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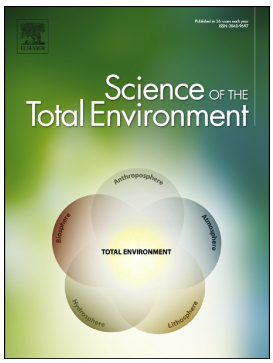

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Microplastics do not increase bioaccumulation of petroleum hydrocarbons in Arctic zooplankton but trigger feeding suppression under co-exposure conditions

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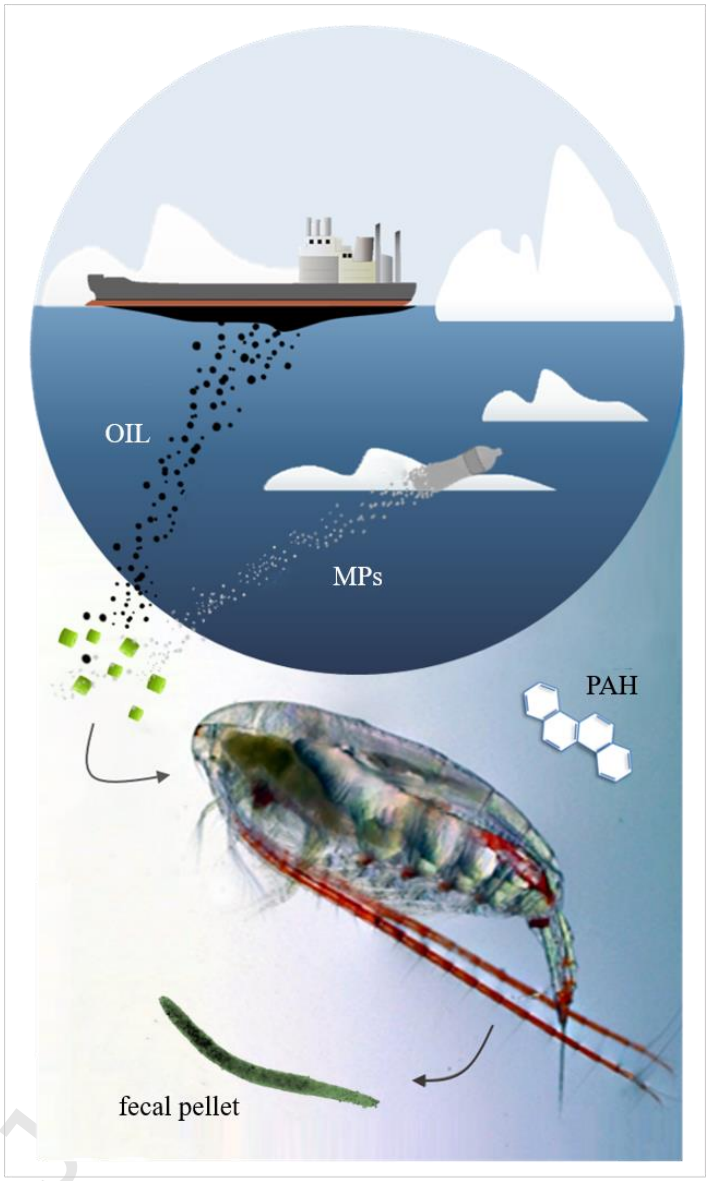
Abstract

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

Key words: crude oil, microplastics, Arctic, zooplankton, PAHs, vertical flux, fecal pellets.

Graphical abstract



1. INTRODUCTION

The Arctic has lost more than 40% of its sea ice in the last four decades, which makes this unique ecosystem one of the most rapidly changing regions on our planet (1). The decrease in Arctic sea ice has opened new opportunities for maritime transportation, cruise tourism and exploration for natural resources in this region (2, 3). An ice- free Arctic summer is projected within a few decades (4), and the shorter shipping routes through the Arctic are expected to be economically viable by 2040 (5). Increased expansion of shipping and oil industries in the Arctic could increase the risk of oil pollution, which is considered a major threat to this sensitive environment (6).

Oil spill responses are particularly challenging in the Arctic due to limited infrastructure. Although the application of chemical dispersants has been proposed as a feasible response to oil spills in the Arctic (7), the environmental benefits of dispersants (e.g., reducing the risk of oil slicks reaching shoreline) may be counteracted by their toxicity to marine pelagic organisms (8-11). The distinctive seasonal conditions and complex life cycle strategies in the Arctic call for a better evaluation of the net effect of chemical dispersants in Arctic ecosystems after oil spills (12).

Microplastics (MPs) have accumulated in the Arctic sea ice for decades by atmospheric deposition and their release will result from sea ice melting (13-16). Although MPs are ubiquitous in the ocean, Arctic sea ice and snow contain concentrations several orders of magnitude higher (>10000 MPs L^{-1}) than commonly found in marine surface waters in other latitudes (<0.1-1 MPs L^{-1}) (14-16). In fact, the Arctic Ocean has the highest average concentration of plastic microfibers in surface waters globally (17). This has increased concerns about the potential environmental impacts of plastic pollution in the Arctic, especially considering the current context of global warming-induced multiple stressors in the region.

Due to the high sorption capacity and lipophilic nature of MPs (18), the concentration of organic pollutants in MPs can be several orders of magnitude higher than in the surrounding waters (19, 20). Polycyclic aromatic hydrocarbons (PAHs), some of the most toxic compounds in crude oil, are sorbed by marine MPs (19, 20). Aside from the direct adverse physical effects (21), contact and ingestion of contaminated MPs could increase the uptake and accumulation of toxic organic pollutants in marine organisms (21-24). Nevertheless, the potential role of marine MPs as vectors of organic pollutants to marine biota is still debated with opposing views published recently (24-28). More research mimicking natural conditions after oil spills is required to understand the role of MPs in the transfer of organic pollutants in marine food webs.

Two key features determine the structure and dynamics of the Arctic ecosystem: a brief but highly productive phytoplankton bloom in spring and a lipid-based food web. The large copepods of the genus *Calanus* (C. *hyperboreus*, *C. glacialis* and *C. finmarchicus*) account for up to 80% of the mesozooplankton biomass and are the most important grazers in Arctic and subarctic food webs (29, 30). During the short spring phytoplankton bloom (1-2 weeks), Arctic copepods efficiently convert carbon from phytoplankton into large lipid reserves (31). As prey, copepods drive the transfer of high-energy lipids and essential fatty acids to higher trophic levels, including fish, birds, and marine mammals throughout the year (32). Moreover, the seasonal migration and deep-water overwintering of lipid-rich Arctic *Calanus* species, such as *C. hyperboreus*, promotes carbon sequestration, acting as a sink in the global carbon cycle (33). Therefore, Arctic *Calanus* species play a pivotal role in Arctic food webs as the main energy drivers, negative impacts on these key planktonic organisms may affect the entire Arctic ecosystem and, consequently the fisheries-dependent local economies. Further, because persistent organic pollutants (e.g., PAHs) accumulate in lipid tissues, the transfer and impact of pollutants in the lipid-rich Arctic food web is a major environmental concern (34).

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

2. MATERIAL AND METHODS

2.1. *Copepod collection*

Zooplankton samples were collected on the slope off Fyllas Banke (64°03' 24" N, 52°10'12" W) in West Greenland during a cruise on the research vessel R/V Sanna in May 2019. The zooplankton were collected using a mid-water ring (MIK) net (1500-µm mesh size) with oblique tows from 150 m depth at a speed of 2 knots. Onboard, the zooplankton samples in the cod-end were transferred to 100 L thermo-box containing *in situ* seawater and specimens of *C. hyperboreus* were immediately sorted out by use of wide-mouth pipettes and spoons. *C. hyperboreus* mature females with red genital somite were identified under the stereomicroscope, carefully separated and placed in beakers with *in situ* <20

µm filtered seawater (FSW) in a tray with ice. The sorted females were transferred to 20-L plastic cool boxes containing FSW, fed *ad libitum* with the diatom *Thalassiosira weissflogii* (12 µm) and kept with gentle aeration in a temperature-controlled room (2 ^{o}C) until returning to the laboratory, where the copepods were placed in an cold room at 2 ^{o}C . Cultures of *T. weissflogii* were grown with B1 medium plus silicates in a 18:6 h light:dark cycle with constant aeration and at room temperature (~15 ^{o}C).

