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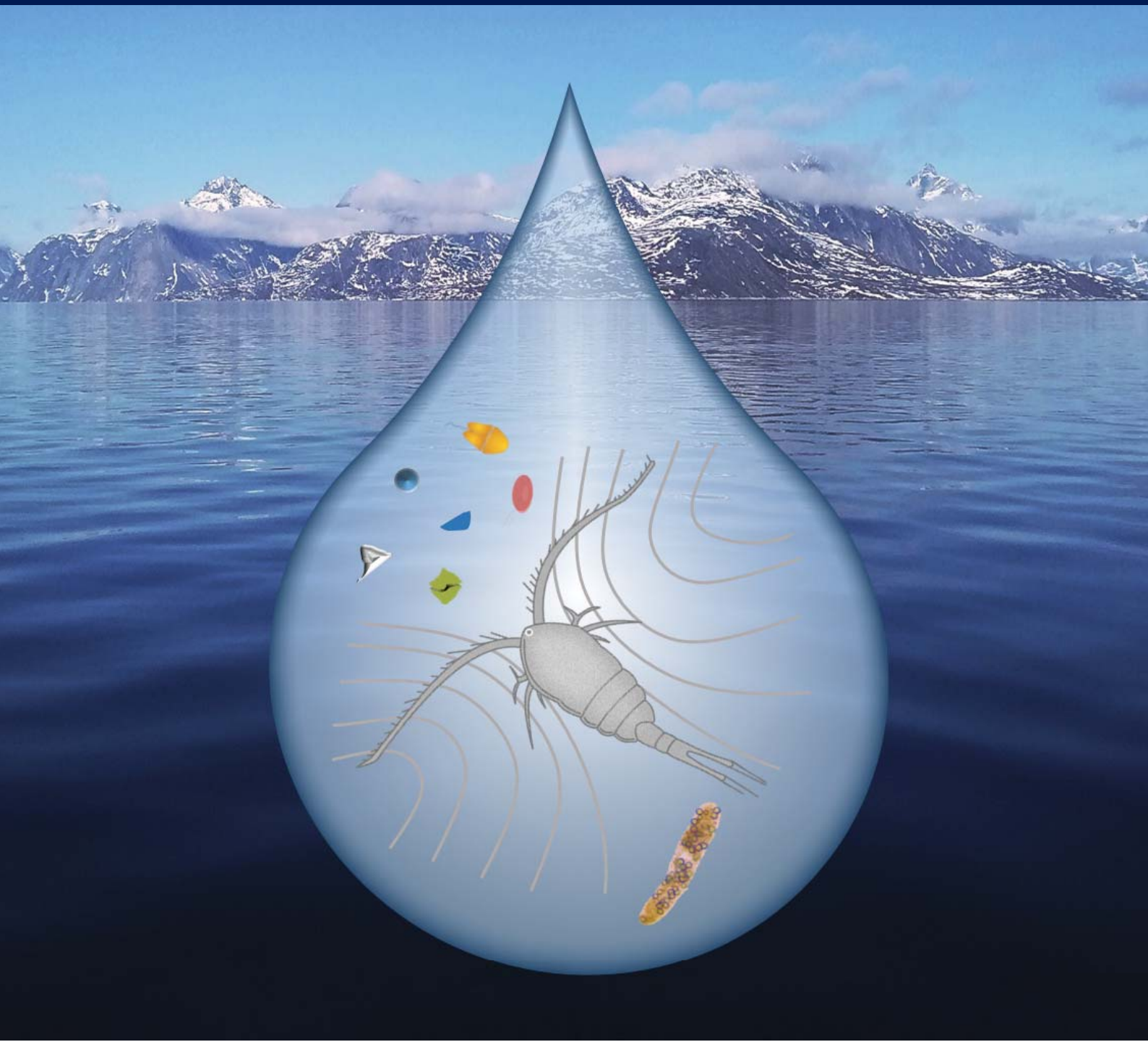
Ingestion and effects of microplastics on marine planktonic food webs

Rocío Rodríguez Torres

PhD Thesis



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Ph.D. Thesis

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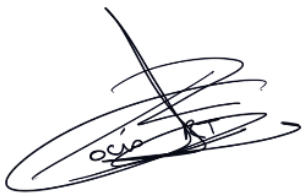
Kongens Lyngby, February 2022

Preface

The work presented in this Ph.D. thesis took place at the National Institute of Aquatic Resources at the Technical University of Denmark to accomplish the requirements for acquiring a Doctor degree in Philosophy. The research was carried out from March 2019 until February 2022 under the supervision of Professor Torkel Gissel Nielsen from DTU Aqua, Senior Researcher Rodrigo Almeda from DTU Aqua/University of Las Palmas de Gran Canaria (ULPGC) and Senior Researcher Nanna B. Hartmann from DTU Environment. This Ph.D. is part of the Danish Center for Research in Marine Plastic Pollution (MarinePlastic), project financed by Villum Foundation (PNO 25084).

This thesis is structured in three parts: first, an introduction showing the current marine plastic pollution scene, followed by an analysis of the ingestion and impact of microplastics in marine planktonic ecosystems, where I put in context the projects carried out during the three years. Secondly, I expose the general conclusions from the investigations accomplished in all the projects, together with some future perspectives that could enrich and complement this work. The last part is the list of papers, numbered below from I to VI which, are the main studies carried out during this Ph.D. thesis. Paper I and III, where I am not first author, are included here because I did major contributions on them to achieve some of the objectives of this thesis. Adding them, contextualizes and provides relevant information to the main topic of this thesis.

Kongens Lyngby, 28th February 2022



Rocío Rodríguez Torres

Acknowledgments

I am thankful to every single person that one way or another has been involved in this Ph.D. project.

Special thanks to my supervisor, Professor Torkel Gissel Nielsen, and co-supervisors: Senior Researcher Rodrigo Almeda and Senior Researcher Nanna B. Hartmann. Thanks for sharing all your knowledge with me. Thank you for your amazing work as supervisors and for being always there when I needed advise to keep making progress. Each one of you is a source of inspiration and together you make a neat combination for a perfect supervision. I also want to give a special thank you to Professor Thomas Kiørboe who welcomed me at the Centre for Ocean Life in 2016. Thank you for the opportunity you gave me to work in science, experience that helped me to pursuit my dream of getting the desired Ph.D.

The Ph.D. dream came true in the Section of Oceans and Arctic lead by Karen Edelvang. Karen, I admire your professionalism and thank you for always looking after my well-being. At DTU Aqua, I have been valued and I found the opportunity I wanted. I even like science more now than I did before, and that is, in part, due to the air breathed in this building full of brilliant and motivated scientists. Thank you to all the coauthors that contributed to improve the articles included in this thesis. Special thanks to Sinja Rist, you are a great role model. I have to thank you for everything I learnt working side by side with you. A big thanks to Tenure Track Nicole R. Posth and Associated Professor Karina Sand from Copenhagen University; the time learning with you boosted my motivation.

I have been lucky to have colleagues like Sei, Camila, Fredrik, Josephine and Delove, among many others, with whom I shared work but also special fun moments. Huge thanks to Jack Melbye for all your help in the laboratory. Thanks to Maj-Britt Willandsen, Lilian Andersen and Karin Stubgaard, key women that make our life easy with her job and especially with her kindness.

These three years were dazzling thanks to my life partner Nicolás, thanks for making this journey easy and for your massages. The love and support of my family and friends was an important factor to never give up in the past and now, to affront the future fearless.

Summary

Plastic is one of the most persistent pollutant in the environment. The current high production of plastic and poor management plastic waste facilitate its entry in the marine environment. The degradation of plastic involves the breakdown of big debris into small pieces that eventually can reach sizes smaller than 5 mm. Those plastic particles are defined as microplastics (MPs). MPs can also be directly manufactured for different purposes in sizes smaller than 5 mm. Independently of their origin, MPs enter the marine environment by different routes, e.g. atmospheric deposition, sewage systems and run-off. MPs have become ubiquitous in marine environments arising global concern about their impacts on the ecosystems.

The size of MPs overlaps with the natural prey of a wide range of marine organisms and consequently pose a potential risk of MP ingestion. Size similarity of small MPs < 100 µm and phytoplankton, makes zooplankton a particularly vulnerable group to MP ingestion. Despite the increasing knowledge about the ingestion and impact of MPs on zooplankton, there is still lack of environmental realistic conditions in the laboratory exposure experiments. The mechanisms behind the interactions between MPs and marine organisms is also understudied. Copepods are the most abundant metazoan in the oceans. They are a key group in the marine food webs as grazers on primary producers and as prey for many economical and environmentally relevant organisms, such as fish larvae. Copepods are also essential part of the biological carbon pump with the transportation of organic matter through fecal pellet production. With this thesis, I provide an overview of the ingestion and impact of MPs on copepods. Furthermore, I elucidate some mechanism behind the copepods-MPs interactions considering realistic MP characteristics, applying small-scale video observations and a trait base approach. In addition, with a literature review, I evaluate the trends in MP research in marine food webs.

Quantification of small size fraction of MPs in the marine environment is challenging due to lack of standardized methodologies and difficulties in comparing data. Besides, some areas are understudied, such as Arctic, Antarctic and Indian Ocean. Therefore, there is still a long way to have a complete overview of the MP concentration and distribution in the oceans. This thesis provides data on the MP concentration and composition for arctic surface waters, with an average of 142 MPs m^{-3}, when sampling with a free-plastic pump system. Given the presence of MPs in the arctic ecosystems, I evaluated the impact of virgin MPs on the most abundant copepods in the Arctic. Our studies show ingestion of MPs (20 µm) in all the studied species, but not impact,

at environmental realistic concentrations. However, when co-exposed to crude oil, the MPs triggered feeding suppression in *Calanus hyperboreus*.

Microplastics in the environment present a high variety of characteristics that can affect their bioavailability and their potential ingestion by planktonic copepods. Using traditional laboratory incubations and small-scale video observations I demonstrated the ability of copepods to select their prey and reject 80% of the encountered MPs, independently of the MP characteristics. Copepods have different feeding behaviors, which allow them to target the prey that provide them with higher energy uptake. This Ph. D. thesis is the first, to our knowledge, evaluating the response of copepods to MPs based on their different feeding strategies. The worldwide distribution of copepods and the high ingestion of MPs by these organisms in previous laboratory studies, led us to consider copepods a potential main entry of MPs in marine food webs. However, in this project, a minimal ingestion of MPs was observed independently of the copepod´s feeding behavior and microplastic characteristics. In consequence, I did not identified globally, areas with high risk of entry of MPs into the marine food webs via copepods.

Microplastics are pollutants of high concern in the marine environment. But, based on the findings from this thesis, planktonic copepods have a low risk of MP ingestion even at concentrations above those found in the environment. Hence, planktonic copepods do not play an important role for the entry of MPs in marine food webs.

Resumé

Plast er et af de mest persistente forurenende stoffer i miljøet. Den nuværende store produktion af plast, kombineret med ineffektiv håndtering af plastaffald, gør det lettere for plast at komme ud i havmiljøet. Plastens forvitringsproces indebærer, at store affaldsrester nedbrydes til små stykker, der i sidste ende kan blive mindre end 5 mm store. Disse plastpartikler defineres som mikroplast (MP). MP kan også fremstilles direkte til forskellige formål i størrelser på under 5 mm. Uafhængigt af deres oprindelse kommer MP ud i havmiljøet ad forskellige veje, f.eks. atmosfærisk nedfald, kloaksystemer og afstrømning. MP er blevet allestedsnærværende i havmiljøer over hele kloden, hvilket har givet anledning til bekymring over deres indvirkning på økosystemerne.

Størrelserne af MP overlapper med en lang række marine organismers naturlige byttedyr og medfører derfor en potentiel risiko for indtagelse af MP. Størrelsesmæssig lighed mellem MP < 100 µm og fytoplankton gør zooplankton til en særlig sårbar gruppe for indtagelse af MP. Trods den stigende viden om indtagelse og indvirkninger af MP på zooplankton mangler der stadig miljørealistiske koncentrationer i laboratorieeksponeringsforsøg. Desuden er der sparsom viden om mekanismerne bag interaktionerne mellem MP og marine organismer. Vandlopper er de hyppigst forekommende metazoer i havene. De er en nøglegruppe i de marine fødekæder idet de græsser på primærproducenter og samtidig er bytte for mange økonomisk og miljømæssigt relevante organismer, som f.eks. fiskelarver. Vandlopper er også en vigtig del af den biologiske kulstofpumpe, hvor de bidrager til transport af organisk materiale gennem produktion af hurtigt synkende fækalier. Gennem denne afhandling giver jeg en oversigt over indtagelse og effekter af MP på vandlopper. Desuden belyser jeg nogle af mekanismerne bag interaktionerne mellem vandlopper og MP, under hensyntagen til realistiske MP-karakteristika, gennem videoobservationer og en tilgang baseret på biologiske træk. Desuden evaluerer jeg, gennem en litteraturgennemgang, tendenserne i forskningen relateret til MP i marine fødekæder.

Det er udfordrende at kvantificere små MP i havmiljøet på grund af mangel på standardiserede metoder og vanskeligheder med at sammenligne data. Desuden er der nogle geografiske områder, som ikke er tilstrækkeligt undersøgt, f.eks. Arktis, Antarktis og Det Indiske Ocean. Der er derfor stadig lang vej til at få et fuldstændigt overblik over koncentrationen og fordelingen af MP i havene. Denne afhandling indeholder data om MP-koncentrationen og -sammensætningen for arktiske overfladevandområder, med et gennemsnit på 142 MP m^{-3} ved prøvetagning med et frit plastisk pumpesystem. På baggrund af tilstedeværelsen af MP i de arktiske økosystemer har jeg evalueret virkningen af rene MP partikler på de mest udbredte vandlopper i Arktis. Min

undersøgelser påviser indtagelse af MP (20 µm) hos alle de undersøgte arter, men vi observere ikke effekter ved miljørealistiske koncentrationer. Ved samtidig eksponering for råolie udløste MP imidlertid reduceret fødeoptag hos *Calanus hyperboreus*.

Mikroplast i miljøet har mange forskellige egenskaber, som kan påvirke deres biotilgængelighed og deres potentielle indtagelse af planktoniske vandlopper. Ved hjælp af traditionelle laboratorieinkubationer og videoobservationer har jegvist, at vandlopperne er i stand til at udvælge deres bytte og dermed afvise 80 % af de MP patrikler, som de møder, uafhængigt af partiklernes egenskaber. Vandlopper har forskellig fødeadfærd, som gør det muligt for dem at målrette sig mod de byttedyr, der giver dem et højere energioptag. Denne ph.d.-afhandling er, så vidt vi ved, den første der evaluerer vandloppers reaktion på MP på grundlag af deres forskellige fødestrategier. Den verdensomspændende udbredelse af vandlopper, kombineret med vandloppers høje indtagelse af MP i tidligere laboratorieundersøgelser, fik os til at anse vandlopper som en potentiel 'hovedindgang' for MP i marine fødekæder. I dette projekt observerede jeg imidlertid en minimal indtagelse af MP uafhængigt af vandloppernes fødeadfærd. Jegidentificerede derfor ingen globale områder med høj risiko for, at MP kommer ind i de marine fødekæder via vandlopper.

Mikroplast er forurenende stoffer i havmiljøet, der giver anledning til stor bekymring. Baseret på mine resultater har planktoniske vandlopper dog en lav risiko for indtagelse af MP, selv ved koncentrationer, der er højere end dem, der findes i miljøet. Planktoniske vandlopper spiller derfor ikke en væsentlig rolle for optag af MP i de marine fødekæder.

List of papers

Paper I: Rist, S., Vianello, A., Winding, M. H. S., Nielsen, T. G., Almeda, R., **Rodríguez-Torres, R.**, Vollertsen, J. (2020). Quantification of plankton-sized microplastics in a productive coastal Arctic marine ecosystem. *Environmental Pollution*, *266*(115248). https://doi.org/10.1016/j.envpol.2020.115248

Paper II: **Rodríguez-Torres, R.**, Almeda, R., Kristiansen, M., Rist, S., Winding, M.S., Nielsen, T.G. (2020). Ingestion and impact of microplastics on arctic *Calanus* copepods. *Aquatic Toxicology*, 228(105631). https://doi.org/10.1016/j.aquatox.2020.105631

Paper III: Almeda, R., **Rodriguez -Torres, R.**, Rist, S., Winding, M.H.S., Stief, P., Hansen, B.H., Nielsen, T.G. (2021). Microplastics do not increase bioaccumulation of petroleum hydrocarbons in Arctic zooplankton but trigger feeding suppression under co-exposure conditions. *Science of Total Environment*, 751(141264). https://doi.org/10.1016/j.scitotenv.2020.141264

Paper IV: Xu, J.†*, **Rodríguez-Torres, R.**†, Rist, S., Nielsen, T.G., Hartmann, N.B., Li, D., Almeda, R. (Environmental Science and Technology). Unpalatable plastic: efficient taste-discrimination of microplastics in planktonic copepods. (Submitted). †Equal contribution

Paper V: **Rodríguez-Torres, R.**, Almeda, R., Xu J., Rist S., Hartmann N.B., Nielsen, T.G. Zooplankton behavior minimizes the entry of microplastics in planktonic food webs (completed draft exists)

Paper VI: **Rodríguez-Torres, R.**, Rist, S., Almeda, R., Nielsen, T.G., Hartmann N.B. Research trends in microplastic uptake and transfer in marine food webs (in preparation).

Table of contents

Abbreviations

MPs	**Microplastics**
MP	**Microplastic**
PP	**Polypropylene**
PE	**Polyethylene**
PET	**Polyethylene terephthalate**
PAHs	**Polycyclic aromatic hydrocarbons**
PS	**Polystyrene**

Background and aims

In the marine environment, plastic debris are found from the surface to the deepest point, the Mariana Trench and its negative potential effects in the ecosystems has been under investigation for decades. The persistence of this material and the non-stopping increasing of plastic production led to a necessity to investigate the potential impacts of this pollutant in the ecosystems. The concern raised with the detection of new small plastic particles in 2004 (Thompson et al., 2004). These particles smaller than 5mm were named microplastics (MPs) (Arthur et al., 2009).

Microplastic presence have been reported in marine environments in water, sediments and several organisms. However, quantification of MP concentrations is still limited. Therefore, the aim of the first article of the thesis (Paper I) is to provide quantitative plankton-sized MPs (>10 µm) concentrations from a relevant but understudied area, the Arctic. The aim was to have a picture of the MP pollution problematic near the most important source of MPs in Greenland: the capital Nuuk. We get to know the presence of MPs in the marine surface waters by using traditional (manta and bongo net) and new plastic-free sampling methods. The impact of MP pollution is also understudied in this pristine remote areas despite MP been detected and quantified (Paper I; Tekman et al., 2020,). With our paper II and III I aim to evaluate the impact of MP in pristine arctic ecosystems, specifically on key arctic species of planktonic copepods. I exposed the three copepod species, that represent approximately the 80% of the biomass of the artic zooplankton, to both environmental relevant (200 MP L^{-1}) and high (20000 MPs L^{-1}) concentrations of MP. I attempted to mimic the strong seasonality in the Arctic by using two food concentrations (high concentrations representing the spring bloom and low for the post/pre bloom). We hypothesized that ingestion of MPs, egg production and fecal pellet production and sinking rates are affected by the ratio food:MP. Unfortunately, marine ecosystems are polluted with several contaminants. Therefore, multiple stressor analysis and co-exposure experiments are key to evaluate MP effects when expose with other pollutants (Oliveira et al., 2013). In the Arctic, the crude oil pollution is a potential risk for the ecosystem in the future due to the increase of maritime and oil extraction activities. Therefore, in paper III, I evaluated the role of MPs as vectors of crude oil in the artic copepod, *Calanus hyperboreus* and the co-exposure effects. The aimed was to see how oil toxicity and bioaccumulation of PAHs in copepods was affected by the presence of MPs. Furthermore, the impact of these pollutants in fecal pellet sinking rates were also evaluated.

While most of the initial studies were using high MP concentrations and clean beads (Cole et al., 2013; Setälä et al., 2014), real MP characteristics were not being assessed. Yet, lately, this issue

was detected and more studies are driving towards realistic conditions. I contributed to this matter with paper IV, where we evaluated the impact of regular, irregular, bio-fouled and polluted MPs on the copepods with the most common zooplanktonic behavior, feeding-current feeders (*Temora longicornis*). For that, I used small-scale video observations and bottle incubations in parallel. This methods allowed us to understand the mechanisms behind the MP ingestion in copepods.

Trait based approach is often use for modelling marine ecosystems processes. This approach allow us to evaluate effects in organisms with the same characteristics/traits instead of individual species (Kiørboe et al., 2018). The objective of Paper V was to use this approach to evaluate if one of the zooplanktonic key traits, feeding behavior, was relevant for the MP ingestion by copepods and therefore, transfer of MPs up the marine food web. We hypothesized that active feeders will be more relevant to the entrance of MPs into marine food webs that ambush feeders, consequently having a major risk of entrance of MP into the food web in areas where active feeders dominate.

Despite several years of MP research, there is a lack of consistent and standardized methodology to collect, analyzed and to report MPs ingestion. It translates into a difficulty to compare studies (Rist et al., 2021; Pinheiro et al., 2020). I conducted a review (Paper VI) with the objective of analyzing the actual tendencies and patterns in MP research. Special attention is given to relevant parameters investigated during this thesis such as, feeding behavior of the studied species or type of MPs used. Thus, we get an overview of the future needs in this research areas.

Specific objectives and hypothesis

Paper I

Objectives: Quantify the microplastic concentration (< 10µm) in the surface waters along a Greenlandic fjord and towards the capital Nuuk to assess its role as a source of plankton-size microplastics.

Hypotheses: Concentration of microplastic are higher near Nuuk, for being the capital an important source of microplastics in Greenland.

Paper II

Objectives: Evaluate the impact of microplastics in three key arctic *Calanus* copepod species (*C. finmarchicus*, *C. glacialis* and *C. hyperboreus*) considering the high primary production seasonality in this polar ecosystems.

Hypotheses: The ratio prey:microplastic will determine the ingestion and sub-lethal effects in arctic copepods. The lower the ratio higher ingestion rate and consequently, higher impact of MPs for the three species.

Paper III

Objectives: Assess the role of microplastics in the bioaccumulation and toxicity of crude oil in arctic copepods. Evaluate if the application of dispersant after an oil spill worsen the effects of microplastic and crude oil co-exposure in copepods.

Hypotheses: Co-exposure of microplastics and crude oil increases PAHs accumulation and sub-lethal effects in the copepods compare with crude oil alone. The application of dispersant increases the transfer of PAHs to the organisms when co-exposed to microplastics and crude oil.

Paper IV

Objective: Identify the feeding behavioral responses of copepods that use the dominant foraging strategy in planktonic copepods, feeding-current feeders. Evaluate these behavioral responses using different microplastic shapes, polymer types and weathering process (biofilm or absorbed pollutant).

Hypotheses: Feeding-current feeders do not discriminate between polymer types but they show a higher rejection for irregular microplastics. The presence of biofilm will increase the ingestion of bio-fouled particles but the presence of sorbed pollutants will increase the rejection of microplastics by these copepod.

Paper V

Objective: From a trait based approach, evaluate the risk of microplastic ingestion and consequently, risk of microplastic entry into marine food webs on planktonic copepods, considering their feeding behavior.

Hypotheses: Active feeders will play a greater role in the entrances of microplastic in the food webs than ambush feeders. The behavior of a mixed feeder will vary depending on the type of prey or microplastic that it is exposed to, reflecting changes in behaviour and microplastic ingestion.

Paper VI

Objective: Evaluate the research carried out in relation to microplastic ingestion in relevant groups of the marine food webs. Identify the current trends in terms of, feeding behavior of the studied species, units used to report microplastic uptake, microplastic characteristics and citation bias, among others.

1. Introduction

1.1. Plastic production and pollution

The production of plastic began in 1940s and it was mainly used for warfare. Since 1950, the production has increased yearly and it is forecast to continue that trend in the future (Fig. 1). What makes plastic stand out from other materials are its unique characteristics such as the low weight and high adaptability for different applications (Andrady, 2011). Nowadays, the driven objective of plastic is packaging, dominated by PP (polypropylene), PE (polyethylene) and PET (polyethylene terephthalate) (Geyer, 2020). Plastic production became an issue due to the poor disposal methods, plastic waste started to accumulate in the environment and its impact in the ecosystems caused concern since late 60's. It is proven that the poor management of plastic waste determines the entrance of plastic into the marine environment (Dąbrowska et al., 2021).

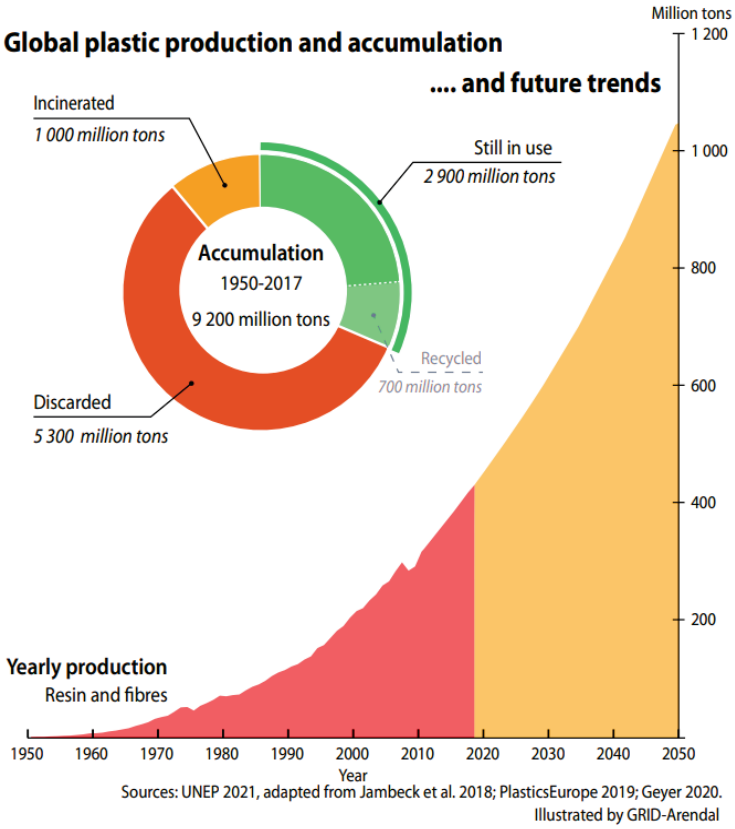


Figure 1. Yearly production of plastic from 1950 until 2018 (red) and future projection until 2050 estimated by Geyer 2020 (yellow). Pie chart shows the cumulative plastic production since 1950 to 2017. Source: UNEP 2021. From pollution to solution report.

From all the produced plastic, it has been estimated that until 2017, 5300 million metric tonnes have been discarded and ending on landfills or dumps as uncontrolled waste streams (Fig. 1). Only in 2020, approximately 30 Mt of post-consumer plastic waste was collected of which, 35% was managed in recycling facilities and 42% was sent to energy recovery operation. Still a 23% was mismanaged in landfills, which potentially end in the oceans (PlasticsEurope, 2021). Li et al., in 2016 estimated that 80% of the plastic in the oceans came from land-based sources and the remaining 20% from marine sources. In average, approximately 8 million tons of plastic enter the oceans yearly (Jambeck et al., 2015). Unfortunately, the problem of plastic waste in the marine environment is now acknowledge as a global issue of high public concern.

Marine plastic got first scientific attention when Carpenter and Smith in 1972 observed floating plastic waste in the Sargasso Sea. But it was in the last two decades, when the scientific effort moved towards the smallest fraction of the plastic waste, the so called micropalstics (MPs). The term “microplastic” started to be used in 2004 (Thompson, 2004). But it is in 2009 when a more precise definition of the term was proposed. Microplastics comprise all plastic particles smaller than 5 mm. (Arthur et al., 2009). Although, there is not yet an international consensus on the definition of MPs (Hartmann et al., 2019).

The origin of MPs is diverse but they have been divided in two main big categories: primary and secondary MPs. Primary MPs are those directly manufactured in sizes smaller than 5 mm for different purposes, such as scrapers, decoration and beauty products, among others. Secondary MPs are originated from fragmentation or degradation of bigger big plastic debris when exposed to environmental stressors (Kershaw, 2015). Photo and thermo-degradation, hydromechanical abrasion and biodegradation are the main responsible factors in the degradation process and consequently in the fragmentation of big plastic waste into small particles.

Microplastics have also been detected in the marine environment globally from pole (Tekmann et al., 2020) to pole (Absher et al., 2019) and from the surface to the deepest point, Mariana Trench (Peng et al., 2018). Most of the MPs in the marine environment come from land sources, from activities like transportation (roads and traffic), landfills, and agriculture (UNEP, 2021). Although, current studies also highlight the relevance of fishing waste as a sea source of MPs in the marine environment (Tan et al., 2020; An et al., 2020) and the atmospheric deposition of airborne MPs transported along the globe (Dris et al., 2016; Brahney et al., 2020). As a consequence of the plastic pollution, oceans are big MP sinks where these particles can accumulate in different habitats such as, beaches or seabed sediments (UNEP, 2021).

1.2. Microplastics in the marine water column

The distribution of MPs in the oceans is patchy with areas containing higher amount of particles than others. Yet, the types of plastics found in the marine environment are also of high variability in terms of sizes, shapes, polymer types, density, chemical composition and weathering stage.

Distribution and abundance

Microplastics have been introduced in practically all the marine ecosystems, even in remote pristine zones such as the Arctic Oceans, including the sea ice (Obbard et al., 2014). The concentrations found in the marine environment vary with the sampled area. For instance, enclosed environments or coastal areas close to MP sources like sewage outlets present higher MP concentrations than open oceans (Cole et al., 2011; Browne et al., 2015).

A wide range of MPs concentrations have been reported in different marine environments around the world. Law et al. in 2010 reported an average of 42 10^{-3} MPs m^{-3} in the North Atlantic. Colingnon et al. in 2014 found 10 times more (422 × 10^{-3} MPs m^{3}) in the Mediterranean Sea. Mean of 32.2 MPs m^{-3} were found in the Baltic Sea (Zobkov et al., 2019). A global range from 0.12 to 7250 × 10^{-3} MPs m^{-3} was reported by Beiras & Schönemann, 2020.In this project, in Paper I, concentrations of 142 MPs m^{-3} and 0.12 MPs m^{-3} were found depending on the method used, in arctic marine surface waters. This high variability in reported concentrations, in some cases, can be explained by the lack of standardization in terms of sampling methods and sample analysis and treatment. Microplastics suspended in the water column could have different fates. They can eventually enter the biota or be transported and accumulated in the sea bottom. MPs have been detected and quantified in water, organism and in the sediments of many marine habitats. However, there is still lack of investigation in the Arctic, Antarctic and Indian Ocean (Ajith, 2020).

Size and shape composition

The small size of MPs (<5mm) increases the risk of MP ingestion by a wide range of marine organisms due to the size overlap with their prey. MPs can be also present in a wide variety of shapes: spheres, fragments, fibers and films (Hartmann et al., 2019). Field studies have collected MPs present in the oceans and reported their sizes and shape (Lusher et al., 2015; Zhang et al., 2017). It has been shown that MP concentration in the environment increases with decreasing size (Paper I; Isobe et al., 2017) and that all type of shapes are present in the oceans, being fibers and fragments the most abundant ones (Paper I; Sun et al., 2017: Tamminga et al., 2019). The most used methods for MP sampling in water are manta, bongo and plankton nets. These nets are often used with mesh sizes of approximately 300µm discarding the small fraction of MPs and

underestimating the MP concentration in the environment (Green et al., 2018; Lindeque et al., 2020). In this thesis, for Paper I, the sampling in Arctic waters was performed with a free-plastic pump system that sorts MPs down to 10µm. In this analysis, MPs were most abundant in the fraction smaller than 100 µm. Sampling of small size-MPs are still scarce in oceans. Once again, the lack of developed and standardized methods hinders the research on MP size distribution in the oceans.

Polymer type

A meta-analysis from 2019 showed than PE (polyethylene) and PP (polypropylene) are the most abundant polymers in surface waters (Erni-cassola et al., 2019). Polymer density is a factor that affects the MP sinking velocity therefore polymer distribution could vary vertically in the water column (Erni-Cassola., 2019). In contrast, other field sampling analysis have shown predominance of polyester on marine surface water (Lusher et al., 2015; Jiang et al., 2020; Paper I). Polystyrene (PS) is the most used polymer type in controlled exposure experiments in laboratory. However, as shown in Paper VI there is a tendency in the last years to broadening the range of polymer types used to evaluate MP ingestion in marine ecosystems.

Weathering: Additives, leachates and biofilm

The weathering affect the physical and chemical properties of the MP, interfering in their interactions with the environment. Plastics are often accompany by other chemicals from different origins, the so-called plastic additives. Some additives are supplied during plastic production to enhance specific characteristics in the final product and others are unintentional sub products result of the production process (Gallo et al., 2018; Mayer et al., 2000). The weathering processes initiated when plastics reach the oceans, potentially facilitates the leach of this chemicals to the water becoming a hazard to the ecosystems (Capolupo et al., 2020). For example, BPA (bisphenol A) or phthalates are common plastic additives that are endocrine disruptors and their effects have already been studied in humans (Wang et al., 2020). However, there is need for more studies evaluating the potential impacts of MP leachates in marine organisms (Gunaalan et al., 2020) and more specifically in copepods.

The weathering process alter the physical and chemical properties of the plastics. It can affect the sorption of chemicals that are dependent on both, environmental and plastic conditions (Arp et al., 2021). For example, MPs absorb polycyclic aromatic hydrocarbons (PAHs), potentially acting as vectors of PAHs and may increase their impact in the ecosystems in co-exposure conditions (Caruso, 2019; Sørensen et al., 2020). Microorganisms colonization also weather the

MP particles to a certain extent, affecting for xample, their sinking velocity and potentially enhancing the risk of ingestion by marine organisms (Wieczorek et al., 2019).

Due to the different properties of the MPs and their interaction with the environment, multiple stressors conditions and co-exposure with other relevant pollutans should be considered to evaluate the effects of MPs in the ecosystems.

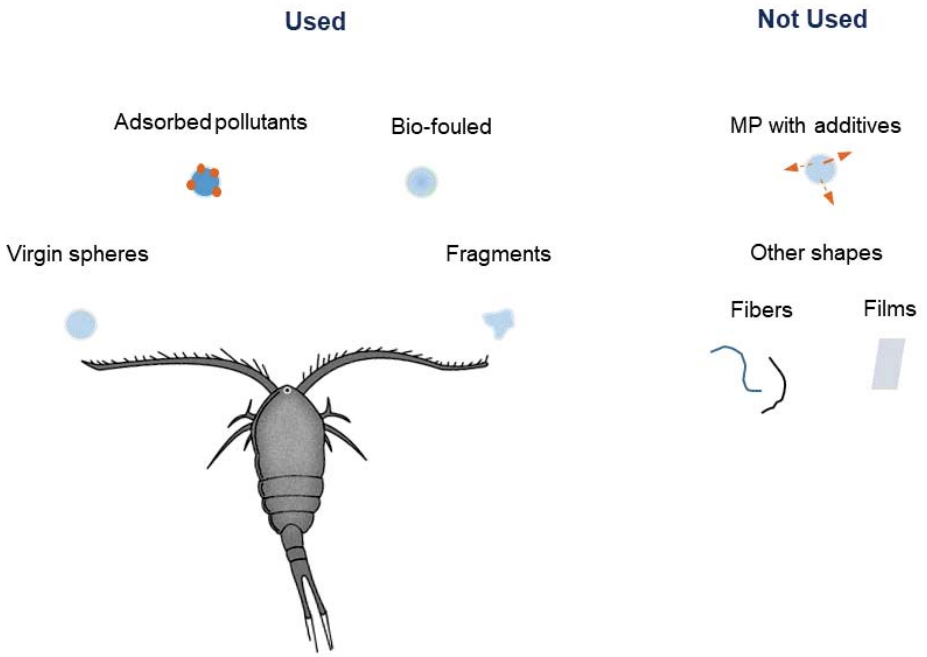


Figure 2. Characteristics of the microplastics used in this thesis (left) and not used (right).
Source of copepod vector: Fredrik Ryderheim.

1.3. Microplastic conditions used to experimentally evaluate MP impact in zooplankton

Despite the known variety of MPs in the oceans displayed above, this diversity is not reflected in the controlled laboratory experiments. Recent studies convey awareness about the need to mimic the environmental conditions (Galloway et. al., 2017; Weiss, 2019; de Ruijter et al., 2020) in order to achieve accurate evaluations of the ingestion and impacts of MPs in the marine environment.

Regarding concentrations of MPs used in the experiments, they can be up to 10 orders of magnitude higher than what is found in the environment. For instance, Fernandez et al., in 1979 used 100000-1000000 particles mL^{-1} or Cole et al., in 2013 used 635 to 3000 particles mL^{-1} or

Setälä et al. 2014 with 1000 to 10000 particles mL^{-1}. The MP sizes used in zooplankton experiments are adapted to the prey size threshold of the exposed organisms. There is a high variability of MPs sizes among studies. However, a trend to broadening the evaluated MP sizes is reflected in Paper VI. Since the beginning of MP impact assessment, PS microbeads have been the polymer type and shape most used in experiments until present (Ayukai, 1987; Lee et al., 2013, Paper VI). Studies with fibers, which are the most abundant MPs shape reported in the marine environment, are still scarce. Few studies evaluating the ingestion (kokalj et al., 2018) and impact of this particles type are being conducted. (Cole et al., 2019).

Lately, the investigations with weathered MPs are also increasing due to its truthful representation of the plastic particles found in nature. Biotic weathering of MPs (Vroom et al., 2017; Procter et al., 2019) and MPs with absorbed pollutants have been considered in recent studies (Guven et al., 2018; Sorensen et al., 2020). A higher impact of MPs in marine organisms could derive from the release of additives to the environment. Some studies are investigating the effects of the leachates in marine organisms, such as mussels (Seuront et al., 2021), sea urchin (Rendell-Batthi, 2021) and copepods (Beiras et al., 2019). But studies are still scarce to be able to understand the hazardousness of these chemicals leached by plastics to planktonic ecosystems.

1.4. Copepods: key organisms in marine food webs

Copepods are the most abundant metazoans on Earth and they occupy a pivotal position in the marine food webs as key grazers of phytoplankton and as essential sources of food for many pelagic predators such as, larvae of species of economic and environmental relevance (Verity and Smetacek, 1996). The life cycle of copepods starts with eggs and ends with and adult female or male copepod passing through six stages of nauplii and five stages of copepodites. The large ontogenetic size range of the copepods is what makes them a perfect food source for a wide variety of predators; from fish larvae to whales.

Copepods are one of the most studied marine zooplankton organisms in connection with the ecological impact of marine microplastic pollution (Boterell et al., 2019; Paper VI). The smallest fraction of MPs overlaps in size with the copepod's prey threshold stressing the importance of the MP-copepods interaction. Ingestion of MP by copepods has been demonstrated in both, the laboratory (Cole et al., 2013; Paper II, III, IV and V) and in the field (Desforges et al., 2015; Sun et al., 2017). Ingestion and impact of MP have been investigated in many copepod species and it

has been shown that MPs can affect their clearance rates (Cole et al., 2015); fecundity (Lee et al., 2013) or moulting process (Cole et al., 2019).

Copepods are considered a potential entry of MPs into marine food webs (Setälä et al., 2014). Although, research about the introduction and transfer of MPs in marine food webs is still scarce, some studies have shown that they can be transferred from copepods to higher trophic levels. For example, to shrimps (Setälä et al., 2014) or jellyfish (Costa et al., 2020). Copepods also have a role in the distribution and fate of the MPs trough the production of fast sinking fecal pellets containing the ingested MP (Cole et al., 2016). Therefore, copepods could ingest the MPs in surface waters together with the food and subsequently egest them via fecal pellets. The fate of the egested fecal pellets containing MPs are the subsurface water layers, and the final destination the seabed. There are evidence of changes in buoyancy and consistency of pellets containing MPs (Cole et al., 2016; Shore et al., 2021), reducing the sinking velocity. In that case, the degradation of the pellets could occur in the surface layers, keeping the MPs in suspension and therefore interrupting the exportation of POM (particulate organic matter) to the benthic community and seabed. In contrast, in our study with arctic copepods, we did not find any effects of MPs in copepod's fecal pellets (Paper II). These findings are highly dependent on the MPs characteristics, e.g. used concentrations and polymer type. Therefore, it is key to evaluate environmental realistic conditions to assess the potential effect of copepod's MP ingestion on global environmental processes like the marine carbon pump in the oceans (Shen et al., 2019).

Feeding behavior of copepods

Pelagic copepods are selective grazers which show different feeding behaviors. Kiørboe (2011) defined three main feeding strategies that include most of the pelagic copepods (Fig. 3): (1) ambush feeders are copepods that stay still and attack the preys that generate a hydro mechanical signal in their surrounding environment or that directly collide with the predator; (2) feeding-current feeders generate a current towards themselves capturing the preys that are coming with the feeding current and; (3) cruising feeders, which swim around in the water while capturing the preys that encounter on their way. Some species are mixed feeders, meaning that they have the ability to switch from active feeders to passive feeders (Kiørboe et al., 2016) depending on food availability.

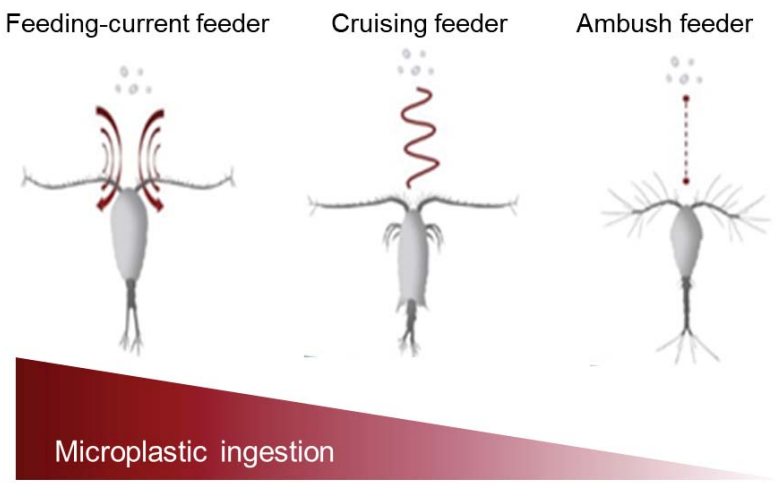


Figure 3. Schematic representation of the three main feeding behaviors of copepods. From left to right: Feeding-current feeders, cruising feeders (active feeders) and ambush feeders (passive feeders). The red triangle represents the initial hypothesis of Paper V expecting active feeders to ingest more microplastics than ambush feeders. (Source copepods vectors: Hans van Someren Gréve).

Certain feeding behaviors are more efficient for a specific prey type (Almeda et al., 2018). For example, Almeda et al., in 2018 demonstrated the low ingestion efficiency of ambush feeders on non-motile prey such as diatoms, compared with active feeders. Ambush feeders need a hydromechanical signal to detect the prey. Since MPs are immotile prey, is expected that ambush feeders ingest less MP and consequently play a minor role in the entry of MPs in marine food webs than feeding-current feeders and cruising feeders. In relation to the feeding mode but from a different perspective, the presence of MPs could also be affecting the copepods feeding responses. For example, the swimming or jumping frequency could change when copepods are exposed to certain plastic particles. It has been shown in previous studies with toxic algae (Xu et al., 2018). Suwaki et al. 2020, reported the effects in the swimming speed of *Temora turbinata* when exposed to microbeads, contrary to our findings in Paper V, where we did not find impact of MP in feeding response.

A high percentage (60%) of all the planktonic copepods used to evaluate the ingestion on MPs are feeding-current feeders (Fig. 4). The ingestion of MP for the most abundant and ubiquitous marine ambush feeder, *Oithona sp*, to our knowledge, it was never studied previous to this thesis. Moreover, other abundant copepods genus such as *Oncaea* spp. and *Microsetella* spp. are being neglected in MP research (Paper VI). Their feeding strategies are suited to solid substrates and

they are classified as “aggregate colonizers” (Koski et al., 2007, 2021). We know little about their feeding behavior and their risk on ingestion of MP. However, it is known that they feed on marine snow which, are potential substrates where MP accumulates (Kvale et al., 2020)

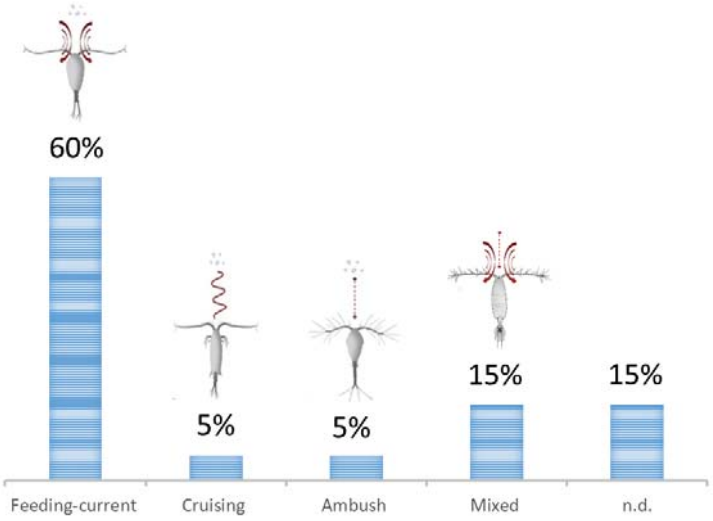


Figure 4. Percentage of the total studied copepod species with a specific feeding behavior (x axis). “n.d.” represents those species that do not have a clear behavioral classification. Data from Paper VI.

1.5. Progress made beyond the state of the art

In paper I, I contributed with trustful values of MP concentrations in Arctic surface waters, which is an understudied area. We used the most advanced methodology available for marine water samples. In the Paper II of this thesis, we evaluated the ingestion and impact of MPs in understudied key arctic copepods. New knowledge on MPs ingestion and impact in relevant *Calanus* species was provided. I used traditional bottle incubations to carry out exposure experiments. The output provided were resulted from representing more realistic conditions compare with previous studies in terms of MPs concentrations used and mimicking food bioavailability in the environment. Impact of MPs in potential scenario of co-exposure with other pollutants (Crude oil) were assessed, paper III.

With paper IV, this thesis make progress towards representation of the variability of MPs present in the marine environment. Different polymer types, shapes, and weathered process (biofilm and absorbed pollutants) were considered. Besides, small-scale observations were, for the first time, applied to get to understand the handling process of MPs by copepods. In marine ecology, small scale observations are applied to gain a better understanding of the mechanisms behind the

interaction between organisms and e.g. their prey (Henriksen et al. 2007; Kiørboe 2008, 2011; Kiørboe et al. 2014). Based on previous results, about prey-predator interactions, we apply the same method to investigate the unknown mechanism behind the MPs-copepods interactions. Xu et al. in 2018 documented the ability of feeding-current feeder copepods to reject toxic algae. In relation to feeding behavior, with these observations we can illustrate how the copepods handle plastic particles. Using this small scale video observations we could directly observed and documented how feeding-current feeders can capture the MPs, taste it and finally decide to reject or ingest the particle. This same methodology allow us, in Paper V, to film and evaluate the feeding responses of a mixed feeder when exposed to different prey and plastic sizes. Overall, the video observations allowed us to discover the processes happening in the ”blind” bottle incubations and understand in detail the mechanism behind the obtained results.

For the first time in MP research, a behavioral trait-based approach was applied. In Paper V we used a trait based approach to assess the influence of the copepods feeding behaviors in MP ingestion. Trait-based approach is widely used in theoretical modelling and it is a simpler alternative to the species analysis, focusing instead, on key traits of the interacting individuals of an ecosystem (Kiørboe et al., 2018). Applying this approach we are able to extrapolate our results to an ecosystem level. As mentioned above, the studied key trait evaluated in this thesis is the feeding behavior. Figure 5 represents the global distribution of the three main feeding modes of planktonic copepods in the oceans. In the laboratory, copepod models are used to represent the three feeding behaviors: *Temora longicornis* for feeding-current feeder; *Centropages hamatus* for cruising feeders and *Oithona davisae* for ambush feeders. For the first time, we aim to identify globally areas with high risk of entrance of MPs in marine food webs via copepods.

