Marine Pollution Bulletin 175 (2022) 113345

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

Toxicity to sea urchin embryos of crude and bunker oils weathered under ice alone and mixed with dispersant

Laura DeMiguel-Jiménez [a,c], Nestor Etxebarria [b,c], Helena C. Reinardy [d,e], Xabier Lekube [a,c], Ionan Marigómez [a,c,*], Urtzi Izagirre [a,c]

[a] BCTA Research Group, Department of Zoology and Animal Cell Biology, Faculty of Science and Technology, University of the Basque Country (UPV-EHU), Sarriena auzoa z/g, E-48940 Leioa-Bizkaia, Basque Country, Spain
[b] IBeA Research Group, Department of Analytical Chemistry, Faculty of Science and Technology, University of the Basque Country (UPV/EHU), Sarriena auzoa z/g, E-48940 Leioa-Bizkaia, Basque Country, Spain
[c] Research Centre for Experimental Marine Biology and Biotechnology (PiE-UPV/EHU), University of the Basque Country (UPV/EHU), Areatza z/g, E-48620 Plentzia-Bizkaia, Basque Country, Spain
[d] Aquaculture Science Department, Scottish Association for Marine Science (SAMS), Scottish Marine Institute, Dunbeg, Oban, Argyll PA37 1QA, Scotland, United Kingdom
[e] Department of Arctic Technology, The University Centre in Svalbard (UNIS), PO Box 156, N-9171 Longyearbyen, Svalbard, Norway

ARTICLE INFO

Keywords:
Oil
Dispersant
Iced seas
Sea urchin embryo
Toxicity
Genotoxicity

ABSTRACT

A multi-index approach (larval lenghthening and malformations, developmental disruption, and genotoxicity) was applied using sea-urchin embryos as test-organisms. PAH levels measured in the under-ice weathered aqueous fraction (UIWAF) were lower than in the low-energy water accommodated fraction (LEWAF) and similar amongst UIWAFs of different oils. UIWAFs and LEWAFs caused toxic effects, more markedly in UIWAFs, that could not be attributed to measured individual PAHs or to their mixture. Conversely, UIWAF was less genotoxic than LEWAF, most likely because naphthalene concentrations were also lower. In agreement, NAN LEWAF, the most genotoxic, exhibited the highest naphthalene levels. Dispersant addition produced less consistent changes in PAH levels and embryo toxicity in UIWAFs than in LEWAFs, and did not modify LEWAF genotoxicity. Overall, under ice weathering resulted in lowered waterborne PAHs and genotoxicity but augmented embryo toxicity, not modified by dispersant application.

1. Introduction

There is increasing interest in Arctic regions because they contain a noteworthy amount of undiscovered oil and gas reserves (Gautier et al., 2009). In parallel, sea ice retreat driven by climate change is enhancing petroleum exploration and maritime transport in Arctic seas, thus increasing the threat of oil spills (OGP, 2013; Yang et al., 2018). Furthermore, Arctic ecosystems are highly vulnerable to oil spills due to their characteristic environmental conditions and remoteness, which can influence oil spill impact and response differently compared to temperate regions.

The low temperature of seawater and the presence of ice-cover cause deferred oil spreading, diminished oil droplet emulsification due to wind-waves flattening, and attenuated physico-chemical weathering and biodegradation (Brandvik and Faksness, 2009; Sørstrøm et al., 2010; Daling et al., 2012). Moreover, in the presence of ice-cover the oil spill impact and response may also vary depending on whether the oil is spilled on or under the ice, as well as on characteristics of the ice-cover. Spreading and encapsulation of the spilled oil, weathering and potential toxicity of spill products are crucial. These processes can be largely modified depending on the duration of the ice-cover season, ice type and concentration, thickness, growth rate, drift velocity, and physical and mechanical properties of the ice-cover (Faksness and Brandvik, 2008; Nordam et al., 2017; Wilkinson et al., 2017).

Remoteness and severe climatological conditions in the Arctic can be major factors hampering clean-up operations after oil spills and therefore the use of chemical dispersants and in-situ burning manoeuvres, together with natural attenuation, are considered suitable alternative

* Corresponding author at: Research Centre for Experimental Marine Biology and Biotechnology (PiE-UPV/EHU), University of the Basque Country (UPV/EHU), Areatza z/g, E-48620 Plentzia-Bizkaia, Basque Country, Spain.
E-mail address: ionan.marigomez@ehu.eus (I. Marigómez).

https://doi.org/10.1016/j.marpolbul.2022.113345
Received 11 November 2021; Received in revised form 30 December 2021; Accepted 10 January 2022
Available online 10 February 2022

response tools (Sørstrøm et al., 2010; Wilkinson et al., 2017). In-situ burning operations are not expected to generate additional residues but the remaining residues will persist on the sea surface or sink (Fritt-Rasmussen et al., 2015), and may produce an atmospheric impact due to the release of large quantities of particles derived from the combustion of oil (McGrattan et al., 1997; Sartz and Aggarwal, 2016). Dispersants reduce interfacial tensions thus enhancing fragmentation of the oil slick into small droplets and allowing hydrocarbon-degrading bacteria to breakdown the oil more rapidly (Prince et al., 2016; Soloviev et al., 2016). On the other hand, these processes can modify the chemical composition of the water-accommodated fraction (WAF) of the spill products and intensify the toxicity to marine biota (Lee et al., 2013; Adeyemo et al., 2015; DeLeo et al., 2016; DeMiguel-Jiménez et al., 2021). Oil-in-ice behaviour (encapsulation, spreading, migration and weathering) has been thoroughly investigated in order to improve oil spill response in iced seas (Fingas and Hollebone, 2003; Brandvik and Faksness, 2009; Afenyo et al., 2016; Boccadoro et al., 2018; Øksenvåg et al., 2019; Nordam et al., 2020; Singsaas et al., 2020). Conversely, the toxicity of under ice weathered oil remains, to our knowledge, unexplored.

The present study, carried out under the framework of the UE-funded project GRACE (Jørgensen et al., 2019), aimed at contributing to the understanding of how under ice weathering modifies oil spill toxicity and this toxicity is influenced by dispersant application. Toxicity of crude and bunker oils representative of prospective oil spill threats in Arctic and Sub-Arctic seas (Naphthenic North Atlantic crude oil, marine gas oil, and an intermediate fuel oil IFO 180), alone and in combination with a third-generation dispersant recommended for iced seas (Finasol OSR52®; Steffek et al., 2016), was tested by means of a sea urchin embryo toxicity assay multi-index approach that included larval size (Beiras et al., 2012), malformation and developmental disruption as endpoints (Carballeira et al., 2012; DeMiguel-Jiménez et al., 2021). Sea urchin embryos are sensitive to oil exposure and have been often used to assess the toxicity of the oil WAF (Fernández et al., 2006; Saco-Álvarez et al., 2008; Bellas et al., 2013; DeMiguel-Jiménez et al., 2021). In parallel, genotoxicity was also determined using the Fast Micromethod® DNA Single-Strand-Break adapted to sea urchin larvae (Scröder et al., 2006; El-Bibany et al., 2014; Reinardy et al., 2016; Reinardy and Bodnar, 2015). Using a well-known toxicity testing model sea urchin species *Paracentrotus lividus* (Bellas et al., 2008, 2013; Saco-Álvarez et al., 2008; Beiras et al., 2012) was seen as a genuine approach to provide data to advance risk assessment of oil spills in the Arctic and Sub-Arctic seas before the use of regionally relevant autochthonous test species is feasible (DeMiguel-Jiménez et al., 2021).

2. Materials and methods

2.1. Test chemicals

Three oils and one dispersant were selected as representative of prospective oil spill threats in Arctic and Sub-Arctic seas (Electronic Supplementary Material, ESM 1): (a) a Naphthenic North Atlantic crude oil (NNA), a very light crude oil of low viscosity, rich in branched and cyclic saturated hydrocarbons; (b) a distillate marine gas oil (MGO), supplemented with the dye Dyeguard Green MC25 (John Hogg Technical Solutions; UK); (c) the intermediate fuel oil IFO 180 (IFO), a heavy bunker oil of high viscosity with low amounts of volatile hydrocarbons (Polaroil, Greenland); and (d) the third-generation dispersant Finasol OSR52® (D) containing >30% non-ionic and 15–30% anionic surfactants (Total Special Fluids, France; SDS no. 30034 2015). Test chemicals were stored in a cool room (4–6 °C).

2.2. UIWAF and LEWAF production, and chemical analysis

The Under-Ice Weathered Aqueous Fraction (UIWAF) of oils alone (NNA UIWAF, MGO UIWAF and IFO UIWAF) or mixed with dispersant (NNA + D UIWAF, MGO + D UIWAF and IFO + D UIWAF) was produced in a cold chamber at −4 ± 2 °C in complete darkness for 2 months. For this purpose, one glass tanks (35 × 25 × 25 cm) per UIWAF type was filled with 15 L filtered seawater (FSW; 32 psu) and capped tightly with a thick ice-cover. The ice-cover was produced using a 25 L polypropylene tank mould (50 × 35 × 11 cm), which was filled with FSW and frozen at −80 °C for a minimum of 3 d. PVC tubes (25 mm Ø) were inserted in the moulding system to produce one ice-free borehole per ice-cover for contaminant delivery into seawater. Two ice-covers of 35 × 25 × 11 cm were obtained from each moulding tank. The ice-cover was carefully placed on top of the seawater in each glass tank and the borderline ice was left melting to tightly close the cap. The three oils (1:200; w oil/v FSW) and their mixture with dispersant (1:10 w D/w oil+D in 1:200; w oil+D/v FSW) were injected into their respective glass tanks through the delivery borehole, which was immediately sealed with melted fresh ice. After 2 months weathering, the UIWAFs of oil and oil+D (100% stock solution) were retrieved from the depth of each glass tank with the aid of a peristaltic pump (Watson-Marlow 323E), using a serological pipette introduced through a newly made retrieval borehole (5 mm Ø).

The specific PAH composition of the UIWAFs was determined by gas chromatography–mass spectrometry (GC–MS) after Prieto et al. (2007). A mix standard solution of 18 PAHs (CRM47543; Supelco, USA) was used for calibration in the GC–MS analysis. A mixture of 5 deuterated compounds was used as internal standard (Norwegian Standard S-4124-200-T; Chiron, Trondheim, Norway). Stir-bars (10 mm length; 0.5 mm film thick; Gerstel GmbH & Co, Germany) were introduced in aqueous samples (35 mL) during 315 min. Once the extraction step was over, stir-bars were rinsed in Milli-Q water to eliminate seawater and dried with paper tissue. Then, they were desorbed using a TDS-2 unit connected to a CIS-4 injector (Gerstel) with the following conditions: desorption time (10 min), desorption temperature (300 °C), desorption flow (23 mL/min), cryo-focusing temperature (−50 °C) and vent pressure (7 psi). The chromatographic conditions were setup as described in Prieto et al. (2007). Detection limits are given in ESM 2.

The Low Energy Water Accommodated Fraction (LEWAF) in FSW of the three oils, alone (NNA LEWAF, MGO LEWAF and IFO LEWAF) or mixed with the dispersant (NNA+D LEWAF, MGO+D LEWAF, IFO+D LEWAF), was produced in the darkness at 10 °C according to Katsumiti et al. (2019), modified after Singer et al. (2000). Briefly, oils (1:200; w oil/v FSW) and their mixtures (1:10 w D/w oil+D in 1:200; w oil+D/v FSW) were poured into filtered seawater in 200 mL glass aspirator bottles and stirred at 200 ± 20 rpm (no vortex; low energy) for 40 h. The LEWAFs of oil and oil+D (100% stock solution) were collected from the bottom glass tap of the aspirator bottles. The specific PAH composition of each LEWAF was characterised following the same procedure than in the case of UIWAFs (Prieto et al., 2007) and reported in a preceding paper in which the same LEWAFs had been used for toxicity testing (DeMiguel-Jiménez et al., 2021). These data (herein provided in ESM3) were used for calculations and comparisons in the present study.

