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Severe Coal Tar Sealcoat Runoff Toxicity to Fish Is Prevented by Bioretention Filtration

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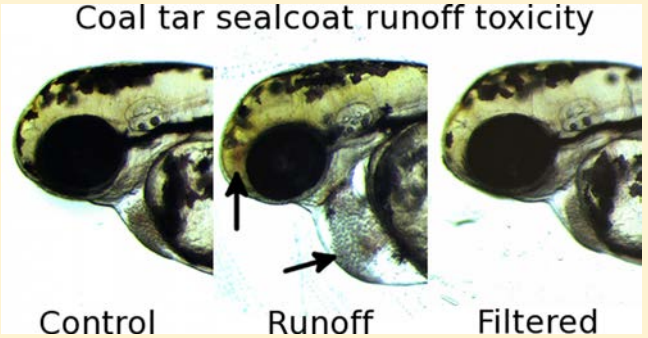
Supporting Information

ABSTRACT: Coal tar sealcoats applied to asphalt surfaces in North America, east of the Continental Divide, are enriched in petroleum-derived compounds, including polycyclic aromatic hydrocarbons (PAHs). The release of PAHs and other chemicals from sealcoat has the potential to contaminate nearby water bodies, reducing the resiliency of aquatic communities. Despite this, relatively little is known about the aquatic toxicology of sealcoat-derived contaminants. We assessed the impacts of stormwater runoff from sealcoated asphalt on juvenile coho salmon (*Oncorhynchus kisutch*) and embryo-larval zebrafish (*Danio rerio*). We furthermore evaluated the effectiveness of bioretention as a green stormwater method to remove PAHs and reduce lethal and sublethal toxicity in both species. We applied a coal tar sealcoat to conventional asphalt and collected runoff from simulated rainfall events up to 7 months postapplication. Whereas sealcoat runoff was more acutely lethal to salmon, a spectrum of cardiovascular abnormalities was consistently evident in early life stage zebrafish. Soil bioretention effectively reduced PAH concentrations by an order of magnitude, prevented mortality in juvenile salmon, and significantly reduced cardiotoxicity in zebrafish. Our findings show that inexpensive bioretention methods can markedly improve stormwater quality and protect fish health.



INTRODUCTION

Coal tar pitch is a byproduct of coking coal for the production of aluminum, commercial carbon, and graphite. Pitch is used in coal tar sealcoats to uniformly color and protect asphalt driveways, parking lots, and playgrounds, predominantly in central and eastern North America. Polycyclic aromatic hydrocarbons (PAHs) are major chemical components of both coal tar pitch and commercial sealcoat products. Stormwater runoff from treated surfaces mobilizes dissolved-phase PAHs[1] as well as particulate-associated PAHs created by gradual vehicle traffic wear.[2] This nonpoint source pollution can contribute substantially to the loading of PAHs and other chemicals to aquatic habitats.[3]

Many PAHs, including four and five ring compounds, are known to be carcinogenic to vertebrates following prolonged environmental exposures.[4] In fish, occurrence and severity of liver lesions have been used as visible morphological indicators of sediment PAH toxicity[5] and health improvement following habitat remediation.[6] Such histopathological injury determinations typically complement conventional biochemical and molecular indicators of PAH exposure, such as the aryl hydrocarbon receptor (AhR)-mediated upregulation of the protective cytochrome P4501A gene (*cyp1a*) or protein (CYP1A) in liver and other tissues.[7,8]

Research on zebrafish (*Danio rerio*) shows that tricyclic PAHs, such as phenanthrene, cause acute cardiovascular toxicity in fish embryos on an exposure time scale of several hours. Compound-specific cardiac abnormalities were initially identified in the developing hearts of early life stage zebrafish.[9] Subsequent studies, predominately on crude oil, have shown that oil-derived PAH mixtures dominated by 2- and 3-ring

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Table 1. Characteristics of Each Simulated Rainfall Event

event	date	simulated rain (cm)	days post-CTSC	antecedent dry period[a] (days)	interval rainfall (cm)	UV index[b]
1	9/19/2013	0.4	0	14	not applicable	4.87
2	9/26/2013	0.4	7	1	3.0	3.58
3	10/2/2013	0.4	13	1	9.6	3.18
4	4/14/2014	0.4	207	6	55.4	5.42
asphalt	9/2/2014	0.4	n.a.	40	24.6	6.40

[a]Days since ≥0.1″ precipitation. [b]For Seattle, WA (58 km from study site); www.cpc.ncep.noaa.gov

compounds disrupt heart development in a wide range of fish species, from cold-adapted Pacific salmon in fresh water[10] to tunas, mahi, and other large subtropical marine predators.[11,12] At a cellular level, this cardiotoxicity is caused by a disruption of excitation-contraction coupling in individual heart muscle cells (cardiomyocytes[13]), a physiological cascade that is highly conserved across vertebrate species. Overall, the developing heart appears to be the most sensitive target organ for tricyclic PAH toxicity in fish.[14,15]

An increasingly mechanistic understanding of PAH toxicity can be used to evaluate the effectiveness of green stormwater infrastructure (GSI), an evolving set of clean water technologies designed to reduce flooding and minimize the impacts of urban stormwater runoff on fish and other aquatic species.[16] In ecological terms, GSI installations are intended to promote biological diversity and resiliency in aquatic communities. This includes watersheds in the built environment and those that are vulnerable to future development and associated changes in land cover. Soil infiltration, or bioretention, is one of the most commonly used methods for treating stormwater.[17]

Filtration through experimental bioretention columns was recently shown to be highly effective in terms of preventing the acutely lethal effects of untreated urban highway runoff on salmon[18,19] and macroinvertebrates.[18] The zebrafish model for studying mechanisms of toxicity,[20] particularly at the embryonic and early larval life stages, has also been used to assess the protectiveness of bioretention.[21] Zebrafish have been a platform for studying the developmental toxicity of PAHs with three,[9] four,[22] and five[23] rings, as well as complex mixtures,[24−26] and are therefore particularly useful for studying GSI treatment effectiveness. In addition to well-described anatomical defects, this includes the availability of molecular markers for PAH exposure and cardiotoxicity.[27]

The present study had two aims. First, we characterized the lethal and sublethal toxicity of untreated runoff from an experimental plot treated with coal tar sealcoat, focusing on the health of juvenile coho salmon and early life stage zebrafish. The sealcoated plot was subject to natural sunlight and rainfall, and we simulated four storm events at intervals spanning seven months. Second, we assessed whether soil bioretention can reduce or eliminate adverse impacts to fish. This approach allowed us to evaluate the performance of an inexpensive GSI treatment method, challenged with an urban stormwater source atypically enriched in PAHs, using assessment end points that are both ecologically relevant (salmon survival) and extremely sensitive (zebrafish cardiac development).

