Earthjustice

Please see attached attachments to Earthjustice Comment Letter, part 3 of 3.

Analytical Resources, Incorporated
Analytical Chemists and Consultants

August 3, 2016

Cynthia Trout
Department of the Army
Directoret of Public Works
2012 Liggett Avenue, Box 339500 M/S-17
Joint Base Lewis McChord, WA 98433

RE: Client Project: Solo Point WWTP NPDES Quarterly
ARI Job Nos. BDK7

Dear Cynthia:

Please find enclosed the original Chain-of-Custody record (COC), sample receipt documentation, and the final data for the project referenced above. Analytical Resources, Inc. (ARI) accepted six samples in good condition on July 14, 2016. For further details regarding sample receipt please refer to the enclosed Cooler Receipt Form.

The samples were analyzed for Total Metals, Cyanide, Phenol, PBDE, NWTPH-Gx and NWTPH-Dx, as requested. The Low Level Mercury was reported under ARI SDG BDM6.

The TPHD surrogate recovery for Influent Grab 1 dilution is out of control low.

An electronic copy of this report and all supporting raw data will remain on file with ARI. Should you have any questions or problems, please feel free to contact me at your convenience.

Respectfully,
ANALYTICAL RESOURCES, INC.

Amanda Volgardsen
-for-
Kelly Bottem
Client Services Manager
(206) 695-6211
kellyb@arilabs.com
www.arilabs.com

eFile: BDK7

Enclosures

4611 South 134th Place, Suite 100 • Tukwila WA 98168 • **206-695-6200** • **206-695-6221** fax

Chain of Custody Record & Laboratory Analysis Request

ARI Assigned Number: BDK7	Turn-around Requested: Standard	Date:	
Joint Base Lewis-McChord	Phone:	Page: 1 of 1	
Cynthia Trout 253-966-1768 Raul Nino 253-477-2806		No. of Coolers:	Cooler Temps: 5.7
Client Project Name: Solo Point WWTP NPDES Quarterly			
Client Project #:	Samplers: Raul Nino		

Analytical Resources, Incorporated
Analytical Chemists and Consultants
4611 South 134th Place, Suite 100
Tukwila, WA 98168
206-695-6200 206-695-6201 (fax)

Sample ID	Date	Time	Matrix	No. Containers	3004L Total Cyanide	3004AH Phenol	3003B NWTPH-Gx	3003F NWTPH-Dx	3005AB Low Level Mercury	3005M *Metals	5003C PDBE Congeners		Notes/Comments
					Analysis Requested								
Influent Composite	7/14/16	1145	W	1	-----	-----	-----	-----	X	X	-----	not	Use TO-39 for 3005M
Influent Grab 1	"	1630	W	8	X	X	X	X	-----	-----	X	used	sample, all others use
Influent Grab 2	"	1645	W	2	X	X	-----	-----	-----	-----	-----	not	TO-38 - LR
Effluent Composite	"	1155	W	1	-----	-----	-----	-----	X	X	-----	used	
Effluent Grab 1	"	1650	W	8	X	X	X	X	-----	-----	X	not	
Effluent Grab 2	"	1710 RW 7/14/16	W	2	X	X	-----	-----	-----	-----	-----	used	
Nothing Approved Below this Line													

Comments/Special Instructions	Relinquished by: (Signature)	Received by: (Signature)	Relinquished by: (Signature)	Received by: (Signature)
	Printed Name: Raul J. Nino	Printed Name: Justin Mays	Printed Name:	Printed Name:
	Company: Nersan	Company: ARI	Company:	Company:
	Date & Time: 7/14/16 1830	Date & Time: 7-14-16 1830	Date & Time:	Date & Time:

Limits of Liability: *ARI will perform all requested services in accordance with appropriate methodology following ARI Standard Operating Procedures and the ARI Quality Assurance Program. This program meets standards for the industry. The total liability of ARI, its officers, agents, employees, or successors, arising out of or in connection with the requested services, shall not exceed the Invoiced amount for said services. The acceptance by the client of a proposal for services by ARI release ARI from any liability in excess thereof, not withstanding any provision to the contrary in any contract, purchase order or co-signed agreement between ARI and the Client.*

Sample Retention Policy: Unless specified by workorder or contract, all water/soil samples submitted to ARI will be discarded or returned, no sooner than 90 days after receipt or 60 days after submission of hardcopy data, whichever is longer. Sediment samples submitted under PSDDA/PSEP/SMS protocol will be stored frozen for up to one year and then discarded.

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Analytical Chemists and Consultants

Cooler Receipt Form

ARI Client: JBLM

Project Name: Solo Pt WWTP NPDES Quarterly

COC No(s): _____ NA

Delivered by: Fed-Ex UPS Courier Hand Delivered Other: _____

Assigned ARI Job No: BDK7

Tracking No: _____ NA

Preliminary Examination Phase:

Were intact, properly signed and dated custody seals attached to the outside of to cooler?	YES	NO
Were custody papers included with the cooler?	YES	NO
Were custody papers properly filled out (ink, signed, etc.)	YES	NO

Temperature of Cooler(s) (°C) (recommended 2.0-6.0 °C for chemistry) 5.7

Time: _____

If cooler temperature is out of compliance fill out form 00070F

Temp Gun ID#: D005276

Cooler Accepted by: JM Date: 7-14-16 Time: 1830

Complete custody forms and attach all shipping documents

Log-In Phase:

Was a temperature blank included in the cooler?		YES	NO
What kind of packing material was used? ... Bubble Wrap Wet Ice Gel Packs Baggies Foam Block Paper Other: _____			
Was sufficient ice used (if appropriate)?	NA	YES	NO
Were all bottles sealed in individual plastic bags?		YES	NO
Did all bottles arrive in good condition (unbroken)?		YES	NO
Were all bottle labels complete and legible?		YES	NO
Did the number of containers listed on COC match with the number of containers received?		YES	NO
Did all bottle labels and tags agree with custody papers?		YES	NO
Were all bottles used correct for the requested analyses?		YES	NO
Do any of the analyses (bottles) require preservation? (attach preservation sheet, excluding VOCs)...	NA	YES	NO
Were all VOC vials free of air bubbles?	NA	YES	NO
Was sufficient amount of sample sent in each bottle?		YES	NO
Date VOC Trip Blank was made at ARI	NA		

Was Sample Split by ARI : NA YES Date/Time: _____ Equipment: _____ Split by: _____

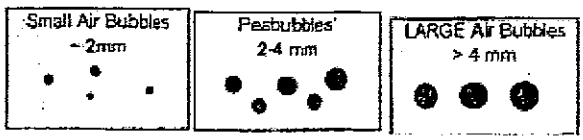
Samples Logged by: TR Date: 7-18-16 Time: 1006

*** Notify Project Manager of discrepancies or concerns ***

Sample ID on Bottle	Sample ID on COC	Sample ID on Bottle	Sample ID on COC

Additional Notes, Discrepancies, & Resolutions:

By: Date:



Small → "sm" (< 2 mm)
Peabubbles → "pb" (2 to < 4 mm)
Large → "lg" (4 to < 6 mm)
Headspace → "hs" (> 6 mm)

BDK7 : 00003

PRESERVATION VERIFICATION 07/18/16
Page 1 of 1

ANALYTICAL RESOURCES INCORPORATED

ARI Job No: **BDK7**

Inquiry Number: NONE
Analysis Requested: 07/18/16
Contact: Trout, Cynthia
Client: Joint Base Lewis McChord
Logged by: TR
Sample Set Used: Yes-481
Validatable Package: No
Deliverables:

PC: Kelly
VTSR: 07/14/16

Project #: #
Project: Solo Point WWTP NPDES Quarterly
Sample Site:
SDG No:
Analytical Protocol: In-house

LOGNUM ARI ID	CLIENT ID	CN >12	WAD >12	NH3 <2	COD <2	FOG <2	MET <2	PHEN <2	PHOS <2	TKN <2	NO23 <2	TOC <2	S2 >9	TPHD <2	BT <2	Fe2+ <2	DMET FLT	DOC FLT	PARAMETER	ADJUSTED TO	LOT NUMBER	AMOUNT ADDED	DATE/BY
16-10711 **BDK7A**	Influent Composite						TOT pass																
16-10712 **BDK7B**	Effluent Composite						TOT pass																
16-10713 **BDK7C**	Influent Grab 1	pass						pass															
16-10714 **BDK7D**	Effluent Grab 1	pass						pass															
16-10715 **BDK7E**	Influent Grab 2	pass						pass															
16-10716 **BDK7F**	Effluent Grab 2	pass						pass															

Checked By TR Date 7-18-16

Sample ID Cross Reference Report

ANALYTICAL RESOURCES INCORPORATED

ARI Job No: BDK7
Client: Joint Base Lewis McChord
Project Event: N/A
Project Name: Solo Point WWTP NPDES Quarterly

	Sample ID	ARI Lab ID	ARI LIMS ID	Matrix	Sample Date/Time	VTSR
1.	Influent Composite	BDK7A	16-10711	Water	07/14/16 11:45	07/14/16 18:30
2.	Effluent Composite	BDK7B	16-10712	Water	07/14/16 11:55	07/14/16 18:30
3.	Influent Grab 1	BDK7C	16-10713	Water	07/14/16 16:30	07/14/16 18:30
4.	Effluent Grab 1	BDK7D	16-10714	Water	07/14/16 16:50	07/14/16 18:30
5.	Influent Grab 2	BDK7E	16-10715	Water	07/14/16 16:45	07/14/16 18:30
6.	Effluent Grab 2	BDK7F	16-10716	Water	07/14/16 17:10	07/14/16 18:30

Analytical Resources, Incorporated
Analytical Chemists and Consultants

Data Reporting Qualifiers

Effective 12/31/13

Inorganic Data

U — Indicates that the target analyte was not detected at the reported concentration

* — Duplicate RPD is not within established control limits

B — Reported value is less than the CRDL but ≥ the Reporting Limit

N — Matrix Spike recovery not within established control limits

NA — Not Applicable, analyte not spiked

H — The natural concentration of the spiked element is so much greater than the concentration spiked that an accurate determination of spike recovery is not possible

L — Analyte concentration is ≤5 times the Reporting Limit and the replicate control limit defaults to ±1 RL instead of the normal 20% RPD

Organic Data

U — Indicates that the target analyte was not detected at the reported concentration

* — Flagged value is not within established control limits

B — Analyte detected in an associated Method Blank at a concentration greater than one-half of ARI's Reporting Limit or 5% of the regulatory limit or 5% of the analyte concentration in the sample.

J — Estimated concentration when the value is less than ARI's established reporting limits

D — The spiked compound was not detected due to sample extract dilution

E — Estimated concentration calculated for an analyte response above the valid instrument calibration range. A dilution is required to obtain an accurate quantification of the analyte.

Q Indicates a detected analyte with an initial or continuing calibration that does not meet established acceptance criteria (<20%RSD, <20%Drift or minimum RRF).

S Indicates an analyte response that has saturated the detector. The calculated concentration is not valid; a dilution is required to obtain valid quantification of the analyte

NA The flagged analyte was not analyzed for

NR Spiked compound recovery is not reported due to chromatographic interference

NS The flagged analyte was not spiked into the sample

M Estimated value for an analyte detected and confirmed by an analyst but with low spectral match parameters. This flag is used only for GC-MS analyses

N The analysis indicates the presence of an analyte for which there is presumptive evidence to make a "tentative identification"

Y The analyte is not detected at or above the reported concentration. The reporting limit is raised due to chromatographic interference. The Y flag is equivalent to the U flag with a raised reporting limit.

EMPC Estimated Maximum Possible Concentration (EMPC) defined in EPA Statement of Work DLM02.2 as a value "calculated for 2,3,7,8-substituted isomers for which the quantitation and /or confirmation ion(s) has signal to noise in excess of 2.5, but does not meet identification criteria" **(Dioxin/Furan analysis only)**

C The analyte was positively identified on only one of two chromatographic columns. Chromatographic interference prevented a positive identification on the second column

P The analyte was detected on both chromatographic columns but the quantified values differ by ≥40% RPD with no obvious chromatographic interference

X Analyte signal includes interference from polychlorinated diphenyl ethers. **(Dioxin/Furan analysis only)**

Z Analyte signal includes interference from the sample matrix or perfluorokerosene ions. **(Dioxin/Furan analysis only)**

Geotechnical Data

A The total of all fines fractions. This flag is used to report total fines when only sieve analysis is requested and balances total grain size with sample weight.

F Samples were frozen prior to particle size determination

SM Sample matrix was not appropriate for the requested analysis. This normally refers to samples contaminated with an organic product that interferes with the sieving process and/or moisture content, porosity and saturation calculations

SS Sample did not contain the proportion of "fines" required to perform the pipette portion of the grain size analysis

W Weight of sample in some pipette aliquots was below the level required for accurate weighting

ANALYTICAL RESOURCES INCORPORATED

ORGANICS ANALYSIS DATA SHEET
Volatiles by P&T GC/MS-Method SW8260C/NWTPHG **Sample ID: Influent Grab 1**
Page 1 of 1 **SAMPLE**

Lab Sample ID: BDK7C
LIMS ID: 16-10713
Matrix: Water
Data Release Authorized:
Reported: 07/27/16

QC Report No: BDK7-Joint Base Lewis McChord
Project: Solo Point WWTP NPDES Quarterly
#
Date Sampled: 07/14/16
Date Received: 07/14/16

Instrument/Analyst: NT2/LH
Date Analyzed: 07/23/16 13:45

Sample Amount: 10.0 mL
Purge Volume: 10.0 mL

CAS Number	Analyte	LOQ	Result	Q	TPHG ID
86290-81-5	**Gasoline Range Hydrocarbons**	0.10	0.12		GRO

Reported in mg/L (ppm)

Volatile Surrogate Recovery

d8-Toluene	98.8%
Bromofluorobenzene	104%

ANALYTICAL RESOURCES INCORPORATED

ORGANICS ANALYSIS DATA SHEET
Volatiles by P&T GC/MS-Method SW8260C/NWTPHG **Sample ID: Effluent Grab 1**
Page 1 of 1 **SAMPLE**

Lab Sample ID: BDK7D
LIMS ID: 16-10714
Matrix: Water
Data Release Authorized:
Reported: 07/27/16

QC Report No: BDK7-Joint Base Lewis McChord
Project: Solo Point WWTP NPDES Quarterly
#
Date Sampled: 07/14/16
Date Received: 07/14/16

Instrument/Analyst: NT2/LH
Date Analyzed: 07/23/16 14:06

Sample Amount: 10.0 mL
Purge Volume: 10.0 mL

CAS Number	Analyte	LOQ	Result	Q	TPHG ID
86290-81-5	Gasoline Range Hydrocarbons	0.10	< 0.10	U	---

Reported in mg/L (ppm)

Volatile Surrogate Recovery

d8-Toluene	97.0%
Bromofluorobenzene	98.2%

ANALYTICAL RESOURCES INCORPORATED

VOA SURROGATE RECOVERY SUMMARY

Matrix: Water

QC Report No: BDK7-Joint Base Lewis McChord
Project: Solo Point WWTP NPDES Quarterly #

ARI ID	Client ID	PV	DCE	TOL	BFB	DCB	TOT OUT
MB-072316A	Method Blank	10	NA	97.6%	96.6%	NA	0
LCS-072316A	Lab Control	10	NA	97.6%	98.4%	NA	0
LCSD-072316A	Lab Control Dup	10	NA	97.0%	100%	NA	0
BDK7C	Influent Grab 1	10	NA	98.8%	104%	NA	0
BDK7D	Effluent Grab 1	10	NA	97.0%	98.2%	NA	0

	LCS/MB LIMITS	QC LIMITS
SW8260C		
(DCE) = d4-1,2-Dichloroethane	(80-129)	(80-129)
(TOL) = d8-Toluene	(80-120)	(80-120)
(BFB) = Bromofluorobenzene	(80-120)	(80-120)
(DCB) = d4-1,2-Dichlorobenzene	(80-120)	(80-120)

Prep Method: SW5030B
Log Number Range: 16-10713 to 16-10714

ANALYTICAL RESOURCES INCORPORATED

ORGANICS ANALYSIS DATA SHEET
Volatiles by P&T GC/MS-Method SW8260C/NWTPHG **Sample ID: LCS-072316A**
Page 1 of 1 **LAB CONTROL SAMPLE**

Lab Sample ID: LCS-072316A
LIMS ID: 16-10713
Matrix: Water
Data Release Authorized:
Reported: 07/27/16

QC Report No: BDK7-Joint Base Lewis McChord
Project: Solo Point WWTP NPDES Quarterly
#
Date Sampled: NA
Date Received: NA

Instrument/Analyst LCS: NT2/LH
LCSD: NT2/LH
Date Analyzed LCS: 07/23/16 10:21
LCSD: 07/23/16 10:42

Sample Amount LCS: 10.0 mL
LCSD: 10.0 mL
Purge Volume LCS: 10.0 mL
LCSD: 10.0 mL

Analyte	LCS	Spike Added-LCS	LCS Recovery	LCSD	Spike Added-LCSD	LCSD Recovery	RPD
Gasoline Range Hydrocarbons	0.90	1.00	90.0%	0.82	1.00	82.0%	9.3%

Reported in mg/L (ppm)

RPD calculated using sample concentrations per SW846.

Volatile Surrogate Recovery

	LCS	LCSD
d8-Toluene	97.6%	97.0%
Bromofluorobenzene	98.4%	100%

ANALYTICAL RESOURCES INCORPORATED

ORGANICS ANALYSIS DATA SHEET
Volatiles by P&T GC/MS-Method SW8260C/NWTPHG **Sample ID: MB-072316A**

Lab Sample ID: MB-072316A
LIMS ID: 16-10713
Matrix: Water
Data Release Authorized:
Reported: 07/27/16

QC Report No: BDK7-Joint Base Lewis McChord
Project: Solo Point WWTP NPDES Quarterly #
Date Sampled: NA
Date Received: NA

Instrument/Analyst: NT2/LH
Date Analyzed: 07/23/16 11:43

Sample Amount: 10.0 mL
Purge Volume: 10.0 mL

CAS Number	Analyte	LOQ	Result	Q	TPHG ID
86290-81-5	Gasoline Range Hydrocarbons	0.10	< 0.10	U	---

Reported in mg/L (ppm)

Volatile Surrogate Recovery

d8-Toluene	97.6%
Bromofluorobenzene	96.6%

ANALYTICAL RESOURCES INCORPORATED

ORGANICS ANALYSIS DATA SHEET
TOTAL DIESEL RANGE HYDROCARBONS
NWTPHD by GC/FID
Extraction Method: SW3510C
Page 1 of 1

QC Report No: BDK7-Joint Base Lewis McChord
Project: Solo Point WWTP NPDES Quarterly
#

Matrix: Water

Date Received: 07/14/16

Data Release Authorized:
Reported: 08/01/16

ARI ID	Sample ID	Extraction Date	Analysis Date	EFV DF	Range/Surrogate	RL	Result
MB-072116	Method Blank	07/21/16	07/29/16	1.00	Diesel Range	0.10	< 0.10 U
16-10713	HC ID: ---		FID4A	1.0	Motor Oil Range	0.20	< 0.20 U
					o-Terphenyl		94.0%
BDK7C	Influent Grab 1	07/21/16	07/29/16	1.00	**Diesel Range**	**0.10**	**20 E**
16-10713	HC ID: **DRO/RRO**		FID4A	1.0	**Motor Oil Range**	**0.20**	**8.6**
					o-Terphenyl		55.5%
BDK7C DL	Influent Grab 1	07/21/16	07/29/16	1.00	**Diesel Range**	**1.0**	**18**
16-10713	HC ID: **DRO/RRO**		FID4A	10	**Motor Oil Range**	**2.0**	**8.9**
					o-Terphenyl		49.6%
BDK7D	Effluent Grab 1	07/21/16	07/29/16	1.00	**Diesel Range**	**0.10**	**0.41**
16-10714	HC ID: **DRO/RRO**		FID4A	1.0	**Motor Oil Range**	**0.20**	**1.1**
					o-Terphenyl		58.9%

Reported in mg/L (ppm)

EFV-Effective Final Volume in mL.
DL-Dilution of extract prior to analysis.
RL-Reporting limit.

Diesel range quantitation on total peaks in the range from C12 to C24.
Motor Oil range quantitation on total peaks in the range from C24 to C38.
HC ID: DRO/RRO indicates results of organics or additional hydrocarbons in ranges are not identifiable.

ANALYTICAL RESOURCES INCORPORATED

TPHD SURROGATE RECOVERY SUMMARY

Matrix: Water

QC Report No: BDK7-Joint Base Lewis McChord
Project: Solo Point WWTP NPDES Quarterly
#

Client ID	OTER	TOT OUT
MB-072116	94.0%	0
LCS-072116	91.0%	0
Influent Grab 1	55.5%	0
Influent Grab 1 DL	49.6%*	1
Effluent Grab 1	58.9%	0

	LCS/MB LIMITS	QC LIMITS
(OTER) = o-Terphenyl	(50-150)	(50-150)

Prep Method: SW3510C
Log Number Range: 16-10713 to 16-10714

ANALYTICAL RESOURCES INCORPORATED

ORGANICS ANALYSIS DATA SHEET
NWTPHD by GC/FID
Page 1 of 1

Sample ID: LCS-072116
LAB CONTROL

Lab Sample ID: LCS-072116
LIMS ID: 16-10713
Matrix: Water
Data Release Authorized:
Reported: 08/01/16

QC Report No: BDK7-Joint Base Lewis McChord
Project: Solo Point WWTP NPDES Quarterly
#
Date Sampled: NA
Date Received: NA

Date Extracted: 07/21/16
Date Analyzed: 07/29/16 13:32
Instrument/Analyst: FID4A/ML

Sample Amount: 500 mL
Final Extract Volume: 1.0 mL
Dilution Factor: 1.00

Range	Lab Control	Spike Added	Recovery
Diesel	2.28	3.00	76.0%

TPHD Surrogate Recovery

o-Terphenyl	91.0%

Results reported in mg/L

ANALYTICAL RESOURCES INCORPORATED

TOTAL DIESEL RANGE HYDROCARBONS-EXTRACTION REPORT

ARI Job: BDK7
Matrix: Water
Project: Solo Point WWTP NPDES Quarterly
Date Received: 07/14/16
#

ARI ID	Client ID	Samp Amt	Final Vol	Prep Date
16-10713-072116MB1	Method Blank	500 mL	1.00 mL	07/21/16
16-10713-072116LCS1	Lab Control	500 mL	1.00 mL	07/21/16
16-10713-BDK7C	Influent Grab 1	500 mL	1.00 mL	07/21/16
16-10714-BDK7D	Effluent Grab 1	500 mL	1.00 mL	07/21/16

ANALYTICAL RESOURCES INCORPORATED

INORGANICS ANALYSIS DATA SHEET
TOTAL METALS

Sample ID: Influent Composite SAMPLE

Lab Sample ID: BDK7A
LIMS ID: 16-10711
Matrix: Water
Data Release Authorized:
Reported: 07/29/16

QC Report No: BDK7-Joint Base Lewis McChord
Project: Solo Point WWTP NPDES Quarterly #
Date Sampled: 07/14/16
Date Received: 07/14/16

Prep Meth	Prep Date	Analysis Method	Analysis Date	CAS Number	Analyte	LOQ	µg/L	Q
200.8	07/19/16	200.8	07/28/16	**7440-38-2**	**Arsenic**	0.2	**1.2**	
200.8	07/19/16	200.8	07/28/16	**7440-43-9**	**Cadmium**	0.1	**0.3**	
200.8	07/19/16	200.8	07/28/16	**7440-47-3**	**Chromium**	0.5	**1.3**	
200.8	07/19/16	200.8	07/28/16	**7440-50-8**	**Copper**	0.5	**63.0**	
200.8	07/19/16	200.8	07/28/16	**7439-92-1**	**Lead**	0.1	**1.3**	
200.8	07/19/16	200.8	07/28/16	**7439-98-7**	**Molybdenum**	0.2	**1.1**	
200.8	07/19/16	200.8	07/28/16	**7440-02-0**	**Nickel**	0.5	**2.2**	
200.8	07/19/16	200.8	07/28/16	7782-49-2	Selenium	0.5	0.5	U
200.8	07/19/16	200.8	07/28/16	7440-22-4	Silver	0.2	0.2	U
200.8	07/19/16	200.8	07/28/16	**7440-66-6**	**Zinc**	4	**97**	

U-Analyte undetected at given LOQ
LOQ-Limit of Quantitation

ANALYTICAL RESOURCES INCORPORATED

INORGANICS ANALYSIS DATA SHEET
TOTAL METALS
Page 1 of 1

Sample ID: Effluent Composite
SAMPLE

Lab Sample ID: BDK7B
LIMS ID: 16-10712
Matrix: Water
Data Release Authorized:
Reported: 07/29/16

QC Report No: BDK7-Joint Base Lewis McChord
Project: Solo Point WWTP NPDES Quarterly
#
Date Sampled: 07/14/16
Date Received: 07/14/16

Prep Meth	Prep Date	Analysis Method	Analysis Date	CAS Number	Analyte	LOQ	µg/L	Q
200.8	07/19/16	200.8	07/28/16	**7440-38-2**	**Arsenic**	0.2	**1.0**	
200.8	07/19/16	200.8	07/28/16	7440-43-9	Cadmium	0.1	0.1	U
200.8	07/19/16	200.8	07/28/16	**7440-47-3**	**Chromium**	0.5	**0.6**	
200.8	07/19/16	200.8	07/28/16	**7440-50-8**	**Copper**	0.5	**30.8**	
200.8	07/19/16	200.8	07/28/16	**7439-92-1**	**Lead**	0.1	**0.4**	
200.8	07/19/16	200.8	07/28/16	**7439-98-7**	**Molybdenum**	0.2	**0.7**	
200.8	07/19/16	200.8	07/28/16	**7440-02-0**	**Nickel**	0.5	**1.8**	
200.8	07/19/16	200.8	07/28/16	7782-49-2	Selenium	0.5	0.5	U
200.8	07/19/16	200.8	07/28/16	7440-22-4	Silver	0.2	0.2	U
200.8	07/19/16	200.8	07/28/16	**7440-66-6**	**Zinc**	4	**45**	

U-Analyte undetected at given LOQ
LOQ-Limit of Quantitation

ANALYTICAL RESOURCES INCORPORATED

INORGANICS ANALYSIS DATA SHEET
TOTAL METALS
Sample ID: METHOD BLANK

Lab Sample ID: BDK7MB
LIMS ID: 16-10712
Matrix: Water
Data Release Authorized:
Reported: 07/29/16

QC Report No: BDK7-Joint Base Lewis McChord
Project: Solo Point WWTP NPDES Quarterly
#
Date Sampled: NA
Date Received: NA

Prep Meth	Prep Date	Analysis Method	Analysis Date	CAS Number	Analyte	LOQ	µg/L	Q
200.8	07/19/16	200.8	07/28/16	7440-38-2	Arsenic	0.2	0.2	U
200.8	07/19/16	200.8	07/28/16	7440-43-9	Cadmium	0.1	0.1	U
200.8	07/19/16	200.8	07/28/16	7440-47-3	Chromium	0.5	0.5	U
200.8	07/19/16	200.8	07/28/16	7440-50-8	Copper	0.5	0.5	U
200.8	07/19/16	200.8	07/28/16	7439-92-1	Lead	0.1	0.1	U
200.8	07/19/16	200.8	07/28/16	7439-98-7	Molybdenum	0.2	0.2	U
200.8	07/19/16	200.8	07/28/16	7440-02-0	Nickel	0.5	0.5	U
200.8	07/19/16	200.8	07/28/16	7782-49-2	Selenium	0.5	0.5	U
200.8	07/19/16	200.8	07/28/16	7440-22-4	Silver	0.2	0.2	U
200.8	07/19/16	200.8	07/28/16	7440-66-6	Zinc	4	4	U

U-Analyte undetected at given LOQ
LOQ-Limit of Quantitation

ANALYTICAL RESOURCES INCORPORATED

INORGANICS ANALYSIS DATA SHEET
TOTAL METALS
Page 1 of 1

Sample ID: LAB CONTROL

Lab Sample ID: BDK7LCS
LIMS ID: 16-10712
Matrix: Water
Data Release Authorized:
Reported: 07/29/16

QC Report No: BDK7-Joint Base Lewis McChord
Project: Solo Point WWTP NPDES Quarterly
#
Date Sampled: NA
Date Received: NA

