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Reconnaissance of contaminants in larval Pacific lamprey (*Entosphenus tridentatus*) tissues and habitats in the Columbia River Basin, Oregon and Washington, USA

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1. Introduction

Pacific lampreys (*Entosphenus tridentatus*) are native to the West Coast of North America and have great ecological and cultural importance. They provide high caloric value sustenance to native peoples and may have acted as a buffer against predation of juvenile salmon and steelhead trout by providing alternative prey to sea lions, northern pike minnow, terns, and gulls (Close et al., 2002; CRITFC, 2011). As larvae, they bioturbate sediments, filter-feed fine organic material, and cycle nutrients and energy between the water column and bed sediments (Close et al., 2002; Sherman, 2014). They die shortly after spawning and, like salmon, return important marine derived nutrients and energy to riverine ecosystems due to their anadromous life history (Close et al., 2002). Culturally, they hold a place of honor for the Tribal people of the Columbia River Basin who have relied on Pacific lampreys for food, medicine, and ceremonial practices for generations (CRITFC, 2011).

After hatching, larval Pacific lampreys spend 4–7 years living in sediment and feeding on detritus and other particulate matter

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ABSTRACT

Pacific lampreys (*Entosphenus tridentatus*) have resided in the Columbia River Basin for millennia and have great ecological and cultural importance. The role of habitat contamination in the recent decline of the species has rarely been studied and was the main objective of this effort. A wide range of contaminants (115 analytes) was measured in sediments and tissues at 27 sites across a large geographic area of diverse land use. This is the largest dataset of contaminants in habitats and tissues of Pacific lamprey in North America and the first study to compare contaminant bioburden during the larval life stage and the anadromous, adult portion of the life cycle. Bioaccumulation of pesticides, flame retardants, and mercury was observed at many sites. Based on available data, contaminants are accumulating in larval Pacific lamprey at levels that are likely detrimental to organism health and may be contributing to the decline of the species.

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(Close et al., 2002). It is not known how far they travel downstream from their nest site before burrowing into sediment. They are thought to be sedentary once they have settled, but they can be dislodged from their burrows during scouring events. The probable age range of larvae analyzed for this study is approximately 1-6 years based on available size and age data (Meeuwig and Bayer, 2005). After the sedentary stage, Pacific lampreys undergo metamorphosis to prepare for parasitizing fish and mammals in salt water. They migrate downstream to the sea during their transformation from the larval to adult stage, and generally complete the migration within a few months (Close et al., 2002). They typically spend 1–3 years in the adult stage, parasitizing at least 15 fish species and several species of whales and other mammals in the ocean (Close et al., 2002), before returning to freshwater to spawn as adults. During their return spawning migration, which lasts at least 1 year, adult lampreys do not feed.

The Columbia River flows more than 1950 km from the Canadian Rockies to the Pacific Ocean, draining more than 670,000 km² of the Pacific Northwest United States and British Columbia. Home to 8 million people, the Columbia River Basin supports important agricultural, commercial fishing, recreation, industrial, and tourism industries in the region. Pacific lampreys were historically abundant throughout the basin before European settlement (Close et al.,







1995). At present, the Willamette River is their only remaining stronghold in the basin (Sherman, 2014), and Willamette Falls supports one of the last traditional Native American harvest sites (CRITFC, 2011; Sheoships, 2014). After surviving as long as several hundred million years, Pacific lamprey populations have declined in recent decades to the point where regional extinction is possible. Perhaps because of their unusual appearance and the fact that invasive sea lamprevs (*Petromvzon marinus*) have caused extensive ecological problems in the Laurentian Great Lakes (e.g., Coble et al., 1990; Bryan et al., 2005), Pacific lampreys have received relatively little concern from non-tribal peoples compared to other Pacific Northwest sentinel species such as salmonids, and have not been a fisheries management priority in the United States (Close et al., 2002) until recently (CRITFC, 2011; Luzier et al., 2011). Reasons for their precipitous declines include passage difficulties at hydroelectric dams and irrigation diversions during upstream and downstream migrations as adults and juveniles (Close et al., 1995; Mesa et al., 2003; Streif, 2008; Moser et al., 2014), habitat loss and degradation, declines of marine host fish (Murauskas et al., 2012), and historical management actions such as intentional kills (Jackson and Kissner, 1997).

Recent research indicates the presence of a variety of contaminants in water (Caton, 2012; Alvarez et al., 2014), sediments (Counihan et al., 2014; Nilsen et al., 2014a), and biota (Caton, 2012; OHA EPH, 2013; Nilsen et al., 2014b) in the Columbia River and its tributaries and has highlighted the importance of understanding their potential effects in the food web (Naiman et al., 2012; Nilsen and Morace, 2014) and relevance to human health (Harper and Harris, 2008). The role of water quality and habitat contamination in Pacific lamprey declines has not previously been studied and was the main focus of this effort. Specifically, this study provides reconnaissance-based information about multiple classes of contaminants of concern in larval Pacific lampreys and their habitats in key areas of the Columbia River Basin with respect to 1) organism health during the sensitive life stages before their transformation to adults, 2) existing data from adult life stages, and 3) human health implications.

