

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY REGION 5 77 WEST JACKSON BOULEVARD CHICAGO, IL 60604-3590

REPLY TO THE ATTENTION OF:

6-91

Stephen Galarneau, Director Office of Great Waters – *Great Lakes and Mississippi River* Wisconsin Department of Natural Resources 101 S. Webster Street P.O. Box 7921 Madison, WI 53707-7921

FE8 1 1 2019

Dear Mr. Galarneau:

Thank you for your November 15, 2018 request to remove the "Fish Tumors or other Deformities" Beneficial Use Impairment (BUI) at the St. Louis River Area of Concern (AOC) located within the cities of Superior, WI and Duluth, MN. As you know, we share your desire to restore all the Great Lakes AOCs and to formally delist them.

Based upon a review of your submittal and the supporting data, the U.S. Environmental Protection Agency (EPA) approves your request to remove this BUI from the St. Louis River AOC. EPA will notify the International Joint Commission (IJC) of this significant positive environmental change at this AOC.

We congratulate you and your staff as well as the many federal, state and local partners who have been instrumental in achieving this environmental improvement. Removal of this BUI will benefit not only the people who live and work in the St. Louis River AOC, but all residents of Wisconsin, Minnesota, and the Great Lakes Basin as well.

We look forward to the continuation of this important and productive relationship with your agency and the Minnesota Pollution Control Agency and Minnesota Department of Natural Resources as we work together to delist this AOC in the years to come. If you have any further questions, please contact me at (312) 353-8320 or your staff can contact Leah Medley at (312) 886-1307.

Sincerely,

Chris Korleski, Director Great Lakes National Program Office Matt Steiger, WDNR Barbara Huberty, MPCA Melissa Sjolund, MNDNR Rick Gitar, Fond du Lac Raj Bejankiwar, IJC

cc:

State of Wisconsin DEPARTMENT OF NATURAL RESOURCES 101 S. Webster Street Box 7921 Madison WI 53707-7921

Scott Walker, Governor Daniel L. Meyer, Secretary Telephone 608-266-2621 Toll Free 1-888-936-7463 TTY Access via relay - 711



November 15, 2018

Mr. Chris Korleski Great Lakes National Program Office U.S. Environmental Protection Agency 77 West Jackson Boulevard (G-17J) Chicago, IL 60604-3507

Subject: Removal of the Fish Tumors or Other Deformities Beneficial Use Impairment in the St. Louis River Area of Concern

Dear Mr. Korleski:

I am writing to request the U. S. Environmental Protection Agency (U.S. EPA) Great Lakes National Program Office's (GLNPO's) concurrence with the removal of the Fish Tumors or other Deformities Beneficial Use Impairment (BUI) in the St. Louis River Area of Concern (SLRAOC).

The SLRAOC team has assessed the status of the management actions for the Fish Tumors and Deformities BUI as outlined in the Remedial Action Plan. A study of 622 White Sucker concluded that tumor incidence rates in the AOC are not significantly different than the reference population. All management actions associated with this impairment have been completed and the delisting target has been met. A public review of the recommendation was conducted from September 21 through October 12, 2018, and public comments were addressed. Therefore, we recommend that the Fish Tumors and Deformities BUI be removed from the SLRAOC's impairments list.

Please find documentation to support this recommendation enclosed, including the Fish Tumors or Other Deformities Beneficial Use Impairment Removal Recommendation document prepared by WDNR and MPCA staff. Also enclosed are letters of support from the St. Louis River Alliance, Mayor Emily Larson, Duluth, Minnesota, and Mayor Jim Paine, Superior, Wisconsin.

We value our continuing partnership in the AOC Program and look forward to working closely with the GLNPO in the removal of BUIs and the delisting of Wisconsin's AOCs.

If you need additional information, please contact Matt Steiger, WDNR, at 715-395-6904, or you may contact me.

Sincerely,

Jah

Stephen Galarneau, Director Office of Great Waters - Great Lakes & Mississippi River Wisconsin Department of Natural Resources 608-266-1956 <u>stephen.galarneau@wisconsin.gov</u>

Naturally WISCONSIN



cc: Leah Medley, SLRAOC Task Force Lead Amy Roe, USGS Technical Resource Lead Matt Steiger, WDNR AOC Coordinator Melissa Sjolund, MN DNR AOC Coordinator Barbara Huberty, MN PCA AOC Coordinator Rick Gitar, Fond du Lac AOC Coordinator

MINNESOTA POLLUTION CONTROL AGENCY

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November 15, 2018

Mr. Chris Korleski Director, Great Lakes National Program Office United States Environmental Protection Agency 77 West Jackson Boulevard Chicago, IL 60604-3507

RE: Approve the request to remove the Fish Tumors and Deformities Beneficial Use Impairment in the St. Louis River Area of Concern

Dear Director Korleski:



The Minnesota Pollution Control Agency (MPCA) and Wisconsin Department of Natural Resources (WDNR) hereby request the approval of the Environmental Protection Agency's (EPA) Great Lakes National Program Office (GLNPO) staff to remove the Fish Tumors and Deformities Beneficial Use Impairment (BUI) in the St Louis River Area of Concern (SLRAOC). The SLRAOC team has assessed the status of the management actions for the Fish Tumors and Deformities BUI as outlined in the 2013 SLRAOC Remedial Action Plan and its subsequent annual updates. All of the management actions associated with this impairment have been completed and a public review of the recommendation has been conducted. Two comment letters were received: the first fully supported the recommendation and no further action was needed. The second letter asked questions about the timing of the removal and future accountability for condition changes. A response to the second letter addressing their concerns was prepared by the SLRAOC Coordinators team and they reached consensus that the comments did not justify further efforts for this BUI. We therefore recommend that the Fish Tumors and Deformities BUI be removed from the SLRAOC's impairments list.

