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Do oil droplets and chemical dispersants contribute to uptake of oil compounds and toxicity of crude oil dispersions in cold-water copepods?

Bjørn Henrik Hansen[a], Dag Altin[b,c], and Trond Nordtug[a]

[a]SINTEF Ocean, Climate and Environment, Trondheim, Norway; [b]BioTrix, Trondheim, Norway; [c]Research Infrastructure SeaLab, Faculty of Natural Sciences, Norwegian University of Science and Technology, Trondheim, Norway

ABSTRACT

Accidental crude oil spills to the marine environment cause dispersion of oil into the water column through the actions of breaking waves, a process that can be facilitated using chemical dispersants. Oil dispersions contain dispersed micron-sized oil droplets and dissolved oil components, and the toxicity of oil dispersions has been assumed to be associated primarily with the latter. However, most hydrophobic, bioaccumulative and toxic crude oil components are retained within the droplets which may interact with marine filter-feeders. We here summarize the findings of 15 years of research using a unique methodology to generate controlled concentrations and droplet size distributions of dispersed crude oil to study effects on the filter-feeding cold-water copepod *Calanus finmarchicus*. We focus primarily on the contribution of chemical dispersants and micron-sized oil droplets to uptake and toxicity of oil compounds. Oil dispersion exposures cause PAH uptake and oil droplet accumulation on copepod body surfaces and inside their gastrointestinal tract, and exposures to high exposure (mg/L range) reduce feeding activity, causes reproductive impairments and mortality. These effects were slightly higher in the presence of chemical dispersants, possibly due to higher filtration of chemically dispersed droplets. For *C. finmarchicus*, dispersions containing oil droplets caused more severe toxic effects than filtered dispersions, thus, oil droplets contribute to the observed toxicity. The methodology for generating crude oil dispersion is a valuable tool to isolate impacts of crude oil microdroplets and can facilitate future research on oil dispersion toxicity and produce data to improve oil spill models.

Introduction

Crude oil is an extremely complex mixture of hydrocarbons and heterocyclic compounds with large variations in hydrophobicity, molecular weight and solubility (Booth et al. 2007; Hughey, Rodgers, and Marshall 2002; Nelson et al. 2019; Sutton, Lewis, and Rowland 2005). When spilled in the marine environment, the spilled crude oil goes through physical, chemical, and biological processes, referred to as weathering process, which continuously change the composition and properties of the oil. One of the most important weathering processes to oil spilled in the marine environment is dispersion. This process, which occurs naturally due to turbulence generated by waves, breaks up surface oil into oil droplets which are transported into the water column. Large droplets will resurface and coalesce forming thin oil films, while smaller droplets in the micron-range will have lower resurfacing velocity and will thus passively drift in the water column. Dispersion increases the surface-to-volume ratio of the oil and facilitates dissolution and degradation (Daling et al. 2014; NRC 2005; Tarr et al. 2016). To enhance this process chemical dispersants are sometimes included as an emergency response action to facilitate the breakup of the oil into small droplets. The dispersant acts as a surfactant lowering the interfacial tension between oil and water and thereby reducing the energy (turbulence) needed to disperse the oil into the water column (NRC 2005). During the Deepwater Horizon spill, dispersants were injected into the release point at the sea floor to disperse the released crude oil and thereby reduce the amount of surfacing oil (Kujawinski et al. 2011). Sensible use of chemical dispersants may reduce the overall environmental impacts of accidental oil spills (Lessard and DeMarco 2000).

CONTACT Bjørn Henrik Hansen bjornhenrik.hansen@sintef.no SINTEF Ocean, Climate and Environment, Postboks 4762 Torgarden, Trondheim 7465, Norway

For marine animals, spilled oil may be toxic, but this depends on the exposure to bioavailable fractions of toxic crude oil compounds in the environment. It has generally been assumed that dissolved compounds represent the main bioavailable and thereby most toxic fraction of crude oil components (Carls and Meador 2009; Carls et al. 2008; Olsvik et al. 2011). Polycyclic aromatic hydrocarbons (PAHs) are usually analyzed in water samples (and sometimes in biota) from experiments studying crude oil toxicity because they are resolvable using gas chromatography (GC). They are toxic to marine organisms, but they only represent a small fraction of the total concentration of compounds found in crude oils, and the toxic potential for the non-PAH fraction is rarely considered (Meador and Nahrgang 2019). All bioavailable crude oil compounds will contribute to overall toxicity (Booth et al. 2007; Meador and Nahrgang 2019; Melbye et al. 2009; Sørensen et al. 2019). The concentration of a crude oil compound (such as a PAH) dissolved in the water depends on its molar fraction in the crude oil, the oil to water loading, and its solubility. The equilibrium distribution of a chemical between water and organic phases (e.g., body lipid or crude oil) is closely related to its hydrophobicity, and a widely used proxy for hydrophobicity is the octanol-water partitioning coefficient (K_{ow}). For PAHs, the logarithmic value of this coefficient ($LogK_{ow}$) ranges ~ 3–7, thus the hydrophobicity and solubility vary significantly, and a water-soluble fraction (WSF) of crude oils will contain more of less hydrophobic PAHs (lower $LogK_{ow}$). Acute toxicity (narcosis) increases with $LogK_{ow}$ of organic compounds, like PAHs (Di Toro and McGrath 2000; Hendriks et al. 2001).

Accurate recreations of crude oil spill scenarios are impossible in the laboratory, and attempts at basing risk assessment on acute toxicity tests run in a laboratory should be done with caution. Toxicity threshold values (e.g., EC_{50}) from laboratory studies may display large variations for different oils, and even for the same oil type, and such variations are often caused by different methodologies used to prepare exposure solutions (Hodson, Adams, and Brown 2019; Redman and Parkerton 2015; Sandoval, Ding, and Gardinali 2017). Preparations of water accommodated fractions (WAF) is the most common practice used to generate crude oil exposure media to conduct toxicity studies to generate toxicity thresholds for crude oils (Singer et al. 2000). Depending on the energy used during preparation, the viscosity of the oil, and whether a chemical dispersant has been added, the resulting solution will contain dispersed oil droplets of varying size distributions. Depending on the droplet size distribution, the residence time prior to use, and the exposure time utilized, droplets will settle toward the surface (Nordtug and Hansen 2021; Sandoval, Ding, and Gardinali 2017). After testing, the toxicity is most often expressed based on the sum of total PAHs (T-PAH) measured in the exposure solution. If the solution contains droplets, and they are not contributing to toxicity, reporting toxicity on a T-PAH basis will overestimate the concentration of the bioavailable fraction and at the same time underestimate the toxicity threshold (Nordtug and Hansen 2021; Parkerton et al. 2023). However, if the droplets are in fact contributing to toxicity, their presence needs to be measured, defined, and accounted for when generating toxicity thresholds. This is particularly challenging for static experimental systems where the presence of droplets in the water will be declining over time due to droplet surfacing. In such static systems, oil droplets may be a source for compounds dissolving to the aqueous phase which may thereafter be depleted through uptake into test organisms (Parkerton et al. 2023; Sandoval, Ding, and Gardinali 2017). Furthermore, determination of toxicity thresholds using summed T-PAH concentrations assumes equal toxic potential (EC_{50}) for all individual PAHs measured. This is incorrect as this procedure ignores the fact that different PAHs have a wide range in toxicological potentials, and, as mentioned above, that there are thousands of other oil compounds that contribute to toxicity, not only PAHs (Booth et al. 2007; Meador and Nahrgang 2019; Melbye et al. 2009). Nevertheless, chemical verification of experiments is a necessity, and PAH compositions are in most cases reported.

Some papers have discussed oil droplets' contribution to toxicity, and their findings vary. Carls et al. (2008) showed that the presence of oil droplets were not necessary to induce embryotoxicity in zebrafish (*Danio rerio*) during WAF exposure. Others have also reported neglectable contribution from oil droplets to PAH bioaccumulation and

toxicity (Nordtug et al. 2011a; Viaene et al. 2013, 2014), whereas other studies suggest that dispersed oil droplets is the primary sources of toxicity (Bobra et al. 1989; Sørhus et al. 2021), and that the toxicity of the oil droplets depends on droplet size (Bobra et al. 1989). Ramachandran et al. (2004) showed that the presence of droplets enhanced PAH bioavailability in chemically enhanced WAFs (CEWAF) compared to WAFs prepared without dispersant application. Such contradictory reports emphasize the importance of establishing laboratory procedures for crude oil toxicity testing where the presence of oil droplets in the exposure solutions is consistent in terms of droplet concentration and size distribution. Indeed, a series of papers have been published the last few years with a plea to improve and standardize test protocols to increase relevancy and comparability between tests (Coelho, Clark, and Aurand 2013; Hodson, Adams, and Brown 2019; Parkerton et al. 2023; Redman and Parkerton 2015). A promising technology, the oil droplet generator (ODG), was developed and published by Nordtug et al (2011a). Using the ODG, oil dispersions can continuously be prepared with and without preincubation of the oil with dispersant, to produce oil dispersions containing oil droplets with defined and reproducible concentration and size distribution. Furthermore, by separating the dispersion in two, where one half is filtered and the other half unfiltered, and thereby exposing organisms to unfiltered and filtered dispersions in parallel setups, the contribution of oil droplets to toxicity can be isolated and addressed. This paper provides and overview of studies conducted over the past 15 years using the ODG technology to provide parameterized data on oil dispersion toxicity, focusing on the toxicological significance of oil droplets and chemical dispersants on the filter-feeding cold-water copepod *Calanus finmarchicus*.

