ASSESSMENT OF THE PHOTOTOXICITY OF WEATHERED ALASKA NORTH SLOPE CRUDE OIL TO JUVENILE PINK SALMON

Final Report
December 2, 2003

Contract No. 955.03.3

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EXECUTIVE SUMMARY

Alaska North Slope (ANS) crude oil is known to have greater toxicity to aquatic organisms in the presence of ultraviolet radiation (UV) compared to toxicity determined in tests performed under standard laboratory lighting with minimal UV. This photoenhanced toxicity of ANS crude oil has only been demonstrated in small translucent organisms, including shellfish embryos, larval and juvenile crustaceans, and larval Pacific herring. Pink salmon are known to be sensitive to ANS crude oil toxicity when chronically exposed as embryos, but in the environment the eggs are shielded from UV during development. Fry and juvenile life stages of pink salmon may be exposed to UV during emergence and migration to the ocean, but their sensitivity to phototoxicity has never been reported. The objective of this study was determine if weathered ANS crude oil would be phototoxic to juvenile pink salmon under conditions of short-term exposures to high levels of oil that may occur during an oil spill, and environmentally relevant levels of UV in natural waters.

Two toxicity tests were performed to determine whether ANS crude oil was likely to be phototoxic to juvenile pink salmon. In Test 1, two separate groups of juvenile pink salmon were assessed that differed in oil exposure in the parental generation to evaluate susceptibility that may be attributed to heritable changes caused by prior oil exposure. Test 1 fish were exposed to several water concentrations of oil followed by exposure to sunlight UV in clean water. Toxicity was evaluated by monitoring mortality and behavioral impairment. In Test 2, a single high oil concentration was evaluated with sunlight UV exposure during the oil exposure. In addition to mortality and behavioral observations, fish gills were assessed for indications of sublethal tissue damage because gills are the most likely site of action for phototoxicity in pigmented juvenile fish.

Fish in the highest treatments of both Tests 1 and 2 exhibited melanosis (darkening of pigment), less mobility, reduced startle response, erratic swimming, and loss of equilibrium. These responses were typical of the acute narcotic toxicity of petroleum. Gills from fish in Test 2 had elevated levels of hydroperoxides in oil-only, UV-only, and oil+UV treatments compared to control fish, which was indicative of increased lipid peroxidation in gill tissue. There was no indication of photoenhanced toxicity as assessed by elevation of mortality, behavioral impairment, or gill lipid peroxidation in oil+UV treatments.

The results of this study indicate that pink salmon are at less risk from photoenhanced toxicity compared to early-life stages of several other Alaska species. Phototoxicity could occur under conditions of higher UV exposure, but additional research is not currently recommended as a high priority.

INTRODUCTION

Alaska North Slope (ANS) crude oil is known to be phototoxic, exhibiting two to greater than one hundred fold greater toxicity to aquatic organisms in the presence of ultraviolet radiation (UV) than under standard laboratory fluorescent lighting (Barron and Ka'aihue, 2001). The mechanism of ANS crude oil phototoxicity is through a process of initial bioaccumulation of specific polycyclic aromatic hydrocarbons (PAHs) and heterocyclic aromatics in life stages of aquatic organisms that are relatively translucent to UV (Barron et al., 2003). UV can then activate the bioaccumulated compounds resulting in the generation of toxic intermediates such as oxygen radicals, and cause rapid tissue damage and greater mortality than would occur in the absence of UV (Landrum et al., 1987; Barron and Ka'aihue, 2001; Barron et al., 2003). Photoenhanced toxicity of a few individual PAHs has been demonstrated in a variety of species and life stages of aquatic organisms. However, the photoenhanced toxicity of ANS crude oil has only been determined in small translucent organisms, including shellfish embryos, larval and juvenile crustaceans, and larval Pacific herring (Pelletier et al., 1997; Duesterloh et al., 2002; Barron et al., 2003). These organisms have limited pigment and have an epidermis of only a few layers, allowing UV to penetrate deeply (Hunter et al., 1980).

