SETAC PRESS

Environmental Toxicology and Chemistry, Vol. 31, No. 6, pp. 1324–1333, 2012
© 2012 SETAC
Printed in the USA
DOI: 10.1002/etc.1828

# TOXICITY OF CRUDE OIL CHEMICALLY DISPERSED IN A WAVE TANK TO EMBRYOS OF ATLANTIC HERRING (*CLUPEA HARENGUS*)

COLLEEN D. GREER,† PETER V. HODSON,*†‡ ZHENGKAI LI,§ THOMAS KING,§ and KENNETH LEE§
†Department of Biology, Queen's University, Kingston, Ontario, Canada
‡School of Environmental Studies, Queen's University, Kingston, Ontario, Canada
§Centre for Offshore Oil, Gas and Energy Research, Bedford Institute of Oceanography, Dartmouth, Nova Scotia, Canada

(*Submitted* 7 *July* 2011; *Returned for Revision* 17 *October* 2011; *Accepted* 20 *January* 2012)

**Abstract**—Tests of crude oil toxicity to fish are often chronic, exposing embryos from fertilization to hatch to oil solutions prepared using standard mixing procedures. However, during oil spills, fish are not often exposed for long periods and the dynamic nature of the ocean is not easily replicated in the lab. Our objective was to determine if brief exposures of Atlantic herring (*Clupea harengus*) embryos to dispersed oil prepared by standard mixing procedures was as toxic as oil dispersed in a more realistic model system. Embryos were first exposed to chemically dispersed Alaska North Slope crude and Arabian light crude oil for 2.4 h to 14 d from fertilization to determine if exposure time affected toxicity. Toxicity increased with exposure time, but 2.4-h exposures at realistic concentrations of oil induced blue-sac disease and reduced the percentage of normal embryos at hatch; there was little difference in toxicity between the two oils. Secondly, oil was chemically dispersed in a wave tank to determine if the resultant oil solutions were as toxic to herring embryos as laboratory-derived dispersed oil using a single exposure period of 24 h. Samples taken 15 min postdispersion were more toxic than laboratory-prepared solutions, but samples taken at 5, 30, and 60 min postdispersion were less toxic. Overall, the laboratory- and wave tank-derived solutions of dispersed oil provided similar estimates of toxicity despite differences in the methods for preparing test solutions, suggesting that laboratory and wave tank data are a reliable basis for ecological risk assessments of spilled oil. Environ. Toxicol. Chem. 2012;31:1324–1333. © 2012 SETAC

**Keywords**—Fish Embryo Crude oil Toxicity Wave tank

## INTRODUCTION

The *Exxon Valdez* oil spill occurred in Prince William Sound, Alaska, USA, on March 24, 1989, coinciding with the return of Pacific herring (*Clupea pallasi*) to spawning grounds in the area [1]. By 1994, the spawning area of Pacific herring had decreased from a range of 106 to 273 km of shoreline to only 12 km, marking a massive population crash and leading to the closure of the roe herring fishery [2]. Although other opinions exist [3], it is assumed that the oil was the leading cause of the crash, corroborating lab-scale tests showing the toxicity of crude oil to Pacific herring [4,5].

As oil sits on water, soluble compounds will partition from the oil, forming the water accommodated fraction of oil in water (WAF) [6]. Chemical dispersants, which were widely used during the Deepwater Horizon oil spill on both surface and subsurface oil [7], decrease the interfacial tension between oil and water, allowing the oil to break into smaller droplets that could be more easily dispersed. Smaller droplets have an increased surface area relative to a slick of oil at the surface, which allows a larger quantity of petroleum compounds to partition into the water, composing the chemically enhanced water accommodated fraction (CEWAF) [6]. Compared to naturally dispersed crude oil, chemical dispersion of oil markedly increases the concentration of oil in water in contact with the droplets [8,9]. Dispersants are applied under the premise that oil will be diluted quickly to subtoxic concentrations, will be more bioavailable to hydrocarbon-degrading microorganisms, and will reduce the risk of oil damage to coastal areas [10]. However, before this can occur, there is a surge in the concentration of oil below the water surface that could induce toxicity in early life stages of fish [11,12].

Polycyclic aromatic hydrocarbons (PAHs) comprise the most chronically toxic fraction of crude oil to fish embryos [13]. By making droplets smaller and increasing the partitioning of oil into water, chemical dispersion of crude oil could increase the exposure of fish embryos to PAH. Exposure of early life stages of fish to PAH can result in blue-sac disease (BSD) [14], signs of which include yolk-sac edema, pericardial edema, spinal curvature, fin rot, and craniofacial deformations [12]. Severe deformities would hinder an embryo's ability to forage for food, escape predators, and swim unimpeded, reducing overall recruitment [1,15].

Oil tanker routes in eastern Canada often pass through fish spawning grounds, particularly those of the Atlantic herring around New Brunswick, Nova Scotia, and Newfoundland, Canada. Atlantic herring may be a useful model for other marine species because they are an accessible source of gametes for four to six months of the year. In particular, the results of research on Atlantic herring could be extended to other *Clupea* species in the North Atlantic and even to Pacific herring (C. Greer, 2011, Master's thesis, Queen's University, Kingston, Ontario, Canada). Atlantic herring spawn in shallow intertidal and subtidal (0–20 m) areas. Although chemical dispersants have not yet been used in these areas, they represent depths where dispersed oil is likely to reach [15].

Most studies of the toxicity of crude oil to fish embryos have used chronic exposure conditions [3,5,9]. Chronic toxicity tests involving continuous exposures of embryos to oil from

All Supplemental Data may be found in the online version of this article.

* To whom correspondence may be addressed (peter.hodson@queensu.ca).

Published online 5 April 2012 in Wiley Online Library (wileyonlinelibrary.com).



EX5035-000001-TRB

fertilization to hatch provide a reproducible and controlled environment and a "worst-case exposure scenario." However, maintaining the same concentration of PAH from fertilization to hatch may not be realistic. In dispersant-effectiveness tests, hydrocarbon concentrations can remain high in the water phase for a few hours to a few days following a spill, but perhaps not to the extent of a chronic toxicity test [16]. Additionally, fish sensitivity is not constant throughout development; fish are most sensitive to oil exposure as gametes and as embryos immediately following fertilization or hatch [12]. The blastula and gastrula stages (within 24–48 h of fertilization) are critical for development, and exposure during this time can decrease hatching success by 40%, whereas exposures 72 h after fertilization do not [1]. By pinpointing the most sensitive stages of development, experiments can be designed to better estimate the potential for toxicity under reasonable oil-exposure scenarios.

According to a National Research Council report [10], oil dispersion in laboratory tests does not naturally model the scale or complexity of conditions at sea, but opportunities to test dispersion during a real spill in the field are rare. As such, a practical intermediate model is needed to validate laboratory studies of CEWAF prepared using the standard protocol of the Chemical Response to Oil Spills: Ecological Effects Research Forum that was reported by Singer et al. [6]. To simulate chemical and natural dispersion of oil spills at sea, a wave tank at the Bedford Institute of Oceanography, Dartmouth, Nova Scotia, Canada, was used to generate plunging breaking waves similar to white caps on a windy day (when the crest of the wave appears white as it breaks). Breaking waves cause velocity shear, increasing the mixing energy among the oil, dispersant, and water, thereby enhancing both the chemical and physical dispersion of the oil [17]. With the use of chemical dispersants, oil-surfactant droplets in the wave tank have a volumetric mean diameter smaller than 50 μm [16]. Compared to laboratory studies, preparation of CEWAF in a wave tank takes into account variable mixing energies and current effects, simulating the natural environment [16].

The main objective of the present study was to determine if oil chemically dispersed within a wave tank could induce similar toxicity to Atlantic herring embryos as laboratory-prepared test solutions. Two oils were tested, Alaska North Slope crude (ANSC), as a reference oil that has been well studied since the *Exxon Valdez* oil spill in 1989, and Arabian light crude (ALC), which is shipped in large volumes to refineries in Atlantic Canada. First, WAF and CEWAF of each oil were analyzed by fluorescence spectrometry to determine if chemical dispersion increased the concentration of oil in the water phase. Second, newly fertilized embryos were exposed to lab-prepared WAF and CEWAF to provide a baseline measure of toxicity and to CEWAF prepared in a wave tank to compare toxicity between the two preparation methods and the two oils. Finally, brief episodic exposures were used to determine the relationships among exposure time, concentration, and toxicity.