BLANK SPIKE QUALITY CONTROL REPORT

Analyte	Analysis Method	Spike Found	Spike Added	% Recovery	Q
Arsenic	200.8	24.4	25.0	97.6%	
Cadmium	200.8	25.6	25.0	102%	
Chromium	200.8	25.4	25.0	102%	
Copper	200.8	26.5	25.0	106%	
Lead	200.8	28.3	25.0	113%	
Molybdenum	200.8	24.5	25.0	98.0%	
Nickel	200.8	25.8	25.0	103%	
Selenium	200.8	77.3	80.0	96.6%	
Silver	200.8	27.0	25.0	108%	
Zinc	200.8	81	80	101%	

Reported in µg/L

N-Control limit not met
Control Limits: 80-120%

ANALYTICAL RESOURCES INCORPORATED

ORGANICS ANALYSIS DATA SHEET
PBDE by GC/ECD Method SW8082
Extraction Method: SW3510C
Page 1 of 1

Sample ID: Influent Grab 1
SAMPLE

Lab Sample ID: BDK7C
LIMS ID: 16-10713
Matrix: Water
Data Release Authorized: MPG
Reported: 08/04/16

QC Report No: BDK7-Joint Base Lewis McChord
Project: Solo Point WWTP NPDES Quarterly #
Date Sampled: 07/14/16
Date Received: 07/14/16

Date Extracted: 07/21/16
Date Analyzed: 07/29/16 04:11
Instrument/Analyst: ECD9/JGR
GPC Cleanup: No
Silica Gel: Yes
Acid Cleanup: No

Sample Amount: 500 mL
Final Extract Volume: 1.0 mL
Dilution Factor: 1.00
Sulfur Cleanup: Yes

CAS Number	Analyte	LOQ	Result
5436-43-1	PBDE-47	0.010	< 0.010 U
189084-64-8	PBDE-100	0.010	< 0.010 U
60348-60-9	**PBDE-99**	**0.010**	**0.024 P**
207122-15-4	PBDE-154	0.010	< 0.010 U
68631-49-2	PBDE-153	0.010	< 0.010 U
41318-75-6	PBDE-28	0.010	< 0.010 U
189084-61-5	PBDE-66	0.010	< 0.010 U
1163-19-5	PBDE-209	0.050	< 0.050 U
2050-47-7	PBDE-15	0.010	< 0.010 U
189084-66-0	PBDE-119	0.010	< 0.010 U
147217-78-5	PBDE-33	0.010	< 0.010 U
446254-22-4	PBDE-46	0.010	< 0.010 U
189084-63-7	PBDE-75	0.010	< 0.010 U
35854-94-5	PBDE-155	0.010	< 0.010 U

Reported in µg/l (ppb)

PBDE Surrogate Recovery

2,2',3,3',4,4',5,6-Octachlorob	69.0%

FORM I

BDK7:00022

ANALYTICAL RESOURCES INCORPORATED

ORGANICS ANALYSIS DATA SHEET
PBDE by GC/ECD Method SW8082
Extraction Method: SW3510C
Page 1 of 1

Sample ID: Effluent Grab 1
SAMPLE

Lab Sample ID: BDK7D
LIMS ID: 16-10714
Matrix: Water
Data Release Authorized:
Reported: 08/03/16

QC Report No: BDK7-Joint Base Lewis McChord
Project: Solo Point WWTP NPDES Quarterly
#
Date Sampled: 07/14/16
Date Received: 07/14/16

Date Extracted: 07/21/16
Date Analyzed: 07/29/16 04:42
Instrument/Analyst: ECD9/JGR
GPC Cleanup: No
Silica Gel: Yes
Acid Cleanup: No

Sample Amount: 500 mL
Final Extract Volume: 1.0 mL
Dilution Factor: 1.00
Sulfur Cleanup: Yes

CAS Number	Analyte	LOQ	Result
5436-43-1	PBDE-47	0.010	< 0.010 U
189084-64-8	PBDE-100	0.010	< 0.010 U
60348-60-9	PBDE-99	0.010	< 0.010 U
207122-15-4	PBDE-154	0.010	< 0.010 U
68631-49-2	PBDE-153	0.010	< 0.010 U
41318-75-6	PBDE-28	0.010	< 0.010 U
189084-61-5	PBDE-66	0.010	< 0.010 U
1163-19-5	PBDE-209	0.050	< 0.050 U
2050-47-7	PBDE-15	0.010	< 0.010 U
189084-66-0	PBDE-119	0.010	< 0.010 U
147217-78-5	PBDE-33	0.010	< 0.010 U
446254-22-4	PBDE-46	0.010	< 0.010 U
189084-63-7	PBDE-75	0.010	< 0.010 U
35854-94-5	PBDE-155	0.010	< 0.010 U

Reported in µg/l (ppb)

PBDE Surrogate Recovery	
2,2',3,3',4,4',5,6-Octachlorob	63.8%

ANALYTICAL RESOURCES INCORPORATED

SW8082/PBDE SOIL/SOLID SURROGATE RECOVERY SUMMARY

Matrix: Water

QC Report No: BDK7-Joint Base Lewis McChord
Project: Solo Point WWTP NPDES Quarterly
#

Client ID	PCB-195	TOT OUT
MB-072116	80.2%	0
LCS-072116	70.6%	0
Influent Grab 1	69.0%	0
Effluent Grab 1	63.8%	0

	LCS/MB LIMITS	QC LIMITS
(PCB-195) = 2,2',3,3',4,4',5,6-Octachlor	(30-160)	(30-160)

Prep Method: SW3510C
Log Number Range: 16-10713 to 16-10714

ANALYTICAL RESOURCES INCORPORATED

ORGANICS ANALYSIS DATA SHEET
PBDE by GC/ECD Method SW8082

Sample ID: LCS-072116
LCS/LCSD

Lab Sample ID: LCS-072116
LIMS ID: 16-10713
Matrix: Water
Data Release Authorized:
Reported: 08/03/16

QC Report No: BDK7-Joint Base Lewis McChord
Project: Solo Point WWTP NPDES Quarterly
#
Date Sampled: NA
Date Received: NA

Date Extracted LCS/LCSD: 07/21/16
Date Analyzed LCS: 07/29/16 00:36
Instrument/Analyst LCS: ECD9/JGR
GPC Cleanup: No
Acid Cleanup: No
Sulfur Cleanup: Yes

Sample Amount LCS: 500 mL
Final Extract Volume LCS: 1.0 mL
Dilution Factor LCS: 1.00
Silica Gel: Yes
Percent Moisture: NA

Analyte	Lab Control	Spike Added	Recovery
PBDE-209	0.286 P	0.250	114%
PBDE-28	0.074	0.100	74.0%
PBDE-47	0.071	0.100	71.0%
PBDE-66	0.073	0.100	73.0%
PBDE-99	0.092	0.100	92.0%
PBDE-100	0.075	0.100	75.0%
PBDE-153	0.072	0.100	72.0%
PBDE-154	0.077	0.100	77.0%

PBDE Surrogate Recovery

2,2',3,3',4,4',5,6-Octach	70.6%

Results reported in µg/l (ppb)
RPD calculated using sample concentrations per SW846.

ANALYTICAL RESOURCES INCORPORATED

ORGANICS ANALYSIS DATA SHEET
PBDE by GC/ECD Method SW8082 **Sample ID: MB-072116**
Extraction Method: SW3510C **METHOD BLANK**

Lab Sample ID: MB-072116
LIMS ID: 16-10713
Matrix: Water
Data Release Authorized:
Reported: 08/03/16

QC Report No: BDK7-Joint Base Lewis McChord
Project: Solo Point WWTP NPDES Quarterly
#
Date Sampled: NA
Date Received: NA

Date Extracted: 07/21/16
Date Analyzed: 07/29/16 00:06
Instrument/Analyst: ECD9/JGR
GPC Cleanup: No
Silica Gel: Yes
Acid Cleanup: No

Sample Amount: 500 mL
Final Extract Volume: 1.0 mL
Dilution Factor: 1.00
Sulfur Cleanup: Yes

CAS Number	Analyte	LOQ	Result
5436-43-1	PBDE-47	0.010	< 0.010 U
189084-64-8	PBDE-100	0.010	< 0.010 U
60348-60-9	PBDE-99	0.010	< 0.010 U
207122-15-4	PBDE-154	0.010	< 0.010 U
68631-49-2	PBDE-153	0.010	< 0.010 U
41318-75-6	PBDE-28	0.010	< 0.010 U
189084-61-5	PBDE-66	0.010	< 0.010 U
1163-19-5	PBDE-209	0.050	< 0.050 U
2050-47-7	PBDE-15	0.010	< 0.010 U
189084-66-0	PBDE-119	0.010	< 0.010 U
147217-78-5	PBDE-33	0.010	< 0.010 U
446254-22-4	PBDE-46	0.010	< 0.010 U
189084-63-7	PBDE-75	0.010	< 0.010 U
35854-94-5	PBDE-155	0.010	< 0.010 U

Reported in µg/l (ppb)

PBDE Surrogate Recovery	
2,2',3,3',4,4',5,6-Octachlorob	80.2%

SAMPLE RESULTS-CONVENTIONALS
BDK7-Joint Base Lewis McChord

ANALYTICAL RESOURCES INCORPORATED

Matrix: Water
Data Release Authorized: W
Reported: 07/25/16

Project: Solo Point WWTP NPDES Quarte
Event: NA
Date Sampled: 07/14/16
Date Received: 07/14/16

Client ID: Influent Grab 1
ARI ID: 16-10713 BDK7C

Analyte	Date Batch	Method	Units	RL	Sample
Total Cyanide	07/21/16 072116#1	EPA 335.4	mg/L	0.005	< 0.005 U
Phenols	07/19/16 071916#1	EPA 420.1	mg/L	0.04	0.23

RL Analytical reporting limit
U Undetected at reported detection limit

SAMPLE RESULTS-CONVENTIONALS
BDK7-Joint Base Lewis McChord

ANALYTICAL RESOURCES INCORPORATED

Matrix: Water
Data Release Authorized:
Reported: 07/25/16

Project: Solo Point WWTP NPDES Quarte
Event: NA
Date Sampled: 07/14/16
Date Received: 07/14/16

Client ID: Effluent Grab 1
ARI ID: 16-10714 BDK7D

Analyte	Date Batch	Method	Units	RL	Sample
Total Cyanide	07/21/16 072116#1	EPA 335.4	mg/L	0.005	0.013
Phenols	07/19/16 071916#1	EPA 420.1	mg/L	0.04	< 0.04 U

RL Analytical reporting limit
U Undetected at reported detection limit

SAMPLE RESULTS-CONVENTIONALS
BDK7-Joint Base Lewis McChord

ANALYTICAL RESOURCES INCORPORATED

Matrix: Water
Data Release Authorized: W
Reported: 07/25/16

Project: Solo Point WWTP NPDES Quarte
Event: NA
Date Sampled: 07/14/16
Date Received: 07/14/16

Client ID: Influent Grab 2
ARI ID: 16-10715 BDK7E

Analyte	Date Batch	Method	Units	RL	Sample
Total Cyanide	07/21/16 072116#1	EPA 335.4	mg/L	0.005	< 0.005 U
Phenols	07/19/16 071916#1	EPA 420.1	mg/L	0.04	0.08

RL Analytical reporting limit
U Undetected at reported detection limit

SAMPLE RESULTS-CONVENTIONALS
BDK7-Joint Base Lewis McChord

ANALYTICAL RESOURCES INCORPORATED

Matrix: Water
Data Release Authorized: LJ
Reported: 07/25/16

Project: Solo Point WWTP NPDES Quarte
Event: NA
Date Sampled: 07/14/16
Date Received: 07/14/16

Client ID: Effluent Grab 2
ARI ID: 16-10716 BDK7F

Analyte	Date Batch	Method	Units	RL	Sample
Total Cyanide	07/21/16 072116#1	EPA 335.4	mg/L	0.005	0.006
Phenols	07/19/16 071916#1	EPA 420.1	mg/L	0.04	< 0.04 U

RL Analytical reporting limit
U Undetected at reported detection limit

METHOD BLANK RESULTS-CONVENTIONALS
BDK7-Joint Base Lewis McChord

ANALYTICAL RESOURCES INCORPORATED

Matrix: Water
Data Release Authorized:
Reported: 07/25/16

Project: Solo Point WWTP NPDES Quarte
Event: NA
Date Sampled: NA
Date Received: NA

Analyte	Method	Date	Units	Blank	ID
Total Cyanide	EPA 335.4	07/21/16	mg/L	< 0.005 U	
Phenols	EPA 420.1	07/19/16	mg/L	< 0.04 U	

STANDARD REFERENCE RESULTS-CONVENTIONALS
BDK7-Joint Base Lewis McChord

ANALYTICAL RESOURCES INCORPORATED

Matrix: Water
Data Release Authorized: J
Reported: 07/25/16

Project: Solo Point WWTP NPDES Quarte
Event: NA
Date Sampled: NA
Date Received: NA

Analyte/SRM ID	Method	Date	Units	SRM	True Value	Recovery
Total Cyanide ERA 030314	EPA 335.4	07/21/16	mg/L	0.132	0.150	88.0%
Phenols ERA #160814	EPA 420.1	07/19/16	mg/L	0.51	0.50	102.0%

REPLICATE RESULTS-CONVENTIONALS
BDK7-Joint Base Lewis McChord

ANALYTICAL RESOURCES INCORPORATED

Matrix: Water
Data Release Authorized:
Reported: 07/25/16

Project: Solo Point WWTP NPDES Quarte
Event: NA
Date Sampled: 07/14/16
Date Received: 07/14/16

Analyte	Method	Date	Units	Sample	Replicate(s)	RPD/RSD
ARI ID: BDK7C Client ID: Influent Grab 1						
Total Cyanide	EPA 335.4	07/21/16	mg/L	< 0.005	< 0.005	NA

MS/MSD RESULTS-CONVENTIONALS
BDK7-Joint Base Lewis McChord

ANALYTICAL RESOURCES INCORPORATED

Matrix: Water
Data Release Authorized: ✓
Reported: 07/25/16

Project: Solo Point WWTP NPDES Quarte
Event: NA
Date Sampled: 07/14/16
Date Received: 07/14/16

Analyte	Method	Date	Units	Sample	Spike	Spike Added	Recovery
ARI ID: BDK7C Client ID: Influent Grab 1							
Total Cyanide	EPA 335.4	07/21/16	mg/L	< 0.005	0.126	0.150	84.0%

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Chemical tracers guide identification of the location and source of persistent organic pollutants in juvenile Chinook salmon (*Oncorhynchus tshawytscha*), migrating seaward through an estuary with multiple contaminant inputs

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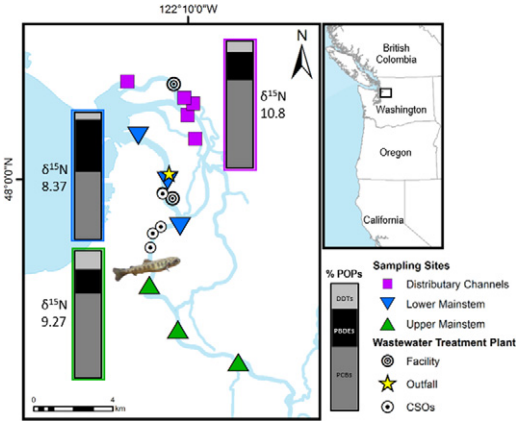
HIGHLIGHTS

- Three chemical tracers identified a contaminant source for seaward migrating salmon.
- Salmon collected near a wastewater outfall had higher contaminant concentrations.
- Salmon near the outfall had distinct combinations of contaminants (fingerprint).
- Altered $\delta^{15}N$ signatures were correlated with distinct contaminant fingerprints.
- Wastewater was the source for both distinct fingerprint and altered $\delta^{15}N$ signature.

GRAPHICAL ABSTRACT



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ABSTRACT

Understanding the spatial extent, magnitude, and source of contaminant exposure in biota is necessary to formulate appropriate conservation measures to reduce or remediate contaminant exposure. However, obtaining such information for migratory animals is challenging. Juvenile Chinook salmon (*Oncorhynchus tshawytscha*), a threatened species throughout the US Pacific Northwest, are exposed to persistent organic pollutants (POPs), including polybrominated diphenyl ether (PBDE) flame retardants and polychlorinated biphenyls (PCBs), in many developed rivers and estuaries. This study used three types of complementary chemical tracer data (contaminant concentrations, POP fingerprints, and stable isotopes), to determine the location and source of contaminant exposure for natural- and hatchery-origin Chinook salmon migrating seaward through a developed watershed with multiple contaminant sources. Concentration data revealed that salmon were exposed to and accumulated predominantly PBDEs and PCBs in the lower mainstem region of the river, with higher PBDEs in natural- than hatchery-origin fish but similar PCBs in both groups, associated with differences in contaminant inputs and/or habitat use. The POP fingerprints of the natural-origin-fish captured from this region were also distinct from other region and

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Wastewater
Stormwater

origin sample groups, with much higher proportions of PBDEs in the total POP concentration, indicating a different contaminant source or habitat use than the hatchery-origin fish. Stable isotopes, independent tracers of food sources and habitat use, revealed that natural-origin fish from this region also had depleted $\delta^{15}N$ signatures compared to other sample groups, associated with exposure to nutrient-rich wastewater. The PBDE-enhanced POP fingerprints in these salmon were correlated with the degree of depletion in nitrogen stable isotopes of the fish, suggesting a common wastewater source for both the PBDEs and the nitrogen. Identification of the location and source of contaminant exposure allows environmental managers to establish conservation measures to control contaminant inputs, necessary steps to improve the health of Chinook salmon and enhance their marine survival.

1. Introduction

An understanding of the spatial extent, magnitude, and source of contaminant exposure in biota is necessary to formulate appropriate conservation measures to reduce or remediate contaminant exposure. In some cases, there is an obvious point source of the contaminants, but in other instances the sources may be cryptic or dispersed, making them more difficult to identify and remediate. Obtaining such information for migratory animals is especially challenging because their routes may traverse habitats exposing them to different contaminants from multiple sources. Persistent organic pollutants (POPs) are contaminants of global concern because of their persistence, bioavailability, and toxicity (Jones and de Voogt, 1999). POPs include a wide variety of toxic chemicals, including polychlorinated biphenyls (PCBs), polybrominated diphenyl ether flame-retardants (PBDEs), and chlorinated pesticides such as dichlorodiphenyltrichloroethane and its metabolites (DDTs). All POPs are slowly metabolized, bioaccumulate in lipid-rich tissues, and biomagnify in the food web (Aguilar et al., 2002; Borrell et al., 2006; Jones and de Voogt, 1999; Tierney et al., 2014).

Proximity to contaminated habitats and the associated POPs in prey are the primary factors for determining the extent to which POPs are accumulated by fishes (Good et al., 2014; O'Neill and West, 2009; West et al., 2008) and marine mammals (Aguilar et al., 2002; Borrell et al., 2006). However, duration of exposure and body condition, including lipid content, reproductive status, and trophic position, can also affect accumulation (Aguilar et al., 1999; Burreau et al., 2006; Fisk et al., 2001; West et al., 2017). For migratory animals, the link between contaminated habitats and POP concentrations can be further obscured by multiple POP inputs (Borrell et al., 2006) as the animals move between habitats.

Although POPs can adversely affect animal health, the proportion of different types of POPs also serve as chemical tracers elucidating information about the trophic ecology, migration patterns, and population structure, for many migratory species (Ramos and González-Solís, 2012) including Atlantic salmon, *Salmo salar* (Svendsen et al., 2009), bluefin tuna, *Thunnus thynnus* (Deshpande et al., 2016a), harbor porpoise, *Phocoena phocoena* (Calambokidis and Barlow, 1991), beluga whales, *Delphinapterus leucas* (Krahn et al., 1999) and killer whales, *Orcinus orca* (Krahn et al., 2007). Additionally, the proportions of different types of POPs have been used to identify sources of POPs in Pacific herring, *Clupea pallasii* (West et al., 2008), bluefish *Pomatomus saltatrix* (Deshpande et al., 2016b), and bottlenose dolphins, *Tursiops truncatus* (Fair et al., 2010).

Current and historical inputs of POPs create environments with distinct chemical proportions or "fingerprints." Given sufficient foraging time, migratory animals accumulate POPs in proportion to their availability in the environments through which they migrate. Furthermore, unlike an individual POP concentration, POP fingerprints are less influenced by individual biological traits (Borrell et al., 2006; Dickhut et al., 2009; Svendsen et al., 2008), such that changes in POP fingerprints in animals along their migration route can indicate different inputs or sources of contaminants in prey along their migration route.

Naturally occurring stable isotopes of carbon, nitrogen, and sulfur also serve as chemical tracers, providing insights into ecological processes and patterns (Boecklen et al., 2011; Newsome et al., 2010; Peterson and Fry, 1987; Thompson et al., 2005). Stable isotopes of nitrogen are frequently used to indicate diet and trophic status (Caut et al., 2009; Olson et al., 2010; Ramos et al., 2011) because consumers accumulate higher levels of $\delta^{15}N$ than their prey. Nitrogen isotopes have also been used to assess exposure to sewage and wastewater inputs (Cabana and Rasmussen, 1996; Loomer et al., 2015; Schlacher et al., 2005), and they can reveal possible exposure to contaminants associated with the wastewater (Spies et al., 1989). Stable isotopes of sulfur and carbon are typically only slightly enriched between trophic levels. Instead, these stable isotopes are typically used as tracers of the types of food sources and have been used to assess habitat use (Connolly et al., 2004; Moore et al., 2016), and migratory patterns (Graham et al., 2010; Hobson, 1999). Sulfur stable isotopes are an especially good source indicator of terrestrial vs. marine producers, with more enrichment of heavier isotopes in marine systems (Thode, 1991; Willacker et al., 2017), and have been used to track residency in estuarine fishes (Fry and Chumchal, 2011) and movements of fish between freshwater and marine systems (Godbout et al., 2010; Moore et al., 2016). Moreover, when stable isotopes of sulfur, nitrogen and carbon are used together they can provide additional information on habitat use and trophic structure than stable isotopes of carbon and nitrogen alone (Connolly et al., 2004). Because stable isotopes fractionate with the organism's metabolism and change with its diet (Hobson, 1999), whereas POPs are not readily metabolized nor eliminated, they provide complementary information about the organism (Fisk et al., 2002; Herman et al., 2005; Ramos and González-Solís, 2012).

Pacific salmon of the genus *Oncorhynchus* exemplify organisms whose migrations take them through multiple habitats, including some where contaminants pose a concern (Johnson et al., 2007a; O'Neill and West, 2009; Ross et al., 2013). Spawned in cool, clear streams and other freshwater habitats, the juveniles feed for a period prior to seaward migration that varies among species, populations, and individuals (Quinn, 2018). Spawning typically takes place high in watersheds where contaminant concentrations are low, but their seaward migration may lead the juveniles through agricultural, industrial, and urbanized areas, each with different classes of contaminants.

In large parts of the southern portion of their native range, Pacific salmon species have experienced declines in abundance sufficient to limit fisheries, resulting in listings under the U.S. Endangered Species Act (ESA), and even lead to extinction. This loss of intra-specific diversity (Gustafson et al., 2007) has many causes, and the relative importance of each varies among watersheds (NRC, 1996) but chemical contaminants can contribute to poor survival of juveniles in populations migrating through contaminated habitats (Johnson et al., 2013; Meador, 2014).

Chinook salmon, *O. tshawytscha*, is listed as Threatened under the US ESA in Puget Sound, Washington, where individuals spawn in a number of large and medium-sized rivers (Myers et al., 1998; Ruckelshaus et al., 2006). The Snohomish River is typical of these, and is characterized by

headwaters in forested land with few major sources of contaminants, with a transition to areas dominated by agriculture and increasingly suburban, urban and industrial areas where they flow into Puget Sound, Washington (Pess et al., 2002). Survival rates of juvenile salmon entering Puget Sound have been low for several decades (Quinn et al., 2005) but vary among rivers (Ruff et al., 2017), indicating that local as well as regional factors affect survival. Some of this variation has been linked to the extent to which the natal estuary has been modified from its natural condition (Magnusson and Hilborn, 2003), including chemical contamination (Meador, 2014). Moreover, natural-origin fish migrate more slowly and reside and feed in estuaries for longer periods than hatchery-origin fish (Levings et al., 1986; Rice et al., 2011), potentially resulting in greater contaminant exposure for natural-origin salmon.

Our goal was to use complementary data types to assess the location and source of contaminant exposure for juvenile Chinook salmon migrating through habitats with multiple contaminant sources, notably wastewater and stormwater. This study was conducted in the Snohomish River Washington, where two previous studies (O'Neill et al., 2015; Sloan et al., 2010) documented elevated levels of PBDEs (a POP class associated with wastewater; Osterberg and Pelletier, 2015) in juvenile Chinook salmon, at concentrations high enough to alter their immune response and increase their susceptibility to naturally occurring diseases, based on laboratory exposure studies (Arkoosh et al., 2010, 2018). The specific objectives were to determine where in their migratory pathway salmon become exposed to potentially harmful concentrations of PBDEs, and to identify potential sources so that corrective actions could be identified. We measured levels of PBDEs, other POPs, and stable isotopes of nitrogen, sulfur, and carbon in salmon collected along their migration routes in the estuarine portions of the Snohomish River. We hypothesized that Chinook salmon caught in the more developed reaches of the river, near wastewater inputs, would exhibit higher concentrations of PBDEs and that their POP fingerprints would have a higher proportion of PBDEs compared to other POPs, more indicative of a wastewater source. We further hypothesized that altered stable isotope ratios of nitrogen would be observed in fish captured in the vicinity of the wastewater inputs, and associated with the amount and type of nitrogen discharged. Additionally, this population includes Chinook salmon spawned naturally in the river and ones produced in a hatchery and we predicted that the natural-origin fish would exhibit higher POP concentrations associated with their higher residence time in the estuaries (Levings et al., 1986; Rice et al., 2011).

2. Material and methods

2.1. Study area

The Snohomish River watershed, in western Washington State, drains approximately 4600 km^2 into Puget Sound (USGS, 2011), and is formed by the confluence of the Skykomish and Snoqualmie rivers. It flows approximately 37 km to Puget Sound via a mainstem and a complex system of deltaic braided distributary channels through Union, Steamboat, and Ebey sloughs (Hall et al., 2018). The Snohomish River estuary's tidal influence extends throughout the distributary channels and up the mainstem to river kilometer (rkm) 27 (Collins and Sheikh, 2005). The maximum extent of saltwater (0.5 ppt) intrusion also extends throughout the distributary channels and to 15.9 rkm on the mainstem channel (Hall et al., 2018). Overall, 75% of the upland areas of Snohomish River basin is forested (Pess et al., 2002). In contrast, land cover in the floodplains and neighboring foothills along the major river channels are much more impacted by human activities, predominantly rural-residential, agricultural, and urban (Pess et al., 2002).

Modern human activities in the Snohomish River estuary have resulted in degradation and loss of juvenile salmonid habitat, considered the primary factor limiting Chinook salmon survival in the basin (Snohomish Basin Salmon Recovery Forum, 2005). Currently available wetland habitat area in the Snohomish estuary is estimated at 1389 ha; roughly 20% of the historical habitat extent in the delta (Beechie et al., 2017; Collins and Sheikh, 2005). The majority of the remaining available rearing habitat for juvenile Chinook salmon is located in the lower estuary (1238 ha) and distributed unevenly between the mainstem (88 ha) and the distributary (1150 ha) portions of the delta (Beechie et al., 2017).

Contaminant inputs likely coincide with the physical habitat loss in the Snohomish estuary. In particular, developed habitats with impervious surfaces adjacent to the river likely increase loadings of contaminants in stormwater to the river, as has been demonstrated for other aquatic systems (Brown and Peake, 2006; Lee et al., 2004; McCarthy et al., 2008). Indeed, stormwater was documented to be a major source of PCB loading to Puget Sound, with developed lands with more impervious surface contributing higher loads of PCBs to the watershed (Osterberg and Pelletier, 2015). Although specific inputs on PCBs to the Snohomish River were lacking, we used impervious surface and road area as proxies for urbanization in this study area and potential inputs of PCBs. The metric utilized for impervious surface was calculated by determining the "percent developed imperviousness", %IS (Fry et al., 2011; Wickham et al., 2013) within predefined watershed catchment areas called Assessment Units (AUs). The %IS values in our study ranged from 0 to 94%, with the most impervious surfaces (41–94%) in the City of Everett, located on the lower section of the mainstem, and the city of Marysville, located in the lower section of Ebey Slough (Fig. 1).

