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Article

Cumulative Impacts of Oil Pollution, Ocean Warming, and Coastal Freshening on the Feeding of Arctic Copepods

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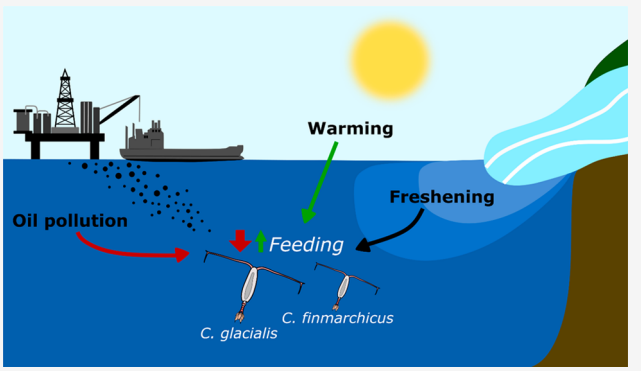
ABSTRACT: The Arctic is undergoing rapid changes, and biota are exposed to multiple stressors, including pollution and climate change. Still, little is known about their joint impact. Here, we investigated the cumulative impact of crude oil, warming, and freshening on the copepod species *Calanus glacialis* and *Calanus finmarchicus*. Adult females were exposed to ambient conditions (control; 0 °C + 33 psu) and combined warming and freshening: 5 °C + 27 psu (Scenario 1), 5 °C + 20 psu (Scenario 2) for 6 days. All three conditions were tested with and without dispersed crude oil. In Scenario 1, fecal pellet production (FPP) significantly increased by 40−78% and 42−122% for *C. glacialis* and *C. finmarchicus*, respectively. In Scenario 2, FPP decreased by 6−57% for *C. glacialis*, while it fluctuated for *C. finmarchicus*. For both species, oil had the strongest effect on FPP, leading to a 68−83% reduction. This overshadowed the differences between climatic scenarios. All variables (temperature, salinity, and oil) had significant single effects and several joint effects on FPP. Our results demonstrate that Arctic copepods are sensitive to environmentally realistic concentrations of crude oil and climate change. Strong reductions in feeding can reduce the copepods' energy content with potential large-scale impacts on the Arctic marine food web.



KEYWORDS: *Calanus, Greenland, temperature, salinity, climate change, multiple stressors*

1. INTRODUCTION

Arctic ecosystems are subject to rapid and dramatic changes due to climate change and other anthropogenic activities.[1,2] Furthermore, many stressors act in concert, which can lead to cumulative effects on organisms. However, to date, such interactive effects are poorly understood and have not been quantified.[3,4] The Arctic is one of the regions in the world most affected by climate change. Air temperatures have increased three times faster than the global average from 1971 to 2019, and the Arctic Ocean is warming more than twice the mean global rate in the upper 2000 m.[2,5] The increasing temperatures accelerate the melting of sea and land ice, and together with increasing precipitation and river runoff, this results in freshening of the Arctic Ocean.[6,7] For Northeast Greenland, a freshening rate of 0.12 psu per year has been reported.[8] In coastal waters of Greenland, the salinity is typically around 33 psu.[9] However, values can drop below 20 psu during summer, with particularly low values in the vicinity of rivers and marine terminating glaciers.[8,10−12]

The current and future decrease in sea ice coverage will open the Arctic for more anthropogenic activities, such as shipping and exploitation of fossil fuels and minerals.[13,14] Since the Arctic holds a substantial amount of oil and gas, extraction and distribution are likely to increase in the future, entailing an elevated risk of oil spills.[15,16] Already to date, small oil spills have been common in the Arctic, averaging for instance 85 spills per year in Alaska.[17] Furthermore, the Arctic has experienced a few major spills in the past, notably the Exxon Valdez disaster in 1989 releasing more than 41 million liters of crude oil.[17] Oil is a highly complex mixture containing thousands of compounds. The majority are hydrocarbons, but it can also contain considerable amounts of metals and other elements.[18] Crude oil is known to be toxic to a wide range of organisms, through direct contact, ingestion, inhalation, or diffusion through membranes.[17,19] For Arctic invertebrates, reported effects include changes in gene expression and enzymatic activity, feeding, reproduction, development, and mortality.[15]

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Copepods of the genus *Calanus* are key components of the Arctic marine food web. In terms of biomass, they are the most abundant herbivores, and they play a significant role in transferring energy from phytoplankton to higher trophic levels.[20−22] Consequently, if the *Calanus* species are adversely affected by climate change and oil pollution, then this could have severe consequences for the functioning of this ecosystem and the services it provides. Previous work has demonstrated the diverse effects of oil and oil-associated compounds on copepods of the genus *Calanus*. On a cellular level, an increase in oxidative stress, as indicated by the increased activity of detoxifying enzymes, has been reported.[23−25] Several studies found a reduction in feeding activity, which may be caused by oil-induced narcosis.[26−29] At high concentrations, narcosis can also strongly hamper swimming of copepods.[27] Miljeteig et al.[30] observed increasing positive phototaxis under oil exposure. Impacts on reproduction have also been reported through reduced egg production and hatching success, though with indications for recovery postexposure.[28,31,32] When comparing the effects between species, previous studies found *Calanus glacialis* to be more tolerant to oil pollution than *Calanus finmarchicus*.[23,24] Microscopic and chemical analyses have revealed the presence of oil droplets and individual hydrocarbons, respectively, in copepods, as well as their fecal pellets after laboratory exposure.[26,32,33] Thus, it is likely that these compounds are ingested and transferred to both higher trophic levels and organisms feeding on fecal pellets.

