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Influence of Petroleum at a Refinery Terminal on Feral Winter Flounder, *Pleuronectes americanus*

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Polycyclic aromatic hydrocarbons (PAHs), components of crude oil, contain toxic, mutagenic and carcinogenic compounds and pose a potential hazard to marine animals. PAHs have been reported to affect marine organisms and sediment appears to be a major source following spills, seeps, atmospheric deposition etc. (McElroy et al. 1989). Aquatic organisms accumulate and bioconcentrate PAHs through food and water and various effects might ensue after chronic exposure (Bender et al. 1988). Biotransformation of these xenobiotic compounds might also occur resulting in benzo-a-pyrenes which have been considered to be carcinogenic (Varanasi, and Stein 1991). Early life-stages of fish tend to be extremely susceptible (Norcross et al. 1996) whereas in older fish subtle changes such as feed intake, blood values, hepatic detoxication enzymes, primarily mixed function oxygenases (MFOs) and histopathology might occur. When high concentrations of PAHs occur in sediment, abnormalities such as skin lesions, hepatic neoplasms and altered activity of the immune system are apparent (Hargis et al. 1989; Kelly-Reay and Weeks-Perkins 1994). Species of flatfish in the marine environment have been reported to be appropriate for biomonitoring studies because of their tendency to be non-migratory and to submerge themselves in sediment when not foraging for food (Fletcher et al. 1981; Scott and Scott 1988). Consequently, bioaccumulation of PAHs can occur following chronic exposure (Hellou et al. 1994). Haensly et al. (1982) reported histopathological abnormalities in the gills, kidney and ovary of plaice, *Pleuronectes platessa,* that were exposed to the Amoco Cadiz oil spill in an isolated inlet off the coast of France but Armstrong et al. (1995) found no differences in lesions in flathead sole, *Hippoglossoides elassodon,* from oiled and non-oiled bays two years after the Exxon Valdez oil spill in the Gulf of Alaska. These differences in results between the two oil spills might be related to isolated in contrast to open marine environments respectively.

Two previous studies conducted near a petroleum refinery in Newfoundland reported a significantly higher level of MFO activity in the liver of Gunner (*Tautogolabrus adspersus*) than in samples from two reference sites and contamination of the sediment with PAHs (Kiceniuk 1992; Payne 1976). A preliminary examination of winter flounder captured recently near the refinery revealed fin necrosis and discoloured liver in some specimens whereas none was

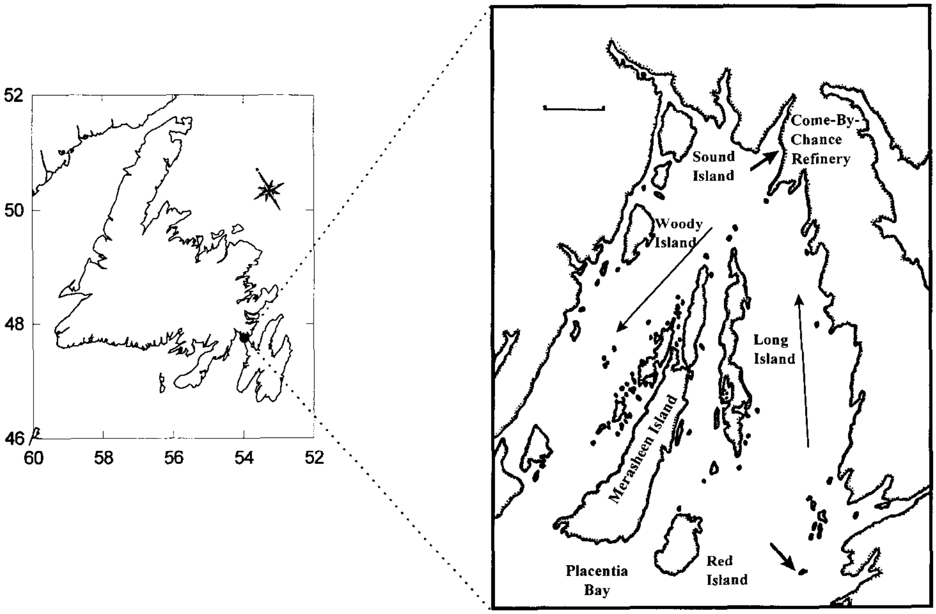


Figure 1. Location of the petroleum refinery at (Come By Chance) and reference site (short arrows) in Placentia Bay, Newfoundland. Longer arrows indicate current flow.

