



City of Covington

Unmatched Quality of Life.

City of Covington

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The City of Covington is a place where community, business, and civic leaders work together with citizens to preserve and foster a strong sense of community.

PLANNING COMMISSION AGENDA

April 18, 2013

6:30 PM

CALL TO ORDER

ROLL CALL

Chair Daniel Key, Vice Chair Paul Max, Sonia Foss, Ed Holmes, Bill Judd, Sean Smith, & Alex White.

PLEDGE OF ALLEGIANCE

APPROVAL OF CONSENT AGENDA

1. Planning Commission Minutes for April 4, 2013.

CITIZEN COMMENTS - *Note: The Citizen Comment period is to provide the opportunity for members of the audience to address the Commission on items either not on the agenda or not listed as a Public Hearing. The Chair will open this portion of the meeting and ask for a show of hands of those persons wishing to address the Commission. When recognized, please approach the podium, give your name and city of residence, and state the matter of your interest. If your interest is an Agenda Item, the Chair may suggest that your comments wait until that time. Citizen comments will be limited to four minutes for Citizen Comments and four minutes for Unfinished Business. If you require more than the allotted time, your item will be placed on the next agenda. If you anticipate, in advance, your comments taking longer than the allotted time, you are encouraged to contact the Planning Department ten days in advance of the meeting so that your item may be placed on the next available agenda.*

PUBLIC HEARING – None

UNFINISHED BUSINESS – None

NEW BUSINESS – None

CONTINUED BUSINESS-

1. Discussion and Guidance to Staff and Consultants on Minimum and Maximum Intensity Alternative Land Use Concepts and Imagery for Hawk Property Subarea Plan and Planned Action EIS (See Attachments)

ATTENDANCE VOTE

PUBLIC COMMENT: (Same rules apply as stated in the 1st CITIZEN COMMENTS)

COMMENTS AND COMMUNICATIONS OF COMMISSIONERS AND STAFF

ADJOURN

*Any person requiring a disability accommodation should contact the City at least 24 hours in advance.
For TDD relay service please use the state's toll-free relay service (800) 833-6384 and ask the operator to dial (253) 480-2400*

Web Page: www.covingtonwa.gov

**CITY OF COVINGTON
Planning Commission Minutes**

April 4, 2013

City Hall Council Chambers

CALL TO ORDER

Vice Chair Max called the regular meeting of the Planning Commission to order at 6:36 p.m.

MEMBERS PRESENT

Vice Chair Paul Max, Ed Holmes, Bill Judd, Sean Smith and Alex White

MEMBERS ABSENT

Chair Key and Sonia Foss

STAFF PRESENT

Richard Hart, Community Development Director
Salina Lyons, Principal Planner
Ann Mueller, Senior Planner
Kelly Thompson, Planning Commission Secretary

APPROVAL OF CONSENT AGENDA

- Ø **1. Commissioner Smith moved and Commissioner White seconded to approve the consent agenda and the corrected minutes for March 7, 2012. Motion carried 5-0.**

CITIZEN COMMENTS – NONE

PUBLIC HEARING – NONE

UNFINISHED BUSINESS –NONE

NEW BUSINESS

CONTINUED BUSINESS

- 1. Discussion of Community Workshop Results on Hawk Property Subarea Plan**

Community Development Director, Richard Hart summarized the results of the Community Workshop held on March 25, 2013. Mr. Hart's memo outlining the process and the results was included in the Planning Commission packet.

The Community Workshop participants seemed very pleased with the forum and the exercise.

Senior Planner, Ann Mueller shared some photos from the forum which showed the different group's concept plans, which include commercial, residential, focal points, and green space.

Commissioner Smith asked whether there was a need for schools in this area as a result of the future development. Mr. Hart responded that 80 of the acres are in the Tahoma school district and the rest are in the Kent School District. Neither Kent nor Tahoma School districts attended the Community Workshop. The Kent School District receives impact fees from single family and multifamily development within the city. There may be a total of 1000 to 1500 residential units.

Commissioner Holmes visited the gravel mine and noted there is still activity on the site. Mr. Hart noted that Lakeside is likely rearranging as much of the gravel as they can on site for reclamation purposes. The less they have to take out, the less costly it is.

A timeline of the Hawk Property Subarea Plan and EIS process was prepared and handed out at the meeting.

ATTENDANCE VOTE

- Ø **Commissioner Judd moved and Commissioner Holmes seconded to excuse Chair Key and Commissioner Foss, motion carried 5-0.**

PUBLIC COMMENT

Kevin Thomas 10925 25th Ave SE, Everett Yarrow Bay Holdings/Oakpointe
The developer will be providing the City a reclamation plan to show how the materials will be placed and compacted. He complimented Salina, Ann and Richard on the great Community Workshop. The one thing he took away was that it was the Community's plan.

COMMENTS AND COMMUNICATIONS FROM STAFF

Ms. Mueller shared that Community Relations Coordinator, Karla Slate will be sending out invitations to a neighborhood HOA meeting to be held on Tuesday April 30, 2013 from 7:00 to 8:30 p.m. The meeting will focus on public safety.

Mr. Hart shared that there will be two Planning Commission Meetings per month in May and June. Staff will present an amendment on Development Agreements, clearing and grading changes, and sign code changes relating to civic government and non-profit signs. The Planning Commission will also discuss the collection of school impact fees.

Commissioner Holmes requested an update on the work plan items mid-year. He also complimented the way the study session was conducted on the Hawk properties. The participants had a lot of enthusiasm for the process.

ADJOURN

The April 4, 2013 Planning Commission Meeting adjourned at 7:37 p.m.

Respectfully submitted,

Kelly Thompson, Planning Commission Secretary

Memo

To: Planning Commission
From: Richard Hart, Community Development Director
CC: Salina Lyons, Principal Planner; Ann Mueller, Senior Planner
Date: April 11, 2013
Re: Finalize alternatives and scope of review in the Hawk Property Subarea Draft EIS

At the Planning Commission's public meeting on April 18, 2013, the commissioners will review the maximum and minimum land use concepts for evaluation as alternatives in the Hawk Property Subarea Plan Draft Environmental Impact Statement (EIS) and imagery related to proposed uses in the subarea. They will provide guidance to staff and the consultant team after reviewing the land use plans that were developed by meeting participants at the March 25, 2013 Hawk Property Subarea Plan Community Workshop and public comments that were submitted during the EIS scoping comment period, as well as from input by city staff, consultants, and the developers of the subarea. The commissioners will also provide guidance on the imagery for the area that was reviewed by participants at the community workshop.

The City is considering designation of a planned action EIS for the Hawk Property Subarea, which will provide a more detailed environmental analysis of land use development components for the subarea. To prepare a planned action EIS, the city begins by identifying alternatives that include the minimum and maximum range of development that is likely to occur in the subarea including a No Action alternative.

Summary of steps to prepare a Planned Action EIS

- Develop alternatives
- Identify scope of review
- Finalize alternatives and scope of review
- Conduct environmental analysis and identify mitigating measures
- Prepare Draft and Final EIS
- Prepare preliminary Planned Action ordinance
- Prepare implementation process

Covington: unmatched quality of life

After completion of the Planned Action EIS, the city may finalize and adopt a planned action ordinance that defines the location, amount and type of development and specific mitigation measures that must be met. Future development proposals consistent with the planned action ordinance and associated performance standards/mitigation measures do not require additional SEPA review. This process does not exempt projects from local, state and federal permitting requirements. If a project is not consistent with the planned action ordinance, the applicant may elect to revise the proposal to meet the requirements of the ordinance or conduct additional environmental review.

During the EIS scoping comment period, March 9 -29, 2013, city staff received comments from Charlene Anderson with the City of Kent; Karen Walter with the Muckleshoot Indian Tribe; Brandon Anderson with Branbar LLC/H&C Enterprises Inc.; Anderson Baugh & Associates, LLC; Gina Hendrickson; and David and Kristy Kiburg. Four comment sheets were also submitted by meeting participants at the community workshop. Based on these comments the scope of review for the EIS was expanded to include the study of the impacts to groundwater and the impacts of a road connection with the neighborhood to the south.

Attached you will find for reference:

Attachment 1

- an aerial with the Hawk Property subarea outlined in blue;
- a drawing showing the existing and proposed local and regional trails and bikeways in the city and connecting into and through the subarea;
- a composite of the minimum development intensity plans produced by participants at the community workshop;
- the minimum development concept plan from each table at the community workshop;
- a composite of the maximum development intensity plans produced by participants at the community workshop;
- the maximum development concept plan from each table at the community workshop; and
- a summary visual preference survey's voting outcome based on the 17 surveys submitted by meeting participants at the community workshop.

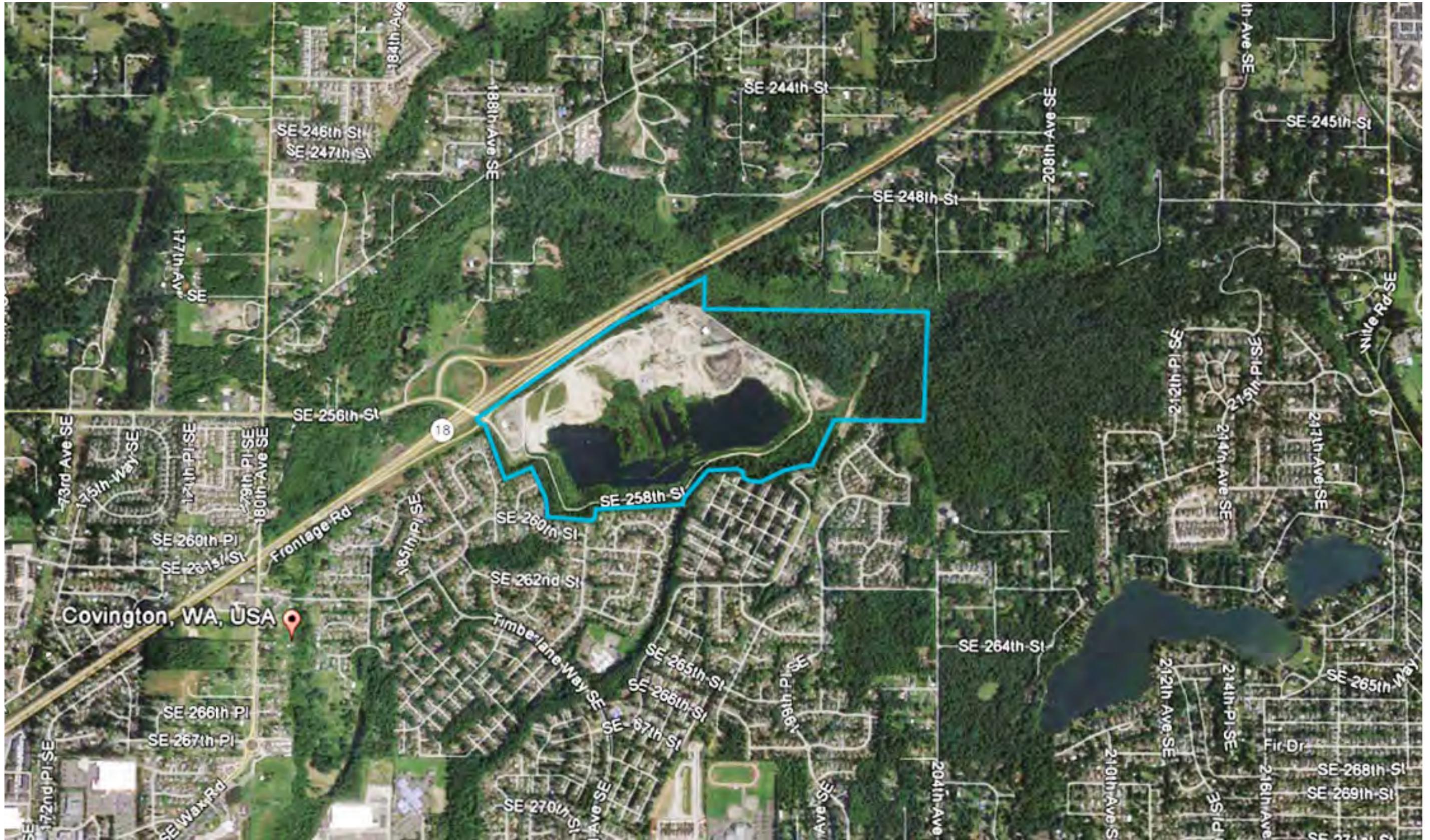
Attachment 2

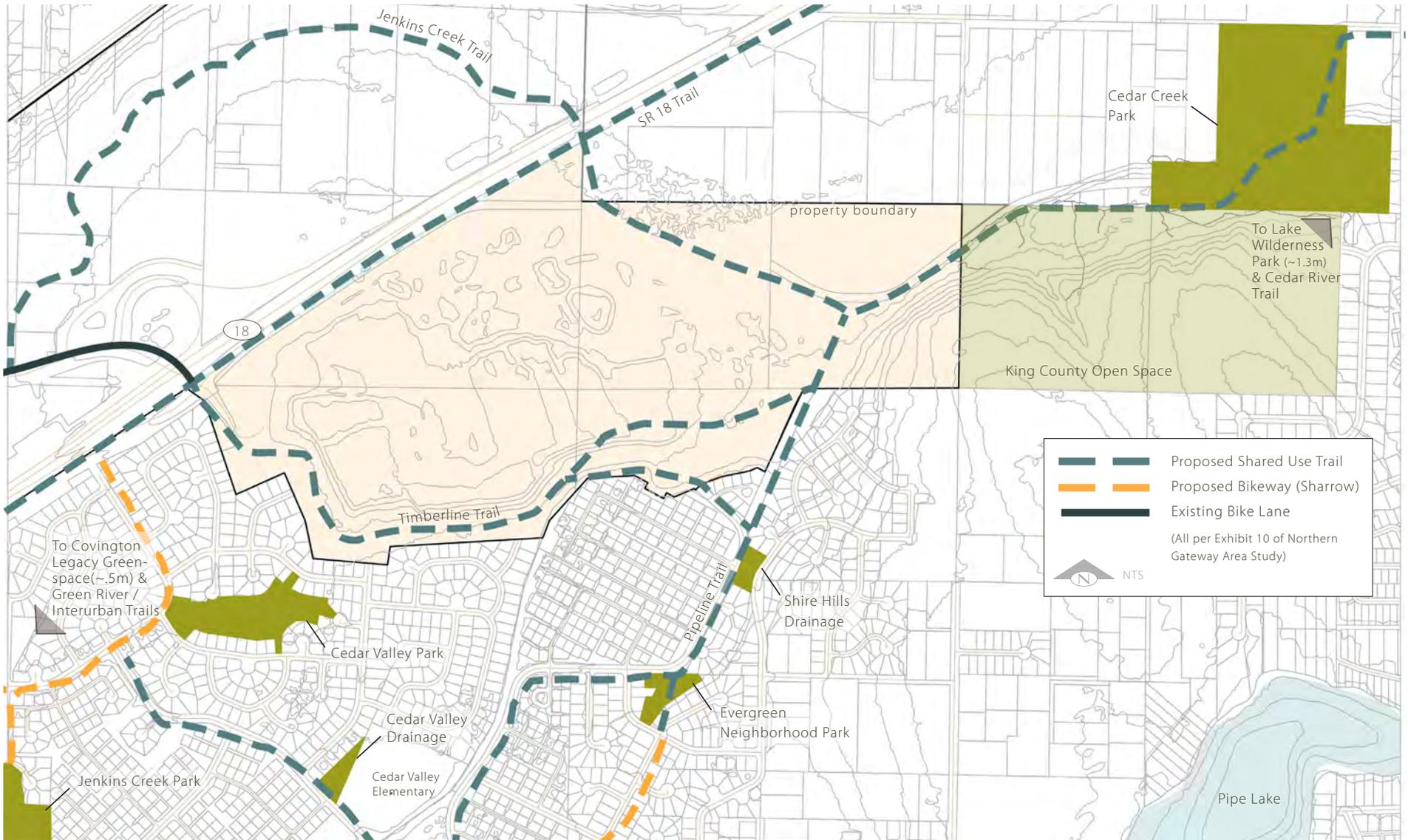
- Written scoping comments received during the EIS scoping period.

Staff's Recommendation for range of development to be reviewed in the Draft EIS:

	Alternative 1 Minimum Intensity Development scenario	Alternative 2 Maximum Intensity Development scenario	No Action Alternative
Commercial	50 acres <i>Big Box -600,000 sf.</i> <i>Retail - 80,000 sf.</i>	55 acres <i>Big Box - 708, 940 sf.</i> <i>Retail - 141,060 sf.</i>	0 acres
Residential	1, 000 units <i>SFR -130 du</i> <i>Townhomes- 270 du</i> <i>MFR -600 du</i>	1,500 units <i>SFR - 200 du</i> <i>Townhomes- 400 du</i> <i>MFR - 900 du</i>	0 acres
Parks	6 acres	9.2 acres	0 acres
Mineral	0 acres	0 acres	210 acres

Attachment 1





PROPOSED TRAILS AND BIKEWAYS MAP

MINIMUM INTENSITY PLAN SOLUTIONS COMPOSITE

SUMMARY:

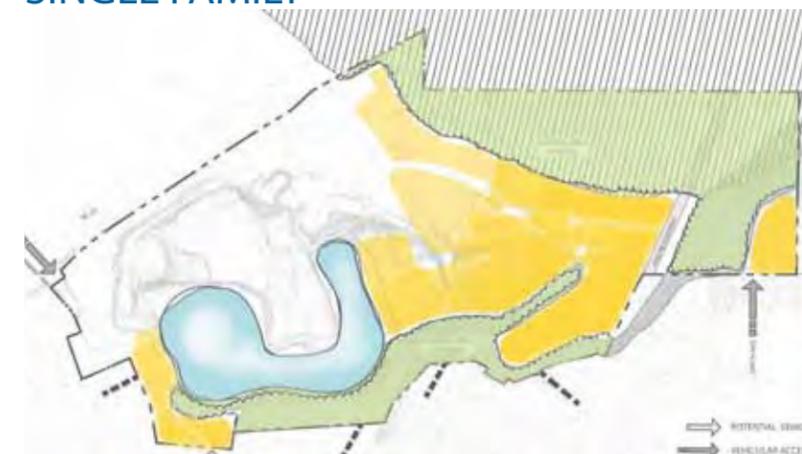
- Commercial - 50 Acres
- Residential - 1,050 Units
- Parks - 6 Acres



PARKS



SINGLE FAMILY



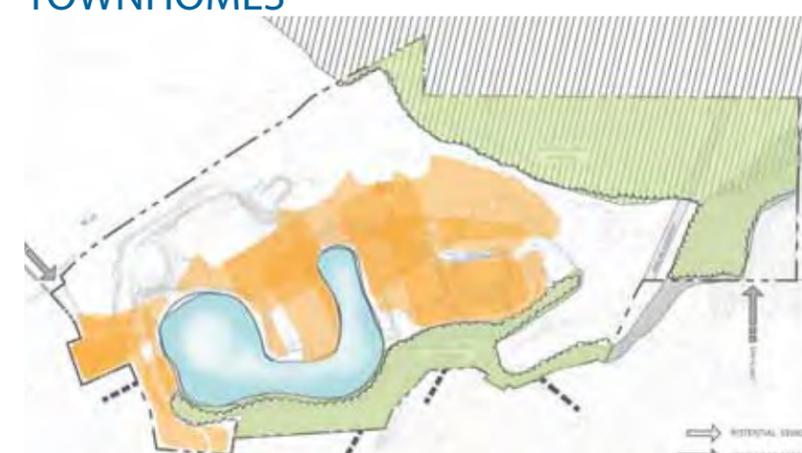
COMMERCIAL



MULTIFAMILY



TOWNHOMES



TEAM 1 MINIMUM INTENSITY PLAN SOLUTION



COMMENTS

- Would like to see restaurants around the lake
- Farmers market as a focal point at a plaza near the lake
- Focal point at the end of the trail
- Housing on top of the commercial at the lake site
- Stack multi-family over commercial with parking below.
- Public access to lake with a trail around it
- Cineplex located in the commercial area
- A trailhead off the new road at the 204th connection end is needed. They'd like it to serve the trail network and a larger park (6-acres+) in that same location.
- They'd like to see a wildlife crossing under the new road in the SE corner.
- Keep the big box retail (& noise) close to the Hwy-18 off-ramp.
- Keep the multi-family near the public areas to minimize crime. (crime concern with multi-family)
- Lower-density residential preferred adjacent to Jenkins Creek (& nearest their properties).
- They want park space to be open to the community, not just the new developments.

