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Sublethal Effects of Crude Oil on a Cold-water Marine Leech, *Johanssonia arctica,* Following Chronic Exposure

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Petroleum polyaromatic hydrocarbons (PAH) are known to affect many species of animals. Mortality and/or morbidity from acute toxicity are usually restricted to the immediate vicinity of a spill, seep or discharge. Subtle effects are sometimes not recognized unless long term observations are made. Several studies have shown that some species of invertebrates tend to accumulate and retain petroleum hydrocarbons for varying periods (Bayne et al 1982), sometimes with detrimental effects. Rossi and Anderson (1977) observed that the gravid female polychete, Neanthes arenacoedentata, accumulated naphthalenes following exposure to No. 2 fuel oil mainly in the lipid-rich eggs. However, there was no evidence of long term effects although zygotes contained high concentrations of hydrocarbons. In a previous communication, it was noted that water soluble fractions (WSF) of a crude oil disturbed digestion and affected reproduction of a hematophagous marine leech, Johanssonia arctica (Kiceniuk and Khan 1983). This leech is a benthic, cold-adapted species that is widely distributed on the continental shelf off eastern Canada especially on the Grand Banks where petroleum reserves have been recently discovered. The present study provides additional evidence that crude oil fractions not only alters egg production but also hatching of eggs and survival of the progeny.

MATERIALS AND METHODS

The leech, J. arctica, was collected from Conception Bay, Newfoundland, as cocoons attached to the legs of a crab, Chionoecetes opilio, or to stones at depths of about 120-180 m and the young that emerged were reared in the laboratory to adulthood (vide Khan 1982). Prior to each experiment, they were allowed to feed on Atlantic cod, Gadus morhua until satiated. The leeches (25 mm in length) were subsequently exposed (10/group) to water soluble fractions of a Venezuelan oil,

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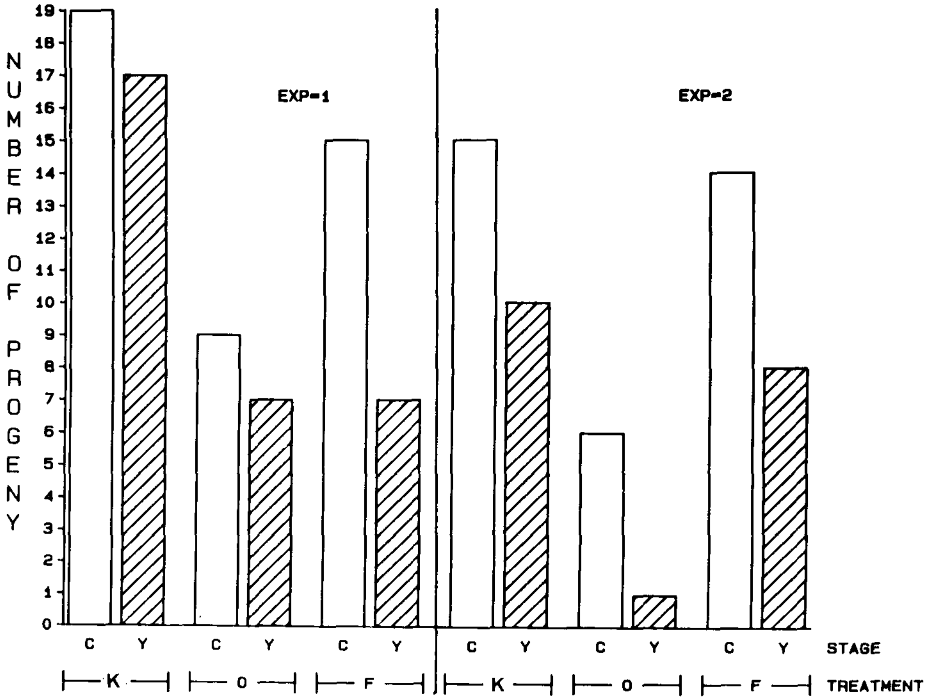

