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Examination of Interaction of Trypanosome Infection and Crude Oil Exposure on Hematology of the Longhorn Sculpin (*Myoxocephalus octodecemspinosus*)

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A high proportion of fish species off the coast of Labrador harbour trypanosome infections (KHAN et al. 1980a, b). The latter are known to induce hematological changes in marine fish (KHAN 1977; KHAN et al. 1980c). Exposure to oil is also known to produce blood changes (KICENIUK et al. 1980; KOTOV 1976). We therefore hypothesized that trypanosome infection and exposure to oil might interact to alter the hematology of fish.

MATERIALS AND METHODS

Adult longhorn sculpins (450-700 g) were collected from Conception Bay, Newfoundland and held in tanks with running sea water for 8 months prior to exposure. They were fed capelin (Mallotus villosus) thrice weekly. In the first experiment sculpins were infected with trypanosomes via leeches (KHAN 1976) and after 42 days , 16 fish were placed in each of two tanks (3000 L) through which sea water flowed (~5 L/min).

One group of fish was exposed to water-accommodated Venezuelan crude oil while the other served as controls. The water-soluble fraction was prepared by introducing one liter of oil into a head tank (80 L) and extracting water soluble compounds with a constant stream of seawater sprayed onto the surface of the head tank. The water extract was drawn from the bottom of the head tank into the treatment tank. Water flow of 2.5 L/min through the head tank and directly into the fish tank provided an oil concentration of 300 ppb initially, measured by florescence spectrophometry, after KEIZER and GORDON 1973. The concentration subsequently decreased to 150 ppb by the end of the week when the old oil was removed from the head tank and 1 L of fresh crude was added. This regime was maintained throughout the entire experiment which was of 64 days duration. Water temperatures varied from 7°C in May, when the experiment commenced, to 11°C in July. Both groups of fish were fed weighed quantities of freshly thawed capelin (Mallotus villosus) ad libitum three times weekly throughout the period of captivity

Hematological parameters which included hemoglobin, hematocrit, total plasma protein, plasma chloride, plasma osmolality and white blood cell counts were determined from cardiac blood at 2 week intervals prior to and after exposure to the oil. At the conclusion

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of the experiment, the total body and organ weights were also determined. Tissue samples for microscopic examination included heart, liver, spleen, kidney, gill, small intestine and gonad. These were fixed in Bouin's fluid, processed by conventional histological techniques and stained with hematoxylin and eosin.

Organ weights were transformed to somatic indices (organ weight/body weight x 100) and these together with condition factor and hematological variables were analyzed by two way analysis of variance for difference between sexes (M ~ F), treatments (C ~ E), and their interaction (M ~ F) x (C ~ E). Means and 95% confidence intervals were calculated at each sample time for the hematological variables and plotted for trends. Hematological variables were also analyzed by one way anova for differences related to treatment at each sample time.

RESULTS AND DISCUSSION

In the trypanosome infected groups, all fish survived except three from the experimental group which died shortly after exposure from a previous bacterial infection. Body condition indices of both oil-exposed and control fish were similar (Table 1). There was a significant difference in the gall bladder somatic index between the two fish groups ($P = 0.002$). Gall bladders of the control group were larger than those of oil-exposed fish. A similar effect was observed in winter flounder, Pseudopleuronectes americanus, exposed to oil-contaminated sediment (FLETCHER et al., in prep.). However, gall bladders of cod, Gadus morhua, (KHAN et al., in prep.), and cunners, Tautogolabrus adspersus, (KICENIUK et al. 1980) were larger than normal in oil-exposed fish. All other somatic indices were similar in both groups of fish and between sexes. There were no significant differences in total plasma protein and plasma osmolality. Plasma chloride levels were lower in oil-treated sculpins ($P = 0.01$). This observation has been reported previously in cunners following long term exposure to Venezuelan crude oil (PAYNE et al. 1978). Hemoglobin concentrations in the present study were also depressed in the last two weeks in oil-exposed sculpins ($P = 0.04$). The difference in hemoglobin levels suggests that trypanosome infection in addition to oil-exposure would reduce the blood oxygen carrying capacity and thereby decrease mobility in active species of fish. There were no differences between the two groups in the other hematological variables sampled during the exposure.

Examination of the tissues for histological changes revealed no apparent alterations. This was unexpected as previous studies on the effects of petroleum products on fish indicated pathological changes in the liver and gill lamellae (HAWKES 1977, KHAN et al. in prep.).

An additional number of longhorn sculpins, previously determined to be free of blood parasites, were exposed to similar concentrations of water-soluble fractions of crude oil for 75 days to ascertain

TABLE 1

Effect of Venezuelan crude oil (≅150-300 ppb) on longhorn sculpins infected with trypanosomes.

Variable	Sex	Control X	Control S.D.	Control N	Oiled X	Oiled S.D.	Oiled N
Hematocrit %	M	16	4.0	9	18	2.2	4
	F	17	4.7	9	13	4.0	7
Hemoglobin (g%)	M	5.3	1.79	9	4.4	0.65	4
	F	5.5	2.00	9	3.7[a]	1.34	7
Plasma Osmolality (mOsm)	M	337	4.1	9	333	1.7	4
	F	330	14.1	9	333	5.6	7
Plasma Cl (mM)	M	157	3.0	9	152	6.4	4
	F	158	3.9	9	154[a]	5.0	7
Plasma Protein (g%)	M	22	8.5	9	24	3.9	4
	F	22	4.9	9	21	6.5	7
Condition Factor (weight/L^3)	M	0.013	0.0016	9	0.012	0.0007	4
	F	0.012	0.0011	9	0.013	0.0020	7
Spleen SI	M	0.15	0.048	9	0.16	0.064	4
	F	0.14	0.057	9	0.12	0.049	7
Liver SI	M	5.9	1.94	9	5.3	0.87	4
	F	5.4	0.70	9	5.3	1.03	7
Heart SI	M	0.16	.031	9	0.17	0.052	4
	F	0.17	0.030	9	0.17	0.020	7
Gonad SI	M	0.50	0.342	9	0.54	0.299	4
	F	4.4	8.04	9	4.1	5.55	7
Gall bladder SI	M	0.20	0.102	9	0.11	0.111	4
	F	0.27	0.168	9	0.06[a]	0.023	7

Somatic indices (SI)=organ weight in grams x body weight $(g)^{-1}$ x 100.

[a] - Significantly different (see results section) between treatments in two way Anova.

its effect on unparasitized fish. No significant differences in hematocrit or hemoglobin levels were observed between control and experimental fish at 26 and at 75 days. However, total plasma protein was significantly lower ($P \leq 0.05$) in oil-treated fish at 75 days. This work suggests that water soluble fractions of Venezuelan crude oil have a minor effect on the hematology of sculpins at these concentrations but that trypanosomes appear to potentiate the effect of oil on blood hemoglobin content of trypanosome infected fish. If this is found to be the case in active species of fish, the decrease in oxygen carrying capacity (reduced hemoglobin) together with gill damage observed by KHAN et al. (1981) would likely limit the aerobic capacity of fish.

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