# RE-DESIGNATION REPORT

## Assessment of Fish Tumors and Other Deformities (BUI #4) in the Detroit River Canadian Area of Concern

Prepared by Dr. Ken Drouillard (Great Lakes Institute for Environmental Research, University of Windsor) for the Detroit River Canadian Cleanup's (DRCC) Monitoring and Research Work Group

2020

DETROIT RIVER CANADIAN CLEANUP
detroitriver.ca

*Suggested Citation: Detroit River Canadian Cleanup (DRCC). 2020. Re-designation Report: Assessment of Fish Tumors and Other Deformities (BUI #4) in the Detroit River Area of Concern. Windsor, Ontario, Canada.*

# CONTENTS

# EXECUTIVE SUMMARY

This report compiles data on liver tumor prevalence in brown bullheads collected from Canadian waters of the Detroit River AOC as well as 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 in the Canadian Stage II Detroit River RAP report is as follows:

> "When the incidence rates of liver tumors 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 Canadian locations in the AOC. The upstream collection location, Peche Island, was previously designated as an in-stream reference site and data from this location were separately examined relative to the midstream (Turkey Creek area) and downstream (Boise Blanc and nearshore areas of Amherstburg Channel) fish collection zones. Over the two survey years, there were 28 fish collected from the reference site, 72 fish from Turkey Creek and 12 fish from Bois Blanc/Amherstburg channel that were in the 3-5 year age range specified by the delisting criteria. In addition, another 16, 11, and 3 fish older than 5 years were also available from the above respective sites. Only one fish from 2002 collected at Boise Blanc Island contained a liver neoplasm giving an absolute tumor prevalence of 1.2% in the combined middle and lower Canadian reaches of the AOC. No tumors were observed at Peche Island which is consistent with its prior designation as a reference site.

The Stage II Report recommended that fish surveys be conducted three years apart; however, the data used for this assessment was collected in 2002 and 2016. The data was combined to increase statistical power to test against the delisting criteria. A weight of evidence (WOE) exposure assessment was performed to justify pooling samples from across survey years and/or fish collection locations. The WOE took into consideration time trends of sediment PAH concentrations in the AOC, differences in sediment PAHs at bullhead sampling locations, differences in chemical signatures in bullheads from different locations and bioaccumulation simulations to predict daily PAH exposures in fish from each collection zone. In addition, sediment PAH concentrations were compared to benchmarks recommended for the protection against fish tumors.

The WOE indicated that there was no evidence for changes in sediment PAH concentrations with time, and likely exposures to carcinogenic PAH compounds by fish, in the AOC as a whole (U.S. and Canadian jurisdictions). Similarly, sediment PAH concentrations were stable through time in combined Canadian waters of the AOC, defined as the upstream, middle stream, and downstream Canadian river reaches as well as at individual bullhead collection sites. This provided support for pooling fish samples collected from the two survey years (2002 and 2016). The WOE was also used to address pooling of samples between the middle and lower Canadian fish collection zones. First, the WOE provided support for the prior designation of Peche Island as a reference location. PAH concentrations in sediments, exceedance of PAH benchmark values, and estimated daily PAH exposure rates by fish were lower at Peche Island compared to the midstream and downstream fish collection areas. Second, sediment PAH contamination and simulated exposures in brown bullhead from the midstream and downstream collection zones were found to be similar to one another. The latter observation provided justification for pooling samples from the midstream and downstream collection zones when addressing tumor prevalence. Third, the WOE indicated that the sediment PAH concentrations measured in the local fish collection zones were consistent with the broader spatial patterns of sediment PAH concentrations present throughout the middle and lower reaches of the Canadian waters of the AOC which were

elevated relative to the upstream reach. Thus, although not all possible brown bullhead habitats of the Canadian waters of the AOC were sampled, the WOE provides support that the fish collection locations used in the present assessment were widely representative of the Canadian waters of the AOC as a whole.

Taking the WOE pooling suggestions into consideration, the BUI #4 delisting criteria was tested with a subset of samples pooled from Turkey Creek and Boise Blanc/Amherstburg Channel over the 2002 and 2016 collection periods. The combined tumor prevalence in these fish was 1.2% (1/84 fish aged 3-5) and not significantly ($p>0.9$) different then the Great Lakes fish tumor reference database. For Peche Island, only 28 fish aged 3-5 were available, with no samples containing liver neoplasms. There were an additional 16 fish from Peche Island and 14 fish from the midstream and downstream collection zones that were older than 5 years of age. Although older fish have a higher probability of achieving liver tumors, none of the age 6+ fish collected in Canadian waters of the AOC were observed to contain liver neoplasms.

It is acknowledged that the combined number of midstream and downstream fish ($n=84$) was less than the target of 100 fish recommended to be used for testing the delisting criteria. However, given that the observed fish tumor prevalence was less than the Great Lakes background reference of 2% and the total number of fish approached the 100 target, it is highly unlikely that the statistical testing used in the assessment generated a false negative or Type II statistical error. Indeed, if the age 6+ fish are added to fish counts, the number of midstream plus downstream fish increases to 98 and the tumor prevalence decreases to 1%.

While the Stage II Report recommended that fish surveys be conducted at least 3 years apart, in this assessment, the 2002 and 2016 data were combined in order to increase statistical power to test the delisting criteria. By pooling the samples across survey years, the temporal component of the experimental design has been removed. However, there are two lines of evidence that support the validity of the current assessment. First, the temporal requirement of the assessment is premised on changes in environmental exposures (either an increase or decrease in environmental contamination) that may have occurred between different fish collection surveys. The WOE indicated that there were no changes in the sediment PAH contamination in Canadian waters of the AOC over the 1999-2013 period implying there is little evidence for change in environmental exposure conditions of fish between survey years. Additionally, there have been no known contributions of contaminants since 2013.Second, an earlier published study by Leadley et al. (1998) documented higher liver tumor prevalence in Canadian caught brown bullhead from Peche Island (tumor prevalence of 4%) and Amherstburg Channel (tumor prevalence of 13%) for fish collected in 1993. This provides support for decreasing trends in brown bullhead liver neoplasm prevalence from the early 1990’s to 2000’s.

Based on the body of evidence collected and considered in this assessment, it is recommended that BUI #4: Fish Tumor and Other Deformities be changed from its current status of Impaired to Not Impaired for the Canadian waters of the Detroit River Area of Concern.

# 1.0 INTRODUCTION

The 51 km long Detroit River is a connecting channel that, along with the St. Clair River and Lake St. Clair, link Lake Huron to Lake Erie. For the past 100 years, the Detroit River and its watershed have experienced large amounts of industrial, urban, and agricultural development, which led to the river becoming highly degraded. As a result, the Detroit River was listed as one of the 43 Areas of Concern (AOC) under the Great Lakes Water Quality Agreement (GLWQA) in 1987. AOCs are sites around the Great Lakes where the aquatic ecosystem has been degraded due to local sources of pollution. This degradation has led to a change in the chemical, physical, or biological integrity sufficient to cause one or more impairments to beneficial uses of the Great Lakes. In each AOC, all levels of government, community, and industry partners undertake a cooperative effort to restore the environmental integrity of the AOC through the implementation of a Remedial Action Plan (RAP). The purpose of these AOC-specific RAPs is to restore beneficial use impairments and ultimately remove the Detroit River from the list of Great Lakes AOCs (Figure 1). Although the Detroit River AOC is technically bi-national, separate Canadian and American RAPs have been developed.

Since 1998, the Remedial Action Plan for the Canadian side of the Detroit River has been implemented through the Detroit River Canadian Cleanup (DRCC) initiative. The success of RAP implementation is measured through the change in status of beneficial use impairments (BUIs) from impaired to not impaired. In 2010, the Detroit River Canadian Remedial Action Plan Stage 2 Report was developed, detailing restoration goals for beneficial use impairments and recommended remedial actions to address them. In the Stage 2 Report, 9 beneficial uses were deemed impaired and 2 required further assessment. Currently, 6 BUIs are impaired, including fish tumors and other deformities, and 1 BUI requires further assessment.

The Fish Tumor and Other Deformities BUI is linked to contaminated sediment, which exists on both sides of the Detroit River. Polynuclear aromatic hydrocarbons (PAHs), in particular, are a class of carcinogenic compounds that were linked to the development of liver tumors in fish in the 1980s. Major sources of PAHs include coal-fired plants, once a common power source around the Great Lakes, and production of coke, aluminum, and asphalt, as well as vehicle exhaust. The lack of consistent studies on the incidence of tumors in the Canadian portion of the Detroit River, and presence of sediment contamination on both sides of the river, led to the Fish Tumors and Other Deformities BUI being deemed impaired in the 2010 Stage 2 Report.


**Figure 1. Location of the Detroit River Area of Concern and its Watershed**

This document provides an analysis and assessment of data related to Beneficial Use Impairment (BUI) #4: Fish Tumors and 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 and publications on fish tumor prevalence in the Detroit River are summarized in order to 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.

## 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 which 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 reported 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 tumor 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 total tumor prevalence in brown bullhead (Baumann 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 the 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 the Trenton Channel, Leadley et al. (1998) reported a total of 6 fish (24%) with biliary neoplasms compared to 1/27 (4%) and 3/23 (14%) caught from Canadian Peche Island and Amherstburg Channel locations, 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 (10% 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 100 fish recommended by Baumann (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 (n=40) 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 (Baumann 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 exceeded the age range recommended by the Canadian delisting criteria.

Table 1 summarizes published studies on brown bullhead tumor prevalence measured in United States and Canadian waters of the Detroit River over the period of 1985-2012. The Trenton Channel had 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 tumor incident rates across studies or a 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 13% in Amherstburg Channel. Both values 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. 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. Summary of published studies on brown bullhead tumor rates in Canadian and U.S. waters of 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<br>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/<br>Not specified | 3-4 /<br>length at age | 3.6±0.14<br>Pectoral spines | 5.6±1.7(3-9) pectoral spines | 5.6±0.3 2011<br>6.5±0.5 2012/<br>Otoliths |
| Neoplasm Type: | | | | | |
| Hepatocellular Adenoma | | NR | NR | | |
| Hepatocellular Carcinoma | + | NR | 0% TT | | |
| Cholangioma | + | 0%, 0%, 4%<br>PI, AC, TT | 10% TT | | 2.5% |
| Cholangio Carcinoma | | 4%, 13%, 20%<br>PI, AC, TT | 5% TT | | 5% |
| Total Neoplasms | 8.8%[*], 12.1%[b] | 4%, 13%, 24% | 15% TT | 5.9% TT | 7.5% TT |

| | | | | | |
|---|---|---|---|---|---|
| | | PI, AC, TT | | | |

*Reported Tumor rate includes neoplasms + total Putative pre-neoplastic lesions
[b] Tumor frequency including total Putative 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

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

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

The Stage II RAP Report also provided guidance on study design for remaining BUIs. These recommendations were generated from DRCC workshops held in 2007 and 2010 and included the following:

1) The background liver tumor 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 changes in sediment contamination because tumors are positively correlated to age

The 2% background tumor prevalence was taken from Baumann (2010) who reviewed brown bullhead tumor prevalence from a wide variety of near-field control locations, far field locations, and urbanized Great Lakes reference areas from the U.S. and Canada to generate a reference brown bullhead tumor prevalence database. Baumann (2010) used the compiled reference database to provide assessments of BUI #4 in 7 Canadian/International AOCs. Given the precedence of this application across several Canadian BUI #4 assessments and its direct referral in the Detroit River Stage II RAP report, the Baumann (2010) approach was adopted as the template on which to evaluate BUI #4 for the Canadian portion of the Detroit River AOC. However, it is recognized that alternative statistical approaches, e.g., those of Rutter (2010), have been adopted in other Areas of Concern. The Rutter (2010) approach was not used in our analyses as we did not have enough fish with tumors to conduct model calibration.

While the Stage II RAP report recommended tumor rates inside the AOC be compared to the 2% rate for the lower Great Lakes, the delisting criteria for the DR AOC only requires a lack of statistical difference between the AOC tumor prevalence and the Great Lakes background prevalence. This leads to some potential confusion in establishing the appropriate experimental design to test the status of BUI #4 since statistical power is related to both the statistical test utilized and sample size (i.e., total number of fish collected). For example, studies that collect only small numbers of fish are less likely to include diseased fish which, even at contaminated sites, reflect a relatively small proportion of the sampled population. In addition, small sample sizes generate large confidence intervals decreasing the statistical power and increasing the likelihood of generating Type II errors (i.e., a false negative or failing to detect a statistical difference in tumor prevalence from background rate when in reality there is a difference). To address these concerns, Baumann (2010) provided further guidance on the statistical test and sample size necessary to test the criteria. He recommended using Fisher's exact test and stipulated, through a power analysis, that a minimum sample size of 100 fish should be targeted. Baumann (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 2).

**Table 2. Contingency Table used in conjunction with Fisher's exact test to determine statistical differences in brown bullhead tumor rates relative to Great Lakes Reference populations**

| Location | # Fish without tumors | # Fish with tumors |
|---|---|---|
| Great Lakes Reference | 1127 | 23 |
| AOC (Impacted Site) | Measured value | Measured value |

The power analysis completed by Baumann (2010) indicated that relatively large sample sizes or very high tumor prevalence at the impacted site are needed to demonstrate statistical differences between the AOC and background tumor prevalence rate.  For example, an AOC with a true tumor prevalence of 5% would require approximately 100 fish in order statistically distinguish this from reference tumor prevalence of 2%. However, when the true tumor prevalence is well above 5%, the number of fish needed to avoid a Type II error becomes lower.  Thus, given a sample size of 100 fish, an observed tumor prevalence of at least 5% would be necessary to achieve an impaired status.

The second item in the Stage II Design and Rationale specifies that at least two tumor prevalence surveys be conducted at least 3 years apart. The rationale for the minimum 3 year gap between surveys was that it would reduce time lag artifacts in successive measures of fish tumor frequency under a condition where environmental contamination and mutagenic substance exposures to fish have changed over time. The gap between successive surveys enables population turnover to occur such that age 3-5 cohorts from the first survey would not be included in the 2$^{nd}$ survey, ensuring independence of the sampled cohorts.  For example, fish from the age 3 cohort from survey one would be 6 years of age by the time a new set of fish were collected in survey 2 conducted 3 years after the first fish.  Therefore, the 3-5 year age cohorts from survey 1 would not be included among fish cohorts sampled and used to measure fish tumor prevalence in the 2$^{nd}$ survey.

An issue that is not covered by the delisting criteria or the Stage II report recommendations is the number and position of sampling locations that should be included in the fish collection surveys. Baumann (2010) had previously designated the upstream Canadian section of the Detroit River as a near field urban reference area and included 2002 collected bullheads from Peche Island (See Figure 2) as part of the Great Lakes brown bullhead liver prevalence reference database. There are limitations in brown bullhead habitats within the Detroit River with potential fragmentation of habitats leading to low ecological connectivity between habitats distributed across the AOC.  The original rationale for specifying Peche Island as a reference site was not described apart from relying on lower tumor prevalence at this location from early-published studies (Leadley et al. 1998). In the present study, brown bullheads were collected at two locations reflecting a mid-stream position of Canadian waters of the Detroit River designated by Turkey Creek and at downstream sections of Canadian waters of the Detroit River near Boise Blanc Island and adjacent nearshore areas of Amherstburg Channel (Figure 2). Under an ideal experimental design, at least 100 fish from each region Peche Island (reference), Turkey Creek (midstream), and Boise Blanc/Amherstburg Channel (downstream) would be collected twice (i.e. a total of 600 fish), separated in time by at least 3 years.

## 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 not 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 (tumor 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 tumors". 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.

## 1.4 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 a sufficient number 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 tumor prevalence delisting criteria. Baumann (2010) pooled fish from sixteen reference locations to establish the reference database used in their Fisher's Exact 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 Baumann'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 Baumann (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 which have movements outside of the AOC, e.g., to Lake St. Clair or western Lake Erie, that could impact the interpretation of the BUI.

Brown bullhead were originally designated an indicator species of fish tumor prevalence because of their sensitivity to neoplasm development, the 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 (Baumann 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 in the Presque Isle Bay of Lake Erie and tracked fish movements over 180 days. All fish were found to remain within Presque Isle 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 Isle AOC but did not appear to move outside of the AOC to Lake Erie. Genetic markers in fish captured from discrete locations within Presque Isle Bay provided further support of within bay fish movements suggesting that fish within Presque Isle 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 tumors is less substantiated (Baumann et al. 1991) relative to other contaminants such as polynuclear aromatic hydrocarbons (PAHs; Baumann 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, and Amherstburg Channel/Bois Blanc)

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 tumors across different reaches of Canadian waters of the AOC.

3) Fish bioaccumulation model to estimate daily PAH uptake rates from sediment PAHs
    i) Apply a food web 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 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 be 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 were 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 covering site locations representative of upstream, midstream, and downstream waters of the AOC (Figure 2). In 2016, fish were collected by electrofishing boat on Aug 16. During this survey, 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). The two collection sites, Boise Blanc Island and Amherstburg Channel, were considered the same location owing to their close proximity (Figure 2).

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 aging. 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.

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. Size at age relationships were generated for the Canadian dataset to exclude or accept fish without age-data in the tumor prevalence assessment. Details of the age assessment are outlined in Section 3.1 of this report.

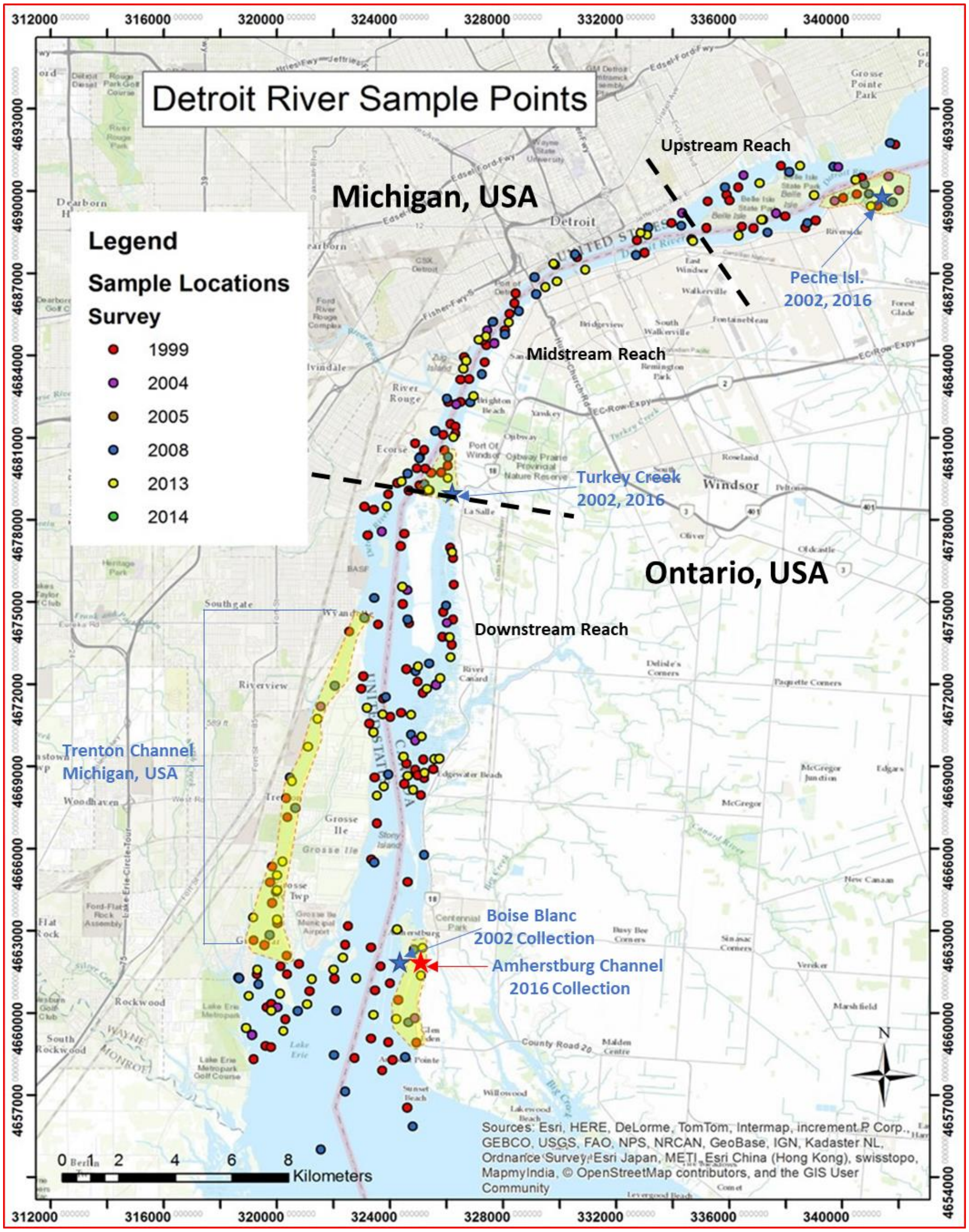


**Figure 2. Sediment sampling (circles) and bullhead collection areas (stars) in the Detroit River AOC. Peche Island (upstream reference), Turkey Creek (mid-stream reach) and Boise Blanc/Amherstburg Channel (downstream reach) represent Canadian collection locations. Yellow shaded polygons denote groups of sediment sites used to establish mean sediment PAH concentrations near bullhead collections zones. Trenton Channel, Michigan U.S. collection area is shown for reference to historical brown bullhead collections outlined in section 1**

## 2.2. Histopathology

Preserved liver samples from 2002 were submitted to Freshwater Institute (Fisheries & Oceans Canada, Winnipeg, MB, Canada) as outlined in Baumann (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 Baumann (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 tumors 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 tumor prevalence counts where fish from across survey years were pooled to keep the data compatible and consistent with the criteria of Blazer et al. (2006).