observed in samples taken downstream (Khan, unpubl. data). Several spills (approximately 10) have occurred since the initial sample was taken (Canadian Coast Guard, unpubl. data). Additional fish were examined more recently after capture near the same oil terminal and a reference site and these results are reported herein.

MATERIALS AND METHODS

The petroleum refinery, located on the southern coast of Newfoundland at Come By Chance, has been operating continually since the 1970s after its construction but continuously since the 1990s (Fig. I). It's ice-free location is ideal for shipping. Surface temperature varies from 0-18°C whereas bottom (depth, 10-15 meters) temperature tends to be somewhat lower. The benthic area near the refinery consisted of soft, dark mud mixed with sand in which the flounder were submerged. Blue mussels (*Mytilus edulis*) were the only macro invertebrates visible. At the reference sites, the bottom was composed of white sand and pebbles and an abundance of seaweed among which were molluscs, crabs and echinoderms. Winter flounder, 5-7 years old, were captured during the month of August by SCUBA divers about 200 meters from the terminal, and at reference sites five km down-current (depth ~10 m, water temperature ~14°C) in 1993 and, as none was available at this location in 1997, about 120 km offshore (depth ~10 m) near Fox Island. All fish were bled subsequently from the caudal artery by means of a heparized syringe and needle to determine hemoglobin levels. At autopsy, gross lesions externally were noted, total body length (1) and eviscerated body weight (w) were recorded to ascertain condition (K) factor (w/l^3) and liver weight for hepatosomatic index (liver weight/eviscerated body weight). Tissues, which included liver, spleen, kidney and gill were fixed in 10% buffered formalin, processed by conventional histological methods and stained with hemotoxylin and eosin whereas additional samples of spleen, liver and kidney were stained for hemosiderin concentration to be determined by digital image analysis (Khan and Nag 1993). Liver samples were taken only in 1993, frozen in dry ice at -60°C for determining ethoxyresorufin o-deethylase (EROD) activity (Porter et al. 1989). Bile was removed from the gall bladder to determine the prevalence (%) and abundance ($\bar{x}$±se) of the myxozoan, *Ceratomyxa acadiemis,* by means of hemocytometer. Stomach contents (species of prey consumed) were recorded and the entire digestive tract removed and examined for the prevalence and abundance of selected metazoan parasites, viz, *Steringophorm furciger* (Digenea) and *Echinorhynchus gadi* (Acanthocephala). Data from the groups of fish including K-factor, hepatosomatic index and parasitic abundance of pooled (1993) or individual sexes/site were compared by ANOVA and Duncan's Multiple Range Test (SPSS™ software package) for differences which were considered significant when $p < 0.05$.

