Trophic Transfer of Microplastic in Invertebrates of Lake Champlain

Student Researchers: Riley S. Masterson, and James Stewart
Faculty Mentors: Danielle Garneau, Ph.D, and Luke Myers
Center for Earth and Environmental Science
SUNY Plattsburgh, Plattsburgh, NY 12901

Introduction

- **Microplastics** (< 5mm) are common pollutants in freshwater systems. Concern is mounting over their potential to harm aquatic organisms.
  - Wastewater treatment plant effluent, stormwater, marine debris, and the breakdown of macroplastics are known sources.
  - Microplastics that can be ingested poses various physicochemical properties that are categorized as fibers, fragments, films, foams, pellets, and nurdles.

- Freshwater organisms are part of complex food webs and forage on a wide diversity of food types, utilizing a variety of different feeding strategies.
  - ➢ Uptake of microplastic occurs when mistaken as food, and can be further conveyed to higher trophic levels (Setälä et al. 2016).
  - ➢ Recent studies suggest *Gammarus* spp. and mysids, both sediment or algae surface feeders, consume the highest microbead abundance of species under investigation (Setälä et al. 2016).

Aquatic Macroinvertebrates

Feeding Habit	Behavior	Taxa	Habitat
Collectors & Gatherers	Physically gather food	*Eurylophella* sp. *Hyalella azteca* *Leucrocuta* *Leptophlebia* *Stenacron*	Lake bottom depositional areas
Scrapers	Have modified mouthparts to scrape food off suitable substrates	*Caenis latipennis* *Phryganea* sp. *Stenonema femoratum* *Neophylax fuscus* *Psephenus* sp.	Shallow areas with sunlight and suitable substrate for algae growth
Herbivores	Have mouthparts adapted for feeding on live plants	*Haliplus* sp. Corixidae	Areas with aquatic macrophytes
Filter Feeders	Spin capture nets, or active filter feeding	*Polycentropus* sp. *Cheumatopsyche* sp. *Hydropsyche* *Hexagenia limbata* *Mysis diluviana* *Dreissena polymorpha*	Wave swept shorelines, or depositional areas
Shredders	Have modified mouthparts to bite, cut and shred coarse organic matter	*Nectopsyche albida* *Pycnopsyche*	Depositional areas where organic matter can accumulate
Predators	Have unique body parts for capturing or ambushing prey species	Gyrinidae Aeshnidae *Belostoma* sp. *Mesovelia* sp. *Ranatra* sp. *Neoplea striola*	All habitat types (mainly shallow)

Field Methodology

- Specimens were collected over a wide range of sites across Lake Champlain (**Fig. 1.**).

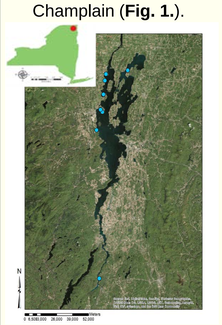

Fig. 1. Lake Champlain and macroinvertebrate sampling sites.

Sources of organisms:

- All of the aquatic invertebrate analyzed were obtain from the invertebrate collection at the Lake Champlain Research Institute (LCRI).
- Collection techniques utilized included hand picking, kick netting, ponar grabs, trawl netting, and vertical net tows.
- Specimens were identified to the lowest taxonomic resolution when possible using recent keys and verified material while paying close attention to changes in taxonomic nomenclature.

Laboratory Methodology

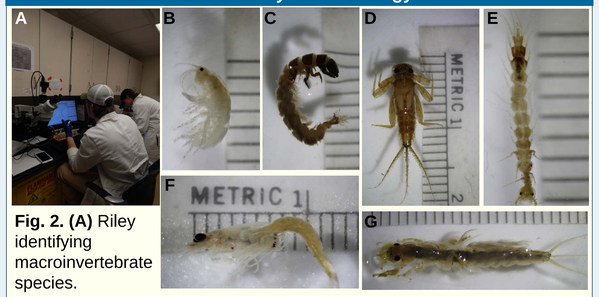

Fig. 2. (A) Riley identifying macroinvertebrate species.

(B) *Hyalella azteca*, **(C)** *Cheumatopsyche* sp., **(D)** *Stenonema femoratum* **(E)** Gyrinidae (larvae), **(F)** *Mysis diluviana*, **(G)** *Hexagenia limbata*.

