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Comparative Study of Oil Well Drill Cuttings and Polycyclic Aromatic Hydrocarbons on Parasitism in Winter Flounder: A Dose-Response Study

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Synthetic-based oil drilling fluids are currently used by drilling rigs offshore to reduce risks in removing petroleum from depths beneath the ocean floor. There is a generally accepted view that drilling fluids that contain aliphatic hydrocarbons possess considerably less toxicity than polycyclic hydrocarbons which are known to produce adverse affects (Neff and Anderson 1981). However, some drilling fluids and mud tend to be toxic to benthic communities whereas others produce only subtle effects (GESAMP 1993; Breuer et al. 2004 and references therein). The latter reports and others have revealed that major effects were apparent only within 250-500 m of the rigs but subtle effects were observed up to 5000 m from development sites in the North Sea (Olsgard and Gray 1995). A synthetic based drilling fluid (IA-35) is currently used in the Newfoundland offshore. Toxicity studies conducted on scallops (*Placopecten megallanicus*), plankton, fish larvae and winter flounder (*Pleuronectes americanus*) suggested a low potential for acute toxicity (Cranford et al. 2000: Armsworthy et al. 2001, Payne et al. 2001a). Additional studies on Microtox and amphipod bioassays suggested that the synthetic fluid cuttings also exhibited a low acute potential (Payne et al. 2001b).

The Hibernia drilling platform off coastal Newfoundland is located in an area of the continental shelf where at least two commercial, sediment-inhabiting flatfish species, viz., American plaice (*Hippoglossoides platessoides*) and yellowtail flounder (*Limanda ferruginea*) are abundant and where there is a potential for release of both oil well drill-cuttings and polycyclic aromatic hydrocarbons (PAHs). The winter flounder has been used previously as a bioindicator species because of its tendency to remain buried in sediment, where hydrocarbons tend to accumulate, when not foraging for food (see Hellou et al. 1994; Payne et al. 1995). During similar studies, we compared the effect of oil well drill-cuttings and crude oil on parasitism in flounder. The rationale of using parasites is based on previous observations that some parasites respond positively or negatively following chronic exposure to toxicants depending on their concentration and, consequently, are useful as biomarkers of environmental contamination (Khan and Thulin 1991; MacKenzie 1999). Three parasites, two external and one internal, were selected because of their widespread occurrence in winter flounder inhabiting inshore localities in Newfoundland (Khan 2003).

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MATERIALS AND METHODS

Winter flounder were captured by SCUBA divers in autumn at a pristine site in Conception Bay (47° 31'N, 53° 05'W) and held subsequently in 300L flow-through aquaria supplied with ambient sea water for about 6 to 12 weeks to acclimate to laboratory conditions prior to exposure. The fish were not fed as they do not feed at this time of the year. Beach sand was obtained also from the same general area of Conception Bay, washed in running sea water for two weeks and removed after into 300L aquaria. Drill mud cuttings (DMCs) from the borehole were obtained from the Hibernia Management and Development Company. Concentrations of the DMCs were prepared by mixing 0, 36, 110, 330, 1000 and 4000 ml with 54L (45kg) of washed beach sand. A homogeneous mixture was prepared by mixing a small quantity of cuttings with sand and then adding progressively the remaining quantity (Payne et al. 1995). Each lot of contaminated sand was then placed in a 300L aquarium, giving a bottom depth of about 5 cm. The flow-through aquaria were supplied with ambient (0-4°C) sea water (4-5 L/min). Another aquarium without any cuttings was used as a control. All six aquaria were mixed with sea water two to three times daily for 10 days to remove oil that was not absorbed. Then, 15 male flounder, about 23 ± 2.1 cm in length, total body weight 153 ± 7.6, were introduced into each aquarium. Analysis of the cuttings conducted previously suggested that the diesel range alkanes (C-10 to C-32) varied from 1, 6, 16, 20, 50 and 210 mg/kg (Payne et al. 2001a). Prior to commencement of the trials, mucus smears were prepared from the pigmented surface of each winter flounder for microscopic estimation for ectoparasites on slides covered with a 24mm^2 cover glass.

Varying quantities of Hibernia crude oil, obtained from an oil well off Newfoundland, was mixed with 45 kg of dry sand and placed in four aquaria to give total hydrocarbon concentrations (THC) of approximately 1.8, 9.0, 18.0 and 33.0 mg/g (see Hellou *et al.* 1994). One aquarium, without oil, served as a control. In this trial, 12 male winter flounder, about 25 ± 2.4 cm in total length, total body weight 165 ± 8.1 g were placed in each aquarium supplied with running, ambient (0-6°C) sea water (flow rate, 5L/min) as reported previously (Hellou et al. 1994). Mucus smears for parasites were prepared prior to the experiment as noted previously. At the termination of the experiments, mucus was removed from the right (upper) side of the body using a 24mm^2 cover glass, placed on a hemocytometer, the number of parasites counted and estimated per mm^2. Following autopsy of all flounder exposed to DMCs and PAHs, the entire digestive tract was removed, dissected, metazoan parasites removed and counted. Since the digenetic treamatode, *Steringophorus furciger* was the most commonly occurring parasite, emphasis was placed on its occurrence and abundance. A sample of spleen was removed from each fish, fixed in 10% buffered formalin and cross sections 8μm in thickness, prepared by conventional histological methods and stained with Perl's Prussian blue for hemosiderin. The latter was estimated by digital image analysis and expressed as a percentage of the area (mm^2) scanned (Khan and Nag 1993).