## METHODS

All experiments were part of an approved Queen's University Animal Care Protocol (Hodson 2007 032) following the guidelines of the Canadian Council on Animal Care (www.ccac.ca).

### *Wave tank*

The wave tank was located at the Bedford Institute of Oceanography and operated by the Centre for Offshore Oil, Gas, and Energy Research, a national Centre of Expertise of the Department of Fisheries and Oceans Canada. The wave tank was made from carbon steel, was 32 m long, 0.6 m wide, and 2 m high, and was located beside the Bedford Basin. It was filled to an average depth of 1.5 m (~29,000 L) with filtered (5 μm) saltwater from the Bedford Basin for each experiment. Using a frequency sweep technique [18], recurrent breaking waves were generated by a computer-controlled flap-type wave maker situated at one end of the tank. A high-frequency, slow-moving wave was superimposed by a low-frequency, fast-moving wave, causing wave heights to increase until the wave broke ($f = 0.85$ Hz for 20 s, followed by $f = 0.50$ Hz for 5 s). The tank provided a useful model of oil dispersion at sea because it simulated deepwater waves (depth ≥0.28 times wavelength), low oil-to-water ratios (300 ml of oil to 29,000 L of water), and low dispersant-to-oil ratios, set at 1:25 to follow past practice for actual spills [16].

The wave tank was equipped with 12 water sampling ports at four horizontal locations (2 m upstream and 2, 6, and 10 m downstream) and three depths (5, 75, and 140 cm from the water surface) from the spot where oil was added (Fig. 1). At time 0, 300 ml of oil was poured onto the surface of the water inside an oil containment ring, and 12 ml of dispersant (Corexit 9500A) was sprayed onto the surface of the oil slick, at a target ratio of 1:25. Water was sampled from each of the 12 sample points at 5, 15, 30, and 60 min during dispersion.

### *Test solutions*

All oils and the dispersant were supplied by the Centre for Offshore Oil, Gas, and Energy Research (Dartmouth, Nova Scotia, Canada). Oils were weathered by evaporation and sparging with air to simulate loss of volatiles at sea: 10% loss by weight of ANSC (final viscosity, 17.5 cP) and 7% loss by weight of ALC (final viscosity, 15.5 cP). The oils were characterized by methods previously described (Table 1) [19,21]. Corexit 9500A (Nalco Energy Services), which has a recommended dispersant-to-oil ratio of 1:50 to 1:10 (U.S. Environmental Protection Agency [U.S. EPA] Technical Product Bulletin D-4), was used to make CEWAF. In contrast to the wave tank, CEWAF prepared in the lab was dispersed at 1:10 to follow the standard lab protocol for CEWAF and WAF described in the Chemical Response to Oil Spills: Ecological Effects Research Forum method that was adapted by Singer et al. [6] and reported previously [12,20]. The intent of this method was standardization among labs, not realism. Briefly, oil was added to water at a ratio of 1:9 and stirred for 18 h. For CEWAF, dispersant was applied to the oil surface after 18 h of stirring and stirring continued for 1.0 h. After stirring, the WAF and CEWAF solutions were allowed to settle for 1.0 h and the solutions below the residual oil slick were decanted and diluted for test solutions.

Positive controls included retene (320 μg/L using methanol as the carrier solvent) and laboratory-prepared CEWAF. Retene (7-isopropyl-1-methyl phenanthrene) is an alkyl-phenanthrene known to cause BSD in early life stages of fish [14]. Negative controls included lab water, wave tank water drawn before oil application, and a dispersant control. The water used in the wave tank was from the Bedford Basin (30 g/L salinity), and samples were diluted with dechlorinated freshwater to 15 g/L before toxicity testing and chemical analyses as this is the optimal salinity for Atlantic herring fertilization and hatching (S. McIntosh, 2009, Master's thesis, Queen's University, Kingston, Ontario, Canada).

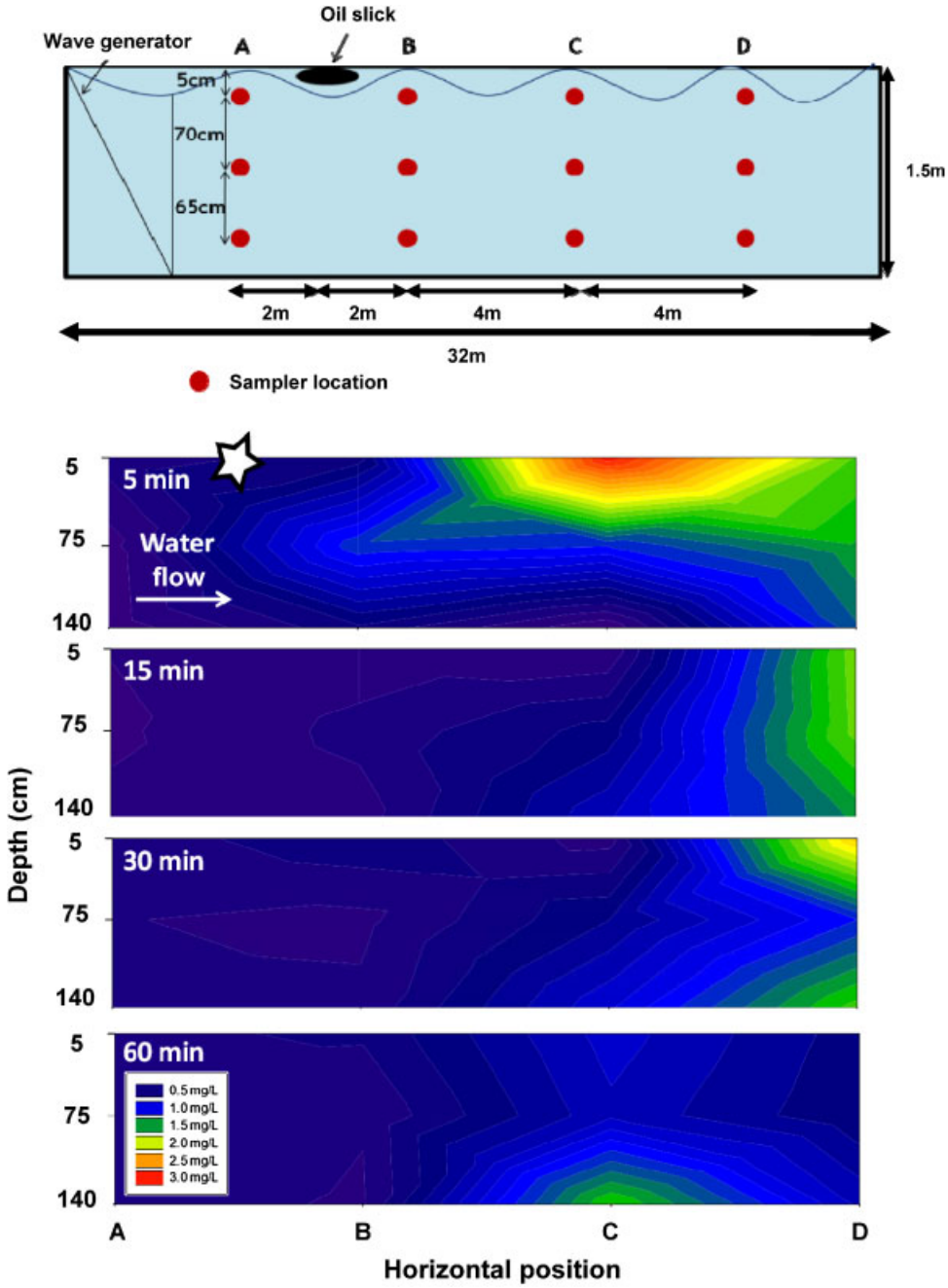


Fig. 1. Schematic of the wave tank with sampling locations (upper panel). The movement of fluorescing hydrocarbons through the wave tank over 5 to 60 min following addition of Corexit 9500 dispersant to Alaska North Slope Crude oil (lower panel). White star represents the location where oil was initially added.

