Draft Report:

Beneficial Use Assessment for BUI #4: Fish Tumours or Other Deformities in the Detroit River Area of Concern

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Executive Summary

This report compiled Canadian data on liver tumour prevalence in brown bullheads and related data on sediment chemistry and fish contaminant levels to assess the delisting criteria for BUI #4: Fish Tumors or Other Deformities in the Detroit River Area of Concern. The delisting criteria identified by the Canadian Stage II Detroit River RAP report is given as follows:

> "When the incidence rates of liver tumours in (3-5 year old) brown bullhead are not statistically different than the Great Lakes background rate."

Brown bullhead were collected in 2002 and 2016 from three locations in Canadian waters of the AOC. There were 67 and 45 fish collected that were in the 3-5 year old age bracket in 2002 and 2016, respectively. Only one fish from 2002 contained a liver neoplasm giving an absolute tumour prevalence 1.5% and 0% for the 2002 and 2016 survey years, or a combined across year prevalence of < 1%. Each estimate was not statistically different than Great Lakes background tumour rate of 2%.

However, because fish were collected at different time points spanning more than a decade apart and from different areas of the AOC, a weight of evidence (WOE) exposure assessment was performed to justify whether or not samples could be pooled across time points and/or fish collection locations. The WOE took into consideration differences in sediment PAH concentrations in the AOC over time, differences in sediment PAHs concentrations at different bullhead sampling locations, compared sediment PAH concentrations to benchmarks recommended for the protection against fish tumours and contrasted chemical signatures in fish collected from different sampling locations. The WOE demonstrated that sediment contamination of carcinogenic PAHs, linked to brown bullhead liver neoplasm prevalence, has not changed over the time period between 1999 to 2013. This provides support for pooling samples across different survey years. However, sediment PAH concentrations and fish chemical signatures were found to differ between collection locations. Specifically, PAHs in the upstream Peche Island area were significantly lower than present at collection locations in the midstream (Turkey Creek) and downstream (Bois Blanc Island) Canadian river reaches. There were no differences in sediment contamination or estimated chemical exposures of fish between the Turkey Creek and Boise Blanc collection areas or between collective waters of the middle and lower Canadian river reaches. This suggests that fish samples could be pooled between Turkey Creek and Bois Blanc collection zones but that these samples should not be pooled with fish collected from the less contaminated Peche Island area.

Taking the WOE pooling suggestions into consideration, the BUI #4 delisting criteria was reassessed using temporally pooled samples from Peche Island and Turkey Creek + Boise Blanc. The authors of this assessment note that although the delisting criteria specifies the age range of fish (aged 3-5) to be used in the assessment, this age range differs from that used by other Canadian AOCs when addressing BUI #4. Other Canadian RAPs assessments have adopted the approach of including all fish age 3 and above in their assessments which is compatible with the

range of fish ages included in the reference database used to estimate the background tumour rate. Because the reference database on brown bullhead lesion preferences includes older fish with a higher likelihood of spontaneous lesion generation, the current delisting criteria may be biased towards Type I error (False negative) when it censors fish to the 3 to 5 year old age bracket. Also, many fish of the fish collected in the AOC were greater than 5 years of age meaning that their removal reduced the statistical power of the analysis since fewer fish were put into contingency tables used for statistical testing.

In order to address these issues, this document generated two sets of delisting statements that could be applied to the upstream waters of the AOC and to the midstream and downstream waters of the AOC. In addition, the delisting criteria was examined using both censored age 3-5 year old fish and all fish over the age of 3 to provide further support for recommendations.

Conclusions for the upstream Canadian waters of the Detroit River:

> 1. Peche Island, representative of the upstream Canadian reach of the Detroit River, had neoplasm incidents in 0/28 aged 3-5 fish and 0/44 aged 3+ fish. The data suggest a low tumour prevalence in this reach of the Detroit River but are insufficient in sample size to test the delisting criteria with sufficient statistical rigor. However, WOE exposure assessment indicates that this area of the river has among the lowest concentration of carcinogenic PAHs throughout the entire AOC. In addition, previous Canadian BUI #4 assessments have classified Peche Island as a Great Lakes reference site. Indeed a portion of the 2002 Peche Island data were included in the reference database values used to establish the Great Lakes Background Tumour rate of 2%. Given that Peche Island is already incorporated into the Great Lakes reference database and there is no counter evidence suggesting elevated liver neoplasms at this location, it is recommended that the upstream Canadian waters of the Detroit River are consistent with an unimpaired assessment status.

Conclusions for the midstream and downstream Canadian waters of the Detroit River:

> 2. Fish collected from Turkey Creek and Bois Blanc, representative of the middle and lower Canadian reaches of the AOC, were pooled between 2002 and 2016 fish surveys. 1/84 fish from this sample pool contained a liver neoplasm yielding a tumour prevalence of 1.2%. Fisher's exact test indicated no significant difference ($p>0.9$) in the tumour prevalence of 3-5 year old brown bullhead from the middle and lower Canadian reaches of the Detroit River compared to the Great Lakes reference dataset. When the data were expanded to include all fish aged 3+ the tumour prevalence fell to 1% (1/98 fish) and was not statistically different then the Great Lakes reference dataset. Based on this evidence it is suggested that brown bullhead tumour prevalence in middle and lower Canadian reaches of the AOC are consistent with the delisting criteria. It is therefore recommended that the middle and lower Canadian reaches of the Detroit River be considered unimpaired with respect to BUI #4.

The authors of this report further note that the Canadian Stage II RAP report provides additional guidance statements towards approaches used when assessing the BUI #4 delisting criteria. It recommended that at least two fish tumour surveys be conducted no less than 3 years apart from one another. Two surveys taken 14 years apart were considered in this report and meet this requirement. However, given that the data from the two surveys had to be combined in order to meet the statistical sample size requirements necessary for testing the criteria, the two surveys can no longer be deemed independent of one another. The single fish collected from the Canadian waters of the Detroit River exhibiting a liver tumour was collected in 2002. There is no corroborating evidence from a sediment chemistry perspective to indicate major change in sediment contamination of the AOC or worsening conditions over time. In addition, most Canadian waters of the AOC have sediment PAHs below the 4 ug/g sum PAH benchmark that predicts increased tumour prevalence in Great Lakes bullhead populations. In contrast, the majority of published studies reporting on fish liver tumours from the Detroit River have focused on U.S. jurisdictions of the AOC, particularly the area of Trenton Channel. In this area, brown bullhead liver tumour prevalence exceed 5-6% in contemporary histopathology surveys, the sediment PAHs are between 6-8 fold higher than observed in equivalent Canadian reaches and exceed the 4 ug/g sum PAH sediment benchmark at more than 90% of sediment sampling stations. However, there is some mixed evidence for improvements in sediment PAH concentrtions in Trenton Channel over time. Overall, evidence inclusive of recent U.S. Geological Survey reports suggests that impairment of BUI#4 for the Detroit River AOC is likely still an on-going issue. However, multiple lines of evidence suggest that impairment of BUI#4 occurs as a result of contamination in the U.S. jurisdiction of the AOC and not a result of local sources and/or legacy inputs to Canadian waters of the Detroit River.

TABLE OF CONTENTS

1.0 Introduction....................1

1.1 BUI # 4 Status and Previous Detroit River Fish Tumor Rate Studies....................1
1.2 BUI #4 Delisting Criteria Assessment....................3
1.3 Diagnostic Criteria and Terminology Related to Proliferative Hepatic Lesions.......5
1.3 Weight of Evidence Fish Exposure Assessment 6

2.0 Methods....................9

2.1 Fish Collection and Field Dissection....................9
2.2. Histopathology....................11
2.3. Fish age, size and sex....................11
2.4. Weight of Evidence Fish Exposure Assessment....................12
2.4.1 Temporal and Spatial Trends in Sediment PAHs....................13
2.4.2 Sediment PAH concentrations compared to benchmark values....................13
2.4.3 Total Daily Intake Rates of PAHs in Brown Bullheads....................15
2.1.4 Chemical Signatures in brown bullheads....................15

3.0 Results....................16

3.1 Brown bullhead age and sex....................16
3.2. Total neoplastic, putative pre-neoplastic and non-neoplastic biliary lesion prevalence....................17
3.3 Weight of Evidence Fish Exposure Assessment....................19
3.3.1 Temporal Changes in sediment PAH concentrations....................19
3.3.2 Spatial Patterns of Sediment PAHs....................21
3.2.3 Exceedance of Sediment PAHs against Benchmark Values....................24
3.2.4 Model estimates of brown bullhead total daily intake of PAHs....................27
3.2.5 Brown Bullhead Chemical Signatures....................29
3.2.6 Weight of Evidence Fish Exposure Assessment Conclusions....................31

4.0. Delisting criteria assessment....................34

5.0 References....................36

LIST OF TABLES

Table 1.1 Summary of published studies on brown bullhead tumor rates in the Detroit River Area of Concern (1991-2012)..3

Table 1.2 Contingency Table used in conjunction with Fisher's exact test to determine statistical differences in brown bullhead tumour rates relative to Great Lakes Reference populations...4

Table 2.4.1. Benchmark values of PAHs reported in the literature...14

Table 3.1.1. Number of brown bullhead collected per age category across locations and survey years...17

Table 3.1.2 Lesion Prevalence in Brown Bullhead from different collection sites and sample years of the Detroit River AOC..18

Table 3.3.1 ∑PAH Concentrations in different river sections of the Detroit River Area of Concern...22

Table 3.3.2. Percent sites exceeding selected PAH sediment benchmark values in the Detroit River...26

Table 3.3.3. Hazard Quotients for PAHs based on selected benchmark values and CCME ISQGs and PELs in the Detroit River..27

Table 3.3.4. Chemicals identified as having strong loadings onto individual principle component axes from brown bullhead chemical signature analysis......................30

Table 3.3.5 Lines of evidence in the WOE assessment of brown bullhead exposures at different sampling locations of the Detroit river..33

LIST OF FIGURES

Figure 2.1. Sediment sampling locations and brown bullhead collection regions in the Detroit River Area of Concern.....10

Figure 3.3.1 Dry weight ∑PAH sediment concentrations (ng/g) by year in the Detroit River AOC.....19

Figure 3.3.2 TOC normalized ∑PAH sediment concentrations (ng/g) by year in the lower Canadian river reach of the Detroit River AOC.....20

Figure 3.3.3 Changes in ∑PAH sediment concentrations (µg/g TOC weight) with time in the Trenton Channel of the Detroit River AOC.....21

Figure 3.3.4 ∑PAH sediment concentrations (µg/g dry weight) in Canadian and U.S. waters of the Detroit River AOC. Boxes present 25-75 percentiles and median.....23

Figure 3.3.5 ∑PAH sediment concentrations (µg/g dry weight) in upper, middle and lower Canadian reaches of the Detroit River AOC.....23

Figure 3.3.6 ∑PAH sediment concentrations (µg/g dry weight) in upper, middle and lower Canadian reaches of the Detroit River AOC.....24

Figure 3.3.7. Model validation of PCB concentrations in brown bullhead collected from Peche Island, Turkey Creek and Boise Blanc in 2016.....28

Figure 3.3.8. Model predicted PAH total daily intake rates (ng/g fish/day) in brown bullhead at each of the bullhead collection locations in the Detroit River.....29

Figure 3.3.9. PCB and Hg concentrations in brown bullhead collected from three Canadian locations in the Detroit River.....29

Figure 3.3.10. Principle component scores across PCA axes 1 and 2 demonstrating differences in chemical signatures of brown bullhead collected from three Canadian sampling locations in the Detroit River.....31

1.0 Introduction

This document provides an analysis and assessment of data related to Beneficial Use Impairment (BUI) # 4: Fish Tumors or Other Deformities for the Canadian waters of the Detroit River Area of Concern. The interpretive focus of this report is on Canadian datasets generated for or related to BUI # 4. However, because the current impairment status for the BUI is based on data collected from U.S. waters of the AOC, related U.S. data are summarized and interpreted to help place Canadian data into context. In addition, because the delisting criteria recommended by the Stage 2 Canadian RAP Report focuses on internal hepatic lesions in indicator fish species, this report does not include a review of external lesions documented in fish from the AOC. Recent reviews of external lesions in Great Lakes fish from U.S. locations in the Detroit River can be found in Blazer et al. (2009a) and Blazer et al. (2014).

1.1 BUI # 4 Status and Previous Detroit River Fish Tumor Rate Studies

BUI #4 is listed as Impaired for the Detroit River AOC as identified in the Canadian Stage II RAP report (Green et al., 2010). The initial impairment was designated based on Macubbin and Ersing's (1991) study who reported high rates of dermal and oral neoplasms in five fish species including brown bullhead collected from U.S. downstream waters of the Detroit River. Brown bullhead (*Ameiurus nebulosus*) collected from Trenton Channel between 1985-1987 had a liver neoplasm prevalence of 8.8% in fish and dermal/oral lesions of 10.2% of collected fish. Bullheads collected during 1987 were aged and age specific liver tumors were also reported. As interpolated from Figure 2 of their study, there were 159 fish captured in 1987 with 119 fish in the age range of 3-5. An estimated 12 fish (interpolated from their figure) had liver neoplasms generating a tumour rate of 10.1% in 3-5 year old fish (Macubbin and Ersing, 1991). However, Macubbin and Ersing conducted their study prior to standardization of histopathological criteria for liver neoplasms in this indicator species and their estimates of liver neoplasms included putative pre-neoplastic hepatocellular lesions which are typically excluded from contemporary studies of of total tumor prevalence in brown bullhead (Bauman 2003; Blazer et al., 2009b).

Subsequent studies have re-affirmed elevated liver neoplasms in Detroit River brown bullheads, particularly those caught in U.S. waters of Trenton Channel. Leadley et al. (1998) reported brown bullhead liver lesions in fish collected from Trenton Channel (n=25 fish) and from two Canadian locations (Amherstburg Channel, n=23 fish and Peche Island, n=27 fish) of the Detroit River. However, only biliary neoplasms were reported by Leadley et al. (1998) and therefore tumor frequencies could underestimate the total tumor frequencies presented given a lack of information about hepatocellular lesions reported by their manuscript. For Trenton Channel, a total of 6 fish (24%) were reported with biliary neoplasms compared to 1/27 (4%) and 3/23 (14%) in Canadian caught fish from Peche Island and Amherstburg Channel, respectively. In the category of Putative pre-neoplastic lesions, two of four types of lesions were reported by Leadley et al. (1998). Total putative pre-neoplastic lesion frequencies were 0, 9 and 4% for Peche Island, Amherstburg Channel and the U.S. Trenton Channel, respectively. Arcand-Hoy and Metcalfe (1999) reported liver neoplasm prevalence from 20 brown bullhead collected from the U.S. Trenton Channel collected in the fall of 1994. Three of 4 types of liver neoplasms were reported generating 2/20 fish (15% total tumor frequency). Owing to different terminology of histopathology used in their study, incident rates of putative pre-neoplastic lesions could not be explicitly identified. However, biliary hyperplasia was present in 2/20 (10%) of fish collected from Trenton Channel. Both of the above studies were limited in the number of fish captured per site and lower than the recommended 100 fish recommended by Bauman (2010) for testing statistical differences between an AOC and the Great Lakes background tumor frequency prevalence. Differences in the types of liver neoplasms reported by Leadley et al. (1998) and Arcand-Hoy and Metcalfe (1999) also prevent pooling fish from the common Trenton Channel location even though the two studies were completed within a year of one another.

