The fate of microplastics in aquatic food webs

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I. Preface

I.I Table of Contents

I.II Acknowledgements

Land acknowledgements

McGill University is located on land which has long served as a site of meeting and exchange amongst Indigenous peoples, including the Haudenosaunee and Anishinabeg nations. Both our laboratory and field sites were located on the unceded territories of the Kanien'kehá꞉ka, Wendake-Nionwentsïo and Ho-de-no-sau-nee-ga nations who long lived as protectors of these lands and waters. I honour, recognize, and respect these nations as the traditional stewards of the lands and waters where my work has been conducted.

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I.III Résumé

Les microplastiques - des particules de plastique de 5 mm ou moins - sont des stresseurs environnementaux émergents qui polluent les écosystèmes et les organismes aquatiques. La concentration de ces particules chimiquement complexes est écologiquement significative en raison de leur persistance dans l'environnement et de leurs interactions avec des processus écologiques clés. Dans le cadre de ma thèse doctorale, je propose d'abord de quantifier la présence des microplastiques dans l'espace et dans le temps en incorporant leur échantillonnage à des protocoles limnologiques normalisés afin d'établir des politiques efficaces pour la gestion de ces déchets. Puis, pour mieux comprendre les mécanismes régulant le cycle des microplastiques dans les réseaux trophiques d'eau douce, j'ai effectué une série d'expériences d'exposition microplastiques sur une communauté modèle représentative d'une chaîne alimentaire aquatique. Je démontre que l'acquisition des microplastiques se fait par différentes voies d'exposition qui inclus les particules en suspension, les particules sédimentées et par les interactions inter-espèces. La charge corporelle de particules est également régulée par les caractéristiques biologiques des organismes et leur capacité à retenir ces particules. Nos résultats indiquent que la concentration et la route d'exposition affectent le taux de contamination subie par chaque organisme, et que les différentes interactions entre ces espèces facilitent l'acquisition et la distribution des microplastiques au sein de la chaîne alimentaire. De plus, cette approche par communauté permet de concevoir des scénarios écologiquement réalistes et complexes tel qu'observer en milieu naturel et permet de comparer l'effet de la composition du réseau trophique sur la capacité des organismes à ingérer et transférer les microplastiques. Finalement, mes travaux démontrent que l'exposition aux conditions de réchauffement climatique et de pollution microplastique actuelles et projetées nuisent à la performance prédatrice du gobie à

tache noire, un prédateur benthique envahissant, ce qui peut engendrer des répercussions potentielles sur la structure et la fonction des réseaux trophiques d'eau douce.

I.IV Abstract

Microplastics—plastic particles of ≤5mm in size—are increasingly found to ubiquitously contaminate aquatic environments and diverse organisms. The concentrations of these chemically complex particles are ecologically significant parameters because of their environmental persistence and interactions with key ecological processes. Here, I propose that an essential step toward informing policy for managing plastic waste is to quantify the presence of microplastics both spatially and temporally by incorporating their sampling into standardized limnological protocols. I also propose that our understanding of the mechanisms regulating the cycling of these particles within freshwater food webs can be advanced using a community-level approach, which I demonstrate using a food web module in a series of laboratory experiments. These experiments showed that microplastic uptake occurs through different routes of exposure (particles in suspension, particles in surficial sediments, and those transferred via interspecies interactions), but is regulated by the life history traits of the organisms and their ability to retain these particles. The contamination load of each organism is affected by the concentration and route of exposure, as well as their biotic interactions. I applied a network approach in designing experiments with realistic environmental conditions, whereby I compared the sensitivity of different food web components to acquiring microplastics from various routes of contamination. Finally, my work has shown that, under current and projected warming as well as microplastic contamination scenarios, these co-occurring stressors negatively impact the predatory performance of an invasive benthic predator, the round goby, and thus have potential repercussions on the structure and function of freshwater food webs.

I.V List of Tables

I.VI List of Figures

I.VII List of Supplementary Tables

I.VIII List of Supplementary Figures

I.IX List of Supplementary Photos

I.X Definitions of common terms used for this thesis

Bioaccumulation: is the net result of all uptake and loss processes resulting in the accumulation or enrichment of contaminants in organisms, relative to that in the environment. It includes dietary uptake, respiration, excretion, diffusion, etc.

Bioconcentration: the accumulation of a chemical substance in an organism from the ambient water so that the concentration in the organism exceeds the concentration of the contaminant in the water.

Biomagnification: the uptake of contaminant via the diet of an organism, leading to higher concentrations in the consumer than in the diet. Subsequently, concentrations of the contaminant increase with trophic position in the food web.

Bioconcentration factor (BCF): ratio of chemical concentration in the organism to the concentration in the water at steady state. For this thesis, we used this factor for microbeads offered in suspension and sedimented because their concentrations are measured as a ratio to water. We also assume all uptake and excretion processes are stable during the time period examined.

Bioaccumulation factor (BAF): the ratio of chemical concentration in the organism to the concentration available from all potential routes of uptake (e.g., water, sedimented, transfer between organisms). In Chapter 3 of this thesis we use the term pseudo-bioaccumulation factor (BAF) to designate the concentration of beads in an organism based on its transfer from species interaction mechanism (biodeposits or predator-prey transfer) and total bioaccumulation factor (tBAF) as the total accumulation of beads from all potential routes of uptake over the total number of beads available to the organism.

Biomagnification factor (BMF): the ratio of chemical concentration in an organism to the concentration offered in its prey. Biomagnification occurs when BMF > 1. In this thesis, this factor would consider the number of microbeads taken up by round gobies via its diet; sothe number of microbeads in round goby digestive tract in relation to the number available in its prey.

Biodeposition: Deposition of biogenic material on the bottom of water resulting from the production of faeces and pseudofaeces

Biomarkers: biological response to a chemical or chemicals that gives a measure of exposure or toxic effects

Contaminant: the presence of an extraneous or unwanted materials in the environment or in an organism

Dose: Amount of a substance that is absorbed by the body surfaces of an animals which enters the tissues or internal structures.

Ecological risk: The risk associated with the actual likelihood that a hazard will occur in the real world, based on potential or expected exposures.

Exposure: In ecotoxicology, it is the qualitative or quantitative representation of a contact between a chemical or physical agent and the surface of an organism (ie. via the body surface or the lungs or gills).

Functional group: organisms which possess similar lifeform, life history traits and assumes a specific position within food web and perform key role in the ecosystem by its relationships and interactions with other species and its environment.

Ingestion: consumption of a substance by an organism; the process of taking in food, drink or other sources of nutrition

Microplastics: synthetic polymers 5mm or less in size which can be manufactured as materials of a specific shape and size for commercial or industrial use (primary microplastic) or the result from the breakdown or erosion of a larger synthetic product (secondary microplastic)

Nanoplastics: synthetic polymers of 1 to <100 nm in size. They can include primary and secondary processes as described for microplastics.

Pollutant: A pollutant is a substance introduced in the environment or in an organism that interferes with natural processes and causes adverse effects.

Pseudofaeces: Specialized mechanism used by filter-feeding mollusks to expel unpalatable particles before the particles pass through the digestive tract of the animal. As opposed to faeces, the particles released in pseudofaeces are captured, wrapped in mucus and expelled, so are never ingested and digested.

Translocation: substance moving from one organ of the body to the next via cell membrane transfer

Uptake: taking up a substance that is available. For the purpose of this thesis, this term includes ingestion, respiration, or adherence.

I.XI Thesis format

This thesis is presented in a manuscript-based format. My work opens with a general introduction, then I present an extensive literature review as a first chapter which is followed by three original manuscripts interspersed with preamble sections acting as connecting statements. The thesis ends with a general discussion and concluding remarks which summarize the main contributions and finding of the thesis and comments on the implications, limitations and future directions. The general introduction provides a brief overview of the research questions addressed in this thesis. The manuscript presented as Chapter 1 is published, Chapter 2 and 4 have been submitted for publication, while Chapter 3 is in preparation for submission to a scientific journal. References and supplementary materials are provided at the end of each manuscript. A bibliography which encompasses all references cited in this thesis (including the general introduction, the manuscript, supplementary materials, the discussion and concluding remarks. The main content of this thesis includes the following contributions:

Chapter 1 | Status: published in the journal Environmental Reviews

D'Avignon, G., Gregory-Eaves, I, and Ricciardi, A. 2021. Microplastics in lakes and rivers: an issue of emerging significance to limnology. Environmental Reviews. 1-17. doi:10.1139/er-2021-0048.

Chapter 2 | Status: in preparation for submission

D'Avignon, G., Hsu, S.S.H., Gregory-Eaves, I., Ricciardi, A. Species interactions, environmental routes and exposure concentration influences the uptake and retention of microplastics in freshwater food webs

Chapter 3 | Status: in preparation for submission

D'Avignon, G., Gregory-Eaves, I., and Ricciardi, A. Using community modules to predict risks and fate of microplastics in freshwater food webs

Chapter 4 | Status: Accepted for publication

D'Avignon, G., Wang, D., Reid, H.B., Gregory-Eaves, I., and Ricciardi, A.
Effects of elevated temperature and microplastic exposure on growth and predatory performance of a freshwater fish, the round goby (*Neogobius melanostomus*). Limnology and Oceanography. In press.

Some of the work conducted for this thesis had to be abandoned due to the COVID outbreak. These projects may be reconducted as PDF projects. Although these contributions are not included in the main body of this thesis, they reflect an important amount of work that was conducted during my PhD.

D'Avignon, G., Gregory-Eaves, I., Ricciardi, A. Can we predict the risk of microplastic contamination using physical, chemical, environmental, and biological data: Microplastic contamination in SLR aquatic food webs? (pending)

Hernandez, L. M., Crew, A., D'Avignon, G., Tufenkji, N., Gregory-Eaves, I., Ricciardi, A. Cellulose acetate microbeads found throughout the St. Lawrence River: environmentally friendly alternative or just another form of anthropogenic litter? (pending)

In parallel to the main content of this thesis, I also had the opportunity to lead, co-lead or participate in the development of side projects on microplastic pollution or aquatic invasion ecology and guided independent researcher and honours students in the creation, design and writing of their dissertations:

Rothberg, L. Using bioindicators to track and monitor microplastic pollution., Dept. Biology, McGill University. 2021-2022.

Pfister, H. Caddisfly larvae facilitate the uptake of microplastics by a freshwater fish (*Ameiurus nebulosus*)., Dept. Biology, McGill University. 2020-2021.

Wang, D. Effects of temperature and microplastic pollution on the health and behaviour of an invasive fish, the round goby. Bieler School of Environment (BSE). McGill University. 2019-2020. (Partial data included in Chapter 4*,* manuscript was submitted to *Limnology and Oceanography*)

Bourgault, J. An invasion under progress: Early invasion of zebra mussel (*Dreissena polymorpha*) in South-Eastern Quebec (Lake Memphremagog and Lake Magog)., Dept. Biology, McGill University. 2019. www.researchgate.net/publication/339212614

Hsu, S.S.H. Evaluation of dose-dependent microplastic ingestion in St. Lawrence River fauna. Dept. Biology, McGill University. 2019 (partial data included in Chapter 2)

Wang, A.M.Y. The use of plastic in case-building by larval caddisflies. Bieler School of Environment, McGill University. 2018.

Thesis projects conducted by Duncan Wang (Chapter 4) and Sophia S.H. Hsu (Chapter 2), were included in my thesis as I played a large role in developing the study design, data analysis and writing, and these students have been included as co-authors to recognize their contributions. The work I co-led with Jasmin Bourgault was published as a technical report (see below) and now serves as benchmark data for an ongoing Master thesis. The work lead by Hélène Pfister and Amy Wang is in preparation for submission.

D'Avignon, G., Bourgault, J., Hsu, S.S.H, et Ricciardi, A. 2020. Évaluation de la distribution et de la structure des populations de moule zébrées au lac Memphrémagog et ses environs, 51 p. doi:10.13140/RG.2.2.12044.64645. [Available from: www.researchgate.net /publication/339212411]

Pfister, H., Wang, A.M.Y., D'Avignon, G., Ricciardi, A. Caddisfly larvae (Trichoptera: Limnephilidae) facilitate the uptake of microplastics by a freshwater fish (*Ameiurus nebulosus*). *In preparation for submission.*

I.XII Contribution of authors

This thesis is composed of my original work. For each chapter, I developed the focal research questions, developed the methodologies, conceptual framework and led the study. Some of my co-authors were undergraduate students who took part in the development of some aspect of the research conducted in chapter 2 and 4 as part of their honour's project. My co-supervisors were involved in the conceptualization of the leading ideas and arguments of each chapter, and to the editing the manuscripts and thesis. Below I describe the main contributions of each author to the four manuscripts of my thesis. The title of each chapter as well as the full names of authors are listed, then I refer to their initials to describe their individual contribution.

Chapter 1 | Microplastics in lakes and rivers: an issue of emerging significance to limnology

D'Avignon, G., Gregory-Eaves, I, and Ricciardi, A. 2021. Microplastics in lakes and rivers: an issue of emerging significance to limnology. Environmental Reviews. 1-17. doi:10.1139/er-2021-0048.

GD contributed to the conceptualization of the leading ideas and arguments, performed the literature review, data acquisition and compilation, created the figures and tables, drafted and edited the manuscript. AR contributed to the development of arguments, the drafting and editing the manuscript. IGE contributed to the development of arguments, and to the editing of the manuscript.

Chapter 2 | Species interactions, environmental routes and exposure concentration influences the uptake and retention of microplastics in freshwater food webs

D'Avignon, Genevieve., Hsu, Sophia Shu Han, Gregory-Eaves, Irene, Ricciardi, Anthony.

In preparation for submission

GD and SH contributed equally to the study conception, experimental design and data acquisition for the uptake experiments. **GD** led the data acquisition for retention and species interaction experiments, data analyses, the creation of figures, tables, and manuscript draft and edits. IGE and AR contributed to conceptualization of study, interpretation of results, and drafting and editing the manuscript.

Chapter 3 | Using community modules to predict risks and fate of microplastics in freshwater food webs

D'Avignon, Genevieve, Gregory-Eaves, Irene, and Ricciardi, Anthony.

In preparation for submission

GD contributed to the study conception, experimental design, data acquisition, data analyses, the creation of figures, tables, and manuscript draft and edits. IGE and AR contributed to conceptualization of study, interpretation of results, and drafting and editing the manuscript.

Chapter 4| Effects of elevated temperature and microplastic exposure on growth and predatory performance of a freshwater fish, the round goby (*Neogobius melanostomus*)

D'Avignon, Genevieve, Wang, Duncan, Reid, Heather B., Irene Gregory-Eaves, Irene, and Ricciardi Anthony. Limnology and Oceanography. In press.

GD and DW contributed equally to the study conception, experimental design, data acquisition, preliminary data analysis, and manuscript draft. **GD** led the revision of 2019 data analyses, completed 2020 experiments and data analyses, figures, tables, and manuscript edits. HBR contributed to specimen acquisition, experimental design of predatory experiments, data analyses, and manuscript edits. IGE and AR contributed to conceptualization of study, interpretation of results, and drafting and editing the manuscript.

I.XIII Contribution to original knowledge

Over the last 30 years, the field of plastic pollution has developed rapidly, and the effects of this emerging pollutant has been shown as a new driver of global change which poses threats to all ecosystems. Most research has been focused on 1) assessing benchmark contamination across aquatic and marine ecosystems, or 2) performing laboratory exposure studies to understand ecotoxicological endpoints of micro (1µm-5mm) and nano (<1µm) plastics. Many authors have criticized the lack of coherence between laboratory studies and field studies, as the first set of studies used concentrations well above those reported from field sampling. For my thesis, I am using food web ecology concepts and methodologies to bridge the gap between these separate types of studies by designing laboratory experiments to answer important ecological questions on the distribution and cycling of microplastics while maintaining environmental relevance.

In Chapter 1, I consolidated laboratory and field studies on microplastic pollution to provide evidence that microplastics are a distinct particle component of freshwater environments whose concentration is an ecologically significant parameter that must be monitored. My co-

authors and I proposed that because this contaminant is present across different matrices and affect aquatic ecosystem processes and functioning, its sampling must become a part of standardized limnological protocols to set benchmark concentrations of this pollutant in inland waters and their variability across spatial and temporal scales.

In Chapter 2, I used a novel experimental model composed of a community module rather than single species to study how microplastics are incorporated and transferred in aquatic food webs. This novel food web approach provides a framework to ask general ecological questions, that can be guided by hypotheses concerning the fate of microplastics using species' natural history as predictors of this contaminant's uptake and retention by organisms and its cycling between recipients of the community. To our knowledge, our study was the first to test and demonstrate that species take up microplastic particles from different environmental matrices (water, sediments) as well as from other species via trophic (predator-prey) and non-trophic interactions (e.g., biodeposition). We also allowed organisms to take up particles based on their natural feeding behaviour rather than injecting them with a fixed dose or preparing their tissue as a spiked food source to investigate microplastic trophic transfer - a new approach to examining this pathway of transfer.

Building off the findings from Chapter 2, I designed experiments in Chapter 3 that allowed me to examine how microbeads are distributed and cycled when organisms are exposed to multiple routes of microbeads simultaneously. Our design allowed us to examine the cycling of microbeads under more realistic conditionscloser to those experienced by organisms in natural settings. We demonstrated that each organism takes up different concentrations of microbeads depending on its availability and the structure of its community. We also showed that modelling

different routes of uptake in single-exposure experiments can help to predict total contamination of each animal by adding the number of beads acquired via each route.

Finally in Chapter 4, we built on the knowledge acquired from our own experiments and field contamination data to assess the effect of microplastic exposure and climate warming on the behaviour of the round goby – the predator used in our community module. Our study is unique because it used microplastic concentrations observed in the field in our exposure scenario (concentrations in water, sediments and mussel prey) as well as the projected changes in water temperatures of the Great Lakes-St. Lawrence River basin to set the baseline and future conditions that are and will be experiencing the fish. Only a few other authors investigated the effect of microplastic pollution and climate change under a multiple-stressor scenario. In this experiment, we showed that under realistic conditions round gobies experienced declines in feeding performance due to microplastic pollution after 37-days exposure, even if they did not display high retention of microbeads in their tissues – suggesting an exposure to microplastic without substantial bioaccumulation and biomagnification potential can still impact fish behaviour. We identified that the effect of increased temperature was more important than microplastic pollution and contributed to a reduction in both growth and predatory performance of juvenile round gobies.

II. General introduction

II.I Plastic – a revolutionary material with a pervasive problem

In our current economic model, growth depends heavily on the production and consumption of goods (Crawford and Quinn 2017). Over the years, this created a trend towards a planned obsolescence of consumer products—whether caused by rapid technological advances, a perceived need to purchase newer or more fashionable items, or a reduction in the quality of manufactured products (Packard 1960; Solczak 2013). Increased consumption had the undesired effect of producing more waste to the detriment of the environmental and social costs (Maycroft 2009). Since the 1950s, there has been an exponential increase in the production of low-cost, versatile and durable synthetic materials like plastics to produce short-term consumables. Although plastic provides many societal benefits (Andrady 2015a), its mass production has caused burgeoning global waste management issues and an innumerable amount of plastic litter to accumulate in ecosystems worldwide (Rochman 2016; Geyer et al. 2017; Barnes 2019). Plastic pollution is a result of our tendencies towards overconsumption, waste mismanagement and inability to assess the entire life cycle of a product in its design and use. There is an urgent need for information on the life cycle of plastic in the environment to improve the management and sustainable use of this material.

II.II Microplastic pollution – a legacy stressor

The issue of waste mismanagement has gained public attention through shocking images of islands of plastic litter floating and accumulating in the five oceanic gyres (Eriksen et al. 2014). Records indicate that the most abundant type of debris in our oceans are plastics, accounting for 60-80% of marine waste (Barnes et al. 2009). The most common component of this waste is

microplastics (MP)—particles below 5 mm in size (Browne et al. 2007; Andrady 2015b; Koelmans et al. 2015). Microplastics are emerging persistent synthetic pollutants, either 1) produced as beads, pellets and fibres for manufacturing purposes or 2) resulting from the degradation of larger pieces exposed to photolytic, mechanical, and biological degradation (Browne et al. 2007; Duis and Coors 2016) and therefore bound to multiply endlessly as they become smaller.

This pollution is of ecological and societal concern because plastics are 1) composed of hazardous ingredients and toxic additives (dyes, paints and carcinogenic molecules; Rochman et al. 2013a); 2) they can sorb contaminants (persistent organic pollutants, heavy metals, pharmaceuticals); 3) be colonized by bacteria and viruses, all of which can leach out to the environment (Teuten et al. 2009; Rochman et al. 2013b; Menéndez-Pedriza and Jaumot 2020). Microplastics are associated with toxic substances that can also become airborne and transported around the world and can be inhaled terrestrial biota including humans (Dris et al. 2016; Brahney et al. 2021). Additionally, microplastics can be mixed with or confused as food by terrestrial, aquatic and marine fauna, and therefore ingested by diverse organisms (Lusher 2015; de Souza Machado et al. 2018; Azevedo-Santos et al. 2021). Small plastic particles can be engulfed by the membranes within organisms (van der Wel et al. 2017) and translocated across tissues and organs (Browne et al. 2008; McIlwraith et al. 2021). This process increases retention of microplastics which can then be transferred across trophic levels (Farrell and Nelson 2013; Chae et al. 2018), thereby exposing consumers, including humans, to a multidimensional contaminant that can cause both chemical and physical stress to organisms (Bucci and Rochman 2022).

II.III Plastic pollution of aquatic environments

For the past 30 years, researchers have invested efforts to study the impacts of this pollution on marine ecosystems and only recently have begun exploring the effect of this pollution on freshwater environments and their community (Wagner et al. 2014; Horton et al. 2017; Provencher et al. 2019). Aquatic ecosystems are already recognized as being among the most threatened ecosystems on the planet because multiple anthropogenic stressors (i.e., invasive species, climate change, habitat fragmentation and degradation, chemical and nutrient pollution) are impacting water quality, biodiversity, and ecosystem functioning (Dubois et al. 2018; Desforges et al. 2022). These ubiquitous particles circulate in the water column, accumulate in sediments, and are transformed and transferred by physical, chemical, and biological processes, thereby interacting with entire biotic communities in inland waters across the globe.

Since 2013, large numbers of microplastics were found in the surface waters and sediments of the Great Lakes-St. Lawrence River Basin (Eriksen et al. 2013; Castañeda et al. 2014; Baldwin et al. 2016; Crew et al. 2020; Earn et al. 2021), highlighting that the largest freshwater ecosystem of the world is substantially contaminated by microplastic pollution. In fact, the level of microplastic contamination of the St. Lawrence River watershed is similar in magnitude to the most polluted Asian rivers (Crew et al. 2020), whereas fish from the Great Lakes host some of the highest concentrations of microplastics in freshwater environments (Munno et al. 2021). These particles were found to be stored in the flesh of fish species that play essential roles in lacustrine food webs (McIlwraith et al. 2021) and contribute to local sport and commercial fisheries (Gewurtz et al. 2011; Dunlop et al. 2019). It is likely that St. Lawrence River fauna living downstream of the Great Lakes could face similar conditions. Preliminary work revealed round gobies (*Neogobius melanostomus*) from ten different sites along the river had ingested

microplastic fibres (Figure S0.1A), whereas both round gobies and yellow perch (*Perca flavescens*) sampled near Bécancour, Quebec, an area of the river whose sediments contained high microbead abundance (~140 000 beads·m^2; Castañeda et al., 2014), had ingested polyethylene microbeads (Figure S0.1B). Furthermore, eleven species of fish and dreissenid mussels from the St. Lawrence River were found to be contaminated by 0-35 suspected microplastics·$individual^{-1}$ (Table S0.1). Considering this basin drains an area over 1,000,000 km^2 and provides water for drinking, transport, and leisure activities for more than 60% of Quebec residents (planstlaurent.qc.ca), understanding the threat of microplastic pollution on this ecosystem and its species could be critical for the protection of habitat quality and ecosystem services.

II.IV Assessing ecological risks

Microplastic concentration has been proposed as a criterion to assess the ecological risk for organisms (Koelmans et al. 2017). However, the abundance of microplastics ingested by aquatic organisms is highly variable because it may depend on physicochemical properties of their environment (Dantas et al. 2012; Nel et al. 2018), their life stage or feeding strategy (Setälä et al. 2016; Scherer et al. 2017), and the physical characteristics of microplastics such as their size, type (Au et al. 2015; Qu et al. 2018; Bucci et al. 2020), and state (e.i., pristine, aged, with a biofilm or associated contaminants; Besseling et al., 2014; Kalcikova et al., 2020). Therefore, the microplastic burden in animals is not explained entirely by environmental contamination (Doucet et al. 2021; Hoellein et al. 2021).

Effective evidence-based legislation and monitoring strategies require data on the ecological risks associated with microplastics contamination, including detailed information on the availability and fate of microplastics in freshwater environments, their pathway of entry into

food webs, and their impacts on aquatic organisms, communities, and ecosystems (Wagner et al. 2014; Horton et al. 2017; Ivleva et al. 2017). Yet, studies of the effects of microplastics on aquatic communities and ecosystem functions are rare (Krause et al. 2021; O'Connor et al. 2022) with the bulk of research to date focusing on effects on individual species and typical laboratory model organisms (Schiavo et al. 2018; Provencher et al. 2019; Wang et al. 2019). To bridge the gap between lab and field studies, experimental food web modules reflecting known species relationships could offer valuable insight into the fate and impacts of microplastics at the community and ecosystem levels.

II.V Thesis outline

In this thesis, I adopt a systematic approach to study to how microplastics are incorporated and transferred in aquatic food webs, with the premise that each biotic component of a food web acts as a recipient and a vector of microplastics through interactions with their environment and other food web components. The goal of my research is to understand the mechanisms involved in the distribution and cycling of microplastics by aquatic organisms and examine the potential impacts of this pollution on aquatic food webs. My thesis objectives were as follows:

1. Synthesize current knowledge the ecological abundance, impacts, and research gaps of microplastic pollution in inland waters (Chapter 1)
2. Assess the effect of microplastic concentration, environmental route and transfer pathways on the uptake and retention of microplastics in organisms (Chapters 2 & 3)
3. Clarify the role of species interactions on microplastic distribution and circulation within food webs (Chapter 2& 3)

4. Determine if single-exposure experiments can be used to predict microplastic contamination in aquatic food webs (Chapter 3)
5. Explore the effects of microplastic exposure and warming on the growth and predatory performance of a benthic freshwater fish (Chapter 4)

As a first step, I have done a comprehensive literature review to identify the ecological impacts and research gaps of microplastic pollution in inland waters (Chapter 1). Some of these gaps in the next chapters of my thesis. Then, I am using a community food web module comprised of a triad of species: dreissenid mussels (*Dreissena bugensis*), amphipods (*Gammarus fasciatus*), and the round goby (*Neogobius melanostomus*), to examine the fate, distribution and cycling of microplastics in aquatic food webs. This module is composed of three abundant and widespread species with distinct roles common in freshwater food webs and thus allows for the experimental study of a suite of trophic and non-trophic interactions. This novel community ecology approach provides added advantages to single-species exposure model by addressing more complex interactive plastic cycling scenarios approaching situations observed in natural conditions. Finally, to keep this research ecologically and socially relevant (Rochman et al. 2016; Koelmans et al. 2017; Bucci et al. 2020), I used realistic projected climatic and microplastic pollution data from environmental and biotic samples reported for the Great Lakes- St. Lawrence River Basin to explore the effects of these stressors on the growth and predatory performance of the round goby—the predator in our community module (Chapter 4).

II.VI Supplementary Materials

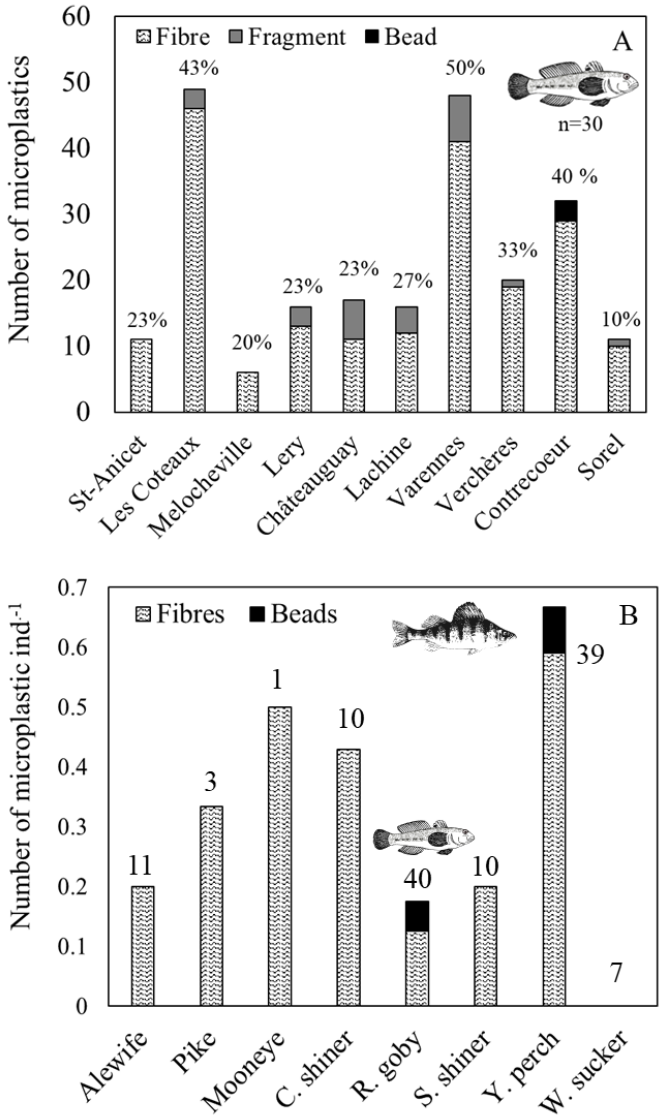

Figure S0.1.1: Number of microplastics reported in fish from the St. Lawrence River A) Total number of microplastic per type found in the stomachs of 30 round gobies and the percentage of fish contaminated. Fish were collected at 10 sites along the St. Lawrence River in 2014. B) Number of microplastic per individual collected at Bécancour and Sorel in 2015. Brusco, B. *unpublished data*. Sample sizes are indicated above the bars. See Table S1 for more details.

Table S0.1.1: Summary of microplastic ingestion by St. Lawrence River (Canada) fauna unpublished data. N = sample size, % Occ. refers to the percentage of individuals with microplastics per sample size and the mean and range refer to the number of suspected microplastic particles per individual. Microplastic shape are described as fibre (FB), fragment (FR), or bead (B). The most common colours of particles ingested are identified as black (B), blue, (BL), red (R), clear (C), orange (O), violet (V), pink (P), white (W), or unknown (U). [a]Anthropogenic particles were identified visually[a] and using Nile Red fluorescence[b]. Beads were tested using hot needle test and reported as PE when they were similar to those reported in sediments by Castañeda et al. 2014[c].

Species	Location	Latitude	Longitude	Year	N	% Occ.	Mean	Range	Size	Shape	Colour	Author
Round goby	Les Coteaux	N45°15'13.89"	W74°12'43.67"	2014	30	43	1.87	0-15	500	FB, FR	BL, R, B	Brusco et al., 2014[a]
Round goby	Varennes	N45°40'43.74"	W73°26'31.57"	2014	30	50	1.3	0-11	500	FB, FR	B	Brusco et al., 2014[a]
Round goby	Contrecoeur	N45°57'22.35"	W73°11'57.42"	2014	30	40	1.03	0-7	500	FB, B	B, R, O	Brusco et al., 2014[a,c]
Round goby	Melocheville	N45°19'9.18"	W73°55'39.79"	2014	30	20	0.23	0-2	500	FB	B, R, V, C	Brusco et al., 2014[a]
Round goby	Léry	N45°20'7.91"	W73°49'6.38"	2014	30	23	0.43	0-3	500	FB, FR	R, B, BL	Brusco et al., 2014[a]
Round goby	Châteauguay	N45°22'31.71"	W73°46'34.25"	2014	30	23	0.36	0-3	500	FB, FR	R, BL, B	Brusco et al., 2014[a]
Round goby	Sorel	N46°2'55.55"	W73°6'4.64"	2014	30	10	0.27	0-4	500	FB, FR	BL, R	Brusco et al., 2014[a]
Round goby	Lachine	N45°25'59.4"	W73°41'08.2"	2014	30	27	0.53	0-3	500	FB, FR	BL, R, B	Brusco et al., 2014[a]
Round goby	St-Anicet	N45°8'29.43"	W74°21'38.61"	2014	30	23	0.31	0-3	500	FB	BL, R, B	Brusco et al., 2014[a]
Round goby	Verchères	N45°27'41.97"	W73°33'36.56"	2014	30	33	0.59	0-4	500	FB, FR	BL, R, B	Brusco et al., 2014[a]
Round goby	Sorel	N46°2'55.55"	W73°6'4.64"	2015	20	22	0.2	0-1	500	FB	BL, R, B	Brusco et al., 2014[a]
Yellow perch	Sorel	N46°2'55.55"	W73°6'4.64"	2015	15	33	0.67	0-4	500	FB, B	B, BL, R	Brusco et al., 2014[a,c]
White sucker	Sorel	N46°2'55.55"	W73°6'4.64"	2015	7	0	0.00	0	500	none	none	Brusco et al., 2015[a]
Alewife	Sorel	N46°2'55.55"	W73°6'4.64"	2015	9	0	0.00	0	500	none	none	Brusco et al., 2015[a]
Common shiner	Sorel	N46°2'55.55"	W73°6'4.64"	2015	10	20	0.27	0-2	500	FB	C, BL	Brusco et al., 2015[a]
Round goby	Bécancour	N46°23'42.96"	W72°20'58.62"	2015	20	15	0.15	0-2	500	FB, B	B, O	Brusco et al., 2015[a,c]
Yellow perch	Bécancour	N46°23'42.96"	W72°20'58.62"	2015	24	29	0.20	0-3	500	FB, B	B	Brusco et al., 2015[a,c]
Alewife	Bécancour	N46°23'42.96"	W72°20'58.62"	2015	2	50	0.70	0-1	500	FB	B	Brusco et al., 2015[a]
Northern pike	Bécancour	N46°23'42.96"	W72°20'58.62"	2015	3	33	0.33	0-1	500	FB	B	Brusco et al., 2015[a]
Mooneye	Bécancour	N46°23'42.96"	W72°20'58.62"	2015	1	100	1.00	1	500	FB	B	Brusco et al., 2015[a]
Spottail shiner	Bécancour	N46°23'42.96"	W72°20'58.62"	2015	10	20	0.20	0-1	500	FB	B	Brusco et al., 2015[a]
Round goby	Bécancour	N46°23'42.96"	W72°20'58.62"	2017	1	100	5.00	5	<100	FR	U	D'Avignon, et al., 2017[b]
Yellow perch	Cap-Santé	N46°37'59.3"	W71°46'32.9"	2017	2	100	20.00	3-35	<100	FR	U	D'Avignon, et al., 2017[b]
Walleye	Cap-Santé	N46°37'59.3"	W71°46'32.9"	2017	3	100	8.30	3-16	<100	FR, B, FB	U	D'Avignon, et al., 2017[b]
Shorthead redhorse	Cap-Santé	N46°37'59.3"	W71°46'32.9"	2017	1	100	7	7	<100	B, FR	U	D'Avignon, et al., 2017[b]
White sucker	Cap-Santé	N46°37'59.3"	W71°46'32.9"	2017	1	100	10	10	<100	FB, FR	U	D'Avignon, et al., 2017[b]
White perch	Cap-Santé	N46°37'59.3"	W71°46'32.9"	2017	1	100	3	3	<100	FR	U	D'Avignon, et al., 2017[b]
Round goby	Varennes	45°41.097'N	73°27.480'W	2017	2	100	21.5	10-33	<100	FR	U	D'Avignon, et al., 2017[b]
Yellow perch	Varennes	45°41.097'N	73°27.480'W	2017	1	100	2	2	<100	FR, FB, B	U	D'Avignon, et al., 2017[b]
Walleye	Varennes	45°41.097'N	73°27.480'W	2017	2	100	5	2-7	<100	FR, FB, B	U	D'Avignon, et al., 2017[b]
Yellow perch	St. Nicolas	N46°44'23.9"	W71°18'15.4"	2017	3	100	6	5-7	<100	FR, FB	U	D'Avignon, et al., 2017[b]
White sucker	St. Nicolas	N46°44'23.9"	W71°18'15.4"	2017	1	100	3	3	<100	FR, FB	U	D'Avignon, et al., 2017[b]
White perch	St. Nicolas	N46°44'23.9"	W71°18'15.4"	2017	2	100	8.5	8-9	<100	FR, FB	U	D'Avignon, et al., 2017[b]
Zebra mussel	Neuville	N46°44'23.9"	W71°18'15.4"	2017	4	100	5	1-12	<100	FR, FB, B	C, P, O	D'Avignon, et al., 2017[b]
Quagga mussel	Iles de la Paix	N45°19.648'	W73°51.534'	2017	12	83	9.42	1-22	<100	FR, FB, B	C, P, O	D'Avignon, et al., 2017[b]
Quagga mussel	Les Coteaux	N45°15'13.89"	W74°12'43.67"	2017	7	100	6	1-15	<100	FR, B, FB	C, P, O	D'Avignon, et al., 2017[b]
Quagga mussel	N-D-L'Ile-Perrot	N45°21.296'	W73°52.501'	2017	4	100	2.5	1-5	<100	FR, FB, B	C, W	D'Avignon, et al., 2017[b]
Quagga mussel	Baie de Valois	N45°26.807'	W73°46.899'	2017	6	100	8.3	3-25	<100	FR, FB, B	C, W, P	D'Avignon, et al., 2017[b]
Quagga mussel	Varennes	N45°41.097'	W73°27.480'	2017	3	67	7.3	0-17	<100	FB, FR, B	C, O, W	D'Avignon, et al., 2017[b]
Quagga mussel	Sorel	N46°03'08.1"	W 73°05'27.0"	2017	5	100	8.8	2-18	<100	FR, FB, B	C, O, P	D'Avignon, et al., 2017[b]

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1. Chapter 1 | Microplastics in lakes and rivers: an issue of emerging significance to limnology

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1.1 Abstract:

Microplastics—plastic particles in the size range of planktonic organisms—have been found in the water columns and sediments of lakes and rivers globally. The number and mass of plastic particles drifting through a river can exceed those of living organisms such as zooplankton and fish larvae. In freshwater sediments, concentrations of microplastics reach the same magnitude as in the world's most contaminated marine sediments. Such particles are derived from a unique biogeochemical cycle that ultimately influences productivity, biodiversity, and ecosystem functioning. Furthermore, microplastics act as vectors of toxic substances to invertebrates, fishes, herpetofauna, and waterfowl. We contend that the concentration of this distinct particle component is an ecologically significant parameter of inland waterbodies because of its ubiquity, environmental persistence, and interactions with key ecological processes. No environmental field survey that has searched for microplastics has yet failed to detect their presence. Standardized limnological protocols are needed to compare spatio-temporal variation in the concentration of microplastics within and across watersheds. Data obtained from such protocols would facilitate environmental monitoring and inform policy for managing plastic waste;

furthermore, they would enable more accurate modeling of contaminant cycling and the development of a global plastic budget that identifies sources, distribution and circulation pathways, reservoir size and retention times.

1.2 Introduction

Plastics are engineered from long repeating chains of carbon molecules derived from oil and natural gas to produce a final product with desirable properties such as strength, rigidity or elasticity, and resistance to temperature and acidity (Crawford and Quinn 2016). Technological advancements have reduced the cost of plastic production, facilitating their increased use in manufacturing, packaging, and single use containers. The mass of plastics in solid waste has been increasing steadily since the 1960s, generating escalating costs of waste management and environmental pollution, because most plastics do not decompose (Geyer et al. 2017) and their chemical components and additives pose barriers to recycling (Barra and González 2018). In the 1970s, attention began to focus on the drawbacks of these innovative materials when researchers reported alarming densities of floating plastics accumulating within oceanic gyres (Ryan 2015) and, later, in freshwater systems (Anderson et al. 2016). Nearly 80% of all plastics ever created has been accumulating in the environment or landfills (Geyer et al. 2017), underscoring a need to improve plastic waste management and maintain efforts to reuse, recycle, incinerate, or increase their biodegradability.

Initial consensus defined microplastic as synthetic polymers 5mm or less (Andrady 2015; Crawford and Quinn 2016; GESAMP 2016). Emerging classifications differentiate plastic particles into macro- (>1 cm), meso- (1 to <10 mm), micro- (1 to <1000 μm), nano- (1 to <100 nm), and sub-micro-sizes (100 to <1000 nm), as well as characterizing particle shape, structure,

and composition (Hartmann et al. 2019), as these factors affect their distribution, circulation, ingestion by biota, and impacts on environments. In this paper, microplastics refer to synthetic particles ≤5mm in size.

Primary microplastics are manufactured as specific materials, of a specific shape and size for commercial or industrial use. These include polyethylene microbeads manufactured as small (5µm to 2mm) spherical particles for use as mild abrasives in cosmetic products (Fendall and Sewell 2009). A 150 mL container of a facial scrub can contain up to 2.8 million microbeads (Napper et al. 2015), and such products are the source of trillions of particles released with effluents daily (Rochman et al. 2015). Over a dozen countries have banned the use of microbeads in cosmetics; however, other types of microplastics are still produced and continue to be released to the environment (e.g., spillage of industrial pellets) (Zbyszewski et al. 2014). All large plastic debris can ultimately degrade into micro-sized or nano-sized particles during their use (e.g., fibres released from garments or textiles, rubber fragments released by abrasion from car tires, plastic mulching, paint flakes, etc.; Horton et al. 2017) or through mechanical stress, photodegradation, and oxidation (Eerkes-Medrano and Thompson 2018). Synthetic fibres from nets, clothing, or textiles are typically predominant in microplastics found in waterbodies and aquatic biota (O'Connor et al. 2020; Lim 2021; Rebelein et al. 2021; Yang et al. 2021). A portion of these originate from the laundering of clothing, as a single synthetic garment can produce thousands of microfibres per wash (Browne et al. 2011; Napper and Thompson 2016; McIlwraith et al. 2019).

All land-based plastic waste (e.g., littering, landfills, plastic mulching, dredge piles, sewage sludge, organic fertilizers from biowaste fermentation and composting), can be released and transported into aquatic systems carried by winds, erosion and surface runoff. Wastewater

treatment plants (WWTP) process domestic, industrial, and commercial effluent, and sometimes surface water runoff. Primary treatment removes 41-93% of microplastic particles, whereas secondary and tertiary treatments remove 54-99.9% and 82-99.9%, respectively (Iyare et al. 2020). Despite their efficacy, owing to the shear volume of water treated, a single WWTP can release 10^4 to 10^8 particles daily (Mason et al. 2016; Kalcikova et al. 2017; Edo et al. 2020). Considering ~ 80% of wastewater worldwide is estimated to be released directly into the environment without treatment (WWAP 2017), grey waters (from domestic sinks, showers, baths, washing machines) are a major source of microplastic to aquatic systems. The retained particles accumulate in WWTP sludge, which are often applied as fertilizer to agricultural fields (Zubris and Richards, 2005; Edo et al 2020); therefore, these microplastics may eventually enter inland waters via agricultural runoff (Figure1.1).

Furthermore, when microplastics become airborne and transported long distances by winds (Enyoh et al. 2019), they can eventually be deposited in areas ranging from a large metropolis (Dris et al. 2016) to a remote mountain catchment (Allen et al. 2019; Figure 1.1). The presence of plastics is therefore not limited to the location at which they enter the environment; they can easily be redistributed by surface runoff and by atmospheric and ocean circulation, such that microplastics have been found to accumulate even in polar regions (Bergmann et al. 2019) and deep ocean trenches (Courtene-Jones et al. 2019).

In aquatic systems, biota play active roles in the transport, temporary storage, and transformation of plastics. Given the general definition of microplastics, these particles overlap in size with coarse particulate organic matter (>1mm; Cummins 1974), fine particulate organic matter (>0.45 to <1000 μm, including seston; Wallace et al. 2007), and dissolved organic matter (< 0.45 μm; Lamberti and Gregory 2007). Many freshwater invertebrates (e.g., “shredders” such

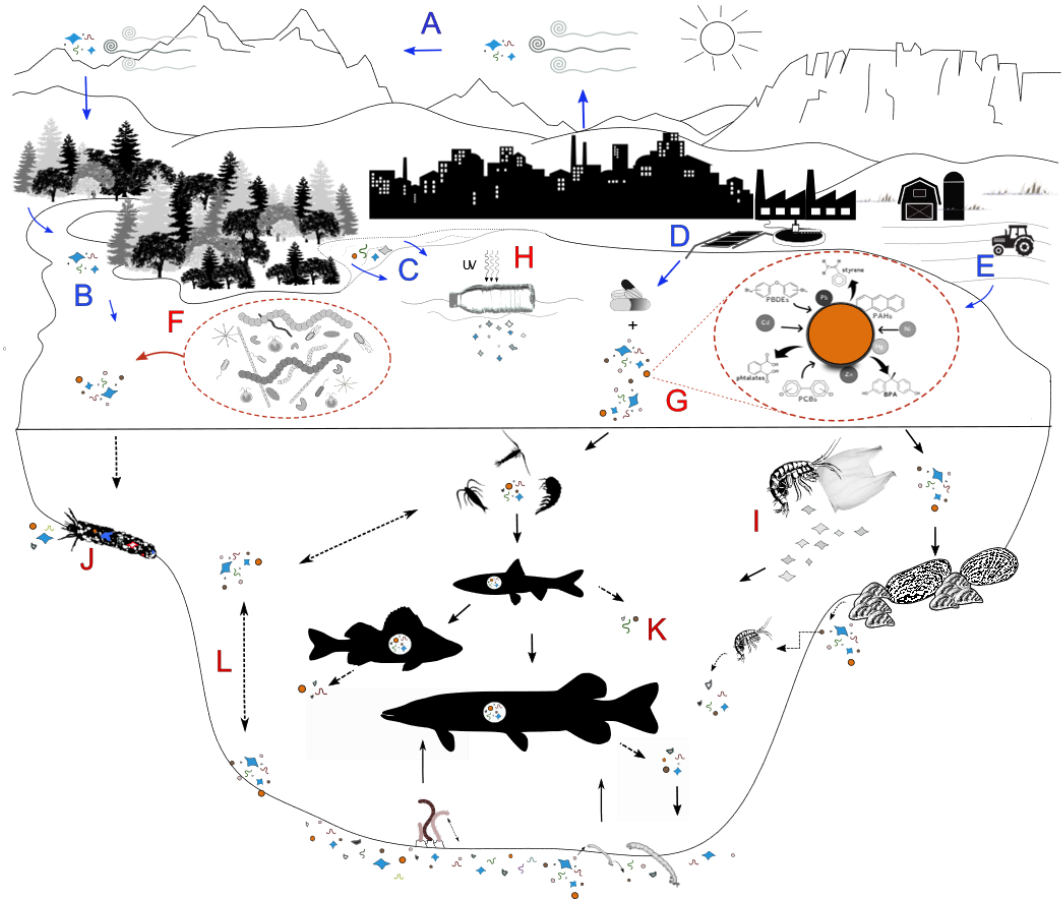

Figure 1.1: The biogeochemical cycle of plastics in inland waters. Blue letters represent microplastic transport pathways to aquatic systems. Microplastics are transported A) via aerial transport and deposition (winds) or B) by tributaries throughout the watershed. Terrestrial plastic waste and debris are carried by water via C) flooding, D) wastewater and stormwater effluents, or E) runoff (e.g., urban, agricultural applications of contaminated sludge or biowaste, dredge piles). Red letters illustrate processes within aquatic environments: F) biofilm formation via colonization by microbial organisms; G) the sorption of associated contaminants (heavy metals, organic pollutants, pharmaceuticals) onto the surface of plastic particles [orange sphere represents a microbead]; H) the physical fragmentation of plastics (due to exposure to UV light, mechanical or chemical erosion), or by I) their interaction with organisms; J) incorporation of microplastics in cases or shelters of organisms; K) introduction and circulation of microplastics in aquatic food webs; and L) vertical movement of microplastics (e.g. change in buoyancy, deposition, re-suspension, burial). Drawn using license-free clipart images and Inkscape vector graphics editor.

as gammarid amphipods, limnephilid caddisfly larvae, and pteronarcyid stonefly nymphs) play vital roles in the breakdown of particulate organic matter and could similarly interact with microplastics. A broad variety of aquatic organisms ingest microplastics including birds (Holland et al. 2016), fish (Jabeen et al. 2017; Azevedo-Santos et al. 2019), bivalves (Su et al. 2018; Baldwin et al. 2020; Wardlaw and Prosser 2020), crustaceans (Iannilli et al. 2020; Simmerman and Wasik 2020), other invertebrates (Nel et al. 2018; Ehlers et al. 2019; Windsor et al. 2019) and can transfer them through aquatic and terrestrial food webs. Plastic debris are also fragmented and transformed as a result of being chewed, shred, grazed upon, or partly digested by various organisms (Hodgson et al. 2018; Jang et al. 2018; McGivney et al. 2020; Po et al. 2020), some of which can metabolize carbon stored in the synthetic polymers (Taipale et al. 2019).

Each polymer has unique affinities to sorb and release heavy metals, persistent organic pollutants, pharmaceuticals products, and antibiotics (Menéndez-Pedriza and Jaumot 2020). The routes taken by these particles to reach aquatic realms (Figure 1.1) dictate their associations with environmental contaminants. Particles circulating via sewers are temporarily retained along with chemicals, pharmaceuticals, bacteria, and viruses common in waste waters, thereby acquiring an assortment of hazardous chemicals and colonizing biota different from those of microparticles cycling via atmospheric circulation or runoffs. Weathering or microbial action on the surface of microplastics enhances the leaching of both additives (e.g., colorants, fillers, plasticizers, stabilisers, flame retardants, bisphenol-A; (Hahladakis et al. 2018) and associated contaminants, which could become bioavailable (Avio et al. 2015; Boyle et al. 2020). Thus, a unique and complex mixture of associated chemicals and biofilms, distinct from surrounding water and sediments (McCormick et al. 2016), can evolve through time as the particle travels through an

aquatic system. Owing to progressive fragmentation, weathering, and biotic interactions with larger size fractions of plastic, microplastic loads will continue to increase, perhaps for decades, even if a sharp decline in plastic production were to occur.

Limnology is concerned with the biological, chemical, physical, and geological characteristics of inland waters and their interactions with surrounding ecosystems. Given the pervasiveness of microplastics, their emerging impacts on aquatic biota, and their unique role in biogeochemical and contaminant cycling in aquatic environments, we suggest that limnologists should recognize them as a distinct particle component that is not derived from the same geological or physico-chemical processes as other inorganic seston, though subjected to similar forces of erosion (i.e. mechanical disintegration, chemical weathering driven by ultraviolet light and high temperatures) and sedimentation.

Here, we present evidence that microplastic concentration is an ecologically-relevant parameter and thus should be integrated within standard limnological surveys and water quality assessments. By incorporating microplastics within standard sampling protocols in limnology, we can address a research priority within the field of plastic pollution and provide policy relevant information on the source, circulation, and distribution of plastics within aquatic realm (Provencher et al. 2020). Floating microplastics can outnumber plankton and larval fish in various rivers and marine systems, at ratios up to ~30:1 (Lechner et al. 2014, Steer et al. 2017, see Table 1.1); therefore their presence cannot be ignored when assessing the health of inland waters. We review the ecological impacts of these particles in inland waters and identify key research gaps concerning their significance in animal physiology, trophic ecology, and aquatic ecosystem function based on current microplastic pollution research. Finally, we make recommendations on future directions that can be adopted to integrate microplastic monitoring in

Table 1.1: Microplastic (MP) contamination (particles ≤ 5mm) of watersheds and their biota. Original data and references are listed in Table S1.1. [a]Numerical superscripts indicate references used to compile microplastic contamination values. [b]Beach densities include samples taken in areas that are never, or only temporarily, submerged (e.g., shoreline, intertidal areas). [c]The concentration of microplastics is reported as numbers of particles per individual; where data are not available, the presence of microplastics is reported as either the proportion of animals contaminated (%) or as the number of particles per g of tissue. [d]Number of zooplankton per liter. [e]Ratio of microplastic to zooplankton concentrations × 100.

Watershed[a]	MP concentration in water (#/L)		MP concentration in sediments (#/kg dw)		MP concentration in biota (#/ind.)[c]				Bioseston[d]	MP/ bioseston[e]
	Surface	Column	Beach[b]	Benthic	Benthos	Fish	Birds	Frogs	ind./L	%
Laurentian Great Lakes Basin, USA & Canada										
Lake Erie & tributaries [2,10,40]	<0.001-0.032		50-391	117-5985		70%	1.8-9.8			
Lake Ontario & tributaries [2,4,7,8,15,37]	0.002-1.5		20-4270	40-27830		50%	1.8-9.8			
Lake Michigan & tributaries[2,20,28]	<0.001-0.007	<0.001-0.003		39-6229		0-19.1				
Kinnickinnic River[20,31]	0.003-0.006	0-0.001		32.9	4-20 /g	0-1242				
Milwaukee River[2,20,28]	0.002-0.017	0.002		1410-2110		4.5-6.5				
Yangtze River Basin, China										
Three Gorges Reservoir[11,45]	4.7-12.6			25-300					1-105000	<0.01-470
Lake Taihu[17,23,32,33,42]	0.53-25.8			11-320	0.2-10.4	0.2-17.2				
Lake Gaoyouhu[33,39]	0.7-3.1			17.6-208.9	1.6-5.0				2.0-13	0.54-155
Lake Poyang[5,23,32,33,44]	0.24-34		11-3153	7.1-506	0.4-1.6	0-18			137.6-219.2	0.11-24.7
Lake Dianshan[23,33]	0.5-1.8			14.8-140						
Lake Chao[23,33]	0.2-1.9			0.6-225	0.4-0.9					
Yangtze River Delta[16,39]	0.5-21.5			35.9-3185	0.4-1.4			0.17-3.51	2.4-117.3	0.43-896
Other watersheds										
Colorado River-Lake Mead, USA[3,36]	0-1.99			88-2040	2-105	2.0-12.0				
Pearl River system, China[14,22,24,38,41,43,46]	0.015-53			20-9597	1.4-7.0	0.2-27.4				
Rhine River, Europe[1,19,21,26,27,30]	0.005-0.022		228-3763	250-11670	0-30 /g	0.2-1.0				
Rize inland waters, Turkey[18]	1.0-13.0		64.2-472					124-489 /g		
Lake Victoria, Tanzania[6,12,13]	0.02-2.19		50-1102	6.5-108		20%				
Braamfontein Spruit, South Africa[9]	0.16-2.08			4-1348	20-97 /g					
Melbourne inland waters, Austral.[29,34,35]	0.03-1.7			4.5-172.7	0.07-1.4	0.7				

limnological research and we demonstrate how these particles could serve as a marker of anthropogenic activities within a catchment area.

In this work, we compiled evidence from articles retrieved through Web of Science using the following search string for years 2010 to 2020, inclusive: *((TS=(microplastic* AND (aquatic OR river* OR lake* OR marine* OR sea OR ocean* OR estuary OR brack* OR *water* OR sediment* OR beach* OR shoreline*)))) AND LANGUAGE: (English) AND DOCUMENT TYPES: (Article)*. Relevant papers among the 3731 articles retrieved were used to summarize some key aspects of our current understanding of microplastic pollution in aquatic environments. Though our focus is on inland waters, data from marine environments were considered for comparison and to gain further insight into ecological impacts likely to occur in freshwater ecosystems. We selected studies with comparable units of microplastic abundance and summarized only those which reported concentrations of plastic particles in the ≤5000µm size range. Finally, we selected the 25 journals that accounted for >70% of the publications on microplastics (according to Web of Science) and used them to calculate research effort on this topic, i.e. the percentage of publications that comprised microplastics studies (Figure 1.2).

1.3 The pervasiveness of microplastics in inland waters

1.3.1 Increasing attention on microplastics in fresh waters

Within the last dozen years, microplastic pollution has become a growing subject of limnological research, beginning with lakes and subsequently expanding to rivers and reservoirs. However, it is clear that marine studies still dominate the microplastic literature, with over 60% of papers published in 2020 focusing on marine systems (Figure 1.2). Likewise, as most published studies have emanated from Asia and Europe, the geographic cover is not

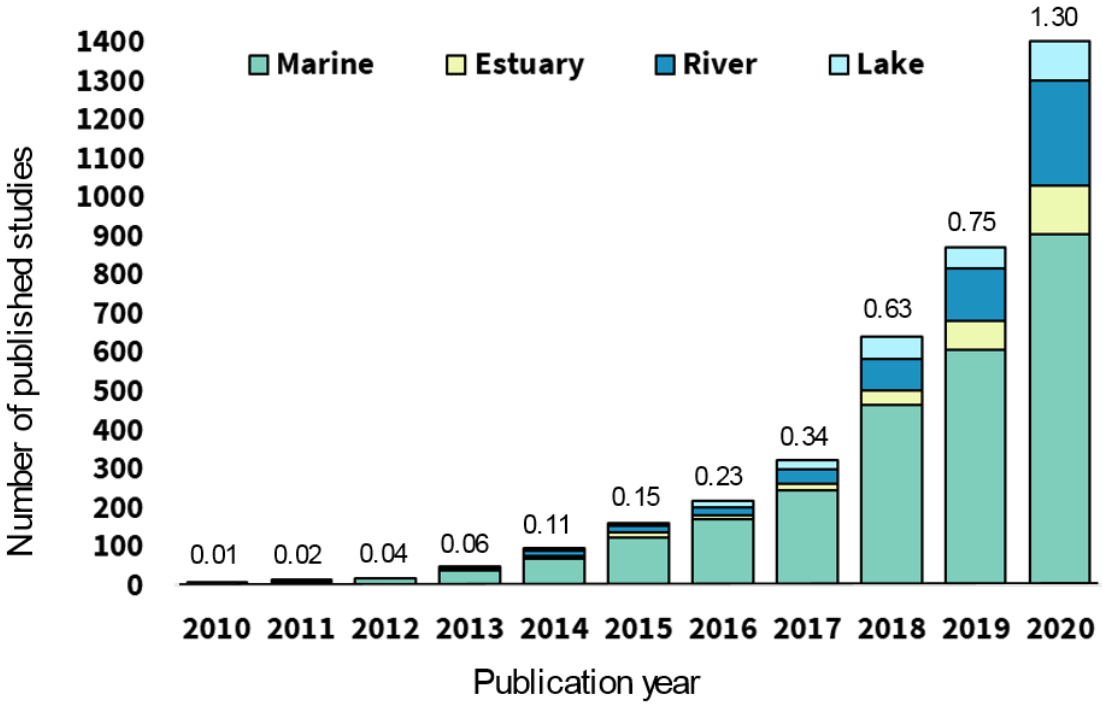

Figure 1.2: Number of studies on microplastic pollution (particles ≤ 5mm) for each water type published between 2010 and 2020, inclusive, yielding a total of 3731 studies. Values above the bars are the percentages (%) of studies on microplastic pollution based on research effort in the 25 journals having the most publications on microplastic pollution (71% of all reviewed studies).

homogeneous. To summarize the state of evidence and illustrate knowledge gaps, we have compiled information acquired since 2010 on reported microplastic densities across matrix types (i.e., on beaches, at the water surface, in the water column, in sediments, and within aquatic organisms; Table 1.1). Our summary shows that the Yangtze River catchment (including Lake Taihu, Lake Poyang, and the Xiangi River) in China is the most extensively studied water body in the world, yet still lacks information on spatiotemporal variation in microplastic concentrations throughout the catchment. Many European countries have reported on microplastic pollution, but less than a quarter of these studies focus on freshwater systems (with

the Rhine River receiving the most attention). In North America, most studies have been conducted in the Great Lakes-St. Lawrence River system. Researchers have begun examining Lake Victoria on the African continent, but many other large inland waters are poorly represented (Tables 1.1 and S1.2). As sources and transport routes of microplastic are more fully described, we can begin to depict the complexity of their biogeochemical cycles in aquatic systems (Figure 1.1). Much work is needed to identify missing or understudied links, including aerial deposits, the role of biota as temporary reservoirs of microplastics, as well as the many possible chemical interactions.