## 2.3. Fish age, size and sex

Tumor prevalence in fish is influenced by fish age, body size, and sex (Macubbin and Ersing, 1991; Baumann, 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; Baumann 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 tumor prevalence studies (Baumann 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 tumor prevalence analysis. Where otoliths were 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 otolith were excluded from the initial tumor 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 than 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 throughout U.S. and Canadian waters of the Detroit River AOC by the Great Lakes Institute for Environmental Research, University of Windsor. The database consisted of 300 sediment samples distributed throughout the entire AOC collected between 1999-2013 (Figure 2). 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 database 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/Amherstburg 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/Amherstburg 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 3. 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 $\mu g \cdot g^{-1}$ ∑PAH dry weight recommended by Johnson et al. (2002) for the protection against neoplasms in marine fish and 4 $\mu g \cdot g^{-1}$ (Baumann 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 $\mu g \cdot g^{-1}$ that are proximate in magnitude to the 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 exceedance 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 3. Benchmark values of PAHs reported in the literature**

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

#### 2.4.4 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 biotransformation 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. Twenty-one fish from the 2002 survey did not have ages assigned to them and 1 fish from the 2016 data set had missing age information. 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 tumor prevalence counts.

The combined samples from different surveys (2002, 2016) 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 ($p>0.3$; Lilliefors test) but body weight was non-normal. However, log transformation of body weight data generated a normal dataset ($p>0.2$; Lilliefors test). 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 ($F_{1,136}$=0.952; p>0.6; ANOVA) on the prediction of fish length after accounting for age nor was there a significant interaction between the method·age on fish length ($F_{1,135}$=0.315; p>0.5; ANOVA). There was also no significant interaction between sex·age on fish length ($F_{1,136}$=1.658; p>0.2; ANOVA) indicating that male and female fish grow at statistically similar rates. Finally, there was no significant interaction between site·age ($F_{1,134}$=0.327; p>0.7), although mean ages and lengths did differ across sites. Overall, neither aging method (pectoral spine versus otolith), fish sex, or collection location provided statistically different predictions of fish length at age relationships for brown bullhead. Similar results were obtained for log normalized body weight.

The linear regressions between body size (length or log weight) and fish ages are given by:

$$\text{Body Length (cm)} = 0.749 \pm 0.126\ \text{Age} + 25.921 \pm 0.601; \quad R^2 = 0.20;\ p<0.001 \qquad (3.1)$$

$$\text{Log Body Weight (g)} = 0.039 \pm 0.0054 \cdot \text{Age} + 2.360 \pm 0.026; \quad R^2 = 0.29;\ p<0.001 \qquad (3.2)$$

Based on Equation 3.1, three year old bullheads have a mean (5-95% confidence interval) body length of 28.2 (27.6-28.7) cm and body weight of 297.2 (281.5 – 313.7) g. Five year old bullheads have a mean (5-95% confidence interval) body length of 29.7 (29.2-30.1) cm and body weight of 356.5 (342.0 – 371.7) 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 27.6 and 30.1 cm and body weights between 281.5 to 371.7 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 tumor 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 tumor prevalence estimates. Of the 21 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 tumor 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. There were 30 fish greater than 6 years of age collected from Canadian locations. Table 4 provides a summary of fish counts by age by collection site and survey year. However, no individual collection site or survey year contained the recommended 100 fish target by Baumann (2010).

**Table 4. 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/Amherstburg Channel (2002; 2016) | 13,0 | 9, 3 | 3,0 | |
| **Total by age/survey** | **20** (19,1) | **112** (67,45) | **30** (12,18) | = **162** (98, 64) |

Sex ratio differences between collection years and locations can potentially influence interpretation of tumor 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/Amherstburg Channel 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 5 summarizes 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 to Baumann (2010)'s assessment. 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 tumor 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 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 5. Lesion prevalence in Canadian Brown Bullheads 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% |
| Amherstburg Channel 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% |

## 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 either a 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.

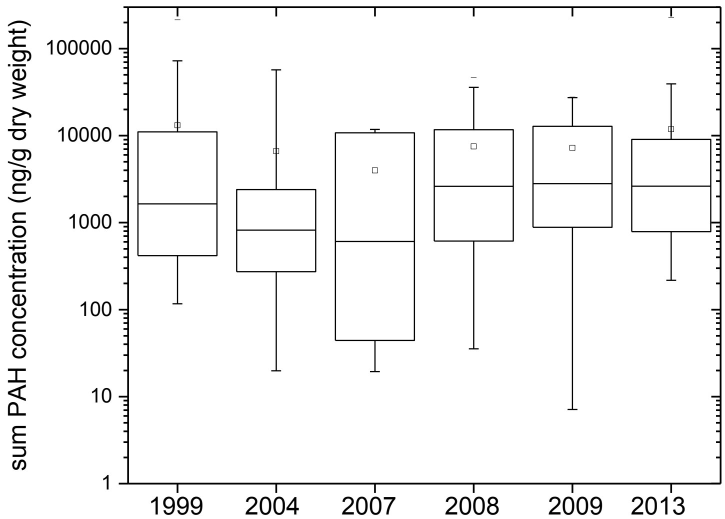


**Figure 3. Dry weight ∑PAH sediment concentrations (ng/g) by year in Canadian and United States jurisdictions of the Detroit River AOC. Boxes present 25-75 percentiles and median. Square presents mean concentration and whiskers present 5-95% confidence intervals of the distribution**

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 4 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.

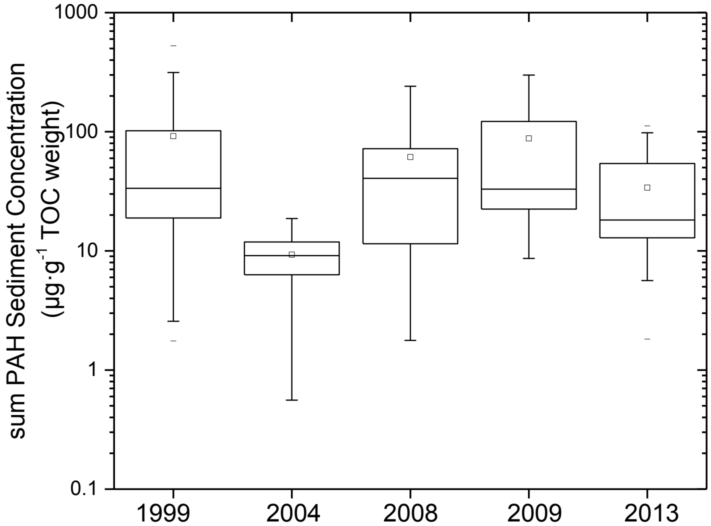


**Figure 4. TOC normalized ∑PAH sediment concentrations (ng/g) by year in the lower Canadian river reach of the Detroit River AOC. Boxes present 25-75 percentiles and median. Square presents mean concentration and whiskers present 5-95% confidence intervals of the distribution**

The last temporal contrast examined differences in ∑PAH sediment concentrations with time at each of the brown 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/Amherstburg Channel 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.

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 provides support for pooling fish 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.

### 3.3.2 Spatial Patterns of Sediment PAHs

Table 6 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 ($p<0.001$; Kruskal-Wallis Test; n=300; Test Statistic = 70.22) and OC-normalized basis ($p<0.001$; Kruskal-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 5 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; See Figure 2) 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 6 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 6. ∑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 |

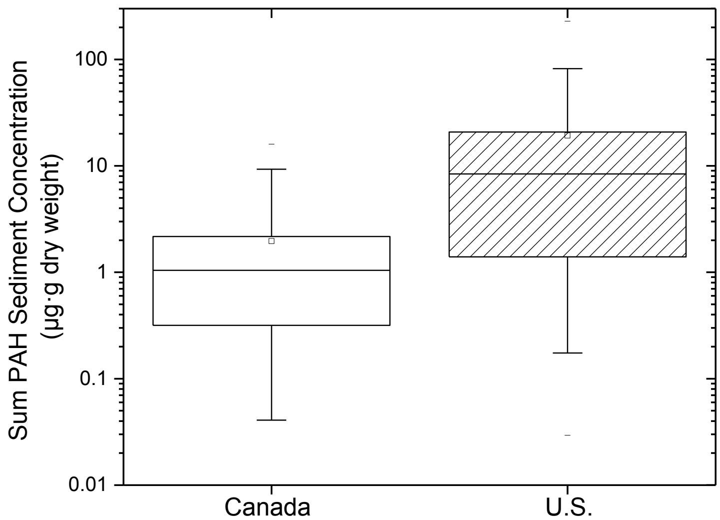


**Figure 5. ∑PAH sediment concentrations (µg/g dry weight) in Canadian and U.S. waters of the Detroit River AOC. Boxes present 25-75 percentiles and median. Square presents mean concentration and whiskers present 5-95% confidence intervals of the distribution**

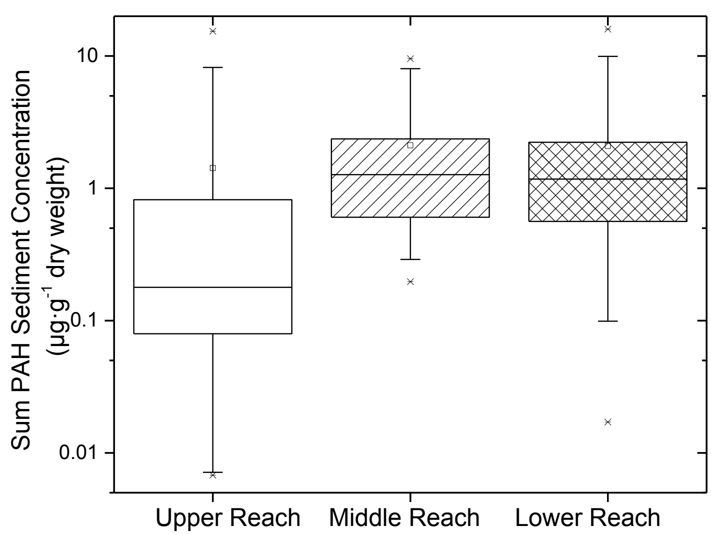


**Figure 6. ∑PAH sediment concentrations (µg/g dry weight) in upper, middle and lower Canadian reaches of the Detroit River AOC. Boxes present 25-75 percentiles and median. Square presents mean concentration and whiskers present 5-95% confidence intervals of the distribution**

For Contrast 3, there were highly significant differences in sediment ∑PAH concentrations between different areas where bullheads have been collected (Figure 7). Peche Island had significantly lower sediment ∑PAHs compared to other brown bullhead collection sites ($p<0.01$ for all contrasts; Conover Inman's tests). For the Turkey Creek and Boise Blanc/Amherstburg 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). 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 and lower stream reaches. Overall, this portion of the weight of evidence exposure assessment provides support for Baumann's recommendation to include Peche Island as an urban Great Lakes reference area.

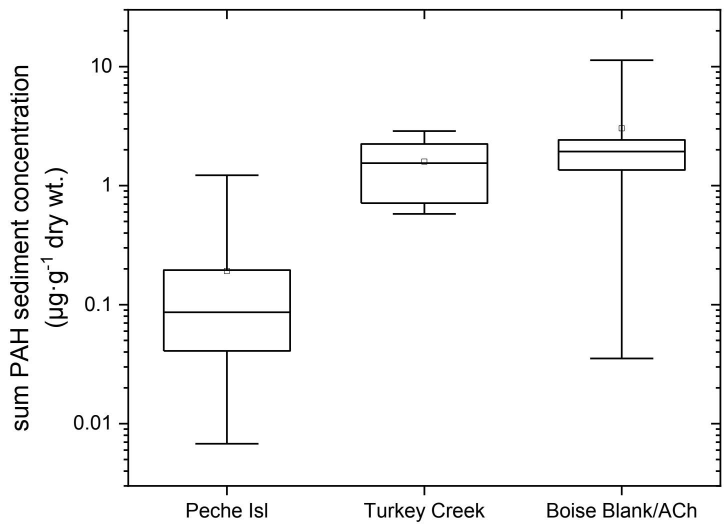


**Figure 7. ∑PAH sediment concentrations (µg/g dry weight) in upper, middle and lower Canadian reaches of the Detroit River AOC. Boxes present 25-75 percentiles and median. Square presents mean concentration and whiskers present 5-95% confidence intervals of the distribution**

### 3.3.3 Exceedance of Sediment PAHs against Benchmark Values

Table 7 summarizes the percentage of sites exceeding selected sediment PAH benchmark values while Table 8 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, exceedances 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/Amherstburg Channel bullhead collection locations. 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 and 1.9 for both Turkey Creek and Boise Blanc/Amherstburg Channel, respectively.

For the 4 $ug \cdot g^{-1}$ ∑PAH benchmark, 38% of Detroit River stations were in excess of this benchmark value. Among Canadian locations, the percentage of exceedances dropped to 12% while in the U.S. the percent exceedances remained above 60%. The exceedances at bullhead collection locations ranged from 0% (Peche and Turkey Creek) to 22% of stations in the vicinity of Boise Blanc/Amherstburg Channel. Median Hazard Quotients for the 4 $ug \cdot g^{-1}$ ∑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 and 0.5 at both Turkey Creek and Boise Blanc/Amherstburg 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, exceedances of this benchmark were relatively rare in Canadian waters and imply that exposures of fish at individual bullhead collection sites were generally lower than 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, exceedances 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% of locations in Turkey Creek and all stations at Boise Blanc/Amherstburg Channel. At brown bullhead collection sites, median HQs were 2.3, 39.3 and 33 for Peche Island, Turkey Creek, Boise Blanc/Amherstburg Channel, respectively.

For PAH PELs, the number of exceedances 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, exceedances of one or more PAH PELs were 0, 0 and 22.2% of stations, at Peche Island, Turkey Creek, Boise Blanc/Amherstburg 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 7. Percent sites exceeding selected PAH sediment benchmark values in the Detroit River**

| Zone | % Exceedances $1 \mu g \cdot g^{-1}$ Benchmark | % Exceedances $4 \mu g \cdot g^{-1}$ Benchmark | % Exceedances PAH ISQGs | % Exceedances 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/Amherstburg Channel | 77.8 | 22.2 | 100 | 22.22 | 9 |

**Table 8. 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/ Amherstburg Channel | 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 |

### 3.3.4 Model estimates of brown bullhead total daily intake of PAHs

Figure 8 summarizes the food web bioaccumulation model calibration as evaluated against individual PCB congeners measured in bullheads from three Canadian locations 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 Turkey Creek 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/Amherstburg Channel 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 8). The combined goodness of fit test, for all congeners and sample locations yielded the following relationship:

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

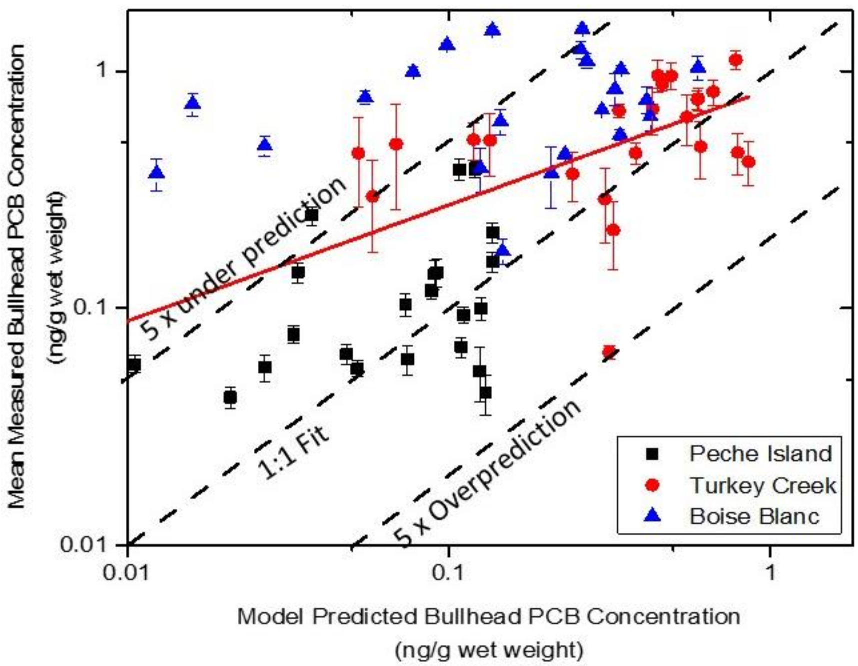


**Figure 8. Model validation of PCB concentrations in brown bullhead collected from Peche Island, Turkey Creek and Boise Blanc/Amherstburg in 2016. Solid red line presents regression fit of measured against predicted PCB concentrations in brown bullhead**

Model estimated PAH daily total uptake rates are provided in Figure 9. The model predicted the lowest daily PAH exposures at Peche Island and intermediate exposures at Turkey Creek and somewhat higher exposure at Boise Blanc/Amherstburg Channel. For the raw model output PAH exposures at Turkey Creek and Bois Blanc were 1.1 and 1.4 fold higher than predicted for Peche Island. For the calibrated model output the exposures were 3.5 and 4.2 fold higher than Peche Island, respectively.