2. Materials and methods

2.1. Collection, shipment, and storage of samples

In 2011, 30 paired samples (15 larval lamprey/15 sediment samples) were collected at 15 sites from Umatilla River Basin, Deschutes River Basin, Fifteenmile Creek, Mill Creek, Hood River, and Willamette River Basin (Fig. 1). In 2012, paired samples were collected from an additional eight sites in the Willamette River Basin and three sites from the Yakima River Basin (Detailed site information is available in Appendix A and sample list in Appendix B1). Lampreys collected from the Yakima River Basin were predominately western brook lamprey (Eubucco richardsoni), since Pacific lampreys were not readily available at those three study sites (out of 26 total sites). Although western brook lampreys are not anadromous, the two species are thought to have a similar life history during their larval stage (Kostow, 2002; Moser et al., 2014). This study focused on Pacific lampreys, for which western brook lampreys were considered to be a surrogate. Comparisons were made to contaminant levels previously measured in adult Pacific lampreys (OHA EPH, 2012). The adult samples were collected at Willamette Falls, Sherars Falls and John Day Dam (Fig. 1).

Larval lampreys were collected using a backpack-mounted electroshocking device. Multiple individuals were collected from each site and composited to reach the minimum sample weight of 5 g needed for analysis. This generally constituted 3–10 individuals, depending on their size. Fish were euthanized using MS-222

(tricaine methane sulphonate; Cat. No. E10521, Sigma—Aldrich, St. Louis, MO, U.S.A.). Body lengths were measured before shipment to the USGS National Water Quality Laboratory (NWQL).

Surface sediments were collected and composited from 3 to 5 locations within the larvae collection bed to obtain a sample that was representative of the site habitat. The collection area was roughly 3 m by 3 m, but the size and shape of the area varied depending on stream characteristics (Ward and Harr, 1990; Lane et al., 2005). The top 2–5 cm of sediment were collected.

Sampling equipment was made of glass, stainless steel, or Teflon[®] and was free of materials that might leach interferences, absorb compounds of interest, or potentially contaminate or degrade the tissue samples. Some of the compounds that were determined in this study are also found in commonly used products, such as soaps, electronics, and textiles; therefore, precautions were followed to avoid contamination (Lewis and Zaugg, 2003). The sampling tools were cleaned with Liquinox[®] and methanol before each sample was collected to prevent cross-contamination between samples. Tissue and sediment samples were stored in certified organics-free (I-CHEM[®] brand) jars. Samples were frozen in the field as soon as possible after collection and shipped on wet or dry ice via overnight service to the USGS NWQL in Denver, CO.

2.2. Laboratory analysis of fish tissue and sediment

Samples received at the NWQL were frozen at -20 °C and thawed just prior to sample preparation. Tissue samples were homogenized using a blender specially fitted with glass, stainless steel, and Teflon[®] parts. A laboratory reagent/sand blank and a spiked sample were prepared with each set (up to 10 environmental samples), and 50 µL of surrogate solution was added to each sample. Spiked samples were fortified with 100 µL of solution containing all method compounds at 2 ng/g. The compounds of interest were extracted from 0.5 to 1.5 g of homogenized tissue sample (wet weight), or 5–10 g of homogenized sediment sample (wet weight) using a pressurized liquid extraction (PLE) system (Dionex ASE TM 200, Sunnyvale, Calif., USA).

The tissue sample extracts were prepared by the procedure described in Nilsen et al. (2014b); PLE settings, extract cleanup procedures, surrogate spiking solution, and internal standard solutions are documented therein. For sediment samples, two analyses were performed. First, sample extracts were prepared as described by Nilsen et al. (2014b) and halogenated compounds were separated by capillary column gas chromatography (GC) and detected by negative ion mass spectrometry (MS), with ammonia as the reaction gas, using selected ion monitoring (Agilent Technologies, Model 5975 GC/MS). Then, a separate extraction was performed on all samples for analysis of 62 anthropogenic waste indicator (AWI) compounds; these extracts were prepared and analyzed by positive ESI gas chromatograph-mass spectrometry (GC-MS) using methods previously described (Burkhardt et al., 2005, 2006). Sample results are reported in nanogram per gram for tissues on a wet weight basis and for sediments on a dry weight basis. The qualitative identification of compounds detected by the mass spectrometer can be verified, although not necessarily reliably quantified, at concentrations less than the method quantitation limit. Any such detection is reported with an 'E' qualifier code (Appendices B2 and B3). Lipid content was determined by ASE extraction as described in Appendix A.

