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Uptake and excretion of polystyrene microplastics in the marine copepod Calanus finmarchicus

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Abstract

Microplastics (MPs) are plastic particles <5 mm widely distributed in the marine environment and ingested by a broad range in marine organisms. The copepod *Calanus finmarchicus* is a key species in the marine food web in the North Atlantic Ocean, and it is one of several copepod species known to ingest MPs. Negative impacts are related to the ingestion of MPs such as decreased intake of microalgae feed and possible hazards related to polymer composition and adsorbed environmental pollutants. In the present study, uptake and excretion of polystyrene (PS) MPs (750 particles ml^{-1}) were studied in two separate experiments. In the uptake study, young adult female *C. finmarchicus* were exposed to MPs in the presence of the microalgae *Rhodomonas baltica* (7,500 cells ml^{-1}) for up to 96 hours. The 10 μm MPs were readily ingested apparently by non-selective filtration. A somewhat lower average number of algae appeared to be ingested in the presence of MPs compared to non-exposed groups, although the difference between the means at each sampling point was not significant at the $p \leq 0.05$ level. During the excretion study a 24-hour exposure phase was followed by depuration for up to 72 hours in filtered seawater (FSW) with/without microalgae. The majority the MPs were egested via the faeces during the first hours of depuration in the presence of microalgae feed. In the absence of microalgae, a lack of sufficient data made it difficult to draw any conclusions about MP excretion via the faeces. MP content in the copepods decreased significantly between 1 and 24 hours, and in the presence of algae a significant lower MP content was found in the copepods at 24 hours compared to copepods not receiving feed. In the latter group the MP content did not appear to decrease further towards 72 hours, and, unexpectedly the MP content also increased in the copepods receiving algae in the same period. Although a small amount of particles was assumed to be retained in the copepods over the period, the increase was assumed to partly be due to other factors such as faecal pellet degradation and re-ingestion of MPs. More studies, with a preferably longer depuration phase, should be conducted to reveal more about a possible retention of MPs in the presence and absence of feed. An assumed sedimentation process was probably causing a discrepancy between measured MP removal and retrieved MPs in the copepods and in their faeces. This was complicating interpretations of results, and emphasizes the need of comprehensive measurements of MPs during experiments. Lastly, faecal pellets loaded with MPs could contribute to increased MP exposure, furthermore as pellets, especially those produced in the absence of algae appeared more fragile and therefore are more prone to degrade and thereby release MPs.

Sammendrag

Mikroplast (MP) er plastpartikler <5 mm og som man kan finne nærmest overalt i det marine miljø, og som i tillegg beites på av en rekke marine organismer. Kopepoden *Calanus finmarchicus* er en nøkkelart i næringsnettet i det nordlige Atlanterhavet, og er samtidig en av flere kopepoder som kan ta opp MP. Eksempler på negative effekter knyttet til MP-opptak er redusert algeopptak, og andre risikoer knyttet til plastpolymeren og til det faktum at miljøgifter kan akkumuleres i og på plasten. I dette studiet ble opptak og utskillelse av polystyren (PS) MP (750 partikler ml^{-1}) undersøkt i to ulike delforsøk. I opptaksstudiet ble unge, voksne hunner av *C. finmarchicus* eksponert for MP med tilgang på mikroalge (*Rhodomonas baltica*) (7500 celler ml^{-1}) i opptil 96 timer. Opptaket av plastpartiklene med størrelse 10 μm skjedde raskt og uten at kopepodene virket å diskriminere mellom MP og alger. Sammenlignet med kontrollgrupper var gjennomsnittlig algeopptak hos eksponerte dyr noe lavere, men forskjellen var ikke signifikant på $p \leq 0.05$-nivå. Under utskillelsesstudiet ble kopepodene etter en 24-timers eksponeringsfase overført til filtrert sjøvann med eller uten alger for utskillelse i opptil 72 timer. Mesteparten av MP ble skilt ut i løpet av de første timene der det var alger tilstede. På grunn av utilstrekkelig datagrunnlag kunne ikke utskillelse av MP via feces beregnes i gruppene uten tilgang på alger. MP-innhold i kopepodene ble signifikant redusert mellom 1 og 24 timer i begge grupper, videre var antall MP i dyrene ved 24 timer signifikant lavere i gruppen med alger tilstede. For grupper uten tilgang på alger virket MP-innholdet i dyrene å forbli uendret fra dette tidspunktet og frem til 72 timer, mens det i samme periode nokså uventet økte i grupper med alger tilstede. Selv om et lavt antall MP trolig var holdt igjen i dyrene, skyldtes trolig denne observasjonen andre faktorer som nedbrytning av feces og påfølgende gjenopptak av partikler. Flere studier behøves derfor for å undersøke retensjon av MP i kopepoder med og uten alger tilstede, og en lengre utskillelsesfase burde også benyttes for å kunne undersøke utviklingen over tid. En antatt sedimentering av partikler var trolig årsaken til avviket mellom målt fjerning av MP fra medium og antall MP funnet igjen i dyr og feces. Dette gjorde tolkning av resultater mer komplisert, samtidig som det understreker viktigheten av å kvantifisere plastpartiklene i alle deler av eksponeringssystemet under hele eksperimentet. Høye antall MP målt i feces viser ellers at opptak og utskillelse av MP i kopepoder kan bidra til en økt eksponering av andre organismer, dette gjelder spesielt feces produsert uten tilgang på alger, da disse virket mer skjøre og trolig lettere kan gå i oppløsning og frigi MP til omgivelsene.

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Abbreviations

B(a)P	Benzo[a]pyrene
Cd	Cadmium
CO_2	Carbon dioxide
DDT	Dichloro-diphenyl-trichloroethane
DOM	Dissolved organic matter
FSW	Filtered seawater
HDPE	High density polyethylene
HOC	Hydrophobic organic chemicals
LDPE	Low density polyethylene
MP	Microplastic
Ni	Nickel
PAH	Polycyclic aromatic hydrocarbon
Pb	Lead
PCB	Polychlorinated biphenyl
PE	Polyethylene
PET	Polyethylene terephthalate
POM	Particulate organic matter
PP	Polypropylene
PS	Polystyrene
PVC	Polyvinyl chloride
rpm	Rotations per minute
SD	Standard deviation
UV	Ultra violet
Zn	Zinc

1. Introduction

1.1 Plastic pollution in the marine environment

Marine plastic litter has become a global issue of concern due to its ubiquity and persistence, and its adverse effects on marine organisms and their habitats (Cózar et al., 2014, Gall and Thompson, 2015). Over 250,000 tons of plastic are estimated to be floating at the surface of the open oceans (Eriksen et al., 2014). The highest concentrations are found in the five large sub-tropical ocean gyres in the North and South Pacific and Atlantic Oceans and in the Indian Ocean, commonly called the five garbage patches (Sebille et al., 2012). A sixth garbage patch is predicted to emerge in the Barents Sea (Sebille et al., 2012), although Cózar et al. (2017) suggest that both the Greenland Sea and the Barents Sea are sinks of plastics transported by branches of the thermohaline circulation. The annual production of plastics in the European Union, Norway and Switzerland in 2015 was 322 million tons (PlasticsEurope, 2016) and it is expected to increase due to a rising demand from a growing human population. Large amounts of the plastics are entering the ocean. In 2010 between 4.8 to 12.7 million metric tons of plastics were estimated to have entered the marine environment from land-based sources, and the input is expected to increase unless a large effort is made in mitigating measures such as improved waste management (Jambeck et al., 2015).

1.2 Microplastics (MPs)

1.2.1 Definition and sources of primary and secondary MPs

The presence of small plastic items in the marine environment has gained increased attention during recent years, both among scientists, politicians and the public, due to their prominence in the environment and intake by a broad range of organisms (Wang et al., 2016, Thompson, 2015). These particles are called microplastics (MPs), referring to their microscopic size. Despite an increased focus on MPs, their size definition is still a subject of debate. The 5 mm upper size boundary is commonly used and is the one used herein. Disagreement also exists regarding the definition of the lower size boundary (Thompson, 2015).

MPs are usually classified as primary or secondary MPs, depending on their origin. Primary MPs are produced in a microscopic size, and are commonly used in cosmetic products, in processes to remove rust and paint (air-blasting), vectors in medical treatment and as resin pellets for further production of plastic products. Accidental spills or leakages during

fabrication or transport and use are pathways for primary MPs to the marine environment (Andrady, 2017, Cole et al., 2011, Ogata et al., 2009). The annual consumption of MP containing personal care products among the U.S. population is estimated to cause an emission of 263 tons of polyethylene MPs to the environment alone (Gouin et al., 2011).

Secondary MPs on the other hand are fragments of larger plastic items, created through physical, chemical and biological degradation processes altering the polymers´ structural integrity (Cole et al., 2011). In the marine environment the most effective route of degradation is through UV initiated photo-oxidation, accompanied by physical wearing by for example waves and sand particles. Thus, if oxygen and light are limiting factors, degradation of plastic will decelerate. Polymer composition, presence of additives and biofouling influence the rate of the degradation of plastics (Andrady, 2015, Andrady, 2017). Synthetic fibres (e.g. acrylic and polyester fibres) from clothes and textiles are also regarded as secondary MPs. They can be released during laundry and enter the marine environment through waste water effluents (Browne et al., 2011).

1.2.2 Abundance and fate of MPs in the marine environment

Based on previous studies (e.g., Eriksen et al. (2014) and Cózar et al. (2014)), large datasets based on global measurements of MPs by plankton net surface-trawling, ocean circulation models and plastic input data, Sebille et al. (2015) estimated that between 93,000 and 236,000 metric tons of MPs are present in the world´s oceans. Fragments are the predominant MP type, whereas fibers and spheres occur to a lesser extent (Kooi et al., 2016). MPs of 0.5 – 5 mm mainly occur in the upper 3 m of the water column, with both the number concentration and mass decreasing exponentially with depth (Kooi et al., 2016). Lighter and smaller particles, especially plastic lines, can be distributed lower in the water column by vertical mixing due to a low buoyant terminal rise velocity (Kooi et al., 2016, Reisser et al., 2015).

A larger estimated global rate of plastic input to the ocean compared to the estimated concentration of plastics present at the ocean surface, indicates that a larger fraction of plastics is removed from the surface (Sebille et al., 2015, Cózar et al., 2014). Several pathways of removal of MPs have been proposed. A likely route is through sinking due to polymer density. Polymers denser than seawater such as polystyrene (PS) and polyvinyl chloride (PVC) will readily sink. Additives and plastic fillers may also alter the particles floating capability (Andrady, 2015). Regardless of density, biofouling commonly increases MP density (Cózar et al., 2014). Incorporation into faecal pellets (Cole et al., 2016) and

phytoplankton aggregates may also alter the vertical transport by either decreasing or increasing the sinking velocity of MPs (Long et al., 2015). Several studies have confirmed the presence of MPs in deep-sea sediments (Woodall et al., 2014, Van Cauwenberghe et al., 2013), and at the sea floor in remote areas in the Arctic (Tekman et al., 2017). Stranding of floating particles along shorelines worldwide is also documented (Browne et al., 2011). Accumulation of MPs in Arctic Sea ice represents yet another sink of MP particles (Obbard et al., 2014). A rapid nano-fragmentation of MPs could also be an explanation for the removal of plastics from the ocean surface (Cózar et al., 2014). Lastly but not less importantly, marine organisms can act as a sink, as a broad range of marine organisms have shown to ingest MPs, from zooplankton (Desforges et al., 2015) and several fish species, to seabirds (Lusher et al., 2013) and large baleen whales (Besseling et al., 2015).

1.2.3 Plastic polymers: types, composition and potential hazards

1.2.3.1 Common plastic polymers

The most applied plastic polymers are polystyrene (PS), high- and low-density polyethylene (HDPE and LDPE respectively), polypropylene (PP), polyvinyl chloride (PVC) and polyethylene terephthalate (PET). These polymers are also the most dominant in the environment (Li et al., 2016, Andrady, 2011). PS, the polymer used in the present study, is used for numerous purposes e.g. in food containers, cups, furniture and building materials because of its transparency and ease of reworking and production (Garrigós et al., 2004).

1.2.3.2 Hazards related to the plastic composition

Despite plastic polymers are regarded as biochemically inert and consequently do not to pose any threats to neither humans or the environment (Lithner et al., 2011), there are potential hazards associated with both the inherent composition of the MPs and their ability to sorb contaminants from the surroundings (Cózar et al., 2014, Wang et al., 2016). The polymerization process can give rise to several impurities in the plastic structure which potentially can migrate from the plastics. One example is reactive and hazardous monomers which can occur if the polymerization reactions are incomplete (Lithner et al., 2011), such as the styrene monomer in PS which can be acute toxic and act as a carcinogen and an endocrine disruptor (Rochman et al., 2013b, Garrigós et al., 2004). However, the hazard of additive-free virgin PS plastics is believed to be negligible as the structure is regarded as stable and

migration of monomers only is considerable after extended periods of weathering and environmental degradation (Cole et al., 2015). To provide polymers with desired properties and features, plasticizers, fillers, stabilizers, flame retardants, pigments, etc. can be added to the polymers (Lithner et al., 2011). Examples of additives are phthalates, bisphenol A and alkylphenols. The additives´ potential to migrate from the plastic polymer is dependent of parameters such as the molecule size, the pore size of the polymer and the surrounding environment (Teuten et al., 2009).

1.2.3.3 Adsorption and desorption of environmental pollutants

Contaminants in the marine environment can sorb to MPs, especially hydrophobic organic chemicals (HOCs) (e.g. PCBs, PAHs and DDT and its metabolites) can accumulate in high concentrations due to the hydrophobic properties of the plastics (Carpenter et al., 1972, Endo et al., 2005, Ogata et al., 2009, Teuten et al., 2009). According to Teuten et al. (2009) factors that govern the adsorption/desorption of chemicals are the properties of both the plastics and the chemical compound, pH, temperature and the presence of dissolved organic compounds in the seawater. For example, the adsorption of PAHs has shown to be strongest for PS compared to most other common polymer types (Rochman et al., 2013b).

MPs can also adsorb metals, as shown for HDPE, LDPE, PVC, PET and PP pellets which accumulated Cd, Ni, Pb and Zn from the ocean (Rochman et al., 2014). Metal concentrations in the plastic increased proportionally with time although variations in concentration between the polymer types were rather small. Furthermore, aged pellets from the environment showed greater accumulation of metals, probably due to changes in the chemical structure during the weathering process and biofilm formation (Holmes et al., 2012).

1.2.3.4 Transfer of chemicals from MPs to organisms

Marine MPs have been regarded as a potential vector both for transport of contaminants in the environment and for transfer of chemicals to organisms that ingest plastics (Lee et al., 2014, Koelmans et al., 2013). The latter has been underpinned by several studies of transfer of chemicals from ingested plastics to marine organisms and subsequent adverse effects (Besseling et al., 2013, Browne et al., 2013, Rochman et al., 2013a). The rate of transfer is dependent on several factors including the physico-chemical properties of the compound, the gastric conditions of the organism, gut retention time of the MPs, the gradient between the

compounds in the MPs and in the lipids of the organism, and the age and weathering status of the polymer (Koelmans et al., 2014, Teuten et al., 2009). However, the relative importance of MPs as a source of contaminants remains a subject of debate (Koelmans et al., 2016, Lohmann, 2017). One of the assertions is that MPs are only a minor source of contaminants compared to other routes of uptake (Koelmans et al., 2016).

1.3 Study species: Calanus finmarchicus

1.3.1 Ecological role and life cycle

Calanus finmarchicus (Gunnerus) is a marine copepod species of high importance in the North Atlantic Ocean due to its high abundance and by being a link between primary producers and higher trophic levels in the marine food chain. The species is important prey for many commercial fish species such as mackerel, salmon, herring and capelin, while the egg and nauplius of *C. finmarchicus* serve as prey for e.g. cod larvae (Leiknes et al., 2014, Melle et al., 2014, Heath and Lough, 2007).

Copepods develop from egg stage through six naupliar stages (NI-NVI) and five copepodite stages (CI-CV) before becoming adults. Usually a single generation is produced per year, although up to three generations can occur. The reproduction and development of copepods are tightly connected to the phytoplankton spring bloom and pre-bloom. Around the onset of the phytoplankton blooms, the copepods ascend from deep water towards the surface to feed and reproduce as adult copepods. The new generation develops to the CV-stage before they return to deeper waters for hibernation during the mid to late summer, or alternatively moult into adult stage and reproduce again (Melle et al., 2014, Broms and Melle, 2007).

1.3.2 Feeding, digestion and faecal pellet production

First feeding starts when *C. finmarchicus* nauplii reach NIII stage (Melle et al., 2014). The species is both described as herbivorous and omnivorous, preying both on phytoplankton such as diatoms, and microzooplankton such as ciliates and copepod nauplii (Leiknes et al., 2014). *C. finmarchicus* is a filter-feeder, also capable of selective feeding (Marshall and Orr, 1972), and can change its feeding strategy dependent on type and abundance of prey (Leiknes et al., 2014). Also prey size can be an important factor for prey selection (Leiknes et al., 2014). When nauplii, copepodites and adult *C. finmarchicus* were offered algae with similar external

morphology, the size appeared to be a determinant factor for selection of prey (Meyer et al., 2002).

Food particles can be broken down into smaller fragments, presumably by the copepods` chitinous teeth and its mandibles before transport into the gut for further digestion by digestive enzymes. Undigested food gathers in the posterior part of the gut where it is packed into faecal pellets. The pellets are often characterized by a distinct membrane which sometimes forms a tail. In *Calanus* species, the entire process from ingestion of food particles, movement through the gut and egestion as faecal pellets takes approximately 20 minutes when food is abundant (Marshall and Orr, 1972). The digestion and assimilation of nutrients have been reported to occur rapidly, although undigested food fragments can be observed in the faeces when food availability is high. During active feeding between 6 and 12 faecal pellets can be produced per hour. When food availability is limited, faecal pellet production is restricted, and the few pellets produced have been characterized as transparent "ghost pellets".

Faecal pellets from copepods play a role in the biological carbon pump in the ocean. The biological pump infers the transport of particulate organic matter (POM) produced by photosynthesis downward in the water column by sinking particles, marine organisms or physical mixing. Zooplankton and fish faecal pellets, marine snow (organic aggregates) and phytodetritus are the main components of POM. However, the importance and efficiency of this carbon transport are variable, but nevertheless regarded as important in the sequestration of CO_2 from the atmosphere (Giering et al., 2014, Turner, 2015).

1.4 Interactions between MPs and copepods

1.4.1 Uptake of MPs by marine copepods

Uptake of PS MPs in marine copepods has been demonstrated in several laboratory experiments (e.g. Cole et al. (2013), Setälä et al. (2014), Lee et al. (2013), Powell and Berry (1990)). Cole et al. (2013) observed uptake of 7.3, 20.6 and 30.6 μm PS microbeads at concentrations 3000, 2240 and 635 beads ml^{-1} respectively among 13 of 15 studied zooplankton species, in the absence of other prey. Four of the species were copepods, whereof two of the species (*Acartia clausi* and *Calanus helgolandicus*) showed a specific preference

for beads of certain sizes. The egestion of particles occurred usually within hours after ingestion, but the excretion could be delayed in the absence of food. According to the authors, the copepods could retain the MPs in their guts for a period of one week. MP uptake has also been demonstrated in the presence of algae, even at concentrations as low as 75 MP ml^{-1} (Cole et al., 2015).

Zooplankton in natural marine environments also has been observed to ingest MPs (Desforges et al., 2015, Frias et al., 2014). Up to 91 % of the individuals in proximity to a densely populated area had ingested MPs (Frias et al., 2014). Desforges et al. (2015) found that the percentage of plastic fibres ingested was higher at a location near land compared to the offshore locations, and that 100 % of the MPs found in *Neocalanus cristatus* and *Euphausia pacifica* at this location were fibres.

1.4.2 Effects of MPs on marine copepods

1.4.2.1 Energy intake and reproduction

Algae ingestion rate for the copepod *Centropages typicus* was significantly reduced in response to increasing concentrations of 7.3 µm PS MP beads (>4000 beads ml^{-1}) (Cole et al., 2013). The copepod *Calanus helgolandicus* reduced algae ingestion by 11 % after 24 hour exposure to 20.0 µm PS microbeads (75 particles ml^{-1}) (Cole et al., 2015). Furthermore, algae ingestion by *Calanus pacificus* nauplii decreased in the presence of PS MPs (Fernández, 1979). Reduced feeding on algae can according to Cole et al. (2013) be due to a lack of ability among the copepods to differentiate between algae and MPs if they are of similar size. The shift to an ingestion of smaller sized algae during exposure to 20.0 µm PS microbeads reduced carbon biomass ingestion by *C. helgolandicus*, and was suggested to be due to an avoidance of the larger beads (Cole et al., 2015).

Exposure to 20 µm PS MPs (75 beads ml^{-1}) for six days had impacts on reproduction by significantly decreasing hatching success at day three of exposure while a significantly decreased egg size was observed from day four. The reproductive effects were assumed to be linked to the observed reduced intake of carbon biomass (Cole et al., 2015). Smaller PS beads (0.5 and 6 µm) in high concentrations (0, 0.125, 1.25, 12.5 and 25 µg ml^{-1} respectively) resulted in reduced fecundity that probably was attributed to a lower energy intake, reduced digestive function or physical hindering of fertilization (Lee et al., 2013).

Other studies have shown a close relationship between algae ingestion and egg production (Kiørboe et al., 1985). *C. finmarchicus* respond rapidly to starvation, resulting in a dramatic decrease in egg production rate before an almost cessation within days. Thus, reproduction is dependent on food availability and not internal lipid reservoirs. A restoration of energetic balance was needed before any resumption of reproduction occurred. The effect varied between mature and immature individuals and duration of starvation, however, the fecundity was assumed to be permanently reduced (Niehoff, 2000).

1.4.2.2 Distribution of faecal pellets

Ingestion of MPs and incorporation into faecal pellets by copepods have been suggested as a sink for MPs in the ocean. The faecal pellets egested by *C. helgolandicus* exposed to PS microbeads, had a significant reduction in density and the related sinking velocity was reduced 2.25-fold (Cole et al., 2016). An increased retention time in the upper parts of the water column could have implications for carbon cycling in terms of reduced downward transport of DOM and increased remineralization in upper parts. Furthermore it increases the possibility of ingestion and fragmentation of faeces for example by coprophagic copepods and microbial degradation of the faecal pellets. Protozoa (mainly ciliates and dinoflagellates) and bacteria are regarded as the most important in the degradation of copepod faecal pellets (Morata and Seuthe, 2014, Poulsen and Iversen, 2008). However, the copepods are also contributing to the degradation and vertical transport of faeces to a smaller extent by coprohexy (fragmentation of faeces), by which the fragmented faeces also become more available to degradation by the protozooplankton and bacteria. Coprophagy (ingestion of faecal pellets) and coprochaly (loosening of faecal pellets) are also mechanisms of faeces removal and degradation. Fragmentation of the faeces further implies a decreased sinking velocity and an increased residence time in the water column. The faeces degradation by these mechanisms are enhanced by an increased concentration of other prey as this will increase suspension feeding, and by increased faeces concentrations due to an increased encounter rate (Iversen and Poulsen, 2007).

1.4.2.3 Accumulation and trophic transfer

A potential issue of MP ingestion by marine organisms is bioaccumulation and transfer of MPs from one trophic level to the next (Rochman et al., 2015, Law and Thompson, 2014). In

controlled experiments, trophic transfer of MPs has been demonstrated from mesozooplankton (*Marenzelleria* spp. and copepods) to macrozooplankton (mysid shrimps) (Setälä et al., 2014). Another example is the transfer of MPs from brine shrimp to zebrafish, where B(a)P associated with the ingested MPs desorbed in the intestines of the fish and eventually transferred to the liver (Batel et al., 2016). The discovery of anthropogenic litter in 67 % of purchased fish and shellfish species in the USA and 55 % of the species purchased in Indonesia is of concern for human health with regard to the chemical transfer from ingested plastics to fish (Rochman et al., 2013a, Batel et al., 2016), and if the organism is eaten whole as is the case for some shellfish (Rochman et al., 2015).

