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The effects of exposure to microplastics and pollutants on the arthropod microbiome

JAVIER EDO VARG

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Abstract

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Anthropogenic pollution is widespread across various ecosystems. This disturbance can alter the interaction between a host and its associated microbiome, with repercussions for hosts traits such as health, behavior, and host evolution. The thesis aim is to understand the effects of inert microplastics and other pollutants, as pesticides, detergents, and toxic metals, on the host-microbiota of different freshwater invertebrates. Specifically, this thesis explores the secondary effects of stress factors on the host, trophic interactions, and free-living microbes. **Chapter I** tested the effects of microplastics and the pesticide esfenvalerate on *Chironomus riparius* survival, emergence, and its microbiome. **Chapter II** tested the effects of microplastics and the pesticide deltamethrin on a trophic chain of three organisms: *Daphnia magna*, damselfly larva *Ischnura elegans*, and wild dragonfly larva *Aeshna cyanea*. **Chapter III** tested the effects of microplastics and sodium dodecyl sulfate on the microbiome of wild water boatman from the family *Corixidae*. **Chapter IV** tested the effects of microplastics and Chromium VI on *Daphnia magna* mortality and its microbiome. The thesis used metagenomic tools to characterize both the host microbiome and its surrounding microcosms. Our results showed that microplastics interact with additive toxicants to affect the host microbiome, however, these effects depend on the type of toxicant, the size of the microplastic, and the host itself.

Keywords: ecotoxicology, host microbiota, freshwater, anthropogenic pollution, microplastics, pesticides, pyrethroids, detergents, toxic metals, daphnids, chironomids, water boatmen, damselflies, dragonflies

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To my Demons

List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

- I Varg, E, J., Kuncce, W., Outomuro, D., Svanbäck. R., Johansson, F. (2021). Single and combined effects of microplastics, pyrethroid and food resources on the life-history traits and microbiome of *Chironomus riparius*. *Manuscript*
- II Varg, E, J., Outomuro, D., Kuncce, W., Kuehrer, L., Svanbäck. R., Johansson, F. (2021). Microplastic exposure across trophic levels: effects on the host microbiota of freshwater organisms. *Manuscript*
- III Varg, E, J & Svanbäck. R. (2021). Multi stress system: microplastics in freshwater and their effects in host microbiota. *Manuscript*
- IV Varg, E, J & Svanbäck. R. (2021). The stressful effects of microplastics associated with chromium (VI) on the microbiota of *Daphnia Magna*. *Manuscript*

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Abbreviations

MPs – Microplastics

DMT – Deltamethrin

Cr(VI) – Chromium (VI)

SDS – Sodium dodecyl sulfate

DNA – Deoxyribonucleic acid

RNA – Ribonucleic acid

PCR – Polymerase chain reaction

ASV – Amplicon sequence variant

PERMANOVA – Permutational Multivariate Analysis of Variance

PCoA – Principal Coordinates Analysis

MANOVA – Multivariate Analysis of Variance

GLM – Generalized Linear Model

GLMM – Generalized Linear Mixed Model

GLS – Generalized Least Squares fit model

1. Introduction

The diversity of microorganisms and microbial communities are ubiquitous, and they are cornerstones for the evolution of life (Foster et al. 2017, Sharpton 2018). All the living assemblage of microorganisms (bacteria, archaea, fungi, algae, small protists), and all genetic content including mobile genetic elements such as viruses, plasmids, and transposons are known as the microbiome. The majority of microbes in the microbiome are essential for the ecosystem function and have coevolved with the macro-organisms they inhabit (Maynard et al. 2012, Stilling et al. 2014, Moran and Sloan 2015, Douglas 2016, Wasielewski et al. 2016, Richardson 2017, Gould et al. 2018, Berg et al. 2020). Macro-organisms hold an immense and diverse community of microbes in and on their bodies. This host-microbiome has been shown to play an important role in many aspects of the host's function; microbiome studies have a bacteriocentric view due to the very high functional bacterial diversity that inhabit the host (Cnit et al. 2014, Marchesi et al. 2015). These microorganisms are very important for physiological processes and life-history traits such as health, growth, and development of the host (Nicholson et al. 2012, Jin et al. 2018, Zha et al. 2018). Similarly, it is well known that the host-microbiome is a key dimension of biochemical processes of every single organism affecting nutrition, immunity maturation, pathogen resistance, and host behavior (Huttenhower et al. 2012, Hanning and Diaz-Sanchez 2015, Roger et al. 2016, Foster et al. 2017).

