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Field and experimental tainting of arctic freshwater fish by crude and refined petroleum products

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Abstract

We have investigated two incidences of tainting of fish from northern Canada: one by effluent spilled from a synthetic crude oil production plant on the Athabasca river in northern Alberta, and one by diesel fuel spilled when a truck overturned near the Cameron River, Northwest Territories. These cases resulted in complaints that whitefish taken downstream from the spill sites became tainted. Supplies of the spilled materials were available, and laboratory tests confirmed the ability of the spilled materials to produce oily taints in fish. These confirmatory experiments lead to a more systematic study of the relationship between exposure of fish to oil in water and tainting as judged by the responses of taste panels. Fish (rainbow trout and arctic char) were exposed experimentally to three mixtures of Norman Wells oil (nominally 3, 12 and 50 ppm) in water over a period of seventy-two hours. Fish were then transferred to clean holding tanks where they were held for a further 600-840 hours. Three fish were removed from each tank at intervals over both uptake and clearance phases in order to measure the production and subsequent loss of the oily taste. Taste panel members were selected for their ability to detect, identify and rank correctly weak mixtures of oil in water. All panellists were able to discriminate between treated and untreated fish during the uptake phase (mostly by the earliest sampling period of four hours) and early clearance phases. Panellists continued to detect taint in the fish at the end of the clearance phase of the experiment (600 and 840 hours). The implication of these taste panel results is that oil tainting is sensitive to the concentration of oil, the duration of exposure, and duration of the clearance phase. Rainbow trout and arctic char can be expected to become tainted after an exposure of only a few hours and to remain tainted for a month or even longer, following removal to clean water.

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Introduction

In February, 1982, a spill occurred at an oil sand extraction plant near Fort McMurray, Alberta, releasing effluent from the plant into the Athabasca River. Following the spill, complaints were received that whitefish taken in a winter fishery downstream from the plant had become tainted with an oily taste. Investigation confirmed the complaints and resulted in closure of the fishery for the remainder of the fishing season. In the laboratory, lake whitefish (*Coregonus clupeaformis*) were exposed experimentally to plant effluent and then fillets were examined for tastes and odours by sensory panels; these panels confirmed petroleum tainting.

Similar complaints were received several weeks after a spill of diesel fuel into the Cameron River, near Yellowknife, N.W.T. in March, 1983 (Lockhart [1]). In this instance also, whitefish were exposed to the spilled fuel in laboratory tests and the ability of the diesel fuel to produce tainting was confirmed. Both of these examples resulted in court prosecutions and convictions under the pollution prevention provisions of the Canadian Fisheries Act.

The published literature has documented numerous examples of tainting of fish and other aquatic organisms by petroleum products (e.g. Mackie [2]; Motohiro[3]; Jardine [4]; Goodlad [5]). Tidmarsh [6] and Hofer [7] listed several pages of references reporting tainting of fish and other fishery products. Following the field examples, we began more systematic experimental exposures of rainbow trout (*Oncorhynchus mykiss*) and arctic char (*Salvelinus alpinus*) to define dosage-tainting relationships and particularly to examine rates of recovery after exposures had ceased.

Methods

Experiments with whitefish (*Coregonus clupeaformis*) showed that exposure to oil sand plant effluent or to diesel fuel for as little as four hours was sufficient to produce a noticeable off-flavour in the fish. We used rainbow trout and arctic char for the experiments reported below because sufficient large whitefish were not available. The fish used in these experiments were reared at the Freshwater Institute's Rockwood Hatchery. The arctic char were raised from eggs obtained in 1977 from Nauyuk Lake, Northwest Territories. Their mean weights and lengths were 729.1 ± 109.2 g and 40.6 ± 2.1 cm, respectively. The rainbow trout were from the Tagwerker strain with mean weights and lengths of 943 ± 189 g and 41.9 ± 2.7 cm, respectively. All the fish were starved for 14 days prior to treatments with oil, and were not fed during the uptake or the clearance phases of the experiments. All the fish tanks were covered with black polyethylene producing a 0-hour photo-period for the entire experiment. Water temperatures in the rainbow trout and arctic char exposures were 10.2 ± 0.9°C and 9.1 ± 0.3°C, respectively; dissolved oxygen ranged from 6.2 - 9.5 ppm and 5.6 - 9.3 ppm, respectively.