Compounds analyzed in both tissues and sediments include 62 chemicals grouped into the following classes: halogenated flame retardants (HFRs), polychlorinated biphenyls (PCBs), currently used pesticides (CUPs), legacy pesticides (LPs), and industrial/personal care products (I/PCPs). For the sediments an additional 57 compounds including polycyclic aromatic hydrocarbons (PAHs) and a

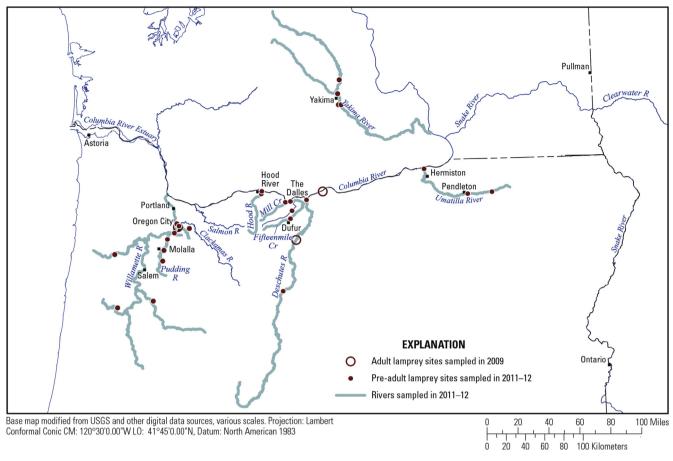


Fig. 1. Site map showing all sites sampled for larval lamprey and sediment in 2011–2012 and adult lamprey sampled as part of the most recent previous study (OHA EPH, 2012).

larger suite of I/PCPs were analyzed. This larger suite of I/PCPs was further classified into fragrances, plasticizers, surfactants, sterols, and I/PCP other. CUPs and I/PCPs are considered to be contaminants of emerging concern (CECs). Appendices B2 and B3 include a full list of compounds and classifications.

2.3. Quality assurance/quality control

The analytical method and results for the environmental samples were validated against a comprehensive set of performancebased quality control parameters including laboratory blanks and spikes, matrix spikes, replicate samples, and surrogate recoveries. Laboratory blanks for both tissue runs (N = 4) and sediment runs (N = 4) consisted of reagent grade sand carried through the extraction, cleanup, and analysis steps. Recoveries for laboratory reagent spikes for tissues (N = 4) and sediments (N = 2) are reported in Appendix B4. For the blanks and spikes, the mean recoveries of the surrogates dibromooctafluorobiphenyl, DDT- d_8 , and PCB 202-¹³C₁₂ were 90%, 116%, and 80% for tissues and 74%, 118%, and 74% for bed sediment, respectively. Thirteen percent of tissue samples and 16% of sediment samples were collected and analyzed in replicate. Relative standard deviations (RSDs) for the major compound classes in the replicate samples were determined for detections at or above the method reporting limits for both matrices. Tissue replicate samples had RSDs of 18.5% for pesticides, and 21.7% for PBDEs. PCB detections were few, and all were well below the method quantification limits and thus highly variable. Sediment replicate samples had RSDs of 14.2% for pesticides. There were few detections of PBDEs and PCBs in sediments, and all were well below quantification limits and thus highly variable. Refer to Nilsen et al. (2014b) for assessment of method performance for detections at or near reporting limits for these compound classes.

Equivalent blanks, matrix spikes, and all other laboratory QC procedures were followed in both years. Information on blank detections is reported in Appendix A. Method detection limits (MDLs) and method reporting limits (MRLs) are reported in Appendix B5.

3. Results

3.1. Site comparisons of contaminants

3.1.1. Sediments

The most prevalent contaminant classes detected in sediments were PAHs and industrial/personal care products (I/PCP). Different spatial patterns were observed for different classes of contaminants detected in surface sediments. For example, PAHs and surfactant compounds were relatively high at only a few sites, whereas fragrance compounds were more widely distributed (Fig. 2, top panel). Legacy pesticides were detected at the majority of the sites (Appendix B2), but currently used pesticides were generally found at higher concentrations than legacy pesticides (Fig. 2, bottom panel). Legacy and currently used pesticides did not always cooccur. Mill Creek and Roza Canal generally had the highest concentrations (Appendix B). Mill Creek and the Yakima River Basin sites had the highest number of compounds detected in sediments. An unidentified source of domestic wastewater to Mill Creek was recently implicated in higher than average counts of the fecal bacteria indicator Escherichia coli (Hanson, 2013) and may account

