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# Dispersant application increases adverse long-term effects of oil on shrimp larvae (*Pandalus borealis*) after a six hour exposure

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## ABSTRACT

The application of chemical dispersants is one option of oil spill response (OSR). Here, Northern shrimp (*Pandalus borealis*) larvae were experimentally exposed for short periods (6 h and 1 h) to a realistic concentration of chemically dispersed oil (CDO) ( $\sim 10 \text{ mg L}^{-1}$  THC), mechanically dispersed oil (MDO) ( $\sim 7 \text{ mg L}^{-1}$  THC), and dispersant only (D). A control (C) with seawater served as reference. Short-term effects on survival and feeding were examined right after exposure and longer-term consequences on survival, feeding, growth and development following 30 days of recovery. Both exposure durations provoked long lasting effects on larval fitness, with 1 h exposure leading to minor effects on most of the selected endpoints. The 6 h exposure affected all endpoints with more adverse impacts after exposure to CDO. This study provides important data for assessing the best OSR option relevant to NEBA (Net Environmental Benefit Analysis).

## 1. Introduction

In the event of an oil spill, responders need to take immediate actions that minimize the damage to the ambient environment. Therefore, operational planning and preparedness needs to be tailored to the environmental conditions on-site. Sub-arctic and Arctic latitudes are becoming more accessible due to warmer climate and therefore, longer ice-free periods. As a consequence, ship traffic (Eguíluz et al., 2016) as well as oil and gas exploration and production activities may increase further in the future. The first offshore oil field in the Barents Sea has been in operation since March 2016 (Offshore Technology, 2019) and recent well drilling events were noted in 2017 and 2018 (Jakobsson, 2018). These activities increase the risk for oil spills.

There is still insufficient knowledge of the consequences of oil spills for northern key species (National Academies of Sciences and Medicine, 2019), as well as of specific oil spill response (OSR) options to mitigate large impacts (Wenning et al., 2018; Wilkinson et al., 2017). At present, there are 6 prominent methods for spill impact mitigation assessment in the Arctic, all grounded in classical environmental risk assessment and the Net Environmental Benefit Analysis (NEBA) approach (Wenning et al., 2018). These are tools to support the response community in choosing the OSR option with the lowest impact on people and the environment (Wenning et al., 2018). For the northern regions, the application of chemical dispersants is one OSR option, and dispersants

such as Corexit 9500, Slickgone NS or Finasol OSR 52, which are listed as global stockpile dispersants, are commonly used (see Bejarano, 2018 for a review). The dispersant (surface active agent) increases the dilution rate of the oil slick into the water column by breaking the oil into smaller droplets (Lessard and DeMarco, 2000) and enhancing its bacterial degradation (Hazen et al., 2016; Krolicka et al., 2017). Total hydrocarbon concentrations (THC) of  $30\text{--}50 \text{ mg L}^{-1}$  have been found within the top few meters of the water column in experimental field trails and dispersant operations during real spills a short time after treatment (Bejarano et al., 2013; Lessard and DeMarco, 2000), which then rapidly decrease to  $< 1\text{--}10 \text{ mg L}^{-1}$  within a few hours (typically  $\leq 4$  h) (Bejarano et al., 2014; Lessard and DeMarco, 2000). Considerable research was conducted to understand the toxicity of dispersant alone (e.g. George-Ares and Clark, 2000; Negri et al., 2018; Scarlett et al., 2005) that, overall, seems to be low to marine organisms (Hemmer et al., 2011). In recent years, more and more studies were published that compare the effects of different OSR options, such as the use of dispersant, on different marine species, including fish (Bender et al., 2018; Frantzen et al., 2015; McConville et al., 2018), scallops (Frantzen et al., 2016), corals (Frometa et al., 2017) and ctenophore (Peiffer and Cohen, 2015). Although zooplankton has an important role in the marine food web, only a limited number of species (mainly copepods) have been used to study the effects of dispersants and chemically dispersed oil so far (Cohen et al., 2014; Hansen et al., 2012;

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Hansen et al., 2015; Toxvård et al., 2018) and particularly key northern species are understudied (Bejarano et al., 2017). A recent investigation using *Pandalus borealis* larvae was published by the present group (Arnberg et al., 2019) and here, the results of a follow-up study using even shorter exposure times are presented.

Overall, few studies showed that the chemically dispersed oil was more toxic than oil alone (Arnberg et al., 2019; K.-W. Lee et al., 2013; Philibert et al., 2019; Rial et al., 2014). However, these studies used exposure durations of 24 h to 96 h, although short exposure times (1–8 h) are considered most representative for oil spill settings (Bejarano et al., 2014). The influence of the exposure time is an important component in evaluating the toxicity of different OSR options, also in field situations, where the patchy distribution of oil and planktonic organisms results in variable exposure durations (National Academies of Sciences and Medicine, 2019). Arnberg et al. (2019) found that 24 h exposure to field realistic concentrations of chemically dispersed oil (CDO) led to reduced survival and feeding as well as slower development in *P. borealis* larvae compared to mechanically dispersed oil (MDO). Here, three consecutive replicate experiments were conducted with the same experimental set-up as in Arnberg et al. (2019), but with shorter exposure times of 6 h and 1 h. Survival and sublethal responses on feeding rate, growth, and development were examined right after exposure and during the recovery period. Compared to the study of Arnberg et al. (2019), we hypothesized that shorter exposure times would cause less effects and that exposure times as short as 1 h would not result in any negative impact of oil exposure on shrimp larvae.

