



Emerging contaminants in juvenile Chinook salmon: patterns of exposure and implications for conservation

Molly Shuman-Goodier^{a,*}, James Meador^b, Andrea Carey^a, Sandra O'Neill^a

^a Washington Department of Fish and Wildlife, Toxics Biological Observation System, PO Box 43200, Olympia, WA, 98504-3200, United States

^b University of Washington, Dept. of Environmental and Occupational Health Sciences, School of Public Health, 4225 Roosevelt Way NE, Suite 100, Seattle, WA, 98105-6099, United States

ARTICLE INFO

Keywords:

Chinook salmon
Pacific Northwest
Salmon recovery
Contaminants of emerging concern
PFAS
Pharmaceuticals
Personal care products

ABSTRACT

Efforts to recover populations of threatened Chinook salmon in the Pacific Northwest may be hindered by exposure to contaminants in the juvenile life stage. Here we leverage a large dataset collected by the Washington Department of Fish and Wildlife's Toxic Biological Observation System to test for the accumulation of 219 contaminants of emerging concern (CECs) in juvenile Chinook salmon and identify chemicals that may affect salmon physiology, behavior, or fitness at current exposure levels. We also highlight results obtained from the region's two most urbanized watersheds, the Green/Duwamish and Puyallup/White, to demonstrate how these data can inform decision making to protect juvenile salmonids. We found that juvenile Chinook salmon sampled in the Puyallup/White watershed had the highest average concentrations of CECs, and that six chemicals appeared to be ubiquitous and were found in all five watersheds spanning a gradient of urbanization. In the subsequent detailed studies in the Green/Duwamish and Puyallup/White watersheds, we observed patterns in accumulation of per- and polyfluoroalkyl substances (PFAS) that revealed regions where certain analytes could be entering the rivers. In contrast, spatial patterns for pharmaceutical and personal care products (PPCPs) were less clear and indicated diffuse sources throughout the migration corridor, such as stormwater or wastewater. We recommend these results be used to target areas for source tracing studies for PFAS, and that future studies test for PPCP residues in commercial fish feed. Finally, the detection of multiple emerging contaminants in almost all composite fish samples, 11 of which exceeded available biological effects thresholds, reinforces the global call for green infrastructure projects that target source control and the removal or reduction of emerging contaminants from stormwater and wastewater.

1. Introduction

Recovery efforts for threatened Chinook salmon in the Salish Sea, an iconic inland sea in the Pacific Northwest, are likely hindered by exposure to chemical contaminants during their juvenile life stage (Pearsall et al., 2021). Chinook salmon may be particularly vulnerable to contaminant exposure compared to other salmon species because juveniles spend more time feeding in estuaries and nearshore marine habitat (Quinn, 2018), which receive higher contaminant inputs than offshore environments. Juvenile salmon also experience significant physiological stress as they undergo smoltification transitioning from freshwater to marine environments, which heightens their susceptibility to other stressors (Bjornsson et al., 2011). Exposure to contaminants during this critical life stage is correlated with decreased first-year growth rates

(Lundin et al., 2021), which is in turn strongly correlated with adult survival and projected salmon population abundance (Claiborne et al., 2011; Duffy and Beauchamp, 2011; Spromberg and Meador, 2005). If we are to successfully recover salmon populations in the Pacific Northwest and elsewhere, contaminants must be considered while planning for habitat restoration and fish passage projects, alongside swift improvements to stormwater and wastewater management.

Numerous studies have documented that juvenile Chinook salmon from urbanized watersheds of the US Pacific Northwest are exposed to legacy contaminants such as polychlorinated biphenyls (PCBs), the insecticide dichlorodiphenyltrichloroethane (DDT), polybrominated diphenyl ether (PBDEs) flame retardants and polycyclic aromatic hydrocarbons (PAHs) during their spring migrations downstream through freshwater streams and rivers to saltwater (Johnson et al., 2013;

* Corresponding author.

E-mail address: Molly.Shuman-Goodier@dfw.wa.gov (M. Shuman-Goodier).

<https://doi.org/10.1016/j.envpol.2025.126639>

Received 2 April 2025; Received in revised form 27 May 2025; Accepted 6 June 2025

Available online 11 June 2025

0269-7491/© 2025 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

Johnson et al., 2007; Meador et al., 2010; O'Neill et al., 2020; O'Neill et al., 2015; Sloan et al., 2010; Stehr et al., 2000; Stein et al., 1995). In some river systems the concentrations observed in fish tissue or water exceed values predicted to cause an adverse impact on salmon survival or growth (Berninger and Tillitt, 2019; Lundin et al., 2021; Meador et al., 2006), metabolism (Meador et al., 2024a), thyroid hormone levels (Arkoosh et al., 2017), immune response (Arkoosh et al., 2010; Arkoosh et al., 2018), and reproductive development (Peck et al., 2011), and may be contributing to reduced marine survival (Meador, 2014).

Unfortunately, legacy contaminants are only the tip of the proverbial contaminant iceberg. A wide range of other contaminants originating from human activity have also been documented in freshwater and marine habitats of Chinook salmon, as well as in their plasma and tissue (James et al., 2016; Meador et al., 2024b; Meador et al., 2020; Meador et al., 2017; Meador et al., 2016; Tian et al., 2020; Yeh et al., 2017). This diverse pool of chemicals is often classified loosely as contaminants of emerging concern (CECs) due to the lack of information surrounding environmental fate, exposure, or toxicity. Examples of CECs include pharmaceutical and personal care products (PPCPs), per- and poly-fluoroalkyl substances (PFAS), alkylphenols, bisphenol A, phthalates, tire related compounds (6PPD and 6PPD-Q), and current-use pesticides. In the Pacific Northwest, several PPCPs and PFAS, the two contaminant classes we focus on in this study, were identified as high priority contaminants likely to be eliciting a biological effect at current exposure concentrations (James et al., 2023). For salmon specifically, characterizing exposure to CECs during the critical juvenile life stage has also been identified as a priority research gap by the Salish Sea Marine Survival Project, a transboundary effort to evaluate threats to the early marine survival of salmon (Pearsall et al., 2021).

In the absence of conventional concentration targets set by regulatory agencies or comprehensive ecotoxicological data, efforts to identify data gaps and prioritize CECs for regulatory action, monitoring, and future study are strengthened by drawing from multiple lines of evidence on adverse effects (Deere et al., 2020; Edwards et al., 2024; Elliott et al., 2022; Jorgenson et al., 2018; Maruya et al., 2016). Various analyses have calculated screening values and species sensitivity distributions from the experimental ecotoxicity data compiled in EPA's ECOTOX knowledgebase (Gefell et al., 2019; Maloney et al., 2025; Posthuma et al., 2019; Pronschinske et al., 2022), which provide valuable means of inferring potential biological effects from CEC exposure. Likewise, tools such as the fish plasma model can be applied to predict potential physiological or behavioral effects in fish in the absence of experimental data for many pharmaceuticals (Huggett et al., 2003; Meador et al., 2024b).

Here, we leverage a comprehensive dataset collected by the Washington Department of Fish and Wildlife's (WDFW's) Toxic Biological Observation System (TBIOS) to test for the accumulation and potential biological effects of 219 emerging contaminants in the tissue of threatened juvenile Chinook salmon throughout five watersheds in Washington State. We also highlight detailed results obtained from the two most urbanized watersheds in the region, the Green/Duwamish and Puyallup/White, to illustrate how these data could be utilized for decision making relevant to remediation to protect juvenile salmonids. Importantly, we build upon previous work to identify CECs most likely to be negatively impacting fish physiology, behavior, or fitness, and evaluate whether concentrations observed in migrating fish reveal geographic regions where contaminants may be entering urbanized river systems.

We asked the following questions.

1. Are juvenile Chinook salmon originating from developed watersheds accumulating higher concentrations and total numbers of CECs compared to populations originating in less developed watersheds?
2. Do concentrations measured in juvenile Chinook salmon exceed concentrations predicted to impact fish physiology, behavior, or survival?

3. Are there spatial trends in the contaminant profiles of juvenile Chinook salmon sampled throughout the region's two most urbanized watersheds that reveal potential sources of chemicals along their migratory pathway?

We hypothesized that the concentrations and numbers of detected contaminants would be higher in juvenile Chinook salmon collected from more urbanized watersheds in Puget Sound as compared to less urbanized watersheds. We further hypothesized that multiple contaminants would exceed the concentrations derived from the literature indicating potential effects to behavior, physiology, or fitness of fish. Last, we predicted that spatial groupings of samples and chemicals would reveal potential sources of some chemicals accumulating in juvenile Chinook salmon along their migration path through the Puyallup/White and Green/Duwamish watersheds.

2. Methods

2.1. Samples and study design

All data compiled for this manuscript were collected by the WDFW between 2013 and 2023 and included 141 composite samples comprising 772 individual subyearling Chinook salmon collected between April and June during the peak of their seaward migration. Fish were captured using beach seines, lampara seines, and an in-river screw trap, with additional collections made directly from hatchery ponds at the Soos Creek Hatchery in Auburn, WA. All collections were conducted under an ESA Section 4(d) permit obtained by WDFW for the collection of listed Chinook salmon. Hatchery-origin fish were identified using fin clips, otolith thermal marks, and coded wire tags; all others were presumed to be of natural origin and are hereafter referred to as wild fish. To meet the 12 g target mass required for chemical analysis in this study and a parallel study on legacy contaminants, individual fish were composited based on collection location, origin (wild or hatchery), and body weight. The average individual fish mass was 5 g (range: 0.41–21.8 g). Final samples consisted of composites of whole-body juvenile Chinook salmon (excluding stomach contents), averaging five fish per composite (range: 2–20). Because stomach contents were removed for a separate study, the contaminant concentrations reported here do not reflect recent dietary exposure.

To first explore whether juvenile Chinook salmon originating from developed watersheds in Puget Sound accumulated higher average concentrations and total numbers of CECs as compared to those in less developed watersheds, we collated a dataset collected across five river systems in 2013. These data consisted of three whole-body composite samples of fish collected from the Nisqually, Puyallup/White, Green/Duwamish, Snohomish and Skagit watersheds (Fig. 1A; 15 total samples) which were tested for 13 PFAS, 34 current use pesticides, four alkylphenols, and 141 PPCPs. Of the three samples collected from each watershed, one was from within the river estuary, and two were from adjacent marine shoreline, hereafter defined as nearshore areas. These five systems were chosen for sampling because they comprise the major rivers that flow into the Southern Salish Sea that support populations of Chinook salmon, and because they span a gradient of urbanization. We used the percentage of impervious surface (USGS, 2024) observed within each river's delta unit as adapted from Stefankiv et al. (2019) to provide a frame of reference of the overall level of urbanization pertinent to each watershed (Table 1). Differences in the average concentrations and percentages of CECs detected above the reporting limits across watersheds were evaluated with linear and generalized linear models using the R package *lme4* (Bates et al., 2015).