In addition to contaminant inputs from stormwater, the cities of Marysville and Everett primarily discharge treated wastewater, a potential source of PBDEs and other contaminants, into the estuarine portion of the Snohomish River. Specific levels of PBDEs discharged into the Snohomish River are unknown, as wastewater dischargers in WA State are not required to monitor PBDEs in their effluent. Everett's Water Pollution Control Facility (WPCF) is adjacent to the mainstem of the Snohomish River. The facility operates two outfalls and 13 combined sewer overflows (CSOs). One outfall and six CSOs discharge into the lower section of the river's mainstem and the others discharge into the marine waters of Port Gardner in Possession Sound (Fig. 1). The WPCF uses an aeration/oxidation pond (lagoon) system for treating the wastewater that discharges to the Snohomish River outfall (WA Dept. Ecology, 2015). Marysville's WWTP is located in the Distributary Channels, adjacent to Ebey Slough and uses an aerated lagoon with a filtration system to treat sewage prior to discharge into an outfall in Steamboat Slough or the marine waters of Port Gardner during the summer (WA Dept. Ecology, 2017). While most of the effluent discharged from these two facilities is treated, CSOs release untreated wastewater. CSOs occur on average, 1–2 times a year.

Discharges of dissolved inorganic nitrogen (DIN) in effluent from municipal WWTPs were used to assess the loads of nitrogen discharged from WWTPs. In total, Puget Sound has 78 municipal WWTPs discharging to Puget Sound – 70 discharge to marine waters and eight discharge to river estuaries. In the year of our study (2016), the Everett WPCF outfall in the Snohomish River had the highest DIN average daily discharge (average of February to July) of the eight facilities that discharge to the estuarine portion of Puget Sound rivers (Table 1). Additionally, the Everett WPCF outfall had the eight highest average daily discharge of DIN of all 78 municipal WWTPs discharging into Puget Sound (Table 1). WWTP loads were originally estimated for the years 1999–2008 using methods described in Mohamedali et al. (2011), and these inputs were updated through mid-2017 as described in Ahmed et al. (2019). During the 6-month migration window for juvenile Chinook salmon (February through July) in 2016, discharged DIN from the Everett WPCF averaged 1006 kg/day (Table 1). The Everett WPCF effluent, however, was atypical compared to other Puget Sound wastewater facilities, and contained a higher proportion of ammonium relative to nitrates and nitrites compared to the effluent from other facilities that discharge into similar waters frequented by juvenile salmon. The

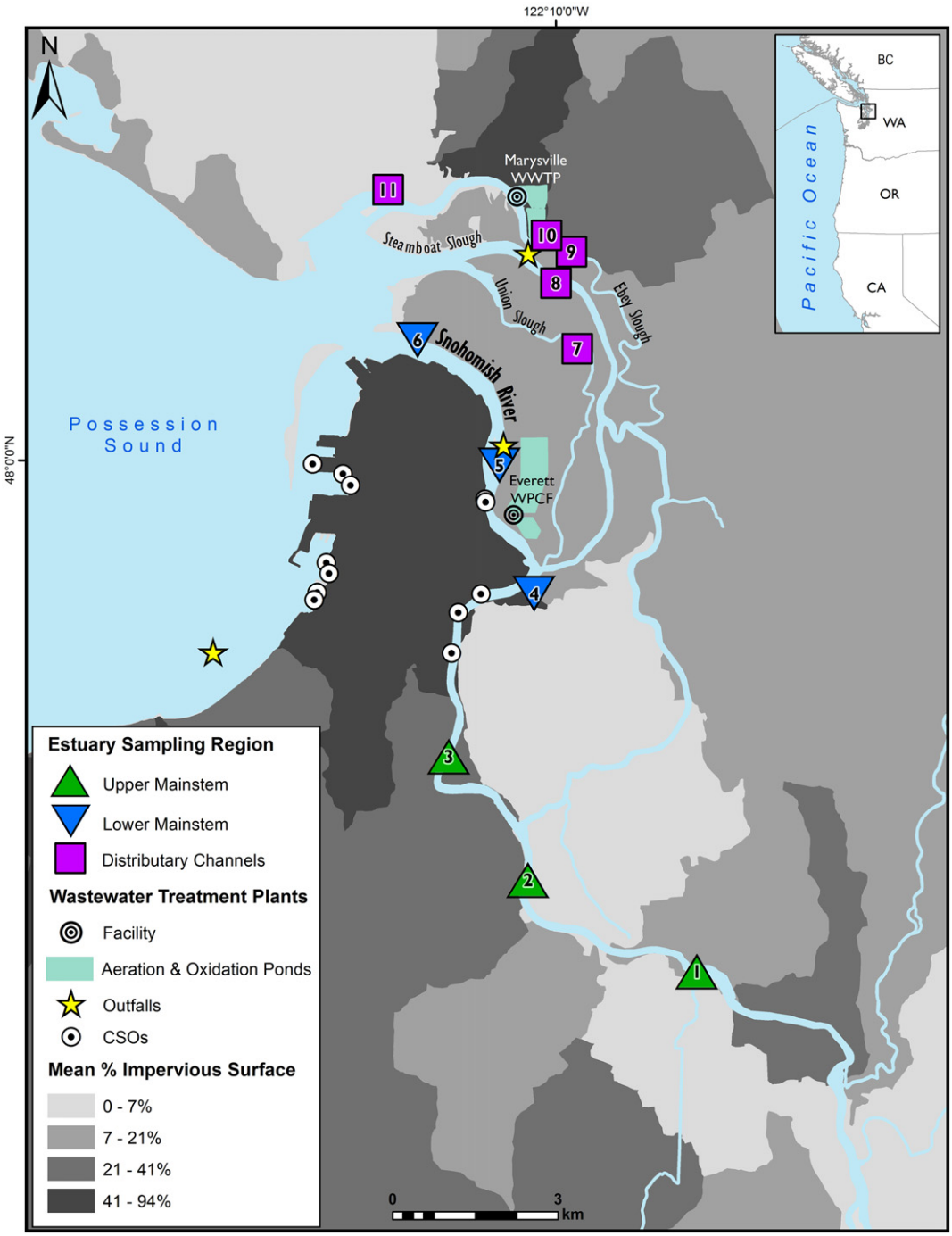

Fig. 1. Location of estuary sampling sites and sampling regions where juvenile Chinook salmon were collected for contaminant and stable isotope analyses (see Table 2 for additional site and sample data). Impervious land-surface is shown as grey scale gradations from <7% (lightest grey) to >40% (darkest grey). WPCF = Water Pollution Control Facility. WWTP = Wastewater Treatment Plant.

only other WWTP facilities discharging into Puget Sound with comparable or higher ammonium loads (>1000 kg/day) were high load facilities (≥250 DIN kg/day) that discharged in deep offshore marine waters, beyond habitats typically used by juvenile salmonids (Table 1).

2.2. Sampling design and fish collections

We sampled juvenile Chinook salmon for chemical tracers from 11 sites along their migration pathway in the estuarine portion of the Snohomish River in 2016. The sites were distributed in three regions: the Upper Mainstem, through which all the fish migrate, and two downstream regions, the Lower Mainstem and the Distributary Channels, that constitute alternative routes by which the fish can enter Puget Sound (Fig. 1). A minimum of three sites per region, distributed along the migration pathway within each region, were sampled to assess the range of stormwater and wastewater inputs that fish were potentially exposed to. Due to limitations on the number of ESA-listed Chinook salmon we were allowed to capture, our sampling design was intended to compare salmon among the three regions, rather than at specific sites. Our three sampling regions roughly represent the major bifurcation in the system based on hydrological properties of the rivers (Collins and Sheikh, 2005; Hall et al., 2018). The Upper Mainstem was the least developed of the three regions, with the most downstream site located 2 to 7 km upstream of the outfalls for the wastewater

Table 1
Mean and range (in parentheses) of daily loads of nitrogen types (DIN, ammonium, and nitrate + nitrite) and ratio of ammonium to nitrate + nitrite in municipal wastewater treatment plant effluent for facilities discharging into Puget Sound rivers and marine waters (Mohamedali et al., 2011; Ahmed et al., 2019), reported for each month from February through July of 2016. Effluent data for the Lower Mainstem and Distributary Channel regions of the Snohomish River sampled in this study are summarized separately from data for other facilities. Facilities discharging mean dissolved inorganic nitrogen (DIN) daily loads >250 kg/day for that six-month window were further categorized as either a freshwater, nearshore or offshore facility depending on the location of their effluent outfall in the receiving waters. All other facilities discharging into nearshore or offshore marine waters with a mean DIN <250 kg/day was placed in the "Other nearshore and offshore marine facilities" category. If load data was missing for a month, it was excluded from the average load calculation.

WWTP	n	DIN (kg/day)	Ammonium (kg/day)	Nitrate + Nitrite (kg/day)	Ratio of Ammonium (kg/day) to Nitrate + Nitrite (kg/day)
Lower Mainstem facility[a]	1	1006 (829–1162)	1002 (822–1161)	4.09 (1.71–6.97)	335 (118–678)
Distributary Channels facility[b]	1	649 (462–816)	628 (452–806)	20.4 (6.56–47.4)	48.3 (14.2–80.2)
Other river facilities (DIN ≥ 250 kg/day)	1	410 (203–583)	325 (126–462)	85.4 (33.2–158)	5.58 (1.64–13.9)
Other river facilities (DIN < 250 kg/day)	5	71.6 (1.61–257)	5.99 (0.005–58.0)	65.7 (1.60–256)	0.019 (0.002–2.14)
Nearshore marine facilities (DIN ≥ 250 kg/day)	3	658 (383–1177)	631 (365–1173)	27.0 (3.63–83.4)	84.7 (5.83–323)
Offshore marine facilities (DIN ≥ 250 kg/day)	13	2004 (229–9543)	1560 (7.87–8684)	445 (0.73–2794)	61.7 (0.0029–1385)
Other nearshore and offshore marine facilities (DIN < 250 kg/day)	54	32.9 (0.0077–352)	17.0 (0.0012–300)	15.9 (0.0012–236)	10.1 (0.00035–416)

[a] Everett Water Pollution Control Facility (see Fig. 1, Everett WPCF).
[b] Marysville Wastewater Treatment Plant (see Fig. 1, Marysville WWTP).

treatment facility and associated CSOs. We assumed that contaminants measured in seaward migrating salmon collected in the Upper Mainstem region represented cumulative exposure from all upstream sources. At each subsequent downstream site in the Lower Mainstem and the Distributary Channels, we assumed contaminant concentrations in salmon indicated additional inputs from stormwater and wastewater to which the salmon were exposed.

Juvenile Chinook salmon were collected in 2016 from April through July, primarily during the peak of downstream migration (April and May) to represent the average contaminant concentrations of the river system's fish populations. All fish were collected with beach seines or fyke nets, following procedures described in Roegner et al. (2009), euthanized, transferred to the laboratory on ice, assigned a unique number, and stored at −80 °C until tissue samples for chemistry and stable isotopes were prepared.

2.3. Sample processing

To process fish for analyses of contaminants and stable isotopes, fish were thawed slightly, fork length (mm) was recorded for each fish, and scales were removed for age determination (sub-yearling vs. yearling). To ensure the gut contents did not influence the contaminant and stable isotope data, they were removed from the stomach and intestine of fish and discarded to create gutted whole body fish samples. Additionally, the brain was removed from each fish for use in a separate study. Each fish was examined for presence of a clipped adipose fin, a coded wired tag (CWT), or thermally marked otoliths, any of which would indicate hatchery-origin fish. Based on thermally marked otoliths, we excluded from our study a few hatchery-origin fish that did not originate from within the Snohomish River, leaving 177 salmon for analyses (Table 2).

Forty-eight composite samples of gutted whole body fish, less the brain, were created by combining 1–8 similarly sized salmon in each sample (Table 2). The samples were homogenized, placed in pre-cleaned glass jars, and stored at −20 °C for subsequent chemical analyses. The proportion of natural-origin fish in each composite sample was used to classify the samples as either predominantly natural-origin (>65%) or hatchery-origin (<35%). In most cases, samples classified as predominantly natural- or hatchery-origin contained only fish of that designation (25 of 30 natural- and 15 of 18 hatchery-origin samples).

2.4. Contaminant analysis

Samples (approximately 2 g from each composite tissue sample) were analyzed for POPs, including 11 PBDEs, 46 PCBs, and six DDTs, using an established gas chromatography/mass spectrometry (GC/MS) method (Sloan et al., 2014). This method comprises three steps: (a) a dichloromethane extraction using an accelerated solvent extractor, (b) cleanup by gravity flow silica/aluminum columns and followed by

Table 2
Number (No.) of individual juvenile Chinook salmon and composite samples (Comps.) sampled in 2016 for contaminant analyses at multiple sites in the estuary habitat of the Snohomish River. Each composite sample is composed of 1–8 individual salmon of similar size and was classified as either natural- or hatchery–origin, based on the proportion of natural fish present. Site numbers refer to the sampling locations depicted in Fig. 1.

Sampling regions	Site No.	Site name	River km	Collection period	Natural origin		Hatchery origin	
					No. fish	No. Comps.	No. fish	No. Comps.
Upper Mainstem	1	Fields Riffle	18.7	April–July	16	5	3	1
	2	Big Tree	14.9	April–July	11	3		
	3	Old Bridge	11.5	April–July	15	4	4	2
Lower Mainstem	4	Old Barge	7.4	April–May	20	4	3	1
	5	Langus Pier	5.2	April–July	31	7	9	3
	6	Lower Mainstem	2.0	May–June			11	3
Distributary Channels	7	Union Slough	5.9	May June	8	2	3	1
	8	Steamboat Slough	4.8	April–May	6	2	3	1
	9	Ebey Slough 1	6.9	April–May	10	2	3	2
	10	Ebey Slough 2	6.4	April–May			1	1
	11	Ebey Slough 3	2.5	May	7	1	12	3
All regions				April–July	124	30	53	18

size-exclusion high-performance liquid chromatography (HPLC) cleanup, and (c) quantitation of POPs using gas chromatography/mass spectrometry (GC/MS) with selected-ion monitoring (SIM). A subsample of each pre-cleaned extract was used to determine percent lipids gravimetrically (Sloan et al., 2014). As part of a performance-based quality assurance program, a solvent (dichloromethane) method blank and National Institute of Standards and Technology (NIST) Lake Michigan fish tissue Standard Reference Material (SRMs, 1947) were analyzed with each batch of field samples and the results of the quality control samples met established laboratory criteria (Sloan et al., 2019). The solvent method blank for each sample batch contained no more than five analytes that exceeded 2 × the lower limit of quantitation (LOQ), which met our laboratory QA criteria. Levels of ≥70% of individual analytes measured in NIST SRM 1947 for each sample batch were within 30% of either end of the 95% confidence interval of the NIST certified values. Surrogate recoveries for the POP analyses ranged from 99 to 116% and met established laboratory criteria (surrogate recoveries are to be between 60 and 130%). Additional details for our laboratory quality assurance measures and criteria for POPs analyses can be found in Sloan et al. (2019). The lower limits of quantitation (LOQ) for individual PBDEs, PCBs, and DDTs measured in the field samples and their associated quality assurance samples ranged from <0.063 to <0.31 ng/g, wet weight.

Analyte data are presented as summed values for PBDEs and DDTs. Summed PBDEs (i.e., $\sum_{11}$PBDEs), were calculated by summing detected concentrations of the congeners 28, 47, 49, 66, 85, 99, 100, 153, 154, 155, and 183. Summed DDTs (i.e., $\sum_{6}$DDTs) were calculated by summing the detected concentrations of *o,p′*-DDD, *o,p′*-DDE, *o,p′*-DDT, *p,p′*-DDD, *p,p′*-DDE, and *p,p′*-DDT. The total PCB concentration (i.e., TPCBs) was estimated using a simple algorithm based on the subset of 17 commonly detected congeners (and coeluting congeners) representing homologues containing three to ten chlorine atoms [congeners 18, 28, 44, 52, 95, 101(90), 105, 118, 128, 138(163,164), 153 (132), 170, 180, 187(159,182), 195, 206, and 209], wherein the sum of the detected values for these 17 (and coeluting) congeners was multiplied by two. The calculated TPCB concentration using this method was previously shown to agree well with the sum of 209 congeners measured by high resolution methods for two fish species (West et al., 2017) and is similar to method used by Lauenstein and Cantillo (1993) discussed in West et al. (2017). Summed or total POP results were expressed as nanogram (ng) per gram of tissue weight (wet weight). Additionally, we calculated POP concentrations on a lipid basis, as ng of contaminant per g of fish lipid (ng/g lipid), to facilitate comparisons with other published studies, including those on adverse critical body residues (CBRs). Published CBRs for POPs are sometimes reported as lipid normalized concentrations because POP toxicity can be inversely dependent on lipid content (Lassiter and Hallam, 1990).

2.5. Stable isotopes analyses

All samples with sufficient tissue mass (41 of 48 samples) were analyzed for stable isotope ratios of nitrogen, carbon, and sulfur. Frozen, non-lipid extracted tissue samples were freeze-dried, ground to a fine powder, and weighed into tin capsules for isotope analyses with a target mass of 0.5 mg of tissue for carbon and nitrogen and 7.5 mg for sulfur. Stable isotope analyses were performed at the University of Washington's IsoLab in Seattle, WA as in Fry et al. (1992) and Fry et al. (2002). A Costech Elemental Analyzer, Conflo III, and Thermo MAT253 isotope ratio mass spectrometer was used for a continuous-flow based measurement of bulk carbon $\delta^{13}C$ and bulk nitrogen $\delta^{15}N$. A Eurovector Elemental Analyzer, Conflo III, and Thermo MAT253 isotope ratio mass spectrometer was used for a continuous flow based measurement of the bulk sulfur $\delta^{34}S$. During analytical runs, these methods provided precisions (1 sigma) of ±0.05‰, ±0.1‰ and ±0.2‰ for carbon, nitrogen and sulfur, respectively.

Stable isotopes of carbon, nitrogen, and sulfur were expressed in standard delta notation ($\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$),

$$\delta\ (‰) = 10^3\ \left[\left(R_{sample}/R_{standard}\right)-1\right],$$

where R is the ratio of heavy and light isotopes in a sample (^{13}C:^{12}C, ^{15}N:^{14}N, and ^{34}S:^{32}S). We expressed stable isotope ratios in units of permil (‰ – parts per thousand) and are relative to international standards: Vienna PeeDee Belemnite (VPDB) for $\delta^{13}C$, atmospheric nitrogen for $\delta^{15}N$, and Vienna–Canyon Diablo Troilite (VCDT) for $\delta^{34}S$. Most (78%) of the fish tissue samples had a C:N ratio greater than the 3.5 threshold for lipid correction (Post et al., 2007), indicating that the lipid content of the samples may have depleted the $\delta^{13}C$ values. Accordingly, we used the following equation from Post et al. (2007),

$$\delta^{13}C_{normalized} = \left(\delta^{13}C_{untreated}-3.32\right) + (0 : 99 \times C : N)$$

to mathematically lipid normalize $\delta^{13}C$ values.

2.6. Data analysis

We applied multiple linear regression (R Development Core Team, 2018) to identify the potential effects of four factors on contaminant concentration for three major POP classes (DDTs, PBDEs, and PCBs). The four predictor variables were: 1) capture region (one of three), 2) fish origin (natural or hatchery), 3) fish size (mean length of fish in composite sample) at capture, and (4) % lipid content. Fish origin was considered a factor because natural-origin fish may reside and feed in estuaries for longer periods than hatchery-origin fish (Levings et al., 1986; Rice et al., 2011), resulting in greater potential exposure. Fish length (an indirect measure of age and duration of exposure) and lipid content were included as factors as they can affect the concentrations of POPs accumulated (West et al., 2017). All POP data were log transformed to meet assumptions of normality and homogeneity of group variances. Additive and interactive effects were evaluated, however, due to limited degrees of freedom, only models with up to three factors were considered. Akaike Information Criterion corrected for small sample size (AICc) and Akaike weights were used to identify the best model to parsimoniously explain the variation in the concentrations of PBDE, PCBs and DDTs data (Akaike, 1974; Burnham and Anderson, 2002). The model with the lowest AICc and highest Akaike weight was considered the best fit. For the best fit model for each POP class, pairwise comparisons were conducted on estimated marginal means using the Holm-Sidak adjustment for multiple comparisons. Test results for pairwise comparisons were considered statistically significant at $p \le 0.05$. Additionally, independent linear regressions were performed for $\sum_{6}$DDT by fish length for each origin.

To illustrate POP fingerprints among the three regions by two origin groups, the proportion of $\sum_{11}$PBDEs and other POPs classes in Chinook salmon samples were compared among the six regions and origin groups using principal component analyses (PCA), as detailed in the software package Primer-E version 6 (Clarke and Warwick, 2006; Clarke and Gorley, 2006). Prior to analyzing with PCA, the POP class data were pretreated by standardizing (i.e., computing the proportional contribution of each POP class concentration to the total POP concentration in each sample) and then transforming the data by taking the square root to reduce the contribution of dominant classes. Similar POP fingerprints among groups would indicate consistent sources of contaminants, whereas dissimilar contaminant patterns would suggest inputs of specific POPs associated with different sources. Pairwise site comparisons of group patterns were conducted with ANOSIM, using the R statistic and p values to identify the main between-group differences. Values of the ANOSIM R statistic range from 0 (i.e., no separation, or complete similarity) to 1.0 (i.e., complete separation, or no similarity) of a population. A p value ≤ 0.05 was used as a guide for determining

whether the measured segregation between groups (i.e., R statistic) was statistically significant.

A two-way ANOVA was used to test for difference in stable isotopes, fish length, and lipid content among regions and between natural and hatchery-origin fish. A Holm-Sidak test was used to conduct pairwise comparisons among group means for each POP class. Probability values were used to help evaluate the significance of differences; a p value ≤ 0.05 was used as a guide to assess whether results for pairwise comparisons were considered statistically significant.

Salmon POP fingerprints (i.e., PCA analyses of the proportion of $\sum_{11}$PBDEs and other POPs), a potential indicator of contaminant source, and δ^{15}N, an indicator of the nitrogen source, were compared to see if they co-varied. For each region and origin sampling group, we used linear regression to test for significant relationships between δ^{15}N and PC1 scores in salmon samples, two independent metrics that can both be affected by wastewater inputs. Additionally, linear regression was used to test for significant relationships between δ^{15}N and $\sum_{11}$PBDEs, and TPCBs.

3. Results

3.1. POPs concentrations

Mean POP wet weight (ww) concentrations in juvenile salmon varied 10-fold for $\sum_{11}$PBDEs as a function of sampling region and origin (naturally or hatchery produced), from 2.4 to 24 ng/g ww, and less so for TPCBs, from 12 to 31 ng/g ww (Table 3). In contrast, mean $\sum_{6}$DDT concentrations were much more similar among regions and origin groups, ranging from 1.7 to 2.9 ng/g ww. Although TPCBs varied less than $\sum_{11}$PBDEs among sampling groups, overall TPCB concentrations were greater than $\sum_{11}$PBDEs, followed by $\sum_{6}$DDTs (Table 3). For example, among fish of the same region, measured mean TPCBs concentrations were 1–3 times greater than $\sum_{11}$PBDE and 5–10 times greater than $\sum_{6}$DDTs for natural-origin fish and 2–6 times greater than $\sum_{11}$PBDEs and 6–16 times greater than $\sum_{6}$DDTs for hatchery-origin fish.

Concentrations of $\sum_{11}$PBDEs and TPCBs in juvenile salmon were best predicted by models that included factors for the collection region and the fish origin, rather than body size (i.e., length) or lipid content, but the importance of these factors varied by POP class (Table S1). Specifically, $\sum_{11}$PBDE concentrations were best predicted by models that included region, origin, and a strong region x origin interaction term (Adjusted r^2 = 0.58; Tables S1, S2). Post-hoc tests identified that natural-origin fish from the Lower Mainstem had the highest $\sum_{11}$PBDE concentrations (p ranged from 0.033 to <0.0001). Overall, concentrations of $\sum_{11}$PBDEs in natural-origin fish from the Lower Mainstem (mean = 24 ng/g ww) were 4–10 times higher than salmon from all other sampling groups, regardless of region or origin (Table 3, Fig. 2). The only other statistically significant difference in $\sum_{11}$PBDE concentrations was between natural- and hatchery-origin salmon from the Distributary Channels (means = 5.7 and 2.4 ng/g ww), representing the second highest and lowest concentrations (Table 3, Fig. 2, p = 0.05). The mean lipid content among samples ranged from 0.97% to 1.6% (Table 3) but did not differ significantly among regions or between fish origins (Two-Way ANOVA, region F = 0.722 and p = 0.492; origin F = 0.783 and p = 0.781). Moreover, models with lipid content as a single factor or in combination with region or origin were poor fits and explained less of the measured variation in $\sum_{11}$PBDEs (Table S1). Although natural-origin fish were smaller than hatchery-origin fish (mean = 65.7 vs. 77.4 mm, Table 3), fish length for combined origins did not differ among regions (Two-Way ANOVA, origin F = 9.910 and p = 0.003, region F = 0.217 and p = 0.806), and models with fish length alone or in combination with region or origin were poor fits and did not contribute substantively to the measured variation of $\sum_{11}$PBDEs (Table S1).

Concentrations of TPCBs were best predicted by sampling region, accounting for 46% of the measured TPCB variation (Table S1). Fish length and lipid content, as individual factors or in combination with region or origin, did not substantively improve the model fit. Overall, fish from the Lower Mainstem had measured TPCB concentrations (30 ng/g ww) approximately twice as high as those from the Upper Mainstem (mean = 13 ng/g ww) and the Distributary Channels (16 ng/g ww; Table 3, Fig. 2, p < 0.0001 for both comparisons), which did not differ from each other (p > 0.05).

Unlike the patterns measured for $\sum_{11}$PBDEs and TPCBs, fish size was significantly correlated with $\sum_{6}$DDT concentrations. The $\sum_{6}$DDT concentration was best predicted by the origin, fish length, and a fish origin-length interaction, accounting for 40% of the measured variation (Table S1). A model with only origin and fish length was not near as good a fit to the actual data (Table S1), indicating that the fish origin-length interaction term was significant. Predicted mean $\sum_{6}$DDT concentrations were higher in natural- than hatchery-origin fish (2.3 vs. 1.7 ng/g ww), based on a mean fish length of 70 mm in the best-fit model regression model (Table S2, Fig. 2c). However, predicted $\sum_{6}$DDT concentrations depicted in Fig. 2c do not fully represent the interaction between fish origin and fish length due to differences in sizes between natural- and hatchery-origin fish. The size of newly emerged natural-origin Chinook salmon prior to exogenous feeding (Beacham and Murray, 1990) are just a few mm smaller than those we sampled from the river, however, hatchery-origin fish are not released to the river until they reach approximately 65 mm, prohibiting full examination of $\sum_{6}$DDT size comparison for fish of both origins from the river. As a result, in addition to the full model, independent linear regressions were performed for $\sum_{6}$DDT by fish length for each origin (Fig. 3). There

Table 3
Arithmetic mean lipid content (Lipids), fork length (FL), and concentrations of $\sum_{11}$PBDE, $\sum$PBDE 47+99, TPCBs, and $\sum_{6}$DDTs of composite samples of Chinook salmon (*Oncorhynchus tshawytscha*). Measured POPs concentrations are reported as ng/g wet weight (ww) and ng/g lipid weight (lw), based on the measured gutted whole body wet weight and lipid content in the fish.

Region	Origin	N	Lipids	FL	$\sum_{11}$PBDE	$\sum_{11}$PBDE	$\sum$PBDE$_{47+99}$[a]	$\sum$PBDE$_{47+99}$	TPCBs	TPCBs[b]	$\sum_{6}$DDTs	$\sum_{6}$DDTs
			(%)	(mm)	ww	lw	ww	lw	ww	lw	ww	lw
Upper Mainstem	Natural	12	1.6	61.3	4.0	270	3.5	240	14	950	2.9	210
	Hatchery	3	0.97	85.7	5.2	640	4.4	540	12	1200	2.0	220
	Natural + hatchery	15	1.4	66.2	4.2	340	3.7	300	13	1000	2.7	210
Lower Mainstem	Natural	11	1.5	66.9	24	1500	20	1200	31	2100	2.9	210
	Hatchery	7	1.5	75.8	5.4	500	4.7	430	29	2600	1.9	140
	Natural + hatchery	18	1.5	70.3	17	1100	14	930	30	2300	2.5	180
Distributary Channels	Natural	7	1.1	71.3	5.7	540	4.9	460	18	1700	2.3	200
	Hatchery	8	1.6	75.8	2.4	190	2.0	160	15	940	1.7	110
	Natural + hatchery	15	1.4	73.7	3.9	350	3.4	300	16	1300	2.0	150
All regions	Natural	30	1.4	65.7	12	790	9.7	660	21	1500	2.8	210
	Hatchery	18	1.5	77.4	4.0	380	3.5	330	20	1600	1.8	140

[a] $\sum$PBDE$_{47+99}$ = sum of detected BDE-47 and BDE-99 congeners used to assess adverse critical body residues (CBR) for exposure to PBDEs.
[b] TPCB used to assess adverse CBR for exposure to PCBs.