## 2. Material and methods

### 2.1. Experimental organisms

Ovigerous shrimp (*Pandalus borealis*) were collected on January 10th, 2018 by bottom trawl from Hillefjord (north of Åmøy, Rogaland County, Norway; 59° 04' 00" N, 5° 45' 00" E), transported to the laboratory facilities and acclimatized as described in Arnberg et al. (2019), before transfer into hatching tanks in a cold temperature room on January 31st, 2018. Water temperature was gradually adjusted to 5 °C. Up to two ovigerous females were kept in one hatching tank (18 L) for several weeks prior to the start of hatching. Shrimp were fed every other day *ad libitum* with 3 mm fish feed pellets (Spirit supreme, Skretting, Norway). Newly hatched larvae were collected within 24 h into separate aquaria and kept there until exposure start using 2 days old larvae (2 days post hatch, 2 dph). Larvae were fed with freshly hatched *Artemia* nauplii and algae (*Thalassiosira weisslogi*) as described in Arnberg et al. (2013).

### 2.2. Experimental set-up

All experimental work was conducted in temperature-controlled rooms at 5 °C under artificial light conditions (10 h low intensity light and 14 h darkness). The experimental system used to generate exposure waters (Fig. S1) is based on a protocol developed by the French Centre of Documentation, Research and Experimentation on Accidental Water Pollution (Cedre) for exposing marine organisms to the dissolved fraction of dispersed oil, and also the oil droplets (Milinkovitch et al., 2011). Briefly, 10 g oil (for mechanical dispersion) or 10 g oil premixed with 0.4 g dispersant (for chemical dispersion) were added to the mixing tanks containing 150 L seawater through a funnel, 24 h prior to use of the exposure waters. For the dispersant only treatment, 0.4 g dispersant was added the same way.

### 2.3. Chemicals

A naphthenic crude oil from the Troll field in the North Sea (provided by the Norwegian Clean Seas Association for Operating

Companies, NOFO) was selected for the experiments. The Troll field is one of the largest oil producing fields on the Norwegian continental shelf, in operation since 1995. Slickgone NS (Dasic International OSD Ltd), a type 3 concentrate dispersant used worldwide, was chosen for chemical dispersion. This dispersant has one of the greatest national stockpiles in Norway and was shown to be an effective dispersant for Troll oil (European Maritime Safety Agency, 2016). Dispersant was added at a ratio of 1:25 (4% w/w) and thorough premixing was performed before adding the oil and dispersant mixture into the tanks for preparation of exposure waters.

### 2.4. Chemical analyses

Chemical analyses were performed by Intertek West Lab AS (Tananger, Norway). Oil and dispersant were added to the mixing tanks 24 h prior to time zero ( $t_0$ ), when exposure water was transferred into exposure bottles and shrimp larvae were added (exposure start). Shrimp larvae were exposed for 1 h ( $t_1$ ) or 6 h ( $t_6$ ), respectively, but samples for water chemistry (approx. 1 L) were only taken at  $t_0$  and  $t_6$ . Water samples were taken in three replicates from the mixing tanks for mechanically dispersed oil (MDO) and chemically dispersed oil (CDO) for THC analysis. One water sample from each experiment was taken at  $t_0$  for PAHs analysis. Samples were taken from the center of the mixing tanks at about 40 cm depth using silicon tubing and acidified (pH < 2) to stop biodegradation prior to transport. THC analysis (C7–C40) was performed according to NS-EN ISO 9377-2 OSPAR 2005-15 using GC-FID with a quantification limit of 0.4 mg L<sup>-1</sup> (Intertek analytical report). Samples for determination of PAH concentrations were analyzed for the 16 Environmental Protection Agency (EPA) priority PAHs and C1–C3 naphthalenes, C1–C3 phenanthrenes and C0–C3 dibenzothio-phenes (NPD) adding up to 26 PAHs in total (sum 26 PAHs). This was done using GC–MS following the standards of ISO 28540:2011. A more detailed description can be found in Arnberg et al. (2019). The quantification limits for PAHs were between 0.01 and 0.02 µg L<sup>-1</sup>. In order to calculate sum 26 PAHs, a value of 0.5 × limit of detection (LOD) was used for single components with values smaller than LOD (Frantzen et al., 2016).

Temperature and dissolved oxygen levels were measured at the start of each experiment in all exposure bottles and monitored every third day before water renewal during recovery. Temperature was 5 ± 0.4 °C and the dissolved oxygen levels remained above 90% saturation throughout the experiments.

### 2.5. Experimental design and endpoints

Three consecutive replicate experiments (Exp. 1–Exp. 3) were conducted in spring 2018. Due to logistical restrictions, only one mixing tank per treatment could be prepared at a time. Therefore, the experiment was repeated three times.

Shrimp larvae were statically exposed for 6 h and 1 h to i) clean seawater (control), ii) dispersant only (D), iii) mechanically dispersed oil (MDO) or iv) chemically dispersed oil (CDO), followed by a recovery phase of 30 days in seawater. Tests were performed with approx. 1 L exposure water in 1 L Schott glass bottles. All experiments were conducted with three replicate bottles per treatment and exposure duration and with 10 shrimp larvae per bottle at the start. After exposure, shrimp larvae were carefully transferred into new bottles filled with seawater for recovery. During the recovery, water was changed every third day by transferring the remaining larvae into newly prepared bottles filled with seawater. Shrimp larvae were fed every other day *ad libitum* with freshly hatched *Artemia* nauplii. Only 24 h prior to the second feeding test at the end of the recovery phase, larvae were transferred into newly prepared bottles and starved. Monitoring of survival and stage determination were performed in parallel to water renewal to avoid extra handling of the larvae.

## 2.6. Survival

Dead shrimp larvae were recorded after the exposure and every third day during recovery. Survival is presented as mean survival (% dead versus initial number of larvae added) over time.