1.4.2.4 Physical damage and toxicity

The expected physical effects of ingested MPs in invertebrates are e.g. blocking of the digestive tract and abrasions following the ingestion of MPs of various shapes (Wright et al., 2013). It has further been shown that PS MPs can adhere to external surfaces of copepods, between the carapace segments, between filament hairs of e.g. the swimming legs and antennules, appendages important for the copepods functionality and behaviour (Cole et al., 2013).

In a two-generation chronic toxicity test with the copepod species *Tigriopus japonicus*, PS MP beads (0.05 μm) caused mortality at concentrations >12.5 μg beads ml^{-1} of both nauplii and copepodites in the F_0 generation. Higher concentrations caused mortality in the following generation (Lee et al., 2013).

1.5 Aims of the study

The main aims of the present master´s thesis were to study the uptake dynamics of polystyrene (PS) microplastics (MPs) in the marine copepod *Calanus finmarchicus* in the presence of the microalgae prey *Rhodomonas baltica*, and excretion dynamics of MPs both in the presence and absence of *R. baltica*.

Sub-objectives included:

- Study the impacts of MPs on algae ingestion
- Study if any preference for either MPs or algae exists
- Study the impact of MPs on the faeces and faecal pellet production
- Determine whether excretion of MPs is affected by the presence/absence of microalgae.
- Study the distribution of the MPs in the exposure system

2. Materials and Methods

2.1 Experimental setup

The study of uptake and excretion dynamics of PS microbeads in the copepod *C. finmarchicus* was performed in March and April 2017 at NTNU Sealab in Trondheim. The experiment was meant to be carried out as one experiment, but had to be separated into two studies, an uptake study and an excretion study, each lasting for up to two weeks. This was a necessity due to limitations of equipment, and also because the time consuming procedure would result in a larger variation in development between the copepods used in the beginning and those used in the end of the experiment.

In the uptake study, the copepods were exposed to both PS microbeads and a microalgae (*R. baltica*) for different time intervals (Figure 2.1). For the excretion study, a 24 hour exposure to PS and *R. baltica* was followed by depuration in either FSW only or FSW with algae (*R. baltica*) for different time intervals (Figure 2.2). At termination, the PS content was measured in both the copepods, the faeces and in the exposure media. Also faecal pellet production and PS remaining on surfaces were measured (Figure 2.3). Both experiments were performed with 1 litre bottles placed on plankton wheels. All exposure bottles were terminated at the time when the samples were drawn. As the termination step was common for both studies, it will be a common description of this step. Algae growth in the absence of copepods was also measured during the uptake study. Three replicate bottles of *R. baltica* (7,500 cells ml^{-1}) in FSW were prepared and treated in the same way as the bottles containing copepods. Samples from the medium were taken at termination after 24, 48 and 72 hours.

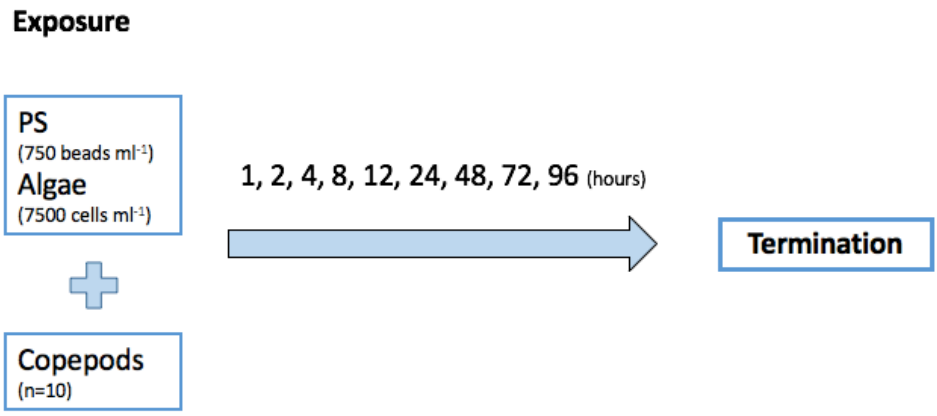

Figure 2.1. Experimental setup for the uptake study. The copepods *C. finmarchicus* (10 individuals per replicate) were exposed to PS microbeads (750 beads ml^{-1}) and algae (*R. baltica*) (7,500 cells ml^{-1}) for 1,2, 4, 8, 12, 24, 48, 72 and 96 h. Each exposure group (time point) comprised 3 replicates

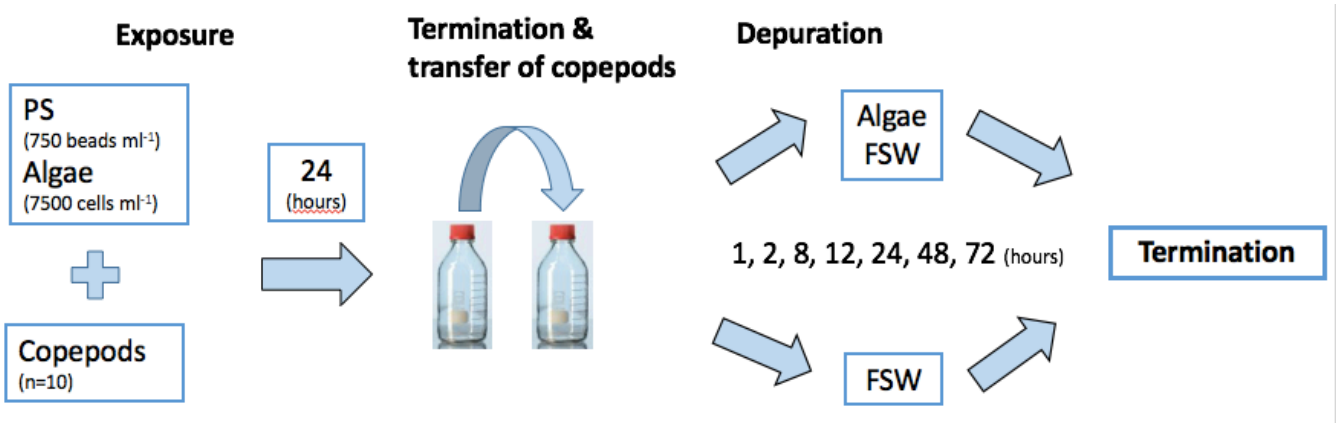

Figure 2.2. Experimental setup for the excretion study. The copepods *C. finmarchicus* (10 individuals per replicate) were exposed to PS microbeads (750 beads ml^{-1}) and algae (*R. baltica*) (7,500 cells ml^{-1}) for 24 h, before transfer to either FSW and algae (*R. baltica*) (7500 cells ml^{-1}) or FSW only for 1, 2, 8, 12, 24, 48 and 72 h. Each exposure group (time point) comprised 3 replicates.

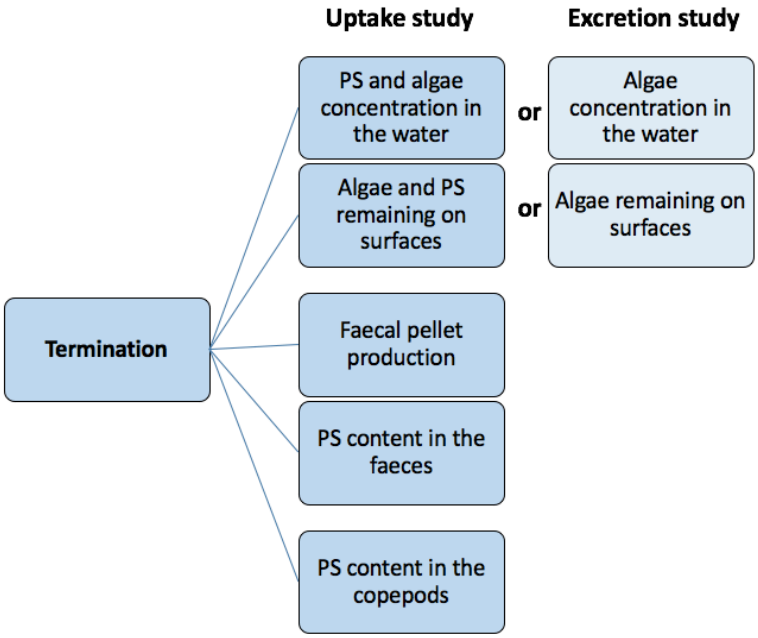

Figure 2.3. Termination and the subsequent analysis in both the uptake and the excretion study. Note that faecal pellet production, PS content in the faeces and PS content in the copepods also were analysed in the excretion study.

2.2 Experimental animals

Female *C. finmarchicus* from the inhouse culture at NTNU SeaLab in Trondheim (Hansen et al., 2007) were used in both experiments. Only non-ovulating female individuals in early adult stage were used. The copepods were sorted out from the culture in the evening before they were used in experiments and stored in buckets with FSW (Millipore Sterivex-GP, 0.22 μm filter unit) at ambient temperature in the same room as the culture. The copepods were fed with the microalgae *R. baltica ad libitum*. Due to the time difference between the uptake and excretion study, copepods from two different culture tanks had to be used in the two experiments, respectively. However, the copepods were from the same generation and were offspring from the same parental culture tank.

2.3 Preparation of solutions

2.3.1 PS MP stock solution

10 μm PS microspheres (1.05 g/cc) were purchased from Cospheric. A working stock solution of 50 ml was prepared at a concentration so that 1 ml could be added to the exposure bottles

to obtain a final concentration of 750 particles ml^{-1}. A Beckman Coulter Multisizer 3 particle analyser was used to verify the particle concentration in the stock solution and to measure the concentration of the particles after they were mixed with FSW. Two different stock solutions were prepared for the two studies, both with a concentration of approximately 847,500 particles ml^{-1}.

2.3.2 Microlgae solution

The cryptophyte *R. baltica* (clone NIVA 5/91) was used in all the experiments, harvested from an in-house culture in exponential growth phase at NTNU SeaLab. Shortly before onset of an exposure, the concentration of algae in a sample from the culture was measured on the Beckman Coulter Multisizer 3. The average of 3 measurements from a single sample was used for calculating the volume of algae stock culture needed to be added to each bottle to obtain a concentration of 7,500 algae cells ml^{-1}, after filling the bottle to the rim with FSW. To ensure the right concentration in the exposure bottles was achieved, a 20 ml sample post mixing was analysed on the coulter counter. However, this was only done for the excretion studies.

2.4 Experimental procedure

2.4.1 Uptake study

The copepods were exposed to PS microbeads (750 particles ml^{-1}) and algae (7,500 algae cells ml^{-1}) for 1, 2, 4, 8, 12, 24, 48, 72 and 96 h. Each exposure group (time point) comprised 3 replicates. The different exposure groups were not started at the same time, but over a period of 1-2 weeks**.** This was done to have more time for the comprehensive termination steps, and to ensure a more feasible time schedule for sampling.

Before the onset of one exposure group the bottles were almost filled up with FSW, but with remaining headspace for addition of algae, particles and copepods. 1 L borosilicate glass bottles were used in both the uptake and excretion study, with an average total volume of 1,130 ml. Before use, the bottles were stored in a temperature controlled room at 10 ℃. Further addition of both MPs, algae and copepods was done in a temperature controlled room at the same temperature.

1 ml of the working PS stock solution was transferred to each of the exposure bottles to give a final concentration of 750 particles ml^{-1}. As the particles settled in the bottles due to their density, the stock solution was stirred properly before transfer. An aliquot of the algae culture was added shortly after to give a set concentration of 7,500 algae cells ml^{-1}. This was done for all 3 replicates at the same time, but the transfer of copepods (n=10) to each replicate was done with an interval of maximum 20 minutes to ensure sufficient time for the sampling during termination procedure. The bottles were gently shaken before the copepods were added, and shortly after the addition of the copepods the bottles were filled to the rim, but left space for a small air bubble and placed randomly on plankton wheels (Figure 2.4) rotating at 0.8 rpm in a temperature controlled room. The rotating plankton wheels (n=6) were all placed in water baths with a recorded water temperature of 8.4±0.4℃, and each could hold 6 exposure flasks.

The control groups consisted of copepods (n=10) only feed with *R. baltica*, (7,500 cells ml^{-1}) for 24, 48 and 72 h. Each control group had 3 replicates.

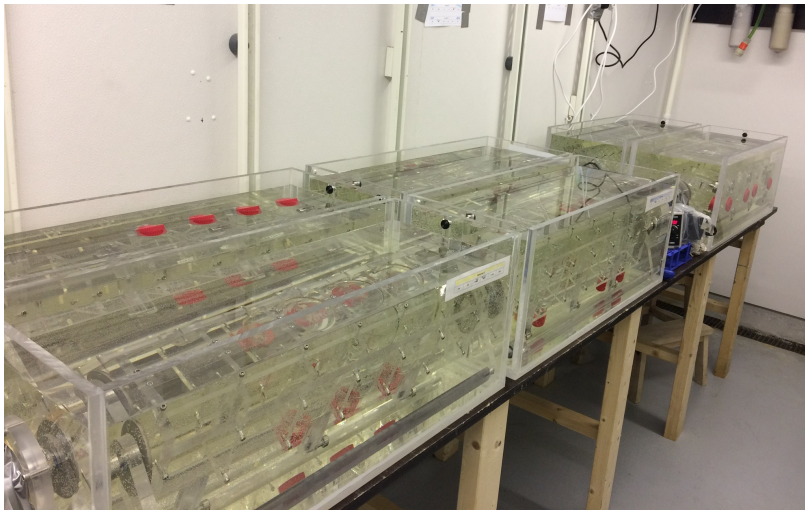

Figure 2.4. The six plankton wheels used in the experiments.

2.4.2 Excretion study

The copepods in the excretion study were first exposed to 750 MPs ml^{-1} and 7,500 algae cells ml^{-1} for 24 hours as described for the uptake study using 3 replicates of 10 copepods each, before transfer to bottles with either FSW or FSW and algae (*R. baltica*) for depuration for 1, 2, 8, 24, 48 and 72 hours. Also in this experiment, the different exposure groups were started at different time points over a period of 1 week. However, groups transferred to FSW with algae and groups transferred to FSW without algae with identical depuration time, were both started at the same day.

After 24 hours exposure, the copepods were transferred, first to a bucket filled with FSW, then to a new, marked flask with either algae (7,500 algae cells ml^{-1}) or FSW only. When

transferring, it was attempted to transfer as little water as possible together with the copepods. The bucket with FSW was changed between each exposure group.

The control groups were only exposed to an algae suspension (7,500 algae cells ml^{-1}) during the 24 hours "exposure" phase, but otherwise treated in the same way as the ones exposed to MPs and algae. 3 replicates containing 10 copepods were transferred to either FSW with or without algae (7,500 algae cells ml^{-1}) for depuration for 24 and 72 hours.

2.4.3 Termination and analysis

2.4.3.1 MP and microalgae concentrations in the exposure media

The Beckman Coulter Multisizer 3 (Figure 2.5) was used for measuring algae and MP concentrations in the exposure flasks that were terminated in the uptake study, and to measure the algae concentration in the terminated flasks used for depuration in the excretion study.

The following method was applied to extract the samples from the bottles in both studies: A 20 ml pipette was used to collect in total 100 ml of the solution from the upper part of the bottle. The reason for sampling in the upper part of the flask was to minimize the contact between MPs and algae on the one side, and the pipette, and hence reduce the loss of particles due to adhering to the pipette. The sample was transferred to a dedicated glass beaker for further analysis using the Beckman Coulter. During analyses the glass stirrer of the instrument was used to keep the algae cells and/or MPs in suspension. The pipette used for the sampling was rinsed first with tap water, before pipetting 20 ml deionised water twice to clean the pipette between every sampling. The glass beaker and stirrer were also washed carefully between each sample.

2.4.3.2 MPs and microalgae on exposure bottle surfaces

After removing the content, the bottle was added 100 ml of FSW and shaken vigorously to disperse particles adhering to the glass wall. The water dispersion was then transferred to the glass beaker and particle concentration measured on the Beckman Coulter during stirring, as described above.

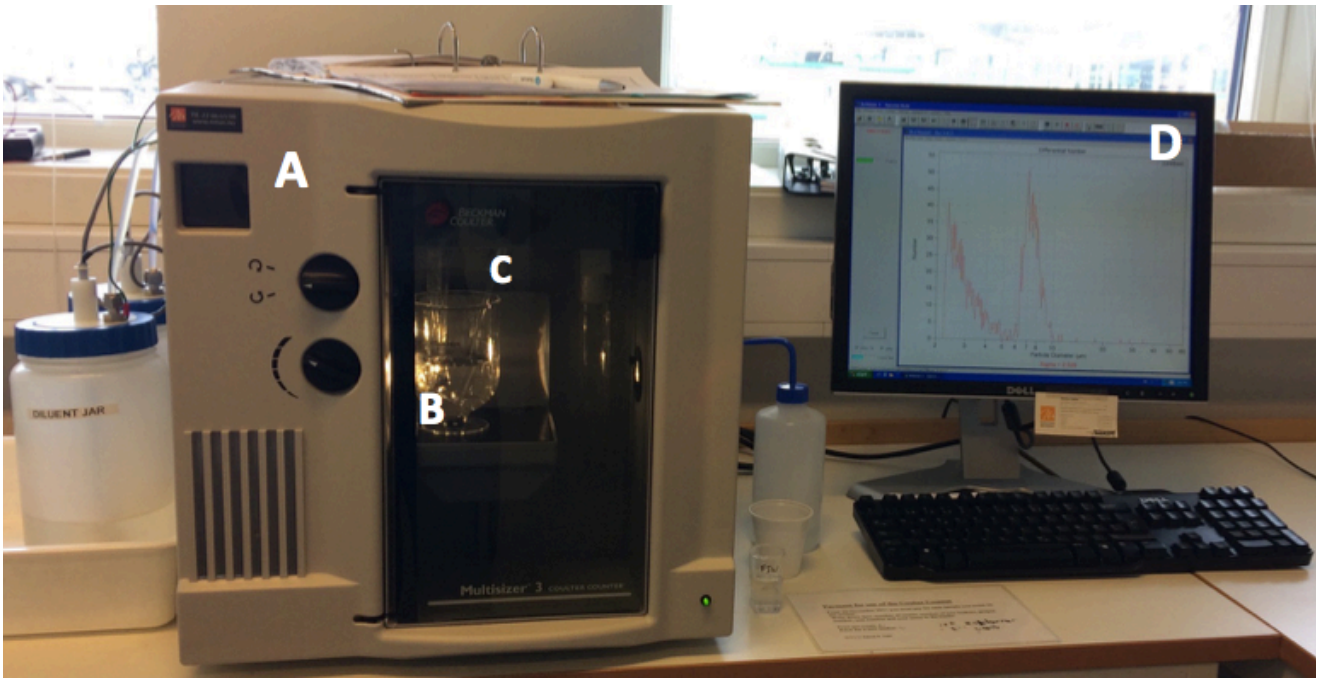

Figure 2.5. The Beckman Coulter Multisizer 3 (A) used for measuring particle (MPs and algae) concentration and diameter. 3 samples of 1 ml were drawn from the glass beaker (B), and the average value of the measurements was used. A glass stirrer (C) was used to keep the particles in suspension. Results were displayed in the program Beckman Coulter Multisizer 3 Control Software (D).

2.4.3.3 Collection of copepods

The content of the exposure flasks was sieved through a 40 μm mesh placed in a large beaker to collect copepods and faeces (Figure 2.6). The copepods were further transferred to a petri dish with a tweezer, and then photographed under a stereomicroscope (Leica MZ APO with 0.63x APO objective) mounted on a transmitted light stand (Leica HL base). The sedative MS-222 (Finquel vet., Argent Labs) was used to immobilize the copepods during photography. A ccd camera (Nikon DS-Fi1/DS-U2 controller) connected to the stereomicroscope by a 1.0x video adapter (Diagnostic Instruments) and controlled by a PC running NIS Elements-F (Nikon, v.4.3) was used for capturing the images (Figure 2.7).

Each of the photographed copepod was tagged with name and number, and placed in a corresponding pre-marked 8 ml scintillation vial containing 0.5 ml hypochlorite (15 %) for dissolution.

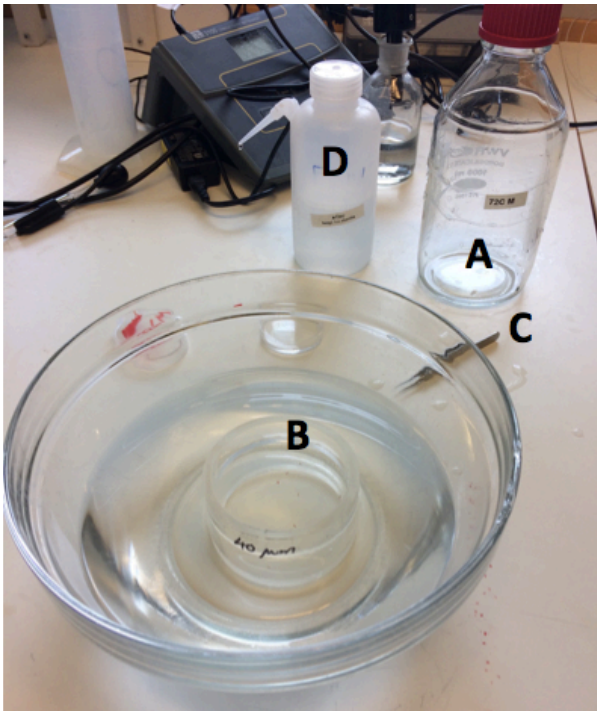

Figure 2.6. The content of the exposure flask (A) was sieved through a 40 μm mesh (B) in a glass beaker. A tweezer (C) was used to collect the copepods, and squeeze bottle with FSW (D) was used to rinse the faecal pellets down in the glass petri dishes.

2.4.3.4 Quantification of faecal pellets

All faeces on the mesh were washed down in glass petri dishes using a squeeze bottle with FSW. To simplify the quantification, the dishes were pre-marked with a cross in the bottom, dividing it into four quadrants. The quantification was done by photographing each quadrant of the petri dish, using a macroscope (Leica Z6 APO with a 0.5x APO objective). To achieve the desired resolution and field of view of the images a Nikon DS-Ri camera was coupled to the macroscope with a 0.5x video adapter (Diagnostic Instruments), controlled by a DS-U2 camera controller (Nikon), connected to a PC running the software NIS-Elements F 4.3 (Nikon). The photographing was complicated by the pellets readily drifting around in response to even soft handling of the dish. Therefore, to smooth the movement of the dish, a motorized stage H101 A (Prior Scientific) was installed on a transmitted light base (Leica TL5000 Ergo) to ease the manipulation of the samples.

The pictures were tagged with name and number, and all pellets in each picture were subsequently quantified by using the ImageJ software (National Institute of Health, Bethesda Maryland, USA).

All faeces from each triplicate were collected in a 40 ml tube, fixed with Lugols solution in 15 % acetic acid and stored at 10℃ until further analysis.

The faecal pellet production in the groups transferred to FSW without algae in the excretion study, was generally limited. Often no faeces could be observed on the mesh, and it was initially not made any attempt collecting the faeces from these groups. However, for following groups with no visible faecal pellets, the sample was washed down in glass petri dishes and studied under the stereomicroscope (Leica MZ APO). Several pale faecal pellets were observed, and therefore fixated the same way as the other faeces.

2.4.3.5 Quantification of MPs in the faeces

For analysing the MP content in the faeces, pellets were pipetted from the fixed samples in the 40 ml tubes, transferred to microscope slides, and studied under a stereomicroscope (Leica MZ APO). The first 25 pellets observed which were intact and with no obvious breaks or damage, were selected for photography and further analysis. The same setup for photography was used as during the photography of the individual copepods, except for the use of a 1.6x objective (Figure 2.7) to get the desired resolution. To have the particles in the faeces distributed in one layer, a small stick was used to gently tap the coverslip. The software ImageJ was used to count the individual particles manually afterwards.

For samples with less than 25 faecal pellets, the total number of pellets was photographed. The content of the glass vials with fixed faeces was poured out into a large glass petri dish placed under the stereomicroscope, and the pellets present were transferred to microscopic slides. They were then counted under the stereomicroscope and photographed for later quantification of MPs.