With Paper VI I evaluated trends and detected knowledgment gaps in relation to the evaluation of MP ingestion in marine food webs. This input will be useful to design future investigations in this field and to make further progress in understanding the effects of microplastics in marine food webs.

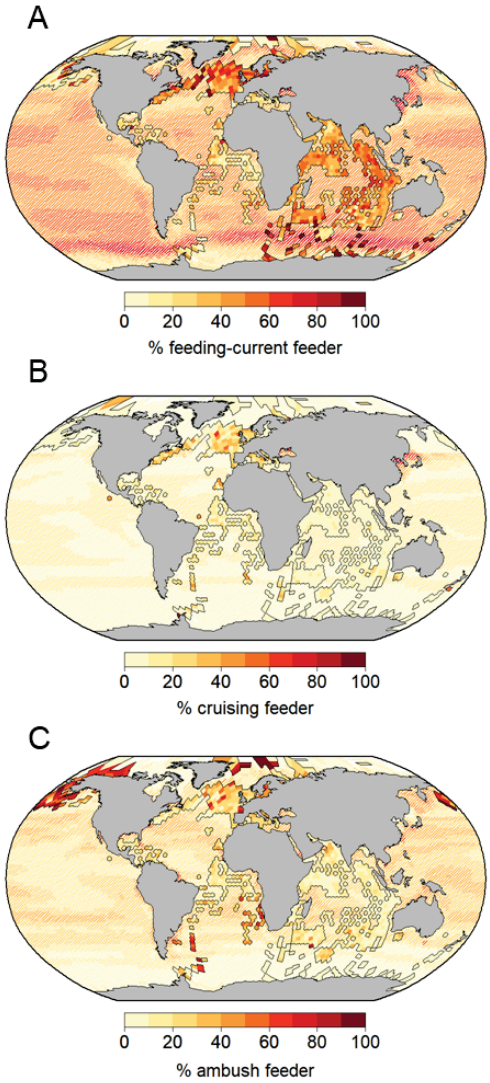


Figure 5. Global distribution of the abundance of each copepod's feeding behavior in the oceans. A: feeding-current feeders; B: cruising feeders and C: ambush feeders. Solid coloured poligons are direct observations and dashed area model-based information.

2. General conclusions

Despite increasing research in microplastic pollution contributing to our knowledge about MP-organisms interactions in the marine environment, we are still far from having a complete understanding of the fate and impact of MPs in marine ecosystems.

This thesis has quantified the amount of MPs in the Arctic environment where MP sampling is scarce. We documented relatively low concentrations of plankton- size MPs in the marine Arctic surface waters (142 MPs m^{-3}), in line with other field sampling studies.

This thesis has shown how ingestion of MP by copepods depends on the MP:prey ratio. High food concentration implies low clearance rates and consequently low MP ingestion. Therefore, given the low MP concentrations in nature, and considering the food availability, the probability of a copepod to encounter a MP in the marine environment is very low. For example, *C. finmarchicus* can clear 160 mL per day, which translate into a maximum encounter of 0.02 MPs per day in the studied Arctic area. Even when a copepod encounter a MP in the oceans, our high speed camera observations show that the copepod reject 80% of the encountered MP particles independently of the MP characteristics. In case of ingestion, the studied particles are egested via fecal pellets and consequently no impact is found on copepods. However, co- exposure of copepods to pollutants, such as crude oil, was found to induce feeding suppression and reduce fecal pellet production in an arctic copepod species (*C. hyperboreus*).

Copepods feeding behavior is a key trait to understand the interaction between copepods and MPs. Low ingestion of MPs was expected for ambush feeders which feeds inefficiently on non-motile prey. However, based on the results of this thesis, the risk of ingestion of MPs by copepods is low for all the analyzed feeding behaviors. Therefore, despite having a global geographical distribution of the behaviors we cannot identify areas with high risk of entrance of MPs into marine food webs via planktonic copepods.

Overall, based on the findings in this thesis, the impacts of MPs on marine planktonic copepods are low even at higher concentrations than those found in the surface waters. Furthermore, given the low ingestion rates from all studied copepods and their behavioral response to MPs, I can conclude that planktonic copepods are not relevant vectors for the entry of MPs in the marine food webs.

3. Future perspectives

To quantify MP in the oceans, and evaluate their potential environmental risk, future studies of MP in the marine environment need to be based on standardized sampling and quantification protocols. This would allow us to collect comparable data among studies and to get a global overview of MP in the oceans. Therefore, it is still necessary to explore and monitor MP in understudied areas, such as the Arctic, Antarctic and Indian Ocean, to get a global understanding of the distribution, composition and concentration of MPs in the oceans.

Future laboratory experiments should consider realistic environmental conditions of MP in order to assess the current effects of MPs in the ecosystems. The review included in this thesis (Paper VI), illustrates the temporal diversification of MP research in relation to polymer type and exposure concentrations. Given the complexity of the marine environment, mimicking the environmental conditions in the laboratory is challenging. However, it could be approached and improved in different ways: (1) using a multiple stressor approach where the influence of physical factors such as temperature and salinity are included, (2) carrying out mesocosms experiments that allow a more *in situ* like setup and, (3) applying trait-based approach and a principal component analysis (PCA) to simplify the plankton diversity and still make general conclusions while considering environmental conditions.

With respect ingestion and impact of MPs on copepods, there are some important groups of zooplankton or copepods that have been nearly ignored (Paper VI). Paper V is, to our knowledge, the first study of ambush feeder *Oithona spp*., a relevant and abundant genus in marine waters. Furthermore, Quantitative important groups of aggregate colonizing associated pelagic copepods with a global distribution, such as the harpacticoid *Microsetella spp.* and the cyclopoid oncaeid copepods, *Oncaea* spp., have also been neglected in MP research. These species live and feed on marine snow, which often contain higher concentrations of MPs than water. Therefore, it is relevant to evaluate the ingestion and, in particular, their role in the exportation of MP from the surface by these quantitatively important groups as well as other zooplankton (gelatinous zooplankton, protozoans) in future investigations.

Lastly, I want to highlight the need to continue investigating the plastic particles in the lower limit of microplastic, the nanoplastics. These particles smaller than 1μm have the potential to cause critical ecological impact due to their small size and therefore ability to migrate into the organism's tissues.

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Paper I

Quantification of plankton-sized microplastics in a productive coastal Arctic marine ecosystem

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Quantification of plankton-sized microplastics in a productive coastal Arctic marine ecosystem☆

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ABSTRACT

Microplastics (MPs) are polluting the Arctic, but our understanding of their abundance, distribution, and sources is limited. This study quantified MPs down to 10 μm in marine waters of the most populated region in Greenland. A new plastic-free pump-filter system was used to collect MPs from surface waters in the fjord Nuup Kangerlua close to Nuuk. Additionally, we took samples by horizontal tows with a bongo net (300 μm mesh-size). The median concentrations were 142 MPs m^{-3} and 0.12 MPs m^{-3} in the pump and bongo samples, respectively. The most abundant polymer was polyester across stations and sampling types. Fibers were the dominant shape in the bongo samples, while non-fibrous particles dominated in the pump samples. MP abundance was lower in the fjord and increased close to Nuuk and towards the open ocean, indicating that Nuuk is an important point source for MPs. In both samples, concentrations of MPs increased with decreasing size, illustrating the importance of quantifying the smallest fraction of MPs. Thus, the use of methods allowing for a quantification of the smallest MPs is vital to reduce the underestimation of MP concentrations in the environment. The smallest size fraction is also most available to plankton-feeding marine invertebrates and an important entry point for MPs into marine food webs. At the found concentrations, immediate adverse effects on the pelagic food webs are unlikely. However, growing anthropogenic activities could increase the risk of MPs to affect the sensitive Arctic ecosystem.

1. Introduction

It is well documented that microplastics (MPs, <5 mm) have already polluted the most remote areas on earth, such as the polar regions (Cincinelli et al., 2017; Lusher et al., 2015; Obbard et al., 2014). In the Arctic, MPs have already been detected in marine waters (Morgana et al., 2018; Tekman et al., 2020), sediments (Bergmann et al., 2017), sea ice (Peeken et al., 2018), and snow (Bergmann et al., 2019). Although data on MP concentrations in the Arctic is still very limited, a wide range of concentrations has been reported for Arctic seawater: from 0.0007 to 31,300 MPs m^{-3} (Table 1). Especially in sea ice and snow surprisingly high concentrations have been documented; for instance up to $1.2 \cdot 10^7$ MP m^{-3} in ice cores of Fram Strait (Peeken et al., 2018). The total load of plastics floating on the surface of the Arctic Ocean has been estimated to range between 100 and 1200 tons (Cózar et al., 2017), and global modeling of the distribution and accumulation of marine debris identified a garbage patch in the Barents Sea (van Sebille et al., 2012). These high levels of plastic pollution in an area with extremely low direct anthropogenic impact can to a great extend be explained by long-range transport of plastics with air and currents (Obbard, 2018). Atmospheric deposition has only recently been identified as a major pathway for global MP transport (Brahney et al., 2020) and can most likely explain high concentrations of MPs in Arctic snow samples (Bergmann et al., 2019). In contrast, long-distance transport by currents is well known and the poleward branch of the Thermohaline Circulation is a major pathway of plastics from the North Atlantic to the Greenland and Barents Sea

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Table 1
Reported MP concentrations in seawater, sea ice and snow samples in the Arctic region.

Location	Matrix and depth	MP conc. (MPs m^{-3})	Size limit (μm)	Sampling method	Dominant polymer	Reference
Nuup Kangerlua, West Greenland	Water, 5 m	142	10	Pump	Polyester	This study
	Water, surface	0.12	300	Bongo net		
Greenland Sea Gyre	Water, surface	800–3740	50	Pump and plankton net	Polyester	[1]
East Greenland	Water, 0–50 m	2.38 (2014)	500	WP-2 net	Polyester	[2]
Northeast Greenland	Water, 6 m	2.4	80	Water intake	Polyethylene	[3]
Greenland & Barents Sea	Water, surface	$6.3 \cdot 10^4$ km^{-2}	500	Manta trawl	–	[4]
Fram Strait	Water, 1–5350 m	95	32	McLane WTS-LV	Polyamide	[5]
Fram Strait, Svalbard	Snow	$1.76 \cdot 10^6$	11	Sampled with a spoon	Acrylates/PUR/varnish	[6]
Svalbard	Water, surface	0.34	333	Manta trawl	Polyester, polyamide	[7]
	Water, 6 m	2.68	250	Onboard pump		
Svalbard*	Water, 0–1000 m	0.0007–0.048	50	Niskin bottles	Paint	[8]
	Sea ice, free float	0.158		Boat hook	Unknown pigment	
Central Arctic Basin	Water, 8.5 m	0.7	250	Water intake	Polyester	[9]
	Water, 8–4369 m	20.8		Niskin bottles		
Arctic	Water, surface	31,300	100	1 L grab samples	Polyester	[10]
Central Arctic	Sea ice	$0.38–2.34 \cdot 10^5$	0.22	Drilling of ice cores	Rayon	[11]
Arctic	Sea ice	$0.11–1.2 \cdot 10^7$	11	Drilling of ice cores	Polyethylene	[12]
Arctic Central Basin	Sea ice	$0.2–1.7 \cdot 10^4$	100	Drilling of ice cores	Polyester	[13]
	Water below ice	0–18	250	Pump and sieve		
Chukchi Sea, Bering Sea	Water, surface	0.23–0.091	330	Manta trawl	Polyethyleneterephthalate	[14]

*Quantified all anthropogenic particles, including non-synthetic.
[1] (Jiang et al., 2020).
[2] (Amélineau et al., 2016).
[3] (Morgana et al., 2018).
[4] (Cózar et al., 2017).
[5] (Tekman et al., 2020).
[6] (Bergmann et al., 2019).
[7] (Lusher et al., 2015).
[8] (von Friesen et al., 2020).
[9] (Kanhai et al., 2018).
[10] (Barrows et al., 2018).
[11] (Obbard et al., 2014).
[12] (Peeken et al., 2018).
[13] (Kanhai et al., 2020).
[14] (Mu et al., 2019).

(Cózar et al., 2017). Model simulations suggest that these areas are accumulation zones for plastics, holding 95% of the estimated plastic load in the Arctic. This was corroborated by field measurements (Cózar et al., 2017). One branch of the Thermohaline Circulation also goes into the Labrador Sea, but except for the study by Cózar et al. (2017), no data on MP pollution is available from the west coast of Greenland.

West Greenland receives plastic debris from the North Atlantic as well as from local point sources. Relevant point sources of plastic pollution include fishing and maritime industry, raw sewage outlets, mismanaged waste, and last but not least Nuuk, the capital and largest city of Greenland with 18,000 inhabitants. Nuuk holds the biggest port of Greenland and the city's wastewater is discharged untreated to the fjord Nuup Kangerlua (Gunnarsdóttir et al., 2013). Thus, there are several unquantified sources of MP pollution in West Greenland. If MPs introduced to the marine ecosystem are taken up by the marine food web, Greenlandic livelihood may be impacted. Fishery alone provides more than half of Greenland's export income (Jacobsen et al., 2018). If the food web efficiency or the quality of the marine products are affected by MPs, it will potentially have a major impact on the local and national economy of Greenland.

The present study is the first to focus on marine MPs in the most populated area of Greenland. Furthermore, we applied a new plastic-free pump-filter system, which allows the collection and full quantification of MPs down to 10 μm. Very few studies have quantified MPs down to that size in the Arctic (Table 1). This abundant and understudied fraction of MPs overlaps in size with the planktonic prey of the dominant secondary producers in the Arctic, the *Calanus* copepods (Cole et al., 2013; Levinsen and Nielsen, 2002). The large lipid-rich *Calanus* copepods have a key position in the Arctic marine ecosystem. They provide food for the important Greenlandic fish stock and are responsible for carbon sequestering through the export of carbon to the deep waters and sediment by the production of large, fast-sinking fecal pellets (Juul-Pedersen et al., 2006). The presence of plankton-sized marine MPs in the highly productive coastal ecosystem around Nuuk gives rise to concern for the quality of the important commercial and recreationally exploited fish stocks (Jacobsen et al., 2018). Therefore, the present study aims to provide a baseline of plankton-sized MPs (>10 μm) from the fjords of Greenland and the coastal area towards the potentially most important point source of MPs in West Greenland: the capital Nuuk.

2. Materials and methods

2.1. Study site

The sampling of six stations in Nuup Kangerlua, in the bay in front of Nuuk, and at the banks off the coast of West Greenland, was done from the research vessel R/V Sanna (Greenland Institute of Natural Resources) between 10 and 12 of May 2019. The stations differed in their proximity to the expected MP point source Nuuk (Fig. 1, see more details in SI). Nuup Kangerlua is located on the southwest coast of Greenland with the capital Nuuk (64°10′N, 51°44′W) located at the mouth of the fjord. The average depth is approximately 250 m (max. depth >600 m) (Mortensen et al., 2011). The main fjord branch is approximately 190 km long, and several sills characterize the outer part of the fjord. The uppermost branch of Nuup Kangerlua connects the marine environment to the

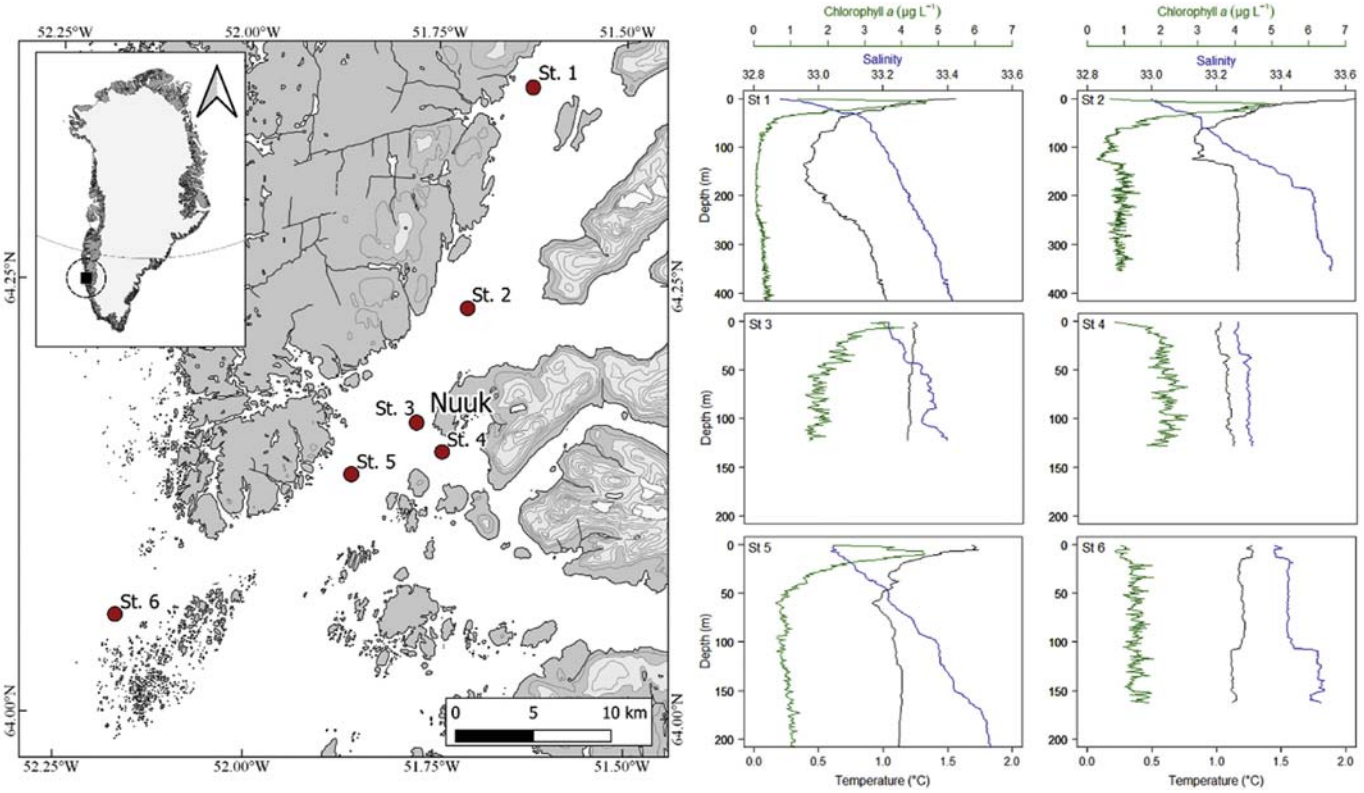

Fig. 1. Sampling stations in Nuup Kangerlua with respective CTD and chlorophyll *a* profiles. (For interpretation of color in this figure, the reader is referred to the Web version of this article.)

margin of the Greenland Ice Sheet. The freshwater input induces a seasonal stratification of the upper part of the water column (Fig. 1), which is further strengthened by solar heating during summer (Mortensen et al., 2013, 2011).

2.2. CTD measurements

On arrival at the sampling station, vertical profiles of water temperature, salinity, density, and fluorescence were obtained using a CTD (SBE 19plus V2 CTD from Sea-Bird Electronics) equipped with a Seapoint chlorophyll *a* fluorometer and a Biospherical/Licor sensor. Depth profiles were recorded from the surface to 5 m above the bottom. Water samples for chlorophyll *a* (chl *a*) measurements were collected at 1, 5, 10, 15, 20, 30, 40, 50, 100, 150, 250, and 300 m depth using a 5 L Niskin bottle between 1 and 2 of May. Each sample was filtered onto a GF/F filter and extracted in 96% ethanol for 12–24 h (Jespersen and Christoffersen, 1987). Fluorescence was measured on a Turner TD-700 fluorometer (Turner Designs, California, USA) before and after acidification. The fluorometer was calibrated against a pure chl *a* standard. The analysis was used for calibration of the CTD fluorometer, and chl *a* concentrations were then calculated from CTD fluorescence profiles at all stations. The phytoplankton biomass was calculated from the average chl *a* in the upper 10 m, a carbon/chl *a* conversion factor of 42.6 (Juul-Pedersen et al., 2006) and a carbon to dry weight conversion factor of 55% (Hansen et al., 1994).

2.3. MP sampling

Two different sampling devices were employed to collect one sample each at the six stations. We collected MPs >10 μm from 5 m depth using a custom made pump-fed filtering device (hereinafter termed "pump samples"). In addition, we took surface samples with a submerged bongo net targeting >300 μm sized MPs (hereinafter termed "bongo samples"). The plastic-free pump-filter system was developed to sample the smallest fraction of MPs, which is often neglected in other studies. The purpose of the bongo samples was to allow comparability to previous studies using this and similar net-sampling techniques. Both samples were taken in the upper 5 m, i.e., above the pycnocline (Fig. 1), in the water impacted by the meltwater from the inner part of the fjord system. Unfortunately, the bongo sample from station 1 was lost during transportation, resulting in only 5 stations for this sampling method.

The plastic-free pump-filter device (UFO system - Universal Filtering Objects system) was composed of a metal hose deployed in the water, a pump controlled by an inverter, and a modular filtering device capable of filtering large volumes of water (Fig. S1, SI). All parts of the system were in metal but for a gasket of rubber in the three filtering cartridges themselves. In brief, the water was pumped through a flexible metal hose, the mouth of which was equipped with a stainless steel metal cage of 5 mm mesh to protect the system against large debris. A positive displacement pump with a brass impeller (Oberdorfer Gearpump N994RE) sent the water through a short metal hose to the filter cartridges. Three separate stainless steel filter cartridges were applied, which each contained a stainless steel filter of 167 mm diameter. The water first passed through a filter of 300 μm mesh to retain bigger items with the purpose of protecting the finer filtering mesh from clogging. The water was then divided onto two parallel units with filters of 10 μm. The outlets were re-combined and connected to a mechanical flowmeter to quantify the filtered volume. The inlet hose was deployed at 5 m depth using a crane, and approximately 1 m^3 of water was filtered at each station. After sampling, the system was evacuated, and the single filter cartridges were opened inside a clean fume hood to prevent plastic contamination. Both the 300 μm

and the two 10 μm enriched filters collected at each station were transferred to a glass petri dish, which subsequently was wrapped in aluminum foil and frozen at −20 °C until sample processing. Three air blanks were taken during the sampling by exposing empty petri dishes with pre-muffled 10 μm steel filters to the surrounding environment during the opening phase of the UFO system. The air-blanks were taken and stored similarly to the samples. Moreover, several paint fragments were collected onboard the ship to assess the potential contamination related to this specific source.

The bongo net, equipped with a flowmeter, was deployed 3 m from the side of the ship using a crane and dragged for 20 min in the upper 0.5 m of the water column. The net was made of nylon, it had a mesh size of 300 μm, and the samples were collected in the cod-end of the net. The bottle at the cod-end was removed from the net, and the content was carefully rinsed onto a 300 μm nylon mesh. The mesh was carefully folded, wrapped in aluminum foil, and stored in a polyethylene plastic zip-lock bag at −80 °C until sample processing.

2.4. Sample processing

The pump samples were processed following a protocol slightly modified from Liu et al. (2019) (see SI for details). In brief, this included six treatment steps: 1) SDS (5% sodium dodecyl sulfate) treatment for 24 h and transfer of samples onto 10 μm steel filters (47 mm diameter), 2) overnight incubation with protease (Sigma, Protease from *Bacillus* sp.) and lipase (Strem Chemicals, Lipozyme® TL 100L), 3) incubation with cellulase (Sigma, Cellulase enzyme blend) and Viscozyme® L (Sigma) for 72 h, 4) oxidation (Fenton reaction) with hydrogen peroxide, sodium hydroxide and iron sulfate overnight, 5) size fractionation with a 500 μm steel sieve, 6) density separation of the fraction <500 μm with sodium polytungstate (1.9 g cm^{-3}). The fraction >500 μm underwent spectroscopic analysis together with the bongo samples. The filter with the sample fraction <500 μm was sonicated and rinsed with 50% ethanol, and all liquid was sequentially transferred to 10 mL glass vials and evaporated in a water bath at 50 °C using a stream of nitrogen (Biotage, TurboVap). When the samples were dry, 5 mL of 50% ethanol were added for subsequent analysis with FPA-μFTIR-Imaging (Focal Plane Array-Fourier-Transform Infrared Imaging-micro Spectroscopy). The collected air-blanks from the ship underwent the same processing and analysis in the laboratory as the pump samples. Thus, the blank samples monitor the combined MP contamination potentially occurring onboard and later during sample processing and analysis.

For the bongo samples we used a simplified protocol. The samples were rinsed off the mesh into glass beakers with a 5% SDS solution and kept at 50 °C with gentle stirring (100 rpm) for 48 h. The samples were then sieved over a 200 μm stainless steel sieve, rinsed with filtered water, and flushed back into the beaker with 200 mL of Tris (trisaminomethane)-HCl buffer (pH 8.3), adding 700 μL of protease, 500 μL of lipase, and incubating at 50 °C with gentle stirring for 72 h. Thereafter, samples were vacuum-filtered onto 10 μm stainless steel filters (47 mm diameter) and stored in glass petri dishes until analysis.

2.5. MP analysis

The pump samples were analyzed using FPA-μFTIR-Imaging, which is, at present, considered the most suitable analytical approach for analysis of small MPs (Liu et al., 2019; Löder et al., 2015; Mintenig et al., 2016; Primpke et al., 2016; Simon et al., 2018; Vianello et al., 2019). A sub-sample of the 5 mL particle suspension (3 aliquots of 1–1.2 mL – corresponding to 60–72% of the total volume) was deposited onto three 13 × 2 mm zinc selenide (ZnSe) infrared windows pre-heated and held in a compression cell (PIKE Technologies, Fitchburg, WI, USA) using a capillary glass pipette (micro-classic, Brand GmbH, Germany). Each enriched window was dried overnight (55 °C) and subsequently analyzed, accounting for the whole active surface (10 mm diameter - 78.5 mm^2). The applied instrument was an Agilent 620 FTIR microscope with a 128 × 128-pixel FPA detector combined with a Cary 670 FTIR spectrometer (Agilent Technologies, Santa Clara, CA, USA). It provides two main outputs: a magnified optical image, and the relative IR map of stitched tiles of 128 × 128 pixels, co-adding several scans. Each IR map pixel contains an FTIR spectrum, allowing the identification of a wide range of organic and inorganic materials (including synthetic polymers) by comparing the unknown spectra with a dedicated database. The analysis was carried out in transmission mode in a spectral range of 3750–850 cm^{-1} at 8 cm^{-1} resolution, using a 15× Cassegrain objective/condenser with 5.5 μm resulting pixel size; 120 co-added scans were collected for the background on a single tile, while 30 co-added scans were recorded when scanning the sample. The beam attenuation was 50%. The scan time at these settings was approximately 4 h.

The bongo samples were visually inspected using a stereo microscope with a connected camera (SteREO Discovery V8 with Axiocam 105 Color, Zeiss GmbH, Oberkochen, Germany). All potential plastic particles were photographed and categorized by shape (fibers vs. all other shapes), length, and width. The material composition of each particle was then analyzed by Attenuated Total Reflection – Fourier Transform Infrared Spectroscopy (ATR-FTIR), using an Agilent Cary 630 FTIR spectroscope (Agilent Technologies, Santa Clara, CA, USA) with a single reflection diamond ATR. The samples were positioned onto the ATR crystal and then compressed using the instrument clamp to achieve optical contact, allowing to record surficial ATR-FTIR spectra. The spectra were recorded at 4 cm^{-1} spectral resolution by co-adding 64 scans, and a background in air was collected before each measurement. Each collected spectrum was exported and compared to multiple spectral databases containing both synthetic polymers and non-synthetic materials (Omnic 8, Thermo Fisher Scientific, Madison, WI, USA).

2.6. Contamination control

The use of plastic materials during sampling and sample processing was avoided if possible, except for the polytetrafluoroethylene (PTFE) stopcock of the separation funnels and the PTFE septa of the vials containing the processed pump samples. Therefore, this material, together with the rubber from the UFO gaskets, was excluded from the MP quantification. Furthermore, we took samples of the ship's paint and excluded matching paint particles in the samples. For the bongo samples, potential contamination could have resulted from the nylon mesh as well as the polyethylene zip-lock bags. All materials were either rinsed with filtered water or muffled at 500 °C and wrapped in aluminum foil until use. Samples were also covered with aluminum foil or glass lids whenever possible. All solutions used for processing the samples were filtered over 0.7 or 1.2 μm GF filters. Sample processing was done in a laminar flow cabinet, and cotton lab coats were worn. As described above, three air-blank samples were collected on the ship during sampling, which underwent the same laboratory processing and analysis as the real samples. They therefore represent the total contamination, from sampling until sample analysis.

2.7. Data analysis

The collected FPA-Imaging data were processed using siMPle (Primpke et al., 2020), an open-source software developed by

Aalborg University and Alfred Wegener Institute, which allows the automated analysis of large μFTIR-Imaging datasets. In brief, it runs a Pearson correlation between each sample spectrum and the database using the raw spectrum, first and second derivative, reconstructing each particle from the FTIR spectra of the pixels it covers, and hence providing a false-color map of the identified materials in the sample. The software also provides morphological and size measurements (Vianello et al., 2019), as well as estimated mass measurements (Liu et al., 2019). With regards to particle shape, we differentiate between fibers and particles (i.e. all other shapes) as described in Vianello et al. (2019) (Fig. S3, SI). The mass of the plastic particles in the pump samples was estimated following an approach described by Simon et al. (2018). In short, the 2-dimensional size and shape of a MP particle obtained by the siMPle analysis are used to estimate a particle volume. The mass is then derived by multiplying with the typical density of the determined polymer.

3. Results and discussion

3.1. MP abundance and size distribution

We found MPs in the water samples from all stations. In the pump samples (>10 μm), we found MP concentrations of 67–278 particles m^{-3}, with a median of 142 particles m^{-3} (Fig. 2a). The MP concentrations (>300 μm) found in the bongo samples were 2–3 orders of magnitude lower than those collected by the pump, ranging between 0.08 and 0.4 MPs m^{-3} with a median concentration of 0.12 MPs m^{-3} (Fig. 2b). Data on MPs in Arctic waters is scarce, and a wide range of concentrations has been reported (Table 1). The results of the bongo samples are in a similar range as what was collected using a Manta net around Svalbard (Lusher et al., 2015) and in the Chukchi and Bering Sea (Mu et al., 2019) as well as from pump samples in the Arctic Central Basin (Kanhai et al., 2018). The MP concentrations from our pump samples are comparable to recently reported values from surface water samples in the Fram Strait, where 113–262 particles m^{-3} were found (except for one station with 1287 particles m^{-3}) (Tekman et al., 2020). Tekman et al. (2020) fully quantified MPs down to 32 μm (the size of the sieve during sampling) and semi-quantified MPs down to 11 μm.

Despite the comparative remoteness of the studied area, the concentrations of MPs found in this study were similar to those reported in locations with higher anthropogenic activity. In Manta trawls close to the Danish coast, the average MP concentration was 0.07 m^{-3} (>300 μm) (Tamminga et al., 2018), and in marine waters around Japan, Isobe et al. (2015) found a median concentration of 0.74 MPs m^{-3} (>350 μm). A quantification of MPs down to 10 μm in a transect from the European coast across the Atlantic to the Sargasso Sea gave 13 to 501 MPs m^{-3}, with the highest concentrations in the English Channel (Enders et al., 2015).

Other studies from the Arctic report both lower and higher MP concentrations in comparison to our findings (Table 1). Several factors, however, complicate comparisons between studies. Firstly, the Arctic covers a vast area and the sampled locations differ greatly with regards to oceanographic regimes and potential MP sources. Since our study is the first to analyze MPs on the west coast of Greenland, the closest available data on MPs comes from East Greenland. There, long-range transport is expected to play a much bigger role, while local sources are more relevant for West Greenland (see MP distribution and sources). Secondly, studies employed widely different methodologies for sampling, sample preparation, and analysis, as well as the analyzed size fractions. The problems with lack of standardization are frequently emphasized within the MP field (e.g., Avio et al., 2017; Mai et al., 2018). The impact of the chosen methodology on the results becomes very apparent when comparing our two sampling methods. The concentrations from the pump and the bongo net differed by 2–3 orders of magnitude. This is likely related to several factors. While the particle identification and quantification of the pump samples were fully automated, the analysis of the bongo samples relied on visual pre-sorting, which entails the risk of missing particles. More importantly though, the lower size limit was very different, with 10 μm for the pump and 300 μm for the bongo net. The results of our pump samples clearly show an increase in particle numbers with decreasing size (Fig. 2e). The size distribution was similar for all stations (Fig. S2, SI). Overall, 93% of all the particles we found were smaller than 300 μm and 68% smaller than 100 μm. Only for the size fraction of 11–25 μm we found very few particles. This is most likely related to the detection limit of the μFTIR approach, which is limited to around 11 μm due to diffraction phenomena. The spectra of very small and thin particles often have a low signal-to-noise ratio, affecting their automatic identification by the siMPle software, probably leading to an underestimation of the smallest MP particles. The size distribution in the bongo samples showed a similar trend of higher particle numbers with decreasing size, with the exception of a large number of very big particles (>5000 μm) (Fig. 2f). These were mainly long fibers, which were also the dominant particle shape in the bongo samples (see MP shape and polymer composition). Similar size distributions as in our pump samples were found in studies on Arctic sea ice (Peeken et al., 2018), snow (Bergmann et al., 2019), and water samples (Tekman et al., 2020), which also analyzed MPs down to 11 μm. This finding clearly illustrates the importance of quantifying the smallest MPs. Since the majority of published studies on MP concentrations in marine waters used a mesh size that is considerably larger, concentrations are generally underestimated at present. Therefore, we encourage the use of pump-filter systems as used in the present study or similar devices, which enable sampling the smallest size fraction of MPs that can be analyzed.

3.2. Blank samples

The blank samples contained both the on-site airborne contamination and the contribution derived from the laboratory. The results showed an average contamination of 6.7 ± 2.5 MPs per sample, corresponding to 4.8 ± 2.5% of the average MP abundance in the analyzed samples. The polymeric composition of the contaminating MPs was 50% polyester, 25% polyamide (nylon), 17% ethylene vinyl acetate, and 8% polystyrene. The results were corrected for contamination by subtracting the averaged contribution of every single polymer found in the blank samples (both by particle number and mass). The polyester and nylon items identified in the blank samples could be related to airborne contamination from textiles, as most of the technical clothes used onboard are made of these polymers. The ship paints were another relevant source of contamination found in the analyzed samples, but not in the blanks. Several paint particles were identified by μFTIR-Imaging analysis as the specific paints collected onboard from different surfaces of the ship during the survey. This specific contribution was excluded from the results. However, this finding stresses the importance of monitoring every source of potential contamination to reduce bias in MP analysis.

3.3. MP shape and polymer composition

Fibers were found in all samples, but while they comprised the minority of shapes in the pump samples (18%), fibers were the dominant shape in the bongo samples (73%) (Fig. 2a and b). Fibers were also the predominant shape in Manta trawls (333 μm mesh)

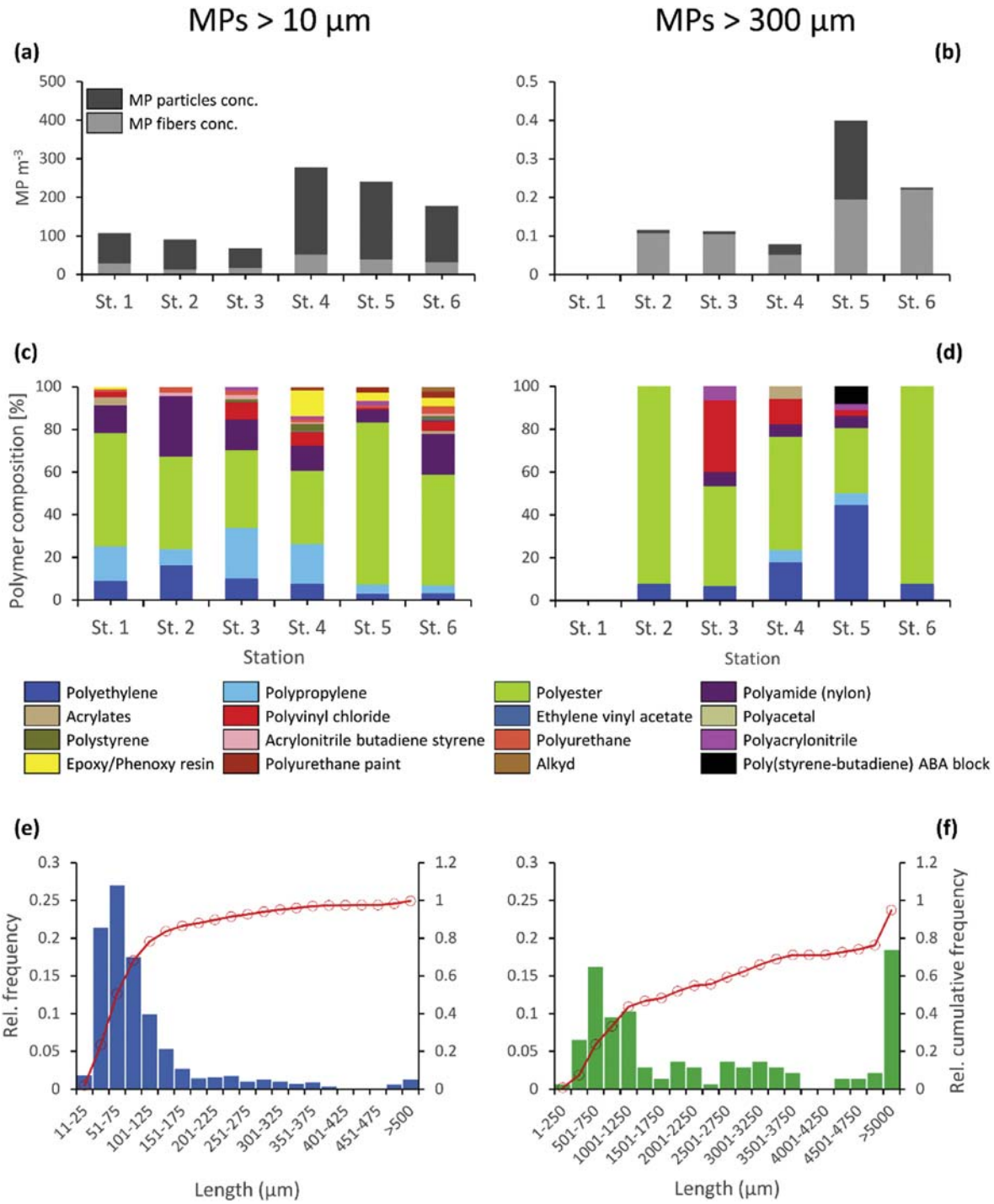

Fig. 2. Microplastics (MPs) from pump (left panels) and bongo (right panels) samples: MP concentration (MPs m^{-3}) in (a) the pump and (b) the bongo samples; Polymer composition (%) in (c) the pump and (d) the bongo samples; Size distribution (length - µm) in (e) the pump and (f) the bongo samples. The red line in (e) and (f) shows the relative cumulative frequency of MPs. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

close to Svalbard (Lusher et al., 2015) and vertical tows with a plankton net (500 µm mesh) in East Greenland (Amélineau et al., 2016). The most common polymer from both sample types was polyester, except for the bongo sample at station 5 (Fig. 2c and d). This is in accordance with other Arctic studies, which found polyester to dominate (Amélineau et al., 2016; Barrows et al., 2018; Kanhai et al., 2020, 2018; Obbard et al., 2014) (Table 1). One likely source for polyester is synthetic textiles, which fits with the finding of fibers. It has been shown that textiles can release thousands of fibers during washing, which subsequently end up in the wastewater (Napper and Thompson, 2016; Pirc et al., 2016). Since Nuuk has no wastewater treatment, all fibers from washing effluent are released directly to the fjord and are a highly likely source for polyester in our samples. Furthermore, polyethylene terephthalate, which is part of the polyester family, is one of the most produced and used plastics, e.g., for plastic bottles and packaging material. The next most common polymers were polyamide/nylon, which is used for fishing nets, polyethylene and polypropylene, which are

among the top 5 high-production volume plastics globally, and epoxy/phenoxy resins, which are for instance applied as adhesives or coatings in boats (Andrady, 2011; Hoge and Leach, 2016; PlasticsEurope, 2019). Overall, the polymer composition reflects important local use of plastics: fleece clothing for low temperatures, plastic packaging, fishing, and boating.

3.4. MP distribution and sources

The relative abundance of MPs between stations was similar for both methods applied (except for station 4), illustrating that both methods give a representative description of the MP distribution. In general, the distribution pattern followed the oceanography of the fjord, with the lowest MP concentrations (68–107 MPs m^{-3}) at the meltwater-impacted stations inside the fjord (stations 1, 2 and 3) while the MP concentration peaked after passing the major point source of the capital Nuuk (Fig. 2a and b). This indicates that Nuuk is an important source for MPs in this area, as illustrated by the increase in MPs along the cruise track from the inner fjord and passing Nuuk towards the sea. Furthermore, data on wind direction and water flow within the fjord during the sampling indicated that the upper water layer was pushed out of the fjord (data not shown). This means that plastics originating from Nuuk were even more likely to be found towards the outer part of the fjord. The importance of local mismanagement of plastic waste in West Greenland was also confirmed by beach litter monitoring, which reported the majority of plastic debris to originate from local sources (PAME, 2019). This underlines the importance of local anthropogenic activities for plastic pollution in comparison to long-range transport with currents, which is expected to be more prominent for Greenland's east coast (PAME, 2019). Airborne transport of MPs has been suggested as an important pathway. A recent study found extremely high concentrations of up to $14.4 \cdot 10^6$ MPs m^{-3} in Arctic snow samples (Bergmann et al., 2019). This is a relevant finding with respect to the present study as the Nuup Kangerlua is fed by meltwater runoff from the Greenland Ice Sheet, terrestrial runoff, meltwater from sea ice, and calved glacial ice (Mortensen et al., 2013). A considerable fraction of the water in the upper layers of Nuup Kangerlua is meltwater. Part of this is old ice from the bottom of the glacier that is expected to be MP-free, but melted snow significantly contributes to freshwater input into the fjord. With a MP contamination of snow as reported from the Fram Strait, we would expect much higher concentrations in the surface waters of Nuup Kangerlua, especially in the inner fjord. Our findings indicate that the MP contamination of snow in West Greenland is most likely several orders of magnitude lower than reported by Bergmann et al. (2019) and the main source of MPs in the fjord is of local origin rather than airborne. However, it is important to note, once MPs enter the water column, they are also vertically exported to the seafloor/deep waters by physical and biological processes (Choy et al., 2019; Long et al., 2015; Möhlenkamp et al., 2018; Peng et al., 2018; Porter et al., 2018). In fact, marine sediments in the deep sea are a major sink for plastic pollution (Woodall et al., 2014). More research is needed to understand the distribution and fate of MPs in the Arctic ecosystem.

3.5. MP mass versus number

For the pump samples, mass concentrations of MPs were determined, which ranged from 9.3 to 51.3 μg m^{-3}, with a median of 28.8 μg m^{-3} (Fig. 3a). The polymer composition in terms of mass differed from the particle number-based composition (Fig. 2c). Polyethylene constituted the highest mass in the stations inside the fjord (stations 1 and 2), polyvinyl chloride dominated at station 3, polyester at stations 4 and 5 close to Nuuk, and polyamide was the polymer with the highest mass at station 6, close to the open ocean (Fig. 3b). Also, the distribution of MPs between stations gave a somewhat different picture than the number-based results. While the stations from Nuuk towards the open ocean (stations 4–6) still had higher concentrations than the more inward stations 2 and 3, station 1, furthest inside the fjord, showed the second-highest MP abundance (Fig. 3a). This speaks for the presence of a few larger (or heavier) plastic particles at that station. Plastic concentrations are rarely reported in terms of mass for water samples, especially for small-sized MPs, even though models of plastic input and abundance in the oceans on a big scale often report mass (Cózar et al., 2017; Jambeck et al., 2015; van Sebille et al., 2015). Cózar et al. (2017) estimated the total load of plastics floating on the surface of the Arctic Ocean to range between 100 and 1200 tons. The Arctic Ocean has an area of $1.4 \cdot 10^7$ km^2. Assuming that the surface area equals the upper 1 m of the water column, the reported mass equals a mass concentration of 7–86 μg m^{-3}. Although this is a rough estimate, our values fall within this range.

Our results illustrate that the decision of whether to report MPs in terms of mass or number will influence the picture that we get of the MP contamination. Both have their strengths and weaknesses. Mass concentrations on the one hand can be more informative about the sources and input of plastics into the environment. On the other hand, number-based concentrations are important in a biological context when considering organisms that interact with individual particles. In the best case, both should be reported. However, if only one is chosen, this choice should be based on a thorough consideration of what the results will be used for.

3.6. Biological implications

The dominant size fraction of MPs found in the fjord is the most available for plankton-feeding marine invertebrates. These particles overlap in size with their natural prey, and the ingestion of MPs between 7 and 150 μm has, for instance, been observed for different copepod species (Cole et al., 2013; Sun et al., 2017; Vroom et al., 2017). The high abundance and vital role of copepods in the Arctic ecosystem illustrate their key position as an entry point for MPs into Arctic food webs. However, compared to their phytoplankton prey, the mass of plankton-sized MPs is more than 3–4 orders of magnitude lower than that of phytoplankton (Fig. 4), which illustrates the low probability for ingestion and impact on zooplankton. The ratio between phytoplankton and MPs depends, however, on the location. While the ratio was high in the inner fjord due to a phytoplankton bloom, it was considerably lower from Nuuk towards the open ocean (stations 4–6) (Fig. 4). This means that the likelihood for a plankton-feeding organism to ingest MPs differs greatly between stations, depending on the phytoplankton biomass "background". Benthic invertebrates like crustaceans and bivalves, as well as fish, have been found to contain MPs (Abbasi et al., 2018; Davidson and Dudas, 2016; Devriese et al., 2015; Li et al., 2018). Furthermore, studies have documented the trophic transfer of MPs (Setälä et al., 2014; Welden et al., 2018). Thus, MP pollution is of concern to marine food webs. However, at the concentrations of MPs found in this study, a minor impact on the Arctic pelagic food web is expected. Still, in a predicted scenario of increasing plastic pollution (Lebreton et al., 2019; Lebreton and Andrady, 2019) and accelerated melting of Arctic sea ice, increased MP pollution, together with other anthropogenic stressors, could negatively affect the Arctic pelagic food web. This could be a major concern for the fishing and seafood sector in Greenland in the future, where the economy heavily depends on unpolluted marine resources (Jacobsen et al., 2018). Therefore, it is of paramount importance to get a better understanding of the sources, distribution, and abundance of MPs in the Arctic waters, and to reduce global plastic pollution that will end up in the Arctic.

