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Effects of crude oil and field-generated burned oil residue on Northern shrimp (*Pandalus borealis*) larvae

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ABSTRACT

In situ burning (ISB) is an oil spill clean-up option used by oil spill responders to mitigate impacts on the marine environment. Despite advantages such as high efficiency and potential applicability for challenging areas such as the Arctic, the actual environmental side effects are still uncertain. Acute and sublethal effects of the water accommodated fractions (WAFs from 25 g oil/L seawater) of a pre-weathered North Sea crude (Oseberg Blend 200 °C+) and field generated ISB residue were evaluated on Northern shrimp (*Pandalus borealis*) larvae. The larvae were first exposed for 96 h to a serial dilution of seven concentrations, and then maintained for two weeks in clean seawater post-exposure. No acute (mortality) or sublethal effects (feeding, development, or growth) were detected in any of the ISB residue concentrations. Significant larvae mortality was found in the three highest concentrations of crude oil (96-h LC50:469 μg/L total petroleum hydrocarbon) but no sublethal effects were found in the surviving larvae post-exposure. This study indicates that applying ISB could mitigate acute impacts of spilled oil on shrimp larvae.

1. Introduction

In situ burning (ISB) is the controlled burning of crude oil or refined products at the location of an oil spill, applicable in open marine waters, coastal and freshwater environments from temperate regions to high northern latitudes. The use of ISB as a response method is controversial (Evans et al., 2001). The volume of oil on the water surface is rapidly reduced during burning, overall reducing the concentration of many toxic compounds such as poly-aromatic hydrocarbon (PAHs). However, by-products are formed and can be categorized as ISB residue, airborne components, and heat energy. The amount and nature of the ISB residue depend on the oil type and burning efficiency (Fritt-Rasmussen et al., 2015). Whether the residue sinks or floats depends on the density of the original oil. There is a correlation between medium and light oils producing floating residues and heavy oils producing sinking residues (Ross, 2002). ISB may increase the relative concentrations of large poly-aromatic hydrocarbons (PAHs), while reducing the concentration of smaller PAHs (Fritt-Rasmussen et al., 2015). ISB residues may pose a risk of toxicity or contamination to organisms living in the water column or at the seafloor. Floating residues may be ingested or cause fouling of feathers in birds or gills of fish (Fritt-Rasmussen et al., 2016), while localized smothering of benthic habitats may be the greatest concern when residues sink (Restoration, 2019). Few studies investigating the acute and long-term effects of unburned oil and ISB residue fractions in marine organisms are published, particularly for Arctic areas (Fritt-Rasmussen et al., 2015). Overall, there is an indication of low to no acute toxicity of ISB residues to aquatic organisms compared to other operative spill response options such as mechanical or chemical dispersion of the oil (Bender et al., 2018; Faksness et al., 2012; Gulec and Holdway, 1999). However, these studies used laboratory-generated residues. Weathering conditions as well as the flame regime might be different in the field compared to the laboratory. Therefore, using field-generated residue will further increase the relevance of the obtained data, also for environmental risk assessment and assessment of different oil spill response options. Recently, Toxværd et al. (2018) and Johann et al. (2020) investigated the effects of field-generated ISB residue. However, exposure duration lasted for 14 days in the first mentioned study and exposure was conducted at 26 °C using zebrafish embryos in the second. Therefore, the relevance for (sub-)arctic conditions is very limited.

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In the present study, we used a field-generated ISB residue to study the acute and long-term effects on Northern shrimp (*Pandalus borealis*) larvae and compared that to effects from the crude oil. Northern shrimp has been chosen as a representative zooplankton species of the marine ecosystem, with a key role in the North Atlantic food web and high commercial value for local fisheries (Bergström, 2000). Adults and shrimp larvae have repeatedly been shown to be sensitive to oil exposure (Arnberg, 2015; Arnberg et al., 2018; Bechmann et al., 2010) and to the use of chemical dispersant to combat oil spills (Arnberg et al., 2019; Keitel-Gröner et al., 2020). Moreover, Keitel- Gröner et al. (2020) showed that both acute and chronic effects from chemical dispersion were still significant beyond the short-term exposure period. Therefore, shrimp larvae were exposed for 96 h, followed by a two-weeks post--exposure phase in clean seawater to measure the long-term effects. The ISB residue was collected during a pilot oil release and subsequent burning in the North Sea in 2018 (Engen et al., 2018; Faksness and Krause, 2018).