In the control groups, 5 pellets were photographed and around 20 pellets were checked for presence of MPs or other abnormalities. As MPs were observed in faeces in some of the control groups, all faeces with MPs were photographed in these groups and the MPs quantified.

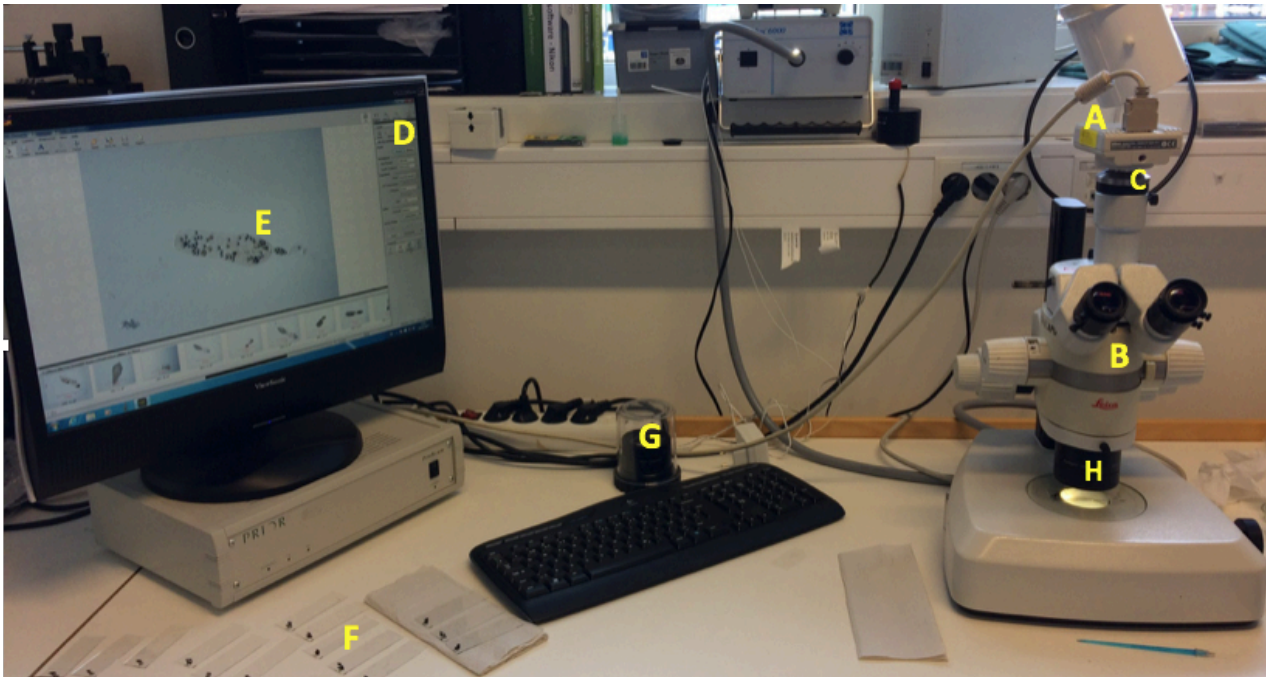

Figure 2.7. The equipment and setup during photography of copepods and faecal pellets in both the uptake and excretion study. The Nikon DS-Fi1 camera (A) was connected to the stereomicroscope Leica MZ APO (B) by a video adapter (1.0x) (Diagnostic Instruments) (C) and controlled by DS-U2 microscope camera controller (Nikon). Images were transferred to the imaging software NIS-Elements F 4.3 (Nikon) (D). A faecal pellet (E) from one of the microscope slides (F) can be seen on the screen. During photography of copepods, a 0.63x objective lens was used (G), while a 1.6x (H) was used for the faeces.

2.4.3.6 Quantification of MPs in the copepods

The copepods were as previously described placed in hypochlorite for dissolution, and stored at room temperature for later analysis of MP content. For the analysis, an inverted microscope Eclipse TE2000-S (Nikon) was used to quantify the particles. However, as the particles were distributed in several layers, the vials were filled up with distilled water and centrifuged (Universal 32, Hettich) at 3,000 rpm for 3 min in order to collect all particles in one layer at the bottom, and hence simplify the quantification in the microscope. To ensure a systematic quantification, and to avoid counting particles several times, a frame (15x15mm) was inserted into the eyepiece of the microscope, returning a 1.5 mm field of view with a 10x objective (Nikon CFI 10X Plan Fluor). Also, a motorized stage (model H117, Prior Scientific) with linear encoders (Renishaw) was installed and controlled with a ProScan II controller (Prior Scientific) by a touch screen controller (model SC152KB, Prior Scientific). This enabled a

systematic stepwise horizontal and vertical movement of the visual field of 1.5 mm at a time (Figure 2.8). Often the manual focus was used instead to scan vertically through the sample.

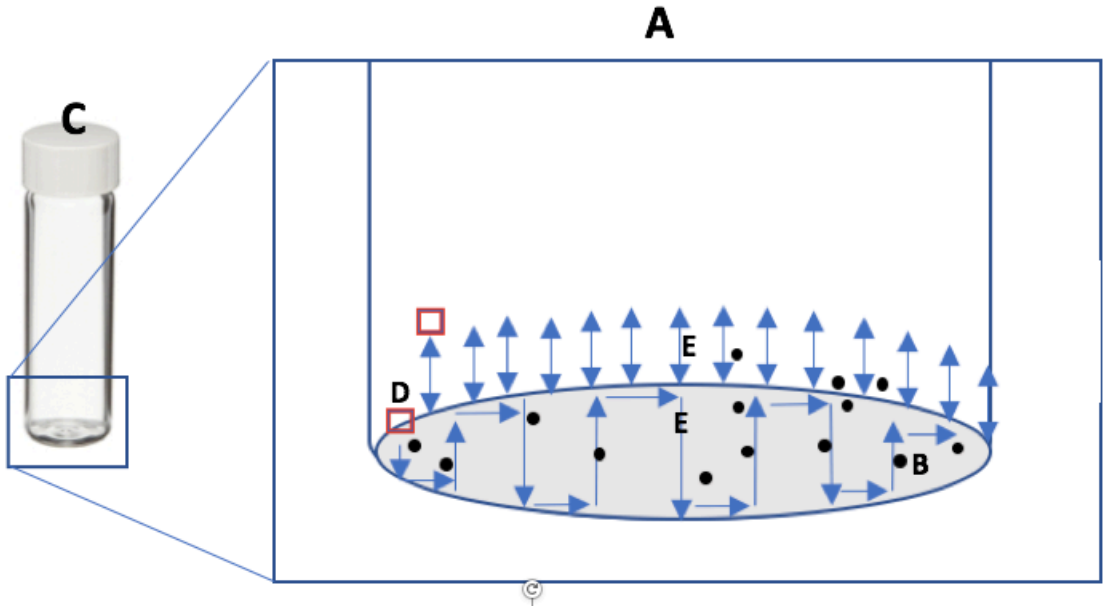

Figure 2.8. Illustration (A) of the procedure for counting the PS particles (B) in the dissolved copepods in the 8 ml scintillation vials (C). A motorized stage H117 (Prior Scientific) connected to a linear encoder (Renishaw), further coupled to a control unit ProScan II (Prior Scientific), controlled by a touch screen controller SC152KB (Prior Scientific), enabled a systematic movement of the 1.5x1.5 mm visual field (D) horizontally and vertically (E) at and near the bottom of the vials

2.5 Calculations and statistics

All sorting of data, calculations and smaller statistical operations (t-tests for comparisons of replicates) were performed in Microsoft Excel (version 2017). Graphing and further statistical analysis were performed in SigmaPlot 13.0 (Systat Software). In both the uptake and excretion study the MP content in the copepods in each replicate was compared to each other (t-test) before the replicates were merged. For all statistical analysis the level of significance was set to 0.05.

2.5.1 Analysis of coulter counter data

The results of the coulter counter measurements of MP and algae concentrations in the bottles were analysed with the Beckman Coulter Multisizer 3 Control Software. The average of the three samples of 1 ml drawn from the sample of the medium was used for further calculations. The peaks of the MPs and the algae were easy to distinguish. Cells were usually in the size

range from around 6.0 μm to around 9.5 μm, whereas MPs were in the size range around 9.7-9.8 to around 11.2 μm.

2.5.2 Removal of MPs and algae from the medium

Estimates of numbers of MPs and algae removed from the medium in the uptake study, and the number of algae removed during the excretion study were made by subtracting the sum of the MPs/algae in the medium at a given sampling point in one replicate plus the MPs/algae attached to surfaces at the corresponding time for the corresponding replicate, from the MPs/algae initially present in the replicate bottle (nominal values) (750 MPs ml^{-1} corresponding to 847,500 MPs $bottle^{-1}$ and 7,500 algae cells ml^{-1} corresponding to 8,475,000 MPs $bottle^{-1}$).

2.5.3 Number of MPs per faeces

The average number of MPs per faeces in a replicate was based on the MP content in 25 faecal pellets randomly selected from all faeces collected from the replicate. However, 25 faeces were not always obtained from the bottles, especially in the excretion study without algae. Occasionally data was not obtained from 1, 2 or all replicates at all. In the uptake study, 25 pellets were analysed for time point 8-96 hours, while 20-21 pellets were analysed at time point 1 hour, 21-25 faeces at time point 2 hours and 7-23 faeces at time point 4 hours. In the excretion study with algae, 25 faeces were analysed for all replicates except 1 replicate at 1 hour where 20 were analysed. With no algae available, at time point 1 hour, 3 replicates of 14-25 faeces were analysed, at 2 hours 1 replicate of 18 faeces, 8 hours 3 replicates of 11-18 faeces, no faeces were collected at 24 hours, at 48 hours 1 replicate of 6 faeces and at 72 hours 3 replicates of 5-8 faeces were analysed.

2.5.4 Cumulative MP excretion and cumulative faeces production

The cumulative MP excretion was calculated by multiplying the average MP content per faeces in one replicate with the corresponding cumulative faeces production per copepod in the same replicate at the same time point. Calculation of the average faeces production between the time points was performed by subtracting the average cumulative faeces production in the previous time point from each of the replicates in the next time point.

2.5.5 MPs per faeces between time points

Calculation of the average number of MPs per faecal pellet produced between samplings in the uptake study was done by dividing the average cumulative MPs excretion per copepod between the sampling points in one replicate by the cumulative faeces production per copepod in the corresponding replicate in the same period. The cumulative MP excretion between the time points was calculated for each replicate by subtracting the average value of the previous time points from each of the replicates of the specific time point.

2.5.6 Replicates removed from the computations

Replicate C72CU and 72CU were removed from the computations as the 72CU replicate most likely was terminated on the time point for C72CU, due to both incorrect marking of the bottles and a misplacement on the plankton wheel.

3. Results

The results from the uptake and excretion study will be presented under three main topics; 1) the uptake of MPs as measured in the copepods and in the faeces during the exposure period, 2) the correspondent removal of MPs and algae from the medium during MP exposure, and 3) the excretion of MPs during the depuration experimental period as measured in the copepods and in the faeces.

3.1 MP uptake in C. finmarchicus

3.1.1 MP uptake measured in *C. finmarchicus*

Only copepods sampled after 1, 12, 24 and 72 hours exposure to 750 PS microbeads ml^{-1} were analysed for MP content (Figure 3.1), and based on these data MP content peaked at 12 hours before declining. The average MP content in the copepods differed significantly between all samplings ($p<0.05$), except between 1 and 72 hours ($p=0.72$) (α).

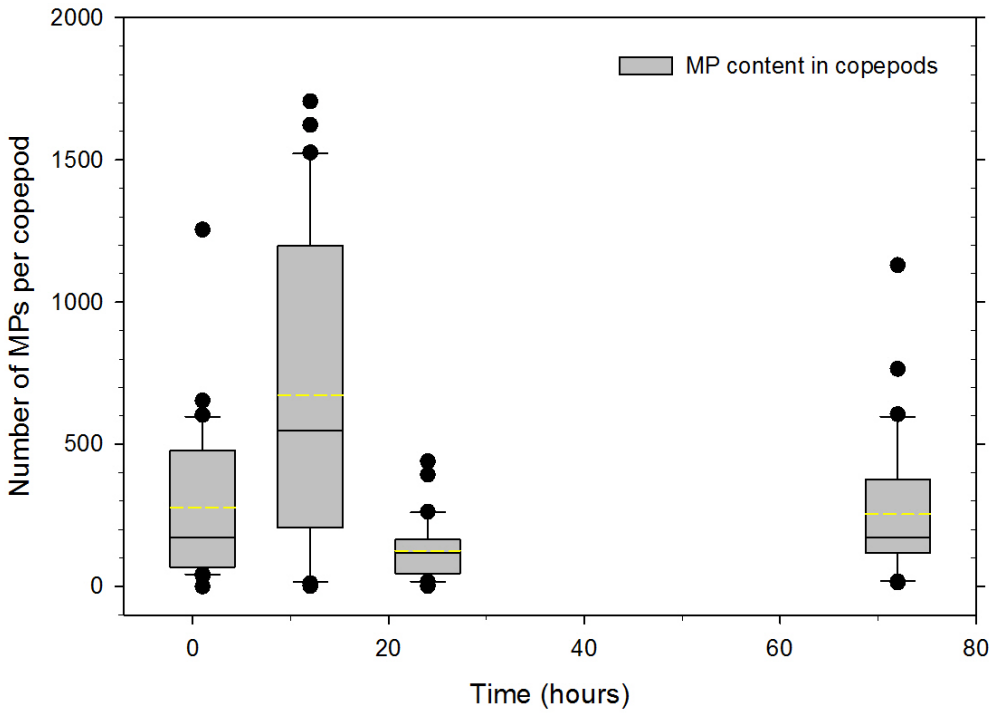

Figure 3.1. Average MP content per *C. finmarchicus* of MPs for each time point 1, 12, 24 and 72 hours (n=30). No significant difference in MP content was found between replicate bottles sampled at each sampling time, and the copepods in the three replicates were therefore pooled (n=30). The solid black line indicates median, the dashed yellow line gives mean. The α indicates groups *not* significantly different from each other ($p>0.05$).

The MP quantification in *C. finmarchicus* was confirmed by visual observations of MP content in the gut of the copepods (Figure 3.2).

Figure 3.2. A faecal pellet loaded with MPs in the rear gut of a young female *C. finmarchicus* in pre-egg laying stage exposed to PS microbeads for 1 hour, photographed shortly after sampling and before anaesthesia with MS-222 and dissolution in hypochlorite (15 %).

3.1.2 MPs measured in the faeces during MP exposure

3.1.2.1 Cumulative faecal production

Cumulative faeces production (per copepod) increased linearly throughout the exposure period ($R^2 = 0.98$) (Figure 3.3). However, as a slight levelling off of faeces production was suspected, therefore an additional polynomial curve was fitted to the data returning a similar good fit ($R^2 = 0.98$). The polynomial curve returned a lower y-intercept than the linear curve, however neither were starting at 0**.** The cumulative faeces production among non-exposed copepods (control) increased in a similar manner as the exposed copepods up to 48 hours. However at 72 hours cumulative faeces production appeared suppressed, both compared to control production 48 hours and to exposed copepods. A negative cumulative increase in the faeces production is not realistic, and it is therefore suggested that faeces production by controls at 72 hours was somehow hampered, or, more likely, the produced faeces had disintegrated.

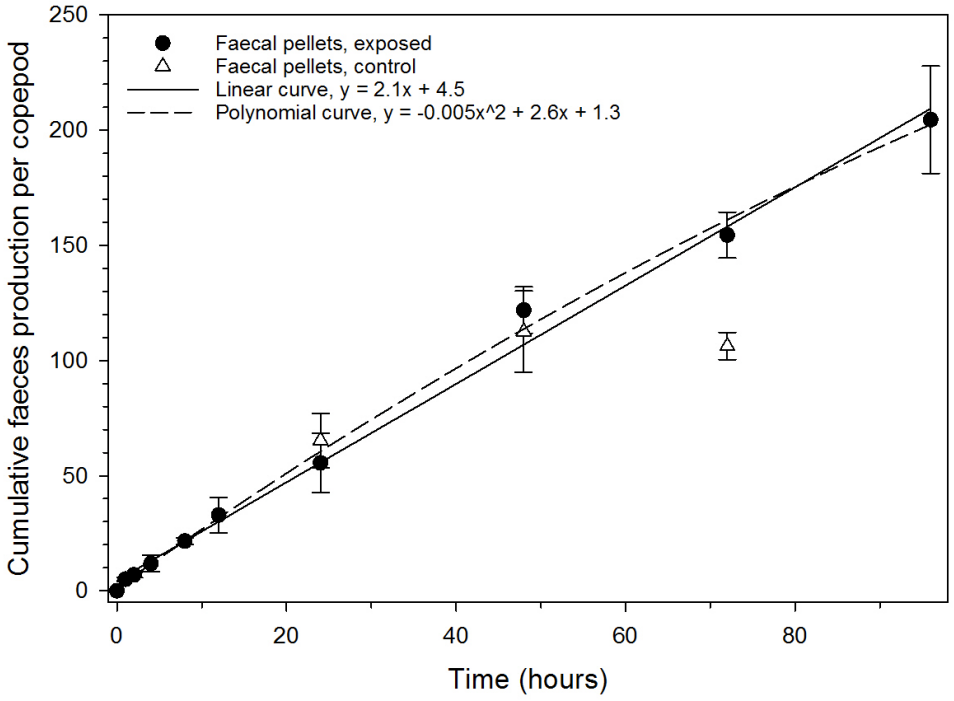

Figure 3.3. Cumulative faeces production during exposure to PS microbeads (black dots) and non-exposed copepods (control, white triangles), all numbers per *C. finmarchicus*. Mean±SD of three replicate bottles are given for each sampling point, each replicate containing 10 *C. finmarchicus* (except in replicate B in control group 72 hours, n=11). Linear and polynomial trend lines fitted to the data for the exposed copepods.

3.1.2.2 Cumulative MP excretion via the faeces

The cumulative MP number excreted by *C. finmarchicus* in the faecal pellets increased throughout the uptake study (Figure 3.4). However, different linear trends with different slopes were observed for time points 0-12 hours (R^2=0.96) and 12-96 hours (R^2=0.77).

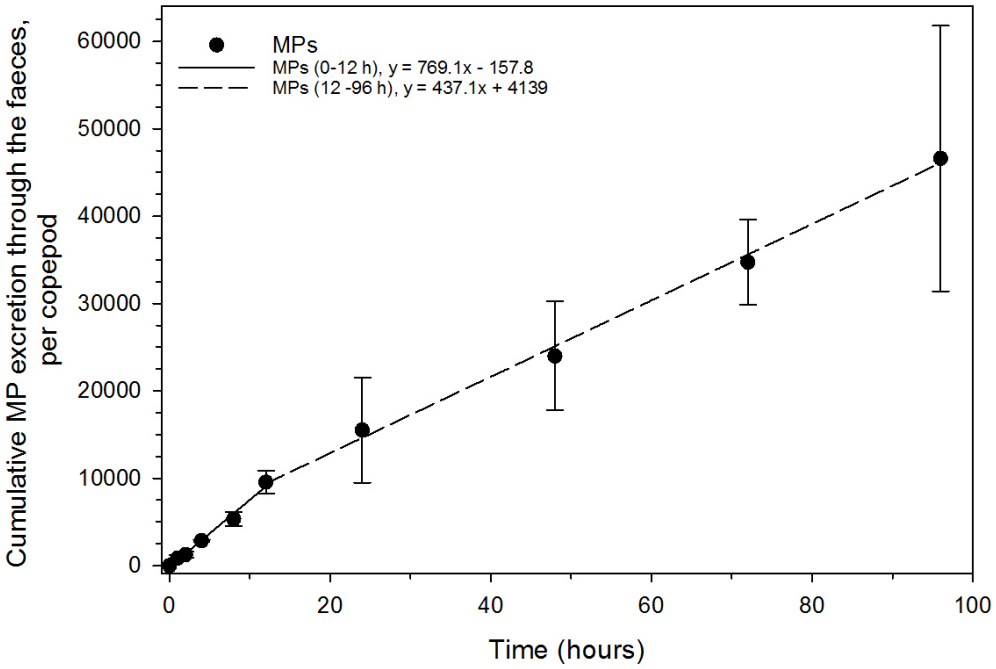

Figure 3.4. Cumulative MP excretion through the faeces (all numbers per *C. finmarchicus*). Mean±SD for three replicate bottles, each containing 10 copepods, at each sampling point during MP exposure. Separate linear curves are fitted for the time points 0-12 hours (solid line) and time points 12-96 hours (dashed line).

3.1.2.3 MP content per faeces between the sampling points

The average MP content per faecal pellets produced by each individual *C. finmarchicus* between the sampling points is shown in Figure 3.5. Large variations among the replicates were observed although the average MP content per faeces was relatively stable throughout the period, with an average of 323 MPs per faeces.

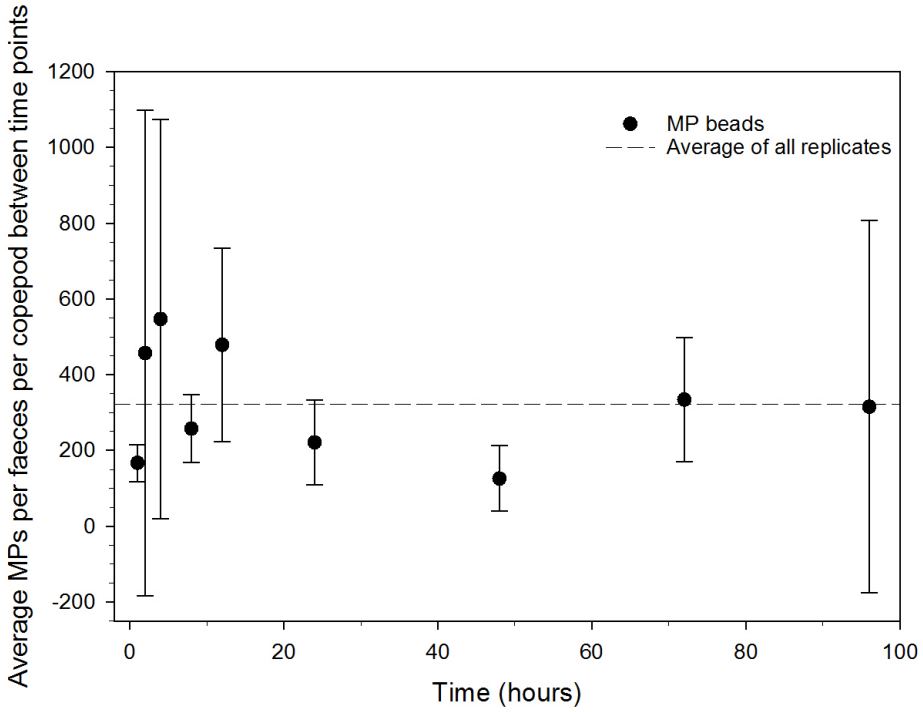

Figure 3.5. Number of MPs per faecal pellet produced by *C. finmarchicus* between the sampling points. Values are calculated by dividing cumulative MP excretion per copepod between the sampling points by cumulative faeces production per copepod in the same period. Mean±SD based on MPs per faeces in the three replicate bottles between each sampling point, each replicate containing 10 *C. finmarchicus*. Dashed line represents the average MP content per faeces of all replicates (323 MPs per faeces).

3.1.2.4 Distribution of MPs in the faeces

A random selection of faecal pellets from the samplings after 1, 12, 24 and 72 hours exposure is shown in Figure 3.6. MPs were either relatively evenly distributed (A and B) or more spread or grouped (C and D) in the faeces. Some of the faeces burst when tapping the cover slip gently in an attempt to spread out the particles more evenly in the faeces (B).

