Supporting Information

Decreased growth rate associated with tissue contaminants in juvenile Chinook salmon out-migrating through an industrial waterway

Jessica I. Lundin[1], Paul M. Chittaro[2], Gina M. Ylitalo[2], John W. Kern[3], David R. Kuligowski[4], Sean Y. Sol[2], Keri A. Baugh[2], Daryle T. Boyd[2], Mary C. Baker[5], Robert M. Neely[5], Kennith G. King[6], and Nathaniel L. Scholz[2]

[1]National Research Council Research Associateship Program, Under contract to Northwest Fisheries Science Center, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, 2725 Montlake Blvd. E., Seattle, WA 98112 USA.

[2]Environmental and Fisheries Sciences Division, Northwest Fisheries Science Center, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, 2725 Montlake Blvd. E., Seattle, WA 98112 USA.

[3]Kern Statistical Services, Inc. 13680 Bete Grise RD, Mohawk, MI 49950 USA.

[4]Conservation Biology Division, Northwest Fisheries Science Center, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, 2725 Montlake Blvd. E., Seattle, WA 98112 USA.

[5]Assessment and Restoration Division, Office of Response and Restoration, National Ocean Service, National Oceanic and Atmospheric Administration, 7600 Sand Point Way N.E., Seattle, WA 98115-0070 USA.

[6]US Fish and Wildlife Service, 510 Desmond Dr SE #102, Lacey, WA 98503 USA.

Number of pages: 22

Number of tables: 12

Number of figures: 5

Supporting text: 3 pages

References: 2 pages

Table of contents

Figure S2. Measured contaminants [polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethanes (DDTs), and polycyclic aromatic hydrocarbons (PAHs)] in tissues of UWR juvenile Chinook salmon by sampling site; *p<0.05, site H as the upstream reference

Figure S3. Measured polycyclic aromatic hydrocarbons (PAHs) in tissues of UWR juvenile Chinook salmon by sampling site, adjusted for percent lipid, *p<0.05, site G-alt as the upstream reference

Figure S4. Contaminants with loadings greater than absolute value of 0.3 for PC2 and PC3 by site, represented by hexa- through nona-chlorinated PCBs (ng/g lipid adjusted), alkylated PAHs (ng/g, wet weight), and parent PAHs (ng/g, wet weight)

Figure S5. Polycyclic aromatic hydrocarbon (PAH) profiles of whole body tissues of juvenile Chinook salmon sampled at sites F and C

Supporting Text

Table S1. Number of individual fish genetically assigned as Upper Willamette Chinook and confirmed to be subyearlings (n=135; 42% of total fish collected) by site

Site	Bank	River mile	GPS coordinates (latitude, longitude)[a]	Presence of an annulus in otolith	Number of UWR Chinook salmon	Percent of total fished sampled from a site
A	East	0.5	45.64479, -122.76813	0	14	24%
B	West	3.5	45.61500, -122.79334	0	2	17%
B-alt	West	4.0	45.60695, -122.78842	0	16	35%
C	East	4.5	45.60343, -122.77689	0	19	43%
D	West	5.0	45.59150, -122.77565	0	1	100%
E	West	7.0	45.57417, -122.74749	0	19	44%
F	East	8.5	45.56149, -122.70712	1	24	47%
G[a]	West	13.5	45.50644, -122.67057	0	0	0%
H	East	14.0	45.49875, -122.66080	0	23	66%
G-alt	West	16.8	45.46151, -122.66685	0	18	55%

[a]Decimal degrees; datum = "WGS 84"

Table S2. Distribution of genetic assignments of 320 Chinook salmon collected

Site	Percent of total catch by site, West Bank					Percent of total catch by site, East Bank			
	G-alt (n=33)	G (n=1)	E (n=43)	D (n=1)	B/B-alt (n=58)	H *(n=35)*	F *(n=51)*	C *(n=44)*	A *(n=54)*
Snake River Fall	**0.0%** *(n=0)*	**0.0%** *(n=0)*	**0.0%** *(n=0)*	**0.0%** *(n=0)*	**3.4%** *(n=2)*	**0.0%** *(n=0)*	**0.0%** *(n=0)*	**0.0%** *(n=0)*	**0.0%** *(n=0)*
Willamette River Spring	**54.5%** *(n=18)*	**0.0%** *(n=0)*	**44.2%** *(n=19)*	**100.0%** *(n=1)*	**31.0%** *(n=18)*	**66.0%** *(n=23)*	**47.0%** *(n=24)*	**43.0%** *(n=19)*	**26.0%** *(n=14)*
West Cascade Spring	**0.0%** *(n=0)*	**0.0%** *(n=0)*	**4.7%** *(n=2)*	**0.0%** *(n=0)*	**3.4%** *(n=2)*	**3.0%** *(n=1)*	**10.0%** *(n=5)*	**9.0%** *(n=4)*	**4.0%** *(n=2)*
Spring Crk Group Fall	**0.0%** *(n=0)*	**0.0%** *(n=0)*	**0.0%** *(n=0)*	**0.0%** *(n=0)*	**17.2%** *(n=10)*	**0.0%** *(n=0)*	**4.0%** *(n=2)*	**5.0%** *(n=2)*	**11.0%** *(n=6)*
Upper Columbia Summer/Fall	**0.0%** *(n=0)*	**0.0%** *(n=0)*	**0.0%** *(n=0)*	**0.0%** *(n=0)*	**1.7%** *(n=1)*	**0.0%** *(n=0)*	**2.0%** *(n=1)*	**0.0%** *(n=0)*	**7.0%** *(n=4)*
West Cascade Fall	**18.2%** *(n=6)*	**100.0%** *(n=1)*	**20.9%** *(n=9)*	**0.0%** *(n=0)*	**25.9%** *(n=15)*	**11.0%** *(n=4)*	**27.0%** *(n=14)*	**20.0%** *(n=9)*	**43.0%** *(n=23)*
Unassigned genetic lineage	**27.3%** *(n=9)*	**0.0%** *(n=0)*	**30.2%** *(n=13)*	**0.0%** *(n=0)*	**17.2%** *(n=10)*	**20.0%** *(n=7)*	**10.0%** *(n=5)*	**23.0%** *(n=10)*	**9.0%** *(n=5)*

Table S3. Percent lipids and proportion lipid class in UWR subyearling Chinook salmon whole body composites by site; mean value (minimum-maximum)