## 2.7. Feeding rates

Two feeding tests were conducted, using three replicates per treatment and exposure duration ( $n = 3$ ). Feeding rates were calculated as number of *Artemia* nauplii eaten per individual per hour ( $\text{artemia eaten ind}^{-1} \text{h}^{-1}$ ). The first feeding test was done directly after exposure. All alive stage I larvae ( $n = 9-10$ , 2 dph) were transferred into feeding test bottles, and 120 *Artemia* nauplii were added. After 21 h, feeding tests ended and remaining *Artemia* nauplii were counted.

After 30 days in recovery, the second feeding test was performed with stage III larvae (33 dph). Since the larger larvae feed more, only up to 5 individuals were used per test bottle, and 150 *Artemia* nauplii were added. The test lasted for 5 h.

## 2.8. Growth

Total length (TL) and dry weight (DW) were determined at the end of each experiment as proxies for larval growth. Larvae were stored individually at  $-20^\circ\text{C}$  until analysis. TL was measured on scaled paper using a stereoscopic microscope in up to 3 individuals per exposure bottle ( $n = 8-9$  per treatment). After TL was taken, individuals were dried to constant weight on pre-weighed aluminum trays at  $60^\circ\text{C}$  (approx. 24 h). DW was measured using a precision scale (Mettler-Toledo XPE205 Delta Range, Oslo, Norway).

## 2.9. Development

Staging of shrimp larvae was performed in parallel to water renewal. Data for 14 days and 17 days old larvae are presented to cover transition from stage I to stage II (12 and 15 days in recovery, respectively) and on 26 days and 29 days old larvae to cover transition from stage II to stage III (24 and 27 days in recovery, respectively). Larval stage determination was done according to Haynes (1979) and Rasmussen and Aschan (2011). The anatomical features used to discriminate stages were stalked eyes for stage II compared to stage I and distinct exopodites on the telson for stage III larvae. Stage II larvae do not have exopodites.

## 2.10. Statistical analysis

The three replicate experiments were designed to increase data robustness and statistical power. Statistical testing revealed that occasionally data within one treatment between experiments were significantly different and therefore, data were not pooled. Data were first

tested for normal distribution (Kolmogorov-Smirnov test) and homogeneity of variances (Leven's test). Parametric data were further analyzed with one-way ANOVA and Tukey HSD post-hoc testing, while non-parametric data were tested with Kruskal-Wallis followed by pairwise Mann-Whitney  $U$  tests. Survival data were analyzed using Kaplan-Meier Log Rank (Mantel Cox) test including pairwise comparisons. The level of statistical significance was set at  $p \leq 0.05$ . All statistical analyses were performed using IBM SPSS Statistics 25 (IBM, Chicago, USA).

## 3. Results

### 3.1. THC and PAH concentrations in exposure water

Total hydrocarbon concentrations (THC) were more variable between experiments than polycyclic aromatic hydrocarbon (PAH) concentrations (Table 1, Fig. S2 and Table S1). THC concentrations in chemically dispersed oil (CDO) were about the same at  $t_6$  and  $t_0$ , while concentrations decreased slightly over time in mechanically dispersed oil (MDO). Exp. 1 and Exp. 3 showed higher THC concentrations in CDO compared to MDO, while in Exp. 2, THC concentrations were slightly lower in CDO. The greatest difference between THC concentrations in the two treatments was found in Exp. 3, where overall the lowest concentrations were measured. At the start of the exposure ( $t_0$ ), THC concentrations ranged from  $1.3$  to  $13.7 \text{ mg L}^{-1}$  in MDO and from  $6.4$  to  $13.0 \text{ mg L}^{-1}$  in CDO. After 6 h in the exposure bottles ( $t_6$ ), concentrations ranged from  $0.4$  to  $12.3 \text{ mg L}^{-1}$  and from  $6.0$  to  $12.0 \text{ mg L}^{-1}$ , respectively. Differences in the PAH concentrations were less pronounced between experiments at  $t_0$ . Sum 26 PAHs ranged from  $221$  to  $335 \text{ } \mu\text{g L}^{-1}$  in MDO and from  $387$  to  $477 \text{ } \mu\text{g L}^{-1}$  in CDO. Sum 26 PAHs were therefore generally higher in CDO than in corresponding MDO treatments. The predominant PAHs were naphthalene and substituted naphthalene homologues ( $84 \pm 2\%$ ), followed by the substituted anthracene/phenanthrene and dibenzothiophene homologues ( $13 \pm 2\%$ ). Acenaphthene, fluorene and phenanthrene represented  $3 \pm 0\%$ . More details on the chemical composition can be found in the supplementary section.

### 3.2. Survival

No mortality occurred during exposure, but within a few days in recovery (Fig. 1), mainly in oil exposed larvae. MDO as well as CDO treatment reduced survival significantly compared to control, with overall lowest survival after 6 h exposure in Exp. 1 (72% and 57%, respectively). In Exp. 3, survival after 6 h MDO exposure was significantly higher than in CDO ( $p \leq 0.05$ , Kaplan-Meier Log Rank test). After 1 h exposure, MDO reduced survival significantly in Exp. 1. In Exp. 2, all treatments were significantly different from the control and in Exp. 3 no differences were found at all.

**Table 1**

Chemical characterization of exposure water at the start of the exposure ( $t_0$ ) and 6 h later in the exposure bottles ( $t_6$ ). Results from the three experiments (Exp. 1–Exp. 3) are presented. Total hydrocarbon (THC) concentrations are given in  $\text{mg L}^{-1}$ , while polycyclic aromatic hydrocarbon (PAH) concentrations are in  $\text{ } \mu\text{g L}^{-1}$ . MDO: mechanically dispersed oil, CDO: chemically dispersed oil. More detailed chemistry results can be found in the supplementary section.  $N = 3$  water samples per experiment for THC measurements,  $n = 1$  water sample for PAH analysis.