- Samples were placed in a drying oven at 55ºC for 72 hours (Fig. 3A). This assured all moisture was removed in order to collect an accurate dry weight.
- Each sample vial was placed inside tin foil cups, and weighed on a microbalance (Fig. 3B).
- 30 mL of 4M KOH was added to sample before heating to 60ºC while stirring to initiate tissue breakdown (Fig. 3C).
- KOH dosed samples were removed from heat and 5 mL of 30% H_2O_2 was added and stirred at 350 rpm for 15 min.
- Samples were then sieved through a 125 µm sieve and rinsed with DI water.
- **Wet-peroxide oxidation:** 20 mL of $FeSO_4$ and 20 mL H_2O_2 were added to samples, heated at 75ºC while stirring at 350 rpm. Aliquots of 20 ml H_2O_2 were added as needed for clearing.
- Digested samples were filtered through 1 mm, 355 µm, and 125 µm sieves for size separation and washed with DI water.
- Microplastics were characterized by type and color (e.g., fiber, film, fragment, foam, pellet/bead, and nurdle) using Leica Ez4 and Zeiss Stemi 2000-c stereomicroscopes, and stored in 5 mL shell vials and DI water.
- Polymeric confirmation will proceed in the future with Fourier Transform Infrared Spectroscopy (FT-IR).

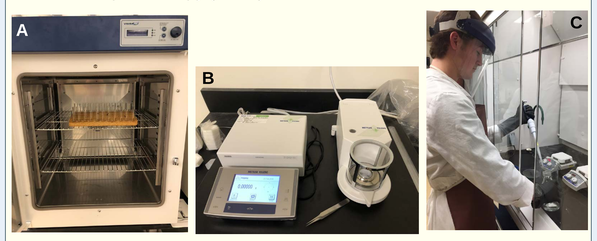

Fig. 3. (A) Macroinvertebrate specimens drying in oven, **(B)** microbalance used for dry weighing, **(C)** Riley performing a digest on macroinvertebrates.

Results

- Microplastics (< 5mm) were present in all aquatic macroinvertebrate taxa, excluding Gyrinidae **(Fig. 4A).**
- There were a total of n = 246 microplastics within 509 individuals of varying in taxa and feeding groups.
- Ranatra were the only invertebrates to have uptaken a pellet **(Fig. 4A).**
- Pychnopsyche were the only group whose particles were dominated by films rather than fibers **(Fig. 4A).**

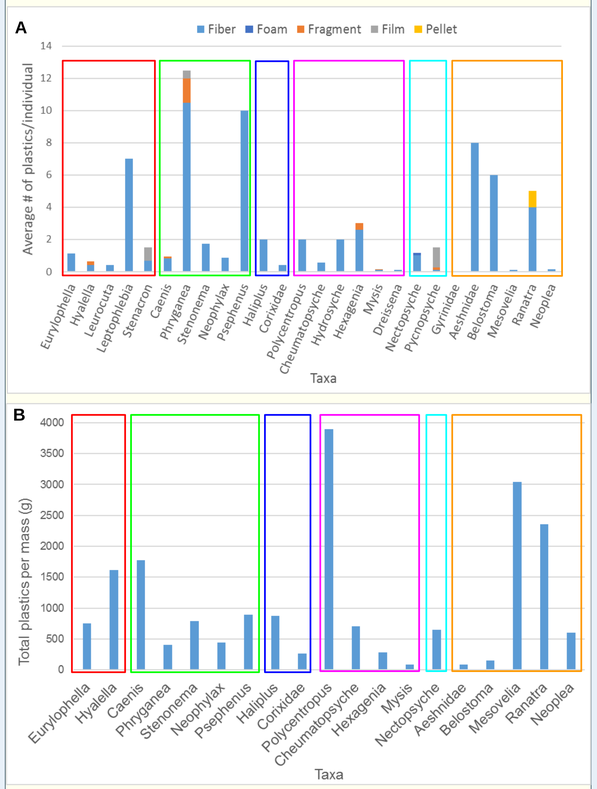

Fig. 4. (A) Mean microplastic abundance by type, **(B)** total plastic per mass (g).

