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journal homepage: www.elsevier.com/locate/envpolInterspecies variation in the susceptibility of adult Pacific salmon to toxic urban stormwater runoff[☆]Jenifer K. McIntyre^{a,*}, Jessica I. Lundin^b, James R. Cameron^c, Michelle I. Chow^d, Jay W. Davis^e, John P. Incardona^f, Nathaniel L. Scholz^f^a Washington State University, Puyallup Research and Extension Center, 2606 W. Pioneer Ave., Puyallup, WA 98371, USA^b National Research Council, under contract to Northwest Fisheries Science Center, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, 2725 Montlake Blvd. E., Seattle, WA 98112, USA^c Earth Resources Technologies, under contract to Northwest Fisheries Science Center, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, 2725 Montlake Blvd. E., Seattle, WA 98112, USA^d University of Washington, School of Aquatic and Fisheries Sciences, 1122 Boat St., Seattle, WA 98105, USA^e U.S. Fish and Wildlife Service, Washington Fish and Wildlife Office, 510 Desmond Dr. S.E., Lacey, WA 98503, USA^f Environmental and Fisheries Science Division, Northwest Fisheries Science Center, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, 2725 Montlake Blvd. E., Seattle, WA 98112, USA

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ABSTRACT

Adult coho salmon (*Oncorhynchus kisutch*) prematurely die when they return from the ocean to spawn in urban watersheds throughout northwestern North America. The available evidence suggests the annual mortality events are caused by toxic stormwater runoff. The underlying pathophysiology of the urban spawner mortality syndrome is not known, and it is unclear whether closely related species of Pacific salmon are similarly at risk. The present study co-exposed adult coho and chum (*O. keta*) salmon to runoff from a high traffic volume urban arterial roadway. The spawners were monitored for the familiar symptoms of the mortality syndrome, including surface swimming, loss of orientation, and loss of equilibrium. Moreover, the hematology of both species was profiled by measuring arterial pH, blood gases, lactate, plasma electrolytes, hematocrit, and glucose. Adult coho developed behavioral symptoms within a few hours of exposure to stormwater. Various measured hematological parameters were significantly altered compared to coho controls, indicating a blood acidosis and ionoregulatory disturbance. By contrast, runoff-exposed chum spawners showed essentially no indications of the mortality syndrome, and measured blood hematological parameters were similar to unexposed chum controls. We conclude that contaminant(s) in urban runoff are the likely cause of the disruption of ion balance and pH in coho but not chum salmon. Among the thousands of chemicals in stormwater, future forensic analyses should focus on the gill or cardiovascular system of coho salmon. Because of their distinctive sensitivity to urban runoff, adult coho remain an important vertebrate indicator species for degraded water quality in freshwater habitats under pressure from human population growth and urbanization.

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1. Introduction

Adult coho salmon (*Oncorhynchus kisutch*) return from the north Pacific Ocean to spawn in lowland streams of northern Japan, eastern Russia, Alaska, British Columbia, the Pacific Northwest, and

northern California. In watersheds impacted by human land use activities (past and present), many coho populations have been extirpated or are at historically low abundances. In urban areas, it is increasingly evident that toxic stormwater runoff is causing the premature mortality of freshwater transitional adults, prior to spawning. The urban spawner mortality syndrome is characterized by a progressive suite of symptoms that lead to death on a timescale of a few hours (Scholz et al., 2011). The typical sequence of symptoms includes a loss of orientation, surface swimming and gaping, and a loss of equilibrium. Rates of spawner losses to the syndrome can be as high as 90% of an entire fall run (Scholz et al., 2011),

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thereby posing a considerable threat of local extinctions within coho metapopulations (Spromberg and Scholz, 2011). The phenomenon has been most extensively investigated in Puget Sound (Washington, U.S.A) where the distinct coho population segment is presently listed as a species of concern under the U.S. Endangered Species Act. The Puget Sound Basin is currently undergoing extensive human population growth and development. To ensure coho conservation in future decades, it will be imperative to 1) identify the cause of the urban spawner mortality syndrome, and 2) implement clean water measures to prevent toxic runoff to coho spawning habitats.

Urban stormwater is a conduit for a very complicated mixture of chemical contaminants – numbering in the thousands (Du et al., 2017) – and the specific agents that cause the syndrome are not yet known. However, a preponderance of evidence implicates runoff from roadways and other impervious surfaces, and specifically chemicals that originate from motor vehicles (Feist et al., 2017). Several non-chemical hypotheses for the syndrome (e.g., poor fish condition, disease, low dissolved oxygen) have been discounted (Scholz et al., 2011). Adult coho are unable to survive controlled exposures to stormwater collected from an urban arterial, showing the same set of symptoms as previously documented in the field (Spromberg et al., 2016). Moreover, these lethal effects of arterial runoff are prevented when the stormwater is pre-treated with bioretention to remove pollutants (Spromberg et al., 2016). Initial land use analyses combined with field surveys identified a positive relationship between coho mortality rates and the amount of impervious surface within a watershed (Feist et al., 2011). More extensive modeling has recently shown that the most robust predictors of the syndrome are metrics for motor vehicle traffic density in proximity to spawning habitats (Feist et al., 2017).