Blazer et al. (2009) reported frequencies of liver neoplasms and putative pre-neoplastic lesions in 34 fish collected from the Trenton Channel in 2000. Total liver tumor and pre-neoplastic lesion frequencies were 5.9% and 5.9% respectively. Additional fish were collected from the same area in 2011-2012 (Blazer et al., 2014). In the 2011-12 survey, total liver neoplasms and putative pre-neoplastic lesions were 7.5% and 5% of collected fish respectively. The above studies meet the modern standards of bullhead tumor histopathology studies. However, the total number of fish remain well below the recommended 100 fish minimum (Bauman 2010) for any given year of collection. Although raw data were made publicly available for the 2011-12 survey data, the raw data were not available from Blazer et al. (2009). Notably, the 2000 data described total neoplasm prevalence in fish aged 3 to 9 years old and exceed the age range recommended by the Canadian delisting criteria.

Table 1.1 summarizes published studies on brown bullhead tumor prevalence measured in the Detroit River over the period of 1985-2012. Trenton Channel had consistently elevated tumor prevalence ranging from 4.9 to 24% over the time period of 1985-2012. Although as indicated earlier, differences in methodologies between surveys preclude combining liver tumour incident rates across studies or legitimate interpretation of temporal patterns. Published brown bullhead tumor frequencies from Canadian waters of the Detroit River are limited to the study of Leadley et al. (1998) conducted in 1993. Reported tumor frequencies in the upstream Peche Island site were 4% and in Amherstburg Channel it was 13%. Both at face value exceed the recommended reference liver lesion rate of 2% although the number of fish captured per site is much lower than the recommended 100 fish needed to address the Canadian Delisting Criteria for BUI #4 in the Detroit River as described in the Canadian Stage II RAP report. Finally, the aging methodology (non-AOC explicit size at age relationships) for brown bullheads used in the Leadley et al. (1998) study are not considered accurate for addressing the BUI #4 delisting criteria.

Table 1.1 Summary of published studies on brown bullhead tumor rates in the Detroit River Area of Concern (1991-2012)

End Point	Maccubin and Ersing 1991	Leadley et al. 1998	Arcand-Hoy and Metcalfe 1999	Blazer et al. 2009	Blazer et al. 2014
Sample Date	1985 - 1987	1993	Fall 1994	June, 2000	May 2011 May 9, 2012
# Fish Collected/Location	306 (123 – 1987 only)/TT	27, 23, 25/ PI, AC, TT	20/ TT	34/TT	40/TT
Mean Age (Range)/Method	3.25 (1-7) 1987/ Not specified	3-4 / length at age	3.6±0.14 Pectoral spines	5.6±1.7(3-9) pectoral spines	5.6±0.3 2011 6.5±0.5 2012/ Otoliths
Neoplasm Type:					
Hepatocellular Adenoma		NR	NR		
Hepatocellular Carcinoma	+	NR	0% TT		
Cholangioma	+	0%, 0%, 4% PI, AC, TT	10% TT		2.5%
Cholangio Carcinoma		4%, 13%, 20% PI, AC, TT	5% TT		5%
Total Neoplasms	8.8%*, 12.1%[b]	4%, 13%, 24% PI, AC, TT	15% TT	5.9% TT	7.5% TT

*Reported Tumour rate includes neoplasms + total Putative pre-neoplastic lesions
[b] Tumour frequency including total Putatitve pre-neoplastic lesions for age 3-7 bullheads extrapolated from Fig. 2 of Macubbin and Ersing (1991).
Locations: PI = Peche Island (Canadian Upstream), AC = Amherstburg Channel (Canadian Downstream), TT = Trenton Channel (U.S. Downstream)

1.2 BUI #4 Delisting Criteria Assessment

The Canadian Stage II Detroit River RAP report (Green et al., 2010) reports the delisting criteria for BUI #4 as:

> "When the incidence rates of liver tumours in (3-5 year old) brown bullhead are not statistically different than the Great Lakes background rate."

The Stage II RAP Report provides further guidance concerning the assessment of the delisting criteria as follows:

1. The background liver tumour prevalence for Great Lakes' brown bullhead used to assess the status of the BUI is 2%
2. A minimum of two sampling events take place 3 years apart to show the changes in sediment contamination and because tumours are positively correlated to age

The background tumour prevalence of 2% identified by the Stage 2 RAP report was taken from Bauman (2010) who reviewed brown bullhead tumour prevalence from a wide variety of near-field control locations, far field locations and urbanized reference areas from the U.S. and

Canada. Bauman (2010) used the combined reference data to provide an assessment of the status BUI #4 in 7 Canadian/International AOCs including Detroit River, Wheatley Harbour, Niagara River, Hamilton Harbour, Toronto and Region, Bay of Quinte and St. Lawrence River. Of the above AOCs, there was insufficient data at the time of writing to support a conclusion on the Detroit River. The remaining AOCs assessed were concluded to be not impaired with the exception of Hamilton Harbour which had a neoplasm prevalence of 9% in 100 fish during 2007 and a combined tumour prevalence of 5.5% in 200 fish collected over 2001-2007. Both the 2007 collection and combined across year collections generated statistically elevated liver neoplasms prevalence above the Great Lakes background tumour rate.

One caveate from the Bauman (2010) report is that the author did not censor fish older than 5 years of age as outlined in the Detroit River BUI#4 delisting criteria. As a result, older fish, more prone to spontaneous neoplasms were included as part of their Canadian BUI#4 assessments. These older fish were also included in the Great Lakes reference database making the comparison compatible. However, this presents a problem when using the same Great Lakes reference database to address the Detroit River delisting criteria since the criteria recommends censoring fish older than age 5. In so doing, this would generate a higher potential of producing a Type 1 error (falsely concluding that there is no difference in tumour incidence rate relative to the Great Lakes background). One option to address this issue would be to censor the Great Lakes reference database to include only fish aged 3-5 years and then compare this with fish collected from the AOC. However, the authors of this report did not have access to the raw database used to generate the Great Lakes reference tumour incidence rate and could not accomplish this. As an alternative, the authors test the delisting criteria twice, once using the censored data set of aged 3-5 year old brown bullhead as stated and a second time using an expanded data set of age 3+ fish for consistency with the actual reference database composition. A second advantage of the latter approach is that by including more fish in the assessment, a higher statistical power is generated for testing the delisting criteria.

Bauman (2010) recommended using Fisher's Exact test to statistically test for differences in tumour prevalences within each AOC relative to the combined reference dataset using a probability criteria for statistical differences of less than 0.05 computed as a two-tailed p value. Bauman (2010) reported the sample sizes of the reference population so that a contingency table for testing with Fisher's exact test could be set up as shown below.

Table 1.2. Contingency Table used in conjunction with Fisher's exact test to determine statistical differences in brown bullhead tumour rates relative to Great Lakes Reference populations.

Location	# Fish without tumours	# Fish with tumours
Great Lakes Reference	1127	23
AOC	Measured value	Measured Vale

A power analysis completed by Bauman (2010) indicated that relatively large sample sizes or very high tumor prevalence at the impacted site are needed to demonstrate statistical differences of fish tumour rates between the AOC and background reference rate. For example, an AOC with a true tumor prevalence of 5% would require at least 100 fish in order statistically distinguish this from reference of 2%. However, when the true tumor prevalence is well above

5% the number of fish needed to avoid a Type II error (i.e. failure to reject a false null hypothesis) becomes lower.

Rutter (2010) recommended a smaller minimum of 30-50 fish be collected to evaluate fish tumour prevalence in Great Lakes AOCs. Rutter (2010) adopted a Bayesian hierarchical logistic regression model that accounts for differences in fish age, length and sex affording greater statistical power than Fisher's Exact test. However, this approach requires an extensive database of fish tumor frequencies at both impacted and non-impacted locations in order to calibrate the model given that sufficient numbers of fish with tumors that vary by size, age and sex are needed for model calibration. Thus, while this approach potentially affords greater statistical power than the Exact Fisher's test recommended by Bauman, it also necessitates the collection of a much more comprehensive database of impacted sites for use in model training and application. Given that this report focuses on data collected within Detroit River and observed tumors in fish are rare, the statistical approach adopted by Bauman (2010) is used here. This same approach was used for BUI #4 delisting criteria assessment at six other Canadian AOCs and is the recommended method in the Canadian Stage II RAP report (Green et al. 2010).

1.3 Diagnostic Criteria and Terminology Related to Proliferative Hepatic Lesions

The terminology and histopathology criteria of Blazer et al. (2006) is used throughout for describing hepatic lesions in brown bullhead (*Ameiurus nebulosus*). These criteria were explicitly applied in histopathological examination studies of 2002 and 2016 collected Detroit River fish. Liver lesions are classified into non-neoplastic biliary lesions, putative pre-neoplastic hepatocellular lesions, neoplastic hepatocellular or neoplastic biliary lesions (Blazer et al., 2006). Only neoplastic lesions (hepatocellular and biliary) are included in counts of liver tumor prevalence. However, Blazer et al. (2006) recommended the inclusion of putative pre-neoplastic hepatocellular lesions in fish tumor monitoring efforts owing to their potential linkages to neoplastic lesion etiology. The authors recommended that monitoring efforts document non-neoplastic biliary lesions as potential toxicopathologic indicators of lesions in fish but noted that such lesions can also be generated by parasite infection and therefore should not be included in total tumor prevalence rates. These indicators are included in the appendix data summary sheets but not explicitly interpreted in the present report since they are included in the actual delisting criteria.

Neoplastic lesions are categorized into 4 types: *hepatocellular adenoma; hepatocellular carcinoma, biliary cholangioma and biliary cholangiocarcinoma*. For monitoring purposes the frequency of each lesion type is documented among fish samples separately. Given that multiple liver sections and slides are prepared for each fish to identify histological alteration of tissues, it is possible that multiple lesions and lesion types will be identified within an individual fish. As such lesion specific and total tumor frequencies are assigned as binary values (tumour presence or absence) for each fish (i.e. if a single fish has multiple neoplastic lesions in its liver, it is given a value of 1; if there are no neoplastic lesions identified it is given a value of 0).

In the 2016 histopathological analysis, a fifth category of neoplastic liver lesions was assessed and identified as "pancreatic islet cell tumours". This information is retained and reported in the supporting documentation as its own category of neoplastic lesions for fish but excluded from the total liver tumor frequency counts to ensure standardization with the 2002 collections and histopathology reports. It should be noted that no fish from the 2016 Detroit River collections contained neoplasms in this category and therefore the effect of such a censor had no impact on the delisting statement interpretation.

Putative pre-neoplastic hepatocellular lesions or foci of cellular alteration are identified as four types. These include *basophilic foci, eosinophilic foci, vacuaolated cell foci and clear cell foci*. The Non-neoplastic biliary lesion includes *bile duct hyperplasia* recommended as a potential toxicopathologic indicator of chemical exposures by fish. Lesion frequencies of these types are reported separately in their own category for the collected Canadian and U.S. datasets but excluded from total neoplasm frequency tallies used for testing the delisting criteria.

1.3 Weight of Evidence Fish Exposure Assessment

An issue that has limited BUI #4 Delisting Criteria Assessment in the Detroit River in the past has been the inability to collect sufficient numbers of fish during a given survey year and at each sampling location necessary to meet the statistical rigor for delisting criteria testing. Given that brown bullhead habitat is fragmented in the Detroit River, fish can only be collected from a limited number of locations in the system. Bullhead habitats in the Detroit River are further fragmented and limited to nearshore areas and wetlands whose connectivity is broken up by fast flowing navigational channels.

There is limited guidance in the literature as to the appropriateness of pooling fish collected between different survey years or from different collection sites when attempting to assess tumour prevalence delisting criteria. Bauman (2010) pooled fish from sixteen reference locations to establish the reference database used in their Fisher's Extact test comparisons. The reference sites included several Great Lakes locations with low neoplasms prevalence rates, far field locations and urbanized reference locations throughout Canada and the U.S. Among the reference locations included in Bauman's reference database were 34 fish from Peche Island located in the upstream Canadian headwaters of the Detroit River AOC. Apart from a lack of neoplasms in the Bauman (2010) compiled data set and evidence presented by Leadley et al. (1998), there was no strong rationale as to why this location was considered an independent reference location from other prospective sampling locations in Canadian waters of the Detroit River. A discussion of the possibility of pooling fish samples necessitates consideration of potential fish spatial movements and likelihood of differences in environmental contaminant exposures between different sub-populations inhabiting the AOC. There are also concerns for widely migrating fish that movements outside of the AOC, e.g. to Lake St. Clair or western Lake Erie, could impact the interpretation of the BUI.

Brown bullhead were originally designated an indicator species of fish tumour prevalence because of their sensitivity to neoplasm development, availability of strong causal inferences relating environmental contamination to liver neoplasm etiology and because they are a considered relatively philopatric fish with limited spatial movements in a given environment (Bauman et al. 1996; Rafferty et al., 2009; Blazer et al. 2009). Telemetry studies on North American populations of Brown Bullheads are available for tagged fish from the Anacostia River, Washington, DC and Presque Isle Bay, Lake Erie, PA (Sakaris et al. 2005; Millar et al., 2009). Sakaris et al. (2005) tagged 35 fish in the Anacostia River and tracked their movements over a 30 day period. Home ranges of fish differed between seasons with larger linear home ranges in the winter and spring compared to the summer. Most brown bullheads exhibited movements within 500 m of their release location with a maximum home range of 3.7 km reported. However, the authors did indicate that after long range movements, bullheads may remain in their new location and therefore should be managed over a broader scale (i.e. 4 km range distance) compared to discrete sections of a river within 500 m of one another. Millard et al. (2009) tagged 49 brown bullheads released to Presque Ilse Bay of Lake Erie and tracked fish movements over 180 days. All fish were found to remain within Presque Ile Bay during tracking, although there was evidence for movement of fish within the Bay. Tagged fish were observed to move between various lagoons and bays within the Presque Ile AOC but did not appear to move outside of the AOC to Lake Erie. Genetic markers in fish captured from discrete locations within Presque Ile Bay provided further support of within bay fish movements suggesting that fish within Preseque Ilse Bay were a panmictic population (Millard et al., 2009). Both telemetry studies imply somewhat limited movements of fish from their release locations. Sarkaris's home range distances of up to 4 km are notably much lower than the length of the Detroit River at 51 km suggesting that upstream, midstream and downstream populations of brown bullheads could remain isolated from one another.

Fish movements between the U.S. and Canadian nearshore locations of the Detroit River remain largely unknown. The Detroit River width ranges from 0.62 km at its narrowest point at the Ambassador Bridge to more than 6 km in width at its downstream end suggesting a possibility of fish movements between U.S. and Canadian nearshore locations in the upper and midstream reaches based on Sarkaris' home range linear distances. However, navigation channels that separate the Canadian and U.S. shorelines could potentially act as barriers that limit cross channel fish movements. There is some evidence from contaminant signatures in brown bullheads for differences in fish exposures between collection locations in the Detroit River. Leadley et al. (1998) demonstrated higher bioaccumulation of PCBs in brown bullhead from Trenton Channel compared to upstream and downstream Canadian locations at Peche Island and Amherstburg Channel. Mean concentrations of Aroclor 1254/1260 were slightly higher in fish from Peche Island but within a factor of 2 of those measured in fish from Amherstburg Channel. Similar differences between Canadian locations were evident for other organochlorine pesticides reported by the authors. Farwell et al. (2012) collected brown bullheads in 2008 from Peche Island, the adjacent U.S. upstream Belle Island, downstream Trenton Channel and an upstream location in Lake St. Clair (Belle River outlet). PCB concentrations were determined in brown bullhead eggs from fish collected from each location. Concentrations where highest in Trenton Channel fish (~550 µg/kg lipid weight) followed by the U.S. Belle Island (280 µg/kg lipid weight) and Peche Island (~70 ug/kg lipid weight; concentration values estimated from published figure 2 in Farwell et al.) and lowest in Belle River fish. Notably, the large difference in PCB concentrations between fish between the adjacent U.S. and Canadian headwater sites implied limited cross channel mixing of fish despite their relatively close proximity of less than 5 km from one another.

