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Effect of Petroleum Aromatic Hydrocarbons on Monogeneids Parasitizing Atlantic cod, *Gadus morhua* L.

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Fish gills appear to be more susceptible than other tissues to toxicants. The latter include petroleum aromatic hydrocarbons, which can induce lesions characterized by excessive mucus secretion, hyperplasia, fusion of secondary gill lamellae and capillary dilation (Haensly et al. 1982; Solangi & Overstreet 1982; Khan & Kiceniuk 1984; Grizzle 1986). Fish are also natural hosts to several species of ectoparasites, especially monogeneans which live among the gill filaments. A previous study on the interrelation of water quality, gill parasites and gill pathology provided evidence that fish living in habitats degraded by pollutants such as Biscayne Bay, Florida, were heavily infested with monogeneids especially when gill lesions were severe (Skinner 1982). Haensly et al. (1982), in their studies on the effects of crude oil spilled by the Amoco Cadiz off the coast of France in 1978, observed that the percentage of peritrichous ciliates on the gills increased over a period of time. Atlantic cod, Gadus morhua, are hosts to monogeneans (Appy & Burt 1982). We reported previously that crude oil fractions induced gill lesions in cod (Khan & Kiceniuk 1984) and also affected some gastrointestinal parasites (Khan and Kiceniuk 1983). In the light of these reports, a study was undertaken to ascertain whether any relationship existed between gill lesions and gill parasites in cod following chronic exposure to petroleum hydrocarbons.

MATERIALS AND METHODS

Atlantic cod (38–60 cm) were obtained from traps in Conception Bay, Newfoundland (47°35'N, 53°02'W) and held in a flow-through sea water system for at least 6 weeks prior to treatment. The fish were subsequently placed in groups in experimental and control tanks (3000 L). The water-soluble extract of Venezuelan oil was prepared by introducing crude oil three times weekly into a head tank (80 L), allowing it to mix with a constant spray of seawater and then drawing off the bottom contents. The flow rate was adjusted to 2.5 L/min. An additional supply of uncontaminated seawater (2.5 L/min) also was provided in the experimental tank. Final hydrocarbon concentrations were determined either by ultraviolet fluorescence or by high

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performance liquid chromatography (Kiceniuk and Khan 1987). The profile of the Venezuelan crude was described before (Khan and Kiceniuk 1984).

The fish were exposed from September to December (water temperature, 9-13°C), 10-14 weeks, to three hydrocarbon concentrations at ~30, ~80 and ~500 ppb. Two groups were held for an additional 16-20 weeks following exposure to oil fractions. In one of these experiments, five cod from each of the oil-treated and control groups were necropsied at 6 and 12 weeks after exposure. All groups of fish were fed capelin, Mallotus villosus, ad libitum three times weekly. At necropsy, a piece of gill, 1.5-2.0 cm, was taken from the first, left branchial arch of each fish fixed in Bouin's fluid, subsequently dehydrated and embedded in paraffin. Sections, 6 μm in thickness, were prepared and stained with hematoxylin and eosin. Some sections were also stained with Schiff's reagent (PAS) for mucopolysaccharides (Pearse 1968).

The number of monogeneans attached to 10 secondary gill lamellae in tissue sections was enumerated for each fish that was examined. The data were analysed using the "NPAR 1 WAY" (Wilcoxon option) procedure of SAS software. This is a non parametric test that corresponds to a Wilcoxon rank sum test or a Mann-Whitney "U" test.

RESULTS AND DISCUSSION

Severity of the lesions was correlated with the level of hydrocarbon concentration. When exposed to concentrations of ~30 and ~80 ppb, mild epithelial hyperplasia occurred at the interlamellar bases, never exceeding 25% of the total length between adjacent secondary lamellae in more than 50% of the fish. These lesions, moreover, were restricted to the distal third of the primary lamellae. Excessive numbers of mucus-secreting cells were also observed in these areas. Exposure of cod to higher hydrocarbon concentrations ~500 ppb, resulted in lesions in 70% of the fish. Epithelial hyperplasia, capillary dilation and lamellae fusion occurred along most of the length in more than 50% of the secondary lamellae. Lamellae troughs especially the distal third of the primary lamellae, exceeded more than one half of the height between adjacent filaments. Without exception, excessive numbers of mucus secreting cells were apparent in all treated fish.

In the northwestern Atlantic Ocean, cod were parasitized by the monogeneids, Gyrodactylus spp. (vide Appy & Burt 1982). Examination of cross sections of gills taken from 120 fish in the summer of 1982 revealed a prevalence of 70% (78) with a mean intensity of 2.4 ± 0.3/cod. Following exposure to oil fractions, we observed that the mean number of monogeneids parasitizing the gills of oil-treated cod, at high (~ 500 ppb), and low (~ 12 ppb) total hydrocarbon concentrations, were ($\bar{x}$, 9.1 ± 4.4 and 8.8 ± 2.0) not significantly different from those of controls ($\bar{x}$, 5.2 ± 1.3 and 8.4 ± 3.4 respectively). In a third experiment (~ 80 ppb), no difference was observed in prevalence (% of fish infected with parasites) or intensity ($\bar{x}$, no.