Fitness components such as survival, fecundity, growth, development and lifespan are also affected by the microbial communities that inhabiting the host (Huttenhower et al. 2012, Tremaroli and Bckhed 2012, McFall-Ngai et al. 2013, Sommer and Bckhed 2013, Rosenberg and Zilber-Rosenberg 2014, Souza et al. 2019). For example, the results of Gould et al. (2018) suggest that microbial composition and the timing of the interaction can highly impact lifespan as well as life-history tradeoffs in the fruit fly *Drosophila melanogaster*. Several studies have reported effects in fitness and life-history traits in diverse host organisms as a result of changes in microbial composition (Seung Chul et al. 2011, Semova et al. 2013, Kohl et al. 2016, Butt and Volkoff 2019). The magnitudes of the interactions in the microbial structure can be a major driver of host physiology (Gould et al. 2018). However, the effects in life-history traits and microbiome can as well

be due to factors such as diet (including the prey microbiome) and host genetics (Bolnick et al. 2014, Dehler et al. 2017, Gould et al. 2018).

1.1 Microplastics

Since microorganisms grow in relatively stable conditions and nutrients, both host-associated and free-living microorganisms can be affected by environmental stressors such as pollutants. Exposure to pollutants such as microplastics (MPs) can affect the host-microbiome (Jin et al. 2018, Lu et al. 2018, Zhu et al. 2018, Qiao et al. 2019). MPs are plastic debris < 1mm with different shapes that include fragments of plastic items that undergo some kind of degradation: such as physical (wind, waves, and sand abrasion), chemical (salting and burial in sand rich in organic matter), photochemical and thermal (Costa et al. 2009, Browne et al. 2011, Cole et al. 2015a). Ingesting MPs instead of food can modify the host environment and its gut and provide a new carbon source in addition to providing a novel surface that only some microbes can colonize (Zettler et al. 2013, McCormick et al. 2014, Lu et al. 2018, Jacquin et al. 2019, Amaral-Zettler et al. 2020). Such changes in the microbial communities, known as dysbiosis, could end up in a loss of important functions for the host. For example, microbiota have a role on the host immune response, development and function of vital organs (e.g. the brain), and production of key elements, e.g. vitamin B12 (Kock and Schmid-Hempel 2011; Bennett et al 2015; Farmer et al 2014; Marchesi et al 2015). Thus, the presence of ingested MPs with novel bacterial gut or skin colonization might change the microbial community and subsequently alter host responses and host metabolisms.

The presence of more than 100 000 microplastic particles per m³ of debris is considered to be an environmental problem (UNEP, 2005; Eerkes-Medrano et al 2015). In fact, a large variety of aquatic organisms have been reported to ingest and accumulate MPs, from zooplankton to fish (Mattsson et al 2015; Cole et al 2015). One of the major concerns about MPs is thus that they can enter food webs via direct ingestion because of their bioavailability in water environments and terrestrial ecosystems (Cole et al 2013; Tanaka 2013). However, there are also indirect ways to be exposed to MPs such as ingestion through predation on organisms that contain MPs. Hence, MPs can be transported in the food chain (Nowack et al 2007; Zhu et al 2008; Ferry et al 2009; Mattsson et al 2015).

The effects of microplastic ingestion can either be physical damage or toxic, depending on the material and whether the plastic is associated with a toxic chemical. This may compromise individual fitness by reducing feeding, energetic reserves, growth rate, fecundity, and survival (Cole et al 2016).

Moreover, the bioavailability of MPs and their possible interactions with other pollutants such as toxic metals, pesticides, and normal household products such as detergents might be synergistic (Tanaka et al. 2013, Rochman et al. 2014, Wang et al. 2016, Wagner and Lambert 2018, Felten et al. 2020). The joint synergic effects of MPs and toxicants on organisms and their microbiome remain unexplored and constitutes one of the new frontiers in toxicology.

1.2 Pesticides

Pesticides are organic chemical compounds that are designed specifically to interfere the organismal biochemistry to kill or prevent the growth of undesired organism i.e. pest and pathogens (Bernhardt et al. 2017). Pyrethroid pesticides such as Deltamethrin (DMT) and esfenvalerate are known for their neurotoxic effects. Pyrethroids are extensively applied in agriculture, aquaculture, and forestry as pest control agents (Mestres and Mestres 1992, Hong et al. 2020), and they can enter the aquatic ecosystem via stormwater run-off from urban landscaping and home gardens as well as wastewater effluent and agricultural run-off (Jiang et al. 2010, Weston et al. 2013). DMT can negatively affect a variety of organisms including mammals and birds, but it is highly toxic to aquatic organisms such as fish and aquatic arthropods (Dawood et al. 2020, Hong et al. 2020). Esfenvalerate has also been shown to have negative effects on the survival and development on many aquatic invertebrates (Beketov 2004, Forbes and Cold 2005, Amweg et al. 2006, Brady et al. 2006, Rasmussen et al. 2013, Rodrigues et al. 2015). Pyrethroids to control pests acts mainly in the excitable membrane focusing on the voltage-gated Na^+ channel of the nervous system, including transduction signals (Mestres and Mestres 1992, Hong et al. 2020). Moreover, the effect of pyrethroids in non-target organisms might be heightened due to the presence of other stressors, nutritional deficiencies, or pollutants such as MPs (Menéndez-Pedriz and Jaumot 2020). Studies on the combination of both MPs and pyrethroids are scarce and do not take into account its effects on the microbial communities both in the environment and in the host (Zettler et al. 2013, Horton et al. 2018, Felten et al. 2020).