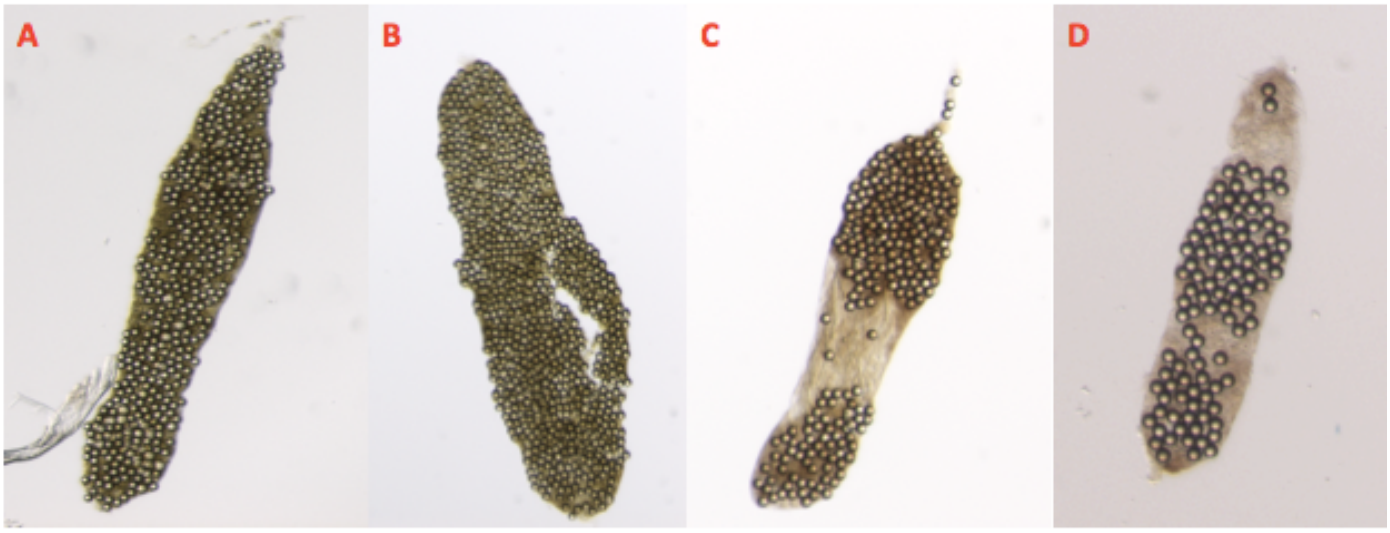

Figure 3.6. Distribution of 10 µm PS microbeads in adult female *C. finmarchicus* faecal pellets randomly selected at 1 hour (A), 12 hours (B), 24 hours (C) and 72 hours exposure to MPs (D). The faecal pellets are pressed between the microscope slide and the cover slip, and where necessary tapped gently to spread the particles more evenly.

3.2 Removal of MPs and microalgae from the medium

3.2.1 MP removal from the medium

Computed numbers of MPs removed from the medium during the uptake study are shown in Figure 3.7, (see the Materials and Methods section for further details on calculations). The measurements at 1-4 hours (especially at 4 hours) were highly variable compared to the subsequent sampling points. From 8 hours onwards the fluctuations appeared to have stabilized and MP removal increased more linearly with time, therefore a separate linear trend line was fitted to sampling points 8-96 hours (R^2 = 0.91). An extrapolation of this trend line back to time zero gave an y-value (no particles removed) in excess of 13,500, indicating an initial loss of MPs. This loss was assumed to be caused by other factors or processes than solely removal of MPs by the copepods. The initial fluctuations, which were not expected as each data point represents a cumulative value, also underpinned that unaddressed processes or factors could have affected the system during the early phase of MP exposure and led to an initial loss of particles. Additionally, a second-order polynomial curve was fitted time points 8-96 hours (R^2=0.94), due to an observed levelling off of the MP removal between 72 and 96 hours. The obtained y-intercept of 8,681 when extrapolating the polynomial trend line back to time zero, indicates a lower loss of particles compared to the linear approach.

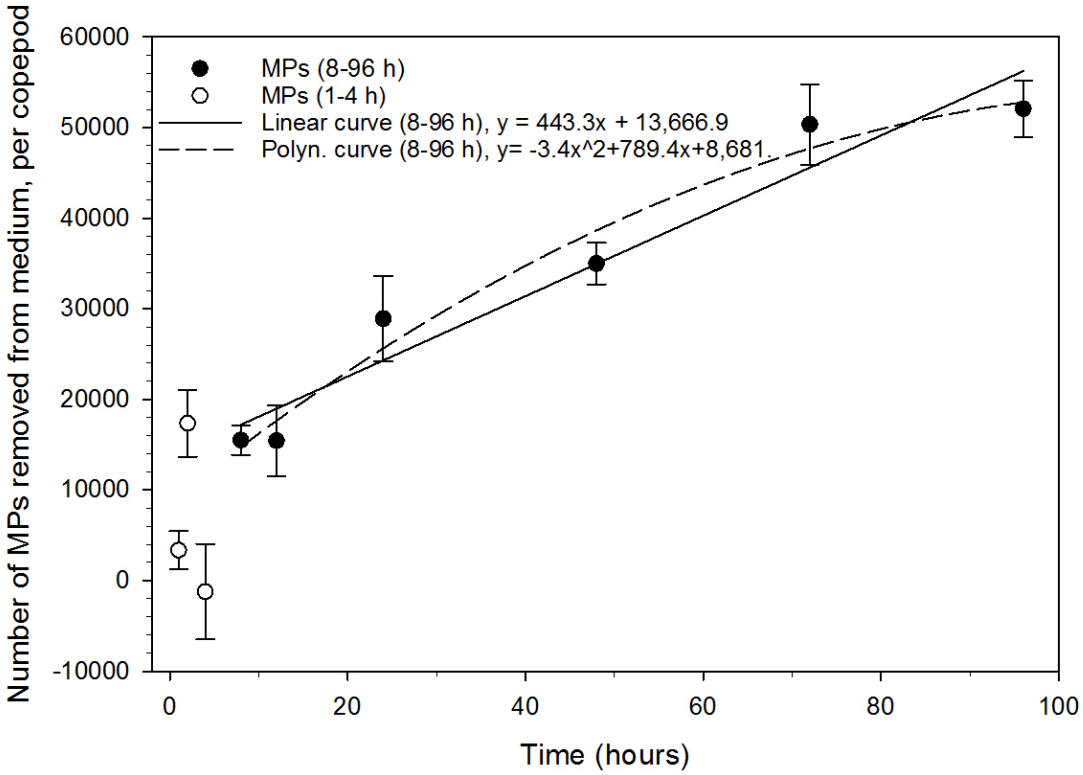

Figure 3.7. Cumulative removal of MPs from the exposure medium per *C. finmarchicus*. Mean±SD of three replicate bottles are given for each sampling point, each replicate containing 10 *C. finmarchicus*. The amounts were calculated by subtracting the total number of MPs remaining in the medium plus the total number of MPs attached to the exposure bottle surfaces, from the initial number of MPs (847,500 beads bottle^{-1}, nominally). A linear trend line was fitted to the time points 8-96 hours. Sampling points 1-4 hours were omitted from the curve fit because the initial data was assumed to be influenced by processes additional to removal by the copepods. A second-order polynomial curve was fitted to the same time points (8-96 hours) due to an observed levelling off of the MP removal between 72 and 96 hours.

A positive correlation was found between the cumulative MP removal from the medium per *C. finmarchicus* and the cumulative MP excretion through the faeces per copepod, both for the sampling points 8-96 hours ($R^2 = 0.74$, $p=0.0006$) and for all sampling points ($R^2 = 0.77$, $p=0.0015$) (Figure 3.8). Two linear trend lines were fitted to the data points as the values of MP removal at time points 1-4 hours were variable and probably influenced by processes additional to MP removal by the copepods (see Figure 3.7 and related text above). The y-intercepts at 8,733 and 14,216 MPs for the curves for all time points and for 8-96 hours respectively, in addition to the variable initial values, further indicate that the loss of MPs

could originate from other factors or processes in the exposure bottles than removal by the copepods.

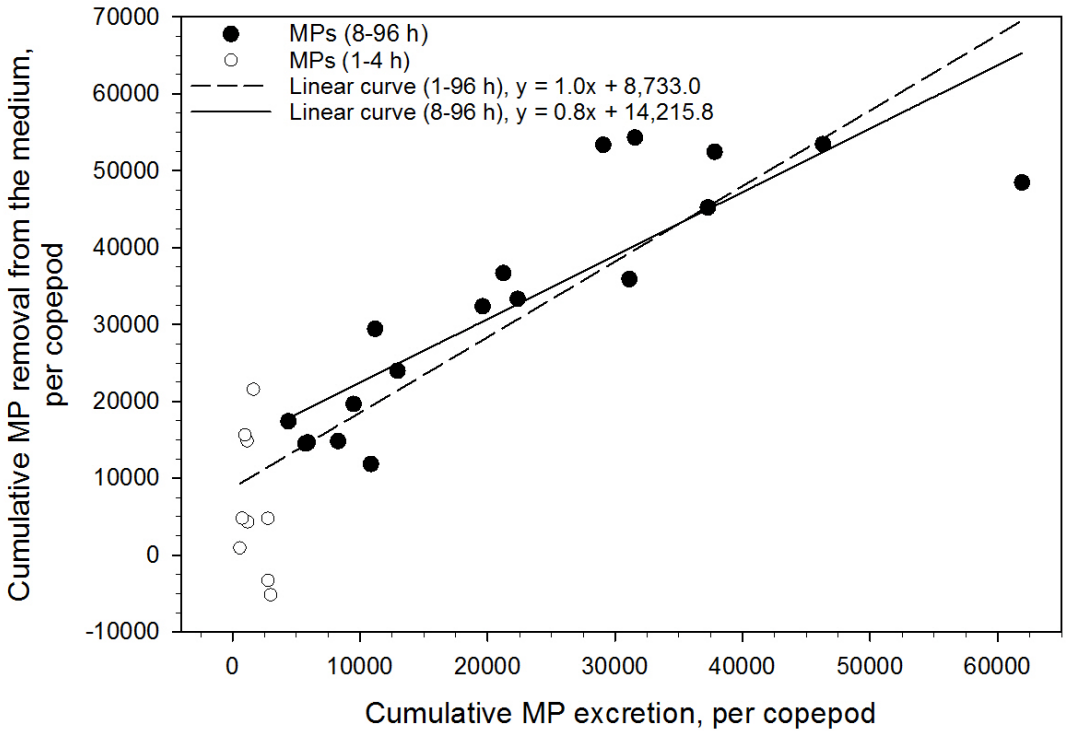

Figure 3.8. Correlation between cumulative numbers of PS microbeads excreted through the faeces per *C. finmarchicus*, and the cumulative numbers of MPs removed from the medium per copepod. Linear trend line fitted to the entire data set (dashed line) and to the data generated between 8-96 hours (solid line).

The cumulative MP removal from the medium, the cumulative MP content in the faeces and in the copepods are presented in Figure 3.9. Ideally all MPs removed from the exposure medium should be retrieved from the faeces and/or the copepods, but it is evident from Figure 11 that at all sampling points, except at 4 hours, a large fraction of the removed MPs is not accounted for. The detailed values of the average discrepancies per sampling point are presented in Table 3.1. If leaving out the measurements from 1-4 hours, which were highly variable, the average discrepancy per copepod was 10,087 MPs. Per bottle this value corresponds to 11.8 % of the initial nominal number of MP present.

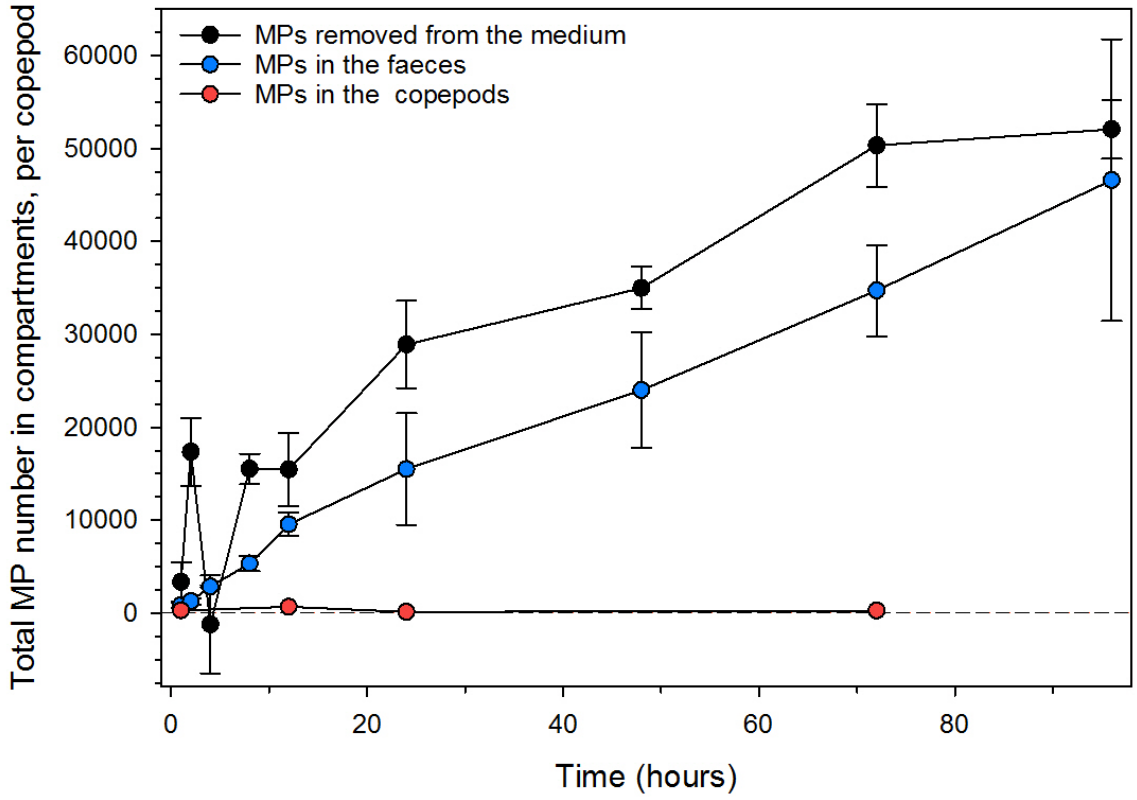

Figure 3.9. Cumulative MP removal from the medium (mean±SD, n=3) (black dots), cumulative values of MP excretion through the faeces (blue dots) and the cumulative MP content in copepods (red dots). All values are expressed as per *C. finmarchicus* (n=10 in each replicate bottle).

Table 3.1. Differences between cumulative MP removal from the medium per *C. finmarchicus* (n=10) and the cumulative MP excretion through the faeces plus the copepods´ MP content (both numbers per copepod). Average values for three replicate bottles are given for each sampling point. * indicates time points with no data of MP content per copepod.

	1	**2***	**4***	**8***	**12**	**24**	**48***	**72**	**96***
Average discrepancy, per copepod	2,225	16,101	-4,073	10,189	5,221	13,290	10,996	15,355	5,471

3.2.2 Removal of microalgae cells from the medium

Estimates of microalgae removal from the medium were done as for MPs (see Materials and Methods for further details of the calculations) (Figure 3.10). The cumulative removal of algae was assumed to increase steadily with time, therefore a linear curve was fitted to all sampling points ($R^2 = 0.92$). However, at 4 and 12 hours the algae removal was elevated compared to the following sampling points, which was unexpected as the values are cumulative. These values were omitted when fitting another linear curve which returned a slightly better curve fit ($R^2 = 0.98$). An extrapolation of both curves generated y-intercepts of 44,158 and 11,758 cells for the curves with all values included and for the curve where 4 and 12 hours were omitted respectively. The y-intercepts further indicate that unknown processes or factors (as suggested for MPs) could have contributed to the initial variations and loss of algae cells. As the cumulative faeces production (Figure 3.3) indicated a possible flattening in faecal pellet production towards the latest time points, a second-order polynomial curve was fitted to the sampling points, except 4 and 12 hours ($R^2 = 0.98$). An extrapolation of the curve generated a lower y-intercept than the linear curves (6,878 algae cells).

The cumulative algae removal in the control groups increased steadily and appeared to be higher at 24 and 48 hours compared to exposed groups. However, by comparing the algae removal in exposed and in control groups (t-test), no significant differences existed for 24 hours ($p=0.155$) and 48 hours ($p=0.0775$). However, the average algae removal at 24 and 48 hours for exposed copepods were 68 and 73 % of the controls respectively (Appendix A).

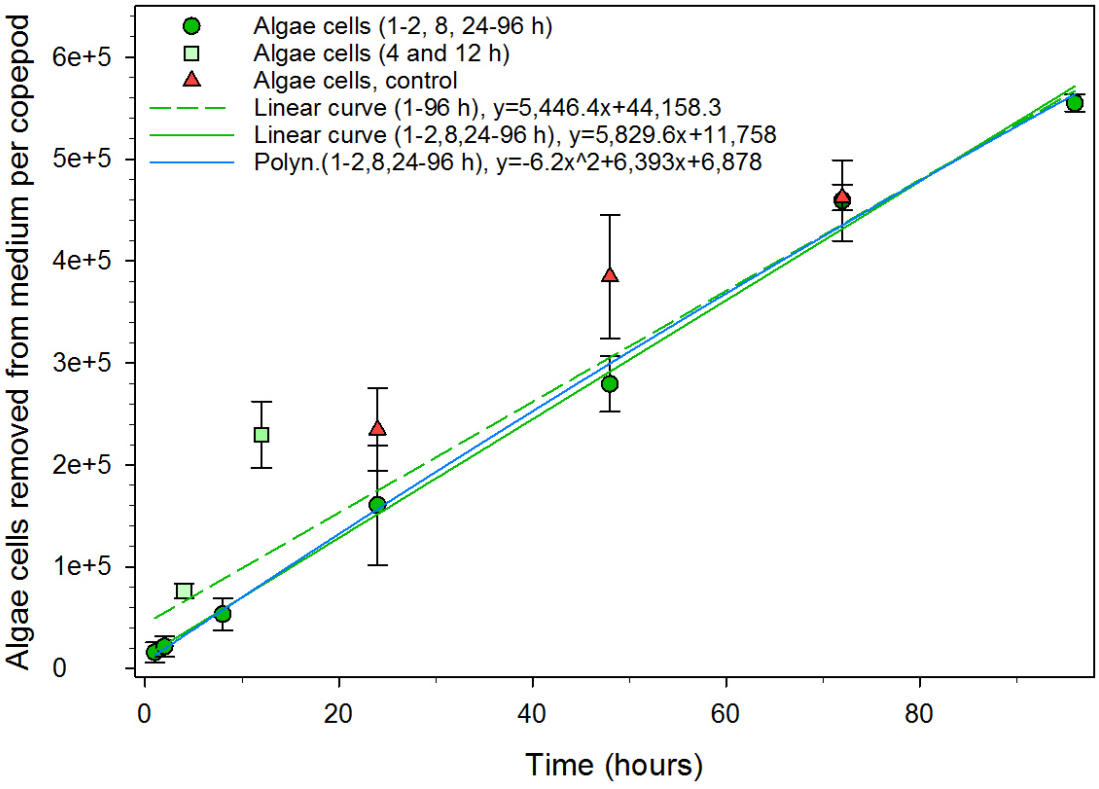

Figure 3.10. Cumulative removal of microalgae (*R. baltica*) from the medium per *C. finmarchicus*. Mean±SD of three replicate bottles are given for each sampling point, each replicate containing 10 *C. finmarchicus*, except one replicate in control group at 72 hours, n=11. The algae removal was calculated by subtracting the total number of algae cells measured in the medium plus the number of cells attached on the exposure bottle surface from the initial nominal concentration of 8,475,000 algae cells bottle^{-1} (7,500 cells ml^{-1}). Both a linear curve (green solid line) and a second-order polynomial curve (blue solid line) were fitted to the data points of exposed copepods (green dots). A second linear curve (dashed line) was fitted all values including values for exposed copepods at 4 and 12 hours (light green squares**).** Data points for controls are marked with red triangles.

3.2.3 Microalgae and MP removal

3.2.3.1 MP and microalgae removal in relation to faeces production

The correlation between the cumulative faeces production and the cumulative MP and algae removal from the medium indicates that the removed particles and cells were ingested by the copepods (Figure 3.11). By extrapolation of the linear trend line fitted to all data points (R^2=0.92), it appears that more than 40,000 MPs and algae already were removed from the exposure medium during the first hours, due to other factors than the copepods alone. As an initial increase in removal at the earliest time points were observed for both algae and especially MPs (Figure 3.7 and 3.10), the values from 1-4 hours were omitted as they were not assumed to relate to the faeces production. The linear curve fitted to time points 8-96 hours (R^2=0.87) indicates an initial removal of around 69,000 MPs which is not associated with faeces production.

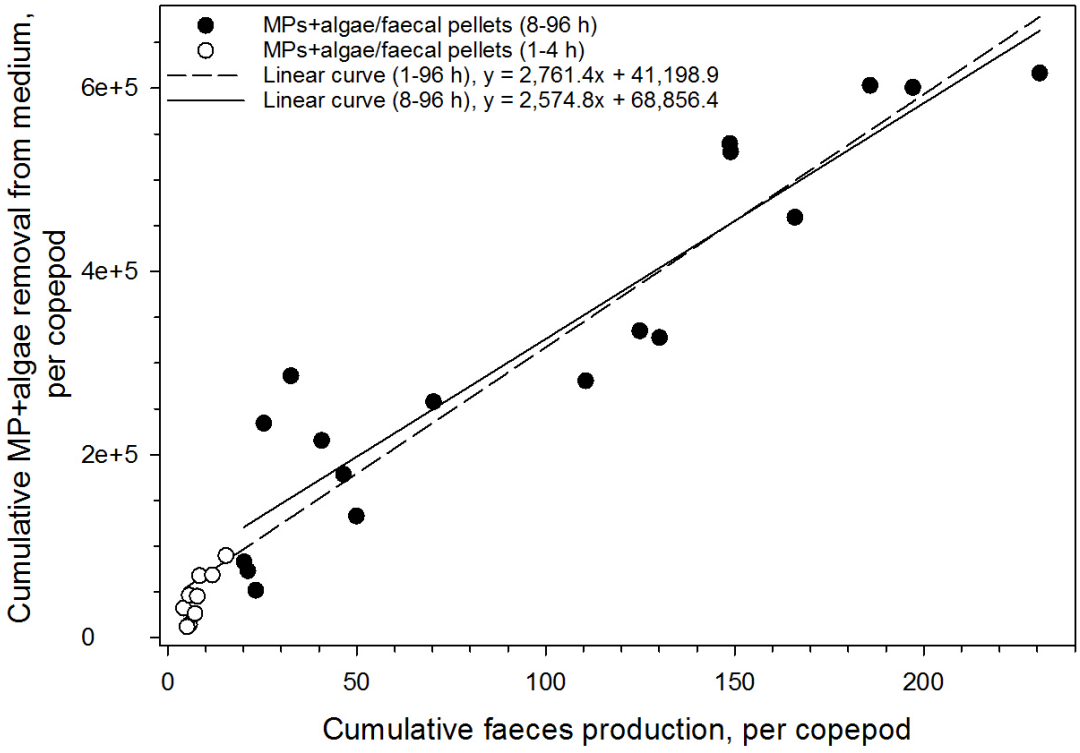

Figure 3.11. Correlation between cumulative faeces production and cumulative removal of MPs and microalgae (*R. baltica*) (all numbers per *C. finmarchicus*). Each data point containing one replicate bottle of 10 copepods, three replicates per time point. Time points 1-4 hours indicated with white dots and 8-96 hours indicated with black dots. Linear trend lines are fitted both all data points, and the data points 8-96 hours.