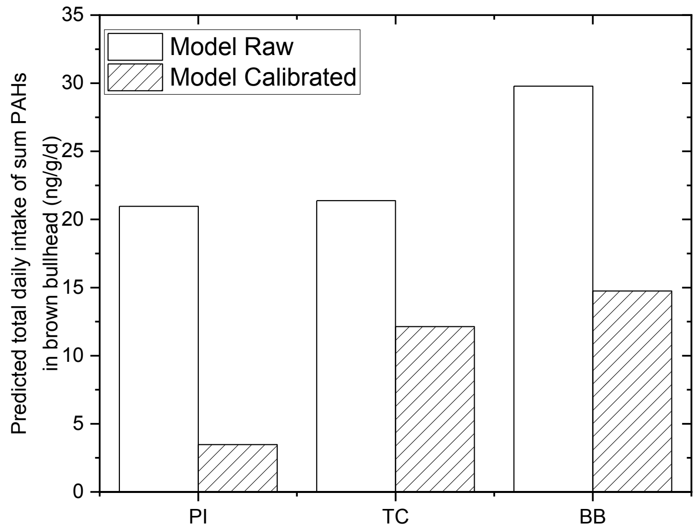


**Figure 9. Model predicted PAH total daily intake rates (ng/g fish/day) in brown bullhead at each of the bullhead collection locations in the Detroit River. Calibrated model refers to raw model output adjusted for the goodness of fit equation generated for PCBs**

### 3.3.5 Brown Bullhead Chemical Signatures

Turkey Creek fish had generally lower mean ∑PCB concentrations relative to Peche Island and Bois Blanc 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 10 presents box and whisker plots of sum PCB and total Hg concentrations across the three Canadian brown bullhead sampling locations.

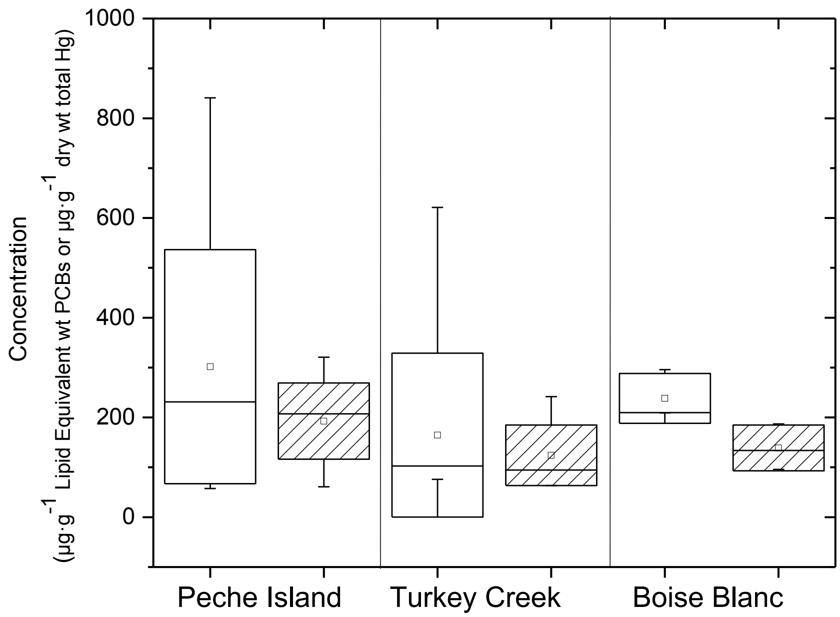


**Figure 10. PCB and Hg concentrations in brown bullhead collected from three Canadian locations in the Detroit River. Boxes present median and standard deviation, whiskers are 5-95 confidence intervals and square is the mean. Hollow boxes present PCB concentrations and lined boxes present total Hg**

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 scree plot and broken stick model. Most organochlorine pesticides and hydrophobic PCB congeners loaded onto PCA 1 (Table 9) 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 Bonferroni correction) whereas chemical signatures of Bois Blanc fish did not differ from the other locations ($p>0.1$; Tukey's HSD with Bonferroni correction). Figure 11 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 9. 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 coefficients 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/Amherstburg Channel) but potentially different chemical exposures occurring at the upstream and downstream locations (Peche Island vs Turkey Creek).

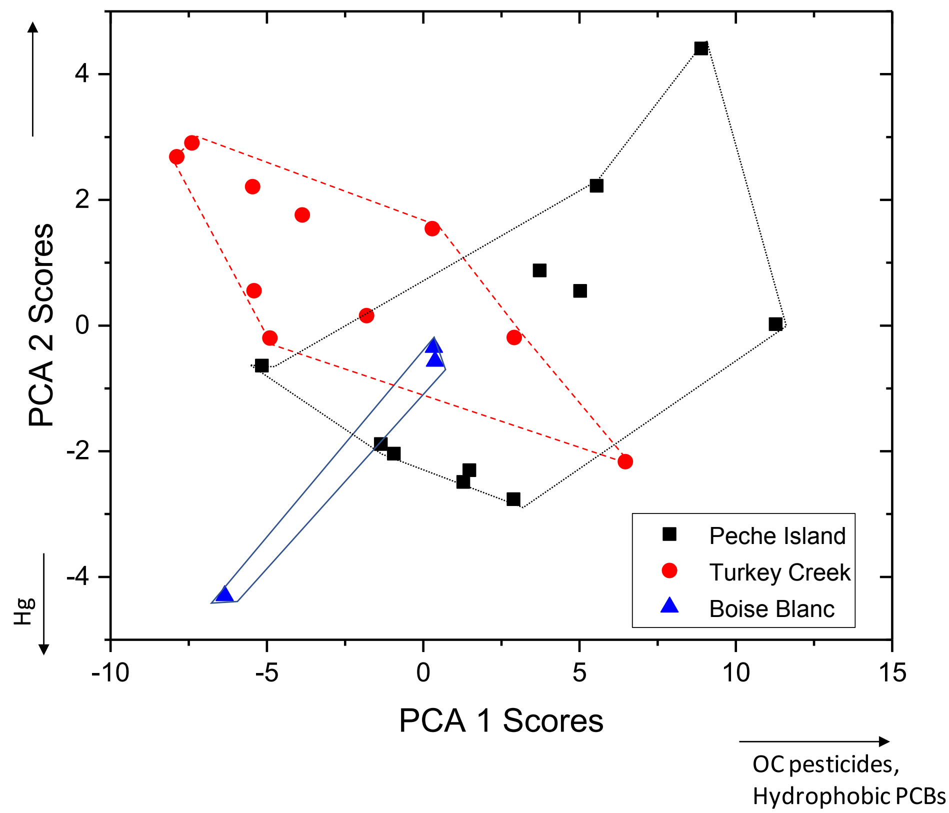


**Figure 11. Principle component scores across PCA axes 1 and 2 demonstrating differences in chemical signatures of brown bullhead collected from three Canadian sampling locations in the Detroit River. Lines present convex hull areas connecting data from each sampling location**

### 3.3.6 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.

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. In addition, sediment PAH concentrations at the Turkey Creek and Boise Blanc/Amherstburg

Channel collection areas were significantly elevated compared to Peche Island. Alternatively, sediment PAH concentrations were statistically equivalent between Turkey Creek and Boise Blanc/Amherstburg Channel 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 exceedances ranged from 7.1% to 77.8% for the 1 µg/g benchmark and from 0-22.2% for the 4µg/g benchmark. Larger numbers of exceedances 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 tumors, they were not further considered in decisions concerning grouping of fish tumor 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/Amherstburg Channel.

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 Amherstburg Channel from either Peche Island or Turkey Creek. However, the sample size from Amherstburg Channel was small at only 3 fish and no definitive conclusions about chemical signatures at this site can be reached.

Table 10 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/Amherstburg Channel locations and that sediment contamination at these locations has not changed over the 1999-2013 time period. The temporal conclusion is anticipated to be extrapolated to the 2002-2016 fish collections used for fish tumor prevalence. However, PAH exposures were lower at Peche Island relative to the midstream and downstream Canadian bullhead 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/Amherstburg Channel areas across the two survey years. The weight of evidence assessment indicates that fish from Peche Island are exposed to lower PAH levels and supports the designation of this sample location as a Near Field Urban Reference as identified by Baumann (2010).

**Table 10. 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/Amherstburg Channel |
|---|---|---|---|
| Median Sediment ∑PAHs µg/g OC weight | 10.2 | 40.8 | 36.6 |
| % Exceedance of 1ug/g ∑PAH in sediment; Median Hazard Quotient | 7.1%<br>0.1 | 75%<br>1.9 | 78%<br>1.9 |
| % Exceedance of 4 µg/g ∑PAH in sediment; Median Hazard Quotient | 0%<br>0.02 | 0%<br>0.5 | 22%<br>0.5 |
| Bullhead Chemical Signatures | Different from TC | Different from PI | Sample size insufficient to distinguish from PI or TC |
| Model Total Daily PAH Intakes (Calibrated model) | 5.9 (7.8) | 26.1 (14.9) | 24.4 (14.7) |

# 4.0 DELISTING CRITERIA ASSESSMENT

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

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

Over the 2002-2016 surveys there were 84 fish collected from Canadian midstream and downstream waters of the AOC that were in the age range of 3 to 5 years and 28 fish aged 3-5 collected from the upstream Peche Island previously classified as a nearfield urban reference location by Baumann (2010). The WOE exposure assessment provided support for combining data between sample years and between the midstream and downstream Canadian collection locations. It also provided support for the original designation of Peche Island as a reference given that sediment PAH exposures at this location are significantly lower compared to other midstream and downstream sections of the Canadian waters of the Detroit River.

A total of 1/84 fish from Canadian midstream and downstream locations contained a liver neoplasm generating an observed tumor prevalence of 1.2%. Fisher's exact test indicated no significant difference ($p>0.9$) in the tumor prevalence of 3-5 year old brown bullhead from middle and lower Canadian reaches of the Detroit River compared to the Great Lakes reference tumor dataset. However, the total number of samples in this age bracket were somewhat less than the recommended sample size of 100 fish. Including fish older than age 5 generated a total of 98 fish with only 1/98 specimens having a liver neoplasm. Older fish have a higher probability of generating liver neoplasms independent of contaminant exposures and this is why there were excluded from the delisting criteria. However, the

Great Lakes background tumor prevalence does not restrict ages of fish to 5 years but includes fish age 3 and above. Therefore, although not specified by the delisting criteria, a comparison of age 3+ fish in the AOC with the Great Lakes reference database is a more appropriate statistical test. Fishers Exact test performed on combined fish older than 3 years failed to detect a significant difference ($p>0.9$) in tumor prevalence in the midstream and lower reaches compared to the Great Lakes reference data set. Although a target of 100 fish was not fully reached in the two above tests, the small decrease in statistical power from 100 to 84 fish or 100 to 98 fish is not unlikely dramatically impact statistical inferences. This is particularly the case in the present assessment because the absolute tumor prevalence of brown bullheads observed from the midstream and downstream Canadian waters was lower than the Great Lakes background tumor prevalence of 2%. Thus the likelihood of having made a Type II error (or false negative) is highly unlikely.

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 tumors are positively correlated to age" Green et al. (2010). In the present report, fish were collected between 2002 and 2016 supporting the minimum requirement 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 power, the combined surveys cannot be considered as two independent tests of the delisting criteria. However, the weight of evidence exposure assessment indicated that there were no major changes in fish exposures to PAHs through the time period encompassing the different fish tumor prevalence surveys. Given the lack of change in sediment PAH concentrations between 1999-2013, it is assumed that exposure conditions of aged 3-5 bullheads and aged 3+ bullheads were comparable between the two different survey years. There is also a previous published study (Leadley et al. 1998) which reported brown bullhead tumor prevalence in Canadian waters of the AOC.  Leadley et al. (1998) identified tumor prevalence’s of 4% and 13% at Peche Island and Amherstburg Channel for samples collected 1993. This is suggestive that tumor prevalence have declined in Canadian waters of the AOC between the early 1990’s to 2000’s and provides support to address the temporal assessment recommendation of the Stage II Design and Rationale for delisting criteria assessment.

In conclusion, this study assessed BUI #4 in the brown bullhead indicator species from Canadian waters of the Detroit River Area of Concern. Based on the data collected and fish pooling as supported by a weight of evidence exposure assessment, the body of evidence indicates that the prevalence of fish tumors in brown bullheads is not significantly different from the Great Lakes background. Prior assessments of BUI #4 in the Detroit River have listed this beneficial use as Impaired primarily based on assessments conducted in U.S. waters of the AOC. The current assessment provides support to recommend that the status of BUI #4 in Canadian waters of the AOC be re-designated to not impaired.

# 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.

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

Baumann 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.

Baumann 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.

Baumann 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.

Baumann PC, JC Harshbarger. 1998. Long term trends in liver neoplasm epizootics of brown bullhead in the Black River, Ohio. Environ. Monitor. Assess. 53:213-223.

Baumann 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.

Baumann 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.

Baumann 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 biphenyls (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. Environ. Contam. Toxicol. (Accepted Feb 27, 2019).

Farwell M, KG Drouillard, DD Heath, TE Pitcher. 2012. Acclimation of life-history traits to experimental changes in environmental 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, from 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. Toxiciol. 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 lesions in brown bullheads (Ameiurus nebulosus) from contaminated habitats. Environ. Toxicol. Chem. 25:3035-3038.

# APPENDIX A. Final Histopathy Report and protocol for 2016 samples

FINAL HISTOPATHOLOGY REPORT
Case #: Detroit River 2016 livers (fish #'s 1-64)
Sample year = 2016
Species = Brown bullhead
Location sampled = Detroit River, Turkey Creek
Means of capture = Electrofishing
Lead pathologist: Laura Baseler, DVM, PhD, Diplomate, A.C.V.P.
Reviewing pathologist: Gary D. Marty, DVM, PhD, Diplomate, A.C.V.P.

Report Date: May 16, 2018

**Diagnoses:** For details of microscopic findings, see the e-mailed spreadsheet <2016_Brown Bullhead_Liver Histopathology #1-64_Final.xlsx>.

**Final Comment:** The majority of the fish do not have lesions that would have significantly impacted their health. However, fish have multiple lesions that might be markers of differences in sex, age, or contaminant exposure.

Livers from 5 fish (3 females, 2 males) have one or more eosinophilic cellular foci. Livers from 4 fish (1 female, 3 males) have one or more clear cellular foci. Eosinophilic cellular foci occur in fish 3-9 years old, while clear cell foci occur in fish 4-6 years old. Each focus of cellular alteration is counted separately when the cells of adjacent foci do not touch each other. Zero fish have other types of altered cellular foci or liver neoplasia of any type.

Other lesions with differences between sex and/or age, include:

1. Cholangitis/pericholangial leukocytes are more prevalent in females (82% of 34 fish) than males (60% of 30 fish). However, this lesion is mild in all fish and is unlikely to be clinically significant.
2. Cestodes are more prevalent in females (50%) than in males (37%).
3. Ascarids are more prevalent in males (37%) than in females (12%).
4. Eosinophilic bile canaliculi are more prevalent in fish 4 years of age or older (29% of 51 fish) than in fish 2-3 years old (8% of 12 fish). The significance of this finding is unknown.
5. The prevalence of fibrous capsule granulomas and cavitary histiocytic hepatitis tends to increase with age. This increase might be related to an increase in the prevalence of parasites in older fish. For example, cestodes are more prevalent in fish 4 years of age or older, and ascarids are more prevalent in fish 6-12 years old, as compared to fish 2-3 years old.
6. Myxosporean plasmodia are more prevalent in fish 4 years of age or older (25% of 51 fish) than in fish 2-3 years old (8% of 12 fish). However, this parasite is likely clinically insignificant because an inflammatory response associated with the parasite occurs in only 1 out of 14 affected fish. In the affected fish, degenerate plasmodia are outside of bile ductule lumens.

The livers from the sixty-four fish have a variety of other lesions, most of which are probably within the range of background lesions that affect populations of brown bullheads, regardless of toxin exposure.

Further characterization of three lesions can be done if unstained paraffin sections are submitted to the Animal Health Centre for special stains:

1. Fish #5 block 4 – hepatocellular cytoplasmic pigment (lipofuscin and iron stains)
2. Fish #57 block 2 – hepatocellular eosinophilic inclusions (PAS stain with and without amylase)
3. Fish #54 block 4 – coccobacilli (Gram stain)

**Histopathology:** H&E stained liver sections from 64 brown bullheads (*Ameiurus nebulosus*) were received for histopathology on January 30th, 2018. Step sections of five separate liver samples from each fish were distributed on ten slides. Most slides contained 3 – 5 serial or step-sections from a single paraffin block. For each examined slide, every liver section was systematically scanned using the 4× objective lens (low power), and then a single liver section was systematically scanned using the 10× objective lens (medium power). Higher magnification (20× and 40× objective lenses) was used as needed. For foci of cellular alteration and neoplasia, the number of foci per liver is recorded. Parasites are scored as absent (0) or present (1). For other lesions, findings are scored on a relative scale as none (0), mild/small amounts (1), moderate (2), or severe/abundant (3). The location of uncommon focal lesions, or unusual characteristics about that lesion, is often identified by a comment added to the spreadsheet cell (e.g., "slide 5-1 or 5-1" designates the slide and "22 x 111" designates the coordinates on the pathologist's microscope). Additional features of the slides are described in the Comments column.

Examination of glass slides for this report was blinded (i.e., during histopathology, no information was known about the fish other than the species, year, and river where the fish were sampled). An interim report from the blinded analysis was e-mailed to Mark McMaster and Jim Bennett on May 1st, 2018. On May 10th, 2018, Gerald Tetreault provided additional information about the fish (age, sex, weight, length, etc.) via e-mail. For this final report, data are presented in three ways: 1) in numerical order by sample number, 2), by sex (male vs. female), and 3) by age. Basic summary statistics (count, mean score, prevalence) are included for each group. Lesion classification generally follows published guidelines (e.g., Wolf and Wolfe 2005).

In some fish, parasites are surrounded by inflammation (fibrous capsule granulomas or cavitary histiocytic hepatitis). When this happens, the lesion is only scored in the appropriate parasite column (CES, ASC, ACN, etc.) rather than being scored as both inflammation (fibrous capsule granuloma or cavitary histiocytic hepatitis) and a parasite. If the parasite within the inflammation is too degenerate to be identified, then the lesion is only scored in the appropriate inflammatory column (fibrous capsule granuloma or cavitary histiocytic hepatitis).

General comments on specific microscopic findings that occur in this group follow in alphabetical order (comments are not included for lesions that do not occur in any fish):

**ACN** = acanthocephalan parasites. Acanthocephalans are common in the abdominal cavity and viscera (e.g., liver) of fish captured from the wild. They are differentiated from other helminths based on a hypodermis thicker than muscle layers, lacunar channels, embryonated eggs, a spiny proboscis, and lack of a digestive tract.

**ASC** = ascarid parasites. Ascarids are a type of nematode parasite that in the liver are usually coiled and have distinctive cuticular longitudinal lateral alae. The parasites are usually surrounded by granulomatous inflammation.

**CCF** = clear cellular foci. Foci of cellular alteration are identified by subtle changes in staining pattern and morphology that differentiate a group of hepatocytes from the bulk of hepatocytes in the liver. Clear cell foci are composed of a well-delineated group of hepatocytes with clear wispy to angular cytoplasmic vacuoles (probably glycogen). Clear cell foci are considered to be preneoplastic in rats, but we are not aware of studies that have made this link in fish.

**CES =** cestode parasites. Cestodes are common in the abdominal cavity and viscera (e.g., liver) of fish captured from the wild. They are differentiated from other helminths based on a parenchymatous body with an internal ring of smooth muscle and no digestive tract; adult cestodes usually have calcareous corpuscles. Because these cestodes can migrate through the liver and cause cavitary histiocytic hepatitis, even single organisms might adversely affect fish health. Hepatic cestodes have previously been identified in brown bullheads by histopathology (Figure 6, "helminth parasites," Blazer et al. 2006).

**CHH** = cavitary histiocytic hepatitis. Lesions in this category are cavities loosely filled with scattered cords and individual hepatocytes, with variable numbers of histiocytes and erythrocytes, a few of which are necrotic. The lesions are probably a result of migration of parasites. In some of the submitted fish, parasites are surrounded by, and likely caused the development of, cavitary histiocytic hepatitis.

**CPL** = cholangitis/pericholangial leukocytes (liver). Inflammation in and around bile ductules (mostly lymphocytes) is evidence of chronic immune stimulation. This type of inflammation can result from bacteria ascending from the intestine to the liver through the biliary system; the cause is usually not determined.

