Organochlorine and Metal Contaminants in the Blood Plasma of Green Sturgeon Caught in Washington Coastal Estuaries

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Abstract
Non-invasive monitoring was used to evaluate the concentrations of 40 contaminants in the blood plasma of the North American Green Sturgeon Acipenser medirostris caught and released from three estuaries in Washington State. The highest contaminant concentrations were found in fish caught in the most urbanized sites. Few statistical differences were found when evaluating contaminant levels according to sex, maturation stage, or distinct population segments of Green Sturgeon. The results indicate that recent exposure to legacy contaminants was reflected in Green Sturgeon plasma. Aldrin, 4,4-DDE, α-HCH, copper, and selenium were the most frequently detected contaminants. This study also explored the challenges of assessing toxicity in threatened species using non-lethal approaches. There is currently a lack of environmental contaminant monitoring data in estuaries frequented by Green Sturgeon and limited plasma to tissue toxicity correlations.

Green Sturgeon are a long-lived, anadromous species, endemic along the west coast of North America. The species consists of two distinct population segments (DPSs) that are genetically differentiated and are defined by the river locations during spawning. The Southern DPS (sDPS), listed as threatened under the US Endangered Species Act (ESA; 71 FR 17,757, April 7, 2006) and the Northern DPS (nDPS), identified as a species of concern (Doukakis 2014). The sDPS includes fish that spawn in the Sacramento River, and the nDPS includes those spawning annually in the Rogue, Klamath, and Eel rivers, and those spawning inconsistently in the Umpqua and Columbia rivers (Farr and Kern 2005; Adams et al. 2007; Webb and Erickson 2007; Schreier et al. 2016; Stillwater Sciences, Wiyot Tribe Natural Resources Department 2017; Schreier and Stevens 2020). Green Sturgeon have an extended range off of the North American coast where adults and sub-adults make long-distance annual migrations (Lindley et al. 2008, 2011; Moser et al. 2016). During the summer, Green Sturgeon will aggregate in estuaries in search of food (Moser and Lindley 2007), particularly the Columbia River estuary, Willapa Bay, and Grays Harbor (Adams et al. 2002; Lindley et al. 2011). These feeding areas have significant anthropogenic activities, potentially exposing sturgeon to a variety of stressors, including fisheries bycatch, increased water temperature, and contaminants (Israel et al. 2009). Gut content studies show that adult and sub-adult Green Sturgeon feed on a variety of benthic fishes, crustaceans, and bivalves while in these coastal estuaries (Dumbauld et al. 2008). In the Columbia River, Green Sturgeon congregate in the turbidity maximum to feed on these organisms (Langness et al. 2014). In Willapa Bay and Grays Harbor, thousands of pits per hectare indicate intensive sturgeon feeding over unvegetated littoral mud flats during high tide (Moser et al. 2017). This disturbance of the substrate while feeding increases their exposure to sediment-associated contaminants.

One specific threat identified in the sDPS Green Sturgeon Recovery Plan (2017) is the possible exposure to contaminants and the resulting impacts on survival and reproduction. Contaminants may stem from agriculture runoff, urban development, discharge from industries, mining, land-use practices, and the application of chemicals.
and pesticides to control burrowing shrimp populations (Moser et al. 2016). While foraging in estuaries, Green Sturgeon may accumulate contaminants through prey, sediment contact, or direct absorption from the water. Green Sturgeon blood plasma taken from twenty-one fish caught in the Columbia River estuary, Willapa Bay and Grays Harbor during 2003–2005, was previously examined for the presence of 17 organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs). Low OC concentrations and no PCB detections were recorded. This preliminary work done by Gundersen (Langness 2007) had insufficient samples to determine statistically significant differences between sex, size, or DPS categories. Previous studies have shown contaminants to have deleterious impacts on sturgeon health. Studies looking at White Sturgeon (Acipenser transmontanus), collected from the same areas occupied by Green Sturgeon, have shown a variety of contaminants in tissues at levels that correlated with negative health effects. Levels of selenium, mercury, cadmium, arsenic, and copper exceeded levels shown to impair health in other fish species (Gundersen et al. 2017). Dichlorodiphenyldichloroethylene (p,p′-DDE), PCBs and methylmercury were frequently detected in White Sturgeon gonad and liver tissues collected from the Columbia River. Levels of these contaminants were negatively correlated with plasma testosterone and 11-ketotestosterone in male White Sturgeon (Feist et al. 2005). Contaminants have been detected in other fish species in estuaries occupied by Green Sturgeon. A study investigating contaminant levels in juvenile Chinook and Coho salmon collected from a variety of estuaries in the Pacific Northwest, found relatively high whole-body levels of PCBs and p,p′-DDE were found in fish collected from Grays Harbor and Willapa Bay (Johnson et al. 2007).

Evaluating toxicity from contaminant accumulation typically requires sacrificing the animals, measuring concentrations and biomarkers of effect in several tissues. However, non-lethal methods of contaminant monitoring have now been employed for many species of wildlife, including birds (Elliott and Shutt 1993; Dhananjayan and Muralidharan 2010), snakes (Bishop and Rouse 2000), turtles (Bucchia et al. 2015), and sturgeon (Gundersen et al. 2008; Jacobs et al. 2014). These previous studies demonstrated correlations between blood and tissue concentrations for lipophilic contaminants. Tissue and blood correlations were found between whole blood and fat from sea turtles (Keller et al. 2004) and between blood plasma, fat, and eggs from snapping turtles (Dabrowska et al. 2006) for chlorinated lipophilic contaminants. A study on White Sturgeon found correlations between plasma and liver OC concentrations (Gundersen et al. 2008) and plasma contaminant concentrations were used to evaluate endocrine disruption in a study on Lake Sturgeon Acipenser fulvescens (Jacobs et al. 2014).