Preparation of crude oil in seawater dispersions using the oil droplet generator

The purpose of developing the oil droplet generator (ODG) was to enable continuous production of defined oil dispersions in terms of oil droplet concentration and size distributions for crude and refined oils that were reproducible over time. The ODG design was based on theoretical considerations using the theory of droplet formation in turbulent water jets (Karabelas 1978; Martínez-Bazán, Montañés, and Lasheras 2002) as described in Nordtug et al. (2011a). This paper also describes the methodology in detail. Briefly, the custom-made ODG is a cylindrical device consisting of a series of chambers inter-connected with small nozzles, and water and oil is pumped into the first chamber through separate inlets. Water is pushed through the chambers by a water pump and oil is added using a syringe pump via a capillary in front of the first nozzle. The oil is subjected to repeated turbulence through the nozzles between each chamber which breaks up the oil into micron-sized droplets to produce a continuous flow of stable oil dispersions in terms of oil droplet concentration and size distributions for crude and refined oils. Differently weathered crude oils varying in physio-chemical properties (e.g., viscosities and weathering degrees) have been used successfully (Hansen et al. 2018; Nordtug et al. ,2011a), but highly viscous weathered field-collected oil emulsions are not dispersible using the ODG. Generation of chemically enhanced dispersion of oils can also be made in the same manner, by premixing the dispersant into the oil prior to generation of the dispersion. Identical dispersions (droplet size distributions and concentrations) can be prepared with and without dispersant to do side-by-side comparative toxicity testing of naturally and chemically dispersed oil where the only difference is the presence of the chemical dispersant (Hansen et al. 2012, 2019; Nordtug and Hansen 2021). Furthermore, to assess and isolate the contribution of oil droplets to toxicity, the dispersion(s) can be separated into two, where one half is filtered and the other half is not filtered. Then, side-by-side exposures to dispersions with and without oil droplets can be conducted in parallel setups. Dilution series of dispersions are prepared using several computer-controlled solenoid valves to accurately dilute a parent dispersion with various amounts of clean water before the (diluted) dispersions are fed into appropriate vessels for exposing marine organisms.

Oil droplets have a positive buoyancy and tend to adhere to surfaces, which is a problem when performing toxicity testing of oil

dispersions in small exposure vessels under static or semi-static conditions. Oil dispersions are generated by the ODG at a flow rate of typically 150–200 mL/min, being optimal for connections to flow-through systems. Although the exposure vessels regularly used in connection with the ODG are large in volume (minimum 5 L for all toxicity tests performed on copepods) and they have flow-through, serious considerations have been made to the design of whole exposure vessel system. To reduce immediate surfacing of oil droplets and to keep homogenous dispersions in the exposure vessels, a balance between turbulence and droplet size distribution must be obtained. Adhesion of oil droplets to wetted surfaces can be minimized by the system design, choice of materials and by reducing volume-to-surface ratios (Nordtug et al. 2011a). The oil droplet mean volumetric size range used in most studies using the ODG has been 10–15 µm, but studies have also been done using mean droplet sizes up to 30 µm (Brakstad, Nordtug, and Throne-Holst 2015). Adjustment of concentrations and droplet sizes can be achieved by manipulating the oil and/or water flow through the generator or by adding chemical dispersants to the oil prior to generation of the dispersion (Nordtug and Hansen 2021; Nordtug et al. 2011a).

Compared to conventional methods for generating oil-in-water dispersions (e.g., WAF systems), the ODG system can produce dispersions of consistent and defined concentrations and droplet size distributions over time. This system thus has a better potential for performing experiments that can enable research on the contribution of droplets to oil dispersion toxicity, and, since exposure concentrations are stable over time, can produce more reliable toxicity thresholds for crude oil dispersions to marine organisms to be used in oil spill models.

The ODG has been used for assessing toxicity of crude oil dispersions on the filter-feeding copepod *Calanus finmarchicus* which is a key ecological species in the Northern Atlantic Ocean and Barents Sea. A summary of the experiments, including details about the treatment, measured water chemistry and toxicologically significant endpoints measured are given in Table 1.

Partitioning of PAHs between crude oil microdroplets and seawater

According to the principles of Raoult´s law (Guggenheim 1937), the equilibrium partitioning of a chemical compound between oil and water is determined by the product of the (supercooled) solubility in water and its molar concentration in the oil phase (Cline, Delfino, and Rao 1991; Lee et al. 1992; Lee, Rao, and Okuda 1992). This implies that during dilution of oil in water, the most soluble components will initially be depleted in the oil due to dissolution. Components with low solubility will to a larger extent be retained in the oil. The result is that the water concentration of the most soluble components may decline almost linearly with the dilution factor whereas the less soluble substances will remain at almost at the same (low) concentrations over a large range of dilutions. Under the assumption that the oil components in micro-sized oil droplets equilibrate fast between the oil and water phases, the oil dispersion generated using the ODG can be filtered to produce an equilibrated water-soluble fraction (WSF). This WSF can be used in direct comparison to the unfiltered dispersion to isolate the contribution of oil droplets to dispersion toxicity (Nordtug et al. 2011b). PAH distribution between the oil and water phase has been characterized for most experiments performed using the ODG (e.g., Hansen et al. 2018), and an example is given in Figure 1a. The two curves illustrate that the components with low solubility (i.e., high K_{ow}) predominantly reside in the oil phase, and most components with high solubility (low K_{ow}) are dissolved in the water phase. At the highest dilution (0.7 mg oil/L. the curve is shifted to the right relative to the lowest dilution indicating a larger fraction of compounds with low solubility (high K_{ow}) in the water phase. Comparing identical crude oil dispersions (same droplet size distribution and concentration) with and without the addition of chemical dispersion demonstrated insignificant effects of the dispersant on the distribution of components between oil and water (Figure 1b) (Nordtug and Hansen 2021).

According to dilution theory, the concentration (and toxicity) of the WSF of a dispersion can only decrease during dilution. However, the reduction

TABLE 1. Overview of Experiments on *Calanus Finmarchicus* to Assess the Toxicity of Crude Oil Dispersions. N.R. = Not Reported

References	Animal state	Duration (days)	Oil type	Effects studied	Dispersant added?	Filtered?	Treat-ment acronym	Oil concen-tration (mg/L)	THC C9-C36 (µg/L)	TPAH (µg/L)	Significant effects (compared to controls)
(Hansen et al. 2009)	Copepodite 5/Adults	4	Naphthenic oil (Troll) washed	Mortality gene expression (GST, CYP330A1), oil droplet adhesion and filtration	No	No	D1	N.R.	N.R.	82.03	GST ↑ (lipid-rich and poor), CYP330A1 ↓ (lipid-poor), mortality ↑
					No	Yes	V1	N.R.	N.R.	19.86	GST ↑, CYP330A1 ↓ (lipid-rich and -poor)
					No	No	D2	N.R.	N.R.	53.37	GST ↑ (lipid-rich and poor), CYP330A1 ↓ (lipid-rich and -poor)
					No	Yes	V2	N.R.	N.R.	4.78	GST ↑ (lipid-rich and poor), CYP330A1A ↑ (lipid-poor), CYP330A1 ↓ (lipid-rich)
					No	No	D3	N.R.	N.R.	29.82	GST ↑ (lipid-rich), CYP330A1 ↓ (lipid-poor)
					No	Yes	V3	N.R.	N.R.	3.93	GST ↑ (lipid-rich and -poor), CYP330A1A ↑ (lipid-poor), CYP330A1 ↓ (lipid-rich)
					No	No	D4	N.R.	N.R.	14.28	GST ↑ (lipid-rich and -poor), CYP330A1 ↓ (lipid-rich and -poor)
					No	Yes	V4	N.R.	N.R.	2.99	GST ↑ (lipid-rich and -poor), CYP330A1 ↓ (lipid-rich)
					No	No	D5	N.R.	N.R.	5.88	CYP330A1 ↓ (lipid-rich and -poor)
					No	Yes	V5	N.R.	N.R.	1.46	CYP330A1A ↑ (lipid-poor), CYP330A1 ↓ (lipid-rich)
(Hansen et al. 2012)	Copepodite 5	4	Naphthenic oil (Troll) 200+ residue	Mortality, algae filtration, algae and oil droplet filtration and excreion	No	No	NDL	0.15	35.7	0.34	Algae filtration ↓
					Yes	No	CDL	0.16	45.4	0.46	Algae filtration ↓
					No	No	NDM	0.81	276.1	5.91	Mortality ↑
					Yes	No	CDM	0.82	336.2	7.84	Mortality ↑
					No	No	NDH	4.05	2782.8	53.78	Mortality ↑, algae filtration ↓
					Yes	No	CDH	3.87	2834.4	60.38	Mortality ↑, algae filtration ↓
(Olsen et al. 2013)	Adults	5	Naphthenic oil (Troll) 200+ residue	Mortality, oil droplet adhesion and filtration, reproduction during recovery	No	No	Low	0.04	N.R.	0.42	
					No	No	Med	1.8	N.R.	55.97	Fecundity day 11 ↓
					No	No	High	16.5	N.R.	304.8	Nauplii prod. day 11 ↓, day 25 ↑, average ↑, Acc. Egg prod. Day 11–25 ↓, Reproducing females ↓, Mortality ↑
(Hansen et al. 2015; Nordtug et al. 2015)	Adults (16% lipid vol)	4	Naphthenic oil (Troll) 200+ residue	PAH body burden, mortality, reproductionduring recovery	No	No	MDL	0.23	266.7	3.91	PAH body burden ↑, algae filtration ↓
					Yes	No	CDL	0.2	176.9	2.75	PAH body burden ↑, algae filtration ↓
					No	No	MDM	1.01	886.9	16.22	PAH body burden ↑, algae filtration ↓
					Yes	No	CDM	0.95	693.8	13.27	PAH body burden ↑, algae filtration ↓, hatching success (day 16–25) ↓
					No	No	MDH	5.16	4346.6	78.05	PAH body burden ↑, algae filtration ↓, egg prod day 16–25 ↑
					Yes	No	CDH	5.44	4571.1	76.94	PAH body burden ↑, algae filtration ↓, egg prod day 1–25 ↓
(Hansen et al. 2017)	Adults	4	Naphthenic oil (Troll) 200+ residue	PAH body burden, oil droplet adhesion, reproduction, maternal PAH transfer, offspring gene expression	No	No	Disp.	1 (nominal)	677	20.39	PAH body burden ↑, maternal PAH transfer ↑, hatching success ↓, offspring gene expression: thioredoxin 3 ↑, xanthine dehydrogenase e ↑, phospholipase A2 act. prot. ↓
						Yes	WSF	0	47	15.13	PAH body burden ↑, offspring gene expression: HSP ↑, cyp2j ↑, thioredoxin 3 ↑, xanthine dehydrogenase ↑, phospholipase A2 act. prot. ↓

(*Continued*)

TABLE 1. (Continued).