Site	# total comps	# TBT comps	% Lipids	TG	FFA	Chol	PL
A	3	0	1.4 (1.2-1.6)	17.2 (11.8-24.1)	19.5 (11.9-27.9)	13.6 (10.2-17.2)	49.7 (30.8-62.3)
B	1	0	2.0	55.7	15.6	12.9	15.8
B-alt	4	1	1.1 (1.0-1.5)	23.6 (11.6-33.3)	18.4 (16.5-20.3)	22.5 (11.7-31.4)	35.6 (23.6-60.3)
C	5	1	0.9 (0.8-1.1)*	19.8 (0.0-44.3)	16.7 (0.0-29.0)*,†	22.9 (11.9-39.0)	40.6 (0-83.7)†
D	1	1	0.9	20.8	32.1	19.0	28.1
E	6	1	1.2 (0.8-1.6)	25.2 (10.4-35.2)	26.1 (15.6-31)	19.2 (12.5-27.9)	29.6 (18.3-44.7)
F	8	3	1.3 (0.8-3.0)	30.9 (7.8-66.3)	23.1 (10.7-29.5)	22.3 (8.6-33.4)	23.7 (14-42.4)
H	6	0	1.2 (0.9-1.9)	35.1 (9.0-54.7)	23.4 (15.3-32.4)	20.9 (13.1-24.8)	20.6 (13.9-35.7)
H+G-alt	1	1	1.7	50.5	23.9	9.4	16.2
G-alt (Ref)	3	0	1.2 (1.1-1.3)	20.7 (13.2-30.9)	20.3 (13.7-24.1)	20.7 (15.3-25.4)	38.2 (20.5-57.8)

*p<0.05, G-alt as the upstream reference site; †p<0.05, upstream site H as the reference**.** Average fork length per composites was included in all analyses and percent lipids was included in analyses of lipid class.

H+G-alt = A single tissue composite created by combining fish collected at sites H and G-alt to reach the requisite mass requirements for TBT analysis in addition to POPs and PAHs

Table S4. Predicted daily growth rate (mm fork length per day) for the 14 days prior to collection from otolith microanalysis of UWR Chinook salmon (model coefficients[a], standard error in parentheses), with upstream site G-alt as the reference. †p<0.05 when upstream site H was used as the reference.

		Average daily growth rate[b] (mm/day), 14 days	p-values	Incremental daily growth rate[c] (mm/day), 14 days	p-values
Site model (Ref G-alt)	Intercept	-0.911 (0.075)	<0.001	-0.747 (0.079)	<0.001
	A	0.135 (0.060)	0.026	0.117 (0.074)	0.114
	B-alt	0.002 (0.058)†	0.978	-0.016 (0.051)†	0.751
	C	0.047 (0.056)†	0.401	0.048 (0.058)†	0.402
	E	0.121 (0.056)	0.032	0.112 (0.062)	0.070
	F	-0.025 (0.056)†	0.662	-0.034 (0.050)†	0.496
	H	0.182 (0.054)	0.001	0.166 (0.056)	0.003
	G-alt	Reference†	x	Reference†	x
	days	NA	NA	-0.040 (0.008)	<0.001
	days*days	NA	NA	0.002 (0.0005)	<0.001
	Fork Length (mm)	0.004 (0.001)	0.003	0.004 (0.001)	<0.001

a: For a 1-unit change in covariate (e.g. PC2), exp(coefficient) equals the ratio of predicted daily growth rate

b: Generalized Linear Model adjusted for fork length at capture

c: Generalized Estimating Equation with repeat measures to account for daily growth measurement for each individual, adjusted for fork length at capture, with an autoregressive correlation structure

Table S5. PCA varimax rotation of COCs, loadings greater than absolute value of 0.3

Descriptor	DDTs	alkyl PAHs, 6-9Cl PCBs	parent PAHs, low TGs, high PL	3-5 Cl PCBs	% lipids, high TGs, low FFAs, low Chol
Component	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**
Eigenvalue, percent variance explained by component	6.9, 33%	3.7, 18%	2.5, 12%	2.3, 11%	1.8, 9%
PCB3				0.566	
PCB4				0.555	
PCB5				0.520	
PCB6		0.366			
PCB7		0.466			
PCB8_9		0.431			
p,p'-DDD	0.380				
p,p'-DDE	0.428				
p,p'-DDT	0.410				
o,p'-DDD	0.377				
o,p'-DDE	0.424				
o,p'-DDT	0.405				
LMWAHs_alkyl		0.438			
LMWAHs_parent			0.512		
HMWAHs_parent			0.450		
HMWAHs_alkyl		0.399			
Percent lipid					0.586
TG			-0.441		0.308
FFA					-0.453
PL			0.455		
Chol					-0.547

Abbreviations: PAHs — polycyclic aromatic hydrocarbons, PCBs — polychlorinated biphenyls, TGs — triglycerides, PL — polar lipids, FFAs — free fatty acids, Chol — cholesterol, PCB3 — trichlorinated PCBs, PCB4 — tetrachlorinated PCBs, PCB5 – pentachlorinated PCBs, PCB6 – hexachlorinated PCBs, PCB7 – heptachlorinated PCBs, PCB8_9 – summed octachlorinated and nonachlorinated PCBs, LMWAHs_alkyl – alkylated low molecular weight PAHs, LMWAHs_parent – parent low molecular weight PAHs, HMWAHs_alkyl – alkylated high molecular weight PAHs, HMWAHs_parent – parent high molecular weight PAHs

Table S6. Incorporation of environmental covariates and fish metric covariates into growth models

Model	AIC value[b]
Site model adjusted for fork length	-250.8
+ hepatosomatic index	-251.1
+ gut fullness (mass stomach contents)	-246.6
Contamination model adjusted for fork length	-241.7
+ hepatosomatic index	-242.5
+ gut fullness (mass stomach contents)	-238.0
+ Lipid class (PCA components; PC1 and PC2)[a]	-238.1

[a]PCA run for just lipid class components (triglycerides, free fatty acids, polar lipids, and cholesterol); two PC were retained representing all 4 classes (data not shown); [b]Smaller AIC values indicate "better" models. A difference in AIC values of less than 2 indicates little difference between the models being compared; a difference in AIC of 2–10 indicates moderate support for a difference between the models, and a difference in AIC of greater than 10 indicates strong support.[1]

Table S7. Percent lipids, sum of 6 dichlorodiphenyltrichloroethanes (DDTs) and sum of 45 polychlorinated biphenyls (PCBs) as ng/g wet weight (ww) in UWR subyearling Chinook salmon whole body composites by site; mean value (minimum-maximum)

Site	% lipids	Sum PCBs ww	Sum DDTs ww
A	1.4 (1.2-1.6)	15.0 (12.7-19.5)	11.5 (9.7-12.4)
B	2.0	18.0	16.9
B-alt	1.1 (1.0-1.5)	40.8 (19.6-65.2)	11.3 (7.0-19.3)
C	0.9 (0.8-1.1)*	22.2 (13.2-36.4)	18.0 (11.4-23.6)
D	0.9	14.7	19.3
E	1.2 (0.8-1.6)	102.6 (25.5-390.8)*,†	211.3 (72.9-497.0)*,†
F	1.3 (0.8-3.0)	50.7 (25.9-82.6)*	16.1 (9.7-23.4)
H	1.2 (0.9-1.9)	28.0 (18.6-54.1)	11.8 (7.7-16.7)
H+G-alt	1.7	14.2	13.7
G-alt (Ref)	1.2 (1.1-1.3)	15.1 (11.6-21.1)	7.7 (7.5-8.1)