The objectives of this work were to use non-lethal methods to evaluate the level of contaminant exposure and to assess whether contaminant burden differs in Green Sturgeon across various size, sex, and stage of maturity. Because sub-adult and adult Green Sturgeon of both DPSs aggregate within major coastal estuaries of Washington during the summer months (Moser and Lindley 2007; Moser et al. 2016), this study had a unique opportunity to evaluate contaminant exposure and compare contaminant burden between the ESA-listed sDPS and nDPS Green Sturgeon.

**Materials and Methods**

**Fish Sampling**

Green Sturgeon were captured by gill net in Grays Harbor, Willapa Bay, and the Columbia River estuary, Washington (Fig. 1), in June–September of 2011 and 2012 following the protocol in Langness et al. (2014). Before release, the fish were examined for injuries, infections, deformities, condition, and pre-existing internal and external tags, with fork length (FL) measured to the nearest cm. If fish were not already tagged, a passive integrated transponder (PIT) tag was applied in accordance with NMFS tagging protocols (Kahn and Morhead 2010). The fish used in this study had 69 kHz VEMCO-coded acoustic transponder tags interperionally implanted as well. An approximately 1 cm² section of pectoral fin was removed for genetic analysis and stored in 95% ethanol solution. Whole blood was drawn from the caudal vein using heparinized Vacutainers. About 1–6 mL of plasma was separated from the whole blood samples by centrifugation at 5000 RPM for 5 min. Aliquots of plasma were transferred to microcentrifuge tubes and stored frozen for sex steroid and chemical analyses.

**Genetics**

Pectoral fin clips, corresponding to the 131 blood plasma samples used for the contaminant analyses, were subsampled for genetic analysis. Genomic DNA was extracted from all samples with DNeasy 96 blood and tissue kits on a BioRobot 3000 (Qiagen, Inc., Valencia, CA, USA). Discrimination of the nDPS and sDPS was accomplished using genotypes from a set of 74 Single Nucleotide Polymorphism (SNP) markers developed for Green Sturgeon and a juvenile
reference dataset from samples of juveniles captured in the Klamath (nDPS) and Sacramento (sDPS) rivers (Anderson et al. 2017). Genotype data were generated using SNP Type allelic discrimination assays in 96.96 Dynamic Arrays on an EP1 system (Fluidigm Corporation, S. San Francisco, CA). Because Green Sturgeon are tetraploid (i.e., have four functional genome copies, not two like most animals), traditional genotype calling methods are not possible. Rather, we used the graphical genotype category and calibration approach of Anderson et al. (2017) to call genotype categories for each individual at each genetic marker. This approach has been shown to identify individuals to the two Green Sturgeon DPSs with 100% accuracy, even when data quality is poor (Anderson et al. 2017). This SNP assay approach also provide more accurate identifications than previously described microsatellite methods (Israel et al. 2009; Schreier et al. 2016).

Steroid Analysis

Steroids (testosterone [T] and estradiol-17β [E2]) were extracted from the plasma (n = 131) following the method of Fitzpatrick et al. (1987). Briefly, 100 µL of plasma was extracted twice with 2 mL of diethyl ether. Tubes were vortexed vigorously with ether, and the aqueous phase removed by snap-freezing in liquid nitrogen. Ether was allowed to evaporate overnight. The extracted steroids were suspended in 1 mL of phosphate-buffered saline with gelatin (PBSG), and 10 or 50 µL was assayed for each steroid. Recovery efficiencies for all steroids were determined by adding tritiated steroids to tubes containing plasma (n = 4), which was extracted as described above. Recovery efficiencies were 91–98% for testosterone (T) and 83–96% for estradiol-17β (E2). All steroid assay results were corrected for recovery.

Steroids (T and E2) were measured by radioimmunoassay (RIA) as described by Fitzpatrick et al. (1986) and modified by Feist et al. (1990). All samples were analyzed in duplicate. Steroid levels were validated by verifying serial dilutions were parallel to standard curves. A slightly more concentrated charcoal solution (6.25 g charcoal and 4.0 g dextran L⁻¹ PBSG) was used for all assays to reduce nonspecific binding. The intra- and inter-assay coefficients of variation for all assays were less than 5%. Quantified plasma sex steroid concentrations below the minimum quantifiable concentration (MQC) of the RIA were assigned the MQC (0.40 ng mL⁻¹ for T and 0.20 ng mL⁻¹ for E2).