**EBC** = eosinophilic bile canaliculi. Bile canaliculi are the first part of the biliary system formed by the cell membranes of adjacent hepatocytes. They are usually not prominent in standard H&E sections of fish livers; 25% of the fish in this submission have prominent canaliculi.

**ECF** = eosinophilic foci of cellular alteration. Foci of cellular alteration are identified by subtle changes in staining pattern and morphology that differentiate a group of hepatocytes from the bulk of hepatocytes in the liver. Eosinophilic cell foci are composed of a well-delineated group of hepatocytes with cytoplasm that stains more eosinophilic than surrounding hepatocytes. Eosinophilic cell foci are considered to be preneoplastic in rats, but we are not aware of studies that have clearly made this link in fish.

**FCG** = fibrous capsule granuloma. Fibrous capsule granulomas are fairly common in fish that are captured from the wild and/or reared outdoors. In some cases, the granulomas represent foci where parasites died long ago; all that is left is the indigestible remnants. Most of these foci are probably of little significance for fish health.

**FPL** = focal/multifocal parenchymal leukocytes. This category is used for foci of leukocytes that are not obviously associated with bile ductules, veins, or arteries. In some cases, foci of parenchymal leukocytes might include increased numbers of biliary preductular epithelial cells. In other cases, many of the cells are hematopoietic (blood forming) cells. The foci probably develop in response to chronic immune stimulation (e.g., focal bacteria, parasites, or other antigens), but the exact cause is rarely determined.

**GLY** = hepatocellular glycogen. Glycogen is a common form of readily available energy that is stored by many fish species in their liver cells (hepatocytes). Lack of hepatocellular glycogen is evidence that food assimilation is not adequate to supply energy needs; it occurs most commonly in fish that go off feed, but it can also be an indicator of inadequate nutrition. In general, glycogen will be depleted within about 2 days in healthy fish that stop feeding. Glycogen depletion is common and probably normal in many fish species during their spawning season.

**IMP** = intraluminal myxosporean plasmodium parasites. Plasmodia of myxosporeans are common in the biliary system of wild fish. As in most fish in this submission, they are generally well adapted to their host and cause minimal lesions. Myxosporean plasmodia have been identified in brown bullheads by histopathology (Figure 6 in Blazer et al. 2006), but further work (e.g., special stains, genetic analysis) is needed to speciate the plasmodia.

**LGR** = granulomatous inflammation, liver. Foci of granulomatous inflammation in the liver are evidence of persistent foreign material. In wild fish, this occurs in response to persistent bacteria, fungi, or parasites; often, though, the exact cause is not determined. Sometimes the foreign material is surrounded by multinucleated giant cell macrophages. When the inflammatory response is mostly a fibrous capsule surrounding a central core with few inflammatory cells, it is not scored here; instead, it is scored as part of FCG.

**NEM** = nematode (not obviously ascarids) parasites. Sections of nematodes are differentiated from other helminths based on the presence of a body cavity (= pseudocoelom). The NEM category is only used for nematodes that do not have obvious features of ascarids.

**PER** = peritonitis/coelomitis. Peritonitis is consistent with a reaction to foreign material; it is common in fish with parasites or inflammatory mediators in the visceral cavity. The plane of section might not include the inciting parasite.

**PIG** = hepatocellular cytoplasmic pigment. Accumulation of yellow-brown to yellow-green pigment (lipofuscin and/or hemosiderin) in hepatocyte cytoplasm is a nonspecific change that can result from a variety of insults, including rancid feed, low levels of antioxidants in the feed, chronic infections, and exposure to organic contaminants. Hepatocellular hemosiderosis has been described in fish from contaminated sites (Malins et al. 1984, Thiyagarajah et al. 1998), but the cause has not been determined under controlled laboratory conditions. In poultry, hepatocellular hemosiderin accumulation can be due to an iron storage disorder, starvation, or a non-specific result of chronic disease.

**PMA** = pigmented macrophage aggregates. Pigmented macrophage aggregates are irregular spherical structures that are a normal component of parenchymatous organs (liver, kidney, and spleen) of fish, amphibians, and some reptiles. Lipofuscin and iron are the most common nonmelanin pigments, and three main functions have been identified (Wolke 1992): 1) immunity, including humoral and cellular responses; 2) storage, destruction, or detoxification of exogenous and endogenous substances; and 3) iron recycling. Accumulation of lipofuscin in the liver is a nonspecific change that can result from a variety of insults, including rancid feed, low levels of antioxidants in the feed, chronic infections, and exposure to organic contaminants; it is more common in older fish. Conditions that lead to moderate to abundant hepatic lipofuscin have been associated with decreased growth and survival in several studies. Accumulation of hemosiderin might result from increased turnover of red blood cells.

**PVL** = perivascular leukocytes, liver. Leukocytic inflammation around vessels in the liver is evidence of chronic immune stimulation. Differentials include a low-grade bacterial infection, but the cause is rarely determined. In this case, the lesions have no obvious infectious agents.

## Literature Cited:

Blazer, V.S., Fournie, J.W., Wolf, J.C., and Wolfe, M.J. 2006. Diagnostic criteria for proliferative hepatic lesions in brown bullhead *Ameiurus nebulosus*. Dis. Aquat. Org. 72:19-30.

Malins, D.C., McCain, B.B., Brown, D.W., Chan, S.L., Myers, M.S., Landahl, J.T., Prohaska, P.G., Friedman, A.J., Rhodes, L.D., Burrows, D.G., Gronlund, W.D., and Hodgins, H.O. 1984. Chemical pollutants in sediments and diseases of bottom-dwelling fish in Puget Sound, Washington. Environ. Sci. Technol. 18(9):705-713.

Thiyagarajah, A., Hartley, W.R., and Abdelghani, A. 1998. Hepatic hemosiderosis in buffalo fish (*Ictiobus spp*.). Mar. Environ. Res. 46(1-5):203-207.

Wolf, J.C., and Wolfe, M.J. 2005. A brief overview of nonneoplastic hepatic toxicity in fish. Toxicologic Pathology. 33(1):75-85.

Wolke, R.E. 1992. Piscine macrophage aggregates: a review. Ann. Rev. Fish Dis. 2:91-108.

## Quality Control:

Sectioning and staining quality is good. Artifact is mild in all fish (n = 64). Liver preservation is generally good, but in all fish, tissues adjacent to major bile ductules and blood vessels often have mild decomposition/autolysis, probably due to bile digestion before formalin fixation. This level of autolysis might obscure some foci of cellular alteration in the digested regions. Bile digestion can be minimized by aspirating bile from the gallbladder before handling the remainder of the liver.

In 97% of the fish (n = 62), some large foci of erythrocytes have precipitates of acid hematin. Acid hematin accumulates as brown birefringent deposits when tissues are not fixed in neutral buffered formalin and when tissues become acidic before or during fixation (as can happen with thick bloody pieces of tissue or with acid decalcification). No livers have postfixation dehydration.

In addition to blinded examination, we used two methods to minimize pathologist bias in assigning scores:

1) To minimize bias in scoring that can occur over time (e.g., the same lesion assigned a score of "1" at the beginning of the study might be assigned a score of "2" at the end of the study), no more than five fish from a given slide box (slides were consolidated to 20 fish per box for analysis) were sequentially examined at one time; once analysis of livers from five fish per box was complete, the next slides to be examined were selected from a different box.
2) As a check of diagnostic quality, for every 10 fish examined by the lead pathologist (LB), the reviewing pathologist (GDM) selected one of the fish and independently examined and scored the lesions. The pathologists then compared diagnoses and scores. The scores agreed on by both pathologists are reported.

# APPENDIX B. Body size, age and condition factors for Brown Bullhead collected from Canadian waters of the Detroit River AOC in 2006 and 2016

| Year | Fish # | Length (cm) | Weight (g) | Age | Retained or excluded based on age | Sex | L3 | k | GSI | LSI | Gonad wt (g) | Liver Wt (g) | Site |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2002 | 02-289 | 25.2 | 95 | 2 | E | F | 16003.01 | 0.563831 | 1.950571 | 3.335919 | 1.76 | 3.01 | PI |
| 2002 | 02-215 | 29 | 300 | 3 | R | M | 24389 | 1.209808 | 0.210127 | 1.464109 | 0.62 | 4.32 | PI |
| 2002 | 02-217 | 32 | 437 | 3 | R | F | 32768 | 1.297943 | 0.952247 | 1.796337 | 4.05 | 7.64 | PI |
| 2002 | 02-220 | 29 | 348 | 3 | R | M | 24389 | 1.402928 | 0.12275 | 1.584054 | 0.42 | 5.42 | PI |
| 2002 | 02-222 | 30.9 | 387 | 3 | R | F | 29503.63 | 1.273267 | 1.006229 | 2.012458 | 3.78 | 7.56 | PI |
| 2002 | 02-225 | 30 | 340 | 3 | R | M | 27000 | 1.237852 | 0.122674 | 1.606726 | 0.41 | 5.37 | PI |
| 2002 | 02-264 | 27.8 | 285 | 3 | R | F | 21484.95 | 1.289461 | 1.22726 | 1.645972 | 3.4 | 4.56 | PI |
| 2002 | 02-302 | 28.1 | 275 | 3 | R | M | 22188.04 | 1.201278 | 0.172582 | 3.001426 | 0.46 | 8 | PI |
| 2002 | 02-405 | 29.3 | 411 | 3 | R | M | 25153.76 | 1.60481 | 0.1635 | 1.65234 | 0.66 | 6.67 | PI |
| 2016 | AOC2016-62 | 27 | 247.2 | 3 | R | M | 19683 | 1.255906 | 0.12 | 1.54 | 0.286 | 3.808 | PI |
| 2002 | 02-285 | 29.6 | 384 | 4 | R | F | 25934.34 | 1.437245 | 1.124108 | 1.896765 | 4.19 | 7.07 | PI |
| 2002 | 02-290 | 31.8 | 386 | 4 | R | M | 32157.43 | 1.180225 | 0.173899 | 1.530841 | 0.66 | 5.81 | PI |
| 2002 | 02-301 | 30.7 | 412 | 4 | R | M | 28934.44 | 1.392665 | 0.173715 | 2.069684 | 0.7 | 8.34 | PI |
| 2002 | 02-303 | 31.5 | 457 | 4 | R | M | 31255.88 | 1.428947 | 0.235094 | 2.086738 | 1.05 | 9.32 | PI |
| 2002 | 02-304 | 29.9 | 308 | 4 | R | M | 26730.9 | 1.131836 | 0.175178 | 1.626177 | 0.53 | 4.92 | PI |
| 2002 | 02-409 | 33.9 | 486 | 4 | R | M | 38958.22 | 1.22462 | 0.148819 | 1.718753 | 0.71 | 8.2 | PI |
| 2002 | 02-218 | 31.4 | 391 | 5 | R | F | 30959.14 | 1.232431 | 0.956624 | 1.520115 | 3.65 | 5.8 | PI |
| 2002 | 02-221 | 33.6 | 509 | 5 | R | F | 37933.06 | 1.306459 | 1.033133 | 1.674805 | 5.12 | 8.3 | PI |
| 2002 | 02-223 | 29.7 | 334 | 5 | R | F | 26198.07 | 1.23948 | 1.065533 | 1.792313 | 3.46 | 5.82 | PI |
| 2002 | 02-224 | 26.4 | 260 | 5 | R | F | 18399.74 | 1.378063 | 0.982016 | 1.557817 | 2.49 | 3.95 | PI |
| 2002 | 02-261 | 31.4 | 405 | 5 | R | F | 30959.14 | 1.26977 | 1.017527 | 2.007072 | 4 | 7.89 | PI |

| Year | Fish # | Length (cm) | Weight (g) | Age | Retained or excluded based on age | Sex | L3 | k | GSI | LSI | Gonad wt (g) | Liver Wt (g) | Site |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2002 | 02-262 | 31.6 | 290 | 5 | R | F | 31554.5 | 0.894611 | 0.913954 | 1.81728 | 2.58 | 5.13 | PI |
| 2002 | 02-288 | 32.2 | 477 | 5 | R | F | 33386.25 | 1.384941 | 1.092175 | 2.069726 | 5.05 | 9.57 | PI |
| 2002 | 02-291 | 30.8 | 397 | 5 | R | F | 29218.11 | 1.315828 | 1.092441 | 2.169276 | 4.2 | 8.34 | PI |
| 2002 | 02-305 | 29.2 | 304 | 5 | R | M | 24897.09 | 1.196284 | 0.194735 | 1.873489 | 0.58 | 5.58 | PI |
| 2002 | 02-406 | 13.2 | 408 | 5 | R | M | 2299.968 | 17.37329 | 0.202713 | 1.9045 | 0.81 | 7.61 | PI |
| 2002 | 02-407 | 33.7 | 603 | 5 | R | M | 38272.75 | 1.552279 | 0.102676 | 1.395388 | 0.61 | 8.29 | PI |
| 2002 | 02-408 | 29 | 338 | 5 | R | M | 24389 | 1.358317 | 0.178097 | 1.850398 | 0.59 | 6.13 | PI |
| 2016 | AOC2016-56 | 30.9 | 431.7 | 5 | R | M | 29503.63 | 1.46321 | 0.18 | 1.65 | 0.798 | 7.121 | PI |
| 2002 | 02-173 | 33.7 | 575 | 6 | E | F | 38272.75 | 1.460229 | 0.92866 | 1.957521 | 5.19 | 10.94 | PI |
| 2002 | 02-286 | 31.6 | 415 | 6 | E | F | 31554.5 | 1.275222 | 1.14317 | 1.990606 | 4.6 | 8.01 | PI |
| 2002 | 02-287 | 32.2 | 451 | 6 | E | M | 33386.25 | 1.32941 | 0.180245 | 1.432949 | 0.8 | 6.36 | PI |
| 2016 | AOC2016-54 | 32.7 | 516.6 | 6 | E | M | 34965.78 | 1.477444 | 0.07 | 1.81 | 0.37 | 9.33 | PI |
| 2016 | AOC2016-55 | 30.1 | 420.2 | 6 | E | M | 27270.9 | 1.540837 | 0.07 | 1.46 | 0.303 | 6.123 | PI |
| 2016 | AOC2016-61 | 31.5 | 523.6 | 6 | E | F | 31255.88 | 1.675205 | 0.55 | 1.93 | 2.881 | 10.1 | PI |
| 2016 | AOC2016-64 | 31.2 | 417.5 | 6 | E | F | 30371.33 | 1.374652 | 0.58 | 1.74 | 2.43 | 7.26 | PI |
| 2002 | 02-216 | 33.3 | 576 | 7 | E | F | 36926.04 | 1.513647 | 0.960764 | 2.093285 | 5.37 | 11.7 | PI |
| 2002 | 02-260 | 35.3 | 555 | 7 | E | F | 43986.98 | 1.229273 | 0.84332 | 1.797603 | 4.56 | 9.72 | PI |
| 2016 | AOC2016-57 | 32.8 | 516.7 | 7 | E | M | 35287.55 | 1.464256 | 0.19 | 2.12 | 1.003 | 10.963 | PI |
| 2016 | AOC2016-58 | 33.8 | 645.2 | 7 | E | F | 38614.47 | 1.670876 | 0.41 | 2.36 | 2.628 | 15.226 | PI |
| 2002 | 02-219 | 31.5 | 521 | 8 | E | F | 31255.88 | 1.622799 | 1.100114 | 1.616655 | 5.58 | 8.2 | PI |
| 2016 | AOC2016-59 | 29 | 345.5 | 8 | E | F | 24389 | 1.416622 | 0.57 | 2.07 | 1.958 | 7.149 | PI |
| 2016 | AOC2016-60 | 29.9 | 323 | 8 | E | F | 26730.9 | 1.208339 | 0.64 | 1.95 | 2.076 | 6.291 | PI |
| 2016 | AOC2016-63 | 32.9 | 445.4 | 8 | E | M | 35611.29 | 1.250727 | 0.13 | 1.10 | 0.56 | 4.902 | PI |
| 2002 | 02-172 | 36 | 693 | 9 | E | F | 46656 | 1.441722 | 1.04066 | 1.984687 | 7 | 13.35 | PI |
| 2002 | 02-384 | 27.6 | 260 | 4 | R | F | 21024.58 | 1.202212 | 1.167115 | 1.697262 | 2.95 | 4.29 | BB |
| 2002 | 02-386 | 25.2 | 206 | 5 | R | F | 16003.01 | 1.255576 | 1.020256 | 1.503011 | 2.05 | 3.02 | BB |

| Year | Fish # | Length (cm) | Weight (g) | Age | Retained or excluded based on age | Sex | L3 | k | GSI | LSI | Gonad wt (g) | Liver Wt (g) | Site |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2016 | AOC2016-47 | 28.9 | 269.6 | 5 | R | M | 24137.57 | 1.116931 | 0.12 | 1.62 | 0.33 | 4.37 | BB |
| 2016 | AOC2016-48 | 29.6 | 385.6 | 5 | R | M | 25934.34 | 1.486832 | 0.06 | 1.67 | 0.24 | 6.43 | BB |
| 2016 | AOC2016-49 | 28.2 | 285.2 | 5 | R | F | 22425.77 | 1.271751 | 0.57 | 1.85 | 1.63 | 5.28 | BB |
| 2002 | 02-385 | 26 | 213 | 6 | E | F | 17576 | 1.17962 | 1.070757 | 1.664014 | 2.22 | 3.45 | BB |
| 2002 | 02-387 | 28.5 | 278 | 6 | E | M | 23149.13 | 1.185228 | 0.138499 | 1.184532 | 0.38 | 3.25 | BB |
| 2002 | 02-410 | 25.8 | 206 | | E | M | 17173.51 | 1.177569 | 0.143401 | 1.720813 | 0.29 | 3.48 | BB |
| 2002 | 02-411 | 30.7 | 374 | | E | F | 28934.44 | 1.261956 | 1.035219 | 1.391247 | 3.78 | 5.08 | BB |
| 2002 | 02-412 | 24.5 | 193 | | E | M | 14706.13 | 1.286811 | 0.14796 | 1.838935 | 0.28 | 3.48 | BB |
| 2002 | 02-413 | 22.7 | 133 | | E | M | 11697.08 | 1.117031 | 0.122455 | 1.668452 | 0.16 | 2.18 | BB |
| 2002 | 02-414 | 28.4 | 298 | | R | F | 22906.3 | 1.256772 | 1.187995 | 2.327359 | 3.42 | 6.7 | BB |
| 2002 | 02-415 | 24.5 | 213 | | E | F | 14706.13 | 1.412609 | 0.943487 | 1.588524 | 1.96 | 3.3 | BB |
| 2002 | 02-416 | 26.4 | 205 | | E | F | 18399.74 | 1.083385 | 1.053476 | 1.785893 | 2.1 | 3.56 | BB |
| 2002 | 02-417 | 25 | 184 | | E | F | 15625 | 1.1488 | 0.930362 | 1.576602 | 1.67 | 2.83 | BB |
| 2002 | 02-418 | 26.5 | 267 | | E | F | 18609.63 | 1.394762 | 0.943905 | 1.922484 | 2.45 | 4.99 | BB |
| 2002 | 02-420 | 24.7 | 208 | | E | F | 15069.22 | 1.339352 | 0.951296 | 2.105733 | 1.92 | 4.25 | BB |
| 2002 | 02-421 | 28 | 299 | | R | F | 21952 | 1.327032 | 1.005801 | 1.633998 | 2.93 | 4.76 | BB |
| 2002 | 02-422 | 28.4 | 302 | | R | M | 22906.3 | 1.300253 | 0.21488 | 1.181843 | 0.64 | 3.52 | BB |
| 2002 | 02-423 | 27.3 | 254 | | E | F | 20346.42 | 1.214219 | 0.914795 | 1.898401 | 2.26 | 4.69 | BB |
| 2002 | 02-424 | 27.9 | 312 | | R | M | 21717.64 | #VALUE! | #VALUE! | #VALUE! | n/a | 5.19 | BB |
| 2002 | 02-425 | 26.5 | 258 | | E | M | 18609.63 | 1.361016 | 0.15398 | 1.70957 | 0.39 | 4.33 | BB |
| 2002 | 02-426 | 28 | 278 | | R | M | 21952 | 1.246857 | 0.14614 | 1.421212 | 0.4 | 3.89 | BB |
| 2002 | 02-427 | 29.5 | 359 | | R | M | 25672.38 | 1.37424 | 0.170068 | 1.587302 | 0.6 | 5.6 | BB |
| 2002 | 02-428 | 27.3 | 258 | | E | F | 20346.42 | 1.236385 | 0.985848 | 1.574177 | 2.48 | 3.96 | BB |
| 2002 | 02-429 | 25.8 | 287 | | R | F | 17173.51 | 1.625061 | 1.286369 | 1.551526 | 3.59 | 4.33 | BB |
| 2002 | 02-430 | 29.1 | 301 | | R | M | 24642.17 | 1.20306 | 0.155164 | 1.37624 | 0.46 | 4.08 | BB |
| 2002 | 02-431 | 30 | 350 | | R | M | 27000 | 1.276333 | 0.11027 | 1.453817 | 0.38 | 5.01 | BB |