3.2.3.2 Comparison of MP and microalgae concentrations

The concentrations of MPs and microalgae (*R. baltica*) as percent of the initial nominal concentrations (750 MPs ml^{-1} and 7,500 algae cells ml^{-1} respectively), calculated for each time point are shown in Figure 3.12. The best fitted trend line for the percentage of initial MP concentrations in the entire exposure period was a hyperbola (single rectangular II, 3 parameter) (R^2=0.86), while a linear trend line was fitted to all percentages for algae in the exposed groups (R^2=0.92). As MP and algae removal (Figure 3.7 and 3.10) partly were based on the same values as the percentages of initial concentrations, corresponding initial variations and drop in concentrations could be observed in these results. Therefore, a second linear trend line was fitted to the sampling points 8-96 hours (R^2=0.90). Furthermore, as the MP removal appeared to flatten between 72 and 96 hours, a second-order polynomial trend line ($y=0.0042x^2 – 0.94x + 89.46$) was fitted to the same data points (R^2=0.94). Also a possible levelling off of the algae removal was observed, therefore both a linear curve (R^2=0.98) and a second-order polynomial trend line ($y=0.0008x^2 – 0.75x + 98.72$) ($R^2$=0.98) were fitted to the data points 1-2, 8, 24-96 hours.

Comparing the decline of MPs and algae when the deviating values were omitted, the percent change in MPs concentration decreased slightly slower (-0.52) compared to the algae concentration in the exposed groups (-0.69). Comparing the polynomial curves, the slopes calculated in the time points 1 and 96 hours are deviating. The slopes for MPs are declining from -0.93 to -0.13 in the period, while the slopes for algae is only decreasing from -0,75 to -0.60. Furthermore, the polynomial curve for MP removal levels off at 112 hours, while this occurs 469 hours for the polynomial curve for algae.

The percentage of MPs and algae attached to the exposure bottle surfaces were below 1.3 and 2.5 % of total concentration of particles/cells respectively (Appendix B), therefore this fraction was considered negligible when comparing MP and algae concentrations.
In a further comparison of the total concentration of MPs and microalgae during MP exposure to the concentration of algae in the controls, the sum only constituted 81 and 82% of the controls at sampling points 24 and 48 hours respectively. At the sampling point 72 hours however, the sum of the MPs and algae concentration was 107 % of the algae concentration in algae control.

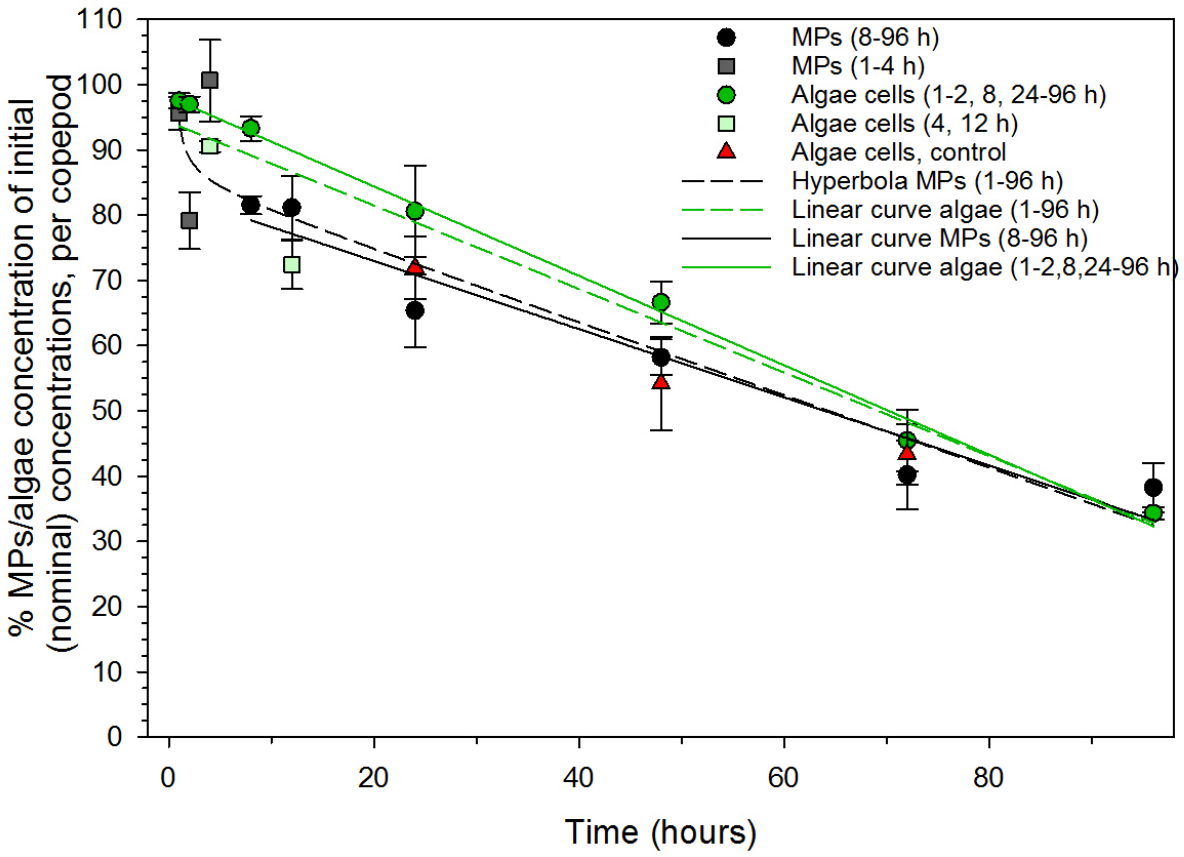

Figure 3.12. Concentrations of PS microbeads (MPs ml^{-1}) (black dots) and microalgae (*R. baltica*) (green dots) (cells ml^{-1}) as percentage of initial nominal concentrations of 750 MPs ml^{-1} and 7,500 algae cells ml^{-1} respectively. Algae concentration in control groups (red triangles) also presented as percentage of initial nominal concentration of 7,500 algae cells ml^{-1}. Mean±SD of three replicate bottles are given for each sampling point, each replicate containing 10 *C. finmarchicus*, except in one replicate in control group at 72 hours, n=11. A hyperbolic (single rectangular II, 3 parameter, y=(85.53x/(-0.1+x))-0.55x) trend line was fitted all data points for MPs (dashed black line) and a linear trend line for all data points for algae (dashed green line, y = -0.64x+94.31). A second, linear trend line was fitted all data points for MPs except at 1-4 hours (solid black line, y=-0.52x+83.43), and a second linear trend line for all data points for algae except 4 and 12 hours (solid green line, y=-0.69x+98.12). Data points for MPs 1-4 hours are marked with black squares, sampling points for algae at 4 and 12 hours are marked with light green squares.

3.3 Excretion of MPs by *C. finmarchicus*

3.3.1 Reduction in MP content in the copepods

The MP content in *C. finmarchicus* pre-exposed to PS microbeads for 24 hours was analysed for the sampling points 1, 24 and 72 hours in the excretion study (Figure 3.13). Between 1 and 24 hours the MP number per copepod decreased significantly both among copepods receiving and not receiving microalgae ($p=0.045$ and $p<0.0001$ respectively). Subsequently there was an unexpected significant increase in MP content at 72 hours compared to that at 24 hours ($p<0.0001$) in the copepods receiving algae. The presence of algae during depuration only affected the MP content in the copepods significantly at 24 hours ($p=0.00072$). However, for the copepods receiving algae, large variations between individuals at the first sampling point may have influenced the statistics.

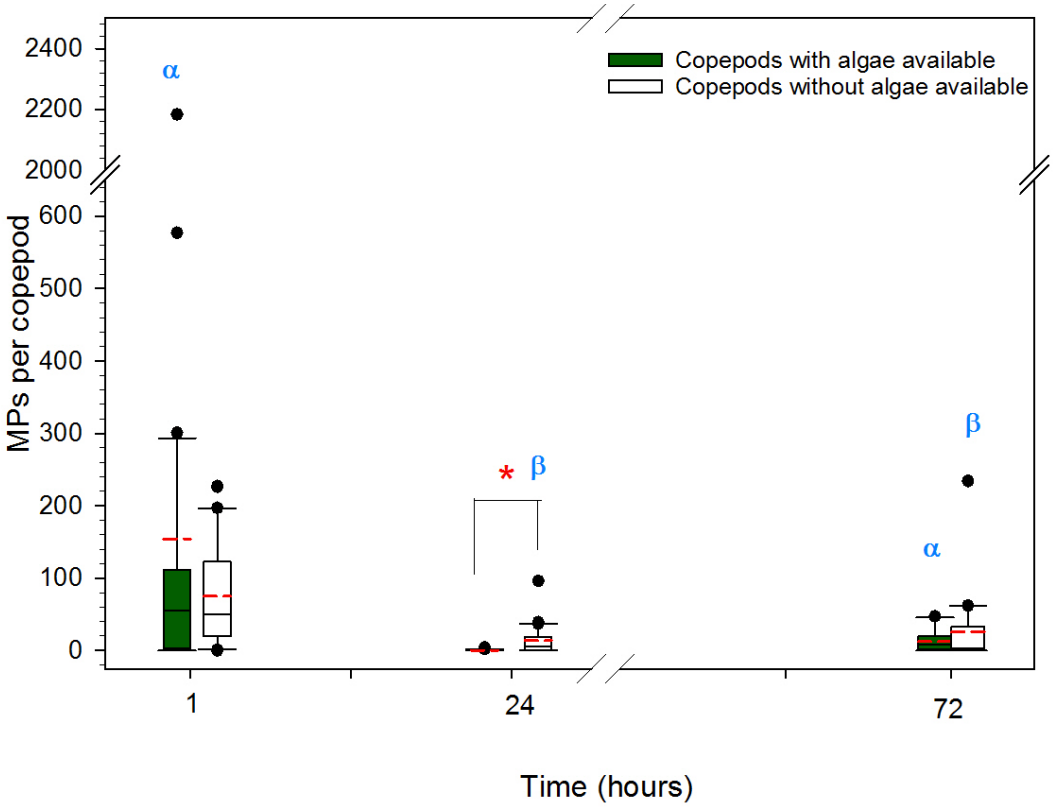

Figure 3.13. Average content per *C. finmarchicus* of MP for each time point 1, 24 and 72 hours of depuration in the presence of microalgae (*R. baltica*) (green boxes) or without algae present (white boxes). N=30, except n=29 for the 72 hours sample with algae present. No significant difference in MP content was found between replicate bottles sampled at each sampling time, and the copepods in the three replicates were merged. The solid black lines indicate the median and dashed yellow lines the average. * indicates significant difference between copepods receiving/not receiving microalgae at each time point. α and β indicates *no* significant difference ($p>0.05$) between time points within groups (with/without algae).

3.3.2 MP excretion through the faeces

3.3.2.1 Cumulative faecal production and microalgae removal

The cumulative faeces production by exposed copepods receiving algae increased steadily towards 48 hours, following a second-order polynomial path ($y = -0.013x^2 + 3.14x + 0.76$) rather than a straight line (Figure 3.14). At 72 hours, however, a consistent drop was observed, down to the level observed at 24 hours. A slight deflection was also evident in the cumulative faeces production by the copepods in the control groups at the same time point. Regarding that this is cumulative data a drop at 72 hours below the previous values seems inconsequent, and could be due to degradation of faeces in the 72 hours samples. To be able to compute cumulative MP excretion, an estimate was made of the theoretical cumulative faeces production at the 72 hours sampling point based on the polynomial equation referred above. The returning value was almost twice the observed value.

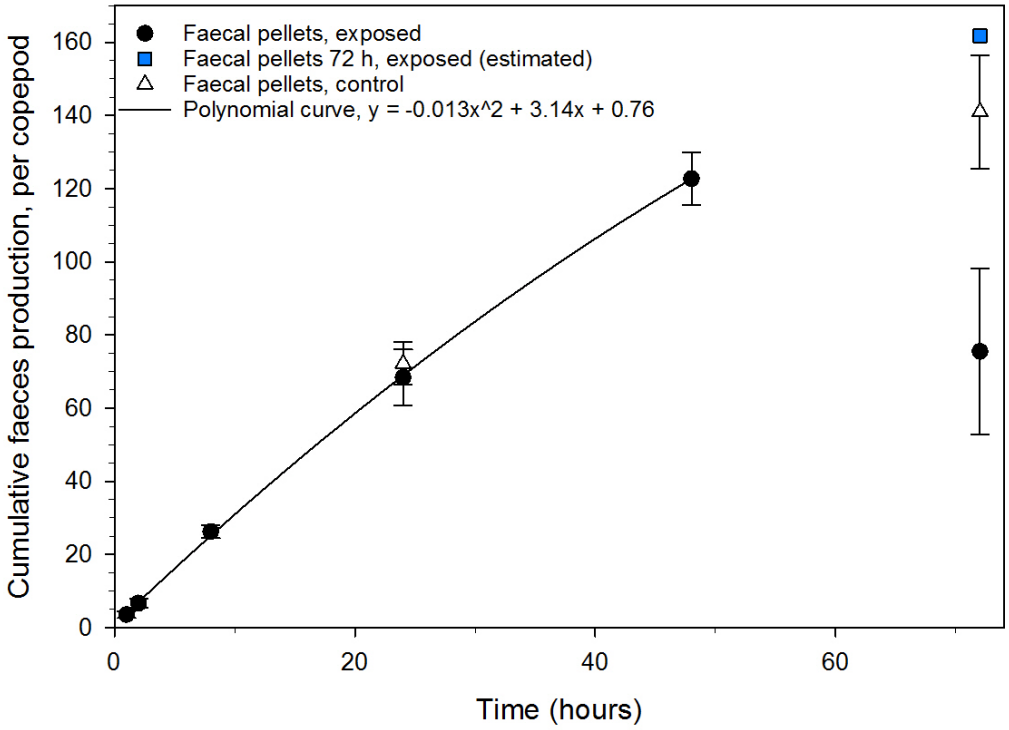

Figure 3.14. Average cumulative faeces production per *C. finmarchicus* during the MP depuration period (black dots) and controls (white triangles), with microalgae (*R. baltica*) available. Mean±SD of three replicate bottles are given for each sampling point, each replicate containing 10 *C. finmarchicus*, except in one replicate at 72 hours, n=9. A theoretical value (blue square) for cumulative faeces production was calculated based on the equation derived from the selected polynomial curve fit between 1-48 hours ($y = -0.013x^2 + 3.14x + 0.76$).

Cumulative algae removal by copepods receiving algae during depuration increased steadily, also following a polynomial path (-48.8x^2 + 12,729.2x + 49,216.9) (R^2=0.989) (Figure 3.15). However, the initial increase in cumulative algae removal from 1-2 hours was suggested to relate to an initial loss of cells similar to that described for algae removal during the uptake study. Therefore an additional second-order polynomial curve was fitted the time points 2-72 hours (y = -35.9x^2 + 11,543.7x + 70,280.6) (R^2=0.990). The removal also appeared to increase linearly during the same period, and a linear curve (y = 8,922.5x + 92,694.3) returned a similar good fit (R^2=0.985). Y-intercepts of regression curves were >0, further indicating an initial loss of cells. In the control groups, a levelling off of the cumulative algae removal was observed at 72 hours, which could be related to the deviation observed in faeces production at the same time point (Figure 3.14).

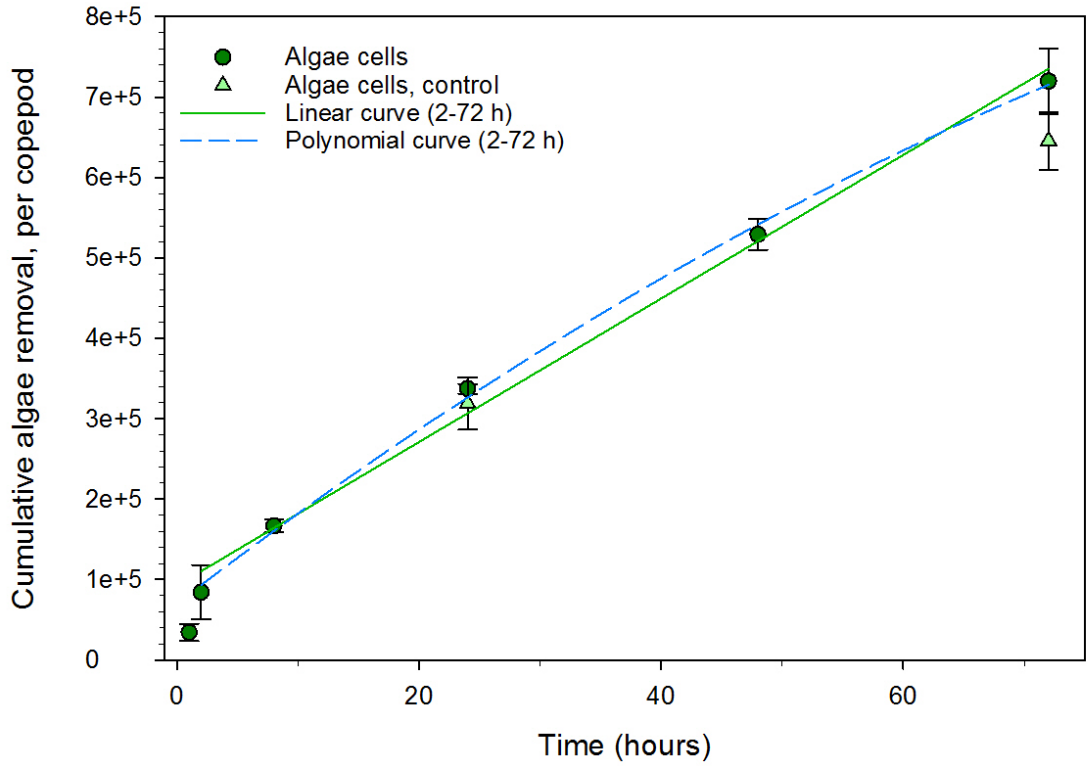

Figure 3.15. Cumulative removal of microalgae (*R. baltica*) from the medium by *C. finmarchicus* during depuration after 24-hour exposure to PS microbeads. Mean±SD of three replicate bottles are given for each sampling point, each replicate containing 10 copepods, except in one replicate at 72 hours, n=9. The algae removal was calculated by subtracting the total number of algae cells measured in the medium plus the number of cells attached on the exposure bottle surface from the initial nominal concentration of 8,475,000 algae cells $bottle^{-1}$ (7,500 cells ml^{-1}). A second-order polynomial trend line (y = -35.9x^2 + 11,543.7x + 70,280.6) (dashed blue line) was fitted the data points 2-72 hours in exposed groups (dark green dots). A linear trend line (solid green line) was fitted the same data points (y = 8,922.5x + 92,694.3). Control groups are indicated by light green triangles.

The cumulative faeces production per copepod was linearly correlated with the algae removal per copepod during the depuration in the presence of algae (R^2= 0.98) (Figure 3.16). A y-intercept at 49,330 indicates that a significant fraction of algae cells was removed from the system by other factors than grazing during the first hours, and hence not associated with ingestion and faeces production. Hence, the initial increase from 1-2 hours was assumed to be due to an increased algae removal from 1-2 hours (Figure 3.15), which probably was associated with the already mentioned initial variations and loss of cells.

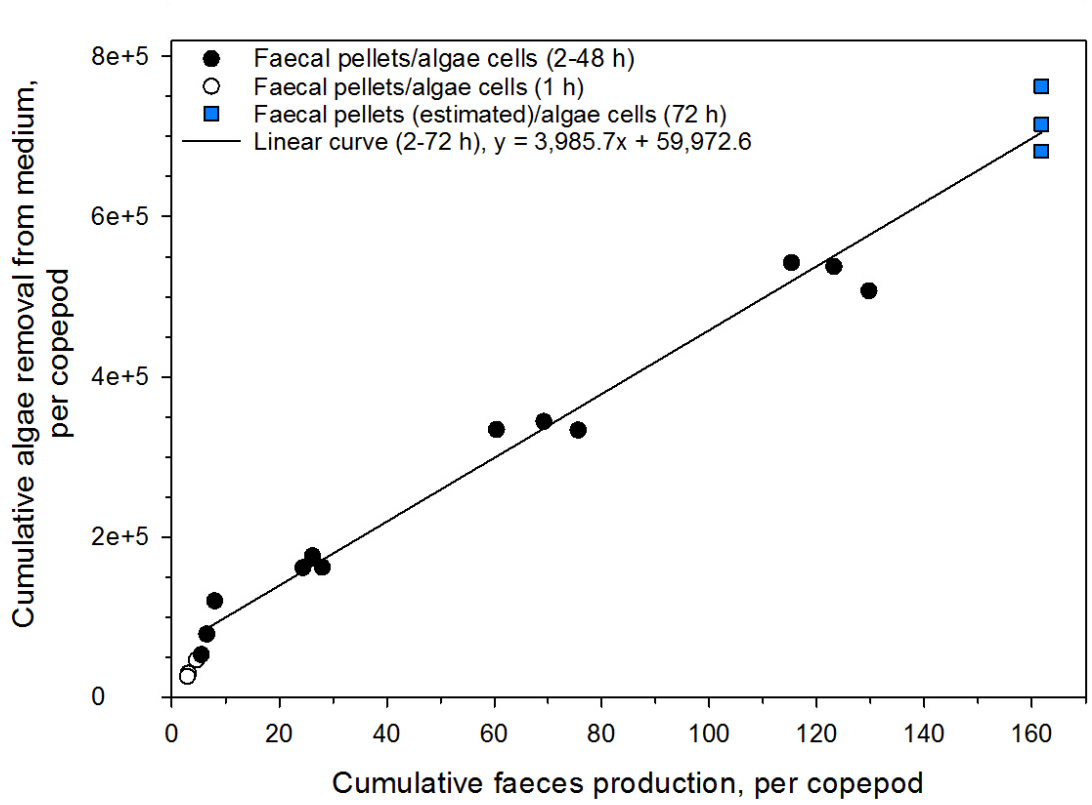

Figure 3.16. Correlation between cumulative faeces production and cumulative algae removal, all numbers calculated per *C. finmarchicus*. All three replicates included for both faeces production and algae removal (10 *C. finmarchicus* individuals in each replicate bottle). Linear regression line fitted for sampling points 2-72 hours (black dots, blue squares for 72 hours). Values for 1 hour (white dots) are omitted because they were assumed to be influenced by processes additional to removal by the copepods. Cumulative faeces production at 72 hours was calculated based on the equation derived from the selected polynomial curve fit between 1-48 hours ($y = -0.013x^2 + 3.14x + 0.76$) (Figure 3.14).

The measured cumulative faeces production by *C. finmarchicus* not receiving microalgae was in general low in both exposed and control groups (Figure 3.17). For the exposed copepods, the total number of faeces increased up to 2 hours, before it declined towards 48 hours and again increased slightly at 72 hours. The decline in cumulative faeces production seems inconsequent, as an eventual cease in faeces production should result in a levelling off of the curve rather than a decline. The faeces production in control groups is more stable but low. However, few sampling points for controls and the lack of faeces at 24 hours in the exposed group hamper the overall interpretations of the results. In general, the faeces produced in the absence of algae tended to be more brittle, often transparent, and hence difficult to observe without a microscope.

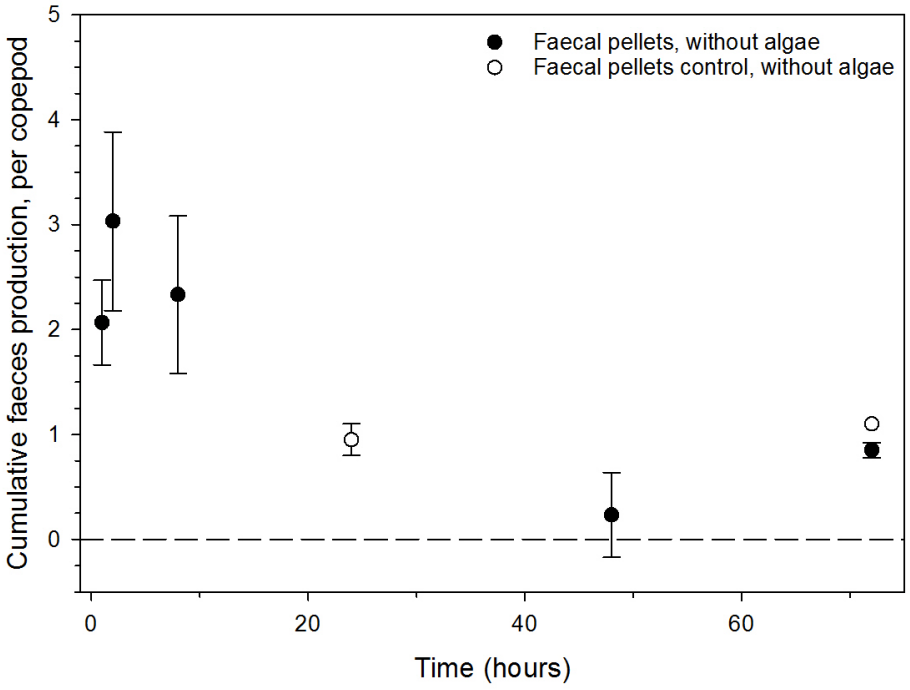

Figure 3.17. Average cumulative faeces production per *C. finmarchicus* during the MP depuration period (black dots) and controls (white triangles), with no algae available (mean±SD, n=3*) (10 *C. finmarchicus* individuals in each replicate bottle) (*no faeces collected at 24 h, and fore replicates 48A and 48B, and C72CU).