Sex and stage of maturity were assigned according to plasma T and E2 concentrations as no gonadal tissue sampling was possible during 2011 and 2012. Sex and stage of maturity assignments based on plasma sex steroid concentrations correspond well with assignments based on histology of gonad tissue (Webb and Talbott 2007; Webb and Erickson 2007). Fish with plasma concentrations of 4–19 ng mL⁻¹ T and E2 concentrations less than 1 ng mL⁻¹ were assigned as non-reproductive males (pre-meiotic), while fish with less than 4 ng mL⁻¹ T and E2 concentrations less than 1 ng mL⁻¹ were assigned as non-reproductive females (pre-vitellogenic). Fish were assigned as spawning-capable males (meiotic) if plasma T concentrations were greater than 20 ng mL⁻¹ and E2 concentrations were less than 1 ng mL⁻¹ and vitellogenic or spawning-capable females if plasma T concentrations were greater than 10 ng mL⁻¹ and E2 concentrations were greater than 1 ng mL⁻¹. Females over 132 cm fork length and males over 109 cm fork length were considered as adults according to Nakamoto et al. (1995).

Contaminant Analysis

Plasma samples were stored at 0 °C until analysis. A total of 131 blood plasma samples were analyzed for metals, and 128 samples were analyzed for OCPs. A list of analytes included for chemical analysis can be found in Table S1.

For metals analysis, ultra-high purity (UHP) water (18 MΩ) from an Elga water purification system was used to prepare all standards and samples. A stock standard of 10 metals, plus an internal standard (yttrium) was prepared from individual metal standards (Sigma-Aldrich Trace-cert® > 99.999% purity) in 2% wt/wt nitric acid. Metals calibration solutions were prepared in 2% wt/wt UHP nitric acid in concentrations ranging from 0.1 to 5000 ppb with 0.2 ppm of the internal standard, yttrium. All metals standard solutions were stored in plastic containers at room temperature. For metals, 300 µL of equilibrated and vortexed plasma were digested at 60 °C for 1 h in 35% wt/wt UHP nitric acid. Samples were then spiked with the internal standard, diluted to 6.0 mL using UHP water, and filtered through a 0.45-micron PTFE syringe filter. An Agilent 5100 Inductively Coupled Plasma–Atomic Emission Spectrometer (ICP–AES) with a 94.74 lines/mm echelle grating and prism with Charged Coupled Device (CCD) detector was used for metals analysis.

All reagents were trace analytical grade including hexanes (Sigma-Aldrich GC residue analysis, > 98.5% purity), sodium sulfate (Sigma-Aldrich, > 99% purity), and nitric acid (Aristar Plus > 99.999% purity). A mix of 18 OCs (Accustandard Z-1014C, 2 mg mL⁻¹) and three mixes consisting of 16 polychlorinated biphenyls (Accustandard PCB C-SCA-01, -02, and -03) were combined into one analytical standard along with two surrogate recovery standards, decachlorobiphenyl and tetrachloroxylene (Restek 3253, 1000 ppm) for the preparation of an external calibration curve using authentic standards. Solutions for the external calibration curve were prepared in hexanes at concentrations ranging from 0.1 to 1000 ng mL⁻¹. All organic standard solutions were stored in glassware at 4 °C. For organic
analysis, 0.5 to 3 mL of plasma was spiked with recovery surrogate standards (50 μL of a 1000 ppm standard), added to 8.0 mL of hexanes, and placed on a tube rotator at 50 rpm for two hours. The organic layer was removed, dried with sodium sulfate, filtered through at 0.45-micron syringe filter, and concentrated using N₂ gas (Turbovap LV, Biotage) to a final volume of 0.50 mL. OCPs and PCBs were analyzed using a Shimadzu 2014 GC equipped with a chlorine-sensitive Electron Capture Detector (ECD) and a 30 m SPB 0608 column (0.25 mm, 0.25 μm). The GC used nitrogen for the makeup gas and helium at 1.5 mL/min as the carrier gas. The separation program was as follows: 150 °C, 4 min hold, ramp at 8 °C/min to 290 °C, followed by a 10 min hold.

**Quality Control for Contaminants Analysis**

Numerous procedures were used to limit laboratory contamination of the samples, including the use of high-quality standards and reagents. For metals, plastic ware was used when possible. Metals were identified using characteristic emission wavelengths and quantified using an internal standard method of calibration with 7-point calibration curves having \( R^2 > 0.995 \). Average analyte recoveries from the calibration verification standards were 98.6 +/- 0.6% (n = 2). Method detection limits (MDLs) were calculated from the standard deviations (SDs) at the 99% confidence level for \( n = 7 \) low concentration spikes at 15 ng mL\(^{-1}\). Individual MDLs can be found in Table S1. There was spectral interference for arsenic in method blanks (n = 4, Table S2). Arsenic was only reported in plasma samples where the concentration in the sample was 10 times the method blank. Positive controls included either a laboratory control sample (LCS) and matrix spike (MS). The LCS recoveries of metals (n = 2) averaged 103 +/- -3% (with SD) and 95.2 +/- -9%, n = 5, for the LCS controls. The relative percent difference (RPD) for copper and selenium were 4% and 10%, respectively. For OCs, baked glassware (450 °C for 4 h) was used to limit contamination. OCs were identified by retention times (RT) established with authentic standards. Analytes were quantified using surrogate-recoveries and 8-point calibration curves having \( R^2 > 0.995 \). MDLs were determined based on \( n = 7 \) low concentration spikes, Table S1. Average analyte recoveries from the calibration verification standards were 102 +/- -8% (n = 10) recoveries. Each batch (15 to 20 samples) consisted of at least two negative controls, matrix-matched method blanks and reagent blanks. OCs detected in method (n = 10) and reagent blanks (n = 12) were below method detection limits, with the exception of δ-HCH, which was detected at an average concentration of 2.8 ng mL\(^{-1}\) in method blanks. As such, all samples were method blank-subtracted to account for δ-HCH. Recoveries (with SD) from MS positive controls were between 101 +/- -18%, n = 8 for OCs. Duplicate plasma samples were included to assess precision, \( n = 5 \). The average RPD ranged from 4 to 38% for most individual analytes. However, because some OCs were detected at concentrations close to the MDLs, there were a few occurrences where an analyte was detected in one sample, but not the duplicate. Quality control sample data can be found in Tables S3 and S4.

