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TOXICITY OF DISPERSED WEATHERED CRUDE OIL TO EARLY LIFE STAGES OF ATLANTIC HERRING (*CLUPEA HARENGUS*)

STEPHEN MCINTOSH,† TOM KING,‡ DONGMEI WU,§ and PETER V. HODSON*†‖
†Department of Biology, Queen's University, Kingston, Ontario, K7L 3N6, Canada
‡Centre for Offshore Oil, Gas and Energy Research, Fisheries and Oceans Canada, Bedford Institute of Oceanography, P.O. Box 1006, Dartmouth, Nova Scotia, B2Y 4A2 Canada
§Institute of Loess Plateau, School of Environmental Science and Resources, Shanxi University, Taiyuan, People's Republic of China 030006
‖School of Environmental Studies, Queen's University, Kingston, Ontario, K7L 3N6 Canada

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Abstract—Reports of the chronic toxicity of dispersed crude oil to early life stages of fish perpetuate uncertainty about dispersant use. However, realistic exposures to dispersed oil in the water column are thought to be much briefer than exposures associated with chronic toxicity testing. To address this issue, the toxicity of dispersed weathered oil to early life stages of Atlantic herring (*Clupea harengus*) was tested for short exposure durations, ranging from 1 to 144 h. Toxicity was a function of concentration and duration of exposure, as well as of the life stage exposed. Medium South American crude oil dispersed with Corexit 9500 caused blue sac disease in embryos, but not in free-swimming embryos. The age of embryos was negatively correlated with their sensitivity to oil; those freshly fertilized were most sensitive. Sensitivity increased after hatch, with free-swimming embryos showing signs of narcosis. Gametes were also tested; dispersed oil dramatically impaired fertilization success. For exposures of less than 24 h, gametes and free-swimming embryos were the most sensitive life stages. For those of more than 24 h, young embryos (<1 d old) were most sensitive. The results are presented as statistical models that could assist decisions about dispersant use in the vicinity of fish spawning habitats. Environ. Toxicol. Chem. 2010;29:1160–1167. © 2010 SETAC

Keywords—Fish embryos Dispersed oil toxicity Exposure time Predictive model

INTRODUCTION

To minimize the damage caused by oil spills, responders may chemically disperse spilled oil into the underlying water before it contacts shorelines and wildlife. Quantifying this strategy's net environmental impact requires analyses of its subsurface aquatic effects. Currently, spill responders lack much of the toxicological data upon which these analyses depend.

After a spill, waves and currents mechanically mix a small fraction of oil below the surface, introducing a water-accommodated fraction (WAF) of oil into the water column. Adding chemical dispersant to slicks enables a larger quantity of oil, the chemically enhanced water accommodated fraction (CEWAF), to mix with water. Toxic constituents of oil, such as three- to five-ringed polynuclear aromatic hydrocarbons (PAH) [1], are more abundant and bioavailable in CEWAF (i.e., dispersed oil) than in WAF (nondispersed oil). Accordingly, CEWAF can be 100- to 1,000-fold more toxic than WAF [2]. Early life stages of fish chronically exposed to CEWAF display signs of blue sac disease (BSD), which is linked to mortality [2]. Signs of BSD have been observed in the embryos of an array of freshwater and marine fish species, including fathead minnows (*Pimephales promelas* [3]), mummichog (*Fundulus heteroclitus* [4]), Atlantic herring (*Clupea harengus*; M. Sweezey. 2005. Bachelor of Science thesis, Queen's University, Kingston, ON, Canada), and Pacific herring (*Clupea pallasi* [5]) exposed to waterborne hydrocarbons contained in WAF or CEWAF. The general sensitivity of the early life stages is well documented (e.g., [6]), and experience with the *Valdez* spill corroborated lab-scale results on an ecological scale. The subsequent collapse of Prince William Sound's herring fishery underscored the interdependency of early life stages, adult populations, and fisheries.

Despite the number of publications on dispersed oil and early life stages of fish [7], most deal with chronic toxicity (i.e., 10–20-d exposures). However, dispersion efficacy tests suggest that appreciable concentrations of waterborne hydrocarbons persist only 1 to 48 h postdispersion, and almost certainly not several days [8]. Lessard and DeMarco [9] reported that in the first hour following a ''typical'' dispersion application, the waterborne hydrocarbon concentration ranges from 40 to 60 mg/L in the upper 10 m of water, but dilutes to 1 mg/L within 5 h. Traditional toxicity tests based on 10- to 20-d exposure regimes may therefore be unrealistic and overly conservative [8,9]. Due to the lack of acute toxicity data, current effects-forecasting models for dispersed oil (OilToxEx, SIMAP) utilize chronic or incipient lethality data to estimate the effects of brief exposures.

In the present study, the acute and latent toxicity of dispersed, weathered crude oil to early life stages (gametes, embryos, free-swimming embryos) of Atlantic herring were characterized following realistic, brief exposures. Stage-specific sensitivities were considered and toxicity was tested during sensitive windows of development. Statistical models were developed to describe the relationships among life stage, exposure duration, and concentration of dispersed oil causing lethal and sublethal effects. These models will help to define the critical exposure conditions needed to protect fish embryos from the adverse effects of chemically dispersed oil.

