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4-23-2020

## Assessing the presence and concentrations of microplastics in the gizzards of Virginia waterfowl

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### Recommended Citation

Bustamante, Thomas, "Assessing the presence and concentrations of microplastics in the gizzards of Virginia waterfowl" (2020). *Research and Creativity Symposium*. 17.

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## Introduction

Microplastics have become a ubiquitous and concerning water pollutant in recent years. Research has shown they can damage tissue and negatively impact growth. Their high surface area makes them capable of transporting plasticizers and organic pollutants, and their small size allows them to move between trophic levels. Therefore, they could have substantial ecological impacts now and in the future. While microplastics have been extensively studied in marine environments, freshwater microplastic studies are in their infancy. Furthermore, methods for microplastic quantification in general need of further refinement and standardization. To investigate microplastic consumption by freshwater waterfowl, as well as aid in the standardization of quantification methods, we assessed microplastic concentrations in the gizzards of Canada Goose (*Branta canadensis*), Ring-necked Duck (*Aythya collaris*), Long-tailed Duck (*Clangula hyemalis*), Bufflehead Duck (*Bucephala albeola*), and Mallard (*Anas platyrhynchos*), hunted in the Piedmont and Coastal Plain of Virginia

## Lab Methods

- Waterfowl were hunted in January 2019 in the Piedmont and Coastal Plain of Virginia
- Gizzards, intestines, and tissue samples were collected and frozen
- After thawing for 24 hours, gizzards were bisected and three portions of their contents were removed with a metal scoop
- Portions were placed in individual glass beakers covered with aluminum foil and dried at 105°C for 12 hours
- Portions had their masses recorded, then underwent density separation in 100 mL of 1.2 g/mL NaCl solution for 30 minutes
- Samples were left to sit following separation for 30 minutes
- The top layer was removed and vacuum filtered using Fisherbrand P4 grade filter paper
- The filter paper was allowed to dry overnight and was then visually inspected under a dissecting and compound microscope
- Infrared Spectroscopy was tested on one microfiber to determine its identity
- Blanks were run to determine contamination levels

## Quantification Methods

- Every plastic was categorized by type and shape, was measured, and had its color identified
- Each sample's concentration in plastics per gram was calculated
- The frequency of occurrence was calculated among all samples