**Statistical Analysis**

Concentrations less than the MDL for contaminants analysis were considered non-detections for totaling and summations. For other statistical purposes, a value of 0.5 MDL was substituted for non-detections on analytes with a detection frequency greater than 60%. In most cases, parametric statistical tests were used, despite normality test failures using the Shapiro–Wilk test, because of large sample sizes. Differences among groups were compared using Student’s t-tests and one-way Analysis of Variance (ANOVA). For t-tests comparing groups with fewer than 30 samples, nonparametric rank-sum methods using medians were applied. Correlations were tested using Pearson Product Moment Correlation. All statistics were performed using SigmaPlot 14.0.

**Results and Discussion**

**Contaminants in Green Sturgeon Plasma**

The sampled population in this study included northern and southern DPS fish from three estuaries, Table 1. Metals were detected in 100% of Green Sturgeon plasma samples (n = 131). Copper and selenium were the most abundant metals with detection frequencies of 97% and 52%, respectively. Average (with SD) copper plasma concentrations were 270 +/- -303 ng mL\(^{-1}\) (n = 126), while selenium concentrations were 227 +/- -57 ng mL\(^{-1}\) (n = 76). The highest copper concentration (3390 ng mL\(^{-1}\)) was found in plasma from a female nDPS fish from Willapa Bay. Other metals, lead and arsenic, were found in less than ten plasma samples, Table 2.

It was expected that copper and selenium would have the highest detection frequencies given that they are essential for biological function. However, the detections are significant since both metals may be harmful to sturgeon at certain concentrations (Vardy et al. 2015; Linares-Casenave et al. 2015). A similar non-invasive monitoring study on sea turtles reported comparable concentrations of copper (1020 +/- -99 ng mL\(^{-1}\)) and selenium (2447 +/- -631 ng mL\(^{-1}\)) in blood (Van De Merwe et al. 2010). This sea turtle study also observed correlations between tissue and blood concentrations for many metals, including selenium, but not copper. Plasma concentrations
of metals have not previously been reported in sturgeon, but other studies have reported copper and selenium accumulation in the liver and gonads (Gundersen et al. 2017). It remains unclear how plasma concentrations of metals might correlate to accumulated metal concentrations in tissue of sturgeon.

OCs were detected in 94% \((n = 120)\) of the 128 plasma samples analyzed. The most frequently detected OCs were (aldrin, α-HCH, γ-HCH, p,p′-DDE, and endrin), Table 3. Additional pesticides were detected in 25 or fewer samples and included heptachlor, δ-HCH, dieldrin, endosulfan II, endosulfan sulfate, and methoxychlor. The median OC concentrations were near the analytical MDLs; however, the concentrations were wide-ranging for several pesticides. The highest contaminant concentrations detected in the plasma were of endosulfan II from two fish, a sDPS male from the Columbia River and a sDPS female from Willapa Bay. A sDPS female fish caught in Grays Harbor had the highest p,p′-DDE concentrations. Total OCPs \((\Sigma_{16}\text{-OCPs})\) were low, averaging 5.5 ± 5.5 ng mL\(^{-1}\). The highest \(\Sigma_{16}\text{-Pest}\) concentration \((48.5\text{ ng mL}^{-1})\) was influenced by anomalous concentration of endosulfan II. PCBs were infrequently detected and found in 15 or less plasma samples, Table 3. Detected PCBs included PCB99, PCB153, PCB180, and PCB194. The OCs found in Green Sturgeon plasma were banned in most countries during the 1970-1980s after the Stockholm Convention; however, the environmental redistribution, prevalence, persistence, and bioaccumulation potential of these lipophilic chemicals have been well documented (Tanabe et al. 1994). Additionally, Green Sturgeon are long-lived fish that feed on sediment-dwelling benthic organisms and can potentially bioaccumulate OCs for decades.

Previous monitoring studies on other sturgeon species frequently detected OCs in the tissues of sacrificed animals (Foster et al. 2001; Feist et al. 2005; Hosseini et al. 2008; Jacobs et al. 2014; Gundersen et al. 2017), and at least two studies have reported OCs in the plasma of sturgeon (Gundersen et al. 2008; Jacobs et al. 2014). These studies reported that p,p′-DDE (the most stable degradation product of the pesticide p,p′-DDT) was the most prevalent and concentrated OC in sturgeon. Plasma concentrations of p,p′-DDE (non-detect to 10.6 ng mL\(^{-1}\)) from the Green Sturgeon were similar to what was reported in plasma for White Sturgeon from the Pacific Northwest, USA where