Exp.	$t_0$ (start of exposure)									$t_6$ (end of exposure)					
	THC ( $\text{mg L}^{-1}$ )			PAH ( $\text{ } \mu\text{g L}^{-1}$ )						THC ( $\text{mg L}^{-1}$ )					
	1	2	3	16 EPA PAH			NPD			Sum 26 PAH					
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
MDO	8.1	13.7	1.3	31	25	21	330	250	210	335	251	211	4.0	12.3	0.4
CDO	13.0	11.7	6.4	37	39	31	470	470	380	477	474	387	12.0	11.0	6.0

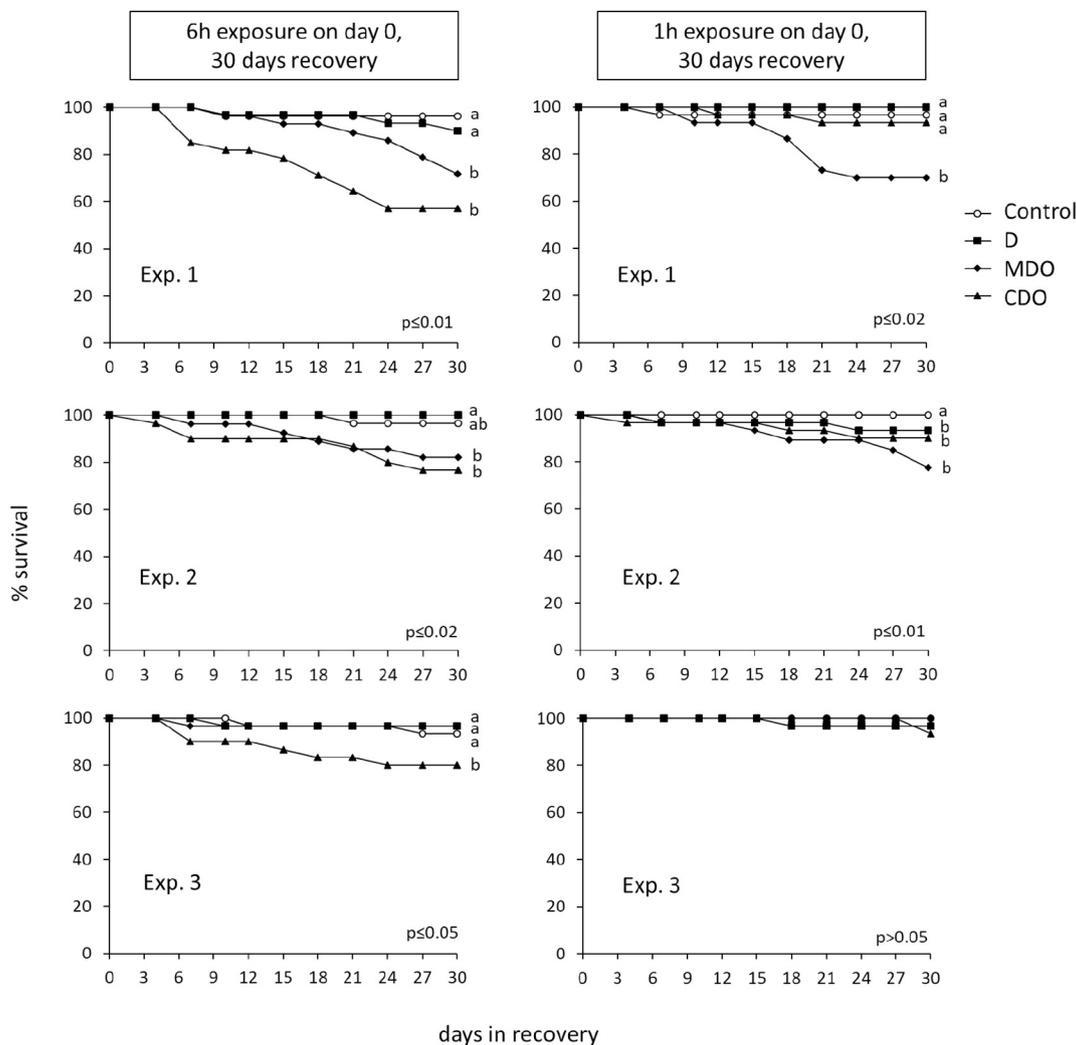


Fig. 1. Mean survival (%) of *Pandalus borealis* larvae during 30 days in recovery after 6 h exposure (left column) or 1 h exposure (right column) to dispersant only (D), mechanically dispersed oil (MDO) or chemically dispersed oil (CDO), in addition to control. Experiments are presented from top down. Different letters indicate statistically significant differences between treatments,  $p \leq 0.05$ .

### 3.3. Feeding rates

Feeding rates (Fig. 2) in control and D groups were about twice as high as in MDO and CDO groups that, however, were significantly reduced compared to control (except for MDO after 1 h exposure in Exp. 3). No significant differences between MDO and CDO were found after 1 h exposure in any experiment, and after 6 h only in Exp. 3 ( $p \leq 0.05$ , Mann-Whitney  $U$  test). Feeding rates of stage III shrimp larvae after 30 days in recovery were not significantly different between treatments and exposure durations and ranged between  $2.44 \pm 1.04$  and  $3.03 \pm 0.94$  *Artemia* eaten per hour (data not shown).