2.3. Sea urchin embryo toxicity (SET) testing

Sea urchin 48-h embryo toxicity assay was carried out according to the International Council for the Exploration of the Sea (ICES, Beiras et al., 2012). Gametes were obtained from sexually mature sea urchins (*P. lividus*) collected from a rocky shore in Armintza (43°26′01.1″N 2°53′56.1″W; Bay of Biscay) in spring (March–May) 2019. Spawning and fertilisation were carried out as described by DeMiguel-Jiménez et al. (2021). Within 30 min after fertilisation, the successfully fertilised eggs were transferred to glass vials containing 10 mL of the test solutions (50 embryos/mL), capped with Teflon lids. Toxicity assays were conducted in complete darkness at 20 °C. Successive dilutions in FSW (0, 8, 21, 34, 55, 89 and 100%) of UIWAF and LEWAF alone or mixed with dispersant were prepared at 20 °C. The dilutions were selected following a Fibonacci dose escalation between 0 and 100% UIWAF or LEWAF,

after excluding some of the lower doses from the dilution series in order to optimise the experimental set up, as in previous investigations (DeMiguel-Jiménez et al., 2021).

After 48 h exposure, larvae were fixed by adding two drops of 40% formaldehyde. The longest dimension of larvae (L in μm; sample size: n = 35 larvae per vial ×3 exposure replicates) and the egg size at t_0 (L_0 in μm; sample size: n = 35 egg per vial ×3 exposure replicates) were measured using a Nikon Di-Qi2 camera attached to an inverted microscope (Nikon Eclipse Ti-2) and NIS-Elements Imaging Software v4.30 (Nikon Instruments BV). Lengthening was calculated as $\Delta L = L\text{-}L_0$ (Beiras et al., 2012). Specific abnormalities of the pluteus larvae were recorded (n = 100 larvae per vial ×3 replicates per experimental group) and integrated in the Toxicity Index (TI, in a 0–100 range; after Carballeira et al., 2012), as detailed in DeMiguel-Jiménez et al. (2021). Sea urchin embryo developmental disruption (SEDD) was measured in terms of Inhibition of Pluteus Larvae Formation Index (IPLFI) and developmental arrest (Cleavage Disruption Index: CDI; Gastrulation Disruption Index: GDI; Metamorphosis Disruption Index: MDI; DeMiguel-Jiménez et al. (2021).

2.4. Genotoxicity assay

Sublethal exposure concentrations were selected according to present results regarding the effect of the test oils on pluteus larvae lengthening (ΔL) as well as to preceding data (DeMiguel-Jiménez et al., 2021): 55% oil UIWAF or oil LEWAF, and 34% oil+D UIWAF or oil+D LEWAF. After 48 h exposure, sea urchin larvae were centrifuged (1800 ×g at 4 °C for 10 min) to obtain pellets made of 500 larvae that were directly frozen in 500 μL of RNAlater® (Life Technologies, Carlsbad, CA, USA) and stored at −80 °C until the genotoxicity assay was performed.

The amount of intact double-stranded DNA (dsDNA) was determined by the Fast Micromethod® DNA Single-Strand-Break Assay (Scröder et al., 2006), adapted to sea urchin larvae (Reinardy and Bodnar, 2015). Samples were assayed in quadruplicate by loading 20 μL (15 larvae) to each replicate well on a black-walled 96-well microplate (USA Scientific, INC., FL, USA), placed on ice. Ca/Mg-free phosphate buffer solution (PBS) was used as blank. Lysis solution (20 μL, 9 M urea 0.1% SDS, 0.2 M EDTA) containing 1:49 Picogreen (P7581, Life Technologies, NY, USA) was added and samples were left to lyse on ice in the dark for 40 min. Then, DNA unwinding solution (20 mM EDTA, 1 M NaOH) was added (200 μL) to initiate alkaline unwinding (pH 12.65 ± 0.02). Fluorescence (intact dsDNA) was recorded at an excitation wavelength of 480 nm and an emission wavelength of 520 nm (POLARstar® Omega Plate Reader, BMG LABTECH, Aylesbury, UK) as relative fluorescent units (RFU) at t_0 in the experimental control group and after 20 min in both control and exposure groups. Blank values were subtracted from all the RFU values before calculations. Then, the strand scission factor (SSF) was calculated according to Scröder et al. (2006):

$$SSF = -log\frac{\%\text{dsDNA}_i}{\%\text{dsDNA}_c};$$

where $\%\text{dsDNA}_i$ is the percentage of dsDNA in each exposure group and $\%\text{dsDNA}_c$ is the percentage of dsDNA in the experimental control group. The %dsDNA values were calculated as RFU for a given sample divided by the RFU recorded in the experimental control group at t_0.

2.5. Toxic units

Toxic units (TUs; Sprague, 1970), the relative contribution of each individual PAH to the TUs of UIWAFs and LEWAFs (RT_i) and the relative concentration of each PAH in the mixtures (RC_i) were calculated according to DeMiguel-Jiménez et al. (2021). For TU calculations, EC50 values of individual PAHs published for marine organisms were used as reference (ESM 4; Ott et al., 1978; Ward et al., 1981; Holcombe et al., 1983; Trucco et al., 1983; Spehar et al., 1999; Lyons et al., 2002; Pillai et al., 2003; Calbet et al., 2006; Bellas et al., 2008; Olsen et al., 2011; Frantzen et al., 2012; Renegar et al., 2017; Knap et al., 2017). RT_i was determined as $RT_i = TU_{PAHi}/\sum TU_{\sum PAHs}$; where TU_{PAHi} is the TU estimated for each individual PAH and $\sum TU_{\sum PAHs}$ is the TUs of the mixture. RC_i was determined as $RC_i = C_{PAHi}/\sum PAHs$; where C_{PAHi} stands for the individual concentration of each PAH. The ratio RT_i/RC_i was calculated as indicative of whether the toxicity of a given individual PAH ("i") in the mixture was, or not, the one expected due to its proportion in the composition of the mixture (assuming the Concentration Addition (CA) model; Altenburger et al., 2003).

2.6. Data treatment and statistical analysis

Statistical analyses were carried out using SPSS statistical package (IBM SPSS Statistics 24.0). Shapiro-Wilk's test and Levene's test were performed to study normality and equality of variances of the datasets, respectively. EC50 values were calculated through Probit analysis. For normal data, differences between control and each exposure group were tested using the parametric one-way ANOVA test followed by post hoc procedures (T Dunett if the variances were homogenous and T3 Dunnett if they were not). For non-normal data sets, the non-parametric Kruskal-Wallis' test was used. Linear regressions were compared using the ANCOVA test. Differences in SSF were tested by one-way ANOVA on arcsine-transformed data, with post hoc Fisher's least significant difference (LSD) test for differences between each treatment and control. Significant differences in chemical data were tested with the Z-score test. Level of significance for all analyses was $p < 0.05$.

3. Results

3.1. Chemical composition of the UIWAF of oils alone and combined with dispersant

The Naph concentration was similar in MGO UIWAF, IFO UIWAF and IFO + D UIWAF, whilst for NNA UIWAF, NNA + D UIWAF and MGO + D UIWAF the values were around three-times higher (Table 1). The concentration of 1-MN was higher in NNA UIWAF and IFO UIWAF than in MGO UIWAF and all the oil+D UIWAFs (Table 1). The concentration of Ace, Flu and Phe was high in all the UIWAFs and dispersant addition increased the concentrations of Flu and Phe in NNA + D UIWAF and MGO + D UIWAF, whilst in IFO + D UIWAF the concentrations of Ace, Flu and Phe decreased (ESM 5; Table 1). Pyr, Fluo, B[a]A + Chr and B[a]P were detected at relatively low concentrations in the three oil UIWAFs whilst Ant was only detected in IFO UIWAF (Table 1). After the addition of dispersant, the concentration of these five individual PAHs in UIWAF decreased in IFO, whilst the concentration of Pyr, Fluo and B[a]A + Chr increased in NNA and MGO (Table 1). Overall, the values of $\sum$PAHs (without Naph), $\sum_{LMW}$PAHs and $\sum_{HMW}$PAHs were low in all the UIWAFs (Table 1); yet, after dispersant addition the values increased in NNA + D UIWAF and MGO + D UIWAF, and decreased in IFO + D UIWAF (Table 1).

3.2. Toxicity of the UIWAF and LEWAF of oils alone

For the three tested oils, ΔL decreased at increasing concentrations of both oil UIWAF and oil LEWAF, more markedly for the former (Fig. 1A, C and E; ESM 6 and 7). In parallel, TI increased linearly at increasing concentrations of both oil UIWAF and oil LEWAF, with a higher slope for the case of UIWAF than for LEWAF (ANCOVA, $p < 0.05$; Fig. 2A, C and E; ESM 6 and 7). CDI increased linearly at increasing concentrations of the UIWAF of the three oils and of MGO LEWAF, but remained unchanged with exposure to NNA and IFO LEWAF (Figs. 3A, 4A and 5A; ESM 6 and 7). Likewise, GDI, MDI and IPLFI increased linearly at increasing concentrations of both oil UIWAF and oil LEWAF, more markedly for the former (ANCOVA; $p < 0.05$; Figs. 3C, E, 4C, E, 5C, E, 6A, C and E; ESM 6 and 7). Finally, SSF increased on exposure to UIWAF and LEWAF of the

Table 1
GC–MS analysis of PAHs (ng/L) in oil UIWAF and oil+D UIWAF samples of NNA, MGO and IFO. Asterisks indicate significant differences in each oil LEWAF type (*Z*-score). (UDL: under detection limits; $_{LMW}$PAHs: Low molecular weight polycyclic aromatic hydrocarbons; $_{HMW}$PAHs: High molecular weight polycyclic aromatic hydrocarbons; #: Total of PAHs without Naphthalene).

	NNA UIWAF	NNA+D UIWAF	MGO UIWAF	MGO+D UIWAF	IFO UIWAF	IFO+D UIWAF
Naph	74,898	107,030	22,700	80,908	31,565	21,025
1-MN	24,180	15,515	8708	10,953	27,530	5270
2-MN	33,663	52,508	8883	30,440	28,385	10,095
Acy [(1)]	UDL	25	28	55	95	15
Ace [(1)]	215	320	350	415	655	120
Flu [(1)]	818	1148	525	1100	660	185
Ant [(1)]	UDL	UDL	UDL	UDL	98	15
Phe [(1)]	563	1698	655	1715	1130	340
Pyr [(2)]	50	93*	40	70	70	30
Fluo [(2)]	33	150	20	70	28	25
B[*a*]A + Chr [(2)]	33	40	UDL	15	73	28
B[*a*]P [(2)]	33	UDL	38	UDL	35	UDL
B[*b*]F + B[*k*]F [(2)]	UDL	UDL	28	UDL	UDL	UDL
B[*g,h,i*]P [(2)]	UDL	UDL	53	UDL	45	UDL
D[*ah*]A[(2)]	UDL	UDL	UDL	UDL	UDL	UDL
I[1,2,3-*cd*]P [(2)]	UDL	UDL	UDL	UDL	UDL	UDL
∑PAHs	134,483	178,525	42,025	125,740	90,368	37,148
$\sum_{LMW}$PAHs $\sum$[(1)]	1595	3190	1558	3285	2638	675
$\sum_{HMW}$PAHs $\sum$[(2)]	148	283	178	155	250	83
$\sum_{Naph}$PAHs	132,740	175,053	40,290	122,300	87,480	36,390
∑PAHs[#]	1743	3473	1735	3440	2888	758
$\sum^{(1)}/\sum^{(2}$	11	11	9	21	11	8
Phe/Ant	–	–	–	–	11.59	22.67
Fluo/Pyr	0.65	1.62*	0.5	1	0.39	0.83