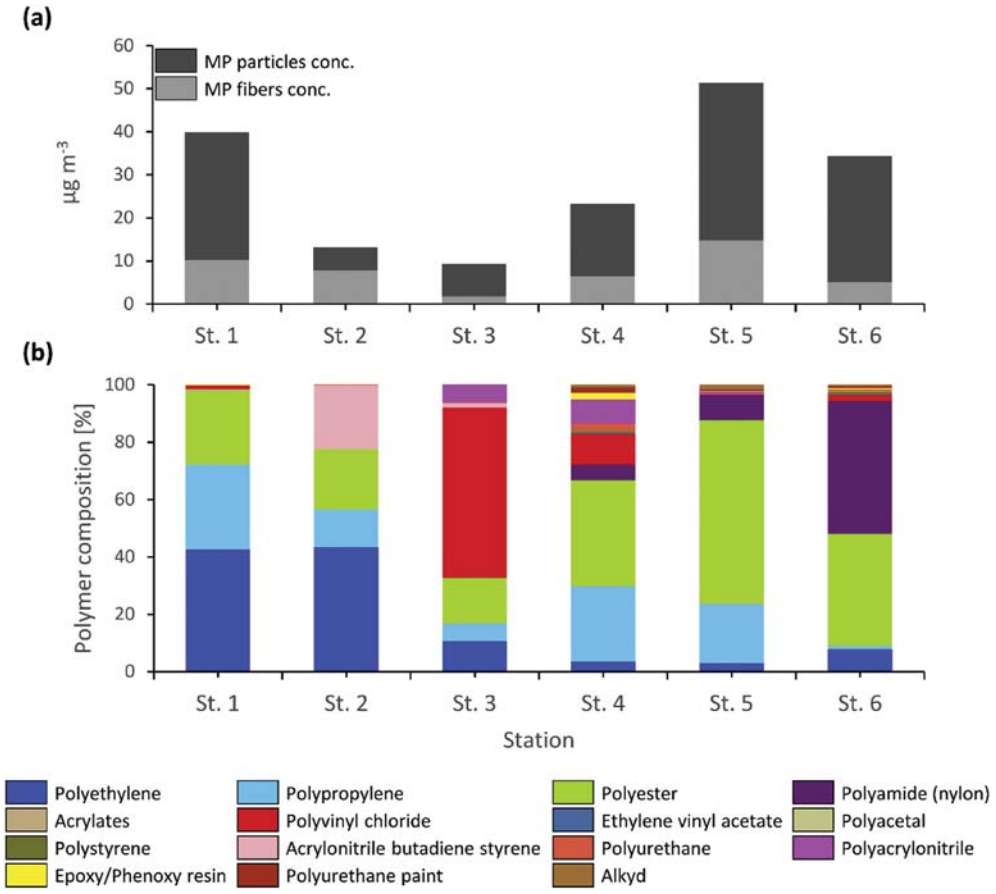

Fig. 3. (a) Mass-based microplastic (MP) concentrations (µg m^{-3}) of the pump samples and (b) polymer composition based on estimated mass. (For interpretation of color in this figure, the reader is referred to the Web version of this article.)

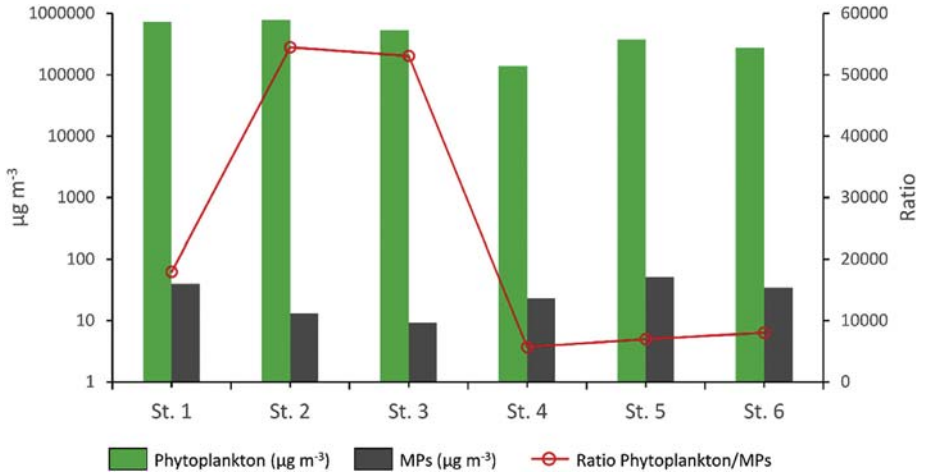

Fig. 4. Mass concentrations (µg m^{-3}) of phytoplankton (green), based on the fluorescence measurements of the CTD, and microplastics (MPs) (grey) at the six sampling stations. The red line shows the ratio of phytoplankton to MPs in terms of mass. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Sinja Rist: Investigation, Writing - original draft. **Alvise Vianello:** Conceptualization, Methodology, Investigation, Visualization, Writing - review & editing. **Mie Hylstofte Sichlau Winding:** Investigation, Visualization, Writing - review & editing. **Torkel**

Gissel Nielsen: Project administration, Conceptualization, Investigation, Writing - review & editing. **Rodrigo Almeda:** Conceptualization, Investigation, Writing - review & editing. **Rocío Rodríguez Torres:** Investigation, Writing - review & editing. **Jes Vollertsen:** Conceptualization, Methodology, Writing - review & editing.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2020.115248.

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Supporting Information: Quantification of plankton-sized microplastics in a productive coastal Arctic marine ecosystem

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S1. Materials and methods

Sampling locations

Table S1. GPS coordinates of the sampled stations.

Station name	Sampling date	Latitude	Longitude
St. 1	11.05.2019	64° 21.916 N	51° 37.537 W
St. 2	12.05.2019	64° 14.199 N	51° 42.667 W
St. 3	11.05.2019	64° 10.256 N	51° 46.257 W
St. 4	10.05.2019	64° 09.174 N	51° 44.529 W
St. 5	12.05.2019	64° 08.372 N	51° 51.672 W
St. 6	10.05.2019	64° 03.397 N	52° 10.204 W

Universal Filtering Objects (UFO) system

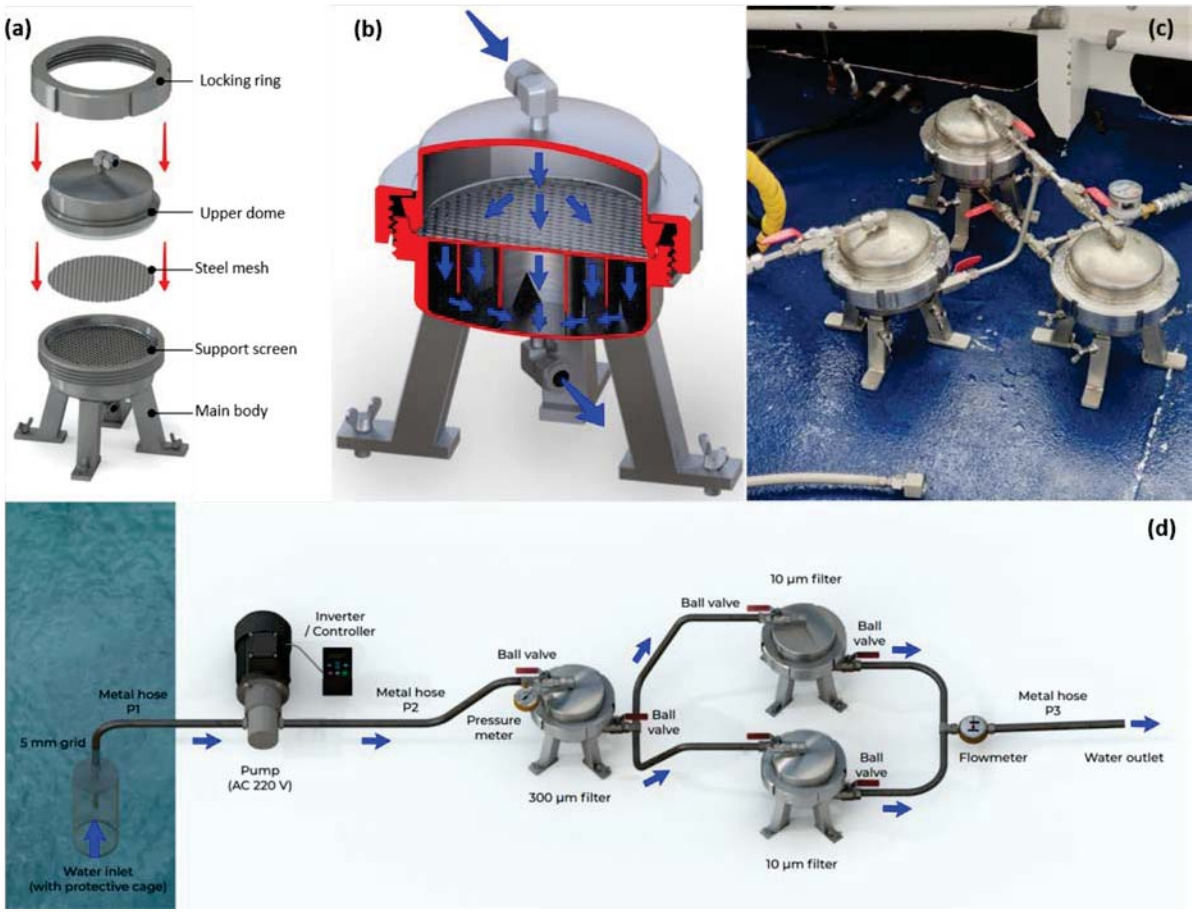

Figure S1. (a) Mounting schematic of a single UFO unit, highlighting the main components; (b) cross-section of a single UFO (the blue arrows illustrate the water flow) credits: Andras Valer Kiss, Lucian Iordachescu; (c) picture of the real setup operating on board during the survey, credits: Alvise Vianello; (d) overall schematics of the setup of the Universal Filtering Objects (UFO) system used to collect the pump samples, credits: Andras Valer Kiss, Lucian Iordachescu

Detailed description of the sample processing of the pump samples

The three filters of a pump sample were individually placed into a crystallizing dish, covered with a 5% sodium dodecyl sulfate (SDS) solution and placed in an ultrasonic bath (Elma, Elmasonic S 50 R) for 3 min to remove particles adhering to the filter. After sonication, the filters were thoroughly rinsed with SDS solution and the liquid transferred to a 1 L glass beaker. The volume of the solution was adjusted to 300 mL, a glass stirrer was added and beakers were covered with glass lids. Samples were kept at 50°C with gentle stirring (100 rpm) for 24h. Subsequently, samples were vacuum-filtered onto 10 µm steel filters (47 mm diameter). The small filters were then transferred back into the beakers, covered with Tris-HCl buffer (pH 8.3) and sonicated as before. All material was rinsed off the filter

with Tris-HCl buffer, reaching a final volume of 300 mL. The cleaned filters were stored in glass petri dished for later use. As for the bongo samples, protease and lipase were added and samples were incubated at 50°C with gentle stirring overnight. Thereafter, the samples were vacuum-filtered onto the same filters as before, put back into the beakers, covered with acetate buffer (pH 4.8) and sonicated, followed by rinsing the filter and adjusting the volume of acetate buffer to 300 mL. To this, we added 500 µL of cellulase (Sigma, Cellulase enzyme blend) and 500 µL of Viscozyme® L (Sigma), and incubated the samples at 50°C with gentle stirring for 72h. After filtering the samples as before, sonication and rinsing was done with filtered water, reaching a final volume of 200 mL. We then added 145 mL 50% hydrogen peroxide (H_2O_2), 65 mL 0.1 M sodium hydroxide (NaOH) and 62 mL 0.1 M iron sulfate ($FeSO_4$) to conduct a Fenton reaction. Within the first 4 h, the samples were kept on ice to keep the temperature between 20°C and 30°C as the reaction is exothermic. After the reaction had slowed down, the samples were left standing at room temperature overnight. Subsequently, the samples were filtered again, however adding a 500 µm steel sieve as a pre-filter to remove bigger particles. This bigger fraction underwent visual analysis together with the bongo samples. As a final step, a density separation was conducted. For this, the steel filters were rinsed with sodium polytungstate (1.9 g cm^{-3}) and all liquid was transferred to a separation funnel. After applying air from below for 30 min to mix the sample, it was left to settle overnight. The settled material was removed and the floatation was repeated with the remaining sample. After again removing the bottom layer, the rest of the solution was filtered. The filter was sonicated and rinsed with 50% ethanol (EtOH) and all liquid was sequentially transferred to 10 mL glass vials and evaporated in a water bath at 50°C using a stream of nitrogen (Biotage, TurboVap). When the samples were dry, 5 mL of 50% EtOH were added for subsequent analysis with FPA-µFTIR-Imaging (Focal Plane Array-Fourier-Transform Infrared Imaging-micro Spectroscopy).

S2. Results

Table S2. Summary of the results of the pump samples.

		St. 1	St. 2	St. 3	St. 4	St. 5	St. 6	Average	Median	Max	Min
Tot. MP conc.	**MP m^{-3}**	107	91	68	278	241	178	160	142	278	68
	μg_{MP} m^{-3}	40	13	9	23	51	34	29	29	51	9
MP particle conc.	**MP m^{-3}**	79	79	52	227	202	146	131	112	227	52
	μg_{MP} m^{-3}	30	5	8	17	37	29	21	23	5	37
MP fibers conc.	**MP m^{-3}**	28	12	16	51	38	32	30	30	51	12
	μg_{MP} m^{-3}	10	8	2	6	15	5	8	7	15	2
Maj_Dim (µm)	**D10**	38	33	37	42	38	39	38	38	42	33
	D50	84	64	76	70	83	67	74	73	84	64
	D90	319	202	197	173	232	231	226	216	319	173
Min_Dim (µm)	**D10**	18	16	21	18	18	19	18	18	21	16
	D50	37	30	33	33	39	35	35	34	39	30
	D90	76	69	55	59	79	60	66	64	79	55

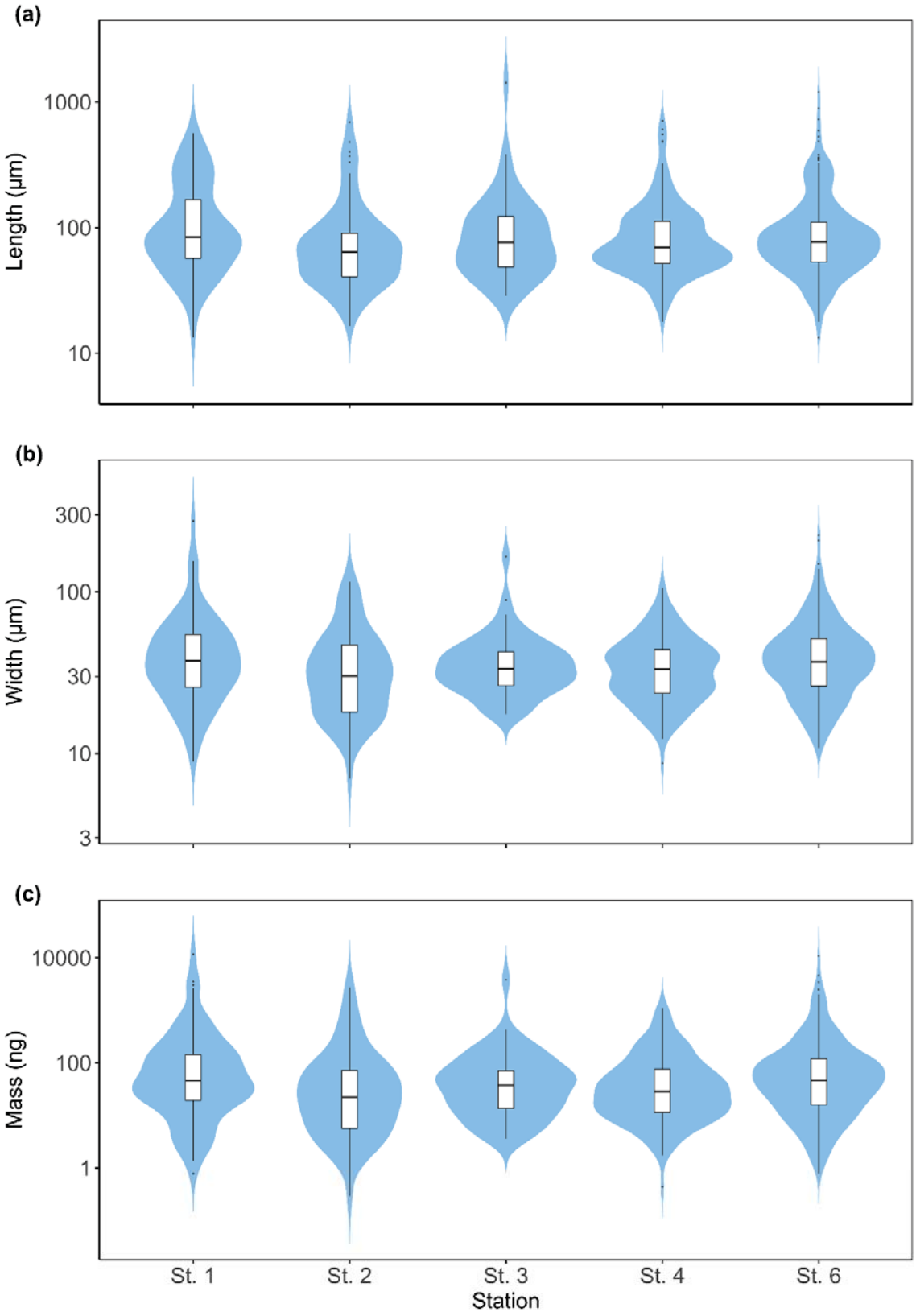

Figure S2. Size (a, b) and mass distribution (c) of all MPs in the pump samples per station. The y-axis is in Log10 scale in all the three plots.

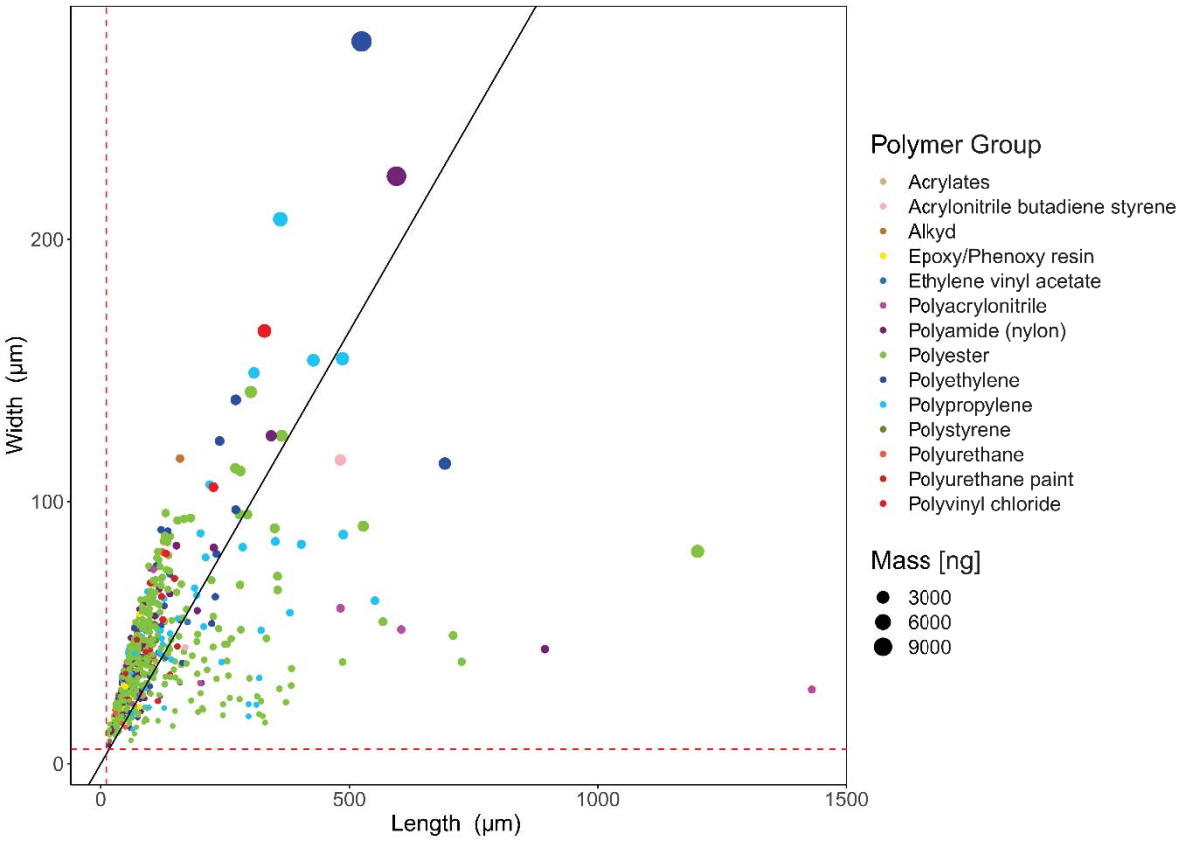

Figure S3. Width vs length scatter plot of all the MPs identified in the pump samples. The size of the dots is related to the particle's mass (ng). The black line divide particles (area below the line to the right) from fibers (area above the line on the left) based on the threshold for fibre classification used by Vianello et al. (2019); the vertical and horizontal dashed lines highlight the limit of detection for length (11 µm) and width (5.5 µm) (2x1 pixels).

Table S3. Summary of the results of the bongo samples.

		St. 1	St. 2	St. 3	St. 4	St. 5	St. 6	Average	Median	Max	Min
MP conc.	MP m^{-3}	0.00	0.12	0.11	0.08	0.40	0.23	0.19	0.12	0.40	0.08
	MP particles m^{-3}	0.00	0.01	0.01	0.03	0.20	0.00	0.05	0.01	0.20	0.00
	MP fibers m^{-3}	0.00	0.11	0.11	0.05	0.19	0.22	0.14	0.11	0.22	0.05
Length (µm)	D10	0	762	398	542	379	1163	649	542	1163	379
	D50	0	1866	1771	1105	778	6136	2331	1771	6136	778
	D90	0	3581	5204	2319	2818	21852	7155	3581	21852	2319
Width (µm)	D10	0	18	15	20	20	24	19	20	24	15
	D50	0	22	32	39	271	32	79	32	271	22
	D90	0	35	258	413	606	47	272	258	606	35

Paper II

Ingestion and impact of microplastics on arctic *Calanus* copepods

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Ingestion and impact of microplastics on arctic *Calanus* copepods

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ABSTRACT

Microplastics (MPs) are contaminants of emerging concern in the Arctic, but knowledge of their potential effects on Arctic plankton food webs remains scarce. We experimentally investigated ingestion and effects of MPs (20 μm polyethylene spheres) on the arctic copepods *Calanus finmarchicus, C. glacialis* and *C. hyperboreus*. These species dominate arctic zooplankton biomass and are relevant target species to investigate the potential impacts of MPs on the Arctic marine ecosystem. Females of each species were exposed to two concentrations of MPs (200 and 20,000 MPs L^{-1}) in combination with different food (diatom) concentrations, reflecting high (3000–5000 cells mL^{-1}, spring phytoplankton bloom) and low (50–500 cells mL^{-1}, pre/post bloom) food conditions. MPs did not affect negatively fecal pellet production rates in any of the species at the studied exposure concentrations. However, egg production rates of copepods exposed to MPs were 8 times higher compared with the controls, which suggests that MP exposure can cause stress-induced spawning in arctic copepods. Microscopic examination of the fecal pellets confirmed ingested MPs in the three species (up to aprox. 1000 MPs cop^{-1} d^{-1}). The number of MPs per pellet decreased exponentially with increasing food concentration. The daily ingestion of MPs per copepod was higher at low- food concentrations (250–500 cells mL^{-1}). At our exposure conditions, the presence of MPs inside *C. hyperboreus* fecal pellets did not affect their sinking rates. Overall, our experimental research show that 1) acute exposure to virgin polyethylene MPs has a low impact on arctic *Calanus* species at environmentally relevant MP concentrations, independent of food availability, and 2) arctic copepods influence the environmental fate of plankton-sized MPs by exporting buoyant MPs from the surface layer to the sea floor via fecal pellets.

1. Introduction

Microplastics (MPs, plastic particles < 5 mm) are found worldwide (Moore, 2008; Arthur et al., 2008; Law and Thompson, 2014), even in the remote polar ecosystems (Obbard et al., 2014; Peeken et al., 2018). In fact, Arctic sea ice and snow currently contain extremely high concentrations of MPs, >10,000 plastic particles L^{-1} (Peeken et al., 2018; Bergmann et al., 2019). Moreover, the highest global concentration of plastic fibers in surface waters has been found in the Arctic (Barrows et al., 2018). Considering global warming-induced stressors in the Arctic, there is a rising concern about the potential impacts of MPs in the Arctic ecosystems (AMAP, 2017). MPs have been trapped in sea ice for decades. Accelerated melting due to global warming would increase the release of MPs to the surrounding waters, increasing the exposure of arctic biota to MPs (Obbard et al., 2014). Furthermore, the decrease of sea ice cover (Arrigo et al., 2008; Stroeve et al., 2012) facilitates anthropogenic activities such as maritime transportation, fisheries and resources exploitation (Dalsøren et al., 2007; Melia et al., 2016). These activities may potentially increase plastic pollution in the sensitive arctic ecosystems.

A large proportion of MPs in the environment are similar in size to phytoplankton (Enders et al., 2015), which makes them bioavailable for a broad range of pelagic marine organisms (Lusher et al., 2015), including planktonic copepods (Cole et al., 2013). Copepods are the most abundant animals (metazoans) in the ocean and they play a pivotal role in the marine food web, both as grazers of phytoplankton and as prey for higher trophic levels (e.g. fish; Castonguay et al., 2008). It has been documented that copepods ingest MPs and that high MP

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concentrations negatively affect their grazing, reproduction and lipid content (e.g., Cole et al., 2013, 2016; Raju et al., 2019). However, knowledge of the potential impacts of MPs on arctic copepods is lacking (AMAP, 2017), particularly in relation to the seasonal change in food availability, which is a crucial feature of the arctic ecosystem (Nielsen and Hansen, 1995; Rysgaard et al., 1999 Leu et al., 2015). Additionally, copepod fecal pellets play an important role in the export of organic matter from the productive surface layers to the sediments (Turner, 2015). This process could be impacted by the presence of MPs inside the fecal pellets (Coppock et al., 2019; Cole et al., 2016). The ingestion and packaging of MPs into fecal pellets may promote the vertical exportation of MPs. In turn, low-density MPs may alter the buoyancy of fecal pellets, affecting the sinking velocity of organic matter and therefore the carbon-sequestering pump (Cole et al., 2016). Knowledge of interactions between arctic zooplankton and MPs under more environmentally relevant conditions is needed to evaluate the role of arctic zooplankton on the fate of plastic pollution in the arctic marine ecosystem.

This study aims to evaluate the acute impact of plankton-sized MPs (20 μm) on the key arctic copepods: *Calanus finmarchicus*, *C. glacialis* and *C. hyperboreus*. These *Calanus* species represent 80 % of arctic zooplankton biomass (Madsen et al., 2008) and influence carbon sequestration by vertical exportation of large, fast-sinking fecal pellets (Turner, 2015). Given the strong seasonality of trophic conditions in the Arctic, we experimentally investigated the impact of virgin MPs on arctic copepods under two food conditions: high food (phytoplankton bloom scenario) and low food availability (pre/post bloom scenarios). For a given MP concentration, we hypothesize that ingestion and sub-lethal effects of MPs on copepods depends on the food concentration; specially we expect a higher impact of MPs at low prey:MP ratio, when copepod clearance rates are higher. First, we estimated the influence of food concentration on the impact of MPs on fecal pellet and egg production rates. Secondly, we investigated MP ingestion in relation to food concentration. Lastly, we tested if the presence of MPs inside the fecal pellets affects their sinking rates.

2. Material and methods

2.1. Collection of experimental organisms

The copepods were collected from Western Greenland waters during a cruise with the research vessel R/V SANNA in May 2019. Hydrography and environmental conditions of the sampling area during our study is presented in Rist et al. (2020). *Calanus hyperboreus* females were obtained from zooplankton samples collected with a large ring-net of 2 m diameter equipped with black netting by oblique tows (150 m depth) at a speed of 2 knots outside the Nuup Kangerlua fjord, on Fyllas Bank (N 64°03′ 24″ N, 52°10′12″ 64 °W). *C. glacialis* and *C. finmarchicus* were obtained from zooplankton samples collected in the Nuup Kangerlua fjord using two gear types: a Bongo net with a mesh size of 500 μm and a WP-2 net of 200 μm mesh size with a large, non-filtering cod end. On deck, the samples collected in the cod ends were emptied in thermo-boxes filled with in situ seawater. Onboard, we sorted and identified the females of the target species (*C. finmarchicus* and *C. glacialis*) under a microscope according to criteria in Nielsen et al., 2014. Identified females were immediately transferred to glass beakers containing surface water and kept in trays with ice. Afterwards, the females were stored in a temperature-controlled room in the vessel at 2℃ in 20 L cold boxes filled with filtered seawater (FSW) with gentle aeration. They were fed with the diatom *Thalassiosira weissflogii* ad libitum until returning to the laboratory on land. Once in the harbor, the transfer of copepods from the cold room at the vessel to the cold room at the laboratory was conducted in large thermo-boxes (70 L) in less than 1 h. The phytoplankton species used to feed the copepods during the experiments was *T. weissflogii* (average equivalent spherically diameter (ESD) =11.6 μm). The diatom culture was kept in exponential growth at 15℃ in culture in B1 medium (Hansen, 1989), grown in a 12:12 light: dark cycle and constant aeration.

2.2. MP type and preparation of MP suspensions

Clear polyethylene (PE) spherical particles with a density of 0.96 g cm^{-3} (Cospheric®), supplied as a dry powder, were used in the experiments. Approximately 1 mg of powder was suspended in 250 mL glass bottles containing distilled water with 0.01 % Tween 80 and mixed through vigorous shaking and rotation of the bottles from that stock suspension (5×10^5 MPs mL^{-1}), the concentration and size of the particles were measured using a Beckman Multisizer III Coulter Counter. The particles had a size range of 13.9–30.3 μm, and the mean ESD was 20.7 μm. From the stock suspension, we prepared two diluted "working suspensions" (10^3-10^4 MPs mL^{-1}), one for each exposure level of MPs (see treatments below). The absence of MP aggregates and the concentration of MPs in the working solutions was verified by manual counting under an inverted microscope with Sedgewick-Rafter counting chambers.

2.3. Experimental set up

An overview of the exposure treatments and analyses is presented in Fig. 1.

The copepods were exposed to the following treatments:

1) Control (no MPs) with low food concentrations (LF).
2) Control (no MPs) with high food concentrations (HF).
3) Low MPs (200 MPs L^{-1}) with low food concentrations (LF).
4) Low MPs (200 MPs L^{-1}) with high food concentrations (HF).
5) High MPs (20,000 MPs L^{-1}) with low food concentrations (LF).
6) High MPs (20,000 MPs L^{-1}) with high food concentrations (HF).

The size of the copepod and the concentrations of phytoplankton used in the low (LF: 50–500 cells mL^{-1}) and high (HF: 3000–5000 cells mL^{-1}) food treatments are indicated in Table 1. In terms of phytoplankton abundance, the high and low diatom concentrations tested can be expected during bloom and pre/post bloom conditions, respectively (Juul-Pedersen et al., 2015; Arendt et al., 2010; Tang et al., 2011).

Prior to the experiments the copepods were kept in a temperature-controlled room at 2℃ and fed with *T. weissflogii* ad libitum. Experiments were conducted in 600 mL Pyrex glass bottles with a lid sealed with polytetrafluoroethylene (PTFE) protection. Four replicates per treatment were conducted, the number of copepod females incubated per bottle were 4 for *C. finmarchicus* and *C. glacialis* and 2 for *C. hyperboreus*. The three *Calanus* species differ in size. *C. hyperboreus* is ~ 3 times larger than *C. glacialis* and C. *finmarchicus,* and *C. glacialis* is slightly larger than *C. finmarchicus* (Table 1).

The bottles were filled with 0.2 μm FSW at 30‰ salinity and the copepods were added. Subsequently, an aliquot of the diatom stock culture and the corresponding MP working suspension were pipetted to each bottle to ensure the desired exposure concentrations for each treatment (Table 1).Tween 80 alone was added to the control bottles at the same concentration than in the experimental bottles. The final concentration of Tween in the incubation bottles was approx. 0.00001 %. Before adding the aliquot of diatoms, the concentration of diatoms in the stock culture was determined under an inverted microscope using Sedgewick-Rafter counting chambers.

Finally, the bottles were filled up with FSW, closed and mounted on a plankton wheel (1 rpm) in a temperature-controlled room at 2℃ in dark.

The exposure media were renewed daily for 6 days. After 24 h, the content from the bottles was filtered through a 40 μm mesh sieve to collect the copepods, eggs and fecal pellets. The copepods were checked for survival and gently transferred to a new bottle with new exposure medium. The concentrated eggs and fecal pellets were transferred to petri dishes by rinsing and fixed with Lugol's solution (1%) for later quantification and analysis.

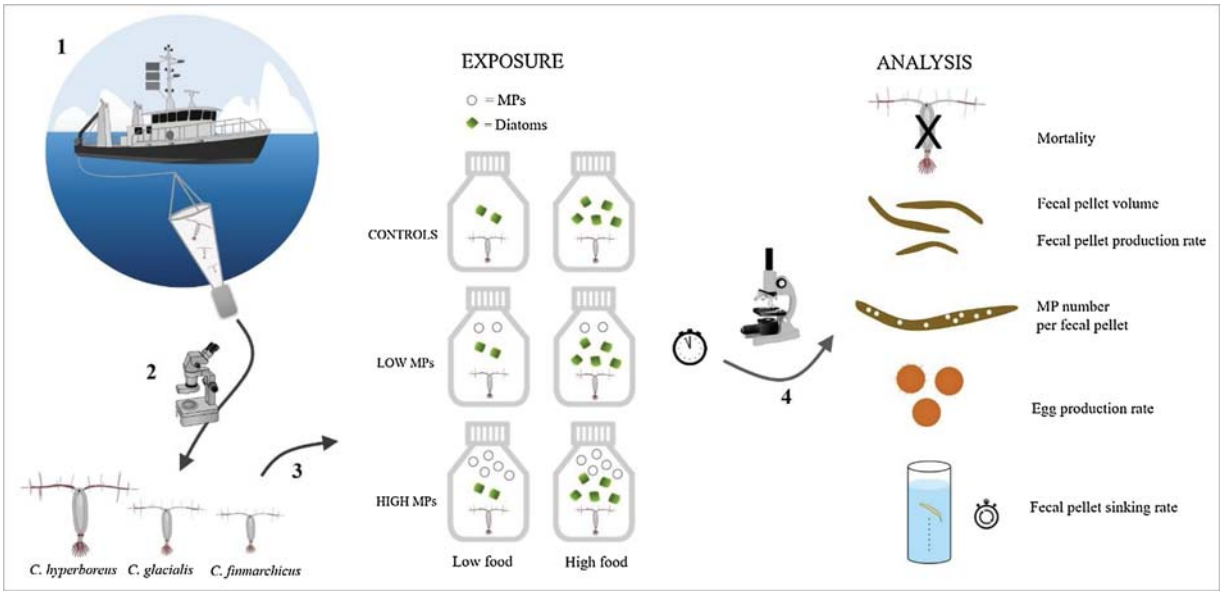

Fig. 1. Schematic overview of the experiment and subsequent analyses used to investigate the impact of MPs (20 μm polyethylene spheres) on arctic *Calanus* copepods. Animals were exposed to different combinations of 0.2 (low) or 20 (high) MPs mL^{-1}, and 50-500 (low food) or 3000-5000 (high food) algal cells mL^{-1} for 6 days.

Table 1
Microplastic concentration (MP conc., MPs L^{-1}) and food exposure concentrations for the threecopepod species. The food concentration is given in cell concentration (cells mL^{-1}) and in biomass (μg C L^{-1}) representing pre/post bloom (low food) and bloom (high food) conditions. The conversion from cell to carbon for *T. weissflogii* was calculated according to Henriksen et al., 2007.

Species	Prosome length (μm)	MP CONC. (MPs L^{-1})			LOW FOOD (cells mL^{-1}) (μg C L^{-1})	HIGH FOOD (cells mL^{-1}) (μg C L^{-1})
		CONTROL	LOW	HIGH		
C. finmarchicus	2713	0	200	20000	50, 250, 500 6, 30, 60	3000, 5000 360, 600
C. glacialis	3301	0	200	20000	50, 250, 500 6, 30, 60	3000, 5000 360, 600
C. hyperboreus	7061	0	200	20000	250, 500 30, 60	3000, 5000 360, 600

2.4. Sample analysis

We measured the following end-points: survival, fecal pellet production rates (FPPR), fecal pellet volume, volume specific FPPR, egg production rates (EPR), ingestion of MPs and fecal pellet sinking rates (Fig.1).

The fecal pellets and eggs were counted daily using a stereomicroscope to estimate the fecal pellet and egg production rates. To estimate the fecal pellet volume, pictures of approximately 20 fecal pellets per treatment were taken using a Leica DMC camera mounted on a stereomicroscope and the length and width of the fecal pellets were measured using Image J software. The fecal pellet volume was calculated assuming a cylindrical shape.

To quantify the MP ingestion in each treatment, ten intact fecal pellets were randomly selected from all replicates. The fecal pellets were placed in a Sedgewick-Rafter chamber and the number of MPs per fecal pellet counted under an inverted microscope, checking different planes and using a magnification of 200×.

The fecal pellets of *C. hyperboreus* were used to investigate if the presence of MPs inside the fecal pellets influences their sinking velocity. We randomly collected and took pictures of 10 unbroken pellets from each treatment under the inverted microscope. Then, the pellet was gently transferred with a glass pipette to a graduated glass cylinder of 4.9 cm diameter and 39 cm height filled with FSW at 15°C. Once the pellet started sinking, we measured the time that took it to settle 5.25 cm (distance between two measuring lines) in the water column without touching the walls of the cylinder. Only free-falling fecal pellets were included into the analysis.

At the end of the 6-day exposure, all copepods were photographed with a Leica camera attached to a stereomicroscope. The prosome length and width was measured with image J software and the volume of the prosome was estimated considering an ellipsoidal shape.

2.5. Statistical analysis

All data were statistically analyzed using IBM SPSS 25.0. When the data followed the assumptions for parametric tests, a one-way analysis of variances (ANOVA, $p < 0.05$) was carried out together with a Bonferroni post hoc test to assess statistical differences between treatments. We used a non-parametric method (Kruskal-Wallis, $p < 0.05$) in cases when the data did not follow the parametric assumptions of normality or homogeneity of variances.

3. Results

3.1. Effects of MPs on survival and fecal pellet production rates of arctic Calanus species

Survival of copepods was not affected by exposure to MPs. The fecal pellet production rate (FPPR, fecal pellets $cop.^{-1}$ d^{-1}) in the treatments with high food concentrations was 3–6 times higher than with low food concentrations (Fig. 2). MP exposure did not affect FPPR in any of the species studied (Fig. 2). We did not find any statistically significant difference between treatments except for *C. finmarchicus,* which showed

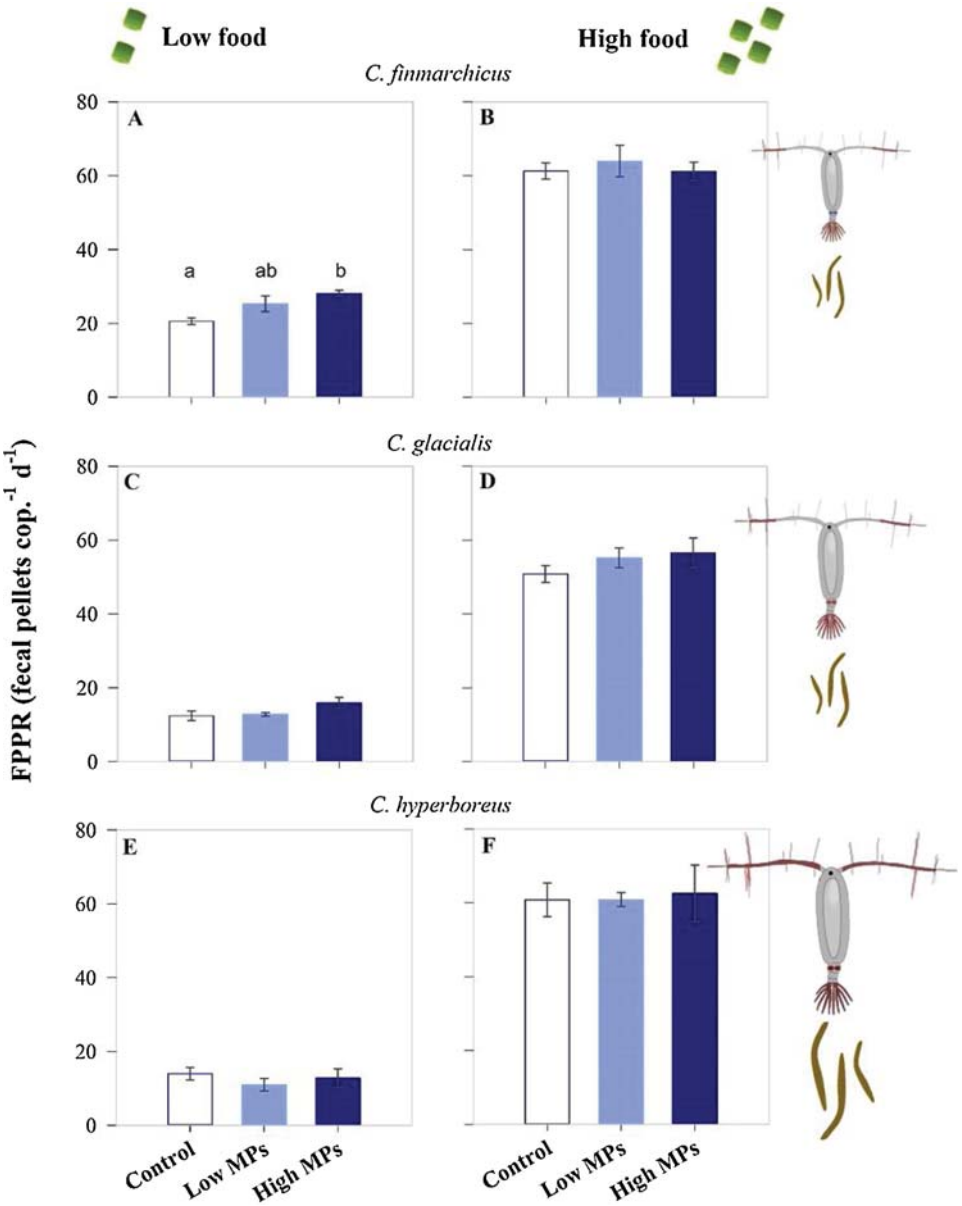

Fig. 2. Fecal pellet production rate (FPPR, fecal pellets copepod^{-1} d^{-1}) of *C. finmarchicus* (A, B), *C. glacialis* (C, D) and *C. hyperboreus* (E, F) in the different treatments: control (no MPs), low MPs (0.2 M Ps mL^{-1}) and high MPs (20 MPs mL^{-1}). Error bars indicate the standard error. The lowercase letters (a, b) indicate different statistical groups according to ANOVA and Post-hoc Bonferroni test analysis ($p < 0.05$). Note that we did not found significant differences among treatments in the results showed in the panels. B-F—.

a small but statistically significant increase in FPPR when exposed to high concentrations of MPs and low food (ANOVA, Bonferreni, $p < 0.05$) (Fig. 2A). Fecal pellet volume (mm^3) of *C. finmarchicus* was not affected by MPs in any of the treatments (Kruskal-Wallis, $p > 0.05$) (Fig. 3 A). However, the fecal pellet volume in *C. glacilis* and *C. hyperboreus* showed some small but statistically significant differences between the control and MP treatments (Kruskal-Wallis, $p < 0.05$) (Fig. 3B, 3C). Considering the fecal pellet size, the volume of fecal pellet produced daily (Vol.-FPPR, mm^3 cop^{-1} d^{-1}) increased with food concentration for all treatments (Fig. 3 D–F). We did not find significant differences in Vol.-FPPR between the control and treatments with MPs for most tested concentrations (ANOVA, $p < 0.05$) (Fig. 3D). Hence, all the data were fitted to a Michaelis-Menten model (Eq. 1).

$$P = B_{max}*C/C_{50}+ C \quad (1)$$

Where, P is the Vol.-FPPR, B_{max} is the saturation level, C is the food concentration and C_{50} is the concentration at which 50 % of the FPPR is reached. The equations for each species are presented in the figure panels (Fig. 3 D–F).

3.2. Effects of MPs on egg production rates (EPR) of arctic Calanus species

C. hyperboreus females do not produce eggs in May, hence only EPR of *C. finmarchicus* and *C. glacialis* were measured (Fig. 4). Accumulative EPR for *C. finmarchicus* was ~3 times higher than for *C. glacialis* (Fig. 4). EPR increased at high food concentrations in both species (Fig. 4). We found higher production rates in the treatments with MPs (Fig. 4 A–C), with the exception of *C. glacialis* at high food concentration (Fig. 4D). Although the EPR was low at low food concentrations, the increase in EPR after exposure to MPs was more notable at low concentrations (up to 8 times higher) (Fig. 4 A, C). No differences in egg size were observed between the control and the treatments with MPs for *C. finmarchicus* (Fig. 4E).

3.3. Effect of food concentration on MP ingestion in arctic copepods

The number of MPs inside the fecal pellets (Fig. 5) decreased exponentially with increasing food concentration, following an exponential decay model (Eq. 2).

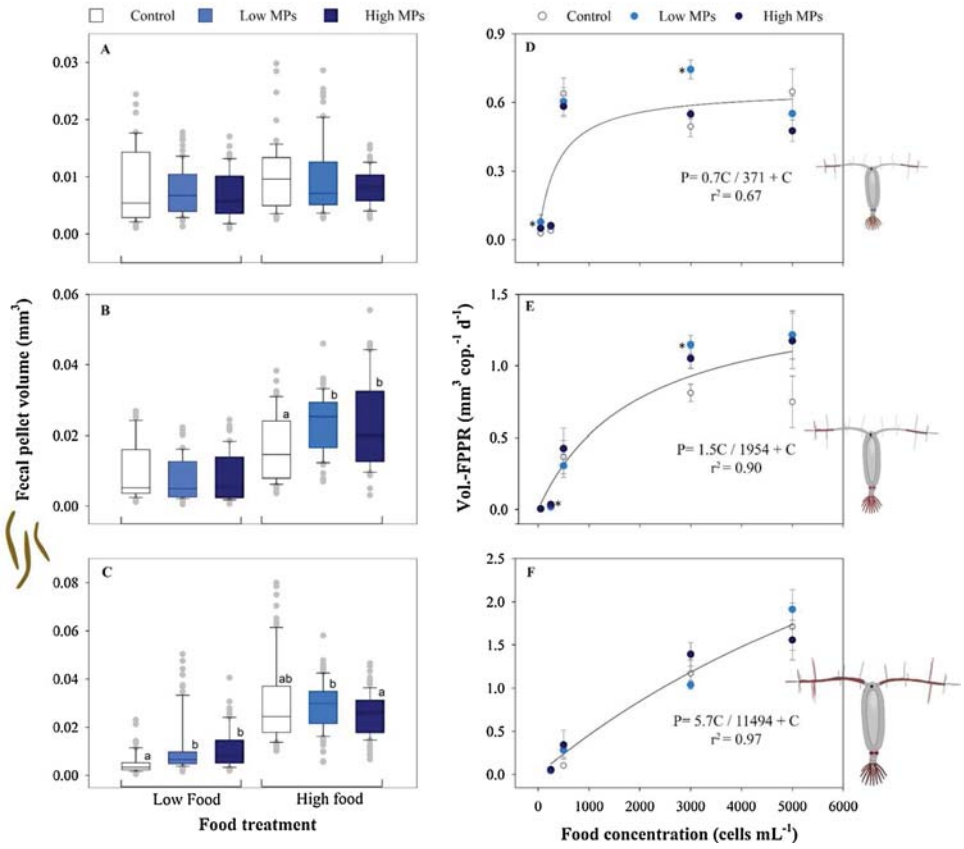

Fig. 3. Left panels: Fecal pellet volume after exposure to the different conditions. Right panels: relationship between volume-specific fecal pellet production rates (P, Vol.-FPPR, volume of fecal pellets produced per copepod per day, mm^3 $copepod^{-1}$ d^{-1}) and food concentration (C, cells mL^{-1}) for the different MP treatments: control (no MPs), low MPs (0.2 M Ps mL^{-1}) and high MPs (20 MPs mL^{-1}). *C. finmarchicus* (A, D), *C. glacialis* (B, E), *C. hyperboreus* (C, F). Error bars indicate the standard error. Right panels, a Michaelis Menten model was fixed to all data since no significant differences were observed among treatments in most of the concentrations (equations are given in the panels). The asterisk indicates significant difference (ANOVA, Bonferroni $p < 0.05$) between that treatment and the control.

$$N = a*\exp(-b*C) \quad (2)$$

where, N is the number of MPs per fecal pellet, a is the number of MPs per fecal pellet in absence of food ($C = 0$), b is the decay factor and C is the food concentration (cells mL^{-1}).

