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Growth of Pacific staghorn sculpin (*Leptocottus armatus*) is reduced at contaminated sites in the Lower Duwamish River, Washington

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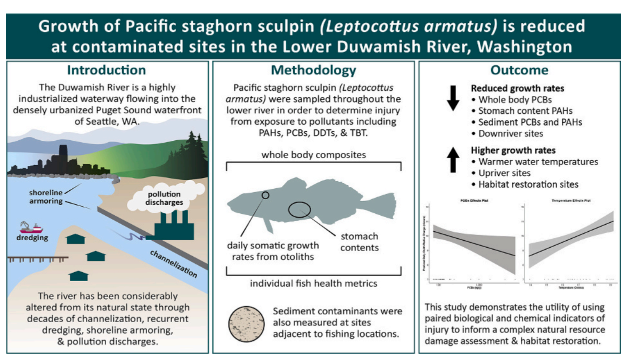
HIGHLIGHTS

- Sediment-associated pollutants impacted juvenile fish in an urbanized waterway.
- Growth rates were negatively correlated with PCBs and DDTs in tissues and sediments.
- Remediated sites produced faster growing fish with lower contaminant body burdens.
- Pacific staghorn sculpin are an effective bioindicator of pollutant impacts.

GRAPHICAL ABSTRACT



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ABSTRACT

The Lower Duwamish River is a highly industrialized waterway flowing into the densely urbanized Puget Sound waterfront of Seattle, Washington, USA. The river has been profoundly altered from its natural state following more than a century of channelization, recurrent dredging, shoreline armoring, and pollution discharges. As part of a Natural Resource Damage Assessment addressing historical pollution at three designated Superfund sites (i.e., the assessment area), juvenile Pacific staghorn sculpin (*Leptocottus armatus*) were sampled throughout the

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lower river in order to evaluate injury from exposure to polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethane (DDTs), and butyltins (BTs). Sculpin live in close association with the river sediments within and upriver of the assessment area. Fish were collected for analysis of contaminant concentrations in composited whole bodies and stomach contents, as well as individual fish health metrics including daily somatic growth rates measured from otoliths. Sediment contaminant concentrations were also measured at sites near to fishing locations. Fish growth rates varied from 0.65 to 1.05 mm/day, and were significantly lower at unremediated downriver sites compared to upriver and remediated locations. Sculpin growth rates were negatively correlated with concentrations of PCBs in fish bodies, PAHs in stomach contents, as well as PCBs, DDTs and PAHs in sediment. Mixed effects models for whole-body and stomach content contaminants showed positive correlations between growth rate and water temperature. Temperature was not a significant confounding variable for the relationship between growth rate and sediment contaminants. Overall, these results show that juvenile sculpin are harmed by contaminant exposure in the Lower Duwamish River. Furthermore, this study demonstrates the utility of using paired biological and chemical indicators of pollutant-induced injury in a resident fish to inform a complex Natural Resource Damage Assessment and associated restoration efforts.

1. Introduction

As in many waterbodies in highly urbanized areas, the Lower Duwamish River (LDR) has a long history of profound alteration to its hydrological and ecological processes. Notable anthropogenic activities impacting the LDR over the past century include channelization, shoreline armoring, repeated dredging of the shipping channel, maritime activity, and pollution discharges from industrial activities (Cummings, 2020). The historical contamination of concern in the LDR has primarily been polychlorinated biphenyls (i.e., PCBs), however dichlorodiphenyltrichloroethane (i.e., DDT and its various metabolites), butyltins (i.e., BTs), and other compounds (i.e., heavy metals) have also raised concerns for their potential to cause adverse biological effects. The primary sources of these chemicals include industrial activities and manufacturing, and despite various legislative actions banning the use of these chemicals decades ago (Davies and Mazurek, 1998), sediment-bound PCBs, DDTs, and BTs remain in elevated concentrations at locations throughout the LDR (LDWG, 2010). Additionally, ongoing and historical inputs of polycyclic aromatic hydrocarbons (i.e., PAHs) are also a potential threat to the health and fitness of exposed fish (Logan, 2007). Combustion-related discharges, industrial activities, stormwater runoff, direct oil spills, and leaching from creosote-treated in-water structures continue to deliver PAHs to the LDR and may result in site-specific concentrations of biological concern (Johnson et al., 2006). Contaminant-related concerns also extend to nearby human populations. Specifically, as a protective human health measure in response to elevated contaminant concentrations of PCBs and mercury, fishing advisories implemented by the Washington State Department of Health to limit the consumption of fish, crab and shellfish caught in the LDR (WADOH, 2003) remain in effect.

Due to the presence of elevated levels of sediment-associated contaminants, particularly PCBs, the Lower Duwamish Waterway, Harbor Island, and Lockheed West Superfund sites were designated between 1983 and 2007. Several targeted remedial actions have occurred over the past decades within these three sites, and several more are nearing implementation. In particular, remediation at specific sites (i.e., early action areas) has been in response to contaminant "hot spots" (i.e., areas with elevated sediment contaminant concentrations) and point-source discharges (NOAA, 2013). The goal of river cleanup and restoration is to improve habitat conditions, and subsequently fish health, by reducing exposure to current and legacy sources of sediment-associated contaminants. Distinct from the Superfund remediation process, a Trustee Council composed of representatives from Federal Agencies, State Government, and Tribal Nations guides a Natural Resource Damage Assessment (NRDA) at these sites, collectively referred to as the assessment area. The NRDA process, as guided by U.S. statute (CERCLA, 1980; OPA, 1990), is intended to quantify natural resource injury due to releases of hazardous substances into the environment and restore the assessment area to the condition that would have existed in the absence of the contaminants.

Several fish species are common throughout the lower stretches of the river. These include forage fish such as shiner perch (*Cymatogaster aggregate*), migratory salmonids (*Oncorhynchus* sp.), flatfish such as starry flounder (*Platichthys stellatus*), and several species of sculpin. Collectively, sculpin are an important food source for upper trophic level species such as piscivorous fish, birds, river otters and seals (Love, 1996; Bjorkland et al., 2015). One of the most common sculpin species in our study site, and the focal species of this study, is the Pacific staghorn sculpin (*Leptocottus armatus*) (Pietsch and Orr, 2015). These marine fish are highly tolerant of low salinity environments in estuaries and rivers throughout the Pacific Northwest. Pacific staghorn sculpin (i.e., sculpin) are omnivorous, consuming a variety of prey including benthic invertebrates, worms, crustaceans, and fish (Armstrong et al., 1995; Whitney et al., 2017). Because resident sculpin live in close association with the sediments and have a relatively small home range, they were identified as an ideal indicator species for this study which is focused on biological effects of exposure to contaminated sediments and prey. Furthermore, we targeted juvenile sculpin up to one year in age because they exhibit relatively high site-fidelity (McPeek et al., 2015) and rear in the nearshore estuarine habitats accessible throughout our study site (Morley et al., 2012) where contaminants are present.