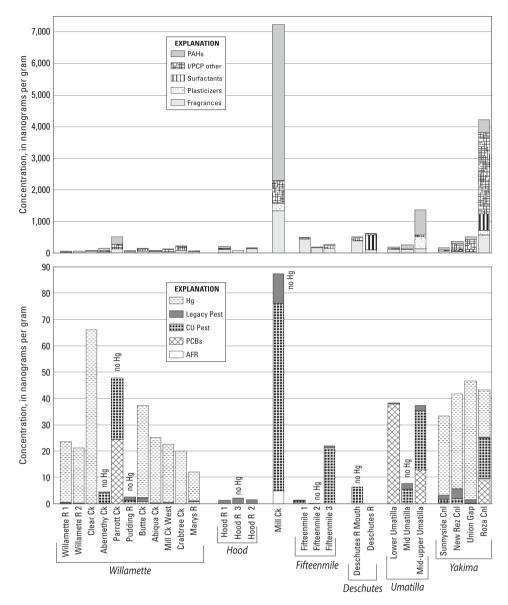


Fig. 2. Summed concentrations of compound categories detected in surface sediments displayed in 2 panels for ease of viewing from downstream (left) to upstream (right). Top panel: polycyclic aromatic hydrocarbons (PAHs), surfactant compounds, plasticizer compounds, fragrance compounds, and other industrial or personal care product compounds (l/ PCP other). Bottom panel: mercury (Hg), legacy pesticides, current use pesticides (CU Pesticides), polychlorinated biphenyls (PCBs), and alternative flame retardants (AFR) in bed sediments of the Columbia River Basin. Subbasins are labeled in italic text. The text 'no Hg' indicates the 2011 samples for which there is no Hg data.

for the relatively high concentrations of some I/PCP contaminants at the site, although probably does not explain the elevated pesticides and PAHs. Higher concentrations of pesticides than flame retardants were detected at most sites where both were present, except at Parrot Creek and Lower Umatilla.

The most widespread halogenated compounds detected in sediments were DDE, several HFRs, and several other organochlorine pesticides. Alternative flame retardants (AFR), which are increasingly used instead of halogenated flame retardants (HFRs), were detected at higher concentrations than HFRs. The currently used pesticide chlorpyrifos was also frequently detected. The sum of pesticides in sediments did not necessarily increase along a downstream gradient. More CEC compounds than halogenated compounds were detected in sediments, except at Mill Creek. Concentrations were comparable to those from recent sediment studies in the Columbia River Basin for compound classes that were common between studies (Counihan et al., 2014; Nilsen et al., 2014a), although the sampling sites were not identical. Mercury was analyzed only in the samples collected in 2012 (Yakima River Basin sites and 8 of the 11 Willamette River Basin sites). Where analyzed, mercury concentrations were higher than those of pesticides, PCBs, and flame retardants (Fig. 2, lower panel). Many of the most frequently detected contaminants in sediments were not measured in tissues because there were not suitable analytical methods. However, they were measured in sediments in order to guide selection of contaminants to be analyzed in tissues in future studies.

3.1.2. Tissues

In larval lamprey tissues, pesticides accounted for a greater proportion of total contaminants than did HFRs (Fig. 3). Legacy pesticides, particularly DDE, accounted for much of the total pesticide concentration in tissues at most sites. Currently used pesticides generally accounted for a lower proportion of pesticide

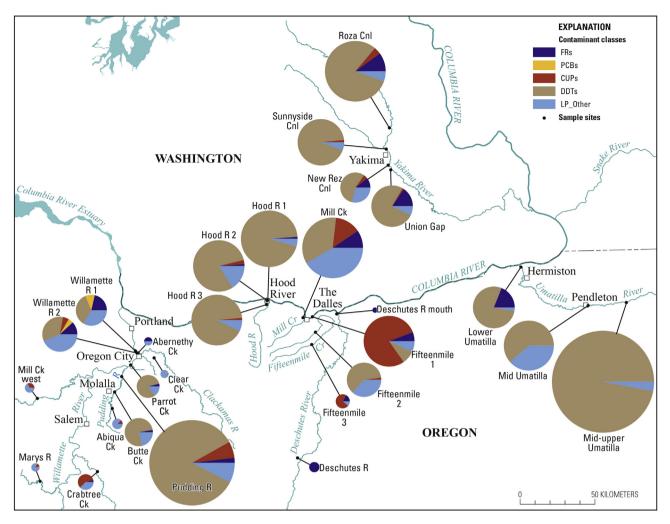


Fig. 3. Map of the study area with pie charts for presence and relative proportions of compound classes detected in larval lamprey tissues. Larger pie charts indicate higher concentrations. Contaminant classes include flame retardants (FR), polychlorinated phenyls (PCBs), currently used pesticides (CUPs), dichlorodiphenyltrichloroethane and its degradates (DDTs), and other legacy pesticides (LP_Other).

concentrations in larval tissues than legacy pesticides. The most notable exception was at Fifteenmile Creek Site 1, where much of the high pesticide concentration detected in larval lamprey was from oxyfluorfen, the active ingredient of the herbicide Goal®, which was spilled near the site in 2000. Of the currently used pesticides, oxyfluorfen and chlorpyrifos were detected the most frequently and at the highest concentrations (Appendix B2). Oxyfluorfen was detected in tissues from the mid-Columbia subbasins and Pudding River, while chlorpyrifos was detected most frequently in the Willamette and Yakima River Basins. Compared to other subbasins, the Deschutes had relatively low concentrations of pesticides in larval tissues. Pesticide concentrations in tissues were also relatively low at the Willamette River Basin sites, except at the Pudding River and at the two lower Willamette River sites. Many of the PCB congeners were also detected in larval tissues at the two lower Willamette sites.