Arctic copepods are exposed to ongoing environmental changes, including warming and freshening. Makri et al.[34] recently determined the tolerance of *C. glacialis* and *C. finmarchicus* to a range of temperatures (0, 5, 10 °C) and salinities (5−60 psu), as well as their combination. They found a stress response in the form of increased feeding outside of 25−40 psu and higher mortality outside of 5−55 psu and 20−45 psu for *C. glacialis* and *C. finmarchicus*, respectively. However, potential cumulative impacts arising from the combination of climate change and oil pollution are currently unknown for Arctic copepods. In fact, very few studies have investigated such a combined effect of warming and contaminants in polar invertebrates.[4]

The aim of this study was to experimentally assess and quantify how climate change, specifically warming and freshening, combined with crude oil, may affect Arctic copepods. The response variables tested were fecal pellet production (FPP) and fecal pellet volume (FPV) as proxies for feeding. We furthermore monitored survival, lipid sac area, and prosome length. The derived responses were compared between the two species *C. glacialis* and *C. finmarchicus*. For the three tested stressors, we hypothesized 1) oil to reduce feeding regardless of differences in temperature and salinity, 2) higher temperature to increase feeding, 3) salinity below 25 psu to have a slightly positive effect on feeding, and 4) the three stressors to interact, resulting in cumulative effects when combined. Furthermore, we expected *C. finmarchicus* to exhibit a stronger response than *C. glacialis* based on previously reported differences in tolerance.

2. MATERIALS AND METHODS

2.1. Study Site and Animal Collection. This study was performed in March 2023 in Disko Bay, West Coast of Greenland. Copepods were collected at two locations, 3.4 km apart (N 69° 14.832′, W 53° 35.428′ and N 69° 14.550′, W 53° 30.357′), by vertical towing of a 300 μm WP3 net with a nonfiltering cod end. Maximum depths were 87 and 67 m. *In situ* temperature and salinity at the surface were −1.6 °C and 33 psu for both locations. Plankton samples were immediately transferred to a cool box with *in situ* surface water and transported to the laboratory where females of the target species *C. glacialis* and *C. finmarchicus* were separated. Sorting was done on ice under a stereomicroscope, and the individuals retained were kept in buckets with *in situ* water and gentle aeration at 0 °C.

2.2. Experimental Design. Two climate change scenarios were tested in comparison to a control under ambient conditions (0 °C and 33 psu). Scenario 1 represented warming to 5 °C and a moderate decrease in salinity to 27 psu. Scenario 2 had the same degree of warming but a stronger decrease in salinity to 20 psu. Both scenarios as well as the control were tested in the absence and presence of crude oil pollution at a concentration of 1 μL L^{-1}, resulting in a total of six treatment groups (Table 1). Each treatment had five replicates, consisting of 302 mL glass bottles with two individuals each. The design was identical for both species. The exposure lasted for 6 days.

Table 1. Tested Treatments, Differing in Temperature, Salinity, and Crude Oil Concentration, for the Exposure Experiments with *C. glacialis* and *C. finmarchicus*[a]

Treatment	Temperature (°C)	Salinity (psu)	Crude oil (μL L^{-1})
Ambient (Ctrl)	0	33	0
Ambient + Oil	0	33	1
Scenario 1	5	27	0
Scenario 1 + Oil	5	27	1
Scenario 2	5	20	0
Scenario 2 + Oil	5	20	1

[a]Each treatment had 5 replicates (n = 5).

2.3. Crude Oil Preparation and Chemical Analysis. *2.3.1. Preparation of Crude Oil.* The crude oil used in the experiments was Light Louisiana Sweet oil, and a suspension of oil droplets was made following the method of Almeda et al.[33] By adding oil to seawater under high-speed magnetic stirring, oil droplets with a mean diameter of 8 μm (95% of droplets 1−20 μm) are formed.[35] This stock suspension was used to add crude oil to the exposure bottles of all oil treatments, reaching a final concentration of 1 μL L^{-1}.

For the chemical analysis, additional glass bottles (1 L, for organic analysis) and plastic tubes (50 mL, for metal analysis) with ambient seawater were spiked with crude oil in the same way as the experimental bottles. The glass bottles were immediately frozen at −20 °C, and the plastic tubes were stored at 4 °C in the dark until analysis.

2.3.2. Analysis of Metals in the Oil Exposure. Analysis of 29 elements (Table S1), extracted from the 50 mL water samples, was performed using inductively coupled mass spectrometry (ICP-MS) (Agilent 7500 cx). For sample introduction and nebulization, a MicroMist nebulizer was used connected to a Scott double pass spray chamber cooled to 2 °C. A carrier gas flow of 0.9 L min^{-1} and a makeup gas flow of 0.2 L min^{-1} were utilized for best sample nebulization. The plasma was run at 1500 W at a torch-to-sampling cone distance of 8 mm.

Elements prone to di- and polyatomic interferences (i.e., K, V, Cr, Fe, As, and Se) were analyzed in collision mode using

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helium as the collision gas at a flow rate of 5 mL min^{-1}. External calibration with a "Merck VI 10580 multi element standard" was used in the concentration intervals of 0.01 to 10,000 $\mu g\ L^{-1}$. Boron was not included in the analysis.

Prior to the analysis, all samples were acidified with 1% concentrated nitric acid (HNO_3) and diluted ten times with 1% HNO_3 in order to minimize any matrix effects. Rh was added as an internal standard after sample dilution.

2.3.3. Analysis of Polycyclic Aromatic Compounds. Polycyclic aromatic compounds (PACs) were extracted and concentrated from the 1 L water samples by solid phase extraction (SPE). Initially, the Oasis HLB cartridges were conditioned with 5 mL of dichloromethane (DCM), followed by 5 mL of methanol and 5 mL of milli-Q grade water, and spiked with internal standard solutions (12.5−50 ng). Methanol (10%, v/v) was added to the water samples (1 L), and the samples were loaded to the cartridges at a flow rate of approximately 5 mL min^{-1}. Each cartridge was centrifuged at 6,000*g* for 10 min to remove most of the residual water in the adsorbent bed and then dried for 30 min using a vacuum pump. Analytes were eluted with 12 mL of DCM. Eluates were evaporated by a gentle stream of nitrogen gas, transferred to GC vials, and solvent exchanged to 0.1 mL of toluene. A recovery standard of deuterium-labeled perylene (50 ng) was added to the GC vials, and vials were stored in −18 °C until gas chromatography mass spectrometry (GC/MS) analysis.