The contaminants of interest here (i.e., PCBs, DDTs, BTs, PAHs) frequently bind to sediments and may become bioavailable following ingestion of contaminated prey and incidental ingestion of contaminated sediments. The links between contaminant exposure and adverse health effects in fish are well established, particularly for the target contaminants in this study. In general, PCBs are endocrine disruptors (Johnson et al., 2014) and immune system suppressors (Arkoosh et al., 2001) that produce numerous adverse health effects in exposed fish (Baldigo et al., 2006; Berninger and Tillitt, 2019). Field studies have shown that elevated tissue PCB concentrations are correlated with increased prevalence of liver lesions in exposed fish (Myers et al., 1998; Barron et al., 2000; Myers et al., 2008). In addition to the iconic adverse effect of egg shell thinning in birds exposed to DDT (Ratcliffe, 1967), more recent experiments in fish have shown decreased growth and endocrine disruption following DDT exposure (Johnson et al., 2014; Martyniuk et al., 2020). Likewise, the endocrine disrupting effects of BTs, especially tributyltin (i.e., TBT), are well known in marine gastropods (reviewed in (Basu and Janz, 2013)), and research shows the potential for TBT to masculinize certain flatfish (Shimasaki et al., 2009). Furthermore, PAH compounds are ubiquitous pollutants from legacy (West et al., 2019) and current (Stein et al., 2006) sources that can have profound environmental impacts. PAHs are known to produce a cascade of adverse biological effects (Collier et al., 2013) including altered cardiac function (Incardona et al., 2015), carcinogenic liver lesions (Myers et al., 2003), and reduced growth (Meador et al., 2006; Lundin et al., 2021). In total, the pollutants that are a focus of this study act through an array of biological mechanisms to adversely impact the health and fitness of individual fish, as well as the population trajectories of exposed fish (Davis et al., 2007; Meador, 2014; Paterson et al., 2016).

In the current study, we use juvenile sculpin as integrators of the biological effects of exposure to multiple environmental contaminants at sites throughout the highly urbanized LDR estuary. This field study was designed to test the assumption that fish growth is negatively correlated with contaminant concentrations in whole-bodies, stomach contents, and sediments. Juvenile sculpin are important sentinels of habitat condition and vital components of the estuarine food web in terms of biomass and contaminant pathways to higher trophic levels. Given these characteristics, Pacific staghorn sculpin is an ideal species for documenting and quantifying natural resource injuries.

2. Methods

2.1. Study location

A geographically stratified random sampling design (Cochran, 1977) was utilized to provide a spatially balanced set of samples for comparing fish from different sampling units (sites) within the assessment area of the LDR. As shown in Fig. 1, the LDR was divided into eight geographic strata which were subdivided into a total of 28 sampling units. Slips (SP; constructed side-channels) and early action areas (EAA) were each grouped into a single stratum despite sites occurring at geographically disparate locations within the river. Otherwise, strata represented geographically contiguous areas. An array of fishing locations within each sampling unit was randomly selected using GIS software, and fishing was attempted if access was available within 15 m of the location's planned GPS coordinates. Fishing locations inaccessible to beach seining (e.g., armored shoreline, private property) or setting traps (e.g., overwater hazards, boat traffic) were marked as such by the fishing crews, who then proceeded to the next accessible pre-determined fishing location. Sculpin caught from multiple net deployments at a given fishing location were summed to represent the total catch from that particular sampling unit. Water quality parameters including temperature, salinity, and dissolved oxygen were measured concurrent with fishing using a hand-held YSI meter, and are presented as averages when multiple locations and/or days within a sampling unit were fished. Chain of custody procedures were followed throughout data collection and analysis.

2.2. Fish collections and sampling

Fishing activities took place during July and August of 2019 using

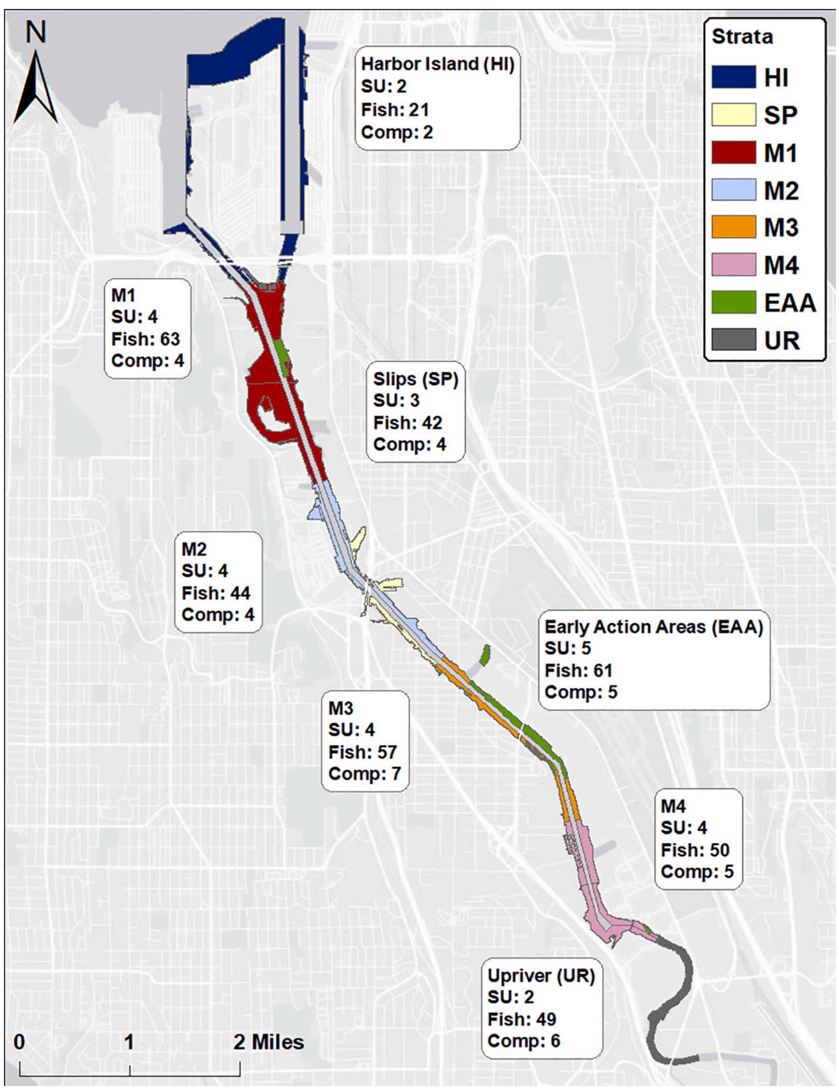

Fig. 1. The Lower Duwamish River was divided into eight strata (i.e., early action areas (EAA), Harbor Island (HI), M1, M2, M3, M4, slips (SP) and upriver (UR)). Inset boxes show the number of sampling units (SU), the total number of sculpin caught (Fish), and the number of whole-body and stomach contents composites (Comp) analyzed for contaminant concentrations (i.e., PCBs, DDTs, PAHs, and BTs) within each strata.

both a beach seine net and minnow traps. Traps were deployed from the *RV Stickelback*, a 17-ft long Boston Whaler. Cylindrical traps were constructed of 0.25-in. metal mesh walls, marked with attached floats, and baited with soft pet food mixed with salmon oil. Bait was placed in small mesh bags so that it could not be consumed by attracted fish. Traps were generally set at low tide and left to soak for at least 4 h before retrieval. Additionally, a beach seine (7.5 m long, 1 m high, 3 cm mesh size) was walked parallel to shore at depths up to 1 m, and pulled to shore where the resulting catch was sorted. Regardless of fishing method, all non-target fish were identified, enumerated, and immediately released at the site of capture. Sculpin within the desired size range (40–120 mm) were assigned a unique identifying code and euthanized in a cooler of dry ice. Fish remained frozen on dry ice for the duration of daily field operations and during transport to NOAA's Northwest Fisheries Science Center (NWFSC) laboratory, where they were immediately stored in a − 80 °C freezer for later analysis.

Sculpin were dissected at the NWFSC over the course of several weeks in October 2019. Fish lengths (i.e., total length (mm)) and weights (g) were taken immediately prior to dissections. Frozen fish were dissected on blocks of dry ice in order to reduce potential tissue degradation caused by repeated freeze-thaw cycles. In order to reduce cross-contamination, separate exterior (i.e., cutting open the body cavity) and interior (i.e., removing liver and stomach contents) dissection tools were used. All dissection tools and cutting surfaces were cleaned with deionized water in between fish caught from within sampling units, while ethanol and deionized water were used to clean tools and cutting surfaces between sampling units. Otoliths were removed, blotted dry, and stored dry in a polypropylene snap-top microcentrifuge tube. Excised livers were weighed (mg), placed into labeled microcentrifuge tubes and snap frozen in liquid nitrogen. Stomachs were removed and contents squeezed into glass scintillation vials. Two eDNA samples were collected from each fish by wiping the internal stomach surface with a foam-tipped sterile swab, and stored individually in polypropylene snap-top microcentrifuge tubes. Whole bodies were individually wrapped in solvent-rinsed aluminum foil and placed in a labeled plastic bag for later chemical analysis. With the exception of otoliths, which were stored at room temperature, all tissues were immediately stored in a − 80 °C freezer for later analysis. Liver and eDNA samples were not analyzed for this study.

