

Urban Roadway Runoff Is Lethal to Juvenile Coho, Steelhead, and Chinook Salmonids, But Not Congeneric Sockeye

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ABSTRACT: We compared the sensitivity of closely related Pacific salmon and steelhead (*Oncorhynchus* spp.) to untreated urban stormwater runoff across three storm events. Juvenile coho, sockeye, steelhead, and Chinook were exposed for 24 h to untreated urban runoff and then transferred to clean water for 48 h. As anticipated from previous studies, coho were highly susceptible to runoff toxicity, with cumulative mortality rates ranging from 92%–100% across the three storms. By contrast, juvenile sockeye were unaffected (100% survival), and cumulative mortality rates were intermediate for steelhead (4%–42%) and Chinook (0%–13%). Furthermore, coho died rapidly following the onset of stormwater exposure (generally <4 h), whereas mortality in Chinook and steelhead was delayed by 1–2 days. Similar to previous findings for coho, steelhead and Chinook did not recover when transferred to clean water. Lastly, significant mortality occurred in coho even when roadway runoff was diluted by 95% in clean water. Our findings extend the urban runoff mortality syndrome in salmonids and point to a near-term need for sublethal studies in steelhead and Chinook to more precisely understand stormwater risks to threatened species recovery efforts in the western United States.

KEYWORDS: urbanization, stormwater, endangered species, *Oncorhynchus*, microplastics, 6PPD-quinone, urban runoff mortality syndrome, tire wear particles



conservation implications for ESA-listed salmon are considerable, given high rates of premature death (up to 90% or more) among adult coho returning to spawn in urban watersheds, as documented in field surveys across multiple years in Puget Sound and elsewhere.^{10,11}

At present, uncertainty around the extent to which roadway runoff toxicants, including 6PPD-q and other tire antioxidant/antiozonant transformation products, impact aquatic taxa beyond coho is spurring a global effort in ecotoxicology.^{3,12,20} Although environmental health data are limited, there appears to be variation in vulnerability to roadway runoff even among closely related species of Pacific salmon belonging to the genus *Oncorhynchus*. This is evident from recent studies, wherein adult coho and chum (*O. keta*) were exposed to urban runoff or tire leachate, with high mortality observed in coho but not chum.^{13,14} These differences in survival were consistent with observations from field surveys that previously documented

INTRODUCTION

Recent advances in analytical methods have made it increasingly possible to fractionate urban stormwater runoff into individual and identifiable toxic components. These efforts have yielded several chemicals of emerging concern (CECs) that are derived primarily from vehicle tires and therefore ubiquitous in roadway runoff.^{1,2} Many are essentially unknown to ecotoxicology despite an apparent prevalence in the environment^{3,4} and rapid mobilization in stormwater runoff.^{5–7} One such CEC is *N*-(1,3-dimethylbutyl)-*N*'-phenyl-*p*-phenylenediamine (6PPD), an antiozonant added during the manufacture of tires to help prevent tread degradation. Ozone abiotically converts 6PPD into 6PPD-quinone, as well as other transformation products.⁸

For the past two decades, researchers in the Pacific Northwest of the United States (U.S.) have been studying a severe urban runoff mortality syndrome in coho salmon (*Oncorhynchus kisutch*). This forensic investigation eventually led to the discovery of 6PPD-quinone (6PPD-q) as the primary causal agent in the urban runoff mortality phenomenon.⁴ Recurring, stormwater-driven dieoffs pose a significant threat to the near-term and long-term conservation of wild coho populations,⁹ several of which are currently managed under the U.S. Endangered Species Act (ESA). The

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high rates of premature spawner mortality in coho but not chum in restored urban catchments.¹⁰ However, the vulnerability of other Pacific salmonids to urban roadway runoff, including sockeye (*O. nerka*), Chinook (*O. tshawytscha*), and steelhead (ocean-going rainbow trout; *O. mykiss*), has not been determined. In the context of the ESA, this represents a major information gap, particularly for stream-type Chinook and steelhead that spend proportionately more time in freshwater habitats as juveniles, where they are more likely to be exposed to runoff from the transportation grid. Moreover, relative to coho, ESA-listed Chinook and steelhead populations encompass a much wider range of river basins in the western U.S., particularly in California (see www.fisheries.noaa.gov/species-directory/threatened-endangered for current ESA range designations).

