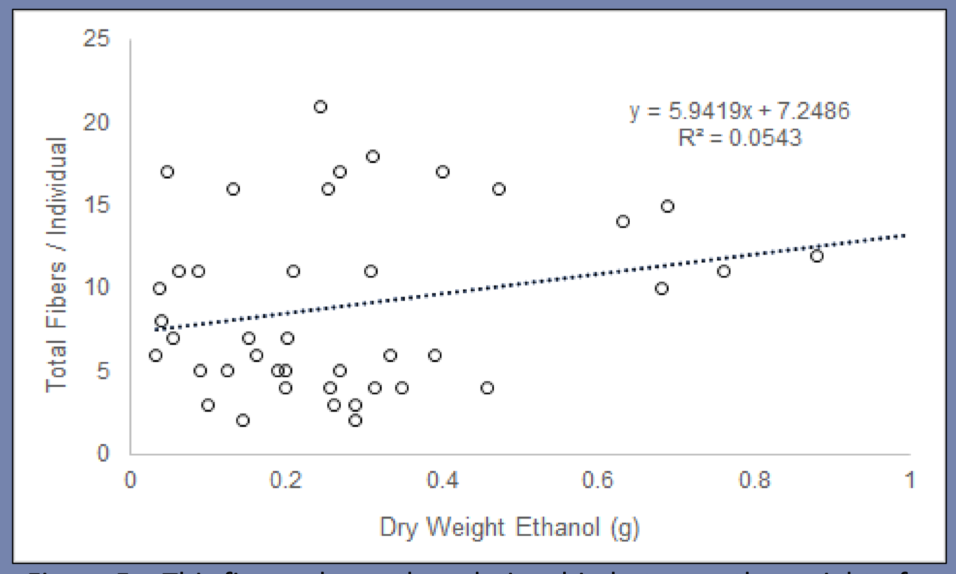


Figure 5 – This figure shows the relationship between the weight of fish stored in ethanol and the total fibers found per individual fish. The $R^2$ value being 0.0543 shows there is not a strong correlation between weight and fibers per individual

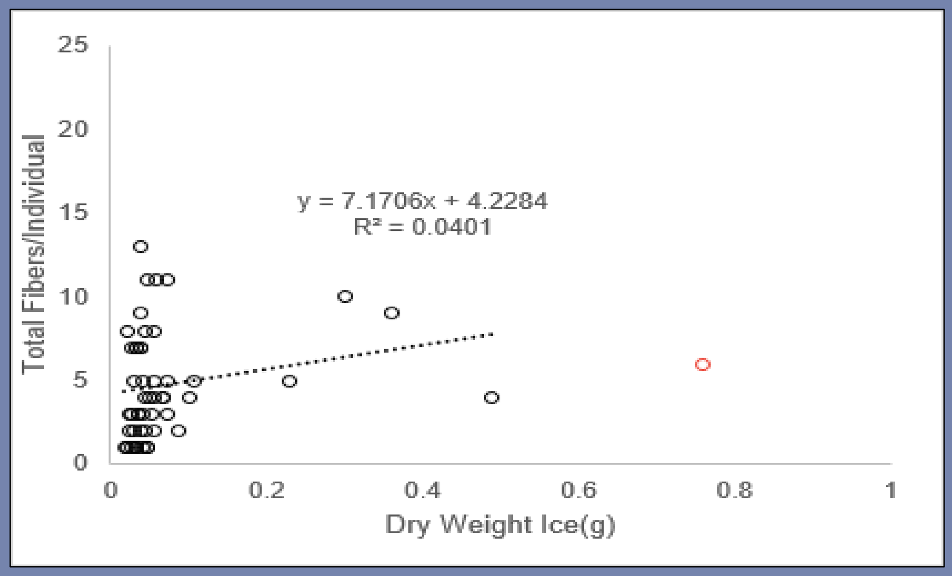


Figure 6 – This figure shows the relationship between the weight of fish stored on-ice and the total fibers found per individual fish. The $R^2$ value being 0.0401 shows there is not a strong correlation between weight and fibers per individual. The 0.76 g fish was not included in the trendline.

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