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Parasitism in Marine Fish after Chronic Exposure to Petroleum Hydrocarbons in the Laboratory and to the Exxon Valdez Oil Spill

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Crude oil or its water soluble components are known to induce histopathological effects in fish following chronic exposure (McCain et al. 1978; Solangi and Overstreet 1982; Haensly et al. 1982; Khan and Kiceniuk 1984). Fish tend to harbor a variety of parasites, most of which under natural conditions cause little or no apparent harm. However, after chronic exposure to petroleum hydrocarbons, the prevalence and intensity of parasitism increases substantially (Skinner 1982; Khan 1987; Khan and Kiceniuk 1988). Trichodinid ciliates are mainly ectoparasitic protozoans on the gills of fish. Since a previous study showed that chronic exposure to crude oil fractions resulted in increased parasitism (Khan and Kiceniuk 1988), a study was initiated to ascertain the relationship between trichodinid infections and exposure of fish to crude oil or its fractions in the laboratory and subsequently, in the Gulf of Alaska following the Exxon Valdez oil spill.

MATERIALS AND METHODS

Adult Atlantic cod (Gadus morhua) 48-62 cm in length were captured in a cod trap set 5 to 10 meters deep in Conception Bay, Newfoundland (47°35'N, 53°02'W) and held in a flow-through sea water system for about 6 mon prior to initiation of the experiment. Longhorn sculpins (Myoxocephalus octodecemspinosus) were collected at 3 to 10 meter depths by SCUBA and held in a similar manner. All fish were fed freshly thawed caplin (Mallotus villosus) two to three times weekly. Prior to exposure, each fish was weighed, measured, and tagged. The cod were placed in 3,000 L tanks (water temperature, 0 to 6°C) and one group was exposed to a slick-free effluent at a flow rate of 5 L/min. The effluent was prepared was introducing 50 ml of crude oil into a head tank (80L), allowing it to mix with a constant spray of sea water and then drawing off the bottom contents (Khan and Kiceniuk 1984). Total hydrocarbon concentrated was 50 to 100 µg/L. After 12 wk, the oil-treated fish were depurated for an additional 14 wk before necropsy. Control fish were treated similarly except no petroleum was administered to them. Longhorn sculpins were exposed to oil-contaminated sediment (2200 µg/g) for 12 wk and subsequently depurated for 20 additional wk. Corresponding control fish were held. At necropsy, gills were fixed in Bouin's fluid.

In Alaska, samples of an intertidal sculpin, *Oligocottus maculosus*, were collected on August 20, 1989 with a dip net (water temperature, 12°C) from two areas, viz., Seward, a site apparently not contaminated by oil and an oiled beach (Wildcat Cove) on the Pye Islands, both located on the Kenai Peninsula, about 5 mon after the Exxon Valdez spill (March 24, 1989). Gill smears and formalin-fixed tissues were prepared, stained and examined. The tissues were processed by conventional histological methods and paraffin-embedded sections, 6µm in thickness were stained with hematoxylin and eosin (Drury et al. 1967). The number of *Trichodina* spp. per 10 gill filaments in sections was enumerated for both oil-treated and control groups. Duncan's multiple range tests were used in statistical analysis of the data (Sokal and Rohlf 1969). Total hydrocarbon concentrations in the fish tanks were determined only in the laboratory experiments by ultraviolet fluorescence method (Keizer and Gordon 1973; Kiceniuk and Khan 1987).

RESULTS AND DISCUSSION

Trichodinid infections on the gills of oil-treated longhorn sculpins and cod increased significantly when compared to the control groups. The mean number of parasites were about 17-fold greater among the oil-treated sculpins than in the control group (Table 1). This increase in prevalence and intensity was associated with severe hyperplasia of the gill lamellae and an excessive secretion of mucus that imparted to the gills a whitish appearance observed at necropsy. Lamellar troughs were substantially extended, with parasites distributed between them. No hyperplasia was observed in control fish. Similarly, in oil-treated cod, both the prevalence and intensity of *Trichodina* sp. were significantly greater than in the control group (Table 1). Eighty eight percent of the oil-treated fish harbored a mean intensity of 102.3 parasites/fish whereas only 9% of the controls were infected with 0.9 parasites/host. The infection was associated with hyperplasia of the lamellae with parasites lying in the intervening troughs of the oiled cod.

Examination of samples of gill tissue from sculpins originating from an oil-free and an oil-contaminated site in Alaska showed that both the prevalence and intensity of Trichodina sp. were significantly greater in the oil-exposed group (Table 1). Seventeen fish from the oil-free site showed no signs of gill abnormalities and only one specimen was parasitized. In contrast, 6 of 14 fish from the oil-contaminated area displayed severe hyperplasia of the branchial epithelium and harbored a mean intensity of 30.0 trichodinids/fish while a mean of 14.3 parasites/fish was recorded for the entire group, which were all parasitized. Since these fish were exposed to oil-contaminated water for periods up to 4 mon, observations on the extent of parasitism and gill hyperplasia appear similar to those noted in laboratory studies on oil-treated sculpins and cod.

Table 1. Influence of crude oil-contaminated sediment (*) or water-soluble oil fractions (ǂ) on trichodinid parasites of fish. C=control, O=oil-treated.

Host Species (fish group)		No. of Fish	% Infected	$\bar{x}$ parasites /fish (± S.E)
Myoxocephalus octodecemspinosus	(C)	21	48	1.1 ± 0.3
	(O)*	20	95	19.0 ± 0.9
Gadus morhua	(C)	23	9	0.9 ± 0.1
	(O)ǂ	24	88	102.3 ± 3.4
Oligocottus maculosus	(C)	17	6	0.2 ± 0.1
	(O)	14	100	14.3 ± 0.6

There is evidence that a variety of pollutants suppress the immune response causing fish to become susceptible to an infection (O'Neill 1981; Zeeman & Brindley 1981; Weeks & Warriner 1984).As a result, fish living in habitats degraded by pollutants show increased prevalence of lesions such as fin rot and tumours and appear to be more susceptible to viral and bacterial infections (Sindermann 1979, 1982). Probably, the increased prevalence and intensity of trichodinid infections noted in fish after exposure to petroleum hydrocarbons in the present study was associated with lowered host resistance caused by the pollutant and also conditions on the gills that were conductive for growth and reproduction. Three other studies also associated increased trichodinid infections with exposure to pollutants and in one instance, mortality by these ciliates (Das & Shrivastava 1984; Dabrowska 1974; Lehtinen et al. 1984). Since more than 50% of the 48 million L of crude oil spilled by the Exxon Valdez in the Gulf of Alaska was not recovered and probably lies trapped on shore and at the bottom of the ocean, it will pose a potential threat to wildlife which survived the spill initially. Future research in this region should focus on the level of parasitism and the prevalence of abnormalities in fish following chronic exposure to the pollutant. Additionally, in view of the death of many seabirds and eagles without evidence of oil on their feathers, studies should take cognizance of the fact that potentially harmful hydrocarbons can be bioaccumulated and passed on through the food chain.

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