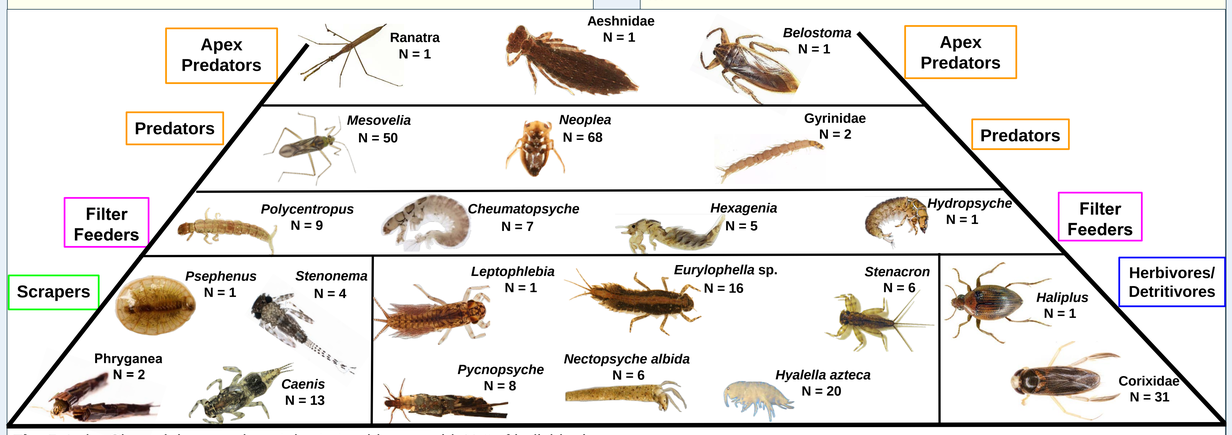

Fig. 5. Lake Champlain macroinvertebrate trophic pyramid, N # of individuals.

Size separation via sieves:

- 1 mm → 42 fibers, 1 fragment, 1 foam
- 355 µm → 49 fibers, 3 fragments, 11 films, 1 pellet
- 125 µm → 122 fibers, 11 fragments, 5 films

Discussion

- Mean microplastic abundance per individual varied among the seven unique feeding behaviors, but all invertebrate samples contained microplastics excluding Gyrinidae analyzed N = 2 **(Fig. 3).**
- Across all species fibers (86.5%), were the most common particle, followed by films (6.5%), fragments (6%), foams (<1%), and pellets (<1%).
- Based on feeding habit, the greatest abundance of total plastics per mass (g) was found within one species of filter feeders (*Polycentropus*), and the predator guild, followed by certain collectors and scrapers (Fig. 4).
  - ➢ Organisms may directly consume microplastics actively (e.g., due to confusion with potential prey) or passively (e.g., during particle filtration) (Collignon et al. 2014).
- Plastic load in aquatic macroinvertebrates may be correlated with their unique life cycle (i.e., time spent in water, lifespan, selective pressures), or spatial distribution.

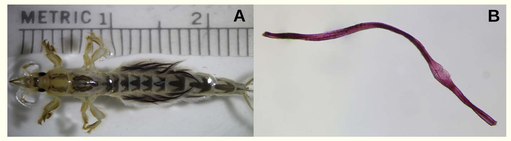

Fig. 6. (A) *Hexigenia limbata* containing N = 13 fibers, N = 2 fragments**, (B)** red fiber found in *Hexigenia limbata.*

- Thus far we have analyzed polymer type with FTIR for zebra mussels (n = 2, tetraethylsilicate- adhesion layer for plastic substrate/copolymer), amphipods (n = 2, nylon), mysids (n = 3, rayon), and *Hexigenia limbata* **(Figs. 6, 7).**

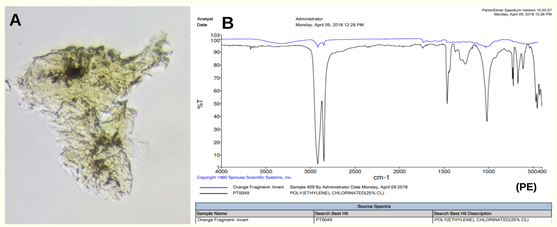

Fig. 7. (A) Orange fragment from *Hexigenia limbata*, **(B)** FT-IR spectrum of the orange fragment (Polyethylene(PE)) in the *Hexigenia limbata* samples.

Future Directions

- Continue categorizing microplastics to polymer type using Fourier Transform Infrared spectroscopy (FT-IR).
- Process more aquatic macroinvertebrates from targeted areas that are collectors and filter feeders to further test the hypothesis that feeding method is an important predictor of plastic load.
- Assess the influence of microplastic ingestion in aquatic macroinvertebrates on survival, behavior, and reproduction in lab setting.

Acknowledgements

Special thanks to Luke Myers and Mark Lamay (LCRI at SUNY Plattsburgh) for providing aquatic macroinvertebrate samples, useful invertebrate and freshwater community expertise, and graphical support. Additional thanks to fellow microplastics researchers for their assistance in technical support and collaborative efforts. Funding for this research was provided by a NOAA funded Lake Champlain Sea Grant and for conference travel by SUNY Plattsburgh's College Auxiliary Service, Arts and Sciences, and the Center for Earth and Environmental Science.

References

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Collignon, A., Hecq, J.-H., Galgani, F., Collard, F., Goffart, C. 2014. Annual variation in neustonic micro- and meso-plastic particles and zooplankton in the Bay of Calvi (Mediterranean–Corsica) *Marine Pollution Bulletin* 79: 293-298.