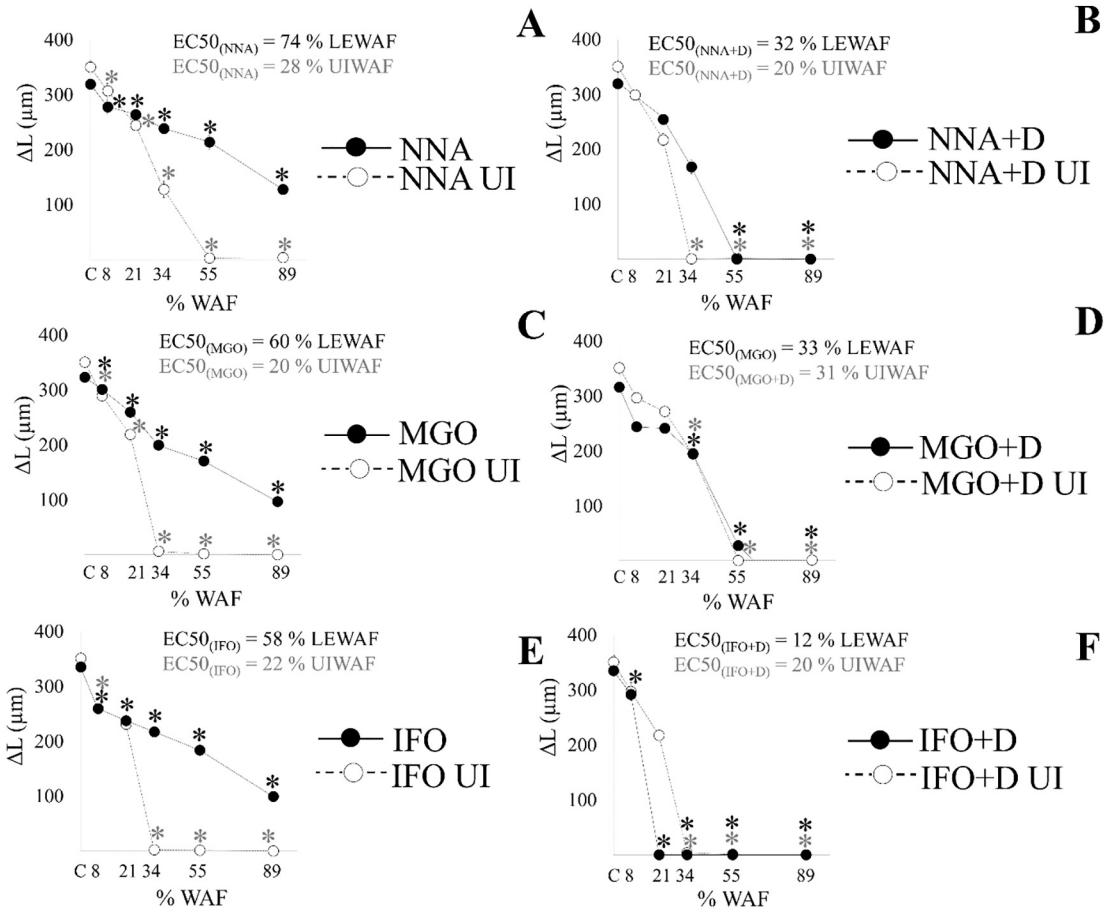

Fig. 1. Lengthening (ΔL in μm) of sea urchin larvae exposured to oil and oil+D WAF weathered under ice (UI) or produced at 10 °C (LEWAF). (A) NNA UIWAF and NNA LEWAF; (B) NNA+D UIWAF and NNA+D LEWAF; (C) MGO UIWAF and MGO LEWAF; (D) MGO+D UIWAF and MGO+D LEWAF; ϵ IFO UIWAF and IFO LEWAF; and (F) IFO+D UIWAF and IFO+D LEWAF. Values are given in μm (means ± SD). Asterisks indicate significant differences amongst concentrations of each treatment and control ($p < 0.05$).

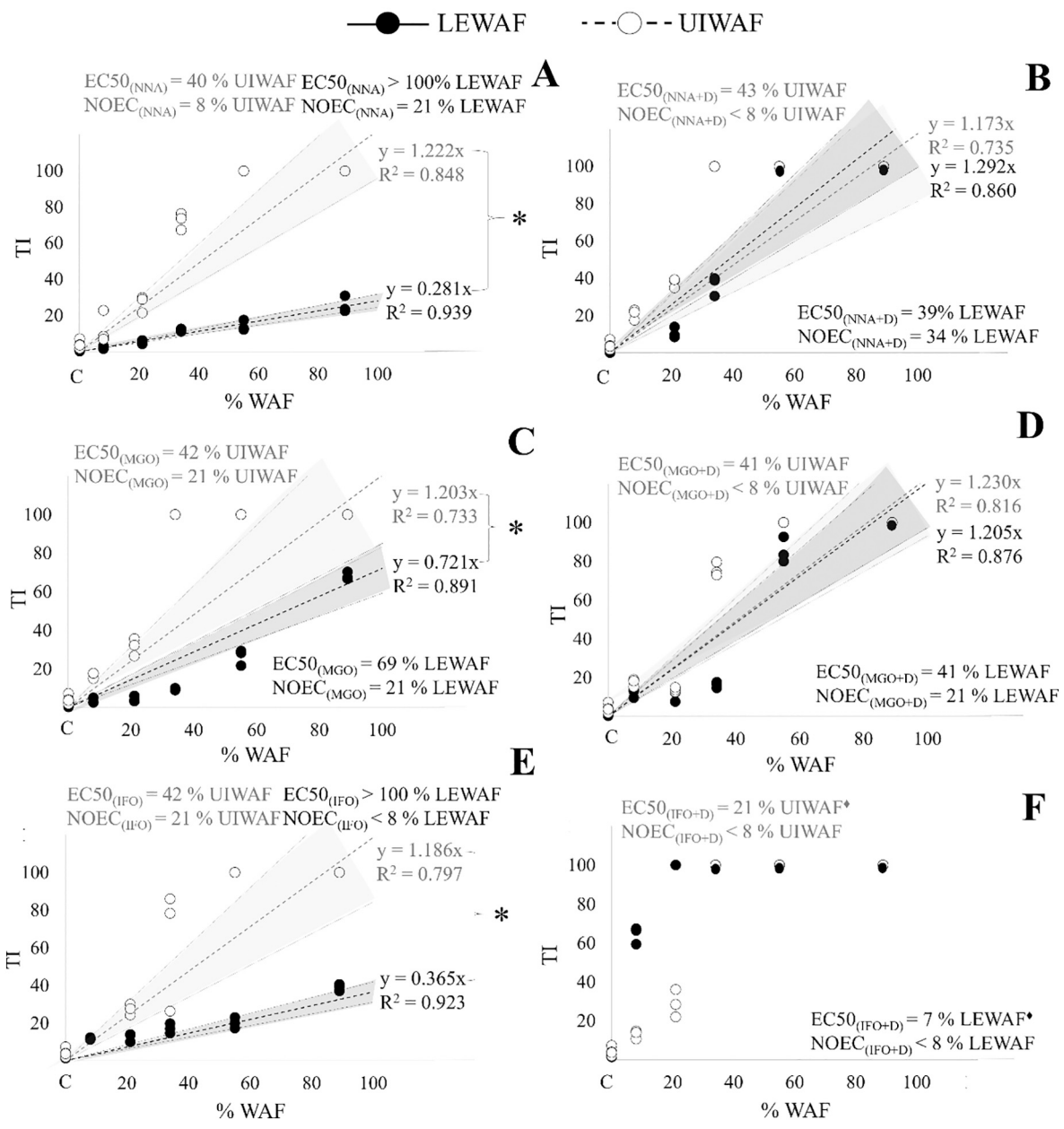

Fig. 2. TI (Toxicity Index) on exposure to oil and oil+D WAF weathered under ice (UI) or produced at 10 °C (LEWAF). (A) NNA UIWAF and NNA LEWAF; (B) NNA+D UIWAF and NNA+D LEWAF; (C) MGO UIWAF and MGO LEWAF; (D) MGO+D UIWAF and MGO+D LEWAF; (E) IFO UIWAF and IFO LEWAF; and (F) IFO+D UIWAF and IFO+D LEWAF. Half-effective concentration (EC50 from regression equations, EC50◆ based on Probit analyses) and NOEC values are shown. Asterisks indicate significant differences between linear regression coefficients (r^2, ANCOVA ($p < 0.05$)).

three oils, more markedly in the LEWAF, especially in the case of MGO (Fig. 7).

Regarding the mixture toxicity assessment, TU values were always very low (<0.20) after oil UIWAF exposure (Table 2). In contrast, on exposure to oil LEWAF these values were higher, most remarkably for NNA (TU > 1; Table 3). RT_i was greater than "1" after exposure to the UIWAF and LEWAF of the three oils, especially on exposure to IFO (Tables 2 and 3). RT_i/RC_i values greater than "1" were found for various individual PAHs after oil UIWAF exposure (Table 2): (a) 2-MN, Pyr and Fluo; (b) Ant (only in IFO); (c) B[*a*]A + Chr (not in MGO); and (d) B[*a*]P. Alike, RT_i/RC_i was also greater than "1" after oil LEWAF exposure for various individual PAHs (Table 3): (a) 2-MN, Pyr, Fluo and B[*a*]A + Chr; (b) Ace, Acy, Phe and B[*a*]P (only in NNA); (c) Phe (only in MGO); and (d) Ant (only in IFO).

3.3. Toxicity of the UIWAF and LEWAF of oils combined with dispersant

After addition of dispersant, ΔL decreased in a comparable manner at increasing concentrations of both UIWAF and LEWAF of the three oils (Fig. 1B, D and F; ESM 6 and 7). Likewise, no differences between UIWAF and LEWAF were found for the other biological endpoints (ANCOVA, $p > 0.05$; Figs. 2-6; ESM 6 and 7). Concretely, a concentration dependent linear increase was observed in TI, CDI, GDI, MDI and IPLFI for NNA + D and MGO + D UIWAF and LEWAF (Figs. 2B, D, 3B, D, F, 4B, D, F, 6B and D; ESM 6 and 7). In contrast, TI, CDI, GDI and IPLFI reached the maximum value of 100 on exposure to 34% IFO + D UIWAF and 21% IFO + D LEWAF (Figs. 2F, 5B, D and 6F; ESM 6 and 7). Meanwhile, on exposure to IFO + D, MDI increased linearly at increasing concentrations of UIWAF but reached the maximum value of 100 on exposure to 21% LEWAF (Fig. 5F; ESM 6 and 7). SSF increased upon exposure to

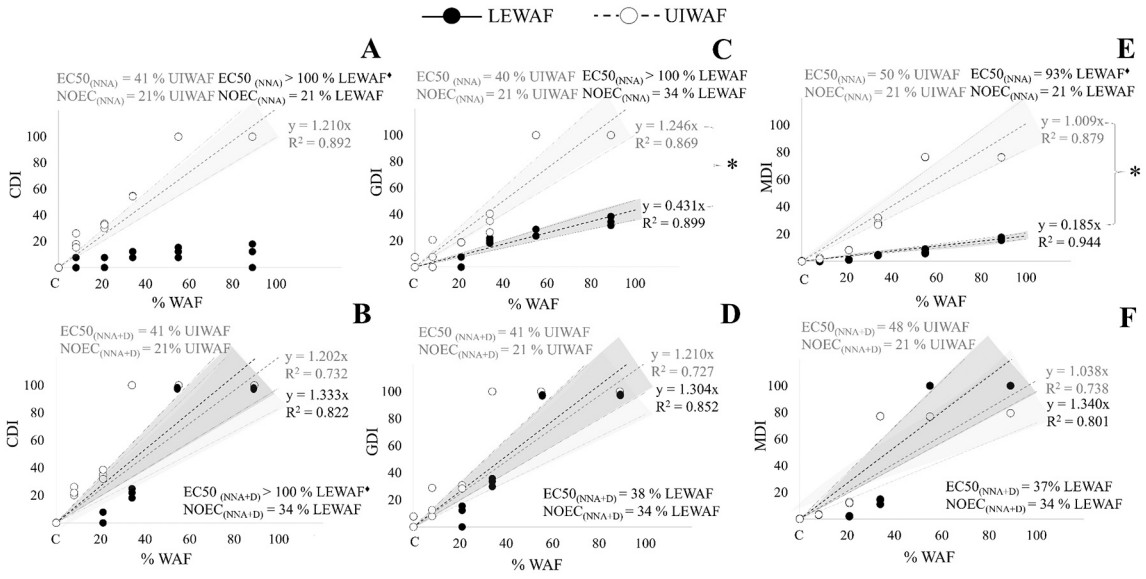

Fig. 3. CDI (Cleavage Disruption Index), GDI (Gastrulation Disruption Index), MDI (Metamorphosis Disruption Index) on exposure to oil and oil+D WAF weathered under ice (UI) or produced at 10 °C (LEWAF). (A) CDI for NNA UIWAF and NNA LEWAF; (B) CDI for NNA+D UIWAF and NNA+D LEWAF; (C) GDI for NNA UIWAF and NNA LEWAF; (D) GDI for NNA+D UIWAF and NNA+D LEWAF; (E) MDI for NNA UIWAF and NNA LEWAF; and (F) MDI for NNA+D UIWAF and NNA+D LEWAF. Legend and abbreviations as in Fig. 2.