2.2. *Experimental setup/design*

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

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

The experiment was designed to answer two main questions:

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

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

Additionally, we conducted a short-term experiment to estimate the effects of oil on the respiration rates of *C. hyperboreus*. We focused only on oil and dispersant as pollutants because MPs did not affect respiration rates in a parallel co-exposure study with other Arctic copepods (Almeda et al., unpublished results). Moreover, acute exposure to virgin MPs does not affect *Calanus helgolandicus* respiration rates (35).

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

2.3. Preparation and characteristics of the pollutants

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

The crude oil used in the exposure experiments was Light Louisiana Sweet oil. The chemical dispersant used was Corexit® 9500A, the main type of dispersant used during the Deepwater Horizon oil spill (36). We used a ratio of dispersant to oil of 1:20. Detailed methods on the preparation of the oil dispersions and dispersant solutions are provided in the Supporting Information (S.I.-Text 1). The diameter of the oil droplets in the dispersion (range: 1–90 µm, 95% of oil droplets were between 1–20 µm, mean = 8 µm) prepared with this methodology was previously determined using an Imaging Particle Analysis system (FlowSight®) (37).

2.4. Experimental conditions and procedures

The PAH composition and concentration in the crude oil and in the exposure media are shown in the Supporting Information (S.I.-Table 1 and Table 2, respectively). The oil and dispersant concentrations we used were in the range of concentrations normally found in the water column after accidental oil spills and/or dispersant applications (38-41). The oil exposure concentration we used (~0.84 ppm) also was environmentally relevant considering "operational pollution" and the legal upper limits for oil discharges from the oil extraction industry (~30 ppm for "produced water") (42) and shipping effluents

(15 ppm) (43). The type of MP used in this study, PE, is the most abundant plastic polymer in surface waters (44) and one of the MPs with the highest affinities for sorption of hydrophobic organic pollutants, including PAHs (18,19). The concentration of MPs used here (20 MPs mL^{-1}) was higher than those currently observed in Arctic surface waters (14), but it could occur in a future if abundant MPs retained in Arctic sea ice and snow (15-16) are released to adjacent waters due to global warming (13).

Copepods were acclimated to the experimental temperature (2 ^{o}C) and diet (*T. weissflogi*i) for 2 days before the experiment. *C. hyperboreus* females were incubated individually in 600-mL glass bottles with red screw caps with polytetrafluoroethylene (PTFE) protected seal. First, the bottles were filled with FSW (salinity=33) and an aliquot of the diatom (*T. weissflogii*) culture was added to ensure a food concentration of 2,000-3,000 cells mL^{-1}. These diatom concentrations are found commonly during the spring bloom in Greenlandic waters (45). Sedgewick-Rafter counting chambers and an inverted microscope were used to determine the concentrations of diatoms in the culture. Second, copepods were placed in a beaker with FSW, sorted gently with a spoon or a wide-mouth pipette and then added individually to the bottles (1 copepod per bottle). Ten random individuals were used to measure the initial size and lipid sac area. Finally, the pollutants were added to the corresponding experimental bottles to obtain the exposure levels indicated above. We used 10 replicates per treatment.

The bottles were mounted on a plankton wheel that rotated at 1 rpm in a temperature-controlled room at 2 ^{o}C. This incubation temperature is similar to the *in situ* temperature that *C. hyperboreus* experiences in west Greenland during spring (46). The food and exposure media were renewed every 24 h for 5 days. The bottle content was carefully poured in a bucket and the copepod was gently collected and transferred with a metal spoon to the new bottle containing food and exposure media. The water from the bottles was filtered through a 40-μm mesh sieve to collect the fecal pellets daily. Fecal

pellets from days 3 and 4 were combined and triplicate samples of 100 fecal pellets from each treatment were sorted and stored in glass vials at -80 ^{o}C for later chemical analysis of PAHs.

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

Before the respiration experiment, thirty females were sorted and placed in a glass beaker containing 1 L of FSW. By use of a 3 mL metal spoon, 2-3 copepods were added to 26-mL acid-washed glass bottles filled with 0.2 µm-FSW. Then the copepods were exposed to the following treatments: 1 µL L^{-1} of oil ("oil-1"); 2 µL L^{-1} of oil ("oil-2"); oil plus dispersant (1 µL L^{-1} of oil and 0.05 µL L^{-1} of dispersant) ("oil +D"); no pollutants ("CTRL"). We also measured the oxygen (O_2) concentration in bottles with FSW and 3 mL of seawater from the beaker from which the copepods were sorted ("FSW"). The bottles (three replicates per treatment=15 bottles) were sealed with butyl rubber stoppers without trapping air bubbles and mounted on a plankton wheel. The bottles were incubated in darkness at 2°C for up to 24 h. O_2 concentration was measured every 4 h, as described in Stief et al. (2016) (47). Respiration rates were calculated from the linear regression of the decrease in O_2 concentration over time in copepod incubations corrected for O_2 concentration changes in the "FSW" bottles.

2.5 Sample analysis and calculations

To estimate copepod size and lipid content, we photographed 10 specimens separated at the beginning of the experiment and of every copepod in the experiment at the end of the exposure period (~5 days). Each copepod was placed in a Petri dish and then photographed from a lateral view. Images were taken

with a camera attached to a stereomicroscope; prosome length (Fig. 2A) and lipid sac area of each copepod (Fig. 3A) were measured with image analysis (Image J). The volume of the prosome was calculated assuming an ellipsoidal shape. We used lipid sac area (A, mm^2) as a proxy for individual total lipid content (TL, mg), which was calculated according to the equation in Vogedes et al. (2010) (48):

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

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

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

For PAH analyses, copepod and fecal pellet samples were extracted and analyzed according to internal, validated protocols at SINTEF Ocean (49, 50). Briefly, samples were solvent extracted and purified as described in Øverjordet et al. 2018 (50) and the analysis of 44 parent and alkylated PAHs was conducted using gas chromatography-tandem mass spectrometry (GC-MS-MS) (51). The PAH contents in the crude oil and in the initial exposure media were profiled after solvent extraction and analysis by gas chromatography-mass spectrometry (GC-MS) (51). Internal standards were utilized to account for losses of analyte during sample extraction.

2.6. Statistical data analysis

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

3. RESULTS AND DISCUSSION

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

Although marine zooplankton are particularly sensitive to crude oil (8-10, 52-54), we did not observe lethal effects on *C. hyperboreus* in any of the treatments. Lethal effects of oil on copepods are expected to decrease with increasing body size and with decreasing temperature (55); therefore, the large size of *C. hyperboreus* (Fig. 2C) and the low water temperature could explain the higher tolerance to oil of this species compared with smaller copepods from warmer waters (8). Lipid-rich copepods commonly have higher survival rates than lipid-poor copepods, probably because the temporary immobilization of lipophilic oil compounds (like PAHs) in large storage lipids may retard toxicokinetics (49, 56).

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

Fecal pellet production rate (FPPR), or egestion rate, is directly related to ingestion rate and commonly used as a proxy for grazing rate in copepods (57). The median fecal pellet volume was not-significantly different between treatments, except for the OIL treatment, in which the fecal pellets were significantly bigger (Kruskal-Wallis Test, $H(3) = 40.3$, $p<0.001$) (Fig. 2D). This can be partly explained by the slightly bigger mean size of the copepods in the OIL treatment than in the control (ANOVA, Bonferroni test, $p=0.03$) (Fig. 2C). The ingestion and subsequently encapsulation of MPs in fecal

pellets did not affect the size of the fecal pellets compared to the control (Fig. 2C), as previously found in *C. helgolandicus* when exposed only to MPs (35).

Reduced ingestion or fecal pellet production rates have been found in other planktonic copepods after exposure to environmentally realistic oil concentrations, as in this study (8, 58, 59). At the exposure concentration used, oil alone did significantly affect daily FPPR during the first two days of exposure (ANOVA, $p>0.05$), but the reduction of FPPR was significant after day 3, 20-27% lower than the control (ANOVA, $p<0.05$) (Fig. 2E).