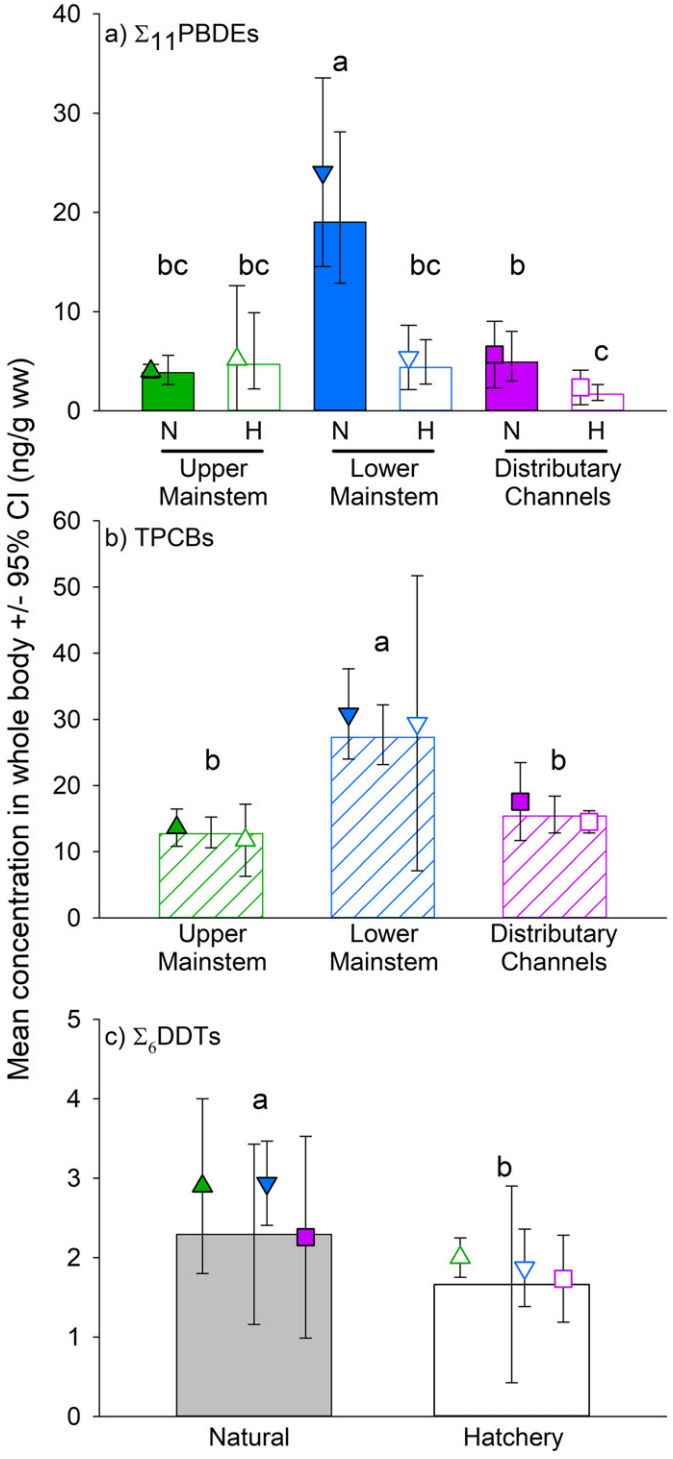

Fig. 2. Measured (symbols) and predicted (bars) concentrations of $\sum_{11}$PBDEs, TPCBs, and $\sum_{6}$DDTs in juvenile Chinook salmon collected from the Snohomish River estuary. Symbols represent the arithmetic mean concentrations for $\sum_{11}$PBDEs, TPCBs, and $\sum_{6}$DDTs where Upper Mainstem, Lower Mainstem, and the Distributary Channels sites are represented by upward triangles, downward triangles, and squares, respectively. Solid filled and open symbols are used to represent natural- and hatchery-origin fish, respectively. Bars are modeled estimated geometric mean concentrations with solid filled, open, and hatched bars used to represent natural-, hatchery- and mixed-origin fish, respectively. Predicted $\sum_{6}$DDTs concentrations were modeled using a grand mean fish length of 70 mm. For each POP class, groups with the same lower case letter are not significantly different from each other.

was an inverse relationship between fish length and $\sum_{6}$DDT for natural-origin fish, ranging from 40.2 to 90.0 mm (Fig. 3). In contrast, $\sum_{6}$DDT was not significantly correlated with fish length for hatchery-origin fish for the limited length range tested (65.1 to 95.1 mm, Fig. 3). Similarly, there is also no significant correlation between length and $\sum_{6}$DDT for natural-origin fish >65 mm ($p = 0.36$; data not shown).

3.2. POP fingerprints

A comparison of POP fingerprints among the samples indicated clear segregation between natural-origin fish from the Lower Mainstem and all but one of the other sampling groups (Fig. 4; Table 4). The natural-origin fish from the Lower Mainstem exhibited distinct POP fingerprints (Fig. 4, filled blue triangles), with the higher proportions of $\sum_{11}$PBDEs in the total POP concentration, compared to other sampling groups. These fingerprints were most different from hatchery-origin fish from the same region (Fig. 4, open blue triangles) and the Distributary Channels (Fig. 4, open pink squares), which exhibited the two lowest relative concentrations of $\sum_{11}$PBDEs (ANOSIM, $R = 0.484$ and 0.596), followed by natural origin fish from the other regions (ANOSIM, $R = 0.467$ and 0.315), with intermediate relative concentrations of $\sum_{11}$PBDEs (Table 4, $p \leq 0.006$ for all pair-wise comparison). The only sampling group that was not clearly segregated from the natural-origin fish from the Lower Mainstem was the hatchery-origin fish from the Upper Mainstem (Fig. 4, open green triangles; ANOSIM, $R = 0.251$, $p = 0.052$); however, the sample size representing this group was small ($n = 3$), so the power to detect difference between these two groups, if it existed, was low.

The unique pattern of POPs in the Lower Mainstem natural-origin fish can be further illustrated by examination of POP fingerprints among the remaining five sampling groups, which were statistically indistinguishable from each other. For example, the POP fingerprints in natural-origin fish from the Upper Mainstem and the Distributary Channels were not different from each other ($R = 0.068$, $p = 0.195$) and nor were the hatchery-origin fish from the three regions different from each other (ANOSIM, R from 0.014 to 0.237, and $p = 0.103$–0.467 for all comparisons). Among these five sampling groups, natural-origin fish were only segregated from hatchery-origin fish in three of six comparisons (ANOSIM, R from 0.272 to 0.43, $p < 0.01$ for all comparisons; Table 4).

Among region and origin sample groups, the variation in congener patterns within the TPCB and $\sum_{11}$PBDEs POP classes was minor in comparison to the variation observed between the TPCBs and $\sum_{11}$PBDEs POP classes. The main PCB congeners contributing to the TPCB concentration in each region and origin sample groups (Table S3) were PCB 153 and 138, followed by 101, 118, and then 28 and 18, collectively accounting for 38–68% of the total concentration. The heavier congeners, 195, 206, and 209, were not detected in any samples and the remaining congeners, when detected, were at low concentration near the LOQ (Table S3). Although the TPCB concentrations were higher in fish collected from the Lower Mainstem compared to those from other regions, the pattern of detected concentrations of PCB congener homologues, was similar among region and origin sample groups (Fig. S1). The calculated values for $\sum_{11}$PBDEs were dominated primarily by contributions from BDE congeners 47 and 99, followed by 100 (Table S4), collectively accounting for 86–100% of the $\sum_{11}$PBDEs for individual fish samples. The BDE congeners 85, 155 and 183 were not detected in any salmon samples and the remaining congeners, when detected were at low concentrations near the LOQ (Table S4). Although natural-origin fish from the Lower Mainstem had higher $\sum_{11}$PBDE concentrations compared to other region and origin sample groups, the pattern of BDE congeners detected was similar among these groups (Fig. S2). Likewise, the DDT and DDT metabolites patterns did not vary among region and origin sample groups. The calculated $\sum_{6}$DDTs concentration was dominated by *p,p′*-DDE, which was detected in 100% of the samples (Table S5). The other DDT compounds were never detected (i.e., *o,p′*-DDE, *o,p′*-DDT) or infrequently (8–21%) detected at concentration near the LOQ (i.e., *o,p′*-DDD, *p,p′*-DDD, and *p,p′*-DDT) in all region and origin sample groups.

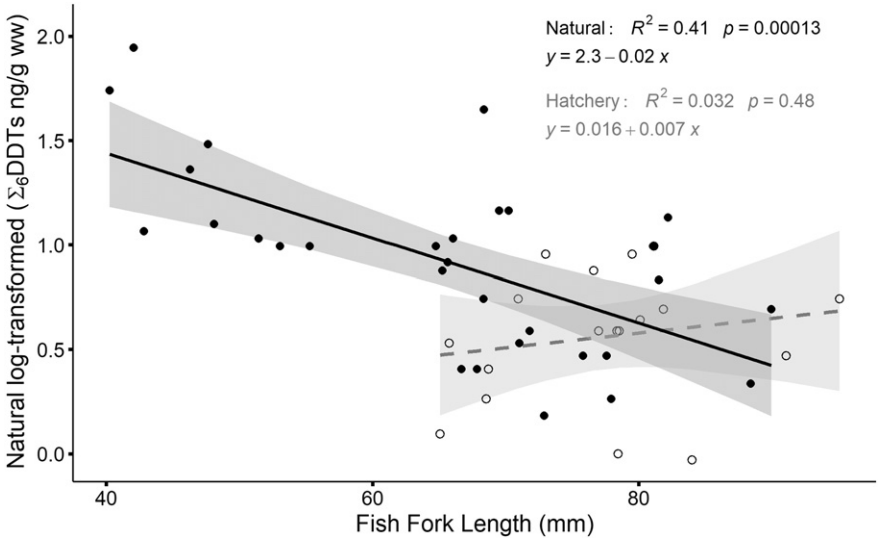

Fig. 3. Relationships between fish length and $\sum_6$DDTs for natural-origin (black solid line ± 95% CI shaded region) and hatchery-origin (dashed line ± 95% CI shaded region) fish. Actual data are plotted using solid filled symbols for natural-origin fish and open symbols for hatchery-origin.

3.3. Stable isotopes

The isotopic values δ^{34}S and δ^{13}C in Chinook salmon generally showed a similar pattern of enrichment from upper to downstream regions of the estuary, and more enrichment in hatchery- than natural-origin fish within each region (Two-Way ANOVA, Table 5; Fig. 5a). Overall, δ^{34}S values (Fig. 5a, vertical axis) in fish from the Distributary Channels (squares) were 2.1 times more enriched than those in the Upper Mainstem (upward triangles; t = 10.278, p < 0.001) and 1.2 times more enriched than those from the Lower Mainstem (downward triangles; t = 4.314, p < 0.001). Measured δ^{34}S in fish from the Lower Mainstem (downward triangles) were also 1.7 times more enriched than those from the Upper Mainstem (upper triangles; t = 6.646, p < 0.001). Overall, δ^{34}S was 1.1 times more enriched in hatchery- than natural-origin fish (open vs. closed symbols; t = 2.561, p = 0.015). A somewhat similar pattern of enrichment was measured for δ^{13}C in fish, although the differences were less pronounced from upstream to downstream (Fig. 5a, horizontal axis). Measured δ^{13}C in fish from the Distributary Channels (squares) were 1.1 times more enriched than those from the Upper Mainstem (upward triangles; t = 4.509, p < 0.001) and the Lower Mainstem (downward triangles; t = 4.141, p < 0.001), which did not differ from each other (t = 1.043, p = 0.304). Overall, δ^{13}C in hatchery-origin fish (open symbols) were 1.1 times greater than those of natural-origin (closed symbols; t = 6.664, p < 0.001).

The patterns of δ^{15}N in Chinook salmon (Fig. 5b, vertical axis) were more complex than those of δ^{34}S and δ^{13}C, with depleted δ^{15}N values in natural-origin fish from the Lower Mainstem (solid downward triangles) compared to other sample groups (Two-Way ANOVA, Table 5). Apart from the natural-origin fish in the Lower Mainstem, as juvenile salmon moved from the Upper Mainstem to the more saltwater influenced region of the Lower Mainstem and the Distributary Channels, values of δ^{15}N and δ^{34}S were positively correlated and increasingly enriched (Fig. 5b). Natural-origin fish from the Distributary Channels (filled squares) were 1.2 times more enriched in δ^{15}N values compared to natural-origin fish from the Upper Mainstem (filled upward triangles; means = 10.8 and 9.3, t = 3.624, p = 0.002). Hatchery-origin fish (open symbols) had more similar δ^{15}N values among regions, but a slight enrichment (1.1 times) was also measured in the downstream

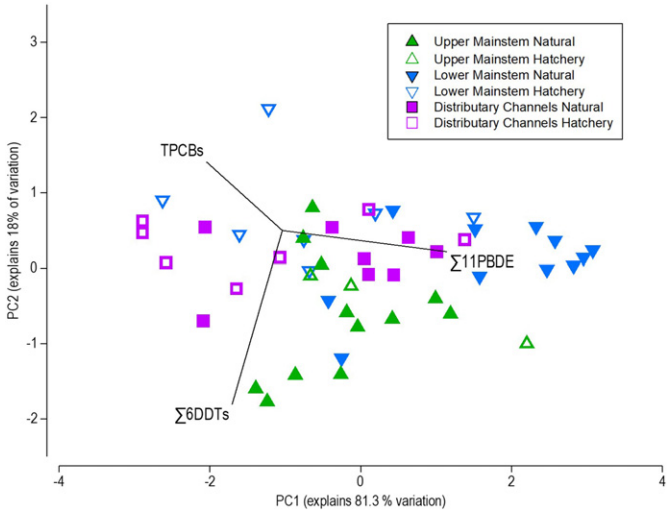

Fig. 4. Plot of the first two principal components (PC) based on the Principal Component Analysis (PCA) of proportions of $\sum_{11}$PBDEs, TPCBs and $\sum_6$DDTs measured in juvenile Chinook salmon collected from three regions of the Snohomish River estuary. Collectively, both PCAs explain 99.3% of the variation, with PC1 accounting for 81.3%, showing higher proportions of $\sum_{11}$PBDEs in natural-origin fish from the Lower Mainstem.

Table 4
ANOSIM statistical results for pairwise comparisons of the proportion of POP classes in juvenile Chinook salmon sampling groups. R varies between 0 and 1, although small negative values close to zero are possible. R values closer to 1 signify a higher degree of separation. Statistically significant differences are noted with an *. LM = Lower Mainstem region, UM = Upper Mainstem region, and DC = Distributary Channels region. Global R for test = 0.306 and p = 0.001.

Sampling group comparisons	R	p	
LM natural vs. DC hatchery	0.596	0.001	*
LM natural vs. LM hatchery	0.484	0.004	*
LM natural vs. UM natural	0.467	0.001	*
LM natural vs. DC natural	0.315	0.006	*
LM natural vs. UM hatchery	0.251	0.052	
UM natural vs. DC natural	0.068	0.195	
UM natural vs. DC hatchery	0.43	0.002	*
UM natural vs. LM hatchery	0.318	0.01	*
UM natural vs. UM hatchery	0.018	0.411	
DC natural vs. DC hatchery	0.272	0.033	*
DC natural vs. UM hatchery	0.127	0.258	
DC natural vs. LM hatchery	0.106	0.097	
UM hatchery vs. DC hatchery	0.237	0.103	
UM hatchery vs. LM hatchery	0.111	0.283	
DC hatchery vs. LM hatchery	−0.014	0.467	

Table 5
Results of a two-way ANOVA with sampling region (i.e. Region) and fish origin (i.e. Origin) as factors affecting stable isotopes of sulfur (δ34S), carbon (δ^{13}C), and nitrogen (δ15N) measured in whole-body samples of juvenile Chinook salmon collected from the estuary of the Snohomish River.

Stable isotopes	Factor	d.f	Sum squared	Mean squared	F value	p value
δ^{34}S	Region	2	187.39	93.695	52.863	<0.001
	Origin	1	11.623	11.623	6.558	0.015
	Region × Origin	2	0.403	0.202	0.114	0.893
	Residual	35	62.035	1.772		
	Total	40	315.047	7.876		
δ^{13}C	Region	2	35.649	17.825	13.299	<0.001
	Origin	1	59.511	59.511	44.402	<0.001
	Region × Origin	2	1.89	0.945	0.705	0.501
	Residual	35	46.91	1.34		
	Total	40	172.28	4.307		
δ^{15}N	Region	2	20.219	10.11	14.779	<0.001
	Origin	1	9.874	9.874	14.435	<0.001
	Region × Origin	2	7.132	3.566	5.213	0.01
	Residual	35	23.942	0.684		
	Total	40	71.463	1.787		

Distributary Channels region compared to those from the Upper Mainstem (means = 11.2 and 9.8, $t = 2.456$, $p = 0.056$). However, in stark contrast, δ^{15}N in natural-origin fish from the Lower Mainstem was significantly more depleted than would be predicted based on their δ^{34}S values (Fig. 5b). Mean δ^{15}N in natural-origin fish from the Lower Mainstem were only 90% of those in natural-origin fish from the Upper Mainstem (means =8.372 and 9.268, $t = 2.284$, $p = 0.029$). A comparison of natural- and hatchery–origin fish within regions also revealed δ^{15}N was only depleted in natural- compared to hatchery-origin fish in the Lower Mainstem (means = 8.372 and 10.595; $t = 5.205$, $p < 0.001$), however, significant differences were not observed from either the Upper Mainstem ($t = 0.973$, $p = 0.337$) or the Distributary Channels ($t = 0.885$, $p = 0.382$).

Nitrogen isotopic signatures of natural-origin fish from the Lower Mainstem were also negatively correlated with higher relative concentrations of $\sum_{11}$PBDEs ($R^2 = 0.68$, $p = 0.003$, slope = −0.74, intercept = 8.15). In natural-origin fish from the Lower Mainstem, the greater the depletion in nitrogen isotopic signature, the higher the proportion of $\sum_{11}$PBDEs (Fig. 6a; proportion of $\sum_{11}$PBDEs measured by PC1 in Fig. 4). The δ^{15}N values were also negatively correlated with absolute concentrations of $\sum_{11}$PBDEs ($R^2 = 0.68$, $p = 0.003$, slope = −8.93 and intercept =100.64) and TPCBs ($R^2 = 0.63$, $p = 0.006$, slope = −6.56 and intercept = 86.52), not shown for brevity. In contrast, for each of the other sampling groups, there was no relationship between δ^{15}N and PC1 (Fig. 6b) or $\sum_{11}$PBDEs, or TPCB (not shown for brevity). Furthermore, samples of natural-origin fish that were presumed to have spent the least amount of time in the Lower Mainstem, based on their lower δ^{34}S, deviated most from the predicted relationship between PC1 score and δ^{14}N (Fig. S3, F = 27.0701, $p = 0.0008$, $R^2 = 0.77$).

4. Discussion

Our study demonstrated the value of three types of complementary chemical tracer data (POP concentrations, POP fingerprints, and stable isotopes), to assess location and source of contaminant exposure for juvenile Chinook salmon migrating seaward through a developed watershed with multiple contaminant sources. Using contaminant concentration data, we first assessed that along their migration pathway through Snohomish River estuary, salmon were exposed predominantly to PCBs and PBDEs in the Lower Mainstem region, with higher $\sum_{11}$PBDEs in natural- rather than hatchery-origin fish but similar TPCBs in both fish origins (Fig. 2). Second, we used POP fingerprints to determine that natural-origin fish captured from the Lower Mainstem had a distinct pattern from other region and origin samples, with a much higher proportion of $\sum_{11}$PBDEs in the total POP concentration, indicating a different contaminant source (Fig. 4). Third, we used stable isotopes, an independent tracer of food sources and habitat use, to document that natural-origin fish from the Lower Mainstem region had depleted δ^{15}N signatures compared to fish from the other region and origin groups (Fig. 5b). Moreover, the $\sum_{11}$PBDE-enhanced POP fingerprint in the natural-origin salmon from the Lower Mainstem was negatively correlated with the δ^{15}N in the salmon (Fig. 6), suggesting a common source for both the high PBDEs exposure and the depleted nitrogen isotopic signal.

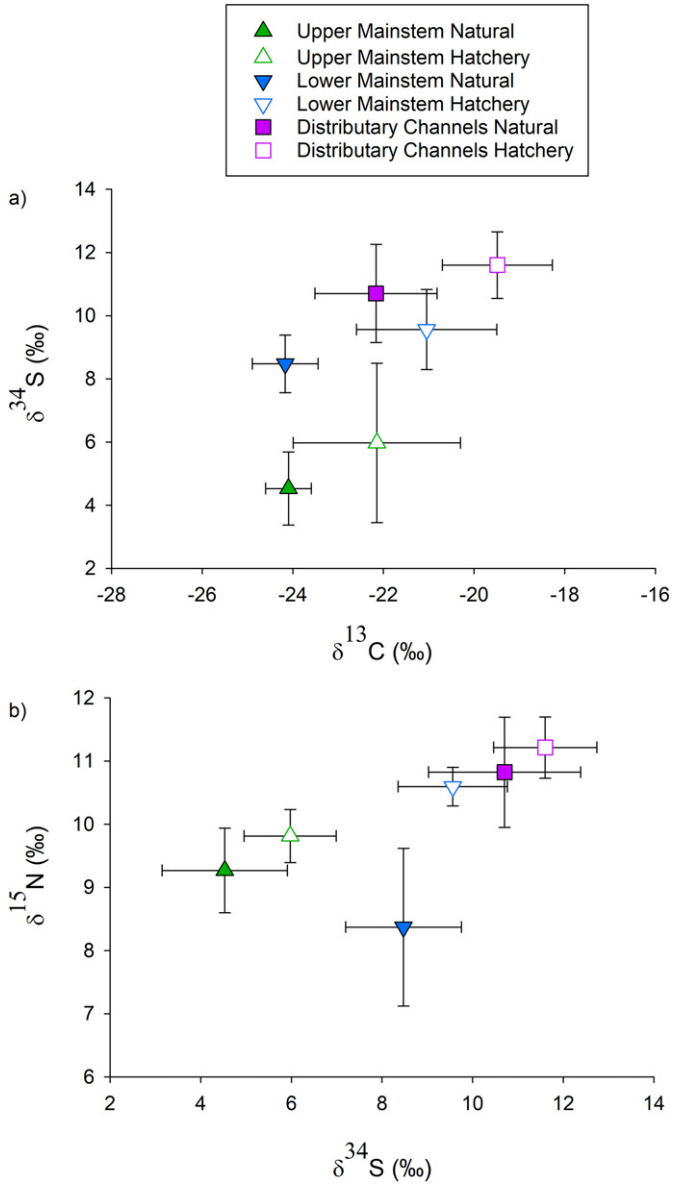

Fig. 5. Stable isotopes of a) sulfur (δ^{34}S) and carbon (δ^{13}C) and b) nitrogen (δ^{15}N) and sulfur, measured in natural- and hatchery-origin juvenile Chinook salmon (mean ± 95% CI) collected from three regions of the Snohomish River estuary.

4.1. POP concentrations

As hypothesized, POPs concentrations, and $\sum_{11}$PBDEs in particular, were greatest in salmon sampled from the Lower Mainstem, nearest a high volume wastewater outfall, suggesting a wastewater source. Natural-origin fish from the Lower Mainstem had $\sum_{11}$PBDE concentrations 4–10 times higher than salmon from other regions, regardless of origin, indicating the natural-origin fish were most exposed in this

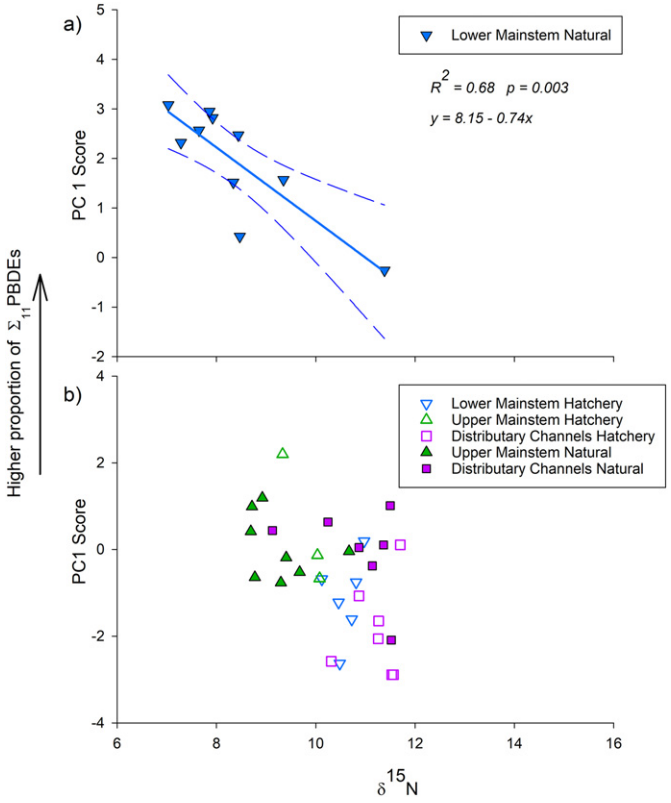

Fig. 6. Relationship between PC1 score and δ^{15}N showing a significant inverse relationship for a) natural-origin fish collected from the Lower Mainstem, but no relationship for b) each of the other region and origin sampling groups (i.e., $p > 0.05$) for each group.

region. Similar but less pronounced patterns were measured for TPCBs; concentrations in fish from the Lower Mainstem were approximately twice as high as those in fish from the less developed Distributary Channels and the Upper Mainstem, however, TPCBs did not differ by fish origins. Unlike $\sum_{11}$PBDEs and TPCBs, $\sum_{6}$DDT concentrations were uniformly low in all regions sampled.

The best-fit models for TPCB and $\sum_{11}$PBDE concentrations measured in juvenile salmon in this study support the conclusion that POP concentrations were determined primarily by the sampling region where the fish were captured (i.e., TPCBs) or the sampling region and the origin of the salmon (i.e., $\sum_{11}$PBDEs), rather than fish size or lipid content (Table S1). Although lipids can affect contaminant uptake (Elskus et al., 2005; West et al., 2017), the small range of lipid values measured in the juvenile Chinook salmon in this study likely dampened the importance of this factor. Likewise, fish length was only a factor for $\sum_{6}$DDT concentrations (Table S1), but this potential effect was obscured by the small range in fish sizes and differential size distributions between natural- and hatchery-origin fish. The inverse relationship between $\sum_{6}$DDT concentrations in the natural–origin salmon (Fig. 3), was consistent with previous studies documenting maternal transfer of DDTs to eggs and fry (Miller, 1994), and subsequent growth dilution. Given the limited size range of hatchery-origin fish collected, we cannot test for the presence of maternal transfer and growth dilution in these fish. POPs in maturing female Pacific salmon are transferred to the developing eggs (deBruyn et al., 2004; Ewald et al., 1998; Miller, 1993). Estimated $\sum_{6}$DDT concentrations in newly emerged Chinook salmon would range from 0.9 and 7 ng/g ww, based on a range of $\sum_{6}$DDT concentrations measured in muscle tissue of adult Chinook salmon (4.3–59 ng/g ww) returning to Puget Sound rivers (West et al., 2001) and correlations between POP concentrations in muscle and fry of Chinook salmon (Miller, 1994). Notably, the estimated maximum $\sum_{6}$DDT concentration encompassed the highest $\sum_{6}$DDT concentrations (i.e., 5.7–7.0 ng/g ww) we measured in small (≤42 mm) natural-origin fish, which are just a few mm larger than newly emerged Chinook salmon prior to exogenous feeding (Beacham and Murray, 1990), supporting the hypothesis that the elevated $\sum_{6}$DDTs in the smaller natural-origin fish we sampled were maternally derived. The lack of relationship between $\sum_{6}$DDT concentrations and fish length in hatchery-origin fish is likely due to the lack of availability of small fish (i.e. hatchery-origin fish are not released until they reach approximately 65 mm) and subsequent sampling of hatchery-origin fish after growth dilution occurred. Moreover, these observations suggest DDTs were not present in the Chinook salmon prey in this system in great enough quantities to overcome growth dilution.