### 3.4. Growth

Six hours exposure to oil affected total length (TL) and dry weight (DW) (Fig. 3) of shrimp larvae. MDO led to significantly reduced TL in Exp. 1 (Fig. 3a), while CDO exposure caused both significantly reduced TL (Fig. 3a) and DW (Fig. 3b) compared to control in all three experiments. Comparing MDO and CDO, a significant difference in TL was found in Exp. 2 and Exp. 3 ( $p = 0.035$ , Mann-Whitney  $U$  test and  $p = 0.036$ , Tukey HSD test, respectively), with shorter larvae in the latter treatment (Exp. 2:  $6.3 \pm 0.5$  mm and  $5.7 \pm 0.5$  mm, Exp. 3:  $6.3 \pm 0.4$  mm and  $5.8 \pm 0.4$  mm). No differences were found in TL and DW of shrimp larvae between control and D treatment (Fig. 3,

average size of  $6.4 \pm 0.4$  mm). Overall, DW displayed a greater variation than TL and a significant difference between MDO and CDO was found in Exp. 2 ( $p = 0.005$ , Tukey HSD test).

Initial exposure of 1 h did not significantly affect TL or DW in shrimp larvae in any treatment after 30 days in recovery (data not shown). TL was about the same in all three experiments, ranging from  $6.0 \pm 0.3$  to  $6.5 \pm 0.4$  mm, whereas DW was slightly higher in the third experiment compared to the two others ( $0.60 \pm 0.14$  to  $0.73 \pm 0.08$  mg in Exp. 3 compared to  $0.47 \pm 0.06$  to  $0.57 \pm 0.15$  mg in Exp. 1 and  $0.49 \pm 0.13$  to  $0.68 \pm 0.13$  mg in Exp. 2).

### 3.5. Development

Shrimp larvae that were exposed to oil for 6 h developed slower from stage I to stage II and from stage II to stage III than control larvae (Table 2). CDO affected larval development more than MDO, and 1 h exposure caused less effect than 6 h exposure (Table 2 and Table 3).

## 4. Discussion

The data show that short-term exposure to field realistic concentrations of mechanically (MDO) and chemically (CDO) dispersed oil has long lasting effects on shrimp larvae fitness. However, survival,

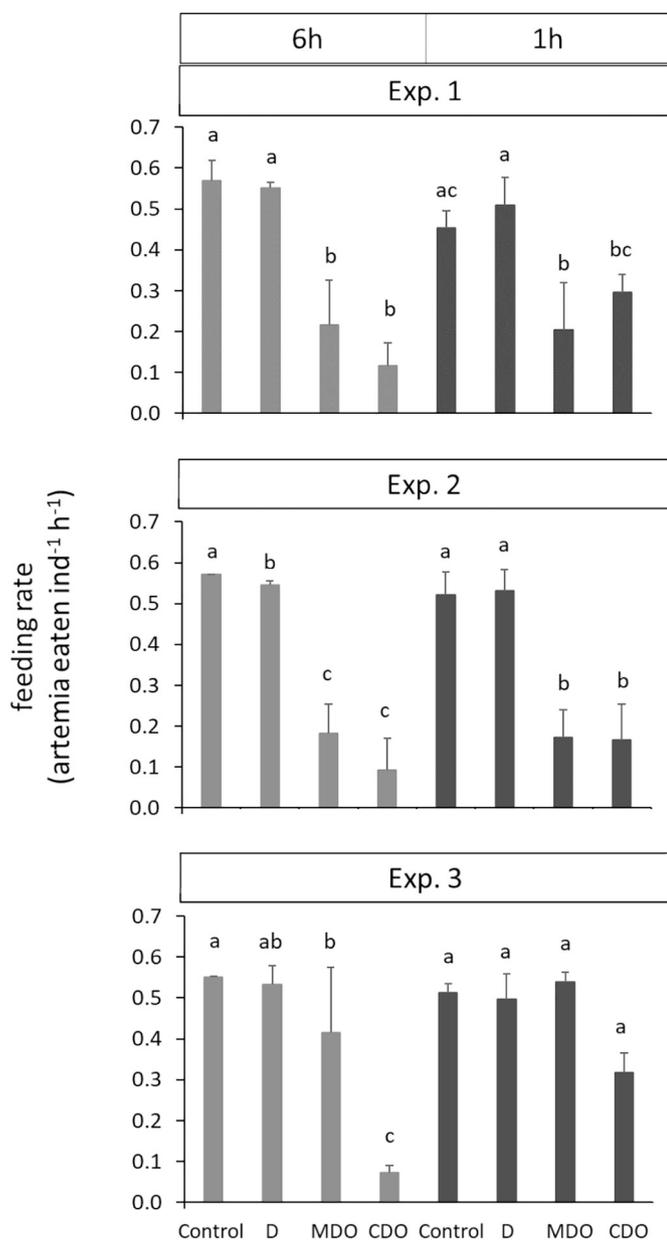


Fig. 2. Mean feeding rates (+SD) (number of artemia eaten per individual per hour) of *Pandalus borealis* larvae in the three replicate experiments. Larvae were initially exposed for 6 h (light grey) or 1 h (dark grey) to dispersant only (D), mechanically dispersed oil (MDO) or chemically dispersed oil (CDO), in addition to control. Different letters indicate statistically significant differences between treatments within one exposure duration,  $p \leq 0.05$ .

growth and development data indicate a greater sensitivity of shrimp larvae to CDO than MDO in the direct comparison of these two oil spill response (OSR) options.

Effect data gained from the three replicate experiments could not be pooled based on statistical analysis, contrary to data from similar experiments generated with the same exposure system (Arnberg et al., 2019). This may arise from differences in exposure concentrations in the three replicate experiments. The unexpectedly low THC concentrations in Exp. 3 may explain that effects were less severe for larvae exposed to oil in Exp. 3. Overall, PAH values were rather similar between experiments and also similar to the high PAH concentration reported in Arnberg et al. (2019).