Pink salmon are an important species in Alaska and are known to be sensitive to ANS crude oil. Chronic embryonic exposures to PAHs derived from ANS crude oil causes malformations, edema, and death in the absence of UV. The potential for phototoxicity in pink salmon embryos is limited because in the environment the eggs are shielded from UV during development (Heintz et al., 1999; Rice et al., 2001). Fry and juvenile life stages of pink salmon may be exposed to UV during emergence and migration to the ocean, but their sensitivity to phototoxicity has never been reported. Previous studies in a heavily pigmented juvenile freshwater fish, the fathead minnow, suggested that juvenile pink salmon might be susceptible to the photoenhanced toxicity of petrogenic PAHs. In the fathead minnow study, the phototoxic PAH fluoranthene caused significant damage in gill tissue, including disruption of mucosal cell membrane function and integrity, only in fish that were also exposed to UV (Weinstein et al., 1997). Tissue injury was attributed to activation of PAHs in the gill tissue exposed to UV during operculation (Weinstein et al., 1997).

A pilot study was initiated to determine if weathered ANS crude oil would be phototoxic to juvenile pink salmon under conditions of short-term exposures to high levels of oil that may occur during an oil spill, and environmentally relevant levels of UV. Experiments were performed in collaboration with the NOAA/NMFS Auke Bay Laboratory in Juneau, Alaska, and the laboratory of Dr. Richard Di Giulio at Duke University. Oil exposures were designed to achieve the maximum tolerated dose of oil for juvenile pink salmon exposed for 24 hr, followed by 5 to 7 hours of UV exposure in ambient sunlight and natural waters. The toxicity and phototoxicity of weathered ANS crude oil was assessed through observations of mortality, behavioral impairment, and evidence of lipid peroxidation in gill tissue as an indicator of sublethal tissue damage.

MATERIALS AND METHODS

Experimental Design

Two tests were performed to evaluate the potential for weathered ANS crude oil to cause photoenhanced toxicity (Fig. 1). Test 1 assessed the acute toxicity (i.e., the ability to cause mortality or impaired mobility) of oil-only, UV-only and oil+UV exposures using five different concentrations of crude oil in water, plus a no-oil control. Two different groups of juvenile pink salmon were tested to evaluate whether prior embryonic exposure affected the acute sensitivity to oil in subsequent generation fish. Group C fish were from parents that had no prior oil exposure. Group H fish were from parents that had been exposed to ANS crude oil as embryos. Previous research indicated that there was significantly reduced marine survival and growth in the group H fish that was caused by petroleum induced heritable changes (Heintz et al., 2000; Rice et al., 2001).
Test 2 assessed both acute toxicity and sublethal toxicity of ANS crude oil caused by injury to gill tissue in fish combined from groups C and H.

Preparation of Water Accommodated Fractions

Water accommodated fractions of oil (WAF) were prepared from ANS crude oil that had been weathered by distillation to a vapor phase temperature of 200 ^{o}C under controlled conditions in the laboratory to simulate evaporative weathering. WAF was prepared in sealed 20 L glass carboys using 40.6 g oil per L of water using 16 L of a 17 parts per thousand ($^{o}/_{oo}$) mixture of fresh water (Auke Lake, AK) and saltwater (Auke Bay, AK). All water was passed through 1 micron polyester filters before use. WAF was vigorously mixed in the dark for 22 hr using a magnetic stir bar and recirculating pump. WAF was allowed to settle for 3 hours, filtered through a 1 micron filter, and slowly stirred prior to use in toxicity tests. WAF for Test 2 was prepared by reusing the Test 1 oil in each carboy. A no-oil control was also prepared in each experiment in a separate 20 L carboy.