MATERIALS AND METHODS

Coal Tar Sealcoat Application. A leaf blower and broom were used to clean debris from an extant plot of asphalt (110 m^2; Figure S1) on the Washington State University Puyallup campus (WSU-P; Puyallup, WA). Stormwater flowed off the experimental plot across a slight grade to a concrete catch basin at the lowest elevation. Prior to sealcoat application, the basin was pressure washed and the outflow was fitted with a PVC cap to facilitate runoff collections during simulated rainfall events. A commercial coal tar sealcoat (SealPave Driveway Sealer; Surface Coatings Company, Auburn Hills, MI) was applied per the manufacturer instructions using a sealcoat recirculating spray system (model ASD-PBK; Asphalt Spraycoating Direct; Douglass, KS) to ensure a well-mixed solution. The sealcoat contained 30−33% coal tar pitch, 17−20% clay, and 45−50% water (Figure S2). Application took place on September 19, 2013 at 1000 h (17 °C, 56% humidity, 0% cloud cover) following a 14-day antecedent dry period. Following manufacturer instructions, each spray pass was overlapped 50%. A single application was used because of relatively low vehicle density at this site, measured by a traffic counter (PicoCount 2500; Pico Counter, St. Louis Park, MN; 68 vehicles per day). The first simulated rain event followed the minimum recommended drying period (2 h).

Rainfall Simulation. Simulated runoff events were conducted on days without precipitation, with antecedent dry periods of 1−40 days (Table 1). A total of four rain events were simulated over a seven month period (Events 1−4), followed by one event on the adjacent asphalt-only control plot (Table 1). Rainfall in the interval between events ranged from 0.6 cm/d for the asphalt-only event to 9.6 cm/d for Event 3. Given changing seasons and variable cloud cover, the UV index on event days ranged from 3.2 to 6.4 (Table 1). A custom sprinkler system was installed above the coated surface to simulate rainfall events. Dechlorinated municipal water was pumped from a high-density polyethylene tote to three parallel lines that were perpendicular to the slope of the plot (Figure S1). Full coverage of the plot with minimum overlap was achieved with a combination of individually manipulated misters and revolving sprinkler heads (DripWorks, Inc., Willits, CA) spaced at 1.5 m intervals along the three lines. A total of 300 L of simulated rainfall was delivered at a rate of 0.25 cm/h. Runoff collected in the catch basin was pumped into a stainless steel tote (MetalCraft Inc., Springfield, MO) using Teflon-lined hoses (HoseCraft USA, Chicago, IL) attached to a Pentair magnetic drive pump (W30HDX; Aquatic Eco-Systems). The runoff was then homogenized within the tote using a pedestal-mount agitator (5000-series; Dynamix Agitators, Richmond, BC, Canada), after which 114 L was divided between six glass carboys. The remaining runoff was filtered through six soil bioretention columns (see below).

Bioretention Treatment. The soil bioretention system consisted of six PVC columns (36 cm diameter). At the base of each column was a 30 cm gravel aggregate drainage layer containing a slotted under-drain (5 cm PVC). The drainage layer was overlain by 60 cm of bioretention soil medium, a mixture of 60% sand and 40% compost (Cedar Grove, WA), topped with 5 cm of bark mulch. Materials were tamped down with uniform pressure every 15 cm during construction. The

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columns were conditioned prior to the experiment to settle the component materials and test prerunoff leaching with three pore volumes of dechlorinated city water. Each 21 L pore volume was applied at 12 L/h, and the effluent was collected from each under-drain into glass carboys for subsequent chemistry analyses for conventional water parameters, dissolved metals, and nutrients.

Following homogenization, simulated rainfall runoff was pumped from the stainless steel tote to the six columns, with each column receiving 22 L runoff at a rate of 12 L/h. Column effluents were collected in glass carboys on ice and then added to 35 L glass aquaria (two carboys per aquarium, triplicate treatments per simulated runoff event). Water from each aquarium was subsampled for analytical chemistry and zebrafish exposures before the onset of toxicity assays with juvenile coho salmon.

Chemical Analyses. One water sample was taken from each triplicate aquaria for analyses of pH, alkalinity, hardness, total suspended solids (TSS), total and dissolved organic carbon, Ca^{2+} and Mg^{2+} ions, nutrients (ammonia, nitrate, orthophosphate, and total phosphorus), dissolved and total metals (Zn, Cu, Ni, Cd, Pb), and PAHs (listed in Table S1). Samples for PAH quantification were placed in amber glass bottles, preserved with 10% methylene chloride, transported on ice to NOAA's Northwest Fisheries Science Center (Seattle, WA) and stored at 4 °C until analysis by GC-MS using established protocols.[28] All other samples were transported on ice within 24 h to outside laboratories for analyses using conventional EPA methods. These included Analytic Resources Inc. (Tukwila, WA) for water samples collected during column conditioning or AmTest Laboratories (Kirkland, WA) for all waters used in toxicity assays. Metals were analyzed by inductively coupled plasma-mass spectrometry (ICP-MS). The QA/QC standards for each analytical method were met for all samples. Water samples for zebrafish exposures (below) were composited across aquaria within each treatment and frozen in glass until testing. We have previously shown that this method of sample storage (−20 °C) does not modify the toxicity of untreated urban runoff to embryo-larval zebrafish.[21]

Juvenile Coho Exposures. Soos Creek juvenile coho salmon (*Oncorhynchus kisutch*) were obtained from the Northwest Fisheries Science Center's hatchery facility and maintained at the WSU-P fish lab on a 12:12 h light:dark regime at 13 °C. The first three simulated rainfall events (Events 1−3) used coho hatched in 2013. The coho for Event 4 and the asphalt-only test hatched in 2014. Average coho size (length and weight) for each test is reported in Table S2.

Test aquaria were placed in random pairs in water baths. When thermal equilibrium (13 °C) was reached, 10 juvenile coho were added to each aquarium. Water temperature, pH, dissolved oxygen (DO), and conductivity were monitored prior to fish addition, and fish behavior and survival were monitored along with water quality every 12−24 h after test initiation. Oxygen was provided with air stones, and temperature, pH, and DO remained within values appropriate for husbandry for the duration of each test. After 96 h, survivors were euthanized with an overdose of MS-222 per an experimental protocol (#00435-001) approved by Washington State University's Institutional Animal Care and Use Committee (IACUC). Control survival was always 100%.

Zebrafish Exposures. Zebrafish were maintained in a breeding colony at the Northwest Fisheries Science Center according to established animal care and husbandry procedures.[29] Toxicity tests were conducted as previously described.[21] Briefly, eggs were produced by spawning pairs of adult (AB) zebrafish, collected midmorning, and embryos were sorted for viability by 2.5 h post fertilization (hpf). Triplicate groups of viable embryos (n = 15 per group) were exposed in glass Petri dishes (15 × 6 mm) containing 10 mL of embryo system water (controls) or runoff, pre- or post-treatment with bioretention. Solutions were renewed daily. At 48 h, embryos were anaesthetized in MS-222, digitally imaged and morphological measurements (embryo length, eye area, and pericardial area) made in ImageJ as previously described.[21,22] Representative images of cardiovascular abnormalities are shown in Figure S3. Embryos with fluid accumulation in the pericardial region were scored as having pericardial edema. Blood accumulated in the head region was scored as cranial hemorrhaging. Blood accumulation caudal and ventral of the pericardium was scored as common cardinal vein (CCV) pooling. Control embryo survival was >90% (93−100%) with low cardiovascular abnormalities (0−8%, $\bar{x}$ < 3%).

