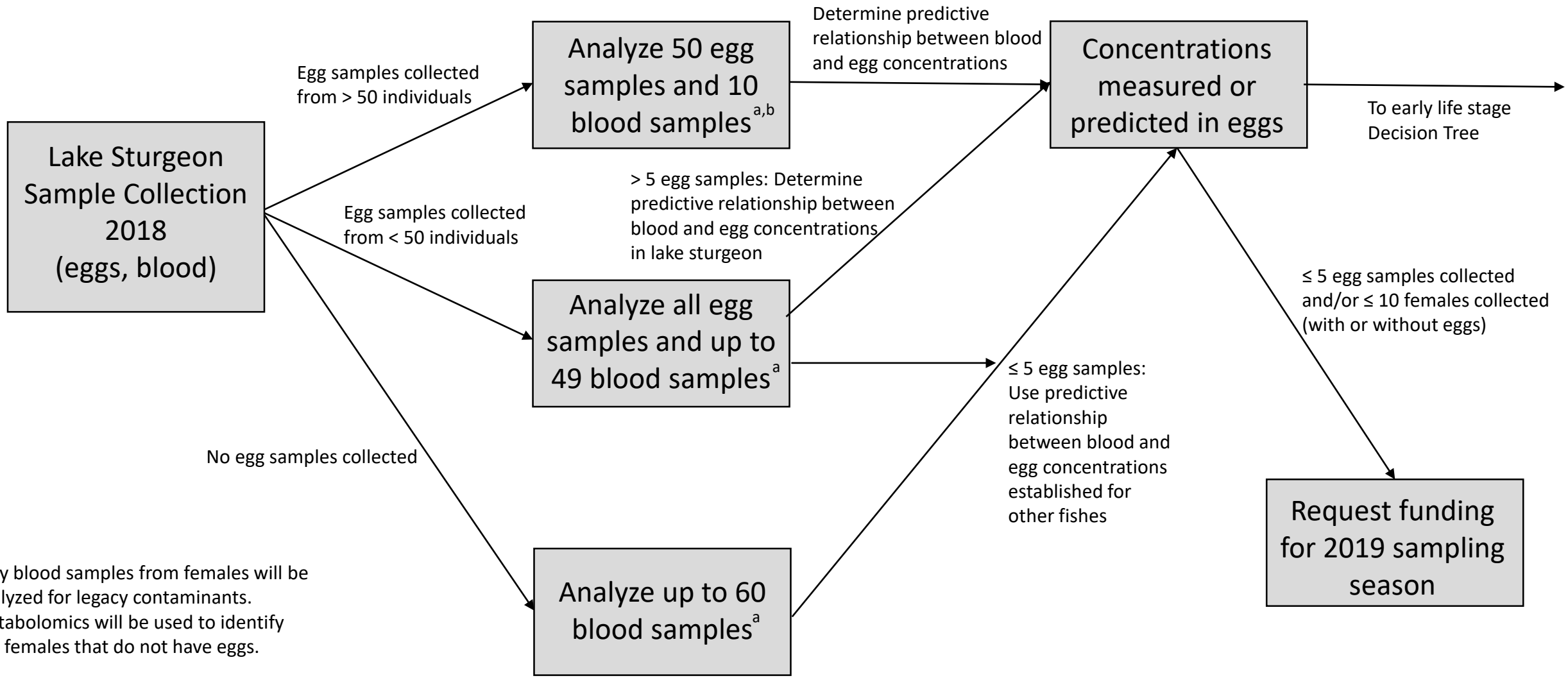
Appendix D

Lake Sturgeon Study Decision Tree

and Final Report

Decision Tree for Sample Selection for Chemical Analysis



[a] Only blood samples from females will be analyzed for legacy contaminants. Metabolomics will be used to identify any females that do not have eggs.

[b] Blood samples will be selected from individuals with a range of egg concentrations (low, medium, high), if a range in egg concentrations is present.

Decision Tree for Assessment of Early Life Stages

Request funding for 2019 sampling season

> 10-fold range in total toxicity of legacy contaminants among individuals

Proceed: Resource management action to be considered

Mortality predicted by model for ≥ 1 individual (Figure 1)

Proceed: Develop Sturgeon-specific Total Toxicity[b]

Stop: Exposure unlikely to cause elevated mortality[c]

No mortality predicted by model (Figure 1)

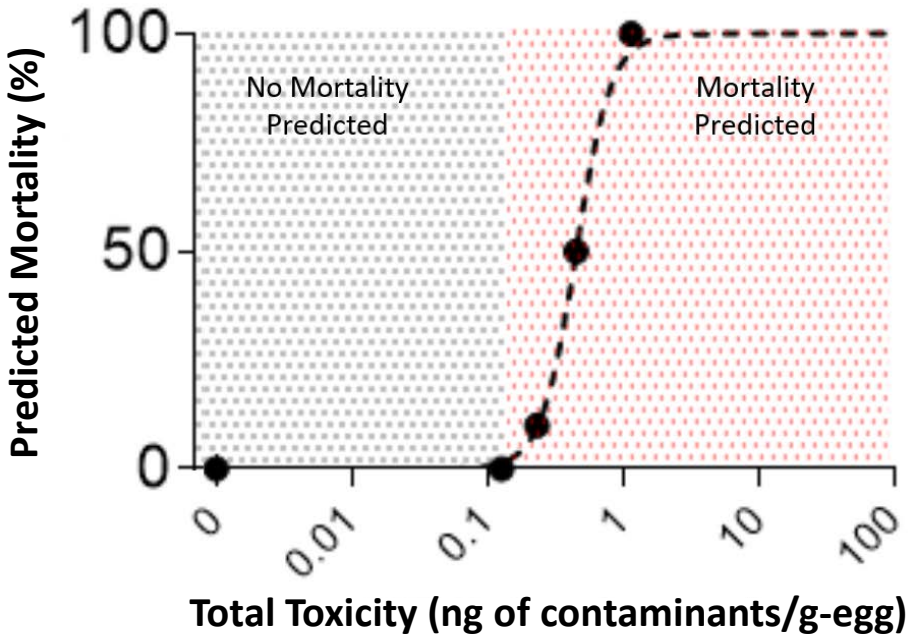
Mortality predicted by model for ≥ 1 individual (Figure 1)