Enclosed please find the documentation to support this recommendation, including the Fish Tumors and Deformities BUI Removal Recommendation document prepared by MPCA and WDNR staff, letters of support from the St. Louis River Alliance, Mayor Emily Larson, Duluth, Minnesota, and Mayor Jim Paine Superior, Wisconsin.

We value our continuing partnership with the GLNPO staff and the funding support provided to the SLRAOC through the Great Lakes Restoration Initiative. It is through your significant involvement and that of all of our federal, state and local partners that will keep us on the path to delisting the SLRAOC by 2025.

Director Korleski Page 2 November 15, 2018

If you need further information about the Minnesota aspects of this request please contact Barb Huberty at 218-302-6630 or <u>barbara.huberty@state.mn.us</u>.

Sincerely,

· SR.

Jobn Linc Stine Commissioner

JLS/BH:nld

Enclosure: St. Louis River Area of Concern Beneficial Use Impairment Removal Recommendation for Fish Tumors and Other Deformities

cc: Leah Medley, SLRAOC Task Force Lead Amy Roe, USGS Technical Resource Lead Matt Steiger, WDNR AOC Coordinator Melissa Sjolund, MN DNR AOC Coordinator Rick Gitar, Fond du Lac AOC Coordinator

St. Louis River Area of Concern

Beneficial Use Impairment Removal Recommendation for

Fish Tumors and Other Deformities

Submitted to

U.S. EPA-Region 5 77 W. Jackson Boulevard Chicago, IL 60604

By

Prepared by St. Louis River Area of Concern Management Agencies

Submitted November 15, 2018



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Purpose

The purpose of this document is to provide the components that support a recommendation to remove the Fish Tumors and Deformities Beneficial Use Impairment (BUI) in the St. Louis River Area of Concern (SLRAOC).

Background and Problem Statement

The St. Louis River creates a 12,000-acre freshwater estuary at the western extent of Lake Superior, forming the Twin Ports of Duluth, MN and Superior, WI. Due to industrial and urban development, legacy contaminants, organic waste, loss of aquatic habitat, and degraded water quality conditions, the lower St. Louis River was designated as an Area of Concern in 1987 under the US-Canada Great Lakes Water Quality Agreement. A Stage I Remedial Action Plan (RAP) identified a series of nine beneficial use impairments (MPCA and WDNR, 1992). Steady progress has been made through development and implementation of the Stage II RAP, RAP updates, and stakeholder developed BUI removal targets. The RAP lists the BUI status and removal strategy, measurable BUI targets, and management actions required for each BUI removal. The current RAP and previous versions can be viewed at WDNR's website at http://dnr.wi.gov/topic/greatlakes/st.louis.html or MPCA's website at https://dnr.wi.gov/topic/greatlakes/st.louis.html or MPCA's website at https://dnr.wi.gov/topic/greatlakes/st.louis-river-area-concern

The SLRAOC is spatially large and geographically complex, spanning the Minnesota and Wisconsin state line and including tribal interests (Figure 1). The SLRAOC is jointly managed by its delegated authorities, the Minnesota Pollution Control Agency (MPCA) and the Wisconsin Department of Natural Resources (WDNR). The Minnesota Department of Natural Resources (MNDNR) and the Fond du Lac Band of Lake Superior Chippewa (FDL) are also partners. Many more stakeholders continue to participate in various aspects and are noted in the RAP.

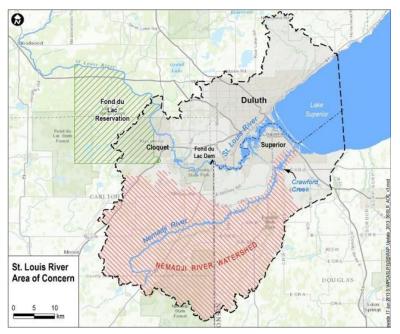


Figure 1. Extent of the St. Louis River AOC, including portions of Carlton County and St. Louis County, MN, Douglas County, WI and the Fond du Lac Reservation

BUI Listing and Removal Criteria

The SLRAOC's RAP describes the rationale for listing this BUI as follows:

Observations at the time of AOC listing suggested that fish tumors and deformities represented an impaired use in the St. Louis River estuary. However, no studies documenting the incidence rates of tumors in fish were available at the time.

Removal Target

The BUI removal target, as established by stakeholders in 2008, is:

Incidence rates of contaminant-related internal and external tumors and deformities in resident benthic fish species, including neoplastic or pre-neoplastic liver tumors, do not exceed incidence rates from unimpaired areas elsewhere in the Great Lakes Basin.

The SLRAOC RAP states that the removal of the Fish Tumors and Other Deformities BUI will be justified when the liver tumor incidence rates in the AOC, as seen in three consecutive samplings of at least 200 white suckers (*Catostomus commersoni*), are statistically similar to, less than, or trending towards the reference site(s) in a six-year period. Comparisons will be made using the variation of tumor incidence rates observed in the reference site(s).

Removal Strategy

The justification for removing this BUI is based on the report: *St. Louis River Area of Concern Fish Tumors and Other Deformities Beneficial use Impairment Study Summary Report* (Blazer, Hoffman, & Mazik, 2017) and is included as Appendix 1. The report is the outcome of implementing the BUI removal strategy and it summarizes data collection and assessment associated with the three actions identified below. In addition, the prevalence of white sucker tumors and deformities and the risk factors for liver and skin neoplasms for data collected in 2011, 2013 and 2015 are applied to the BUI target.