Different components of water bodies contain varying levels of contamination. Microplastic concentration at the water surface is highly dynamic and altered by flow regimes, precipitation, seasonality, and proximity to points of entry (e.g., sewage or storm water effluent, sludge discharge, road or agricultural runoff, litter) (Browne 2015; Horton et al. 2017). Recent environmental analyses show that concentrations in surface inland waters (mean value ~1.9 particles L^{-1}) are lower than estuarine (3.1 particles L^{-1}) or marine environments (16 particles L^{-1}) (Figure 1.3). However, when comparing median concentrations of these same compartments, results ranged from 0.007 particles L^{-1} detected in lotic systems to ~1 particles L^{-1} in lentic systems (Figure 1.3). There is substantial heterogeneity in surface water microplastic abundances in highly modified waterways or areas with high human densities. For example, in the Pearl River (China) concentrations of 8 to 53 particles L^{-1} have been recorded in urban sections, compared with much smaller concentrations (<1 particles L^{-1}) elsewhere along the river (see Tables 1.1 & S1.2; Yan et al. 2019; Fan et al. 2019). Stations along the Gallatin River (USA) recorded 1–68 particles L^{-1} (Barrows et al. 2018); and in Patagonian lakes (Argentina) concentrations were <0.001 particles L^{-1}, with some individual samples reaching 44 L^{-1} (Alfonso

et al. 2020). Water samples from the canals of Amsterdam recorded 48-187 particles L^{-1} (Leslie et al. 2017), while median microplastic concentrations in all lotic environments are below 0.01 particles L^{-1} (Figure 1.3).

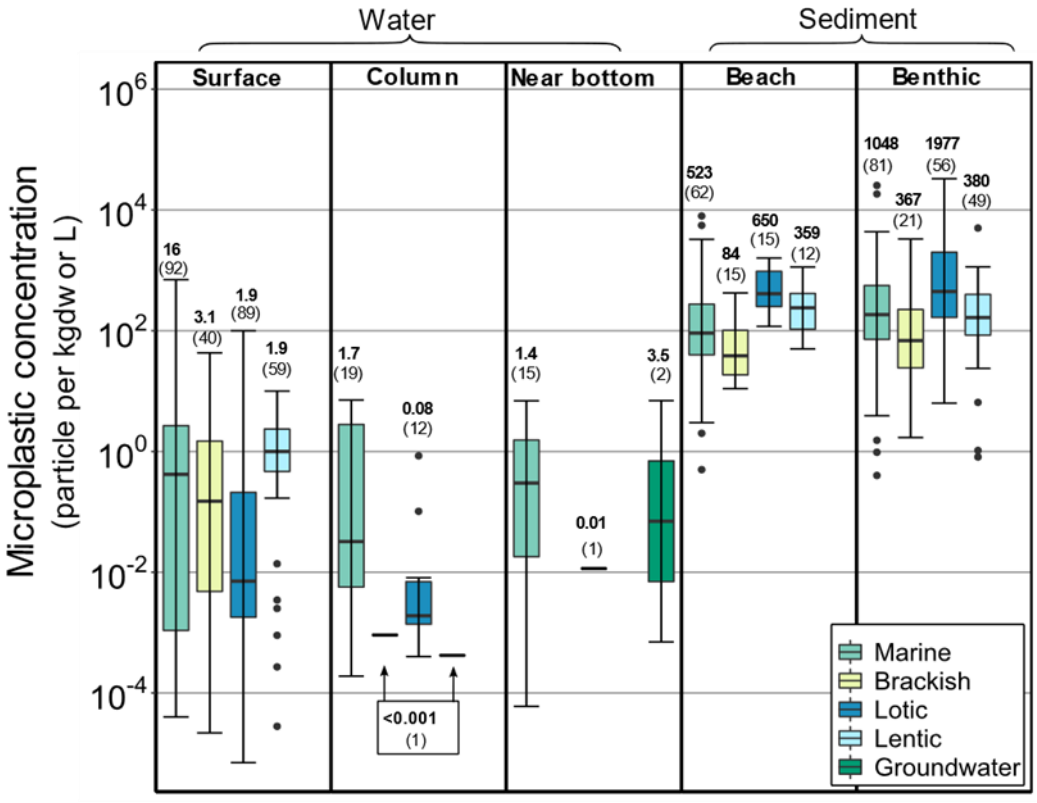

Figure 1.3: Microplastic concentrations (particles ≤ 5mm) in various aquatic environmental matrices from 642 records (n=220 articles). Box-and-whisker plots were constructed from a compilation of mean concentrations provided in the articles; when only a range of values was provided instead of a mean, the lowest values (if above zero) were included. Concentrations are reported as the number of particles per kg dry weight of sediments for beaches and benthic samples, whereas in water samples concentrations are reported as the number of microplastics per liter of water at the surface, within the water column, or near the bottom of the water column. Median lines are shown within the boxplot. Means are indicated as bold values above the boxplot, and values in parentheses indicate sample sizes. Original data and references are available in Table S1.2.

Specific hydrological conditions and sampling season can also play an important role in the abundances reported. Higher flow regimes in streams carry more particles per unit of time, but on average yield lower concentrations than areas with lower flows (Watkins et al. 2019). Seasonal changes in flow regimes (e.g., snow melt, flooding, drought), extreme rainstorms, or anthropogenic control of waterways (e.g., via dams, spillway gates) can alter the transport and concentration of microplastics. Spatial and temporal variation of particle abundance in surface and water column highlight the need for repeated and broader sampling to establish more representative baseline concentrations. Such intensive sampling has rarely been conducted, owing in part to both the time-intensive effort required and the use of incomparable sampling methods. Investments in effort and harmonization of methods is encouraged, so that a comprehensive understanding of microplastic pollution can be achieved. Limnologists would do well to coordinate with marine science colleagues to render data more easily comparable across realms.

An important insight is that the abundance of microplastics floating at the surface is not a reliable indicator of concentrations throughout the water column. For example, microplastics concentrations in six South Korean bays were four times higher at the surface than in the rest of the water column (Song et al. 2018). Over 85% of microplastic studies sample surface waters, and comparisons of abundance along depth profiles are not common (Figure 1.3). In a study that quantified the vertical and longitudinal distribution of microplastics along the Lake Michigan watershed, concentrations of particles in surface waters were found to be generally higher than those measured deeper in the water column, but lower than those in the sediments (Lenaker et al. 2019). The pool of literature we reviewed (Figure 1.3) indicates that lentic systems have higher densities of floating plastics than lotic systems, while the highest concentrations of microplastics

are found along the shorelines and in the benthic sediments. Furthermore, the timing and geographic location of sampling programs also affect surface measurements: the salinity and temperature of water will influence biofilm colonization (Kaiser et al. 2017), causing microplastics to remain afloat longer during colder seasons or at higher latitudes (Chen et al. 2019b). Naturally occurring spring and fall mixing of lakes can also cause shifts in the vertical distribution of particles, suggesting the importance of implementing seasonal sampling protocols. We recommend that different ecosystem compartments be sampled repeatedly along the river continuum from freshwater to marine systems, to produce reliable baseline pollution data in inland waters.

1.3.2 Inland waters as sources and sinks of microplastics

Evidence suggests that rivers contribute substantively to ocean inputs (Schmidt et al. 2017; Meijer et al. 2021), and it has been estimated that rivers shuttle between six thousand to 1.5 million metric tonnes of microplastics to the ocean annually (Boucher and Friot 2017; Weiss et al. 2021). Most plastic transport models assume that no significant retention of plastics occurs along the river network from inland waters to the ocean (Jambeck et al. 2015; Weiss et al. 2021), in spite of ubiquitous river impoundment and myriad other anthropogenic and natural hydrological conditions that can cause the deposition of large numbers of plastic particles within the watershed. Buoyant particles can become trapped on the shorelines of lakes and rivers (Zbyszewski et al. 2014); median concentrations (per kg dry mass) along river shorelines are four to ten times higher than along marine/estuarine coastal beaches (Figure 1.3). Furthermore, microplastics can accumulate on the riverbed, where their concentrations are as much as four to five orders of magnitude higher than in the overlying water column (Figure 3; Castaneda et al. 2014; Crew et al. 2020; Scherer et al 2020). Although a portion of microplastics stored in

riverbeds and on shorelines can be resuspended after dredging activities, storm disturbance, or seasonal flooding events, and be transported downstream (Ji et al. 2021), current mass transport models likely greatly overestimate the flux of plastic to the oceans.

1.3.3 Benthic microplastics as an anthropogenic marker

The quantification and characterization of microplastics within sediments could serve as an indicator of anthropogenic pressure on inland waters. Areas where burial rates allow the preservation of plastics within the sediment layer without mineralization or fragmentation (Hoellein and Rochman 2021), such as the depositional zones of river beds and lakes, can function as a long-term or permanent storage for such particles. Analyses of lake sediment cores have been used to track temporal dynamics in microplastics, which date back to the early 1970s in Lake Ontario sediments (Corcoran et al. 2015) and to the 1950s in a lake in north London, U.K. (Turner et al. 2019). The presence of plastics in sediment layers are sufficiently pervasive and globally distributed that they can be used as an anthropogenic marker horizon in geological and paleolimnological records (Barnosky 2014; Bancone et al. 2020). Plastic debris is already used as a stratigraphic marker in archaeological studies (Zalasiewicz et al. 2016).

1.3.4 Aquatic biota are transient reservoirs for microplastics

From inland waters to marine systems, a growing diversity of aquatic organisms (algae, macrophytes, zooplankton, insects, crustaceans, molluscs, fish, amphibians, birds, and mammals; Figure 4) have been reported to take up microplastics via feeding, drinking, respiration, swimming, and random adherence, among other processes. Contamination levels (number of particles per kg of tissue) of freshwaters and estuarine taxa are within of the same order of magnitude as their marine counterpart (Figure 1.4). According to Covernton et al. (2021),

freshwater fishes are more frequently found with plastics in their guts and with a higher microplastic load per individual than marine fishes. Recently, fish from the Great Lakes recorded the highest load ever reported (Munno et al. 2021), illustrating the need for freshwater biologists to explore the impacts of this emerging stressor.

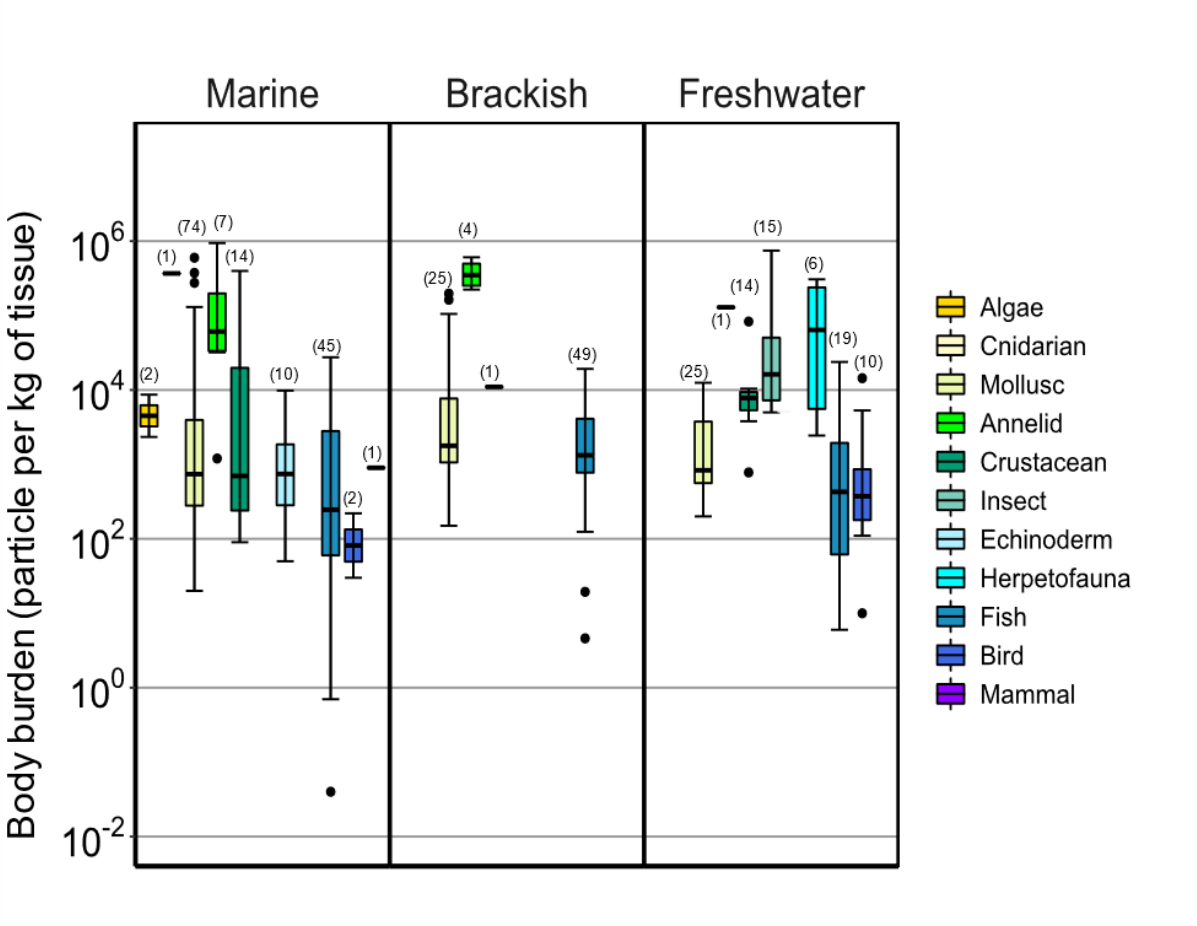

Figure 1.4: The abundance of microplastic particles (≤ 5mm) per kg of tissue recorded for aquatic organisms of different waterbody types. Box-and-whisker plots were constructed from a compilation of mean concentrations provided in the articles; when only a range of values was provided instead of a mean, the lowest values and the highest values were included. Reports of body burden below 0.01 particle per kg were excluded. Numbers in parentheses indicate sample size per group. Original data and references are available in Table S1.3.

Although the mechanisms by which aquatic organisms acquire and retain plastics from their environment are still poorly documented, a comparison of body burdens within a taxonomic group suggest feeding mechanisms and the habitat preference along the watershed continuum could influence the contamination risk for aquatic organisms (Figures 1.5). Benthic invertebrates, especially those associated with depositional areas of rivers, may be more vulnerable to microplastic pollution, being restricted to environments which accumulate and stored microplastics (Figure 1.5). Midge larvae *Chironomus* sp. and oligochaete worms *Tubifex tubifex*, were found with the highest burdens with 370–1200 particles g^{-1} and 129 ± 65.4 particles g^{-1}, respectively (Hurley et al. 2017; Nel et al. 2018). Nearly 60% of larval caddisflies *Lepidostoma basale* use plastic materials to construct their cases incorporating an average of 0.36 ± 0.09 particles per mg of case (Ehlers et al. 2019)- an addition which negatively affects case integrity in ways that could potentially reduce larval survivorship (Ehlers et al. 2020).

Biota act as transient reservoirs for these particles which, after entering an organism, can continue cycling within the animal, be egested, or be transferred through a food web. Retention time within the body depends on particle size and shape, metabolic activity, and the complexity of the animal's digestive tract or gill structure. Particles ≤500 µm in maximum dimension were found in the liver and filets of freshwater fish, suggesting they were translocated from the gut to other organs (Collard et al. 2018; McIlwraith et al. 2021); whereas, by comparison, clay size particles (<5µm) can cross cell membranes and enter the bloodstream, where they can remain for 20 to 48 days (Browne et al. 2008; Farrell and Nelson 2013). Animals with high metabolic rates, like daphniid or gammarid crustaceans, can take up high concentrations of microplastics but usually expel them in their faeces quite rapidly (Mateos-Cardenas et al. 2019; Elizalde-Velazquez et al. 2020). However, particles tend to be retained for longer periods of time in fish

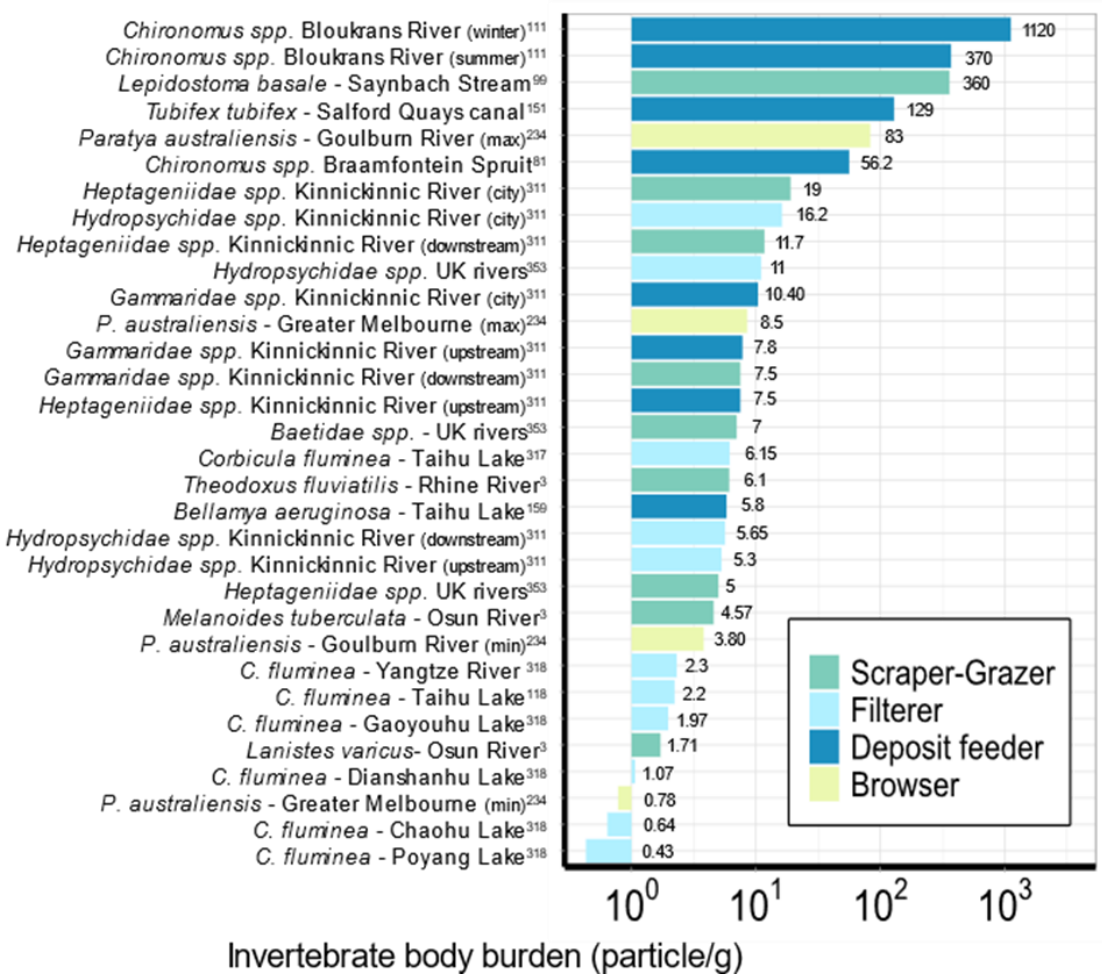

Figure 1.5: Comparison of the body burden (microplastic particle per g of tissue) of freshwater invertebrates found along rivers. Only organisms which incorporated microplastics (particles ≤ 5mm) to their body via ingestion or essential structures (e.g. the case of *Lepidostoma basale*) were used for this figure. Colours represent different functional groups. Species are listed individually with the locations sampled and the reference number. Original data and complete references are available in Table S1.3.

with irregular body shapes (Hoang and Felix-Kim 2020) or organisms with complex digestive tracts (Welden and Cowie 2016). When no translocation occurs, plastic particles are egested within 24–72 h (Scherer et al. 2017; Redondo-Hasselerharm et al. 2018), which is sufficient time for a contaminated animal to be eaten by a predator and thus transfer their plastic load to the next

trophic level (Chae et al. 2018). Cedervall et al. (2012) demonstrated trophic transfer of 25 nm polystyrene particles that were taken up by green algae (*Scenedesmus* sp.) and passed on to herbivorous water fleas (*Daphnia magna*), which were subsequently consumed by fish. Regardless of the mechanism, once microplastics are egested, the cycle can begin anew and the same particle can be re-ingested (Hoang and Felix-Kim 2020) or passed from one individual to another for an indeterminant period of time. Therefore, even without evidence of biomagnification (Covernton et al. 2021), a single plastic particle could cycle multiple times in and out of food webs with unknown consequences to its hosts.

1.4 Ecological impacts on freshwater ecosystems

Microplastics affect many biological and physico-chemical processes of significance for organisms, communities, and ecosystems. Dose-dependent biotic responses to plastic pollution have been shown for diverse groups including algae (Gambardella et al. 2018), suspension feeders (Pedersen et al. 2020), deposit feeders (Fueser et al. 2019), detritivores (Au et al. 2015), and predators (Kim et al. 2019). Yet, meta-analyses of the ecotoxicological effects of virgin plastics on organisms find that acute endpoints generally occur at doses higher than those typically observed in natural habitats (Foley et al. 2018; Cunningham and Sigwart 2019; Bucci et al. 2020) and biotic responses are modulated by the duration, particle size, types of exposure conditions, and associated contaminants.

We provide a non-exhaustive summary of the physical, physiological, and ecotoxicological effects reported for freshwater taxa in Table 2. Not all organisms tested displayed negative effects, but even small effects at the cellular or molecular levels can have repercussions at the community or ecosystem levels. For example, increased oxidative stress in plastic-exposed cyanobacteria promotes microcystin synthesis and release, thereby inducing

toxic algal blooms (Feng et al. 2020). Other small changes, such as delays in aquatic insect emergence and reduced numbers of adults following exposure to environmentally-relevant microplastic concentrations (Ziajahromi et al. 2018), or the ontogenic transfer of plastics from mosquito larvae to terrestrial adults (Al-Jaibachi et al. 2019), further highlight potential repercussions of this form of pollution across the aquatic-terrestrial ecotone. A full accounting of the presence of microplastics and their associated chemicals is crucial to understand the impacts of these stressors on freshwater systems.

1.4.1 Biodiversity

The surfaces of plastic particles host communities of micro-organisms whose composition is sensitive to polymer type, size, and environmental conditions. Among these aggregates, bacterial communities on plastics have lower species abundance and diversity (richness, evenness) than those from surrounding natural substrates (McCormick et al. 2016; Miao et al. 2019) - except for microplastic-biofilms in oligo-mesotrophic lakes, whose functional richness was found to be higher than biofilms on natural substrates (Arias-Andres et al. 2018). Plastic biofilms tend to be dominated by particular taxa, including polymer-degrading bacteria (e.g., *Pseudomonas*), bacterial pathogens (e.g., *Arcobacteria*, *Vibrio*), antibiotic-resistant bacteria, as well as parasitic and saprophytic fungi (Kettner et al. 2017; Sun et al. 2020). Plastic waste offers novel media on which some micro-organisms thrive and could thus signal an attractive food source for higher-level consumers (Battin et al. 2003). However, plastic-bound biofilms may not offer the same food quality as biofilms on natural materials (Vosshage et al. 2018).

Considering that their plastic substrate is durable by design, colonizers have a stable surface on which to develop, rendering buoyant plastic debris of all sizes as potential transport

vectors for non-native species, pathogens, and drug-resistant bacteria (Wang et al. 2021). Freshwater environments subjected to effluent from WWTP receive regular inputs of microplastics, with estimated daily discharges ranging from 50,000 to nearly 15 million particles in the United States (Mason et al. 2016). The plastic-associated bacterial communities from these WWTP exhibit higher gene exchanges, making microplastic a suitable environment for the development of antibiotic- and metal-resistant genes, as well as vectors to disperse these bacteria downstream (Eckert et al. 2018); in fact, multidrug-resistant *Escherichia coli* strains (Song et al. 2020) were found to be carried by microplastics across different environments.

Additionally, evidence from both marine and freshwater habitats suggests that continuous exposure to high microplastic concentrations near effluents can reduce community diversity. Repeated exposure to 80 $\mu g\ L^{-1}$ of microplastics caused minimal impacts on oyster health and biological functioning, but the benthic community within oyster beds experienced a 1.5-fold decline in numerical abundance (Green 2016). Similarly, benthic communities exposed to micro-synthetic polymers for 15 months had altered community composition, whereby some species' abundances were affected positively, and the more sensitive taxa were affected negatively (Redondo-Hasselerharm et al. 2020). Benthic communities are expected to be disproportionately affected, as their habitats typically contain the most contaminated aquatic matrix.

1.4.2 Ecosystem productivity and functioning

The mineralization of plastics can alter concentrations of key nutrients that affect the growth and composition of primary producers of aquatic systems. Bacterial strains, fungi, microbial assemblages, and biofilm communities can mineralize microplastics and reduce their mass by up to 20% (Yuan et al. 2020). In the photic zone, DOC compounds are released from plastics into the water as a by-product of photodegradation, which in turn can stimulate the

activity of heterotrophic bacteria that degrade natural and anthropogenic polymers (Romera-Castillo et al. 2018; Zhu et al. 2020). Heterotrophic bacterial communities originating from boreal humic lakes containing recalcitrant sources of carbon are effective at mineralizing and using plastic-derived carbon for cell growth. Once released, plastic-derived carbon has been found in the cell membrane fatty acids of mixotrophic algae and herbivorous cladocerans, demonstrating that the microbial community can transform polyethylene molecules into nutritional biomolecules and pass them onto higher trophic levels (Taipale et al. 2019). Under specific conditions and microbial assemblage, a small fraction of the microplastic load accumulating in aquatic systems can become a new source of carbon to their food webs.

The type and density of polymers found in freshwater systems can further influence nutrient availability. For example, some polymers (e.g., polyurethane foams and polyactic acid) promote nitrification and denitrification processes in sediments, whereas others (e.g., polyvinyl chloride) inhibit both processes (Seeley et al. 2020). Changes in denitrification activity depends on whether the plastic surface and anaerobic conditions combine to promote the growth of denitrifying bacteria (Li et al. 2020a), which can accelerate the conversion of nitrate to nitrite and subsequently to N_2O, NO, or ultimately N_2. As the biofilm disintegrates, it releases P and N from its plastic substrate (Chen et al. 2020). The presence of specific plastic polymers in the riverbed sediment along Brisbane River negatively correlated with total N and P levels, while higher abundances of microplastics positively influenced the total carbon concentration levels measured (He et al. 2020). Overall, the presence of some microplastic polymers will induce the formation of specific biofilms, which can alter nutrient ratios in freshwater systems.

The presence of plastics also affects the performance of aquatic primary producers and herbivores (Table 1.2). Increasing exposure to microplastics is linked to lower rates of leaf litter

decomposition by caddisflies as well as by microbial and fungal detritivores (Seena et al. 2019; Lopez-Rojo et al. 2020). Laboratory exposures to high doses of nanoplastics (<1µm) have been observed to reduce population growth, chlorophyll content, and photosynthetic activity of freshwater algae (Besseling et al. 2014; Li et al. 2020b). Plastic-induced reductions in the growth, development, and reproduction of zooplankton and small invertebrates can limit the abundance of secondary producers (Besseling et al., 2014; Ziajahromi et al., 2018). Conversely, plastic leachates induced increased photosynthetic activity in some microalgal species (Chae et al. 2020), emphasizing the complexity of potential responses to these pollutants (see Table 1.2). Although this has not been studied directly, plastic-induced changes in the feeding behaviour and habitat use by consumers (Cedervall et al. 2012; Chae et al. 2018), changes in shoaling behaviour (Mattsson et al. 2017), and the performance of top predators (de Sa et al. 2015), could conceivably alter trophic interactions sufficiently to affect ecosystem productivity in areas of high microplastic concentrations.

1.4.3 Nutrient cycling

Small plastics (300-4400µm) tend to aggregate with biogenic materials or suspended sediments (Mohlenkamp et al. 2018); thus, they are often colonized by micro-organisms and accumulate metals or minerals. The microbiome biomass alters the density of the microplastics and can accelerate the sinking of nutrients and other chemicals bound to these particles (Long et al. 2015). Nevertheless, the formation of biofilms can be insufficient to sink particles. For example, in a stratified reservoir, particles remained buoyant until a seasonal mixing event resuspended enough organic materials, cyanobacteria, and iron particles from deeper waters to allow colonization and aggregation of these particles, thereby inducing the sinking of floating plastic debris (Leiser et al. 2020).

Impacts related to the buoyancy of microplastics are also being revealed in freshwater invertebrates. The ingestion of microplastics by the sessile cnidarian *Hydra attenuata* can reduce the animal's specific gravity to the point where it loses its ability to remain attached to substrate (Murphy and Quinn 2018). Similarly, zooplankton faecal pellets in marine systems were observed to sink more slowly when plastics were incorporated into the waste material via ingestion (Cole et al. 2016). Changes in buoyancy of particulate matter imply potential broader impacts on sedimentation rates and nutrient cycling for profundal communities, which depend on nutrient inputs from the pelagic zone.

The highest concentrations of microplastics are found in benthic sediments where maximum values can exceed 10 000 particles kg^{-1} dry mass in rivers and 5000 particles kg^{-1} dry mass in lake sediments (Figure 1.3; Table S1.2). Such concentrations can negatively affect the growth of chironomid larvae, reducing their body length and head capsule size (Ziajahromi et al. 2018), which could impact their bioturbation activities in areas of lakes that tend to be oxygen limited. When offered a choice, ephemerid mayfly larvae preferred burrowing amongst microplastic substrates instead of natural sediments (Gallitelli et al. 2021), and tubificid worms retained ingested microplastics for longer periods than other particulate materials within sediments (Hurley et al. 2017). These results further demonstrate that key bioturbating species interact distinctly with plastic contaminated sediments. Among these species, tubificid worms could prove to be important biomonitors of plastic pollution in benthic habitats; they can accumulate higher loads while suffering negligible effects from polyethylene particle exposure (Redondo-Hasselerharm et al. 2018; Scopetani et al. 2020).

Bivalves are key players in shuttling suspended plastics and associated contaminants to benthic habitats. Through filtration and biodeposition, they transfer micro- and nanoplastics from

Table 1.2: The ecotoxicological effects of microplastic exposure on inland water organisms compiled per taxonomic group. Superscript numbers indicate the reference(s) associated with these outcomes. Complete references are provided in Table S3 (Supplementary Material). Symbols indicate the direction of the effects observed: ↑ indicates an increase in the effect, ↓ indicates a reduction in the effect, • indicates no effect was detected, Δ indicates that a change in the parameter was observed (other than increase or decrease), and ✓ indicates the exposure to microplastics caused the effect. The presence of multiple symbols indicate multiple conditions were observed across trials or experiments.

	Movement	Feeding	Digestion	Predatory performance	Respiration	Growth	Development	Protection/Defence	Cell/ organ damage	Reproduction	Mortality	Oxidative stress	Inflammation control	Antinviral protection	Toxicity control	Metabolic activities	Gene damage	Neurotoxicity	Productivity	Nutrient cycling	Toxic blooms
Duckweeds [94, 169, 217] *(Lemna, Spirodela spp.)*						↑•													↑•		
Submerged plant [328, 369] *(Elodea, Myriophyllum, Utricularia spp.)*						↓													↓•		
Cyanobacteria [87, 108, 109, 357] *(Microcystis, Synechoccus spp.)*						↓			✓			✓							↓	↑	↑
Green algae [28, 32, 48, 55-7, 189, 202, 204, 214, 241, 312, 357] *(Chlamydomonas, Chlorella, Pseudokirchneriella, Scenedesmus spp.)*						↓↑•					•				•				↓↑•	↑	
Daphnids [28, 55, 72, 86, 143, 157, 161, 164, 180, 205-6, 235, 243, 271, 274, 279, 299, 300, 372] *(Ceriodaphnia, Daphnia spp.)*	↓	↓				↓↑	Δ			↓↑	↑↓	✓							↓↑		
Hydrozoan *(Hydra sp.)* [230]		↓	↓																Δ	Δ	
Dipterans *(Culex, Chaoborus spp.)* [4, 5, 80]		•		•			•			↓	•										
Chironomids *(Chironomus spp.)* [308, 373]						↓				↓	↑							↓			
Caddisflies [101, 209] *(Sericostoma, Lepidostoma spp.)*		↓			↓	•		↓			↑								↓	↓	
Coleoptera (Cybister sp.) [181]	↑	↓		↓																	
Isopod *(Asellus sp.)* [269]						•					•										
Amphipods *(Gammarus, Hyalella, spp.)* [15, 34, 186, 217, 269, 316, 345]	↓	•	↓↑		↓	•↓	•	↓↑		↓	↑•					↓↑•					
Snails *(Potamopyrgus sp.)* [156]							•			•											
Bivalves [131, 213, 246, 256, 269, 283] *(Corbicula, Dreissena, Sphaerium spp.)*		↓			•	•			•	↓•	•	√•					•	•✓		↓	
Annelids [151, 269, 297] *(Lumbriculus, Tubifex spp.)*			↓↑			•	•			•	•	•									
Carps *(Barbodes, Carassius spp.)* [55, 284]		↓	↓						✓				✓								
Zebra fish [22, 166, 195, 204, 211, 251, 335] *(Danio rerio)*			↓			•	•		✓•			↑	✓			↓•					
Tilapia *(Oreochromis niloticus)* [92]																		✓			
Ricefish *(Oryzias spp.)* [56, 250]	↑Δ					↓									✓						
Discus *(Symphysodon aequifasciatus)* [351]												✓									
Chub *(Zacco temminckii)* [56]	Δ								✓												
Sturgeon *(Acipenser transmontanus)* [283]		Δ																			
Bass *(Dicentrarchus labrax)* [104]												✓		↓							
Minnow *(Pimephales promelas)* [102]																					
Goby *(Pomatoschistus microps)* [245]			↓								↓					✓		✓			

the water column to the sediments, thus acting as a biological pump (Van Cohen et al. 2021). Their normal activities—which contribute significantly to nutrient dynamics in lakes and rivers (Vaughn and Hakenkamp 2001)—could be altered through plastic exposure (Table 1.2). For example, bivalves have lower recruitment success (Sussarellu et al. 2016) and reduced filtration rates (Pedersen et al. 2020) in the presence of microplastics. Therefore, changes in bivalve biomass and functioning in response to plastic pollution could affect water column turbidity and alter the amount of organic and inorganic material deposited to benthic habitats. Since bivalves are among the organisms reported to have the longest internal retention of microplastic particles (Table S1.3) and are rather tolerant to plastic contamination (Magni et al. 2018); their bodies could also serve as incubation chambers for the desorption of toxic substances associated with plastic particles, but this hypothesis needs to be examined further (Hoellein et al. 2021).

1.4.4 Contaminant cycling

Smaller weathered polymer particles have a greater surface area-to-volume ratio than larger, unweathered plastics, thereby offering proportionally more substrate for microbial colonization and the sorption of pollutants (Menéndez-Pedriza and Jaumot 2020). The ecotoxicity of microplastic particles varies depending on their characteristics (e.g., shape, size, crystallinity, chemical composition) and adsorbed substances (Lambert et al. 2017). Toxicological risks stem from the particles themselves, their biofilm (Rummel et al. 2017), the release of contaminants (persistent organic pollutants, heavy metals, pharmaceuticals) adsorbed by the plastic, and the leaching of additives or chemicals associated with its polymer matrix (Rochman et al. 2013b; Menéndez-Pedriza and Jaumot 2020).

An important research gap is the influence of micro- and nano-sized plastics on contaminant transfer to animals. Under laboratory conditions, microplastics loaded with

benzo[a]pyrene, a polycyclic aromatic hydrocarbon (PAH), were transferred trophically from contaminated *Artemia* nauplii to zebrafish, and showed evidence of desorption within the predator's intestine (Batel et al. 2016). This example demonstrates the possibility of plastic-mediated contaminant transfer within freshwater food webs. In some cases, co-exposure of plastics and pollutants increased contaminant transfer to experimental fish by as much as 2.6 times the concentration found in the head and viscera when exposed to bisphenol A alone (Chen et al. 2017). The co-exposure of microplastics with antidepressants also amplified the drug's bioaccumulation factor by 10-fold in another freshwater fish (Qu et al. 2019). With over 200 organic chemicals being reported to associate with marine plastics in the field (Hong et al. 2017), it seems likely freshwater plastics would also sorb an array of chemicals, though few studies have thus far demonstrated it. Given that the majority of the contaminants able to sorb to plastics are mutagenic, carcinogenic, teratogenic, or endocrine disruptors (Alimi et al. 2018; Fred-Ahmadu et al. 2020), the ecotoxicological potential of small plastic particles (<5000μm) merits increased attention.

Context dependencies challenge risk evaluation of the role of microplastics in contaminant cycling. The sorption-desorption response is governed by ambient conductivity, pH, salinity (Holmes et al. 2014; Llorca et al. 2018) and dissolved organic matter content in water (Chen et al. 2019a). For example, Ziajahromi et al. (2019) observed that polyethylene particles reduced the availability of a chemical insecticide (bifenthrin) to chironomid larvae, because most of the chemical compound was sorbed to the plastic. However, when the microplastics were present with organic carbon, the toxicity of the pesticide was no longer reduced, suggesting water chemistry and DOC concentrations can mediate the role of microplastics as chemical vectors.

In comparison with sediments, the sorption of trace metals (e.g., Cd, Cs, Zn) is lower (Holmes et al. 2014; Johansen et al. 2018; Besson et al. 2020), PAHs are equal or higher (Teuten et al. 2007; Bartonitz et al. 2020), and mercury concentrations are at least one order of magnitude higher on plastics (Graca et al. 2014). However, the sorption of several elements (Holmes et al. 2014) and antibiotics (Li et al. 2018; Guo and Wang 2019) varies with salinity, suggesting that microplastics in freshwater environments may be more effective vehicles for some metals and for the spread antibiotic resistance. Likewise, the microplastic-associated biofilm community can induce higher dissipation rates of contaminants (e.g., DDTs, PAHs) and enhance their biotransformation (Wu et al. 2017).

1.5 Integrating microplastics into limnology

Given the ubiquity, pervasiveness and emerging impacts of microplastics in lakes and rivers, we contend that they should be recognized by limnologists as a distinct particle component whose concentration is an ecologically-relevant parameter. A plastic lexicon is slowly being developed (Haram et al. 2020), but standard definitions remain to be developed and consistently applied. To promote strong policies applied beyond the boundaries of a single nation or discipline, the adoption of an international framework for plastic debris is justified (Hartmann et al. 2019). One way to encourage limnologists to incorporate microplastics in their standardized sampling protocol is to integrate the topic into their lexicon (perhaps using a distinct term, e.g. *plaston*, to distinguish this particle type from seston, plankton, or neuston).

Mitigating the environmental impacts of plastic pollution will require a multidisciplinary limnology that integrates, *inter alia*, socioeconomics (sources of microplastics), hydrology (physical dynamics of non-biodegradable particles), environmental chemistry, and ecotoxicology. Proposed guidelines are emerging to direct microplastic research with the aim of

increasing reproducibility and comparability between studies (Cowger et al. 2020). We recommend these as a starting point for integrating microplastics research into limnology.

To develop accurate risk assessments that describe the impacts of microplastics independent of interactions with other aquatic stressors, we must have sufficient data to establish reliable exposure scenarios that can be repeated and examined under controlled conditions. Currently, there is a large discrepancy between doses of microplastics used in laboratory assays and the levels recorded in the field (Cunningham and Sigwart 2019; Bucci et al. 2020; O'Connor et al. 2020).

Another issue with choosing environmentally-relevant concentrations for experimental studies is that field concentrations are typically based on samples from a single matrix (e.g., water surface, benthic sediments), and therefore do not account for organisms interacting with more than one matrix. Exposures to 100 particles L^{-1} or per kg of sediment are implicitly considered to be realistic scenarios (Cunningham and Sigwart 2019), but the highest concentrations recorded in a single compartment of the environment can be a misleading representation of the bioavailability of microplastics. For example, a fish with an ontogenetic diet shift (e.g., yellow perch, *Perca flavescens*), may feed on zooplankton in the water column during its larval stage; on benthic invertebrates on the sediments during its juvenile stage; and on small pelagic fishes when it reaches sufficient adult size. Thus, throughout its life it interacts with multiple potential sources of plastic contamination in the water, sediments, and in contaminated prey, sometimes simultaneously. A useful goal would be the compilation of realistic natural exposures within a plastic budget, by isolating sources, pathways and recipient organisms (Horton and Dixon 2018; Bank and Hansson 2019; Waldschlager et al. 2020; Hoellein and Rochman 2021).

To date, few water bodies have been monitored with sufficient resolution to encompass the various matrices in which organisms and microplastics interact (see Table 1.1), especially for those animals using multiple habitats during their life cycle. Despite increasing numbers of studies published on microplastic pollution in marine and inland waters (Figure 1.2), there are repeated studies for only a few model organisms—mainly daphniid waterfleas, bivalves, and zebrafish (Table 1.2). Experimental studies have generally been conducted on individual organisms using smaller particles sizes and greater concentrations than what is recorded in the field, and these are presented as pristine particles or associated with a single sorbed contaminant. Studies incorporating multiple foodweb links as well as realistic concentrations and contaminant exposure are needed to understand how biota retain, bioaccumulate, biomagnify, and transfer plastics and their contaminants in aquatic food webs (Schiavo et al. 2018; Provencher et al. 2019; Wang et al. 2019). Only under these circumstances will it be possible to bridge the gap between laboratory and field studies. However, some conditions, such as controlling the colonization of plastics by micro-organisms, may be more difficult to apply. We must explicitly account for the scope and limitations of conditions in each experiment to assess the risk posed by microplastics as an individual stressor.

1.5.1 Toward standard practices and biomonitoring

Limnologists routinely collect water, plankton, and sediment samples, along with a plethora of environmental parameters; the incorporation of microplastic sampling would reveal baseline levels of contamination as well as temporal and spatial differences in the risk of exposure to organisms. However, determination of the abundance and distribution of microplastics is constrained by our ability to capture and detect the particles in various environmental matrices. Consequently (owing to the limitations of standard plankton meshes,

sediment sieves, or other equipment selected by researchers), 30% of samples collected in the water column to date have been limited to particles > 300µm, whereas 40% of sediment samples use sieves of ~60µm (Table S1.2). Given that a filtration mesh pore size of 300µm is typically used for processing water samples, this can lead to an underestimate of smaller microplastic concentrations by up to four orders of magnitude (Covernton et al. 2019). We are certainly underestimating the fraction comprised by nanoplastics (particles <1 µm) in many reported samples.

The continuous disintegration of plastics into progressively smaller particles in the aquatic environment suggests that the abundance of microplastics would be several-fold more numerous as particle sizes decrease, a phenomenon demonstrated in samples from surface water (Kooi and Koelmans 2019), sediments (Yang et al. 2021) and biota (Roch et al. 2019). New analytical techniques allow more effective characterization of micropolymers under 20µm (e.g., µ- Raman, RT-Raman) but they have been rarely used in the past because of time requirements, costs, and cross-laboratory reliability (Cabernard et al. 2018; Muller et al. 2020). Considering that aquatic invertebrates are reported to retain higher numbers of particles that can be translocated within their bodies and suffered stronger negative effects when ingesting smaller particles < 63µm (Jeong et al. 2016; Ziajahromi et al. 2018; Franzellitti et al. 2019), researchers must strive to harmonize sampling techniques and quantify smaller fractions of plastics in natural environments.

There is a need to standardized practices and enforce strong quality assurance and control measures to allow data to be reproducible and comparable across aquatic systems. Many authors have recommended guidelines and best practices to improve initial study design and ensure a minimum standard quality for microplastic data (Twiss 2016, Connors et al. 2017, Hung et al.

2020, and Miller et al. 2021). Cowger et al. (2020) compiled a checklist of elements for researchers to provide comparable information. We summarized the main recommendations under nine steps.

1) Provide basic information on the subject or environmental matrix of interest (e.g., watershed characteristics, limnological parameters, full taxonomic name), the timing and location (coordinates) of sampling.
    a. Collect duplicate or triplicate environmental samples.
    b. For sediment samples, basic characterization should be performed (% organic content, granulometry of riverbed) (Enders et al. 2019).
    c. Pilot studies should be performed to develop estimates of measurement precision (eg. ensure sample sizes are adequate to address the research question).
2) Describe sampling techniques and equipment used (e.g., mesh size, volume, surface area, flow rate, mass, duration of collection, depth) to allow conversions across samples measured.
3) Report sufficiently detailed methodological steps of the extraction procedure to allow replication.
4) Clarify the quality assurance and control procedures followed (e.g., cotton lab coats, washing/decontamination procedures, use of air filtration unit, application of blanks, use of positive/negative controls) and report contamination levels.
5) Classify plastic particles based on their morphological features: colour, shape and size and provide definitions or reference for the classification criteria.
6) Verify polymer composition (e.g., polystyrene, polyethylene, polyester, nylon) and provide details on the analytical technique, analysis employed, and data transformation (Andrade et al. 2020).

7) Measure the efficacy of the lab methodology used by reporting retention rates after spiking subsamples with known polymers of relevant size and shape.

8) Specify if results presented (tables, figures, text) were corrected for contamination or adjusted based on method efficacy retention rates of the methods, and account for the size range of the particles defined by each size classes.

9) Declare main findings within the limits of the experiment and account for limitations of the study.

To account for potential spatiotemporal changes to plastic concentrations in the environment, biomonitoring should employ appropriate sentinel organisms. Potential taxa that have been identified to monitor the presence of particles of <300μm sizes include mosses (Capozzi et al. 2018), bivalves (Su et al. 2018; Merzel et al. 2020), chironomids (Nel et al. 2018), tubificid worms (Redondo-Hasselerharm et al. 2018; Scopetani et al. 2020), and fishes (Su et al. 2019). Appropriate sentinels should be selected based on their relative abundance in different sectors (lakes, rivers, estuaries) and ecosystem compartments (benthos, plankton), their site fidelity, lifespan, and ability to tolerate contamination a few orders of magnitude higher than currently found in nature. It is also essential to know the organism's dose-response to microplastic pollution and whether its natural history characteristics influences uptake, retention and egestion of these particles. Further studies are needed to establish how well the organism indicates ambient pollution conditions and thus whether they could truly serve as sentinels (Doucet et al. 2021; Hoellein et al. 2021).

1.5.2 Interactions between microplastics and multiple stressors: a major research gap

Given the burgeoning influence of anthropogenic stressors (e.g., climate change, urbanization, river impoundment, nutrient pollution, invasive species) on aquatic environments, synergies between stressors and the fate of plastics should be explored. Under climate change,

for example, increased frequency of extreme weather events (e.g., strong winds or heavy precipitation) can exacerbate the propagation of microplastics globally via wind dispersion (Figure 1.1A), increased surface runoff (Figure 1.1BC, E), increased untreated waste water release (Figure 1.1D), or by increased flooding events and erosion which can re-suspend some of the microplastics stored on shorelines or in riverbeds, and transport them downstream (McCormick and Hoellein; 2016; Tibbetts et al. 2018; Hitchcock 2020; Ockelford et al. 2020). Some bateria, virus or invasive micro-organisms colonizing plastic, could be distributed faster and farther downstream as a result of increased flow, thereby altering the distribution of potential pathogens (Hoellein et al. 2017) and invaders.

Researchers have begun to test the ecological impacts of microplastics under elevated temperatures expected under climate warming. As temperature increases beyond the optimal thermal regimes, key functional groups could become more sensitive to microplastic pollution. Under elevated temperatures, the tolerance of daphnids to microplastic exposure decreased by three to five orders of magnitude (Jaikumar et al. 2018), while short-term exposure to environmentally-relevant concentrations altered the metabolism of a freshwater detritivore *Gammarus pulex*, (Kratina et al. 2019).

Elevated water temperatures also amplify the colonization of suspended particle by micro-organisms (Villanueva et al. 2011). Higher colonization rates by assemblages capable of mineralizing microplastics would enhance their role in degrading plastics (Figure 1G). This fragmentation can be further exacerbated by stronger currents, more intense sunlight, thus more physical weathering of particles. Moreover, elevated water temperatures could conceivably select for stress-resistant communities and accentuate microplastic-biofilm interactions that influence nutrient and contaminant cycling. Finally, the relationships between climate change, sediment re-

suspension, and microplastic pollution, could conceivably lead to mutual reinforcement and magnified eutrophication in shallow lakes (Zhang et al. 2020). Clearly, the interactions of microplastics and other stressors in inland waters is a potentially fertile area of research highly relevant to limnologists.

1.6 Conclusions and recommendations

Microplastics are increasingly prevalent in the waters and sediments of the world's lakes and rivers. Given their ubiquity and environmental persistence, microplastics in the water and sediments of aquatic environments can be recognized as a distinct particle component whose concentration is an ecologically significant parameter that should be monitored routinely by limnologists. Standardized limnological protocols are needed to measure micro- and nano-sized plastic particle concentrations. Such data are crucial for *i*) environmental assessments; *ii*) informing policy for managing plastic pollution; and *iii*) building accurate models of habitat quality, the fate and transport of plastic pollution, and contaminant transfer to freshwater biota. To facilitate the integration of microplastics into limnological research, we propose the following objectives:

- Develop harmonized sampling and extraction protocols that account for the diverse forms of plastics, and that are applicable across environmental matrices, to generate comprehensible and reproducible data.
- Encourage multidisciplinary approaches to studying microplastic pollution (e.g., socioeconomics, landscape ecology, environmental chemistry, ecotoxicology), thereby fostering collaborations toward understanding the sources, transport, fluxes, and fate of

plastic in inland waters. These efforts should include adopting standard definitions internationally and a universal lexicon for plastic debris.

- Identify appropriate model sentinel species to monitor the spread, distribution and accumulation of plastics, and to assess risks on different components of aquatic ecosystems.
- Create plastic budget models to estimate the true bioavailability of plastics to organisms and include associated pollutants and organisms in ecotoxicological studies to bridge the gap between field and laboratory exposure conditions.
- Investigate synergistic interactions between aquatic biota, microplastic pollution and other anthropogenic stressors (e.g., climate change, physical habitat/landscape/hydrological alterations, nutrient pollution, chemical pollution).

Microplastics in aquatic systems should not be the exclusive domain of ecotoxicologists but should be recognized by aquatic scientists in general—and thus be included in the fundamental training of students in the field. Limnology courses and workshops are the most obvious starting points for encouraging best practices in monitoring and reporting microplastic concentrations and for promoting an understanding of their significance. However, interdisciplinary communication and analyses are needed to set the issue of microplastics in inland waters into a more global context.

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1.8 Chapter 1 Supplementary Materials

All Supplementary Materials for Chapter 1 can be found with the published journal available at: https://cdnsciencepub.com/doi/10.1139/er-2021-0048

Table S1.1: Data used to compile Table 1.1. Reference numbers are in column A and full reference information is described under column N.

Table S1.2: Data describing the concentrations of microplastics reported from field studies on environmental aquatic matrices (at the water surface, in the water column, in the water near the bottom, in beach sediments or benthic sediments). Concentrations were reported in original units than converted to the number of microplastic per litre for water samples or per kg dry weight for sediments (column U). When concentrations were available in published figures, values were extracted using the DigitizeIt software. To convert sediment records from a number of microplastic particles per kg of dry sediments to a number of particles per cubic meter of sediments, we used the density of sediments 2.17 kg per dm^3 reported from the Elbe River (Scherer et al. 2020). Values are presented under column Z. We acknowledge there is a potential range of error around this conversion factor with some rivers having higher sediment densities and others having lower densities. Size limits (column M) represent the limitation of the study based on sampling methodologies (e.g., a water sample collected with a mesh of 355 microns would have a value of 355, while a bulk sample digested and filtered using a mesh of 1 micron has a value of 1).

Table S1.3: Data on microplastic concentrations in biota. Studies from 2010-2020 were compiled and specific information for each study was recorded. Microplastic uptake by aquatic organisms are listed per species. Subsets of this dataset was used to produce figures 1.4, 1.5, and tables 1.1 and 1.2. Metadata for this table is listed under S2.4.

S1.4 Metadata information for supplementary Tables S1.1 to S1.3.

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Connecting statement between Chapter 1 & 2

Following my review of the literature (Chapter 1), it was evident that microplastics are abundant in different environmental matrices of aquatic systems (e.g., water, sediments), while aquatic organisms act as transient reservoirs for these particles. As organisms often interact with both environmental and biotic components of aquatic systems, the fate of microplastics could be regulated by the presence and interactions of species of a food web. In Chapter 1, I advocated the design of laboratory experiments that are environmentally relevant by using community modules to clarify microplastic interactions and vectors of contamination within food webs. With these goals in mind, for Chapter 2, I used a community module composed of three abundant and widespread species with distinct roles common in freshwater food webs. This approach considers differences in natural history and known interactions amongst species. Herein, I conducted experiments for each pathway of uptake and transfer, which allowed me to explore 1) the effects of microplastic concentration, environmental route, and transfer pathways on the uptake and retention of microplastics in organisms; and 2) the role of species interactions on the fate of microplastics. Because the ecotoxicological risks of microplastic depend on the capacity of organisms to acquire and retain these particles, experiments exploring the dose-response and retention time of microplastics can provide valuable information to understand future effects of this pollution. The choice of the community module further allows us to clarify the role of species interactions on microplastic distribution and circulation within food webs.

2. Chapter 2 | Species interactions, environmental routes and exposure concentration influences the uptake and retention of microplastics in aquatic food webs

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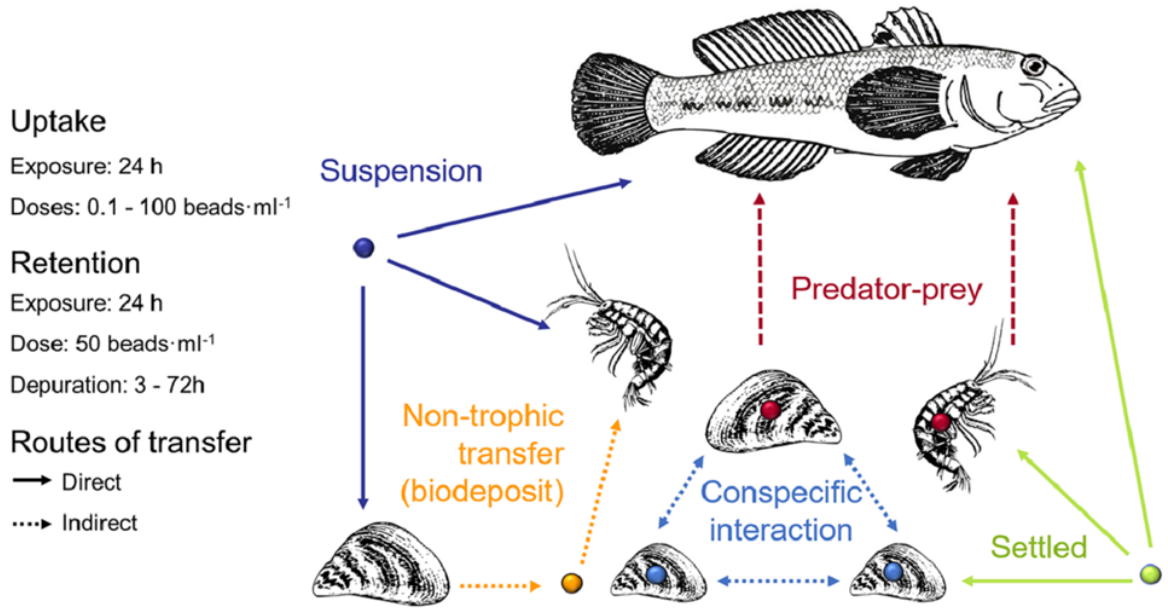
Keywords: food web, microplastic, uptake, retention, species interactions, trophic transfer

Graphical abstract:



2.1 Abstract:

Plastics are pervasive in aquatic environments, in which they circulate in the water column, accumulate in sediments, and are taken up, retained, and exchanged with their environment via trophic and non-trophic activities. Identifying and comparing such organismal interactions are necessary steps to improve monitoring and impact assessment of microplastics. Here, we use a community module approach to test how different abiotic and biotic interactions

determine the fate of microplastic particles in an aquatic ecosystem. Using single-exposure trials on a trio of interacting animal species (dreissenid mussels, gammarid amphipods, fish), we quantified the uptake of microplastics from environmental routes (water, sediment) and transferred via trophic (predator-prey) and behavioural (effects of conspecifics, commensalistic) interactions. Body burdens were consistent with the trophic ecology of the model species and exhibited dose-dependent relationships. Under 24-h exposures, each organism of our community module acquired beads from both environmental sources; the body burden of filter feeders was higher when exposed to particles in suspension, whereas detritivores retained more beads from surficial sediments. Mussels successfully transferred microbeads to amphipods, while both invertebrates transferred beads to the round goby. Predators displayed low contamination from all routes (suspension, sedimented, species interactions) but obtained a higher microbead load from consuming contaminated mussels. Mussel density (up to 10–15 mussel per aquaria ~200–300 mussels$\cdot m^2$) did not affect the microbead burdens of mussels exposed to conspecifics or the load transferred gammarids via biodeposits. Our community module approach revealed that trophic and non-trophic species interactions and multiple environmental routes contribute to the microplastic burden of animals. Using these modules and multiple pathways of uptake, rather than a single organism and a single route, captures more food web complexity and advances our understanding of the fate of microplastics in aquatic ecosystems.

