PAHs AND OTHER CONTAMINANTS IN EFFLUENTS FROM ARTIFICIALLY WEATHERED OIL ON GRAVEL

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ABSTRACT

Several recent studies report that low parts per billion (ppb) concentrations of petroleum polycyclic aromatic hydrocarbons (PAH) are toxic to marine fish embryos and that crude oil toxicity increases as it weathers. Such claims for Pacific herring embyros derive from two experiments by Carls et al. (1999) in which herring eggs were exposed to seawater passed through gravel coated with artificially weathered Alaska North Slope crude oil. The experiments differed in the extent of weathering of the oil on gravel. Carls et al. reported that developmental abnormalities in herring embryos occur during chronic exposure to PAH levels as low as 0.4 ppb in seawater passed through the oiled gravel. Earlier studies had shown that effects are observed at low PAH levels only when oil droplets or films adhered to the herring eggs. To better understand Carls et al. experiments, we examined effluent from a gravel bed prepared following Carls et al. and report that ammonia, sulfides, and oil droplets were present in the effluent from oiled gravel generators that were shut down between two 16-day trials (as was done by Carls et al.). Oil droplets (0.5 to 1 mm) were intermittently present in effluent from oiled gravel generators even when the flow was continuous. Two hours after restarting flow, low dissolved oxygen, ammonia, and sulfides were present in the generators and in the effluent. Droplets, ammonia, and sulfides all induce developmental abnormalities of the types seen by Carls et al. The presence of ammonia and sulfide in the effluent after shutdown is a laboratory artifact and constitutes clear evidence of anaerobic biodegradation of the oil on gravel. Evidence of anaerobic biodegradation suggests that the exposure regime of Carls et al. did not effectively simulate field conditions. Our results demonstrate that the presence of confounding toxicants in the Carls et al. experiments cannot be dismissed. There is no basis to conclude that aqueous exposure to low ppb PAH levels affects herring eggs or that weathering increases oil toxicity to fish eggs without additional experiments that specifically account for the potential confounding factors and all chemicals in effluents from oiled gravel columns.

INTRODUCTION

Since the Exxon Valdez Oil Spill (EVOS) in 1989, debates have continued concerning the extent to which Pacific herring in Prince William Sound (PWS) were exposed to oil during EVOS (Pearson et al. 1999, Short et al. 1999). The results of two recent experimental studies are being put forward to claim that effects on herring eggs can occur at much lower levels of exposure than previously supposed. Carls et al. (1999) reports effects on herring embryos from exposures as low as 0.4 ppb from aqueous exposure to artificially weathered Alaska North Slope (ANS) crude oil. Previously, exposures to herring embryos producing effects at the ppb level were seen only in treatments in which oil droplets or films were observed adhering to the eggs (Pearson et al. 1985, Hay et al. 1995).

The results of other recent experiments have only increased the uncertainty about the levels and kinds of exposure inducing adverse effects in fish embryos. For exposure to the water soluble fraction (WSF) of ANS crude oil artificially weathered and then biodegraded by bacteria, Middaugh et al. (1996, 1998) report effects on fish embryos at 1370 ppb of total neutral fraction for Pacific herring, *Clupea pallasi*, and 750 ppb for silversides, *Menidia beryllina*. For exposure of Atlantic salmon eggs to water from sand beds contaminated by unweathered ANS crude oil and artificially weathered ANS crude oil, Anderson and Zitko (2000) reports depressed hatching success for the initial PAH concentrations in treatment water from fresh and weathered crude oil at 220 ppb and 130 ppb, respectively. The fresh oil proved more toxic than the weathered. Also, a large part of the uncertainty derives from the distinct possibility that the results of Carls et al. (1999) may be due to a confounding toxicant other than PAH produced in their system.