1.3 Detergents

Detergents are highly surface-active chemical compounds (i.e. surfactants) with a complex structure that are able to disorganize cell membranes and denature proteins (Baker et al. 1941, Sobrino-Figueroa 2018). The effects of detergents and their toxicity in aquatic organisms have been studied since 1950 (Czyżewska 1975), yet detergents continue to be used daily worldwide

and released in aquatic environments. Prolonged exposure to detergents such as Sodium dodecyl sulfate (SDS) can induce paralysis, affect biological function, and end in the death of the organism (Czyzewska 1975, Sobrino-Figueroa 2018, Gerasimidis et al. 2019). MPs and detergents can be manufactured together or can converge in water environments after disposition (Sibley and Hanson 2011). Though it is well known that detergents also function as bactericides (Baker et al. 1941, Brandt et al. 2001, Mousavi and Khodadoost 2019), we lack knowledge on how detergents can affect host-associated microorganism as well as the possible interactions that detergents might have with MPs.

1.4 Metals

Metal ions are known to be highly toxic and can occur in food, water, or air (Tchounwou et al. 2012). Because of its toxicity, chromium is one of the toxic metal ions ranked as a priority with great public health significance (Tchounwou et al. 2012). Chromium, specifically the state VI (Cr(VI)), has been attributed to highly carcinogenic, nephrotoxic, hepatotoxic, neurotoxic, and mutagenic effects along with other pathophysiological effects (Zeng et al. 2016, Bojarski et al. 2021, Guo et al. 2021). Cr(VI) is the most toxic state of chromium and it is found in water environments because of several industrial processes such as leathering tanning, electroplating manufacturing of dye, metal finishing, and production of paint and paper (Pérez-Candela et al. 1995, Vargas and Dussán 2016, Guo et al. 2021). Cr(VI) can affect macro-organism due to its oxidizing properties and ability to cross biological membranes causing an acute exposure. In microorganisms, the toxicity is mainly to the direct damage in biological membranes, cell structure, and protein function (Babich et al. 1980, Breton et al. 2013, Lemire et al. 2013, Hose et al. 2016, Assefa and Köhler 2020, Bojarski et al. 2021). The presence of MPs together with metal ions is well known (Pietrojusti et al. 2015, Häder et al. 2020). However, studies of the synergic effects of MPs and metal ions on host physiology and host-microbiome dysbiosis are needed.

1.5 Aims of the thesis

In this thesis I use laboratory experiments on a variety of aquatic arthropods to answer the following questions:

1. Is the diversity of the host-microbiome affected by single and mixed exposure to MPs and toxicants? (I - IV)
2. How does diet affect the host-microbiome in single and mixed exposure to MPs and toxicants? (I and II)
3. How do single and mixed exposure to MPs and toxicants affect host life-history traits? (I, III and IV)
4. How do single and mixed exposure to MPs and toxicants affect feeding behavior? (II)
5. How do single and mixed exposure to MPs and toxicants affect host-microbiome through the trophic chain? (II)
6. Do single and mixed exposure to MPs and toxicants affect the free-living microbiome in the same way as the host-microbiome? (II-IV)

2. Methods

2.1 Microcosms

I tested the effects and interactions of MPs and toxicants (esfenvalerate, deltamethrin (pyrethroids) or sodium dodecyl sulfate or chromium VI) on *Chironomus riparius* (Meigen 1804), *Daphnia magna* (Straus, 1820), damselfly larva *Ischnura elegans* (Vander Linden, 1820), a wild dragonfly larva *Aeshna cyanea* (Müller, 1764), a water boatman from the family Corixidae (Leach, 1815), and their microbiome. All the aforementioned pollutants are of high environmental and biological importance because they converge in water sources thus affecting non-target water arthropods. **Chapter I** used clay as bottom substrate for microcosms. **Chapter II-IV** used fresh water to perform the microcosms (Figures 1-4). I performed the experiments with 1 μm polystyrene-based latex spheres MPs (CAS# 0009003536, Polysciences, Inc.). But in **chapter II, I** used 3 μm MPs.