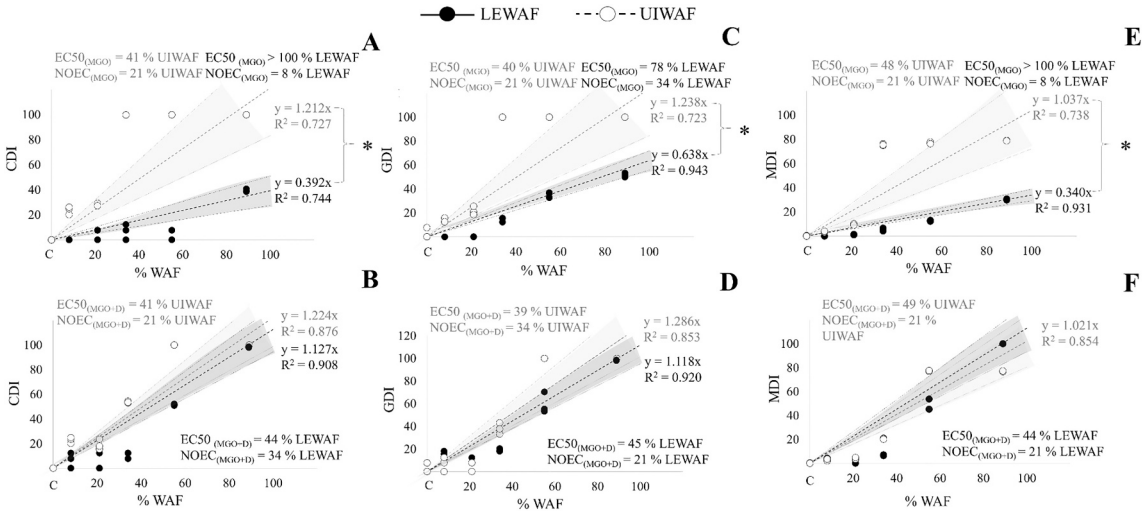

Fig. 4. CDI (Cleavage Disruption Index), GDI (Gastrulation Disruption Index), MDI (Metamorphosis Disruption Index) on exposure to oil and oil+D WAF weathered under ice (UI) or produced at 10 °C (LEWAF). (A) CDI for MGO UIWAF and MGO LEWAF; (B) CDI for MGO+D UIWAF and MGO+D LEWAF; (C) GDI for MGO UIWAF and MGO LEWAF; (D) GDI for MGO+D UIWAF and MGO+D LEWAF; (E) MDI for MGO UIWAF and MGO LEWAF; and (F) MDI for MGO+D UIWAF and MGO+D LEWAF. Legend and abbreviations as in Fig. 2.

UIWAF and LEWAF of the three oils, with and without dispersant, except for IFO + D UIWAF, DNA damage being always more marked in the case of LEWAF than in UIWAF (Fig. 7).

TU values were always very low (<0.20) after oil+D UIWAF exposure (Table 2) and relatively high after oil+D LEWAF exposure (TU > 1; Table 3). RT_i was greater than "1" after exposure to the UIWAF and LEWAF of the three oils combined with the dispersant (Tables 2 and 3). RT_i/RC_i values greater than "1" were found for various individual PAHs after oil+D UIWAF exposure (Table 2): (a) 2-MN, Pyr and Fluo; and (b) Ant (only in IFO + D). Alike, RT_i/RC_i was also greater than "1" after oil+D LEWAF exposure for various individual PAHs (Table 3): (a) 2-MN, Pyr, Fluo and B[*a*]A + Chr; (b) Phe (only in MGO + D); and (c) Ant and B[*a*]P (only in IFO + D).

4. Discussion

4.1. PAH concentration and profile in aqueous fractions of under-ice weathered oil

The ∑PAHs (with and without Naph) in UIWAF was always lower than in the corresponding LEWAF (DeMiguel-Jiménez et al., 2021), which can be explained because the conditions to produce LEWAF (short-term, low-energy stirring, closed system, 10 °C; Katsumiti et al., 2019, after Singer et al., 2000) and UIWAF (long-term −2 months–, static, open ice-cover system, −4 ± 2 °C) were different. Unlike LEWAF production, long-term under ice weathering includes evaporation, partitioning through ice, and specific photoxidation and biodegradation of oil compounds (Payne et al., 1991; Faksness et al., 2008, 2011; Desmond et al., 2019; Saltymakova et al., 2020). Thus, after long-term under ice

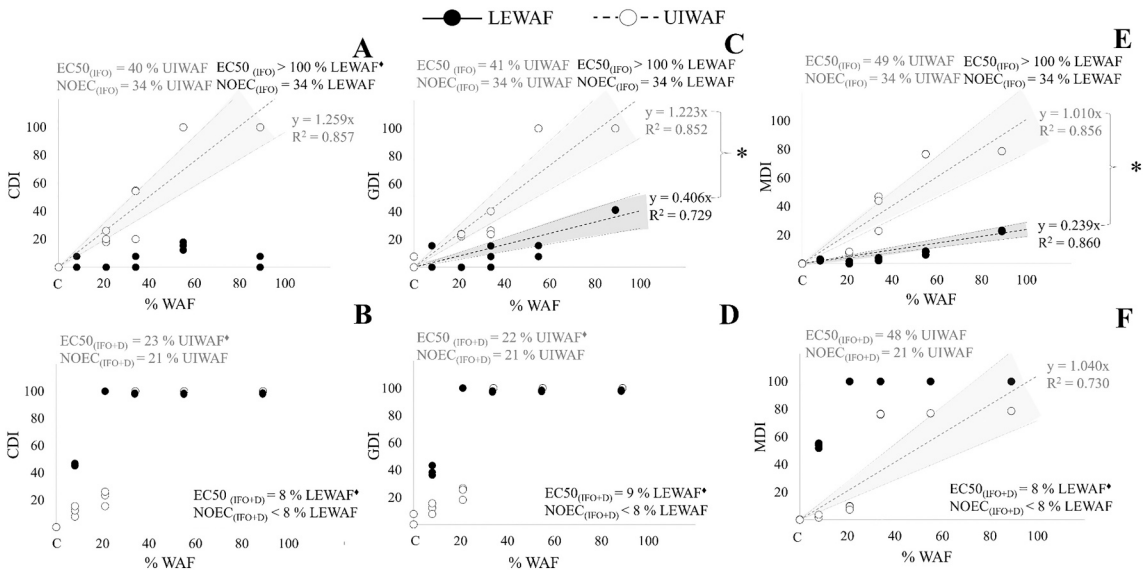

Fig. 5. CDI (Cleavage Disruption Index), GDI (Gastrulation Disruption Index), MDI (Metamorphosis Disruption Index) on exposure to oil and oil+D WAF weathered under ice (UI) or produced at 10 °C (LEWAF). (A) CDI for IFO UIWAF and IFO LEWAF; (B) CDI for IFO+D UIWAF and IFO+D LEWAF; (C) GDI for IFO UIWAF and IFO LEWAF; (D) GDI for IFO+D UIWAF and IFO+D LEWAF; (E) MDI for IFO UIWAF and IFO LEWAF; and (F) MDI for IFO+D UIWAF and IFO+D LEWAF. Legend and abbreviations as in Fig. 2.

weathering the majority of the hydrocarbons of a light-medium crude oil was found to be in the ice (70.2%) or evaporated (19%), despite of the presence of ice (Desmond et al., 2019). Evaporation was relevant for aliphatic compounds, low molecular weight (LMW) alkylbenzenes and some water soluble compounds (e.g., Naph), but less relevant for high molecular weight (HMW) compounds (Desmond et al., 2019). As a result, only a 2.5% of the hydrocarbons (mainly PAHs, in particular, Naph and 1-MN, 2-MN and 3-MN-; and LMW alkylbenzenes and aryl-isoprenoids) were in the water column (Desmond et al., 2019). In agreement, the PAH concentrations recorded in the present study in UIWAFs were lower than in LEWAFs. The values of ∑PAHs (with Naph) were in the range of 42 to 134 ng/mL depending on the oil, which are comparable to the 48 ng PAH/mL reported after weathering under ice (Desmond et al., 2019) and 1–2 fold lower than the 100–1000 ng/mL reported when WAF was produced in absence of ice (Neff et al., 2000; Faksness et al., 2008; Forth et al., 2017; Bender et al., 2018; Johann et al., 2020b; DeMiguel-Jiménez et al., 2021). Furthermore, the high similarity found amongst the PAH levels measured in the UIWAFs of different oils, in contrast with the differences found amongst LEWAFs (DeMiguel-Jiménez et al., 2021), could be a consequence of long-term weathering under ice. The presence of ice-cover is known to reduce viscosity, flash point and pour point of water-in-oil emulsions (Brandvik and Faksness, 2009), which are relevant parameters regarding oil behaviour and weathering, especially at low temperatures (Faksness et al., 2008).

Although the PAH concentrations recorded in UIWAFs were lower than in LEWAFs of their corresponding oil, UIWAFs and LEWAFs exhibited comparable oil-specific PAH profiles. The chemical profile of a WAF is very unlike that of the parent oil; however, for a given oil load, if the mixing time and temperature allow reaching the equilibrium, each oil type WAF seems to have a characteristic PAH profile irrespective of how the WAF is produced (Faksness et al., 2008). Herein, as reported for the LEWAFs of the same oils (DeMiguel-Jiménez et al., 2021), naphthalenes were also at high concentrations in all the UIWAFs; however, the concentrations were overall much lower in UIWAF than in LEWAF (20–110 vs. 50–400 μg Naph/L, 5–30 vs. 50–200 μg 1-MN/L, and 10–50 vs. 50–350 μg 2-MN/L). Similar Naph concentrations in the range of 100–300 μg/L have been reported in the LEWAF of a variety of oils (Neff et al., 2000; Gardiner et al., 2013; Faksness et al., 2008; Johann et al., 2020b). The lower concentrations recorded in the UIWAFs can be the result of evaporation, which occurs even under ice conditions. Indeed, Naph evaporation is known to prevail during under ice weathering because, despite its high solubility, this compound quickly migrates up through the ice before its dissolution is started (Desmond et al., 2019). Likewise, the profile of PAHs excluding naphthalenes was also oil specific in all the UIWAFs, and was modified by dispersant addition except for the case of IFO. The concentration of Ace, Flu and Phe was high in the UIWAFs. These three PAHs were also at high concentrations in the LEWAF of diverse crude and diesel oils with concentrations comparable to the ones presently recorded (Neff et al., 2000; DeMiguel-Jiménez et al., 2021): 200–3000 ng Ace/L, 600–3000 ng Flu/L, and 100–5000 ng Phe/L. After dispersant addition, Flu and Phe increased in NNA and MGO UIWAF, and Ace, Flu and Phe decreased in IFO UIWAF. In contrast, in the case of LEWAF (DeMiguel-Jiménez et al., 2021), dispersant addition caused a raise in the concentration of Ace, Flu and Phe in NNA and IFO but no change in the PAH profile of MGO. Pyr, Fluo, B[*a*]A + Chr and B[*a*]P in the three oil UIWAFs and Ant in IFO UIWAF were detected at low concentrations and after the addition of dispersant, they tended to decrease, unlike in the case of LEWAF in which IFO + D LEWAF presented much higher concentrations of these PAHs than IFO LEWAF (DeMiguel-Jiménez et al., 2021). Pyr and Ant were also detected at low concentrations in other oil LEWAFs (Neff et al., 2000; DeMiguel-Jiménez et al., 2021), although these were slightly higher than the ones presently recorded in UIWAF: 30–140 ng Pyr/L and 200 ng Ant/L.