Next, to prioritize CECs based on potential impacts to fish health and examine spatial trends, we collated datasets that encompassed the entire rearing and downstream migration habitat of juvenile Chinook salmon in the two most urbanized watersheds in Puget Sound, Washington; the Green/Duwamish and Puyallup/White (Fig. 1B and C). The collection

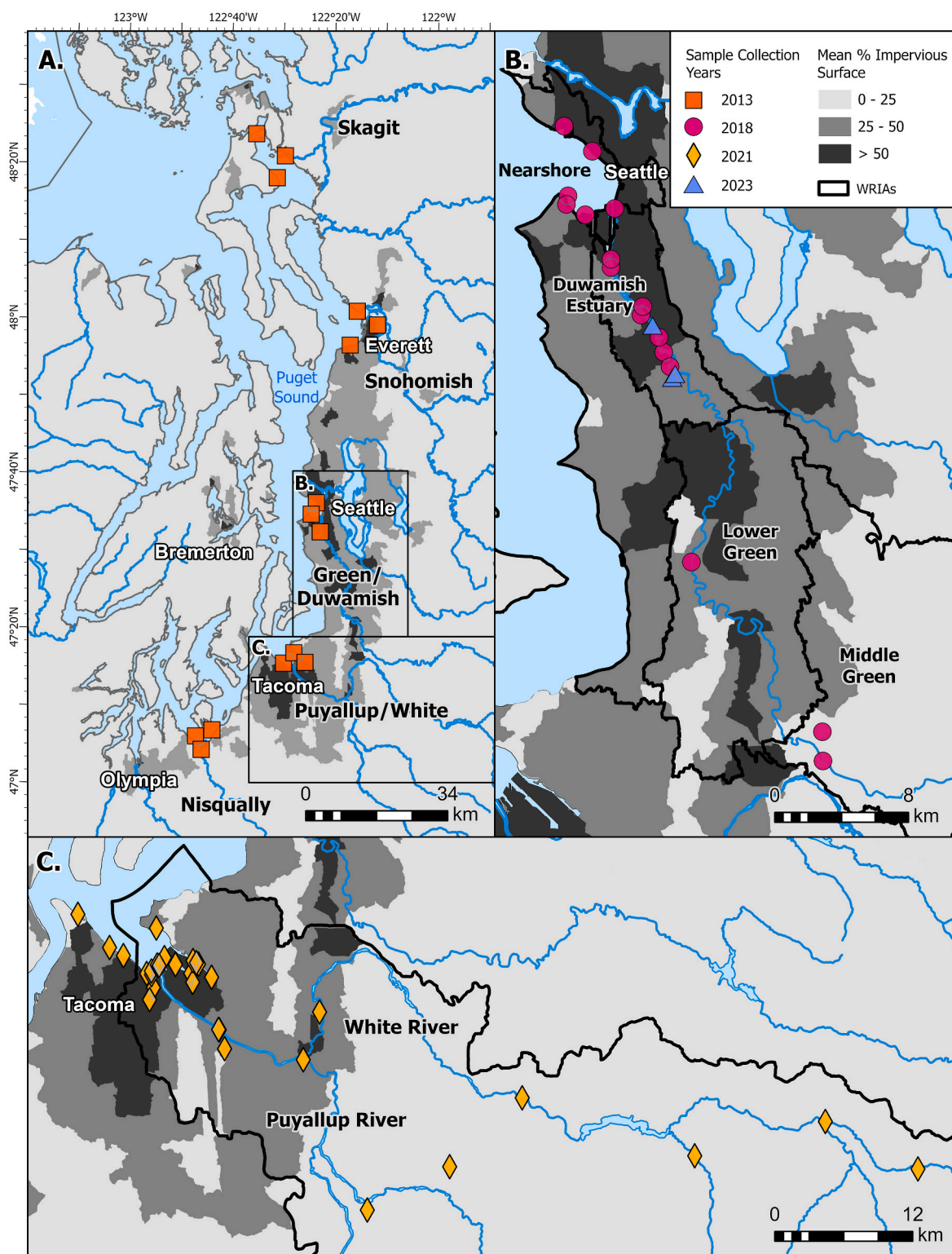


Fig. 1. Sampling locations where juvenile Chinook salmon were collected for CEC analyses across five major watersheds of Puget Sound, Washington in 2013 (A), throughout the Green/Duwamish watershed in 2018 and 2023 (B) and throughout the Puyallup/White watershed in 2021 (C). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

sites were chosen based on contaminant levels previously measured in juvenile Chinook salmon, known areas of historic contamination or potential point sources (i.e., superfund sites and WWTP outfalls), reference areas upstream of contaminant sources and accessibility for sampling. The chemistry datasets compiled in the two most urbanized watersheds included 1) 37 samples analyzed for 141 PPCPs and four

alkylphenols (4n-OP, 4-NP, NP1EO, NP2EO) in the Green/Duwamish watershed in 2018, 2) 67 samples analyzed for 40 PFAS compounds in the Green/Duwamish in 2018 and 2023, and 3) 67 samples analyzed for 40 PFAS compounds in the Puyallup/White watershed in 2021. Overviews of the compiled samples and analytes tested are provided in [Tables S1 and S2](#). Analyses of sample splits taken in 2013 and re-

Table 1

Summary data from each of the five watersheds sampled in 2013 including % impervious surface (IS), % of total chemicals tested that were not detected (ND), % of total chemicals tested that were detected (% D), minimum, median, geometric mean, and maximum concentrations of detected chemicals (no substitution for non-detects) and summary reporting limits in ng/g wet weight. Samples obtained were tested for 141 PPCPs, 13 PFAS, 4 alkylphenols, 32 current-use pesticides.

Watershed	% IS ^a	% ND	% D	Concentration (ng/g ww)				Reporting Limit (ng/g ww) ^b		
				Min	Median	Mean	Max	Min	Mean	Max
Skagit	2.52	96	4	0.023	1.57	3.01	247	0.002	1.08	241
Snohomish	8.64	95	5	0.016	1.07	2.49	629	0.002	1.23	210
Green/Duwamish	72.9	93	7	0.015	2.28	3.55	370	0.004	1.14	455
Puyallup	63.2	94	6	0.044	2.17	5.66	2030	0.011	1.31	495
Nisqually	2.18	96	4	0.014	2.12	3.21	427	0.002	1.02	382

^a % Impervious surface (USGS, 2024) was estimated within each river delta, with adaptations noted by Stefankiv et al. (2019).

^b SGS AXYS determined reporting limits for each chemical and sample based on the sample mass and lowest calibration standard analyzed. Effects of the sample matrix and recovery were accounted for and final reporting limits for each chemical and sample were calculated either as the lowest concentration in the calibration standard or as the sample specific detection limit (SDL), whichever value was higher.

analyzed with later samples in 2021 for quality control indicated that more CECs were detected when using newer methods due to analytical improvements resulting in lower reporting limits (Document S1). Because of these analytical improvements and differences in the numbers of analytes tested each year across the two river systems, each of the three datasets listed above were considered as not directly comparable and were analyzed separately. This also ensured that comparisons across samples and sampling locations within watersheds were made using a complete set of tested analytes.

2.2. Chemical analysis

All chemical analyses were completed by the commercial laboratory SGS AXYS Analytical Laboratory (Sydney, B.C.). Multiple established extraction and analytical methods were used to test for a range of CECs including 141 PPCPs, four alkylphenols, 34 current-use pesticides, and 40 PFAS. These methods are referenced in Document S1 alongside further discussion of quality control screening criteria for % recovery, lab flags, method blanks, and reporting limits. Method documents are available from SGS AXYS upon request. Briefly, fourteen chemicals were detected in the method blanks, which consisted of solvent (Table S4). Most blank detections were at low concentrations near reporting limits (<1 ng/g), and likely reflected trace residues on analytical equipment or presence at low background concentrations in materials used for chemical analyses. However, two chemicals, DEET and nonylphenol (4-NP), were occasionally observed at higher concentrations in the blanks (up to 15 ng/g) and were observed consistently across lab sets. 4-NP, a degradant product of non-ionic surfactants, is a commonly reported laboratory contaminant (Salgueiro-Gonzalez et al., 2012). The insecticide DEET has also been reported in field and lab blanks (Kolpin et al., 2013; Thomas et al., 2007), perhaps due to its ubiquity in the environment after decades of use. To account for background contamination, concentration estimates for these fourteen chemicals were adjusted by subtracting 1x the measured concentration in the lab set blank from the measured concentration in the corresponding sample. This approach was preferred to ensure concentration estimates that accounted for the specific background conditions defining each set of extractions and machine runs for comparisons with fish health effects thresholds.

Reporting limits defined in this study were determined by SGS AXYS for each chemical and sample and were defined as either the lowest concentration in the calibration standard (lowest level calibration concentration x extract volume)/sample size), or as the Sample Specific Detection Limit (SDL), if it was higher. The SDL was calculated by converting 3x the estimated chromatographic noise height to a concentration and accounted for effects of sample matrix and recovery achieved through the analytical work up. Because observed reporting limits improved significantly with time due to methodological advances (Document S1) we generated summary data and conducted multivariate analyses separately for each study year, except for the PFAS data collected in the Green/Duwamish in 2021 and 2023, which was

obtained utilizing the same newer analytical method.

For each analyte we summarized detection frequencies, geometric means and concentration ranges based solely on measured values; no substitutions were made for non-detects. For the multivariate spatial analyses (see section 2.5), values that were not detected above the analytical reporting limit were replaced by a constant factor of 0.2 times the mean reporting limit for each analyte. Percent lipid content was also measured and ranged from 0.5 to 7 % across samples. Concentration data were not normalized as is common practice for lipophilic chemicals (e.g., PCBs) because many of the CECs tested in this study preferentially bind to proteins rather than lipids (Alesio et al., 2022), are poorly correlated with lipid content (Ramirez et al., 2009), or do not have well characterized toxicokinetic properties.

2.3. Biological effects thresholds

Unlike legacy chemicals, which have established regulatory thresholds set by EPA, most of the CECs tested in this study lack similar consensus thresholds that are intended to be protective of the environment and provide biological context. To address this knowledge gap and provide insight about potential impacts to threatened juvenile Chinook salmon, we compared concentrations of detected chemicals to four different biological effects thresholds derived from the literature (Gefell et al., 2019; Meador et al., 2024b; Posthuma et al., 2019; Pronschinske et al., 2022). Two of these available lines of evidence including screening values (SVs) and hazardous concentrations (HC₅s) (Gefell et al., 2019; Posthuma et al., 2019) were selected to remain consistent with and expand upon a regional prioritization effort for CECs that occurred in 2023 (James et al., 2023), while the fish plasma model (Meador et al., 2024) and screening values derived in Pronschinske et al. (2022) provide new lines of evidence not previously utilized.

Each of the effects thresholds we obtained, including SVs, HC₅s, and values derived from the fish plasma model, are defined and described in detail in Document S1, along with any calculations made. In total, we obtained effects threshold data for 137/185 chemicals tested (Table S2). We did not include comparisons to *in vitro* assays, such as Activity Concentrations at Cut off (ACC) values generated using EPA's TOXCAST program, due to the unclear relationships observed between *in vitro* versus *in vivo* toxicity data for many chemicals (Rodea-Palomares and Bone, 2024; Schaupp et al., 2023). For PFOA and PFOS, measured concentrations in tissue were also compared to the final chronic aquatic life criteria for whole body fish that EPA issued in 2024 of 6.49 mg/kg ww and 0.201 mg/kg ww (EPA, 2024a; EPA, 2024b).

2.4. Inferring potential biological effects

Hazardous concentrations and screening values obtained from the literature (reflective of concentrations in the water column) were converted to whole body concentration estimates expressed as ng/g ww using bioconcentration factors (BCFs). BCFs were generated using

OPERA, a widely used modeling tool produced by the Organization for Economic Cooperation and Development that predicts physical chemical properties including BCFs based on quantitative structure activity relationships (QSARs) (Mansouri et al., 2018). For PFAS compounds, which are not well characterized by QSAR models that rely heavily on n-octanol–water partition coefficients ($\log K_{ow}$), we used BCFs compiled in a meta-analysis (Burkhard, 2021). For the fish plasma model, the plasma concentrations estimated to cause a behavioral or physiological effect in vertebrates were converted to tissue concentrations by multiplying the concentrations in plasma known to produce therapeutic effects in humans and rodents (termed C_{max}) by the volume of distribution. We then compared the concentrations of detected chemicals in whole body juvenile Chinook salmon to the concentrations estimated to produce an adverse effect using all the available lines of evidence. For the purposes of this study, we flagged chemicals as having the potential to cause a biological effect if fish tissue concentrations exceeded any one of the biological effects thresholds.

2.5. Spatial trends

To test for spatial trends in the contaminant profiles of PPCPs and PFAS detected in juvenile Chinook salmon migrating through the Green/Duwamish and Puyallup/White watersheds we used MetaboAnalyst 6.0, an R-based software package (Pang et al., 2024). MetaboAnalyst is primarily used to analyze omics data; however, it contains many statistical methods that are widely applicable for multivariate data analysis and exploring relationships between spatially oriented samples and observed values. We used hierarchical clustering analyses to identify patterns in contaminant profiles and spatial associations among sampling locations, which consisted of replicate composite samples. Euclidean distance was used as the distance measure between samples, Ward's linkage was used as the clustering algorithm, and data were normalized by autoscaling. Analytes occurring in only one composite sample above the RL were excluded, and values that were not detected below the analytical reporting limit (RL) were replaced by a constant factor of 0.2 times the mean RL for each analyte. This data filtering approach was chosen to address the large number of blank cells due to low detection frequencies of many CECs and issues with data normalization. This is one of several established approaches utilized by MetaboAnalyst 6 to address missing values and left censored data.