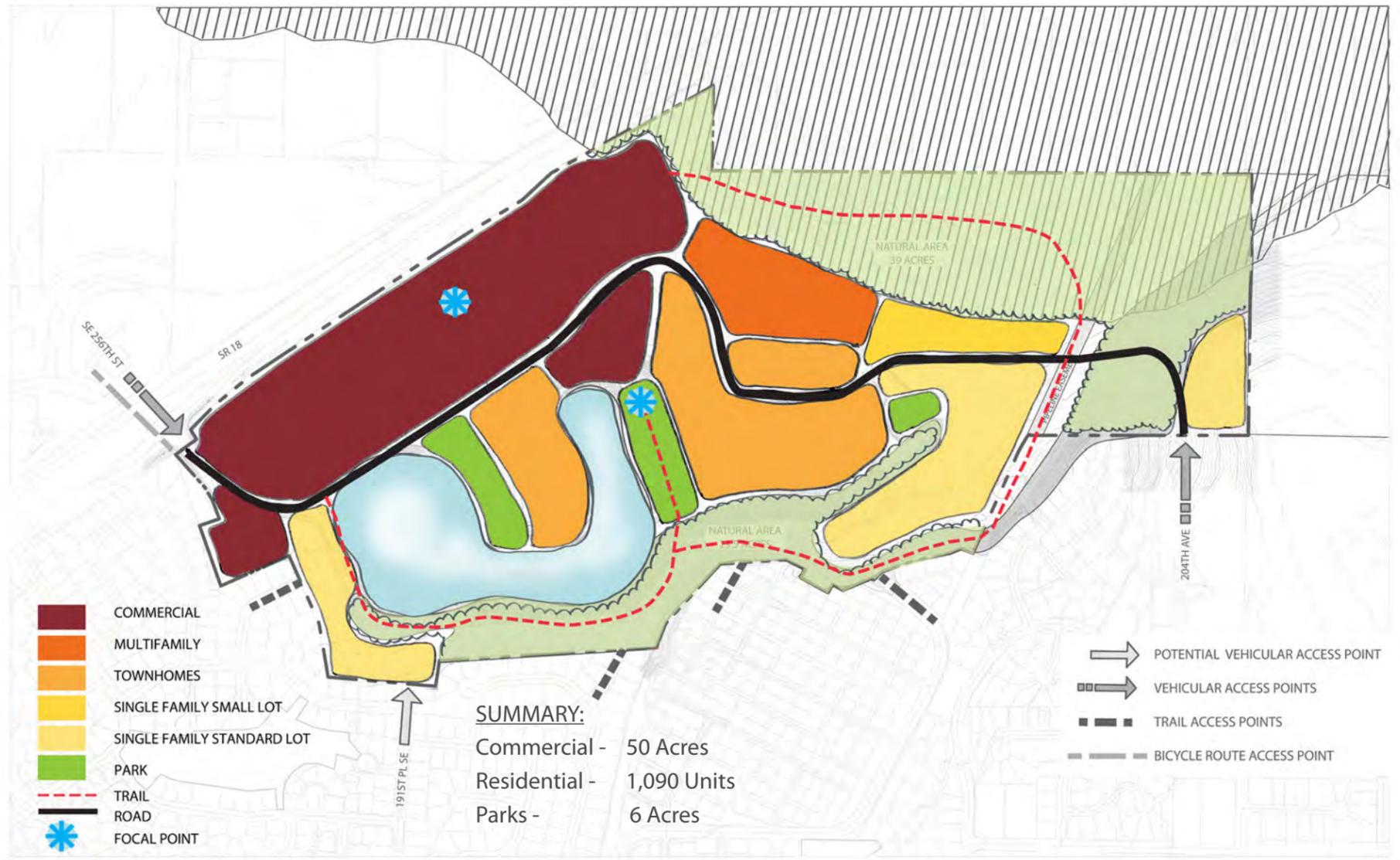
TEAM 2 MINIMUM INTENSITY PLAN SOLUTION



COMMENTS

- Provide more activities for the youth. Youth center, movie theater, bowling, climbing tower, etc.
- Provide kayaking and activities on the lake
- Would like to see a Community Center, YMCA or Boys and Girls club
- Provide parks along the lake with a trail around it. Parks should provide activities for different age groups
- Like the connection to 191st street, for fire and access to the center and hwy 18th
- Would like to see a daycare
- Mix residential with commercial
- Would like to see medical office or clinic
- Like 5 story multi-family with structured parking better than 3 story with surface parking
- Wanted a mix of uses along the lake

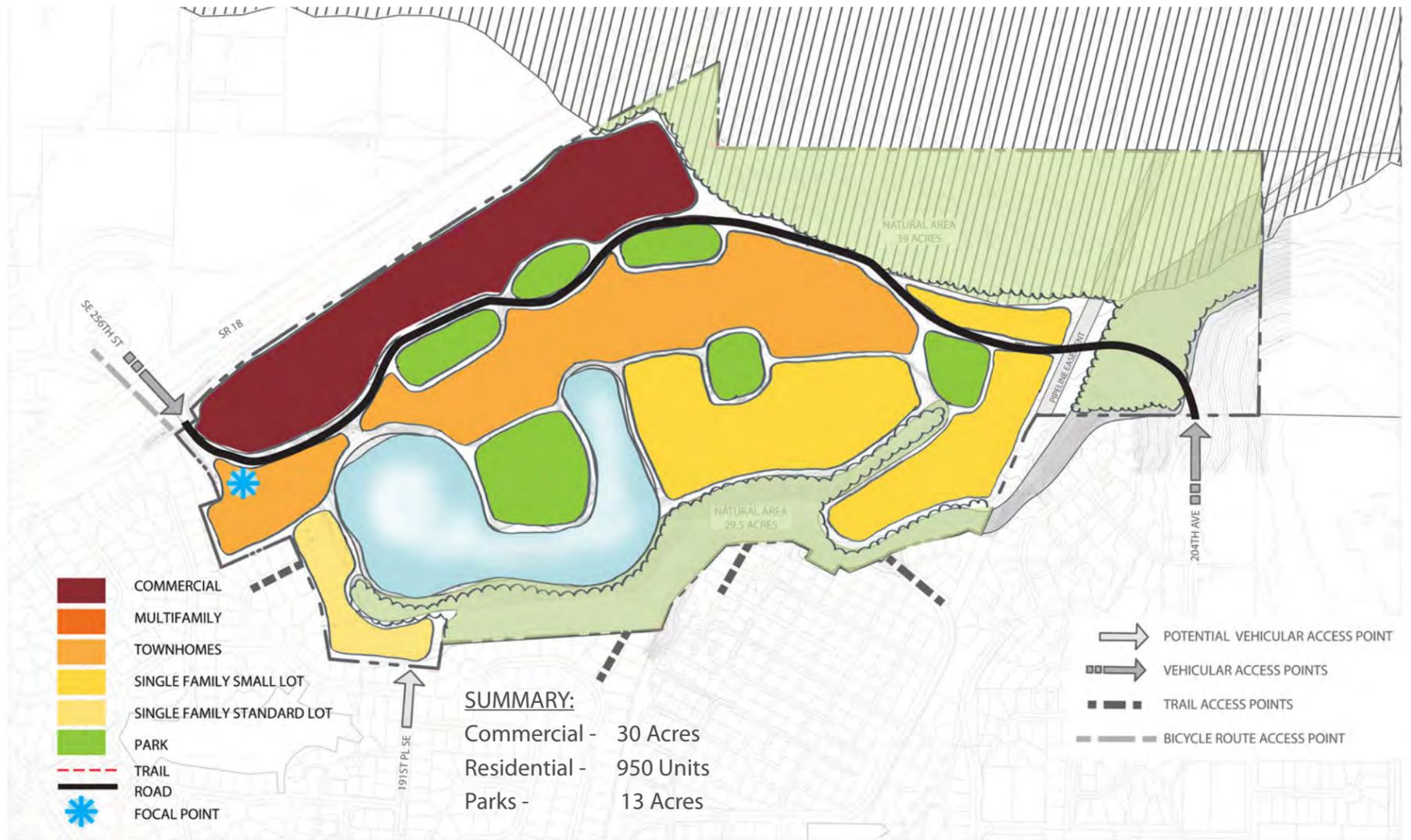
TEAM 3 MINIMUM INTENSITY PLAN SOLUTION



COMMENTS

- The road connection to the neighborhood should be only for emergency vehicles.
- Single family homes should be located along the boundary with the adjacent neighborhood.
- Industrial uses should not be allowed, including continuation of the batch plant.
- The main road should meander to discourage speeding vehicles.
- 5 and 4-story multifamily housing is acceptable
- City needs more multifamily housing and this would be a good location as long as there are amenities for residents like commercial, retail and entertainment uses.
- Two focal points and the one in the commercial/retail area should be large
- The area should be an urban village.

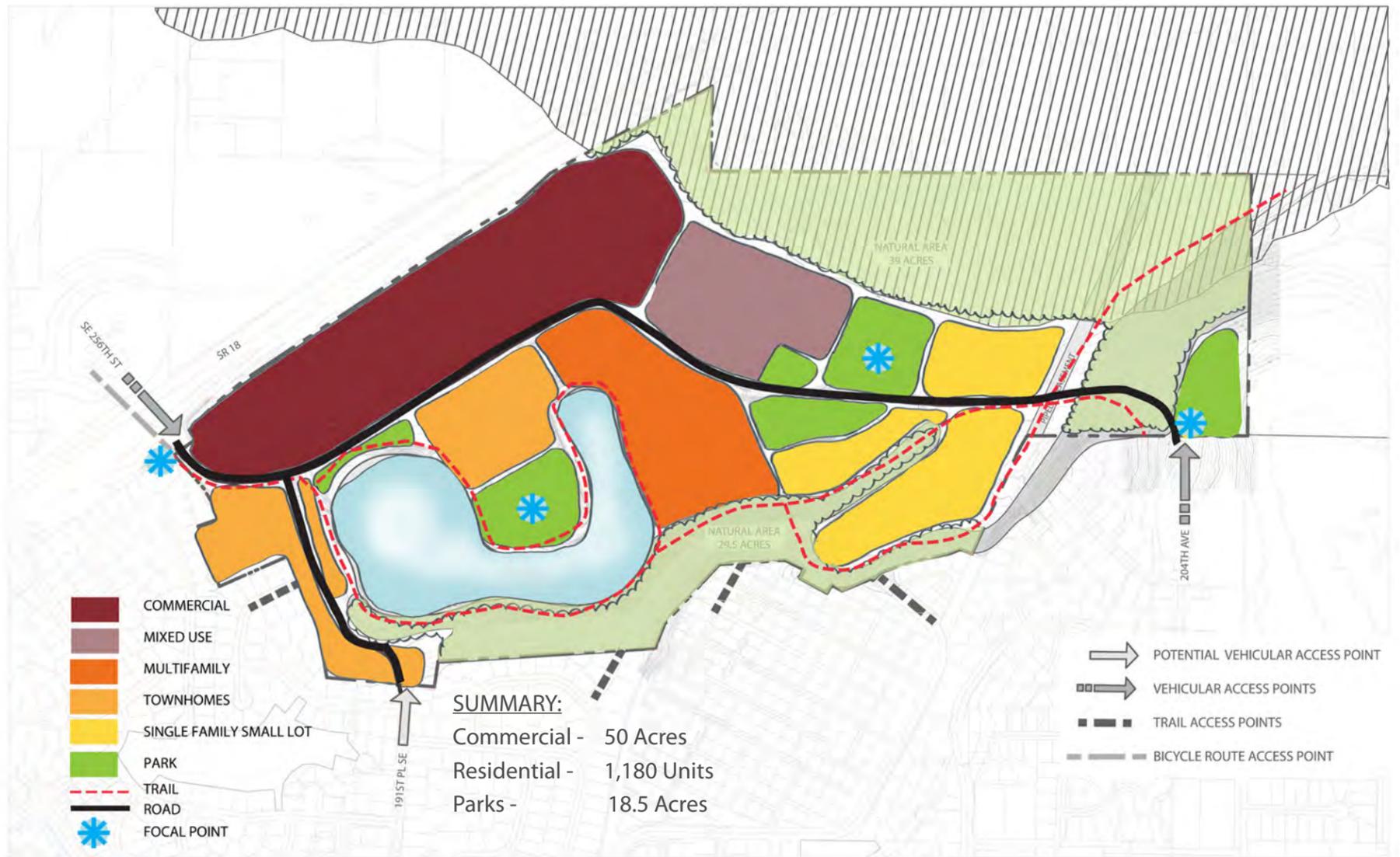
TEAM 4 MINIMUM INTENSITY PLAN SOLUTION



COMMENTS

- Would prefer no multifamily housing; all single-family or townhomes.
- Willing to see additional height in the commercial areas to make more room for residences.
- Use the road and a series of parks to create a buffer between the commercial area and single-family.
- Generally opposed to flats over commercial and worried that multifamily housing will increase crime.
- Prefer to keep commercial and residential units separated.
- Focal point near entry to act as a gateway.

TEAM 5 MINIMUM INTENSITY PLAN SOLUTION



COMMENTS

- Want to see mixed-use buildings with housing above retail and parking underneath
- Would like to see a trail around the lake and trail connections to Timberlane as well as to regional trails

MAXIMUM INTENSITY PLAN SOLUTIONS COMPOSITE

SUMMARY:

Commercial - 55 Acres
Residential - 1,575 Units
Parks - 9.2 Acres

- COMMERCIAL
- MULTIFAMILY
- TOWNHOMES
- SINGLE FAMILY
- PARKS
- TRAILS



PARKS



SINGLE FAMILY



COMMERCIAL



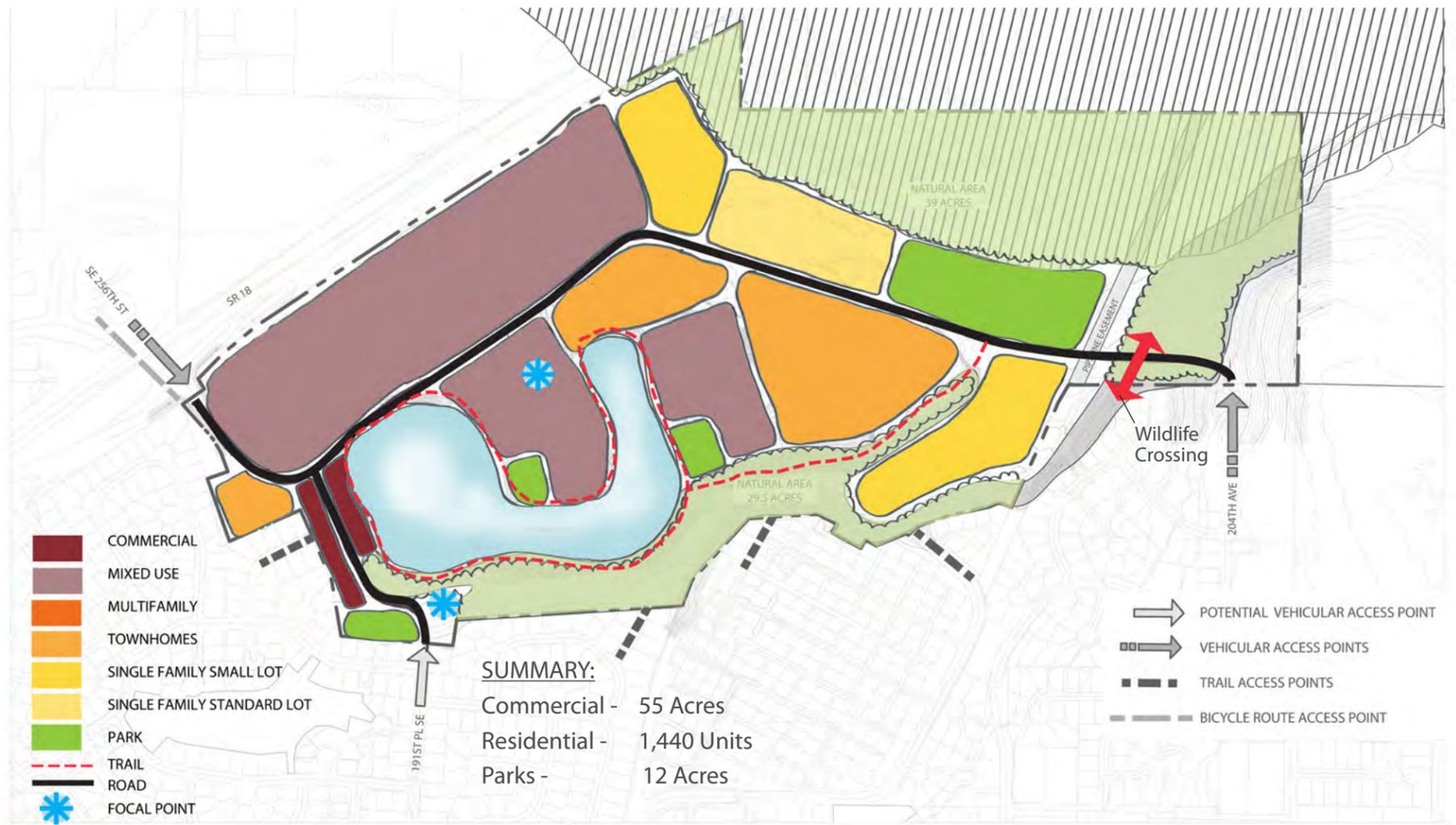
MULTIFAMILY



TOWNHOMES



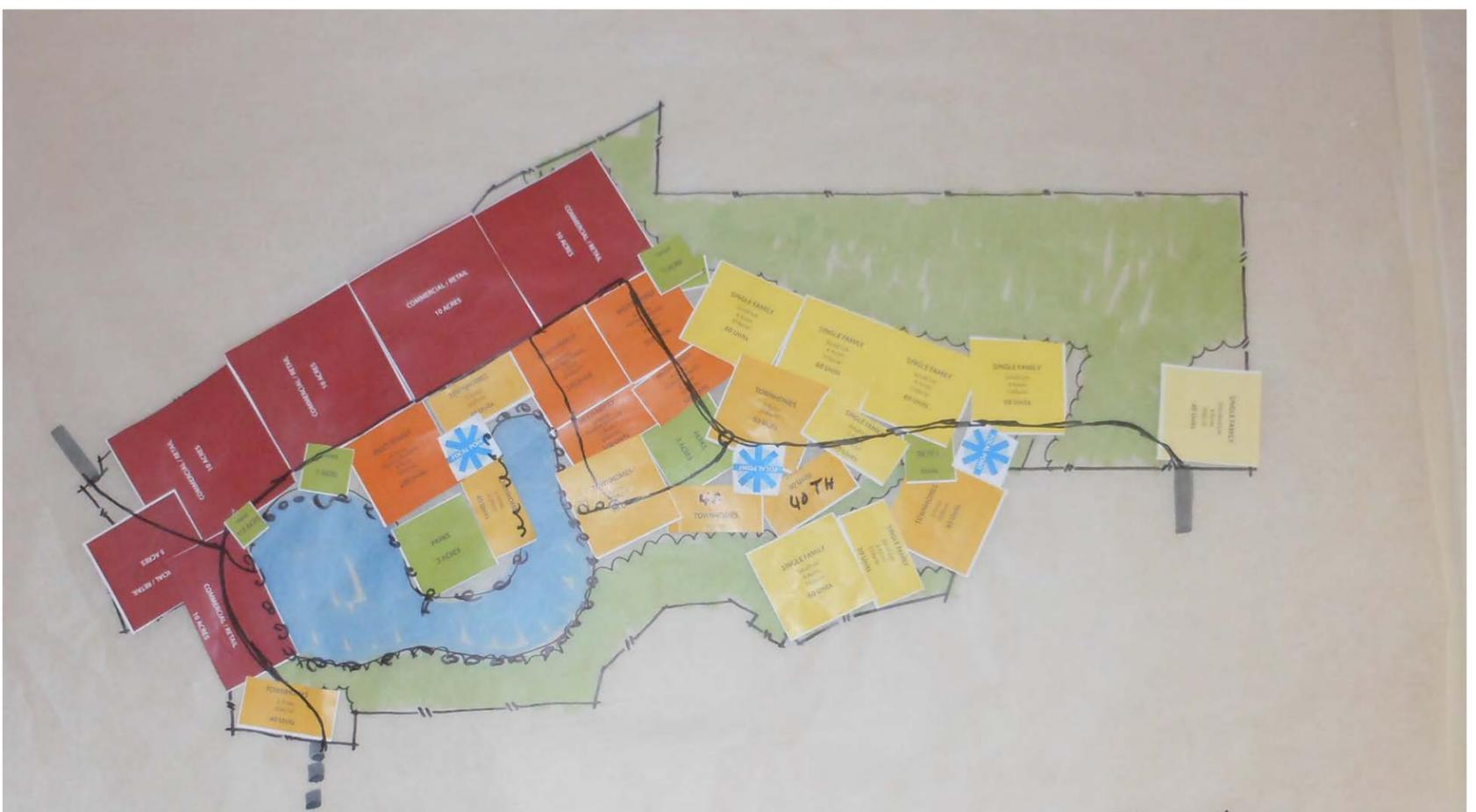
TEAM 1 MAXIMUM INTENSITY PLAN SOLUTION



COMMENTS

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- Farmers market as a focal point at a plaza near the lake
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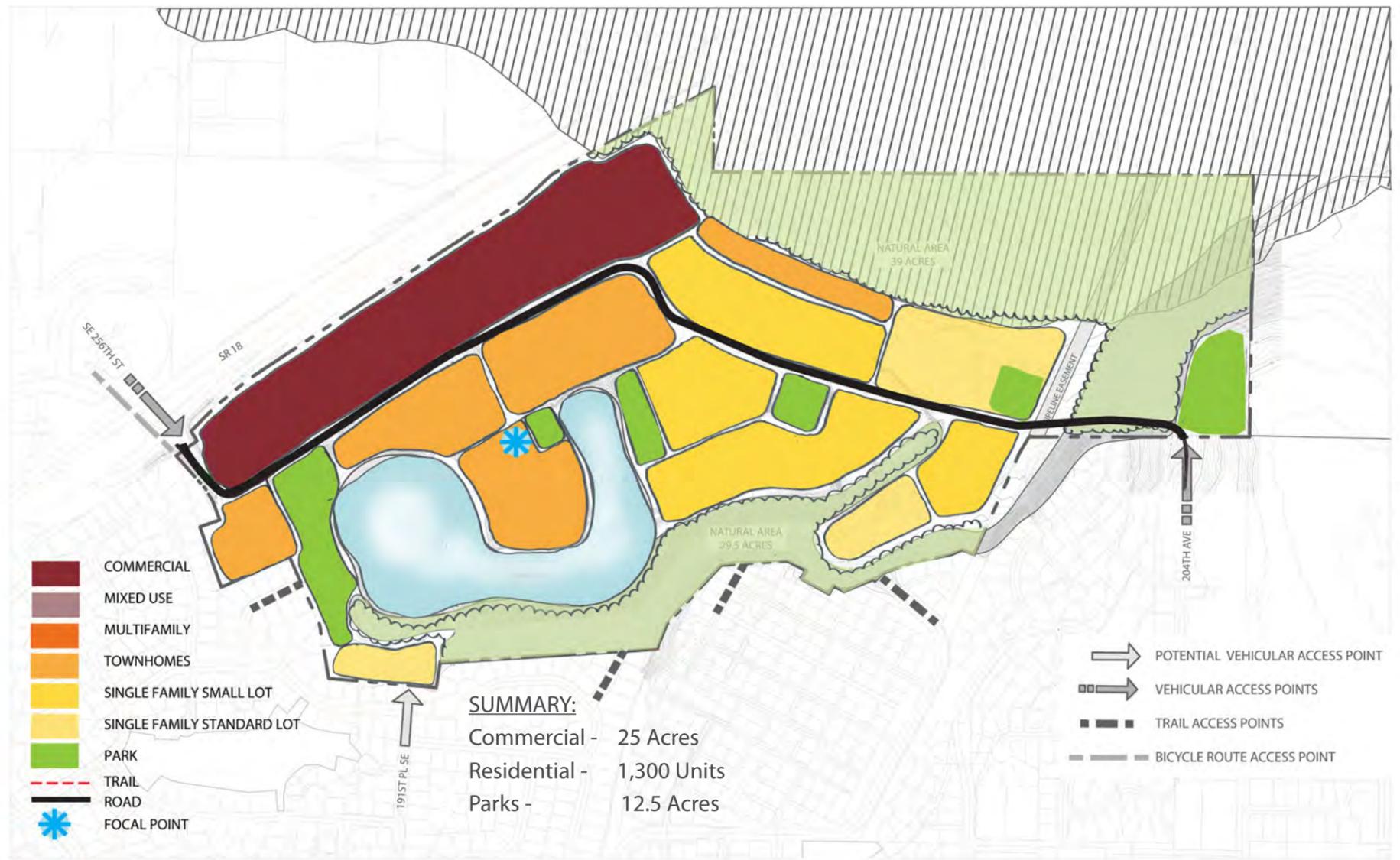
TEAM 2 MAXIMUM INTENSITY PLAN SOLUTION



COMMENTS

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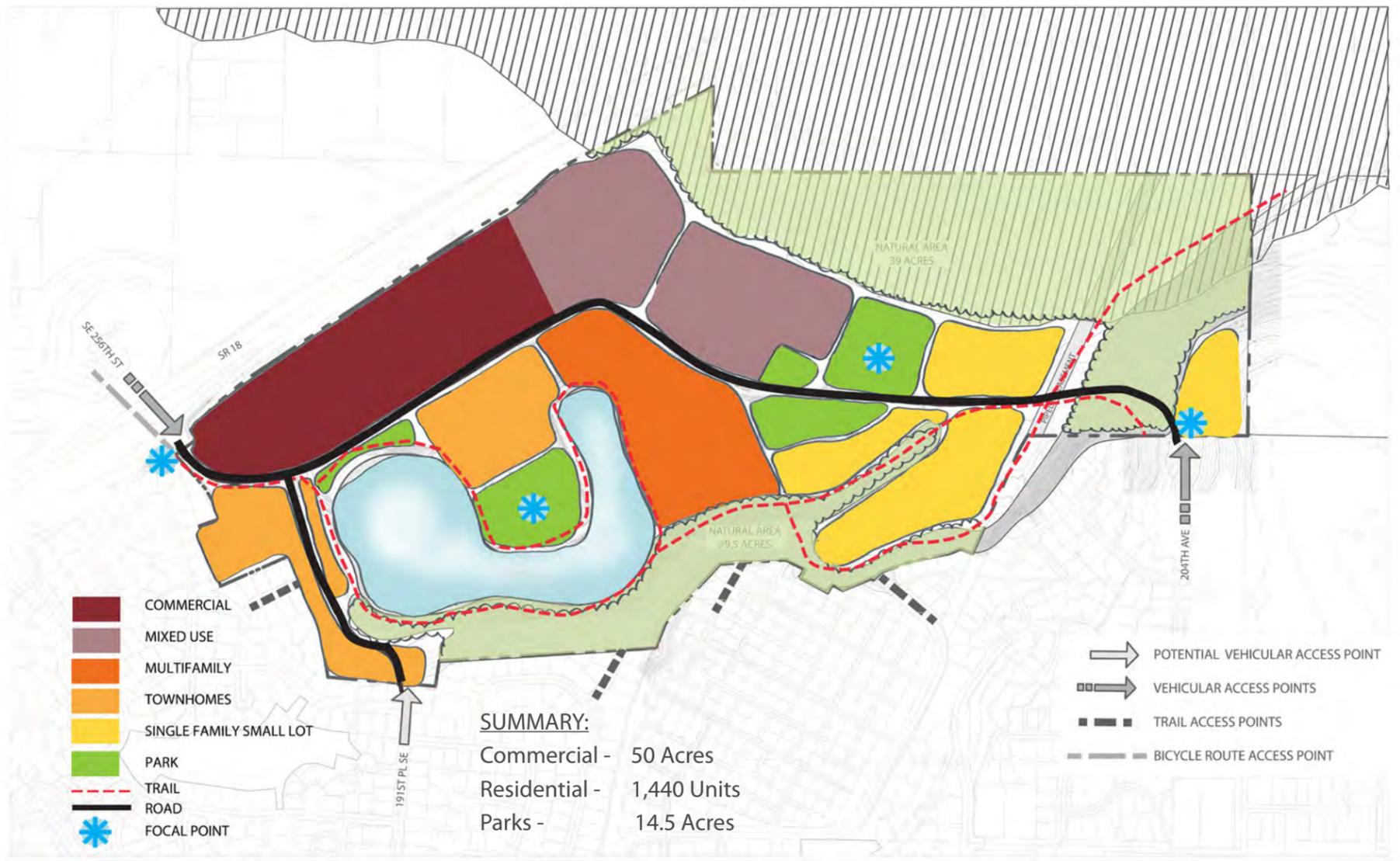
TEAM 4 MAXIMUM INTENSITY PLAN SOLUTION



COMMENTS

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TEAM 5 MAXIMUM INTENSITY PLAN SOLUTION



COMMENTS

- Want to see mixed-use buildings with housing above retail and parking underneath
- Would like to see a trail around the lake and trail connections to Timberlane as well as to regional trails

SINGLE-FAMILY

Small Lot



1 8.5 du/ac



2 12 du/ac



3 8 du/ac



4 10 du/ac



5 12 du/ac



6 14 du/ac

Standard Lot



7 6 du/ac



8 6 du/ac



9 8 du/ac



10 7 du/ac

* **#** = number of votes received **#** = image number

TOWNHOMES



2

1



4

2



6

3



4

7



3

5



1

6



2

8



6

9

* # = number of votes received # = image number

MULTI-FAMILY

4-Story with Parking Beneath



1

2



3

4

3-Story with Surface Parking



5

7



6

7

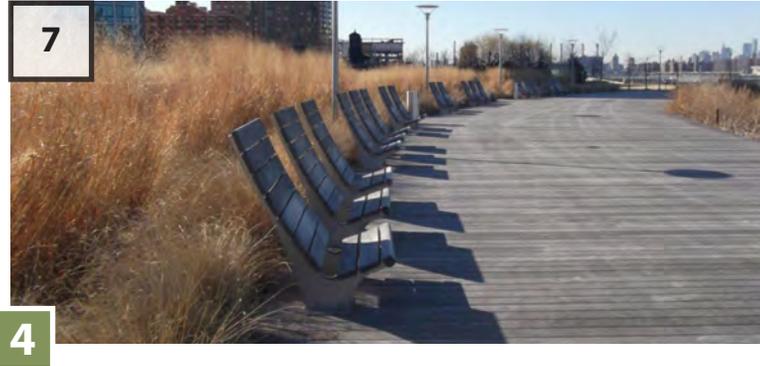
* # = number of votes received # = image number

COMMERCIAL / RETAIL



* # = number of votes received # = image number

AMENITIES / RECREATION



* # = number of votes received # = image number

Attachment 2

From: [Gina Hendrickson](#)
To: [Ann Mueller](#)
Cc: [sherrie Rosling](#); [erin harrington](#); [David and Kristy Kilburg](#); [angie johnnie](#)
Subject: hawk properties
Date: Friday, March 29, 2013 5:00:37 PM

I am a homeowner that would be affected by any development that would take place in Hawk properties, looking at the website I would like to give my input that we as a neighborhood (located at 189th ave se and 258th st) we would not be happy to have any businesses as well as any multi-family developments be built, our streets are already too narrow and many children including one with special needs would be impacted by this. Thank you for your consideration into this matter.

Gina Hendrickson

From: [David and Kristy Kilburg](#)
To: [Ann Mueller](#)
Subject: Hawk Property
Date: Friday, March 29, 2013 5:00:54 PM

Ann,

I am a homeowner that would be affected by any development that would take place in the Hawk Property subarea. From what I have gathered from your website, I would like to put in my input that I would not be happy to see any businesses or multi-family dwelling be built on the property as I can imagine more traffic would go down my street (258th Street/189th Street) as well as increased crime. Our street would not be practical for increased traffic as our streets are already narrow and is a family street with many children out at play.

I would appreciate you considering our requests.

Thank you!
David and Kristy Kilburg

From: [Anderson Baugh](#)
To: [Richard Hart](#); [Ann Mueller](#)
Subject: EIS Scoping Comments - 3-29-13
Date: Friday, March 29, 2013 4:50:44 PM
Attachments: [EIS Scoping letter to City of Covington 3-29-13.docx](#)

Richard and Ann: Please find attached our EIS scoping letter requests for the Stalzer Phase II, Sub-Area Study.

--

Anderson Baugh & Associates, LLC
All Mail: PO Box 58792
Tukwila, Washington 98138
Street Address:
18215 72nd Ave SE, Suite 127
Kent, Washington 98199
425-656-7491

ANDERSON, BAUGH & ASSOCIATES, LLC.

**PROFESSIONAL SERVICE CONSULTANTS
P.O. BOX 58792, TUKWILA, WA. 98138-1792
18215 72ND AVE SE, No. 127, KENT, WASHINGTON 98032
EMAIL: ANDERSON.BAUGH@GMAIL.COM
425-656-7491**

March 29, 2013

Richard Hart, Com Dev. Dir.
Ann Mueller, Sr Proj Plnr.
City of Covington
16720 SE 271st St
Covington, WA. 98042

RE: EIS Scoping for Stalzer Phase II
Northern Gateway Sub-Area Plan
Site Conditions

As the City is aware, we are Land Use Consultants to the contiguous Northern component, North of Hwy 18, and as such, are submitting our request to receive all materials generated from the City's and Proponent's Land Contractees' either by email or mail when the drawings are too large.

We would also like to be kept abreast of all meetings, public interactions, and any other City outreach so that we may attend and participate.

Thank you for your consideration.

Yours very truly,

Anderson, Baugh & Associates

From: brandon@branbar.com
To: [Ann Mueller](#); [Richard Hart](#)
Subject: Public scoping comment submission for the Northern Gateway Area Study
Date: Friday, March 29, 2013 4:58:21 PM

BranBar, LLC / H&C Enterprises Inc.

A WASHINGTON STATE CO-TENANCY

P.O. Box 7157 Covington, WA 98042
Ph. (425) 656-7477 fax. (253) 638-0026
branbar.llc@gmail.com

March 29, 2013

City of Covington

Attn: Anne Mueller (amueller@covingtonwa.gov)

16720 SE 271st Street, Suite 100

Covington, WA 98042-4964

Re: Public scoping comment submission for the Northern Gateway Area Study

BranBar, LLC, and H&C Enterprises, Inc., as an adjacent property owner/neighbor of the Lakeside Gravel pit for 8+ years, as well as 40+ year residents of Kent/Covington, wish to express our input of the Northern Gateway Area Study.

The 31 million dollar tax-payer investment, that is the 256th Ave SE / Highway 18 interchange, has sat fallow for 11 years, and we feel any development associated with bringing an economic impulse from that interchange is an extremely important step for the region and it's residents. However, it is our concern that the planned development sits in an existing permitted gravel mine, with an extremely large surface body of water, and an even bigger unknown subsurface water table that, in our opinion needs severe scrutiny before any development is to begin. We feel that more extensive environmental study and comment periods would allow for a better public understanding of the planned development.

Thank you,

Brandon Anderson

--

BranBar, LLC

Professional Service Consultants

PO BOX 7157 - Covington, WA 98042

Mobile (206)371-5215 Office (425)656-7491
brandon@branbar.com

Ann Mueller

From: Anderson, Charlene [CAnderson@kentwa.gov]
Sent: Monday, March 18, 2013 4:27 PM
To: Ann Mueller; Richard Hart
Cc: Peterson, Kelly; Gibson, Hope; Satterstrom, Fred
Subject: Hawk Property Subarea Plan

Thank you for the opportunity to comment on the EIS scoping of the Hawk Property Subarea Plan. The City of Kent has the following comments:

1. The proposed Hawk Property Sub Area Plan is immediately north and adjacent to the City of Kent's Armstrong Springs Wellhead Protection Area. Kent respectfully requests Covington include groundwater, including quantity and quality, to the list of elements of the environment to be studied in the Planned Action EIS. Kent's Wellhead Protection Program, identifies residential and urban development as a potential source of contamination to Armstrong Springs, an important source of municipal water. Please address the impacts of groundwater recharge with the increased impervious surfaces that may result from this proposal.
2. It would be good to consider bike and pedestrian connections to the regional trail system as part of the transportation analysis.

Ann Mueller

From: Richard Hart
Sent: Tuesday, March 19, 2013 4:14 PM
To: Bill Stalzer; Ann Mueller; Salina Lyons; 'Lisa Grueter'
Subject: FW: Hawk Property Subarea Plan
Attachments: Phase 1 WHPA.pdf

Here is the response I received from the City of Kent after I responded to their initial comments. You can see all of the communication below.

Richard

From: Anderson, Charlene [mailto:CAnderson@kentwa.gov]
Sent: Tuesday, March 19, 2013 3:11 PM
To: Richard Hart
Subject: FW: Hawk Property Subarea Plan

Hi, Richard...see follow-up e-mail below from Kelly Peterson in our Environmental Engineering section of Public Works.

From: Peterson, Kelly
Sent: Tuesday, March 19, 2013 2:38 PM
To: Anderson, Charlene; Gibson, Hope
Subject: RE: Hawk Property Subarea Plan

Charlene-

While the Armstrong Springs property is located to the south of Kent Kangley Road, the Wellhead Protection Area extends beyond the property lines. The attached Aquifer Susceptibility map is a compilation of the Armstrong, Kent and Clarks Springs municipal water supply sources which are include in the Phase I Wellhead Protection Program. This map shows high susceptibility for soil permeability, geologic materials, depth to water and topography. As previously noted, the proposed land use are potential sources of contamination.

The Phase II sources are located within the Kent municipal boundaries.

If Richard has questions, let him know he can contact me while you are on vacation.

Thanks,

Kelly



Kelly Peterson, AICP *Environmental Conservation Supervisor*
Environmental Engineering | Public Works Department
220 Fourth Avenue South, Kent, WA 98032
Phone **253-856-5547** | Cell **253-740-8066**
KPeterson@KentWA.gov

www.KentWA.gov   

PLEASE CONSIDER THE ENVIRONMENT BEFORE PRINTING THIS E-MAIL

From: Anderson, Charlene
Sent: Monday, March 18, 2013 5:29 PM
To: Peterson, Kelly; Gibson, Hope
Subject: FW: Hawk Property Subarea Plan

From: Richard Hart [<mailto:rhart@covingtonwa.gov>]
Sent: Monday, March 18, 2013 5:18 PM
To: Anderson, Charlene
Subject: RE: Hawk Property Subarea Plan

Charlene:

Thanks for the two comments. We certainly will include them in our analysis during this scoping exercise. I do have one question and comment.

On your first point, I think your Armstrong Springs Wellhead Protection Area is located south of Kent-Kangley Road (SE 272nd) and east of Wax Road. This is at the southern boundary of Covington, if it's the one I'm aware of. I checked your Kent Water System Plan and maps and found the aforementioned Armstrong Springs Wellhead Protection Area. Our Hawk Property Subarea Plan and Planned Action EIS is not immediately north and adjacent to your wellhead site. The 210 acres for which we are doing our subarea plan and Planned Action EIS is located about two miles to the north at the northern end of Covington and has fully developed subdivisions on its southern and southeastern boundary within the city limits of Covington. To the east is a King County Park, to the north and northwest is unincorporated King County. Possibly you thought our Hawk Property Subarea was further to the south near the SE 272nd intersection and interchange of Highway 18. However, we will make sure that groundwater recharge impacts are addressed in relation to increased impervious surfaces, and that we are cognizant of all wellhead protection areas anywhere near our study area during our environmental analysis. If I have misspoken, please let me know.

On your second point, the comment about bike and pedestrian connections within our study area being linked to the regional trail system as part of the transportation analysis is certainly a valid point and one we do want to consider and maximize.

The email I sent to you this morning had an attachment of 5 pages which has a good air photo of our study area, the concept plan for the area, a proposed trail system plan and connections to the regional trail plan and typical intensities of land development which the developer has provided to us as part of his initial analysis of the site. Check out these attachments if you didn't get to do so this morning and maybe they will help to clarify some of my comments.

Thanks again for your comments and interest.

