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Exposure of pink salmon embryos to dissolved polynuclear aromatic hydrocarbons delays development, prolonging vulnerability to mechanical damage

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ABSTRACT

Exposure to dissolved polynuclear aromatic hydrocarbons (PAHs) from crude oil delays pink salmon (*Oncorhynchus gorbuscha*) embryo development, thus prolonging their susceptibility to mechanical damage (shock). Exposure also caused mortality, edema, and anemia consistent with previous studies. Hatching and yolk consumption were delayed, indicating the rate of embryonic development was slowed by PAH exposure. The net result was that exposed embryos were more susceptible to shock than normal, unexposed embryos. Susceptibility to shock was protracted by 4–6 d for more than a month in embryos exposed to exponentially declining, dissolved PAH concentrations in water passed through oiled rock; the initial total PAH concentration was 22.4 $\mu g\,L^{-1}$ and the geometric mean concentration was 4.5 $\mu g\,L^{-1}$ over the first 20 d. Protracted susceptibility to shock caused by exposure to PAHs dissolved from oil could potentially increase the reported incidence of mortality in oiled stream systems, such as those in Prince William Sound after the *Exxon Valdez* oil spill, if observers fail to discriminate between direct mortality and shock-induced mortality.

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1. Introduction

Exposure of pink salmon (*Oncorhynchus gorbuscha*) embryos to polynuclear aromatic hydrocarbons (PAHs) dissolved in water results in mortality and physiological damage near the time of emergence (Marty et al., 1997a,b; Heintz et al., 1999, 2000; Carls et al., 2005). However, none of these studies examined the influence of PAH exposure on rate of embryo development and physiological effects near the time of hatch. Studies involving embryos of other teleost species provide evidence that development rates may be affected by PAHs including slowed embryo development (Kuhnhold, 1974; Carls and Rice, 1990), premature or delayed hatching (Kuhnhold, 1974; Linden, 1978; Kocan et al., 1996; Carls et al., 1999), and shorter larvae at hatch (Linden, 1976, 1978; Farwell et al., 2006). Pre-emergent pink salmon fry with oil-induced edema (ascites) were shorter and heavier than controls (Marty et al., 1997b) and exposure to PAHs caused reduced growth rate in older salmonid fry, whether exposed as embryos or as fry (Wang et al., 1993; Carls et al., 1996, 2005; Heintz et al., 2000). These changes in development rate, coupled with a suite of obvious physiological defects such as edema (Linden, 1978; Incardona et al., 2004; Sundberg et al., 2005) that affect swimming ability (Carls et al., 1999) and long-term survival in natural settings (Heintz et al., 2000), suggested that salmonid embryos would be similarly affected before hatch. Indeed, physiological damage in trout embryos exposed to PAHs has been reported, including hemorrhage, edema, and yolk–sac asymmetry (Sundberg et al., 2005). We hypothesized that pink salmon embryos exposed to PAHs would not only develop morphological abnormalities, they would develop more slowly, evident by increased incubation time and slower yolk consumption, hence they would experience protracted susceptibility to mechanical damage (shock). Susceptibility to shock would be protracted if development rate were reduced because the vitelline membrane, the initial protective barrier between surrounding water and the yolk, is easily damaged by mechanical disturbance (Jensen and Alderdice, 1989; Jensen, 1997) until reinforced with epidermal tissue as embryos develop.

The hypothesized increase in the amount of time alevins are susceptible to mechanical damage is of particular interest in the interpretation of observed differences in embryo survival among oiled and reference streams in Prince William Sound (PWS), Alaska, for several years after the *Exxon Valdez* oil spill (Bue et al., 1996, 1998). The incidence of embryo mortality, determined by hydraulically pumping eggs from natural substrate, remained greater in oiled streams than in reference streams before merging about 5 yr post-spill. Two principal hypotheses have been advanced to explain this phenomenon: (1) direct mortality as a result of oil exposure (Rice et al., 2001; Carls et al., 2003) and (2) differential responses in shock resistance based on hypothetical differences in run timing among oiled and reference streams (Brannon and Maki, 1996). The goal of this study is to examine the possibility

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that protracted susceptibility to mechanical damage caused by oil-induced, developmental delays indirectly contributed to the observed disparity in mortality between oiled and reference streams in PWS.

2. Methods

2.1. Column preparation

Pink salmon embryos were incubated in oiled-rock columns or clean gravel columns as in preceding experiments (Marty et al., 1997a,b; Heintz et al., 1999; Carls et al., 2005). Dried 6 mm rock was coated with weathered oil to yield two oil treatment levels (1 or 3 g oil kg^{-1} rock). Alaska North Slope crude oil was weathered by heating at 70 °C for 12 h until approximately 20% evaporated (Bence and Burns, 1995; Marty et al., 1997a,b), sprayed onto tumbling rock, and air dried on plywood for 1 d. Each of the three treatments (control, low, high) was replicated three times in 20 × 60 cm polyvinylchloride incubator columns with 25 kg rock in each. Fresh water entered column bottoms at 1.5 L min^{-1} and exited at the top, providing flow through the gravel and upwelling conditions for the eggs isolated above the gravel with perforated aluminum plates. Twice a day the salinity was increased to 29 ppt with seawater for 1 h periods to approximate natural tidal cycles. The temperature, monitored daily, declined from about 7.9 to 3.4 °C. Incubators were flushed with water (at 1.5 L min^{-1}) for 1 d before eggs were introduced. Visual absence of particulate oil after washout was verified spectrophotometrically before egg introduction. Salt water treatments were discontinued coincident with hatch onset 80 d post-fertilization (dpf) [546 accumulated temperature units (ATUs) $\equiv \sum$°C · day; day = 1].

