INTRODUCTION

Orimulsion-400® (PDVSA-BITOR, Caracas, Venezuela) is a Venezuelan fuel, marketed for use in thermal generation stations as an economical and environmentally friendly alternative to Fuel Oil No. 6 or coal. Orimulsion-400 is a surfactant-stabilized emulsion consisting of approximately 70% Orinoco Belt bitumen and 30% water [1]. The only power plant in North America that presently burns Orimulsion-400 is located in Dalhousie (NB, Canada), where the New Brunswick Power Corporation (NB Power) has committed to a 20-year contract for tanker delivery of up to 800,000 metric tons of Orimulsion-400 annually [2]. On November 1, 2001, NB Power announced that it had filed an application with regulatory agencies to refurbish a second power generating station at Coleson Cove (NB, Canada) to begin burning Orimulsion-400 by 2004. If spilled in water, the physical properties of Orimulsion-400 result in behavior quite different from that of conventional fuel oils [1]. Because of the surfactant, Orimulsion-400 initially disperses and forms a cloud of suspended individual bitumen particles throughout the water column. The aqueous component, which contains most of the hydrocarbons that can be solubilized from the bitumen particles as well as the surfactant, eventually diffuses throughout the receiving body. The behavior of the suspended bitumen particles over time depends largely on salinity, temperature, and turbulence. In fresh water, the bitumen particles tend to sink, whereas at high salinities, the bitumen particles tend to coalesce and rise to the surface. In brackish water, studies have concluded that the movement of the bitumen in the water column is very difficult to predict [1,3] because even small changes in temperature and salinity have drastic effects on the relative densities. Agitation from waves or currents affects the rate at which all processes take place, and in a dynamic receiving environment, the bitumen particles can spend considerable time mixed in the water column. If the bitumen particles coalesce and rise to the surface, their movement will be influenced primarily by wind, similar to other hydrocarbon slicks. Cleanup at this stage can be complicated by the extremely high viscosity of the bitumen, rendering many conventional cleanup methods ineffective. The marketing company Bitor (Boca Raton, FL, USA) has recently demonstrated what it considers to be effective recovery technology, but that process requires high temperatures and air injection to be effective. Concern has been expressed by some that this might not be a practical solution for anything but minor spills. Given the potential for extensive dispersal of bitumen in the event of an Orimulsion-400 spill, research examining the potential effects on organisms in the receiving environment is clearly needed.

The present study examines the effects of Orimulsion-400 on eggs and larvae of the Atlantic herring (Clupea harengus), a commercially important pelagic fish. The Baie des Chaleurs area adjacent to Dalhousie, where the NB Power generating station is located, is a valuable spawning area for the Atlantic herring [4]. Herring eggs are attached to seaweed and substrate in the intertidal and subtidal zones [5,6], with highest egg concentrations at depths of 1.4 to 4.0 m [7]. Given the spawning period and incubation time, herring eggs would be vulnerable to exposure to Orimulsion-400 as many as three months of the year in the event of a spill.

MATERIALS AND METHODS

Eggs of three female herring were stripped, mixed, and fertilized using pooled sperm of five males. Fertilized eggs were allowed to adhere to the bottom of petri dishes, with approximately 250 eggs per dish [8]. Petri dishes containing approximately 35 ml of filtered seawater (salinity 30%) were incubated at 5°C, with daily water changes using prechilled, filtered seawater. Blastula-stage herring eggs were exposed to five concentrations of Orimulsion-400 in the following manner. Oil-in-water dispersions of Orimulsion-400 were prepared by adding seawater to a weighed quantity of the emulsion [9]. Nominal concentrations of 0.1, 1, 10, 100, and 1,000 mg/L were prepared, although the final concentrations were somewhat lower because of the tendency of the Orimulsion-400 to
adhere to the sides of containers. Once the eggs reached the blastula stage, three replicate petri dishes were exposed to each concentration of Orimulsion-400 for 24 h. Exposures were static, with no water changes. The choice of the exposure time followed experimental results from Jokuty et al. [9], in which substantial reductions in concentration of suspended bitumen was observed after 24 h. Three control dishes of eggs were exposed to filtered seawater only, resulting in a total of 18 dishes. After the exposure period, each dish was rinsed three times with fresh seawater and refilled.

Daily mortality counts were performed and time until hatch was noted for all embryos. The first 30 larvae (approximately) that hatched from each petri dish were anesthetized by MS-222 and photographed with image analysis software (IMAQ Vision, LabVIEW® Austin, TX, USA). These images were used to obtain the following measurements: total length, notochord length, yolk sac depth and length, head depth, and depth of myomeres adjacent to the pectoral buds. The yolk sac depth was divided by the depth of myomeres adjacent to the pectoral bud to categorize larvae according to the staging system for Atlantic herring devised by Doyle [10]. Recently hatched, normal herring larvae are expected to have a ratio of yolk sac depth to the depth of myomeres adjacent to the pectoral bud sac of 2.5 to 3.75 [10]. For the purposes of this paper, larvae with a ratio greater than 3.75 were considered to have hatched prematurely. Any abnormalities also were categorized and enumerated.

Treatment groups were compared using one-way analysis of variance, with tests of normality of residuals followed by post hoc means tests to provide specifics of any observed significant differences. If residuals were not normally distributed, the Kruskal–Wallis nonparametric test was used, followed by post hoc tests of mean ranks. Significance level was 0.05 in all cases. The percentage of abnormal larvae was compared to the control treatment by the proportions test [11].