*$p<0.05$ with site G-alt as the reference site; †$p<0.05$, upstream site H as the reference. Average fork length per composites and percent lipids was included in all analyses; H+G-alt = A single tissue composite created by combining fish collected at sites H and G-alt to reach the requisite mass requirements for TBT analysis in addition to POPs and PAHs

Table S8. Summed values of polycyclic aromatic hydrocarbons (PAHs), low molecular weight PAHs (LMWAHs), and high molecular weight PAHs (HMWAHs) as ng/g wet weight in UWR subyearling Chinook salmon whole body composites by site; mean value (minimum-maximum)

Site	Sum PAHs	Sum LMWAHs	Sum HMWAHs	Sum Parent PAHs	Sum Alkyl PAHs
A	13.6 (12.6-14.6)	13.4 (12.3-14.2)†	0.2 (0-0.4)	2.9 (2.5-3.3)	10.7 (9.7-11.3)†
B	15.0	14.4	0.7	3.9	11.1
B-alt	14.2 (10.0-17.1)†	12.9 (9.5-16.6)†	1.3 (0.5-2.8)*,†	3.9 (2.5-5.5)†	10.3 (7.4-13.2)†
C	21.9 (11.3-28.8)*,†	18.9 (9.6-24.8)†	3.1 (1.6-4.4)*,†	11.9 (4.3-18.9)*,†	10.1 (7.0-11.8)†
D	14.7	14.2	0.5	3.5	11.2
E	10.3 (8.2-13.2)	10.2 (8.2-13.2)	0.1 (0-0.5)	2.2 (1.7-2.8)	8.1 (6.5-11.1)
F	26.8 (18.7-44.6)*,†	25.1 (16.9-43.5)*,†	1.6 (0.7-3.1)*,†	5.6 (4.0-9.1)*,†	20.4 (13.9-30.1)*,†
H	8.7 (6.0-11.2)*	8.4 (5.6-10.8)*	0.3 (0.0-0.7)	2.2 (1.7-2.8)	6.5 (4.1-8.9)*
H+G-alt	11.2	10.6	0.6	2.6	8.6
G-alt (Ref)	14.8 (13.6-16.7)†	14.7 (13.6-16.2)†	0.2 (.00-0.5)	2.8 (2.5-3.4)	12.0 (11.1-13.3)†

*p<0.05 with site G-alt as the reference site; †p<0.05, upstream site H as the reference**.** Average fork length per composites and percent lipids was included in all analyses; H+G-alt = A single tissue composite created by combining fish collected at sites H and G-alt to reach the requisite mass requirements for TBT analysis in addition to POPs and PAHs

Table S9. Summed values of 45 polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethanes (DDTs), and 42 polycyclic aromatic hydrocarbons (PAHs) determined in UWR subyearling Chinook salmon stomach contents (ng/g wet weight) by site

Site	Sum PCBs	Sum DDTs	Sum PAHs
A	27	19	77
B-alt	53	15	286
C	35	21	593
E	46	142	104
F	59	13	837
H+G-alt	33	17	196

H+G-alt = A single stomach contents composite created by combining fish collected at sites H and G-alt to reach the requisite mass requirements for POPs and PAHs analyses

Table S10. Proportions of polychlorinated biphenyl (PCB) homologues to summed PCBs in whole bodies, by sampling sites

	PCB3	PCB4	PCB5	PCB6	PCB7	PCB8_9
A	0.09 (0.06-0.13)	0.16 (0.13-0.18)	0.31 (0.28-0.32)†	0.31 (0.29-0.33)	0.11 (0.11-0.12)†	0.02 (0.02-0.03)†
B	0.09	0.15	0.27	0.33	0.14	0.03
B-alt	0.08 (0.03-0.15)	0.11 (0.05-0.16)	0.29 (0.21-0.37)†	0.37 (0.32-0.46)	0.12 (0.06-0.18)†	0.03 (0.02-0.04)†
C	0.07 (0.04-0.1)	0.12 (0.08-0.13)	0.25 (0.23-0.27)*,†	0.35 (0.33-0.38)	0.18 (0.13-0.23)*	0.04 (0.03-0.05)†
D	0.18	0.2	0.24	0.24	0.09	0.04
E	0.05 (0.02-0.10)	0.22 (0.11-0.51)*,†	0.28 (0.24-0.31)†	0.29 (0.09-0.40)	0.13 (0.03-0.17)†	0.03 (0.01-0.04)†
F	0.04 (0.02-0.06)*	0.08 (0.05-0.13)	0.23 (0.19-0.3)*	0.38 (0.35-0.41)	0.23 (0.14-0.27)*	0.05 (0.02-0.06)
H	0.06 (0.02-0.07)	0.09 (0.05-0.15)	0.20 (0.17-0.26)*	0.35 (0.31-0.38)	0.23 (0.2-0.27)*	0.06 (0.05-0.08)*
H+G-alt	0.09	0.11	0.29	0.34	0.14	0.04
G-alt (Ref)	0.08 (0.05-0.09)	0.10 (0.08-0.11)	0.34 (0.31-0.40)†	0.34 (0.34-0.35)	0.11 (0.08-0.13)†	0.03 (0.02-0.05)†

*p<0.05 with site G-alt as the reference site; †p<0.05, upstream site H as the reference**.** Average mass per composites was included in all analyses; H+G-alt = A single tissue composite created by combining fish collected at sites H and G-alt to reach the requisite mass requirements for TBT analysis in addition to POPs and PAHs

Table S11. Proportions of dichlorodiphenyltrichloroethanes (DDTs) isomers to summed DDTs in whole bodies, by sampling site