References	Animal state	Duration (days)	Oil type	Effects studied	Dispersant added?	Filtered?	Treatment acronym	Oil concentration (mg/L)	THC C9-C36 (µg/L)	TPAH (µg/L)	Significant effects (compared to controls)
(Hansen et al. 2017, 2018)	Copepodite 5/Adults	4	Naphthenic (Troll) 150+ residue	PAH body burden, oil droplet adhesion, mortality, algae filtration and excretion, metabolomics	No	No	LD	0.08	73.2	0.37	PAH body burden ↑
						Yes	LW	0.04	40.6	1.28	PAH body burden ↑
						No	MD	0.69	551.3	8.52	PAH body burden ↑, fecal pellet production ↓, acetylcholine ↓, creatine ↓
						Yes	MW	0.03	47.9	6.42	PAH body burden ↑
						No	HD	4.12	2976.5	58.79	PAH body burden ↑, mortality ↑, fecal pellet production ↓, algae filtration ↓, homarine ↓, acetylcholine ↓
						Yes	HW	0.04	104.5	20.49	PAH body burden ↑, fecal pellet production ↓, algae filtration ↓, creatine ↓
			Waxy (Alvheim) 150+ residue	PAH body burden, oil droplet adhesion, mortality, algae filtration and excretion, metabolite profiling	No	No	LD	0.1	44.7	0.42	PAH body burden ↑, lactate ↓
						Yes	LW	0.04	83	0.31	PAH body burden ↑, homarine ↓, acetylcholine ↓, creatine ↓, lactate ↓
						No	MD	0.85	496.1	6.74	PAH body burden ↑, fecal pellet production ↓, lactate ↓
						Yes	MW	0.04	78.2	2.38	PAH body burden ↑, lactate ↓
						No	HD	5.63	3281.8	43.85	PAH body burden ↑, fecal pellet production ↓,algae filtration ↓, Mortality ↑, algae filtration ↓, lactate ↓
						Yes	HW	0.06	106.8	6.78	PAH body burden ↑, lactate ↓
(Hansen et al. 2018)	Copepodite 5/Adults	4	Paraffinic (MC252) 150+ residue	PAH body burden, mortality, algae and oil droplet adhesion and filtration	No	No	LD	0.05	33	0.18	PAH body burden ↑
						Yes	LW	0.04	149	0.25	PAH body burden ↑
						No	MD	1.17	726	11.52	PAH body burden ↑
						Yes	MW	0.05	99.8	4.56	PAH body burden ↑
						No	HD	6.99	6157	88.3	PAH body burden ↑, mortality ↑, algae filtration ↓
						Yes	HW	0.07	176.4	10.03	PAH body burden ↑. algae filtration ↓,

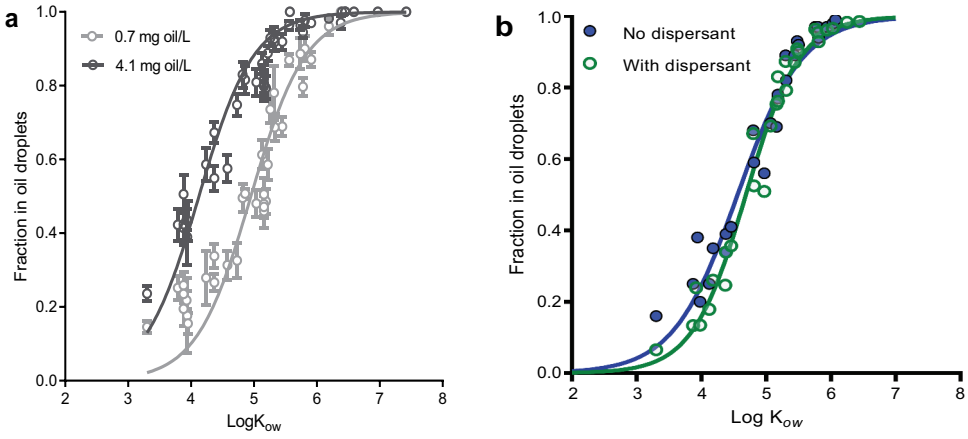

FIGURE 1. Fraction of individual PAH's retained in the oil phase (droplets) of crude oil dispersions in sea water as a function of the Log K_{ow} of the PAHs. A: at different dispersion concentrations, i.e., at oil loadings of 0.7 (grey) and 4.1 (black) mg oil/L seawater. B: for dispersions generated with (green) and without (blue) addition of chemical dispersant (4% Dasic NS) at identical oil loading (20 mg/L). Solid lines generated by a three-parameter non-linear dose-response (min/max restrictions: 0–1). Data replotted for a naphthenic crude oil from Hansen et al. (2018) (A) and Nordtug and Hansen (2021) (B).

in toxicity is less than the dilution factor because the dilutions contain a larger fraction of hydrophobic oil components which have a higher potential for bioconcentration and higher acute toxicity per mass unit than less hydrophobic compounds.

The non-linear dissolution of oil compounds at different oil-to-water ratios has some important implications for toxicity testing. When using conventional testing of toxicity for dispersions or WSF ´s of different oil loadings to generate toxicity thresholds like effect concentrations (EC_{50}) or lethal concentration (LC_{50}), the WSF at low oil concentrations may appear more toxic (lower EC/LC_{50}) than higher concentrations. Assuming that the toxicity is mainly caused by dissolved components, this reflects that the specific toxicity (per mol or weight unit) of the quantified components is higher in the diluted dispersion. This is simply an indication that the more soluble and less toxic components have been depleted and not an indication that the exposure medium has become more toxic. Mass specific toxicity thresholds (LC/EC_{50}) do not account for the loss of mass during the dilution process. The actual toxicity of a water volume can be estimated by calculating the toxic unit (TU) which is the total water concentration divided by a defined effect concentration (for instance LC_{50}) for individual compounds measured (Di Toro, McGrath, and Stubblefield 2007). The TU thus indicates how much the solution must be diluted (or concentrated) to cause a defined effect. However, for oil dispersions estimating TUs are not straightforward due to the problems of determining the biological relevant concentration which may be the toxicity of the dissolved fraction (WSF) plus a potential contribution of the oil droplets (Redman et al. 2012).

Partitioning of PAHs between crude oil dispersions and copepods

Interactions between oil droplets and biota may occur through direct fouling of an organism (Sørhus et al. 2015) or through ingestion or filtration of oil droplets from the water (Lee, Koster, and Paffenhofer 2012; Conover 1971; Gyllenberg 1981; Rodrigo et al. 2014). To investigate the partitioning of PAHs between crude oil dispersions and biomass, Hansen et al. (2018) exposed *C. finmarchicus* to dispersions (with droplets) and filtered dispersions (WSF, without droplets) for 96 hours (Figure 2). Experiments were conducted by exposing the copepods in a flow-through system with three dispersion concentrations containing oil droplets with a mean spheric size of 10–14 µm and their corresponding in-line filtered WSFs (Hansen et al. 2018; Nordtug et al. 2011a). Three oils, classified as paraffinic, naphthenic, and waxy (Daling et al. 1990), respectively, were tested at comparable oil loadings and oil droplet sizes. Further details are given in Table 1. Figure 2 shows the bioconcentration factors (for WSFs) and accumulation factors

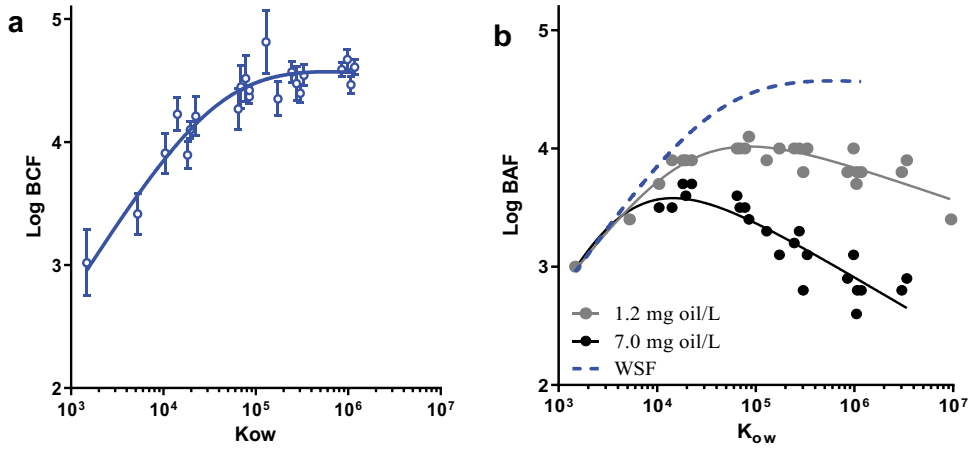

FIGURE 2. Partitioning of PAH between water and biomass for WSFs (A) and dispersions (B) where the copepod *C. finmarchicus* was exposed to WSF (at different loadings, average ± STDEV) (A) or oil dispersions (B) of a paraffinic crude oil for 4 days and analyzed for PAH body burden. Bioconcentration/accumulation factors for PAHs calculated as the ratio between body burden (cb) and water concentration (cw) are plotted as a function of the Log K_{ow} of the PAHs. A: for water soluble fraction of a (filtered) dispersion (data from different oil loadings and oil types, mean values for $n = 6$). B: for dispersions at two different oil-to-water loadings (1.2 (grey) and 7.0 (black) mg crude oil/L). The WSF line from a is inserted into the figure for comparison. Curves are fitted by a bilinear model (Kubinyi 1977), and data and figures are adapted from Hansen et al. (2018).