*Test species*

"Ripe and running" stage VI herring were acquired from the roe herring fishery in Eastern Passage, Nova Scotia, Canada [22,23]. Ripe herring have transparent eggs, and the eggs and sperm are released from the fish with little external pressure [23]. Gonads were removed from the fish at the wharf using a scalpel and transported in resealable plastic bags in a cooler to the Bedford Institute of Oceanography. Eggs and sperm were used within 24 h of capture, to ensure freshness.

*Egg fertilization*

Freshly fertilized eggs were used for these experiments because they are the most sensitive life stage for herring [12]. Each experiment, from egg fertilization to scoring hatched embryos, was maintained at 10°C (± 1°C) with a 2:22 h dim light:dark photoperiod. Approximately 50 to 100 eggs were spread onto glass slides and separated into groups of two or three using a dissection needle. Once the eggs were attached to glass slides, the slides were placed into a milt solution that was prepared by mashing one-fifth of a testis, from a pool of all testes collected (approximately six males), in approximately 500 ml of saltwater (15 g/L salinity). Slides were left in the milt solution for approximately 15 min, after which they were rinsed with clean saltwater to remove excess milt and placed into test solutions. Individual experiments used eggs from a single female that had been fertilized by sperm from multiple males.

Table 1. Physical and chemical characterization of Alaska North slope crude (ANSC) and Arabian light crude (ALC) oils[a]

| Oils | ANSC | ALC |
|---|---|---|
| Type | Medium | Light |
| %Weathered by weight | 10 | 7 |
| Density (g/ml) | 0.8607 | 0.8691 |
| Viscosity (20°C, cStk) | 17.5 | 15.5 |
| %Alkanes | 32.0 | 32.7 |
| %Resins | 24.4 | 46.9 |
| %Asphaltenes | 4.3 | 1.5 |
| %Aromatics | 39.3 | 18.9 |
| Σ Naphthalenes (μg/g) | 4,665 | 3,221 |
| Σ Fluorenes (μg/g) | 461 | 540 |
| Σ Phenanthrenes (μg/g) | 1,687 | 1,086 |
| Σ Pyrenes (μg/g) | 284 | 202 |
| Σ Chrysenes (μg/g) | 562 | 188 |
| Σ Dibenzothiophenes (μg/g) | 1727 | 3788 |
| Σ Naphthobenzothiophenes (μg/g) | 851 | 3683 |
| Σ Alkyl-PAH (μg/g) | 90.07 | 96.33 |
| Σ Nonalkyl PAH (μg/g) | 126.7 | 8.3 |
| Total PAH (μg/g) | 10,363 | 12,716 |
| Total PAH (% of oil) | 1.03 | 1.27 |

[a] Oils were weathered by evaporation and sparging with air to simulate loss at sea. Density and viscosity were measured after weathering (Lee et al. [19]). The aromatics include the polycyclic aromatic hydrocarbons (PAHs), which were characterized by gas-chromatographymass spectrometry as described previously [20] and presented in detail in Supplementary Data Table S1.

*Experimental setup*

To assess the effect of exposure time on oil toxicity, slides of herring eggs were exposed to dilutions of WAF for 14 d or CEWAF for 2.4 h, 8 h, 24 h, or 14 d. After exposure, embryos were transferred to clean Mason jars containing fresh saltwater (15 g/L). After 24 h, water and test solutions for 14-d exposures of WAF and CEWAF were renewed daily until hatch.

To assess the toxicity of oil dispersed in the wave tank, exposure solutions were drawn from the 12 sampling ports at 5, 15, 30, and 60 min after oil and dispersant application. Solutions were diluted 1:1 with dechlorinated freshwater to reach a salinity of 15 g/L. Slides of herring eggs were exposed in the lab to the diluted wave tank samples for 24 h, after which the embryos were transferred to clean saltwater and raised to hatch. Embryos remained attached to the glass slides until hatch. Seven days postfertilization, unfertilized eggs were removed from the slides and fertilized embryos were randomly culled to approximately 20 per slide. Every other day from this point to hatch, dead embryos were removed and survivors were monitored to determine the day of hatch.

Although each slide contained 20 eggs, the slides were the unit of experimentation (i.e., $n = 1$). The measurement of oil concentrations in each of a wide array of test concentrations enabled the calculation of regressions and of median lethal concentrations (LC50s) and median effective concentrations (EC50s) without replication. The size of these experiments (50–70 treatments) and initial difficulties in obtaining fertilized eggs made replication impractical.

Because herring were captured by a commercial fishery, fish were usually not at the peak of fertility, and many experiments failed because eggs were not fertilized. High variability was observed among and within females in terms of egg quality and in terms of response to oil exposure. It is not uncommon that 50% of naturally spawned herring in the wild exhibit morphological deformities [5,24], a background response rate that would reduce reproducibility among experiments. As a result, we only included slides in the 14-d experiment that had a minimum of five fertilized eggs. All slides in the wave tank and exposure-time experiments had at least 18 eggs. Experiments with abnormally high rates of malformed embryos in the controls (<75% normal) were discarded. Future experiments would be more successful if herring were captured live and held in fresh seawater until ready to spawn (S.D. Rice, National Oceanographic and Atmospheric Administration, Juneau, AK, USA, personal communication).

*Scoring signs of toxicity*

Within 24 h of hatch, embryos were scored for signs of BSD. These included pericardial edema (score 0–3), yolk sac edema (0–3), spinal curvature (0–3), fin rot (0–1), craniofacial deformities (0–1), and mortality. A graduated scale was used for scoring pathologies: 3 indicated the most severe response and 0 indicated no response. Fin rot and craniofacial deformities were scored as either present (1) or absent (0) because it was assumed they would have less of a lasting impact on the fish, and the responses were subtle and could not be scored on a graduated scale. Edemas and spinal curvature were the most sensitive responses to oil exposure and could clearly be scored on a graduated scale. Mortality of embryos is often a result of edema, and edema and spinal curvatures can prevent an embryo from swimming effectively, escaping predators, or foraging for food [25,26]. We assumed that fish with lower BSD scores were more likely to survive and contribute to the next generation, despite the deformities.

Embryos that died from day 7 to hatch received the highest possible BSD score (11.5) to indicate the most severe response beyond sublethal toxicity. The BSD Severity Index was the average BSD score for all embryos within a treatment, normalized to the maximum score, and included both lethal (mortality) and sublethal (BSD) signs of toxicity (modified from Villalobos et al. [27]). Alternatively, embryos were considered normal if they were alive at hatch, did not show any signs of BSD, and swam normally. These fish would be most likely to grow, reproduce, and contribute to the next generation.

*Water chemistry*

Water samples were drawn from exposure solutions to quantify the concentration of hydrocarbons by fluorescence (detailed operating procedure in Supplemental Data, SOP S1; Fig. S1). Fluorescence measures compounds in oil with conjugated double bonds that fluoresce when bombarded with ultraviolet light [28]. Water samples (3 ml) were drawn from exposure solutions at the start (time, 0 h) and at the end of the exposure period, then averaged to determine concentration. They were added to 3 ml of absolute ethanol in 7-ml glass scintillation vials with foil-lined caps and stored at 4°C. Samples were sonicated for 3 min before chemical analysis to reduce the loss of hydrocarbons to the container from storage. On mixing with ethanol, salt from the water samples precipitated from the solution. To reduce interference by salt particles, samples were centrifuged at 9,100 *g* for 10 min to separate the salt from the sample but not to remove hydrophobic compounds. An aliquot of the supernatant was placed in a quartz cuvette and analyzed for total fluorescence using the RF-5301PC scanning spectrofluorometer (Shimadzu) with Panorama fluorescence 1.1 software (LabCognition). The excitation (278 nm) and emission (300–450 nm) wavelengths used for the experiments were the same for both ALC and ANSC. The fluorometric response was measured by integrating the area

under the curve from 300 to 450 nm. A comparison of fluorescence to a standard curve prepared from undispersed whole oil dissolved in 50:50 ethanol:salt water (15 ppt) provided an estimate of the compounds in the test solution that fluoresce (Supplemental Data, Fig. S2). The limit of detection, or method detection limit, is the concentration of chemical that can be determined above the level of the blank and reported with 99% confidence (U.S. EPA method 40 CFR 136, Appendix B, revision 1.11; www.epa.gov/region9/qa/pdfs/40cfr136_03.pdf). The limit of detection is $t \times S$, where $t$ is the Student's $t$ value at the 99% confidence level for $n - 1$ degrees of freedom and $S$ is the standard deviation of 10 replicates of a sample. The limit of detection was 0.028 mg/L.

