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Tissue contaminants and wild fish health in the St. Clair River Area of Concern – Part 2: Spatial trends and temporal declines in organics

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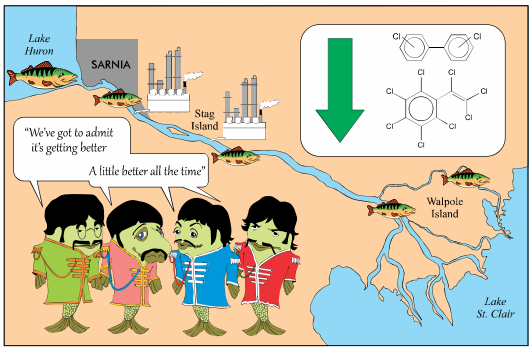
HIGHLIGHTS

- Fish tissue concentrations of persistent organics have decreased over last 12-years.
- Little evidence of contaminant-linked health effects in fish in 2014.
- Non-legacy PCBs may be increasing in yellow perch in the region of Stag Island.
- Tissue concentrations reflected life history and food chain position of fish species.

GRAPHICAL ABSTRACT



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ABSTRACT

We explored tissue concentrations of polychlorinated biphenyls (PCBs), chlorinated pesticides, and relevant organochlorines and fish health in the following adult wild fish in the St. Clair River Area of Concern (Ontario, Canada): shorthead redhorse (*Moxostoma macrolepidotum*), yellow perch (*Perca flavescens*), and emerald shiner (*Notropis atherinoides*). We collected adult fish from sites within the river's industrial zone (Stag Island), a downstream site adjacent to Walpole Island (Chenal Écarte), and an upstream reference site in Lake Huron in 2002/2003 and 2014. We tested for trends in tissue concentrations of organic contaminants across sites and over time; we assessed the potential effects of contaminants on morphological indicators of fish health across sites by year. Over the 12-year period, the tissue concentrations of most PCBs declined at the river sites, except for some non-legacy PCBs (PCB11 and 185), which increased in yellow perch at Stag Island, a new observation for fish in the St. Clair River AOC. There was little difference between the concentrations of calculated toxic equivalents (TEQs) of the Lake Huron and the St. Clair River fish in 2014, except for emerald shiners from Stag Island which had elevated ΣPCB and TEQs. Each fish species at all sites exceeded the Canadian tissue residue guideline for PCBs for the protection of mammalian wildlife consumers of aquatic biota, but fish-derived TEQs indicated little potential health risk to fish. Over time, hexachlorobutadiene and hexachlorobenzene concentrations increased in some fish at Stag Island by about 8- and 4-fold, respectively, whereas they decreased at other sampling locations. Principal Component Analysis followed by Linear Discriminant Analysis of the 2014 SHRH data suggested that although the fish

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separated by site, tissue concentrations of PCB and organochlorine contaminants did not have consistent relationships to the morphological health indicators, including egg production in females, which implied the absence of causative relationships.

1. Introduction

The St. Clair River is an international boundary water that separates Canada from the United States of America and serves as a major shipping channel. It was designated an Area of Concern (AOC) by the International Joint Commission in 1985 due to long-term contaminant loadings from extensive industrialization (e.g., refineries, petrochemical facilities, a chlor-alkali plant, organic and inorganic chemical manufacturers, paper companies, salt producers, and coal-fired generating facilities) that has occurred along the river since the 1940s and has led to beneficial use impairments (BUIs). Many of the environmental impacts are not unique to the St. Clair River and thus this study is widely applicable to numerous other Areas of Concern, Superfund sites, and pollution hotspots around the world.

Historical discharges from industrial and municipal wastewater treatment systems and sewers, spills, as well as non-point sources have contributed to contamination of St. Clair River sediments with organic contaminants. Examples of such contaminants are polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), volatile hydrocarbons (e.g., trichloroethane and perchloroethylene), and semi-volatile organochlorine by-products (e.g., hexachlorobutadiene (HCBD)), a number of chlorinated benzenes such as hexachlorobenzene (HCB), and octachlorostyrene (OCS) (King and Sherbin, 1986; Marsalek, 1986; Oliver and Pugsley, 1986). Although contaminant loading of the river has generally decreased over the last four decades, historically contaminated bottom sediments continue to be a source of industrial chlorinated compounds to the water column, fish, and downstream environments (Gewurtz et al., 2010; Jia et al., 2010; Richman and Milani, 2010; Richman et al., 2017).

Monitoring data (Schneider et al., 1998; Weis, 2004) showed that concentrations of total-(Σ)PCB, OCS, HCB, and total-dichlorodiphenyltrichloroethane (ΣDDT) in sports fish tissues generally decreased from the early 1970's to mid-1980's, but stabilized in the 1990's. Tissue concentrations of PCBs and DDT related compounds in spottail shiner (*Notropis hudsonius*) decreased at various sites in the St. Clair region between 1978 and 1994 (Suns et al., 1993; Schneider et al., 1998). Concentrations in the St. Clair River shiners, however, were significantly higher than in those from Lake Huron or Lake St. Clair. Thus, non-atmospheric sources of those chemicals, likely from contaminated sediment, remain in the St. Clair River. Bottom sediment concentrations of OCS, and HCB have remained higher in the upper compared to the lower zones of the St. Clair River (Richman and Milani, 2009), whereas suspended sediment and water concentrations of PCBs were higher in the lower St. Clair River (St. Clair River RAP Team, 2006) when compared to upstream reference sites. Non-legacy or unintentionally produced PCBs (i.e. not found in the original technical PCB products) are an emerging concern in the Great Lakes. There is evidence that atmospheric inputs of PCB 11 (3,3′-dichlorobiphenyl) have not declined from 2004 to 2015 (Hites, 2018) and represent a significant proportion of total PCBs in air (Khairy et al., 2015). The non-legacy PCBs, which appear to be mainly derived from paint pigments, include, in addition to mono- and dichloro congeners, a nonachlorobiphenyl (PCB 206) and decachlorobiphenyl (PCB 209) (Hu and Hornbuckle, 2010; Grossman, 2013). There may be other sources from waste combustion (Ishikawa et al., 2007). Although well documented in Great Lakes air and sediments, the temporal trends of non-legacy PCBs in Great Lakes fishes have not been specifically examined to our knowledge, although some individual congeners, e.g. PCB 206, 209 have been routinely determined (Zhou et al., 2018).

Although some BUIs are no longer impaired, human consumption of fish and wildlife and degradation of benthic communities remain listed as "impaired", and many other BUIs require further assessment (Canadian Remedial Action Plan Implementation Committee (CRIC), 2013). In an accompanying paper (Muttray et al., 2019), we report spatial trends and temporal differences of metal tissue concentrations in the same wild fish species and related these trends to morphological measures of fish health. The selected fish species had different life histories and positions in the food chain. Shorthead redhorse (*Moxostoma macrolepidotum*, SHRH) is a bottom-feeding benthivorous fish, yellow perch (*Perca flavescens*, YP) is a pelagic and predatory fish, and emerald shiner (*Notropis atherinoides* Rafinesque, ES) is a small bentho-pelagic planktivorous fish with high site fidelity (Scott and Crossman, 1998). We found an improvement in metal tissue concentrations over time, with no consistent correlation between metal tissue concentrations and morphological health indicators. In the present paper we ask several questions related to spatial trends and temporal differences of PCBs and organochlorine contaminants (OCs), including pesticides and industrial by-products in tissues of the fish from Part 1 (Muttray et al., 2019). Specifically, we tested three hypotheses. Are there differences in tissue concentrations of organic contaminants between fish from the upstream reference site Lake Huron and fish from the St. Clair River near both Stag Island (industrial zone) and Walpole Island (downstream site)? Have tissue concentrations of organic contaminants declined in wild fish since 2002? Are there contaminant-associated effects on fish health and reproduction at Walpole and Stag Islands? Because of the large amount of data, we divided our work into two reports. In the companion paper (Part 1, Muttray et al., 2019) we tested the aforementioned hypotheses on tissue concentrations of metal contaminants in the same fish.

2. Materials and methods

Much of the methodology for the assessment of organic tissue concentrations and fish health within the St. Clair River AOC was similar to that presented in the companion article (Muttray et al., 2019); deviations from that methodology are described below.

The tissue of the same fish from three different species, yellow perch (YP), shorthead redhorse (SHRH), and emerald shiner (ES) were analyzed herein. Sampling sites (Lake Huron - upstream reference site, Stag Island - industrial zone, and Walpole Island - downstream site) and sample processing details are described in the companion paper (Fig. 1 and Table 1 in Muttray et al., 2019). Our methods of fish collection and processing complied with Canadian Council on Animal Care guidance and the study was approved by the GLLFAS-WSTD Animal Care Committee (Government of Canada).

2.1. Analysis of PCBs and organochlorine contaminants

Fifteen ES (from Lake Huron and Stag Island) or 30 ES (from Port Lambton, as they were smaller) were homogenized and pooled such that each pool contained a minimum of 35 g of homogenate, which was not further subsampled prior to analysis in triplicate. Subsampled carcass homogenates of eight individual SHRH fish and three pools of three YP, per sex per site were submitted for analysis. PCBs and OCs were determined by means of US EPA Methods 1668 (US EPA, 2010)