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

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

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METHODS

Experimental design

To assess the risk of chemically dispersed oil in fish spawning habitats, gametes, embryos, and free-swimming embryos of Atlantic herring were exposed to CEWAF of weathered Medium South American (MESA) crude oil. Toxicity to unfertilized gametes was assessed by combining eggs and sperm in CEWAF (0.0032–3.2% v/v) for 1 h, after which fertilization success was recorded. Embryos aged 0 to 12 d were exposed to 0.32 and 1% CEWAF for 48 h to identify the most sensitive stage(s) of embryonic life. The results, which suggested that sensitivity to CEWAF decreases with age, were corroborated by responses of 0- to 11-d-old embryos exposed to 0.032 to 3.2% v/v for 48 h. To assess the toxicity of CEWAF to embryos during their most sensitive phase of development, freshly fertilized embryos (5–8 h postfertilization) were exposed to 0.01 to 3.2% v/v CEWAF for durations of 1 to 144 h. After exposures, fish were transferred to uncontaminated water until hatch. Toxicity was assessed by cumulative mortality and the occurrence of BSD at the end of the experiments, shortly after hatch.

Freshly hatched, free-swimming embryos were also exposed to CEWAF. The concentration and duration series used for free-swimming embryo exposures were 0.00032 to 3.2% v/v, and 1 to 144 h, respectively. Chronic exposure (from fertilization to hatch) to 3.2% CEWAF provided positive control results for embryo testing. Water controls, as well as water spiked with 0.0032% v/v Corexit 9500 dispersant, accounted for any toxic effects not imposed by components of MESA in CEWAF.

Oil, dispersant, and CEWAF

Both MESA crude oil and Corexit 9500 (Ondeo Nalco Energy Services) were supplied by the Centre for Offshore Oil, Gas and Energy Research in Dartmouth, Nova Scotia, Canada. Corexit 9500 is the only dispersant preapproved for use in Canada (K. Lee, Fisheries and Oceans Canada, Dartmouth NS, Canada, personal communication). The oil was weathered by sparging with air for 130 h to simulate the evaporation of volatile components at sea after a spill, representing approximately 14 % by weight [10].

Corexit 9500 was used to disperse MESA into 15‰ sea water to prepare CEWAF according to Ramachandran et al. [10], with one modification: dispersant was added to the oil–water mixture at a ratio of 1 part dispersant/10 parts oil, rather than 1 part dispersant/20 parts oil. This increase in dispersant concentration increased the total hydrocarbon concentration (THC) of the resulting CEWAF by approximately 50%.

Gonad shipment

Viable gametes were obtained from landed adult herring of at least a 25-cm fork length and stage VI of sexual maturity [11]. Twenty ripe female and male herring gonads were received on September 26, 2007, from Eastern Shore, Nova Scotia, Canada, and on May 13, 2008, from Escuminac (NB, Canada). Approximately half of the gonads of each sex were left in vivo, whereas the remainder were excised and stored in sealed glass Mason jars. All were shipped in coolers with freezer packs from sea to Queen's University in Kingston (ON, Canada) via air cargo within approximately14 h of landing (i.e., within 15–22 h of catch). Excised testes were pooled in one Mason jar. In contrast, all ovaries were stored separately and used for separate replicates to account for variable egg qualities among females following existing methods ([12], http://www.ac.wwu.edu/~spmc/).

Fertilization procedure

The optimum salinity for fertilization was 15‰ (data not shown), consistent with that for Pacific herring from San Francisco Bay, and approximately twice that for Baltic Sea herring (*Clupea harengus membras* [13]). Tolerance of non-optimal salinity is significantly higher for Atlantic herring than for Pacific or Baltic Sea herring [13].

A total of 15‰ artificial saltwater was prepared using Kent Sea Salt (Kent Marine) and water from Lake Ontario (alkalinity = 90 mg/L $CaCO_3$). The water was cooled to 10°C, and with the exception of final scoring, all experimental procedures took place at 10°C with a 2:22-h dim light to darkness photoperiod. Semen was expressed from testes and diluted in saltwater to a concentration of 5‰ v/v. Approximately 50 to 75 eggs from the ripest excised ovaries were expressed into 50 ml Petri dishes containing 30 ml salt water. Enough dishes were prepared to provide three replicates for each exposure, using the eggs from a different female for each replicate. One milliliter of milt was subsequently added to each Petri dish. After 1 h, the milt solutions were decanted. Because the eggs adhered to the dishes, it was possible to rinse them with salt water to remove excess milt, before refilling the dishes with 30 ml saltwater. The comparatively low milt density and higher milt–egg contact time allowed high fertilization success (95%) shared by other methods that used higher sperm concentrations. After 5 h, unfertilized eggs, distinguished by an undifferentiated appearance and lack of a distinct chorion, were discarded.

Gamete exposures

To determine if eggs can be fertilized in the presence of dispersed oil, a solution of milt containing 0.0032 to 3.2% v/v CEWAF was added to Petri dishes containing eggs. After 1 h, the solutions were exchanged with clean saltwater and the eggs rinsed. At least 5 h later, the percentage of fertilized eggs in each Petri dish was recorded, and the average fertilization success associated with each CEWAF exposure concentration was calculated.

Embryo exposures

In all experiments, 95% of each test solution, including controls, was renewed daily until hatch (~12 d postfertilization [dpf]), when free-swimming embryos were anesthetized (Tricaine Methanesulfonate 100 mg/L, Argent Laboratories Canada) and responses quantified. Dead embryos were removed daily. Water chemistry for all experiments was measured every second day to ensure conditions were within a suitable range (temperature: 8.8 ± 0.3°C [mean ± standard deviation]; dissolved oxygen: 98.9 ± 5.5%; pH = 7.8 ± 0.3; conductivity 23.9 ± 1.8 mS/cm).

For the first set of exposures, groups of 25 healthy, fertilized eggs were placed, unbound to one another, in 500-ml glass Mason jars with 200-ml aerated saltwater. Enough jars were prepared for triplicate semistatic, daily renewal exposures of 1 and 0.32% v/v CEWAF, beginning on each day of embryonic life (0–11 dpf) and lasting 48 h. Eggs from a different female herring were used for each replicate. An age of 0 dpf was considered to start 5 h after fertilization, and 1 dpf started 24 h thereafter. After 48 h exposure to CEWAF, embryos were transferred to clean water until hatch, when responses were measured.