RESULTS AND DISCUSSION

Some differences were observed between samples of winter flounder examined in 1993 near the refinery terminal and the reference site. One of six fish showed evidence of fin necrosis near the refinery but none at the reference site. Samples captured near the refinery were significantly greater in length ($\bar{x}$34±1.2 cm) and weight ($\bar{x}$364±23 g) than at the reference site (length, $\bar{x}$28±0.9 cm; weight, $\bar{x}$217±14g) whereas K-factor and HSI were significantly greater in flounder from the reference site ($\bar{x}$1.23±0.02x10-2, $\bar{x}$1.65±0.03x10^{-2} respectively) than near the terminal ($\bar{x}$0.93±0.01x10^{-2}, $\bar{x}$1.36+0.02x10^{-2} respectively). However, levels of EROD activity were greater in samples taken near the terminal ($\bar{x}$287±21 p/mm/mol/mg) than at the reference site ($\bar{x}$61±8 p/min/mol/mg). The liver of reference samples were yellowish in color and examination of histological sections revealed pale-staining hepatocytes, suggestive of lipid storage (Fig. 2). Examination of tissues of samples taken near the oil terminal revealed in the liver basophilic-staining hepatocytes, pericholangitis, macrophage aggregates with hemosiderin and clear cell foci (Figs. 3-4). Hemosiderosis (two fish) also occurred in the spleen (Fig. 5) and kidney (two samples) and slight hyperplasia (two fish) in the distal portion of the secondary gill lamellae was apparent in flounder taken near the terminal in contrast to none in reference samples (Figs. 6-7). These anomalies, moreover, were not restricted to any one sample but occurred at random. No differences in hemoglobin levels were observed.

More pronounced differences were observed among samples taken near the terminal and the reference site located offshore in 1997. Six (30%) of 20 flounder

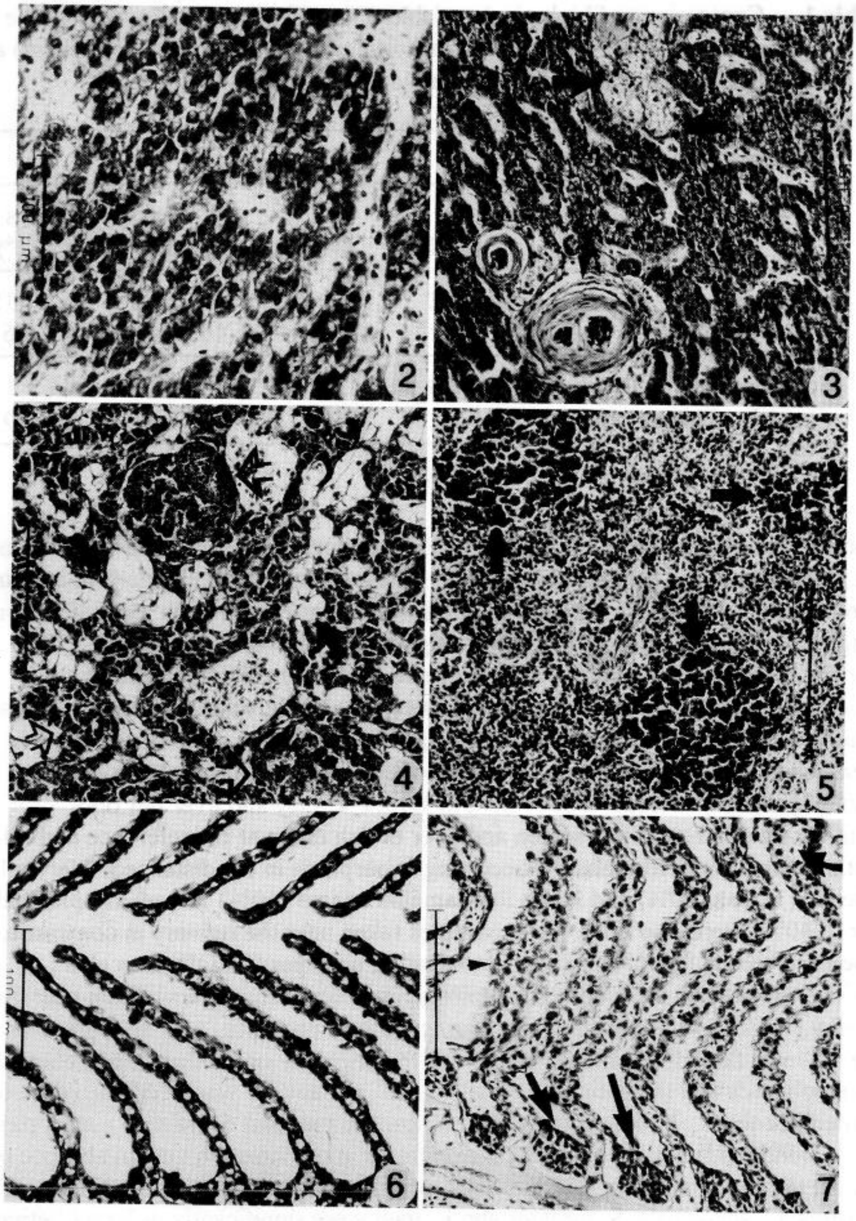
Table 1. Comparison of biological variables ($\bar{x} \pm$ se) of winter flounder (male and female, n = 10/sex/site) sampled at a reference site (Fox Island) and near an oil refinery terminal in Newfoundland in 1997.