The following null hypotheses were tested: (1) WAF obtained from crude oil is more acutely toxic to shrimp larvae than WAF from ISB residue and (2) there are no long-term effects of WAF from ISB residue.

2. Material and methods

2.1. Test organism

Ovigerous Northern shrimps (*Pandalus borealis*) were collected on January 27th, 2020 by bottom trawl from Kvitsøyfjorden (Rogaland County, Norway; N59.4, E5.34) at about 160 m depth and transported to the laboratory facilities within 2 h (for more details see Bechmann et al., 2020). Shrimps were kept for a week in 500 L tanks with flow-through of sand filtered seawater pumped from a depth of 75 m from the adjacent fjord (Byfjord, ambient temperature approx. 7 °C, salinity 34 psμ) before temperature was gradually reduced to 5 °C during one week of acclimatization. Shrimps were fed a mixture of 3 mm pellets of fish feed (Nutra Olympic, Skretting, Norway) and 1 mm shrimp feed (reference diet, Skretting, Norway) *ad libitum*.

Upon acclimatization to 5 °C, females were transferred individually into hatching aquaria (18 L) and kept there under the same conditions as described above until hatching started at the beginning of March. All larvae hatched within 24 h were collected into separate aquaria and fed with 1-day old *Artemia* nauplii and algae (*Thalassiosira weisslogii*) as described in Arnberg et al. (2013) until used for toxicity testing 4 days post-hatch (dph).

All experimental procedures were approved by the Norwegian Animal Research Authority (FOTS 22860).

2.2. Test oil and WAF preparation

A pre-weathered North Sea crude oil (Oseberg 200 °C+, representing oil at sea for 1–4 days; referred to as NSC in the following) and the ISB residue (referred to as ISBR) were provided by NOFO (Norwegian Clean Seas Association for Operating Companies). The oil is a light (API gravity: 39.6), intermediate low-sulfur (0.20%) oil (Leirvik and Myrhaug Resby, 2007). The ISBR sample was obtained during an ISB experiment conducted by NOFO and the Norwegian Coastal Administration in June 2018, where 6 m3 of oil were released into a fire-resistant oil boom and ignited by a drone. Further details, including physical-chemical characterization can be found in Engen et al. (2018) and Faksness and Krause (2018).

Low-energy water accommodated fractions (WAFs) of NSC and ISBR were prepared following CROSERF (Chemical Response to Oil Spills: Ecological Effects Research Forum) guidelines (Aurand and Coelho, 2005) with modifications described in Faksness and Altin (2019) and Hansen et al. (2011). WAFs were generated at 13 ± 1 °C and passively acclimated to the exposure temperature (5 ± 1 °C) following Hansen et al. (2011) Two glass jars with a tap at the bottom were filled with 9 L sand filtered natural seawater, and 225 g of either NSC or ISBR were carefully added to the surface as described in Hansen et al. (2011), resulting in an oil-to-water loading of 1:40 (25 g oil/L water). This oil loading has been recommended for oil toxicity testing under subarctic conditions before (Barron and Ka'aihue, 2003) and represents a saturated, conservative estimate of potential concentrations during an oil spill (Faksness and Altin, 2019). The ISBR was heated to 50 °C (approx. 40 min) prior to use to increase homogeneity and to ease handling, then weighed and added to the jar with water. Stirring without vortex was maintained for 72 h at approx. 13 °C in darkness and then the WAFs were used immediately without further settling time (Hansen et al., 2011). The aqueous phase was tapped slowly from the bottom of the jar. The different concentrations were prepared by dilution of the original (100%) WAFs with seawater (5 °C) to i) avoid accumulation of error due to serial dilution and ii) allow extrapolation of chemistry results in 100% WAFs to lower dilution levels (Barron and Ka'aihue, 2003; Gardiner et al., 2013). In total, seven WAF concentrations were prepared (100%, 59%, 35%, 20%, 12%, 7% and 4%) for NSC (WAF_{NSC}) and ISBR (WAF_{ISBR}).

2.3. Chemical analyses

Water samples for chemical analyses were taken at the start (t_0) and end of the exposure (t_{96}). WAF samples were acidified (0.5 mL 15% HCl per 100 mL sample) to avoid biodegradation before analyses. Samples for analysis of volatile organic compounds were collected in 40 mL glass vials without headspace, while all other parameters (total petroleum hydrocarbon and semi-volatile organic compounds) were analyzed from 800 mL samples in 1 L glass jars. All chemical analyses were conducted by SINTEF Ocean AS (Trondheim, Norway). Details on the analyses and lists of target compounds can be found in Faksness et al. (2012) with minor modification. Here, the GC/FID analyses were performed according to an updated EPA protocol (EPA Method 8015D, US EPA, 2003) compared to Faksness et al. (2012) and a total of 35 target volatile analytes in the C5 to C10 range were determined (see supplementary data for details).