Total Toxicity of Legacy Contaminants[a]

No mortality predicted by model (Figure 1)

Stop: Exposure unlikely to cause elevated mortality[c]

Figure 1: Model Output for Toxicity to Lake Sturgeon

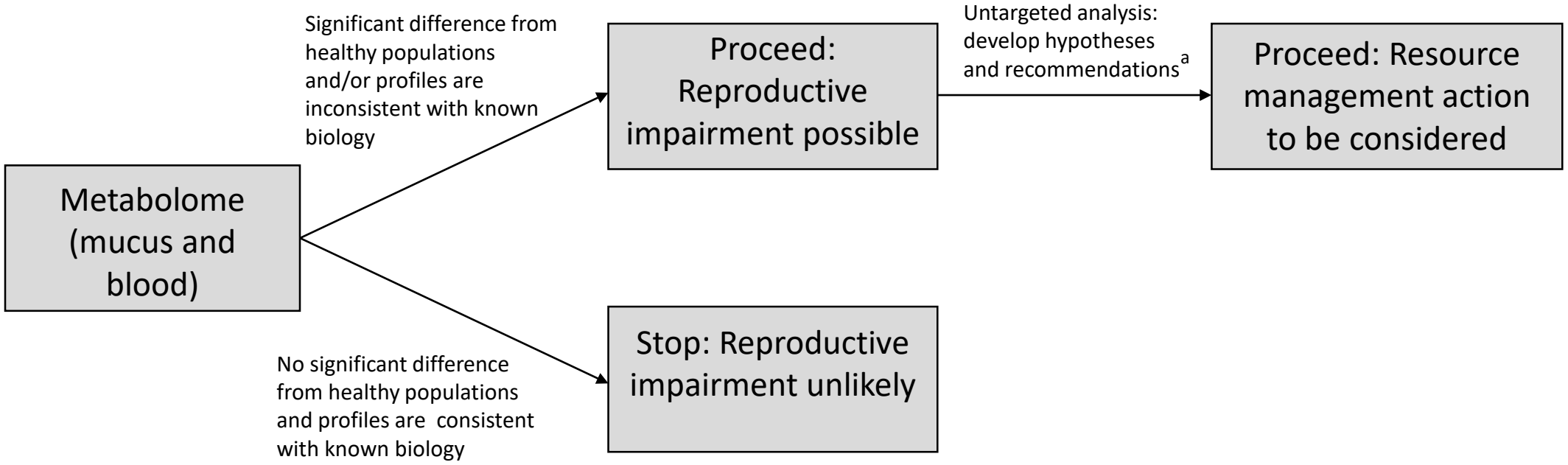


[a] Total toxicity of the mixture of legacy contaminants is calculated using established potencies based largely on rainbow trout and which are intended for initial screening purposes only.

[b] Specific chemicals chosen for development of sturgeon-specific potency values will be based on expert opinion and take into consideration the concentrations measured, contribution to total toxicity, known differences in potency of different chemicals among species, and frequency of detection among individuals.

[c] Assessment only predicts mortality caused by exposure to chlorinated aromatic hydrocarbons (dioxins/furans and PCBs) and does not consider other contaminants (ex. metals, DDT, etc).

Decision Tree for Assessment of Adults



[a] Since metabolomics is an untargeted analysis that can measure thousands of metabolites it is not possible to develop concrete hypotheses at this time. Expert opinion will be used to interpret the data in order to make hypotheses and provide regulatory recommendations. Possible information derived from metabolomics include phenotypic sexing (even with no external sex characteristics), evidence of reproductive health or reproductive dysfunction, evidence of delayed maturation, and evidence of exposure to other chemicals of potential concern, such as polycyclic aromatic hydrocarbons, wastewater effluents, and others.

Possible Scientifically Defensible Recommendations Resulting from this Research:

1) **No egg mortality predicted, no indications of reproductive impairment:** Recruitment failure is likely a result of factors unrelated to the Area of Concern.

2) **Elevated egg mortality predicted, no indications of reproductive impairment:** Recruitment failure is likely a result of bioaccumulated legacy contaminants being maternally transferred to embryos.

3) **No egg mortality predicted, indications of reproductive impairment:** Recruitment failure is likely a result of reduced reproductive potential of adults. Depending upon results of metabolomics, this reproductive impairment could be predicted to result from exposure to legacy contaminants, present day contaminants, or some other biotic or abiotic stressor. It is possible that results will suggest a delayed maturation and that healthy reproduction should occur in the future.

4) **Elevated egg mortality predicted, indications of reproductive impairment:** Recruitment failure is likely a combined result of maternally transferred legacy contaminants and reduced reproductive potential of adults. Suggestive of severe, contaminant related pressures on recruitment in lake sturgeon.

Assessing whether maternal transfer of legacy dioxin-like chemicals is limiting lake sturgeon natural recruitment in the St. Louis River Area of Concern

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ABSTRACT: The purpose of this research is to answer questions about whether impairment of natural recruitment of lake sturgeon (*Acipenser fulvescens*) in the St. Louis River (SLR) Area of Concern (AOC) could be related to exposure to and bioaccumulation of legacy contaminants, such as polychlorinated dibenzo-*p*-dioxins/furans (PCDD/Fs) and polychlorinated biphenyls (PCBs), which are collectively known as dioxin-like chemicals (DLCs). Lake sturgeon are identified as an indicator species with specific population targets linked to the removal of SLR-AOC Beneficial Use Impairment (BUI) 2: Degraded Fish and Wildlife Populations. Despite past efforts to recover SLR-AOC lake sturgeon populations via fingerling stocking, recruitment is not being observed at anticipated levels and is not trending towards BUI targets. Therefore, resource managers and fisheries experts working in the SLR-AOC have identified a need to assess potential factors limiting lake sturgeon recovery and determine whether limiting factors are influenced by legacy contamination. In particular, DLCs can accumulate in sturgeons and be maternal transferred to embryos leading to decreased survival of early life-stages. Therefore, samples of eggs were collected from SLR-AOC lake sturgeon using a combination of egg mats and releases from ripe females collected during the MN DNR 2017 to 2019 sturgeon spawning assessments. Additionally, plasma was collected from adult female, male, and immature individuals. Concentrations of the 15 dioxin-like PCDD/Fs and 4 dioxin-like PCBs were quantified in each sample. Toxic equivalents (TEQs) in eggs and plasma were calculated using World Health Organization (WHO) toxic equivalency factors (TEFs) for fish and predicted lake sturgeon-specific TEFs developed from a quantitative adverse outcome pathway (qAOP) model. The calculated TEQs for eggs were compared to lake sturgeon mortality curves and TEQs for plasma were compared to a predicted effect threshold for adults. These comparisons determined that bioaccumulation and maternal transfer of DLCs is below levels that would be expected to cause any known toxicities in lake sturgeon. This evidence suggests that legacy contamination of DLCs is unlikely to be a factor in recruitment failure and provides support for other hypothesized drivers, possibly unrelated to the SLR-AOC.