| Year | Fish # | Length (cm) | Weight (g) | Age | Retained or excluded based on age | Sex | L3 | k | GSI | LSI | Gonad wt (g) | Liver Wt (g) | Site |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2002 | 02-161 | 28.5 | 347 | 2 | E | M | 23149.13 | 1.467658 | 0.117734 | 2.016188 | 0.4 | 6.85 | TC |
| 2002 | 02-334 | 28.6 | 303 | 2 | E | F | 23393.66 | 1.250724 | 1.001401 | 2.556478 | 2.93 | 7.48 | TC |
| 2002 | 02-338 | 26.7 | 241 | 2 | E | F | 19034.16 | 1.225901 | 1.229965 | 2.052798 | 2.87 | 4.79 | TC |
| 2002 | 02-340 | 28.7 | 312 | 2 | E | M | 23639.9 | 1.294633 | 0.133965 | 1.810162 | 0.41 | 5.54 | TC |
| 2002 | 02-342 | 27.2 | 246 | 2 | E | M | 20123.65 | 1.199633 | 0.153266 | 1.748063 | 0.37 | 4.22 | TC |
| 2016 | AOC2016-23 | 27.1 | 254.7 | 2 | E | M | 19902.51 | 1.279738 | 0.07 | 1.16 | 0.19 | 2.97 | TC |
| 2002 | 02-250 | 28.2 | 317 | 3 | R | F | 22425.77 | 1.377032 | 0.854895 | 1.797222 | 2.64 | 5.55 | TC |
| 2002 | 02-252 | 26.2 | 242 | 3 | R | M | 17984.73 | 1.32568 | 0.109051 | 1.392501 | 0.26 | 3.32 | TC |
| 2002 | 02-253 | 27.5 | 251 | 3 | R | M | 20796.88 | 1.188255 | 0.157818 | 1.412269 | 0.39 | 3.49 | TC |
| 2002 | 02-257 | 24.2 | 219 | 3 | R | M | 14172.49 | 1.522457 | 0.139037 | 1.357927 | 0.3 | 2.93 | TC |
| 2002 | 02-307 | 28.9 | 334 | 3 | R | F | 24137.57 | 1.346904 | 0.996586 | 1.737873 | 3.24 | 5.65 | TC |
| 2002 | 02-311 | 28.9 | 320 | 3 | R | F | 24137.57 | 1.293958 | 1.01175 | 1.443986 | 3.16 | 4.51 | TC |
| 2002 | 02-312 | 29 | 281 | 3 | R | M | 24389 | 1.136045 | 0.184069 | 1.234345 | 0.51 | 3.42 | TC |
| 2002 | 02-325 | 27 | 320 | 3 | R | F | 19683 | 1.578926 | 1.122981 | 1.843748 | 3.49 | 5.73 | TC |
| 2002 | 02-326 | 26.8 | 297 | 3 | R | M | 19248.83 | 1.505598 | 0.224285 | 2.256651 | 0.65 | 6.54 | TC |
| 2002 | 02-327 | 26.6 | 295 | 3 | R | F | 18821.1 | 1.521644 | 0.838018 | 2.168372 | 2.4 | 6.21 | TC |
| 2002 | 02-328 | 27.5 | 257 | 3 | R | M | 20796.88 | 1.213548 | 0.154529 | 1.676044 | 0.39 | 4.23 | TC |
| 2002 | 02-329 | 29.9 | 336 | 3 | R | F | 26730.9 | 1.216233 | 1.033496 | 2.316139 | 3.36 | 7.53 | TC |
| 2002 | 02-330 | 29.7 | 309 | 3 | R | M | 26198.07 | 1.162872 | 0.20023 | 1.227638 | 0.61 | 3.74 | TC |
| 2002 | 02-333 | 31.1 | 374 | 3 | R | M | 30080.23 | 1.216114 | 0.183155 | 2.055712 | 0.67 | 7.52 | TC |
| 2002 | 02-336 | 25.9 | 267 | 3 | R | F | 17373.98 | 1.499196 | 0.979 | 1.528007 | 2.55 | 3.98 | TC |
| 2002 | 02-337 | 26.7 | 270 | 3 | R | M | 19034.16 | 1.391918 | 0.16985 | 1.740017 | 0.45 | 4.61 | TC |
| 2002 | 02-343 | 30.5 | 328 | 3 | R | M | 28372.63 | 1.138457 | 0.15789 | 1.386954 | 0.51 | 4.48 | TC |
| 2016 | AOC2016-07 | 27.3 | 259.4 | 3 | R | M | 20346.42 | 1.274917 | 0.11 | 1.39 | 0.29 | 3.60 | TC |
| 2016 | AOC2016-17 | 27.2 | 263.3 | 3 | R | F | 20123.65 | 1.308411 | 0.47 | 1.55 | 1.23 | 4.09 | TC |
| 2016 | AOC2016-24 | 27.2 | 278 | 3 | R | F | 20123.65 | 1.381459 | 0.35 | 1.36 | 0.97 | 3.79 | TC |

| Year | Fish # | Length (cm) | Weight (g) | Age | Retained or excluded based on age | Sex | L3 | k | GSI | LSI | Gonad wt (g) | Liver Wt (g) | Site |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2016 | AOC2016-31 | 25.0 | 200.8 | 3 | R | M | 15625 | 1.28512 | 0.72 | 0.95 | 1.44 | 1.91 | TC |
| 2016 | AOC2016-32 | 26.9 | 261.1 | 3 | R | M | 19465.11 | 1.341374 | 0.12 | 1.35 | 0.32 | 3.52 | TC |
| 2016 | AOC2016-34 | 25.8 | 261.8 | 3 | R | F | 17173.51 | 1.524441 | 1.18 | 1.40 | 3.10 | 3.66 | TC |
| 2016 | AOC2016-35 | 27.3 | 279.6 | 3 | R | M | 20346.42 | 1.374198 | 0.32 | 1.22 | 0.89 | 3.40 | TC |
| 2016 | AOC2016-36 | 29.2 | 381.2 | 3 | R | M | 24897.09 | 1.531103 | 0.09 | 1.46 | 0.33 | 5.56 | TC |
| 2016 | AOC2016-43 | 33.0 | 542.6 | 3 | R | F | 35937 | 1.509864 | 0.39 | 2.61 | 2.14 | 14.17 | TC |
| 2016 | AOC2016-44 | 25.4 | 244.9 | 3 | R | M | 16387.06 | 1.494471 | 0.07 | 1.78 | 0.17 | 4.35 | TC |
| 2002 | 02-254 | 27.5 | 280 | 4 | R | M | 20796.88 | 1.323516 | 0.152589 | 1.573115 | 0.42 | 4.33 | TC |
| 2002 | 02-255 | 27.6 | 284 | 4 | R | F | 21024.58 | 1.314652 | 1.070912 | 1.678726 | 2.96 | 4.64 | TC |
| 2002 | 02-256 | 26.9 | 216 | 4 | R | F | 19465.11 | 1.082501 | 0.854255 | 1.656305 | 1.8 | 3.49 | TC |
| 2002 | 02-258 | 26.9 | 243 | 4 | R | F | 19465.11 | 1.215868 | 0.912663 | 1.761947 | 2.16 | 4.17 | TC |
| 2002 | 02-259 | 29.8 | 352 | 4 | R | F | 26463.59 | 1.294118 | 0.753351 | 2.029375 | 2.58 | 6.95 | TC |
| 2002 | 02-308 | 27.5 | 286 | 4 | R | F | 20796.88 | 1.342894 | 0.859353 | 1.546835 | 2.4 | 4.32 | TC |
| 2002 | 02-310 | 28.4 | 301 | 4 | R | M | 22906.3 | 1.288816 | 0.210013 | 1.747849 | 0.62 | 5.16 | TC |
| 2002 | 02-313 | 29 | 272 | 4 | R | M | 24389 | 1.097954 | 0.194189 | 1.381731 | 0.52 | 3.7 | TC |
| 2002 | 02-324 | 27 | 240 | 4 | R | M | 19683 | 1.189351 | 0.213584 | 2.306707 | 0.5 | 5.4 | TC |
| 2002 | 02-331 | 29.2 | 285 | 4 | R | F | 24897.09 | 1.115793 | 0.917927 | 1.673866 | 2.55 | 4.65 | TC |
| 2002 | 02-339 | 27.7 | 354 | 4 | R | M | 21253.93 | 1.623558 | 0.165184 | 2.422697 | 0.57 | 8.36 | TC |
| 2002 | 02-341 | 32.1 | 442 | 4 | R | M | 33076.16 | 1.309765 | 0.138498 | 1.888186 | 0.6 | 8.18 | TC |
| 2016 | AOC2016-08 | 26.7 | 275.1 | 4 | R | F | 19034.16 | 1.445296 | 0.49 | 2.42 | 1.35 | 6.67 | TC |
| 2016 | AOC2016-14 | 29.6 | 360.3 | 4 | R | M | 25934.34 | 1.389278 | 0.12 | 1.69 | 0.45 | 6.07 | TC |
| 2016 | AOC2016-15 | 28.7 | 318.2 | 4 | R | F | 23639.9 | 1.346029 | 0.52 | 1.83 | 1.64 | 5.81 | TC |
| 2016 | AOC2016-16 | 30.1 | 421 | 4 | R | F | 27270.9 | 1.54377 | 0.37 | 1.90 | 1.54 | 8.00 | TC |
| 2016 | AOC2016-18 | 28.8 | 356.6 | 4 | R | M | 23887.87 | 1.492808 | 0.16 | 1.69 | 0.57 | 6.01 | TC |
| 2016 | AOC2016-21 | 28.2 | 297.4 | 4 | R | M | 22425.77 | 1.326153 | 0.08 | 0.87 | 0.24 | 2.59 | TC |
| 2016 | AOC2016-22 | 25.1 | 228 | 4 | R | M | 15813.25 | 1.441829 | 0.09 | 1.49 | 0.21 | 3.39 | TC |

| Year | Fish # | Length (cm) | Weight (g) | Age | Retained or excluded based on age | Sex | L3 | k | GSI | LSI | Gonad wt (g) | Liver Wt (g) | Site |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2016 | AOC2016-25 | 26.5 | 259.8 | 4 | R | F | 18609.63 | 1.396052 | 0.45 | 1.62 | 1.18 | 4.21 | TC |
| 2016 | AOC2016-27 | 29.0 | 335.5 | 4 | R | F | 24389 | 1.37562 | 0.48 | 1.54 | 1.62 | 5.18 | TC |
| 2016 | AOC2016-28 | 29.1 | 325.1 | 4 | R | F | 24642.17 | 1.319283 | 0.52 | 1.90 | 1.70 | 6.19 | TC |
| 2016 | AOC2016-29 | 29.6 | 359.2 | 4 | R | F | 25934.34 | 1.385036 | 0.43 | 1.69 | 1.56 | 6.08 | TC |
| 2016 | AOC2016-42 | 30.2 | 409.3 | 4 | R | M | 27543.61 | 1.486007 | 0.17 | 1.60 | 0.68 | 6.54 | TC |
| 2016 | AOC2016-45 | 29.2 | 390.5 | 4 | R | F | 24897.09 | 1.568457 | 0.38 | 1.54 | 1.48 | 6.02 | TC |
| 2016 | AOC2016-50 | 30.6 | 405.8 | 4 | R | F | 28652.62 | 1.416276 | 0.40 | 1.58 | 1.64 | 6.42 | TC |
| 2016 | AOC2016-51 | 29.7 | 297 | 4 | R | F | 26304.07 | 1.129103 | 0.32 | 2.08 | 0.97 | 6.16 | TC |
| 2016 | AOC2016-52 | 28.4 | 316.9 | 4 | R | F | 22906.3 | 1.383462 | 0.46 | 1.48 | 1.47 | 4.68 | TC |
| 2002 | 02-249 | 24.8 | 264 | 5 | R | F | 15252.99 | 1.689439 | 0.776126 | 1.672552 | 2 | 4.31 | TC |
| 2002 | 02-251 | 26 | 260 | 5 | R | F | 17576 | 1.44117 | 0.908014 | 1.737071 | 2.3 | 4.4 | TC |
| 2002 | 02-332 | 30.5 | 279 | 5 | R | F | 28372.63 | 0.948872 | 1.277765 | 2.354951 | 3.44 | 6.34 | TC |
| 2016 | AOC2016-01 | 28.8 | 405.9 | 5 | R | F | 23887.87 | 1.699189 | 0.48 | 1.55 | 1.96 | 6.30 | TC |
| 2016 | AOC2016-03 | 31.1 | 415.9 | 5 | R | M | 30080.23 | 1.382636 | 0.11 | 1.47 | 0.46 | 6.11 | TC |
| 2016 | AOC2016-04 | 30.9 | 456.4 | 5 | R | M | 29503.63 | 1.546928 | 0.15 | 1.60 | 0.70 | 7.31 | TC |
| 2016 | AOC2016-05 | 31.2 | 408.8 | 5 | R | M | 30371.33 | 1.346006 | 0.12 | 1.73 | 0.47 | 7.06 | TC |
| 2016 | AOC2016-09 | 30.1 | 410 | 5 | R | F | 27270.9 | 1.503434 | 0.36 | 2.09 | 1.49 | 8.58 | TC |
| 2016 | AOC2016-10 | 31.2 | 348.7 | 5 | R | F | 30371.33 | 1.148122 | 0.50 | 1.99 | 1.74 | 6.94 | TC |
| 2016 | AOC2016-11 | 29.2 | 311.4 | 5 | R | F | 24897.09 | 1.250749 | 0.44 | 1.57 | 1.38 | 4.90 | TC |
| 2016 | AOC2016-12 | 29.8 | 370.4 | 5 | R | F | 26463.59 | 1.399659 | 0.45 | 2.18 | 1.66 | 8.09 | TC |
| 2016 | AOC2016-13 | 30.6 | 388.5 | 5 | R | F | 28652.62 | 1.355897 | 0.52 | 1.32 | 2.01 | 5.11 | TC |
| 2016 | AOC2016-26 | 28.5 | 314.1 | 5 | R | F | 23149.13 | 1.356855 | 0.33 | 1.28 | 1.04 | 4.02 | TC |
| 2016 | AOC2016-40 | 29.9 | 349 | 5 | R | F | 26730.9 | 1.305605 | 0.41 | 1.88 | 1.44 | 6.56 | TC |
| 2016 | AOC2016-46 | 28.8 | 390.8 | 5 | R | F | 23887.87 | 1.635977 | 0.40 | 1.46 | 1.57 | 5.69 | TC |
| 2016 | AOC2016-53 | 30.8 | 370.5 | 5 | R | M | 29218.11 | 1.268049 | 0.09 | 1.49 | 0.33 | 5.53 | TC |
| 2002 | 02-335 | 30 | 346 | 6 | E | F | 27000 | 1.246333 | 1.120323 | 1.699801 | 3.77 | 5.72 | TC |

| Year | Fish # | Length (cm) | Weight (g) | Age | Retained or excluded based on age | Sex | L3 | k | GSI | LSI | Gonad wt (g) | Liver Wt (g) | Site |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2016 | AOC2016-30 | 30.0 | 381 | 6 | E | M | 27000 | 1.411111 | 0.12 | 1.25 | 0.45 | 4.75 | TC |
| 2016 | AOC2016-38 | 30.4 | 370 | 6 | E | M | 28094.46 | 1.316985 | 0.07 | 1.17 | 0.27 | 4.34 | TC |
| 2016 | AOC2016-39 | 30.3 | 352.8 | 6 | E | F | 27818.13 | 1.268238 | 0.35 | 1.41 | 1.24 | 4.98 | TC |
| 2016 | AOC2016-41 | 30.7 | 410.5 | 6 | E | M | 28934.44 | 1.418724 | 0.08 | 1.80 | 0.35 | 7.40 | TC |
| 2002 | 02-309 | 31 | 421 | 7 | E | F | 29791 | 1.369743 | 0.967995 | 2.203107 | 3.95 | 8.99 | TC |
| 2016 | AOC2016-19 | 31.0 | 425.6 | 7 | E | F | 29791 | 1.428619 | 0.31 | 1.43 | 1.31 | 6.10 | TC |
| 2016 | AOC2016-20 | 34.6 | 651.9 | 7 | E | M | 41421.74 | 1.573811 | 0.22 | 1.72 | 1.40 | 11.23 | TC |
| 2016 | AOC2016-02 | 35.1 | 535.9 | 8 | E | M | 43243.55 | 1.23926 | 0.16 | 1.81 | 0.88 | 9.71 | TC |
| 2016 | AOC2016-37 | 30.2 | 354.6 | 9 | E | F | 27543.61 | 1.287413 | 0.57 | 1.73 | 2.03 | 6.14 | TC |
| 2016 | AOC2016-06 | 30.3 | 411.7 | 12 | E | F | 27818.13 | 1.47997 | 0.46 | 2.37 | 1.91 | 9.76 | TC |
| 2016 | AOC2016-33 | 28.7 | 346.4 |  | R | M | 23639.9 | 1.465319 | 0.04 | 1.37 | 0.14 | 4.74 | TC |

Notes:
1.NA = Not Available
2.E = Excluded from tumor prevalence due to age outside of 3-5 year interval; R = Retained in tumor prevalence counts
3.Site Names: BB = Boise Blanc Island (downstream collection location) ; PI = Peche Island (Upstream Reference); TC = Turkey Creek (Mid-stream region); AC = Amherstburg Channel (Downstream collection point)