Experiment of Carls et al. (1999)

In Carls et al. (1997, 1999), Pacific herring eggs artificially spawned on glass slides were exposed in two experiments to seawater contaminated with artificially- weathered oil. Oil-contaminated seawater was produced by flowing seawater upward through vertical columns containing oiled gravel. The gravel was oiled with five treatments (control, trace, low, medium and high) of artificially weathered ANS crude oil. Weathering was accomplished by heating the oil to 70(C overnight. Heating produced a 20% loss in mass. Two exposures were performed with the same oiled gravel. In the first, the less-weathered oil (LWO) exposure, herring eggs on slides were exposed to effluents from the oiled gravel columns for 16 days. In the second, the more-weathered oil (MWO) exposure, herring eggs were exposed to effluents from the columns with the same oiled gravel being reused. Between the exposures, the flow to the oiled gravel was stopped for 14 days. After restarting the seawater flow for the MWO exposure through the oiled gravel, flow was continued for 1 day before the MWO egg exposure began.

Carls et al. (1999) reported that the MWO exposure proved more toxic to herring eggs than the LWO exposure, even though seawater flow in the LWO exposure had reduced the hydrocarbon levels. Egg mortality and abnormalities in the hatched larvae were observed in both LWO and MWO but at lower concentrations of total polycyclic aromatic hydrocarbons (TPAH) in the MWO. The larval abnormality, yolk sac edema, was the most sensitive abnormality showing a lowest observed effect concentration (LOEC) of 9.1 ppb in LWO and of 0.41 ppb in MWO. Carls et al. (1999) attributed the greater observed toxicity of MWO to changes in hydrocarbon composition between LWO and MWO. In light of their findings, Carls et al. (1999) called into question the adequacy of the State of Alaska water quality standard of 10 ppb TPAH.

Because Carls et al. (1999) stopped water flow to their gravel bed for 2 weeks between their first and second experiment, there is substantial reason to believe that anaerobic conditions could well have produced toxic agents other than oil that accounted for the increased severity of effects observed in the second experiment. For example, it is well recognized that marine sediment contains sulfur-reducing bacteria, which in the presence of petroleum and saltwater, reduce the sulfate in saltwater to hydrogen sulfide (Bak and Widdel 1986, Aeckersberg et al. 1991, Coates et al. 1997, Rothermich et al. 2002). Also, ammonia can be produced under such conditions. Both ammonia and sulfides exposure can produce abnormalities in larvae hatching from exposed fish eggs (Adelman and Smith 1970, Smith and Oseid 1972, McCormick et al. 1984). Also, oil droplets could be present despite statements by Carls et al. (1999) that no oil droplets were visible. Alkane concentrations can be indicative of oil present as droplets (Verbruggen et al. 2000). Total alkane hydrocarbons were between 2 ppb and 5 ppb in the low treatment of the LWO exposure (Carls et al. 1997). Pearson et al. (1985) and Hay et al. (1995) report that oil droplets adhering to herring eggs induce larval abnormalities

To address these issues, we examined effluent from a gravel bed prepared following Carls et al. (1997, 1999) and assessed whether other toxicants were present. We report here that ammonia, sulfides, and oil droplets were present in the effluent from oiled gravel generators that were shut down between the two 16-day trials, and that oil droplets were present in effluent from oiled gravel generators where the flow was continuous (i.e., without shutdown between 16-day trials).

MATERIALS AND METHODS

Following Carls et al. (1997, 1999), contaminated seawater generators were built from polyvinyl chloride (PVC) pipes (30-cm diameter and 122-cm length) placed vertically with inflow at bottom and outflow at top. Epoxy-coated stainless-steel screening was placed inside the pipe at the bottom to hold the gravel and to provide a plenum. A trap fitted at the generator's outflow prevented slick overflow. Each generator provided flow into a 40-L aquarium fitted with a standpipe. The top of each generator was covered with opaque black plastic. Sampling ports on each generator enabled water sampling at three locations: bottom, midpoint, and top (the water 2 cm above the gravel).