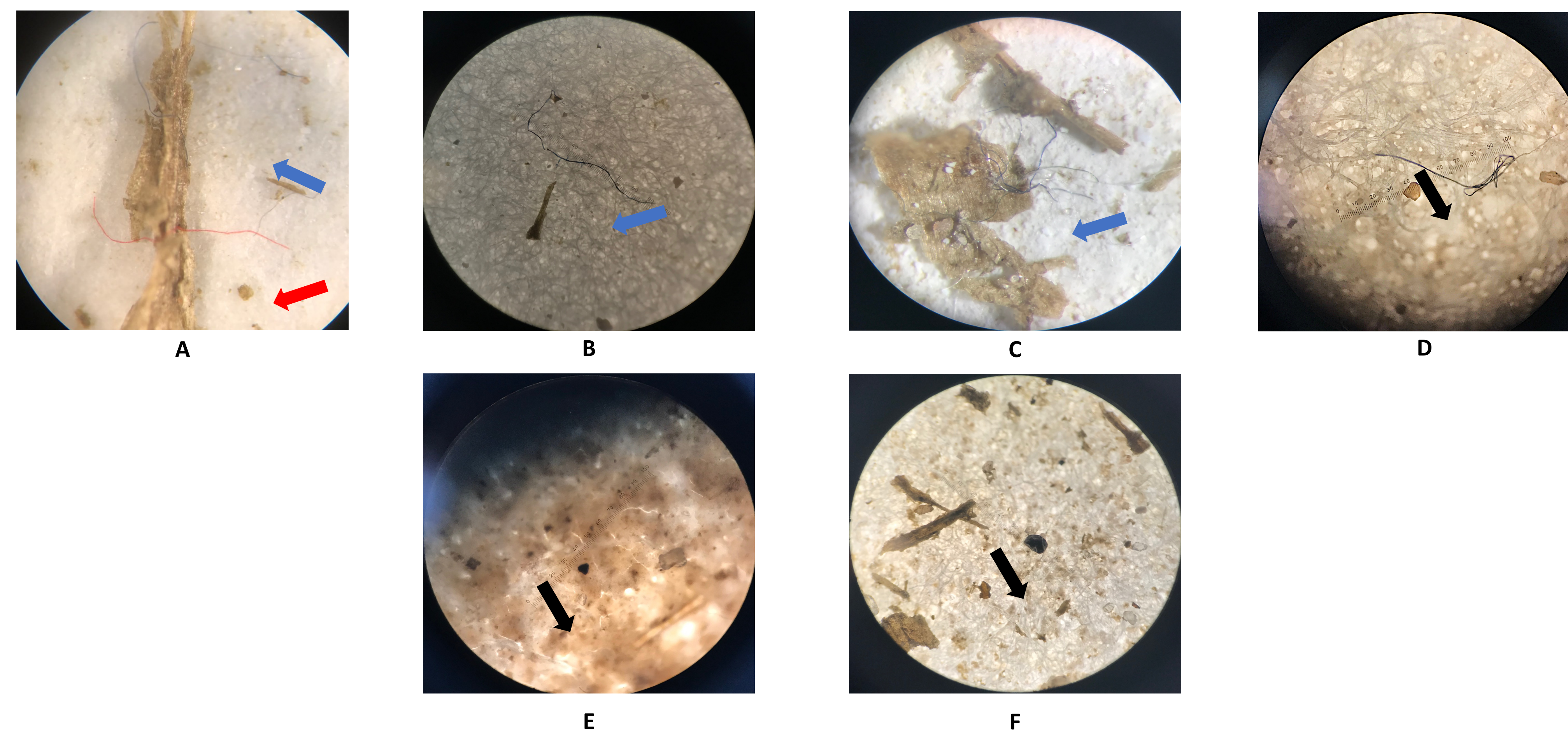


Figure 1. Photos of microplastic fibers found in the gizzards of waterfowl under a compound microscope (B and D) and dissecting microscope (A and C) as well as some of the black fragments (E and F)

Bird #	Species	County	Sex	# Plastic Fibers	Mean (± SD) Concentration (fibers/g gizzard contents)
1	Canada Goose	Westmoreland	Male	78	8.02 (10.37)
2	Canada Goose	Westmoreland	Female	1	0.14 (0.24)
3	Canada Goose	Westmoreland	Female	3	0.19 (0.33)
4	Canada Goose	Westmoreland	Female	0	0 (0)
5	Canada Goose	Westmoreland	Male	1	0.08 (0.13)
6	Canada Goose	Westmoreland	Male	6	0.50 (0.44)
7	Longtail Duck	Westmoreland	Male	2	0.69 (0.62)
8	Mallard	Culpeper	Male	18	11.49 (7.01)
9	Ringneck Duck	Culpeper	Male	3	1.02 (0.91)
10	Bufflehead Duck	Culpeper	Female	3	3.33 (5.77)

Table 1. General information regarding plastic loads found in each individual studied up to this point with contamination taken into account. Plastic concentrations had extremely high variation within and between samples. The highest raw number of plastic fibers was found in a Canada Goose (n=78), but the highest concentration was found in a Mallard (11.49 fibers/g).

