AGENDA

Stormwater Technical Meeting
Thursday, April 25th, 2013, 1:00 - 3:00 pm
EPA Building - Seattle, WA
15th Floor Conference Rooms (15P, 15Q, & 15S)
Check-in at Service Center on the 12th Floor

Time	Item	Lead Presenter
1:00 pm	Welcome, Introductions and Opening Remarks	Melissa Whitaker
1:10 pm	Interagency Agreements Update	Jay Davis
1:20 pm	Big Picture Stormwater Research Objectives, Overview of Stormwater Center and Recent Media Attention & Publications	John Stark and Nat Scholz
1:30 pm	Impacts on Various Lifestages of Coho Salmon and Identifying Watersheds at Risk	Nat Scholz
2:00 pm	Bioeffectiveness of Emerging LID Technologies	Jen McIntyre
2:30 pm	Questions and Discussions	All
2:45 pm	Draft Statements of Work for Future Collaborations on Stormwater Issues in Puget Sound	Nat Scholz and Jay Davis
3:00 pm	Closing Remarks and Adjournment	Michael Rylko

Manage these Agreements → Btwn EPA & Services

Nisqually Estuary Restoration Monitoring / Puget Sound SW Related PreSpawn Mortality

Cumulative impacts of SW on salmon & their habitats

Puget Sound Biological Effectiveness of Emerging LID Technologies + Pollution Source Control

WSU EPA
USFWS
NOAA
Puyallup
~~SPU~~
King County
~~Sequamish~~
Tribe

3 studies

ID at risk wtrsheds

Assess green infrastructure effectiveness

WSU Green Infrastructure — Puyallup WSU Extension
national BMP

Allows replication of other studies

permeable pavements
primarily urban freeways
green roof — w/cistern, can look at atmospheric deposition
"I. P. Zone" which got state $ means must w/private
In an old chicken house, a salmon/ future zebra fish
renovation to include office/labs etc.

Whats the problem / whats the solution

Nat'l Ocean Policy
Non point source pollution is the target.
NOAA toxics office is unique to this NOAA office

Anything that goes into SW

What is a sentinel species?
wide distribution / lives in urban habitat / sensitive to toxics

COHO — they overwinter, they like lowland streams
more likely to be exposed to SW

Species of Concern

Freshwater phase (1-2 yrs) is the crucial phase
(Longfellow Creek projects using actual surface flow)

Embryo → Vascular problems on embryos → affects development

Juvenile Stage:

SW
indirect effects on the foodweb

What is integrity of foodweb? Most urban streams are not good quality
BIBI = gradient of WQ correlated w/ urbanization
is it habitat? or is it exposure to SW

Healthy Stream = Cedar River (set up "Bug Condos"
vs Longfellow
= SW limits food (bugs) for Coho

Recent Study results
Sept + Oct 2012 ~> correlates
storm, high SW dose ~> Bugs leave Creek
mayflys etc.
so: Bugs don't like SW
but, existing Juveniles are actually doing ok — comparable to non urban streams

[recent good news also]

Does it only hit Coho?
Phenomena of Coho prespawn mortality / but not the juveniles
adults

Coho + Chum in same Pipers Creek — 100% mortality of the Coho, 90% Chum survival

Fury's But Out
respawn mortality
Chinook
Chum in Issaquah etc.

Coho mortality widespread & recurrent in Puget Sound urban streams

in 2012 50 day dry period so

it's been hard to find symptomatic fish alive

common suite of symptoms
gaping, disoriented

mortality in urban may drag down populations in non urban wtrsheds

40-90% mortality ~ wild stocks cannot survive

the only thing keeping it alive are stray's coming in

Adult spawners
consistently die each fall (CA - Canada)
Phenomenon is widespread in urban watersheds
toxic runoff is smoking gun

predictive modelling based on land use

There are many
↳ Piper's Creek ↗
↳ different land uses
Longfellow Creek ↘ cover

Coho likely widely impacted throughout Central P.S.

Spawner mortality correlates to land use
% impervious, roads

roads = impervious
132 models w/in the research model

Can we predict where it will/is happening

A tool, we want to be able to tell communities where based on landcover they'll find these fish

Next yr Objectives: Common source = Cars
expose adults to mixtures metals + ~~PAHs~~ PAHs

Expose juvenile & adult coho in urban runoff

Refine extend population & land use modelling

Collect gill, liver, heart tissues for genomic analysis

Services can

Grover's Creek Hatchery w/ Snoqualmie

exposing Coho to "cocktails" of crude oil +

Cu 7.0 ug/L

Can't kill fish just w/ these premade cocktails Metals + oil PAHs

1st 2-3 wks is when Coho die

is it a total rainfall exposure? of fall

Coho survive later in fall/winter

looking also for Climate change impacts
when it rains relatively to life

Exposed	nonexposed
4 fish each	4 fish

10-15
10-29
4 storms

100% survival of nonexposed

Stages

1st	2nd	3rd	4th
embryo mortality			prespawn mortality

"Whole SW" is toxic causing problems

What's happening in this life stage?

NMFS can provide a protocol for looking at Coho rates in streams

~~test~~ what are Coho prespawn mortality

additional sites
Update land use layers / precip data — Climate / rainfall

Regional population models — as pop / where are Coho pop. at risk?

~~PH~~ PAH + Metals isn't enuf to kill em

pinpoint precisely why they are dying
(looks like a heart attack) gill / liver / heart

technology available to look at gene expression
now, wasn't available 7 yrs ago
tissues can be evaluated in weeks
not years

Next step
look at
14 dying fish Longfellow
12 healthy fish Grove
16 tank exposed fish
16 control tank exposed fish

NOAA also studying Chemistry of SW changes thru the year @ the freeway on ramp near their office

what are they eating?
mainly midge larvae / Coho eating snails
in the urban setting
warm temps

ratio
ind
per weight / value

midge isn't necessarily "good food" compared
but Coho aren't malnourished — they are good
size, good ability to switch prey
midge are plentiful

we thought it was metals / PAHs but it's not

(We thought Coho would be starving in urban environments
but they seem to be doing OK on midge larvae)

more correlation to PSM roads, to use of pesticides

switch from organophosphates to pyrethins
despite change in consumer use of chemicals
no evidence severity / change

can't get enough of Coho Brains samples
mixtures matter

Media Attention.
NW Indian Fisheries
Media made a big deal of it
(WSDOT figured it out)

Re: Coho juveniles = despite good news in Creeks, the spawner survey, show symptomatic some juveniles

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Testing Biological Effectiveness & GSI Green SW Info

Focus
Characterize Highway Runoff

526 Highway >60,000 ADT
Downspout onto NOAA parking lot

Captured first flush

C. dubia
Acute Lethality
7d growth / impairment

zebrafish model
Survival / morphology
Cardiovascular
Neurotoxicity

Zebrafish Research Model

its a good useful species for research

Genome completely sequenced

Invertebrate Survival & Fecundity

5 storms ~ relatively Survival

Acute mortality

August Jan May are lethal

other months = severe impacts

Developing fish

proportion hatched ~~was~~ was severe vs. control

Swim bladder devlpmt

Length

eye size

Looked at Dilution of Runoff

50% strength runoff

= heart defects

accumulation of blood

deformed hearts

inability to hatch

common abnormality

sublethal effects of runoff on developing fish

inability/delay hatch

developmt delay

small eye phenotype

cardiovascular edema of the heart

Deformed jaws & hearts

hatch delay occured at 5% runoff

Runoff alters genetic Cardiac & Detox. Messages

QPCR How much is gene saying "go replicate this cell"

PAHs = theres a toxic here
need to
of exposure to PAHs
Heart is a target

is gene altered?
how much & where

in the epidermis (skin) and vasculature

Whats in SW causing this

Atmosphere
Roads
Autos

Metals
O&G
Surfactants
Deicing

Can
~~Dust~~ contribute
toxicity

No pattern to levels of metals or PAHs between 5 months of storms

Zn - Cu

Runoff - Zn maybe sufficient to cause lethality

Are metals in highway runoff neurotoxic

Dissolved Zn – neurons die at hi concentrations

No neurotoxicity evident in these events

despite hi Cu — but hi DOC can protect against hi levels of metals

10–20x Cu concentration w/ DOC

metals may not be causing toxicity

PAHs — Crude Oils pH fingerprint

Flounders Cardiotoxic — exposure to Phenanthrene = edema, small eyes

Detox enzyme show up in skin / heart in healthy but not in SW runoff exposed fish

toxicity fingerprint attenuates over time — ie, store it, it gets less toxic

Bio effectiveness of GSI

are reductions sufficient to ↓ bio effects

runoff is severe lethal & sublethal

metals may not be bio available

Test Bio. Effectiveness of LID

4 Bioretention reduced met

Daphid survival
Zfish embryo
Mayfly Survival
Coho

forcee runoff thru LID
Soil treatment

this storm wasnt unusual for Metals & PAHs

Biological Effectiveness

normal development
normal hearts
normal eyes w/ plants

% reduction in dissolved Metals
(no difference btwn soil vs planted soil

lots of reduction for most metals

for PAHs —

% reduction reduced by Bioretention
75-97% reduction

Is that enough to protect species?

Relative survival

Sig mortality to untreated runoff

mayfly —

100% ~~exposure~~ mortality of Coho to untreated runoff

But — treated bioretention

0% mortality relative to control

① Validate pilot results on effectiveness

② Can Bioretention prevent (Oho PSM?
eliminate

dream that treating ③ Are PAHs driving runoff toxicity

runoff from coal tar sealcoat mixtures

Can bioretention prevent harm from such PAH impacts

④ Resilience to episodic exposure

Long term test of runoff on simulated stream channels + fish

making the ~~XPAH~~ experimental cocktail of PAHs is admittedly different from real highway runoff

tool box will expand
use the oil spill in Gulf
and relate it to effects of Puget sound

its not only exposure to raw oil causing PSM - there has to be something about car engine combustion

Are the pesticides used bioavailable to hurt bugs/fish
look at tissue residue studies

physical filtration
absorption in
microbial communities are undefined

Some plants are good at metals absorption
what type of plants — grass types

metabolizm by microbes is responsible for reduction of PAHs during residence time

What's the compost mixtures in these studies showing elevated metals — there's a lot of confusion

What's the composition of the soils that work

what part do the microbial

what is the fate of organic contaminants

WSDOT
Tribes

Baseline data in Longfellow Creek

Thorton Creek Coho mortality is 100%

how much research on the problem vs on the solution?

Total P. reduction
organophosphate

↳ no longer need to characterize the problem.

what will it take to have healthy Coho & People?

SPU is a partner

Putting a biological spin on the research

Scope of work for follow up research

6% overhead

Stormwater Related Pre-Spawn Mortality: Cumulative Impacts of Toxic Stormwater Runoff on Puget Sound Salmonids and Their Habitat

Project Components

Task 1: Linking the effects of stormwater on multiple coho life stages to impacts on coho population productivity in a spatially explicit system.

Nearly a decade of laboratory and field research at NOAA's NWFSC (together with the USFWS, the City of Seattle, Suquamish Tribe, USEPA, and many other partner organizations) has shown that non-point source pollution has important consequences for coho salmon and the habitat on which they depend. The most dramatic example of this was the discovery in the late 1990's that adult coho returning to spawn in restored urban drainages are prematurely dying in high numbers when exposed to stormwater runoff (Figure 1). Spawner surveys on various urban streams over the past decade have consistently revealed very high rates of coho pre-spawn mortality (PSM) – i.e., ~ 30 – 90% of the entire run.

Figure 1. Example of a pre-spawn female that returned to an urban creek and died before spawning. Note the large amount of eggs present.

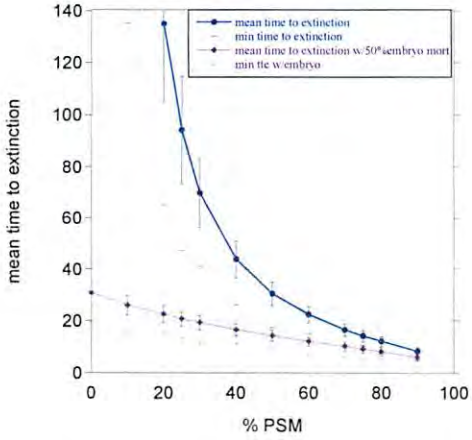

Figure 2. Estimated time to localized extinction for isolated coho populations experiencing various levels of pre-spawn mortality (PSM) using density dependent models. Models incorporate variable survival rates and fecundity, with a constant annual level of pre-spawn mortality ranging from 0 to 90%. The graph shows the mean time to extinction, standard deviation, and minimum time to extinction for PSM alone (● and -) and for PSM and embryo mortality(◆ and x) (1000 repetitions). Models were run out to 200 years, so the mean time to extinction was not determined for 10% PSM.

Initial coho pre-spawn mortality models were developed that incorporated the effects on coho populations of reductions in adult spawner survival. These models predicted rapid declines in adult abundances for isolated populations affected by PSM. Importantly, local population extinctions were predicted across the range of PSM rates that have been recently documented in Puget Sound urban streams (20-90%; Figure 2). When embryo mortality levels observed in experimental and field studies were incorporated, time to extinction was shortened even further (Figure 2). Direct and indirect effects on juvenile survival were not identified in the field and experimental studies, so this life stage remained unchanged in the model. Our analysis shows that stormwater poses an extinction risk for local coho populations on a time scale of a few years to a few decades. Incorporating metapopulation dynamics mitigates some of these impacts, but to a limited extent. This task will expand the population models to incorporate spatially explicit metapopulation dynamics and stormwater impacts as informed by the land-use models from Task 2.

Project Writeups for future funding, (2) opportunities

Task 2: Expand predictive coho population modeling to look at watersheds identified as having coho most at risk, now and in the future from changes in land use and climate patterns.

While interannual rates of coho PSM have varied across the years observed, the severity of the adult mortality has been consistently higher in some Seattle-area drainages (e.g. Longfellow Creek, Thornton Creek) relative to others (Pipers Creek). These differences in PSM between watersheds suggest that the mortality phenomenon may correlate with various metrics describing the built urban environment (e.g., impervious surface area, measured and modeled vehicle traffic density, street network classification, etc.). To investigate possible relationships between land cover/use and coho PSM, the NOAA-USFWS research group initiated a study to evaluate whether inter-watershed variation in PSM could be predicted from specific landscape and traffic density metrics. Despite small data sets for some urban streams (few coho spawners), we found a strong relationship between several selected metrics and PSM. Watersheds that have a higher percentage of arterial roadways as a percentage of the total street network have consistently higher rates of coho PSM.

This finding is important for several reasons. First, it suggests that motor vehicles and street runoff are the source of contaminants that are causing the recurrent coho die-offs. Although motor vehicles are sources of many toxic contaminants (predominantly petroleum hydrocarbons and metals), this narrows the list of causative agents considerably. Second, it suggests that we can use existing land cover information to predict where PSM may be occurring in Puget Sound watersheds for which no survey information currently exists. A pilot example for catchments in Kitsap, Pierce, King, and Snohomish counties is shown in Figure 3. The black points indicate the sites for which we have acquired PSM data from tribes, cities, counties, and non-profit organizations to refine the initial model. We can use these relationships between PSM and different metrics of urbanization to identify potential "hot spots" for PSM, and then focus limited monitoring resources to validate these findings. The new model will allow predictions of which coho populations are most vulnerable to future development activities (e.g. transportation infrastructure).

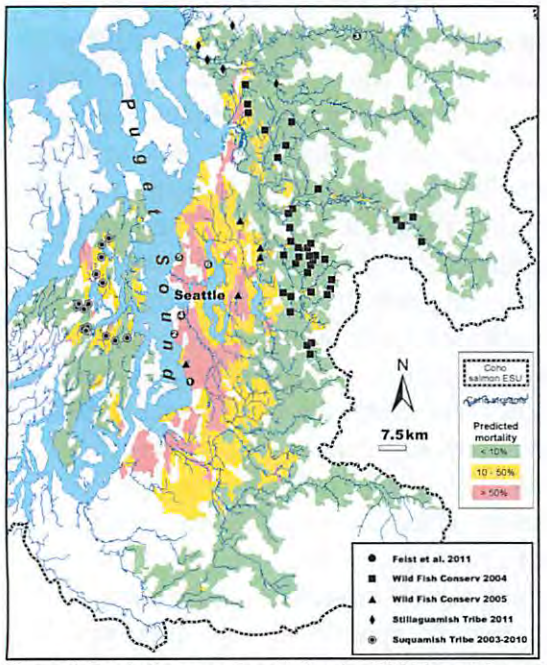

Figure 3.Predictive map for adult coho PSM in catchments from Pierce, King and Snohomish Counties. Points indicate new data being integrated in the next version of the model.

For this task, we will use a GIS-based approach (at the catchment scale) with commonly available GIS data layers, including impervious surfaces, land use type, road density and precipitation, to identify watersheds (or catchments within watersheds) where the likelihood of coho PSM is relatively high. This information will be communicated to local communities, resource agencies, tribal organizations, fisheries enhancement groups, watershed restoration groups, and others who are currently prioritizing and implementing stream habitat monitoring, restoration, and stormwater mitigation activities at the local level. We will also use the model to evaluate the potential future impacts of various regional

growth scenarios on wild coho populations that are currently healthy but at risk from future development and increasing toxic runoff. Planned urban growth information and projected precipitation changes will be represented by projected GIS data layers to represent future scenarios and the models rerun to estimate changes in potential PSM. The aim of this task is to improve the identification of watersheds most at risk of PSM due to urbanization, now or in the future.

Task 3: Identify the underlying cause of adult mortality in urban drainages.
Several lines of scientific evidence suggest that toxic urban runoff, likely from motor vehicles, is responsible for the recurring, acute spawner die-offs that have been observed in Seattle-area streams. As noted above, there is a close correlation between metrics for impervious surfaces and vehicle traffic and the severity of PSM. Also, alternative hypotheses (pathogens, fish condition, dissolved oxygen, etc.) have largely been ruled out. Further, there is also a correlation between PSM and rainfall patterns, in that PSM is more severe when fall storm events are preceded by long periods without rainfall. Lastly, affected coho show a common suite of symptoms (surface swimming, gaping, etc.) and they die within a few hours. These symptoms were reproduced in controlled exposures conducted in the fall of 2012 in which returning adult coho were exposed to runoff collected from an elevated highway.

Despite the lines of evidence above, we have not definitively shown which toxics in urban runoff are causing PSM. To address this question, we will investigate the mechanism by which the coho are dying and use this to inform which chemicals could be involved. Adult migratory coho salmon are logistically difficult to work with from an experimental standpoint, and there are dozens (if not hundreds) of candidate contaminants in stormwater to consider. A set of returning adults will be exposed to untreated water, to stormwater runoff that we know induced PSM in otherwise returning, healthy adult salmon, or to stormwater treated in various ways in order remove different classes of contaminants and thus remove the symptomology and lethality of the runoff. The endpoints of behavior and mortality will be used, as well as other biological endpoints of effect that may indicate the physiological pathways being disrupted due to the exposure. Additionally, direct sequencing will be done on previously obtained tissues collected from symptomatic adults in the field and from runoff-exposed and control fish. This process identifies changes in the patterns of gene expression, due to different classes of contaminants triggering specific transcriptional responses that correspond to a particular injury phenotype – e.g., PAH-induced cardiovascular toxicity. Identifying the injury phenotype will help us determine which components of stormwater are responsible for coho pre-spawn mortality.

Project Timeline

Table 1. Project components schedule of activities, 2011-2013.

FY Year	2013	2014				2015			
Tasks / Quarter	4	1	2	3	4	1	2	3	4
1.0 Linking the effects of stormwater on multiple coho life stages to impacts on coho population productivity in a spatially explicit system.									
2.0 Expand predictive coho population modeling to look at watersheds identified as having coho most at risk, now and in the future from changes in land use and climate patterns.									
3.0 Identify the underlying cause of adult mortality in urban drainages.									

Approximate Budget Request: $500,000 (Total) = $250,000 (for work conducted in FY14) and $250,000 (for work conducted in FY15).

Biological Effectiveness of Green Stormwater Infrastructure

Our recent research has established that untreated runoff can cause mortality in exposed fish and invertebrates, reproductive impairment in invertebrates, and sublethal cardiovascular toxicity in developing fish. There is uncertainty about the effectiveness of bioretention for reducing contaminants and preventing toxicity in waters receiving urban stormwater runoff. Our proposed work builds on our previous findings to answer important questions about the biological effectiveness of biotention.

Project Components

Task 1: Validate pilot results of bioretention effectiveness

In a pilot test of bioretention effectiveness at Washington State University in Puyallup, WA, we saw significant reductions of contaminants in runoff. Untreated runoff caused significant mortality and sublethal effects in juvenile coho, zebrafish embryos, and aquatic invertebrates. Unfiltered runoff caused 100% mortality in juvenile coho within 12 h of exposure. Runoff treated with bioretention was no longer acutely toxic to fish or invertebrates. We observed 100% survival of juvenile coho salmon following runoff treatment with bioretention.

Figure 1. Large bioretention columns at Washington State University in Puyallup, WA used to test biological effectiveness of bioretention.

This pilot experiment will be repeated with runoff from various storms to confirm our preliminary findings and further describe the ability of bioretention to filter contaminants and prevent sublethal effects in fish and invertebrates, particularly juvenile coho salmon. Treatment will use the same bioretention columns from the pilot test (Figure 1), enabling an additional comparison of effectiveness over time.

Task 2: Test the effectiveness of bioretention treatment to prevent PSM in adult coho

Prespawn mortality (PSM) in adult coho (Figure 2) is linked to land development and urban or urbanizing land uses. The high mortality rates (up to 90%) observed in basins with PSM are a serious threat to the resilience of coho in developing areas like the Puget Sound basin. In 2012 we determined that exposure to unfiltered highway runoff was sufficient to trigger PSM in adult coho returning to the Suquamish Tribe's Grover's Creek Salmon Hatchery in Poulsbo, WA.

Figure 2. Female coho spawner in Longfellow Creek in Seattle, WA following a storm event during October 2012.

We propose testing whether bioretention can prevent mortality in adult coho exposed to highway runoff. For this task, we will construct bioretention columns at Grover's Creek Salmon Hatchery. Similar to the columns used in pilot testing at Washington State University, bioretention columns will be filled with a drainage layer of gravel aggregate overlain by a mixture of 60% sand: 40% compost, as recommended in the Washington Stormwater Manual.