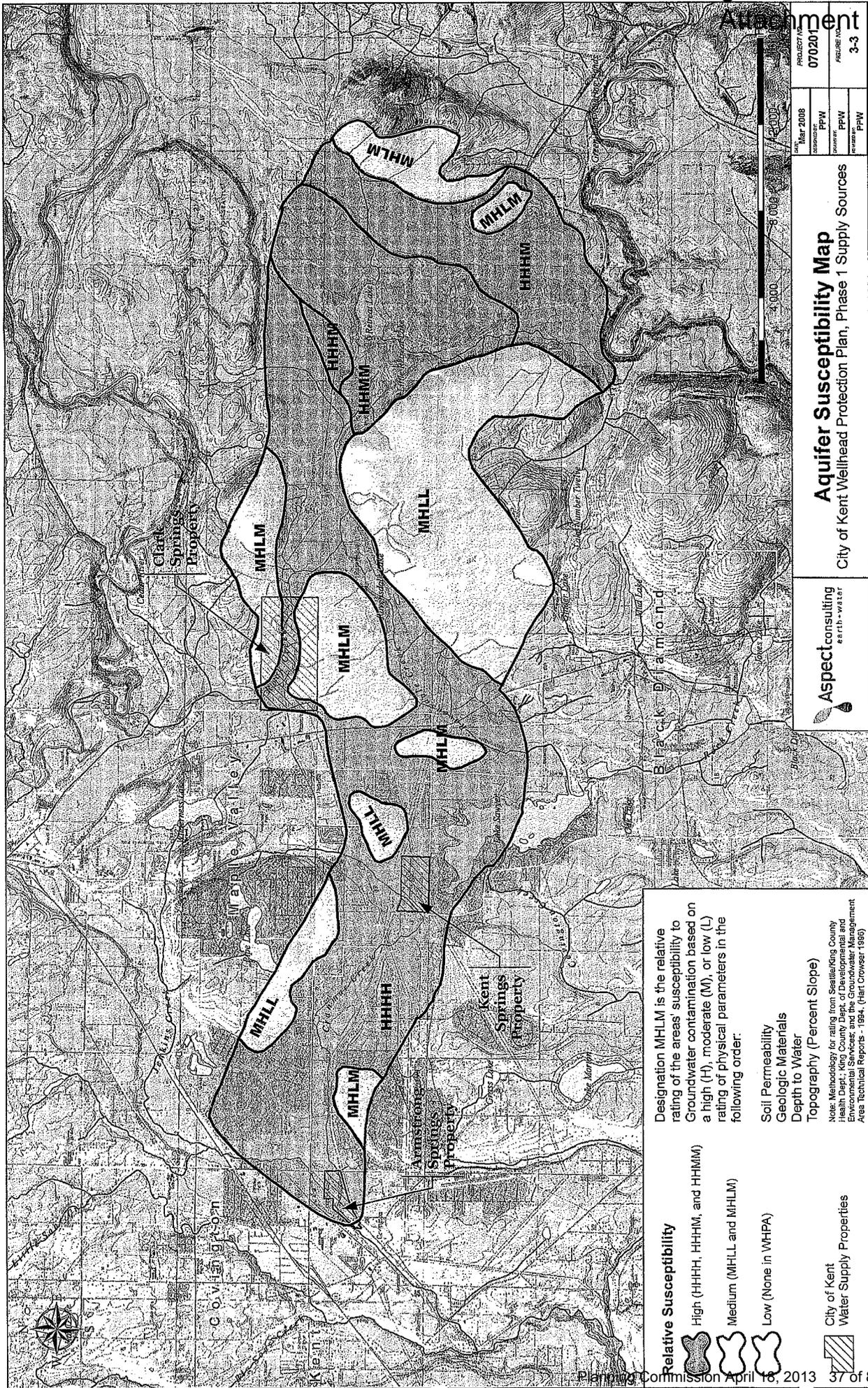
Richard

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Thank you for the opportunity to comment on the EIS scoping of the Hawk Property Subarea Plan. The City of Kent has the following comments:

Agenda Item 1 Attachment 2

1. The proposed Hawk Property Sub Area Plan is immediately north and adjacent to the City of Kent's Armstrong Springs Wellhead Protection Area. Kent respectfully requests Covington include groundwater, including quantity and quality, to the list of elements of the environment to be studied in the Planned Action EIS. Kent's Wellhead Protection Program, identifies residential and urban development as a potential source of contamination to Armstrong Springs, an important source of municipal water. Please address the impacts of groundwater recharge with the increased impervious surfaces that may result from this proposal.
2. It would be good to consider bike and pedestrian connections to the regional trail system as part of the transportation analysis.



PROJECT NO.	070201
DATE	Mar 2008
DATE	PPW
DATE	PPW
DATE	PPW

Aquifer Susceptibility Map

City of Kent Wellhead Protection Plan, Phase 1 Supply Sources



Designation MHLM is the relative rating of the areas' susceptibility to groundwater contamination based on a high (H), moderate (M), or low (L) rating of physical parameters in the following order:

- Soil Permeability
- Geologic Materials
- Depth to Water
- Topography (Percent Slope)

Note: Methodology for rating from Seattle/King County Health Dept., King County Dept. of Developmental and Environmental Services, and the Groundwater Management Area Technical Reports - 1984. (Patt Crowder 1986)

Relative Susceptibility

- High (HHHH, HHHM, and HHMM)
- Medium (MHLL and MHLM)
- Low (None in WHPA)

City of Kent
Water Supply Properties

From: Karen Walter [<mailto:KWalter@muckleshoot.nsn.us>]
Sent: Friday, March 29, 2013 10:44 AM
To: Ann Mueller
Subject: FW: Hawk Property Subarea Plan, Determination of Significance and Scoping Notice

Ann,

My apologies; the previous email we sent did not include the technical reports we referenced in the email below.

Karen Walter
Watersheds and Land Use Team Leader

*Muckleshoot Indian Tribe Fisheries Division
Habitat Program
39015 172nd Ave SE
Auburn, WA 98092
253-876-3116*

From: Karen Walter
Sent: Friday, March 29, 2013 10:43 AM
To: Ann Mueller (amueller@covingtonwa.gov)
Subject: Hawk Property Subarea Plan, Determination of Significance and Scoping Notice

Ann,

We have reviewed the City's Determination of Significance and Scoping Notice for the Proposed Hawk Property Subarea Plan. We offer the following comments in response to this scoping notice.

The project area/subarea includes or is adjacent to Jenkins Creek, a salmon-bearing tributary in the Soos Creek basin. We recommend that the DEIS analyze and discuss the following:

1. Details about the plans to reclaim the Lakeside gravel mine (assuming the mine will be closed to accommodate future land use) and protection/restoration of the large wetland (1D) shown in the gravel mine on King County's IMAP;
2. Details regarding how Jenkins Creek and its large associated wetland will be protected and potentially restored (where needed) as part of the subarea plan;
3. Details regarding how stormwater will be managed in the subarea, including the opportunities to fully treat and infiltrate stormwater using enhanced treatment methods that reduce or significantly limit the amount of metals and PAHs in stormwater that may be discharged to Jenkins Creek. Also, the opportunities to implement low impact development techniques and retention of trees. As part of this analysis, the DEIS should consider the available data in the attached papers regarding stormwater impacts to salmon, particularly coho, a species found in Jenkins Creek.
4. The DEIS authors should contact WDFW and the Muckleshoot Indian Tribe Fisheries Division to obtain the most current information regarding salmon populations in Jenkins Creek and the Soos Creek basin prior to completing any analysis of potential impacts.

We appreciate the opportunity to provide comments to the Scoping Notice and look forward to reviewing the DEIS that addresses our recommendations above. Please let me know if you have any questions.

Karen Walter
Watersheds and Land Use Team Leader

Muckleshoot Indian Tribe Fisheries Division
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Low-level copper exposures increase visibility and vulnerability of juvenile coho salmon to cutthroat trout predators

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²*NOAA Fisheries, Northwest Fisheries Science Center, 2725 Montlake Boulevard East, Seattle, Washington 98112 USA*

Abstract. Copper contamination in surface waters is common in watersheds with mining activities or agricultural, industrial, commercial, and residential human land uses. This widespread pollutant is neurotoxic to the chemosensory systems of fish and other aquatic species. Among Pacific salmonids (*Oncorhynchus* spp.), copper-induced olfactory impairment has previously been shown to disrupt behaviors reliant on a functioning sense of smell. For juvenile coho salmon (*O. kisutch*), this includes predator avoidance behaviors triggered by a chemical alarm cue (conspecific skin extract). However, the survival consequences of this sublethal neurobehavioral toxicity have not been explored. In the present study juvenile coho were exposed to low levels of dissolved copper (5–20 µg/L for 3 h) and then presented with cues signaling the proximity of a predator. Unexposed coho showed a sharp reduction in swimming activity in response to both conspecific skin extract and the upstream presence of a cutthroat trout predator (*O. clarki clarki*) previously fed juvenile coho. This alarm response was absent in prey fish that were exposed to copper. Moreover, cutthroat trout were more effective predators on copper-exposed coho during predation trials, as measured by attack latency, survival time, and capture success rate. The shift in predator–prey dynamics was similar when predators and prey were co-exposed to copper. Overall, we show that copper-exposed coho are unresponsive to their chemosensory environment, unprepared to evade nearby predators, and significantly less likely to survive an attack sequence. Our findings contribute to a growing understanding of how common environmental contaminants alter the chemical ecology of aquatic communities.

Key words: alarm behavior; coho salmon; copper; cutthroat trout; olfaction; predation; skin extract; sublethal; survival.

INTRODUCTION

Various forms of water pollution are known to interfere with chemical communication in aquatic habitats (Sutterlin 1974). There are senders and receivers of chemical signals both within and among species in aquatic communities, and certain contaminants are directly toxic to the olfactory, mechanosensory, or gustatory sensory neurons of receivers. This form of sublethal ecotoxicity has been termed info-disruption (Lurling and Scheffer 2007) because it diminishes or distorts the sensory inputs that convey important information about an animal's surrounding environment. Contaminant-exposed receivers thereby respond inappropriately (or not at all) to cues that signal the proximity and status of predators, mates, food, and other factors that can influence growth, survival, distribution, or reproduction.

One of the most extensively studied examples of info-disruption is the neurotoxicity of dissolved copper to the peripheral olfactory system of fish (Tierney et al. 2010). Olfactory receptor neurons are located in the epithelium of the olfactory rosette, within the nasal cavity. Cilia containing odor receptors extend from the apical surfaces of olfactory neurons into the nasal cavity, separated from ambient waters by a thin layer of mucous. Olfactory receptor neurons are continuously exposed to ambient waters and are therefore highly vulnerable to dissolved toxicants in aquatic habitats.

Copper is a widely occurring pollutant in association with diverse human activities, including agricultural, industrial, commercial, and residential land uses. For example, copper is used in various agriculture and homeowner pesticide formulations, in building materials, as an antifoulant in hull paints for vessels, and in motor vehicle friction materials (i.e., brake pads). As a consequence, copper is commonly transported to aquatic systems in land-based stormwater runoff (Davis et al. 2001). Copper contamination is also associated with hard rock mining and municipal wastewater discharges.

Similar to fish mechanosensory receptor neurons (i.e., lateral line; Linbo et al. 2006), olfactory receptor

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neurons undergo cell death in response to dissolved copper concentrations above approximately 20 µg/L (Julliard et al. 1996, Hansen et al. 1999). At lower concentrations in the 2–20 µg/L range, dissolved copper reversibly inhibits the physiological responsiveness of olfactory receptor neurons in a concentration-dependent manner (Baldwin et al. 2003, Sandahl et al. 2004). The loss of sensory function occurs rapidly, within the first few minutes of copper exposure (Baldwin et al. 2003). In most fish species that have been studied to date, peripheral sensory neurons do not acclimate to copper during exposures lasting days (Julliard et al. 1996, Linbo et al. 2006) or weeks (Saucier et al. 1991, Saucier and Astic 1995).

Chemical signals of predation risk are an ecologically important category of olfactory information for fish (Wisenden 2000, Ferrari et al. 2010). For many species (Chivers and Smith 1998), including juvenile salmonids, an olfactory alarm cue released via mechanical tearing of the skin (e.g., during a predation event) triggers predator avoidance behaviors by nearby conspecifics. Juvenile salmon and trout, for example, become motionless in response to the alarm cue (Brown and Smith 1997, Berejikian et al. 1999, Scholz et al. 2000). This reduces their visibility and corresponding vulnerability to attack by motion-sensitive predators such as piscivorous fishes and birds (Webb 1986, Martel and Dill 1995). Numerous studies have demonstrated a survival benefit for alarm-cue-responsive prey (Mirza and Chivers 2001, 2003, Chivers et al. 2002).

Previous studies have shown that peripheral olfactory toxicity and diminished sensory responsiveness correspond to a disruption in alarm behaviors in copper-exposed fish (Beyers and Farmer 2001, Sandahl et al. 2007). For individual juvenile coho salmon (*Oncorhynchus kisutch*), loss of alarm behavior triggered by an ecologically relevant olfactory alarm cue is directly correlated with loss of olfactory function at copper exposures ranging from 2 to 20 µg/L (Sandahl et al. 2007).

Copper's effect on chemical communication in aquatic systems has broad implications for the chemical ecology and conservation of aquatic species and communities. In the case of salmon, subtle but important impacts on sensory physiology and behavior at the juvenile life stage could increase predation mortality and thus increase losses from wild salmon populations, many of which remain at historic lows in large river basins throughout the western United States (Good et al. 2005). Conversely, improving water quality conditions (i.e., by reducing copper loading) could potentially improve juvenile survival and abundance, thereby enhancing ongoing efforts to recover depressed stocks. However, the cascading effects of copper across biological scales, from salmon physiology and behavior to predator-prey interactions and survival, have not been empirically determined.

Here we explored the influence of environmentally relevant copper exposures on juvenile coho salmon (see Plate 1) predator avoidance and survival during encounters with coastal cutthroat trout (*O. clarki clarki*). Cutthroat trout are visual foragers (Henderson and Northcote 1985, Mazur and Beauchamp 2003) that commonly prey on juvenile salmon in stream, lake, and nearshore marine habitats (Nowak et al. 2004, Duffy and Beauchamp 2008). We used a range of sublethal copper exposures (5–20 µg/L) and a duration (3 h) previously shown to impair both peripheral olfaction and alarm behavior in juvenile coho (Sandahl et al. 2007). In a subset of trials, predators were also exposed to dissolved copper (10 µg/L for 3 h).

METHODS AND MATERIALS

Animals

Juvenile coho.—

1. *Behavior experiments.*—In 2007, wild juvenile coho salmon were collected as needed by seining a side channel of Big Beef Creek at the University of Washington's Big Beef Creek Research Station (Seabeck, Washington, USA). Coho were maintained on well water (Table 1) in indoor raceways under natural light regime and fed pellets daily (1–2 mm extruded; Silver Cup Fish Feed, Murray, Utah, USA). Coho grew slightly throughout the experimental period, from April–May (39–49 mm total length [TL], \bar{x} = 42.8, SD = 3.3, n = 13) to June–July (36–60 mm TL, \bar{x} = 48.7, SD = 5.6, n = 79).

2. *Predation experiments.*—In 2008, juvenile coho were produced from eggs fertilized at the Big Beef Creek Research Station. Hatchlings were maintained outdoors in 1-m³ net pens suspended in a 5 m diameter circular tank continuously supplied with well water. One net pen of juveniles (approximately 1000 fish) provided the experimental prey. Coho were fed pellets daily. Coho grew slightly throughout the experimental period; random samples in April–May were 30–40 mm TL (\bar{x} = 36.2, SD = 2.5, n = 24) and in June–July were 35–46 mm TL (\bar{x} = 41.3, SD = 2.7, n = 64). During predation trials, there was a significantly higher attack rate on the larger coho in June–July compared to those used in April–May (t_{29} = -2.136, P = 0.041), likely related to the slightly larger size and therefore visibility of coho in the second set of predation trials. Other predator prey metrics were not affected (P = 0.084–0.625).

Cutthroat trout.—

1. *Behavior experiments (response to upstream predator).*—During April 2007, wild cutthroat trout (sizes 178–245 mm TL, \bar{x} = 205, SD = 18, n = 16) for use as predators were obtained from Big Beef Creek in smolt traps at a weir operated by Washington Department of Fish and Wildlife. Predators were maintained outdoors in flow-through circular holding tanks supplied with well water. On experimental days, predators were fed one juvenile coho each. Other days, predators were fed one fish each every other day. Predators were divided

TABLE 1. Conventional water chemistry characteristics, including total organic carbon (TOC), for source (well) water at the Big Beef Creek Research Station (Seabeck, Washington, USA).

Parameter	Units	D.L.	N	Mean	SE
pH			11	7.5	0.3
Alkalinity	mg/L CaCO ₃	1.0	11	46.7	0.7
Hardness	mg/L CaCO ₃	1.0	11	56.0	0.0
Bicarbonate	mg/L	1.0	11	46.7	0.7
Calcium	mg/L	0.05	11	18.00	0.00
Potassium	mg/L	0.10	11	0.50	0
Magnesium	mg/L	0.05	11	2.67	0.03
Sodium	mg/L	0.05	11	11.00	0.00
Chloride	mg/L	1.0	11	15.7	0.3
Sulfate	mg/L	1.0	11	2.0	0
TOC	mg/L	0.1	7	0.07†	0.01
0 Cu	µg/L	0.04	6	0.16	0.04
5 Cu	µg/L	0.04	2	4.54	0.07
10 Cu	µg/L	0.04	6	9.21	0.13
10 Cu‡	µg/L	0.04	8	8.94	0.54
10 Cu§	µg/L	0.04	4	8.06	0.34
20 Cu	µg/L	0.04	2	17.25	0.55

Notes: Also shown are measured copper concentrations for the different exposures; copper measurements are for exposure aquaria unless otherwise noted. D.L. stands for instrument detection limit.

† An eighth sample had anomalously high TOC (0.68 mg/L) and was excluded

‡ Experimental arenas for predator + prey trials.

§ Predator holding tanks for predator + prey trials.

randomly into four groups of four. On experimental days, predators within a group were randomly assigned to one of four arenas. Groups were rotated such that each predator was exposed to each treatment.

2. *Predation experiments.*—During April 2008, wild cutthroat trout for use as predators (sizes 150–215 mm TL, \bar{x} = 183, SD = 18, n = 32) were again obtained from Big Beef Creek and divided into three groups: groups 1 and 2 contained 8 predators each and were used in predation trials, while group 3, containing 16 predators, was held in reserve. Between the first set of predation trials (15–30 May) and the second set (25 June–3 July), predators in groups 1 and 2 were replaced with inexperienced fish from group 3. On experimental days, predators in Group 1 and Group 2 were fed one juvenile coho each during the predation trial. On other days, fish in all three groups were fed one fish each, every other day. For six days prior to collecting experimental data, predators were trained daily by simulating the experimental sequence. Trout were acclimated in the tank behind the divider for 1 h. The divider was then lifted, allowing the predators to locate, attack, and consume up to two prey fish.

Experimental arenas and alarm cue delivery

Behavior experiments with upstream predator.—Outdoor raceways (0.84 m width) were divided into segments (1.2 m long) with steel mesh barriers to create one experimental arena per raceway. A PVC sheet (1/16 inch [\sim 0.16 cm]; Calsak Plastics, Kent, Washington, USA) subdivided by gridlines (5 cm²) was placed at the bottom of each arena. Well water flowed into the raceway (2 L/s) from an underwater pipe upstream of the arena. A standpipe downstream of the arena maintained a water depth of 25 cm. Dividers partitioned

each arena into an upstream predator-containing compartment (46 × 84 cm) and an adjacent downstream compartment containing prey (76 × 84 cm). Dividers were frames (13 cm wide) constructed from PVC sheets (1/16 inch) and covered with window screen.

Well water or skin extract was delivered to the prey compartment through evenly spaced holes in a tube (Tygon tubing, 1/4 inch outer diameter [\sim 0.63 cm]) crossing the upstream divider, approximately 5 cm below the surface. Even dispersion was confirmed visually by dye tests. A three-way valve connected to a syringe allowed for injection of water or water plus alarm odor from outside the visual field of the fish.

Predation experiments.—Circular fiberglass tanks (bottom diameter = 130 cm, height = 90 cm) were used as experimental arenas. Gridlines were drawn at 5-cm intervals on the tank bottom to track fish location via video. An external standpipe maintained water depth (30 cm, 400 L). A sheet of PVC (90 × 60 cm) suspended vertically was used to divide cutthroat trout predators into a small sub-area (34 L) of the arena during acclimation. Juvenile coho prey were introduced into the arena and allowed to acclimate within a clear acrylic cylinder (25 cm inner diameter, 38 cm tall; U.S. Plastic Corp, Lima, Ohio, USA). The acclimation chamber was placed in one of the quadrants opposite the predator divider, within 15 cm from the tank edge. Predator dividers and acclimation chambers were attached by rope to overhead pulleys so they could be gently raised without the observer coming into view of the fish.

Skin extract was introduced to the prey acclimation chamber via Tygon® tubing just below the water surface connected to a three-way valve fitted with two syringes outside the tank. The skin extract solution was immediately flushed from the line with well water (60 mL).

Skin extract alarm cue

An alarm cue-containing skin extract from juvenile coho was prepared as previously described (Sandahl et al. 2007).

Behavior experiments with upstream predator.—In each flow-through arena, 1 mL of concentrated skin extract (160 cm² juvenile coho skin/L) was diluted in 50 mL of well water to a final concentration of 2 cm²/L. This solution was introduced over 60 s into an average flow of 2 L/s for an exposure of approximately 1 × 10⁻³ cm²·L⁻¹·s⁻¹. Pilot trials confirmed a behavioral reaction to the alarm cue at this diluted concentration (\bar{x} activity reduction = 51%, SD = 15%, n = 8).