Figure 1. Comparison of the effect of WSF of crude oil (50-100 μg/L) on cocoon production and emergence of young of the marine leech, Johanssonia arctica, with untreated controls and groups that fed on Atlantic cod, Gadus morhua, 92 days after exposure to WSF. In the upper line, stage of development is indicated by C= cocoon and Y = young. In the lower line, control (k) and oil-treated (O) leeches fed on untreated cod whereas a third group (F) of leeches fed on cod that had been exposed to WSF but these were held in uncontaminated seawater subsequently.

purchased from a commercial distributor, or a crude oil (Hibernia), originating from the Grand Banks off Newfoundland, at total hydrocarbon concentrations of ~50 or 150 μg/L at 0°C in a flow-through, sea water system as described previously (Khan and Kiceniuk 1984) for 49 to 92 days and then depurated. They were held in a pliable, sieve-like hardware cloth containers 8 x 8 x 8 cm (pore size, 1 mm^2). Four plastic strips 80 x 20 x 2 mm were glued to the top to provide rigidity. The container was suspended by a string in a 3000 L tank so that its top was slightly (0.5 cm) above the water surface. Leeches invariably attached to the hard plastic strip and deposited their cocoons on it. Following exposure to WSF, the leeches, including the containers, were removed and held in clean, running

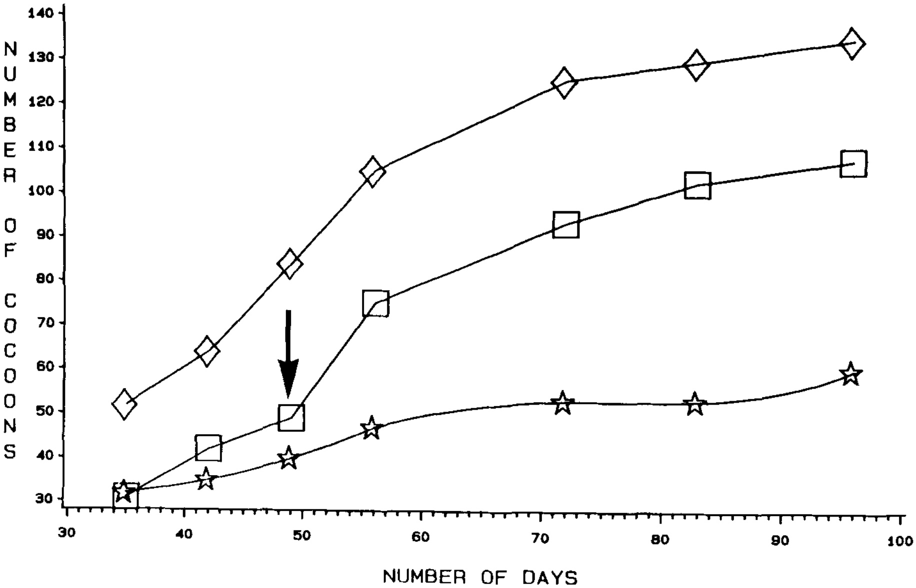

Figure 2. Effect of WSF (Hibernia crude oil), ~50 and ~150 µg/L, on cocoon production of J. arctica during (7 wk, period to left of arrow) and after exposure (diamond = control, square = ~50 µg/L; star = ~150 µg/L).

seawater until the blood meal was digested (80-135 days). Blood meal digestion was determined by the leech's color which changed from dark red after feeding to a pale brown following complete digestion. Control leeches were held in similar containers but were not exposed to oil-fractions. In one trial only a third group of leeches, that fed on cod which had been exposed previously to WSF (~100 µg/L) for 92 days were also held in oil-free water. In a second trial, the effect of water-accomodated oil fractions of Hibernia crude oil on cocoon production and egg viability as measured by emergence of young was investigated by exposing two groups of leeches for 7 weeks to two concentrations (~50 and ~150 µg/L). A third group of leeches, placed in uncontaminated sea water, served as controls. Survival of the young up to 35 days after emergence was also determined. A third trial examined the effect of WSF (~50 µg/L) on cocoon deposition after removing the leeches at 9, 15, 26, 38, 53 and 67 days after exposure. Controls were held in oil-free water. Cocoons of treated and untreated leeches were enumerated at intervals during and after exposure (9-96 days). They were subsequently maintained at 0°C for 67-135 days in uncontaminated, running sea water and the number of young emerging and their longevity after emergence were recorded. The data (vide Fig. 3) were