Highway runoff transported to Grover's Creek will be filtered on-site and adult coho spawners will be exposed to one of three treatments across multiple storms; unfiltered runoff, filtered runoff, or well water. This task will allow us to determine the biological effectiveness of bioretention for preventing PSM in adult spawners exposed to urban road runoff.

Task 3: Assess the contribution of PAHs to stormwater runoff toxicity

Exposure of developing fish embryos to road runoff produces symptoms similar to those caused by exposure to oil. The effects of oil are driven by PAHs dissolved in the water fraction (Figure 3). We will focus on PAHs as drivers of the toxicity of urban runoff to fish and invertebrates. Coal tar based asphalt sealcoat is a commonly applied product in the most populated regions of the United States and Canada. Runoff from coal tar sealcoated surfaces contains a complex mixture of PAHs similar to that produced by oil spills and road runoff. We will push the performance of bioretention systems with runoff from sealcoated surface. If these systems can be biologically effective at high levels of complex PAHs present in urban runoff, we can rely on them to be effective at the lower concentrations more commonly present in runoff from various land uses. This task is national is scope for various land uses.

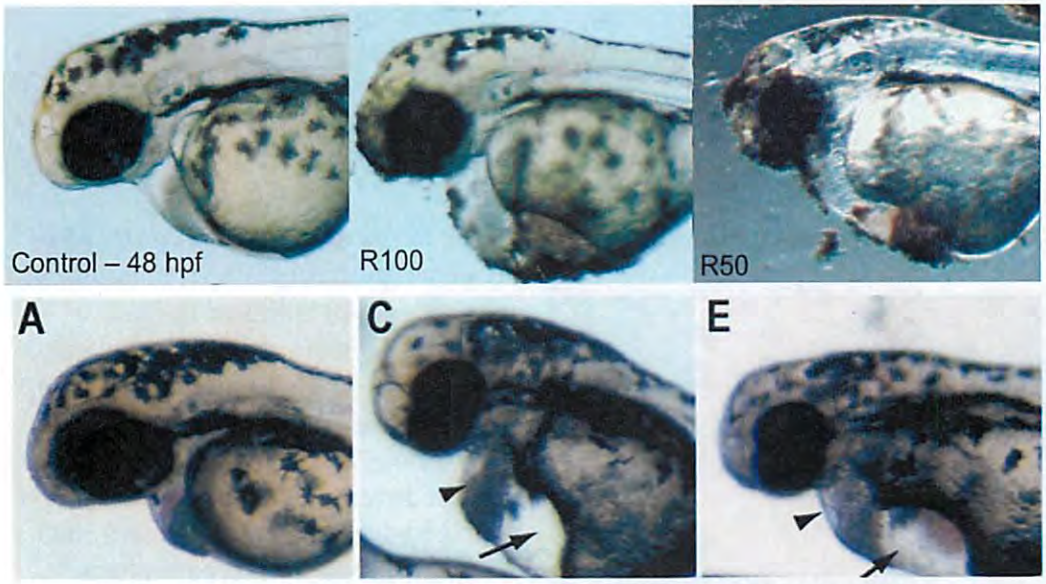

Figure 3: Zebrafish embryos exposed to control water (top left and bottom left), dilutions of highway runoff (R50 = 50% runoff)(top two right photos), or exposed to the PAH phenanthrene (bottom two right photos). Embryos exposed to highway runoff or phenanthrene for 48 h showed significant heart deformities and cardiovascular defects.

Task 4: Determine the resilience of salmonid food webs to episodic exposure of stormwater runoff

Bioretention ultimately needs to perform at a level that preserves ecological resilience. From our previous research at an urban creek in Seattle (Longfellow Creek), we know that urban runoff can seriously impact aquatic ecosystems, causing elevated mortality and deformity of developing coho embryos and increased drift of native aquatic invertebrates (Figure 4).

Simulated stream channels will be built alongside bioretention systems for this task. Stream channels will contain coho embryos and native aquatic invertebrates in gravel from a pristine watershed (Cedar River). Over several months, the stream channels will be episodically exposed to urban runoff. The survival and health of coho embryos as well as the distribution and survival of invertebrates will be monitored over the exposure period. This task will allow us to explore biological effectiveness of bioretention at a higher level of ecological relevance.

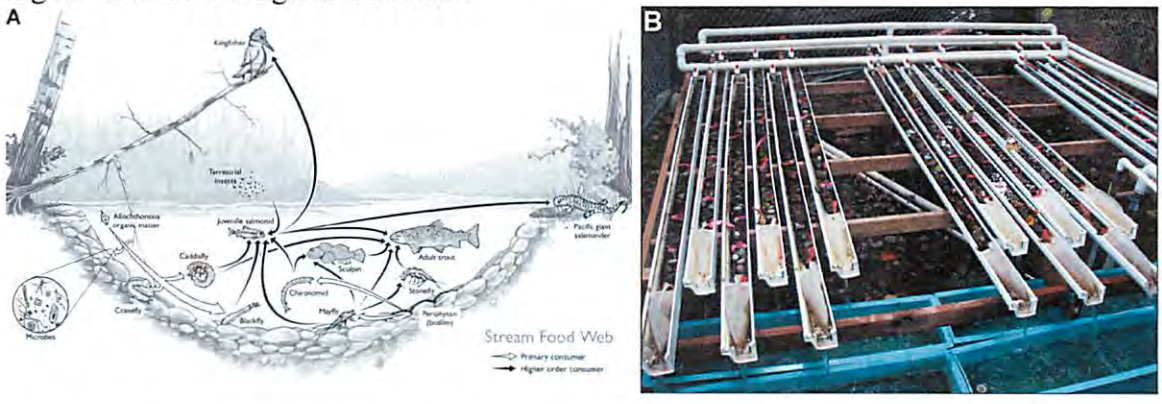

Figure 4. (A) Representative salmonid stream food web in the Pacific Northwest. (B) Experimental stream channels at Longfellow Creek.

Project Timeline

Table 1. Project components schedule of activities, 2013-2015.

	FY Year	2013	2014				2015			
Tasks	**Quarter**	4	1	2	3	4	1	2	3	4
1.0 Verifying and validating the biological effectiveness of bioretention										
2.0 Bioretention to prevent PSM in adult coho spawners										
3.0 Exploring PAHs as drivers of toxicity										
4.0 Ecological relevancy of episodic exposure to runoff										

Approximate Budget Request: $600,000 (Total) = $300,000 (for work conducted in FY14) and $300,000 (for work conducted in FY15).

Integrated Environmental Assessment and Management — Volume 7, Number 4—pp. 648–656

Research published (this year) using past Grant $

Estimating the Future Decline of Wild Coho Salmon Populations Resulting from Early Spawner Die-Offs in Urbanizing Watersheds of the Pacific Northwest, USA

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(Submitted 19 January 2011; Returned for Revision 28 February 2011; Accepted 20 April 2011)

ABSTRACT

Since the late 1990s, monitoring efforts evaluating the effectiveness of urban stream restoration projects in the greater metropolitan area of Seattle, Washington, USA, have detected high rates of premature mortality among adult coho salmon (*Oncorhynchus kisutch*) in restored spawning habitats. Affected animals display a consistent suite of symptoms (e.g., disorientation, lethargy, loss of equilibrium, gaping, fin splaying) that ultimately progresses to death on a timescale of a few hours. Annual rates of prespawn mortality observed over multiple years, across several drainages, have ranged from approximately 20% to 90% of the total fall run within a given watershed. Current weight-of-evidence suggests that coho prespawn mortality is caused by toxic urban stormwater runoff. To evaluate the potential consequences of current and future urbanization on wild coho salmon, we constructed life-history models to estimate the impacts of prespawn mortality on coho populations and metapopulations. At the low (20%) and high (90%) ends of the range of observed mortality, model results indicated the mean time to extinction of localized coho populations in 115 and 8 y, respectively. The presence of productive source populations (i.e., unaffected by prespawn mortality) within a metapopulation reduced local extinction risk. However, as more populations within a metapopulation become affected by spawner die-offs prior to spawning, the source population's productivity declined. These simple models demonstrate the potential for rapid losses from coho populations in urbanizing watersheds. Because the models do not account for possible impacts of toxic runoff to other coho life stages, they likely underestimate the cumulative impacts of nonpoint source pollution on wild populations. Integr Environ Assess Manag 2011;7:648–656. © 2011 SETAC

Keywords: Nonpoint source pollution Stormwater runoff Urbanization Population model Coho salmon

INTRODUCTION

The decline of Pacific salmon (*Oncorhynchus spp.*) in the Pacific Northwest has been attributed to many factors. These include the physical, biological, and chemical degradation of freshwater and estuarine habitats as well as fishing pressure, hatchery production, and hydropower operations (National Research Council 1996; Good et al. 2007). To reverse salmon declines due to loss or deterioration of freshwater habitat, many lowland urban streams have been the focus of active restoration efforts (Katz et al. 2007). These projects have generally included postproject monitoring to assess the effectiveness of the restoration effort in terms of habitat use by salmon spawners and their progeny. Beginning in the late 1990s, spawner surveys in streams within the greater Seattle, Washington, USA, metropolitan area discovered that adult coho salmon (*O. kisutch*) returning to spawn in small urban catchments were behaving abnormally. Affected animals were found gaping and swimming in circles at the surface of these creeks. The symptoms typically progressed to a loss of equilibrium and death within a few hours. In most cases, egg retention in dead females was nearly 100% (City of Seattle 2007; McCarthy et al. 2008; Wild Fish Conservancy 2008).

This phenomenon has been referred to as coho prespawn mortality (PSM) (McCarthy et al. 2008; Wild Fish Conservancy 2008) and has now been documented in several lowland urban streams around Puget Sound, Washington. The discovery that returning coho salmon were dying in restored urban streams prompted the National Oceanic and Atmospheric Administration (NOAA Fisheries), the US Fish and Wildlife Service (USFWS), the City of Seattle (Seattle Public Utilities), the Wild Fish Conservancy, and others to expand and coordinate fall spawner surveys. In addition, a forensic investigation was initiated by NOAA Fisheries to determine possible causes of PSM (McCarthy et al. 2008; Wild Fish Conservancy 2008). Conventional water quality parameters (i.e., temperature, dissolved O_2, ammonia, pH) are favorable for salmon survival (City of Seattle 2007), and PSM-affected animals show no consistent signs of pathogen infection or disease (N.L. Scholz, personal observation). Therefore, the urban coho mortality phenomenon is dissimilar from other documented circumstances of adult salmon dying prematurely at the spawner life stage because of disease, predation, altered flows, inaccessible spawning habitat, and other nonchemical stressors (St-Hilaire et al. 2002; Kocan et al. 2004; Keefer et al. 2010).

All of the streams where coho prespawn mortality has been observed receive urban stormwater runoff in the fall when coho return to spawn. The severity of the fish kills correlates

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Published online in Wiley Online Library (wileyonlinelibrary.com).
DOI: 10.1002/ieam.219

with both fall patterns of rainfall (McCarthy et al. 2008) as well as with the amount of impervious surface within the drainage (B.E. Feist, personal communication). The weight of evidence to date therefore suggests that coho are dying from exposure to chemical pollutants in nonpoint source urban runoff. Urban stormwater contains a very diverse mix of contaminants (Eriksson et al. 2007), including metals (Tiefenthaler et al. 2008), polycyclic aromatic hydrocarbons (Stein et al. 2006), pesticides (Hoffman et al. 2000), and other chemicals. The precise agent, whether alone or as a component of a chemical mixture, that causes coho PSM has not yet been identified and is the focus of ongoing investigation.

The Pacific Northwest is currently undergoing substantial human population growth and development. By 2025, 1.4 million persons are expected to join the 4 million persons currently residing in the Puget Sound region (Puget Sound Action Team 2007). Similar growth patterns are projected for the Willamette Basin in Oregon, USA (Baker et al. 2004) and the lower Columbia River region. Much of the projected urban and residential growth will occur in watersheds that currently support both productive and at-risk wild coho populations. Streams in these systems serve as sentinels for anthropogenic impacts (Williamson et al. 2008). Coho populations belonging to the lower Columbia River evolutionarily significant unit (distinct population segment) were recently listed as threatened under the US Endangered Species Act (ESA). Puget Sound coho have declined from historical levels and are presently designated as a "species of concern" in the context of the ESA (for current designations, see http://www.nwr.noaa.gov/ESA-Salmon-Listings/Salmon-Populations/Coho/Index.cfm). The ongoing and future conversion of forested and agricultural lands for residential and commercial uses is likely to increase the loading of nonpoint source pollutants to lakes, rivers, and streams that have historically supported viable coho populations. This, in turn, is likely to expand the geographical range over which adult coho are affected by PSM.

In the present study, we used a modeling approach to forecast the potential impacts of increasing PSM on wild coho stocks. Our aim was to assess extinction risks for isolated populations and evaluate how these risks might be modified by straying between different populations within a larger metapopulation. This approach allowed us to relate effects on coho within an urbanizing catchment to the dynamics of a metapopulation across a larger watershed. The models were parameterized with empirical life history data for coho, and basic assumptions were used to produce modeled populations that were initially stable and viable. To simulate the effects of changes in land use and the intermittent nature of stormwater runoff, the severity of PSM was held steady, varied, or gradually increased over a timespan of a few decades. The results provide insights into how coho salmon populations may respond to complex and rapidly changing human land use activities throughout urbanizing areas of the Pacific Northwest.

METHODS

Projection matrices integrating age-specific survival and reproductive demographic rates were selected as the modeling environment for investigating effects of PSM on solitary coho populations and metapopulations. A metapopulation is a group of populations connected by migration or dispersal (a "population of populations") (Hanski and Gyllenburg 1993). Salmon dispersal takes the form of adults straying to spawn in habitats other than their stream of origin. This produces a series of genetic linkages between individual populations within a watershed or collection of watersheds. Projection matrix methods followed Caswell (2001) and were used to 1) calculate the transition matrix, 2) determine the intrinsic population growth rate (λ) and stable age distribution, and 3) conduct a sensitivity analysis. These analyses were performed for control (baseline; no PSM) and constant PSM scenarios outlined below using MATLAB (The Math Works, version 6.5). Model projections were run by year for 200 y and incorporate various levels of prespawn mortality. RAMAS® Metapop (Applied Biomathematics, version 5.0) was used for conducting the projections.

The modeled coho population assumes a 3-y lifespan for individual fish. Spawning occurs in late fall and early winter with fry emergence occurring from March to May. Fry rear for 14 to 18 months in freshwater before they smolt and then migrate to the ocean. They spend 16 to 20 months in the ocean before returning to freshwater streams to spawn. For the purpose of the model, vital rates for survival and reproduction were drawn from field data collected for wild coho populations as summarized by Knudsen et al. (2002). Adult upstream migration survival was 0.97 ($\pm$0.01). The average fecundity of each female was 4510 eggs with a standard deviation (SD) of 500. The male to female ratio was 1:1. Survival from spawning to emergence was 0.335 ($\pm$0.07). Survival from emergence to smolt was 0.0296 ($\pm$0.0029) and marine survival was 0.0505 ($\pm$0.01). All parameters followed a normal distribution (Knudsen et al. 2002).

The availability of food and freshwater rearing habitat are both known to influence the 1st-year survival rate in density-dependent salmon populations (DeAngelis et al. 1980; Bradford et al. 1997; Kareiva et al. 2000). Therefore, the models were constructed using 1st-year survival (S1) as the density-dependent factor. We assumed a ceiling density dependence that did not provide compensatory growth rates for low abundances. This was based on age 1 (1st-year) abundance with a carrying capacity of 2 900 000 individuals and a low SD of 2900 for each population. This produced an equilibrium spawning abundance of approximately 3300 age 3 adults. This value falls within the range of documented abundances for small catchments in the Puget Sound region (Weitkamp et al. 1995). Natural variability was incorporated by randomly selecting each vital rate value in the projection matrix for each year from a normal distribution within the reported means and SD (Knudson et al. 2002). Straying of individuals in and out of the modeled solitary populations was assumed to be equal, with zero net effect on population abundance and population fitness. Modeled initial conditions were calculated from the stable age distribution of the control transition matrix scaled to produce approximately 3300 age 3 adults annually. This resulted in a distribution of 2 199 120 1st year, 64 900 2nd year, and 3300 3rd year individuals. The transition elements and SD for the control coho model are provided in Table 1.

The direct impacts of PSM were incorporated in the model as a reduction in the percentage of female adults successfully spawning. It was assumed that mean survival rates for embryos, fry, and smolts were unaffected. Model outputs included adult abundance and time to local extinction (abundance <1 individual). The modeled scenarios simulated

Table 1. Matrix transition elements (SD) for S1, S2, and R3 for control conditions[a]

Transition element	Control matrix values	Sensitivity values	Sensitivity changes from PSM
S1	0.0296 (0.0029)	0.143	–
S2	0.0505 (0.01)	18.67	–
R3	732.8 (51.25)	0.0001	+

PSM = prespawn mortality; R3 = reproduction by 3rd-year adults; SD = standard deviation; S1 = 1st-year survival; S2 = 2nd-year survival.
[a]Sensitivity analysis on models incorporating PSM showed decreased sensitivity of λ to changes in survival (S1 and S2) and increased sensitivity to changes in reproduction (R3).

solitary coho populations impacted by PSM with different degrees of severity. Populations in the constant PSM scenarios experienced a steady rate of spawner die-off over the entire 50-y model projection at the PSM rates shown in Table 2. Another set of scenarios explored the consequences of gradually increasing the level of PSM to simulate a gradual urbanization of a watershed. This was modeled in 2 ways: 1) a relatively rapid increase in PSM (from 0% to 75% PSM over 20 y) and 2) a slower and less severe impact (from 0% to 25% PSM over 30 y). In both cases, changes in adult abundance and time to local extinction were predicted. The impact on population growth rate for each PSM scenario was calculated as the percent of the control intrinsic population growth rate. In addition, 200-y projections were run to determine mean time to extinction, minimum time to extinction, and SD. All projection scenarios were repeated 1000 times to incorporate the variation reported in the literature.

To assess the relative impacts of prespawn mortality on populations connected by straying (dispersal), we constructed a metapopulation model in RAMAS Metapop consisting of 5 linked populations. The model simulated individual populations within a watershed (Figure 1), with each connected to the others by a low and consistent rate of spawner straying. We assumed that 5% of the age 3 individuals in each population would stray in equal numbers to the other 4 populations within the metapopulation, resulting in 1.25% of spawners in a given population moving to each of the other 4 populations. Mortality rates for straying spawners matched the PSM rate for the destination population. The straying rates and their distribution across populations reflect documented straying observed for coho in coastal watersheds of the Pacific Northwest (Quinn 1993; VanderHaegen and Doty 1995). Demographic rates, carrying capacities, and initial conditions for all 5 populations were assumed to be equal and were incorporated into the individual population matrix model as described above. The populations in the metapopulation are defined based on their spawning stream location and carrying capacity. Therefore, changes to survival or reproduction, such as PSM, resulting from conditions in a particular stream habitat become intrinsic to the population demographics.

The metapopulation modeling approach was used to forecast the impacts of increasing urbanization (i.e., increasing PSM) on coho salmon in large watersheds and to estimate changes in the abundance of the linked individual populations as well as the larger metapopulation over time. Constant PSM

Table 2. Isolated populations affected by constant or gradually increasing levels of PSM[a]

PSM (%)	Percent of 1000 model runs extinct within 50 y	Average years to extinction (SD)	Minimum years to extinction	Change in λ (%)
0	0	NO	NO	0
10	0	>200	135	−3.4
20	0	135 (30)	65	−7.2
25	1	94 (21)	50	−9.1
30	6	70 (13)	41	−11.2
40	74	44 (7)	26	−15.6
50	100	30 (5)	17	−20.6
60	100	22 (3)	15	−26.3
70	100	16 (2)	10	−33.0
75	100	14 (2)	8	−37.0
80	100	12 (2)	7	−41.5
90	100	8 (2)	5	−53.6
0–25 over 30 y	0	115 (21)	68	NA
0–75 over 20 y	100	30 (2)	24	NA

NO = no extinction deserved; NA = not applicable because the population growth rate ranges over the projection; PSM = prespawn mortality; SD = standard deviation.
[a]The values reflect the incorporation of natural variability in survival rates and fecundity as well as density dependence during 1st-year survival.

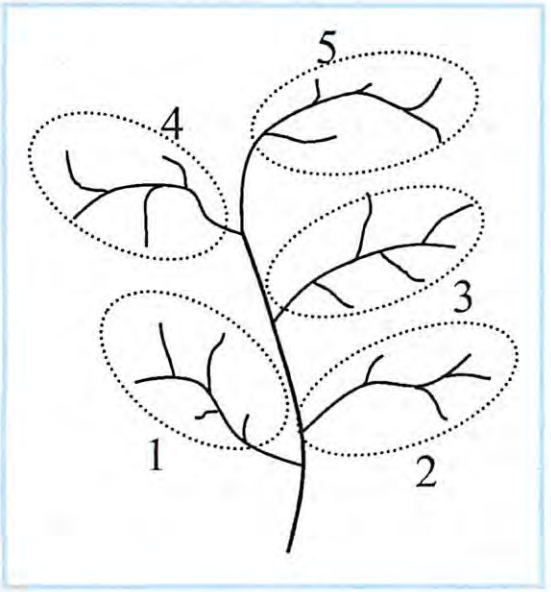

Figure 1. Ovals in the cartoon represent locations of populations in a watershed based on their spawning grounds. Straying between the populations links them into a metapopulation.

levels of 25%, 50%, 75%, and 100% were applied to 1, 2, 3, and 4 of the 5 populations to simulate the gradual and recurrent prespawn die-offs across a watershed that supported an initially viable coho metapopulation. The importance of stray rate for model outcomes was evaluated by comparing results from stray rates of 3%, 5%, and 10%, levels that have been observed for wild coho (Quinn 1993). An additional set of model scenarios investigated how the proportion of the metapopulation impacted by PSM may influence both the population and metapopulation abundance over time. These scenarios also let us assess whether the distribution of the affected proportion across populations affected the responses. The proportion was calculated as the relative equilibrium abundance for each population at its carrying capacity. The metapopulation models discussed above have populations with equal carrying capacities and a population ratio of 1:1:1:1:1. The impacts of PSM to 20%, 40%, 60%, and 80% of the metapopulation were represented as effects on 1, 2, 3, and 4 of the 5 populations, respectively. To determine whether the responses to PSM were influenced by the distribution of the population carrying capacities in the metapopulation, we changed the carrying capacities of the 5 populations to a ratio of 4:2:2:1:1. The total metapopulation abundance at equilibrium was maintained. This allowed us to investigate whether impacts on 40% of the metapopulation consisting of 1 population produced the same results as when 40% was made up of 2 or 3 populations. Simulations for 25% and 75% PSM were run for all possible population combinations to simulate impacts on 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, and 90% of the metapopulation. All of the metapopulation models were run for 50 y and repeated 500 times to integrate natural variability as described above. The output was the adult abundance for each population at year 50. Further analysis was conducted with other carrying capacity ratios with similar results to the 2 sets presented here (data not shown).