Analysis of 59 PACs was performed using an Agilent 7890A GC coupled to a 5975C low-resolution MS. Analytes (1 μL) were injected into the GC in the splitless mode and separated with an Agilent Select PAH (30 m × 0.25 mm, 0.15 μm film thickness) capillary column. Oven temperatures are specified in the SI. Identification and quantification of the PACs in the samples were done using quantification mixtures including all 59 PACs in addition to stable isotopically labeled internal standards.

2.4. Exposure Experiments. *2.4.1. Salinity Acclimatization.* Prior to the exposure experiment, the copepods were acclimatized stepwise to the experimental salinities. The decreased salinity was achieved by mixing seawater (33 psu) with freshwater and confirmed using a CTD probe (Sea & Sun Technology, CTD 48M). We used unfiltered, field-collected seawater and freshwater from a nearby spring. A subset of copepods (for Scenarios 1 and 2) was first transferred to water with 30 psu for 12 h. Then, half of the individuals (for Scenario 1) were transferred to water with 27 psu, while the other half was transferred to 25 psu. After 18 h, the copepods in 25 psu water were transferred to 20 psu (Scenario 2). To avoid any impact of the handling during transfer, all subsets of copepods were transferred to new buckets in parallel. Those that had already reached their final salinity were transferred to buckets with the same salinity. All buckets had a volume of 5 L and were kept at 0 °C. The experiment was initiated 18 h after the final transfer.

2.4.2. Preparation of Exposure Bottles. Exposure experiments were conducted in 250 mL glass bottles, which were completely filled (final volume of 302 mL) and closed air-free with lids with a polytetrafluoroethylene-protected seal. A stock suspension of crude oil was prepared before the bottles. The diatom *Thalassiosira weissflogii* was added as food at a final concentration of 3,000 cells mL^{-1}, ensuring saturation of the copepods.[36,37] Two individuals were added per bottle. The bottles were kept in the dark in temperature-controlled rooms of 0 and 5 °C, respectively. The exposure lasted for 6 days with a daily renewal of the bottles. During the day, the bottles were inverted approximately every 4 h.

2.4.3. Treatment Effects on Food Algae. To confirm that the diatoms were not affected by the treatments, potentially resulting in differing food concentrations for the copepods, we exposed *T. weissflogii* alone to the six treatments at the same density (3,000 cells mL^{-1}) for 24 h. Cell densities were measured at the start and end of the exposure by fixing a subsample with Lugol's solution and counting the cells in a Sedgewick rafter counting chamber with a microscope.

2.4.4. Exposure Renewal and Measured End Points. For renewal, the content of each bottle was gently poured through a 20 μm filter that was submerged in water to collect the copepods and fecal pellets. The filter was then backwashed into a Petri dish using a spray bottle, and the Petri dish was immediately examined under a stereomicroscope. The copepods were checked for their physical condition and survival, and transferred to a new bottle that was freshly prepared as described before. All fecal pellets (FPs) were counted, and fecal pellet production (FPP) was calculated as FPs $ind.^{-1}$ day^{-1}. The procedure of bottle renewal was repeated daily during the 6 days of exposure.

Additionally, pictures of fecal pellets were taken on days 1, 4, and 6 using a stereomicroscope (Olympus SZ40) with a connected camera (Leica EC3) to measure their size. Based on the length and width, the fecal pellet volume (FPV) was calculated assuming a cylindrical shape. FPP and FPV together were used to determine the effects on feeding. However, we did not address the assimilation efficiency.

We also took pictures of copepods to measure the prosome length and the size of the lipid sac.[38] At the start of the experiment, we took pictures of 30 individuals of each species from the same pool of sorted copepods, which represent the starting conditions (10 from each salinity acclimatization). These individuals were not used for the experiment afterward. At the end of the exposure, pictures of all of the individual copepods were taken. All image analyses of fecal pellets and copepods were done in ImageJ.[39]

2.5. Data Analysis. Averages are given with standard deviation (SD), unless otherwise stated. Analysis of variance (ANOVA) was used to test for differences between group means in the condition of copepods (prosome length and lipid sac area) at the start and end of exposure and for the algal cell densities in the different treatment groups. The Shapiro-Wilks test was used to test for normality of the data, and the Fligner-Kileen test was used for the homogeneity of variances. In the case of a violation of the assumptions, the Kruskal−Wallis test was used as a nonparametric alternative.

To assess cumulative impacts of climatic conditions and oil pollution on the copepods' feeding, we examined the single and joint effects of temperature, salinity, and oil on the FPP and FPV using Generalized Additive Mixed Models (GAMMs, see the SI for details). We assessed model performance by comparing the observed and predicted level of FPP (or FPV) on the basis of the underlying responses to salinity, temperature, and oil. We also visualized the cumulative impacts of climate change and pollution on copepod FPP by predicting the species responses to all combinations of stressors (including combinations not tested experimentally) and displayed the outcomes using 3D plots. All statistical analyses and visualizations were performed with the software R (version 3.6.3),[40] using the package "mgcv",[41] "ggplot2",[42] and "scatterplot3d".[43]

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Table 2. Concentrations of Metals and Polycyclic Aromatic Compounds in the Treatments with Crude Oil (1 μL L^{-1})[a]