2.4. Acute toxicity and sublethal effects

The 4-dph shrimp larvae were exposed for 96 h (static, non-renewal) to the different WAF concentrations and four replicates were used. A seawater control with four replicates was used for comparison. After the exposure, the larvae were kept for two weeks in clean seawater for post-exposure evaluation. Exposure bottles (0.5 L glass bottles) were sealed with Teflon lined screw caps to mitigate loss of the most volatile compounds, and post-exposure bottles (1 L glass bottles) were covered but not sealed to allow air exchange. A total of 10 shrimp larvae was gently added to each exposure bottle at start. No food was provided during exposure, while post-exposure larvae were fed daily with 1-day old *Artemia* nauplii. Mortality was monitored every 24 h during exposure and every third day post-exposure during water renewal, and both oxygen and temperature were measured in a random subsample of five bottles each time. Oxygen saturation was high (98.7 ± 1%) and temperature stable (5.0 ± 0.1 °C) throughout the experiment. A summary of test conditions can be found in Table S1. Stage determination of remaining stage I and stage II larvae was conducted simultaneously with water renewal at 14 and 17 dph (6 and 9 days post-exposure, respectively) as described in Keitel-Gröner et al. (2020). Feeding rates were determined twice, in the control group as well as in the surviving WAF-treated groups (4%, 20% and 35% WAF_{NSC} and $WAF_{ISBR.}$)The first feeding test was conducted within 21 h after exposure, while the second feeding test was performed at the end of the post-exposure phase according to Keitel-Gröner et al. (2020) with minor modifications. The number of shrimp larvae used in the tests had to be modified, depending on mortality rates. In the first feeding test, 4 to 10 stage I larvae were used, whilst 3 to 5 stage II larvae were used in the second test. The larvae

were given 120 *Artemia* nauplii per replicate bottle.

2.5. Statistical analyses

All statistical analyses were performed using GraphPad Prism statistic software version 8.4.3 (GraphPad Software, San Diego, CA, USA). Non-linear regressions (sigmoid curve fitting) were applied to the data to determine the 96-h LC50 value. Data were tested for normal distribution using Shapiro-Wilk tests. Then, differences in observed effects between the different treatments were tested using either one-way ANOVA (normally distributed data) or Kruskal-Wallis when the normality test failed. Statistical significance was tested at $p < 0.05$.

3. Results

3.1. Chemical composition of WAFs

Table 1 and Fig. 1 give a summary of concentrations of selected compounds in the original (100%) WAFs. The measured concentrations of oil compounds in the WAFs were very similar at t_0 and t_{96}. There was less than 2% difference in the concentrations measured at the start and end of the exposure (Table S4, SVOC and S5, VOC), hence the mean concentrations measured at t_0 and t_{96} are presented in Fig. 1. The total petroleum hydrocarbon (TPH) concentration in the original WAF_{NSC} was about 20 times greater compared to WAF_{ISBR}. Also, while WAF_{NSC} contained about 13 times more semi-volatile compounds in total than WAF_{ISBR}, NSC/ISBR ratios for PAH concentrations were relatively similar with ratios ranging from 9 for naphthalenes, 6 for 2–3 ring PAHs to 2 for 4–6 ring PAHs. However, volatile organic compounds were around 20 times greater in WAF_{NSC}. The smallest difference in concentration was found for 4–6 ring PAHs, while C0–C5 phenols had the greatest difference. See supplementary data for more details on the chemical compositions of the two WAFs (Table S2–S5).

3.2. Acute toxicity and post-exposure survival

Mortality of larvae during exposure and post-exposure is shown in Fig. 2. Exposure to 100% WAF_{NSC} was lethal to all shrimp larvae within 48 h. Within 72 h, all larvae exposed to 59% WAF_{NSC} died and exposure to 35% WAF_{NSC} caused significant mortality over time as well. The estimated lethal WAF concentration was 37.4% and the 96-h LC50 was 469.3 μg/L TPH (Table S6). Exposure to ISBR did not cause any significant mortality and post-exposure survival was not significantly different from control in any remaining treatment.

