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Effect of Oil-Contaminated Sediment on the Longhorn Sculpin (*Myoxocephalus octodecemspinosus*) Following Chronic Exposure

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One of the most common pollutants in coastal marine areas is petroleum that is discharged continually from bilges and tankers. Fish which inhabit the littoral zone are usually exposed to a variety of pollutants, including petroleum, that originate from urban and industrial waste. The longhorn sculpin, Myoxocephalus octodecemspinosus, is one of the fish species that inhabits littoral areas adjacent to wharves and fish-processing plants where it feeds on discarded offal (Scott and Scott 1988). Discharged crude oil has been reported to contaminate and persist in sediment for long periods and is known to affect fish in a variety of ways (Connell and Miller 1981). A previous study noted that chronic exposure to water-soluble fractions of crude oil produced minor effects in the longhorn sculpin (Kiceniuk et al. 1982). The present study was conducted to ascertain the effect of oil-contaminated sediment, following long-term exposure, on body weight, organs, tissues and parasitofauna of the sculpin and the potential use of its parasites as indicators of pollution.

MATERIALS AND METHODS

Adult longhorn sculpins (length 36 ± 2 cm and weight, 557 ± 52 g), mostly females (n = 17), were collected in June 1988 by SCUBA divers from Conception Bay, Newfoundland, and held in 300-L aquaria supplied with ambient (-1.5 to 6°C), running sea water (dissolved oxygen 6.8 - 11.8 ml/L; salinity ~32°/oo). The fish were fed thrice weekly freshly-thawed caplin, Mallotus villosus, for a period of about 3 months until exposure. At this time, the fish were divided into two groups and exposed to uncontaminated and oil-contaminated (1 L of crude oil / 45 kg washed sand) sediment in 300-L aquaria with a sea water flow rate of 5 L/min. The crude oil originated from the Hibernia field located on the Grand Banks off Newfoundland and was made available through the courtesy of Mobil Oil Canada Ltd. Total hydrocarbon concentration was estimated at 2 to 3 mg/g (Fletcher et

al. 1981). The fish were fed caplin, once to twice/wk.

The length and weight of each sculpin were recorded prior to experimentation and at the time of autopsy/necropsy when, in addition, organ weights and blood factors were determined. Tissue samples for microscopic examination included gill, skin, liver, spleen, heart, kidney trunk muscle and intestine. These were fixed in Bouin's fluid or 10% formalin, processed by conventional histological methods and sections 10 μm in thickness stained with hematoxylin and eosin or Perl's Prussian blue for hemosiderin. The number of melanomacrophage centers in the spleen was estimated using 10 fields (x 100) per spleen section. Parasites on the gills were removed by immersion in 1:4000 formalin for about 5 min and fixation subsequently in 70% alcohol. The intestinal tract was also examined for naturally occurring parasites which were removed and enumerated following fixation. Blood smears and bile were also examined for parasites. Sampling of the fish occurred twice at approximately 3 and 6 mon after exposure. Two additional trials involving the exposure (3 to 4 mon) of longhorn sculpins to oil-contaminated sediment (2-3 mg/g) and controls were conducted in August to December, 1989 following the previously outlined protocol.

Condition (K) factor (W/L^3) and organ somatic indices (organ weight/body weight), hematological variables, numbers of parasites, freezing and melting point temperatures of sera were compared between control and oil-treated fish by the one way anova for differences related to treatment. Differences were considered significant when $P \leq 0.05$.

RESULTS AND DISCUSSION

Subtle changes were apparent in the oil-treated longhorn sculpins. Approximately 6 wk following exposure, control female sculpins commenced spawning in the aquarium in January and in the following 2 wk, a total of 17 egg masses were observed. No oil-treated fish spawned until 5-6 wk later and only six egg masses were observed. At this time (March 9) 10 oil-treated fish died following a rapid decline of the ambient sea water temperature from 0 to -1.5°C. No external lesions were observed. Following autopsy, four of 10 oil-treated females failed to spawn whereas all of the controls had spawned. Statistical comparison of the two groups revealed that at 3 mon ovarian somatic indices, lymphocyte levels and the number of melanomacrophage centres in the spleen were significantly greater in the oil-treated group than in the controls ($P \leq 0.05$, Table 1). However, the number of Trichodina sp. on the gills was significantly greater ($P \leq 0.05$) in the oil-treated

Table 1. Effect of crude oil-contaminated sediment (2-3 mg/g) on the longhorn sculpin at 3 and 6 months after exposure.