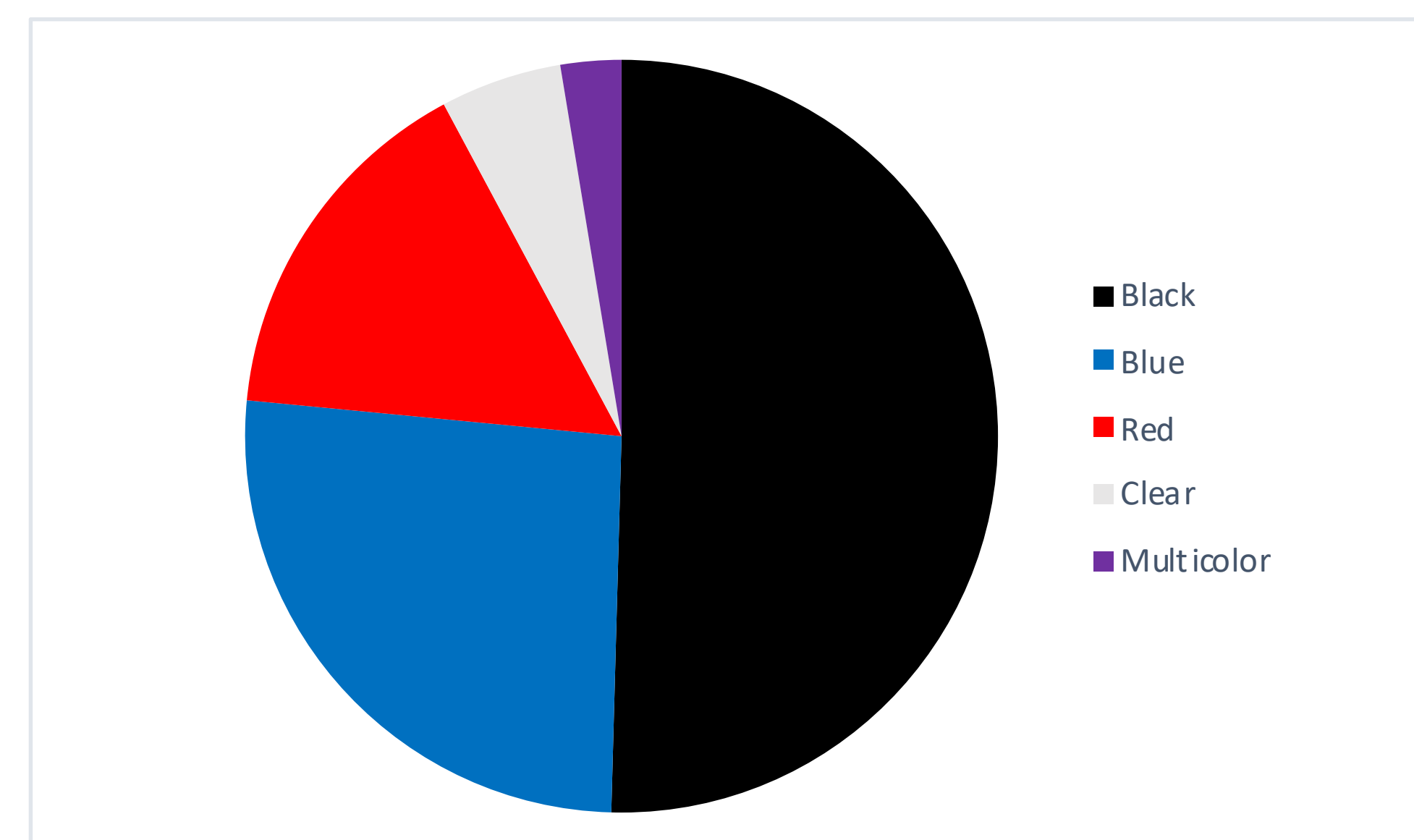


Figure 2. Pie chart showing the percentage of each color of fiber. The most abundant color is black (50%) followed by blue (26%)