## APPENDIX C. Body size, age, condition factor and histopathology data for Brown Bullhead collected from Canadian waters of the Detroit River in 2002 and 2016

| | | | | | | | | | | | | | | Neoplastic lesions | | | | | Putative pre-neoplastic lesions | | | | Non-neoplastic biliary lesions | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Year | Fish # | Length (cm) | Weight (g) | Age | Retained or excluded based on age | Sex | L3 | k | GSI | LSI | Gonad wt (g) | Liver wt (g) | #Slides | Hepatocellular Adenoma | Hepatocellular Carcinoma | Cholangioma | Cholangio Carcinoma | Pancreatic islet cell tumor | Basophilic Focus | Eosinophilic Focus | Clear Cell Focus | VacuolatedCell Focus | BileDuct Hyperplasia | Cholangiofibrosis | Parasites | Site |
| 2002 | 02-384 | 27.6 | 260 | 4 | R | F | 21024.58 | 1.20 | 1.17 | 1.70 | 2.95 | 4.29 | 2 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 1 | BB |
| 2002 | 02-385 | 26 | 213 | 6 | E | F | 17576 | 1.18 | 1.07 | 1.66 | 2.22 | 3.45 | 2 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | BB |
| 2002 | 02-386 | 25.2 | 206 | 5 | R | F | 16003.01 | 1.26 | 1.02 | 1.50 | 2.05 | 3.02 | 2 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | BB |
| 2002 | 02-387 | 28.5 | 278 | 6 | E | M | 23149.13 | 1.19 | 0.14 | 1.18 | 0.38 | 3.25 | 2 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | BB |
| 2002 | 02-410 | 25.8 | 206 | NA | E | M | 17173.51 | 1.18 | 0.14 | 1.72 | 0.29 | 3.48 | 2 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | BB |
| 2002 | 02-411 | 30.7 | 374 | NA | E | F | 28934.44 | 1.26 | 1.04 | 1.39 | 3.78 | 5.08 | 2 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | BB |
| 2002 | 02-412 | 24.5 | 193 | NA | E | M | 14706.13 | 1.29 | 0.15 | 1.84 | 0.28 | 3.48 | 2 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | BB |
| 2002 | 02-413 | 22.7 | 133 | NA | E | M | 11697.08 | 1.12 | 0.12 | 1.67 | 0.16 | 2.18 | 2 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | BB |
| 2002 | 02-414 | 28.4 | 298 | NA | R | F | 22906.3 | 1.26 | 1.19 | 2.33 | 3.42 | 6.7 | 2 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 1 | BB |
| 2002 | 02-415 | 24.5 | 213 | NA | E | F | 14706.13 | 1.41 | 0.94 | 1.59 | 1.96 | 3.3 | 2 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | BB |
| 2002 | 02-416 | 26.4 | 205 | NA | E | F | 18399.74 | 1.08 | 1.05 | 1.79 | 2.1 | 3.56 | 2 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | BB |
| 2002 | 02-417 | 25 | 184 | NA | E | F | 15625 | 1.15 | 0.93 | 1.58 | 1.67 | 2.83 | 2 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | BB |
| 2002 | 02-418 | 26.5 | 267 | NA | E | F | 18609.63 | 1.39 | 0.94 | 1.92 | 2.45 | 4.99 | 2 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 1 | BB |
| 2002 | 02-420 | 24.7 | 208 | NA | E | F | 15069.22 | 1.34 | 0.95 | 2.11 | 1.92 | 4.25 | 2 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 1 | BB |
| 2002 | 02-421 | 28 | 299 | NA | R | F | 21952 | 1.33 | 1.01 | 1.63 | 2.93 | 4.76 | 2 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | BB |
| 2002 | 02-422 | 28.4 | 302 | NA | R | M | 22906.3 | 1.30 | 0.21 | 1.18 | 0.64 | 3.52 | 2 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | BB |
| 2002 | 02-423 | 27.3 | 254 | NA | E | F | 20346.42 | 1.21 | 0.91 | 1.90 | 2.26 | 4.69 | 2 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 1 | BB |
| 2002 | 02-424 | 27.9 | 312 | NA | R | M | 21717.64 | 1.43 | n/a | n/a | n/a | 5.19 | 2 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 1 | BB |
| 2002 | 02-425 | 26.5 | 258 | NA | E | M | 18609.63 | 1.36 | 0.15 | 1.71 | 0.39 | 4.33 | 2 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | BB |
| 2002 | 02-426 | 28 | 278 | NA | R | M | 21952 | 1.25 | 0.15 | 1.42 | 0.4 | 3.89 | 2 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | BB |
| 2002 | 02-427 | 29.5 | 359 | NA | R | M | 25672.38 | 1.37 | 0.17 | 1.59 | 0.6 | 5.6 | 2 | 0 | 0 | 0 | 1 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | BB |
| 2002 | 02-428 | 27.3 | 258 | NA | E | F | 20346.42 | 1.24 | 0.99 | 1.57 | 2.48 | 3.96 | 2 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | BB |
| 2002 | 02-429 | 25.8 | 287 | NA | R | F | 17173.51 | 1.63 | 1.29 | 1.55 | 3.59 | 4.33 | 2 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | BB |
| 2002 | 02-430 | 29.1 | 301 | NA | R | M | 24642.17 | 1.20 | 0.16 | 1.38 | 0.46 | 4.08 | 2 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | BB |
| 2002 | 02-431 | 30 | 350 | NA | R | M | 27000 | 1.28 | 0.11 | 1.45 | 0.38 | 5.01 | 2 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | BB |
| 2002 | 02-172 | 36 | 693 | 9 | E | F | 46656 | 1.44 | 1.04 | 1.98 | 7 | 13.35 | 2 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 1 | PI |
| 2002 | 02-173 | 33.7 | 575 | 6 | E | F | 38272.75 | 1.46 | 0.93 | 1.96 | 5.19 | 10.94 | 2 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 1 | PI |
| 2002 | 02-215 | 29 | 300 | 3 | R | M | 24389 | 1.21 | 0.21 | 1.46 | 0.62 | 4.32 | 2 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 1 | PI |
| 2002 | 02-216 | 33.3 | 576 | 7 | E | F | 36926.04 | 1.51 | 0.96 | 2.09 | 5.37 | 11.7 | 2 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | PI |
| 2002 | 02-217 | 32 | 437 | 3 | R | F | 32768 | 1.30 | 0.95 | 1.80 | 4.05 | 7.64 | 2 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | PI |

| | | | | | | | | | | | | | Neoplastic lesions | | | | | Putative pre-neoplastic lesions | | | | Non-neoplastic biliary lesions | | | Site |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Year | Fish # | Length (cm) | Weight (g) | Age | Retained or excluded based on age | Sex | L3 | k | GSI | LSI | Gonad wt (g) | Liver wt (g) | #Slides | Hepatocellular Adenoma | Hepatocellular Carcinoma | Cholangioma | Cholangio Carcinoma | Pancreatic islet cell tumor | Basophilic Focus | Eosinophilic Focus | Clear Cell Focus | Vacuolated Cell Focus | BileDuct Hyperplasia | Cholangio fibrosis | Parasites | |
| 2002 | 02-218 | 31.4 | 391 | 5 | R | F | 30959.14 | 1.23 | 0.96 | 1.52 | 3.65 | 5.8 | 2 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 1 | PI |
| 2002 | 02-219 | 31.5 | 521 | 8 | E | F | 31255.88 | 1.62 | 1.10 | 1.62 | 5.58 | 8.2 | 2 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | PI |
| 2002 | 02-220 | 29 | 348 | 3 | R | M | 24389 | 1.40 | 0.12 | 1.58 | 0.42 | 5.42 | 2 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | PI |
| 2002 | 02-221 | 33.6 | 509 | 5 | R | F | 37933.06 | 1.31 | 1.03 | 1.67 | 5.12 | 8.3 | 2 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 1 | PI |
| 2002 | 02-222 | 30.9 | 387 | 3 | R | F | 29503.63 | 1.27 | 1.01 | 2.01 | 3.78 | 7.56 | 2 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 1 | PI |
| 2002 | 02-223 | 29.7 | 334 | 5 | R | F | 26198.07 | 1.24 | 1.07 | 1.79 | 3.46 | 5.82 | 2 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 1 | PI |
| 2002 | 02-224 | 26.4 | 260 | 5 | R | F | 18399.74 | 1.38 | 0.98 | 1.56 | 2.49 | 3.95 | 2 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | PI |
| 2002 | 02-225 | 30 | 340 | 3 | R | M | 27000 | 1.24 | 0.12 | 1.61 | 0.41 | 5.37 | 2 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | PI |
| 2002 | 02-260 | 35.3 | 555 | 7 | E | F | 43986.98 | 1.23 | 0.84 | 1.80 | 4.56 | 9.72 | 2 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 1 | PI |
| 2002 | 02-261 | 31.4 | 405 | 5 | R | F | 30959.14 | 1.27 | 1.02 | 2.01 | 4 | 7.89 | 2 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 1 | PI |
| 2002 | 02-262 | 31.6 | 290 | 5 | R | F | 31554.5 | 0.89 | 0.91 | 1.82 | 2.58 | 5.13 | 2 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 1 | PI |
| 2002 | 02-264 | 27.8 | 285 | 3 | R | F | 21484.95 | 1.29 | 1.23 | 1.65 | 3.4 | 4.56 | 2 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 1 | PI |
| 2002 | 02-285 | 29.6 | 384 | 4 | R | F | 25934.34 | 1.44 | 1.12 | 1.90 | 4.19 | 7.07 | 2 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 1 | PI |
| 2002 | 02-286 | 31.6 | 415 | 6 | E | F | 31554.5 | 1.28 | 1.14 | 1.99 | 4.6 | 8.01 | 2 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 1 | PI |
| 2002 | 02-287 | 32.2 | 451 | 6 | E | M | 33386.25 | 1.33 | 0.18 | 1.43 | 0.8 | 6.36 | 2 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 1 | PI |
| 2002 | 02-288 | 32.2 | 477 | 5 | R | F | 33386.25 | 1.38 | 1.09 | 2.07 | 5.05 | 9.57 | 2 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 1 | PI |
| 2002 | 02-289 | 25.2 | 95 | 2 | E | F | 16003.01 | 0.56 | 1.95 | 3.34 | 1.76 | 3.01 | 2 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | PI |
| 2002 | 02-290 | 31.8 | 386 | 4 | R | M | 32157.43 | 1.18 | 0.17 | 1.53 | 0.66 | 5.81 | 2 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 1 | PI |
| 2002 | 02-291 | 30.8 | 397 | 5 | R | F | 29218.11 | 1.32 | 1.09 | 2.17 | 4.2 | 8.34 | 2 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 1 | PI |
| 2002 | 02-301 | 30.7 | 412 | 4 | R | M | 28934.44 | 1.39 | 0.17 | 2.07 | 0.7 | 8.34 | 2 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 1 | PI |
| 2002 | 02-302 | 28.1 | 275 | 3 | R | M | 22188.04 | 1.20 | 0.17 | 3.00 | 0.46 | 8 | 2 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 1 | PI |
| 2002 | 02-303 | 31.5 | 457 | 4 | R | M | 31255.88 | 1.43 | 0.24 | 2.09 | 1.05 | 9.32 | 2 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | PI |
| 2002 | 02-304 | 29.9 | 308 | 4 | R | M | 26730.9 | 1.13 | 0.18 | 1.63 | 0.53 | 4.92 | 2 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | PI |
| 2002 | 02-305 | 29.2 | 304 | 5 | R | M | 24897.09 | 1.20 | 0.19 | 1.87 | 0.58 | 5.58 | 2 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 1 | PI |
| 2002 | 02-405 | 29.3 | 411 | 3 | R | M | 25153.76 | 1.60 | 0.16 | 1.65 | 0.66 | 6.67 | 2 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 1 | PI |
| 2002 | 02-406 | 13.2 | 408 | 5 | R | M | 2299.97 | 17.4 | 0.20 | 1.90 | 0.81 | 7.61 | 2 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 1 | PI |
| 2002 | 02-407 | 33.7 | 603 | 5 | R | M | 38272.75 | 1.55 | 0.10 | 1.40 | 0.61 | 8.29 | 2 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | PI |
| 2002 | 02-408 | 29 | 338 | 5 | R | M | 24389 | 1.36 | 0.18 | 1.85 | 0.59 | 6.13 | 2 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 1 | PI |
| 2002 | 02-409 | 33.9 | 486 | 4 | R | M | 38958.22 | 1.22 | 0.15 | 1.72 | 0.71 | 8.2 | 2 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 1 | PI |
| 2002 | 02-161 | 28.5 | 347 | 2 | E | M | 23149.13 | 1.47 | 0.12 | 2.02 | 0.4 | 6.85 | 3 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 1 | 1 | TC |
| 2002 | 02-249 | 24.8 | 264 | 5 | R | F | 15252.99 | 1.69 | 0.78 | 1.67 | 2 | 4.31 | 2 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 1 | TC |
| 2002 | 02-250 | 28.2 | 317 | 3 | R | F | 22425.77 | 1.38 | 0.85 | 1.80 | 2.64 | 5.55 | 2 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 1 | TC |
| 2002 | 02-251 | 26 | 260 | 5 | R | F | 17576 | 1.44 | 0.91 | 1.74 | 2.3 | 4.4 | 2 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 1 | TC |

| | | | | | | | | | | | | | | Neoplastic lesions | | | | | Putative pre-neoplastic lesions | | | | Non-neoplastic biliary lesions | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Year | Fish # | Length (cm) | Weight (g) | Age | Retained or excluded based on age | Sex | L3 | k | GSI | LSI | Gonad wt (g) | Liver wt (g) | #Slides | Hepatocellular Adenoma | Hepatocellular Carcinoma | Cholangioma | Cholangio Carcinoma | Pancreatic islet cell tumor | Basophilic Focus | Eosinophilic Focus | Clear Cell Focus | VacuolatedCell Focus | BileDuct Hyperplasia | Cholangio fibrosis | Parasites | Site |
| 2002 | 02-252 | 26.2 | 242 | 3 | R | M | 17984.73 | 1.33 | 0.11 | 1.39 | 0.26 | 3.32 | 2 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 1 | TC |
| 2002 | 02-253 | 27.5 | 251 | 3 | R | M | 20796.88 | 1.19 | 0.16 | 1.41 | 0.39 | 3.49 | 2 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 1 | TC |
| 2002 | 02-254 | 27.5 | 280 | 4 | R | M | 20796.88 | 1.32 | 0.15 | 1.57 | 0.42 | 4.33 | 2 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 1 | TC |
| 2002 | 02-255 | 27.6 | 284 | 4 | R | F | 21024.58 | 1.31 | 1.07 | 1.68 | 2.96 | 4.64 | 2 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | TC |
| 2002 | 02-256 | 26.9 | 216 | 4 | R | F | 19465.11 | 1.08 | 0.85 | 1.66 | 1.8 | 3.49 | 2 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 1 | TC |
| 2002 | 02-257 | 24.2 | 219 | 3 | R | M | 14172.49 | 1.52 | 0.14 | 1.36 | 0.3 | 2.93 | 2 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | TC |
| 2002 | 02-258 | 26.9 | 243 | 4 | R | F | 19465.11 | 1.22 | 0.91 | 1.76 | 2.16 | 4.17 | 2 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 1 | TC |
| 2002 | 02-259 | 29.8 | 352 | 4 | R | F | 26463.59 | 1.29 | 0.75 | 2.03 | 2.58 | 6.95 | 2 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 1 | TC |
| 2002 | 02-307 | 28.9 | 334 | 3 | R | F | 24137.57 | 1.35 | 1.00 | 1.74 | 3.24 | 5.65 | 2 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | TC |
| 2002 | 02-308 | 27.5 | 286 | 4 | R | F | 20796.88 | 1.34 | 0.86 | 1.55 | 2.4 | 4.32 | 2 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | TC |
| 2002 | 02-309 | 31 | 421 | 7 | E | F | 29791 | 1.37 | 0.97 | 2.20 | 3.95 | 8.99 | 2 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 1 | TC |
| 2002 | 02-310 | 28.4 | 301 | 4 | R | M | 22906.3 | 1.29 | 0.21 | 1.75 | 0.62 | 5.16 | 2 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | TC |
| 2002 | 02-311 | 28.9 | 320 | 3 | R | F | 24137.57 | 1.29 | 1.01 | 1.44 | 3.16 | 4.51 | 2 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 1 | 0 | 0 | 0 | TC |
| 2002 | 02-312 | 29 | 281 | 3 | R | M | 24389 | 1.14 | 0.18 | 1.23 | 0.51 | 3.42 | 2 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | TC |
| 2002 | 02-313 | 29 | 272 | 4 | R | M | 24389 | 1.10 | 0.19 | 1.38 | 0.52 | 3.7 | 2 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 1 | TC |
| 2002 | 02-324 | 27 | 240 | 4 | R | M | 19683 | 1.19 | 0.21 | 2.31 | 0.5 | 5.4 | 2 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | TC |
| 2002 | 02-325 | 27 | 320 | 3 | R | F | 19683 | 1.58 | 1.12 | 1.84 | 3.49 | 5.73 | 2 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | TC |
| 2002 | 02-326 | 26.8 | 297 | 3 | R | M | 19248.83 | 1.51 | 0.22 | 2.26 | 0.65 | 6.54 | 2 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | TC |
| 2002 | 02-327 | 26.6 | 295 | 3 | R | F | 18821.1 | 1.52 | 0.84 | 2.17 | 2.4 | 6.21 | 2 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | TC |
| 2002 | 02-328 | 27.5 | 257 | 3 | R | M | 20796.88 | 1.21 | 0.15 | 1.68 | 0.39 | 4.23 | 2 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | TC |
| 2002 | 02-329 | 29.9 | 336 | 3 | R | F | 26730.9 | 1.22 | 1.03 | 2.32 | 3.36 | 7.53 | 2 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 1 | TC |
| 2002 | 02-330 | 29.7 | 309 | 3 | R | M | 26198.07 | 1.16 | 0.20 | 1.23 | 0.61 | 3.74 | 2 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | TC |
| 2002 | 02-331 | 29.2 | 285 | 4 | R | F | 24897.09 | 1.12 | 0.92 | 1.67 | 2.55 | 4.65 | 2 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 1 | TC |
| 2002 | 02-332 | 30.5 | 279 | 5 | R | F | 28372.63 | 0.95 | 1.28 | 2.35 | 3.44 | 6.34 | 2 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 1 | TC |
| 2002 | 02-333 | 31.1 | 374 | 3 | R | M | 30080.23 | 1.22 | 0.18 | 2.06 | 0.67 | 7.52 | 2 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | TC |
| 2002 | 02-334 | 28.6 | 303 | 2 | E | F | 23393.66 | 1.25 | 1.00 | 2.56 | 2.93 | 7.48 | 2 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | TC |
| 2002 | 02-335 | 30 | 346 | 6 | E | F | 27000 | 1.25 | 1.12 | 1.70 | 3.77 | 5.72 | 2 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 1 | TC |
| 2002 | 02-336 | 25.9 | 267 | 3 | R | F | 17373.98 | 1.50 | 0.98 | 1.53 | 2.55 | 3.98 | 2 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | TC |
| 2002 | 02-337 | 26.7 | 270 | 3 | R | M | 19034.16 | 1.39 | 0.17 | 1.74 | 0.45 | 4.61 | 2 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | TC |
| 2002 | 02-338 | 26.7 | 241 | 2 | E | F | 19034.16 | 1.23 | 1.23 | 2.05 | 2.87 | 4.79 | 2 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | TC |
| 2002 | 02-339 | 27.7 | 354 | 4 | R | M | 21253.93 | 1.62 | 0.17 | 2.42 | 0.57 | 8.36 | 2 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 1 | TC |
| 2002 | 02-340 | 28.7 | 312 | 2 | E | M | 23639.9 | 1.29 | 0.13 | 1.81 | 0.41 | 5.54 | 2 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 1 | TC |
| 2002 | 02-341 | 32.1 | 442 | 4 | R | M | 33076.16 | 1.31 | 0.14 | 1.89 | 0.6 | 8.18 | 2 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 1 | TC |