RESULTS

The modeled dynamics for solitary coho populations in the absence of PSM (control) produced an intrinsic population growth rate (λ) of 1.03. This resulted in a slow increase in population abundance until the carrying capacity defined by the model (approximately 3300 spawners) was reached. Control projections incorporating natural variability resulted in a range of abundances around an equilibrium with no populations going extinct (Table 2).

When realistic rates of PSM were incorporated into the model, the population growth rate declined in a nonlinear manner. This is indicated as a reduced population growth rate (as the percent of control) in Table 2. Sensitivity analysis showed that PSM reduced the extent to which changes in 1st- and 2nd-year survival contribute to changes in λ. PSM increased the relative importance of reproductive output to changes in λ (Table 1). The demographic rates of this coho salmon model made the population particularly sensitive to impacts on adult reproduction (the stage affected by PSM). Prespawn mortality also altered the age distribution of the population by increasing the proportion of 2nd- and 3rd-year individuals (results not shown).

Solitary and initially viable populations influenced continuously by PSM at a rate of 20% went locally extinct within 135 y. At higher PSM rates of 60%, 70%, 80%, and 90%, initially viable populations declined rapidly (Figure 2A shows output for 60% and 80%) with mean times to extinction of 22, 16, 12, and 8 y, respectively (Figure 2B and Table 2).

To simulate the population-scale impacts of PSM in response to varying rates of urbanization within a watershed, rates of PSM were gradually increased over time. A scenario that simulated rapid urbanization incorporated an increase in PSM from 0% to 75% over 20 y. A second scenario represented a more moderate rate of urban development with a less severe impact (0%–25% PSM) over a longer time interval (30 y; Figure 3). With all initial conditions equal, coho populations predictably persisted longer when the rate of PSM increased more slowly. When PSM rates were increased to 75% over 20 y, the model output predicted the extinction of a solitary coho population in 30 y. This was extended to 115 y for a population experiencing a lower rate of PSM (25%) over 30 y (Table 2).

Connecting 5 individual coho model populations through straying created a metapopulation and afforded individual populations some degree of protection from localized extinction when at least 1 unaffected population served as a source of straying emigrants to the other populations. Whereas constant rates of PSM drove solitary coho populations to extinction, the larger metapopulation and the populations within it declined but did not go extinct when only 1 to 3 of the 5 component populations were impacted by PSM at these same rates. Transient, localized extinctions of the affected component populations were observed when 4 of 5 populations experienced $\geq$20% PSM. When the rate of PSM across 4 populations was 25% and the 5th experienced 10% PSM, approximately half (52%) of the model outputs resulted in metapopulation-wide extinctions. As expected, gradually increasing the number of populations experiencing a given level of PSM produced a decreasing equilibrium adult abundance in both the affected and unaffected populations (Figure 4 and Table 3). These results show that individuals straying from source populations to PSM-affected populations within a metapopulation can prevent the extinction of

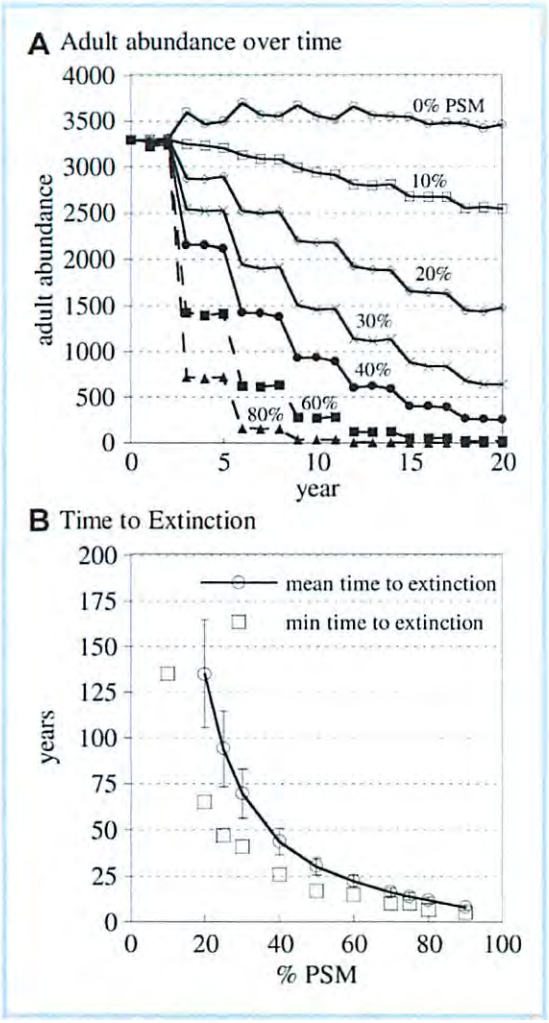

Figure 2. (A) Mean coho adult abundance for solitary populations experiencing density dependence, natural variability in survival and fecundity, and constant rates of prespawn mortality (PSM). (B) Estimated mean time to localized extinction for isolated coho populations experiencing various levels of PSM using density-dependent models with no straying. Mean time to extinction (○), standard deviation, and minimum time to extinction (–) for 1000 repetitions.

the latter (as predicted by the model for solitary populations). For example, a solitary population experiencing 75% PSM can be expected to decline to extinction in an average of 14 y. However, if the same affected population is connected to a source population by straying, the model predicted a significant decline (to <6% of control) but no extinction over the 50-y run of the model using a 5% stray rate (Table 3).

When individual populations within the metapopulation became sink populations, with more adult spawners straying in than out, a net (indirect) effect was a reduction in the abundance of the remaining source populations. The unaffected population (Population 5) declined due to a net loss of strays as other populations were increasingly impacted by PSM (Figure 4). In this example, the decrease in source population abundance (model Population 5) over time due to 75% PSM in 1, 2, 3, and 4 of the other populations resulted in equilibrium abundance reductions to 94%, 87%, 80%, and 71% of control abundances, respectively (Figure 4 and Table 3). At the scale of the entire metapopulation, even

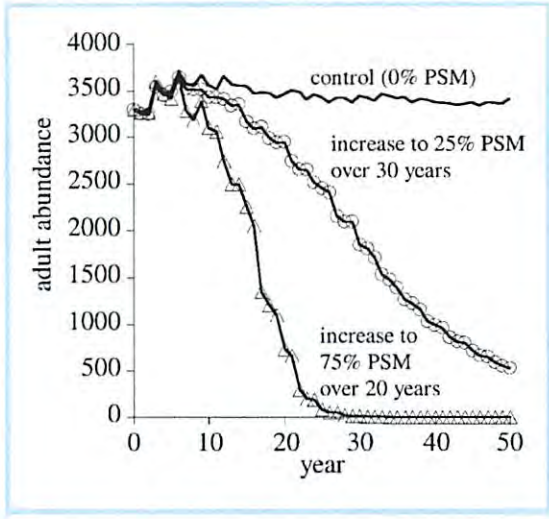

Figure 3. Adult abundance for isolated coho populations exposed to gradually increasing levels of prespawn mortality (PSM). Slow increases to 25% PSM over 30 y (○) and a rapid change to 75% PSM over 20 y (Δ).

low rates of PSM affecting a subset of populations produced large reductions in equilibrium abundance due to these source-sink dynamics (Table 3).

The proportion of the metapopulation experiencing PSM (i.e., the number of sinks) was a stronger driver for loss of potential productivity than the relative rate of PSM. For example, a single population experiencing 50% PSM reduced the overall metapopulation abundance by 23%, whereas 2 populations experiencing PSM at half the rate (25%) reduced the metapopulation abundance by 38% (Table 3). Similarly, the proportion of the metapopulation experiencing PSM was a more important determinant of the final metapopulation abundance than the rate of PSM (Figure 5). This was shown by changing the relative proportions of the carrying capacity and initial abundances of the 5 populations from a ratio of 1:1:1:1:1 to a ratio of 4:2:2:1:1. The modeled rates of PSM

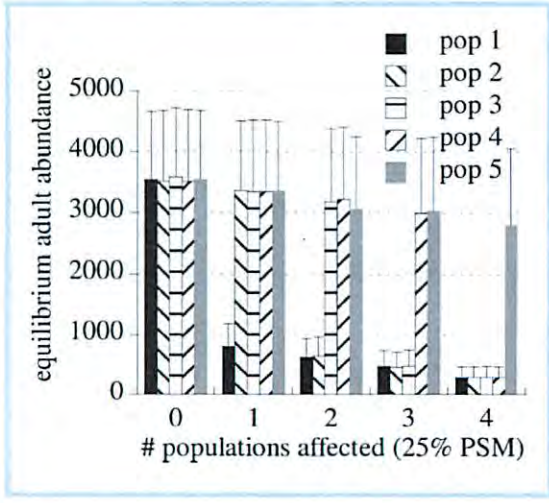

Figure 4. Equilibrium adult abundance of each population (pop) within the metapopulation after constant 25% prespawn mortality (PSM) affecting 0 to 4 populations for 50 y. Coho metapopulation models incorporate variable annual survival and fecundity, 1st-year density dependence, and 5% straying within the metapopulation.

Table 3. Impacts of PSM showing direct and indirect effects on each of the 5 populations and the whole metapopulation as the percentage of the control abundances (SD)[a]

Scenario	Control abundance (%)		
	Impacted populations	Unimpacted populations	Metapopulation
1 population affected (20% of metapopulation)			
25% PSM	23 (10)	94 (34)	82 (15)
50% PSM	9 (3)	94 (34)	78 (14)
75% PSM	6 (2)	94 (32)	76 (14)
Range 0%–25% PSM	52 (28)	98 (33)	89 (16)
Range 25%–75% PSM	11 (3)	93 (33)	78 (14)
Range 50%–100% PSM	6 (2)	93 (34)	76 (14)
2 populations affected (40% of metapopulation)			
25% PSM	18 (8)	89 (34)	62 (13)
50% PSM	7 (3)	88 (34)	56 (13)
75% PSM	4 (2)	87 (35)	55 (13)
Range 0%–25% PSM	48 (27)	93 (33)	76 (15)
Range 25%–75% PSM	8 (3)	89 (35)	57 (13)
Range 50%–100% PSM	4 (2)	87 (34)	54 (13)
3 populations affected (60% of metapopulation)			
25% PSM	13 (7)	85 (34)	42 (12)
50% PSM	4 (2)	80 (35)	35 (11)
75% PSM	3 (1)	80 (35)	34 (11)
Range 0%–25% PSM	44 (27)	92 (33)	64 (14)
Range 25%–75% PSM	5 (2)	80 (36)	35 (12)
Range 50%–100% PSM	3 (1)	80 (35)	34 (12)
4 populations affected (80% of metapopulation)			
25% PSM	8 (5)	79 (37)	23 (9)
50% PSM	2 (1)	71 (37)	17 (8)
75% PSM	1 (1)	71 (37)	15 (8)
Range 0%–25% PSM	39 (25)	88 (34)	49 (14)
Range 25%–75% PSM	2 (1)	73 (37)	16 (10)
Range 50%–100% PSM	1 (1)	59 (39)	16 (9)

PSM = prespawn mortality; SD = standard deviation.
[a]Each scenario was run for 50 y and repeated 1000 times. The percentage of initial metapopulation carrying capacity that is impacted is indicated at the top of each section.

were then adjusted to 25% and 75%. This relatively large difference in PSM rate produced only slight differences in the relationship between the proportion impacted and metapopulation abundance. The SD about the mean metapopulation abundances for the 25% and 75% PSM overlapped (suggesting no significant difference) and the linear regressions for abundances at the 2 rates of PSM had similar slopes (−97.167 and −104.37 for 25% and 75%, respectively) (Figure 5).

The influence of stray rates on the responsiveness of a coho metapopulation to PSM was investigated by varying straying between populations (3%, 5%, and 10%) while holding PSM constant at 75%. At lower rates of straying, PSM-affected populations were more isolated and therefore more likely to

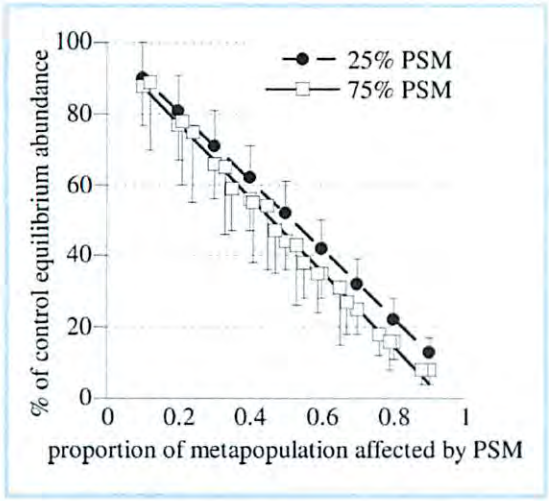

Figure 5. The influence of the level of prespawn mortality (PSM; 25% and 75%) relative to the proportion of the metapopulation that was affected by PSM. The percent of control metapopulation equilibrium abundance after 50 y experiencing 25% (●) or 75% (□) PSM. The *x* axis shows the proportion of the metapopulation carrying capacity that is exposed to PSM.

experience localized extinction when 3 or more linked populations were affected. A higher stray rate provided more emigrants from source populations and maintained PSM-affected populations at higher abundances (Figure 6). However, the larger migrant exchange connecting the populations came at a cost in terms of reducing the total metapopulation abundance.

DISCUSSION

The coho prespawn mortality models developed for this study predicted rapid declines in adult abundances for isolated populations affected by PSM. Importantly, local population extinctions were predicted across the range of PSM rates that have been recently documented in Puget Sound urban streams (20%–90%) (McCarthy et al. 2008; Wild Fish Conservancy 2008; City of Seattle 2007). Interannual variation in spawner die-offs may slightly delay predicted population declines and extinction. Nevertheless, models simulating slow, gradual increases in PSM predicted significant declines in the abundance of solitary wild coho populations. Overall, our analysis shows that PSM could pose an extinction risk for local coho populations on a timescale of a few years to a few decades.

Under natural conditions, up to 10% of adult coho may stray to nearby populations each spawning season. When straying was integrated into the metapopulation models, PSM-affected populations within the larger metapopulation were less likely to go extinct. As expected, migrant strays from unaffected populations provided directly impacted populations a degree of protection from extinction. Accordingly, rates of PSM that drove isolated populations extinct had less impact on populations linked by straying. However, by creating sink populations that drain nearby source populations, PSM has the potential to reduce local abundance of coho in habitats of a watershed that are unaffected by development or polluted runoff (Figures 4 to 6). We therefore expect straying to have 2 important consequences for wild coho metapopulations affected by PSM. First,

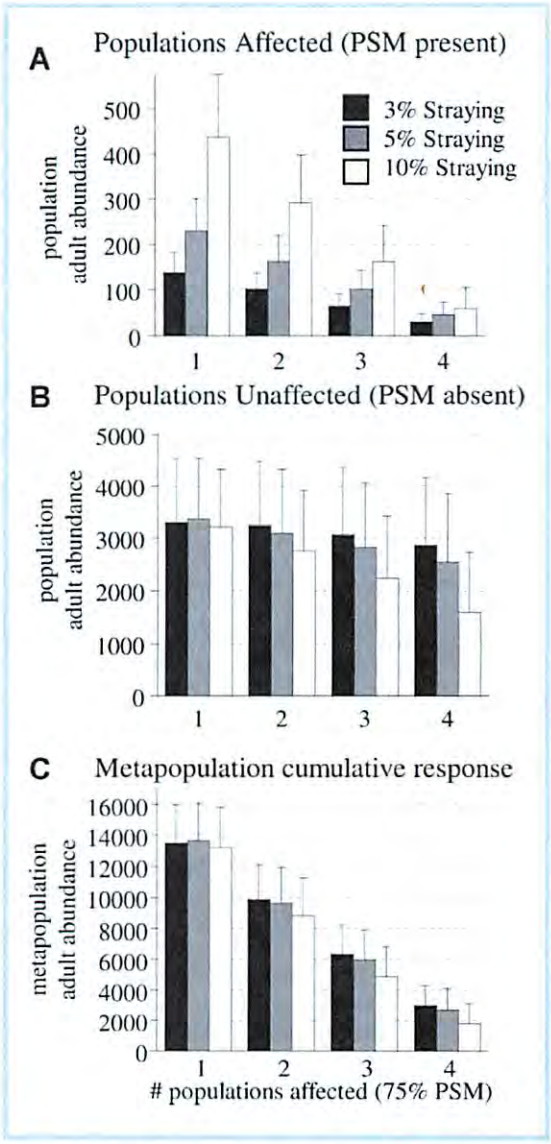

Figure 6. Adult abundances (mean and standard deviation [SD]) for (A) populations experiencing 75% prespawn mortality (PSM), (B) unimpacted populations, and (C) metapopulation for 1 to 4 populations affected by PSM that exhibit 3%, 5%, and 10% straying rates. One impacted and 1 unimpacted population is depicted for each scenario because the remaining populations have similar means to their respective counterparts. SD subtracted from the mean was not shown for image clarity.

straying will provide a buffer against local extinction in urbanizing stream catchments. Second, the source–sink dynamics of the metapopulation will draw down coho spawner abundances in geographic areas where freshwater habitat conditions are favorable.

The number and relative size of PSM-affected populations within a metapopulation was a more important determinant of declining metapopulation abundance than the rate of PSM. For example, a relatively large shift in PSM rate (25% to 75%; Table 3 and Figure 5) produced only a slight reduction in the overall number of spawners in the metapopulation. The relative size of individual populations within a metapopulation is determined by local carrying capacity (i.e., availability of spawning and rearing habitat). Therefore, PSM impacts on

Puget Sound coho will be more significant if the occurrence of PSM is distributed across multiple linked populations, or if the affected populations occupy habitats that would otherwise be highly productive for the metapopulation. Although our current understanding of adult spawner mortality is drawn from streams where die-off rates are high (up to 90%), this finding points to the ongoing, incremental, and distributed development of Puget Sound lowland areas (Alberti et al. 2007) as a potentially important driver for future coho metapopulation declines.

The relative impacts of adult spawner die-offs on source and sink populations were strongly influenced by different rates of straying. With increased straying, the supplementary spawners from source populations effectively masked the local impacts of polluted terrestrial runoff. By delaying local extinctions, straying has the potential to make it more difficult to accurately assess the location-specific impacts of PSM at a population scale. For example, it may be difficult during the course of conventional census activities within an urbanizing watershed to detect the initial impacts of PSM on local coho abundance. Conversely, the indirect drains of PSM on source populations may increase their vulnerability to emerging stressors that are unrelated to urban growth and development. These include, for example, future climate change and freshwater quantity for river systems in the Pacific Northwest (Mote et al. 2005; Battin et al. 2007; Williamson et al. 2008).

Our model results suggest that the Seattle-area urban streams that have been surveyed for spawners over the last decade are all sinks. Self-sustaining wild coho runs have largely disappeared from the streams where coho PSM has been documented to date. PSM-affected wild coho have been observed in urban streams (McCarthy et al. 2008; Wild Fish Conservancy 2008). However, most of the adults that return to spawn in these drainages are products of artificial propagation (as identified by coded wire tags and the absence of adipose fins). Regional hatchery operations have therefore served as "source" populations for these restored urban streams. Daily spawner surveys for PSM in streams that traverse current urban growth boundaries have not been conducted. Therefore, at present, we do not have an accurate understanding of the source-sink dynamics for wild Puget Sound coho that are likely to be increasingly impacted by PSM over the timescales considered in this study. More work in this area is needed.

Given our finding that even moderate rates of spawner mortality (approximately 40%) can lead to rapid declines in the local abundance of adults in only a few years, it may be difficult to restore coho to Pacific Northwest streams where urban runoff and associated PSM are likely to occur. This has several implications for future stream restoration projects, particularly in the vicinity of regional urban growth boundaries. First, an increase in natural coho production should not necessarily be an expected outcome for projects in highly urbanized watersheds, particularly if these projects do not specifically address water quality and nonpoint source runoff. Second, reconnecting urban stream segments (e.g., via culvert removal) can have the unintended consequence of drawing coho spawners into contact with urban runoff (Jackson and Pringle 2010). If PSM ensues, this would have the net effect of transforming the restored habitat into a sink habitat for coho (an attractive nuisance habitat; Simenstad et al. 2005). Third, consideration should be given to PSM before new restoration projects are implemented in urbanizing areas. This might include, for example, preproject surveys for local spawner mortality. Baseline information on PSM will help ensure the physical objectives of stream habitat restoration (Bernhardt and Palmer 2007; Katz et al. 2007) are not undermined by degraded water quality.

Incorporating population source-sink dynamics into management decisions could also help identify appropriate areas in a watershed that should be protected from urban sprawl. The protected habitats could support source populations that could then maintain PSM-affected populations whereas remediation efforts may be established for those runs. Protected upstream habitat areas within urbanizing watersheds may still see losses at the metapopulation level because PSM-affected populations will drive the unaffected populations and metapopulation downward, especially as the impacted proportion of the metapopulation increases. Protecting habitat that supports large proportions of the metapopulation's spawners will be more effective than protecting headwaters alone.

In the larger picture of cumulative toxic stormwater impacts on wild coho populations, our modeled predictions only incorporate losses at the spawner stage, and do not aggregate impacts across the entire coho salmon life cycle. As noted earlier, coho spend more than 1 y rearing in freshwater environments, including small streams that are receiving waters for urban runoff containing metals, pesticides, petroleum hydrocarbons, plasticizers, and other nonpoint source pollutants. Low-level exposures to these contaminants can adversely affect juvenile salmon physiology and behavior (Sandahl et al. 2005, 2007), and they also pose potentially important but poorly understood threats to stream food webs that support coho growth and survival (Macneale et al. 2010). Given this potential for toxic-driven losses at other life stages, our model outputs here are likely underestimates of the overall population-scale effects of toxic runoff in urban and urbanizing watersheds. Future modeling efforts should therefore be expanded to capture potential toxicity to coho embryos, alevins, parr, and smolts.