INTRODUCTION

Historical municipal and industrial waste disposal and improper land-use practices before the onset of modern environmental protection laws have created a complex set of issues in the St. Louis River (SLR), a tributary of Lake Superior situated in Minnesota and Wisconsin. In 1987 the SLR was identified as a Great Lakes Area of Concern (AOC), leading to development of a comprehensive remedial action plan (RAP) to restore all beneficial use impairments (BUIs), including BUI-2: degraded fish and wildlife populations (EPA, 2020). As part of removal of BUI-2, lake sturgeon (*Acipenser fulvescens*) were identified as an indicator species with specific population targets (EPA, 2020). The population of lake sturgeon in the SLR were considered extirpated in the early 1900's due to a combination of habitat degradation, impaired water quality, and overharvest (Auer, 1996; Schram et al., 1999). Beginning in 1983, a restoration stocking program was initiated by the Minnesota Department of Natural Resources (MNDNR) and Wisconsin Department of Natural Resources (WDNR) to support recovery of the SLR lake sturgeon population in conjunction with restoration of spawning habitat, improved water quality through treating domestic and industrial effluent, and reduced exploitation through conservative fishing regulations (Schram et al., 1999). Fry and fingerling lake sturgeon from the Wolf River of the Lake Michigan watershed were stocked into the SLR from 1983 to 1994 and from the Sturgeon River of the Lake Superior watershed from 1998 to 2000 (Estep et al., 2020; Schram et al., 1999). In total, 781,000 fry and 142,180 fingerling lake sturgeon were stocked into the SLR (Estep et al., 2020). However, more than 30 years since the first fish were stocked, recruitment is not trending towards BUI-2 targets (SLR-AOC Fish Tech Team, personal communication). As a result, resource managers and fisheries experts working in the SLR-AOC have identified a need to assess potential factors that could be limiting lake sturgeon recovery.

Legacy contaminants could be one factor limited lake sturgeon natural recruitment in the SLR-AOC. Elevated levels of sediment-associated legacy contaminants have been documented in the SLR-AOC, including complex mixtures of polychlorinated biphenyl (PCB), polychlorinated dibenzo-*p*-dioxin (PCDD), and polychlorinated dibenzofuran (PCDF) congeners (Crane & MacDonald, 2003). A total of 12 PCBs, 7 PCDDs, and 10 PCDFs are collectively known as dioxin-like chemicals (DLCs) and have the greatest toxicity to wildlife due to their planar structure which allows them to bind with relatively great affinity to the aryl hydrocarbon receptor (AHR) (Denison & Nagy, 2003). The AHR is a ligand-activated transcription factor that regulates a range of physiological processes in vertebrates and whose dysregulation can cause adverse biological effects, including wasting syndrome, hepatotoxicity, immune suppression, impaired endocrinology, carcinogenesis, developmental deformities, and early life mortality (Elonen et al., 1998; Giesy et al., 2002; King-Heiden et al., 2012; Spitsbergen et al., 1986, Walter et al., 2000). DLCs can bioaccumulate in wildlife, undergo biomagnification through trophic levels, and be maternally transferred to embryos (Lohmann & Jones, 1998; Opperhuizen & Sijim, 1990). Lake sturgeon are uniquely susceptible to bioaccumulation of DLCs because they live in close association with sediments where these contaminants are most persistent and feed primarily on benthic organisms (Hochleithner & Gessner, 2001). Additionally, lake sturgeon are extremely long-lived (>100 years), do not spawn until late in life (12 to 27 years), and then only spawn intermittently (every 2 to 6 years). These attributes of lake sturgeon also mean that embryos have increased likelihood of being exposed to elevated concentrations of bioaccumulated DLCs via maternal transfer which might cause increased early life mortality.

The objective of the present study was to assess whether maternal transfer of legacy DLCs could be limiting lake sturgeon natural recruitment in the SLR-AOC. Assessments of ecological risk posed by complex environmental mixtures of PCB, PCDD, and PCDF congeners are performed using the TCDD-equivalency factor (TEF) approach (Van den Berg et al., 1998). TEFs are order of magnitude consensus values for the potency of a DLC congener relative to the prototypical reference congener, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD), which can be used in combination with tissue residue measurements to calculate TCDD-equivalents (TEQs) of a mixture of DLCs in terms of potency equivalents to 2,3,7,8-TCDD (Van den Berg et al. 1998). Fish TEFs have been published by the World Health Organization (WHO) based entirely on results of early life toxicity assays with salmonids (Van den Berg et al. 1998). However, the potency of DLCs relative to 2,3,7,8-TCDD can differ by more than an order of magnitude among species which introduces significant uncertainty when interpreting species-specific risk (Eisner et al., 2016). Development of TEFs specific for lake sturgeon using traditional early life toxicity assays is largely impractical because performing these assays for a suite of DLCs would be expensive, time consuming, and require large numbers of this difficult to acquire species; in addition to challenges related to unique life history characteristics which make sturgeon difficult to use in early life toxicity assays (Tompsett et al., 2014). However, 21st century advances in predictive ecotoxicology have resulted in the development of mechanism-based biological models, known as quantitative adverse outcome pathways (qAOPs), capable of accurately predicting species-specific relative potencies for DLCs using only cell-based assays that do not require lethal samples (Doering et al., 2018). Therefore, the specific objectives of the present study were to calculate TEQs in eggs and plasma collected from SLR-AOC lake sturgeon using WHO TEFs for fish and predicted lake sturgeon-specific relative potencies. The calculated TEQs

could be compared to lake sturgeon mortality curves to determine whether maternal transfer would be expected to cause elevated early life mortality and potentially explain the observed recruitment failure.