2.2 Introduction

Microplastics are synthetic polymers <5mm, either designed as beads, pellets and fibers for manufacturing purposes, or produced by the degradation of larger pieces exposed to photolytic, mechanical, and biological forces (Browne et al. 2007; Duis and Coors 2016)—and therefore bound to multiply endlessly as they become smaller (Barnes et al., 2009). These

ubiquitous particles circulate in the water column, accumulate in sediments, and are transformed and transferred by physical, chemical and biological processes, thereby interacting with entire biotic communities via multiple pathways.

Acting as a sedimentary component after circulating for a lengthy period by hydrodynamical forces (Kumar et al., 2021), microplastics become available to diverse organisms functionally adapted to target food items amongst particulate matter (D'Avignon et al., 2022). Microplastic acquisition across diverse taxa depends on the concentration of exposure in their nearby environment (Pedersen et al., 2020; Redondo-Hasselerharm et al., 2018); but some organisms are more susceptible to contamination owing to their habitat preference and feeding mechanisms (Adeogun et al., 2020; McNeish et al., 2018; Scherer et al., 2017). For instance, planktivorous clupeid fishes are more contaminated with microplastics than other fishes of higher trophic levels (Covernton et al., 2021), whereas filter-feeding invertebrates retain more plastic than either benthic predators or omnivores (Setälä et al., 2016; Sfriso et al., 2020)

Negative effects associated with microplastic pollution depend on the concentration of exposure and the bioavailability of microplastics to organisms. Exposure to high concentrations of microplastics in depositional areas of rivers, for example, induced delayed maturation in aquatic invertebrates (Stankovic et al., 2020; Ziajahromi et al., 2018) and altered community composition (Green, 2016; Redondo-Hasselerharm et al., 2020. When ingested, microplastics can cause intestinal blockage, reduce foraging ability and food acquisition, diminish nutrition (Bucci et al., 2020), and impair growth and reproduction (Ziajahromi et al., 2018). The chemicals associated with the particles during fabrication (e.g., flame retardants, endocrine disrupting molecules), or sorbed from the environment (e.g., heavy metals, pharmaceutical products, and pesticides), can cause further negative impacts on aquatic communities (Wilkinson et al., 2017).

Predicting ecological risks of microplastic pollution requires a comprehensive understanding of the bioavailability and fate of microplastic particles to food webs and, more specifically, how organisms within a community acquire, transfer, and retain these particles.

Table 2.1: Hypotheses (H) tested in this study. Table S2.2 documents the models used to test each hypothesis.

H1: Individual body burden will depend on the animal, the route, and the concentration of exposure (the 3-way interaction term in the model will be significant).
H2: Body burden will display a positive dose-dependence relationship.
H3: Each animal from the benthic module will retain beads long enough (≥24 h) for trophic transfer to occur. Invertebrate prey will retain beads longer than their predators.
H4: At a given concentration, mussels as filter feeders, will have a higher body burden when beads are offered in suspension rather than sedimented.
H5: At a fixed concentration and route of exposure, an increase in mussel abundance will increase individual body burden, owing to collective filtration.
H6: At a given concentration, gammarids, as deposit-feeders, will have a higher body burden when exposed to sedimented beads than suspended beads.
H7: Gammarids will have a higher body burden when they are exposed to higher mussel abundances, owing to increased biodeposits.
H8: The body burden of round gobies will be higher from feeding on contaminated prey than by accidentally uptake from environmental routes.

To examine relationships among the acquisition and retention of microplastics in food webs, we propose an approach that considers differences in natural history and known

interactions amongst species. We used a common food web module composed of organisms of different functional roles: the quagga mussel (*Dreissena bugensis*), a filter feeder; the gammarid amphipod (*Gammarus fasciatus*), a deposit feeder; and the round goby (*Neogobius melanostomus*), a benthivorous fish that feeds on mussels and amphipods. Presuming the feeding mode and habitat use of each module component dictate its interaction with microplastics, we expect these traits to influence which route of contamination is dominant and, together with the exposure concentration, predict the microplastic body burden of each animal. We also predict that the body burden of microplastics will increase with ambient concentration and be influenced by trophic and non-trophic species interactions (Table 2.1).

2.3 Materials and Methods

2.3.1 Choice of model organisms and pathways of contamination

The invasion of the Great Lakes–St. Lawrence River system by dreissenid mussels likely facilitated subsequent colonization by its natural predator, the round goby (Ricciardi, 2005), with which the mussels share an evolutionary history. In their invaded range, the round goby now serves as an abundant food source for several native piscivorous fishes including walleye, yellow perch, largemouth bass, smallmouth bass, and lake sturgeon (Taraborelli et al., 2010; Jacobs et al., 2017). The round goby is also a frequent prey item for waterfowl such as the double-crested cormorant (Johnson et al., 2015), thereby linking aquatic and terrestrial food webs. Several studies reported the presence of microplastics in our study animals from natural environments (Table S1.1), suggesting this module could be valuable to assess contamination risk beyond the Great Lakes–St-Lawrence system.

The quagga mussel was chosen because it is a highly efficient filter feeder with the capacity to process water at 0.01–0.4 L mussel^{-1} h^{-1} (Baldwin et al., 2002). It can sort particles of 7–348 microns in size (Tang et al., 2014) and deposit undigested material (such as silt, algae and microbeads) as faeces or pseudofaeces (Vaughn, 2018), thereby shuttling nutrients from the water column to the sediments. Furthermore, collective filtration activity is enhanced as the abundance of mussels increases within a colony (Yu and Culver, 1999), causing individual mussels to be exposed to a greater number of particles and potentially resulting in greater uptake of microplastic. Gammarids are closely associated with dreissenid mussel colonies, using the interstitial spaces between clumped shells as shelter and mussel biodeposits (e.g. pseudofaeces, faeces) as nourishment (Ricciardi et al., 1997; González and Burkart, 2004). Round gobies are a dominant predator of dreissenid mussels (Perello et al., 2015) and gammarids (Diggins et al., 2002; González and Burkart, 2004); they can acquire beads from contaminated prey, and while foraging amongst benthic sediments, or from the water column during respiration (Hurt et al., 2020).

2.3.2 Model organism collection and care

Mussels, gammarids and fish were captured in tributaries, canals, or fluvial lakes of the St. Lawrence River at sites where the three species co-occurred, in the summers of 2018 to 2020. Quagga mussels were manually removed from rocks by a scuba diver in the Soulanges Canal near Pointe-des-Cascades, Quebec (45°19'51.99"N, 73°58'4.11"W). Gammarids were collected in deployed traps (cement bricks wrapped with nylon mesh) left overnight along the shoreline at Pointe-des-Cascades (45°20'5.71"N, 73°57'09.0"W). Round gobies were captured with a beach seine net and with minnow traps left overnight on the north shore of Lac St. Louis at Notre-Dame-de-l'Île-Perrot, Quebec (45°20'44.74"N, 73°54'20.23"W), and along the south shore of

Lake St. Louis between Melocheville (45°19'08.7"N 73°55'38.3"W) and Léry (45°20'48.50"N, 73°48'33.67"W), Quebec.

Captured animals were placed into aerator-mounted coolers filled with ambient water and transported to McGill University. All acclimation, housing, and experiments were conducted in temperature-controlled Conviron® phytotron growth chambers that were maintained at ~19°C and a 12h light/12h dark light cycle. Once at the facility, mussels and fish were left to acclimate until the water in coolers reached room temperature (18°C), at which point they were transferred to large aquariums of 70L filled with dechlorinated water. Each aquarium was mounted with an aerator and fitted with a AquaClear 50 Power Filter (with activated carbon, sponge and ceramic bio rings) for at least two weeks before the onset of experiments.

Healthy animals were selected for experimental trials based on the following characteristics: 1) actively swimming gammarids; 2) filtering mussels with open siphons and that responded to touch; and 3) fish displaying normal swimming and breathing behaviour. All animals were fasted for ~48h before the start of the experiment. Each test aquarium was mounted with an aerator and one bio-ring were deposited at the bottom, before being filled with dechlorinated water. A PVC tube was added as a shelter to each fish aquarium. Gammarids were placed individually in 10 ml wells of ice-cube trays. A single gravel pellet was placed in each well to serve as substrate for the animal. Seachem Stability bacterial solution was added (1 ml per 8 L), and water quality was monitored daily (ammonia, nitrate, nitrite, pH, temperature) until the onset of experiments (Suppl. Mat. 2.9).

2.3.3 Treatment preparation for exposure experiments

A series of microbead exposure experiments examined 1) the uptake of suspended and sedimented beads across concentrations for each organism, 2) bead depuration, 3) mussel interaction with conspecifics, 4) commensalistic interaction (mussels to amphipod), and 5) predator-prey interactions (mussels to goby, amphipods to goby).

The experimental design employed 8 L aquaria for exposure experiments involving round gobies, mussels, and species interactions. Each aquarium was fitted with an aerator to oxygenate the water and create a small current. The experimental aquaria were filled with 5 or 7 litres of chemically dechlorinated tap water, to which 1 ml of Seachem Stability bacterial solution was added. One bio-ring was placed in each aquarium to help maintain water quality. Ice cube trays with 21 × 10 ml wells were used for amphipod exposure and amphipod pre-contamination procedures for predator-prey interaction experiments. Dechlorinated water treated with beneficial bacteria (1 ml per 5 L) was used to fill the wells and to create bulk microbead solutions.

Fluorescent Cospheric 63–75 μm polyethylene (PE) beads selected because previous research indicated that particles of this range could be taken up by gammarids (Redondo-Hasselerharm et al., 2018; Straub et al., 2017), dreissenids (White and Sarnelle, 2014) and gobies (Nagelkerke et al., 2018). Microplastic exposure treatments were prepared to have these beads either held in suspension or sedimented to the bottom of the aquarium. Microbead concentrations for each route of exposure were determined by calculating the mass of beads required to obtain the desired concentrations for our experimental 5 L or 8 L aquaria (fish, mussel; Table S2.13) or ice-cube trays with 10 ml wells (amphipods; Table S2.14). For the preparation of treatments for gammarids conducted in 10 ml ice cube wells, we created a stock solution of suspended or settled beads then used dilutions to create the desired concentrations (Table S2.14).

In pilot trials, over 40% of Cospheric 1.00g/cc PE beads sedimented within 24 h after they were added to water. To ensure beads remained in suspension during our 24-h suspended trials, the desired mass of violet or orange beads were placed in vials and treated with a Tween80 surfactant solution prepared according to the manufacturer's guidelines. After the surfactant was applied, 1 ml of algal solution and 5 ml of dechlorinated water was added to the vial, shook for 30 seconds, and left to interact for 48 h to stimulate biofilm formation. For experiments with round gobies, two food pellets were also dissolved into the vials. These treatments were prepared two to three days before the start of the exposure experiments. To conduct trials with suspended beads, animals were placed in the aquaria or wells before the desired concentration of beads were added.

For treatments requiring sedimented beads, green beads (1.025 g/cc) were weighed, placed into vials, and exposed to the bacterial-algae solution to induce biofilm formation. Beads were introduced to the experimental containers and left to sediment for 48 h before the start of experiments. Using a UV light, we confirmed ~90% of beads had sedimented before introducing the animals.

In preliminary trials, we observed that beads often aggregated along the walls of the aquarium and could be expelled outside the aquarium by the bubbling of water produced by the aerators. Upon introduction, the aggregated beads were sprayed with dechlorinated water until they were separated, and no further aggregation occurred. Glass covers were placed over each aquarium and ice cube tray, and these covers were rinsed periodically to ensure concentrations in solutions were maintained.

2.3.4 Uptake experiments

Treatments were prepared by weighing 6.1 mg ± 0.2 mg, 1.21 mg± 0.2 mg, 60.5 mg ± 0.2 mg, and 12.05 mg ± 0.2 mg of beads on a 0.001 g scale into glass vials. These yielded concentrations of 5, 10, 50, and 100 beads·ml^{-1}, respectively, once added in 7 L of water (based on the estimated concentration of 5.81×10^6 beads·g^{-1} provided by the manufacturer; see Table S2.12). To prepare lower concentrations of beads (e.g., 0.1 and 1 bead·ml^{-1}), a solution of 100 beads·ml^{-1} was prepared and diluted to the desired concentration (Table S2.12).

For each uptake trial, a different animal from each species was selected and exposed to one of 12 treatments combining a concentration (0.1, 1, 5, 10, 50, or 100 beads·ml^{-1}) and a route of exposure (suspended or sedimented beads) for 24 h. The experimental aquaria and ice cube trays were set up as described above. Beads prepared for sedimented treatments were added to their experimental container and left to sediment for 48 h, before adding the test subject (mussel, amphipod, or round goby).

Mussels were placed in groups of 5 or 7 per aquarium, whereas round gobies and gammarids were examined individually. Five to six replicates were conducted for mussels (N=415) while five to twelve replicates were examined per route for round gobies (N=143). Each ice cube tray was assigned one concentration and contained 21 wells (N=356; see Table S2.7). For suspended treatments, animals were added first, followed by the bead concentration. Bead aggregations were separated, and covers were added.

After the introduction of animals to their respective experiments, mussels were offered 5 ml of a premixed concentrated algae solution (*Scenesdesmus sp.*, *Pseudokirchneriella sp.* and PhytoPlex), while gammarids were offered 0.1 ml of the same mixture. Round gobies were

offered three Nutrafin granules (Bug Bites Bottom Feeder Formula 1.4-1.6 mm, Suppl. Mat. 2.93). Animals were left to interact with microbeads (or no beads in controls) for 24 h.

After each experiment was completed, mussels from the same aquaria were collected, triple rinsed and placed together into a labelled bag, while gammarids were placed individually in Eppendorf vials filled with 60% ethanol. The invertebrates were then placed in the freezer to be euthanized. Fish were collected by net, triple rinsed, and euthanized by immersion in 10% eugenol followed by cervical dislocation. All animals were kept in the freezer before performing dissections.

2.3.5 Depuration experiments

The experimental design was the same as for the uptake experiment, except those treatments were prepared based on the best route of transfer observed from the uptake experiments; mussels were exposed to suspended beads, gammarids to sedimented beads, and gobies were exposed to either route. Animals were exposed to a concentration of 50 beads·ml^{-1} for 24 h, then randomly selected to be collected immediately (time zero) or moved to a clean aquarium to depurate for 3, 6, 9, 12, 24, 48 or 72 h (sample size available in Table S2.8). To avoid re-ingestion during the depuration phase, animals were moved to a clean aquarium every 24 h. After each depuration period, animals were collected, rinsed and stored as described in section 2.3.4.

2.3.6 Species interaction experiments

Uptake experiments revealed ≤90% of organisms sampled were contaminated when exposed to a concentration of 100 beads·ml^{-1}, so all experiments examining species interactions were conducted at this exposure level. To test the effect of conspecific on mussels' uptake, we

exposed one (n=10), five (n= 5), seven (n=5), or fifteen mussels (n=10) per 8 L aquaria (i.e., densities of ~20, 100, 140, and 300 mussels·m^{-2} respectively) to violet or orange suspended (1.00g/cc) beads for 24 h. After exposure, mussels were collected, rinsed, and stored as described in section 2.3.4.

To assess biodepositional transfer from mussels to gammarids, seven mussels per aquarium were pre-exposed to a concentration of 100 beads·ml^{-1} for 24 h to take up beads naturally. After this period, mussels were collected, tripled rinsed to remove beads adhering to the shell, and placed in a clean 8 L aquarium at a density of one, five or ten pre-contaminated mussels (~20, 100, 200 mussels·m^2). Ten gammarids were placed in each aquaria for 24 h or 48 h (see Table S2.10 for sample size).

In predator-prey experiments, gobies were exposed to one of four pre-contaminated prey assemblage for 24 h: 1) five pre-contaminated mussels, 2) five pre-contaminated gammarids, 3) five pre-contaminated gammarids and five pre-contaminated mussels, or 4) a combination of 15 prey items composed of five pre-contaminated gammarids, five juvenile mussels (shell length ≤ 10 mm), and five adult mussels (shell length ≥ 15 mm). Mussels were pre-exposed to 100 suspended orange beads·ml^{-1}, while amphipods were pre-exposed to 100 green sedimented beads·ml^{-1}. After the invertebrates were left to acquire beads from their respective routes for 24 h, they were cleaned then moved to a clean 8 L aquarium to be offered as prey to gobies. Mussel shells were pre-opened to facilitate feeding for gobies of all sizes. Gammarids were offered alive and moving. At the end of each trial, all live animals were collected to examine the number of prey items ingested and the number of beads they retained.

2.3.7 Microbead extraction

To quantify the number of beads taken up by each animal, an extraction protocol was designed (Suppl. Mat. 2.12). The soft tissues of mussels were removed from the shell by sliding a blade between the valves and detaching the retractor and adductor muscles. Both the whole body and digestive tract of round gobies were weighed. The digestive tract, from the esophagus to the anus of the fish was removed, and the rest of the body (emptied of all organs) was placed in separate vials filled with a 10% KOH solution. The digestion vials for the tissues of fish and mussels were placed in an oven at 40°C for 48 h. The entire body of gammarids was placed either in a 10% KOH solution for 7 days in a 40°C oven or digested with 20% KOH, following 24 h to 48 h of freezing and another 48 h at room temperature. A 125 µm sieve was used to pre-filter the digested samples from the 63–73 µm PE beads of interest. The sieve was triple-rinsed, and the filtrate was then processed by vacuum filtration over a 1µm glass filter (4.7 mm). The beads were usually released from organic material and left on the filter, except in the case of gammarids, whose exoskeleton was not always fully digested. The remaining exoskeleton was broken down with tweezers to release the beads held within their bodies. The number of beads ingested by each animal was counted under a stereoscope (Nikon SMZ 800) at a total magnification of 20× lit by an ultraviolet (UV) led light.

2.3.8 Quality assurance

A rigorous protocol was applied to prevent contamination during these experiments. All equipment (e.g., aquaria, hand nets, vials) used during exposure trials or microplastic extraction protocols were triple-rinsed and visually examined under the UV light to detect beads before use. Glass vials and tools for the dissection and digestion procedures were washed thoroughly and triple rinsed with ultrapure 0.1 mm filtered water, then placed in a 230°C oven for 3 h, before

being used. All dissection, digestion, filtration, and extraction procedures were performed in an isolated room under a laminar flow hood, and researchers wore a cotton lab coats. An ultraviolet light was used to verify that no fluorescent Cospheric bead contamination was present before using the equipment.

To track contamination across trials, we alternated the suspended and sedimented trials which were conducted using different bead colours to detect prior contamination of equipment. Mesh hand nets used to remove animals, after experimental trials (for gobies and mussels) were assigned to each dose level and bead colour.

Animals were removed from experimental trials starting with the control trial and followed by trials of increasing concentration. In pilot experiments we observed that: 1) fish could expel beads in the eugenol solution during euthanasia procedures, and 2) beads that had initially adhered to fish bodies could be released into the solution and subsequently be respired or ingested. Fish increased their respiration rates during the eugenol treatment, suggesting that this procedure could be a source of contamination. We euthanized fish from lower exposures first and applied washing procedures between each treatment. After euthanasia, animals were dissected per route and in order of concentration of exposure from the lowest to the highest.

2.3.9 Controls and data correction

For each exposure experiment, one control aquarium or ice cube tray was set up per trial using the same protocols as for the exposure experiments, except that no microbeads were added. Thus, 5-7 mussels, 21 amphipods and one fish were used as controls per trial. Animals from these trials were processed following the same protocols described above. These controls helped monitor contamination from the exposure trials. Procedural filtration blanks were run before and

after processing samples from a treatment and for each route per animal group and used to account for contamination during the microplastic extraction phase.

During our uptake exposure experiments, we noted the presence of contamination in our controls and procedural blanks. We subtracted the number of beads found in procedural blanks from the total bead count per individual. In some cases, we found a higher number of beads in animals exposed to control treatments than in experimental trials of the lower range of concentrations offered (i.e.,0.1 or 1 bead ml^{-1}). Since there is no standard method to correct for contamination when multiple controls are reported per trial, we analyzed our data using different control-corrected methods to measure the body burden of animals (Suppl. Mat.2.13). We chose to use the positive counts to represent background contamination levels. Significant differences between the control and other concentration levels indicated when the treatment surpassed the threshold detection level. For the depuration and species interaction analyses, controls were relatively clean, so the mean number of beads in the controls of each trial was subtracted from the observed counts.

2.3.10 Data analyses

We compared the uptake and retention of microplastics by quantifying the *body burden*—defined as a number of particles per organism or per gram of tissue (Collard et al., 2019; Covernton et al., 2021; O'Connor et al., 2020). The total number of whole beads found per individual per treatment was counted and used as the response variable (Figure S2.1). To standardize body burden across animals we measured the number of beads divided by the mass (g) of the digested tissue of each animal.

Data exploration and analyses were performed using R version 4.0.3 (R Development Core Team, 2022). Models were created based on our hypotheses (Table 2.1) of the expected effect of

the concentration and route of microplastic on each animal or the predicted effect in species interactions. The *gamlss* package (Rigby and Stasinopoulos 2005) and *lme4* package for mixed models were used for our analyses (see Table S2.2 for model choice). Model assumptions were confirmed visually using plots of the normalized quantile residuals while the normal residual distribution was assessed with the Shapiro-Wilk test. Models respecting homogeneity and normality of residuals and with the lowest Akaike information criterion scores were chosen to estimate marginal means per treatment using the *emmeans* package (Lenth et al., 2022). Type III ANOVA tables comparing the main effects amongst groups followed by sidak-adjusted multiple comparison of treatments were performed for each model.

2.4 Results

2.4.1 Effect of feeding mechanism, concentration and route of exposure on the body burden

The animal's functional ecology was most influential in explaining variation in body burden of each recipient of the food web module (H1). The greatest contrast in mean body burden was among each animal group ($F_{(2,49)} = 136.5$, $p<0.0001$) then, among concentrations ($F_{(6,49)}= 51.5$, $p<0.0001$). Although the route of microbeads origin did not contribute significantly to explaining variation in the body burden by itself ($F_{(1,49)} = -0.01$, $p=1.0$), all two-way and three-way interactions were significant ($p <0.05$, Table S2.3). The effect of the route of plastic transfer on individual body burden measurements depended on the feeding mechanism of the animal tested, so we considered the body burden dose-response relationship of each animal type separately. We observed a significant increase in the body burden with increasing exposure concentration for the invertebrates as predicted by H2, but not for the predatory fish (Figure 2.1).

For filter-feeding mussels, the body burden was significantly affected by the concentration of microbeads ($\chi^2_{(6,414)}$=123.6, p<0.0001) and the interactions between concentration and route ($\chi^2_{(6,414)}$=16.6, p=0.011), when controlling for the effect of different mussel abundances in each aquarium ($\chi^2_{(6,414)}$=8.64, p=0.003). To account for variation among replicates of a treatment, individual mussels were nested per aquarium as a random effect (accounting for 51% of the model's variance; Table S2.4). As predicted by H4, mussels retained more microplastics when exposed to suspended beads than sedimented beads, with significant differences across routes being observed at each concentration (p<0.05; Figure 2.1A). Filter feeders exhibited a positive response to exposure with a higher body burden at higher exposure levels under both routes.

For deposit-feeding gammarids, the concentration ($F_{(6,356)}$ =18.08, p<0.0001) drove the acquisition of beads, although the route influenced the body burden under certain exposure levels (interaction between concentration and route: $F_{(6,356)}$ =5.92, p=0.0001; Table S2.5). The body burden of gammarids was higher when they were exposed to sedimented versus suspended beads, but the difference between routes was only significant at a concentration of 100 beads·ml^{-1} (Figure 2.1B). A positive concentration response was nonetheless observed for both routes. However, gammarids took up significantly more beads than in control treatments only when suspended concentrations were >5 beads ml^{-1} or when sedimented concentrations were >10 beads ml^{-1} (Figure 2.1B).

The predators (round gobies) had the lowest body burden amongst all animals examined (Figure 2.1), which was influenced by the concentration ($F_{(6,122)}$ =5.95, p<0.0001) and the route ($F_{(1,122)}$ = 10.1, p= 0.0015; Table S2.6). Whether gobies were offered beads in suspension or sedimented, their body burden did not differ for a given concentration ($F_{(6,122)}$ =1.78, p=.099; Figure 2.1C).

The mean body burden in goby increases with concentration only when exposed to sedimented beads rather than suspended beads (Figure 2.1C).

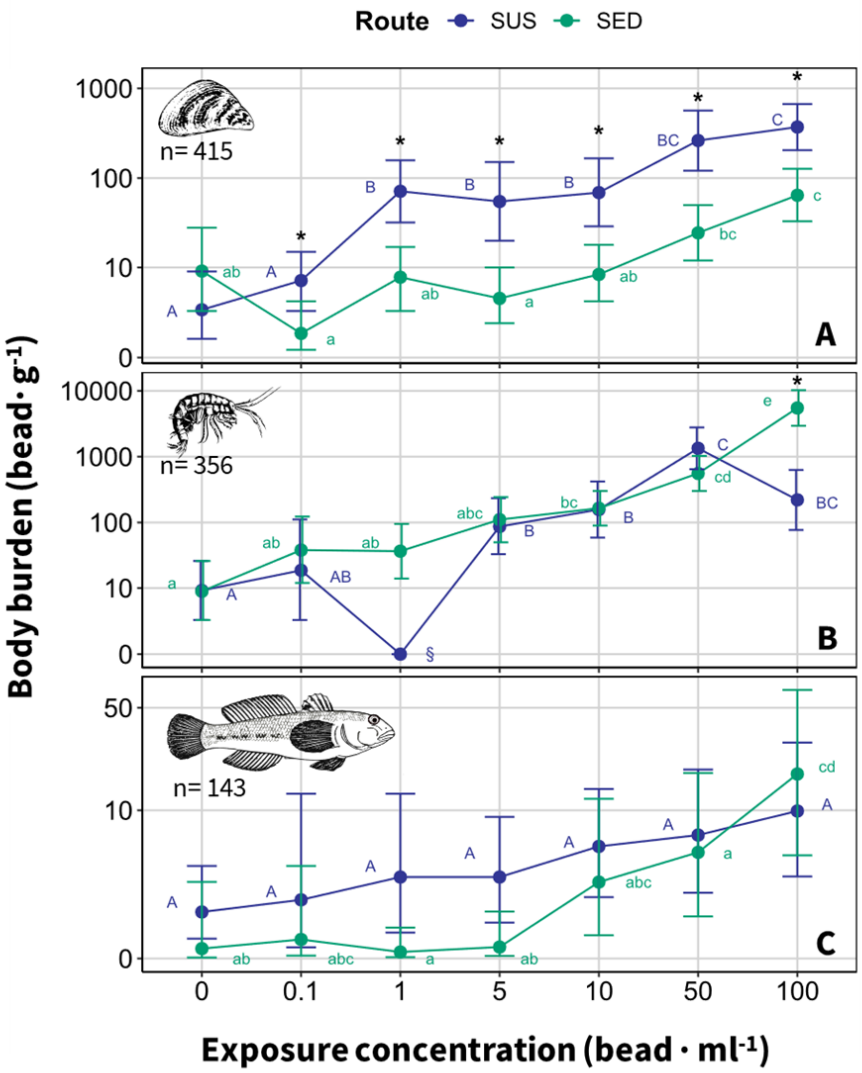


Figure 2.1: Estimated mean body burden of A) mussels, B) gammarids, C) round gobies, exposed for 24 h to one of seven microbead concentration offered in suspension (SUS) or sedimented (SED). A unique letter code indicates differences (p<0.05) across doses under each route, whereas asterisks illustrate differences between routes (p<0.05). Error bars represent the 95% confidence intervals around the mean. The § symbol indicates no bead was observed. The y-scale was pseudo-$\log_{10}$-transformed to visualize body burden values of zero. Table S2.7 reports means and confidence intervals per model.

2.4.2 Comparing depuration amongst food web recipients

Differences in retention times after acute contamination were observed among animal groups (Figure 2.2). In addition, the initial body burden varied among animals after being exposed to 50 beads·ml^{-1} for 24 h: the body burden in gammarids was an order of magnitude higher (700-2000 beads·g^{-1}) than mussels (~127 beads·g^{-1}) and round gobies (~7 beads·g^{-1}; Table S2.8).

Contrary to our prediction (H3), mussels were the only animal to maintain a relatively constant body burden for 24 h (Figure 2.2A). About half of the mussels depurated all beads after 48 h, but their body burden was only significantly reduced from initial contamination after 72 h (Figure 2.2A). Although gammarids had the highest initial body burden, ~50% of the individuals depurated all beads between 6- and 12 h post-exposure (Figure 2.2B). We also observed a significant decline of one to two orders of magnitude in gammarids' body burden after 24 h. Among the animals tested, round gobies displayed the lowest initial body burden and the fastest depuration time, with only 10–25% of animals retaining beads after 3-h of depuration in a clean environment (Figure 2.2C).

2.4.1 The role of species interactions

2.4.1.1 Predator-prey interactions

To examine the effects of predator-prey interactions on the body burden of gobies, we compared different prey transfer scenarios to environmental routes of contamination (suspended versus sedimented; Figure 2.3). When gobies were offered mussels individually or with gammarids, they did not consume all the prey offered; so we retained only trials where gobies consumed 100% of the prey offered, to allow the data to be comparable with environmental

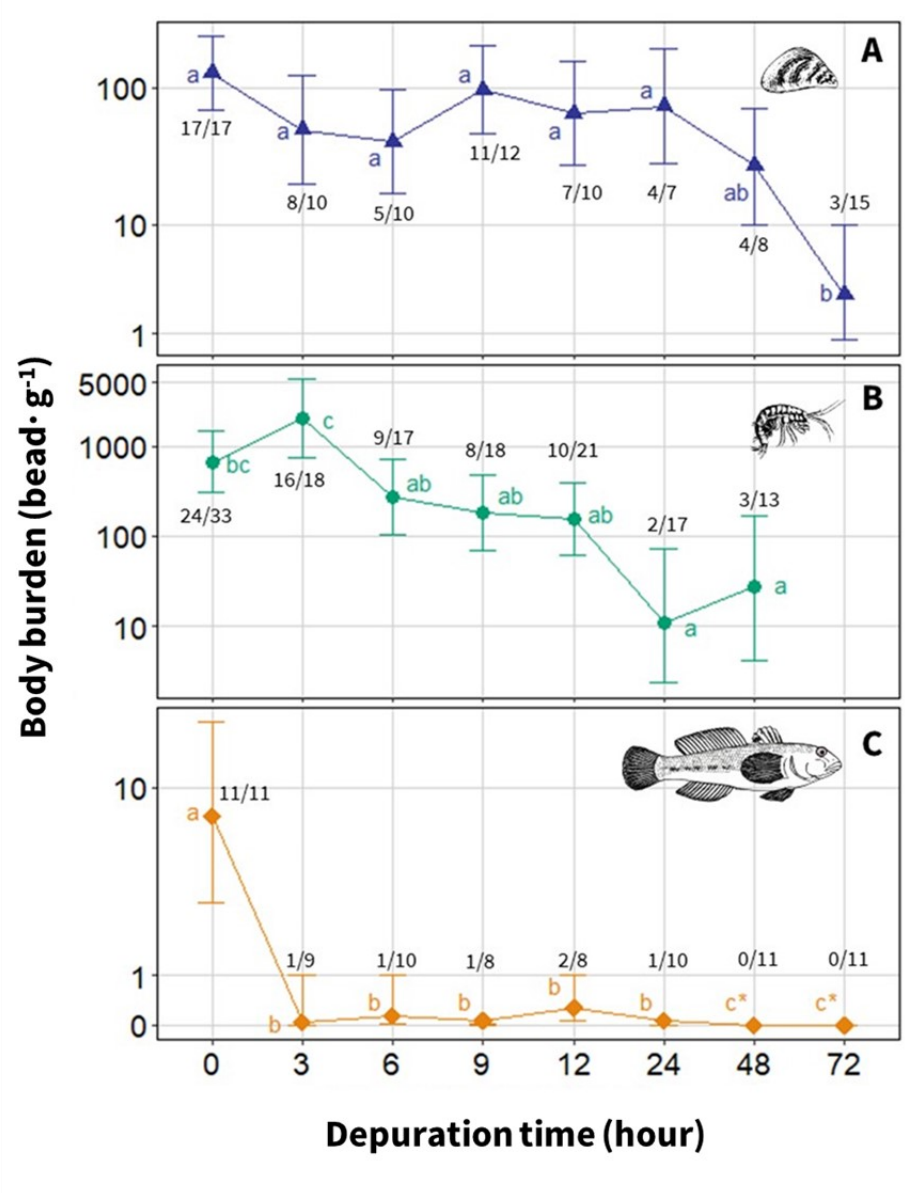


Figure 2.2: Estimated mean body burden retained by A) quagga mussels, B) gammarids, C) round gobies after depuration in a clean environment. The fractions near the mean indicate the number of animals observed with beads (numerator) and the total sample size examined (denominator). Error bars show the confidence intervals. Letters indicate differences in body burden between depuration times. The asterisk indicates no animals were found with beads; therefore, the upper confidence interval cannot be estimated. Table S2.8 reports means and confidence intervals per model.

routes and test how the route of contamination influenced predator body burden. In this case, the number of beads observed in gobies from five mussels was significantly higher (315 beads·g^{-1}) than the other routes of contamination, partially supporting our hypothesis that prey were better vectors of microplastics than beads provided via environmental routes (H7; Figure 2.3). However, exposure to pre-exposed gammarids (27 beads·g^{-1}) or a combination of prey items did not yield a higher body burden than environmental routes, contrary to our prediction.

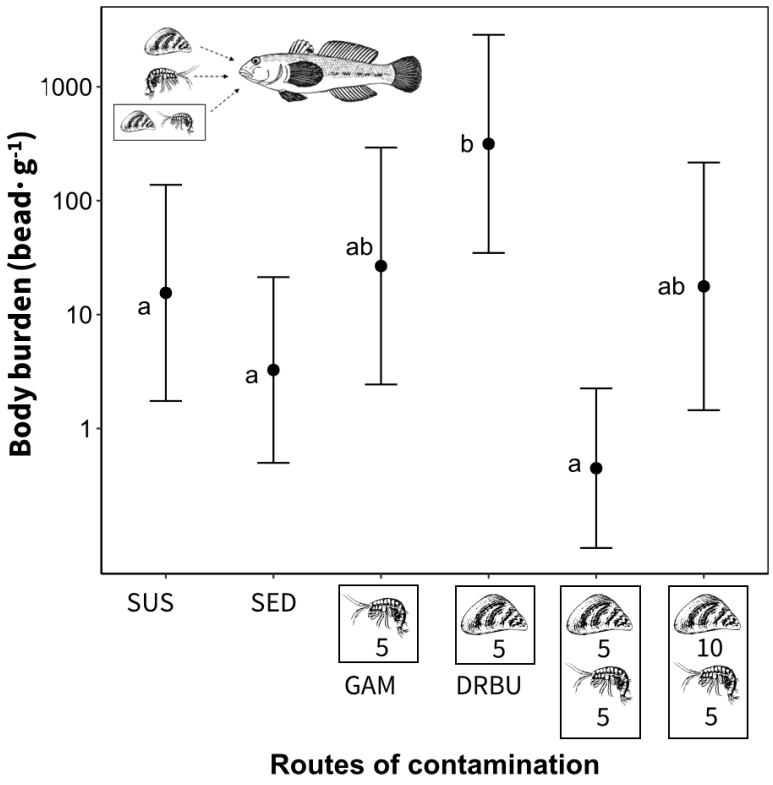


Figure 2.3: Estimated marginal means and confidence intervals of the Poisson-Inverted Gaussian model comparing the effect of different routes of contamination on round goby body burden (number of beads per gram of tissue) after an exposure of 24 h. Each prey combination (5 gammarids, 5 mussels, 5 gammarids and 5 mussels, or 5 gammarids and 10 mussels) was pre-exposed to 100 beads·g^{-1} for 24 h. Error bars show the confidence intervals. Letters indicate differences in body burden between routes. See Table S2.10 for estimated means and confidence intervals.

We determined how the transfer of beads in each predator-prey interaction scenario was influenced by the proportion of prey consumed (number of prey consumed in relation to number of prey offered). First, we tested the effect of body size (length) as a proxy for gape size (Karlson et al., 2007; Matern et al., 2021), which is known to play a role in prey-size selection; we did not find that the length of the fish ($F_{(1,56)}$=0.54, p=0.43) or its interaction with the proportion of prey consumed influenced body burden (Table S2.9). Our best model showed that the proportion of prey eaten ($F_{(1,56)}$=12.83, p=0.0003) and prey scenario ($F_{(3,56)}$=11.15, p≤0.0001) were the most influential. By adjusting for the proportion of prey eaten, we observed similar results as before—where the scenario offering five prey of each type results in the lowest body burden, whereas the transfer from mussels produced the highest body burden in round gobies (Figure 2.4).

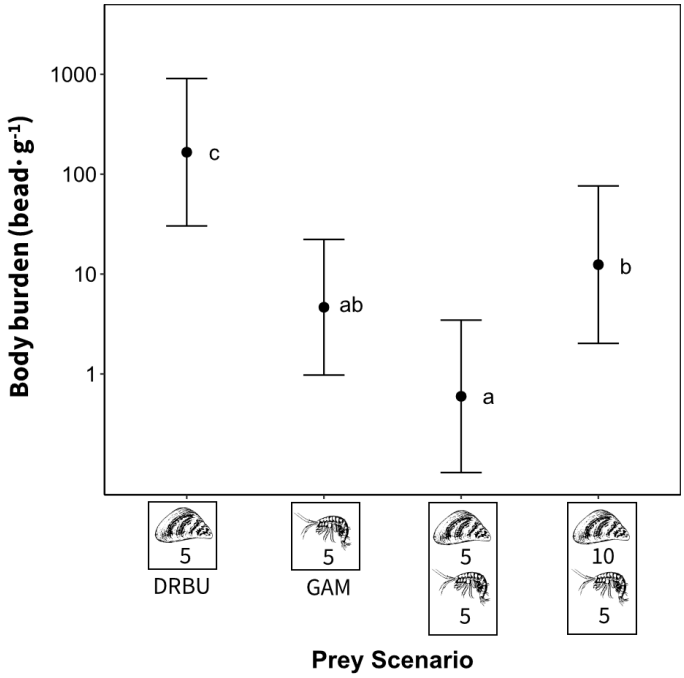


Figure 2.4: Mean estimated body burden per predator-prey interaction scenario for all trials where gobies were permitted to feed on live mussels, gammarids, or a mixture of both prey types. Error bars show the confidence intervals. Letters indicate differences in body burden between each prey scenarios.

2.4.1.2 Commensalism

The commensal relationship scenario examined whether contaminated mussels could transfer beads to deposit feeders (gammarids) via biodeposition. We observed that 14–29% of gammarids took up beads from mussels in the first 24-h period and 0–5% had body burdens of 1–4 beads·g^{-1} after 48 h exposure to pre-contaminated mussels. Body burden was generally low for this route of uptake in comparison with beads acquired from environmental routes (suspended, sedimented). A mean of ~16 beads·g^{-1} were observed in gammarids from non-trophic transfer from one (~20 mussels·m^2) and five mussels (~100 mussels·m^2). When gammarids were exposed to ten mussels (~200 mussels·m^2) at once for 24 h, they retained 38 beads·g^{-1} (Figure 2.5). Although we observed an increase in body burden for gammarids exposed to the highest mussel density both after 24 h and 48 h exposures (Figure 2.5; Table S2.10), this increase was not significant ($F_{(2,143)}$=1.415, p=0.243). However, a significant decrease in body burden was observed between gammarids exposed to mussels for 48 h versus 24 h ($F_{(1, 143)}$ = 7.46, p=0.006), suggesting that they depurate faster than mussels. Based on estimated retention means for each animal, gammarids lost 98% of beads acquired within 24 h, while mussels depurated only 42% of their initial burden (Figure 2.2; Table S2.8).

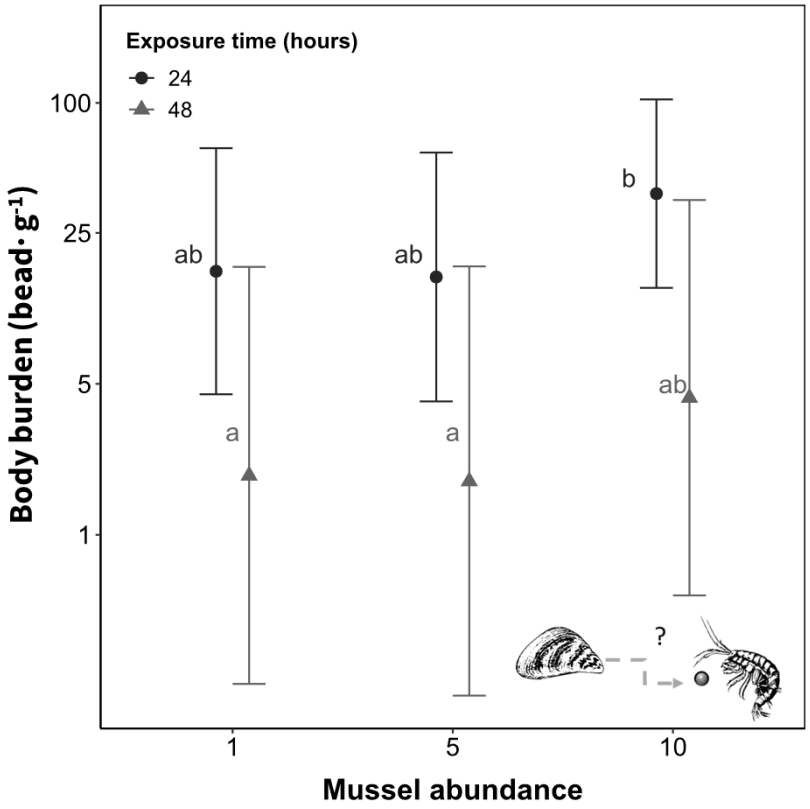


Figure 2.5: Type I Negative Binomial model-estimated mean body burden of gammarids exposed for 24 h (dark circle) or 48 h (light triangle) to abundances of one, five and ten mussels (representing densities of ~20, 100 and 200 mussels·m^2, respectively). Error bars display the confidence intervals. Different letters indicate significant differences ($p \leq 0.05$) between treatments. See Table S2.10 for estimated means and confidence intervals.

2.4.1.3 Influence of conspecifics

To examine the effect of mussel abundance on the body burden of individual mussels, a mixed model was used to account for a nesting effect per aquaria among replicates. We detected substantial variation within treatment; so although the abundance of conspecifics in the experimental aquaria appeared to affect the mean body burden (e.g., a mean of 930 beads·g^{-1} at a density of five mussels (~100 mussels·m^{-2}) and a mean of 85 beads·g^{-1} at a density of seven

mussels (~140 mussels·m^{-2}), these differences are not statistically significant (p>0.05) (Figure 2.6; Table S2.10).

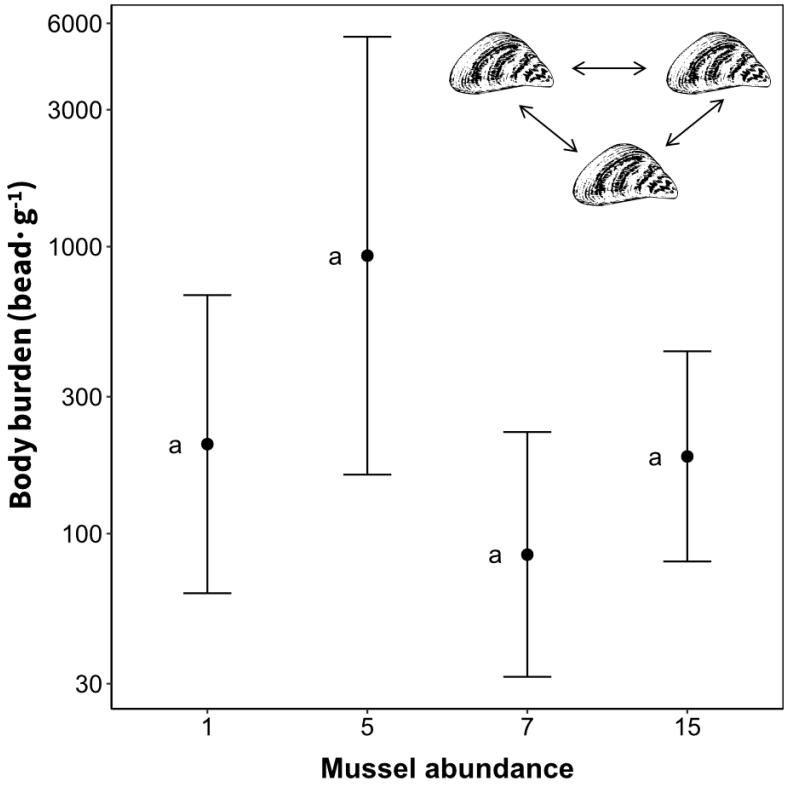


Figure 2.6: Type I Negative Binomial model-estimated marginal means and confidence intervals comparing the effect of mussel abundance on individual body burden (number of beads per gram of tissue) after an exposure of 24 h. Body burdens were controlled for the significant random effect of aquarium. Different letters indicate significant differences ($p \leq 0.05$) between mussel abundances. See Table S2.10 for estimated means and confidence intervals.

2.5 Discussion

2.5.1 Natural history influences uptake and retention

The responses of a trio of species representing different trophic groups and collectively comprising a common freshwater community module suggests that microplastic contamination of organisms is influenced by the concentration and route of exposure, and the feeding mode of the animal. Knowledge of the natural history and behaviour of a species can help assess its risk of contamination if coupled with empirical data on dose response in different biotic and abiotic environmental contexts. Collectively, this information increases our understanding of how groups of interacting species take up, retain, and redistribute these particles within their community.

Despite the well-documented natural history of each taxon in our study, some of their responses to exposure to microbeads were unexpected. For example, although the body burden of the filter-feeding species was higher at each concentration when beads were offered in suspension, we also observed significant uptake of beads from surficial sediments. We suspect that mussels could acquire sedimented beads because their siphonal currents cause such beads to be locally re-suspended. Additionally, beads were often found around the byssus of the mussel (Photo S2.1), suggesting that particles could have been acquired via pedal feeding. Dreissenid mussels can both ingest food and eject pseudofaeces by the posterior inhalant siphon and through the pedal gape (Nichols et al., 2005). For example, the ejection of the *Microcystis* algae through the pedal gape is a known response of mussels to non-palatable toxic particles (Juhel et al., 2006). The presence and accumulation of beads on the byssus could be a result of either feeding or excretion of the beads.

The body burden of gammarids was similar across routes, suggesting that they encounter suspended beads as often as they encounter beads recently deposited on the sediments. However, in our experiments, gammarids were exposed to beads exclusively in small wells of 10 ml, which could have restricted movements and forced gammarids to encounter more beads in suspension than they would in habitats of greater volume. We also provided algae as a food source for the gammarids during the 24-h exposure trials. Gammarids exhibit broad feeding plasticity that includes foraging on coarse particulate organic material (as shredders) and fine particulate organic material (as suspension feeders and deposit feeders) (Delong et al., 1993; MacNeil et al., 1997). Therefore, gammarids use diverse feeding mechanisms to capture food particles, thereby increasing the likelihood of accidental capture and ingestion of microplastics (Blarer and Burkhardt-Holm, 2016; Cole et al., 2013).

The predator species had the lowest body burden of all animals examined. Both the mussel and the gammarid are adapted to sort small particles of food within the size range of the beads used in our experiments (63–73µm), whereas the round goby feeds on macroscopic invertebrate prey items. We suspect that it acquired beads accidentally from its environment and, because it can egest the beads rapidly within 24 h (Figure 2.2) beads were not readily retained, regardless of concentration or route of exposure.

It is important to note that background contamination in the controls of our trials were important, with 40-50% of the controls for gobies and mussels having positive counts. As such, the body burdens we presented may be overestimated in comparison to particle acquisition rates that would be observed in the field.

2.5.2 Species interaction contributes to body burden and transfer

Our experiments revealed that microplastics can be acquired and transferred by aquatic organisms via both trophic and non-trophic interactions. Organisms within food webs are both recipients and vectors of microplastics, and sometimes (e.g., mussels) are a more important route of contamination to species than environmental routes.

2.5.2.1 Predator-prey interactions

For predator-prey interactions, we predicted all prey scenarios to increase the body burden of round gobies (H8). However, aquatic organisms do not capture and retain all the beads available to them and have a low capture efficiency (Table S2.11). In our experiments, all prey took up beads on their own when exposed to 100 beads·ml^{-1}. We estimate mussels captured ~0.013% of beads available in suspension, whereas gammarids captured 0.009% of sedimented beads (Table S2.11). Based on these estimates, the number of beads in prey was orders of magnitude lower than those available to gobies through 100 beads·ml^{-1} environmental route (~ 700 000 beads per aquaria). Although gobies were exposed to lower numbers of available beads under trophic transfer, their acquisition of beads from their prey suggests that predation is an important pathway to consider when assessing risk of microplastic contamination, especially when an animal feeds primarily on prey that can retain particles for long periods of time.

Indeed, the scenario where five mussels were offered to round gobies contributed to the highest mean transfer of microbeads. We hypothesize that mussels have a higher body burden per individual because they have a higher mass (~0.26 g of tissue) than gammarids (~0.012 g); therefore, even if the body burden of gammarids exposed to 100 beads·ml^{-1} is higher than mussels (5260 beads·g^{-1} and 355 beads·g^{-1}, respectively), the total number of beads per individual is lower for gammarids (Table S2.11).

Our predator-prey interaction experiments, coupled with the observed retention rates, suggest mussels play an important role in acquiring and distributing microplastics to the benthic community. Mussels contributed most to trophic transfer when acting as a single prey item for round gobies. We also observed that a mixture of contaminated prey items can result in more complex interactions that do not necessarily lead to increased body burden of a predator. For example, diet switching between amphipod and dreissenid mussel prey was previously observed in round gobies, which favour amphipods when both prey items are available and visibility is high (Diggins et al., 2002). In a similar setting as our experiment (ambient water without substrate), gobies consumed 5.2 dreissenids·h^{-1} when mussels were offered alone, and 14.3 amphipods·h^{-1} when amphipods were offered alone. When both prey items were offered together, gobies had lower consumption rates of both preys eating <2 dreissenids·h^{-1} and 12.8 amphipods·h^{-1} (Diggins et al., 2002). Smaller gobies from the St. Lawrence River adopt a generalist diet and shift more towards a dreissenid prey when the fish grow beyond 130 mm (Miano et al., 2021). In the scenario with ten mussels and five gammarids offered as prey, we offered a mixture of five small (<10 mm) and five large (>15 mm) mussels. Gobies larger than ~100 mm can feed on the larger class of mussels (Naddafi and Rudstam, 2014), whereas smaller gobies (mean ± standard deviation: 67.7 mm± 17.3 mm) preferentially selected smaller quagga mussels (6.0–9.9 mm shell length; Perello et al. 2015). The round gobies used in this experiment had a mean length of 82.2 mm ± 14.7 mm, suggesting they would feeding on gammarids when both prey were offered simultaneously, thereby ingesting a lower body burden than if they fed exclusively on a mussel diet. This would occur because gammarids depurate more rapidly than mussels within the first 24 h post-exposure to microbeads (Figure 2.2). Unlike mussels, gammarids can swim in attempt to avoid predation, and as they flee the predator, they could

release fecal pellets that include some or all the beads they had ingested (G.D., *pers. obs.*; Photo S2.2). If we consider the average daily consumption of macroinvertebrates by round gobies, trophic transfer may shift depending on the ontogeny of the fish, where smaller fish favour amphipod prey and thus become less contaminated than larger adult fish that are more molluscivorous.

2.5.2.2 Non-trophic transfer and circulation of microplastics in benthic communities

Quagga mussels can filter up to 400 $ml \cdot hr^{-1}$ (Wong et al., 2010). Their filtration activity can control primary production in lakes (Caraco et al., 2006; Wong et al., 2003) and, through biodeposition, shifts nutrients and energy from pelagic to benthic environments (Higgins and Zanden, 2010; Vanderploeg et al., 2002). Furthermore, mussel biodeposition and attachment provide smaller, co-occurring invertebrates with nourishment (in the form of feces and pseudofaeces) and shelter (interstitial spaces between clumped shells) (Ricciardi et al., 1997; Ward and Ricciardi 2007).

Collective filtration of clustered individuals creates a stronger current that allows mussels to process more particles (Yu and Culver, 1999). Although we did not observe an increase in body burden per individual when mussels were in higher abundances, nor an effect of abundance on transfer to gammarids via bio-deposition, the densities selected for our experiments were perhaps below the required threshold for this phenomenon to occur; previous studies used densities >1000 $mussels \cdot m^{-2}$ (Yu and Culver, 1999; Zaiko and Daunys, 2012).

Additionally, for mussels to benefit from the current or pre-filtration of other conspecifics, and for gammarids to hide amongst shells, mussels need to be locally aggregated. Mussels in our experiments were randomly distributed in the aquaria rather than placed in clusters. Yu and Culver (1999) reported higher per capita clearance rate of food particles when mussels were in

clusters, especially when food is limited. In our design, mussels in each treatment were exposed to the same concentration of beads and to the same amount of food particles; therefore, it is possible higher mussel density depleted the food reserves faster. At lower densities, the presence of these non-palatable items could clog the inhaling siphon and thus cause a reduction in filtration rate (Zaiko and Daunys, 2012). Perhaps for an abundance of five mussels in 7 L of water, the microplastic and food concentrations offered provided the most optimal filtration conditions.

Despite these experimental limitations, we demonstrated that mussels serve as a conduit for the transfer of microplastics as they directly contributed to a higher body burden in their predator and circulated microbeads via biodeposition to gammarids. The biodeposition of microplastics in mussel feces and pseudofaeces has been observed in marine bivalves (Khan and Prezant, 2018). Because quagga mussels are highly interactive within benthic communities, we expect non-trophic transfers of microplastic to involve diverse taxa and functional groups living amongst mussel colonies (Ward and Ricciardi, 2007), whereas trophic transfer could increase body burdens of consumers such as crayfish, benthic fishes, and various other animals that feed on quagga mussels or their associated invertebrates (Naddafi and Rudstam, 2014; Watzin et al., 2008). As mussels are known to bioaccumulate contaminants (Bruner et al., 1994; Gossiaux et al., 1998), their contribution to the plastic cycle could enhance the transfer of microplastic-associated contaminants through freshwater food webs.

Non-trophic transfer of microplastics from mussels to gammarids via biodeposition was observed in our experiments. In comparison with environmental exposure, this mechanism of transfer is low, albeit the size of aquaria differed between these sets of exposures. A 7 L aquarium was used to examine the transfer of beads from mussels to gammarids (in contrast to

the 10 ml wells that were used to examine the effects of concentration and environmental routes), and this greater area may reduce the chance of gammarids encountering beads. As food was limited for mussels in our system, so was the quantity of food filtered, processed, and transferred via biodeposition, thereby likely limiting the quantity of beads circulating in this manner. In contrast, mussel populations in rivers and lakes have access to more stable nutrient sources and can thus provide a continuous supply of particulate material to associated benthic communities. We expect this route of contaminant transfer to add to the ecological impacts of dreissenid mussel invasions.

2.5.3 Incorporating all routes of microplastic contamination in risk assessment

Assessing risks associated with microplastics under current environmental concentrations can be challenging, owing to difficulties in replicating relatively low exposure scenarios in laboratory settings. Furthermore, contextual factors including seasonality (Nel et al., 2018), the life stage of the animal and its feeding strategy (Scherer et al., 2017; Setälä et al., 2016), the size, the type and the condition of particles (Au et al., 2015; Qu et al., 2018; Kalcikova et al., 2020) influence the abundance of microplastics available for ingestion. Our experiments clarified the relationship between concentration and possible routes of contamination within a trio of interacting species that are becoming increasingly common in food webs within the Great Lakes–St. Lawrence River system and the results might better inform risk assessment.

At present, microplastic risk models are typically based on concentrations found in surface waters. Yet, as demonstrated in our study, microplastic particles retained by aquatic organisms can originate from multiple routes of contamination: water, sediments, and species interactions. We assert that it is necessary to account for the presence of microplastic in each of these environments and how they are circulated within food chains, to predict risk of contamination.

We demonstrated that, despite the well-known natural history and behaviour of our trio of species, each animal took up and retained microbeads from both environmental routes. Yet, the availability of these routes can change. For instance, sedimented beads can re-enter food chains because of species interactions with benthic environments (as observed in our study), or be re-suspended by flooding or dredging in natural environments (Ji et al., 2021).

We also confirmed that each member of this trio acted both as a temporary reservoir for microplastics and as a vector transferring and circulating particles via species interactions. In addition to trophic transfer of microplastics (Chae et al., 2018; Elizalde-Velázquez et al., 2020), we have shown that species interactions shuttle microplastics via non-trophic routes (e.g., biodeposits) that could significantly add to microplastic body burdens.

