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Assessment of the phototoxicity of weathered Alaska North Slope crude oil to juvenile pink salmon

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Abstract

Petroleum products are known to have greater toxicity to the translucent embryos and larvae of aquatic organisms in the presence of ultraviolet radiation (UV) compared to toxicity determined in tests performed under standard laboratory lighting with minimal UV. This study assessed the acute phototoxicity of the water accommodated fractions of weathered Alaska North Slope crude oil (ANS) to juvenile pink salmon, which are a heavily pigmented life stage. Fish in the highest ANS treatments exhibited melanosis, less mobility, reduced startle response, erratic swimming, and loss of equilibrium. Gills from fish exposed to ANS 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. Under the test conditions of moderate salinity, low UV and high short-term oil exposure there were no indications of photoenhanced toxicity as assessed by elevation of mortality, behavioral impairment, or gill lipid peroxidation in oil + UV treatments. The results of this study suggest that pink salmon may be at less risk from photoenhanced toxicity compared to the translucent early-life stages of several other Alaska species.
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Keywords: Oil; Phototoxicity; Salmon; Ultraviolet radiation

1. Introduction

Alaska North Slope (ANS) crude oil is known to be phototoxic, exhibiting two to greater than 100-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;

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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; Barron et al., 2003; Duesterloh et al., 2002). 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 (Peterson et al., 2003; Barron et al., 2004). The potential for phototoxicity in pink salmon embryos is limited because in the environment the eggs are shielded from UV during development by stream gravel (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. Pink salmon fry and juveniles are pigmented and may have a lower potential for UV exposure, although their habitat preference at the earliest marine life stage may lead to greater vulnerability. Emergent fry of pink salmon may be exposed to UV shortly after emergence where they inhabit shallow inshore marine waters that can be contaminated with stranded oil on shorelines (Rice et al., 2001). At mid-tides, foraging fry may be in proximity to both the surface of water and stranded oil. Observations of phototoxic responses 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).

This study investigated whether weathered ANS crude oil would be phototoxic to heavily pigmented 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. Oil exposures were designed to achieve the maximum tolerated dose of oil for juvenile pink salmon exposed for 24 h, followed by 5–7 h 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.

An additional objective of this study was to compare the acute toxicity and phototoxicity of two groups of pink salmon that differed in prior oil exposure in the parental generation. Previous research had shown heritable changes and reduced marine survival in pink salmon from chronic embryonic exposures to weathered ANS (Heintz et al., 2000; Rice et al., 2001). A limited laboratory investigation was performed to determine if prior oil exposure in the parental generation affected tolerance to the acute toxicity and phototoxicity of ANS.

2. Materials and methods

2.1. 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

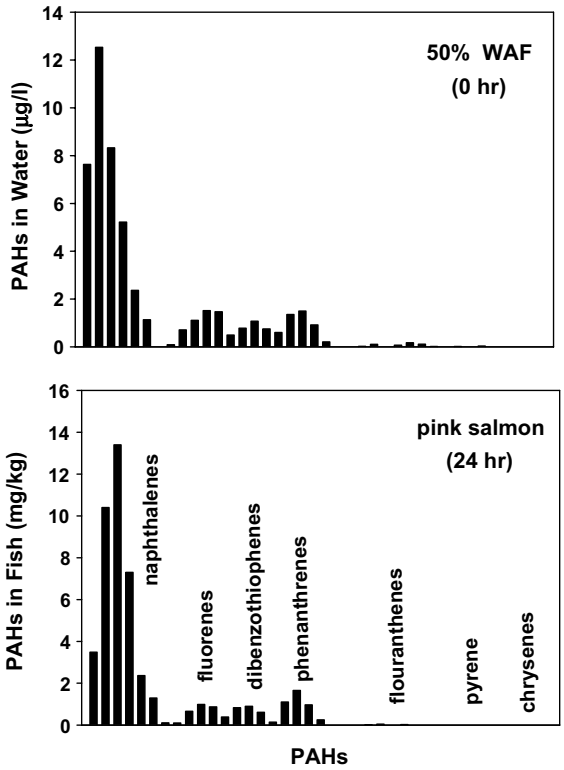

Fig. 1. Parent and alkyl polycyclic aromatic hydrocarbons in exposure water (top panel: µg/l in 50% water accommodated fraction) and pink salmon (bottom panel: mg/kg wet weight in whole body) exposed for 24 h.

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 1 evaluated whether prior oil exposure in the parental generation affected tolerance to the acute toxicity and phototoxicity of ANS. 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. As detailed below, UV exposure was directly quantified with radiometric measurements inside the fish test chambers and PAH exposure was measured through analysis of both exposure water and fish tissue.