At low MP exposure concentrations, the number of microplastics per pellet at low food concentrations was approximately 3 times higher than at high food concentrations (Fig. 5A, C, E). When exposed to high MP concentrations, the decrease in the number of MPs per pellet in relation to food concentration was even more pronounced, more than one order of magnitude between low and high food concentrations (Fig. 5 B, D, F). The maximum number of MPs per fecal pellets was 143 for *C. hyperboreus* in conditions of low food and exposed to 20 MPs mL^{-1}. In terms of MP ingestion rates, *C. hyperboreus* was also the species with the highest MP ingestion, up to ca. 1000 MPs per copepod per day (Table 2).

An estimation of the daily ingestion of MPs based on the content of MPs per pellet and FFPR showed that MP ingestion was higher at high plastic concentrations and varied depending on the food level (Table 2). *C. hyperboreus*, the largest studied copepod, was the species that ingested the higher amount of MPs per copepod per day, up to approximately 1000 MPs d^{-1} (Table 2). The maximum ingestion was typically at low food concentrations (250–500 cells mL^{-1}) for *C. finmarchicus* and *C. glacialis*. However, we did not find any differences in MP ingestion rates between food concentrations for *C. hyperboreus* (Table 2). Daily mass-specific ingestion rates of plastic ($\mu g_{plastic}$ $mgC_{copepod}$ d^{-1}) varied depending on the species and exposure concentrations, with maximum values found for *C. finmarchicus* at low food concentrations (approx. 25 $\mu g_{plastic}$ $mgC_{copepod}$ d^{-1}) (S.I., Table 1).

3.4. Effect of MPs on sinking rates of fecal pellets

Sinking rates of *C. hyperboreus* fecal pellets estimated in the laboratory varied from approx. 30 to 200 m d^{-1} depending on the fecal pellet volume (Fig. 6). We observed a significant positive relationship between sinking rates and fecal pellet volume in the control treatments (Fig.6). However, we did not find any effect of MPs on fecal pellet sinking rates in any of our experimental treatments (Fig. 6).

4. Discussion

MPs can have negative physical impacts on marine organisms after ingestion or inhalation, such as gut abrasion, obstruction, decreased assimilation rates and reduced brachial function (Wright et al., 2013; Watts et al., 2016). Additionally, MPs - even virgin MPs - can release potentially toxic "leachates" to aquatic biota (Schiavo et al., 2018; Seuront, 2018). Given the importance of zooplankton in marine ecosystems (Banse, 1995; Turner, 2015; Visser et al., 2017), negative effects of MPs on copepods can impact higher trophic levels, vertical export of organic matter, and carbon sequestration (Cole et al., 2013, 2016; Galloway et al., 2017; Coppock et al., 2019).

4.1. MP type and exposure levels tested in this study

The diversity of plastic polymers found in the marine ecosystem is large (Erni-Cassola et al., 2019). The polymer used in this study, polyethylene (PE), is typically the most abundant found in surface waters (Erni-Cassola et al., 2019) and therefore relevant for exposure tests. The size of the MPs tested is also relevant since the size fraction <25 μm is the most abundant in marine environmental samples, including in the Arctic (Enders et al., 2015; Peeken et al., 2018; Bergmann et al., 2019; Rist et al., 2020). Virgin spherical plastic particles as used here serve as a useful model to investigate MP-biota interactions, and have been used in many previous studies (Cole et al., 2013,2015; Setâla et al., 2014; Vroom et al., 2017), allowing comparison between organisms. Yet, more research on field-collected MPs is needed because weathering modifies

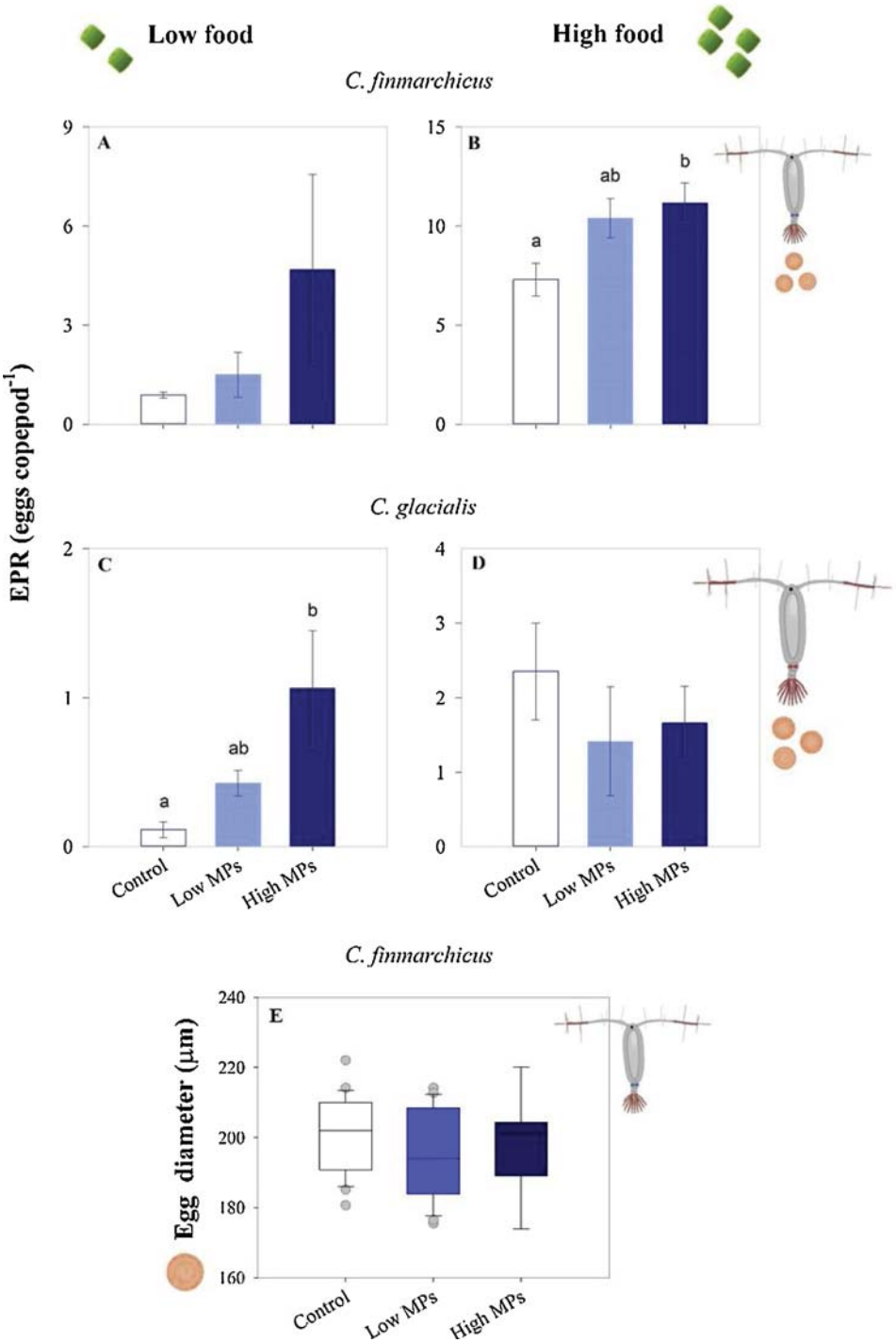

Fig. 4. Cumulative egg production rates (EPR, eggs cop^{-1}) of *C. finmarchicus* (A, B) and *C. glacialis* (C, D) after 6 days of exposure in the different treatments: control (no MPs), low MPs (0.2 M Ps mL^{-1}) and high MPs (20 MPs mL^{-1}). Egg size of *C. finmarchicus* with high food concentrations after exposure to different MP concentrations (E). The error bars represent the standard error. The lowercase letters (*a* and *b*) indicate different statistical groups according to Post-hoc Bonferroni test analysis ($p < 0.05$).

plastic properties, affecting the interactions between MPs and zooplankton (Vroom et al., 2017; Botterell et al., 2019).

Adverse effects of virgin MPs on planktonic copepods have only been observed at higher concentrations (75–4,000 MPs mL^{-1}) than used in this study. With this in mind, the MP exposure levels used in our experiments were selected considering two environmentally relevant concentrations: (1) the maximum concentration found in upper layers of oceanic marine waters to date (0.2 MPs mL^{-1}) (Song et al., 2015); (2) the MP concentrations recently reported in arctic sea ice and snow (20 MPs mL^{-1}) (Peeken et al., 2018; Bergmann et al., 2019), which could be temporally found in Arctic sea-ice adjacent waters in an accelerating ice-melting situation induced by climate change (Obbard et al., 2014).

4.2. Effect of MP exposure on fecal pellet production rates in arctic Calanus

Fecal pellet production rate (FPPR) is an appropriate proxy for feeding rates in copepods since the egestion rate is directly related to ingested food quantity (Paffenhöfer and Knowles, 1978; Butler and Dam, 1994). Previous studies found a notable decrease in ingestion rates in copepods after exposure to very high concentrations of 7.3 µm fluorescent polystyrene (PS) virgin microspheres (e.g., 4000 MPs mL^{-1}, Cole et al., 2013). However, the reduction of ingestion was moderate (6–11 % reduction in cell ingestion) when the 20 µm PS MP microspheres concentration was ≤ 100 MPs mL^{-1} (Cole et al., 2015; Coppock et al., 2019).

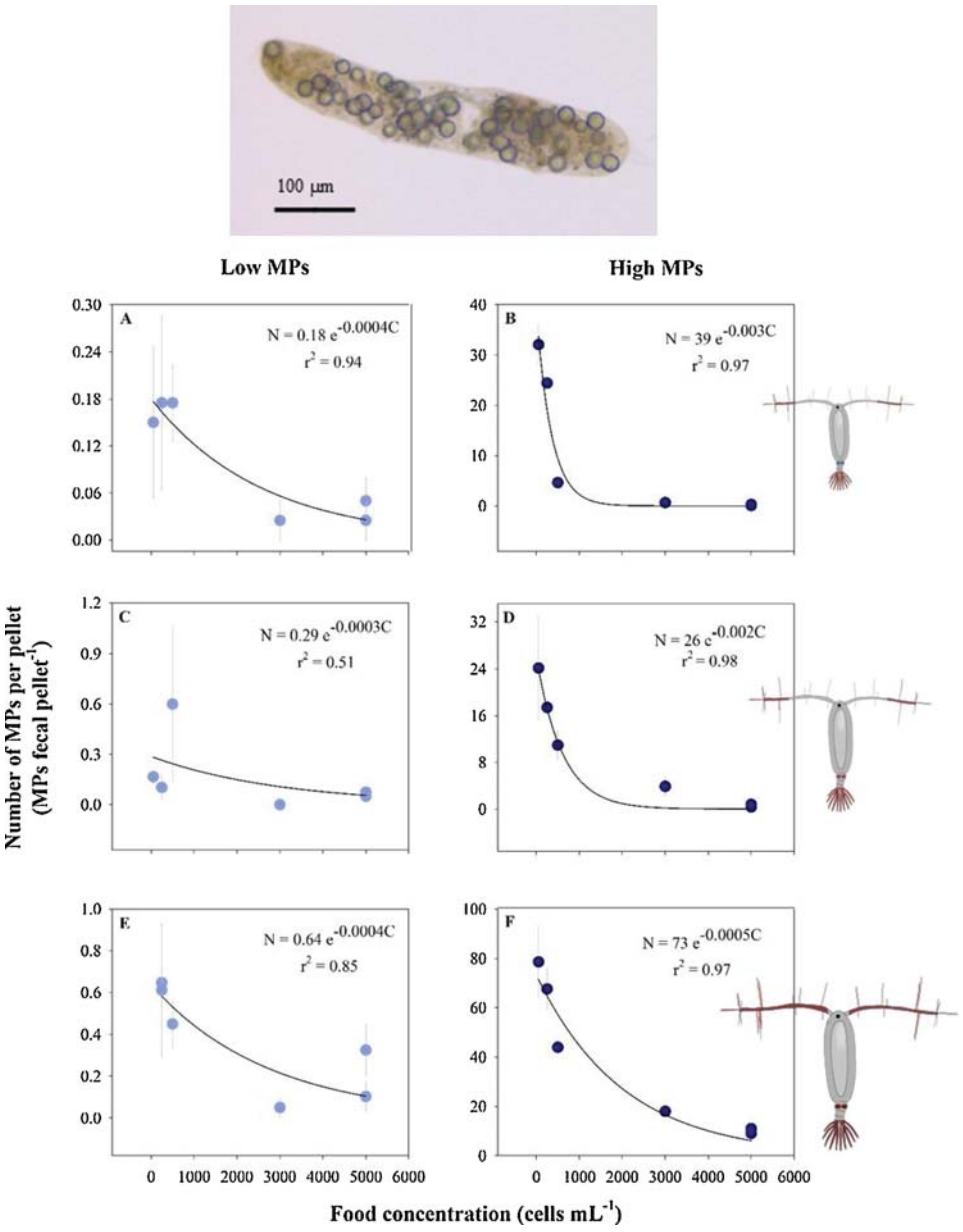

Fig. 5. The photograph in the top is a microscope image showing a *Calanus* fecal pellet with MPs inside. Relationship between the number of MPs per fecal pellet (N, MPs fecal pellet^{-1}) and the food concentration (C, cell mL^{-1}) for *C. finmarchicus* (A, B), *C. glacialis* (C, D) and *C. hyperboreus* (E, F) after exposure to low (0.2 M Ps mL^{-1}, left panels) and high (20 MPs mL^{-1}, right panels) concentrations of MPs. The error bars represent the standard error. An exponential decay model was fixed for all the treatments (equations are given in the panels).

Table 2
Average ± standard error (n = 4) of the number of MPs ingested per copepod per day (MPs cop.$^{-1}$ d^{-1}) in the different food and MP conditions. LF: low food; HF: high food. The lowercase letters (a, b, c, d) indicate different statistical groups according to ANOVA, post-hoc Bonferroni test ($p < 0.05$).

Exposure conditions			*C. finmarchicus*	*C. glacialis*	*C. hyperboreus*
MP level	Food level	Food conc. (cells mL^{-1})	MPs cop.$^{-1}$ d^{-1}	MPs cop.$^{-1}$ d^{-1}	MPs cop.$^{-1}$ d^{-1}
Low MPs (0.2 M Ps mL^{-1})	Low food (Pre/post bloom)	50	1.1 ± 0.7	0.3 ± 0.1	–
		250	3.3 ± 0.1	1.0 ± 0.6	4.3 ± 1.5
		500	7.0 ± 2.1	13.5 ± 10.8	6.5 ± 1.7
	High food (Bloom)	3000	1.6 ± 1.6	0 ± 0	2.6 ± 2.6
		5000	2.9 ± 1.1	3.1 ± 1.0	10.6 ± 3.4
High MPs (20 MPs mL^{-1})	Low food (Pre/post bloom)	50	292 ± 55 ^{a}	46 ± 34 $^{a, b}$	–
		250	537 ± 67 ^{b}	264 ± 59 $^{a, c}$	700 ± 89
		500	204 ± 63 $^{a, c}$	226 ± 67 ^{a,c}	501 ± 102
	High food (Bloom)	3000	36 ± 8 $^{c, d}$	180 ± 77 ^{a,b,c}	991 ± 262
		5000	9 ± 4 ^{d}	32 ± 9^{b,c}	626 ± 92

At the exposure conditions tested here, MPs did not negatively affect FPPR (nor volume-specific FPPR), showing that virgin MPs did not interfere with ingestion or egestion rates in Arctic *Calanus* copepods.

4.3. Effect of MP exposure on egg production rates in arctic Calanus

The three species used in this study have different reproduction

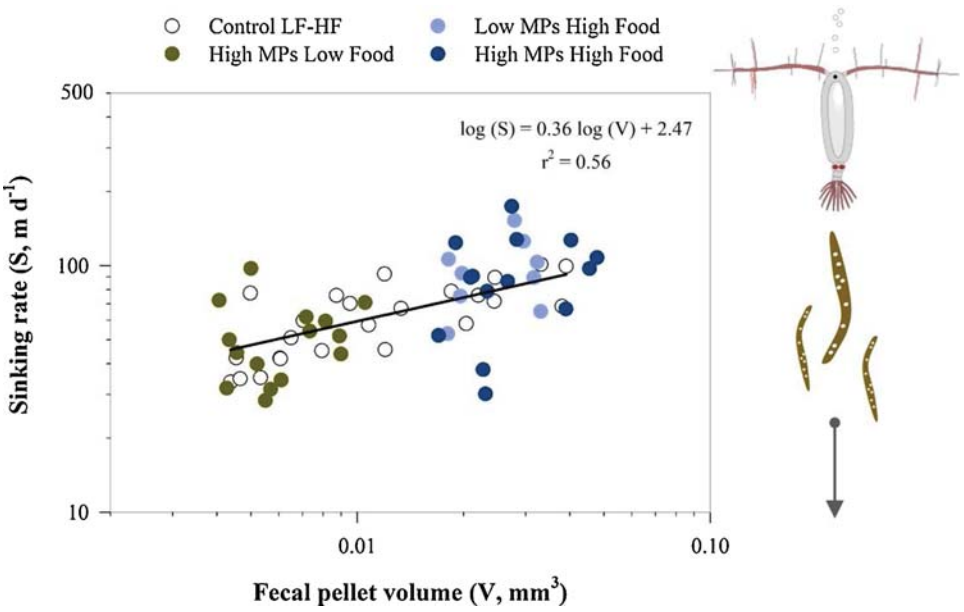

Fig. 6. Relationship between sinking rate (S, m d^{-1}) and fecal pellet volume (V, mm^3) of *C. hyperboreus* for four different treatments. (LF: low food and HF: high food). The blue dots represent treatments with low MPs (0.2 M Ps mL^{-1}, light) and high MPs (20 MPs mL^{-1}, dark). The line represents the linear regression equation fitted to the logarithmically transformed data; the equation is given in the figure. Note that the fecal pellets from the low MPs and low food treatments were not used because they were soft and got broken with the handling.

strategies. *C. finmachicus* is mostly an income breeder (Varpe et al., 2009), producing offspring based on concurrent food intake, mainly during the spring phytoplankton bloom. In contrast, *C. hyperboreus* is a capital breeder, meaning that it produces offspring utilizing stored resources (Varpe et al., 2009). *C. glacialis* has a flexible reproductive strategy depending on the feeding environment (Daase et al., 2013). EPR were lower than observed in other studies (Madsen et al., 2008; Swalethorp et al., 2011) and, as expected, EPR increased with increasing food concentration.

Most previous experimental studies on impacts of MPs on copepods have found either no effects or a decrease in egg production and fecundity, depending on the MP exposure concentration. For example, Cole et al. (2015) did not find any significant effects of 20 μm PS microspheres (75 MPs mL^{-1}) on *C. helgolandicus* EPR, but the eggs were smaller and the hatching decreased, which caused a negative effect on the overall fecundity. However, we did not find any effect of MPs in *C. finmarchicus* egg size (Fig. 4E). Interestingly, we found that egg production rates increased after exposure to MPs in three out of four treatments (Fig. 4), suggesting that MPs can trigger stress-induced spawning in arctic copepods. Both natural (e.g., acute changes in temperature and UV light) and anthropogenic stressors (pollution) can induce spawning in marine animals (Loosanoff., 1945; Schreck et al., 2001). As far as we know, this is the first study reporting that MPs can promote stress- induced spawning in marine invertebrates. It is unclear how MP–induced spawning can affect population dynamics. The studied species mainly reproduce during the spring bloom, when nauplii are expected to have enough food, so a mismatch between nauplii and food resources could occur. More research is needed to understand the consequences of MP- induced stress responses on copepods and their population dynamics.

4.4. Ingestion of MPs in relation to food concentration

Ingestion of plastic particles by zooplankton in the laboratory is well documented (reviewed in Botterell et al., 2019), and there is some supporting evidence from field studies (Sun et al., 2017; Desforges et al., 2015). Several properties affect the bioavailability of MPs to zooplankton, including the size and concentration of MPs (Botterell et al., 2019). Particles of 20 μm diameter are within the prey size-range of the *Calanus* species (Frost, 1972; Levinsen et al., 2000), and our observation of MPs inside the fecal pellets confirm the ingestion of MPs of this size. As expected, ingestion of MPs increased with increasing MP concentration, independently of food availability (Fig. 5; Table 2). The observed influence of phytoplankton concentration on MP ingestion can be explained by the relationship between copepod clearance rates ("filtration rates") and food concentration (Frost, 1972). Clearance rates decrease with increasing food concentration, which causes a reduction in MPs per pellet observed in our study at high food levels (Fig. 5). The presence of MPs inside fecal pellets, particularly at low food levels, suggests that the studied copepods have a limited capability to avoid the ingestion of small MPs (20 μm) in the presence of phytoplankton. However, little is known about mechanistic discrimination and selection of plastic particles of different characteristics.

4.5. Sinking rates of fecal pellets containing MPs

Zooplankton fecal pellets play an important role in the recycling and vertical transportation of carbon in the water column (Wieczorek et al., 2019). Cole et al. (2016) found that incorporation of ingested MPs into fecal pellets reduced their sinking rates in the laboratory. In our study, fecal pellet sinking rates increased with increasing pellet volume (depending on food concentrations, Butler and Dam, 1994), but were not affected by the presence of MPs inside the fecal pellets. This discrepancy may be due to differences in MP exposure concentrations between studies, since our MP concentrations were two to two orders of magnitude lower than in Cole et al. (2016) (PS, 20.6 μm, 1.000 MPs mL^{-1}). The density of the MPs used in this study was 0.96 g cm^{-3}, so they are positively buoyant in seawater. However, in our study MPs only represented a small fraction (from < 1%–5%) of the total volume of the fecal pellet in the three species. Yet, fecal pellets of *C. hyperboreus* containing MPs can be vertically exported at relatively high rates (up to 100 m d^{-1} at 15 ℃) due to their large size (up to 3 mm). Nevertheless, given the influence of temperature on water viscosity and density, the fecal pellet sinking rates are expected to be slower at in situ temperatures than estimated here in the laboratory at 15 °C (~ 70 % slower at 0 °C than at 15 °C, Bach et al., 2012).

4.6. Ecological implications

Extrapolation of laboratory results to field conditions must always be done cautiously. In the field, interactions between MPs and zooplankton depend on many factors, and some of them (e.g., currents, turbulence and vertical migration) are difficult to simulate in the laboratory. Furthermore, MPs are weathered in the marine environment (e.g,

formation of biofilms, polymer degradation), which affects the perception, ingestion and effects of MPs on zooplankton (Liu et al., 2020). Nevertheless, exposure experiments under controlled conditions are an essential tool to investigate the impact of MPs on marine biota.

The impact of MPs on Arctic copepods is expected to be minor since MP concentrations currently found in Arctic surface waters are very low, generally <1 MP L^{-1} (Lusher et al., 2015; Kanhai et al., 2017). We did not find any negative effects of virgin MPs on FPPR even at high concentrations, and the concentrations of MPs that induce spawning of *Calanus* were three to four orders of magnitude higher than those we currently reported in marine waters, including West Greenland waters (0.15 MPs L^{-1}, Rist et al., 2020). However, considering increasing global plastic production and accelerating global warming, MP pollution could increase in the Arctic, particularly in sea ice adjacent waters, which can become potential hot spots of plastic pollution (Obbard et al., 2014; Coppock et al., 2019; Bergmann et al., 2019). Even though our results suggest that PE virgin MPs spheres alone have a low impact on arctic copepods, the combination of MPs with other stressors (e.g., other pollutants, Almeda et al., 2020) require further investigation to evaluate potential synergetic effects of multiple stressors on the sensitive arctic ecosystem, one of the regions most affected by global warming.

Ingestion of MPs by zooplankton is considered the main entry of MPs into marine food webs and their potential transfer to higher trophic levels (Setälä et al., 2014). Our results suggest that, for a given MP concentration, the ingestion of MPs by zooplankton will be higher during the early post- phytoplankton bloom when the copepods are abundant and their clearance rates are higher due to the low food concentration. MPs had a low impact on the studied copepods, but they could act as vectors of plastic transfer to higher or other trophic levels in different ways: (1) with MPs in the gut when eaten by predators and (2) via fecal pellets. Since gut evacuation time of copepods are fast (from minutes to 2 h, Ellis and Small, 1989; Hansen et al., 1990; Barquero et al., 1998; Maar et al., 2002), MPs would stay in the copepod guts for short periods. However, the residence time of MPs inside the fecal pellets is expected to be longer than in the copepod, which emphasizes the potential role of the fecal pellet in the bio-transfer of MPs in the food webs. Fecal pellets from copepods are an important source of energy for coprophage zooplankton (Paffenhöfer & Knowless, 1978; Paffenhöfer and Strickland, 1970; Iversen and Poulsen, 2007; Green et al., 1992). Furthermore, some protozoans (e.g, dinoflagellate, *Noctiluca scintillans*) can feed on fecal pellets (Kiørboe, 2003). However, there is still little experimental and field evidence of the bio-transfer of MPs, either via copepods or fecal pellets, in marine planktonic food webs.

Exportation of MPs via fecal pellets can contribute to the removal of buoyant MPs from surface waters (Cózar et al., 2017). When MP-contaminated fecal pellets reach the seafloor, they can be either ingested by benthic organisms or integrated into the sediments. For example, based on our estimations and after correcting the temperature-effect on sinking rates (Bach et al., 2012), a fecal pellet (volume = 0.05 mm^3) of *C. hyperboreus* with 20 MPs inside will reach the sea floor (50 m depth, Arendt et al., 2010) in less than 1 day in the sampling site, Fyllas Bank. However, the same fecal pellet would reach the seafloor of Nuup kangerlua fjord (600 m average depth, Weidick et al., 2012) in approx. 10 days. During this time, degradation and recycling of fecal pellets in the water column could affect the vertical exportation and distribution of MPs via fecal pellets. More research and field studies on the role of zooplankton fecal pellets in the distribution of MPs is needed to better understand the ultimate fate of plastic pollution in marine environments.

5. Conclusions

Exposure to polyethylene virgin MPs did not affect egestion rates of the three dominant arctic *Calanus* species. However, high concentrations of MPs caused stress-induced spawning in two of the studied copepod species. Arctic copepods ingested plankton-sized MPs and the uptake depended on the phytoplankton concentration. The presence of MPs in the fecal pellets did not affect their sinking rates. MP-contaminated fecal pellets act as vehicles of MPs, potentially contributing to the distribution and fate of plastic pollution in the environment. Overall, our experimental results suggest that virgin MPs have a low impact on arctic *Calanus* species considering current environmental concentrations of MPs found in surface waters.

CRediT authorship contribution statement

Rocío Rodríguez-Torres: Conceptualization, Methodology, Formal analysis, Investigation, Writing - original draft, Writing - review & editing. **Rodrigo Almeda:** Conceptualization, Methodology, Formal analysis, Investigation, Writing - original draft, Writing - review & editing, Project administration, Funding acquisition. **Michael Kristiansen:** Investigation, Writing - review & editing. **Sinja Rist:** Methodology, Formal analysis, Investigation, Writing - review & editing. **Mie S. Winding:** Investigation, Methodology, Writing - review & editing. **Torkel Gissel Nielsen:** Conceptualization, Writing - review & editing, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.aquatox.2020.105631.

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Supporting material

Supporting information (Table 1). Daily biomass of plastic ingested per copepod (average ±SE) ($\mu g_{plastic}$ $mgC_{copepod}$ d^{-1}) in the different treatments. The lowercase letters (a, b, c, d) indicate different statistical groups according to ANOVA, post-hoc Bonferroni test ($p<0.05$).

Exposure conditions			*C. finmarchicus*	*C. glacialis*	*C. hyperboreus*
MP level	**Food level**	Food cells mL^{-1}	µgMPs mgC^{-1} d^{-1}	µgMPs mgC^{-1} d^{-1}	µgMPs mgC^{-1} d^{-1}
Low MPs (0.2 MPs mL^{-1})	Low food (Pre/post bloom)	50	0.04 ± 0.03	0.01 ± 0.002	-
		250	0.12 ± 0.08	0.02 ± 0.01	0.02 ± 0.006
		500	0.27 ± 0.08	0.27 ± 0.22	0.02 ± 0.006
	High food (Bloom)	3000	0.06 ± 0.06	0 ± 0	0.01 ± 0.01
		5000	0.12 ± 0.04	0.05 ± 0.02	0.03 ± 0.01
High MPs (20 MPs mL^{-1})	Low food (Pre/post bloom)	50	13.54 ± 2.55 [a]	0.89 ± 0.66 [a, b]	-
		250	24.84 ± 3.11 [b]	5.10 ± 1.15 [a, c]	2.74 ± 0.35
		500	9.47 ± 2.95 [a,c]	4.36 ± 1.29 [a, c]	1.96 ± 0.40
	High food (Bloom)	3000	1.71 ± 0.39 [c,d]	3.48 ± 1.50 [a,b,c]	4.16 ± 1.10
		5000	0.41 ± 0.20 [d]	0.61 ± 0.18 [b,c]	2.62 ± 0.39

Paper III

Microplastics do not increase bioaccumulation of petroleum hydrocarbons in Arctic zooplankton but trigger feeding suppression under co-exposure conditions

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Microplastics do not increase bioaccumulation of petroleum hydrocarbons in Arctic zooplankton but trigger feeding suppression under co-exposure conditions

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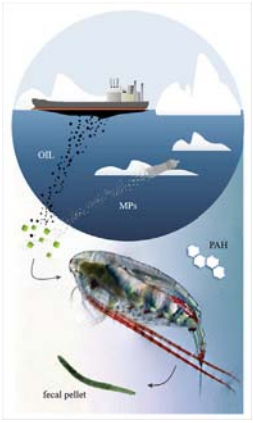
HIGHLIGHTS

- Co-exposure to oil droplets and MPs induced feeding suppression in *C. hyperboreus*.
- MPs did not increase bioaccumulation of PAHs under co-exposure conditions.
- Feeding suppression resulted in a lower bioaccumulation of PAHs.
- Dispersant increased PAH bioaccumulation in arctic copepods and fecal pellets.
- Phenanthrene and ≥4 ring-PAHs were the most bioaccumulated PAHs.

GRAPHICAL ABSTRACT



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ABSTRACT

Arctic sea ice has alarmingly high concentrations of microplastics (MPs). Additionally, sea ice reduction in the Arctic is opening new opportunities for the oil and maritime industries, which could increase oil pollution in the region. Yet knowledge of the effects of co-exposure to MPs and crude oil on Arctic zooplankton is lacking. We tested the influence of MPs (polyethylene, 20.7 μm) on polycyclic aromatic hydrocarbon (PAH) bioaccumulation and oil toxicity in the key arctic copepod *Calanus hyperboreus* after exposure to oil with and without dispersant. Up to 30% of the copepods stopped feeding and fecal pellet production rates were reduced after co-exposure to oil (1 μL L^{-1}) and MPs (20 MPs mL^{-1}). The PAH body burden was ~3 times higher in feeding than in non-feeding copepods. Copepods ingested both MPs and crude oil droplets. MPs did not influence bioaccumulation of PAHs in copepods or their fecal pellets, but chemical dispersant increased bioaccumulation, especially of ≥4 ring-PAHs. Our results suggest that MPs do not act as vectors of PAHs in Arctic marine food webs after oil spills, but, at high concentrations (20 MPs mL^{-1}), MPs can trigger behavioral stress responses (e.g., feeding suppression) to oil pollution in zooplankton.

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1. Introduction

The Arctic has lost more than 40% of its sea ice in the last four decades, which makes this unique ecosystem one of the most rapidly changing regions on our planet (Intergovernmental Panel on Climate Change [IPCC], 2018). The decrease in Arctic sea ice has opened new opportunities for maritime transportation, cruise tourism and exploration for natural resources in this region (Dalsøren et al., 2007; Melia et al., 2016). An ice-free Arctic summer is projected within a few decades (Screen and Deser, 2019), and the shorter shipping routes through the Arctic are expected to be economically viable by 2040 (C.Ø. Hansen et al., 2016). Increased expansion of shipping and oil industries in the Arctic could increase the risk of oil pollution, which is considered a major threat to this sensitive environment (Arctic Council, 2019).

Oil spill responses are particularly challenging in the Arctic due to limited infrastructure. Although the application of chemical dispersants has been proposed as a feasible response to oil spills in the Arctic (Wilkinson et al., 2017), the environmental benefits of dispersants (e.g., reducing the risk of oil slicks reaching shoreline) may be counteracted by their toxicity to marine pelagic organisms (Almeda et al., 2014a; Almeda et al., 2014b; Almeda et al., 2014c; Pančić et al., 2019). The distinctive seasonal conditions and complex life cycle strategies in the Arctic call for a better evaluation of the net effect of chemical dispersants in Arctic ecosystems after oil spills (IPIECA-IOGP, 2015).

Microplastics (MPs) have accumulated in the Arctic sea ice for decades by atmospheric deposition and their release will result from sea ice melting (Obbard et al., 2014; Bergmann et al., 2017; Peeken et al., 2018; Bergmann et al., 2019). Although MPs are ubiquitous in the ocean, Arctic sea ice and snow contain concentrations several orders of magnitude higher (>10,000 MPs L^{-1}) than commonly found in marine surface waters in other latitudes (<0.1–1 MPs L^{-1}) (Bergmann et al., 2017; Peeken et al., 2018; Bergmann et al., 2019). In fact, the Arctic Ocean has the highest average concentration of plastic microfibers in surface waters globally (Barrows et al., 2018). This has increased concerns about the potential environmental impacts of plastic pollution in the Arctic, especially considering the current context of global warming-induced multiple stressors in the region.

Due to the high sorption capacity and lipophilic nature of MPs (O'Connor et al., 2016), the concentration of organic pollutants in MPs can be several orders of magnitude higher than in the surrounding waters (Rochman et al., 2013a; Mai et al., 2018). Polycyclic aromatic hydrocarbons (PAHs), some of the most toxic compounds in crude oil, are sorbed by marine MPs (Rochman et al., 2013a; Mai et al., 2018). Aside from the direct adverse physical effects (Wright et al., 2013), contact and ingestion of contaminated MPs could increase the uptake and accumulation of toxic organic pollutants in marine organisms (Wright et al., 2013; Rochman et al., 2013b; Hartmann et al., 2017; Pittura et al., 2018). Nevertheless, the potential role of marine MPs as vectors of organic pollutants to marine biota is still debated with opposing views published recently (Bakir et al., 2016; Koelmans et al., 2016; Hartmann et al., 2017; Besseling et al., 2017; Rodrigues et al., 2018). More research mimicking natural conditions after oil spills is required to understand the role of MPs in the transfer of organic pollutants in marine food webs.

Two key features determine the structure and dynamics of the Arctic ecosystem: a brief but highly productive phytoplankton bloom in spring and a lipid-based food web. The large copepods of the genus *Calanus* (*C. hyperboreus*, *C. glacialis* and *C. finmarchicus*) account for up to 80% of the mesozooplankton biomass and are the most important grazers in Arctic and subarctic food webs (Nielsen et al., 2007; Falk-Petersen et al., 2009). During the short spring phytoplankton bloom (1–2 weeks), Arctic copepods efficiently convert carbon from phytoplankton into large lipid reserves (Lee et al., 2006). As prey, copepods drive the transfer of high-energy lipids and essential fatty acids to higher trophic levels, including fish, birds, and marine mammals throughout the year (Dahl et al., 2003). Moreover, the seasonal migration and deep-water overwintering of lipid-rich Arctic *Calanus* species, such as *C. hyperboreus*, promotes carbon sequestration, acting as a sink in the global carbon cycle (Visser et al., 2017). Therefore, Arctic *Calanus* species play a pivotal role in Arctic food webs as the main energy drivers, negative impacts on these key planktonic organisms may affect the entire Arctic ecosystem and, consequently the fisheries-dependent local economies. Further, because persistent organic pollutants (e.g., PAHs) accumulate in lipid tissues, the transfer and impact of pollutants in the lipid-rich Arctic food web is a major environmental concern (De Laender et al., 2010).

Oil and MPs may be present simultaneously after oil spills or oily shipping pollution in the Arctic, but we lack knowledge of the effects of co-exposure to these pollutants on Arctic zooplankton. Furthermore, it is necessary to assess if the application of chemical dispersants after oil spills affects the potential role of MPs as vectors of PAHs in Arctic food webs. Here, we evaluate the impacts of co-exposure to crude oil and MPs on Arctic zooplankton. Most studies focus on dissolved PAHs; however, this is the first attempt to experimentally assess the influence of MPs as vectors of PAHs when Arctic copepods are exposed to crude oil dispersions. Specifically, the main goal of this study is to address two questions: 1) Can microplastics increase the toxicity and bioaccumulation of oil after spills? 2) In case of an oil spill, when co-exposure to MPs and oil is expected, would application of chemical dispersants increase the impacts of MP + oil? To answer these questions, we tested how MPs affect PAH bioaccumulation and oil toxicity to the Arctic copepod *C. hyperboreus* after exposure to crude oil with and without chemical dispersant. We also quantified the amount of MPs and PAHs inside the fecal pellets as well as the fecal pellet sinking rates. Lastly, we evaluated the ecological significance of our experimental results.

2. Material and methods

2.1. Copepod collection

Zooplankton samples were collected on the slope off Fyllas Banke (64°03′ 24″ N, 52°10′12″ W) in West Greenland during a cruise on the research vessel R/V Sanna in May 2019. The zooplankton were collected using a mid-water ring (MIK) net (1500-μm mesh size) with oblique tows from 150 m depth at a speed of 2 knots. Onboard, the zooplankton samples in the cod-end were transferred to 100 L thermo-box containing in situ seawater and specimens of *C. hyperboreus* were immediately sorted out by use of wide-mouth pipettes and spoons. *C. hyperboreus* mature females with red genital somite were identified under the stereomicroscope, carefully separated and placed in beakers with in situ <20 μm filtered seawater (FSW) in a tray with ice. The sorted females were transferred to 20-L plastic cool boxes containing FSW, fed ad libitum with the diatom *Thalassiosira weissflogii* (12 μm) and kept with gentle aeration in a temperature-controlled room (2 °C) until returning to the laboratory, where the copepods were placed in an cold room at 2 °C. Cultures of *T. weissflogii* were grown with B1 medium plus silicates in a 18:6 h light:dark cycle with constant aeration and at room temperature (~15 °C).

2.2. Experimental setup/design

C. hyperboreus females were exposed to the following four treatments:

Control (without pollutants) = "CTRL"; crude oil (as a suspension of oil droplets) = "OIL"; crude oil + plastic particles (MPs) = "OIL + MP"; oil + MPs + chemical dispersant (D) = "OIL + MP + D". (Fig. 1). The exposure concentrations were 1 μL of oil L^{-1}, 20 MPs mL^{-1} and 0.05 μL of dispersant L^{-1}.

The experiment was designed to answer two main questions:

1) Can microplastics increase the toxicity and bioaccumulation of oil after spills? To test this, we compared the treatments of oil only (OIL) and OIL + MPs.

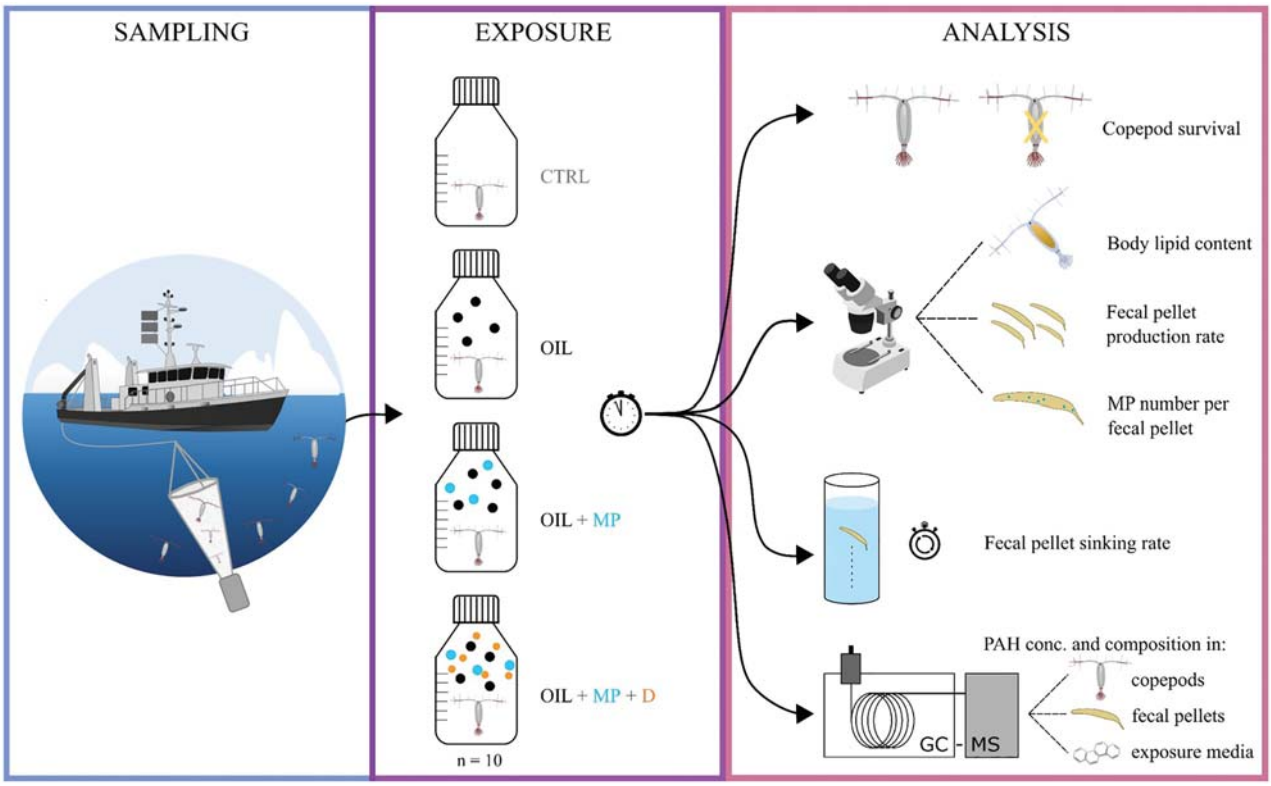

Fig. 1. Overview of the study, including the exposure treatments and the subsequent analyses.

2) In an oil spill, when co-exposure of MPs and oil is expected, would the application of dispersant (D) increase the impacts of MP + OIL? To answer this, we compared the treatments with OIL+ MP and Oil + MP + D.

The following endpoints and variables were determined: copepod survival, fecal pellet production rates, body lipid content, PAH body burden, the concentrations of PAHs and MPs in the fecal pellets and the sedimentation rates of fecal pellets in the different treatments (Fig. 1).

Additionally, we conducted a short-term experiment to estimate the effects of oil on the respiration rates of *C. hyperboreus*. We focused only on oil and dispersant as pollutants because MPs did not affect respiration rates in a parallel co-exposure study with other Arctic copepods (Almeda et al., unpublished results). Moreover, acute exposure to virgin MPs does not affect *Calanus helgolandicus* respiration rates (Cole et al., 2016).

In a parallel study under similar experimental conditions, we did not find any effects of MPs alone on survival or fecal pellet production rates of *C. hyperberous* at 20 MPs mL^{-1} tested in this study (Supporting Information, S.I.-Fig. 1).

2.3. Preparation and characteristics of the pollutants

Clear polyethylene (PE, Cospheric®) spherical particles with a density of 0.96 g cm^{-3} were used as MPs. To prepare the stock suspension, approximately 1 mg of plastic particles was suspended in Milli-Q water with Tween 80 (0.01%). The size (ESD = 20.7 μm; range: 14–30 μm) and concentration of the particles in the stock suspension were determined using a Beckman Multisizer III Coulter Counter. The concentration of MPs in the stock suspension was corroborated by counting subsamples in Sedgewick-Rafter counting chambers under an inverted microscope.

The crude oil used in the exposure experiments was Light Louisiana Sweet oil. The chemical dispersant used was Corexit® 9500A, the main type of dispersant used during the Deepwater Horizon oil spill (National Commission on the BP Deep Ocean Horizon Oil Spill and Offshore Drilling, 2011a). We used a ratio of dispersant to oil of 1:20. Detailed methods on the preparation of the oil dispersions and dispersant solutions are provided in the Supporting Information (S.I.-Text 1). The diameter of the oil droplets in the dispersion (range: 1–90 μm, 95% of oil droplets were between 1 and 20 μm, mean = 8 μm) prepared with this methodology was previously determined using an Imaging Particle Analysis system (FlowSight®) (Almeda et al., 2015).

2.4. Experimental conditions and procedures

The PAH composition and concentration in the crude oil and in the exposure media are shown in the Supporting Information (S.I.-Tables 1 and 2, respectively). The oil and dispersant concentrations we used were in the range of concentrations normally found in the water column after accidental oil spills and/or dispersant applications (McAuliffe et al., 1981; Lichtenthaler and Daling, 1985; Mukherjee and Wrenn, 2009; National Commission on the BP Deep Ocean Horizon Oil Spill and Offshore Drilling, 2011b). The oil exposure concentration we used (~0.84 ppm) also was environmentally relevant considering "operational pollution" and the legal upper limits for oil discharges from the oil extraction industry (~30 ppm for "produced water") (OSPAR, 2010) and shipping effluents (15 ppm) (IMO, 2016). The type of MP used in this study, PE, is the most abundant plastic polymer in surface waters (Erni-Cassola et al., 2019) and one of the MPs with the highest affinities for sorption of hydrophobic organic pollutants, including PAHs (O'Connor et al., 2016; Mai et al., 2018). The concentration of MPs used here (20 MPs mL^{-1}) was higher than those currently observed in Arctic surface waters (Bergmann et al., 2017), but it could occur in a future if abundant MPs retained in Arctic sea ice and snow (Peeken et al., 2018; Bergmann et al., 2019) are released to adjacent waters due to global warming (Obbard et al., 2014).

Copepods were acclimated to the experimental temperature (2 °C) and diet (*T. weissflogii*) for 2 days before the experiment. *C. hyperboreus* females were incubated individually in 600-mL glass bottles with red

screw caps with polytetrafluoroethylene (PTFE) protected seal. First, the bottles were filled with FSW (salinity = 33) and an aliquot of the diatom (*T. weissflogii*) culture was added to ensure a food concentration of 2000–3000 cells mL^{-1}. These diatom concentrations are found commonly during the spring bloom in Greenlandic waters (Lafond et al., 2019). Sedgewick-Rafter counting chambers and an inverted microscope were used to determine the concentrations of diatoms in the culture. Second, copepods were placed in a beaker with FSW, sorted gently with a spoon or a wide-mouth pipette and then added individually to the bottles (1 copepod per bottle). Ten random individuals were used to measure the initial size and lipid sac area. Finally, the pollutants were added to the corresponding experimental bottles to obtain the exposure levels indicated above. We used 10 replicates per treatment.

The bottles were mounted on a plankton wheel that rotated at 1 rpm in a temperature-controlled room at 2 °C. This incubation temperature is similar to the in situ temperature that *C. hyperboreus* experiences in west Greenland during spring (Swalethorp et al., 2011). The food and exposure media were renewed every 24 h for 5 days. The bottle content was carefully poured in a bucket and the copepod was gently collected and transferred with a metal spoon to the new bottle containing food and exposure media. The water from the bottles was filtered through a 40-μm mesh sieve to collect the fecal pellets daily. Fecal pellets from days 3 and 4 were combined and triplicate samples of 100 fecal pellets from each treatment were sorted and stored in glass vials at −80 °C for later chemical analysis of PAHs.

Fecal pellet sinking rates were estimated on day 4 of the exposure experiment. First, we sorted intact fecal pellets under a stereomicroscope. Second, the fecal pellets were placed individually on a glass slide to be photographed with a camera attached to a microscope. Then, we gently pipetted single intact pellets into a graduated glass cylinder (4.9 cm diameter, 39 cm height) filled with FSW. The sinking rate of each fecal pellet was measured for a distance of 5.25 cm in seawater at a room temperature of 15 °C. Only fecal pellets that did not touch the rim or sides of the graduated cylinder were considered for analysis. The sinking rates of at least 20 fecal pellets were measured per treatment.