In this study, 20-40% of *C. hyperboreus* females did not produce fecal pellets (= non-feeding) in the treatments with co-exposure to oil and MPs with or without dispersants (Oil + MPs and Oil + MPs + D) (Fig. 2F). In contrast, all copepods in the control and OIL treatments produced fecal pellets (Fig. 2F). Pollution-induced feeding suppression in *C. hyperboreus* also was reported after exposure to seawater from oil seeps (60). In a parallel study, however, MPs alone (20 MPs mL^{-1}) did not cause feeding suppression or significantly affect *C. hyperboreus* FPPR (ANOVA, F2,9, =0.267, p=0.77) (S.I. Fig. 1). This indicates that the presence of MPs can intensify this behavioral stress response of *C. hyperboreus* to oil, which emphasizes the importance of investigating the effects of both single and combined pollutants on zooplankton.

The daily FPPR for the entire exposure period (A-FPPR, Fig. 2G) were significantly lower in the treatments with Oil + MPs and Oil + MPs + D than in the control and oil treatments (ANOVA, $F_{3,36}=6.25$, $p=0.002$; Bonferroni test, $p<0.05$) (Fig. 2G). By excluding non-feeding copepods and considering the fecal pellet and copepod size, volume- specific FPPR in the treatments with Oil + MPs and Oil + MPs + D were about 50% lower than in the control and oil treatments (ANOVA, $F_{3,31}=19.8$, $p<0.001$; Bonferroni test, $p<0.05$) (Fig. 2H). Therefore, the decrease in FPPR in the co-exposure treatments was due to a suppression of feeding in some individuals, and to a reduction in the ingestion rates. This decrease could be due to potential adverse effects of oil droplets and plastic particles on the

feeding behavior (e.g., selectivity, handling time, etc.) (61, 62), together with a certain degree of oil-induced narcosis (63).

The addition of chemical dispersant did not increase the negative effect of oil and MPs together on FPPR (Fig. 2). Hansen et al. (2012) also found small differences in acute toxicity between mechanically and chemically dispersed oil on *C. finmarchicus* (64). This suggests that large *Calanus* species may have a higher tolerance to acute exposure to chemical dispersants than smaller lipid-poor copepods from warmer waters (8).

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

The lipid sacs of *C. hyperboreus* females used in the experiments were thin and elongated, indicating they were not completely filled (Fig. 3A); the individual lipid content was positively correlated with body volume (Fig. 3B). The median volume-specific lipid content ranged from 0.042 to 0.056 mg mm^{-3} without significant differences among treatments (ANOVA, $F_{4,39} = 1.18$, p=0.331) (Fig. 3C). Oxygen consumption in the experimental bottles with *C. hyperboreus* was higher than in those from the "FSW" treatment, which contained water that the copepods were kept in before the test (Fig. 3D). Respiration rates (nmoles O_2 $copepod^{-1}$ h^{-1}) increased with copepod body size (Fig. 3E) and were in the range of respiration rates found previously for this species (60, 65) and, more generally, in Arctic zooplankton (66). Daily C-specific respiration rates were low, only 2-3% (Fig. 3F). Although there was a trend towards increased respiration rates in the presence of pollutants, these differences among treatments were not significant different (ANOVA, $F_{3,8}$ =1.46, p=0.295) (Fig. 3F). The absence of effects of acute exposure to oil-contaminated waters on respiration rates in *C. hyperboreus* also has been reported (60). Despite the decrease in food uptake (based on FPPR, Fig. 2H), the low respiratory carbon losses of *C. hyperboreus* (Fig. 3F) can partly explain why the total lipid storage did not differ among treatments.

3.4. *Bioaccumulation of PAHs in copepods and fecal pellets*

We found bioaccumulation of PAHs in *C. hyperboreus* in the three experimental treatments (ANOVA, $F_{3, 36}$ =10.04, p=0.001; Bonferroni test, p<0.05) (Fig. 4A). The median total PAH (T-PAH) body burden of *C. hyperboreus females* was ~1-2 µg g^{-1} (Fig. 4A). PAHs are considered to be the most toxic compounds in crude oil (67, 68). The main routes of PAH uptake in marine organisms are passive diffusion from the water, ingestion of contaminated prey (dietary intake) and, in the case of oil dispersions, contact (fouling) and direct ingestion of oil droplets (62, 69-71). When exposed to oil dispersions, ingestion of oil droplets is a significant contributor to PAH body burden (70). In agreement with this, we found that the T-PAH body burden in feeding individuals was ~ 3 times significantly higher than in non-feeding copepods (t-test, t (15.21)= 5.748, p<0.001) (Fig. 4B). The concentration of less soluble PAHs (≥ 4 rings) was ~16 times higher in feeding than in non-feeding copepods (t-test, t (14.06)=6.603, p<0.001) (Fig. 4D). This indicates that, when copepods are exposed to oil dispersions, dietary uptake (ingestion of oil droplets and PAH-contaminated food) is the main route for accumulation of PAHs, particularly for less soluble PAHs (≥ 4 rings) (4B, 4D) which are primarily found in the droplet fraction (70). The presence of numerous oil droplets in the fecal pellets corroborated the ingestion of small oil droplets by *C. hyperboreus* (Fig. 5H). The concentration of T-PAHs in the fecal pellets ranged from ~0.10 to 0.35 µg g^{-1} with significant differences among treatments (ANOVA, $F_{3, 8}$ =33.59, p<0.001; Bonferroni test, p<0.05) (Fig. 5A).

We found differences in PAH compositions among the exposure media, copepods and fecal pellets (Fig. 6). The exposure solution had predominantly PAHs with high solubility (e.g., naphthalenes, 2 rings), whereas PAHs with lower solubility (3-6 rings) tended to be accumulated in copepods and fecal pellets (Fig. 6). Phenanthrene was the most abundant PAH in the copepod and fecal pellet samples (Fig. 6B, 6C). The observed partitioning of parent PAHs between oil dispersions and copepods tended to follow the PAH octanol/water partition coefficient (K_{ow}) and bioconcentration factor (BCF) relationship observed in a previous study for *C. finmarchicus* (70).

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

We found that *C. hyperboreus* ingested MPs, which after passage through the copepod gut, are expelled inside fecal pellets (Fig. 5). The median T-PAH body burden in the treatment with oil + MPs was 30% lower than in the treatment with oil alone, but the difference between treatments was not statistically significant (ANOVA, Bonferroni test, $p>0.05$) (Fig. 4). The T-PAH concentration in fecal pellets in the treatment with oil + MPs was similar to the treatment with oil alone (ANOVA, Bonferroni test, $p>0.05$) (Fig. 5A). Therefore, the ingestion of MPs did not facilitate PAH bioaccumulation in the copepods (or fecal pellets) in our study (Fig. 5A). The fact that we did not observe any effect of MPs in PAH bioaccumulation was not related to the co-exposure time or the polymer type (PE) used because it has a high affinity for PAHs (77) including phenanthrene (78), the most abundant PAH in the copepods and fecal pellets in our study (Fig. 6). Additionally, in the case of small PE MPs, the sorption of PAHs is even higher at low temperatures (76).

Several laboratory studies have found that MPs can transfer sorbed organic pollutants to marine biota after contact or ingestion, especially when gut conditions promote desorption of organic pollutants (28, 72-74). Other studies, however, suggest that MPs may act as "passive samplers" of organic pollutants, rather than as vectors and, consequently MPs may reduce the amount of bioavailable PAHs (79, 76). In our study, copepods were exposed to food concentrations commonly found during the phytoplankton bloom in the Arctic. The biomass of phytoplankton, which also absorbs PAHs (69), is several orders of magnitude higher than the concentration of MPs typically found in surface waters (14), which minimizes the role of MPs as vectors of PAHs. Therefore, our results support the view that the quantitative role of MPs as vectors of pollutants to marine biota is minor compared to other routes of uptake, such as passive diffusion from the water and uptake from contaminated food (80).