Twenty-two arctic char and twenty-four rainbow trout were placed in each of four 770-L circular tanks for the 72-hour exposure phase. One tank was untreated and three tanks were treated with Norman Wells crude oil (0.003,

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0.012, 0.050 ml L^{-1}, referred to as 3, 12 and 50 ppm) mixed with de-chlorinated City of Winnipeg water. At the end of the 72-hour exposure period, fish were transferred to clean tanks with water flow rates of 20 L min^{-1} which maintained dissolved oxygen levels at 9.5 ppm for the entire clearance phase.

Fish of this size and number would rapidly exhaust the dissolved oxygen in the tanks, and aeration would cause volatilization of the low-boiling components (Lockhart [8]). Consequently, a flow-through dosing apparatus was used to maintain acceptable oxygen levels, and keep concentrations of oil constant throughout the experiment. The dosing and mixing apparatus used consisted of a 20-L mixing tank suspended 1.5 m above each fish tank. Four litres per minute of de-chlorinated City of Winnipeg tap water were mixed with the required volumes of crude oil, which was dispensed by a Harvard Model # 1201 peristaltic pump. The water and oil were rapidly mixed in each mixing tank by means of large magnetic stirrers. Swing arms located at the bottom of each mixing tank dispensed the mixture by gravity to the fish tanks. This arrangement effectively prevented oil, which accumulated at the water surface of the head tank, from being dispensed into the fish tanks (Davis [9]). Flow rates of water and oil were monitored regularly throughout the exposure period and the total volumes dispensed to each tank were recorded and used in determining the final oil : water mixing ratios. The water in each fish tank was circulated with a submersible pump to aid mixing. Three fish were sampled from each tank at 4, 24, 48, and 72 hours during the uptake phase and again at 24, 288, 600, and 840 hours during the clearance phase. (There were insufficient char to include sampling at 840 hours and so the char experiment ended at 600 hours.)

Following removal from the experimental tanks, fish were weighed, measured, and filleted. The washed fillets were vacuum-sealed individually in 1-mil polyethylene-mylar laminated pouches, coded, and stored at -35 C until sensory analysis. Ten panel members experienced in the evaluation of fish products were selected according to their ability to perceive hydrocarbon off-flavours in five water solutions, and to rank those solutions in the correct order of concentration. Solutions used were prepared from dilutions of the WSF of Norman Wells crude oil. The rating procedure was a modification of quantitative descriptive analysis (Stone [10]); a 15-cm line was provided with end points marked weak and strong. Panellists were asked to record their response to each liquid cocktail by placing a mark on the line at a point corresponding to their perceived intensity of any petroleum off-flavour in the sample. Responses were then converted to numerical scores with a template marked in mm.

Fish samples were prepared daily for taste panel presentation. The vacuum-packed fillets were thawed in cold, running water, removed from the pouches, and chopped into 0.63-cm cubes. The pieces were mixed and seven-gram portions were wrapped in aluminum foil and numerically coded for identification during sensory analysis. The taste panels were run according to prescribed methods (Reilly [11]). For presentation to the ten panellists, the wrapped, randomly coded samples were cooked over steam for five minutes, randomly placed on warming trays, and presented to panellists in a sensory-testing laboratory equipped with individual booths. Panellists were instructed to chew unsalted soda crackers and to rinse

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thoroughly with tap water before and after each sample, to minimize transfer of off-flavours from one sample to another.

This design yielded 10 scores for each fish and the arithmetic mean of those was taken to be the final score for an individual fish. Scores of the judges tended to be more consistent with more highly tainted samples. Comparing standard deviations to means, there was a tendency of standard deviations to increase with the means, for samples without a strong taint, but the variation became smaller with more highly tainted samples (Figure 1, upper panel). When viewed as relative standard deviations, there was a clear tendency to lower RSD values with increasingly severe tainting (Figure 1, lower panel). Judges agreed more closely with each other when evaluating highly contaminated samples.

Results

The amounts of oil mixed with the water were based on volumetric measurements of amounts of oil and water added to the head tanks. Most of the oil added to the head tanks remained there with only the finely dispersed or dissolved fractions reaching the fish tanks. There is no fully satisfactory way to measure the amount of oil that actually reached the fish tanks. Extraction and measurement of hydrocarbons is perhaps the most reproducible measurement and we have performed those measurements a number of times on oil/water mixtures to estimate how much oil was incorporated into the water phase. Generally only a small fraction of the oil added (<1 – 3 %) was recovered from the water after initial mixing (Murray [12]). The implication of this is that the fish were actually exposed to only a small fraction of the amount of oil described as concentrations of 50, 12 and 3 ppm, in agreement with Ernst [13].