MATERIALS AND METHODS

Sample collection

Collection of lake sturgeon for sampling was performed as described previously (Estep et al., 2020). Briefly, lake sturgeon were captured in the SLR estuary between the Fond du Lac dam and Highway 23 in the springs of 2017, 2018, and 2019 during the sturgeon spawning season when water temperatures reach 8 to 10 °C. Lake sturgeon were captured via a combination of boat electrofishing, dip-netting, backpack electrofishing, and angling. All captured fish were measured for total length, girth, and weight. A small pelvic fin clip was taken from each captured lake sturgeon for genetic stock identification as reported previously (Estep et al., 2020). The sex of each individual was determined visually using manual extrusion of gametes. From each female, a sample of the extruded oocytes were collected and transported on dry ice before being stored at -80 °C until analyzed. Approximately 6 mL of blood was collected from each individual through the caudal vein using a 21 G, 1” needle, attached to a 6 mL heparinized vacutainer. Blood was transported on wet ice before being centrifuged at 1750 g for 10 min at 10 °C to separate plasma. Plasma was stored at -80 °C until analyzed.

Exposure assessment

Concentrations of the selected dioxin-like PCDDs, PCDFs, and PCBs were measured in eggs and plasma collected from SLR-AOC lake sturgeon by use of high-resolution isotope-

dilution mass spectrometry (HRMS) according to Methods 1668A and 1613B. Total lipid content (as % wet weight) was measured by use of the microgravimetric of Radin method. Lipid content was used for lipid normalization in order to directly compare concentrations of selected dioxin-like PCDDs, PCDFs, and PCBs between eggs and plasma, which differ significantly in lipid content. Lipid normalization was performed by dividing the chemical concentration of the sample by the total lipid content of the sample.

Hazard assessment and risk characterization

Measured concentrations of the selected dioxin-like PCDDs, PCDFs, and PCBs in eggs and plasma of SLR-AOC lake sturgeon were multiplied by WHO TEFs or lake sturgeon-specific TEFs predicted using the qAOP for each chemical in each sample (Table 1). The qAOP was developed and previously validated for application to lake sturgeon and is known to produce accurate predictions for this species (Doering et al., 2018). Predictions were based on lake sturgeon AHR function reported previously (Doering et al., 2015). In cases where the selected dioxin-like PCDD, PCDF, or PCB were not detected in the sample, each TEF was multiplied by the detection limit (as reported) to produce the most conservative estimate of possible exposure. Previously published concentrations of the selected dioxin-like PCDDs, PCDFs, and PCBs in eggs from lake sturgeon from a known healthy population (Tillitt et al., 2017) were also multiplied by WHO TEFs (Van den Berg et al., 1998) or lake sturgeon-specific TEFs predicted using the qAOP to act as a reference. The toxicity of each chemical in each sample were summed to produce TEQs in terms of potency of the mixture relative to 2,3,7,8-TCDD for each sample using both sets of TEFs. The TEQs in eggs were compared to the predicted toxicity curve of 2,3,7,8-TCDD for lake sturgeon predicted using the qAOP (Doering et al., 2018) and effect

concentrations for 2,3,7,8-TCDD in limited toxicity assays performed with lake sturgeon (Tillitt et al., 2017). The qAOP uses not lipid normalized egg TEQs and therefore lipid normalized values were not used for these comparisons.

RESULTS AND DISCUSSION

The objective of the present study was to assess whether maternal transfer of legacy DLCs could be limiting lake sturgeon natural recruitment in the SLR-AOC. To assess maternal transfer of bioaccumulated DLCs to embryos, eggs were collected for chemical analysis from 1 female lake sturgeon from the SLR-AOC in 2017, 3 females in 2018, and 3 females in 2019. DLCs were detected in eggs collected from all 7 female lake sturgeon caught in the SLR-AOC (Table 2). The detected DLCs were predominantly PCDFs and PCBs, with 2,3,7,8-TCDD and 1,2,3,4,6,7,8-heptachlorodibenzo-*p*-dioxin (1,2,3,4,6,7,8-HCDD) being the only 2 detected PCDDs, and only in 1 of the egg samples (Table 2). The most frequently detected DLCs in SLR-AOC lake sturgeon eggs were 2,3,7,8-tetrachlorodibenzofuran (2,3,7,8-TCDF), 1,2,3,6,7,8-hexachlorodibenzofuran (1,2,3,6,7,8-HCDF), 3,3',4,4'-tetrachlorobiphenyl (PCB 77), and 3,3',4,4',5-pentachlorobiphenyl (PCB 126) (Table 2). The DLC with the overall greatest measured concentration in SLR-AOC lake sturgeon eggs was PCB 126, however PCB 77 was the DLC with the greatest measured concentration in 4 of the 7 egg samples (Table 2).

To assess possible toxicities of exposure to maternally transferred DLCs, TEQs were calculated using both WHO TEFs for fish and lake sturgeon-specific TEFs predicted using the qAOP. TEQs in SLR-AOC lake sturgeon eggs ranged from 2.9 to 9.0 pg of 2,3,7,8-TCDD/g of egg using WHO TEFs and from 10.3 to 76.8 pg of 2,3,7,8-TCDD/g of egg using lake sturgeon-

specific TEFs (Table 3). Most of the TEQs calculated for eggs from SLR-AOC lake sturgeon using either set of TEFs were greater than TEQs calculated for eggs from lake sturgeon from a healthy reference population (Figure 1). For most of the egg samples from SLR-AOC lake sturgeon, the greatest contributor to the TEQs using WHO TEFs was 1,2,3,6,7,8-HCDF, while using lake sturgeon-specific TEFs the greatest contributors to the TEQs were 1,2,3,6,7,8-HCDF, PCB 126, and 2,3,7,8-TCDF (Table 1; Table 2). The TEQs calculated using lake sturgeon-specific TEFs were on average 5-fold greater than TEQs calculated using WHO TEFs (Table 3). The greater TEQs using lake sturgeon-specific TEFs is driven heavily by increased potency of PCB 126 and 2,3,7,8-TCDF. These differences illustrate how using WHO TEFs as is standard in risks assessments for DLCs could lead to underestimation of risk in some cases and for certain species, as has been suggested previously (Doering et al., 2014; Eisner et al., 2016).