Chemical dispersants increase oil dispersion by formation of smaller oil droplets resulting in faster PAH equilibration between oil droplets and water (81). This can promote PAH uptake through passive

diffusion from the water to phytoplankton and then to copepods, or directly from the water to fecal pellets. Although we did not find significant differences in the T-PAH body burden among experimental treatments (ANOVA, Bonferroni test, $p>0.05$) (Fig. 4A), the concentration of less soluble PAHs (≥4 rings, log K_{ow}>4.5) in feeding copepods was 45% higher in the treatment with dispersants than in the treatment only with oil and MPs (ANOVA, $F_{3, 31} = 18.09$, $p<0.001$; Bonferroni test, $p<0.05$) (Fig. 4C). This suggests higher ingestion rates of small chemically dispersed oil droplets. In contrast, uptake of ≥4 ring PAH was comparable in *C. finmarchicus* exposed to oil dispersion with and without the addition of dispersants (82). Our study, however, shows that the concentration of T-PAHs per gram of fecal pellets with dispersants was ~3 times higher than in the other treatments with oil (ANOVA, $F_{3, 8} = 33.59$, $p<0.001$; Bonferroni test, $p<0.05$) (Fig. 5A). This suggests that the amount of bioavailable PAHs increased with the addition of dispersants, and that the increase in accumulation rate was higher than the PAH sorption rate MPs in our study. Therefore, the use of dispersants after oil spills can increase the entry and bioaccumulation of toxic PAHs in the Arctic food webs via zooplankton and fecal pellets.

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

The presence of oil droplets and MPs inside the fecal pellets did not have any clear effect on their sinking rates (Supporting Information, S.I. Fig. 2), likely due to their small volume compared with the denser material in the fecal pellets. We estimated sinking rates of fecal pellets at 15 ^{o}C in the laboratory (SI; Fig. 2) but, given the influence of temperature in water viscosity and density, the fecal pellet sinking rates are expected to be slower at in situ temperatures (~ 70% slower at 0 ^{o}C than at 15 ^{o}C) (83). When the impact of the studied pollutants on FPPRs is taken into account, we estimated that the amount of PAHs defecated was lower in the presence of MPs than with oil alone but higher in the presence of dispersants (Fig. 5B, 5C). Our experimental food concentration (2000-3000 cells mL^{-1}) represents spring bloom densities; we estimated that copepods could potentially ingest and defecate up

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

3.7. Ecological implications

Exposure experiments under laboratory-controlled conditions are an essential tool to investigate the interactions between MPs and marine biota, but direct extrapolation to the field should always be considered carefully. In nature, the ingestion and effects of MPs on copepods will depend on multiple environmental factors that are difficult to mimic in the lab, e.g., currents and turbulence, which may affect encounter rates between MPs and zooplankton. Moreover, once in the environment, MPs are weathered (e.g, bio-fouled), which can affect the interactions between copepods and MPs (84). Yet, our eco-toxicological exposure experiment is a reliable means of detecting effects of virgin MPs on zooplankton and their potential role in the bioaccumulation of PAHs.

Our results indicate that exposure to environmentally relevant concentrations of oil can cause sub-lethal effects on a key secondary producer in the Arctic. *C. hyperboreus* has the largest lipid reserves and the longest life span (3-5 years) among the Arctic *Calanus* species (30), and a slow depuration rate of oil components (85). Therefore, the bioaccumulation of PAHs in this species could increase the residence time and biotransfer of these persistent organic pollutants in Arctic food webs. Additionally, the mobilization of PAH-contaminated lipid reserves during overwintering could negatively affect the survival of *C. hyperboreus* and reproduction during this critical period of the life cycle (86).

High concentrations of MPs (20 particles mL^{-1}) can enhance suppression of feeding as a behavioral stress response to oil pollution in *C. hyperboreus*. In the short term, this behavioral response to acute exposure to pollutants seems positive for these individuals since it reduces the uptake of oil droplets and the accumulation of toxic PAHs, but prolonged feeding suppression can lead to reduced lipid

storage and starvation. The influence of MPs on the toxicity and bioaccumulation of oil in Arctic copepods is expected to be minor when considering the concentrations of MPs currently found in Arctic surface waters (87,88). However, in an accelerating global warming scenario, higher concentrations of MPs could be temporarily found in Arctic sea-ice adjacent waters (15-18), which requires further field research and monitoring.

The use of chemical dispersants after oil spills can increase the accumulation of toxic PAHs in zooplankton and their fecal pellets. Since *Calanus* copepods are the main prey of fish, birds, and marine mammals in the Arctic, the potential trophic transfer of PAHs via zooplankton is a major concern. PAHs can be subject to trophic dilution in the marine food webs (89,90). However, trophic transfer and magnification of PAHs via contaminated zooplankton could be relevant for very hydrophobic PAHs (91). Particularly for Arctic planktivorous seabirds (e.g., little auk, one of the most numerous seabirds in the world) that feed directly on *Calanus* copepods (92).

Zooplankton fecal pellets can play a role in the sedimentation of PAHs (93). Additionally, vertical exportation of small low-density MPs via copepod fecal pellets is one of the processes that may explain why the deep sea is a major sink for MP debris (94). The potential impacts of accumulation of MPs on benthic sediment communities in the Arctic are still not well known and need further research.

The Arctic has the world's largest remaining reservoirs of crude oil (95). Oil industry activities in the Arctic may rise over the next decades considering the expected increase in oil demand (96) and the loss of sea ice cover, which will facilitate oil industry accessibility and maritime transportation in the Arctic (2-3). Strong regulations and controls on oil pollution and better knowledge about the net environmental benefits of different oil spill responses in the Arctic are priority issues to reduce the impacts of oil pollution in the Arctic (7). In addition, MPs are ending up in the Arctic sea ice, which is becoming a major sink of global plastic pollution (15). Therefore, reducing global plastic pollution will prevent increased anthropogenic impacts on the unique but multiple-stressed Arctic ecosystem.

4. CONCLUSIONS

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

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

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

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

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

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Declaration of interests:

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

Sample CRediT author statement:

Rodrigo Almeda: Conceptualization, Methodology, Formal analysis, Investigation, Writing - Original Draft, Writing - Review & Editing. Project administration, Funding acquisition. **Rocío Rodriguez-Torres**: Investigation, Writing - Review & Editing. **Sinja Rist**: Investigation, Writing - Review & Editing. **Mie H.S. Winding**: Investigation, Writing - Review & Editing. **Peter Stief**: Investigation, Writing - Review & Editing. **Bjørn Henrik Hansen**: Resources, Formal analysis, Writing - Review & Editing. **Torkel Gissel Nielsen**: Methodology, Investigation, Writing - Review & Editing, Project administration, Funding acquisition.