(AFs for dispersions) of PAHs as the concentration measured in the biomass relative to that of the water phase. The curve showing partitioning between WSFs and copepods (Figure 2a) is based on WSFs filtered from dispersions with oil loadings in the range between 0.5 and 7 mg/L from all three oils (150 data points). The lipid solubility, i. e. hydrophobicity, of a component has traditionally been related to its octanol/water partition coefficient (K_{ow}) with the assumption that octanol is a suitable proxy for lipids in organisms which would display a linear relationship between Log bioconcentration factor and Log K_{ow}. It is evident from Figure 2a, and from theoretical and empirical studies (e.g., Connell and Hawker 1988; Seto and Handoh 2009), that the most hydrophobic PAHs deviates from linearity. This may be related to insufficient time (4-day experiments were conducted) to attain steady state concentrations for the most hydrophobic oil compounds, or it may be related to steric hindrance of larger molecules that may severely hamper their diffusion through biological membranes resulting in reduced uptake rates and long equilibrium times (Øverjordet et al. 2018; Sujit and Baughman 1991). Limited duration of the exposure may therefore underestimate the potential bioaccumulation of these components.

When comparing the accumulation factors from dispersions with the corresponding BCFs from WSFs (Figure 2b) it is apparent that the trajectories of the accumulation factors deviate from BCFs of the WSF above approximately $10^{3.5}$ and $10^{4.2}$ for the high and medium dispersion, respectively. According to Figure 1 this corresponds to the lower K_{ow} limits of the range where about 25–100% of the individual PAHs are retained in the oil droplets. PAHs contained in oil droplets in the water phase will contribute to the analytical concentrations of the exposure solutions and reduce the apparent accumulation factor for the biomass. Contrary to this, the oil droplet associated with the biomass, whether they are associated with the copepod surface or consumed/filtered, will contribute to a higher accumulation factor.

In contrast to the reduced accumulation factors compared to the WSF exposure, the corresponding total PAH concentration associated with the copepods was higher by a factor of 1.6 (medium loading) and 2.6 (high loading) in the dispersion (data from Hansen et al. 2018). This increase in body residue is partly related to oil droplets associated with the biomass (Figure 3), but may also to some extent be caused by increased tissue uptake due to direct contact with the oil droplets, as shown for fish embryos (Sørensen et al. 2019; Sørhus et al. 2021). According to Figure 1, PAHs in the K_{ow} range 10^6 to 10^7 will initially almost exclusive be found in the oil phase and may therefore

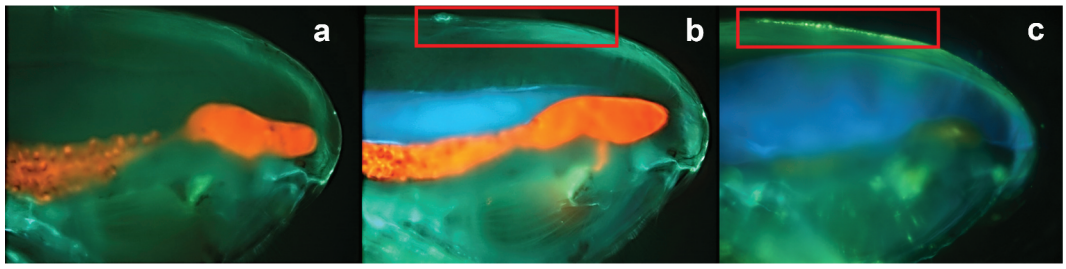

FIGURE 3. Side view of copepods (*Calanus finmarchicus*) imaged using fluorescence microscopy. A: image of an unexposed (control) copepod: B: copepod exposed to water-soluble fraction of crude oil (filtered dispersion, 5 mg crude oil/L). C: copepod exposed to oil dispersion (5 mg crude oil/L). The red square in B and C shows the areas where oil droplets on the copepod prosome surface are visible as yellow dots. This is not visible in the WSF-exposed copepod. Ingested food (algae) is visible as red fluorescence in the intestines of control (A) and WSF-exposed copepod (B), but not in the dispersion-exposed copepod (C). Images taken by Dag Altin (BioTrix) for experiments described previously (Hansen et al. 2018).

potentially be used as a proxy for the partitioning of oil droplets between water and biota (Nordtug et al. 2011b, 2015). This, however, assumes that these PAHs are far from an equilibrium where their concentration in the body lipid according to chemical partitioning theory is expected to be almost equal to that of the oil. Modelling of uptake from oil WSFs based on 45 days uptake/depuration study with stage C5 *Calanus hyperboreus* (average weight 12,5 mg and 28% lipid content) estimated the time to reach 50% of the equilibrium concentration ($T_{1/2)}$ for uptake of 6 PAHs with K_{ow} above $10^{5.7}$ to be on average 40,1 (±6.6) days (Øverjordet et al. 2018). The corresponding $T_{1/2}$ for the C3 stage (average weight 0,5 mg and 5% lipid content) was 2.4 (±0.4) days. The large differences in uptake kinetics illustrate one of the challenges in determining the relative contribution of oil droplets and uptake from dissolved components in short exposure studies.

Clearly, oil droplets interact with copepods during exposure, as body burden analysis of hydrophobic PAHs, associated primarily with oil droplets, are measured in exposed copepods (Figure 2). Fluorescence microscopy imaging validated oil droplet presence on copepod surfaces (Figure 3). However, oil droplet accumulation is reduced as a function of concentration, suggesting reduction in oil droplet filtration at high concentrations (Nordtug et al. 2015). Furthermore, filtration of oil droplets appears to be somewhat higher during application of chemical dispersant to disperse the crude oil (Nordtug et al. 2015).

Toxicity of crude oil dispersions

Oil exposure may cause toxic effects to marine biota, from microbial communities (Harayama, Kasai, and Hara 2004) to large sea mammals (Rainer Engelhardt 1983). Experiments using ODG-generated crude oil dispersions for toxicity testing have been conducted primarily using cold-water marine copepods, which are summarized in this review) and early life stages of marine fish (e.g. Olsvik et al. 2011; Sørhus et al. 2015).

Exposure of the copepod *C. finmarchicus* to crude oil dispersions caused impaired feeding behavior (algae filtration), development, reproductive effects and mortality. Being a highly efficient filter-feeder (Meyer et al. 2002), this copepod species has been shown to filter, ingest and egest oil droplet (Hansen et al. 2009, 2017; Olsen et al. 2013). Dispersion exposure (0.1–5.6 mg oil/L, 4 days) caused a concentration-dependent reduction in feeding activity, measured as algae uptake, gut filling (examples visualized by fluorescence microscopy in Figure 3) and fecal pellet production (Hansen et al. 2017). Two different crude oils were tested, and reduced feeding activity was comparable for both. Compared to starved controls, copepods fed algae and exposed to oil dispersions simultaneously displayed similar starvation-type responses such as reduced metabolites (homarine, acetylcholine, creatine and lactate) (Hansen et al. 2017). Acute toxicity, measured as mortality after four days exposure, was high (>75%) after exposure to 2.7–2.8 mg oil/L (53.8–60.4 µg/L TPAH), and an estimated LC_{50} based on TPAH was 16.1 (13.5–19.1) µg/L for mechanically dispersed oil and

slightly lower for chemically dispersed oil (10 (8.4–12.8) µg/L) (Hansen et al. 2012). Interestingly, in a different study, exposure to oil dispersions up to 5 mg/L oil (78 µg TPAH/L) caused no significant differences in mortality compared to control (Hansen et al. 2015). A difference between these two studies were that in the first study (Hansen et al. 2012), copepods were lean due to being fed lower amounts of algae prior to the exposure, whereas in the second study (Hansen et al. 2015), the copepods were fed well and therefore lipid-rich at onset of the experiment. In both experiments, copepods were fed algae during exposure. Thus, pre-exposure feeding conditions and lipid content of exposed copepods are important aspects to consider when conducting toxicity testing of these copepods. Studies also show that lipid-rich copepods survive longer than lipid-poor copepods during exposures to WAFs of crude and refined oils (Hansen et al. 2011, 2013). These observations suggest that low lipid status caused higher oil sensitivity and/or that higher lipid content somehow protects against acute toxicity of oil exposure. A modeling approach to identify possible toxicokinetics differences between lipid-rich and lipid-poor copepods showed that although a large lipid sac might retard toxicokinetics, the differences in lipid volumes could not completely explain differences in toxicity (Hansen et al. 2016). In a different study, exposing stage copepodite 3 (C3) and C5 of *Calanus hyperboreus* to WSFs of crude oil, significant differences were found between the two stages. At stage C3 (with very low lipid sac), the animals were significantly more sensitive to acute exposure than C5 (with large lipid sacs) (Øverjordet et al. 2018), and comparable to what was observed in *C. finmarchicus* (Jager et al. 2016). Of course, some of the differences may be attributed to stage-specific differences observed in *C. hyperboreus*, e.g., intrinsic sensitivity, body size and swimming activity, but lipid-rich copepods were shown to have lower elimination rates, thus reaching steady state more slowly, a process that might cause a delay in the onset of toxic effects (Øverjordet et al. 2018). A long retention time for PAHs in lipid storage of late copepodite stage (C5) could have implications during diapause and gonad maturation when the lipid store is utilized for maintenance and reproduction, respectively. This could involve maternal PAH transfer into eggs and subsequent impacts on offspring (Hansen et al. 2017; Toxværd et al. 2019). Reproductive effects were observed in a concentration-dependent manner for *C. finmarchicus* females exposed for four days to oil dispersions ranging 1.8–16.5 mg oil/L (Olsen et al. 2013). Significant reduction in reproduction rates shortly after dispersion exposure were, however, compensated by higher reproduction rates after 14 days recovery suggesting that although short-term exposure can temporarily suspend reproduction, copepods may recover and still produce viable offspring after recovery in clean water (Olsen et al. 2013). Similar observations were observed in a later study, where *C. finmarchicus* were exposed to oil dispersions in the range 1.0–5.4 mg oil/L for four days exhibited delayed initiation of reproduction. These also displayed compensatory reproduction during the last 10 days of a 25-day recovery period reaching control level fecundity (Hansen et al. 2015).

Exposure of *C. finmarchicus* to oil dispersions causes acute toxicity (mortality), reduction in feeding and reproduction, however, such effects occur at relatively high dispersion concentrations (in mg/L range), and for impacts on feeding and reproduction, compensatory mechanisms have been displayed during recovery.