2.2. 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 °C under controlled conditions in the laboratory to simulate evaporative weathering. WAF was prepared in sealed 20 l glass carboys using 16 l of dilution water and 40.6 g oil per liter of water. The dilution water was a 17 parts per thousand (‰) mixture of fresh water (Auke Lake, AK) and saltwater (Auke Bay, AK). All water was passed through 1 μm polyester filters before use in toxicity testing. WAF was vigorously mixed in the dark for 22 h using a magnetic stir bar and recirculating pump. WAF was allowed to settle for 3 h, filtered through a 1 μm 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 with the 17‰ dilution water.

2.3. Fish exposures

Juvenile pink salmon (0.20–0.26 g/fish) were cultured from artificially spawned adults that returned to the National Marine Fisheries Service 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 ± 1‰ salinity, 5 ± 1 °C, and greater than 65% dissolved oxygen saturation. Fish were not fed 16 h prior to and during testing. Test chambers were 3 l glass jars filled with 2.5 l of water, which was replaced every 24–48 h. 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 groups C and H that had prior oil exposure as embryos in the parental generation. Fish were first exposed for 22 h to oil in water under static conditions in the laboratory, then to a total of 5 h of natural sunlight (full sun; no clouds) in clean water (Table 1). Mortality and impaired mobility were assessed for 96 h 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 h to a single high concentration of oil in water under static conditions in the laboratory, then to 7 h of natural sunlight (heavy clouds, haze, light rain). Static oil in water exposures continued for a total of 48 h 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 exposures, 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 Photometries Livingston, Scotland). The radiometer was calibrated against standards traceable to the National Physical Laboratory and the British Standard

Table 1
Experimental design summary

Test	Fish group[a]	Fish per replicate (replicates per group)	Oil exposure		Sunlight exposure	
			% WAF[b]	Duration (h)	Intensity[c] ($mW\ m^{-2}$)	Duration[c]
1	C and H	10 (1)	0, 6.25, 12.5, 25, 50, 100	22	UVA: 0.229 UVB: 0.0035	0, 4.8 h
2	Combined	10 (4)	0, 50	48	UVA: 0.0648 UVB: 0.0009	0, 6.9 h

[a] 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.

[b] Water accommodated fraction; high test concentration produced light sheening in each experiment.

[c] Each replicate treatment included exposure to either sunlight at the average intensity shown, or fluorescent light (0 h sunlight): UVA: 0.002 $mW\ cm^{-2}$; UVB: 0.0001 $mW\ cm^{-2}$.

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 (mW h cm^{-2}) were computed from the intensity and duration of sunlight exposures in each test.

2.4. Analytical chemistry

Samples of pink salmon consisting of 20–40 fish were collected after 24 h of oil exposure, rinsed, blotted dry, and frozen at −20 °C. Exposure water samples (0.5 l; 25% and 50% WAF) were extracted two times with 20 ml dichloromethane and the extract was frozen at −20 °C. Tissue and water samples were analyzed for petrogenic PAHs by the method of Short et al. (1996). Total PAHs (tPAH) in water and fish samples were determined as the sum of detected concentrations of parent PAHs and their alkyl homologs (Short et al., 1996).

2.5. Gill lipid peroxidation

In Test 2, whole gills were dissected from randomly sampled fish from each oil and UV treatment at 48 h 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 thiocyanate 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 thiocyanate 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 milligram of gill tissue. Significant differences in gill hydroperoxide levels were determined by analysis of variance and Fisher's least squares difference post hoc test for individual comparisons.

3. Results and discussion

3.1. Acute toxicity and chemistry

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 acute morphological and behavioral responses in pink salmon. The 50% WAF treatment resulted in tPAH concentrations of 50 μg/l as an initial exposure water concentration and 48 mg/kg in fish after 24 h of exposure (Table 2). The predominant PAHs in both water and fish were alkyl naphthalenes, which are not phototoxic (Fig. 2). Concentrations of known phototoxic chemicals were very low, including anthracene which was below 0.006 μg/l in water and 0.005 mg/kg in whole fish (Fig. 1). Concentrations of

Table 2
Total PAH concentrations in exposure water (μg/l) and pink salmon (mg/kg wet weight whole body)

Matrix	Sample[a]	Total PAHs[b]
Water	Control	<0.04 μg/l
	25% WAF (Experiment 1)	19.8 μg/l
	50% WAF (Experiment 2)	50.4 μg/l
Fish	Control	<0.2 mg/kg
	25% WAF (Experiment 1)	15.1 mg/kg
	50% WAF (Experiment 2)	47.9 mg/kg

[a] WAF: water accommodated fraction.
[b] Sum of detected parent and alkyl homlogs.