This preliminary experiment was refined to assess mortality and occurrence of BSD of 0 to 5 and 11-d-old embryos exposed to a broader range of CEWAF concentrations (0.032–3.2% v/v) at a single exposure time of 48 h. A third experiment measured lethal and sublethal effects (BSD) of 0.01 to 3.2% v/v CEWAF on 0- and 1-d-old embryos exposed for 1 to 144 h. Although the methodology was otherwise consistent with previous experiments, time constraints required that embryos be exposed to 30 ml of CEWAF directly in the Petri dishes in which they were originally fertilized, rather than being transferred to Mason jars for exposure. Enough eggs were removed from each Petri dish until only 20 fertilized eggs, free from contact with one another, adhered to the bottom of each dish.

Free-swimming embryo exposures

Triplicate groups of 10 newly hatched, free-swimming embryos were exposed for 1 to 144 h to 0.00032 to 3.2% v/v CEWAF in aerated 2-L stainless steel bowls, with daily solution renewal. Cumulative mortality was monitored.

Response quantification

Using a rating system from 0 (minimum) to 3 (maximum), each embryo was assessed for pericardial and yolk sac edemas (*PE*, *YE*), spinal curvature (*SC*), and swimming ability (*SA*). Individuals that moved very little, even when prodded, had *SA* scores of 0 to 1; faster and more active swimmers had scores of 2 to 3. Craniofacial malformation (*CF*) and fin rot (*FR*) were rated categorically as 0 (absent) or 1 (present). These signs of BSD were integrated with mortality to form a slightly modified version of the Scott and Hodson [14] BSD Severity Index (*SI*):

$$\text{BSD } SI = \frac{\Sigma PE + \Sigma YE + \Sigma SC + \Sigma CF + \Sigma FR - (2 \times SA) + (11.5 \times D)}{\text{Maximum possible } SI \text{ Score}}$$

where D refers to the total number dead for the treatment and the maximum SI score equals $n \times 11.5$. Dead individuals were assigned a score of 11.5 (0.5 higher than the maximum sublethal score). For example, if 20 exposed individuals are considered, of which five have a *PE* of 3 and two others have *PE* scores of 2; 3 have spinal curvatures of 1, four are dead, and the average movement of individuals is two, the BSD *SI* for this treatment would equal $([5 \times 3] + [3 \times 2] + [3 \times 1] - [2 \times 2] + [11.5 \times 4]) \div (20 \cdot 11.5)$, or 0.3. All signs of toxicity observed corresponded to exposure to CEWAF, and of these signs, pericardial and yolk sac edemas, and reduction in movement were most frequent.

Characterization of hydrocarbon concentrations in test solutions

Daily, 1.5-ml samples from exposure solutions were diluted 50% with ethanol and stored at 4°C in scintillation vials (7 ml) for analysis by scanning spectrofluorometry (Quanta-Master Fluorescence Spectrometer; Photon Technology International). Fluorescence of solutions excited by coherent light (297-nm wavelength) was measured for emission wavelengths of 305 to 480 nm; fluorescence of solutions excited at 356 nm was measured for emission wavelengths of 220 to 350 nm. Felix software (Photon Technology International) was used to integrate the average fluorescence profiles of each sample, along with a standard curve of MESA diluted in ethanol. These analyses indicated that container choice (Petri dish or Mason jar) had little effect on THC of solutions.

At 0 and 24 h after preparation, PAH and THC were measured in samples of CEWAF test solutions (0.1 and 1% v/v), the CEWAF stock, and controls solutions. A surrogate standard solution (midrange of experiment concentrations) was prepared gravimetrically from deuterium-labeled d_8-naphthalene, d_{10}-acenaphthene, d_{10}-phenanthrene, d_{12}-chrysene, d_{12}-benzo[*a*]-pyrene, d_{12}-perylene, d_{26}-*n*-dodecane, d_{34}-*n*-hexadecane, d_{42}-*n*-eicosane, d_{50}-*n*-tetracosane, and d_{62}-*n*-triacontane. One milliliter of surrogate standard solution was added per liter of sample to determine individual sample recovery control. Hydrocarbons were extracted in dichloromethane and the extracts dried with anhydrous sodium sulphate prior to solvent exchange into 2,2,4-trimethylpentane for analysis by Agilent 6890N gas chromatograph/5973N mass spectrometer (Agilent Technologies) in the selective ion-monitoring mode [15,16].

Statistics

Nonlinear regression analyses, Dunnett's tests, Bonferroni's Multiple Comparison tests, Pearson Correlation tests, extra sum-of-squares F tests, median lethal concentration (LC50) and median effective concentration (EC50) calculations, and associated descriptive statistics for fertilization success, percent mortality, and BSD *SI* were performed using GraphPad Prism software (ver 4.02, GraphPad Software). Based on average water control and positive control/maximal response values, top and bottom regression constraints were 100 and 0% for percent mortality, and 1 and 0.2 for BSD *SI*. TableCurve 3D (ver 4.0, Systat Software) was used to conduct multiple nonlinear curve fitting analyses. Three-dimensional surface plots were generated using TableCurve 3D, and formatted in SigmaPlot (ver 11.0, Systat Software). Upper and lower 95% confidence intervals for data were calculated as follows:

$$95\% \text{ confidence limits} = \text{mean} \pm t_{0.05,\,N-1} \times (\text{EMS}/n)^{0.5}$$

where t represents the critical Student t value (two-tailed), $N-1$ refers to error degrees of freedom from the analysis of variance (ANOVA), EMS is the error mean square from the ANOVA (pooled variance), and n is the number of replicates per treatment.