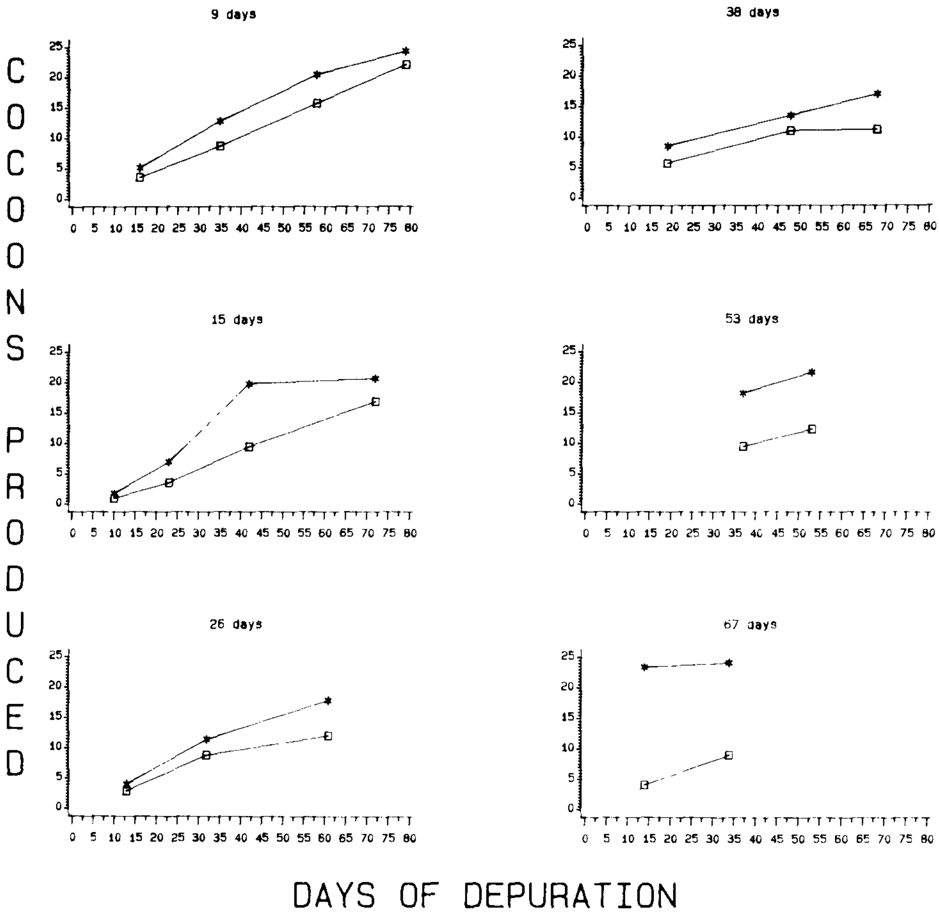

Figure 3. Effect of varying periods (days) of exposure to oil-fractions (~50 μg/L) on cocoon deposition by _J. arctica_ following depuration. (Upper lines are controls, lower are oil-treated).

analysed by Wilcoxon's signed rank test and analysis of covariance (Sokal and Rohlf 1969).

RESULTS AND DISCUSSION

The effect of oil fractions on cocoon production and emergence of young was investigated in two separate experiments. In the first trial, a greater number of cocoons was deposited by both the control group and the group that had fed on oil-treated cod than by those exposed directly to oil fractions (Fig. 1). Although blood meals were digested within 92 days in the two oil-free groups, 135 days elapsed before it was completed in the oil-treated group. Additionally, the