Metals	LOD (μg L^{-1})	Oil 1 (μg L^{-1})	Oil 2 (μg L^{-1})	Oil 3 (μg L^{-1})	Mean (μg L^{-1})	SD	RSD (%)
208 Pb	0.3	0.5	0.5	0.8	0.6	0.1	20.9
16 priority PAHs	**LOD (ng L^{-1})**	**Oil 1 (ng L^{-1})**	**Oil 2 (ng L^{-1})**	**Oil 3 (ng L^{-1})**	**Mean (ng L^{-1})**	**SD**	**RSD (%)**
Naphthalene	100	1380	250	310	650	640	98
Acenaphthylene	1	1.6	1.7	2.7	2.0	0.61	30
Acenaphthene	1	5.7	5.2	7.2	6	1.0	17
Fluorene	1.2	35	35	43	38	4.6	12
Phenanthrene	3	77	77	79	78	1.2	1.5
Fluoranthene	1	3.3	3	3.3	3.2	0.17	5.4
Pyrene	1.2	4.2	3.8	4.7	4.2	0.45	11
Benzo[a]anthracene	1	1.2	1.1	1.4	1.2	0.15	13
Chrysene	1	4.0	3.7	4.0	3.9	0.17	4.4
Benzo[b]fluoranthene	1	1.5	1.3	1.4	1.4	0.15	11
Other PAHs	**LOD (ng L^{-1})**	**Oil 1 (ng L^{-1})**	**Oil 2 (ng L^{-1})**	**Oil 3 (ng L^{-1})**	**Mean (ng L^{-1})**	**SD**	**RSD (%)**
Biphenyl	3.3	72	68	81	74	6.7	9.0
4H-Cyclopenta[def]phenanthrene	1	6.7	6.3	7.5	6.8	0.61	9.0
Benzo[a]fluorene	1	16	13	22	17	4.1	27
Benzo[c]fluorene	2.1	120	99	130	120	16	13
Triphenylene	1	6.5	6.0	7.3	6.6	0.66	9.9
Benzo[e]pyrene	1	1.8	1.4	2.1	1.8	0.35	20
Perylene	1	3.0	2.4	3.7	3.0	0.65	21
Alkylated PAHs and dibenzothiophenes	**LOD (ng L^{-1})**	**Oil 1 (ng L^{-1})**	**Oil 2 (ng L^{-1})**	**Oil 3 (ng L^{-1})**	**Mean (ng L^{-1})**	**SD**	**RSD (%)**
2-Methylnaphthalene	22	380	360	440	398	42	10
1-Methylnaphthalene	12	290	270	310	291	20	7.0
1,6-Dimethylnaphthalene	7.2	350	340	390	362	26	6.9
2,3,5-Trimethylnaphthalene	2.7	89	94	110	100	11	11
Dibenzothiophene	1.2	7.2	7.0	8.2	7.6	0.64	8.5
2-Methyldibenzothiophene	1	5.0	4.9	6.0	5.4	0.61	11
2-Methylphenanthrene	2.4	46	45	50	47	2.6	5.6
2-Methylanthracene	1	2.00	1.5	1.7	1.6	0.25	15
2,4,7-Trimethyldibenzothiophene	1	2.6	2.2	3.3	2.7	0.56	20
1,2,8-Trimethylphenanthrene	1	5.3	4.7	5.9	5.3	0.60	11
1,2,6-Trimethylphenanthrene	1	3.9	3.4	4.5	3.9	0.55	14
1-Methylfluoranthene	1	1.2	1.1	1.1	1.1	0.06	5.2
3-Methylchrysene	1	3.9	3.0	5.1	4.0	1.1	26
2-Methylchrysene	1	5.2	4.3	7.1	5.6	1.4	25
1-Methylchrysene	1	3.3	2.7	4.3	3.5	0.81	23
sum 16 priority PAHs		1510	380	460	790	631	80
sum other PAHs		230	200	250	230	25	11
sum alkylated PAHs		1180	1130	1330	1220	104	8.5
sum dibenzothiophene and alkylated dibenzothiophenes		15	14	18	15.7	1.8	11

[a]Triplicates of 'Ambient + Oil' exposure bottles were measured (Oils 1, 2, 3). The limit of detection (LOD) was defined as three times the concentration found in the blank (ambient seawater). When a compound was absent in the blank, the LOD was 1 ng L^{-1}. SD = standard deviation, RSD = relative standard deviation.

3. RESULTS

3.1. Chemical Analysis. We detected 32 PACs at elevated concentrations in the oil exposure bottles (Table 2). Of those, 10 belong to the 16 priority polyaromatic hydrocarbons (PAHs) defined by the US EPA,[44] seven are other PAHs, and 15 are alkylated PAHs and dibenzothiophenes. In sum, alkylated PAHs were the most concentrated, reaching on average 1220 ng L^{-1}. The sums of the priority and other PAHs were 790 and 230 ng L^{-1}, respectively. Dibenzothiophenes and alkylated dibenzothiophenes had a mean concentration of 15.7 ng L^{-1}. The single compound with the highest concentration was naphthalene. However, this was mainly due to one replicate which had a concentration four times higher (1380 ng L^{-1}) than the other two replicates (310 and 250 ng L^{-1}). Other compounds at high concentrations were 2-methylnaphthalene (398 ng L^{-1}), 1,6-dimethylnaphthalene (362 ng L^{-1}), 1-methylnaphthalene (291 ng L^{-1}), benzo[c]fluorene (120 ng L^{-1}), and 2,3,5-trimethylnaphthalene (100 ng L^{-1}).

Only one metal was found at an elevated concentration in the oil exposure bottles: lead, with a mean concentration of 0.6 μg L^{-1} (Table 2).