CONCLUSIONS

In conclusion, our current results show that degraded stormwater quality has the potential to drive local coho populations to extinction on a timeline of a few years to a few decades based on the observed, real-world range of spawner mortalities in Puget Sound urban streams. Moreover, focal coho spawner losses in developed catchments can constrain population abundances in undeveloped regions of river basins through source–sink metapopulation dynamics. Spawner mortality rates are closely associated with land cover attributes within catchments (impervious area, road density, and so forth; B.E. Feist, personal communication). It will therefore be possible in future studies to estimate water quality driven declines in coho abundances for specific population segments that spawn along discrete gradients of urbanization in western North America (e.g., the threatened Lower Columbia River ESU). This, in turn, will inform current efforts to conserve and recover ESUs in large river basins where human population growth and development represent persistent, ecosystem-scale forcing pressures on freshwater habitat quality.

Acknowledgment—This study was supported by the NOAA Coastal Services Center (Coastal Storms and Ocean Research

Priority Programs), USEPA Region 10, and grants from the King Conservation District and the US Fish and Wildlife Service's National Contaminants Program.

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PLoS one

Landscape Ecotoxicology of Coho Salmon Spawner Mortality in Urban Streams

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Abstract

In the Pacific Northwest of the United States, adult coho salmon (*Oncorhynchus kisutch*) returning from the ocean to spawn in urban basins of the Puget Sound region have been prematurely dying at high rates (up to 90% of the total runs) for more than a decade. The current weight of evidence indicates that coho deaths are caused by toxic chemical contaminants in land-based runoff to urban streams during the fall spawning season. Non-point source pollution in urban landscapes typically originates from discrete urban and residential land use activities. In the present study we conducted a series of spatial analyses to identify correlations between land use and land cover (roadways, impervious surfaces, forests, etc.) and the magnitude of coho mortality in six streams with different drainage basin characteristics. We found that spawner mortality was most closely and positively correlated with the relative proportion of local roads, impervious surfaces, and commercial property within a basin. These and other correlated variables were used to identify unmonitored basins in the greater Seattle metropolitan area where recurrent coho spawner die-offs may be likely. This predictive map indicates a substantial geographic area of vulnerability for the Puget Sound coho population segment, a species of concern under the U.S. Endangered Species Act. Our spatial risk representation has numerous applications for urban growth management, coho conservation, and basin restoration (e.g., avoiding the unintentional creation of ecological traps). Moreover, the approach and tools are transferable to areas supporting coho throughout western North America.

Citation: Feist BE, Buhle ER, Arnold P, Davis JW, Scholz NL (2011) Landscape Ecotoxicology of Coho Salmon Spawner Mortality in Urban Streams. PLoS ONE 6(8): e23424. doi:10.1371/journal.pone.0023424

Editor: Howard Browman, Institute of Marine Research, Norway

Received January 31, 2011; **Accepted** July 17, 2011; **Published** August 17, 2011

Funding: This work was supported by the National Oceanic and Atmospheric Administration - Coastal Storms Program; U.S. Fish and Wildlife Service - National Contaminants Program; City of Seattle (Seattle Public Utilities); and the U.S. Environmental Protection Agency - Region 10. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

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Introduction

In recent decades, human population growth and development have continued to increase along the coastal margins of North America [1]. The associated changes in land cover and human land use have elevated land-based sources of pollution, and toxic stormwater runoff in particular, to become one of the most important threats to the biological integrity of basins, lakes, estuaries, and nearshore marine environments [2]. In the United States, concerns related to non-point source pollution have gained momentum over the past decade (e.g., [3,4]). This has culminated most recently in the designation of "water quality and sustainable practices on land" as one of nine National Priority Objectives for the newly established National Ocean Council, together with ecosystem-based management, marine spatial planning, climate change and ocean acidification, and changing conditions in the Arctic [2]. For toxic runoff, however, the connections between unsustainable practices on land and the decline of ecological resilience in aquatic habits remain poorly understood.

In western North America, semelparous anadromous salmonids (*Oncorhynchus* spp.) typically migrate thousands of kilometers in their lifetimes. They hatch and rear in freshwater, migrate seaward to capitalize on the productivity of the oceans to grow rapidly and reach sexual maturity, and then return to their natal streams to spawn and die. Certain salmonids, including pink (*O. gorbuscha*) and chum (*O. keta*) migrate to the ocean relatively soon after hatching. Others, however, such as Chinook (*O. tshawytscha*), steelhead, (*O. mykiss*), sockeye (*O. nerka*), and coho (*O. kisutch*) may spend one or more years in freshwater lakes, rivers and streams. Because of this extended freshwater residency, juveniles of these species are potentially more vulnerable to anthropogenic modifications of freshwater habitat quality [5].

In contrast to the high mortality experienced by juvenile salmonids, mortality at the adult spawner life stage is relatively low. Familiar natural causes of mortality include predation, disease [6,7,8,9], stranding (following high flows), elevated stream temperatures, and competition – e.g., in habitats with abundant salmon returns and limited spawning substrate. Various human activities such as recreational and commercial fishing, stream dewatering, and the placement of migration barriers can also increase salmon spawner mortality. In general, however, salmon spawner mortality has not been attributed to toxic chemical contaminants in stormwater runoff – a data gap that may be due, in part, to 1) the relative rarity of salmon spawners in urban basins with poor water quality, and 2) the logistical difficulty of implementing toxicity studies on migratory, seawater-to-freshwater transitional adults.

The exception is a recently documented phenomenon of returning adult coho salmon dying at high rates in urban and urbanizing streams in lowland Puget Sound region, which includes

the greater Seattle metropolitan area [10]. Coho return to small coastal stream networks to spawn each fall. Entry into freshwater is triggered by early autumn rainfall and rising stream flows. Since there had been extensive habitat degradation and loss in these lowlands, many basins were targeted for stream restoration projects in the 1990s. Subsequent surveys to evaluate project effectiveness discovered that many coho salmon were dying in newly-accessible stream reaches before they were able to spawn – i.e., female carcasses were found in good condition (ocean bright colors) with skeins (membrane or sac that contains the eggs within the fish) filled with unspawned eggs [10]. In addition, affected coho from several different urban basins showed a similar progression of symptoms leading up to death, including disorientation, lethargy, loss of equilibrium, mouth gaping, and fin splaying. Systematic daily spawner surveys in recent years (2002–2009) have shown that adult mortality rates in urban streams are consistently high (relative to spawning coho salmon in more pristine areas), ranging from ~25–90% of the total fall runs [10]. Mortality rates of this magnitude likely have important negative consequences for maintaining viable coho populations [11]. Consistent with this, most coho mortalities observed over the past decade were spawners that strayed (did not home to their natal stream reaches) into these restored urban freshwater habitats.

The precise underlying cause of recurrent coho die-offs remains under investigation. An initial weight-of-evidence forensic study has systematically ruled out stream temperature, dissolved oxygen, poor overall spawner condition, tissue pathology (e.g., gill), pathogen prevalence or disease, and other factors commonly associated with fish kills in freshwater habitats (Scholz et al., unpublished data). These findings, together with the rapid onset of the syndrome, the nature of the symptoms (e.g., gaping and disequilibrium), and the consistent re-occurrence within and between urban basins over many years together point to toxic stormwater runoff from urban landscapes as the likely cause of coho spawner mortality. Urban runoff and stormwater-influenced combined sewer overflows (CSOs) contain an exceptionally complex mixture of chemical contaminants. Specifically, urban streams are receiving waters for runoff and discharges containing pesticides [12], metals [13], petroleum hydrocarbons [14], plasticizers, flame-retardants, pharmaceuticals, and many other potentially toxic chemicals. The list of possible causal agents is therefore long.

The above chemical complexity notwithstanding, there are several reasons to suspect motor vehicles as sources of toxics that are killing returning coho. Vehicles deposit many compounds on road surfaces via exhaust emissions, leaking fluids, and the wearing of tires, brake pads and other friction materials [15]. Emissions contain nitrogen and sulfur dioxide, benzene, formaldehyde, and a large number of polycyclic aromatic hydrocarbons (PAHs). Fluids, including antifreeze and motor oil, contain ethylene and propylene glycol and PAHs. Tire wear releases zinc, lead, and PAHs onto road surfaces [16], and brake pad wear is a major source of copper, zinc, nickel, and chromium [16,17]. Collectively, these contaminants accumulate on streets and other impervious surfaces until they are mobilized by rainfall and transported to aquatic habitats via runoff. Polycyclic aromatic hydrocarbons and metals such as copper are known to be toxic to fish, although acute lethality usually occurs at exposure concentrations that are higher (by orders of magnitude) than those typically detected in urban streams. It is likely that fall stormwater pulses contain higher concentrations than winter and spring due to the potential buildup of contaminants during the relatively dry summer months.

Although the adult die-off phenomenon has been observed in all Seattle-area urban streams where coho salmon occur, the overall rate of mortality has varied among basins. In qualitative terms, a higher proportion of returning animals have survived to spawn in basins that have more open space (e.g., parks and woodlands). Conversely, mortality rates have been consistently higher in basins with proportionately greater "urban" land cover and land uses. This raises the possibility of a quantitative relationship between discrete basin characteristics and coho survival and spawning success. Such a relationship would be important for several reasons. First, if coho mortality is significantly correlated with one or more land cover or land use variables, the latter could be used to identify unmonitored lowland basins where coho populations are at greatest risk. Second, it could provide a means to evaluate how future human population growth and development might impact wild coho populations in Puget Sound (and elsewhere) that are currently healthy. Finally, it could narrow the list of potentially causative pollution sources in urban basins, thereby focusing future toxicological studies to identify the specific contaminants involved.

In this study we performed a spatial analysis to identify landscape variables that correlate most closely with surveyed rates of coho spawner mortality across six different basins in Puget Sound. The variables included land use and land cover, tax parcel types, roadways, and impervious surfaces. We then used the information from these correlations to generate spatially explicit predictions of recurrent spawner losses in unmonitored basins throughout the four most densely populated counties in the greater Seattle metropolitan area.

Materials and Methods

Study Sites

We characterized habitat conditions within the drainage basins from streams at six sites in the Puget Sound lowlands (Figure 1). We chose these sites because coho spawner mortality has been monitored at these locations for several years (2000–2009; [10]). The sites represent a wide range of anthropogenic alteration, from highly urbanized (e.g., Longfellow Creek) to relatively undisturbed (e.g., Fortson Creek). Fortson Creek is considered a non-urban site, whereas the other five sites are urban streams and have varying degrees of development. The urban streams have all been a focus of varying restoration project efforts aimed at enhancing habitat quality for anadromous Pacific salmon. With the exception of the relatively unaltered Fortson Creek site, all site basins had impervious surface proportions well above the levels (5–10%) commonly associated with the decline of biological integrity in streams [18,19].

Confirmed observation of the coho spawner mortality syndrome (see below) within a stream system was a key factor in study site selection. Importantly, natural production of coho in Seattle-area urban streams is very low. Not unexpectedly, recent modeling has shown that local coho population abundance declines precipitously at rates of spawner mortality documented for these drainages [11]. The adult returns to these streams are thus likely to be animals straying into sink or attractive nuisance habitats. Conversely, the syndrome has not been documented in streams where coho are relatively abundant – i.e., non-urban basins, as confirmed by a full season of daily stream surveys on Fortson Creek. Therefore, to evaluate the phenomenon in relation to land cover, we were constrained to streams where coho are affected, even if adult returns to these basins were low in certain years. Lastly, there is no evidence that the mortality syndrome is related to the origin of the spawners (i.e., hatchery vs. wild fish). For example, artificially propagated coho that return as adults to regional hatchery facilities in non-urban basins are unaffected.

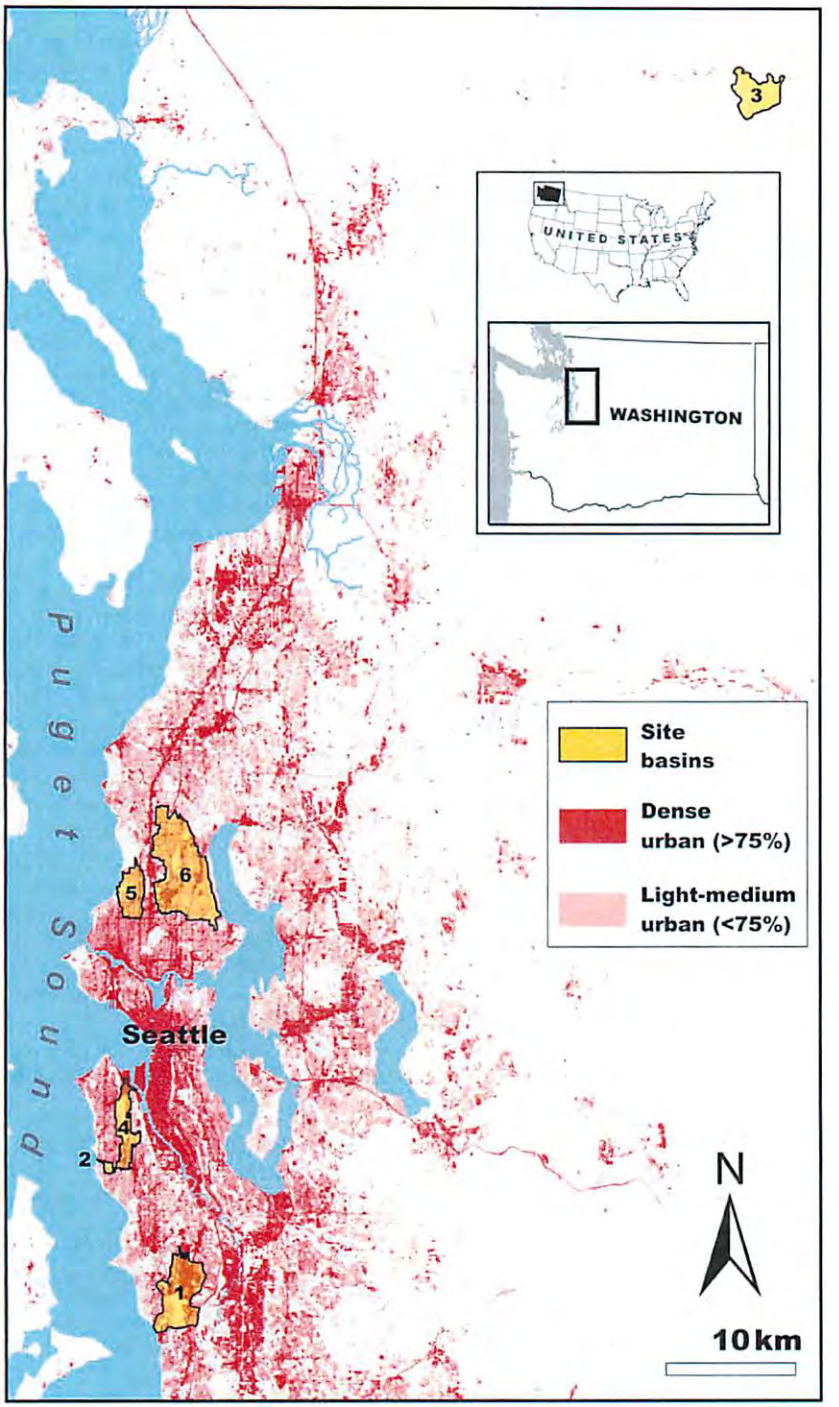

Figure 1. Six study sites where coho spawner mortality was monitored and landscape conditions were quantified. Main map depicts the Greater Seattle Metropolitan Area in Washington State, which is within the Puget Sound/Georgia Basin of the Pacific Northwest, United States of America (USA). Inset map illustrates location of the study sites within Washington State and the location of Washington State within the USA. For reference, red shading on main map represents the relative intensity of urbanization (light-medium and dense urban [23,24]). Drainage basins depicted in yellow shaded polygons represent the total basin flowing into a given stream reach site. Key for site numbers: 1 = Des Moines; 2 = Fauntleroy; 3 = Fortson; 4 = Longfellow; 5 = Piper's; and, 6 = Thornton Creek.
doi:10.1371/journal.pone.0023424.g001

Study Subjects

Coho salmon in this study were all within the Puget Sound/Strait of Georgia Evolutionarily Significant Unit (ESU). An ESU is defined as a group of populations that 1) are substantially reproductively isolated from conspecific populations and 2) collectively represent an important component in the evolutionary

legacy of the species [20]. Currently, Puget Sound/Strait of Georgia coho are designated a "species of concern" under the U.S. Endangered Species Act [21].

Coho typically spawn in small (lower order) streams in the Puget Sound lowlands in late fall and early winter and their fry emerge from stream substrates from March to May. Fry reside in riverine habitats for 14–18 months, smolt, migrate to marine environments where they grow rapidly and mature (16–20 months), and finally migrate to their natal basins where they spawn and die [22]. The adult spawners from the six study basins were both marked (adipose fin clipped) and unmarked, suggesting a mix of hatchery and wild origins.

Coho Spawner Mortality

We used existing monitoring data collected as part of daily and weekly spawner surveys in each of the six study locations (Table 1). Data were collected during the fall spawning season from 2000–2009 by Seattle Public Utilities (SPU), the Wild Fish Conservancy, and the Northwest Fisheries Science Center (NWFSC). Streams were checked every few days in the early fall (usually the first or second week in October, depending on rainfall) until the first adult coho was observed. The streams were then surveyed daily for the duration of the fall run, until the last carcass was documented, typically in the first or second week of December. For several years, biologists working for the City of Seattle (Wild Fish Conservancy) also surveyed many of the same urban streams for coho spawner mortality on a weekly basis. Side-by-side comparisons of daily and weekly survey data (e.g., for Longfellow Creek in 2005 and 2007) revealed practically no loss of carcasses to scavengers. Accordingly, we included the weekly survey data in our analyses.

The entirety of the available spawning habitat within a given urban drainage was surveyed for premature adult coho mortality. For some streams, including Longfellow Creek, mid-stream barriers to upstream migration confined adults to the lower portions of the drainage. This made it possible, in the course of a few hours as part of a daily survey, to inspect all sections of the stream that 1) had a gravel substrate suitable for redds (spawning "nests" built by females), and 2) were focal areas for repeated (year-to-year) redd building during successive spawner runs.

Monitoring data were not collected at all sites for all years (Table 1). Mortality among returning coho was quantified only for females on the basis of egg retention – i.e., the number of partially spawned or unspawned female carcasses observed in streams over an entire spawning season. Notably, the total number of returning adults was low for some years and some basins (Table 1). Nevertheless, the aggregate spawner survey data used in this analysis are the most comprehensive currently available.

Geospatial Datalayers

We used existing geospatial datalayers as our source of potential predictor variables and as a proxy for habitat type and condition. The datalayers were generated by a variety of organizations for planning and analytical purposes, making them suitable for running spatial analyses on habitat. They were also available over the entire spatial domain of our predictive model. We used four geospatial datalayers: Land-cover of the Greater Puget Sound Region [23,24]; impervious and impacted surfaces [25]; property type (compiled from King [26], Kitsap [27], Pierce [28] and Snohomish county [29] tax parcel databases), and roadways (Puget Sound Regional Council; PSRC [30]).

The Land-cover of Puget Sound datalayer is the highest quality and most accurate depiction of land use and land cover in the Puget Sound lowlands. The datalayer used 30 m gridded LANDSAT TM imagery from 2002, which was extensively analyzed and corrected to produce an accurate (83% overall accuracy, [24]) depiction of land use and land cover conditions. To reduce the total number of potential predictor variables, we only used the dense urban (>75%); light to medium urban (<75%); and grass, crops and/or shrubs categories. We also combined the mixed and deciduous forest with the coniferous forest category and named it forests.

The impervious and impacted surfaces datalayer was derived from a 2001 LANDSAT TM image with 30 m pixels and an accuracy of 83–91% [25]. This datalayer depicts high to completely impermeable surfaces such as building roofs; concrete or asphalt roads and parking lots; concrete, asphalt or brick sidewalks, pedestrian walkways, and malls; etc.

One of the limitations of these two datalayers was that the pixel size of the source LANDSAT TM imagery is 30 m, so smaller

Table 1. Coho spawner mortality proportion and cumulative number of female carcasses enumerated (in parentheses) by site (columns) and year (rows).

	Des Moines	Fauntleroy	Fortson[1]	Longfellow	Piper's	Thornton
2000	-	0.25 (12)	-	0.74 (135)	0.18 (17)	0.88 (33)
2001	-	0.22 (9)	-	0.61 (111)	0.70 (37)	0.82 (11)
2002	-	0.00 (1)	0.01 (114)[a]	0.86 (57)[a]	0.60 (10)	080 (5)
2003	-	(0)	-	0.67 (18)[a]	0.00 (1)	1.00 (2)
2004	0.63 (30)[a]	(0)	-	0.89 (9)[a]	0.33 (3)	1.00 (1)
2005	-	0.75 (4)	-	0.72 (75)[a]	0.75 (4)	0.50 (8)
2006	-	(0)	-	1.00 (4)[a]	1.00 (9)[a]	1.00 (4)
2007	-	0.75 (4)	-	0.73 (41)[a]	0.20 (5)	0.80 (5)
2008	-	-	-	0.67 (12)[a]	-	1.00 (2)
2009	-	-	-	0.78 (36)[a]	-	-
Overall	0.63 (30)	0.37 (30)	0.01 (114)	0.72 (498)	0.57 (86)	0.83 (71)

A dash (-) indicates survey was not conducted for that year/site.
[a]Northwest Fisheries Science Center (NWFSC) daily surveys, all others were weekly and collected by Seattle Public Utilities (SPU) or the Wild Fish Conservancy [51,52].
[1]Non-urban site.
doi:10.1371/journal.pone.0023424.t001

features, such as roads and precise land cover boundaries, were not adequately captured. In order to address this deficiency, we analyzed property types and roadways, as they are represented as precise polyline and polygon delineations of the corresponding land cover variables. The boundaries in these geospatial datalayers were derived from precise survey data from major metropolitan areas, collected over many years by King, Kitsap, Pierce and Snohomish Counties.