Molecular Indicators of PAH Exposure and Cardiotoxicity. Cytochrome P4501A (*cyp1a*) encodes a key component of the AhR-mediated PAH metabolism pathway[30] and was used as an indicator of PAH exposure from sealcoat runoff. B-type natriuretic peptide (*nppb*) is a small polypeptide hormone that is synthesized and secreted by atrial and ventricular cardiomyocytes of vertebrate hearts during cardiogenesis.[31] It is highly responsive to cardiac stress, preventing cardiomyocyte hypertrophy through inhibition of calcium (Ca^{2+}) influx.[32] In 48 hpf zebrafish embryos, *nppb* is expressed throughout the ventricle and in the outer curvature myocardium of the atrium.[31] *Nppb* was targeted as a candidate molecular indicator of cardiotoxicity given its cardiac-specific expression, previous incorporation in a luciferase transgenic reporter line for zebrafish hypertrophic cardiomyopathy,[31] and established use as cardiac stress and cardiac failure biomarker in humans.[33]

RNA Extraction, cDNA Synthesis, and Quantitative PCR. RNA extraction, cDNA synthesis, and quantitative PCR (qPCR) analysis were conducted as previously described for zebrafish early life stages.[27] Briefly, RNA was extracted in TriZol (Ambion, Inc.) by mechanical homogenization of snap-frozen larvae (n = 25−30) using stainless steel beads (Qiagen, Inc.) and TissueLyser (Invitrogen, Inc.), and subsequently purified and DNase treated using Zymo DirectZol columns (Zymo Inc.). First strand cDNA was synthesized from 2 μg of total RNA using High Capacity RNA-to-cDNA kit (Life Technologies Inc.). qPCR reactions were run in duplicate 20 μL reactions containing Fast SYBR Green chemistry (Applied Biosystems Inc.), 250 nM each primer, and 4 ng cDNA template on a Viia7 Real-Time qPCR Detection System (Applied Biosystems Inc.) using manufacturer's fast-cycling condition (95 °C for 2 min, followed by 40 cycles of 95 °C for 1 s and 60 °C for 20 s). Dissociation curves were generated as the terminal step of all qPCR reactions to verify single product amplification. Previously published qPCR primers were used to quantify *nppb*[31] and *cyp1a*[34] expression. We used WD and tetratricopeptide repeats 1 (*wdtc1*) as a reference gene. Although uncharacterized during zebrafish embryogenesis, *wdtc1* is expressed in cardiac tissue of humans[35] and 1-month-old juvenile zebrafish (data not shown). Primers for qPCR quantification of *wdtc1* (F: 5′-GCAGCGCTCT-TCTCCAAAAC-3′, R: 5′-CGACTCCTTCCGGCTGAAAT-3′) were designed using Primer3[36] and verified by Primer-BLAST.[37] All primer pairs demonstrated acceptable efficiency

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(90−105%)[38] and relative expression for *nppb* and *cyp1a* were normalized to the expression of *wdtc1*,[39] which was unperturbed by exposure to coal tar sealcoat runoff (one-sample z test; $Z(34) = 0.613$, $p = 0.272$).

Statistical Analyses. For each coal tar sealcoat runoff event, significant differences in metrics among treatments (control water, unfiltered sealcoat runoff, filtered sealcoat runoff) were assessed using a multivariate general linear model (GLM). This is a multivariate analysis of variance that allows for categorical independent variables. For tests with significant differences among treatments, a Dunnett posthoc analysis compared metrics for fish in unfiltered runoff and filtered runoff to clean water controls. For the asphalt-only runoff test, runoff was not filtered; therefore *t* tests were used to compare end points for unfiltered runoff to clean water controls. Survival and morphometric measurements were expressed relative to controls; for each event, interval data (survival, length, eye size, pericardial area) for runoff-exposed embryos were divided by the control mean, whereas for ratio data (proportion of embryos with cardiovascular abnormalities, pericardial edema, cranial hemorrhaging, or CCV pooling) the control mean was subtracted from the values for runoff-exposed. Gene expression results (fold-change relative to controls) were $\log_2$-transformed prior to analysis. All statistics were performed in SPSS v. 22 (IBM) with a significance value of $\alpha < 0.05$ unless otherwise stated.

RESULTS

Stormwater Chemistry. Measured concentrations for conventional water quality parameters, nutrients, and metals are reported in Table S2. Conditioning with clean water prior to runoff treatment flushed TSS and nutrients (nitrate, TP) from the bioretention columns. Nutrients remained elevated relative to the unfiltered coal tar runoff, but TSS was equivalent after Event 2 (Table S2). The column soils were a prolonged source of DOC, dissolved Cu, and dissolved Ni to the filtered runoff (Table S2). Dissolved Zn was elevated in the dechlorinated city water used to generate runoff (Table S2), but was reduced 60−88% in the resulting unfiltered runoff and was additionally reduced 70−90% by bioretention filtration.

For the first simulated storm event (Event 1; 2 h postapplication), untreated runoff from the coated plot contained 1311 μg/L total PAH (∑PAH). By Event 2 (7 days postapplication), PAH concentrations decreased by an order of magnitude to 145 μg/L ∑PAH. Thereafter, ∑PAH concentrations did not change appreciably for Event 3 (13 days postapplication) or Event 4 (207 days postapplication; Table S2). Total PAHs were another order of magnitude lower in runoff collected from the adjacent, unsealed asphalt plot (11 μg/L ∑PAH). Runoff from the sealcoated plot contained a higher proportion of the cardiotoxic tricyclic PAHs (37−57% of ∑PAH) relative to runoff from asphalt alone (23%) (Figure S4). In all cases, the tricyclic fraction was dominated by phenanthrene (Table S2). Filtering runoff from the sealcoated plot through the experimental soil columns reduced ∑PAH by >99% (Table S2).

Lethal Toxicity. Unfiltered runoff from coal tar sealcoat was highly toxic to juvenile coho salmon. The first runoff event (Event 1) was the most acutely lethal, with no coho surviving after 5 h of exposure (Figure 1). Mortality decreased substantially for subsequent simulated storms and remained significantly different from controls for Event 2 (20% mortality; $F(2,9) = 25.000$, Dunnett $p = 0.002$) and Event 4 (55%; $F(2,9) = 55.102$, $p < 0.001$), but not for Event 3 (10%; $F(2,9) = 3.000$, $p = 0.132$). Fish showed loss of equilibrium (Event 2; Video S1) and surface swimming/gaping (Event 4; Video S2) prior to mortality. In contrast, no control fish showed these symptoms. Mortality across different events was correlated with the proportion of tricyclic PAHs in the sealcoat runoff (Figure 1). In contrast, juvenile coho exposed to runoff from the adjacent unsealed asphalt plot showed no mortality during the 96-h exposure (Figure 1). No mortality was observed among juvenile coho controls held in clean water from the WSU-P fish holding facility.

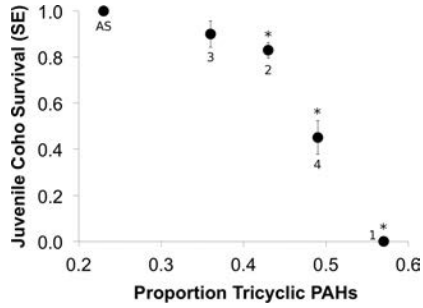

Figure 1. Proportion of tricyclic PAHs relative to mortality among juvenile coho salmon exposed to unfiltered runoff from the coal tar sealed (Event 1−4) or asphalt-only plot (AS). Asterisks indicate survival values significantly different from controls.