3. Results & discussion

3.1. Comparison across five watersheds

Out of the 192 PPCPs, PFAS, and current-use pesticides tested across five watersheds in Puget Sound in 2013, 45 were detected. Detection frequencies for most compounds were low (0–14 %), and the majority (27/45) of detected chemicals were observed in less than 3 samples (Table S7). However, there were 18 chemicals detected in three or more samples (Table 2) and six of those were observed in every watershed at high relative concentrations, with detection frequencies ranging from 47 to 100 %: 4-nonylphenol (4-NP) and nonylphenol diethoxylate (NP2EO), iopamidol, perfluorooctane sulfonic acid (PFOS), the antibiotic oxolinic acid, and the insecticide N,N-diethyl-meta-toluamide (DEET). 4-NP and its degradation product NP2EO are surfactant derivatives used in industrial manufacturing of a wide variety of products ranging from drugs to explosives. Both are prevalent in the environment (Acir and Guenther, 2018) and can inhibit growth in rainbow trout (*Oncorhynchus mykiss*) at ng/mL concentrations (Ashfield et al., 1998). Iopamidol is an X-ray contrast agent that is also frequently detected in wastewater and surface water around the world because it is not metabolized by humans or removed from wastewater treatment plants. PFOS is the one of most heavily studied fluorinated compounds and has been released into the environment for decades due to industrial manufacturing and use and discarding of products that contain PFAS

(Paul et al., 2009). DEET is frequently detected in environmental media, although some uncertainty has been raised about its ubiquity because concentrations measured in the environment are higher than predicted from known uses (Marques Dos Santos et al., 2019). We detected DEET at low concentrations in several method blanks, and the possibility that target analyses pick up DEET mimics has been discussed, though none have been identified (Merel et al., 2015; Merel and Snyder, 2016). Nevertheless, the accumulation of these chemicals in fish from five different river systems indicates widespread exposure, and all but oxolinic acid have been previously flagged as likely to produce biological effects at current concentrations in Puget Sound (James et al., 2023). Detections are a function of the detection limits, and so we emphasize that there are almost certainly more CECs that may accumulate in tissue than were detected in this early study due to marked improvements in analytical methods since 2013.

Contaminant profiles in juvenile Chinook salmon appeared to differ across watersheds, (Fig. S3), though small sample sizes precluded in-depth quantitative analyses across chemical classes. The highest percentages of detected chemicals were observed in juvenile Chinook salmon from the Puyallup/White and Green/Duwamish systems, and the highest average and maximum concentrations were observed in samples from the Puyallup/White (Table 1, Fig. S4). No significant differences were observed in the total numbers of CECs detected across the five watersheds, but geometric mean concentrations of CECs were higher in fish sampled in the Puyallup/White River than in the Skagit and Snohomish, which raises concerns (Table S10). Other studies also indicate that the estuary at the mouth of the Puyallup/White River, referred to as Commencement Bay, is a hot spot for CECs, potentially associated with the Tacoma Central wastewater treatment plant outfall approximately 370 m northwest from the mouth of the Blair Waterway (Fig. S6). CECs including amphetamine, azithromycin, fluoxetine, miconazole, nor-fluoxetine, sertraline, sulfadimethoxine, triclosan, and nonylphenol have been observed in juvenile Chinook salmon and caged mussels at higher concentrations and detection frequencies in the Puyallup/White river estuary relative to other regions in Puget Sound (Meador et al., 2016; Swam-Jaramillo et al., 2024). Overall, our findings were only in partial support of our hypothesis that the frequency of CEC detections and average concentrations would be greatest in Puget Sound's most urbanized watersheds. Bioaccumulation of some CECs in juvenile Chinook salmon is also occurring in less developed watersheds.

3.2. Detailed study in two urbanized watersheds

Our data suggests juvenile Chinook salmon are exposed to multiple PPCPs and PFAS during their downstream migration through the regions' most urbanized watersheds; the Green/Duwamish and Puyallup/White. Summary concentration data, detection frequencies, and reporting limits are provided in Table 2 for chemicals detected in a minimum of 3 samples, and in Table S7 for all chemicals tested.

3.2.1. Pharmaceuticals and personal care products

Of the 145 PPCPs tested, 36 were detected in the juvenile Chinook salmon collected in 2018 in the Green/Duwamish watershed. The most frequently detected chemicals were 4-NP, NP2EO, hydrocortisone, oxytetracycline, 4-epioxytetracycline, and diazepam, and the highest geometric mean concentration was 464 ng/g ww (oxytetracycline). Of the detected PPCPs, 11 were observed at concentrations in fish from the Green/Duwamish River that could potentially cause an adverse biological response based on either the fish plasma model, HC_{50} s, or SVs derived from the literature (Fig. 2). These included the antibiotics oxytetracycline [OTC], tetracycline [TC], and erythromycin-H2O, the insect repellent DEET, and the pharmaceuticals metformin, ibuprofen, valsartan, norfluoxetine, citalopram, caffeine, and hydrocortisone. Except for hydrocortisone, which is a synthetic glucocorticoid that may be conflated in these analyses with endogenously produced cortisol, the remaining 10 chemicals raise potential concerns. These CECs have been

Table 2

Summary data for chemicals detected in a minimum of three composite samples of whole-body juvenile Chinook salmon for A) the study conducted across five watersheds in 2013, B) the focus study conducted in the Green/Duwamish watershed testing for PPCPs in 2021 and PFAS in 2021 and 2023 and C) the focus study conducted in the Puyallup/White watershed testing for PFAS in 2021. Data includes the number of samples where a chemical was detected (#D), number of samples tested (N), % detection (%D), minimum, median, geometric mean, and maximum values of detected chemicals (no substitution for non-detects) and summary reporting limits provided by SGS AXYS in ng/g wet weight. See [Table S2](#) for associated CAS numbers.

Analyte	CEC Category	N	#D	% D	Concentration (ng/g ww)				Reporting limit (ng/g)		
					Min	Med	Mean	Max	Min	Mean	Max
A) Comparison across five river systems (141 PPCPs, 13 PFAS, 4 alkylphenols, 32 current-use pesticides - 2013)											
4-NP	Industrial	13	13	100	6.80	23.3	22.4	62.4	0.498	0.712	1.34
Iopamidol	Pharmaceutical	15	15	100	144	236	276	629	31.0	43.7	76.9
NP2EO	Industrial	13	12	92	0.615	1.13	1.754	70.2	0.442	0.483	0.505
PFOS	PFAS	14	11	79	1.12	2.03	2.99	14.2	0.905	1.06	1.62
DEET	Pesticide	15	9	60	0.56	33.7	43.5	2.03E+03	0.31	0.38	2.0
Oxolinic Acid	Antimicrobials	15	7	47	0.345	1.03	1.06	3.02	0.273	1.14	3.06
Octachlorostyrene	Industrial	15	6	40	1.4E-02	0.020	0.024	0.072	2.2E-03	7.5E-03	0.139
Citalopram	Pharmaceutical	15	6	40	0.347	0.475	0.568	2.17	0.155	0.404	2.95
4n-OP	Industrial	13	4	31	0.555	0.857	0.834	1.32	0.468	0.553	0.928
Diphenhydramine	Pharmaceutical	15	4	27	0.354	0.657	0.660	1.38	0.233	0.289	1.19
Sertraline	Pharmaceutical	15	4	27	0.382	0.649	0.901	4.15	0.234	0.352	0.503
Erythromycin-H2O	Antimicrobials	15	4	27	1.00	1.23	1.26	1.67	0.891	0.913	0.966
Etoposide	Pharmaceutical	15	4	27	1.71	3.95	3.41	5.06	0.781	2.53	13.1
Sulfadiazine	Antimicrobials	15	4	27	2.68	3.22	3.93	8.59	0.588	0.892	1.46
PFOSA	PFAS	14	3	21	0.657	0.936	0.875	1.09	0.543	0.589	0.628
Desmethyldiltiazem	Pharmaceutical	15	3	20	0.079	0.333	0.227	0.446	0.058	0.064	0.164
Fluoxetine	Pharmaceutical	15	3	20	0.686	0.894	1.09	2.1	0.581	0.681	2.06
Anhydrochlortetracycline	Antimicrobials	15	3	20	8.72	9.62	10.1	12.1	6.47	9.08	12.8
B) Green/Duwamish River (141 PPCPs & 4 alkylphenols - 2018, 40 PFAS - 2021 & 2023)											
PFOS	PFAS	69	69	100	0.447	1.49	1.86	21.4	0.088	0.104	0.189
PFOSA	PFAS	69	38	55	0.103	0.215	0.285	3.38	0.088	0.104	0.189
4-NP	Industrial	35	34	97	1.86	9.99	10.1	40.7	0.448	0.494	1.11
Hydrocortisone	Hormones	37	33	89	9.82	24.3	25.1	213	2.26	3.92	11.7
PFNA	PFAS	69	31	45	0.101	0.184	0.203	0.798	0.088	0.104	0.189
Oxytetracycline [OTC]	Antimicrobials	37	30	81	24.8	359	465	2.25E+04	2.22	4.21	71.4
NP2EO	Industrial	35	27	77	0.506	0.906	0.988	5.51	0.448	0.485	0.588
4-Epioxytetracycline [EOTC]	Antimicrobials	37	26	70	10.3	47.4	68.8	3250	2.24	4.82	71.4
PFDoA	PFAS	69	26	38	0.08	0.18	0.20	0.91	0.07	0.09	0.15
PFDA	PFAS	69	26	38	0.099	0.175	0.183	0.511	0.088	0.104	0.189
Diazepam	Pharmaceutical	37	23	62	0.205	0.334	0.340	0.705	0.186	0.208	0.322
PFMBA	PFAS	69	23	33	0.096	0.240	0.241	0.560	0.088	0.104	0.189
PFTTrDA	PFAS	69	21	30	0.104	0.237	0.243	0.704	0.088	0.104	0.189
PFTeDA	PFAS	69	21	30	0.099	0.143	0.184	1.00	0.088	0.106	0.339
N-EtFOSE	PFAS	69	17	25	0.757	0.987	1.24	6.77	0.693	0.888	1.89
PFDS	PFAS	69	17	25	0.112	0.146	0.181	0.335	0.088	0.104	0.189
PFHxS	PFAS	69	15	22	0.100	0.192	0.204	0.713	0.088	0.104	0.189
6:2 FTS	PFAS	69	13	19	0.337	0.505	0.546	0.842	0.316	0.374	0.679
PFHxA	PFAS	69	13	19	0.108	0.125	0.160	0.433	0.088	0.120	0.436
4n-OP	Industrial	35	12	34	0.490	0.906	0.818	1.32	0.448	0.523	2.13
2-Hydroxy-ibuprofen	Pharmaceutical	37	12	32	1.75	2.37	2.55	6.25	1.48	1.57	1.68
PFUnA	PFAS	69	11	16	0.142	0.322	0.303	0.599	0.088	0.104	0.189
Ibuprofen	Pharmaceutical	37	8	22	1.62	2.03	2.22	3.86	1.48	1.57	1.63
PFOA	PFAS	69	7	10	0.099	0.123	0.156	0.273	0.088	0.104	0.189
Sulfathiazole	Antimicrobials	37	6	16	0.615	2.61	2.16	4.03	0.560	0.886	7.54
Ormetoprim	Antimicrobials	37	6	16	0.236	0.521	0.605	3.86	0.222	0.236	0.268
Caffeine	Pharmaceutical	37	4	11	7.96	10.6	13.9	41.8	5.60	6.16	11.3
Sulfadimethoxine	Antimicrobials	37	4	11	0.426	2.87	1.54	5.31	0.111	0.378	6.37
Erythromycin-H2O	Antimicrobials	37	4	11	0.900	0.985	0.982	1.07	0.852	0.902	0.935
Warfarin	Pharmaceutical	37	4	11	0.189	0.200	0.216	0.286	0.148	0.161	0.223
N-MeFOSA	PFAS	69	4	6	0.119	0.130	0.151	0.259	0.088	0.115	0.197
Tetracycline [TC]	Antimicrobials	37	3	8	37.2	83.1	70.4	113	2.22	3.02	6.21
4-Epitetracycline [ETC]	Antimicrobials	37	3	8	25.0	57.8	49.4	83.2	2.22	2.66	5.96
Metformin	Pharmaceutical	37	3	8	1.45	1.83	2.00	3.00	0.251	0.298	0.445
Sulfamethazine	Antimicrobials	37	3	8	0.808	2.87	1.97	3.28	0.222	0.445	3.56
PFBA	PFAS	69	3	4	0.723	0.905	1.00	1.51	0.351	0.415	0.755
C) Puyallup/White River (40 PFAS - 2021)											
PFOS	PFAS	67	61	91	0.140	0.823	0.829	8.25	0.089	0.110	0.180
PFPeA	PFAS	67	27	40	0.203	0.399	0.436	2.46	0.178	0.232	0.560
PFDoA	PFAS	67	22	33	0.086	0.124	0.156	0.494	0.071	0.088	0.144
PFUnA	PFAS	67	21	31	0.110	0.149	0.177	0.531	0.089	0.110	0.180
PFDA	PFAS	67	20	30	0.111	0.189	0.203	0.906	0.089	0.110	0.180
PFOSA	PFAS	67	19	28	0.104	0.198	0.200	0.421	0.089	0.110	0.180
PFNA	PFAS	67	18	27	0.099	0.183	0.184	0.464	0.089	0.110	0.180
N-EtFOSE	PFAS	67	17	25	1.01	1.33	1.49	2.63	0.889	1.10	1.80
PFDS	PFAS	67	9	13	0.110	0.134	0.223	1.89	0.089	0.110	0.180
PFTTrDA	PFAS	67	9	13	0.119	0.183	0.189	0.306	0.089	0.110	0.180
PFTeDA	PFAS	67	8	12	0.111	0.175	0.180	0.352	0.089	0.110	0.180