FIGURE 1

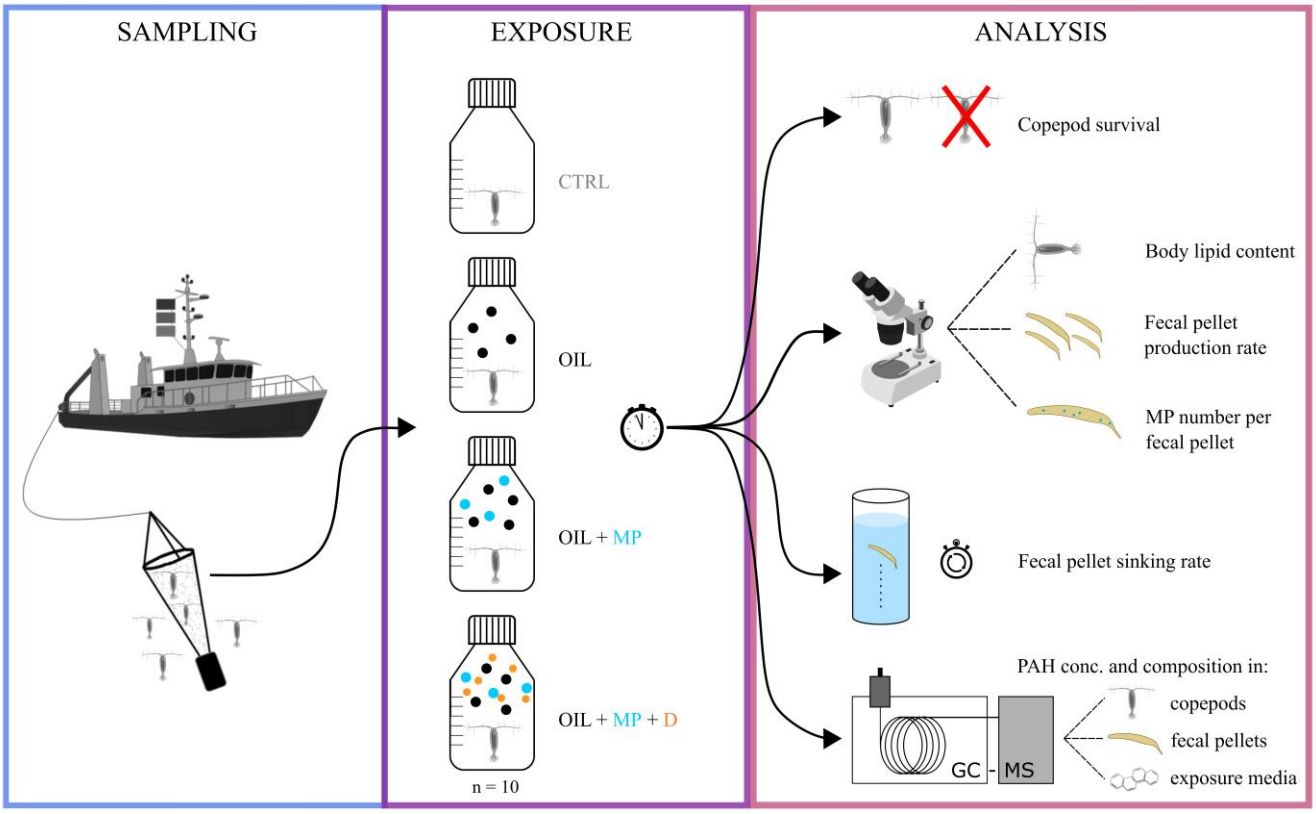


Figure 1. Overview of the study, including the exposure treatments and the subsequent analyses.

FIGURE 2

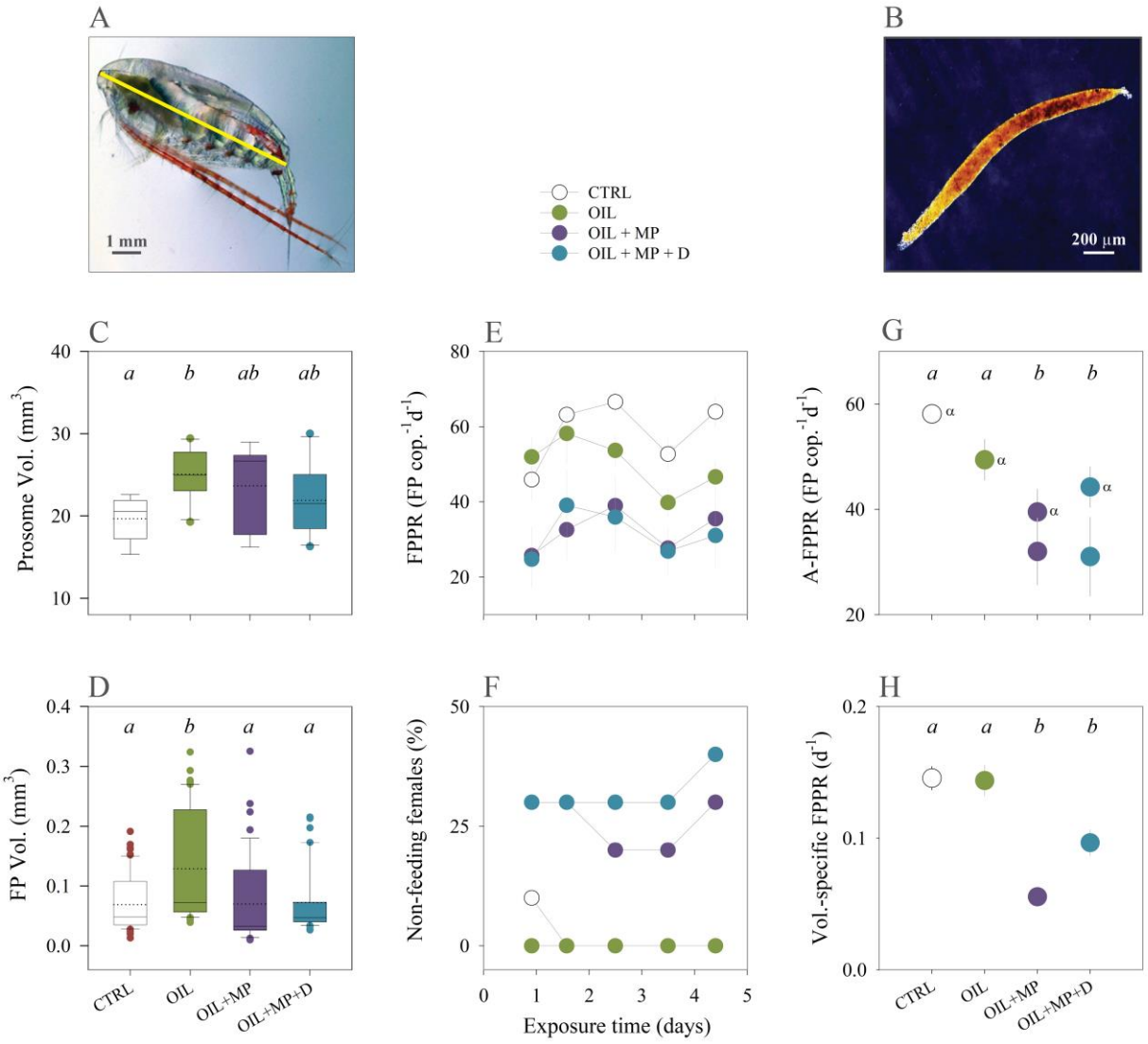


Figure 2. A: Female of *Calanus hyperboreus* where the prosome length is indicated with a yellow line. **B**: fecal pellet of *C. hyperboreus*. **C**: prosome volume of the specimens used in the different treatments. **D**: fecal pellet (FP) volume in the different treatments. **E**: mean daily fecal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