Variable	Fish Groups					
	3 months			6 months		
	Control	Oiled	P-value	Control	Oiled	P-value
No. of fish	10	10	—	7	7	—
Weight (g)	559 ± 51	530 ± 70	.75	371 ± 47	345 ± 51	.75
Length (cm)	37 ± 1	36 ± 1	.61	34 ± 1	34 ± 3	.82
K Factor[+] ($x10^4$)	108 ± 3	106 ± 6	.83	92 ± 4	88 ± 10	.66
Liver si[†] ($x10^2$)	3.0 ± 0.2	2.8 ± 0.2	.55	2.6 ± 0.2	2.4 ± 0.7	.73
Heart si ($x10^3$)	2.3 ± 0.2	4.6 ± 0.1	.06	1.7 ± 0.1	1.4 ± 0.2	.14
Spleen si ($x10^3$)	1.7 ± 0.2	1.3 ± 0.2	.09	1.5 ± 0.2	1.1 ± 0.2	.25
Gut si ($x10^2$)	6.6 ± 0.3	5.6 ± 0.3	.06	6.3 ± 0.7	5.2 ± 0.8	.34
Ovary si ($x10^2$)	7.6 ± 1.1	22.7 ± 4.6	.04	5.2 ± 1.4	2.2 ± 0.8	.15
Gall bladder si ($x10^3$)	4.1 ± 0.5	4.1 ± 0.4	.99	2.0 ± 0.7	2.2 ± 0.4	.83
Hemoglobin (g/%)	5.3 ± 0.2	4.5 ± 0.4	.18	4.3 ± 0.5	3.9 ± 0.7	.69
Hematocrit (%)	—	—	—	20 ± 1.5	22 ± 2.7	.49
Lymphocytes*	16.8 ± 2	9.2 ± 1.6	.02	17.1 ± 1.2	10.1 ± 1.8	.04
Melting point (0°C)	0.77 ± 0.06	0.80 ± 0.05	.56	—	—	—
Freezing point (0°C)	0.83 ± 0.06	0.87 ± 0.04	.62	—	—	—
Melano-macrophage centers	30.4 ± 2.4	18.5 ± 2.1	.04	—	—	—

[+] W/L^3
[†] si = somatic index
*per 1 $x10^3$ erythrocytes

fish than in the controls (Table 2). This increase in numbers was associated with hyperplasia in the gill lamellae and excessive mucus secretion. No tissue abnormalities were apparent in the liver, heart, kidney, stomach, intestine or ovary. Differences were also not observed in the melting and freezing point temperatures between the two groups of fish.

The experiment was terminated 6 mon after it commenced. Following necropsy, it was observed that K-factor, organ somatic indices and blood values were similar between the control and oil-treated sculpins (Table 1). Examination of tissue sections revealed severe hyperplasia and an increase in the diameter of the gill lamellae as well as a greater number of trichodinids (Table 2). Hemosiderin was dispersed in a somewhat reticulate manner through the splenic tissue instead of within discrete melanomacrophage centers in both groups of fish and differences were not seen. No other tissue abnormalities were apparent. Additionally, digenetic trematodes were observed in all control sculpins whereas none was apparent in the oil-treated group. The remaining parasitofauna of the two groups of sculpins were of little use as indicators of exposure to oil as their prevalence and intensity were extremely low and inconsistent. These taxa included Myxozoa (Myxidium incurvatum/oviforme), Monogenea (Gyrodactylus spp.), Nematoda (Anisakis sp., Contracaecum sp.), Acanthocephala (Echinorhynchus sp.) and Hirudinea (Malmiana brunnea and Oceanobdella microstoma).