Fish Exposures

Juvenile pink salmon (0.20 to 0.26 g/fish) were cultured from artificially spawned adults that returned to the Little Port Walter field station (Baranoff Island, AK) under flow-through conditions (Test 1: groups C and H; Test 2: combined groups). Water quality conditions during culture, and oil and UV exposures were: 17 $\pm$ 1 $^{o}/_{oo}$ salinity, 5 $\pm$ 1 ^{o}C, and greater than 65% dissolved oxygen saturation. Fish were not fed 16 hr prior to and during testing. Test chambers were 3 L glass jars filled with 2.5 L of water, which was replaced every 24 to 48 hr. Ten fish per jar were tested and fish loading was less than 1 g/L.

In Test 1 separate tests were performed with fish from group C and group H that had prior oil exposure as embryos in the parental generation. Fish were first exposed for 22 hours to oil in water under static conditions in the laboratory, then to a total of 5 hours of

natural sunlight (full sun; no clouds) in clean water (Table 1). Mortality and impaired mobility were assessed for 96 hours following the initiation of oil exposure to allow observation of any delayed mortality.

In Test 2, fish combined from groups C and H were tested. Fish were initially exposed for 24 hours to a single high concentration of oil in water under static conditions in the laboratory, then to 7 hours of natural sunlight (heavy clouds, haze, light rain). Static oil in water exposures continued for a total of 48 hours in Experiment 2, then gills of fish from each treatment were dissected for the assessment of gill damage. Gills were sampled because this is the tissue most likely to be affected by phototoxicity in pigmented juvenile life stages of fish (Weinstein et al., 1997).

During laboratory and sunlight exposure, the intensity (W/m^2) of total UVB (280-320 nm), UVA (320-400 nm), and visible light (400-700 nm) were measured with a Macam UV-203 broad wavelength radiometer (Macam Photometrics Livingston, Scotland). The radiometer was calibrated against standards traceable to the National Physical Laboratory and the British Standard Institution. The sunlight exposure system in both tests consisted of test chambers placed in a temperature controlled 1.0 m diameter × 19 cm high aluminum-lined basin filled with filtered laboratory seawater. UVA in the test chambers was 17% of ambient UVA in surface sunlight, 3.7% of surface UVB, and 14% of surface visible light. Total UV exposures ($mWXhXcm^{-2}$) were computed from the intensity and duration of sunlight exposures in each test.

Analytical Chemistry

Samples of pink salmon consisting of 10 to 40 fish were collected after 1 or 2 days of oil exposure, rinsed, blotted dry, and frozen at -20 ^{o}C. Water samples (0.5 L) were extracted two times with 20 mL dichloromethane and the extract was frozen at -20 ^{o}C. Tissue and water samples are being analyzed by NOAA's Auke Bay Laboratory for petrogenic PAHs by the method of Short et al. (1996), but the results will not be available until 2004. The chemistry data will not be included in the final report to RCAC, but will be incorporated into the final manuscript submitted to a scientific journal.

Gill Lipid Peroxidation

In Test 2, whole gills were dissected from randomly sampled fish from each oil and UV treatment at 48 hours after initiation of oil exposure. Fish were initially anesthetized in MS222, then rinsed in ice cold control water. Gills were excised under a stereoscope after removal of the opercular plate, rinsed in ice cold saline, placed in a cryovial, and flash frozen in liquid nitrogen.

Lipid peroxidation in pink salmon gills was quantified using a lipid hydroperoxide assay kit (Cayman Chemical, Ann Arbor, MI) by measuring hydroperoxides in tissue that directly utilize ferrous ion redox reactions. Unstable hydroperoxides react with ferrous ions to produce ferric ions that are then detected by using thiocynate ion as the chromogen. Gill tissue (average weight = 8.3 mg wet weight) was homogenized using a

glass homogenizer in 500 µl of deionized water. Lipid hydroperoxides were then extracted from the samples using a chloroform/methanol extraction. The absorbance at 500 nm was measured on a plate reader using the thiocynate chromogen and values were compared to a standard curve. The concentration of hydroperoxide in each sample (µM) was calculated by correcting for the volume of the original sample homogenate used for the chloroform/methanol extraction and the volume of the chloroform/methanol extracted sample used to measure absorbance (350 µl). Final values were corrected for the original tissue weight, and were reported as the concentration of hydroperoxide (µM) per mg of gill tissue.