Predation experiments.—Initial range-finding tests indicated that 2 × 10⁻⁵ cm² of homogenized skin extract per liter of water was the minimum concentration to evoke an alarm response (\bar{x} activity reduction = 77%, SD = 24%, n = 4). This agrees closely with previously published thresholds for conspecific skin extract evoking predator avoidance behavior in salmonids (1.85 × 10⁻⁵ cm²/L in *O. mykiss* [Mirza and Chivers 2003]; 2 × 10⁻⁵ cm²/L in *O. kisutch* [Sandahl et al. 2007]). In static arenas, diluted skin extract (1 cm²/L) was prepared daily from a frozen aliquot of concentrated skin extract (22 cm²/L). At the end of the 15-min prey acclimation, 257 μL of diluted skin extract in 50 mL of well water was injected into the prey acclimation chamber (12.9 L) for a final skin concentration of 2 × 10⁻⁵ cm²/L. Dye tests indicated that injected water did not diffuse from the acclimation chamber prior to the chamber being lifted from the experimental arena.

Copper exposures

Juvenile coho were exposed to dissolved copper prior to experimental trials. Exposures took place in 30-L glass aquaria wrapped in black plastic and supplied with an airstone. Aquaria were filled with 15 L of well water (controls) or well water containing varying copper concentrations (conventional water quality parameters shown in Table 1). Copper was added to the aquaria just prior to the onset of the 3-h exposures. Copper chloride stock solution (0.15 g Cu/L) was diluted to achieve nominal concentrations of 0, 5, 10, or 20 μg/L.

Experimental sequence

Behavior experiments with upstream predator.—Individual predators were placed in the predator compartment of each arena, upstream of the prey compartment, the evening before a trial and allowed to acclimate (>13 h). The following morning, juvenile coho (1 prey/predator) were exposed to either well water or well water containing 20 μg/L copper for 3 h. They were then transferred to the prey compartment of the experimental arena (one prey per arena) and allowed to acclimate for 30 min prior to the injection of stimulus solutions (water or water plus skin extract).

Predation experiments.—The timeline for predation trials is delineated in Table 2. For trials in which only

TABLE 2. Predation trial timeline.

Timeline	Duration	Event
-3 h 15min	3 h	prey exposure
-1 h 0 min	1 h	predator acclimation
-15 min	15 min	prey acclimation
0 min	10 s	skin extract injected
30 s	10 s	prey released
50 s	5 s	predators released

juvenile coho prey were exposed to copper, predators (two per arena) were acclimated behind the divider during the last hour of the 3-h prey exposure interval. Exposed prey were then transferred to the acrylic chamber (two fish per arena) for 15 min, an interval brief enough to minimize olfactory recovery in clean water and yet long enough to produce reliably robust control activity (swimming speed ~5 cm/s). Filming began at the time of prey transfer. Following prey acclimation, skin extract was administered and given 30 s to disperse (verified with dye tests) before the chamber was gently lifted and removed from the experimental arena. Thereafter, predators were released from their enclosure. Two consecutive sets of trials using a different group of predators were run each day, and the arenas were drained and filled between sets.

For trials in which both prey and predators were exposed to copper, both exposures were for 3 h, including acclimation time in the experimental arena. Predators were exposed to copper for 2 h in their holding tanks followed by a 1-h exposure in the experimental arena. Prey were exposed to copper in the exposure aquarium for 2.75 h. This was followed by 15 min in the acclimation chamber of the experimental arena.

Water chemistry analyses

Conventional water quality parameters and total organic carbon (TOC) were measured in water samples collected in 2008 between 20 May and 3 July. This interval spans most of the experimental period (16 May–3 July). Concurrently, dissolved copper (DCu) concentrations were measured in 28 samples that were representative of the different copper exposures. For conventional parameters, samples were stored at 4°C in polyethylene bottles until analysis by standard methods at an EPA-certified laboratory (AmTest Laboratories; Redmond, Washington, USA). Samples for TOC were stored in glass vials at -20°C until analysis by combustion catalytic oxidation/NIDR method with a Shimadzu TOC-VCSH (University of Washington, Oceanography Technical Services, Seattle, Washington, USA). Samples for dissolved copper were stored at 4°C for up to 72 h prior to analysis by inductively coupled plasma mass spectrometry (Frontier Global Sciences, Seattle, Washington, USA).

The well water at BBC used in all experiments had low ion and organic carbon content (Table 1), which is similar to Pacific Northwest streams west of the

Cascades (e.g., Fig. 2 in McIntyre et al. 2008). The background copper concentration was very low (mean of 0.16 µg/L) and samples from copper exposures were 81–91% of nominal concentrations.

Video data acquisition

The four experimental arenas were sheltered outdoors beneath a wooden scaffolding to which cameras and pulleys were attached. The stand was covered by blue tarps to prevent direct lighting. Prey acclimation and predation trials were filmed with digital video cameras (SONY Exwave HAD SSC-M383) fitted with auto-iris lenses (2M-2812A, F1.4 DC AutoIris, 1/3" varifocal 28–12 mm, angle of view 95.6–22.1 degrees; Sony, Tokyo, Japan) mounted over each arena. Video footage for the four concurrent trials were recorded on a digital video recorder (Pro 8-CH DVR; SecurityCameraWorld.com, Cooper City, Florida, USA) at 30 frames per second (FPS).

Data analysis

Coho activity.—

1. *Behavior experiments.*—Following the 30-min acclimation, the activity of juvenile coho was quantified for 5 min by measuring swimming speed, approximated by the sum of vertical and horizontal line crossings on the 5-cm² grid of the prey compartment.

2. *Predation experiments.*—We quantified prey activity after coho were released from the acclimation chamber, during the 10 s prior to releasing the predators. Average swimming speed across the 10-s period was determined by tracking each prey fish in two-dimensional space with image analysis software. Using Quicktime Pro (version 7.6; Apple, Cupertino, California, USA), video was exported as an image sequence at 1 frame per second. In Image J, the position (*x*, *y*) of each prey fish was tracked between images, converting changes in position into swimming speed (cm/s) by standardizing the pixels to the bottom tank dimensions (software available online).⁴ We assumed that movement between frames was linear.

For most prey pairs (69/76), the two fish were equally active, and we averaged the swimming speed of the two prey each second. In the remaining 10% of cases, one prey was significantly more active (Kolmogorov-Smirnov distribution test, *P* < 0.05), and the more active prey was attacked first in seven of the eight cases. For these pairings, we used only the activity record for the more active prey in calculating prey activity.

Predation trial metrics.—Predator–prey interactions were analyzed from video recordings of each predation trial. Only attacks and captures of the first prey of the prey pair were quantified. Metrics were time to first attack (δA), time to capture (δC), time between first attack and capture ($\delta C - \delta A$), number of attacks (*A*),

and attack frequency (attacks per second during attack period; $A/[\delta C - \delta A]$). For each copper concentration and predator exposure combination, 16 predation trials were conducted for a total of 112 data trials. Not all metrics could be quantified for all trials.

Statistical analyses

Coho prey activity.—For the experiments in 2007, a two-factor ANOVA was used to explore whether copper exposure (0 vs. 20 µg/L) affected the behavioral response (activity level) to predation risk (no risk, upstream predator, upstream predator plus skin extract). Simple main effects analysis used a Bonferroni adjustment for multiple comparisons. For 2008, single-factor ANOVA was used to test the effect of the various copper treatments on prey activity in the combined presence of predators and skin extract. Dunnett’s post-hoc was used to compare activity in the copper treatments to the control treatment. Statistical analyses were conducted in SPSS 16.0 for MacIntosh (IBM, Armonk, New York, USA).

Predator–prey interactions.—Data for predator–prey interactions were not normally distributed and were positively skewed, being bounded by zero. Log-transformation resulted in normally distributed δA , δC , and *A*, which were analyzed by ANOVA followed by Dunnett’s post-hoc for comparing copper treatments to controls. Log-transformation did not normalize $\delta C - \delta A$ and attack frequency. Differences in central tendency of $\delta C - \delta A$ and attack frequency were tested by Kruskal-Wallis nonparametric multiple comparison. For the separate set of predation trials in which predators were also exposed to copper, Tukey’s post-hoc test was used following the ANOVA to compare among the three treatments (controls, prey exposed to 10 µg/L copper, predator + prey exposed to 10 µg/L copper).

The relationship between capture success probability (capture on first attack) and copper treatment was tested by linear regression of the natural log of the odds ratio for capture success weighted by sample size. This method transforms curvilinear data in a probability distribution to a linear function of the independent variable. We transformed capture success probability at each copper concentration to the log_e odds ratio (OR) as follows:

$$\log_e(\text{OR}) = \ln\left(\frac{\text{CSR}}{1 - \text{CSR}}\right) \quad (1)$$

where CSR is the capture success ratio across trials within each copper concentration.

Survival curves.—Time to capture of the first prey fish for each trial was used to assess differences in the distribution of survival times (δC) among treatments. Within each treatment, survival time was ranked across trials and each trial was assigned a decreasing proportion of the total survival of the first prey as per Vilhunen (2006). For example, the first prey captured among control trials had a survival time of 6 seconds. Up to 6 s,

⁴ <http://rsbweb.nih.gov/ij/>

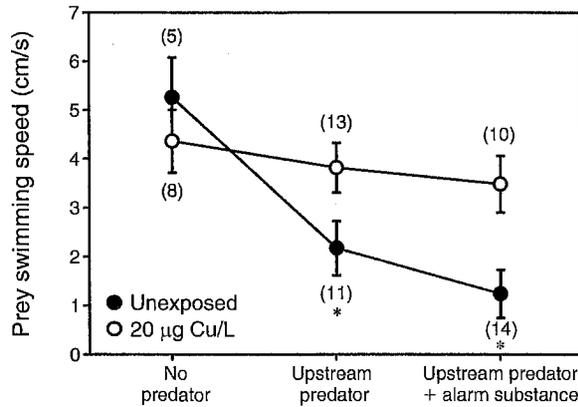


FIG. 1. Activity levels for control (unexposed) and copper-exposed (20 µg Cu/L for 3 h) juvenile coho downstream from one of three levels of predation risk; a compartment with a predator absent, a cutthroat trout predator present, and predator present plus the addition of juvenile coho skin extract. Swimming speed was recorded over 5 min at the end of the 30-min prey acclimation period. Significant differences ($P < 0.05$) from unexposed control are marked with an asterisk. Numbers by each symbol are the sample sizes. Error bars indicate \pm SE.

prey survival was 100%. At 6 s, survival across control trials dropped to 15/16, or 93.75%.

For each treatment, the proportion surviving was analyzed as a function of survival time by non-linear regression using the following sigmoid equation:

$$P(T) = \frac{1}{1 + e^{k(T - ST50)}} \quad (2)$$

where k was the slope of the linear portion of the curve, indicating how quickly survival declined with time, T was time in \log_{10} (number of seconds), and $ST50$ was the midpoint of the curve, the \log_{10} survival time for 50% of trials—analogous to the median survival time. For significantly different distributions, a t test assessed differences in the slope and midpoint among treatments. The benefit of using this method over simply comparing the central tendency of survival time among treatments was that we could compare not only the median survival time, but also the shape of the relationship between survival and time.

To calculate survival probabilities for copper treatments relative to the control treatment, we solved Eq. 2 for survival time, T , using the control slope (k) and midpoint ($ST50$) from Table 4:

$$T = k^{-1} \times \ln\left(\frac{1}{P} - 1\right) + ST50. \quad (3)$$

For given control survival probabilities (0.95 and 0.5), we used Eq. 3 to calculate the associated prey survival time. These times were then used in Eq. 2 with the respective slopes and midpoints for various copper exposures to estimate the related survival probability at that time for coho in each copper exposure.

RESULTS

Copper-exposed coho prey are behaviorally unresponsive to alarm cues.—We found a significant interaction between copper exposure and upstream predator cues with respect to their effect on coho activity ($F_{2,55} = 6.083$, $P = 0.054$; Fig. 1). In the absence of proximal predator cues, i.e., no upstream predator or conspecific skin extract, coho swam at an average speed of 5.2 cm/s (control condition; Fig. 1). A significant alarm response (tendency toward motionlessness) was elicited by the presence of a predator (2.1 cm/s; $F_{1,55} = 4.813$, $P = 0.032$) and a predator together with an upstream introduction of skin extract (1.2 cm/s; $F_{1,55} = 8.738$, $P = 0.005$). When the prey was exposed to copper, upstream predator cues had no effect on activity (combined 3.9 cm/s; $F_{2,55} = 0.518$, $P = 0.599$). Exposure to copper (20 µg/L) alone did not significantly affect baseline swimming activity (predator absent; 4.3 cm/s, $F_{1,55} = 0.734$, $P = 0.395$). Based on previous work (Baldwin et al. 2003), juvenile coho would be expected to recover ~20% of lost olfactory function during the 30 min acclimation interval in clean water used in these behavioral experiments. Nevertheless, copper-exposed fish were still unresponsive to chemical predator cues.

Similar to flow-through trials, control coho in static trials showed a strong alarm response to skin extract, as indicated by a reduction in swimming speed to 1.0 cm/s (Fig. 2). The magnitude of this alarm response decreased with increasing copper exposure. The average swimming speed of coho exposed to copper at 20 µg Cu/L was 4.9 cm/s and comparable to the baseline swimming speed of unexposed control fish in the flow-through trials (5.2 cm/s; Fig. 1). The loss of the alarm response was

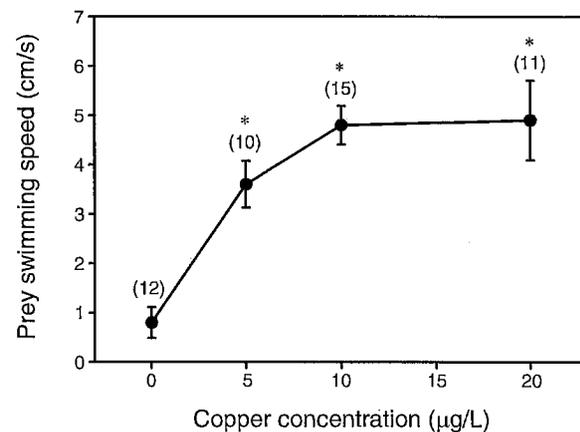


FIG. 2. Alarm behavior in juvenile coho prey at the outset of predation trials. Predators were located within the trial arena behind an opaque divider. Prey swimming speed was recorded at the end of the 15-min prey acclimation, after the presentation of conspecific skin extract. An asterisk indicates that juvenile coho unexposed to copper (0 µg/L) were significantly less active (i.e., were alarmed) relative to copper-exposed coho at all copper exposure concentrations ($P < 0.05$). Error bars indicate \pm SE.

TABLE 3. Median values (min, max) for time to first attack (δA), time to first capture (δC), time between δA and δC , number of attacks to δC (A), and frequency of attacks.

[Cu] [‡] ($\mu\text{g/L}$)	δA (s)	δC (s)	$\delta C - \delta A$ (s)	A	Attack frequency (s^{-1}) [§]
May					
0	29.4 (4.2, 218.4)	41.7 (6, 256.8)	3.3 (0, 106.2)	2 (1, 5)	0.75 (0.029, 16.67) [¶]
5	8.4 (0, 102)*	13.2 (3, 175.8) [†]	3 (0, 73.8)	3 (1, 7)	1.11 (0.054, 16.67)
10	6 (1.8, 97.2) [†]	9.3 (3, 422.4)*	2.7 (0, 422.47)	2 (1, 6)	1.25 (0.007, 16.67)
20	4.5 (0.6, 426.6)*	9.6 (1.2, 426.6)*	3 (0, 6)	3 (1, 6)	1.15 (0.667, 16.67)
June					
0	22.2 (4.2, 156)	23.4 (5.4, 159)	1.8 (0, 7.2)	3 (1, 6)	1.67 (0.555, 16.67)
10	3 (0, 114)*	6.9 (0.6, 124.8)*	3 (0, 12)	3 (1, 6)	1.5 (0.222, 16.67)
10#	5.4 (1.2, 27)*	9 (1.2, 34.8)*	2.1 (0, 28.8)	3 (1, 10)	1.57 (0.347, 16.67)

* $P < 0.05$; $\dagger P < 0.1$.

[‡] Copper exposures for 3 h prior to predation trial.

[§] $A/(\delta C - \delta A)$.

[¶] To calculate attack frequency for $\delta C - \delta A = 0$, number of attacks was divided by 0.06 s.

Predators also exposed to copper.

significant among copper-exposed coho relative to controls ($F_{3,44} = 14.27$, $P < 0.001$; Dunnett's post hoc test, $P \leq 0.001$).

Copper-exposed coho are more vulnerable to predation.—Prior copper exposure significantly affected time to first attack (ANOVA, $F_{3,58} = 3.550$, $P = 0.020$) and time to first capture ($F_{3,58} = 4.33$, $P = 0.008$) of juvenile coho by predators (Table 3). Time to attack (δA) and time to capture (δC) were reduced for all copper treatments compared to controls (Dunnett's post hoc test (0 vs. 5, 10, 20 $\mu\text{g/L}$): $P_{\delta A} = 0.031, 0.069, 0.014$; $P_{\delta C} = 0.062, 0.020, 0.004$). Other predator-prey interactions were unaffected by copper exposure (Table 3), including time between first attack and capture (Kruskall-Wallis $\chi^2_{3,63} = 2.43$, $P = 0.488$), number of attacks ($F_{3,58} = 0.624$, $P = 0.602$), and attack frequency ($\chi^2_{3,63} = 6.00$, $P = 0.111$).

Time to attack and time to capture were positively correlated because time to capture includes time to first attack ($\delta C = \delta A + [\delta C - \delta A]$). The correlation between time to attack and time to capture was very strong ($r_s = 0.959$, $n = 63$, $P < 0.001$). When log-transformed to allow calculation of a coefficient of determination, time to attack explained nearly all the variation in time to capture ($r^2 = 0.912$). Capture-attack interval ($\delta C - \delta A$) was not significantly different among treatments ($\chi^2_{3,63} = 2.43$, $P = 0.488$, median = 3 s), and was not correlated with δA ($r_s = 0.094$, $n = 63$, $P = 0.470$), suggesting that the primary component of the predation sequence affected by copper was prey detection leading to attack (δA).

Although the number of attacks to capture (A) was not different among treatments (Table 3), the capture success rate (probability of capturing prey on the first attack) increased with copper concentration (Fig. 3). Capture success rate was significantly correlated with increasing copper exposure concentration ($F_{1,3} = 60.060$, $P = 0.016$, $r^2 = 0.968$) following the equation $\log_e(\text{OR}) = 0.062[\text{Cu}] - 2.039$, where $[\text{Cu}]$ is dissolved copper concentration in $\mu\text{g/L}$. Standard error for the slope was 0.008 and was 0.092 for the intercept.

Exposing predators to copper does not improve the evasion success of prey.—In a separate set of predation trials, we determined the effect of co-exposing predators and prey to copper at 10 $\mu\text{g/L}$ (Table 3). Similar to the first set of predation trials, copper exposure affected time to attack ($F_{2,42} = 8.639$, $P = 0.001$) and time to capture ($F_{2,42} = 6.368$, $P = 0.004$). However, these metrics were not significantly different from experiments in which prey alone were exposed (Tukey's post hoc, δA , $P = 0.340$; δC , $P = 0.715$). Number of attacks ($F_{2,42} = 1.429$, $P = 0.251$), time between first attack and capture ($\chi^2_{2,45} = 0.732$, $P = 0.693$), and attack frequency ($\chi^2_{2,45} = 0.318$, $P = 0.853$) were not affected by copper exposure (prey exposed and predators plus prey exposed were similar to controls). In addition, exposing predators to copper did not change the likelihood of capturing prey on the first attack (25% for exposed prey only vs. 31% for co-exposed predators and prey; $\chi^2_1 = 0.643$, $P = 0.423$).

Copper exposure reduces prey survival.—Survival curves for each treatment were constructed from the

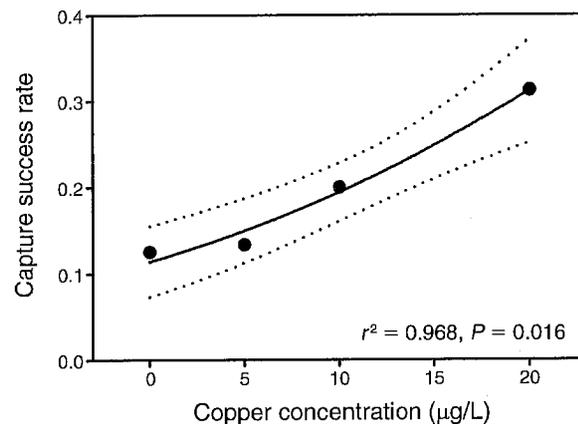


FIG. 3. Proportion of trials for which prey were captured on the first attack (capture success rate). Dashed lines are 95% confidence bands for the logistic regression. Capture success rate is described by the equation $e^F/(1 + e^F)$, where $F = 0.062[\text{Cu}] - 2.039$ (see Results for associated statistics).