Early life toxicities of exposure to DLCs in fishes is well established and great differences in sensitivity are known to exist (Doering et al., 2013). The sensitivity of lake sturgeon has previously been assessed using predictive methods and in embryo toxicity assays (Doering et al., 2015; 2018; Tillitt et al., 2017). These studies have demonstrated lake sturgeon to be a moderately sensitive species with a 50% lethal dose (LD50) of 2,3,7,8-TCDD of 610 pg/g-egg and the no observed effect level (LOEL) is 360 pg/g-egg (Tillitt et al., 2017). Based on these studies, the lake sturgeon is approximately 10-fold less sensitive than the most sensitive known species of fish, the lake trout (*Salvelinus namaycush*) (Doering et al., 2013). In eggs from SLR-AOC lake sturgeon, TEQs calculated using either set of TEFs were all below levels predicted to cause any toxicities in early life stages based on predictive methods or embryo toxicity assays (Figure 1). Further, all TEQs were below known effect concentrations for sublethal endpoints in

lake sturgeon based on results of embryo toxicity assays, including effects on long-term growth, developmental anomalies, swim performance, or behavior (Figure 1). Therefore, the TEQs measured in eggs from SLR-AOC lake sturgeon in context with results of existing studies and predictions from the qAOP suggest that neither lethal nor sublethal adverse effects would be expected to occur in early life stages as a result of maternally transferred dioxin-like PCDDs, PCDFs, or PCBs.

Eggs from only 7 female SLR-AOC lake sturgeon were able to be collected for chemical analysis between 2017 and 2019. Therefore, to get a broader scope of possible exposure to and bioaccumulation of dioxin-like PCDDs, PCDFs, and PCBs among SLR-AOC lake sturgeon, plasma samples from females, males, and immature individuals were also collected for analysis. Plasma was not collected from any fish in 2017. In 2018, plasma was collected and analyzed from 1 female, 2 male, and 3 immature SLR-AOC lake sturgeon (Table 4-6). In 2019, plasma was collected and analyzed from 5 female and 7 immature SLR-AOC lake sturgeon (Table 4-6). As was the case with the eggs, the detected DLCs were predominantly PCDFs and PCBs, with PCDDs being detected in only 1 female caught in 2019 (Table 4) and 1 immature individual caught in 2019 (Table 6). The most frequently detected DLCs in plasma from female SLR-AOC lake sturgeon were 2,3,7,8-TCDF and 1,2,3,4,6,7,8-HCDF, but the DLC with the greatest measured concentration was 1,2,3,6,7,8-HCDF (Table 4). Three DLCs were detected in plasma from male SLR-AOC lake sturgeon, specifically 2,3,7,8-TCDF, 1,2,3,6,7,8-HCDF, and PCB 126; with PCB 126 having the greatest measured concentration in both males (Table 5). In common with female and male SLR-AOC lake sturgeon, the most frequently detected DLCs in

plasma from immature individuals were 2,3,7,8-TCDF, 1,2,3,4,6,7,8-HCDF, and PCB 126; with PCB 126 having the greatest measured concentration (Table 6).

In common with eggs, TEQs for plasma were calculated using both WHO TEFs for fish and lake sturgeon-specific TEFs predicted using the qAOP. However, to directly compare TEQs between eggs and plasma, TEQs were calculated following lipid normalization for plasma (Table 7). Lipid normalization was subsequently performed on concentrations measured in eggs and lipid normalized TEQs calculated (Table 7). Lipid normalized TEQs in SLR-AOC lake sturgeon eggs ranging from 0.4 to 1.4 pg of 2,3,7,8-TCDD/g of egg using WHO TEFs and from 1.3 to 8.4 pg of 2,3,7,8-TCDD/g of egg using lake sturgeon-specific TEFs (Table 7). However, lipid normalized TEQs in SLR-AOC lake sturgeon plasma were greater than in eggs, with a range from 2.6 to 10.5 pg of 2,3,7,8-TCDD/g of plasma using WHO TEFs and from 10.7 to 29.3 pg of 2,3,7,8-TCDD/g of plasma using lake sturgeon-specific TEFs (Table 7). Again, the TEQs calculated using lake sturgeon-specific TEFs were greater and driven heavily by the increased potency of PCB 126 and 2,3,7,8-TCDF.

Less is known about toxicities of exposure to DLCs in adult fish and nothing is known about toxicities in adult lake sturgeon. However, long-term exposure studies to environmentally relevant concentrations of DLCs in rainbow trout (*Oncorhynchus mykiss*) have shown the potential for toxicities in adult fishes, including decreased survival, altered behavior, and impaired reproduction (Giesy et al., 2002). These toxicities in adults were shown to begin to occur at body burdens that result in maternal transfer of doses that cause toxicities in early life stages (Giesy et al., 2002). Using this knowledge, a plasma TEQ (lipid normalized) that is

predicted to represent a threshold for sublethal or lethal effects in adult lake sturgeon was predicted by converting the embryo TEQ for threshold for effects to the plasma TEQ. This was done by dividing the embryo effect threshold of 360 pg TCDD/g-egg (Tillet et al., 2017) by the average egg lipid content (8.3%) (Table 2) allowing extrapolation across tissues. This results in a predicted lipid normalized TEQ effect threshold for sublethal or lethal effects on adults of 43 pg TCDD/g of tissue. The TEQs calculated in plasma from SLR-AOC lake sturgeon ranged from 2.6 to 10.5 pg of 2,3,7,8-TCDD/g of plasma using WHO TEFs and from 10.7 to 28.5 pg of 2,3,7,8-TCDD/g of plasma using lake sturgeon-specific TEFs (Table 7). All these TEQs measured in plasma from adult SLR-AOC lake sturgeon are below the predicted effect threshold of 43 pg TCDD/g of plasma which suggests that neither lethal nor sublethal adverse effects would be expected to occur in female, male, or immature adult individuals as a result of bioaccumulated dioxin-like PCDDs, PCDFs, or PCBs.