3.2. Condition of the Copepods. We observed neither mortality of copepods nor any obvious changes in their behavior during the exposure. Furthermore, there were no changes in prosome length and lipid sac area (Figures S1−S3, Tables S2−S3). Females of *C. glacialis* and *C. finmarchicus* had prosome lengths of 3.3−3.6 and 2.6−2.7 mm, respectively. The lipid sac areas ranged from 0.59 to 0.88 mm^2 for *C. glacialis* and from 0.42 to 0.54 mm^2 for *C. finmarchicus*. There were no significant differences in the prosome length or lipid sac area

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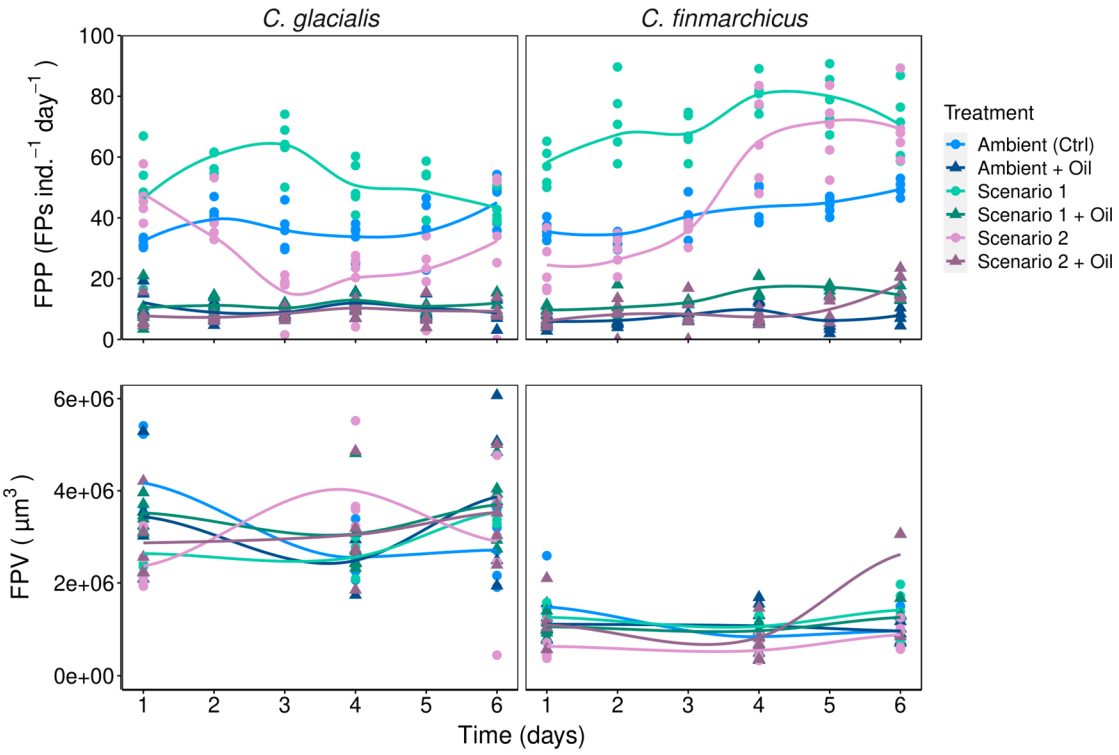

Figure 1. Fecal pellet production (FPP, top row) and fecal pellet volume (FPV, bottom row) of *C. glacialis* (left) and *C. finmarchicus* (right) during the 6 days of exposure to different combinations of crude oil, temperature, and salinity. Lines show overall trends visualized by LOESS regressions. Ambient conditions (0 °C, 33 psu), Scenario 1 (5 °C, 27 psu), Scenario 2 (5 °C, 20 psu), + Oil = addition of crude oil (1 μL L^{-1}).

between the three salinity acclimatization groups at the start of the exposure, except for the 27 psu group of *C. finmarchicus* which had a slightly lower prosome length (X^2 = 12.7, df = 2, p = 0.002) (Figure S1). At the end of the 6-day exposure period, there were no significant differences between treatment groups for prosome length or lipid sac area in either species (Figure S3). We cannot make a direct comparison of start and end conditions for each individual since we measured prosome length and lipid sac area at the start in a group of individuals that were not used for the experiment (to reduce stress).

3.3. Fecal Pellet Production (FPP) and Volume (FPV).

Copepods in all treatment groups were feeding and produced fecal pellets throughout the entire duration of the exposure (Figure 1). According to our model results (Table 3), individual copepods produced an average of 40.7 fecal pellets per day across treatments, with *C. glacialis* yielding slightly lower numbers (−7.1) than did *C. finmarchicus*. Both species demonstrated significant differences in FPP for all treatments relative to the control (Table 3, Tables S5−S6), with the exception of Scenario 2 that fluctuated both above and below the mean levels under ambient conditions (Figure 1).

For *C. glacialis*, the FPP across all days in the control group was 37.3 ± 5.3 FPs ind.$^{-1}$ day^{-1}, with a minimum and maximum of 31.7 ± 1.4 and 45.3 ± 7.9 FPs ind.$^{-1}$ day^{-1}, respectively. Copepods in Scenario 1 (5 °C, 27 psu) consistently had a higher FPP than the control (by 40−78%), except for day 6, ranging between 42.4 ± 4.8 and 64.1 ± 8.9 FPs ind.$^{-1}$ day^{-1}, with an overall average of 52.5 ± 8.0 FPs ind.$^{-1}$ day^{-1}. Except for day 1, copepods in Scenario 2 (5 °C, 20 psu) had a 6−57% lower FPP than the control, with a range of 15.6 ± 8.0 to 46.3 ± 7.2 FPs ind.$^{-1}$ day^{-1}. The three treatment groups with oil consistently had a very low FPP with an average of 10.1 ± 1.6, 11.2 ± 1.5, and 8.4 ± 1.6 FPs ind.$^{-1}$ day^{-1} for the Ambient + Oil, Scenario 1 + Oil, and Scenario 2 + Oil treatment groups, respectively (Figure 1). This is an average decrease of 73, 70, and 78% in comparison to the control, respectively.