Before the respiration experiment, thirty females were sorted and placed in a glass beaker containing 1 L of FSW. By use of a 3 mL metal spoon, 2–3 copepods were added to 26-mL acid-washed glass bottles filled with 0.2 μm-FSW. Then the copepods were exposed to the following treatments: 1 $\mu L\ L^{-1}$ of oil ("oil-1"); 2 $\mu L\ L^{-1}$ of oil ("oil-2"); oil plus dispersant (1 $\mu L\ L^{-1}$ of oil and 0.05 $\mu L\ L^{-1}$ of dispersant) ("oil +D"); no pollutants ("CTRL"). We also measured the oxygen (O_2) concentration in bottles with FSW and 3 mL of seawater from the beaker from which the copepods were sorted ("FSW"). The bottles (three replicates per treatment = 15 bottles) were sealed with butyl rubber stoppers without trapping air bubbles and mounted on a plankton wheel. The bottles were incubated in darkness at 2 °C for up to 24 h. O_2 concentration was measured every 4 h, as described in Stief et al. (2016). Respiration rates were calculated from the linear regression of the decrease in O_2 concentration over time in copepod incubations corrected for O_2 concentration changes in the "FSW" bottles.

2.5. Sample analysis and calculations

To estimate copepod size and lipid content, we photographed 10 specimens separated at the beginning of the experiment and of every copepod in the experiment at the end of the exposure period (~5 days). Each copepod was placed in a Petri dish and then photographed from a lateral view. Images were taken with a camera attached to a stereomicroscope; prosome length (Fig. 2A) and lipid sac area of each copepod (Fig. 3A) were measured with image analysis (Image J). The volume of the prosome was calculated assuming an ellipsoidal shape. We used lipid sac area (A, mm^2) as a proxy for individual total lipid content (TL, mg), which was calculated according to the equation in Vogedes et al. (2010):

$$TL = 0.197 * A^{1.38}$$

To calculate daily fecal pellet production rates (FPPRs, fecal pellets $copepod^{-1}\ d^{-1}$) and volume-specific FPPR (total volume of fecal pellet produced per copepod volume per day, d^{-1}), the fecal pellets produced after ~24 h were counted in Petri dishes under a stereomicroscope. Images of approximately 20 fecal pellets per treatment were taken with a camera attached to a microscope on days 3, 4 and 5. The length and width of the fecal pellets (Fig. 2B) were measured with image analysis (Image J) to calculate an average fecal pellet volume per treatment assuming a cylindrical shape.

The concentration of MPs inside the fecal pellets was determined on day 4. Intact fecal pellets were placed in Sedgewick-Rafter counting chambers and examined with an inverted microscope. The number of MPs inside the fecal pellets was determined for 20 fecal pellets per replicate.

For PAH analyses, copepod and fecal pellet samples were extracted and analyzed according to internal, validated protocols at SINTEF Ocean (Sørensen et al., 2016; Øverjordet et al., 2018). Briefly, samples were solvent extracted and purified as described in Øverjordet et al. 2018 (Sørensen et al., 2016) and the analysis of 44 parent and alkylated PAHs was conducted using gas chromatography-tandem mass spectrometry (GC–MS-MS) (Faksness et al., 2012). The PAH contents in the crude oil and in the initial exposure media were profiled after solvent extraction and analysis by gas chromatography–mass spectrometry (GC–MS) (Faksness et al., 2012). Internal standards were utilized to account for losses of analyte during sample extraction.

2.6. Statistical data analysis

To test statistically for significant differences between treatments, Bonferroni posthoc tests were conducted when the one-way analyses of variance (ANOVAs) were significant ($p < 0.05$). Independent-samples *t*-tests were used for cases in which the means of only two independent groups were compared ($p < 0.05$). Shapiro-Wilks and Levene tests checked for normal distribution and variance homogeneity of the data, respectively. When the data did not follow any of the assumptions for parametric tests, Kruskal-Wallis tests with pairwise comparisons were used to test for significant differences between treatments ($p < 0.05$). All statistical tests were conducted using the statistics software IBM SPSS v. 24.

3. Results and discussion

3.1. Lethal effects of the studied pollutants on C. hyperboreus

Although marine zooplankton are particularly sensitive to crude oil (Lee, 1977; Almeda et al., 2013; Almeda et al., 2014a; Almeda et al., 2014b; Almeda et al., 2014c; Almeda et al., 2016a), we did not observe lethal effects on *C. hyperboreus* in any of the treatments. Lethal effects of oil on copepods are expected to decrease with increasing body size and with decreasing temperature (Jiang et al., 2012); therefore, the large size of *C. hyperboreus* (Fig. 2C) and the low water temperature could explain the higher tolerance to oil of this species compared with smaller copepods from warmer waters (Almeda et al., 2014a). Lipid-rich copepods commonly have higher survival rates than lipid-poor copepods, probably because the temporary immobilization of lipophilic oil compounds (like PAHs) in large storage lipids may retard toxicokinetics (B.H. Hansen et al., 2016; Øverjordet et al., 2018).

3.2. Effects of the studied pollutants on FPPR of C. hyperboreus

Fecal pellet production rate (FPPR), or egestion rate, is directly related to ingestion rate and commonly used as a proxy for grazing rate in copepods (Besiktepe and Dam, 2002). The median fecal pellet volume was not-significantly different between treatments, except for the OIL

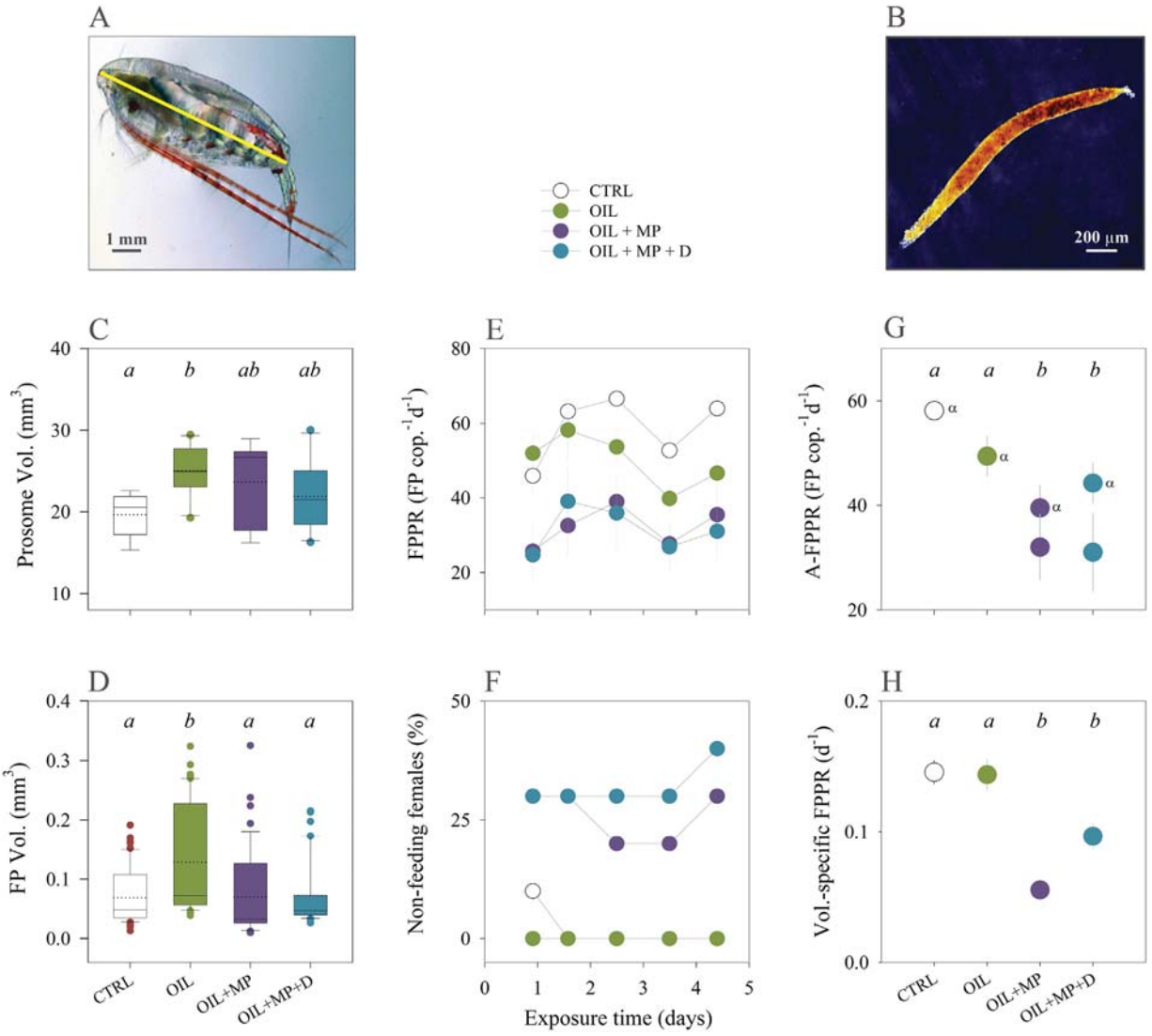

Fig. 2. A: Female of *Calanus hyperboreus* where the prosome length is indicated with a yellow line. B: fecal pellet of *C. hyperboreus*. C: prosome volume of the specimens used in the different treatments. D: fecal pellet (FP) volume in the different treatments. E: mean daily fecal pellet production rate (FPPR, fecal pellets copepod^{-1} d^{-1}) in each treatment along the experiment. F: percent of females in each treatment that did not produce fecal pellets (non-feeding) during the experiment. G: mean "accumulative" FPPR (A-FPPR) calculated as the total fecal pellets produced per copepod divided by the total incubation time (days). α indicates the A-FFPR excluding the replicates where the FPPR was zero (non-feeding females). H: mean volume-specific FFPR (d^{-1}) for the different treatments calculated from A-FFPR excluding the non-feeding females. Lowercase italics letters (*a*, *b*) indicated different statistical groups according to the Post-hoc Bonferroni test ($p < 0.05$). Error bas (E, G, H) are SE. In the box-plots (C–D), boxes encompass the interquartile range, horizontal continues bar shows the median, dotted bar shows the mean and whiskers are 1.5 times the interquartile range and dots outside the whiskers are outliers.

treatment, in which the fecal pellets were significantly bigger (Kruskal-Wallis Test, H (Melia et al., 2016) = 40.3, $p < 0.001$) (Fig. 2D). This can be partly explained by the slightly bigger mean size of the copepods in the OIL treatment than in the control (ANOVA, Bonferroni test, $p = 0.03$) (Fig. 2C). The ingestion and subsequently encapsulation of MPs in fecal pellets did not affect the size of the fecal pellets compared to the control (Fig. 2C), as previously found in *C. helgolandicus* when exposed only to MPs (Cole et al., 2016).

Reduced ingestion or fecal pellet production rates have been found in other planktonic copepods after exposure to environmentally realistic oil concentrations, as in this study (Hansen et al., 2011; Almeda et al., 2014a; Hansen et al., 2017). At the exposure concentration used, oil alone did significantly affect daily FPPR during the first two days of exposure (ANOVA, $p > 0.05$), but the reduction of FPPR was significant after day 3, 20–27% lower than the control (ANOVA, $p < 0.05$) (Fig. 2E).

In this study, 20–40% of *C. hyperboreus* females did not produce fecal pellets (= non-feeding) in the treatments with co-exposure to oil and MPs with or without dispersants (Oil + MPs and Oil + MPs + D) (Fig. 2F). In contrast, all copepods in the control and OIL treatments produced fecal pellets (Fig. 2F). Pollution-induced feeding suppression in *C. hyperboreus* also was reported after exposure to seawater from oil seeps (Gillfillan et al., 1986). In a parallel study, however, MPs alone (20 MPs mL^{-1}) did not cause feeding suppression or significantly affect *C. hyperboreus* FPPR (ANOVA, F2,9, =0.267, $p = 0.77$) (S.I. Fig. 1). This indicates that the presence of MPs can intensify this behavioral stress response of *C. hyperboreus* to oil, which emphasizes the importance of investigating the effects of both single and combined pollutants on zooplankton.

The daily FPPR for the entire exposure period (A-FPPR, Fig. 2G) were significantly lower in the treatments with Oil + MPs and Oil + MPs + D than in the control and oil treatments (ANOVA, $F_{3,36} = 6.25$, $p = 0.002$; Bonferroni test, $p < 0.05$) (Fig. 2G). By excluding non-feeding copepods and considering the fecal pellet and copepod size, volume- specific FPPR in the treatments with Oil + MPs and Oil + MPs + D were about 50%

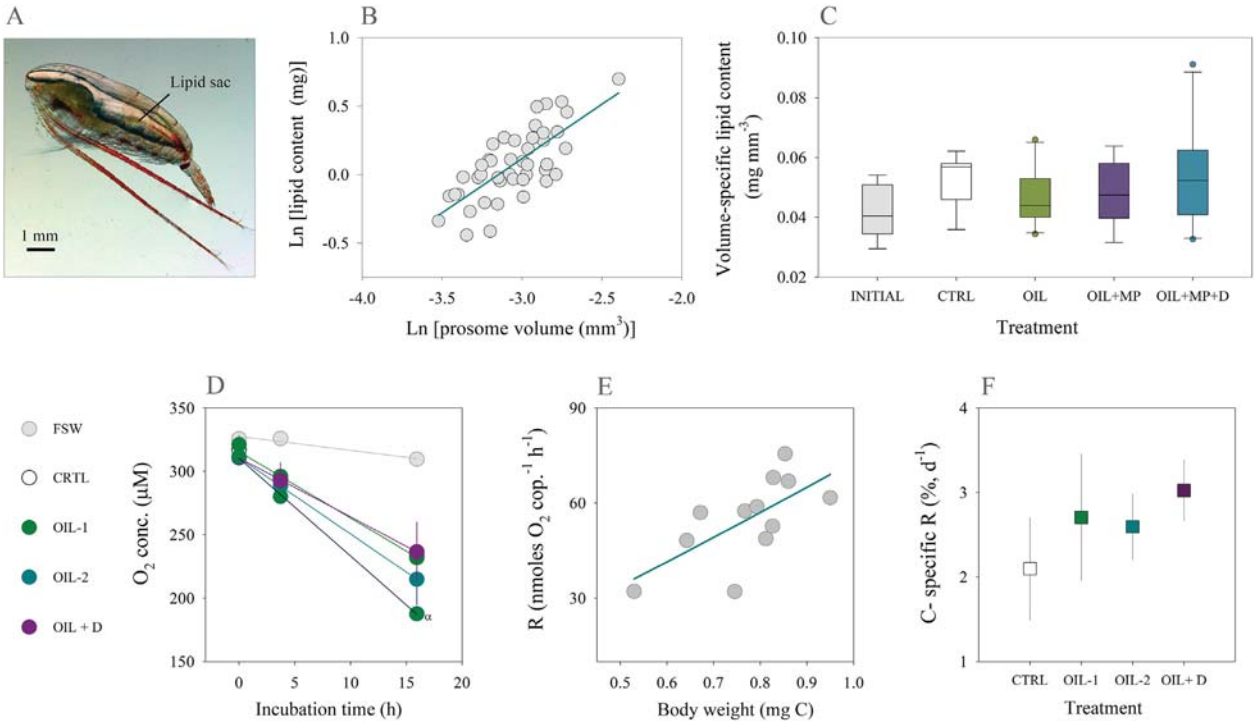

Fig. 3. A: Female of *Calanus hyperboreus* with a visible lipid sac. B: Correlation between body total lipid content (L, mg) and the prosome volume (V, mm^3) in *C. hyperboreus* considering all the experimental specimens. Linear regression: $\ln [L] = 2.50 + 0.79 \ln [V]$, $r^2 = 0.53$, $p < 0.001$. C: Volume-specific lipid content of *C. hyperboreus* in the different treatments. D: Temporal evolution of the average oxygen concentration (O_2 conc.) in the different treatments. Error bars are the standard deviation. The lines are the linear regressions fitted to the data to estimate the slopes (=oxygen consumption rate) for each treatment.α indicates an experimental replicate where three copepods per bottle were used (two copepods per bottle were used in the other replicates). E: Correlation between respiration rates (R, nmoles O_2 copepod^{-1} h^{-1}) and carbon body weight (W, mg C) considering all the experimental specimens. Regression: $R = 73*W^{1.1}$, $r^2 = 0.40$, $p < 0.05$. F: Average daily carbon-specific respiration rates (C-specific R, % d^{-1}) of *C. hyperboreus* in the different treatments. Error bars are the standard deviation.

lower than in the control and oil treatments (ANOVA, $F_{3,31} = 19.8$, $p < 0.001$; Bonferroni test, $p < 0.05$) (Fig. 2H). Therefore, the decrease in FPPR in the co-exposure treatments was due to a suppression of feeding in some individuals, and to a reduction in the ingestion rates. This decrease could be due to potential adverse effects of oil droplets and plastic particles on the feeding behavior (e.g., selectivity, handling time, etc.) (Nordtug et al., 2015; Uttieri et al., 2019), together with a certain degree of oil-induced narcosis (Saiz et al., 2009).

The addition of chemical dispersant did not increase the negative effect of oil and MPs together on FPPR (Fig. 2). Hansen et al. also found small differences in acute toxicity between mechanically and chemically dispersed oil on *C. finmarchicus* (Hansen et al., 2012). This suggests that large *Calanus* species may have a higher tolerance to acute exposure to chemical dispersants than smaller lipid-poor copepods from warmer waters (Almeda et al., 2014a).

3.3. Effects of the studied pollutants on lipid content and respiration rates

The lipid sacs of *C. hyperboreus* females used in the experiments were thin and elongated, indicating they were not completely filled (Fig. 3A); the individual lipid content was positively correlated with body volume (Fig. 3B). The median volume-specific lipid content ranged from 0.042 to 0.056 mg mm^{-3} without significant differences among treatments (ANOVA, $F_{4,39} = 1.18$, $p = 0.331$) (Fig. 3C). Oxygen consumption in the experimental bottles with *C. hyperboreus* was higher than in those from the "FSW" treatment, which contained water that the copepods were kept in before the test (Fig. 3D). Respiration rates (nmoles O_2 copepod^{-1} h^{-1}) increased with copepod body size (Fig. 3E) and were in the range of respiration rates found previously for this species (Gillfillan et al., 1986; Conover and Cota, 1995) and, more generally, in Arctic zooplankton (Alcaraz et al., 2010). Daily C-specific respiration rates were low, only 2–3% (Fig. 3F). Although there was a trend towards increased respiration rates in the presence of pollutants, these differences among treatments were not significant different (ANOVA, $F_{3,8} = 1.46$, $p = 0.295$) (Fig. 3F). The absence of effects of acute exposure to oil-contaminated waters on respiration rates in *C. hyperboreus* also has been reported (Gillfillan et al., 1986). Despite the decrease in food uptake (based on FPPR, Fig. 2H), the low respiratory carbon losses of *C. hyperboreus* (Fig. 3F) can partly explain why the total lipid storage did not differ among treatments.

3.4. Bioaccumulation of PAHs in copepods and fecal pellets

We found bioaccumulation of PAHs in *C. hyperboreus* in the three experimental treatments (ANOVA, $F_{3,\ 36} = 10.04$, $p = 0.001$; Bonferroni test, $p < 0.05$) (Fig. 4A). The median total PAH (T-PAH) body burden of *C. hyperboreus females* was ~1–2 µg g^{-1} (Fig. 4A). PAHs are considered to be the most toxic compounds in crude oil (Van Brummelen et al., 1998; Jensen et al., 2008). The main routes of PAH uptake in marine organisms are passive diffusion from the water, ingestion of contaminated prey (dietary intake) and, in the case of oil dispersions, contact (fouling) and direct ingestion of oil droplets (Berrojalbiz et al., 2009; Nordtug et al., 2015; Almeda et al., 2016b; Hansen et al., 2018). When exposed to oil dispersions, ingestion of oil droplets is a significant contributor to PAH body burden (Hansen et al., 2018). In agreement with this, we found that the T-PAH body burden in feeding individuals was ~3 times significantly higher than in non-feeding copepods (*t*-test, $t\ (15.21) = 5.748$, $p < 0.001$) (Fig. 4B). The concentration of less soluble PAHs (≥4 rings) was ~16 times higher in feeding than in non-feeding copepods (*t*-test, $t\ (14.06) = 6.603$, $p < 0.001$) (Fig. 4D). This indicates that, when copepods are exposed to oil dispersions, dietary uptake (ingestion of oil droplets and PAH-contaminated food) is the main route for accumulation of PAHs, particularly for less soluble PAHs (≥4 rings) (4B, 4D) which are primarily found in the droplet fraction (Hansen et al., 2018).

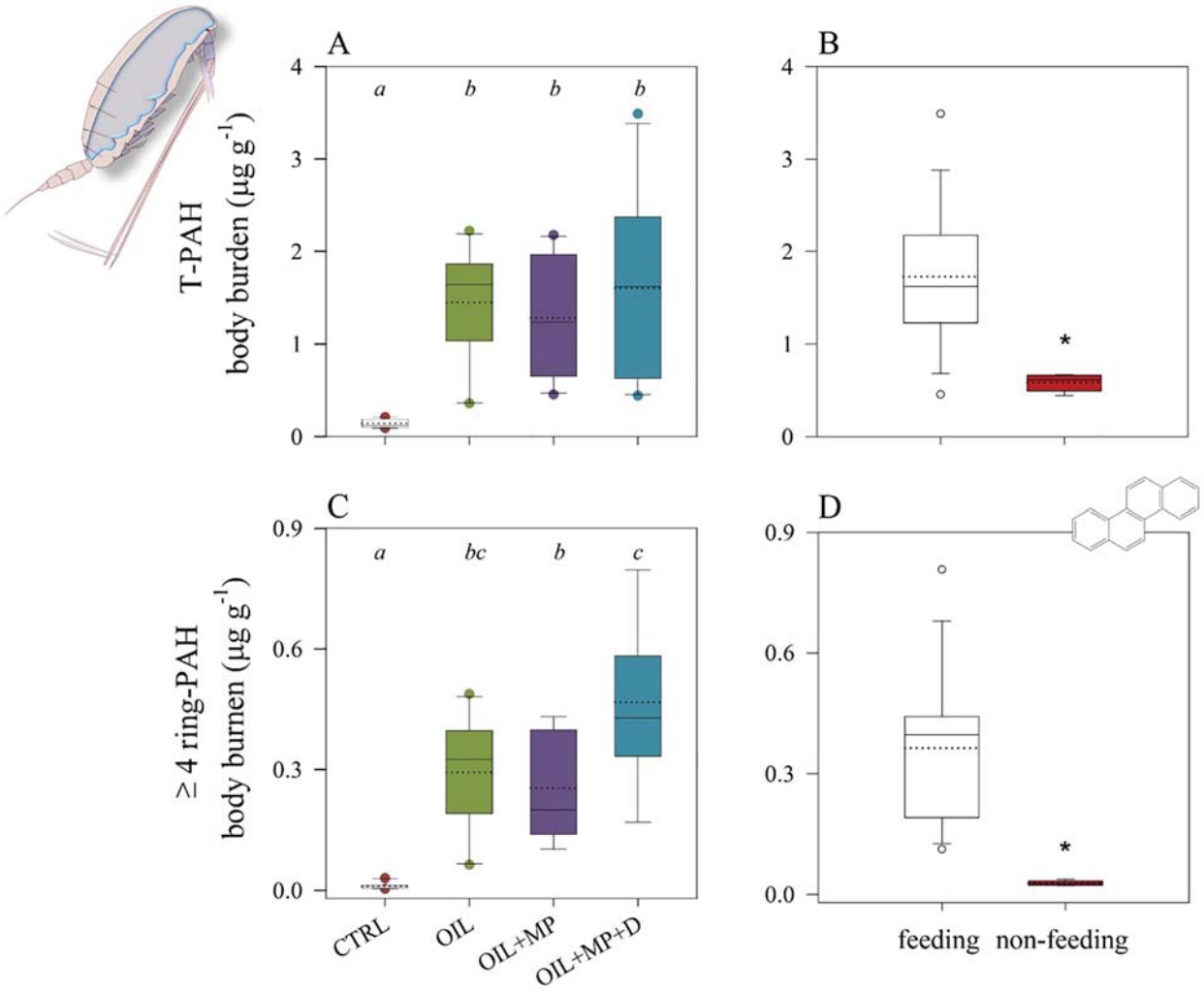

Fig. 4. A: Total PAHs (T-PAH) body burden in *Calanus hyperboreus* after 4.5 days of exposure to the different treatments. B: T-PAH body burden in non-feeding and feeding specimens from the treatments were feeding suppression was observed (OIL + MP; OIL + MP+ D) (Fig. 1F). C: Body burden of ≥4 ring-PAHs (log $K_{ow} > 4.5$) in feeding copepods. D: Body burden of ≥4 ring-PAHs in non-feeding and feeding specimens. Boxes encompass the interquartile range, horizontal solid line shows the median, dotted line shows the mean, whiskers are 1.5 times the interquartile range and dots outside the whiskers are outliers. Lowercase italics letters (*a*, *b*, *c*) indicate different statistical groups according to the Post-hoc Bonferroni test. Treatments that do not share the same letter are significantly different from each other. The asterisks (*) indicate a significant difference between feeding and non-feeding specimens (Independent Samples *t*-Test).

The presence of numerous oil droplets in the fecal pellets corroborated the ingestion of small oil droplets by *C. hyperboreus* (Fig. 5H). The concentration of T-PAHs in the fecal pellets ranged from ~0.10 to 0.35 µg g^{-1} with significant differences among treatments (ANOVA, $F_{3, 8} = 33.59$, $p < 0.001$; Bonferroni test, $p < 0.05$) (Fig. 5A).

We found differences in PAH compositions among the exposure media, copepods and fecal pellets (Fig. 6). The exposure solution had predominantly PAHs with high solubility (e.g., naphthalenes, 2 rings), whereas PAHs with lower solubility (3–6 rings) tended to be accumulated in copepods and fecal pellets (Fig. 6). Phenanthrene was the most abundant PAH in the copepod and fecal pellet samples (Fig. 6B, C). The observed partitioning of parent PAHs between oil dispersions and copepods tended to follow the PAH octanol/water partition coefficient (K_{ow}) and bioconcentration factor (BCF) relationship observed in a previous study for *C. finmarchicus* (Hansen et al., 2018).

3.5. Influence of MPs on PAH bioaccumulation after exposure to oil with and without dispersant

We found that *C. hyperboreus* ingested MPs, which after passage through the copepod gut, are expelled inside fecal pellets (Fig. 5). The median T-PAH body burden in the treatment with oil + MPs was 30% lower than in the treatment with oil alone, but the difference between treatments was not statistically significant (ANOVA, Bonferroni test, $p > 0.05$) (Fig. 4). The T-PAH concentration in fecal pellets in the treatment with oil + MPs was similar to the treatment with oil alone (ANOVA, Bonferroni test, $p > 0.05$) (Fig. 5A). Therefore, the ingestion of MPs did not facilitate PAH bioaccumulation in the copepods (or fecal pellets) in our study (Fig. 5A). The fact that we did not observe any effect of MPs in PAH bioaccumulation was not related to the co-exposure time or the polymer type (PE) used because it has a high affinity for PAHs (Wang and Wang, 2018) including phenanthrene (Wang et al., 2018), the most abundant PAH in the copepods and fecal pellets in our study (Fig. 6). Additionally, in the case of small PE MPs, the sorption of PAHs is even higher at low temperatures (Sørensen et al., 2020).

Several laboratory studies have found that MPs can transfer sorbed organic pollutants to marine biota after contact or ingestion, especially when gut conditions promote desorption of organic pollutants (Browne et al., 2013; Rochman et al., 2013c; Bakir et al., 2014; Batel et al., 2018; Rodrigues et al., 2018). Other studies, however, suggest that MPs may act as "passive samplers" of organic pollutants, rather than as vectors and, consequently MPs may reduce the amount of

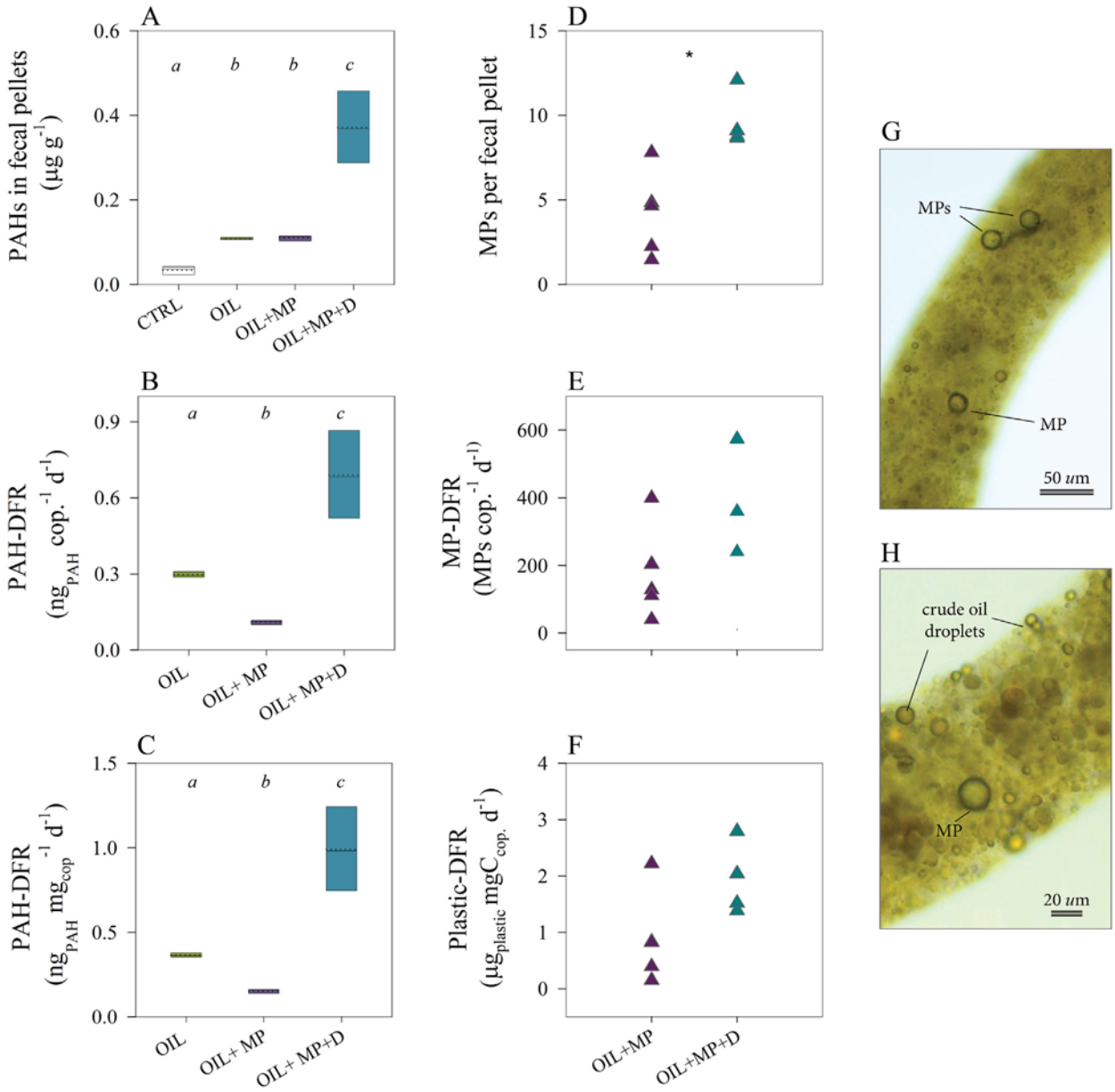

Fig. 5. A: Concentration (μg g^{-1}) of total PAHs in fecal pellets of *Calanus hyperboreus* in the different treatments. B: Daily defecation rate of PAHs (PAH-DFR) per copepod. C: weight-specific PAH-DFR. D: average number of MPs per fecal pellets. E: Daily defecation rate of MPs (MP-DFR) per copepod. F: weight-specific MP-DFR. Fecal pellet density for calculations was 1.12 g cm^{-3} (Urban et al., 1993). G–H: microscope images showing the presence of MPs and oil droplets inside fecal pellets. The asterisk (*) indicates a significant difference between treatments according to the Independent Samples *t*-Test (D). Lowercase italics letters (*a*, *b*, *c*) indicated different statistical groups according to the Post-hoc Bonferroni test.

bioavailable PAHs (Herzke et al., 2016; Sørensen et al., 2020). In our study, copepods were exposed to food concentrations commonly found during the phytoplankton bloom in the Arctic. The biomass of phytoplankton, which also absorbs PAHs (Berrojalbiz et al., 2009), is several orders of magnitude higher than the concentration of MPs typically found in surface waters (Bergmann et al., 2017), which minimizes the role of MPs as vectors of PAHs. Therefore, our results support the view that the quantitative role of MPs as vectors of pollutants to marine biota is minor compared to other routes of uptake, such as passive diffusion from the water and uptake from contaminated food (Lowman, 2017).

Chemical dispersants increase oil dispersion by formation of smaller oil droplets resulting in faster PAH equilibration between oil droplets and water (Greer et al., 2012). This can promote PAH uptake through passive diffusion from the water to phytoplankton and then to copepods, or directly from the water to fecal pellets. Although we did not find significant differences in the T-PAH body burden among experimental treatments (ANOVA, Bonferroni test, $p > 0.05$) (Fig. 4A), the concentration of less soluble PAHs (≥4 rings, log $K_{ow} > 4.5$) in feeding copepods was 45% higher in the treatment with dispersants than in the treatment only with oil and MPs (ANOVA, $F_{3,\ 31} = 18.09$, $p < 0.001$; Bonferroni test, $p < 0.05$) (Fig. 4C). This suggests higher ingestion rates of small chemically dispersed oil droplets. In contrast, uptake of ≥4 ring PAH was comparable in *C. finmarchicus* exposed to oil dispersion with and without the addition of dispersants (Hansen et al., 2015). Our study, however, shows that the concentration of T-PAHs per gram of fecal pellets with dispersants was ~3 times higher than in the other treatments with oil (ANOVA, $F_{3,\ 8} = 33.59$, $p < 0.001$; Bonferroni test, $p < 0.05$) (Fig. 5A). This suggests that the amount of bioavailable PAHs increased with the addition of dispersants, and that the increase in accumulation rate was higher than the PAH sorption rate MPs in our study. Therefore, the use of dispersants after oil spills can

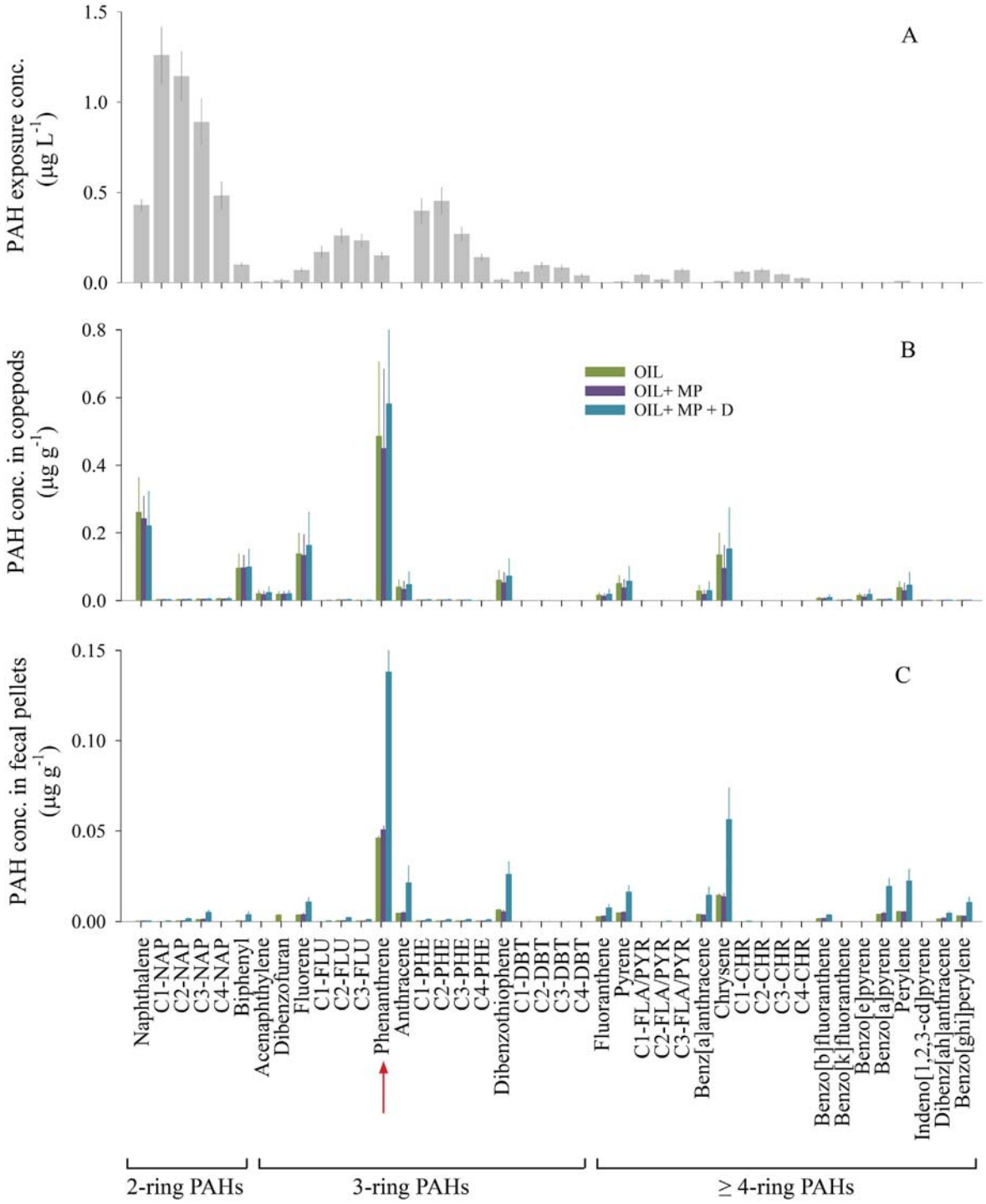

Fig. 6. Composition and concentration of PAHs in the exposure media (A), copepods (B) and fecal pellets (C). The red arrow indicates the most abundant PAHs found in copepod and fecal pellet samples. Error bars are standard deviation.

increase the entry and bioaccumulation of toxic PAHs in the Arctic food webs via zooplankton and fecal pellets.

3.6. Sinking rates of fecal pellets containing oil droplets and MPs

The presence of oil droplets and MPs inside the fecal pellets did not have any clear effect on their sinking rates (Supporting Information, S.I. Fig. 2), likely due to their small volume compared with the denser material in the fecal pellets. We estimated sinking rates of fecal pellets at 15 °C in the laboratory (SI; Fig. 2) but, given the influence of temperature in water viscosity and density, the fecal pellet sinking rates are expected to be slower at in situ temperatures (~70% slower at 0 °C than at 15 °C) (Bach et al., 2012). When the impact of the studied pollutants on FPPRs is taken into account, we estimated that the amount of PAHs defecated was lower in the presence of MPs than with oil alone but higher in the presence of dispersants (Fig. 5B, C). Our experimental food concentration (2000–3000 cells mL^{-1}) represents spring bloom densities; we estimated that copepods could potentially ingest and defecate up

to 600 MPs per day at the studied plastic concentrations (Fig. 5E), which in terms of mass is up to 3 μg of plastic per mg C of copepod per day (Fig. 5F). Therefore, *Calanus* fecal pellets can be carriers of both MPs and oil droplets and promote the vertical transfer of these buoyant pollutants from surface waters to the sediments.

3.7. Ecological implications

Exposure experiments under laboratory-controlled conditions are an essential tool to investigate the interactions between MPs and marine biota, but direct extrapolation to the field should always be considered carefully. In nature, the ingestion and effects of MPs on copepods will depend on multiple environmental factors that are difficult to mimic in the lab, e.g., currents and turbulence, which may affect encounter rates between MPs and zooplankton. Moreover, once in the environment, MPs are weathered (e.g., bio-fouled), which can affect the interactions between copepods and MPs (Vroom et al., 2017). Yet, our eco-toxicological exposure experiment is a reliable means of detecting effects of virgin MPs on zooplankton and their potential role in the bioaccumulation of PAHs.

Our results indicate that exposure to environmentally relevant concentrations of oil can cause sub-lethal effects on a key secondary producer in the Arctic. *C. hyperboreus* has the largest lipid reserves and the longest life span (3–5 years) among the Arctic *Calanus* species (Falk-Petersen et al., 2009), and a slow depuration rate of oil components (Agersted, 2018). Therefore, the bioaccumulation of PAHs in this species could increase the residence time and biotransfer of these persistent organic pollutants in Arctic food webs. Additionally, the mobilization of PAH-contaminated lipid reserves during overwintering could negatively affect the survival of *C. hyperboreus* and reproduction during this critical period of the life cycle (Toxværd et al., 2019).

High concentrations of MPs (20 particles mL^{-1}) can enhance suppression of feeding as a behavioral stress response to oil pollution in *C. hyperboreus*. In the short term, this behavioral response to acute exposure to pollutants seems positive for these individuals since it reduces the uptake of oil droplets and the accumulation of toxic PAHs, but prolonged feeding suppression can lead to reduced lipid storage and starvation. The influence of MPs on the toxicity and bioaccumulation of oil in Arctic copepods is expected to be minor when considering the concentrations of MPs currently found in Arctic surface waters (Everaert et al., 2018; Rist et al., 2020). However, in an accelerating global warming scenario, higher concentrations of MPs could be temporarily found in Arctic sea-ice adjacent waters (O'Connor et al., 2016; Barrows et al., 2018; Peeken et al., 2018; Bergmann et al., 2019), which requires further field research and monitoring.

The use of chemical dispersants after oil spills can increase the accumulation of toxic PAHs in zooplankton and their fecal pellets. Since *Calanus* copepods are the main prey of fish, birds, and marine mammals in the Arctic, the potential trophic transfer of PAHs via zooplankton is a major concern. PAHs can be subject to trophic dilution in the marine food webs (Gray, 2002; Wan et al., 2007). However, trophic transfer and magnification of PAHs via contaminated zooplankton could be relevant for very hydrophobic PAHs (Mackay et al., 2018). Particularly for Arctic planktivorous seabirds (e.g., little auk, one of the most numerous seabirds in the world) that feed directly on *Calanus* copepods (Mehlum and Gabrielsen, 1993).

Zooplankton fecal pellets can play a role in the sedimentation of PAHs (Prahl and Carpenter, 1979). Additionally, vertical exportation of small low-density MPs via copepod fecal pellets is one of the processes that may explain why the deep sea is a major sink for MP debris (Woodall et al., 2014). The potential impacts of accumulation of MPs on benthic sediment communities in the Arctic are still not well known and need further research.

The Arctic has the world's largest remaining reservoirs of crude oil (Gautier et al., 2009). Oil industry activities in the Arctic may rise over the next decades considering the expected increase in oil demand (Energy Information Administration, 2019) and the loss of sea ice cover, which will facilitate oil industry accessibility and maritime transportation in the Arctic (Dalsøren et al., 2007; Melia et al., 2016). Strong regulations and controls on oil pollution and better knowledge about the net environmental benefits of different oil spill responses in the Arctic are priority issues to reduce the impacts of oil pollution in the Arctic (Wilkinson et al., 2017). In addition, MPs are ending up in the Arctic sea ice, which is becoming a major sink of global plastic pollution (Peeken et al., 2018). Therefore, reducing global plastic pollution will prevent increased anthropogenic impacts on the unique but multiple-stressed Arctic ecosystem.

4. Conclusions

Can microplastics increase the toxicity and bioaccumulation of oil after spills?

Co-exposure to MPs and oil negatively affects *C. hyperboreus*. The combination of these pollutants induced feeding suppression and a reduction in fecal pellet production at rates higher than when the copepods were exposed to each pollutant individually. When copepods are exposed to food concentrations commonly found during the Arctic phytoplankton bloom, the ingestion of MPs does not increase the bioaccumulation of PAHs in copepods or their fecal pellets. This suggests that MPs are not acting as vectors of PAHs under environmentally relevant conditions.

In an oil spill, would the application of dispersant increase the impacts of MPs + oil?

At the used concentrations, chemical dispersant did not increase the toxicity of Oil + MPs on *C. hyperboreus*. However, the addition of dispersant increased the bioaccumulation of toxic PAHs in copepods and their fecal pellets, which is a negative environmental aspect of the application of dispersants as a response to marine oil spills.

Overall, our results indicate that crude oil is a pollutant of higher concern to Arctic zooplankton than MPs, but the combination of both pollutants can synergistically increase negative impacts of pollution on Arctic copepods. Consequently, when viewed in the context of global warming-induced multiple stressors, reducing and preventing oil and plastic pollution is critical in order to diminish the anthropogenic impact on the sensitive Arctic ecosystem.

CRediT authorship contribution statement

R. Almeda: Conceptualization, Methodology, Formal analysis, Investigation, Writing - original draft, Writing - review & editing, Project administration, Funding acquisition. **R. Rodriguez-Torres:** Investigation, Writing - review & editing. **S. Rist:** Investigation, Writing - review & editing. **M.H.S. Winding:** Investigation, Writing - review & editing. **P. Stief:** Investigation, Writing - review & editing. **B.H. Hansen:** Resources, Formal analysis, Writing - review & editing. **T. Gissel Nielsen:** Methodology, Investigation, Writing - review & editing, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2020.141264.

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Supporting information

S.I. - Text 1:

To prepare the crude oil dispersions, 1 L of 0.2 μm-FSW was poured in a 2 L glass beaker with a magnetic stir bar, which was sealed with aluminum foil to prevent oil absorption. The glass beaker containing the seawater was placed on a magnetic stirrer plate and stirred at 900 rpm at room temperature (15°C). Then, 1 mL of oil was added to the FSW using an automatic pipette with a glass pipette as a tip. The glass pipette was exhaustively rinsed to remove the crude oil that could be attached to the pipette tip. The beaker was covered with aluminum foil and the oil was emulsified by keeping the stir rate at 900 rpm. The used stirring speed caused a vortex, which extended from the bottom of the beaker to the water surface, allowing the formation of a dispersion of homogeneously distributed oil droplets in seawater during mixing. After 5 min and keeping the mixing, aliquots of the dispersion (600 μL) were added to the experimental bottles (600 mL) to obtain the desired exposure concentrations (1 μL L^{-1}). The size (diameter) of the oil droplets in the dispersion (range: 1–90 μm, 95% of oil droplets being between 1–20 μm, mean= 8 μm) prepared with this methodology was previously determined using an Imaging Particle Analysis system (FlowSight®) (37). Three additional bottles were prepared to determine the initial PAH concentration and composition in the exposure media (S.I., Table 2). To prepare the dispersant solutions, 50 μL of chemical dispersant was added to 1 L of FSW and stirred at 900 rpm for 5 min at 15 °C as in the preparation of oil dispersions. Aliquots (600 μL) were added to the corresponding treatment bottles to obtain the desired exposure nominal concentrations (0.05 μL L^{-1}). We used a ratio of dispersant to crude oil of 1:20.

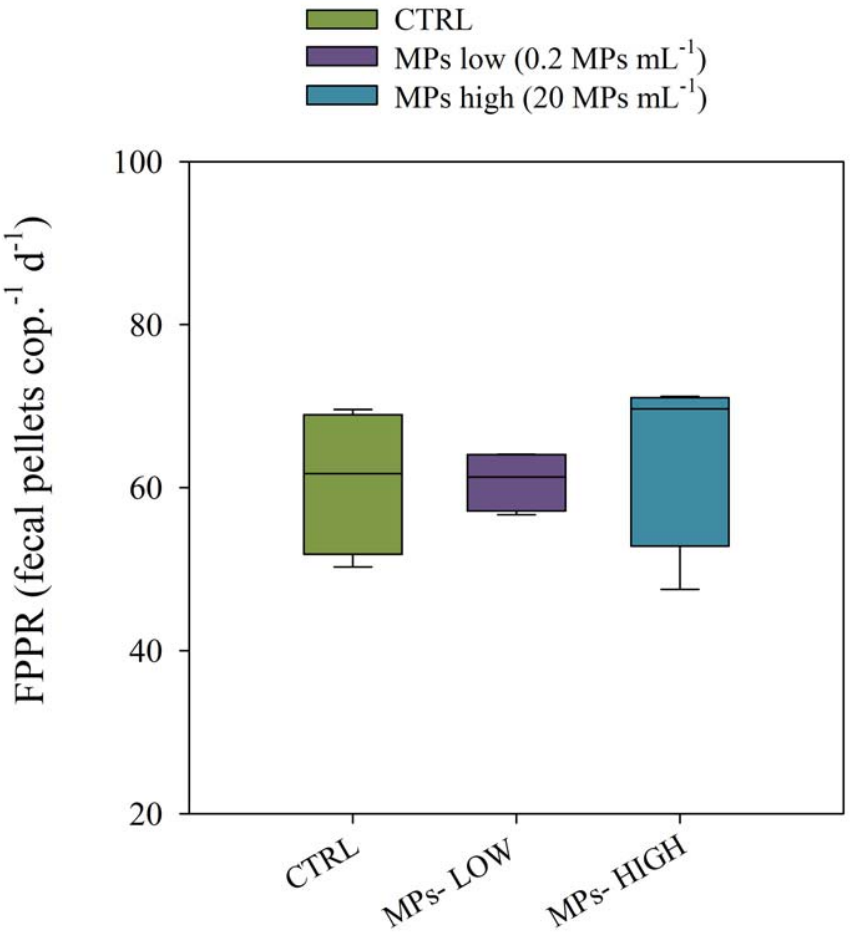
S.I.-TABLE 1. PAH composition and concentration (conc.) in the crude oil used in the experiments. K_{ow}: octanol/water partition coefficient.