Contribution of chemical dispersants to crude oil dispersion toxicity

The use of chemical dispersants can in some cases be an effective spill response option to prevent oil from reaching shorelines as these chemicals facilitate dispersion of spilled oil to the water column. However, by preventing surfacing of the spilled oil using dispersants inevitably increases the risk of exposure of pelagic biota. Dispersants alone display low acute toxicity compared to the oil itself, with 4-day LC_{50} values above 10 mg/L reported for the chemical dispersants Dasic NS, Corexit 9500A and Gamlen OD4000 (Hansen et al. 2014).

To compare the aquatic toxicity of oil with and without dispersant application, several studies have utilized CROSERF-methodology and compared conventional WAFs generated with and without dispersant application (Singer et al. 2000; Gardiner et al. 2013; Ramachandran et al. 2004).

This method is based on stirring a water volume with a defined amount of oil added on top in a closed bottle for a defined time and collecting the water phase after a settling period. When using the same stirring velocity to generate WAFs with dispersant application (chemically enhanced WAF; CEWAF), the solution will inevitably contain a much higher proportion of microdroplets that, due to their low surfacing velocity, tend to reside in the water phase. Thus, despite using the same oil to water loading, the droplet concentrations and droplet size distributions in the compared WAF and CEWAF will be different and will vary over time (Sandoval, Ding, and Gardinali 2017). Making conclusions on the contribution of chemical dispersants to dispersion toxicity using such experimental designs needs to be done with caution as both the physical and chemical properties as well as temporal changes of the two solutions are different. Comparing the toxicity of the two solutions reveals the effects related to the different physical properties of the solutions, but provides little or no information about the potential toxicity of the dispersant itself. Using the ODG system, however, it is possible to generate oil dispersions that are comparable in terms of oil loading and droplet size range with and without the application of a chemical dispersant (Hansen et al. 2012, 2016; Olsvik et al. 2012). Figure 1b shows the comparable distribution of PAHs between oil and water for dispersions generated with a loading 20 mg oil/L with and without the use of dispersant when the ODG has been used. Thus, the two dispersions are identical except for the presence of the dispersant, and potential differences in toxicity can be assigned to the dispersant.

In exposure, experiments with *C. finmarchicus* using the ODG to generate dispersions, comparable oil dispersions (similar oil droplet concentrations and size ranges) were used with dispersants (chemically dispersed – CD) and without dispersants (mechanically dispersed – MD) to isolate the potential impact of chemical dispersant to crude oil dispersion toxicity (Hansen et al. 2012, 2015; Nordtug et al. 2015). For *C. finmarchicus*, acute 4-day-exposure to mechanically dispersed (MD) and chemically dispersed (CD, with 4% Dasic) oils provided a slightly lower (1.6 fold) LC_{50} for CD compared to MD suggesting a slight contribution of dispersant to oil dispersion toxicity (Hansen et al. 2012). Reduced feeding was also observed as a function of dispersion concentration, but not significantly different between MD and CD (Hansen et al. 2012). In a follow-up study, Nordtug et al (2015), studied filtration rates in *C. finmarchicus* exposed to three concentrations of oil dispersions ranging from 0.25 to 5.6 mg/L with a constant supply of microalgae for a period of 4 d. Filtration rates, as well as accumulation of oil droplets, decreased with increasing exposure concentration, thus resulting in higher amount of oil associated with the copepod biomass for the two lowest exposures compared to the highest exposure. Furthermore, exposure to the two lowest concentrations resulted in higher oil uptake in CD compared to MD. After exposure, reproductive output was monitored for 25 days in pre-exposed copepods, and although lower initial production of eggs/nauplii for both MD and CD exposures was observed for the two highest concentrations, copepods exposed to MD exhibited compensatory reproduction during the last 10 days of the recovery period, and the cumulative egg and nauplii production reached control levels at the end of the 25-day reproduction period. Interestingly, the copepods exposed to CD did not display this compensatory effect and never reached the same cumulative egg/nauplii production as the MD-treated copepods (Hansen et al. 2015).

Studies comparing toxicity of mechanically and chemically dispersed crude oil have shown a dispersant-dependent increase in acute toxicity and reproductive toxicity (at high oil loadings), which may be attributed to higher oil droplet filtration during exposure to oil dispersions containing dispersant.

Contribution of oil droplets to crude oil dispersion toxicity

Using the ODG, parallel filtered and unfiltered dispersions were used to assess the contribution of oil droplets to dispersion toxicity in four separate studies on copepods (Hansen et al. 2009, 2017, 2017, 2018). Expression of glutathione S-transferase (GST) and cytochrome P450 330A1 following exposure of lipid-poor and lipid-rich copepods to an artificially weathered naphthenic crude oil, suggested that that the lipid-content of the copepods was more important for transcription

of these genes than the presence of droplets (Hansen et al. 2009). While GST expression was comparable with and without the presence of droplets regardless of copepod lipid content, CYP330A1 exhibited opposite concentration-dependent responses for lipid-rich and lipid-poor copepods in the presence of oil droplets, i.e., upregulation in lipid-poor copepods and downregulation in lipid-rich copepods. While GST is involved in the xenobiotic biotransformation processes (Roncalli et al. 2015), the CYP330A1 gene, being induced by ecdysone in shore crab (*Carcinus maenas*) (Rewitz, Styrishave, and Andersen 2003), probably has a function in the molting process (Hansen et al. 2008). Somewhat higher mortality was observed in the highest dispersion concentration compared to the corresponding WSF, which may have been caused by adhesion of oil droplets to filtering apparatus and other copepod surfaces (Hansen et al. 2009). Although cold-water copepods like *C. finmarchicus* can survive prolonged periods without food, e.g., during diapause (Hirche 1996), even short-term (days) food deprivation affects their GST gene expression (Soloperto et al. 2022), metabolism (Hansen et al. 2017; Helland et al. 2003), development (Campbell et al. 2001) and reproductive potential (Niehoff 2000). Exposure to parallel treatments of dispersions (ranging 0.08–5.6 mg oil/L) and corresponding WSFs showed a clear concentration-dependent effect of the presence of droplets for algae filtration, gut filling and fecal pellet production, and impacts on food consumption was observed at dispersion concentration as low as 0.08 mg oil/L (Hansen et al. 2017). These effects were only observed in the presence of droplets, i.e., not observed for filtered dispersions (WSFs). The same study also included a non-feed starved control, and comparable effects on gut filling were observed between starved copepods and copepods subjected to 0.7–0.8 mg oil/L (see examples in Figure 3). Using metabolomics to assess molecular impacts of oil exposure, verified a starvation-like response (loss of metabolites and free amino acids) in the presence of oil droplets. These effects were shown for two different oil types, suggesting the responses not to be oil type specific (Hansen et al. 2017) which is in line with observations for PAH partitioning between water and copepods (Hansen et al. 2018).

The presence of oil droplets on feeding appendages and inside the gastrointestinal tract may facilitate uptake of oil components into tissues, and subsequent partitioning into organs rich in lipids like the lipid sac and gonads, but the long-term effect of such accumulation is unknown. Pyrene exposure during simulated overwintering conditions caused reduced survival, reproductive output and strong delayed effects on grazing rate and lipid accumulation in *C. hyperboreus* (Toxværd et al. 2019). In an exposure experiment where *C. finmarchicus* females were exposed to an oil dispersion (1 mg oil/L) and the corresponding WSF for four days followed by recovery for 8 days, hatching of eggs were significantly delayed. 48 h after eggs were laid, 94% of controls were hatched, whereas for eggs from mothers exposed to oil dispersion only 86% were hatched. After 96 h, there were, however, no significant differences between controls and the dispersion treatment. Nauplii of mothers from the three treatments (control, dispersion and WSF) were subjected to RNA extraction and transcriptomics analyses, revealing differentially expressed genes through pairwise comparisons between treatments. Some of the differentially expressed genes have known roles in responses to chemical stress including xenobiotic metabolism enzymes, antioxidants, chaperones, and components of the inflammatory response. This suggests that maternal transfer of oil compounds cause transgenerational effects. However, the impact of droplets were somewhat unclear, and a relatively small number of DEGs suggested a minor long-term effect on offspring following maternal exposure (Hansen et al. 2017).

In summary, the studies focusing on isolating potential contribution of oil droplets to toxicity states clearly that the oil droplets are interacting with copepods through adhesion and filtration causing an increase in mortality, altered gene expression, reduction in feeding and show potential for reproductive effects and maternal transfer of oil compounds. As mentioned above, dispersion concentrations causing toxic effects in copepods are high compared to observations in cold-water marine fish eggs where presence of oil droplets appear to be a key driver for embryotoxicity (Hansen, Sørhus, and Nordtug 2023; Sørhus et al. 2015, 2021).

Concluding remarks and suggestions for future research

The ODG technology is capable of generating oil-in-water dispersions of controlled oil concentrations and droplet size distributions for use in flow-through exposure experiments. Comparable dispersions can be made with and without dispersants, making direct comparisons between mechanically and chemically dispersed oil possible, thus isolating the potentially added contribution of a dispersant to dispersion toxicity. The methodology can also be used to isolate the contribution of oil droplets to uptake and toxicity by preparing parallel dispersions with inline filtration (and dilution) steps. In total, the ODG has proven a promising alternative to conventional methods to assess crude oil toxicity (Parkerton et al. 2023).

In this mini review, we have summarized the findings from studies using the ODG to assess bioavailability and toxicity of crude oil dispersions on the filter-feeding copepod *Calanus finmarchicus* which is a key species in the ecosystems of the Northern Atlantic Ocean and Barents Sea and a representative model species for marine filter-feeding organisms.