FIGURE 3

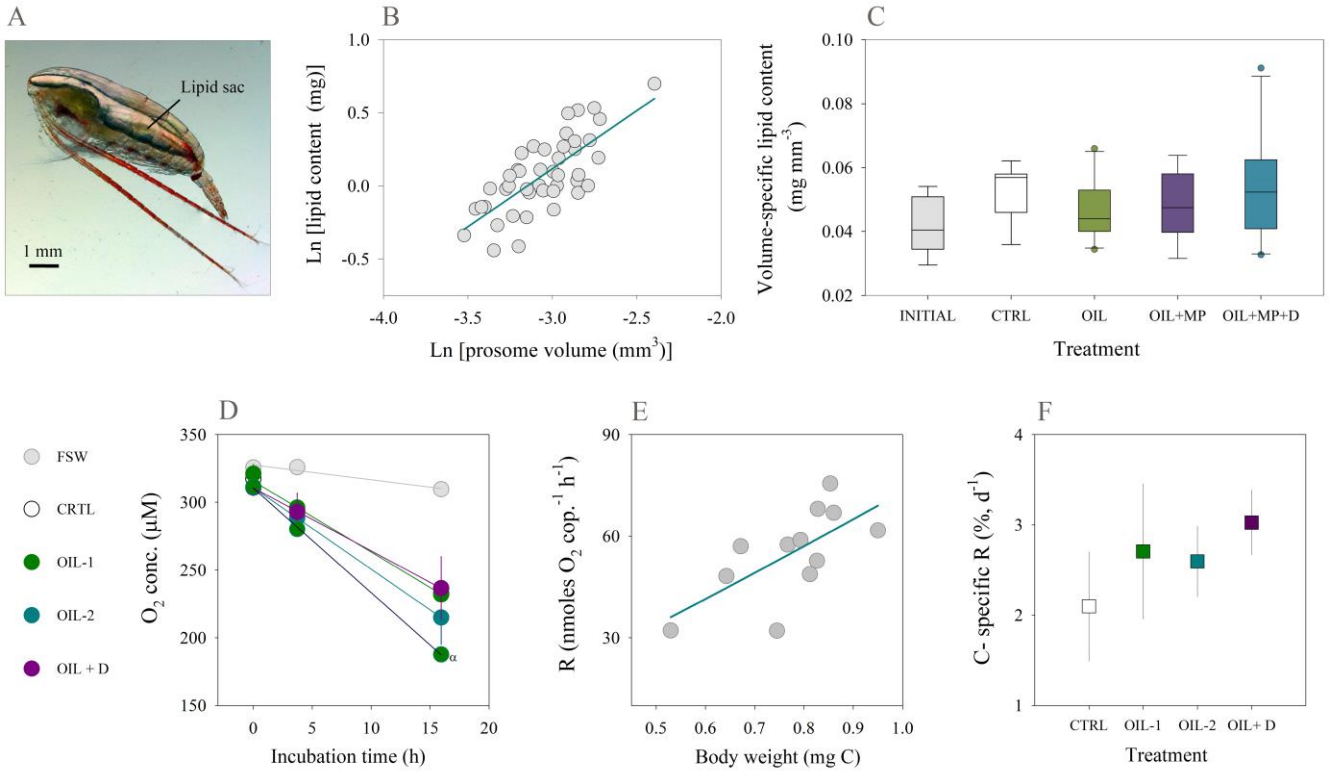


Figure 3. **A**: Female of *Calanus hyperboreus* with a visible lipid sac. **B**: Correlation between body total lipid content (L, mg) and the prosome volume (V, mm^3) in *C. hyperboreus* considering all the experimental specimens. Linear regression: ln [L] = 2.50 + 0.79 ln [V], r^2=0.53, p<0.001. **C**: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

FIGURE 4

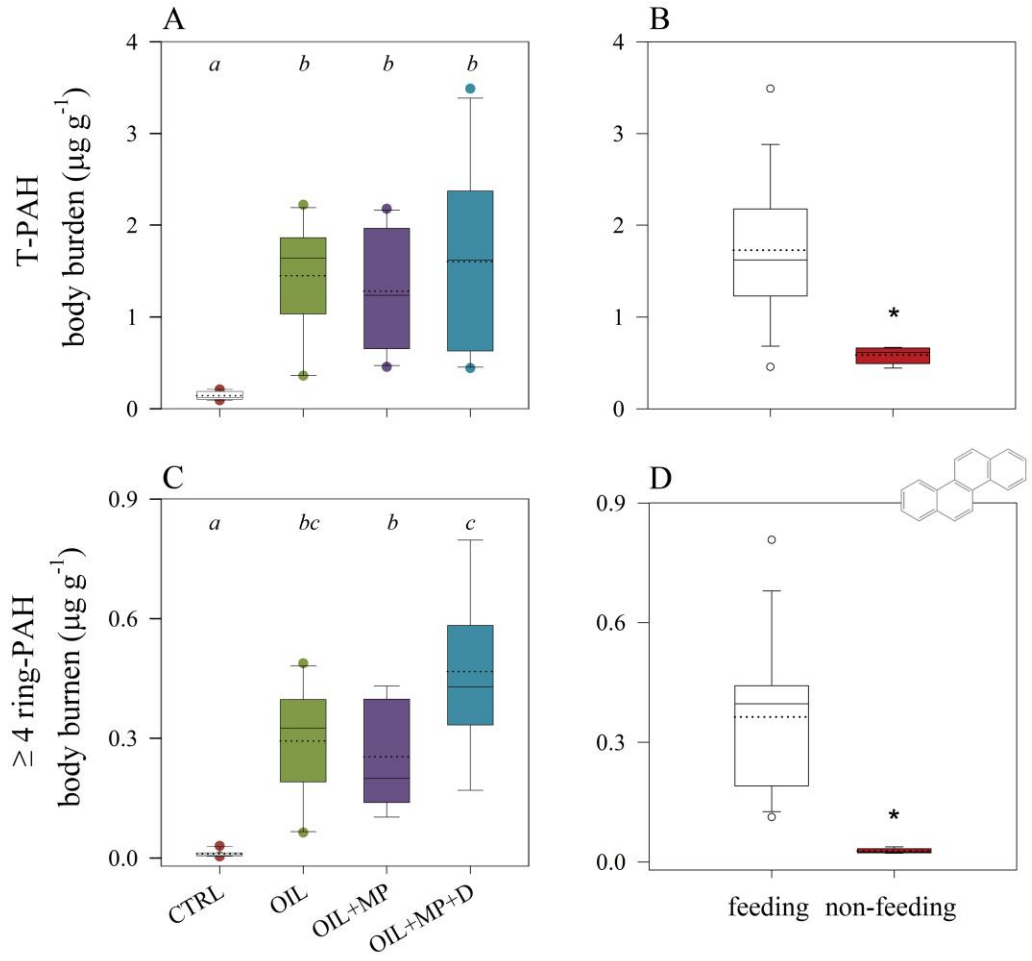


Figure 4. **A**: Total PAHs (T-PAH) body burden in *Calanus hyperboreus* after 4.5 days of exposure to the different treatments. **B**: T-PAH body burden in non-feeding and feeding specimens from the treatments were feeding suppression was observed (OIL + MP; OIL + MP+ D) (Fig. 1F). **C**: Body burden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