RESULTS AND DISCUSSION

Acute Toxicity

No treatment related mortality was observed in fish exposed to WAF prepared with 40 g/L of weathered oil and vigorous mixing. The highest tested oil mixtures (Test 1: 100% WAF; Test 2: 50% WAF) produced water exposures with a perceptible sheen that approximated the maximum tolerated dose based on morphological and behavioral responses in pink salmon. Fish in the highest treatments of both Test 1 and 2 exhibited melanosis (darkening of pigment), less mobility, reduced startle response, erratic swimming, and loss of equilibrium. These responses were indicative of narcosis that is typical of the acute toxicity of high short-term oil exposure (Rice et al., 1976). Fish transferred to clean water exhibited signs of rapid recovery in both pigmentation and behavioral impairment. There were no apparent differences in acute toxicity of ANS crude oil between Group C fish with no prior oil exposure, and Group H fish that offspring of fish that had been exposed as embryos in the parental generation.

Photoenhanced Toxicity

Increased mortality or behavioral impairment was not apparent in either Test 1 with sunlight exposure in clean water (UVA: 1.1 mWXhXcm^{-2}), or in Test 2 with concurrent WAF and sunlight exposure (UVA: 0.45 mWXhXcm^{-2}). These UV levels were lower than previously shown to be phototoxic to Pacific herring and calanoid copepods (Table 2). Low UV exposures were caused in part by the high UV attenuation (83 to 96% reduction) of the Auke Lake water. This water appeared to be naturally high in dissolved organic carbon, a known UV attenuator, as indicated by the intense yellow color in water passed through a one micron filter.

The photoenhanced toxicity of PAHs generally follows a reciprocity relationship, with higher PAH exposures able to elicit phototoxicity under low UV (Barron and Ka'aihue, 2001). Oil dosing was designed to provide high PAH exposures, but pink salmon and other fish are known to rapidly metabolize PAHs, so actual PAH exposures may be lower. Water and tissue chemistry results will not be included in the final report to RCAC; these data will be incorporated into the final manuscript submitted to a scientific journal. Determination of tissue residues of PAHs will allow for a more definitive

assessment of the degree of PAH exposure in the pink salmon phototoxicity studies, and an assessment of relative susceptibility of juvenile salmonids and life stages of susceptible Alaskan species.

Sublethal Gill Damage

Lipid peroxidation is a critical step in the onset of phototoxicity in aquatic organisms. Choi and Oris (2000) reported that photoenhanced toxicity of the PAH anthracene resulted in increased production of superoxide anion and lipid peroxidation. Weinstein et al. (1997) reported that photoenhanced toxicity of fluoranthene caused gill damage in juvenile fathead minnows (~1 g), including disruption of mucosal cell membrane function and integrity. Initial gill damage was attributed to rapid lipid peroxidation reactions in tissue exposed to UV during operculation.

Increased lipid peroxidation in pink salmon gill tissue was observed in fish exposed to oil, UV, and oil+UV, but there was no evidence of synergistic toxicity in oil+UV exposures. Gill lipid hydroperoxide was elevated three to four fold in the oil-only and oil+UV treatments ($p<0.05$), and the UV-only treatment ($p=0.06$) compared to a control response of 0.55 uM/mg wet weight. UVA doses to pink salmon were similar to the 0.6 $mWXhXcm^{-2}$ reported by Weinstein et al. (1997) to cause gill damage in juvenile fathead minnows exposed to the phototoxic PAH fluoranthene. UVB doses to pink salmon were lower by 3 to 8 times than Weinstein et al. (1997). UVA is considered the predominant region of the light spectra responsible for inducing phototoxicity, but recent research suggests that UVB is also a likely factor in the photoenhanced toxicity of weathered ANS crude oil (Barron et al., 2003).