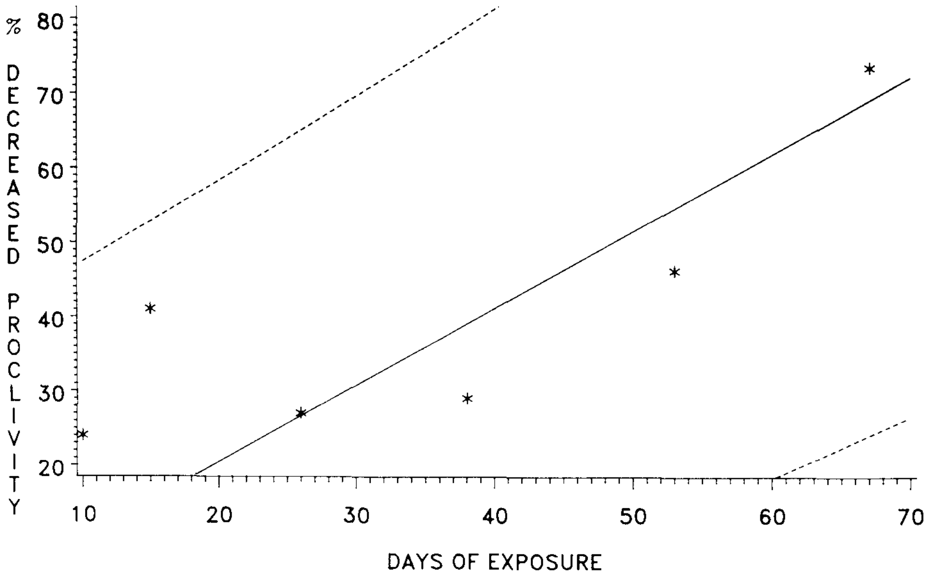

Figure 4. Relationship between days of exposure to oil (WSF, ~50 µg/L) and cocoon production by J. arctica. (Dashed lines are 95% confidence intervals).

number and percentage of young that emerged after a period of about 8-10 months were greatest in the control group than those that were either exposed to oil or fed on the oil-treated cod (vide Fig. 1). In the second trial which involved exposure of two groups to different oil concentrations, oil-treated leeches deposited fewer cocoons than controls especially at the higher (~150 µg/L) concentration (Fig. 2). Moreover, a higher mean number of young emerged from the control (10.7) than from the oil-treated groups exposed to the higher (1.2) or lower (2.5) oil concentrations. Survival of the young, 35 days after emergence, was also greater in the control (~60%) than among the progeny that originated from the oil-treated leeches (~10%). A third trial ascertained the effect of duration of exposure to WSF on reproductive capacity. Fewer cocoons were deposited by the oil-treated leech groups after exposure for 9, 15, 26, 38, 53 and 67 days than by the corresponding controls (Fig. 3). Analysis of covariance of the slopes of the pooled data for all exposure times gave a $p = 0.03$ value. Since cocoon production of the oil-exposed groups was always lower than that of control groups, it is evident from Wilcoxon's signed rank's test that any six pairs (control-exposed) is significantly different at the 5% level (Sokal and Rohlf 1969). The blood meal was not digested in more than 60% of the oil-treated leeches 90 days after depuration although it was complete in more than 95% of the control groups. It was also observed that the decrease in the rate of cocoon deposition was dose dependent (Fig. 4).

The present study confirms previous observations (Kiceniuk and Khan 1983) that chronic exposure of adult J. arctica to water-accomodated fractions of crude oil affects reproduction. However, this effect hinges on the period of exposure, varying from no apparent to slight (< 9 days exposure) to a significant reduction in reproductive capacity (38 to 67 days of exposure. The number of young that emerged and their survival subsequently was dependent on the hydrocarbon concentration to which they were exposed. These subtle effects are in agreement with observations made from field studies that petroleum hydrocarbons disturb growth and reproduction of some organisms (Gilfillan et al. 1978; Percy 1978; Mahoney & Noyes 1982). Moreover, productivity was not only dose-dependent but also related to the period of exposure to oil fractions.

Most aquatic organisms accumulate petroleum hydrocarbons following exposure and many can metabolize and excrete them as judged by the presence of mixed function oxidase activity (Rossi 1977; Lee 1981; Stegeman 1980; Neff et al. 1976). In some polychetes, such as N. arenacoedentata, the hydrocarbons are located in eggs as fat depots, which are later mobilized for nutrition and growth of the larval stages. Assuming that bioaccumulation also occurs in the leech, J. arctica, it is likely that release of the aromatic hydrocarbons and their metabolites reduced viability of the embryos and decreased survival of the young.

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