Using community modules rather than single species offers a more realistic model of contamination risk. Food web structure and trophic pathways play crucial roles in regulating ecological processes in lakes and rivers (Covernton et al., 2021; Vander Zanden et al., 1999). Microplastics do not appear to biomagnify in food webs (Covernton et al., 2021; O'Connor et al., 2022; *this study*), but contaminants associated with these particles could behave differently. As such, it may be important to consider the food web structure and the number of trophic links within a food chain in different communities when modelling the distribution and movements of microplastics and their associated contaminants.

2.6 Acknowledgements

McGill University is located on land which has long served as a site of meeting and exchange amongst Indigenous peoples, including the Haudenosaunee and Anishinabeg nations. We would honour, recognize, and respect these nations as the traditional stewards of the lands and waters where our work has been conducted. We would like to thank Lorena Vidal, Wendy Huang, Hélène Pfister, Duncan Wang, and Nicole Moore for their help in caring for the animals and performing microplastic extractions. LV also participated in conducting trials and in the processing of samples. Experiments using live animals were regulated by the McGill Animal Care Committee under the permit Animal Use Permit 2017-7913. Animals were collected under the Quebec's Ministère de la Forêt, de la faune et des parc under the SEG permits 2018-7-31-2467-06-16-SP, 2019-7-8-2665-06-16-SP and 2020-07-13-2862-05-06-16-SP.

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2.8 Chapter 2 Supplementary Materials: Figures, Tables, Photos

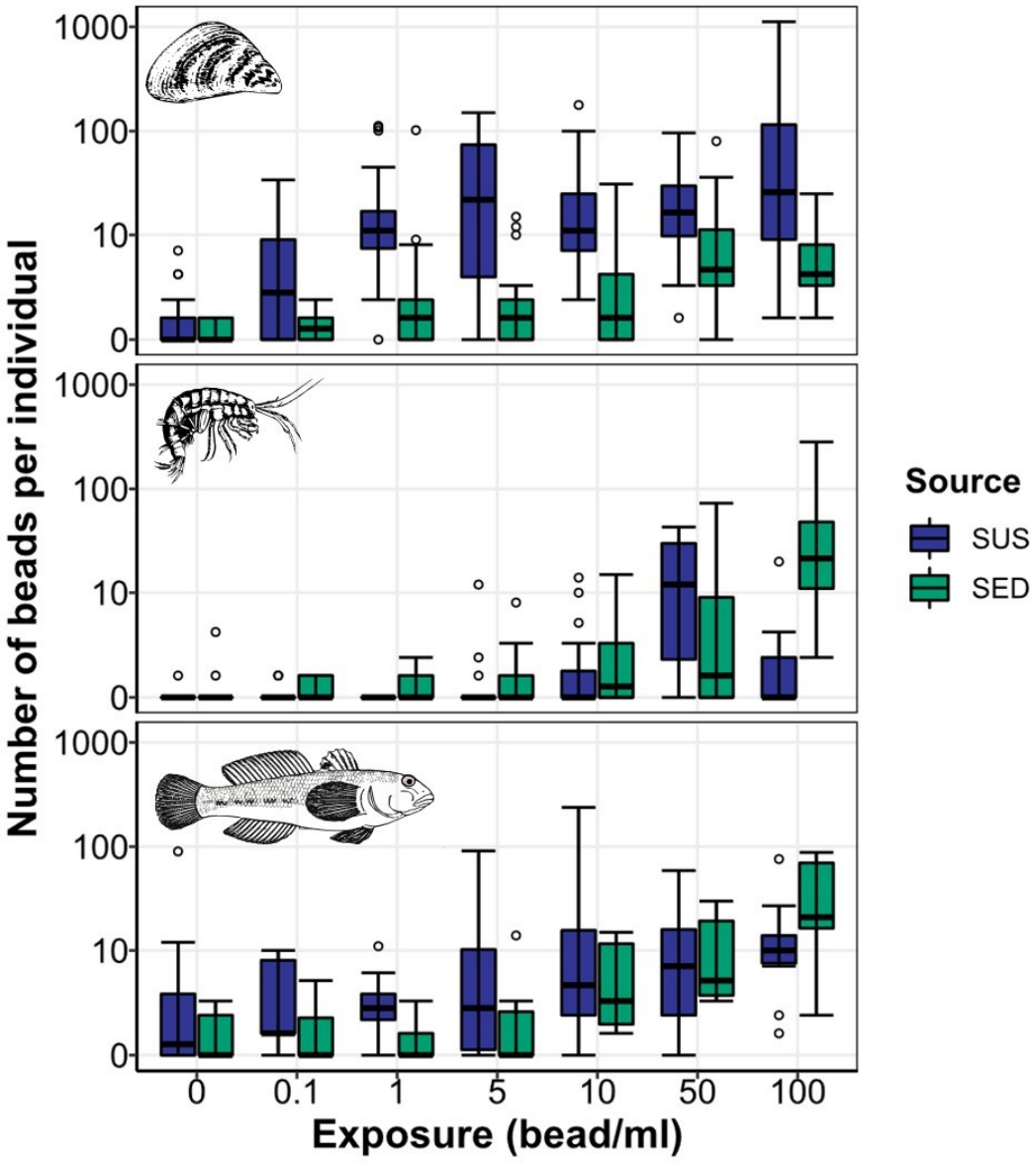


Figure S2.1: Boxplots show the number of beads per individuals acquired by each animal via each concentration of exposure and source of beads. Treatments offering suspended beads are in violet while treatments assessing the uptake of beads in sedimented beads are in green. The median uptake is shown by the mid-box segment and the errors include the interquartile range of the data.

Table S2.1: Summary of articles with recorded evidence of microplastic uptake from natural environments for the focal taxonomic group: round goby (Neogobius melanostoma), amphipods, and dreissenid mussels. Note that SLR in location refers to the St. Lawrence River. N = sample size, % Occ. refers to the percentage of individuals with microplastics per sample size and the No/ ind, refers to the number of particles per individual. Microplastic shape are described as fibre (FB), Fragments (FR), beads (B), foam (FM) or film (FL). The 3 most common colours of particles ingested are identified as black (B), blue, (BL), gray (GR), green (G), red (R), clear (C), orange (O), violet (V), pink (P), white (W), or yellow (Y). The common acronyms of polymers were used AC= acrylates, AN= acrylonitrile, CE= cellulose, PA= nylon, PAN=polyacrylonitrile, PE= polyethylene, PET= polyethylene terephthalate, POM= polyacetal, PP= polypropylene, PU= polyurethane, PVA= polyvinyl acetate, and RY=rayon.

Taxon	Location	Year	N	% Occ.	No/ind.	Microplastic characteristics				Reference
						Size (µm)	Shape	Colour	Polymer	
Neogobius melanostoma	Rhein, Germany	2014-2018	*na*	25	0.3	51-4986	FB, FR	B, BL	*na*	Roch et al., 2019[a]
	Altrhein, Germany	2014-2018	*na*	15	0.3	51-4986	FB, FR	B, BL, R	*na*	Roch et al., 2019[a]
	Neckar, Germany	2014-2018	*na*	8	0.1	51-4986	FR	W, C	*na*	Roch et al., 2019[a]
	Muskegon River, USA	2016	*na*	100	20	1750-5000	FB	BL, GR, R	POM, PVA, PET	McNeish et al., 2018
	Milwaukee River, USA	2016	*na*	100	22.9	1750-500	FB, FR	BL, GR, R	PE, POM, PAN	McNeish et al., 2018
	St. Joseph River, USA	2016	*na*	100	10.5	1750-5000	FB	BL, GR, R	PAN	McNeish et al., 2018
	Lake Ontario, Canada	2015	84	*na*	31	*na*	FB	BL, B, C	PE, PET, PP	Munno et al. 2021 *
	Lake Michigan, USA	1994-1999	5	40	1.2	560-1120	FB	C, BL, B	PET, AC, CE	Hou et al., 2020*
	Lake Michigan, USA	2005	5	60	1.8	280-2800	FB	C, BL, GR	PET, AC, CE	Hou et al., 2020*
	Lake Michigan, USA	2010-2011	5	60	0.6	840-1960	FB	B, GR, BL	CE, PU	Hou et al., 2020*
	Lake Michigan, USA	2018	17	65	2.06	280- 3360	FB	C, BL, R	CE, PE	Hou et al., 2020*
	Sorel, SLR, Canada	2015	18	22	0.2	500	FB	BL, R, B	*na*	Brusco et al., 2005[b**]
	Gentilly, SLR, Canada	2015	20	15	0.15	500	FB, B	*na*	*na*	Brusco et al., 2005[b**]
	Les Coteaux, SLR, Canada	2015	30	43	1.87	500	FB, FR	BL, R, BL	*na*	Brusco et al., 2005[b**]
	Varennes, SLR, Canada	2015	30	50	1.3	500	FB, FR	B	*na*	Brusco et al., 2005[b**]
	Contrecoeur, SLR, Canada	2015	30	40	1.03	500	FB, B	B, R, O	PE	Brusco et al., 2005[b**]
	Melocheville, SLR, Canada	2015	30	20	0.23	500	FB	B, R, V	*na*	Brusco et al., 2005[b**]
	Léry, SLR, Canada	2015	30	23	0.43	500	FB, FR	R, B, BL	*na*	Brusco et al., 2005[b**]
	Châteauguay, SLR, Canada	2015	30	23	0.36	500	FB, FR	R, BL, B	*na*	Brusco et al., 2005[b**]
	Sorel, SLR, Canada	2015	30	10	0.27	500	FB, FR	BL, R	*na*	Brusco et al., 2005[b**]
	Lachine, SLR, Canada	2015	30	27	0.53	500	FB, FR	BL, R, B	*na*	Brusco et al., 2005[b**]
	St-Anicet, SLR, Canada	2015	30	23	0.31	500	FB	B, R, BL	*na*	Brusco et al., 2005[b**]
	Verchères, SLR, Canada	2015	30	33	0.59	500	FB ,FR	B, R, BL	*na*	Brusco et al., 2005[b**]
	Basel, Rhine River, Switzerland	2018-2019	194	*na*	0.15	243-8897	FB	W-Y	PU	Bosshart et al. 2020
	Cologne, Rhine River, Germany	2018-2019	30	*na*	0.60	243-8897	FB	W-Y	PU	Bosshart et al. 2020
	Rees, Rhine River, Germany	2018-2019	193	*na*	0.13	243-8897	FB	W-Y	PU	Bosshart et al. 2020
Amphipod	Albano Lake, beach 1, Italy	2017	10	*na*	2.20	25-55	FR	*na*	PA, PE, RY	Iannilli et al. 2020
spp.	Albano Lake, beach 2, Italy	2017	10	*na*	1.80	25-55	FR	*na*	PA, PE, RY	Iannilli et al. 2020
	Bracciano Lake, beach 1, Italy	2017	10	*na*	5.00	25-55	FR	*na*	PE	Iannilli et al. 2020
	Bracciano Lake, beach 2, Italy	2017	10	*na*	4.60	25-55	FR	*na*	PA, PE, RY	Iannilli et al. 2020
	Italian Beaches, Italy	2010-2011	200	*na*	presence	8-25	*na*	W, BL , G	PE, PE, CE	Ugolini et al. 2013
Dreissena spp.	Port of Milwaukee (ref1), USA	2018	100	*na*	0.6	*na*	FB, FR	BL, C, B	none	Hoellein et al., 2021[c]
	McKinley Park (ref 0), USA	2018	300	*na*	0.36	*na*	FB, FR, B	BL, C, B	PET, CE, AN	Hoellein et al., 2021[c]
	Veterans Park (Ref 4), USA	2018	300	*na*	0.76	*na*	FB, FR	BL, C, B	PE, CE, PA	Hoellein et al., 2021[c]
	Lake Park (ref 5), USA	2018	300	*na*	0.43	*na*	FB, FR	C, BL, B	CE, PET, PP	Hoellein et al., 2021[c]
	Milwaukee River (R6), USA	2018	200	*na*	0.57	*na*	FB, FR	C, BL, GR	CE, PET	Hoellein et al., 2021[c]
	Jones Island -WWTP (17), USA	2018	200	*na*	1.11	*na*	FB, FR	C, BL, GR	CE, PET	Hoellein et al., 2021[c]
	Neuville, SLR, Canada	2017	4	100	5	*na*	FR, FB, B	C, P, O	*na*	D'Avignon, et al., 2017[d**]
	Iles de la Paix, SLR, Canada	2017	12	83	9.42	*na*	FR, FB, B	C, P, O	*na*	D'Avignon, et al., 2017[d**]
	Les Coteaux, SLR, Canada	2017	7	100	6	*na*	FR, B, FB	C, P, O	*na*	D'Avignon, et al., 2017[d**]
	N-D-L'Ile-Perrot, SLR, Canada	2017	4	100	2.5	*na*	FR, FB, B	C, W	*na*	D'Avignon, et al., 2017[d**]
	Baie de Valois, SLR, Canada	2017	6	100	8.3	*na*	FR, FB, B	C, W, P	*na*	D'Avignon, et al., 2017[d**]
	Varennes, SLR, Canada	2017	3	67	7.3	*na*	FB, FR, B	C, O, W	*na*	D'Avignon, et al., 2017[d**]
	Sorel, SLR, Canada	2017	5	100	8.8	*na*	FR, FB, B	C, O, P	*na*	D'Avignon, et al., 2017[d**]
	Las Vegas Bay, USA	2017	11	*na*	13	>125	FB, FR, FM	*na*	*na*	Baldwin et al. 2020
	Overton Arm, USA	2017	10	*na*	2.7	>125	FL, FB	*na*	*na*	Baldwin et al. 2020

[a] Values were not reported per species but per fish community, MP validated by hot needle test

[b] particles were identified visually, beads were tested using hot needle test, PE cosmetic beads similar to those in sediments (Castañeda et al. 2014)

[c] Counts were estimated from figures from manuscript using DigitizeIt

[d] Counts of anthropogenic particles identified using Nile Red fluorescence, sizes of particles not yet measured range can be from 1-5000µm

* Supporting information

** Unpublished data

Table S2.2: Summary of models used to answer each hypothesis with the corresponding chosen distribution and R package. The standard deviation of our counts being greater than the mean implied overdispersion of our data. A type I Negative binomial distribution (mean = μ and variance = $\mu+\sigma\mu^2$) usually provided a good fit. The Poisson-Inverted Gaussian distribution (P-IG) was chosen when count were highly skewed with a high initial peak and a long right tail. The estimated variance could be constant or vary per concentration (constant sigma vs. sigma ~ concentration). To measure body burden, the total bead count was standardized by the weight of the digested tissue of the animal in grams, represented by the offset in the models. When different models were selected per animal, these are indicated in subscript by DRBU for mussels, GAM for gammarids and NEME for round gobies.

H1	Count ~ concentration + source + animal + concentration:source + concentration:animal + source: animal + concentration:source:animal +offset *P-IG*, *sigma~concentration (gamlss)*
H2	Count~ concentration + source + animal + concentration:source + concentration:animal + source: animal + concentration:source:animal +offset *P-IG, sigma~concentration (gamlss)*
H3	$Count_{ret}$ ~ Depuration time + offset $NBI_{(DRBU, NEME)}$, $P\text{-}IG_{(GAM)}$ *(gamlss)*
H4	$Count_{DRBU}$~ concentration + source + concentration:source + random(1|aquaria) + offset *Neg.Binomial (lme 4)*
H5	$Count_{DRBU}$ ~ abundance + random(1|aquaria) + offset *NBI (lme4)*
H6	$Count_{GAM}$ ~ concentration + source +concentration:source + offset *P-IG*, *constant sigma (gamlss)*
H7	$Count_{GAM}$ ~ mussel abundance + exposure time + offset *NBI (mean = μ and variance = $\mu+\sigma\mu^2$) (gamlss)*
H8	$Count_{NEME}$ ~ route of exposure + ln(condition) + offset *P-IG*, *constant sigma; routes = 6 levels; condition= scaled & centered (gamlss)*

Table S2.3: Type III ANOVA table comparing estimated marginal mean body burden (number of beads per gram of tissue) between treatments computed from the best fitting P-IG model, where sigma ~ concentration. A significant p-value ($p< 0.05$) indicates the variable is influential on predicting the abundance of microbeads.

	DF	**F-ratio**	***p*-value**
Concentration	6	51.52	<0.001
Animal	2	136.5	<0.001
Route	1	-0.01	1.0
Concentration:Animal	12	2.26	0.007
Concentration:Route	6	3.05	0.005
Animal:Route	2	4.69	0.009
Concentration:Animal:Route	12	3.54	<0.001

Table S2.4: Model output for the mixed NBI model calculated with lme4 package. The model described the effects of concentration, source, and their interaction while controlled for the abundance of mussels. The nesting of mussels within aquaria was used as a random effect amongst replicates and accounted for 51% of the model's variance.

Terms	**Coeff.**	**Std error**	**Z-value**	***p*-value**
0 SUS	1.14	0.54	2.10	0.036
0.1 SUS	0.82	0.67	1.23	0.221
1 SUS	3.13	0.68	4.57	<.001
5 SUS	2.87	0.75	3.81	<.001
10 SUS	3.10	0.71	4.36	<.001
50 SUS	4.43	0.65	6.78	<.001
100 SUS	4.78	0.57	8.40	<.001
Route (SED)	1.07	0.77	1.39	0.165
Abundance	-0.34	0.12	-2.94	0.003
0.1 : Route	-3.29	0.96	-2.86	0.004
1 : Route	-3.61	0.90	-3.66	<.001
5 : Route	-3.18	1.05	-3.43	0.001
10 : Route	-3.44	0.97	-3.28	0.001
50 : Route	-2.82	0.97	-3.56	<.001
100 : Route	-2.66	0.77	-3.64	<.001
Random effect	Variance	1.11	Std. Dev.	1.052

Table S2.5: Type III ANOVA table comparing estimated marginal mean body burden (number of beads per gram of tissue) between treatments computed from the best fitting P-IG model, with a constant sigma. A significant p-value ($p < 0.05$) indicates the variable is influential on predicting body burdens.

Terms	**DF**	**F-ratio**	***p*-value**
Concentration	6	18.08	<.0001
Route	1	-0.536	1.000
Concentration:Route	6	5.923	0.0001
AIC		1229	
SBC		1287	
R^2		52.68	

Table S2.6: Type III ANOVA table comparing estimated marginal mean body burden (beads$\cdot g^{-1}$) between treatments computed from the best fitting P-IG model, with a constant sigma. A significant p-value ($p < 0.05$) indicates the variable is influential on predicting body burdens.

Terms	**DF**	**F-ratio**	***p*-value**
Concentration	6	5.95	<.0001
Route	1	10.12	0.002
Concentration:Route	6	1.78	0.099
AIC		752	
SBC		794	
R^2		34.42	

Table S2.7: Summary of the body burden estimated means (beads·g^{-1}) from best fitting models per species showing sidak-adjusted multiple contrasts between treatment means with their associated standard error, lower and upper confidence intervals. Sample size (N) per treatment is also provided.

Treatment	[Bead]	N	Mean	SE	LCI	UCI	Contrast
DRBU	0	27	3.1	1.7	1.1	9.0	A
SUS	0.1	28	7.1	2.8	3.3	15.3	A
	1	27	71.3	28.9	32.3	157.6	B
	5	28	54.9	28.4	19.9	151.5	B
	10	29	69.0	31.0	28.6	166.3	B
	50	32	262.3	103.0	121.5	566.2	BC
	100	54	370.9	111.9	205.3	670.1	C
DRBU	0	20	9.1	5.3	2.9	28.2	ab
SED	0.1	18	1.3	0.7	0.4	3.9	a
	1	29	7.7	3.1	3.5	17.0	ab
	5	28	4.4	1.9	1.9	10.1	a
	10	33	8.3	3.3	3.8	18.2	ab
	50	28	24.5	8.9	12.0	50.0	bc
	100	33	64.4	22.3	32.7	126.9	c
GAM	0	18	9.2	*4.9**	*3.2**	*26.1**	A
SUS	0.1	18	18.7	17.0	3.15	111.1	AB
	1	19	0	*13.8**	0	*13.8**	§
	5	20	87.1	43.1	20.64	367.5	B
	10	20	176.1	70.1	55.4	559.9	B
	50	23	1379.5	503.8	477.3	3987.1	C
	100	18	251.7	130.8	55.6	1140.1	BC
GAM	0	31	8.9	4.7	1.9	41.2	a
SED	0.1	20	37.2	21.9	6.7	205.4	ab
	1	32	36.0	17.2	9.0	144.8	ab
	5	33	107.8	42.2	34.5	336.4	abc
	10	36	162.5	46.5	70.7	373.5	bc
	50	33	543.8	165.4	224.7	1316.1	cd
	100	34	5257.8	1603.9	2166.5	12759.9	e
NEME	0	12	0.3	0.3	0.0	2.8	A
SUS	0.1	5	0.6	0.6	0.1	4.0	A
	1	8	0.2	0.2	0.0	1.0	A
	5	10	0.4	0.3	0.1	1.6	A
	10	12	3.0	2.1	0.7	12.0	A
	50	13	5.1	3.3	1.4	18.1	A
	100	11	17.7	11.8	4.8	65.6	A
NEME	0	5	1.6	0.7	0.6	4.0	ab
SED	0.1	7	2.1	1.9	0.3	12.7	abc
	1	10	3.3	2.3	0.8	13.0	a
	5	8	3.3	1.7	1.2	9.1	ab
	10	7	5.6	2.6	2.2	14.0	abc
	50	7	6.7	3.5	2.4	18.6	bc
	100	7	9.9	5.5	3.3	29.5	c

Table S2.8: Summary of the body burden means (beads·g^{-1}) at each depuration time estimated from best fitting retention models per species with their associated standard error, lower and upper confidence intervals and sample size (N). Sidak-adjusted multiple contrasts between treatment means are displayed by letter codes.

Animal	Depuration time (hours)	N	Mean	SE	LCI	UCI	Constrast
$DRBU_{NBI}$ (SUS)	0	17	127.0	39.7	68.8	234.5	a
	3	10	49.2	23.1	19.6	123.5	a
	6	10	40.5	17.9	17.1	96.2	a
	9	12	96.2	36.5	45.8	202.2	a
	12	10	65.1	29.2	27.1	156.6	a
	24	7	72.9	36.0	27.7	192.0	a
	48	8	27.1	13.2	10.4	70.6	ab
	72	15	2.8	1.8	0.8	9.6	b
GAM_{PIG} (SED)	0	33	669.3	262.6	310.2	1443.9	bc
	3	18	2020.1	1014.8	754.7	5407.1	c
	6	17	274.8	133.7	105.9	713	ab
	9	18	182.2	89.5	69.57	477.2	ab
	12	21	154.5	71.6	62.3	383.2	ab
	24	17	10.9	10.5	1.65	72.1	a
	48	13	27.7	25.6	4.51	170	a
$NEME_{NBI}$ (SUS & SED)	0	11	7.51	3.58	2.95	19	a
	3	9	0.05	0.06	0.01	1	b
	6	10	0.18	0.13	0.04	1	b
	9	8	0.08	0.07	0.01	0	b
	12	8	0.35	0.26	0.08	1	b
	24	10	0.08	0.07	0.01	0	b
	48	11	0.00	0.00	0.01	Inf	c*
	72	7	0.00	0.00	0.01	Inf	c*

Table S2.9: Panel A described the joint test analyses (Type III ANOVA) showing the effect of fish length on the body burden of round gobies and its influence on the percentage of prey ingested vs. the number of prey offered. Panel B shows the results of the joint test and best P-IG model coefficients with their respective *p*-value. Length was centered and ln-transformed.

A	**TERMS**	**DF**	**F-ratio**	***p*-value**
Length (NBI)	Treatment	3	10.38	<.0001
	P. of prey cons.	1	3.79	0.05
	c.log.length	1	2.33	0.13
	P.of prey cons.* length	1	2.33	0.13
Length (P-IG)	Treatment	3	10.49	<.0001
	P. of prey cons.	1	2.29	0.13
	c.log.length	1	0.54	0.46
	P.of prey cons.* length	1	0.63	0.43

B	**TERMS**		**DF**	**F-ratio**	***p*-value**
Best model (P-IG)	Treatment		3	11.15	<.0001
	P.tot.avail		1	12.83	0.0003
		Coefficient	**SE**	**t-value**	***p*-value**
	DRBU	1.38	1.4	1.24	0.219
	GAM	-3.57	0.7	-4.74	<.0001
	5 DRBU + 5 GAM	-5.63	1.1	-5.05	<.0001
	10 DRBU + 5 GAM	-2.59	0.7	-3.56	0.0008
	P. of prey cons.	0.045	0.0	3.58	0.0007

Table S2.10: Summary of the body burden estimated means (beads·g^{-1}) with their associated standard error, lower and upper confidence intervals from best fitting models per species interaction experiment. A) Effect of conspecific on per capita mussel body burden, B) Effect of mussel abundance and exposure time on gammarid body burden via non-trophic transfer, C) Comparison of body burden between environmental exposure and predator-prey interaction scenarios. Sidak-adjusted multiple contrasts (MC) between treatment means are indicated with a letter code. The sample size (N), the observed occurrence of individuals with beads (%). The number of gobies which consumed 100% of prey items are indicated under the colums "Prey cons." in experiment C.

A		**Abundance**	**N**	**%**	**Mean**	**SE**	**LCI**	**UCI**	**MC**
		1	9 (10)	90	205.1	125.2	62	678	a
		5	23 (8)	100	930.5	833.9	160.6	5390	a
		7	31 (10)	97	84.5	42.3	31.7	226	a
		15	141 (10)	83	185.8	79.9	80	432	a
B	**Time**	**Abundance**	**N**	**%**	**Mean**	**SE**	**LCI**	**UCI**	**MC**
	24	1	30	17	16.61	8.29	4.47	61.7	ab
		5	20 (4)	14	15.63	7.88	4.15	58.9	ab
		10	40 (4)	29	38	14.51	13.92	103.8	b
	48	1	30	4	1.88	1.59	0.20	17.4	a
		5	20 (4)	5	1.77	1.54	0.18	17.5	a
		10	20 (2)	0	4.31	3.45	0.52	35.5	ab
C	**Treatments**	**Prey cons.**	**N**	**%Risk**	**Mean**	**SE**	**LCI**	**UCI**	**MCt**
	SUS	10	10	100	15.50	17.28	1.74	137.84	a
	SED	12	12	58	3.26	3.13	0.50	21.33	a
	GAM	17	17	76	26.70	32.61	2.44	292.60	ab
	DRBU	6	22	95	315.36	354.82	34.76	2860.91	b
	5DRBU_5GAM	8	9	33	0.45	0.37	0.09	2.25	a
	10DRBU_5GAM	4	9	100	17.69	22.60	1.45	216.25	ab

Table S2.11: Summary of metrics comparing the capacity of each organism to acquire microbeads. The total number of beads per individual is calculated based on the mean body burden estimated from Table S2.7 multiplied by the mean mass of the animal (in gram). The capture efficiency (reported in %) corresponds to the percentage of beads found in each animal after 24 h of exposure to 100 beads·ml^{-1} based on the total available at this concentration (~700 553 beads).

Prey	**Route**	**Bead g^{-1}**	**Digested mass (g)**	**Bead ind.$^{-1}$**	**% Efficiency**
DRBU	SUS	371	0.26 ± 0.2	97	0.014
GAM		252	0.012 ± 0.01	3	0.0004
NEME		18	4.28 ± 1.93	77	0.011
DRBU	SED	65	0.26 ± 0.2	17	0.002
GAM		5258	0.012 ± 0.01	63	0.009
NEME		10	4.28 ± 1.93	43	0.006

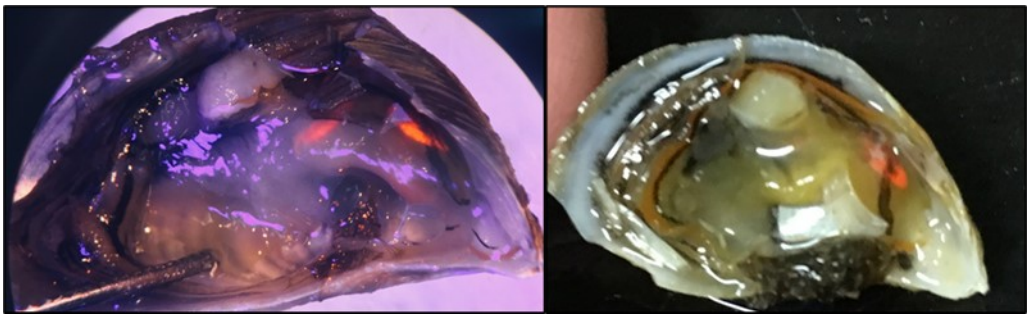


Photo S2.1: Example of bead aggregation in quagga mussels (*Dreissena bugensis*) after 24-h exposure to 100 PE beads·ml^{-1}. Orange beads accumulate on the internal byssal threads.

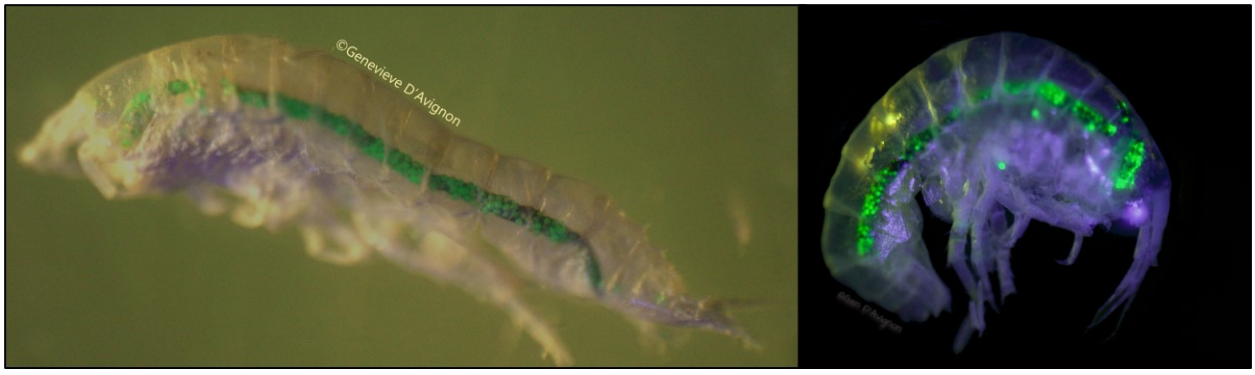


Photo S2.2: Examples of microbead retention inside the digestive tract of gammarid amphipods exposed to 100 beads·ml^{-1} for 24 h. Beads will eventually be released trapped with the fecal pellet produced after digestion of food items.

2.9 Supplementary Materials: Collection and maintenance of live specimen

2.9.1 General animal acclimation and care

Captured animals were placed into aerator-mounted coolers filled with ambient water and transported in these coolers to the animal facility within three hours of capture. All housing and experiments were conducted in temperature controlled Conviron® phytotron growth chamber or temperature-controlled room, maintained between 18-19°C at a 12h light/12h dark light cycle. Once at the facility, mussels and fish were left to acclimate until the water in coolers reached room temperature (18°C), at which point they were transferred to large aquariums of 20-70L mounted with an aerator and three-part filter for at least two weeks before the onset of experiments.

Aquarium water was prepared ahead of time by filling a large 30-gallon drum with tap water and using Seachem Prime solutions to condition and dechlorinate the water at least 24-h before use. The drum was held at 18-20°C. When conditioned water was added to an aquarium, 5ml of Seachem Stability was added per 40L to establish a healthy biofilter. Water used for gammarid trials was prepared as described above but held in a 35-L acid-washed carboy. Five ml of Seachem Stability was added directly to the carboy. The water held in the carboy was used to fill the 500ml containers holding the amphipods during the acclimation period lasting from 5 to seven days to avoid molting, reproduction, and death.

Water quality parameters (pH, ammonia, nitrite, nitrate) were monitored three times a week until aquarium cycling has been achieved, then these were monitored weekly. The removal of accumulated debris, digested foods and fecal material in aquaria was performed with an aquarium pump each week, followed by the replacement of 25-30% of the water volume with new dechlorinated water.

2.9.2 Algal Culture and feeding mixture preparation

A new algae culture is started from a seeding stock of *Scenedesmus* sp. and *Pseudokirchneriella* sp. every month. To prepare the culture, approximately 20 mL of concentrated Bold Basal Medium (Sigma-Aldrich B5252 -500mL) is added to a 1000ml clean flask and sterilized via a 25-minute autoclave liquid cycle at 121°C and 15 psi. Once the media has cooled, a small amount (2–5 mL) of inoculum from the culture provided are added. The new culture will be stored in a temperature (18˚– 19˚C) under a 16-h light to 8-h dark period. An aquaria stone bubbler fixed to an air pump is added to the flask to provide proper growing conditions. All material used to grow the culture is cleaned and autoclave to prevent contamination. Under the current setting, both algae reach optimum growth after 14 days. Once optimum growth is achieved, the culture is transferred to a 4°C fridge under low light to be kept as inoculum for future sub-sampling. At least 4 x 55mL flasks containing inoculum of the newly grown culture is stored each month. The freshest culture showing the best condition (under the scope) is sub-sampled to prepare fixed algae concentration solutions, while the majority of the culture is used to feed the mussels. Algae is stored for no more than 3 months.

Once the live algae cultures were ready, an algal feeding mixture was prepared for the invertebrates and stored at 4˚C. The mixture was composed of an equal volume of live algae and of coral and fine-filter feeder foods (either PhytoPlex Plankton or Microvert food from Kent Marine). We pre-mixed 25ml of *Scenedesmus* sp., 25ml of *Pseudokirchneriella* sp., and 25 ml of PhytoPlex into a new container 100ml vial before each feeding event. Unused solution were kept refrigerated at 4˚C.

2.9.3 Fish care

Fish care followed the CCAC protocol 2017-7913 based on McGill SOP 519. Fish-holding aquariums were filled with gravel substrate and one PVC tube was added per fish to serve as a shelter during acclimation. Round gobies were fed until satiation every second day with protein/carbohydrate sinking granules (Nutrafin Bug Bites Bottom Feeder Formula 1.4-1.6 mm). The health of the fish was visually monitored daily. Fish showing signs of poor health or distress - e.g., lack of respiratory activity (mouth and gill movement) or lethargy (lack of swimming activity), they were isolated and kept under observation during acclimation. If we did not observe improvement over 24h, the fish were euthanized by immersion in a 50-350 mg/kg fish solution of clove oil followed with cervical dislocation as per McGill SOP 303. All euthanized animals were disposed in closed plastic bags and discarded as biohazard waste.

2.9.4 Invertebrate care

Mussels were acclimated in large 50-70L aquaria mounted with an aerator and three-part filter for at least two weeks before the onset of experiments. Every second day, mussels were fed with the live and PhytoPlex algae mixture solution by adding 5ml per 50L of water.

Gammarids were held in a temperature-controlled room at 18°C and placed in small groups in 500 ml containers with gravel and mesh pieces of 2x2cm for shelter. Natural leaves taken from the field were also provided to the gammarids for the first few days to serve as food and shelter. When leave material had been consumed, gammarids were fed with 1ml of algae mixture solution. Gammarids were acclimated for five to seven days before the onset of experiments. Debris and waste were removed using a pipette and about 30% of the water was replaced with clean 18°C dechlorinated water daily.

2.9.5 Animal care during the experimental phase

Healthy animals were selected for experimental trials assessed, based on the following characteristics: 1) actively swimming gammarids; 2) filtering mussels with open siphons and that responded to touch; and 3) fish displaying normal swimming and breathing behaviour (monitored throughout the holding period). All animals fasted for ~48h before the start of experiment and fed after the start of the experimental trials as described in Supplementary Material S2.9.3-S2.9.5.

Most trials for mussels and gobies were run in ~8-L aquaria that were set-up at least 3 days in advance. However, 16-L aquaria were used for six gobies ≥ 90 mm and ≥ 9g because they showed signs of stress in 8-L settings, and for predator-prey experiments where both invertebrates were offered to fish. Mussels were placed in groups of five or seven per aquaria, whereas round gobies were placed individually. Each test aquarium was mounted with an aerator and a bio-bead was deposited at the bottom, before being filled with five or seven liters of dechlorinated water (in 8L aquaria, but 15L for 16-L aquaria). A PVC tube was added as a shelter to each aquarium used to examine the response of round gobies. Seachem Stability bacterial solution was added (1ml per 8L), and water quality was monitored daily (ammonia, nitrate, nitrite, pH, temperature) until the onset of experiments. Gammarids were placed individually in 10-ml wells of ice-cube trays. A single gravel pellet was placed in each well to serve as a shelter for the animal.

2.10 Supplementary Materials: Treatment preparation

Table S2.12: Procedures to prepare settled bead treatments for mussels and gobies for aquaria holding 7-L of water.

Desired [beads·ml^{-1}]	**7L aquaria**	**Estimated No. beads**	**Measured mass (mg)**
0.1 ±0.03	Take 1ml of (7000 beads·ml^{-1}) and add 10 ml of water. Take 1 ml of the new solution.	713 ± 90	0.12±0.02 (est.)
1 ±0.2	Measure 0.1205g ±0.001mg of beads, add to 100ml of water. Take 1ml.	7 117 ± 256	1.2±0.1 (est.)
5±0.7	0.0061±0.0001g	35 464 ± 581	6.1±0.1
10±1.3	0.0121±0.0002g	70 346 ± 1163	12.1±0.2
50±6	0.0605±0.0002g	35 1730 ± 1163	60.5±0.2
100 ±11	0.1205g±0.0002g	700 553 ± 1163	120.5±0.2

Some uptake experiment trials for mussels and gobies were conducted in 5-L aquaria. For these treatments, beads were prepared by creating a suspended stock solution and then, using dilutions. We weighed 500 ± 0.1mg of PE beads which were treated with Tween80, and then diluted in 400ml ±1ml of filtered reverse-osmosis water using a 50ml±1ml cylinder to create a suspended stock solution of 7267 ± 33 beads·ml^{-1}. To create desired concentrations, we used a 0.01ml pipette or a 10ml± 1ml graduated cylinder and followed procedures described in Table S2.13 for 5-L aquaria. For gammarid trials, 12.1 ± 0.1 mg of Tween 80-treated beads were added to 700 ±1 ml to create a stock solution of 100 beads·ml^{-1} (Table S2.14).

Table S2.13: Procedures to prepare suspended bead treatments scenarios for mussels and gobies for 5 or 7L aquarium. Error for beads is estimated as the number of beads prepared before being added to experimental aquarium. Different volumes of aquarium were used depending on the trials.

Desired [mp/ml]	5 L aquaria			7 L aquaria		
	No beads	Volume measured (ml)	Estimated mass of beads (mg)	No beads	Volume measured (ml)	Estimated mass of beads (mg)
0.1	509 ± 75	0.07 ± 0.01	0.09±0.1	654 ± 76	0.09±0.01	0.11 ± 0.01
1	5014 ± 96	0.69 ± 0.01	0.80 ±0.1	6976 ± 104	0.96±0.01	1.2 ± 0.02
5.5	27615 ± 198	3.80 ± 0.01	4.7±0.1	38516 ± 247	5.30±0.01	6.6 ± 0.03
10	50143 ± 7526	6.90 ± 1.00	8.6 ±1	69765 ± 7615	9.6±1	12 ± 1.3
100	501433 ± 9562	69.0 ± 1.00	86±1	697646 ± 10447	96 ±1	120 ± 1.6

Table S2.14: Procedures for the preparation of treatments for gammarid exposures. Note that d-water refers to dechlorinated water. Tween80 was added for treatments in suspension. Solutions were well mixed before sampling desired volumes from the stock concentration using a 0.01ml graduated pipette.

Concentrations beads·ml^{-1}	Steps	Estimated No. beads	Estimated mass of beads (mg)
0.1 ± 0.01	Measure 0.01ml of the stock solution. Add 9.99ml of d-water to the well.	1 ± 0.1	0.0002 ± 0.0001
1 ± 0.04	Measure 0.1ml of the stock solution and add it to 9.9 ml of d-water.	10 ± 0.4	0.0017 ± 0.0005
5 ± 0.16	Measure 0.5ml of the stock solution and add it to the well. Add 9.5 ml of d-water to the well.	50 ± 2	0.009 ± 0.002
10 ± 0.4	Measure 1ml of the stock solution and add it to the well. Add 9ml of d-water to the well.	100 ± 3	0.017 ± 0.005
50 ± 20	Measure 5ml of the stock solution and add to well. Add 5ml of d-water to the well.	500 ± 118	0.086 ± 0.03
100 ± 27	Weigh 12.1±0.1mg of beads. Add to 700±1ml of water to produce the stock solution of 100 beads·ml^{-1}. Fill well with 10ml.	1000 ± 132	0.17 ± 0.05

2.11 Supplementary Material: Microplastic extraction and counts

2.11.1 Summary

Extraction of microplastic were adapted from the digestion-and-filtration method proposed by Karami et al. (2017) and the quality insurance protocol suggested by Hermsen et al. (2018). Each organ and tissue of interest will be removed under a laminar flow using pre-cleaned metal dissecting tools. For smaller animals, the entire body will be selected, while in for larger animals, organs or tissues can be selected individually. A procedural blank will be recorded per groups of animals to assess air contamination during dissections. Each group corresponds to animal tested for the same treatment for a single trial date. A 10% or 20% solution of potassium hydroxide (KOH) was used as chemical agent to digest organic materials from the sample for 48 h in a 40°C oven. This method is the most efficient and least damaging to microplastics reported in the literature (Table S3.11.1). Digested samples will be poured on a A/E glass fiber PALL 1µm filter (0.47mm, product #61631) and drained using a vacuum pump. Reverse-osmosis (RO) water filtered at 0.1µm was used to wash and rinse all materials.

2.11.2 Personal Protective Safety Equipment

- Please wear the appropriate cotton clothing as soon as you enter the microplastic lab
- Use protective goggles when handling chemicals
- Wear protective nitrile gloves when handling samples or chemical
- All chemicals should be handled underneath the fume hood. When using KOH under the laminar flow hood, wear a mask to prevent inhaling fumes
- If the noise is too loud (both laminar and vacuum pump are on), wear earplugs

2.11.3 Dissection and digestions

Material Preparation

- Fill the RO 10L jugs
- Wash all glass material and metal dissection equipment with soap and rinse 3 times with RO water.
- Check under UV light for presence of fluorescent orange or green beads
- Place under the laminar flow to dry
- One or two hours before dissections, bring bags out of the freezer and place in cold tap water in a container for the specimen to defrost

Table S 2.15: Summary of digestion methods to extract microplastics from the gastro-intestinal tract of fish and bivalves' tissues. Superscripts indicate the effects of the digestion agent on the polymer, - indicate a decrease in mass, + indicate an increase in mass, * indicate a change in mass but no significant trends.

Protocol	Digestion agent	Efficiency (%)	Effects on polymer types	Recovery (%)	Total time	Polymer ID
Karami et al. (2017)	KOH (10%)	97.61	LDPE, HDPE, PP, PS, PET, PVC, PA6, PA66^{+}	85-104	24h	RAMAN, Slight change in intensity for PA
Karami et al. (2017)		98.6	LDPE, HDPE, PP, PS, PET, PVC, PA6, PA66	93-105	48h	RAMAN, no effect
Kuhn et al. (2017)	KOH (10%)	n.a	PBT, EVA VA19%, PE-LLD, SAN, GPPS, PC, ABS	n.a	48h	no effect
Roch and Brinker (2017)	NaOH 1mol/L	Na 90±2.9	HDPE, LDPE^{-}, PET^{+}, EPS, PS^{-}, PP, PA*, PVC-U^{+}/P^{+}	95	1-2h	FTIR, no effect
Catarino et al. (2017)	Corolase 7089	100	PET, HDPE, PVC+, PA	82-104	1-12h*	FTIR, no effect
Courtene-Jones et al. (2017)	Trypsin	88	PET, HDPE, PVC, PP, PS, PA	n.a.	<1h	ATR-FTIR, no effect
Cole et al. (2013)	Proteinase K	88.9-97	PS, PET, PE, PA, PVC			FTIR, no effect

Material

Make sure you have all the material available before starting. Get everything you need (fill RO water, clean vials, etc).

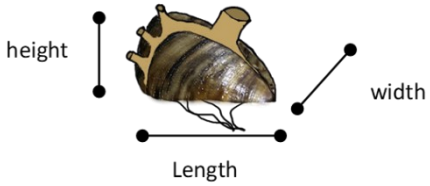
- Clean glassware (20ml or 30 ml glass vial for GI tract of fish, amphipod, and mussels)
- Clean dissection tools
- Full 10L RO water container
- RO squirt bottles
- Weighing Scale
- Measuring board, rulers, calipers

Dissections and extraction of organs and tissues

- Place a glass petri dish under the laminar flow hood and fill it with water – this will serve as contamination blank – at the end of all dissection pour the content in a jar and triple rinse it with RO water. Label it as blank with the date of the day. * If you are continuing to digestion, add 20mL of KOH and place with other samples in the oven, otherwise place it on the table.
- Put on the cotton clothes provided and other PPE, and turn on the laminar flow hood

Mussels

1. Take animals out of the bag, record the experimental details (date of experiment, type of experiment, time of exposure, concentration, retention time, etc.)
2. Weigh entire mussel including the shell and tissue onto the 0.01g scale. Write the information on the datasheet.
3. Using a caliper measure the length, width and height of the shell in mm. Write the information on the datasheet.



4. Place a clean 20 ml vial on the scale and tare.
5. Slide blade between the two valves to cut the muscles holding the valves shut.
6. Extract all the soft tissues and place in the vial on the scale.
7. Weigh the soft tissues and record the weight on the datasheet. Close the lid

Amphipods

1. Take animals out of the Eppendorf, record the experimental details (date of experiment, type of experiment, time of exposure, concentration, retention time, etc)
2. Using the stereoscope, measure the full length of the amphipod, from the base on its antenna to the end segment of the tail.
3. If a 0.001g scale is available, weigh the animal and record its mass on the datasheet.
4. Place the animal in a small cleaned 20ml vial. Label the jar with the appropriate label

Round gobies

- Copy the info from the bag to the datasheet
- Measure the total length of the fish in mm using a ruler and record the value on the datasheet
- Weigh the fish on the 0.01g scale and record the value on the datasheet
- Weigh an empty 20 ml vial and label with animal number and GI.
- Place the fish to be dissected so the head faces left.
- Make an incision starting from the anus to the side of the gill and open the skin to see the organs.
- Cut the digestive tract (GI) by removing the tube from the esophagus to the anus, and place in the pre-weighed vial, and close the lid.
- Weigh and record the mass of the gi tract (if required, weigh other organs and record the value)
- Verify the gonads for sex identification and maturity status. Confirm sex with the shape of the anal pore (V-shape for male, rectangular-flat for female).
- Slice the skull near the base, collect the otolith, and store in a labelled bag or in an Eppendorf for ageing.
- Remove all organs from the body and dispose in biohazard bin.
- Weigh the 30 ml vial and label it with fish number and body.
- Place the carcass of the fish in the pre-weighed 30 ml vial and weigh again to record the mass of the carcass. Close the lid.
- Proceed to digestion

2.11.4 Digestion

Material preparation

- Wash all glass material and metal dissection equipment with soap and rinse 3 times with pure distilled water. Place in the oven at 230°C for 6 h to sterilize items (clean oven). If using plastic items or items that are not resistant to heat, clean them with soap and water, rinse them 3 times with RO water and place in the acid bath (follow acid bath protocol, acid bath is under the fume hood). Note that glass items must remain 3 days in the acid bath and plastic items can soak overnight.
- Triple rinse all metal equipment with RO water and dry immediately using cotton cloths
- Fill the RO 10L jugs
- Prepare the KOH digestion solutions, the Alcojet detergent solution and the oil or calcium chloride solution for microplastic separation (see Table S3.11.2)
- Get the samples out of the freezer 15 minutes to 1 h before starting and let them thaw under the laminar hood (you may take this time to ensure you have all the material ready. Smaller organ will thaw faster)

Table S 2.16: Description of solution preparation for digestion and microplastic separation.

Digestion Method	**Solution Preparation**	**Personal Protective Equipment Required**
20% KOH (200 g/L)	Add 200g of KOH pellets per liter. We are preparing 500mL at a time so that implies 100g of KOH will be added to 500mL of RO water. Use magnetic stir bar to mix solution. Prepare KOH solution under the fume hood at the beginning of the day.	Safety glasses Lab coat Rubber dish gloves Overhead fume hood
10% KOH	Add 100g of KOH pellets per liter (1000mL). We are preparing 500mL at a time so that implies 50g of KOH. Use magnetic stir bar to mix solution. Prepare KOH solution under the fume hood at the beginning of each week and repeat when needed.	Safety glasses Lab coat Rubber dish gloves Overhead fume hood
$CaCl_2$ 1.4g/mL	Wash and dry stir bar and place in triple rinsed 500mL beaker. Cover with foil. Under the fume hood, weigh 200g of CaCl2 (saturated is around 1117.5g/1500g water at 20ºC) and place in the 500mL beaker.Add 300mL of RO water. Cover with aluminum foil. Place beaker on the stir bar magnet and switch the power on. Wait until liquid becomes colourless and all CaCl2 dissolves. Remove beaker from stir bar magnet and switch power off. Transfer to labelled brown bottle and cover with foil. Close the lid for transport. Store bottle in fume hood until needed.	Safety glasses Lab coat Rubber dish gloves Overhead fume hood
Alcojet Detergent Soak	Following methods of Crichton (2017), prepare 4% Alcojet solution; 40g Alcojet /L. Under the laminar flow hood, add 20g of Alcojet in 500mL of RO water.	Lab coat Nitrile gloves Safety glasses Laminar flow hood
Oil	Filter canola oil under vacuum filtration set-up under the laminar flow. Use a 0.1µm filter to prevent microplastic contamination.	Lab coat Nitrile gloves Safety glasses Laminar flow hood

Material

- Clean glass jars and graduated cylinders
- Full 10L RO water container
- RO water squirt bottles
- Alcojet squirt bottle
- 10% and 20% KOH solutions

Mussels

- Open the labelled vial with the soft tissue of the mussels and add 3 times the volume of the tissues. (check mass for estimate) or add at least 5 ml of 10% KOH.
- Verify the label the glass vial (use one colour per treatment).
- Place all samples of a group in the oven at 40ºC.
- Remove sample from oven 48 h later and proceed to filtration.

Amphipods

- Open the lid of the glass vial containing the amphipod.
- Add 3 times the volume of the animal of 20% KOH and place in the oven at 40ºC for at least 48 h.
- Remove sample from oven 48 h later and observe the state of degradation of the amphipod. Normally, the KOH digestion does not degrade the entire keratine exoskeleton but degrades the internal gut tissues. You can let the KOH digest the animal further for a few days if necessary.
- Once degradation is complete (no more amphipod shell visible) or when the digestive tract is visible within the exoskeleton of the animal, you can proceed to filtration.

Gobies

- Under the laminar flow, open the vials with the digestive tract and add three times the volume of the gi tract of KOH 10% solution. Close the lid.
- Open the vials with the carcass of the fish and add three times the volume of body tract of KOH 20% solution. Close the lid.
- Mix the vials for a group and place in the oven at 40ºC for 48 h.
- Proceed to filtration if digestion is complete

- Label the jar and cover with the appropriate label

2.11.5 Filtration

Material preparation

- Wash all glass material and metal dissection equipment with soap and rinse 3 times with water.
- Look for the presence of fluorescent beads, if none, let the vials dry under the laminar flow
- Fill the RO 10L jugs
- Set-up the vacuum pump (see figure)

Material

- Clean glass jars and graduated cylinders
- Full 10L RO filtered at 0.1µm water container
- RO squirt bottles
- Ethanol squirt bottle
- Vacuum pump material (vacuum pump, air tubes, tube backwash filter, manifold, funnel, stopper, parafilm)
- Fibreglass 1um filters
- Lighter (make sure it has fuel)
- Filter forceps
- 2x 125 µm 4" diameter sieves (+ 2 cut Nalgene bottle holder for sieves)
- Petri dishes
- Oil for pump (if required)

Examine the digested solution

1. Turn on the laminar flow hood
2. Remove samples from oven or take samples that were digested for at least 48 h
3. Set-up vacuum pump

a. Set up the apparatus as described by Figure S2.2 for regular filtrations and like Figure S2.3 if oil separation was performed or for faster filtration. Add a backwash filter if needed and cover all joints with parafilm.

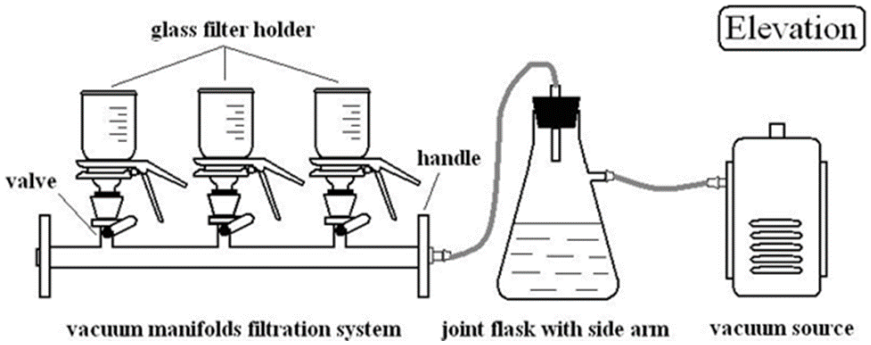


Figure S2.2: Multiple manifold vacuum pump set-up for regular filtration

b. Turn on the vacuum pump to a pressure of approximately -5 mmHg and wait 5 minutes before connecting it to the flask (if it is an oil-based pump, make sure to add the proper amount of oil before starting).

Note: Do not exceed the pressure past -0.2 bar; too much pressure can break the filter and/or lyse the cells. Check with grad student if you are unsure about the pump's condition

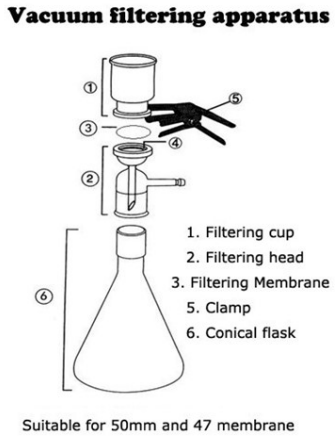


Figure S2.3: Vacuum filtration apparatus set-up after oil separation of for faster filtration.

4. For all samples, run one blank before and between each treatment group

a. Place a clean A/E glass fiber PALL 1 µm filter (0.47mm, product #61631) on the setup
b. Fill the funnel with 200 ml of RO water and rinse through
c. Remove it and label as BLANK the date YYYY-MM-DD and whether it is performed before or at the end of a series of treatment and indicate which treatment

5. Examine the digested solution clarity, density and for the presence of undigested material

Note: normally digestion solution of the animals with little soft tissues will be translucent. This includes: dreissenid mussels, amphipods, or the GI tract of gobies. The digestion of the body will leave behind bones.

6. Procedures for amphipods, mussels and goby gi tract
    a. Using sterile tweezers (flamed with EtOH) take a new filter and place the filter on the funnel stem – shiny side of the filter (1µm pore size) facing up.
    b. Mix the digested sample well by inverting it up and down several times or stirring it using a clean glass rod
    c. Pour the sample slowly into the glass funnel until all content has passed through
    d. Rinse the vial and cover 3 times with RO water over the funnel
    e. When all liquid has passed through, triple rinse the funnel with RO water
    f. When all liquid has passed through the stone filter, remove the clamp and rinse the bottom contour of the funnel onto the filter (over the circle where material has been)
    g. Wait for all liquid to pass and remove the filter from the manifold using the forceps (you may need to stop the pump before doing this if the vacuum is really strong)

    Note: be careful to place the forceps on an area of the filter without deposits

    h. Place the filter in a petri dish and label both the dish and the cover
    i. Repeat for all samples that were under the same treatment, then repeat the blank (e.g. after you processed all animals exposed to a given concentration or exposure time) -step 4
    j. Remove the funnel and wash it with a soapy cotton cloth and rinse it 3 times with RO water

7. Procedures for goby bodies (carcass)
    a. Mix the digested sample well by inverting it up and down several times or stirring it using a clean glass rod
    b. Set up the 125µm sieve onto the cut Nalgene bottles to fit over the glass funnel.
    c. Pour the sample onto the sieves and rinse abundantly with RO water
    d. Rinse the vial and lid 3 times with RO water
    e. Rinse each sieve piece 3 times with RO water.
    f. Triple rinse the funnel over the filter
    g. Repeat steps f to j from step 6.

2.11.6 Microbead count

- Under the laminar flow, mount the Nikon SMZ 800 stereoscope with the UV fluorescent light.
- Place a petri dish with its filter under the scope and remove the cover
- Place the stereoscope magnification to 2x (eye piece is 10x) for a total of 20x
- Move the stage to count all beads and record the counts on the datasheet.
- For Amphipods: After filtration, bring the filter under a stereoscope and slowly dissect or break apart the external exoskeleton to release the microplastic beads from the animal digestive tract or faeces.
- Count the number of particles on the filter. If there is a large number of particles, do a first count and note to recount after plastification.

2.11.7 Filter preparation and storage

- Prepare transparency sheets by labelling it per experiment and treatment.
- Cut plastic book cover material in 2” by 2” squares
- Sterilize tweezers (by flaming with EtOH) after washing and triple rinsing with RO water
- Once the filters of a group have been counted, transfer one filter onto the labelled transparent sheet with the sterile tweezers

- Place the book cover square over the filter and slowly remove the paper from one corner to the next
- Apply an even pressure as the paper is removed to place the plastic book cover over the filter.
- Remove air bubbles and flatten folds as you go.
- Once the filter is covered, label the filter on the sheet or cover and add old label.
- Repeat for each filter of a group.
- Bring the sheet to the stereoscope and repeat the count.
    - When there are a lot of beads, use a dry erase marker to track bead count.
- Record the final count onto the transparent sheet beside the filter.