The measured hydrocarbon concentrations corresponding to nominal CEWAF concentrations (% v/v) were used to express EC50s and LC50s in terms of total hydrocarbons and PAH, assuming that the proportional chemical composition of CEWAF did not change with changes in concentration.

RESULTS

Gamete toxicity

Exposing gametes to 0.32% v/v CEWAF for 1 h significantly reduced fertilization success (Dunnett's Test, negative control = 95 %, $p < 0.01$; Fig. 1). The 1 h EC50 for fertilization success was 0.19% v/v (nonlinear decay regression, $R^2 = 0.99$).

Embryotoxicity

The preliminary embryo sensitivity test demonstrated that percent mortality was negatively correlated with age at initial exposure (Pearson Correlation Test, $p < 0.05$). On days 0 to 2 of embryonic life, sensitivity to 1% v/v CEWAF (48-h exposure duration) significantly exceeded that of other days (Bonferroni's Multiple Comparison Test, $p < 0.05$; Supplemental Data, Fig. S1).

Embryos exposed for 48 h to a broader range of CEWAF concentrations corroborated the finding that the sensitivity of embryos decreased as they aged, as indicated by increasing 48-h median effective concentrations (EC50s) for the BSD index (Fig. 2). The EC50s were calculated from sigmoidal exposure–

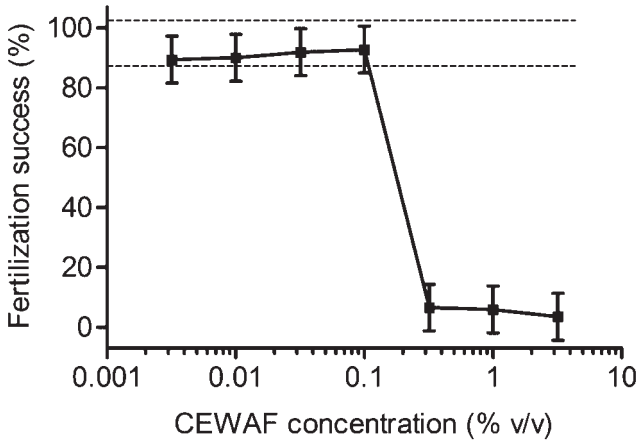

Fig. 1. Fertilization success of Atlantic herring eggs coexposed to milt and to the chemically enhanced water accommodated fraction (CEWAF) of weathered Medium South American crude oil for 1 h. Error bars are 95% confidence limits ($n = 3$) and the dashed lines represent the 95% confidence intervals for water control fish.

response relationships for exposures starting 0 to 11 dpf. For exposures 0 to 5 dpf, $R^2 = 0.80$, 0.92, 0.96, 0.84, 0.96, 0.90, respectively, but an EC50 could not be calculated for exposures starting 11 dpf because there was no toxicity. A multiple nonlinear logistic dose–response regression linked BSD to CEWAF exposure concentration and embryo age, a relationship shown as a response surface in Figure 2B.

The effect of increasing exposure duration and increasing CEWAF concentration on the toxicity of CEWAF to embryos first exposed at 0 dpf was analyzed as indicated above (Supplemental Data, Fig. S2). The EC50s decreased as the duration of exposure increased following an exponential decay regression (Fig. 3A). Regressions of BSD *SI* in response to 3- and 48-h exposures were quite variable ($R^2 = 0.27$ and 0.28, respectively) and not used to generate EC50 values. Reordering this equation provided a way to estimate the median effective time (ET50) for a 50% increase in the BSD *SI* at each concentration of CEWAF (y [% v/v]; $R^2 = 0.99$):

$$\text{BSD } SI \text{ ET50} = -9.66 \ln\{(\log_{10}[\text{y}] + 1.28)/2.25\}$$

Multiple dose–response curve-fitting was used to generate a single equation for predicting the BSD *SI* from CEWAF concentration and exposure time for exposures starting 0 dpf (Fig. 3B).

Free-swimming embryo toxicity

Without displaying signs of BSD, free-swimming embryos died in an exposure-dependent fashion after exposure to CEWAF (Supplemental Data, Fig. S3). The concentration of CEWAF contributed to mortality roughly four times as much as did exposure duration (two-way ANOVA, $p < 0.01$). The LC50s were related to exposure duration by the equation in Figure 4A. When this equation was reordered, the LT50s for each CEWAF concentration (y [% v/v]; $R^2 = 0.95$) could be calculated as follows:

$$\text{LT50} = -86.2 \ln\{(\log[y] + 2.31)/1.95\}$$

The three-way interactions among percent mortality, CEWAF concentration, and exposure duration were represented by a logistic three-dimensional response surface that could be described mathematically to predict toxicity (Fig. 4B).