| | | | | | | | | | | | | | | Neoplastic lesions | | | | | Putative pre-neoplastic lesions | | | | Non-neoplastic biliary lesions | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Year | Fish # | Length (cm) | Weight (g) | Age | Retained or excluded based on age | Sex | L3 | k | GSI | LSI | Gonad wt (g) | Liver wt (g) | #Slides | Hepatocellular Adenoma | Hepatocellular Carcinoma | Cholangioma | Cholangio Carcinoma | Pancreatic islet cell tumor | Basophilic Focus | Eosinophilic Focus | Clear Cell Focus | VacuolatedCell Focus | BileDuct Hyperplasia | Cholangio fibrosis | Parasites | Site |
| 2002 | 02-342 | 27.2 | 246 | 2 | E | M | 20123.65 | 1.20 | 0.15 | 1.75 | 0.37 | 4.22 | 2 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 1 | TC |
| 2002 | 02-343 | 30.5 | 328 | 3 | R | M | 28372.63 | 1.14 | 0.16 | 1.39 | 0.51 | 4.48 | 2 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 1 | TC |
| 2016 | AOC2016-47 | 28.9 | 269.6 | 5 | R | M | 24137.57 | 1.12 | 0.12 | 1.62 | 0.33 | 4.37 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | AC |
| 2016 | AOC2016-48 | 29.6 | 385.6 | 5 | R | M | 25934.34 | 1.49 | 0.06 | 1.67 | 0.24 | 6.43 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | AC |
| 2016 | AOC2016-49 | 28.2 | 285.2 | 5 | R | F | 22425.77 | 1.27 | 0.57 | 1.85 | 1.63 | 5.28 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | AC |
| 2016 | AOC2016-54 | 32.7 | 516.6 | 6 | E | M | 34965.78 | 1.48 | 0.07 | 1.81 | 0.37 | 9.33 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | PI |
| 2016 | AOC2016-55 | 30.1 | 420.2 | 6 | E | M | 27270.9 | 1.54 | 0.07 | 1.46 | 0.303 | 6.123 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | PI |
| 2016 | AOC2016-56 | 30.9 | 431.7 | 5 | R | M | 29503.63 | 1.46 | 0.18 | 1.65 | 0.798 | 7.121 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | PI |
| 2016 | AOC2016-57 | 32.8 | 516.7 | 7 | E | M | 35287.55 | 1.46 | 0.19 | 2.12 | 1.003 | 10.963 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | PI |
| 2016 | AOC2016-58 | 33.8 | 645.2 | 7 | E | F | 38614.47 | 1.67 | 0.41 | 2.36 | 2.628 | 15.226 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | PI |
| 2016 | AOC2016-59 | 29 | 345.5 | 8 | E | F | 24389 | 1.42 | 0.57 | 2.07 | 1.958 | 7.149 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | PI |
| 2016 | AOC2016-60 | 29.9 | 323 | 8 | E | F | 26730.9 | 1.21 | 0.64 | 1.95 | 2.076 | 6.291 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | PI |
| 2016 | AOC2016-61 | 31.5 | 523.6 | 6 | E | F | 31255.88 | 1.68 | 0.55 | 1.93 | 2.881 | 10.1 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | PI |
| 2016 | AOC2016-62 | 27 | 247.2 | 3 | R | M | 19683 | 1.26 | 0.12 | 1.54 | 0.286 | 3.808 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | PI |
| 2016 | AOC2016-63 | 32.9 | 445.4 | 8 | E | M | 35611.29 | 1.25 | 0.13 | 1.10 | 0.56 | 4.902 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | PI |
| 2016 | AOC2016-64 | 31.2 | 417.5 | 6 | E | F | 30371.33 | 1.37 | 0.58 | 1.74 | 2.43 | 7.26 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | PI |
| 2016 | AOC2016-01 | 28.8 | 405.9 | 5 | R | F | 23887.87 | 1.70 | 0.48 | 1.55 | 1.96 | 6.30 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | TC |
| 2016 | AOC2016-02 | 35.1 | 535.9 | 8 | E | M | 43243.55 | 1.24 | 0.16 | 1.81 | 0.88 | 9.71 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | TC |
| 2016 | AOC2016-03 | 31.1 | 415.9 | 5 | R | M | 30080.23 | 1.38 | 0.11 | 1.47 | 0.46 | 6.11 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | TC |
| 2016 | AOC2016-04 | 30.9 | 456.4 | 5 | R | M | 29503.63 | 1.55 | 0.15 | 1.60 | 0.70 | 7.31 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | TC |
| 2016 | AOC2016-05 | 31.2 | 408.8 | 5 | R | M | 30371.33 | 1.35 | 0.12 | 1.73 | 0.47 | 7.06 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | TC |
| 2016 | AOC2016-06 | 30.3 | 411.7 | 12 | E | F | 27818.13 | 1.48 | 0.46 | 2.37 | 1.91 | 9.76 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | TC |
| 2016 | AOC2016-07 | 27.3 | 259.4 | 3 | R | M | 20346.42 | 1.27 | 0.11 | 1.39 | 0.29 | 3.60 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | TC |
| 2016 | AOC2016-08 | 26.7 | 275.1 | 4 | R | F | 19034.16 | 1.45 | 0.49 | 2.42 | 1.35 | 6.67 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | TC |
| 2016 | AOC2016-09 | 30.1 | 410 | 5 | R | F | 27270.9 | 1.50 | 0.36 | 2.09 | 1.49 | 8.58 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | TC |
| 2016 | AOC2016-10 | 31.2 | 348.7 | 5 | R | F | 30371.33 | 1.15 | 0.50 | 1.99 | 1.74 | 6.94 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | TC |
| 2016 | AOC2016-11 | 29.2 | 311.4 | 5 | R | F | 24897.09 | 1.25 | 0.44 | 1.57 | 1.38 | 4.90 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | TC |
| 2016 | AOC2016-12 | 29.8 | 370.4 | 5 | R | F | 26463.59 | 1.40 | 0.45 | 2.18 | 1.66 | 8.09 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | TC |
| 2016 | AOC2016-13 | 30.6 | 388.5 | 5 | R | F | 28652.62 | 1.36 | 0.52 | 1.32 | 2.01 | 5.11 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | TC |
| 2016 | AOC2016-14 | 29.6 | 360.3 | 4 | R | M | 25934.34 | 1.39 | 0.12 | 1.69 | 0.45 | 6.07 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | TC |
| 2016 | AOC2016-15 | 28.7 | 318.2 | 4 | R | F | 23639.9 | 1.35 | 0.52 | 1.83 | 1.64 | 5.81 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | TC |
| 2016 | AOC2016-16 | 30.1 | 421 | 4 | R | F | 27270.9 | 1.54 | 0.37 | 1.90 | 1.54 | 8.00 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | TC |
| 2016 | AOC2016-17 | 27.2 | 263.3 | 3 | R | F | 20123.65 | 1.31 | 0.47 | 1.55 | 1.23 | 4.09 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | TC |

| | | | | | | | | | | | | | | Neoplastic lesions | | | | | Putative pre-neoplastic lesions | | | | Non-neoplastic biliary lesions | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Year | Fish # | Length (cm) | Weight (g) | Age | Retained or excluded based on age | Sex | L3 | k | GSI | LSI | Gonad wt (g) | Liver wt (g) | #Slides | Hepatocellular Adenoma | Hepatocellular Carcinoma | Cholangioma | Cholangio Carcinoma | Pancreatic islet cell tumor | Basophilic Focus | Eosinophilic Focus | Clear Cell Focus | VacuolatedCell Focus | BileDuct Hyperplasia | Cholangio fibrosis | Parasites | Site |
| 2016 | AOC2016-18 | 28.8 | 356.6 | 4 | R | M | 23887.87 | 1.49 | 0.16 | 1.69 | 0.57 | 6.01 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | TC |
| 2016 | AOC2016-19 | 31.0 | 425.6 | 7 | E | F | 29791 | 1.43 | 0.31 | 1.43 | 1.31 | 6.10 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | TC |
| 2016 | AOC2016-20 | 34.6 | 651.9 | 7 | E | M | 41421.74 | 1.57 | 0.22 | 1.72 | 1.40 | 11.23 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | TC |
| 2016 | AOC2016-21 | 28.2 | 297.4 | 4 | R | M | 22425.77 | 1.33 | 0.08 | 0.87 | 0.24 | 2.59 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | TC |
| 2016 | AOC2016-22 | 25.1 | 228 | 4 | R | M | 15813.25 | 1.44 | 0.09 | 1.49 | 0.21 | 3.39 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | TC |
| 2016 | AOC2016-23 | 27.1 | 254.7 | 2 | E | M | 19902.51 | 1.28 | 0.07 | 1.16 | 0.19 | 2.97 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | TC |
| 2016 | AOC2016-24 | 27.2 | 278 | 3 | R | F | 20123.65 | 1.38 | 0.35 | 1.36 | 0.97 | 3.79 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | TC |
| 2016 | AOC2016-25 | 26.5 | 259.8 | 4 | R | F | 18609.63 | 1.40 | 0.45 | 1.62 | 1.18 | 4.21 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | TC |
| 2016 | AOC2016-26 | 28.5 | 314.1 | 5 | R | F | 23149.13 | 1.36 | 0.33 | 1.28 | 1.04 | 4.02 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | TC |
| 2016 | AOC2016-27 | 29.0 | 335.5 | 4 | R | F | 24389 | 1.38 | 0.48 | 1.54 | 1.62 | 5.18 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | TC |
| 2016 | AOC2016-28 | 29.1 | 325.1 | 4 | R | F | 24642.17 | 1.32 | 0.52 | 1.90 | 1.70 | 6.19 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | TC |
| 2016 | AOC2016-29 | 29.6 | 359.2 | 4 | R | F | 25934.34 | 1.39 | 0.43 | 1.69 | 1.56 | 6.08 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | TC |
| 2016 | AOC2016-30 | 30.0 | 381 | 6 | E | M | 27000 | 1.41 | 0.12 | 1.25 | 0.45 | 4.75 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | TC |
| 2016 | AOC2016-31 | 25.0 | 200.8 | 3 | R | M | 15625 | 1.29 | 0.72 | 0.95 | 1.44 | 1.91 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | TC |
| 2016 | AOC2016-32 | 26.9 | 261.1 | 3 | R | M | 19465.11 | 1.34 | 0.12 | 1.35 | 0.32 | 3.52 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | TC |
| 2016 | AOC2016-33 | 28.7 | 346.4 | ns | R | M | 23639.9 | 1.47 | 0.04 | 1.37 | 0.14 | 4.74 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | TC |
| 2016 | AOC2016-34 | 25.8 | 261.8 | 3 | R | F | 17173.51 | 1.52 | 1.18 | 1.40 | 3.10 | 3.66 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | TC |
| 2016 | AOC2016-35 | 27.3 | 279.6 | 3 | R | M | 20346.42 | 1.37 | 0.32 | 1.22 | 0.89 | 3.40 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | TC |
| 2016 | AOC2016-36 | 29.2 | 381.2 | 3 | R | M | 24897.09 | 1.53 | 0.09 | 1.46 | 0.33 | 5.56 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | TC |
| 2016 | AOC2016-37 | 30.2 | 354.6 | 9 | E | F | 27543.61 | 1.29 | 0.57 | 1.73 | 2.03 | 6.14 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | TC |
| 2016 | AOC2016-38 | 30.4 | 370 | 6 | E | M | 28094.46 | 1.32 | 0.07 | 1.17 | 0.27 | 4.34 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | TC |
| 2016 | AOC2016-39 | 30.3 | 352.8 | 6 | E | F | 27818.13 | 1.27 | 0.35 | 1.41 | 1.24 | 4.98 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | TC |
| 2016 | AOC2016-40 | 29.9 | 349 | 5 | R | F | 26730.9 | 1.31 | 0.41 | 1.88 | 1.44 | 6.56 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | TC |
| 2016 | AOC2016-41 | 30.7 | 410.5 | 6 | E | M | 28934.44 | 1.42 | 0.08 | 1.80 | 0.35 | 7.40 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | TC |
| 2016 | AOC2016-42 | 30.2 | 409.3 | 4 | R | M | 27543.61 | 1.49 | 0.17 | 1.60 | 0.68 | 6.54 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | TC |
| 2016 | AOC2016-43 | 33.0 | 542.6 | 3 | R | F | 35937 | 1.51 | 0.39 | 2.61 | 2.14 | 14.17 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | TC |
| 2016 | AOC2016-44 | 25.4 | 244.9 | 3 | R | M | 16387.06 | 1.49 | 0.07 | 1.78 | 0.17 | 4.35 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | TC |
| 2016 | AOC2016-45 | 29.2 | 390.5 | 4 | R | F | 24897.09 | 1.57 | 0.38 | 1.54 | 1.48 | 6.02 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | TC |
| 2016 | AOC2016-46 | 28.8 | 390.8 | 5 | R | F | 23887.87 | 1.64 | 0.40 | 1.46 | 1.57 | 5.69 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | TC |
| 2016 | AOC2016-50 | 30.6 | 405.8 | 4 | R | F | 28652.62 | 1.42 | 0.40 | 1.58 | 1.64 | 6.42 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | TC |
| 2016 | AOC2016-51 | 29.7 | 297 | 4 | R | F | 26304.07 | 1.13 | 0.32 | 2.08 | 0.97 | 6.16 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | TC |
| 2016 | AOC2016-52 | 28.4 | 316.9 | 4 | R | F | 22906.3 | 1.38 | 0.46 | 1.48 | 1.47 | 4.68 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | TC |
| 2016 | AOC2016-53 | 30.8 | 370.5 | 5 | R | M | 29218.11 | 1.27 | 0.09 | 1.49 | 0.33 | 5.53 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | TC |

| | | | | | | | | | | | | | | **Neoplastic lesions** | | | | | **Putative pre-neoplastic lesions** | | | | **Non-neoplastic biliary lesions** | | | Site |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Year** | **Fish #** | **Length (cm)** | **Weight (g)** | **Age** | **Retained or excluded based on age** | **Sex** | **L3** | **k** | **GSI** | **LSI** | **Gonad wt (g)** | **Liver wt (g)** | **#Slides** | **Hepatocellular Adenoma** | **Hepatocellular Carcinoma** | **Cholangioma** | **Cholangio Carcinoma** | **Pancreatic islet cell tumor** | **Basophilic Focus** | **Eosinophilic Focus** | **Clear Cell Focus** | **VacuolatedCell Focus** | **BileDuct Hyperplasia** | **Cholangiofibrosis** | **Parasites** | |
| | | | | | | | | | | | | | | | | | | | | | | | | | | |
| NA = Not available | | | | | | | | | | | | | | | | | | | | | | | | | | |
| E = Excluded from tumor prevalence due to age outside of 3-5 year interval; R = Retained in tumor prevalence counts | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Site Names: BB = Boise Blanc Island (downstream collection location) ; PI = Peche Island (Upstream Reference); TC = Turkey Creek (Mid-stream region); AC = Amherstburg Channel (Downstream collection point) | | | | | | | | | | | | | | | | | | | | | | | | | | |

# APPENDIX D. Review comments from U.S. Fish and Wildlife Service on the Beneficial Use Assessment for BUI #4: Fish Tumors and Other Deformities in the Detroit River Area of Concern

**Comment**: Thank you for the opportunity to review this BUI Assessment Report. The report was well written and provided a nuanced examination of evidence that could be considered during re-designation determination. The Weight of Evidence approach combined with a thorough review of relevant brown bullhead movement literature provided a solid justification for examining brown bullhead from Canadian and US waters separately, and for BUI re-designation for the Canadian portion of the Area of Concern (AOC). The US Fish and Wildlife Service (USFWS) provides, for your consideration, suggestions for strengthening and clarifying the recommendation for re-designation to “not impaired”, and we include suggestions for a few minor editorial revisions to the Re-designation Recommendation Report.
**Response**: Thank you the comments were helpful and were used to revise the report.

**Comment:** We recommend re-titling and re-framing the report to clarify that this is a recommendation for re-designation rather than an assessment of the BUI. We discuss this further in the following paragraphs. To help highlight the recommendation for re-designation, we suggest moving details that are not crucial (e.g., detailed methods) to appendices. Much of the information contained in the report was presented with a greater level of detail than necessary to communicate the findings to the general public.
**Response**: The executive summary was streamlined to omit specific details of the assessment process and generate stronger statements about the status of BUI #4 concluding it to be unimpaired. It should be noted that this document is a technical report that provides supporting information to DRCC. It was not intended as a public communication.

**Comment:** We recommend clarifying support for re-designation to “not impaired”. Ample evidence has been presented to support this recommendation, however, discussions about a secondary criterion throughout the report and slight inconsistencies in wording referring to this criterion call into question if the report truly recommends re-designation to “not impaired”. While we value the nuance presented in discussing shortcomings in the data, we also suggest strengthening and/or clarifying the recommendation to avoid confusion. To clarify our suggestion, we discuss examples, below.
The report objectively examines the data and compares the results against the wording regarding the Fish Tumor and Other Deformities Beneficial Use Impairment (BUI) re-designation in the Canadian Stage II RAP. While concluding that the rate of tumors in the Detroit River AOC is not different from the Great Lakes background rate, the report also notes that the sampling did not meet a secondary re-designation criterion (BUI assessment based on two sampling events ≥3 years apart). We recognize that the Detroit River Canadian Stage II RAP (2010) does not describe the frequency of sampling as a requirement (i.e., part of the criterion), but rather in the “Design and Rationale” section following the “Delisting Criterion” (pg. 40 of the RAP). The Stage II RAP describes the “Design and Rationale” section as, “…a description of the rationale for the revisions and proposed design considerations for future studies in order to achieve scientifically sound delisting targets.” (pg. 23 of the RAP). However, on page 35 of this report, it notes that this secondary criterion is a “delisting criteria guidance statement”. Thus, it is unclear to us whether the guidance in the “Design and Rationale” section was interpreted as a recommendation or is essential to clarify intent of the delisting criterion.

**Response:** The Executive Summary, Section 1.2, 2.3, 3.3 and 4.0 were revised and rewritten. It is acknowledged that the reality of data available for the current assessment does not match an ideal design. The sample size requirements of an ideal design are articulated in the revised section 1.2 of the report. We also note that the Stage II RAP report provides flexibility and refers to the Rationale and Design section as a recommended guidance. Sections 1.4 and 2.4 of the report detail the WOE exposure assessment. The purpose of adding the WOE exposure assessment was to compensate for the lack of sample size specified in the ideal design. Through the WOE, sample pooling between mid-reach and lower reach sampling locations was deemed to be acceptable and enabled statistical testing of the delisting criteria with appropriate statistical power.

**Comment:** There is confusion about the final recommendation of the report. For example, on page ii of the Executive Summary it is, "…recommended that the middle and lower Canadian reaches of the Detroit River be considered unimpaired with respect to BUI #4." However, on page iii of the Executive Summary the author notes that the data examined, "do not fully meet the temporal component of survey implementation specified by the Stage II RAP report." This statement appears to question the scientific validity of a recommendation for BUI re-designation based on the current information.
**Response:** Both the Executive Summary and Section 4 have been edited to be more conclusive regarding the impairment status.

**Comment:** While the "4.0. Delisting criteria assessment" section technically includes a recommendation that "Canadian upstream waters of the Detroit River AOC are likely to be not impaired", and "the middle and lower reaches of the Canadian waters of the Detroit River meet the conditions specified by the BUI #4 delisting criteria" these statements are not prominent among a discussion of weakness in the data. To avoid concluding the body of the report with a paragraph discussing weaknesses of the data, we suggest adding a final paragraph or Recommendation section with a conclusive recommendation for re-designation, which does not include extensive caveats about deficiencies in the data.
**Response**: Section 4 was re-written to both address how the assessment met the conditions of the Stage II Design and Rationale, clarification of the weaknesses related to data availability and the supporting evidence to indicate that the weaknesses are unlikely to contribute to Type II errors. While it is acknowledged that combining results from 2002 and 2016 surveys prevents their use as two independent surveys to address the temporal component of the recommended design, we draw on the earlier published study from Leadley et al. (1998) to satisfy this condition.