2.12 Supplementary Materials: Detailed data correction and analyses

2.12.1 Body burden using different data correction methods

The best microbead uptake model was described by a Poisson Inverted Gaussian (P-IG) regression with a parameter estimate per concentration to account for overdispersion of our data (sigma ~ concentration) for unadjusted and adj.trial data, while a negative bionomial (NB) model best described adj.mean data.

Table S2.17: Type III ANOVA table comparing estimated marginal mean body burden (number of beads per gram of tissue) between treatments computed from the best fitting model (F-ratio). A significant *p*-value ($p< 0.05$) indicates the variable is influential on predicting body burdens. Grey highlights show significant terms across methods used: unadjusted data, adjusted data by removing the mean contamination in control per trial (adj.trial) and adjusted data by removing the total mean contamination across all controls (adj.mean).

		Unadj. data		**Adj. trial**		**Adj.mean**	
	Df	**F**	***p*-value**	**F**	***p*-value**	**F**	***p*-value**
Dose	6	51.52	<0.001	24.92	<0.001	19.07	<0.001
Animal	2	136.5	<0.001	81.22	<0.001	5.40	0.01
Route	1	-0.01	1.0	-0.25	1.0	0.1	0.92
Dose: Animal	12	2.26	0.007	2.89	<0.001	5.40	<0.001
Dose: Route	6	3.05	0.005	2.12	<0.05	4.01	<0.001
Animal: Route	2	4.69	0.009	1.92	0.15	4.79	0.008
Dose: Animal: Route	12	3.54	<0.001	3.03	<0.001	7.78	<0.001

Contrasts in the variance based on estimated mean body burden (Table S2.17) shows the animal and concentration contribute most to the observed body burden our experiments, regardless of the correction method employed. Our hypothesis that the animal's behaviour, the environmental concentration and route of exposure all influence the body burden of aquatic organisms also holds true across methods with a significant 3-way interaction (p<0.001, Table S2.17).

Microbead uptake in filter feeders

After data exploration of the acquisition of beads in mussels we observed length was correlated with height (R=0.86, p<0.001), width (R=0.81, p <0.001), weight (R=0.84, p<0.001) and tissue weight of the mussels (R=0.67, p<0.001). The length is associated with the age and maturity of mussels significantly affected body burden, however, because we are already standardizing counts per mass, we did not include other physiological measurements as parameters in our final models.

Table S2.18: P-IG model output describing the effects of concentration, route, their interaction on the body burden of mussels with random effect of aquaria and controlling for length (representing age class).

Terms	**Coeff.**	**Std error**	**t-value**	***p*-value**
0 SUS	304	2.61	5.95	<.0001
0.1 SUS	5.85	1.49	4.44	<.0001
1 SUS	32.62	1.45	9.43	<.0001
5 SUS	24.91	1.46	8.44	<.0001
10 SUS	32.88	1.46	9.30	<.0001
50 SUS	91.55	1.45	12.26	<.0001
100 SUS	74.72	1.42	12.28	<.0001
Route (SED)	2.99	1.70	2.06	0.004
Log Length	0.17	1.36	-5.62	<.0001
0.1 : Route	0.043	2.05	-4.39	<.0001
1 : Route	0.042	1.84	-5.15	<.0001
5 : Route	0.03	1.88	-5.57	<.0001
10 : Route	0.037	1.82	-5.46	<.0001
50 : Route	0.051	1.80	-5.01	<.0001
100 : Route	0.14	1.79	-3.33	0.0009
R^2= 72.31%	AIC	2459		

When accounting for the grouping of mussels per aquaria, we observe a significant effect of the concentration (p<0.0001) on the retention of beads which varies per route (Table S2.18). The P-IG distribution allows to account for extreme counts therefore has larger estimated means and may over-estimate body burden when extreme values are present. Although the P-IG model

had a better fit with our observed data for mussels, this distribution within the *gamlss* package does not allow us to effectively compute group means with reliable standard errors and confidence intervals. We chose to use a more conservative approach with the lme4 package and the NB distribution for these analyses (see Table S2.4 in Suppl. Mat. 2.9).

Microbead retention by deposit-feeders

All regression models regardless of the method chosen for managing the presence of beads in controls revealed the same pattern for the deposit feeding gammarid (Table S2.19). A significant positive concentration response ($p<0.0001$) drives the acquisition of beads. The body burden differs between routes at a concentration of 100 microbead·ml^{-1}. Beads were found in one gammarid from the control group, while no beads were found in individuals at concentrations of 1 bead·ml^{-1}. To estimate standard errors and confidence intervals for the estimated means of these treatments, we added one bead to one individual to the 1 bead·ml^{-1} group, which allowed to compute standard errors. The values computed in this manner were italicized in Table S2.7 and highlighted by an asterisk. The pairwise contrast matrix could not be produced based on the body burden of zero observed at a concentration of 1 for gammarids exposed to suspended beads, therefore we did not provide a letter code for this treatment mean.

Table S2.19: Comparison of the effect of the concentration, route and their interactions on estimated marginal mean body burden (number of beads per gram of tissue) of gammarids computed from the best fitting models using three data correction methods. "Unadjusted" data were not corrected for positive counts in control (as in manuscript), "adj.trial", data were corrected by removing the mean contamination in control per trial and adding 1 bead at a concentration of 1 bead·ml^{-1} in suspension to correct for missing values, while "adj.mean" method removed the total mean contamination across all controls from each observation. The best model for the adj.mean method was computed with a NB distribution, the others used P-IG distributions. A significant p-value ($p< 0.05$) indicates the variable is influential on predicting the uptake of microbeads.

		Unadjusted		Adj. trial		Adj.mean*	
Terms	**Df**	**F**	***p*-value**	**F**	***p*-value**	**F**	***p*-value**
Concentration	6	18.08	<.0001	19.83	<.0001	7.95	<.0001
Route	1	-0.536	1.000	2.79	0.10	0.000	0.993
Conc.: Route	6	5.923	0.0001	5.19	<.0001	4.850	0.0001
AIC		1229		124		1025	
SBC		1287		1298		1083	
R^2		52.68		52.45		43.83	

Microbead retention in predators

For the predator, we observed low bead counts under both sources and across concentrations. Increasing exposure level increases the body burden of gobies regardless of the control correction method employed for the P-IG model (Table S2.20). However, there is a significant effect of the route of exposure for unadjusted and means adjusted data but not when contamination in controls is removed per trial. This likely indicates that some trials had higher control contamination than others and that the concentration of exposure has a greater effect than the route on the body buden for this fish.

Table S2.20: Comparison of the effect of the concentration, route and their interactions on estimated marginal mean body burden (number of beads per gram of tissue) of round gobies computed from P-IG models using three data correction methods. "Unadjusted" data were not corrected for positive counts in control (as in manuscript), "adj.trial", data were corrected by removing the mean contamination in control per trial, and "adj.mean" where we removed the total mean contamination across all controls from each observation. A significant *p*-value ($p < 0.05$) indicates the variable is influential on predicting the uptake of microbeads.

		Unadjusted		Adj. trial		Adj.mean	
Terms	**Df**	**F**	***p*-value**	**F**	***p*-value**	**F**	***p*-value**
Concentration	6	5.95	<.0001	7.15	<.0001	6.13	<.0001
Route	1	10.12	0.002	0.4	0.529	7.310	0.007
Conc.: Route	6	1.78	0.099	0.53	0.783	1.830	0.089
AIC		752		666		701	
SBC		794		709		743	
R^2		34.42		40.74		36.53	

2.12.2 Depuration time

For retention analyses, we first ran a model with all data (Body burden ~ Animal + Time +Animal*Time+ offset) and found that there were differences in depuration rates between animals. We decided to subset data per species to create best fit models for each to have more robust estimates of how the body burden changed with increasing depuration time. Individual models and estimated mean body burden are reported in Table S2.8 (Suppl. Mat. 2.9).

Although we conducted experiments to examine the depuration time of gammarids after 72 h, our results were very high for trial 5 looking at depuration at 24 h (n=3), 48 h (n=7) and 72 h (n=30). We suspect initial exposure concentration was 100 beads·ml^{-1} instead of 50 beads·ml^{-1}; therefore, this trial was removed from the analyses. Figure S2.4 shows a summary of results obtained when including data from trial 5 for gammarids (other species are not affected).

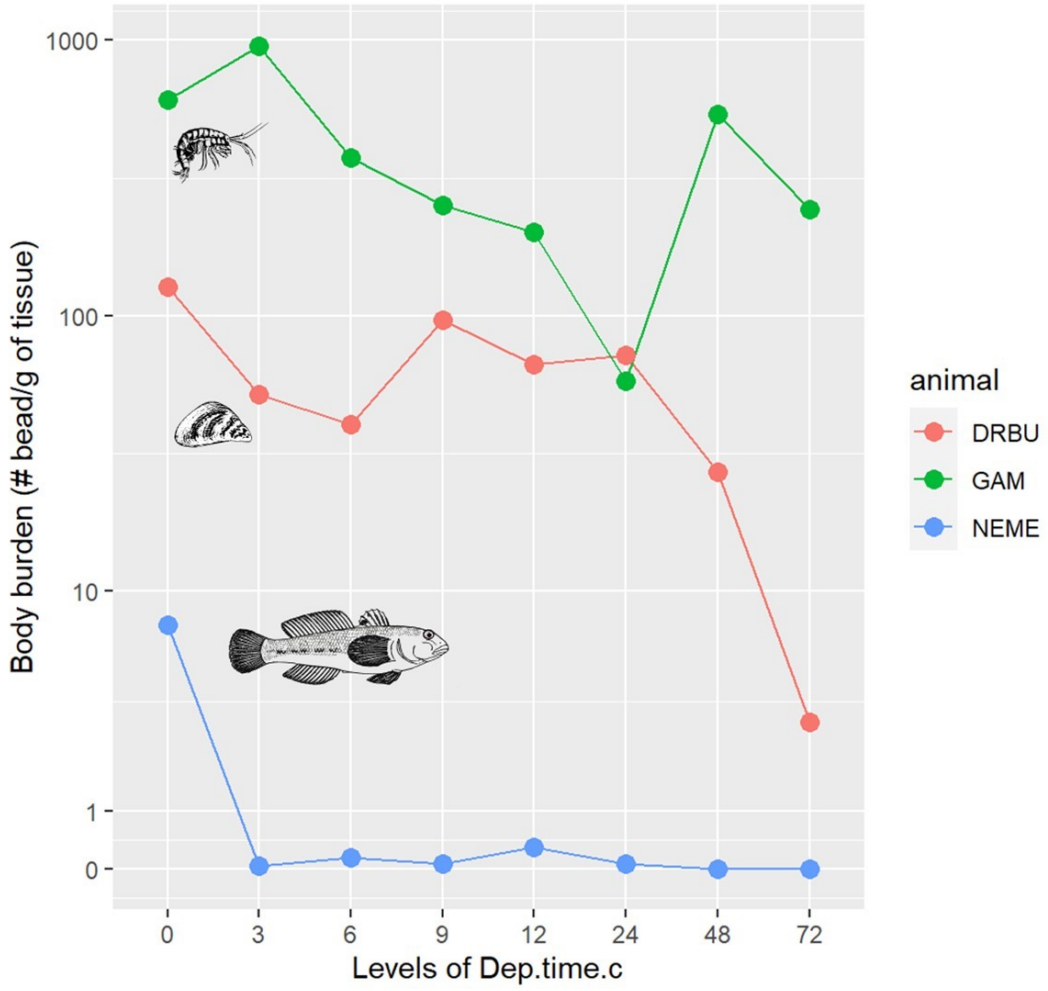


Figure S2.4: Comparison of men microbead body burden of gammarids (green), mussels (pink), and round gobies (blue) at different times after acute exposure to 50 beads·ml^{-1} using data from all trials.

2.12.3 Species interactions

We examined the effect of the length of fish (as a proxy for gape size) on the body burden of gobies and its interaction with the proportion of prey consumed out of the prey offered in each scenario. Results of negative binomial and P-IG models are described in the top panel of Table S2.9 (Suppl. Mat. 2.9). The best model to describe how prey scenario and percentage of prey consumed influenced the body burden of gobies followed a P-IG distribution and is presented in

the bottom panel of Table S2.9 (Suppl. Mat. 2.9). Even though multiple gammarids were placed in the same aquarium to examine the non-trophic transfer of beads from mussels to gammarids, the effect of this nesting per aquarium accounted for ~0.02% of the model variance so had no effect on the parameter estimates or estimated means. We kept the simpler model using only mussel abundance and the time of exposure as terms with a NB distribution.

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Connecting statement between Chapters 2 and 3:

Chapter 2 demonstrated that species with different roles within a food web take up microbeads that are 1) suspended in the water column, 2) sedimented out of the water column, or 3) transferred from a contaminated organism via predation or biodeposition, when they are exposed to each route at once. Under natural conditions, organisms of a food web are exposed to all these sources simultaneously, which could affect their ability to acquire and bioaccumulate microplastics. Given most studies have explored the uptake of particles from a single route of exposure, I wanted to determine if knowledge from these experiments could be used to understand how microplastics are acquired and transferred under natural conditions—where multiple sources are available at once.

The community ecology approach used in Chapter 2 provides added advantages over single-species exposure models because it addresses some of the more complex interactive plastic cycling scenarios, thus approaching situations observed under more natural conditions. In Chapter 3, I take the next step to determine if single-exposure experiments (such as those conducted in Chapter 2 and by multiple authors on an array of organisms) can be used to reconstruct interactions within food web and thus predict microplastic contamination under more complex food web scenarios.

3. Chapter 3 | Using community modules to predict risks and fate of microplastics in aquatic food webs

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Keywords: Food web, microplastic fate, community module, network, microbead

3.1 Abstract:

Species play active roles in the cycling of microplastics within aquatic food webs, yet multi-species trophic interactions are rarely considered when assessing microplastic contamination pathways. Here, we use a network approach to study the fate and cycling of microplastics in a freshwater food web. Informed by previous experiments (chapter 2), we created an empirical model by combining the results from single exposure experiments testing individual routes of microplastic contamination within a community module of interacting species. The module consisted of the quagga mussel (*Dreissena bugensis*), a gammarid amphipod (*Gammarus fasciatus*), and the round goby (*Neogobius melanostomus*). We then exposed animals as a community to four routes (in sediment, in suspension, in pre-contaminated amphipods, and in pre-contaminated mussels) simultaneously. We compared the risk of occurrence, body burden, proportion of beads per route, ecotoxicological metrics, and total contamination across models. Our objectives we to assess (1) the contribution of each route for each animal under different food web scenarios, (2) the effect of exposure time, and (3) whether the single exposure model can predict microplastic burden of animals in the community module exposed to multiple routes simultaneously.

3.2 Introduction

Microplastics are a group of emerging contaminants with unique properties and toxicities that vary depending on their size, shape, polymer type and on the chemicals (e.g., persistent organic pollutants, heavy metals, pharmaceutical products) sorbed onto their surfaces (Bucci and Rochman 2022). As these particles are long lived, they can carry their toxic load with them as throughout their passage through ecosystems. In Canada, microbeads were listed under the List of Toxic Substances under the Canadian Environmental Protection Act in 2016 (CEPA, 2016), thus limiting their use and application in this country. Nonetheless, they have subsequently been found in high concentrations in the benthic sediments of the St. Lawrence River (Crew et al. 2020). Microbeads have also been observed in the digestive tracts of freshwater fishes (round gobies *Neogobius melanostomus* and yellow perch *Perca flavescens*) collected from the St. Lawrence River, although fibres were the most common microplastics in these fish (Figure S0.1B). Clearly, microplastics remain a major environmental issue despite legislation implemented to date.

Model organisms are typically used to define key thresholds and acute impacts of plastic pollution. Contamination models constructed in the laboratory often target a single route of uptake (Ma and You 2021), consider few taxonomic groups as model organisms (e.g., daphnids, zebrafish, bivalves; D'Avignon, et al. 2022, Chapter 1), or study simple linear trophic transfers (Krause et al. 2021). A burgeoning number of taxa have been found to interact with microplastics in the water column and sediments, especially in littoral areas of rivers and lakes, but also through relationships with other members of the biotic community. Microplastics can be acquired from multiple routes depending on the animal's feeding strategy and habitat (Chapter 2). Under field conditions where organisms are exposed to multiple routes of microplastic

simultaneously through complex interactions with their environment; microplastic fate may be altered along with concentrations of exposure within food webs.

3.2.1 Mechanisms of microplastic uptake by organisms

A first step to gaining a better understanding of plastic cycling in food webs is to acquire information on the acquisition and retention of particles in multiple organisms. Although there is little evidence for biomagnification of microplastics in food webs (Miller et al. 2020; McIlwraith et al. 2021), plastic particles along with their array of toxic substances are ingested and retained by large diversity of aquatic organisms (D'Avignon et al. 2022, Chapter 1).

Accidental uptake from feeding, drinking, respiration, swimming, or non-trophic activities are possible. Microplastics can be ingested because the particles are confused for food (de Sa et al. 2015), or accidentally by feeding on contaminated food items (Scherer et al. 2017). Particles can also be acquired as contaminated water passes through the gills of fish (e.g., Red tilapia *Oreochromis niloticus*; Ding et al. 2018) or crabs (e.g., European green crab *Carcinus maenas*; Watts et al., 2014). Caddisflies also use plastic in their environment to construct their larval cases (Ehlers et al. 2019), and mayfly nymphs may be exposed to beads as they dig burrows in littoral sediments (Gallitelli et al. 2021). Microplastic-contaminated organisms at lower levels within a food chain can transfer their loads to their predators (Cedervall et al. 2012; Chae et al. 2018).

3.2.2 Retention and translocation

Though some organisms possess diverse mechanisms to reject undesired particles both before and after uptake (Evan Ward and Shumway 2004; Ward et al. 2019), the lipophilic properties of certain polymers can slow this process as particles can adhere to the tissues or appendages of copepods or mussels during sorting, and thereby increase the quantity of particles

acquired (Cole et al. 2013; Kolandhasamy et al. 2018). Most invertebrates and fished species have been reported to retain microplastics for approximately 48 h (Au et al. 2015; Blarer and Burkhardt-Holm 2016; Murphy and Quinn 2018). However, a portion of particles were retained for up to six days by goldfish *Carassius auratus* (Grigorakis et al. 2017), whereas marine mussels *Mytilus edulis* held particles for more than 49 days (Browne et al. 2008). Once on or in an organism, particles of 1–400 μm in size were found to cross cell membranes and translocate to other organs or to the haemolymph of animals (McIlwraith et al. 2021), thus prolonging their retention time. Longer retention times increase the risk of trophic transfer between organisms of a food web. With their different abilities to uptake and retain particles, each organism plays a unique role as a recipient and source of microplastic to its community.

3.2.3 Circulation of microplastics in food webs

Secondary pathways of uptake are difficult to monitor and have been poorly studied. Our knowledge of ecological interactions between species suggests microplastic particles, given their long-life and integrity, could be ingested and egested by more than one animal, allowing them to cycle almost indefinitely in an ecosystem. For instance, particles that are re-suspended in the water column (Karakolis et al. 2018) or encased in pseudofaeces or faecal pellets (Khan and Prezant 2018; Chapter 2) could be re-ingested more readily. In fact, previous ingestion by another organism may increase the availability of microplastics that cycle within a food web (Hoang and Felix-Kim 2020).

Trophic position and food chain length significantly influence the degree of contamination in fish from freshwater lakes (Cabana et al. 1994; Vander Zanden and Rasmussen 1996) and could similarly affect how microplastics are acquired and transferred in food web recipients. Each lake and river section hosts communities in which the discrete trophic level of

any constituent species can change depending on the organisms involved in its network. Plastic loads could similarly be influenced by the number of trophic links and potential routes they can use to travel and accumulate in each organism of a food web. Difference in community/food web structure could explain why plastic concentrations in organisms do not always reflect their dietary trophic levels calculated from large databases (Covernton et al. 2021; McIlwraith et al. 2021), but rather reflect the trophic links present in the community of interest.

Previous work showed microplastics could be transferred trophically from a contaminated prey to its predator (Chae et al. 2018; Zhu et al. 2018; Van Colen et al. 2020; Chapter 2), non-trophically via biodeposits (Chapter 2), and even from the larval cases of caddisflies to a predator as it struggles to liberate its embedded prey (*unpubl. data*). These observations demonstrate how various species interactions distribute and circulate microplastics within food webs. Clarifying the contribution of each route is invaluable to improve microplastic contamination risk models.

To our knowledge, no studies have yet compared microplastic uptake from diverse potential contamination routes. To address this gap, we used a network approach modelled by a community module—the quagga mussel-gammarid-round goby trio—as a proof-of-concept to examine how microplastics cycle in food webs and whether single-exposure experiments conducted in the laboratory can be used to predict the fate of microplastics in more complex systems.

An implicit assumption of trophic transfer studies is that food web transfer is the additive result of a series of interactions, and experiments conducted as single-exposure trials can thus be combined to infer real-world contamination potential. To examine this assumption, we combined the results of a series of single-route exposure experiments describing potential interactions

within our community module as an additive empirical model to test if it can predict the microplastic contamination when multiple simultaneous contamination routes (depending on community structure and time of exposure) are implicated. Starting with the premise that the behaviour, role, and interactions of each animal within the community will influence its respective microplastic contamination load (Chapter 2), we expect to observe differences in the body burden and proportion of beads from each route when comparing single and multiple-exposure scenarios for each animal. Using the models described in Table 3.1, we aimed to test the reliability of using a network approach to understand microplastic distribution in more complex food webs.

3.1 Methods

3.1.1 Description of the conceptual model

The quagga mussel *Dreissena bugensis* and the round goby *Neogobius melanostomus* originate from the Black and Caspian Seas region and was successfully introduced to estuaries, rivers and lakes across Europe following the construction of reservoirs and canals (Kornis et al. 2012; Marescaux et al. 2016). They were introduced to North America through the discharge of ballast water of transoceanic ships and have since become dominant in the Great Lakes-St. Lawrence River Basin (Ricciardi and MacIsaac 2000). Dreissenid mussels are natural prey for the round goby and may have facilitated its dominance in the Great Lakes (Ricciardi 2005). Dreissenid mussel beds provide interstitial habitat and biodeposits as nourishment to gammarid amphipods (Ricciardi et al. 1997; González and Burkart 2004). This trio of species is abundant, widespread, and interactive in the Great Lake-St Lawrence River basin as well as in Europe, and thus is an appropriate experimental module for our studies.

In previous studies, gammarids ingested microplastics in the size range of 32–250 µm (Scherer et al. 2017; Straub et al. 2017; Redondo-Hasselerharm et al. 2018) and preferentially consume ~32–63 µm particles, whereas dreissenid mussels preferentially select particles less than 80–150 µm in size (Horgan and Mills 1997; White and Sarnelle 2014). Based on these reported values, we used particles in the 63–75 µm size range for our experiments, with the expectation that they would be ingested by macroinvertebrates and can pass through the gills of the round goby (Nagelkerke et al. 2018). Indeed, this size range of microbeads was shown to be transferred via predator-prey interactions (gammarid to goby; mussel to goby) and commensal interactions (mussel biodeposits to gammarids) (Chapter 2).

Table 3.1: Description of the different food web scenarios examined. S refers to single-exposure scenarios (each food web link is examined individually) while M= multiple-exposure scenarios where sources of microbeads are from suspension, sediment, pre-contaminated mussels (DRBU) and pre-contaminated gammarids (GAM) all at once (note: NEME = round goby). Number of animals used per aquarium and the number of replicates per scenario are also indicated. Specific calculations are described in data analyses section.

Modelled scenario description and assumptions		**Exposure time (hour)**	**Number of animals**			**Number of replicates**
			DRBU	**GAM**	**NEME**	
S	24-h single-exposure experiments for each route per animal (see 3.1.7 for details) taken from Chapter 2. We assumed each link is additive to estimate cumulative microplastic contamination.	24	5- 8	1-10	1	7 to 33 per route
M 1	24-h multiple-exposure scenario composed of an equal number of pre-contaminated mussels and gammarids equivalent to the mean number used in the single-exposure model. We assumed results for this model to be like those predicted by the single-exposure model.	24	7	7	1	5
M 2	24-h multiple-exposure scenario with more gammarids offered than mussels to observe if prey switching occurs.	24	10	14	1	10
M 3	24-h multiple-exposure scenario with more mussels than gammarids and the presence of a competitor to examine how it influenced prey acquisition and microbead transfer.	24	15	10	2	10
M 4	48-h multiple-exposure scenario with more mussels than gammarids and the presence of a competitor. This scenario will be compared to M3 to examine the influence of exposure time.	48	15	10	2	10

3.1.2 Animal collection and care

All animals (mussels, gammarids and gobies) were collected from nearshore areas of Lac St. Louis, a fluvial lake in the St. Lawrence River, between June and September of 2018, 2019, and 2020. All three species occurred at each of our collections sites and were captured with artificial substrates, minnow traps, seine net, or manual removal by scuba divers. Once captured, animals were placed in ambient water within 70 L coolers mounted with an aerator. They were transported to an animal holding facility within 3 h of capture. Gobies and mussels were placed in aerated and filtered 75 L aquaria held inside temperature-controlled Conviron® growth chambers that were maintained at ~19°C under a 12 h light/12 h dark light cycle for a 2-week acclimation period. Gammarids were placed in groups of 10 per 1 L Tupperware containers within a temperature-controlled room (18°C) and provided with leaf detritus from the collection site. Mesh (5 cm × 5 cm) and gravel were added to provide shelter. Gammarids were acclimated for two to five days before the onset of experiments. Every second day, mussels and gammarids were offered an equal mixture of live algae *Scenedesmus* sp., *Pseudokirchneriella* sp., and PhytoPlex Plankton from Kent Marine (Suppl. Mat. 2.9), whereas round gobies were fed with ~1.5 mm protein/carbohydrate sinking granules (Fluval Bug Bites) until they were satiated. While being acclimated, animal health was monitored daily (CCAC 2017-7913). Water quality parameters were monitored every second day and waste was removed weekly.

3.1.3 Experimental design of multiple exposure scenarios

Multiple exposure experiments were conducted using 20 L aquaria placed randomly within a temperature-controlled Conviron® growth chamber maintained at ~19°C under a 12h light/12h dark light cycle (see Table 3.1 for replicates per scenario). Each aquarium was fitted

with an AquaClear 30 Power Filter to allow water circulation, but filter media was removed to prevent microbead aggregation outside the aquarium. Five ceramic bio rings were placed in each aquarium to maintain water quality. An aerator was placed in each aquarium to oxygenate the water and create a small current. A thin layer of Super Naturals aquarium gravel substrate (~1cm × 1cm) was added to cover the floor of the aquarium, followed by the addition of 15 L of dechlorinated tap water.

To prepare the multiple microplastic exposure scenarios, we used 63–75 µm fluorescent Cospheric polyethylene beads of four colours, one per route. We followed the bead preparation protocols described in Chapter 2. We weighed 0.1205g ± 0.0004g of beads into a vial, before applying a surfactant coating (Tween80) to the blue (1.13 g/cc) and orange (1.00 g/cc) beads. To induce biofilm formation, 1 ml of a live algae/bacteria solution and 5 ml of dechlorinated water were added to each vial in which beads were left to soak for 48h.

To pre-contaminate gammarids, a concentration of 100 red beads·ml^{-1} (1.090 g/cc) were added to 10 ml wells and were left to settle for 48 h. Gammarids were then added individually to red-bead contaminated wells a 24 h exposure period. To pre-contaminate mussels, we placed seven individuals in 7 L aquarium and exposed them to a concentration of 100 orange beads·ml^{-1}(1.00 g/cc) held in suspension during the same 24 h as the gammarid exposure period. Invertebrates were fed 3 ml of algae per 7 L mussel aquarium, and 0.1 ml of algae per well. After a 24 h exposure to food and beads, invertebrates were collected, triple-rinsed to remove externally adhering beads and placed in a clean 1 L container for transport to the experimental chambers.

The sedimented beads were added to the aquarium first by adding a concentration of ~50 green beads·ml^{-1} (1.025 g/cc). After 48 h, ~90% of the beads were sedimented to the bottom and

one polyvinyl chloride tube (5 cm diameter, 10 cm length) was added per fish to serve as shelter. The pre-contaminated gammarids (red beads) and pre-contaminated mussels (orange beads) were added to the aquarium, shortly followed by the introduction of round gobies, based on the scenarios described in Table 3.1. Once all animals were placed into the aquarium, the blue surfactant-coated beads were added to provide ~50 beads·ml^{-1} of suspended beads. Animals were left to interact with each microplastic route and the available prey for 24 h or 48 h, depending on the scenario (see Table 3.1 for sample size and replicates per scenario).

At the end of each scenario, fish were collected by net, triple rinsed, and euthanized by immersion in 10% eugenol followed by cervical dislocation. Unconsumed mussels were collected, triple rinsed, and placed together into a labelled bag, while uneaten gammarids were placed individually in Eppendorf vials filled with 60% ethanol. All animals were placed in a larger labelled bag and kept in the freezer. Experimental bags were thawed for a few hours under a laminar flow hood before proceeding to microplastic extraction.

One aquarium per scenario was used as a control, where the same protocol was followed to prepare aquarium, but no beads were added in sediments or in suspension. Gammarids and mussels were placed in a clean aquarium without microplastics for 24 h at the same time as contaminated invertebrates, and afterwards transferred to experimental aquaria. Equivalent number of prey and predators (as described in Table 3.1) were placed in the aquaria and left to interact for 24 h. At the end of the experiments, animals were euthanized as described above, place in a freezer and processed using the same microplastic extraction protocol (see below). The total number of beads found in the tissues of each species was considered as contamination in terms of treatment results, and this number was subtracted from the total number of beads counted in microbead-exposed organisms.

3.1.4 Microplastic extraction

We applied the same microbead extraction, quality control and quality assurance protocols described in Chapter 2 (Suppl. Mat. 2.11). Briefly, the soft tissues of mussels, the goby digestive tract, the goby's carcass were dissected and separately weighted, then placed in a 10% potassium hydroxide (KOH) solution for 48 h in an oven at 40°C. The body of amphipods was placed in a 20% KOH solution in an oven at 40°C for 48 h, then left to digest for another 48 h at room temperature. The digestate of each animal tissue was individually sifted through a 125 µm sieve and processed by vacuum filtration over a 1 µm glass filter (4.7 mm). Procedural filtration blanks were run before and after each animal from a given scenario was processed. Then, the beads of each colour remaining on the filter were counted under a stereoscope (Nikon SMZ 800) at a total magnification of 20× and lit by an ultraviolet led light. As a quality insurance measure, we subtracted the total number of beads of each colour found in the procedural blanks and in the controls from the final counts.

3.1.5 Contamination prevention and control

To prevent contamination during the experiments, all used equipment (e.g., aquarium, mesh hand nets, gravel, fish shelters, euthanasia containers) was washed thoroughly and triple rinsed with 0.1µm reverse osmosis (µRO) water. An ultraviolet light was used to verify no contamination was present by visually inspecting for the presence of fluorescent beads. Other disposable equipment (e.g., bio-beads, charcoal bags, airlines, pipettes, and pipette tips) were discarded and replaced with new equipment. All glass material and metal dissection equipment were washed with soap, triple rinsed with µRO water and sterilized. Glass containers were placed in the oven at 230°C for 6 h, while dissecting equipment were sterilized by ethanol flaming before each use. When animals were removed from the experimental aquaria or wells,

they were triple rinsed with µRO water and placed in a clean container. A control was run per trial and always processed first to avoid cross contamination.

3.1.6 Contamination metrics

To compare the microplastic contamination of organisms within our food web, we applied common metrics including (1) *risk of occurrence*—the number of organisms within a sample that are contaminated with microbeads; (2) *body burden*—the number of beads per gram of tissue; (3) *proportion of beads per route*—total number of beads of a given route (colour coded) divided by the total number of beads found in each animal at the end of the exposure trial; and (4) *total contamination*—total number of microbeads retained per animal for each route of exposure. For this work, we adapted the following common ecotoxicological metrics to assess bioconcentration, bioaccumulation, and biomagnification potential (Arnot and Gobas, 2006; Borgå, 2013), which have been rarely applied to microplastics:

Bioconcentration factor (BCF)—the concentration of microbeads (µg) in the body of an animal (per kg of digested tissue) relative to the microbead concentration (µg) per volume (L) of media (water or surficial sediments).

Pseudo-bioaccumulation factor (BAF)—the concentration of beads (µg) in a host organism (kg of tissue) relative to the concentration of beads (µg) in the animal (kg of tissue) which serves as a source (e.g., mussel or gammarids, via species interactions). For the transfer of beads via biodeposits, the BAF denominator was multiplied by the number of mussels available to transfer particles. For the predator-prey interactions, the BAF's numerator is multiplied by the number of prey ingested, while the denominator is multiplied by the number of prey available.

Biomagnification factor (BMF)—the concentration of beads (µg) in an animal (kg of digestive tract tissue) relative to the concentration in its diet (µg·kg^{-1} of prey). The mass of beads was either measured directly (by weighing beads with a scale) or estimated based on the manufacturer's specification (i.e., 5,813,720 beads·g^{-1}). See Table S3.2 for estimated means and confidence intervals calculated for each metric.

Total bioaccumulation factor (tBAF)—total contamination of beads from all potential routes of uptake over the total number of beads available to the organism (Elizalde-Velázquez et al. 2020). See Table S3.3 for estimated means, standard error and confidence intervals.

3.1.7 Single-exposure scenarios

The data used for the single-exposure scenario were obtained from previous experiments examining the uptake of microbeads for each route individually (Chapter 2). The number of beads acquired by each organism within the module, as well as the estimated body burden, were extracted from original data to allow comparison with the first multiple exposure scenario (M1). The schematic of the steps used to produce mean and confidence intervals of body burden and contamination metrics are shown in Figure S3.1; we used results from experiments conducted at an exposure of 50 beads·ml^{-1} for suspended and sedimented routes, and the number of beads and body burden acquired from exposure to five pre-contaminated mussels and five pre-contaminated gammarids. A grand means was recalculated from all trials examining the transfer from mussel to gammarids via biodeposits, because mussel abundance was not significant (see section 2.4.3.2).

To calculate the proportion of beads acquired via each route, we assumed that the acquisition would be additive. The estimated mean number of beads acquired per organism for each route of exposure from Chapter 2 was used. The proportion of beads per organism was

calculated by dividing the mean uptake of a given route by the sum of all routes of transfer per organism.

To calculate the mean number of beads remaining in each animal following pre-contamination, we used the estimated mean number of beads remaining after 24 h depuration. To account for differences in initial concentrations, we multiplied the mean number of beads obtained by the ratio of the mean number of beads observed at time zero of depuration (50 beads·ml^{-1}) to the mean number of beads after an exposure to 100 beads·ml^{-1}.

The total count was calculated by adding the means of each route per animal. Table S3.1 summarizes the estimated means, standard error and confidence intervals predicted by the regression models for contamination and ecotoxicological metrics.

3.1.8 Data analyses

Preliminary analyses revealed that the animal and the number and types of interactions depicted in each scenario influenced the number of beads acquired, the body burden (beads per gram), and the contamination metrics of each animal. We created regression models per animal to estimate means and confidence intervals for each route of exposure and scenario. Counts followed a negative binomial or Poisson-inverted Gaussian distribution modelled with the *gamlss* package in R (Rigby and Stasinopoulos 2005). The best regression model was selected following hierarchical selection of significant terms choosing the lowest Akaike information criterion and Schwartz Bayesian criterion. Model assumptions were validated visually using residual plots for normality and homogeneity of variance.

3.2 Results and discussion

3.2.1 Food chain interactions influence microbead acquisition, distribution, and cycling

The single-exposure experiments offered a controlled environment to assess microplastic contamination by each route, but they do not characterize multispecies interactions within freshwater communities. Multiple-exposure scenarios mimic more realistic conditions experienced by the community by presenting a variety of mussel sizes, refugia for gammarids, and in scenarios M3 and M4 allowed round gobies to compete for space and resources. Comparing the effects of such conditions on the fate of microplastics is crucial to understand how distribution and cycling of these particles are altered by the behaviour of the animals within a module.

In this study, we found microbead contamination of organisms was context dependent. When comparing the effect of multiple-exposure scenarios on the body burden of animals per route of exposure, we found variation across individuals to be important, accounting for 42–48% of the variation explained by our regression models. The number of beads acquired, and the body burden were both significantly influenced by the interactions defining the scenario, the animals' role in the module, and the routes of microbead exposure ($p<0.05$). The total number of beads acquired by members of the community were affected by the role of the animal ($F_{(2,138)}=21.42$, $p<0.001$), their relative size ($F_{(1,138)}=43.0$, $p<0.001$) and the scenario examined ($F_{(2,138)}=8.16$, $p<0.001$).

In the multiple-exposure experiments, all gammarids offered to round gobies were consumed, except for six individuals from the M1 scenario. The number of suspended beads retained in the tissue of gammarids was two times higher in single-exposure experiments (S) than under M1 (544 beads$\cdot g^{-1}$ and 250 beads$\cdot g^{-1}$ respectively; Figure 3.1). This higher contamination

occurred when animals were exposed in small 10 ml wells (S) rather than when they could move freely in a 7000 ml aquarium. By the end of M1, nearly 50% of gammarids retained ~5 beads·individual^{-1} from their initial exposure (Table S3.1). Gammarids also acquired similar concentrations of beads from mussels' depuration processes, leading to similar body burdens: 21 beads·g^{-1} for S and 28 beads·g^{-1} for M1 (orange routes in Figure 3.1).

Interestingly, beads were taken up by gammarids from all routes (Figure 3.2 M1): suspended (blue), sedimented (green), and biodeposited transfers from mussels (orange) in the multiple-exposure setting. We hypothesize that in this scenario (M1), gammarids are actively hiding from the predator amongst gravel or mussels' shells, thus increasing their interactions with sedimented beads and mussel biodeposits. Indeed because gammarids use interstitial habitat and feed on deposited materials in multiple exposure scenarios, they encounter fewer beads in suspension than in scenario S. The smaller habitat (10 ml wells) in S may increase interactions with suspended beads. Despite the differences in body burdens and proportion of beads predicted by the single-exposure scenario, the total number of beads retained in gammarids did not differ between the single and multiple-exposure scenario (p=0.702; Figure 3.3B).

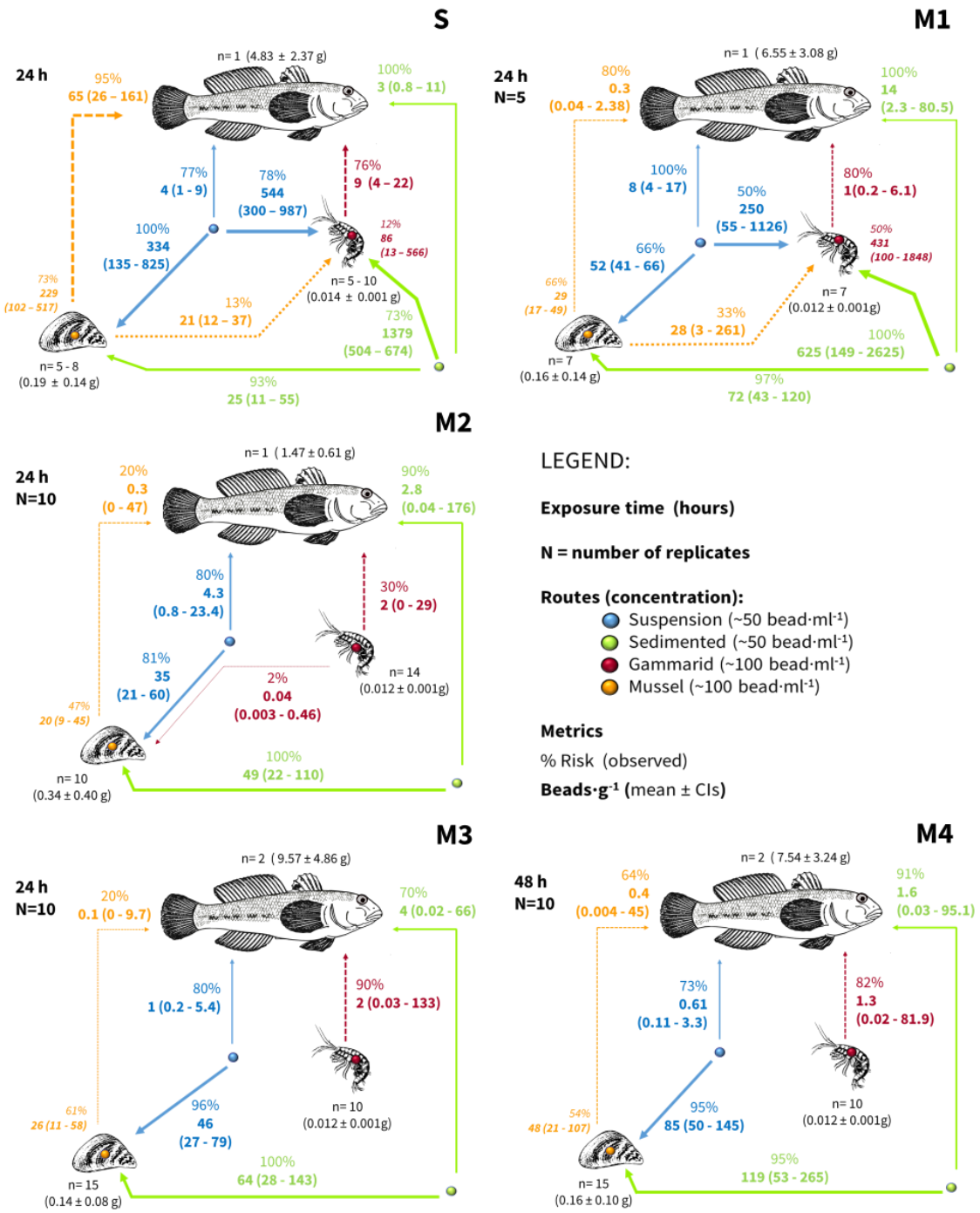

Figure 3.1: Summary of scenario design with observed occurrence of contamination (% risk), and the estimated mean body burden (beads·gram^{-1} of tissue) with lower and upper confidence intervals for each route examined. In multiple exposure scenarios M2-M4, all gammarids were consumed by round gobies, so no values are expressed for this animal. See Table S3.1 for exact values.

For mussels, a greater microbead burden resulted from exposure to suspended beads under the single-exposure scenario (resulting in 334 beads·g^{-1} in mussels). In contrast, mussel acquired more sedimented beads than suspended beads under multiple exposure scenarios (49 and 199 beads·g^{-1}; Figure 3.1). Differences in relative importance of exposure routes between the single and multiple-exposure tests were not uncommon for the invertebrates. Based on the single-exposure experiments model, we observed that both mussels and gammarids acquired a higher proportion of beads from suspension versus other routes, whereas the round goby acquired more beads from pre-contaminated mussel prey (Figure 3.2S). However, when comparing the proportion of beads acquired during M1, all benthic animals acquired ~50% of their load from sedimented beads (SED; Figure 3.2). The presence of sedimented beads in organisms remained important in the other multiple-exposure scenarios as well with >40% of total beads being found in mussels and 20–53% of beads in gobies (Figure 3.2 M1-M4). We suspect that adding mobile gammarids, which may use mussel shells as refuge in the presence of a predator that hunts gammarids, can lead to more re-suspension of sedimented beads during predator foraging activities under multiple-exposure settings. In turn, the movements and interactions of organisms with one another would promote re-suspension of sedimented beads making them more bioavailable to all animals.

The behaviour of organisms is altered by the presence of interactive species. For example, mussels are capable of pedal feeding (Nichols et al. 2005). Therefore, if disturbed or threatened by a predator, they might avoid fully exposing their siphons and opt for the pedal feeding. Both situations—re-suspension and the presence of a predator—could enhance uptake of sedimented particles captured by the mussels (Figure 3.1) leading to a greater proportion being observed across multiple-exposure scenarios (Figure 3.2).

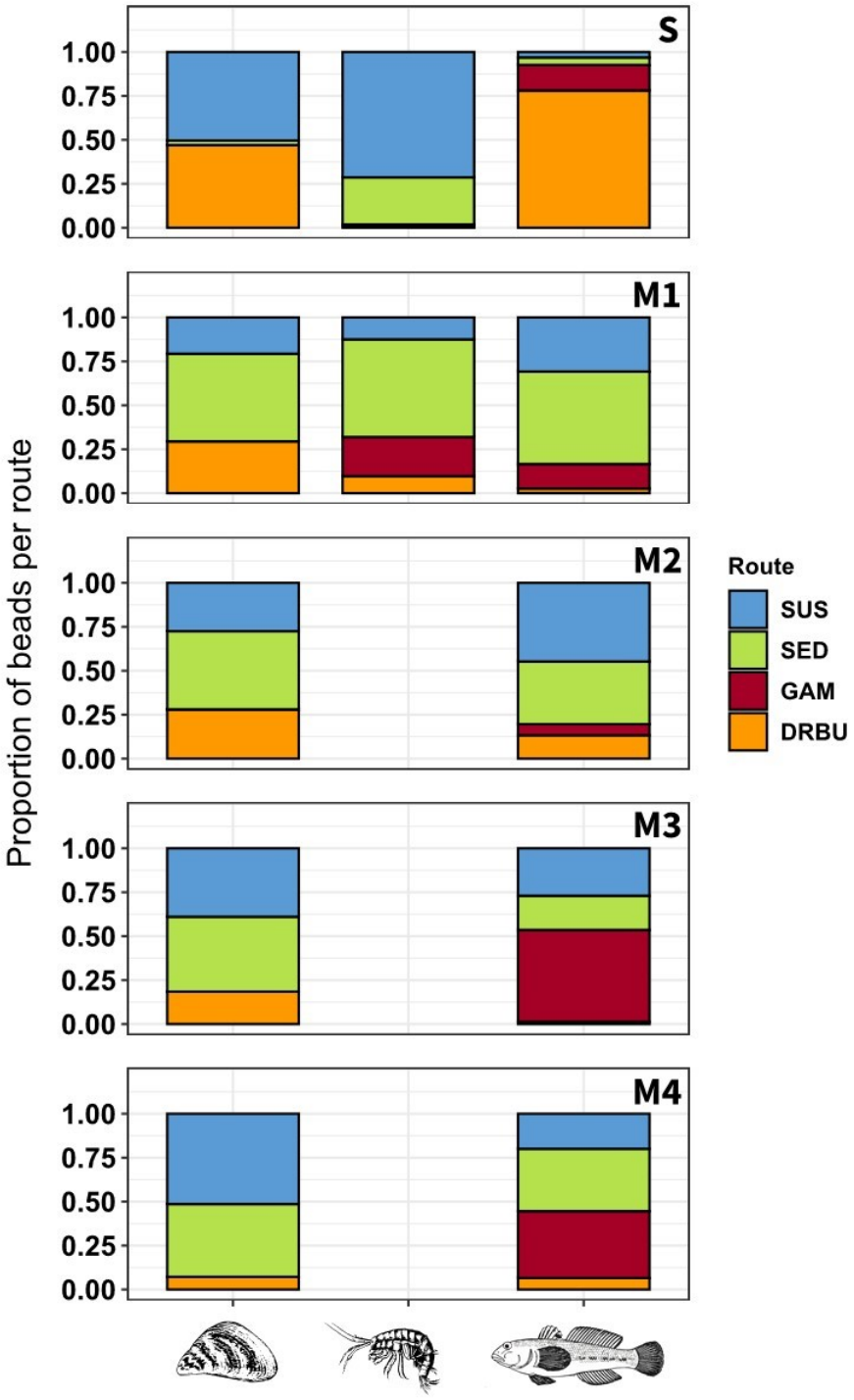

Figure 3.2: Mean proportion of beads acquired per individual from each route of contamination reported per animal and scenario. Numerical values of proportion are reported in Table S3.1.

The ability of organisms to take up and depurate microplastics differed depending on surrounding exposures. Gammarids have previously been found to rapidly take up and egest microplastics (López-Rojo et al. 2020). In our experiments, ~50% of the individuals tested egested all the PE beads they had ingested within the first 12 h of depuration (Chapter 2). Based on these depuration experiments, gammarids were predicted to have <0.5% of their total load composed of red beads (from pre-contamination) after 24 h, but we observed 22% of their burden to be red beads in M1 (Figure 3.2). Gammarids continuously exposed to microplastics retain particles in their digestive tract thereby reducing the amount of food they can assimilate (Blarer and Burkhardt-Holm 2016). Such a phenomenon is observed when amphipods are exposed to high concentrations of microplastics or for longer periods (Mateos-Cárdenas et al. 2020), such as under scenario M1. The presence of a predator may further reduce gammarid feeding activities to avoid being hunted, causing amphipods to retain food and beads in their guts under this period of starvation (Bärlocher and Kendrick 1975). These conditions may cause the slower depuration rate observed in M1 than under single-exposure laboratory studies.

Mussels exposed to high concentrations of microplastic (1250–100 000 particles ml^{-1}) had lower clearance rates (Harris and Carrington 2020 ; Weber et al. 2021), suggesting they are unable to filter new particles over a given contamination threshold. The microbead load retained by mussels in our experiments was an order of magnitude higher when they depurated in a clean environment (Figure 3.1S, mean: 229 beads$\cdot g^{-1}$) than under continuous exposure over the same period (M1-3; means of 20-29 beads$\cdot g^{-1}$). Their body burden was even higher when a longer exposure of 48 h was used (M4; mean: 48 beads$\cdot g^{-1}$). The proportion of beads remaining in mussels after 24 h of depuration also declined from 47% under clean environment to 29% under M1 (Figure 3.2; Table S3.1). Furthermore, a decline in the proportion of beads remaining from

initial contamination was observed over time, decreasing from 18% to 7% while the proportion of suspended beads increased from 39 to 51%.

Reductions in the initial contamination load would suggest a constant exposure to microplastics may enhance filtration and depuration processes of mussels and that the ambient concentrations offered did not impact their clearance rates. In addition, the replacement of orange beads with blue suspended beads indicates that filtration activity and depuration processes are cycling the portion of suspended beads retained by mussels. On the other hand, the relatively constant proportion of sedimented beads across time suggests beads acquired via sedimented particles have slower depuration processes.

Interestingly, under increasing mussel densities we observed an 11% decline in the proportion of orange beads per mussel (original contamination) and a 19% increase in the proportion of blue suspended beads (Figure 3.2, M1–M3). This increase in mussel density can induce stronger currents from collective filtration. This would allow individuals to filter a greater volume of water (Yu and Culver 1999), so taking up more suspended beads, and to clear their siphons more effectively (Zaiko and Daunys 2012), thereby releasing more orange beads.

For the predatory round goby, we observed opposing responses in the dominant routes of transfer between single and multiple-exposure scenarios. Under single-exposure experiments, we observed a stronger contamination and proportion of beads from prey than environmental routes. The mean body burden of round gobies was 65 beads$\cdot g^{-1}$ from mussel prey (Figure 3.1S), which contributed to 78% of their total microbead load (Figure 3.2S). However, under M1, M3, and M4 scenarios (Figure 3.1) sedimented particles yield a higher body burden accounting for 53, 70 and 91% of the total microbead load (Figure 3.2; Table S3.1).

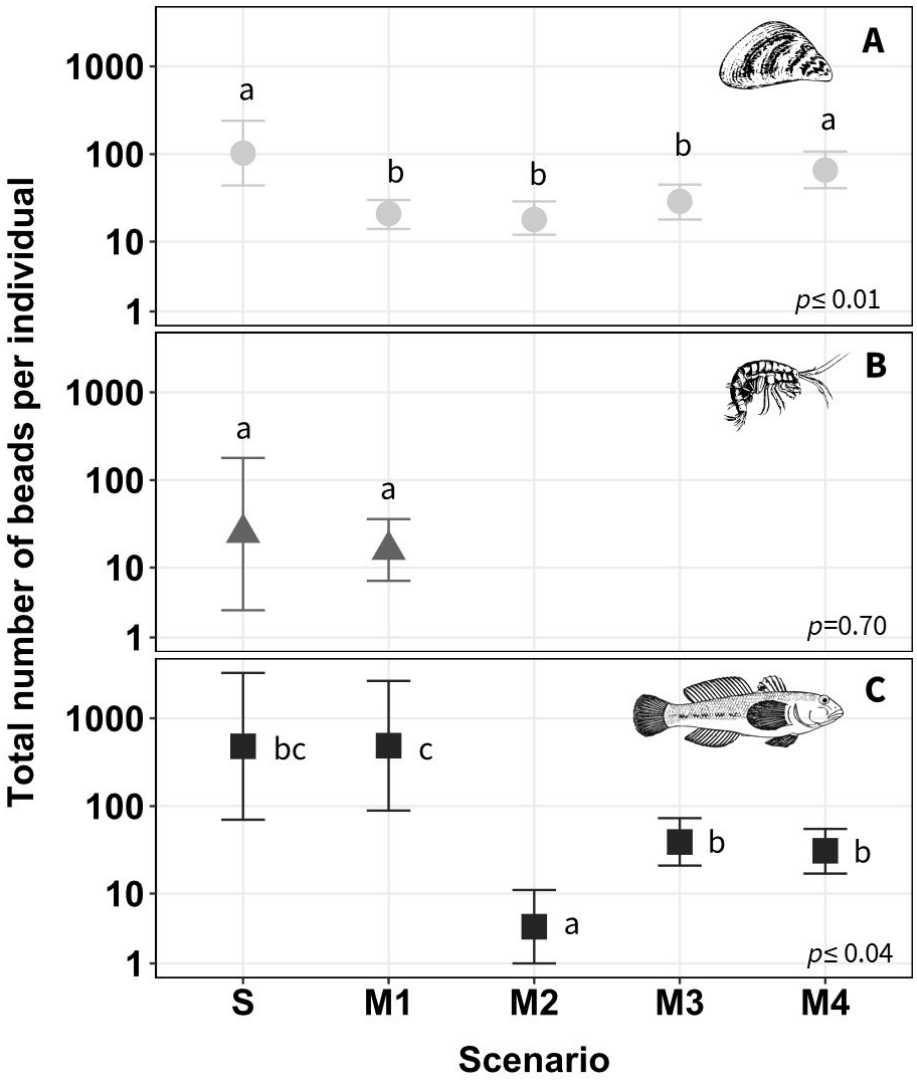

Figure 3.3: Estimated total number of beads acquired per animal: mussel (A), gammarid (B), and round goby (C) for each scenario. Points represent the type I negative binomial regression model marginal means and error bars display the estimated confidence intervals. Letter codes identify Sidak-adjusted pairwise contrasts between scenarios per species with significant differences detected when $p \leq 0.05$. Numerical values are reported in Table S3.3.

Continuous exposure to multiple routes yielded similar daily total contamination of beads in round gobies when the community assemblage was the same. For example, when fish were exposed to an equivalent abundance of contaminated prey (scenarios S and M1), the total

number of beads they retained was not significantly different with 480 and 488 beads·individual^{-1}, respectively (Figure 3.3C; Table S3.3). Likewise, gobies exposed to the same food web assemblage (10 gammarids and 15 mussels) over two exposure periods, 24 h (M3) and 48 h (M4), had similar body burden with 39 and 31 beads·individual^{-1}, respectively. Fish had the most variable total bead contamination amongst scenarios that differed in animal abundance (i.e.; S and M1 contrasted with scenarios M3 & M4; Figure 3.1 & 3.3 C). The total number of beads acquired per individual was significantly influenced by the food web assemblage scenario ($F_{(4,37)}$=8.83, p<0.001) and the proportion of prey eaten during the exposure period ($F_{(1,37)}$=8.98, p=0.003). The contamination potential of round gobies was likely affected by the number of invertebrate prey offered and the percentage of each prey type that was eaten (Table S3.4). Indeed, we observed larger changes in the proportion of beads acquired per fish than per mussels across multiple-exposure scenarios (Figure 3.2, M1-M4), as the latter may be less influenced by the presence of other species when filtering.

The number and type of prey offered under multiple-exposure scenarios can affect the capacity for round gobies to capture each type of prey and thus affect the quantity of beads they obtain from their prey. For example, in the single-exposure experiments, pre-opened mussels were offered to the fish to facilitate its access to the contaminated soft tissues, and 100% of the mussels being ingested. In multiple-exposure experiments, we selected a variety of mussel sizes for predator-prey transfer, and these were all alive (none were pre-opened). Some of the mussels might have required longer handling time or were too large to crush by the fish.

Prey selection in round gobies depends not only on fish length but also on the number and type of prey offered. Generally, gobies of all sizes prefer feeding on small-sized quagga mussels

(<10 mm; Naddafi and Rudstam, 2014; Perello et al. 2015). The mean sizes of round gobies and mussels used in each scenario differed (Table S3.5), but the majority of mussels used were between 10 and 20 mm in length, while round gobies were ~80 mm (Tables S3.5-3.7). Based on these measures, round gobies should favour an amphipod diet when visibility is high and refugia are rare, but switch to a mussel diet when gammarids can escape and hide (Diggins et al. 2002).

In our experiments, environmental microbeads can create a slightly more turbid environment during the multiple-exposure scenarios as a result of re-suspension of sedimented beads. Considering that only larger round gobies (>130 mm) opt for a dreissenid diet, largely because they are less limited by their gape size (Miano et al. 2021), the low predation rate on mussels observed in our multiple-exposure scenarios (7–30%) is to be expected. The presence of shell fragments in the gut of an 80 mm goby (M3) indicate smaller gobies can sometimes overcome gape limitation when feeding on 20 mm mussels (e.g., through shell crushing prior to consumption; Angradi 2018). However, gobies under multiple exposure scenarios had more beads from gammarid preys (Figure 3.1 and Figure 3.2), suggesting hunting gammarids was preferred over the capture and the crushing of sessile mussel preys.

Selection of one prey over another can depend on the presence of a competitor (M3 and M4) and the exposure time. In scenarios where two round gobies compete for resources, they acquired 52% of their contamination from gammarids after a 24 h exposure (M3), and 38% after a 48 h period (M4); by contrast, when a single goby is present per aquarium only 6–14% were contaminated (Figure 3.2; M2 and M1, respectively). Although fewer gammarids were available per fish in scenarios M3 and M4 (10 gammarids for two fish vs. 7 for M1, and 14 for M2), more beads from gammarid prey were retained by round gobies. Gammarids remain more accessible to gobies of all sizes, so the presence of a competitor may have incited each fish to capture

gammarids more rapidly, and therefore increase the proportion of beads from this prey. Regardless of the food web assemblage, we observed intact carcasses of gammarids holding high numbers of red beads in five of the fish stomachs (Photo S3.1).