All larvae in 100% and 59% WAF_{NSC} died within the exposure time and hence were not used further in the post-exposure measurements.

Table 1
Chemical composition and concentration of original (100%) WAF (μg/L) of North Sea Crude (NSC), and the in situ burn residue (ISBR) at start (t_0) and end (t_{96}) of exposure. Ratios for the different chemical groups were calculated. Further details can be found in the supplementary section.

	NSC t_0 (μg/L)	NSC t_{96} (μg/L)	ISBR t_0 (μg/L)	ISBR t_{96} (μg/L)	Ratio NSC/ ISBR t_0	Ratio NSC/ ISBR t_{96}
TPH	1239	1272	56.3	66.6	22	20
Sum SVOC	330	334	25.5	25.5	13	13
Sum decalins	0.336	0.320	0.042	0.042	8	8
Naphthalenes	179	179	20.1	20.0	9	9
2–3 ring PAHs	20.1	20.0	3.34	3.35	6	6
4–6 ring PAHs	0.189	0.232	0.100	0.090	2	3
C0–C5 phenols	131	134	1.91	1.92	67	70
Sum VOC[a]	267	266	13.7	13.9	19	19
BTEX	160	153	8.64	8.59	19	18
C3-benzenes	94.9	98.2	4.62	4.99	21	20
Other VOC	12.0	14.6	0.335	0.353	36	41

TPH: total petroleum hydrocarbons; SVOC: semi-volatile organic compounds; PAHs: polycyclic aromatic hydrocarbons; VOC: volatile organic compounds; BTEX: benzene, toluene, ethylbenzene, xylene.
[a] C4–C5 benzenes not included.

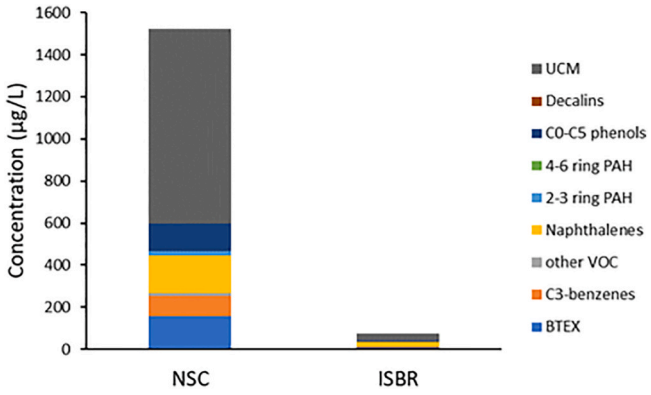

Fig. 1. Chemical concentration (μg/L) of the original (100%) WAF of North Sea crude (NSC) and the in situ burn residue (ISBR) for selected (aromatic) groups, including unresolved complex materials (UCM). Mean of concentrations measured at start and end of the 96 h exposure.

3.3. Sublethal effects

3.3.1. Feeding rates and larval length

No significant differences were found in feeding rates at the end of exposure with stage I larvae (Kruskal-Wallis test, $p = 0.181$) and at the end of the post-exposure period with stage II larvae (ANOVA, $p = 0.494$) (Figure S1 and Figure S2). The mean feeding rate of stage I larvae was 0.36 ± 0.16, 0.43 ± 0.07 and 0.39 ± 0.08 artemia/larvae/hour, in control, 35% WAF_{NSC} and 35% WAF_{ISBR}, respectively. Mean feeding rates of stage II larvae were 0.92 ± 0.59, 1.46 ± 1.05 and 0.62 ± 0.33 artemia/larvae/hour in the same treatments. Also, the total length of stage II larvae did not differ significantly between treatments (Kruskal-Wallis test, $p = 0.267$, data not shown).

3.3.2. Development

Development in surviving larvae was not significantly affected in any treatment compared to control (Fig. 3). However, at 14 dph, there were fewer stage II larvae with increasing concentrations of WAF_{NSC} (Kruskal-Wallis test, $p = 0.06$), but this was not observed in WAF_{ISBR} (Kruskal-Wallis test, $p = 0.951$). At 17 dph almost all shrimp larvae had reached stage II in all treatments.