1. Reference Site Determination

Mountain Bay, Ontario was reviewed as a reference site. It was determined Mountain Bay was not an acceptable reference site due to a small sample size, the lack of isotope data to show diet range or migration information, and the lack of tumors found in 2006 which is inconsistent with earlier studies.

The BUI technical team developed a method for the refence area in conjunction with the residency method using stable isotope data to show the habitat usage of fish that are collected in the estuary during spawning. The white sucker population that is sampled in the St. Louis River during spawning includes migratory individuals that are believed to inhabit Western Lake

Superior. The BUI technical team determined that the white sucker that feed largely from western Lake Superior would be an acceptable reference population (reference site being Western Lake Superior) because Lake Superior habitats have low levels of contaminants and the fish could be collected and analyzed using the same methods as SLRAOC fish (Marvin et al. 2004). It is important to note that migratory white sucker routinely inhabit Lake Superior to feed, but the precise location of their foraging habitat within western Lake Superior is not known.

2. Fish Residency Determination

Because white sucker are migratory, the capture location is not necessarily representative of their long-term habitat use. Exposure to contaminated sediments is thought to increase the risk of tumor incidence (Blazer et al. 2014). Therefore, it was important to understand the fraction of diet that white sucker are getting from areas of the SLRAOC and Lake Superior. This information was then used to infer the relative exposure to contaminated sediments.

The stable isotopes of carbon and nitrogen are a diet-based tracer. In adult fish, these isotopes have a temporal resolution of 1 to 2 years. In the SLRAOC, the stable isotopes of carbon and nitrogen can be used to determine the feeding grounds of fish; systematic isotopic variation within the SLRAOC owing to underlying spatial changes in the river biogeochemistry can be used to separate fish primarily feeding in one of three zones: western Lake Superior, St. Louis Bay and Superior Bay, and the Lower River (Figure 2). In total, 619 fish from 2011, 2013, and 2015 were successfully analyzed for the isotopic composition. Generally, the diet contribution from these three zones was assigned using stable isotope analysis to potentially migratory white sucker captured in the river between Lake Superior and Fond du Lac dam; those captured in the Upper River are land-locked and thus assumed to be Upper River residents because the Fond du Lac dam lacks fish passage facilities (Figure 2).

The study used a model to evaluate the isotope composition (habitat usage) in relation to tumor prevalence. A reference comparison was built into the logistic regression test by including two factors: % Lower River contributing to diet and % Lake Superior contributing to diet (Appendix 1. p. 9).

Most of the white sucker had a stable isotope composition that indicated they were feeding in a mix of zones (Lake Superior, Superior and St. Louis Bay combined and the Lower River). The study found no relationship between the isotopic composition of the fish and its capture location; that is, the locations where the white sucker were captured during the spawning run was not representative of the longer-term habitat from which the white sucker was feeding (Figure 3).

3. Fish Sampling and Analysis of White Suckers

Fish were sampled three times (2011, 2013, 2015) with a year in between each sampling. Although the time between sampling dates equals less than six years, this study fits the intent of sampling three rounds with an off year between sampling events. Adult white sucker were collected during spawning periods: May 3-23, 2011, May 29-30, 2013, and May 6-8, 2015. The total fish collected was 200 in 2011, 172 in 2013, and 250 in 2015. All fish were collected in the river and stable isotope data were used to determine if the fish had been residing in certain river zones or Western Lake Superior (Figure 2). Fish were euthanized, weighed, measured, and visually assessed for abnormalities. Any visual gross abnormalities as well as liver pieces were removed and preserved for further analysis. In addition, a section of the dorsal muscle was removed for stable isotope analysis and otoliths were removed from the inner ear for aging.



Figure 2. River zones in St. Louis River where C and N stable isotope ratios can be used to reliably distinguish where fish reside (feed). (Blazer et al., 2017)

Supporting Data and Analysis

The SLRAOC undertook three rounds of sampling to collect and analyze white sucker data associated with tumors and deformities to better understand if this is a system wide impairment. The full report titled, *St. Louis River Area of Concern Fish Tumors and Other Deformities Beneficial Use Impairment Study Summary Report (Blazer et al., 2017)* is provided in Appendix 1. In addition, the reference section provides the sources related to research in

support of this work. What follows is a summary of the data collection, analysis and conclusions in relation to this BUI for the SLRAOC.

In total, 619 of the 622 white sucker collected were examined (one sample from each of St. Louis Bay, the Lower River, and the Upper River were not successfully analyzed). These fish ranged in age from 2 to 25 years; the mean age of the sample years ranged from 6 to 8 years (Table 1).

Most of the white sucker were feeding in a mix of zones. The study found no relationship between the isotopic composition of the fish and its capture location (Figure 3).