Comparing the present results to those from the 24 h exposure conducted by Arnberg et al. (2019) underlines the importance of

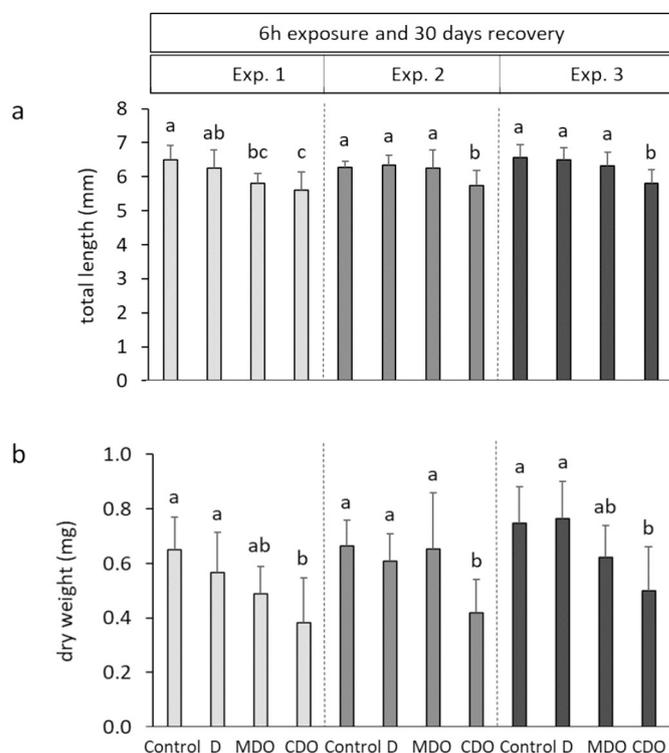


Fig. 3. Mean total length (a, in mm) and dry weight (b, in mg) (+SD) of *Pandalus borealis* larvae stage III after 30 days in recovery in clean seawater. Larvae were initially exposed for 6 h to dispersant only (D), mechanically dispersed oil (MDO) or chemically dispersed oil (CDO) in addition to control. Results from the three replicate experiments are presented (light grey: Exp. 1, medium grey: Exp. 2 and dark grey: Exp. 3). Different letters indicate statistically significant differences between treatments within one experiment,  $p \leq 0.05$ ,  $n = 8-9$ .

exposure duration on larval effects, as pointed out in several recent publications (Bejarano et al., 2014; Bejarano, 2018; K. Lee et al., 2013; National Academies of Sciences and Medicine, 2019). Overall, it was shown that the shorter the exposure time, the smaller the effects on lethal as well as sublethal responses and that 6 h exposure to MDO and CDO affects long-term shrimp larval fitness parameters. The consequences of such short exposures are seldom reported in the literature, which often overlooks short realistic exposure duration and focus on standard duration test. Further, in standard laboratory tests recovery time is not considered and the long-term consequences of exposure are not included. Here, mortality was observed first beyond the exposure period and a few days after exposure and increased during recovery. Feeding rates were significantly reduced directly after exposure and comparable to control at the end of recovery. These effects would have been overlooked without prolonged observations during recovery.

In contrast to the working hypothesis, the results show that oil exposure as short as 1 h can negatively affect survival and feeding in shrimp larvae. After 6 h exposure, all endpoints were significantly different to control, and generally shrimp larvae show a greater sensitivity to CDO compared to MDO. Also earlier studies with various crustacean species show their sensitivity to oil exposure and that the addition of chemical dispersants increase the negative effects (Almeda et al., 2014a; Almeda et al., 2014b; Arnberg et al., 2019; Hansen et al., 2012; Hansen et al., 2015; K.-W. Lee et al., 2013; Nordtug et al., 2015). Exposure to dispersant only (D) was found to reduce survival and feeding in shrimp larvae after 24 h (Arnberg et al., 2019). In the present study, 6 h exposure to D affected development and feeding in one of the three experiments. The effects observed in D treatments are possibly due to the composition of the dispersant mixture containing hydrocarbons of the solvent. Dasic NS dispersant consists of 30–60% petroleum

**Table 2**

Development of *Pandalus borealis* larvae during recovery. Larvae were exposed for 6 h to dispersant only (D), mechanically dispersed oil (MDO) and chemically dispersed oil (CDO), in addition to control. Mean % stage II or % stage III larvae are shown at selected days post-hatch (dph). Results from the three replicate experiments are presented (Exp. 1–Exp. 3). Different letters indicate statistically significant differences in % larval stages between treatments for each experiment and observations,  $p \leq 0.05$ .

Exp.	Development from stage I to II						Development from stage II to III					
	14 dph (% stage II)			17 dph (% stage II)			26 dph (% stage III)			29 dph (% stage III)		
	1	2	3	1	2	3	1	2	3	1	2	3
Control	62 <sup>a</sup>	93 <sup>a</sup>	93 <sup>a</sup>	100 <sup>a</sup>	100 <sup>a</sup>	100 <sup>a</sup>	47 <sup>a</sup>	69 <sup>a</sup>	80 <sup>a</sup>	86 <sup>a</sup>	82 <sup>a</sup>	87 <sup>a</sup>
D	76 <sup>a</sup>	69 <sup>b</sup>	80 <sup>a</sup>	100 <sup>a</sup>	100 <sup>a</sup>	100 <sup>a</sup>	46 <sup>a</sup>	47 <sup>ab</sup>	65 <sup>a</sup>	79 <sup>a</sup>	66 <sup>ab</sup>	79 <sup>a</sup>
MDO	0 <sup>b</sup>	47 <sup>c</sup>	69 <sup>a</sup>	42 <sup>b</sup>	92 <sup>a</sup>	93 <sup>a</sup>	0 <sup>b</sup>	32 <sup>bc</sup>	28 <sup>b</sup>	37 <sup>b</sup>	69 <sup>ab</sup>	65 <sup>ab</sup>
CDO	0 <sup>b</sup>	3 <sup>d</sup>	11 <sup>b</sup>	19 <sup>b</sup>	45 <sup>b</sup>	61 <sup>b</sup>	0 <sup>b</sup>	0 <sup>c</sup>	4 <sup>b</sup>	27 <sup>b</sup>	31 <sup>b</sup>	38 <sup>b</sup>

(Frantzen et al., 2016). Nonetheless, toxicity of dispersants alone is in general low (George-Ares and Clark, 2000).