Bile PAH metabolites have also been reported in brown bullhead from the Detroit River. Biliary PAHs provide a short term (less than 1 day) integrated exposure of fish to water, sediment and/or ingested food PAH contamination (Leadley et al., 2009). Arcand-Hoy and Metcalfe (1999) failed to detect biliary PAH metabolites in 20 fish from the Trenton Channel. Likewise, bile metabolites of benzo[a]pyrene from Trenton Channel fish were reported as moderate by Yang and Baumann (2006). However, both studies were restricted in their collections to just one Detroit River location precluding the use of these data for analysis of between site exposure differences. Leadly et al. (1999) caged brown bullheads at three Detroit River locations that included the U.S. Trenton Channel, the Canadian midstream Turkey Island and Canadian headwaters at Peche Island. After 8 days, bile PAH metabolites were 3600, 950 and 700 ng BAP/mL from each respective location providing support for elevated PAH exposures at Trenton Channel compared to the Canadian locations and relatively similar PAH exposures between the Canadian upstream and midstream site. These between Canadian location PAH exposure differences were comparable to differences in PCB signatures in fish collected in Canadian upstream and downstream locations (Leadley et al., 1998).

A more thorough investigation of spatial differences in brown bullhead exposure to potentially carcinogenic contaminants throughout Canadian waters of the AOC is warranted and included as an element of this assessment report. While PCBs are readily bioaccumulated by fish and detected in Detroit River bullhead populations, their causal inference to fish tumours is less substantiated (Bauman et al. 1991) relative to other contaminants such as polynuclear aromatic hydrocarbons (PAHs; Bauman et al., 1987, 1991; Baumann and Harshbarger, 1995, 1998; Brown et al., 1973; Harshbarger et al., 1984; Leadley et al., 1999; Pinkney et al., 2001, 2004a; Pyron et al., 2001; Smith et al., 1994; Rafferty et al. 2009; Blazer et al. 2009). As such, this report provides additional focus on spatial and temporal patterns of sediment PAHs in the Detroit River in a weight of evidence (WOE) assessment of differences in chemical exposures by fish from different fish collection sites and regions of Canadian waters of the AOC.

The weight of evidence approach adopted in this report addresses potential fish exposures to carcinogenic PAHs from sediments coupled with differences in bioaccumulative fish chemical signatures. The interpretative value of the WOE is to justify whether or not brown bullheads sampled from different collection locations and different survey years can be pooled together or not for evaluation of the BUI #4 delisting criteria.

The following elements are incorporated into the WOE fish PAH exposure assessment:

1) Spatial/Temporal Patterns of Detroit River Sediment PAHs (1999-2013)
    i) Determine temporal changes to sediment PAH concentrations in the AOC
    ii) Determine if sediment PAHs differ between different reaches (upstream, midstream and downstream) reaches of Canadian waters of the AOC
    iii) Determine if sediment PAHs differ between bullhead collection locations (Peche Island, Turkey Creek, Amherstburg Channel and U.S. Trenton Channel)

2) Fish tumor hazard assessment based on Detroit River Sediment PAHs
    i) Determine level of exceedance of sediment PAH benchmarks recommended for the protection of fish against tumours across different reaches of Canadian waters of the AOC and between Canadian and U.S. brown bullhead sampling locations.

3) Fish bioaccumulation model to estimate daily PAH uptake rates from sediment PAHs

i) Apply a foodweb bioaccumulation model to predict differences in PAH uptake rates from ingested sediments/benthic invertebrates from different reaches of Canadian waters of the AOC and between different Canadian and U.S. brown bullhead sampling locations.

4) Compare chemical signatures of bioaccumulative contaminants in brown bullheads from different brown bullhead sampling locations.

i) Use the chemical fingerprint in fish collected from different brown bullhead collection sites to determine whether fish from different locations exhibit a common exposure to environmental contaminants.

WOE elements 1-4 were subsequently used to support decisions about whether to pool or to separately analyze fish from different sampling survey years and different collection sites used in the delisting criteria assessment. In cases where sediment PAHs exposures (WOE items 1-3) and chemical signatures (WOE #4) show no differences across time and between sample locations, fish samples will be pooled between sites and/or survey years when addressing the delisting criteria assessment. Where PAH exposures and chemical signatures show differences between years or sampling locations, site specific samples will analyzed separately when evaluating the delisting criteria.

2.0 Methods

2.1 Fish Collection and Field Dissection

Brown bullheads were collected in Canadian waters of the Detroit River in 2002 and 2016. In 2002, 3 specimens were collected on Sep 24-26 and 95 bullheads collected between Oct 1- 10 by electrofishing boat. Thirty four fish were collected from Peche Island, 39 fish from Turkey Creek and 25 fish from Boise Blanc Island (formerly described as Grosse Isle) covering site locations representative of upstream, midstream and downstream waters of the AOC (Figure 2.1). In 2016, fish were collected by electrofishing boat on Aug 16. During this survey year there were 64 brown bullheads collected, 11 from Peche Island, 49 from Turkey Creek and 3 from the Canadian nearshore waters of Amherstburg Channel adjacent to Boise Blanc Island (Boblo dock).

Following capture, fish were placed in a live well under aeration until euthanasia and dissection which was completed on the same day. Fish were anaesthetized in a water bath of clove oil (~0.05% clove oil with 0.025% ethanol as an emulsifier) and euthanized by anesthetic overdose. Physical abnormalities on the skin and barbels were assessed visually and written in field notes but skin biopsies were not collected or submitted for histopathological examination. Fish fork length (cm) and total weight (±0.1 g) were measured for each fish. For 2002 fish, pectoral fin rays were collected for aging while in 2016 otoliths were obtained for ageing. The liver was dissected and separated into sections for histopathology. Liver sections were placed in plastic cassettes, labelled and stored in Davidson's Fixative. After 1-4 weeks of collection the preserved tissues were transferred to a solution of 70% ethanol followed by submission of preserved tissue samples to histopathology labs.

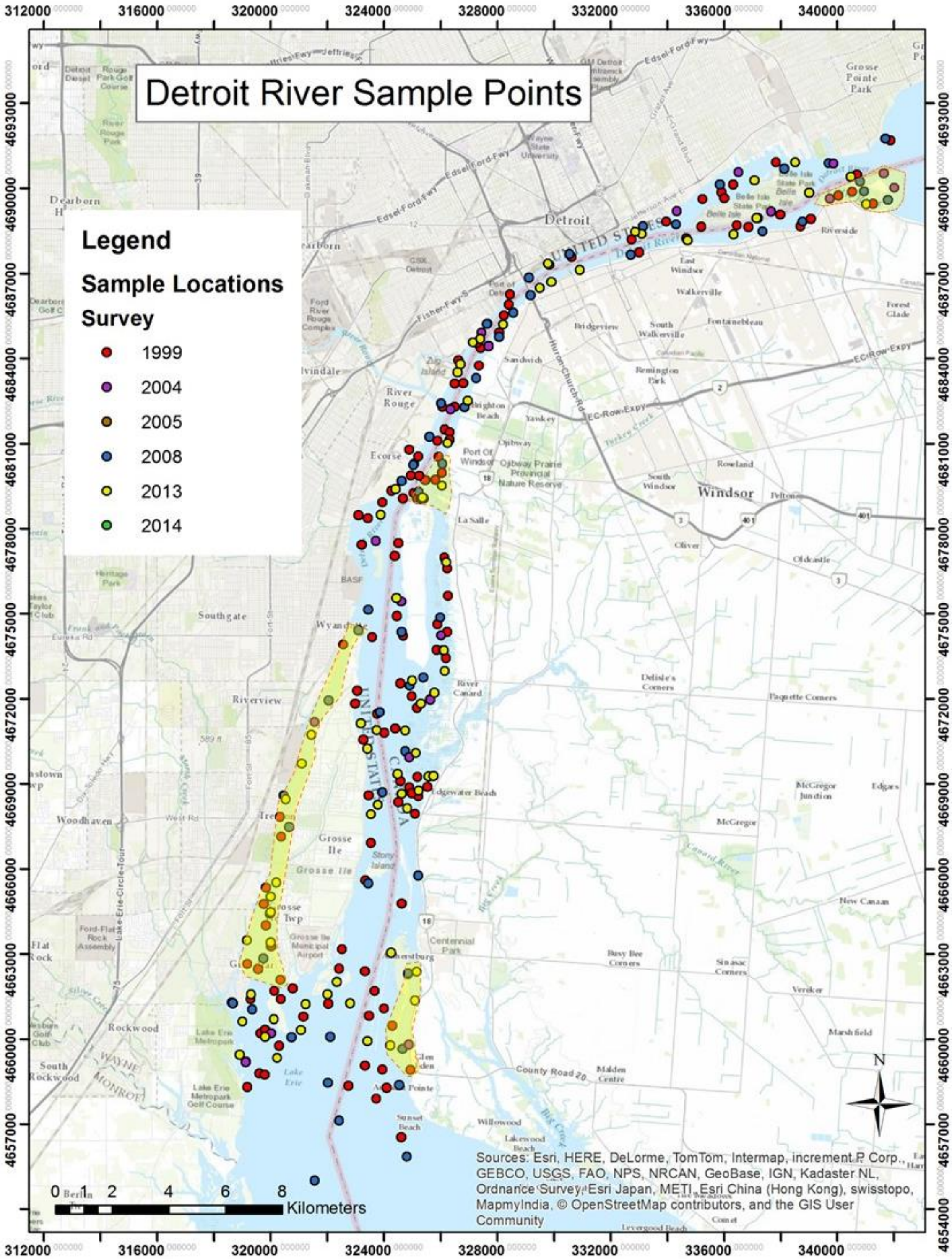

Figure 2.1. Sediment sampling locations in the Detroit River AOC. Yellow polygon areas identify bullhead collection areas and sediment sample sites used to compare PAH concentrations at fish collection zones.

There were differences in aging methodology for fish collected between survey years. For 2002, age was determined by reading annual rings of pectoral spines. The left pectoral spine was removed as close to the body as possible and placed in a scale envelope. If broken during removal, the right spine was used. Twenty one fish from the 2002 Bois Blanc site did not have age data associated with them. In 2016, Otoliths were used for fish aging. All fish from 2016 were aged except for one specimen where data were reported as missing.

2.2. Histopathology

Preserved liver samples from 2002 were submitted to Freshwater Institute (Fisheries & Oceans Canada, Winnipeg, MB, Canada) as outlined in Bauman (2010). The 2016 samples were submitted to the University of British Columbia and Animal Health Centre for histopathological examination. Both histopathology labs adopted the criteria and terminology of Blazer et al. (2006) for describing lesions and Wolf and Wolfe (2005) for 2016 sample submissions.

Protocols for 2002 collections are described in Bauman (2010) and briefly summarized here. Multiple liver sub-samples were processed in a routine ethanol/toluene series and individually embedded in paraffin blocks. The embedded tissues were sectioned at 4 – 6 microns and one slide, each with three tissue sections, was prepared from each block. The slides were stained with Harris hematoxylin and eosin. Slides were examined with a Zeiss Photomicroscope III with Plan lenses and an Olympus Q-Color 3 digital camera in blinded fashion. Image capture and brightness/contrast adjustments were performed using Image-Pro Plus software (Version 7.0 for Windows) at 2082 x 1536 pixel resolution.

Histopathology protocols for 2016 samples are added as an Appendix to this report. Briefly, five liver sections per fish were distributed across ten slides. Each slide was scanned using a 4x objective lens and then a single liver section was systematically scanned using the 10 x objective lens. Higher magnifications (20 x and 40 x objective lenses) were utilized as needed for lesion characterization. Slides were analyzed in blinded fashion. As a diagnostic check, for every 10 fish examined by the lead pathologist, one fish was independently examined and scored by the reviewing pathologist.

A difference noted between the histopathology reports from 2002 and 2016 collections was the inclusion of neoplastic liver lesions identified as pancreatic islet cell tumours that was not included in the neoplastic lesion categories identified in 2002. This information is retained in the data spreadsheets but neoplasms of this type were excluded in the total tumour prevalence counts where fish from across survey years were pooled so as to keep the data compatible and consistent with the criteria of Blazer et al. (2006).

2.3. Fish age, size and sex

Tumour prevalence in fish is influenced by fish age, body size and sex (Macubbin and Ersing, 1991; Bauman, 1992, Rutter 2010). The delisting criteria also explicitly identifies that brown bullhead between ages 3-5 be used for the delisting criteria assessment. Female fish have a higher prevalence of certain lesions compared to males (Blazer et al. 2009; Bauman 2010).

Therefore it is important to determine if sex ratio differences occur between collection locations or survey years prior to pooling data.

A complication related to the data examined in this document was the use of different fish aging methodologies in the 2002 (pectoral spine) and 2016 (otolith) survey years. It is generally acknowledged that otoliths are the preferred method of aging brown bullhead in tumour prevelance studies (Bauman 2003; Blazer et al., 2009). Aging by otolith will generally yield higher age estimates for older fish compared to pectoral spines. This generates the potential for inclusion of fish greater than age 5 in the delisting criteria assessment when pectoral spines were used for aging. Blazer et al. (2009) compared age estimates in 345 brown bullheads that were aged by both otolith and pectoral spine methods. They reported that age 2-4 categorized fish had between 80-85% agreement between spine and otolith age estimates. However, agreement between the two aging methods decreased to 65.5% for age 5 fish and to 0% for fish aged 12. For age 5 fish, the authors reported no consistent bias in the age estimates generated for fish. Half of the fish age 5 had older estimated ages by pectoral spine while half the fish had younger estimated ages by pectoral spines (Blazer et al. 2009). After age 7, the pectoral spine method was found to consistently underage fish relative to otolith aging techniques.

The following approach was adopted to classifying the age of individual fish used for tumour prevalence analysis. Where otolith ageing was used to measure fish age, it was accepted as the default age of the fish. Where pectoral spines were used to measure fish age, it was accepted as the default age of the fish if a given fish had an assessed age of between 1-5 years of age. Fish aged 6 and above as determined by pectoral spine or otilith were excluded from the initial tumour prevalence analysis. In cases where there was no age reported for a given fish, the length at age relationship generated using combined Canadian and U.S. Geological Survey data was used to generate an age estimate for fish. Linear regression on length vs age or log body weight vs age were established. For each size metric, the 95% lower confidence limit for age 3 fish and 95% upper confidence limit for age 5 was used to discriminate non-aged fish as being less then 3 years of age, greater than 5 years of age or within the 3-5 year age bracket, respectively.

To test for differences in sex ratio of fish collected from different years we used Fisher's Exact Test. Differences in the proportion of male and female fish collected in 2002 versus 2016 were computed on the combined fish collections and at each collection site separately.