| conected within the | | . Louis River Area of Concern in 2011 – 2015 (Blazer et al., 2017) | | | |
|---------------------|-----|--|-------------|--------------|---------------|
| | Sex | Sample size | Age (years) | Length (mm) | Weight (gm) |
| All sites (2011) | F | 94 | 7.9 ± 2.1 | 427.1 ± 46.7 | 923.4 ± 321.1 |
| | М | 106 | 8.5 ± 2.7 | 408.4 ± 48.5 | 736.0 ± 185.8 |
| Site Compari | son | | | | |
| Superior Bay | | 50 | 7.3 ± 2.3 | 395.8 ± 65.1 | 678.9 ± 209.1 |
| St. Louis Bay | | 50 | 8.6 ± 2.5 | 412.2 ± 36.5 | 756.9 ± 212.8 |
| Lower River | | 50 | 8.8 ± 2.8 | 430.0 ± 42.5 | 959.6 ± 334.6 |
| Upper River | | 50 | 8.1 ± 1.9 | 430.8 ± 36.2 | 900.9 ± 231.5 |
| | | | | | |
| All sites (2013) | F | 90 | 6.6 ± 2.5 | 413.2 ± 58.0 | 749.2 ± 246.7 |
| | М | 82 | 6.4 ± 3.2 | 379.9 ± 42.7 | 561.8 ± 177.9 |
| Site Compari | son | | | | |
| Superior Bay | | 89 | 6.0 ± 1.9 | 380.8 ± 37.1 | 584.9 ± 189.7 |
| Lower River | | 30 | 8.8 ± 4.3 | 440.3 ± 40.5 | 864.2 ± 233.2 |
| Upper River | | 53 | 5.9 ± 2.4 | 404.6 ± 60.1 | 670.1 ± 239.5 |
| | | | | | |
| All sites (2015) | F | 125 | 7.6 ± 2.8 | 432.4 ± 49.6 | 974.3 ± 409.0 |
| | М | 125 | 7.2 ± 2.5 | 399.9 ± 33.5 | 724.0 ± 188.4 |
| Site Compari | son | | | | |
| Superior Bay | | 37 | 7.4 ± 1.8 | 397.7 ± 41.4 | 924.8 ± 484.1 |
| St. Louis Bay | | 87 | 8.1 ± 2.8 | 417.4 ± 39.4 | 874.5 ± 348.1 |
| Lower River | | 75 | 7.4 ± 2.6 | 422.1 ± 38.6 | 864.8 ± 267.1 |
| Upper River | | 51 | 6.2 ± 2.5 | 396.5 ± 53.6 | 728.0 ± 278.8 |

Table 1. Morphometric and age characteristics (mean \pm standard deviation) of white suckercollected within the St. Louis River Area of Concern in 2011 – 2015 (Blazer et al., 2017)



Fish collection tent and data collection work 2014 (Photo Credit: Diane Desotelle, MPCA)

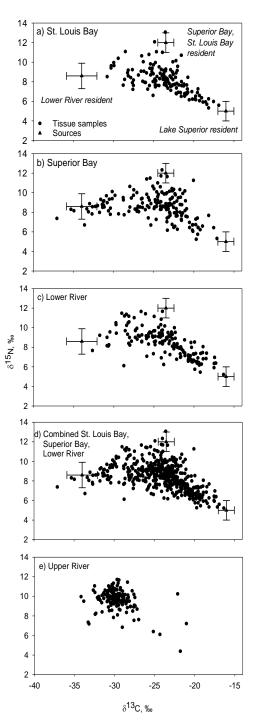


Figure 3. Distribution of ${}^{13}C$ and ${}^{15}N$ values in muscle samples of white sucker collected at the sampling areas and below Fond du Lac dam (where white sucker are migratory) combined. The source values with error bars (± 1 standard deviation) for putative resident fish are shown (Blazer et al., 2017)

All liver sections were examined for the presence of bile duct (cholangioma and cholangiocarcinoma) and hepatic cell (hepatic cell adenoma and hepatic cell carcinoma) neoplasms, all of which were considered as hepatic neoplasms. Liver sections were also examined for altered foci which are indicators of contaminant exposure and may be considered preneoplastic. Bile duct hyperplasia was also noted which has been considered in some instances to be preneoplastic but can also be induced by parasites and noncarcinogenic chemicals. Pieces of raised external lesions were also examined microscopically to determine if they were neoplasms (papillomas or squamous cell carcincomas) or nonneoplastic lesions such as hyperplasia (an increase in the number of normal cells).

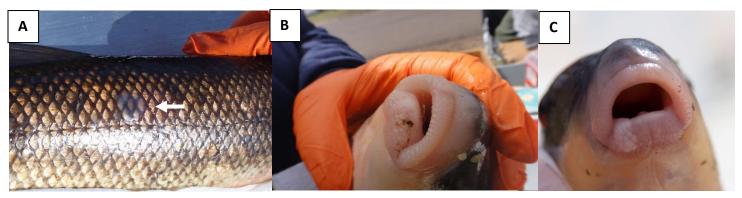
The prevalence of white sucker collected with lesions is summarized in Table 2. The external lesions included raised skin lesions, growths on the lips, and mucoid lesions on the body and fins. The raised lesions on body and lips as well as some of the mucoid lesions were benign neoplasms. The majority of the mucoid lesions were composed of hyperplastic skin cells.

Although the study documented additional information such as total external raised lesions, the BUI removal was evaluated based on two groupings, skin neoplasm and liver neoplasm (Table 3).

| | External Ob | External Observations | | Microscopic Liver Lesions | | |
|---------------|-----------------------------|------------------------------|-----------------------------|---------------------------|----------------------|--|
| Site | External Raised Lesions* | Orocutaneous Neoplasms | Bile duct Proliferation* | Altered Foci* | Hepatic Neoplasms | |
| 2011 | | | | | | |
| Superior Bay | 10.0 | 0 | 52.0 | 8.0 | 4.0 | |
| St. Louis Bay | 44.0 | 12.0 | 40.0 | 6.0 | 4.0 | |
| Lower River | 32.0 | 4.0 | 58.0 | 4.0 | 6.0 | |
| Upper River | 38.0 | 2.0 | 46.0 | 0 | 4.0 | |
| All sites | 31.0 | 4.5 | 49.0 | 3.5 | 4.5 | |
| 2013 | | | | | | |
| Superior Bay | 2.2 | 1.1 | 42.7 | 4.5 | 4.5 | |
| Lower River | 10.0 | 10.0 | 73.5 | 3.3 | 16.7 | |
| Upper River | 11.3 | 3.8 | 45.3 | 1.9 | 1.9 | |
| All sites | 6.4 | 3.5 | 48.8 | 3.5 | 5.8 | |
| 2015 | | | | | | |
| Superior Bay | 10.8 | 5.4 | 48.6 | 2.7 | 0.0 | |
| St. Louis Bay | 16.1 | 4.6 | 55.2 | 5.7 | 4.6 | |
| Lower River | 14.7 | 8.0 | 64.0 | 0.0 | 6.7 | |
| Upper River | 7.8 | 2.0 | 33.3 | 2.0 | 2.0 | |
| All sites | 13.2 | 5.2 | 53.2 | 2.8 | 4.0 | |

Table 2. Prevalence (percentage) of white sucker collected from the SLRAOC with selected microscopic lesions 2011 – 2015 (Blazer et al., 2017)

*documented observations included in the study but are not considered neoplasms and therefore not included in BUI evaluation.