Clearly, oil droplets interact with copepods during exposure, as evidenced through uptake of hydrophobic PAHs and fluorescence microscopy imaging validating presence of oil droplets on copepod surfaces and within their gastrointestinal tract. However, oil droplet accumulation is reduced as a function of concentration, suggesting a reduction in oil droplet filtration at high concentrations. Reduced algae filtration was also clearly shown with increasing dispersion exposure. Furthermore, accumulation of oil droplets appears to be somewhat higher during application of chemical dispersant, which may be the reason for increased mortality during exposure to chemically dispersed oil than mechanically dispersed oil. This may also be the reason why compensatory reproduction was only observed after exposure to mechanically dispersed crude oil. As dispersants alone have very low toxicity (>10 mg/L) in this species, and dispersant to oil ratios used were low (1:25), the added toxicity observed in chemically dispersed oil (compared to mechanically dispersed oil) are probably attributed to indirect effects of dispersants (increased filterability of chemically dispersed microdroplets of crude oil) rather than direct effects. Importantly, however, toxic effects were observed at high dispersions concentrations (mg/L range), suggesting that *C. finmarchicus* is less sensitive to exposure than fish eggs, which has been shown to be affected at oil concentrations in the µg/L range. Limited toxicity was observed for filtered dispersions (WSFs) compared to dispersions containing oil droplets clearly showing that presence of oil droplets is contributing to the toxic responses (increased mortality, altered gene expression and reduction in feeding), and that droplet exposure is also necessary to cause reproductive effects and maternal transfer of oil compounds within the dispersion concentrations used in the published experiments.

The contribution of oil droplets on dispersion toxicity displayed from our studies on *C. finmarchicus* are important on a general basis for filter-feeding organisms in the marine environment. As our studies have focused exclusively on late life stages of copepods, future work should focus on earlier life stages which may show higher sensitivity (Jager et al. 2016). As exposure concentrations used in our studies are in the high end of field measurements during acute oil spills, lower exposure concentrations should also be considered in future studies. Other filter-feeding organisms should also be considered for comparison. The same applies for early life stages of cold-water marine fish which not only have displayed high sensitivity to dispersion exposure, but also droplet-driven dispersion toxicity (Hansen, Sørhus, and Nordtug 2023; Sørhus et al. 2021). The methodology for generating dispersion described and used in the cited publications is a valuable tool to isolate impacts of crude oil microdroplets and/or chemical dispersants on marine organisms. Data from these and further studies will facilitate a better understanding of oil toxicity and produce data to improve future oil spill models used to assist environmental risk assessment, oil spill contingency planning, net environmental benefit analyses and natural resource damage assessments.

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References

Bobra, A. M., W. Ying Shiu, D. Mackay, and R. H. Goodman. 1989. "Acute Toxicity of Dispersed Fresh and Weathered Crude Oil and Dispersants to Daphnia Magna." *Chemosphere* 19 (8–9): 1199–1222. https://doi.org/10.1016/0045-6535(89)90068-4.

Booth, A. M., P. A. Sutton, C. A. Lewis, A. C. Lewis, A. Scarlett, W. Chau, J. Widdows, and S. J. Rowland. 2007. "Unresolved Complex Mixtures of Aromatic Hydrocarbons: Thousands of Overlooked Persistent, Bioaccumulative, and Toxic Contaminants in Mussels." *Environmental Science & Technology* 41 (2): 457–464. https://doi.org/10.1021/es0615829.

Brakstad, O. G., T. Nordtug, and M. Throne-Holst. 2015. "Biodegradation of Dispersed Macondo Oil in Seawater at Low Temperature and Different Oil Droplet Sizes." *Marine Pollution Bulletin* 93 (1–2): 144–152.

Campbell, R. G., M. M. Wagner, G. J. Teegarden, C. A. Boudreau, and E. G. Durbin. 2001. "Growth and Development Rates of the Copepod Calanus Finmarchicus Reared in the Laboratory." *Marine Ecology-Progress Series* 221:161–183. https://doi.org/10.3354/meps221161.

Carls, M. G., L. Holland, M. Larsen, T. K. Collier, N. L. Scholz, and J. P. Incardona. 2008. "Fish Embryos are Damaged by Dissolved PAHs, Not Oil Particles." *Aquatic Toxicology (Amsterdam, Netherlands)* 88 (2): 121–127. https://doi.org/10.1016/j.aquatox.2008.03.014.

Carls, M. G., and J. P. Meador. 2009. "A Perspective on the Toxicity of Petrogenic PAHs to Developing Fish Embryos Related to Environmental Chemistry." *Human and Ecological Risk Assessment* 15 (6): 1084–1098. https://doi.org/10.1080/10807030903304708.

Cline, P. V., J. J. Delfino, and P. S. C. Rao. 1991. "Partitioning of Aromatic Constituents into Water from Gasoline and Other Complex Solvent Mixtures." *Environmental Science & Technology* 25 (5): 914–920. https://doi.org/10.1021/es00017a013.

Coelho, G., J. Clark, and D. Aurand. 2013. "Toxicity Testing of Dispersed Oil Requires Adherence to Standardized Protocols to Assess Potential Real World Effects." *Environmental Pollution* 177:185–188. https://doi.org/10.1016/j.envpol.2013.02.004.

Connell, D. W., and D. W. Hawker. 1988. "Use of Polynomial Expressions to Describe the Bioconcentration of Hydrophobic Chemicals by Fish." *Ecotoxicology and Environmental Safety* 16 (3): 242–257. https://doi.org/10.1016/0147-6513(88)90054-1.

Conover, R. J. 1971. "Some Relations Between Zooplankton and Bunker C Oil in Chedabucto Bay Following the Wreck of the Tanker Arrow." *Journal of the Fisheries Research Board of Canada* 28 (9): 1327–1330. https://doi.org/10.1139/f71-202.

Daling, P. S., P. Johan Brandvik, D. Mackay, and O. Johansen. 1990. "Characterization of Crude Oils for Environmental Purposes." *Oil and Chemical Pollution* 7 (3): 199–224. https://doi.org/10.1016/S0269-8579(05)80027-9.

Daling, P. S., F. Leirvik, I. Kjersti Almås, P. Johan Brandvik, B. Henrik Hansen, A. Lewis, and M. Reed. 2014. "Surface Weathering and Dispersibility of MC252 Crude Oil." *Marine Pollution Bulletin* 87 (1): 300–310. https://doi.org/10.1016/j.marpolbul.2014.07.005.

Di Toro, D. M., and J. A. McGrath. 2000. "Technical Basis for Narcotic Chemicals and Polycyclic Aromatic Hydrocarbon Criteria. II. Mixtures and Sediments." *Environmental Toxicology and Chemistry* 19 (8): 1971–1982. https://doi.org/10.1002/etc.5620190804.

Di Toro, D. M., J. A. McGrath, and W. A. Stubblefield. 2007. "Predicting the Toxicity of Neat and Weathered Crude Oil: Toxic Potential and the Toxicity of Saturated Mixtures." *Environmental Toxicology and Chemistry* 26 (1): 24–36. https://doi.org/10.1897/06174r.1.

Gardiner, W. W., J. Q. Word, J. D. Word, R. A. Perkins, K. M. McFarlin, B. W. Hester, L. S. Word, and C. M. Ray. 2013. "The Acute Toxicity of Chemically and Physically Dispersed Crude Oil to Key Arctic Species Under Arctic Conditions During the Open Water Season." *Environmental Toxicology and Chemistry* 32 (10): 2284–2300. https://doi.org/10.1002/etc.2307.

Guggenheim, E. A. 1937. "The Theoretical Basis of Raoult's Law." *Transactions of the Faraday Society* 33:151–156. https://doi.org/10.1039/tf9373300151.

Gyllenberg, G. 1981. "Ingestion and Turnover of Oil and Petroleum Hydrocarbons by Two Planctonic Copepods in the Gulf of Finland." *Annales zoologici Fennici* 18 (4): 225–228. http://www.jstor.org/stable/23734539.

Hansen, B. H., D. Altin, K. Bonaunet, and I. B. Øverjordet. 2014. "Acute Toxicity of Eight Oil Spill Response Chemicals

to Temperate, Boreal and Arctic Species." *Journal of Toxicology and Environmental Health, Part A* 77 (9–11): 495–505. https://doi.org/10.1080/15287394.2014.886544.

Hansen, B. H., D. Altin, K. M. Hessen, U. Dahl, M. Breitholtz, T. Nordtug, and A. J. Olsen. 2008. "Expression of Ecdysteroids and Cytochrome P450 Enzymes During Lipid Turnover and Reproduction in Calanus Finmarchicus (Crustacea: Copepoda)." *General and Comparative Endocrinology* 158 (1): 115–121. https://doi.org/10.1016/j.ygcen.2008.05.013.

Hansen, B. H., D. Altin, T. Nordtug, I. Beathe Øverjordet, A. J. Olsen, D. Krause, I. Størdal, and T. R. Størseth. 2017. "Exposure to Crude Oil Micro-Droplets Causes Reduced Food Uptake in Copepods Associated with Alteration in Their Metabolic Profiles." *Aquatic Toxicology (Amsterdam, Netherlands)* 184:94–102. https://doi.org/10.1016/j.aquatox.2017.01.007.

Hansen, B. H., D. Altin, A. J. Olsen, and T. Nordtug. 2012. "Acute Toxicity of Naturally and Chemically Dispersed Oil on the Filter-Feeding Copepod Calanus Finmarchicus." *Ecotoxicology and Environmental Safety* 86:38–46. https://doi.org/10.1016/j.ecoenv.2012.09.009.

Hansen, B. H., D. Altin, I. B. Overjordet, T. Jager, and T. Nordtug. 2013. "Acute Exposure of Water Soluble Fractions of Marine Diesel on Arctic Calanus Glacialis and Boreal Calanus Finmarchicus: Effects on Survival and Biomarker Response." *Science of the Total Environment* 449:276–284. https://doi.org/10.1016/j.scitotenv.2013.01.020.

Hansen, B. H., D. Altin, S. F. Rorvik, I. B. Overjordet, A. J. Olsen, and T. Nordtug. 2011. "Comparative Study on Acute Effects of Water Accommodated Fractions of an Artificially Weathered Crude Oil on Calanus Finmarchicus and Calanus Glacialis (Crustacea: Copepoda)." *The Science of the Total Environment* 409 (4): 704–709. https://doi.org/10.1016/j.scitotenv.2010.10.035.

Hansen, B. H., T. Jager, D. Altin, I. B. Øverjordet, A. J. Olsen, I. Salaberria, and T. Nordtug. 2016. "Acute Toxicity of Dispersed Crude Oil on the Cold-Water Copepod Calanus Finmarchicus: Elusive Implications of Lipid Content." *Journal of Toxicology and Environmental Health, Part A* 79 (13–15): 549–557. https://doi.org/10.1080/15287394.2016.1171981.

