

## Evidence for Exposure of Fish to Oil Spilled into the Columbia River

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### ABSTRACT

*On March 19, 1984, more than 170 000 gallons of oil were spilled into the Columbia River. We had recently developed analytical methods for estimating the exposure of fish to aromatic compounds by measuring the concentrations of metabolites of these contaminants in fish bile. The oil spill provided an opportunity to field test our methods in assessing the exposure of fish to petroleum aromatic compounds from the spilled oil. Our findings indicated that, within 5 days after the spill, mean concentrations of metabolites of aromatic compounds in the bile of white sturgeon (*Acipenser transmontanus*) captured 57 miles downstream from the spill were significantly higher than those of sturgeon caught upriver.*

### INTRODUCTION

On March 19, 1984, the tanker *Mobiloil* ran aground near St. Helens, Oregon, releasing more than 170 000 gallons of high density residual and industrial oil into the Columbia River (Weston & Morson, 1985). Much of the downriver Washington State shoreline was oiled as a result of the spill, and some of the oil was noted on Pacific Ocean beaches 50 miles north of the mouth of the Columbia River. Oil attributed to this spill remained in the river in the form of tarballs and oiled vegetation at least through August, 1985.

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The Washington State Department of Ecology (WDOE) activated the Marine Resource Damage Assessment team, a coalition of eleven state and federal agencies, to study the spill and assess the damage. As part of this study, fish were captured from Columbia River sites both upriver and downriver from the spill for the purpose of analyzing them for evidence of oil contamination.

The uptake by fish of aromatic compounds, such as aromatic hydrocarbons (AHs) from petroleum, cannot usually be assessed by analyzing directly for these compounds in fish tissues and fluids, because fish rapidly convert AHs to a variety of metabolic products (Varanasi & Gmur, 1981; Krahn *et al.*, 1982). Ten years ago, Statham *et al.* (1976) suggested that analyzing for metabolites in bile could prove useful as a 'qualitative monitoring aid for certain types of xenobiotics in water' because of large bioconcentration factors for certain aromatic compounds. However, that study, and other, more recent, laboratory studies of AH metabolism (e.g. Varanasi & Gmur, 1981), used radiolabelled compounds; thus, these methods are clearly not suitable for studying the fate of complex mixtures of aromatic compounds from an oil spill. Some researchers have developed gas chromatographic/mass spectrometric (GC/MS) methods to detect derivatized metabolites of certain AHs in hydrolyzed bile (Solbakken *et al.* 1980; Solbakken & Palmork, 1981; Gmur & Varanasi, 1982), but such work has generally been limited to a single parent compound.

Recently, we have developed non-radiometric methods using high-performance liquid chromatography (HPLC) with fluorescence detection to estimate the exposure of English sole (*Parophrys vetulus*) from contaminated sites in Puget Sound to complex mixtures of aromatic compounds by measuring the concentrations of metabolites of these contaminants in fish bile (Krahn *et al.*, 1984; 1986). The oil spill gave us the opportunity to apply our techniques in evaluating the exposure of white sturgeon (*Acipenser transmontanus*) to AHs from the oil.

## EXPERIMENTAL

The sites for field collection of the fish were established WDOE sampling sites; none of these sites was known to have any previous petroleum contamination (Weston & Morson, 1985; Lewey Kittle, pers. comm.).

TABLE 1

Concentrations of Metabolites of Aromatic Compounds (Measured at the Benzo[*a*]pyrene (BaP), Phenanthrene (PHN) and Naphthalene (NPH) Wavelength Pairs) in the Bile of White Sturgeon from the Columbia River<sup>a</sup>

River mile <sup>c</sup>	Location	Sampling date	n	$\bar{X} \pm SE^b$ ng/g, wet weight)		
				BaP	PHN	NPH
30	Downriver	24 March, 1984	7	2 100 ± 680 <sup>d</sup> 6/7 <sup>e</sup>	210 000 ± 73 000 <sup>d</sup> 7/7 <sup>e</sup>	200 000 ± 52 000 <sup>d</sup> 6/7 <sup>e</sup>
87	Oil spill (19 March, 1984)					
100	Upriver	26 March, 1984	4	64 ± 30	9 700 ± 4 100	32 000 ± 15 000
125	Upriver	2 April, 1984	4	200 ± 190	9 600 ± 8 800	17 000 ± 13 000

<sup>a</sup> Wavelength pairs (excitation/emission) are as follows: BaP, 380/430 nm; PHN, 256/380 nm; NPH, 290/335 nm.

<sup>b</sup> Standard error of the mean.

<sup>c</sup> Measured from the mouth of the Columbia River.

<sup>d</sup> Significantly different from values for sturgeon caught at the combined upriver sites (Student's *t*-test;  $p \leq 0.005$  for BaP,  $p \leq 0.01$  for PHN and  $p \leq 0.0025$  for NPH).