**Comment:** Although Baumann (2010) was referenced for the 2% background rate, other recent assessment and proposed re-designation of Fish Tumor BUIs at other Canadian AOCs (2018 assessment of the St. Marys River Fish Tumor BUI, pre-2019 drafts of the St. Clair Fish Tumor BUI Re-designation Proposal) have referenced a different background rate (5%). To provide a consistency in BUI re-designations among AOCs, we suggest investigating the reasons for this difference and providing a brief explanation of why a different background rate was used here.
**Response:** The difference is articulated in section 1.2. The Stage II RAP specifically refers to Baumann (2010) in its experimental design and rational guidance section. We followed the advice of the Stage II RAP report by adopting the Baumann method. The absolute value of the background tumor prevalence rate is only partially relevant to the delisting criteria given that the criteria statement is concerned with detecting a statistical difference from the background prevalence not the absolute prevalence in the AOC itself. Using the guidance by Baumann (2010), the recommended Fisher's exact test was applied coupled with a sample size target of 100 fish used to test the delisting criteria. According to Baumann's Power analysis, when a sample size of 100 fish are collected from the impact site, a tumor frequency of 5% or higher would be necessary to statistically distinguish from the Great Lakes background. Thus,

while the background tumor frequency is stated to be 2%, a threshold tumor prevalence of 5% or more is necessary to conclude impairment of the BUI. This apparent discrepancy in the absolute prevalance (2%) vs relative prevalance (5%) needed to distinguish impact vs non-impact arises dependent on how the delisting criteria is worded.

**Comment:** Amherstburg Channel appears to be part of or grouped with the Boise Blanc study site for analyses, but this is not initially clear when Amherstburg Channel is mentioned early in the document. For example, on page 8, weight of evidence determinations are listed as being based on bullhead collection locations including the Amherstburg Channel (and not Boise Blanc), and on page 9, bullhead collection locations included the Amherstburg Channel which is described as "adjacent to Boise Blanc Island", but on page 13 bullhead collection locations include Boise Blanc and not the Amherstburg Channel.
**Response**: Boise Blanc samples and Amherstburg Channel were grouped as the same location owing to the proximity to one another and the fact that both collection areas fell within the downstream reach of the Detroit River. Their positions are now identified in the revised Figure 2 for clarity.

**Comment**: Throughout the document, there is a lack of consistency and clarity of the site category (least impacted reference site, adjacent binational AOC comparison site, or Canadian AOC sites for assessment), especially concerning the Trenton Channel, Peche Island, Amherstburg Channel, and Gross Isle. It is especially important to clearly identify the Trenton Channel as an adjacent binational AOC comparison site since the fish tumor BUI within the US Detroit River AOC will be separately assessed. As a courtesy to those involved in the future US fish tumor BUI removal process, we recommend clarifying that data from the Trenton Channel or other US waters within the US Detroit River AOC is for comparison only and the Canadian Detroit River AOC fish tumor BUI re-designation proposal is not intended to have any bearing on the assessment of the fish tumor BUI within the US Detroit River AOC.
**Response:** Apart from the literature review in Section 1.1, data of fish tumors from the U.S. have now been removed from the revised assessment report. Regarding the reference site. We maintain at the outset, that the primary reference for testing the delisting criteria is Great Lakes background tumor prevalence database generated by Baumann (2010). It wasn't clear why Peche Island, which is located within Canadian waters of the AOC, was originally selected as an Urban Great Lakes Reference site by Baumann and we did not want to pre-judge this location as being not-impacted at least until the WOE provided support for lower exposure conditions at this location. Ultimately, the WOE substantiated and supported Baumann's decision. The wording in the document was changed to reflect this stance. The testing of the delisting criteria at Peche Island was therefore removed. We also added the 2016 Peche Island data to the contingency table for the Great Lakes Reference database used to test the combined middle and lower reach tumor prevalence to make it compatible with the original addition of the 2002 Peche Island data to the database by Baumann.

**Comment:** As the report currently reads, Peche Island is a least impacted reference site as well as one of the assessment sites used to represent the current condition of the AOC. Because this site falls within the boundaries of the AOC but fish tumor data from this site were used to calculate the 2% fish tumor reference rate (Baumann 2010), the use of data from this site must be consistent and explicitly stated throughout. Although this BUI Assessment Report discusses the inclusion of Peche Island in Baumann's (2010) reference database (pg. 6, Section 1.3) there is not a clear conclusion of how data from Peche Island will be treated in the report. We recommend clarifying this even in the Introduction where previous information collected near Peche Island is used to provide historical context for this assessment. From that point forward in the report we recommend framing all comparisons of data from Peche Island with other data as a justification for maintaining Peche Island as a least impacted reference

site. If Peche Island is truly a reference site, then it should not be pooled with the non-reference sites' data, for example as a way to meet the recommended sample size, as is mentioned on page 17 (second paragraph), nor should the these data be used to calculate an overall neoplasm prevalence for the AOC, as is mentioned in the Delisting Criteria Assessment on page 34 (within the first paragraph; 112 fish include those collected from Peche Island). Another example of the inconsistency in the treatment of Peche Island as a reference site is that, on page 19, Figure 3, boxplots of PAH concentrations are shown that, presumably, pool data from the adjacent binational AOC comparison site and the least impacted reference sites as well as sites to be assessed within the AOC, confusing the role of these sites in determining re-designation. Similarly, on page 23, Figure 6 appears to pool sediment data from the relatively least impacted Peche Island reference site with the other Canadian sites, despite Peche Island's "significantly lower ∑PAHs compared to all other brown bullhead collection sites" (last paragraph on page 23).
**Response:** The report has been revised to reflect this change. With respect to examination of sediment PAH contamination and boxplots. We completed an analysis according to a hierarchical scheme. This commenced with a comparison of temporal patterns of PAHs in the Detroit River as a whole, followed by U.S. and Canadian jurisdictions in isolation, then upstream, midstream and downstream segments followed by comparison of temporal trends at the individual bullhead collection sites. With each analysis the statistical power changes dramatically with the lowest statistical power occurring at the individual collection site comparisons. The scheme of changing the scale of analysis for temporal patterns provides reinforcement for the final test, ultimately that PAH concentrations are stable through time not only where bullheads were collected but across all portions of the Canadian waters of the Detroit River.

**Comment:** Whereas ample context is provided for past studies conducted that are relevant to Fish Tumor rate assessment, we suggest adding geographic and historic context about the AOC as well as for the importance of addressing fish tumor rates and the linkage to the designation of the Fish Tumor BUI as "impaired". A map depicting the extent of the AOC, the delineation between Canadian and US waters, and all sampled locations (for fish and sediment) could help readers orient themselves to the locations that are discussed later in the report. A description of the anticipated delisting timeline could help readers understand the role of this re-designation in the AOC delisting process and in the context of broader Detroit River restoration and conservation efforts.
**Response:** The report has been revised to include reflect this suggestion.

**Minor editorial and clarifying suggestions**
Please note the correct spelling of "Baumann" with two "n"s throughout the document.
**Response:** Spelling corrected.

Pg. 1, Introduction, last paragraph
Suggest either revising the fraction or percentage of total tumor frequency for three of four types of liver neoplasms so these number match (2/20 ≠ 15%). If there is an extra step of calculations translating 2 of 20 fish having tumors to a 15% tumor frequency, we suggest adding an explanation to lead the reader through this thought process.

**Response:** This was an error of the text, 2/20 = 10% not 15%. It was corrected.

**Comment:** Page. 2, Introduction, second paragraph, third and fourth sentences
For consistency with reference to the Blazer et al 2009 study, we suggest including the number of fish collected (40) in 2011-2012 in the Trenton Channel from Blazer et al 2014.

**Response:** Sample number added to document as suggested.

**Comment :** Page 5, Introduction, Section 1.2, first paragraph on page
Suggest revision to the first paragraph to address the following issues:
Rutter (2010) did not recommend collection of 30 to 50 fish as noted in the first sentence. As stated in Rutter (2010), 30-50 fish met the minimum length requirement of 250 mm and longer and were selected for necropsy (Rutter personal comm. 2019 and Rutter 2010). Rutter (personal comm. 2019) indicated that the Rutter (2010) approach still works when sample size is low as it combines information about the effects of size and gender.

While not mentioned in the paragraph, USFWS notes that the Rutter (2010) Bayesian hierarchical approach is also an appropriate approach for sampling designs that include multiple sampling locations and multiple years. Samples collected from multiple sites and years within a location, such as an AOC, are not independent samples, and it is important to correctly incorporate the sampling design, including this lack of independence, into the statistical approach (Rutter personal comm. 2019).

In addition, the stated requirement of an extensive database is not correct. An extensive database was used in Rutter (2010) to determine possible appropriate Least Impacted Control Sites for comparison to the Presque Isle Bay AOC. Related to Least Impacted Control Sites, possible control sites should be located in the same system and/or watershed (Rutter personal comm. 2019).

Rutter (personal comm. 2019) indicated that logistic regression can account for the differences in size or age of field-collected fish, between sites and samples, and that may not be balanced across lengths, ages, and sexes. Model calibration, or a better term, “model fitting” is the process of determining if length, age, or sex are factors that affect tumor incidence at the sites sampled (Rutter personal comm. 2019). It is important to recognize if the age/length/sex composition are different to properly account for them in the statistical design (Rutter person comm. 2019).
**Response:** We removed most of the text concerning the Rutter (2010) study and simply reference it as an alternative statistical method in the revised document. Authors note that this study was informed by the Canadian Stage II RAP assessment sites Baumann (2010) in its design and rationale section for BUI #4. We attempted to follow this template as closely as possible.

**Comment**: Page 5, Introduction, Section 1.3, first paragraph of this section, last sentence
Suggest changing:

> “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.” to
> “These indicators are included in the appendix data summary sheets but not explicitly interpreted in the present report since they are **not** included in the actual delisting criteria.”

**Response:** Thank you, wording changed as indicated.

**Comment:** Recommend removing further mention of lesion types in the following paragraphs, as they are not part of the BUI criteria should be available for review in the referenced appendix.
**Response:** Text left in as it may address a reader question.

**Comment:** Page 7, last paragraph and elsewhere throughout the document
Suggest clearly indicating which sites (e.g., Trenton Channel, Peche Island) are Canadian versus US here and throughout the document to help orient those less familiar with these specific locations.

**Response:** We removed Trenton Channel throughout the document and simulations except for the Introduction material. All data analysis focus on Canadian Waters except for broad patterns of U.S. vs Canadian sediment contamination comparisons.

**Comment:** Page 9, Section 2.1, first paragraph of this section and Figure 2 (page 10)
The location of Boise Blanc Island, including whether it is within Canadian versus US waters, is unclear. Verify that Boise Blanc Island is indeed the former Grosse Isle site, and define "far field collection site". Clearly show Boise Blanc Island on a map. Grosse Isle as shown in Figure 2 is within US waters and includes the Trenton Channel, a highly contaminated area within the US Detroit River AOC as later described in the report. Additionally, waters along the western edge of Grosse Isle, which include the Trenton Channel, are highlighted with a yellow polygon indicating bullhead used in BUI assessment were collected here. If this is not true, we suggest adding clarification within the text, figure, and figure legend. If fish were sampled from the Trenton Channel and other waters along the western edge of Grosse Isle, suggest clarifying the purpose of collecting these data.
**Response.** In the 2002 Canadian bullhead collections report mistakenly referred to the Boise Blanc site as Grosse Isle. Fortunately the report supplied coordinates which place the collection location on the immediate west side of the narrow southern stretch of Boise Blanc (See Revised Figure 2.). The 2016 downstream collections occurred at the same latitude as the 2002 collections but along the Canadian Shoreline and is referred to as Amherstburg Channel, specifically located at the old Boblo dock. The locations of bullhead collection coordinates are now superimposed on Figure 2. The location of Trenton Channel is retained on the Figure as it provides value for interpreting the historic collections of bullheads from the AOC as documented in Section 1.1.

**Comment:** To clarify the extent of sampling conducted within this section and throughout the report, we suggest adding a table with the location, year of sampling, number of samples, and type of analyses.
**Response:** This information is already presented across individual sample tables. Fish sample numbers by site are clearly defined5 in Table 4 and 5. Sediment sample numbers by region and sample site are defined in Table 4.

**Comment:** Page 10, Figure 2 Suggest labeling yellow polygons with corresponding text names and clearly indicate US versus Canadian sites. Suggest labeling Trenton Channel (upper portion of polygon) as it is frequently referenced. Label lower, middle, and upper areas of the AOC for later references in sections referencing PAH analyses. Do the lower, middle, and upper correspond to Bois Blanc, Turkey Creek, and Peche Island, respectively?
**Response:** Figure 2 is modified to show each Canadian collection location by site and year. The figure caption is modified to explain features of the figure. Trenton Channel as a polygon is retained for reference to Section 1 and is identified as U.S. waters.

**Comment:** Page 12, Section 2.4, first paragraph of this section
Suggest clarifying that the sediment chemistry data was from both Canadian and US Detroit River AOC sampling locations. Please also add this clarification in either a direct blanket statement at the beginning of the report (e.g., "the AOC refers to the Canadian Detroit River AOC unless otherwise stated") or to other sections of the assessment report that state "all waters of the AOC" or "the AOC" (e.g., pages i, 1, 4, 6, 7, 8, 13, 19, 31, 35).
**Response:** Changed as suggested.

**Comment:** Page 15, Section 2.1.3

Suggest addressing the use of Trenton Channel data in this modeling, as results are presented for Trenton Channel in the corresponding results section (pages 28-29, Section 3.2.4).
**Response:** Trenton Channel removed from sediment chemistry analysis and simulations. We did retain the generic U.S. vs Canadian contrasts as a point of reference.

**Comment:** Page 16, Section 3.1, first paragraph of this section
Suggest clearly stating Trenton Channel in the US was used as an adjacent binational AOC comparison site and not included in dataset used for assessments within the Canada Detroit River AOC. This is unclear as written.
**Response:** Changed as suggested.

**Comment:** Page 16, Section 3.1, second paragraph of this section
We suggest not pooling fish from the least impacted reference site and adjacent binational AOC comparison site with the fish collected to assess the questionable portions of the AOC when estimating size at age relationships without first verifying no significant difference among these groups of sites. Recent research of brown bullhead in the Detroit River support the need for this preliminary step in analysis. Farwell et al. (2012) found female brown bullhead among locations in the Detroit River (including Trenton Channel, Belle Island, Belle River, and Peche Island) to have no difference in age but differences in body condition (Fulton's condition factor incorporating body mass and length). They also found that a treatment of female brown bullhead removed from the river and reared in uncontaminated conditions for one year to have a different body condition than those sampled directly from sites in the Detroit River, with no difference in mean age between these two treatments.
**Response:** We have excluded the Trenton Channel US Geological Survey data and retested the length x age and body weight by age relationships for the Canadian data. A series of ANCOVA's were performed to test interactions (slopes of the length x age or weight x age relationship) between aging method, sex and collection site. None of the interaction terms were found to be significant. The revised document has been updated with new relationships to reflect this change in data analysis. The authors recognize some differences in the findings of this assessment with those of Farwell et al. (2012; the consultant who wrote this report is a co-author of Farwell et al). The differences in body condition shown by Farwell et al likely reflect the more extreme case of differences between Peche Island and Trenton Channel collected fish from their study. Since we now removed Trenton Channel fish from the data analysis this should have no impact on the relationships derived. Body condition and liver/somatic plus gonado/somatic indices were available across the sites but were not explicitly tested between sites, as it is not part of the delisting criteria.

**Comment:** Suggest changing, "…prevalence in fish aged 3+ for comparison the Bauman (2010)'s assessment criteria." to "prevalence in fish aged 3+ for comparison **to** ~~the~~ **Baumann's** (2010) assessment ~~criteria~~."
**Response:** Wording changed as suggested.

**Comment:** Page 18, Table 5
Suggest including in the Table title "**Canadian and United States**" Detroit River AOC (or something similar). Suggest noting in the legend that information from the US Trenton Channel is included for comparisons only.
**Response:** US data removed.

**Comment:** Page 19, Figure 3
Suggest including in the figure caption "**Canadian and United States**" Detroit River AOC, if this figure reflects data pooled from Canada and US waters.
**Response:** Caption altered as suggested.

**Comment:** Page 21. Figures 3.3.3 Suggest removing as this is a US location. Data from US waters should not be used in this BUI assessment. If data collected from US water are used for comparisons, clearly state that is the case and label as collected from US locations.
**Response:** Figure and associated text relating to Trenton Channel were removed.

**Comment:** Page 22. Suggest referencing Figure 2 for corresponding river reaches (upstream, middle, and lower Detroit River) to accurately reference locations of PAH sampling.
**Response:** Figure 2 was revised and is referred to in text as suggested.

**Comment:** Page 23, Figure 7
Assuming the boundaries of Upper, Middle, and Lower reaches are depicted and labeled in Figure 2 in future report versions, we suggest referencing Figure 2 for Upper, Middle, and Lower reaches. In the current version, it is unclear where sediment samples were collected in relation to each other and the US/Canada boundary.
**Response:** Boundaries are now delineated in Figure 2.

**Comment:** Page 27, Figure 3.3.3
Suggest noting in the legend that the Trenton Channel is in the US and only used for comparison but not in analyses. In the "Zone" column, add "US" to the Trenton Channel zone. If greater consistency is desired, add "CA" to all other zones without country specified (i.e., Peche Island **CA**, Turkey Creek **CA**, Boise Blanc **CA**). Reference updated Figure 2 for location and extent of Upper, Middle and Lower reaches.
**Response:** Trenton Channel has been removed from figures.

**Comment**: Page 31, Section 3.2.6, first paragraph in section
Suggest removing reference to Trenton Channel as not part of the Canadian Assessment or clearly define it as a US site, which was used for comparison to Canadian waters.
**Response:** Trenton Channel data now removed. Generalized comparison of U.S. vs Canadian sediment PAH contamination were retained for general information purposes.

**Comment:** Page 35, Delisting criteria assessment, second paragraph
Suggest revising "It identified tumor prevalence's of 4 and 13% at Peche Island and Amherstburg Channel (near Bois Blanc), respectively in samples collected 1993 suggestive that tumor frequencies have declined in Canadian waters of the AOC over time." to "It identified tumor prevalence's of 4 and 13% at Peche Island and Amherstburg Channel (near Bois Blanc), respectively<u>,</u> in samples collected **<u>in</u>** 1993**<u>, which is</u>** suggestive that tumor frequencies have declined in Canadian waters of the AOC over time."
**Response:** Paragraph has been altered as suggested in the revised report.

# APPENDIX E: Fish Tumor and Other Deformities BUI Assessment Report Tracking

This report has been presented to the Detroit River Canadian Cleanup's (DRCC's) Monitoring and Research Work Group and Steering and Implementation Committee, the public, Indigenous communities, and the Four Agency Management Committee for comments. See below for the timeline associated with this review process.

| | |
|---|---|
| DRCC Monitoring and Research Work Group | Draft assessment presented on May 14, 2019; comments addressed. Decision to move assessment forward to DRCC Steering and Implementation Committee. |
| DRCC Steering and Implementation Committee (SIC) | Draft assessment presented to SIC June 19, 2019; comments addressed. Decision to move forward with re-designation to 'not impaired'. |
| Public Review | Presented to DR PAC at June 26, 2019 meeting, comments requested; one comment received.<br><br>Assessment and associated fact sheets posted on DRCC website for public comment period July 5, 2019 – August 31, 2019 (Facebook reminders to comment on July 4, 10, 22, 31 and August 21, 29; periodic Twitter reminders; notice in July and August newsletter). No comments received. |
| Indigenous Review | Reports and fact sheets were sent to Aamjiwnaang and Caldwell First Nations on September 3, 2019. No comments were received. |
| Four Agency Management Committee | Comments received from Michigan Environment, Great Lakes, and Energy on September 3, 2019; no concerns identified.<br><br>Comments received from US EPA and US EPA GLNPO on September 10, 2019; comments addressed. |
| COA AOC Annex Leads | Submitted for formal re-designation April 2020. |