2.4. Weight of Evidence Fish Exposure Assessment

Detroit River sediment chemistry data were obtained from multiple sediment chemistry surveys conducted in the Detroit River AOC by GLIER, University of Windsor. The database consisted of 300 sediment samples distributed throughout the entire AOC collected between 1999-2013 (Figure 2.1). Sediment chemistry data included concentrations of total organic carbon, PAHs (16 U.S. EPA Priority PAH compounds), PCBs, selected organochlorine pesticides and trace metals. A full description of the sampling design and analytical methodology of the sediment chemistry surveys can be found in Drouillard et al. (2006), Szalinska et al. (2013) and Drouillard et al. (2019 In Press). Individual surveys contributing to the sediment quality data base were conducted in years 1999, 2004, 2008, 2009 and 2013 to provide a temporal perspective of changes in sediment contamination at regional and local scales within the AOC.

2.4.1 Temporal and Spatial Trends in Sediment PAHs

The sediment chemistry data were examined for temporal patterns of ∑PAHs concentrations by combining sampling locations by site according to individual tests and testing for differences between survey years. As the data were non-normal and remained that way after log-transformation, non-parametric Kruskal Wallis tests were performed to compare differences in ∑PAH concentrations between groups of sample sites. Conover-Inman pairwise comparisons were used as non-parametric post-hoc tests to compare differences between individual regions or bullhead collection sites. Where temporal differences were determined on a by-year basis, additional linear regression on ln transformed concentration data were evaluated to examine for broader temporal trends. Measures of central tendency and variation reported in the text refer to median and 5-95 percentiles to be consistent with rank-order statistical tests used statistical contrasts. The following contrasts were performed to determine temporal patterns of sediment PAHs in the Detroit River AOC:

1) Determine if AOC-wide ∑PAH sediment concentrations changed over time (1999-2013)
2) Determine if ∑PAH sediment concentrations changed over time in Canadian waters of the of AOC
3) Determine if ∑PAH sediment concentration changed over time in either the upstream, middle or lower river reaches of Canadian jurisdictions of the AOC
4) Determine if ∑PAH sediment concentration changed over time at each bullhead collection area (Peche Island, Turkey Creek, Bois Blanc, Trenton Channel).

Contrasts 1-4 above were completed on both dry weight ∑PAH concentrations and organic carbon normalized ∑PAH sediment concentrations. The following contrasts were performed to determine spatial patterns of sediment PAHs in the Detroit River AOC:

1) Determine differences in ∑PAH sediment concentrations between U.S. and Canadian jurisdictions
2) Determine differences in ∑PAH sediment concentrations between upstream, middle and downstream reaches of the Canadian waters of the AOC
3) Determine differences in ∑PAH sediment concentrations between individual bullhead collection zones (Peche Island, Turkey Creek, Bois Blanc, Trenton Channel).

2.4.2 Sediment PAH concentrations compared to benchmark values.

Benchmark ∑PAH and individual PAH sediment concentrations were compiled from different sources that were developed for the protection of fish against tumors and protection of aquatic life. Benchmark values from the literature are summarized in Table 2.4.1. Recommended benchmarks for protection of fish against neoplasms ranged from 1 $\mu g\cdot g^{-1}$ to 10 $\mu g\cdot g^{-1}$ dry weight ∑PAHs. In addition, individual PAH sediment quality guidelines (SGQs) for the protection of aquatic life were obtained from CCME (2001). The CCME guidelines report both interim sediment quality guidelines (ISQG's) and probable effect level (PEL) values for benthic invertebrate toxicity. For purposes of the hazard assessment, two ∑PAHs benchmark values were contrasted against measured PAH concentrations in Detroit River sediments along with individual PAH ISQGs and PELs recommended by CCME. The ∑PAH benchmarks applied in the hazard assessment were 1 µg·g^{-1} ∑PAH dry weight recommended by Johnson et al. (2002) for the protection against neoplasms in marine fish and 4 µg·g^{-1} (Bauman and Harshbarger, 1995) representing the change point reported by the authors for increased neoplasm frequencies in Brown Bullhead from the Black River, Ohio. Summing the 13 individual ISQGs and PELs from CCME generates a ∑PAH concentration of 0.41 and 6.5 µg·g^{-1} that are proximate in magnitude to the of recommended benchmarks for protection of fish neoplasms.

For each benchmark, ISQG and PEL, hazard quotients were generated by dividing the measured ∑PAH or individual PAH concentration at a given sampling location by the benchmark. Hazard quotients (HQ) greater than 1 indicate local PAH concentrations in excess of the benchmark. Sites were grouped into whole river, by country, by river reach (6 zone area) or by bullhead collection area. For %Exceedences, the total number of sites where HQs exceeded a value of 1 was compiled. In the case of ISQGs and PELs, 1 exceedence was reported for a given site when one or more individual PAHs exhibited an HQ greater than 1. In addition, quantitative measures of HQ and ∑HQ were compiled by reporting the median and 5-95 percentiles of HQ or ∑HQ.

Table 2.4.1. Benchmark values of PAHs reported in the literature.

Benchmark	Species	Chemical	Reference
1.0 µg·g^{-1}	Marine & estuarine fish, protection against neoplasms	Total PAHs	Johnson et al. 2002
2.8 µg·g^{-1}	English sole (*P. vetulus*) neoplasms	Total PAHs	Horness et al. 1998
2.9 µg·g^{-1}	English sole, threshold effect neoplasms	Total PAHs	Johnson et al.
4.3 µg·g^{-1}	Brown Bullhead, Black River, neoplasms	Total PAHs - TEL	Bauman and Harshbarger 1995
10 µg·g^{-1}	Brown Bullhead Black River, neoplasms	Total PAHs	Baumann cited as Personal Comm. In Raferty et al. 2009
0.0067, 0.089 µg·g^{-1} 0.0059, 0.128 µg·g^{-1} 0.0469, 0.245 µg·g^{-1} 0.0317, 0.385 µg·g^{-1} 0.0319, 0.782 µg·g^{-1} 0.0571, 0.862 µg·g^{-1} 0.0062, 0.135 µg·g^{-1} 0.111, 2.355 µg·g^{-1} 0.0212, 0.144 µg·g^{-1} 0.0346, 0.391 µg·g^{-1} 0.0419, 0.515 µg·g^{-1} 0.053, 0.875 µg·g^{-1}	Protection of Aquatic Life ISQG, PEL	Acenaphthene Acenaphthylene Anthracene Benz(a)anthracene Benzo(a)pyrene Chrysene Dibenz(a,h)anthracene Fluoranthene Flurene Naphthalene Phenanthrene Pyrene	CCME, 2001

2.4.3 Total Daily Intake Rates of PAHs in Brown Bullheads

A one-compartment steady state food web bioaccumulation model developed for hydrophobic organic contaminants (Arnot and Gobas 2004) was used to estimate total daily uptake rates (ng/g/d) of PAHs by bullheads in the Detroit River (Kashian et al., 2010; McLeod et al., 2015). The model integrates predictive algorithms and parameter estimates into the general concepts provided by Thomann and Connolly (1984) and is a well-established food web bioaccumulation model used within the literature (Arnot and Gobas, 2004; McLeod et al., 2015; Li et al., In Press). The model was previously applied to predict polychlorinated biphenyl (PCB) congener concentrations Detroit River sport fish using sediment and water PCB concentrations as model inputs. The predicted PCB concentrations were compared to measured concentrations in a variety of Detroit River sport fish and generaly found to be within a factor of 10 of empirical observations (Kashian et al., 2010; Li et al., In Press). Details of the model structure and calibration are outlined in McLeod et al. (2015) and Li et al. (In Press). Given that PAHs are rapidly biotransformed by fish, only the uptake rate of PAHs predicted by the model was used in order to provide total daily exposure (TDI) of brown bullheads to PAHs from sediments.

The model was parameterized using site specific PCB and PAH congener TOC-normalized concentrations present in sediment. For each model zone or bullhead collection area the median PCB or PAH concentration in sediment was used as the model input. Exposure of chemicals to water was ignored given a lack of data on dissolved PAH concentrations in different bullhead sampling locations. Prior to calculating PAH TDI's, the model was first evaluated for its ability to predict congener specific PCB concentrations in brown bullheads collected at Peche Island, Turkey Creek and Boise Blanc and verified using chemical data measured in 2016 collected fish. Site specific sediment organic carbon content and chemical specific K_{OW} values were applied in the model. For PCBs, K_{OW} values were derived from Hansen et al. (1999). For PAHs, K_{OW} values were obtained from Sahu and Pandit (2003). Model bias in the brown bullhead PCB predictions were used to calibrate the model on a site specific basis to subsequently adjust PAH exposures estimates in fish from each collection location. This assumes that the bioavailability of PCBs and PAHs was similar to one another at different fish collection locations.

2.1.4 Chemical Signatures in brown bullheads

Twenty four brown bullheads from 2016 were submitted for PCB, organochlorine pesticide and total mercury (Hg) analysis. PAHs were not analyzed in fish owing to rapid biotrasformation of these chemicals by fish and limited bioaccumulation potential (Leadely et al., 1993). However, given that sediment PCBs and OC pesticides are correlated with PAHs in the Detroit River Area of Concern (Drouillard et al., 2006), differences in, or lack of, hydrophobic organic chemical exposures by fish between sampling locations provides additional support for differences in PAH exposures by fish across the sites and/or fish movement potentials.

Lipid equivalent PCB and dry weight total Hg concentrations in bullheads were normal after log correction (Lillefors test; $p>0.2$) and therefore ANOVA was performed to test for site specific differences in fish contamination for each chemical separately. Multivariate ordination was subsequently performed on the full PCB congener dataset, individual organochlorine pesticides and total Hg to examine for chemical signature differences in fish between sampling locations. Tetrachlorobenzenes, mirex, hexachlorocyclohexanes, PCB 191 and PCB 205 were excluded because of insufficient detections (<50% of samples) among fish samples. For the other chemicals, non-detected values were replaced with the detection limit to generate a complete data matrix. Principle component analysis (PCA) was performed on log transformed lipid equivalent data for organic chemicals or dry weight total Hg concentrations using a correlation matrix. Multivariate analysis of variance was performed on PCA scores for the first 2 PCA axes to test for significant differences between chemical signatures between sample locations.

3.0 Results

3.1 Brown bullhead age and sex

A total of 162 fish were available across three Canadian locations in the 2002 and 2016 surveys and additional raw data were obtained for 40 fish from the 2011-2012 U.S. Geological Survey datasets from Trenton Channel. Of the Canadian fish submitted for histopathology, 21 fish from the 2002 collections did not have ages assigned to them and 1 fish from the 2016 data set had missing age information on its age. Unfortunately, the only fish from the Canadian samples identified to contain a liver neoplasm was not aged and therefore its general age had to be estimated in order to consider it for inclusion in the tumour prevalence counts.

The combined samples from different surveys (2002, 2016 and U.S. Geological Survey Data) was used to estimate size at age relationships for Detroit River brown bullheads in order to estimate the ages of fish which had been submitted for histopathology but did not have age data associated with them. Body length data followed a normal distribution but body weight had to be log transformed in order to normalize it. Although differences between aging by spine and otolith have been reported by others (Blazer et al., 2009), the data from the present study did not show a significant effect of aging method ($F_{1,176}$=2.682; p>0.1; ANOVA) on the prediction of fish length after accounting for age nor was there a significant interaction between the method · age on fish length (F1,75=0.486; p>0.4; ANOVA). There was also no significant interaction between sex·age on fish length (F1,175=0.572; p>0.4; ANOVA) indicating that male and female fish grow at statistically similar rates. Similar results were obtained for fish body weight. Overall, neither aging method (pectoral spine versus otolith) of fish or fish sex provided significant prediction of fish size of brown bullhead after accounting for age. The linear regressions between body size (length or log weight) and fish ages are given by:

$$\text{Body Length (cm)} = 0.755 \pm 0.083\ \text{Age} + 25.904 \pm 0.426; \quad R^2 = 0.32;\ p<0.001 \qquad (3.1)$$

$$\text{Log Body Weight (g)} = 0.039 \pm 0.004 \cdot \text{Age} + 2.360 \pm 0.022; \quad R^2 = 0.31;\ p<0.001 \qquad (3.2)$$

Based on Equation 3.1, three year old bullheads have a mean (5-95% confidence interval) body length of 28.2 (27.75-28.59) cm and body weight of 299.9 (285.1 – 315.5) g. Five year old bullheads have a mean (5-95% confidence interval) body length of 29.7 (29.4-30.0) cm and body weight of 358.9 (345.9 – 371.5) g. The lower and upper confidence limits of 3 and 5 year old fish respectively were used to establish the size boundaries to delineate fish in the 3-5 year old age range for all non-aged samples in the database. Thus, non-aged fish with body lengths between 27.8 and 30.0 cm and body weights between 285.1 to 371.5 g were considered to be in the age 3 to 5 range. Fish falling outside of the above ranges for both length and body weight were excluded from tumour prevalence calculations during delisting criteria assessment. Fish that had one size measure within acceptable ranges (either length or body weight) were still retained for use in tumour prevelance estimates. Of the 22 non-aged fish from 2002, 9 were judged to be in the size range consistent with 3-5 year old Detroit River fish and 11 were excluded. The one fish exhibiting a liver neoplasm from Boise Blanc Island in 2002 had a body length of 29.5 cm and body weight of 359 g and was considered age appropriate for inclusion in the tumour prevalence estimate for the delisting criteria assessment. The single fish from 2016 which lacked an age measurement was considered of appropriate size for an age 3-5 year old fish. Its body dimensions were 28.7 cm length, although its weight was low at 246.4 g.

Overall, a total of 112 brown bullheads captured in Canadian waters of the AOC between 2002 and 2016 were in the 3-5 year age category. In terms of fish older than 3 years of age, there were a total of 142 fish collected from Canadian locations aged 3 and above. Table 3.1.1 provides a summary of fish counts by age by collection site and survey year. Although the total number of fish collected in Canadian waters of the jurisdiction meet the minimum number of fish recommended by Bauman (2010), there were insufficient numbers of brown bullhead in the correct age category for any given collection location and survey year.

Table 3.1.1. Number of brown bullheads collected per age category across locations and survey years.

Location (Year)	< Age 3	Age 3-5	> Age 6
Peche Island (2002; 2016)	1,0	26, 2	7,9
Turkey Creek (2002; 2016)	5,1	32, 40	2, 9
Boise Blanc (2002; 2016)	13,0	9, 3	3,0
Total:	20	112	30

Sex ratio differences between collection years and locations can potentially influence interpretation of tumour prevalence when pooling samples across sites or years. Fisher's Exact Test was used to determine if there was a difference in the sex ratio of fish collected across Canadian locations in 2002 and 2016. There was no significant difference ($p>0.9$) in the sex ratio of fish collected between 2002 and 2016 when samples were combined across sample locations. Similar results were obtained on a site-specific basis. Sex ratios were not significantly different between 2002 and 2016 at Peche Island ($p>0.7$; Fishers' Exact Test); Turkey Creek ($p>0.6$; Fishers Exact Test) or Boise Blanc Area ($p>0.5$, Fisher's Exact Test).

3.2. Total neoplastic, putative pre-neoplastic and non-neoplastic biliary lesion prevalence.

Raw data of histopathology compilations are provided in an Appendix included with this report. Table 3.2.1 summaries lesion prevalence by lesion category in age 3-5 bullheads from each survey location and year. The table also lists lesion prevalence in fish aged 3+ for comparison the Bauman (2010)'s assessment criteria. Only 1/162 Canadian collected fish was identified to have a liver neoplasm characterized as cholangio carcinoma. This fish, assessed to be 3-5 years of age by its size, was captured in the vicinity of Boise Blanc Island located in the downstream Canadian waters of the AOC during 2002. However, given that only 9 fish aged 3-5 year fish were collected from this location in 2002 and only 12 fish older than age 3, this would imply a site and year specific tumour prevalence of 11.0 and 8.3%, respectively. The total number of fish collected at this location and all other locations within a given survey year, however, was much lower than the required 100 fish to statistically assess the delisting criteria.