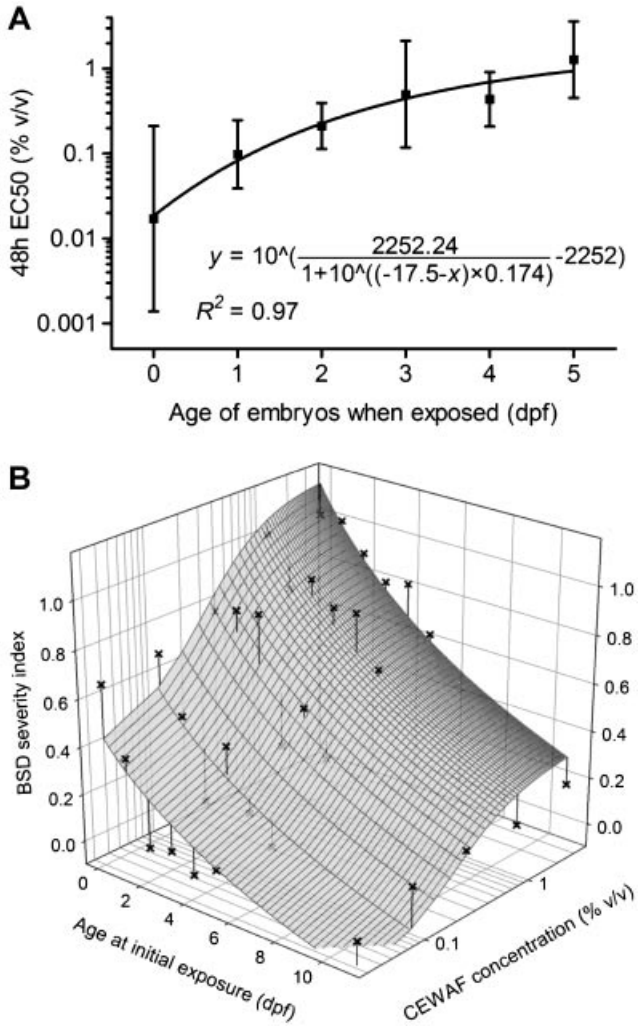

Fig. 2. The effect of age at first exposure (days postfertilization [dpf]) on the severity of blue sac disease (BSD) of Atlantic herring embryos exposed for 48 h to the chemically enhanced water accommodated fraction (CEWAF) of weathered Medium South American crude oil as shown by (**A**) 48-h median effective concentrations (EC50s; error bars represent 95% confidence intervals), and (**B**) interactions among the BSD Severity Index (*SI*), embryo age at first exposure, and CEWAF concentration:

$$Z = a + \frac{b}{1+\left(\frac{c}{x}\right)^d} + \frac{e}{1+\left(\frac{f}{y}\right)^g} + \frac{h}{\left(1+\left(\frac{c}{x}\right)^d\right)\left(1+\left(\frac{f}{y}\right)^g\right)}$$

where Z = BSD *SI*, x = time of initial exposure (dpf), y = CEWAF concentration (% v/v), $a = -0.77$, $b = 1.2$, $c = 12$, $d = -0.96$, $e = 0.18$, $f = 0.25$, $g = 1.5$, $h = 0.57$ ($R^2 = 0.87$).

Waterborne hydrocarbon analyses

Hydrocarbons were readily measurable in test solutions of dispersed MESA oil, and concentrations reflected the dilution series (Table 1; Supplemental Data, Fig. S4). However, the extraction/recovery of surrogate standards from 1% v/v CEWAF was poor for samples taken shortly after the time of spiking (~14%; data not shown), likely because the surfactant interfered with the extraction. The PAH were mostly alkylated (>90% of total PAH), dominated by alkyl napthalenes, alkyl dibenzothiophenes, and alkyl phenanthrenes, which together comprised more than 80% of alkyl PAH (Supplemental Data, Table S1).

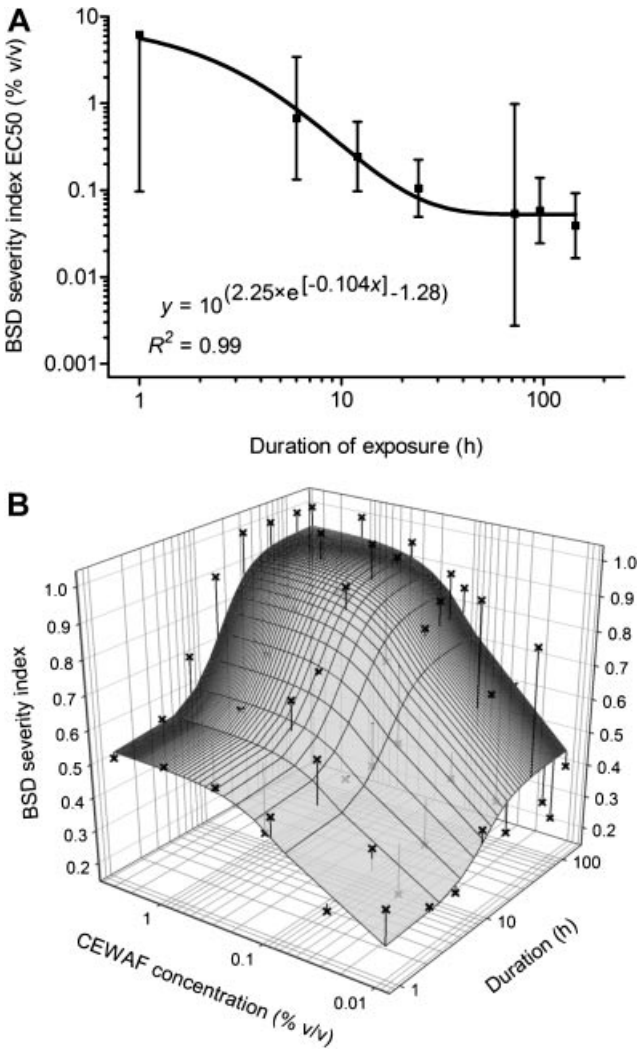

Fig. 3. The effect of exposure duration on the severity of blue sac disease (BSD) in Atlantic herring embryos exposed immediately after fertilization to the chemically enhanced water accommodated fraction (CEWAF) of weathered Medium South American crude oil, as shown by: (**A**) median effective concentration (EC50s; error bars represent 95% confidence intervals) and (**B**) the interactions among the BSD Severity Index (*SI*), exposure duration, and CEWAF concentration ($n = 3$; 95% confidence intervals = mean ± 0.18). Vertical lines indicate the distance of data points from a response surface of the logistic model:

$$Z = a + \frac{b}{1+\left(\frac{c}{x}\right)^d} + \frac{e}{1+\left(\frac{f}{y}\right)^g} + \frac{h}{\left(1+\left(\frac{c}{x}\right)^d\right)\left(1+\left(\frac{f}{y}\right)^g\right)}$$

where Z = BSD *SI*, x = duration of exposure (h), y = CEWAF concentration (% v/v), $a = 0.22$, $b = 0.13$, $c = 12$, $d = 2.5$, $e = 0.31$, $f = 0.053$, $g = 1.4$, and $i = 0.29$ ($R^2 = 0.73$).