3.3.3 MPs per faeces

The average number of MPs per faecal pellet at each sampling point during the depuration period in the presence and absence of algae is presented in Figure 3.18. Numbers from excretion with and without microalgae are shown. After 1 hour of depuration in the presence of algae the average MP content per faeces was 235 MPs, while the average number was 176 MPs per faeces in groups not receiving algae. Thereafter the MP content per faeces in the presence of algae dropped to 79 MPs before declining further. In the absence of algae the decline in MP content occurred mainly from 8 hours onwards, however less steep compared to the copepods that received algae. Larger variations between the replicates occurred especially at 8 hours without algae and at 2 hours with algae present. In general, the data from the excretion without algae was scarce, often with only a few measurements of one replicate. Hence any conclusions drawn from these data warrant caution. It should also be noted that the average numbers of MPs per faeces were based on randomly selected faeces collected from a pool of faeces produced up to the point of sampling.

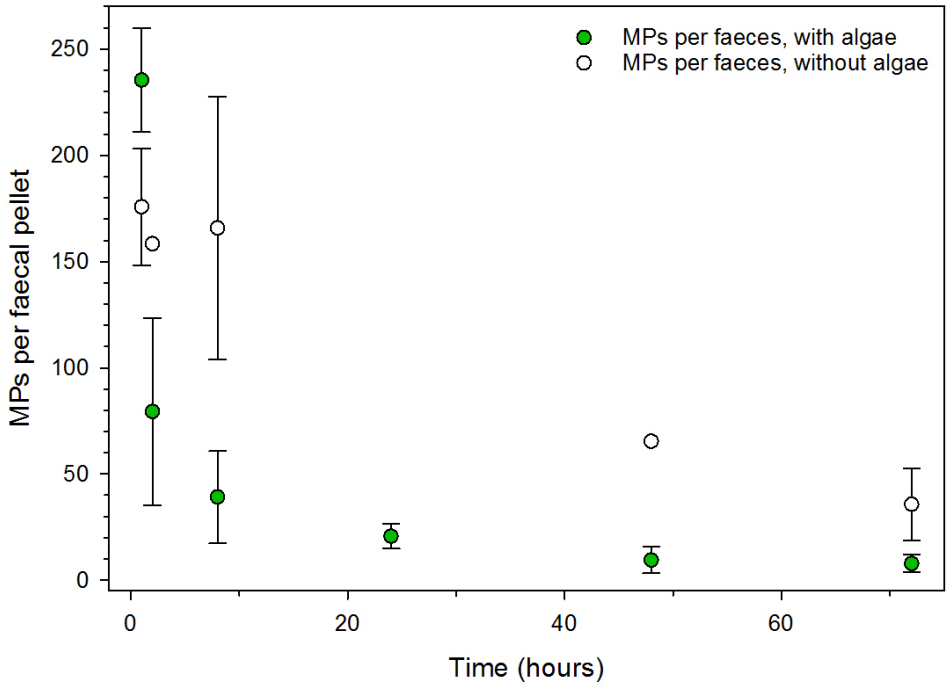

Figure 3.18. Average numbers of MPs in faecal pellets at each sampling point during the depuration period. Both data for depuration period with algae (*R. baltica*) (green dots) and without algae (white dots) are shown. Each data point represents the average of the mean number of MPs per faecal pellet in three parallel bottles±SD. The mean MP number per faeces in each bottle is based on 25* randomly selected faecal pellets produced by 10 *C. finmarchicus*, except one replicate (with algae) at 72 hours, n=9). (*no faeces collected at 24h, and for replicates 48A and 48B, 2B and 2C).

3.4 Cumulative MP excretion

Further calculations of cumulative MP excretion in the groups not receiving algae during depuration was regarded to be misleading due to the lack of sufficient and reliable data, and was therefore not conducted.

A more stable faeces production during depuration in the presence of algae (Figure 3.14) enabled a calculation of cumulative MP excretion per copepod (Figure 3.19). The results indicate a levelling off of the cumulative MP excretion between 11,000-15,000 MPs per copepod, although the accuracy of this estimate may be dubious due to large variations between samples. However, these values were higher than 970 MPs per copepod, the sum of the average MP content per copepod at sampling point 1 hour, plus the average MPs numbers excreted with the faeces during the first hour, and which was assumed to be the total number of MPs brought into the system.

The system was analysed further by multiplying the measured number of faeces at each sampling point (except estimated values at 72 hours) with calculated average MP content in faeces at the same points. The calculated numbers were derived from a model predicting average particle content in faeces at a given time by simple dilution (in additional faecal particles) and under the assumption that the cumulative number of MPs present should level off at the original number of particles present. Input parameters to the model were the number of MPs per faeces present after 1 hour, and a fraction of this number added for each of the subsequent periods. The model may therefore also be used to study the excretion dynamics of the MPs. The computed fractions indicated a rapid decline in MP content in the copepods, with about 83 % of the MPs excreted during the first hour of depuration, 12.6 % during the next hour, 2.6 % during the following 6 hours, 1.2 % during the next 16 hours, and less than 0.3 % during the remaining period up to 72 hours.

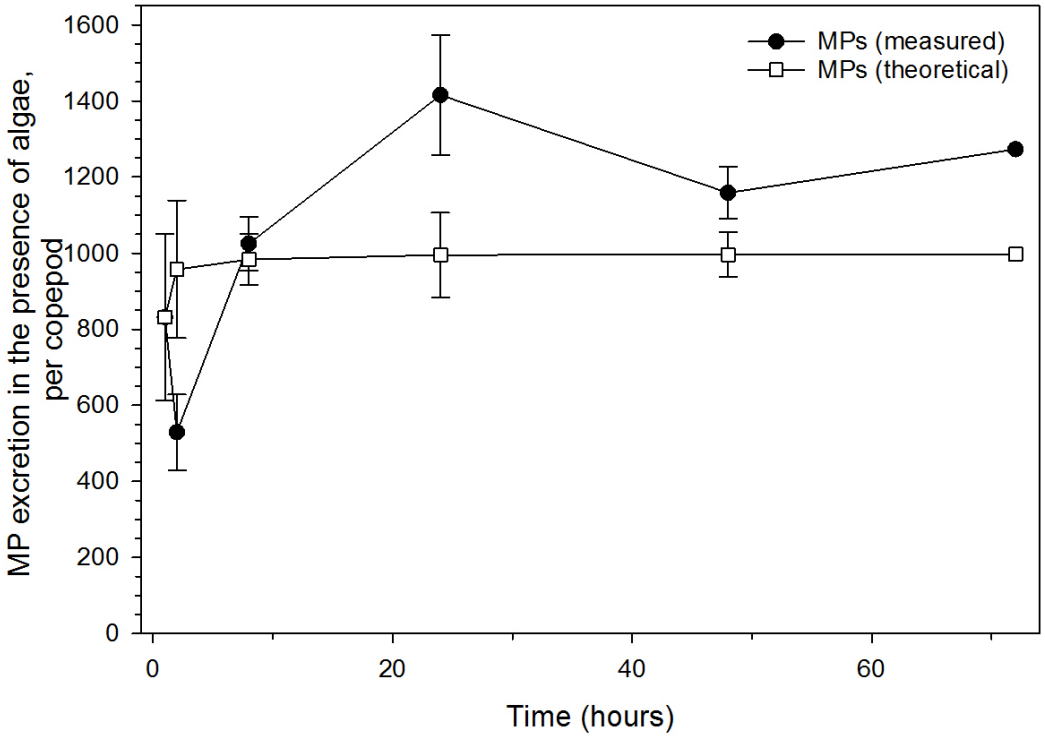

Figure 3.19. Cumulative MP excretion through the faeces per *C. finmarchicus*, calculated from measured values only (black dots) and measured faeces numbers and modelled particles content at each sampling time point (white squares). Each data point refers to the average of the mean numbers±SD of MPs excreted in three parallel bottles, calculated by multiplication of the average MP number per faeces in each bottle (based on 25 randomly selected faecal pellets or calculated), with the total number of faecal pellets present. There were 10 *C. finmarchicus* in each bottle, except one replicate at 72 hours where 9 copepods were present. The cumulative faeces production at 72 hours used in both occasions was calculated as described in section 3.3.2.1.

3.5 Distribution of MPs in the faeces

In Figure 3.20, a random selection of photographed faecal pellets produced by *C. finmarchicus* collected at the sampling points 1 hour with microalgae (A) and without algae (B), and 72 hours with algae (C) and without algae (D) is presented, to illustrate the appearance and distribution of the MPs in the faeces.

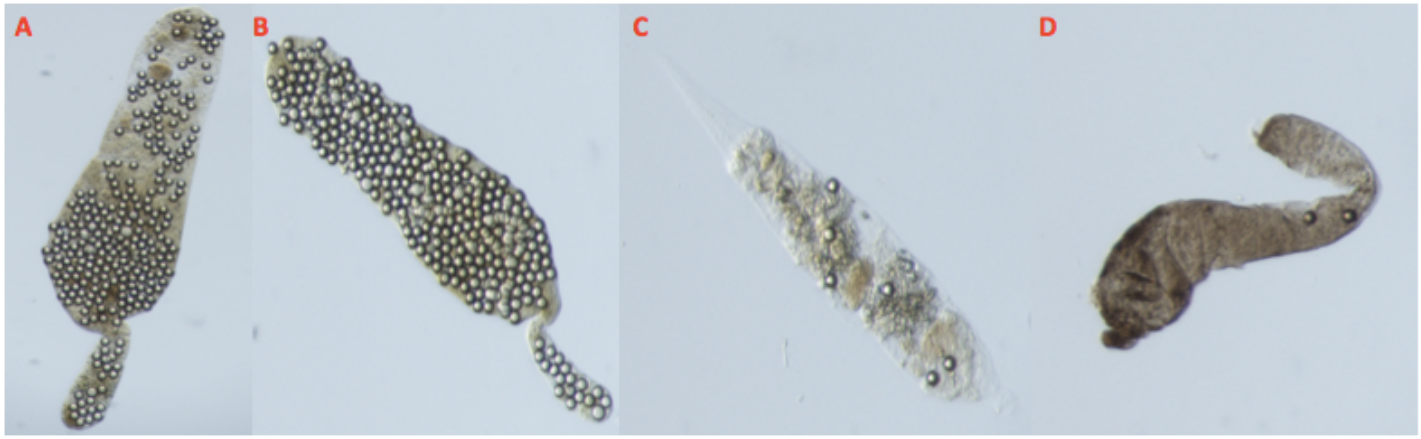

Figure 3.20. Distribution of 10 µm PS microbeads in adult female *C. finmarchicus* faecal pellets randomly selected from the photographed faeces at sampling points 1 hour with microalgae (*R. baltica*) (A) and without algae (B), and 72 hours with algae (C) and without algae (D). The faeces are pressed between the microscope slide and the cover slip, and if necessary tapped gently to spread the particles more evenly.

4. Discussion

The uptake and excretion of PS microbeads by *C. finmarchicus* in the presence of microalgae (*R. baltica*) were confirmed by the observation of MPs in the dissolved copepods, by visual inspections of the anesthetized copepods during image capture and by the presence of MPs in the faecal pellets. The observations are in line with previous observations of ingestion of PS microbeads (7.3-30.0 μm) by copepods in the presence of algae (Cole et al., 2013, Cole et al., 2015, Cole et al., 2016, Vroom et al., 2017) Furthermore, the observations indicated that the copepods fed indiscriminately on algae and MPs offered. Mean algae removal was somewhat lower in the presence of MPs compared to control at all three time points measured, but the difference was not significant at any occasion. In the excretion study the MPs were excreted rapidly through the faeces in the presence of algae. However, measurements of MPs in the copepods indicated a possible limited retention even beyond 72 hours, both in the presence and absence of algae, which has also been observed earlier in copepods in the absence of algae (Cole et al., 2013). Data of faeces production in the absence of algae was insufficient and could therefore not be used for further studies of MP excretion through the faeces.

4.1 Uptake of MPs

The MPs were readily ingested by the copepods, illustrated by an average MP load per copepod after 1 hour of 278 microbeads and with 844 MPs egested through the faeces during the first hour of exposure. During the entire exposure period of 96 hours more than 46,500 MPs were excreted via the faeces, while on average 332 MPs were present in the copepods during the period 1-72 hours. During the first 12 hours, there are indications of enhanced MP uptake seen as a higher rate of MP excretion through the faeces. This coincided with an increased content of MPs in the copepods at 12 hours, further indicating an elevated ingestion of MPs. These findings will be discussed more in detail below.

To study the dynamics of MP and algae uptake, the initial concentrations of both MPs and algae had to be sufficiently high to maintain filtration of both MPs and algae throughout the exposure period. This was confirmed to be achieved by the selected concentrations of both algae and MPs, however with a slight levelling off with time especially for the MP-removal

(cumulative curve, Figure 3.7). A continuous faeces production throughout the study also indicated a stable ingestion and excretion of algae. Accordingly, the MP content in both the copepods and their excreted faeces was expected to remain relatively constant or slightly decrease (due to reduced concentrations in the medium) during the exposure part of the experiment.

4.1.1 Indications of elevated MP removal and variations between sampling points

Increased cumulative MP excretion through the faeces towards 12 hours of exposure in combination with an elevated average content of MPs per copepod at 12 hours, indicated increased ingestion of MPs during this period. Furthermore, the average numbers of MPs per faeces excreted between samplings were in general higher during the same period. However these findings are not supported by the MP removal from the medium. When omitting the initial fluctuating values (1-4 hours) (discussed later), MP removal from 8-96 hours is close to stable (Figure 3.7). Furthermore, stable algae removal and faeces production are not indicating enhanced uptake during this period either, if non-selective feeding is assumed.

These contradictive findings could be due to an uneven distribution of MPs in the exposure bottles, which could have been a consequence of sedimentation processes which will be discussed in detail in section 4.2.1.1. If assuming an uneven distribution of the MPs in the exposure bottles, the copepods behaviour could have influenced both the number of MPs found in the copepods and in their excreted faeces. If the copepods were filtrating more along the bottle surfaces during the first hours of exposure where the sedimented particles were assumed to accumulate, they would non-selectively filtrate more MPs simply due to a higher abundance and encounter rate of MPs. As some individuals possibly fed more extensively and prolonged than others in layers of high MP concentrations, this hypothesis is also in agreement with high standard deviations in MP content per faeces in the period, and very high MP content in some individuals at 12 hours.

An initial enhanced metabolism could also have occurred, without seemingly affecting faeces production. Increased metabolism would increase the fraction of MPs in the pellets (MPs do not disintegrate) and thereby also explain the elevated number of MPs per faeces excreted between the time points. However this alone cannot explain the results as the MP numbers in the copepods were elevated, which means an increased removal (of MPs and algae) had taken place either during the entire period up to 12 hours or at some point during this period. No

increased removal is assumed due to stable values of MP removal during samplings 8-96 hours. When interpreting the results from the measurements of MPs in the copepods it should also be considered that few sampling points are available for MP content in copepods, and that initial particle concentrations were not verified in each bottle.

If considering the variations in the values of MPs in the copepods and the numbers of MPs per faeces excreted between the time points separately, these values could also partly be ascribed to chance related to high variability in uptake between individuals. This is in line with previous findings of individual variations in MP uptake among copepods (Vroom et al., 2017, Wilson, 1973). Additionally, the non-verified start concentrations could have added to this variation, meaning some time points had more/less than 750 MPs ml^{-1} available initially. Incorrect start concentrations could be caused by improper mixing of the MP stock solution before pipetting. Importantly, measurements of MP content in the copepods are available for few sampling points only. Analysis of more time points could probably also have revealed more of the trends and strengthen the statistics. Regarding the number of MPs in the faeces excreted during the first hour, the low numbers were probably caused by replacement of the initial gut content (of algae remains only), with a mixture of MPs and algae from the exposure medium, as the copepods were fed on algae only up to the point of transfer to the exposure bottles. Accordingly this could partly explain the increase in the number of MPs in the copepods between 1 and 12 hours.

4.2 MP and microalgae removal from the medium during the uptake study

4.2.1 Comparison of MP and microalgae removal from the medium

The copepods were expected to feed indiscriminately on the MPs and the algae offered, both due to the observations of stable faeces production and due to previous findings documenting non-selective feeding when copepods are offered MPs and algae of similar size (Cole et al., 2013). However an assumed dynamic sedimentation process of the MPs in addition to uncertainties of initial concentrations and a levelling off of MP concentrations are complicating overall interpretations of the results. These subjects will be discussed in relation to the expectation of non-selective feeding in the sections below.

4.2.1.1 Indications of sedimentation or loss of MPs and microalgae

It is assumed that the observed initial reduction of MPs was primarily related to sedimentation processes, although grazing by the copepods could have modified the process to some extent (see section 4.1.1 above). This assumption is based on regression analysis and an evaluation of initial deviations from the general trend (see section 3.2.1)

The observed large initial fluctuating values (1-4 hours) for MP removal (Figure 3.7) are assumed not to be solely related to copepod filtering activity, especially as cumulative values sometimes appear to decrease with time, which is unlikely. Since, however, the values at each time point represent discrete populations (in separate bottles), variation could be explained by inaccuracies in initial concentrations of MPs and algae in the bottles, i.e. above or below the nominal start concentrations of 750 MPs ml^{-1} and 7,500 cells ml^{-1}, respectively. This could further be explained by insufficient mixing of the MP stock solution, or the algae culture aliquot, by miscalculations of the dilution of the algae culture aliquot and/or transfer of an incorrect volume.

In contrast to the initial values a strong and positive correlation between MP removal and cumulative MP excretion was found from 8-96 hours, indicating that removal of MPs during this period was largely due to grazing by the copepods. However, by omitting the initial fluctuating values, the y-intercepts of both the MP removal/time regression line, and the regression line of MP removal vs. MP excretion are >0. Additionally, the generated y-intercepts from Figure 3.12 are between 83 and 89 % of initial concentrations, corresponding to a 11-17 % difference. Furthermore, a similar discrepancy (in average 12 %) between MP removal and the MPs retrieved from the copepods and the faeces at time points 8-96 hours is observed. Considering the cumulative MP excretion measured in the faeces (Figure 3.4), the value of the generated y-intercept from the trend line for time points 8-96 hours is around 4,100 MPs per copepod. This value implicates that the copepods in the period before 8 hours on average had processed around 41,000 MP (if measured per replicate bottle), or around 5 % of the number of MPs present in the bottles initially, probably by grazing sedimented particles with higher density (see section 4.1.1 above). These 5 % are making up almost 30 % of the fraction of about 16-17 % which was obtained from the y-intercept generated from the linear trend line (8-96 hours) in Figure 3.12. In other words, the observation of an initial increase in MP excretion can probably account for around 30 % of the registered initial loss of MPs. Also if adding 5 % to the average of 12 % discrepancy between MP removal and MP excretion, the

number amounts to 17 %, further underpinning the theory that grazing by the copepods add to explain the discrepancies observed.

Anyways, it is likely that a considerable fraction of the MPs had sedimented and was subsequently lost from the system, as they could not be retrieved from the copepods or the faeces. It is also probable that the initial fluctuating values partly could originate from the same processes or factors involved in the loss of MPs, in addition to possible variations caused by incorrect initial concentrations. The missing fraction was probably not recorded due to a methodological flaw. Assuming that the particles sedimented (particles quantified at surfaces, Appendix B) and that at any time a fraction of these sedimented particles was loosely associated with the exposure bottle surfaces, these particles would not be measured during the initial sampling from the medium, nor found as attached to surfaces after emptying the bottles. They could instead be loosened by stretch forces and decanted with the water when the bottles were emptied during termination. Unfortunately no measurements of particle content in the decanted water were made.

The same pattern of initial fluctuating values was not or only slightly observed for algae removal, however the values at 4 and 12 hours were rather strongly deviating from the general trend (Figure 3.10). As these values are cumulative values observed decreases with time cannot occur, and the same reasoning as for the MP removal is therefore applicable. The y-intercept generated from the regression of algae concentrations of initial concentrations and time, indicates an initial loss of 2 %, which is considerably lower than the corresponding y-intercept for MPs. The discrepancy from MP particle numbers could be explained by the swimming activity of the algae, which may actively counteract sedimentation. Also, differences in buoyancies and surface properties between MPs and the algae may also add to explain the differences. In addition during the excretion study, an initial loss is assumed illustrated by the initial increase in algae removal between 1-2 hours (Figure 3.15). However, more algae cells are apparently removed (elevated y-intercepts), but this probably due to the generally higher algae removal during the excretion study.

4.2.1.2 Are the copepods grazing indiscriminately on MPs and algae?

As mentioned above, the assumed sedimentation of MPs is reflected in Figure 3.12, where MP and algae concentrations of initial concentrations are presented. The observed discrepancies between MP and algae concentrations are therefore not believed to be caused by selective feeding on MPs. Further, the differing slopes of MP and algae concentrations of initial concentrations are assumed to be related to the dynamics of the sedimentation process and to variable initial concentrations. If assuming that a dynamic equilibrium between particles in the sedimented fraction and particles freely dispersed in the medium existed, this could have affected the measured levelling off of MP concentrations in the medium, and also caused variations. If the MPs gradually migrated from the sedimented layer to the medium during the 96-hour period, this could explain a slightly less steep slope for MP concentrations at the later time points. This would further imply that the measured MP concentrations at these time points levelled off, not because a lower filtering activity by the copepods, but due to the increased input of particles to the medium from the sedimented fraction.

In addition to the above mentioned dynamic sedimentation process, an apparent levelling off after 72 hours is thought to be a result of variations in initial concentrations, i.e. the initial concentration was higher than 750 MPs ml^{-1} for the 96-hour bottles. As no sampling points exist after 96 hours it is difficult to predict the further development. Sampling points after 96 hours are therefore necessary to reveal more about the overall trend and should be considered in future studies. However, if assuming an actual levelling off in the removal of particles, this could be indicative of selective feeding. This again could be due to either a lower encounter rate, or to a shift in preference when the concentration of MPs was reaching a certain level (Leiknes et al., 2014). Regarding a lower encounter rate the concentration of MPs at 72 hours was in average 302 MPs ml^{-1}, which is considerably higher than the concentration of 75 MPs ml^{-1}, of which non-selective feeding on PS microbeads and algae has been demonstrated (Cole et al., 2015). The relationship between algae and MPs in that study was also similar to that of the present study. A shift in preference due to a decreased abundance of MPs could therefore be more likely, as this has been shown to be important for selection between living prey for *C. finmarchicus* (Leiknes et al., 2014). On the other hand, the copepods are probably not able to differentiate between particles as small as 10 μm. This has been demonstrated for the copepod *Centropages typicus*, which fed non-discriminately on 7.3 μm PS microbeads in the presence of similar sized algae, probably due to a lack of ability to sense and respond to any difference in size of such small items (Cole et al., 2013). Furthermore, *C. finmarchicus*

did not discriminate between ciliates below about 30 µm (Leiknes et al., 2014), and to the author`s knowledge selective feeding on particles or cells <10 µm has not been demonstrated for *C. finmarchicus*.

If the levelling of not was due to a selective feeding at the latest time points but rather incorrect start concentrations, this must be considered when interpreting the values of the y-intercepts (especially for the linear trend lines) and the fraction of sedimented MPs (Figure 3.7 and 3.12).