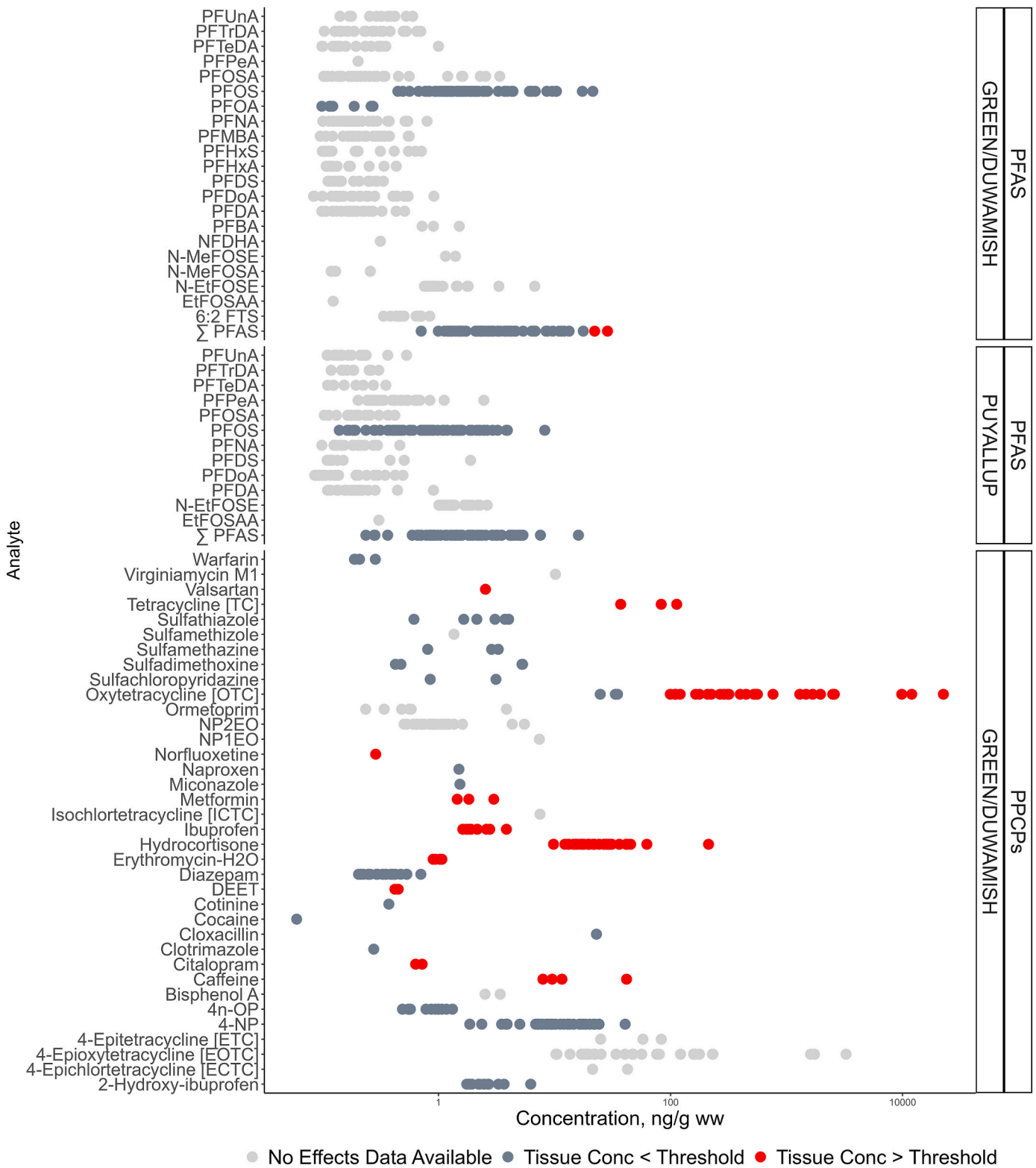


Fig. 2. Concentrations of contaminants of emerging concern (CECs) detected above reporting limits in whole body composite samples of juvenile Chinook salmon in the Green/Duwamish and Puyallup/White watersheds. Points represent sample concentrations, and multiple points indicate a chemical was detected in multiple samples. Colors represent whether concentrations exceeded compiled thresholds derived from the fish plasma model, Hazardous Concentrations (HC₅s), or Screening Values that indicate potential biological effects. See [Table S2](#) for associated CAS numbers. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

documented to affect behavior (norfluoxetine, citalopram, caffeine - Chiffre et al., 2016; Steele et al., 2018; Yan et al., 2023), development (ibuprofen - David and Pancharatna, 2009), and metabolic function (DEET, metformin - Colas-Ruiz et al., 2023; Zhang et al., 2024) in various fish species, and have the potential to similarly affect juvenile Chinook salmon, which could reduce their probability of survival to adulthood. Additional work is needed to test for links with reduced growth or survival in the field and better understand the relevant exposure durations of these priority chemicals.

The consistent detection of oxytetracycline and several of its degradation products in juvenile Chinook salmon sampled at the Soos Creek hatchery was associated with the application of medicated feed treatment that was occurring at the time of sampling in April 2018 to treat disease. It is not a surprise that oxytetracycline and associated metabolites were detected at high concentrations in fish from the hatchery that were being actively treated. However, concentrations observed in hatchery origin fish sampled at sites farther downstream in the Green/Duwamish watershed remained unexpectedly high. This could be due to additional exposure sources downstream as oxytetracycline is a widely prescribed antibiotic or unexpectedly slow elimination rates following exposure in hatcheries. Notably, most samples collected downstream of the hatchery facility also exceeded concentrations that indicate the potential for direct negative effects on juvenile Chinook salmon health from two separate lines of evidence; the fish plasma model and the HC₅ derived from chronic exposure toxicity studies that included several fish species. Of the 37 samples collected and tested for PPCPs, 30 consisted of hatchery origin fish and 7 consisted of wild fish. Wild fish were not found at most of the sampling sites, precluding direct comparisons to hatchery origin fish, except for three sites downstream in the estuary (Elliott Bay). At those three sites, oxytetracycline was detected in all 5 hatchery origin fish (Fig. S13), but was also detected in wild fish (in 2 out of 7 samples) further supporting the possibility of additional sources in the watershed. We also detected additional antibiotics including ormetoprim, sulfonamides, and cloxacillin that point towards additional sources of antibiotic exposure which could be from wastewater, stormwater, or residues in fish feed. Commercial fish feed has been found to contain other chemical contaminants (Johnson et al., 2010; Kelly et al., 2008; Maule et al., 2007), and may contain additional antibiotic or pharmaceutical residues, although to our knowledge this has not yet been tested. We suggest that identifying these additional sources of antibiotics could be a priority for future work.

Our finding of consistently high concentrations of several antibiotics in juvenile Chinook salmon sampled outside of the hatchery also raises concerns about promoting antibiotic-resistant or pathogenic bacteria harmful to juvenile salmon. Many antibiotics such as azithromycin, ciprofloxacin, erythromycin, oxytetracycline, and tetracycline exhibit predicted no effect concentrations (PNEC) for resistance at aqueous exposure concentrations in the 1 µg/L range (Bengtsson-Palme and Larsson, 2016), which would result in low ng/g tissue concentrations due to their low BCF values. Consequently, many of the antibiotics detected in this study occurred at concentrations that would be considered a concern for fostering bacterial resistance. Preliminary studies of antimicrobial resistance and resistant genes within Puget Sound demonstrate their presence in southern resident killer whales (Melendez et al., 2019; Raverty et al., 2017), marine waters (Vingino et al., 2021) and sediments (Herwig et al., 1997), as well as waters, wastewater, and nearshore waters (Wallace et al., 2018). The connections between antimicrobial exposure and the development of antimicrobial-resistant bacteria in marine species reinforces the need to identify additional exposure sources and to minimize residues of antibiotics entering the environment.

3.2.2. Per- and polyfluoroalkyl substances

Of the 40 PFAS tested, 21 were detected in fish migrating through the Green/Duwamish watershed and 12 in fish migrating through the Puyallup/White watershed, estuary, and adjacent nearshore marine

waters. The most frequently detected PFAS chemicals in fish from the Green/Duwamish were PFOS, PFOSA, and PFNA and the highest geometric mean concentration was 1.3 ng/g ww (PFOS) and the maximum concentration observed was 21.4 ng/g ww (PFOS). When we considered the sum of all PFAS detected in each sample ($\sum$ PFAS), the geometric mean was 3.1 ng/g ww and the maximum was 28.6 ng/g ww. In the Puyallup/White, PFOS and PFPeA were the most frequently detected and the highest geometric mean concentration was 0.8 ng/g ww (PFOS) and the maximum concentration was 8.2 (PFOS) ng/g ww. The geometric mean concentration of $\sum$ PFAS was 1.6 ng/g ww and the maximum concentration was 16.1 ng/g ww. PFAS concentrations were higher on average in fish collected from the Green/Duwamish watershed as compared to fish from the Puyallup/White (Table 2, Fig. 3).

The median $\sum$ PFAS concentration we observed in anadromous juvenile Chinook salmon (2.2 ng/g ww) was lower than those observed in freshwater fish fillets across the United States (9.5 ng/g) summarized using data from the National Rivers and Streams Assessment and the Great Lakes human health fish tissue studies (Barbo et al., 2022; Stahl et al., 2023). These differences are likely even larger as both meta-analyses were based on fillet data, while our samples were whole bodies, which typically have concentrations 1.4–2.5 times higher than fillets (Fair et al., 2019). Another study in the Pacific Northwest (Meador et al., 2016) reported concentrations of three PFAS (PFOS, PFOSA, and PFDA) out of 13 analyzed in juvenile Chinook salmon collected in Sinclair Inlet, WA (max $\sum$ PFAS = 35 ng/g ww) but not in fish from the Puyallup River estuary. However, tissue RLs ranged from 0.5 to 1 ng/g for Meador et al. (2016) whereas the RLs for this study were 2 to 10x lower due to analytical improvements.

None of the individual PFAS detected in fish from the Puyallup/White or Green/Duwamish watersheds were observed at concentrations that exceeded the predicted biological effects thresholds (Fig. 2) or EPA's final chronic aquatic life tissue criteria for PFOA or PFOS (EPA, 2024b). However, EPA's chronic aquatic life criteria were based on data from freshwater fish, and it is uncertain whether they are protective of anadromous species. This is an important consideration because estuaries undergo large fluctuations in salinity and temperature, which can influence toxicity of PFAS chemicals (Burcham et al., 2024; Chung et al., 2024). In one sample from the Green/Duwamish River, PFOS was detected at 21 ng/g ww, just below the concentration derived from the HC₅ from Posthuma et al. (2019). Given the composite samples analyzed represent the average concentration across multiple fish, it is highly likely that a small percentage of individuals in the Green/Duwamish have concentrations of PFOS that would exceed the HC₅ derived from species sensitivity distributions. If mixtures or the indirect effects of PFAS on invertebrate prey, which have been observed to be more sensitive to PFAS than fish (EPA, 2024b), were considered, conclusions could further shift. For instance, if we assume comparable toxicity across analytes and compare the $\sum$ PFAS to the HC₅ for PFOS, four composite samples had concentrations that indicate potential concerns. The current data based on individual chemicals suggest exposure to a limited number of PFAS may not be affecting the majority of juvenile Chinook traveling downstream in two highly urbanized watersheds, but we caution that a large amount of uncertainty remains as we tested for only 40 analytes out of the hundreds of PFAS that are listed as active by EPA (Ankley et al., 2021).