Measured lengths and weights were used to calculate Fulton's condition factor (K) using the equation: (weight (g)/ (total length (mm))ˆ3)) x 100,000

Individual liver and fish weights were used to calculate hepatosomatic index (HSI) using the equation: (liver weight (g)/fish weight (g)) x 100

2.3. Compositing schemes for organic chemical analyses

Single whole-body composites, comprised of individual fish bodies excluding dissected otoliths, livers, and stomach contents, were created for each sampling unit in the assessment area. The target mass of each composite sample was at least 15 g (wet weight). Contaminant concentrations of whole-body composite samples ($n = 2$ to 6) from sampling units within each stratum were averaged to yield a single tissue chemistry value for each stratum, expressed as an area-weighted geometric mean value. Each seine-caught tissue composite sample consisted of the five fish closest to the median length of all fish captured from that sampling unit. Trap-caught sculpin were composited separately to yield an additional three composite samples from stratum M3, and one composite sample each from strata M4 and SP. Each of the 5 trap-caught composites consisted of all of the fish caught (n = 2 or 3) from the sampling unit. At the upstream reference stratum, which contained 2 sampling units, 6 tissue composite samples were made of 2–5 fish each. Stomach content composites were made by combining all of the individual fish stomach contents from each sampling unit. Contaminant concentrations of stomach content composites (n = 2 to 6) from sampling units within each stratum were averaged to yield a single stomach content value for each stratum, expressed as an area-weighted geometric mean.

2.4. Otolith microstructure preparation and growth determination

Otoliths from individual sculpin ($n = 374$) were prepared following procedures described previously (Stevenson and Campana, 1992; Chittaro et al., 2015; Chittaro et al., 2020). Briefly, otoliths were mounted on glass microscope slides with thermoplastic glue and polished until the cores and rings, which represent daily growth increments, were visible. Digital images of each otolith were captured using a digital camera mounted on a compound microscope. Measurements of otolith radius (distance from core to edge, O_c, measured in μm) and radius at 28 days before capture (distance from core to daily increments from edge, O_a) were made on each otolith image using imaging software (Image Pro Plus 7.0, Media Cybernetics Inc.).

Total fish length at 28 days prior to capture (L_a) was estimated using the Fraser-Lee equation: $L_a = d + ((L_c - d)/O_c) \times O_a$, where d is the intercept (−5.89) of the regression between fish length and otolith radius ($R^2 = 0.83$) and where L_c is total fish length (mm) at capture.

Average daily somatic growth rate (mm per day) was calculated for each fish for the 28-day period prior to capture (a) using the equation $(L_c - L_a)/a$. In addition to average daily somatic growth rates, statistical analyses were also performed on daily otolith increment width measurements taken for each fish between the otolith edge and 28 days prior to capture. Microstructure measurements could not be performed on 14 otoliths from sculpin caught at multiple strata due to natural vaterite deformities or over-polishing. Quality control procedures were performed on approximately 10 % of otoliths to ensure consistency of analysis. As described in Chittaro et al. (2020), repeat measurement of the average increment width across the last seven increments prior to capture was performed. The averages between replicate measurements were then compared using a student's t-test to check for significant differences.

2.5. Sediment collections

Sediments were collected during two separate sampling efforts in 2018 and 2020 from sites throughout the LDR. Details of sediment sampling can be found in the 2018 Lower Duwamish Waterway Baseline Surface Sediment Collection and Chemical Analyses Quality Assurance Project Plan (https://semspub.epa.gov/work/10/100098029.pdf) and in the 2020 Sediment Sampling and Analysis Plan (https://pub-data.diver.orr.noaa.gov/admin-record/5501/LDR%20Sediment%20Sampling%20and%20Analysis%20Plan_2020_0417%20%281%29.pdf). In 2018, a spatially balanced random sampling design was used to select sampling locations. Surface grab samples were collected from 168 cells distributed throughout the LDR assessment area using a pneumatic sampler operated from a boat. The top 10-cm layer of sediment from individual grab samples was homogenized in a stainless-steel bowl and stored in 8-oz glass jars at 4 °C until later chemical analysis (i.e., PCBs, TBT and PAHs). An additional sediment sampling event occurred in 2020 to expand the spatial extent of sediment analyses in the LDR assessment area. Fifty samples were collected from sites near Harbor Island (i.e., river miles 0–3) and upriver (i.e., river miles 10–12) according to established protocols (EPA, 1997). Sediments collected from the navigational channel are not included here. Briefly, a pneumatic sampler operated from a research vessel was used to collect surface sediments. The top 10-cm sediment layer was removed from each grab, homogenized in a stainless-steel bowl, and stored in glass jars at 4 °C for later analysis of conventional parameters (i.e., total organic carbon, percent solids, and grain size) and contaminants (i.e., PCBs, DDTs, TBT, and PAHs).

2.6. Analytical chemistry

Whole-body (n = 37) and stomach content (n = 35) composite samples were analyzed at the NWFSC (Seattle, WA) for concentrations of PCBs, DDTs, and PAHs by gas chromatography–mass spectrometry (GC–MS) methods (Sloan et al., 2014). Briefly, individual fish were homogenized and combined according to the compositing scheme described in the previous section. Subsamples of composited samples were extracted with dichloromethane using an accelerated solvent extractor (ASE). Sample extracts were precleaned on an alumina/silica column, lipids were removed using size-exclusion liquid chromatography, and analyzed by low-resolution GC–MS. The target analyte list included 45 PCB congeners, 6 DDT isomers, and 42 PAHs (both parent compounds and alkylated homologues). Concentrations of constituents within those three chemical groups were summed and presented as totals. The full list of PCB, DDT, and PAH compounds analyzed, as well as details of the quality assurance protocols, can be found in Sloan et al. (2014 and 2019). Percent lipids in whole-body composites were measured gravimetrically following dichloromethane extraction. Lipid classes (triglycerides, free fatty acids, polar lipids, cholesterol) were measured using thin-layer chromatography/flame ionization detection, and are not presented here. All chemical and lipid analyses met established quality assurance criteria (Sloan et al., 2019).

Whole-body composites (n = 37) were analyzed for total butyltins (BTs) by ALS Environmental Laboratory (Kelso, WA). Analyses were performed according to the lab's National Environmental Laboratory Accreditation Program (NELAP)-approved quality assurance program. The complete analytical report can be found in Supplemental Material (S1). Analysis of butyltins (i.e., n-Butyltin, Di-n-butyltin, Tri-n-butyltin, and Tetra-n-butyltin) followed published methods (Unger et al., 1986). Tissue composite samples were acidified and extracted using methylene chloride followed by a Grignard reaction. The extract was eluted through silica and a Florisil column, and analyzed by GC-PFD (gas chromatography-pulsed flame detector). Individual analytes from both 1× and 10× dilutions were summed and presented as a total butyltin concentration for each tissue sample.