4.2. Genotoxicity and embryo toxicity of aqueous fractions of under-ice weathered oil

Crude oil aqueous fraction and individual PAHs such as e.g. Pyr and B[*a*]P produce oxidative stress mediated genotoxicity in marine invertebrates (Wessel et al., 2007; Banni et al., 2010; Han et al., 2017; Xie et al., 2017). Accordingly, sea urchin larvae exposed to the aqueous fractions of the three oils investigated herein exhibited DNA damage. IFO was seemingly less genotoxic than NNA and MGO, which is in agreement with the results obtained after applying the micronucleus test to zebrafish liver cell cultures (Johann et al., 2020a). NNA was the most genotoxic and IFO the least, which correlated positively with the concentrations of Naph in the LEWAFs (Johann et al., 2020a). Likewise, UIWAFs, which presented lower concentrations of Naph compared to LEWAFs, were less genotoxic than these latter. In agreement with

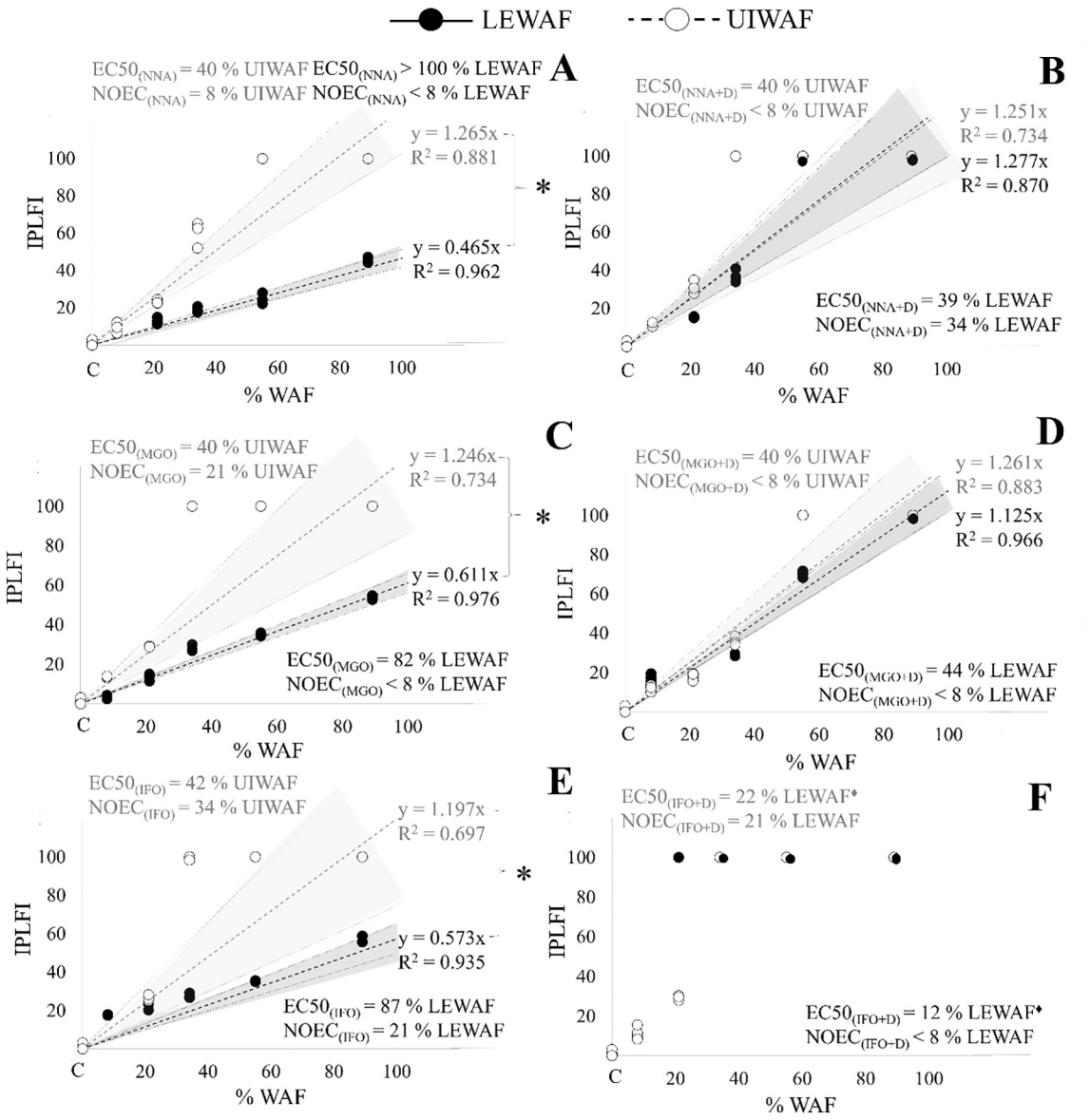

Fig. 6. IPLFI (Inhibition Pluteus Larvae Formation Index) on exposure to oil and oil+D WAF weathered under ice (UI) or produced at 10 °C (LEWAF). (A) NNA UIWAF and NNA LEWAF; (B) NNA+D UIWAF and NNA+D LEWAF; (C) MGO UIWAF and MGO LEWAF; (D) MGO+D UIWAF and MGO+D LEWAF; (E) IFO UIWAF and IFO LEWAF; and (F) IFO+D UIWAF and IFO+D LEWAF. Legend and abbreviations as in Fig. 2.

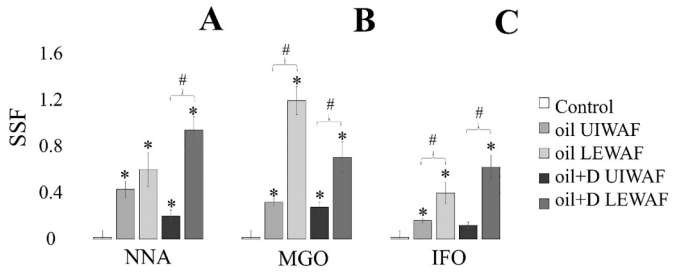

Fig. 7. DNA damage measured in Strand Scission Factor (SSF; mean ± SD) of sea urchin larvae exposed to oil LEWAF (55%), oil UIWAF (55%), oil+D LEWAF (34%) and oil+D UIWAF (34%) of NNA, MGO and IFO. Asterisks indicate significant differences between each treatment and control (ANOVA $p < 0.05$). Pads indicate significant differences between each oil condition (LEWAF or UIWAF) (Student's *t*-test; $p < 0.05$).

previous studies (Johann et al., 2020a), the addition of dispersant did not seem to modify genotoxicity consistently in any case.

It is known that exposure to aqueous fraction of diverse oils provokes toxic effects on pluteus larvae of various sea urchin species (Fernandez et a., 2006; Lv and Xiong, 2009; Bellas et al., 2013; Rial et al., 2013; Alexander et al., 2017; Pereira et al., 2018; DeMiguel-Jiménez et al., 2021). However, no toxicity data to marine organisms exposed to the aqueous fraction of under-ice weathered oil is available, to our knowledge. In the present study, exposure to the oil UIWAF and LEWAF of the three tested oils caused reduced lengthening (ΔL), abnormalities (TI) and development impairment (CDI, GDI, MDI, IPLFI) in pluteus larvae of *P. lividus*. Overall, even though the PAH concentrations recorded in UIWAFs were lower than in LEWAFs of their corresponding oil, toxic effects were more marked after UIWAF exposure, especially for NNA and IFO. It seems therefore that long-term under-ice weathering can contribute to enhance toxicity of oil aqueous fractions irrespective of the measured PAH concentrations. In a previous study, the toxicity of the 1:40 LEWAFs of the oils herein studied was only to a low degree

Table 2
Summary of the TU analysis of the toxicity of under ice weathered WAF (UIWAF) based on the mixture of identified PAHs. The sum of TUs (∑TU) for each toxicity endpoint (ΔL, TI, IPLFI, CDI, GDI and MDI) is "1" if there is additive toxicity, ">1" if there is synergistic effecst and "<1" if the toxicity is not caused by the mixture assuming the CA joint action. The sum of the TUs of individual PAHs vs. the TUs of the sum of PAHs ($\sum TU_{PAHi}/TU_{\sum PAHs}$) is "1" if all the PAHs in the mixture exert the same toxicity, ">1" if there are one or more individual PAHs with more toxicity than expected from its contribution to the mixture according to the CA model; and "<1" otherwise. The balance between the relative contribution of an individual PAH to the toxicity of the mixture and its relative contribution to the chemical composition of the mixture (RT_i/RC_i) is "1" if the individual toxicity of this PAH is the one expected due to its proportion in the mixture (CA model); "<1" if it is not a contributor to the mixture toxicity; and ">1" if there this PAH exerts toxicity beyond the one expected as a part of the mixture. ΔL: pluteus larvae lengthening; CDI: cleavage disruption index; GDI: gastrulation disruption index; MDI: metamorphosis disruption index; TI: toxicity index; IPLFI: inhibition of pluteus larvae formation index.

	NNA UIWAF	NNA+D UIWAF	MGO UIWAF	MGO+D IUWAF	IFO UIWAF	IFO+D UIWAF
$\sum TU_{\Delta L}$	0.05	0.07	0.01	0.07	0.05	0.02
$\sum TU_{TI}$	0.11	0.16	0.03	0.09	0.10	0.03
$\sum TU_{IPLFI}$	0.10	0.15	0.03	0.09	0.10	0.03
$\sum TU_{CDI}$	0.11	0.15	0.03	0.09	0.09	0.03
$\sum TU_{GDI}$	0.11	0.15	0.03	0.09	0.10	0.03
$\sum TU_{MDI}$	0.12	0.18	0.04	0.11	0.12	0.04
$\sum TU_{PAHi}/TU_{\sum PAHs}$	1.63	1.74	1.49	1.49	2.20	1.76
RT/RC_{Naph}	0.08	0.10	0.12	0.12	0.08	0.10
$RT/RC_{1\text{-}MN}$	0.59	0.79	0.91	0.92	0.62	0.78
$RT/RC_{2\text{-}MN}$	2.16	2.87	3.34	3.34	2.26	2.83
RT/RC_{Acy}	–	0.57	0.66	0.66	0.45	0.56
RT/RC_{Ace}	0.48	0.63	0.74	0.74	0.50	0.62
RT/RC_{Flu}	0.19	0.25	0.29	0.29	0.19	0.24
RT/RC_{Ant}	–	–	–	–	19.22	24.05
RT/RC_{Phe}	0.86	1.14	1.32	1.33	0.90	1.12
RT/RC_{Pyr}	2.84	3.78	4.40	4.41	2.98	3.73
RT/RC_{Fluo}	1.45	1.93	2.24	2.25	1.52	1.90
$RT/RC_{B[a]}$	36.69	48.72	–	56.86	38.44	48.11
$RT/RC_{B[a]P}$ A+Chr	2.45	–	3.78	–	2.56	–

Table 3
Summary of the TU analysis of the toxicity of LEWAF based on the mixture of identified PAHs. The sum of TUs (∑TU) for each toxicity endpoint (ΔL. TI. IPLFI. CDI. GDI and MDI) is "1" if there is additive toxicity. ">1" if there is synergistic effecst and "<1" if the toxicity is not caused by the mixture assuming the CA joint action. The sum of the TUs of individual PAHs vs. the TUs of the sum of PAHs ($\sum TU_{PAHi}/TU_{\sum PAHs}$) is "1" if all the PAHs in the mixture exert the same toxicity. ">1" if there are one or more individual PAHs with more toxicity than expected from its contribution to the mixture according to the CA model; and "<1" otherwise. The balance between the relative contribution of an individual PAH to the toxicity of the mixture and its relative contribution to the chemical composition of the mixture (RT_i/RC_i) is "1" if the individual toxicity of this PAH is the one expected due to its proportion in the mixture (CA model); "<1" if it is not a contributor to the mixture toxicity; and ">1" if there this PAH exerts toxicity beyond the one expected as a part of the mixture. ΔL: pluteus larvae lengthening; CDI: cleavage disruption index; GDI: gastrulation disruption index; MDI: metamorphosis disruption index; TI: toxicity index; IPLFI: inhibition of pluteus larvae formation index.

	NNA LEWAF	NNA+D LEWAF	MGO LEWAF	MGO+D LEWAF	IFO LEWAF	IFO+D LEWAF
$\sum TU_{\Delta L}$	1.43	0.84	0.20	0.07	0.41	0.14
$\sum TU_{TI}$	1.55	1.03	0.23	0.09	0.70	0.08
$\sum TU_{IPLFI}$	1.61	1.03	0.27	0.10	0.61	0.14
$\sum TU_{CDI}$	2.01	2.64	0.33	0.10	0.70	0.09
$\sum TU_{GDI}$	1.57	1.00	0.26	0.10	0.70	0.10
$\sum TU_{MDI}$	1.87	0.98	0.33	0.10	0.70	0.09
$\sum TU_{PAHi}/TU_{\sum PAHs}$	2.31	2.22	1.46	1.46	2.22	3.15
RT/RC_{Naph}	0.25	0.08	0.12	0.12	0.08	0.06
$RT/RC_{1\text{-}MN}$	1.92	0.61	0.94	0.93	0.62	0.43
$RT/RC_{2\text{-}MN}$	7.01	2.24	3.41	3.41	2.25	1.58
RT/RC_{Acy}	1.39	0.44	0.67	0.67	0.44	0.31
RT/RC_{Ace}	1.55	0.50	0.75	0.75	0.50	0.35
RT/RC_{Flu}	0.60	0.19	0.29	0.29	0.19	0.14
RT/RC_{Ant}	–	–	–	–	19.11	13.45
RT/RC_{Phe}	2.79	0.89	1.35	1.35	0.89	0.63
RT/RC_{Pyr}	9.24	2.96	4.49	4.49	2.96	2.08
RT/RC_{Fluo}	4.71	1.51	2.29	2.29	1.51	1.06
$RT/RC_{B[a]}$	119.22	38.13	57.98	57.92	38.22	26.89
$RT/RC_{B[a]P}$ A+Chr	7.95	–	–	–	–	1.79

attributable to the measured PAHs and it was seemingly caused by individual or combined toxic action of other non-identified compounds present in the LEWAFs (DeMiguel-Jiménez et al., 2021).