It was recently shown that juvenile coho, in addition to spawning adults, are highly susceptible to the mortality syndrome.¹⁵ Salmonids other than coho, including *O. mykiss*, also appear vulnerable.¹² In the present study, we extended these earlier observations by coexposing juvenile coho, sockeye, Chinook, and steelhead to runoff collected during multiple rain events. We focused on small subyearlings (coho, sockeye, Chinook) as well as larger age 1+ juveniles (coho, steelhead), and used coho as a positive control for the baseline toxicity of untreated stormwater (i.e., as confirmation of the mortality syndrome). Our study had three primary objectives: (1) determine whether ESA-listed species other than coho are vulnerable to acutely lethal stormwater toxicity, (2) evaluate the time course for mortality across species and whether affected fish recover in clean water, and (3) assess the role of dilution as a factor influencing survival, using juvenile coho as an indicator species.

MATERIALS AND METHODS

Urban Roadway Runoff Collection and Transport.

Urban stormwater was collected from downspouts draining a short section of elevated urban arterial (west-bound onramp to State Route 520 from Montlake Boulevard in Seattle, Washington). Runoff from six storms was collected at different times of the year in 2018 (August 26, October 25, and December 17) and 2019 (March 12, March 25, and April 4). During each storm event, runoff was filtered through a fiberglass window screen to remove coarse debris and collected in 900 L or 1325 L stainless steel collection totes (Custom Metalcraft Inc., Springfield MO) for subsequent transport at ambient temperature to the Washington State University Research and Extension Center in Puyallup (WSU-P). Juvenile salmonid exposures were initiated within 24 h of each storm event. Note that 6PPD-q levels in runoff from this location have been previously measured across nine storms in 2017–2019,^{4,19} including the October and December collection events used here in 2018; all storms contained 6PPD-q at concentrations expected to be lethal to juvenile coho (Table S3).

Juvenile Salmon and Steelhead. Juvenile coho, sockeye, and Chinook salmon as well as an ocean-migrating stock of *O. mykiss* (hereafter referred to as steelhead) were obtained from local hatcheries, held in a recirculating freshwater system at WSU-P on a 12:12 h dark:light cycle, and fed daily with commercial fish food (BioVita, Bio-Oregon). Fish rearing water consisted of municipal water treated with reverse osmosis and adjusted to pH 7.6 and a conductivity of 1500 $\mu\text{S}/\text{cm}$, then passed through a bead filter, UV reactor,

bioreactor, and chiller. Sockeye prefer cooler waters (8 °C optimum), and thus exposure temperatures were maintained at 5.0–10.3 °C for the cross-species vulnerability experiments involving sockeye and the three other salmonids. For the dilution experiments (coho only), temperatures ranged from 10.0 to 12.1 °C. Fish sizes and water quality measurements for all experiments are described in the Supporting Information (Tables S3 and S4); experimental protocols were approved by Washington State University's Institutional Animal Care and Use Committee.

Stormwater Exposures to Assess Species-Specific Sensitivity. For the multispecies comparisons, replicate groups of juvenile salmonids were exposed to undiluted roadway runoff (100% stormwater) for 24 h following each of the three 2019 storm events and then transferred to clean (rearing) water for an additional 48 h (Table S1). Coho served as positive controls for the urban mortality syndrome in side-by-side exposures using subyearlings (coho, sockeye, and Chinook) and larger age 1+ salmonids (coho and steelhead). We selected an ocean-migrating stock of *O. mykiss* to differentiate the steelhead life history from domesticated, freshwater-resident strains of rainbow trout.

Static exposures were carried out in 35 L glass aquaria containing either 30 L of clean water or 100% stormwater. For the smaller fish (subyearling coho, Chinook, and sockeye), 9–10 fish were placed in each of the three replicate exposure tanks. For the larger (age 1+) coho and steelhead, $n = 6$ fish were placed in each of four replicate exposure tanks. A few fish escaped into the surrounding water bath and were excluded (Table S1).

Exposure tanks were supplied with air stones for oxygenation (>10 mg/L) and placed in flow-through water baths with chillers to maintain temperatures. Across all three storms, mortality was monitored throughout exposure periods at regular intervals (2, 4, 8, and 24 h). Final reported mortality counts included moribund fish that did not react to gentle prodding and were removed and euthanized with MS-222 (400 mg/L) followed by severing the spinal cord.

All fish surviving the 24 h exposure were transferred to larger (57 L) tanks containing clean water for a 48 h depuration. For the smaller fish (subyearling coho, Chinook, and sockeye), individuals were removed from the three replicate exposure tanks and grouped into a single depuration tank with a maximum number of 30 fish per tank. For the larger age 1+ coho and steelhead, fish from individual exposure tanks were divided evenly into two depuration tanks ($n = 12$ maximum per tank, to adjust for larger fish). Moribund and dead fish were monitored and removed midway through depuration (48 h) and at the end of depuration (72 h).