PAH concentrations in sediments collected in 2018 (n = 168) were measured by the Environmental Protection Agency (EPA) Manchester Environmental Laboratory (Port Orchard, WA) using GC–MS methods (SW3541) and a Florisil cleanup step. Butyltins were measured by Analytical Resources, Inc. (Tukwila, WA) using GC–MS. Samples were extracted using methylene chloride and hexane, hydrolyzed with hydrochloric acid, then subjected to silica gel cleanup. Sediment PCBs in samples collected in both 2018 (n = 168) and 2020 (n = 50) were analyzed by Axys Analytical (Sydney, BC, Canada) using GC–MS method 1668C. Briefly, samples were extracted with dichloromethane, followed by cleanup using Bio-Bead copper and Florisil chromatographic columns. Sediments collected in 2020 (n = 50) were analyzed by ALS Environmental Labs (Kelso, WA) for PAHs, butyltins, and conventional parameters using standard methodologies. Analytical methods and lists of analytes can be found in the 2018 Lower Duwamish Waterway Baseline Surface Sediment Collection and Chemical Analyses Quality Assurance Project Plan (https://semspub.epa.gov/work/10/100098029.pdf) and in the 2020 Sediment Sampling and Analysis Plan (https://pub-data.diver.orr.noaa.gov/admin-record/5501/LDR%20Sediment%20Sampling%20and%20Analysis%20Plan_2020_0417%20%281%29.pdf). Sediment contaminant concentrations from multiple locations within each stratum were averaged and presented as geometric means in ng/g dry weight units.

2.7. Data and statistical analysis

Comparisons of fish parameters (length, weight, K, HSI, percent lipids), environmental parameters (temperature and salinity), and growth rates were analyzed with weighted linear regression to identify significant differences between strata and the upriver reference stratum (UR; $p < 0.05$) in R (version 4.0.5) using the *lm* function from the stats package (R Core Team, 2022). Regressions utilized mean values of each sampling unit, weighted by sampling unit area. Normality was assessed using Shapiro-Wilkes tests from the stats package (R Core Team, 2022). An ANOVA with Tukey's post-hoc ($p < 0.001$) was used to compare the average growth rates from sampling units within the EAA and SP strata. Additionally, mixed effects models were run in R (version 4.0.5) using the *lmer* function from the lme4 package (Bates et al., 2015) to determine relationships between contaminant concentrations and growth rates. Model fit was compared using Akaike's information criterion (AIC) coefficients, with the smallest coefficient indicating the best fit model (Akaike, 1973). AIC coefficients were calculated using the AIC function from the stats package (R Core Team, 2022). Correlation between chemical concentrations, water quality parameters, and fish metrics were evaluated using the *cor* function from the stats package (R Core Team, 2022). Because fish length and weight were strongly correlated, only length was included in mixed effects models.

Mixed effects models utilized growth rate measurements from the 163 fish that comprised the whole-body chemistry composites (n = 37). Additionally, contaminant concentrations in stomach content composites (n = 35) and sediments (n = 241 for PAHs, n = 255 for PCBs) were analyzed against growth rate measurements from all fish (n = 374). Within the mixed effects models, growth (i.e., daily otolith radius change measured in μm) was the dependent variable. To account for the primary experimental units (i.e., sampling units) and repeated measurements of growth within individual fish, the mixed effects models included random effects for sampling unit and fish within sampling unit. The upriver stratum (UR) was used as the reference. In addition to the stratum variable, covariates considered for each model included total length, weight, temperature, salinity, percent lipids, hepatosomatic index (HSI), and condition factor (K). To avoid problems with multicollinearity of predictors in the regression model, whole-body PCBs, DDTs, PAHs, and BTs were evaluated in separate models because they were highly correlated with each other ($p < 0.05$).

3. Results

3.1. Fish health metrics and growth

Of the 391 fish collected for this study, four fish were removed from the data set prior to data and statistical analysis. Three were excluded due to a data recording discrepancy, and one was excluded because it was determined to be greater than one year of age by otolith analysis. Beach seining was the more productive fishing method, yielding the vast majority (97 %) of the fish utilized in this study. Average fish lengths and weights across strata ranged from 68.4 to 87.8 mm and 3.8 to 7.3 g, respectively. Ranges of lengths and weights from individual fish in each stratum are shown in Table 1. Fish were juveniles within our target size (40–120 mm total length) and age (< one year) ranges. Average values of HSI varied from 1.1 to 1.7, average values of K varied from 1.0 to 1.1, and percent lipids ranged between 0.38 % and 0.75 % (Table 1). All fish and environmental parameters were normally distributed ($p < 0.05$). Across all fish metrics and environmental parameters, several strata were significantly different than the upriver stratum (UR; linear model $p < 0.05$).

Average 28-day somatic growth rates (i.e., average daily growth rate over the 28-day period immediately prior to capture) showed significant strata-specific differences (Fig. 2). Average growth rates ranged from a high of around 1.0 mm/day at the UR, M3, M4, and EAA strata, to a low of around 0.7 mm/day at the HI and SP strata. Fish from strata M1 and M2 showed intermediate growth rates of just over 0.8 mm/day.

3.2. Tissue and sediment chemistry

Whole-body contaminant concentrations showed strata-specific differences (Fig. 3) with a pattern of higher concentrations at locations

Table 1
Measured and calculated fish metrics and environmental conditions at each strata. Values are arithmetic means with ranges in parenthesis. Significant difference from UR (upriver) stratum, denoted by *, determined by a linear model ($p < 0.05$).

Strata	Number of Fish	Number of chemistry composite samples	Total Length (mm)	Weight (g)	Total Lipids (%)	Condition Factor (K)	Hepatosomatic Index (HSI)	28-day Somatic Growth Rate (mm/day)	Temperature (°C)	Salinity (ppt)
HI	21	2	87.8 (59–108)	7.3 (2–12.1)	0.38* (0.36–0.42)	1.0 (0.8–1.1)	1.3 (0.9–1.9)	0.65* (0.5–0.8)	15.0* (13.6–16.2)	23.8* (21.4–26.9)
SP	42	4	74.8* (52–107)	5.2 (1.8–14.8)	0.75 (0.47–1.0)	1.1* (0.9–1.4)	1.7* (0.8–3.4)	0.70* (0.5–0.8)	17.1* (13.8–20.8)	15.0* (3.7–27.1)
M1	63	4	68.4* (50–96)	3.8* (1.4–10)	0.4 (0.3–0.46)	1.1 (0.8–1.4)	1.1 (0.3–1.7)	0.83* (0.7–1.1)	15.2* (12.7–17.5)	19.6* (0.5–26.3)
M2	44	4	79.7 (55–110)	6.2 (1.9–14.3)	0.44* (0.37–0.56)	1.1* (1.0–1.8)	1.2 (0.7–2.0)	0.81* (0.6–1.0)	16.7* (13.5–18.5)	15.5* (6.8–25.7)
M3	57	7	76.4 (50–101)	5.5 (1.3–11.9)	0.69* (0.48–1.0)	1.1 (0.7–1.4)	1.3 (0.2–1.8)	1.05 (0.8–1.4)	16.8* (13.3–20.8)	12.1* (0–24.5)
M4	50	5	82.5 (45–125)	7.0 (1.1–19.2)	0.62 (0.57–0.67)	1.1* (0.8–1.4)	1.3 (0.5–2.2)	0.94 (0.7–1.2)	18.5 (14.6–21.3)	7.1* (0.7–24.0)
EAA	61	5	76.8 (50–107)	5.8 (1.4–13.2)	0.49 (0.43–0.59)	1.1* (0.8–1.6)	1.1 (0.1–1.8)	0.95 (0.7–1.1)	16.6* (12.3–20.1)	11.8* (0.3–27.5)
UR	49	6	87.1 (66–123)	7.3 (3.1–21.1)	0.54 (0.42–0.7)	1.0 (0.8–1.2)	1.2 (0.8–1.9)	0.97 (0.7–1.3)	19.0 (17.8–19.8)	1.8 (0.1–7.7)