3.2.3. Mixtures

Our results also reflect the reality that environmental exposures in urban watersheds are comprised of mixtures of multiple chemicals. Multiple PPCPs were detected in every sample obtained from the Green/Duwamish watershed, with a maximum of 16 detected in one sample from the Green River hatchery (GR02) and 10 from station LDR06 in the Lower Duwamish River (Fig. S7). Notably, 73 % (27/37) of the fish originating from the Green/Duwamish had two or more PPCPs detected at concentrations that exceeded the available biological effects thresholds, the majority of which were antibiotics. Multiple PFAS were also

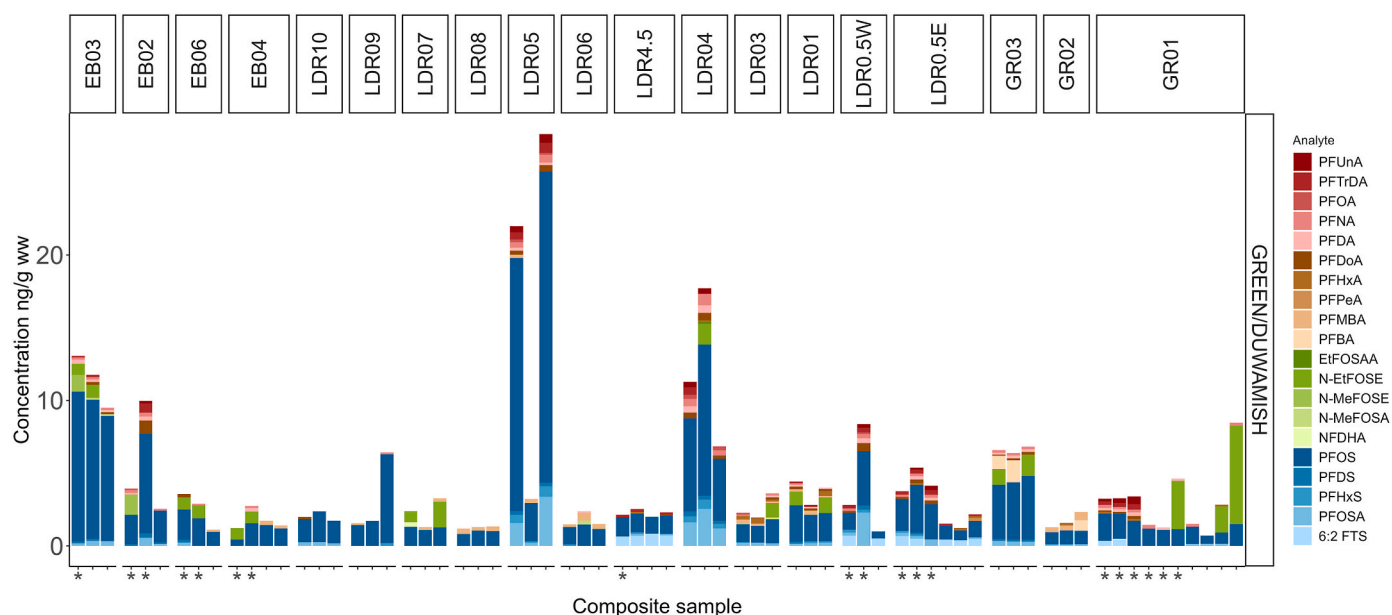


Fig. 3. Total concentrations of perfluoroalkyl and polyfluoroalkyl substances (PFAS) observed in whole-body composite samples of juvenile Chinook salmon collected in 2018 and 2023 from the Green/Duamish rivers, estuary, and adjacent nearshore waters of Elliott Bay. A total of 40 PFAS were tested across samples. Samples are grouped by sampling site (top panels) and arranged from upstream (right) in the Green River to downstream (left) in Elliott Bay (see Fig. S5 for a detailed map of sampling locations and Table S2 for associated CAS numbers). Colors represent detected PFAS, which are grouped by class: perfluoro carboxylic acids in shades of red and brown, PFAS derived via electrochemical fluorination in shades of green, perfluoroalkyl sulfonates in shades of blue. Composite samples of wild origin fish are marked with an *. All other samples were hatchery origin fish. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

detected in every sample from the Green/Duamish and most samples (55 out of 67) from the Puyallup/White watersheds (Figs. S7 and S8). In the Green/Duamish, the highest numbers of distinct PFAS chemicals were detected in samples collected at stations LDR04 (Rhône Poulenc; 16 distinct chemicals) and LDR05 (Slip 4; 13 distinct chemicals), which fall within the Lower Duamish Waterway Superfund site. In the Puyallup/White watershed, the highest numbers of distinct PFAS chemicals were detected in samples collected from the screw trap at station RW02 (13 distinct chemicals), and in the Hylebos waterway and Commencement Bay at stations WHY02 and CBC01 (10 distinct chemicals). Results from the spatial analyses below further support that these are regions in both the Green/Duamish and Puyallup/White where certain mixtures of PFAS appear to be entering waterways (Figs. 3 and 4, S10, S12). Together these results demonstrate the average juvenile Chinook migrating downstream in an urbanized watershed accumulates multiple CECs in its tissue by the time it reaches saltwater. This is in addition to the accumulation of a myriad of legacy contaminants (Meador et al., 2010; O'Neill et al., 2015), which were not tested in this study, and any exposures to non-accumulative contaminants in river water, food, or sediment. There are many additional uncertainties surrounding the effects of mixtures of chemicals on salmon health that we were unable to address in this study but which we discuss further below.

3.2.4. Uncertainties inferring fish health effects

We found there were no syntheses of biological effects data available for 48 of the PPCPs and 38 of the PFAS chemicals tested in this study, including 10 out of the 36 detected PPCPs and 19 out of the 21 detected PFAS (Fig. 3). This reinforces the need for the scientific community to generate ecologically relevant toxicity data for many understudied CECs. Tissue-based toxicological data is especially needed because it provides a more accurate estimation of the potential risk for CECs and other chemicals (McCarty et al., 2011; Meador et al., 2008). While the biological impacts of many PFAS and PPCPs remain unclear, their presence in the environment is certain and should not be ignored.

There are many other uncertainties associated with comparing tissue

concentrations to biological effects thresholds compiled from multiple sources that should be described with transparency. First, the toxicity thresholds we gathered from the literature are not specific to salmonids and were generated largely from experiments with model organisms and freshwater species (SVs and HC5s) or, in the case of the fish plasma model, using read-across assumptions about the conservation of receptors in humans and fish. Second, converting toxicity data derived from water column experiments to tissue concentration equivalents using modeled BCFs introduces additional uncertainty to the biological effect threshold estimates. Last, the samples analyzed in this study were composites that represent the average concentrations across multiple fish. Composite samples do not capture the maximum and minimum values that might occur within individual members of a population. As a result, while we have high confidence around the mean tissue concentrations, our findings do not fully represent the complete range of the true exposure distribution for juvenile Chinook salmon. Despite these uncertainties and in the absence of detailed toxicity datasets specific to salmonids, we believe this study provides an important means of inferring the potential biological relevance for a daunting list of emerging contaminants which would otherwise be difficult to prioritize. Follow up work should confirm the degree to which the chemicals identified as having the potential to cause adverse effects in this study are affecting populations of wild salmonids.

Comparisons in this study were also based on single chemical comparisons (PPCPs) or single chemicals and the sum of all detected chemicals (PFAS). Since contaminants in the environment occur as complex mixtures, it is important to consider adverse effects from multiple chemicals instead of as single-chemical cause and effect outcome, especially for pharmaceuticals (Kidd et al., 2024). Multiple compounds in such classes as macrolide antibiotics, selective serotonin reuptake inhibitors (SSRIs), and endocrine receptor agonists often occur in surface waters and are frequently similar in potency and receptor interaction. For example, additivity has been demonstrated for antidepressants (Bisesi et al., 2015) and endocrine disruptors (Hinfray et al., 2016). At a minimum, related compounds could be considered as

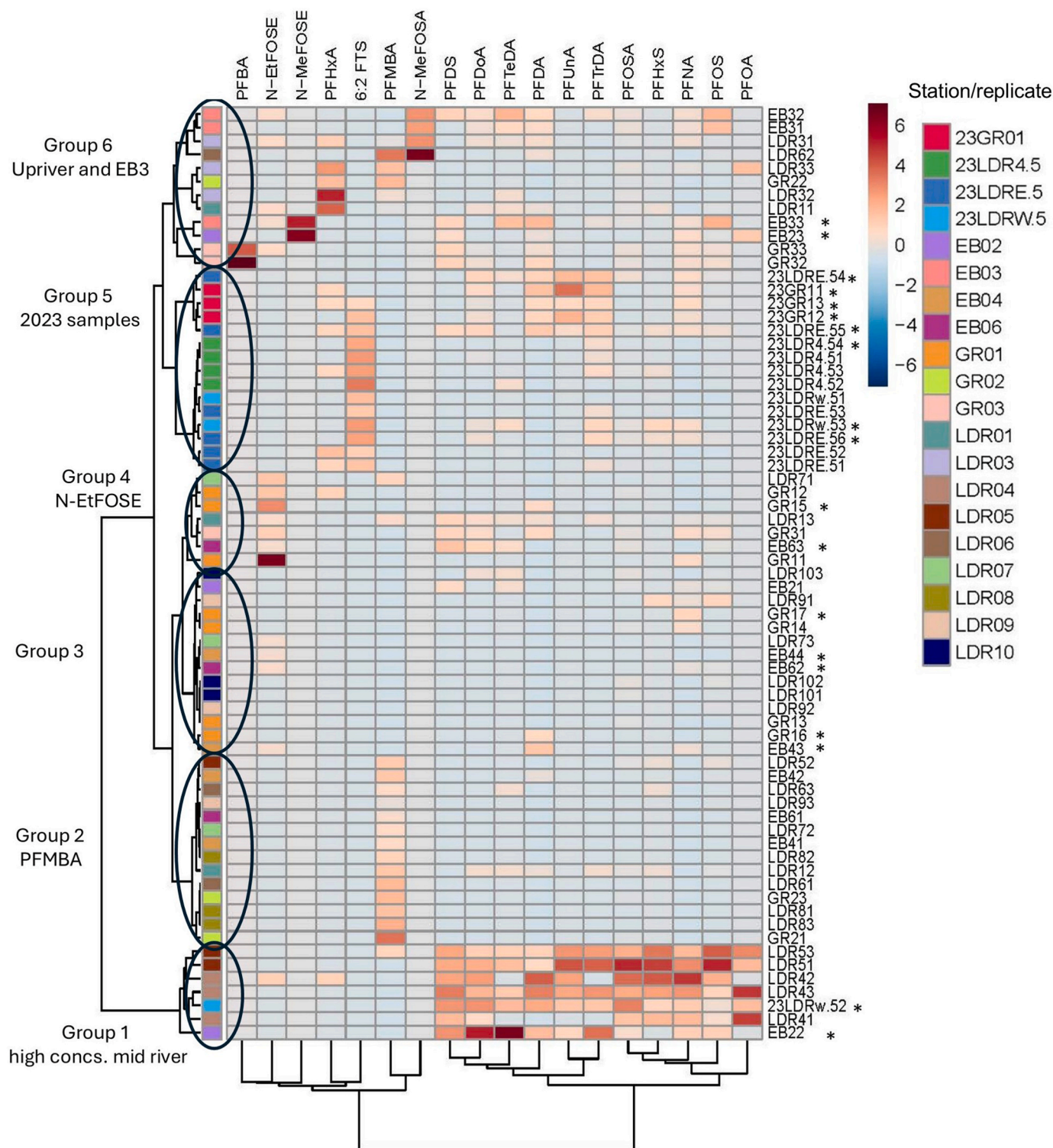


Fig. 4. Spatial clustering results shown as a heatmap (distance measure using Euclidean and clustering algorithm using ward.D) for samples of whole-body composite juvenile Chinook salmon collected from the Green/Duwamish in 2018 and 2023 and tested for 40 PFAS. Colored boxes show relative concentrations of detected analytes for each replicate sample. Red is relatively high, and blue is relatively low. Composite samples of wild origin fish are marked with an *. All other samples were hatchery origin fish. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

additive. A recent meta-analysis of mixture toxicity found that additivity was the most common result (60 %) for studies of active pharmaceutical ingredient (API) mixtures and 18 % were considered synergistic (Martin et al., 2021). However, the data on mixture toxicity for PFAS is limited and contradictory. One study (Soderstrom et al., 2022) found potentiation of the peroxisome proliferator-activated receptor (PPAR), a

nuclear receptor that plays a key role in energetics. A PFOS-PFOA mixture induced a synergistic activation whereas no activation was observed with either PFAS alone. Other studies conclude that there is no trend for PFAS interactions including additivity, synergism, and antagonism (Tanabe et al., 2024). Considering mixtures with chemicals grouped by their mechanisms of action represents an excellent next step

but is difficult to carry out in practice due to the large amount of research needed to characterize similar mechanisms or describe adverse outcome pathways (AOPs).