Location	Sex	Variable			
		Length	Weight	K-factor (10^{-4})	Liver s.i. ($x10^{-4}$)
Fox Island	M	34 ± 1.2	630 ± 72*	133 ± 4*	165 ± 15
Come By Chance	M	24 ± 1.2*	133 ± 19	109 ± 3	75 ± 6
Fox Island	F	34 ± 0.8	618 ± 47*	156 ±13*	134 ± 11
Come By Chance	F	24 ± 1.3*	151 ± 27	105 ± 6	88 ± 9

1 $p< 0.05$ than other group of same sex

captured near the oil terminal showed evidence excessive fin necrosis and in two fish, only rays of the caudal fin remained. The liver was small and dark in colour in comparison with that of reference samples which were larger with a yellowish tint. Length, weight, K-factor and HSI were significantly lower in the sample taken near the oil terminal than at the reference site (Table 1). While differences in hemoglobin values were not apparent between the two groups, there was evidence of histopathological changes in the liver such as an absence of lipid, observed in reference fish, and a higher concentration of pericholangitis and clear cell foci (Table 2). Hemosiderosis was evident in spleen, kidney and liver but only significantly different in the spleen and liver of fish taken at the reference and oil-contaminated sites. Moderately-occurring hyperplasia in the distal secondary gill lamellae (12 fish, 60%) and in the interlamellar spaces (60%) as well as epithelial lifting (40%) were also observed in samples taken near the refinery in contrast to none in reference fish. Activity of detoxicating enzymes (EROD) was also significantly greater in combined samples of both sexes (n = S/sex) taken near the refinery ($\bar{x}$426 ± 48 p/min/mol/mg) than at the reference site ($\bar{x}$85 ± 26 p/min/mol/mg). Winter flounder at the reference site fed primarily on molluscs, polychetes and amphipods and the entire intestinal tract of all samples was tilled with digested food and/or feces. In contrast, fish taken near the terminal contained a dark paste-like unidentified mass devoid of food organisms in the stomach and an absence of feces in the intestinal tract. Additionally, prevalence and abundance of three parasites, C. acadiensis, S. furciger and E. gadi were significantly different between the two groups of fish (Table 3).

Results from the present study suggest significant differences between samples taken at the reference sites and the oil terminal and also at the latter site between 1993 and 1997. A study on the total hydrocarbon concentration (THC) in sediment conducted near the refinery in 1992 revealed 0.3 µg/g (Kiceniuk 1993) Since this time, there has been several spills in the area and it is likely that the increase between 1993 and 1997 in external lesions, paucity of food in the



Figures 2-7. Histological sections of tissues of winter flounder sampled at a reference site and near a petroleum refinery terminal in 1993. Dimension of scale bar ( ) in µm Fig. 2. Reference liver (100). Fig. 3. Liver of flounder taken by the refinery exhibiting bile ductule hyperplasia (thin arrow) and hemosiderin deposits in macrophage aggregates (larger arrows, 250). Fig. 4. Clear cell foci (arrows) in the liver (250). Note macrophage aggregates (open arrows). Fig. 5. Macrophage aggregates (solid arrows) in the spleen (500). Fig. 6. Reference gill (100). Fig. 7. Gill of flounder taken near refinery showing hyperplasia in the secondary lamellae (arrow head) and interlamellar spaces (arrows) and epithelial lifting (curved arrow, 100).