### Table 1 Characterization of sampled population of Green Sturgeon

| Location of catch and release, Washington State, USA | n = | DPS assignment |
|------------------------------------------------------|-----|---------------|
|                                                      |     | sDPS | nDPS |
| Columbia River (CR)                                  | 44  | 20   | 24   |
| Willapa Bay (WB)                                     | 43  | 17   | 26   |
| Gray’s Harbor (GH)                                   | 44  | 32   | 12   |
| Totals                                               | 131 | 69   | 62   |

| Sex   | n = | Life stage |
|-------|-----|------------|
|       |     | Sub-adult  |
|       |     | Adult      |
| Male  | 24  | 1          |
| Female| 98  | 66         |
| Not assigned\(^a\) | 9 | NA |

| Fork length (cm) | Avg | SD | Min | Max |
|------------------|-----|----|-----|-----|
| Male (n=24)      | 146 | 16 | 108 | 171 |
| Female (n=98)    | 122 | 24 | 84  | 199 |

\(^a\)Life stage is assigned according to gender and fork length

### Table 2 Frequency and range of detection for metals

**Summary of metals results**

| Analyte   | % Detection | Average (ppb) with SD, \(n = 131\) | Min–Max (ppb) |
|-----------|-------------|------------------------------------|---------------|
| Arsenic (As) | 1           | 509                                | ND-509        |
| Cadmium (Cd) | 0           | ND                                 | –             |
| Chromium (Cr) | 0           | ND                                 | –             |
| Copper (Cu)  | 97          | 270 (+/−) 303                      | ND-3387       |
| Nickel (Ni)  | 0           | ND                                 | –             |
| Lead (Pb)    | 5           | 168 (+/−) 45                       | ND-232        |
| Selenium (Se)| 52          | 227 (+/−) 57                       | ND-359        |
| Tin (Sn)     | 0           | ND                                 | –             |
| Titanium (Ti)| 0           | ND                                 | –             |
| Vanadium (V) | 0           | ND                                 | –             |

\(ND\) non-detection
Table 3 Frequency and range of detection for OCPs and PCBs

| Analyte          | Summary of organochlorine results | Min–Max (ppb) | % Detection | Average (ppb) with SD n=131 |
|------------------|-----------------------------------|---------------|-------------|-----------------------------|
| α-HCH            | 65                                | 2.27 (+/− 1.7) | ND-8.10     |                             |
| β-HCH            | 0                                 | ND            | −           |                             |
| δ-HCH            | 6                                 | 2.07 (+/− 1.9) | 6.5         |                             |
| γ-HCH            | 37                                | 1.03 (+/− 1.0) | ND-6.58     |                             |
| Heptachlor       | 16                                | 1.01 (+/− 0.8) | ND-3.42     |                             |
| Heptachlor epoxide | 0                               | ND            | −           |                             |
| Aldrin           | 73                                | 1.70 (+/− 0.9) | ND-5.89     |                             |
| Dieldrin         | 11                                | 0.31 (+/− 0.1) | ND-0.57     |                             |
| Endrin           | 37                                | 0.57 (+/− 0.3) | ND-1.39     |                             |
| Endrin ketone    | 0                                 | ND            | −           |                             |
| Endosulfan I     | 0                                 | ND            | −           |                             |
| Endosulfan II    | 2                                 | 24.77 (+/− 23.5) | ND-48.48 |                             |
| Endosulfan sulfate | 9                            | 0.74 (+/− 1.1) | ND-3.95     |                             |
| p,p’-DDD         | 0                                 | ND            | −           |                             |
| p,p’-DDE         | 69                                | 1.05 (+/− 1.4) | ND-10.56    |                             |
| Methoxychlor     | 5                                 | 3.18 (+/− 1.5) | ND-4.81     |                             |
| PCB77            | 0                                 | ND            | −           |                             |
| PCB87            | 0                                 | ND            | −           |                             |
| PCB99            | 12                                | 1.64 (+/− 0.9) | ND-3.50     |                             |
| PCB101           | 0                                 | ND            | −           |                             |
| PCB105           | 0                                 | ND            | −           |                             |
| PCB118           | 0                                 | ND            | −           |                             |
| PCB128           | 0                                 | ND            | −           |                             |
| PCB153           | 7                                 | 0.84 (+/− 0.4) | ND-1.64     |                             |
| PCB156           | 0                                 | ND            | −           |                             |
| PCB169           | 0                                 | ND            | −           |                             |
| PCB170           | 0                                 | ND            | −           |                             |
| PCB180           | 2                                 | 6.86 (+/− 4.0) | ND-9.70     |                             |
| PCB183           | 0                                 | ND            | −           |                             |
| PCB194           | 2                                 | 12.04 (+/− 9.3) | ND-22.5    |                             |

ND non-detection

The ΣDDTs (lipid normalized) reached 150 ng mL−1 (Gundersen et al. 2008). Lake sturgeon from the Midwestern USA had similar reported concentrations of p,p’-DDE, ranging from 7.20 to 47.82 ng mL−1 (Jacobs et al. 2014). It should be noted that typically ΣDDTs are reported; however, p,p’-DDT and p,p’-DDD were not detected in this study.