PAH	Rings	Log K_{ow}	Conc. (g kg^{-1})
Naphthalene	2	3.17	0.7264
C1-NAP	2	3.72	2.1090
C2-NAP	2	4.26	2.5234
C3-NAP	2	4.81	1.8743
C4-NAP	2	5.36	0.9596
Biphenyl	2	3.76	0.2124
Acenaphthylene	3	3.94	0.0105
Acenapthene	3	4.15	0.0092
Dibenzofuran	3	4.21	0.0307
Fluorene	3	4.02	0.1257
C1-FLU	3	4.15	0.3082
C2-FLU	3	5.11	0.4267
C3-FLU	3	5.24	0.3514
Phenanthrene	3	4.35	0.2360
Anthracene	3	4.35	0.0118
C1-PHE	3	4.89	0.6176
C2-PHE	3	5.44	0.7097
C3-PHE	3	5.99	0.4500
C4-PHE	3	6.53	0.2196
Dibenzothiophene	3	4.29	0.0261
C1-DBT	3	4.84	0.1002
C2-DBT	3	5.39	0.1610
C3-DBT	3	5.93	0.1242
C4-DBT	3	6.48	0.0697
Fluoranthene	4	5.93	0.0050
Pyrene	4	5.93	0.0091
C1-FLA/PYR	4	5.48	0.0696
C2-FLA/PYR	4	6.03	0.1102
C3-FLA/PYR	4	6.57	0.1175
Benz[a]anthracene	4	5.52	0.0075
Chrysene	4	5.52	0.0178
C1-CHR	4	6.07	0.1139
C2-CHR	4	6.59	0.1232
C3-CHR	4	6.98	0.0856
C4-CHR	4	7.99	0.0343
Benzo[b]fluoranthene	5	6.11	0.0044
Benzo[k]fluoranthene	5	6.11	0.0000
Benzo[e]pyrene	5	6.11	0.0061
Benzo[a]pyrene	5	6.11	0.0026
Perylene	5	6.11	0.0147
Dibenz[ah]anthracene	5	6.70	0.0013
Indeno[1,2,3-cd]pyrene	6	6.70	0.0000
Benzo[ghi]perylene	6	6.70	0.0013

S.I.-TABLE 2. PAH concentration (µg L^{-1}) in exposure oil dispersions (#1-3, triplicates).

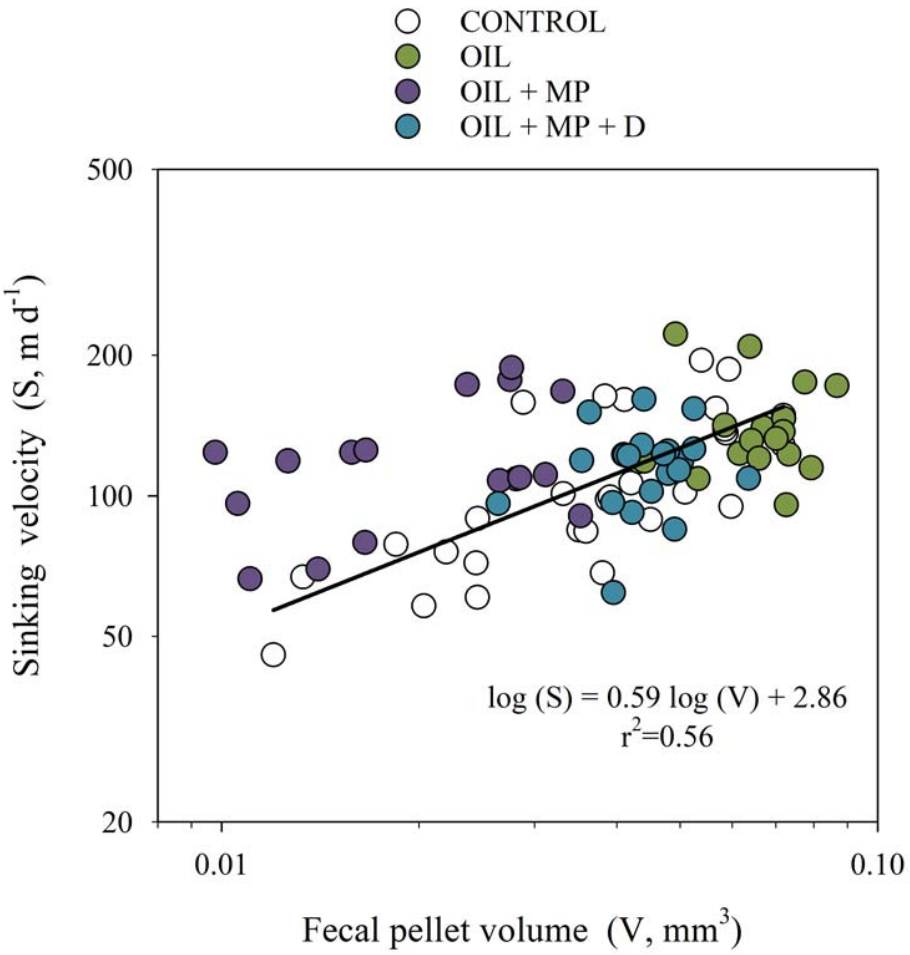
PAH	#1	#2	#3
Naphthalene	0.19	0.19	0.17
C1-NAP	0.29	0.29	0.26
C2-NAP	0.29	0.27	0.23
C3-NAP	0.35	0.3	0.25
C4-NAP	0.32	0.26	0.21
Biphenyl	0	0	0
Acenaphthylene	0.46	0.43	0.4
Acenapthene	1.4	1.29	1.09
Dibenzofuran	1.27	1.16	1
Fluorene	1.02	0.89	0.76
C1-FLU	0.56	0.48	0.41
C2-FLU	0.11	0.1	0.09
C3-FLU	0.01	0.01	0
Phenanthrene	0.01	0	0
Anthracene	0.02	0.01	0.01
C1-PHE	0.08	0.07	0.06
C2-PHE	0.2	0.17	0.14
C3-PHE	0.3	0.26	0.22
C4-PHE	0.27	0.23	0.2
Dibenzothiophene	0.17	0.15	0.13
C1-DBT	0	0	0
C2-DBT	0.47	0.39	0.33
C3-DBT	0.53	0.45	0.38
C4-DBT	0.31	0.27	0.23
Fluoranthene	0.16	0.14	0.12
Pyrene	0.02	0.02	0.01
C1-FLA/PYR	0.07	0.06	0.05
C2-FLA/PYR	0.11	0.1	0.08
C3-FLA/PYR	0.1	0.08	0.07
Benz[a]anthracene	0.05	0.04	0.03
Chrysene	0	0	0
C1-CHR	0.01	0.01	0
C2-CHR	0.05	0.04	0.04
C3-CHR	0.02	0.02	0.01
C4-CHR	0.08	0.07	0.06
Benzo[b]fluoranthene	0	0	0
Benzo[k]fluoranthene	0.01	0.01	0.01
Benzo[e]pyrene	0.07	0.06	0.05
Benzo[a]pyrene	0.08	0.07	0.06
Perylene	0.05	0.05	0.04
Dibenz[ah]anthracene	0.03	0.02	0.02
Indeno[1,2,3-cd]pyrene	0	0	0
Benzo[ghi]perylene	0	0	0
TOTAL PAHs (µg L^{-1})	**9.55**	**8.47**	**7.23**
Avg. total PAHs (µg L^{-1})		8.42	

S.I.-FIGURE 1



S.I. Figure 1. Average fecal pellet production rate (FPPR, fecal pellet copepod^{-1} d^{-1}) of *Calanus hyperboreus* after 6 days of exposure to 2 concentrations of MPs (low: 0.2 MPs mL^{-1} and high: 20 MPs mL^{-1}) (data from Rodríguez-Torres et al. 2020, unpublished results)

S.I.-FIGURE 2



S.I.-Figure 2. Relationship between sinking velocity (S) and volume (V) of *Calanus hyperboreus* fecal pellets in the different treatments. Note that the linear regression was significant ($p<0.001$) only for fecal pellets from the control treatment; the line represents the linear regression equation fitted to the logarithmically transformed data; the equation is given in the figure.

Paper IV

Unpalatable plastic: efficient taste-discrimination of microplastics in planktonic copepods

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Unpalatable plastic: efficient taste-discrimination of microplastics in planktonic copepods

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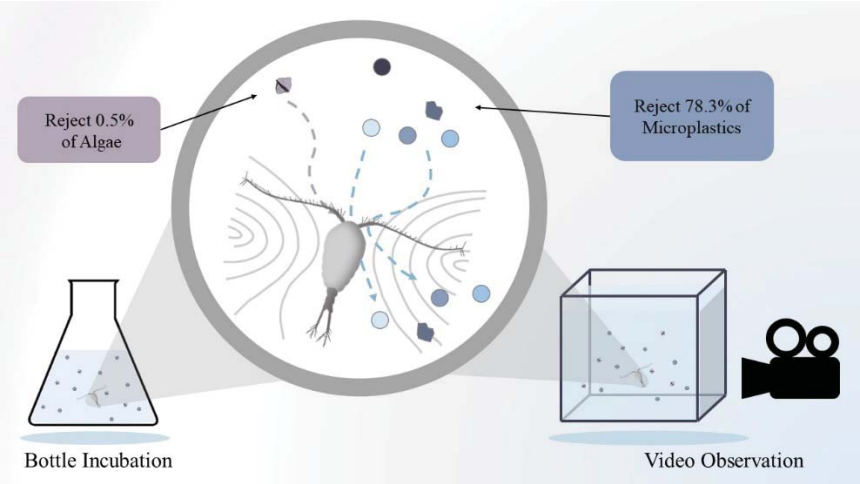
Abstract

Planktonic copepods are the most abundant animals in the ocean and key players in global biochemical processes. Recent modelling suggests that zooplankton ingestion of microplastics (MPs) can disrupt the biological carbon pump and accelerate a global loss of oceanic oxygen. Here we investigate the behavioural responses and ingestion rates of a model feeding-current generating copepod when exposed to MPs of different characteristics by small-scale video observations and bottle incubations. We found that copepods rejected 80% of the MPs after touching them with their mouths parts, in essence exhibiting a kind of taste discrimination. High rejection rates of MPs were independent of polymer type, shape, presence of biofilms, or sorbed pollutant (pyrene), indicating that MPs are unpalatable for feeding-current feeding copepods and that post-capture taste discrimination is a main sensorial mechanism in rejection of MPs. In an ecological context, taking into account the behaviours of planktonic copepods and the concentrations of MPs found in marine waters, our results suggest a low risk of MP ingestion by zooplankton and a low impact of MPs on the biological carbon pump.

Keywords: Zooplankton, Copepods, Feeding Behavior, Microplastic, Taste Discrimination,

Synopsis: The behavioral reaction of copepods to microplastics were rarely reported. This study simulates the feeding behaviors of copepods exposure to different types of microplastics in the nature.

TOC:



Introduction

Microplastics (MPs) are ubiquitous pollutants in the marine environment [1,2]. Understanding the consequences of plastic pollution in marine ecosystems is therefore of major societal and scientific concern. Most biological oceanographic processes are directly linked to the presence and activities of planktonic organisms [3], which are exposed to MPs in the water column. This makes zooplankton of particular interest in relation to potential global environmental impacts of MP pollution. It has been hypothesized that the ingestion of MPs instead of organic prey by zooplankton may change the sinking velocity of their fecal pellets [4], consequently affecting the vertical transportation of carbon and weakening the marine biological carbon pump [5,6]. It is even predicted to accelerate global loss of ocean oxygen through reduced grazing on primary producers [7]. Among planktonic organisms, copepods are the most abundant animals in the ocean and the dominant zooplankton group [8,9]. Ingestion of MPs by copepods is potentially the main route by which plankton-sized MPs enter marine food webs and are transferred to higher trophic levels [10,11]. A better knowledge of the interactions between copepods and MPs is

therefore essential to understand the fate and impacts of marine plastic pollution.

It is well documented that marine macro- and megafauna ingest plastic debris with more than 900 recorded cases of vertebrate species, including sea mammals, sea birds, marine turtles and fishes, being entangled by, or having consumed plastics [12,13]. Additionally, laboratory research has clearly demonstrated that zooplankton, including copepods, ingest plankton-sized MPs when exposed to high concentrations [10,11,14–16]. However, evidence of MP consumption by copepods in the natural environment and its consequences is lacking. Several field surveys have reported ingestion of MPs by copepods [17–21]. However, for some of these studies, the reported size of ingested MPs is outside of the size-range of natural prey, and in some cases, it is even larger than the mouth of the copepods, suggesting entanglement or sample contamination rather than actual ingestion. In any case, these field studies indicate a low occurrence of MP ingestion in natural zooplankton communities, which contradicts the high probability of MP ingestion predicted from laboratory studies.

One reason for this mismatch in the findings of laboratory and field research may be the methods used in the laboratory. Most laboratory research on the ingestion of MPs by zooplankton has been conducted using virgin spherical MPs [10] whereas investigations of zooplankton preferences for different shapes [4], aging states [22] and other characteristics of MPs are still lacking. In addition, most laboratory research involves only bottle incubations that allow little insight the mechanisms of the interactions between different types of MPs and copepods. A practical tool to open the “black box” of bottle incubations when investigating the feeding behavior of zooplankton is small-scale video observation. This technique has successfully been applied to study selective feeding behavioral responses of copepods to different species of

harmful algae [23].

In this study, we investigated how different characteristics of MPs affect the behavioral responses and ingestion rates of the feeding-current feeder, *Temora longicornis*. *Temora* species are distributed worldwide from coastal to oceanic waters [24]. Moreover, feeding-current feeding is one of the three dominant feeding modes of planktonic copepods [25,26]. Through modelling we represent the global distribution of the feeding-current feeding mode with the aim of reflecting the relevance of this feeding mode in the world oceans [27,28]. Furthermore, we used direct small-scale video observations and parallel bottle incubations to quantify the feeding behavioral responses of copepods to diverse MP characteristics: plastic polymer type (polystyrene (PS) vs polyethylene (PE)), shape (irregular vs sphere), presence of biofilms (bio-fouled vs “clean” microspheres) and sorption of organic pollutants (microspheres with sorbed pyrene vs “clean” microspheres). We hypothesized that feeding-current generating copepods (i) do not discriminate between plastic polymer types; (ii) show a higher rejection of MPs when plastic particles are irregular (different from normal prey which are typically of regular shape); (iii) have a higher ingestion of bio-fouled MPs than virgin MPs; and (iv) show a higher rejection of MPs with sorbed chemicals pollutants. Our results will provide a better understanding of how plastic properties and weathering processes influence the risk of MPs to enter the marine food webs via zooplankton.

Materials and Methods

Spatial modelling

To represent the global distribution of feeding-current feeders, the global ocean was discretized

into roughly 5000 polygons of similar area and feeding-current feeders were assumed to be represented by the world most abundant genera: *Paracalanus*, *Pareucalanus*, *Parvocalanus*, *Rhincalanus*, *Pseudocalanus*, *Calocalanus*, *Nannocalanus*, *Temora, Acartia*, *Calanus*, *Centropages*, *Pleuromamma*, and *Euchaeta*. Observation-based estimates were derived polygon-wise as community-weighted means using abundance observations [29], body length data [30], and the procedure described by Brun et al. [31]. For model extrapolations we fitted generalized additive models [32,33], assuming beta distribution and using average and range of monthly sea surface temperature (derived from the HadISST1 product [34]) and average chlorophyll a concentration (derived from http://www.globcolour.info/) as predictors.

General experimental approach

To test our hypothesis, we conducted four studies:

a) "Polymer type" (PS vs PE), where copepods (*T. longicornis*) were exposed to either (i) algae and virgin, spherical PS MPs or (ii) algae and virgin, spherical PE MPs.

b) "Shape" (spheres vs irregular fragments), where copepods were exposed to either (i) algae and virgin, spherical PS MPs or (ii) algae and virgin, irregular PS fragments.

c) "Biofilms" (bio-fouled vs "clean" microspheres), where copepods were exposed to either (i) algae and spherical PE MPs with biofilms or (ii) algae and virgin, spherical PE MPs without biofilms ("clean").

d) "Sorbed pollutants" (MPs with sorbed pyrene vs MPs without sorbed pyrene) where copepods were exposed to either (i) algae and spherical PE MPs with sorbed pyrene, (ii) algae and spherical PE MPs without sorbed pyrene, (iii) algae and spherical PS MPs with sorbed pyrene or (iv) algae and spherical PS MPs without sorbed pyrene.

In all experiments, copepods were exposed to a nominal prey:MP ratio of 1:1 with concentrations of 200 cells mL^{-1} and 200 MPs mL^{-1}. Measured experimental concentrations are shown in Table S1. Feeding behavioral responses of individual copepods, including prey detection, capture, handling, rejection and ingestion of prey and different MP types were examined by small-scale video observations, as described in details below. Feeding rates of copepods on the studied prey and MPs were calculated from both video observations and bottle incubations as described below.

Experimental organisms

The culture of our model copepod species, *T. longicornis*, originates from samples from the Gullmars fjord (Sweden) and Øresund (Denmark) in 2016. The copepods were subsequently maintained in a continuous laboratory culture at the Technical University of Denmark. They are grown in 30 L tanks with filtered seawater (salinity= 30 psu) at 18℃ in the dark. Copepod cultures were fed a mixed diet consisting of cultured phytoplankton (*Heterocapsa steinii* (formerly known as *H. triquetra), Thalassiosira weissflogii, Rhodomonas salina*) and a heterotrophic dinoflagellate (*Oxyrrhis marina*). The phytoplankton and *O. marina* cultures were maintained in the lab [25].

The day before the experiments, healthy copepod females of similar size (prosome length approximately 740 μm) were sorted under a stereo microscope and kept overnight in glass beakers with 0.2 μm-filtered seawater. From the sorted copepod stock, we picked females for both bottle incubations and video experiments.

The dinoflagellate *H. steinii* was the model prey used in the feeding experiments. The cultures

of *H. steinii* were maintained in autoclaved 0.2 µm-filtered seawater with B1 medium at 16℃, 150 µmol photons m^{-2} s^{-1}, 12 h light: 12 h dark cycle, and a salinity of 30 psu. The size distribution and concentration of *H. steinii* were measured with a Beckman Multisizer Coulter Counter before the experiment. Only cultures in exponential growth phase were used as prey. The equivalent spherical diameter (ESD) of algal cell was approximately 20 µm on average.

Preparation of the different types of microplastics

Study 1: "Polymer type"

We used virgin spherical MPs of the two different plastic polymers, PS and PE. 20 µm PS microspheres suspended in water with tween 80 were purchased from Degradex®. 20 µm PE microspheres were purchased from Cospheric® as powder. In both cases, the MPs were suspended in a 0.01% tween 80 miliQ water solution to prepare the working suspensions.

Study 2: "Shape"

To obtain irregular MPs, PS pellets (500 µm in diameter, purchased from Cospheric®) were frozen with liquid nitrogen and subsequently ground using an IKA A11 basic analytical mill. The resulting MP fragments were suspended in a 0.01% tween 80 solution. The suspension of MPs was then filtered through nylon filters with 30 µm and 15 µm mesh sizes in sequence to obtain irregular MPs with an average size of approximately 20 µm. Upon filtration, the fragments were collected and re-suspended in a 0.01% tween 80 solution. 20 µm PS microspheres were used as spherical MPs to compare with irregular MPs of the same size and polymer but with different shape.

Study 3: "Biofilms"

Seawater, containing natural microbial communities, was collected from a Danish estuary, Limfjorden, and filtered through 8 µm polycarbonate filters. To produce bio-fouled MPs, 20 µm PE microspheres were added to 600 mL Pyrex bottles containing the 8 µm-filtered seawater, in a concentration of 50 MPs mL^{-1}. The bottles were placed in a plankton wheel at 1 rpm and incubated with the following conditions: temperature of 18 ℃, light intensity of 100 µmol photons m^{-2} s^{-1} and a photoperiod of 12 h light: 12 h dark cycle. B1 medium (1 mL L^{-1})[35] was added to all the bottles to avoid nutrient depletion. After four weeks of incubation, the presence of a biofilm on the MPs was confirmed using DAPI (4', 6-Diamidino-2-Phenylindole, Hydrochloride) staining and examination under an epifluorescence microscope with UV light (Fig. 1).

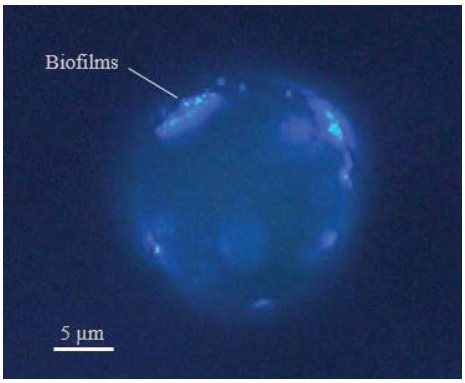


Figure 1. Epifluorescence microscope image of a bio-fouled PE MP stained with DAPI under UV illumination. Bright blue fluorescent areas correspond to the DNA of biofouling microorganisms growing on the surface of the MP particle.

Study 4: "Sorbed pollutants"

To obtain MPs with a sorbed hydrophobic pollutant, PE and PS microspheres respectively were exposed to a pyrene solution. Pyrene powder was diluted in methanol to prepare a stock pyrene solution of 100 µg mL^{-1}. 10 mg of MPs were added to acid-washed glass bottles (68 mL) with a pyrene solution of 50 µg L^{-1}. Additionally, 10 mg of MPs were added to bottles with methanol

alone as a control treatment. The bottles were incubated for 72 hours in a plankton wheel at 5 rpm at 18 ℃ in the dark. Upon incubation, we filtered the bottle contents through 5 µm polycarbonate filters, separating the MPs (residue) from the pyrene solution (filtrate). A subsample of the collected MPs was re-suspended in filtered seawater to be used in the bottle incubations and filming experiments, respectively. Another subsample was stored at -80 ℃ for later pyrene sorption analysis. The concentration of pyrene sorbed by the MPs was measured in triplicates of MP samples with an Agilent 6890 gas chromatograph. Extraction was performed by adding 4 mL n-hexane: acetone (6:4) directly to the vials. The extraction time was 24 h. Chromatographic separation was achieved on an Agilent 6890 gas chromatograph equipped with a 60 m × 0.25 mm inside diameter × 0.25 µm film thickness DB-5ms column (Agilent Technologies). A 2 µL sample was injected in splitless mode with the sample inlet held at 300 °C. The oven was programmed to 70 °C, then 20 °C/min to 300 °C, and then 50 °C/min to 325 °C held for 10 min. Helium was used as carrier gas with a 1 mL/min constant flow. Detection was achieved on an Agilent 5975C triple-axis mass-selective detector operated in SIM mode with the MS source at 230 °C and the quadrupole at 150 °C.

The average concentration of sorbed pyrene in the PE MPs was 0.059 ± 0.009 µg mg^{-1}. This value is the same order of magnitude as observed by Wang and Wang [36].

Particle sizes

The size (equivalent spherical diameter, ESD) and concentration of each type of MP in the prepared stock suspensions were measured using a Multisizer Coulter Counter. The average size of PE and PS microspheres was 20.5 ± 0.2 µm and 20.6 ± 0.5 µm, respectively. The average size of the obtained irregular MPs was 20.6 ± 1.7 µm. *H. steinii* used for the experiment

had an average ESD of 17 ± 0.4 μm.

Video observation

Before the video recordings, copepods were tethered to a needle from their dorsal surface [37]. Tethering does not affect the feeding selective behavior of *T. longicornis* [23,38]. The video observation was conducted in a thermo-constant filming room (at 16℃). A 10×10×10 cm^3 transparent container was placed between an infrared light and a high-speed camera (Phantom V210). In each treatment, 800 mL of the MP-alga suspension was added to the container and gently stirred by a magnetic stirrer. Then, a single tethered copepod was attached to a micromanipulator by the other edge of the tether immersed in the mixed particle suspension. Subsequently, the tethered copepod was adjusted to the center of screen field in focus. A 3-hour video recording (resolution: 1024×512 pixels; frame rate: 100 Hz) was started instantly after preparing the set up. Due to limited storage space on the camera, each video lasted for maximum 100s. Thus, with 28 recorded videos, a total of 3h were saved for analysis. All the experimental operations were conducted outside the filming room and the room was kept in darkness throughout the entire process to minimize any interruption. Three copepod females from each treatment were filmed separately.

The capture, ingestion and rejection events of *T. longicornis* were counted from the videos. The copepods beat their feeding appendages constantly to maintain the feeding current (percentage of time beating = 99.7% ± 0.1%) and scan the surrounding water. When prey particles were drawn into their detection range, contractions of swimming appendages were observed, in many cases followed by a successful capture of the particle. A behavioral event

was defined as “ingestion” when the captured particle was handled, tasted and finally eaten by the copepod (Movie S1 and S2). On the contrary, a behavioral event was defined as “rejection” when the particle was actively “kicked” away by the copepod after tasting (Movie S3). Although the used prey and MPs have similar sizes, it was easy to visually distinguish dinoflagellates from MPs in the video (Movie S1 and S2) due to their specific morphological characteristics.

Bottle incubation experiments

All the glassware used for these experiments was acid-washed with 10% HCl and rinsed three times with miliQ water. Experiments were conducted in triplicates in 600 mL Pyrex glass bottles with lids lined with polytetrafluoroethylene (PTFE) protection. Six copepod females were incubated in each bottle. For all the treatments, we prepared 3 initial bottles (time=0), 3 control bottles (without copepods) and 3 experimental bottles (with copepods). The bottles were first filled with 0.2 μm-filtered seawater (salinity = 30 psu), Aliquots of MPs and algae working suspensions were added to each bottle to obtain the desired exposure concentrations for each treatment (200 MPs mL^{-1} and 200 cells mL^{-1}). Subsequently, the copepods were added to the experimental bottles. Finally, the bottles were filled up with filtered seawater, closed with a lid and wrapped in aluminum foil. The bottles were mounted on a plankton wheel (1 rpm) in a temperature-controlled incubation room at 16 ℃ for 24 hours.

At the beginning of the incubation (time= 0), for each treatment, 25 mL samples of MP-alga mixture were collected from the three initial bottles to measure the precise concentration of MPs and algae added (Table S1). After the 24 hours of incubation, 25 mL samples were collected from three experimental and three control bottles, respectively, to measure the final

concentration of MPs and algae. All 25 mL samples were immediately fixed with 1% of Lugol's solution and subsequently MPs and algae were counted under an inverted microscope using Sedgewick-Rafter counting chambers. At the end of the experiment, copepods were examined under a stereomicroscope to verify that there was no mortality during the experiment. We did not observe mortality in any treatment. The ingestion and clearance rates were calculated according to Frost [39]. Selective feeding was evaluated using the electivity index (E) [40]. Electivity index of the particle type I (E_i) was calculated as:

$$E_i = \frac{Wi-(1/n)}{Wi+(1/n)}$$

with *n* as the total number of particle types in a given bottle (n=2), and the coefficient *Wi* as

$$W_i = \frac{Fi}{\sum Fi}$$

Where, Fi is the clearance rate of the particle type i, and ∑ Fi is the sum of clearance rates on all food types. The electivity index (E) ranges between -1 and +1, where 0 indicates no electivity (no selective grazing), negative values correspond to avoidance and positive values represent selection.

Statistical analysis

Statistical analysis was performed using IBM-SPSS v25. For each treatment, we statistically analyzed the significant differences between algae and MPs ingestion and clearance rates. Furthermore, we tested the statistical differences in feeding rates on algae and MP types among the treatments. One-way ANOVA was applied followed by a pairwise multiple comparison using Bonferroni test. In treatment (T7), of the incubation experiment, the number of replicates was only two due to the loss of one sample during the analysis, in this case, we used a t-test analysis

to evaluate the difference between ingestion of algae and MPs. Significant difference was determined at $P<0.05$.

Results

Global distribution of feeding-current feeding copepods

Our results show that feeding-current feeding copepods are commonly found across the global ocean, in particular in high and middle latitudes (Fig. 2a). In low latitudes, this feeding mode represent approximately the 40% of the copepods on average, while in some areas of high latitudes it reaches the 80% (Fig. 2b).

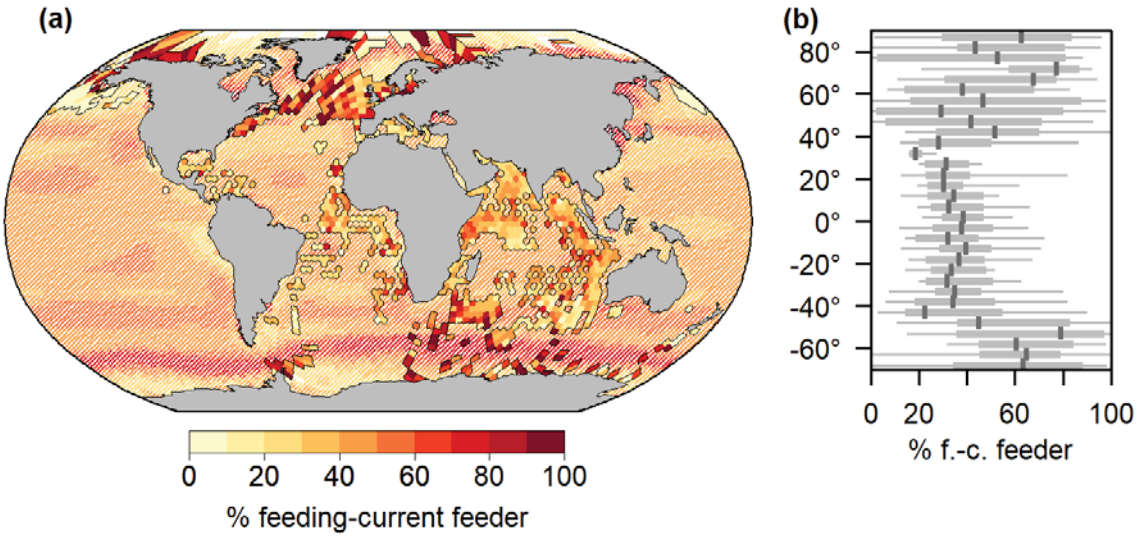


Figure 2. Global (a) and latitudinal (b) distribution of the fraction of feeding-current feeding copepods. Dashed areas represent model extrapolations and solid colors/latitudinal boxplots are observation-based estimates.

Video observations: capture rates, ingestion rates, and rejection percentages of algae and MPs

Video observations showed that the copepod *T. longicornis* did not differentiate between algae

and MPs before capture. Within each treatment, the capture rates for algae and MPs were not significantly different (P>0.05, Fig. 3a and 3b). In addition, there were no significant differences in capture rates on algae between treatments (P=0.167). Capture rates on algae ranged from 2014 to 4423 cells ind.$^{-1}$ d^{-1} (Fig. 3a), and capture rates on MPs ranged from 1445 to 5249 MPs ind.$^{-1}$ d^{-1} (Fig. 3b).

Overall, ingestion rates on algae or MPs did not significantly differ across all treatments (P=0.166 and 0.184, respectively). The average ingestion rate on algae was 2995 cells ind.$^{-1}$ d^{-1} (Fig. 3c), which was 5 times higher than on MPs (Fig. 3d) and similar to the capture rate of algae (Fig. 3a).

All examined copepods presented a significantly higher rejection rate of MPs than algal cells. The averaged percentage of rejected algae and MPs, considering all the treatments, was 0.5% ± 0.2% and 78.3% ± 3.2%, respectively (Fig. 3 e-i). Generally, an algal cell or a MP particle was captured and then handled by the copepod for approximately 120 ms before it was tasted. Afterwards, most of the algal cells and a few MP particles were ingested, whilst the majority of MPs were spit out after being tasted for an average of approximately 500 ms.

We did not find significant differences in the rejection percentage of MPs between polymer types (P=0.304). The average percent of rejection of virgin PS and PE microspheres was 71.9% ± 11.3% and 82.6% ± 4.4%, respectively (Fig. 3e). Similarly, the shape of MPs did not affect the percentage of rejection (P=0.964). Compared to spherical PS, irregular PS was only 0.5% more rejected by *T. longicornis* on average (Fig. 3f). The attachment of a biofilm and the sorption of pyrene did not change the proportion of rejected MPs either. 82.5% ± 10.2% of bio-fouled PE microspheres were rejected by *T. longicornis*, which was very close to the ratio of clean PE

microspheres (Fig. 3g). PE microspheres with pyrene, and to a lower degree PS with pyrene, appeared to be rejected less by *T. longicornis* than their control treatments. However, the differences were found not to be statistically significant (Fig. 3h and 3i).

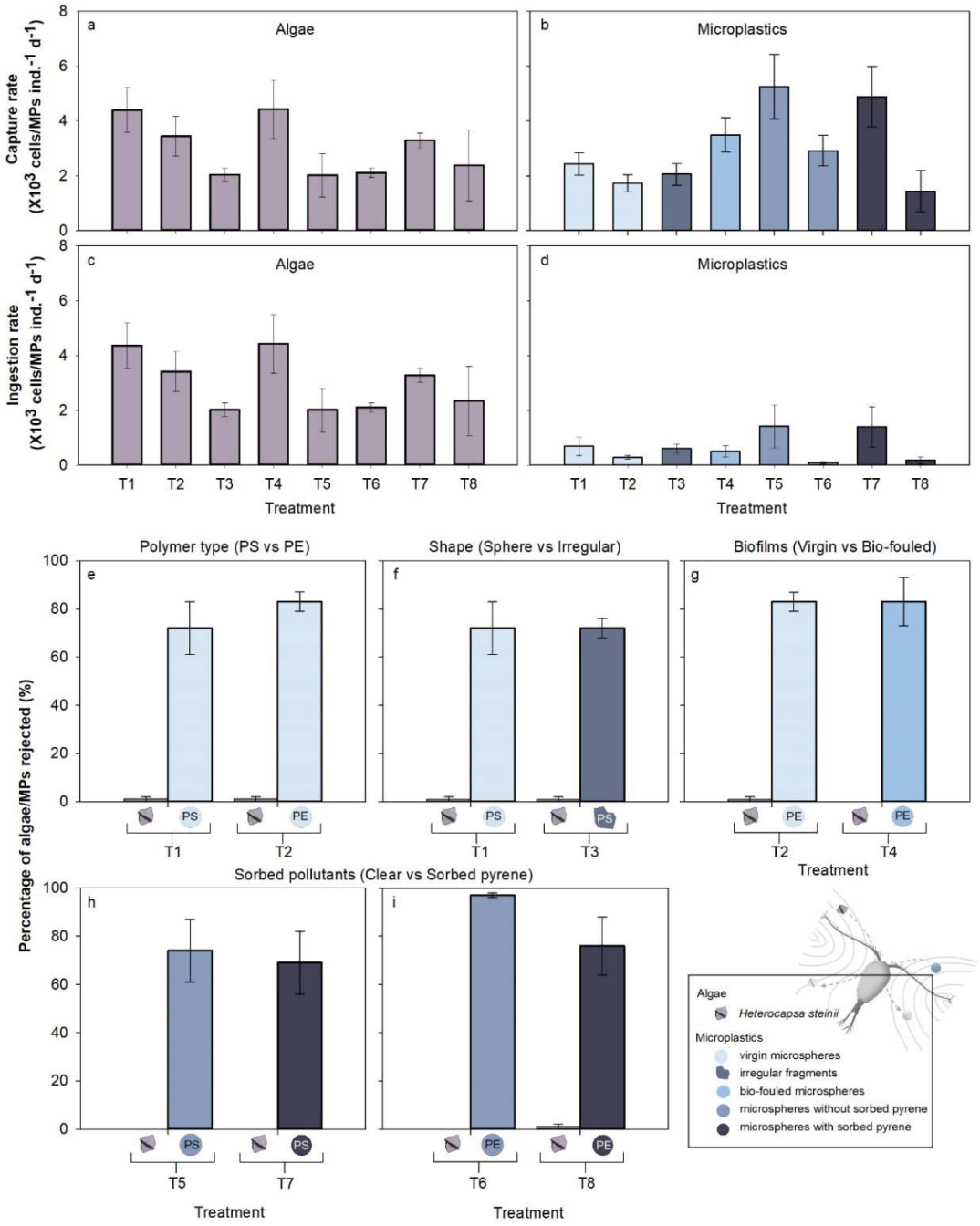


Figure 3. Feeding behaviors of *T. longicornis* on algae and MPs in the different treatments recorded by video camera. Capture rates of *T. longicornis* on *H. steinii* (a) and MPs (b). Ingestion rates of *T. longicornis* on *H. steinii* (c) and MPs (d). Percentage of *H. steinii* and MPs

that were rejected by *T. longicornis* when supplied simultaneously (e-i). Comparison between treatments added with (e) virgin PS and PE microspheres, (f) spherical and irregular PS, (g) virgin and bio-fouled PE microspheres, and (h-i) clean and pyrene-polluted PS/PE microspheres. Error bars show standard errors (n=3). Note that algae and MPs were offered together in each treatment.

Bottle incubations: ingestion and selection of algae and MPs

The daily ingestion and clearance rates of *T. longicornis* on algae and MPs were also calculated from 24-hour bottle incubations (Fig. 4a, 4b and Table S2). Ingestion of MPs only occurred in 7 of 24 bottles. Besides, in all the treatments the ingestion of algae was significantly different from the ingestion of MP. Ingestion rates of algae (Fig. 4a) were 1.4-12.6 times higher than of MPs (Fig. 4b). In general, *T. longicornis* presented a distinct preference for algae and largely avoided eating MPs when exposed to alga-MP mixtures. Overall, no significant differences occurred between algae ingestion rates among all treatments with the exception of the treatment with bio-fouled MPs (T4). The low ingestion rate on algae in T4 leads to a non-significant difference between MP and algae ingestion in that treatment. The electivity index (E) of algae varied from 0.21 to 0.33 among treatments, with positive E values indicating selection. By contrast, the electivity index of MPs varied from -0.49 to -1.00, with negative E values indicating avoidance (Fig. 4c).

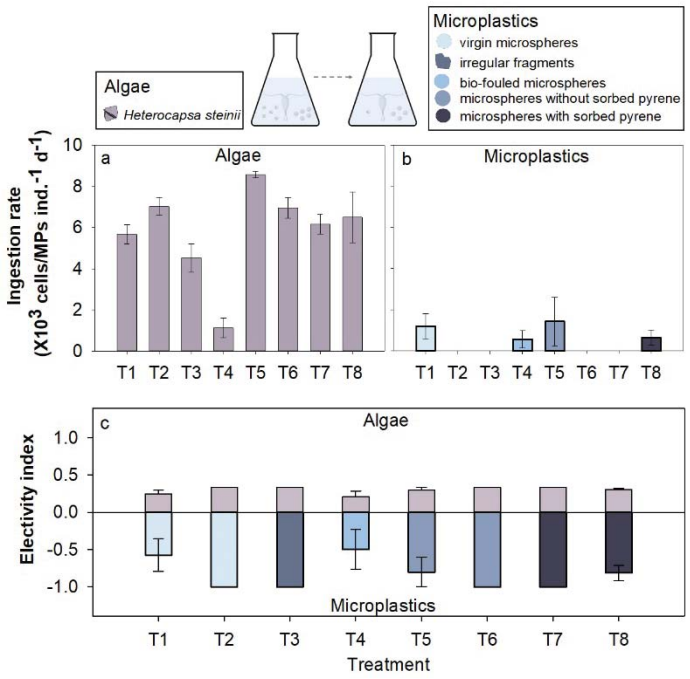


Figure 4. Feeding behaviors of *T. longicornis* on algae and MPs in the different treatments recorded from bottle incubations. Ingestion rates of *T. longicornis* on *H. steinii* (a) and MPs (b). Electivity index (c) of *T. longicornis* among mixtures of algae (top bars) and MPs (bottom bars). Error bars show standard errors (n=3). Note that algae and MPs were offered together in each treatment.

Discussion

Behavior and feeding rates of *T. longicornis* exposed to algae and MPs

Current knowledge about the ingestion and effects of MPs on copepods is largely based on bottle incubations [10,15,16,41,42]. Most studies use ingestion rates as the main parameter to describe MP consumption and selection by copepods, but this is not sufficient to reveal the underlying mechanisms. On one hand, promoting ingestion of MPs, copepods could either selectively graze on plastic particles or indiscriminately ingest the particles with the natural prey. On the other hand, ingestion of MPs could be impeded by rejection of MPs by copepods, a

reduction in feeding rates due to physical impacts/interference of MPs or by chemical toxicity associated with plastics (leachates or sorbed pollutants) [43]. During a 'black box' bottle incubation, the different mechanisms or processes might lead to similar overall ingestion rates. However, the detection, handling and rejection rates of MPs need to be evaluated to understand the impact on the grazer. Here we did not observe any behavioral abnormalities when *T. longicornis* was exposed to MPs with a similar sized microalga. We conclude that the lower ingestion rates of MPs compared to similar sized natural prey was due to an active selective behavior of the planktonic copepods.

Copepods have been shown to possess diverse sensors on their antennae, feeding appendages, or body surfaces for detecting either hydro-mechanical or chemical signals created by their prey [44]. The capacity of copepods for remote chemoreception is controversial and discussed in literatures [45–48]. According to the calculation by Tiselius [48], distant detection was only feasible for prey that is unusually large and leaking chemicals to the environment, whilst it was more common to observe nearby or touch detection of prey cells within a radius of around 10-50 µm. In the present study, the feeding response (i.e. ingestion or rejection) of *T. longicornis* occurred only after capturing the alga or MP particle. This demonstrates a nearby- or touch detection, which is similar to observations from previous investigations [23,27]. In addition, the similar encounter and capture rates of algae and MPs (Fig. 3a and 3b) suggest that *T. longicornis* does not carry out any pre-capture selection between algae and MPs. Thus, all 20 µm MPs and algae were equally perceived and captured when they were very close to the antennae or feeding appendages of *T. longicornis*.

The evaluation and selection of prey by the copepods occurred post-capture when prey touched

the setae on the feeding appendages and in the mouth. The duration of the subsequent handling time is mainly caused by the position of the prey particle when it is first captured [49]. In our study, the duration was variable but it did not show any statistically significant difference between prey types. The handled particle was pushed into the mouth, tasted and either ingested immediately by *T. longicornis* or spat out. In many cases, MPs were handled, tasted, and spat out several times until finally being pushed away by *T. longicornis.* Tasting was therefore the main mechanism used by *T. longicornis* to discriminate MPs from normal prey.

Results further showed that algal ingestion by the copepod was not impeded by the presence of MPs at the studied concentrations (≈ 200 MPs mL^{-1}). According to Dam and Lopes [50], when *T. longicornis* was given dinoflagellate *H. steinii* as the sole food, the ingestion rate increased linearly with the algal concentration. An ingestion rate of 4320 cells $ind.^{-1}$ d^{-1} was estimated, with an initial algal concentration of 200 cells mL^{-1}, which is very close to the rates measured in this study (Fig. 3c and 4a). Early studies have similarly demonstrated that algal ingestion by other copepods, for example *Acartia clausi* and *Calanus pacificus*, were not affected by the presence of virgin plastic microspheres [51,52]. Similarly, fecal pellet production rates of arctic copepods, which are directly related to ingestion rates, were not affected by the presence of virgin MPs at a concentration of 20 MPs mL^{-1} [16].

Effects of shape and polymer type on MP ingestion

The shape of MPs is one of the characteristics that may regulate copepods' selective ingestion of MPs. Botterell [11] further hypothesized that different feeding strategies of copepods might lead to different preferences for MP shapes. According to their experiments, feeding-current feeders ingested more fragments than fibers, suggesting differences in selectivity depending on MP

shape. In the present study, we hypothesized that copepods will reject microspheres to a smaller extent than irregular fragments due to their similar shape to the natural prey. However, the ingestion rates of the feeding-current feeder *T. longicornis* on different shapes of MPs showed no significant difference: upon capture, spherical and irregular PS particles were rejected in the same proportion (≈ 70%). Meanwhile, the algae offered together with MPs were rarely rejected (≈ 1%).

The plastic polymer type is another factor that may affect the ingestion of MPs by copepods [18]. Polymers differ in several physical and chemical characteristics, like hardness and density [53]. We measured the selection of two polymer types (PS vs PE) in this study, and found no significant difference between ingestion rates on virgin PS and PE microspheres. The similar high percentage of rejected MPs by copepods (Fig. 3e) suggests that copepods select similarly strictly against the different polymer types corroborating our hypothesis.

The high rejection rate of all types of MPs in our experiments indicates that shape and polymer type may not be crucial factors for *T. longicornis* to selectively reject a specific MP particle. The reasons for the high rejections may be that copepods dislike the chemical composition of virgin plastic or that virgin MPs lack the organic signals, which help the copepods to recognize the particles as food. However, more studies on other MP physical characteristics (colors, additional shapes, etc.) could be very relevant to give a better overview of the effects of different MP types on zooplankton.

Effects of weathering on MP ingestion

Weathered MPs are more bioavailable for marine organisms and potentially harmful for aquatic ecosystems due to their biofilm or absorbed pollutants [22,54–56]. When a primary MP enters the

aquatic environment, bacteria quickly colonize the surface, and within the subsequent weeks, the dominant bacterial species could entirely change and create a new biofilm community depending on environmental conditions [57]. The organisms growing on the plastic surface, releasing metabolic products, that can make MPs smell and taste more like food particles [58]. For example, it was observed that MPs with biofilm were preferred by some copepod species over virgin MPs [22]. Another study showed that MPs infused with dimethyl sulfide or dimethylsulfoniopropionate (DMS, DMSP), compounds that are naturally synthesized by marine phytoplankton, were ingested to a larger extent by *Calanus helgolandicus* and *Acartia tonsa* compared to clean MPs [11]. Therefore, we hypothesized that bio-fouled MPs would be ingested to a higher degree than clean ('virgin') plastic particles in our experiment. We observed, however, that only a few bio-fouled MPs were ingested by *T. longicornis*. Similar to virgin MPs, large proportions of bio-fouled MPs were rejected by the copepods after tasting, which implies that they can easily be recognized through a post-capture selection by copepods. Possibly the biofilms on our MPs had an organic signal that copepods cannot detect or biofilms were not thick enough to completely cover the polymer signal. The ingestion and impact of bio-fouled MPs is of high interest for example due to their role as available surface for invasive species or for antibiotic resistant bacteria [59,60]. Therefore, further studies are needed to evaluate the impact of this biofilm coated MPs.

Microplastics are potential vectors of harmful chemicals sorbed from the environment [61]. Since plastics were reported to absorb high concentrations of PAHs (polycyclic aromatic hydrocarbons) like pyrene [36,61] and copepods have the ability to avoid diesel oil in water [62], we hypothesized that *T. longicornis* also has the ability to avoid plastic particles contaminated with

pyrene. However, no significant difference was observed between capture rates of pyrene-contaminated MPs and virgin MPs. This indicates that either the concentration of signals from pyrene-contaminated MPs was not sufficient to stimulate the remote chemosensitivity of copepods, or the compounds associated with our pyrene-contaminated MPs are not perceivable to *T. longicornis*.