HFRs were detected in tissues at most sites (Appendix B2). BDE 47 and BDE 99 were detected at the highest concentrations and BDE 100 was also frequently detected, but at lower concentrations. HFRs in tissues were generally highest at sites downstream in subbasins or sites with larger drainage areas. PCBs were rarely detected in larval lamprey, except at the two lower Willamette River sites. Concentrations of DDTs, HFRs and PCBs in adult lamprey tissues were slightly lower than those recently measured in resident

largescale sucker (*Catostomus macrocheilus*), while concentrations of PCBs in larval lamprey tissues were substantially lower than those recently measured in largescale suckers in the lower Columbia River (Nilsen et al., 2014b). It is likely that PCBs are bioaccumulated through the parasitic diet of mature lampreys. However, since PCBs were present in larvae at two sites, it is also possible that if PCBs were present at higher concentrations in sediments, they would also bioaccumulate in larvae. Mercury is not shown in Fig. 3 since it was analyzed only in the samples collected in 2012, but, where analyzed, mercury concentrations were higher than those of the other compounds (Appendix B2). Mercury was highest in larvae from Yakima and Willamette Rivers, DDTs were highest in larvae from Umatilla and Hood Rivers, and other legacy pesticides were highest in larvae from Mill Creek.

4. Discussion

4.1. Sediment-tissue contaminant comparisons

Halogenated contaminants and some currently used pesticides were analyzed in both tissues and sediments. Concentrations of several flame retardants and pesticides were several hundred times higher in larval tissues than in the paired sediment samples (Fig. 4). Because it is unknown how long larvae stay in specific stream

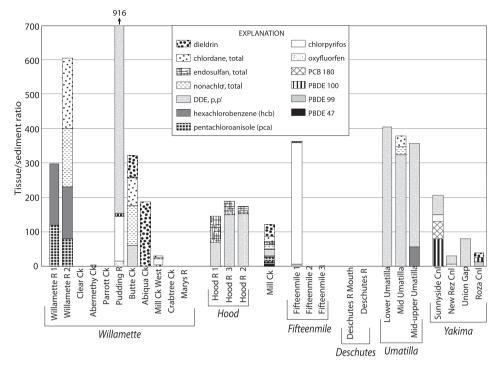


Fig. 4. Sediment-tissue comparisons of individual compounds detected in both media for larval tissues and associated sediment substrate. DDE = dichlorodiphenyldichloroethylene; PCB = polychlorinated biphenyl; PBDE = polybrominated diphenyl ether.

locations before moving downstream, it may not be possible to directly compare tissue and sediment contaminant levels at a particular site. However, the protracted, sedentary life of lamprey larvae in the benthos and their relatively high lipid content predispose them to contaminant exposure and uptake, which can occur via interstitial porewater, surface water, suspended sediments, and the consumption of contaminated sediment and organic matter. Larvae can be exposed to toxics through dermal, ingestion, and/or gill uptake pathways, and the concentration patterns in the two media may indicate bioaccumulation of pesticides, flame retardants, and mercury at certain sites; alternatively, larval lamprey may have bioaccumulated the contaminants at upstream locations before migrating downstream.

Contaminants detected most frequently and at the highest concentrations in both media were: the legacy pesticides DDT, chlordane, endosulfan, dieldrin, hexachlorobenzene, and/or their degradation products: the currently used pesticides chlorpyrifos and oxyfluorfen; HFRs including BDE 47, 99, and 100 (Fig. 4); and mercury. Many of the same contaminants were also detected most frequently and at the highest concentrations in adult lamprey tissues from the Deschutes and Willamette River Basins and at the John Day Dam on the Columbia River. Despite having been banned for decades, several legacy contaminants, such as PCBs and chlorinated pesticides, including DDT, are still present in the Columbia River Basin (U.S. EPA, 2009; Nilsen et al., 2014b). However, while legacy pesticides are clearly bioaccumulating in larval Pacific lamprey in the basin, PCBs were only detected at the two Willamette River sites and, to a lesser extent, at Roza Canal in the Yakima River Basin. PCBs were detected even less frequently in sediments. Currently used pesticides are widely applied for agriculture in the region, HFRs reach the Columbia River through municipal wastewater (Schreder and La Guardia, 2014), and mercury has natural and anthropogenic sources to the basin (U.S. EPA, 2009).