The higher $\sum_{11}$PBDE concentrations in natural-origin fish from the Lower Mainstem compared to the natural-origin salmon from other regions, suggests a higher input of PBDEs into this region of the Snohomish River estuary. However, the higher $\sum_{11}$PBDE concentrations in natural-origin fish from the Lower Mainstem compared to the hatchery-origin fish from the same regions suggests fish of different origins were not equally exposed to the higher inputs of PBDEs. Natural-origin juvenile Chinook salmon were primarily exposed to and accumulated $\sum_{11}$PBDEs at two sites within the Lower Mainstem of the Snohomish River estuary, both located in the immediate vicinity of an Everett WPCF outfall and multiple CSOs. In contrast, hatchery-origin salmon from the same region accumulated lower $\sum_{11}$PBDE concentrations, likely because they moved through the estuary more rapidly than natural-origin fish (Levings et al., 1986; Rice et al., 2011) or they spent less time in the tidally influenced mesohaline area of the estuary (Davis et al., 2018) where wastewater was discharged. Davis et al. (2018) documented that seaward migrating juvenile Chinook from another river estuary in Puget Sound exhibited distinct habitat use patterns; natural-origin fish were more frequently captured in the tidally influenced freshwater and mesohaline habitats whereas hatchery-origin fish were more frequently captured in the nearshore intertidal habitat (Davis et al., 2018).

Concentrations of TPCBs were similarly elevated in natural- and hatchery-origin juvenile Chinook salmon, suggesting that although TPCBs inputs were greater in the more developed Lower Mainstem region of the estuary compared to other regions, the inputs were likely from more dispersed sources throughout the region, and not high enough to disproportionately elevate concentrations for natural-fish that likely resided in the area for a longer time.

Previous contaminant studies in juvenile Chinook salmon have also documented elevated levels of POPs in this species, especially those sampled from moderately to highly urbanized rivers and estuaries of Puget Sound (Johnson et al., 2007a; Meador et al., 2010; O'Neill et al., 2015; Olson et al., 2008; Sloan et al., 2010) and the lower Columbia River and Washington and Oregon coasts (Johnson et al., 2013; Johnson et al., 2007b; Sloan et al., 2010). The $\sum_{11}$PBDE concentrations we measured in natural-origin Chinook salmon in the Lower Mainstem were 2 to 24 times higher than concentrations in natural- and hatchery-origin fish from other Puget Sound estuaries and nearshore marine habitats (O'Neill et al., 2015; Sloan et al., 2010), but they were lower than the highest concentrations measured in samples collected from the Columbia River near areas with high inputs of wastewater (Sloan et al., 2010). Additionally, the Snohomish River estuary appears to be a consistent but possibly decreasing PBDE hotspot for seaward migrating juvenile Chinook salmon. Mean concentration of $\sum_{11}$PBDEs in the natural-origin Chinook salmon in the Lower Mainstem in this study (29 ng/g ww) were similar to those measured in natural-origin fish at the same location in 2013 (24 ng/g ww) but half (1100 vs. 2400 ng/g lipid weight) those measured in 2006 by Sloan et al. (2010), potentially indicating a decline in PBDEs as has been observed for other fish species in Puget Sound (West et al., 2017). Alternatively, the higher PBDE concentrations measured by Sloan et al. (2010) could be associated with differences in the mean fish length (100 vs. 66.9 mm) or sampling time (August vs April–July) compared to the present study. Concentrations of TPCBs in juvenile Chinook salmon from our study were similar

to those measured in 2013 (30 vs. 27 ng/g ww) at the same sampling location in the Lower Mainstem (O'Neill et al., 2015). The TPCB concentrations we measured in salmon were higher than those measured at rural river and estuary sites in the Pacific Northwest (Johnson et al., 2013; Johnson et al., 2007a; Johnson et al., 2007b), but below those generally observed at heavily urbanized estuaries in Puget Sound (Johnson et al., 2007a; Meador et al., 2010; Olson et al., 2008) and the Columbia River (Johnson et al., 2013; Johnson et al., 2007b). In contrast to TPCBs and $\sum_{11}$PBDEs, the $\sum_{6}$DDT concentrations measured in juvenile Chinook salmon from the Snohomish River estuary were not elevated compared to other sites in Puget Sound in 2013 (O'Neill et al., 2015). Higher DDT concentrations were measured in juvenile Chinook salmon from the Columbia River basin from 2005 to 2009, approximately 8 to 12 times higher than those we measured in the Snohomish River, possibly associated with the high degree of agricultural activity in the interior Columbia River as well as Willamette basins and point sources within Portland Harbor (Johnson et al., 2013).

Concentrations of $\sum_{11}$PBDEs, and to a lesser extent TPCBs, we measured in juvenile Chinook salmon in the Snohomish River estuary were high enough to pose a conservation threat. Based on published laboratory exposure studies (Arkoosh et al., 2010, 2018; Meador et al., 2002), the concentrations of these POPs in some Chinook salmon were within ranges of adverse CBRs known to impair their health. Approximately 73% and 14% of the natural-origin Chinook sampled from the Lower Mainstem and the Distributary Channels, the two regions receiving wastewater effluent discharges, had concentrations of BDE congeners 47 and 99 (Table 3), the two congeners detected most frequently and at the highest concentrations, within the range of concentrations found to alter their immune response and increase disease susceptibility (Arkoosh et al., 2010, 2018). In contrast, none of the natural-origin Chinook salmon from the Upper Mainstem or hatchery-origin Chinook salmon from this study had $\sum$PBDE$_{47+99}$ levels high enough to predict altered immune response.

Impairment of immune response is of particular concern for salmonids because a properly functioning immune system is vital for both individual survival and population productivity (Segner et al., 2003). Seaward migrating salmonids are exposed to a number of naturally occurring pathogens and parasites, including the trematode *Nanophyetus salmincola* (Arkoosh et al., 2004). Exposure to PBDEs and other POPs may reduce the marine survival of juvenile salmonids due to immune suppression, thus increasing their susceptibility to naturally occurring infectious and parasitic diseases, causing direct mortality or indirect mortality via predation by larger fish, birds and mammals. For example, Hostetter et al. (2011) reported steelhead (*O. mykiss*) smolts that tested positive for pathogens were more likely to have poor external condition (i.e., external signs of disease or more scale loss). Moreover, tagged fish with poor external condition were subsequently observed to have lower overall marine survival (Hostetter et al., 2011), associated with increased avian predation (Hostetter et al., 2012). In addition to directly impairing the immune function of salmonids, exposure to POPs has been documented to work in conjunction with naturally occurring parasites (i.e., trematode exposure) further increasing their susceptibility to a naturally occurring marine bacterial pathogen (Jacobson et al., 2003), potentially leading to population level effects (Arkoosh et al., 1998; Loge et al., 2005; Meador, 2014; Spromberg and Meador, 2005). Chen et al. (2018) suggested the exposure to POPs and *N. salmincola* serve as mortality cofactors for juvenile steelhead from Puget Sound, with the proximate cause of death involving bacterial pathogens or selective predation of infected cohorts.

Based on lipid normalized TPCB concentrations (ng/g lw) measured in salmon from the Upper Mainstem, Lower Mainstem and Distributary Channels, 0%, 27%, and 29%, respectively of the natural–origin fish and 0%, 14% and 0%, respectively of the hatchery-origin fish, had concentrations above an adverse CBR threshold for total PCBs (Meador et al., 2002). Published CBR thresholds based on individual congeners were not available for salmon. These lipid normalized values likely underestimate the number of impaired fish because juvenile salmon rapidly metabolize lipids as they migrate downstream, typically achieving lipid concentrations of 1% or less by the time they move from the estuary to marine waters (Arkoosh et al., 2011; O'Neill et al., 2015). For example, modeling a 1% lipid content for the natural–origin fish from the Lower Mainstem to predict their increased risk after lipids have been metabolized, would increase the number of fish above the PCB CBR from 27% to 64% for natural-origin fish and 14% to 29% for hatchery-origin fish, potentially increasing the likelihood of reducing their marine survival. Indeed, Meador (2014) documented that hatchery Chinook salmon originating from Puget Sound rivers with contaminated estuaries, including the Snohomish River, have lower marine survival than those originating from uncontaminated rivers.

4.2. POP fingerprints

Analyses of POP fingerprints in salmon from the three regions support the hypothesis that salmon in the Lower Mainstem are exposed to a contaminant source influenced primarily by wastewater rather than stormwater. Except for the hatchery fish from the Upper Mainstem, natural-origin Chinook salmon from the Lower Mainstem had distinct POP fingerprints from all other sampling groups (Fig. 4, Table 4), with high relative concentrations of $\sum_{11}$PBDEs. The POP fingerprints in natural-origin fish from the Lower Mainstem overlapped with those of hatchery-origin fish from the Upper Mainstem ($R = 0.251$) and the p value was 0.052, suggesting that the difference between these groups may not be statistically significant. However, the small sample size ($n = 3$) representing the hatchery-origin fish from the Upper Mainstem, limited our ability to adequately evaluate a significant difference between these groups should one exist. Although POPs can enter the Snohomish River estuary via various sources such as WWTPs, stormwater, or atmospheric deposition, wastewater is considered to be the primary source for PBDEs in Puget Sound, whereas stormwater is the greater source for PCBs (Osterberg and Pelletier, 2015). Modeled loading of contaminants to Puget Sound indicated that most PBDEs enter Puget Sound via publically owned WWTPs, followed by stormwater related surface runoff, and then atmospheric deposition (9.91, 4.56, and 3.49 kg/year, respectively) (Osterberg and Pelletier, 2015). In contrast, Osterberg and Pelletier (2015) concluded that most PCBs enter Puget Sound via stormwater surface runoff (4.17 kg/yr), with considerably less entering via publically owned WWTPs and atmospheric deposition (0.32 and 0.43 kg/yr). In the year we conducted our study, the Lower Mainstem received wastewater DIN loads 1.5 times higher than those in the Distributary Channels (Table 1), and the Upper Mainstem region did not receive direct input of wastewater effluent. Although we do not have estimates of stormwater loads to the three regions of the Snohomish River estuary sampled by our study, loadings from surface runoff are likely highest in the Lower Mainstem region, based on the high percentage (41–94%) of impervious surface area in the lands adjacent to this region of the river (Fig. 1), potentially contributing to the higher concentrations of TPCBs in both natural-and hatchery-origin fish from this location. However, stormwater loadings to the Snohomish River are likely lower than those of more urbanized rivers because measured PCBs in juvenile Chinook from the Snohomish are much less than those measured in other more urbanized estuaries in the Puget Sound (Johnson et al., 2007a; Meador et al., 2010; O'Neill et al., 2015; Olson et al., 2008) and the Columbia River (Johnson et al., 2013; Johnson et al., 2007a; Johnson et al., 2007b).

Contaminant fingerprints are well established chemical tracers for providing information about the sources of POPs and movement patterns of migratory animals (Ramos and González-Solís, 2012), but typically over a broader geographic areas than evaluated in this study. For example, Krahn et al. (2007) used ratios of PCBs and DDTs acquired by migratory killer whales, to discriminate differences in feeding areas and contaminant sources for three pods of whales that forage along

the west coast of North America. In contrast, we used variation in POP fingerprints in juvenile salmon sampled over <30 rkm to identify a PBDE contaminant-source, indicating the robustness of POPs fingerprint at discriminating contaminant sources along a contaminant gradient.

4.3. Stable isotopes

Isotopic signatures of salmon, especially $\delta^{15}N$, from three regions of the Snohomish estuary (Fig. 5) also support the hypothesis that natural-origin salmon from the Lower Mainstem region were exposed primarily to a wastewater source rather than a stormwater contaminant source. Stable isotopic signatures of nitrogen in biota are tools to assess assimilation of wastewater-derived sources of nitrogen into aquatic food webs (deBruyn and Rasmussen, 2002; Savage, 2005). In addition to the ambient nitrogen load in the river, nitrogen in wastewater is incorporated into aquatic food webs though the uptake of sewage-derived nutrients by primary producers or consumption of particulate-organic matter by primary consumers (Tucker et al., 1999), and then subsequently transferred through the food web (McClelland et al., 1997; Vander Zanden et al., 2005). Incorporation of wastewater-derived nitrogen sources into the food web, beyond the background river nitrogen, causes shifts in nitrogen stable isotopes in aquatic organisms when compared to background or reference values in both freshwater (deBruyn and Rasmussen, 2002; Hicks et al., 2017; Loomer et al., 2015; Steffy and Kilham, 2004) and marine systems (Savage, 2005; Schlacher et al., 2005; Tucker et al., 1999). However, the extent to which biota exposed to wastewater have altered $\delta^{15}N$ values depends on the treatment processes used at the plant, effluent quality (e.g., concentration and load of ammonia/ammonium), and the characteristics of the receiving waters (Hicks et al., 2017).

Depleted $\delta^{15}N$ in natural-origin fish from the Lower Mainstem suggests they were exposed to sewage characterized by relatively high nutrient concentrations. In contrast, the $\delta^{15}N$ in the hatchery-origin fish from this region was not depleted, suggesting they were less exposed to nutrient rich wastewater effluent. Complex treatment processes determine the amount of nutrient removal, and whether dissolved inorganic nitrogen in effluent is discharged as ammonia/ammonium, nitrite or nitrate (Metcalfe et al., 2003). WWTPs designed to optimize removal of nutrients from wastewater typically use nitrification (conversion of ammonia to nitrate) followed by de-nitrification (conversion of nitrate to nitrogen gas) processes to remove nitrogen. In contrast, WWTPs designed without specific nutrient removal, discharge effluent with more ammonium than nitrates (Hicks et al., 2017; Loomer et al., 2015). Furthermore, nitrification, denitrification, as well as volatilization of wastewater, can alter the concentration and the nitrogen isotopic signature of the pools of ammonia/ammonium and nitrate/nitrite they act upon (Heaton, 1986; Valiela et al., 2000), as well as the resulting effluent released to the aquatic systems (Toyoda et al., 2011). Overall, biota exposed to untreated and primary treated sewage, or secondary sewage with insufficient nutrient removal, typically exhibit a depleted $\delta^{15}N$ signal (deBruyn and Rasmussen, 2002; Hicks et al., 2017; Loomer et al., 2015), as we observed in natural-origin Chinook salmon from the Lower Mainstem. Indeed, the form of DIN discharged by the Everett's WPCF is atypical compared to other Puget Sound wastewater facilities that discharge into rivers and nearshore marine receiving waters, with a higher proportion of ammonium compared to nitrates and nitrites (Table 1). Conversely, biota exposed to secondary and tertiary sewage treatment that removes excess nitrogen with nitrifying and denitrifying bacteria typically have an enriched $\delta^{15}N$ signal compared to background values (Heaton, 1986; Savage, 2005; Valiela et al., 2000).

In contrast to nitrogen isotopes, carbon and sulfur stable isotopes were enriched in salmon as they moved downstream (see Fig. 5a), consistent with the frequency and amount of saltwater intrusion into the downstream regions of the Snohomish River (Hall et al., 2018) and a gradual shift to downstream food sources, as noted in salmon from other rivers (Moore et al., 2016). Sulfur and carbon isotopes provide information regarding food sources for consumers, with marine food webs typically more enriched in $\delta^{34}S$ and $\delta^{13}C$ than freshwater systems (Peterson and Fry, 1987), and thus reveal the prey base and movements of animals (Hobson, 1999). Based on Hall et al. (2018), there is a continuum from freshwater in the Upper Mainstem region to more saline waters in both the Lower Mainstem and Distributary Channels regions. The higher $\delta^{34}S$ and $\delta^{13}C$ we measured in salmon in these downstream regions, reflects this salinity gradient and the salmons' changing food supply that is incorporated into their tissues as they migrate downstream. Similarly, Moore et al. (2016) documented that natural-origin juvenile Chinook salmon from the relatively undeveloped watershed of the Skeena River in British Columbia, Canada, became enriched in both $\delta^{13}C$ and $\delta^{34}S$ as they migrated from the headwaters of the river to nearshore marine waters. The slight enrichment of $\delta^{34}S$ and $\delta^{13}C$ in hatchery-origin fish, compared to natural-origin fish from the same region, may be due to the residual influence of the diet of hatchery fish, prior to release from the hatchery (Weber et al., 2002). In the hatchery, fish are fed commercial diets dominated by protein from marine sources enriched in $\delta^{34}S$, whereas, natural-origin fish consume freshwater prey with more depleted $\delta^{34}S$, and the muscle tissue of fish reflect these sources (Weber et al., 2002). However, tissue differences in $\delta^{34}S$ between hatchery- and natural-origin fish will rapidly be masked by the freshwater diet consumed by hatchery-origin fish after they leave the hatchery, given the rapid turnover rates of liver and muscle tissue of juvenile salmonids (Heady and Moore, 2013).

4.4. Complementary chemical tracers

We used multiple, complementary chemical tracers to infer nutrient and contaminant sources to seaward migrating juvenile salmon, more discernable information than either tracer provided individually. Collectively, the isotope tracers and POP fingerprints indicated that natural-origin salmon were exposed to and assimilated both nitrogen and POPs from wastewater in the Lower Mainstem. The $\sum_{11}$PBDE enhanced POP fingerprints in natural-origin fish from the Lower Mainstem were inversely correlated with their $\delta^{15}N$ (Fig. 6), suggesting similar sources for both; the more fish were exposed to the ammonia/ammonium rich effluent, the more depleted they were in $\delta^{15}N$ and the greater their relative $\sum_{11}$PBDE concentrations. Concentrations of $\sum_{11}$PBDEs and TPCBs were also each negatively correlated with $\delta^{15}N$ in salmon. However, the slope of these relationships were steeper for $\sum_{11}$PBDEs (8.93 vs. 6.55), supporting our previous conclusion that the wastewater the fish were exposed to had a greater load of PBDEs than PCBs. Additionally, based on their $\delta^{34}S$, the natural-origin fish that had spent the least amount of time in the Lower Mainstem where the wastewater discharged, deviated most from the predicted relationship between PC1 score and $\delta^{15}N$ (Fig. S3), further supporting our conclusion that fish were exposed to and accumulated PBDEs from a wastewater source in the Lower Mainstem.

These results highlight the role of wastewater as a vector of toxic contaminants to aquatic consumers, as demonstrated previously (Meador et al., 2016; Spies et al., 1989), and raises additional concerns about juvenile salmon exposure to other contaminants in wastewater not evaluated in this study. Effluent from WWTPs are major sources of industrial chemicals (Servos, 1999), pharmaceutical and personal care products, (PPCPs) (Metcalfe et al., 2010), and natural and synthetic hormones (Ternes et al., 1999). Adverse effects observed in aquatic biota exposed to wastewater include endocrine disruption in individuals (Tyler and Jobling, 2008; Vajda et al., 2011), and alterations in species communities (Tetreault et al., 2013). Most pertinent to our study, Chinook salmon collected from wastewater impacted sites had modeled fish plasma concentrations for a variety of PPCPs in the range expected to produce adverse effects in fish (Meador et al. 2017); mitochondrial dysfunction, which is adverse for growing juvenile fish (Yeh et al., 2017); and altered blood chemistry parameters, a potential early indicator of metabolic disruption (Meador et al., 2018).

5. Conclusions

Our study demonstrated the utility of multiple chemical tracers to document the spatial extent, magnitude, and source of contaminant exposure in juvenile Chinook salmon, information necessary to formulate appropriate conservation measures to reduce or remediate contaminant exposure. Three types of complementary chemical tracer data, POP concentrations, POP fingerprints, and stable isotopes, allowed us to 1) identify where in their migration pathway threatened Chinook salmon were exposed to and accumulated PBDEs (and to a lesser extent PCBs), at concentrations high enough to impair their health, and 2) reveal that wastewater discharging into the river was the likely source of these POPs. These results highlight the importance of understanding the role that wastewater may play as a vector of toxic contaminants to aquatic consumers.

Data from this study can be used to guide and prioritize management actions to reduce threats from wastewater and other habitat stressors to juvenile salmon migrating through the Snohomish River estuary to Puget Sound. Specifically, identifying the region within the Snohomish watershed where salmon are most exposed to PBDEs, as well as the source (i.e., wastewater or stormwater), allows environmental managers to establish corrective actions to control PBDE inputs. Ultimately, reductions in PBDE exposure should improve Chinook salmon health and enhance their marine survival. The Snohomish River is the second largest contributor of Chinook salmon to the Puget Sound evolutionarily significant unit (Jonathan Carey, National Marine Fisheries Service, Personal communication); consequently, reductions in salmon survival due to wastewater-contaminant exposure could affect the recovery of the ESA-listed Chinook salmon from Puget Sound. Furthermore, exposure to contaminants in wastewater may thwart substantial habitat remediation efforts underway throughout the US Pacific Northwest to improve survival of natural-origin salmon. For example, between 2005 and 2017 approximately $ 90,000,000 US has been spent to improve the freshwater, estuarine and nearshore marine rearing habitat for natural-origin Chinook salmon originating from the Snohomish River (Snohomish Basin Salmon Recovery Forum, 2019), with the ultimate goal of improving their overall survival. The efficacy of this effort could be reduced if juvenile salmon have increased susceptibility to disease because of exposure to wastewater-derived contaminants. More broadly, Chinook and other salmon species are at risk in much of the southern part of their North American range (Gustafson et al., 2007), where interactions with many anthropogenic factors affect them, including contaminants (Lundin et al., 2019; Meador, 2014).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary Material

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Chemical tracers guide identification of the location and source of persistent organic pollutants in juvenile Chinook salmon (*Oncorhynchus tshawytscha*), migrating seaward through an estuary with multiple contaminant inputs

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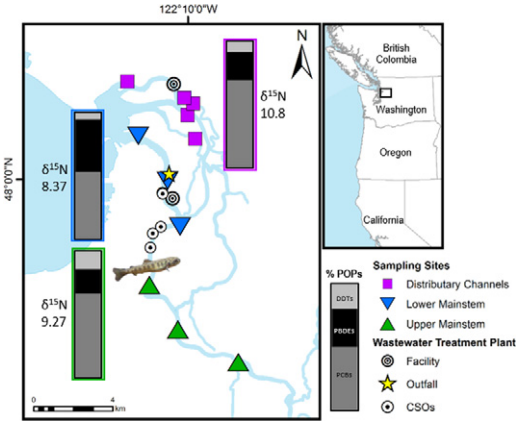
HIGHLIGHTS

- Three chemical tracers identified a contaminant source for seaward migrating salmon.
- Salmon collected near a wastewater outfall had higher contaminant concentrations.
- Salmon near the outfall had distinct combinations of contaminants (fingerprint).
- Altered $\delta^{15}N$ signatures were correlated with distinct contaminant fingerprints.
- Wastewater was the source for both distinct fingerprint and altered $\delta^{15}N$ signature.

GRAPHICAL ABSTRACT



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ABSTRACT

Understanding the spatial extent, magnitude, and source of contaminant exposure in biota is necessary to formulate appropriate conservation measures to reduce or remediate contaminant exposure. However, obtaining such information for migratory animals is challenging. Juvenile Chinook salmon (*Oncorhynchus tshawytscha*), a threatened species throughout the US Pacific Northwest, are exposed to persistent organic pollutants (POPs), including polybrominated diphenyl ether (PBDE) flame retardants and polychlorinated biphenyls (PCBs), in many developed rivers and estuaries. This study used three types of complementary chemical tracer data (contaminant concentrations, POP fingerprints, and stable isotopes), to determine the location and source of contaminant exposure for natural- and hatchery-origin Chinook salmon migrating seaward through a developed watershed with multiple contaminant sources. Concentration data revealed that salmon were exposed to and accumulated predominantly PBDEs and PCBs in the lower mainstem region of the river, with higher PBDEs in natural- than hatchery-origin fish but similar PCBs in both groups, associated with differences in contaminant inputs and/or habitat use. The POP fingerprints of the natural-origin-fish captured from this region were also distinct from other region and

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Wastewater
Stormwater

origin sample groups, with much higher proportions of PBDEs in the total POP concentration, indicating a different contaminant source or habitat use than the hatchery-origin fish. Stable isotopes, independent tracers of food sources and habitat use, revealed that natural-origin fish from this region also had depleted $\delta^{15}N$ signatures compared to other sample groups, associated with exposure to nutrient-rich wastewater. The PBDE-enhanced POP fingerprints in these salmon were correlated with the degree of depletion in nitrogen stable isotopes of the fish, suggesting a common wastewater source for both the PBDEs and the nitrogen. Identification of the location and source of contaminant exposure allows environmental managers to establish conservation measures to control contaminant inputs, necessary steps to improve the health of Chinook salmon and enhance their marine survival.

1. Introduction

An understanding of the spatial extent, magnitude, and source of contaminant exposure in biota is necessary to formulate appropriate conservation measures to reduce or remediate contaminant exposure. In some cases, there is an obvious point source of the contaminants, but in other instances the sources may be cryptic or dispersed, making them more difficult to identify and remediate. Obtaining such information for migratory animals is especially challenging because their routes may traverse habitats exposing them to different contaminants from multiple sources. Persistent organic pollutants (POPs) are contaminants of global concern because of their persistence, bioavailability, and toxicity (Jones and de Voogt, 1999). POPs include a wide variety of toxic chemicals, including polychlorinated biphenyls (PCBs), polybrominated diphenyl ether flame-retardants (PBDEs), and chlorinated pesticides such as dichlorodiphenyltrichloroethane and its metabolites (DDTs). All POPs are slowly metabolized, bioaccumulate in lipid-rich tissues, and biomagnify in the food web (Aguilar et al., 2002; Borrell et al., 2006; Jones and de Voogt, 1999; Tierney et al., 2014).

Proximity to contaminated habitats and the associated POPs in prey are the primary factors for determining the extent to which POPs are accumulated by fishes (Good et al., 2014; O'Neill and West, 2009; West et al., 2008) and marine mammals (Aguilar et al., 2002; Borrell et al., 2006). However, duration of exposure and body condition, including lipid content, reproductive status, and trophic position, can also affect accumulation (Aguilar et al., 1999; Burreau et al., 2006; Fisk et al., 2001; West et al., 2017). For migratory animals, the link between contaminated habitats and POP concentrations can be further obscured by multiple POP inputs (Borrell et al., 2006) as the animals move between habitats.

Although POPs can adversely affect animal health, the proportion of different types of POPs also serve as chemical tracers elucidating information about the trophic ecology, migration patterns, and population structure, for many migratory species (Ramos and González-Solís, 2012) including Atlantic salmon, *Salmo salar* (Svendsen et al., 2009), bluefin tuna, *Thunnus thynnus* (Deshpande et al., 2016a), harbor porpoise, *Phocoena phocoena* (Calambokidis and Barlow, 1991), beluga whales, *Delphinapterus leucas* (Krahn et al., 1999) and killer whales, *Orcinus orca* (Krahn et al., 2007). Additionally, the proportions of different types of POPs have been used to identify sources of POPs in Pacific herring, *Clupea pallasii* (West et al., 2008), bluefish *Pomatomus saltatrix* (Deshpande et al., 2016b), and bottlenose dolphins, *Tursiops truncatus* (Fair et al., 2010).

Current and historical inputs of POPs create environments with distinct chemical proportions or "fingerprints." Given sufficient foraging time, migratory animals accumulate POPs in proportion to their availability in the environments through which they migrate. Furthermore, unlike an individual POP concentration, POP fingerprints are less influenced by individual biological traits (Borrell et al., 2006; Dickhut et al., 2009; Svendsen et al., 2008), such that changes in POP fingerprints in animals along their migration route can indicate different inputs or sources of contaminants in prey along their migration route.

Naturally occurring stable isotopes of carbon, nitrogen, and sulfur also serve as chemical tracers, providing insights into ecological processes and patterns (Boecklen et al., 2011; Newsome et al., 2010; Peterson and Fry, 1987; Thompson et al., 2005). Stable isotopes of nitrogen are frequently used to indicate diet and trophic status (Caut et al., 2009; Olson et al., 2010; Ramos et al., 2011) because consumers accumulate higher levels of $\delta^{15}N$ than their prey. Nitrogen isotopes have also been used to assess exposure to sewage and wastewater inputs (Cabana and Rasmussen, 1996; Loomer et al., 2015; Schlacher et al., 2005), and they can reveal possible exposure to contaminants associated with the wastewater (Spies et al., 1989). Stable isotopes of sulfur and carbon are typically only slightly enriched between trophic levels. Instead, these stable isotopes are typically used as tracers of the types of food sources and have been used to assess habitat use (Connolly et al., 2004; Moore et al., 2016), and migratory patterns (Graham et al., 2010; Hobson, 1999). Sulfur stable isotopes are an especially good source indicator of terrestrial vs. marine producers, with more enrichment of heavier isotopes in marine systems (Thode, 1991; Willacker et al., 2017), and have been used to track residency in estuarine fishes (Fry and Chumchal, 2011) and movements of fish between freshwater and marine systems (Godbout et al., 2010; Moore et al., 2016). Moreover, when stable isotopes of sulfur, nitrogen and carbon are used together they can provide additional information on habitat use and trophic structure than stable isotopes of carbon and nitrogen alone (Connolly et al., 2004). Because stable isotopes fractionate with the organism's metabolism and change with its diet (Hobson, 1999), whereas POPs are not readily metabolized nor eliminated, they provide complementary information about the organism (Fisk et al., 2002; Herman et al., 2005; Ramos and González-Solís, 2012).