The extent to which other species of Pacific salmon are vulnerable to the urban spawner mortality syndrome is not well understood. This is due, in part, to differences in habitat use. In Puget Sound, for example, Chinook salmon (*O. tshawytscha*) spawn primarily in the relatively non-urbanized upper reaches of major river basins. By contrast, coho prefer small lowland streams that are more likely to receive non-point source discharges. Run timing is another factor. Chum salmon (*O. keta*) often spawn in the same streams as coho, but returning adults often arrive weeks or months later. Nevertheless, there is limited evidence that species differences may be more important than run timing. For example, in 2006 there was an early chum return to a restored urban stream in Seattle. Among the overlapping spawners, nearly all of the chum survived to spawn while all of the coho were killed by the urban stream syndrome (Scholz et al., 2011).

The present study had two central aims. Our first goal was to compare the presence or absence of behavioral symptomatology in adult coho and chum spawners concurrently exposed to urban stormwater runoff, to determine whether coho are more vulnerable to the mortality syndrome. Second, we profiled the hematology of both species to better understand the pathophysiology underlying the syndrome. Measured blood parameters included pH, blood gases, lactate, plasma ions, hematocrit (% red blood cells in whole blood), and glucose. Consistent with prior field observations, chum did not develop overt symptoms of the pre-spawn mortality phenomenon. We report and discuss major dysregulations in the blood of coho but not chum spawners.

2. Materials and methods

2.1. Runoff collection

Stormwater runoff was collected in a 900-L stainless steel tote (Custom Metalcraft, U.S.A.) from a downspout on a westbound on-

ramp to State Route 520 in Seattle, WA as described previously (McIntyre et al., 2014; Spromberg et al., 2016). This section of highway is elevated and the on-ramp has an average daily traffic density of 15,000 vehicles. Runoff was pre-filtered with fiberglass window screen to remove debris and used for exposure assays within 72 h of collection.

2.2. Adult salmon

Adult coho and chum salmon at the pre-spawn stage were provided by the Suquamish Tribe from their Grovers Creek Salmon Hatchery (Indianola, WA). Adults returned to the hatchery pond at the facility after migrating upstream approximately 0.5 km from Miller Bay in Puget Sound. Fish were present in the pond for up to three days prior to exposures. Individual fish were removed from the pond by seining, placed directly into ventilated PVC containment tubes (Spromberg et al., 2016), and submerged in a stream-fed raceway until the onset of the experimental exposure (0.5–4 h, mean = 2.6 h). All fish appeared healthy and outwardly normal. Males comprised 81% of the coho and 39% of chum. Average length and weight of coho spawners was 390 ± 21 mm (mean \pm standard error) and 1.6 ± 0.3 kg in the control treatment and 422 ± 29 mm and 2.1 ± 0.5 kg in runoff. For chum spawners, length and weight were 638 ± 12 mm and 6.0 ± 0.3 kg in control water, and 636 ± 12 mm and 6.0 ± 0.4 kg in runoff. Most of the coho spawners (31/43) had a clipped adipose fin, indicating a hatchery origin. By contrast, only 2% (1/50) of the chum were clipped. Notably, prior field assessments in coho spawning habitats have shown that the urban mortality syndrome affects male and female coho of hatchery and wild origin (Scholz et al., 2011).

2.3. Exposures

Exposures were conducted in high-density polyethylene tanks at the Grovers Creek Hatchery. Tubes containing fish were submerged in 400 L of either well water (controls) or undiluted stormwater runoff. Each tank was equipped with pumps (Lifeguard Aquatics 5000) to recirculate water and airstones attached to air compressors (Super Luft Pump SL-38) to supplement dissolved oxygen (DO). Exposure waters were pumped directly to each PVC tube to ensure adequate movement over fish gills (Spromberg et al., 2016). Tanks were outdoors but sheltered from rain and direct sunlight. It should be noted that the concentrations of certain ions were consistently lower in stormwater relative to well water (e.g., as hardness; Table S4). This is unlikely to have influenced toxicity, as exposure to synthetic waters containing ions (e.g., Na^+ , K^+ , Ca^{2+}) at concentrations measured in road runoff do not cause the mortality syndrome nor the changes in blood parameters observed in coho exposed to runoff (juvenile coho salmon; M. Chow and J. McIntyre, unpublished results).

For most tests, a total of 16 PVC tubes containing salmon were distributed across four exposure tanks - two containing control water and two containing stormwater runoff. This resulted in the co-placement of two chum and two coho per tank. At the time of the first storm event, chum had not yet returned to the hatchery, and thus only two tanks were used with $n = 4$ coho in clean water and $n = 4$ coho in runoff (Table S1). Water quality (temperature, pH, dissolved oxygen) was monitored prior to test initiation and at periodic intervals during the exposure.

Fish behavior was monitored periodically throughout the exposure to determine whether individuals were showing familiar symptoms of the mortality syndrome (Spromberg et al., 2016). Containment tubes were gently lifted from the water and the front gate was opened to observe individual fish. Spawners that were upright were re-submerged. Those lying on their sides or struggling

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