<sup>e</sup> The number of fish out of the total captured downriver from the oil spill that showed elevated concentrations of the metabolites in bile. The concentrations of metabolites in bile were considered to be elevated if the values were higher than the upper 95% confidence interval for the mean concentration of bile metabolites from the eight upriver (reference) fish.

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However, after the spill, oil and tarballs were found throughout the water column and in the sediment of the sampling site 57 miles downriver from the spill.

Five days after oil was spilled into the Columbia River, seven subadult white sturgeon were captured 57 miles downstream from the spill site (mile 30; Table 1). Two days later, four additional sturgeon were collected 13 miles above the spill (mile 100). Over the next week, four sturgeon were collected at a site 38 miles upstream from the oil spill (mile 125). Average lengths ( $\bar{X} \pm SD$ ) for the sturgeon were  $81 \pm 14$  cm (downriver) and  $73 \pm 20$  cm (upriver). After capture, all fish were frozen and delivered to the Northwest and Alaska Fisheries Center in Seattle, Washington.

The frozen fish were thawed and bile samples were removed from the gall bladders. Previously reported direct-injection HPLC techniques with fluorescence detection (Krahn *et al.*, 1984; 1986) were used to estimate the concentrations of metabolites of selected aromatic compounds in bile. Fluorescence was measured at the benzo[*a*]-pyrene (BaP; 380/430 nm, excitation/emission), phenanthrene (PHN; 256/380 nm) and naphthalene (NPH; 290/335 nm) wavelength pairs. Integrated areas of peaks eluting after 7 min in the HPLC chromatograms were summed, and this area sum was converted to the concentration (ng/g, wet weight) of the particular reference standard (BaP, PHN or NPH) that would be present if the integrated area were attributed only to that compound. This conversion of area sums to concentrations in terms of a reference standard does not mean that metabolites of that standard are the only ones present. Metabolites of other aromatic compounds can also fluoresce at the wavelength pair of the standard (see Krahn *et al.*, 1984), and, therefore, if other metabolites are present, they also contribute to the area sum measurement.

## RESULTS

Figure 1 shows the HPLC/fluorescence chromatograms (at the three wavelength pairs) of the bile from two white sturgeon—one captured 57 miles downriver from the spill area and the other 38 miles upriver from the spill. A number of metabolite peaks appear in the chromatograms of bile from the downriver sturgeon. Note, however, that few metabolites