Concentrations of contaminants in tissues and sediments were compared to 2006 National Land Cover Data (Fry et al., 2011) to explore whether there were correlations between chemical patterns and upstream land use as have been found previously (Harding et al., 1998; Kratzer et al., 2006). However, it was determined that larger sample numbers and geographic extent may be necessary to discern meaningful correlations of contaminant patterns with sources based on upstream land use and land cover (Appendix A).

4.2. Concentrations of contaminants in larval Pacific lamprey compared to adults and to other species

Larval lamprey tissue data from this study were compared to contaminant data from adult Pacific lampreys collected at three sites in 2009 (OHA EPH, 2012). Although this is not an ideal comparison due to temporal, spatial, and sampling differences, little information on contaminants in Pacific lampreys exists and comparisons may provide some insight on contaminant uptake at different life stages. Only compounds that overlapped in the two studies were considered. All samples in both studies are composites of multiple individuals. In addition, multiple composited samples were averaged for each basin for simplicity. Mercury, DDTs, and other legacy pesticide concentrations in adults tended to be more consistent across sites than for larvae (Fig. 5). Some compounds had higher concentrations in larval lamprey and some in adults, depending on the basin. In the Willamette River Basin, mercury and DDT concentrations were higher in larvae, while PCB concentrations were higher in adults (Fig. 5). Lipid contents were 2-6x higher in adults. Lipid-normalized concentrations of all contaminants combined were highest in larval lampreys from the Willamette and Yakima River Basins, primarily due to higher mercury concentrations from those basins. In another study of adults collected at Willamette Falls in 2004, mercury and DDTs had similar concentrations to those measured in adults in 2009 (138 and 30.6 ng/g, respectively), while PCB concentrations were higher in the earlier study (44.8 ng/g) (ODHS, 2005). All PCB congeners were

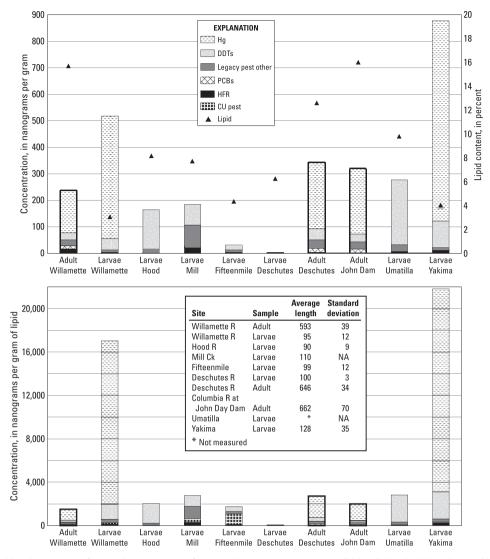


Fig. 5. Top panel: Adult and larval comparison of summed concentrations of compound categories: Mercury (Hg), dichlorodiphenyltrichloroethane and its degradates (DDTs), other legacy pesticides (Legacy pest other), polychlorinated biphenyls (PCBs), halogenated flame retardants (HFR), current use pesticides (CU pest) in lamprey tissues displayed from downstream (left) to upstream (right). Percent lipid content also shown. Bottom panel: Same categories shown as lipid-normalized concentrations. Average length (millimeters) and standard deviation of lamprey composited shown in inset. No length data was available for Umatilla samples. Larval data are from this study; adult data are from OHA EPH (2012). Only compounds that overlapped in the two studies are included: BDE congeners 47, 99, 100, 154, 66, 153, 85, 183, 138; PCB congeners 138, 118, 101, 18, 187, 149, 110, 146, 151, 183, 170, 177, 174, 194, 206; legacy pesticides hexachlorobenzene (hcb), oxychlordane, DDT, DDE, DDD, nonachlor, endosulfans, dieldrin; and mercury (Hg). Hg not measured for Hood, Mill, Fifteenmile, Deschutes, or Umatilla samples.

consistently detected in adults, whereas few PCBs were detected in larvae, except at the two most downstream Willamette River larval sites (out of 11 sites in the Willamette River Basin). Deschutes River Pacific lamprey larvae had consistently low concentrations of all contaminants.

Salmonids provide a useful comparison to Pacific lampreys since they overlap in range, share an anadromous life history (Hess et al., 2014), and juveniles have similar lipid contents (Johnson et al., 2007a; Arkoosh et al., 2010). Adult Pacific lamprey were shown to have the highest average concentrations of total pesticides, DDE, chlordanes, and mercury compared to other anadromous fish species, including salmonids and Eulachon (U.S. EPA, 2002). Adult Pacific lampreys had lower concentrations of legacy pesticides, PCBs, and other compounds than did resident species such as pike minnow, mountain whitefish, largescale sucker, and others (Johnson et al., 2007a; Nilsen et al., 2014b). However, persistent organic pollutants measured in larval Pacific lamprey during the current study were in some instances higher than thresholds determined for adverse health effects in juvenile salmonids (Johnson et al., 2007b). Higher contaminant levels in juvenile salmonids have been correlated to longer residence time in the Columbia River Estuary (Johnson et al., 2013), which is relevant to the longer residence time of larval Pacific lampreys in fresh water compared to salmonids.