	***p,p'*-DDE**	***p,p'*-DDT**	***p,p'*-DDD**	***o,p'*-DDE**	***o,p'*-DDT**	***o,p'*-DDD**
A	0.80 (0.78-0.80)	0.07 (0.07-0.08)	0.09 (0.08-0.10)	0.00 (0.00-0.01)	0.02 (0.02-0.02)	0.02 (0.02-0.02)
B	0.65	0.18	0.09	0.01	0.04	0.03
B-alt	0.78 (0.73-0.83)	0.09 (0.07-0.11)	0.09 (0.07-0.09)	0.01 (0.00-0.01)	0.02 (0.02-0.03)	0.02 (0.01-0.03)
C	0.76 (0.71-0.85)	0.10 (0.06-0.14)*,†	0.07 (0.06-0.09)	0.01 (0.00-0.02)	0.03 (0.02-0.05)*,†	0.03 (0.01-0.08)†
D	0.78	0.10	0.08	0.00	0.02	0.02
E	0.60 (0.54-0.71)*,†	0.15 (0.10-0.24)*,†	0.12 (0.05-0.19)†	0.02 (0.01-0.03)*,†	0.07 (0.04-0.11)*,†	0.04 (0.02-0.07)*,†
F	0.82 (0.75-0.85)	0.07 (0.06-0.11)	0.08 (0.06-0.08)	0.00 (0.00-0.01)	0.02 (0.01-0.03)	0.01 (0.01-0.03)
H	0.82 (0.78-0.84)	0.07 (0.06-0.09)	0.07 (0.07-0.09)	0.01 (0.00-0.01)	0.02 (0.01-0.02)	0.01 (0.01-0.02)
H+G-alt	0.80	0.08	0.07	0.01	0.02	0.02
G-alt (Ref)	0.82 (0.79-0.83)	0.06 (0.06-0.07)	0.08 (0.07-0.10)	0.01 (0.01-0.01)	0.02 (0.01-0.02)	0.01 (0.01-0.02)

*p<0.05 with site G-alt as the reference site; †p<0.05, upstream site H as the reference**.** Average mass per composites was included in all analyses; H+G-alt = A single tissue composite created by combining fish collected at sites H and G-alt to reach the requisite mass requirements for TBT analysis in addition to POPs and PAHs

Table S12. Tributyltin (TBT) data. Italics represent measures that were < limit of detection (LOD), listed values are half the lower LOD

Site	TBT cation (ng/g ww)
B-alt	*1.05*
H + G-alt	*0.75*
F	2.1
F	*0.85*
F	1.9
C	*0.9*

Figure S1. Plots of PC1-5 as predictors of average daily growth for the recent 14 days prior to collection; model from Table 4

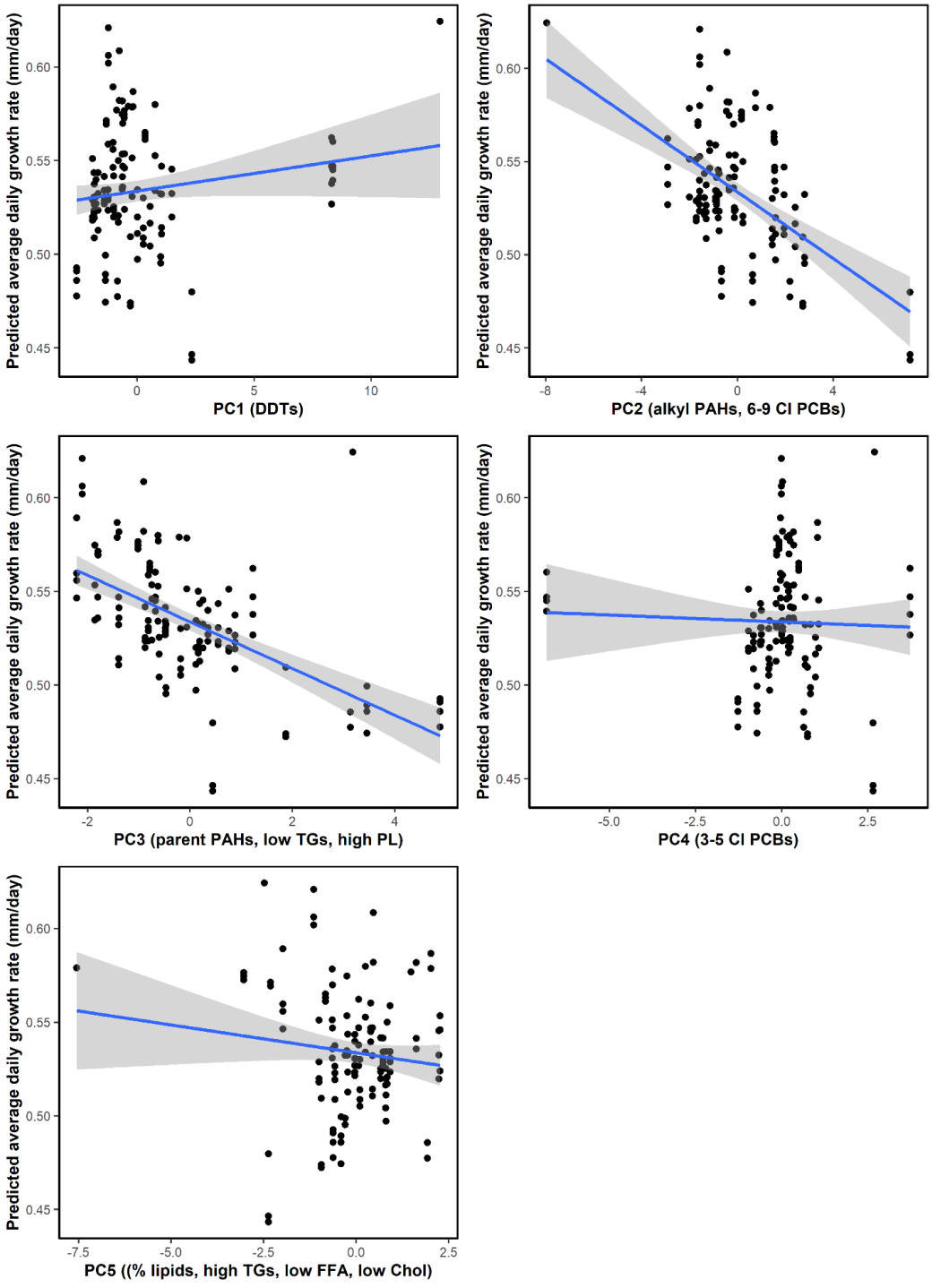
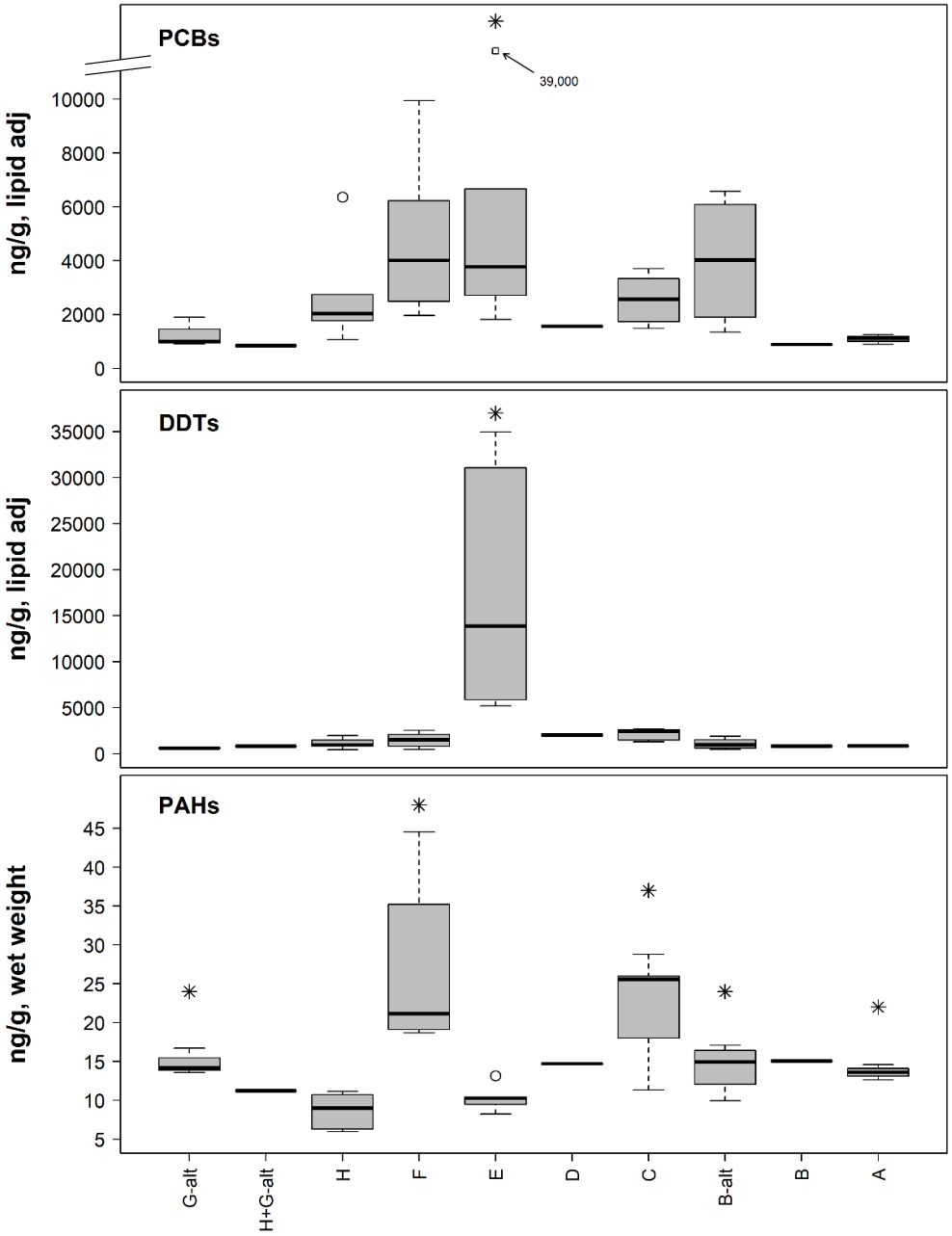