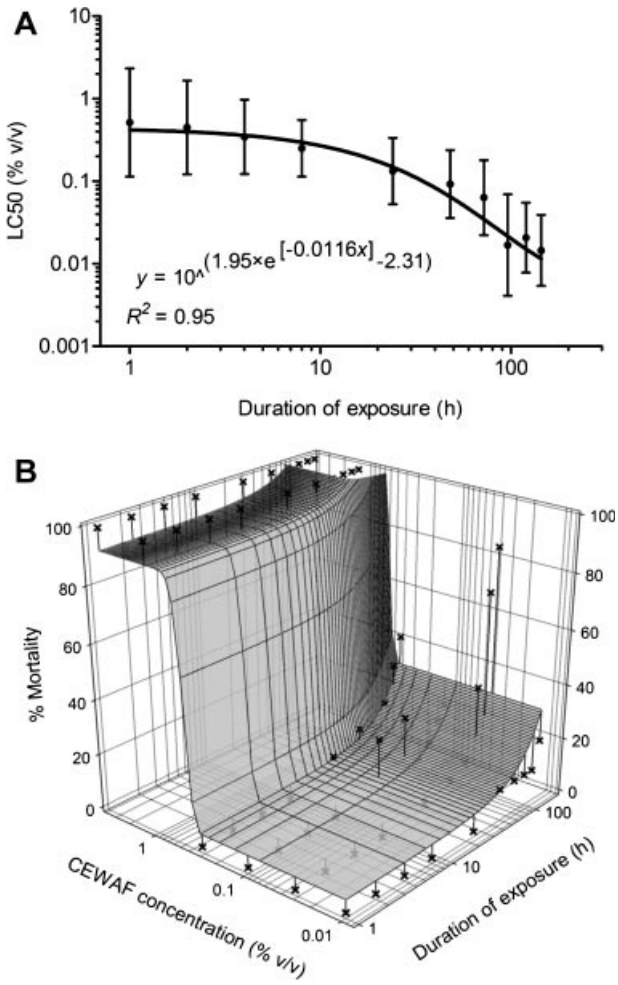

Fig. 4. The effect of exposure duration on the mortality of free-swimming Atlantic herring embryos exposed immediately after hatch to the chemically enhanced water accommodated fraction (CEWAF) of weathered Medium South American crude oil, as shown by (**A**) median lethal concentration (LC50s; error bars represent 95% confidence intervals) and (**B**) the interactions among percent mortality, exposure duration, and CEWAF concentration:

$$Z = a + bx + cx^2 + \frac{d}{1+\left(\frac{e}{y}\right)^f}$$

where Z = % mortality, x = duration of exposure (h), y = CEWAF concentration (% v/v), $a = 4.7$, $b = 0.053$, $c = 0.0010$, $d = 88$, $e = 0.56$, and $f = 22$. $R^2 = 0.93$; $n = 3$; 95% confidence intervals = mean 7.5); vertical lines display residual distance of the data points from the response surface.

DISCUSSION

Early life stages of Atlantic herring exposed to CEWAF of weathered MESA crude oil were sensitive to realistic oil spill scenarios, with clear differences among developmental stages in sensitivity (Table 2). Gametes responded dramatically to exposures far shorter than those toxic to other life stages. Sensitivity of embryos was greatest during the first 24 h after fertilization, but diminished rapidly with age; there was no visible effect on 11-d-old embryos. Free-swimming embryos did not display signs of sublethal toxicity, but succumbed to what presented as narcosis after brief exposures to CEWAF. Depending on the exposure time, each of the three stages could be considered the most sensitive (Table 2).

The period of high sensitivity immediately after fertilization identified by the present work is corroborated by studies of other chemicals and test species (e.g., [17][18]). Despite evidence for this sensitivity, embryotoxicity tests often omit the first 12 to 24 h of life in the experimental design. In some instances, embryo exposures start one to several dpf and continue until hatch (e.g., [19][20]). Given the sensitivity of embryos during the first 24 h of development, studies commencing one to several dpf may underestimate toxicity. Additionally, toxicity tests of identical chemicals, test species, and exposure regimes could generate entirely different results if the exposures started at different stages of embryonic development. For instance, in other tests of fish embryos, the initial age at exposure ranged from 24 h to several days postfertilization [19,21,22]. Some

Table 1. Total alkanes, alkylated polynuclear aromatic hydrocarbons (PAH), and unsubstituted PAH concentrations, corrected for % recovery of surrogate standards, in three samples of the chemically enhanced water accommodated fraction (CEWAF) of weathered Medium South American crude oil[a]

Sample concn. (% v/v); age (h)	Recovery of surrogate standards (% ± SE)[b]	Total alkane concn. (mg/L)	Total alkyl PAH concn. (mg/L)	Total unsubstituted PAH concn. (mg/L)
1; 24	95 ± 9.1	318	121	10.1
0.1; 0	74 ± 9.7	34	12.9	0.97
0.1; 24	86 ± 6.3	22	2.0	0

[a] Concentrations of individual PAH are listed in the Supplemental Data, Table S1.
[b] Standard error.

embryos may therefore have been exposed during a critically sensitive period, while others were exposed when more resistant. Hence, there is a high risk of underestimating toxicity (false negatives), depending on experimental design.