Putative pre-neoplastic lesions were also detected in age 3-5 year bullheads in 1/32 fish from Turkey Creek in 2002 and 4/40 fish from the same location in 2016 as well as in 3 additional aged 6+ fish from 2016. Putative pre-neoplastic lesions were not detected at the other Canadian sampling locations in aged 3-5 fish apart from 2 fish from Peche Island aged 6 and 7. Non-neoplastic biliary lesions were only detected in 1 Canadian caught fish that was age 2. Non-neoplastic biliary lesions were much more commonly reported in U.S. captured brown bullheads (Blazer et al., 2014).

Table 3.1.2 Lesion Prevalence in Brown Bullhead from different collection sites and sample years of the Detroit River AOC.

Location & Survey Year	Age 3-5 Fish			All fish older than 3 years old		
	Neoplastic Lesions	Putative Pre-Neoplastic Lesions	Non-Neoplastic Biliary Lesions	Neoplastic Lesions	Putative Pre-Neoplastic Lesions	Non-Neoplastic Biliary Lesions
Peche Island 2002	0 (n=26) 0%	0 (n=26) 0%	0 (n=26) 0%	0 (n=33) 0%	0 (n=33) 0%	0 (n=33) 0%
Peche Island 2016	0 (n=2) 0%	0 (n=2) 0%	0 (n=2) 0%	0 (n=11) 0%	2 (n=11) 18.2%	0 (n=11) 0%
Turkey Creek 2002	0 (n=32) 0%	1 (n=32) 3.1%	0 (n=32) 0%	0 (n=34) 0%	1 (n=34) 2.9%	0 (n=34) 0%
Turkey Creek 2016	0 (n=40) 0%	4 (n=40) 10%	0 (n=40) 0%	0 (n=49) 0%	7 (n=49) 14.3%	0 (n=49) 0%
Bois Blanc 2002	1 (n=9) 11.1%	0 (n=9) 0%	0 (n=9) 0%	1 (n=12) 8.3%	0 (n=12) 0%	0 (n=12) 0%
Bois Blanc 2016	0 (n=3) 0%	0 (n=3) 0%	0 (n=3) 0%	0 (n=3) 0%	0 (n=3) 0%	0 (n=3) 0%
Trenton Channel 2000	NA	NA	NA	2 (n=34) 5.9%	2 (n=34) 5.9%	NA
Trenton Channel 2011-12	1 (n=24) 4.2%	1 (n=24) 4.2%	11 (n=24) 45.8%	3 (n=40) 7.5%	2 (n=40) 5%	18 (n=40) 45%

3.3 Weight of Evidence Fish Exposure Assessment

3.3.1 Temporal Changes in sediment PAH concentrations

Sediment PAH concentrations were compiled for all waters of the AOC collected over the time period of 1999-2013. There were 147 sediment sampling sites collected in 1999, 17 sites from 2004; 6 sites from 2007; 32 and 33 sites from 2008 and 2009 and 65 sites from 2013. Kuriskal-Wallis non parametric tests were used to detect differences in the ∑PAH sediment concentration across years. There were no significant differences in the AOC-wide ∑PAH sediment concentrations by year when data were expressed on a either dry weight basis ($p>0.2$; Kruskal-Wallis test; n=300 cases, test statistic = 7.06) or on an organic carbon normalized basis ($p>0.1$; Kruskal-Wallis Test; n=300 cases, test statistic = 7.68). A box and whisker plot of river wide ∑PAH sediment concentration by year is presented in Figure 3.3.1.

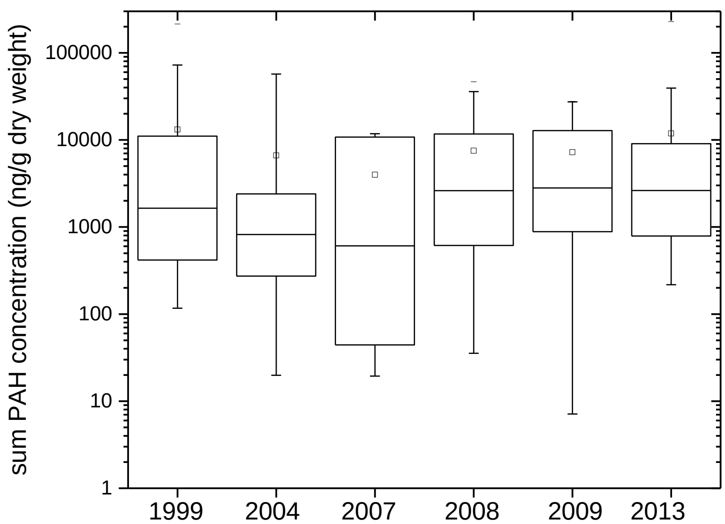

Figure 3.3.1 Dry weight ∑PAH sediment concentrations (ng/g) by year in the Detroit River AOC. Boxes present 25-75 percentiles and median. Square presents mean concentration and whiskers present 5-95% confidence intervals of the distribution.

Sediment ∑PAH concentrations were subsequently grouped into sites collected from Canadian waters of the AOC. This reduced the total number of samples sites from 300 to 142. As in the case of the river wide contrast, there was no significant differences in ∑PAH sediment concentrations between years for samples from the Canadian jurisdiction on a dry weight ($p>0.1$ Kruskal-Wallis Test; n=142 Test Statistic = 8.58) and OC weight basis ($p>0.05$; Kruskal-Wallis Test; n=142; Test Statistic = 10.08).

For contrast 3, temporal trends were tested in each river reach separately within the Canadian jurisdiction. There were no significant differences in dry weight ∑PAH sediment concentrations in any of the individual river reaches ($p>0.4$, n=28, Test Statistic=4.66; $p>0.1$, n=25, Test Statistic =6.75; $p>0.1$, n=89; Test Statistic = 8.77; Kruskal Wallis Tests for upstream, middle and lower reaches, respectively). Similar results were observed for OC normalized ∑PAH sediment concentrations in the upstream and middle reaches. However, there was a significant difference in the lower reach OC-normalized ∑PAH sediment concentration ($p<0.05$; $n=89$, Test Statistic = 12.50) with year. Notably, the between year differences observed in the lower river reach OC normalized PAH concentrations did not exhibit a consistent temporal trend and subsequent attempts to perform a linear regression of ln PAH concentration with time did not yield a significant relationship ($p>0.15$; ANOVA). Figure 3.3.2 presents the TOC normalized ∑PAH sediment concentrations by year in the lower Canadian reach. Although concentrations were lower in 2004 this is considered an artifact of the small sample size of sites collected in this reach and year. Thus, the between year differences in ∑PAH concentrations observed in the Canadian lower reach are considered an artifact of different sampling intensity across years but not reflective of actual changes in sediment PAHs occurring in Canadian strata over time.

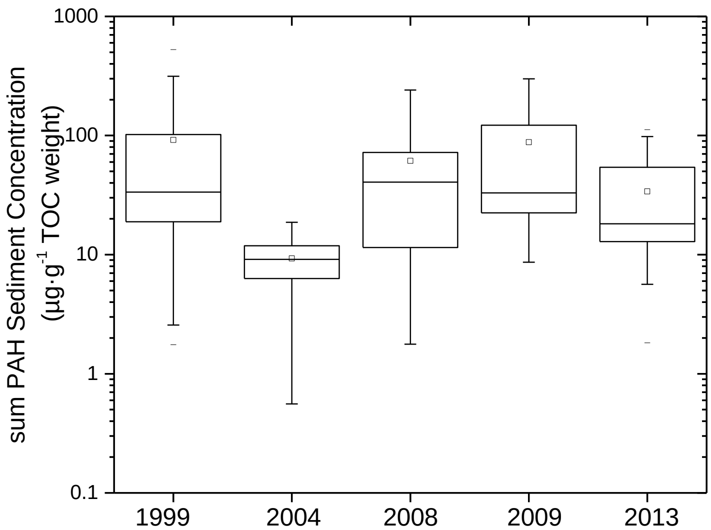

Figure 3.3.2 TOC normalized ∑PAH sediment concentrations (ng/g) by year in the lower Canadian river reach of the Detroit River AOC. Boxes present 25-75 percentiles and median. Square presents mean concentration and whiskers present 5-95% confidence intervals of the distribution.

The last temporal contrast examined differences in ∑PAH sediment concentrations with time at each of the bullhead collection areas. For Peche Island there were 15 samples taken between 1999-2013. Kruskal-Wallis test indicated no-significant difference in sediment ∑PAHs with time on a dry weight ($p>0.1$; $n=15$; Test Statistic = 7.925) or TOC weight ($p>0.05$; $n=15$; Test Statistic = 10.74) basis. Data were insufficient to test for temporal changes in ∑PAHs at Turkey Creek given that only 5 samples were available within this region across different years. For Boise Blanc collection areas, there was no-significant difference in sediment ∑PAHs with time on a dry weight ($p>0.05$; $n=9$; Test Statistic = 4.67) or TOC weight ($p>0.2$; $n=9$; Test Statistic = 5.77) basis. For the U.S. Trenton Channel area, there were no significant differences in sediment ∑PAHs with time on a dry weight ($p>0.1$; $n=37$; Test Statistic = 8.95). However, there was a significant difference with time when data were TOC-normalized ($p<0.05$; $n=37$; Test Statistic = 13.25). In this case, the linear regression between ln PAH concentrations with time yielded a highly significant decreasing trend ($p<0.001$) with time corresponding to a half life of 9.4 years. Figure 3.3.3 presents temporal trends in TOC normalized ∑PAH sediment concentrations with time for the Trenton Channel region of the Detroit River.

Overall, the weight of evidence indicates that PAH concentrations in sediments within Canadian jurisdictions of the Detroit River, including brown bullhead collection locations, have remained stable between 1999-2013. This provide supports for pooling fish data collected from 2002 and 2016 given a lack of change in sediment chemistry of key carcinogenetic substances in the environment known to elicit liver tumors in fish. However, there is some evidence to support a decrease in sediment PAH concentrations in the U.S. Trenton Channel region of the Detroit River over time where brown bullheads were collected as part of U.S. Geological Surveys.

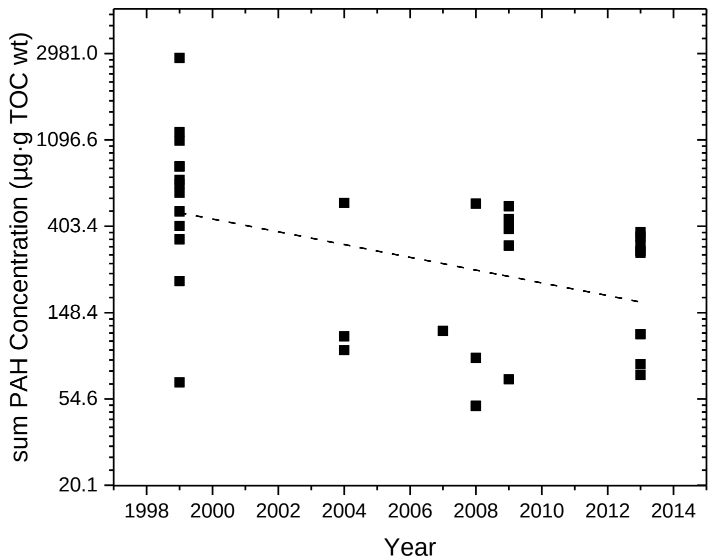

Figure 3.3.3 Changes in ∑PAH sediment concentrations (µg/g TOC weight) with time in the Trenton Channel of the Detroit River AOC. Dashed line presents linear regression fit to the data.

3.3.2 Spatial Patterns of Sediment PAHs

Table 3.3.1 summarizes median and 5-95 percentile distributions of sediment ∑PAH concentrations in different sections of the Detroit River. For the first comparison, ∑PAHs were compared between U.S. and Canadian waters after grouping samples across time points. There were highly significant differences in sediment ∑PAH concentrations between U.S. and Canadian waters on both a dry weight ($p<0.001$; Kurskal-Wallis Test; n=300; Test Statistic = 70.22) and OC-normalized basis ($p<0.001$; Kurskal-Wallis Test; n=300; Test Statistic = 76.61). Median ∑PAH concentrations in the Canadian jurisdiction were 8.1 fold lower compared to those found within U.S. waters. Figure 3.3.4 presents box and whisker plots of the distribution of sediment ∑PAH concentrations (µg/g dry weight) in Canadian and U.S. waters of the Detroit River.

For Contrast 2, there were highly significant differences in ∑PAH concentrations between the different Canadian reaches (upstream, middle and lower Detroit River) on both a dry weight ($p<0.001$; n=142, Test Statistic = 17.25) and OC weight ($p<0.001$; n=152, Test Statistic = 12.20). Conover-Inman's tests were subsequently applied as post-hoc comparisons to establish differences between individual river reaches. For both dry and OC weight contrasts, the upstream reach was significantly lower ($p<0.001$; Conover-Inman test) than both the middle and lower reach. However, there was no significant difference ($p>0.5$ and $p>0.1$; Conover-Inman test) between dry or OC weight ∑PAH concentrations between the middle and lower reach sediments. Figure 3.3.5 presents box and whisker plots of the distribution of sediment ∑PAH concentrations (µg/g dry weight) in the upper, middle and lower Canadian reaches of the Detroit River.

Table 3.3.1 ∑PAH Concentrations in different river sections of the Detroit River Area of Concern

Zone	Median ∑PAH Sediment Concentration $\mu g \cdot g^{-1}$ Dry Wt. (5-95 Percentile)	Median ∑PAH Sediment Concentration $\mu g \cdot g^{-1}$ OC Wt. (5-95 Percentile)	n
Riverwide (1999-2013)	1.84 (0.09-48.22)	70.96 (4.46-1199.25)	300
Canada (1999-2013)	1.04 (0.04-9.25)	24.80 (2.14-298.47)	142
U.S. (1999-2013)	8.38 (0.19-77.17)	234.02 (10.12-1718.28)	158
Canada Upstream	0.18 (0.01-7.21)	11.18 (1.06-165.96)	28
Canada Middle Stream	1.27 (0.30-7.56)	42.78 (13.90-323.67)	25
Canada Downstream	1.17 (0.10-9.68)	26.19 (2.19-290.98)	89
U.S. Upstream	0.87 (0.07-60.16)	27.08 (4.92-2776.18)	36
U.S. Middle Stream	27.73 (0.28-192.80)	532.68 (16.07-2151.33)	27
U.S. Downstream	9.52 (0.46-58.59)	280.11 (23.12-1207.20)	95
Canada – Peche Island Area	0.09 (0.02-0.57)	7.72 (1.60-25.00)	15
Canada – Turkey Creek Area	1.55 (0.61-2.75)	42.68 (25.50-66.27)	5
Canada – Bois Blanc Area	1.94 (0.33-8.92)	33.55 (8.94-210.16)	9
U.S. – Trenton Channel	12.74 (2.03-39.06)	346.44 (62.93-1109.72)	37

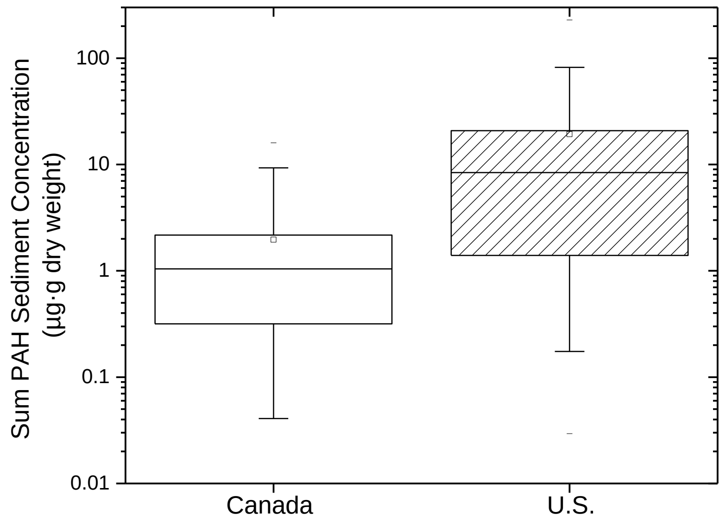

Figure 3.3.4 ∑PAH sediment concentrations (µg/g dry weight) in Canadian and U.S. waters of the Detroit River AOC. Boxes present 25-75 percentiles and median. Square presents mean concentration and whiskers present 5-95% confidence intervals of the distribution.