Regarding the decline in MP and algae concentrations as percentage of initial concentrations, it could have been that the algae concentration was declining more rapidly than MPs concentrations due to natural mortality of the algae. However, algae controls without copepods illustrated that the algae numbers were relatively stable during the period of 96 hours (Appendix C), indicating that there are no measurable constraints on the algae. As for the declining algae concentrations, a slight deflection of the algae removal (polynomial curve fit) was also observed (Figure 3.10), however it was by far as prominent as that for MPs. However, if assuming an equilibrium between the sedimented fraction of MPs and the MPs free in the medium, and if this fraction is accounted for when computing the concentrations of MPs as percentage of initial concentrations, the slope is elevated by 20 %. If computing new values for the corresponding values of algae, it is evident that the difference between the respective slopes is reduced by 47 %. Hence, the assumed migration of MPs (and algae to a certain degree) could therefore explain around half of the observed difference between decrease in MP and decrease in algae concentrations.

4.2.2 MPs and algae on surfaces

As the percentage of MPs and algae adhered to bottle inner surfaces in all replicates were below 2.5 % of the total number of MPs or algae present initially (nominal values) during the uptake and the excretion study, these numbers were regarded as too small to be influencing the comparison of removal of MPs and algae (Figure 3.12). Also the differences between MPs and algae adhering to the walls were negligible. However, more MPs tended to adhere to surfaces when both MPs and algae were present, which could be related to a more prominent sedimentation of MPs compared to algae. Regarding the algae adhering to the surfaces, more

variable values were obtained in the absence of MPs, with elevated values in the pre-exposed groups initially.

4.2.3 Effects of MP exposure on algae removal and faeces production

The presence of MPs resulted in a 32 and 27 % lower algae removal in exposed groups compared to the algae removal in the corresponding control groups at 24 and 48 hours (Appendix A). Also when comparing the total removal of MPs and algae with algae removal in control groups for the given time points these values are both 85 % of control when the estimated amount of sedimented MPs (y-intercept linear curve, Figure 3.7) is accounted for and it is assumed that the sedimented fraction remains unchanged throughout the period. However, the average differences were proven not to be significant. As a MP concentration as low as 75 MPs ml^{-1} has shown to reduce algae ingestion significantly in a study by Cole et al. (2015) the obtained results in the present study could be explained by the larger variations between the replicates affecting statistical computations.

A possible lower algae removal could be explained by a slightly larger diameter of the MPs that will fill up more of the gut compared to algae, further leading to a faster satiation. Compared to MPs the algae may be metabolised and lose water, and hence take up even less space in the gut. Also the MPs could have affected the filtering capacity of the copepods, lowering the number of algae ingested (Cole et al., 2013).

The indications of reduced algae ingestion by exposed copepods are not reflected in the cumulative faecal production, which is similar to that in control groups. If assuming a lower algae removal in exposed groups, this could indicate enhanced metabolism among copepods in the control groups or a decreased metabolism among exposed copepods. Otherwise, the copepods in the control groups may have excreted larger pellets or the exposed copepods were excreting more and smaller pellets as a consequence of the present MPs. As the faecal pellet size was not studied this cannot be verified within the present study.

Higher algae removal in the excretion study compared to the uptake study was associated with a levelling off of the cumulative algae removal at later time points and also a corresponding levelling off of the cumulative faeces production. The observation could be explained by filtering capacity limitations when concentration of feed particles is reduced, but could also be

the result of a trade-off between increased filtering energetic expenditure and energetic gain with decreasing concentration of feed particles.

4.3 Excretion of MPs in the presence and absence of microalgae

Excretion of MPs through the faeces in the presence of algae occurred rapidly, the observation was supported by continuous algae filtration correlated with a stable faeces production. Furthermore, other studies have found the same rapid excretion of MPs in the presence of algae (Cole et al., 2013, Vroom et al., 2017). In the absence of algae, the data on faecal production was flawed and could not be used for further studies of MP excretion. Measurements of MP content in the copepods indicated a possible retention beyond 72 hours, both in the presence and absence of algae. This observation was unexpected due to the findings of a rapid excretion through the faeces in the copepods receiving algae, but could be ascribed to degradation of faeces and subsequent ingestion of MPs. In the absence of algae, the retention of MPs was more in line with expectations, as MPs have been found to remain in copepods for up to 7 days in the absence of feed (Cole et al., 2013).

4.3.1 MP excretion measured in the copepods in the presence and absence of microalgae

The lack of feed did not interfere with the MP content in the copepods after 1 hour of depuration. The elevated values for two individuals in the group with algae could be due to increased filtration by these individuals (Vroom et al., 2017, Wilson, 1973) or other factors influencing the quantification (see discussion of method). The higher values are probably also affecting the statistics as MP content at 1 and 72 hours with algae are not significantly different.

From 1 to 24 hours the MP content of the copepods in both the fed and unfed groups was significantly reduced. The following modest but significant increase in the MP content observed in copepods at 72 hours in groups receiving algae was thus unexpected, as no MPs were added the medium initially. However, if degradation of the faecal pellets by either copepod coprophagy (Iversen and Poulsen, 2007)or by the activity of protozoan such as ciliates (Poulsen and Iversen, 2008) had occurred, this could lead to direct uptake of MPs with ingested faeces remains, or uptake due to filtering and ingestion of MP particles released to the water from the faecal pellets. Regarding a possible presence of ciliates, they could be

introduced with the algae and with short generation times and exponential growth reached a sufficiently large number during the first days of depuration. As the algae aliquot used for these particular replicates only was used for this time point, it is difficult to compare to similar effects at other time points. If degradation of faeces was mainly the result of coprophagy by the copepods, a steadily increasing grazing over the entire exposure period should be assumed, since grazing on faeces has been shown to increase proportional to the concentration of faeces particles in the water (Iversen and Poulsen, 2007). As grazing on faeces cannot be ruled out, it is not supported by the present results which show a sudden drop in cumulative faeces numbers at 72 hours. The degradation proposed for the faeces at 72 hours could therefore be due to microorganism activity.

For copepods not receiving algae during depuration, no statistical difference between the 24 hours and 72 hours samples may indicate a retention of MPs in the copepods. However, degradation of faeces by the same processes as mentioned above could also have occurred, although coprophagy or coprohexy are more prominent when prey is present due to higher filtration activity and when the faecal pellet concentration is higher, which leads to a higher encounter rate (Iversen and Poulsen, 2007).

4.3.2 MP excretion via the faeces in the presence and absence of microalgae

4.3.2.1 Cumulative MP excretion in the presence of microalgae

The cumulative MP excretion based on the measured values levelled off at a higher value than the average total number of 970 MPs measured in the copepods plus the faeces excreted during the first hour (Figure 3.19), which is assumed to be the average total number of MPs carried by each of the exposed copepods. This was the reason for making a numerical model which generated a levelling off slightly above 990 MPs, and thereby was assumed to return more accurate results than if based on measured values only. However, the model predicted average numbers of MPs in the faeces at each sampling point that did not deviate much from the quantified number of MPs in the faeces at each time point, further indicating the precision of the model. A slight adjustment of the MP number per faeces demonstrates that even a minor flaw of either factor would result in significant deviations in the calculated maximum cumulative number.

The model predicted that the excretion of MPs occurs predominantly during the first hour, and more than 90 % of the MPs originally present in the copepods were depurated during the first two hours.

4.3.2.2 MPs per faeces in the absence of algae

As the cumulative MP excretion in the absence of algae not was possible to compute due to insufficient data of faecal production, only the average numbers of MPs per faeces were evaluated. The numbers decreased steadily towards 72 hours in agreement with dilution of early MP-containing pellets with new ones containing few or no MPs, indicating that at least some pellets were produced throughout the period. However, not only does the assumed loss of faeces impose a statistical bias to the calculation, the low number of faeces found at several sampling points also made it necessary to reduce the sample size to < 25 pellets, further weakening the precisions of the calculations. Possible explanations for the loss of faeces are discussed in the section below and in section 4.5.2.2.

4.3.2.3 The significance of the presence of microalgae prey on faecal pellets and faecal pellet production

That the faecal production in the groups receiving and not receiving algae differed was not surprising, as no feed was supplied in the latter group, and the copepods therefore reduced or completely stopped their faeces production (Marshall and Orr, 1972). Furthermore the faeces appeared more transparent and fragile in the non-fed groups (Figure 3.20 C), in line with the description by Marshall and Orr (1972) of such pellets as “ghost pellets”. Faeces containing MPs can also be more prone to fragmentation in general (Cole et al., 2016).

The decreased faeces production measured after 72 hours in the presence of algae was probably caused by faeces degradation as suggested above, and not decreased faeces production by the copepods. In addition, a lower quantification of faeces due to methodological mistakes is possible (see discussion section 4.5.2.2). The computed value for 72 hours correlated well with the algae removal from the medium, further indicating that the decreased value not was caused by a decreased faeces production by the copepods.

In the absence of algae, the measured cumulative faeces production decreased after 2 hours and was therefore regarded as untrustworthy. The lack of suitable feed may nevertheless

ultimately lead to decreased or ceased faeces production (Marshall and Orr, 1972), and increased retention of gut content. Both are in agreement with the present results. However, already produced faeces should remain in the bottles, and the cumulative values should at best level off. Therefore a loss of faeces is assumed, and may be related to degradation and fragmentation of faeces by coprophagy/coprohexy and/or microbial degradation. However, neither alternative feed or a high abundance of pellets were present in these bottles, factors which have been shown to enhance degradation by the copepods themselves. Therefore it is not likely that these processes occurred at high rates herein. As the faecal pellets in this group were small and brittle, and probably more likely to fragment (Cole et al., 2016), some could also have been crushed and remains lost during the collection process (see discussion of method, section 4.5.2.2). Due to the uncertain values of cumulative faeces production, cumulative excretion of MPs through the faeces was therefore not possible to compute in the groups not receiving algae.

4.4 Ecological relevance

The MP concentration used in the current study is arguably higher than what is commonly observed in the marine environment (Phuong et al., 2016). On the other hand, there is little consensus about the actual concentrations of MPs <100 μm in the marine environment, as no standardized methods for quantification are established (Huvet et al., 2016). Furthermore, the study shows that *C. finmarchicus* most likely are prone to feed non-discriminately on MPs and algae if they are in similar size range and <10 μm. This is of concern due to the importance of the species in the marine food web in the North Atlantic Ocean (Melle et al., 2014).

Although the uptake of algae in the presence of MPs did not deviate significantly from controls at any of the three time points measured, mean uptake was nevertheless less than control values at all sampling points. A lower uptake is in agreement with previous findings where copepods exposed to MPs at a concentration an order of magnitude lower than the concentration used during present study reduced algae uptake significantly (Cole et al., 2015). During prolonged MP exposure reductions in algae ingestion could have implications for energy allocation and thereby also reproduction (Cole et al., 2015).

As it has been shown that *C. finmarchicus* ingest biofouled MPs at higher numbers than pristine MPs (Vroom et al., 2017), the actual number of ingested MPs would probably be higher in the marine environment.

Although the results from the depuration study were difficult to interpret due to lack of sufficient and reliable data for groups not receiving algae, it is assumable that at least a small number of MPs are retained in the copepods beyond 72 hours especially when algae are absent. As the availability and concentration of prey can be variable in the ocean (Melle et al., 2014) this could imply both an increased risk of desorption of chemicals from the polymers (Teuten et al., 2009), and a higher probability of predators to ingest copepods containing MPs, thereby transferring the MPs from one trophic level to the next. If copepods contain a high number of MPs when descending to deeper water for hibernation, consequences could be even more pronounced.

Furthermore, faecal pellets loaded with MPs could contribute to enhanced MP exposure of organisms that ingest or degrade faeces, and other organisms which do not degrade faeces but are exposed to the MPs after their release from the pellets. The pellets containing MPs, and especially those produced when the simultaneous ingestion of algae were low or none, are probably more fragile and therefore more prone to dissociation or fragmentation earlier during the vertical transport of faeces. Moreover, as faecal pellets containing PS microbeads can have a reduced sinking rate, this can imply an increased risk of MP exposure and ingestion (Cole et al., 2016). Studies of faecal pellet sinking rates were not within the scope of the present study. However, it should be mentioned given the high numbers of MPs measured in the pellets.

4.5 Discussion of method

4.5.1 Experimental setup

The division of the study into two main experiments, as mentioned in the section of Materials and Methods was a necessity to ensure enough time and equipment for the conduction of the experimental procedures. Also the use of copepods from different culture tanks was considered not to significantly affect the results, as they were from same generation and same parental culture. Furthermore the use of copepods raised in culture tanks could probably be an advantage as this could reduce stress related to the experimental conditions. In spite of the arrangement the individual studies were demanding and time-consuming. Also, some of the copepods reached egg-laying phase during the experiment, as eggs occasionally were observed in the petri dishes during photography of the copepods. A simultaneous onset of all exposure sample bottles would possibly have limited variations between the time points as the same algae culture aliquot could have been used. Verification of the initial MP and algae concentrations in the exposure media in each bottle during the uptake study would also have reduced the uncertainties and probably the variations between the groups. However, simultaneous onset of all exposure groups was not feasible as the plankton wheel setup only held a maximum of 36 bottles. Continuous rotation on the plankton wheel was needed to keep particles and algae in suspension. However, the faecal pellets were also kept in suspension, which could have increased degradation or modification by the copepods (Iversen and Poulsen, 2007). A device separating the faeces and the copepods would have excluded such uncertainties.

4.5.2 Experimental conduction

4.5.2.1 Quantification of MPs in the copepods

The use of hypochlorite was successful for the analysis of MP content in the copepods as they dissolved within a short period of time, and the PS beads were not affected by the strong oxidant. Even after two months in hypochlorite solution there were no visible signs of degradation of the MPs. However, occasionally parts of the copepods, such as antennae, did not dissolve completely and MPs were observed to adhere to some of these fragments and could to a certain degree complicate MP quantification. Aggregation of particles could also have contributed to somewhat inaccurate measurements. Centrifugation of the vials was presumably only partly successful in gathering the MPs at the bottom, as the particles still

were unevenly distributed at different depths in the medium which also complicated the quantification. However, under these circumstances the ability to systematically scan through the medium in both vertical and horizontal directions enabled nevertheless relatively accurate measurements of MP numbers. Occasionally the vertical scanning through the medium was done manually and could have brought more uncertainties to the accuracy of the measurements. As MPs have shown to adhere to the copepods external surface smaller amounts of MPs could have followed when the copepods were transferred to the vials for dissolution, and an intermediate step of transfer to FSW could have removed external MPs. Furthermore, it cannot be excluded that some particles adhered to the glass surfaces (Long et al., 2017) as observed in the exposure bottles, and were hence not counted.

Occasionally, copepods got injured during the transfer from the mesh to the petri dishes for photographing, and thereby lost some of their gut content. Furthermore, some faeces and probably MPs were lost, as faecal pellets were observed in the petri dishes during photography, however this number is assumed to be low.

4.5.2.2 Quantification of faeces and MPs in the faeces

The image capturing and storage of faeces for later quantification were time saving, but it also had some disadvantages. Firstly a lower number of faecal pellets were adhering to the walls of the petri dishes, and due to optical challenges they were not possible to quantify. Movements of the content in the petri dishes during photography sometimes reduced the quality of the photographs, hence complicating the interpretation and possibly lowering the accuracy of the quantification. A loss of faeces could have occurred during the transfer from the petri dishes to the vials as this involved an intermediate transfer to a small beaker as some faecal pellets could have remained in both. During the additional step conducted in the uptake study for the first exposure groups (4 hours and replicate A at 12 hours) (see material and methods), faeces could have been lost or disrupted, this could possibly be the reason for a lower observed number of faeces for the quantification of MPs per faeces at the 4-hour time point.

A majority of the faeces collected for further quantification of MP content was obtained from either the bottom or the middle of the vial. However, a considerable number of faeces were floating at the surface, but as they adhered to the outside of the pipette they were quite difficult to get hold of. If the ability to float is affected by the MP content as observed with PS MPs previously (Cole et al., 2016), the selected faeces may not have been representative for

all faeces in the bottles. The transparent faeces seen in groups not receiving algae during depuration were also difficult to observe on the mesh, and many could therefore have been overlooked during collection. As they were brittle they possibly were more prone to get damaged during transfer to the mesh and during the further handling. During the preparation of microscopic slides faeces may have been damaged or lost when placing on the cover slip. As only intact pellets could be used for determination of MP content this further limited the number of faeces for quantification.

In all, the quantification of MPs in the individual pellets allowed an accurate measurement of the MP content, as the microbeads were easy to distinguish and to count. However, a better method of obtaining the sample should be regarded in future studies, as it was suspected that the selection of pellets was not fully representative due to the above-mentioned challenges. Furthermore, a larger sample size for analysis should probably be considered to increase the power of the statistical testing, although this is compromised by the time-consuming counting procedure of MPs in the pellets. If the faeces were collected at intervals underway, this could have given an accurate number of MPs in the faeces at each time point, instead of cumulative values which were obtained in this study.

4.5.2.3 Contamination by MPs

Even though all equipment in contact with the MPs were cleaned between samplings and replicates within the same time point, smaller numbers of MPs may have been removed as PS beads are highly hydrophobic, and easily adsorbed to glassware (Long et al., 2017). Contamination was observed in the controls at some time points as they contained a low number of MPs.

Faeces from different time points and replicates could also have been interchanged by inappropriate cleaning of the mesh.

5. Conclusions and future recommendations

In the present study *C. finmarchicus* filtrated PS microbeads apparently indiscriminately in the presence of microalgae *R. baltica*. Subsequent excretion after a 24-hour exposure period occurred rapidly according to estimates of MPs excretion through the faecal pellets, but with some MPs still being excreted at 72 hours of depuration in the presence of algae. Regarding MP content in the copepods, the presence of algae resulted in significantly less MPs in the copepods at 24 hours compared to copepods with no algae available. However increasing numbers after 72 hours, complicates overall interpretations. Although an observable lower algae removal in exposed groups compared to algae removal in all control groups, the differences were not proved to be significant. Importantly, an assumed sedimentation of MPs and the dynamics of this sedimentation process were probably partly causing an observable discrepancy between the measured removal of MPs and the retrieved number of particles obtained from the copepods and their faeces, and further variations in the data.

The findings of the present study illustrate the importance of quantification of MPs not only in the medium, but in all compartments to verify the actual exposure concentration. It is furthermore necessary to establish standard methods of quantification of MP content in copepods and in their excreted faeces, which diminish variations and improve accuracy of the measurements. In future studies it is further important to study the uptake and excretion of a combination of both MP size, shape, type, concentrations, concentrations of feed and the impact of biofouling. In addition, it is necessary to improve methods of MP quantification and characterization in the environment to better understand the actual concentrations and composition of MPs to enable relevant experimental designs in the future.

6. List of references

ANDRADY, A. L. 2011. Microplastics in the marine environment. *Marine Pollution Bulletin,* 62**,** 1596-1605.

ANDRADY, A. L. 2015. Persistence of Plastic Litter in the Oceans. *In:* BERGMANN, M., GUTOW, L. & KLAGES, M. (eds.) *Marine Anthropogenic Litter.* Cham: Springer International Publishing.

ANDRADY, A. L. 2017. The plastic in microplastics: A review. *Marine Pollution Bulletin,* 119**,** 12-22.

BATEL, A., LINTI, F., SCHERER, M., ERDINGER, L. & BRAUNBECK, T. 2016. Transfer of benzo[a]pyrene from microplastics to Artemia nauplii and further to zebrafish via a trophic food web experiment: CYP1A induction and visual tracking of persistent organic pollutants. *Environmental Toxicology and Chemistry,* 35**,** 1656-1666.

BESSELING, E., FOEKEMA, E. M., VAN FRANEKER, J. A., LEOPOLD, M. F., KÜHN, S., BRAVO REBOLLEDO, E. L., HESS, E., MIELKE, L., IJZER, J., KAMMINGA, P. & KOELMANS, A. A. 2015. Microplastic in a macro filter feeder: Humpback whale Megaptera novaeangliae. *Marine Pollution Bulletin,* 95**,** 248-252.

BESSELING, E., WEGNER, A., FOEKEMA, E. M., VAN DEN HEUVEL-GREVE, M. J. & KOELMANS, A. A. 2013. Effects of Microplastic on Fitness and PCB Bioaccumulation by the Lugworm Arenicola marina (L.). *Environmental Science & Technology,* 47**,** 593-600.

BROMS, C. & MELLE, W. 2007. Seasonal development of Calanus finmarchicus in relation to phytoplankton bloom dynamics in the Norwegian Sea. *Deep Sea Research Part II: Topical Studies in Oceanography,* 54**,** 2760-2775.

BROWNE, M. A., CRUMP, P., NIVEN, S. J., TEUTEN, E., TONKIN, A., GALLOWAY, T. & THOMPSON, R. 2011. Accumulation of Microplastic on Shorelines Woldwide: Sources and Sinks. *Environmental Science & Technology,* 45**,** 9175-9179.

BROWNE, MARK A., NIVEN, STEWART J., GALLOWAY, TAMARA S., ROWLAND, STEVE J. & THOMPSON, RICHARD C. 2013. Microplastic Moves Pollutants and Additives to Worms, Reducing Functions Linked to Health and Biodiversity. *Current Biology,* 23**,** 2388-2392.

CARPENTER, E. J., ANDERSON, S. J., HARVEY, G. R., MIKLAS, H. P. & PECK, B. B. 1972. Polystyrene Spherules in Coastal Waters. *Science,* 178**,** 749.

COLE, M., LINDEQUE, P., FILEMAN, E., HALSBAND, C. & GALLOWAY, T. S. 2015. The Impact of Polystyrene Microplastics on Feeding, Function and Fecundity in the Marine Copepod Calanus helgolandicus. *Environmental Science & Technology,* 49**,** 1130-1137.

COLE, M., LINDEQUE, P., FILEMAN, E., HALSBAND, C., GOODHEAD, R., MOGER, J. & GALLOWAY, T. S. 2013. Microplastic ingestion by zooplankton. *Environ Sci Technol,* 47.

COLE, M., LINDEQUE, P., HALSBAND, C. & GALLOWAY, T. S. 2011. Microplastics as contaminants in the marine environment: A review. *Marine Pollution Bulletin,* 62**,** 2588-2597.

COLE, M., LINDEQUE, P. K., FILEMAN, E., CLARK, J., LEWIS, C., HALSBAND, C. & GALLOWAY, T. S. 2016. Microplastics Alter the Properties and Sinking Rates of Zooplankton Faecal Pellets. *Environmental Science & Technology,* 50**,** 3239-3246.

CÓZAR, A., ECHEVARRÍA, F., GONZÁLEZ-GORDILLO, J. I., IRIGOIEN, X., ÚBEDA, B., HERNÁNDEZ-LEÓN, S., PALMA, Á. T., NAVARRO, S., GARCÍA-DE-LOMAS, J., RUIZ, A., FERNÁNDEZ-DE-PUELLES, M. L. & DUARTE, C. M. 2014. Plastic debris in the open ocean. *Proceedings of the National Academy of Sciences,* 111**,** 10239-10244.

CÓZAR, A., MARTÍ, E., DUARTE, C. M., GARCÍA-DE-LOMAS, J., VAN SEBILLE, E., BALLATORE, T. J., EGUÍLUZ, V. M., GONZÁLEZ-GORDILLO, J. I., PEDROTTI, M. L., ECHEVARRÍA, F., TROUBLÈ, R. & IRIGOIEN, X. 2017. The Arctic Ocean as a dead end for floating plastics in the North Atlantic branch of the Thermohaline Circulation. *Science Advances,* 3.

DESFORGES, J.-P. W., GALBRAITH, M. & ROSS, P. S. 2015. Ingestion of Microplastics by Zooplankton in the Northeast Pacific Ocean. *Archives of Environmental Contamination and Toxicology,* 69**,** 320-330.

ENDO, S., TAKIZAWA, R., OKUDA, K., TAKADA, H., CHIBA, K., KANEHIRO, H., OGI, H., YAMASHITA, R. & DATE, T. 2005. Concentration of polychlorinated biphenyls (PCBs) in beached resin pellets: Variability among individual particles and regional differences. *Marine Pollution Bulletin,* 50**,** 1103-1114.