Pacific salmon of the genus *Oncorhynchus* exemplify organisms whose migrations take them through multiple habitats, including some where contaminants pose a concern (Johnson et al., 2007a; O'Neill and West, 2009; Ross et al., 2013). Spawned in cool, clear streams and other freshwater habitats, the juveniles feed for a period prior to seaward migration that varies among species, populations, and individuals (Quinn, 2018). Spawning typically takes place high in watersheds where contaminant concentrations are low, but their seaward migration may lead the juveniles through agricultural, industrial, and urbanized areas, each with different classes of contaminants.

In large parts of the southern portion of their native range, Pacific salmon species have experienced declines in abundance sufficient to limit fisheries, resulting in listings under the U.S. Endangered Species Act (ESA), and even lead to extinction. This loss of intra-specific diversity (Gustafson et al., 2007) has many causes, and the relative importance of each varies among watersheds (NRC, 1996) but chemical contaminants can contribute to poor survival of juveniles in populations migrating through contaminated habitats (Johnson et al., 2013; Meador, 2014).

Chinook salmon, *O. tshawytscha*, is listed as Threatened under the US ESA in Puget Sound, Washington, where individuals spawn in a number of large and medium-sized rivers (Myers et al., 1998; Ruckelshaus et al., 2006). The Snohomish River is typical of these, and is characterized by

headwaters in forested land with few major sources of contaminants, with a transition to areas dominated by agriculture and increasingly suburban, urban and industrial areas where they flow into Puget Sound, Washington (Pess et al., 2002). Survival rates of juvenile salmon entering Puget Sound have been low for several decades (Quinn et al., 2005) but vary among rivers (Ruff et al., 2017), indicating that local as well as regional factors affect survival. Some of this variation has been linked to the extent to which the natal estuary has been modified from its natural condition (Magnusson and Hilborn, 2003), including chemical contamination (Meador, 2014). Moreover, natural-origin fish migrate more slowly and reside and feed in estuaries for longer periods than hatchery-origin fish (Levings et al., 1986; Rice et al., 2011), potentially resulting in greater contaminant exposure for natural-origin salmon.

Our goal was to use complementary data types to assess the location and source of contaminant exposure for juvenile Chinook salmon migrating through habitats with multiple contaminant sources, notably wastewater and stormwater. This study was conducted in the Snohomish River Washington, where two previous studies (O'Neill et al., 2015; Sloan et al., 2010) documented elevated levels of PBDEs (a POP class associated with wastewater; Osterberg and Pelletier, 2015) in juvenile Chinook salmon, at concentrations high enough to alter their immune response and increase their susceptibility to naturally occurring diseases, based on laboratory exposure studies (Arkoosh et al., 2010, 2018). The specific objectives were to determine where in their migratory pathway salmon become exposed to potentially harmful concentrations of PBDEs, and to identify potential sources so that corrective actions could be identified. We measured levels of PBDEs, other POPs, and stable isotopes of nitrogen, sulfur, and carbon in salmon collected along their migration routes in the estuarine portions of the Snohomish River. We hypothesized that Chinook salmon caught in the more developed reaches of the river, near wastewater inputs, would exhibit higher concentrations of PBDEs and that their POP fingerprints would have a higher proportion of PBDEs compared to other POPs, more indicative of a wastewater source. We further hypothesized that altered stable isotope ratios of nitrogen would be observed in fish captured in the vicinity of the wastewater inputs, and associated with the amount and type of nitrogen discharged. Additionally, this population includes Chinook salmon spawned naturally in the river and ones produced in a hatchery and we predicted that the natural-origin fish would exhibit higher POP concentrations associated with their higher residence time in the estuaries (Levings et al., 1986; Rice et al., 2011).

2. Material and methods

2.1. Study area

The Snohomish River watershed, in western Washington State, drains approximately 4600 km^2 into Puget Sound (USGS, 2011), and is formed by the confluence of the Skykomish and Snoqualmie rivers. It flows approximately 37 km to Puget Sound via a mainstem and a complex system of deltaic braided distributary channels through Union, Steamboat, and Ebey sloughs (Hall et al., 2018). The Snohomish River estuary's tidal influence extends throughout the distributary channels and up the mainstem to river kilometer (rkm) 27 (Collins and Sheikh, 2005). The maximum extent of saltwater (0.5 ppt) intrusion also extends throughout the distributary channels and to 15.9 rkm on the mainstem channel (Hall et al., 2018). Overall, 75% of the upland areas of Snohomish River basin is forested (Pess et al., 2002). In contrast, land cover in the floodplains and neighboring foothills along the major river channels are much more impacted by human activities, predominantly rural-residential, agricultural, and urban (Pess et al., 2002).

Modern human activities in the Snohomish River estuary have resulted in degradation and loss of juvenile salmonid habitat, considered the primary factor limiting Chinook salmon survival in the basin (Snohomish Basin Salmon Recovery Forum, 2005). Currently available wetland habitat area in the Snohomish estuary is estimated at 1389 ha; roughly 20% of the historical habitat extent in the delta (Beechie et al., 2017; Collins and Sheikh, 2005). The majority of the remaining available rearing habitat for juvenile Chinook salmon is located in the lower estuary (1238 ha) and distributed unevenly between the mainstem (88 ha) and the distributary (1150 ha) portions of the delta (Beechie et al., 2017).

Contaminant inputs likely coincide with the physical habitat loss in the Snohomish estuary. In particular, developed habitats with impervious surfaces adjacent to the river likely increase loadings of contaminants in stormwater to the river, as has been demonstrated for other aquatic systems (Brown and Peake, 2006; Lee et al., 2004; McCarthy et al., 2008). Indeed, stormwater was documented to be a major source of PCB loading to Puget Sound, with developed lands with more impervious surface contributing higher loads of PCBs to the watershed (Osterberg and Pelletier, 2015). Although specific inputs on PCBs to the Snohomish River were lacking, we used impervious surface and road area as proxies for urbanization in this study area and potential inputs of PCBs. The metric utilized for impervious surface was calculated by determining the "percent developed imperviousness", %IS (Fry et al., 2011; Wickham et al., 2013) within predefined watershed catchment areas called Assessment Units (AUs). The %IS values in our study ranged from 0 to 94%, with the most impervious surfaces (41–94%) in the City of Everett, located on the lower section of the mainstem, and the city of Marysville, located in the lower section of Ebey Slough (Fig. 1).

In addition to contaminant inputs from stormwater, the cities of Marysville and Everett primarily discharge treated wastewater, a potential source of PBDEs and other contaminants, into the estuarine portion of the Snohomish River. Specific levels of PBDEs discharged into the Snohomish River are unknown, as wastewater dischargers in WA State are not required to monitor PBDEs in their effluent. Everett's Water Pollution Control Facility (WPCF) is adjacent to the mainstem of the Snohomish River. The facility operates two outfalls and 13 combined sewer overflows (CSOs). One outfall and six CSOs discharge into the lower section of the river's mainstem and the others discharge into the marine waters of Port Gardner in Possession Sound (Fig. 1). The WPCF uses an aeration/oxidation pond (lagoon) system for treating the wastewater that discharges to the Snohomish River outfall (WA Dept. Ecology, 2015). Marysville's WWTP is located in the Distributary Channels, adjacent to Ebey Slough and uses an aerated lagoon with a filtration system to treat sewage prior to discharge into an outfall in Steamboat Slough or the marine waters of Port Gardner during the summer (WA Dept. Ecology, 2017). While most of the effluent discharged from these two facilities is treated, CSOs release untreated wastewater. CSOs occur on average, 1–2 times a year.

Discharges of dissolved inorganic nitrogen (DIN) in effluent from municipal WWTPs were used to assess the loads of nitrogen discharged from WWTPs. In total, Puget Sound has 78 municipal WWTPs discharging to Puget Sound – 70 discharge to marine waters and eight discharge to river estuaries. In the year of our study (2016), the Everett WPCF outfall in the Snohomish River had the highest DIN average daily discharge (average of February to July) of the eight facilities that discharge to the estuarine portion of Puget Sound rivers (Table 1). Additionally, the Everett WPCF outfall had the eight highest average daily discharge of DIN of all 78 municipal WWTPs discharging into Puget Sound (Table 1). WWTP loads were originally estimated for the years 1999–2008 using methods described in Mohamedali et al. (2011), and these inputs were updated through mid-2017 as described in Ahmed et al. (2019). During the 6-month migration window for juvenile Chinook salmon (February through July) in 2016, discharged DIN from the Everett WPCF averaged 1006 kg/day (Table 1). The Everett WPCF effluent, however, was atypical compared to other Puget Sound wastewater facilities, and contained a higher proportion of ammonium relative to nitrates and nitrites compared to the effluent from other facilities that discharge into similar waters frequented by juvenile salmon. The

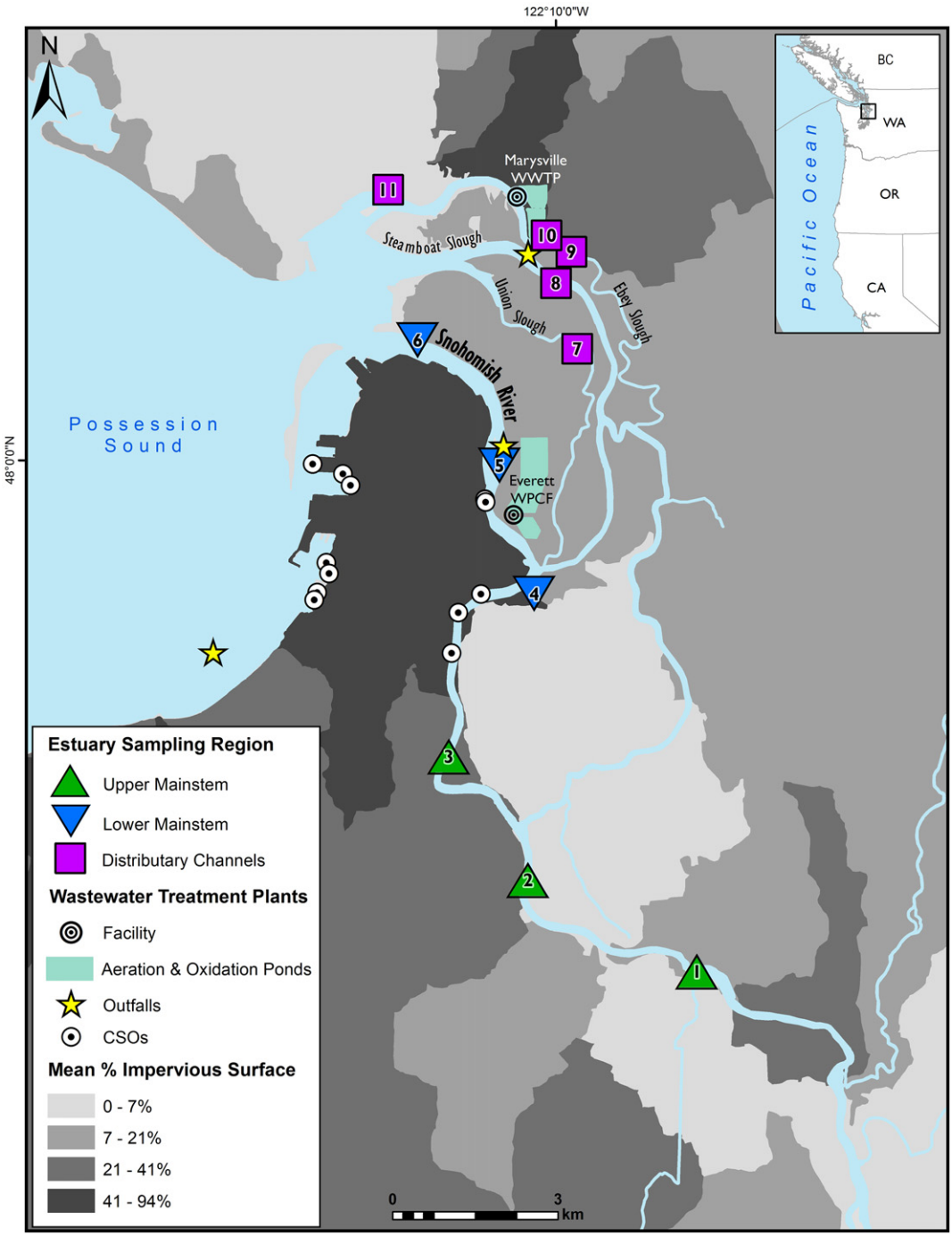

Fig. 1. Location of estuary sampling sites and sampling regions where juvenile Chinook salmon were collected for contaminant and stable isotope analyses (see Table 2 for additional site and sample data). Impervious land-surface is shown as grey scale gradations from <7% (lightest grey) to >40% (darkest grey). WPCF = Water Pollution Control Facility. WWTP = Wastewater Treatment Plant.

only other WWTP facilities discharging into Puget Sound with comparable or higher ammonium loads (>1000 kg/day) were high load facilities (≥250 DIN kg/day) that discharged in deep offshore marine waters, beyond habitats typically used by juvenile salmonids (Table 1).

2.2. Sampling design and fish collections

We sampled juvenile Chinook salmon for chemical tracers from 11 sites along their migration pathway in the estuarine portion of the Snohomish River in 2016. The sites were distributed in three regions: the Upper Mainstem, through which all the fish migrate, and two downstream regions, the Lower Mainstem and the Distributary Channels, that constitute alternative routes by which the fish can enter Puget Sound (Fig. 1). A minimum of three sites per region, distributed along the migration pathway within each region, were sampled to assess the range of stormwater and wastewater inputs that fish were potentially exposed to. Due to limitations on the number of ESA-listed Chinook salmon we were allowed to capture, our sampling design was intended to compare salmon among the three regions, rather than at specific sites. Our three sampling regions roughly represent the major bifurcation in the system based on hydrological properties of the rivers (Collins and Sheikh, 2005; Hall et al., 2018). The Upper Mainstem was the least developed of the three regions, with the most downstream site located 2 to 7 km upstream of the outfalls for the wastewater

Table 1
Mean and range (in parentheses) of daily loads of nitrogen types (DIN, ammonium, and nitrate + nitrite) and ratio of ammonium to nitrate + nitrite in municipal wastewater treatment plant effluent for facilities discharging into Puget Sound rivers and marine waters (Mohamedali et al., 2011; Ahmed et al., 2019), reported for each month from February through July of 2016. Effluent data for the Lower Mainstem and Distributary Channel regions of the Snohomish River sampled in this study are summarized separately from data for other facilities. Facilities discharging mean dissolved inorganic nitrogen (DIN) daily loads >250 kg/day for that six-month window were further categorized as either a freshwater, nearshore or offshore facility depending on the location of their effluent outfall in the receiving waters. All other facilities discharging into nearshore or offshore marine waters with a mean DIN <250 kg/day was placed in the "Other nearshore and offshore marine facilities" category. If load data was missing for a month, it was excluded from the average load calculation.

WWTP	n	DIN (kg/day)	Ammonium (kg/day)	Nitrate + Nitrite (kg/day)	Ratio of Ammonium (kg/day) to Nitrate + Nitrite (kg/day)
Lower Mainstem facility[a]	1	1006 (829–1162)	1002 (822–1161)	4.09 (1.71–6.97)	335 (118–678)
Distributary Channels facility[b]	1	649 (462–816)	628 (452–806)	20.4 (6.56–47.4)	48.3 (14.2–80.2)
Other river facilities (DIN ≥ 250 kg/day)	1	410 (203–583)	325 (126–462)	85.4 (33.2–158)	5.58 (1.64–13.9)
Other river facilities (DIN < 250 kg/day)	5	71.6 (1.61–257)	5.99 (0.005–58.0)	65.7 (1.60–256)	0.019 (0.002–2.14)
Nearshore marine facilities (DIN ≥ 250 kg/day)	3	658 (383–1177)	631 (365–1173)	27.0 (3.63–83.4)	84.7 (5.83–323)
Offshore marine facilities (DIN ≥ 250 kg/day)	13	2004 (229–9543)	1560 (7.87–8684)	445 (0.73–2794)	61.7 (0.0029–1385)
Other nearshore and offshore marine facilities (DIN < 250 kg/day)	54	32.9 (0.0077–352)	17.0 (0.0012–300)	15.9 (0.0012–236)	10.1 (0.00035–416)

[a] Everett Water Pollution Control Facility (see Fig. 1, Everett WPCF).
[b] Marysville Wastewater Treatment Plant (see Fig. 1, Marysville WWTP).

treatment facility and associated CSOs. We assumed that contaminants measured in seaward migrating salmon collected in the Upper Mainstem region represented cumulative exposure from all upstream sources. At each subsequent downstream site in the Lower Mainstem and the Distributary Channels, we assumed contaminant concentrations in salmon indicated additional inputs from stormwater and wastewater to which the salmon were exposed.

Juvenile Chinook salmon were collected in 2016 from April through July, primarily during the peak of downstream migration (April and May) to represent the average contaminant concentrations of the river system's fish populations. All fish were collected with beach seines or fyke nets, following procedures described in Roegner et al. (2009), euthanized, transferred to the laboratory on ice, assigned a unique number, and stored at −80 °C until tissue samples for chemistry and stable isotopes were prepared.

2.3. Sample processing

To process fish for analyses of contaminants and stable isotopes, fish were thawed slightly, fork length (mm) was recorded for each fish, and scales were removed for age determination (sub-yearling vs. yearling). To ensure the gut contents did not influence the contaminant and stable isotope data, they were removed from the stomach and intestine of fish and discarded to create gutted whole body fish samples. Additionally, the brain was removed from each fish for use in a separate study. Each fish was examined for presence of a clipped adipose fin, a coded wired tag (CWT), or thermally marked otoliths, any of which would indicate hatchery-origin fish. Based on thermally marked otoliths, we excluded from our study a few hatchery-origin fish that did not originate from within the Snohomish River, leaving 177 salmon for analyses (Table 2).

Forty-eight composite samples of gutted whole body fish, less the brain, were created by combining 1–8 similarly sized salmon in each sample (Table 2). The samples were homogenized, placed in pre-cleaned glass jars, and stored at −20 °C for subsequent chemical analyses. The proportion of natural-origin fish in each composite sample was used to classify the samples as either predominantly natural-origin (>65%) or hatchery-origin (<35%). In most cases, samples classified as predominantly natural- or hatchery-origin contained only fish of that designation (25 of 30 natural- and 15 of 18 hatchery-origin samples).

2.4. Contaminant analysis

Samples (approximately 2 g from each composite tissue sample) were analyzed for POPs, including 11 PBDEs, 46 PCBs, and six DDTs, using an established gas chromatography/mass spectrometry (GC/MS) method (Sloan et al., 2014). This method comprises three steps: (a) a dichloromethane extraction using an accelerated solvent extractor, (b) cleanup by gravity flow silica/aluminum columns and followed by

Table 2
Number (No.) of individual juvenile Chinook salmon and composite samples (Comps.) sampled in 2016 for contaminant analyses at multiple sites in the estuary habitat of the Snohomish River. Each composite sample is composed of 1–8 individual salmon of similar size and was classified as either natural- or hatchery–origin, based on the proportion of natural fish present. Site numbers refer to the sampling locations depicted in Fig. 1.

Sampling regions	Site No.	Site name	River km	Collection period	Natural origin		Hatchery origin	
					No. fish	No. Comps.	No. fish	No. Comps.
Upper Mainstem	1	Fields Riffle	18.7	April–July	16	5	3	1
	2	Big Tree	14.9	April–July	11	3		
	3	Old Bridge	11.5	April–July	15	4	4	2
Lower Mainstem	4	Old Barge	7.4	April–May	20	4	3	1
	5	Langus Pier	5.2	April–July	31	7	9	3
	6	Lower Mainstem	2.0	May–June			11	3
Distributary Channels	7	Union Slough	5.9	May June	8	2	3	1
	8	Steamboat Slough	4.8	April–May	6	2	3	1
	9	Ebey Slough 1	6.9	April–May	10	2	3	2
	10	Ebey Slough 2	6.4	April–May			1	1
	11	Ebey Slough 3	2.5	May	7	1	12	3
All regions				April–July	124	30	53	18

size-exclusion high-performance liquid chromatography (HPLC) cleanup, and (c) quantitation of POPs using gas chromatography/mass spectrometry (GC/MS) with selected-ion monitoring (SIM). A subsample of each pre-cleaned extract was used to determine percent lipids gravimetrically (Sloan et al., 2014). As part of a performance-based quality assurance program, a solvent (dichloromethane) method blank and National Institute of Standards and Technology (NIST) Lake Michigan fish tissue Standard Reference Material (SRMs, 1947) were analyzed with each batch of field samples and the results of the quality control samples met established laboratory criteria (Sloan et al., 2019). The solvent method blank for each sample batch contained no more than five analytes that exceeded 2 × the lower limit of quantitation (LOQ), which met our laboratory QA criteria. Levels of ≥70% of individual analytes measured in NIST SRM 1947 for each sample batch were within 30% of either end of the 95% confidence interval of the NIST certified values. Surrogate recoveries for the POP analyses ranged from 99 to 116% and met established laboratory criteria (surrogate recoveries are to be between 60 and 130%). Additional details for our laboratory quality assurance measures and criteria for POPs analyses can be found in Sloan et al. (2019). The lower limits of quantitation (LOQ) for individual PBDEs, PCBs, and DDTs measured in the field samples and their associated quality assurance samples ranged from <0.063 to <0.31 ng/g, wet weight.

Analyte data are presented as summed values for PBDEs and DDTs. Summed PBDEs (i.e., $\sum_{11}$PBDEs), were calculated by summing detected concentrations of the congeners 28, 47, 49, 66, 85, 99, 100, 153, 154, 155, and 183. Summed DDTs (i.e., $\sum_{6}$DDTs) were calculated by summing the detected concentrations of *o,p′*-DDD, *o,p′*-DDE, *o,p′*-DDT, *p,p′*-DDD, *p,p′*-DDE, and *p,p′*-DDT. The total PCB concentration (i.e., TPCBs) was estimated using a simple algorithm based on the subset of 17 commonly detected congeners (and coeluting congeners) representing homologues containing three to ten chlorine atoms [congeners 18, 28, 44, 52, 95, 101(90), 105, 118, 128, 138(163,164), 153 (132), 170, 180, 187(159,182), 195, 206, and 209], wherein the sum of the detected values for these 17 (and coeluting) congeners was multiplied by two. The calculated TPCB concentration using this method was previously shown to agree well with the sum of 209 congeners measured by high resolution methods for two fish species (West et al., 2017) and is similar to method used by Lauenstein and Cantillo (1993) discussed in West et al. (2017). Summed or total POP results were expressed as nanogram (ng) per gram of tissue weight (wet weight). Additionally, we calculated POP concentrations on a lipid basis, as ng of contaminant per g of fish lipid (ng/g lipid), to facilitate comparisons with other published studies, including those on adverse critical body residues (CBRs). Published CBRs for POPs are sometimes reported as lipid normalized concentrations because POP toxicity can be inversely dependent on lipid content (Lassiter and Hallam, 1990).

2.5. Stable isotopes analyses

All samples with sufficient tissue mass (41 of 48 samples) were analyzed for stable isotope ratios of nitrogen, carbon, and sulfur. Frozen, non-lipid extracted tissue samples were freeze-dried, ground to a fine powder, and weighed into tin capsules for isotope analyses with a target mass of 0.5 mg of tissue for carbon and nitrogen and 7.5 mg for sulfur. Stable isotope analyses were performed at the University of Washington's IsoLab in Seattle, WA as in Fry et al. (1992) and Fry et al. (2002). A Costech Elemental Analyzer, Conflo III, and Thermo MAT253 isotope ratio mass spectrometer was used for a continuous-flow based measurement of bulk carbon $\delta^{13}C$ and bulk nitrogen $\delta^{15}N$. A Eurovector Elemental Analyzer, Conflo III, and Thermo MAT253 isotope ratio mass spectrometer was used for a continuous flow based measurement of the bulk sulfur $\delta^{34}S$. During analytical runs, these methods provided precisions (1 sigma) of ±0.05‰, ±0.1‰ and ±0.2‰ for carbon, nitrogen and sulfur, respectively.

Stable isotopes of carbon, nitrogen, and sulfur were expressed in standard delta notation ($\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$),

$$\delta\ (‰) = 10^3\ \left[\left(R_{sample}/R_{standard}\right)-1\right],$$

where R is the ratio of heavy and light isotopes in a sample (^{13}C:^{12}C, ^{15}N:^{14}N, and ^{34}S:^{32}S). We expressed stable isotope ratios in units of permil (‰ – parts per thousand) and are relative to international standards: Vienna PeeDee Belemnite (VPDB) for $\delta^{13}C$, atmospheric nitrogen for $\delta^{15}N$, and Vienna–Canyon Diablo Troilite (VCDT) for $\delta^{34}S$. Most (78%) of the fish tissue samples had a C:N ratio greater than the 3.5 threshold for lipid correction (Post et al., 2007), indicating that the lipid content of the samples may have depleted the $\delta^{13}C$ values. Accordingly, we used the following equation from Post et al. (2007),

$$\delta^{13}C_{normalized} = \left(\delta^{13}C_{untreated}-3.32\right) + (0 : 99 \times C : N)$$

to mathematically lipid normalize $\delta^{13}C$ values.

2.6. Data analysis

We applied multiple linear regression (R Development Core Team, 2018) to identify the potential effects of four factors on contaminant concentration for three major POP classes (DDTs, PBDEs, and PCBs). The four predictor variables were: 1) capture region (one of three), 2) fish origin (natural or hatchery), 3) fish size (mean length of fish in composite sample) at capture, and (4) % lipid content. Fish origin was considered a factor because natural-origin fish may reside and feed in estuaries for longer periods than hatchery-origin fish (Levings et al., 1986; Rice et al., 2011), resulting in greater potential exposure. Fish length (an indirect measure of age and duration of exposure) and lipid content were included as factors as they can affect the concentrations of POPs accumulated (West et al., 2017). All POP data were log transformed to meet assumptions of normality and homogeneity of group variances. Additive and interactive effects were evaluated, however, due to limited degrees of freedom, only models with up to three factors were considered. Akaike Information Criterion corrected for small sample size (AICc) and Akaike weights were used to identify the best model to parsimoniously explain the variation in the concentrations of PBDE, PCBs and DDTs data (Akaike, 1974; Burnham and Anderson, 2002). The model with the lowest AICc and highest Akaike weight was considered the best fit. For the best fit model for each POP class, pairwise comparisons were conducted on estimated marginal means using the Holm-Sidak adjustment for multiple comparisons. Test results for pairwise comparisons were considered statistically significant at $p \le 0.05$. Additionally, independent linear regressions were performed for $\sum_{6}$DDT by fish length for each origin.

To illustrate POP fingerprints among the three regions by two origin groups, the proportion of $\sum_{11}$PBDEs and other POPs classes in Chinook salmon samples were compared among the six regions and origin groups using principal component analyses (PCA), as detailed in the software package Primer-E version 6 (Clarke and Warwick, 2006; Clarke and Gorley, 2006). Prior to analyzing with PCA, the POP class data were pretreated by standardizing (i.e., computing the proportional contribution of each POP class concentration to the total POP concentration in each sample) and then transforming the data by taking the square root to reduce the contribution of dominant classes. Similar POP fingerprints among groups would indicate consistent sources of contaminants, whereas dissimilar contaminant patterns would suggest inputs of specific POPs associated with different sources. Pairwise site comparisons of group patterns were conducted with ANOSIM, using the R statistic and p values to identify the main between-group differences. Values of the ANOSIM R statistic range from 0 (i.e., no separation, or complete similarity) to 1.0 (i.e., complete separation, or no similarity) of a population. A p value ≤ 0.05 was used as a guide for determining

whether the measured segregation between groups (i.e., R statistic) was statistically significant.