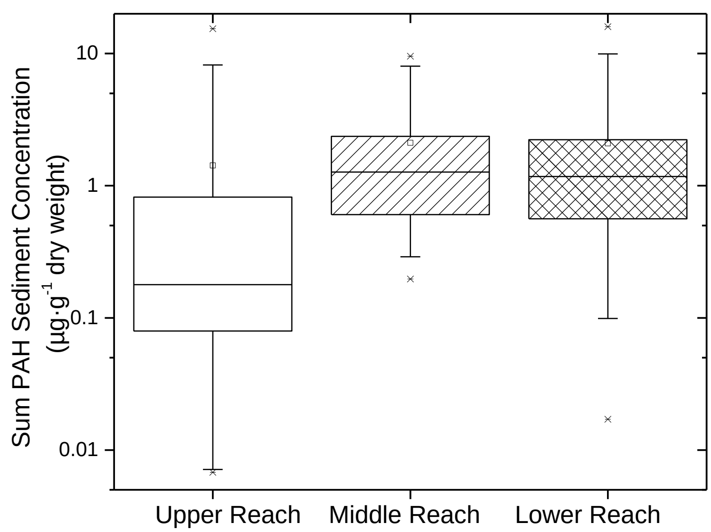

Figure 3.3.5 ∑PAH sediment concentrations (µg/g dry weight) in upper, middle and lower Canadian reaches of the Detroit River AOC. Boxes present 25-75 percentiles and median. Square presents mean concentration and whiskers present 5-95% confidence intervals of the distribution.

For Contrast 3, there were highly significant differences in sediment ∑PAH concentrations between different areas where bullheads have been collected. Peche Island had significantly lower ∑PAHs compared to all other brown bullhead collection sites ($p<0.01$ for all contrasts; Conover Inman's tests) while sediment ∑PAH concentrations in the U.S. Trenton Channel were significantly higher compared to all other collection sites ($p<0.001$ for all contrasts; Conover Inman's tests). For the Turkey Creek and Boise Blanc locations, there were no significant differences in sediment ∑PAH concentrations when expressed on either a dry weight or OC normalized basis (p>0.6; for both contrasts). Overall ∑PAH concentrations at Trenton channel were nearly 142 fold higher compared to Peche Island and between 6.6 to 8.2 fold higher than sediment PAHs at Turkey Creek and Boise Blanc. The differences between ∑PAHs at Peche Island and Turkey Creek/Boise Blanc were on the order of 17.2-21.6 fold. These data suggest that exposure conditions of Detroit River brown bullheads were equivalent at the Turkey Creek and Bois Blanc sample locations. However, Peche Island bullheads have lower exposures to sediment PAHs than fish from Canadian middle stream and lower stream reaches. Finally, Trenton Channel from the U.S. lower downstream reach generates the highest sediment PAH exposures compared to other bullhead sampling sites. Figure 3.3.6 presents box and whisker plots of the distribution of sediment ∑PAH concentrations (µg/g dry weight) at each bullhead collection zone in the Detroit River.

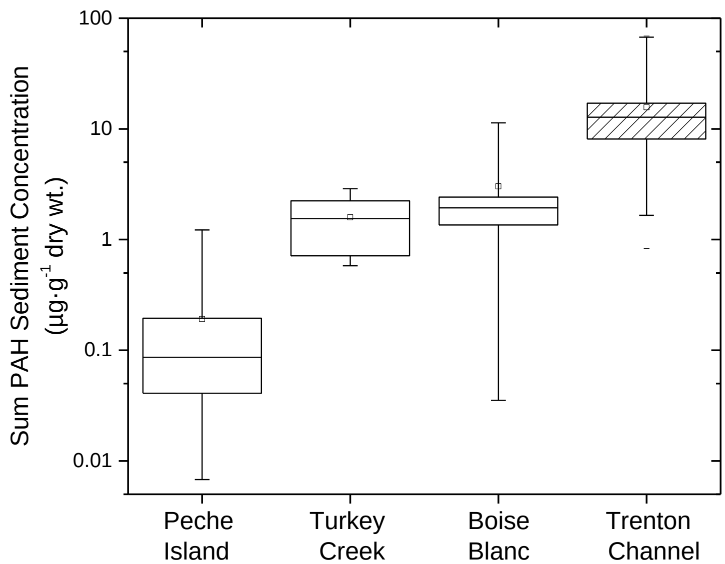

Figure 3.3.6 ∑PAH sediment concentrations (µg/g dry weight) in upper, middle and lower Canadian reaches of the Detroit River AOC. Boxes present 25-75 percentiles and median. Square presents mean concentration and whiskers present 5-95% confidence intervals of the distribution.

3.*2.3 Exceedance of Sediment PAHs against Benchmark Values*

Table 3.3.2 summarizes the percentage of sites exceeding selected sediment PAH benchmark values while Table 3.3.3 presents the median and 5-95 percentiles of hazard quotients. For the 1 $\mu g \cdot g^{-1}$ ∑PAH benchmark, 64.3% of Detroit River locations exceeded the benchmark. When partitioned into Canadian or U.S. locations, 50% of Canadian and 77.2% of US stations exceeded the benchmark. As described for sediment chemistry studies, exceedences of the benchmark were higher in the middle and lower Canadian reaches compared to the upper reach. A similar observation was made for Peche Island compared to Turkey Creek and Boise Blanc bullhead collection locations. In the U.S. Trenton Channel, 97% of stations exceeded the 1 $ug \cdot g^{-1}$ bench mark value. The median HQ for the 1 $\mu g \cdot g^{-1}$ ∑PAH benchmark for the entire Detroit River was 1.85 and 1.04 and 8.23 for the Canadian and U.S. waters, respectively. For the bullhead collection locations, the median HQs were 0.1 at Peche Island, 1.9 for both Turkey Creek and Boise Blanc and 13.6 for the U.S. Trenton Channel, respectively.

For the 4 $ug \cdot g^{-1}$ $\sum$PAH benchmark, 38% of Detroit River stations were in excess of this benchmark value. Among Canadian locations, the percentage of exceedences dropped to 12% while in the U.S. the percent exceedences remained above 60%. The exceedences at bullhead collection locations ranged from 0% (Peche and Turkey Creek) to 22% of stations in the vicinity of Boise Blanc Island and 91% of stations in the U.S. Trenton Channel. Median Hazard Quotients for the 4 $ug \cdot g^{-1}$ $\sum$PAH benchmark were 0.46 on a riverwide basis and 0.26 and 2.06 for sites grouped into Canadian and U.S. locations, respectively. At bullhead collection sites, median hazard quotients were 0.02 at Peche Island, 0.5 at both Turkey Creek and Boise Blanc and 3.4 at Trenton Channel. Given that the 4 $\mu g \cdot g^{-1}$ benchmark was derived specifically for brown bullhead tumor frequencies in Great Lakes tributaries, this benchmark most likely best corresponds with higher risk of neoplasms in the indicator species. Overall, the HQ from the 4 $ug \cdot g^{-1}$ $\sum$PAH benchmark indicate a much higher potential for neoplasms in fish from U.S. locations of the Detroit River particularly those in the middle and lower strata and Trenton Channel Areas. Alternatively, exceedences of this benchmark were relatively rare in Canadian waters and imply that exposures of fish at individual bullhead collection sites were lower then the benchmark.

CCME ISQGs and PELs were also contrasted against measured PAH congeners. CCME ISQGs were developed for the protection of aquatic life and use endpoints of mortality and chronic toxicity in invertebrates but do not necessarily reflect carcinogenic and fish tumor endpoints. Therefore, exceedences of CCME ISQGs and PELs should be interpreted in the context of potential to generate biological toxicity rather than being linked to BUI#3. For ISQGs, 92% of stations in the Detroit River exceeded one or more chemical specific ISQG values and ranged from 88.7 to 94.9% in Canadian and U.S. jurisdictions respectively. One or more PAH ISQGs were exceeded at 35.7% of sites at Peche island, 33 locations in Turkey Creek and all stations at Boise Blanc and U.S. Trenton Channel collection areas. At brown bullhead collection sites, median HQs were 2.3, 39.9, 33 and 253.4 for Peche Island, Turkey Creek, Boise Blanc and U.S. Trenton Channel, respectively.

For PAH PELs, the # of exceedences were 54.7% (River wide) and from 34.5 to 72.8% of sites in Canada and the U.S. waters, respectively. At individual brown bullhead collection sites, exeedences of one or more PAH PELs were 0, 0, 22.2 and 93.9% of stations, at Peche Island, Turkey Creek, Boise Blanc and Trenton Channel, respectively. On the basis of PELs, PAHs appear to be a widespread issue throughout the Detroit River and even within Canadian waters many stations have the potential to elicit chronic toxicity to benthic invertebrates based on the CCME guideline values.

Table 3.3.2. Percent sites exceeding selected PAH sediment benchmark values in the Detroit River.

Zone	% Exceedences $1 \mu g \cdot g^{-1}$ Benchmark	% Exceedences $4 \mu g \cdot g^{-1}$ Benchmark	% Exceedences PAH ISQGs	% Exceedences PAH PELs	# Sites
Upper Canadian	25.0	10.7	71.4	42.9	28
Middle Canadian	60.0	16.0	100.0	28.0	25
Lower Canadian	55.1	11.2	91.0	33.7	89
Upper US	44.4	27.8	83.3	50.0	36
Middle US	81.5	70.4	100	81.5	27
Lower US	88.4	71.6	97.9	78.9	95
All Canadian	50	12.0	88.7	34.5	142
All US	77.2	61.4	94.9	72.8	158
Riverwide	64.3	38.0	92.0	54.7	300
Peche Island	7.1	0	35.7	0	14
Turkey Creek	75	0	33.0	0	4
Boise Blanc	77.8	22.2	100	22.22	9
U.S. Trenton Channel	97.0	90.9	100	93.9	33

Table 3.3.3. Hazard Quotients for PAHs based on selected benchmark values and CCME ISQGs and PELs in the Detroit River

Zone	Median HQ (5-95%) 1μg·g^{-1} Benchmark	Median HQ (5-95 %) 4 μg·g^{-1} Benchmark	Median ∑HQ (5-95 %) PAH ISQGs	Median ∑HQ (5-95 %) PAH PELs	# Sites
Upper Canadian	0.2 (0.01-7.2)	0.04 (0-1.80)	4.4 (0.5-147.4)	0.3 (0.1-10.6)	28
Middle Canadian	1.27 (0.30-7.21)	0.32 (0.07-1.89)	25.1 (5.2-153.8)	1.8 (0.4-11.7)	25
Lower Canadian	1.17 (0.10-9.68)	0.29 (0.03-2.42)	21.4 (2.0-173.5)	1.4 (0.1-11.5)	89
Upper US	0.88 (0.08-59.56)	0.22 (0.02-14.89)	16.6 (1.0-1320)	1.5 (0.1-93.4)	36
Middle US	27.73 (0.28-192.80)	6.93 (0.07-48.20)	520.9 (7.3-3015.1)	40.6 (0.6-218.9)	27
Lower US	9.52 (0.46-58.59)	2.38 (0.11-14.65)	185.1 (8.6-950.5)	13.0 (0.6-62.6)	95
All Canadian	1.04 (0.04-9.25)	0.26 (0.01-2.31)	18.8 (1.2-167.4)	1.3 (0.1-12.1)	142
All US	8.23 (0.19-76.88)	2.06 (0.05-19.22)	158.7 (4.2-1490.8)	10.5 (0.3-109.9)	158
Riverwide	1.85 (0.09-47.87)	0.46 (0.02-11.97)	35.2 (2.2-892.6)	2.6 (0.1-68.1)	300
Peche Island	0.1 (0.01-0.64)	0.02 (0.01-0.16)	2.3 (0.2-12.1)	0.2 (0.02-0.8)	14
Turkey Creek	1.9 (0.8-2.8)	0.5 (0.2-0.7)	39.9 (15.9-52.5)	3.2 (1.2-3.7)	4
Boise Blanc	1.9 (0.3-8.9)	0.5 (0.1-2.2)	33.0 (6.2-178.6)	2.2 (0.4-12.1)	9
Trenton Channel	13.6 (1.9-46.1)	3.4 (0.5-11.5)	253.4 (36.8-708.3)	16.9 (2.6-48.8)	33

3.2.4 Model estimates of brown bullhead total daily intake of PAHs

Figure 3.3.7 summarizes the food web bioaccumulation model calibration as evaluated against individual PCB congeners measured in bullheads from three Canadian collection location taken in 2016. At Peche Island, the food web bioaccumulation model behaved as expected producing model predictions that had equivalent accuracy as described for other Detroit River sport fish species (Li et al. In Press). The geometric mean model bias (Observed/Predicted Concentration) at PI was 1.30. Eighty nine percent of model predictions were within a factor of 10 of observed PCB concentrations measured in individual fish and 74.7% were within a factor of 5. Similar results were observed for the model calibration at Turkey Creek. At TC the geometric mean model bias was 1.24. A total of 83% and 78% of observations were within a factor of 10 and 5 of model predictions.

However, model performance at Boise Blanc was poorer than the other sites. The geometric mean model bias was 3.81 indicating the model tended to underestimate PCB concentrations in fish at this site. Closer examination of the data indicated that the model severely underpredicted the PCB concentrations for highly hydrophobic PCBs (congeners 158, 170-180, 183, 187, 194 and 195/208). However, the majority of tri-hexa chlorinated PCBs apart from PCB 158 were predicted with similar accuracy to other sampling locations (Figure 3.3.7). The combined goodness of fit test, for all congeners and sample locations yielded the following relationship:

$$\text{Log } C_{PCB(obs)} = 0.49 \pm 0.09 \cdot \log C_{PCB(pred)} - 0.08 \pm 0.09; R^2 = 0.28; p<0.001$$

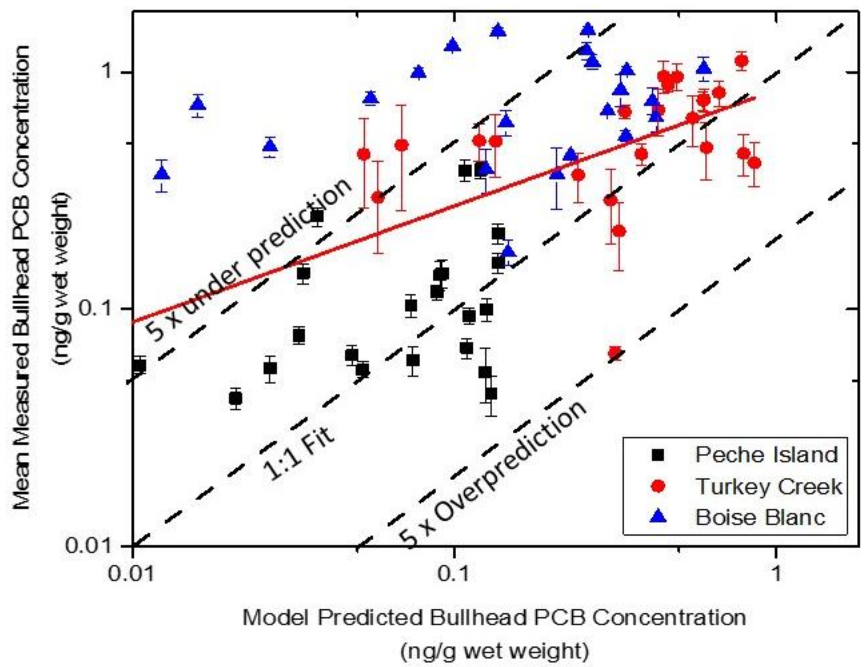

Figure 3.3.7. Model validation of PCB concentrations in brown bullhead collected from Peche Island, Turkey Creek and Boise Blanc in 2016. Solid red line presents regression fit of measured against predicted PCB concentrations in brown bullhead.