Table 3. Summary Statistics of the Final GAMM for Fecal Pellet Production (FPP) Showing the Intercept and Parametric Coefficients for Species and Treatments as Fixed Effect Factors as well as the Smooth Terms Accounting for Temporal Variation across Days by Treatment[a]

Parametric coefficients:				
	Estimate	SE	t-value	p-value
Intercept	40.70	3.83	10.64	<2e-16***
Species (*C. glacialis*)	−7.06	1.05	−6.73	7.62e-11***
Ambient + Oil	−28.36	5.36	−5.29	2.22e-07***
Scenario 1	34.83	5.36	6.50	2.99e-10***
Scenario 1 + Oil	−23.66	5.36	−4.41	1.37e-05***
Scenario 2	−5.40	5.36	−1.01	0.32
Scenario 2 + Oil	−29.66	5.36	−5.53	6.39e-08***

Approximate significance of smooth terms:				
	edf	ref.df	F-value	p-value
S(Day, Treatment)	9.54	12	4.09	6.02e-08***
R-sq.(adj) = 0.81	Deviance explained = 82.5%			
GCV = 107	Scale est. = 99.03	*n* = 360		

[a]edf is the estimated degrees of freedom for the model smooth terms (s) (i.e., edf >1 indicates a nonlinear relationship). GCV is the generalized cross validation score, and *n* is the number of observations used for model fitting. (See Tables S5−S6 for species-specific models.)

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For *C. finmarchicus*, the FPP in the control ranged between 32.5 ± 2.4 and 49.7 ± 2.5 ind.$^{-1}$ day^{-1}, with an overall mean of 41.1 ± 6.2 ind.$^{-1}$ day^{-1}. Again, the FPP in Scenario 1 was considerably higher than in the control, with a relative increase of 42−122% (57 ± 6.4−80.6 ± 5.7 FPs ind.$^{-1}$ day^{-1}). Individuals exposed to Scenario 2 exhibited a FPP that fluctuated around the values in the control, going from a minimum of 23.8 ± 8.8 to a maximum of 70.1 ± 11.5 FPs ind.$^{-1}$ day^{-1}. The three treatment groups with crude oil showed a similar decrease in FPP to that in *C. glacialis*. Average values were 7.0 ± 2.2, 13.5 ± 3.1, and 9.1 ± 4.5 FPs ind.$^{-1}$ day^{-1} in the Ambient + Oil, Scenario 1 + Oil, and Scenario 2 + Oil treatment groups, respectively (Figure 1). This is an average decrease of 83, 67, and 78% in comparison to the control, respectively.

In contrast to FPP, the estimates of FPV did not vary significantly between treatments (Figure 1, Table S4). The only noticeable difference is the significantly larger volume of fecal pellets from *C. glacialis* compared to that from *C. finmarchicus*. *C. glacialis* exhibited a minimum FPV of 4.4 × 10^5 μm^3 and a maximum of 6.1 × 10^6 μm^3. In *C. finmarchicus*, FPV ranged from 3.3 × 10^5 μm^3 to 3.1 × 10^6 μm^3. Hence, we observed responses of feeding to treatments primarily in terms of the number of fecal pellets but not in terms of their size.

After having established that significant differences in FPP exist between treatments, we investigated the single and joint responses to temperature, salinity, and oil for both species together (Table 4) or separately (Tables S7−S8). (Note that since FPV did not demonstrate any responses to treatments the analysis was confined to FPP only.) The model results showed significant single effects for all variables, indicated by a positive response of FPP to temperature, notably for *C. finmarchicus* (Table S7), but also a marked negative effect of oil for both species (i.e., an average decrease in FPP by 21.5). In terms of salinity, we found a dome-shaped nonlinear response, characterized by saturating or declining FPP at higher salinities (as indicated by the significant negative squared terms). With regard to the interactions among variables, we found significant pairwise effects between temperature, salinity, and oil for both species, although *C. finmarchicus* showed a significant interaction only for the squared term of salinity (similar to the single effect). Finally, the joint interaction among all three variables was significant, at least for the squared component of salinity (Table 4). Therefore, no model reduction of lower order effects (i.e., removal of single or pairwise terms) was warranted.

The predicted values of FPP for all combinations of salinity, temperature, and oil (including also treatments not tested experimentally) demonstrate the joint responses and cumulative effects on climatic conditions and oil for both species together (Figure 2) or separately (Figure S5). More specifically, it shows a higher FPP at higher temperatures, especially for *C. finmarchicus*. Salinity modulates FPP in both directions, depending on the temperature. Finally, we show a marked decline in FPP for both species if exposed to oil, regardless of climatic conditions, despite the slight positive effects of increasing temperature and salinity (Figure 2). In terms of model performance, the fitted models explained a high degree of deviance overall (82.5%) and well predicted the observed levels of FPP for each treatment on the basis of the underlying variables (temperature, salinity, oil) used for model fitting (Figure S6).

Table 4. Summary Statistics of the Final GAMM for Fecal Pellet Production (FPP) Showing the Intercept and the Parametric Coefficients for Species, as well as the Single and Joint Effects of Temperature, Salinity, and Oil[a]

Parametric coefficients:				
	Estimate	SE	t-value	p-value
Intercept	26.46	2.51	10.54	<2e-16***
Species (*C. glacialis*)	−7.06	1.05	−6.73	7.61e-11***
Temperature (L)	7.16	2.42	2.96	0.003**
Salinity (L)	4.76	2.51	1.90	0.058.
Salinity (Q)	−9.83	3.11	−3.17	0.002**
Oil (L)	−21.53	3.50	−6.16	2.10e-09***
Temperature (L): Salinity (L)	−4.81	1.57	−3.06	0.002 **
Temperature (L): Salinity (Q)	−11.67	2.15	−5.44	1.03e-07***
Temperature (L): Oil (L)	−6.82	3.42	−1.99	0.047*
Salinity (L): Oil (L)	−4.40	3.55	−1.24	0.22
Salinity (Q): Oil (L)	10.64	4.39	2.42	0.02*
Temperature (L): Salinity (L): Oil (L)	2.83	2.21	1.28	0.20
Temperature (L): Salinity (Q): Oil (L)	10.95	3.03	3.61	0.0004***
Approximate significance of smooth terms:				
	edf	ref.df	F-value	p-value
S(Day, Treatment)	9.54	12	4.05	3.77e-08***
R-sq.(adj) = 0.81	Deviance explained = 82.5%			
GCV = 107	Scale est. = 99.03			*n* = 360

[a]L and Q denote the linear and squared component for each effect. Also shown are the smooth terms accounting for temporal variation across days by treatment. edf is the estimated degrees of freedom for the model smooth terms (s) (i.e., edf >1 indicates a nonlinear relationship). GCV is the generalized cross validation score, and *n* is the number of observations used for model fitting (see Table S7−S8 for species-specific models).