Figure S2. Measured contaminants [polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethanes (DDTs), and polycyclic aromatic hydrocarbons (PAHs)] in tissues of UWR juvenile Chinook salmon by sampling site; *p<0.05, site H as the upstream reference



Sum of (top) 45 PCBs (ng/g lipid adjusted) (middle) 6 DDTs (ng/g lipid adjusted) and (bottom) 42 PAHs and PAH homologues measured (ng/g wet weight). Open square for site E denotes outlier beyond y-axis. G-alt/H = A single tissue composite created by combining fish collected at sites H and G-alt to reach the requisite mass requirements for TBT analysis in addition to POPs and PAHs

Figure S3. Measured polycyclic aromatic hydrocarbons (PAHs) in tissues of UWR juvenile Chinook salmon by sampling site, adjusted for percent lipid, *p<0.05, site G-alt as the upstream reference

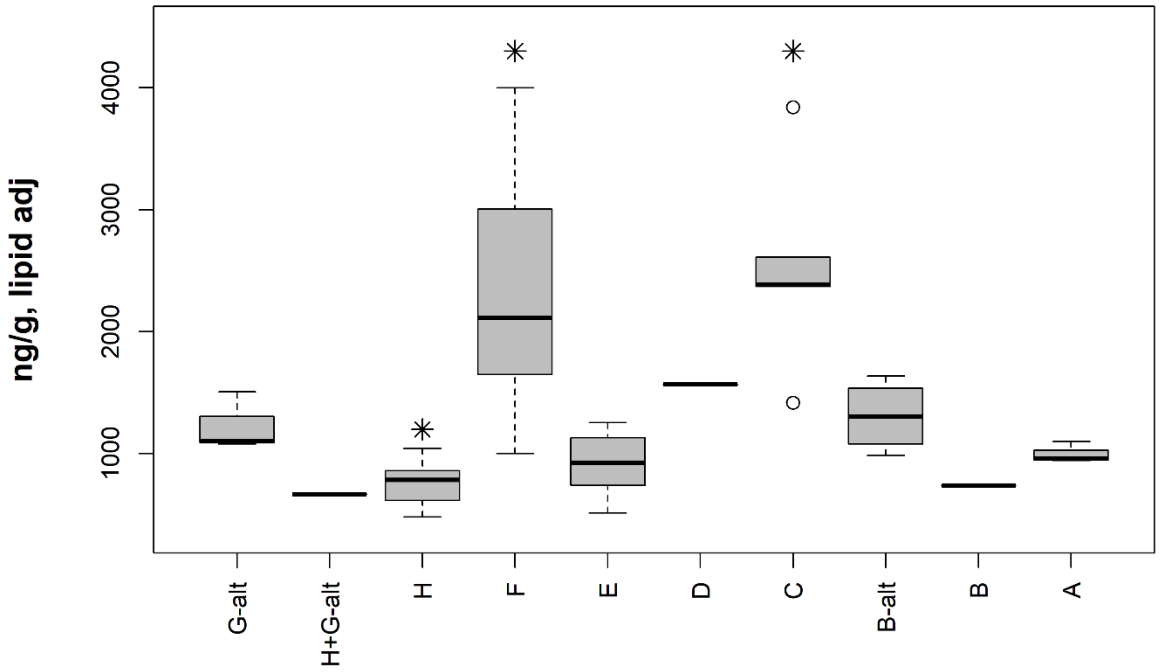


Figure S4. Contaminants with loadings greater than absolute value of 0.3 for PC2 and PC3 by site, represented by hexa- through nonachlorinated PCBs (ng/g lipid adjusted), alkylated PAHs (ng/g, wet weight), and parent PAHs (ng/g, wet weight)