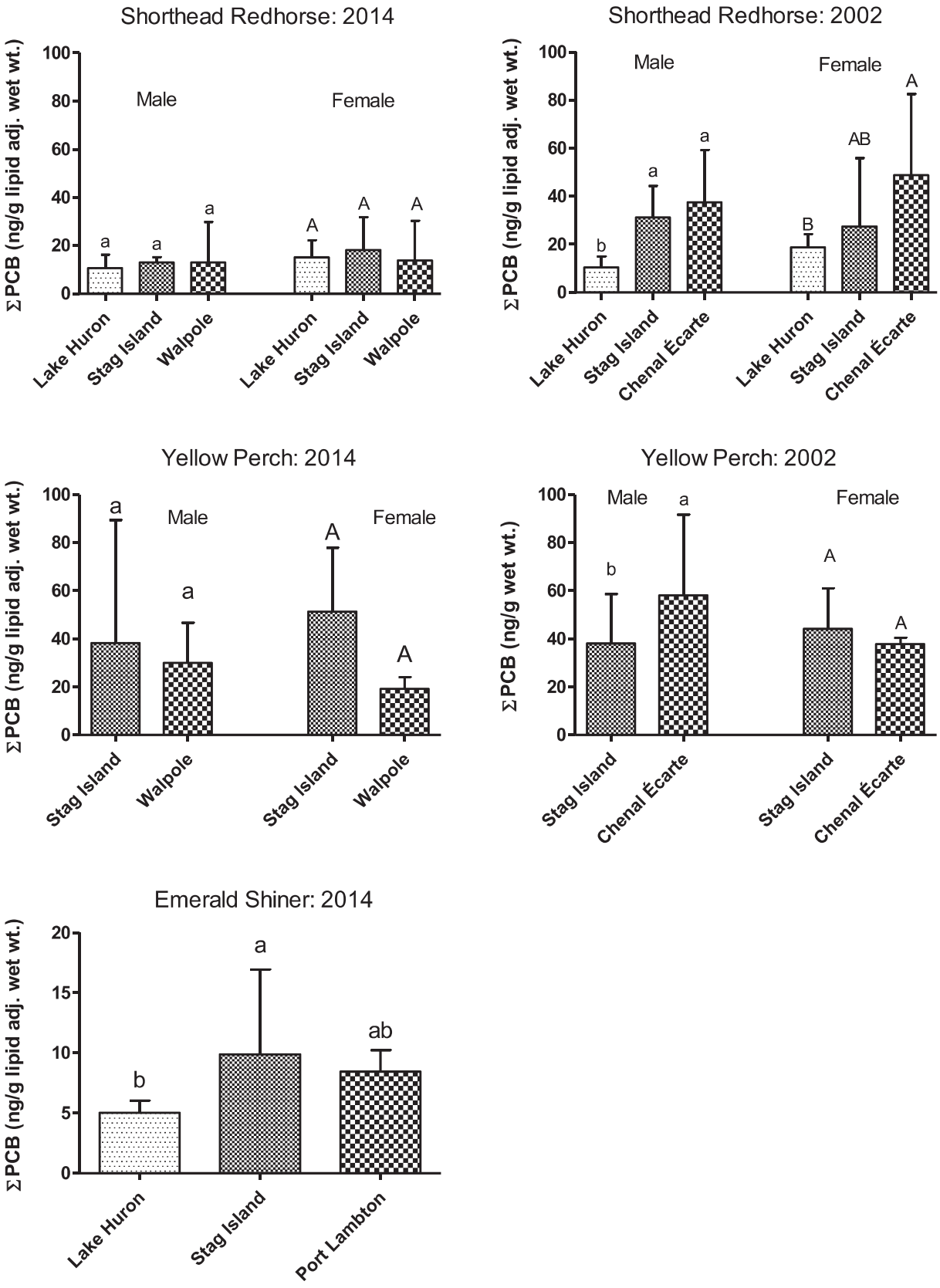

Fig. 1. Mean tissue concentration of ΣPCBs in SHRH, YP, and ES in 2014 and 2002/03 at sites within the St. Clair River AOC. Error bars represent 95% confidence interval. Congener concentrations below the detection limit were treated as half the detection limit. Results for lower and upper case letters represent homogeneous groups within each sex (Tukey's HSD $p < 0.05$).

Table 1
Fold change between 2002/03 and 2014 in the concentration of PCBs, organochlorines, and pesticides in SHRH from the Lake Huron reference site and three sites in the St. Clair River AOC. Arrows indicate direction of change; * indicates the change was significant (Tukey's HSD $p < 0.05$).

Contaminant	Fold change (2014/2002(3))					
	Lake Huron		Stag Island		Chenal Écarte/Walpole	
	Male	Female	Male	Female	Male	Female
∑PCB	1.01↑	1.28↓	2.33↓*	1.44↓	3.19↓*	3.86↓*
Dioxin-like PCBs						
77	1.35↑	1.67↑	3.13↓*	1.91↓	1.49↓	1.97↓
105	1.12↑	1.51↓	2.43↓*	1.54↓	3.26↓*	3.65↓*
118	1.10↑	1.49↓	2.26↓*	1.43↓	3.12↓*	3.79↓*
126	1.92↑	1.36↑	1.32↓	2.00↓	1.48↓	1.76↓
156/7	1.05	1.22↓	2.82↓*	1.34↓	3.51↓*	3.75↓*
167	1.04↓	1.32↓	2.67↓*	1.32↓	3.57↓*	4.02↓*
169	3.98↑*	2.18↑	71.4↓*	3.99↓*	1.33↑	1.90↓
114	1.49↓	1.51↓	5.34↓*	1.36↓	4.58↓*	3.22↓*
123	1.50↑	1.32↓	4.41↓*	1.23↓	2.02↓	4.67↓*
189	1.1↑	1.09	2.25↓*	1.18↓	3.25↓*	3.79↓*
TEQ (WHO)	2.85↑*	1.15↓	1.11↑	1.02	1.78↓	2.16↓*
TEQ (fish)	1.46↑	1.04	2.33↓*	1.39↓	1.92↓	2.05↓
Non-legacy PCBs						
209	1.07↑	1.25↑	1.73↓	2.00↑	1.93↓	2.60↓*
185	1.17↑	1.34↑	1.69↓	1.34↓	1.13↑	3.37↓*
11	2.36↑	1.21↑	1.23↑	3.01↓*	5.35↓*	2.04↑
29	1.92↓*	2.24↓*	2.24↓	1.86↓	2.55↓*	3.23↓*
Organochlorine contaminants						
Hexachlorobenzene	1.86↓*	1.45↓	1.03	1.06	1.22↓	3.34↓*
Hexachlorobutadiene	4.96↓*	5.94↓*	2.69↑	8.15↑*	5.49↓*	6.41↓*
Octachlorostyrene	1.31↑	1.03↑	1.16↓	2.03↑	3.32↓*	7.17↓*
4,4′-DDE	1.02	2.12↓*	3.24↓*	1.67↓*	2.57↓*	4.09↓*
4,4′-DDD	1.92↓*	2.35↓*	3.52↓*	1.48↓	3.42↓*	4.04↓*
4,4′-DDT	1.2↑	2.21↓*	2.62↓*	1.02	3.04↓*	2.68↓*
Dieldrin	1.38↓*	2.14↓*	2.22↓*	2.26↓*	2.99↓*	2.71↓*

and 1699 (US EPA, 2007) by ALS Environmental (Burlington, ON). Briefly, a 10 g wet weight (w.w.) sub-sample of the tissue homogenates was Soxhlet extracted with dichloromethane. A laboratory blank consisting of all reagents, a National Institute of Standards and Technology (NIST) reference material (fish muscle SRM 1946), and a laboratory control sample (corn oil) spiked with the analytes, were analyzed with each batch of 20 samples. The extracts were then rotary evaporated under vacuum, exchanged into DCM:hexane (1:1) applied to a gel permeation chromatography (GPC) column (60 g Biobeads SX3) to remove lipids and other biogenic materials. The GPC eluate was reduced to 1 mL under vacuum. Percent lipid was determined gravimetrically on a subsample of the extract. The GPC eluate was split into separate PCB and OC fractions. The OC fraction was chromatographed on a 2% deactivated silica gel column then reduced to 0.05 mL for analysis. The PCB fraction was cleaned up on an acid-silica gel column (45% *w*/w H_2SO_4 on Silica Gel topped with neutral Silica Gel) then reduced to 0.04 mL for analysis. The extracts were analyzed for 31 OC-related compounds by means of GC-HRMS at ≥10,000 resolution (US EPA, 2007). The extracts were also analyzed for 209 individual and co-eluting PCB congeners, including co-planar PCBs, by GC-HRMS (US EPA, 2010).

Mono-ortho and non-coplanar PCBs were summed to give ΣPCB. Selected non-legacy congeners (PCB 11, 29, 185, and 209) were included in ΣPCB but are discussed separately. Total toxic equivalents for mammals that consume fish (TEQ_m) or for fish (TEQ_f) were calculated from measured coplanar dioxin like (dl-)PCBs concentrations and the 2005 published World Health Organization (WHO) toxic equivalency factors (TEFs) for mammalian species (Van den Berg et al., 2006) or the TEFs for fish (Van den Berg et al., 1998). The TEQ_m were compared to the Canadian tissue residue guideline for PCBs for the protection of mammalian wildlife consumers of aquatic biota (0.79 pg TEQ/g diet w.w., Canadian Council of Ministers of the Environment (CCME), 2001). Laboratory blanks were generally less than instrument detection limits (defined as a signal to noise ratio of approximately 2.5:1) except for HCBD, several chlorobenzenes, and PCB 11(Appendix A Table A-1), in which case the results were blank-corrected. Blank-corrected results less than zero were set to a nominal value of 0. Analyte concentrations below the detection limit were treated as half the detection limit. To facilitate comparisons across species and sites, analyte concentrations were lipid-normalized using sample lipid concentration (reported as pg/g lipid-adjusted w.w.). Tissue analyte concentrations (not normalized) and lipid concentrations are tabulated in the Environment and Climate Change Canada open data portal (https://open.canada.ca/data/en/dataset?organization=ec).

2.1.1. Quality assurance

A recovery spike using corn oil as a sample matrix demonstrated good recoveries of 64 organochlorine analytes (71–121%) (Appendix A Table A-1). Taking these factors into account, no corrections for recovery were made. Analysis of the reference materials (NIST SRM 1946) showed good agreement with all analytes quantified to within ±25% of certified values of organochlorines (17 compounds) and PCBs (29 congeners).

2.2. Statistical analysis

Statistical analysis was performed as described in the accompanying Part 1 (Muttray et al., 2019). Briefly, data were first examined in box whisker plots to assess structure and to identify potential outliers. The Shapiro-Wilk test, Levene's test, and residuals by fitted values plots were used to assess normality and homogeneity of variances. When data did not conform to the assumptions, adjusted logarithmic transformations (Log_{10}, Ln, square root, or inverted square root, as appropriate) were applied. If an outlier remained after that transformation for the purpose of the statistical test it was replaced by either the next highest or lowest (as appropriate) value in the sample group plus or minus (as appropriate) 30–50% of that value (Winsorized mean). Occasionally it was necessary to use the Kruskal-Wallis analysis of variance (ANOVA) followed by Dunn's HSD test for nonparametric samples. If the sample groups were heteroscedastic, we used Welch's ANOVA followed by Dunnett's Modified Tukey-Kramer Pairwise Multiple Comparison Test (DTK).

Statistical analysis of morphometric data and fish health indices (fecundity; condition factor, K; gonadosomatic index, GSI; hepato- (liver-) somatic index, LSI) and deformities, erosions, lesions, or lumps (DELLs) are presented in Part 1 (Muttray et al., 2019). We used ANOVAs to test whether there were changes in tissue concentrations of contaminants of interest across time. The data were analyzed in a general multifactor ANOVA using sex, site, and year as factors (3-way ANOVA) which was followed by Tukey's HSD. Within the ANOVA model, Simple Effects Tests were used to assess for each sex the effect of site within year and the effect of year within site. Sex differences were not explored further. For all tests, alpha was set at 0.05. The analyses were undertaken in Statistix10 (Analytical Software, Tallahassee, FL) and STATA11 (Stata Corp., College Station, TX) except for the DTK test which was run in R; plots were prepared in GraphPad Prism6 (GraphPad Software Inc.). To facilitate calculation of geometric means, concentrations of blank-corrected analytes with nominal value of zero were substituted with a small value (0.1 or 0.5).