The four treatments applied here were the following:

1. *Oiled with shutdown:* Treatment 1 replicates the "Low" level from Carls et al. (1999). Gravel (45 kg) was coated with artificially weathered oil. Treatment 1 received continuously flowing seawater (6 L/min and 5°C) for 16 days, then no flowing seawater for 14 days, then continuously flowing seawater (5 L/min and 6°C) for 17 days. Total PAH concentration in seawater of Treatment 1 was 15.6 ng/L on Day 0.
2. *Oiled without shutdown:* This treatment replicates Treatment 1 except that it received continuously flowing seawater without a period of shutdown. Gravel (45 kg) was coated with artificially weathered oil. This treatment received continuously flowing seawater (6 L/min and 5°C) for 16 days and then continuously flowing seawater (5 L/min and 6°C) for 17 days. Total PAH concentration in seawater of Treatment 2 was 15.2 ng/L on Day 0.
3. *Control with shutdown:* Treatment 3 was the control for Treatment 1. Gravel (45 kg) prepared as in Treatments 1 and 2, except no oil was added. Treatment 3 received continuously flowing seawater (6 L/min and 5°C) for 16 days, then no flowing seawater for 14 days, then continuously flowing seawater (5 L/min and 6°C) again for 17 days.
4. *Control without shutdown:* Treatment 4 was the control for Treatment 2. Gravel (45 kg) was prepared as in Treatments 1 and 2, except no oil was added. Treatment 4 was without a period of shutdown. Treatment 4 received continuously flowing seawater (6 L/min and 5°C) for 16 days and then continuously flowing seawater (5 L/min and 6°C) for 17 days.

Monitoring of seawater flow and temperature showed that flow and temperature conditions here matched those of Carls et al. (1999).

Oiled Gravel Preparation

Pea gravel was rinsed with fresh water, sieved with a 2-mm sieve, and air-dried. Gravel size ranged from 2 mm to 10 mm with the major portion between 3.35 mm and 8.0 mm. Treatment oil was fresh Exxon Valdez crude oil (EVCO) that was collected in 1989 and held under nitrogen. The EVCO was heated in glass beakers for 19 hours at 70°C with stirring on a stirring hot plate. Heating reduced the initial mass by 19%. An aliquot of 100 kg of gravel for Treatments 3 and 4 (control) was tumbled in a pre-cleaned and dried cement mixer for 15 min, and then 45 kg were loaded into each generator for the control treatments. An aliquot of 100 kg of gravel for Treatments 1 and 2 (oiled) was added to the cement mixer and tumbled. A paint sprayer was used to coat the tumbling gravel with about 1 kg of the artificially weathered oil. Before spraying, the artificially weathered oil was heated in a water bath to 40°C and held at that temperature during spraying by a heating mantle attached to the sprayer reservoir. The paint sprayer provided an even coating of oil without recourse to any chemical additives. After tumbling for 15 min, the oiled gravel was split into aliquots, and 45 kg were loaded into each generator for the oiled treatments.

Measurements

Visual inspection of each aquarium for the presence of oil was made daily. Seawater flow, temperature, salinity, dissolved oxygen (DO), and pH in the aquaria were monitored routinely. Seawater was sampled periodically for analyses of TPAH by gas chromatography in selected ion mode (GC-SIM), saturate hydrocarbons (SHC) by gas chromatography flame ionization detection (GC-FID), total soluble sulfides by colorimetry, and total ammonia by indophenol colorimetry. To confirm the presence of oil droplets in the aquaria, seawater samples were filtered with glass fiber filters. Filters were examined under a dissecting microscope and analyzed by GC-SIM and GC-FID. The oiled gravel was sampled for TPAH and SHC analyses at the beginning and end of the experiment.

RESULTS

Seawater Flow and Water Quality

Under continuous flowing conditions, salinity in the aquaria for all treatments varied between 30 psu and 32 psu; DO, between 6.7 mg/L and 8.3 mg/L; and pH, between 7.6 and 7.9. In the treatments with shutdown, low DO was observed in the seawater from the generators just before restart and in the receiving aquaria after restart. About 1 to 3 hours after restart of flow, the DO in the aquaria of the control and oiled with shutdown treatments was 6.1 mg/L and 2.5 mg/L, respectively. One day after restart, the DO in the aquarium of the control with shutdown treatment had returned to ambient (8.0 mg/L), whereas the DO in the aquarium of the oiled with shutdown treatment was slightly depressed (7.6 mg/L).