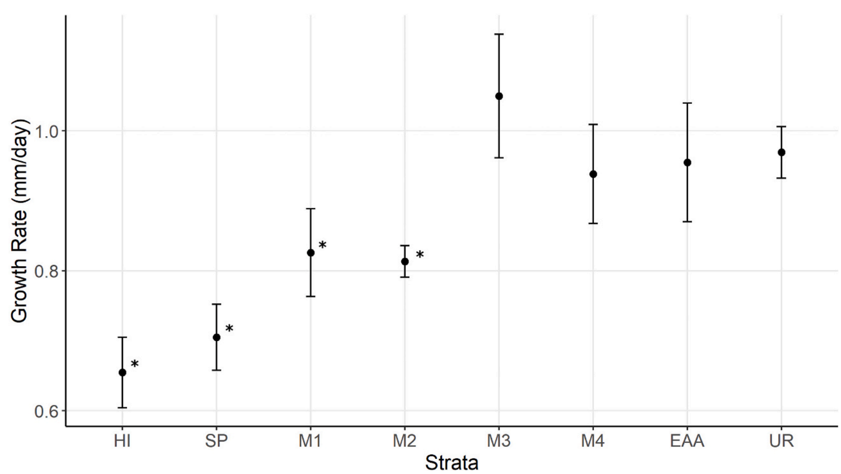

Fig. 2. Average somatic growth rate (i.e., growth rate averaged over the 28 days immediately preceding fish capture) was significantly different among river strata. The highest growth rates were measured in fish from strata furthest upriver (M3, M4 and UR) as well as at habitat remediation sampling units (EAA). Number of fish per strata varied from 21 to 63. Filled circles are average daily somatic growth rates (mm/day), error bars are 2× standard error. Asterisk denotes a significant difference from UR (linear model, $p < 0.05$). UR = upriver, SP = slips, HI = Harbor Island, EAA = early action areas.

furthest downriver and closest to identified Superfund sites (i.e., HI and SP strata). Across all sites, total PCB concentrations in whole bodies ranged from 75.5 to over 7000 ng/g wet weight (Table S2) with the highest concentrations at the HI, SP, and M2 locations. Concentrations of PCBs in stomach contents showed similar site-specific patterns but were generally lower than whole-body concentrations. Sediment PCBs at strata M4 and UR (i.e., the furthest upriver) and EAA (i.e., remedial sites) were 10 to 100-fold lower than both whole body and stomach content concentrations at those same strata (Fig. 3). Total PAH concentrations were markedly lower in whole bodies compared to both stomach contents and sediments within each strata. The lowest total PAH concentrations in fish tissues were at the M4, UR, and EAA strata, with concentrations at all other strata near 1200 ng/g wet weight. Stomach content PAH concentrations varied between 100 and 1000 ng/g wet weight. Total DDT concentrations measured in whole-body and stomach content composites were highest at the SP stratum (about 60 ng/g wet weight), but otherwise did not show a trend with strata. Whole-body BT concentrations were similar to those measured in sediments, and many samples were below method detection limits. The highest average sediment BT concentration was about 25 ng/g dry weight at the HI stratum, and the highest whole-body concentration measured 15 ng/g wet weight also at the HI stratum. Throughout this study, all error bars in chemistry data plots are 2× standard error in order to show likely significant differences between data points.

3.3. Mixed effects models

Mixed effects model analysis showed that fish growth was negatively correlated with whole-body PCB concentrations and positively correlated with water temperature (Table 2; Fig. 4, A and B). Growth was negatively associated with whole-body concentrations of DDTs, however that relationship was not significant ($p = 0.3$; Table 2). For all model scenarios using whole-body contaminant concentrations, the best fit models (i.e., the model with the lowest AIC coefficient) were those that included contaminant concentrations and temperature (Table S1). Stomach content PAHs were the only contaminants to show a significant correlation with fish growth ($p < 0.05$; Table 2). Growth rates were negatively correlated with stomach content PAHs and positively correlated with temperature (Fig. 4 C and D). The best fit model predicting growth included both stomach content PAHs and temperature (Table S1). Fish growth rates were negatively correlated with sediment concentrations of PCBs, PAHs, and BTs (Fig. 5). These correlations were

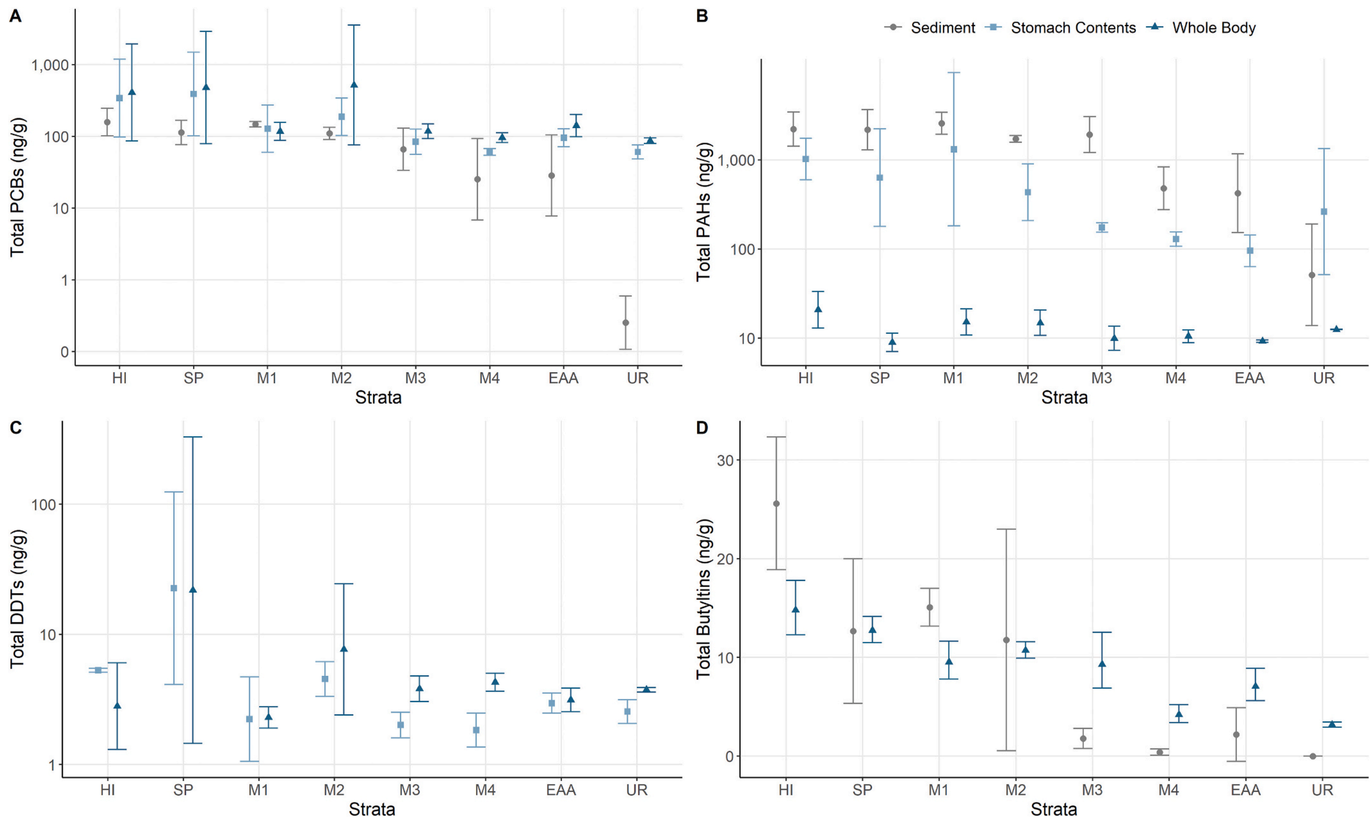

Fig. 3. Average concentrations of A) total PCBs and B) total PAHs in sediments, stomach contents, and whole-body composites; C) total DDTs in stomach content and whole-body composites; and D) total BTs in sediments and whole-body composite samples at each of the eight strata. Stomach content and whole-body concentrations are ng/g wet weight, sediment concentrations are ng/g dry weight. Symbols are area-weighted geometric means of 2–7 composite samples of 5 fish each within each strata, error bars are 2× standard error.

