2021 South Sound Science Symposium Project Summary

Title: Acute Cerebrovascular Effects in Juvenile Coho Salmon Exposed to Roadway Runoff

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Project Summary: Urban stormwater runoff is a primary entryway for pollutants into aquatic environments around the Puget Sound. Stormwater pollutants are responsible for recurrent die-offs of pre-spawning coho salmon in urban streams, referred to as coho urban runoff mortality syndrome. While the putative stormwater toxicant was recently identified as a tire-related chemical (i.e., 6PPD-quinone), toxicology data is lacking for this chemical and the toxic mode of action in coho urban runoff mortality syndrome has remained a mystery for decades. Since extreme hematocrit values appeared closely related to acute mortality of runoff-exposed coho in previous studies, we explored potential mechanisms of hematocrit rise. To do this, we exposed juvenile coho salmon to roadway runoff diluted to 50% using clean fish system water until reaching loss-of-equilibrium. Blood was sampled and analyzed for total hemoglobin concentration of whole blood and hematocrit following centrifugation. Blood plasma was isolated via centrifugation for determination of total protein, total antioxidant power and thiols. Mean cell hemoglobin concentrations were calculated based on total hemoglobin concentrations and hematocrit. Runoff-exposed coho showed increased total hemoglobin and reduced mean cell hemoglobin concentration of the blood, relative to controls, indicating that hematocrit rises were due to increased concentration of red blood cells (hemoconcentration) and red cell swelling.

Because red cell swelling is a common fish stress response, we hypothesized that the unusually high hematocrit rises in runoff-exposed coho was due to loss of plasma volume. We tested whether osmoregulatory dysfunction may have led to a loss of plasma water but did not observe a significant change in blood plasma constituents (i.e., antioxidant power, thiols), discounting osmoregulatory dysfunction as a major driver of hemoconcentration. To test for plasma leakage from blood vessels, Evans Blue dye complexed with bovine serum albumin (EDB-BSA) was injected into the heart of anesthetized fish and circulated for five minutes, followed by a 15-minute saline washout of the blood vessels. Coho salmon exposed to roadway runoff showed substantial and widespread accumulation of EBD-BSA in brain and olfactory rosette regions compared to controls, demonstrating plasma leakage from the

cerebrovasculature. While we focused on cerebrovascular effects, we also noted plasma leakage coming from the gills in runoff-exposed coho, which was absent in controls. Our results suggest blood-brain barrier disruption underlies extreme hematocrit rises and acute mortality in coho salmon exposed to roadway runoff. Due to a high conservation concern in the Puget Sound, sublethal effects in juvenile Chinook based on blood-brain barrier disruption will be evaluated in upcoming experiments.

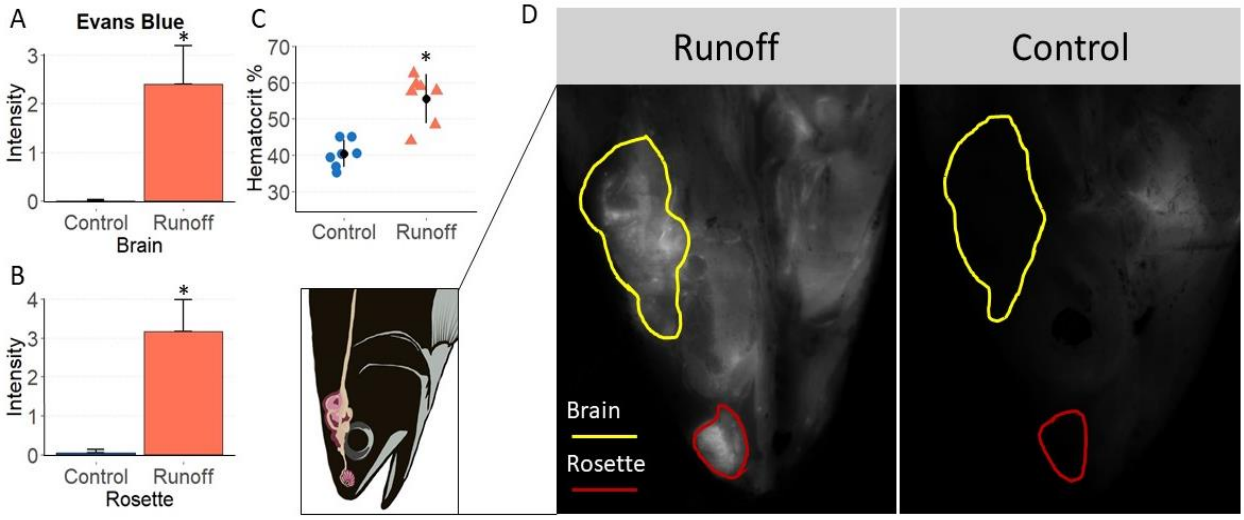

Figure. Hematocrit and blood-brain barrier tracer results for juvenile coho lethally exposed to roadway runoff (n=7). Fluorescence intensities of tracer Evans Blue bound to bovine serum albumin for brain (A) and olfactory rosette (B) regions show extensive accumulation of the tracer in runoff-treated fish. Concurrently, runoff-exposed coho show severe hematocrit rises (bars show mean ± s.d.) (C), relative to controls. * indicates p-value < 0.001 based on Bonferroni confidence intervals. Images of cryosections through the center of coho heads (D) demonstrate post-washout retention of Evans Blue-albumin fluorescence in brain and rosette regions of runoff-treated fish, and absence of Evans Blue-albumin in control tissues. (Blair et al. 2021).