To aid in spatial interpretation of organic contaminant concentration in three fish species at three locations, we illustrated tissue concentrations in "heat maps" using Excel conditional formatting, where colors (red-yellow-green spectrum) were applied to data cells in accordance with mean tissue concentration of one analyte in all species (including male and female) and all sampling sites (standard deviation of the mean was not considered as conditional formatting can only be applied to one number.)

We examined the standardized 2014 SHRH metal and organic contaminants as well as fish health data by Principal Component Analysis

(PCA) in StataSE11. After transformation, where required, to stabilize variances and/or normalize the data, we used Linear Discriminant Analysis (LDA) in StataSE11 to explore the separation of fish in ordination space. If there was no site separation, links among health and chemistry variables were not further explored. The LDA was based on the explanatory variables together with some variables of key interest, particularly health and chemistry variables: post estimation tests included sample classification, MANOVA, univariate ANOVA, and correlation analysis (pooled and within group). Non-explanatory variables were removed from the PCA/LDA in an iterative process.

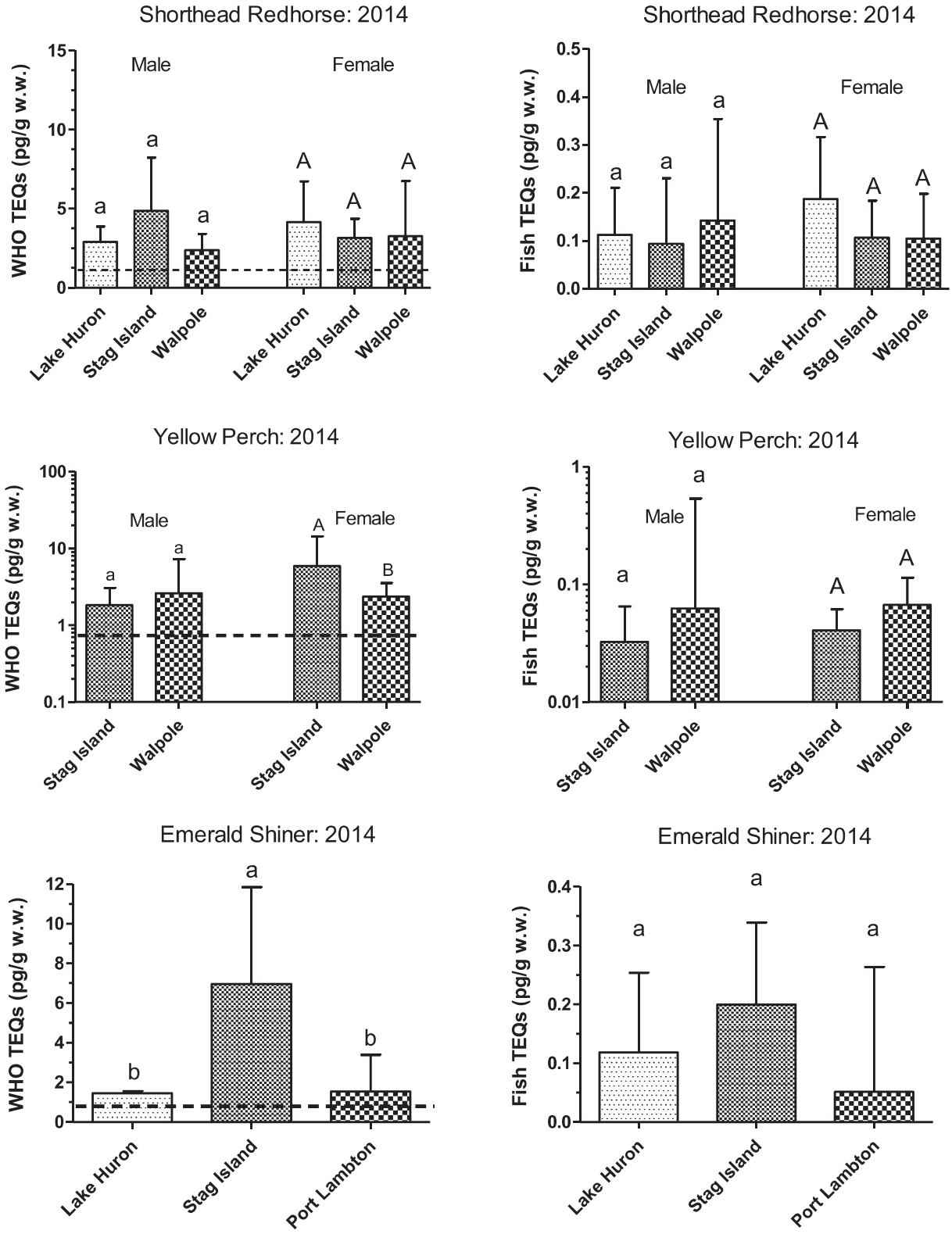

Fig. 2. Mean toxic equivalency for mammals consuming fish (WHO TEQ) and for fish (Fish TEQ) for coplanar dl-PCB in SHRH, YP, and ES taken from sites in the St. Clair AOC and the Lake Huron reference site in 2014. The error bars represent the 95% confidence interval. Lower and upper case letters represent homogeneous groups within each sex (Tukey's HSD $p < 0.05$). The horizontal line represents the Canadian tissue residue guideline for PCBs for the protection of mammalian wildlife consumers of aquatic biota (0.79 pg TEQ/g diet w.w.).

3. Results

3.1. Shorthead redhorse

3.1.1. PCBs

ΣPCB concentrations in male and female SHRH (approximately 15 ng/g lipid-adjusted w.w.) were homogeneous among sites in 2014 (Fig. 1). In contrast, ΣPCB concentrations in fish captured in 2002 were significantly higher at Stag Island and Chenal Écarte compared to Lake Huron.

The concentrations of almost all dl-PCBs were lower in 2014 than in 2002 in SHRH at both Stag Island and Chenal Écarte/Walpole (Table 1): in about 60% of cases the differences were significant (Appendix D). That temporal difference was influenced by several dl-PCBs (PCB 77, 105, 118, 156/7, 167, 169, 114, 123, and 189), which were relatively elevated in fish tissues at Stag Island and Chenal Écarte/Walpole in 2002 (Table 1; Appendix B Figs. B.1, B.2). Concentrations of PCB 169 decreased significantly in male and female SHRH at Stag Island, but showed an increasing trend (4 fold) in SHRH at the reference site (Table 1). In contrast, there were no other changes in tissue concentrations of dl-PCBs in reference site SHRH between 2002 and 2014 (Table 1).

The mean concentration of non-legacy PCBs (PCB 11, 29, 185, 209) in SHRH tissues were homogeneous among sites in 2014, except for PCB 209 in male SHRH, which was significantly higher in tissues from Walpole compared to tissues from the reference Lake Huron location ($F_{(2,83)} = 3.6$, $p = 0.037$), and PCB 11, which was significantly lower in females at Stag Island ($F_{(2,83)} = 3.48$, $p = 0.036$; Appendix D and Appendix B Fig. B.6). In contrast, the spatial distributions in 2002 indicated lowest concentrations for Lake Huron SHRH, and increased concentrations at Stag Island and Chenal Écarte, with trends being significant for PCB 209 (both sexes), PCB185 (females, $F_{(2,83)} = 4.3$, $p = 0.017$), and PCB11 (males, $F_{(2,83)} = 15.53$, $p = 0.0$) (Appendix B Fig. B.7).

There was a temporal 2-fold decrease between 2002 and 2014 in tissue concentration of PCB 29 in Lake Huron reference SHRH. All four nl-PCBs showed significant 1.2–5.4-fold decreases inconsistently between male and female SHRH at Chenal Écarte/Walpole (Table 1 and Appendix D). At Stag Island, only PCB 11 decreased significantly 3-fold in female SHRH.

When calculated based on lipid adjusted wet weights, TEQ_m and TEQ_f were similar across all sites in 2014 (Appendix B Figs. B.8 and B.9). In 2002/03, TEQ_m and TEQ_f were significantly higher in male SHRH at Stag Island and Walpole compared to the reference site. TEQ_m's showed little change over time in SHRH from Stag Island, however they increased significantly (2.8-fold) in male SHRH from Lake Huron. TEQ_m's were lower at Chenal Écarte/Walpole, but only significantly (2-fold) in females. TEQ_f's only decreased in male SHRH from