Conclusions

The results of this study indicate that pink salmon are at lower risk from petroleum phototoxicity than other species and life stages such as bivalve embryos, larval crustaceans, and larval fish. It is possible that phototoxicity may occur under conditions of more prolonged oil and higher UV exposure, but additional research is not currently recommended as a high priority. Previous research indicated that there was significantly reduced marine survival and growth in fish with prior oil exposure caused by petroleum induced heritable changes, but there were no apparent differences in acute toxicity or phototoxicity to offspring of parents exposed to ANS crude oil as embryos. Despite the absence of measurable photoenhanced toxicity, the results of these studies are informative and will assist risk managers with interpreting the potential for photoenhanced toxicity of spilled oil in Prince William Sound.

Acknowledgments

Thanks to the my collaborators in this research, including M. Carls, J. Short, J. Rice, and R. Heintz (Auke Bay Laboratory), and M. Rau and R. Di Giulio (Duke University). Thanks to L. Ka'aihue (PWS RCAC) for assistance in report production and review, and

to Steve Diamond (U.S. EPA) for peer review. This study was funded by the PWS RCAC.

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Table 1. Experimental design summary.

Test	Fish Group[1]	Fish per replicate (replicates per group)	Oil Exposure		Sunlight Exposure	
			% WAF[2]	Duration (hr)	Intensity[3] ($mWXcm^{-2}$)	Duration[3]
1	C and H	10 (1)	0, 6.25, 12.5, 25, 50, 100	22	UVA: 0.229 UVB: 0.0035	0, 4.8 hr
2	combined	10 (4)	0, 50	48	UVA: 0.0648 UVB: 0.0009	0, 6.9 hr

1. Group C: no oil pre-exposure; Group H: chronic embryonic exposure to weathered ANS crude oil in parental generation. Combined: fish from both groups C and H.
2. Water accommodated fraction; high test concentration produced light sheening in each experiment.
3. Each replicate treatment included exposure to either sunlight at the average intensity shown, or fluorescent light (0 hr sunlight): UVA: 0.002 $mWXcm^{-2}$; UVB: 0.0001 $mWXcm^{-2}$.

Table 2. Photoenhanced toxicity of weathered Alaska North Slope crude oil in aquatic species exposed to Alaska relevant test conditions of temperature, salinity, and sunlight.

Species (life stage)	**Life History**	**Origin[1]**	**UV Exposure ($mWXhXcm^{-2}$)**	**Phototoxic Concentration**	**Citation**
Pink salmon (juvenile)	anadromous fish	FP	A: 1.1 B: 0.017	no phototoxicity (high nominal oil exposure)[2]	this study
Pacific herring (juvenile)	marine fish	FP	A: 2.2 - 3.6 B: 0.019 - 0.041	2.5 ug/L 0.7 mg/kg	Barron et al. (2003)
Calanus marshallae (juvenile)	copepod zooplankton	FJ	A: 6.2 B: 0.14	2.0 ug/L tPAH 16 mg/kg	Deusterloh et al. (2002)

1. Origin of test organisms: field collected parents from Alaska, and spawned in the laboratory (FP), field collected juveniles (FJ).
2. Maximum tolerated dose for acute toxicity (40 g oil per L water); analytical chemistry data not available for final report.

FIGURE LEGENDS

Figure 1. Experimental design of exposures of pink salmon to oil (black line), ultraviolet radiation (UV; yellow bar), and no oil (blue line) in two experiments with weathered crude oil. Percent of the water accommodated fraction in each oil treatment is shown in parentheses. Group C: no prior oil exposure history; Group H: offspring of fish exposed as embryos to weathered ANS crude oil.