The property types (parcels) datalayer was based on ground surveyed delineations of property, which are used for taxation purposes, with positional accuracy of +/−12 m or less [26,27,28,29]. The original number of parcel types described by each county was between 103 and 292. Using the descriptions in the documentation that accompanied the datalayers, we were able to place each of the original parcel types into one of the five following categories: apartments and condominiums; commercial; industrial; parks and open space; and, residential.

The roadways datalayer was based on ground surveyed road and street centerlines. Each segment had a corresponding functional classification (FC##) code and width, as defined by the Federal Highway Administration [31] Highway Performance Monitoring System, and the Puget Sound Regional Council [30], respectively. We reduced the original nine functional classification types down to two categories: 1) heavily used roads (rural minor collector [FC08]; urban principal arterial - interstate [FC11]; urban principal arterial - other freeways and expressways [FC12]; urban principal arterial - other [FC14]; urban or rural minor arterial [FC16 or FC06]; urban collector [FC17]); and, 2) urban or rural local access roads (FC09 or FC19). We then calculated the total area (total length of given street centerline segment multiplied by its width) of each street functional classification for each corresponding site basin.

Spatial Analyses

We defined the area of influence of the surrounding landscape for each site as the total area draining into that site (basin). Drainage basins for each site were generated using the 'flowaccumulation' command in Environmental Systems Research Institute (ESRI) ArcGIS (v. 9.3). We used a United States Geological Survey (USGS) 10 m digital elevation model (DEM) as the underlying terrain for generating basins. We then intersected the corresponding basin boundary for each of the six sites with each of the geospatial datalayers and their associated categories using ArcGIS. We quantified each geospatial datalayer and its associated category in a given basin as the fraction or proportion of the total area of the basin occupied by that geospatial datalayer or category. Longfellow Creek stood apart from the other sites in terms of the accuracy of the flow accumulation model because an unknown fraction of stormwater runoff in this drainage is diverted into the municipal sewer system. Therefore, the theoretical basin area, based on the terrain represented in the DEM, was not as representative of the true basin area compared with the other five sites.

Statistical Analyses

We used generalized linear mixed-effects models (GLMMs; [32,33]) to test the relationships between geospatial variables and coho spawner mortality. The response was binomial (observed number of female spawner mortalities each year, given the total number of female coho that returned to each site) and the models used a logit link function. All models included a random effect of site on the intercept, which accounts for nonindependence of the repeated samples taken at each site. We constructed a set of 139 candidate models by considering all combinations of the 12 predictors taken one, two, three or four at a time, with the restriction that a model could include at most one predictor from each of the four geospatial datalayers (land cover, impervious surfaces, property types, and roadways). We also excluded combinations of predictors that had a pairwise Spearman rank correlation exceeding 0.9 in absolute value. The candidate set included an intercept-only model as a no-effect baseline against which we could assess the predictive power of the geospatial variables.

We fitted the models using the Laplace approximation to the marginal likelihood [32] in the lme4 package in R [34,35]. We then used Akaike's information criterion, corrected for sample size (AIC_c) to rank the strength of evidence for each candidate model based on the data. Akaike's information criterion is a weight-of-evidence measure that reflects the balance between a model's goodness-of-fit to the data and its parsimony (i.e., number of parameters). Lower AIC_c values indicate greater support, and are reported as differences (ΔAIC_c) relative to the best (smallest) value in the candidate set. We computed Akaike weights [36], which represent the relative support for each model, normalized so the weights sum to unity across the candidate set. We used these weights to compute model-averaged estimates and unconditional standard errors (SEs) for the fixed regression coefficients, and we quantified the relative importance of each predictor using variable weights (i.e., the summed Akaike weights of all models that included that predictor; [36]). These model averaging calculations were based on the 95% confidence set of models (i.e., the top-ranked models whose cumulative Akaike weight is 0.95), after re-normalizing the weights.

Mapping coho spawner mortality

Using the fitted models, we built a map of predicted coho spawner mortality throughout the four counties (King, Kitsap, Pierce and Snohomish) representing much of the Puget Sound lowlands, by applying the GLMM equations to geospatial data from unmonitored basins. We used basins delineated in the National Hydrography Dataset Plus [37] as the underlying mapping unit (300 ha mean, 466 ha SD) and intersected the NHDPlus datalayer with each of the geospatial datalayers used in the statistical analyses. Within the four-county region, we only made spawner mortality predictions in basins where coho salmon presence has been documented, based on current geospatial datalayers generated by the Washington Department of Fish and Wildlife [38]. We then calculated the proportion of each basin that was covered by the selected landscape feature. We generated predicted values of the proportion of mortalities from each model in the 95% confidence set and then model-averaged these values using the normalized Akaike weights [36]. These predictions apply to the average basin in the Puget Sound coho ESU with some given set of habitat conditions, in the sense that the random effect of site was set to zero. To be conservative in representing the precision of the predicted values, we divided the calculated rates of likely coho spawner mortality into three bins: <10%, 10–50%, and >50%. These break points were chosen somewhat arbitrarily to represent low, medium and high spawner mortality rates.

Results

We found strong associations between land use and land cover attributes and rates of coho spawner mortality. Across the 95% confidence set of fitted models, three variables were particularly important for predicting mortality based on high variable weights: impervious surfaces, local roads, and commercial property type (Table 2 and Figure 2). There was substantial model selection

Table 2. AIC weights, model averaged parameter estimates and unconditional confidence intervals for each variable, ranked by AIC_c weight.

Datalayer	Variable	AIC_c weight	Model Averaged coefficient	Unconditional SE
Impervious	Impervious surfaces	0.7158	16.8425	14.5376
Roadways	Local roads	0.5647	−15.6199	68.3331
Property type	Commercial	0.5107	7.9375	8.2616
Land cover	Dense urban	0.3865	−7.7776	16.1614
Property type	Apartments & condominiums	0.2409	−9.5330	31.1917
Roadways	Heavily used roads	0.2019	5.3445	31.5073
Land cover	Forest	0.1163	−0.7793	6.2249
Land cover	Light to medium urban	0.1149	0.3250	2.9751
Land cover	Grass, shrubs & crops	0.0993	0.1664	5.4517
Property type	Residential	0.0975	0.0738	16.8920
Property type	Industrial	0.0547	−0.2475	4.7008
Property type	Parks & open space	0.0000	0.0000	0.0000

doi:10.1371/journal.pone.0023424.t002

uncertainty, reflected in a large 95% confidence set and large number of models with $\Delta AIC_c < 2.0$ (37 and 8 of 139 candidate models, respectively; Table 3). In addition, although we excluded highly multicollinear combinations of variables ($|r| > 0.9$), many variables were still strongly correlated, resulting in unstable parameter estimates and large unconditional SE estimates (Table 2). Nonetheless, predictive models that included land use and land cover attributes as predictors were clearly superior to the intercept-only model ($\Delta AIC_c = 20.4$; Table 3), supporting the association of these variables with coho mortality.

While the multicollinearity among potential predictors made causal interpretation of the models difficult, it did not preclude predictions of where coho salmon are likely to be affected along an urbanization gradient. Not surprisingly, the highest predicted mortality rates were clustered around the major metropolitan areas of eastern Puget Sound, contained within Snohomish, King, Kitsap, and Pierce counties (Figure 3). In addition, there is a significantly sized area in Eastern Puget Sound that has considerable proportions of the variables (local roads, impervious surface and commercial parcels) most correlated with substantial mortality rates. It is important to note that these predicted values have substantial associated uncertainty and should therefore be interpreted cautiously; however, it is reasonable to use them for assigning the break points for the low, medium, and high mortality rate categories represented on the map.

Discussion

Overall, we have used conventional tools in landscape ecology to shed light on an unusually complex ecotoxicological challenge. Our analyses strongly suggest that specific characteristics of basins in the Puget Sound lowlands are linked to the die-offs of coho spawners that have been widely observed in recent years. Across basins, the strength of the association is greatest for impervious surfaces, local roads, and commercial property. We did not evaluate hydrologic or geomorphic basin characteristics as part of our analysis. Nevertheless, our findings support the hypothesis that coho are being killed by as-yet unidentified toxic chemical contaminants that originate from these types of surfaces and are transported to salmon spawning habitats via stormwater runoff.

Our results extend a large body of scientific information linking urbanization (broadly defined) and degraded water quality to a loss of biological integrity (sensu Karr [39]) and productivity in freshwater stream networks [18,40,41]. Previous studies have generally related land use and land cover variables to macroinvertebrate assemblages in streams [42], or to the relative abundance of salmon and other fish (e.g., [22,43,44]). The present analysis is novel because it relates basin characteristics directly to salmon health and survival, versus species presence or absence. Moreover, it offers new insights on the water quality aspects of urban runoff. The focus of most salmon restoration projects is physical characteristics of spawning and rearing habitat [45]. Most salmon specific restoration projects are deemed successful if they simply restore the physical habitat to a suitable state for a given species [46]. Our study suggests that suitable spawning and rearing habitat may not be supportive of coho salmon persistence when the surrounding landscape is urbanized. The linkages between increased impervious coverage within a basin, increased stormwater runoff, altered hydrologic processes, and ecological decline are well established (e.g., [18]). However, stormwater impacts encompass both physical and chemical drivers of decline, and it can be difficult to distinguish between these via *in situ* assessments because stream invertebrate communities integrate both stressor categories. Coho salmon spawners, by contrast, appear to be promising and specific sentinels for the degraded water quality aspect of urban runoff. Compared to macroinvertebrate sampling and taxa identification, the coho mortality syndrome is relatively easy and inexpensive for non-specialists to monitor in the form of digital video recordings of symptomatic fish, or the presence of unspawned female carcasses in streams.

Interestingly, the mortality syndrome appears to be specific to coho salmon. For example, there were temporally overlapping runs of coho and chum salmon (*O. keta*) in Piper's Creek in the fall of 2006. Whereas all of the adult coho succumbed to the mortality syndrome, the chum were unaffected, with nearly all surviving to spawn (130 of 135 spawned out female carcasses; Scholz et al., unpublished data). Consistent with this, the survey

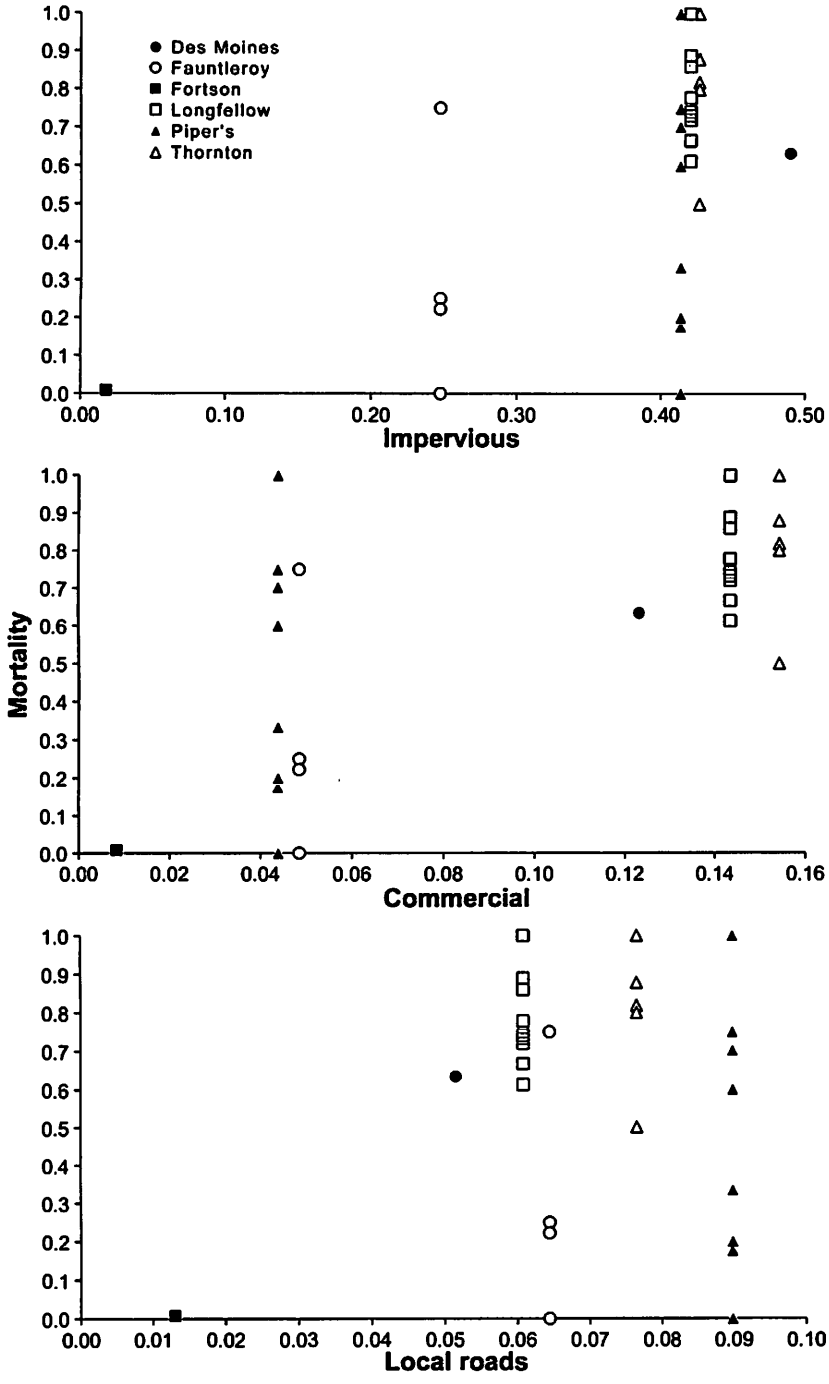

Figure 2. Female coho spawner mortality as a function of the proportion of each of the top three predictors in a given site basin, at the six study sites. Individual points correspond to specific years for each site. Mortality expressed as proportion of all returning females that died in a given year. Solid circle = Des Moines; hollow circle = Fauntleroy; solid square = Fortson; hollow square = Longfellow; solid triangle = Piper's; hollow triangle = Thornton Creek.
doi:10.1371/journal.pone.0023424.g002

teams have not observed the characteristic symptoms (e.g., surface swimming, gaping) among other fish species that inhabit urban streams such as sticklebacks and cutthroat trout. Not only are coho unusual in this respect, the phenomenon appears to be restricted to the adult life stage. In the fall of 2003, surface flows from Longfellow Creek were diverted through streamside sheds housing aquaria that contained individual juvenile coho from the NWFSC hatchery. The juveniles (n = 20) were maintained and observed daily throughout the fall spawner run. Overall juvenile survival was 100%, and the juveniles behaved normally, even on days when symptomatic adults were observed in the nearby stream (Scholz et al., unpublished data). The underlying reasons

Table 3. Summary of the 95% confidence set (37 of a total of 139 candidate models) of candidate models used to generate map of mortality rates, showing intercepts, estimated coefficients, ΔAIC_c and w_{AICc}. Intercept only model included at bottom for reference.

Model	Equation	ΔAIC_c	w_{AICc}
a+b	−4.5664+19.76(a)+44.41(b)	0.000	0.0933
c+d+b	−3.9215−109.56(b)+48.75(c)−29.98(d)	0.046	0.0912
c+e+f	−3.9355+12.94(c)−40.15(e)+38.61(f)	0.372	0.0775
c+d+a	−4.4921+12.61(a)+14.03(c)−7.54(d)	0.579	0.0698
c+g+a	−4.4858+14.31(a)+5.23(c)+3.62(g)	0.669	0.0668
h+a+b	−2.6065+15.89(a)+30.87(b)−2.38(h)	1.150	0.0525
c+a+b	−4.6629+16.37(a)+35.26(b)+2.70(c)	1.357	0.0473
d+a+b	−4.7001+17.52(a)+43.83(b)+1.62(d)	1.576	0.0424
c+e	−4.5943+19.70(c)−53.28(e)	2.425	0.0277
c+d+i+b	−3.0628−83.44(b)+56.38(c)−40.28(d)−7.82(i)	2.485	0.0269
c+j+i+b	−7.3055−130.72(b)+21.23(c)+19.12(i)+10.65(j)	2.543	0.0262
c+d+k+b	−3.9266−94.52(b)+43.32(c)−25.00(d)−1.60(k)	2.613	0.0253
j+a+b	−4.5174+20.03(a)+43.79(b)−0.52(j)	2.752	0.0236
c+d+a+b	−4.0864+3.99(a)−76.44(b)+38.23(c)−23.27(d)	2.885	0.0221
c+d+a+f	−4.7368+15.57(a)+16.88(c)−9.22(d)−22.10(f)	2.925	0.0216
c+d+e+b	−3.9607−100.49(b)+46.40(c)−27.43(d)−5.54(e)	2.954	0.0213
c+d+e+f	−3.8347+12.37(c)+0.49(d)−40.69(e)+39.28(f)	3.280	0.0181
c+g+e+f	−3.8534+12.93(c)−40.45(e)+38.73(f)−0.18(g)	3.294	0.0180
c+j+e+f	−3.9360+12.94(c)−40.28(e)+39.36(f)−0.31(j)	3.326	0.0177
c+g+a+f	−4.6143+16.25(a)+5.79(c)−13.40(f)+4.06(g)	3.378	0.0172
c+d+i	−1.1996+64.26(c)−55.97(d)−24.83(i)	3.423	0.0168
h+i+b	9.3911−153.97(b)−17.49(h)+15.89(i)	3.858	0.0136
h+e+f	2.2747−27.99(e)+47.38(f)−7.31(h)	3.931	0.0131
h+a	1.2512+8.63(a)−6.13(h)	4.028	0.0124
c+j+a+b	−4.5887+16.71(a)+34.25(b)+2.72(c)−0.75(j)	4.299	0.0109
h+k+b	5.8364−27.35(b)−11.39(h)−5.97(k)	4.837	0.0083
c+j+e	−4.4356+18.70(c)−50.31(e)+1.33(j)	4.915	0.0080
c+j+k+b	−2.4511−52.30(b)+20.45(c)−13.34(j)−10.60(k)	4.937	0.0079
c+d+e	−4.7362+20.37(c)−0.45(d)−53.43(e)	5.141	0.0071
c+e+b	−4.4680−1.36(b)+19.52(c)−52.48(e)	5.158	0.0071
c+g+e	−4.5797+19.68(c)−53.23(e)−0.02(g)	5.188	0.0070
h+e+b	8.1285−20.52(b)−45.07(e)−14.67(h)	5.509	0.0059
c+k	−4.3426+13.30(c)−5.31(k)	5.649	0.0055
c+i+b	−5.6775−141.73(b)+22.77(c)+17.24(i)	5.821	0.0051
c+k+b	−3.9708−12.84(b)+14.63(c)−6.46(k)	5.896	0.0049
h+a+f	0.4930+6.87(a)+19.67(f)−5.22(h)	6.083	0.0045
c+d+i+f	−1.0499+68.65(c)−59.91(d)−6.04(f)−26.58(i)	6.343	0.0039
Intercept only	N/A	20.428	0

Model weights shown here are re-normalized for the set of 37 top-ranked models shown. a = commercial; b = local roads; c = impervious; d = dense urban; e = apartments and condominiums; f = heavily used roads; g = light to medium urban; h = forest; i = residential; j = grass, crops and/or shrubs; and, k = industrial.
doi:10.1371/journal.pone.0023424.t003

for the syndrome's surprising uniqueness to adult coho are not yet known.

Daily or weekly stream surveys are labor intensive, and for this reason only a subset of urban drainages in Puget Sound have been monitored to date. The GIS-based mapping tool developed for this study can be used to focus future monitoring efforts on basins with a higher likelihood of coho die-offs based on land cover attributes. In addition to the basins we have identified within the range of the Puget Sound/Georgia Basin ESU, this approach could be extrapolated to other geographic areas where coho return to spawn along a gradient of urban growth and development. This includes, for example, coho from the Lower Columbia River ESU, a threatened population segment with a spawner range encompassing the greater metropolitan area of Portland, Oregon. Overall, future surveys will ground-truth initial model outputs and provide additional data that can be used to improve the predictive accuracy of the mapping tool.

Our findings have two near-term applications. First, they identify likely "hotspots" for coho spawner mortality throughout central Puget Sound. Given that recurring adult losses at a rate greater than approximately 10% are likely to substantially reduce local population abundances, the high mortality basins in Figure 3 (10–50% and >50% predicted mortality categories) may represent sink habitats for the Puget Sound/Georgia Basin ESU. This is an important consideration for coho recovery planning at the local, county, and regional scales. Second, our results indicate areas where toxic runoff could potentially undermine stream restoration efforts - specifically, strategies that improve physical and biological habitat conditions (flow, connectivity, channel complexity, riparian function, etc.) as a means to boost coho population productivity.

The potential influence of rainfall, including timing, frequency, and individual storm intensity, remains an area of active investigation. Throughout the years of stream surveys, it has been qualitatively evident that rainfall influences the mortality syndrome. For example, salmon that arrive and enter a stream during an extended dry interval (a week or more) often survive and then become symptomatic and die when it next rains (Scholz et al., unpublished data). One of our aims in surveying Longfellow Creek (the stream with the most abundant overall returns) for more than a decade was to evaluate inter-annual variation in coho spawner mortality in relation to rainfall. However, a quantitative analysis has proven problematic due to highly variable rainfall patterns in combination with low adult returns in some years. It is clear, however, that the syndrome is not a simple first-flush phenomenon. In most years, both egg retaining and spawned out carcasses were observed across the 8–10 week fall run, irrespective of the number and size of rain events over that interval.

Over the longer term, an approach similar to the one developed here could be used to forecast the likely impacts of future human population growth and development on Puget Sound coho populations that are currently healthy. For example, the expansion of local road networks is a core focus for urban growth planning, and these projections could serve as a basis for evaluating how and where coho spawner mortality will increase under different growth management scenarios. This, in turn, would inform strategies to reduce or mitigate toxic runoff in highly productive basins, in advance of expanding transportation infrastructure – i.e., prevention vs. costly retrofits to the built environment. Also, our modeling approach could be expanded to include the timing and intensity of rainfall as potential drivers for coho spawner mortality. Rainfall patterns may be a key determinant of stormwater quality, although more work in this area is needed. Climate change is expected to shift regional rainfall patterns, and it should be possible to explore how this will interact with changing land cover (urbanization) to influence stormwater quality and toxic runoff to coho spawning habitats.