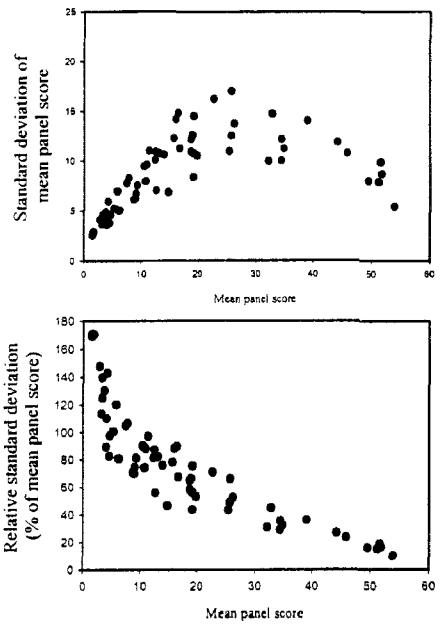

Figure 1. Standard deviation as a function of mean panel score (above) and relative standard deviation of panel scores as a function of mean panel score (below).

Mean scores from the sensory panel judges for arctic char fillets during the uptake phase of the experiment are shown in the upper panel of Figure 2. Exposure for as little four hours resulted in dosage-dependent tainting; the control value at that time was 1.7 while the treatment groups ranged from 4.5 at the 3-ppm exposure to 32.2 in the 50-ppm exposure. The intensity of taint detected by the

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judges was directly related to the nominal concentration of oil added to the exposure water at all sampling times. The intensity of taint increased over time to twenty-four hours exposure, reaching a value of 51.5 in the 50-ppm group. There was little further change between twenty-four and seventy-two hours exposure, with the maximum value for the 50-ppm group increasing to only 53.8. This failure to increase over the final two exposure periods suggests that equilibrium may have been established between fish and tainting substances within the first twenty-four hours.

Following transfer to clean flowing water, the char from all treatment groups showed declines in the intensity of tainting over sampling periods extending as long as 600 h after the transfer (lower panel, Figure 2). The 50-ppm treatment group declined from a mean tainting score of 53.8 at the time of transfer to 19.2 at 600 hours. However, the lowest treatment group declined proportionately less, from 12.6 to 8.9. All treatment groups continued to score higher panel ratings than untreated controls throughout the entire clearance phase. We cannot determine from this data the time required for the char to become free of taint. The profile of panel score against time suggests that further loss of tainting was very slow by the termination of the experiment. Consequently, the time required for the exposed fish to become free of detectable tainting may have been much longer than the duration of these experiments.

Tainting of rainbow trout during the uptake phase is plotted in the upper panel of Figure 3. The time-course of taint production was very similar to that observed with arctic char, and the final values reached were about the same. Again there was a clear relationship between nominal dosage and tainting intensity. The highest dosage (50 ppm) had reached essentially the same level of tainting by 24 h as at subsequent sampling times, but the 12 ppm group increased steadily from 4 to 72 h, with little indication of the plateau shape typical of the other doses with both species.

Loss of taint by the rainbow trout in the clearance phase is shown in the lower panel of Figure 3. Trout from the two higher dosages clearly lost tainting intensity over the clearance phase, especially during the first 24 h. The 50-ppm exposure dropped from a score of 51.2 to 38.9 and the 12-ppm exposure dropped from 34.8 to 25.8 in that time. In contrast, the 3 ppm exposure remained relatively unchanged, dropping only from 18.8 to 12.5 over the entire clearance period of 840 h.