Stormwater Exposures to Assess the Influence of Dilution on the Mortality Syndrome. To evaluate dilution, juvenile coho were exposed to a graded stormwater concentration series for 24 h following three storms in 2018 (Table S2). Juvenile coho (age 1+; Table S4) were placed in static exposure chambers as described above. Glass aquaria (35 L) were filled with either 30 L of clean water or urban stormwater diluted to 25%, 11.2%, 5%, 2.2%, or 1% with clean water. For the August and December storms, individual fish ($n = 8$, adjusted for size) were placed in each of three replicate tanks per concentration. For the October storm, a mechanical failure (chiller) necessitated a shift in experimental design, whereby $n = 10$ fish were exposed in each of the two replicate

tanks per dilution. Mortality was enumerated as described above.

Statistical Analyses. Statistical analyses were developed using R (<https://www.r-project.org/>) and RStudio (<https://www.rstudio.com>). Mortality was calculated in proportion to the total number of individuals across all tanks within a given treatment (e.g., exposure, species, time, and/or dilution; see data in [Tables S1 and S2](#)). For each observed proportion, the 95% confidence interval was calculated using the BinomCI function from the DescTools R package with the “modified Wilson” method to account for proportions near 0 and 1 (presented as percentages in [Figure 1](#)). Differences among treatments were determined by comparing confidence intervals around the observed proportions.

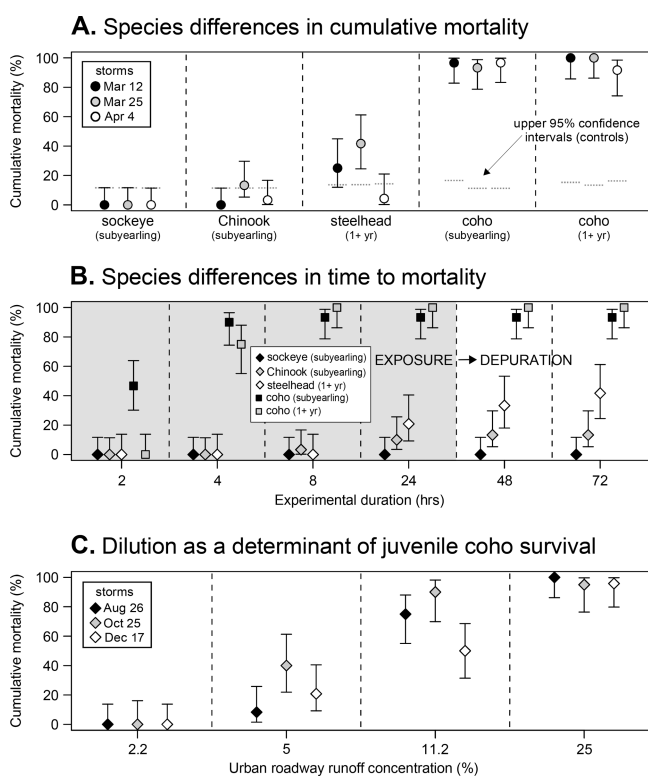


Figure 1. Cumulative mortality (% and 95% confidence interval) of juvenile Pacific salmonids exposed to urban roadway runoff. (A) Species differences across three storms following 24 h exposure and 48 h depuration. Upper 95% confidence intervals of controls are shown as dotted lines. (B) Species differences in mortality over time for a single storm (March 25) after 24 h exposure (gray shaded area) and 48 h depuration (unshaded area). (C) Mortality in coho (1+ yr) exposed for 24 h to different dilutions of roadway runoff over three storms. No mortality was observed in controls. (A–C) Data from all control and exposed fish are shown in [Tables S1 and S2](#). Fish age is noted as subyearlings or 1+ yrs.

RESULTS AND DISCUSSION

Varying Mortality in Response to Untreated Stormwater Across Salmonid Species. Juvenile sockeye, Chinook, and steelhead, as well as two size classes of juvenile coho (positive controls), were exposed to untreated roadway runoff from three separate storm events ([Figure 1A](#)). As anticipated from earlier studies,^{4,15,16} subyearling and larger (age 1+) coho were highly susceptible to toxic runoff, as evidenced by >90% cumulative mortality ([Figure 1A](#); right-

most panels) across all three 2019 storms. Conversely, all juvenile sockeye survived 24 h exposures to runoff, irrespective of the storm, similar to previous observations of 100% survival for chum.^{13,14} The response of juvenile Chinook was less consistent, with only modest mortality in response to the second (March 25) storm. Steelhead were intermediate between sockeye (no deaths) and coho (almost no survivors), with proportionately higher mortality for the first and second storms ([Figure 1A](#)).