4. Discussion

4.1. Relevance

The results of the present study provide insight into the comparative toxicity effects of field-generated burned oil residue and the initial crude oil on a sensitive shrimp life stage. Most studies published so far used either laboratory-generated residues (e.g. Bender et al., 2018; Faksness et al., 2012; Cohen and Nugegoda, 2000) or a model species with little relevance for arctic conditions (Johann et al., 2020). Toxværd et al. (2018) used a highly relevant species (*Calanus glacialis*) and exposure water collected from mesocosms set up in Van Mijenfjorden in Svalbard. However, their focus was on the effects of ice on the PAH release of oil and the exposure lasted for 14 days. The comparability of results obtained from laboratory-generated to field-generated residue may be limited due to weathering in the field as well as potential flame regime differences. Broch et al. (2020) recently simulated crude oil exposure in North Atlantic *Calanus finmarchicus* populations and found overall minor effects on population level due to a limited spatial and temporal overlap

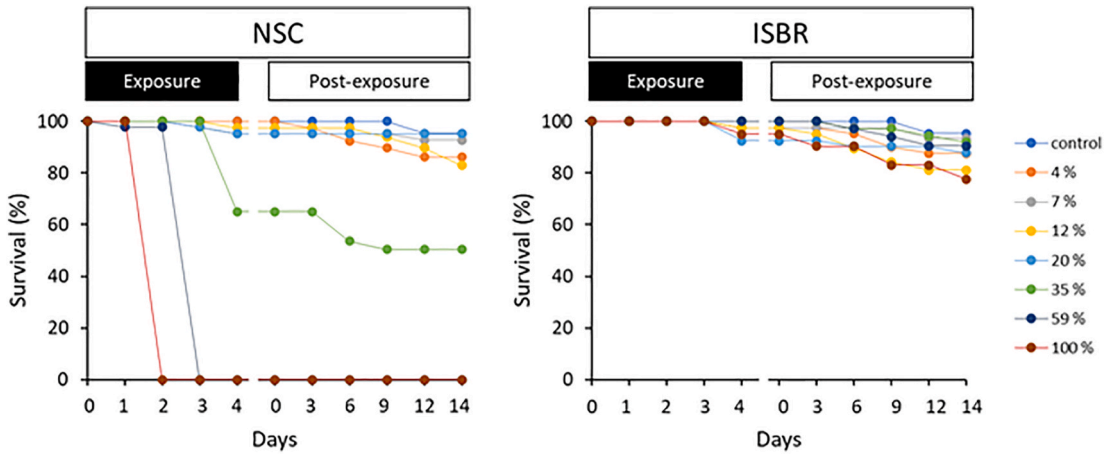

Fig. 2. Survival of *Pandalus borealis* larvae during and after exposure to the different WAF concentrations (4–100%) of North Sea crude (NSC) and the in situ burn residue (ISBR).

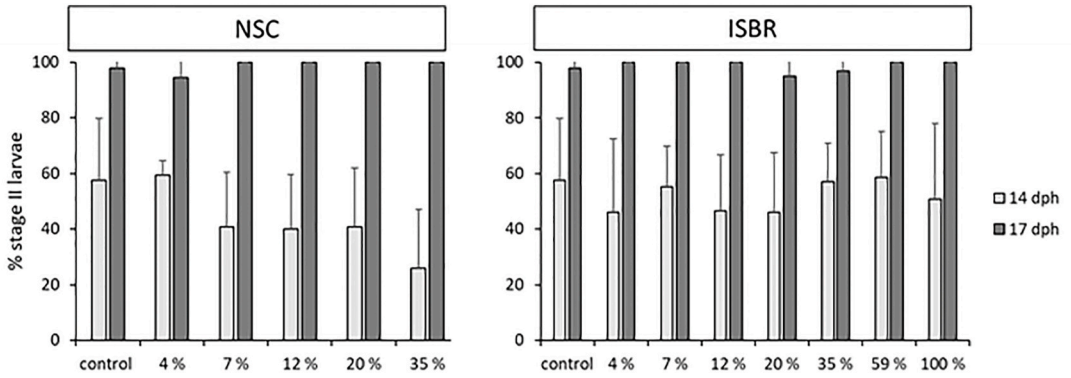

Fig. 3. Percentage stage II larvae (mean + SD) of *Pandalus borealis* 14 days post-hatch (dph) and 17 dph after exposure to increasing concentrations of North Sea crude (NSC) and the in situ burn residue (ISBR). N = 4.

between copepods and oil in the environment.