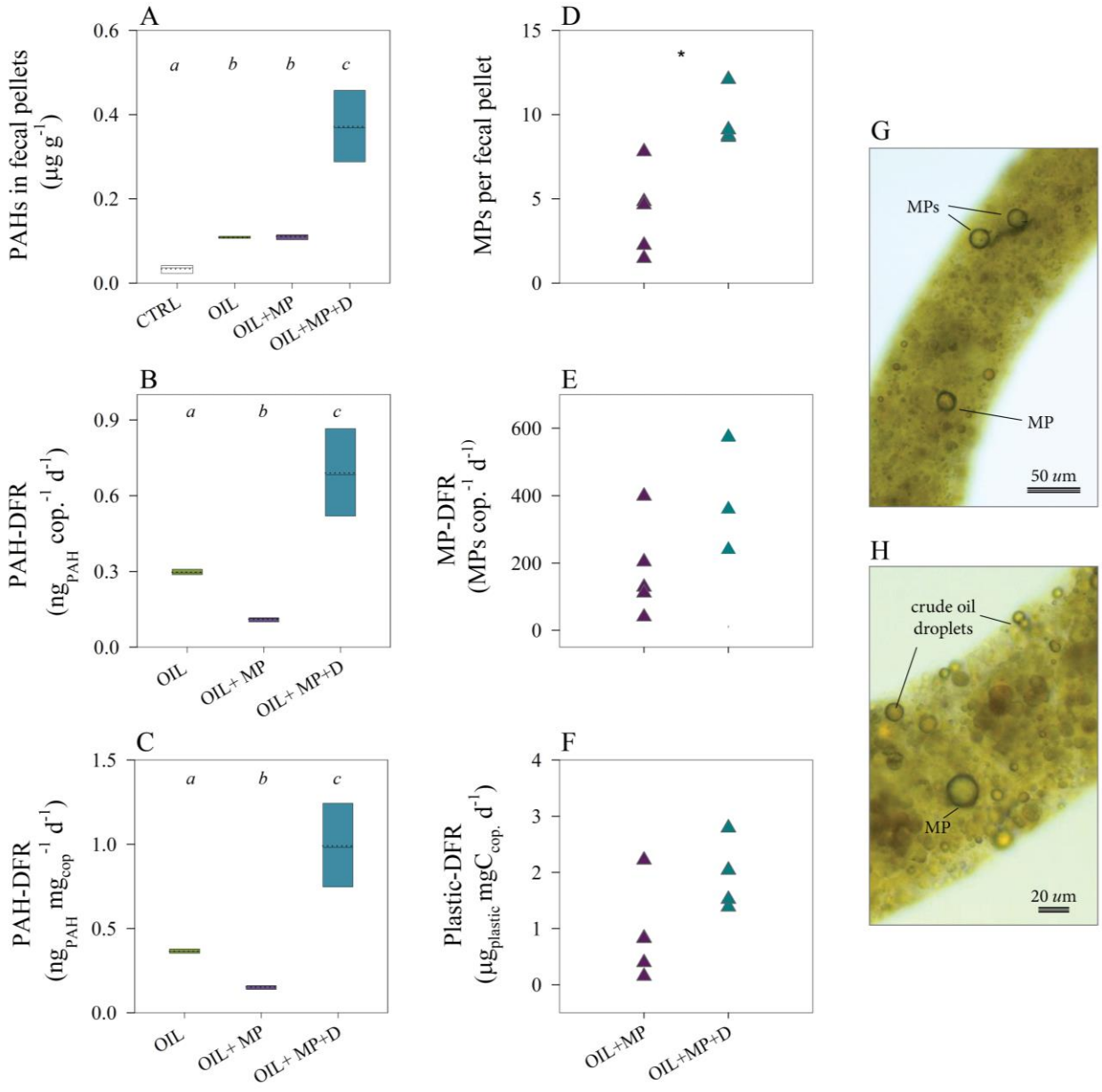


FIGURE 5

Figure 5. **A**: Concentration (µg g^{-1}) of total PAHs in fecal pellets of *Calanus hyperboreus* in the different treatments. **B**: Daily defecation rate of PAHs (PAH-DFR) per copepod. **C**: weight- specific PAH-DFR. **D**: average number of MPs per fecal pellets. **E**: Daily defecation rate of MPs (MP-DFR) per copepod. **F**: weight-specific MP-DFR. Fecal pellet density for calculations was 1.12 g cm^{-3} [97]. **G-H**: microscope

images showing the presence of MPs and oil droplets inside fecal pellets. The asterisk (*) indicates a significant difference between treatments according to the Independent Samples t-Test (D). Lowercase italics letters *(a,b,c)* indicated different statistical groups according to the Post-hoc Bonferroni test.

FIGURE 6

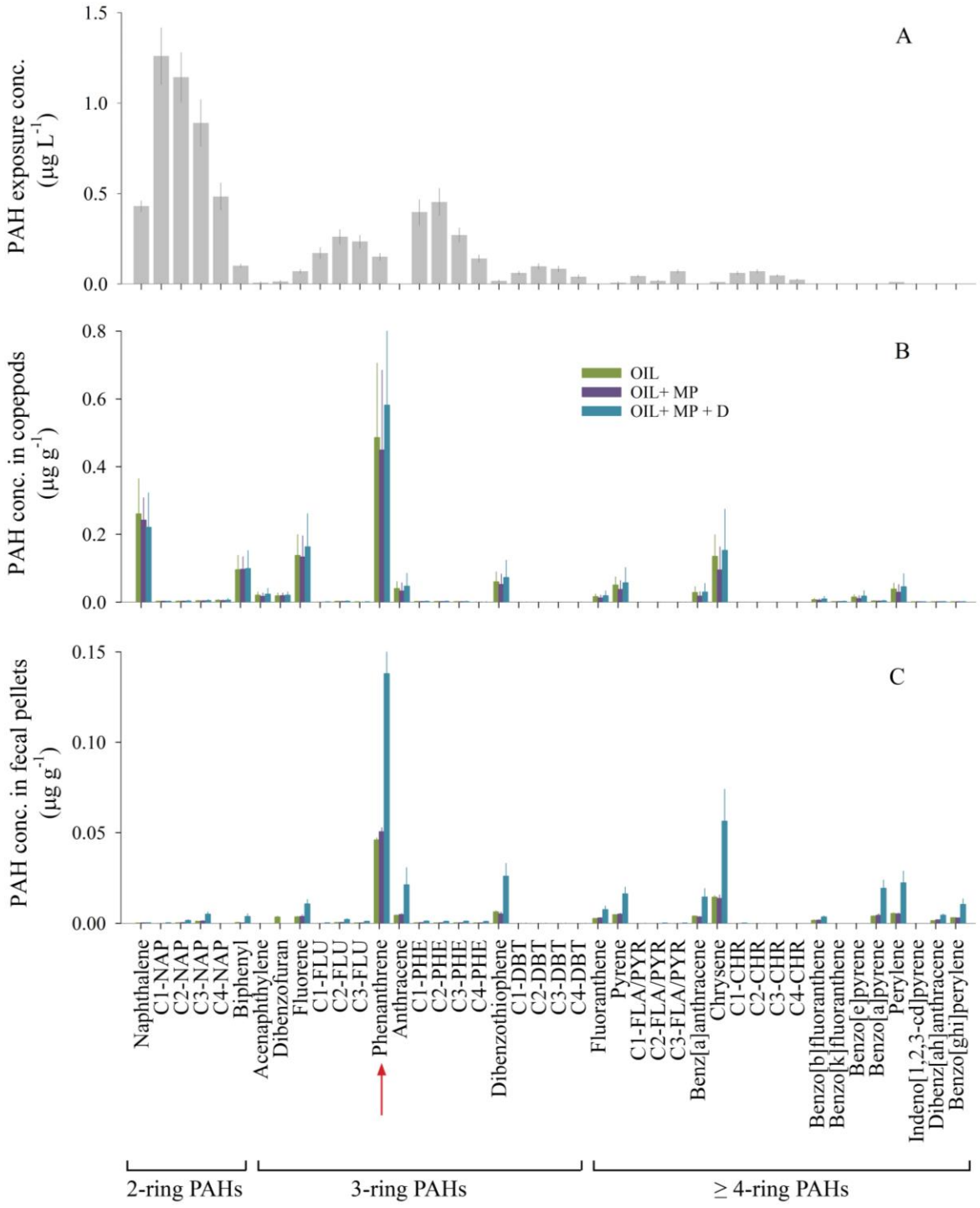


Figure 6: Composition and concentration of PAHs in the exposure media (**A**), copepods (**B**) and fecal pellets (**C**). The red arrow indicates the most abundant PAHs found in copepod and fecal pellet samples. Error bars are standard deviation.

Supporting information

S.I. - Text 1:

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

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

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

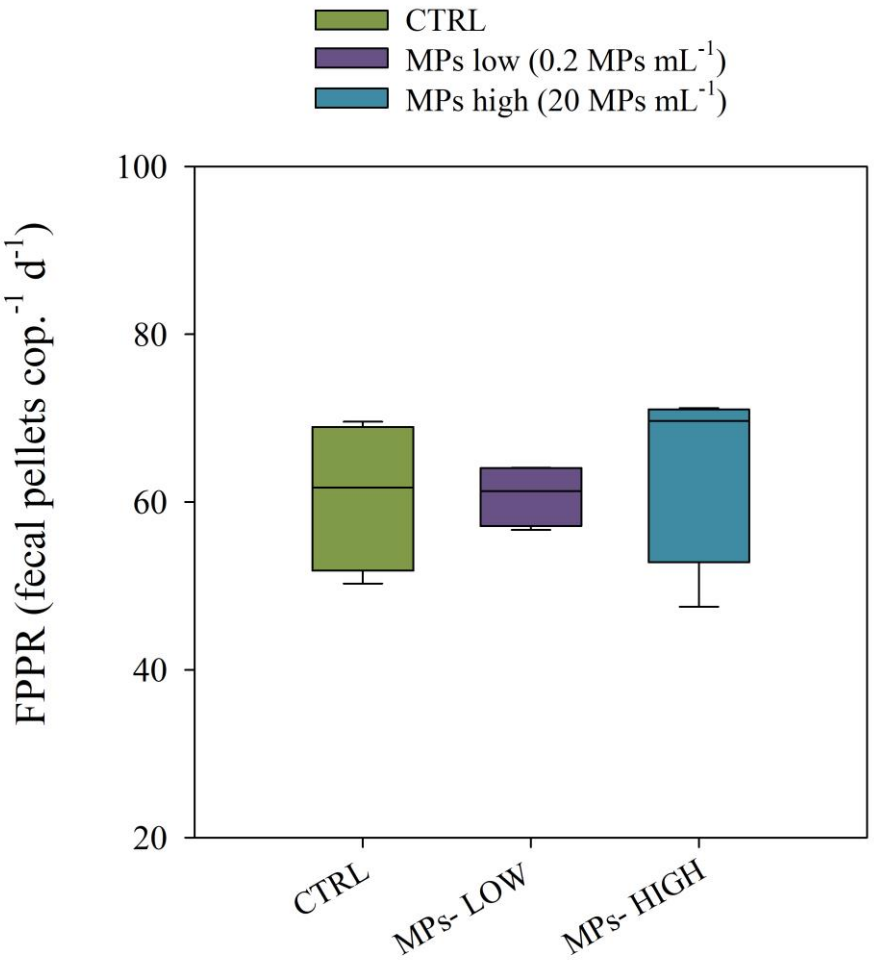
Dibenz[ah]anthracene	5	6.70	0.0013
Indeno[1,2,3-cd]pyrene	6	6.70	0.0000
Benzo[ghi]perylene	6	6.70	0.0013