Table 2
Results of mixed effects model runs for 28-day growth rate (mm/day) and contaminants in whole bodies, stomach contents and sediments. In each case only the model with the best (i.e., lowest) AIC score is shown. Significant *p* values are in bold. Predictors marked with * are plotted in Figs. 4 and 5.

Model	Predictors	Estimates	P value
Whole body PCBs	Intercept	4.1	0.1
	PCBs*	−0.4	**0.03**
	Temperature*	0.5	**0.001**
Whole body PAHs	Intercept	2.1	0.4
	PAHs	−0.06	0.8
	Temperature	0.5	**0.001**
Whole body DDTs	Intercept	1.6	0.5
	DDTs	−0.2	0.3
	Temperature	0.5	**< 0.001**
Whole body BTs	Intercept	2.1	0.5
	BTs	−0.03	0.9
	Temperature	0.5	**0.005**
Stomach content PAHs	Intercept	4.3	0.06
	PAHs*	−0.5	**0.02**
	Temperature*	0.4	**0.001**
Sediment PCBs	Intercept	11.39	**< 0.001**
	PCBs*	−0.85	**0.008**
Sediment PAHs	Intercept	14.2	**< 0.001**
	PAHs*	−1.38	**0.01**
Sediment BTs	Intercept	10.6	**< 0.001**
	BTs*	−0.1	**< 0.001**

highly significant ($p < 0.01$). For each contaminant, the best fit mixed effects model contained only that chemical (Table S1). Temperature was not a significant confounding variable explaining the relationship between fish growth rates and sediment contaminant concentrations.

3.4. Environmental parameters

Water temperatures and salinities across the study site ranged from 12.3 to 21.3 °C and 0 to 27 ppt, respectively (Table 1). These parameters varied at any given site depending on the tidal cycle, but water temperature generally increased upriver. Within the SP and EAA strata, which both contained non-contiguous sampling points from throughout the river, average temperatures varied between 15 and 18 °C. Fish growth rates at sampling locations in the EAA stratum generally increased commensurate with increasing temperature (Fig. 6) to a maximum growth rate of over 1.0 mm/day at 17.5 °C. However, fish growth rates within the SP stratum were comparatively lower overall and did not show a pattern with temperature (Fig. 6). Salinity did not show a clear pattern of variation among river strata or with fish growth.

4. Discussion

This study documented slower growth rates in juvenile sculpin collected from sites with higher contaminant concentrations in whole-bodies, stomach contents, and sediments. Here, we showed that in addition to being useful indicators of aquatic habitat condition, juvenile sculpin effectively revealed the adverse impacts of contaminants on fish in the LDR system. Similar studies have likewise shown the utility of fish as sentinels of environmental health in highly urbanized habitats (Collier et al., 1998; Johnson et al., 2014). Additionally, growth was the primary measure of adverse biological effect in contaminant-exposed fish. Growth is a useful endpoint because it represents a whole-organism effect that is integrative of multiple mechanisms of toxicity following contaminant exposure (Johnson et al., 2014). Consistent with the current study, previous field studies have shown that lower fish growth is associated with elevated contaminant concentrations in other

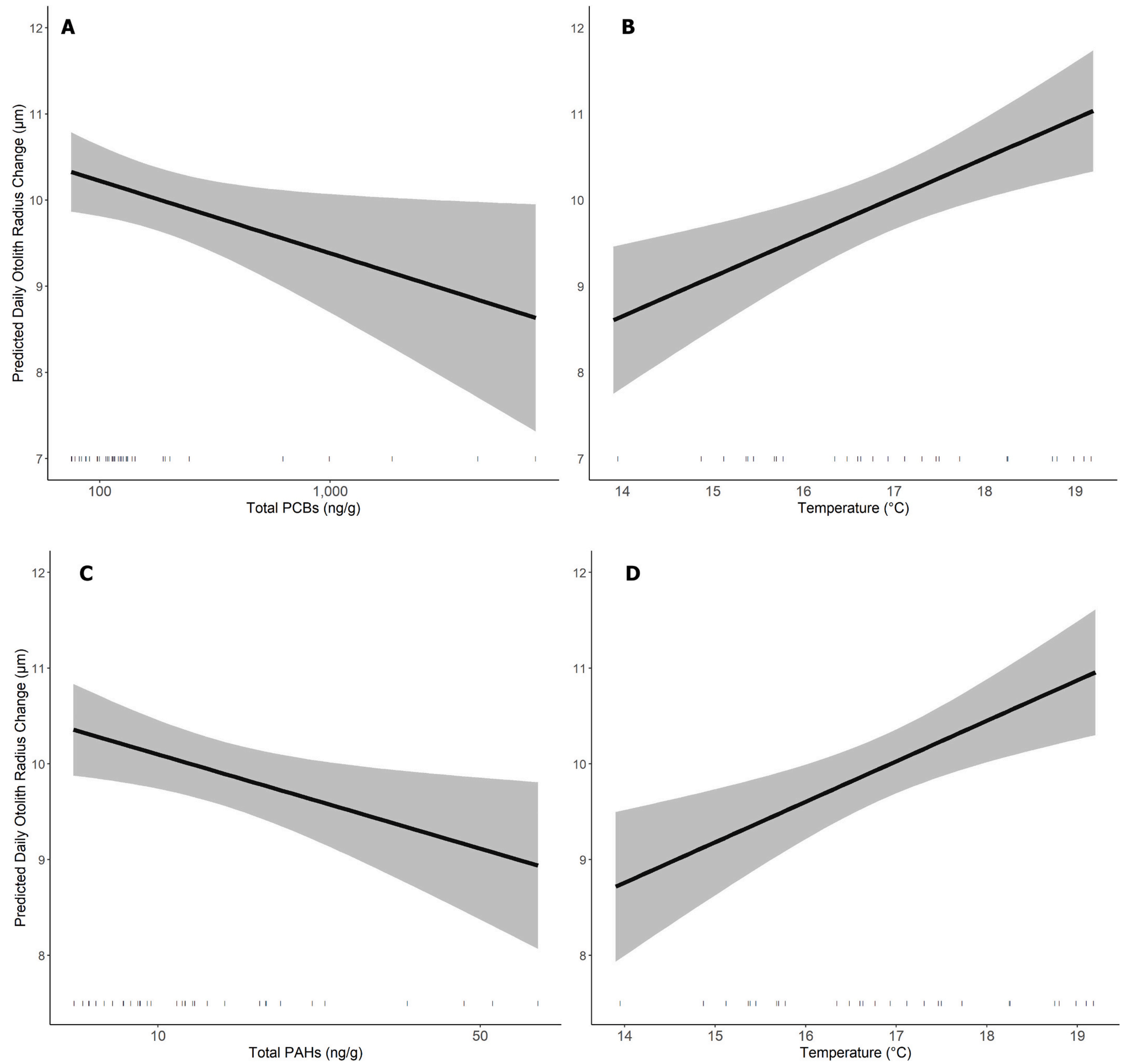

Fig. 4. Mixed-effects model results for PCBs show the negative association between fish growth (daily otolith radius change, μm) and A) whole-body PCB concentrations (ng/g wet weight) along with the positive association between fish growth and B) water temperature. Mixed-effects model results for PAHs show the negative association between fish growth and C) stomach content PAHs (ng/g wet weight) along with the positive association between fish growth and D) water temperature. Black lines are the model prediction lines, while the grey bands are the confidence intervals. Vertical tick marks at bottom of plots indicate measured data points.

species including juvenile Chinook salmon (Lundin et al., 2021), adult *Fundulus* (Black et al., 1998), and juvenile sole (Amara et al., 2007). These studies occurred in a range of habitats impacted by various anthropogenic and environmental stressors and show the utility of growth as an integrative measure of fish injury. In addition to individual-level impacts, lower fish growth rates resulting from pollutant exposure have been linked to reduced fitness and health at the population level (Baldwin et al., 2009; Hamilton et al., 2016; Lundin et al., 2019). While population-level impacts were not directly assessed in the current study, it is reasonable to conclude that lower sculpin growth rates could alter population demographics (e.g., increased prevalence of smaller fish), resulting in reduced reproductive output and lower biomass as observed in other studies (Hamilton et al., 2016). Therefore, the cascading effects of reduced growth at the individual level and potential population-level impacts may ultimately affect the piscivorous birds and marine mammals that rely on sculpin as a food source (Luxa and Acevedo-Gutierrez, 2013; Buckner et al., 2022) in this impacted environment.