Data on the prevalence (%) and mean abundance of the parasites were compared by the least squares difference method with no correction on the alpha value, using

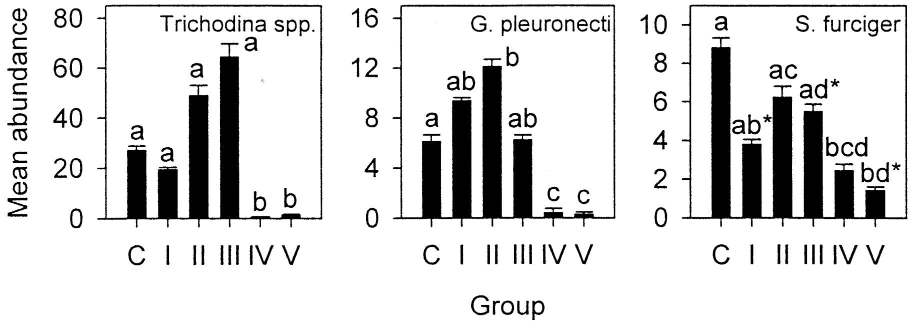

Figure 1. Influence of gradient concentrations of sediment contaminated with drill-mud cuttings on three parasites of winter flounder following chronic exposure. Significant differences between fish groups are indicated by different letters.

SPSS software. Differences were considered significant when $p < 0.05$. Terminology on prevalence and abundance follows that outlined by Bush et al. (1997).

RESULTS AND DISCUSSION

Differences in both the prevalence and mean abundance of three parasites were observed in flounder after chronic exposure to both DMCs and PAHs. Mucus examined from the pigmented surface of all fish, prior to exposure, revealed an extremely low abundance (< one / 22 mm^2) of trichodinids while monogeneids were sparse. Two ectoparasites, viz., *Trichodina* spp. (Ciliophora) and *Gyrodactylus pleuronecti* (Monogenea) were more prevalent on control flounder and three groups (I, II and III) at the lower concentrations of the cuttings than on the two groups exposed at higher levels with the prevalence varying from 33 to 53%. A similar trend was noted with the endoparasite, the prevalence of *S. furciger* which decreased substantially at the two higher concentrations (20 to 40%). Striking differences were observed when the mean abundance was compared among the groups. Trichodines and *G. pleuronecti* were most abundant in either groups III or II founder respectively and also significantly greater than in the control or the groups IV or V (Fig. 1). However, *S. furciger* was greatest in the control group but only significantly greater than the highest concentrations, groups IV and V.

Differences were observed in the level of parasitism and hemosiderin deposits in the spleen among the groups of winter flounder exposed to gradient levels of crude oil-contaminated sand. After 15 weeks, the mean abundance of *Trichodina* spp. was significantly greater in the control and groups I and II than the two groups at higher concentrations (Fig. 2). However, mean abundance of *G. pleuronecti* was significantly greater in the oil-contaminated groups, I and II, than in the controls which in turn were greater than the groups III & IV. Prevalence of both parasites were significantly greater in the controls than in III and IV but not from each other. A different pattern was noted in fish harbouring *S. furciger* as only the group IV