External skin and lip lesions observed on white sucker captured in the St. Louis River Area of Concern. **A**. Slightly raised mucoid lesion (arrow) on the lateral body surface. **B** and **C**. Raised lip lesions. (Photo Credit: Vicki Blazer, USGS)

The prevalence of white sucker collected with a neoplasm prevalence are summarized in Table 3. Neoplasms are an abnormal mass of tissue that results when cells divide more than they should or do not die when they should. Neoplasms may be benign (not cancer) or malignant (cancer).

| Site | Sample size | Skin Neoplasm# Observed (%) | Liver neoplasm# Observed (%) |
|---------------|----------------|--------------------------------|---------------------------------|
| Superior Bay | 176 | 3 (1.7%) | 6 (3.4%) |
| St. Louis Bay | 137 | 10 (7.3%) | 6 (4.4%) |
| Lower River | 155 | 11 (7.1%) | 13 (8.4%) |
| Upper River | 154 | 3 (1.9%) | 4 (2.6%) |
| All Sites | 622 | 27 (4.3%) | 29 (4.7%) |

Table 3. Summary of neoplasm prevalence at sites within the SLRAOC 2011-2015 (Blazer et al.,2017)

Logistic regression was used to test relationships between biological variables, habitat usage (i.e., the feeding range as measured by the amount of diet derived from a certain area based on stable isotope signatures), and incidence of either skin or liver neoplasia. The factors considered in both logistic regression models were sampling year, age, sex, % Lower River contributing to diet and % western Lake Superior contributing to the diet (following Blazer et al.

2014). Sampling year was not a significant factor in either model; therefore, the data were reanalyzed excluding this variable.

For both types of neoplasia, we found that increased feeding in the AOC over the past 1-2 years (i.e., the percent diet based on the stable isotope composition) did not result in a significant increase in neoplasia incidence relative to feeding in western Lake Superior (reference population) (Tables 4 and 5). This indicates that feeding location (residency) is not a significant factor in incidence of tumors in the SLRAOC and BUI removal is justified.

We found that sex and age were significantly associated with skin neoplasia incidence, whereas neither sampling year nor either habitat variable (% Lake, % Lower River) were significant (Table 4). With increasing age, white sucker were significantly more likely to have skin neoplasia, and female white sucker less likely than males. We found that only age was significantly associated with liver neoplasia incidence (Table 5). As with skin neoplasia, older white sucker were significantly more likely to have liver neoplasia than younger white sucker.

| Table 4. Logistic regression model of skin neoplasia incidence excluding sampling year as a |
|---|
| factor, including parameter estimates, Z scores, and associated p-values (p <0.05 in bold). The |
| full model p = 0.001. (Blazer et al., 2017) |

| Parameter | Estimate (±SE) | Z | p-value |
|---------------|----------------|-------|---------|
| Constant | 4.37 (0.92) | 4.77 | 0.000 |
| Sex (F) | 1.34 (0.57) | 2.34 | 0.019 |
| Age | -0.24 (0.07) | -3.40 | 0.000 |
| % Lake | 0.66 (1.20) | 0.55 | 0.581 |
| % Lower River | -0.04 (1.37) | -0.03 | 0.975 |

Table 5. Logistic regression model of liver neoplasia incidence excluding sampling year as a factor, including parameter estimates, Z scores, and associated p-values (p <0.05 in bold). The full model p = 0.027. (Blazer et al., 2017)

| | Estimate | | |
|---------------|--------------|-------|---------|
| Parameter | (±SE) | Z | p-value |
| Constant | 4.44 (0.90) | 4.92 | 0.000 |
| Sex (F) | 0.40 (0.46) | 0.87 | 0.385 |
| Age | -0.20 (0.07) | -2.98 | 0.003 |
| % Lake | -0.05 (1.17) | -0.04 | 0.969 |
| % Lower River | -0.09 (1.35) | -0.07 | 0.946 |

Fin Erosion Issues Not Included in SLRAOC BUI Removal Criteria

From 1997-2005, WDNR, USEPA and Murphy Oil led remediation projects at the Hog Island Inlet and Newton Creek to address petroleum contaminated sediments. In 2016, WDNR water resources staff completed a post-remediation assessment of the project. They found that Hog Island Inlet and Newton Creek continue to meet the established sediment clean-up goals related to legacy contamination. However, they also documented partial to complete fin loss in several species of fish surveyed in Newton Creek. After discovering the fin abnormalities, the removal of the Fish Tumors and Deformities BUI was put on hold until it could be determined whether the abnormalities were a legacy issue.

Newton Creek is the only location where fin loss has been documented to this extent within the boundaries of the St. Louis River AOC. In 2017, WDNR worked collaboratively with USEPA and dischargers to Newton Creek to conduct additional monitoring and assessment to help identify the potential source and cause of the fin abnormalities and chronic aquatic toxicity. Based on the information to date from the 2017 study, WDNR has concluded that the cause of the fin abnormalities is not related to legacy contamination in the AOC, and therefore removal of the BUI is appropriate.