Discussion

These results indicate empirically, that the severity of fish tainting was related both to the concentration of oil and to the duration of exposure. The threshold for tainting by Norman Wells crude oil was below our lowest nominal exposure of 3 ppm. The incorporation of tainting substances into fish muscle occurred very

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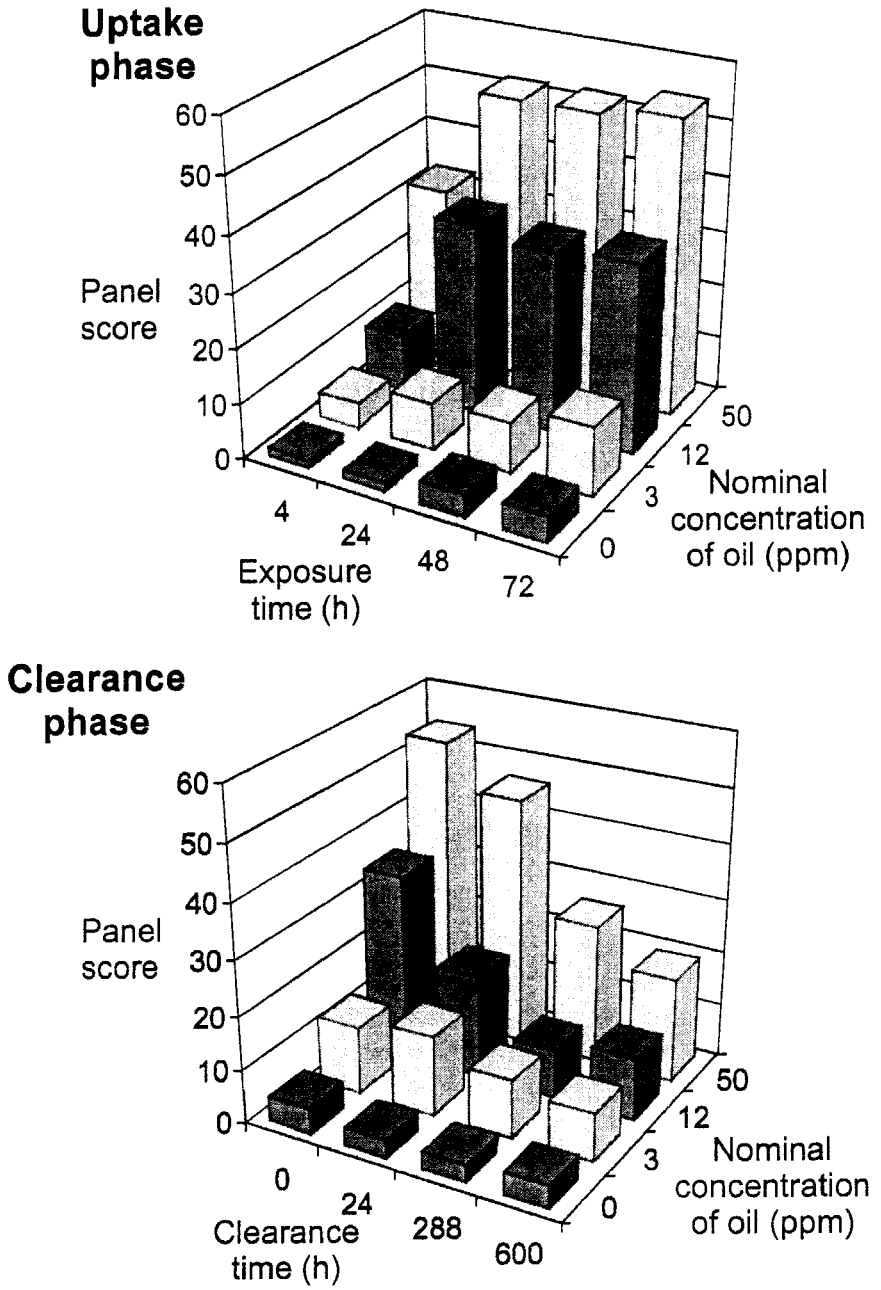

Figure 2. Uptake (above) and clearance (below) of oily taint by arctic char during and after experimental, laboratory exposure to Norman Wells crude oil.