Commercial models that estimate toxicity of dispersed oil use this available body of chronic toxicity data, and are thus subject to these potential errors in estimates of toxicity. Models may also underestimate toxicity if they assume responses of fish exposed throughout embryo–larval life can predict responses of embryos or larvae exposed for a day or less. The observed sensitive windows of development and stage-specific differences in sensitivity to CEWAF among early life stages of Atlantic herring challenge that assumption.

Summarizing 54 field experiments on the efficacy of dispersants, Nichols and Parker [23] reported that dispersed spills generate waterborne hydrocarbon concentrations of 1 to 67 mg/L, with 10 mg/L being typical. Depths at which those concentrations occurred, as well as the duration they persisted, were variable. McAuliffe [24] predicted the most likely hydrocarbon concentrations under a dispersed spill (depth of 7 m) to be much higher: between 50 and 150 mg/L. Assuming these concentrations persisted 1 to 3 h, gametes and free-swimming embryos of herring would be at risk (Table 2). However, Lessard and DeMarco [9] insisted that the upper 10 m of the water column would contain 40 to 60 mg/L for no more than 1 h, suggesting only gametes would be affected by dispersion (Table 2). MacNeill et al. [25] also found 60 mg/L hydrocarbon under a spill dispersed in an experimental wave basin, but in contrast to Lessard and DeMarco [9], speculated that the duration would depend on case-specific conditions (wave energy, temperature, oil type, etc.). In corroboration, Humphrey et al. [26] recorded concentrations greater than 50 mg/L for at least 16 h at a depth of 10 m, while an undispersed control spill generated only 1 mg/L hydrocarbon in the water column. With peak hydrocarbon concentrations of 150 mg/L persisting approximately 1.5 h at 10 m of depth, the group described dispersion as ''a massive injection of oil into the water column.'' This exposure would be detrimental to herring gametes, embryos (0 dpf), and free-swimming embryos (Table 2).

The relevance of these test spills to realistic large-volume spills is generally undisclosed. Ballou et al. [27] suggested that dispersing a spill of 100 to 1,000 barrels (14–140 tons) would sustain a 50-mg/L hydrocarbon concentration 1 to 3 m deep for 24 h. This spill volume is a fraction of what the International Tanker Owners Pollution Federation considers ''major'' (>700 tons; www.itopf.com/information-services/data-and-statistics/statistics/index.html), but dispersing it would nevertheless lead to exposure concentrations exceeding the EC/LC50s for embryos and free-swimming embryos of herring. One may deduce that major, large-scale spills have even more extensive detrimental impacts when dispersed around spawning habitats.

Exposure of newly spawned herring depends on the depths of their spawning habitats. Along George's Bank of Eastern Canada and the United States, spawning occurs 40 to 80 m deep, which is likely below the range of dispersed oil, especially on the southern flank of the bank where the water is physically stratified during herring spawning seasons [28]. Likewise, spring-spawning Norwegian herring spawn 250 m below the surface [29]. However, on Canada's Nova Scotian shelf, herring spawn 15 to 60 m below the surface [30], which may be within the range of dispersed oil pollution. Moreover, Pacific herring off British Columbia and the Western United States preferentially spawn in shallow water, 0.5 to 8 m below mean tidal depths [30]. Similarly, Baltic herring spawn 0 to 4 m subsurface [31], and North Sea herring populations spawn 2 to 20 m deep [32]. As discussed, dispersed oil can reach toxic concentrations relevant to many of these spawning depths.

Oil used in the present study was weathered prior to dispersion and bioassays (according to Singer et al. [33]), voiding it of many narcotic constituents [34]. The SIMAP model estimates oil weathered for 10 h to be three times less toxic than its nonweathered counterpart. Given the sensitivity of early life stages of herring to CEWAF of weathered MESA, it follows that they could be much more sensitive to the nonweathered version, assuming that the volatile constituents cause toxicity. However, although volatile compounds may enhance the acute lethal effects observed in free-swimming embryos, they would likely

Table 2. Most sensitive early life stages of Atlantic herring exposed to the chemically enhanced water accommodated fraction (CEWAF) of weathered Medium South American crude oil according to exposure duration[a]

Exposure duration (h)	Most sensitive stage	EC/LC50		
		CEWAF (% v/v)	TPAH (mg/L)	THC (mg/L)
1	Gametes	0.19	21	79
3	Free-swimming embryos	0.35	38	146
10	Free-swimming embryos	0.18	20	75
24	Embryos	0.08	8.5	32

[a] Median effective and lethal concentrations (EC50s/LC50s) are presented in terms of CEWAF concentration, total polynuclear aromatic hydrocarbon concentrations (TPAH), and total hydrocarbon concentration (THC; estimated from the Supplemental Data, Fig. S4). Respective responses of gametes, free-swimming embryos, and 0-day-old embryos were fertilization inhibition, mortality, and blue sac disease (BSD). Gametes were tested for only a single duration (1 h).

be dissipated in a real spill long before affecting the chronic toxicity to eggs caused by the more persistent alkyl PAH [1].

Although the present study confirms the importance of expressing toxicity in terms of actual (mg/L) rather than nominal (% v/v) concentrations, the application of gas chromatography-mass spectrometry can be costly for large experiments. Fluorescence spectroscopy is more cost-effective, and demonstrated that changes in nominal concentrations parallel changes in actual hydrocarbon concentrations. Samples measured by gas chromatography-mass spectrometry were thus limited to reduce costs, but were necessary to confirm results and for quality assurance monitoring. Alternative dose-metric analyses and indicators of PAH exposure, such as whole-body EROD activity bioassays and Western blotting of cytochrome P450 1A (CYP1A) proteins, were not sufficiently sensitive to demonstrate exposure dependency in herring embryos (data not shown).