Species-Specific Timelines for the Mortality Syndrome. Among Pacific salmonids, the stormwater mortality phenomenon has primarily been studied in coho, where the progression of the syndrome—from asymptomatic fish to visible distress (disorientation, loss of equilibrium) to death—occurs over a span of a few hours in fish exposed to 100% stormwater. In accord with earlier published results,¹⁵ juvenile coho began dying soon during exposure (2–4 h), with near-maximal cumulative mortality within 8 h ([Figure 1B](#); stormwater exposure window in shaded panels at left). Relative to coho (positive controls), the progressions of symptoms in Chinook and steelhead were qualitatively the same (surface swimming and gaping, loss of equilibrium) albeit with a delayed onset and a longer window for mortality thereafter, with fish generally dying toward the end of the 24 h exposure or after subsequent transfer to clean water. Hence, similar to coho,¹⁵ the distress syndrome in Chinook and steelhead appears irreversible.

Influence of Dilution on Coho Survival. Our sourcing of stormwater from an urban arterial with high traffic volume (relatively concentrated runoff) is well suited for studies of green infrastructure effectiveness^{16,17} but is less representative of exposure conditions in salmon habitats where stormwater is diluted by receiving waters. To evaluate the influence of dilution on survival, juvenile coho were exposed to runoff from three separate storm events for 24 h, at concentrations ranging from 1% to 25%. Mixtures containing 5% or more stormwater were generally lethal to juvenile coho ([Figure 1C](#)). Note that the upper end of the exposure range (25% runoff diluted in clean water) was chosen based on maximal coho lethality in pilot experiments; untreated surface runoff is likely to represent more than 25% of receiving surface water volumes in many urban watersheds.

Implications. In the U.S. Pacific Northwest, the urban runoff mortality phenomenon has been studied intensively for the past two decades, with a primary focus on adult coho returning from the ocean to spawn in urbanized Puget Sound watersheds.^{10,18} Wild coho populations are unlikely to withstand the high rates of dying spawners previously and consistently documented in urban stream reach surveys.⁹ The mortality syndrome was subsequently extended to juvenile coho,¹⁵ which enabled relatively high-throughput testing of stormwater fractions and set the stage for the eventual discovery of 6PPD-q as the causative agent.⁴ Because the parent compound (6PPD) and other chemicals similar in structure and function are ubiquitous in motor vehicle tires worldwide, there has been an expanding effort to identify other fish species at risk from exposure to untreated roadway runoff. Our current findings extend the urban runoff mortality phenomenon to steelhead and Chinook but with phenotypic nuances in severity (less) and time course (longer) relative to coho. Nevertheless, the characteristics of the syndrome in coho exposed to roadway runoff (e.g., the present study; Chow et al.¹⁵) or 6PPD-q alone (e.g., Tian et al.⁴) are the same,

implying a common underlying mechanism. The discussion that follows therefore hypothesizes that the coho, steelhead, and Chinook toxicity observed here was caused by 6PPD-q across the different stormwater collections, an assumption further supported by recent findings for *O. mykiss*.¹² For context, coho are exceptionally sensitive to 6PPD-q toxicity, with lethality (LC50) in response to exposure concentrations less than 0.1 parts per billion.¹⁹ In the present study, 6PPD-q levels were likely to have exceeded this acute lethality threshold for coho across all storms by an order of magnitude. This is supported by published 6PPD-q measurements in runoff from the same source (SR520 elevated bridge) across nine separate rain events in 2017–2019,^{4,19} including the October and December stormwaters collected for the dilution experiments in Figure 1C (Table S3).