Untreated runoff from Event 1 was also acutely lethal to zebrafish embryos, resulting in 100% mortality within 48 h. When Event 1 stormwater was diluted in series, the median lethal dilution for 50% zebrafish embryo mortality was 34% ($\chi^2(25) = 89.8$, $p < 0.001$; Figure S5). No significant mortality relative to controls was observed for zebrafish exposed to unfiltered runoff from Events 2−4 or the asphalt runoff ($F(3,12) = 3.266$, $p = 0.080$). Control survival was >92% for each exposure.

Sublethal Toxicity. Sublethal toxicity was assessed in zebrafish exposed for 48 h during embryonic development. Unfiltered coal tar sealcoat runoff caused a reduction in embryo length and eye size (microphthalmia), and resulted in a range of visible cardiovascular abnormalities including pericardial edema, CCV pooling, and cranial hemorrhaging. Serial dilutions of unfiltered Event 1 runoff were also sublethally toxic ($F(4,14) = 25.913−79.256$, $p < 0.001$) to zebrafish embryos (Figure S6), with thresholds of 25% runoff (Dunnett posthoc, $p < 0.001$) for length, 12% for pericardial area ($p = 0.038$), 12% for cardiac abnormalities ($p = 0.044$), and 3% for eye area ($p = 0.028$). At quarter-strength runoff (25%), the most common cardiovascular defects among abnormal embryos were pericardial edema (85%), cranial hemorrhaging (53%) and CCV blood pooling (24%). At 12% runoff, the most prevalent defects were CCV blood pooling (87%) and pericardial edema (78%). Embryos that survived exposure to half-strength (50%) Event 1 runoff were severely developmentally delayed with prominent visible evidence of heart failure (severe edema).

The runoff from subsequent simulated storms (Events 2−4) was not diluted. The collected stormwater from all three events caused expected abnormalities (Figure 2), including small eyes ($F(2,6)$ for Events 2−4 = 5.792−17.906, $p = 0.003−0.040$, Dunnett posthoc $p = 0.002−0.036$), enlarged pericardial area ($F(2,6) = 45.271−834.441$, $p \leq 0.002$; Dunnett $p \leq 0.002$), and cardiac abnormalities ($F(2,6) = 20.115−442.654$, $p \leq 0.002$, Dunnett $p \leq 0.002$). Cranial hemorrhaging was the most

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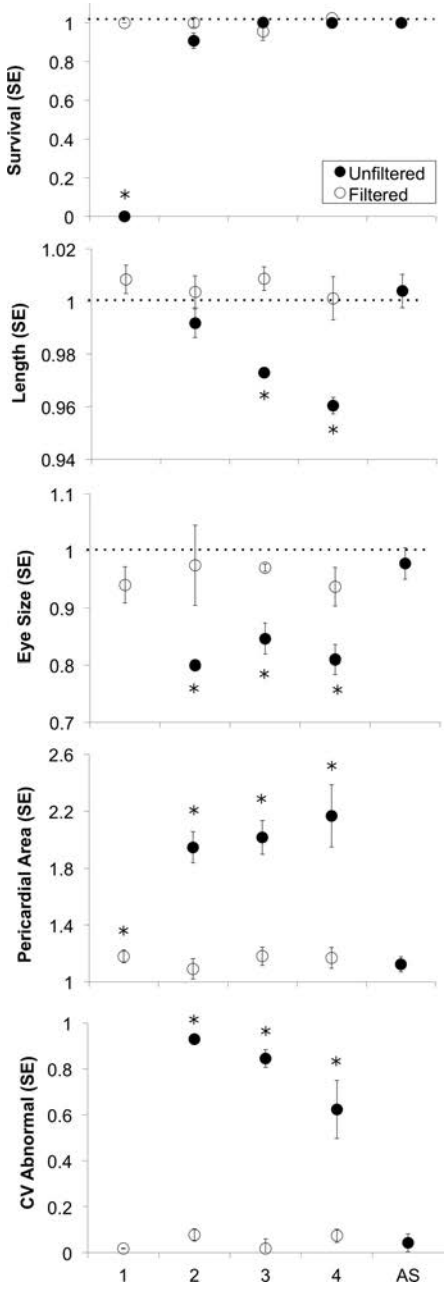

Figure 2. Survival and morphological defects in zebrafish embryos exposed to unfiltered coal tar sealcoat runoff (Events 1−4; closed circles) or the same runoff after filtration through experimental soil columns (open circles). Bioinfiltration substantially reduced all forms of visible toxicity, and in most cases embryos were indistinguishable from those exposed to runoff from the adjacent untreated asphalt plot (closed circle, AS). Values are relative to clean water controls. A dashed line indicates the control mean when the *x*-axis does not. Error bars are ± SEM. Asterisk is significantly different from controls at $p < 0.05$. CV = cardiovascular.

prevalent cardiovascular abnormality in embryos exposed to unfiltered runoff from Event 2 (67%) and Event 3 (72%), but was equally as prevalent (39%) as pericardial edema (35%) in Event 4. Significant reductions in length were evident following exposure to unfiltered runoff from Event 3 ($F(2,6) = 202278.130$, $p \leq 0.001$; Dunnett $p = 0.004$) and Event 4 ($F(2,6) = 14.847$, $p = 0.005$; Dunnett $p = 0.006$), but not Event 2 ($F(2,6) = 1.453$, $p = 0.306$). Runoff from the asphalt-only plot did not affect lethal or sublethal metrics (Figure 2).

Transcriptional Responses. Molecular markers for exposure to PAHs (*cyp1a*) and cardiac stress (*nppb*) were significantly induced in zebrafish exposed to unfiltered sealcoat runoff from each Event (Table 2). *Cyp1a*, expressed throughout the embryo, responded to diluted runoff from Event 1 at concentrations of 6% runoff and greater ($F(4,25) = 68.973$, Dunnett $p \leq 0.001$), with 128-fold upregulation at the highest concentration tested (25% runoff; Figure 3). High mortality rates prevented collecting sufficient embryos for molecular analysis at higher concentrations of Event 1 runoff (Figure S5). Exposure to unfiltered runoff from Events 2−4 also significantly induced *cyp1a* (65.3−97.2-fold; $F(2,15) = 170.696-418.590$; Dunnett $p < 0.001$). Runoff from the asphalt-only plot also caused significant induction of *cyp1a* (3.7-fold; $t(6) = -4.267$, $p = 0.005$), albeit at lower levels than unfiltered runoff from the coal tar sealcoat plot (>60-fold induction; Table 2).

Cardiac-specific *nppb* was significantly induced in 25% Event 1 runoff ($F(4,25) = 8.722$, Dunnett $p = 0.001$), but was not significantly induced at lower concentrations ($p = 0.528-0.927$) despite visible cardiovascular toxicity (Figure S6). Exposure to unfiltered runoff from subsequent events significantly upregulated *nppb* (1.9−6.3-fold; $F(2,15) = 8.384-117.172$, Dunnett $p \leq 0.048$). *Nppb* was also mildly upregulated in embryos exposed to asphalt-only runoff (1.8-fold; $t(6) = -3.04$, $p = 0.023$), similar to that for embryos exposed to unfiltered Event 4 runoff (Table 2).