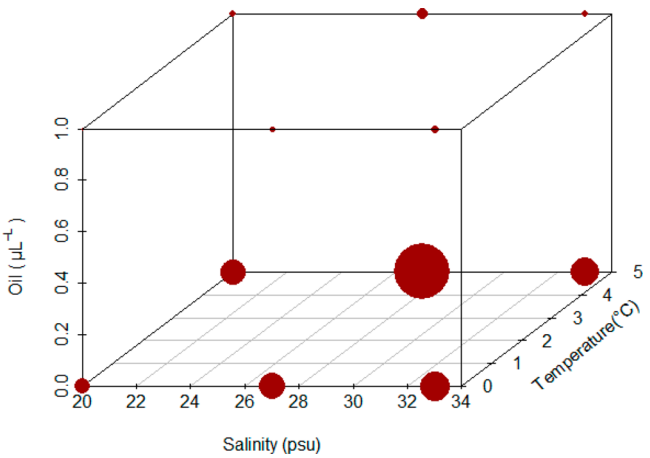

Figure 2. Cumulative impacts of climatic conditions and crude oil on fecal pellet production (FPP) shown as the predicted values of FPP for all combinations of temperature, salinity, and oil, including also model predictions for combinations not tested experimentally. The size of the dots shows the relative difference in FPP between dots (i.e., ranging from 4.5 to 72.7). (See Figure S5 for species-specific predictions.)

4. DISCUSSION

4.1. Stressors Do Not Affect the Condition of Copepods. The experimental exposure did not affect the

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prosome length or lipid sac area of the copepods. A change in prosome length was not expected since adults do not molt. We monitored it primarily to confirm that there were no significant differences in size between treatment groups that could have impacted their response in terms of feeding. Although knowledge on the impact of stressors on the lipid storage is still very limited, there are some indications that lipid metabolism can be affected by stress exposure.[45−47] While we cannot draw conclusions on a biochemical level, our observations of the lipid sacs give no indication of an effect of our treatments on the copepods' lipid storage. A longer exposure period than the 6 days of our experiment may however more likely impact the oil sac size, and future studies should ideally perform start and end measurements of the same individual. We can conclude that the observed differences in feeding between treatment groups were not caused by differing amounts of lipids, since the lipid sac area did not differ between groups.

4.2. Crude Oil Reduces Copepod Feeding. Oil was the strongest stressor for both copepod species, leading to an immediate decrease in FPP by up to 83% (Figure 1). At the same time, the FPV remained unchanged. This means that the copepods ingested much less algae, which supports our first hypothesis. The decrease in feeding was not related to a negative impact of oil on the food source (*T. weissflogii*) since we confirmed that the exposure of the feed algae alone did not result in different cell concentrations between treatments (Figure S4). Reduced feeding in response to oil exposure was observed in previous studies. Hansen et al.[26] reported lower clearance rates of lab-cultured *C. finmarchicus* at concentrations of 1.99 and 13.9 μL L^{-1} of mechanically dispersed crude oil. A later study with the same culture found a decrease in FPP by approximately 80% at a concentration of 0.77 μL L^{-1} and 90% at 4.58 μL L^{-1} of crude oil droplets.[27] These results are consistent with our findings. However, another study that analyzed effects of oil on feeding in *C. finmarchicus* and *C. glacialis* collected close to Svalbard and in the southern Barents Sea did not find a significant effect on FPP in either species, even at the highest tested concentrations of 7 and 10.4 μg L^{-1} (16 priority PAHs).[28] This is approximately 10 times the concentration of our exposure (i.e., 0.79 μg L^{-1} of 16 priority PAHs). One factor that might explain some of this discrepancy is the difference in stage or age of copepods, since that study used copepodites instead of adults of *C. finmarchicus*, and their experiments took place later in the year (late spring and summer in contrast to early spring in our case). It is well-known that sensitivity to stressors can be stage- or age-dependent.[48−50] Furthermore, Jensen and Carroll[28] used the water accommodated fraction (WAF) of crude oil instead of oil droplets. This may indicate that the oil droplets drive the observed response, which is in accordance with the findings by Hansen et al.[27] who reported a strong reduction in FPP only in response to dispersed oil, not the WAF. In contrast, Lemcke et al.[29] found up to 86% lower FPP of field-collected (Greenland Sea in late August) *C. finmarchicus* exposed to a WAF of 0.55 mg L^{-1} (total hydrocarbons). Other studies that used the same crude oil and exposure concentration as in the present study found reduced FPP (by 20−27%) in the Arctic species *Calanus hyperboreus* collected in West Greenland in May[33] and several temperate copepod species.[51]

Reduced feeding in the presence of oil has previously been attributed to narcosis.[33,51] However, we did not observe any signs of changed behavior when inspecting the individuals during daily renewal. Similarly, Hansen et al.[27] saw narcosis (i.e., measured by reduced swimming activity) of *C. finmarchicus* only at a higher oil concentration (4.58 μL L^{-1}). Nevertheless, it is plausible that a subtle level of narcosis, such as reduced filtering activity, could impact feeding also in our experiment, even if we could not detect such behavioral impacts based on visual inspection.