Model estimated PAH daily total uptake rates are provided in Figure 3.3.8. The model predicted the lowest daily PAH exposures at Peche Island, intermediate PAH exposures at Turkey Creek and Boise Blanc and highest PAH exposures in fish exposed to U.S. Trenton Channel. For the raw model output PAH exposures at TC and BB were 4.3 and 4.1 fold higher than predicted for PI. For the calibrated model output the exposures were 1.91 and 1.87 fold higher than PI respectively. With regard to U.S. Trenton Channel locations, predicted PAH daily uptake rates were between 5.4 and 34 fold higher compared to Peche Island and from 2.8 to 8.2 fold higher than predicted exposures at Turkey Creek and Boise Blanc, respectively.

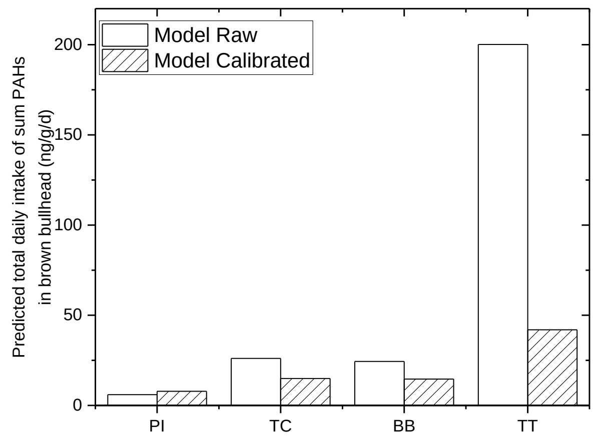

Figure 3.3.8. Model predicted PAH total daily intake rates (ng/g fish/day) in brown bullhead at each of the bullhead collection locations in the Detroit River. Calibrated model refers to raw model output adjusted for the goodness of fit equation generated for PCBs.

3.2.5 Brown Bullhead Chemical Signatures

Turkey Creek fish had generally lower mean ∑PCB concentrations relative to PI and BB fish, however, ANOVA revealed no significant differences ($p>0.1$; $F_{2,21}=2.103$) between sampling locations. Similarly, total Hg concentrations were not significantly different between the sampling locations ($p>0.1$; $F2,21=2.498$). Figure 3.3.9 presents box and whisker plots of sum PCB and total Hg concentrations across the three Canadian brown bullhead sampling locations.

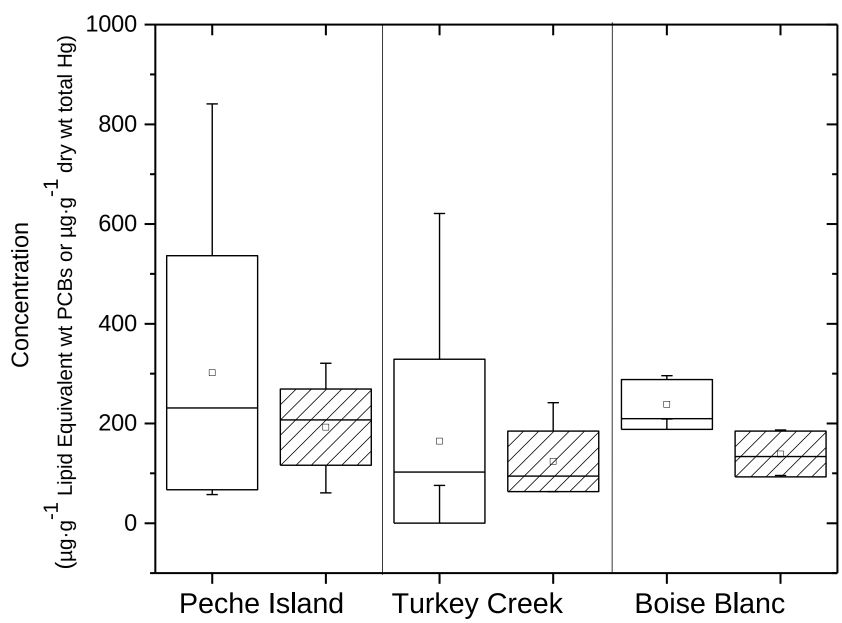

Figure 3.3.9. PCB and Hg concentrations in brown bullhead collected from three Canadian locations in the Detroit River. Boxes present median and standard deviation, whiskers are 5-95 confidence intervals and square is the mean. Hollow boxes present PCB concentrations and lined boxes present total Hg.

Multivariate analysis was used to establish differences in the chemical fingerprints of fish collected from the different Canadian sampling locations. The first 2 PCA axes explained 73.8% of the variation of the data and were the only significant axes as determined by a spree plot and broken stick model. Most organochlorine pesticides and hydrophobic PCB congeners loaded onto PCA 1 (Table 3.3.4) while Hg and tri-tetrachlorobiphenyls exhibited strong to marginal loadings onto PCA2. MANOVA, performed on PCA scores for the first 2 axes indicated significant differences ($p<0.05$) in chemical signatures between the sites. Pairwise comparisons indicated that site specific differences occurred between Peche Island and Turkey Creek ($p<0.05$; Tukey's HSD with bonferonni correction) whereas chemical signatures of Bois Blanc fish did not differ from the other locations ($p>0.1$; Tukey's HSD with bonferonni correction). Figure 3.3.10 presents a plot of the PCA scores across axes 1 and 2 and convex hull areas designating overlap in chemical signatures between the sites.

Table 3.3.4. Chemicals identified as having strong loadings onto individual principle component axes from brown bullhead chemical signature analysis.

PCA Axis	Chemicals with Strong and *Marginal* loading onto a given axis
PCA1	OCS, trans-nonachlor, p,p'-DDE; cis-chlordane, p,p'-DDD, cis-nonachlore; PCBs 66/95, 101, 99, 87, 110, 151/82, 149, 118, 153, 105/132, 138, 158, 187, 183, 128, 177, 156/171, 180, 170/190, 199, 195/208, 194, 206, 209; *marginal loadings for trans-chlordane*
PCA2	PCB 49; *marginal loadings for total Hg (negative); HCB, PCB 52*

* Chemicals with correlation coefficients greater than 0.7 were considered strongly loaded onto a given axis. Chemicals with correlation coefficeints between 0.6 and 0.7 were considered marginally strongly loaded onto the axis.

Overall, the chemical signature analysis supports the results of the site specific sediment chemistry analysis which indicated a lack of difference in chemical exposures between the downstream bullhead sampling locations (Turkey Creek and Bois Blanc) but potentially different chemical exposures occurring at the upstream and downstream locations (Peche Island vs Turkey Creek).

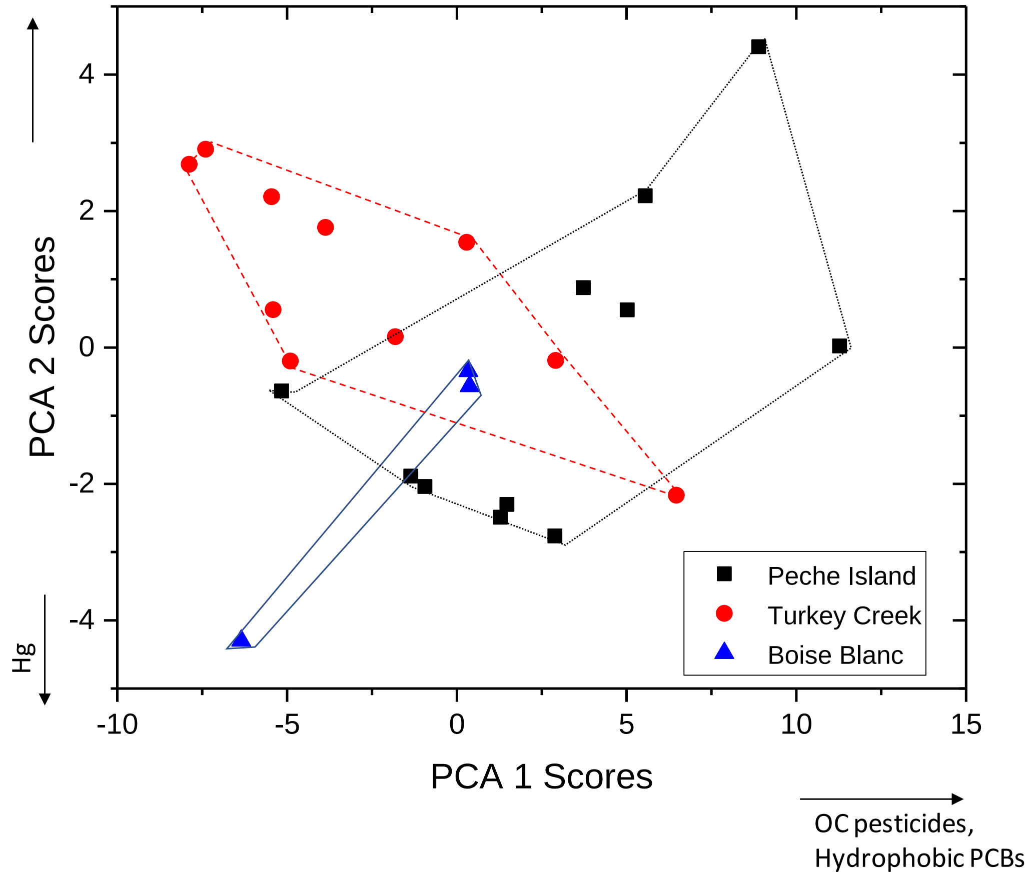

Figure 3.3.10. Principle component scores across PCA axes 1 and 2 demonstrating differences in chemical signatures of brown bullhead collected from three Canadian sampling locations in the Detroit River. Lines present convex hull areas connecting data from each sampling location.

3.2.6 Weight of Evidence Fish Exposure Assessment Conclusions

Examination of PAH concentrations in sediments of the Detroit River indicated no major temporal differences in PAH concentrations within the AOC over the period of 1999-2013. This provides support for combining samples from different sediment survey years to bolster sample sizes for statistical testing of the delisting criteria for BUI #4. The one exception appears to be Trenton Channel where there is some evidence for a decline in OC-normalized sediment PAH concentrations with time. The estimated half life of PAHs in Trenton Channel was 9.4 years. However, such patterns were not evident when PAHs were examined on a dry weight basis.

With regards to spatial patterns of sediment PAHs, there were highly significant differences between sediment PAH concentrations in U.S. and Canadian waters of the AOC. There were also highly significant differences in sediment PAH concentrations between different river reaches of the Canadian waters of the Detroit River. Specifically, upstream waters of the Detroit River had significantly lower PAH concentrations compared to middle and lower reaches. These patterns were mirrored when sediment chemistry data were confined to sites in proximity to brown bullhead collection areas within the Detroit River. As observed on a river wide basis, the

U.S. Trenton Channel was highly significantly elevated in sediment PAHs relative to Canadian brown bullhead collection locations. This indicates that Trenton Channel collected brown bullheads should not be pooled with Canadian caught fish when assessing the delisting criteria. In addition, sediment PAH concentrations at the Turkey Creek and Boise Blanc collection areas were significantly elevated compared to Peche Island. Alternatively, sediment PAH concentrations were statistically equivalent between Turkey Creek and Boise Blanc as well as between the middle and lower Canadian reaches of the Detroit River.

Sediment PAH concentrations were subsequently contrasted against benchmark sediment PAH values used to assess probability of fish tumors. Two benchmark values consisting of 1 µg/g dry total PAHs and 4 µg/g dry total PAHs were applied. The former was recommended for the protection of marine fish species while the 4 µg/g was generated based on the inflection point for increased tumor frequency measured in a Great Lakes brown bullhead population. Fifty and twelve percent of Canadian sediment collection locations exceeded the 1 and 4 µg/g sediment PAH benchmarks, respectively. At Canadian brown bullhead collection locations the benchmark exceedences ranged from 7.1% to 77.8% for the 1 µg/g benchmark and from 0-22.2% for the 4µg/g benchmark. In contrast, the U.S. Trenton Channel exceeded the two benchmarks at 97 and 91% of sediment collection sites, respectively. Larger numbers of exceedences were noted for the CCME ISQG and PELs both in Canadian and U.S. waters. However, because these benchmarks relate to benthic invertebrate toxicity rather than fish tumours, they were not further considered in decisions concerning grouping of fish tumour prevalence samples by location.

The food web bioaccumulation model was applied to estimate the daily PAH intake rates of bullheads from the different bullhead collection sites. Following calibration to PCBs measured in bullheads from each location, the model predicted lowest daily PAH exposures at Peche Island, intermediate, but equivalent, exposures at Turkey Creek and Bois Blanc and high exposures in the U.S. Trenton Channel region of the AOC.

Finally, brown bullhead from the three Canadian collection zones obtained in 2016 were examined for bioaccumulative contaminants to examine for between site differences in chemical fingerprints. Although PAHs are not detected in fish, both sediment PCB and organochlorine pesticides are correlated with sediment PAHs in the Detroit River. Thus, the bioaccumulation of PCB and organochlorine pesticides was used as a proxy for potential sediment exposures to PAHs by bullheads from the different collection sites. There were no significant differences in PCB or Hg concentrations in bullheads across the three Canadian bullhead collection sites. However, multivariate analysis of chemical signatures did indicate different chemical fingerprints among the collection areas. Peche Island fish had significantly different chemical signatures compared to Turkey Creek collected fish. No differences were apparent in the chemical signature of fish at Boise Blanc from either Peche Island or Turkey Creek. However, the sample size at Boise Blanc was small at only 3 fish and no definitive conclusions about chemical signatures at this site can be reached.

Table 3.3.5 provides a summary of the different findings from each line of evidence used in the WOE. Overall, multiple lines of evidence support the finding that bullhead exposures to PAHs are equivalent at Turkey Creek and Boise Blanc locations and that sediment contamination at these locations has not changed over the 199-2013 time period. The temporal conclusion is anticipated to be extrapolated to the 2002-2016 fish collections used for fish tumour prevalence. However, PAH exposures were lower at Peche Island and much elevated at the U.S. Trenton Channel fish collection locations. Based on these lines of evidence and lack of temporal trends in sediment chemistry it is suggested that bullhead histopathology studies can combine fish collected from Turkey Creek and Bois Blanc areas across the two survey years. It is recommended that fish from Peche Island not be pooled with fish collected from other locations but could be combined from the two survey years at this location. Similarly, fish from Trenton Channel should be analyzed separately with respect to assessing the BUI #4 delisting criteria. The WOE support for combining Trenton Channel fish from the 2001 and 2012 sample surveys is mixed and warrants caution owing to a decline in organic carbon normalized sediment PAH concentration with time at this location of the river.