In agreement with previous studies (DeMiguel-Jiménez et al., 2021), the toxic effects of the oil LEWAFs were much higher after the application of dispersant in the three oils, reaching toxicity levels comparable to those recorded after UIWAF exposure. Dispersant application further decreased larvae lengthening, and increased the values of the Toxicity Index and the Inhibition of Pluteus Larvae Formation Index; especially on exposure to NNA + D and IFO + D LEWAF, and less markedly on exposure to MGO + D LEWAF. Dispersant enhanced toxicity was especially remarkable in the cases of IFO + D UIWAF and LEWAF, in which an all-or-nothing response was found instead of a linear dose-response. Overall, adding dispersant to crude oils enhances the toxicity of the WAF (Epstein et al., 2000; Lee et al., 2013; Rial et al., 2014; Dussauze et al., 2015; DeLeo et al., 2016; Katsumiti et al., 2019; Johann et al., 2020b; DeMiguel-Jiménez et al., 2021), which can be explained because dispersant addition may increase the amount of PAHs and alter the PAH profile in the LEWAFs (Yamada et al., 2003; DeLorenzo et al., 2017). However, although chemical dispersion is effective even in presence of 90% ice-coverage (Brandvik et al., 2010) and in nearly freezing water (Belore et al., 2009), the toxicity of the UIWAF of oils alone or in combination with dispersant was similar. It is likely that under long-term weathering under ice the dispersant effect is less evident because the aqueous fraction reaches its full stability in both cases and differences in the chemical profiles of the two stable conditions would be minimal. Indeed, whilst the PAH levels and composition in the LEWAFs changed upon the addition of dispersant in comparison with the LEWAFs of oils without dispersant (DeMiguel-Jiménez et al., 2021), changes were minimal and less consistent in the case of UIWAFs.

4.3. PAH contribution to toxicity of aqueous fractions of under-ice weathered oil

TU values were always very low (<0.20) after UIWAF exposure. In contrast, on exposure to LEWAF these values were higher, both with and without dispersant, most remarkably for NNA (TU > 1). Oil toxicity is frequently attributed only to the identified compounds (e.g., PAHs) known to be toxic; yet, other hydrocarbons and organic compounds are likely contributors to crude oil toxicity (Melbye et al., 2009). Presently, the higher toxicity of UIWAF in comparison with LEWAF is not related to the concentrations of measured PAHs (ESM 6). The EC50s obtained as a function of ∑PAH concentrations varied for every endpoint in a range of several folds. Therefore, the mixture of the measured PAHs is not sufficient to explain the toxicity exerted by the UIWAF and LEWAF of the tested oils alone and in combination with dispersant. Moreover, EC50s were comparable amongst the tested oil aqueous fractions in terms of % UIWAF (20–30% for ΔL, sensitive; and 40–50% for other endpoints, less sensitive) but disparate in terms of ∑PAH concentrations (ESM 6A). A similar inconsistency was evident when the toxicity of oil and oil+D LEWAFs were compared (ESM 6B). Moreover, NNA + D LEWAF was more toxic than NNA LEWAF (in terms of %LEWAF) but similarly toxic in terms of ∑PAH concentrations; and IFO + D LEWAF was more toxic than IFO LEWAF in terms of %LEWAF but similarly toxic in terms of ∑PAH concentrations (ESM 6B). Yet, MGO + D LEWAF was slightly more toxic than MGO LEWAF in terms of %LEWAF, but much less toxic

(5× times) in terms of ∑PAH concentrations (ESM 6B). Thus, EC50s as a function of ∑PAH concentrations cannot explain (or explain only partially) the observed toxicity. Therefore, PAHs do not seem to be the major determinants of oil WAF toxicity to sea urchin early life stages, in agreement with previous studies (Barron et al., 1999; Johann et al., 2020b; DeMiguel-Jiménez et al., 2021). In the present study, only a few representative PAHs (USEPA 16 list) were analysed assuming the widespread practice in environmental monitoring that the oil toxicity to marine organisms is due to the aromatic hydrocarbon fraction (Pelletier et al., 1997). Yet, PAHs constitute less than 1% of the total petroleum hydrocarbons in crude oil; the 99% is made of polar compounds and the unresolved complex mixture (UCM; >250,000 compounds), which are resistant to weathering and toxic (Barron et al., 1999; Neff et al., 2000; Booth et al., 2007; Lang et al., 2009; Melbye et al., 2009; Sammarco et al., 2013).

DeMiguel-Jiménez et al. (2021) also found that the relative contribution of the measured PAHs (16 USEPA list) to the toxicity of LEWAFs of the three oils studied herein, alone or in combination with dispersant, was low. According to the present results, contribution of these PAHs to the toxicity of the UIWAFs seems to be even lower. After weathering, HMW PAHs and their hydroxyl and alkyl derivatives, which are at relatively low proportion of the WAF compounds (Carls et al., 1999), could become relevant contributors to the toxicity of the mixture (e.g. as shown for fish embryos; Heintz et al., 1999). Hydroxyl PAHs, pyrenol and phenanthrol formed during oil weathering are known to be more toxic than parental PAHs to larvae of sea urchin, *P. lividus* (Saco-Álvarez et al., 2008). Likewise, the WAF of weathered Prestige fuel oil had lower PAH concentrations but higher toxicity to sea urchin embryos than the WAF of fresh fuel oil (Saco-Álvarez et al., 2008); and long-term weathering (80 d) caused up to eightfold increase in toxicity to sea urchin and mussel embryos that was unrelated to the PAH concentrations (Bellas et al., 2013).

Nevertheless, the potential risk posed by the toxicity of the identifed PAHs should not be neglected a priori, especially in the long-term and after weathering (Incardona et al., 2004, 2005, 2009; Hendon et al., 2008; Frantzen et al., 2012). Present RT_i values higher than "1" suggest that one or more individual PAHs could exhibit more toxicity than the one predicted for the mixture toxicity (DeMiguel-Jiménez et al., 2021). This is the case of 2-MN, Ant, Pyr, Fluo and B[*a*]A + Chr in both UIWAFs and LEWAFs of the three oils, with and without dispersant, B[*a*]P for all the UIWAFs, and Ace, Acy and Phe for all the LEWAFs ($RT_i/RC_i > 1$). In marine organisms, 2-MN is toxic via non-polar narcosis (Falk-Petersen et al., 1982; De Hoop et al., 2011; Olsen et al., 2011). Pyr causes severe oxidative stress, genotoxicity, immunotoxicity, neurotoxicity, peroxisome proliferation, reproductive disruption and behavioral alterations (Frantzen et al., 2012). Fluo produces acute toxicity (Rossi and Neff, 1978; Horne and Oblad, 1983; Gendusa, 1990; Suedel et al., 1993). Pyr, Fluo and B[*a*]A affect spicule formation and larvae lengthening in sea urchin larvae (Bellas et al., 2008; Sekiguchi et al., 2018). B[*a*]P is genotoxic, carcinogenic (via the AhR-CYP1 pathway) and endocrine disruptor (Banni et al., 2010; Booc et al., 2014; Alharthy et al., 2017). Phe and Chr are carcinogenic and their metabolites exert endocrine disrupting effects (USEPA, 2000). Yet, the effective concentrations reported for these individual PAHs are 1–3 orders of magnitude higher than the ones corresponding to the EC50 values calculated herein. Therefore, it is quite unlikely that these individual PAHs showing RT_i/RC_i ratios higher than "1" might constitute a realistic concern regarding UIWAF toxicity.

5. Concluding remarks

A multi-index approach of the SET test that included larval size increase, larval malformation and developmental disruption as endpoints was applied. It was concluded that the long-term weathering under ice contributed to enhance the toxicity of the aqueous fraction of crude and bunker oils representative of prospective oil spill threats in Arctic and Sub-Arctic seas, irrespective of the use of a third generation dispersant. The PAH levels measured in UIWAFs were lower than in the corresponding LEWAF, and they were similar amongst the UIWAFs of different oils tested, which would be the direct consequence of long-term weathering under ice. Moreover, the addition of dispersant produced minimal and less consistent changes in the PAH levels, composition and toxicity in the UIWAFs in comparison with the LEWAFs. Yet, UIWAF was always more toxic than LEWAF for the three tested oils, with and without dispersant. Certainly, both UIWAF and LEWAF caused length reduction, abnormalities, and development impairment in sea urchin embryo, but the effects were more marked in the former case. Conversely, UIWAF was less genotoxic than LEWAF and the addition of dispersant did not modify genotoxicity consistently.

CRediT authorship contribution statement

Laura DeMiguel-Jiménez: Methodology, Investigation, Visualization, Writing – original draft, Writing – review & editing. **Nestor Etxebarria:** Methodology, Formal analysis. **Helena C. Reinardy:** Methodology, Formal analysis. **Xabier Lekube:** Methodology, Investigation. **Ionan Marigómez:** Conceptualization, Funding acquisition, Formal analysis, Supervision, Writing – original draft, Writing – review & editing. **Urtzi Izagirre:** Conceptualization, Investigation, Writing – original draft, Supervision.

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.

Acknowledgments

The authors would like to gratefully acknowledge financial support from the European Union's Horizon 2020 research and innovation program EU H2020-BG-2005-2 under grant agreement No 679266 (project GRACE), from the Spanish Ministry of Education, Culture and Sport through pre-doctoral fellowship FPU15/05517, and from the Basque Government through Consolidated Research Group GIC IT810-13 & IT1302-19. The authors acknowledge Total Spain for kindly providing the dispersant.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2022.113345.

References

Adeyemo, O.K., Kroll, K.J., Denslow, N.D., 2015. Developmental abnormalities and differential expression of genes induced in oil and dispersant exposed Menidia beryllina embryos. Aquat. Toxicol. 168, 60–71.

Afenyo, M., Khan, F., Veitch, B., Yang, M., 2016. Modeling oil weathering and transport in sea ice. Mar. Pollut. Bull. 107, 206–215.

Alexander, F.J., King, C.K., Reichelt-Brushett, A.J., Harrison, P.L., 2017. Fuel oil and dispersant toxicity to the Antarctic Sea urchin (Sterechinus neumayeri). Environ. Toxicol. Chem. 36, 1563–1571.

Alharthy, K.M., Albaqami, F.F., Thornton, C., Corrales, J., Willett, K.L., 2017. Mechanistic evaluation of benzo[a]pyrene's developmental toxicities mediated by reduced Cyp19a1b activity. Toxicol. Sci. 155, 135–147.

Altenburger, R., Nendza, M., Schüürmann, G., 2003. Mixture toxicity and its modeling by quantitative structure-activity relationships. Environ. Toxicol. Chem. 22, 1900–1915.

Banni, M., Negri, A., Dagnino, A., Jebali, J., Ameur, S., Boussetta, H., 2010. Acute effects of benzo[a]pyrene on digestive gland enzymatic biomarkers and DNA damage on mussel Mytilus galloprovincialis. Ecotoxicol. Environ. Saf. 73, 842–848.

Barron, M.G., Podrabsky, T., Ogle, S., Ricker, R.W., 1999. Are aromatic hydrocarbons the primary determinant of petroleum toxicity to aquatic organisms? Aquat. Toxicol. 46, 253–268.

Beiras, R., Durán, I., Bellas, J., Sánchez-Marín, P., 2012. Biological effects of contaminants: Paracentrotus lividus sea urchin embryo test with marine sediment elutriates. ICES TIMES 51, 1–13.