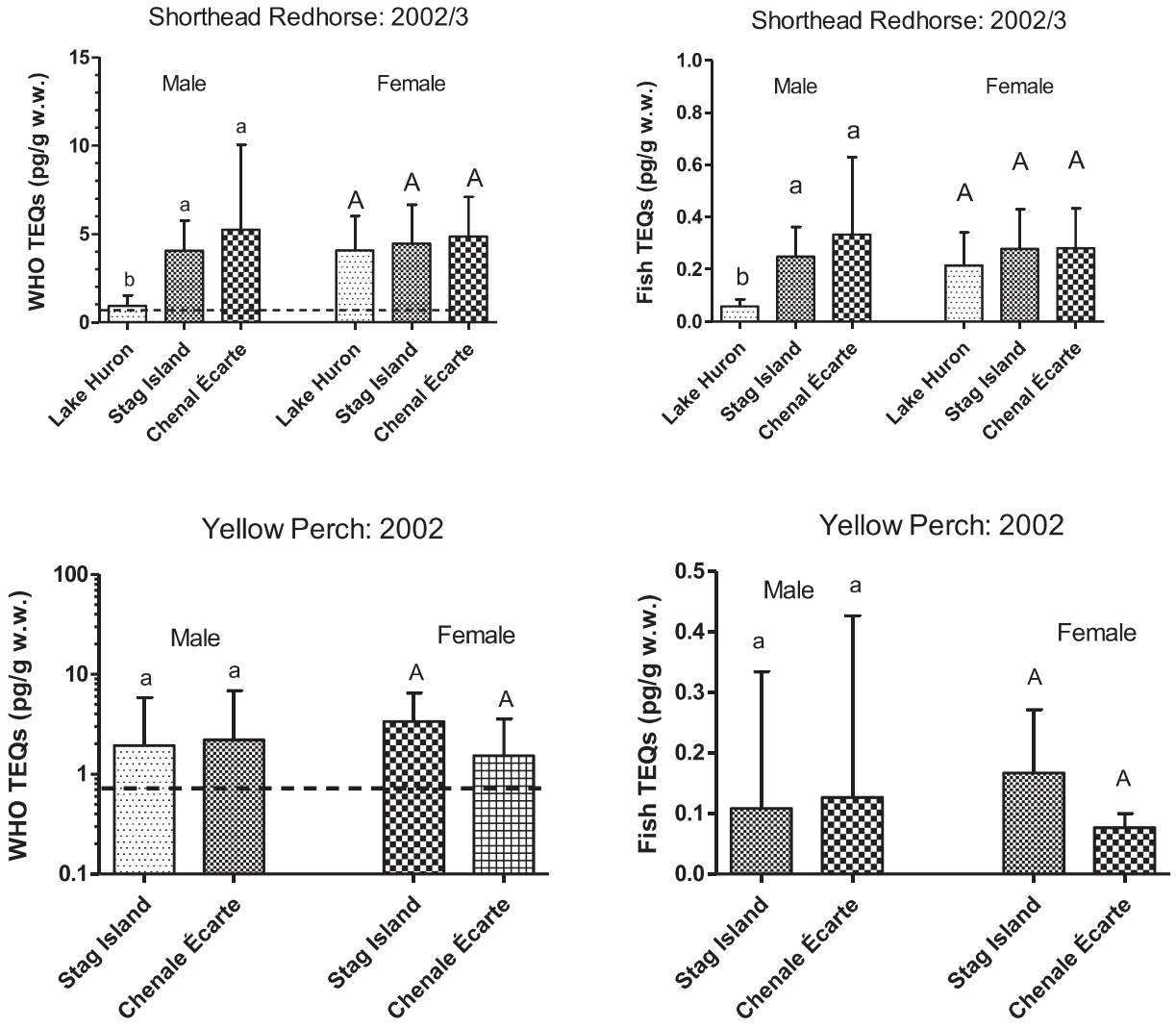

Fig. 3. Mean toxic equivalency for mammals consuming fish (WHO TEQ) and for fish (Fish TEQ) for coplanar dl-PCB in SHRH and YP taken from sites in the St. Clair AOC and the Lake Huron reference site in 2002/2003. The error bars represent the 95% confidence interval. Lower and upper case letters represent homogeneous groups within each sex (Tukey's HSD $p < 0.05$). The horizontal line represents the Canadian tissue residue guideline for PCBs for the protection of mammalian wildlife consumers of aquatic biota (0.79 pg TEQ/g diet w.w.).

Stag Island (2-fold) (Table 1 and Appendix D). The CTRQ for PCBs for the protection of mammalian wildlife consumers of aquatic biota (0.79 pg TEQ/g diet w.w., CCME (2001)) was exceeded in SHRH collected from the three sites in 2002 and 2014 (Figs. 2 and 3).

3.1.2. Organochlorine contaminants

There were some site-specific differences in tissue concentrations of HCB and HCBD in SHRH in 2014 and in 2002. Concentrations of HCB in males (2014) and in females (2002) from Chenal Écarte/Walpole were

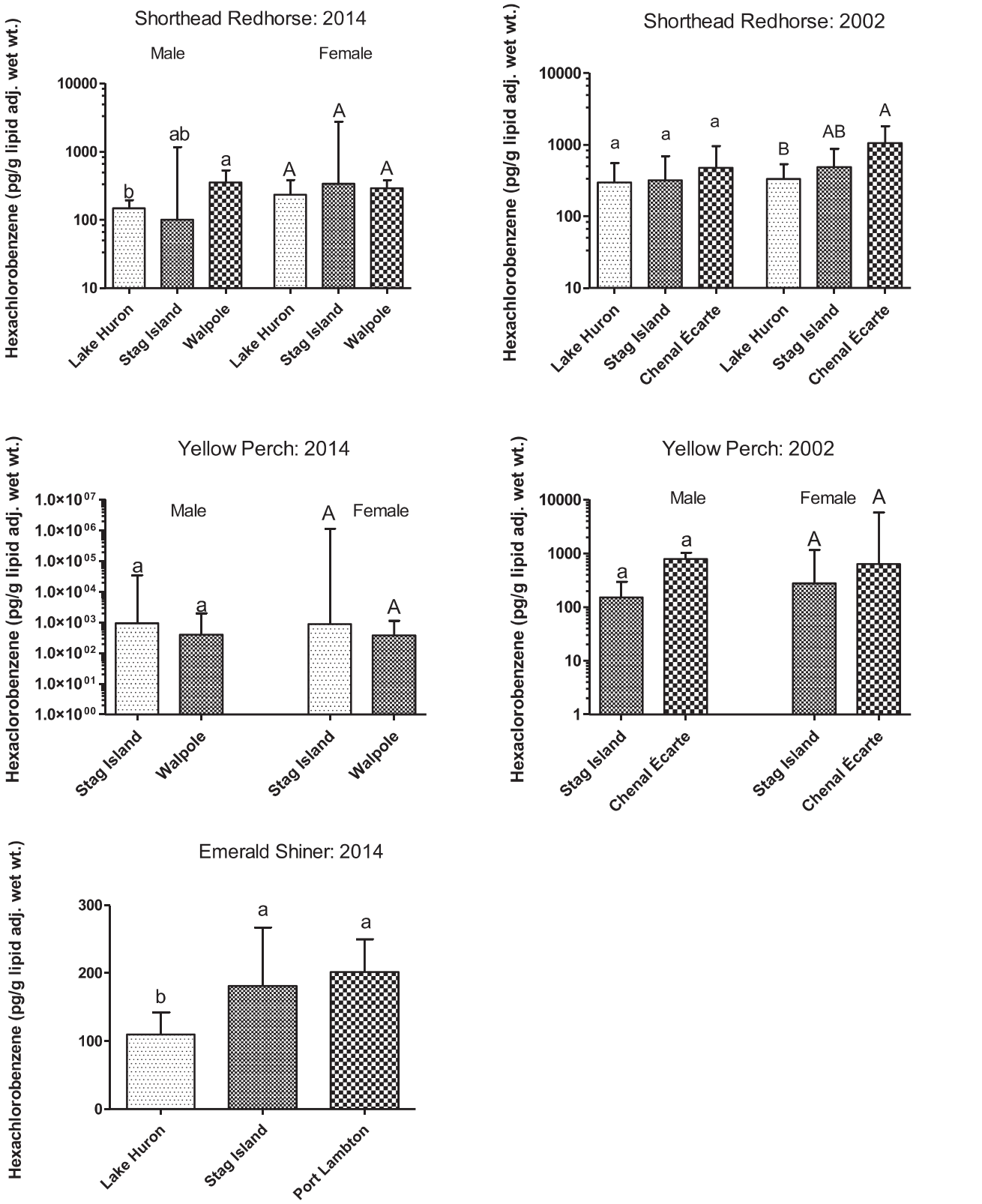

Fig. 4. Mean tissue concentration of HCB in SHRH, YP, and ES from sites in the St. Clair AOC and the Lake Huron reference site in 2014 and 2002/03. Error bars represent the 95% confidence intervals. Data were analyzed by three-way ANOVA with year, sex, and site as main factors. The results are presented as simple effects by year. Lower and upper case letters represent homogeneous groups within each sex (Tukey's HSD $p < 0.05$).

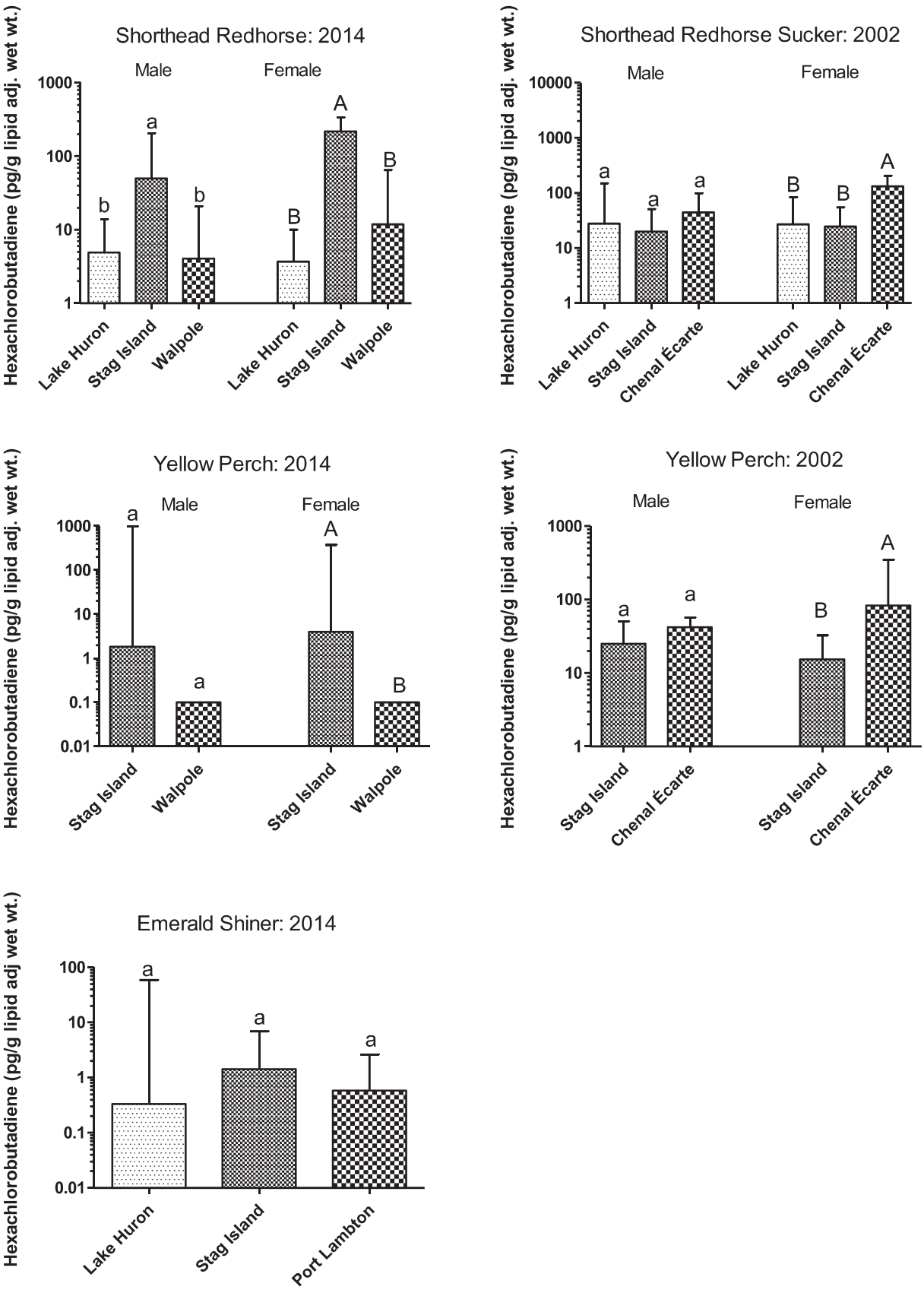

Fig. 5. Mean tissue concentration of HCBD in SHRH, YP, and ES from sites in the St. Clair AOC and the Lake Huron reference site in 2014 and 2002/03. Error bars represent the 95% confidence intervals. Data were analyzed by three-way ANOVA with year, sex, and site as main factors. The results are presented as simple effects by year. Lower and upper case letters represent homogeneous groups within each sex (Tukey's HSD $p < 0.05$).