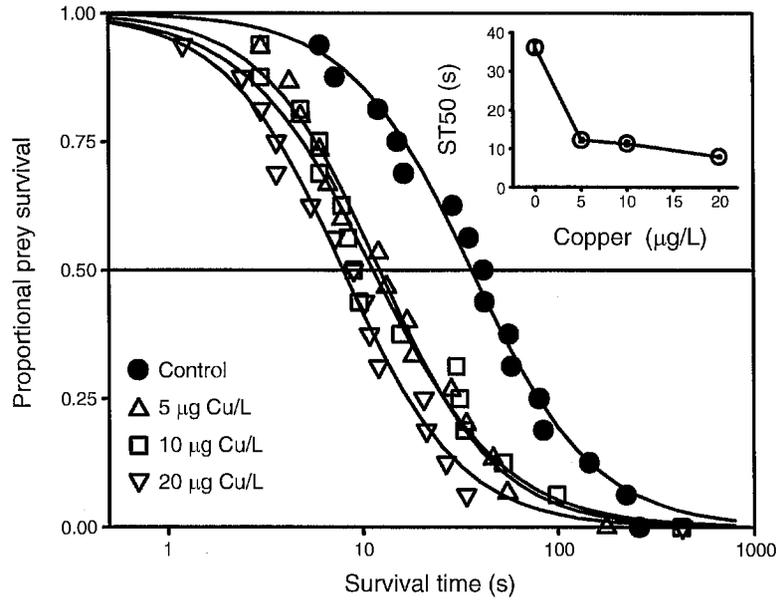


FIG. 4. Survival curves for control and copper-exposed coho in predation trials. Each point represents one predation trial, and survival times are based on the first prey fish consumed. The inset shows the midpoints of each curve, representing median survival time (ST50) for each treatment as a function of copper exposure.

time to first capture among trials (Figs. 4 and 5). Slopes, midpoints, and coefficients of determination for these curves are presented in Table 4.

Survival curves for copper treatments (Fig. 4) were significantly different from the control curve (F test, all $P < 0.001$). This was due to differences in midpoint (t

test, all $P < 0.001$), as slope between survival and time for each copper treatment was similar to the slope of the control curve (t test, all $P > 0.480$). Among copper treatments, 5 $\mu\text{g/L}$ and 10 $\mu\text{g/L}$ produced similar survival curves ($F_{2,27} = 2.222$, $P = 0.128$), with similar slopes (t_{27} , $P = 0.314$) and midpoints (t_{27} , $P = 0.274$),

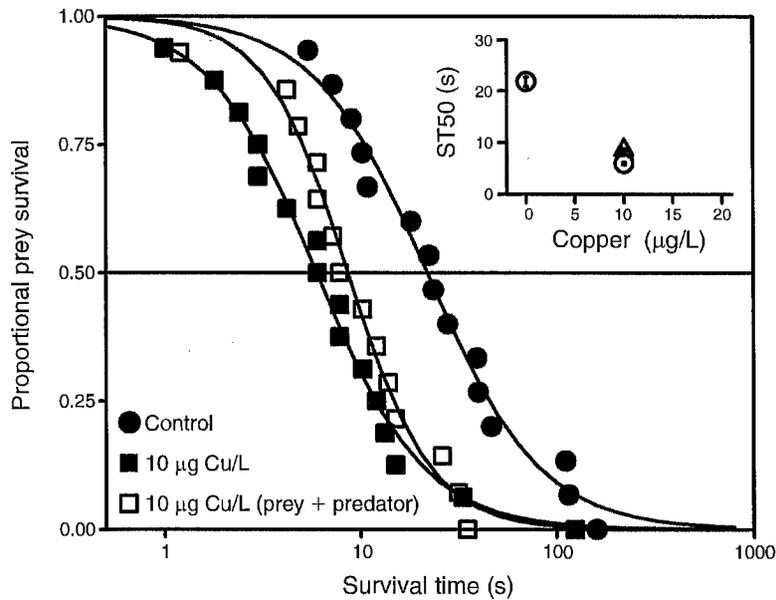


FIG. 5. Survival curves for predation trials in which prey alone or predators and prey were both exposed to copper (10 $\mu\text{g/L}$). Each point represents one trial, and survival times are based on the first prey fish consumed. Insets show the midpoints of each curve, representing median survival time (ST50) for each treatment as a function of copper exposure. The triangle symbol in the inset represents the ST50 for trials in which both predator and prey were exposed to copper.

TABLE 4. Sigmoid regression parameters for the survival curves.

[Cu] ($\mu\text{g/L}$)	r^2	ST50†	SE	K ‡	SE	N
May						
0	0.984	1.557	0.016	3.219	0.186	16
5	0.988	1.085	0.014	3.36	0.166	15
10	0.965	1.052	0.026	3.042	0.262	16
20	0.987	0.898	0.014	3.333	0.17	16
June						
0	0.983	1.338	0.016	3.493	0.213	15
10	0.985	0.774	0.014	3.659	0.203	16
10§	0.985	0.935	0.012	4.768	0.302	14

Note: All $P < 0.001$.

† Log of time to 50% survival across trials, midpoint of curve, measured in seconds.

‡ Slope of the sigmoid regression curve.

§ Predators and prey both exposed to copper.

whereas these curves had significantly different midpoints (Table 4) than the curve for 20 $\mu\text{g/L}$ (both $P < 0.004$).

For the predation trials in which both predators and prey were exposed (Fig. 4), survival curves for copper treatments (10 $\mu\text{g/L}$) were again different from the control curve (F test, both $P < 0.001$). Prey alone exposed to 10 $\mu\text{g/L}$ resulted in a survival curve that had a similar slope (t_{27} , $P = 0.577$), but different midpoint (t_{27} , $P < 0.001$) than the control curve. Exposing predators and coho to 10 $\mu\text{g/L}$ affected both the slope (t_{25} , $P = 0.002$) and the midpoint (t_{25} , $P < 0.001$) of the survival curve compared to the control curve. The predator + prey copper curve also had a different slope (t_{26} , $P = 0.005$) and midpoint (t_{26} , $P < 0.001$) compared to the prey-only copper exposures. Therefore, exposing predators to copper resulted in a subtle change in the shape of the survival curve, although it was not strong enough to alter predator-prey metrics (see *Exposing predators to copper does not improve the evasion success of prey*).

We calculated survival probabilities for copper exposures relative to controls using Eqs. 1 and 2. At 4.4 s, 95% of control coho were alive. Relative survival probabilities for copper-exposed coho were 82% for 5 $\mu\text{g/L}$, 78% for 10 $\mu\text{g/L}$, and 70% for 20 $\mu\text{g/L}$. The median survival time for controls was 36.1 s (50% survival; Table 4). Corresponding survival probabilities for copper exposures were 17%, 18%, and 10% for 5 $\mu\text{g/L}$, 10 $\mu\text{g/L}$, and 20 $\mu\text{g/L}$ treatments, respectively.

DISCUSSION

We have evaluated the effects of copper exposure on juvenile coho predator avoidance behaviors and the related consequences for coho survival during encounters with predatory wild cutthroat trout. We find that relatively brief (3 h) exposures to copper at 5–20 $\mu\text{g/L}$ eliminated the behavioral alarm response in coho prey, leading in turn to increased detection, reduced evasion, and reduced survival during predation trials.

The magnitude of the coho alarm response was greatest when the presence of an upstream predator was paired with skin extract, consistent with previous studies (e.g.,

Lautala and Hirvonen 2008). Our results showing a copper-induced loss of antipredator behavior reinforces and extends previous observations for juvenile coho. Sandahl et al. (2007) found that hatchery-raised coho become motionless (freeze) following presentation of a conspecific skin extract, and that this alarm response is reduced or abolished by copper exposure (3h; 2–20 $\mu\text{g/L}$). We have extended this behavioral toxicity to wild coho, and shown that copper also renders coho unresponsive to possibly distinct chemical cues emanating from a proximal upstream predator. This is consistent with copper's broad neurotoxicity across non-overlapping olfactory receptor neuron populations in the salmon olfactory epithelium (Baldwin et al. 2003).

Copper-exposed prey were easier for predators to identify, attack, and capture. This was due primarily to higher activity than alarmed controls, leading to a more rapid detection by cutthroat trout. For juvenile salmon, activity critically determines the likelihood of detection by visually guided predators such as larger salmonids, piscivorous birds, and river otters. For example, in predation trials with Mergansers, attacks on active juvenile coho were 15 times more frequent than attacks on inactive coho (Martel and Dill 1995). In the current study, copper also negatively influenced evasion of a predator once an attack was initiated, i.e., it became increasingly likely that prey would be captured on the first attack at higher copper exposure concentrations. Evasion success depends in part on whether the prey fish is aware of proximal danger (Lima and Dill 1990). In the current study the threat awareness of unexposed controls was heightened via the introduction of conspecific skin extract prior to the onset of the trial. By comparison, copper-exposed coho were unresponsive to the chemical alarm cue, thus unaware of the impending threat, and less prepared to evade once an attack sequence was initiated.

Copper toxicity to the coho lateral line mechanosensory system may have contributed to the observed reduction in evasion success. As with olfactory receptor neurons, copper is toxic to lateral line neurons that are directly exposed to contaminated waters (Linbo et al.



PLATE 1. Juvenile coho salmon are sensitive to olfactory alarm cues. Photo credit: Morgan Bond.

2006). The lateral line system in salmon and other fish responds to water displaced by an approaching predator and triggers a well-studied sequence of evasive behaviors (the C-type startle reflex: reviewed by Bleckmann 1993). Conversely, predators can capture prey without a functioning lateral line system. For predatory bass (*Micropterus salmoides*) and muskellunge (*Esox masquinongy*), prey capture success rate was unaffected by cobalt exposures at concentrations toxic to the lateral line (New 2002). Despite similar prey capture success, some aspects of the attack sequence were altered in cobalt-exposed predators relative to controls, including shorter distance to strike (both predators) and mean angular approach (muskellunge). We found a subtle shift in the midpoint and slope of the prey survival curve when predators were co-exposed to copper, possibly due to copper neurotoxic effects on the lateral line of cutthroat trout predators. Additional behavioral studies with a focus on lateral line function are warranted, particularly for predator-prey encounters under low visibility conditions.

Prey may make compensatory behavioral changes to improve their likelihood of surviving an attack (Lima

and Dill 1990, Lind and Cresswell 2005); however, we saw no evidence of this among copper-exposed coho. Also, co-exposing predators and prey to copper did not eliminate the reduced survival time of prey relative to exposing prey alone. This indicates that sublethal copper toxicity will have a disproportionate impact on prey in predator-prey dynamics, irrespective of whether the visually guided predators occupy the same contaminated surface waters (e.g., cutthroat trout and other piscivorous fish) or attack from the air above (e.g., Kingfishers and other birds).

The arena used for the predation trials lacked substrate, making it easier for cutthroat trout to detect and successfully capture alarmed coho relative to an encounter under natural conditions. Substrate complexity improves juvenile coho crypsis (Donnelly and Dill 1984) and provides refuge. Turbidity in streams can further constrain visual detection (Mazur and Beauchamp 2003). Thus, our observed differences in predation vulnerability between copper-exposed and unexposed prey would likely be magnified in natural stream habitats where survival rates for alarmed (predator aware) coho are higher.

Our findings likely extend to other fish species. For example, Baldwin et al. (2011) recently showed that the olfactory toxicity of copper is comparable in coho and steelhead, and also comparable among fish raised in hatchery and natural environments. Numerous other studies have demonstrated the olfactory-mediated neurobehavioral toxicity of copper for alarm behavior (reviewed by Tierney et al. 2010) in both controlled laboratory settings (e.g., Beyers and Farmer 2001, Jaensson and Olsen 2010) and in situ in copper-contaminated habitats (McPherson et al. 2004, Mirza et al. 2009). Copper impacts on chemosensory function also extends to other taxa; for example, disruption of the kairomone-mediated morphological predation defense of zooplankton (*Daphnia pulex*) and altered olfactory-based feeding behaviors of leeches (*Nepheleopsis obscura*; Pyle and Mirza 2007) have similar toxicity thresholds (~5 µg/L).

The toxic effects of copper have been remarkably consistent in coho salmon across biological scales, from the functional responsiveness of receptor neurons in the olfactory epithelium (Baldwin et al. 2003, Sandahl et al. 2004, 2007, McIntyre et al. 2008, Baldwin et al. 2011) to the olfactory-mediated behavior of individual animals (Sandahl et al. 2007; this study) to coho survival in predator-prey interactions (this study). Across these studies, the thresholds for neurobehavioral toxicity have been in the range of 2–5 µg/L (although this will shift upward in waters with relatively high dissolved organic carbon content: McIntyre et al. 2008). Notably, this is very close to the toxicity threshold reported for rainbow trout olfaction more than 35 years ago (7 µg/L: Hara et al. 1976). Olfactory disruption as measured at the olfactory epithelium is therefore a reliable proxy for behavioral impairment and reduced survival.

In conclusion, our findings are an example of how chemical habitat degradation in the form of water pollution can have nuanced but important impacts on the behavioral ecology of salmon. The effects of copper on coho survival are context-dependent and likely to go unnoticed in conventional field surveys of juvenile salmon abundance, habitat use patterns, and physical habitat quality. New biological indicators of copper toxicity, including diagnostic changes in gene expression within the salmon olfactory epithelium (e.g., Tilton et al. 2008), may eventually reveal the extent of sensory isolation in wild salmon under natural exposure regimes. In the interim, copper control strategies will likely improve juvenile salmon survival and minimize the disruption of a range of chemosensory-dependent behaviors. This includes, for example, legislation recently enacted in Washington State (SB6557) and California (SB346) to phase out the use of copper and other metals in motor vehicle brake pads.

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Copper: Adverse Effects on Salmonids

Scientific Abstracts and References

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The following information was collected from recent peer reviewed scientific publications. The full text of each article is available from the journal and publisher cited. Cu = copper.
* Indicates annotations by C. Woody for clarification or explanation. Questions or comments or criticisms greatly appreciated. For information on the importance of olfaction to fish see the Salmon Ecology 101 Fact Sheet.

Pyle, GG, and RS Mirza. 2007. Copper-impaired chemosensory function and behavior in aquatic animals. Human and Ecological Risk Assessment. 13 (3): 492 – 505.

Abstract: Chemosensation is one of the oldest and most important sensory modalities utilized by aquatic animals to provide information about the location of predators, location of prey, sexual status of potential mates, genetic relatedness of kin, and migratory routes, among many other essential processes. The impressive sophistication of chemical communication systems among aquatic animals probably evolved because of the selective pressures exerted by water as a "universal solvent." Impairment of chemosensation by toxicants at the molecular or cellular level can potentially lead to major perturbations at higher levels of biological organization. We have examined the consequences of metal-impaired chemosensory function in a range of aquatic animals that represents several levels of a typical aquatic ecosystem. In each case, low, environmentally relevant metal concentrations were sufficient to cause chemosensory dysfunction. Because the underlying molecular signal transduction machinery of chemosensory systems demonstrates a high degree of phylogenetic conservation, we speculate that metal-impaired chemosensation among phylogenetically disparate animal groups probably results from a common mechanism of impairment. We propose developing a chronic chemosensory-based biotic ligand model (BLM) that maintains the advantages of the current BLM approach, while simultaneously overcoming known difficulties of the current gill-based approach and increasing the ecological relevance of current BLM predictions.

'Safe' heavy metals hit fish senses. 18:16 09 April 2007.
NewScientist.com news service. Aria Pearson

Pollution far below the level seen as dangerous for aquatic life has nevertheless dramatically altered animal behaviour in North American lakes. Heavy metals are knocking out the sense of smell in organisms from bacteria to fish. Even we may not be immune.

Nathaniel Scholz, at the Northwest Fisheries Science Center in Seattle, Washington, and colleagues found that salmon lose their sense of smell if there are even low levels of copper in the water they are swimming

in. The fish could die as a result, because they cannot smell chemicals that would warn of a nearby predator.

All over the world, storm water run-off shuttles heavy metals such as copper and zinc from industry, mines and built-up areas into natural water courses. The concentrations are generally low - too low for polluters to bother about, or so many of them seem to have thought. "Now we're going after [this] 'So what?' question," says Scholz.

Scholz's team kept young coho salmon in tanks with different concentrations of copper for 3 hours, then measured their movements when a drop of salmon skin extract was added to the water. In the wild, the skin would be a cue that a predator may have injured a fish nearby.

Unexposed salmon stopped swimming, sank to the bottom of the tank and kept still - typical tactics for avoiding predators. But fish exposed to concentrations of copper as low as 2 parts per billion (ppb) just stopped for a few seconds, or merely slowed down, while fish exposed to 10 or more ppb didn't notice the cue at all (*Environmental Science and Technology*, DOI: 10.1021/es062287r).

The US Environmental Protection Agency has set the maximum safe level of copper for aquatic life at 13 parts per billion, well above that needed to wipe out the salmon's ability to sense chemical cues. Yet Greg Pyle, at Nipissing University in North Bay, Ontario, Canada, has found chemosensory problems at three levels of the food chain at or below 5 ppb, the limit set by Ontario's water quality standards. "The phenomenon is ubiquitous," he says.

Leeches lost their ability to smell food, zooplankton were unable to evade predators, and fathead minnows couldn't recognize their eggs: the fish ate them instead of protecting them. The contamination in these lakes is much too weak to kill these organisms outright, Pyle says, yet their populations are suffering.

Metals may have the same effect in humans. The makers of the cold remedy Zicam, which contains zinc, recently settled out of court for \$12 million with people who reported losing their sense of smell after spraying the product into their noses. The company maintains the remedy is safe. Studies have not been conducted to test whether zinc destroys human sensory abilities, but given what's happening in aquatic ecosystems, Pyle believes it could. "Don't squirt metals up your nose," he says. "That would be my advice."

Sandahl, JF, DH Baldwin, JJ Jenkins and NL Schlotz. 2007. A sensory system at the interface between urban stormwater runoff and salmon survival. *Environ. Sci. Technol.* 41:2998-3004.

Abstract: Motor vehicles are a major source of toxic contaminants such as copper, a metal that originates from vehicle exhaust and brake pad wear. Copper and other pollutants are deposited on roads and other impervious surfaces and then transported to aquatic habitats via stormwater runoff. In the western United States, exposure to non-point source pollutants such as copper is an emerging concern for many populations of threatened and endangered Pacific salmon (*Oncorhynchus spp.*) that spawn and rear in coastal watersheds and estuaries. To address this concern, we used conventional neurophysiological recordings to investigate the impact of ecologically relevant copper exposures (0-20 µg/L for 3 h) on the olfactory system of juvenile coho salmon (*O. kisutch*). These recordings were combined with computer-assisted video analyses of behavior to evaluate the sensitivity and responsiveness of copper-exposed coho to a chemical predation cue (conspecific alarm pheromone). The sensory physiology and predator avoidance behaviors of juvenile coho were both significantly impaired by copper at concentrations as low as 2 µg/L. Therefore, copper-containing stormwater runoff from urban landscapes has the potential to cause chemosensory deprivation and increased predation mortality in exposed salmon.

Baldwin, DH, JF Sandahl, JS Labenia, and NL Schlotz. 2003. Sublethal effects of copper on coho salmon: impacts on nonoverlapping

receptor pathways in the peripheral olfactory nervous system. Environmental Toxicology and Chemistry. 10:2266–2274.

Abstract: The sublethal effects of copper on the sensory physiology of juvenile coho salmon (*Oncorhynchus kisutch*) were evaluated. In vivo field potential recordings from the olfactory epithelium (electro-olfactograms) were used to measure the impacts of copper on the responses of olfactory receptor neurons to natural odorants (L-serine and taurocholic acid) and an odorant mixture (L-arginine, L-aspartic acid, L-leucine, and L-serine) over a range of stimulus concentrations. Increases in copper impaired the neurophysiological response to all odorants within 10 min of exposure. The inhibitory effects of copper (1.0–20.0 mg/L) were dose dependent and they were not influenced by water hardness. Toxicity thresholds for the different receptor pathways were determined by using the benchmark dose method and found to be similar (a 2.3–3.0 mg/L increase in total dissolved copper over background). Collectively, examination of these data indicates that copper is broadly toxic to the salmon olfactory nervous system. Consequently, short-term influxes of copper to surface waters may interfere with olfactory-mediated behaviors that are critical for the survival and migratory success of wild salmonids.