Bellas, J., Saco-Álvarez, L., Nieto, Ó., Beiras, R., 2008. Ecotoxicological evaluation of polycyclic aromatic hydrocarbons using marine invertebrate embryo–larval bioassays. Mar. Pollut. Bull. 57, 493–502.
Bellas, J., Saco-álvarez, L., Nieto, T., Bayona, J.M., Albaigés, J., Beiras, R., 2013. Evaluation of artificially-weathered standard fuel oil toxicity by marine invertebrate embryogenesis bioassays. Chemosphere 90, 1103–1108.
Belore, R.C., Trudel, K., Mullin, J.V., Guarino, A., 2009. Large-scale cold water dispersant effectiveness experiments with alaskan crude oils and corexit 9500 and 9527 dispersants. Mar. Pollut. Bull. 58, 118–128.
Bender, M., Frantzen, M., Camus, L., Le Floch, S., Palerud, J., Nahrgang, J., 2018. Effects of acute exposure to dispersed oil and burned oil residue on long-term survival, growth, and reproductive development in polar cod (Boreogadus saida). Mar. Environ. Res. 140, 468–477.
Boccadoro, C., Krolicka, A., Receveur, J., Aeppli, C., Floch, S.L., 2018. Microbial community response and migration of petroleum compounds during a sea-ice oil spill experiment in Svalbard. Mar. Environ. Res. 142, 214–233.
Booc, F., Thornton, C., Lister, A., MacLatchy, D., Willett, K.L., 2014. Benzo[a]pyrene effects on reproductive endpoints in Fundulus heteroclitus. Toxicol. Sci. 140, 73–82.
Booth, A.M., Sutton, P.A., Lewis, C.A., Lewis, A.C., Scarlett, A., Chau, W., Widdows, J., Rowland, S.J., 2007. Unresolved complex mixtures of aromatic hydrocarbons: thousands of overlooked persistent, bioaccumulative and toxic contaminants im mussels. Environ. Sci. Technol. 41, 457–464.
Brandvik, P.J., Faksness, L., 2009. Weathering processes in Arctic oil spills: meso-scale experiments with different ice conditions. Cold Reg. Sci. Technol. 55, 160–166.
Brandvik, J., Myrhaug, J.L., Daling, P.S., Leirvik, F., Fritt-Rasmussen, J., 2010. Meso-scale weathering of oil as a function of ice conditions. Oil properties, dispersibility and in situ burnability of weathered oil as a function of time. In: SINTEF Materials and Chemistry, Oil in Ice JIP, Rep. No. 19.
Calbet, A., Saiz, E., Barata, C., 2006. Lethal and sublethal effects of naphthalene and 1,2-dimethylnaphthalene on the marine copepod paracartia grani. Mar. Biol. 151, 195–204.
Carballeira, C., Ramos-Gómez, J., Martín-Díaz, L., DelValls, T.A., 2012. Identification of specific malformations of sea urchin larvae for toxicity assessment: application to marine pisciculture effluents. Mar. Environ. Res. 77, 12–22.
Carls, M.G., Rice, S.D., Hose, J.E., 1999. Sensitivity of fish embryos to weathered crude oil: part I. Low-level exposure during incubation causes malformations, genetic damage, and mortality in larval pacific herring (Clupea pallasi). Environ. Toxicol. Chem. 18, 481–493.
Daling, P.S., Brandvik, P.J., Singsaas, I., Lewis, A., 2012. Dispersant Effectiveness Testing of Crude Oils Weathered Under Various Ice Conditions. Interspill London.
De Hoop, L., Schipper, A.M., Leuven, R.S.E.W., Huijbregts, M.A.J., Olsen, G.H., Smit, M. G.D., Hendriks, A.J., 2011. Sensitivity of polar and temperate marine organisms to oil components. Environ. Sci. Technol. 45, 9017–9023.
DeLeo, D.M., Ruiz-Ramos, D.V., Baums, I.B., Cordes, E.E., 2016. Response of deep-water corals to oil and chemical dispersant exposure. Deep-Sea Res. II 129, 137–147.
DeLorenzo, M.E., Key, P.B., Chung, K.W., Pisarski, E., Shaddrix, B., Wirth, F., Pennington, P.L., Wade, J., Franco, M., Fulton, M.H., 2017. Comparative toxicity to two chemical dispersants and dispersed oil in estuarine organisms. Arch. Environ. Contam. Toxicol. 74, 414–430.
DeMiguel-Jiménez, L., Etxebarria, N., Lekube, X., Izagirre, U., Marigómez, I., 2021. Influence of dispersant application on the toxicity to sea urchin embryos of crude and bunker oils representative of prospective oil spill threats in Arctic and sub-Arctic seas. Mar. Pollut. Bull. 172, 112922.
Desmond, D.S., Saltymakova, D., Neusitzer, T.D., Firoozy, N., Isleifson, D., Barber, D.G., Stern, G.A., 2019. Oil behavior in sea ice: changes in chemical composition and resultant effect on sea ice dielectrics. Mar. Pollut. Bull. 142, 216–233.
Dussauze, M., Pichavant-Rafini, K., Le Floch, S., Lemaire, P., Theron, M., 2015. Acute toxicity of chemically and mechanically dispersed crude oil to juvenile sea bass (Dicentrarchus labrax): absence of synergistic effects between oil and dispersants. Environ. Toxicol. Chem. 34, 1543–1551.
El-Bibany, A.H., Bodnar, A.G., Reinardy, H.C., 2014. Comparative DNA damage and repair in echinoderm coelomocytes exposed to genotoxicants. PLoS ONE 9, e107815.
Epstein, N., Bak, R.P.M., Rinkevich, B., 2000. Toxicity of third generation dispersants and dispersed egyptian crude oil on red sea coral larvae. Mar. Pollut. Bull. 40, 497–503.
Faksness, L., Brandvik, P.J., 2008. Distribution of water soluble components from oil encapsulated in Arctic Sea ice: summary of three field seasons. Cold Reg. Sci. Technol. 54, 106–114.
Faksness, L., Brandvik, P.J., Sydnes, L.K., 2008. Composition of the water accommodated fractions as a function of exposure times and temperatures. Mar. Pollut. Bull. 56, 1746–1754.
Faksness, L.G., Brandvik, P.J., Daae, R.L., Leirvik, F., Børseth, J.F., 2011. Large-scale oil-in-ice experiment in the Barents Sea: monitoring of oil in water and MetOcean interactions. Mar. Pollut. Bull. 62, 976–998.
Falk-Petersen, I., Sæthre, L.J., Lönning, S., 1982. Toxic effects of naphthalene and methylnaphthalenes on marine plankton organisms. Sarsia 67, 171–178.
Fernández, N., Cesar, A., Salamanca, M.J., DelValls, T.A., 2006. Toxicological characterisation of the aqueous soluble phase of the prestige fuel-oil using the sea-urchin embryo bioassay. Ecotoxicology 15, 593–599.
Fingas, M.F., Hollebone, B.P., 2003. Review of behaviour of oil in freezing environments. Mar. Pollut. Bull. 47, 333–340.
Forth, A.P., Mitchelmore, C.L., Morris, J.M., Lay, C.L., Lipton, J., 2017. Characterization of dissolved and particulate phases of water accommodated fractions used to conduct aquatic toxicity testing in support of the Deepwater horizon natural resource damage assessment. Environ. Toxicol. Chem. 36, 1460–1472.
Frantzen, M., Falk-Petersen, I., Nahrgang, J., Smith, T.J., Olsen, G.H., Hangstad, T.A., Camus, L., 2012. Toxicity of crude oil and pyrene to the embryos of beach spawning capelin (Mallotus villosus). Aquat. Toxicol. 108, 42–52.
Fritt-Rasmussen, J., Wegeberg, S., Gustavson, K., 2015. Review on burn residues from in situ burning of oil spills in relation to arctic waters. Water Air Soil Pollut. 226, 329.
Gardiner, W.W., Word, J.Q., Word, J.D., Perkins, R.A., McFarlin, K.M., Hester, B.W., Word, L.S., Ray, C.M., 2013. The acute toxicity of chemically and physically dispersed crude oil to key Arctic species under Arctic conditions during the open water season. Environ. Toxicol. Chem. 32, 2284–2300.
Gautier, D.L., Bird, K.J., Charpentier, R.R., Grantz, A., Houseknecht, D.W., Klett, T.R., Moore, T.E., Pitman, J.K., Schenk, C.J., Schuenemeyer, J.H., Sørensen, K., Tennyso, M.E., Valin, Z.C., Wandrey, C.J., 2009. Assessment of undiscovered oil and gas in the Arctic. Science 324, 1175–1179.
Gendusa, A.C., 1990. Toxicity of Chromium and Fluoranthene From Aqueous and Sediment Sources to Selected Freshwater Fish. PhD Thesis. University of North Texas, Denton, TX, 138 pp.
Han, J., Kim, H., Kim, I., Kim, S., Hwang, U., Lee, J., 2017. Effects of water accommodated fractions (WAFs) of crude oil in two congeneric copepods tigriopus sp. Ecotoxicol. Environ. Saf. 145, 511–517.
Heintz, R.A., Short, J.W., Rice, Sd., 1999. Sensitivity of fish embryos to weathered crude oil: part ii. Increased mortality of pink salmon (Oncorhynchus gorbuscha) embryos incubating downstream from weathered exxon Valdez crude oil. Environ. Toxicol. Chem. 18, 494–503.
Hendon, L.A., Carlson, E.A., Manning, S., Brouwer, M., 2008. Molecular and developmental effects of exposure to pyrene in the early life-stages of Cyprinodon variegatus. Comp. Biochem. Physiol. 147, 205–215.
Holcombe, G.W., Phipps, G.L., Fiandt, J.T., 1983. Toxicity of selected priority pollutants to various aquatic organisms. Ecotoxicol. Environ. Saf. 7, 400–409.
Horne, J.D., Oblad, B.R., 1983. Aquatic toxicity studies of six priority pollutants. In: Final Report/Task II. US Environmental Protection Agency, Contract No. 68-01-6201. NUS Corporation, Houston, TX, 196 pp.
Incardona, J.P., Collier, T.K., Scholz, N.L., 2004. Defects in cardiac function precede morphological abnormalities in fish embryos exposed to polycyclic aromatic hydrocarbons. Toxicol. Appl. Pharmacol. 196, 191–205.
Incardona, J.P., Carls, M.G., Teraoka, H., Sloan, C.A., Collier, T.K., Scholz, N.L., 2005. Aryl hydrocarbon receptor-independent toxicity of weathered crude oil during fish development. Environ. Health Perspect. 113, 1755–1762.
Incardona, J.P., Carls, M.G., Day, H.L., Sloan, C.A., Bolton, J.L., Collier, T.K., Scholz, N. L., 2009. Cardiac arrhythmia is the primary response of embryonic Pacific herring (Clupea pallasi) exposed to crude oil during weathering. Environ. Sci. Technol. 43, 201–207.
Johann, S., Goßen, M., Behnisch, P.A., Hollert, H., Seiler, T.B., 2020a. Combining different in vitro bioassays to evaluate genotoxicity of water-accommodated fractions from petroleum products. Toxics 8, 45.
Johann, S., Nüßer, L., Goßen, M., Hollert, H., Seiler, T.B., 2020b. Differences in biomarker and behavioral responses to native and chemically dispersed crude and refined fossil oils in zebrafish early life stages. Sci. Total Environ. 709, 136–174.
Jørgensen, K.S., Kreutzer, A., Lehtonen, K.K., Kankaanpää, H., Rytkönen, J., Wegeberg, S., Gustavson, K., Fritt-Rasmussen, J., Truu, J., Kõuts, T., Lilover, M.J., Seiler, T.B., Hollert, H., Johann, S., Marigómez, I., Soto, M., Lekube, X., Jenssen, B. M., Ciesielski, T.M., Wilms, L.B., Högström, R., Pirneskoski, M., Virtanen, S., Forsman, B., Petrich, C., Phuong-Dang, N., Wang, F., 2019. The EU horizon 2020 project GRACE: integrated oil spill response actions and environmental effects. Environ. Sci. Eur. 31, 44.
Katsumiti, A., Nicolussi, G., Bilbao, D., Prieto, A., Etxebarria, N., Cajaraville, M.P., 2019. In vitro toxicity testing in hemocytes of the marine mussel Mytilus galloprovincialis (L.) to uncover mechanisms of action of the water accommodated fraction (WAF) of a naphthenic North Sea crude oil without and with dispersant. Sci. Total Environ. 670, 1084–1094.
Knap, A., Turner, N.R., Bera, G., Renegar, D.A., Frank, T., Sericano, J., Riegl, B.M., 2017. Short-term toxicity of 1-methylnaphthalene to Americamysis Bahia and five deep-sea crustaceans. Environ. Toxicol. Chem. 36, 3415–3423.
Lang, D.A., Bastow, T.P., van Aarssen, B.G.K., Warton, B., Davis, G.B., Johnston, C.D., 2009. Polar compounds from the dissolution of weathered diesel. Ground Water Monit. Remidiat. 29, 85–93.
Lee, K., Shim, W.J., Yim, U.H., Kang, J., 2013. Acute and chronic toxicity study of the water accommodated fraction (WAF), chemically enhanced WAF (CEWAF) of crude oil and dispersant in the rock pool copepod Tigriopus japonicus. Chemosphere 92, 1161–1168.
Lv, F., Xiong, D., 2009. Effect of water-accommodated fractions of no.0 diesel oil on embryos and larvae development of sea urchin, Hemicentrotus pulcherrimus. In: Proceed. 3rd Internat Conf Bioinf Biomed Eng, pp. 1–4.
Lyons, B.P., Pascoe, C.K., McFadzen, I.R.B., 2002. Phototoxicity of pyrene and benzo[a] pyrene to embryo–larval stages of the pacific oyster Crassostrea gigas. Mar. Environ. Res. 54, 627–631.
McGrattan, K.B., Walton, W.D., Evans, D.D., 1997. Smoke plumes from in-situ burning of crude oil. In: Proceed Internat Oil Spill Conf, 1, pp. 137–147.
Melbye, A.G., Brakstad, O.G., Hokstad, J.N., Gregersen, I.K., Hansen, B.H., Booth, A.M., Rowland, S.J., Tollefsen, K.E., 2009. Chemical and toxicological characterization of an unresolved complex mixture-rich biodegraded crude oil. Environ. Toxicol. Chem. 28, 1815–1824.
Neff, J.M., Ostazeski, S., Gardiner, W., Stejskal, I., 2000. Effects of weathering on the toxicity of three offshore australian crude oils and a diesel fuel to marine animals. Environ. Toxicol. Chem. 19, 1809–1821.