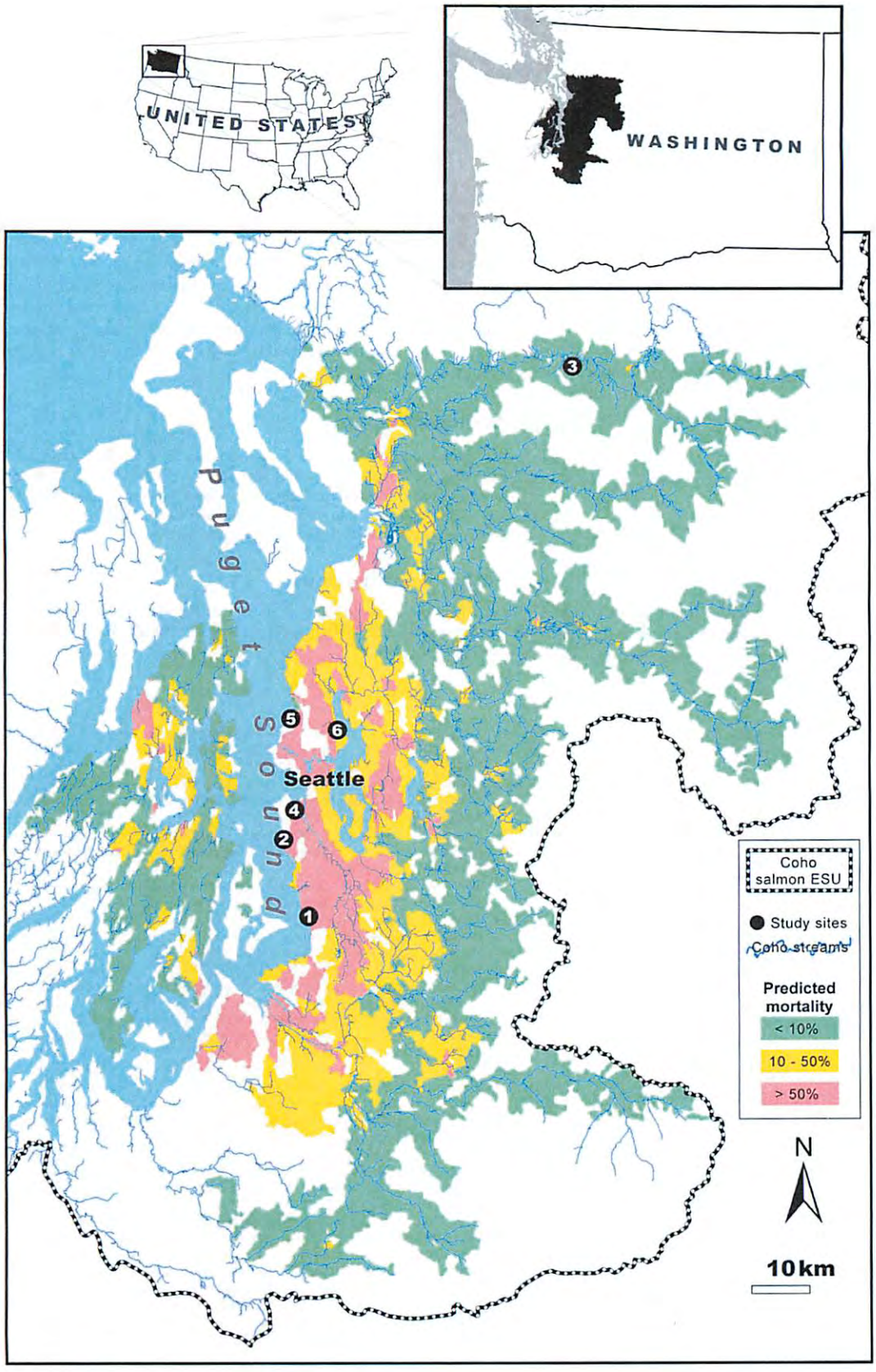
UNITED STATES
WASHINGTON
Puget Sound
Seattle
1
2
3
4
5
6
Coho salmon ESU
Study sites
Coho streams
Predicted mortality
< 10%
10 - 50%
> 50%
N
10km

Figure 3. Predictive map of modeled coho spawner mortality rates within the Puget Sound lowlands. Mortality rates are a function of the proportion of key landscape variables within a given basin. Green, yellow and red areas indicate basins with predicted rates of spawner mortality (as a percentage of total fall runs) of <10%, 10–50%, and >50%, respectively. Black dots denote locations of the six study sites that were the basis for this analysis. Thick dashed black line depicts the southern boundary of the coho salmon Puget Sound/Georgia Basin Evolutionarily Significant Unit (ESU). Basins that do not have documented presence of coho salmon [38] are not represented on the map, even if they have landscape conditions associated with coho spawner mortality. Key for site numbers: 1 = Des Moines; 2 = Fauntleroy; 3 = Fortson; 4 = Longfellow; 5 = Piper's; and, 6 = Thornton Creek.
doi:10.1371/journal.pone.0023424.g003

While not definitive, our results reinforce the parsimonious explanation that coho deaths are caused by one or more contaminants originating from motor vehicles. As noted earlier, this is important because it narrows the list of candidate toxics in complex urban landscapes. Future toxicological studies should focus on two ubiquitous urban runoff contaminant classes in particular. The first are metals in brake pads and other vehicle friction materials. Copper, zinc, and other metals are known to specifically target the fish gill, thereby disrupting respiration and osmoregulation [47]. The second, PAHs, [14,48,49] are taken up across the fish gill, and can impair cardiac function and respiration [50]. The symptoms displayed by affected coho (surface swimming, gaping, loss of equilibrium, etc.) are consistent with a disruption of respiration, osmoregulation, or circulation, or some combination of these.

Notably, PAHs and metals usually cause the above toxicological effects at concentrations well above those typically detected in urban streams. However, the majority of conventional toxicology studies using salmonids focus on freshwater species (e.g., rainbow trout) or the freshwater life stages of juvenile anadromous species. There are practically no toxicity data for coho salmon at the adult spawner stage. Many important osmoregulatory changes take place during the transition from seawater prior to spawning, and these may render adult coho more vulnerable to metals and PAHs than freshwater-resident salmonids. Adding to this complexity is the possibility of interactive toxicity (e.g., synergism) among contaminant mixtures. Studies that experimentally reproduce the familiar symptomology and mortality in adult coho, under controlled exposure conditions with environmentally realistic mixtures of metals and PAHs, will likely be necessary to definitively implicate motor vehicles.

Acknowledgments

We thank John Williams and an anonymous reviewer for significantly improving previous drafts of this manuscript. Disclaimer: the findings, conclusions and views expressed herein are those of the authors and do not necessarily represent those of the National Oceanic and Atmospheric Administration or the U.S. Fish and Wildlife Service.

Author Contributions

Conceived and designed the experiments: BEF JWD NLS. Performed the experiments: BEF ERB PA. Analyzed the data: BEF ERB PA. Wrote the paper: BEF ERB NLS.

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KCTS 99 Oct 17 2012

OPEN ACCESS Freely available online

PLoS one

Recurrent Die-Offs of Adult Coho Salmon Returning to Spawn in Puget Sound Lowland Urban Streams

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Abstract

Several Seattle-area streams in Puget Sound were the focus of habitat restoration projects in the 1990s. Post-project effectiveness monitoring surveys revealed anomalous behaviors among adult coho salmon returning to spawn in restored reaches. These included erratic surface swimming, gaping, fin splaying, and loss of orientation and equilibrium. Affected fish died within hours, and female carcasses generally showed high rates (>90%) of egg retention. Beginning in the fall of 2002, systematic spawner surveys were conducted to 1) assess the severity of the adult die-offs, 2) compare spawner mortality in urban vs. non-urban streams, and 3) identify water quality and spawner condition factors that might be associated with the recurrent fish kills. The forensic investigation focused on conventional water quality parameters (e.g., dissolved oxygen, temperature, ammonia), fish condition, pathogen exposure and disease status, and exposures to metals, polycyclic aromatic hydrocarbons, and current use pesticides. Daily surveys of a representative urban stream (Longfellow Creek) from 2002–2009 revealed premature spawner mortality rates that ranged from 60–100% of each fall run. The comparable rate in a non-urban stream was <1% (Fortson Creek, surveyed in 2002). Conventional water quality, pesticide exposure, disease, and spawner condition showed no relationship to the syndrome. Coho salmon did show evidence of exposure to metals and petroleum hydrocarbons, both of which commonly originate from motor vehicles in urban landscapes. The weight of evidence suggests that freshwater-transitional coho are particularly vulnerable to an as-yet unidentified toxic contaminant (or contaminant mixture) in urban runoff. Stormwater may therefore place important constraints on efforts to conserve and recover coho populations in urban and urbanizing watersheds throughout the western United States.

Citation: Scholz NL, Myers MS, McCarthy SG, Labenia JS, McIntyre JK, et al. (2011) Recurrent Die-Offs of Adult Coho Salmon Returning to Spawn in Puget Sound Lowland Urban Streams. PLoS ONE 6(12): e28013. doi:10.1371/journal.pone.0028013

Editor: Howard Browman, Institute of Marine Research, Norway

Received August 5, 2011; **Accepted** October 29, 2011; **Published** December 14, 2011

Funding: This project received agency funding from the National Oceanic and Atmospheric Administration (Coastal Storms Program), the U.S. Fish and Wildlife Service National Contaminants Program, the U.S. Environmental Protection Agency Region 10, and the King Conservation District. Funds were delivered via interagency agreements, so grant numbers are not applicable. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

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Introduction

In lowland Puget Sound, many urban streams in the vicinity of Seattle were a focus of extensive physical and biological restoration activities in the 1990s. These projects, sponsored by the City of Seattle and other regional municipalities, served multiple purposes such as the creation of public green space, the removal of culverts and other impassable barriers for fish, the placement of large woody debris and gravel substrate, the removal of noxious weeds, and the planting of native vegetation. A related aim was to evaluate the extent to which adult salmon would return to spawn in the newly available and improved habitats. This post-project effectiveness monitoring was carried out via fall spawner surveys that were conducted weekly from 1999–2001, with a primary focus on coho (*Oncorhynchus kisutch*), Chinook (*O. tshawytscha*) and chum (*O. keta*) salmon.

These early monitoring efforts in 1999–2001 identified an unusual syndrome of pre-spawn mortality among adult coho returning to restoration sites to spawn. Coho typically spawn in small lowland streams in October through December. Eggs incubate in gravel nests (redds) from which fry emerge in the spring (March through May). Juveniles rear in freshwater for approximately a year and then outmigrate to estuaries the following spring. Coho spend at least one full year in the ocean before returning to their natal watersheds to spawn, after which they die (semelparous life history). Adult migration into freshwater is triggered by fall rain events that produce transient high flows in streams. Coho spawning in Seattle-area streams are often a mix of hatchery and natural origins, with hatchery fish distinguishable by a clipped adipose fin and, less commonly, the presence of a rostral-implant coded wire tag.

Affected coho spawners observed in post-restoration effectiveness monitoring surveys showed a consistent suite of symptoms that included surface swimming, gaping, loss of equilibrium, and pectoral fin splaying (Video S1). The onset of the syndrome was rapid, and stricken fish typically died within a few hours. Pre-spawn mortality was confirmed by a near-total retention of eggs in female carcasses inspected during the surveys.

The recurrent die-off of coho in urban drainages appears to be a phenomenon distinct from other types of pre-spawn mortality that have previously been reported for other species of salmon. These include, for example, sockeye salmon in the Fraser River and watersheds of Bristol Bay, as well as Chinook salmon in the Klamath River. In those non-urban freshwater habitats, pre-spawn mortality is described as a chronic process where fish are weakened by a low energy status, poor physical condition, wasting, and eventual death. This process occurs over a protracted timeframe (i.e., weeks). The causes vary and include an abnormally early arrival on spawning grounds, thermal stress, and increased susceptibility to the myxosporean parasite *Parvicapsula minibicornis* (Fraser River sockeye; [1,2,3]); high spawner density, low water level, high water temperature, and low dissolved oxygen levels (Bristol Bay sockeye; [4,5]); and low flows, increased water temperature, high spawner densities, and diseases caused by the pathogenic ciliate *Ichtyophthirius multifiliis* and *Flavobacterium columnare*, the bacterial agent for columnaris in fish (e.g., Klamath River Chinook salmon; [6]).

Here we report the results of an eight-year investigation (2002–09) to characterize the frequency and geographical extent of coho mortality, and to identify associated water quality and spawner condition factors. We conducted daily surveys of multiple creeks to assess rates of pre-spawning mortality across the entire duration of fall coho runs. We assessed the physical condition, pathogen exposure status, and disease status of affected female coho for comparison to 1) unaffected wild adult females collected from a non-urban reference stream, 2) unaffected adult females returning to several area hatcheries, and 3) seawater-phase adults collected from Elliott Bay along the Seattle waterfront, prior to their entry to restored freshwater habitats in urban drainages. Fish from a subset of these locations were profiled using biomarkers of exposure to common toxic contaminants in urban runoff, including metals, homeowner use insecticides, and petroleum hydrocarbons. Lastly, we monitored conventional water quality information (e.g., temperature, dissolved oxygen, specific conductance, and pH) for urban streams during adult coho die-off events.

Materials and Methods

A paucity of coho spawners in urban streams throughout this study placed important constraints on sample collection for the purposes of a forensic analysis. Spawner abundances were generally low and unpredictable in urban streams where the die-off phenomenon occurs. By contrast, in non-urban streams where coho are relatively abundant, spawners were unaffected. Therefore, tissue collections from coho in urban streams were ad hoc and opportunistic. The streams surveyed during the course of this study and associated samples collected are listed in Table 1.

To ensure sample integrity, tissues were not collected from the decomposing carcasses found in streams. Conversely, we did not sacrifice live, non-symptomatic fish in urban streams because these coho may or may not have survived to successfully spawn. As a consequence, some types of samples (e.g., gill metals, bile PAHs; see below) had to be collected from spawners that were either overtly symptomatic or very recently dead, as evidenced by gill coloration (see below). As noted earlier, symptomatic fish progress to death rapidly. Stream surveys were generally less than two hours in a given day, and thus encounters with symptomatic fish were infrequent. This accounts for a relatively small sample size for some tissues despite an intensive overall survey effort.

Study locations

Daily or weekly spawner surveys (including tissue collections) were conducted on several Seattle-area streams from 2002–2009. These included Longfellow, Thornton, Piper's, Des Moines, Taylor, and Fauntleroy Creeks (Figure 1). Detailed descriptions of each of these drainages (except Des Moines) can be found in a recent City of Seattle report on urban waterways [7]. Longfellow Creek in West Seattle, the urban stream found to have the highest numbers of adult-entry coho in preliminary assessments (1999–2001), was the focus of daily surveys in each of the eight years of monitoring. Des Moines Creek to the southwest of Seattle was surveyed daily in 2004 and Piper's Creek in northwest Seattle was surveyed daily in 2006. In other years, these two urban streams were monitored approximately weekly, as were Taylor, Thornton, and Fauntleroy Creeks [8]. To assess the prevalence of pre-spawn mortality among wild coho salmon returning to spawn in a non-urban drainage, we surveyed Fortson Creek (a tributary to the North Fork Stillaguamish River north of Seattle; Figure 1) daily in the fall of 2002.

Table 1. Summary of survey locations and associated tissue samples.

Location	Category	Years Sampled	Survey Frequency	Water Quality	Tissue Sampling				
					gills	brain	bile	pathogen screen	histopathology
Longfellow Creek	urban stream	2002–2009	daily	✓[b]	✓[c]	✓[a]	✓[a,b]	✓[b,c]	✓[b,c]
Piper's Creek	urban stream	2006	daily						
Des Moines Creek	urban stream	2004	daily	✓[b]	✓			✓	✓
Fortson Creek	forested stream	2002	daily			✓	✓		
University of Washington Hatchery	urban hatchery	2002	one day			✓			
Stillaguamish Hatchery	rural hatchery	2002	one day			✓			
Issaquah Creek Hatchery	urban hatchery	2002, 2003	one day			✓[a]		✓	✓
Wallace River Hatchery	rural hatchery	2003, 2008	one day		✓[d]		✓	✓	✓
Elliot Bay	estuary	2003	one day				✓	✓	✓

[a]sampled only in 2002;
[b]sampled only in 2003;
[c]sampled only in 2004;
[d]sampled only in 2008.
doi:10.1371/journal.pone.0028013.t001

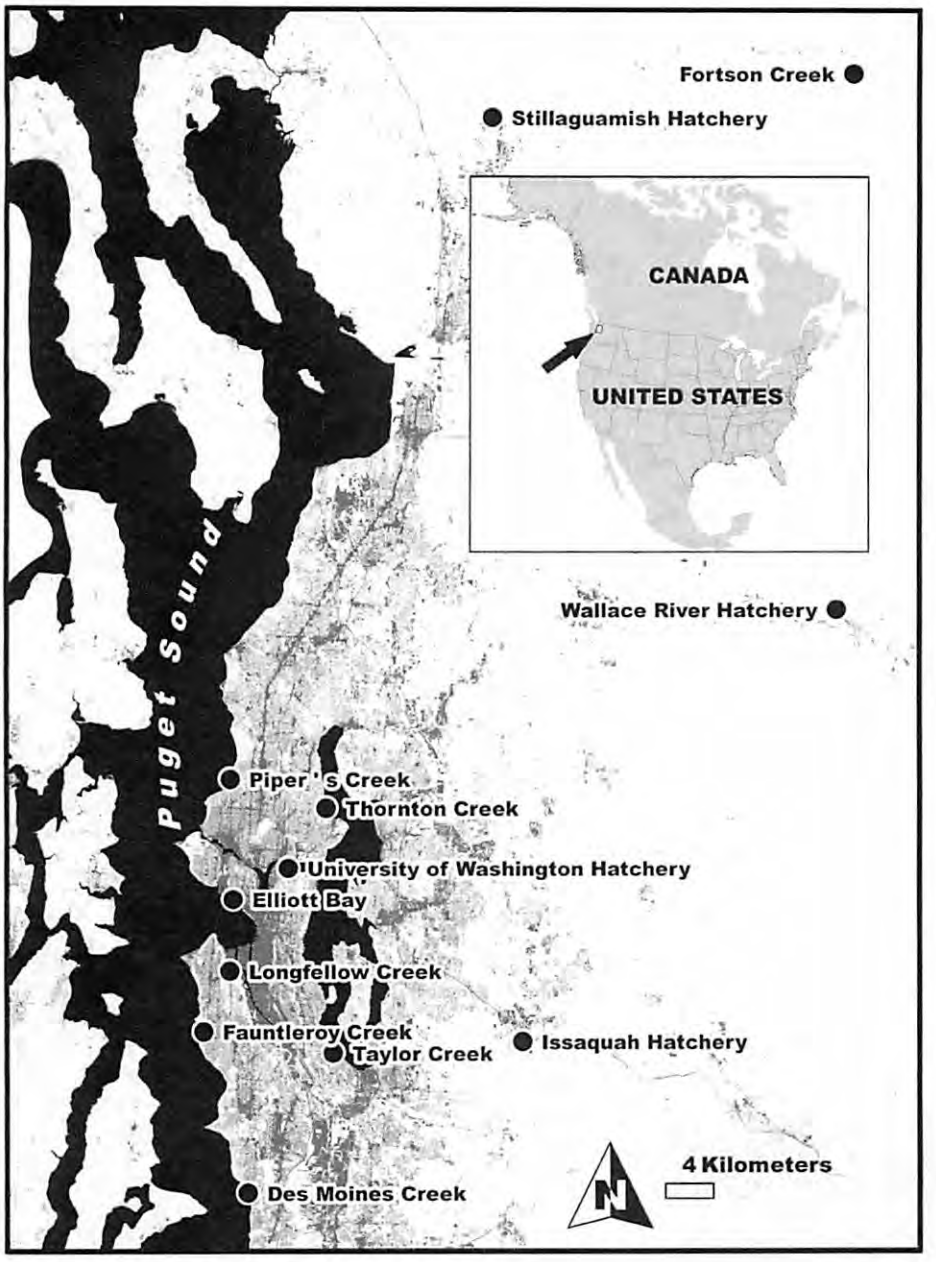

Figure 1. Stream survey and sample collection locations. The map indicates the greater Seattle metropolitan area, with gray shading representing the relative intensity of urbanization. Shown are the urban and non-urban creeks surveyed for coho spawner mortality, regional hatcheries, and the location of seawater-phase adult coho collections along the Seattle waterfront (Elliott Bay).
doi:10.1371/journal.pone.0028013.g001

Tissue samples were also collected from regional hatcheries, including the Stillaguamish Tribal Hatchery (2002), the University of Washington Research and Teaching Hatchery (2002), the Wallace River State Hatchery (2003), and the Issaquah Creek State Hatchery (2002 & 2003). Salmon returning to both the University of Washington and Issaquah hatcheries traverse an urbanized landscape through a series of lakes separated from Puget Sound by a single set of locks. The Stillaguamish and Wallace River hatcheries are located on tributaries to the North Fork of the Stillaguamish River and the Snohomish River, respectively (Figure 1); returning coho pass through primarily rural and forested landscapes.

Adult seawater-phase coho salmon were collected in Elliott Bay (Seattle waterfront, lower Duwamish waterway) prior to freshwater entry. Animals were captured by gillnet in coordination with Muckleshoot tribal fishing operations in the early fall (September 11) of 2003. Adult coho were transferred live or recently dead (<3 hrs) to the NOAA research vessel *Harold W. Streeter* for immediate necropsy and sample storage.

Spawner survey procedures

Coho typically return to Puget Sound urban streams in the early fall (i.e., early October), depending on the timing and intensity of rain events. Coho returned to the non-urban tributary of the

North Fork Stillaguamish River (Fortson Creek) later in the fall relative to coho returning to urban creeks. Thus, daily surveys on Fortson Creek extended into late December in 2002 (approximately three weeks later than for surveys on urban creeks).

Daily surveys involved a visual inspection of most of the accessible freshwater habitat within a given stream. In some instances, poor visibility due to turbidity, high flows, or deep pools and wetlands precluded visual access. Surveyors began at the bottom of the reach and moved upstream, inspecting the stream channel for live adult salmon and carcasses. Where possible, the stream banks were also searched for carcasses that may have been dragged into the riparian zone by predators (predominantly river otters) or scavengers. Urban stream surveys spanned the entirety of the available spawning habitat within a given drainage. In some systems such as Longfellow Creek, impassable barriers restricted returning coho to spawning sites in the lower reaches of the stream.