In conclusion, the present study demonstrates that SLR-AOC lake sturgeon are bioaccumulating dioxin-like PCDDs, PCDFs, and PCBs to levels greater than at a reference site, but concentrations being maternally transferred to embryos are well below concentrations known to cause any lethal or sublethal toxicities in this species. Additionally, TEQs measured in plasma from adult SLR-AOC lake sturgeon also suggest that neither lethal nor sublethal adverse effects would be expected to occur in adult female, male, or immature individuals. Therefore, these results support that bioaccumulated or maternally transferred DLCs are unlikely to be a contributing factor to the recruitment failure of lake sturgeon in the SLR-AOC. As a result, other limiting factors that could cause the observed recruitment failure should be assessed, such as

potential toxicity from other contaminants of concern, competition or predation from invasive species, habitat suitability, or the extremely long generation time of this species.

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Table 1. Comparison of WHO TEFs for fish and TEFs specific for lake sturgeon.

DLC Congener	WHO Fish TEFs[a]	Lake Sturgeon TEFs	Fold-difference
PCDDs			
2,3,7,8-TCDD	1.0	1.0	1
1,2,3,7,8-PCDD	1.0	1.0	1
1,2,3,4,7,8-HCDD	0.5	0.5	1
1,2,3,6,7,8-HCDD	0.01	0.01	1
1,2,3,7,8,9-HCDD	0.01	0.01	1
1,2,3,4,6,7,8-HCDD	0.01	0.01	1
PCDFs			
2,3,7,8-TCDF	0.05	1	20
1,2,3,7,8-PCDF	0.05	0.5	10
2,3,4,7,8-PCDF	0.5	2.5	5
1,2,3,4,7,8-HCDF	0.1	1	10
1,2,3,6,7,8-HCDF	0.1	1	10
1,2,3,7,8,9-HCDF	0.1	1	10
2,3,4,6,7,8-HCDF	0.1	1	10
1,2,3,4,6,7,8-HCDF	0.01	0.1	10
1,2,3,4,7,8,9-HCDF	0.01	0.1	10
Non-*ortho* PCBs			
PCB 77	0.0001	0.05	500
PCB 81	0.0005	0.1	200
PCB 126	0.005	0.1	20
PCB 169	0.00005	0.01	200

[a] TEFs as shown previously (Van den Berg et al., 1998).

Table 2. DLCs measured in eggs (pg DLC/g egg) collected from SLR-AOC lake sturgeon in 2017, 2018, and 2019.[a]

Analyte	2017	2018			2019		
	Female 1	Female 1	Female 2	Female 3	Female 1	Female 2	Female 3
Lipid (%)	4	9	11	10	8	8	8
PCDDs							
2,3,7,8-TCDD				0.4			
1,2,3,7,8-PCDD							
1,2,3,4,7,8-HCDD							
1,2,3,6,7,8-HCDD							
1,2,3,7,8,9-HCDD							
1,2,3,4,6,7,8-HCDD				0.6			
PCDFs							
2,3,7,8-TCDF		2.7		8.1			3.9
1,2,3,7,8-PCDF		2.2			0.7		
2,3,4,7,8-PCDF							
1,2,3,4,7,8-HCDF		2.5					
1,2,3,6,7,8-HCDF	18.0	21.0		58.0			
1,2,3,7,8,9-HCDF							
2,3,4,6,7,8-HCDF							
1,2,3,4,6,7,8-HCDF	1.7						
1,2,3,4,7,8,9-HCDF							
Non-*ortho* PCBs							
PCB 77	58.0	41.0			54.8	10.1	10.2
PCB 81							
PCB 126	24.0	91.0	120.0				
PCB 169						6.2	8.8

[a] DLCs that were not detected in eggs are left blank. Detection limit was 1 pg/g-egg for PCDD/PCDFs and 5 pg/g-egg for PCBs.

Table 3. TEQs of SLR-AOC lake sturgeon eggs calculated using WHO TEFs or lake sturgeon-specific TEFs show as pg of 2,3,7,8-TCDD/g of egg.

Year	Individual	WHO TEQ	Lake Sturgeon-specific TEQ
2017	Female 1	5.4	33.7
2018	Female 1	6.3	46.2
	Female 2	4.2	23.5
	Female 3	9.0	76.8
2019	Female 1	2.9	10.6
	Female 2	3.2	10.3
	Female 3	3.9	12.8