This field study was designed to investigate the relationship between fish growth rates and contaminant concentrations. Because the study was by necessity observational as opposed to a controlled laboratory experiment, it was anticipated that environmental conditions other than contaminant exposure, and variables related to the fish themselves, could be associated with growth, the primary biomarker in this study.

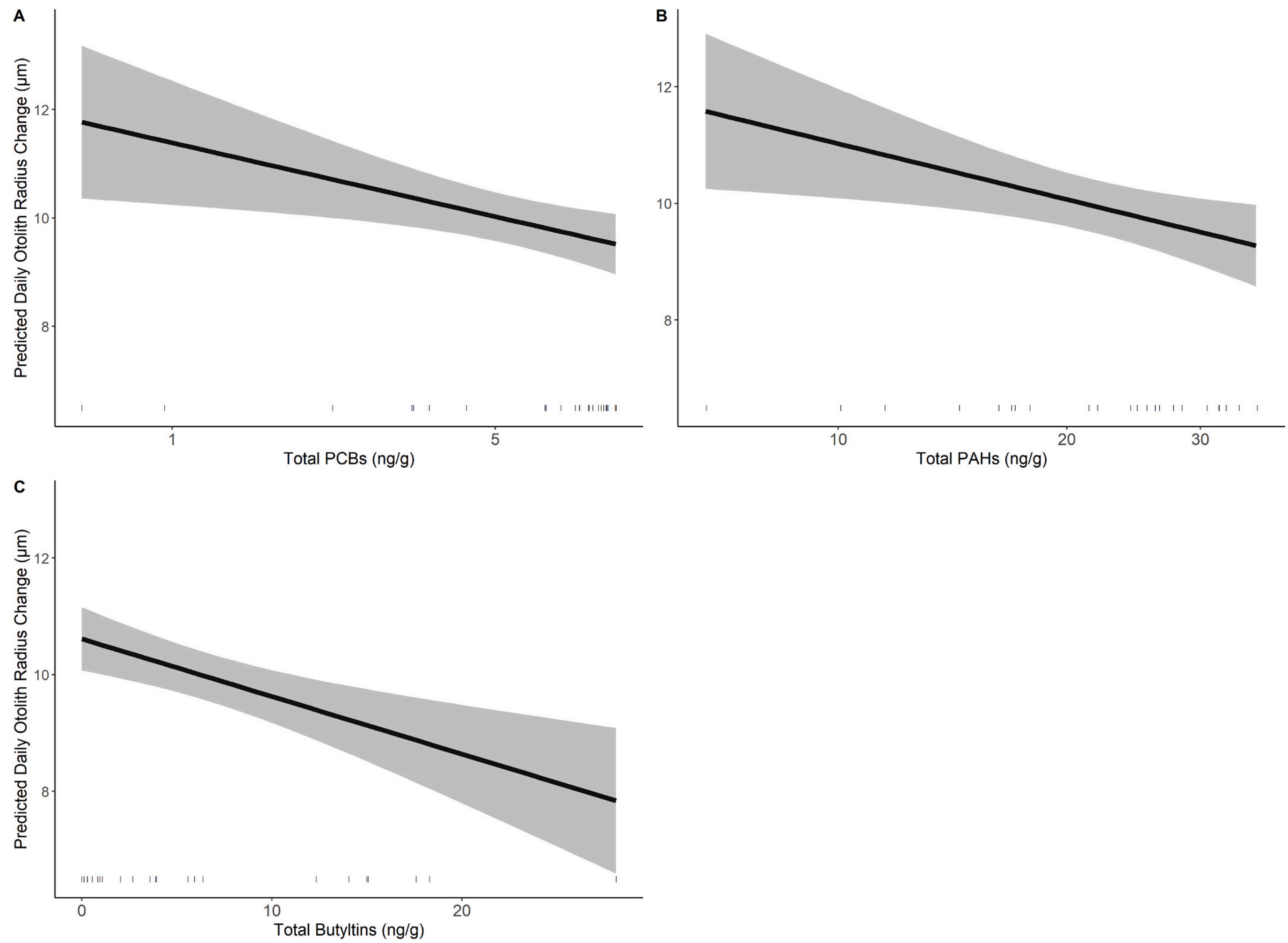

Fig. 5. Mixed-effects model results show a negative association between fish growth (daily otolith radius change, μm) and sediment concentrations of A) total PCBs, B) total PAHs, and C) total butyltins. Chemical concentration units are ng/g dry weight. Black lines are the model prediction lines, while grey bands are the confidence intervals. Vertical tick marks at the bottom of each plot indicate measured data points.

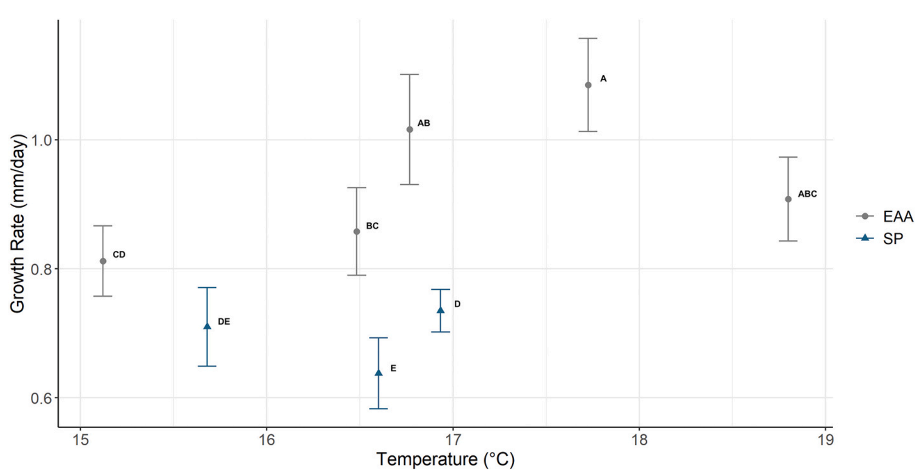

Fig. 6. Significantly different sculpin growth rates occurred at sampling units within and between Early Action Area (EAA, $n = 5$) and Slips (SP, $n = 3$) strata. Water temperatures at these locations varied between 15 and 19 °C. Symbols show average somatic growth rate (i.e., growth rate averaged over the 28 days immediately preceding fish capture), error bars are 2× standard error. Letters indicate significant differences as determined by ANOVA with Tukey's post-hoc ($p < 0.001$).

Because confounding factors could not be fully controlled in the design of this field study, we measured the most likely confounders and accounted for their effects on fish growth statistically by including them in mixed effects models (Gelman and Hill, 2007). To understand the statistical importance of contaminant exposures relative to potentially confounding variables, three situations were represented by mixed effects models. Namely, fish growth as a function of 1) contaminant concentrations, 2) environmental and/or biological variables, and 3) both contaminant concentrations and environmental and/or biological variables. The best fit models were those that predicted fish growth based on contaminants alone (i.e., sediment PCBs, DDTs, and PAHs) or contaminants and environmental variables (i.e., stomach content PAHs and temperature, whole-body PCBs and temperature). The strength of these correlations points to the overall influence of these contaminants on the growth of resident juvenile sculpin. Noting that multiple environmental stressors likely impact the health of fish in this urbanized estuary (Toft et al., 2018), this study highlights the measurable influence of PCB, DDT, and PAH pollution on sculpin growth.