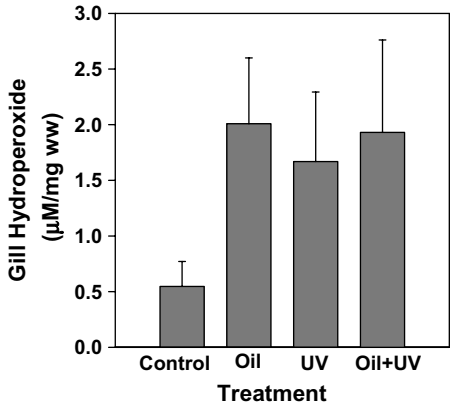

Fig. 2. Hydroperoxide in gills (mean, SD) of control pink salmon (no-oil or UV exposure; $n = 2$), or fish exposed in oil-only, UV-only, or UV + oil treatments ($n = 5$). Gills were from Test 2 fish.

four and five ring PAHs were less than 0.2 μg/l in water and less than 0.05 mg/kg in tissue. Bioconcentration factors (fish:water) for tPAH ranged from 760 to 950.

There was no treatment-related mortality after 1–2 days of exposure, but fish in the highest treatments of both Tests 1 and 2 exhibited melanosis, 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 to naphthalene dominated mixtures (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 had survived as offspring of fish that had been exposed as embryos in the parental generation. These results suggested that prior oil exposure in the parental generation did not appreciably affect tolerance to the acute toxicity of ANS under the conditions of this experiment.

3.2. Sublethal gill damage

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 effects in oil + UV exposures. Gill lipid hydroperoxide was elevated 3–4-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 μM/mg wet weight (Fig. 2). Lipid peroxidation is a critical step in the onset of phototoxicity in aquatic organisms. For example, 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. UVA doses to pink salmon were similar to the 0.6 mW h cm^{-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 3–8 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).

3.3. Photoenhanced toxicity

Phototoxicity as evidenced by increased mortality or behavioral impairment was not apparent in either Test 1 with sunlight exposure in clean water (UVA: 1.1 mW h cm^{-2}), or in Test 2 with concurrent WAF and sunlight exposure (UVA: 0.45 mW h cm^{-2}). However, these UV levels were lower than previously shown to be phototoxic to Pacific herring and calanoid copepods (Table 3). Low UV exposures were caused in part by the high UV attenuation (83–96% 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 1 μm filter.

Photoenhanced toxicity experiments were performed under conditions of high PAH exposures, with 48 mg/kg tPAH measured in pink salmon after 24 h exposure to 50 μg/l WAF. 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; Marwood et al., 2003). The phototoxic components of oil appear to be restricted to specific polycyclic aromatic compounds with three to five fused benzene or heterocycle rings (Barron and Ka'aihue, 2001). Barron et al. (2003)

Table 3
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 history	Pigmentation	Origin[a]	UV exposure (mW h cm^{-2})	Phototoxic concentration	Citation
Pink salmon	Juvenile anadromous fish	Pigmented	FP	A: 1.1 B: 0.017	No phototoxicity[b] 50 μg/l 48 mg/kg	This study
Pacific herring	Larval marine fish	Translucent	FP	A: 2.2–3.6 B: 0.019–0.041	2.5 μg/l 0.7 mg/kg	Barron et al. (2003)
Calanus marshallae	Juvenile copepod zooplankton	Translucent	FJ	A: 6.2 B: 0.14	2.0 μg/l tPAH 16 mg/kg	Duesterloh et al. (2002)

[a] Origin of test organisms: field collected parents from Alaska, and spawned in the laboratory (FP) or field collected juveniles (FJ).
[b] Maximum tolerated dose for acute toxicity.

previously demonstrated that the phototoxicity of ANS occurred through a photosensitization mechanism under environmentally relevant light conditions.

4. Conclusions

Under the combination of moderate salinity, low UV and high PAH exposure, there was no indication of photoenhanced toxicity in heavily pigmented juvenile pink salmon as assessed by elevation of mortality, behavioral impairment, or gill lipid peroxidation in oil + UV treatments. These results suggest that pink salmon may be at lower risk from petroleum phototoxicity than more translucent early-life stages such as bivalve embryos, larval crustaceans, and transparent planktonic larval fish. Phototoxicity may possibly occur under conditions of more prolonged oil and higher UV exposure. Previous research indicated that there was significantly reduced marine survival and growth in fish with prior oil exposure caused by petroleum induced heritable changes (Heintz et al., 1999; Rice et al., 2001). In the limited assessment reported here, there were no apparent differences in acute toxicity or phototoxicity to the offspring of parents that were only exposed to ANS crude oil as parental generation embryos. Additional research is needed to definitively assess any differences in sensitivity to short-term petroleum exposure caused by prior generational exposures.

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