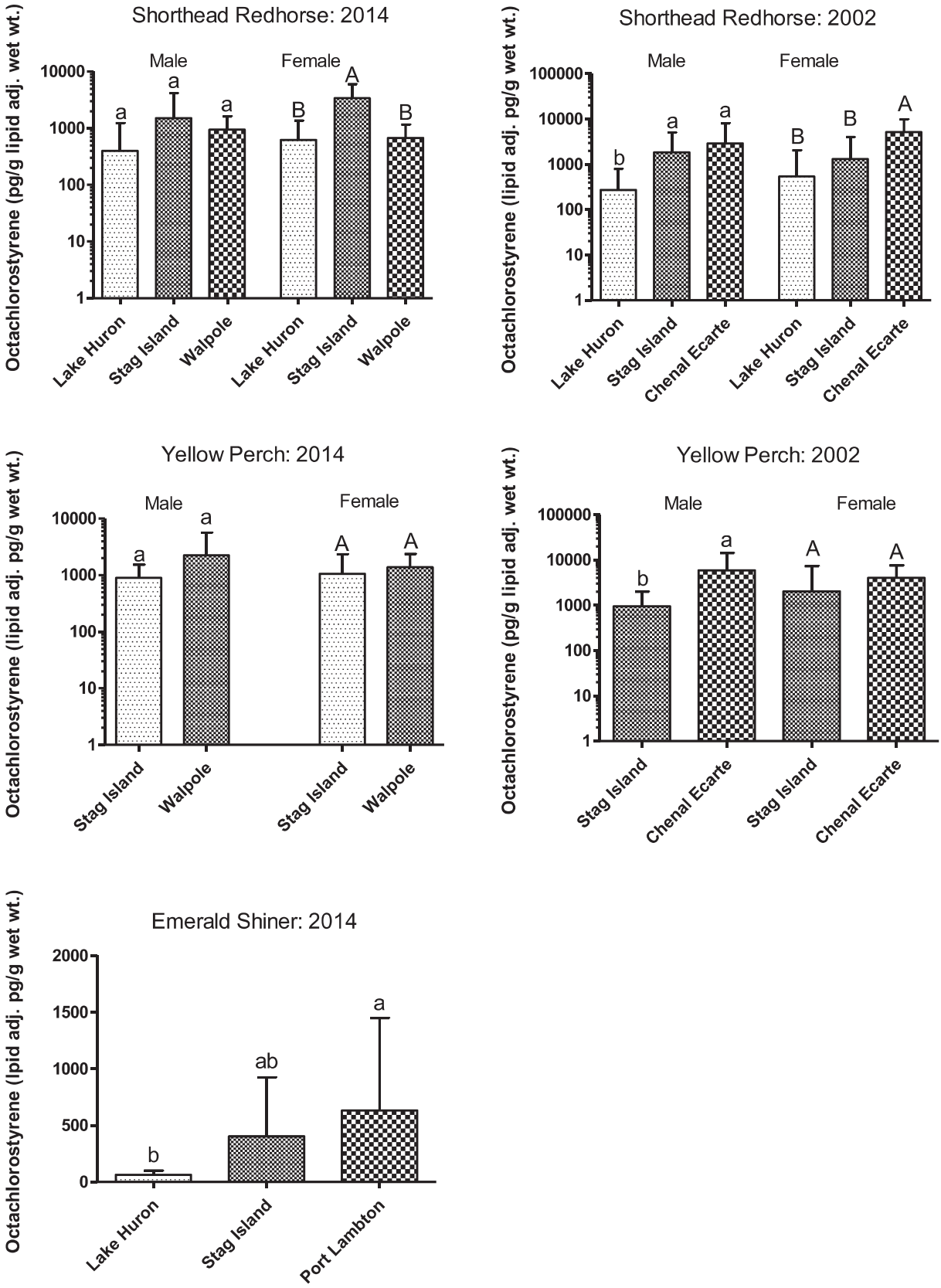

Fig. 6. Mean tissue concentration of OCS in SHRH, YP, and ES from sites in the St. Clair AOC and the Lake Huron reference site in 2014 and 2002/03. Error bars represent the 95% confidence intervals. Data were analyzed by three-way ANOVA with year, sex, and site as main factors. The results are presented as simple effects by year. Lower and upper case letters represent homogeneous groups within each sex (Tukey's HSD $p < 0.05$).

higher than in fish from Lake Huron (Fig. 4). Temporal analysis indicated that a significant decline in concentrations of HCB in male SHRH tissues from Lake Huron (2-fold; $F_{(1,83)} = 5.28$, $p = 0.024$) and in female SHRH from Chenal Écarte/Walpole (3-fold; $F_{(1,83)} = 7.61$, $p = 0.007$), with no change at Stag Island (Table 1).

In 2014, concentrations of HCBD were significantly higher in tissues of male ($F_{(2,83)} = 6.22$, $p = 0.03$) and female ($F_{(2,83)} = 21.88$, $p = 0.0$) SHRH from Stag Island compared to either Walpole or Lake Huron (Fig. 5). In contrast, the only spatial difference observed in 2002 was in female SHRH, which had three times higher tissue concentrations at Chenal Écarte/Walpole. Temporal analysis of tissue concentrations of HCBD between 2002 and 2014 indicate an increase of 8.1-fold (female) at Stag Island, and significant decreases of approximately 5- to 6-fold in tissue concentrations at Lake Huron and Chenal Écarte/Walpole (Table 1 and Appendix D).

Tissue concentrations of OCS in 2014 were significantly higher in female SHRH from Stag Island compared to either Walpole or Lake Huron (4-fold, $F_{(2,83)} = 8.5$, $p = 0.004$; Fig. 6). Spatial trends in 2002 showed that concentrations increased from Lake Huron through Stag Island to downstream Chenal Écarte/Walpole. Temporal analysis indicated that although concentrations of OCS did not decline in SHRH from Lake Huron or Stag Island, there was a significant decrease in concentrations of 7-fold and 3-fold for females and males respectively at Chenal Écarte/Walpole (Table 1 and Appendix D).

Tissue concentrations of dieldrin in both male and female SHRH decreased significantly between 1.4 and 3-fold over time at the reference and St. Clair River sites. In 2014, the highest tissue concentrations of dieldrin were observed at Lake Huron for both male and female SHRH, with concentrations at Walpole being significantly lower for both sexes, whereas only males at Stag Island had lower tissue concentrations (Appendix B Fig. B.10). There were no significant differences among sites in 2002 for dieldrin concentrations (Appendix B Fig. B.11).

There were no spatial trends observed in tissue concentrations of 4,4′-DDE, 4,4′-DDE, and 4,4′-DDT in SHRH in 2014 (Appendix B Fig. B.12), whereas the St. Clair River sites tended to have significantly higher in concentrations than the Lake Huron reference site in 2002/03 (Appendix B Fig. B.13). This shift in spatial trends was predominantly (but not exclusively) due to decreases in concentrations at the St. Clair River sites since 2002/03. The concentrations of all four pesticides decreased significantly by 1.7 to 4-fold at all sites between 2002 and 2014 for male and female SHRH (Table 1).

3.2. Yellow perch

3.2.1. PCBs

In 2014, ΣPCB tissue concentrations in female YP were significantly lower at Walpole than at Stag Island ($F_{(1,16)} = 13.97$, $p = 0.018$; Fig. 1; Lake Huron was not sampled). That was in contrast to YP collected in 2002 at Stag Island and Chenal Écarte, when ΣPCB tissue concentrations were homogeneous between sites for female YP, whereas male YP had significantly higher tissue concentrations at Chenal Écarte ($F_{(1,16)} = 5.43$, $p = 0.033$). Compared to 2002, ΣPCB concentrations were significantly lower (2-fold at Chenal Écarte/Walpole, Table 2) in 2014, which was influenced by decreases in the concentrations of the following dl-PCBs: PCB 77, 105, 118, 156/7, 167, 114, and 123 (Appendix B Figs. B.3 and B.4 and Appendix D). There was a similar, but non-significant, temporal decline in dl-PCB tissue concentrations in YP of both sexes from Stag Island; PCB 126 had noteworthy and significant decreases of high magnitude (17-fold in male and 24-fold decreases in female YP). Interestingly, PCB 126 showed no significant temporal change in YP from Chenal Écarte/Walpole Island.

Non-legacy PCBs 185, 11, and 29 were significantly higher in YP tissues in 2014 at Stag Island compared to Chenal Écarte/Walpole, although the differences were inconsistent between sexes (Appendix B Fig. B.6 and Appendix D). In contrast, nl-PCB 209 tissue concentrations were significantly higher at Chenal Écarte/Walpole compared to Stag Island. This was reminiscent of the spatial distribution of PCB 209 in 2002 (Appendix B Fig. B.7): its tissue concentrations were largely unchanged apart from a 1.6-fold decrease in male SHRH at Chenal Écarte/Walpole (Table 2). Other nl-PCBs also tended to be higher at Chenal Écarte/Walpole in 2002, but have since increased at Stag Island (2.5 to 4.6-fold for PCB 185 and 20 to 32-fold for PCB 11). The results for PCB 11, however, included the low measured tissue concentrations in 2002 compared to other nl-PCBs. Tissue concentrations of PCB 29 decreased significantly in male and female YP at Chenal Écarte/Walpole over the measurement period.

We found no significant temporal differences in lipid normalized TEQ_m and TEQ_f in YP, except for female YP at Stag Island, where TEQ_m increased significantly over time ($F_{(1,16)} = 5.44$, $p = 0.033$); (Table 2). The CTRQ for the protection of mammalian wildlife consumers of aquatic biota was exceeded in YP collected from the two river sites in 2002 and 2014 (Figs. 2 and 3).

3.2.2. Organochlorine contaminants

HCB concentrations in tissue did not significantly differ between sites in 2002/03 and in 2014 (Fig. 4). However, temporal analysis indicated that there was a significant increase in concentrations of HCB in male YP from Stag Island (4.5-fold; $F_{(1,16)} = 6.4$, $p = 0.022$; Table 2) over the study period.

In 2014, HCBD tissue concentrations were lower in female YP sampled at Chenal Écarte/Walpole compared to Stag Island (Fig. 5), which was not the case in 2002, when concentrations were higher at Chenal Écarte compared to Stag Island (Fig. 5). Temporal analysis indicated that there were significant decreases in HCBD concentrations at Stag Island (5.3-fold in males) and at Chenal Écarte/Walpole (844-fold for females and 420-fold for males) (Table 2 and Appendix D). That temporal difference in female YP was the largest decrease for any of

Table 2
Fold change between 2002/03 and 2014 in the concentration of PCBs, organochlorines, and pesticides in YP from the Lake Huron reference site and two sites in the St. Clair River AOC. Arrows indicate direction of change; * indicates the change was significant (Tukey's HSD $p < 0.05$).