Contaminant Differences According to Sex and Maturity

A sex-specific trend was not observed for copper, selenium, the Σ16-OCPs, and most individual OCPs when all female and male fish were compared (n=122). Some earlier sturgeon studies reported males with higher contaminant concentrations of persistent, bioaccumulative contaminants than females. A possible explanation is that, at reproductive maturity, females are able to release contaminants in their eggs lowering their overall body burden (Foster et al. 2001; Gundersen et al. 2017). In this study, four female fish were considered capable of spawning, but contaminant concentrations were not different (p>0.05) than those of other female fish or the male fish, with one exception. Only α-HCH concentrations were significantly different (p=0.038) between male and female fish. Plasma concentrations of α-HCH for males were 2.47+/−2.1 ng mL−1 and female plasma concentrations were 1.48+/−1.4 ng mL−1. Additionally, differences were not observed between the sexes when contaminant concentrations were compared between male and female sub-adult and adult fish, Table 4. The absence of sex-specific differences may also be explained by a sampled population skewed by more sub-adult fish (Gundersen et al. 2017) or the larger number of female fish (n=98) compared to male fish (n=24) included in this study, Table 4. The influence of size according to fork length was also investigated between the sexes (see Table 1), but no trends were apparent. P-values for all sex differences can be found in Table S5.

Table 4 Comparison of blood plasma contaminant concentrations (ng mL−1) as mean+/−SD, (Min–Max)

| Contaminant | Sub-adult | Adult |
|------------|----------|-------|
|            | Male, n=1 | Female, n=66 | Male, n=23 | Female, n=32 |
| Cu         | 187      | 258+/−403, (ND-3387) | 310+/−151, (135−798) | 257+/−134, (ND-648) |
| Se         | 306      | 108+/−112, (ND-355) | 140+/−130, (ND-345) | 132+/−126, (ND-359) |
| α-HCH      | 3.52     | 1.22+/−1.1, (ND-5.21) | 2.42+/−2.1, (ND-8.10) | 2.06+/−1.9, (ND-7.78) |
| Aldrin     | 1.27     | 1.45+/−0.84, (ND-4.41) | 1.45+/−1.1, (ND-4.59) | 1.31+/−1.2, (ND-5.89) |
| p,p’-DDE   | 0.16     | 0.84+/−1.1, (ND-7.57) | 0.38+/−0.25, (ND-0.97) | 0.89+/−1.9, (ND-10.6) |
| Σ16-OCPs   | 14.2     | 5.41+/−6.1, (1.8−50.3) | 5.70+/−3.5, (1.81−16.8) | 6.80+/−5.4, (1.63−26.8) |
Contaminant Differences According to Site of Catch and Release

With few exceptions, contaminants were statistically different according to location, Table 5. For metals, there were site differences for selenium in the Green Sturgeon, but not for copper. Bucchia et al. (2015) observed something similar in loggerhead turtles. Site-specific concentration differences were not observed for most metals (Cu, As, Pb or Cd), but they did find higher concentrations of zinc and mercury in the loggerhead turtles from industrialized waters (Bucchia et al. 2015). They did not report data for selenium. It is known that some toxic metals like mercury bioaccumulate in wildlife (Van De Merwe et al. 2010), and previous studies have indicated that selenium can bioaccumulate in protein-rich sturgeon tissues (Schwarz et al. 2006; Gundersen et al. 2017). Plasma monitoring may be an indicator of recent exposure for bioaccumulative metals, but maybe not for other metals. With so few studies on the monitoring of metals in wildlife plasma, it remains difficult to interpret the inconsistent trends. To date, less research has been done on inorganic contaminants in wildlife plasma, and inorganics would not be expected to biomagnify like OCs and other persistent, bioaccumulative contaminants.

The greatest differences in the present study were for OCPs. For α-HCH, Green Sturgeon caught in Grays Harbor had different plasma concentrations (p < 0.001) than Green Sturgeon from the Columbia River and Willapa Bay. The average p,p’-DDE concentrations from the Columbia River plasma samples were the highest and different from Grays Harbor (p = 0.04) and Willapa Bay (p = 0.006). Aldrin, on the other hand, had similar concentrations as the Columbia River and Grays Harbor but were different than Willapa Bay (p < 0.04). The Σ16-OCPs concentrations followed the same pattern as aldrin and were not statistically different in fish caught in Grays Harbor and the Columbia River. The Σ16-OCPs for fish caught in Willapa Bay were statistically different (p < 0.02). The only contaminant that did not differ according to location was copper (Table 5). The most discernible observation was that fish caught in Willapa Bay had the lowest contaminant concentrations.

Table 5 Contaminant concentrations by location (ng mL⁻¹) as mean ± SD, (Min–Max)

| Contaminant | Columbia River (44) | Willapa Bay (n = 41) | Grays Harbor (n = 43) |
|------------|---------------------|----------------------|----------------------|
| Copper     | 217+/−98, (ND-451)  | 272+/−140, (ND-798) | 228+/−130, (ND-648) |
| Selenium   | 129b+/−119, (ND-344)| 80.33a+/−104, (ND-291)| 147a+/−127, (ND-359) |
| α-HCH      | 1.45+/−1.5, (ND-7.78)| 0.96a+/−0.7, (ND-3.31)| 2.5a+/−1.9, (ND-8.10) |
| p,p’-DDE   | 1.23+/−1.3, (ND-7.57)| 0.41b+/−0.3, (ND-1.15)| 0.63b+/−1.58, (ND-10.56) |
| Aldrin     | 1.62b+/−1.0, (ND-4.59)| 1.03b+/−0.6, (ND-2.49)| 1.50b+/−1.0, (ND-5.89) |
| Σ16-OCPs   | 6.67+/−7.3, (1.81–50.29)| 3.75b+/−4.0, (1.63–26.76)| 6.98+/−3.5, (1.81–16.50) |

Letters indicate a significant difference (p < 0.05) between contaminant concentrations across locations when letters differ as determined by ANOVA.
One possible explanation for contaminant differences between estuaries could be differences in DPS compositions. In a previous study, Schreier et al. (2016) found that sDPS Green Sturgeon dominate collections from the Columbia River estuary and Willapa Bay, and that nDPS Green Sturgeon are more common in Grays Harbor than in the other two estuaries, during 2010–2012. However, the plasma samples used in this study were selected to provide adequate and mostly equal samples of fish from both DPSs from each estuary, and across all locations.