Table 2. Influence of crude oil-contaminated sediment on numbers of trichodinids parasitizing one gill (arch) and the numbers of digenetic trematodes in the intestinal tract of the longhorn sculpin at 3 (n = 10) and 6 (n = 7) months following exposure.

	No. of parasites/fish group					
	3 months			6 months		
Parasite	Control	Oiled	P-value	Control	Oiled	P-value
Trichodina sp.	54 ± 24	119 ± 18	.006	24 ± 7	76 ± 22	.005
Digenea	---	---	--	6.9 ± 0.4	0	--

Two additional trials were conducted on a mixture of female and male longhorn sculpins following a similar protocol but the exposure period in the oil-contaminated sediment was approximately three and four months respectively. No mortality or differences in condition factor, hematocrit or hemoglobin levels as well as organ somatic indices, were observed. However, indices of the gonads in female oil-treated fish, when pooled

(n = 15), were significantly less ($P \leq 0.05$; $\bar{X}$, 2.3 ± 1.1) than those of the control fish ($\bar{X}$, 6.4 ± 0.9). Additionally, the prevalence and intensity of trichodinids on the gills, after the samples were pooled, were significantly greater ($P \leq 0.05$) in the oil-treated fish (100%; $\bar{X}$, 84 ± 14) than in the controls (65%; $\bar{X}$, 21 ± 10).

The cause of mortality among some of the longhorn sculpins exposed to oil-contaminated sediment is unknown as no external lesions were apparent and post-mortem changes which occurred after the fish became frozen in the frigid northwest Atlantic waters precluded a detailed histopathological examination. Some species of fish avoid freezing in winter by producing antifreeze glycoproteins or elevated electrolyte levels (Davies et al. 1988). However, no differences were observed in the meeting or freezing point levels between the control and oil-treated fish. It is possible, however that fish exposed to pollutants, become less tolerant to environmental changes than those held under uncontaminated conditions. This view is supported by our unpublished observations that sculpins collected by SCUBA from habitats adjacent to locations of a petroleum refinery or a pulp and paper mill are less likely to survive in captivity than those taken from apparently unpolluted areas.

The present study has shown that crude oil-contaminated sediment appears to have had minimal effects on condition factor, most organ somatic indices, hemoglobin and hematocrit levels of adult, female longhorn sculpins following chronic exposure. A previous study reported similar results after the sculpins were exposed to water-soluble fractions of crude oil (Kiceniuk et al. 1982). In contrast, both field and laboratory studies indicate that retardation of growth, severe organ and tissue damage and impairment of reproduction occurred in species of flatfishes after exposure to oiled sediment (Fletcher et al. 1981; Haensly et al. 1982). These differences might be related to behavior of the fish. Most species of flatfish, unlike sculpins, submerge themselves in sediment and probably absorb greater levels of hydrocarbons through the skin. Consequently, the pollutant is likely to have more of an effect on them than in fish which are located above the contaminated substrate. Additionally, most species of flatfish tend to be more sedentary than sculpins. In spite of these differences, the sculpins still showed signs of exposure to hydrocarbons as indicated by lesions in the gills which probably impaired their function and restricted their foraging activities. An increase of melanomacrophage centers in the spleen also suggests some erythrocytic destruction via cytotoxicity

but not to the extent to cause anemia. Moreover, while gonadal maturation might superficially appear to be a minor effect, there are long-term consequences of chronic exposure to petroleum hydrocarbons. There is evidence that eggs of oil-treated animals are less viable, have a lower rate of hatching success and low survival of the offspring (Khan and Kiceniuk 1989). A decrease in lymphyocyte levels, observed in oil-treated sculpins in the present study might be associated with immunosuppression. There are reports that a number of pollutants, which act as stressors, can cause an elevation of cortisol levels which in turn suppress lymphocyte production and impair the defence systems (Fries 1986; Pickering and Pottinger 1989). Consequently, a variety of opportunistic organisms take advantage of the hosts' increased susceptibility and increase in numbers after establishment. It is, therefore, not surprising that many fish hosts harbor more ectoparasites following chronic exposure to pollutants than those taken from apparently uncontaminated habitats (Skinner 1981; Lehtinen et al. 1984). Similar observations were made in fish chroni-cally exposed to pollutants in the laboratory (Khan and Kiceniuk 1988; Khan 1990). Ingestion of the pollutant might cause the death or voiding of intestinal para-sites as determined from both field and laboratory studies (Khan and Kiceniuk 1983; Valtonin and Koskivaara 1987). However, not all pollutants cause enteric parasites to be shed as there are reports of increased parasitism in fish taken from degraded habi-tats (Möller-Buchner 1981). Thus, there appears to be a body of progressively increasing evidence which supports the view that the prevalence and intensity of parasites are additional indicators of pollutants following chronic exposure.