Nordam, T., Dunnebier, D.A.E., Beegle-Krause, C., Reed, M., Slagstad, D., 2017. Impact of climate change and seasonal trends on the fate of Arctic oil spills. Ambio 46, 442–452.
Nordam, T., Litzler, E., Skancke, J., Singsaas, I., Leirvik, F., Johansen, Ø., 2020. Modelling of oil thickness in the presence of an ice edge. Mar. Pollut. Bull. 156, 111229.
OGP, 2013. Environmental Management in Arctic Oil and Gas Operations: Good Practice Guide. (No. 449). International Association of Oil and Gas Producers, London, United Kingdom.
Øksenvåg, J.H.C., Fossen, M., Farooq, U., 2019. Study on how oil type and weathering of crude oils affect interaction with sea ice and polyethylene skimmer material. Mar. Pollut. Bull. 145, 306–315.
Olsen, G.H., Smit, M.G.D., Carroll, J., Jæger, I., Smith, T., Camus, L., 2011. Arctic versus temperate comparison of risk assessment metrics for 2-methyl-naphthalene. Mar. Environ. Res. 72, 179–187.
Ott, F.S., Harris, R.P., O'Hara, S.C.M., 1978. Acute and sublethal toxicity of naphthalene and three methylated derivatives to the estuarine copepod, Eurytemora affinis. Mar. Environ. Res. 1, 49–58.
Payne, J.R., Mcnabb Jr., G.D., Clayton Jr., J.R., 1991. Oil weathering behavior in Arctic environments. Polar Res. 10, 631–662.
Pelletier, M.C., Burgess, R.M., Ho, K.T., Kuhn, A., McKinney, R.A., Ryba, S.A., 1997. Phototoxicity of individual polycyclic aromatic hydrocarbons and petroleum to marine invertebrate larvae and juveniles. Environ. Toxicol. Chem. 16, 2190–2199.
Pereira, T., Merçon, J., Passos, L., Coppo, G., Lopes, T., Cabral, D.S., Scherer, R., Chippar, Gomes A., 2018. Effects of the water-soluble fraction of diesel oil (WSD) on the fertilization and development of a sea urchin (Echinometra lucunter). Ecotoxicol. Environ. Saf. 162, 59–62.
Pillai, M.C., Vines, C.A., Wikramanayake, A.H., Cherr, G.N., 2003. Polycyclic aromatic hydrocarbons disrupt axial development in sea urchin embryos through a β-catenin dependent pathway. Toxicology 186, 93–108.
Prieto, A., Zuloaga, O., Usobiaga, A., Etxebarria, N., Fernández, L.A., 2007. Development of a stir bar sorptive extraction and thermal desorption -gas chromatography-mass spectrometry method for the simultaneous determination of several persistent organic pollutants in water samples. J. Chromatogr. 1174A, 40–49.
Prince, R.C., Kelley, B.A., Butler, J.D., 2016. Three widely-available dispersants substantially increase the biodegradation of otherwise undispersed oil. J. Mar. Sci. Res. Dev. 6, 183.
Reinardy, H.C., Bodnar, A.G., 2015. Profiling DNA damage and repair capacity in sea urchin larvae and coelomocytes exposed to genotoxicants. Mutagenesis 30, 820–839.
Reinardy, H.C., Chapman, J., Bodnar, A.G., 2016. Induction of innate immune gene expression following methyl methanesulfonate-induced DNA damage in sea urchins. Biol. Lett. 12, 2015105.
Renegar, D.A., Turner, N.R., Riegl, B.M., Dodge, R.E., Knap, A.H., Schuler, P.A., 2017. Acute and subacute toxicity of the polycyclic aromatic hydrocarbon 1-methylnaphthalene to the shallow-water coral Porites divaricata: application of a novel exposure protocol. Environ. Toxicol. Chem. 36, 212–219.
Rial, D., Radović, J.R., Bayona, J.M., Macrae, K., Thomas, K.V., Beiras, R., 2013. Effects of simulated weathering on the toxicity of selected crude oils and their components to sea urchin embryos. J. Hazard. Mater. 260, 67–73.
Rial, D., Vázquez, J.A., Murado, M.A., 2014. Toxicity of spill-treating agents and oil to sea urchin embryos. Sci. Total Environ. 472, 302–308.
Rossi, S.S., Neff, J.M., 1978. Toxicity of polynuclear aromatic hydrocarbons to the polychaete Neanthes arenaceodentata. Mar. Pollut. Bull. 9, 220–223.
Saco-Álvarez, L., Bellas, J., Nieto, O., Bayona, J.M., Albaigés, J., Beiras, R., 2008. Toxicity and phototoxicity of water-accommodated fraction obtained from prestige fuel oil and marine fuel oil evaluated by marine bioassays. Sci. Total Environ. 394, 275–282.
Saltymakova, D., Desmond, D.A., Isleifson, D., Firoozy, N., Neusitzer, T.D., Xu, Z., Lemes, M., Barber, D.G., Stern, G.A., 2020. Effect of dissolution, evaporation, and photooxidation on crude oil chemical composition, dielectric properties and its radar signature in the Arctic environment. Mar. Pollut. Bull. 151, 110629.
Sammarco, P.W., Kolian, S.R., Warby, R.A.F., Bouldin, J.L., Subra, W.A., Porter, S.A., 2013. Distribution and concentrations of petroleum hydrocarbons associated with the BP/Deepwater horizon oil spill, Gulf of Mexico. Mar. Pollut. Bull. 73, 129–143.
Sartz, P., Aggarwal, S., 2016. Gaseous emissions from herding agent-mediated in-situ burning for arctic oil spills. In: Technical Seminar Arctic and Marine Oilspill Program (AMOP), Environment and Climate Change Canada, Ottawa, ON, pp. 735–750.
Scröder, H.C., Batel, R., Schwertner, H.R., Boreiko, O., Müller, W.E.G., 2006. Fast micromethod DNA single-strand-break assay. In: Henderson, D.S. (Ed.), Methods in Molecular Biology: DNA Repair Protocols Mammalian System. Humana Press Inc, pp. 287–305.
Sekiguchi, T., Yachiguchi, K., Kiyomoto, M., Ogiso, S., Wada, S., Tabuchi, Y., Hong, C.S., Srivastay, A.K., Archer, S.D.J., Pointing, S.B., Hayakawa, K., Suzuki, N., 2018. Molecular mechanism of the suppression of larval skeleton by polycyclic aromatic hydrocarbons in early development of sea urchin hemicentrotus pulcherrimus. Fish. Sci. 84, 1073–1079.
Singer, M.M., Aurand, D., Bragin, G.E., Clark, J.R., Coelho, G.M., Sowby, M.L., Tjeerdema, R.S., 2000. Standardization of the preparation and quantitation of water-accommodated fractions of petroleum for toxicity testing. Mar. Pollut. Bull. 40, 1007–1016.
Singsaas, I., Leirvik, F., Daling, P.S., Guénette, C., Sørheim, K.R., 2020. Fate and behaviour of weathered oil drifting into sea ice, using a novel wave and current flume. Mar. Pollut. Bull. 159, 111485.
Soloviev, A.V., Haus, B.K., McGauley, M.G., Dean, C.W., Ortiz-Suslow, D., Laxague, N.J. M., Özgökmen, T.M., 2016. Surface dynamics of crude and weathered oil in the presence of dispersants: laboratory experiment and numerical simulation. J. Geophys. Res. Oceans 121, 3502–3516.
Sørstrøm, S.E., Brandvik, P.J., Buist, I., Daling, P., Dickin, D., Faksness, L.G., Potter, S., Rasmussen, J.F., Singsaas, I., 2010. Joint industry program on oil spill contingency for Arctic and ice-covered waters. In: SINTEF Materials and Chemistry, Oil in Ice. JIP Report, 32, p. 40.
Spehar, R.L., Poucher, S., Brooke, L.T., Hansen, D.J., Champlin, D., Cox, D.A., 1999. Comparative toxicity of fluoranthene to freshwater and saltwater species under fluorescent and ultraviolet light. Arch. Environ. Contam. Toxicol. 37, 496–502.
Sprague, J.B., 1970. Measurement of pollutant toxicity to fish. II. Utilizing and applying bioassay results. Water Res. 4, 3–32.
Steffek, T., Bittler, K., Guarino, A., 2016. Comparative Testing of Corexit EC9500A, Finasol OSR 52, Accell Clean DWD, and ZI 400 at Ohmsett in a Simulated Arctic Environment. U.S. Dept Interior, Bureau of Safety and Environmental Enforcement, 71 pp.
Suedel, B.C., Rodgers Jr., J.H., Clifford, P.A., 1993. Bioavailability of fluoranthene in freshwater sediment toxicity tests. Environ. Toxicol. Chem. 12, 155–165.
Trucco, R.G., Engelhardt, F.R., Stacey, B., 1983. Toxicity, accumulation and clearance of aromatic hydrocarbons in Daphnia pulex. Environ. Pollut. 31A, 191–202.
USEPA, 2000. Toxic Release Inventory Public Data Release. Office of environmental information, United States Environmental Protection Agency, Washington, DC.
Ward, G.S., Parrish, P.R., Rigby, R.A., 1981. Early life stage toxicity tests with a saltwater fish: effects of eight chemicals on survival, growth, and development of sheepshead minnows (Cyprinodon variegatus). J. Toxicol. Environ. Health 8, 225–240.
Wessel, N., Rousseau, S., Caisey, X., Quiniou, F., Akcha, F., 2007. Investigating the relationship between embryotoxic and genotoxic effects of benzo[a]pyrene, 17α-ethinylestradiol and endosulfan on Crassostrea gigas embryos. Aquat. Toxicol. 85, 133–142.
Wilkinson, J., Beegle-Krause, C.J., Evers, K.U., Hughes, N., Lewis, A., Reed, M., Wadhams, P., 2017. Oil spill response capabilities and technologies for ice-covered Arctic marine waters: a review of recent developments and established practices. Ambio 46, 423–441.
Xie, J., Zhao, C., Han, Q., Zhou, H., Li, Q., Diao, X., 2017. Effects of pyrene exposure on immune response and oxidative stress in the pearl oyster, Pinctada martensii. Fish Shellfish Immunol. 63, 237–244.
Yamada, M., Takada, H., Toyoda, K., Yoshida, A., Shibata, A., Nomura, H., Wada, M., Nishimura, M., Okamoto, K., Ohwada, K., 2003. Study on the fate of petroleum-derived polycyclic aromatic hydrocarbons (PAHs) and the effect of chemical dispersant using an enclosed ecosystem, mesocosm. Mar. Pollut. Bull. 47, 105–113.
Yang, H., Ma, M., Thompson, J.R., Flower, R.J., 2018. Transport expansion threatens the Arctic. Science 359, 646–647.