Theoretically, aging of MPs (biofilm formation and sorption of chemicals) could either promote or impede MP ingestion by copepods. However, based on the high rejection rates of all types of MPs observed in this study, the influence of MP aging appears limited for planktonic copepods with an efficient tasting-discrimination technique, like *T. longicornis*.

Ecological implications

Feeding behavior is a key trait to understand the entry of MPs into marine food webs. Zooplankton, having an important trophic role in connecting primary producers and higher trophic levels, are considered one of the main vectors for small MPs into marine food webs [63]. However, there is so far little evidence to support this hypothesis [64,65]. Approximately 11.500 copepod species are known worldwide [66] and they can be grouped into three main feeding modes: ambush feeders, cruising feeders and feeding-current feeders. Ambush feeders need a physical disturbance in the surrounding water to detect their prey. Cruising feeders swim and feed on the particles they encounter on their way. Feeding-current feeders, like *T. longicornis,* create a feeding current to draw and scan prey within their current. Active feeders (feeding-current feeders and cruising feeders) are one order of magnitude more efficient than ambush feeders at getting non-motile prey (e.g., diatoms) [25,26]. Since MPs are non-motile and captured in the feeding current at the same rate as motile prey, feeding-current generating copepods are

more susceptible to encounter and ingest MPs than ambush feeding copepods. Therefore, feeding current feeders play a particularly important role in enable MPs to enter into marine food webs. More importantly, feeding-current feeding copepods represent a dominant abundance in many oceanic areas (Fig. 2). Hence, foraging behavior of zooplankton is the key trait to understand the entry of MPs into marine food webs.

Ingestion of MPs by planktonic copepods in the natural environment is expected to be much lower than predicted from laboratory experiments.

Although, data from laboratory experiments have shown a high degree of MP ingestion by copepods [14,64], the concentrations used were 4-5 orders of magnitude higher (10-1000 MPs mL^{-1}) than what is currently observed in marine surface waters (0.0001-0.01 MPs mL^{-1}) [67–71]. Consequently, the chance of encountering and capturing a MP particle by a copepod in the natural environment is much lower than suggested by those studies. As discussed above, we showed that copepods like *T. longicornis* can detect the plastics, evaluate their edibility and actively reject 80% of all captured MPs. High ingestions, reported in laboratory studies with high MP concentrations, could be due to the percentage that the copepods still mistake for food after handling and selection (20% in this study). Therefore, especially when exposed to low concentrations, the risk of MP ingestion by feeding-current feeding copepods appears to be minor. However, it is important to note that the entrance of MPs into marine food webs can still happen through organisms that do not have the ability to discriminate between natural prey and MPs or that use other mechanisms, e.g. visual detection, to select their prey (e.g. fish larvae) [13,72].

Can zooplankton ingestion of MPs disrupt the biological carbon pump?

The biological carbon (C) pump is the mechanism by which inorganic carbon fixed through photosynthesis is exported out of the surface layer via biological processes. The biological C pump is crucial for the sequestration of CO_2 and climate regulation [73]. Planktonic copepods are keystone components of the biological C pump by ingestion of primary production, export of particulate organic matter via fecal pellets and carcasses production, vertical migrations and respiration during hibernation [74,75] (Fig. 5). The adverse biological effects of MPs, shown in laboratory studies, have raised concerns about the impact of MP pollution on the C cycle [76].

Kvale [7] predicts that a physical effect of MP pollution via zooplankton negatively affect the biological C pump and consequently the global ocean oxygenation. Kvale´s model assumes that the ingestion and selection of MPs by zooplankton is only driven by the ratio of MPs to natural food. However, this is not the case for planktonic copepods, where foraging behavior and prey selection capability of copepods are key aspects that determine the ingestion of MPs as demonstrated here. Due to the capture mechanisms (ambush feeder) and taste-discrimination (feeding current feeders) of copepods, the ingestion of MPs is expected to be low and therefore also their impacts on planktonic copepods. Grazing of zooplankton is not negatively affected by ingestion of virgin MPs at *in situ* concentrations of MPs (0.0001-0.01 MPs mL^{-1}) [14,77,78]. In our studies, the ingestion rates of copepods on algae were not affected by the presence of MPs and were similar to those in the absence of MPs [79]. Production and sinking rates of fecal pellets are also key processes in the biological C pump. Assuming that the presence of MPs inside the fecal pellets increases their buoyancy, the pellets would be recycled in the water column reducing the C sequestration in the bottom waters. However, similar to grazing rates, fecal pellet production and sinking rates are not expected to be affected by

ingestion of MPs under natural conditions [16]. Therefore, it is unlikely that physical impacts of MPs can disrupt the role planktonic copepods play in the global biological carbon cycle.

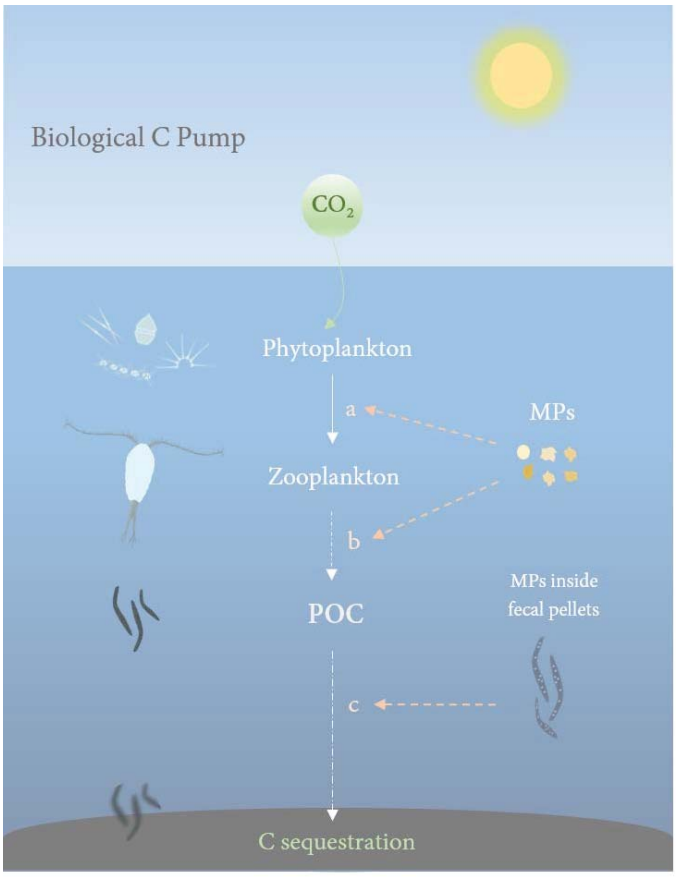


Figure 5. Simplified scheme of the key role of zooplankton in the “biological carbon (C) pump” and the potential impacts of microplastics (MPs) on the transfer and exportation of carbon: decreased zooplankton grazing (a), reduced fecal pellet production (b), and lower sinking velocity of fecal pellets containing ingested MPs (c). POC (Particulate Organic Carbon).

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microplastic pollution in marine ecosystems) supported by the Innovation Fund Denmark (IFD, 9087-00006B), a research grant from the 2019 Maritime DTU-Orients Fund to RA, a grant from the Spanish Ministry of Science and Innovation through the Ramón y Cajal Program to RA (RYC2018-025770-I). This study was furthermore supported by the H2020 CLAIM project (Cleaning Litter by developing and Applying Innovative Methods in European seas; Grant Agreement No. 774586) to TGN. Thanks to Mikael Emil Olsson for chemical analysis and to Hans Van Someren Gréve for the copepod vector graphic.

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Supporting Information

Table S1. List of actual MP and algae concentrations used in both incubation and filming experiments, based on counts in the initial bottles. Concentrations were measured in triplicate. PE: polyethylene; PS: polystyrene; SE: standard error.

Treatments	Microplastics		Algae
	Type	Concentration ± SE (MPs mL^{-1})	Concentration ± SE (cells mL^{-1})
T1	virgin PS microspheres	214 ± 18	197 ± 11
T2	virgin PE microspheres	322 ± 22	200 ± 8
T3	irregular PS fragments	232 ± 12	195 ± 5
T4	Bio-fouled PE microspheres	242 ± 20	188 ± 9
T5	PS microspheres without sorbed pyrene	214 ± 18	197 ± 11
T6	PE microspheres without sorbed pyrene	312 ± 15	188 ± 6
T7	PS microspheres with sorbed pyrene	388 ± 2	225 ± 9
T8	PE microspheres with sorbed pyrene	305 ± 18	223 ± 13

Table S2. Bottle incubation data. Ingestion (I, cells/MPs $cop.^{-1}$ d^{-1}) and clearance (F, mL $cop.^{-1}$ d^{-1}) rates with their standard error (SE) for the eight treatments.

Treatments	I_algae ± SE	F_algae ± SE	I_MPs ± SE	F_MPs ± SE
T1	5687.7 ± 466.0	27.6 ± 2.8	1211.5 ± 612.7	6.2 ± 3.2
T2	7039.0 ± 431.7	35.7 ± 2.9	0	0
T3	4531.4 ± 694.1	23.3 ± 4.3	0	0
T4	1132.9 ± 463.4	6.2 ± 2.7	577.9 ± 422.6	3.2 ± 2.4
T5	8578.2 ± 164.2	53.3 ± 0.2	1433.2 ± 1170.2	4.8 ± 3.9
T6	6963.6 ± 509.3	46.6 ± 0.8	0	0
T7	6172.8 ± 473.4	31.3 ± 2.7	0	0
T8	6503.3 ± 1235.4	32.5 ± 7.2	646.7 ± 370.4	2.5 ± 1.4

Paper V

Zooplankton behavior minimizes the entry of microplastics in planktonic food webs

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This paper is in the last stage of preparation to be submitted

Zooplankton behavior minimizes the entry of microplastics in planktonic food webs

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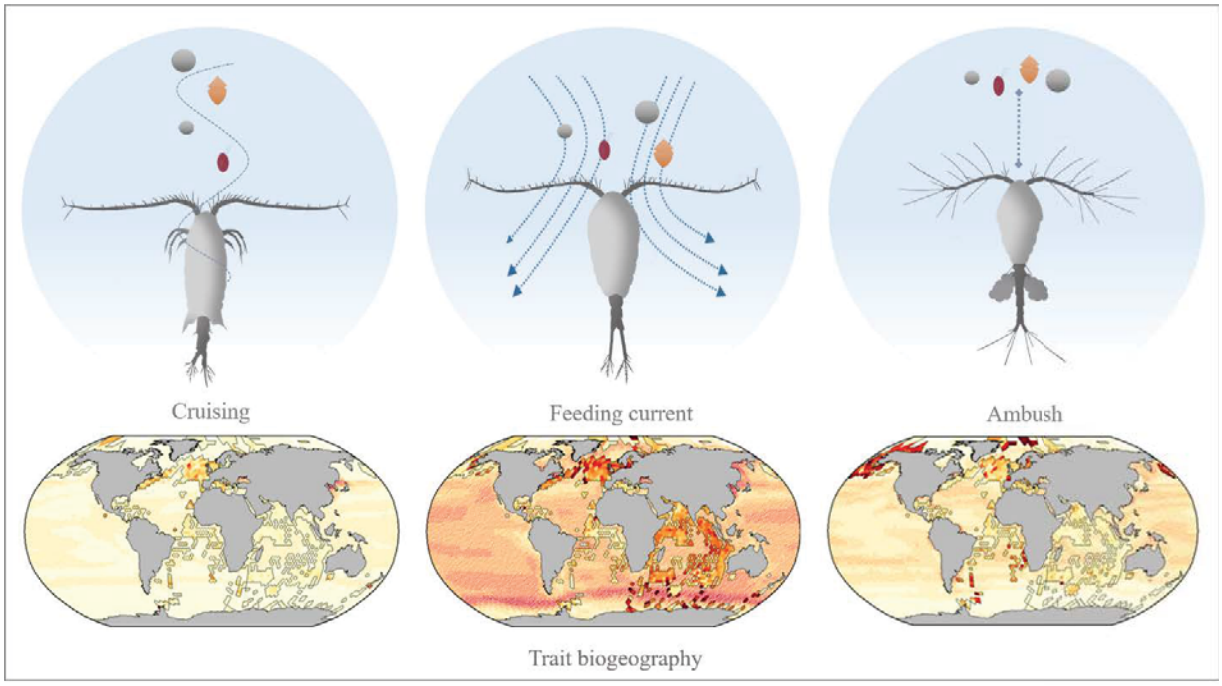
ABSTRACT

The entry of microplastics (MPs) into marine food webs is a major environmental concern. We investigated how planktonic copepods behavior influences the risk of MPs to enter marine food webs, by applying a trait-based approach and by combining experiments with biogeographical analyses. We aim to evaluate which type of feeding behavior is most risky in terms of MP ingestion, and which marine geographical areas are more susceptible to MP ingestion by planktonic copepods. We used different planktonic copepods as models of the main foraging behaviors in planktonic copepods: feeding-current, cruising, ambush and mixed behavior feeding. All the behaviors show a similar low risk of MP ingestion, up to one order of magnitude lower than for similar sized microalgae. We did not observe any influence of the prey type or MP size (8 µm and 20 µm) on MP ingestion for all the behaviors. By estimating the global distribution of feeding behaviors, we showed that feeding current feeding is the most common behavior, but the risk of MP ingestion remain similarly low across the global ocean, independently of the predominant behavior. Overall, our results suggest low risk of MP ingestion by planktonic copepods and therefore a minimal risk of MP trophic transfer via copepods or fecal pellets in marine ecosystems.

Key words: microplastics, copepods, trait-based approach, feeding behavior, ingestion.

Synopsis: We applied a trait-based approach to investigate the relevance of zooplankton feeding behavior in the entry of MPs in marine food webs.

GRAPHICAL ABSTRACT



1. INTRODUCTION

Microplastics (MPs) are now ubiquitous in the oceans (Cózar et al., 2014) and the amount of plastic litter in aquatic ecosystems is projected to triple by 2040 (UNEP, 2021). The consequences of plastic pollution on marine ecosystems are, therefore, of global environmental concern. MPs range in size between 1 μm and 5 mm (Frias and Nash, 2019) with the size fraction < 100 μm being the most abundant in marine waters (Rist et al., 2020). MPs < 100 μm are similar in size to phytoplankton, which makes them potentially available for ingestion by zooplankton (Cole et al., 2013; Botterell et al., 2019, 2020). Given the key trophic position of zooplankton in marine food webs (Banse 1995, Castonguay et al., 2008), knowledge of the risk of MP ingestion by zooplankton is essential to assess the fate of MPs in marine ecosystems.

Planktonic copepods are the dominant zooplankton group (Verity & Smetacek, 1996) and the most abundant animals in the ocean. Copepods are both grazers and prey; therefore, they may influence the entry and biotransfer of MPs in marine food webs. Additionally, copepods can contribute to vertical exportation of MPs via fecal pellets. Microplastic ingestion by planktonic copepods has been observed in laboratory experiments using high concentrations of MPs (Cole et al., 2013, Botterell et al., 2019, 2020; Rodríguez-Torres et al., 2020; Almeda et al., 2021). However, field studies indicate a low occurrence of MP ingestion in natural zooplankton communities (Zheng et al., 2020), which contradicts the high-risk assessment of MP ingestion predicted from laboratory studies. It is not feasible to study the interactions between MPs and all 11,000 copepod species. A trait-based approach on the other hand proposes to represent the thousands of species with few well-picked organisms that reflect the existing diversity in the most important traits. In the context of zooplankton ecology (Litchman et al., 2013), it is well documented that foraging behavior is a key trait due to its strong influence on feeding rates and predation risk (Kiørboe, 2011; Almeda et al., 2017; 2018). Additionally, functional traits such as feeding behavior in marine copepods vary across the global ocean as a function of the environment (Brun et al., 2016). A trait-based approach using feeding behavior as key trait is, therefore, a useful tool to evaluate the risk of ingestion and biotransfer of MPs via zooplankton on a global scale.

Suspension-feeding planktonic copepods have three main types of foraging behaviors: (1) “feeding-current feeding” where they create a current to harvest prey; (2) “cruising” where they encounter prey while swimming through the water, and (3) “ambush” where they wait motionless

for motile prey to enter their sensory reach, or otherwise capture those prey that directly collide with them (Tiselius and Jonsson 1990; Kiørboe 2011, 2016). The two first feeding behaviors are categorized as "active feeders". Some planktonic copepods can be "mixed behavior feeders", which are able to switch between behaviors depending on the prey type and food availability (Landry 1981; Tiselius and Jonsson, 1990; Kiørboe et al. 1996). For example, *Acartia tonsa* can either generate a feeding current to harvest small or non-motile prey or behave as an ambush feeder to capture large motile prey (Kiørboe 1996). Despite the importance of behavior to evaluate the risk of MP ingestion of zooplankton, behavior is still an understudied variable in MP pollution (Rodríguez-Torres et al., 2022, in preparation). For instance, it is unknown if ambush feeding copepods (e.g. *Oithona*) ingest MPs despite their abundance and wide distribution across the oceans (Galliene and Robins, 2001). Therefore, research about the influence of zooplankton behavior on MP ingestion is needed to better assess the risk of entrance and transfer of MPs in marine food webs.

The general aim of this study is to evaluate how planktonic copepods behavior influences the risk of MPs to enter marine plankton food webs. We used a trait-based approach and planktonic copepods as model organisms. Our specific objectives and hypotheses are to: (1) estimate the risk of MP ingestion associated with the three main feeding behaviors of suspension-feeding copepods: feeding-current feeding, cruising and ambushing. We hypothesize that ambushing is the least risky behavior in terms of MP ingestion since ambushers are inefficient in detecting non-motile prey (Almeda et al, 2018); (2) investigate the influence of prey switching behavior on MP ingestion in a mixed behavior feeder. We hypothesize that presence of a large motile prey will induce ambushing, which in turn, will reduce the ingestion of MPs in a mixed behavior feeder; (3) evaluate the global risk of ingestion of MPs in zooplankton by intersecting the outcome from the experiments with estimates of the global distribution of the feeding behaviors. We hypothesize that areas dominated by active feeders are more susceptible to the entry of MPs into food webs than areas dominated by ambush feeders.

2. MATERIAL AND METHODS

2.1. Experimental organisms

The trait of relevance in this study is foraging behavior, and thus the experimental organisms were selected in order to compare among contrasting foraging behaviors. The following species of copepods were used as models of the different foraging behaviors: *Temora longicornis* (feeding-current feeder); *Centropages hamatus* (cruising feeder), *Oithona davisae*, (ambush feeder) and *Acartia tonsa* (mixed behavior feeder). To avoid body size effects on the MP ingestion we ensured similar body size of the experimental organisms. To this end, we used copepodites I and II of *C. hamatus* and *T. longicornis* and females of *O. davisae* to ensure a similar body size between experimental organisms. To investigate the prey switching behavior we used adults of *A. tonsa*. In all the experiments, specimens were selected from the main cultures the day before the experiment and kept isolated overnight in glass beakers without food.

Copepods were obtained from stock cultures at the Technical University of Denmark (DTU AQUA). Cultures of *T. longicornis* and *C. hamatus* were established from specimens originally collected in Gullmarsfjorden (Sweden) and Øresund (Denmark); *O. davisae* was collected from the Mediterranean Sea (Barcelona harbor, Spain) and *A. tonsa* from the Øresund (Denmark). All copepod species are cultivated at DTU in dim light, at 18°C and a salinity of 30‰, and they are fed ad libitum three times per week. *T. longicornis* and *C. hamatus*, were fed a mixture of the diatom *Thalassiosira weissfloggi*, the flagellate *Rhodomonas salina* and two dinoflagellates, *Heterocapsa steinii* and *Oxyrrhis marina*. The copepod *O. davisae* was fed only with the dinoflagellate *O. marina* and *A. tonsa* fed on *R. salina*.

The phytoplankton prey used in this study were *H. steinii* and *R. salina*. We used these two species because their average size is 17 µm and 7 µm, respectively, which fit the size range of MP beads used in this experiment (20 µm and 8 µm MP beads). Both species were grown in pasteurized filtered seawater (FSW) with a salinity of 30‰ with B1 medium (Hansen, 1989) at 16°C in a 12:12 dark:light cycle with a radiation of 150 µmol photons m^{-2} s^{-1}.

2.2. *Microplastics*

Transparent polyethylene (PE) spherical beads with a density of 0.96 g cm^{-3} were used as MPs in this study. The spherical beads were supplied as a dry powder (Cospheric®). To prepare the MP

suspensions, a small amount of the powder was suspended in 250 mL glass bottles containing distilled water with 0.01 % Tween 80 (Cospheric®). We mixed the suspensions through vigorous hand shaking until the particles appeared dispersed and any clusters broke into individual beads, as verified through microscopy. The size of both algae and MPs was measured using a Beckman Multisizer III Coulter Counter. Two size ranges of PE particles were used: the larger particles had a size range of 13.9–30.3 μm, with a mean ESD of 20.7 μm and the size of the smaller particles ranged from 5–16 μm, and the mean ESD was 7.9 μm. From the stock suspension, we prepared the following diluted working suspensions for each MP size: 20 μm MPs = 52080 MPs mL^{-1}, 8 μm MPs = 57400 MPs mL^{-1}. The absence of MP aggregates and the concentration of MPs in the working solutions was verified by manual counting under an inverted microscope using Sedgewick-Rafter counting chambers.

2.3. *General experimental set-up*

To estimate the influence of foraging behaviors on the ingestion of MPs (objective 1), we conducted bottle incubation experiments with model species of each feeding behavior offering together MPs and a similar sized microalgae. We evaluated the ingestion of MPs associated with different behaviors using two different MPs and prey sizes: 1) MPs of 20 μm and similar sized microalgae *(H. stineii*) and 2) MPs of 8 μm and a similar sized microalgae (*R. salina*) (Fig. 1).

To assess the influence of prey switching behavior on MP ingestion in a mixed behavior feeder (objective 2), we exposed *A. tonsa* to microalgae alone (T1 and T2), MPs alone (T3 and T4) , and in combination with microalgae of different size/motility: large MPs and large prey (T5), small MPs and large prey (T6), large MPs and small prey (T7) and small MPs and small prey (T8) (Fig. 1). For each treatment, we determined motile behavioral responses using video observations and ingestion rates by bottle incubations. We used *H. stineii* as large motile prey and *R. salina* as small low motile prey.

2.4. Bottle incubation experiments and sample analysis

The bottle incubation experiments were carried out in 34 mL Pyrex bottles sealed with a lid protected with polytetrafluoroethylene (PTFE). The bottles were half filled with 0.2 μm FSW. Aliquots of the MP working suspensions were added to obtain the desired test concentrations.

Afterwards, the corresponding algae were added. The algae stocks were previously counted under the microscope using a Sedgewick-Rafter counting chamber in order to calculate the stock suspension volume needed to obtain the desired concentration in the incubation bottles. The nominal concentration of microalgae and MPs in the incubations where both types of particles were offered together was 200 MPs mL^{-1} and 200 cells mL^{-1}. In the prey switching behavior experiment where *A. tonsa* was also exposed to single particles (T1-T4), the nominal concentration was 400 MPs or cells mL^{-1} to keep the same amount of total particles in all treatments. For each treatment, we prepared triplicates of "Initial bottles", where we determined the concentration of algae and MPs at time=0; "Control bottles" that were incubated with no grazers to follow changes in prey concentrations during the 24 hours and "Experimental bottles" that were incubated with the copepods to estimate the ingestion rates after 24 h. Due to the differences in clearance rates among species and between prey types, the number of copepods per bottle was adjusted to ensure a recommended decrease in prey concentration of 30% (Almeda et al. 2019): 12 and 18 ind. $bottle^{-1}$ when *O. davisae* was exposed to 20 µm prey and 8 µm prey respectively; 8 ind. $bottle^{-1}$ for *A. tonsa*, and 4 ind. $bottle^{-1}$ for *C. hamatus and T. longicornis*. Lastly, after adding the copepods to the bottles, we filled the bottles with the test particle suspensions and closed them. The bottles were wrapped in aluminum foil to keep them dark and then mounted on a plankton wheel rotating at 1 rpm in a temperature-controlled room at 16°C for approximately 24 hours. After the incubation, copepod mortality was checked with a microscope and a 25 mL subsample was taken from each bottle. Mortality was not observed in any of the experiments. Particle suspension subsamples (25 mL) were fixed with 1% Lugol´s solution and stored at 4°C for later analysis. The concentration of MPs and algae in the tested particle suspensions were counted under an inverted microscope (x20 magnification) using Sedgewick-Rafter chambers. We calculated ingestion (I) and clearance rates (F) on MPs and algae according to Frost (1972).

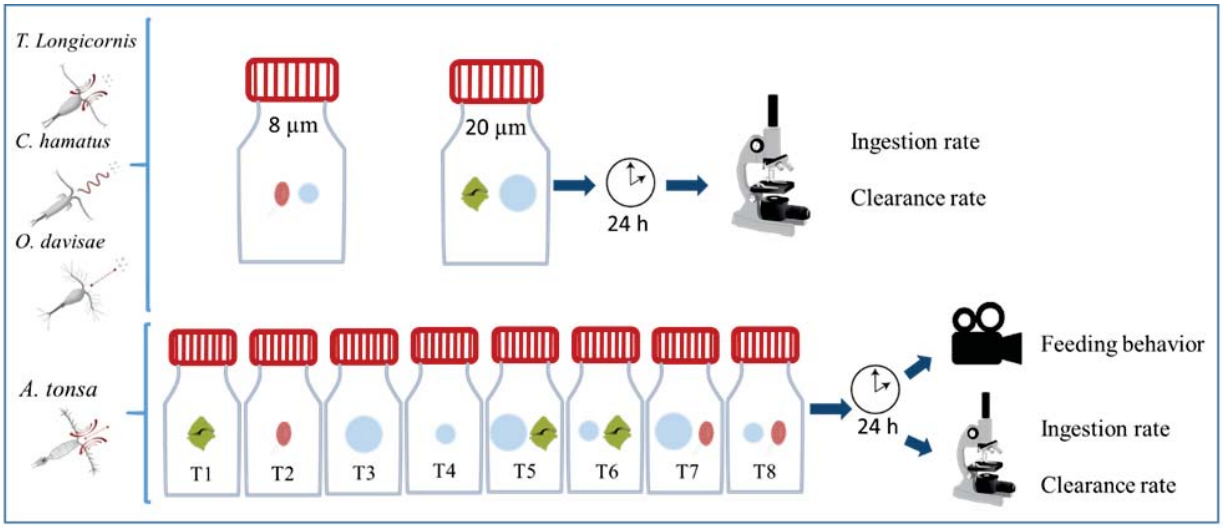


Figure 1. Schematic overview of the experimental studies. The upper bottles represent the bottle incubation experiments of the three main feeding behaviors when exposed to *R. salina* (red alga) and MPs of 8 µm or *H. steinii* (green algae) and MPs of 20 µm. The lower row indicates the incubation and video filming treatments used in the experiment with a mixed behavior feeder, *A. tonsa*, exposed to different combinations of microalgae and MP sizes.

2.5. Video observations set-up and behavior analysis

The video observations were conducted in a temperature-controlled room at 16°C. The different combinations of algae and MPs (Fig. 1) were prepared in 50 mL cell culture flasks with eight female adults of *A. tonsa* per flask. The flask was placed between an infrared light and a high-speed camera (Phantom V210). The camera was set at a frame rate of 100 fps, a resolution of 800×600 pixels, and a field of view of 40×30 mm^2. Each flask was recorded for three hours and one 60-second video was saved every hour. The flask was mixed every half an hour to minimize particle settlement. Each treatment was conducted in triplicate. The filming was done in a closed room in darkness to avoid any light and temperature change or noise that could affect the behavior of the copepods.

Copepods' trajectories were extracted from videos using a tracking plugin in ImageJ (version 1.52n) and behaviors were further analyzed by running an R-script in R Studio (version 1.3.959). The parameters in the script were set based on previous manual frame-by-frame analysis to match the threshold of each type of behavior (Kiørboe et al., 2018). Between 61 and 119 tracks were

analyzed per treatment and four different types of behaviors were categorized: swim, sink, jump and hop. “Swim” is the movement made by copepods when actively swimming; “sink” refers to when passive moves downwards in the water column; “jump” are active, big and rapid moves usually to capture prey, and “hop” are active, short jumps for the copepod to relocate back to its original position. The hop-like motion was distinguished from jump by its slower velocity. Hop is considered a movement that enables copepods to adjust their body orientation or switch their position in a short range rather than to escape from threats or quickly scan the water column like with the jumps (Henriksen et al., 2007; Hong et al., 2012; Jiang and Paffenhöfer, 2004). The time budget (fraction of time of swim, sink, jump or hop), the duration of each motion bout, the frequency and the velocity of these motions were calculated for all treatments.

2. 7. Statistical anaylisis for experimental data

Statistical analyses were performed using the free statistical computing software R (version 3.6.3) and IBM-SPSS v25SPS. The assumptions of normality of residuals and homogeneity of variances were tested with the Shapiro-Wilks-W-Test and Fligner-Killeen test, respectively. When normality and homogeneity assumptions were valid, differences between three or more groups were analyzed with a one-way analysis of variances (ANOVA, $p < 0.05$) followed by a Tukey’s HSD post hoc. Differences between two groups were analyzed with t-tests. When normality and homogeneity of variance assumption were violated, non-parametric Kruskal-Wallis tests was performed.

2.6. *Spatial modelling of the dominance of the three main feeding behaviors.*

We estimated the weight fractions of feeding current feeders, cruising feeders, and ambush feeders from global compilations of abundance and body length data, using data-based estimates where available and model-based interpolations elsewhere. The data was prepared following the approach described in Brun et al. (2016). We first discretized the global ocean into roughly 5000 polygons of similar area. Then, we intersected these polygons with abundance observations from the Coastal and Oceanic Plankton Ecology, Production and Observation Database (COPEPOD, O’Brien 2010). After preprocessing and filtering of the raw data (see Brun et al. 2016), we estimated abundance and weight fractions of each taxon in each polygon, multiplying abundance fractions with cubed body length to obtain weight fractions. Body-length data originated from Brun et al. (2017). Then, we identified which taxa in a polygon belonged to each feeding behavior

by considering the world's most abundant representative genera as a reference. Feeding-current feeders were represented by: *Acartia*, *Calanus*, *Calocalanus*, *Euchaeta, Nannocalanus*, *Paracalanus*, *Pareucalanus*, *Parvocalanus*, *Pleuromamma*, *Pseudocalanus*, *Rhincalanus*, and *Temora*. Cruising feeders were assumed to include *Centropages, Clausocalanus*, and *Metridia*, and ambush feeders were represented by *Acartia* and *Oithona*. Note that due to its mixed feeding strategy, the genus *Acartia* was assigned to both, feeding current and ambush feeders. Finally, we calculated both abundance fraction and weight fraction of each feeding behavior relative to all remaining planktonic copepods in each polygon. In total, abundance estimates were available for 584 taxa (species and genera), and 564 of them could be matched with body length information. Feeding current feeders were represented with 103 taxa, cruising feeders with 42 taxa, and ambush feeders with 32 taxa.

We used statistical modelling to interpolate observation-based estimates to regions of the global ocean that lacked observations. We fitted six generalized additive models (Hastie et al. 1990), one for each combination of abundance/weight fraction and feeding behavior as response. As predictors we considered smooth terms of polygon-wise averages of annual mean and annual range of monthly sea surface temperature (derived from the HadISST1 product, Cowles and Strickier, 1983), as well as average chlorophyll *a* concentration (derived from http://www.globcolour.info/). We used the R package mgcv (Wood, 2011) to fit generalized additive models and assumed errors to follow a beta distribution.

3. RESULTS

3.1. *Ingestion of MPs in relation to feeding behavior*

The incubation experiments comparing the three main feeding behaviors showed that ingestion (I, particles cop^{-1} d^{-1}) and clearance rates (F, mL cop^{-1} d^{-1}) of algae was one order of magnitude higher than for MPs in all treatments, independently of particle size (Fig. 2). Active feeders (cruising and current-feeding feeders) had higher ingestion and clearance rates of algae than ambush feeder. However, there was no difference among the three species regarding the ingestion of MPs. All of them showed low ingestion and clearance rates of MP particles (on average, 8 µm: 32.4 MPs cop^{-1} d^{-1} and 20 µm: 39.2 MPs cop^{-1} d^{-1}) compared to similar sized microalgae (S.I. Table 1).

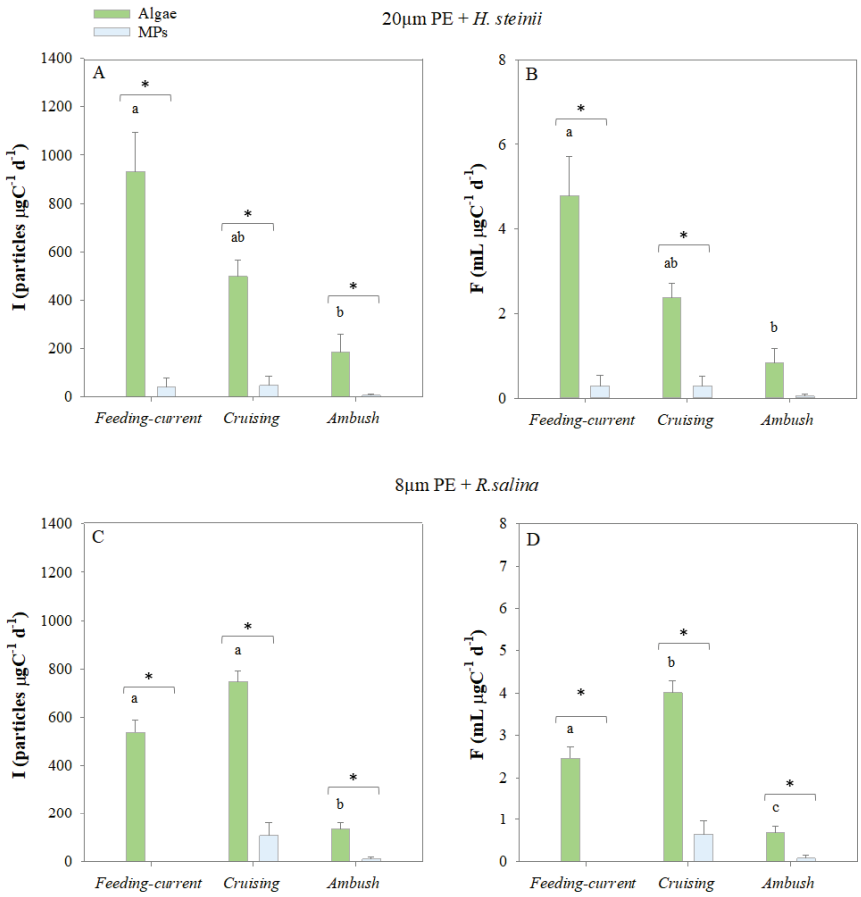


Figure 2. Ingestion (I, particles µgC^{-1} d^{-1}) and clearance rates (F, mL µgC^{-1} d^{-1}) of algae (green bars) and MPs (blue bars) for the three main feeding behaviors of copepods. Top panels (A, B) show the data for particles and algae of 20 µm diameter and the bottom panels (C, D) show the data for 8 µm particles and algae. Data is presented as means of triplicates with the standard error. Asterisks (*) represent a statistical significant difference between algae and MP ingestion or clearance rate within each behavior. Letters indicate significance differences among behaviors.

3.2. Influence of prey type on behavior and MP ingestion in the a mixed behavior feeder

Behavioral responses from video observations

The accumulated fraction (0-1) of the duration of the four behavior responses clearly reflects the dominance of sinking or swimming behavior in all treatments (Fig. 3 A and B). The accumulated fraction of swimming was higher in treatment 1, 5 and 6 than in the other treatments. Those are

the treatments in which *H. steinii* was present and the ones that had higher ingestion and clearance rates (Fig. 4). The accumulated duration of the jump was equally distributed in all treatments without significant differences.

Sink and swim were the most frequent movements (Fig. 3 C-F). There were no significant differences among treatments for jump frequency. However, for sink, swim and hop we found significant differences between several treatments (S.I. Table 2). The frequency of sink, swim and hop were always higher for the treatments with *H. steinii*. Sink, swim and hop frequencies never differed significantly in treatments with *R. salina*.

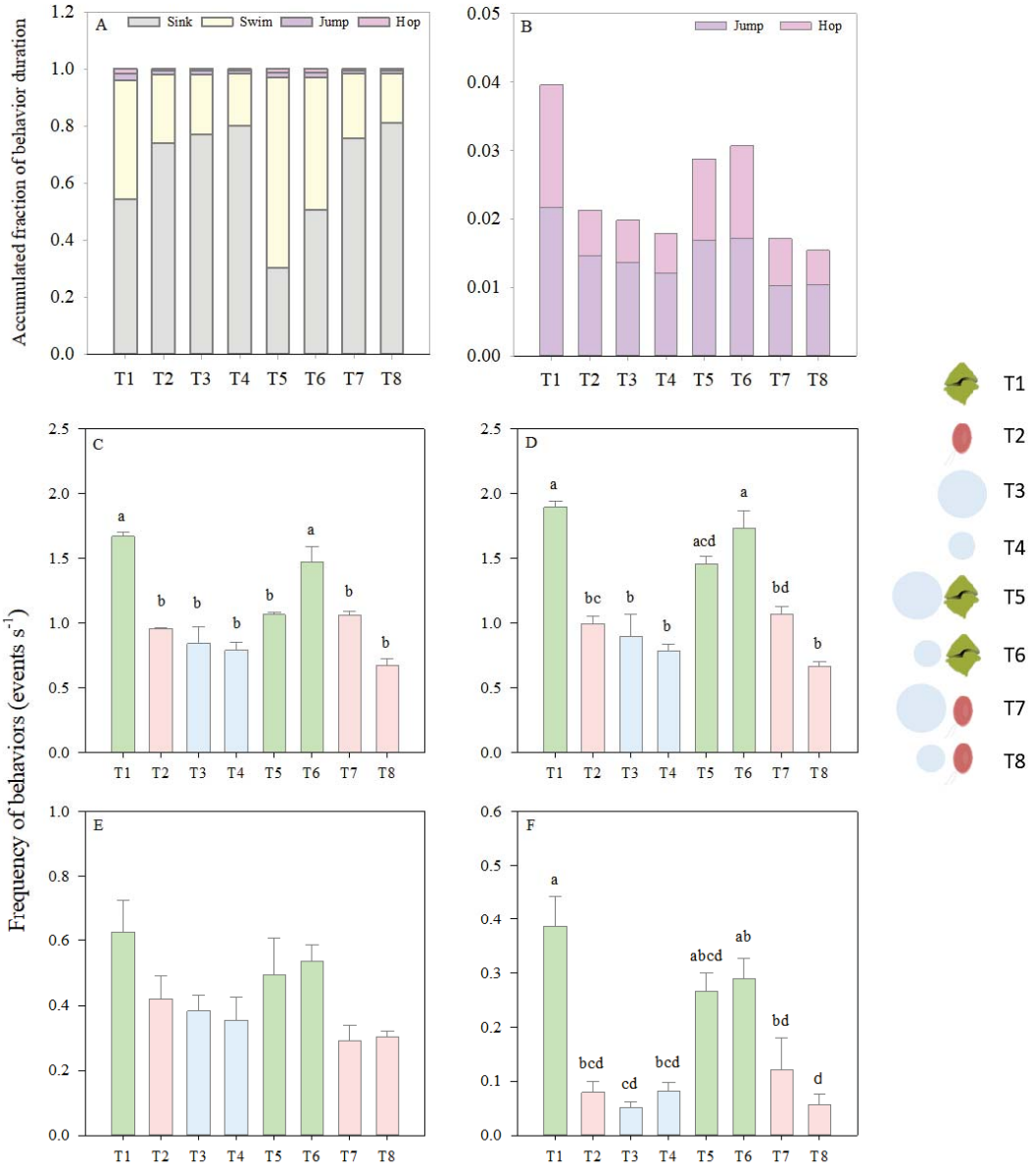


Figure 3. Accumulated fraction (A-B) of the durations of the four behaviors and frequency (events s^{-1}) for sink (C), swim (D), jump (E) and hop (F) in the eight different treatments (T1-T8). Chart A shows the accumulation of the four behaviors and chart B is a magnification of hop and jump data for a clearer observation. Error bars represent the standard error.

Regarding the duration of the sinking bouts, they were shorter in the treatments with *H. steinii* (S.I. Fig. 2A). For swimming bouts, only treatment 5 showed statistically significant differences with

T1, T3, T4 and T7 (S.I. Fig. 2B). The duration of the individual bouts of jumps and hops did not vary significantly among treatments (S.I. Fig. 2C and D). We observed that jumps were by far the fastest movement but in most cases, there were no significant differences (S.I. Fig. 3).

Ingestion and clearance rates from bottle incubation: A.tonsa

A. tonsa ingested both algae species when offered alone, albeit eight times more of *H. steinii* than *R. salina* (Fig. 4 A and B). In contrast, the ingestion and clearance rates of both MP sizes were zero when exposed to MPs alone (Fig. 4 A and B). When *A. tonsa* was simultaneously exposed to MPs and algae, the copepods ingested both types of particles (Fig. 4). However, the ingestion and clearance rates of MPs were 2-10 times lower than of algae (Fig. 4).

Ingestion and clearance rates of *R. salina* were significantly lower than of *H. steinii* when offered together with MPs (Fig. 4C, D). The ingestion (0-274 particles μgC^{-1} d^{-1}) and clearance (0-1.8 mL μgC^{-1} d^{-1}) rates of MPs were generally low and did not differ significantly between treatments with different MP and microalgae combinations (Fig. 4C, D).

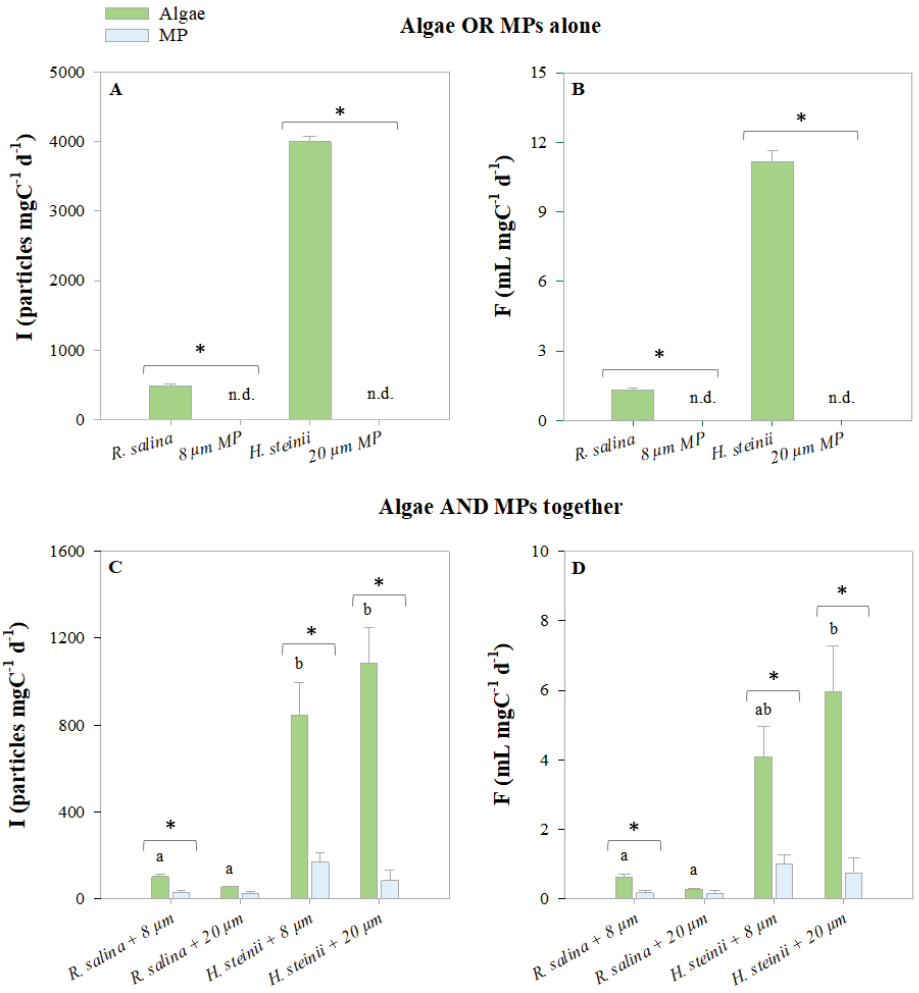


Figure 4. Ingestion (I, particles µgC^{-1} d^{-1}) and clearance rates (F, mL µgC^{-1} d^{-1}) of *Acartia tonsa* when they were fed with single prey (A and B) or two preys simultaneously (C, D): algae (*R. salina* or *H. steinii*) in green and MPs (8 µm or 20 µm PE beads) in blue. Data is presented as means of triplicates with the standard error. Letters indicate significant difference among MP ingestion or clearance rates in each treatment. Asterisks (*) represent a statistically significant difference between algae and MP ingestion or clearance rate within each treatment.

3.3. Global distribution of the three main feeding behaviors

The three feeding behaviors (feeding-current feeders, cruising feeders and ambush feeders) are present all around the globe but feeding-current feeders are the most abundant (Fig. 5) followed by ambush feeders. Feeding-current feeders are particularly abundant above 40° latitude in both

hemispheres (Fig. 5A,B). Cruising feeders show comparably most individuals in temperate latitudes but stable fractions of around 10% also occur in subtropical and tropical areas. Above 60° latitude cruising feeders become rare (Fig. 5C, D). Model-based estimates of the relative abundance of ambush feeders peaks between 60°N and 30°S at around 25% of individuals and declines towards higher latitudes. However, in the northern hemisphere higher relative abundances of ambush feeders were repeatedly observed at higher latitudes (Fig. 5E,F). When weight fractions instead of abundance fractions are compared the patterns remain similar but the fractions of feeding current feeders generally increase, while the fractions of ambush feeders decrease (S.I. Fig. 1).

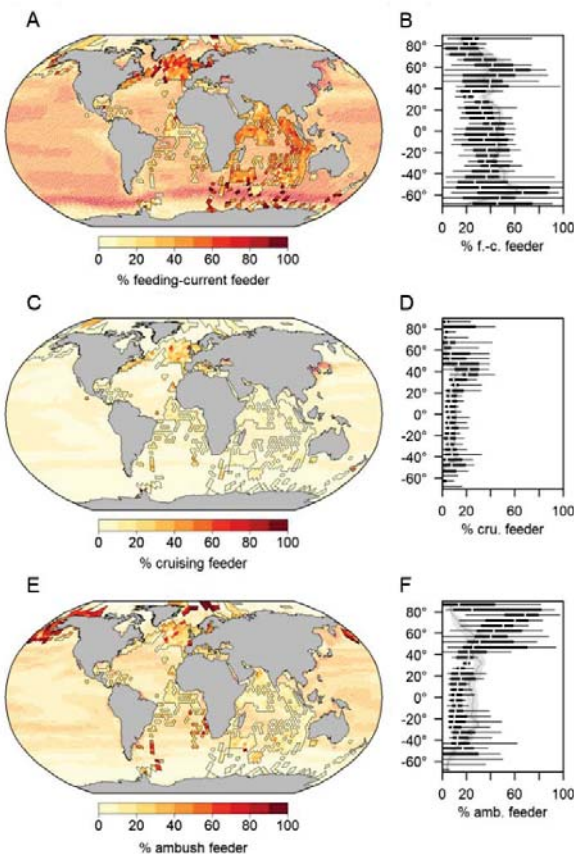


Figure 5. Global (left) and latitudinal (right) distribution of the abundance fraction of each feeding behavior: feeding-current feeders (A, B); cruising feeders (C, D) and ambush feeders (E, F). Dashed areas in maps represent model extrapolations and solid colors represent observation-based estimates. Latitudinal boxplots are observation-based estimates, where central lines represent medians, boxes represent interquartile ranges and whiskers represent 95% confidence intervals. Underlying polygons represent interquartile ranges of model-based estimates and underlying grey line represents median of model-based estimates.