Several compounds widely measured during this study have been shown to be harmful in early life stage development of other fish species. PAHs were a prevalent class of compounds detected in sediments across all sites, and are known to be toxic to the early life stages of fish. They specifically disrupt the normal function and morphogenesis of the fish heart (Brette et al., 2014; Incardona et al., 2014). The cellular targets for tricyclic PAHs in fish cardiomyocytes are conserved across species (Incardona et al., 2014) and therefore likely impact lamprey early life stages. Chlorpyrifos was also frequently detected during this study. Chlorpyrifos exposure has been shown to cause behavioral impairment in juvenile coho salmon at environmentally realistic concentrations (Sandahl et al.,

Table 1

Detected concentrations of select contaminants in adult lamprey tissue and Acceptable Tissue Levels for humans (tribal or subsistence consumers). Methyl mercury screening level guidance is from Oregon DEQ Table 40, Human Health Water Quality Criteria for Toxic Pollutants based on consumption of the organism only. Data from Oregon Department of Environmental Quality. [µg/Kg, micrograms per kilogram].

| Site | Detected concentrations (µg/Kg) | | | | | |
|---|---------------------------------|------------------|----------|------------------|----------------------|-----------------|
| | Chlordane ^a | ΣDDT^{b} | Dieldrin | HCB ^c | PCB ^d 118 | Mercury (total) |
| Willamette R. at Willamette Falls | 11.2 | 22.0 | 2.54 | 6.46 | 1.69 | 60 |
| Willamette R. at Willamette Falls | 11.9 | 33.3 | 2.99 | 8.03 | 3.32 | 260 |
| Willamette R. at Willamette Falls | 7.35 | 24.1 | 2.68 | 6.36 | 1.56 | 160 |
| Deschutes R. at Sherars Falls Fish Ladder | 13.2 | 42.4 | 2.62 | 6.53 | 3.24 | 250 |
| Columbia R. at John Day Dam | 10.7 | 33.3 | 2.9 | 6.82 | 2.45 | 450 |
| Columbia R. at John Day Dam | 11.0 | 25.5 | 3 | 7.08 | 2.43 | 130 |
| Columbia R. at John Day Dam | 11.2 | 30.3 | 2.99 | 6.92 | 2.59 | 160 |
| Acceptable Tissue Level, carcinogen (μ g/Kg, wet weight) | 3.3 | 3.4 | 0.072 | 0.72 | 0.25 | 40 ^e |

^a Chlordane includes cis-chlordane, cis- and trans-nonachlor, and oxychlordane; no data for trans-chlordane.

^b ΣDDT includes DDT, p,p'; DDE, p, p'; and DDD, p,p'.

^c Hexachlorobenzene.

^d Polychlorinated biphenyl.

^e Water Quality Criteria for mercury is for methylmercury.

2005), and chlorpyrifos interacts with other organophosphate insecticides in mixtures to produce synergistic toxicity (Laetz et al., 2009). PBDEs have also been shown to cause increased susceptibility of juvenile salmonids to infectious diseases with dietary exposures at concentrations consistent with those found in the Willamette River (Arkoosh et al., 2010). Dietary exposure to mercury causes negative effects on growth and reproduction in multiple fish species at relevant concentrations (Depew et al., 2012). In short, although contaminant studies have been lacking for Pacific lampreys, other anadromous species in overlapping or similar habitats provide information about what the exposures observed in the current study may mean for Pacific lamprey health.

Adult Pacific lamprey lipid content ranged from 12 to 16% (OHA EPH, 2012). For comparison, Hamilton et al. (2005) found a wider range of lipid contents (4-20%) in adult salmon of various species including both farmed and wild fish. Wild Pacific salmon averaged just 6.4% lipid (Hamilton et al., 2005). In a previous study, adult lampreys had the highest lipid content of the anadromous fish species sampled (U.S. EPA, 2002). Lipid contents in larvae measured in this study were lower and more variable than adults and ranged from 3 to 10%. Lipid content is an important factor in helping to understand observed contaminant patterns. Many of the persistent organic pollutants analyzed are lipophilic and therefore will accumulate to a greater degree in organisms with higher lipid content. The more variable lipid contents observed in larvae may partially explain the more variable contaminant concentrations also observed. However, sources of contaminants to the different basins are also important, reflected in the fact that the highest contaminant concentrations were not always associated with the highest lipid contents (Fig. 5).