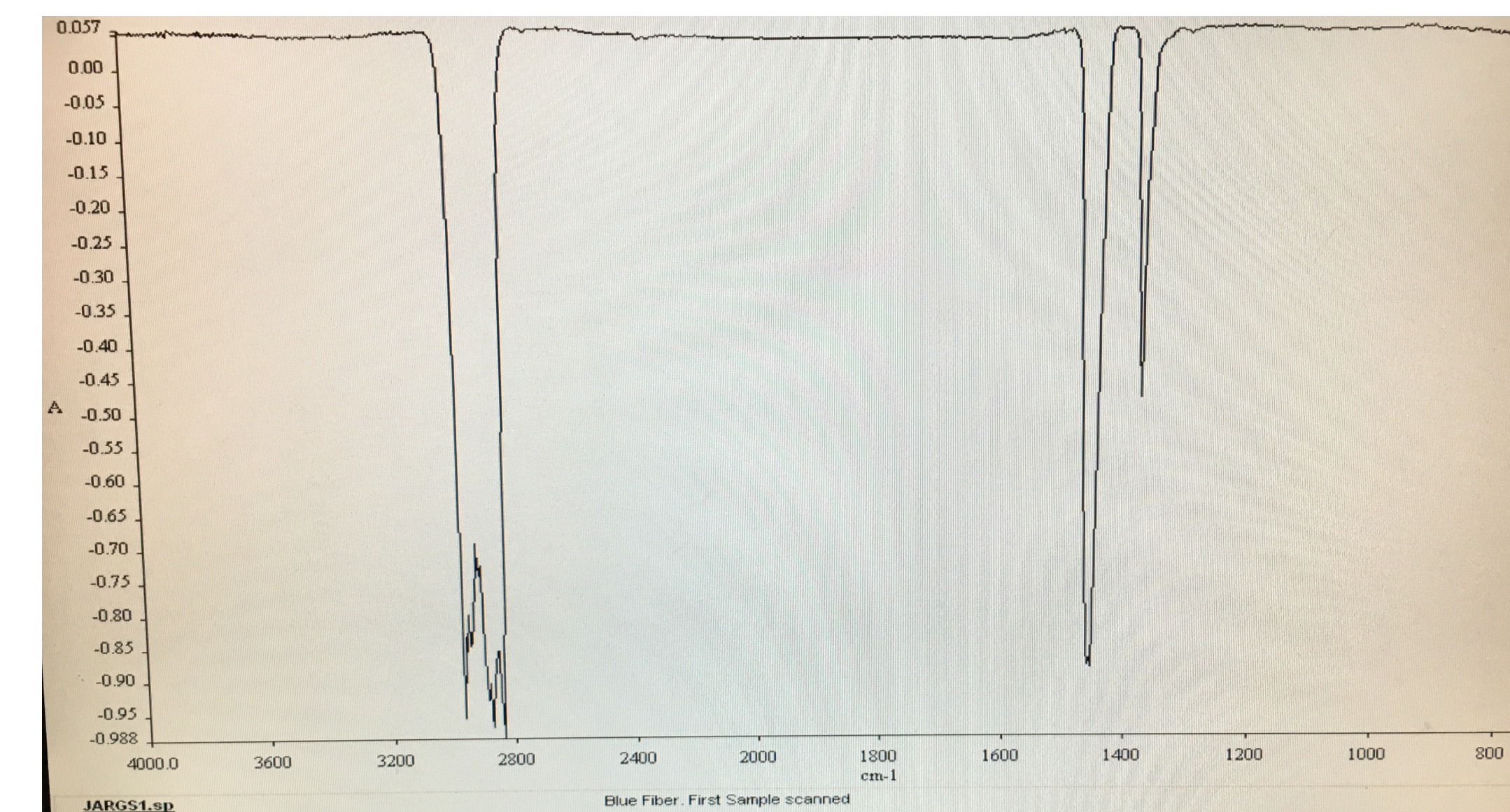


Figure 3. Results of the Infrared spectroscopy done on one of the blue fibers that came from a Canada Goose. The peaks show that the fiber is pure polyethylene.

## Results

- We found 115 plastic fibers in 7 samples (Fig. 1)
- We found many black fragments, it is uncertain if they are plastic (Fig. 1)
- Fiber size ranged from 25-6250 μm (0.0025- 0.625 cm)
- Most birds sampled contained microplastics to some degree (n=10, frequency of occurrence= 90%) all plastics were secondary
- Plastic concentrations had extremely high variation (2.55±4 SD, Table 1)
- The highest concentration came from a Mallard located in Culpeper County (11.49 fibers/g, Table 1)
- The most abundant color was black (50%, Fig. 2)
- IR spectroscopy was done on one sample; was found to be pure polyethylene (Fig. 3)

## Discussion

Almost every bird we sampled contained microplastics. These results are more drastic than other studies assessing microplastics in freshwater birds. Concentrations had high variation, which may be attributed to species, location, or recency of consumption. The high concentrations are likely attributed to the level of urbanization and high human populations in our sites. Microplastic fibers were present in gizzard contents. This shows that waterfowl are consuming microplastics and retaining them in their systems.

All plastics were secondary fibers, meaning they resulted from the breakdown of larger plastics. While an exact source cannot be pinpointed, common sources of fibers include synthetic clothing, rope, and netting. The evidence showing that one fiber is polyethylene may rule out clothing, since these are typically composed of polymers like polyester.

## Future Studies

In the future we plan to refine our methodology by utilizing chemical digestion and a more efficient chemical analysis. We will continue to assess gizzards and compare concentrations between locations and species. We also plan to assess intestinal samples for microplastics in the lumen and intestinal wall.

## Acknowledgments

We thank the University of Mary Washington as well as Larry G. Valade for funding this research. We also thank Adrienne Matuté for aiding in plastic counting

## References

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