digestive tract, low condition factor and virtual absence of parasites are indicative of exposure to xenobiotics. According to Kiceniuk (unpubl. data), levels of THC varied considerably near the refinery since 1993 and at times exceeded 50 µg/g depending on the quantity of oil spilled and the time of collection of the sediment sample after the spill. It is possible, then, that acute exposure of winter flounder to PAHs occurred after one or more spills and was responsible for the severe fin necrosis and elevated EROD levels reported in the present study. Moreover, these observations are consistent with laboratory studies that report similar and additional changes including mortality, feeding and growth in winter flounder following chronic exposure to petroleum-contaminated sediment (Fletcher et al. 1981; Khan 1995; Khan and Kicenuck 1988).

Table 2. Abundance ($\bar{x}$±se) and prevalence (%) of lesions in the liver and hemosiderin concentration in three organs of winter flounder (n = 20/site) sampled near a petroleum refinery in Newfoundland in 1997.

Location	Liver		Hemosiderin (%/mm^2)		
	P†	CCF	Spleen	Liver	Kidney
Fox Island‡	0.08±0.01* (88)	0	2.7±0.3*	0.28±0.02*	0.36±0.02
Come By Chance	0.20±0.02 (92)	10	6.5±0.2	0.81±0.02	0.18±0.01

P† = pericholangitis/mm^2, CCF = clear cell foci.
‡reference site.
*significantly different (P < 0.05) from other group.

Table 3. Abundance ($\bar{x}$ ± se) and prevalence (%) of *Ceratomyxa acadiemis, Echinorhynchus gadi* and *Steringofphorus furciger,* in winter flounder (n = 20/site) sampled near a petroleum refinery in Newfoundland in 1997.

Location	Parasite species [$\bar{x}$±se; (%)]		
	Ceratomyxa	Echinorhynchus	Steringophorus
Fox Island†	1.8±0.2x10^{2*} (100)	0.9±0.1* (88)	4.6±0.4* (88)
Come By Chance	<0.10 (40)	<.01 (10)	0 (0)

†reference site.
*significantly different (p < 0.05) from other group.

Lesions that were predominant in the liver of winter flounder sampled near refinery included pericholangitis (bile ductule hyperplasia), hemosiderosis and less often clear cell foci. Schiewe et al. (1991) reported 11 distinct types of histological lesions in the liver of English sole (Parophrys vetulus) following exposure to an extract of an urban marine sediment containing aromatic hydrocarbons including the three previously mentioned anomalies. However, eight of these lesions were not observed in winter flounder in the present study and might be related to the concentration of PAHs and also to the period of exposure. For example, some lesions such as hepatocellular neoplasms might take two or more years to develop in flatfish species (Schiewe et al. 1991) and have been observed mainly in large and presumably older specimens (Murchelano and Wolke 1991).

Significant differences in prevalence and abundance of three parasites between winter flounder sampled at the reference site and the refinery, where there was a paucity of macroscopic invertebrates and endozoic metazoans in winter flounder, is an indication of environmental change. Several studies have reported differences in the parasitofauna of fish inhabiting sites degraded by pollutants and reference iocations (Khan and Thulin 1991). Laboratory studies involving the exposure of winter flounder to oil-contaminated sediment resulted in a lower prevalence and abundance of *S. furciger* than in the control group (Khan and Kiceniuk 1988). Additionally, flounder captured in the vicinity of a paper mill were infected with fewer *E. gadi* than at a reference site (Barker et al. 1994). These results, in addition to differences in body condition and lesions, both external and internal, in winter flounder sampled at the refinery and a reference site in 1997 suggest a connection between spilled petroleum and the anomalies observed.

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