4.3. Organism and human health implications

4.3.1. Contaminant effects on lampreys

Very little is known about contaminant uptake by and effects on lamprey. But the few existing data suggest that contaminants are a threat to lamprey. Bettaso and Goodman (2010) found that lamprey larvae bioaccumulate total- and methylmercury more efficiently than do pearl mussels in the Trinity River, CA. Pacific lamprey larvae were found to be relatively sensitive to pentachlorophenol compared to other aquatic species (Andersen et al., 2010). In the Klamath River Basin, toxics were identified as a limiting factor for Pacific lamprey populations and a potential threat to the humans who consume them (Petersen Lewis, 2009).

With regard to other lamprey species, the hatchability of

European river lamprey (Lampetra fluviatilis) roe and survival of larvae decreased with increasing metal contamination in a Finnish river (Myllynen et al., 1997). In another study, PCBs and polychlorinated diphenyl ethers (PCDEs) were shown to bioaccumulate in river lamprey in Finland (Soimasuo et al., 2004). Soimasuo et al. (2004) noted that the potential for developmental damages to lampreys from contamination of persistent organic pollutants is of concern, but had rarely been studied. A decade later, there is still little data on contaminant levels and effects on lamprev species. especially the Pacific lamprey. More recently, Tapie et al. (2011) found PCBs and PBDEs at levels of concern in several species of European lampreys in the Gironde estuary, France. The current study is the first to analyze a large suite of contaminants in larval Pacific lamprey, to compare these results to the limited adult data available, and to consider the role of contaminants in observed Pacific lamprey declines.

4.3.2. Concentrations detected in this study compared to human health guidance levels

Screening values for human consumption were calculated for each contaminant using the most current oral reference dose, which is an estimate of a daily chemical dose to the human population below which non-cancer health effects are not expected (OHA EPH, 2012). None of the chemicals tested in the study exceeded the calculated screening values for human consumption. Mercury and PCBs were the two contaminants of greatest concern for human health, but concentrations indicated a safe consumption rate for adult lampreys of four meals per month (OHA EPH, 2012). Considering recommended fish consumption rates for recreational fishers, no fish advisory was issued based on these data. Concentrations did exceed the strictest human consumption guidance for subsistence fisher populations (Table 1) and mercury risk-based action levels based on tribal fish ingestion rates (Harper and Harris, 2008), indicating that these levels may be detrimental to humans who consume substantial quantities of adult lamprey tissue on a regular basis (i.e., more than four meals per month).

Based on the most prevalent contaminants in larval and adult tissues and other risk factors, such as bioaccumulative potential and endocrine disruption, the following contaminants may be of special concern with respect to Pacific lampreys in the Columbia River Basin: DDT and its degradates, DDE and DDD; chlordane and its degradate, nonachlor; hexachlorobenzene (HCB); dieldrin; chlorpyrifos; endosulfan; PAHs; Hg; and BDE 47 and 99 (Appendix B6).

5. Conclusions and implications

This study constitutes the largest dataset of contaminants in habitats and tissues of Pacific lampreys in North America and provides new insights about a multistressor risk to this imperiled species. This is the first study to compare contaminant bioburden during different life stages of Pacific lampreys and to establish that bioaccumulation of some contaminants, such as pesticides and flame retardants, occurs at the larval stage, while others such as PCBs may be taken up primarily during the anadromous, adult portion of the life cycle. This study identified the individual compounds detected most frequently and at the highest concentrations in both sediments (flame retardants, PAHs, pesticides, Hg, and CECs) and tissues (pesticides, flame retardants, and Hg) at sites across a large geographic area including multiple subbasins and diverse land use. The observed higher concentrations in tissues compared to sediments likely indicate bioaccumulation of pesticides (chlorpyrifos, oxyfluorfen, DDT, chlordane, endosulfan, dieldrin, hexachlorobenzene, and/or their degradation products), flame retardants (BDE 47, 99, and 100), and mercury at many sites. Contaminant concentrations and lipid contents in larval Pacific lampreys tended to be more variable among sites than previous data from adult Pacific lampreys. Based on studies showing effects on other species, contaminants are accumulating in larval Pacific lampreys at levels that are likely to be detrimental to organism health and this contamination could be contributing to the unexplained decline of the species. However, additional sampling is needed across life histories and life stages, perhaps including controlled experiments, to better refine where and at what rate certain contaminants are taken up, and what risks they pose to these organisms.

Of the 34 lamprey species in the Northern Hemisphere, 10 are endangered, 9 are vulnerable, and one is extinct (Renaud, 1997; Moser et al., 2014). The documented detrimental effects of contaminants on lamprey in other parts of the world, effects of contamination on anadromous salmonid health in the Pacific Northwest, along with the concentrations of concern measured in this study, suggest that water quality and contamination could be playing an important role in the observed decline of Pacific lampreys in the Columbia River Basin. Further study of threats to this iconic Pacific Northwest species, including habitat contamination, is warranted.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.envpol.2015.03.003.

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