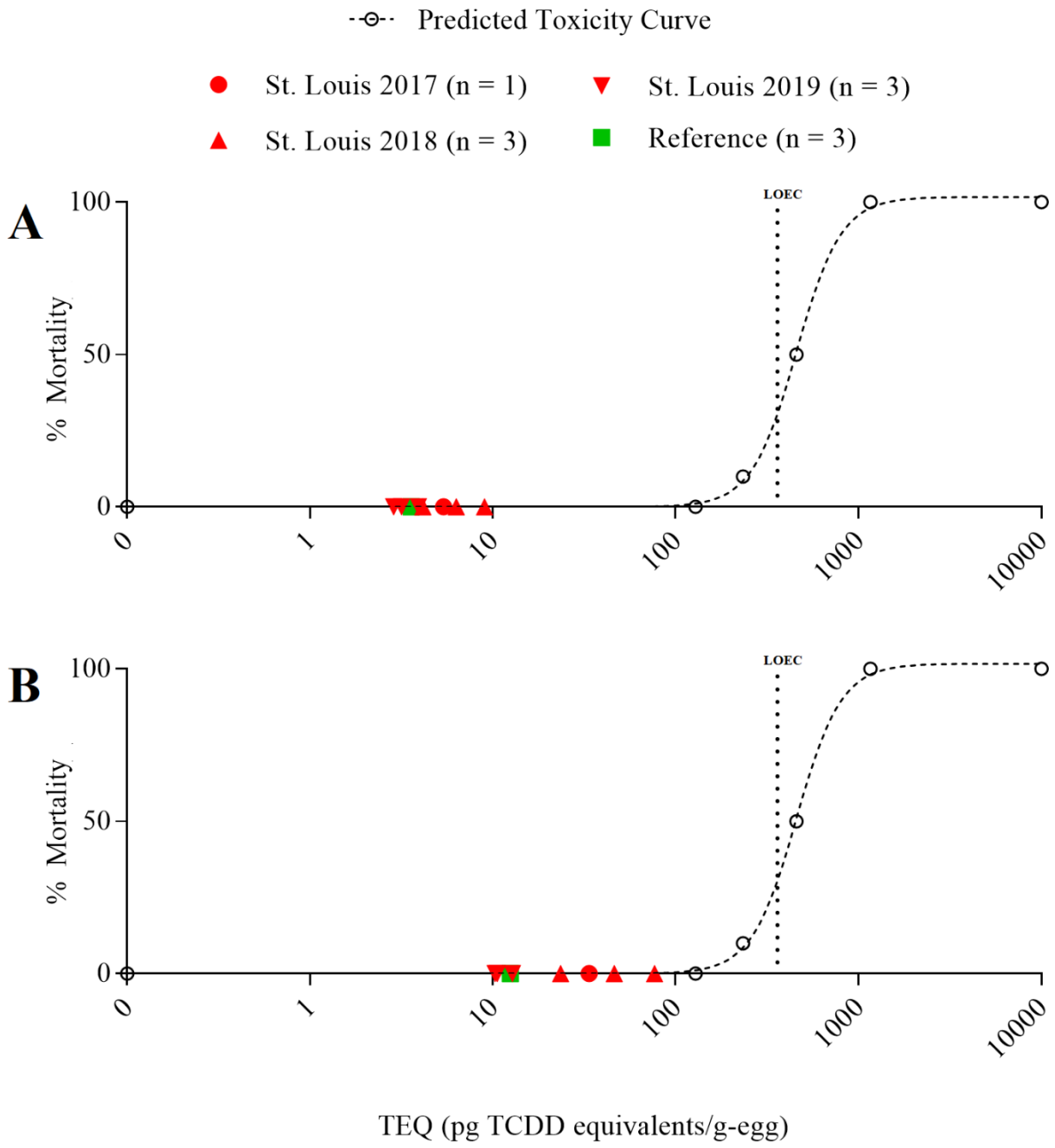

Figure 1. TEQs of SLR-AOC lake sturgeon eggs calculated using WHO TEFs (A) or lake sturgeon-specific TEFs (B) relative to predicted mortality response curve (open circles connected by curved dotted line). Lowest observed effect concentration (LOEC) in lake sturgeon following laboratory toxicity testing is shown (vertical dotted line) (Tillitt et al., 2017). TEQs calculated for lake sturgeon eggs from a healthy population is shown as a reference (Tillitt et al., 2017).

Table 4. DLCs measured in plasma (pg DLC/g plasma) collected from female SLR-AOC lake sturgeon in 2018 and 2019.[a]

Analyte	2018	2019				
	Female 1	Female 1	Female 2	Female 3	Female 4[b]	Female 5[b]
Lipid (%)	1.2	1.3	1.3	1.3	1.3	1.3
PCDDs						
2,3,7,8-TCDD						2.6
1,2,3,7,8-PCDD						4.6
1,2,3,4,7,8-HCDD						
1,2,3,6,7,8-HCDD						
1,2,3,7,8,9-HCDD						
1,2,3,4,6,7,8-HCDD						
PCDFs						
2,3,7,8-TCDF	1.3	3.3				
1,2,3,7,8-PCDF						
2,3,4,7,8-PCDF						2.5
1,2,3,4,7,8-HCDF						2.5
1,2,3,6,7,8-HCDF	9.0					2.2
1,2,3,7,8,9-HCDF						
2,3,4,6,7,8-HCDF						2.8
1,2,3,4,6,7,8-HCDF	0.6		3.4			4.1
1,2,3,4,7,8,9-HCDF						
Non-*ortho* PCBs						
PCB 77						
PCB 81						
PCB 126						
PCB 169						

[a] DLCs that were not detected in plasma are left blank. Detection limit was 1 pg/g-egg for PCDD/PCDFs and 5 pg/g-egg for PCBs.

[b] Plasma sample, but no egg sample, collected from these individuals.

Table 5. DLCs measured in plasma (pg DLC/g plasma) collected from male SLR-AOC lake sturgeon in 2018.[a]

Analyte	2018	
	Male 1	Male 2
Lipid (%)	1.5	1.1
PCDDs		
2,3,7,8-TCDD		
1,2,3,7,8-PCDD		
1,2,3,4,7,8-HCDD		
1,2,3,6,7,8-HCDD		
1,2,3,7,8,9-HCDD		
1,2,3,4,6,7,8-HCDD		
PCDFs		
2,3,7,8-TCDF	0.5	0.6
1,2,3,7,8-PCDF		
2,3,4,7,8-PCDF		
1,2,3,4,7,8-HCDF		
1,2,3,6,7,8-HCDF	4.3	5.3
1,2,3,7,8,9-HCDF		
2,3,4,6,7,8-HCDF		
1,2,3,4,6,7,8-HCDF		
1,2,3,4,7,8,9-HCDF		
Non-*ortho* PCBs		
PCB 77		
PCB 81		
PCB 126	18	14
PCB 169		

[a] DLCs that were not detected in plasma are left blank. Detection limit was 1 pg/g-egg for PCDD/PCDFs and 5 pg/g-egg for PCBs.