Temperature is known to have a consequential impact on fish growth, especially in poikilothermic fish such as sculpin where metabolism and growth are heavily influenced by the temperature of the ambient environment (Heath, 2019). Accordingly, this study confirmed an overall positive correlation between temperature and sculpin growth rate regardless of strata. However, this study also revealed that temperature alone did not explain growth differences among sites, especially at those sites with similar temperature regimes. Sampling locations at the mouth of the LDR tended to be colder and saltier (Table 1) than locations further upriver due to the strong tidal influx of cold ocean water from Elliott Bay and Puget Sound (McKeon et al., 2021). Though this study was not designed to distinguish the effect of temperature from that of contamination on juvenile sculpin growth, data collected from two strata (i.e., SP and EAA) provided important insight. The SP and EAA strata included sampling units (i.e., fishing locations) at proximate locations with similar temperatures (i.e., 15–18 °C; Fig. 6) but different levels of contaminant concentrations (Fig. 3). Specifically, juvenile sculpin from the more contaminated SP locations had consistently lower 28-day growth rates than the less contaminated EAA locations despite these locations having similar water temperatures (Fig. 6). More specifically, within a narrow temperature range of 16.5 to 17 °C at two SP and two EAA sampling locations, a 10-fold increase in whole-body PCB concentration was measured commensurate with a 35 % reduction in growth rate. Therefore, this comparison of EAA and SP locations, which isolated differences in contaminant concentrations from variation in environmental conditions (i.e., temperature), highlights the influence of contaminants on sculpin growth in the LDR.

The contaminants of concern in the LDR that were a focus of this study have long been known to be bad actors in aquatic habitats. In particular, bioaccumulative PCBs have a long history as a persistent and pervasive threat to fish health in urbanized coastal environments (Davis et al., 2007; Pan et al., 2016; Paterson et al., 2016; West et al., 2017). A recent review (Berninger and Tillitt, 2019) of laboratory and field studies investigating impairment to fish growth and reproduction found that total PCB tissue concentrations of 1 μg/g resulted in 15 % inhibition of fish growth. This analysis agrees well with our study showing an approximate 20 % decrease in sculpin growth rate between the reference strata (UR) and strata HI, SP, and M1 where the highest whole-body concentrations exceeded 1 μg/g (Table 1). Additionally, at nearly all strata, PCBs showed a consistent pattern of lowest PCB concentrations in sediments, intermediate concentrations in stomach contents, and highest concentrations in whole-bodies (Fig. 3A). This observed pattern suggests biomagnification between trophic levels through the consumption of contaminated prey (Ericson et al., 2008; Johnson et al., 2014), and reinforces the importance of understanding contaminant pathways through complex food webs as well as their potential impact on higher trophic levels including piscivorous fish (O'Neill and West, 2009; Meador et al., 2010), birds (Jarman et al., 1996), and marine mammals (Ross et al., 2000; Wainstein et al., 2022). Notably, spatial movements of fish through heterogenous environments may influence the biomagnification of PCBs, as predicted in a food web model of a contaminated riverine system (McLeod et al., 2014). Contrary to this observed pattern of PCB biomagnification at the majority of the LDR sites, three strata in "cleaner" areas, (UR, M4, and EAA) contained sediment PCBs at concentrations much lower than those measured in stomach contents and whole bodies (Fig. 3A). At these strata, sculpin may have either moved off-site to consume contaminated prey from nearby locations, or contaminated prey was transported to these less-contaminated strata by tidal currents and surface water flows. Despite extensive remedial actions to remove PCB-contaminated sediments from the river and control upland sources of PCBs along the LDR (LDWG, 2010), sediment-bound and bioavailable PCBs continue to accumulate in sculpin and their prey. Interestingly, metabolism of accumulated PCBs was observed in *Myoxocephalus thompsoni*, a common freshwater species of sculpin (Stapleton et al., 2001). The degree to which Pacific staghorn sculpin may likewise have the ability to utilize a novel biochemical pathway to metabolize PCBs remains unstudied.

Throughout the LDR, juvenile sculpin are exposed to PAHs from upland combustion sources, stormwater runoff, upland releases of oil, and leaching from in-river sources such as creosote pilings. Our observation of PAHs in stomach contents confirms that ingestion of contaminated prey is a route of exposure for sculpin in the LDR, consistent with previous findings in ESA-listed salmon (Johnson et al., 2006; Meador et al., 2006) and flatfish (Myers et al., 2008) from the LDR and nearby Puget Sound. Because PAHs are metabolized by fish (Collier et al., 2013), it is not surprising that concentrations of PAHs measured in whole bodies were substantially lower than those in stomach contents of sculpin throughout our study site. Additionally, the extent of impaired sculpin health following PAH exposure, including liver lesions (Myers et al., 2003) and increased disease susceptibility (Arkoosh et al., 2001) throughout our study site is a concern. One recent study in a similarly contaminated river found reduced growth of outmigrating juvenile salmon at PAH concentrations similar to those measured here (Lundin et al., 2021). Also concerning in terms of fish health, yet unexplored in sculpin, are possible PAH-induced impacts to early life-stage fish (Barron et al., 2004; Incardona et al., 2015) and reductions in population abundance (Lundin et al., 2019) that have been observed in other species.

As they mature, Pacific staghorn sculpin are likely to move into the deeper marine waters of Elliott Bay and Puget Sound (Toft et al., 2007; Reum and Essington, 2011). Regardless of this range expansion, the LDR remains an important habitat for foraging and rearing of early life stage sculpin. Habitat restoration actions within the LDR, including removing over-water structures, softening shorelines, and planting native vegetation, are expected to increase the abundance of invertebrate prey available to sculpin (Morley et al., 2012), and may even lessen the characteristics of degradation associated with urban waterways (Booth et al., 2016). Remediating contaminated sediments is expected to reduce the chance that fish will be exposed to the contaminants of concern in the LDR, thereby improving the health and fitness of resident sculpin and other fish. For example, marked improvement in the health of English sole followed pollution remediation efforts at a site contaminated with PAHs from a decommissioned creosote wood treatment facility (Myers et al., 2008), and ecosystem health has improved in the Chesapeake Bay following concerted restoration efforts (Carey, 2021). The LDR is a dynamic system with multiple stressors adversely impacting resident fish, which adds complexity to planning effective restoration efforts and reducing pollution (Teichert et al., 2016). Habitat restoration and remediation of contaminated sediments may therefore be vital components of improving fish health and productivity even in heavily urbanized waterways.

In this field study, we have identified Pacific staghorn sculpin as a suitable bioindicator of environmental health in an urbanized environment. Reductions in sculpin growth rate were significantly correlated with the identified contaminants of concern, leading to potential consequences for sculpin survival, reproductive output, and overall population productivity (Hamilton et al., 2016). Additionally, sculpin are a likely vector for moving bioaccumulative contaminants such as PCBs from the benthos to higher trophic levels such as seabirds (Buckner et al., 2022) and river otters (Wainstein et al., 2022) at this estuarine site. While this study is not alone in documenting adverse impacts to fish health following exposure to persistent and pervasive pollution (Davis et al., 2007; Meador, 2014; Paterson et al., 2016; West et al., 2017), it highlights the importance of measuring fish injury from chronic pollution in the context of Natural Resource Damage Assessments. Furthermore, this study emphasizes the enduring concerns of current and legacy contaminants in urbanized waterways, and underscores the benefit of pollution reduction actions to improve environmental conditions for resident fish.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2023.168365.

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.

Data availability

Data will be made available on request, or at https://www.ncei.noaa.gov/archive/accession/0283629.

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