Bioretention Filtration. Bioretention treatment prevented all mortality in both juvenile coho and developing zebrafish. Moreover, stormwater infiltration reversed nearly all visible signs of sublethal toxicity in zebrafish embryos (Figure 2). The one exception was filtered runoff from Event 1, which caused mild pericardial edema ($F(1,6) = 13.583$, Dunnett $p = 0.021$; Figure 2). At a molecular level, soil bioinfiltration did not completely remove all AhR agonists from the chemical mixture, as evidenced by a modest (3.5−12.1-fold) but significant ($p \leq$

Table 2. Transcriptional Response (Fold-Increase) Of Genes Responsive to Contaminant Exposure (*cyp1a*) or Cardiac Stress (*nppb*) in Zebrafish Embryos Exposed to Unfiltered or Filtered Coal Tar Sealcoat Runoff (Event 1-4) or unfiltered Asphalt-Only (AS) Runoff for 48 h[a]

gene/Event	unfiltered	filtered
cyp1a		
1	128.0*[bc]	8.3*
2	97.2*	3.7*
3	76.3*	4.0*
4	65.3*	3.2*
AS	3.7*	na
nppb		
1	4.0*[b]	1.0
2	4.3*	1.3*
3	6.3*	1.0
4	1.9*	0.7
AS	1.8*	na

[a]All values are relative to clean water controls. na, not applicable; asphalt-only runoff was not filtered. [b]response at 25% runoff for unfiltered Event 1; all others are 100%. [c]asterisks indicate significantly different from control.

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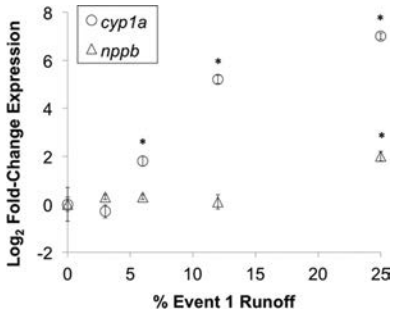

Figure 3. *Cyp1a* and *nppb* induction in 48 hpf zebrafish embryos exposed to dilutions of Event 1 runoff throughout embryogenesis (0–48 h). Expression is relative to controls, with asterisks indicating a significant upregulation of gene expression. Error bars are ± SEM.

0.001) induction of *cyp1a* in embryos exposed to filtered runoff from Events 1–4 (Table 2). The corresponding marker for cardiac stress, *nppb*, was also mildly (1.3-fold) but significantly (p = 0.030) induced in filtered runoff from Event 2 (Figure 3), but not from Events 1 (p = 0.944), Event 3 (p = 0.975), or Event 4 (p = 0.325). Overall, pretreating sealcoat runoff with bioretention filtration prevented most adverse health impacts in salmon and zebrafish.

DISCUSSION

The use of coal tar-based sealcoat products on roadways and other impervious surfaces has been controversial in recent years. Throughout the U.S., concerns over potential adverse impacts to aquatic life have led to bans at the state (Washington and Minnesota) and local levels (e.g., cities and counties in seven states). However, only a few studies have assessed the extent to which chemicals are mobilized in runoff from coated surfaces following a real-world application. We have shown here that initial runoff from newly coated asphalt was 100% lethal to juvenile coho salmon and zebrafish embryos. While consistent with a very high total PAH (∑PAH) concentration of 1311 ug/L, sealcoat runoff is a complex chemical mixture and we cannot rule out a role for other, as-yet unidentified contaminants in the observed mortality. These findings complement and extend similar results for larval fathead minnows (*Pimephales promelas*) and daphnids (*Ceriodaphnia dubia*), which were also unable to survive exposures to runoff from freshly coated asphalt (5 h postapplication, 357 ug/L ∑PAH[40]). Therefore, fresh coal tar sealcoats are a source of bioavailable and highly toxic chemical contaminants in urban stormwater runoff.

We also monitored the health impacts of sealcoat runoff up to seven months after application. Although lethality is a relatively insensitive biological end point, we nevertheless observed significant salmon mortality following runoff exposures at 7 days (17%) and 207 days (55%) postsealcoat application. This is similar to the mortality documented among fathead minnows and daphnids exposed to runoff up to 36 days postapplication.[40] Across events, juvenile coho mortality was related to the relative enrichment of tricyclic (3-ring) compounds in the PAH mixture, with death occurring when the proportion of tricyclics exceeded 40%. These runoff samples were dominated by phenanthrene, a well-known cardiotoxic agent in fish.[9] Moreover, phenanthrene is substantially more acutely toxic to fish than five other aromatic hydrocarbons found in coal tar-based seal coat runoff,[41] including the azaarenes quinoline and acridine. Nevertheless, as noted above, coal tar-based sealcoat contains hundreds or even thousands of different chemicals, including O-, N-, and S-substituted heterocycles that can contribute significantly to aromatic hydrocarbon toxicity.[42] The correlation between tricylic PAH concentration and juvenile salmon mortality does not preclude a causal role for one or more of these unidentified agents.

The elevated salmon mortality at 207 days in our study may be attributable to photomodification of aromatic hydrocarbons in coal tar sealcoat runoff. Whereas unfiltered runoff for Event 4 had similar ∑PAH concentrations as Events 2–3, the toxicity associated with Event 4 to salmon was relatively higher. In addition to a higher proportion of tricyclic PAHs, Event 4 had a higher UV index during runoff collection compared to Events 2–3. Under UV radiation, PAHs in aqueous solutions can be oxidized to new and more toxic chemical species, both before[43] and after[44−46] uptake by cells. Consistent with this, UV treatment significantly increased mortality in day-old fathead minnow exposed for 48 h to a nonlethal coal tar sealcoat runoff - even months after sealcoat application.[40] Similarly, runoff samples that were not genotoxic to rainbow trout liver cells induced DNA damage following brief coexposure with UV.[47] The role of UV in exacerbating sublethal toxicity of coal tar sealcoat runoff to developing fish has yet to be directly addressed.

We used zebrafish to explore sublethal toxicity because of many previous studies on PAH-induced ontogenetic defects in this model experimental species. Unlike the mortality end point, indicators of PAH-driven developmental cardiotoxicity are very sensitive in zebrafish.[14] PAHs derived from weathered crude oil block excitation-contraction coupling in isolated fish cardiomyocytes, with blocking activity correlating to tricyclic PAH concentrations but not total PAHs.[13] Disruption of cardiomyocyte repolarization and internal calcium cycling is the likely cause of abnormalities in heart rhythm and contractility, respectively, that have been reported for a wide range of fish species exposed embryonically to petrogenic PAHs.[12,14] As expected from the relatively high measured concentrations of tricyclic PAHs, untreated sealcoat runoff produced a high incidence (>60%) of cardiovascular abnormalities in developing zebrafish, including pericardial edema, CCV pooling, and cranial hemorrhaging, as well as decreases in embryo length and eye size. These effects were observed across all simulated rain events on the coal tar sealcoat, even in the absence of significant mortality. Pericardial edema is a visible indication of heart failure,[24,48] and grossly abnormal fish are unlikely to survive to later life stages. Therefore, wild fish populations that spawn in water bodies receiving runoff from impervious surfaces coated with coal tar-based products are particularly at risk of developmental toxicity and delayed mortality.