The location and general or atypical behavior of live salmon were recorded. For all dead or moribund fish, information on collection location, species, gender, fork length, weight (with and without ovaries for females), condition (e.g., signs of physical injury), and egg retention (females only) were recorded. Because males may spawn multiple times (or not at all) within a season, a determination of pre-spawn mortality was only made for females, and the reported rates of coho mortality for different streams are based on data from females only. Although we classified all female carcasses with >50% egg retention as pre-spawn mortalities, in most cases retention was closer to 100% (representative female in Figure 2). After examination, carcasses were left in the stream and marked with dated flagging tape. Even during high flow events, the day-to-day movement of flagged carcasses within streams was minimal (a few meters at most), and there was no evidence that pre- and post-spawn carcasses were disproportionately recoverable.

We collected tissue samples only from animals that were either overtly symptomatic or freshly dead (i.e., <3 hrs post-harvest or gill coloration was red to pink). Due to the limited availability of animals in some years, we collected tissues from symptomatic males as well as females.

Figure 2. Representative adult female coho carcass with characteristically high egg retention. Rates of premature spawner mortality within and across urban drainages were quantified on the basis of egg-retaining female carcasses. Shown is a female affected by the mortality syndrome in Longfellow Creek in the fall of 2005. In most cases, egg retention was nearly 100%. Photo by Tiffany Linbo, NOAA Fisheries.
doi:10.1371/journal.pone.0028013.g002

Throughout the study we observed a mix of marked and unmarked fish returning to urban streams. In 2002, marked coho were identified by the absence of adipose fins. From 2003 through 2009, carcasses were scanned with a hand-held coded wire tag scanner (Northwest Marine Technology, Inc., Shaw Island, WA). To determine source hatcheries, retrieved tags were processed by U.S. Fish and Wildlife Service staff in the Fisheries Division of the Washington Fish and Wildlife Office (Lacey, WA).

Fish condition

Condition factor was determined for pre- and post-spawn female coho salmon collected from Longfellow Creek (2002–09), Des Moines Creek (2004), and Fortson Creek (2002) as well as for female coho from the University of Washington and Stillaguamish Hatcheries (2002). Pre-spawn mortalities were weighed, the gonads removed, and then the fish were weighed again. All post-spawn and hatchery fish were weighed without gonads. Weight of the fish without gonads was used as a standard index to compare condition factor between pre- and post–spawn mortalities and hatchery fish. Condition factor was calculated using Fulton's condition factor ($K = [\text{weight (g)}/\text{length (cm)}^3] \times 100$; [9]).

Fish histopathology

Tissues were collected for histopathology in 2003 and 2004 from affected coho on Longfellow Creek (N = 21 animals) and Des Moines Creek (N = 22), healthy coho returning to the Wallace River (N = 20) and Issaquah (N = 24) hatcheries, and pre-freshwater entry coho from the Muckleshoot tribal fishery in Elliott Bay (N = 27). Samples of liver, head and trunk kidney, exocrine pancreas, pyloric caeca, small or upper intestine, large or lower intestine, stomach, heart, spleen, gonad, brain, and gill were preserved in Davidson's fixative [10]. Portions of each organ (3–7 mm in thickness) were excised from salmon bodies *in situ* and placed in 200 ml bottles filled with approximately 120 ml of fixative. Field-collected samples were transferred to the Northwest Fisheries Science Center, trimmed to a 3–4 mm thickness, and placed in tissue cassettes labeled with a unique fish identification number. Tissues were processed according to routine methods for paraffin embedding in Polyfin (Triangle Biomedical Sciences, Durham, NC) using a Shandon Hypercenter XP automated tissue processor (Shandon Lipshaw, Pittsburg, PA). Embedded sections were then cut to a 4–5 μm thickness, stained with hematoxylin and eosin-phloxine [11], and examined by light microscopy. Histopathologic diagnoses were coded as published previously [12].

The Fisher's Exact Test [13] was used to test whether the prevalences of certain histopathologic conditions in coho exhibiting pre-spawning mortality syndrome from the urban creeks were significantly higher than those for normal spawners from either the Wallace River or Issaquah hatcheries, or the pre-freshwater entry animals collected by gillnet from Elliott Bay. The critical level of significance was set at $p \leq 0.05$.

Pathogen detection

Fish collected in 2003 and 2004 were screened for infectious non-viral pathogens commonly observed in Pacific salmon, especially those pathogens affecting osmoregulatory tissues such as trunk kidney and gill. In 2003, fish were analyzed for the myxosporean parasite *Parvicapsula minibicornis* (gill and kidney), the larval digenetic trematode parasite *Nanophyetes salmincola* (kidney), the bacterium *Renibacterium salmoninarum* (kidney), the myxosporean parasite *Tetracapsula bryosalmonae* (gill), the microsporidian parasite *Loma salmonae* (gill), and the myxosporean parasite *Ceratomyxa shasta* (posterior-most large intestine). In 2004, fish were analyzed for *P. minibicornis*, *R. salmoninarum*, *T. bryosalmonae*, and *L. salmonae*.

For *C. shasta* analysis, up to 5 mm of the posterior-most portion of the large intestine was placed immediately into extraction buffer [14] without proteinase K and placed on ice in the field. Within a few hours, samples were transported back to the lab and stored at 4°C. DNA extraction and analysis were completed by adding proteinase K and performing PCR with primers Cs1 and Cs3 by the method of Palenzuela et al. [14].

For *N. salmincola*, trunk kidney tissue was collected into a sample bag (Whirl-Pak™, Nasco, Modesto, CA) and immediately stored on ice. Within a few hours these tissues were transported back to the lab and stored at −20°C until analysis by light microscopy [15].

For *T. bryosalmonae*, *P. minibicornis*, *R. salmoninarum*, and *L. salmonae*, trunk kidney and gill tissues were placed into separate sample bags and immediately stored on ice. Within a few hours these tissues were transported back to the lab where they were stored at −20°C. DNA was extracted from approximately 25 mg of tissue using the Qiagen DNeasy tissue kit (Valencia, CA) with the following modification of the kit protocol. Samples were lysed in buffer (20 mM Tris-Cl, pH 8.0; 2 mM EDTA; 1.2% Triton X-100; 20 mg/ml lysozyme) for >45 m at 37°C and digested in 25 µl of proteinase K with 200 µl of buffer AL at 55°C overnight. Sample processing thereafter followed the kit protocol for animal tissues. PCR for *T. bryosalmonae* was performed with primers PKX5f and PKX6r [16] and for *P. minibicornis* with primers Parvi-1f and Parvi-2r [17]. *R. salmoninarum* was detected by nested PCR [18]. PCR for *L. salmonae* was performed with primers LS-1 and LS-2 [19]. To confirm the specificity of detection of these pathogens by PCR, representative amplification products were subjected to cycle sequencing by BigDye Terminator (version 3.1) reactions and analyzed on an ABI 3100 (Applied Biosystems, Foster, CA).

Current use pesticide exposure (brain acetylcholinesterase activity)

Current use pesticides are commonly detected in urban streams [20]. Some of the most frequently detected insecticides, including diazinon, malathion, chlorpyrifos, and carbaryl are known to be neurotoxic to salmon [21,22]. These chemicals inhibit the brain enzyme acetylcholinesterase (AChE), thereby disrupting chemical forms of synaptic communication in the salmon nervous system. Measures of brain AChE activity in fish are commonly used to diagnose anticholinesterase poisoning in response to insecticide exposure (e.g., [23]).

To assess the extent to which coho in urban streams may have been exposed to potentially toxic concentrations of insecticides, brains were collected from affected fish from Longfellow Creek (N = 32) as well as the non-urban reference stream (Fortson Creek; N = 18 pre-spawn, 38 post-spawn) and the Stillaguamish (N = 15 pre-spawn), University of Washington (N = 21 pre-spawn), and Issaquah (N = 21 pre-spawn) hatcheries. All samples were collected in the fall of 2002. Brains were dissected *in situ* from fresh carcasses (recently killed, or red to pink gills for fish collected from Longfellow Creek), flash frozen in liquid nitrogen, and then transported to the Northwest Fisheries Science Center for storage at −80°C. For analyses of AChE activity, tissues were homogenized in 50 mg ml^{-1} PBS-T (10 mM phosphate buffered saline containing 1% Triton X-100) and assayed on a 96-well plate using previously published methods for coho salmon [22]. Differences in brain AChE activity among locations were analyzed using a one-way ANOVA and Tukey-Kramer HSD posthoc test (JMP 8.0, SAS Institute, Inc., Cary, NC). The significance level was set at $p \leq 0.05$.

Metal exposure

Gill tissue was collected opportunistically from affected coho from Longfellow Creek and Des Moines Creek in the fall of 2004 and from Wallace River Hatchery spawners in the fall of 2008. Samples were collected with plastic forceps and titanium scissors to avoid metal contamination. Upon collection, gill tissue was placed in plastic bags on ice in coolers and transported to the King County Environmental Laboratory (KCEL). Samples were stored at −20°C until analysis. Prior to analysis, samples were homogenized in blenders that were rinsed with methanol and wiped down prior to and between samples.

All samples were analyzed for arsenic, cadmium, chromium, copper, lead, nickel, and zinc. Total metals were measured by inductively coupled plasma-mass spectrometry (ICP-MS) using KCEL standard operating procedures. Tissue was digested with nitric acid in conjunction with hydrogen peroxide to remove the analytes from the sample matrix and then further digested in nitric and sulfuric acid in the presence of potassium permanganate and potassium persulfate. Sodium chloride hydroxylamine hydrochloride was added after digestion to reduce the sample and stannous chloride was added immediately before analysis.

Measures for quality assurance/quality control (QA/QC) included checking measurement accuracy against certified reference materials such as DORM-2 (dogfish muscle) from the Institute for National Measurement Standards (Ottawa, Canada). Further QA/QC procedures included the measurement of background metal levels with method blanks, monitoring variability with duplicate laboratory samples, and measuring recovery of total metals from spiked samples without (spike blank) and with (matrix spike) the sample matrix. Accepted variability for laboratory duplicates was 20%, ±15% for spike blanks, and ±25% for matrix spikes.

Gill tissue metal concentrations were normalized using a $\log_{10}$ transformation. For each metal, differences in concentrations due to location were analyzed using a one-way ANOVA and Tukey-Kramer HSD posthoc test with the level of significance set at $p \leq 0.05$.

Polycyclic aromatic hydrocarbon exposure

Bile was collected from the gallbladders of returning adult coho during the 2002–2004 field seasons and analyzed for metabolites of polycyclic aromatic hydrocarbons (PAHs) using established methods [24]. Samples were collected from fish in an urban stream (Longfellow Creek in 2002 and 2003), a non-urban stream (Fortson Creek in 2002), a non-urban hatchery (Wallace River Hatchery in 2002), and seawater-phase fish prior to their entry into Seattle-area urban streams (Elliott Bay in 2003). Briefly, bile was injected directly onto a C_{18} reverse- phase column (Phenomenex Synergi Hydro, Torrance, CA) and eluted with a linear gradient from 100% water (containing a trace amount of acetic acid) to 100% methanol at a flow of 1.0 mL/min. Chromatograms were recorded at two fluorescence wavelength pairs: 1) 260/380 nm where several 3–4 ring compounds [e.g., phenanthrene (PHN)] fluoresce and 2) 380/430 nm where many 4–5 ring PACs [e.g., benzo[a]pyrene (BaP)] fluoresce. The concentrations of fluorescent aromatic compounds in bile were determined using PHN or BaP as external standards and converting the fluorescence response of bile to phenanthrene (ng PHN equivalents/g bile or ng PHN equivalents/mg protein) or benzo[a]pyrene (ng BaP equivalents/g bile or ng BaP equivalents/mg protein) equivalents. Total biliary protein was determined using the method of Fryer et al. [25]. Copper sulfate (in alkaline solution) and Folin reagent were added to each diluted bile sample (1:1000 v:v with distilled water). The absorbance of each sample

was measured at 620 nm using a plate-reader spectrophotometer and was compared to the absorbance of bovine serum albumin measured at this wavelength. Total biliary protein values are reported as mg protein/mL bile.

The HPLC/fluorescence system was calibrated prior to analyzing field samples by analyzing a PHN/BaP calibration standard numerous times (N = 5) until a relative standard deviation <15% was obtained for each PAC as previously described [26]. As part of the QA plan, a method blank and a fish bile control sample (bile from Atlantic salmon exposed to 25 µg of Monterey crude oil per mL of water for 48 hours) were analyzed with each salmon bile sample set.

Concentrations of PHN and BaP equivalents, as well as protein values, were $\log_{10}$-transformed to increase the homogeneity of variances. Analysis of variance (ANOVA) and the Tukey-Kramer HSD test were used to determine if mean concentrations of PHN and BaP equivalents and protein content of bile varied among collection years or collection sites. The level of significance was set at $p \leq 0.05$.

Conventional water quality monitoring

Field meters were used to continuously monitor conventional water quality parameters during the fall of 2003 on both Longfellow Creek and Des Moines Creek. A 4a Minisonde™ (Hydrolab, Austin, TX) was installed by the City of Seattle in Longfellow Creek, in a pond at the terminus of the survey reach on this stream (just below an impassable culvert). A YSI 6600 multi-sonde unit (YSI Inc., Yellow Springs, OH) was installed by King County on Des Moines Creek. The unit was located in the stream channel below a footbridge in a community park, about 500 feet above the point at which the stream flows directly into Puget Sound. Both meters were programmed to measure and record water temperature, pH, dissolved oxygen, and specific conductance at 15-minute intervals. The Hydrolab was serviced and calibrated during the deployment period according to U.S. Geological Survey protocols [27]. The YSI meter was serviced weekly according to King County Environmental Lab standard operating procedures.

Mortality in relation to rainfall patterns

We surveyed Longfellow Creek over eight consecutive years in part to evaluate the influence of rainfall on spawner mortality within and between fall coho runs. Daily and total rainfall data were collected as the sum of 1-minute interval detections from the nearest City of Seattle rain gauge (Rain Gauge 17, or RG17), located approximately 5 km southeast of the upstream terminus of the surveyed portion of the stream. Rainfall was quantified from one week prior to encountering the first live fish in Longfellow Creek until the day the last carcass was found. When data were not available at RG17 they were transposed from the next nearest rain gauge (RG18, distance approximately 13 km).

The relationship between inter-annual spawner mortality and total rainfall was assessed using binary logistic regression. For the correlation coefficient, the natural log of the odds ratio [(% pre-spawn/(1 - % pre-spawn)] for each year was weighted by sample size and regressed (simple linear regression) against total rainfall. In 2006, only 4 females were encountered, and data from this year (100% mortality) were excluded from the linear regression because they produced an undefined odds ratio. Both analyses were performed using JMP version 8 (SAS Institute Inc., Cary, NC).

Results

Behavior, condition, and origin of affected coho spawners

Consistent with initial observations of overtly symptomatic fish during early surveys in 1999–2001, we observed the same suite of behaviors in affected spawners during daily surveys from 2002–2009. These included circular surface swimming (loss of orientation), gaping, pectoral fin splaying, and loss of equilibrium (Video S2, S3). Symptomatic coho encountered during the course of a survey usually died by the end of the survey (i.e., within 1–2 hrs). Those that were still alive were found as pre-spawn carcasses the next day. Symptoms were displayed by both male and female spawners, were consistent from year to year, and were consistent across urban drainages.

Numerous adult coho carcasses were found in all monitored streams (Figure 1 and Table 2). For the urban streams, the frequency of egg retention among dead females (Figure 2) was high. For example, for Longfellow Creek, pre-spawn mortality ranged between 70–90% of the overall run in years where returning coho were relatively abundant (Table 2).

The size and condition of affected fish from urban streams were comparable to those of wild coho returning to Fortson Creek and

Table 2. Rates of coho pre-spawn mortality in Puget Sound lowland streams surveyed daily from 2002–2009.

Creek	Year	N*	% Wild**	% Pre-Spawn Mortality Wild	% Pre-Spawn Mortality Total
Longfellow	2002	57	4	100	86
	2003	18	28	20	67
	2004	9	89	88	89
	2005	75	72	72	72
	2006	4	75	100	100
	2007	41	10	75	73
	2008	12	0	n.a	67
	2009	44	0	n.a.	79
Piper's	2006	9	78	100	100
Des Moines	2004	30	33	60	63
Fortson (non-urban)	2002	114	100	0.9	0.9

*Sample size reflects female coho of known spawning condition, with no signs of predation.
**Presumed wild origin based on presence of adipose fin and absence of a coded wire tag.
doi:10.1371/journal.pone.0028013.t002

unaffected hatchery coho returning to regional hatcheries (Table 3). For example, the condition factor for affected fish from Longfellow Creek in 2002 was not significantly different than the condition factor for wild coho from the non-urban reference stream (Fortson Creek; t-test, $p = 0.12$) and it was higher than the condition factors for the unaffected hatchery fish (e.g., Issaquah Hatchery; Table 3).

The spawner mortality syndrome appears to be specific to coho in urban drainages. We observed no symptoms and less than 1% pre-spawn mortality among wild coho returning to spawn in the non-urban reference stream in 2002 (Fortson Creek; Table 2). This is consistent with a widely reported absence of the syndrome among coho spawners from non-urban catchments. For example, in 2003–2004, Washington Trout (now the Wild Fish Conservancy) surveyed 29 Washington Department of Fish and Wildlife index reaches (<10% developed land cover) for coho spawner success in the Snohomish River basin north of Seattle. Of more than 1,000 intact adult female carcasses inspected, less than 0.5% died with an egg retention rate of >50% [28].

We did not observe corresponding die-offs of resident fish in urban streams (e.g., sticklebacks, sculpins, or cutthroat trout), nor did we find the syndrome in other species of migratory salmon return to these same urban streams to spawn in the fall. Also, the phenomenon appears to be specific to adult coho. In 2003, water from Longfellow Creek was diverted into a flow-through streamside shed facility with juvenile coho housed individually in separate aquaria ($N = 24$). The juveniles were fed daily and monitored throughout the duration of the fall spawner run. Despite the presence of symptomatic adults in the adjacent stream, juveniles exposed to the same surface flows showed no overt symptoms, with 100% survival across the experimental group (data not shown).

Throughout the study, the general dearth of coho salmon returning to Seattle-area urban streams posed a challenge in terms of collecting tissues for forensic analyses. Longfellow Creek was chosen as a site for long-term monitoring in part because of the proportionally higher number of coho that typically enter this drainage relative to the other urban creeks in Seattle. Coded wire tag analysis of >50 tags collected from coho in Longfellow Creek (2003–2008) showed that many of these fish are hatchery strays originating from a net pen facility operated in Elliott Bay by the Muckleshoot and Suquamish Tribes. This facility serves to transition approximately 500,000 juvenile coho each year from the Soos Creek hatchery (Washington State Department of Wildlife) to the saltwater environment. Importantly, however, for certain high-return years (e.g., 2002 and 2005: Table 2), most of the stricken coho spawners were unmarked and presumably of wild origin. The mortality syndrome therefore appears to affect wild and hatchery coho alike.

Table 3. Spawner condition for female coho salmon collected from urban and non-urban locations.

Site	Year	N	Mean Condition*	SD
Fortson Creek	2002	20	0.893	0.111
University of Washington Hatchery	2002	21	0.816	0.103
Stillaguamish Hatchery	2002	5	0.840	0.047
Issaquah Hatchery	2002	21	0.814	0.06
Longfellow Creek	2002	47	0.856	0.077
	2003	10	0.920	0.137
	2004	8	1.018	0.103
	2005	54	1.057	0.105
	2006	4	1.084	0.078
	2007	21	0.995	0.153
	2008	7	1.032	0.122
	2009	30	0.930	0.235
Des Moines Creek	2004	19	1.109	0.268
Piper's Creek	2006	9	1.055	0.107

*Condition Factor was Fulton's K = (weight/(length^3))*100. Weights were for gravid females (i.e., with ovaries containing eggs).

doi:10.1371/journal.pone.0028013.t003

Coho mortality is not correlated with common pathogen-associated disease or noninfectious lesions

A systematic survey of histopathological conditions in pre-spawn carcasses (urban streams) and unaffected fish from Elliott Bay and two regional hatcheries (Wallace River and Issaquah Creek) was conducted in 2003 and 2004. Various infectious, parasitic and idiopathic (of unknown etiology) diseases were detected in the gill, heart, trunk kidney, gastrointestinal tract and liver of adult spawners. These findings are summarized in Table S1 and Text S1. No significant lesions were detected in the gonad, brain, spleen or exocrine pancreas of adult coho regardless of origin. None of the observed disease conditions were specific to animals that succumbed to the mortality syndrome, nor were they unique to the urban streams where die-off rates were high.

Among the six pathogens screened by PCR or microscopy, *L. salmonae* in the gill and *R. salmoninarum* in the kidney were detected among fish from all sampling sites, with the highest prevalence observed among Longfellow Creek fish (*L. salmonae*) or Des Moines Creek fish (*R. salmoninarum*) (Table S1). In contrast, *P. minibicornis* in the gill or kidney was detected among fish from all sites except Des Moines Creek. Prevalence of *T. bryosalmonae* in kidney, *N. salmincola* in kidney, and *C. shasta* in lower intestine varied widely among the sites, ranging from 0% to 48%. Although no single pathogen was consistently associated with pre-spawn mortality, infection or infestation by multiple pathogens occurred more frequently among Longfellow Creek fish. In 2003 fish from Longfellow Creek exhibited a significantly higher number of infections or infestations per fish (median = 3) than fish from Elliott Bay (median = 2), Issaquah Hatchery (median = 1), or Wallace River Hatchery (median = 1) (Fisher Exact Probability, $p<0.0001$; Figure S1).

Prematurely dying spawners show no indication of exposure to common insecticides

A reduction in the enzymatic rate of brain acetylcholinesterase is a bioindicator of exposure to common carbamate and organophosphate insecticides. As shown in Figure 3, measured brain enzyme activity affected coho spawners from Longfellow Creek was not significantly lower than corresponding brain enzyme activities in fish from a non-urban reference stream (Fortson Creek) or from regional hatcheries. Rather, AChE activity in the brains of affected fish was slightly but significantly higher than for coho spawners from Fortson Creek and the Stillaguamish Tribal Hatchery (one-way ANOVA; $p<0.05$).