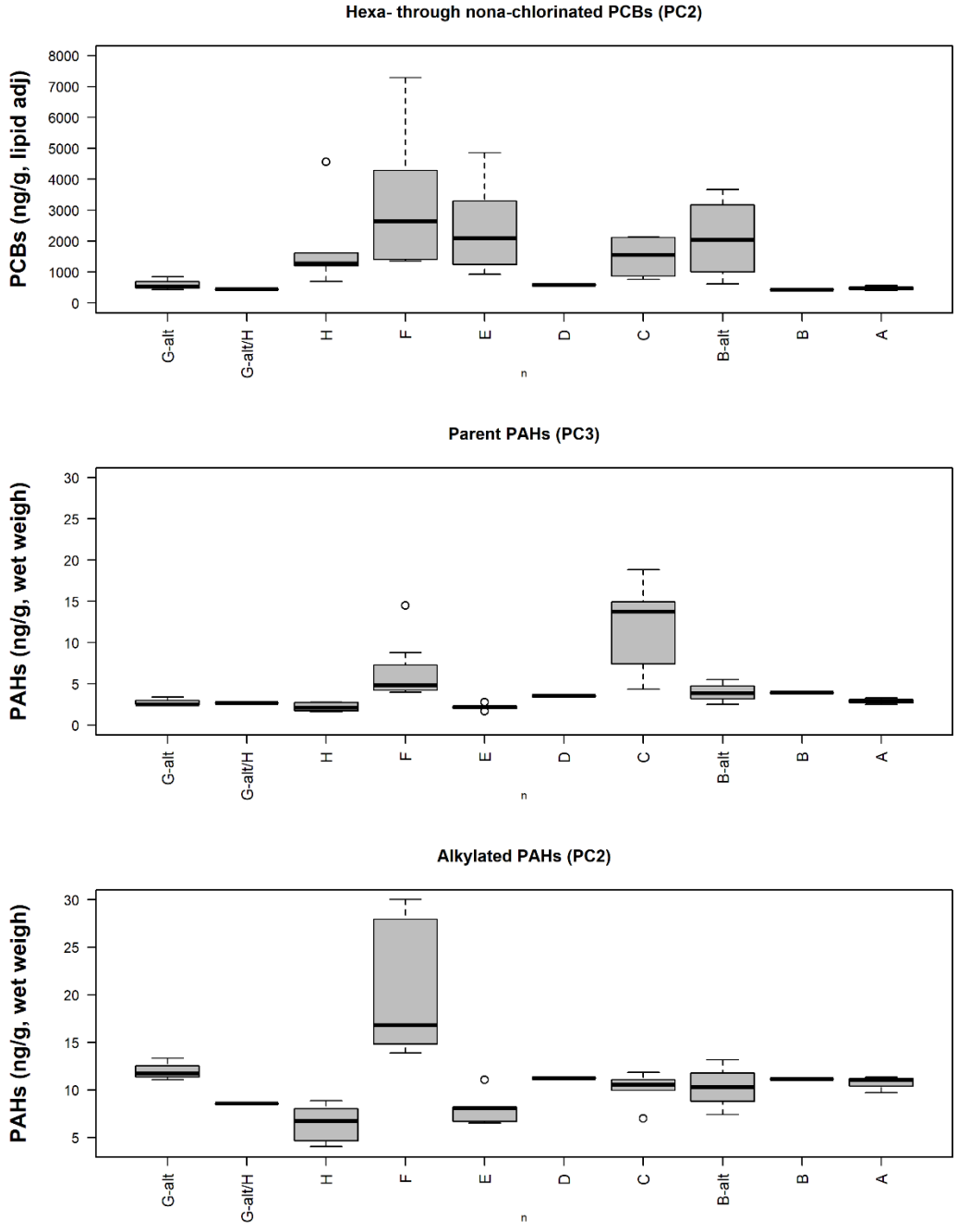
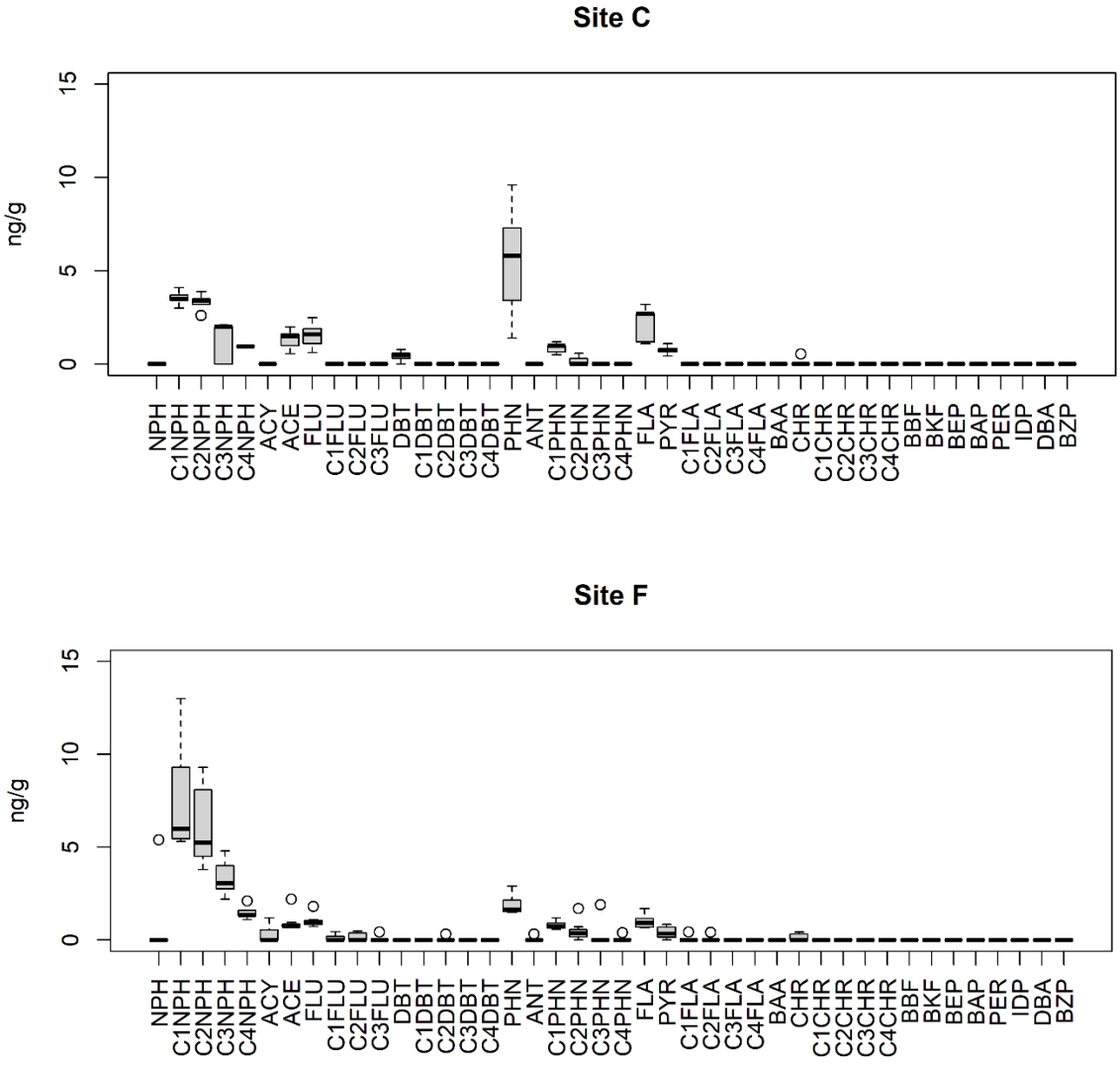