ERIKSEN, M., LEBRETON, L. C. M., CARSON, H. S., THIEL, M., MOORE, C. J., BORERRO, J. C., GALGANI, F., RYAN, P. G. & REISSER, J. 2014. Plastic Pollution in the World's Oceans: More than 5 Trillion Plastic Pieces Weighing over 250,000 Tons Afloat at Sea. *PLoS ONE,* 9**,** e111913.

FERNÁNDEZ, F. 1979. Particle selection in the nauplius of Calanus pacificus. *Journal of Plankton Research,* 1**,** 313-328.

FRIAS, J. P. G. L., OTERO, V. & SOBRAL, P. 2014. Evidence of microplastics in samples of zooplankton from Portuguese coastal waters. *Marine Environmental Research,* 95**,** 89-95.

GALL, S. C. & THOMPSON, R. C. 2015. The impact of debris on marine life. *Marine Pollution Bulletin,* 92**,** 170-179.

GARRIGÓS, M. C., MARÍN, M. L., CANTÓ, A. & SÁNCHEZ, A. 2004. Determination of residual styrene monomer in polystyrene granules by gas chromatography–mass spectrometry. *Journal of Chromatography A,* 1061**,** 211-216.

GIERING, S. L. C., SANDERS, R., LAMPITT, R. S., ANDERSON, T. R., TAMBURINI, C., BOUTRIF, M., ZUBKOV, M. V., MARSAY, C. M., HENSON, S. A., SAW, K., COOK, K. & MAYOR, D. J. 2014. Reconciliation of the carbon budget in the ocean/'s twilight zone. *Nature,* 507**,** 480-483.

GOUIN, T., ROCHE, N., LOHMANN, R. & HODGES, G. 2011. A Thermodynamic Approach for Assessing the Environmental Exposure of Chemicals Absorbed to Microplastic. *Environmental Science & Technology,* 45**,** 1466-1472.

HANSEN, B. H., ALTIN, D., NORDTUG, T. & OLSEN, A. J. 2007. Suppression subtractive hybridization library prepared from the copepod Calanus finmarchicus exposed to a sublethal mixture of environmental stressors. *Comparative Biochemistry and Physiology Part D: Genomics and Proteomics,* 2**,** 250-256.

HEATH, M. R. & LOUGH, R. G. 2007. A synthesis of large-scale patterns in the planktonic prey of larval and juvenile cod (Gadus morhua). *Fisheries Oceanography,* 16**,** 169-185.

HOLMES, L. A., TURNER, A. & THOMPSON, R. C. 2012. Adsorption of trace metals to plastic resin pellets in the marine environment. *Environmental Pollution,* 160**,** 42-48.

HUVET, A., PAUL-PONT, I., FABIOUX, C., LAMBERT, C., SUQUET, M., THOMAS, Y., ROBBENS, J., SOUDANT, P. & SUSSARELLU, R. 2016. Reply to Lenz et al.: Quantifying the smallest microplastics is the challenge for a comprehensive view of

their environmental impacts. *Proceedings of the National Academy of Sciences,* 113**,** E4123-E4124.
IVERSEN, M. H. & POULSEN, L. K. 2007. Coprorhexy, coprophagy, and coprochaly in the copepods Calanus helgolandicus, Pseudocalanus elongatus, and Oithona similis. *Marine Ecology Progress Series,* 350**,** 79-89.
JAMBECK, J. R., GEYER, R., WILCOX, C., SIEGLER, T. R., PERRYMAN, M., ANDRADY, A., NARAYAN, R. & LAW, K. L. 2015. Plastic waste inputs from land into the ocean. *Science,* 347**,** 768-771.
KIØRBOE, T., MØHLENBERG, F. & HAMBURGER, K. 1985. Bioenergetics of the planktonic copepod Acartia tonsa: relation between feeding, egg production and respiration, and the composition of specific dynamic action. *Marine Ecology - Progress Series,* 26**,** 85-97.
KOELMANS, A. A., BAKIR, A., BURTON, G. A. & JANSSEN, C. R. 2016. Microplastic as a Vector for Chemicals in the Aquatic Environment: Critical Review and Model-Supported Reinterpretation of Empirical Studies. *Environmental Science & Technology,* 50**,** 3315-3326.
KOELMANS, A. A., BESSELING, E. & FOEKEMA, E. M. 2014. Leaching of plastic additives to marine organisms. *Environmental Pollution,* 187**,** 49-54.
KOELMANS, A. A., BESSELING, E., WEGNER, A. & FOEKEMA, E. M. 2013. Plastic as a Carrier of POPs to Aquatic Organisms: A Model Analysis. *Environmental Science & Technology,* 47**,** 7812-7820.
KOOI, M., REISSER, J., SLAT, B., FERRARI, F. F., SCHMID, M. S., CUNSOLO, S., BRAMBINI, R., NOBLE, K., SIRKS, L.-A., LINDERS, T. E. W., SCHOENEICH-ARGENT, R. I. & KOELMANS, A. A. 2016. The effect of particle properties on the depth profile of buoyant plastics in the ocean. *Scientific Reports,* 6**,** 33882.
LAW, K. L. & THOMPSON, R. C. 2014. Microplastics in the seas. *Science,* 345**,** 144.
LEE, H., SHIM, W. J. & KWON, J.-H. 2014. Sorption capacity of plastic debris for hydrophobic organic chemicals. *Science of The Total Environment,* 470**,** 1545-1552.
LEE, K.-W., SHIM, W. J., KWON, O. Y. & KANG, J.-H. 2013. Size-Dependent Effects of Micro Polystyrene Particles in the Marine Copepod Tigriopus japonicus. *Environmental Science & Technology,* 47**,** 11278-11283.
LEIKNES, Ø., STRIBERNY, A., TOKLE, N. E., OLSEN, Y., VADSTEIN, O. & SOMMER, U. 2014. Feeding selectivity of Calanus finmarchicus in the Trondheimsfjord. *Journal of Sea Research,* 85**,** 292-299.
LI, W. C., TSE, H. F. & FOK, L. 2016. Plastic waste in the marine environment: A review of sources, occurrence and effects. *Science of The Total Environment,* 566–567**,** 333-349.
LITHNER, D., LARSSON, Å. & DAVE, G. 2011. Environmental and health hazard ranking and assessment of plastic polymers based on chemical composition. *Science of The Total Environment,* 409**,** 3309-3324.
LOHMANN, R. 2017. Microplastics are not important for the cycling and bioaccumulation of organic pollutants in the oceans—but should microplastics be considered POPs themselves? *Integrated Environmental Assessment and Management,* 13**,** 460-465.
LONG, M., MORICEAU, B., GALLINARI, M., LAMBERT, C., HUVET, A., RAFFRAY, J. & SOUDANT, P. 2015. Interactions between microplastics and phytoplankton aggregates: Impact on their respective fates. *Marine Chemistry,* 175**,** 39-46.
LONG, M., PAUL-PONT, I., HÉGARET, H., MORICEAU, B., LAMBERT, C., HUVET, A. & SOUDANT, P. 2017. Interactions between polystyrene microplastics and marine phytoplankton lead to species-specific hetero-aggregation. *Environmental Pollution,* 228**,** 454-463.

LUSHER, A. L., MCHUGH, M. & THOMPSON, R. C. 2013. Occurrence of microplastics in the gastrointestinal tract of pelagic and demersal fish from the English Channel. *Marine Pollution Bulletin,* 67**,** 94-99.
MARSHALL, S. M. & ORR, A. P. 1972. *The Biology of a Marine Copepod: Calanus finmarchicus (Gunnerus)*, Springer-Verlag Berlin Heidelberg.
MELLE, W., RUNGE, J., HEAD, E., PLOURDE, S., CASTELLANI, C., LICANDRO, P., PIERSON, J., JONASDOTTIR, S., JOHNSON, C., BROMS, C., DEBES, H., FALKENHAUG, T., GAARD, E., GISLASON, A., HEATH, M., NIEHOFF, B., NIELSEN, T. G., PEPIN, P., STENEVIK, E. K. & CHUST, G. 2014. The North Atlantic Ocean as habitat for Calanus finmarchicus: Environmental factors and life history traits. *Progress in Oceanography,* 129, Part B**,** 244-284.
MEYER, B., IRIGOIEN, X., GRAEVE, M., HEAD, R. & HARRIS, R. 2002. Feeding rates and selectivity among nauplii, copepodites and adult females of Calanus finmarchicus and Calanus helgolandicus. *Helgoland Marine Research,* 56**,** 169-176.
MORATA, N. & SEUTHE, L. 2014. Importance of bacteria and protozooplankton for faecal pellet degradation**This work is a contribution to the Arctos Network and Conflux project. *Oceanologia,* 56**,** 565-581.
NIEHOFF, B. 2000. Effect of starvation on the reproductive potential of Calanus finmarchicus. *ICES Journal of Marine Science / Journal du Conseil,* 57**,** 1764-1772.
OBBARD, R. W., SADRI, S., WONG, Y. Q., KHITUN, A. A., BAKER, I. & THOMPSON, R. C. 2014. Global warming releases microplastic legacy frozen in Arctic Sea ice. *Earth's Future,* 2**,** 2014EF000240.
OGATA, Y., TAKADA, H., MIZUKAWA, K., HIRAI, H., IWASA, S., ENDO, S., MATO, Y., SAHA, M., OKUDA, K., NAKASHIMA, A., MURAKAMI, M., ZURCHER, N., BOOYATUMANONDO, R., ZAKARIA, M. P., DUNG, L. Q., GORDON, M., MIGUEZ, C., SUZUKI, S., MOORE, C., KARAPANAGIOTI, H. K., WEERTS, S., MCCLURG, T., BURRES, E., SMITH, W., VELKENBURG, M. V., LANG, J. S., LANG, R. C., LAURSEN, D., DANNER, B., STEWARDSON, N. & THOMPSON, R. C. 2009. International Pellet Watch: Global monitoring of persistent organic pollutants (POPs) in coastal waters. 1. Initial phase data on PCBs, DDTs, and HCHs. *Marine Pollution Bulletin,* 58**,** 1437-1446.
PHUONG, N. N., ZALOUK-VERGNOUX, A., POIRIER, L., KAMARI, A., CHATEL, A., MOUNEYRAC, C. & LAGARDE, F. 2016. Is there any consistency between the microplastics found in the field and those used in laboratory experiments? *Environmental pollution (Barking, Essex : 1987),* 211**,** 111-23.
PLASTICSEUROPE. 2016. *Plastics - the Facts 2016* [Online]. Available: http://www.plasticseurope.org/Document/plastics---the-facts-2016-15787.aspx?FolID=2 [Accessed 15 Jul 2017].
POULSEN, L. K. & IVERSEN, M. H. 2008. Degradation of copepod fecal pellets
key role of protozooplankton. *Marine Ecology Progress Series,* 367**,** 1-13.
POWELL, M. D. & BERRY, A. J. 1990. Ingestion and regurgitation of living and inert materials by the estuarine copepod Eurytemora affinis (Poppe) and the influence of salinity. *Estuarine, Coastal and Shelf Science,* 31**,** 763-773.
REISSER, J., SLAT, B., NOBLE, K., DU PLESSIS, K., EPP, M., PROIETTI, M., DE SONNEVILLE, J., BECKER, T. & PATTIARATCHI, C. 2015. The vertical distribution of buoyant plastics at sea: an observational study in the North Atlantic Gyre. *Biogeosciences,* 12**,** 1249-1256.
ROCHMAN, C. M., HENTSCHEL, B. T. & TEH, S. J. 2014. Long-Term Sorption of Metals Is Similar among Plastic Types: Implications for Plastic Debris in Aquatic Environments. *PLoS ONE,* 9**,** e85433.

ROCHMAN, C. M., HOH, E., KUROBE, T. & TEH, S. J. 2013a. Ingested plastic transfers hazardous chemicals to fish and induces hepatic stress. *Scientific Reports,* 3**,** 3263.
ROCHMAN, C. M., MANZANO, C., HENTSCHEL, B. T., SIMONICH, S. L. M. & HOH, E. 2013b. Polystyrene Plastic: A Source and Sink for Polycyclic Aromatic Hydrocarbons in the Marine Environment. *Environmental Science & Technology,* 47**,** 13976-13984.
ROCHMAN, C. M., TAHIR, A., WILLIAMS, S. L., BAXA, D. V., LAM, R., MILLER, J. T., TEH, F.-C., WERORILANGI, S. & TEH, S. J. 2015. Anthropogenic debris in seafood: Plastic debris and fibers from textiles in fish and bivalves sold for human consumption. 5**,** 14340.
SEBILLE, E. V., CHRIS, W., LAURENT, L., NIKOLAI, M., BRITTA DENISE, H., JAN, A. V. F., MARCUS, E., DAVID, S., FRANCOIS, G. & KARA LAVENDER, L. 2015. A global inventory of small floating plastic debris. *Environmental Research Letters,* 10**,** 124006.
SEBILLE, E. V., H. ENGLAND, M. & FROYLAND, G. 2012. Origin, dynamics and evolution of ocean garbage patches from observed surface drifters. *Environmental Research Letters,* 7**,** 044040.
SETÄLÄ, O., FLEMING-LEHTINEN, V. & LEHTINIEMI, M. 2014. Ingestion and transfer of microplastics in the planktonic food web. *Environmental Pollution,* 185**,** 77-83.
TEKMAN, M. B., KRUMPEN, T. & BERGMANN, M. 2017. Marine litter on deep Arctic seafloor continues to increase and spreads to the North at the HAUSGARTEN observatory. *Deep Sea Research Part I: Oceanographic Research Papers,* 120**,** 88-99.
TEUTEN, E. L., SAQUING, J. M., KNAPPE, D. R., BARLAZ, M. A., JONSSON, S., BJORN, A., ROWLAND, S. J., THOMPSON, R. C., GALLOWAY, T. S., YAMASHITA, R., OCHI, D., WATANUKI, Y., MOORE, C., VIET, P. H., TANA, T. S., PRUDENTE, M., BOONYATUMANOND, R., ZAKARIA, M. P., AKKHAVONG, K., OGATA, Y., HIRAI, H., IWASA, S., MIZUKAWA, K., HAGINO, Y., IMAMURA, A., SAHA, M. & TAKADA, H. 2009. Transport and release of chemicals from plastics to the environment and to wildlife. *Philos Trans R Soc Lond B Biol Sci,* 364.
THOMPSON, R. C. 2015. Microplastics in the Marine Environment: Sources, Consequences and Solutions. *In:* BERGMANN, M., GUTOW, L. & KLAGES, M. (eds.) *Marine Anthropogenic Litter.* Cham: Springer International Publishing.
TURNER, J. T. 2015. Zooplankton fecal pellets, marine snow, phytodetritus and the ocean's biological pump. *Progress in Oceanography,* 130**,** 205-248.
VAN CAUWENBERGHE, L., VANREUSEL, A., MEES, J. & JANSSEN, C. R. 2013. Microplastic pollution in deep-sea sediments. *Environmental Pollution,* 182**,** 495-499.
VROOM, R. J. E., KOELMANS, A. A., BESSELING, E. & HALSBAND, C. 2017. Aging of microplastics promotes their ingestion by marine zooplankton. *Environmental Pollution,* 231**,** 987-996.
WANG, J., TAN, Z., PENG, J., QIU, Q. & LI, M. 2016. The behaviors of microplastics in the marine environment. *Marine Environmental Research,* 113**,** 7-17.
WILSON, D. S. 1973. Food Size Selection Among Copepods. *Ecology,* 54**,** 909-914.
WOODALL, L. C., SANCHEZ-VIDAL, A., CANALS, M., PATERSON, G. L. J., COPPOCK, R., SLEIGHT, V., CALAFAT, A., ROGERS, A. D., NARAYANASWAMY, B. E. & THOMPSON, R. C. 2014. The deep sea is a major sink for microplastic debris. *R Soc Open Sci,* 1.
WRIGHT, S. L., THOMPSON, R. C. & GALLOWAY, T. S. 2013. The physical impacts of microplastics on marine organisms: A review. *Environmental Pollution,* 178**,** 483-492.

Appendix

Appendix A

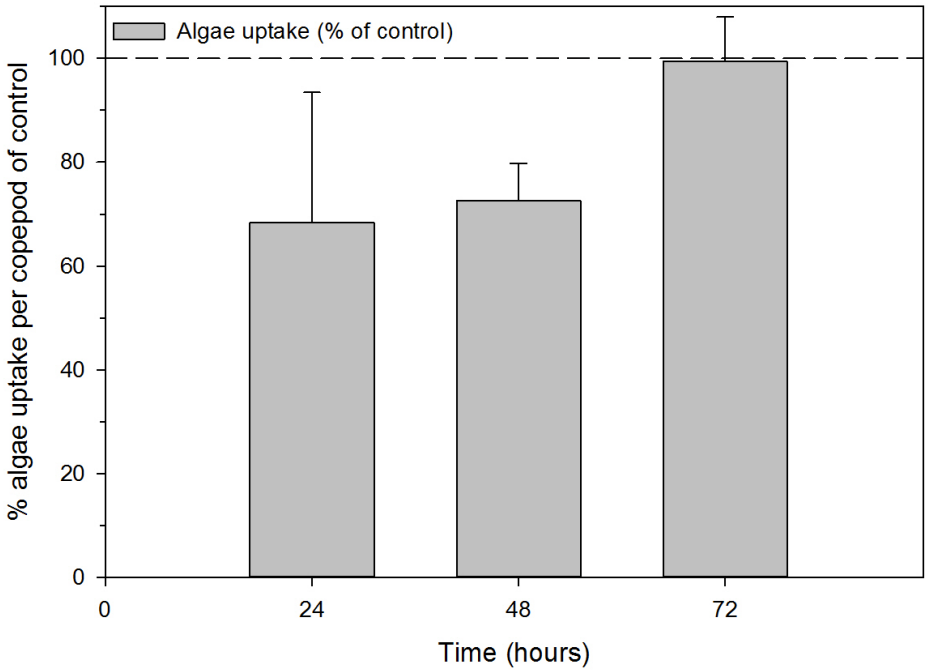

Figure A. Cumulative algae removal by MP exposed *C. finmarchicus* as percent of cumulative algae removal in non-exposed copepods (control) at sampling points 24, 48 and 72 hours. Mean±SD of three replicate bottles are given for each sampling point, each replicate containing 10 copepods, except one replicate in control group at 72 hours, n=11. The algae removal was calculated by subtracting the total number of algae cells measured in the medium plus the number of cells attached on the exposure bottle surface from the initial nominal concentration of 8,475,000 algae cells bottle^{-1} (7,500 cells ml^{-1}).

Appendix B

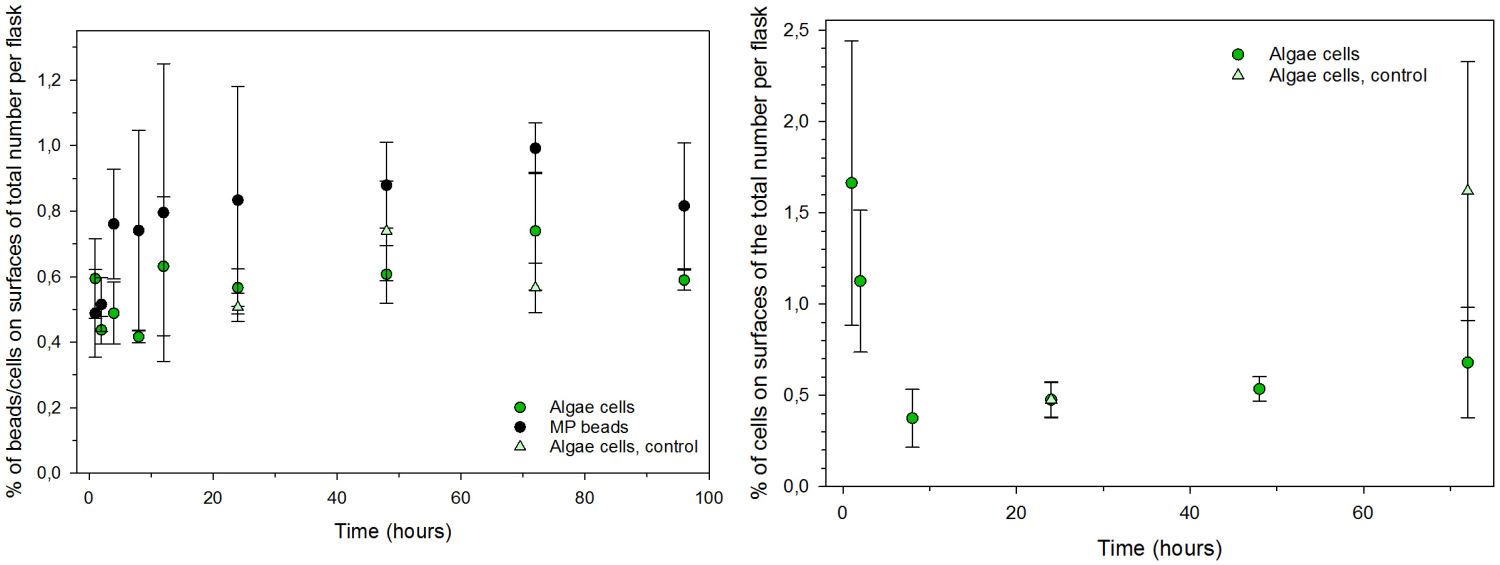

Figure B. Number of microalgae (*R. baltica*) (green dots) and PS microbeads (black dots) obtained from exposure bottle surfaces as percent of initial nominal numbers of algae (7,500 cells ml^{-1}) and particles (750 MPs ml^{-1}) during uptake study (left figure) and excretion study (algae only) (right figure). Percentages of initial nominal concentrations in controls are indicated by light green triangles. Mean±SD of three replicate bottles are given for each sampling point, each replicate containing 10 copepods, except one replicate in control group at 72 hours, n=11.

Appendix C

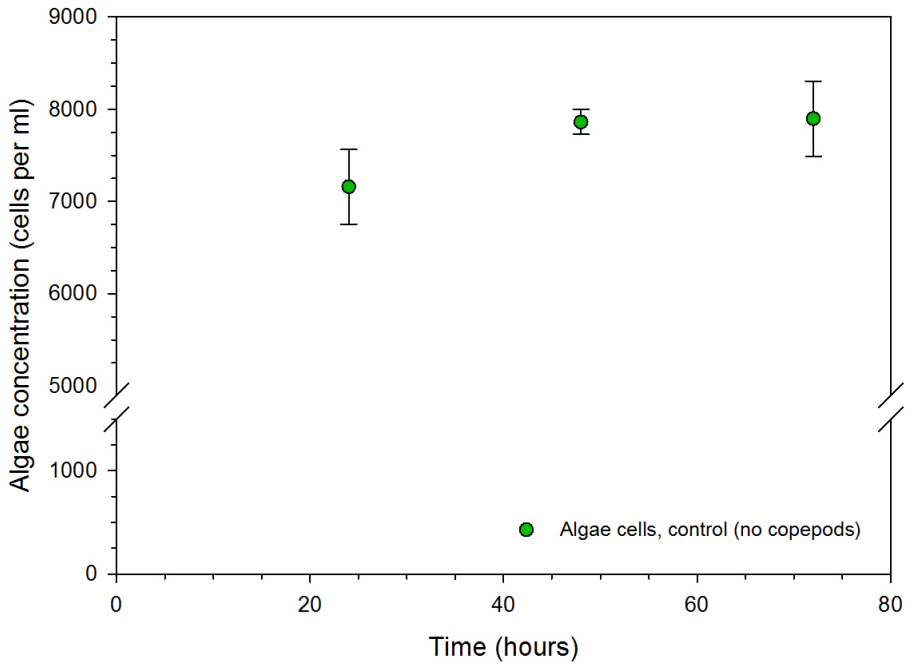

Figure C. Concentrations of microalgae *R. baltica* (cells ml^{-1}) in FSW measured at 24, 48 and 72 hours. Mean±SD of three replicate bottles are given for each sampling point.