RESULTS

Egg and hatching effects

No significant differences were found among treatments in percent hatch (mean = 39%). Time until hatch data suggest a nonlinear dose–response relationship, where exposure to low doses (0.1, 1, 10, and 100 mg/L) of Orimulsion-400 significantly increased time until hatch, compared to controls, and exposure to the 1,000 mg/L concentration significantly reduced time to hatch (Fig. 1). Time until hatch for all treatment groups ranged from 11.8 to 13.8 d.

Larval effects

Total length and notochord length. A number of significant differences among treatments in both total length (Fig. 2a) and notochord length at hatch were seen. No consistent pattern to the differences emerged, except that, in both cases, the measurements for the 1,000 mg/L treatments (median 3.57 and 3.49 mm for total length and notochord length, respectively) are dramatically lower than was measured for all other treatments (range 5.96–7.56 mm total length, 5.82–7.40 mm notochord length).

Yolk sac depth and length. The average yolk sac depth (Fig. 2b) at the 100 mg/L and 1,000 mg/L concentrations was significantly larger than that of all lower concentrations and the control. The average yolk sac length results were similar, with the measurements from the larvae treated with 100 and 1,000 mg/L significantly larger than all other treatments.
Varied larval deformities were categorized and enumerated (Fig. 3): slight flexion, one bend of the notochord; severe flexion, two or more bends; curled, the larva is curled in a complete but open circle; wrapped, the larva has hatched, but the body of the larva is still tightly wrapped around the yolk sac; other, clubbed tails or less severe versions of the other categories. The 0.1 mg/L and the 10 mg/L treatments had significantly fewer abnormalities than did the control (Fig. 4). The 1,000 mg/L treatment had significantly more abnormalities than did the control group and appeared to have more abnormal larvae than other treatments (Fig. 5).
Effects of Orimulsion-400 on herring eggs and larvae

A reduction in time until hatch might serve as an adaptive response to stress that enables larvae to leave a source of contamination quickly [15]. However, several potential costs are associated with premature hatch. First, premature larvae will spend a longer than normal time in the vulnerable yolk sac stage. If more time is spent in this stage, the likelihood of predation increases substantially [16,17]. Smaller larvae could also have diminished swimming ability, which could decrease their ability to capture food or their ability to maintain position in the water column [18,19]. Early developmental stages have been identified as the most influential stages in the establishment of fish year classes [20,21]; therefore, any potential effect is of concern.

We found that exposure to 1,000 mg/L of Orimulsion-400 resulted in over 80% abnormal larvae, which was significantly greater than that of control exposures. Some of these were obviously the result of premature hatch, such as the wrapped category. Others were of normal size but had developed abnormalities in shape. Although we did not attempt to maintain larvae beyond the yolk sac stage, it is unlikely that any of these malformed larvae would survive through to metamorphosis. Gassman [22] exposed seatrout (Cynoscion nebulosus) eggs and larvae to an earlier formulation of Orimulsion, Orimulsion-100. Reporting percentage of malformed larvae, Gassman [22] calculated a 24-h median effective concentration of 234 mg/L for pregastrulation eggs exposed to oil-in-water dispersions of Orimulsion-100. This is comparable to the results of the present study. Gassman [22] also reported that later stages of eggs and larvae are more sensitive to exposure to Orimulsion-100. Future research with Orimulsion-400 should include other life history stages of larval fish, as well as longer exposure times. The 24-h exposure time from the present study is probably a conservative estimate of the time herring eggs would be exposed to Orimulsion-400 in the case of a spill. If the salinity-temperature-current conditions favor sedimentation, a large percentage of spilled bitumen could end up on the bottom [1], thereby extending potential exposure times for eggs attached to the substrate.

The surfactant causes Orimulsion-400 to initially mix in water, the extent and duration of which depends on factors such as salinity, temperature, currents, wind, and waves. A spill of 10,000 tons could result in levels of 1,000 mg/L in 10,000,000 cubic meters of water. Floating booms have been developed to contain spills of Orimulsion; however, these containment devices are not effective at current velocities in excess of 0.75 knots [23]. In the vicinity of the existing terminal at Dalhousie, currents routinely reach 1 to 2 knots (chart 4426, Canadian Hydrographic Services, Ottawa, ON, Canada). In the macrotidal Saint John harbor, the proposed off-loading terminal for the new Orimulsion-400 plant in New Brunswick, currents are rarely less than 1 knot [24].

Proponents of Orimulsion-400 generally point out that their product has comparable toxicity to that of other fuels such as Bunker C and Fuel Oil No. 6, and the present study supports this contention. However, the different behavior of Orimulsion-400 in water, coupled with the difficulty in predicting bitumen movement in brackish water, makes direct comparisons of toxicity with those of floating fuels somewhat questionable [25]. Clearly, more research is needed to investigate behavior of Orimulsion-400 in conditions relevant to the off-loading sites in Atlantic Canada, incorporating an evaluation of toxicity of Orimulsion-400 to commercially important marine and estuarine species.

Fig. 4. Percentage of newly hatched larvae that had at least one morphological abnormality from each exposure concentration of Orimulsion-400 (PDVSA-BITOR). Asterisks indicate significantly different percentages than control values. Ninety larvae examined from each exposure concentration.

Fig. 5. Classification of abnormal larvae from each exposure concentration of Orimulsion-400 (PDVSA-BITOR) into different categories. Slight flexion, one pronounced bend in notochord; severe flexion, two pronounced bends in notochord; curled, larva in circle but with space around yolk sac; wrapped, larva wrapped around yolk sac with whole body in contact with yolk sac; other, a catch-all category including all other abnormalities, including slight bends, clubbed tails, and so on. Ninety larvae examined from each exposure concentration.
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REFERENCES