Figure 2. Hydroperoxide in gills (mean, SD) of control pink salmon (no oil or UV exposure; n=2), or fish exposed in oil-only, UV-only, or UV+oil treatments (n=5). Gills were from Test 2 fish.

Figure 1

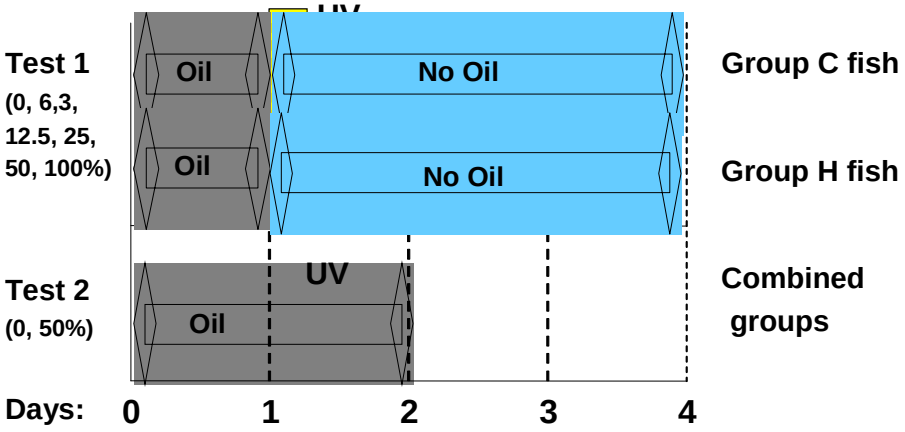
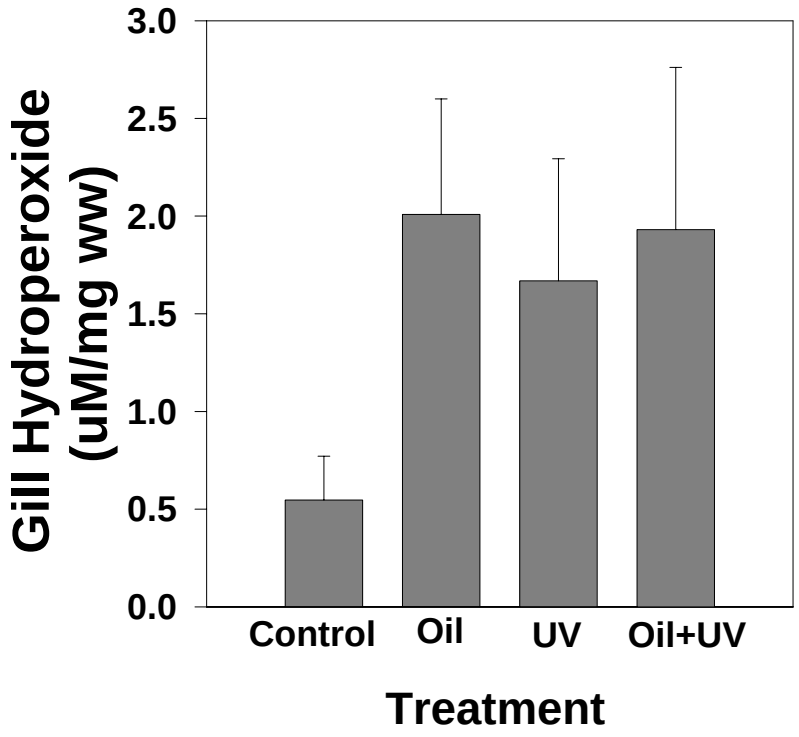

Test 1
(0, 6,3,
12.5, 25,
50, 100%)
Oil
No Oil
Oil
No Oil
Group C fish
Group H fish
Test 2
(0, 50%)
UV
Oil
Combined
groups
Days:
0
1
2
3
4

Figure 2


Gill Hydroperoxide (uM/mg ww)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Control
Oil
UV
Oil+UV
Treatment