Hansen, B. H., K. K. Lie, T. R. Størseth, T. Nordtug, D. Altin, and P. A. Olsvik. 2016. "Exposure of First-Feeding Cod Larvae to Dispersed Crude Oil Results in Similar Transcriptional and Metabolic Responses as Food Deprivation." *Journal of Toxicology and Environmental Health, Part A* 79 (13–15): 558–571. https://doi.org/10.1080/07317131.2016.1171985.

Hansen, B. H., T. Nordtug, D. Altin, A. Booth, K. M. Hessen, and A. J. Olsen. 2009. "Gene Expression of GST and CYP330A1 in Lipid-Rich and Lipid-Poor Female Calanus Finmarchicus (Copepoda: Crustacea) Exposed to Dispersed Oil." *Journal of Toxicology and Environmental Health-Part A-Current Issues* 72 (3–4): 131–139. https://doi.org/10.1080/15287390802537313.

Hansen, B. H., A. J. Olsen, I. Salaberria, D. Altin, I. Beathe Øverjordet, P. Gardinali, A. Booth, and T. Nordtug. 2018. "Partitioning of PAHs Between Crude Oil Microdroplets, Water, and Copepod Biomass in Oil-In-Seawater Dispersions of Different Crude Oils." *Environmental Science & Technology* 52 (24): 14436–14444. https://doi.org/10.1021/acs.est.8b04591.

Hansen, B. H., T. Parkerton, T. Nordtug, T. R. Størseth, and A. Redman. 2019. "Modeling the Toxicity of Dissolved Crude Oil Exposures to Characterize the Sensitivity of Cod (Gadus Morhua) Larvae and Role of Individual and Unresolved Hydrocarbons." *Marine Pollution Bulletin* 138 (January): 286–294. https://doi.org/10.1016/j.marpolbul.2018.10.065.

Hansen, B. H., I. Salaberria, A. Johny Olsen, K. Ella Read, I. Beathe Øverjordet, K. Hammer, D. Altin, and T. Nordtug. 2015. "Reproduction Dynamics in Copepods Following Exposure to Chemically and Mechanically Dispersed Crude Oil." *Environmental Science & Technology* 49 (6): 3822–3829. https://doi.org/10.1021/es504903k.

Hansen, B. H., E. Sørhus, and T. Nordtug. 2023. *Using an Oil Droplet Generator to Study Contribution of Crude Oil Micro-Droplets to Dispersion Toxicity in Cold-Water Pelagic Marine Organisms*, 1–14. Edmonton, Canada: Environment and Climate Change Canada.

Hansen, B. H., A. M. Tarrant, I. Salaberria, D. Altin, T. Nordtug, and I. B. Overjordet. 2017. "Maternal Polycyclic Aromatic Hydrocarbon (PAH) Transfer and Effects on Offspring of Copepods Exposed to Dispersed Oil with and without Oil Droplets." *Journal of Toxicology and Environmental Health, Part A* 80 (16–18): 881–894. https://doi.org/10.1080/15287394.2017.1352190.

Harayama, S., Y. Kasai, and A. Hara. 2004. "Microbial Communities in Oil-Contaminated Seawater." *Current Opinion in Biotechnology* 15 (3): 205–214. https://doi.org/10.1016/j.copbio.2004.04.002.

Helland, S., J. C. Nejstgaard, H. J. Fyhn, J. K. Egge, and U. Bamstedt. 2003. "Effects of Starvation, Season, and Diet on the Free Amino Acid and Protein Content of Calanus Finmarchicus Females." *Marine Biology* 143 (2): 297–306. https://doi.org/10.1007/s00227-003-1092-x.

Hendriks, A. J., A. van der Linde, G. Cornelissen, and D. T. H. M. Sijm. 2001. "The Power of Size. 1. Rate Constants and Equilibrium Ratios for Accumulation of Organic Substances Related to Octanol-Water Partition Ratio and Species Weight." *Environmental Toxicology and Chemistry* 20 (7): 1399–1420. https://doi.org/10.1002/etc.5620200703.

Hirche, H. J. 1996. "Diapause in the Marine Copepod, Calanus Finmarchicus - a Review." *Ophelia* 44 (1–3): 129–143. https://doi.org/10.1080/00785326.1995.10429843.

Hodson, P. V., J. Adams, and R. S. Brown. 2019. "Oil Toxicity Test Methods Must Be Improved." *Environmental Toxicology and Chemistry* 38 (2): 302–311. https://doi.org/10.1002/etc.4303.

Hughey, C. A., R. P. Rodgers, and A. G. Marshall. 2002. "Resolution of 11 000 Compositionally Distinct Components in a Single Electrospray Ionization Fourier Transform Ion Cyclotron Resonance Mass Spectrum of Crude Oil." *Analytical Chemistry* 74 (16): 4145–4149. American Chemical Society. https://doi.org/10.1021/ac020146b.

Jager, T., D. Altin, C. Miljeteig, and B. Henrik Hansen. 2016. "Stage-Dependent and Sex-Dependent Sensitivity to Water-Soluble Fractions of Fresh and Weathered Oil in the Marine Copepod *Calanus Finmarchicus*." *Environmental Toxicology and Chemistry* 35 (3): 728–735. https://doi.org/10.1002/etc.3237.

Karabelas, A. J. 1978. "Droplet Size Spectra Generated in Turbulent Pipe Flow of Dilute Liquid/Liquid Dispersions." *AIChE Journal* 24 (2): 170–180. https://doi.org/10.1002/aic.690240203.

Kubinyi, H. 1977. "Quantitative Structure-Activity Relations. 7. The Bilinear Model, a New Model for Nonlinear Dependence of Biological Activity on Hydrophobic Character." *Journal of Medicinal Chemistry* 20 (5): 625–629. https://doi.org/10.1021/jm00215a002.

Kujawinski, E. B., M. C. Kido Soule, D. L. Valentine, A. K. Boysen, K. Longnecker, and M. C. Redmond. 2011. "Fate of Dispersants Associated with the Deepwater Horizon Oil Spill." *Environmental Science & Technology* 45 (4): 1298–1306. https://doi.org/10.1021/es103838p.

Lee, L. S., M. Hagwall, J. J. Delfino, and P. S. C. Rao. 1992. "Partitioning of Polycyclic Aromatic Hydrocarbons from Diesel Fuel into Water." *Environmental Science & Technology* 26 (11): 2104–2110. https://doi.org/10.1021/es00035a005.

Lee, R. F., M. Koster, and G. A. Paffenhofer. 2012. "Ingestion and Defecation of Dispersed Oil Droplets by Pelagic Tunicates." *Journal of Plankton Research* 34 (12): 1058–1063. https://doi.org/10.1093/plankt/fbs065.

Lee, L. S., P. S. C. Rao, and I. Okuda. 1992. "Equilibrium Partitioning of Polycyclic Aromatic Hydrocarbons from Coal Tar into Water." *Environmental Science & Technology* 26 (11): 2110–2115. https://doi.org/10.1021/es00035a006.

Lessard, R. R., and G. DeMarco. 2000. "The Significance of Oil Spill Dispersants." *Spill Science & Technology Bulletin* 6 (1): 59–68. https://doi.org/10.1016/s1353-2561(99)00061-4.

Martínez-Bazán, C., J. L. Montañés, and J. C. Lasheras. 2002. "Statistical Description of the Bubble Cloud Resulting from the Injection of Air into a Turbulent Water Jet." *International Journal of Multiphase Flow* 28 (4): 597–615. https://doi.org/10.1016/S0301-9322(01)00078-7.

Meador, J. P., and J. Nahrgang. 2019. "Characterizing Crude Oil Toxicity to Early-Life Stage Fish Based on a Complex Mixture: Are We Making Unsupported Assumptions?" *Environmental Science & Technology* 53 (19): 11080–11092. https://doi.org/10.1021/acs.est.9b02889.

Melbye, A. G., O. G. Brakstad, J. N. Hokstad, I. K. Gregersen, B. H. Hansen, A. M. Booth, S. J. Rowland, and K. E. Tollefsen. 2009. "Chemical and Toxicological Characterization of an Unresolved Complex Mixture-Rich Biodegraded Crude Oil." *Environmental Toxicology and Chemistry* 28 (9): 1815–1824. https://doi.org/10.1897/08-545.1.

Meyer, B., X. Irigoien, M. Graeve, R. N. Head, and R. P. Harris. 2002. "Feeding Rates and Selectivity Among Nauplii, Copepodites and Adult Females of Calanus Finmarchicus and Calanus Helgolandicus." *Helgoland Marine Research* 55 (3): 169–176. https://doi.org/10.1007/s10152-002-0105-3.

Nelson, R. K., K. M. Gosselin, D. J. Hollander, S. A. Murawski, A. Gracia, C. M. Reddy, and J. R. Radović. 2019. "Exploring the Complexity of Two Iconic Crude Oil Spills in the Gulf of Mexico (Ixtoc I and Deepwater Horizon) Using Comprehensive Two-Dimensional Gas Chromatography (GC × GC)." *Energy & Fuels* 33 (5): 3925–3933. American Chemical Society. https://doi.org/10.1021/acs.energyfuels.8b04384.

Niehoff, B. 2000. "Effect of Starvation on the Reproductive Potential of Calanus Finmarchicus." *ICES Journal of Marine Science* 57 (6): 1764–1772. https://doi.org/10.1006/jmsc.2000.0971.

Nordtug, T., and B. H. Hansen. 2021. "Chapter 6 - Assessment of Oil Toxicity in Water." In *Marine Hydrocarbon Spill Assessments*, edited by O. Makarynskyy, 199–220, Amsterdam, Netherlands: Elsevier.