Round gobies can take up more environmental beads through their gills or swallow them accidentally while hunting and consuming gammarid prey than if they are resting, thereby increasing beads from environmental routes. An additional load can be transferred from their prey because both gammarids and mussels acquired suspended and sedimented beads under multiple-exposure scenarios (Figure 3.2)— a phenomenon that is not possible when a single route of exposure is offered. As exposure time increases, the transfer of beads from predator-prey relationships may be more difficult to track as prey will have gained more beads of environmental origin.

3.2.2 Effect of exposure time on microplastic contamination

The number of microbeads acquired and retained by dreissenid mussels depends on time of exposure, concentration of available microbeads, and abundance of food (Weber et al. 2021). Mussels can identify microbeads as undesirable but could still retain such beads during the depuration process when particles are wrapped in mucus and transported to the mantle to be expelled as pseudofaeces (Ward et al. 2019). With a longer exposure to microbeads, encounter rates via feeding, respiration, or species interactions increases. Indeed, a 48 h microbead exposure nearly doubled the microbead burden of mussels from each route (Figure 3.1 M4 vs. M3) and increased total contamination level (Figure 3.4). Mussels exposed to multiple routes of microbeads for two consecutive days also had more beads than during a single day (i.e., ~66 beads·individual^{-1} vs. 29 beads·individual^{-1}; Figure 3.3; Table S3.3). Coupled with the increased proportion of suspended beads (from 39% to 51%) and a reduction in the proportion of pre-

contaminated orange beads (from 18% to 7%; Figure 3.2), our results indicate that under multiple-exposure scenarios, mussels filter and retain particles faster than they can depurate them; increasing their body burden over time. The mussel's filtration and depuration activities cycled beads acquired from suspension (replacing orange beads with blue beads), whereas the proportion of sedimented beads remained relatively constant—indicating either slower depuration processes or better retention of these particles. Even if dreissenids can sort particles and select palatable food items amongst particulate materials (Evan Ward and Shumway 2004; Tang et al. 2014), evidence from our experiments offer support for the conclusion of Merzel et al. (2020) that dreissenids are not as efficient at sorting and egesting microplastic beads as they do for natural particles.

On the second day of exposure, a greater number of fish were found with orange beads (from mussels; Figure 3.1 M4) but none of the fish stomachs contained shell fragments. Yet, one to two mussels were missing at the end of these trials. We suspect the missing mussels could have been ingested by one of the gobies, but their shell was crushed and egested so were not found in the stomach. The orange microbeads from mussels could have originated both from predator-prey interactions and from depuration events. Over longer exposures, beads retained in mussels from original contamination (orange) were replaced with environmental beads (blue and green beads; Figure 3.2 M3 & M4), indicating depuration mechanisms are at play. As mussel more depurate beads, these particles become available to other organisms. This might explain why, despite low mussel predation by gobies, fish acquired four times more beads from mussels in M4 than M3 (4 beads·individual^{-1} vs. 1 bead·individual^{-1}; Table 3.1).

3.2.3 Bioaccumulation and biomagnification potential

One of the presumed risks of microplastic pollution in aquatic environments is the potential to bioaccumulate in organisms and biomagnify up trophic levels (Krause et al. 2021; McIlwraith et al. 2021). Bioaccumulation potential can be evaluated using an experimental community module by adapting ecotoxicological metrics for microplastic contamination. Our results suggest that the animal's trophic position and mechanism of uptake influence its capacity to bioconcentrate microplastics in their environment.

The invertebrates had the highest potential to accumulate microbeads from water and sediments, with BCF >1 $L \cdot kg^{-1}_{ww}$ for environmental routes across all scenarios (circles in Figure 3.4). This implies that the concentration of microbeads in 1 kg of animal tissue is greater than the concentration in 1 L of water. Mussels acquired ~100·beads·$individual^{-1}$ via single-exposure scenarios and BCF values of 11 $L \cdot kg^{-1}_{ww}$ and 28 $L \cdot kg^{-1}_{ww}$ for sedimented and suspended routes. The number of beads acquired was nearly an order of magnitude greater than those retained by mussels when they were exposed to multiple routes at once (~19 – 29·beads·$individual^{-1}$; Figure 3.3A); BCF values were nearly an order of magnitude lower, indicating that results of single-exposure experiments do not represent microplastic uptake potential for mussels within a community.

However, mussels maintained similar BCFs from sedimented particles (1.4 – 2.85 $L \cdot kg^{-1}_{ww}$) and suspended particles (1.02 – 2.04 $L \cdot kg^{-1}_{ww}$) across all multiple-exposure scenarios (Figure 3.4A), suggesting bioaccumulation potential may be more robust to variations in food web assemblages. Because mussels acquired more beads from their environment (Figure 3.2 M1-M3) over time, they progressively increased their total contamination (Figure 3.3 M4). As a result their ingestion rate exceeds their egestion rate so they bioaccumulate microbeads (*sensu*

Nordberg et al. 2009). Based on this observation, we can confirm that bioconcentration is taking place. However, to be considered bioaccumulative, a contaminant needs to have BCF or BAF values above 1000 and 5000, respectively (Arnot and Gobas 2006; Borgå 2013; Elizalde-Velázquez et al. 2020), which is three to four orders of magnitude above the reported factors examined. Given that microplastics are complex sometimes behaving as natural sediment (Waldschläger et al. 2022), other times behaving like a chemical substance (Bucci and Rochman 2022), this criterion may need to be revised when more information is known about how these particles are distributed and circulate in food webs.

To assess the bioaccumulation potential from species interactions, we calculated a pseudo-BAF for particles transferred via mussel biodeposits or from predator-prey interactions (BAF; triangles in Figure 3.4). Gammarids had a higher potential to bioaccumulate microbeads from mussels (orange triangles, Figure 3.4B) under the multiple-exposure scenario than predicted by single exposure. Perhaps the presence of a predator forces gammarids to seek refuge amongst the substrate and mussel shells, thereby increasing their access to beads stored in mussel biodeposits, a known source of food (Ricciardi et al. 1997; González and Burkart 2004). On the other hand, the BAFs of gobies from the ingestion of beads originating from prey were the lowest (Figure 3.4C; Table S3.2).

The biomagnification factor (BMF) of the fish was significantly influenced by the choice of prey (or route, $F_{(1,110)}=128.6$, $p<0.0001$), the food web assemblage scenario ($F_{(4,110)}=15.7$, $p<0.0001$), and the proportion of prey they consumed ($F_{(1,110)}=13.8$, $p<0.0002$). We observed greater biomagnification factors when round gobies consumed mussels (orange squares, BMF ~ 2.7 $\mu g \cdot kg^{-1}$ for M1, M3 and M4), but not gammarids (red squares, BMF $\leq$ 0.0079 $\mu g \cdot kg^{-1}$) under multiple-exposure scenarios (Figure 3.4C). Based on this metric, it appears the consumption of

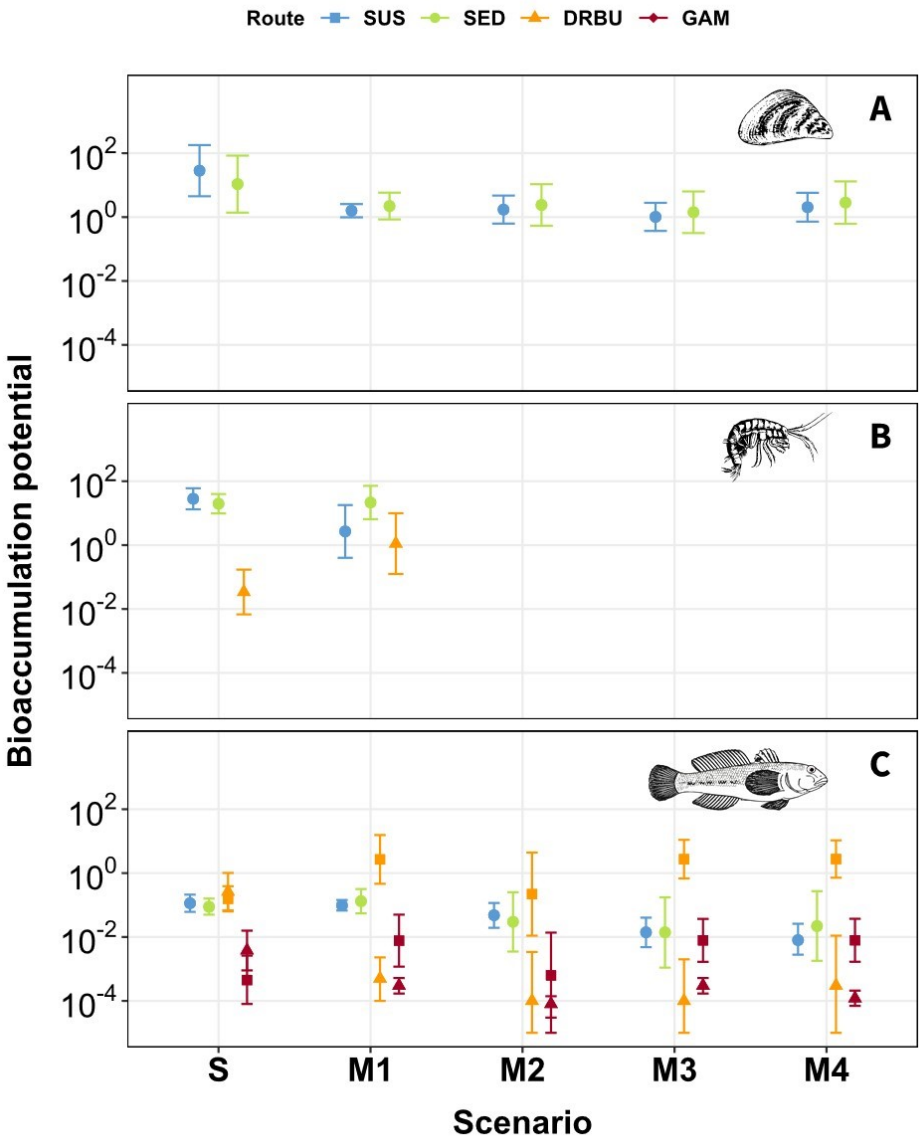

Figure 3.4: Mean bioconcentration (BCF), bioaccumulation (BAF) and biomagnification factors (BMF) measured per route of transfer and food web assemblage scenario for each animal (A) Mussel, (B) gammarids, (C) round goby. Mean BCFs (●) represent microplastic accumulation per $L \cdot kg^{-1}$, mean BAFs (▲) describe the accumulation of beads from the transfer of another organism, and BMFs (■) show the concentration of beads accumulated per kg of GI tract from eating 1 kg of prey. Error bars represent the lower and upper confidence intervals. See Table S3.2 for specific values.

mussels can lead to biomagnification of microbeads by round gobies under the given concentration, time and scenario of exposure. However, microbead uptake being concentration-dependent (Chapter 2), biomagnification is more likely to be observed under laboratory settings where concentrations used are orders of magnitude higher than those reported in aquatic environments (D'Avignon et al. 2022; Chapter 1).

If biomagnification of microbeads occurred, we would expect to observe a larger total number of beads acquired by round gobies than by invertebrates. In our experiments, we observed much higher body burden in gobies from the single-exposure trials and M1 models with means of 480 and 488 beads·individual^{-1}, respectively (Figure 3.3C; Table S3.3). However, in the other multiple-exposure scenarios (M2-M4), the total number of beads acquired per fish were generally lower than for mussels (Figure 3.3; Table S3.3). The BAFs were significantly lower for the round gobies than for gammarids (z=4.88, p<0.0001) and mussels (z=5.72, p<0.0001) across all scenarios (Figure 3.5). Even if we note a higher total number of beads per fish than per invertebrates under scenarios S, M1 and M3 (Figure 3.3, Table S3.3), the tBAF suggests biomagnification is not happening in this short food web. Round gobies are not efficiently bioaccumulating the microbeads that are available from their prey or from other sources in their environment, even though they have more routes of microplastic transfer than lower trophic levels in this community module.

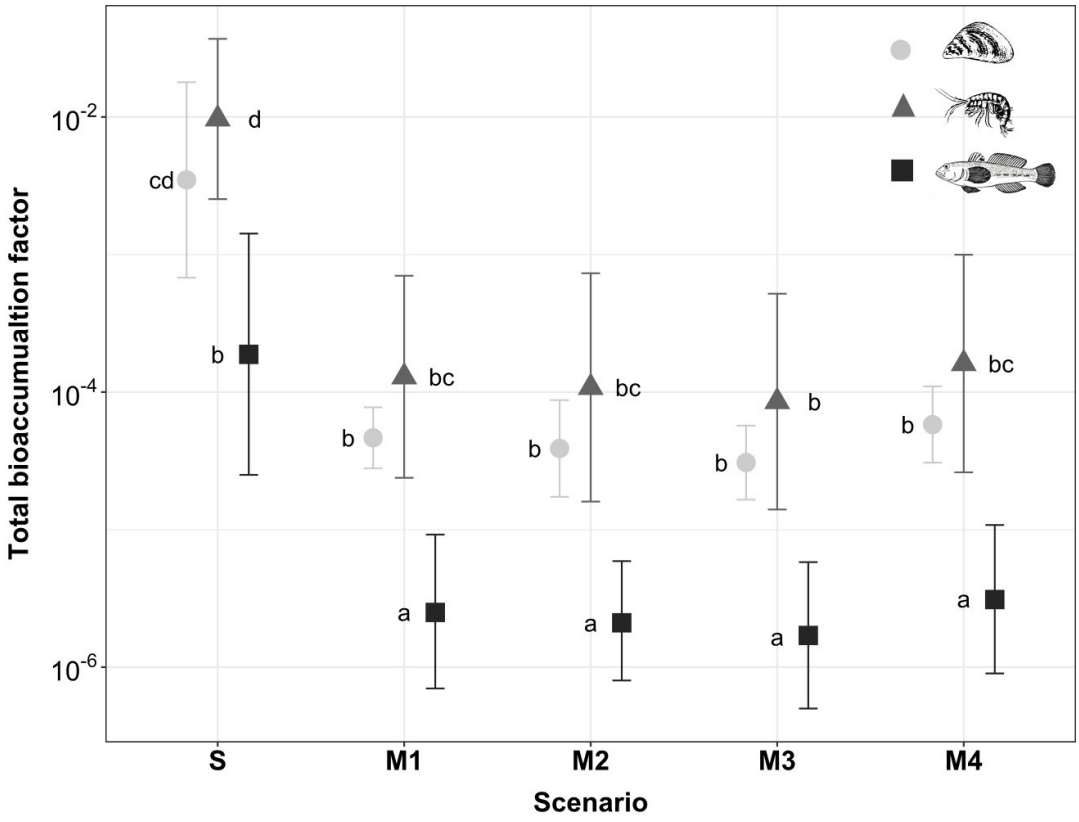

Figure 3.5: Estimated mean bioaccumulation factors (including all routes of uptake) per animal for each food web assemblage scenario modelled. Symbols represent the different animal where mussels (●), gammarids (▲) and round gobies (■). Error bars show the lower and upper confidence limits. A unique letter code is used to identify significant difference (p>0.05) between BAF means. Numerical values are reported in Table S3.3.

3.2.4 Can single exposure experiments be used to predict microplastic contamination in food webs?

In this study, we found that the nature of species assemblage influences the contribution of each route taken by microplastics to circulate within a food web as species interactions creates novel conditions absent from single-exposure experiments. The behaviour of each animal is

influenced by the members of its community through changes in environmental conditions (e.g., increasing re-suspension), the stress levels (competition or predation), and the availability of food and microplastics.

Neither the body burden (Figure 3.1) nor the proportion of beads per route (Figure 3.2) predicted by S corresponded to the contamination reported by multiple exposure scenarios- even under similar species assemblages. The predicted BCFs of sedimented beads in mussels were three to seven times higher in S than multiple exposure models. Similarly, the BCF value for suspended beads were one order of magnitude greater (Figure 3.4A).

For gammarids, we observed the greatest difference between suspended beads in the single and M1 scenarios with an order of magnitude reduction in their bioconcentration factor (Figure 3.4B). As for the total bioaccumulation factors, the single-exposure scenario predicted a significantly higher potential than what was observed under multiple scenarios for each animal (Figure 3.5). Considering multiple-exposure scenarios are more representative of a food web community observed in natural settings, the results obtained from these models may be more reliable than what is observed or inferred from single-exposure experiments. However, the total number of beads measured in gammarids, and round gobies were similar in the single and multiple-exposure scenarios with equal prey numbers offered (S & M1 in Figure 3.3). This suggests that if all microbead routes in a food web are tested with single-exposure experiments, the sum of all routes may predict total contamination under natural settings (multiple exposure simultaneous routes).

However, designing laboratory experiments that model each contamination pathways of complex food webs is challenging because it requires *a priori* knowledge of the food web structure and interactions and a good understanding of microplastic cycling within its network.

For species (such as the round gobies in our community module) influenced by the food web structure and abundance of prey, single-exposure experiments must account for the prey selection behaviour of the species.

We showed that total contamination of gobies subjected to the same community assemblages (i.e., S and M1; M3 and M4) was stable across scenarios (Figure 3.3). When prey items differed total contamination and the microbead contribution per route also differed. These observations suggest that single-exposure models can inform hypotheses on the potential contamination of microplastics, when we can model each food web link and estimate the abundance and consumption of each prey, otherwise our predictions of microplastic contaminations in complex food webs will remain biased.

Finally, we suggest that predictive models of microplastic distribution and transfer within food webs could be improved by applying community ecology concepts and isotopic or fatty acid tracers in combination with the quantification of microplastic burdens, thereby integrating laboratory and field experimental approaches.

3.3 Chapter 3 Supplementary Materials

Table S3.1: Summary of results showing the sample size (N); observed risk of occurrence (%); mean proportion of beads per individual (%); the estimated means, standard error (SE), lower (LCI) and upper (UCI) confidence intervals for the number of beads per individual; and the body burden per animal, route of exposure and scenario. GAM refers to gammarid, DRBU to mussel, NEME to the round goby, SED to sedimented beads and SUS to suspended beads. Scenario S used results of chapter 2. See Table 3.1 for descriptions of scenarios.

				Risk	Proportion	Number of beads individual $^{-1}$				Body burden (bead g^{-1})			
Scenario	Animal	Route	N	%	% of total	Mean	SE	LCI	UCI	Mean	SE	LCI	UCI
S	DRBU	DRBU	7	73	47	19.77	5.553	8.69	44.99	229.20	63.66	101.66	516.90
S	DRBU	SED	28	93	3	5.00	2.074	2.29	11.31	24.79	10.17	11.09	55.40
S	DRBU	SUS	32	100	50	100.00	46.644	39.76	249.39	333.87	154.02	135.17	824.63
S	GAM	DRBU	158	13	1	0.33	0.1004	0.18	0.60	21.44	5.98	12.41	37.00
S	GAM	SED	33	73	27	6.74	1.9331	3.85	11.83	1379.49	503.80	674.32	2822.10
S	GAM	SUS	23	78	71	17.90	6.519	8.76	36.54	543.84	165.40	299.66	987.00
S	GAM	GAM	17	12	0	0.08	0.05023	0.01	0.46	85.63	82.48	12.96	566.39
S	NEME	DRBU	22	95	78	265.61	123.9	106.47	662.70	65.39	30.11	26.52	161.23
S	NEME	SED	7	100	4	14.79	8.432	4.84	45.21	2.93	2.00	0.77	11.19
S	NEME	SUS	13	77	3	10.55	4.158	4.87	22.84	3.55	1.75	1.35	9.35
S	NEME	GAM	17	76	14	49.09	21.33	20.95	115.00	9.27	4.00	3.98	21.59
M1	DRBU	DRBU	32	66	29	4.34	1.13	3.45	5.46	29.16	1.15	17.49	48.62
M1	DRBU	GAM	32	0	0	0.01	2.75	0.00	0.06	0.05	2.75	0.01	0.50
M1	DRBU	SED	32	97	50	10.38	1.45	8.20	13.15	72.22	1.15	43.51	119.9
M1	DRBU	SUS	32	66	21	7.51	1.13	5.93	9.51	51.73	1.13	40.71	65.74
M1	GAM	SUS	6	50	13	3.00	1.81	0.67	13.52	250.00	151.00	55.48	1126.0
M1	GAM	SED	6	100	56	7.50	4.32	1.79	31.50	625.00	360.00	148.82	2625.0
M1	GAM	GAM	6	50	22	5.20	3.02	1.20	22.17	430.60	252.00	100.34	1848.0
M1	GAM	DRBU	6	33	10	0.33	0.3	0.03	3.14	27.80	25.00	2.95	261.0
M1	NEME	SUS	5	100	31	72.34	1.43	35.93	145.64	8.08	1.45	3.91	16.72
M1	NEME	SED	5	100	53	121.32	1.67	22.04	667.86	13.73	1.70	2.34	80.50
M1	NEME	GAM	5	80	14	9.00	1.7	1.57	51.53	1.01	1.72	0.17	6.10
M1	NEME	DRBU	5	80	3	2.70	1.96	0.36	20.44	0.30	1.99	0.04	2.38
M2	DRBU	DRBU	53	47	28	3.91	1.15	2.95	5.18	19.86	1.15	8.85	44.55
M2	DRBU	GAM	53	2	0	0.01	2.75	0.00	0.06	0.04	2.75	0.00	0.46
M2	DRBU	SED	53	100	45	9.34	1.16	6.99	12.47	49.18	1.15	22.02	109.81
M2	DRBU	SUS	53	81	27	6.76	1.62	5.08	8.99	35.23	1.16	20.60	60.23
M2	NEME	SUS	10	80	45	8.58	1.62	1.65	44.66	4.30	1.64	0.79	23.36
M2	NEME	SED	10	90	36	5.64	2.02	0.10	319.26	2.81	2.05	0.04	176.29
M2	NEME	GAM	10	30	6	0.62	1.96	0.01	53.93	0.30	2.01	0.00	28.71
M2	NEME	DRBU	10	20	13	0.66	1.95	0.01	90.48	0.31	1.99	0.00	46.83
M3	DRBU	DRBU	67	61	18	3.45	1.16	2.58	4.60	25.80	1.50	11.43	58.19
M3	DRBU	GAM	67	0	0	0.01	2.75	0.00	0.05	0.05	2.75	0.00	0.60
M3	DRBU	SED	67	100	43	8.24	1.16	6.15	11.03	63.88	1.15	28.45	143.43
M3	DRBU	SUS	67	96	39	5.96	1.64	4.48	7.93	45.76	1.17	26.62	78.67
M3	NEME	SUS	10	80	27	12.65	1.61	2.48	64.53	1.01	1.63	0.19	5.40
M3	NEME	SED	10	70	19	14.02	2.48	0.26	743.41	1.10	2.49	0.02	65.92
M3	NEME	GAM	10	90	52	27.69	1.98	0.50	1537.36	2.17	2.01	0.03	132.60
M3	NEME	DRBU	10	20	1	1.01	1.96	0.01	112.88	0.08	1.99	0.00	9.68
M4	DRBU	DRBU	66	55	7	7.14	1.15	5.38	9.47	47.99	1.50	21.42	107.52
M4	DRBU	GAM	66	0	0	0.01	2.75	0.00	0.10	0.09	2.75	0.01	1.11
M4	DRBU	SED	66	95	41	17.07	1.16	12.81	22.74	118.85	1.15	53.30	265.03
M4	DRBU	SUS	66	95	51	12.35	1.16	9.35	16.30	85.14	1.16	49.86	145.37
M4	NEME	SUS	11	73	20	6.63	1.61	1.29	33.97	0.61	1.63	0.11	3.33
M4	NEME	SED	11	91	35	10.58	2.69	0.20	547.49	1.62	2.73	0.03	95.14
M4	NEME	GAM	11	82	38	18.53	2.46	0.34	1007.27	1.32	2.50	0.02	81.89
M4	NEME	DRBU	11	64	7	4.29	2.29	0.04	418.21	0.40	2.33	0.00	44.46

Table S3.2: Summary of estimated marginal means and confidence intervals of bioaccumulation potential metrics: A) bioaccumulation factor (BCF), B) bioaccumulation factor (BAF), and C) biomagnification factor (BMF) per animal, route and scenario. Animals include mussels (DRBU), gammarids (GAM), and the round goby (NEME). Routes of contamination are from beads in suspension (SUS), sedimented beads (SED), or beads originating from pre-contaminated mussels (DRBU) or pre-contaminated gammarids (GAM). See Table 3.1 for descriptions of scenarios.

A. Bioconcentration factor (BCF)

Scenario	Animal	Route	Mean	LCI	UCI
S	DRBU	SED	10.80	1.380	84.31
S	DRBU	SUS	28.45	4.500	179.8
S	GAM	SED	19.77	9.831	39.70
S	GAM	SUS	28.16	13.18	60.20
S	NEME	SED	0.088	0.050	0.160
S	NEME	SUS	0.114	0.061	0.212
M1	DRBU	SUS	1.592	0.988	2.564
M1	DRBU	SED	2.220	0.845	5.830
M1	GAM	SUS	2.677	0.400	17.92
M1	GAM	SED	21.56	6.478	71.77
M1	NEME	SUS	0.098	0.068	0.144
M1	NEME	SED	0.132	0.055	0.318
M2	DRBU	SED	2.402	0.537	10.74
M2	DRBU	SUS	1.722	0.628	4.725
M2	NEME	SUS	0.048	0.019	0.116
M2	NEME	SED	0.030	0.004	0.252
M3	DRBU	SED	1.420	0.317	6.361
M3	DRBU	SUS	1.018	0.370	2.797
M3	NEME	SUS	0.014	0.005	0.040
M3	NEME	SED	0.014	0.001	0.175
M4	DRBU	SED	2.850	0.619	13.12
M4	DRBU	SUS	2.043	0.723	5.771
M4	NEME	SUS	0.008	0.003	0.026
M4	NEME	SED	0.022	0.002	0.272

B. Bioaccumulation factor (BAF)

Scenario	Animal	Route	Mean	LCI	UCI
S	NEME	GAM	0.0038	0.0009	0.0158
M1	GAM	DRBU	1.1144	0.1248	9.9480
M1	NEME	DRBU	0.0005	0.0001	0.0023
M1	NEME	GAM	0.0003	0.0002	0.0005
M2	NEME	DRBU	0.0001	0.0000	0.0034
M2	NEME	GAM	0.0001	0.0000	0.0001
M3	NEME	DRBU	0.0001	0.0000	0.0020
M3	NEME	GAM	0.0003	0.0002	0.0005
M4	NEME	DRBU	0.0003	0.0000	0.0110
M4	NEME	GAM	0.0001	0.0001	0.0002

C. Biomagnification factor (BMF)

Scenario	Animal	Route	Mean	LCI	UCI
S	NEME	DRBU	0.1566	0.0632	0.3885
S	NEME	GAM	0.0005	0.0001	0.0026
M1	NEME	DRBU	2.6871	0.4640	15.5594
M1	NEME	GAM	0.0077	0.0012	0.0501
M2	NEME	DRBU	0.2204	0.0110	4.4078
M2	NEME	GAM	0.0006	0.0000	0.0137
M3	NEME	DRBU	2.7377	0.6810	11.0093
M3	NEME	GAM	0.0078	0.0017	0.0369
M4	NEME	DRBU	2.7609	0.7180	10.6205
M4	NEME	GAM	0.0079	0.0017	0.0372

Table S3.3: Summary of estimated marginal means, standard error (SE), lower (LCI) and upper (UCI) confidence intervals for the total number of beads per individual and the total bioaccumulation factor (tBAF) per animal for each scenario. Animals include mussels (DRBU), gammarids (GAM), and the round goby (NEME). See Table 3.1 for descriptions of scenarios.

		Total number of beads				Total Bioaccumulation factor (tBAF)			
Scenario	**Animal**	**Mean**	**SE**	**LCI**	**UCI**	**Mean**	**SE**	**LCI**	**UCI**
S	DRBU	103	34	44	240	0.00349	0.00195	0.00068	0.01790
S	GAM	25	68	3	179	0.00968	0.00444	0.00253	0.03700
S	NEME	480	472	70	3289	0.00019	0.00013	0.00003	0.00142
M1	DRBU	21	3	14	30	0.00005	0.00001	0.00003	0.00008
M1	GAM	16	24	7	36	0.00013	0.00008	0.00002	0.00070
M1	NEME	488	423	89	2667	<0.00001	0.00000	<0.00001	0.00001
M2	DRBU	18	3	12	29	0.00004	0.00001	0.00002	0.00009
M2	GAM	0	0	0	0	0.00011	0.00007	0.00002	0.00073
M2	NEME	4	2	1	11	<0.00001	0.00000	<0.00001	0.00001
M3	DRBU	29	5	18	45	0.00003	0.00001	0.00002	0.00006
M3	GAM	0	0	0	0	0.00009	0.00005	0.00001	0.00052
M3	NEME	39	12	21	73	<0.00001	0.00000	<0.00001	0.00001
M4	DRBU	66	12	41	107	0.00006	0.00001	0.00003	0.00011
M4	GAM	0	0	0	0	0.00016	0.00010	0.00003	0.00100
M4	NEME	31	9	17	55	<0.00001	0.00000	<0.00001	0.00001

Table S3.4: Number of animals per scenario, number of replicates for each scenario, percentage of individuals eaten by the predator over the time of exposure, and number of replicates in which no animals of a given species (DRBU = mussel; GAM=gammarids) were eaten.

	GAM	DRBU	NEME	Replicate	% GAM	Zero	% DRBU	Zero
S	5	5	1	17 -22	100	0	0-100	3
M1	7	7	1	5	30-100	0	0-30	3
M2	10	14	0	10	100	0	0-10	6
M3	15	10	2	10	100	0	0-20	2
M4	15	10	2	10	100	0	0-15	2

Table S3.5: Lengths and weights of animals used in each scenario. N, sample size; std, standard deviation.

	Mussel						Goby						Gammarids	
Length (mm)	**N**	**mean**	**std**	**median**	**min**	**max**	**N**	**mean**	**std**	**median**	**min**	**max**	**mean**	**std**
S	60	18.93	3.77	18.55	13.40	29.30	58	81.9	12.8	82.5	53.0	103.0	8.3	2.55
M1	128	17.05	4.87	17.50	8.00	25.00	20	80.6	13.5	85.0	64.0	95.0	7.8	2.55
M2	76	16.40	2.91	15.25	13.3	22.70	40	49.6	6.3	51.5	40.0	60.0	7.8	2.55
M3	80	12.17	2.14	12.40	7.50	15.00	40	89.3	15.5	87.0	65.0	112.0	7.8	2.55
M4	72	12.55	2.92	12.60	7.50	20.00	44	83.2	12.0	83.0	63.0	101.0	7.8	2.55
Weight (g)														
S	60	0.19	0.14	0.14	0.04	0.75	59	4.8	2.4	4.4	1.2	12.5	0.014	0.001
M1	128	0.16	0.14	0.14	0.01	0.73	20	6.5	3.1	7.4	3.000	10.4	0.012	0.001
M2	76	0.34	0.40	0.12	0.04	1.48	40	1.5	0.6	1.6	0.6	2.5	0.012	0.001
M3	80	0.14	0.08	0.12	0.01	0.35	40	9.6	4.9	8.1	3.4	17	0.012	0.001
M4	72	0.16	0.01	0.16	0.02	0.41	44	7.5	3.2	7.7	3.00	12.7	0.012	0.001

Table S3.6: Numbers of mussels in each size class (length in mm) per scenario (S, M1-M4).

Size class	**S**	**M1**	**M2**	**M3**	**M4**
5-10	0	4	0	16	8
10-15	10	44	28	56	52
15-20	25	28	36	8	8
20-25	21	40	12	0	0
>25	2	0	0	0	0

Table S3.7: Numbers of round gobies in each size class (length in mm) per scenario (S, M1-M4).

Size class	**S**	**M1**	**M2**	**M3**	**M4**
40- 99.9	55	20	40	28	36
100-130	3	0	0	12	8
>130	0	0	0	0	0

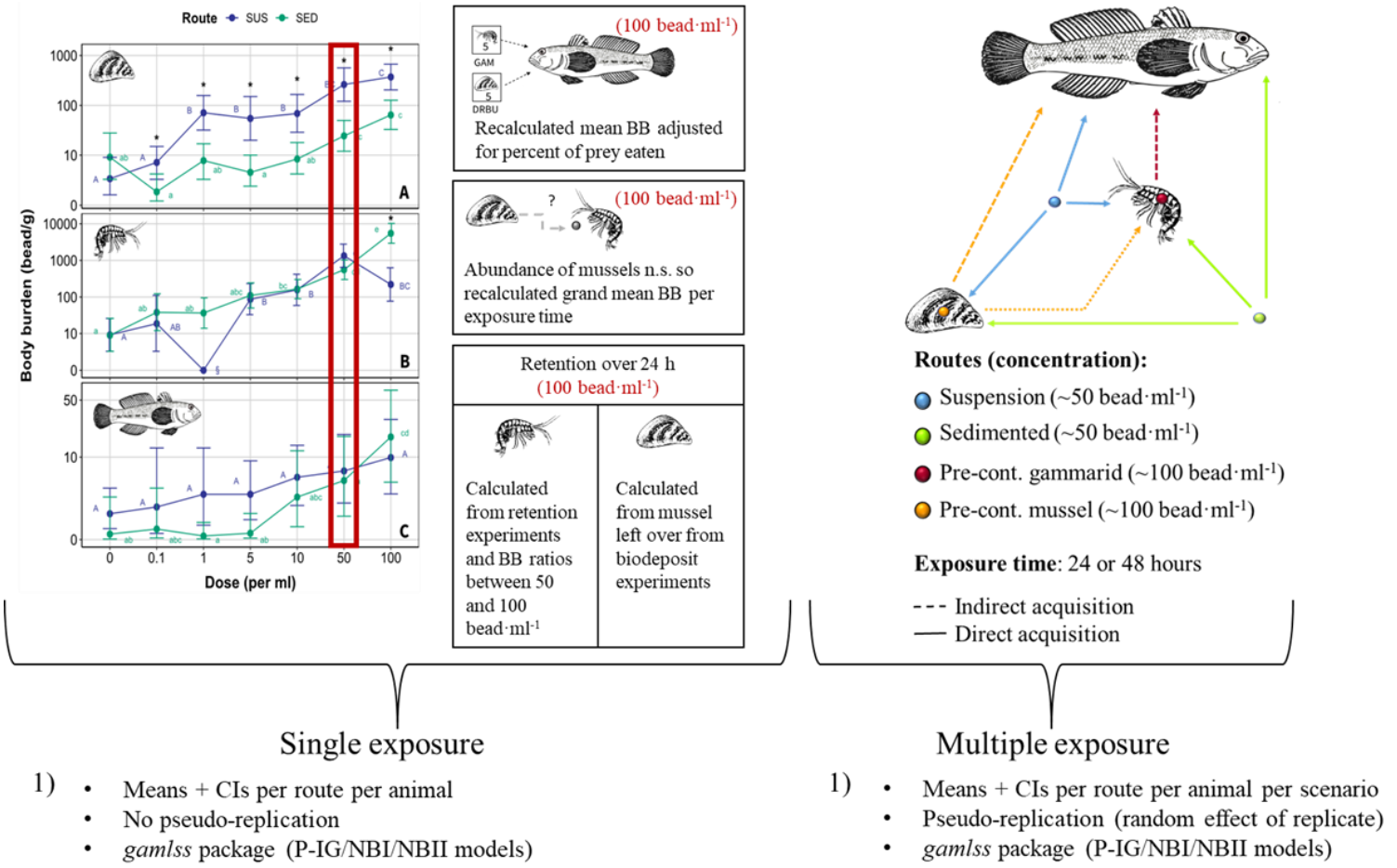

Figure S3.1: Schematic of the background calculations to compute the means and confidence intervals of the metrics. Original number of beads, animal weights and estimated numbers of beads available were taken from the original data from Chapter 2 for the single-exposure model. Multiple-exposure scenarios were based on the results of the current study.

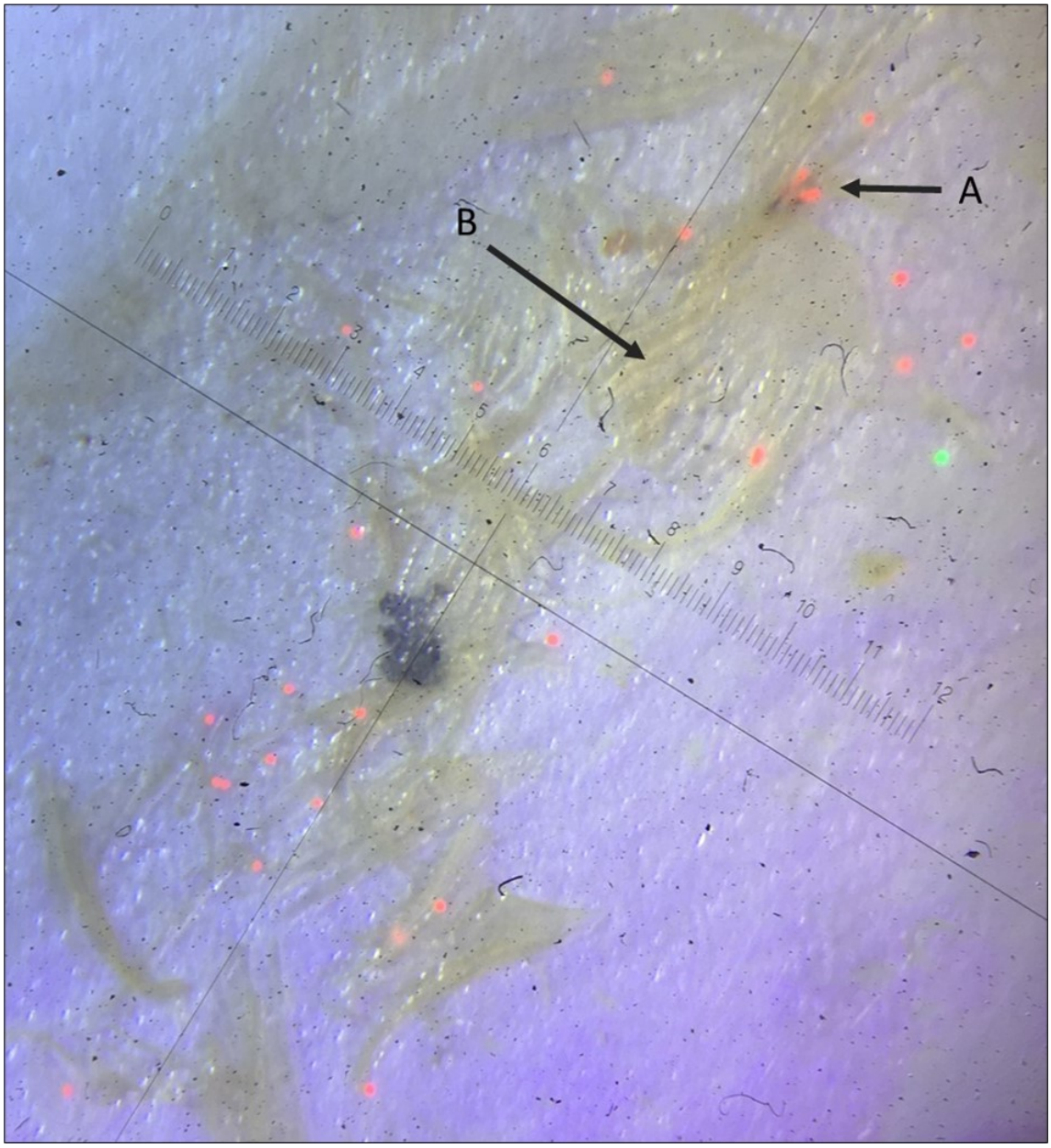

Photo S3.1: Photo of a gammarid body found in the digestive tract of round gobies. Arrow A indicates red beads in the exoskeleton of the gammarid originating from initial contamination, while arrow B shows the broken-down exoskeleton.

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Connecting statement between Chapters 3 and 4

Among the key gaps identified in Chapter 1 is that microplastics are part of a network of environmental stressors that are threatening aquatic ecosystems and, therefore, it is most ecologically relevant to study the effect of this pollution under multiple-stressor scenarios. Using the information gained from Chapters 2 and 3, which show that microplastic contamination can arise from different routes of transfer for the round goby, I am using this fish as a model organism to investigate the individual and combined effects of microplastic pollution under a multiple-stressor scenario. The design of the experiment involves realistic environmental microplastic exposures based on data from environmental and biotic samples reported for the Great Lakes-St. Lawrence River basin. I also used surface water temperature projections for nearshore areas of the basin to explore the effects of two anthropogenic stressors (microplastic exposure and climate warming) on the growth and predatory performance of the round goby.

4. Chapter 4 | Effects of elevated temperature and microplastic exposure on growth and predatory performance of a freshwater fish, the round goby (*Neogobius melanostomus*)

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4.1 Abstract

Freshwater ecosystems are increasingly exposed to co-occurring anthropogenic stressors that can alter food web interactions and organismal life histories. We examined the individual and combined effects of climate warming and microplastic pollution on the growth rate and predatory performance of an invasive freshwater fish, the round goby (*Neogobius melanostomus*). In temperature-controlled chambers, we exposed 160 juvenile gobies to one of six scenarios over 37 days, combining three environmentally relevant microplastic concentrations with two temperature regimes representing contemporary (18°C) and projected mean summer maxima (26°C) in their current range in the Great Lakes–St. Lawrence River basin. Exposure to elevated temperature reduced the growth and predatory performance of round gobies. Their decline in predatory performance was greatest at the highest microplastic concentration, regardless of temperature. The effects of environmentally relevant microplastic

concentrations on the growth and performance of gobies were weaker than the effects of thermal stress. Given that the round goby is an abundant and widely distributed bottom-dwelling fish in nearshore areas of the Great Lakes–St. Lawrence River basin, its responses to these co-occurring stressors could have cascading effects on food webs.

4.2 Introduction

Multiple anthropogenic stressors can interact synergistically, additively, or antagonistically to affect community dynamics and ecosystem functioning (Jackson et al. 2016). The world's largest freshwater ecosystem, the Great Lakes–St. Lawrence River basin (hereafter the "Great Lakes Basin"), faces unprecedented environmental risks from climate change and novel contaminants such as microplastics (Smith et al. 2015; Earn et al. 2021). In recent years, scientists discovered that the water, sediments, and aquatic organisms of the Great Lakes Basin contain some of the highest microplastic concentrations documented in the world (Crew et al. 2020; Munno et al. 2021). In general, large freshwater systems are accumulating microplastics (D'Avignon et al. 2022) and experiencing rapid warming (O'Reilly et al. 2015), making them potentially vulnerable to the additive or synergistic effects of these stressors. Microplastics—plastic particles less than 5 mm—are synthetic pollutants that can sorb toxic substances, including heavy metals, persistent organic pollutants, and pharmaceuticals. These associated contaminants can be released as microplastics travel and interact with physical, chemical or biological processes in aquatic environments, thereby rendering them available to be inhaled or ingested by aquatic organisms (Menéndez-Pedriza and Jaumot 2020). Microplastics that are not excreted can infiltrate various tissues and bioaccumulate within organisms (McIlwraith et al. 2021) or can be further transferred to other consumers through food web interactions (Mateos-Cárdenas et al. 2022). As a result of this cycle of exposure to microplastic pollutants, aquatic

organisms can experience adverse physical and ecotoxicological effects that impair their ability to reproduce, grow, feed, or perform essential ecosystem functions (D'Avignon et al. 2022).

The risk posed by microplastic pollution in the Great Lakes Basin is additionally complicated by the growing stress of global warming. Although summer temperatures modelled for the Great Lakes vary greatly depending on the lake and the depth, nearshore temperatures were recently estimated to be in the range of 12–24°C (Xue et al. 2022; NOAA-GLERL 2022), while Hudon et al. (2010) reported summer temperatures of 15–24°C for the St. Lawrence River around the Montreal area (1992–2002). The mean summer surface water temperature is estimated vary around ~18°C for the current period 2011–2040, and in future scenarios, it is predicted to rise by 4–10°C by 2070 (Trumpickas et al. 2009; 2015), with a mean summer water maximum near ~26°C (Trumpickas et al. 2015; Zhang et al. 2018).

Rising water temperatures increase the residence time and toxicity of sorbed contaminants associated with microplastics (Noyes et al. 2009; Patra et al. 2015). The estimated 4-10 °C warming of the Great Lakes, combined with higher plastic toxicity, could cause aquatic organisms to become less tolerant to currently sublethal levels of pollution (Jaikumar et al. 2018). Higher water temperatures reduce dissolved oxygen, increase thermal stress amongst organisms, and trigger shifts in species distribution limits (Jane et al. 2021). Species with wider thermal tolerance limits, like many invasive species, could experience minimal negative impacts or even improve their performance under climate warming, whereas thermally-sensitive native species could experience range contractions and become locally extirpated if they cannot adapt or migrate to areas within their tolerance limits (Kelley 2014; Patra et al. 2015). Changes in thermal limits provoked by a warming environment could further alter the growth, performance, and metabolic response mechanisms of aquatic species (Mazumder et al. 2015).

While evidence suggests that co-occurring environmental stressors may generate unanticipated ecosystem impacts (Jackson et al. 2016), few studies have explored the combined effects of microplastic pollution and climate change on a freshwater species under realistic stress levels. To address this gap, we examined the effects of exposure to realistic microplastic concentrations and elevated temperature (from 18 to 26°C) on the growth and predatory performance of an invasive bottom-dwelling fish in the Great Lakes Basin, the round goby (*Neogobius melanostomus*).

The round goby is amongst the most successful invaders of temperate aquatic systems, owing to its adaptation to multiple stressors in novel environments including extreme thermal fluctuations (Drouillard et al. 2018) and various levels of pollution (McCallum et al. 2014). Both the round goby and its natural prey, the quagga mussel (*Dreissena bugensis*), are broadly distributed and co-occurring in the Great Lakes Basin (Vanderploeg et al. 2002; Reid and Orlova 2002). The round goby's key roles as a competitor/predator/prey species (Kornis et al. 2012; McCallum et al. 2014), and its propensity to ingest microplastics from various environmental routes (McNeish et al. 2018; Hou et al. 2021) and trophic transfer from mussel prey (unpublished data), make it a valuable model organism for testing the effects of synthetic pollutants under current and future thermal conditions experienced in the Great Lakes Basin. Recent studies investigating the response of aquatic organisms to temperature and microplastic pollution showed that higher exposure to microplastics acted synergistically on the feeding, growth, and fitness of aquatic organisms (Kratina et al. 2019; Lyu et al. 2021). Exposure to rising temperatures increases nearly all rates of biological activity, including metabolism, which can lead to increased consumption (Schulte 2015; Morley et al. 2019). In the case of the round goby, maximal consumption has been observed to be between 23°C and 26°C (Lee and Johnson 2005;

Kornis et al. 2012); therefore, we expected that the exposure of round gobies to elevated temperature (~26°C) predicted by Trumpickas et al. (2009, 2015) would increase their growth rate and their energetic requirements, contributing to a higher predatory performance than at ambient conditions. Alternatively, because the tolerance of aquatic organisms to microplastic pollution and their associated contaminants is reduced under elevated temperatures (Fonte et al. 2016; Jaikumar et al. 2018), an antagonistic interaction might occur for gobies exposed to both high temperatures and high microplastic concentrations. Therefore, we predicted a greater reduction in predatory performance and growth rate in gobies exposed to combined elevated temperature and microplastic concentration. In addition to growth and predatory performance, we also recorded the source and number of particles retained by the gobies post-exposure, with the expectation of a higher body burden under higher microplastic exposure. We tested these predictions in multifactorial experiments under temperatures and microplastic concentrations that reflect ambient and elevated levels.

4.3 Methods

4.3.1 Animal collection, acclimation and care

Round gobies and quagga mussels were collected from Lake St. Louis, a fluvial lake in the upper St. Lawrence River near Montreal (Quebec, Canada), at sites where these species co-occur in high abundance. Quagga mussels were collected from the Soulanges Canal at Pointe-des-Cascades, Quebec (45°19'51.99"N, 73°58'4.11"W), while fish were collected using either beach seine sweeps or minnow traps left overnight near the shoreline at Parc Bourcier (45°19'08.7"N 73°55'38.3"W) and at the Beauharnois Marina (45°18'59.6"N 73°52'34.1"W). To standardize fish size across years for the growth experiment, a subsample of 160 juvenile fish

weighing ~2–3g were selected from specimen collected in September-October 2019 and in June-July 2020.

Immediately after their capture, animals were placed in 70-L coolers filled with ambient water and equipped with portable aerators. Gobies and mussels were transported to 75 L aquaria, filled with filtered and aerated water, and contained within temperature-controlled Conviron® growth chambers at ~19°C under a 12hL/12hD light cycle for a 2-week acclimation period. While being acclimated, animal health was monitored daily. Round gobies were fed every second day until satiation with ~1.5 mm protein/carbohydrate sinking granules (Fluval Bug Bites), while mussels were fed a mixture of live algae. Water quality parameters were monitored every second day and deposited wastes were removed weekly.

To identify individual fish, each goby was sedated and marked by injecting a colour-coded fluorescent elastomer dye (Visible Implant Elastomer VIE tag, Northwest Marine Technology) between the skin and flesh of the fish (Photo S4.1). After recovery from anesthesia, five fish were placed together per 20-L aquarium for the 37-day exposure trial, because gobies displayed healthier behaviour when held in groups during captivity (G.D., *pers. observation*). Plastic tubes (~4 cm diameter) were added to the aquaria, so each fish had a shelter during the experiment. Fish were fed three times a week with Enterra sinking pellets. Aquarium temperature and water quality parameters were monitored weekly (Table S4.1), followed by waste removal through a 30% water change. After each cleaning event, dechlorinated water was added to compensate for removed water. Seachem Stability bacterial blend was added to maintain water quality. Glass covers were placed on each aquarium to reduce the risk of microplastic contamination between treatment groups and to prevent fish from escaping. See Suppl. Mat. 4.6.1 for detailed fish care procedures.

Quagga mussels were selected to offer a trophic route of microplastic contamination to round gobies because they are a dominant prey item in goby diets (Raby et al. 2010) and can be artificially contaminated with microbeads. After collection, mussels were brought to the lab, where they were opened manually to expose their tissues. Tissues were left connected to one valve and artificially contaminated by using a hypodermic syringe to inject a fixed concentration of 10 or 50 orange microbeads into the soft tissues for the low and high treatments, respectively. Each contaminated mussel was deposited into individual wells of an ice-cube tray and placed in the freezer until needed.

4.3.2 Experimental design

To examine the effects of co-occurring stressors on fish growth and predatory behaviour, we first ran a pilot experiment in 2019 using 80 gobies randomly divided into one of four treatments: no (control) or low microplastics exposure crossed with two temperature scenarios ~18°C or ~26°C (Figure 4.1). Preliminary results indicated that the low microplastic treatments slightly decreased growth while samples sizes for the follow-up predatory experiments were too low to validate a trend. In 2020, we captured new gobies from the same field sites to increase sample size (n=40) and these fish were tested across four treatments (2 replicates of each treatment tested, Figure 4.11). The same year, we captured 40 additional gobies to examine the effect of a higher microplastic exposure treatment. Across all years, we had six replicates for the no (control) and low microplastic contamination treatments and four replicates for the high treatment (the latter more modest due to space limitations in 2020 brought on by SARS-COV-2 outbreak; Figure 4.1).

Our experiments were designed to represent realistic environmental conditions of the Great Lakes Basin in terms of nearshore summer temperatures and microplastic concentrations in

the field. The exposure scenarios were chosen based on temperature regimes (mean summer maxima) experienced currently (18°C) and projected for the near future (~26°C; Trumpickas et al. 2009, 2015; Zhang et al. 2018). Microplastic exposure treatments concentrations were selected based on observed concentrations from the St. Lawrence River and Great Lakes (Crew et al. 2020; see Supplementary Information). The 'low' exposure treatment is based on mean sediment concentrations recently reported from the St. Lawrence River (~800 microplastics·kg^{-1} dry mass), whereas the 'high' exposure treatment is the highest local concentration measured in the river's sediments (~4500 microplastic·kg^{-1} dry mass) (Crew et al. 2020). These concentrations also represent realistic contamination levels recorded in other freshwater environments: a mean of ~2000 microplastic·kg^{-1} dry mass was estimated from benthic samples from 56 rivers in various watersheds, whereas the mean microplastic concentrations observed in the surface of freshwater environments worldwide is ~1 microplastic·L^{-1} (D'Avignon et al. 2022), which also represented the maximum concentrations reported in St. Lawrence surface waters (Crew et al. 2020; Supplemental information). To maintain realistic exposure conditions, 1 bead·L^{-1} per day were added to both the low and high scenarios for the 37-day of the experiments. In addition, for contrast, a 'zero' level was used to represent a pristine environment (i.e., zero microbeads added but some present, possibly due to prior contamination).

The concentration of microplastics chosen to be injected into each mussel was informed by microplastic counts from quagga mussels collected from the St. Lawrence River, which ingested 2–11 microplastic pieces per individual (Table S4.2). Given that round gobies consume 30–40 dreissenid mussels daily (Naddafi and Rudstam 2014), they would be exposed to at least 18–24 microplastic particles daily from trophic transfer, assuming quagga mussel body burden is similar to that reported in the Great Lakes (Hoellein et al. 2021). Because our high benthic

contamination treatment was 5-times that of the low treatment, we assumed the increase in contamination in mussels would be of the same magnitude.

To prepare microplastic treatments, three colours of Cospheric fluorescent polyethylene microbeads of 63–75 µm diameter were added to three matrices representing potential natural exposure pathways: green beads to the sediment (UVPMS-BG-1.025g/cc), blue beads to the water column (UVMS-BB-1.13g/cc), and orange beads injected into quagga mussels (UVMS-BO-1.00g/cc). Microbead concentrations for each route of exposure were determined by calculating the mass of beads required to obtain the desired number of beads based on the estimated concentration of 5.81×10^6 beads·g^{-1} provided by the manufacturer. To adjust the microplastic concentration in sediments, the mass of beads was weighed; whereas, for the preparation of beads in suspension and beads to be injected in mussels, a fixed mass of beads was diluted in water to produce bulk solutions of known concentrations. Note that for 2019 trials, our suspension solution was contaminated with orange beads that were also used to contaminate the mussels, so the distinction between these two routes could not be identified in 2019 fish.

To prepare the exposure scenarios, sediments were prepared first by mixing either ~1440 or ~8100 green microbeads with 1.8 kg of Super Naturals aquarium gravel substrate (~1cm × 1cm) to achieve concentrations of 800 beads·kg^{-1} dry mass and 4500 beads·kg^{-1} dry mass for the low and high microplastic treatments, respectively. These microplastic-contaminated gravel mixtures were placed on the bottom of 20-L aquaria then, we added 15 L of chemically dechlorinated tap water .. Each aquarium was fitted with an AquaClear 30 Power Filter to allow water circulation, but filter media was removed to prevent microbead aggregation in the external filter. The AquaClear 55g activated carbon insert and five ceramic bio rings were placed in each aquarium to maintain water quality. The tanks were then left to stand for 48 h. After fish were

introduced into the experimental aquaria, submersible heaters were added to those aquaria selected for the higher temperature treatments, and gradually adjusted over 48h to acclimate fish to ~26°C. Temperatures in each aquarium were recorded weekly, resulting in means and standard deviations of 18.8°C ± 0.8 and ~25°C ± 0.8 for the 5-week duration.

To create suspended microbeads, we weighed blue beads and added them to a small vial where they were treated with a surfactant (Tween80, prepared according to the manufacturer's guidelines). After the surfactant was applied, 1ml of algal solution and 5ml of dechlorinated water was added to the vial, shook for 30 seconds, and left to interact for 48 hours to create a biofilm. This solution was diluted with dechlorinated water to create a bulk solution of 15 blue microbead·mL^{-1}.

Throughout the 37-day exposure trials, a daily concentration of 1 bead·ml^{-1} was added in suspension. In addition, either one uncontaminated mussel (control), one pre-contaminated mussel with 10 orange microbeads (low-microplastic treatment), or one pre-contaminated mussel with 50 orange microbeads (high microplastic treatment) was offered to each fish weekly. Our analyses were based on trials conducted in 2019 and 2020 (Figure 4.1). Because suspended beads and microbead contaminated mussels were added throughout the experiment, the number of beads from these routes increased with exposure time. We estimated the maximum number of beads across all routes of exposure in each aquarium after 37 days to be 2245 for the low microplastic treatment and 9905 beads for the high treatment. The removal of beads during weekly aquarium maintenance was measured by filtering the water over a 30 µm mesh, double rinsing the mesh over a vacuum filtration apparatus (1µm filter) and counting all beads under a 20× stereoscope. Upon completion of the experiments, all the water from each aquarium was filtered using this method (see Suppl. Mat. 4.6.3 for more details). Controls were slightly

contaminated, so we provided estimated concentrations at the end of our exposure period per aquarium in Table S4.3.