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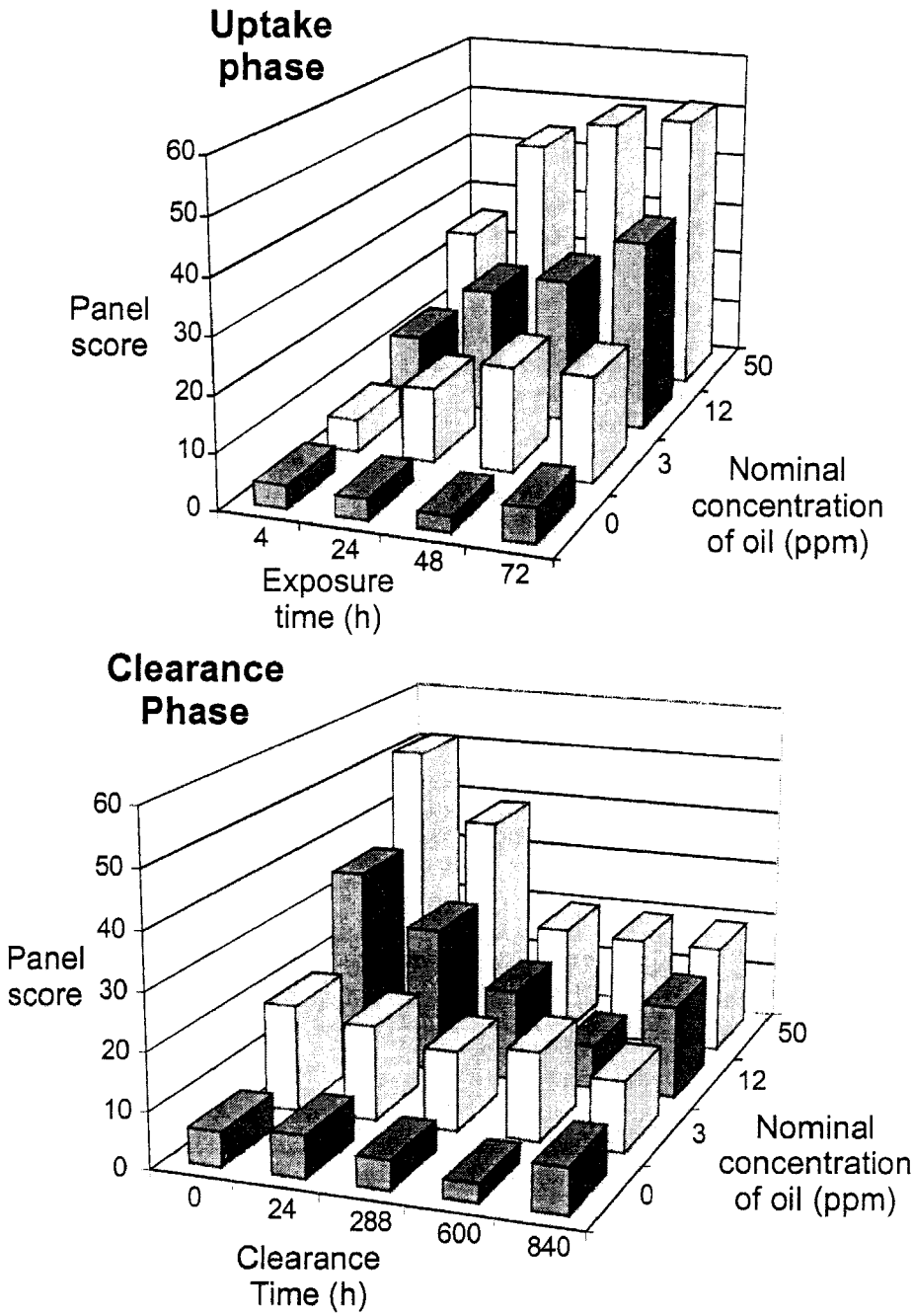

Figure 3. Uptake (above) and clearance (below) of oily taint by rainbow trout during and after experimental, laboratory exposure to Norman Wells crude oil.

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rapidly – in four hours or less. The intensity of tainting of both rainbow trout and arctic char reached a plateau during the uptake phase in the highest exposure concentration (50 ppm). That could be explained by the accumulation of tainting substances having reached equilibrium, or perhaps by the taste sensitivity in the judges being unable to distinguish further degrees of tainting above a certain level of severity.

The oily off-flavour in both arctic char and rainbow trout cleared much more slowly than it accumulated. This is perhaps the most significant observation derived from the study. Following an oil spill and closure of a fishery, the duration of the closure has servious consequences for the fishing industry and for settlement of any claims for financial compensation. This is also true for a subsistence fishery, although there may be smaller or no claims for financial compensation. Tainting was cleared by both rainbow trout and arctic char, to about half of its original intensity, during the first two weeks following the exposures to the two higher concentrations. The ability of the fish to clear the oil taint appears to be greater at the higher exposure concentrations. Reducing an intense taint to a moderate was accomplished more readily than reducing a moderate taint to none. This has a serious implication for a northern fishery since it implies that products may not be suitable for consumption for periods considerably longer than our study.

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