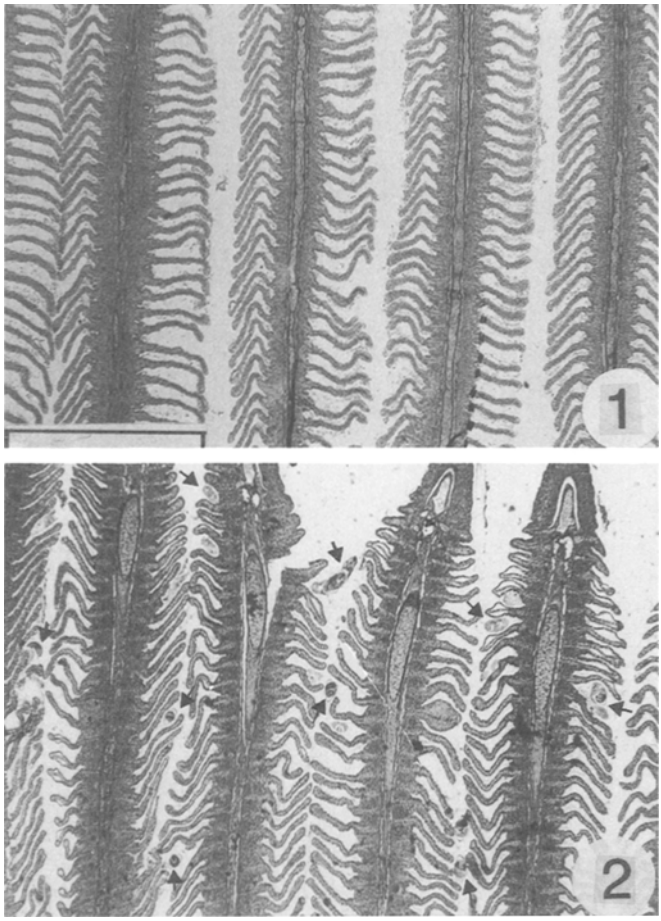

Figure 1. Cross section of gill from control cod.

Figure 2. Gill section from oil-treated cod showning monogeneids (arrows) between the secondary lamellae. Scale bar = 100 μm.

of parasites per fish) of parasitism between oil-treated and control fish necropsied 6 or 12 weeks after exposure. However, in the group of cod retained an additional 16 weeeks after oil treatment, all of 34 fish were infested with monogeneids in contrast to 45% infestation among the controls. Also, the intensity of monogeneans (Figs. 1 and 2) were significantly higher (P = .0001, Wilcoxon's) in cod exposed to oil than in the controls (Fig. 3). Similar results were observed in a second group (n=14) of cod exposed to water-accommodated oil

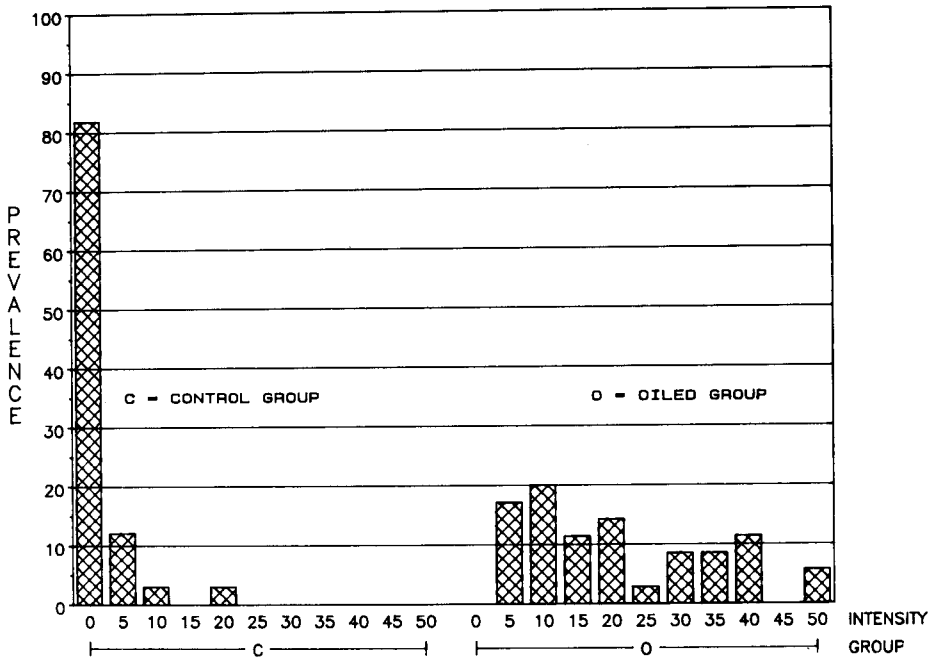

Figure 3. Prevalence (%) and intensity (no.) of monogeneids occurring on the gills of Atlantic cod following exposure (12 wk) to oil fractions and subsequent depuration (16 wk). C = control/group, O = oil-treated group.

fractions for 13 weeks and depurated for 20 weeks (Fig. 4). Prevalence and intensity of monogeneids were greater among the oil-treated group (100%, $\bar{x}$ = 10.2/fish) than in the controls (60%, $\bar{x}$ = 2.2/fish). More than 50% of the monogeneans occurred in the distal third of the secondary lamellae. Alterations produced by the monogeneans appeared to be mechanical, occurring primarily as deflection of the lamellae. There was no evidence of excessive hyperplasia or epithelial sloughing in the immediate vicinity of the adhering parasites, although lesions typical of cod exposed to low oil concentrations were observed.