Our current findings are the first demonstration of urban runoff-driven mortality in Chinook, and our sockeye results reinforce what are apparently stark differences in vulnerability across closely related salmonids. For example, whereas coho (*O. kisutch*) are highly sensitive,¹⁹ several field and laboratory studies have shown that congeneric chum salmon (*O. keta*) are not.^{10,13} Similar to chum, zebrafish (*Danio rerio*) and medaka (*Oryzias latipes*) appear relatively insensitive to 6PPD-q,^{20,21} along with Arctic char (*Salvelinus alpinus*) and white sturgeon (*Acipenser transmontanus*).¹² Conversely, Brinkmann et al.¹² recently showed that brook trout (*Salvelinus fontinalis*), like coho, are also highly sensitive to the acutely lethal toxicity of 6PPD-q. Intriguingly, in the same study, the time to death for rainbow trout (*O. mykiss*) was intermediate, with mortality occurring over a more protracted timeline of a few days. On the basis of these initial findings, the Salmonidae appear to sort into roughly three categories of vulnerability to 6PPD-q acute toxicity: high (coho salmon and brook trout), low (chum and sockeye salmon, Arctic char), and intermediate (steelhead/rainbow trout, Chinook salmon).

In the context of threatened species management, the recent *O. mykiss* results are particularly noteworthy. In addition to being a model test organism in aquatic toxicology, rainbow trout are an important aquaculture species for recreational fishing.²² In the western United States, several distinct population segments (or evolutionarily significant units) of ocean-migrating *O. mykiss* (steelhead) are currently listed for protection under the U.S. Endangered Species Act (ESA). Accordingly, freshwater and estuarine habitats critical for steelhead conservation and recovery have been designated across major metropolitan areas in the coastal and interior regions of California, Oregon, and Washington. The overlap between these recovery domains and the expanding transportation grid, at the watershed and basin scales, is extensive. The ESA requires that federal natural resource managers consider the impacts of federal actions on listed species, and stormwater contaminants (e.g., 6PPD-q) represent a habitat factor that was largely unknown to science when steelhead were listed.

For *O. mykiss*, the initial evidence suggests that life history plasticity (i.e., freshwater residence vs ocean migration) is not a determinant of susceptibility, albeit premised on two important assumptions: (1) Variation in genetics or husbandry/culture practices between the *O. mykiss* stocks used here and those sourced for Brinkmann et al.¹² did not influence observed toxicity, and (2) 6PPD-q was the causative agent for *O. mykiss* deaths here, thus facilitating a direct comparison of mortality across the two studies. Irrespective, more work is needed—

particularly experimental designs that consider phenotypic and genetic differences across wild steelhead stocks. The intermediate and delayed nature of the mortality syndrome in steelhead and Chinook (relative to coho) also raises the potential significance of sublethal toxicity. The recent discovery of 6PPD-q should expedite functional studies of neurobehavioral and cardiorespiratory dysregulation, to match the consistently dominant features of the distress phenotype (e.g., surface swimming and gaping, loss of orientation and equilibrium).

Our findings also directly address the potential role of dilution in receiving waters, as a conventional management strategy for reducing toxic impacts to aquatic communities. Recent laboratory study designs^{14–17} have used urban runoff from a relatively intensively trafficked arterial, in part as a strategy to establish a clear baseline of toxicity in fish and invertebrates prior to green infrastructure treatments to evaluate pollutant removal and improved organismal health. While this approach was useful for challenging bioinfiltration soil systems,^{16,17} exposures to undiluted arterial roadway runoff are not necessarily representative of receiving water quality conditions in large rivers or lakes or in habitats downstream from a site-specific stormwater discharge. Field surveys have consistently demonstrated high rates of coho spawner mortality across all urban watersheds where the phenomenon has been studied closely.¹¹ Therefore, a protective role for dilution has (to date) seemed unlikely, given these indirect lines of evidence.^{10,11} Consistent with this, arterial runoff was acutely lethal to juvenile coho salmon, even when diluted in 95% clean water. Additional factors working against “dilution as the solution” to the urban runoff mortality syndrome include the ubiquitous and diffuse nature of stormwater inputs to salmon habitats (e.g., serial and repeated discharges along migration corridors), ongoing climate change (i.e., reduced receiving water volumes), and the possibility of sublethal toxicity at the lower end of the dilution exposure range examined here. To address these factors, future studies can focus on a few related chemicals (antiozonants and associated transformation products, as opposed to whole urban runoff), using an extensive and established set of tools for studying cardiorespiratory and neurobehavioral toxicity in fish (e.g., Blair et al.²³). These studies are needed to more precisely define sublethal toxicity thresholds in ESA-listed steelhead and Chinook and to better understand what appear to be striking sensitivity differences across closely related species of salmon, trout, and char.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acs.estlett.2c00467>.

Table S1: All data for the species comparison exposure studies. Table S2: All data collected for the exposure studies evaluating dilution effect. Table S3: Storm information and water quality measurements. Table S4: Information related to the fish such as age, size, and hatchery of origin. (XLSX)

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Notes

The authors declare no competing financial interest.

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