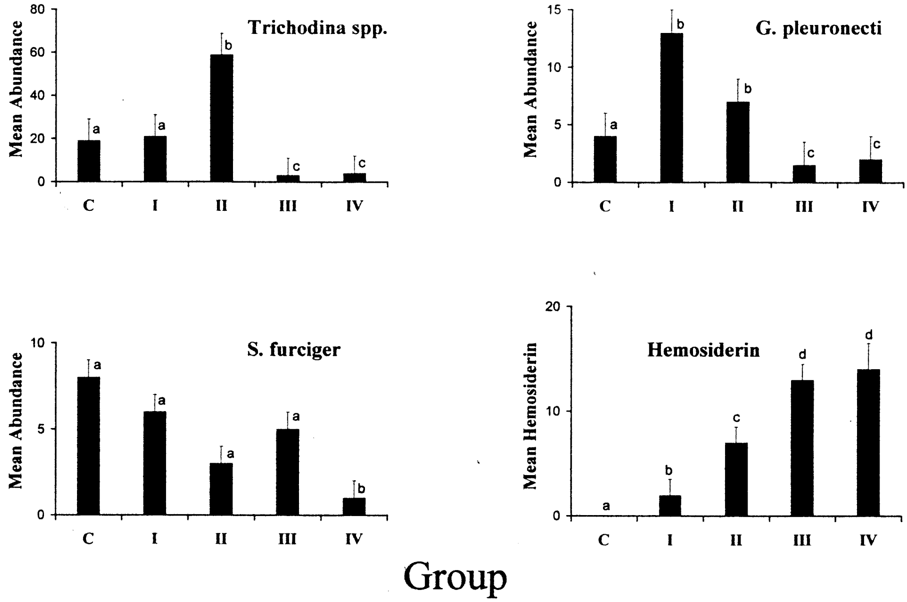

Figure 2. Effect of gradient concentrations of crude oil-contaminated sediment on three parasites and hemosiderin deposits in the spleen of winter flounder following chronic exposure. Significant differences between fish groups are indicated by different letters.

differed significantly from all other groups which appeared similar. Hemosiderin deposits were greatest in flounder exposed to the two highest concentrations of crude oil, groups III and IV, than the other two (oil-contaminated) groups (I and II) which also differed from each other and the controls.

The results from the present study indicated that ectoparasites increased to a threshold level and then declined at the highest concentration of the drill cuttings and crude oil. The endoparasite responded differently, declining progressively as the concentration increased. Both laboratory and field studies have revealed that ectoparasites such as trichodinid ciliates and monogeneans increase while endoparasitic digeneans and acanthcephalans decline following chronic exposure to PAHs (Khan and Thulin 1991 and references therein; Khan and Payne 1997; Marcogliese et al. 1998; Moles and Wade 2001; Khan 2003). More recently, metacercariae of a digenetic trematode, *Cryptocotyle lingua*, have been shown to be more prevalent and abundant in the skin of winter flounder captured near an oil refinery than at a reference site (Khan 2003). Previous studies on winter flounder sampled near a pulp and paper mill, an oil refinery and a PCB-naval facility have also revealed that the digene, *S. furciger*, was significantly greater at the reference than at the contaminated sites (Khan and Payne 1997; Khan 2003). Additionally, Steyermark *et al.* (1999) noted a greater prevalence (100%; n, 37) of a larval cestode, *Proteocephalus* sp., in the brown bullhead (*Amieurus nebulosus*) sampled at a reference site than from a river receiving urban and industrial waste (2%; n, 44). This observation was in agreement with two other observations, cited by the authors, that noted a significantly lower prevalence of the cestode infection at contaminated than reference sites. These authors (Steyermark et al. 1999) speculate that the parasite was restricted in its occurrence because of the sensitivity of the intermediate host to the contaminants.

Gradient studies have suggested that some fish parasites respond differently to varying concentrations of pollutants (Khan and Payne 1997; Khan 2003). A threshold of response by the skin-inhabiting trichodine and monogene and the gastrointestinal digene on/in winter flounder exposed to different oil drill cuttings and PAH concentrations was also observed in the present study. Broeg et al. (1999) reported that flounder (*Platichthys flesus*), inhabiting the North Sea including the German Bight contaminated with the organochlorines, PCBs and DDT, revealed that its parasite species exhibited a gradient of responses in diversity between the polluted and reference sites. Prevalence and mean abundance of *Trichodina* sp. infecting the gills, were significantly greater at the contaminated than at three other less polluted sites. Moreover, parasitic levels correlated with other biomarkers such as EROD activity. It is recommended that future studies using parasites as biomarkers should focus on several sampling sites along a gradient from the centre of contamination.

Most studies investigating the use of fish parasites as bioindicators of hydrocarbon contamination have focussed on PAHs. Chronic exposure of winter flounder to PAH-contaminated sediment resulted in bioaccumulation in muscle tissue, liver enlargement, pathological changes in the gills and elevated levels of hepatic detoxifying enzymes, mixed function exygenases (Payne et al. 1988; Payne, unpubl. data). Moreover, their gastrointestinal parasites were voided most likely after ingestion of the toxic contaminants (Khan and Thulin 1991). In contrast, the aliphatic component of oil well drill cuttings were not toxic to winter flounder and detoxifying enzymes and tissue changes including hemosiderin deposits in the spleen were not apparent following chronic exposure, even at the highest concentrations (Payne et al. 1995). However, similar parasitic responses were observed at the highest concentrations (present study). It appears, then, that differences in response of the endoparasites after chronic exposure of their hosts to PAHs and aliphatic hydrocarbons are not apparent but the pathological responses differ. Since PAHs released during drilling operations tend to evaporate from the water column, residual hydrocarbons in sediment will probably be comprised of the aliphatic components and also metals from the oil drill cuttings (Breuer et al. 2004). Consequently, a study on the parasites and tissue changes, especially hemosiderin levels in macrophage aggregates of the spleen, of flatfish species in the vicinity of two drilling rigs on the Grand Banks off the coast of Newfoundland might be useful not only to determine the impact of the pollutants on fish health but also to distinguish between the effects of PAHs and aliphatic hydrocarbons using digital image analysis (Khan and Nag 1993).

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