Analyte	Fold change (2014/2002(3))			
	Stag Island		Chenal Écarte/Walpole	
	Male	Female	Male	Female
∑PCB	1.0	1.16↑	1.94↓*	1.97↓*
Dioxin-like PCBs				
77	2.44↓*	4.34↓*	6.47↓*	2.07↓
105	1.20↓	1.13↓	2.02↓*	2.28↓*
118	1.03↓	1.02↓	1.74↓*	2.17↓*
126	17.31↓*	23.99↓*	1.63↑	2.84↑
156/7	1.11↓	1.09	2.02↓*	2.02↓*
167	1.09↑	1.14↑	1.69↓*	1.74↓
169	4.38↓	1.01	1.01	1.45↓
114	2.06↓*	1.69↓	4.01↓*	2.36↓
123	2.72↓*	1.24↑	2.14↓*	1.29↓
189	1.24↑	1.18↑	1.66↓	1.59↓
TEQ (WHO)	1.05↓	2.55↑*	2.19↑	3.00↑
TEQ (fish)	3.03↓	2.65↓	1.06	1.29↑
Non-legacy PCBs				
209	1.14	1.16	1.60↓*	1.11
185	4.63↑*	2.50↑*	1.47↓	1.11
29	1.62↓	1.39↑	3.76↓*	3.78↓*
11	32.5↑*	20.4↑*	1.19	5.89↑
Organochlorine contaminants				
Hexachlorobenzene	4.53↑*	1.29↑	2.12↓	1.48↓
Hexachlorobutadiene	5.31↓*	2.20↓	420↓*	844↓*
Octachlorostyrene	1.05	1.93↓	2.66↓*	2.97↓
4,4′-DDE	1.24↓	1.63↓*	1.39↓	2.03↓
4,4′-DDD	1.29↓	1.29↓	1.80↓*	3.30↓*
4,4′-DDT	2.47↓*	2.26↓*	3.13↓*	4.60↓*
Dieldrin	1.83↓*	1.80↓*	2.61↓*	3.11↓*

the organic contaminants analyzed in the present study and was influenced by the blank correction of the 2014 data.

In 2014, there was no difference between sites in YP tissue concentrations of OCS (Fig. 6). This contrasts with 2002, when OCS concentrations were significantly higher in male YP at Chenal Écarte compared to Stag Island. Temporal analysis indicated that there was no change in concentrations of OCS except for male YP (2.7-fold decrease) at Chenal Écarte/Walpole (Table 2 and Appendix D).

There were no spatial trends in YP tissue concentrations of dieldrin during either 2002 or 2014 (Appendix B Figs. B.10 and B.11). Concentrations of 4,4′-DDE, 4,4′-DDD, and 4,4′-DDT were significantly lower in female, but not male, YP at Chenal Écarte/Walpole compared to Stag Island in 2014 and 2002 (with the exception of 4,4′-DDD in 2002; Appendix B Figs. B.12 and B.13). The concentrations of dieldrin and 4,4′-DDT decreased significantly at both locations between 2002 and 2014 (1.8 to 4.6-fold), and 4,4′-DDD decreased significantly at Chenal Écarte/Walpole as well. 4,4′-DDE had generally lesser decreases in concentrations than the other pesticides (Table 2 and Appendix D).

3.3. Emerald shiner

3.3.1. PCBs

We present spatial trends of ΣPCBs (Fig. 1), dl-PCBs (Appendix B Fig. B.5), and nl-PCBs (Appendix B Fig. B.6) for ES tissues from 2014. ΣPCBs was significantly higher at Stag Island compared to the reference site (ANOVA $F_{(2,6)} = 6.4$, $p = 0.0325$). Individual dl-PCB tissue concentrations of some pooled fish samples were higher at Walpole than at Lake Huron (PCB 105, 118, 156/7, 167, and 189). Dioxin-like PCBs 156/7, 167 and 189 were at significantly higher concentrations at Stag Island and Walpole compared to the reference site (Appendix D). Only PCB 209 of the non-legacy PCBs was significantly higher at the river sites compared to the reference site (ANOVA $F_{(2,6)} = 73.09$, $p = 0.0001$). In contrast to SHRH and YP, where lipid concentrations did not differ among sites, lipid concentrations in ES were significantly different, with ES from Lake Huron having the highest, and ES from Walpole having the lowest lipid concentrations.

ES collected from the three sites exceeded the CTRQ guideline for the protection of mammalian wildlife consumers of aquatic biota, with significantly higher concentrations observed at Stag Island, while Port Lambton tissue concentrations were similar to concentrations at the reference site, which only marginally exceeded the guideline (Fig. 2). There was a similar spatial trend for fish-derived TEQs.

3.3.2. Organochlorine contaminants

HCB tissue concentrations were significantly higher at Stag Island and Port Lambton, and OCS concentrations were significantly higher at Port Lambton, compared to Lake Huron, while there was no significant difference between sites in tissue concentrations of HCBD (Figs. 4, 5, and 6, Appendix D). There were also no statistically significant differences in concentrations of pesticides 4,4′-DDE, 4,4′-DDE, 4,4′-DDT, and dieldrin among sample sites in 2014 (Appendix B Figs. B.10 and B.12).

4. Discussion

4.1. PCBs

The measurement of tissue concentrations of PCBs in two fish species from the St. Clair AOC revealed marked reductions between 2002/2003 and 2014, which was particularly evident in SHRH. While site-specific differences in tissue concentrations at river sites vis-à-vis the reference site were observed in 2002/2003, those differences had either largely disappeared or diminished in 2014.

ΣPCB concentration in SHRH decreased to <15 ng/g lipid-adjusted w.w. at the river sites in 2014, which was equivalent to the concentrations in fish from the reference site (Fig. 1). YP collected at Walpole/Chenal Écarte showed a two-fold decrease in lipid-adjusted concentration of ΣPCBs during this period; however, the change in concentration of ΣPCBs was not quite as dramatic in YP at Stag Island. Concentrations of ΣPCBs in ES were also highest at Stag Island (average of 10 ng/g lipid-adjusted w.w.).

The relative contribution of the individual dl-PCB congeners was similar between the sampling years and among fish species, with the penta-chlorinated PCB105 and PCB 118 contributing the majority of the dl-PCB tissue concentration. The sum of dl-PCBs represented at most 0.07% of ΣPCBs, which was similar to the proportion found in Wheatley Harbor, ON (Gilroy et al., 2012). When the ten dl-PCB congeners were recalculated as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) toxic equivalents TEQs, the average concentrations in fish from Stag Island and Walpole were between 1 and 8.6 TEQ_m pg/g w.w. (0.03 to 0.2 TEQ_f pg/g w.w.) in 2014. TEQ_m was significantly higher in ES from Stag Island compared to ES from the reference site or the downstream Port Lambton site. All average TEQ_m values, including those in fish from the reference site, were above the CTRQ guideline for the protection of wildlife consumers of aquatic biota and many were above the guideline for the protection of birds consuming aquatic biota (2.4 pg TEQ/g w.w. diet) (Fig. 2). To evaluate whether there was a potential for risk to fish from dl-PCBs, TEQ_f values were calculated (Van den Berg et al., 1998): the average concentrations in fish from all three sites were between 0.03 and 0.2 TEQ_f pg/g w.w. There appears to be no consistent evidence that such low levels of PCBs in fish tissues cause toxic effects in fish (Henry, 2015). Despite the persistence of low concentrations of PCBs in fish over long periods, concentrations have tended to decrease in the Great Lakes in the absence of active sources (Bhavsar et al., 2007). Our calculated TEQ values did not include polychlorinated dibenzo-p-dioxins/dibenzofurans; however, dioxin-like (dl-) PCBs typically contribute >70% to the total TEQs in the Great Lakes (Bhavsar et al., 2008), suggesting that the inclusion of polychlorinated dibenzo-p-dioxins/dibenzofurans would be unlikely to result in >30% change.

Non-legacy PCB 185 and 209 (hepta- and deca-chlorinated, respectively) dominated the nl-PCB tissue concentration in the three fish species, while di-chlorinated PCB 11, a major nl-PCB (Hu and Hornbuckle, 2010), had the lowest contribution. The general trend in the nl-PCB data was that site-specific differences in tissue concentrations observed in 2002/2003 had largely disappeared in 2014. Although generally not-significant in statistical tests, it appeared that concentrations were higher at Walpole/Chenal Écarte compared to Stag Island in 2002/03. This spatial trend had reversed by 2014 because tissue concentrations of most nl-PCBs had decreased significantly at Walpole/Chenal Écarte in both YP and SHRH. PCB 29 concentrations decreased 2-fold in SHRH from the reference site, but other nl-PCB tissue concentrations have been stable in Lake Huron since 2002/03. Tissue concentrations of PCB 11 increased over the same period up to 32-fold in YP at the river sites, although PCB 11 tissue concentrations were lower than other nl-PCBs. PCB 185 which, like PCB 11, is one of the congeners found in pigment (Bartlett et al., 2010) also increased up to 4.6-fold in YP at Stag Island. The temporal changes may be due to continued inputs to the St. Clair River from the atmosphere and wastewaters as contaminants in pigments and unintended by-products of incineration (Sakai et al., 1993; Ishikawa et al., 2007; Grossman, 2013). Overall, the spatial trends of nl-PCBs were generally similar between male and female SHRH and YP.

Overall, YP had the highest tissue concentrations of all nl- and most dl-PCBs compared to SHRH and ES (Fig. 7): the concentrations were highest in YP at Stag Island. This is in contrast to PCB tissue concentrations for SHRH, which were often higher at Walpole/Chenal Écarte compared to Stag Island. These differences among species were likely due to the different habitat requirements and foraging strategies. The partly piscivorous YP likely have higher lipid-adjusted tissue concentrations of PCBs as they biomagnify in the food chain.