3.2.5. Spatial trends

Multivariate analyses revealed patterns in the contaminant profiles of juvenile Chinook salmon that would otherwise have been overlooked. For PFAS, distinct sample groupings in both the Green/Duwamish and Puyallup/White watersheds revealed regions that are likely serving as entry points for specific analytes and could be targeted for future source tracing efforts. In the Green/Duwamish, five distinct groups were identified. Group 1 exhibited high relative concentrations of multiple PFAS, including PFOS, particularly at stations LDR4 and LDR5, as well as in Elliott Bay (Figs. 3 and 4). Group 2 was characterized by high relative concentrations of PFMBAs at stations LDR6–LDR8, suggesting a potential PFMBA source in that area. Notably, Group 5 was defined by elevated 6:2 FTS levels in 2023 samples, which were below detection in 2018 due to higher analytical detection limits. Similarly, in the Puyallup/White watershed, six groups were identified (Fig. S12). Group 1 consisted of wild fish that had similar chemical profiles and elevated concentrations of PFDA, PFNA, and PFUnA at stations RP02 and 03 and WML01 (Milwaukee waterway), pointing to potential sources and a difference in the chemical profiles of wild versus hatchery origin fish at these locations. Group 2 showed elevated PFOS and n-EtFOSE in samples from Commencement Bay and the lower White River. Hatchery samples grouped with low PFAS concentrations compared to downstream samples (Group 6), and the highest concentration was observed in a sample of wild fish from the White River screw trap (station RW02-W04). These groupings highlight areas for future PFAS source tracing efforts, notably near the Lower Duwamish Superfund Region (Stations LDR04 & 05) in the Green/Duwamish and near the White and Puyallup River screw traps (RW02, RP02, RP03) in the Puyallup/White.

In contrast, spatial patterns for PPCPs were less distinct and indicated potential diffuse sources throughout the migration corridor, such as stormwater or wastewater. Elevated levels of several chemicals (nonylphenols, sulfathiazole, metformin, bisphenol A, and erythromycin) were also observed in two groups of samples from the lower Duwamish River (Fig. S9). However, chemical profiles were highly variable across samples and sampling locations. This variability likely results from differences in juvenile Chinook salmon behavior during migration, as well as temporal and spatial differences in contaminant sources. An exception was observed at the Green River hatchery, where fish prior to release showed distinct chemical profiles, primarily from high levels of oxytetracycline used to treat disease, compared to downstream fish.

3.2.6. Contaminants in context for salmon recovery

Our findings suggest that exposure to CECs may add to the detrimental effects of legacy contaminants on Chinook salmon that have been documented by previous efforts. A limited number of studies have attempted to predict the population-level impacts of contaminant exposure on Chinook salmon, and these have focused primarily on well-characterized chemicals such as PCBs and pesticides (Baldwin et al., 2009; Landis et al., 2020; Lundin et al., 2019; Mitchell et al., 2021; Spromberg and Meador, 2005). The CEC exposure observed in this study indicates that contaminant burdens in urban rivers may be more extensive than previously recognized, and cumulative impacts may be underestimated in current population-level models that consider only legacy contaminants.

Contaminant exposure is just one of many factors contributing to the declines in marine survival of Chinook salmon in the Salish Sea, alongside climate change, shifts in food web dynamics, water quality, and habitat availability (Pearsall et al., 2021). These factors are dynamic and interconnected, with potential compounding effects on salmon health. For example, changes in abiotic factors related to climate change like rising temperature and acidification can alter toxicity (Noyes et al.,

2009). In addition to direct impacts on fish health, exposure to contaminants can further impact fish survival indirectly by causing shifts in food webs, changes in prey availability, altered predation rates, and increased disease and pathogen dynamics (Fleeger et al., 2003; Saaristo et al., 2018). One study across Puget Sound found that juvenile Chinook migrating through contaminated river systems had a 45 % lower survival rate compared to those in less contaminated systems (Meador, 2014). While the decrease in survival could be attributed to several factors autocorrelated with contaminants in urbanized river systems such as decreased habitat availability and prey availability, the study provides a concerning perspective on the cumulative impact of multiple stressors on juvenile Chinook survival.

Looking ahead, inputs of contaminants into vulnerable aquatic habitats throughout the Salish Sea are predicted to increase with development and increased intensity and duration of storms driven by climate change (Borris et al., 2013; Levin et al., 2020). Despite these challenges, salmon recovery may yet be successful in the Pacific Northwest if adaptive management actions recognize these many interacting threats. To address concerns related to contaminants, we recommend targeted regulations considering potential mortality or "take" of listed salmonids, coupled with source tracing studies, and source control. Rapid investments in green infrastructure and green urban spaces are also needed to mitigate contaminant inputs by enhancing stormwater and wastewater management. These actions, while challenging, are essential to increase the odds of successful salmon recovery.

4. Conclusions

This study provides a broad-scale assessment of contaminants of emerging concern in juvenile Chinook salmon across Puget Sound, highlighting spatial patterns of exposure and identifying priority compounds in urbanized watersheds. Although most CECs were below detection limits, six were ubiquitous across sites, and 11 pharmaceuticals and personal care products exceeded literature-based thresholds for biological effects in the region's most urban river systems. While PFAS concentrations did not exceed current EPA or predicted effect thresholds, their near-ubiquitous detection underscores ongoing exposure.

To better protect threatened salmonids and promote population recovery efforts, we recommend this study be used to inform source identification efforts and strategies to remove or reduce CECs in stormwater and wastewater. The 11 CECs identified as a potential concern could be prioritized for future research to clarify the impacts on salmonid growth or survival. We also encourage researchers to fill toxicity data gaps for chemicals that were detected in this study but lack synthesis of biological effects in the literature or data specific to salmonids. Last, given their bioactivity in vertebrates, we propose that PPCPs should be explicitly incorporated into regional management frameworks aimed at reducing contaminant inputs and promoting salmon recovery.

CRedit authorship contribution statement

Molly Shuman-Goodier: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Formal analysis, Data curation. **James Meador:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Funding acquisition, Formal analysis, Conceptualization. **Andrea Carey:** Writing – review & editing, Visualization, Methodology, Funding acquisition, Data curation, Conceptualization. **Sandra O'Neill:** Writing – review & editing, Visualization, Validation, Project administration, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Open research statement

All data are publicly available: <https://doi.org/10.5061/dryad.pc866t212>.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Molly Shuman-Goodier reports financial support was provided by King County. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We would like to acknowledge the Salish Sea Indigenous Nations whose ancestors have been stewards of the lands and water that are the focus of this study for millennia, and who lead and inspire the work to promote salmon recovery today. We specifically thank the Skagit River System Cooperative, Tulalip Tribe, NOAA NWFSC (Josh Chamberlin, Casey Rice, Mark Myers, Sean Sol, Dan Lomax who helped with 2013 and 2018 collections), USGS, Snohomish County, Muckleshoot Indian Tribe, King County, University of Washington SAFS (Jason Toft), Puyallup Tribe of Indians, and Nisqually Tribe for their support and assistance in the collection of juvenile Chinook salmon for this study. We extend our thanks to Qing He for designing the graphical abstract and to the staff and volunteers at WDFW who assisted with sample collection and preparation (James West, Jennifer Lanksbury, Robert Fisk, Danielle Nordstrom, Andrew Beckman, and Mariko Langness). We also thank the reviewers who provided feedback on the manuscript (Iris Kemp, Kirt Hughes, Rae Eaton, Tim Kuzman, Wes Flynn, and Rachel Rubin), as well as the three anonymous reviewers for Environmental Pollution, and SGS AXYS Analytical Services Ltd for their expertise in chemical analyses. This work was funded by the State of Washington, USA and through a grant from King County (Award Number 4922017).

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envpol.2025.126639>.

Data availability

All associated data are publicly available: <https://doi.org/10.5061/dryad.pc866t212>.