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

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

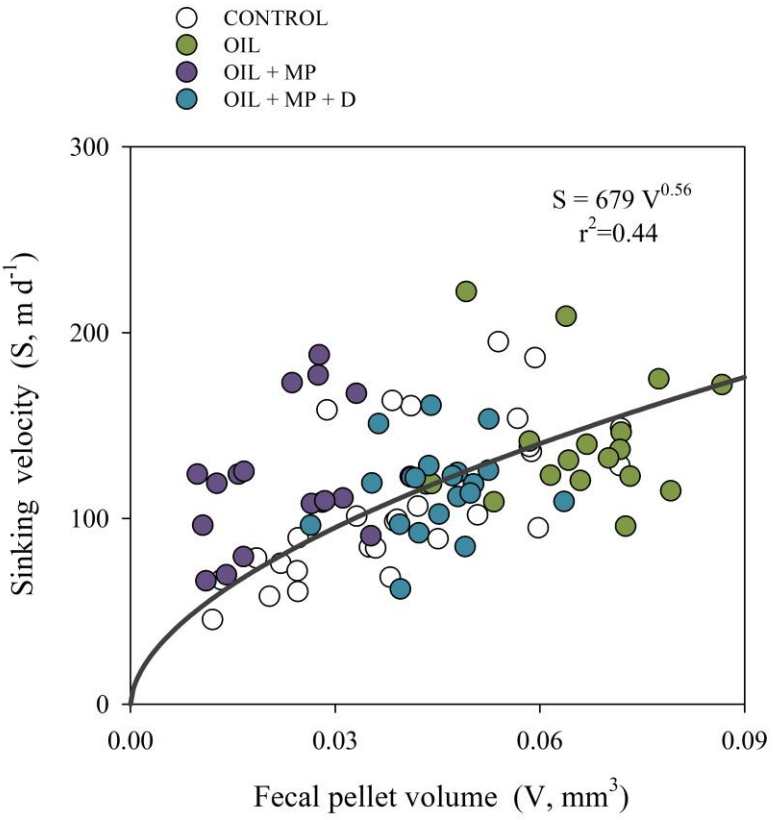
Indeno[1,2,3-cd]pyrene	0	0	0
Benzo[ghi]perylene	0	0	0
TOTAL PAHs (µg L^{-1})	**9.55**	**8.47**	**7.23**
Avg. total PAHs (µg L^{-1})		8.42	

S.I.-FIGURE 1



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

S.I.-FIGURE 2



S.I.-Figure 2. Relationship between sinking velocity (S) and volume (V) of *Calanus hyperboreus* fecal pellets in the different treatments. Power regression was significant ($p<0.001$) only for fecal pellets from the control treatment.

Sample CRediT author statement:

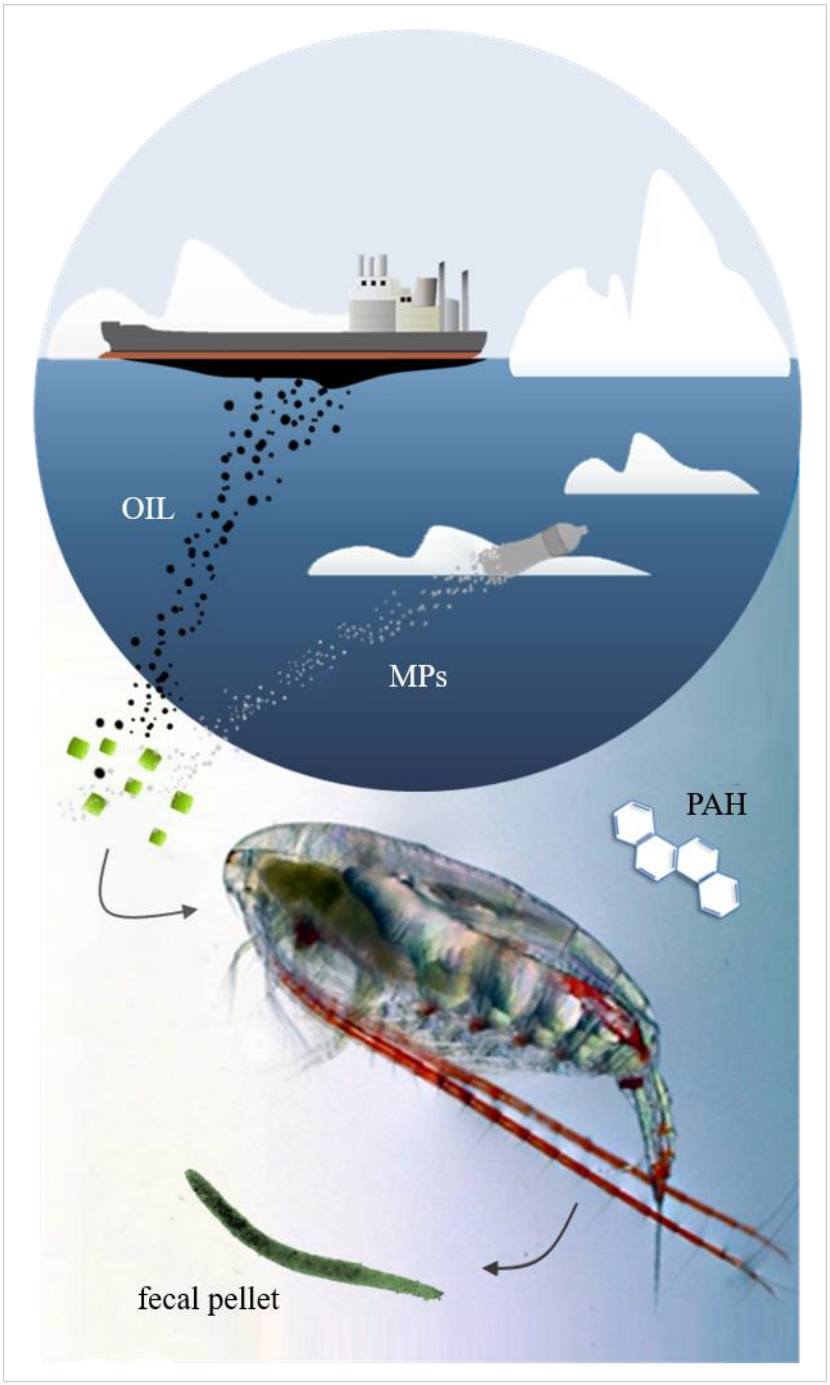
Rodrigo Almeda: Conceptualization, Methodology, Formal analysis, Investigation, Writing - Original Draft, Writing - Review & Editing. Project administration, Funding acquisition. **Rocío Rodriguez-Torres**: Investigation, Writing - Review & Editing. **Sinja Rist**: Investigation, Writing - Review & Editing. **Mie H.S. Winding**: Investigation, Writing - Review & Editing. **Peter Stief**: Investigation, Writing - Review & Editing. **Bjørn Henrik Hansen**: Resources, Formal analysis, Writing - Review & Editing. **Torkel Gissel Nielsen**: Methodology, Investigation, Writing - Review & Editing, Project administration, Funding acquisition.

Declaration of interests:

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

Microplastics do not increase bioaccumulation of petroleum hydrocarbons in Arctic zooplankton but trigger feeding suppression under co-exposure conditions.
R. Almeda, R. Rodriguez-Torres, S. Rist, M.H.S. Winding, P. Stief, B. H. Hansen and T. Gissel Nielsen.

GRAPHICAL ABSTRACT



STOTEN-D-20-08848R1

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

Highlights

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