**Fig. 2** Comparison of contaminant concentrations between northern and southern North American Green Sturgeon distinct population segments (DPSs) at each location, means with standard deviations for a Columbia River (CR) nDPS fish (n = 20) and sDPS fish (n = 24), b Willapa Bay (WB) nDPS fish (n = 17) and sDPS fish (n = 26), c Grays Harbor (GH) nDPS fish (n = 32) and sDPS fish (n = 12). Significant differences are indicated on the figure by inclusion of the p-value. Data tables and p-values can be found on Table S6 & S7

**Contaminant Differences Between Green Sturgeon DPSs**

A main objective of this study was to compare contaminant concentrations between distinct populations segments of Green Sturgeon, the nDPS (n = 69) and the ESA-listed sDPS (n = 62). For all contaminants, only copper plasma concentrations differed by DPS (p = 0.04). The sDPS fish had slightly higher concentrations of plasma copper (262 +/- 145 ng mL^-1) than nDPS fish (216 +/- 100 ng mL^-1). Comparisons were also made among females and males according to DPS. No significant
differences were found between nDPS male \((n = 7)\) and female \((n = 56)\) fish, but sDPS males \((n = 17)\) had higher concentrations of copper \((p = 0.001)\) and \(\alpha\)-HCH \((p = 0.028)\) compared to the female \((n = 42)\) fish (Table S5).

Because there were notable site-specific differences (Table 5), contaminant concentrations in nDPS versus sDPS were compared according to site, Fig. 2. The sDPS samples collected from Grays Harbor had higher copper concentrations \((p = 0.05)\) than nDPS samples from Grays Harbor. The \(\alpha\)-HCH concentrations from fish caught in the Columbia River \((p = 0.007)\) and Willapa Bay \((p = 0.019)\) were also higher in sDPS fish. It is noteworthy that the highest concentrations of most contaminants (\(\alpha\)- and \(\gamma\)-HCH, p,p\(^{′}\)-DDE, endosulfan II, endosulfan sulfate, PCB 180, PCB 194, PCB 194, Ar, Pb, and Se) were found in the ESA-listed sDPS fish. Accordingly, \(\Sigma_{16}\)-OCPs and \(\Sigma\)PCBs were also highest from sDPS fish. The only exceptions of an nDPS fish having higher individual contaminant concentrations were for Cu, heptachlor, aldrin, methoxychlor, and PCB99.

Although the distinct population segments intermix in the open ocean and during feeding in the non-natal estuaries, they spawn and spend the first few years in freshwater, regionally separated by their natal rivers and estuaries. Most Green Sturgeon spawn in southern Oregon to northern California or central California for the nDPS and sDPS, respectively (Adams et al. 2007). The observed differences in contaminants may reflect long-term accumulation, given that the sDPS fish spawn and rear in watersheds with more urbanization and agriculture. It is also possible that the reduced sample size after separating the samples by DPS and location reduced the power of the analysis. Age (using size as a proxy) may also be a confounding factor, as older individuals would have had a longer amount of time to bioaccumulate contaminants. Southern DPS fish were larger on average \((p < 0.001)\). The average fork lengths for the fish in this study were 138 and 108 cm for sDPS and nDPS, respectively. These DPS size averages and distributions closely matched those observed for all Green Sturgeon captured during the 2010–2012 tagging operations (Schreier et al. 2016). However, overall, the average contaminant concentrations were similar for the northern and southern distinct population segments.

### Implications for Toxicity

Monitoring contaminant concentrations using non-lethal samples, such as blood plasma, limits toxicity assessments. However, the OCs included in the present study are highly lipophilic, and the presence of these analytes in blood suggests accumulation in fatty tissues such as the liver or gonads. Studies across several species have correlated the bioaccumulation of OCPs and PCBs in the blood to concentrations of the contaminants in tissues, a matrix for which toxicity thresholds are typically based. Results from polar bears (Bernhoft et al. 1997), several turtle species (Keller et al. 2004; Dabrowska et al. 2006; Van De Merwe et al. 2010; Bucchia et al. 2015) and, most importantly, White Sturgeon (Gundersen et al. 2008) indicate that monitoring OCs in blood is a viable non-lethal way to assess contaminant concentrations in the tissues. Some researchers have also demonstrated linear relationships in sea turtles between blood plasma and tissue concentrations for several metals (Van De Merwe et al. 2010). On the other hand, there are limited studies on the extrapolation of blood concentration to tissue concentrations in sturgeon, and some contaminants may not adhere to linear relationships, as was the case for some toxic metals and OCs in green sea turtles (Van De Merwe et al. 2010). Nonetheless, previous studies do provide a means to estimate tissue concentrations and to convert tissue concentration to plasma concentration, allowing comparisons to traditional toxicity benchmarks.