The LSI range for all fish samples was 0.94 to 1.77% (Muttray et al., 2019), which is typical for fish, where the liver represents approximately 1 to 2% of total body weight (Gingerich, 1982). The adjusted

		Shorthead Redhorse						Yellow Perch				Emerald Shiner		
		Male			Female			Male		Female				
Analyte	Units	Lake Huron	Stag Island	Walpole	Lake Huron	Stag Island	Walpole	Stag Island	Walpole	Stag Island	Walpole	Lake Huron	Stag Island	Walpole
TotalPCB	ng/g	11.19	13.08	15.06	15.66	19.38	15.84	38.28	30.00	51.31	19.19	5.02	9.88	8.47
WHO TEQ	pg/g	0.42	0.43	0.62	0.58	0.54	0.50	1.09	2.35	3.46	1.85	0.14	0.81	0.29
Fish TEQ	pg/g	0.14	0.15	0.23	0.22	0.13	0.23	0.02	0.06	0.02	0.05	0.01	0.02	0.01
Dioxin-like PCBs:														
PCB 77	pg/g	17.42	10.53	24.71	21.77	19.06	20.16	10.30	2.92	8.03	7.75	13.41	12.24	11.59
PCB 105	pg/g	201.60	250.26	243.00	298.88	356.78	246.21	764.22	538.25	1060.90	354.90	120.86	165.16	178.15
PCB 118	pg/g	510.39	667.88	692.15	737.56	1011.58	726.94	1960.80	1613.30	2503.90	1005.10	275.64	429.80	493.16
PCB 126	pg/g	2.09	1.33	2.27	4.14	1.29	2.35	0.28	7.65	0.29	6.71	1.53	3.60	1.16
PCB 156/7	pg/g	78.98	91.47	108.84	120.64	130.56	113.95	298.55	253.36	378.92	143.94	32.16	62.77	65.60
PCB 167	pg/g	33.95	41.21	51.65	47.41	58.71	52.59	127.92	108.67	156.97	56.56	14.25	31.01	30.24
PCB 169	pg/g	1.82	0.25	1.15	3.19	0.58	2.08	0.24	1.17	0.99	0.71	0.37	1.38	0.77
PCB 114	pg/g	10.63	12.79	17.35	18.13	26.50	18.93	44.67	31.69	39.44	21.45	7.01	10.63	8.88
PCB 123	pg/g	7.93	12.92	16.14	14.21	14.71	8.58	22.78	29.45	61.80	20.49	6.56	7.86	6.96
PCB 189	pg/g	9.44	10.95	15.03	14.32	14.33	15.43	35.94	27.95	40.07	17.95	3.41	6.57	6.63
Non-legacy PCBs:														
PCB 209	pg/g	55.87	80.65	150.42	105.15	168.07	132.74	136.25	280.23	117.60	253.02	12.79	34.53	74.89
PCB 185	pg/g	14.73	13.53	18.09	19.90	19.72	20.74	59.59	40.52	75.65	31.36	6.76	12.11	9.67
PCB 11	pg/g	1.30	2.69	1.70	2.04	1.09	4.08	11.34	2.25	6.28	2.89	1.41	1.87	5.44
PCB 29	pg/g	7.46	9.36	8.89	8.51	17.67	11.72	10.13	7.73	21.14	6.22	3.53	7.85	6.47
Organochlorine Contaminants:														
OCS	pg/g	575.39	1913.05	1028.10	760.86	3567.66	749.62	899.40	2230.10	1057.70	1373.00	66.40	406.00	634.30
HCB	pg/g	153.61	399.06	383.34	264.15	777.66	302.65	678.17	369.82	342.94	371.25	109.90	181.10	201.70
HCBD	pg/g	6.96	62.49	8.66	5.22	218.77	20.84	4.77	0	7.06	0	0.68	0.82	0.36
Dieldrin	pg/g	410.20	289.47	243.61	396.55	307.68	264.79	438.84	266.82	504.22	247.85	206.30	134.50	182.60
DDT	pg/g	291.00	389.58	219.48	184.88	371.96	209.17	421.01	176.78	554.94	112.11	5.39	4.86	13.89
DDE	pg/g	1632.68	1953.89	1991.13	1679.96	2042.68	1720.68	4320.10	3526.40	5753.50	2217.60	945.80	1279.10	2645.60
DDD	pg/g	289.27	444.22	298.68	268.29	590.88	341.82	374.62	350.09	624.64	205.66	131.65	145.13	214.29

Fig. 7. Heatmap of mean PCB, organochlorine, and pesticide tissue concentrations (ng/g or pg/g lipid-adjusted w.w.) for SHRH, YP, and ES in 2014 at the Lake Huron reference site and at downstream locations in the St. Clair River AOC (Stag Island, Walpole/Chenal Écarte, Port Lambton). Colors correlate with geometric mean concentration (red = highest, green = lowest) of each analyte at all sites across all species sampled.

mean liver weight (ANCOVA) was significantly higher in male and female SHRH from Walpole and Stag Island compared to Lake Huron in 2014 and 2003, respectively, whereas dl-PCB concentrations were largely similar among all three sites in 2014, indicating no apparent relationship between three adjusted mean liver weight and dl-PCB concentrations. In 2003 Stag Island SHRH, however, dl-PCBs 114 and 189 were significantly elevated compared to Lake Huron fish. Others have shown that fish liver weight can be increased in response to exposure to dl-PCBs (Ahlborg et al., 1994). This may be attributable to a higher liver mixed-function oxygenase activity in response to plasma PCB concentrations (Gilroy et al., 2012). An increase in enzyme activity has energetic costs associated with it (Bains and Kennedy, 2004) and thus may require an increased level of glycogen storage in the liver. For instance, rainbow trout (*Oncorhynchus mykiss*) exposed to β-naphthoflavone increased plasma glucose initially after injection, suggesting enhanced glycogenolysis to meet increased energy demands (Al-Hameedi, 2008; Tintos et al., 2008). Thus, higher dl-PCB levels in SHRH in 2003 at Stag Island may have contributed to an elevated ANCOVA-adjusted mean liver weight. Factors other than PCBs, however, likely affected mean liver weight in SHRH in 2014 at Walpole. Whether glycogen storage is responsible for the increase in ANCOVA-adjusted mean liver weight (and LSI) observed in the SHRH at Walpole and Stag Island, however, requires further elucidation.

4.2. Organochlorine contaminants

Generally, concentrations of organochlorine pesticides and by-products have declined significantly in tissues of SHRH and YP at Chenal Écarte/Walpole over the measurement period (Tables 1 and 2; Figs. 4, 5, and 6). This may be due to long-term effects of remediation actions that have taken place in the Upper St. Clair River since contamination by organochlorines was first identified. Fish tissue contamination, however, appeared to remain higher for some organochlorines and have even increased at Stag Island, where HCBD had significantly increased 8-fold in SHRH, and HCB had increased 4.5-fold (in males) in YP. In comparison, previously high concentrations of OCS and HCB measured in YP and other species in Lake St. Clair in the 1980s had declined steadily to near or at MDL concentrations in the early 2000s (Gewurtz et al., 2010). In ES, we observed no significant differences in HCBD concentrations among sites. HCB and OCS were significantly higher in ES tissues from Stag Island and Port Lambton, respectively, compared to Lake Huron (ANOVA, $p < 0.05$). Spatial trends of organochlorines in the AOC in the early 2000s were also analyzed by Gewurtz et al. (2010) using young-of-the-year spottail shiners: OCS and HCB concentrations were generally higher in the St. Clair River when compared to Lake St. Clair or Lake Huron, in particularly at sites near Suncor, Talfourd Creek (Shell), and Lambton Generating Station. Interestingly, concentrations of OCS and HCB were also elevated at Stag Island immediately downstream of the confluence of Talfourd Creek and the St. Clair River in the present study, similar to the study by Gewurtz et al. (2010). This is despite difference in species, age of fish (young-of-year versus adult), anthropogenic events since 2010, or differences in sampling location around Stag Island, as shiners have high site fidelity.

In the present study, ES generally had lower tissue concentrations of organochlorines than SHRH and YP. HCB (Moermond and Verbruggen, 2013) and OCS (Veith et al., 1979; Kaminsky and Hites, 1984) bioaccumulate and biomagnify with trophic level, whereas HCBD does not biomagnify (Lecloux, 2004; United Nations Environment Programme, 2012). Assuming that ES are a potential food item for YP, our present data support biomagnification through the food chain. On the other hand, ES and YP are pelagic fish, while SHRH are benthic fish, and different organochlorine tissue concentrations may result from differences in habitat and prey preference, such that SHRH may have higher exposure to organochlorine inputs from sediments compared to ES and YP. In addition, older age contributes to a higher tissue concentration. The average lifespan of ES is 4 years (Froese and Pauly, 2018), shorter than the range of age measured for the SHRH in the present study (6.7 to 9.0 years), which likely led to the higher tissue concentration observed in SHRH compared to ES. Similarly, the average age of SHRH was twice that of YP across all sites suggesting a greater potential for bioaccumulation in SHRH (Muttray et al., 2019).

As previously documented by Gewurtz et al. (2010), OCS was phased out from the chlorine industry starting in the 1970s (Kaminsky and

Hites, 1984) and industrial discharges of OCS to the St. Clair River were terminated in 1993 (St. Clair River RAP Team, 2006). The increase in concentrations since 2002/2003 for HCBD in female SHRH and HCB in male YP at Stag Island are difficult to explain. Releases of HCB in Ontario decreased by 62% between 1988 and 2000 (Environment Canada and US EPA, 2004). Sediment was dredged to reduce the load of chlorinated organic compounds (and mercury) in the Upper St. Clair River in 1996 and 2002–2004 (St. Clair River RAP Team, 2006; Richman and Milani, 2009). Loadings of HCBD from the Cole Drain are no longer a factor since the 1998 remediation and decommissioning of the upstream land fill and restoration of the drain itself (Environment Canada, 2000; Lecloux, 2004). HCBD and HCB, however, are among the most persistent environmental pollutants because of their chemical stability and resistance to biotic and abiotic degradation (ATSDR, 1994; United Nations Environment Programme, 2012; ATSDR, 2015). In addition, HCBD has a high bioconcentration factor in fish, which can be as high as 17,000 (rainbow trout), and preferentially partition to sediments and biota over water (ATSDR, 1994; ATSDR, 2015). It is unlikely that recent atmospheric sources of organochlorine contaminants are responsible for the significant increase in HCBD and HCB tissue concentrations observed at river sites in SHRH and YP, respectively, as there was no change or a decrease in tissue concentrations over the same time period at Lake Huron (for SHRH). Rather, residual sediment contamination and sediment resuspension may have contributed to the bioavailability of HCB and HCBD to the food chain and to the observed temporal increase in HCBD and HCB concentration in SHRH and YP tissues at Stag Island. HCBD elevation in the particulate fraction of waters in the St. Clair – Detroit River corridor near Port Lambton in relation to an upstream reference site at the confluence with Lake Huron, was an indication of sources downstream of the reference site (Burniston et al., 2006; Richman and Milani, 2009). Despite those potential inputs and observed increases in HCBD fish tissue concentrations at Stag Island, both SHRH and YP have shown significant decreases in tissue concentrations of other organochlorines at Chenal Écarte and Walpole since 2002/03.