In Exp. 1, where sum PAHs concentrations were highest, survival in oil exposed larvae was overall lowest. Significant mortality occurred after 1 h exposure to MDO in Exp. 1, although THC and PAHs concentrations were about the same or even lower in MDO than in CDO. PAHs in cellular membranes could lead to alterations of membrane structures resulting in mild toxic effects (narcosis) and mortality in later stages of invertebrates (Douben, 2003; Klaassen, 1996). Meador and Nahrgang (2019) concluded in their recent review on the toxicity of crude oil to fish early life stages that available data support the hypothesis that the syndrome of effects typically found is likely the result of baseline toxicity (not receptor based) due to membrane disruption and resulting alterations in ion homeostasis. A comparable mechanism is likely to apply to shrimp larvae, possibly explaining the delayed effects post-exposure.

Feeding rates were reduced in almost all experiments directly after exposure to MDO and more severely after exposure to CDO. This could be related to the formation and size of oil particles, with CDO likely resulting in the formation of smaller particles. Oil particles can coat the feeding apparatus of aquatic species (Hansen et al., 2009) or be ingested by pelagic invertebrates (Hansen et al., 2009; Hansen et al., 2012; Lee et al., 2012; Nordtug et al., 2015). Different zooplankton species such as copepods and tunicates were shown to ingest oil droplets following chemical dispersion (Hansen et al., 2009; Lee et al., 2012). However, after 30 days in clean seawater, no more differences in feeding rates were found between treatments, indicating the potential of surviving larvae to partly recover from 6 h sublethal exposures. Only 24 h in clean seawater were not enough to increase feeding rates of formerly exposed shrimp larvae to those of the control group (Arnberg et al., 2019). Arnberg (2015) exposed shrimp larvae from the day of hatch until 7 days post hatch to a low oil concentration (0.5 mg L<sup>-1</sup> nominal oil; PAHs concentration approx. 5 µg L<sup>-1</sup>) using a flow-through system and found that oil exposure reduced feeding by 70% in stage I larvae directly after exposure compared to control. Feeding rates

of stage III larvae were still affected, while stage IV larvae had similar feeding rates compared to control (Arnberg et al., 2018), demonstrating that the surviving shrimp larvae potentially recover from certain sublethal effects over time. Exposure to MDO and CDO also reduced feeding in first-feeding cod larvae (Hansen et al., 2016), although this study did not find a difference in toxicity between MDO and CDO. Reduced feeding due to oil exposure was also reported in other invertebrate larvae. Arnberg et al. (2017) found feeding and motility in later calyptopis-stage larvae of Northern krill (*Meganyctiphanes norvegica*) to be significantly impaired at exposure of 0.1 mg L<sup>-1</sup> oil. Despite no effect on feeding rates at the end of the recovery, growth was reduced in larvae exposed to CDO for 6 h (and MDO in Exp. 1) after 30 days in recovery. This may be related to the poor feeding of the surviving larvae at the start of their life during exposure. In contrast, 1 h exposure did not affect growth long-term, indicating that shrimp larvae recovered from the potential stress caused by this very short exposure time. Sea urchin larvae grown with scarce food needed more time to complete larval development and metamorphosed into smaller juvenile sand dollars relative to larvae grown with abundant food (Hart and Strathmann, 1994). The reduced feeding activity could result from reduced behavioral activity or weak (reversible) condition with larvae experiencing signs of narcosis (Alford et al., 2015). Survival and development are equally dependent on sufficient food uptake, especially during periods of massive transitions due to metamorphosis. Crustacean larvae rely on the ingestion of exogenous planktonic food. Exposure for 24 h led to poor feeding and decreased larval developmental rates after 9 days in recovery with around 10% of larvae successfully molting to stage II after exposure to MDO (THC concentration around 5 mg L<sup>-1</sup>) and CDO (medium and high THC concentration of around 1.7 and 16 mg L<sup>-1</sup>), compared to 97% in the control group (Arnberg et al., 2019). Here, shorter exposure times showed that most of the exposed larvae needed up to three days more to reach stage II and stage III after 6 h exposure to MDO, while significant differences remained after exposure to CDO at the end of the experiment. A developmental lag was found after 6 h exposure to CDO, while larval development following

**Table 3**

Development of *Pandalus borealis* larvae during recovery. Larvae were exposed for 1 h to dispersant only (D), mechanically dispersed oil (MDO) and chemically dispersed oil (CDO), in addition to control. Mean % stage II or % stage III larvae are shown at selected days post-hatch (dph). Results from the three replicate experiments are presented (Exp. 1–Exp. 3). Different letters indicate statistically significant differences in % larval stage between treatments for each experiment and observations,  $p \leq 0.05$ .