4.2. Water concentrations of oil compounds

Chemical monitoring data of experimental oil releases as well as after real oil spills show that water concentrations of oil compounds vary in space and time. Boehm et al. (2007) summarized studies after the Exxon Valdez incident and concluded that petroleum hydrocarbons were scattered but up to 42 μg/L total polyaromatic hydrocarbon (TPAH) was found in a subset of samples (9 out of 1288 water samples had concentrations > 10 μg/L TPAH). Faksness et al. (2011) conducted monitoring of the water-soluble oil components during an experimental oil released (7 m^3) in the marginal ice zone in the Barents Sea and found up to 1.5 μg/L dissolved hydrocarbons and up to 32 μg/L total hydrocarbons. Diercks et al. (2010) found PAH concentrations of 29 μg/L and 189 μg/L at two near wellhead sites of Deepwater Horizon. The authors concluded that subsurface exposure to PAH at levels considered to be toxic to marine organism would have occurred in discrete depth layers and extending at least 13 km. Hence, exposure levels of NSC found in the present study were in a field relevant range. Lower concentrations and a shift towards larger PAHs were expected for the ISBR treatment due to the removal of mainly lighter compounds during the burning process. In a mesocosm based study on oil in ice, Toxværd et al. (2018) compared the effects of the oil spill response options ISB, chemical dispersion and natural attenuation on the physiological performance of the Arctic copepod *Calanus glacialis* and, in agreement with the present study, found that ISB resulted in the lowest PAH exposure concentration as a result of the removal of 80–90% crude oil volume during the incineration process.

The preparation of exposure waters with protocols other than standardized guidelines such as CROSERF might hamper a direct comparison of results, but trends can be compared. However, using a low-energy mixing approach without vortex to avoid the formation of oil droplets does not necessarily reflect a realistic exposure of pelagic organisms in the field and therefore, the present results should be treated with some precaution as they might underestimate actual toxicity resulting from a combination of dissolved and dispersed oil. This applies particularly for the crude oil. One can assume that the much thicker ISB residue would result in less droplets under any reasonable mixing protocol. Furthermore, reviewing the available literature highlights that different oils and their ISB residues vary considerably due to variations in composition. Also, the execution of the burning operation and its efficiency will influence the composition of the residue and its physical and chemical properties. Thus, several variables, difficult to standardize for laboratory experiments, can influence the final toxicity of ISB residues to marine organisms.

As expected, chemistry results showed that the actual WAF compositions and hence exposures were very different between NSC and ISBR. The greatest difference was found in C0–C5 phenol concentrations, a volatile fraction, whilst the smallest difference was found in 4–6 ring PAHs. The relative concentration of PAHs was higher in ISBR because volatile compounds were removed from the burning. Still, their absolute concentration was much lower, including a lower absolute concentration of naphthalenes, providing a likely explanation for the lower toxicity found in ISBR compared to NSC. However, relating toxicity to mainly PAH concentration to explain the non-toxicity of ISBR on shrimp

larvae is not sufficient. Besides solvents (sum decalins), 2–3 ring PAHs and 4–6 ring PAHs had the smallest NSC/ISBR ratio, also compared to BTEX and other VOCs. These results indicate that shrimp larvae might have died of narcotic effects of BTEX and other VOCs in the exposure phase of the NSC treatment rather than of direct toxic effects of the PAHs alone. In a recent review, Meador and Nahrgang (2019) also postulated that baseline toxicity might be the primary mechanism for fish embryo toxicity when expose to crude oil, rather than PAHs being the main causative agents.

4.3. Effects of crude oil vs burned oil (WAF_{NSC} vs WAF_{ISBR})

Acute mortality was observed in larvae exposed to NSC, but not in those exposed to ISBR. A direct comparison of the relative impacts, however, is limited by the lack of comparable TPH concentrations. The TPH concentration in the lowest WAF_{NSC} (4%) was calculated to be equivalent to 82% WAF_{ISBR}. This difference in concentrations was expected when deciding the experimental set-up with the same oil-to-water ratio in both treatments to increase the field-relevance of the experiment.

Marine crustacean LC50 data for WAFs show a wide range depending on oil type, test species, test period and experimental set-up (Hansen et al., 2011), as well as reported data. For the larvae of the cold-water species Tanner crab (*Chionocetes bairdi*), a LC50 for a Prudhoe Bay crude WAF was reported to be 9.61 mg volatile organic compounds (VOC)/L (Perkins et al., 2005). Hansen et al. (2012) reported a 96-h LC50 of 0.8 mg/L TPH and 0.02 mg/L PAHs for the copepod *Calanus finmarchicus* exposed to a naphthenic crude oil. For single oil compounds, 96-h LC50s have been reported in the range of 0.29 mg/L (2-methylnaphthalene) to 14.8 mg/L (toluene) in adult Northern shrimp (Bytingsvik et al., 2020), indicating a greater sensitivity of early life stages, when compared to the present data. However, the 96-h LC50 toxicity of NSC and the sensitivity of Northern shrimp larvae in the present study was within the range found for other oils and pelagic (Arctic) species (Table 2).