Figure 1. Experiment design of **chapter I**. The experiment was carried out in 165 mL glass jars containing artificial sediment and dechlorinated tap water to provide a ratio of sediment to overlying water of 1:4. The vessels were maintained at $19\pm 1^\circ\text{C}$ and a 18:6 hour (light:dark) photoperiod. Due to fungal contamination the number of replicates ends as follow for the high food level: Control 11, MPs 11, esfenvalerate 16, and MPs+Esfenvalerate 13 replicates. Low food level: Control 28, MPs 27, esfenvalerate 25, and MPs+Esfenvalerate 25 replicates.

Figure 2. Overview of the experiment design of **chapter II**. *D. magna* was exposed to a control without MPs and a $3\mu\text{m}$ of polystyrene-base latex MPs in a concentration of 7.8×10^5 . *I. elegans* were exposed and not exposed to $0.2\text{ }\mu\text{g/L}$ of deltamethrin and fed with *D. magna* exposed to or not to MPs. *A. cyanea* were feed with *I. elegans* exposed to the three treatments. A total of 15 *A. cyanea* were tested individually per treatment. Dechlorinated tap water was used to perform the experiment.

Figure 3. Graphical representation of the experimental design of **chapter III**. Heteroptera water boatmen from the family Corixidae were exposed to $1\mu\text{m}$ polystyrene-base latex MPs, sodium dodecyl sulfate (SDS), and the combination of both MPs and SDS. Water boatmen in the control were not exposed to MPs or SDS. The lake treatment is the direct metagenomics of the water boatman and the $165\mu\text{m}$ filtered lake water (i.e. No microcosm was made for the lake treatment), 6 replicates per treatment were used.

Figure 4. Experimental design of **chapter IV**. *D. magna* was exposed to 1 μ m polystyrene-base latex MPs, two concentrations of Cr(VI), and the combination of both MPs and Cr(VI). Daphnids were not exposed to MPs or Cr(VI) in the control. Water from the river Fyrisån, Uppsala Sweden was used in the experiment and 5 replicates per treatment were used.

2.2 DNA extraction and library preparation

All organisms were rinsed with Mili Q water prior to microbial extraction and analysis to avoid microbes from the water. The microbiome from the water was extracted from each replicate filtered with 0.2 μ m filter. DNA was extracted from the tested organism and the filter using DNeasy Powersoil (Qiagen, No./ID: 12888-10) as recommended in the manufacturer's protocol with an additional incubation at 65°C for 10 min after adding the C1 solution and additional 30 minutes of the bead homogenizer step. The 16S ribosomal RNA gene was amplified in a two-step PCR using primer pair 515F and 805R that flanks the hypervariable region V4. For the first step, PCRs were performed in triplicate using Phusion High-Fidelity DNA polymerase (Thermo Fisher Scientific, No./ID: F-530XL). Thirty cycles were performed following the Phusion polymerase protocol. Negative controls were run during DNA extraction and 16S PCR amplification to check for contamination. Triplicate PCR products of each sample were pooled and subsequently purified using AMPure XP magnetic beads (Beckman Coulter, No./ID: A63882). For the second step, Illumina adaptor sequences and barcodes were attached to the PCR primers to provide each sample with a unique identifier. Samples were then purified again using magnetic beads. An equal concentration of DNA from each sample was pooled and run through agarose gel. Then, the 400-500 bp band was excised and purified using the QIAquick gel extraction kit (Qiagen, No./ID: 28104). PCR products were sequenced on Illumina-

MiSeq to obtain 250 bp paired-end reads at Science for Life Laboratory (SciLifeLab, Uppsala, Sweden).

2.3 Sequencing data statistical analysis

In all the **chapters I- IV**, the tested organisms microbiome amplicon sequence variant (ASV) tables were created using demultiplexed data sequenced by the SciLifeLab and following the DADA2 pipeline 1.8 (Callahan et al. 2016). The alpha diversity and the most abundant phyla were calculated using the R packages lattice (Sarkar, 2008) and MASS (Venables and Ripley, 2002). A diversity analysis (phylogenetic, Shanon and Chao) was performed to obtain ASV phylogenetic biodiversity, ASV evenness, and ASV richness, using the R package fossil (Vavrek 2011), vegan (Oksanen et al. 2018), ape (Paradis and Schliep 2019), and picante (Kembel et al. 2010). To test the effects of the exposure to MPs and the associated toxicant on the microbial alpha diversity, linear models were carried out using the diversity indexes as the response variable and the exposure to MPs and the associated toxicant (presence/absence) as fixed effects. Due to lack of normality, data were transformed (log10 or square root transformed) or tested through permutation analysis with 9999 permutations to confirm the robustness of the parametric model (Hothorn et al. 2006). Post-hoc tests were carried out for pairwise comparisons using the R packages FSA (Ogle et al. 2020) and rcompanion (Mangiafico, 2020).