References

- Acir, I.H., Guenther, K., 2018. Endocrine-disrupting metabolites of alkylphenol ethoxylates - a critical review of analytical methods, environmental occurrences, toxicity, and regulation. *Sci. Total Environ.* 635, 1530–1546.
- Alesio, J.L., Slitt, A., Bothun, G.D., 2022. Critical new insights into the binding of poly- and perfluoroalkyl substances (PFAS) to albumin protein. *Chemosphere* 287, 131979.
- Ankley, G.T., Cureton, P., Hoke, R.A., Houde, M., Kumar, A., Kurias, J., et al., 2021. Assessing the ecological risks of Per- and polyfluoroalkyl substances: current state-of-the science and a proposed path forward. *Environ. Toxicol. Chem.* 40, 564–605.
- Arkoosh, M.R., Boylen, D., Dietrich, J., Anulacion, B.F., Ylitalo, G., Bravo, C.F., et al., 2010. Disease susceptibility of salmon exposed to polybrominated diphenyl ethers (PBDEs). *Aquat. Toxicol.* 98, 51–59.
- Arkoosh, M.R., Van Gaest, A.L., Strickland, S.A., Hutchinson, G.P., Krupkin, A.B., Dietrich, J.P., 2017. Alteration of thyroid hormone concentrations in juvenile Chinook salmon (*Oncorhynchus tshawytscha*) exposed to polybrominated diphenyl ethers, BDE-47 and BDE-99. *Chemosphere* 171, 1–8.
- Arkoosh, M.R., Van Gaest, A.L., Strickland, S.A., Hutchinson, G.P., Krupkin, A.B., Hicks, M.B.R., et al., 2018. Dietary exposure to a binary mixture of polybrominated diphenyl ethers alters innate immunity and disease susceptibility in juvenile Chinook salmon (*Oncorhynchus tshawytscha*). *Ecotoxicol. Environ. Saf.* 163, 96–103.
- Ashfield, L.A., Pottinger, T.G., Sumpter, J.P., 1998. Exposure of female juvenile rainbow trout to alkylphenolic compounds results in modifications to growth and ovosomatic index. *Environ. Toxicol. Chem.* 17, 679–686.
- Baldwin, D.H., Spromberg, J.A., Collier, T.K., Scholz, N.L., 2009. A fish of many scales: extrapolating sublethal pesticide exposures to the productivity of wild salmon populations. *Ecol. Appl.* 19, 2004–2015.
- Barbo, N., Stoiber, T., Naidenko, O.V., Andrews, D.Q., 2022. Locally caught freshwater fish across the United States are likely a significant source of exposure to PFOS and other perfluorinated compounds. *Environ. Res.* 115165.
- Bates, D., Mächler, M., Bolker, B., Walker, S., 2015. lme4: linear mixed-effects models using Eigen and S4. R package version 1.1–8. CRAN.
- Bengtsson-Palme, J., Larsson, D.G., 2016. Concentrations of antibiotics predicted to select for resistant bacteria: proposed limits for environmental regulation. *Environ. Int.* 86, 140–149.
- Berninger, J.P., Tillitt, D.E., 2019. Polychlorinated biphenyl (PCB) tissue-concentration thresholds for survival, growth, and reproduction in fish. *Environ. Toxicol. Chem.* 38, 712–736.
- Bisesi, J.H., Sweet, L.E., Van Den Hurk, P., Klaine, S.J., 2015. Effects of an antidepressant mixture on the brain serotonin and predation behavior of hybrid striped bass. *Environ. Toxicol. Chem.* 35, 938–945.
- Bjornsson, B.T., Stefansson, S.O., McCormick, S.D., 2011. Environmental endocrinology of salmon smoltification. *Gen. Comp. Endocrinol.* 170, 290–298.
- Borris, M., Viklander, M., Gustafsson, A.-M., Marsalek, J., 2013. Simulating future trends in urban stormwater quality for changing climate, urban land use and environmental controls. *Water Sci. Technol.* 68, 2082–2089.
- Burcham, L.E., Hoskins, T.D., Allmon, E.B., Scherer, M.N., Bushong, A.G., Hamilton, M. T., et al., 2024. Does salinity mediate the toxicity of perfluorooctanesulfonic acid (PFOS) in an estuarine fish? *Mar. Pollut. Bull.* 203.
- Burkhard, L.P., 2021. Evaluation of published bioconcentration factor (BCF) and bioaccumulation factor (BAF) data for per- and polyfluoroalkyl substances across aquatic species. *Environ. Toxicol. Chem.* n/a.
- Chiffre, A., Clerandau, C., Dwoinikoff, C., Le Bihan, F., Budzinski, H., Geret, F., et al., 2016. Psychotropic drugs in mixture alter swimming behaviour of Japanese medaka (*Oryzias latipes*) larvae above environmental concentrations. *Environ. Sci. Pollut. Res. Int.* 23, 4964–4977.
- Chung, K.W., Key, P.B., Tanabe, P., DeLorenzo, M.E., 2024. Effects of temperature and salinity on perfluorooctane sulfonate (PFOS) toxicity in larval estuarine organisms. *Toxics* 12.
- Claiborne, A.M., Fisher, J.P., Hayes, S.A., Emmett, R.L., 2011. Size at release, size-selective mortality, and age of maturity of Willamette river hatchery yearling Chinook salmon. *Trans. Am. Fish. Soc.* 140, 1135–1144.
- Colas-Ruiz, N.R., Courant, F., Gomez, E., Lara-Martin, P.A., Hampel, M., 2023. Transcriptomic and metabolomic integration to assess the response of gilthead sea bream (*Sparus aurata*) exposed to the most used insect repellent: DEET. *Environ. Pollut.* 316, 120678.
- David, A., Pancharatna, K., 2009. Developmental anomalies induced by a non-selective COX inhibitor (ibuprofen) in zebrafish (*Danio rerio*). *Environ. Toxicol. Pharmacol.* 27, 390–395.
- Deere, J.R., Moore, S., Ferrey, M., Jankowski, M.D., Primus, A., Convertino, M., et al., 2020. Occurrence of contaminants of emerging concern in aquatic ecosystems utilized by Minnesota tribal communities. *Sci. Total Environ.* 724, 138057.
- Duffy, E.J., Beauchamp, D.A., 2011. Rapid growth in the early marine period improves the marine survival of Chinook salmon (*Oncorhynchus tshawytscha*) in puget sound, Washington. *Can. J. Fish. Aquat. Sci.* 68, 232–240.
- Edwards, M.A., Kimbrough, K., Fuller, N., Davenport, E., Rider, M., Freitag, A., et al., 2024. An assessment and characterization of pharmaceuticals and personal care products (PPCPs) within the Great Lakes Basin: mussel watch program (2013–2018). *Environ. Monit. Assess.* 196, 345.
- Elliott, S.M., Gefell, D.J., Kiesling, R.L., Hummel, S.L., King, C.K., Christen, C.H., et al., 2022. Multiple lines of evidence for identifying potential hazards to fish from contaminants of emerging concern in Great Lakes tributaries. *Integrated Environ. Assess. Manag.* 18, 1246–1259.
- EPA, 2024a. Final Freshwater Ambient Water Quality Criteria and Acute Saltwater Life Benchmark for Perfluorooctanoic Acid (PFOA). Office of Water, United States Environmental Protection Agency.
- EPA, 2024b. Final Freshwater Aquatic Life Ambient Water Quality Criteria and Acute Saltwater Benchmark for Perfluorooctane Sulfonate (PFOS). Office of Water, United States Environmental Protection Agency.
- Fair, P.A., Wolf, B., White, N.D., Arnott, S.A., Kannan, K., Karthikraj, R., et al., 2019. Perfluoroalkyl substances (PFASs) in edible fish species from Charleston Harbor and tributaries, South Carolina, United States: exposure and risk assessment. *Environ. Res.* 171, 266–277.
- Fleeger, J.W., Carman, K.R., Nisbet, R.M., 2003. Indirect effects of contaminants in aquatic ecosystems. *Sci. Total Environ.* 317, 207–233.
- Gefell, D.J., Annis, M., Banda, J.A., Bellamy, A., Choy, S., Hummel, S.L., Jorgenson, Z., Moore, J.N., Secord, A.L., Tucker, W.A., 2019. Ecological hazard assessment of contaminants of emerging concern in the U.S. Great Lakes Basin. *U.S. Fish Wildl. Serv. A* (Volume I of II).
- Herwig, R.P., Gray, J.P., Weston, D.P., 1997. Antibacterial resistant bacteria in surficial sediments near salmon net-cage farms in Puget Sound, Washington. *Aquaculture* 149, 263–283.
- Hinfray, N., Tebby, C., Garoche, C., Piccini, B., Bourguine, G., Ait-Aïssa, S., et al., 2016. Additive effects of levonorgestrel and ethinylestradiol on brain aromatase (cyp19a1b) in zebrafish specific in vitro and in vivo bioassays. *Toxicol. Appl. Pharmacol.* 307, 108–114.
- Huggett, D.B., Cook, J.C., Ericson, J.F., Williams, R.T., 2003. A theoretical model for utilizing mammalian pharmacology and safety data to prioritize potential impacts of human pharmaceuticals to fish. *Hum. Ecol. Risk Assess.* 9, 1789–1799.
- James, C.A., Miller-Schulze, J.P., Ultican, S., Gipe, A.D., Baker, J.E., 2016. Evaluating contaminants of emerging concern as tracers of wastewater from septic systems. *Water Res.* 101, 241–251.