Water samples (100 ml) were also drawn from a subset of exposures to quantify the concentration of total petroleum hydrocarbons. Samples were preserved with 10 ml of dichloromethane and sent to the Centre for Offshore Oil, Gas, and Energy Research, Bedford Institute of Oceanography, to be analyzed by gas chromatography-flame ionization detection (GC-FID, [20]). The relationship between total petroleum hydrocarbon concentrations measured by GC-FID and by fluorescence was used to estimate total petroleum hydrocarbon concentrations in other exposures characterized by fluorescence (Supplemental Data, Fig. S2).

*Statistics*

SigmaPlot 11.0 (Systat Software) and GraphPad Prism 5.00 (GraphPad Software) were used to derive nonlinear regressions from a four-parameter logistic equation [29] and to estimate LC50s and EC50s (details are in the Supplemental Data, Table S2). For logistic equations, the responses of negative control fish and the maximum possible responses of oil-exposed fish were used to establish the upper and lower limits for regressions. Statistical comparisons were made by comparing the confidence intervals (CIs) for overlap and by using the extra sum-of-squares $F$-test function in GraphPad Prism, where parameters were shared to determine if data were more strongly correlated when combined or as separate functions.

## RESULTS

*Water chemistry*

Chemical dispersion of crude oil (CEWAF) caused an approximately 100-fold increase in concentrations of fluorescent petroleum hydrocarbons in the saltwater phase compared to physically dispersed oil (WAF; $F$ test, $p < 0.001$). Above the detection limit of 0.028 mg/L, there were linear relationships between measured concentrations of fluorescent petroleum hydrocarbons and nominal loadings of oil (% v/v) for ANSC and ALC oils of both WAF and CEWAF (Fig. 2). All lines were parallel ($F$ test, $p = 0.39$) and shared a slope of 1.06. At $x = 1$, the y values were significantly different between ANSC and ALC CEWAF ($F$ test, $p < 0.001$), but the y values were not significantly different between the WAFs ($F$ test, $p = 0.98$).

To test the efficiency of the fluorescence method, GC-FID was also used to analyze the concentration of total petroleum hydrocarbons in some exposure solutions. When compared to the concentrations obtained through spectrofluorometry, a linear relationship of log-transformed data was observed (Supplemental Data, Fig. S2), demonstrating that as total petroleum hydrocarbons increased, so did the fluorescent signal. Although a decrease in linearity was observed at low concentrations, the two methods were in agreement at total petroleum hydrocarbon concentrations >0.028 mg/L. While the fluorescence method

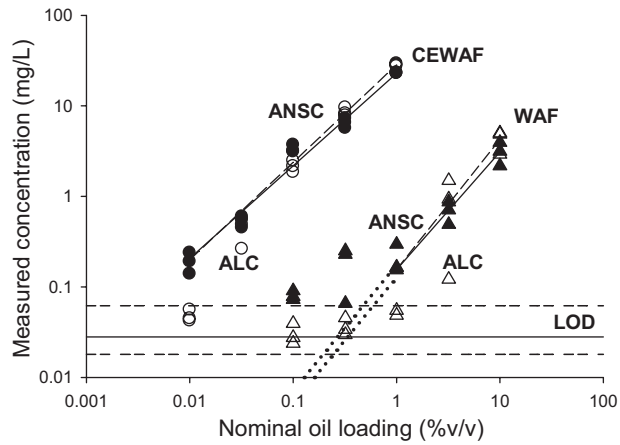


Fig. 2. The concentration of petroleum hydrocarbons in test solutions at different loadings of water accommodated fraction (WAF) or chemically enhanced water accommodated fraction (CEWAF). Open circles and triangles represent Arabian light crude (ALC) CEWAF ($y = 10^{(1.08*\log(x) + 1.45)}$, $r^2 = 1.0$) and WAF ($y = 10^{(1.44*\log(x) - 0.81)}$, $r^2 = 0.89$), respectively. Closed circles and triangles represent Alaska North Slope crude (ANSC) CEWAF ($y = 10^{(1.02*\log(x) + 1.36)}$, $r^2 = 0.99$) and WAF ($y = 10^{(1.29*\log(x) - 0.80)}$, $r^2 = 0.89$), respectively. Dotted lines show the extrapolated concentration of the WAF samples below the limit of detection (solid horizontal line, 0.028 mg/L). Data points close to the limit of detection deviate from linearity and were not used to calculate linear regressions. Upper and lower 95% confidence limits of the limit of detection (LOD) are shown as dashed lines.

targets PAH, it may underestimate other components of oil that do not dissolve in water to the same extent. In both cases, the preservation of samples with either ethanol (fluorescence) or dichloromethane (GC-FID) would solubilize oil droplets so that the assays measured total oil in test solutions.

*Comparison of oils*

Atlantic herring embryos responded to dilutions of laboratory-prepared WAF and CEWAF of ANSC and ALC in an exposure-dependent manner (Fig. 3). Based on measured oil concentrations by fluorescence and the combined data for WAF and CEWAF, ANSC appeared to be no more toxic than ALC, as evidenced by LC50s (Table 2). Assuming that the proportion of total PAH in exposure solutions was the same as that in whole oil (Table 1), the 14-d LC50s for ANSC and ALC were equivalent to 20 and 26 μg/L total PAH, respectively. Similarly, for percentage of hatch and the BSD Severity Index, EC50s for the two oils did not differ markedly. The estimated total PAH concentrations associated with 14-d EC50s for the BSD Severity Index were 0.4 and 0.3 μg/L for ANSC and ALC, respectively. For percentage of normal, the EC50s based on fluorescence were higher for ALC than for ANSC for three of four sampling times, suggesting that ALC was somewhat less toxic than ANSC. All the differences, however, were relatively small and statistically insignificant (overlapping 95% CI), suggesting no real difference in toxicity between the two oils.

*Exposure time*

The toxicities of CEWAF prepared from ANSC and ALC increased linearly with exposure times from 2.4 to 24 h (Fig. 4, Table 2), and there was no significant difference between the slopes ($F$ test, $p = 0.70$) or intercepts ($F$ test, $p = 0.33$) of the lines of best fit of the $EC50_{\%Normal}$ values versus exposure time (Fig. 5). For ANSC CEWAF there was a significant difference among the $EC50_{\%Normal}$ values for all exposure times (Fig. 5; $F$ test, $p < 0.001$), but for ALC there was no significant