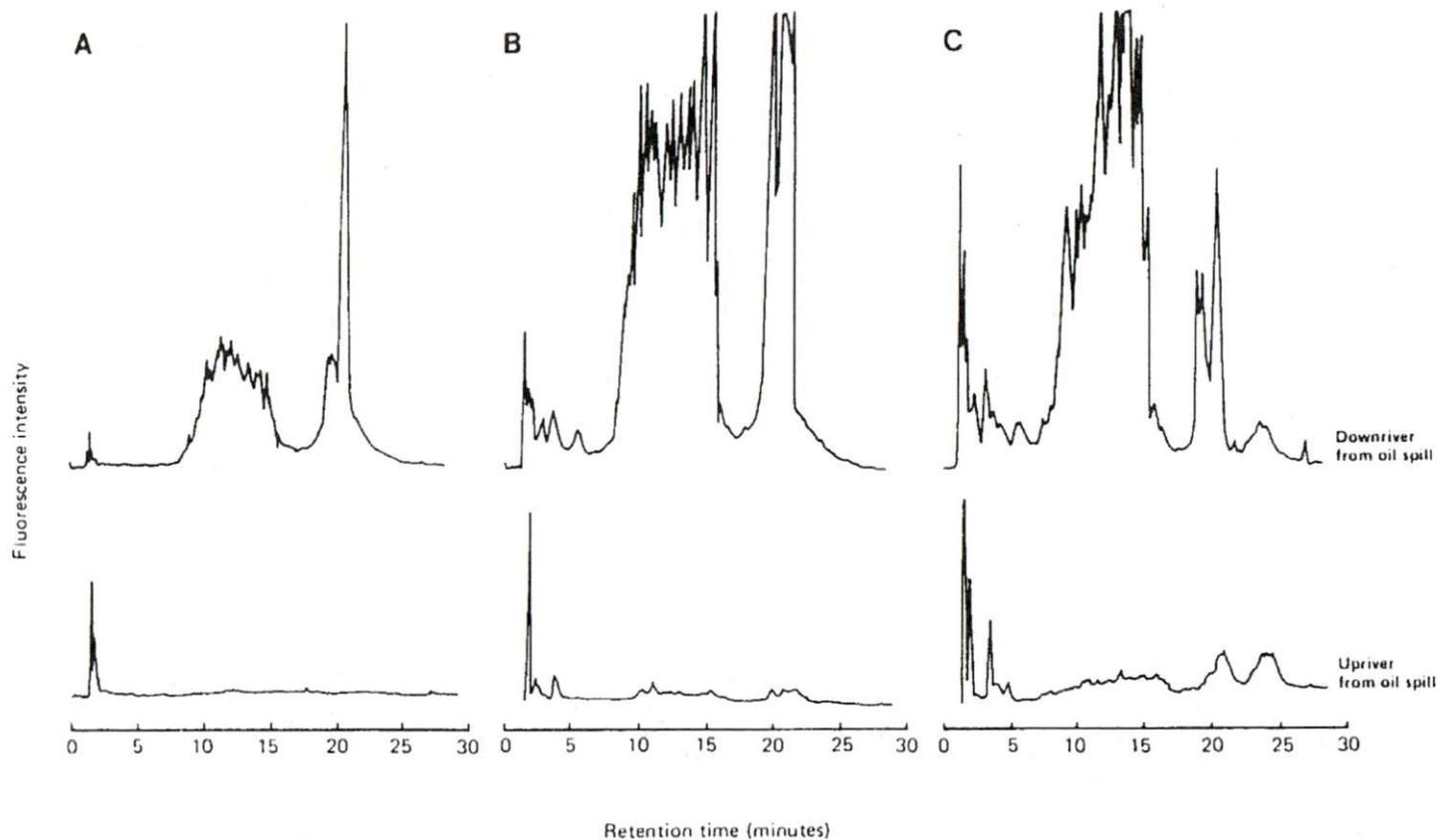


Fig. 1. HPLC/fluorescence chromatograms of bile from a white sturgeon captured downriver (mile 30, upper chromatograms) and a white sturgeon captured upriver (mile 125, lower chromatograms) from the oil spill. Fluorescence recorded at excitation/emission wavelength pairs of (A) BaP, 380/430 nm; (B) PHN, 256/380 nm; and (C) NPH, 290/335 nm.

of aromatic compounds at retention times greater than 7 min were found in bile of the upriver sturgeon at any of the wavelength pairs examined.

Concentrations of metabolites of aromatic compounds in sturgeon bile are given in Table 1. Sturgeon captured 57 miles downstream from the spill had mean concentrations of metabolites in bile measured at the BaP, PHN or NPH wavelength pairs that averaged 16, 22 and 8 times, respectively, those of the upriver sturgeon. Results of Student's *t*-test (one-tailed, Zar, 1984) showed that mean concentrations of metabolites in bile of the sturgeon captured downriver (mile 30) from the spill were significantly different (higher) than those of the sturgeon from the combined upriver (reference) sites at miles 100 and 125 ( $p \leq 0.005$ ,  $p \leq 0.01$  and  $p \leq 0.0025$  for BaP, PHN and NPH wavelengths, respectively).

## DISCUSSION

During the past few years, we have developed analytical methods to provide evidence of exposure of fish from chronically polluted marine environments to aromatic contaminants (Krahn *et al.*, 1984; 1986). The oil spill in the Columbia River gave us an opportunity to test these methods in assessing the exposure of fish to petroleum hydrocarbons from the spilled oil. We found that, just 5 days after oil was spilled into the Columbia River, white sturgeon captured 57 miles downriver from the spill site already had elevated mean concentrations of metabolites of aromatic compounds in their bile. These were similar to mean concentrations found in bile of English sole captured from Eagle Harbor, a site in Puget Sound heavily contaminated with aromatic compounds ( $2100 \pm 1500$  ng/g at the BaP wavelength pair, Malins *et al.*, 1985; Krahn *et al.*, 1986). At the same time, concentrations of metabolites of aromatic compounds in the bile of sturgeon from the upriver sites were comparable to those obtained for English sole from relatively clean sites in Puget Sound (e.g.  $100 \pm 89$  ng/g at the BaP wavelength pair for President Point, Malins *et al.*, 1985). While it is likely that these sturgeon may also have been exposed to low levels of some aromatic compounds before the spill, this contribution would be small compared to that from exposure to the spill, based on our estimates of concentrations of bile metabolites in fish from the reference sites.

Our HPLC/fluorescence method is a simple and rapid means of determining exposure of fish to aromatic hydrocarbons acquired from

spilled oil or from other environmental sources. The bile collected from fish can be processed rapidly due to the simplicity of the direct injection technique (Krahn *et al.*, 1984). Then, the quantitation of the fluorescence response in terms of the reference standard permits statistical comparisons to be made between exposures of fish from contaminated and reference sites. Previously described GC/MS methods (Solbakken *et al.*, 1980; Solbakken & Palmork, 1981; Krahn *et al.*, 1984) could be used to quantitate individual metabolites in the bile, but these methods are time-consuming and more expensive. Thus, our techniques provide a rapid and relatively inexpensive means of evaluating, in part, the impact of an oil spill on fish in its pathway. This method could possibly be applied to evaluate other point source discharges, such as those from municipal or industrial waste.

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