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Effects of the Water–Soluble Fractions of No. 2 Fuel Oil on the Cytokinesis of the Quahog Clam (*Mercenaria mercenaria*)

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The effects of petroleum and petroleum products on the embryonic and larval stages of marine zooplankton have been studied extensively over the past decade (Renzoni 1975; Byrne and Calder 1977; Nicol et al 1977; Falk-Petersen 1979; Smith and Cameron 1979; Koster and Van Den Biggelaar 1980; Lonning and Falk-Petersen 1982). Research has concluded that these stages are among the most sensitive to petroleum pollution in the marine environment.

However, one of the areas of toxicological study which has received little attention has been that of the distortions of cellular components within the developing stages of the marine zooplankton in response to petroleum hydrocarbon contamination (National Academy of Sciences 1975; National Research Council 1985). The purpose of this report is to examine the cytokinetic effects of exposure of the water-soluble fractions of No. 2 fuel oil on the embryonic development of the quahog clam Mercenaria mercenaria.

MATERIALS AND METHODS

Quahog clams Mercenaria mercenaria were shipped from the Virginia Institute of Marine Sciences and spawned following the methods of Loosanoff and Davis (1963). Upon spawning the eggs were filtered through a series of stainless-steel sieves with mesh openings of 120, 74, and 35 μ. The smaller sieves retained the quahog eggs and the largest freed them from extraneous fecal materials. The eggs were concentrated by sieving and resuspended in a small volume of seawater, fertilized, and allowed to develop for 45 minutes to reach the first cleavage stage (Loosanoff and Davis 1963).

The petroleum pollutant of interest in this study was the water-soluble fraction (WSF) of No. 2 fuel oil, which is an American Petroleum Institute (API) reference oil. The water-soluble fraction was prepared by an introduction of 100 mL of oil to 800 mL

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of filtered (0.45 μ natural seawater (27 o/oo S) in a 1000 mL equilibrating flask. The flask was secured on a gyratory shaker and agitated at 200 rpm for 12 h, after which time the flask was allowed to equilibrate for 24 h. The aqueous phase was drawn off through a side arm near the bottom of the flask. The petroleum hydrocarbons dissolved in the WSF of the No. 2 fuel oil was quantitated gravimetrically. A 100 mL aliquot of the stock WSF concentrate was extracted three times in a separatory funnel with doubly-distilled chloroform. The solvent was reduced on a rotary evaporator, transferred to a pre-weighed 1 dram vial, and reduced under a stream of purified nitrogen. The petroleum hydrocarbon residue was weighed to within 0.01 mg on a MicroMettler M 5 analytical balance. The concentration of the 100% WSF No. 2 fuel oil concentrate was determined to be 22.9 ± 1.6 mg/L (ppm). This stock concentrate was used to prepare a modified geometric series of 10-mL test solutions (1% = 0.23 mg/L, 5% = 1.15 mg/L, 10% = 2.30 mg/L, 25% = 5.75 mg/L, 50% = 11.5 mg/L, and 100% = 23.0 mg/L). A concentration of 300 embroys were added to each test solution. All test solutions were maintained at 25°C in filtered seawater (27 o/oo). Duplicate solutions were prepared at each test concentration of the WSF together with four untreated control solutions. The entire experimental test series was repeated in replicate. At end of the 48 h test period, the embryos should have developed into the free-swimming, straight-hinged larval stage.

In addition, embryos were heat treated in seawater at 60°C to determine cytological differences between the effects of the WSF itself and autolysis. The effects on the cytokinesis were observed by means of a Sedgewick-Rafter plankton cell and an Olympus microscope fitted with a Pentax 35 mm camera attachment.

A median lethal concentration (LC50) for the 48-h exposures was determined by counting the number of embryos in each of the replicate solutions that had developed to the normal straight-hinged larval stage. This number was subtracted from the total number of fertilized embryos (n) to obtain the number of embryos that had responded to the WSF stress (r). The resulting response ratio of r:n of each set of replicates were summed and an average value calculated. It was from this ratio that the median lethal concentration (LC50) and its 95% confidence limits (C.I.) were determined by the use of the trimmed Spearman-Karber method (Hamilton et al 1977). Mortality in control solutions was always corrected according to Abbott's formula (Finney 1971).

RESULTS AND DISCUSSION

During the cytokinesis of marine organisms, there is a formation of a complex type of cleavage, which is referred to as "spiral cleavage". 'There is an increase in cell surface due mainly to the apposition of new membranes present in the cytoplasm to the progressing cell furrow. These old and new cell membranes form a continuum, which is in a highly dynamic state' (Brachet 1985). In

the control test solutions, the eggs of Mercenaria mercenaria (Figure 1) were fertilized with viable sperm suspension. The first cleavage stage was reached within 45 min (Figure 2). Within 48 h, 297 of the 300 embryos had developed into free-swimming, straight-hinged veliger larvae (Figure 3). A similar series of embryonic solutions were exposed to the water-soluble fractions of No. 2 fuel oil. Within 3 h of exposure to concentrations WSF of the fuel oil greater than 5 mg/L (ppm), 270 of the 300 embryos had experienced cellular disruption along the developing furrow line, followed by cytoplasm leakage (Figures 4 & 5). One hundred percent mortality occurred within 48 h of exposure (Table 1). A conventional trimmed Spearman-Karber 48-h LC50 was calculated to be 0.59 mg/L with 95% confidence limits of 0.48 to 0.69 mg/L.