Affected coho have elevated levels of metals in gill tissues

The measured concentrations of arsenic, cadmium, chromium, copper, lead, nickel, and zinc in the gill tissue of adult salmon collected from two urban sites (Des Moines and Longfellow

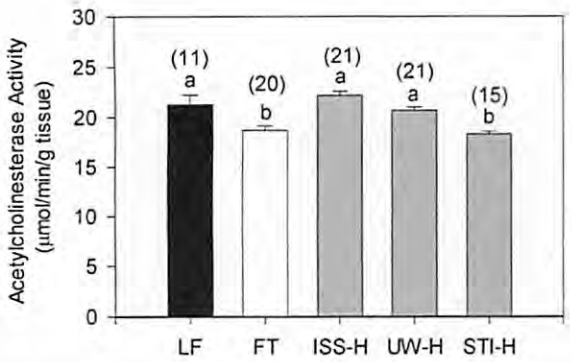

Figure 3. Prematurely dying spawners do not show evidence of neurotoxic pesticide exposure. Shown are relative rates of brain acetylcholinesterase (AChE) activity, a target enzyme for common homeowner use insecticides, in adult coho salmon. The brain enzyme activities of affected fish from an urban stream (Longfellow Creek; LF) were not significantly inhibited relative to unaffected fish from a non-urban stream (Fortson Creek; FT) and three regional hatcheries; Issaquah (ISS-H), University of Washington (UW-H), and Stillaguamish (STI-H). Error bars are 1 standard error of the mean. Sample size is indicated in parentheses and letters indicate significant differences between locations (one-way ANOVA, Tukey's HSD; $p<0.05$).
doi:10.1371/journal.pone.0028013.g003

Creeks) and one non-urban site (Wallace River Hatchery) are shown in Figure 4. There were significant differences among the three sites for cadmium, lead, nickel, and zinc (one-way ANOVA and Tukey-Kramer HSD; $p<0.05$). Fish from the two urban streams had similar levels of cadmium, lead, and nickel, and these were significantly higher than corresponding levels in the gills of coho from the non-urban location. For zinc, fish from the non-urban hatchery had slightly but significantly higher gill concentrations relative to fish from one of the two urban streams (Des Moines Creek).

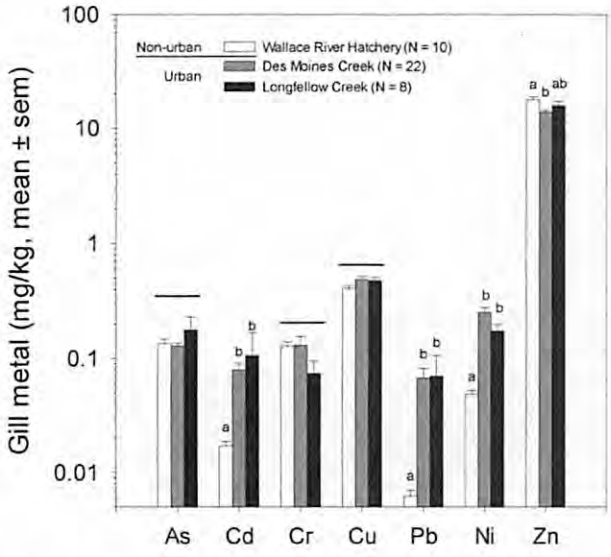

Figure 4. The gill tissues of prematurely dying coho contain elevated levels of cadmium, lead, and nickel. The concentrations of metals in the gills of affected coho from two urban streams (Longfellow and Des Moines Creeks) are plotted relative to samples collected from a non-urban hatchery (Wallace River). Error bars are 1 standard error of the mean. Letters indicate significant differences in measured levels of metals between sampling locations (one-way ANOVA, Tukey's HSD; $p<0.05$) and horizontal bars indicate no significant differences ($p>0.05$).
doi:10.1371/journal.pone.0028013.g004

Bile analysis indicates elevated exposure to petroleum hydrocarbons in fish from urban streams

In the fall of 2002, bile was collected from symptomatic and recently dead coho from Longfellow Creek, as well as from adults returning to spawn in the non-urban reference stream (Fortson Creek). Relative exposures to polycyclic aromatic hydrocarbons (PAHs) were quantified by measuring mean concentrations of phenanthrene (PHN) and benzo(a)pyrene (BaP) metabolites in bile. As shown in Figure 5, affected fish from the urban stream had significantly higher biliary levels of both PHN and BaP equivalents (one way ANOVA, Tukey-Kramer HSD, $p<0.05$).

In 2003, the biliary levels of PAHs in fish from Longfellow Creek were compared to PAH levels in seawater-phase adults collected from a gillnet fishery in Elliott Bay, prior to freshwater entry, and adults returning to a non-urban hatchery (Wallace River Hatchery). As in 2002, fish from the urban stream showed significantly higher exposures to both PHN and BaP relative to the non-urban sampling location (Figure 5; one way ANOVA, Tukey-Kramer HSD, $p<0.05$). PAH levels in the bile of seawater-phase coho collected from Elliott Bay were slightly but not significantly elevated relative to the non-urban location.

Stream temperature, dissolved oxygen, and other conventional water quality parameters do not appear to be causal factors for the mortality syndrome

Monitoring results for conventional surface water quality parameters in urban drainages where premature coho mortality is prevalent have been published previously [7]. During the fall months, urban streams were cool and well mixed. For example, fixed station surface temperature monitoring on Longfellow Creek between October and December in 2002 (86% pre-spawn mortality across the entire run that year; Table 2) revealed maximum daily temperatures ranging from about 6–11°C. Over the same interval, surface water concentrations of dissolved oxygen ranged from about 9–11 mg/L. This fall and winter pattern of relatively cool, oxygen-rich surface flows is also typical of other urban streams where coho die-offs commonly occur (e.g., Piper's Creek; [7]). Monitored conditions for other conventional water quality parameters were also favorable for salmon health and survival. The average level of ammonia-N in water samples collected from Longfellow Creek during storm events (0.04 mg/L) was more than an order of magnitude below the pH-adjusted benchmark criterion for chronic ammonia toxicity (0.43–2.1 mg/L). Moreover, pH levels were normal (pH 6.5–8.5) for Longfellow Creek in the survey years included in this study [7].

Mortality is qualitatively but not quantitatively influenced by rainfall

During the first year of annual surveys on Longfellow Creek (2002), fall coho returns were several weeks late due to an unusually dry October and early November. The first significant rains in the second week of November triggered a large influx of spawners. As the rains continued over the next two weeks, every fish entering the drainage succumbed to the mortality syndrome, with many observations of overt symptomology during daily surveys (Figure 6). Fish only survived to spawn in the weeks following the mid-November storms.

Based in part on the apparent strength of this association between rainfall and mortality in 2002, we continued with daily

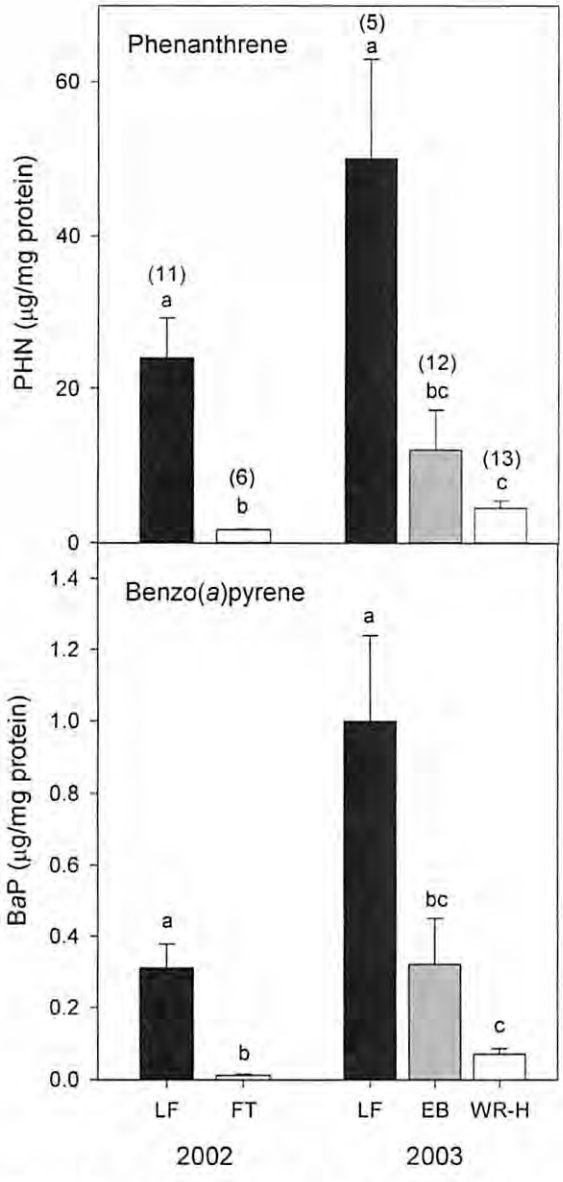

Figure 5. Analysis of bile from affected coho spawners indicates exposure to polycyclic aromatic hydrocarbons (PAHs). Concentrations of fluorescent PAH metabolites (as phenanthrene [PHN] and benzo-*a*-pyrene [BaP] equivalents) in the bile of coho collected in an urban stream (Longfellow Creek; LF) are shown relative to fish from a non-urban stream (Fortson Creek; FT) and a non-urban hatchery (Wallace River Hatchery; WR-H). In 2003, seawater-phase coho (pre-freshwater entry) were also sampled from urban Elliott Bay (EB). The bile data demonstrate a significant increase in PAH exposure after coho spawners transition from a highly urbanized estuary to freshwater spawning habitats. Sample sizes, the same for PHN and BaP, are indicated over each bar. Error bars are 1 standard error of the mean.
doi:10.1371/journal.pone.0028013.g005

surveys on Longfellow Creek in successive years (2003–2009). The relationship between inter-annual variation in total rainfall and the severity of spawner mortality was evaluated using binary logistic regression. The results suggest a pattern of higher coho survival in wetter years where more water moves through the watershed before many of the adults arrive on the spawning grounds. However, there was a large amount of inter-annual variability in both rainfall (timing and amount) and coho returns (timing and number). As a consequence, the logistic regression was not significant at $p \leq 0.05$ (not shown; $\chi 2(1) = 1.70$, $p = 0.19$). The slope for the regression was -0.021 (se = 0.016, $p = 0.19$) and the intercept was 1.674 (se = 0.403, $p < 0.0001$). Across years, rainfall explained 29% of the variability in the spawner mortality syndrome (log odds ratio weighted linear regression, $r^2 = 0.288$).

Notably, in each of the eight survey years, the first carcass found was always a pre-spawn mortality. Conversely, in six of the seven years in which at least one fish survived to spawn, the last carcass found was a successful spawner.

Discussion

We have documented a distinct mortality phenomenon among adult coho salmon returning to spawn in urban watersheds of central Puget Sound. The syndrome has been recurrent for more than a decade, with a consistent symptomology across years and survey locations. The annual die-offs have claimed a large proportion of the fall runs in the drainages monitored during the course of this study. These high mortality rates (e.g., 60–100%) are likely to preclude sustainable natural production in urban drainages more generally [29], and the coho we monitored during the course of this study were fish that appear to have strayed into ecological traps [30] in search of spawning habitat. Our findings fit a general pattern for Puget Sound, in which adult coho are very few in number in watersheds where the mortality syndrome has been observed. Conversely, in (non-urban) watersheds where coho spawners are relatively abundant, they appear to be unaffected.

Factors that are known to cause spawner mortalities in other species of salmon do not appear to be involved in the coho pre-spawn syndrome that we have explored here. The temperatures and dissolved oxygen content of urban streams during mortality events were not unusually high or low, respectively. Although all fish of the fish we examined showed evidence of infection with common pathogens, there was no correlation with the high rates of mortality in urban drainages or the observed symptomology. Lastly, the stricken coho were generally in good physical condition, and we found no evidence that origin (i.e., wild or hatchery) influences an animal's susceptibility.

The weight of evidence therefore suggests that adult coho salmon are unusually vulnerable to the toxic effects of one or more chemical contaminants, most likely delivered to urban spawning habitats via stormwater runoff. The rapid progression of the syndrome and the specific nature of the symptoms are consistent with acute cardiorespiratory toxicity. Our current findings support this hypothesis, albeit indirectly by ruling out alternative, non-chemical explanations.

We found that affected coho show elevated exposure to metals and petroleum hydrocarbons, the latter after spawners transition to freshwater from a highly urbanized estuary (Elliott Bay). Evidence of exposure to metals and PAHs does not imply causality, but future studies should address these toxics, as they are specifically known to disrupt respiratory, osmoregulatory, and cardiovascular physiology in fish. The abrasion of vehicle tires and brake pads releases aluminum, barium, cadmium, cobalt, copper, lead, nickel, zinc, and other elements onto impervious surfaces [31]. Copper and other metals disrupt ionoregulation via binding to ligands in the fish gill [32] without causing overt cytotoxicity. Moreover, metals are generally more bioavailable and thus more toxic to fish in soft waters such as stormwater [33]. Motor vehicles are also sources of PAHs via exhaust and leaking crankcase oil. Certain PAHs are cardiotoxic to fish [34], including specifically the tricyclics phenanthrene and dibenzothiophene (e.g., [35]).

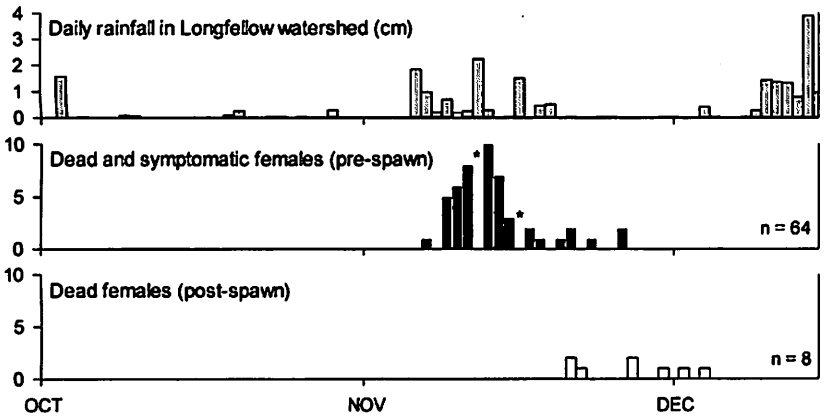

Figure 6. Pre-spawn mortality and survival to spawn in relation to rainfall. Shown are the results of daily stream surveys throughout the 2002 coho spawning season in Longfellow Creek in relation to daily rainfall. Asterisks (*) indicate days when stream flows were too high to survey the creek.
doi:10.1371/journal.pone.0028013.g006

It is important to note, however, that the toxicological context (i.e., established literature) for anticipating possible acutely lethal toxic effects of stormwater contaminants on coho spawners is practically nonexistent. On the one hand, urban runoff typically contains organic chemicals and metals in the low parts per billion to parts per trillion range (e.g., [36,37]), well below levels that would be expected to cause fish kills based on established median lethal (LC_{50}) concentrations for rainbow trout and other salmonids. On the other hand, to our knowledge, there have been no toxicological studies on freshwater-transitional adult coho. When adults return from saltwater to freshwater in preparation for spawning, they undergo osmoregulatory adjustments that include shifts in plasma osmolality, gill sodium-potassium ATPase activity, and the density of chloride cells in the gill (e.g., [38]), as well as changes in the circulation of stress and reproductive hormones [2]. These changes may render adult animals particularly vulnerable to toxics that interfere with the physiological processes that underlie freshwater acclimation. Coho have recently been shown to be considerably more vulnerable to chemical toxicity when they make the opposite transition from freshwater to saltwater [39].

Sensitivity related to freshwater transition might explain our observations of affected adults and unaffected juveniles exposed to the same surface waters, but not our observations of affected coho spawners side-by-side with unaffected spawners of other salmon species. For example, in 2006 there were temporally overlapping runs of coho and chum spawners in Piper's Creek. Whereas all of the coho succumbed, the egg retention rate for chum carcasses was <4% (5 of 135 females; data not shown). Moreover, symptomatic coho were observed in the stream side-by-side with healthy chum, the latter actively digging and defending redds (Video S4). The underlying reason for the interspecific difference in sensitivity remains to be determined.

More work is also needed to define the influence of rainfall on the spawner mortality syndrome. The clearest indication that rainfall plays a role was the 2002 survey results for Longfellow Creek. That year was characterized by an unusually long antecedent dry interval (presumably allowing a proportionally greater accumulation of pollutants on impervious surfaces within the watershed), a relative abundance of returning spawners, and consistent rainfall for approximately two weeks at the beginning of the compressed run. As in 2002, in subsequent years we observed a general tendency towards higher survival later in the run, after multiple fall rain events. However, the relationship was not statistically significant across the survey years, due in part to highly variable rainfall patterns, longer run durations, and very low spawner numbers in some years. Notably, the mortality syndrome is not a simple first-flush phenomenon, as spawned and unspawned carcasses were usually intermixed throughout the duration of each run in 2003–2009.

Additional evidence implicating urban runoff was recently provided by a spatial land use analysis of the watersheds surveyed during the course of this study. Feist et al. [40] found that inter-watershed rates of coho spawner mortality correlate closely and positively with the relative proportion of local roads, impervious surfaces, and commercial property within a basin. These and other correlations were then used to predict areas of possible coho spawner die-offs in unmonitored drainages throughout central Puget Sound [40]. The link to roads and other impervious surfaces further implicates motor vehicles as the likely source of causal toxics, but this remains to be demonstrated directly – e.g., by reproducing the mortality syndrome in otherwise healthy adult coho via exposures to environmentally relevant mixtures of metals and PAHs in freshwater.

Recent population-scale modeling has shown the potential for rapid local declines in coho population abundance across the range of spawner mortality rates observed in urban drainages during the course of this study [29]. Regional human population growth and land use changes that increase the proportion of impervious surfaces within watersheds may therefore pose an important future threat to wild coho populations if 1) toxic urban runoff is the underlying cause of the mortality phenomenon, and 2) wild coho are similar in their vulnerability to the hatchery and unmarked (and presumably wild) coho that were found unspawned in 2002–2009. This is above and beyond the established and widely documented stormwater-driven threats to aquatic habitats (e.g., [41,42,43]).

In closing, past efforts to restore salmon habitats in Seattle-area urban watersheds have revealed unexpected challenges for improving coho spawner abundance and survival. These restoration projects have been successful in numerous other ways, including revitalizing urban green spaces, extending watershed connectivity, enhancing public education and involvement, and improving habitat conditions for otters, waterfowl, amphibians, stream invertebrates, native plants, and other fish species. Restored urban streams have also provided an experimental setting to study what may become a very important threat to wild

coho populations in the decades ahead as some healthy stream networks gradually acquire the land cover characteristics of the Longfellow Creek system and similar urban drainages. The next generation of urban watershed improvements is now underway, including the catchment-scale implementation of natural drainage systems (using green infrastructure and other emerging technologies), floodplain restoration, and new pollution mitigation activities such as vacuum sweeping of roadways. Moreover, Washington recently became the first state in the U.S. to legislatively mandate a phased reduction of metals in vehicle brake pads and other friction materials (SB6557). Future improvements in the survival of adult coho in urban streams will be an important indicator of success for these and other pollution reduction strategies.

Supporting Information

Text S1 A detailed description of the histopathology results from tissue samples collected during the study from urban and non-urban sites.
(DOC)

Figure S1 Number of pathogens per fish detected by pathogen screening methods for fish collected in 2003. Horizontal bar is positioned at the median. Longfellow Creek fish differ from fish from all other locations (Chi-square test, $p<0.0001$).
(TIFF)

Table S1 Prevalence of infectious (parasitic/bacterial) and idiopathic conditions detected by histopathology and by pathogen screening (molecular and microscopic) in adult coho salmon sampled from several creeks and hatcheries in the Puget Sound region in 2003 and 2004. H = histopathology; PS = pathogen screening methods; − = analysis not performed.
(DOC)

Video S1 Symptomatic female coho salmon in Piper's Creek in 2000. The fish appears to be in good physical condition, with ocean-bright (silver) coloration. Characteristic symptoms include loss of equilibrium, gaping, and pectoral fin splaying.
(MP4)

Video S2 Early onset symptomology of an affected adult coho salmon in Longfellow Creek in 2002. The fish has lost orientation (surface swimming) and is gaping.
(MP4)

Video S3 Late-stage symptomology of an affected adult coho salmon in Longfellow Creek in 2002. The fish has lost equilibrium and is gaping, with pectoral fins splayed.
(MP4)

Video S4 A symptomatic coho spawner in Piper's Creek in 2006, just downstream of unaffected chum spawners displaying normal spawning behavior.
(MP4)

Acknowledgments

Among staff from the NWFSC, we thank Gladys Yanagida for assistance with bile analyses, Kym Jacobsen and Todd Sandell for help with pathogen and parasite screening, Blake Feist for generating the map showing sampling locations (Figure 1), and David Baldwin for statistical advice. Sandie O'Neill, Julann Spromberg, David Baldwin, and Keith Kurko provided helpful comments on draft versions of the manuscript. We also thank Lorin Reinelt for providing valuable input to the initial project design. Mike Mahovalich from the Muckelshoot Tribe assisted with the collection of seawater-phase adult coho from Elliott Bay. The Fisheries Division of the U.S. Fish and Wildlife Service's Washington Fish and Wildlife Office retrieved and read coded wire tags from salmon carcasses. We also greatly appreciate the efforts of more than 60 volunteers who assisted with stream surveys from 2002–2009.

The conclusions expressed in this article are those of the authors and do not necessarily represent the views of the U.S. Fish and Wildlife Service or the National Marine Fisheries Service.

Author Contributions

Conceived and designed the experiments: NLS MSM GMY LDR BM DW LR KDL JWD TKC. Performed the experiments: NLS MSM SGM JSL JKM LDR CAL CMS BLF BM DW LR SD JWD. Analyzed the data: NLS MSM SGM JSL GMY LDR CAL BLF BM DW LR KDL JWD TKC. Contributed reagents/materials/analysis tools: BM DW LR KDL SD JWD. Wrote the paper: NLS MSM JSL JKM GMY LDR CAL CMS BLF TKC. Led salmon spawner survey operations, including field team coordination and sample collection: SGM JSL JKM CAL CMS BLF SD. Interagency principal investigators: NLS MSM GMY LDR BM DW KDL JWD TKC.

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