Table 6. DLCs measured in plasma (pg DLC/g plasma) collected from SLR-AOC lake sturgeon in 2018 and 2019 that could not be sexed.[a]

Analyte	2018			2019						
	Fish 1	Fish 2	Fish 3	Fish 1	Fish 2	Fish 3	Fish 4	Fish 5	Fish 6	Fish 7
Lipid (%)	0.7	0.5	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6
PCDDs										
2,3,7,8-TCDD										
1,2,3,7,8-PCDD										
1,2,3,4,7,8-HCDD										
1,2,3,6,7,8-HCDD										
1,2,3,7,8,9-HCDD										
1,2,3,4,6,7,8-HCDD				1.6						
PCDFs										
2,3,7,8-TCDF	0.7			2.3	3		3.7	3.1	3.2	2.9
1,2,3,7,8-PCDF										
2,3,4,7,8-PCDF										
1,2,3,4,7,8-HCDF										
1,2,3,6,7,8-HCDF	3.0	2.3	2.8		0.7	0.9				0.9
1,2,3,7,8,9-HCDF										
2,3,4,6,7,8-HCDF										
1,2,3,4,6,7,8-HCDF										
1,2,3,4,7,8,9-HCDF										
Non-*ortho* PCBs										
PCB 77	6.8									
PCB 81										
PCB 126	11	24	14							
PCB 169										

[a] DLCs that were not detected in plasma are left blank. Detection limit was 1 pg/g-egg for PCDD/PCDFs and 5 pg/g-egg for PCBs.

Table 7. TEQs of SLR-AOC lake sturgeon plasma calculated using WHO TEFs or lake sturgeon-specific TEFs show as lipid normalized pg of 2,3,7,8-TCDD/g of plasma or egg.

Year	Individual	WHO TEQ		Lake Sturgeon-specific TEQ	
		Egg	Plasma	Egg	Plasma
2017	Female 1	1.4		8.4	
2018	Female 1	0.7	3.7	5.1	16.9
	Female 2	0.4	0.9	2.1	7.7
	Female 3	0.9		7.7	
	Male 1		2.6		10.7
	Male 2		3.7		15.3
	Fish 1		5.5		20.9
	Fish 2		7.3		29.3
	Fish 3		5.9		22.7
2019	Female 1	0.4	4.7	1.3	13.9
	Female 2	0.4	1.3	10.5	28.5
	Female 3	0.5	4.6	1.6	15.0
	Female 4		3.7		11.5
	Female 5		6.8		22.8
	Fish 1		5.9		20.5
	Fish 2		5.3		21.4
	Fish 3		6.8		22.5
	Fish 4		7.3		26.3
	Fish 5		7.4		26.7
	Fish 6		7.9		24.2
	Fish 7		6.7		24.9

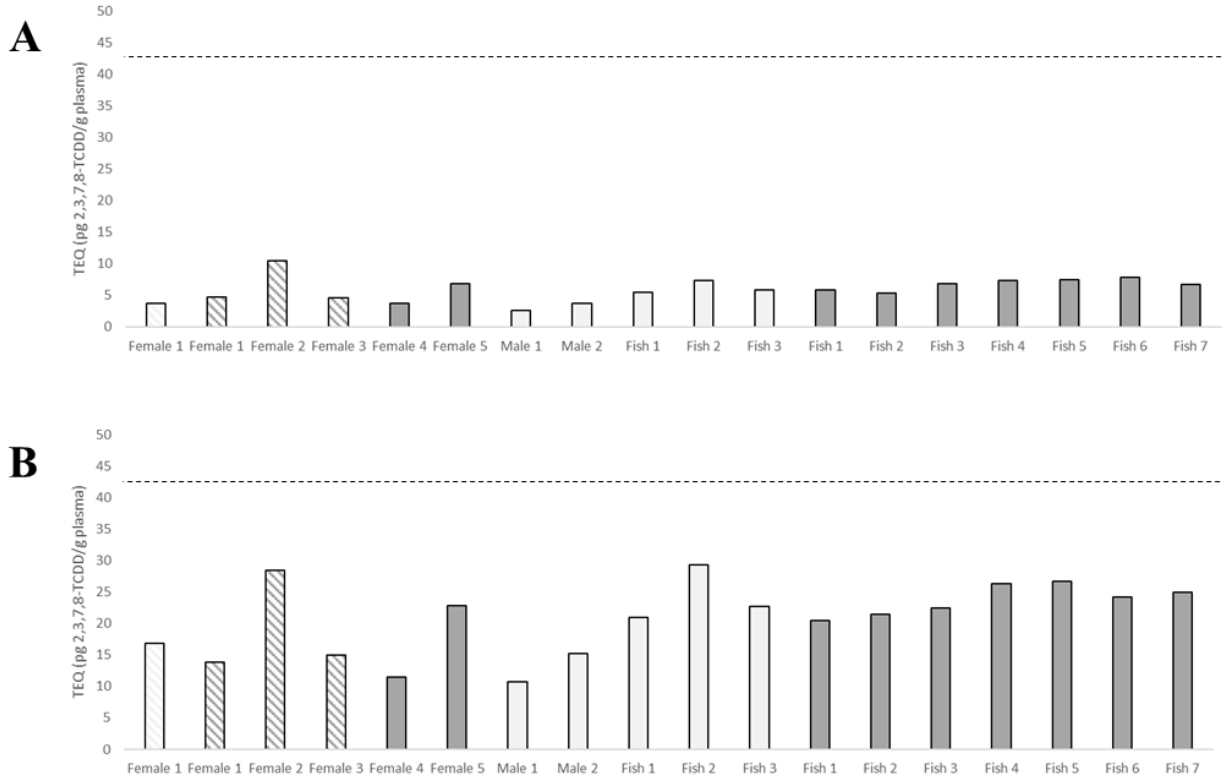

Figure 2. Lipid normalized TEQs of SLR-AOC lake sturgeon plasma calculated using WHO TEFs (A) or lake sturgeon-specific TEFs (B) from females, males, and individuals that could not be sexed that were caught in 2018 (light bars) and 2019 (dark bars). Females with both plasma and egg TEQs are highlighted (hashed bars). Labels for each individual fish as presented previously (Table 7). The predicted effect threshold of 43 pg TCDD/g of plasma is indicated (dotted horizontal line).