The toxicity of burned oil residue is mostly reported as lower or comparable to the initial oil. However, Faksness and Altin (2019) found 72-h LC50 values for *Calanus finmarchicus* nauplii to be 0.27 mg/L WAF (sum VOC and TPH) for unburned oil and 0.12 mg/L WAF for the ISB residue. The authors stated that when comparing toxicity based on analytical mass, the composition of the WAFs, that can vary greatly between oils, are ignored. Therefore, WAFs from different oils with the same concentrations can have different toxicities. In their exposure study, the authors found the ISB residue to be more toxic compared to the fresh oil based on the specific toxicity (acute toxicity normalized to the total WAF concentration – LC50 in mg/L). Presenting toxicity data as measured concentrations facilitates the comparison to field observations, as indicated by the previous example, which in turn can be used in environmental risk assessment and support risk evaluations such as the Net Environmental Benefit Analysis (Gardiner et al., 2013), now included in Spill Impact Mitigation Assessment (SIMA) (Ipieca-Api-Iogp, 2017).

There were no significant effects on development rate, feeding rate or growth of shrimp larvae in the two weeks following 96 h exposure to 4–35% WAF_{NSC} or 4–100% WAF_{ISBR}. However, the percentage of stage II larvae decreased with increasing NSC concentrations 14 dph. Delayed development associated with oil exposure has been observed for shrimp larvae (Keitel-Gröner et al., 2020), copepods and other crustaceans such as lobster (Almeda et al., 2013) before.

Both, the acute effects and the indication of developmental effects in NSC but not ISBR can be explained by the higher concentrations of the oil components in the WAF_{NSC}. Cohen and Nugegoda (2000) found a higher proportion of heavier molecular weight hydrocarbons in the burned oil WAF (C8–C32 in oil compared to C18–C32 in burned oil) and concluded that the lighter aliphatics in the crude oil WAF were more water-soluble and dissolve more easily into the water column and therefore could explain the difference in toxicity found in their study with Australian Bass. Fingas (2016) examined PAH concentrations in laboratory test burns as well as at-sea burns of crude oil and found, when considering the mass balance of the burn, most PAHs were removed by the fire, with some remaining with the residue. Again, a slight increase in the concentration of multi-ringed (5 or 6 rings) PAHs was found in the burn residue.

Toxværd et al. (2018) performed a mesocosm study with oil in ice in Svalbard to compare the effects of ISB, chemical dispersion and natural attenuation on the physiology of the Arctic copepod *Calanus glacialis*. The authors collected the seawater from the mesocosms and used it in laboratory incubation experiments. No negative effects on the physiological performance of the female copepods were found. However, indirect effects on the development of next generation nauplii were noted in the dispersed oil treatment. Recently, Johann et al. (2020) compared the toxicity of field-generated ISB residue to the initial and dispersed heavy fuel oil using zebrafish (*Danio rerio*) embryos. The ISB residue did not induce greater toxicity compared to the initial oil, whereas the application of chemical dispersant increased the acute toxicity. Zebrafish is a well-established ecotoxicological model species with many advantages for use in laboratory studies. However, testing was conducted at 26 °C and is therefore not representative for (sub)arctic conditions (0–5 °C) (Johann et al., 2020). Additionally, a heavy fuel oil (IFO 180) was tested. Compared to crude oils and refined distillates, the dissolved hydrocarbon content of IFO 180 in the water column is relatively low (Brown et al., 2016). Potentially, after an oil spill, the exposure of the pelagic community to water-soluble fractions of heavy fuel oils, and hence the ecotoxicological risk, is limited compared to lighter refined products (Fritt-Rasmussen et al., 2018).