Polycyclic aromatic hydrocarbons represent a predominant class of chemical contaminants in urban stormwater runoff. Independent of sealcoated surfaces, PAHs are ubiquitous in runoff from highways and other roadways where motor vehicles are significant sources. Major efforts are currently underway to identify clean water technologies that effectively remove PAHs and other contaminants (e.g., metals) from urban runoff. Filtration using bioretention has proven very effective at reducing both pollution loadings to receiving waters and protecting the health of aquatic biota.[18,19,21,27] A goal of the present study was to challenge conventional bioretention systems with relatively high concentrations of PAHs from sealcoat runoff. If our experimental columns proved effective under these circumstances, stormwater managers should have

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greater confidence that similar approaches will protect aquatic communities from conventional forms of urban runoff containing considerably lower concentrations of PAHs. Bioretention treatment reversed nearly all of the observed adverse health effects, including mortality in both species and sublethal effects in zebrafish. This high degree of protection was conveyed across four sequential treatments using the same soil media. Hydrocarbons are generally retained in the top 10 cm of bioretention soils[17,49] where they are biodegraded by microbial communities.[50] Degradation rates vary with microcosm conditions such as temperature, moisture, and microbial community composition.[51−53] However, rates of hydrocarbon degradation generally exceed input rates.[50] In the present context, our biological indicators of fish health support this general assumption, even when PAH inputs to soil mixtures are exceptionally high.

Lastly, our molecular biomarker results are an example of the usefulness of biologically based screening approaches for assessing clean water technology effectiveness. Molecular indicators of PAH exposure (*cyp1a*) and cardiac stress (*nppb*) were significantly elevated in fish exposed to unfiltered coal tar sealcoat runoff, as well as runoff from the asphalt-only plot, and were significantly reduced following bioretention treatment. The observed *cyp1a* induction was expected given the presence of AhR-activating PAHs in both coal tar sealcoat and asphalt-only runoff, the latter presumably originating from motor vehicles. Coal tar sealcoat also releases azaarenes[1]-nitrogen-substituted heterocyclic aromatic hydrocarbons that are also cardiotoxic[54] and induce *cyp1a*.[55] The P450 pathway is extremely sensitive to aromatic hydrocarbon exposure.[30] Exposure to low concentrations of *cyp1a*-inducing compounds was evident in the absence of most visible signs of toxicity in the asphalt-only runoff and bioretention-filtered coal tar runoff. Similar results were found for zebrafish embryos exposed to urban highway runoff after treatment with bioretention filtration,[27] representing breakthrough of low concentrations of mobile aromatic hydrocarbons or other AhR agonists.

While indicating exposure to aromatic hydrocarbons, *cyp1a* induction is not necessarily correlated with toxicity[56] because not all *cyp1a*-inducers are cardiotoxic. For example, chrysene is a strong AhR agonist,[57] but is not toxic to fish either before[9] or after UV exposure.[45] In contrast, the natriuretic peptide *nppb* is a molecular marker for cardiac stress in zebrafish[31] and cardiac failure in humans.[58] Moreover, *nppb* correlates with the incidence of cardiovascular abnormalities in zebrafish embryos exposed to urban highway runoff.[27] We found that *nppb* was also significantly induced in zebrafish embryos exposed to cardiotoxic coal tar sealcoat runoff. In 75% of samples, *nppb* induction corresponded with visible cardiovascular toxicity. However, *nppb* may not be more sensitive than visual indicators of cardiotoxicity for complex environmental mixtures enriched in PAHs; for example, fish exposed to 12% runoff from Event 1 had significant cardiovascular abnormalities (39% of embryos), but no *nppb* induction despite similar ∑PAH as Events 2−4. More work is needed to understand how this cardiac stress marker relates to cardiac injury in fish exposed to complex chemical mixtures.

In summary, our findings add to a growing body of evidence that coal tar-treated surfaces are sources of toxic contaminants in stormwater for months following application. As anticipated from previous studies, the developing zebrafish cardiovascular system was exceptionally sensitive to degraded water quality in coal tar sealcoat runoff at the transcriptional, anatomical, and functional scales. Simple stormwater treatment methods based on bioinfiltration were highly effective in terms of reducing PAH loadings and reversing both lethal and sublethal toxicity to fish. Given the success of soil bioretention treatment under these high PAH input conditions, the approach holds considerable promise for more conventional forms of runoff in the built urban environment.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.5b04928.

Supporting Information includes lengths and weights of juvenile coho used in the experiments (Table S1), complete analytical chemistry results for conventional water quality parameters, nutrients, metals, and PAHs (Table S2), and a map of the sealcoated and asphalt-only plots (Figure S1). Figure S2 is the MSDS for the sealant used in the current study. Representative images of zebrafish embryos with cardiovascular abnormalities are shown in Figure S3. Figure S4 shows distribution of PAHs by number of aromatic rings for untreated runoff from each event. Also available are zebrafish embryo results for the dilution series of Event 1 runoff, including mortality (Figure S5), and sublethal metrics (Figure S6) (PDF). Videos are available (Video S1 (MOV) and S2 (MOV)) showing behaviors of affected juvenile coho exposed to coal tar sealcoat runoff.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Mahler, B.; Van Metre, P.; Foreman, W. Concentrations of polycyclic aromatic hydrocarbons (PAHs) and azaarenes in runoff from coal-tar- and asphalt-sealcoated pavement. *Environ. Pollut.* **2014**, *188*, 81−87.

(2) Mahler, B.; Van Metre, P.; Bashara, T.; Wilson, J.; Johns, D. Parking lot sealcoat: An unrecognized source of urban polycyclic aromatic hydrocarbons. *Environ. Sci. Technol.* **2005**, *39* (15), 5560−5566.

(3) Yang, Y.; Van Metre, P.; Mahler, B.; Wilson, J.; Ligouis, B.; Razzaque, M.; Schaeffer, D.; Werth, C. Influence of coal-tar sealcoat and other carbonaceous materials on polycyclic aromatic hydrocarbon loading in an urban watershed. *Environ. Sci. Technol.* **2010**, *44* (4), 1217−1223.