Newton Creek will remain on the WI 303(d) list, which is a Clean Water Act designation separate from the AOC BUI designation. As such, WDNR will use its existing authorities to resolve the problem, including further monitoring to assess conditions in the creek over time and, if impairments remain, further assessments to determine the cause and necessary actions to address unresolved concerns.

Findings and Conclusions

The SLRAOC BUI removal target is to compare external tumors and deformities as well as liver tumors to an unimpaired area (i.e., western Lake Superior). The study conducted from 2011-2015 found that the overall incidence of both skin and liver neoplasms was below 5% and that habitat zone was not a significant factor on tumor incidence. From this, we conclude that tumor incidence was similar and low (<5%) for white sucker sampled in the St. Louis River whether the fish were feeding in the reservoirs, the estuarine portion of the SLRAOC, or in western Lake Superior (the reference area).

Researchers have studied benthic fish in AOC and non-AOC areas across the Great Lakes. Often, a least-impacted site will have a tumor prevalence of <5%; and some AOCs use 5% as their removal target (Blazer, personal communication, Simmons et al, 2012). The fact that the SLRAOC tumor incidence is <5% is another line of evidence that supports BUI removal.

As an additional line of evidence, using identical methods, white sucker from two Wisconsin AOCs (Sheboygan River and Milwaukee Estuary) and the Kewaunee River as a "least impacted site" were sampled in 2011 through 2013 (Blazer et al. 2016). The prevalence of skin tumors was higher (p < 0.0001) at the Kewaunee River than at the SLRAOC, while there was no significant difference (p = 0.5570) between the sites as far as liver neoplasms. Both Kewaunee and St. Louis River had significantly lower liver tumor prevalence when compared to the Sheboygan and Milwaukee AOCs (Milwaukee, WI is 15% and Sheboygan, WI is 8.3%).

Generally, SLRAOC liver and skin neoplasms were highest in white sucker captured in the Lower River and lowest for those captured in the Upper River; however, age accounts for some of these spatial differences. The areas with the lowest neoplasm prevalence (Superior Bay, Upper River) also had the youngest fish. Statistically, age and sex were significantly associated with skin neoplasia, whereas neither the feeding grounds nor the sampling year were significant. With increased age, the fish are more likely to have skin neoplasia, and females are less likely than males. As the fish age, however, they also have a greater diet contribution from western Lake Superior. As for liver neoplasia, only age was a significant factor. Because the feeding range was not a significant factor for potentially migratory white sucker captured in the estuary, skin and liver neoplasia incidence was compared between the zones in the estuary (Superior Bay, St. Louis Bay, Lower River) to that above the Fond du Lac Dam (Upper River), and there was not a significant difference between the regions above and below the dam (Appendix 1, Table 8 and Table 9). It is also interesting to note that SLRAOC skin and liver tumor incidence rates were compared to a least impacted site in Wisconsin, the Kewaunee River as an additional line of evidence. The prevalence of skin tumors was lower in the SLRAOC than the Kewanuee River: 4.3% to 21.0% respectively.

This SLRAOC study found that the removal target has been met. Incidence rates of contaminant-related internal and external tumors and deformities in resident benthic fish species, including neoplastic or pre-neoplastic liver tumors, do not exceed incidence rates from unimpaired areas elsewhere in the Great Lakes Basin.

These data show the SLRAOC to have a very low tumor incident rate and concludes that the AOC wide condition of white sucker is acceptable and meets the BUI removal criteria. The study was not intended to evaluate site specific conditions.

Delisting the SLRAOC will require cleaning up sites where sediment contamination is of concern to human health and the environment (e.g., U.S. Steel, MN Slip, Ponds behind Erie Pier, several slips in Superior Bay) under the Restrictions on Dredging BUI as well as addressing sediment contamination at several habitat restoration sites (Crawford Creek, Pickle Pond, Mud Lake and U.S. Steel) under the Loss of Fish and Wildlife BUI; the goal is to remove the BUI by 2025.

Public participation

Findings of the tumor study have been presented to public audiences including at the St. Louis River Summit in 2016 and as a poster in 2017. The results of the tumor study were presented to the St. Louis River Alliance Board on September 19, 2018.

A draft of this document was posted for public comment from September 21- October 12, 2018. The document, fact sheet and comment form were posted on the Wisconsin DNR's website: http://dnr.wi.gov/topic/greatlakes/st.louis.html. Paper copies of the draft document, fact sheet and comment form were available for review at the Superior Public Library (1530 Tower Ave.) and the Duluth Public Library (520 W Superior St.).

A GovDelivery bulletin and Press release promoting the public input opportunity were released by WDNR and MPCA on September 21, 2018 and September 26, 2018. An invitation to participate in the comment period was sent to multiple SLRAOC distribution lists via email on September 21, 2018.

The fact sheet, comment form, press release, GovDelivery bulletin and email invitation are included in appendix 2.

Public interest consisted of two written comments received by email during the comment period. One comment was in support of the BUI removal and one comment was from an organization that had some questions about the study and wanted BUI removal to occur post remediation. A formal response was sent on November 5, 2018 to the organization addressing their questions and communicating the approach for remediation in the AOC. The comments and response can be found in appendix 3.

Coordination with the Fond du Lac tribe is an ongoing function of the monthly AOC Coordinator meetings. Throughout the BUI removal process, the Fond du Lac AOC Coordinator is responsible for apprising the tribe of AOC progress and communicating any tribal concerns to the AOC team. No concerns were expressed.

No changes were made to the draft document after the public comment period.