Nordtug, T., A. Johny Olsen, D. Altin, S. Meier, I. Overrein, B. Henrik Hansen, and Ø. Johansen. 2011a. "Method for Generating Parameterized Ecotoxicity Data of Dispersed Oil for Use in Environmental Modelling." *Marine Pollution Bulletin* 62 (10): 2106–2113. https://doi.org/10.1016/j.marpolbul.2011.07.015

Nordtug, T., A. J. Olsen, D. Altin, I. Overrein, W. Storøy, B. H. Hansen, and F. De Laender. 2011b. "Oil Droplets Do Not Affect Assimilation and Survival Probability of First Feeding Larvae of North-East Arctic Cod." *Science of the Total Environment* 412 (December): 148–153. https://doi.org/10.1016/j.scitotenv.2011.10.021

Nordtug, T., A. J. Olsen, I. Salaberria, I. B. Øverjordet, D. Altin, I. F. Størdal, and B. Henrik Hansen. 2015. "Oil Droplet Ingestion and Oil Fouling in the Copepod Calanus Finmarchicus Exposed to Mechanically and Chemically Dispersed Crude Oil." *Environmental Toxicology and Chemistry* 34 (8): 1899–1906. https://doi.org/10.1002/etc.3007.

NRC. 2005. *Oil Spill Dispersants: Efficacy and Effects*. Washington D.C: The National Academies Press.

Olsen, A. J., T. Nordtug, D. Altin, M. Lervik, and B. H. Hansen. 2013. "Effects of Dispersed Oil on Reproduction in the Cold Water Copepod Calanus Finmarchicus (Gunnerus)." *Environmental Toxicology and Chemistry* 32 (9): 2045–2055. https://doi.org/10.1002/etc.2273.

Olsvik, P. A., B. H. Hansen, T. Nordtug, M. Moren, E. Nolen, and K. K. Lie. 2011. "Transcriptional Evidence for Low Contribution of Oil Droplets to Acute Toxicity from Dispersed Oil in First Feeding Atlantic Cod (Gadus Morhua) Larvae." *Comparative Biochemistry and Physiology C-Toxicology & Pharmacology* 154 (4): 333–345. https://doi.org/10.1016/j.cbpc.2011.07.002.

Olsvik, P. A., K. K. Lie, T. Nordtug, and B. H. Hansen. 2012. "Is Chemically Dispersed Oil More Toxic to Atlantic Cod (Gadus Morhua) Larvae Than Mechanically Dispersed Oil? A Transcriptional Evaluation." *BMC Genomics* 13 (1): 702. https://doi.org/10.1186/1471-2164-13-702.

Øverjordet, I. B., R. Nepstad, B. Henrik Hansen, T. Jager, J. Farkas, D. Altin, U. Brönner, and T. Nordtug. 2018. "Toxicokinetics of Crude Oil Components in Arctic Copepods." *Environmental Science & Technology* 52 (17): 9899–9907. https://doi.org/10.1021/acs.est.8b01812.

Parkerton, T. F., M. Boufadel, T. Nordtug, C. L. Mitchelmore, K. A. Colvin, D. Wetzel, M. Barron, G. E. Bragin, B. de Jourdan, and J. Loughery. 2023. "Recommendations for Advancing Media Preparation Methods Used to Assess Aquatic Hazards of Oils and Spill Response Agents." *Aquatic Toxicology (Amsterdam, Netherlands)* 255:106518. https://doi.org/10.1016/j.aquatox.2023.106518.

Parkerton, T. F., D. French-McCay, B. de Jourdan, K. Lee, and G. Coelho. 2023. "Adopting a Toxic Unit Model Paradigm in Design, Analysis and Interpretation of Oil Toxicity Testing." *Aquatic Toxicology (Amsterdam, Netherlands)* 255 (February): 106392. https://doi.org/10.1016/j.aquatox.2022.106392.

Rainer Engelhardt, F. 1983. "Petroleum Effects on Marine Mammals." *Aquatic Toxicology (Amsterdam, Netherlands)* 4 (3): 199–217. https://doi.org/10.1016/0166-445X(83)90018-8.

Ramachandran, S. D., P. V. Hodson, C. W. Khan, and K. Lee. 2004. "Oil Dispersant Increases PAH Uptake by Fish Exposed to Crude Oil." *Ecotoxicology and Environmental Safety* 59 (3): 300–308. https://doi.org/10.1016/j.ecoenv.2003.08.018.

Redman, A. D., J. A. McGrath, W. A. Stubblefield, A. W. Maki, and D. M. Di Toro. 2012. "Quantifying the Concentration of Crude Oil Microdroplets in Oil–Water Preparations." *Environmental Toxicology and Chemistry* 31 (8): 1814–1822. https://doi.org/10.1002/etc.1882.

Redman, A. D., and T. F. Parkerton. 2015. "Guidance for Improving Comparability and Relevance of Oil Toxicity Tests." *Marine Pollution Bulletin* 98 (1): 156–170. https://doi.org/10.1016/j.marpolbul.2015.06.053.

Rewitz, K., B. Styrishave, and O. Andersen. 2003. "CYP330A1 and CYP4C39 Enzymes in the Shore Crab Carcinus Maenas: Sequence and Expression Regulation by Ecdysteroids and Xenobiotics." *Biochemical & Biophysical Research Communications* 310 (2): 252–260. https://doi.org/10.1016/j.bbrc.2003.09.063.

Rodrigo, A., S. Baca, C. Hyatt, and E. J. Buskey. 2014. "Ingestion and Sublethal Effects of Physically and Chemically Dispersed Crude Oil on Marine Planktonic Copepods." *Ecotoxicology* 23 (6): 988–1003. https://doi.org/10.1007/s10646-014-1242-6.

Roncalli, V., M. C. Cieslak, Y. Passamaneck, A. E. Christie, P. H. Lenz, and V. N. Uversky. 2015. "Glutathione S-Transferase (GST) Gene Diversity in the Crustacean Calanus Finmarchicus – Contributors to Cellular Detoxification." *Plos One* 10 (5): 5. https://doi.org/10.1371/journal.pone.0123322.

Sandoval, K., Y. Ding, and P. Gardinali. 2017. "Characterization and Environmental Relevance of Oil Water Preparations of Fresh and Weathered MC-252 Macondo Oils Used in Toxicology Testing." *Science of the Total Environment* 576:118–128. https://doi.org/10.1016/j.scitotenv.2016.09.171.

Seto, M., and I. C. Handoh. 2009. "Mathematical Explanation for the Non-Linear Hydrophobicity-Dependent Bioconcentration Processes of Persistent Organic Pollutants in Phytoplankton." *Chemosphere* 77 (5): 679–686. https://doi.org/10.1016/j.chemosphere.2009.07.048.

Singer, M. M., D. Aurand, G. E. Bragin, J. R. Clark, G. M. Coelho, M. L. Sowby, and R. S. Tjeerdema 2000. "Standardization of the Preparation and Quantitation of Water-Accommodated Fractions of Petroleum for Toxicity Testing." *Marine Pollution Bulletin* 40 (11): 1007–1016.

Soloperto, S., D. Altin, A. Hallmann, E. Skottene, B. H. Hansen, B. M. Jenssen, and T. M. Ciesielski. 2022. "Oil-Mediated Oxidative-Stress Responses in a Keystone Zooplanktonic Species, Calanus Finmarchicus." *Science of the Total Environment* 806:806. https://doi.org/10.1016/j.scitotenv.2021.151365.

Sørensen, L., B. Henrik Hansen, J. Farkas, C. E. Donald, W. J. Robson, A. Tonkin, S. Meier, and S. J. Rowland. 2019. "Accumulation and Toxicity of Monoaromatic Petroleum Hydrocarbons in Early Life Stages of Cod and Haddock." *Environmental Pollution* 251 (August): 212–220. https://doi.org/10.1016/j.envpol.2019.04.126.

Sørhus, E., C. E. Donald, D. da Silva, A. Thorsen, Ø. Karlsen, and S. Meier. 2021. "Untangling Mechanisms of Crude Oil Toxicity: Linking Gene Expression, Morphology and PAHs at Two Developmental Stages in a Cold-Water Fish." *Science of the Total Environment* 757 (February): 143896. https://doi.org/10.1016/j.scitotenv.2020.143896.

Sørhus, E., R. B. Edvardsen, Ø. Karlsen, T. Nordtug, T. Van Der Meeren, A. Thorsen, C. Harman, S. Jentoft, S. Meier, and I. Corsi. 2015. "Unexpected Interaction with Dispersed Crude Oil Droplets Drives Severe Toxicity in Atlantic Haddock Embryos." *PLoS ONE* 10 (4): e0124376. https://doi.org/10.1371/journal.pone.0124376.

Sujit, B., and G. L. Baughman. 1991. "Bioconcentration Factors and Lipid Solubility." *Environmental Science & Technology* 25 (3): 536–539. https://doi.org/10.1021/es00015a024.

Sutton, P. A., C. A. Lewis, and S. J. Rowland. 2005. "Isolation of Individual Hydrocarbons from the Unresolved Complex Hydrocarbon Mixture of a Biodegraded Crude Oil Using Preparative Capillary Gas Chromatography." *Organic Geochemistry* 36 (6): 963–970. https://doi.org/10.1016/j.orggeochem.2004.11.007.

Tarr, M. A., P. Zito, E. B. Overton, G. M. Olson, P. L. Adhikari, and C. M. Reddy. 2016. "Weathering of Oil Spilled in the Marine Environment." *Oceanography*

29 (3): 126–135. Oceanography Society. https://doi.org/10.5670/oceanog.2016.77.

Toxværd, K., K. V. Dinh, O. Henriksen, M. Hjorth, and T. G. Nielsen. 2019. "Delayed Effects of Pyrene Exposure During Overwintering on the Arctic Copepod Calanus Hyperboreus." *Aquatic Toxicology (Amsterdam, Netherlands)* 217. https://doi.org/10.1016/j.aquatox.2019.105332.

Viaene, K., C. R. Janssen, L. De Hoop, A. J. Hendriks, and F. De Laender. 2013. "Models That Ignore Oil Droplet Uptake are Sufficiently Accurate to Predict the Bioaccumulation of Oil-Associated PAHs." *Communications in Agricultural and Applied Biological Sciences* 78 (1): 111–113.

Viaene, K. P., C. R. Janssen, L. de Hoop, A. J. Hendriks, and F. De Laender. 2014. "Evaluating the Contribution of Ingested Oil Droplets to the Bioaccumulation of Oil Components—A Modeling Approach." *Science of the Total Environment* 499:99–106. https://doi.org/10.1016/j.scitotenv.2014.08.040.