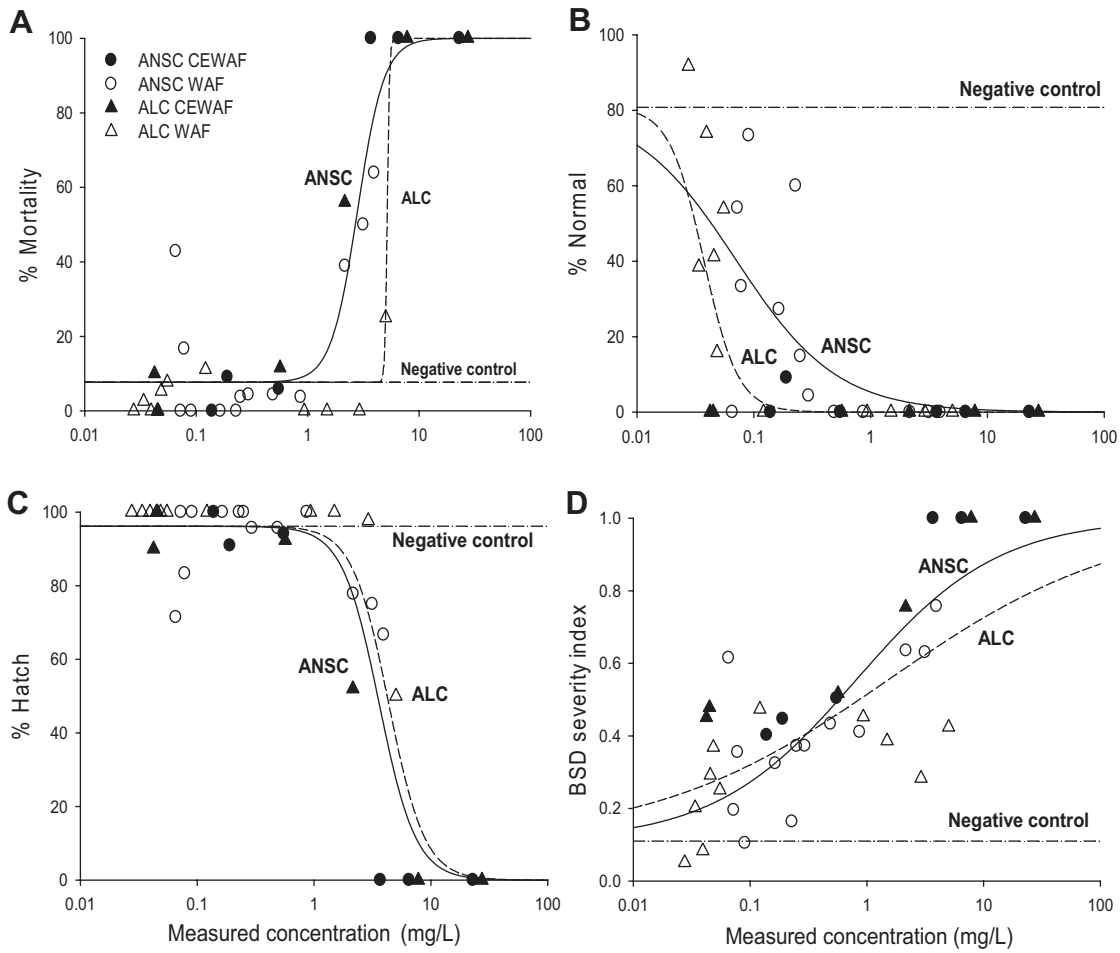


Fig. 3. Toxicity of the water accommodated fractions (WAF) and chemically enhanced water accommodated fractions (CEWAF) of Alaska North Slope crude (ANSC) and Arabian light crude (ALC) oils prepared in the lab to Atlantic herring embryos expressed as (**A**) %mortality, (**B**) %normal, (**C**) %hatch, and (**D**) blue-sac disease (BSD) Severity Index in relation to the measured concentration of fluorescing compounds. Regression lines and median lethal concentration (LC50) and median effective concentration (EC50) values were calculated using WAF (open symbols) and CEWAF (closed symbols). Upper and lower constraints for nonlinear regressions were control and maximal (**A** = 100%, **B** and **C** = 0%, **D** = 1.0) responses. Dashed lines represent the response to ALC exposure, and solid lines represent the response to ANSC exposure. $n = 20$ embryos per treatment.

difference between 8 and 24 h for CEWAF exposures ( $F$ test, $p = 0.86$). For mortality, LC50s were higher than the highest concentrations tested, except for the 14-d exposures, which produced an LC50 of 1.9 mg/L (95% CI too wide to estimate) for ANSC and 2.1 mg/L (95% CI 1.9–2.3 mg/L) for ALC (Supplemental Data, Fig. S3). The $EC50_{\%Hatch}$ values were also higher than the highest concentrations tested, except for the 14-d exposures, which produced an $EC50_{\%Hatch}$ of 0.86 mg/L (95% CI 1.5–5.9 mg/L) for ANSC and 2.2 mg/L (95% CI 1.9–2.7 mg/L) for ALC (Supplemental Data, Fig. S3).

*Wave tank*

Hydrocarbon concentrations produced by dispersing ANSC and ALC oils in the wave tank were too low to affect %hatch, the BSD Severity Index, or mortality (Supplemental Data, Fig. S4). For percentage of normal, the 24-h EC50 for ANSC CEWAF calculated by combining all wave tank samples was 16 mg/L (95% CI 0.32–761 mg/L), while the $EC50_{\%Normal}$ for ALC CEWAF was higher than the highest concentration tested (>10.4 mg/L) (Fig. 6). The toxicity of ANSC CEWAF was especially evident in samples drawn from the tank at 15 min, with an $EC50_{\%Normal}$ of 1.2 mg/L (95% CI 0.61–2.5 mg/L). In contrast, the toxicity of laboratory-prepared ANSC CEWAF was sixfold lower ($EC50_{\%Normal} = 6.6$ mg/L, 95% CI 4.9–8.8 mg/L; $F$ test, $p = 0.026$) when tests were run with embryos from the same female as the wave tank experiment. However, exposures drawn at other sampling times did not show a significant deviation from the toxicity of laboratory-prepared CEWAF. While ANSC CEWAF from the wave tank appeared at times more toxic than laboratory-prepared solutions, concentrations of ALC CEWAF from the tank were lower than the laboratory-prepared $EC50_{\%Normal}$ and did not cause toxicity.

*Quality assurance/quality control*

Hatched embryos exposed to crude oil exhibited exposure concentration- and time-dependent signs of toxicity, including yolk-sac edema, pericardial edema, spinal curvature, fin rot, and craniofacial deformities. The mean responses of controls were as follows: %mortality = 3.2 ± 3.7%; %normal = 86.3 ± 6.1%; %hatch = 98.2 ± 2.0%; and BSD Severity Index = 0.058 ± 0.049.

## DISCUSSION

The present study is the first to compare the embryotoxicity of dispersed oil solutions generated in an outdoor wave tank with those generated by standard laboratory protocols. As such, it provides perspective on the applicability of lab-derived toxicity data to ecological risk assessment of chemical dispersion of oil.

Table 2. Effect of exposure time on the toxicity of the chemically enhanced water accommodated fraction (CEWAF) of Alaska North Slope crude (ANSC) and Arabian light crude (ALC) oils[a]

| | | Atlantic herring (LC50s and EC50s in mg/L) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Oil | Exposure time | %Mortality | CI | %Normal | CI | %Hatch | CI | BSD Severity Index | CI |
| ANSC | | | | | | | | | |
| FL | 14 d | 1.95 | Very wide | <0.14 | — | 0.86 | Very wide | 0.44 | 0.16–1.24 |
| TPH | | 7.12 | Very wide | <1.41 | — | 4.31 | Very wide | 2.85 | 1.51–5.39 |
| FL | 24 h | >24.15 | — | 0.74 | 0.35–1.57 | >24.15 | — | 63.70 | Very wide |
| TPH | | >33.11 | — | 3.94 | 2.49–6.23 | >33.11 | — | 60.48 | 22.31–163.9 |
| FL | 8 h | >26.28 | — | 4.24 | 3.26–5.53 | >26.28 | — | 40.09 | 8.27–194.3 |
| TPH | | >35.48 | — | 11.48 | 9.76–13.51 | >35.48 | — | 45.53 | 17.29–119.9 |
| FL | 2.4 h | >26.93 | — | 14.66 | 6.02–33.70 | >26.93 | — | 39.77 | 16.02–98.66 |
| TPH | | >35.65 | — | 24.56 | 14.22–42.41 | >35.65 | — | 45.30 | 25.94–79.11 |
| ALC | | | | | | | | | |
| FL | 14 d | 2.07 | 1.88–2.28 | <0.04 | — | 2.25 | 1.89–2.70 | 0.25 | 0.02–2.63 |
| FL | 24 h | >28.51 | — | 2.44 | 0.36–16.56 | >28.51 | — | 84.38 | Very wide |
| FL | 8 h | >30.96 | — | 2.79 | 1.02–7.62 | >30.96 | — | 73.70 | Very wide |
| | 2.4 h | >31.79 | — | 29.73 | 16.02–55.18 | >31.79 | — | 419.7 | Very wide |

[a] Median lethal concentrations (LC50s) represent lethal toxicity, specifically an increase in the mortality of embryos. Median effective concentrations (EC50s) represent sublethal toxicity, specifically the concentrations causing a reduction in the percent normal and percentage of hatch and an increase in the blue-sac disease (BSD) Severity Index of Atlantic herring embryos. Values are based on measured concentrations of fluorescing (FL) petroleum hydrocarbons of CEWAF or estimations of total petroleum hydrocarbon (TPH) concentrations (relationship shown in Supplemental Data, Fig. S3) and shown with 95% confidence intervals (CIs). " > " and " < " indicate that the LC50 or EC50 was greater than the highest concentration tested or less than the lowest concentration tested.