Removal Recommendation

The SLRAOC staff have consulted with the BUI technical team, stakeholders and U.S. EPA staff on the data collection and analysis in accordance with the SLRAOC RAP BUI target, actions, and removal objectives for the fish tumors and deformities BUI. The results of the scientific assessment and the support of the St. Louis River Alliance, and stakeholders leads the RAP implementing agencies to approve this recommendation to remove the Fish Tumors and Deformities BUI from the SLRAOC.

Therefore, the Minnesota Pollution Control Agency and the Wisconsin Department of Natural Resources recommend the removal of the Fish Tumors and Deformities BUI for the St. Louis River Area of Concern.

References

- Blazer, V. S., J. Hoffman, P. Mazik. 2017. St. Louis River Area of Concern Fish Tumors and Other Deformities Beneficial Use Impairment Study Summary Report. Appendix 1 of this Document.
- Blazer, V.S., H. L. Walsh, R. P. Braham, C. M. Hahn, P. Mazik and P. B. McIntyre. 2016. Tumours in white suckers from Lake Michigan Tributaries: pathology and prevalence. Journal of Fish Diseases. 40:377-393. <u>https://onlinelibrary.wiley.com/doi/abs/10.1111/jfd.12520</u>
- Blazer, V. S., J. Hoffman, H. L. Walsh, R. P. Braham, C. Hahn, P. Collins, Z. Jorgenson and T. Ledder. 2014. Health of white sucker within the St. Louis river Area of Concern associated with habitat usage as assessed using stable isotopes. Ecotoxicology, 23:236-251.
- Marvin, C., Painter, S., Williams, D., Richardson, V., Rossman, R., Van Hoof, P. 2004. Spatial and temporal trends in surface water and sediment contamination in the Laurentian Great Lakes. Environ Poll. 129:131-144.
- Minnesota Pollution Control Agency. St. Louis River Area of Concern Implementation Framework: Roadmap to Delisting (Remedial Action Plan Update), by LimnoTech. St. Paul, Minnesota. July 15, 2013. http://www.pca.state.mn.us/index.php/viewdocument.html?gid=19677.
- Minnesota Pollution Control Agency and Wisconsin Department of Natural Resources. St. Louis River Area of Concern Remedial Action Plan Annual Updates. <u>http://dnr.wi.gov/topic/greatlakes/st.louis.html</u>.
- Simmons, D. B. D., N. C. Bols, B. P. Duncker, M. McMaster, J. Miller, and J. P. Sherry. 2012. Proteomic Profiles of White Sucker (Catostomus commersonii) Sampled from within the Thunder Bay Area of Concern Reveal Up-Regulation of Proteins Associated with Tumor Formation and Exposure to Environmental Estrogens. Environmental Science & Technology. 46(3): 1886-1894.

Appendix 1- St. Louis River Area of Concern Fish Tumors and Other Deformities Beneficial Use Impairment Study

St. Louis River Area of Concern Fish Tumors and Other Deformities Beneficial Use Impairment Study Summary Report

Submitted by

Vicki S. Blazer, PhD and Patricia Mazik, PhD U.S. Geological Survey

Joel Hoffman, PhD U.S. Environmental Protection Agency

> September 30, 2016 Revised April 11, 2017





About this Document:

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I. Purpose

A. Beneficial Use Impairment (BUI) 3 Background

The results reported here are intended to assist Minnesota, Wisconsin and the Fond du Lac Band of Lake Superior Chippewa in assessing progress towards the removal of BUI 3 – Fish Tumors and Other Deformities in the St. Louis River Area of Concern (SLRAOC).

The St. Louis River watershed has been profoundly changed by over 150 years of human development that began with settlement by Euro-Americans and which resulted in widespread pollution and habitat degradation, especially around the St. Louis River estuary near Lake Superior. In 1987, the U.S. Environmental Protection Agency designated the SLRAOC primarily due to historical degradation of the St. Louis River, which included inappropriate discharge of untreated wastewater and debris from industrial and municipal facilities, as well as poor community land-use practices. The SLRAOC includes the lower 39 miles of the St. Louis River from just upstream of Cloquet, MN to the river's mouth at the Duluth MN/Superior WI Harbor (Figure 1). The section of the river addressed for this BUI includes the 20-mile stretch from the Fond du Lac Dam in west Duluth to the mouth at the Superior entrance. (Figures 1, 2).

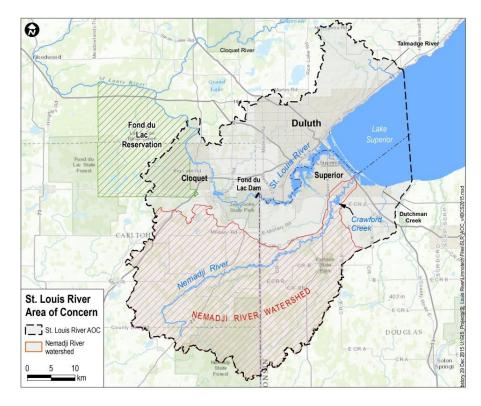


Figure 1. Geographic extent of the St. Louis River Area of Concern.

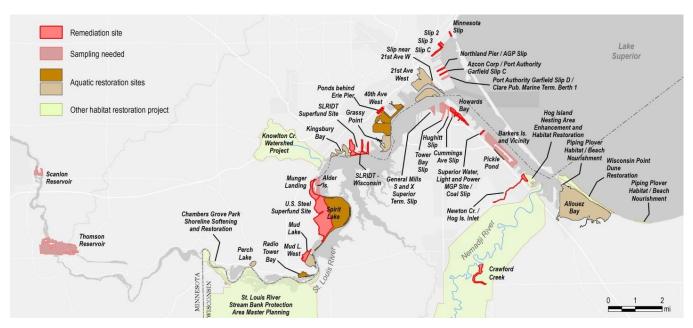


Figure 2. St. Louis River Area of Concern associated sources of contaminants (remediation sites) and aquatic restoration projects.