It has previously been found that *C. glacialis* had a higher tolerance to oil than *C. finmarchicus*.[23,24] Thus, we hypothesized stronger effects on *C. finmarchicus*. Our results show a pronounced negative effect of oil on both species (Figure S5, Tables S7−S8) but with *C. finmarchicus* demonstrating a relatively stronger effect on FPP (−27.3) compared to *C. glacialis* (−15.3). Hence, our results are in line with previous studies and confirm our initial hypothesis that *C. glacialis* is more tolerant of oil, as assessed through FPP.

4.3. Climate Change Has Varying Effects on Copepod Feeding. In accordance with our second hypothesis, higher temperatures enhanced the FPP of both species (Figure 2, Table 4). An increase in temperature accelerates biochemical reactions and hence metabolic rates, which is most likely the underlying mechanism of this finding.[52] Previous studies have also reported a temperature-driven increase in FPP for the two species.[34,53,54] Both Grote et al.[53] and Kjellerup et al.[54] reported increased feeding for *C. glacialis* at higher temperatures within a range of 0−10 °C. This pattern was also found for *C. finmarchicus,* though with higher sensitivity compared to *C. glacialis*, seen by a significantly higher FPP.[54] Makri et al.[34] also found a similar response for *C. finmarchicus* within the same temperature range (0−10 °C) and with salinities ranging between 25 and 40 psu. However, they observed the FPP of *C. glacialis* to peak at 5 °C, contradicting previous observations.[53,54]

In terms of salinity, Makri et al.[34] found no significant impact on FPP across a salinity range of 25−40 psu. At 20 psu, they observed an increase in FPP at a temperature of 0 °C but no difference at 5 °C. Contrary to their findings, our study demonstrated a higher FPP at 27 psu for both species, while at 20 psu FPP decreased for *C. glacialis* and fluctuated or slightly increased for *C. finmarchicus* (Figure 1, Figure S5). Hence, our third hypothesis was not supported. Our results clearly show that the response in FPP changed with exposure time (Figure 1). For instance, in the 20 psu treatments, differences to the control treatment only emerged after 3−4 days. This time lag could explain the difference between our results and those of Makri et al., who measured FPP after 42−48 h only. It also highlights the necessity to carefully consider the exposure time when interpreting results. The reduced feeding of *C. glacialis* at low salinities in our study could be attributed to osmotic stress at 20 psu. In contrast, *C. finmarchicus* appears to exhibit a somewhat higher degree of tolerance. This is surprising since *C. glacialis,* being native in Arctic shelfs, might be expected to be better adapted to regular melting and subsequent freshening. More work is needed to understand how salinity affects the physiology of these species, particularly osmoregulation.

4.4. Cumulative Impact of Crude Oil and Climate Change. The three tested parameters of crude oil, temperature, and salinity had a cumulative impact on the copepods' feeding, as the GAMM identified both two- and three-way interactions among the variables (Table 4, Tables S7−S8). This supports our fourth hypothesis. Despite oil exerting the

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strongest single impact, the effect was still modulated with changes in temperature and salinity (Figure 2). Synergistic interactions between pyrene (used as a proxy for oil) and warming have been described by several studies with copepods.[55−57] For instance, Hjorth and Nielsen[55] found the FPP of *C. finmarchicus* to decrease at low pyrene concentrations when temperature increased. Furthermore, the tolerance of 15 subtropical copepod species to crude oil was seen to significantly decrease with higher water temperatures.[58] The importance of multiple stressor research on pollutants and warming has recently been highlighted, as well as the need for more studies on Arctic invertebrates specifically.[4] Our study contributes to filling this knowledge gap by providing data on the interactive effects of crude oil, temperature, and salinity on two central species in Arctic planktonic food webs.

4.5. Ecological Implications. Oil pollution of marine environments is of global concern, including more remote areas like the Arctic.[59] The Arctic is already experiencing frequent occurrences of small oil spills,[17] and with the projected rise in petroleum extraction and transport, biota will increasingly get exposed to oil. The chosen oil concentration (1 μL L^{-1}, ∼0.84 ppm) is environmentally realistic in the vicinity of spills.[60] Furthermore, the concentration is relevant as it is far below the limits of permitted discharge of oil in produced water (30 ppm) and shipping effluents (15 ppm).[61−63]

Oil is expected to be the most problematic for copepods during the time they feed. For instance, Almeda et al.[33] showed that nonfeeding copepods had lower concentrations of hydrocarbons in their tissues after exposure to dispersed crude oil. This suggests that Arctic copepods could be the most susceptible to oil pollution during the spring bloom. The here observed reduction in feeding under oil exposure has serious implications for the copepods' energy budget since it reduces the aerobic scope (i.e., the energy beyond basic maintenance costs) which could result in impairments of processes such as growth, activity, and reproduction.[64] Additionally, under climate change, more energy may be required for osmoregulation (in response to freshening) and for higher metabolic rates at increasing temperatures, potentially exacerbating the strain on the copepods' energy budget. Another effect of reduced feeding is that it hampers the buildup of lipid reserves which are necessary for overwintering of Arctic copepods and for some species central for reproduction in the following spring.[65,66] Since it is the juvenile stages of *C. glacialis* and *C. finmarchicus* that usually undergo overwintering, it would be relevant to test if juveniles show a response similar to that of adult females. The same stored lipids by copepods are also vital for the transfer of energy to higher trophic levels.[22,67] Furthermore, sinking fecal pellets lead to large transports of carbon from the surface to deeper layers (i.e., the biological carbon pump).[68,69] This implies that oil exposure could have consequences for the Arctic food web as well as for carbon sequestration. Previous work suggests that copepods can recover from the effect of oil exposure;[31] but the window for feeding is short in the Arctic, and the recovery potential for feeding remains to be tested.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.3c09582.

Chemicals and materials for the analysis of polycyclic aromatic compounds; Analysis of polycyclic aromatic compounds; Elemental analysis; Data analysis with GAMMs; Conditions of copepods at the start and end of exposure; Exposure of *Thalassiosira weissflogii* to experimental treatments; Predicted FPP for individual species; Predicted vs observed FPP; Statistical results (PDF)

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Notes

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