Table 3.3.5 Lines of evidence in the WOE assessment of brown bullhead exposures at different sampling locations of the Detroit River.

Line of Evidence	Peche Island	Turkey Creek	Boise Blanc	Trenton Channel
Median Sediment ∑PAHs μg/g OC weight	10.2	40.8	36.6	308.8
% Exceedence of 1ug/g ∑PAH in sediment; Median Hazard Quotient	7.1% 0.1	75% 1.9	78% 1.9	97% 13.6
% Exceedence of 4 μg/g ∑PAH in sediment; Median Hazard Quotient	0% 0.02	0% 0.5	22% 0.5	91% 3.4
Bullhead Chemical Signatures	Different from TC	Different from PI	Sample size insufficient to distinguish from PI or TC	NA
Model Total Daily PAH Intakes (Calibrated model)	5.9 (7.8)	26.1 (14.9)	24.4 (14.7)	200.1 (41.9)

4.0. Delisting criteria assessment

The Canadian Stage II Detroit River RAP report (Green et al., 2010) reports the delisting criteria for BUI #4 as:

> "When the incidence rates of liver tumours in (3-5 year old) brown bullhead are not statistically different than the Great Lakes background rate."

Over the 2002-2016 surveys there were 112 fish collected from Canadian waters of the AOC that were in the age range of 3 to 5 years old. Of these fish, only 1 contained a liver neoplasm giving a total liver neoplasm prevalence of <1%, lower than the Great Lakes background rate of 2%.

However, although the WOE exposure assessment provides support for combining data between sample years the results were mixed for combining samples across sample collection locations. Based on the Weight of Evidence exposure assessment outcomes, contingency tables were set up to test the delisting criteria based on pooled samples. Sample pooling was completed for subsets of pooled samples and Fisher's exact tests were used in conjunction with the reference data contingency table proportions described by Bauman (2010). This led to the following tests:

1. Fish collected from Peche Island were pooled between 2002 and 2016.
   Samples in this category consisted of 28 fish aged between 3-5 years. No fish were observed to have liver neoplasms. Fisher's Exact Test Indicated No Significant Difference (p>0.9) in the tumour prevalence at Peche Island compared to the reference data. However, the sample size of fish was small at this location and considerably less than the 100 sample minimum recommended by Bauman (2010). Addition of fish older fish than 5 years of age increased the sample size to 44 fish. Fisher's Exact Test failed to demonstrate a significant difference in the tumour prevalence at this location relative to the Great Lakes Tumour reference. However, the sample size was still considered insufficient at this site to test the delisting criteria with statistical rigor.

   The authors attempt to provide additional lines of evidence for consideration of the data quality at the Peche Island location. It is noted that Bauman (2010) originally classified Peche Island as a reference location and included data from the 2002 sample collections in the Great Lakes reference data set used to determine the Great Lakes background tumour rate. Leadley et al. (1993) also showed that this location had the lowest incidence of tumours relative to other locations in the Detroit River. Sediment PAHs, which have causal linkage to fish neoplasms, are also among the lowest in this location the levels measured at Peche Island are comparable to the entire upstream Canadian waters of the AOC. Finally, PAH concentrations tend to be lower than sediment quality benchmarks issued for the protection against fish tumours at this location. Thus, despite not achieving sufficient sample size for statistical testing at this location, the body evidence suggests that tumour prevalence in Canadian upstream waters of the Detroit River AOC are likely to be not impaired.

2. Fish collected from Turkey Creek and Bois Blanc area were pooled between 2002 and 2016. Samples in this category consisted 84 fish aged 3-5 years. 1/84 fish contained a liver neoplasm generating a tumour prevalence of 1.2%. Fisher's exact test indicated no significant difference ($p>0.9$) in the tumour prevalence of 3-5 year old brown bullhead from middle and lower Canadian reaches of the Detroit River compared to the Great Lakes reference tumour dataset. However, the total number of samples in this age bracket were somewhat less than the minimum recommended sample size of 100 fish. Including fish older than age 5 yields a total of 98 fish with only 1/98 specimens having a liver neoplasm. Fishers Exact test performed on fish older than 3 years failed to detect a significant difference ($p>0.9$) in tumour prevalence in the midstream and lower reaches compared to the reference data set. Given the proximity of this test to minimum sample requirements and also given that the absolute tumour prevalence was lower than 2%, it is recommended that the middle and lower reaches of the Canadian waters of the Detroit River meet the conditions specified by the BUI #4 delisting criteria.

A final guidance provided by the Canadian Stage 2 RAP report on BUI #4 delisting criteria assessment was that "a minimum of two sampling events take place 3 years apart to show the changes in sediment contamination and because tumours are positively correlated to age" Green et al. (2010). In the present report, fish were collected between 2002 and 2016 supporting the minimum of two sampling events taken 3 years apart. However, because samples had to be pooled between survey years to address issues of low sample size and statistical testing, the combined surveys cannot be considered independent of one another. There was also no evidence for changes in sediment contamination for carcinogenic PAH compounds between the time points in which surveys were undertaken. Only one other study (Leadley et al. 1998) reported data on brown bullhead tumour prevalence in Canadian waters of the AOC. It identified tumour prevalence's of 4 and 13% at Peche Island and Amherstburg Channel (near Bois Blanc), respectively in samples collected 1993 suggestive that tumour frequencies have declined in Canadian waters of the AOC over time.

5.0 References

Arcand-Hoy LD, CD Metcalfe. 1999. Biomarkers of exposure of brown bullheads (*Ameiurus nebulosus*) to contaminants in the lower Great Lakes, North America. Environ. Toxicol. Chem. 18:740-479.

Arnot JA, FAPC Gobas. 2004. A food web bioaccumulation model for organic chemicals in aquatic ecosystems. Environ. Toxicol. Chem. 23:2343-2355.

Bauman PC. 1992. The use of tumors in wild populations of fish to assess ecosystem health. J. Aquat. Ecosyst. Stress Recovery. 1:135-146.

Bauman P. 2003. Detroit River tumor data: problems in comparing different studies. *In* Rafferty (Ed.), *Fish Tumors Related to Great Lakes Areas of Concern Conference Proceedings*,

Cosponsored by PA Dept. Environ. Protection, U.S. EPA Region III and Pennsylvania Sea Grant, Jan 21-22, 2003, Erie, PA, USA, pp. 23-34.
Bauman PC. 2010. Data analysis and fish tumor BUI assessment for the lower Great Lakes and Interconnecting Waterways. Report submitted to Environment Canada, Mar 31, 2010.
Bauman PC, JC Harshbarger. 1995. Decline in liver neoplasms in wild brown bullhead catfish after coking plant closes and environmental PAHs plummet. Environ. Health Perspect. 103:168-170.
Bauman PC, JC Harshbarger. 1998. Long term trends in liver neoplasm epizootics of brown bullhead in the Black River, Ohoio. Environ. Monitor. Assess. 53:213-223.
Bauman PC, WD Smith, WK Parland. 1987. Tumor frequencies and contaminant concentrations in brown bullheads from an industrialized river and a recreational lake. Trans. Amer. Fish. Soc. 116:79-86.
Bauman PC, MJ Mac, SB Smith, JC Harshbarger. 1991. Tumor frequencies in walleye (*Stizostedion vitreum*) and brown bullhead (*Ictalurus nebulosus*) and sediment contaminants in tributaries of the Laurentian Great Lakes. Can. J. Fish. Aquat. Sci. 48:1804-1810.
Bauman PC, IR Smith, CD Metcalfe. 1996. Linkages between chemical contaminants and tumors in benthic Great Lakes fish. J. Great Lakes Res. 22:131-152.
Blazer VS, JW Fournie, JC Wolf, MJ Wolfe. 2006. Diagnostic criteria for proliferative hepatic lesions in brown bullhead *Ameiurus nebulosus*. Dis. Aquat. Org. 72:19-30.
Blazer VS, SD Rafferty, PC Baumann, SB Smith, EC Obert. 2009a. Assessment of the tumors or other deformities beneficial use impairment in brown bullhead: I. Orocutaneous tumors. J. Great Lakes Res. 35:517-526.
Blazer VS, SD Rafferty, PC Baumann, SB Smith, EC OBert. 2009b. Assessment of the tumors and other deformities beneficial use impairment in brown bullhead: II. Liver tumors. J. Great Lakes Res. 35:527-537.
Brown ER, JJ Hazdra, L Keith, I Greenspan, JBG Kwapinski, P Beamer. 1973. Frequency of fish tumors found in a polluted watershed as compared to nonpolluted Canadian waters. Can. Res. 33:189-198.
CCME (Canadian Council of Ministers of the Environment). 2001. Canadian Environmental Quality Guidelines. www.ccme.ca/en/resources/canadian_environmental_quality_guidelines/ (Accessed Dec 18, 2018).
Drouillard KG, M Tomczak, S Reitsma, GD Haffner. A river-wide survey of polychlorinated biphneyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), and selected organochlorine pesticide residues in sediments of the Detroit River – 1999. J. Great Lakes Res. 32:209-226.
Drouillard KG, Y Qian, J Lafontaine, N Ismail, K McPhedran, E Szalinska, A Grgicak-Mannion. In Press. Polybrominated diphenyl ethers (PBDEs) in sediments of the Huron-Erie corridor. Bull. Enviorn. Contam. Toxicol. (Accepted Feb 27, 2019).
Farwell M, KG Drouilaard, DD Heath, TE Pitcher. 2012. Acclimation of life-history traits to experimental changes in evnrionmental contaminant concentrations in brown bullhead (*Ameiurus nebulosus*). Environ. Toxicol. Chem. 31:863-869.
Green, N, L Cargnelli, T Briggs, R Drouin, M Child, J Esbjerg, M Valiante, T Henderson, D McGregor, D Munro. 2010. *Detroit River Canadian Remedial Action Plan Stage 2*

Report. Detroit River Canadian Cleanup, Publication No. 1, Essex, Ontario, Canada, 170 pp.

Hansen BG, AB Paya-Perez, M Rahman, BR Larsen. 1999. QSARs for K_{OW} and K_{OC} of PCB congeners: A critical examination of data, assumptions and statistical approaches. Chemosphere 39:2209-2228.

Harshbarger JC, LJ Cullen, MJ Calabrese, PM Spero. 1984. Epidermal, hepatocellular and cholangiocellular carcinomas in brown bullheads, *Ictalurus nebulosus*, fom industrially polluted Black River, Ohio. Mar. Environ. Res. 14:535-536.

Johnson, LL, TK Collier, JE Stein. 2002. An analysis in support of sediment quality thresholds for polycyclic aromatic hydrocarbons (PAHs) to protect estuarine fish. Aquat. Conserv. Mar. Freshw. Ecosyst. 12:517-538.

Kashian DR, K Drouillard, D Haffner, A Krause, Z Liu, L Sano. 2010. What are the causes, consequences and correctives of fish contamination in the Detroit River AOC that cause health consumption advisories? Final Report to Michigan Sea Grant, 523 pp.

Leadley TA, G Balch, CD Metcalfe, R Lazar, E Mazak, J Habowsky, GD Haffner. 1998. Chemical accumulation and toxicological stress in three brown bullhead (*Ameiurus nebulosus*) populations of the Detroit River, Michigan, USA. Environ. Toxicol. Chem. 17:1756-1766.

Leadly TA, LD Arcand-Hoy, GD Haffner, CD Metcalfe. 1999. Fluorescent aromatic hydrocarbons in bile as a biomarker of exposure of brown bullheads (*Ameiurus nebulosus*) to contaminated sediments. Environ. Toxicol. Chem. 18:750-755.

Li J, K McPHedran, E Szalinska, AM McLeod, SP Bhavsar, J Bohr, A Grgicak-Mannion, KG Drouillard. In Press. Characterizing PCB exposure pathways from sediment and water in aquatic life using a food web bioaccumulation model. Int. Assess. Environ. Manage. Accepted Jan, 2019.

Maccubbin AE, N Ersing. Tumors in fish from the Detroit River. Hydrobiologia. 219:310-306.

McLeod AM, JA Arnot, K Borga, H Selck, DR Kashian, A Krause, G Paterson, GD Haffner, KG Drouillard. Quantifying uncertainty in the trophic magnification factor related to spatial movements of organism in a food web. Int. Environ. Assess. Manage. 11:306-318.

Millard MJ, DR Smith, EC Obert, JL Grazio, M Bartron, C Wellington, S Grise, SD Rafferty, R Wellington, S Julian. 2009. Movement of brown bullheads in Presque Isle Bay, Lake Erie, Pennsylvania. J. Great Lakes Res. 35:613-619.

Pinkney AE, JC Harhbarger, EB May, MJ Melancon. 2002. Tumor prevalence and biomarkers of exposure in brown bullheads (*Ameiurus nebulosus*) from the tidal Potomac River, USA, watershed. Environ. Toxicol. Chem. 20:1196-1205.

Pinkney AE, JC Harshbarger, EB May, WI Reichert. Tumor prevalence and biomarkers of exposure and response in brown bullhead (*Ameiurus nebulosus*) from the Anacostia River, Washington, DC and Tuckahoe River, Maryland, USA> Environ. Toxciol. Chem. 23:638-647.

Pyron M, EC Obert, R Wellington. 2001. Tumor rates and population estimates of brown bullhead (*Ameiurus nebulosus*) in Presque Isle Bay, Lake Erie. J. Great Lakes Res. 27:185-90.

Rafferty SD, VS Blazer, AE Pinkney, JL Grazio, EC Obert, L Boughton. 2009. A historical perspective on the fish tumors or other deformities beneficial use impairment at Great Lakes Areas of Concern. J. Great Lakes Res. 35:496-506.

Rutter MA. 2010. A statistical approach for establishing tumor incidence delisting criteria in areas of concern: A case study. J. Great Lakes Res 36:646-655.

Sahu, SK, GG Pandit. 2003. Estimation of octanol-water partition coefficients for polycyclic aromatic hydrocarbons using reverse-phase HPLC. J. Liquid Chromatogr. Related Technol. 26:135-146.

Sakaris PC, RV Jesien, AE Pinkney. 2005. Brown bullhead as an indicator species: seasonal movement patterns and home ranges within the Anacostia River, Washington, D.C. Trans. Amer. Fish. Soc. 134:1262-1270.

Smith SB, MA Blouin, MJ Mac. 1994. Ecological comparisons of Lake Erie tributaries with elevated incidence of fish tumors. J. Great Lakes Res. 20:701-716.

Szalinska E, A Grgicak-Mannion, GD Haffner, KG Drouillard. 2013. Assessment of decadal changes in sediment contamination in a large connecting channel (Detroit River, North America). Chemosphere 93:1773-1781.

Thoman RV, JP Connolly. 1984. Model of PCB in the Lake Michigan lake trout food chain. Environ. Sci. Technol. 18:65-71.

Wolfe JC, MJ Wolfe. 2005. A brief overview of nonneoplastic hepatic toxicity in fish. Toxicologic Pathology 33:75-85.

Yang X, J Meier, L Chang, M Rowan, PC Baumann. 2006. DNA damage and external esions in brown bullheads (*Ameiurus nebulosus*) from contaminated habitats. Enviorn. Toxicol. Chem. 25:3035-3038.