Figure S5. Polycyclic aromatic hydrocarbon (PAH) profiles of whole body tissues of juvenile Chinook salmon sampled at sites F and C



Supporting Text

Genetic analysis for stock assignment of individual fish

Genomic DNA was extracted from field-collected juvenile fin tissue and amplified for 192 single nucleotide polymorphism (SNP) markers using established methods.[2] SNP sequencing was carried out using a Miseq (Illumina) platform and genotypes were generated using custom perl scripts developed from Campbell et al. (2015).[2] *S*tandard GSI (Genetic Stock Identification) methods[3, 4] were then used to assign individuals to their genetic stock of origin. A genetic baseline for Chinook salmon compiled from the FishGen database (www.fishgen.net) consisting of 79 populations and 185 SNP loci[5] was used. Stock assignments of individual fish were made using the computer program ONCOR,[6] which employs the likelihood model of Rannala and Mountain.[7] Allocations to individual baseline populations were summed to estimate contributions of regional genetic stock groups. Ten genetic reporting groups for Chinook salmon were used (Willamette River spring, West Cascade spring, West Cascade fall, Spring Creek Group spring, Middle/Upper Columbia spring, Snake River spring, Deschutes River fall, Upper Columbia River summer/fall, Snake River fall, Rogue River) representing known genetic lineages within the Columbia River basin, as described in Teel et al.[8] excluding Washington and the Oregon Coast. Power analyses indicate that the 185 locus SNP baseline can be used to estimate the proportions of Columbia River basin stock groups in estuary mixtures of 200 fish. From this the assignments have >99% accuracy for Upper Willamette River spring, and 98% accuracy for other stocks (with the exception of Mid and Upper Columbia River Spring at 93%), similar to the findings in Hess et al. (2014).[5] Individuals with an assignment probability of 0.8 have been shown to have a 98% accuracy of stock assignment.[9] For this study, an assignment probability of 0.8 or greater was used to assign a fish to a designated genetic stock group.[9] Fish falling below this probability cutoff were classified as unassigned.

Analytical Chemistry

The target analyte list includes 45 PCBs (PCBs 17, 18, 28, 31, 33, 44, 49, 52, 66, 70, 74, 82, 87, 95, 99, 101/90 (co-elute), 105, 110, 118, 128, 138/163/164 (co-elute), 149, 151, 153/132 (co-elute), 156, 158, 170/190 (co-elute), 171, 177, 180, 183, 187/182/159 (co-elute; grouped with the 7 chlorine homologues), 191, 194, 195, 196, 199, 200, 201, 202, 205, 206, 207, 208, and 209) and six DDTs (o,p'-DDD; o,p'-DDE; o,p'-DDT; p,p'-DDD; p,p'-DDE; p,p'-DDT). Six analytes (PCBs 191, 200, 205, 207, 208, 209) were less than the lower limit of quantitation (LOQ) for more than 80% of the samples and were therefore excluded (i.e., assigned a value of zero). For all retained analytes below the quantitation limit, a value of half the lower LOQ was used.

Sum "low molecular weight PAHs" (sum LMWAHs) was calculated by summing the concentrations of naphthalene, C1- through C4-naphthalenes, acenaphthylene, acenaphthene, fluorene, C1- through C3-fluorenes, anthracene, phenanthrene, C1- through C4-phenanthrenes/anthracenes, dibenzothiophene, and C1-through C4-dibenzothiophenes. Sum "high molecular weight PAHs" (sum HMWAHs) was calculated by summing the concentrations of fluoranthene, pyrene, C1- through C4-fluoranthenes/pyrenes, benz[*a*]-anthracene, chrysene + triphenylene (coelute), C1- through C4-chrysenes/ benz[*a*]anthracenes, benzo[*b*]fluoranthene, benzo[*j+k*]fluoranthenes (coelute), benzo[*e*]pyrene, benzo[*a*]pyrene, perylene, indeno[1,2,3-*cd*]pyrene, dibenz[*a,h+a,c*]anthracene (coelute), and benzo[*ghi*]perylene. Total PAH concentrations were calculated by summing the levels of sum LMWAHs and sum HMWAHs. Concentrations of zero were assigned to analytes below the LOQ, or individual parent PAHs or alkylated homologue groups that were lower than three times the level measured in a method blank analyzed in the same sample set.

TBT analysis and results

TBT analysis was conducted on the six high-mass composite samples at ALS Environmental (Kelso, WA) using standard operating procedures. In brief, samples were analyzed using solvent extraction, derivatization, and extract cleanup by elution through alumina and silica columns, and instrumental analysis by GC Flame Photometric Detector. Analyses were performed according to the laboratory's National Environmental Laboratory Accreditation Program, or NELAP, approved quality assurance program (www.alsglobal.com).

Although analysis for TBTs was limited due to sample mass availability, two of the three samples from site F in central Portland Harbor were above the limit of detection (LOD; range = 1.5 – 2.1) at 2.1 and 1.9 ng/g ww (Table S12). TBT was not detected in a third sample from site F or any other samples analyzed in this study.

Methods/quality assurance for fish tissue and stomach content composites analyzed at NOAA's Northwest Fisheries Science Center (Seattle, WA) for levels of PCBs, DDTs, and PAHs using a gas chromatography/mass spectrometry method (GC/MS).[10, 11]

As part of the NWFSC's performance-based quality assurance program, a solvent (dichloromethane) method blank and two National Institute of Standards and Technology (NIST) Standard Reference Materials (fish tissue SRM 1947 for POPs and mussel tissue SRM 1974c for PAHs) were analyzed with each sample batch. The percent recoveries of the surrogate standard in the field and associated quality assurance samples met established laboratory criteria (between 60 to 130%), with values ranging from 94% to 119% for the POPs and 78% to 129% for the PAHs. Method blanks did not have any POPs and only a single PAH that exceeded two times the LOQ for each analyte; the laboratory QA criteria specify that no more than 5 POPs and 5 PAHs (excluding NPH and alkylated NPHs) should be more than twice the LOQ for each analyte. The LOQs ranged from < 0.03 to < 0.30 ng/g wet weight for the PCBs and DDTs, and < 0.09 to < 1.2 ng/g wet weight for the PAHs. For each sample set, the reference materials met laboratory QA criteria, with SRM 1947 having 98% of analytes within the acceptable range of values, and SRM 1974c having 73-80% of analytes within the acceptable range; the laboratory criteria state that concentrations of ≥ 70% of individual analytes with certified values measured in the NIST SRMs were within 30% of either end of the 95% confidence interval range of the published NIST certified concentrations. Replicate field samples could not be analyzed for these sample batches due to limited sample mass; however, if the reference materials are evaluated as replicates, the SRMs passed our laboratory QA criteria for replicate analyses (RSDs of analytes with concentrations > 1 ng/g wet weight should be ≤ 15%). Other quality control measures (e.g. continuing calibration) also met established laboratory criteria.[12]