Total Ammonia

Before shutdown, total ammonia concentrations in the two shutdown treatments were below the detection limit of 0.018 mg/L. After shutdown and restarting water flow to the oiled treatment generator, total ammonia concentrations were 5 mg/L to 9 mg/L in the generator and 2 mg/L in the aquarium. After shutdown and restarting flow to the control treatment generator, total ammonia concentrations were 0.04 to 0.05 mg/L in the generator and 0.02 mg/L in the aquarium. One day after restarting the flow, total ammonia concentrations in both treatments fell to below the detection limit of 0.018 mg/L. Ammonia was not detected in effluents from oiled or control generators without shutdown.

Total Soluble Sulfides

After shutdown and about 1 to 3 hours after restart of seawater flow, total soluble sulfides were in the generator and effluent of the oiled-with-shutdown treatment. Sulfides concentrations were above 0.4 mg/L to 1.8 mg/L in the generator and at 0.08 mg/L in the aquarium upon restarting water flow to the oiled generator. One day after restarting the flow, sulfides concentrations fell to below the detection limit of 0.0007 mg/L. Total soluble sulfides were not detected in the control with shutdown treatment. Also, total soluble sulfides were not detected in effluents from oiled- or control-without-shutdown treatments.

PAH and SHC

The PAH concentrations in the aquaria of the oiled treatments fell exponentially with time under flow. The initial PAH concentrations of about 15 ppb in the oiled treatments were slightly higher than the 10 ppb initial concentration of the Low Treatment reported in Carls et al. (1999). The PAH concentrations decreased to 0.17 ppb and 0.48 ppb in the oiled-without- and oiled-with-shutdown treatments, respectively. The composition of the PAH profile was dominated by napthalenes at the beginning. At the end, the napthalenes had washed out.

The SHC concentrations in the aquaria of the oiled treatments did not show exponential decay but varied over the course of the experiment. The initial concentration of alkanes in Carls et al. (1997) was 5.5 ppb, higher than the 0.9 ppb SHC concentration initially found here. At the end of the experiment, SHC concentrations were 0.50 ppb in the oiled-without-shutdown treatment and 11.0 ppb in the oiled-with-shutdown treatment. At the end of the experiment, the alkane concentration in the Low Treatment of Carls et al (1997) was 0.9 ppb.

Observations of Oil Present in Aquaria

Micro-droplets of oil were intermittently present in the aquaria receiving effluent from both oiled gravel treatments (both with and without shutdown). Water from the aquaria was filtered, and examination of filters under a dissecting microscope revealed oil droplets of 0.5 mm to 1 mm in diameter (Figure 1). These droplets were only marginally visible to the naked eye and tended to concentrate on the aquarium bottom. There were some intermittent observations of oil droplets rising to the water surface. Analyses of the filters for TPAH content confirmed the presence of PAH on the filters.

Examination of Oiled Gravel

The total PAH in the oiled gravel at the beginning of the experiment was 140,000 ng/g in the oiled-with-shutdown treatment and 134,000 ng/g in the oiled-without-shutdown treatment. At the end of the experiment, mean PAH levels in the gravel of the oiled-with-shutdown treatment had fallen to 43,100 ng/g or about 31% of its initial value. Mean PAH levels in the oiled-without-shutdown treatment had fallen to 57,833 ng/g or about 43% of its initial value. Extrusion and examination of the oiled gravel after the experiment revealed that the upper few inches of the gravel column had visibly less oiling than did the lower portions. There was no visible evidence of channeled flow through the gravel column.

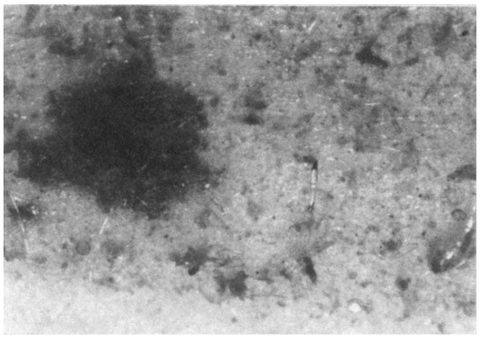

FIGURE 1. OIL MICRO-DROPLETS RETAINED ON GLASS FILTER AFTER FILTERING EFFLUENT FROM THE OILED-GRAVEL GENERATOR.