DOI: 10.1021/acs.est.5b04928
Environ. Sci. Technol. 2016, 50, 1570−1578

(4) Xue, W.; Warshawsky, D. Metabolic activation of polycyclic and heterocyclic aromatic hydrocarbons and DNA damage: A review. *Toxicol. Appl. Pharmacol.* **2005**, *206* (1), 73−93.
(5) Myers, M.; Johnson, L.; Collier, T. Establishing the causal relationship between polycyclic aromatic hydrocarbon (PAH) exposure and hepatic neoplasms and neoplasia-related liver lesions in English sole (*Pleuronectes vetulus*). *Hum. Ecol. Risk Assess.* **2003**, *9* (1), 67−94.
(6) Myers, M.; Anulacion, B.; French, B.; Reichert, W.; Laetz, C.; Buzitis, J.; Olson, O.; Sol, S.; Collier, T. Improved flatfish health following remediation of a PAH-contaminated site in Eagle Harbor, Washington. *Aquat. Toxicol.* **2008**, *88* (4), 277−288.
(7) Nisbet, I.; Lagoy, P. Toxic equivalency factors (TEFs) for polycyclic aromatic hydrocarbons (PAHs). *Regul. Toxicol. Pharmacol.* **1992**, *16* (3), 290−300.
(8) Malins, D.; Anderson, K.; Stegeman, J.; Jaruga, P.; Green, V.; Gilman, N.; Dizdaroglu, M. Biomarkers signal contaminant effects on the organs of English sole (*Parophrys vetulus*) from Puget Sound. *Environ. Health Perspect.* **2006**, *114* (6), 823−829.
(9) Incardona, J.; Collier, T.; Scholz, N. Defects in cardiac function precede morphological abnormalities in fish embryos exposed to polycyclic aromatic hydrocarbons. *Toxicol. Appl. Pharmacol.* **2004**, *196* (2), 191−205.
(10) Incardona, J.; Carls, M.; Holland, L.; Linbo, T.; Baldwin, D.; Myers, M.; Peck, K.; Tagal, M.; Rice, S.; Scholz, N. Very low embryonic crude oil exposures cause lasting cardiac defects in herring and salmon. *Sci. Rep.* **2015**, *5*, 13499.
(11) Incardona, J.; Gardner, L.; Linbo, T.; Swarts, T.; Esbaugh, A.; Mager, E.; Stieglits, J.; French, B.; Labenia, J.; Laetz, C.; Tagal, M.; Sloan, C.; Elizur, A.; Benetti, D.; Grosell, M.; Block, B.; Scholz, N. Deepwater Horizon crude oil toxicity to the developing hearts of large predatory pelagic fish. *Proc. Natl. Acad. Sci. U. S. A.* **2014**, *111*, E1510−E1518.
(12) Edmunds, R.; Gill, J.; Baldwin, D.; Linbo, T.; French, B.; Brown, T.; Esbaugh, A.; Mager, E.; Stieglits, J.; Hoenig, R.; Benetti, D.; Grosell, M.; Scholz, N.; Incardona, J. Corresponding morphological and molecular indicators of crude oil toxicity to the developing hearts of mahi mahi. *Sci. Rep.* **2015**, *5*, 17326.
(13) Brette, F.; Machado, B.; Cros, C.; Incardona, J.; Scholz, N.; Block, B. Crude oil impairs cardiac excitation-contraction coupling in fish. *Science* **2014**, *343* (6172), 772−776.
(14) Scholz, N.; Incardona, J. In response: scaling polycyclic aromatic hydrocarbon toxicity to fish early life stages: a governmental perspective. *Environ. Toxicol. Chem.* **2015**, *34* (3), 459−61.
(15) Incardona, J.; Collier, T.; Scholz, N. Oil spills and fish health: exposing the heart of the matter. *J. Exposure Sci. Environ. Epidemiol.* **2011**, *21* (1), 3−4.
(16) Ahiablame, L.; Engel, B.; Chaubey, I. Effectiveness of low impact development practices: Literature review and suggestions for future research. *Water, Air, Soil Pollut.* **2012**, *223* (7), 4253−4273.
(17) Davis, A.; Hunt, W.; Traver, R.; Clar, M. Bioretention technology: Overview of current practice and future needs. *J. Environ. Eng.* **2009**, *135* (3), 109−117.
(18) McIntyre, J.; Davis, J.; Hinman, C.; Macneale, K.; Anulacion, B.; Scholz, N.; Stark, J. Soil bioretention protects juvenile salmon and their prey from the toxic impacts of urban stormwater runoff. *Chemosphere* **2015**, *132*, 213−219.
(19) Spromberg, J.; Baldwin, D.; Damm, S.; McIntyre, J.; Huff, M.; Sloan, C.; Anulacion, B.; Davis, J.; Scholz, N. Widespread coho salmon spawner mortality in western U.S. urban watersheds: Lethal storm-water impacts are reversed by soil bioinfiltration. *J. Appl. Ecol.* **2015**, DOI: 10.1111/1365-2664.12534.
(20) Hill, A.; Teraoka, H.; Heideman, W.; Peterson, R. Zebrafish as a model vertebrate for investigating chemical toxicity. *Toxicol. Sci.* **2005**, *86* (1), 6−19.
(21) McIntyre, J.; Davis, J.; Incardona, J.; Anulacion, B.; Stark, J.; Scholz, N. Zebrafish and clean water technology: Assessing soil bioretention as a protective treatment for toxic urban runoff. *Sci. Total Environ.* **2014**, *500−501*, 173−180.
(22) Incardona, J.; Day, H.; Collier, T.; Scholz, N. Developmental toxicity of 4-ring polycyclic aromatic hydrocarbons in zebrafish is differentially dependent on AH receptor isoforms and hepatic cytochrome P4501A metabolism. *Toxicol. Appl. Pharmacol.* **2006**, *217* (3), 308−321.
(23) Incardona, J.; Linbo, T.; Scholz, N. Cardiac toxicity of 5-ring polycyclic aromatic hydrocarbons is differentially dependent on the aryl hydrocarbon receptor 2 isoform during zebrafish development. *Toxicol. Appl. Pharmacol.* **2011**, *257* (2), 242−249.
(24) Incardona, J.; Swarts, T.; Edmunds, R.; Linbo, T.; Aquilina-Beck, A.; Sloan, C.; Gardner, L.; Block, B.; Scholz, N. Exxon Valdez to Deepwater Horizon: Comparable toxicity of both crude oils to fish early life stages. *Aquat. Toxicol.* **2013**, *142*, 303−316.
(25) Billiard, S.; Timme-Laragy, A.; Wassenberg, D.; Cockman, C.; Di Giulio, R. The role of the aryl hydrocarbon receptor pathway in mediating synergistic developmental toxicity of polycyclic aromatic hydrocarbons to zebrafish. *Toxicol. Sci.* **2006**, *92* (2), 526−536.
(26) Fleming, C.; Di Giulio, R. The role of CYP1A inhibition in the embryotoxic interactions between hypoxia and polycyclic aromatic hydrocarbons (PAHs) and PAH mixtures in zebrafish (*Danio rerio*). *Ecotoxicology* **2011**, *20* (6), 1300−1314.
(27) McIntyre, J.; Edmunds, R.; Redig, M.; Mudrock, E.; Davis, J.; Incardona, J.; Stark, J.; Scholz, N. Confirmation of stormwater bioretention treatment effectiveness using molecular indicators of cardiovascular toxicity in developing fish. *Environ. Sci. Technol.* **2016**, DOI: 10.1021/acs.est/5b04786.
(28) Sloan, C.; Anulacion, B.; Baugh, K.; Bolton, J.; Boyd, D.; Boyer, R.; Burrows, D.; Herman, D.; Pearce, R.; Ylitalo, G. Northwest Fisheries Science Center's analyses of tissue, sediment, and water samples for organic contaminants by gas chromatography/mass spectrometry and analyses of tissue for lipid classes by thin layer chromatogrphy/flame ionization detection; NMFS-NWFSC-125. *NOAA Technol. Memo* **2014**, 61.
(29) Linbo, T. Zebrafish (*Danio rerio*) husbandry and colony maintenance at the Northwest Fisheries Science Center; NMFS-NWFSC-100. *NOAA Technol. Memo* **2009**, 62.
(30) Andreasen, E.; Spitsbergen, J.; Tanguay, R.; Stegeman, J.; Heideman, W.; Peterson, R. Tissue-specific expression of AHR2, ARNT2, and CYP1A in zebrafish embryos and larvae: Effects of developmental stage and 2,3,7,8-tetrachlorodibenzo-p-dioxin exposure. *Toxicol. Sci.* **2002**, *68* (2), 403−419.
(31) Becker, J.; Robinson, T.; Sachidanandan, C.; Kelly, A.; Coy, S.; Peterson, R.; MacRae, C. In vivo natriuretic peptide reporter assay identifies chemical modifiers of hypertrophic cardiomyopathy signalling. *Cardiovasc. Res.* **2012**, *93* (3), 463−470.
(32) Nishikimi, T.; Maeda, N.; Matsuoka, H. The role of natriuretic peptides in cardioprotection. *Cardiovasc. Res.* **2006**, *69* (2), 318−328.
(33) Braunwald, E. Biomarkers in heart failure. *N. Engl. J. Med.* **2008**, *358* (20), 2148−2159.
(34) Zhang, Y.; Wang, C.; Huang, L.; Chen, R.; Chen, Y.; Zuo, Z. Low-level pyrene exposure causes cardiac toxicity in zebrafish (*Danio rerio*) embryos. *Aquat. Toxicol.* **2012**, *114*, 119−124.
(35) Megy, K.; Audic, S.; Claverie, J. Heart specific genes revealed by EST sampling. *Genome Biol.* **2002**, *3* (12), research0074.1−0074.11.
(36) Rozen, S.; Skaletsky, H. Primer3 on the WWW for general users and for biologist programmers. *Methods Mol. Biol.* **2000**, *132*, 365−86.
(37) Ye, J.; Coulouris, G.; Zaretskaya, I.; Cutcutache, I.; Rozen, S.; Madden, T. Primer-BLAST: A tool to design target-specific primers for polymerase chain reaction. *BMC Bioinf.* **2012**, *13*, 134.
(38) Schmittgen, T.; Livak, K. Analyzing real-time PCR data by the comparative C-T method. *Nat. Protoc.* **2008**, *3* (6), 1101−1108.
(39) Edmunds, R.; McIntyre, J.; Luckenbach, J.; Baldwin, D.; Incardona, J. Toward enhanced MIQE compliance: reference residual normalization of qPCR gene expression data. *J. Biomol. Tech* **2014**, *25* (2), 54−60.
(40) Mahler, B.; Ingersoll, C.; Van Metre, P.; Kunz, J.; Little, E. Acute toxicity of runoff from sealcoated pavement to *Ceriodaphnia dubia* and *Pimephales promelas*. *Environ. Sci. Technol.* **2015**, *49* (8), 5060−5069.