Tissue concentrations of organochlorine pesticides 4,4′-DDD, 4,4′-DDE, and particularly of 4,4′-DDT and dieldrin, have significantly decreased in SHRH and in YP since 2002/03 in Lake Huron and at the AOC sites Stag Island and Chenal Écarte/Walpole (Tables 1 and 2; Appendix B Figs. B.10 to B.13).

Interestingly, 2014 levels of dieldrin in SHRH were significantly lower in the St. Clair River compared to Lake Huron. Other pesticides (4,4′-DDD, 4,4′-DDE, and 4,4′-DDT) appeared not to be different in concentration in SHRH tissues across sites. There were no site-specific differences in pesticide tissue concentrations in YP in 2002/2003 either; however, in 2014, female YP had significantly lower pesticide tissue concentrations at Walpole compared to females at Stag Island, whereas concentrations in males were similar. Of the three fish species examined, pesticide tissue concentrations were lowest in ES and showed no differences across sites. This is in contrast to observations from 2010, where DDT concentrations were highest in young-of-the-year spottail shiners collected at Sarnia Bay, Suncor, and Talfourd Creek when compared to Lake Huron reference sites (Gewurtz et al., 2010), which suggests that there may be a recent (2010 to 2014) improvement in relative DDT tissue concentrations in the AOC. The present study, however, excluded young-of-the-year ES, and thus shiner species and life history/age may account for some or all of the observed differences in trends.

Although dieldrin has not been used as a pesticide in Canada since 1984 (Health Canada, 1995), residues continue to be detectable in the food chain due to its high biological and chemical stability. Detection of dieldrin in the environment may result from the application of either aldrin or dieldrin as aldrin is rapidly metabolised to dieldrin by plants and animals (Burniston et al., 2006). The finding that dieldrin was significantly higher in tissues of SHRH sampled from Lake Huron was not supported by the relative concentrations of dieldrin found in water in Lake Huron and the St. Clair River AOC in 2006 (Burniston et al., 2006). SHRH are bottom feeders and thus would more likely reflect sediment-borne concentrations rather than water concentrations. The observations may reflect a greater temporal decline of dieldrin at the St. Clair River sites compared to Lake Huron.

4.3. Correlations among morphological health indicators, metals and organic contaminants in 2014 SHRH

Muttray et al. (2019) described the multivariate correlations among tissue concentrations of metals in SHRH and the following health indices: fecundity, GSI, LSI, and K. Despite significant differences in tissue concentrations of metals among sites, the measured metals had inconsistent relationships to the measures of fish health and the observed inter-site differences in those variables (Muttray et al., 2019). Because of the potential for interactions among inorganic and organic tissue contaminants, we included the metal data from our companion publication in the present multivariate analysis of tissue concentrations of organic contaminants and the various measures of health of SHRH.

We used PCA to reduce the number of variables without losing separation of fish by site in the LDA analysis. The first four (male) to six (female) principle components explained up to 86% of the observed variability in the data. For male and female SHRH, the reduced variable set in the LDA showed good site separation based on the explanatory variables without any misclassification of fish by site (Appendix Figs. C.1 and C.8). Thus, the sampled fish were representative of their sites based on their measured characteristics and those sites were well separated in ordination space (MANOVA for female SHRH: $F_{(28,16)} = 5.08$, $p = 0.0007$, Wilk's $\Lambda = 0.0102$; and male SHRH: $F_{(32,12)} = 5.82$, $p = 0.0012$, Wilk's $\Lambda = 0.0037$).

For female SHRH, the post-estimation univariate ANOVA showed site differences for tissue concentrations of OCs, HCBD, GSI, K, and LSI ($p < 0.05$; Appendix C Table C.1). Those explanatory variables along with Hg, V, Ni and Zn were included in the LDA. Generally, correlations observed between chemical variables and indicators of fish health in pooled samples from all three sites (Appendix C Table C.2) were not supported when the data were broken out by site (Appendix C Tables C.3, C.4, C.5), possibly because of the reduced sample size at each site or because of site specific effects.

At Stag Island, there was a positive correlation between GSI/LSI in female SHRH and lipid-normalized concentrations of PCB 169 (GSI: $r = 0.78$, $p = 0.023$; LSI: $r = 0.74$, $p = 0.036$; Appendix C Table C.3, Appendix Figs. C.3 and C.4). There was a weak positive relationship between fecundity and PCB 169 in the pooled data from all sites ($r = 0.58$, $p = 0.003$) (Appendix C Table C.2, Appendix Fig. C.2). Concentrations of PCB 11 were also correlated with GSI and K in the pooled sample ($r = 0.60$, $p = 0.002$, and $r = 0.48$, $p = 0.017$, respectively), but this relationship was not observed at any of the individual sites. Moreover, tissue concentrations of PCB 169, which were lower at Stag Island compared to other sites, were also lower than several other dl-PCB congeners (e.g. 105, 118, 156/7, and 167). Thus, the relationship between PCB 169 and those indicators of fish health at Stag Island might not have been causative and may have been confounded by other interacting variables, such as by HCBD (Appendix C Tables C.2, C.3, and C,7). Vanadium also correlated positively with GSI in female SHRH at Stag Island ($r = 0.71$ $p = 0.049$). Although PCB 169 was uncorrelated to other contaminants in female SHRH at Stag Island it was negatively correlated with HCBD in Lake Huron female fish ($r = -0.7438$; $p = 0.03439$), as well as in pooled data for male ($r = -0.5033$, $p = 0.01218$) and female ($r = -0.6137$, $p = 0.00142$) fish.

There was negative correlations between OCS, HCB, dieldrin, and K in female SHRH from Lake Huron; whereas only at Walpole was dieldrin negatively correlated with K (Appendix Figs. C.5, C.6, and C.7). There were weak negative correlations between OCS/HCBD and GSI/K in the pooled dataset. Also in the pooled dataset, HCB and dieldrin had weak negative correlations with K. OCS (Fig. 6) and dieldrin (Appendix B Fig. B.10) concentrations in tissues were significantly lower in female

SHRH at Lake Huron and at Walpole, respectively, compared to Stag Island. The absence of relationships between these chlorinated organics and K in the female SHRH at Stag Island might have been due to confounding interactions with other variables that were also elevated in those fish.

For male SHRH, the post-estimation univariate ANOVA (Appendix C Table C.6) revealed site differences for HCBD, dieldrin, metals (Mg, Ni, Zn, V, Sr, and Fe), and LSI. We also noted differences among sites for Mn and Ba in the 3-way ANOVA (Muttray et al., 2019). As shown by Muttray et al. (2019), there were weak negative relationships in the pooled data between K and LSI and the following metals: Ba, Mg, Mn, Zn, V, and Sr. There were also a weak positive relationship between LSI and Hg in the pooled data. Also in the pooled data set, we observed a weak positive relationship between HCBD and K ($r = 0.44$, $p = 0.034$) and a weak negative relationship between dieldrin and LSI ($r = -0.48$, $p = 0.018$) in male SHRH (Appendix C Table C.7). The respective correlations between HCBD/dieldrin and K/LSI, however, were not apparent when the data were broken out by site (Appendix C Tables C.8, C.9, and C.10): in the case of HCBD ($r = 0.67$ $p = 0.08$) that was likely because of the lower sample size at Stag Island compared to the pooled samples.

At Lake Huron, there was a positive correlation between PCB 169 and K ($r = 0.71$ $p = 0.05$; Appendix C Table C.8). Tissue concentrations of PCB 169 in male SHRH were higher in Lake Huron in 2014, where they had increased 4-fold since 2002/03, than at the other sites (Appendix B Fig. B.1). PCB 169 is unlikely to affect K negatively since it has a comparatively small contribution to toxicity (TEF of 0.03; Van den Berg et al., 2006). Moreover, there was no relationship between PCB 169 and fish health indicators at Walpole, where male SHRH had similar tissue concentrations of PCB 169 to the Lake Huron fish. At Lake Huron, there was a negative correlation between PCB 169 and V ($r = -0.77591$; $p = 0.02895$), which in turn had a negative and strong correlation with K ($r = -0.902$; $p = 0.00219$): those relationships suggest the potential for confounding interactions among the measured variables.

The pooled datasets indicated that the morphological health indicators of female SHRH tended to better correlate with tissue concentrations of organic contaminants; whereas health indicators of male SHRH tended to be correlated with metal contaminants. As in the case of the metal contaminants (Muttray et al., 2019), however, the organic contaminants had inconsistent relationships with the health indicators in SHRH of both sexes in 2014. While it is likely that the significant correlations in the pooled data were strengthened by increased sample size ($n = 24$), as with all correlations analyses, there is the potential for confounding and coincidental factors.

5. Conclusion

The St. Clair River is an international boundary water that is representative of industrially impacted rivers around the world. Most of the measured PCB, organochlorine pesticide and by-products in fish tissue decreased between 2002/2003 and 2014 in the St. Clair River AOC, indicative of an overall improvement in tissue concentrations. Non-legacy PCBs not previously investigated (PCB 11 and 185) represented interesting exceptions in YP tissues at the Stag Island industrial zone, although concentrations were low. Based on multivariate analysis of morphometric variables (condition, GSI, fecundity, and LSI), we found no evidence of obvious, consistent, or significant adverse relationships between tissue concentrations of organic contaminants and growth or reproduction of SHRH in 2014. The present study revealed inter-species differences in tissue concentrations of PCBs and organochlorines: those differences appeared to be not only due to a contaminant's association with bottom sediments. PCBs and organochlorines tended to be at higher concentrations in YP, which are located higher in the food chain, by virtue of their partly piscivorous nature, than ES and SHRH. The larger and benthic SHRH, which are at a lower trophic level, had somewhat lesser tissue concentrations of organic contaminants than YP. Overall, the smaller, shorter-lived, and pelagic ES had the lowest tissue concentrations of the three species.

The tissue concentrations of dl-PCBs tended to mostly decline or not change in SHRH and YP over the 12-year period with the exception of PCB 169 in male SHRH in Lake Huron. Mammalian-derived TEQ_m's calculated for dl-PCBs showed that the CTRQ guideline for PCBs for the protection of mammalian wildlife consumers of aquatic biota was exceeded at all sites in all sampling years. Fish-derived TEQ_f's, however, indicated low potential health risk to fish.

Tissue concentrations of organochlorine contaminants and pesticides either mostly declined or did not change in SHRH and YP over the 12-year period. We observed, however, increased tissue concentrations of HCBD and HCB since 2002/2003 at Stag Island, the origins of which might be due to legacy contamination of sediments since those organochlorines are persistent and bioaccumulative, or an unidentified pollution event. Despite those increased tissue concentrations, the post-LDA estimation tests showed no correlations between HCBD and morphological health indicators in male or female SHRH at Stag Island. Future monitoring of tissue concentrations of organochlorines in fish at Stag Island is recommended to establish whether these data represent trends or isolated pollution events.

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Declaration of competing interest

The authors declare that they do not have actual or potential conflicts of interest.

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