The present study may be the first to evaluate the effects of CEWAF on gametes. Carls et al. [35] found that the fertilization of Pacific herring eggs was unaffected by prior exposure of the parent fish to oil, but the exposure of eggs was indirect. In the present study, egg fertilization decreased precipitously at CEWAF concentrations above 0.10% v/v (40 mg/L THC). Because eggs and sperm were exposed together, it was impossible to determine if the primary effect was on eggs, sperm, or their interaction during fertilization. For example, CEWAF may inhibit the motility of sperm and/or block access to the micropylar region of eggs, regardless of exposure duration. Without the guidance of the micropyle, fertilization is significantly impeded [36]. The in situ observation of dimorphic spawning behavior by herring [37] suggested that a lag time of 1 h or more may occur before eggs are fertilized, during which exposure to dispersed oil could occur. Gametes were exposed to CEWAF in the present study for only 1 h, and further work is needed to define the interactive effects of exposure time and concentration on this critical reproductive event.

High sensitivity immediately after fertilization suggests that water hardening of the chorion imparts resistance to waterborne xenobiotics by creating a barrier to diffusion [18][38]. The increased sensitivity of embryos immediately after hatch further supports the proposal that the chorion protects against exposure and toxicity. It is interesting that free-swimming embryos died after exposure to high concentrations of CEWAF without obvious signs of BSD, suggesting that there are critical periods of development sensitive to the BSD-inducing constituents of oil such as PAH [31].

The BSD responses of embryos from fall-spawning adults of Escuminac ranged from 0 (negative control) to 1 (Fig. 2), whereas those from spring-spawning adults of Eastern Passage (NS, Canada) ranged from approximately 0.2 (negative control) to 1 (Supplemental Data, Fig. S2). No differences were noted in the appearance of fish carcasses or the gametes inside, but obtaining samples of spawning adults and producing embryos was much more successful in the fall, confirming reports that the fecundity of spring spawners is lower than that of fall spawners [27]. However, the EC50s for fall-spawned embryos (Fig. 2) were slightly less than those for spring-spawned embryos (Fig. 3), indicating greater sensitivity of fall-spawned embryos.

Concentration and duration of exposure had roughly equal effects on the toxicity of CEWAF to embryos, but concentration affected toxicity to free-swimming embryos much more than duration of exposure. Estimates of toxicity by SIMAP and OilToxEx models are based on logarithmic relationships between mortality and hydrocarbon concentration, and second-order (exponential) relationships between mortality and duration of exposure, which seems inconsistent with the results of the present study. It appears these models may put too much emphasis on the effect of exposure duration on toxicity.

Responses of fish exposed to dispersant alone were not significantly different from those of water controls. Although dispersant alone can be toxic [7], dispersant molecules in CEWAF are unlikely to contribute directly to toxicity because their hydrophobic interactions with oil droplets reduces their bioavailability. For instance, Ramachandran [39] reported that a mineral oil–dispersant mixture was three times less toxic to medaka embryos than dispersant alone, indicating that the dispersant was sequestered with oil droplets and not free in solution.

Although no negative controls died throughout the 144-h experiment with free-swimming embryos, their yolk sacs were almost exhausted, and swim-up was imminent. Therefore, starvation might have played a role in the observed mortality following CEWAF exposure. Feeding might have provided the energy needed to detoxify hydrocarbons, or even to avoid catabolizing contaminated tissues. On the other hand, if prey items were also contaminated by CEWAF, exogenous feeding could have increased toxicity. Responses of feeding larvae (not tested) may otherwise parallel the responses of free-swimming embryos.

Estimates of toxicity to free-swimming embryos by 3D curve-fitting (Fig. 4B) were generally less than estimates by sigmoidal regressions (Fig. 4A). For example, the 3D regression suggested that CEWAF concentrations less than 0.32% v/v were generally nontoxic. Meanwhile, traditional sigmoidal regressions indicated that 0.32% v/v is close to the EC50 for durations less than 10 h, and well above the EC50 for greater exposure durations (Fig. 4A). The 3D regression for free-swimming embryos may therefore underestimate toxicity.

The sensitivity of Atlantic herring embryos to waterborne hydrocarbons from MESA is similar to that of mummichog embryos, 10 times less than that of rainbow trout embryos, and 100 times less than for Japanese medaka embryos [2]. Compared to Pacific herring, Atlantic herring appeared equally sensitive to oil; Alaska North Slope Crude oil was toxic to Pacific herring at concentrations of total aqueous PAH as low as 0.4 μg/L during continuous exposure throughout 16 d of development [40]. In the present study, increasing exposure time of Atlantic herring embryos to dispersed oil from 1 to 4 d increased toxicity by more than 25-fold (Fig. 4). Exposure for the entire period of embryonic development would likely yield the same sensitivity as observed with Pacific herring. Thus, all available evidence indicates that early life stages of other species spawning in different seasons are also at risk from dispersed oil.

CONCLUSION

Early life stages of Atlantic herring were sensitive to realistic exposures to dispersed weathered oil. Sensitivity fluctuated throughout development, and was highest for gametes, newly fertilized embryos, and newly hatched embryos. The statistical models that relate effects to concentrations of CEWAF and to exposure duration will be particularly useful to oil spill responders if incorporated into oil dispersion models. Lethal and sublethal responses of newly spawned fish to dispersed oil must be estimated to calculate the cost to fisheries and net environmental effects of dispersion. These responses are outputs of the statistical models presented in the present study.

SUPPLEMENTAL DATA

Figs S1–S3.
Table S1. (223 KB PDF)

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