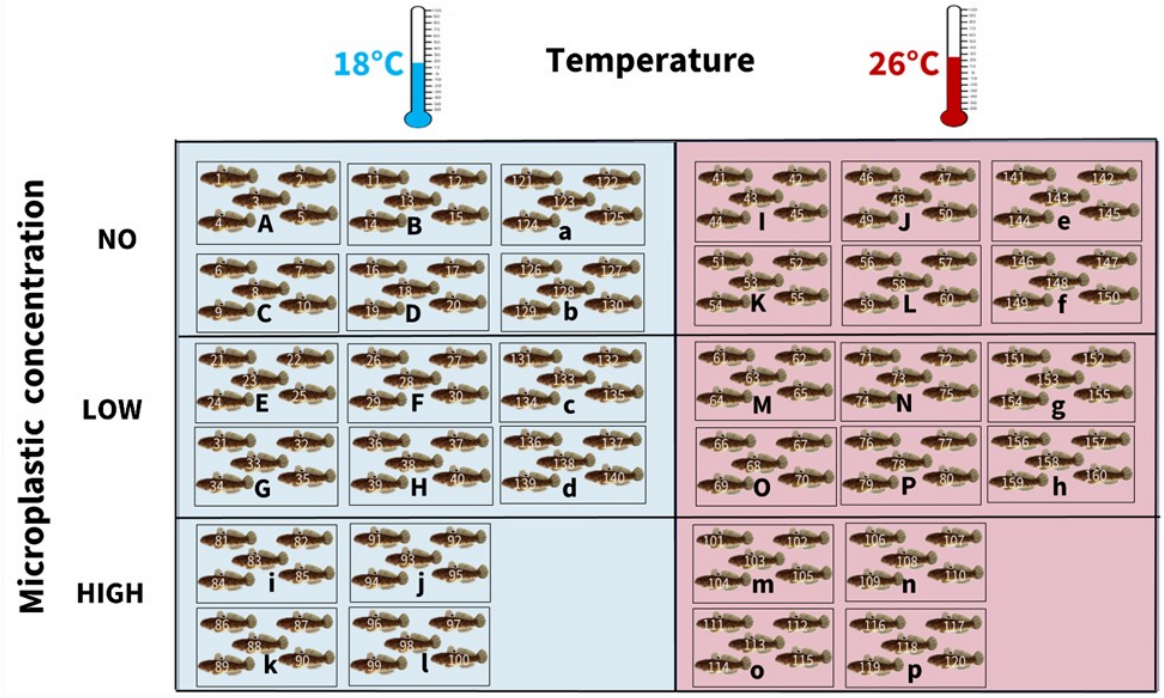

Figure 4.1: Experimental design examining the effects of microplastic exposure under two temperature regimes: "18°C" (18.8 ± 0.8 °C) and "26°C" (25 ± 0.8 °C). Aquarium are represented by the rectangles, where capital letters represent aquaria holding fish collected in 2019 and small letters represent 2020 fish. Fish numbers represent different individuals (identified with a VIE elastomer colour code).

4.3.3 Physiological and behavioural responses

Specific Growth Rate (SGR) was chosen as a physiological response because it is a reliable metric to compare the change in growth of small fish exposed to different stressors (e.g., food regime, temperature, water chemistry) over a short period of time (Hopkins 1992; Crane et al. 2020). The length and weight of each goby was measured before the onset of the experiment

and after the 37-day exposure. To ensure the validity of our assessments, we also modelled the change in mass as a linear function over 37 days and quantified the relative change in mass. All models depicted similar trends as the SGR, so this metric was maintained and used as a proxy of the net energy intake of each fish over the duration of the experiment, which was calculated as follows (Crane et al. 2020):

$$SGR = 100\left[\left(\left(\frac{W_{t_F}}{W_{t_I}}\right)^{\frac{1}{\Delta t}}\right) - 1\right] \quad (1)$$

where SGR = the daily percent change in mass (grams), W = the mass of the fish in grams at the onset of the experiment (t_I) and at the end of the experiment (t_F), Δt = the duration of the experiment in days. The total initial length from the tip of the snout to the end of the caudal fin was measured in mm (range 35–75mm) and the fish initial wet mass in grams was recorded (W_{t_I}1.07–5.35g). Initial measures were recorded during elastomer marking procedure before the onset of the exposure trials, and final measures were recorded after the 37-day exposure.

As a behavioural response, we used the predatory performance of gobies by adapting the method of de Sá et al. (2015). Maximum feeding rate was determined in a pilot study by presenting a subset of individuals with various densities of blood worms (*Chironomus* larvae), each density being offered for a period of two hours. Numbers of worms consumed in each trial were used to construct a functional response curve relating prey consumption to prey supplied, the asymptote of which indicated a maximum feeding rate of ~70 worms (~400 mg). In 2019, 140 chironomids (~800 mg) were offered to ensure maximum feeding rate, but this number was reduced to 80 worms (~460 mg in 2020) after feeding trials indicated that it exceeded the maximum feeding rates observed among the gobies. Ultimately, each fish was offered blood

worms in excess of the maximum feeding rate and was allowed to feed for 2 h; thus, predatory performance was measured as the total number of prey consumed in the 2-h period. The predatory performance was assessed on ten randomly selected gobies per treatment in 2019 and 2020 for the no and low microplastic exposures, while 20 gobies per temperature were examined for the high microplastic treatment (2020), for a total of 120 individuals.

Predatory performance trials were conducted in new aquaria without microplastics. All predatory performance aquaria were prepared a minimum of 48 h before the end of the 37-day growth trial period. To achieve mean water temperatures of 18.5°C and 25.5°C ± 0.5°C, sixteen 9.5 L aquaria held in temperature-controlled chambers set to either 19°C or 26°C were filled with 7 L of tap water that had been chemically dechlorinated and left to rest for 24 h. The next day, 1 mL of Seachem Stability bacterial solution was added to the water with one biobead to host the bacterial colonies. An aerator was installed, and a PVC shelter tube was placed in each aquarium. On day 37 of the growth trial, the water quality and temperature of each aquarium was verified. Fish were first removed from their respective exposure trials, rinsed in clean dechlorinated water, measured, and weighed, and finally individually placed in one of the 9.5 L microbead-free aquaria either in the 18°C or 26°C chambers according to the temperature of exposure during the growth trial. Gobies were acclimated to this environment for 24 h and were not fed during this period. Following this acclimation period, a fixed number of thawed chironomid larvae (*Chironomus* sp., previously kept frozen) was introduced to each aquarium, and the fish were left for two hours to feed. The predatory performance trials were conducted for two hours, and at the end of this time, the gobies and the worms were removed. Remaining chironomids were collected and counted, while gobies were triple rinsed and immersed in a 10% eugenol solution

for euthanasia. Each fish was measured, weighed, and carried in a sealed sterilized container to the lab, where they were dissected under a laminar flow hood to examine microplastic retention.

4.3.4 Quantification of microplastic acquisition

To investigate the potential for round gobies to acquire and retain microbeads under long-term exposure to relevant microplastic concentrations, we counted the number of beads found both in the fish gastrointestinal (GI) tract and in the carcass of the fish (body and gills together but without organs). Bead colour was recorded in 2020 to note the origin of the beads (this was not possible in 2019, due to contamination). When organisms are exposed to microplastics, they can take up particles via respiration or ingestion for different periods of time prior to egestion (expulsion of faeces). Retention duration within the body can vary depending on both microplastic characteristics (e.g., size, shape, polymer), and if particles are translocated to other organs.

Euthanized fish were measured, weighed, and then dissected to remove the GI tract from the esophagus to the anus. Fish were classified as male, or female based on the shape of the anal pore and their gonads. The liver often liquified and mixed with other organs within the body cavity when the fish were thawed. Because no beads were observed in the liver, heart, or gonads during exposure studies (G.D., *per. obs.*), only the GI tract and fish carcass were weighed, and then placed in a clean jar for further processing. Each tissue sample were placed in a 10% KOH solution and left to digest for 48 h in an oven at 40°C. The GI tract and the goby carcass were subsequently passed over a 125 µm sieve to separate any incompletely digested skeletal remains and to remove the visible implant elastomer identification tag from the digested materials. The filtrate was then rinsed with reverse osmosis water and passed through a vacuum filtration apparatus over a 47 mm A/E PALL glass filter of 1 µm. The filters were inspected under a 20×

stereoscope mounted with a UV fluorescent light to count and identify the different fluorescent microplastic beads that remained in the fish tissues.

4.3.5 Quality control and assurance

All equipment used in the study was triple rinsed with reverse-osmosis water (0.1µm filter) and visually inspected under a UV light for the presence of fluorescent beads before use. Glass jars and tools for the dissection and digestion procedures were also washed, triple rinsed, and placed in a 230°C oven for 3 h, before being used. All dissections, digestions, filtrations, and prey counting procedures were performed under a laminar flow hood in an isolated room. Each researcher wore a cotton lab coat during these procedures. To avoid cross contamination, control samples were processed first with their own labelled and decontaminated equipment, followed by samples from the low and then high microplastic exposure treatments. After exposure trials, fish were removed from their aquaria and carried individually in their own containers. A different pre-labelled hand net was used to handle fish from each treatment. These nets were triple rinsed and visually inspected under a UV light before and after each use, and then stored in a clean Ziploc bag.

In previous experiments, we found that fish could take up beads through their gills or ingest them during the euthanasia procedures if they were exposed to contaminated water. To prevent this problem, fish were euthanized individually in 2 L plastic containers using a new eugenol solution for each fish. To ensure accurate measurements of microbead exposure, fish were triple rinsed before being placed in individual containers. To mitigate microplastic contamination between groups, all samples were processed under a laminar flow hood, and procedural blanks were conducted between the filtration of GI and carcasses, as well as between fish from different exposure groups. Samples were processed in the following order: controls

(gobies exposed to no microbeads), fish from the low microplastic treatment, and lastly fish from the high microplastic treatment.

4.3.6 Data analyses

To account for differences in the initial physical characteristics and growth patterns of gobies that were captured using different sampling techniques over two years at two sites, we compared their weight-length relationship to 140 other round gobies captured by co-authors using similar field techniques in Lake St. Louis between 2018 and 2020. Using the FSA package in R (Ogle et al. 2022), we applied a linear model to compute the parameters α and β of the weight-length relationship, while controlling for the sex of the fish (Supplementary Information). The results showed that fish exhibited allometric growth with a uniform condition across their length range (Table S4.4) (Froese 2006). However, fish collected from both years could be grouped into three clusters based on condition indices (see below) that indicated body shapes and thus different age cohorts. Fish belonging to these clusters could have different growth rates. Our analyses also revealed differences in growth patterns between males and females, even though all fish had immature gonads. These analyses informed us that there could variation among fish caused by their condition, the year sampled, or their sex. Each of these parameters was therefore used in our analytical models. The initial weight-length relationships of the fish before the onset of the experiment were binned into three different age cohorts which corresponded to Fulton's condition factor, K_I:

$$K_I = 100\left(\frac{W_I}{{L_I}^3}\right) \quad (2)$$

where, W_I= is the initial mass of the fish in grams and L_I= in the initial total length of the fish in cm (Ricker 1975). In the analyses, Fulton condition was used as a covariate

within the model to control for differences in initial body shape and growth patterns of gobies within the fish sampled.

Table 4.1: Estimated conditional or marginal means resulting from the best fitted model predicting SGR, predatory performance, and microplastic acquisition (beads·individual^{-1}) in round gobies with their standard error and their confidence intervals (CI).

	Specific Growth Rate (%)		**Predatory Performance**		**Microbead acquisition**	
Treatment	*Means*	*95% CI*	*Means*	*95% CI*	*Means*	*95% CI*
No MP 18°C	0.48	0.35 - 0.61	30.20	24.72 - 35.60	0.40	0.15 - 1.06
Low MP 18°C	0.33	0.20 - 0.45	30.70	25.29 - 36.10	0.93	0.40 - 2.20
High MP 18°C	0.36	0.21 - 0.52	15.40	9.23 - 21.60	0.92	0.36 - 2.37
No MP 26°C	0.30	0.17 - 0.44	16.60	10.75 - 22.50	0.14	0.04 - 0.57
Low MP 26°C	0.15	0.02 - 0.28	16.40	10.65 - 22.10	0.10	0.02 - 0.55
High MP 26°C	0.19	0.04 - 0.34	11.20	4.28 - 18.10	1.31	0.51 - 3.36

Data exploration and analyses were performed using R version 4.0.3 (R Development Core Team, 2022); for additional information on analyses and results see Supplementary Information. All fish were used in the SGR and microplastic retention datasets (n=160), but only 120 fish were selected for predatory performance trials. Five fish died or were unhealthy at the onset of the feeding experiment and were removed from the analyses (n=115). For each analysis, a full theoretical model was initially built and included year sampled, sex, fish condition as fixed effects, while tank number was applied as a nested factor (random effect). Least-significant terms were removed one at a time until the best-fit model was obtained. Our selection of the best model was guided by choosing the lowest values of Akaike and Bayesian Information Criteria while meeting model assumptions and maximize the R-square of the model. Diagnostic plots were visually assessed for normality and homogeneity of residuals. The influence of outliers was assessed using Cook's distance; data points were kept if their presence did not reduce model fit, cause violations of assumptions, or yield different outcomes (Suppl. Mat. 4.6.6). Means, standard

error and confidence intervals were computed using the *emmeans* package followed by pairwise comparisons or multiple comparison with Sidak-adjustments using an alpha of 0.05 and adjusting for uneven sampling (Table 4.1).

4.4 Results

4.4.1 Specific growth rate

The percent daily change in mass (g) of juvenile gobies was significantly reduced by warming ($F_{(1,29)}$ =14.1, p<0.001) and by the exposure to environmentally-relevant concentrations of microplastic ($F_{(2,25)}$ =4.40, p=0.023). The sex status of the fish significantly impacted the SGR ($F_{(2,140)}$ = 4.50, p=0.013) but the condition of the fish ($F_{(1,135)}$ =2.82, p=0.095) and date of experiments ($F_{(1,71)}$ =0.23, p=0.633) were not important. We detected a decline of 0.18 in percent daily mass between the baseline and warming scenarios not exposed to microplastic and 0.29 to 0.33 reductions under both warming and microplastic exposures (Table 4.1). A significant effect of temperature (p=0.012) with a large effect size (d=0.92; see Supplementary Table S4.8) was observed at each microplastic exposure concentration. Although the addition of microplastic decreases SGR at each temperature (Figure 4.2), the reduction in SGR was marginally significant (p=0.107) with a medium to large effect size (d=0.61-0.81) between the no and low microplastic treatments at both temperatures. The largest effect size (d > 1) and most significant difference among treatments was between gobies in pristine conditions (no microplastic) at 18°C and gobies held at 26°C under low (p =0.0012) or high microplastic treatment (p =0.014; Figure 4.2; Table S4.8). The fixed effects (temperature, microplastic, condition and sex of the fish) explained 32.9% of the variance, while an additional third (12.8%) was caused by variances between aquarium (Table S4.7). Given that ~54% of the variance was not explained by our model, these

results are to be treated cautiously and other factors beyond those examined and recorded in our study (e.g., history of thermal exposure of each fish; individual behaviour) could be important in regulating SGR under these stressful conditions.

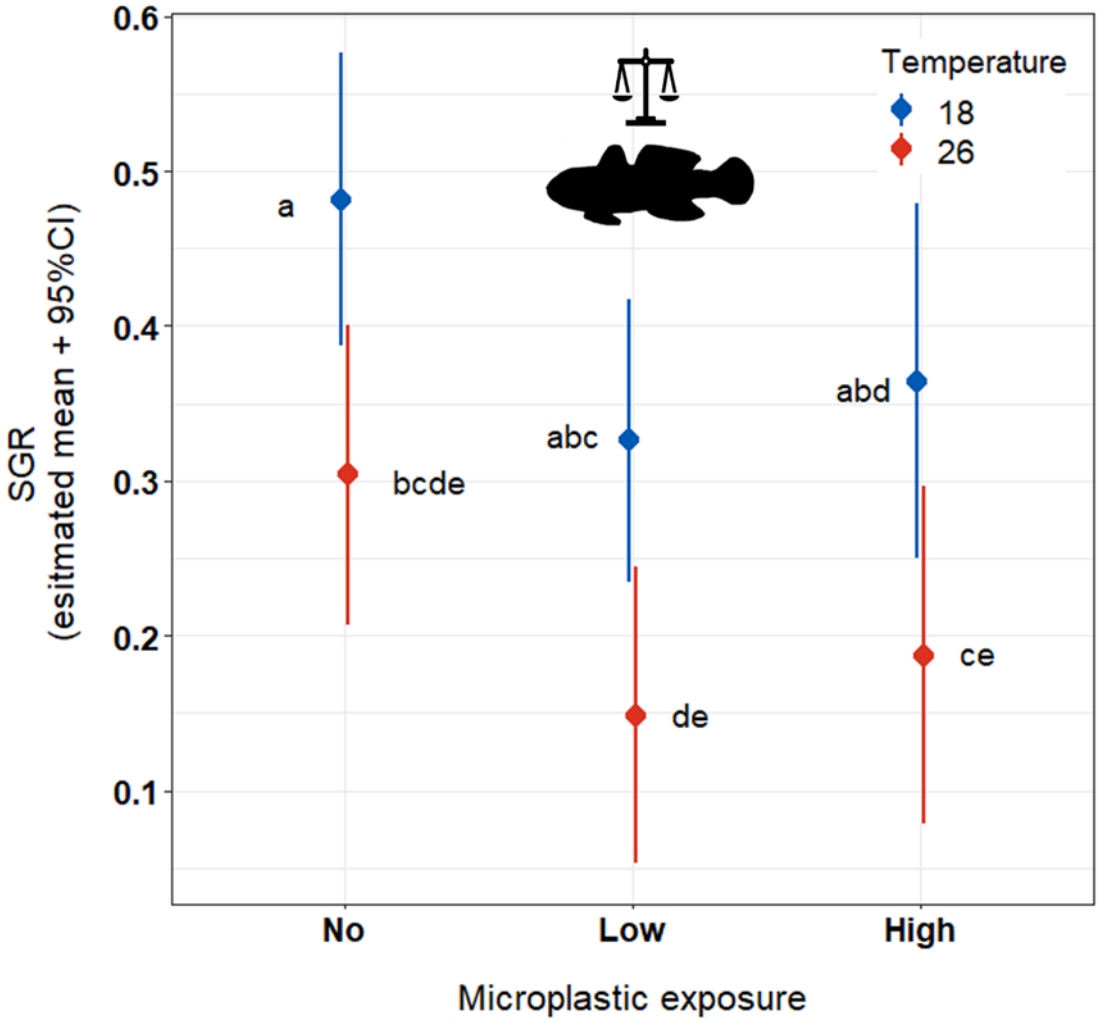

Figure 4.2: Specific growth rate (SGR, based on mass) linear predictions based on marginal means and 95% confidence intervals of the effect of temperature on the SGR under each microplastic exposure scenario. Different letters indicate significant differences in mean SGR among treatments (p <0.05), based on Sidak-adjusted multiple comparisons.

4.4.1 Post-exposure predatory performance

The best regression model explaining predatory performance of gobies (R^2= 0.58, W= 0.98 p >0.05) indicated significant main effects of temperature ($F_{T\ (1)} = 21.7$, $p < 0.001$) and microplastic exposure ($F_{T\ (2)} = 15.6$, $p < 0.001$), as well as an interaction between these treatments ($F_{T\ (2)} = 3.38$, $p = 0.038$), while controlling for the sex and date of sampling (Supplementary Table S4.9). Prey consumption by round gobies was significantly reduced under elevated temperatures of 26°C (Figure 4.3), and was most pronounced when compared with the baseline and low contamination scenarios at a temperature of 18°C.

In comparison with baseline conditions (no microplastic exposure at 18°C), the mean number of prey items consumed by juvenile gobies declined by 45% under a warming climatic scenario, whereas the number declined by 63% when gobies were exposed to the high microplastic exposure (Table 4.1). However, when gobies were exposed to both stressors, the effect of temperature on feeding rates was decoupled at the high microplastic exposure (Figure 4.3). Under optimum temperatures, the number of prey items consumed within two hours declined by ~45% when exposed to high microplastic exposure, whereas at the elevated temperature the effect of high microplastic exposure accounted for a 32% decline (Table 4.1).

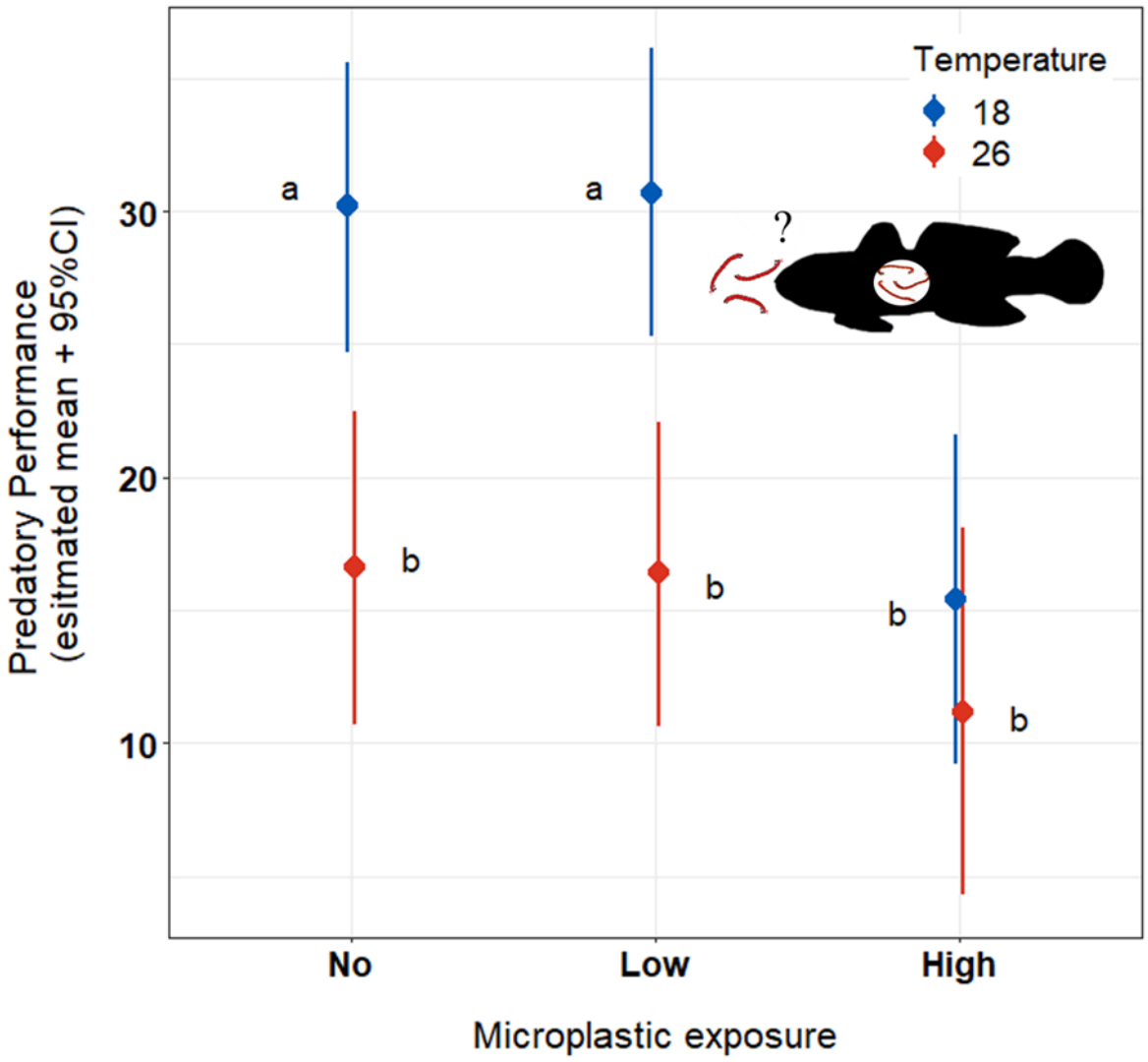

Figure 4.3: Predicted feeding rate of juvenile gobies (after 2 h) based on marginal means and 95% confidence intervals of the effect of temperature on predatory performance under each microplastic exposure scenario. Different letters indicate significant differences in mean predatory performance among treatments (p <0.05), based on weighted Sidak-adjusted multiple comparisons averaged over sex and date.

4.4.2 Microplastic retention in gobies

Total microplastic count per gobies including the sum of the beads from sediments, beads from water, and beads from a mussel) were used for analyses (see Supplementary Table S4.11

for details per route of contamination). Gobies exposed to microbeads during the 37-day growth trials, retained beads in their bodies even after experiencing a 26-hour depuration period which included the 24-hour acclimation and 2-hour predatory performance trial. The year of trials ($\chi^2_{1,160}$ =16.44, p<0.001) affected the abundance of microplastics in gobies, and there was a significant interaction between temperature and microplastic exposure level ($\chi^2_{1,160}$ =12.53, p=0.002) when data was pooled across years (Supplementary Table S4.13). The retention predicted by the model's coefficient further identified the highest microplastic exposure to be significantly different from treatments without microbeads (p=0.0035) or under low microplastic exposure (p=0.052).

Table 4.2: Negative binomial model summaries for the microplastic acquisition for the 2019 and 2020 gobies. Significant p-values are bolded. SE = standard error.

2019 Model	**Coef.**	**SE**	***Z-value***	***p*-value**
Baseline	-1.39	0.69	-2.013	**0.04**
Temp. (26°C)	-1.00	0.63	-0.003	0.99
Low MP	0.59	0.47	0.634	0.53
Temp * Low MP	-0.59	0.87	0.000	0.99
2020 Model				
Baseline	-0.36	0.47	-0.76	0.44
Temp. (26°C)	-0.34	0.70	-0.48	0.63
Low MP	0.54	0.61	0.88	0.38
High MP	1.19	0.53	2.26	**0.02**
Temp (26°C) * Low MP	-0.54	0.96	-0.56	0.58
Temp(26°C) * High MP	0.67	0.78	0.86	0.39

We noted two differences in methods between 2019 and 2020. First, orange beads were found in the 2019 suspension solution so the route could not be distinguished from the beads injected in the mussels; secondly, the high exposure treatment was tested only in 2020. To mitigate these differences in our analyses, we fitted negative binomial regression models to the total microplastic counts in gobies from each year separately. Neither temperature nor exposure

to microplastic affected the mean abundance of microplastics in 2019 gobies (Table 4.2; $p > 0.5$). However, gobies examined in 2020 showed a significantly higher microplastic body burden after being exposed to the highest microplastic treatment under both temperatures (Table 4.2; $Z=2.26$, $p=0.02$).

When pooling fish and controlling for the difference across years in our model, a significantly higher mean microbead load per individual goby was observed at high microplastic concentration for fish under the warmer scenario (Figure 4.4A). Ninety percent of gobies had at least one bead in their body after our chronic exposure to the high-exposure treatment (Figure 4.4A; Table 4.1). Gobies exposed to microplastic treatments at 18°C retained more beads than they did under the control, but this difference was not significant ($p=0.75$; Figure 4.4A).

To examine the origin of the beads (e.g., suspension, sediment, mussel) and where they were found in the bodies of the gobies, we used 2020 fish only (Figure 4.4B). Regardless of temperature, the fish retained more beads when exposed to higher environmental (sedimented or suspended beads) and prey (via mussels) microbead contamination levels (Figure 4.4B). The number of beads detected in the digestive tracts (GI) of the juvenile gobies in 2020 was relatively small (Figure 4.4B). Most microbeads found in the bodies of gobies originated from prey or from sediments across treatments (Figure 4.4B), whereas gobies exposed to the highest microbead concentrations retained most microbeads from the water column.

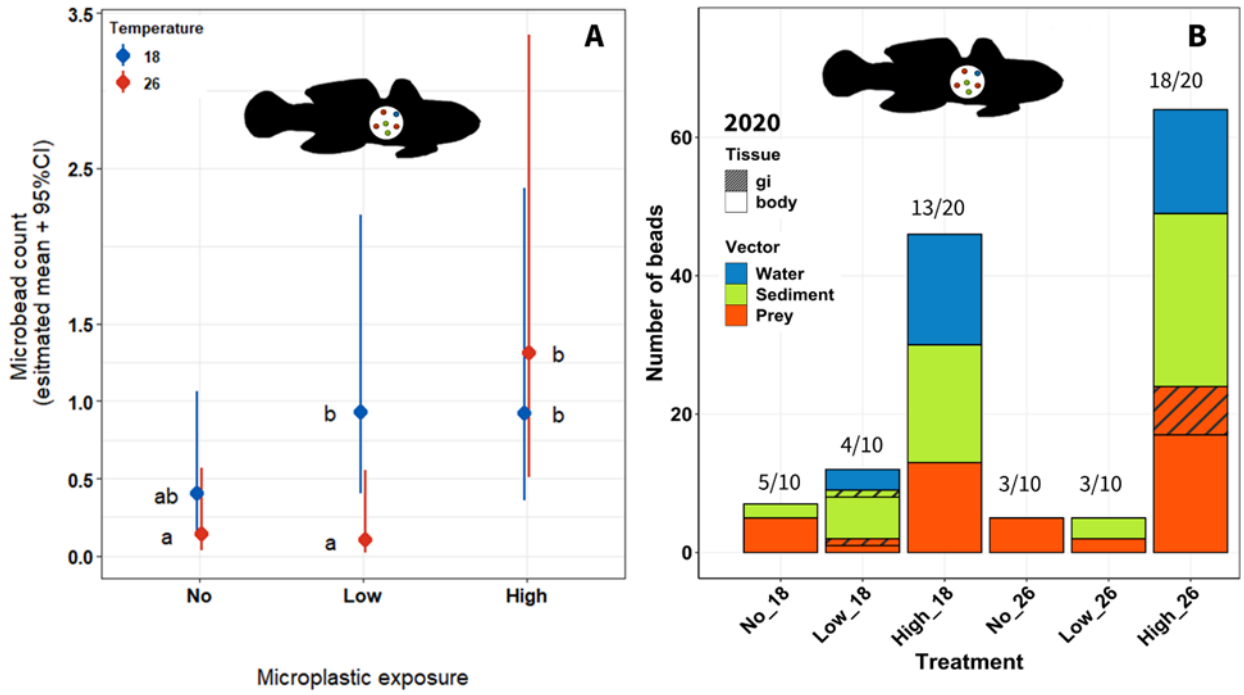

Figure 4.4: (A) Regression model estimated marginal mean microplastic count per fish resulting from each temperature and microplastic treatment (values are indicated in Table 4.1). Different letters indicate significant differences in mean microbead count between treatments (p <0.05) based on sidak-adjusted multiple comparisons. Panel A combines pooled data across 2019 and 2020. (B) Cumulative number of beads observed in the tissues of 2020 juvenile gobies per treatment based on the route of contamination. Fractions above the bar plots are the number of individuals contaminated with microbeads (numerator) and sample size of fish assessed (denominator).

4.5 Discussion

Given current trends in microplastic pollution and climate warming, research on these stressors has been identified as a critical priority by the United Nations (UNEP 2016). Research relevant for environmental and regulatory purposes must consider complex ecological relationships that govern the potential fate and impacts of microplastics. Previous studies reporting microplastics in fish have primarily focused on field-caught specimens (McNeish et al.

2018; Hou et al. 2021), short experimental exposure periods (Foley et al. 2018), and elevated microplastic concentrations beyond environmentally observed levels (Cunningham and Sigwart 2019). Our study measured the retention of microbeads after long-term exposure (37 days) from a variety of natural routes of uptake and under realistic exposure concentrations (estimated concentrations per aquarium are presented in Table S4.3).

The effects of prolonged exposure to microplastics and elevated temperatures on juvenile gobies largely contradicted our initial hypotheses. Elevated temperatures reduced, rather than increased, SGR and predatory performance. However, microplastic exposure lowers tolerance of species to stress (Fonte et al. 2016; Jaikumar et al. 2018). Our study demonstrated that microplastic exposure can produce a synergistic response with warmer temperature that significantly reduces SGR in comparison with baseline conditions (18°C and no microplastic). Furthermore, exposure to realistic microplastic concentrations induced mixed effects on predatory performance that depended on microplastic concentration and temperature. At a temperature of 18°C, round gobies had a reduced feeding rate when exposed to high microplastic concentration, whereas at 26°C the additional stress caused by microplastic exposure was less perceptible than the reduction in prey consumption under elevated temperature. At the end of all experiments, gobies exposed to the high microplastic treatment—which was equivalent to the maximum concentration observed in the St. Lawrence River (Crew et al., 2020)—retained more beads in their body (1–1.5·beads individual^{-1}) than under lower exposure treatments (0.15–0.75·beads·individual^{-1}). The observed contamination load in fish in our laboratory experiments was similar to that recorded in round gobies collected along the shorelines of southern Lake Michigan (Great Lakes Basin) between 1990 and 2018, which had 0.6–2.06 microplastic·individual^{-1} (Hou et al., 2021).

4.5.1 Warming reduces growth and feeding rates of round gobies

Optimal temperatures for fitness and performance depend on an organism's development stage and the environmental conditions to which it is adapted (Mazumder et al. 2015). The estimated thermal limit for round goby growth, ~28.9°C (Kornis et al., 2012), is close to the limit beyond which feeding ceases (~30°C). Peak adult consumption rates are estimated to occur at 23–26°C, followed by an exponential decrease beyond a thermal threshold of 26°C (Lee and Johnson 2005). Based on these estimates and the reported thermal tolerance range of the round goby (-1°C to 30°C; Ng and Gray 2011), we expected the growth rate and feeding performance to be enhanced at ~26°C. However, elevated temperatures reduced both growth and feeding of gobies compared to a baseline of ~18°C, suggesting that 26°C is not an optimal performance temperature for juvenile gobies from the Lake St. Louis (St. Lawrence River) population, and in fact imposes considerable thermal stress. The relatively small size and immature state of fish used in our study (2.58 ± 0.73 g; Table S4.5) were much less variable than those used by Lee and Johnson (2005), whose fish ranged from 2–86 g and comprised both juveniles and adults, though most of their fish were adults. Early life stages often exhibit limited thermal tolerance ranges and lower lethal limits compared to adults within the same species (Pörtner et al. 2006); given that our study focused exclusively on small juveniles, we were able to discern patterns that are potentially specific to this life stage. Furthermore, Lee and Johnson (2005) used fish from Lake Erie and Lake St. Clair, which are located approximately 3° south from our sampling location in the St. Lawrence River. A previous study testing the critical thermal maximum of adult round gobies from the St Lawrence River populations, including our Lake St. Louis population, found that these fish had significantly lower thermal tolerances than goby populations from the lower Great Lakes, and found that feeding rates of Lake St. Louis gobies peaked at 24°C, rather than

26°C (Reid and Ricciardi 2022). This same study recorded variable summer temperatures experienced by round gobies at different locations within the Great Lakes-St. Lawrence River Basin, which could result in population-level differences in thermal responses because recent thermal history could impact a population's ability to acclimate to novel conditions (Somero 2010; Reid and Ricciardi 2022). Further studies should therefore consider life stage, population of origin, and recent thermal history experienced by collected fish when examining the effects of multiple stressors.

Phenotypic plasticity can allow organisms to shift their preferences and optima to maintain performance. Through acclimation, the range of temperatures that an organism can tolerate without loss of performance can be shifted (Morley et al. 2019). For example, a population of Danish round gobies increased their critical thermal maxima with increasing acclimation temperature (10–28°C), and generally maintained stable metabolic rates across the same temperature range (Christensen et al. 2021). However, there are limits to physiological plasticity, and exposure to elevated temperatures beyond an organism's ability to maintain metabolic function can lead to physiological stress and potentially death if environmental conditions or behaviours do not change (Schulte 2015). The stress response often causes an increase in the metabolic and gastric evacuation rates, which in turn lowers the assimilative efficiency of food (Mazumder et al. 2015). The observed decline in SGR of Lake St. Louis juvenile round gobies under thermal stress suggest the energy assimilated through feeding could not maintain metabolic activity and thus limited the energy available for growth. This decline may have been exacerbated by the limited amount of food offered in our experiment. Reid and Ricciardi (2022) observed reduced feeding in adults of the same population at higher temperatures, and juveniles might be even more susceptible to thermal stress. Under low food

availability and warmer conditions, juvenile round gobies likely could not meet the energy demands to maintain growth. Reduced feeding performance and growth during early life stages could have cascading negative effects on goby performance later in life as well, especially since gross conversion efficiency is typically maximal in the youngest life stages (Lee and Johnson 2005).

Exposure to warmer temperatures significantly reduced growth and predatory performance of round gobies, which may be the result of thermal compensation. A few studies that compared energy allocation or metabolism of freshwater fish under elevated temperatures revealed that basal energy requirements to maintain body function are plastic to thermal stress after prolonged exposures of three to eight weeks (Sandblom et al. 2016; Nyboer and Chapman 2017). Our fish experienced a thermally stressful environment for approximately five weeks with minimal food. Juvenile gobies might have adjusted to this thermal stress and low energy input by altering their feeding behaviour; they could have reduced their daily food intake because large meals require more energy to digest (Norin and Clark 2017), resulting in lower feeding rates which, in turn, slowed down growth.

The behaviour of individual fish can affect their growth and ability to compete for resources under the constraints of their experimental environment. This could explain why we observed greater variability in growth response amongst fish exposed to the same treatment and present in the same aquarium than in the responses of these same fish during the predatory performance experiments conducted in isolation. Gobies display territorial behaviour such that bolder and more aggressive fish can displace others and compete with them for resources (Ward et al. 2006). Even when fish match in terms of phenotype (length, mass, or size class), their competitive ability may differ (Milinski and Parker 1991). Gobies that display bolder and more

aggressive behaviour can also have a higher resting metabolic rate (Myles-Gonzalez et al. 2015), and subsequently may require more energy to maintain constant metabolism and thermoregulation. Given the possibility that the presence of conspecifics influenced the response of fish within the same treatment in our growth experiments, we recommend that future studies consider fish personality or intra-specific competition in the design of experiments.

4.5.2 Ecological impacts of thermal stress

The thermal stress imposed on round gobies could impact their recruitment and performance. Although juvenile gobies exhibit faster specific growth rates than adults (Lee & Johnson 2005), under warmer conditions our gobies displayed reduced growth that could delay their maturation and make them more susceptible to predation. As many predators have adapted to feed on the round goby since its invasion of the Great Lakes Basin (e.g. burbot, *Lota lota*; Madenjian et al. 2011), a decline in juvenile goby abundance and future recruitment could have broad repercussions for freshwater food webs. In addition, the reduced predatory performance of juveniles under higher microplastic scenarios and thermal stress could lead to reduced consumption of benthic prey, which might further contribute to reduced growth rates, incidental starvation, and elevated mortality (Mazumder et al. 2014). Such a trade-off in the energy available for growth or maturation might further alter population fitness.

Given that the round goby is a highly adaptable invasive species capable of re-allocating energy to reproduction or growth where needed (Houston et al. 2014), its adaptive response to warming could be more efficient than that of some co-occurring native fishes (Christensen et al. 2021) and, if so, could drive further displacement and population declines of vulnerable native species (Záhorská 2016). However, our study of thermal effects on round gobies of a single life stage under two temperature scenarios may have limited applicability to different life stages,

wider temperature ranges, and littoral habitats affected by hydrological alterations (e.g., lower water levels and flow rates) induced by climate change (Roche et al. 2020). Future studies should investigate how physiological and behavioural responses of the round goby to temperature and hydrological extremes could vary with maturation, life stage, and condition.

4.5.3 Microplastic exposure has weak effects on growth rate

Previous experiments that exposed fish to environmentally relevant microplastic concentrations reported slight changes in mass for juvenile fish (Naidoo and Glassom 2019) and inhibitory effects on the growth of fish larvae (Xia et al. 2020). At higher concentrations, orders of magnitude above natural concentrations, the ingestion of microplastics also induced artificial fullness or inhibited the functioning of the digestive tract (Wright et al. 2013; Parker et al. 2021). Our use of concentrations reported in the field could explain why microplastic exposure only marginally reduced the growth rate of round gobies. Although an effect of microplastic was detected, multiple comparisons indicated that significant differences in SGR only existed between the baseline conditions and microplastic treatments under elevated temperatures. One possibility is that fish become acclimated or adapted to these concentrations of microplastics through long-term exposure over multiple generations. Marginal differences were observed between treatments without microplastics and the current microplastic inputs reported in the St-Lawrence River (low microplastics), but not between the higher treatments. The effect of microplastic on growth may be obscured under elevated temperatures, or by greater between-subject variation in SGR, as discussed previously.

Few beads were observed in the digestive tract of gobies in comparison to their presence in other tissues (Figure 4.4B; Table S4.11), suggesting that digestive tract interference was unlikely. Beads present in fish carcasses might have originated from those adhering to the gills or

the external body, or perhaps they were translocated to flesh from other organs; such transfers were observed for polyethylene particles within the size range of beads used in our experiment (McIlwraith et al. 2021).

The effects of microplastic pollution under concentrations currently found in the field might require a longer exposure time and a necessity to monitor post-exposure conditions after depuration. For example, in an experiment exposing freshwater zebrafish and marine medaka to environmentally relevant concentrations of microplastics (1% polyethylene powder 11-13 μm, embedded in food), Cormier et al. (2021) found no change in body length or weight between control and treated fish after two months, but significant differences were observed after four months. In our experiment, we noticed a significant reduction in the feeding rate of fish after the 37 days of exposure, but non-significant changes in growth. We hypothesize that the decline in predatory performance we observed would eventually impair growth owing to insufficient energy uptake. Furthermore, microplastic exposure disrupts digestive processes (e.g., through microbiota dysbiosis, inflammation of the gastrointestinal tract, enzymatic activity) in several freshwater fish species (Jin et al. 2018; Wen et al. 2018), which may mean that effects require more time to manifest and thus necessitate post-exposure monitoring. For example, when common carp were exposed for 30 days to 32-40 μm polystyrene, their growth was not affected during this exposure period, but declined during the 30-day depuration phase (Ouyang et al. 2021).

4.5.4 Retention and effect of microplastics depend on exposure times and concentrations

Each species has a unique dose-response and retention time for microplastics which influences the scope of effects that can be observed in a contaminated animal. Our experiment tested sub-chronic effects at environmentally relevant microplastic exposures from different

vectors (water, sediment, prey) and failed to detect a significant growth or feeding response at low microplastic concentrations. Previous experiments on acute 24-h dose response and retention of this same type and size of microbead by Lake St. Louis round gobies also showed low contamination under realistic bead concentrations and a slight increase with the concentration of exposure acquired from sedimented beads. Additionally, round gobies had low retention rates, with >50% of fish egesting all beads acquired within 12 h when left to depurate in a clean environment (Chapter 2). These results suggest round gobies are ingesting beads accidentally and egesting them rapidly, thereby maintaining a low body burden. Yet, under the prolonged exposures examined in this multiple stressor experiments, fish exposed to the higher microbead concentration retained more beads at either temperature regime, with a significantly greater body burden when exposed to both warm temperatures and higher amounts of microbeads. Comparing the results from acute exposures to those of this study suggests that chronic levels of microplastic contamination currently observed in the St. Lawrence River meet the minimum threshold required for retention in round gobies. Overall, our results highlight that a chronic exposure, even at low concentrations, may induce significant effects and lead to greater retention of microplastics than an acute high exposure scenario.

4.5.5 Realistic microplastic exposure reduced predatory performance

Under realistic exposures coupled with a 26-h depuration period, we found that 65-90% of round gobies exposed to the highest microplastic concentration retained at least one bead in their body and exhibited reduced predatory performance at a temperature of 18 °C. When comparing the effects of each stressor to those observed under baseline conditions (a temperature of 18°C and no microplastic exposure), the effect of warming (to 26°C) caused a 45% decline in prey consumption, whereas the exposure to the high microbead treatment reduced predatory

performance by 49% (Table 4.1). As microplastic exposure increases, the feeding rate of gobies decreased. We speculate that gobies handle and digest food less efficiently when they retain more plastic particles, as occurred when they were exposed to higher concentrations of microbeads. Our results suggest that while short-term exposure may not result in high and consistent microplastic ingestion rates, the exposure and retention of plastic beads under concentrations currently present in the St. Lawrence River will nonetheless negatively impact juvenile round goby behaviour, even if microplastics are rapidly egested and retained at low rates. The presence of beads in fish after 24 h could also facilitate the trophic transfer of microplastics from round gobies to diverse Great Lakes predators such as the burbot (*Lota lota*), walleye (*Sander vitreus*), largemouth and smallmouth basses (*Micropterus* spp.), yellow perch (*Perca flavescens*), and lake sturgeon (*Acipenser fulvescens*) (Taraborelli et al. 2010; Crane et al. 2015), and can link freshwater to terrestrial food webs when ingested by piscivorous birds such as double-crested cormorants (*Nannopterum auritum*) (Johnson et al. 2015).

4.5.6 Ecological impacts of stressor interaction

Our study revealed a weak interactive effect of the combined stressors and a stronger effect of thermal stress on growth and predatory performance. Thermal stress reduced round goby growth and predatory performance across all microplastic treatments. Significant growth reduction occurred under environmentally-relevant microplastic exposure scenarios only at the elevated temperature, whereas predatory performance was affected at the highest concentration. Under the multiple stressor scenario, the effects of microplastics on predatory performance of juvenile gobies are apparent at 18°C, while the response to thermal stress seems to be masking the effect caused by microplastic pollution at elevated temperatures. Similarly, thermal stress has been shown to have a stronger effect on the behaviour, immune function, and metabolism of

dreissenid mussels co-exposed to polystyrene microplastic and warm temperatures of 23°C and 27°C, but did not display significant interactions (Weber et al. 2020). Our results suggest that the impact of current microplastic contamination levels—even at heavily contaminated sites— are not as important as the thermal stress that will be experienced by juvenile gobies under climate warming. However, if concentrations of microplastic continue to increase beyond currently observed maximum concentrations, feeding rates may continue to decline. Under such scenario, the interaction amongst stressors could become more important and synergistically impact growth and feeding behaviour of round gobies.

Shallower rivers, streams and shoreline habitats are more likely to experience periodically extreme temperatures (Drouillard et al. 2018). Nearshore sites along Lake Erie and Lake Ontario in 2020 were already experiencing mean summer temperatures near or above 25°C for 59 days in Toledo (Ohio, USA) and 32 days in Hamilton Harbour (Ontario, Canada) (Reid and Ricciardi, 2022). In summer 2020, there were only nine days where the mean temperature at our sampling site in Lake St. Louis (Melocheville) was at or above 25°C (Reid and Ricciardi, 2022), but daily maxima reached or exceeded 25°C for 32 days across July and August (H.B. Reid, *unpubl. data*). Our results therefore indicate that juvenile round gobies from the St. Lawrence River are more sensitive to projected temperature scenarios, which could affect their future performance. Thermal stress coupled with increasing microplastic pollution might further reduce predatory performance and growth under daily exposures.

The observed responses of the round goby to climate change and microplastic pollution in the Great Lakes can inform resource management by revealing potential impacts of these co-occurring stressors. We recommend that future research explore a wider range of measures of growth, digestion and nutritional uptake processes. Likewise, future work could assess

physiological and behavioural responses in round gobies and consider how other benthic fishes change with life stage and maturation, and under a longer exposure period and using a wider range of microplastic concentrations and temperature regimes.

4.6 Acknowledgements

McGill University is located on land which has long served as a site of meeting and exchange amongst Indigenous peoples, including the Haudenosaunee and Anishinabeg nations. We would honour, recognize, and respect these nations as the traditional stewards of the lands and waters where our work has been conducted. We thank Jessamine Trueman, Wendy Huang, and Hélène Pfister for their assistance. GD acknowledges financial support from GRIL, the NSERC CREATE Ecolac Program, the Arthur Willey Memorial Fellowship, the Trottier Graduate Fellowship, and the Lawrence Light Fellowship. DW acknowledges support from the Bieler School of Environment and the Helen Guoyi Li Gao Science Undergraduate Research Award. IGE acknowledges support from the Canada Research Chairs program. AR acknowledges support from the McGill Trottier Institute for Science and Public Policy, and the Natural Sciences and Engineering Research Council of Canada.

4.7 Supplementary Materials

4.7.1 Fish acclimation and care

Fish collection and transport methods followed the conditions stipulated in the Quebec ministerial permit for scientific, educational or wildlife management purposes (SEG #2019-7-8-2665-06-16-S-P and 2020-07-13-2862-05-06-16-S-P), while animal care procedures and experiments were approved by the McGill Animal Care Committee and respect Canadian Council on Animal Care guidelines (Protocol #2017-7913). Protocols are detailed below, for more details, please contact the authors.

All animals captured from the field were acclimated to laboratory conditions for at least 2.5 weeks because fish can show signs of disease or pathogen and display delayed responses to stress up to 14 days after collection. The pH, conductivity, and water temperatures at field sites were recorded, and ambient water was used to fill coolers used to transport live animals. Coolers were mounted with an aerator and transported in temperature-controlled vehicles. During transport from the field and for the first week of acclimation, 1 ml of Seachem StressGuard was added daily per 40L of water to reduce fish stress. This creates a healthy slime coat protection around the fish that helps to heal potential injuries and protects them from infections during acclimation. Once at the facility, fish were acclimated to chamber conditions for a few days in the coolers, and until the water reached the temperature of the chamber. Water quality was monitored daily, and water was changed progressively from river water to dechlorinated tap water. A 3-part filtration unit was also added to the cooler to help maintain water quality. Once temperature in the cooler matched the temperature of the chamber, fish were transferred to an aerated and filtered 75-L aquaria for a two-week acclimation period. Given that these fish are

cryptic and favour benthic cover (Jude et al. 1992), all holding aquaria were filled with gravel substrate and one PVC tube per fish was provided to serve as a shelter.

During the two-week acclimation, pH, ammonia, nitrites, nitrate, conductivity, and temperature were verified and recorded daily. Water parameters were maintained within the range of values in Table S4.1 by conducting water exchanges and by removing organic wastes. To prepare dechlorinated water, 1ml of Seachem Prime solution was added per 40 L of water at least 8 h before use. After each water removal, the same volume of new dechlorinated water was added to each aquarium. Then, one capful of Seachem stability (beneficial bacteria) was added to maintain healthy levels of bacterial fauna that contribute to aquarium cycling and healthy water quality. Fish were monitored daily for the presence of disease or signs of poor health.

Table S4.1: Acceptable range of water quality values to maintain the health of round gobies.

Temperature	Ammonia	Nitrites	Nitrate	pH	Ca^{2+}
18-26°C	0-0.25ppm	0	0-5 ppm	7.6-8.4	25-40 ppm

After the 2-3 weeks acclimation, each goby was first temporarily sedated using a diluted eugenol solution (0.28mL eugenol·L^{-1} distilled water) as anesthetic, and then visually marked by injecting a colour-coded florescent elastomer dye (Visible Implant Elastomer VIE tag, Northwest Marine Technology) between the skin and flesh layers of the abdominal region above the anal fin, near the tail of the fish, or on the back of the fish near the dorsal fin (Photo S4.1). While sedated, the total length from the tip of the snout to the end of the caudal fin was measured in mm (L_I; 35–75mm) and the fish wet mass in grams was recorded (W_F; 1.07–5.35g). After marking and measuring, the sedated gobies were monitored in aerated 9.5 L aquaria until full recovery of respiration and movement was noted.

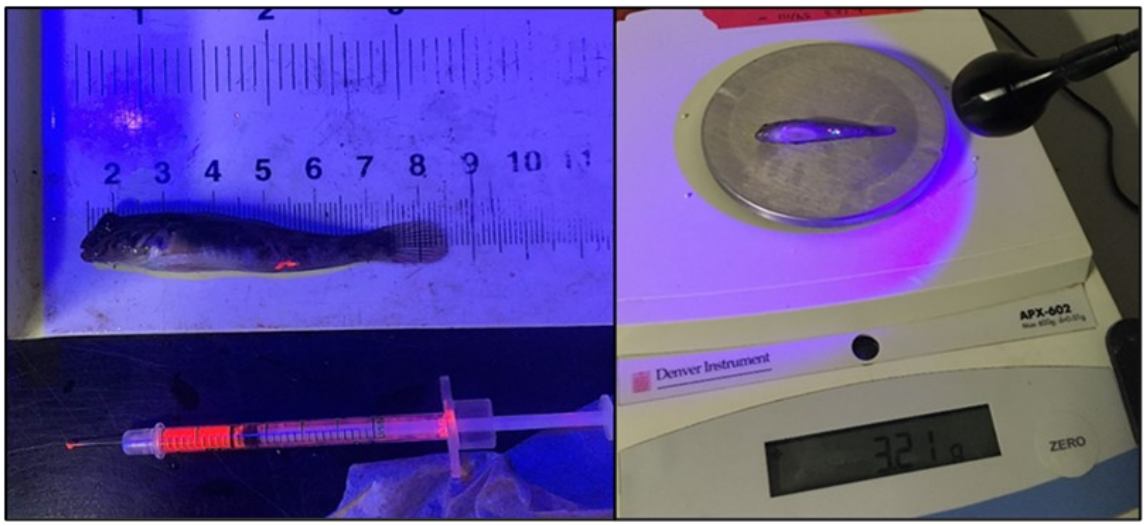

Photo S4.1: Round goby (*Neogobius melanostomus*) marked with Visible Implant Elastomer VIE tag for identification (left) and the identification and weighing of gobies at the end of the experimental trial (right).

4.7.2 Microplastic treatment preparation

Microplastic concentrations were estimated from concentrations measured in the St-Lawrence River or other freshwater bodies of the world. The low concentration represents the mean benthic sediment concentrations reported from the St. Lawrence River (Crew et al. 2020), while the high concentration was measured by calculating the mean of the four highest concentrations of plastic reported in Table S1 of the supplemental material provided by Crew et a. 2020. The highest concentrations in sediments were 7561, 5289, 2854 and 2051 microplastic·kg^{-1} dry mass (dw); ~4500 microplastic·kg^{-1}dw. Using the raw data from Crew et al. 2020, the number of beads found in the St-Lawrence River varied from 0-2978 beads·kg^{-1} dw when clear beads were excluded while concentrations ranged from 27 to 52 6036 beads·kg^{-1} dw. Based on these concentrations, we consider our exposure represent realistic conditions.

As for concentrations in water, the mean microplastic concentrations observed in the surface of freshwater environments worldwide is estimated ~1 microplastic·L^{-1} (D'Avignon et al. 2022), the same as the mean of the four highest microplastic concentrations reported in the St-Lawrence River in Table S4 (Crew et al. 2020). Highest concentrations in Table S4 were 2.37, 1.03, 0.38 and 0.38 microplastic·L^{-1}. We used the same concentration for the low and high treatments also because solutions with a lower concentration had very high error. As such, to maintain realistic exposure conditions we added an average of 1 bead·L^{-1} per day for the 37-day exposure trials for the low and high treatments. The concentration of microplastics injected into each mussel was informed by microplastic counts from quagga mussels collected along the St. Lawrence River which ingested 2-11 microplastic pieces per individual in the St. Lawrence River (*G. D'Avignon pers.comm*., Table S4.2).

Table S4.2: Summary of suspected microplastic ingestion by St. Lawrence River (Canada) quagga mussels G. D'Avignon *unpublished data*. N = sample size, % Occ. refers to the percentage of individuals with microplastics per sample size. The mean and range refer to the number of suspected microplastic particles per individual. Microplastic shape are described as fibre (FB), fragment (FR), or bead (B). The most common colours of particles ingested are identified as clear (C), orange (O), pink (P), or white (W)). Anthropogenic particles were identified visually and using Nile Red fluorescence.

Location	**Year**	**N**	**% Occ.**	**Mean**	**Range**	**Size**	**Shape**	**Colour**
Iles de la Paix	2017	12	83	9.4	1-22	<100	FR, FB, B	C, P, O
Les Coteaux	2017	7	100	6	1-15	<100	FR, B, FB	C, P, O
N-D-L'Ile-Perrot	2017	4	100	2.5	1-5	<100	FR, FB, B	C, W
Baie de Valois	2017	6	100	8.3	3-25	<100	FR, FB, B	C, W, P
Varennes	2017	3	67	7.3	0-17	<100	FB, FR, B	C, O, W
Sorel	2017	5	100	8.8	2-18	<100	FR, FB, B	C, O, P

4.7.3 Exposure concentration at the end of the trial

Our experiment was designed to have continuous (chronic) inputs of microplastics throughout the 5 weeks duration. Assuming all inputs of microplastic beads remained and accumulated in each aquarium over the course of the experiment, the total number of beads per aquarium was estimated to be 2,245 for the low microplastic concentration and 9,905 beads for the high treatment after 37 days. However, to maintain water quality, we performed weekly water changes by removing deposited organic material using a hand pump and adding new dechlorinated water. This procedure removed 20-50% of the water in each aquarium. To estimate the number of beads removed from each tank during this procedure, the water removed during water changes for each aquarium was collected two to four times over the 6 weeks. A different pump and bucket were used to clean and collect water from the control aquaria versus the aquaria with microplastics. All material was triple rinsed and inspected for the presence of fluorescent beads under a UV light.

We collected the water using a hand pump and poured it into a graduated container to record the volume removed, and then sieved the water through a 125 µm and a 30µm mesh to keep particles within this range. We rinsed the material into a clean vial and added 10% KOH for organic digestion for 48 h. After this period, the remaining material was sieved through the 30µm mesh again to separate the beads from the digested material. Particles that were left on the 30µm mesh were rinsed onto a vacuum filtration apparatus holding a 47mm A/E glass filter of 1 µm (PALL). All sieves and vials were inspected under a UV light and rinsed at least three times or until all fluorescent beads were transferred to the glass filter. All beads collected were counted under stereoscope coupled to a UV light and reported per color for each volume of water collected (Table S4.3). At the end of the exposure trials, we filtered the entire volume of water

from the control aquaria to capture the total number of beads present in each aquarium to account for potential contamination. Note that violet beads represent contamination from previous experiments conducted with the equipment and thus represent procedural contamination from the material.

Table S4.3: Estimated number of beads remaining at the end of the 37 days exposure (2020) in relation to the number of beads expected for each treatment and the percentage difference between these values. The mean number of beads collected per litre of water filtered based on multiple samples taken from each aquarium. Green beads were added to sediments, blue beads to the water (in 2020), orange beads to prey, violet beads were not used in this experiment and originate from previous experiments conducted with these aquaria, filters, and gravel, so originate from contamination.