Chemical dispersion of both ALC and ANSC oils increased the concentration of waterborne hydrocarbons by approximately 100-fold, and the concentration of fluorescent hydrocarbons in the water phases of dispersed ALC and ANSC were similar. Little difference in toxicity was seen between ANSC and ALC when embryos were exposed from fertilization until hatch, consistent with the very similar concentrations of PAH in the two oils. The toxicity of oil to early life stages of fish has been associated with concentrations of alkyl PAH [13], implying that a greater quantity of waterborne PAH (or aromatics), particularly the alkyl phenanthrenes, will result in a higher degree of toxicity [20,30]. Both oils contained just over 1.0% PAH by weight, and the concentrations of most classes of alkyl PAH were similar between the two. The exceptions were the sulfur heterocycles, alkyl dibenzothiophenes, and naphthobenzothiophenes, which were about two to four times more concentrated in ALC than in ANSC (Table 1). Because the toxicity of the two oils was not correlated with the relative concentrations of thiophenes, the sulfur heterocycles may not contribute much to oil chronic toxicity. The similarity in fluorescence between the two oils may also relate to the relative amounts of all fluorescent compounds in each oil, as many organic molecules contain conjugated double bonds and will fluoresce (e.g., high molecular weight asphaltenes) [28]. Additionally, compounds vary in their ability to fluoresce according to their chemical composition, and ring substitutions can increase fluorescence; aniline can be 50 times more fluorescent than benzene [31].

In actual oil spills, exposures of embryos may only last a few hours to days if oil is dispersed rapidly by currents and waves [32,33]. As a result, estimates of oil toxicity may be unrealistic if embryos are exposed chronically from fertilization to hatch [12]. The effect of exposure time was examined using laboratory-prepared CEWAF to simulate pulse exposure scenarios, in which embryos were exposed to oil in water over increasing exposure times. In accordance with McIntosh et al. [12], increasing exposure time to CEWAF of ANSC decreased the percentage of normal embryos at hatch. Even the shortest exposure times (2.4 h) caused toxicity, and the longer the embryos were exposed, the higher the degree of embryo malformation and the greater the risk of failure to recruit to future generations. This relationship however, was not as consistent for ALC CEWAF because there was no significant difference in toxicity between 8- and 24-h exposures. This could indicate that the toxic agents in the 24-h exposure had been depleted from solution by 8 h or that uptake had reached equilibrium with excretion and metabolism. Signs of toxicity (%normal, %hatch, %mortality, BSD Severity Index) were more pronounced in embryos exposed chronically (14 d) because soluble hydrocarbons were replenished by the daily static renewal protocol.

Although lethality depends on both exposure time and concentration [12,32], toxicity resulting from exposure times of 24 h or less was primarily determined by sublethal end points, because LC50s could not be estimated within the concentration range tested; %normal and, to a lesser extent, BSD Severity Index were the most sensitive end points used for determining toxicity. In contrast, all end points could be used to estimate toxicity to Atlantic herring embryos exposed for 14 d. However, short-term episodic exposures are likely to be more typical during an oil spill.

Oil is a complex mixture, and the kinetics of weathering and uptake are difficult to mimic in the laboratory. While CEWAF prepared in a wave tank provided more realistic spill and dispersion conditions, solutions of ALC dispersed in the wave tank did not cause toxicity, likely because the concentrations of hydrocarbons were less than those observed to cause toxicity in the lab. In contrast, solutions of ANSC dispersed in the wave tank and drawn from the tank at 15 min postdispersion were approximately six times more toxic than laboratory-prepared CEWAF. Solutions drawn from the tank at 5 and 30 min postdispersion showed little toxicity, most likely because the concentrations were below those found to be toxic in laboratory-prepared CEWAF. The significant increase in toxicity of wave tank solutions of ANSC sampled at 15 min may be due to interactions among droplet dispersion, oil–water partitioning, and exposure to fish, that is, peak concentrations of PAH dissolved in water may have been produced at 15 min. However, because sample preservation solubilized oil droplets, measured

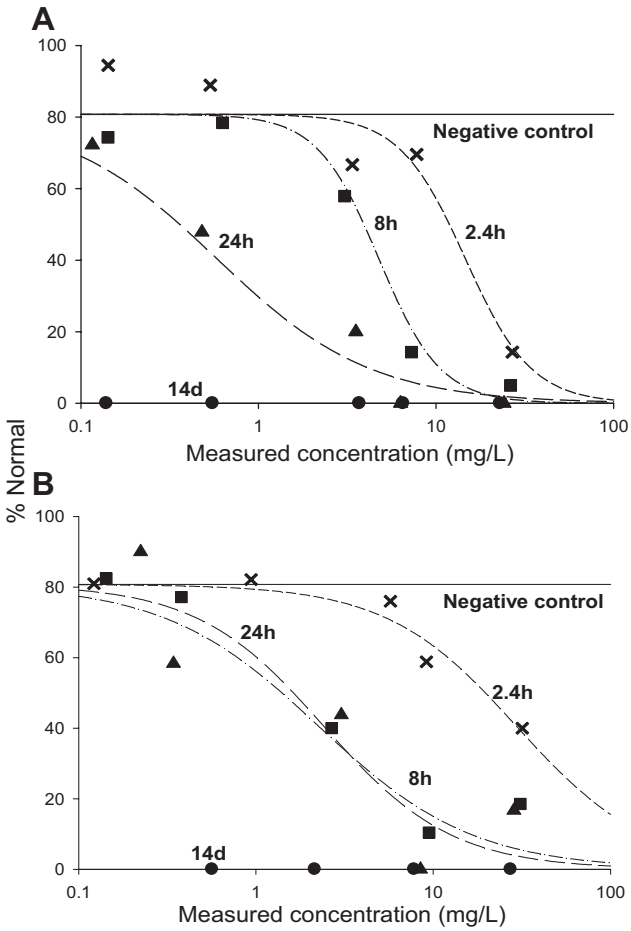


Fig. 4. Effect of exposure time and petroleum hydrocarbon concentration on the percentage of normal embryos at hatch for herring exposed to lab-prepared Alaska North Slope crude (ANSC; **A**) and Arabian light crude (ALC; **B**). %Normal indicates percentage of embryos alive and showing no signs of toxicity at hatch. Embryos were exposed immediately following fertilization for 2.4 (x's), 8 (squares), or 24 h (triangles) or 14 d (circles) (static daily renewal). Upper and lower constraints for nonlinear regressions were control responses and zero (maximum possible response). $n = 20$ embryos per treatment.

concentrations of oil in water do not allow discrimination between dissolved and droplet oil.

While toxicity was greatest at 15 min (Fig. 6), hydrocarbon concentrations within the wave tank were highest toward the end of the tank and at the sampling times immediately following dispersion (5 min). Previous testing using the wave tank has shown that average droplet size decreased quickly from 300 to 50 μm within 10 min of adding oil and dispersant [16]. Smaller droplets would increase partitioning and make hydrocarbons more bioavailable to fish embryos, which could explain why the wave tank exposures were more toxic at 15 min than at 5 min. Although the research done by Li et al. [16] was performed in a wave tank using a flow-through mode, they also observed quick dilution of oil within the wave tank and rapid movement of oil to the end of the tank. In a batch mode operation, the distribution of dispersed oil in the wave tank would be nonuniform, with the highest concentration measured near the surface at the end of the wave tank because of horizontal transport due to Stokes drift and vertical transport from the buoyancy of oil droplets; this distribution was not due to edge effects. This suggests that even though a spill may not occur directly over fish spawning grounds, chemical dispersion, currents, and wave action could quickly move the hydrocarbons to those areas.