- James, C.A., Sofield, R., Faber, M., Wark, D., Simmons, A., Harding, L., et al., 2023. The screening and prioritization of contaminants of emerging concern in the marine environment based on multiple biological response measures. *Sci. Total Environ.* 886, 163712.
- Johnson, L., Anulacion, B., Arkoosh, M., Olson, O.P., Sloan, C., Sol, S.Y., et al., 2013. Persistent organic pollutants in juvenile Chinook salmon in the Columbia River Basin: implications for stock recovery. *Trans. Am. Fish. Soc.* 142, 21–40.
- Johnson, L.L., Willis, M.L., Olson, O.P., Pearce, R.W., Sloan, C.A., Ylitalo, G.M., 2010. Contaminant concentrations in juvenile fall Chinook salmon from Columbia River hatcheries. *N. Am. J. Aquac.* 72, 73.
- Johnson, L.L., Ylitalo, G.M., Arkoosh, M.R., Kagley, A.N., Stafford, C., Bolton, J.L., et al., 2007. Contaminant exposure in outmigrant juvenile salmon from Pacific Northwest estuaries of the United States. *Environ. Monit. Assess.* 124, 167–194.
- Jorgenson, Z.G., Thomas, L.M., Elliott, S.M., Cavallin, J.E., Randolph, E.C., Choy, S.J., et al., 2018. Contaminants of emerging concern presence and adverse effects in fish: a case study in the Laurentian Great Lakes. *Environ. Pollut.* 236, 718–733.
- Kelly, B.C., Fernandez, M.P., Ikononou, M.G., Knapp, W., 2008. Persistent organic pollutants in aquafeed and Pacific salmon smolts from fish hatcheries in British Columbia. *Can. Aquacult.* 285, 224–233.
- Kidd, K.A., Backhaus, T., Brodin, T., Inostroza, P.A., McCallum, E.S., 2024. Environmental risks of pharmaceutical mixtures in aquatic ecosystems: reflections on a decade of research. *Environ. Toxicol. Chem.* 43, 549–558.
- Kolpin, D.W., Blazer, V.S., Gray, J.L., Focazio, M.J., Young, J.A., Alvarez, D.A., et al., 2013. Chemical contaminants in water and sediment near fish nesting sites in the Potomac River basin: determining potential exposures to smallmouth bass (*Micropterus dolomieu*). *Sci. Total Environ.* 443, 700–716.
- Landis, W.G., Chu, V.R., Graham, S.E., Harris, M.J., Markiewicz, A.J., Mitchell, C.J., et al., 2020. Integration of chlorpyrifos acetylcholinesterase inhibition, water temperature, and dissolved oxygen concentration into a regional Scale multiple stressor risk assessment estimating risk to chinook salmon. *Integrated Environ. Assess. Manag.* 16, 28–42.
- Levin, P.S., Howe, E.R., Robertson, J.C., 2020. Impacts of stormwater on coastal ecosystems: the need to match the scales of management objectives and solutions. *Phil. Trans. Biol. Sci.* 375, 20190460.
- Lundin, J.I., Chittaro, P.M., Ylitalo, G.M., Kern, J.W., Kuligowski, D.R., Sol, S.Y., et al., 2021. Decreased growth rate associated with tissue contaminants in juvenile Chinook salmon out-migrating through an industrial waterway. *Environ. Sci. Technol.* 55, 9968–9978.
- Lundin, J.I., Spromberg, J.A., Jorgensen, J.C., Myers, J.M., Chittaro, P.M., Zabel, R.W., et al., 2019. Legacy habitat contamination as a limiting factor for Chinook salmon recovery in the Willamette Basin, Oregon, USA. *PLoS One* 14, e0214399.
- Maloney, E.M., Corsi, S.R., Pronschinske, M.A., Decicco, L.A., Nott, M.A., Frisch, J.R., et al., 2025. Derivation and characterization of environmental hazard concentrations for chemical prioritization: a case study in the Great Lakes tributaries. *Environ. Toxicol. Chem.*
- Mansouri, K., Grulke, C.M., Judson, R.S., Williams, A.J., 2018. OPERA models for predicting physicochemical properties and environmental fate endpoints. *J. Cheminf.* 10.
- Marques Dos Santos, M., Hoppe-Jones, C., Snyder, S.A., 2019. DEET occurrence in wastewaters: seasonal, spatial and diurnal variability - mismatches between consumption data and environmental detection. *Environ. Int.* 132, 105038.
- Martin, O., Scholze, M., Ermler, S., McPhie, J., Bopp, S.K., Kienzler, A., et al., 2021. Ten years of research on synergisms and antagonisms in chemical mixtures: a systematic review and quantitative reappraisal of mixture studies. *Environ. Int.* 146, 106206.
- Maruya, K.A., Dodder, N.G., Mehinto, A.C., Denslow, N.D., Schlenk, D., Snyder, S.A., et al., 2016. A tiered, integrated biological and chemical monitoring framework for contaminants of emerging concern in aquatic ecosystems. *Integrated Environ. Assess. Manag.* 12, 540–547.
- Maule, A.G., Gannam, A.L., Davis, J.W., 2007. Chemical contaminants in fish feeds used in federal salmonid hatcheries in the USA. *Chemosphere* 67, 1308–1315.
- McCarty, L.S., Landrum, P.F., Luoma, S.N., Meador, J.P., Merten, A.A., Shephard, B.K., et al., 2011. Advancing environmental toxicology through chemical dosimetry: external exposures versus tissue residues. *Integrated Environ. Assess. Manag.* 7, 7–27.
- Meador, J.P., 2014. Do chemically contaminated river estuaries in puget sound (Washington, USA) affect the survival rate of hatchery-reared Chinook salmon? *Can. J. Fish. Aquat. Sci.* 71, 162–180.
- Meador, J.P., Ball, S.C., James, C.A., McIntyre, J.K., 2024a. Exposure of juvenile Chinook salmon to effluent from a large urban wastewater treatment plant. Part 2. Metabolomic profiling. *Aquaculture and Fisheries.*
- Meador, J.P., Ball, S.C., James, C.A., McIntyre, J.K., 2024b. Using the fish plasma model to evaluate potential effects of pharmaceuticals in effluent from a large urban wastewater treatment plant. *Environ. Pollut.* 348, 123842.
- Meador, J.P., Bettcher, L.F., Ellenberger, M.C., Senn, T.D., 2020. Metabolomic profiling for juvenile Chinook salmon exposed to contaminants of emerging concern. *Sci. Total Environ.*, 141097.
- Meador, J.P., McCarty, L.S., Escher, B.I., Williams, J.A., 2008. 10th anniversary critical review: the tissue-residue approach for toxicity assessment: concepts, issues, application, and recommendations. *J. Environ. Monit.* 10, 1486–1498.
- Meador, J.P., Sommers, F.C., Ylitalo, G.M., Sloan, C.A., 2006. Altered growth and related physiological responses in juvenile Chinook salmon (*Oncorhynchus tshawytscha*) from dietary exposure to polycyclic aromatic hydrocarbons (PAHs). *Can. J. Fish. Aquat. Sci.* 63, 2364–2376.
- Meador, J.P., Yeh, A., Gallagher, E.P., 2017. Determining potential adverse effects in marine fish exposed to pharmaceuticals and personal care products with the fish plasma model and whole-body tissue concentrations. *Environ. Pollut.* 230, 1018–1029.
- Meador, J.P., Yeh, A., Young, G., Gallagher, E.P., 2016. Contaminants of emerging concern in a large temperate estuary. *Environ. Pollut.* 213, 254–267.
- Meador, J.P., Ylitalo, G.M., Sommers, F.C., Boyd, D.T., 2010. Bioaccumulation of polychlorinated biphenyls in juvenile chinook salmon (*Oncorhynchus tshawytscha*) outmigrating through a contaminated urban estuary: dynamics and application. *Ecotoxicology* 19, 141–152.
- Melendez, D., Roberts, M.C., Greninger, A.L., Weissman, S., No, D., Rabinowitz, P., et al., 2019. Whole-genome analysis of extraintestinal pathogenic *Escherichia coli* (ExPEC) MDR ST73 and ST127 isolated from endangered southern resident killer whales (*Orcinus orca*). *J. Antimicrob. Chemother.* 74, 2176–2180.
- Merel, S., Nikiforov, A.I., Snyder, S.A., 2015. Potential analytical interferences and seasonal variability in diethyltoluamide environmental monitoring programs. *Chemosphere* 127, 238–245.
- Merel, S., Snyder, S.A., 2016. Critical assessment of the ubiquitous occurrence and fate of the insect repellent N,N-diethyl-m-toluamide in water. *Environ. Int.* 96, 98–117.
- Mitchell, C.J., Lawrence, E., Chu, V.R., Harris, M.J., Landis, W.G., von Stackelberg, K.E., et al., 2021. Integrating metapopulation dynamics into a bayesian network relative risk model: assessing risk of pesticides to chinook salmon (*Oncorhynchus tshawytscha*) in an ecological context. *Integrated Environ. Assess. Manag.* 17, 95–109.
- Noyes, P.D., McElwee, M.K., Miller, H.D., Clark, B.W., Van Tiem, L.A., Walcott, K.C., et al., 2009. The toxicology of climate change: environmental contaminants in a warming world. *Environ. Int.* 35, 971–986.
- O'Neill, S.M., Carey, A.J., Harding, L.B., West, J.E., Ylitalo, G.M., Chamberlin, J.W., 2020. 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. *Sci. Total Environ.* 712, 135516.
- O'Neill, S.M., Carey, A.J., Lanksbury, J.A., Niewolny, L.A., Ylitalo, G.M., Johnson, L.L., et al., 2015. Toxic contaminants in juvenile Chinook salmon (*Oncorhynchus tshawytscha*) migrating through estuary, nearshore and offshore habitats of Puget Sound. Washington Department of Fish and Wildlife, Olympia, Washington, p. 132.
- Pang, Z., Lu, Y., Zhou, G., Hui, F., Xu, L., Vlau, C., et al., 2024. MetaboAnalyst 6.0: towards a unified platform for metabolomics data processing, analysis and interpretation. *Nucleic Acids Res.* 52, W398–W406.
- Paul, A.G., Jones, K.C., Sweetman, A.J., 2009. A first global production, emission, and environmental inventory for perfluorooctane sulfonate. *Environ. Sci. Technol.* 43 (386), 92.
- Pearsall, L., Schmidt, M., Kemp, I., Riddell, B., 2021. Synthesis of findings of the Salish Sea Marine survival project. Version 1.0.
- Peck, K.A., Lomax, D.P., Olson, O.P., Sol, S.Y., Swanson, P., Johnson, L.L., 2011. Development of an enzyme-linked immunosorbent assay for quantifying vitellogenin in Pacific salmon and assessment of field exposure to environmental estrogens. *Environ. Toxicol. Chem.* 30, 477–486.
- Posthuma, L., van Gils, J., Zipp, M.C., van de Meent, D., de Zwart, D., 2019. Species sensitivity distributions for use in environmental protection, assessment, and management of aquatic ecosystems for 12 386 chemicals. *Environ. Toxicol. Chem.* 38, 905–917.
- Pronschinske, M.A., Corsi, S.R., DeCicco, L.A., Furlong, E.T., Ankley, G.T., Blackwell, B. R., et al., 2022. Prioritizing pharmaceutical contaminants in great lakes tributaries using risk-based screening techniques. *Environ. Toxicol. Chem.*
- Quinn, T.P., 2018. The Behavior and Ecology of Pacific Salmon and Trout, second ed. University of Washington Press, Seattle, Washington.
- Ramirez, A.J., Brain, R.A., Usenko, S., Mottaleb, M.A., O'Donnell, J.G., Stahl, L.L., et al., 2009. Occurrence of pharmaceuticals and personal care products in fish: results of a national pilot study in the United States. *Environ. Toxicol. Chem.* 28, 2587–2597.
- Raverty, S.A., Rhodes, L.D., Zabek, E., Eshghi, A., Cameron, C.E., Hanson, M.B., et al., 2017. Respiratory microbiome of endangered southern resident killer whales and microbiota of surrounding sea surface microlayer in the Eastern North Pacific. *Sci. Rep.* 7.
- Rodea-Palomares, I., Bone, A.J., 2024. Predictive value of the ToxCast/Tox21 high throughput toxicity screening data for approximating in vivo ecotoxicity endpoints and ecotoxicological risk in eco-surveillance applications. *Sci. Total Environ.* 914, 169783.
- Saaristo, M., Brodin, T., Balshine, S., Bertram, M.G., Brooks, B.W., Ehlman, S.M., et al., 2018. Direct and indirect effects of chemical contaminants on the behaviour, ecology and evolution of wildlife. *Proc. Biol. Sci.* 285, 20181297.
- Salgueiro-Gonzalez, N., Concha-Grana, E., Turnes-Carou, I., Munategui-Lorenzo, S., Lopez-Mahia, P., Prada-Rodriguez, D., 2012. Blank and sample handling troubleshooting in ultratrace analysis of alkylphenols and bisphenol A by liquid chromatography tandem mass spectrometry. *Talanta* 101, 413–419.
- Schaupp, C.M., Maloney, E.M., Mattingly, K.Z., Olker, J.H., Villeneuve, D.L., 2023. Comparison of in silico, in vitro, and in vivo toxicity benchmarks suggests a role for ToxCast data in ecological hazard assessment. *Toxicol. Sci.* 195, 145–154.
- Sloan, C.A., Anulacion, B.F., Bolton, J.L., Boyd, D., Olson, O.P., Sol, S.L., et al., 2010. Polybrominated diphenyl ethers in outmigrant juvenile Chinook salmon from the Lower Columbia River and estuary and Puget Sound, Washington. *Arch. Environ. Contam. Toxicol.* 58, 403.
- Soderstrom, S., Lille-Langoy, R., Yadetie, F., Rauch, M., Milinski, A., Dejaegere, A., et al., 2022. Agonistic and potentiating effects of perfluoroalkyl substances (PFAS) on the Atlantic cod (*Gadus morhua*) peroxisome proliferator-activated receptors (Ppars). *Environ. Int.* 163, 107203.
- Spromberg, J.A., Meador, J.P., 2005. Relating results of chronic toxicity responses to population-level effects: modeling effects on wild chinook salmon populations. *Integrated Environ. Assess. Manag.* 1, 9–21.

- Stahl, L.L., Snyder, B.D., McCarty, H.B., Kincaid, T.M., Olsen, A.R., Cohen, T.R., et al., 2023. Contaminants in fish from U.S. rivers: probability-based national assessments. *Sci. Total Environ.* 861, 160557.
- Steele, W.B., Mole, R.A., Brooks, B.W., 2018. Experimental protocol for examining behavioral response profiles in larval fish: application to the neuro-stimulant caffeine. *J. Vis. Exp.*
- Stefankiv, O., Hall, J., Timpane-Padgham, B., Nicol, C., Fogel, C., Beechie, T.J., et al., 2019. Salmon Habitat Status and Trends: Monitoring Protocols. U.S. Department of Commerce.
- Stehr, C.M., Brown, D.W., Hom, T., Anulacion, B.F., Reichert, W.L., Collier, T.K., 2000. Exposure of juvenile chinook and chum salmon to chemical contaminants in the Hylebos Waterway of Commencement Bay, Tacoma, Washington. *J. Aquatic Ecosyst. Stress Recovery* 7, 215–227.
- Stein, J.E., Hom, T., Collier, T.K., Brown, D.W., Varanasi, U., 1995. Contaminant exposure and biochemical effects in outmigrant juvenile chinook salmon from urban and nonurban estuaries of Puget Sound, Washington. *Environ. Toxicol. Chem.* 14, 1019–1029.
- Swam-Jaramillo, L.M., Pugh, A., Rider, M.M., Apeti, D.A., Pisarski, E., 2024. A 2019 assessment of contaminants of emerging concern in the Pacific Northwest. NOAA Technical Memorandum. 346. NOS NCCOS, Silver Spring, MD, p. 73.
- Tanabe, P., Key, P.B., Chung, K.W., Pisarski, E.C., Reiner, J.L., Rodowa, A.E., et al., 2024. Mixture effects of per- and polyfluoroalkyl substances on embryonic and larval sheepshead minnows (*Cyprinodon variegatus*). *Toxics* 12, 91.
- Thomas, J.C., Lutz, M.A., Bruce, J.L., Graczyk, D.K., Richards, K.D., et al., 2007. Water-Quality Characteristics for Selected Sites Within the Milwaukee Metropolitan Sewerage District Planning Area, Wisconsin, February 2004–September 2005. U.S. Geological Survey. <https://doi.org/10.3133/sir20075084>.
- Tian, Z., Peter, K.T., Gipe, A.D., Zhao, H., Hou, F., Wark, D.A., et al., 2020. Suspect and nontarget screening for contaminants of emerging concern in an urban estuary. *Environ. Sci. Technol.* 54, 889–901.
- USGS, 2024. Annual NLCD collection 1 science products. U.S. Geol. Surv. Data Release. <https://doi.org/10.5066/P94UXNTS>.
- Vingino, A., Roberts, M.C., Wainstein, M., West, J., Norman, S.A., Lambourn, D., et al., 2021. Surveillance for antibiotic-resistant *E. coli* in the Salish Sea ecosystem. *Antibiotics* 10, 1201.
- Wallace, J.C., Youngblood, J.E., Port, J.A., Cullen, A.C., Smith, M.N., Workman, T., et al., 2018. Variability in metagenomic samples from the puget sound: relationship to temporal and anthropogenic impacts. *PLoS One* 13, e0192412.
- Yan, Z., Chen, Y., Zhang, X., Lu, G., 2023. The metabolites could not be ignored: a comparative study of the metabolite norfluoxetine with its parent fluoxetine on zebrafish (*Danio rerio*). *Aquat. Toxicol.* 257, 106467.
- Yeh, A., Marcinek, D.J., Meador, J.P., Gallagher, E.P., 2017. Effect of contaminants of emerging concern on liver mitochondrial function in Chinook salmon. *Aquat. Toxicol.* 190, 21–31.
- Zhang, Z., Shi, H., Zhang, K., An, R., Wang, C., Wang, P., et al., 2024. Transcriptome-guided characterization of the environmental toxicity of metformin: disruption of energy homeostasis and inhibition of embryonic development of zebrafish at environmentally relevant concentrations. *Environ. Sci. Technol.* 58, 17580–17591.