Treatment	Development from stage I to II						Development from stage II to III					
	14 dph (% stage II)			17 dph (% stage II)			26 dph (% stage III)			29 dph (% stage III)		
	Exp. 1	Exp. 2	Exp. 3	Exp. 1	Exp. 2	Exp. 3	Exp. 1	Exp. 2	Exp. 3	Exp. 1	Exp. 2	Exp. 3
Control	73 <sup>a</sup>	83 <sup>a</sup>	93	96	100	100	55 <sup>a</sup>	70 <sup>a</sup>	70 <sup>a</sup>	79	82	73
D	72 <sup>a</sup>	83 <sup>a</sup>	77	86	100	93	48 <sup>ac</sup>	64 <sup>a</sup>	68 <sup>a</sup>	83	82	90
MDO	57 <sup>a</sup>	56 <sup>ab</sup>	93	90	97	100	13 <sup>bc</sup>	35 <sup>ab</sup>	57 <sup>ab</sup>	69	70	80
CDO	27 <sup>b</sup>	45 <sup>b</sup>	87	93	93	100	22 <sup>c</sup>	15 <sup>b</sup>	43 <sup>b</sup>	71	78	66

1 h exposure was recovered. Delayed development associated with oil exposure has been observed in copepods and other crustaceans such as lobsters, potentially caused by alterations in the lipid metabolism, including steroid metabolism, causing reproduction and developmental anomalies (Almeda et al., 2013 and references therein). Delayed development is generally associated to a higher risk of decline in the pool of larvae for population recruitment through a mismatch of food, predation or disease. In bivalve larvae, it was estimated that a 5 day delay to metamorphosis and settlement could cause a 63% decline in spat (Kennedy, 1996).

It was proposed elsewhere that smaller droplets, more dissolved oil components in the water and the properties of the CDO droplets increase the availability of the oil compounds in copepods (Hansen et al., 2015) and that dispersants can increase the solubility of the higher molecular weight, more potent PAHs (Couillard et al., 2005; Wolfe et al., 1998). Measuring the dissolved fraction, hence bioavailable hydrocarbons in exposure waters, as suggested by Redman and Parkerton (2015), would help to interpret the findings and increase the comparability with other study results.

The endpoints investigated in this study are ecologically important for the recruitment and population dynamics of marine invertebrates such as species with strong spawning seasonality and distinct seasonal peaks for egg and planktonic larvae abundance (Highfield et al., 2010; Thorson, 1950). Should an oil spill event occur with dispersant application as OSR during the spawning season of these marine zooplankton species, the toxic effects on larval survival and performance could potentially adversely influence recruitment for the following year (Almeda et al., 2014b). Toxicity data are part of the NEBA assessment and needed for the approval process on the application of dispersant in the event of an oil spill. However, to fully understand the consequences of a spill on the population level is not trivial. To obtain a complete picture of the consequences at the population level requires the toxicity data to be combined with key factors such as the magnitude of the spill, the abundance and distribution of the organisms, their key vital development rates and the use of modelling. All this information is seldom available, especially for most Arctic organisms. Gallaway et al. (2017) combined a fecundity-hindcast model incorporating acute toxicity data, data from field studies of Arctic cod larval distribution and abundance, natural mortality estimates for eggs and larvae, and an oil spill fate model in Alaska Beaufort Sea and concluded that the effect of dispersing a large oil spill on the regional cod population is expected to be insignificant. Hence, effect responses (lethal or sublethal) of individuals do not necessarily translate into population level impacts and therefore a complex integration of toxicology studies with other factors determining population viability is needed to assess the actual environmental risk of an oil spill (National Academies of Sciences and Medicine, 2019).

Improving the experimental set-up and characterization of the exposure waters will further increase the robustness and comparability of this experimental work and the interpretation of (sub-) lethal effects. To further support spill impact mitigation assessments with NEBA for northern latitudes other key species should be tested using comparable set-ups in terms of exposure concentrations, durations and endpoint evaluations. Testing different life stages is important to account for species and temporal variations in spill impact mitigation evaluation. As pointed out by Aune et al. (2018), large proportions of a given population are potentially at risk if an oil spill occurs at times of biological peak production. For instance, an oil spill in early spring would have a higher risk of significantly affecting a copepod (*Calanus glacialis*) population than at a different time of the year. Additionally, these data could be used for species sensitivity distribution (SSD) models, as shown by Sanni et al. (2017), who demonstrated a link between biomarker and whole organism responses related to oil discharges for risk and environmental impact assessment based on SSD.

From the present research and the results of these series of experiments with shrimp larvae, the consequences of the application of

chemical dispersants is worse for early life stages of shrimp compared to no dispersant use. This warrant considering seasonality in OSR planning, since spring/early summer is the period when larval stages are present.

## 5. Conclusions

Field relevant exposure concentrations and exposure durations were used in a series of laboratory experiments to mimic a real-world oil spill scenario in higher northern latitudes. This study demonstrates that regional and seasonal aspects need to be considered in OSR to ensure recruitment of larval stages, and that decisions should consider seasonality and OSR options to mitigate the long-term effects on key species of zooplankton. To understand the actual consequences for recruitment and resilience of a local shrimp population, further endpoints such as fecundity, as well as survival and fitness of offspring of exposed Northern shrimp are needed. This would be relevant for NEBA and population risk assessment to help spill responders in taking the OSR decision with the best net environmental benefit.

## CRedit authorship contribution statement

**Frederike Keitel-Gröner:** Conceptualization, Investigation, Formal analysis, Writing - original draft, Writing - review & editing. **Maj Arnberg:** Conceptualization, Writing - review & editing. **Renée K. Bechmann:** Conceptualization, Writing - review & editing. **Emily Lyng:** Investigation, Writing - review & editing. **Thierry Baussant:** Conceptualization, Supervision, Writing - review & editing, Funding acquisition.

## Declaration of competing interest

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

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.marpolbul.2020.110892>.

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