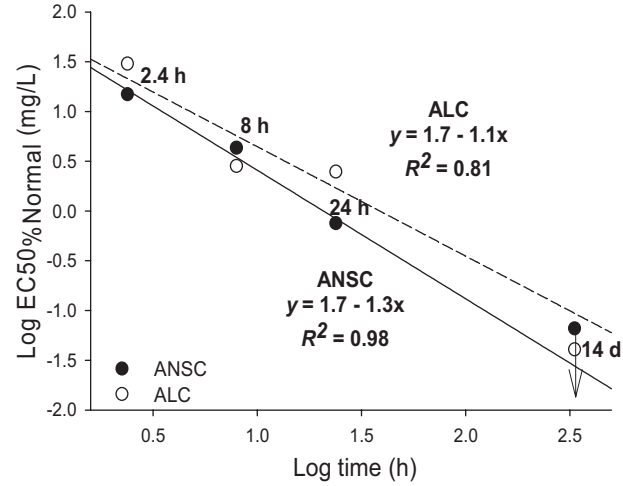


Fig. 5. Effect of exposure time on median effective concentrations ($EC50_{\%Normal}$) of Alaska North Slope crude oil (ANSC) chemically enhanced water accommodated fraction (CEWAF) and Arabian light crude oil (ALC) CEWAF affecting %normal. The lowest concentration tested is shown for the 14-d data point as there was still 100% response at the lowest concentrations; arrow indicates that the actual EC50 would be lower than this concentration. Therefore, linear regressions were calculated using only three data points (2.4, 8, and 24 h).

Wave tank-prepared and laboratory-prepared CEWAF may exhibit differences in the types of hydrocarbons within the water column, the degree of weathering and dilution, and the chemical characteristics and physical composition of the oil. Further chemical analysis and a change in experimental conditions would be required to assess these possibilities. Increasing the volume of oil added to the wave tank for dispersion may increase the concentration of dissolved oil generated by the tank to better pinpoint LC50s and EC50s but may stray from environmentally realistic wave-tank conditions. Further, a difference in toxicity could result from the different dispersant-to-oil ratios used in the wave tank and laboratory tests; the volume of dispersant in laboratory tests could be adjusted to parallel wave tank experiments. If samples were tested undiluted at 30 ppt salinity, the concentration of oil in the water samples would be double those of samples diluted to 15 ppt as was done in the present experiments. Preliminary tests at 30 ppt showed a higher range of toxicity, presumably because samples were undiluted; but there was no difference in survival or rates of pathology among controls between salinities of 15 and 30 ppt.

The wave tank system provides a model for oil spills in the field; but the ocean is a dynamic system, and each oil spill would occur under different conditions and regimes. The wave tank experiments were designed to simulate specific high-energy conditions in the ocean on a windy day and to generate a droplet size distribution similar to that expected at sea. Physical and chemical measurements have verified that the tank is a success in this regard [16]. The demonstration that solutions of oil from the wave tank had effects on herring embryos consistent with those observed with other fish species in field and laboratory studies of oil toxicity [1,34] indicates that the wave tank can be applied successfully to studies of the biological impacts of spilled oil.

## CONCLUSION

Atlantic herring embryos were sensitive to exposure to chemically dispersed crude oil, with 0.1- to 14-d LC50s above 1 mg/L total hydrocarbons. The EC50s for chronic toxicity

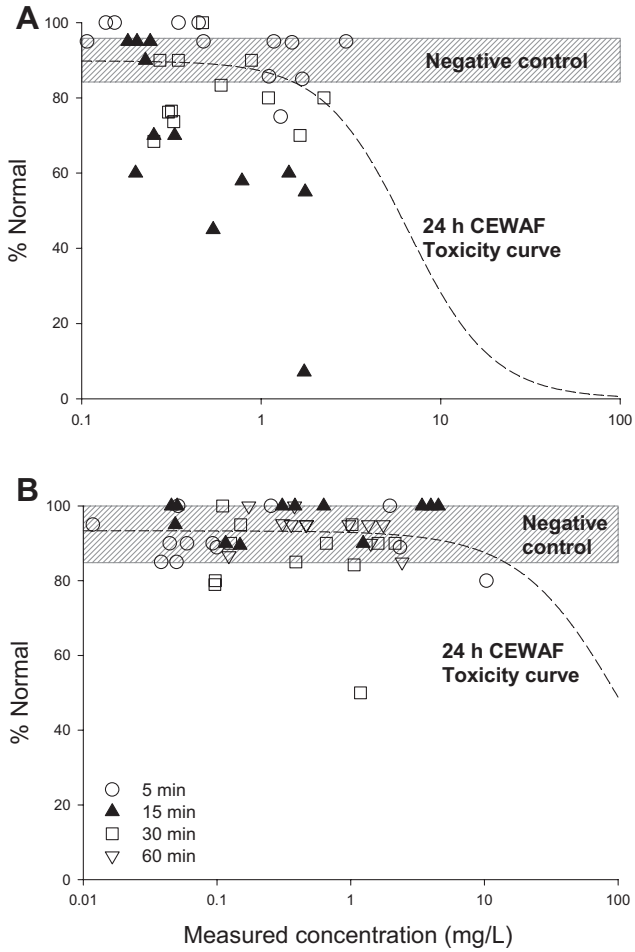


Fig. 6. Change in %normal of herring embryos exposed to water samples drawn from the tank after chemical dispersion of Alaska North Slope crude oil (ANSC; **A**) and Arabian light crude oil (ALC; **B**). Effects on Atlantic herring of 24-h exposure of the corresponding laboratory-prepared CEWAF exposures have been superimposed for comparison (dashed lines). Measured concentrations were derived from samples of toxicity test solutions. $n = 20$ embryos for each exposure.

typical of PAH exposure fell between 0.04 and 84 mg/L, depending on exposure time and end point. Exposure times as short as 2.4 h (0.1 d) caused toxicity at concentrations similar to those observed near oil spills. The estimated total PAH concentrations corresponding to these EC50s ranged from 0.4 to 840 μg/L, and ANSC was no more toxic to Atlantic herring embryos than ALC, reflecting similar concentrations of alkyl PAH.

The wave tank experiments demonstrated that chemical dispersants increased the amount of petroleum hydrocarbons in the water column to toxic concentrations. They also demonstrated that toxicity did not persist, supporting the use of dispersants to rapidly dilute concentrated oil. The risk of toxicity under these circumstances will depend on the amount of oil dispersed, the wave action and mixing energy acting on the dispersed oil, and the proximity of fish embryos to the spill site. While there were differences in toxicity between wave tank and laboratory solutions, they were neither large nor consistent in direction. Thus, data from both laboratory and wave tank experiments could be used to support ecological risk assessments of oil spills. Additional trials at higher concentrations of oil in the wave tank are required to fully understand the chemistry of spilled oil in the wave tank, and these experiments could be extended to achieve a greater degree of realism by conducting toxicity tests entirely in the wave tank. By suspending slides of eggs within the tank during oil dispersion under continuous-flow conditions, the effects of brief pulses of exposure could be measured to support computer modeling of potential spill effects.

*Acknowledgement*—The present study was funded by the Prince William Sound Regional Citizens' Advisory Council (Anchorage, AK, USA) and the Centre for Offshore Oil, Gas, and Energy Research, Fisheries and Oceans Canada (Dartmouth, NS). S. Courtenay, S. Johnson, M. Boudreau, C. McIntyre, and C. Ready provided technical assistance. M. Williston, G. Donaldson, and H&H Seafoods provided roe herring.