Hansen, JA, JD Rose, RA Jenkins, KG Gerow, HL Bergman. 1999. Chinook salmon (*Oncorhynchus tshawytscha*) and rainbow trout (*Oncorhynchus mykiss*) exposed to copper: neurophysiological and histological effects on the olfactory system. Environmental Toxicology and Chemistry. 9:1979-1991.

Abstract: Olfactory epithelial structure and olfactory bulb neurophysiological responses were measured in chinook salmon and rainbow trout in response to 25 to 300 µg copper (Cu)/L. Using confocal laser scanning microscopy, the number of olfactory receptors was significantly reduced in chinook salmon exposed to greater than or equal to 50 µg Cu/L and in rainbow trout exposed to greater than or equal to 200 µg Cu/L for 1 h. The number of receptors was significantly reduced in both species following exposure to 25 µg Cu/L for 4 h. Transmission electron microscopy of olfactory epithelial tissue indicated that the loss of receptors was from cellular necrosis. Olfactory bulb electroencephalogram (EEG) responses to 10(-3) M L-serine were initially reduced by all Cu concentrations but were virtually eliminated in chinook salmon exposed to greater than or equal to 50 µg Cu/L and in rainbow trout exposed to greater than or equal to 200 µg Cu/L within 1 h of exposure. Following Cu exposure, EEG response recovery rates were slower in fish exposed to higher Cu concentrations. The higher sensitivity of the chinook salmon Olfactory system to Cu-induced histological damage and neurophysiological impairment parallels the relative species sensitivity observed in behavioral avoidance experiments. This difference in species sensitivity may reduce the survival and reproductive potential of chinook salmon compared with that of rainbow trout in Cu-contaminated waters.

Dethloff, GM, D Schlenk, JT Hamm, and HC Bailey. 1999. Alterations in physiological parameters of rainbow trout (*Oncorhynchus mykiss*) with exposure to copper and copper/zinc mixtures. Ecotoxicology and Environmental Safety. 42(3):253-264.

Abstract: Rainbow trout (*Oncorhynchus mykiss*) were exposed to sublethal concentrations of copper (Cu, 14 µg/liter or parts per billion) and zinc (Zn, 57 and 81 µg/liter or ppb) for a 21-day period. The four treatments included a control, a Cu control, a Cu and low-Zn treatment and a Cu and high-Zn treatment. Selected parameters [e.g., hemoglobin (Hb), hematocrit (Hct), plasma glucose, lactate and cortisol, differential leukocyte count, respiratory burst, tissue metal concentrations, hepatic metallothionein (MT), brain acetylcholinesterase (AChE)] were evaluated at 2, 7, 14, and 21 days of exposure. Whole blood and

plasma parameters were not altered by exposure to metals. The percentage of lymphocytes was consistently decreased in the three metal treatments, while percentages of neutrophils and monocytes were increased. Respiratory burst activity was elevated in all metal treatments. Gill Zn concentration was highly variable, with no significant alterations occurring. Gill Cu concentration was elevated above control levels in all metal treatments, Gill Cu concentration in the two Cu/Zn treatments was also elevated above levels in the Cu control. Hepatic metal concentrations and MT levels were not altered from control values. Measurements of brain AChE indicated an elevation in this parameter across metal treatments. In general, alterations in physiological parameters appeared to be due to Cu, with Zn having no interactive effect.

Hansen JA, Lipton J, Welsh PG. 2002. Environmental toxicology and chemistry. 21 (3): 633-639.

Abstract: Bull trout (*Salvelinus confluentus*) were recently listed as threatened in the United States under the federal Endangered Species Act. Past and present habitat for this species includes waterways contaminated with heavy metals released from mining activities. Because the sensitivity of this species to copper was previously unknown, we conducted acute copper toxicity tests with bull (*bull trout are an endangered type of charr like Dolly Varden) and rainbow trout (*Oncorhynchus mykiss*) in side-by-side comparison tests. Bioassays were conducted using water at two temperatures (8 degrees C and 16 degrees C) and two hardness levels (100 and 220 mg/L as CaCO₃). At a water hardness of 100 mg/L both species were less sensitive to copper when tested at 16 degrees C compared to 8 degrees C. The two species had similar sensitivity to copper in 100-mg/L hardness water, but bull trout were 2.5 to 4 times less sensitive than rainbow trout in 220-mg/L hardness water. However, when our results were viewed in the context of the broader literature on rainbow trout sensitivity to copper, the sensitivities of the two species appeared similar. This suggests that adoption of toxicity thresholds that are protective of rainbow trout would be protective of bull trout; however, an additional safety factor may be warranted because of the additional level of protection necessary for this federally threatened species.

Brix KV, DeForest DK, Adams WJ. 2001. Assessing acute and chronic copper risks to freshwater aquatic life using species sensitivity distributions for different taxonomic groups. Environmental Toxicology and Chemistry. 20 (8): 1846-1856.

Abstract: Using copper as an example, we present a method for assessing chemical risks to an aquatic community using species sensitivity distributions (SSDs) for different taxonomic groups. This method fits probability models to chemical exposure and effects data to estimate the percentage of aquatic species potentially at risk and expands on existing probabilistic risk assessment methodologies. Due to a paucity of chronic toxicity data for many chemicals, this methodology typically uses an acute-chronic ratio (ACR) to estimate the chronic effects distribution from the acute effects distribution. We expanded on existing methods in two ways. First, copper SSDs were developed for different organism groups (e.g., insects, fish) that share similar sensitivities or ecological functions. Integration of exposure and effects distributions provides an estimate of which organism groups may be at risk. These results were then compared with a site-specific food web, allowing an estimation of whether key food web components are potentially at risk and whether the overall aquatic community may be at risk from the perspective of ecosystem function. Second, chronic SSDs were estimated using the relationship between copper ACRs and acute toxicity (i.e., the less acutely sensitive a species, the larger the ACR). This correction in the ACR removes concerns previously identified with use of the ACR and allows evaluation of a significantly expanded chronic data set with the same approach as that for assessing acute risks.

Goldstein, JN, DF Woodward, and AM Farag. 1999. Movement of adult Chinook salmon during spawning migration in a metals-contaminated system, Coeur d'Alene River, Idaho. *Transactions of the American Fisheries Society* 128:121–129.

Abstract: Spawning migration of adult male chinook salmon *Oncorhynchus tshawytscha* was monitored by radio telemetry to determine their response to the presence of metals contamination in the South Fork of the Coeur d'Alene River, Idaho. The North Fork of the Coeur d'Alene River is relatively free of metals contamination and was used as a control. In all, 45 chinook salmon were transported from their natal stream, Wolf Lodge Creek, tagged with radio transmitters, and released in the Coeur d'Alene River 2 km downstream of the confluence of the South Fork and the North Fork of the Coeur d'Alene River. Fixed telemetry receivers were used to monitor the upstream movement of the tagged chinook salmon through the confluence area for 3 weeks after release. During this period, general water quality and metals concentrations were monitored in the study area. Of the 23 chinook salmon observed to move upstream from the release site and through the confluence area, the majority (16 fish, 70%) moved up the North Fork, and moved up the North Fork, and only 7 fish (30%) moved up the South Fork, where greater metals concentrations were observed. Our results agree with laboratory findings and suggest that natural fish populations will avoid tributaries with high metals contamination.

Dethloff, GM, and HC Bailey. 1998. Effects of Copper on Immune System Parameters of Rainbow Trout (*Oncorhynchus mykiss*). *Environmental Toxicology and Chemistry*. 17(9):1807-1814.

Abstract: Agricultural, urban, industrial, and mining sources release metals into waterways. The effects of sublethal concentrations of metals on integrated physiological processes in fish, such as immunocompetency, are not well understood. The objective of this study was to determine the physiological effects of a range of sublethal copper concentrations (6.4, 16.0, and 26.9 $\mu\text{g Cu/L}$) on Shasta-strain rainbow trout (*Oncorhynchus mykiss*) exposed in soft water. Trout were sampled after 3, 7, 14, and 21 d of exposure to copper. The percentage of monocytes was consistently elevated at 26.9 $\mu\text{g Cu/L}$, and the percentage of lymphocytes was decreased. A consistent increase in the percentage of neutrophils occurred at 26.9 and 6.4 $\mu\text{g Cu/L}$. Respiratory burst activity was decreased for all concentrations at all sampling days, but a significant reduction occurred only at 14 and 21 d of exposure to copper. B-like cell proliferation was decreased (In short, all this means that the immune system of fish was affected by Cu exposure. Woody) by exposure to the higher copper concentrations. Proliferation results, however, had high variability. T-like cell proliferation and phagocytosis were not altered. Hepatic copper concentration was consistently elevated in trout exposed to 26.9 $\mu\text{g Cu/L}$; no correlation was found between hepatic copper concentration and the immune system responses investigated. Consistent alterations in immunological parameters suggest that these parameters could serve as indicators of chronic metal toxicity in natural systems.

Buhl, KJ and SJ Hamilton. 1991. Relative sensitivity of early life stages of arctic grayling, coho salmon, and rainbow trout to nine inorganics. *Ecotoxicology and Environmental Safety*. 2:184-197.

Abstract: The acute toxicity of nine inorganics associated with placer mining sediments to early life stages of Arctic grayling (*Thymallus arcticus*), coho salmon (*Oncorhynchus kisutch*), and rainbow trout (*O. mykiss*) was determined in soft water (hardness, 41 $\text{mg liter}^{-1} \text{CaCO}_3$) at 12°C. The relative toxicities of the inorganics varied by four orders of magnitude; from most toxic to least toxic, the rank order was cadmium,

silver, mercury, nickel, gold, arsenite, selenite, selenate, and hexavalent chromium. In general, juvenile life stages of the three species tested were more sensitive to these inorganics than the alevin life stage. Among juveniles, no single species was consistently more sensitive to the inorganics than another; among alevins, Arctic grayling were generally more sensitive than coho salmon and rainbow trout. Based on the results of the present study, estimated no-effect concentrations of arsenic and mercury, but not cadmium, chromium, gold, nickel, selenium, or silver, are close to their concentrations reported in streams with active placer mines in Alaska. Thus, arsenic (as arsenite(III)) and mercury may pose a hazard to Arctic grayling and coho salmon in Alaskan streams with active placer mines.

Saiki, MK, DT Castleberry, TW May, BA Martin, and FN Bullard. 1995. Copper, cadmium, and zinc concentrations in aquatic food-chains from the upper Sacramento River (California) and selected tributaries. Archives of Environmental Contamination and Toxicology. 29(4):484-491.

Abstract: Metals enter the Upper Sacramento River above Redding, California, primarily through Spring Creek, a tributary that receives acid-mine drainage from a US EPA Super-fund site known locally as Iron Mountain Mine. Waterweed (*Elodea canadensis*) and aquatic insects (midge larvae, Chironomidae; and mayfly nymphs, *Ephemeroptera*) from the Sacramento River downstream from Spring Creek contained much higher concentrations of copper (Cu), cadmium (Cd), and zinc (Zn) than did similar taxa from nearby reference tributaries not exposed to acid-mine drainage. Aquatic insects from the Sacramento River contained especially high maximum concentrations of Cu (200 mg/kg dry weight in midge larvae), Cd (23 mg/kg dry weight in mayfly nymphs), and Zn (1,700 mg/kg dry weight in mayfly nymphs). Although not always statistically significant, whole-body concentrations of Cu, Cd, and Zn in fishes (threespine stickleback, *Gasterosteus aculeatus*; Sacramento sucker, *Catostomus occidentalis*; Sacramento squawfish, *Ptychocheilus grandis*; and chinook salmon, *Oncorhynchus tshawytscha*) from the Sacramento River were generally higher than in fishes from the reference tributaries.

Baatrup, E. Structural and Functional-Effects of Heavy-Metals on the Nervous-System, Including Sense-Organs, of Fish. Comparative Biochemistry and Physiology C-Pharmacology Toxicology & Endocrinology. 1991; 100(1-2):253-257.

Abstract: Today, fish in the environment are inevitably exposed to chemical pollution. Although most hazardous substances are present at concentrations far below the lethal level, they may still cause serious damage to the life processes of these animals. Fish depend on an intact nervous system, including their sense organs, for mediating relevant behavior such as food search, predator recognition, communication and orientation. Unfortunately, the nervous system is most vulnerable and injuries to its elements may dramatically change the behavior and consequently the survival of fish.

Heavy metals are well known pollutants in the aquatic environment. Their interaction with relevant chemical stimuli may interfere with the communication between fish and environment. The affinity for a number of ligands and macromolecules makes heavy metals most potent neurotoxins. The present Mini-Review highlights some aspects of how trace concentrations of mercury, copper and lead affect the integrity of the fish nervous system; structurally, physiologically and biochemically.

Oregon study shows copper from brake pads affects salmon

CORVALLIS, Ore., Oregon State University issued the following news release:

Copper deposited on roads by the wearing of brake pads is transported in runoff to streams and rivers, where it may play a key role in increasing predation of threatened and endangered salmon throughout California and the Pacific Northwest. According to a study released this week in *Environmental Science and Technology*, levels of copper as low as 2 parts per billion have a direct impact on the sensory systems of juvenile coho salmon. The skin of juvenile salmon is equipped with a special kind of warning system, said Nat Scholz, a researcher at the Northwest Fisheries Science Center, a branch of the National Oceanic and Atmospheric Association (NOAA) Fisheries Service. When a salmon is attacked by a predator, a chemical cue is released from the skin that signals danger to nearby fish. These fish smell the predation cue and take behavioral measures to avoid being eaten.

Oregon State University researchers working with scientists from NOAA Fisheries, found that fish exposed to low, environmentally realistic levels of copper had an impaired sense of smell and were less responsive to the chemical alarm signal. At elevated concentrations of copper, these predator avoidance behaviors were largely abolished.

Copper naturally occurs in aquatic environments at trace amounts as a background element. However, fluctuations due to run-off from storm events can increase the level of copper in the water from close to zero to more than 60 parts per billion in some instances, said Jason Sandahl, who co-authored the study while working as an OSU doctoral research assistant at the NOAA research laboratory.

'There is a fine line between active copper uptake and copper toxicity,' said Sandahl. 'We see problems when copper is pulsed into the water, temporarily elevating the copper higher than the natural background level. The olfactory, or scent, neurons are not able to maintain the normal regulation of copper, and the neurons are either disrupted or killed.' Salmon are known to avoid environmental gradients of copper, such as those created by point-source discharges. However, copper in stormwater is a diffuse form of non-point source pollution, and it is unlikely that juvenile fish could reduce their exposure through avoidance behaviors, said the researchers.

As a result of automobile braking and exhaust, higher levels of copper contamination have been observed in streams close to roads and highways. Building materials and certain pesticide formulations are also important sources of copper in western landscapes, said Scholz.

Recent monitoring of northern California streams following storm events found dissolved copper levels averaging 15.8 parts per billion per liter of water. Salmon exposed to copper at concentrations well below this average showed significant impairment to both their sensory physiology and predator avoidance behavior, said Sandahl, whose work on the study was funded in part by a National Institute of Environmental Health Sciences grant to OSU. The work was also supported by NOAA's national Coastal Storms Program.

Since the duration of storm events that cause elevated levels of copper in streams can be relatively short, investigators exposed juvenile coho salmon to copper for only a few hours. In earlier studies they found the onset of copper neurotoxicity to salmon olfactory systems occurs within a matter of minutes. Loss of sensory function is likely reversible, but may take hours or days of the fish being in clean water, said the researchers. If copper exposures are high enough to cause the death of olfactory sensory neurons, it will take several weeks to months for the fish to regenerate new neurons and recover.

The levels of copper contaminant used in the study were at or below current federal regulatory guidelines for heavy metals, said Jeff Jenkins, an environmental toxicologist in OSU's College of Agricultural Sciences. 'It's just like they were poisoned,' said Jenkins. 'Of all the chemicals we have looked at, this effect was clearly happening at levels well below the current copper standards for water quality. It raises the question of whether the current standards are as protective as we thought.'

The current study is an example of how contaminants can disrupt the chemical ecology of aquatic organisms. In the case of salmon, a sublethal loss of sensory function may increase predation mortality in urbanizing watersheds. The influence of copper on predator-prey interactions is the focus of ongoing research, with the eventual aim of linking individual survival to the productivity of wild salmon populations, said Scholz.

Though the study was conducted on juvenile salmon, the results are applicable to fish species in urban watersheds worldwide, said the researchers. Dissolved copper has been shown to affect the olfactory systems of chinook salmon, rainbow trout, brown trout, fathead minnow, Colorado pikeminnow and tilapia.

Barry, KL, JA Grout, CD Levings, BH Nidle, and GE Piercy. 2000. Impacts of acid mine drainage on juvenile salmonids in an estuary near Britannia Beach in Howe Sound, British Columbia. Can. J. Fish. Aquat. Sci. 57: 2032–2043.

Abstract: The abandoned copper mine at Britannia Beach, British Columbia, has been releasing acid mine drainage (AMD) into Howe Sound for many years. To assess the impacts of AMD on juvenile salmonids in the Britannia Creek estuary, we compared fish abundance, distribution, and survival at contaminated sites near the creek with uncontaminated areas in Howe Sound. Water quality near Britannia Creek was poor, particularly in spring when dissolved Cu exceeded $1.0 \text{ mg}\cdot\text{L}^{-1}$ and pH was less than 6. Beach seine surveys conducted during April–August 1997 and March–May 1998 showed that chum salmon (*Oncorhynchus keta*) fry abundance was significantly lower near Britannia Creek mouth ($0\text{--}1.2\cdot 100 \text{ m}^{-2}$) than in reference areas ($11.5\text{--}31.4\cdot 100 \text{ m}^{-2}$). Laboratory bioassays confirmed that AMD from Britannia Mine was toxic to juvenile chinook (*Oncorhynchus tshawytscha*) and chum salmon (96-h LC50 = 0.7–29.7% in freshwater and 12.6–62.2% in 10 ppt water). Chinook salmon smolts transplanted to surface cages near Britannia Creek experienced 100% mortality within 2 days. These results demonstrated that juvenile salmonids are vulnerable to AMD from Britannia Creek: their abundance peaks during spring when Cu concentrations are highest and toxicity is greatest in surface freshwater, which matches their preferred vertical distribution.

**Eisler, R. COPPER HAZARDS TO FISH, WILDLIFE, AND INVERTEBRATES:
A SYNOPTIC REVIEW. U.S. Geological Survey, Laurel, MD 20708**

Excerpt specific to fish:

Fishes Adverse sublethal effects of copper on behavior, growth, migration, and metabolism occur in representative species of fishes at nominal water concentrations between 4 and 10 µg/L. In sensitive species of teleosts, copper adversely affects reproduction and survival from 10-20 µg Cu/L (Hodson et al. 1979; Table 5). Copper exerts a wide range of physiological effects in fishes, including increased metallothionein synthesis in hepatocytes, altered blood chemistry, and histopathology of gills and skin (Iger et al. 1994). At environmentally realistic concentrations, free copper adversely affects resistance of fishes to bacterial diseases; disrupts migration (that is, fishes avoid copper-contaminated spawning grounds); alters locomotion through hyperactivity; impairs respiration; disrupts osmoregulation through inhibition of gill Na⁺-K⁺-activated ATPase; is associated with tissue structure and pathology of kidneys, liver, gills, and other hematopoietic tissues; impacts mechanoreceptors of lateral line canals; impairs functions of olfactory organs and brain; and is associated with changes in blood chemistry, enzyme activities, and corticosteroid metabolism (Hodson et al. 1979).

Copper-induced cellular changes or lesions occur in kidneys, lateral line, and livers of several species of marine fishes (Gardner and LaRoche 1973). Copper-induced mortality in teleosts is reduced in waters with high concentrations of organic sequestering agents and in genetically resistant species (Hodson et al. 1979). At pH values less than 4.9 (that is, at pH values associated with increased aluminum solubility and toxicity), copper may contribute to the demise of acid-sensitive fishes (Hickie et al. 1993). Copper affects plasma Na⁺ and gill phospholipid activity; these effects are modified by water temperature and hardness (Hansen et al. 1993). In red drum, copper toxicity is higher at comparatively elevated temperatures and reduced salinities (Peppard et al. 1991).