The beta diversity was assessed also for all **chapters I-IV** using Permutational Multivariate Analysis of Variance (PERMANOVA) using the R package vegan (Oksanen et al. 2018). The ASV distance matrix was used as a response variable, including the exposure to MP and DMT (presence/absence) as factors. To observe how the microbial communities cluster between treatments, a Principal Coordinates Analysis (PCoA) was performed. A Multivariate Analysis of Variance (MANOVA) was assessed using the top 4 or top 6 most abundant phyla as a response variable and MPs and the associated toxicant as fixed factors. To observe the effects of MPs and the associated toxicant in the relative abundance of the individual most abundant phyla, a Generalized Linear Model (GLM) with a quasi-Poisson family were performed. All the statistical analyses were executed in R statistical Computing Language 3.6.2 (R Core Team 2020). The phylogenetic tree and the taxonomy plots were created using Qiime 1.9.9 (Caporaso et al. 2012).

2.4 Life history statistical analyses

In **chapter I**, Binomial generalized linear models with a logit link function were performed to assess the effects of MPs and esfenvalerate on the survival, emergence, and sex ratio on Chironomid larvae. A Generalized Linear Mixed Model (GLMM) with family Poisson and random effects was run to test the effects of the MPs and esfenvalerate on the Chironomid development time using vessel as a random effect. In **chapter II**, to test the effects of the MPs and the DMT on the damselfly survival against dragonfly predation at 10, 20, 30, 60, 120, 180, 840, and 1440 minutes a Generalized Linear Mixed Models (GLMM) with multivariate normal random effects, using Penalized quasi-likelihood was performed using MASS (Venables and Ripley 2002) and nlme R packages (Pinheiro et al. 2020). Predated damselfly was used as the response variable, time was entered as a covariate, and MP and DMT as fixed factors. Dragonfly ID was entered as a random effect and to account for repeated measures an autocorrelation was included with time as continuous covariate and dragonfly ID as a grouping effect. In **chapter III**, to analyze the effect of MPs and SDS individually and their combined effects on the mortality (%) of the water boatman at 24, 48, 72, and 168 h a Poisson GLM was performed at each individual time. In **chapter IV**, the effects of MPs (two-level factor: presence/absence) and Cr(VI) (three-level factor 0,2,5 ppm) on the *D. magna* mortality at 12, 18, 24, 28, 30, 40, 52, 66, and 72 hours was tested using Generalized Least Squares fit model (GLS) using nlme R packages (Pinheiro et al. 2020). To account for repeated measures an autocorrelation was included with time as a continuous covariate and *D. magna* vessel as a grouping effect. An additional quasipoisson Generalized Linear Model (GLM) was used to assess the effects of MPs (two-level factor: presence/absence) and Cr(VI) (three-level factor 0,2,5 ppm) at 40h and 72h individually.

In all cases post-hoc test were performed to observe differences between treatments using the functions emtrends and emmeans of the R package emmeans (Lenth et al. 2020). All the models were also executed in R statistical Computing Language 3.6.2(R Core Team 2020).

3. Results and Discussion

3.1 Effects of MPs alone on the host-microbiome

There were host-specific effects of MPs on host-microbial communities. For *C. riparius*, there was no major effect due to MPs, and I found that only Shannon microbial diversity index was affected significantly due to the MPs (**Chapter I**). Significant changes in diversity and abundance of gut microbiome due to MPs have been shown before in vertebrates, in contrast to the low effects that I found in *C. riparius* (Lu et al. 2018, Jin et al. 2019). Interestingly, in **chapter I**, I also found that the microbial diversity was highly dependent on the level of food (high or low, Figure 5a). Similar effects of diet in the microbiome have been found in other aquatic organisms such as perch and arctic char (Nyman et al. 2017, Zha et al. 2018). In contrast, the experiments in **chapter II** showed that the MPs strongly affected the microbiomes of *D. magna*, the damselfly *I. elegans*, and dragonfly *A. cyanea*, Figure 5b. For the water boatmen microbiome in **chapter III**, I found effects of MPs on cyanobacteria, but did not find effects on microbial diversity (Figure 6a). I did not find significant effects of MPs on individual phyla on the *D. magna* microbiome in **chapter IV** (Figure 6b). I hypothesize that these differences among organisms could be explained in part by the size of the MPs as well as the MPs potential to be used as a carbon source and as a new colonization substrate (Zettler et al. 2013, Horton et al. 2018). It has been found previously that the effects of MPs vary depending on the size, the shape and material of the MPs (Besseling et al. 2014, Ma et al. 2016, Rist et al. 2017, Aljaibachi and Callaghan 2018, Felten et al. 2020). In **chapter II**, the differences observed could also be due to food chain effect where the severe changes in the microbiome found in the higher trophic levels could be due to the initial stress that the prey *D. magna* experience. Even if the MPs might or might not be in the individual after consumption is still the effect of the MPs on the consumer that is affecting individual's host microbiome. However, if the cause is due to direct effects of microplastic transfer or any indirect effect carried over via the predation remains to be investigated.