Site-specific contamination profiles

Whole body tissue concentrations of PCBs, DDTs, and PAHs generally reflected site-specific contamination profiles despite uncertainty in arrival and residency timing at different locations (Figure 4). The proportions of DDT isomers, PCB homologues, and PAH compounds in the fish tissues varied by site. Homologue profiles indicate a significantly high proportion of tetrachlorinated PCBs at west bank site E (22% of the summed concentration of all PCBs measured) relative to both upstream reference sites (sites G-alt and H each < 10%), and heptachlorinated PCBs at east bank site F (23%), relative to west bank site G-alt upstream of Portland Harbor (11%, respectively) (Table S10; $p<0.05$). The isomers of DDTs also provide an indication of DDT source. The DDT formulation for industrial production and

application was enriched in *p,p'*-DDT, thereby showing an increase in the *o,p'*-isomer ratio.[13] A high proportion of *p,p'*-DDT at site E within Portland Harbor (15% of the summed concentration of all 6 DDT isomers; $p<0.05$ relative to each upstream reference site), with an *o,p'*-isomer ratio of 47%, indicates an industrial production or storage source of DDT (Table S11). No other site had a proportion of *p,p'*-DDT greater than 10%, or *o,p'*-isomer ratio greater than 33%. Tissue mean sum PAH concentrations were statistically higher at east bank site F (mean, 27 ng/g ww) and site C (mean, 22 ng/g ww) relative to the upstream reference sites ($p<0.05$; east bank site H, 9 ng/g ww; west bank G-alt, 15 ng/g ww) (Figure 4, Table S8). However, the composition of PAH compounds at these two sites varied. The LMWAHs, particularly alkylated compounds (Figure S5, Table S8, $p<0.05$ relative to each upstream reference site) dominated at site F, whereas at site C phenanthrene and fluoranthene were the predominant PAHs ($p<0.05$ relative to each upstream reference site). Overall, the differences in composition of DDTs, PCBs, and PAHs in fish tissues across sites indicate a spatial difference in the mixture of compounds, likely related to different sources and transport patterns of the contaminants.

References

1. Burnham, K. P.; Anderson, D. R., A practical information-theoretic approach. Model Selection and Multimodel Inference, 2nd ed. Springer, New York 2002, 2.

2. Campbell, N. R.; Harmon, S. A.; Narum, S. R., Genotyping-in-Thousands by sequencing (GT-seq): A cost effective SNP genotyping method based on custom amplicon sequencing. Molecular Ecology Resources 2015, 15, (4), 855-867.

3. Anderson, E. C.; Waples, R. S.; Kalinowski, S. T., An improved method for predicting the accuracy of genetic stock identification. Canadian Journal of Fisheries and Aquatic Sciences 2008, 65, (7), 1475-1486.

4. Manel, S.; Gaggiotti, O. E.; Waples, R. S., Assignment methods: matching biological questions with appropriate techniques. Trends Ecol Evol 2005, 20, (3), 136-142.

5. Hess, J. E.; Whiteaker, J. M.; Fryer, J. K.; Narum, S. R., Monitoring Stock-Specific Abundance, Run Timing, and Straying of Chinook Salmon in the Columbia River Using Genetic Stock Identification (GSI). North American Journal of Fisheries Management 2014, 34, (1), 184-201.

6. Kalinowski, S. T.; Manlove, K. R.; Tapper, M. L., ONCOR. Department of Ecology, Montana State University Bozeman MT 59717 2007.

7. Rannala, B.; Mountain, J. L., Detecting immigration by using multilocus genotypes. Proceedings of the National Academy of Sciences 1997, 94, (17), 9197-9201.

8. Teel, D. J.; Bottom, D. L.; Hinton, S. A.; Kuligowski, D. R.; McCabe, G. T.; McNatt, R.; Roegner, G. C.; Stamatiou, L. A.; Simenstad, C. A., Genetic Identification of Chinook Salmon in the Columbia River Estuary: Stock-Specific Distributions of Juveniles in Shallow Tidal Freshwater Habitats. N Am J Fish Manage 2014, 34, (3), 621-641.

9. Moran, P.; Bromaghin, J. F.; Masuda, M., Use of genetic data to infer population-specific ecological and phenotypic traits from mixed aggregations. PLOS ONE 2014, 9, (6), e98470.

10. Sloan, C. A.; Anulacion, B. F.; Baugh, K. A.; Bolton, J. L.; Boyd, D.; Boyer, R. H.; Burrows, D. G.; Herman, D. P.; Pearce, R. W.; Ylitalo, G. M., Northwest Fisheries Science Center's analyses of tissue, sediment, and water samples for organic contaminants by gas chromatography/mass spectrometry and analyses of tissue for lipid classes by thin layer chromatography/flame ionization detection. NMFS-NWFSC-125. U.S. Dept. Commer., NOAA Tech. Memo. Seattle, Washington, USA. 2014.

11. Sloan, C. A.; Brown, D. W.; Pearce, R. W.; Boyer, R. H.; Bolton, J. L.; Burrows, D. G.; Herman, D. P.; Krahn, M. M., Extraction, Cleanup, and Gas Chromatography/Mass Spectrometry Analysis of Sediments and Tissues for Organic Contaminants. NMFS-NWFSC-59. U.S. Dept. Commer., NOAA Tech. Memo. Seattle, Washington, USA. 2004.

12. Sloan, C. A.; Anulacion, B. F.; Baugh, K. A.; Bolton, J. L.; Boyd, D.; Chittaro, P. M.; da Silva, D. A. M.; Gates, J. B.; Sanderson, B. L.; Veggerby, K.; Ylitalo, G. M., Quality Assurance Plan for Analyses of Environmental Samples for Polycyclic Aromatic Hydrocarbons, Persistent Organic Pollutants, Dioctyl Sulfosuccinate, Estrogenic Compounds, Steroids, Hydroxylated Polycyclic Aromatic Hydrocarbons, Stable

Isotope Ratios, and Lipid Classes. U.S. Department of Commerce, NOAA Technical Memorandum NMFS-NWFSC-147. https://doi.org/10.25923/kf28-n618. 2019.

13. Beckvar, N.; Lotufo, G. R. DDT and other organohalogen pesticides in aquatic organisms. Environmental Contaminants in Biota: Interpreting Tissue Concentration, 2nd ed.; CRC Press: Boca Raton, Florida, 2011; 23, pp 47-101, DOI: 10.1201/b10598-3.