## REFERENCES

1. Kocan RM, Hose JE, Brown ED, Baker TT. 1996. Pacific herring (*Clupea pallasi*) embryo sensitivity to Prudhoe Bay petroleum hydrocarbons: Laboratory evaluation and in situ exposure at oiled and unoiled sites in Prince William Sound. *Can J Fish Aquat Sci* 53: 2366–2375.
2. Funk F. 1994. Preliminary summary of 1994 Alaska roe sac herring fisheries. Alaska Department of Fish and Game, Juneau, AK, USA.
3. Pearson WH, Elston RA, Bienert RW, Drum AS, Antrim LD. 1999. Why did the Prince William Sound, Alaska, Pacific herring (*Clupea pallasi*) fisheries collapse in 1993 and 1994? Review of hypotheses. *Can J Fish Aquat Sci* 56:711–737.
4. Paine RT, Ruesink JL, Sun A, Soulanille EL, Wonham MJ, Harley CDG, Brumbaugh DR, Secord DL. 1996. Trouble on oiled waters: Lessons from the *Exxon Valdez* oil spill. *Annu Rev Ecol Syst* 27:197–235.
5. Hose JE, McGurk MD, Marty GD, Hinton DE, Brown ED, Baker TT. 1996. Sublethal effects of the *Exxon Valdez* oil spill on herring embryos and larvae: Morphological, cytogenetic, and histopathological assessments, 1989–1991. *Can J Fish Aquat Sci* 53:2355–2365.
6. Singer MM, Aurand D, Bragin GE, Clark JR, Coelho GM, Sowby ML, Tjeerdema RS. 2000. Standardization of the preparation and quantitation of water-accommodated fractions of petroleum for toxicity testing. *Mar Pollut Bull* 40:1007–1016.
7. Kujawinski EB, Kido Soule MC, Valentine DL, Boysen AK, Longnecker K, Redmond MC. 2011. Fate of dispersants associated with the Deepwater Horizon oil spill. *Environ Sci Technol* 45:1298–1306.
8. Cohen AM, Nugegoda D, Gagnon MM. 2001. The effect of different oil spill remediation techniques on petroleum hydrocarbon elimination in Australian bass (*Macquaria novemaculeata*). *Arch Environ Contam Toxicol* 40:264–270.
9. Couillard CM, Lee K, Légaré B, King TL. 2005. Effect of dispersant on the composition of the water-accommodated fraction of crude oil and its toxicity to larval marine fish. *Environ Toxicol Chem* 24:1496–1504.
10. National Research Council. 2005. *Oil Spill Dispersant: Efficacy and Effects*. National Academies Press, Washington, DC, USA.
11. Wolfe DA, Hameedi MJ, Galt JA, Watabayashi G, Short J, O'Claire C, Rice S, Michel J, Payne JR, Braddock J, Hanna S, Sale D. 1994. The fate of the oil spilled from the *Exxon Valdez*: The mass balance is the most complete and accurate of any major oil spill. *Environ Sci Technol* 28: 560–568.
12. McIntosh S, King T, Dongmei W, Hodson PV. 2010. Toxicity of dispersed weathered crude oil to early life stages of Atlantic herring (*Clupea harengus*). *Environ Toxicol Chem* 29:1160–1167.
13. Hodson PV, Khan CW, Saravanabhavan G, Clarke L, Brown RS, Hollebone B, Wang Z, Short J, Lee K, King T. 2007. Alkyl PAH in crude oil cause chronic toxicity to early life stages of fish. *Proceedings*, 28th Arctic and Marine Oil Spill Program Technical Seminar, Edmonton, AB, Canada, June 4-7, pp 291–300.
14. Billiard SM, Querbach K, Hodson PV. 1999. Toxicity of retene to early life stages of two freshwater fish species. *Environ Toxicol Chem* 18: 2070–2077.
15. Smith RL, Cameron JA. 1979. Effect of water soluble fraction of Prudhoe Bay crude oil on embryonic development of Pacific herring. *Trans Am Fish Soc* 108:70–75.
16. Li Z, Lee K, King T, Boufadel MC, Venosa AD. 2009. Evaluating crude oil chemical dispersion efficacy in a flow-through wave tank under regular non-breaking wave and breaking wave conditions. *Mar Pollut Bull* 58:735–744.
17. Li Z, Boufadel MC, Venosa AD, Lee K. 2006. A wave tank facility to assess chemical oil dispersant effectiveness as a function of energy dissipation rate. *Proceedings*, 2006 Interspill Conference, London, UK.

18. Funke ER, Mansard EP. 1979. SPLSH: A program for the synthesis of episodic waves. Hydraulics Laboratory Technical Report LTR-HY-65. National Research Council, Ottawa, ON, Canada.
19. Lee K, King T, Robinson B, Li Z, Burridge L, Lyons M, Wong D, MacKeigan K, Courtenay S, Johnson S, Boudreau M, Hodson P, Greer C, Venosa A. 2011. Toxicity effects of chemically-dispersed crude oil on fish. *Proceedings*, 2011 International Oil Spill Conference, Portland, OR, Paper 163, 16 pp.
20. Wu D, Wang Z, Hollebone B, McIntosh S, King T, Hodson PV. 2012. Comparative toxicity of four chemically-dispersed and undispersed crude oils to rainbow trout embryos. *Envrion Toxicol Chem* 31:754–765.
21. Schein A, Scott JA, Mos L, Hodson PV. 2009. Oil dispersion increases the apparent bioavailability and toxicity of diesel to rainbow trout (*Oncorhynchus mykiss*). *Environ Toxicol Chem* 28:595–602.
22. Dinnel PA, Schwark NT, Balderas A, Cotter M, Middaugh DP. 2002. Development of embryo and larval Pacific herring, *Clupea pallasi*, bioassay protocols: Phase II. Shannon Point Marine Center, Western Washington University, Anacortes, WA, USA.
23. Hay DE. 1985. Reproductive biology of Pacific herring (*Clupea harengus pallasi*). *Can J Fish Aquat Sci* 42:111–126.
24. Purcell JE, Grosse D, Glover JJ. 1990. Mass abundances of abnormal Pacific herring larvae at a spawning ground in British Columbia. *Trans Am Fish Soc* 119:463–469.
25. Carls MG, Rice SD, Hose JE. 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.
26. Marty GD, Hose JE, McGurk MD, Brown ED. 1997. Histopathology and cytogenetic evaluation of Pacific herring larvae exposed to petroleum hydrocarbons in the laboratory or in Prince William Sound, Alaska, after the *Exxon Valdez* oil spill. *Can J Fish Aquat Sci* 54:1846–1857.
27. Villalobos SA, Papoulias DM, Meadows J, Blankenship AL, Pastva SD, Kannan K, Hinton DE, Tillitt DE, Giesy JP. 2000. Toxic responses of medaka, d-rR strain, to polychlorinated naphthalene mixtures after embryonic exposure by in ovo nanoinjection: A partial life-cycle assessment. *Environ Toxicol Chem* 19:432–440.
28. Williams RT, Bridges JW. 1964. Fluorescence of solutions: A review. *J Clin Pathol* 17:371–394.
29. Motulsky H, Christopoulos A. 2004. *Fitting Models of Biological Data Using Linear and Nonlinear Regression*. Oxford University Press, New York, NY, USA, p 15.
30. Barron MG, Carls MG, Heintz R, Rice SD. 2004. Evaluation of fish early life-stage toxicity models of chronic embryonic exposures to complex polycyclic aromatic hydrocarbon mixtures. *Toxicol Sci* 78:60–67.
31. Bridges JW, Williams RT. 1962. Fluorescence of some substituted benzenes. *Nature* 196:59–61.
32. French-McCay DP. 2002. Development and application of an oil toxicity and exposure model, OilToxEx. *Environ Toxicol Chem* 21:2080–2094.
33. Lessard RR, Demarco G. 2000. The significance of oil spill dispersants. *Spill Sci Technol B* 6:59–68.
34. Incardona J, Ylitalo G, Myers M, Scholz N, Collier T, Vines C, Griffin F, Smith E, Cherr G. 2008. The 2007 *Cosco Busan* oil spill: Assessing toxic injury to Pacific herring embryos and larvae in the San Francisco estuary. Draft Report, NOAA Fisheries, Northwest Fisheries Science Center, Seattle, WA, USA.

Copyright of Environmental Toxicology & Chemistry is the property of Wiley-Blackwell and its content may not be copied or emailed to multiple sites or posted to a listserv without the copyright holder's express written permission. However, users may print, download, or email articles for individual use.