Table 1. Percentage of surviving straight-hinged veliger larvae after exposure of 48-h to the WSF of No. 2 fuel oil

Concentration of WSF (mg/L)	% of surviving veliger larvae
23	0.0 ± 0.0
11.5	0.0 ± 0.0
5.75	0.0 ± 0.0
2.30	8.5 ± 10.0
1.15	34.5 ± 31.1
0.23	85.3 ± 4.9
Control	99.3 ± 2.3

Values are arthimetric mean ± standard deviation

Goldacre (1968) observed similar effects on the membrane surfaces of the amoeba, Amoeba dubia, when exposed to a variety of detergents and oils. The initial site of attack by these compounds was the tips of the advancing pseudopods. The effects included the expansion of the cell membrane and the increased thickening of the hyaline layer. At high concentrations of the chemicals, the granular cytoplasm contracted away from the membrane, bursting the membrane, which resulted in the death of the organism.

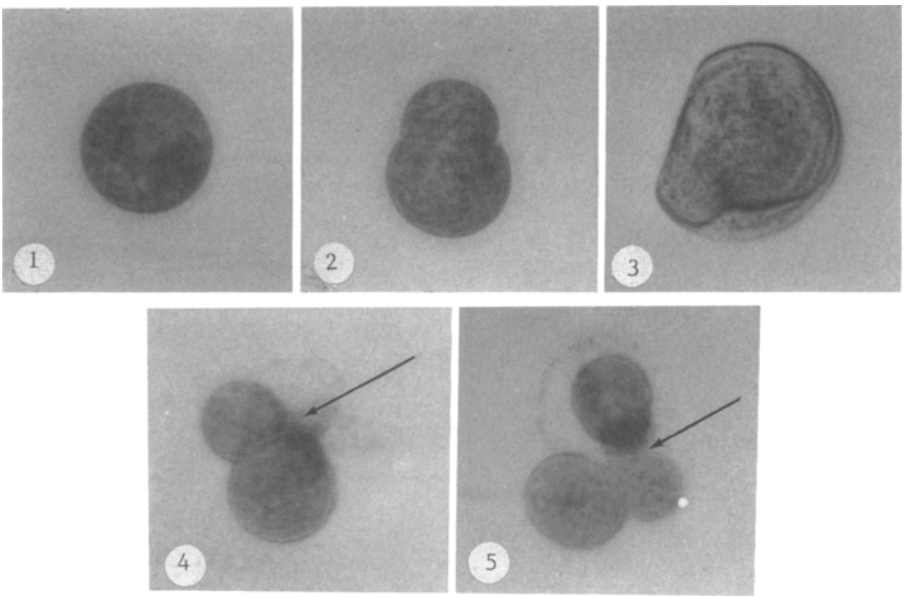

Figure 1. Control egg of the quahog clam Mercenaria mercenaria (X 200).
Figure 2. Control embryo at first cleavage stage - 45 min (X 200).
Figure 3. Control larva - 48 h (X 100).
Figure 4 & 5. Embryos exposed to water-soluble fractions of No. 2 fuel oil (11.5 mg/L) for 3 h, showing cytolysis along the furrow formation (arrows) (X 200).

The water-soluble fractions of No. 2 fuel oil have been shown to be particularly toxic to the embryonic stages of marine zooplankton. Renzoni (1975) observed abnormalities in the development of the larvae of the mollusc Mulinia lateralis exposed to the WSFs of Kuwait, Nigerian, and Prudhoe Bay crude oils. The abnormalities included the distortion of embryonic shape, the degeneration of cellular cytoplasm, and the occurrence of vacuolated intercellular spaces. Nicol et al (1977) noted that at concentrations greater than 600 ppb the WSF of No. 2 fuel oil produced a number of deleterious effects to the fertilization of the eggs of the sand dollar Melitta quinquesperforata. These effects included the reduction in fertilization of the eggs, the elevation of the fertilization envelope, a delay in the completion of the cleavage stages, and incomplete cell cleavage. Falk-Petersen (1979) reported significant irregularity in the development of the sea urchin Strongylocentrotus droebachiensis exposed to the oil product fractions of Ekofisk crude oil: heavy gas oil, light gas oil, kerosine, BP fuel oil No. 6, and BP gasoline. The embryos cleaved irregularly after the second cell stage and the blastulae and

gastrulae filled with degenerated cells. At high concentrations cytolysis resulted in the uncleft eggs. Koster and Van Den Biggelaar (1980), reporting on the impact of the Amoco Cadiz oil spill on the embryonic development of the marine scaphopoda Dentalium vulgare, noted similar effects which included first cleavage abnormalities, premature regression of the polar lobe constriction, and regression of the cleavage furrow. The effects noted on the development of these marine organisms confirm the sensitivity of the cell furrow to exposure to soluble petroleum hydrocarbons.

Although the concentrations of the WSFs of the No. 2 fuel oil used in these experiments were in the parts-per-million levels, concentrations at these levels have been reported in the vicinity of coastal oil spills (Hess et al 1978). This could produce additive stress to pre-existing syngeristic conditions, which could affect local population recruitment (Gilfillan and Vandermeulen 1978; Koster and Van Den Biggelaar 1980).

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