In an earlier study on the effects of mechanically and chemically dispersed oil on Northern shrimp larvae, comparable PAH concentrations in the mechanically dispersed oil caused sublethal effects after 6 h exposure. Both, feeding and growth were significantly reduced (Keitel-Gröner et al., 2020). However, one main difference in the exposure set-up was that a high energy mixing was applied in the experimental setup, potentially resulting in oil particles/droplets of different size. It is possible that the shrimp larvae ingest oil droplets together with prey items as found for other pelagic species (Hansen et al., 2017, Lee et al., 2012). Oil components associated with oil droplets may then become bioavailable. The fraction and composition of oil associated with dispersion (oil droplets) differ largely compared to WAF, which mostly represents the most waters soluble compounds ($K_{OW}<3$). Particularly heavier components are almost only associated with the particulate phase in oil dispersions (Almeda et al., 2014, 2016). The WAF preparation based on CROSERF guidelines is essentially free of oil droplets. Therefore, the absence of oil droplets likely explains

Table 2
Comparison of 96-h median lethal concentrations (LC50) of different pelagic species.

Species	Oil	96-h LC50		Reference
		TPH (mg/L)	PAHs (mg/L)	
Arctic cod (*Boreogadus saida*)	Alaska North Slope crude oil	1.6 ± 0.4	0.03 ± 0.01	Gardiner et al. (2013)
Sculpin (*Myoxocephalus* sp.)		2.3 ± 1.0	0.04 ± 0.01	
Australian Bass (*Macquaria novemaculeata*)	Bass Strait crude oil	0.59		Cohen and Nugegoda (2000)
	Bass Strait burnt oil	0.83		
Copepod (*Calanus finmarchius*)	Naphthenic crude oil	0.80	0.02	Hansen et al. (2012)
Northern shrimp (*Pandalus borealis*) larvae	Oseberg Blend crude	0.47	0.07	present study

differences in study outcomes and the role of oil droplets to shrimp larvae toxicity should be further studied to distinguish between starvation-like effects resulting from oil droplet ingestion and actual toxic effects resulting from other cellular/physiological disruptions. Overall, the contribution of oil droplets to oil dispersion toxicity should be considered in oil toxicity study as this could potentially lead to an underestimation of the actual effects and hence bias the prediction outcomes i.e. the relative impacts, on resources at risk that is part of the NEBA/SIMA methodology used by stakeholders and spill responders.

5. Conclusion

The presence of sensitive organisms (life stages) and the toxicity of untreated as well as treated oil are important information to evaluate the potential environmental impact by the oil itself and the side effects of applied oil spill response technologies (Wegeberg et al., 2017). ISB is one of the response options in the aftermath of an oil spill but the use as a response method is still controversial due to uncertain environmental consequences. Overall, research results indicate that burned oil residues, whether field- or laboratory-generated, have a lower or comparable toxicity compared to the initial oil. In the present study, no acute nor sublethal effects of ISBR exposure were found on shrimp larvae, while the NCS was acutely toxic at higher concentrations. However, as pointed out by Johann et al. (2020), it has to be considered that ISB potentially does not reduce the concentration of toxic oil compounds that have already been dissolved into the water column before the burning process. Therefore, the acute toxicity of an oil spill is likely not eliminated by ISB but reduced due to the removal of large oil volumes by combustion.

The results show that both initial working hypotheses could be confirmed. WAF_{NSC} was acutely toxic in the range of 35–100% to shrimp larvae whilst WAF_{ISBR} was not toxic at any concentration. Also, no significant long-term effects of neither the WAF_{NSC} nor the WAF_{ISBR} were found. Thus, when considering response vs no response ("reference") in case of an oil spill, ISB appears to be a better option as the outcome (toxicity) for zooplankton species seems to be mitigated compared to the "no response" outcome in a SIMA perspective. Hence, the present laboratory data can be used in environmental risk assessments and by oil spill responders, to evaluate the best OSR method for the marine ecosystem and resources. Yet, this study did not consider the other environmental trade-offs of ISB such as airborne components and heat energy, which also need to be appraised in the response decision, to achieve a consensus in the final decision of best response options in SIMA process. Further research is warranted to enhance the confidence in choosing one OSR option over another such as by testing a greater variety of crude oils, and marine fuel oils, and their ISB residues as well as a larger number of key species from different trophic levels, particularly those sustaining important role and function for Arctic ecosystems.

CRediT authorship contribution statement

Frederike Keitel-Gröner: Conceptualization, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Funding acquisition. **Renée K. Bechmann:** Conceptualization, Writing – review & editing. **Frode Engen:** ISB field operations, Conceptualization, Resources, Writing – review & editing. **Emily Lyng:** Investigation, Writing – review & editing. **Ingrid C. Taban:** ISB field operations, Conceptualization, Resources, Writing – review & editing. **Thierry Baussant:** Conceptualization, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

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

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Appendix A. Supplementary data

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