The present study provides evidence that the prevalence and intensity of monogenean parasites were greater in cod following chronic

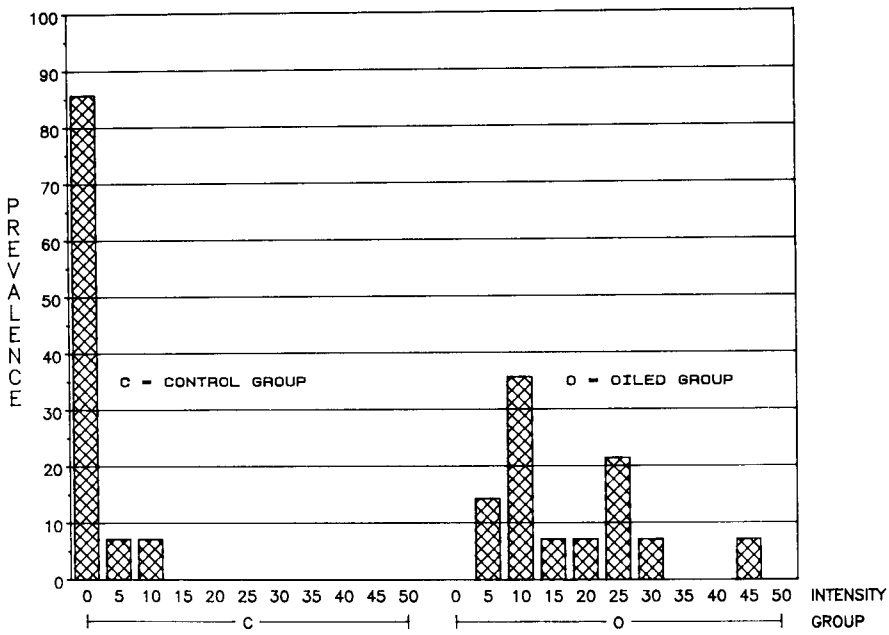

Figure 4. Prevalence and intensity of monogeneids on the gills of cod following exposure (13 wk) to oil fractions and depuration (20 wk) subsequently.

exposure (12-13 weeks) to low concentrations of petroleum hydrocarbons only after they were retained for an additional 16-20 weeks. Since there was no difference between oil-treated and control fish immediately following the exposure period, presumably additional infestation occurred furing the period of depuration. Pollutants have been shown to induce excessive mucus secretion and coagulation (Burton et al. 1972). They are also known to cause physiological stress, induce disease and suppress immune responses (Sindermann 1979; Fries 1986). Most species of Gyrodactylus are viviparous, reproduce directly on their hosts and have short periods of growth from the time of emergence to maturity. Probably, the toxic components in the water-soluble oil-fraction which induced branchial irritation resulting in epithelial and mucus-cell hyperplasia provided a habitat conducive for parasitic infestation and reproduction. While an increase of gill parasitism has been reported after exposure to oil (Hawkes 1977; Haensly et al. 1982), only one study has provided evidence that an increase in intensity of monogenean

parasites is associated with habitats degraded by agricultural, industrial and urban wastes (Skinner 1982). Our study revealed no relationship between the severity of gill lesions and intensity of parasitism because the cod at the highest hydrocarbon concentration were not depurated. However, Skinner (1982) observed that heavy infestation was associated with severe lesions.

A variety of pollutants, including hydrocarbons, heavy metals and pesticides as well as unionized ammonia and low oxygen levels are capable of producing gill lesions in fish (Eller 1975; Hawkes 1977; Haensly et al. 1982; Mallatt 1985). Discovery of major reserves of petroleum hydrocarbons on the Grand Banks raises the possibility of oil discharge into the ecosystem following drilling operations and potential gill damage to cod. It is unlikely that coastal pollutants would occur in this area of the Banks to induce gill pathology in cod because the ocean currents circulate in an offshore to inshore manner and not vice versa (Sutcliffe et al. 1976). Therefore, major changes in gill morphology and increased numbers of monogeneids in cod, following drilling operations could be attributed primarily to petroleum aromatic hydrocarbons. It is unlikely that gill changes would be caused by parasites solely since our study and another (Eller 1975) have suggested that only mechanical damage, resulting in slight epithelial hyperplasia which was restricted to the axes of infested filaments, was associated with parasitism.

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