Figure 5. Phylum-level taxa diversity of a) the microbiome of *C. riparius*; the experiment was run at low and high food levels, b) *Daphnia*, Damselfly and Dragonfly's microbiome including the relative abundance of the water microbiome. Treatments were exposure to MPs and/or the toxicants esfenvalerate (Esfen), or DMT. The control replicates were not exposed to either MPs or any toxicants.

3.2 Effect of single toxicants in the host-microbiome

The pesticide esfenvalerate did not show significant effects in the microbiome of the chironomids (Figure 5a). Though there were observable changes in the microbiome, pesticide concentration may not have been high enough to affect the host microbiome and the host as microbial environment (**Chapter I**). On the other hand, in the experiments of **chapter II-IV** the toxicants highly affected the host-microbiome of the aquatic organism tested (Figure 5b, 6a and b). This is not surprising, since DMT is known for its neurotoxic effects that can cause stress in the host which affects its microbiome (Mestres and Mestres 1992, Toshio 1992, Hong et al. 2020), SDS is known for bactericidal and bacteriostatic effects which will directly affect the microbiome (Baker et al. 1941, Brandt et al. 2001, Mousavi and Khodadoost 2019), and Cr(VI) is known for being able to cross and disturb biological membranes affecting microorganism in the microbiome with no resistance mechanisms (Babich et al. 1980, Breton et al. 2013, Hose et al. 2016, Assefa and Köhler 2020, Bojarski et al. 2021). We argue that this effect depends directly on the toxicity of the toxicant itself. However, after inspecting the microcosm water used in **chapter II-IV**, I found that these toxicants influence the water microbiome in a similar way or even with more significant effects in diversity and abundance than in the host-microbiome. The severe observed dysbiosis in the water compared to the one observed in the host-microbiome might be explained due to the hosts ability to adjust the host environment, and resist the pollutants in the environment through mechanisms of multidrug resistances (Kurelec 1992). Due to its intrinsic characteristics, the host regulates the microbiome, being under strong natural selection to control its microbiota as it is the environment colonized by the microbiota (Foster et al. 2017, Berg et al. 2020). In general, changes in the host environment jeopardize the host itself so under pollutant stress the host has defense mechanisms to expel the stressor and maintain this environment(Kurelec 1992).

Figure 6. Phylum-level taxa diversity of a) Water boatman microbiome including the relative abundance of the water microbiome, and b) *D. magna* microbiome including the relative abundance of the water microbiome. Treatments were exposure to MPs and/or the toxicants SDS, or Cr(VI). The control replicates were not exposed to either MPs or any toxicants.

3.3 Combined effects of MPs and toxicants on the host-microbiome

In **chapter I**, I found no effects in the host-microbiome abundance, richness, and diversity in response to the combined MPs and esfenvalerate (Figure 5a). One explanation could be that microplastics might adsorb esfenvalerate from the water, reducing the concentration and exposure to larvae and microbes (Liu et al. 2020, Menéndez-Pedriz and Jaumot 2020). Nevertheless, the combined MPs and toxicant did show significant effects on some of the six top individual phyla of the host-microbiome (**Chapter I-IV**). I observed that the combined exposure of MPs and toxicants on the host-microbiome negatively affected the abundance in damselflies, dragonflies, and water boatmen of Bacteroidetes but positively affected the abundance of Proteobacteria in daphnids, damselflies, dragonflies, and water boatmen (Figure 5 and 6). Previous studies have shown that a decrease in Bacteroidetes abundance might result in abnormal intestinal permeability and pro-obesity phenotype (Joly Condet et al. 2015, Fang et al. 2018, Yuan et al. 2019). In addition, the increase of Proteobacteria might influence inflammation, lipid metabolism disorder, increase the susceptibility to infections, generate motor disabilities and gut diseases (Nasuti et al. 2016, Yuan et al. 2019). Therefore, such microbial changes might have a large effect on host health.

3.4 Effect of MPs in host life-history traits

I found that polystyrene-based latex spherical MPs that I used in my experiments did not have a strong effect on survival of the organisms tested here (**Chapter I-IV**) (Figure 7b, 8a and b). Depending on the material, MPs can be inert particles without any direct toxic effect (Cole et al. 2015b, Carbery et al. 2018, Wagner and Lambert 2018). Interestingly, the exposure to MPs reduced *C. riparius* emergence at low food levels (Figure 7a). This is a very important finding because even though mortality is low, MPs can still negatively impact adult emergence. Since mortality is usually the main endpoint used to assess if a pollutant is acceptable, I argue that other crucial life-history traits such as successful metamorphosis should be taken into consideration.