Copper is acutely toxic to freshwater teleosts in soft water at concentrations between 10 and 20 µg/L (NAS 1977). In rainbow trout, copper toxicity is markedly lower at high salinities (Wilson and Taylor 1993). Comparatively elevated temperatures and copper loadings in the medium cause locomotor disorientation of tested species (Kleerekoper 1973). Copper may affect reproductive success of fish through disruption of hatch coordination with food availability or through adverse effects on larval fishes (Ellenberger et al. 1994). Chronic exposure of representative species of teleosts to low concentrations (5 to 40 µg/L) of copper in water containing low concentrations of organic materials adversely affects survival, growth, and spawning; this range is 66 to 120 µg Cu/L when test waters contain enriched loadings of organic materials (Hodson et al. 1979). Larval and early juvenile stages of eight species of freshwater fishes are more sensitive to copper than embryos (McKim et al. 1978) or adults (Hodson et al. 1979). But larvae of topsmelt (*Atherinops affinis*) are increasingly sensitive to copper with increasing age. Topsmelt sensitivity is associated with increasing respiratory surface area and increasing cutaneous and branchial uptake of copper (McNulty et al. 1994). Sublethal exposure of fishes to copper suppresses resistance to viral and bacterial pathogens (Rougier et al. 1994) and, in the case of the air-breathing catfish (*Saccobranchus fossilis*), affects humoral and cell-mediated immunity, the skin, and respiratory surfaces (Khangarot and

Tripathi 1991). Rainbow trout exposed to 50 µg Cu/L for 24 h—a sublethal concentration—show degeneration of olfactory receptors that may cause difficulties in olfactory-mediated behaviors such as migration (Klima and Applehans 1990). The primary site of sublethal copper toxicity in rainbow trout is the ion transport system of the gills (Hansen et al. 1993). Dietary copper is more important than waterborne copper in reducing survival and growth of larvae of rainbow trout (Woodward et al. 1994). Simultaneous exposure of rainbow trout to dietary and waterborne copper results in significant copper assimilation. Diet is the main source of tissue copper; however, the contribution of waterborne copper to tissue burdens increases as water concentrations rise (Miller et al. 1993). Rate and extent of copper accumulations in fish tissues are extremely variable between species and are further modified by abiotic and biological variables. Copper accumulations in fish gills increase with increasing concentrations of free copper in solution, increasing dissolved organic carbon (DOC), and decreasing pH and alkalinity (Playle et al. 1993a, 1993b). Starved Mozambique tilapia accumulate significantly more copper from the medium in 96 h than did tilapia fed a diet containing 5.9 mg Cu/kg DW ration (Pelgrom et al. 1994). The bioconcentration factor for whole larvae of the fathead minnow was 290 after exposure for 30 h, but only 0.1 in muscle of bluegills after 660 h (USEPA 1980). Prior exposure of brown bullheads (*Ictalurus nebulosus*) to 83 sublethal copper concentrations for 20 days before exposure to lethal copper concentrations produces higher copper concentrations in tissues of dead bullheads than in those not previously exposed; however, the use of tissue residues is not an acceptable autopsy procedure for copper (Brungs et al. 1973). Rising copper concentrations in blood plasma of catfish (*Heteropneustes fossilis*) seem to reflect copper stress, although the catfish appear outwardly normal. Plasma copper concentrations of catfish increase from 290 µg Cu/L in controls at start to 380 µg Cu/L in survivors at 72 h (50% dead); a plasma copper concentration of 1,060 µg Cu/L at 6 h is associated with 50% mortality (Banerjee and Homechaudhuri 1990). In rainbow trout, copper is rapidly eliminated from plasma; the half-time persistence is 7 min for the short-lived component and 196 min for the long-lived component (Carbonell and Tarazona 1994). Attraction to waters containing low (11 to 17 µg/L) concentrations of copper occurs in several species of freshwater teleosts, including goldfish (*Carassius auratus*) and green sunfish (*Lepomis cyanellus*); however, other species, including white suckers (*Catostomus commersonii*), avoid these waters (Kleerekoper 1973). In avoidance/attraction tests, juvenile rainbow trout avoided waters containing 70 µg Cu/L but were significantly attracted to water containing 4,560 µg Cu/L; a similar pattern was observed in tadpoles of the American toad, *Bufo americanus* (Birge et al. 1993). Copper concentrations in the range of 18 to 28 µg/L interfere with bluegill growth and prey choice (Sandheinrich and Atchison 1989). Copper interferes with the ability of fish to respond positively to L-alanine, an important constituent of prey odors; concentrations as low as 1 µg Cu/L inhibit this attraction response in some species (Steele et al. 1990). Increased tolerance to copper was observed in fathead minnows after prolonged exposure to sublethal concentrations, but tolerance was not sustained on removal to clean water. Copper tolerance in fathead minnows is attributed to increased production of metallothioneins (Benson and Birge 1985). Copper tolerance in rainbow trout seems dependent on changes in sodium transport and permeability (Lauren and McDonald 1987a).

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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.

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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.

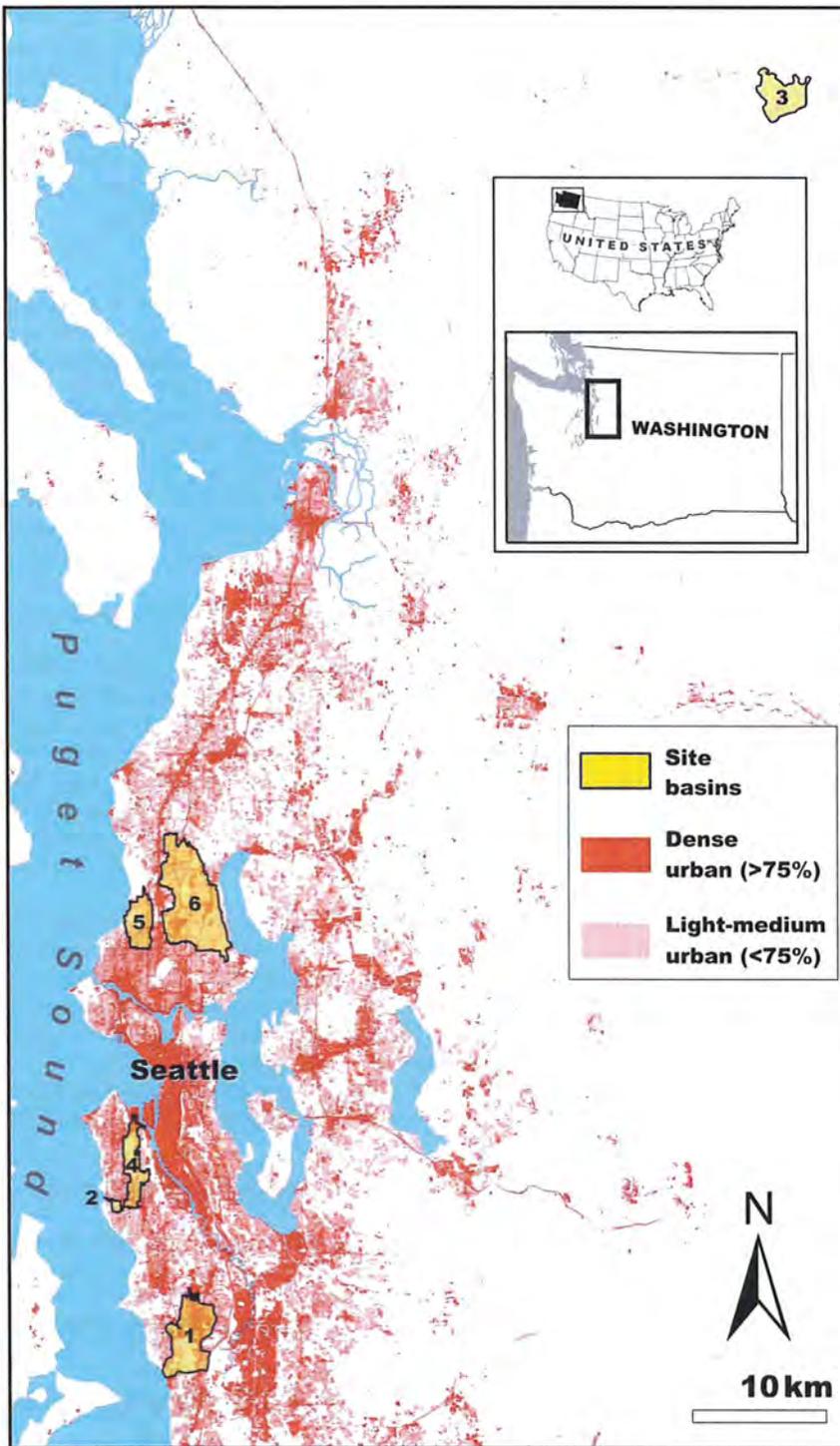


Figure 1. Six study sites where coho spawner mortality was monitored and landscape conditions were quantified. Main map depicts the Greater Seattle Metropolitan Area in Washington State, which is within the Puget Sound/Georgia Basin of the Pacific Northwest, United States of America (USA). Inset map 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 = Fautleroy; 3 = Fortson; 4 = Longfellow; 5 = Piper's; and, 6 = Thornton Creek.
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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)	0.80 (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.

^aNorthwest Fisheries Science Center (NWFSC) daily surveys, all others were weekly and collected by Seattle Public Utilities (SPU) or the Wild Fish Conservancy [51,52].

¹Non-urban site.

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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

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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

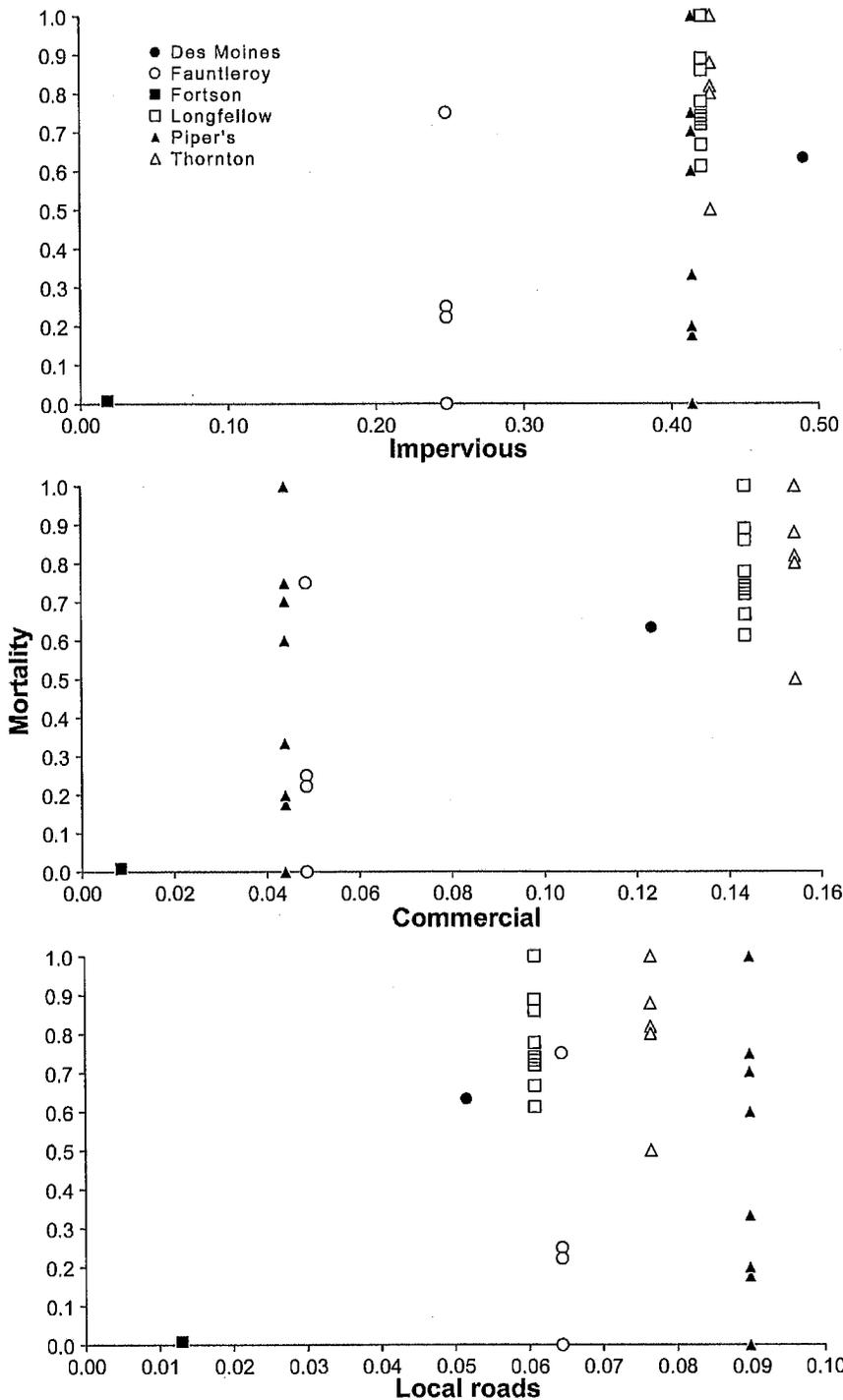


Figure 2. Female coho spawner mortality as a function of the proportion of each of the top three predictors in a given site basin, at the six study sites. Individual points correspond to specific years for each site. Mortality expressed as proportion of all returning females that died in a given year. 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_{AIC_c} . Intercept only model included at bottom for reference.

Model	Equation	ΔAIC_c	w_{AIC_c}
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.

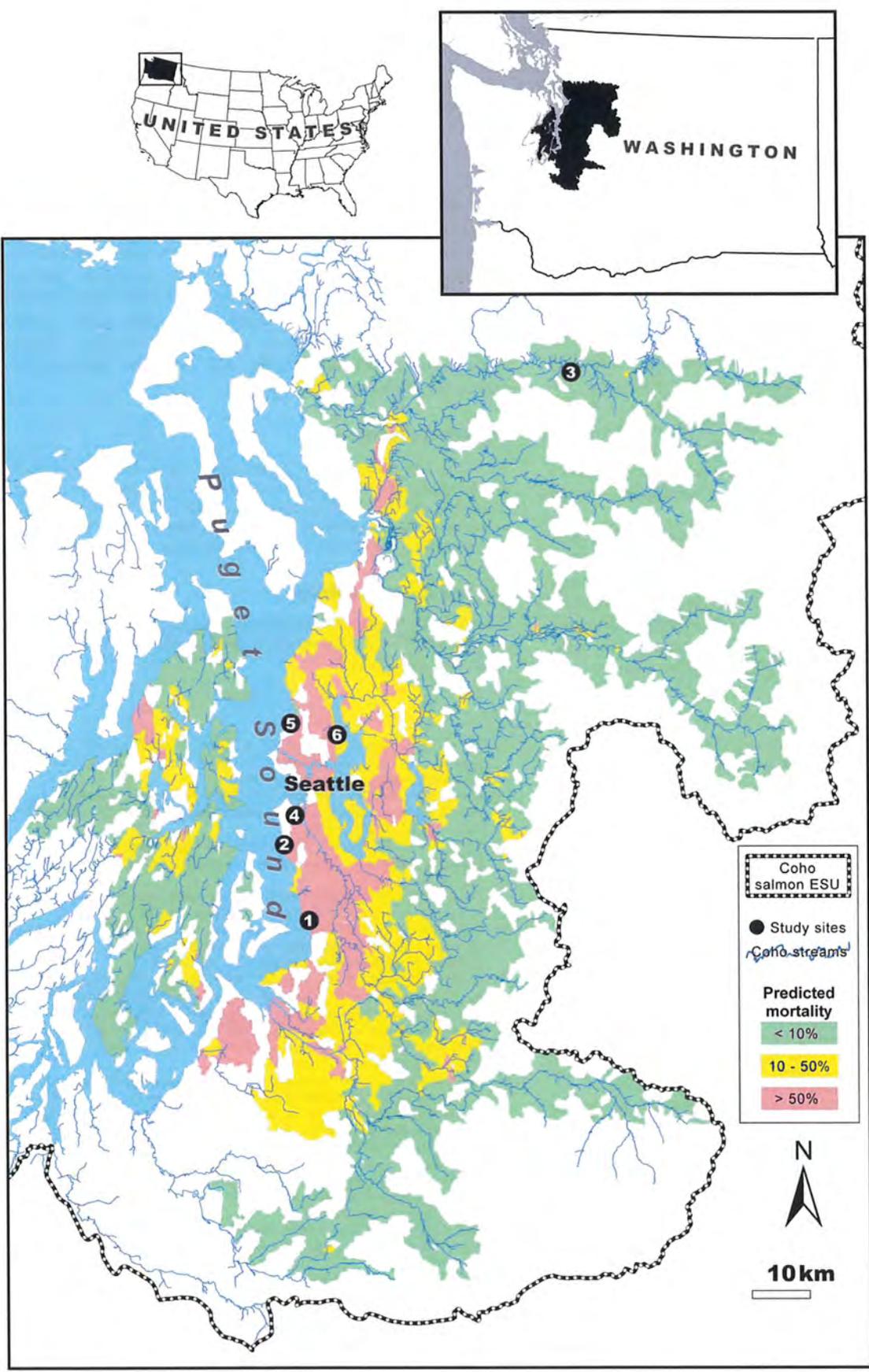


Figure 3. Predictive map of modeled coho spawner mortality rates within the Puget Sound lowlands. Mortality rates are a function of the proportion of key landscape variables within a given basin. Green, yellow and red areas indicate basins with predicted rates of spawner mortality (as a percentage of 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=Fautleroy; 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.

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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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Hawk Property Subarea Plan

Northern Gateway Study - Phase 2

Monday, March 25, 2013

Community Workshop Comment Form

Thank you for attending tonight's Community Workshop on the Hawk Property Subarea Plan. The City of Covington wants to hear from you. Please write your comments on this form. You may either put your completed form in the comment box during tonight's workshop or return it to the address listed on the back.

What do you want to see happen in the future in the subarea? Please list any concerns you have for the future of the Hawk Property Subarea. Please feel free to offer any additional comments, concerns or ideas that you feel may be important subarea planning process.

Comments:

PLEASE RESPOND TO QUESTIONS!

1) WILL THE CITY ANNEX ADDITIONAL PROPERTIES TO THE EAST? (FROM URBAN MAPLE VALLEY)

2) ROAD ACCESS NOT ON HWY 18 WILL THERE BE IMPROVEMENTS TO 180th ST 256th TO KENT KANGRO? IT'S BAD NOW.

3) PLAN FOR POLICE PROTECTION. WHAT IS IT?

(turn over for additional information & space)

The following information is optional.

Name: RUSS & JERRI GARDNER Organization: _____

Address: PO BOX 665

City: MAPLE VALLEY WA State: WA Zip: 98038

Phone: 425-432-6045 E-mail: therussgardner@hotmail.com

Parcel # 202206-9159-04

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What do you want to see happen in the future in the subarea? Please list any concerns you have for the future of the Hawk Property Subarea. Please feel free to offer any additional comments, concerns or ideas that you feel may be important subarea planning process.

- Comments:
- ① Some retail with housing above + parking below
 - ② A good grocery store. I live on the eastside of Covington and hate coming in to Covington to grocery shopping. Over half the time I go to Maple Valley
 - ③ A park by the water with trails (walking, running), picnic area - some covered areas
 - ④ Better access besides Hwy 18
 - ⑤ Old Frontage Road? Is it usable?

(turn over for additional information & space)

The following information is optional.

Name: Mary Fritchard Organization: citizen
 Address: _____
 City: _____ State: _____ Zip: _____
 Phone: _____ E-mail: pepat@wolfenet.com

Note: Consistent with the Public Records Act, Chapter 42.56 RCW, all records within the possession of the City may be subject to a public disclosure request and may be distributed or copied. Records include and are not limited to sign-in sheets, emails, comments, correspondence, etc. Use of lists of individuals or directory information (including address, phone or E-mail) may not be used for commercial purposes.



Hawk Property Subarea Plan

Northern Gateway Study - Phase 2

Monday, March 25, 2013

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Comments:

- Consider making parks as large as possible with large play areas
Small, tiny, HOA-style parks are not well-liked and not well-used.
- Please consider paving your trail so that moms jogging with strollers or rollerbladers or bike riders can also use the trail.
Loose gravel restricts that use.
- As much as possible, encourage walk-ability between stores and general spaces. Big, lighted sidewalks are highly desirable. As this is also an area with many families and small children, please consider putting sidewalks and safe walking areas in parking lots so moms can safely move their children from (turn over for additional information & space) car to store.

The following information is optional.

Name: _____ Organization: _____

Address: _____

City: _____ State: _____ Zip: _____

Phone: _____ E-mail: _____

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Comments:

as far as I go, I would like see to
Thing Single Family

(turn over for additional information & space)

The following information is optional.

Name: _____ Organization: _____
Address: _____
City: _____ State: _____ Zip: _____
Phone: _____ E-mail: _____

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