The average Green Sturgeon plasma concentrations were less than the estimated thresholds shown in Table 6. The \(\Sigma_{16}\)-OCPs average \((n = 128)\) was 5.47 ng mL\(^{-1}\) with a standard deviation of 5.5 ng mL\(^{-1}\) and ranged from non-detect to 48.5 ng mL\(^{-1}\). Only two female fish (one sDPS fish from Willapa Bay and one nDPS fish from the Columbia River) exceeded the estimated threshold of 40.9 ng mL\(^{-1}\). The \(\Sigma\)DDTs (only p,p\(^{′}\)-DDE in this study) ranged from non-detect to 10.6 ng mL\(^{-1}\) and averaged 1.05 \((+/-)\) 1.4 ng mL\(^{-1}\) falling well below the threshold. OC contamination is typically associated with reproductive effects in wildlife, and the toxicity benchmarks for OCs refer to endocrine disruption from White Sturgeon (Feist et al. 2005). This data suggests that few Green Sturgeon had accumulated toxic levels of OCs.

### Table 6 Contaminant toxicity thresholds

| Analyte  | Estimated threshold plasma concentration (ppb\(^{a}\)) | Threshold tissue parameter | Threshold tissue concentration (ppm) | Reference                  |
|----------|--------------------------------------------------------|----------------------------|--------------------------------------|---------------------------|
| \(\Sigma_{16}\)-OCPs | 40.9 ng mL\(^{-1}\)                                      | Liver, White Sturgeon       | 5.6 μg g\(^{-1}\) lipid              | Feist et al. (2005)       |
| \(\Sigma\)DDTs | 48.9 ng mL\(^{-1}\)                                      | Liver, White Sturgeon       | 9.5 μg g\(^{-1}\) lipid              | Feist et al. (2005)       |

\(^{a}\)Tissue thresholds were converted to plasma concentrations using regression equations from White Sturgeon liver correlations (μg g\(^{-1}\) lipid) for OCPs according to Gundersen et al. (2017)

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Other contaminants are not as easily adapted to tissue threshold concentrations. For instance, the copper data is remarkable but also challenging to interpret. Copper concentrations had a sizable range—four orders of magnitude—in the Green Sturgeon plasma. Toxicity studies have shown that sturgeon are particularly sensitive species of fish to waterborne copper (Ivey et al. 2019), although it is unclear if copper in Green Sturgeon plasma is derived from dietary uptake or uptake across the gills. Average selenium concentrations exhibited a narrower concentration range in the blood plasma (non-detect to 359 ng mL\(^{-1}\)), but at environmentally relevant concentrations, selenium can be a concern for all life stages of sturgeon (Linares-Casenave et al. 2015). Toxic effects of selenium in Green Sturgeon have been shown to range from death to endocrine disruption and include teratogenic effects through maternal transfer to the eggs (Silvestre et al. 2010). It is currently unknown how Cu and Se concentrations in the blood relate to tissue concentrations or environmental exposure concentrations. Future studies should study selenium and copper bioaccumulation and the plasma/tissue relationships in order to better understand the range of concentrations observed in the Green Sturgeon plasma. Additionally, all contaminants that were included in this study (10 metals, 16 OCPs, and 14 PCBs) have known toxic effects, but given the absence of toxicity benchmarks and the challenges of converting plasma to tissue concentrations, further toxicity interpretations are not appropriate. Lastly, how other contaminants (not included in this study) and mixtures of contaminants may affect sturgeon health and survival requires further study.

### Conclusions

This is one of the first reports of contaminants in Green Sturgeon from the Pacific Northwestern United States. Green sturgeon are a benthic dwelling species that feed in estuaries, typically areas that accumulate contaminants in sediments. Non-lethal sampling provided an effective means to study contaminant exposure in the sturgeon. Parts per billion levels of OCPs, metals, and PCBs were found in the blood plasma of nDPS and ESA-listed sDPS Green Sturgeon caught in the Columbia River, Grays Harbor, and Willapa Bay. There were minimal differences between average contaminant concentrations for sDPS and nDPS Green Sturgeon or between the sexes. Feeding location may be a better indicator of contaminant concentrations. These data, in combination with tissue correlation studies, suggest that plasma could be used to assess tissue bioaccumulation for some contaminants, but maybe not for many metals, including copper. Few fish exceeded tissue-based toxicity thresholds; however, comparisons were made after correlating plasma to tissue concentrations. Chemical contamination as a contributing reason for population decline and a hinderance for recovery requires further investigation, including information on other contaminants or genetic differences in the susceptibility of contamination, and the relationship between contaminant levels and disease pathology. Further work should address data gaps in the understanding of contaminant redistribution between blood and tissues for OCs and metals; and, plasma/tissue correlations. In order to strengthen the weight of evidence for toxicity assessments using non-lethal monitoring, future studies could also report plasma toxicity threshold values or provide plasma to tissue correlations. In addition, larger volumes of plasma could be collected or pooled to allow for calculations of plasma percent lipid content.

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**Declarations**

**Conflict of interest** The authors declare that they have no conflicts of interest associated with the study reported in this manuscript.

**Ethics approval** In addition to the federal 4(d) permit process for handling an ESA-listed species, the Washington Department of Fish and Wildlife has the authority to do and to permit research activities on fish and wildlife within the state in compliance with state law.

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