Aquaria	Treatment	Expected	Estimated	%	Mean number of beads per litre			
				Difference	Green	Blue	Orange	Violet
1B	High-26	9905	9380	5.3	10.4	3.1	8.1	0.6
2B	Low -26	2245	1498	33.3	11.7	8.8	14.5	0.6
3B	High-26	9905	7971	19.5	65.1	6.2	7.1	1.1
4B	No -26	0	361		2.7	0.4	3.8	0.5
5B	High-26	9905	9045	8.7	16.3	2.2	26.2	10.3
6B	No-26	0	126		0.6	0.3	1.1	0.4
7B	High-26	9905	8785	11.3	17.1	4.8	24.4	1.4
8B	Low -26	2245	1610	28.3	10.8	5.2	73.5	0.6
9B	High-18	9905	8018	19.1	84.5	2.6	4.3	15.9
10B	High-18	9905	8385	15.3	54.5	6.4	2.7	2.9
11B	Low-18	2245	1655	26.3	21.9	3.8	5.7	7.8
12B	No -18	0	297		3.7	0.0	1.3	1.3
13B	High-18	9905	9085	8.3	35.7	12.6	28.9	44.3
14B	Low- 18	2245	1879	16.3	6.9	2.8	6.1	1.1
15B	High-18	9905	9500	4.1	6.8	6.8	2.7	0.1
16B	No-18	0	62		0.0	0.0	1.7	0.0

4.7.4 Data verification: Lake St. Louis goby population characteristics

In 2019, round gobies were sampled from Parc Boursier and Beauharnois Marina using seine or traps, while in 2020, all gobies were captured by traps at Parc Boursier so could display different characteristics impacting their growth. To assess whether the initial physical characteristics of the fish and their projected growth patterns were similar using (1) weight-length relationship, (2) length frequency, and (3) Fulton's condition index.

The length-at-weight relationship of the fish used for this study were compared to 140 other round gobies captured on the shorelines of Lake St. Louis between 2018 and 2020. We grouped fish assemblages per day of capture and location. We found a subsample of fish captured in 2019 for our study (StLouis_S19) had different growth patterns than all other fish from the lake (red circle, Figure S4.2).

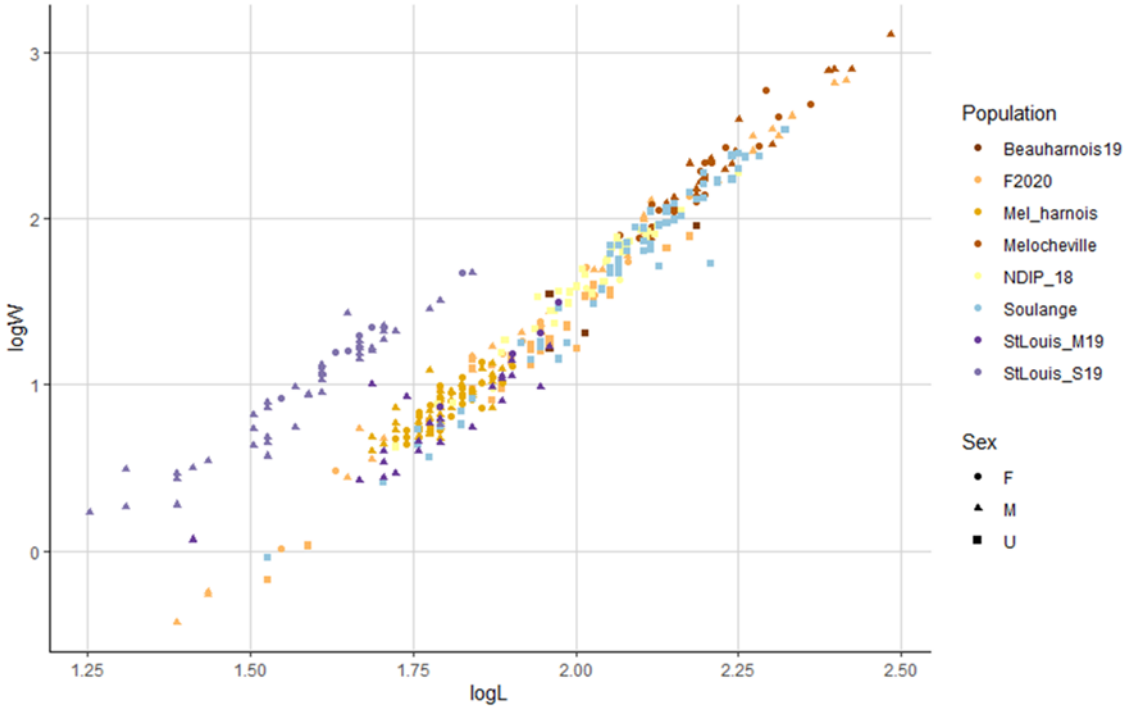

Figure S4.1: Length to weight relationship of Lake St-Louis fish.

Using the FSA package in R (Ogle et al. 2022), we applied a linear model (controlling for the sex of the fish) to compute the parameters α and β of the weight-length relationship of the fish used in our study using Equation S4.1. A linear transformation was applied to produce Equation S4.2 which was used to create a linear model to examine whether the Sex and Population association affect the condition of the fish as it grows.

Equation S4.1: $W_i = aL_i^b\, e^{\epsilon i}$ **Equation S4.2**: $\ln(W_i) = \ln(a) + b \cdot \ln(L_i) + \epsilon_i$

where parameters a and b are constants that are estimated using the FSA package based on the initial massin grams W_i, and the initial length of the fish in cm L_i. Note that ln (a) represents the intercept, while b represents the slope of the linear relationship.

Using the linear form of the weight-length relationship, we built a best-fitting model adjusting for the difference in growth per population and sex. Based on this model, round gobies used in this study exhibit allometric growth with an exponent parameter (b) between 2.3 and 2.67, with 95% confidence (Table S4.4), which indicates a healthy population with a uniform condition throughout its length range (Froese 2006). In Table S4.4, we note the two assemblages have different parameters estimating their growth rates (b). As one group had a high mass per unit of length (were more plump), we decided to use a condition factor to control for the difference in initial body shape of the fish in our analyses.

Table S4.4: Description of coefficients of the weight to length relationship controlling for sex and population. Parameter a in Equation S4.2 represent the intercept, while parameter b represents the slope.

	Mean	**SE**	**2.5%**	**97.5%**	**DF**	**T-stats**	***p*-value**
Intercept (a)	-3.641	0.144	-3.926	-3.355	155	-25.216	< 0.0001
Slope (b)	2.509	0.079	2.353	2.666		31.717	< 0.0001
Population	0.716	0.024	0.668	0.764		29.517	< 0.0001
Other							
Sex Male	-0.008	0.019	-0.046	0.030		-0.413	0.68
Sex Unknown	-0.085	0.020	-0.124	-0.045		-4.207	< 0.0001

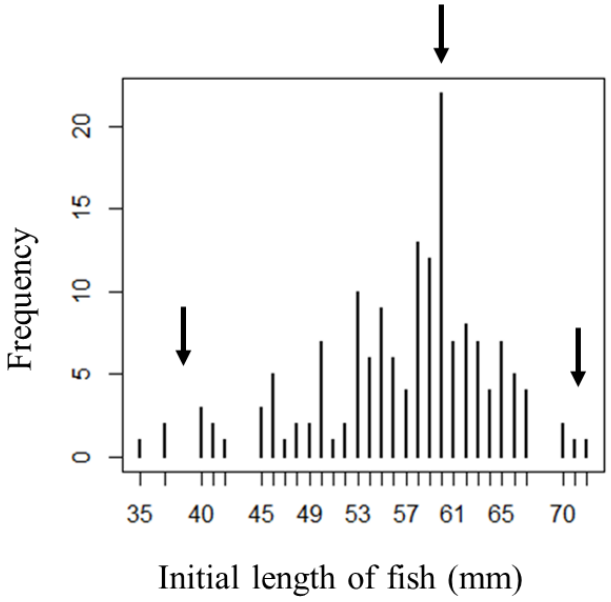

Figure S4.2: Frequency of the initial length (mm) of round gobies sampled from Lac St. Louis (n=160) in 2019 and 2020. The three peaks represent fish from different groups based on the body condition.

Further exploration of the initial length-weight relationships of the fish before the onset of the experiment could be grouped into three different groups, as identified by three peaks in the length frequency distribution of captured fish (Figure S4.2). Duemler et al. (2016) identified

peaks as age cohorts, but we also found these groups were associated to differences in body shapes which were well described by Fulton's condition factor (K) (Ricker, 1975), with mean body conditions of 1.06, 2.25 and 3.05 for the first, second and third cohorts, respectively. As such, Fulton's condition was used as a surrogate of cohort and included in initial data analyses as a covariate to examine whether the initial condition (K_I) of the fish influenced its specific growth rate, predatory performance, or microbead contamination. Descriptive statistics of fish from each cohort and pooled cohorts are described in Table S4.5.

Table S4.5: Descriptive statistics of the three cohorts identified by the length-weight relationship and the pooled data. Values of N represent the sample size, the mean initial length (L_I) and mass (W_I) and final length (L_F) and mass (W_F) of fish and their respective standard deviations are reported. Fulton K is measured by Equation S4.2, SGR stands for specific growth rate as described by Equation 5.1, PP represents the Predatory Performance in number of worms ingested, and MP is the total number of microbeads found in round gobies at the end of all experiments.

	Cohort 1	**Cohort 2**	**Cohort 3**	**Pooled**
N	105	52	3	160
L_I (mm)	60.79 ± 3.80	50.15 ± 5.73	41.3 ± 9.29	57.0 ± 7.11
W_I (g)	2.41 ± 0.46	2.93 ± 0.96	2.36 ± 1.59	2.58 ± 0.73
Fulton K	1.06 ± 0.11	2.25 ± 0.21	3.05 ± 0.21	1.49 ± 0.61
SGR	0.34 ± 0.39	0.23 ± 0.36	-0.10 ± 0.49	0.29 ± 0.38
L_F (mm)	63.67 ± 4.15	66.1 ± 6.35	57.0 ± 8.89	64.3 ± 5.24
W_F (g)	2.74 ± 0.61	3.18 ± 1.06	2.15 ± 1.06	2.88 ± 0.81
PP (n=115)	14.0 ± 13.0	25.0 ± 14.0	28	17.0 ± 14.0
MP	1.34 ± 2.14	0.23 ± 0.36	0	0.96 ± 1.89

4.7.5 Data analysis: Survival

Four fish died and one fish was considered unhealthy (heavy breathing and poor reflex) after being transferred to the clean aquaria and before conducting the predatory performance test. We normally prepared water at least 48 h prior to the experiment, water was added to these aquaria <24 h before the onset of the trial due to laboratory access restrictions during SARS-COV-2 outbreak. Water quality was monitored before the onset of the trial, and we did not observe issues with ammonia, nitrate, nitrite, pH, or temperatures. Although all unhealthy fish were from the High MP treatment, we suspect that the mortalities were attributed to issues with the new water which was added in the new aquaria, because fish in our holding tanks which received water changes on the same day also died. Considering death occurred after the growth trial, it did not affect the results of that experiment, but the five unhealthy fish were removed from the predatory performance analyses.

4.7.6 Data analysis and Results

We used exploratory mechanism to examine the effects of sex, sampling date, the initial condition, cohort, and the interaction between microplastic and temperature as covariates. The terms microplastic and its interaction with temperature, and fish condition and cohort displayed high multicollinearity with variance inflation factor above 5, so one term was kept. Initial mass and condition were centered at the grand mean of the pooled data. We removed collinear terms: interaction and cohort first, then, we performed hierarchical selection by removing the least significant terms one at a time guided by choosing the lowest Akaike and Bayesian Information Criteria to ensure the best model was selected.

Table S4.6: Specific growth rate Analysis of variance table (Type III) with Satterwaite's method and model output using full dataset.

ANOVA		**SS**	**MSS**	**DF**	**F-value**	**p-value**
Temperature		0.438	0.434	1 24	6.87	0.0143
Microplastic		0.042	0.021	2 26	0.33	0.7202
Condition		0.100	0.100	1 143	1.56	0.0213
Sex		0.194	0.097	2 148	1.52	0.2226
Date		0.006	0.006	1 68	0.10	0.7521
MODEL OUTPUT						
Random effects			**Variance**	**Std. dev**		
Aquaria			0.017	0.130		
Residuals			0.064	0.253		
Fixed effects		**Coef.**	**SE**	**DF**	**T-value**	**p-value**
No MP 18°C		0.507	0.086	35	5.860	<0.001
Temp. (26°C)		-0.169	0.064	27	-2.621	0.0143
Low MP		-0.054	0.071	23	-0.758	0.4563
High MP		-0.054	0.094	24	-0.574	0.5713
Condition		-0.058	0.047	143	-1.250	0.2133
Female		-0.094	0.058	138	-1.657	0.1060
Unknown sex		-0.139	0.102	145	-1.363	0.1748
2019		-0.036	0.114	68	-0.317	0.7521
AIC	**BIC**	**mar R^2**	**cond R^2**	**ICC** (adj.)	(cond.)	
82	113	0.178	0.351	0.21	0.173	
Shapiro test:		W = 0.974, p-value = 0.004				

Our model SGR = MP + Temp + Sex + Condition + Date + 1 | Aquaria respected the assumptions of homoscedasticity but had lightly skewed residuals leading to poor estimates of the tails and deviated from a normal distribution of residuals (Shapiro-Wilk, W=0.9736, *p*=0.004, Figure S4.3; Table S4.6). Diagnostic plots showed the presence of outliers which were further examined using Cook's distance to evaluate the influence of these outliers on model fit. Nine values significantly influenced the model and were ultimately dropped as their presence caused higher error and poorer fit of the model.

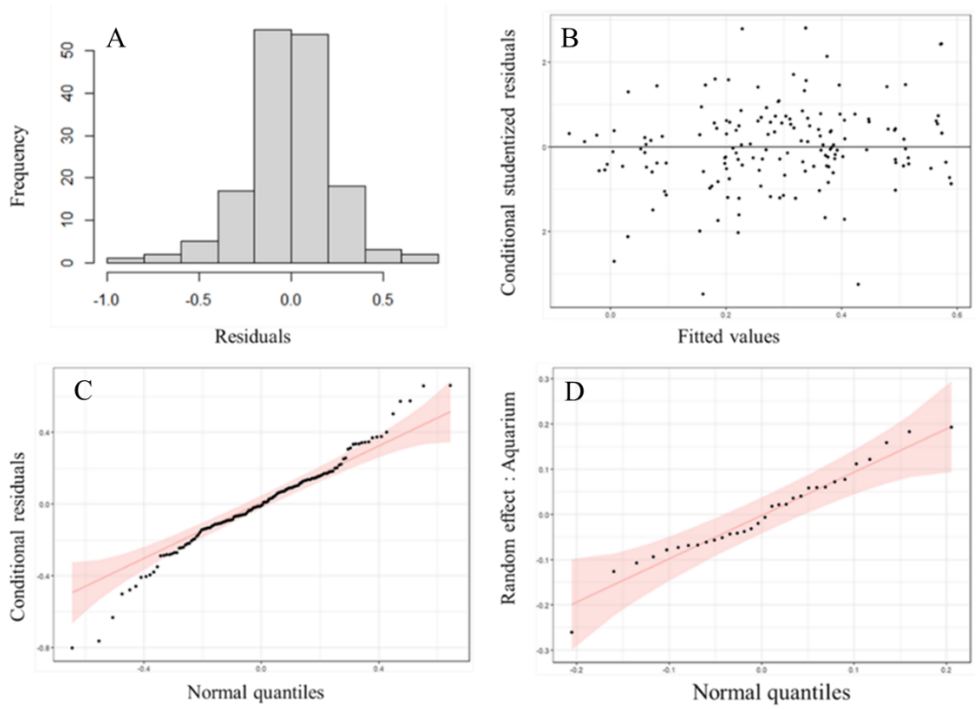

Figure S4.3: Diagnostic plots of the best SGR model based on the coefficient described in Table S4.6. A) Frequency distribution of specific growth rates, B) homogeneity of conditional standardized residuals, C) quantile-quantile plot of the conditional residuals showing poor fit at both tails, and D) residuals of random effect within the expected confidence interval.

The revised model showed significant effects of temperature, sex, and microplastic (Table S4.7). Fish condition was marginally significant (p=0.095) while date of sampling was not affecting SGR. When removing the least significant term: date, fish condition became more important, and was maintained in our model. The simpler model was kept because it met assumptions, had similar R-squared and slightly lower AIC and BIC values. Fixed effects now explained 33% of the variance (rather than 17.8%) while the variation between aquarium declined from 17.3% to 12.8%. The random effect quantile plot shows poorer fit (Figure S4.4 vs.

S4.3). To further improve the model, we removed non-significant terms to obtain a best-fit model used to estimate marginal means per treatments reported in Table 4.1 and Figure 4.2.

Table S4.7: Specific growth rate marginal analysis of variance table (Type III) with Satterwaite's method and output of the simplified best-fit model used to estimate marginal means and confidence intervals for Table 4.1 and Figure 4.2. Multiple comparisons were applied to this model.

ANOVA		**SS**	**MSS**	**DF**	**F-value**	**p-value**
Temperature		0.530	0.530	1 26	14.10	<0.001
Microplastic		0.323	0.162	2 28	4.40	0.023
Condition		0.155	0.155	1 142	2.82	0.043
Sex		0.446	0.223	2 128	4.50	0.003
MODEL OUTPUT						
Random effects			**Variance**	**Std. dev**		
Aquaria			0.0087	0.093		
Residuals			0.0372	0.193		
Fixed effects		**Coef.**	**SE**	**DF**	**T-value**	**p-value**
No MP 18°C		0.589	0.069	40	8.729	<0.001
Temp. (26°C)		-0.177	0.049	29	-3.755	<0.001
Low MP		-0.155	0.054	25	-2.884	<0.008
High MP		-0.117	0.072	26	-1.788	0.0855
Condition		-0.069	0.037	135	-1.679	0.0954
Female		-0.139	0.046	134	-2.947	0.0038
Unknown sex		-0.183	0.078	137	-2.023	0.0450
AIC	**BIC**	**mar R^2**	**cond R^2**	**ICC** (adj.)	(cond.)	
-1.56	25.48	0.329	0.457	0.190	0.128	
Shapiro test:	W = 0.996, p-value = 0.941					

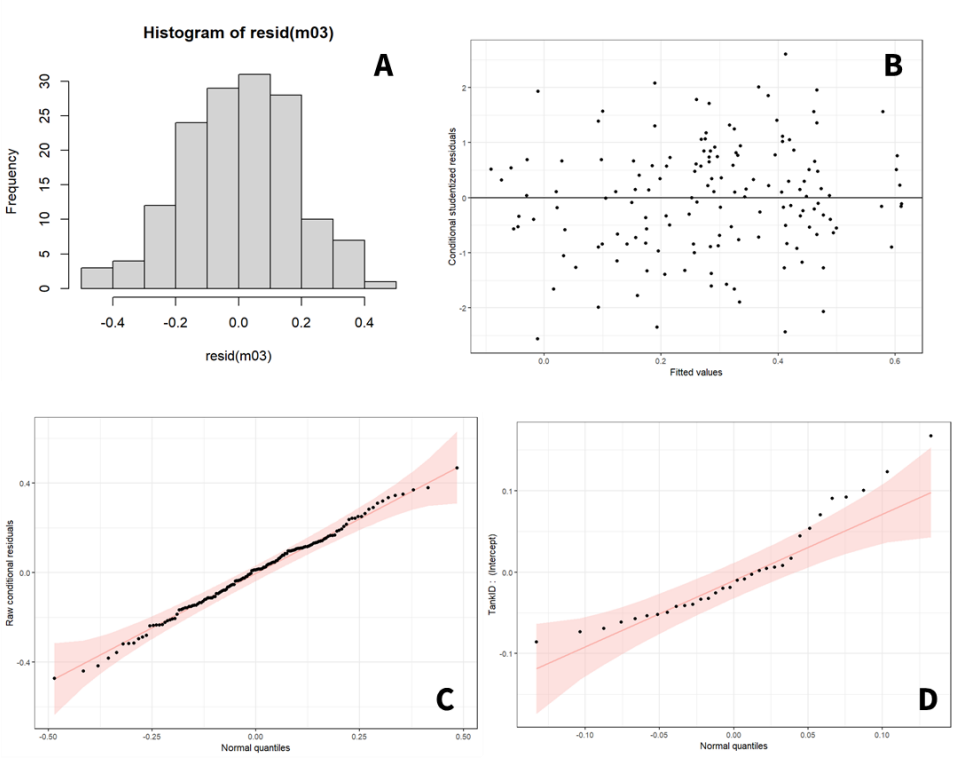

Figure S4.4: Diagnostic plots of the best SGR model based on the coefficient described in Table S4.7. A) Frequency distribution of specific growth rates, B) homogeneity of conditional standardized residuals, C) quantile-quantile plot of the conditional residuals within the expected confidence interval, and D) residuals of random effect showing poor fit at both tails.

To compare the differences observed between the estimated means for each treatment to each other (Figure 4.2), we computed pairwise comparison and Cohen's d effect sizes which are presented in Table S4.8. Large effect sizes are found between the baseline temperature without microplastic (No 18) and the low microplastic treatment under elevated temperature (Low 26) and between the highest microplastic under baseline temperatures (High 18) and the lowest microplastic treatments under elevated temperatures (No and Low 26).

Table S4.8: Summary of pairwise comparison and effect sizes based on Cohen's d. Note that Cohen's d values below 0.2 show a small effect size, a value of 0.5 is a medium effect size and a value of 0.8 or greater is a large effect size. L= low microplastic, N= no microplastic and H= high microplastic treatments, while temperatures are indicated as 18 for 18°C and 26 for 26°C. Significant pairwise comparison and large effect size are in bold.

Treatments	**Pairwise comparison**					**Cohen's effect size**				
	Means	**SE**	**DF**	***t*-value**	***p*-value**	**D**	**SE**	**DF**	**LCI**	**UCI**
N18*L18	0.16	0.05	26.9	2.89	0.107	**0.81**	0.28	27.1	0.23	1.39
N18*H 18	0.12	0.07	31.0	1.73	0.769	0.61	0.35	28.3	-0.11	1.33
N18*N 26	0.18	0.05	26.9	3.77	**0.012**	**0.92**	0.25	28.4	0.41	1.43
N18*L 26	0.33	0.07	26.8	4.64	**0.001**	**1.73**	0.39	28.2	0.94	2.52
N18*H 26	0.29	0.08	28.8	3.70	**0.014**	**1.53**	0.42	27.6	0.66	2.39
L18*H 18	-0.04	0.07	30.4	-0.58	1.000	-0.20	0.35	27.1	-0.91	0.51
L18*N 26	0.02	0.07	27.1	0.31	1.000	0.12	0.37	27.1	-0.64	0.87
L18*L 26	0.18	0.05	26.9	3.77	**0.012**	**0.92**	0.25	27.1	0.41	1.43
L18*H 26	0.14	0.08	28.5	1.77	0.744	0.72	0.41	27.1	-0.12	1.56
H18*N 26	0.06	0.08	30.3	0.71	1.000	0.31	0.44	28.3	-0.59	1.22
H18*L 26	0.22	0.08	29.7	2.56	0.212	1.12	0.44	28.2	0.21	2.03
H18*H 26	0.18	0.05	26.9	3.77	**0.012**	**0.92**	0.25	27.6	0.41	1.43
N26*L 26	0.16	0.05	26.9	2.89	0.107	**0.81**	0.28	28.2	0.23	1.39
N26*H 26	0.12	0.07	31.0	1.73	0.769	0.61	0.35	27.6	-0.12	1.33
L26*H26	-0.04	0.07	30.4	-0.58	1.000	-0.27	0.35	27.6	-0.905	0.50

A linear model without random effect was found to be the most appropriate model for predicting the predatory performance of gobies post-exposure. Both the normality and homogeneity of variance of the residuals were respected (Table S4.9 and Figure S4.5). We also examined the importance of influential values based on Cook's distance. When removing the nine influential values we noticed an increase in the importance of the interaction between temperature and high microplastic concentrations and a decrease in the effect of sex (Table S4.10). Although both information criterion and the r-squared were lower when these values

were removed, residuals no longer displayed normal distribution. Estimated means and contrasts between treatments were similar for both models, so we kept the results based on the full dataset.

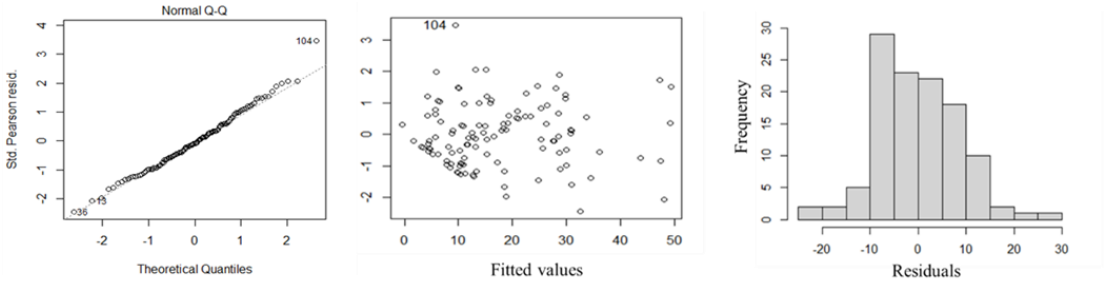

Figure S4.5: Diagnostics plots of the predatory performance model. Although the residuals appear slightly left-skewed, Shapiro test of normality revealed residuals respected normal distribution assumption (W=0.99, p >0.05).

Table S4.9: Post-exposure predatory performance Analysis of variance table (Type III) and model output. Multiple comparisons were applied to this model.

Intercept		8600	1	**105.7**	<0.001
Temperature		1765	1	21.70	<0.001
Microplastic		2537	2	15.59	<0.001
Sex		1369	2	8.41	<0.001
Date		2613	1	32.12	<0.001
Temp *MP		550	2	3.383	0.038
Residuals		8923	105		
Fixed effects		**Coef.**	**SE**	***t*-value**	***p*-value**
Baseline*		25.40	2.47	10.28	<0.001
Temp. (26°C)		-13.56	2.91	-4.66	<0.001
Low MP		0.53	2.88	0.18	0.854
High MP		-14.79	3.04	-4.87	<0.001
Sex F		-2.47	2.10	-1.17	0.243
Sex U		-18.90	4.65	-4.06	<0.001
Date 2019		23.80	4.20	5.67	<0.001
Temp * Low MP		-0.75	4.06	-0.19	0.853
Temp*High MP		9.34	4.23	2.21	0.029
AIC	**BIC**	**Adj. R^2**	**R^2**		
843	871	0.579	0.609		
Shapiro test:		W = 0.982, *p*-value = 0.126			

Table S4.10: Post-exposure predatory performance Analysis of variance table (Type III) and model output when influential values based on Cooks distance were removed from the data (9 individuals removed).

Model	**SS**	**DF**	**F-value**	***p*-value**
Intercept	8393	1	170.23	<0.001
Temperature	2319	1	47.03	<0.001
Microplastic	4408	2	44.71	<0.001
Sex	359	2	3.64	<0.03
Date	774	1	15.70	<0.001
Temp *MP	1337	2	13.56	<0.001
Residuals	4782	97		
Fixed effects	**Coef.**	**SE**	***t*-value**	***p*-value**
Baseline*	27.77	2.13	13.05	<0.001
Temp. (26°C)	-16.23	2.37	-6.86	<0.001
Low MP	-0.76	2.55	-0.30	0.765
High MP	-21.49	2.56	-8.38	<0.001
Sex F	-1.69	1.74	-0.97	0.332
Sex U	-13.48	5.00	-2.69	<0.008
Date 2019	18.35	4.63	3.96	<0.001
Temp * Low MP	0.66	3.36	0.19	0.845
Temp*High MP	16.02	3.43	4.66	<0.001

AIC	**BIC**	**Adj. R^2**	**R^2**
725	751	0.692	0.715

Shapiro test: W = 0.973, *p*-value = 0.03

Microplastic acquisition from beads in the sediments, in the water and from a prey were pooled into a total microplastic count per gobies for the analyses (see **Table S4.11** for details). The best negative binomial model estimating microplastic counts per gobies revealed the retention of microplastic in gobies were significantly affected ($\chi^2_{(1, 160)}$ =16.20, p<0.001) by the date of trials with a significant interaction between temperature and microplastic exposure level ($\chi^2_{(1,160)}$ =9.26, p=0.01) when data were pooled across years (**Table S4.12**).

Table S4.11: Summary of the number of microplastic particles found in goby tissues post-experiments. The sample size (N), the number of particles in the digestive tract (GI), in the body (B) and the number of animals contaminated per matrix (Cont.) are expressed. The total number of beads found in all fish per treatment (Tot.), the means and standard deviation per treatment and the percentage of fish which retained microplastic (% Occ.) are also reported. Treatments are described as N, L or H for no, low and high microplastic concentrations and 18 or 26 represent the temperature regimes.

	N	Sediment			Water			Trophic			Tot.	Mean	SD	% Occ..
		GI	B	Cont.	GI	B	Cont.	GI	B	Cont.				
N 18	30	0	6	5	0	0	0	0	6	5	12	0.40	0.72	27
L 18	30	1	12	3	0	3	2	3	2	5	21	0.70	1.70	27
H 18	20	0	17	7	0	16	8	0	13	5	46	2.30	2.96	65
N 26	30	0	0	0	0	0	0	0	5	4	5	0.17	0.53	10
L 26	30	1	3	3	0	0	0	0	2	3	6	0.17	0.53	13
H 26	20	0	25	12	0	15	9	7	17	10	64	3.20	2.28	90

Table S4.12: Negative binomial model showing the effect of temperature, microplastic and date of trials on the retention of microbeads by round gobies 26 h post-exposure using full data.

Model	χ^2	**DF**	***p*-value**
Temperature	2.78	1	0.095
Microplastic	3.82	2	0.148
Date	16.20	1	<0.001
Temp *MP	9.26	2	0.0097
Fixed effects	**Coef.**	**SE**	***p*-value**
Baseline (No MP at 18°C)	-1.66	0.43	<0.001
Temp. (26°C)	-1.00	0.63	0.110
Low MP	0.59	0.47	0.204
High MP	0.94	0.48	0.048
Date 2020	1.55	0.40	<0.001
Temp * Low MP	-0.59	0.87	0.494
Temp*High MP	1.34	0.73	0.068

AIC	**BIC**	**Shapiro test:**	
350	374	W= 0.990	*p*=0.35

The evaluation of outliers revealed nine values had influential Cook's distance. When these values were removed it slightly reduced AIC and BIC values from 350 and 374 to 317 to 341 respectively, while increasing Nagelkerke's R^2 by 1.7% (Table S4.13) while meeting assumptions of normality and homogeneity of residuals. We chose to apply the model without influential values. Due to the significant effect across years, we fitted negative binomial models on the microbead count for each year. Diagnostic plots are displayed in Figures S4.6 and S4.7 for the 2019 and 2020 models.

Table S4.13: Negative binomial model showing the effect of temperature, microplastic and date of trials on the retention of microbeads by round gobies 26 h post-exposure, without influential outliers. Multiple comparisons were applied to this model.

Model	χ^2	**DF**	***p*-value**
Temperature	2.94	1	0.086
Microplastic	3.73	2	0.154
Date	16.44	1	<0.001
Temp *MP	12.53	2	0.002
Fixed effects	**Coef.**	**SE**	***p*-value**
Baseline (No MP at 18°C)	-1.72	0.45	<0.001
Temp. (26°C)	-1.05	0.64	0.099
Low MP	0.84	0.49	0.089
High MP	0.83	0.49	0.090
Date 2020	1.68	0.43	<0.001
Temp * Low MP	-1.18	0.97	0.221
Temp*High MP	1.41	0.74	0.060

AIC	**BIC**	**Shapiro test:**	
317	341	W= 0.990	*p*=0.40

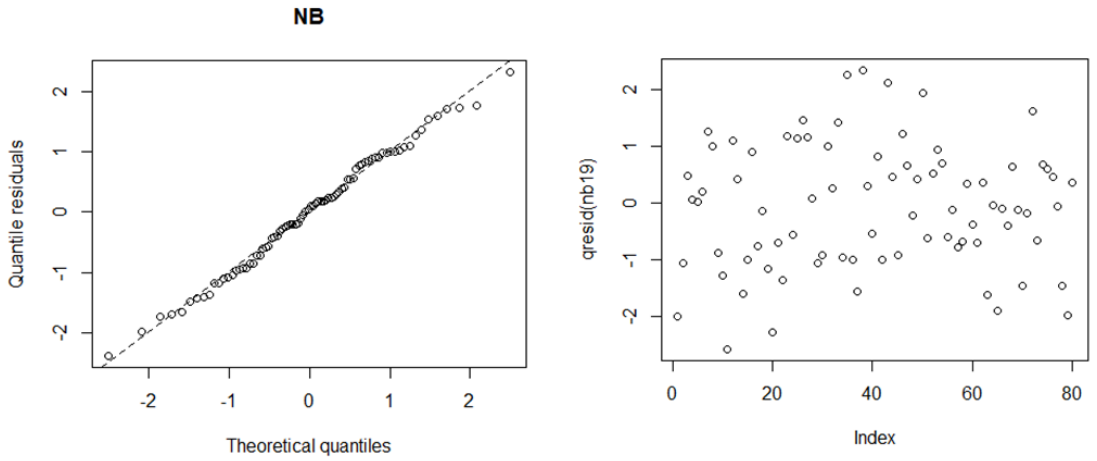

Figure S4.6: Diagnostic plots for the negative binomial model for 2019 microplastic contamination data. A) Quantile-Quantile plots of residuals and B) homogeneity of residuals. Shapiro test shows normality of model's residuals (W=0.99, *p* =0.96).

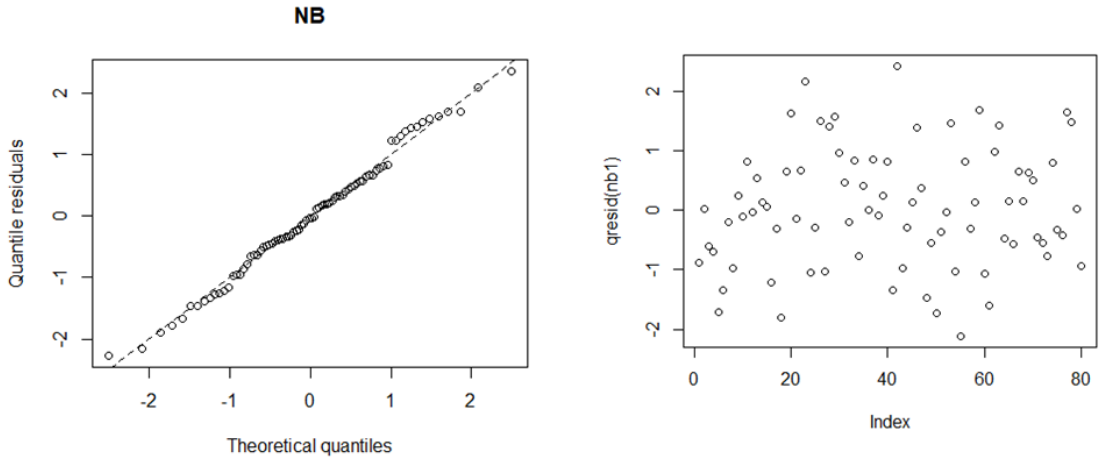

Figure S4.7: Diagnostic plots for the negative binomial model for 2020 microplastic contamination data. A) Quantile-Quantile plots of residuals and B) homogeneity of residuals. Shapiro test shows normality of model's residuals (W=0.98, p =0.66).

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5. General discussion and concluding remarks

5.1 Summary of main findings & contributions

Research on plastic pollution is rapidly evolving. As we learn more about the risks posed by this contaminant, researchers are adopting multidisciplinary approaches to tackle gaps in knowledge. Most studies view microplastic pollution under an ecotoxicological lens that can simplify the complexity of the plastic cycle in aquatic environments. Based on a community ecology perspective, my thesis investigated the mechanisms involved in the bioaccumulation of microplastics by aquatic organisms and examined their impacts and fate in aquatic food webs.

To summarize current knowledge about microplastic pollution and identify gaps that need to be addressed, I conducted a comprehensive review of the literature. Among the gaps and limitations described in our review (Chapter 1), I identified five core research areas that are further addressed in my thesis (Figure 5.1). Briefly, I highlighted the fact that microplastics are acting as a unique contaminant of inland waters; therefore, to facilitate environmental monitoring and inform policy for managing and reducing plastic waste, standardized benchmark sampling of waterbodies across spatial and temporal scales is required. The resulting knowledge would refine the scope of microplastic pollution, enabling the creation of more accurate contaminant cycling models for risk assessment.

Then, my co-authors and I paved the way forward by using a community module as an experimental model to study how microplastics are incorporated and transferred in aquatic food webs (Chapters 2 and 3). I advocate the application of such community modules, not only because they are more realistic than single-exposure scenarios, but also because they allow researchers to 1) examine multiple hypotheses concerning the fate of microplastics, 2) addresses

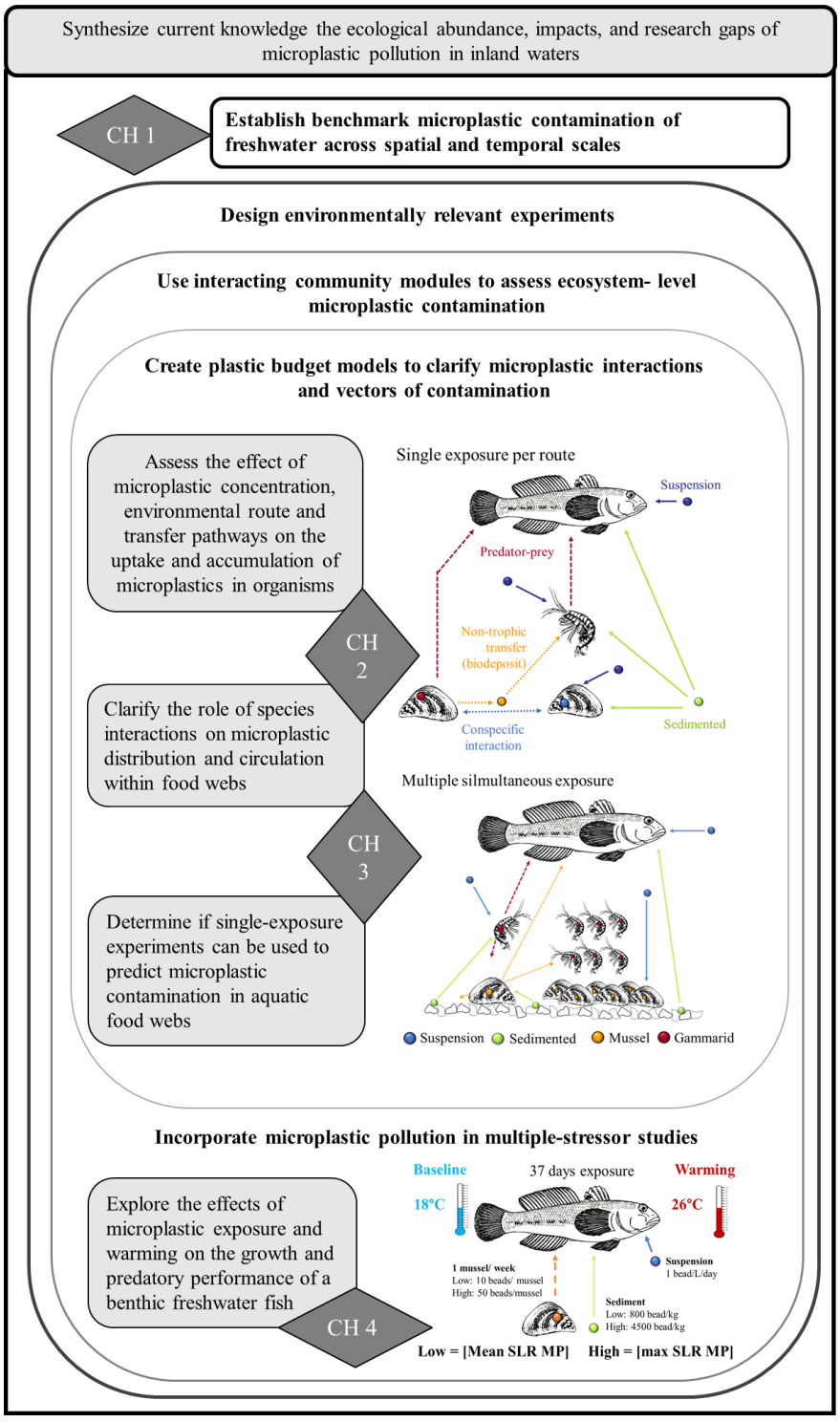

Figure 5.1: Summary of the conceptual foundations of this thesis. Research objectives (grey boxes), research gaps (bold font), and relevant chapters (diamonds) are indicated.

more complex interactive plastic cycling scenarios that approach situations observed in natural field conditions, and 3) predict pathways of interactions and potential cascading effects. By addressing some food web complexity, the community module approach helps to bridge a gap between field and lab studies.

I demonstrated that microplastic uptake occurs through different routes of exposure: particles in suspension, particles in surficial sediments, and particles transferred via species interactions. My work also showed that organismal contamination is largely regulated by the natural history of the implicated species and their ability to retain these particles (Chapter 2). I also confirmed that the concentration and route of exposure affect the contamination load experienced by each organism. I further established that species within a food web act as both recipients and vectors of microplastics, and that they circulate these particles through their interactions with their environment and other food web components (Chapters 2 and 3). My results highlighted the importance to account for different microplastic reservoirs (water, sediments, and organisms) as potential vectors of pollution. I demonstrated that animals of different trophic levels take up microplastics from multiple origins when exposed to each matrix individually (Chapter 2) and simultaneously (Chapters 2 and 3).

In Chapter 3, I also showed that single-exposure experiments conducted in the laboratory can help to identify routes of microplastic transfer within a food web and the potential level of contamination experienced by an organism. Indeed, microplastic contamination predicted by adding single routes did not significantly differ from the contamination observed under simultaneous exposure to all routes. Therefore, laboratory experiments can account for a fraction of the complex interactions between an organism and its physical and biological environment when all pathways are included. Under these conditions, it is possible to predict contamination

levels from the sum of all routes predicted by the single-exposure scenario. Although each recipient of our community module can take up and retain microbeads from its environment and from trophic and non-trophic species interactions, round gobies (and probably most predators in any food web) did not biomagnifying microplastics particles. Albeit the number of microplastics acquired by goby was highly context-dependent and varied according to the behaviour of the species present within its community.

Using our knowledge of how round goby takes up microplastic particles from its environment and its prey (Chapters 2 and 3), I used microplastic concentrations observed in the St. Lawrence River (Castañeda et al. 2014; Crew et al. 2020) and in resident species (Table S0.1) to design an environmentally relevant study that evaluated the potential effects of microplastic pollution under different climate warming scenarios. I found that temperature had a stronger influence than microplastic pollution on the growth and feeding rates of the round goby. However, despite observing low microplastic burdens in fish, the 37-day exposure to realistic microplastic concentrations reduced the predatory performance of round gobies. Current and near-future multiple stressor scenarios should incorporate microplastic pollution when evaluating potential additive, antagonistic, and synergistic impacts of climate change and other stressors on freshwater biota.

Overall, my thesis has shown that our knowledge of microplastics cycling in aquatic food webs is advanced by the application of a community ecology approach in experimental design, thus allowing more accurate comparison between laboratory findings and field realities. We applied concepts and methods from community ecology to ecotoxicological questions—an approach rarely adopted, but necessary to understand the impacts of synthetic pollutants (in this case microplastics) on ecosystems (Bernhardt et al. 2017). Our module strategy clarified 1) how

components of food webs acquired and retained microplastics, 20 identified main routes of transfer for key benthic community species, and 3) evaluated how current and projected increases in plastic pollution and water temperatures can affect the stability of riverine ecosystems.

5.2 Implications, limitations, and future directions

Studies addressing the source, fate, and impacts of microplastic pollution constitute a burgeoning multidisciplinary area of research that is confronting challenges to science and management that have served as a driving force for further research. For example, at the start of my thesis in 2017, only 17% of the 900 studies published on microplastic pollution focused on inland waters, whereas by the end of 2020 this fraction had increased to 27% (Figure 1.2). As the number of studies grows, the ubiquity of this pollution across ecosystems is increasingly evident. However, despite its rapid growth this science is still in its infancy and several gaps in knowledge have emerged that impede consensus on the ecological risks of this pollution.

5.2.1 Multi-dimension contaminant requires a multidisciplinary approach

A first consideration is that microplastics are multidimensional contaminants whose risks and threats are affected by the shape, size, polymer type of the particles, as well as their interactions with other substances (Bucci and Rochman 2022). Therefore, the study of microplastic pollution requires increasingly multidisciplinary approaches. It also represents a valuable opportunity to bring together areas of research that traditionally exist in silos (e.g., socioeconomic, landscape ecology, environmental chemistry), where diverse expertise can be employed to examine all facets of this new stressor. However, owing to the uniqueness of this contaminant, most or virtually all microplastic studies differ in fundamental ways, such that the

results of one study cannot be easily compared with others or used to draw broad conclusions beyond the context of the study design. The results we presented in terms of microplastic dose responses and retentions are specific to the organisms' responses to pristine micro-polyethylene beads of a narrow size range (53–63µm). We recommend future studies examine how microplastic particles of different shapes (e.g., fibres, fragments), sizes (1–1000 µm), polymer composition (other than PE) are taken up and distributed by the organisms of this community module as well as a diversity of key species from different food web configurations.

5.2.2 Improve scope and quality of environmental microplastic sampling

Experts in the field of microplastic science have proposed to develop standardized protocols and control measures to allow data to be reproducible and comparable across ecosystems and across studies (see Chapter 1). The lack of standardized methods has been problematic when trying to compare microplastic abundance and diversity across taxa or ecosystems. For example, as I compiled evidence of microplastic accumulation across aquatic, estuarine and marine systems (Chapter 1), I was forced to exclude many studies which did not report data in comparable units, had insufficient sample size, or did not verify the composition of the particles found. To alleviate these issues, we strongly advocate that microplastic sampling of inland waters is integrated into standard limnological sampling protocols (Chapter 1) that would account for the presence and diversity of this pollution. Recently, there was an effort to develop standardized limnological field methods for an extensive survey across Canada (NSERC Canadian Lake Pulse Network 2021), and it would be ideal if microplastics sampling could be added to an updated version. Future sampling must also be done across a broader diversity of aquatic habitats and capture the different matrices in which these particles accumulate and interact (i.e., water surface, water column, riparian/beach sediments, bottom sediments, etc.).

More sampling is needed across temporal (including seasonal) scales, perhaps aided by sediment coring techniques, which are an underexploited tool to track the presence and diversity of plastics over time (e.g., Turner et al. 2019).

Under Canada's Plastics Science Agenda, experts in the field dedicated to sediment sampling identified the need to establish benchmark levels of plastics across environmental compartments, identifying lakes, rivers and wetlands as priority areas (Plastic Pollution Science Framework Café Backgrounder documents presented by Environment and Climate Change Canada on November 18, 2021, *pers. comm*.). In my thesis, I outlined the importance of microplastic storage in freshwater sediments and offered examples demonstrating how sediment-associated biota is already impacted by this pollution (Chapter 1). Yet, most sediment sampling efforts to quantify microplastics in sediments have been in the Great Lakes Basin (Chapter 1, Table 1.1), supporting the idea that more sampling is required to understand the extent of this pollution in Canada. To tackle such a costly effort, microplastic laboratories could collaborate with limnologists and other specialists that previously sampled lakes, rivers and wetlands. For example, sediment cores were sampled from over 664 lakes across all ecoregions of Canada under the NSERC Canadian Lake Pulse Network (Huot et al. 2019). Subsamples of these cores could be used to quantify microplastic concentrations and rates of accumulation in lake sediments and thus provide a valuable benchmark for microplastic pollution over space and time. Given the breadth of this large-scale interdisciplinary project, many other environmental and ecological data were collected (e.g., nutrient concentration, plankton samples, dissolved gases, subfossils, environmental DNA; see Huot et al. 2019) which could be coupled with microplastic concentrations to reveal indicators and drivers associated with high contamination levels and identify hotspot areas of microplastic accumulation across the country.

5.2.3 Handling contamination

The nature of microplastics as unique contaminants and the ongoing development of experimental protocols also causes difficulties in using laboratory studies to draw conclusions about the behaviour of microplastics in nature. For example, we noted the presence of microplastic contamination in the controls of our experiments (Chapters 2 and 4) despite the application of a rigorous quality assurance protocol. There is no standard method in the literature for correcting this contamination (e.g., removing mean or maximum values, etc.), even though the method chosen can have important repercussions on research outcomes and data analyses.

When organisms are exposed to high concentrations, the presence of contamination (or any correction method chosen) will not have a significant impact on the outcome—as the retention is relatively high in these animals. However, we see that the presence of even 1 microbead per individual in the control treatment can be amplified and yield high body burdens. For example, the shaded violet and green bars in Figure 5.2 (for suspended and sedimented beads, respectively) indicate the threshold concentration at which the body burden significantly differs from the body burden from the controls. These results suggest the microplastic burdens of animals under environmentally relevant concentrations ≤ 0.1 microplastics$\cdot$ml^{-1} (Cunningham and Sigwart 2019), represented by the red dotted line in Figure 5.2, do not differ from environments deprived of microplastics. Although we do not expect to find a habitat completely devoid of microplastic particles, the presence of contamination in controls indicate our experiments cannot be used to precisely predict body burdens of organisms under natural contamination levels. Assuming microplastic contamination is common under controlled experiments, identifying contamination becomes challenging when assessing a diversity of particle shapes, types, and polymers of unknown nature as those from field samples.

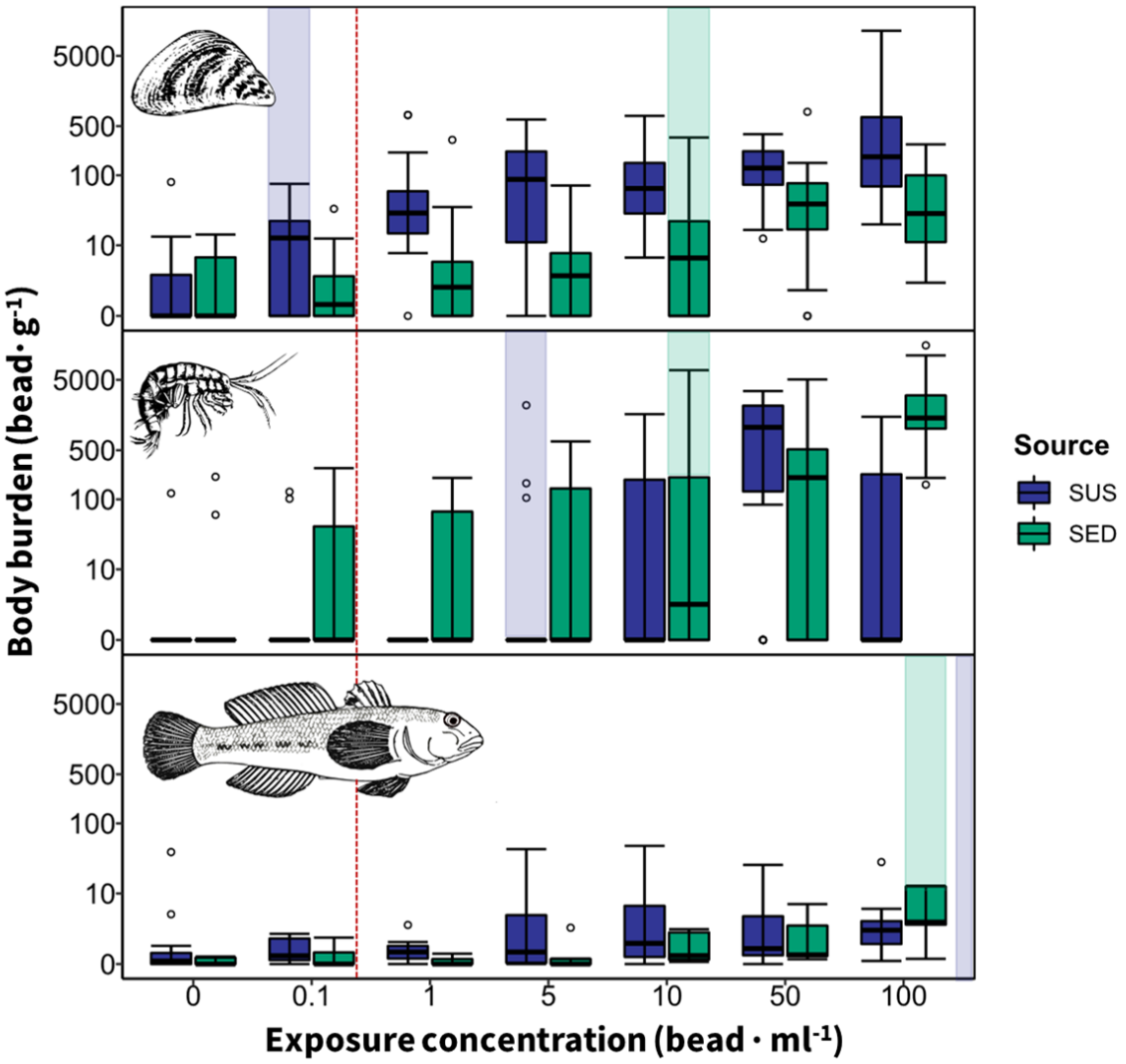

Figure 5.2: Body burden data acquired from single exposures to suspended (violet) or sedimented (green) microbeads across 7 concentrations for mussels (top), gammarids (middle) and round goby (bottom). Highlighted bars indicate where the body burden of a particular exposure level is significantly different from that of the controls (a concentration of zero). Concentrations below the red dotted line represent environmentally relevant exposure levels.

I advocate that authors publishing their work should be more transparent on how the data were acquired, transformed (e.g., contamination correction), and analyzed (e.g., statistical design and analyses). Such steps would enable early career researchers, in particular, to develop more

rigorous studies, better data analyses and correction practices, and promote the incorporation of this information as standard practice in published work.

Future work should also compare the use of the most common protocols to standardize microplastic assessments and provide some mechanism allowing calibration of adjustment factors to allow comparison. Recently, quality assurance guidelines (Cowger et al. 2020) and microplastics metadata templates (Jenkins et al. 2021) have been proposed. Such initiatives are strongly encouraged but require more promotion so that they are used by scientists. We invite current and future work should attempt to use these checklists and metadata template when they design their experiments.

5.2.4 Designing environmentally relevant experiments

The design of environmentally relevant experiments implies the inclusion of the complexity of the plastic cycle and the use of environmentally relevant concentrations. Microplastics are present in all matrices including sediments and water (Chapter 1), but also in species that act as transient recipient and potential vectors of microplastics (Chapters 1, 2, and 3). The results of our experiments emphasize the need to acknowledge the contribution of each matrix to the levels claimed to be an environmentally relevant exposure. We recommend that future work couple biological field surveys of microplastics with analyses of microplastic from surface waters and sediments to examine spatiotemporal variation in environmental contamination, and its relation to the body burden of different functional groups. This would elucidate the role of abiotic and biotic contexts in the uptake of microplastics.

In addition to sampling environmental matrices, community modules relevant to different waterbodies should be employed as sentinels of microplastic pollution in the field after exploring their different routes of transfer and cycling under laboratory or mesocosm experiments. We

targeted freshwater food webs and used a short community module. Similar mechanisms are at play in all ecosystems (terrestrial, marine) and a module approach could be useful to track pollution within an environment of interest over time and study potential effects of this pollution under laboratory conditions.

Both trophic and non-trophic species interactions (Chapter 2) and the composition of species assemblages (Chapter 3) influence the availability, retention, and transfer of microplastics within the community; therefore, it is necessary to account for interactions between environmental matrices and species within the community to understand the distribution and cycling of microplastic in food webs. The natural history of organisms can help inform hypotheses on how microplastics are acquired and distributed (Chapter 2). However, species behaviour is adaptable and flexible to the context and the presence of this stressor (Chapter 3), which can induce responses that differ from those observed for other chemicals or environmental stressors. Understanding the natural history of animals can improve our modelling of microplastic distribution and accumulation in food webs. Techniques used in trophic ecology could be usefully applied here. For example, stable isotopic signatures are commonly used to validate community structure in a group of organisms comprised of species from different trophic levels (Post 2002; Layman et al. 2012; Middelburg 2014). Likewise, fatty acid composition in the tissues of targeted species can also be used to elucidate trophic relationships and consumer diet (Iverson 2009; Growns et al. 2020; Twining et al. 2021; Góra et al. 2022). These methods could be incorporated when sampling species in the wild, and these results can be combined with observed organismal concentrations of microplastic particles to clarify the roles of different species within a community. Such information could also be used to parametrize

bioaccumulation models that are better suited than laboratory experiments to explore the transfer of microplastics within complex food webs (O'Connor et al. 2022).

The design of environmentally relevant experiments implies the inclusion of realistic microplastic exposure scenarios. This necessitates stepwise knowledge of the presence of microplastic in different matrices of the system of interest (e.g., microplastic concentrations from field data) and a preliminary understanding of how the organism acquires, retains and evacuates the particles (Chapter 2). We recognize that to maintain realistic conditions in laboratory experiments (by allowing organisms take up particles naturally rather than injecting or spiking foods artificially) may bring higher variability to the results obtained (Chapters 2 and 3). A tradeoff is necessary to better reflect the complexity of natural systems. It is also virtually impossible to replicate all stressors experienced by organisms in the field under laboratory settings. However future work could try to add complexity to experiments by adding the presence of associated contaminants that are acquired by exposing the microplastics to field conditions via "natural" sorption. These plastics could then be used to compare the dose-response, retention, accumulation and transfer results in the same community module.

5.2.5 Fate of freshwater ecosystems under multiple-stressor scenarios

Microplastic pollution will continue to be coupled to other anthropogenic stressors (e.g., climate change affecting land use change) and may act synergistically to threaten the health and functioning of freshwater systems. Our results found that even with relatively low retention rates and no observed biomagnification, fish exposed to realistic microplastic concentrations under current and elevated temperatures representing conditions experienced in the St. Lawrence River show declines in their feeding rates (Chapter 4). If this reduced predator performance in the

laboratory accurately mirrors impact in the field, it could conceivably have cascading effects on aquatic food webs.

Changes in land use, such as increases in urbanization or agricultural activities in a watershed, have been shown to cause accelerated sedimentation in lakes across the globe (Baud et al. 2021). These and other anthropogenic global changes could drive further sedimentation and altered flow regimes in various lentic and lotic systems, which would likely change the distribution and local retention of microplastics. I recommend pursuing research on the effects of combined stressors to understand their potential consequences on the abundance, distribution, and impacts of microplastics in aquatic ecosystems.

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