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REFERENCES

Connell DW, Miller GJ (1981) Petroleum hydrocarbons in aquatic ecosystems-behaviour and effects of sublethal concentrations: part 2. CRC Critical Rev Environ Cont 12:105-162

Davies PL, Hew CL, Fletcher GL (1988) Fish antifreeze proteins: physiology and evolutionary biology. Can J Zool 66:2611-2617

Fletcher GL, Kiceniuk JW, Williams UP (1981) Effects of oiled sediments on mortality, feeding and growth of winter flounder (Pseudopleuronectes americanus). Mar Ecol Prog Ser 4:91-96

Fries CR (1986) Effects of environmental stressors and immunosuppressants on immunity in Fundulus heteroclitus. Am Zool 26:271-282
Haensly WE, Neff JM, Sharp JR, Morris AC, Bedgood MF, Beom PD (1982) Histopathology of Pleuronectes platessa L. from Aber Wrac'h and Aber Benoit, Brittany, France: long-term effects of the Amoco Cadiz crude oil spill. J Fish Dis 5:365-391
Khan RA (1990) Parasitism in marine fish after chronic exposure to petroleum hydrocarbons in the laboratory and to the Exxon Valdez oil spill. Bull Environ Contam Toxicol 44:759-763
Khan RA, Kiceniuk J (1983) Effects of crude oil on the gastrointestinal parasites of two species of marine fish. J Wildl Dis 19:253-258
Khan RA, Kiceniuk JW (1988) Effect of petroleum aromatic hydrocarbons on monogeneids parasitizing Atlantic cod, Gadus morhua L. Bull Environ Contam Toxicol 41:94-100
Khan RA, Kiceniuk JW (1989) Sublethal effects of crude oil on a cold-water marine leech, Johanssonia arctica, following chronic exposure. Bull Environ Contam Toxicol 43:590-596
Kiceniuk JW, Khan RA, Dawe M, Williams U (1982) Examination of interaction of trypanosome infection and crude oil exposure on hematology of the longhorn sculpin (Myoxocephalus octodecemspinosus). Bull Environ Contam Toxicol 28:435-438
Lehtinen KJ, Notini M, Landler L (1984) Tissue damage and parasite frequency in flounders Platichthys flesus chronically exposed to bleached kraft pulp mill effluents. Ann Zool Fenn 21:23-28
Möller-Buchner VJ (1981) Utersuchungen zum Parasitenbefall von Pomatoschistus microps (Gobiidae, Pisces) in der Unterelbe. [Investigations on parasite infection of Pomatoschistus microps (Gobiidae, Pisces) from the Elbe Estuary. Arch Hydrobiol Suppl 61:59-83
Pickering AD, Pottinger TG (1989) Stress responses and disease resistance in salmonid fish: effects of chronic elevation of plasma cortisol levels. Fish Physiol Biochem 7:253-258
Scott WG, Scott MG (1988) Atlantic fishes of Canada. Can Bull Fish Aquat Sci 219: 731 p
Skinner RH (1981) The interrelation of water quality, gill parasites, and gill pathology of some fishes from South Biscayne Bay, Florida. Fish Bull 80:269-280
Valtonen ET, Koskivaara M (1989) Effects of effluent from a paper and pulp mill on parasites of the roach in central Finland. In: Parasites of the North-West Europe. Petrozavodsk, USSR :163-168

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