DISCUSSION

Oil-contaminated seawater generators constructed and employed following methods in Carls et al. (1999) were clearly found to produce three other toxicants beyond the dissolved TPAH and SHC measured by Carls et al. Upon restarting water flow after the shutdown, the oiled gravel generator and its effluent contained ammonia and sulfide. Both with and without shutdown, oiled-gravel treatments produced micro-droplets of oil. In addition to the observed toxicants, byproducts from bacterial degradation during shutdown cannot be dismissed as a confounding toxicant. Also, under anaerobic conditions, bacteria isolated from marine sediment degrade hydrocarbons in transformation of sulfate to sulfide (Bak and Widdel 1986, Aeckersberg et al. 1991, Coates et al. 1997, Rothermich et al. 2002). Under anaerobic conditions, bacterial activity may also increase formation of small oil droplets (Aeckersberg et al. 1991).

The toxicants, ammonia, sulfides, and oil micro-droplets are all known to produce adverse effects in fish eggs. For effects of ammonia on fish eggs, the lowest observed effect concentration (LOEC) can range from 378 µg/L to 46,000 µg/L total ammonia. Scoliosis and pericardial edema are observed at LOEC of 20,000 µg/L total ammonia (McCormick et al. 1984). For effects of sulfides on fish eggs, the LOEC can range from 0.6 µg/L to 411 µg/L (Smith and Oseid 1972). Low DO can increase the effects of sulfides on fish eggs. The LOEC for scoliosis and edema was 20 µg/L to 24 µg/L at a DO of 2 mg/L, and 18 µg/L to 59 µg/L at DO 6 mg/L (Adelman and Smith 1970).

Pearson et al. (1985) and Hay et al. (1995) report that direct contact between crude oil droplets and Pacific herring eggs is a major determinant of toxic effects. Pearson et al. (1985) found that both fresh and weathered oil treatment filtered to remove droplets had the same abnormality rates as controls, whereas unfiltered, undispersed fresh oil had produced a significant increase in abnormalities at 4 ppb total aromatic hydrocarbons with oil droplets adhered to eggs. Chemically dispersed unfiltered weathered oil had increased abnormalities at 818 ppb aromatic hydrocarbons with oil film on eggs. Hay et al. (1995) collected field samples of herring eggs deposited on clean and oiled algae during an oil spill and then incubated the samples in the laboratory. Eggs in direct contact with oil were dead or died during incubation. Eggs on oiled substrate, but not in direct contact, had elevated rates of larval abnormalities.

The byproducts from anaerobic degradation can be toxic to fish eggs. In Middaugh et al. (1998), weathered ANS crude oil was inoculated with hydrocarbon-degrading organisms from PWS and nutrients, and the degraded hydrocarbons then recovered. Exposure of Pacific herring eggs to a WSF of the degraded hydrocarbons revealed significant increases in larval abnormalities (scoliosis and edema), which occurred at and above 1370 µg/L.

SUMMARY

The results of the experiment reported here offer compelling evidence that the presence of confounding toxicants in the experiments of Carls et al. (1999) simply cannot be dismissed. Oil micro-droplets were present in effluents intermittently over the course of our experiments. After restart from shutdown, low DO, ammonia, and sulfides were present within generators and in aquaria but faded under continuous seawater flow. The anaerobic conditions during shutdown and the presence of low DO, ammonia, and sulfides at restart all indicate the occurrence of bacterial degradation. Oil droplets, ammonia, sulfides, and degradation byproducts are all known to induce larval abnormalities of the types seen by Carls et al. (1999). The implication of the results here is that the presence of the observed confounding factors is a laboratory artifact that indicates that the exposure regime of Carls et al. (1999) did not adequately or realistically simulate field exposures. The results indicate that there is no basis to conclude that aqueous exposure to low ppb PAH levels affects herring eggs or that weathering increases oil toxicity to fish eggs without additional experiments that specifically account for the potential confounding factors and all chemicals in effluents from oiled gravel columns.

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