A two-way ANOVA was used to test for difference in stable isotopes, fish length, and lipid content among regions and between natural and hatchery-origin fish. A Holm-Sidak test was used to conduct pairwise comparisons among group means for each POP class. Probability values were used to help evaluate the significance of differences; a p value ≤ 0.05 was used as a guide to assess whether results for pairwise comparisons were considered statistically significant.

Salmon POP fingerprints (i.e., PCA analyses of the proportion of $\sum_{11}$PBDEs and other POPs), a potential indicator of contaminant source, and δ^{15}N, an indicator of the nitrogen source, were compared to see if they co-varied. For each region and origin sampling group, we used linear regression to test for significant relationships between δ^{15}N and PC1 scores in salmon samples, two independent metrics that can both be affected by wastewater inputs. Additionally, linear regression was used to test for significant relationships between δ^{15}N and $\sum_{11}$PBDEs, and TPCBs.

3. Results

3.1. POPs concentrations

Mean POP wet weight (ww) concentrations in juvenile salmon varied 10-fold for $\sum_{11}$PBDEs as a function of sampling region and origin (naturally or hatchery produced), from 2.4 to 24 ng/g ww, and less so for TPCBs, from 12 to 31 ng/g ww (Table 3). In contrast, mean $\sum_{6}$DDT concentrations were much more similar among regions and origin groups, ranging from 1.7 to 2.9 ng/g ww. Although TPCBs varied less than $\sum_{11}$PBDEs among sampling groups, overall TPCB concentrations were greater than $\sum_{11}$PBDEs, followed by $\sum_{6}$DDTs (Table 3). For example, among fish of the same region, measured mean TPCBs concentrations were 1–3 times greater than $\sum_{11}$PBDE and 5–10 times greater than $\sum_{6}$DDTs for natural-origin fish and 2–6 times greater than $\sum_{11}$PBDEs and 6–16 times greater than $\sum_{6}$DDTs for hatchery-origin fish.

Concentrations of $\sum_{11}$PBDEs and TPCBs in juvenile salmon were best predicted by models that included factors for the collection region and the fish origin, rather than body size (i.e., length) or lipid content, but the importance of these factors varied by POP class (Table S1). Specifically, $\sum_{11}$PBDE concentrations were best predicted by models that included region, origin, and a strong region x origin interaction term (Adjusted r^2 = 0.58; Tables S1, S2). Post-hoc tests identified that natural-origin fish from the Lower Mainstem had the highest $\sum_{11}$PBDE concentrations (p ranged from 0.033 to <0.0001). Overall, concentrations of $\sum_{11}$PBDEs in natural-origin fish from the Lower Mainstem (mean = 24 ng/g ww) were 4–10 times higher than salmon from all other sampling groups, regardless of region or origin (Table 3, Fig. 2). The only other statistically significant difference in $\sum_{11}$PBDE concentrations was between natural- and hatchery-origin salmon from the Distributary Channels (means = 5.7 and 2.4 ng/g ww), representing the second highest and lowest concentrations (Table 3, Fig. 2, p = 0.05). The mean lipid content among samples ranged from 0.97% to 1.6% (Table 3) but did not differ significantly among regions or between fish origins (Two-Way ANOVA, region F = 0.722 and p = 0.492; origin F = 0.783 and p = 0.781). Moreover, models with lipid content as a single factor or in combination with region or origin were poor fits and explained less of the measured variation in $\sum_{11}$PBDEs (Table S1). Although natural-origin fish were smaller than hatchery-origin fish (mean = 65.7 vs. 77.4 mm, Table 3), fish length for combined origins did not differ among regions (Two-Way ANOVA, origin F = 9.910 and p = 0.003, region F = 0.217 and p = 0.806), and models with fish length alone or in combination with region or origin were poor fits and did not contribute substantively to the measured variation of $\sum_{11}$PBDEs (Table S1).

Concentrations of TPCBs were best predicted by sampling region, accounting for 46% of the measured TPCB variation (Table S1). Fish length and lipid content, as individual factors or in combination with region or origin, did not substantively improve the model fit. Overall, fish from the Lower Mainstem had measured TPCB concentrations (30 ng/g ww) approximately twice as high as those from the Upper Mainstem (mean = 13 ng/g ww) and the Distributary Channels (16 ng/g ww; Table 3, Fig. 2, p < 0.0001 for both comparisons), which did not differ from each other (p > 0.05).

Unlike the patterns measured for $\sum_{11}$PBDEs and TPCBs, fish size was significantly correlated with $\sum_{6}$DDT concentrations. The $\sum_{6}$DDT concentration was best predicted by the origin, fish length, and a fish origin-length interaction, accounting for 40% of the measured variation (Table S1). A model with only origin and fish length was not near as good a fit to the actual data (Table S1), indicating that the fish origin-length interaction term was significant. Predicted mean $\sum_{6}$DDT concentrations were higher in natural- than hatchery-origin fish (2.3 vs. 1.7 ng/g ww), based on a mean fish length of 70 mm in the best-fit model regression model (Table S2, Fig. 2c). However, predicted $\sum_{6}$DDT concentrations depicted in Fig. 2c do not fully represent the interaction between fish origin and fish length due to differences in sizes between natural- and hatchery-origin fish. The size of newly emerged natural-origin Chinook salmon prior to exogenous feeding (Beacham and Murray, 1990) are just a few mm smaller than those we sampled from the river, however, hatchery-origin fish are not released to the river until they reach approximately 65 mm, prohibiting full examination of $\sum_{6}$DDT size comparison for fish of both origins from the river. As a result, in addition to the full model, independent linear regressions were performed for $\sum_{6}$DDT by fish length for each origin (Fig. 3). There

Table 3
Arithmetic mean lipid content (Lipids), fork length (FL), and concentrations of $\sum_{11}$PBDE, $\sum$PBDE 47+99, TPCBs, and $\sum_{6}$DDTs of composite samples of Chinook salmon (*Oncorhynchus tshawytscha*). Measured POPs concentrations are reported as ng/g wet weight (ww) and ng/g lipid weight (lw), based on the measured gutted whole body wet weight and lipid content in the fish.

Region	Origin	N	Lipids	FL	$\sum_{11}$PBDE	$\sum_{11}$PBDE	$\sum$PBDE$_{47+99}$[a]	$\sum$PBDE$_{47+99}$	TPCBs	TPCBs[b]	$\sum_{6}$DDTs	$\sum_{6}$DDTs
			(%)	(mm)	ww	lw	ww	lw	ww	lw	ww	lw
Upper Mainstem	Natural	12	1.6	61.3	4.0	270	3.5	240	14	950	2.9	210
	Hatchery	3	0.97	85.7	5.2	640	4.4	540	12	1200	2.0	220
	Natural + hatchery	15	1.4	66.2	4.2	340	3.7	300	13	1000	2.7	210
Lower Mainstem	Natural	11	1.5	66.9	24	1500	20	1200	31	2100	2.9	210
	Hatchery	7	1.5	75.8	5.4	500	4.7	430	29	2600	1.9	140
	Natural + hatchery	18	1.5	70.3	17	1100	14	930	30	2300	2.5	180
Distributary Channels	Natural	7	1.1	71.3	5.7	540	4.9	460	18	1700	2.3	200
	Hatchery	8	1.6	75.8	2.4	190	2.0	160	15	940	1.7	110
	Natural + hatchery	15	1.4	73.7	3.9	350	3.4	300	16	1300	2.0	150
All regions	Natural	30	1.4	65.7	12	790	9.7	660	21	1500	2.8	210
	Hatchery	18	1.5	77.4	4.0	380	3.5	330	20	1600	1.8	140

[a] $\sum$PBDE$_{47+99}$ = sum of detected BDE-47 and BDE-99 congeners used to assess adverse critical body residues (CBR) for exposure to PBDEs.
[b] TPCB used to assess adverse CBR for exposure to PCBs.

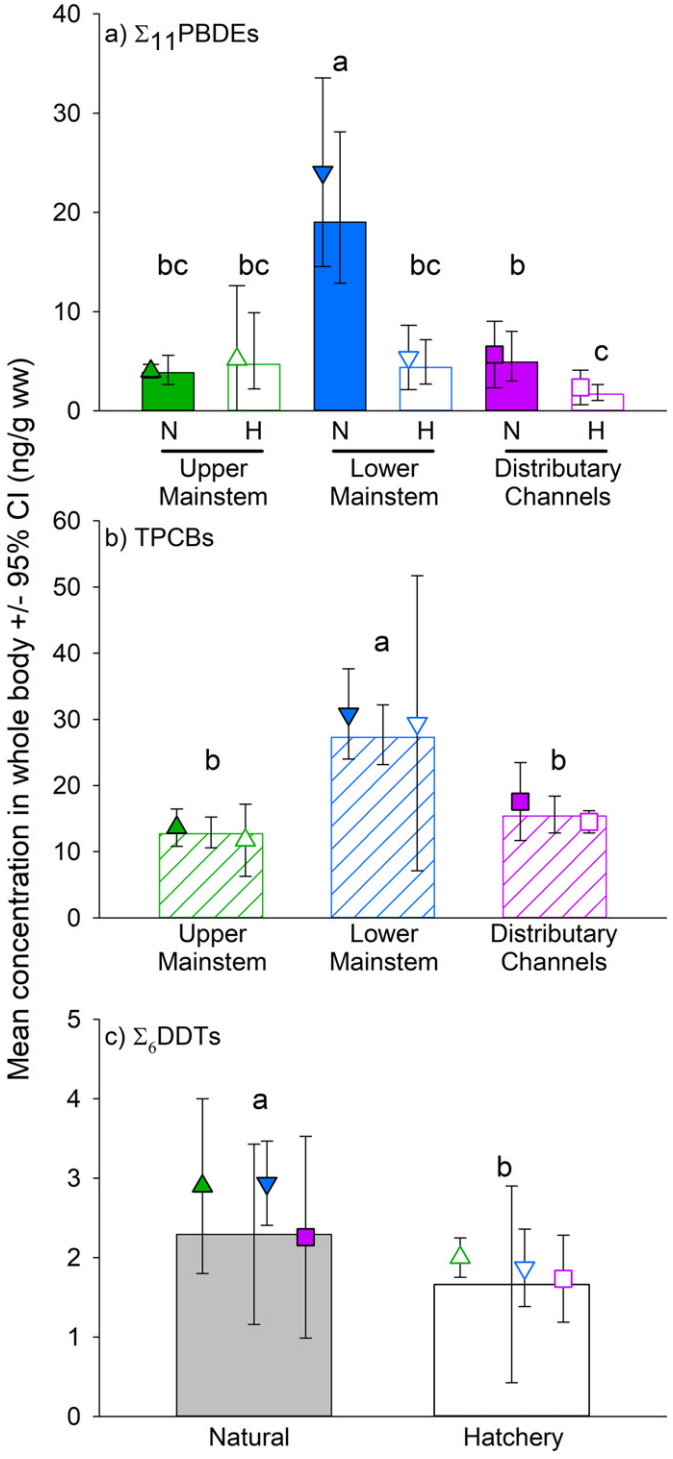

Fig. 2. Measured (symbols) and predicted (bars) concentrations of $\sum_{11}$PBDEs, TPCBs, and $\sum_{6}$DDTs in juvenile Chinook salmon collected from the Snohomish River estuary. Symbols represent the arithmetic mean concentrations for $\sum_{11}$PBDEs, TPCBs, and $\sum_{6}$DDTs where Upper Mainstem, Lower Mainstem, and the Distributary Channels sites are represented by upward triangles, downward triangles, and squares, respectively. Solid filled and open symbols are used to represent natural- and hatchery-origin fish, respectively. Bars are modeled estimated geometric mean concentrations with solid filled, open, and hatched bars used to represent natural-, hatchery- and mixed-origin fish, respectively. Predicted $\sum_{6}$DDTs concentrations were modeled using a grand mean fish length of 70 mm. For each POP class, groups with the same lower case letter are not significantly different from each other.

was an inverse relationship between fish length and $\sum_{6}$DDT for natural-origin fish, ranging from 40.2 to 90.0 mm (Fig. 3). In contrast, $\sum_{6}$DDT was not significantly correlated with fish length for hatchery-origin fish for the limited length range tested (65.1 to 95.1 mm, Fig. 3). Similarly, there is also no significant correlation between length and $\sum_{6}$DDT for natural-origin fish >65 mm ($p = 0.36$; data not shown).

3.2. POP fingerprints

A comparison of POP fingerprints among the samples indicated clear segregation between natural-origin fish from the Lower Mainstem and all but one of the other sampling groups (Fig. 4; Table 4). The natural-origin fish from the Lower Mainstem exhibited distinct POP fingerprints (Fig. 4, filled blue triangles), with the higher proportions of $\sum_{11}$PBDEs in the total POP concentration, compared to other sampling groups. These fingerprints were most different from hatchery-origin fish from the same region (Fig. 4, open blue triangles) and the Distributary Channels (Fig. 4, open pink squares), which exhibited the two lowest relative concentrations of $\sum_{11}$PBDEs (ANOSIM, $R = 0.484$ and 0.596), followed by natural origin fish from the other regions (ANOSIM, $R = 0.467$ and 0.315), with intermediate relative concentrations of $\sum_{11}$PBDEs (Table 4, $p \leq 0.006$ for all pair-wise comparison). The only sampling group that was not clearly segregated from the natural-origin fish from the Lower Mainstem was the hatchery-origin fish from the Upper Mainstem (Fig. 4, open green triangles; ANOSIM, $R = 0.251$, $p = 0.052$); however, the sample size representing this group was small ($n = 3$), so the power to detect difference between these two groups, if it existed, was low.

The unique pattern of POPs in the Lower Mainstem natural-origin fish can be further illustrated by examination of POP fingerprints among the remaining five sampling groups, which were statistically indistinguishable from each other. For example, the POP fingerprints in natural-origin fish from the Upper Mainstem and the Distributary Channels were not different from each other ($R = 0.068$, $p = 0.195$) and nor were the hatchery-origin fish from the three regions different from each other (ANOSIM, R from 0.014 to 0.237, and $p = 0.103$–0.467 for all comparisons). Among these five sampling groups, natural-origin fish were only segregated from hatchery-origin fish in three of six comparisons (ANOSIM, R from 0.272 to 0.43, $p < 0.01$ for all comparisons; Table 4).

Among region and origin sample groups, the variation in congener patterns within the TPCB and $\sum_{11}$PBDEs POP classes was minor in comparison to the variation observed between the TPCBs and $\sum_{11}$PBDEs POP classes. The main PCB congeners contributing to the TPCB concentration in each region and origin sample groups (Table S3) were PCB 153 and 138, followed by 101, 118, and then 28 and 18, collectively accounting for 38–68% of the total concentration. The heavier congeners, 195, 206, and 209, were not detected in any samples and the remaining congeners, when detected, were at low concentration near the LOQ (Table S3). Although the TPCB concentrations were higher in fish collected from the Lower Mainstem compared to those from other regions, the pattern of detected concentrations of PCB congener homologues, was similar among region and origin sample groups (Fig. S1). The calculated values for $\sum_{11}$PBDEs were dominated primarily by contributions from BDE congeners 47 and 99, followed by 100 (Table S4), collectively accounting for 86–100% of the $\sum_{11}$PBDEs for individual fish samples. The BDE congeners 85, 155 and 183 were not detected in any salmon samples and the remaining congeners, when detected were at low concentrations near the LOQ (Table S4). Although natural-origin fish from the Lower Mainstem had higher $\sum_{11}$PBDE concentrations compared to other region and origin sample groups, the pattern of BDE congeners detected was similar among these groups (Fig. S2). Likewise, the DDT and DDT metabolites patterns did not vary among region and origin sample groups. The calculated $\sum_{6}$DDTs concentration was dominated by *p,p′*-DDE, which was detected in 100% of the samples (Table S5). The other DDT compounds were never detected (i.e., *o,p′*-DDE, *o,p′*-DDT) or infrequently (8–21%) detected at concentration near the LOQ (i.e., *o,p′*-DDD, *p,p′*-DDD, and *p,p′*-DDT) in all region and origin sample groups.

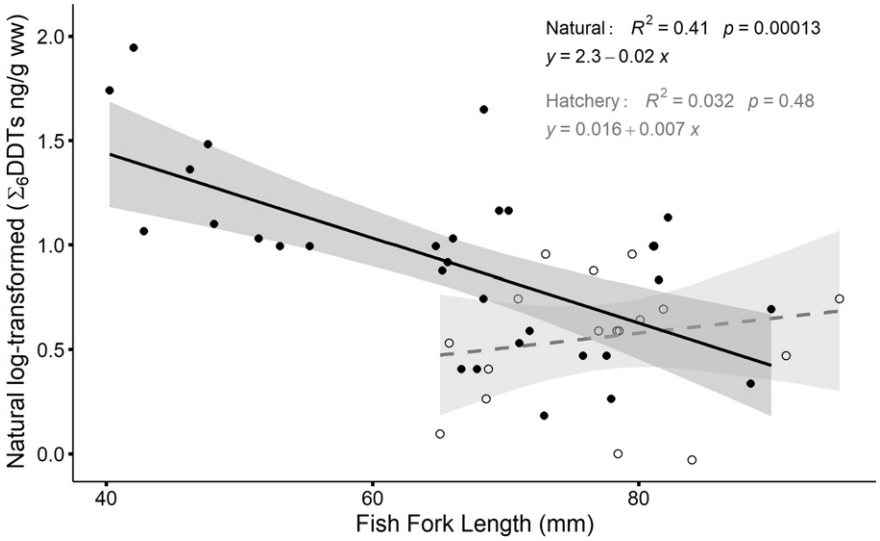

Fig. 3. Relationships between fish length and $\sum_6$DDTs for natural-origin (black solid line ± 95% CI shaded region) and hatchery-origin (dashed line ± 95% CI shaded region) fish. Actual data are plotted using solid filled symbols for natural-origin fish and open symbols for hatchery-origin.

3.3. Stable isotopes

The isotopic values δ^{34}S and δ^{13}C in Chinook salmon generally showed a similar pattern of enrichment from upper to downstream regions of the estuary, and more enrichment in hatchery- than natural-origin fish within each region (Two-Way ANOVA, Table 5; Fig. 5a). Overall, δ^{34}S values (Fig. 5a, vertical axis) in fish from the Distributary Channels (squares) were 2.1 times more enriched than those in the Upper Mainstem (upward triangles; t = 10.278, p < 0.001) and 1.2 times more enriched than those from the Lower Mainstem (downward triangles; t = 4.314, p < 0.001). Measured δ^{34}S in fish from the Lower Mainstem (downward triangles) were also 1.7 times more enriched than those from the Upper Mainstem (upper triangles; t = 6.646, p < 0.001). Overall, δ^{34}S was 1.1 times more enriched in hatchery- than natural-origin fish (open vs. closed symbols; t = 2.561, p = 0.015). A somewhat similar pattern of enrichment was measured for δ^{13}C in fish, although the differences were less pronounced from upstream to downstream (Fig. 5a, horizontal axis). Measured δ^{13}C in fish from the Distributary Channels (squares) were 1.1 times more enriched than those from the Upper Mainstem (upward triangles; t = 4.509, p < 0.001) and the Lower Mainstem (downward triangles; t = 4.141, p < 0.001), which did not differ from each other (t = 1.043, p = 0.304). Overall, δ^{13}C in hatchery-origin fish (open symbols) were 1.1 times greater than those of natural-origin (closed symbols; t = 6.664, p < 0.001).

The patterns of δ^{15}N in Chinook salmon (Fig. 5b, vertical axis) were more complex than those of δ^{34}S and δ^{13}C, with depleted δ^{15}N values in natural-origin fish from the Lower Mainstem (solid downward triangles) compared to other sample groups (Two-Way ANOVA, Table 5). Apart from the natural-origin fish in the Lower Mainstem, as juvenile salmon moved from the Upper Mainstem to the more saltwater influenced region of the Lower Mainstem and the Distributary Channels, values of δ^{15}N and δ^{34}S were positively correlated and increasingly enriched (Fig. 5b). Natural-origin fish from the Distributary Channels (filled squares) were 1.2 times more enriched in δ^{15}N values compared to natural-origin fish from the Upper Mainstem (filled upward triangles; means = 10.8 and 9.3, t = 3.624, p = 0.002). Hatchery-origin fish (open symbols) had more similar δ^{15}N values among regions, but a slight enrichment (1.1 times) was also measured in the downstream

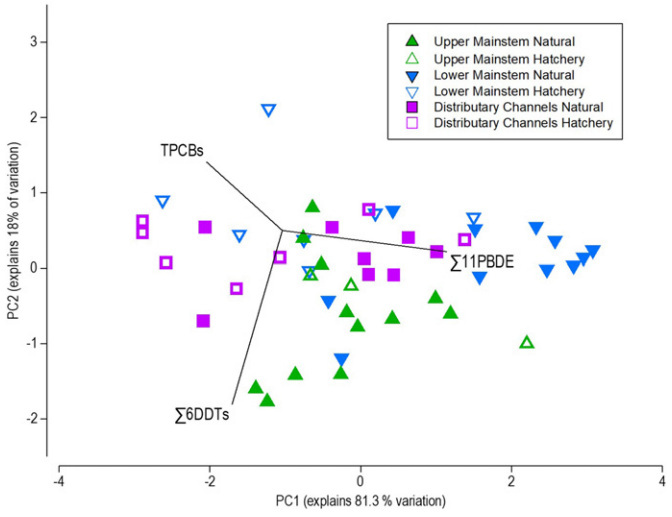

Fig. 4. Plot of the first two principal components (PC) based on the Principal Component Analysis (PCA) of proportions of $\sum_{11}$PBDEs, TPCBs and $\sum_6$DDTs measured in juvenile Chinook salmon collected from three regions of the Snohomish River estuary. Collectively, both PCAs explain 99.3% of the variation, with PC1 accounting for 81.3%, showing higher proportions of $\sum_{11}$PBDEs in natural-origin fish from the Lower Mainstem.

Table 4
ANOSIM statistical results for pairwise comparisons of the proportion of POP classes in juvenile Chinook salmon sampling groups. R varies between 0 and 1, although small negative values close to zero are possible. R values closer to 1 signify a higher degree of separation. Statistically significant differences are noted with an *. LM = Lower Mainstem region, UM = Upper Mainstem region, and DC = Distributary Channels region. Global R for test = 0.306 and p = 0.001.

Sampling group comparisons	R	p	
LM natural vs. DC hatchery	0.596	0.001	*
LM natural vs. LM hatchery	0.484	0.004	*
LM natural vs. UM natural	0.467	0.001	*
LM natural vs. DC natural	0.315	0.006	*
LM natural vs. UM hatchery	0.251	0.052	
UM natural vs. DC natural	0.068	0.195	
UM natural vs. DC hatchery	0.43	0.002	*
UM natural vs. LM hatchery	0.318	0.01	*
UM natural vs. UM hatchery	0.018	0.411	
DC natural vs. DC hatchery	0.272	0.033	*
DC natural vs. UM hatchery	0.127	0.258	
DC natural vs. LM hatchery	0.106	0.097	
UM hatchery vs. DC hatchery	0.237	0.103	
UM hatchery vs. LM hatchery	0.111	0.283	
DC hatchery vs. LM hatchery	−0.014	0.467	

Table 5
Results of a two-way ANOVA with sampling region (i.e. Region) and fish origin (i.e. Origin) as factors affecting stable isotopes of sulfur (δ34S), carbon (δ^{13}C), and nitrogen (δ15N) measured in whole-body samples of juvenile Chinook salmon collected from the estuary of the Snohomish River.

Stable isotopes	Factor	d.f	Sum squared	Mean squared	F value	p value
δ^{34}S	Region	2	187.39	93.695	52.863	<0.001
	Origin	1	11.623	11.623	6.558	0.015
	Region × Origin	2	0.403	0.202	0.114	0.893
	Residual	35	62.035	1.772		
	Total	40	315.047	7.876		
δ^{13}C	Region	2	35.649	17.825	13.299	<0.001
	Origin	1	59.511	59.511	44.402	<0.001
	Region × Origin	2	1.89	0.945	0.705	0.501
	Residual	35	46.91	1.34		
	Total	40	172.28	4.307		
δ^{15}N	Region	2	20.219	10.11	14.779	<0.001
	Origin	1	9.874	9.874	14.435	<0.001
	Region × Origin	2	7.132	3.566	5.213	0.01
	Residual	35	23.942	0.684		
	Total	40	71.463	1.787		

Distributary Channels region compared to those from the Upper Mainstem (means = 11.2 and 9.8, $t = 2.456$, $p = 0.056$). However, in stark contrast, δ^{15}N in natural-origin fish from the Lower Mainstem was significantly more depleted than would be predicted based on their δ^{34}S values (Fig. 5b). Mean δ^{15}N in natural-origin fish from the Lower Mainstem were only 90% of those in natural-origin fish from the Upper Mainstem (means =8.372 and 9.268, $t = 2.284$, $p = 0.029$). A comparison of natural- and hatchery–origin fish within regions also revealed δ^{15}N was only depleted in natural- compared to hatchery-origin fish in the Lower Mainstem (means = 8.372 and 10.595; $t = 5.205$, $p < 0.001$), however, significant differences were not observed from either the Upper Mainstem ($t = 0.973$, $p = 0.337$) or the Distributary Channels ($t = 0.885$, $p = 0.382$).

Nitrogen isotopic signatures of natural-origin fish from the Lower Mainstem were also negatively correlated with higher relative concentrations of $\sum_{11}$PBDEs ($R^2 = 0.68$, $p = 0.003$, slope = −0.74, intercept = 8.15). In natural-origin fish from the Lower Mainstem, the greater the depletion in nitrogen isotopic signature, the higher the proportion of $\sum_{11}$PBDEs (Fig. 6a; proportion of $\sum_{11}$PBDEs measured by PC1 in Fig. 4). The δ^{15}N values were also negatively correlated with absolute concentrations of $\sum_{11}$PBDEs ($R^2 = 0.68$, $p = 0.003$, slope = −8.93 and intercept =100.64) and TPCBs ($R^2 = 0.63$, $p = 0.006$, slope = −6.56 and intercept = 86.52), not shown for brevity. In contrast, for each of the other sampling groups, there was no relationship between δ^{15}N and PC1 (Fig. 6b) or $\sum_{11}$PBDEs, or TPCB (not shown for brevity). Furthermore, samples of natural-origin fish that were presumed to have spent the least amount of time in the Lower Mainstem, based on their lower δ^{34}S, deviated most from the predicted relationship between PC1 score and δ^{14}N (Fig. S3, F = 27.0701, $p = 0.0008$, $R^2 = 0.77$).

4. Discussion

Our study demonstrated the value of three types of complementary chemical tracer data (POP concentrations, POP fingerprints, and stable isotopes), to assess location and source of contaminant exposure for juvenile Chinook salmon migrating seaward through a developed watershed with multiple contaminant sources. Using contaminant concentration data, we first assessed that along their migration pathway through Snohomish River estuary, salmon were exposed predominantly to PCBs and PBDEs in the Lower Mainstem region, with higher $\sum_{11}$PBDEs in natural- rather than hatchery-origin fish but similar TPCBs in both fish origins (Fig. 2). Second, we used POP fingerprints to determine that natural-origin fish captured from the Lower Mainstem had a distinct pattern from other region and origin samples, with a much higher proportion of $\sum_{11}$PBDEs in the total POP concentration, indicating a different contaminant source (Fig. 4). Third, we used stable isotopes, an independent tracer of food sources and habitat use, to document that natural-origin fish from the Lower Mainstem region had depleted δ^{15}N signatures compared to fish from the other region and origin groups (Fig. 5b). Moreover, the $\sum_{11}$PBDE-enhanced POP fingerprint in the natural-origin salmon from the Lower Mainstem was negatively correlated with the δ^{15}N in the salmon (Fig. 6), suggesting a common source for both the high PBDEs exposure and the depleted nitrogen isotopic signal.