4. DISCUSSION

Influence of the feeding behavior on MP ingestion

Our first hypothesis (ambush feeding is the least risky behavior in terms of MP ingestion) was not validated since the three behaviors showed a similar low ingestion of MPs compared to a similar sized prey. As expected, ingestion rates of MPs were very low in strict ambush feeders, which rely on hydromechanical signals to detect their prey (Kiørboe and Visser 1999) and have low clearance rates on non-motile prey (Paffenhöfer and Mazzocchi 2002; Almeda et al., 2018). The non-motile nature of MPs makes them non-detectable to ambushers. Ambush feeders may still perceive MPs if they directly intercept/collide with the copepod feeding/sensorial structures as observed for other non-motile particles (Kiørboe and Visser 1999; Almeda et al., 2018). This mechanism may account for the non-zero ingestion of MPs by ambushers observed in our experiments. In the case of feeding-current feeders, where both motile and not motile prey enter their feeding current, the ingestion of MPs were lower than expected. In this case, a post-captured discrimination of MPs can explain the low ingestion of MPs, as recently demonstrated in Xu et al., 2022 (under review).The feeding currents created by copepods act as "scanning currents" where the prey entered the feeding current and they are perceived and handled individually (Kiørboe, 2011). The prey is perceived as it is touched, or nearly touched, by the setae on the feeding appendages (Gonçalves and Kiørboe, 2015). Chemical discrimination occurrs by tasting as observed for toxic algae (Xu et al., 2017) and MPs (Xu et al., 2022, under review). Therefore, an efficient active rejection of MPs by tasting (chemical discrimination) explains the low ingestion of MPs in feeding-current feeders observed here. Taste-discrimination in feeding current feeder *T. longicornis* also occur when MPs are covered with biofilms, suggesting that the synthetic plastic polymer make the MP unpalatable to these planktonic copepods (Xu et al., 2022; under review). However, even at low rates, feeding current feeders still ingested accidentally some MPs, which can be due to the high concentration of MPs used in the experiments and the optimization of food intake by reducing handling time. Strict cruise feeding is a less common behavior in zooplankton. Passive interception is inefficient in the aquatic environment and cruising feeders depend on remote prey detection by hydromechanical, tactile, and chemical cues (Kjellerup & Kiørboe, 2012). Since virgin MPs do generate any of these cues, the detection and capture of MPs is expected to be low, which could explain the low ingestion of MPs observed in the studied cruising

feeder. Our results agree with field studies, which indicate a low occurrence of MP ingestion in natural zooplankton communities (Outram et al., 2020, Zheng et al., 2020)

Influence of prey type on behavior and MP ingestion in the mixed behavior feeder

MPs were not ingested by the mixed behavior feeder *A. tonsa* when they were offered alone, without prey. This indicates that chemical and hydromechanical cues of prey induce feeding and that the ingestion of MPs occurs accidently in the presence of prey. The presence of small prey, which has a low motility, did not modify the motile behaviors. However, the large motile prey caused an increase in activity, like jumps and hops, which are related to an ambush feeding behavior. Still, the ingestion of MPs was not affected by these changes in behaviors, since in all cases, the ingestion of MPs was low compared to the normal prey. Therefore, our second hypothesis (presence of a large motile prey induces ambushing, which in turn, will reduce MP ingestion) was not proven valid. The mechanisms behind the low ingestion are the same that we described above for the different behaviors: low detection when ambushing, and post-capture chemical discrimination when behaving as a feeding-current feeder.

Behavior distribution and global risk of entrance of MPs in marine food webs

The three main feeding behaviors of planktonic copepods are present in the world oceans showing specific latitudinal distributions. Using the current available data, we estimated the spatial distribution of the three main behavior to identify areas with high risk of entrance of MPs into marine food webs. However, given the low ingestion of MPs found in the laboratory experiments by the three analyzed feeding behaviors, we cannot defined such areas. Therefore, the risk of entrance of MP in the marine food web via planktonic copepods is globally low in all the oceans.

In this study, feeding-current feeders are the most abundant planktonic copepods. Although, the database used in this analysis is likely underestimating the ambush feeders abundance due to the lack of data available in the size fraction smaller than 200µm (O'Brien, 2010). Availability of more accurate field data would allow us to improve the existing databases resulting in an improvement in the spatial distributions. Furthermore, including other abundant and relevant copepods taxa such as harpaticoids, *Oncaea* and *Microsetella,* would enrich the knowledge about the role of copepods in the entry of MPs in marine food webs.

Ecological implications

The risk of ingestion of MPs in copepods, which are a key link between low and high trophic levels in marine food webs, is very low due to their behavior. Therefore, the biotransfer of MPs via copepods is unlikely, particularly if we consider the concentration of MPs commonly found marine surface waters (< 1 MPs L^{-1}; e.g., Rist et al. 2020; Beiras & Schönemann, 2021). The low MP ingestion in copepods also implies a reduced vertical exportation of MPs via fecal pellets and minor consequences on the biological carbon pump.

Since we did not find differences in MP ingestion among foraging behaviors, we cannot predict marine biogeographic areas with higher risk of MP ingestion by planktonic copepods based on their behavior. Still, the risk of MP ingestion in planktonic copepods is expected to be globally low. Our study includes the main behaviors of suspension feeding copepods, which dominated zooplankton communities. However, we know little about the risk on ingestion of MPs in aggregate colonizing copepods (e.g., *Microsetella* and *Oncaea*), which feed on marine snow. MPs (e.g., fibers and plastic fragments) can aggregate and concentrate in marine snow (Kvale et al., 2020) and therefore, copepods with this understudied behaviour can be exposed to higher concentration of MPs.

Marine vertebrates have a higher risk of ingestion of MPs than planktonic copepods. In contrast with planktonic copepods, where there is little evidence of ingestion of MPs in the field, ingestion of MPs have been frequently observed in sea mammals, sea birds, marine turtles and fishes (Boterell et al., 2019, Kühn and Franeker, 2020). The risk seems higher for visual predators, which can mistake MPs as food items, particularly if they have the same color (Ory et al., 2017, Zhu et al., 2019; Cimmaruta et al., 2022). Therefore, there is a risk of entry of MPs in the marine food webs, but planktonic copepods are not expected to be a major entry route.

It is important to note that this study focuses only on the risk of MP ingestion in relation to the zooplankton behavior but not on the potential effects of MP exposure. In this context, there is increasing evidence that MPs can have an impact on marine biota due to the lixiviation of toxic plastic additives (Gallo et al., 2018; Tian et al., 2020) , therefore negative effects can happen to zooplankton even without ingesting MPs.

5. CONCLUSIONS

Marine planktonic copepods with different foraging behaviors showed similar low ingestion rates of MPs compared to similar size prey. The mechanisms behind the observed low ingestion of MPs differ among behaviors: low detection of non- motile MPs in ambush feeders, post-capture chemical discrimination (tasting) in feeding-current feeders, and lack of chemical or hydromecanical cues in MPs to trigger the capture by cruising feeders. The low ingestion of MPs in planktonic copepods was independent of the MP size and the type of offered prey. Overall, our results indicate that the risk of entry of MPs in marine food webs via planktonic copepods is globally low when considering the current concentration of MPs in surface waters and the behavioural responses of copepods to MPs.

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CRediT authorship contribution statement

R. Rodríguez-Torres: Conceptualization, Methodology, Formal analysis, Investigation, Writing- original draft, Writing - review & editing. **R. Almeda**: Conceptualization, Methodology, Investigation, Writing- original draft, Writing - review & editing, funding acquisition. **J. Xu:** Conceptualization, Methodology, Formal analysis, Investigation, Writing - review & editing, funding acquisition; **N.B. Hartmann:** Conceptualization, Methodology, Writing - review & editing. **S. Rist**: Investigation, Formal analysis, Writing - review & editing. **Philipp Brun:** Investigation, Writing - review & editing. **T. Gissel Nielsen**: Conceptualization, Writing - review & editing, funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

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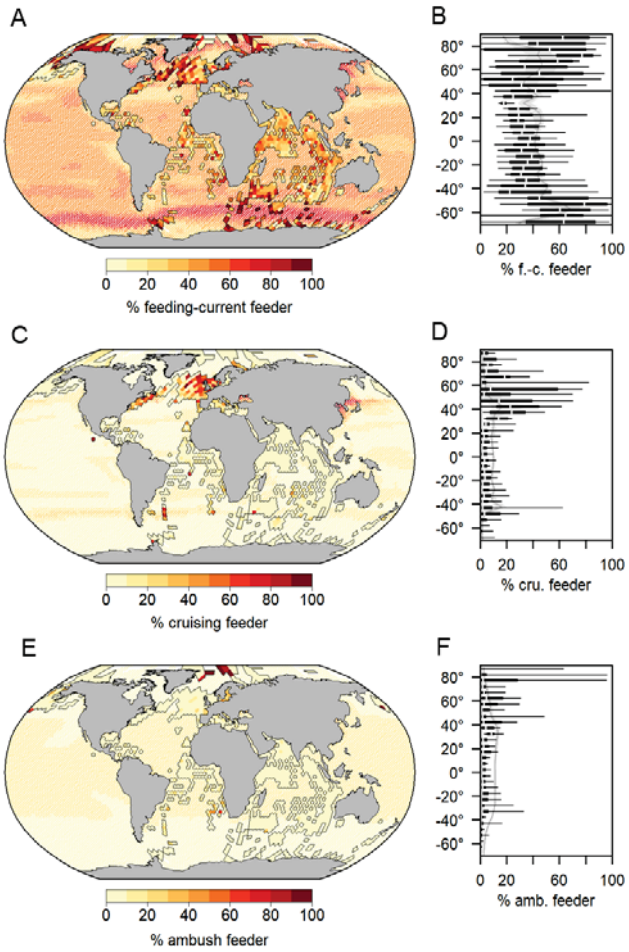
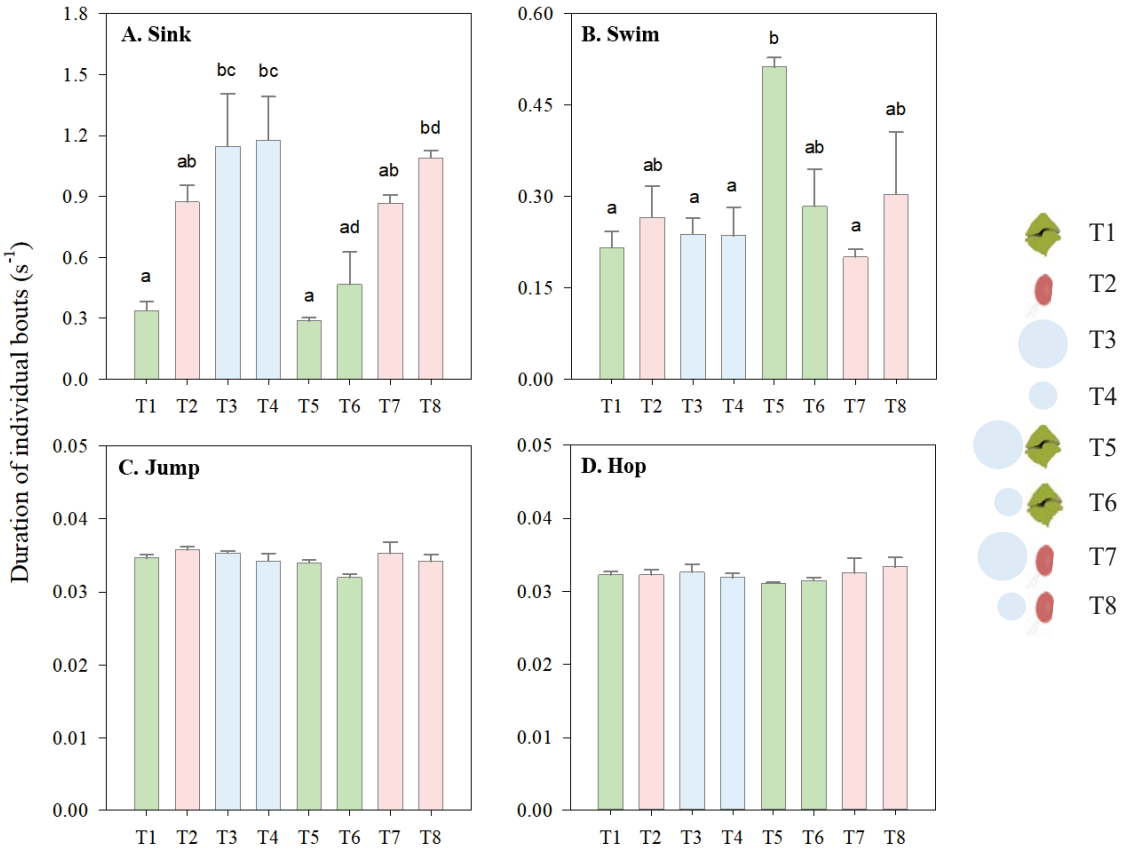
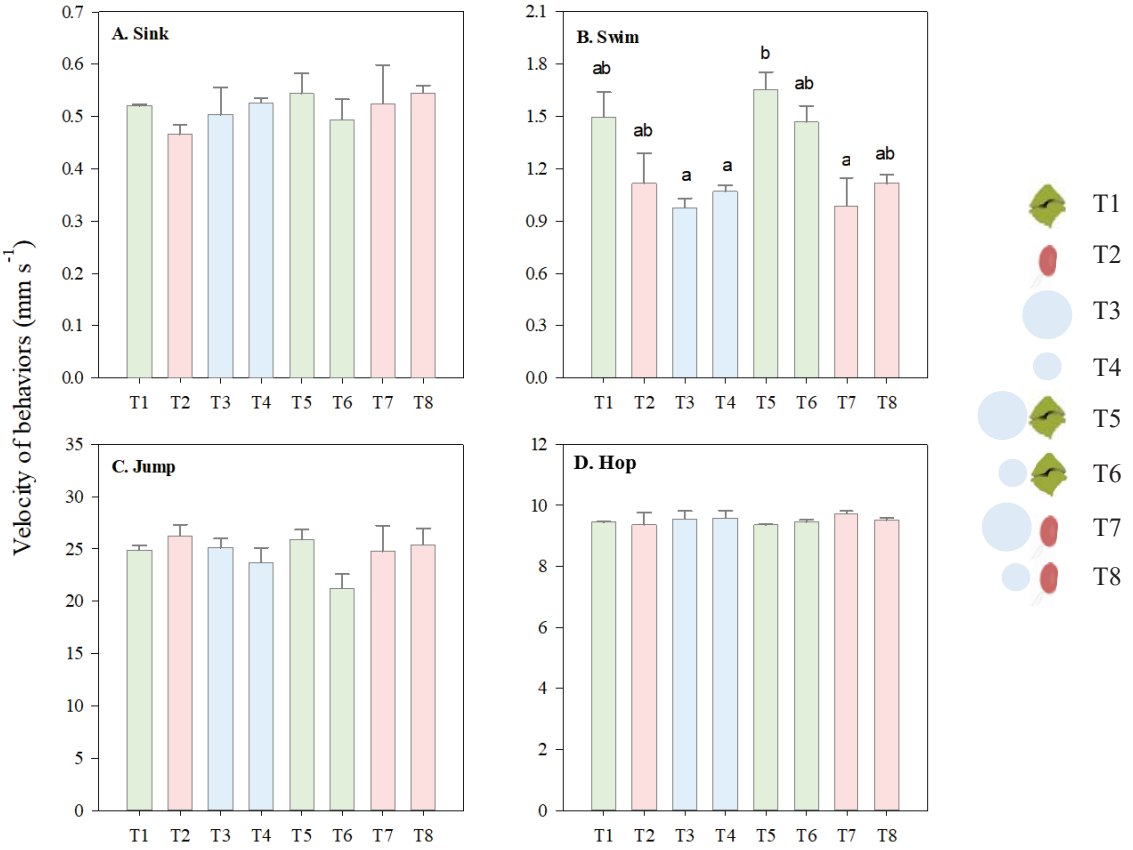
Supplementary information



S.I. Figure 1. Global (left) and latitudinal (right) distribution of the biomass of each feeding behavior: feeding-current feeders (A, B); cruising feeders (C, D) and ambush feeders (E, F). Dashed areas represent model extrapolations and solid colors/latitudinal boxplots are observation-based estimates.



S.I. Figure 2. Duration of individual swim, sink, jump and hop bouts (s^{-1}); that is the average of the duration of each movement individually per type of behavior: sink (A) swim (B) jump (C) or hop (D). The x-axes show the treatments explained in table 2 that copepods were exposed to. Error bars represent the standard error.



S.I. Figure 3. Average velocity (mm s^{-1}) at which copepods sink (A) swim (B) jump (C) or hop (D). In the x-axes are the treatment that copepods were exposed to and they are displayed in table 2. Error bars represent the standard error.

S.I. Table 1. Ingestion (I, particles cop.$^{-1}$ d^{-1}) and clearance rates (F, mL cop.$^{-1}$ d^{-1}) per individual with the standard errors (±SE) for all the incubation treatments.

Species	Treatment	F_ Algae (mL cop^{-1} d^{-1})	I_Algae (Cells cop.$^{-1}$ d^{-1})	F_MP (mL cop^{-1} d^{-1})	I_MP (MPs cop.$^{-1}$ d^{-1})
T. longicornis	*H. steinii* + 20 µm MP	4±0.8	782±137.6	0.3±0.2	35.8±29.2
C. hamatus		2.2±0.3	467.9±62.8	0.3±0.2	45±36.7
O.davisae		0.4±0.12	81.4±31.8	0.02±0.02	3±2.4
T. longicornis	*R. salina* + 8 µm MP	2.1±0.2	451.4±41.6	n.d.	n.d.
C. hamatus		2.8±0.2	516±31	0.4±0.2	73.7±36
O.davisae		0.2±0.05	42.7±8.45	0.03±0.02	3.5±2.9
A. tonsa	H. steinii	35.5±1.50	12704±243.1	-	-
	R. salina	4.20±0.3	1563.6±84.2		
	20µm MPs	-	-	n.d.	n.d.
	8µm MPs	-	-		
	H. steinii+ 8 µmMPs	13±2.7	2680±481.3	3.2±0.9	540±134.9
	R. salina+ 8 µmMPs	2±0.3	329±37.4	0.6±0.2	94.7±32.6
	H. steinii+20 µm MPs	19±4.2	3440±532.2	2.4±1.4	270±53
	R. salina+ 20 µmMPs	0.9±0.04	181.2±6.4	0.5±0.3	71.5±43.1

S.I. Table 2. Statistics output from the general analysis of each behavior in the *A. tonsa* video observations. It does not include the values for the interactions among treatments (S.I. Table 2)

Parameter	Behavior	df	F-value	P-value
Frequency of behaviors (events s-1)	Sink	7	16.81	2.83E-06
	Swim	7	17.63	2.04E-06
	Jump	7	1.86	1.44E-01
	Hop	7	8.42	2.27E-04
Accumulated fraction duration	Sink	7	15.82	4.25E-06
	Swim	7	14.11	9.12E-06
	Jump	7	1.73	1.72E-01
	Hop	7	10.79	5.07E-05
Individual bouts duration (s-1)	Sink	7	7.07	6.10E-04
	Swim	7	3.68	1.47E-02
	Jump	7	0.93	5.11E-01
	Hop	7	Kruskal Wallis	5.08E-01
Velocity (mm s-1)	Sink	7	0.50	8.24E-01
	Swim	7	5.39	2.55E-03
	Jump	7	1.30	3.12E-01
	Hop	7	Kruskal Wallis	5.15E-01

S.I. Table 3. Statistics output (p-values) from the analysis of each parameter in the *A.tonsa* video observations. It includes the specific values for the interactions among treatments.

	Frequency of behaviors (events s-1)			**Accumulated fraction duration**			**Individual bouts duration (s-1)**		**Velocity (mm s-1)**
Interaction	Sink p-value	Swim p-value	Hop p-value	Sink p-value	Swim p-value	Hop p-value	Sink p-value	Swim p-value	Swim p-value
T2-T1	0.000	0.000	0.003	0.110	0.194	0.001	0.181	0.999	0.305
T3-T1	0.000	0.000	0.001	0.048	0.095	0.001	0.014	1.000	0.071
T4-T1	0.000	0.000	0.003	0.020	0.043	0.000	0.010	1.000	0.202
T5-T1	0.002	0.140	0.542	0.033	0.029	0.131	1.000	0.018	0.974
T6-T1	0.714	0.957	0.773	0.999	0.995	0.445	0.997	0.985	1.000
T7-T1	0.002	0.001	0.011	0.069	0.141	0.001	0.192	1.000	0.079
T8-T1	0.000	0.000	0.002	0.015	0.036	0.000	0.023	0.938	0.296
T3-T2	0.970	0.998	1.000	1.000	1.000	1.000	0.844	1.000	0.984
T4-T2	0.835	0.853	1.000	0.979	0.987	1.000	0.768	1.000	1.000
T5-T2	0.982	0.117	0.124	0.000	0.000	0.224	0.114	0.055	0.061
T6-T2	0.009	0.004	0.061	0.037	0.056	0.054	0.462	1.000	0.406
T7-T2	0.983	1.000	0.997	1.000	1.000	1.000	1.000	0.985	0.990
T8-T2	0.304	0.414	1.000	0.959	0.977	0.990	0.941	0.999	1.000
T4-T3	1.000	0.994	0.999	1.000	1.000	1.000	1.000	1.000	0.998
T5-T3	0.577	0.036	0.054	0.000	0.000	0.131	0.008	0.027	0.011
T6-T3	0.001	0.001	0.025	0.016	0.025	0.029	0.048	0.998	0.103
T7-T3	0.582	0.950	0.943	1.000	1.000	1.000	0.828	0.999	1.000
T8-T3	0.839	0.778	1.000	0.998	0.999	1.000	1.000	0.981	0.986
T5-T4	0.339	0.009	0.134	0.000	0.000	0.098	0.006	0.027	0.037
T6-T4	0.001	0.000	0.065	0.006	0.011	0.021	0.036	0.998	0.278
T7-T4	0.343	0.610	0.998	0.997	0.997	1.000	0.750	0.999	0.999
T8-T4	0.971	0.991	1.000	1.000	1.000	1.000	1.000	0.981	1.000
T6-T5	0.0500	0.611	1.000	0.098	0.106	0.990	0.979	0.090	0.930
T7-T5	1.000	0.245	0.351	0.000	0.000	0.224	0.121	0.010	0.013
T8-T5	0.067	0.002	0.065	0.000	0.000	0.054	0.014	0.145	0.059
T7-T6	0.049	0.009	0.192	0.022	0.039	0.054	0.481	0.938	0.115
T8-T6	0.000	0.000	0.031	0.005	0.009	0.011	0.079	1.000	0.395
T8-T7	0.069	0.216	0.964	0.991	0.993	0.990	0.932	0.839	0.991

Paper VI

Research trends in microplastic uptake and transfer in marine food webs

Rocío Rodríguez-Torres, Sinja Rist, Rodrigo Almeda, Torkel Gissel Nielsen, Nanna Hartmann

This paper is a first draft of a literature review in progress

Research trends in microplastic uptake and transfer in marine food webs

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Abstract

[To be written]

1. Introduction

Microplastics (MPs) are ubiquitous throughout the marine environment. Due to their persistence and suspected bioavailability to a broad range of organisms, they have become a topic of increasing environmental concern. Over recent years, the ingestion of MPs has been documented in laboratory studies as well as field-collected organisms. Microplastic ingestion has been evaluated in many different taxa demonstrating that most marine organisms are capable of ingesting MPs (Egbeocha et al, 2018; Sun et. al, 2017). Ingestion can be considered as the initial stage, enabling MPs to cause harm as well as creating a potential entry point for MPs into food webs. Thus, quantification of MP ingestion strengthen the understanding of which types of marine animals are possibly at the highest risk of being negatively affected by MPs in the ocean.

Although MP ingestion has thus, already been studied in different marine organisms, there are many factors neglected and understudied. For example, only few studies have assessed how feeding strategies and feeding behavior influence MP ingestion. At the same time, exposure scenarios in current laboratory studies poorly reflect the complexity of 'real world' MPs and environmental conditions, both of which can affect their interactions with organisms. Finally, it has previously been described how exposure concentrations often exceed any environmentally relevant concentrations by orders of magnitude (Lenz et al., 2016; Koelmans et al., 2019).

As an example, a number of laboratory studies have demonstrated high levels of MP ingestion by copepods (Cheng et al., 2020; Cole et al, 2016) leading to the interpretation of copepods being one of the most relevant organisms for the entrance and transfer of MPs in marine food webs (Setälä et al., 2014). However, the conditions of those studies are not considered environmentally realistic and a more in-depth assessment of data in the available literature is needed to support or reject such a claim. Despite increasing focus in recent years on including environmental realism as a guiding principle for the experimental design, it is unclear to what extend this is being implemented in practice.

In addition, despite numerous studies on MP ingestion by marine organisms, there are no common protocols or standard reporting units to express the results. This applies not only to MP ingestion

(i.e. quantification of MPs inside organisms), but to concentrations of MPs in all environmental matrices. As a result, there is little consistency in the units for reporting MP concentrations (Rist et al., 2021). This hampers comparability between studies, making it difficult to assess properly which MP properties and environmental factors primarily influence ingestion.

To explore this topic further, the aims of this study are: (1) to investigate the ingestion of MPs in different organisms of the marine food webs, (2) to analyze the temporal trends in used MP types, studied organisms, reporting units and citation rates, (3) to evaluate the consistency in reporting units, and (4) to provide recommendations to give direction to future research in this field.

To achieve these aims we perform a systematic review of current literature. We focus on three organism groups: holoplankton, meroplankton and invertebrate benthos as they contain key species in the marine ecosystems. Most of the species within these groups belong to the first steps of the marine food webs being sources of food for other relevant organisms. Besides, they are key players in biological oceanographic processes, such us the biological carbon pump

[In this draft version, only the literature on holoplankton has been reviewed. All results, graphs and discussions are therefore based on the reviewed holoplankton literature.
We assume that trends from this group can be somewhat extrapolated to meroplankton and invertebrate benthos, but this will be explored further in a later version of the manuscript]

2. Methods

2.1. Systematic literature review

A systematic review of articles published prior to January 14th, 2022 was performed using the search engine "Web of Science". We conducted three individual searches, one for each organism group, which this review focuses on: (1) holoplankton, organisms that spend their whole life cycle in the pelagic environment; (2) meroplankton, pelagic young life stages of invertebrates that live in the benthos as adults, and (3) invertebrate benthos, organisms that live in or on the seabed or other surfaces. The search terms (microplastic* OR nanoplastic*), (ingestion OR uptake) and (marine OR sea) were used for all groups since the focus of the study is on the ingestion of MPs or nanoplastics by marine organisms.

The specific search terms for each group were chosen with the aim to encompass the most relevant organisms within each group (Table 1).

2.2. Refining the output and extracting information

In the initial search output, some papers were present in more than one organism group. These articles were checked and only kept in the correct group. However, some papers belonged to more than one group due to multiple organisms or life stages used in the study. Also, as part of the initial screening, articles not relevant for the current study were removed (e.g. studies focused on ingestion of MPs in fish), resulting in a final number of 55 articles for holoplankton, *[XX, to be done]* for meroplankton and *[YY, to be done]* for invertebrate benthos. Review articles were not included as such, but they were used to identify other research articles that were not captured by the original search. The final lists of articles can be found in Supporting information *[to be made upon completing the screening of articles]*.

Table 1. Search terms used in Web of Science for each group of organisms. The initial output is listed as well as the final number of articles used in this review. The reduction in articles is based on an evaluation of relevance in terms of the topic of the study as well as actual classification of the tested organisms.

	Search terms	Initial output	Final no. of papers
Holoplankton	(Microplastic* OR nanoplastic*) AND (ingestion OR uptake) AND (marine OR sea) AND (((pelagic OR free-swimming OR plankton*) AND (polychaet* OR rotifer* OR crustacean* OR cnidari* OR gastropod* OR tunicat* OR jelly fish*)) OR (copepod* OR krill* OR ctenophor* OR arrow worm* OR euphasid* OR cladoceran* OR chaetognat* OR appendicularian*))	98	55
Meroplankton	(Microplastic* OR nanoplastic*) AND (ingestion OR uptake) AND (marine OR sea) AND (meroplankton OR larva* OR embryo*) AND (mollusc* OR bivalve* OR gastropod* OR polychaet* OR crustacean* OR echinoderm* OR urchin* OR tunicat* OR bryozoa* OR phoronid* OR platyhelminthe* OR brachiopod* OR nemertea* OR fish* OR planktivorous)	100	*[To be done]*
Invertebrate Benthos	(Microplastic* OR nanoplastic*) AND (ingestion OR uptake) AND (marine OR sea) AND (mollusc* OR crustacean* OR bivalve* OR echinoderm* OR decapod* OR nematod* OR polychaet* OR urchin* OR planktiovorous OR tunicat*)	245	*[To be done]*

We went through all articles one by one and extracted a pre-defined set of information. Regarding the tested MPs in the studies, we extracted information on polymer type, concentration, size and shape. The studied organism, MP intake with the corresponding units, the organism's feeding behavior and the number of citations per article were the main information collected for later analysis. Some of the mentioned parameters have a high variety of values (e.g. MP size) and were grouped into ranges for easier analysis and grouping of studies (e.g. for MP size: <1µm, 1-5 µm, 5-50µm, 50-100µm and >100µm).

2.3. Statistics

[To be written. All statistical analyses included in this review, will be computed using IBM-SPSS v25SPS SPSS. Level of significance is set to $p < 0.05$.]

3. Results

3.1. Developments in the number of studies

The number of scientific articles, evaluating the ingestion of MPs in holoplankton, has been generally increasing since 2013, and the increase is steeper from 2017 (Figure 1). Of the 55 articles, included on this review, 21 were published in the period 2020-2021.

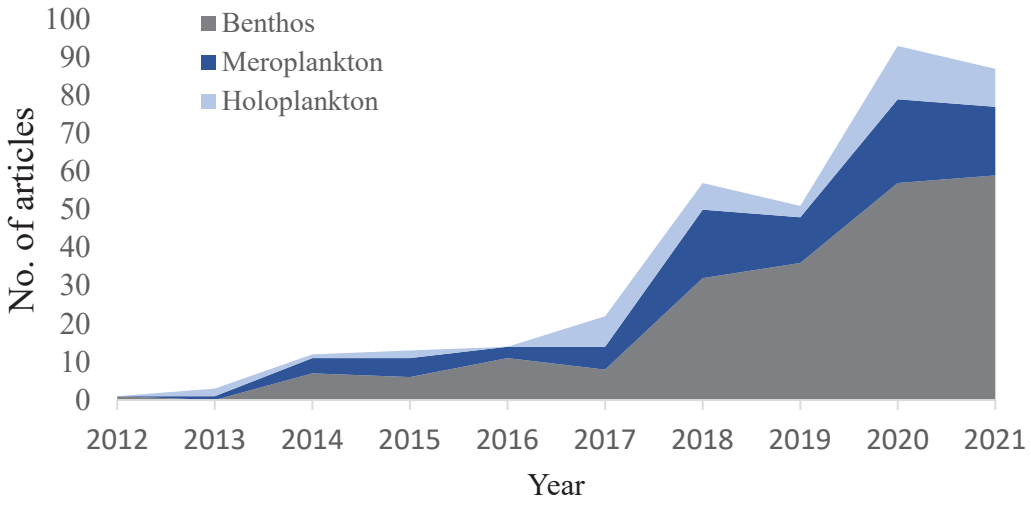

Figure 1. Number of articles resulting from the search terms in Table 1 published in the period 2012-2021. *[In this graph the holoplankton group includes the final amount of papers after removing freshwater organisms, reviews and other articles that, despite containing the searching words, were not about ingestion of microplastics in marine holoplankton. Meroplankton and Invertebrate benthos groups so far include all articles from the first output without the review papers. These two groups will be refined as a next step.]*

[To be written: additional description of Figure 1, regarding the amount of articles published for meroplankton and invertebrate benthos per year]

3.2. Types of studies

We observed a prevalence of laboratory studies over field. Only one of the investigations evaluated ingestion of MPs in the field simultaneously to laboratory experiments (2%, Lab & Field). A quantitative assessment is the most commonly used way to demonstrate the ingestion of MPs in both, lab and field studies. Yet, a high percentage in the laboratory studies are showing the ingestion of MPs in a qualitative way, mostly showing images of the animal gut or fecal pellets containing fluorescent MPs after exposure. In a lower percentage (9.3 %), some studies examined the MP ingestion via image analysis, and provided quantitative results.

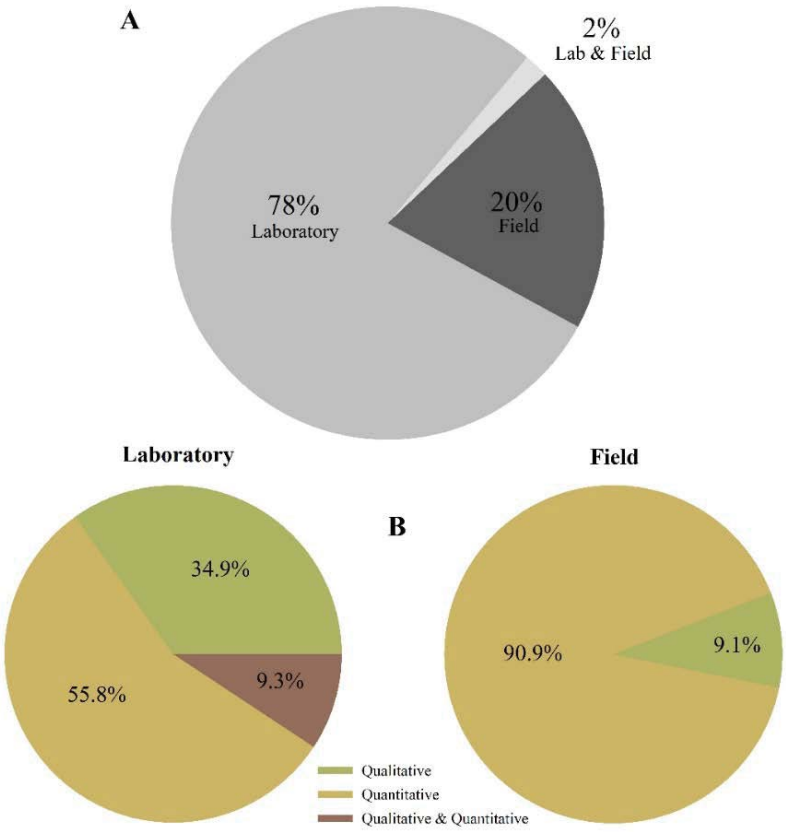

Figure 2. This graph is made based on the final total 55 articles that have assessed the ingestion of MPs by holoplankton in the field or lab (see Table 1). The graph shows (A) the percentage of articles on holoplankton that are conducted as either lab studies, field studies or a combination and (B) the percentage of lab and field studies, respectively, that provide qualitative, quantitative or both types of data on MP ingestion.

For studies providing quantitative data, the reporting unites are listed in Table 2. Qualitative results are those that 1) consider the use of pictures (visual observations) to corroborate ingestion of particles as well as those that 2) use as a measure the percentage of exposed/collected animals with particles inside without specifying the amount of particles per individual. Therefore, the unit describing the percentage of individuals with ingested MPs is considered a qualitative measure and is not included as a unit in Table 2.

Table 2. Variations in units used to report ingestion of microplastics in field and lab studies on holoplankton *[the table with be updated with data from meroplankton and benthos articles]*. Clearance rate is considered a way to measure ingestion of particles, which is why there are units in the laboratory experiments of volume per individual (ind.) per time (d^{-1} or h^{-1})

Field	Lab
particles $ind.^{-1}$	particles $ind.^{-1}$
Total ingested particles	Total ingested particles
% particles ingested	% particles ingested
particles g^{-1}	particles $ind.^{-1}$ d^{-1}
pieces mL^{-1} of tissue digestate	particles $ind.^{-1}$ h^{-1}
pieces m3	particles $taxa^{-1}$
	mL $ind.^{-1}$ h^{-1}
	nl h^{-1} $ind.^{-1}$
	ng mg^{-1} h^{-1}
	ng C^{-1} h^{-1}

[To be written: A more detailed explanation of Table 2, including how these different units are defined and used]

Based on the overview of reporting units, listed in Table 2, there are some overlaps in units between field and laboratory studies. The data illustrates the large variety in reporting units, making data comparability difficult. In some cases, units may be converted to achieve a consistent dataset, allowing for the combination and comparison of data from several studies. For example, the conversion from mass-based (e.g. µg/individual) to number-based units (e.g. particles/individual), and vice versa, would require information on polymer density and particle

dimensions (Leusch & Ziajahromi, 2021). However, units that differ for example exclusively in the measured time would be easily converted.

[A quantitative analysis of the frequency of each reported units would be performed to better evaluate the comparability among studies]

3.3. Lab exposure vs. environmental concentrations

Figure 3 illustrates the variation of MP exposure concentrations with years, and how this compares to the concentrations of MPs in the natural environment. For the purpose of this figure, we only considered exposure concentrations in laboratory experiments. Experiments with nanoplastics where not included since there is currently no estimation of nanoplastic concentrations in the ocean for comparison. Due to the difficulties to convert units, for this graph, we only consider the articles reporting the exposure concentrations in particles per mL (the most frequent unit used).

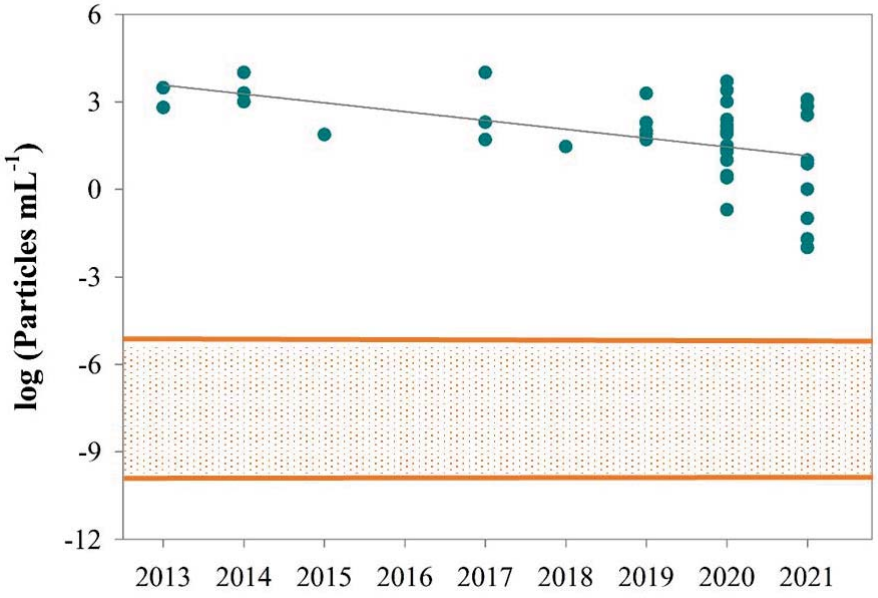

Figure 3: Concentrations of microplastics used in laboratory studies (blued dots) and its trend with time (grey line). Orange lines represent the range of reported mean concentrations of MPs in natural surface waters estimated by Beiras & Schönemann (2020). This range includes data from Mediterranean, Atlantic, Arctic, Pacific, Australia and Southern Ocean.

3.4. Test materials vs. real world microplastics

[To be written: A description of Figure 4 showing temporal variations in MPs characteristics used in laboratory experiments. Figure 4A shows 'polymer type vs year' and Figur 4B shows 'MP size vs year'. This can also be expanded upon to compare 'shape vs year' as well as polymer type found to be ingested by holoplankton in the field vs MP polymer used in laboratory tests.]

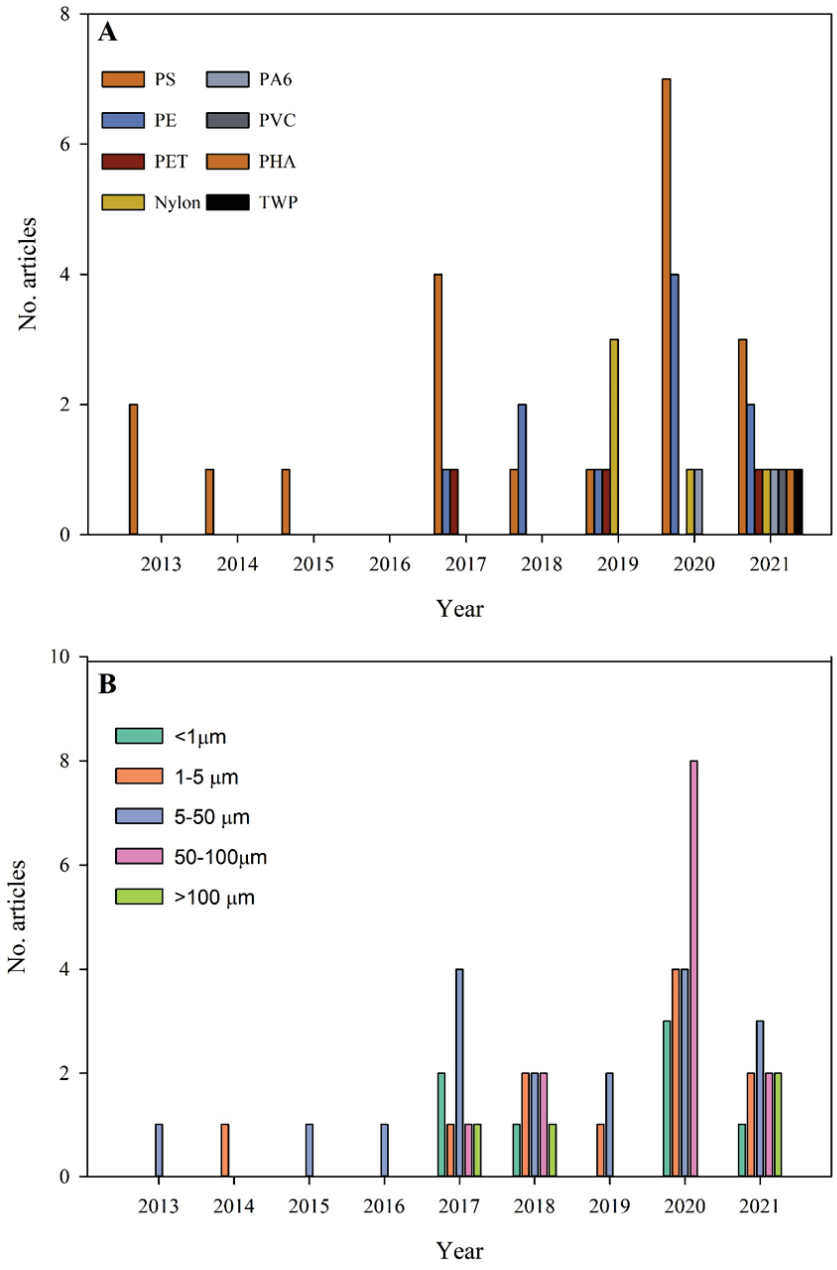

Figure 4. Temporal variations in terms of the used polymer types (A) and MP sizes (B) in laboratory experiments with holoplankton. MP sizes were divided into five size ranges: <1µm, 1-5 µm, 5-50µm, 50-100µm and >100µm

3.5 Feeding behavior of test organisms

[To be written: Continue the classification of the studied organisms by feeding behavior and analysis of the tendencies with time. The purpose of this is to determine if there are any overstudied/understudied feeding behaviours, as well as to explore any diversification over time.]

Forty studied organisms or taxa have been identified in the holoplankton group. Some studies evaluated more than one species and for this behavior analysis we excluded protozoans and microalgae. Within all the species, 50 % are copepods. Feeding-current feeding, cruising feeding and ambush feeding are the three main feeding behaviors for copepods. We observed that most of the analyzed copepods species belong to the first group; only one is cruising feeder and one ambush, which are not abundant in marine waters. We also detected the absence of studies evaluating ingestion of MP in relevant abundant copepod genus such as, *Oncaea* spp. and *Microsetella* spp. (S.I. Table 1).

3.6 Citation bias

[To be written: Analysis of reviewed articles to investigate if there is a citation bias towards articles with high MP concentration or articles with higher impact. The underlying hypothesis is that studies with "positive" and/or statistically significant results for MP ingestion are cited more frequently than others. This has previously been investigated for studies on MP uptake in fish (Müller, 2021). Although citation bias could not be verified, there was marginal trend toward a significant positive relationship. In this case, the analysis was hampered by a lack of studies reporting only minor or no uptake of MP in fish. A similar situation might occur for the organism groups, covered by this review study. However, a similar approach to that of Müller (2021) will be applied to investigate this issue. Citations will be normalized as 'number of citations / years since publication'. For now, only a discussion of the top 5 cited holoplankton papers is included, but this will be expanded to all articles in all three groups of organisms in the final version]

4. Discussion

[To be written: The following topics will be discussed (aligned with results sections):

-The general developments in literature over time for the three organisms groups. Discussion of why some groups studies earlier/later and more/less than others. Discuss the exposure of each group to MP pollution in nature and maybe argue if justifies why e.g. invertebrate benthos is/should be more investigated.

- The distribution between lab and field studies in the literature. What factors might determine that lab studies by far outweigh the field studies?

- The use of varying units in data reporting. Discuss if there are any predominant units and why these might be preferred. Discuss the need of standardization when reporting ingestion of MPs to be able to compare among studies.

- Lab exposure vs. environmental concentrations. Discuss the actual MP concentrations found in marine surface waters and compare them with exposure concentrations in laboratory experiments. Discuss any trends in exposure concentrations over time: has an increased focus on environmental realism in MP studies resulted in lower exposure concentrations?

- Test materials vs. real world microplastics. Based on the trends of MP type in lab experiments and what is found in nature, discuss if there has been a diversification of tested MPs in the lab and to what extend (what parameters?) this has to be broadened further.

- Feeding behavior of test organisms. Point out the relevance of the key trait feeding behavior in the ingestion of MPs and highlight the few articles that already consider it and the need of including it in future research.

- Citation bias. Discuss the usefulness of available studies: how many of these report 'no uptake'? Is there an underlying publication bias i.e. only studies with "positive" and/or statistically significant results for MP ingestion are being reported? Based on data from the three organism groups in this review, can any positive correlation between citations and reported MP ingestion be verified? Result will be compared to previous investigation of citation bias for studies of MP uptake in fish (Müller, 2021).

5. Conclusion

[To be written]

6. Acknowledgements

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Supplementary information

Table 1. Marine zooplankton species associated with its corresponding feeding behaviors from the total analyzed articles for the holoplankton group. The behavior of species that are not strictly planktonic or its behavior is not clearly defined, are classified as n.d. (not determined).

Marine Zooplankton	Foraging behavior
Acartia tonsa	Mixed feeder
Acartia clausi	Mixed feeder
Acartia longiremis	Mixed feeder
Acartia spp.	Mixed feeder
Artemia franciscana	n.d.
Aurelia aurita	n.d.
Barchionus plicatilis	Filter feeder
Bosmina coregoni maritima	Feeding current feeder
Calanus finmarchicus	Feeding-current feeder
Calanus glacialis	Feeding-current feeders
Calanus helgolandicus	Feeding current feeder
Calanus hyperboreus	Feeding-current feeder
Calanus pacificus	Feeding-current feeder
Centropages typicus	Cruising feeder
Diaphanosoma celebensis	Filter feeder
Dolioletta gegenbauri	Feeding-current feeder
Doliolidae	Feeding-current feeder
Eucalanus pileatus	n.d.
Euphasia superba	n.d.
Euphasiidae sp.	n.d.
Eurytemora affinis	Feeding current feeder
Evadne nordmannii	Feeding current feeder
Fritillaria borealis	Feeding-current feeder
Limnocalanus macrurus	Feeding-current feeder
Mysis mixta	Filter or raptorial feeder
Mysis relicta	Filter or raptorial feeder
Neomysis integer	Filter or raptorial feeder
Obelia sp.	n.d.
Oikopleura dioica	Feeding-current feeder
Paracyclopina nana	Ambush feeder
Parasagita sp.	Ambush (predator)
Parvocalanus crassirostris	Feeding-current feeder
Pseudocalanus spp.	Feeding-current feeder
Pseudodiaptomus annandalei	Feeding-current feeders
Siphonophorae	n.d.
Synchaeta spp.	Feeding current feeder
Temora longicornis	Feeding-current feeder
Temora turbinata	Feeding-current feeder
Tigriopus fulvus	n.d.
Tigriopus japonicus	n.d.

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