Figure 7. a) Cumulative emergence of *C. riparius* adults (males and females) over the course of 34 days in the low nutrient sediment and 28 days in the high nutrient sediment, b) Damselfly survival from dragonfly predation over time. The treatments were exposure to MPs, a toxicant; esfenvalerate (Esfen) or DMT, and combined exposure to MPs and toxicant or to none of these stressors (Control).

3.5 Effect of single toxicants in the host life-history traits

All the three tested toxicants had significant effects on the host mortality-survival (**Chapter I-IV**). Figure 7a shows that at low food levels, *C. riparius* larvae exposed to esfenvalerate had a significantly lower emergence than the controls (**Chapter I**). Negative effects on survival of *C. riparius* due to esfenvalerate exposure have been shown before (Kunce et al. 2015). Figure 7b shows that the damselfly exposed to DMT had higher mortality compared to the control (**Chapter II**). This result fits with the effects that DMT has over the voltage-gated Na^+ channel of the nervous system (Mestres and Mestres 1992, Hong et al. 2020), affecting the damselfly ability to escape from the dragonfly predator. For the water boatman, prolonged/chronic exposure is required for a significant effect of SDS alone (**Chapter III**). This chronic effect of detergents had been reported previously in nematode *Caenorhabditis elegans* (Harada et al. 2007), making detergent toxic even under low concentrations if the exposure period in the environment is long. For *D. magna*, I found that both concentrations 2ppm and 5 ppm of Cr(VI) are highly toxic (Figure 8b, **chapter IV**). I expected high mortality because these concentrations of Cr(VI) are based on previous mortality reports in aquatic organisms exposed to Cr(VI) (Kim et al. 2002, Vargas and Dussán 2016, Zhou et al. 2021). I find that this mortality correspond to the lethal concentration 50 at 24h for a concentration of 5 ppm of Cr(VI).

3.6 Combined effects of MPs and toxicants in host life-history traits

In **chapter I**, I showed that the combination of MPs and esfenvalerate lowered emergence when the level of food is low (Figure 7a) but I did not detect any additive negative effects. Conversely, Felten et al. (2020) did report additive negative effects on survival of *D. magna* in response to the combined exposure of MPs with DMT. Interestingly, I found in **chapter II** that in *D. Magna* microbial communities were significantly affected, specifically beta diversity and three of the six top phyla. However, I did not assess the survival of *D. magna* in my experiment. When I evaluated predation risk of *A. cyanea* by *I. elegans*, I did not find an interactive effect between MPs and DMT on damselfly survival (Figure 7b). In **chapter III** observed higher mortality in treatments with MPs combined with SDS at 24 and 48 hours (Figure 8a). I suggest that this interaction reduces the time needed for SDS to cause mortality. Though MPs could interact with organic toxicants (Skjolding et al. 2016, Wagner and Lambert 2018), the interaction between MPs and detergents remains unexplored. In **chapter IV**, I found a synergis-

tic effect between MPs combined with a sublethal concentration of 2 ppm of Cr(VI) that increased the mortality of *D. Magna* at 72 hours (Figure 8b). However, these studies are scarce, and similar studies only focused in the interaction effects between MPs combined with hydrophobic organic chemicals (Rochman et al. 2014, Felten et al. 2020). Since the interaction between MPs and toxic metal was the most toxic interaction, research on the effects of MPs combined with metal ions must be prioritized in future studies.

Figure 8. a) Mortality of water boatman, and b) mortality of *D. Magna*. The treatments were exposure to MPs, and toxicant; SDS or Cr(VI), and a combined exposure to MPs and toxicant or to none of these stressors (Control).

4. Conclusions

This thesis highlights the importance of understanding the cocktail effects of pollutants on host fitness and its microbiome. I showed that microplastics could cause indirect effects on the predator microbiome when a top predator fed on prey that has been exposed to this pollutant. I also observe microbial perturbations or dysbiosis at the top and intermediate predator when the prey had been exposed to a cocktail of a toxicant and the microplastics. I have evidence that hosts such as *Daphnia magna* and the chironomids consume the microplastics though I do not have evidence that the microplastics are transferred to the other trophic levels. I hypothesize that MPs in the *D. magna* could increase stress in the individual itself. This means that the changes in the microbiome of the top predator could be due to the stress response in the prey together with the effects of the microplastics. Thus, even if the MPs are not in the individual after consumption, MPs can indirectly affect the predator individual's gut microbiome. This thesis is of importance for recognizing the hazards of MPs combined with a toxicant as well as for assessing other ways that multiple pollutants can affect an organism such as host microbiome, metamorphosis, and trophic chain effects in aquatic environments.