2.2. Egg exposures

Pink salmon eggs were collected from nine females at Auke Creek, Alaska, on August 28, 2008 and fertilized with milt from two males. Water was added and all eggs and milt were mixed together for 2 min thereafter, redistributed into nine buckets (corresponding to the three treatment × three replicate experimental design), and allowed to water harden 1 h. (Eggs were not hardened in a single group to minimize mechanical damage when distributed to incubators.) Roughly 2000 eggs were then placed in each incubator (range 1179–2509; n did not vary significantly among treatments; $p = 0.987$). Fertility was about 93% and did not vary significantly among treatments ($p = 0.615$). Incubation continued through hatch and until near yolk resorption.

Different subgroups of fertilized eggs were periodically collected with a spoon from each replicate and shocked by dropping them from a height of 0.7 m into water. No egg was shocked more than once. The median number of eggs examined per replicate each observation day was 73, including viable embryos, infertile blanks, and dead eggs. Non-shock egg death was recorded at the time of egg collection; eggs were held 24 h in effluent buckets before shocking to ensure collection damage was not confused with standardized shock events. (Eggs shocked by collection, 0.3% viable eggs + 3.7% infertile eggs, were not included in reported measurements. However, the same conclusions were reached with and without inclusion of these eggs.) Shocked eggs were returned to effluent buckets for 24 h before assessment, allowing dead eggs to whiten. After initial scoring, dead eggs were transferred to salt water, causing them to change from opaque white to translucent orange; the presence or absence of developing embryos was then recorded. Shocking continued every 3 or 4 d from September 11, 2007 through November 16.

2.3. Evaluation of deformities

Beginning November 8, surviving post-shock eggs were retained to observe hatching and to assess alevin condition. These were maintained by treatment and replicate in nets above the rock columns, then transferred to corresponding overflow buckets after completion of shock testing on November 16. Exposure to effluent water (oiled or control) continued throughout the study for these alevins. Hatching was scored daily (in overflow buckets) between November 16 until complete [December 5; 99 dpf, 628 accumulated thermal units (ATU)]. Replicate groups of alevins were anesthetized with tricaine methane sulfonate and assessed four times without replacement between December 15 and December 23 (about 23–31 d after half the control alevins hatched) for edema, yolk shape, hemorrhaging, anemia, and defects of the spine, jaw, and eye. Lengths (caudal peduncle to snout) of all alevins with edema were measured to the nearest 0.1 mm between December 18 and December 21. A subset of alevins without edema, typically five per replicate, was randomly measured for comparison. Assessed alevins were preserved in phosphate-buffered formalin for 24 h, transferred to physiological saline, then methanol.

Relative physiological age at the end of the experiment was qualitatively estimated near the time of yolk resorption (174 dpf) by qualitatively assessing the width of the belly slit. Groups of 32–69 alevins were scored; observations were replicated three (low treatment) to seven times (high treatment), depending on alevin availability. Alevins for this observation (only) were pooled by treatment prior to November 8 and were maintained in uncontaminated fresh water beginning November 8.

2.4. Chemical analyses

To assess aqueous PAH concentrations, effluent water was extracted with dichloromethane at the beginning of exposure and roughly every 10 d thereafter. Extraction volumes increased from 180 ml initially to 2100 ml at the endpoint. Equal water volumes from each of the three replicates were combined to provide single concentration estimates at each time.

Hydrocarbon concentrations were measured per the methods of Short et al. (1996). Samples were extracted twice with dichloromethane after addition of six internal standards. Extracts were reduced in volume, exchanged with hexane over a steam bath, and fractionated and purified by alumina/silica gel chromatography. PAHs were measured by gas chromatography/mass spectrometry (GC/MS) using a mass selective detector. For quality control, a method blank, spiked method blank, and two reference samples were analyzed with every 12 samples. Method detection limits were about 1–8 ng L^{-1} in water; concentrations < method detection limits were treated as 0. Total PAH (TPAH) concentrations were calculated by summing concentrations of 39 individual PAHs, ranging from 2 to 5 rings [parent and alkyl-substituted naphthalenes (N0–N4), biphenyl (BP), acenaphthylene (AC), acenaphthene (AE), fluorenes (F0–F4), dibenzothiophenes (D0–D4), phenanthrenes (P0–P4), anthracene (AN), fluoranthene (FL), pyrene (PY), fluoranthenes/pyrenes (FP1–FP4), chrysenes (C0–C4), benzo(b)fluoranthene (BBF), benzo(k)fluoranthene (BKF), benzo(e)pyrene (BEP), benzo(a)pyrene (BAP), perylene (PER), indeno-123-cd-pyrene (IDP), dibenzo-a,h-anthracene (DBA), and benzo-g,h,i-perylene (DZP)]. Reported doses are based on initial TPAH concentrations, and are preceded by a "$\leqslant$" because exposure concentrations decreased exponentially.

2.5. Data processing and statistical analysis

Infertile eggs were not included in egg shock and mortality estimates; mean infertility (6.9%) was used to correct data collected