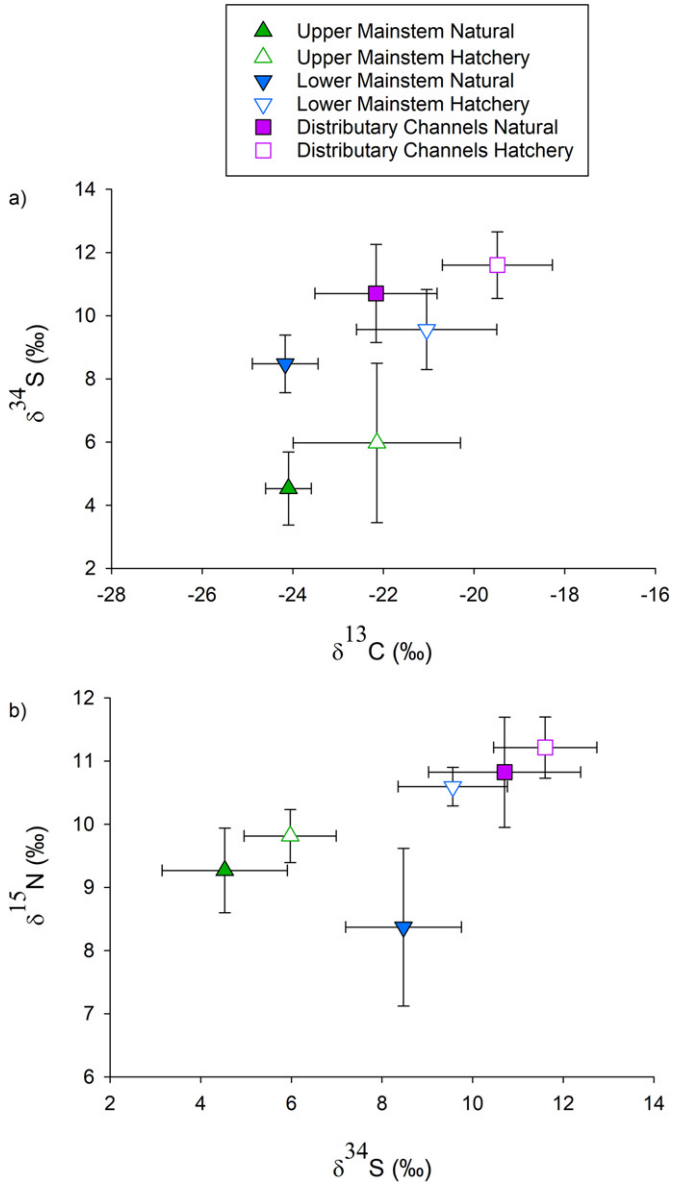

Fig. 5. Stable isotopes of a) sulfur (δ^{34}S) and carbon (δ^{13}C) and b) nitrogen (δ^{15}N) and sulfur, measured in natural- and hatchery-origin juvenile Chinook salmon (mean ± 95% CI) collected from three regions of the Snohomish River estuary.

4.1. POP concentrations

As hypothesized, POPs concentrations, and $\sum_{11}$PBDEs in particular, were greatest in salmon sampled from the Lower Mainstem, nearest a high volume wastewater outfall, suggesting a wastewater source. Natural-origin fish from the Lower Mainstem had $\sum_{11}$PBDE concentrations 4–10 times higher than salmon from other regions, regardless of origin, indicating the natural-origin fish were most exposed in this

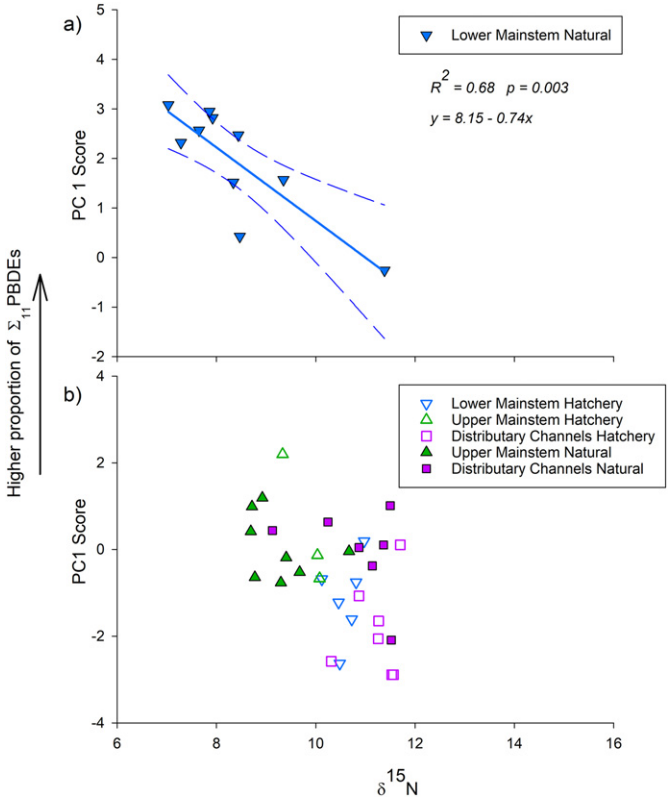

Fig. 6. Relationship between PC1 score and δ^{15}N showing a significant inverse relationship for a) natural-origin fish collected from the Lower Mainstem, but no relationship for b) each of the other region and origin sampling groups (i.e., $p > 0.05$) for each group.

region. Similar but less pronounced patterns were measured for TPCBs; concentrations in fish from the Lower Mainstem were approximately twice as high as those in fish from the less developed Distributary Channels and the Upper Mainstem, however, TPCBs did not differ by fish origins. Unlike $\sum_{11}$PBDEs and TPCBs, $\sum_{6}$DDT concentrations were uniformly low in all regions sampled.

The best-fit models for TPCB and $\sum_{11}$PBDE concentrations measured in juvenile salmon in this study support the conclusion that POP concentrations were determined primarily by the sampling region where the fish were captured (i.e., TPCBs) or the sampling region and the origin of the salmon (i.e., $\sum_{11}$PBDEs), rather than fish size or lipid content (Table S1). Although lipids can affect contaminant uptake (Elskus et al., 2005; West et al., 2017), the small range of lipid values measured in the juvenile Chinook salmon in this study likely dampened the importance of this factor. Likewise, fish length was only a factor for $\sum_{6}$DDT concentrations (Table S1), but this potential effect was obscured by the small range in fish sizes and differential size distributions between natural- and hatchery-origin fish. The inverse relationship between $\sum_{6}$DDT concentrations in the natural–origin salmon (Fig. 3), was consistent with previous studies documenting maternal transfer of DDTs to eggs and fry (Miller, 1994), and subsequent growth dilution. Given the limited size range of hatchery-origin fish collected, we cannot test for the presence of maternal transfer and growth dilution in these fish. POPs in maturing female Pacific salmon are transferred to the developing eggs (deBruyn et al., 2004; Ewald et al., 1998; Miller, 1993). Estimated $\sum_{6}$DDT concentrations in newly emerged Chinook salmon would range from 0.9 and 7 ng/g ww, based on a range of $\sum_{6}$DDT concentrations measured in muscle tissue of adult Chinook salmon (4.3–59 ng/g ww) returning to Puget Sound rivers (West et al., 2001) and correlations between POP concentrations in muscle and fry of Chinook salmon (Miller, 1994). Notably, the estimated maximum $\sum_{6}$DDT concentration encompassed the highest $\sum_{6}$DDT concentrations (i.e., 5.7–7.0 ng/g ww) we measured in small (≤42 mm) natural-origin fish, which are just a few mm larger than newly emerged Chinook salmon prior to exogenous feeding (Beacham and Murray, 1990), supporting the hypothesis that the elevated $\sum_{6}$DDTs in the smaller natural-origin fish we sampled were maternally derived. The lack of relationship between $\sum_{6}$DDT concentrations and fish length in hatchery-origin fish is likely due to the lack of availability of small fish (i.e. hatchery-origin fish are not released until they reach approximately 65 mm) and subsequent sampling of hatchery-origin fish after growth dilution occurred. Moreover, these observations suggest DDTs were not present in the Chinook salmon prey in this system in great enough quantities to overcome growth dilution.

The higher $\sum_{11}$PBDE concentrations in natural-origin fish from the Lower Mainstem compared to the natural-origin salmon from other regions, suggests a higher input of PBDEs into this region of the Snohomish River estuary. However, the higher $\sum_{11}$PBDE concentrations in natural-origin fish from the Lower Mainstem compared to the hatchery-origin fish from the same regions suggests fish of different origins were not equally exposed to the higher inputs of PBDEs. Natural-origin juvenile Chinook salmon were primarily exposed to and accumulated $\sum_{11}$PBDEs at two sites within the Lower Mainstem of the Snohomish River estuary, both located in the immediate vicinity of an Everett WPCF outfall and multiple CSOs. In contrast, hatchery-origin salmon from the same region accumulated lower $\sum_{11}$PBDE concentrations, likely because they moved through the estuary more rapidly than natural-origin fish (Levings et al., 1986; Rice et al., 2011) or they spent less time in the tidally influenced mesohaline area of the estuary (Davis et al., 2018) where wastewater was discharged. Davis et al. (2018) documented that seaward migrating juvenile Chinook from another river estuary in Puget Sound exhibited distinct habitat use patterns; natural-origin fish were more frequently captured in the tidally influenced freshwater and mesohaline habitats whereas hatchery-origin fish were more frequently captured in the nearshore intertidal habitat (Davis et al., 2018).

Concentrations of TPCBs were similarly elevated in natural- and hatchery-origin juvenile Chinook salmon, suggesting that although TPCBs inputs were greater in the more developed Lower Mainstem region of the estuary compared to other regions, the inputs were likely from more dispersed sources throughout the region, and not high enough to disproportionately elevate concentrations for natural-fish that likely resided in the area for a longer time.

Previous contaminant studies in juvenile Chinook salmon have also documented elevated levels of POPs in this species, especially those sampled from moderately to highly urbanized rivers and estuaries of Puget Sound (Johnson et al., 2007a; Meador et al., 2010; O'Neill et al., 2015; Olson et al., 2008; Sloan et al., 2010) and the lower Columbia River and Washington and Oregon coasts (Johnson et al., 2013; Johnson et al., 2007b; Sloan et al., 2010). The $\sum_{11}$PBDE concentrations we measured in natural-origin Chinook salmon in the Lower Mainstem were 2 to 24 times higher than concentrations in natural- and hatchery-origin fish from other Puget Sound estuaries and nearshore marine habitats (O'Neill et al., 2015; Sloan et al., 2010), but they were lower than the highest concentrations measured in samples collected from the Columbia River near areas with high inputs of wastewater (Sloan et al., 2010). Additionally, the Snohomish River estuary appears to be a consistent but possibly decreasing PBDE hotspot for seaward migrating juvenile Chinook salmon. Mean concentration of $\sum_{11}$PBDEs in the natural-origin Chinook salmon in the Lower Mainstem in this study (29 ng/g ww) were similar to those measured in natural-origin fish at the same location in 2013 (24 ng/g ww) but half (1100 vs. 2400 ng/g lipid weight) those measured in 2006 by Sloan et al. (2010), potentially indicating a decline in PBDEs as has been observed for other fish species in Puget Sound (West et al., 2017). Alternatively, the higher PBDE concentrations measured by Sloan et al. (2010) could be associated with differences in the mean fish length (100 vs. 66.9 mm) or sampling time (August vs April–July) compared to the present study. Concentrations of TPCBs in juvenile Chinook salmon from our study were similar

to those measured in 2013 (30 vs. 27 ng/g ww) at the same sampling location in the Lower Mainstem (O'Neill et al., 2015). The TPCB concentrations we measured in salmon were higher than those measured at rural river and estuary sites in the Pacific Northwest (Johnson et al., 2013; Johnson et al., 2007a; Johnson et al., 2007b), but below those generally observed at heavily urbanized estuaries in Puget Sound (Johnson et al., 2007a; Meador et al., 2010; Olson et al., 2008) and the Columbia River (Johnson et al., 2013; Johnson et al., 2007b). In contrast to TPCBs and $\sum_{11}$PBDEs, the $\sum_{6}$DDT concentrations measured in juvenile Chinook salmon from the Snohomish River estuary were not elevated compared to other sites in Puget Sound in 2013 (O'Neill et al., 2015). Higher DDT concentrations were measured in juvenile Chinook salmon from the Columbia River basin from 2005 to 2009, approximately 8 to 12 times higher than those we measured in the Snohomish River, possibly associated with the high degree of agricultural activity in the interior Columbia River as well as Willamette basins and point sources within Portland Harbor (Johnson et al., 2013).

Concentrations of $\sum_{11}$PBDEs, and to a lesser extent TPCBs, we measured in juvenile Chinook salmon in the Snohomish River estuary were high enough to pose a conservation threat. Based on published laboratory exposure studies (Arkoosh et al., 2010, 2018; Meador et al., 2002), the concentrations of these POPs in some Chinook salmon were within ranges of adverse CBRs known to impair their health. Approximately 73% and 14% of the natural-origin Chinook sampled from the Lower Mainstem and the Distributary Channels, the two regions receiving wastewater effluent discharges, had concentrations of BDE congeners 47 and 99 (Table 3), the two congeners detected most frequently and at the highest concentrations, within the range of concentrations found to alter their immune response and increase disease susceptibility (Arkoosh et al., 2010, 2018). In contrast, none of the natural-origin Chinook salmon from the Upper Mainstem or hatchery-origin Chinook salmon from this study had $\sum$PBDE$_{47+99}$ levels high enough to predict altered immune response.

Impairment of immune response is of particular concern for salmonids because a properly functioning immune system is vital for both individual survival and population productivity (Segner et al., 2003). Seaward migrating salmonids are exposed to a number of naturally occurring pathogens and parasites, including the trematode *Nanophyetus salmincola* (Arkoosh et al., 2004). Exposure to PBDEs and other POPs may reduce the marine survival of juvenile salmonids due to immune suppression, thus increasing their susceptibility to naturally occurring infectious and parasitic diseases, causing direct mortality or indirect mortality via predation by larger fish, birds and mammals. For example, Hostetter et al. (2011) reported steelhead (*O. mykiss*) smolts that tested positive for pathogens were more likely to have poor external condition (i.e., external signs of disease or more scale loss). Moreover, tagged fish with poor external condition were subsequently observed to have lower overall marine survival (Hostetter et al., 2011), associated with increased avian predation (Hostetter et al., 2012). In addition to directly impairing the immune function of salmonids, exposure to POPs has been documented to work in conjunction with naturally occurring parasites (i.e., trematode exposure) further increasing their susceptibility to a naturally occurring marine bacterial pathogen (Jacobson et al., 2003), potentially leading to population level effects (Arkoosh et al., 1998; Loge et al., 2005; Meador, 2014; Spromberg and Meador, 2005). Chen et al. (2018) suggested the exposure to POPs and *N. salmincola* serve as mortality cofactors for juvenile steelhead from Puget Sound, with the proximate cause of death involving bacterial pathogens or selective predation of infected cohorts.

Based on lipid normalized TPCB concentrations (ng/g lw) measured in salmon from the Upper Mainstem, Lower Mainstem and Distributary Channels, 0%, 27%, and 29%, respectively of the natural–origin fish and 0%, 14% and 0%, respectively of the hatchery-origin fish, had concentrations above an adverse CBR threshold for total PCBs (Meador et al., 2002). Published CBR thresholds based on individual congeners were not available for salmon. These lipid normalized values likely underestimate the number of impaired fish because juvenile salmon rapidly metabolize lipids as they migrate downstream, typically achieving lipid concentrations of 1% or less by the time they move from the estuary to marine waters (Arkoosh et al., 2011; O'Neill et al., 2015). For example, modeling a 1% lipid content for the natural–origin fish from the Lower Mainstem to predict their increased risk after lipids have been metabolized, would increase the number of fish above the PCB CBR from 27% to 64% for natural-origin fish and 14% to 29% for hatchery-origin fish, potentially increasing the likelihood of reducing their marine survival. Indeed, Meador (2014) documented that hatchery Chinook salmon originating from Puget Sound rivers with contaminated estuaries, including the Snohomish River, have lower marine survival than those originating from uncontaminated rivers.

4.2. POP fingerprints

Analyses of POP fingerprints in salmon from the three regions support the hypothesis that salmon in the Lower Mainstem are exposed to a contaminant source influenced primarily by wastewater rather than stormwater. Except for the hatchery fish from the Upper Mainstem, natural-origin Chinook salmon from the Lower Mainstem had distinct POP fingerprints from all other sampling groups (Fig. 4, Table 4), with high relative concentrations of $\sum_{11}$PBDEs. The POP fingerprints in natural-origin fish from the Lower Mainstem overlapped with those of hatchery-origin fish from the Upper Mainstem ($R = 0.251$) and the p value was 0.052, suggesting that the difference between these groups may not be statistically significant. However, the small sample size ($n = 3$) representing the hatchery-origin fish from the Upper Mainstem, limited our ability to adequately evaluate a significant difference between these groups should one exist. Although POPs can enter the Snohomish River estuary via various sources such as WWTPs, stormwater, or atmospheric deposition, wastewater is considered to be the primary source for PBDEs in Puget Sound, whereas stormwater is the greater source for PCBs (Osterberg and Pelletier, 2015). Modeled loading of contaminants to Puget Sound indicated that most PBDEs enter Puget Sound via publically owned WWTPs, followed by stormwater related surface runoff, and then atmospheric deposition (9.91, 4.56, and 3.49 kg/year, respectively) (Osterberg and Pelletier, 2015). In contrast, Osterberg and Pelletier (2015) concluded that most PCBs enter Puget Sound via stormwater surface runoff (4.17 kg/yr), with considerably less entering via publically owned WWTPs and atmospheric deposition (0.32 and 0.43 kg/yr). In the year we conducted our study, the Lower Mainstem received wastewater DIN loads 1.5 times higher than those in the Distributary Channels (Table 1), and the Upper Mainstem region did not receive direct input of wastewater effluent. Although we do not have estimates of stormwater loads to the three regions of the Snohomish River estuary sampled by our study, loadings from surface runoff are likely highest in the Lower Mainstem region, based on the high percentage (41–94%) of impervious surface area in the lands adjacent to this region of the river (Fig. 1), potentially contributing to the higher concentrations of TPCBs in both natural-and hatchery-origin fish from this location. However, stormwater loadings to the Snohomish River are likely lower than those of more urbanized rivers because measured PCBs in juvenile Chinook from the Snohomish are much less than those measured in other more urbanized estuaries in the Puget Sound (Johnson et al., 2007a; Meador et al., 2010; O'Neill et al., 2015; Olson et al., 2008) and the Columbia River (Johnson et al., 2013; Johnson et al., 2007a; Johnson et al., 2007b).

Contaminant fingerprints are well established chemical tracers for providing information about the sources of POPs and movement patterns of migratory animals (Ramos and González-Solís, 2012), but typically over a broader geographic areas than evaluated in this study. For example, Krahn et al. (2007) used ratios of PCBs and DDTs acquired by migratory killer whales, to discriminate differences in feeding areas and contaminant sources for three pods of whales that forage along

the west coast of North America. In contrast, we used variation in POP fingerprints in juvenile salmon sampled over <30 rkm to identify a PBDE contaminant-source, indicating the robustness of POPs fingerprint at discriminating contaminant sources along a contaminant gradient.

4.3. Stable isotopes

Isotopic signatures of salmon, especially $\delta^{15}N$, from three regions of the Snohomish estuary (Fig. 5) also support the hypothesis that natural-origin salmon from the Lower Mainstem region were exposed primarily to a wastewater source rather than a stormwater contaminant source. Stable isotopic signatures of nitrogen in biota are tools to assess assimilation of wastewater-derived sources of nitrogen into aquatic food webs (deBruyn and Rasmussen, 2002; Savage, 2005). In addition to the ambient nitrogen load in the river, nitrogen in wastewater is incorporated into aquatic food webs though the uptake of sewage-derived nutrients by primary producers or consumption of particulate-organic matter by primary consumers (Tucker et al., 1999), and then subsequently transferred through the food web (McClelland et al., 1997; Vander Zanden et al., 2005). Incorporation of wastewater-derived nitrogen sources into the food web, beyond the background river nitrogen, causes shifts in nitrogen stable isotopes in aquatic organisms when compared to background or reference values in both freshwater (deBruyn and Rasmussen, 2002; Hicks et al., 2017; Loomer et al., 2015; Steffy and Kilham, 2004) and marine systems (Savage, 2005; Schlacher et al., 2005; Tucker et al., 1999). However, the extent to which biota exposed to wastewater have altered $\delta^{15}N$ values depends on the treatment processes used at the plant, effluent quality (e.g., concentration and load of ammonia/ammonium), and the characteristics of the receiving waters (Hicks et al., 2017).

Depleted $\delta^{15}N$ in natural-origin fish from the Lower Mainstem suggests they were exposed to sewage characterized by relatively high nutrient concentrations. In contrast, the $\delta^{15}N$ in the hatchery-origin fish from this region was not depleted, suggesting they were less exposed to nutrient rich wastewater effluent. Complex treatment processes determine the amount of nutrient removal, and whether dissolved inorganic nitrogen in effluent is discharged as ammonia/ammonium, nitrite or nitrate (Metcalfe et al., 2003). WWTPs designed to optimize removal of nutrients from wastewater typically use nitrification (conversion of ammonia to nitrate) followed by de-nitrification (conversion of nitrate to nitrogen gas) processes to remove nitrogen. In contrast, WWTPs designed without specific nutrient removal, discharge effluent with more ammonium than nitrates (Hicks et al., 2017; Loomer et al., 2015). Furthermore, nitrification, denitrification, as well as volatilization of wastewater, can alter the concentration and the nitrogen isotopic signature of the pools of ammonia/ammonium and nitrate/nitrite they act upon (Heaton, 1986; Valiela et al., 2000), as well as the resulting effluent released to the aquatic systems (Toyoda et al., 2011). Overall, biota exposed to untreated and primary treated sewage, or secondary sewage with insufficient nutrient removal, typically exhibit a depleted $\delta^{15}N$ signal (deBruyn and Rasmussen, 2002; Hicks et al., 2017; Loomer et al., 2015), as we observed in natural-origin Chinook salmon from the Lower Mainstem. Indeed, the form of DIN discharged by the Everett's WPCF is atypical compared to other Puget Sound wastewater facilities that discharge into rivers and nearshore marine receiving waters, with a higher proportion of ammonium compared to nitrates and nitrites (Table 1). Conversely, biota exposed to secondary and tertiary sewage treatment that removes excess nitrogen with nitrifying and denitrifying bacteria typically have an enriched $\delta^{15}N$ signal compared to background values (Heaton, 1986; Savage, 2005; Valiela et al., 2000).

In contrast to nitrogen isotopes, carbon and sulfur stable isotopes were enriched in salmon as they moved downstream (see Fig. 5a), consistent with the frequency and amount of saltwater intrusion into the downstream regions of the Snohomish River (Hall et al., 2018) and a gradual shift to downstream food sources, as noted in salmon from other rivers (Moore et al., 2016). Sulfur and carbon isotopes provide information regarding food sources for consumers, with marine food webs typically more enriched in $\delta^{34}S$ and $\delta^{13}C$ than freshwater systems (Peterson and Fry, 1987), and thus reveal the prey base and movements of animals (Hobson, 1999). Based on Hall et al. (2018), there is a continuum from freshwater in the Upper Mainstem region to more saline waters in both the Lower Mainstem and Distributary Channels regions. The higher $\delta^{34}S$ and $\delta^{13}C$ we measured in salmon in these downstream regions, reflects this salinity gradient and the salmons' changing food supply that is incorporated into their tissues as they migrate downstream. Similarly, Moore et al. (2016) documented that natural-origin juvenile Chinook salmon from the relatively undeveloped watershed of the Skeena River in British Columbia, Canada, became enriched in both $\delta^{13}C$ and $\delta^{34}S$ as they migrated from the headwaters of the river to nearshore marine waters. The slight enrichment of $\delta^{34}S$ and $\delta^{13}C$ in hatchery-origin fish, compared to natural-origin fish from the same region, may be due to the residual influence of the diet of hatchery fish, prior to release from the hatchery (Weber et al., 2002). In the hatchery, fish are fed commercial diets dominated by protein from marine sources enriched in $\delta^{34}S$, whereas, natural-origin fish consume freshwater prey with more depleted $\delta^{34}S$, and the muscle tissue of fish reflect these sources (Weber et al., 2002). However, tissue differences in $\delta^{34}S$ between hatchery- and natural-origin fish will rapidly be masked by the freshwater diet consumed by hatchery-origin fish after they leave the hatchery, given the rapid turnover rates of liver and muscle tissue of juvenile salmonids (Heady and Moore, 2013).

4.4. Complementary chemical tracers

We used multiple, complementary chemical tracers to infer nutrient and contaminant sources to seaward migrating juvenile salmon, more discernable information than either tracer provided individually. Collectively, the isotope tracers and POP fingerprints indicated that natural-origin salmon were exposed to and assimilated both nitrogen and POPs from wastewater in the Lower Mainstem. The $\sum_{11}$PBDE enhanced POP fingerprints in natural-origin fish from the Lower Mainstem were inversely correlated with their $\delta^{15}N$ (Fig. 6), suggesting similar sources for both; the more fish were exposed to the ammonia/ammonium rich effluent, the more depleted they were in $\delta^{15}N$ and the greater their relative $\sum_{11}$PBDE concentrations. Concentrations of $\sum_{11}$PBDEs and TPCBs were also each negatively correlated with $\delta^{15}N$ in salmon. However, the slope of these relationships were steeper for $\sum_{11}$PBDEs (8.93 vs. 6.55), supporting our previous conclusion that the wastewater the fish were exposed to had a greater load of PBDEs than PCBs. Additionally, based on their $\delta^{34}S$, the natural-origin fish that had spent the least amount of time in the Lower Mainstem where the wastewater discharged, deviated most from the predicted relationship between PC1 score and $\delta^{15}N$ (Fig. S3), further supporting our conclusion that fish were exposed to and accumulated PBDEs from a wastewater source in the Lower Mainstem.

These results highlight the role of wastewater as a vector of toxic contaminants to aquatic consumers, as demonstrated previously (Meador et al., 2016; Spies et al., 1989), and raises additional concerns about juvenile salmon exposure to other contaminants in wastewater not evaluated in this study. Effluent from WWTPs are major sources of industrial chemicals (Servos, 1999), pharmaceutical and personal care products, (PPCPs) (Metcalfe et al., 2010), and natural and synthetic hormones (Ternes et al., 1999). Adverse effects observed in aquatic biota exposed to wastewater include endocrine disruption in individuals (Tyler and Jobling, 2008; Vajda et al., 2011), and alterations in species communities (Tetreault et al., 2013). Most pertinent to our study, Chinook salmon collected from wastewater impacted sites had modeled fish plasma concentrations for a variety of PPCPs in the range expected to produce adverse effects in fish (Meador et al. 2017); mitochondrial dysfunction, which is adverse for growing juvenile fish (Yeh et al., 2017); and altered blood chemistry parameters, a potential early indicator of metabolic disruption (Meador et al., 2018).

5. Conclusions

Our study demonstrated the utility of multiple chemical tracers to document the spatial extent, magnitude, and source of contaminant exposure in juvenile Chinook salmon, information necessary to formulate appropriate conservation measures to reduce or remediate contaminant exposure. Three types of complementary chemical tracer data, POP concentrations, POP fingerprints, and stable isotopes, allowed us to 1) identify where in their migration pathway threatened Chinook salmon were exposed to and accumulated PBDEs (and to a lesser extent PCBs), at concentrations high enough to impair their health, and 2) reveal that wastewater discharging into the river was the likely source of these POPs. These results highlight the importance of understanding the role that wastewater may play as a vector of toxic contaminants to aquatic consumers.

Data from this study can be used to guide and prioritize management actions to reduce threats from wastewater and other habitat stressors to juvenile salmon migrating through the Snohomish River estuary to Puget Sound. Specifically, identifying the region within the Snohomish watershed where salmon are most exposed to PBDEs, as well as the source (i.e., wastewater or stormwater), allows environmental managers to establish corrective actions to control PBDE inputs. Ultimately, reductions in PBDE exposure should improve Chinook salmon health and enhance their marine survival. The Snohomish River is the second largest contributor of Chinook salmon to the Puget Sound evolutionarily significant unit (Jonathan Carey, National Marine Fisheries Service, Personal communication); consequently, reductions in salmon survival due to wastewater-contaminant exposure could affect the recovery of the ESA-listed Chinook salmon from Puget Sound. Furthermore, exposure to contaminants in wastewater may thwart substantial habitat remediation efforts underway throughout the US Pacific Northwest to improve survival of natural-origin salmon. For example, between 2005 and 2017 approximately $ 90,000,000 US has been spent to improve the freshwater, estuarine and nearshore marine rearing habitat for natural-origin Chinook salmon originating from the Snohomish River (Snohomish Basin Salmon Recovery Forum, 2019), with the ultimate goal of improving their overall survival. The efficacy of this effort could be reduced if juvenile salmon have increased susceptibility to disease because of exposure to wastewater-derived contaminants. More broadly, Chinook and other salmon species are at risk in much of the southern part of their North American range (Gustafson et al., 2007), where interactions with many anthropogenic factors affect them, including contaminants (Lundin et al., 2019; Meador, 2014).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary Material

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