DOI: 10.1021/acs.est.5b04928
Environ. Sci. Technol. 2016, 50, 1570−1578

(41) Millemann, R.; Birge, W.; Black, J.; Cushman, R.; Daniels, K.; Franco, P.; Giddings, J.; McCarthy, J.; Stewart, A. Comparative acute toxicity to aquatic organisms of components of coal-derived synthetic fuels. *Trans. Am. Fish. Soc.* **1984**, *113* (1), 74−85.
(42) Wincent, E.; Jonsson, M.; Bottai, M.; Lundstedt, S.; Dreij, K. Aryl Hydrocarbon Receptor Activation and Developmental Toxicity in Zebrafish in Response to Soil Extracts Containing Unsubstituted and Oxygenated PAHs. *Environ. Sci. Technol.* **2015**, *49* (6), 3869−3877.
(43) Shemer, H.; Linden, K. Photolysis, oxidation and subsequent toxicity of a mixture of polycyclic aromatic hydrocarbons in natural waters. *J. Photochem. Photobiol., A* **2007**, *187* (2−3), 186−195.
(44) Ankley, G.; Erickson, R.; Phipps, G.; Mattson, V.; Kosian, P.; Sheedy, B.; Cox, J. Effects of light intensity on the phototoxicity of fluoranthene to a benthic macroinvertebrate. *Environ. Sci. Technol.* **1995**, *29* (11), 2828−2833.
(45) Schirmer, K.; Chan, A.; Greenberg, B.; Dixon, D.; Bols, N. Ability of 16 priority PAHs to be photocytotoxic to a cell line from the rainbow trout gill. *Toxicology* **1998**, *127* (1−3), 143−155.
(46) Willis, A.; Oris, J. Acute photo-induced toxicity and toxicokinetics of single compounds and mixtures of polycyclic aromatic hydrocarbons in zebrafish. *Environ. Toxicol. Chem.* **2014**, *33* (9), 2028−2037.
(47) Kienzler, A.; Mahler, B.; Van Metre, P.; Schweigert, N.; Devaux, A.; Bony, S. Exposure to runoff from coal-tar-sealed pavement induces genotoxicity and impairment of DNA repair capacity in the RTL-W1 fish liver cell line. *Sci. Total Environ.* **2015**, *520*, 73−80.
(48) Miura, G.; Yelon, D., A guide to analysis of cardiac phenotypes in the zebrafish embryo. In *Methods Cell Biol.*, 3rd ed.; Detrich, H., Westerfield, M., Zon, L., Eds.; **2011**; Vol. *101*, pp 161−180.10.1016/B978-0-12-387036-0.00007-4
(49) DiBlasi, C.; Li, H.; Davis, A.; Ghosh, U. Removal and fate of polycyclic aromatic hydrocarbon pollutants in an urban stormwater bioretention facility. *Environ. Sci. Technol.* **2009**, *43* (2), 494−502.
(50) LeFevre, G.; Hozalski, R.; Novak, P. The role of biodegradation in limiting the accumulation of petroleum hydrocarbons in raingarden soils. *Water Res.* **2012**, *46* (20), 6753−6762.
(51) Song, H.; Wang, X.; Bartha, R. Bioremediation potential of terrestrial fuel spills. *Appl. Environ. Microbiol.* **1990**, *56* (3), 652−656.
(52) Margesin, R.; Schinner, F. Efficiency of indigenous and inoculated cold-adapted soil microorganisms for biodegradation of diesel oil in Alpine soils. *Appl. Environ. Microbiol.* **1997**, *63* (7), 2660−2664.
(53) Nocentini, M.; Pinelli, D.; Fava, F. Bioremediation of a soil contaminated by hydrocarbon mixtures: the residual concentration problem. *Chemosphere* **2000**, *41* (8), 1115−1123.
(54) Davis, C.; Farb, A.; Thorgeirsson, S.; Virmani, R.; Synderwine, E. Cardiotoxicity of heterocyclic amine food mutagens in cultured myocetes and in rats. *Toxicol. Appl. Pharmacol.* **1994**, *124* (2), 201−211.
(55) Barron, M.; Heintz, R.; Rice, S. Relative potency of PAHs and heterocycles as aryl hydrocarbon receptor agonists in fish. *Mar. Environ. Res.* **2004**, *58* (2−5), 95−100.
(56) Wassenberg, D.; Nerlinger, A.; Battle, L.; Di Giulio, R. Effects of the polycyclic aromatic hydrocarbon heterocycles, carbazole and dibenzothiophene, on in vivo and in vitro CYP1A activity and polycyclic aromatic hydrocarbon-derived embryonic deformities. *Environ. Toxicol. Chem.* **2005**, *24* (10), 2526−2532.
(57) Jung, D.; Klaus, T.; Fent, K. Cytochrome P450 induction by nitrated polycyclic aromatic hydrocarbons, azaarenes, and binary mixtures in fish hepatoma cell line PLHC-1. *Environ. Toxicol. Chem.* **2001**, *20* (1), 149−159.
(58) Bhalla, V.; Willis, S.; Maisel, A. B-type natriuretic peptide: the level and the drug-partners in the diagnosis of congestive heart failure. *Congestive Heart Failure* **2004**, *10* (1 Suppl 1), 3−27.

DOI: 10.1021/acs.est.5b04928
Environ. Sci. Technol. 2016, 50, 1570−1578