5. Svensk sammanfattning

I många ekosystem växelverkar/interagerar ett djurs biologi ofta med olika antropogena föroreningar. Mer specifikt är interaktionen mellan ett värd djur och dess associerade mikrobiom en av huvudnycklarna för att förstå effekterna av miljö-reglerade störningar på egenskaper så som hälsa, beteende, och evolution av värd djuret. Antropogena aktiviteter resulterar ofta i stress-responser hos djur vilket kan ha en stark påverkan på djurets mikrobiom, inklusive mikroorganismerna i mag-tarmkanalen. Tidigare studier har visat att kemikalier i omgivningen och andra stress-inducerande faktorer kan skapa stora förändringar i magens mikrobiom hos djur. Därför är det viktigt att studera just hur förändringar i världens mikrobiom, framförallt mångfald och sammansättning, påverkas av miljöföroreningar.

Mikrobiomstudier är viktiga eftersom mikrobiomet fungerar som en biologisk barriär och kan påverka till exempel immunförsvarsreglering, matsmältning, näringsupptagning, tarmrörelser, och även beteende hos värd-organismen. Den här avhandlingen bidrar med att reda ut de multidimensionella effekterna av mikrobiomet på stress och hälsa hos några utvalda vattenlevande leddjur. Målet med forskningen i den här avhandling är att förstå effekterna av mikroplast-föroreningar och giftiga föroreningar så som insektsgift, tvättmedel, metaller, och interaktionerna mellan dessa två grupper på värd-mikrobiomet hos olika modell-organismer. Jag studerade även de sekundära effekterna av stressfaktorerna samt hur effekterna kan spridas i födo-kedjor. För att testa detta gjorde jag mikrokosmexperiment där jag utsatte olika vattenlevande insekter och kräftdjur för mikroplaster tillsammans med olika typer av miljö-gifter.

Jag började med att testa effekterna av mikroplaster och insektsgiftet esfenvalerate (en syntetisk pyretroid) på fjädermygglarven *Chironomus riparius* överlevnad, tillväxt, och mikrobiom. Efter det testade jag effekterna av mikroplaster och insektsgiftet deltamethrin (en pyretroid ester) på en trofisk kedja (födo-kedja) med tre organismer: *Daphnia magna* (vattenloppa), *Ischnura elegans* (flickslända), och *Aeshna cyanea* (trollslända). I den tredje studien testade jag effekterna av mikroplaster och natriumlaurylsulfat (som finns i tvättmedel) på dödlighet hos värd djuret och effekterna på värd djurets mikrobiom hos buksimmare från *Corixidae*-familjen. Slutligen testade jag effekterna av mikroplaster och Krom(VI) på *Daphnia magna*s dödlighet och

på dess mikrobiom. Jag använde mig av metagenomiska verktyg för att analysera både värd-mikrobiomet, och vatten-mikrobiomet i de mikrokosm jag undersökte. Mina resultat visade att mikroplaster kan interagera med additiva gifter och förändra värd-mikrobiomet. De specifika effekterna på mikrobiomets diversitet och sammansättning beror på typen av gift, storleken på mikroplasterna, och vilken värdorganism som studerades. Mina studier belyser vikten av att samstudera interaktiva faktorer (cocktail-effekter) som gifter för att bättre förstå hur dessa påverkar djurens hälsa och mikrobiom. Jag visade dessutom att mikroplaster kan orsaka indirekta effekter på mikrobiomet när rovdjur ansamlar dessa genom sina byten. Jag observerade mikrobiella störningar så kallad dysbios från den andra trofiska nivån när rovdjur konsumerade byten utsatta för diverse gifter och mikroplaster.

Den här avhandlingen visar komplexiteten i interaktionerna mellan mikroplaster och additiva gifter som ett blandat system och dess effekter på värd-mikrobiomet. Mina studier utforskar samband mellan mikrobiomet, mikroplaster, och värd-hälsa, och hur dessa påverkas av additiva gifter.

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It will not be good to have some more years as a PhD student? –What? Are you crazy? Of course it will! I loved this time of my life. First, I would like to acknowledge that I am afraid of what is coming and I think that is the main reason that I am excited to start this new phase in my life. –It is just life, so do not worry. A new phase that hopefully will be mostly full of good energy and happiness because I will have all you with me. Maybe also you! –Are you talking to the reader? Are you OK? Yes, you that does not know me but took the time to read this thesis that is part of me, but I am afraid to say that is not the best part of me –So, what was your point with that? Are you trying to tell us that we are the best part of you?

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AVERNO

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