When the SLRAOC was designated, the Fish Tumors and Other Deformities, BUI 3 was listed as one of nine BUIs. BUI 3 was identified as an impairment based on observations at the time; however, no studies to document the types, severity or prevalence of fish tumors were conducted until this assessment was initiated in 2011 (Blazer et al. 2014). The SLRAOC Remedial Action Plan (RAP) includes the updated list of actions the SLRAOC partners and stakeholders deemed necessary to determine if BUI removal targets have been achieved. This report summarizes data collection and assessment associated with BUI 3 which includes the studies behind the prevalence of white sucker (*Catostomus commersonii*) fish tumors and deformities and the risk factors for liver and skin neoplasms for data collected in 2011, 2013 and 2015.

B. BUI 3 Removal Target

The BUI removal target, as established by stakeholders in 2008, is:

Incidence rates of contaminant-related internal and external tumors and deformities in resident benthic fish species, including neoplastic or pre-neoplastic liver tumors, do not exceed incidence rates from unimpaired areas elsewhere in the Great Lakes Basin.

As previously recognized (Rafferty et al. 2009), there has been difficulty addressing this BUI throughout the Great Lakes because of inconsistent and sometimes inaccurate definitions and the lack of scientific evidence to address removal targets as defined. There are many studies that document higher skin and liver tumor rates in benthic fishes, particularly brown bullhead (*Ameiurus nebulosus*) and white sucker, at sites contaminated with industrial wastes or effluents compared to less impacted sites (Rafferty et al. 2009). However, many neoplasms have multifactorial "causes" and risk factors may include both chemical exposure and biological (viral, parasite) agents. Hence, the certainty that internal or external tumors are contaminant-related is currently lacking.

In addition, the term "pre-neoplastic liver tumors" is inappropriate. Tumor, by definition, is a raised area or swelling. Neoplasia, as defined by the National Cancer Institute is "an abnormal mass of tissue that results when cells divide more than they should or do not die when they should. Neoplasms may be benign (not cancer) or malignant (cancer)." Liver "tumors" in brown bullhead or white sucker are rarely observed by visual inspection, but rather liver neoplasms are diagnosed microscopically. While some investigators have included bile duct proliferation or hyperplasia and foci of cellular alteration as preneoplastic lesions, there is no good experimental evidence to suggest either of these are actually preneoplastic lesions in white sucker or brown bullhead. Foci of cellular alterations include basophilic, eosinophilic, vacuolated and clear cell foci. While these lesions are contaminantrelated they may not be preneoplastic (i.e., progressing to a neoplasm). Conversely, bile duct proliferation may be related to contaminant exposure but may also be due to the presence of parasites. Hence the consensus of a group of fish pathologists conducting fish tumor studies was that these lesions should be documented but should not be included as "neoplastic or preneoplastic liver tumors" (Blazer et al. 2006). The data for foci of cellular alterations and bile duct proliferation are presented separately from the neoplasms.

C. BUI 3 Removal Strategy

Three actions were identified in the RAP related to the removal strategy.

1. Reference Site Determination

The first step in addressing the removal strategy was to determine if Mountain Bay, Ontario was an appropriate reference site. It was determined this was not an acceptable site for the following reasons:

- The data available are from 2006, which is five to nine years earlier than the data collected on the St. Louis River;
- Only 100 white sucker were examined and neither age data nor stable isotope data to indicate residency (that is, whether fish were primarily feeding in in the AOC or outside the AOC) were available; and
- No liver tumors were reported (Mahmood et al. 2014), which is not consistent with studies conducted in 1985-1990 reported by Baumann et al. (1996). In those studies, 3.6% of the white suckers (n = 304) had external tumors while 2.6% (n = 75) had liver neoplasms (Baumann et al. 1996).

In 2015, it was decided white sucker that reside in western Lake Superior could be used to determine the reference tumor prevalence. Western Lake Superior was chosen as the reference location owing to the relatively low concentrations of legacy contaminants, particularly polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs), in sediments. White sucker that reside in Lake Superior, however, are difficult to capture while in Lake Superior, but are readily captured in the St. Louis River during the spawning migration, when they mix with white sucker that reside in the St. Louis River. From the beginning, the assessment included acquiring residency information (specifically, location of feeding as inferred from stable isotope analysis) to separate river residents and lake migrants captured in the course of the sampling. This approach allowed us to address the BUI target by asking whether increased feeding in the SLRAOC (versus outside the SLRAOC, in western Lake Superior) is associated with increased neoplasia prevalence.

2. Fish Residency Determination

White sucker are migratory and can move long distances to suitable riverine spawning habitat during the spring. Unlike well-documented migratory species such as Pacific and Atlantic salmon or shad and herring, the details of white sucker migrations in the Great Lakes are not well-known. Both intrinsic (e.g., soft tissue stable isotope ratios of carbon and

nitrogen, otolith microchemistry) and extrinsic (e.g., floy tags) tags can be used to track fish migrations. Unlike extrinsic tags, intrinsic tags can be used to retrospectively reconstruct wild fish migrations (Hoffman 2016). Otolith microchemistry can provide information over the whole life-history of an individual fish, but can have poor spatial resolution among adjacent habitats and is extremely time-consuming to process samples. Stable isotope ratios can have high spatial resolution and tissue samples are relatively quick to process, but the data can only provide information about an individual fish's movements over the past one- to two-years. In the St. Louis River, C and N stable isotope ratios in soft tissues (e.g., dorsal muscle) can be used to reliably distinguish among fish that reside (feed) in Lake Superior from either those that reside in the lower river (i.e., generally, above St. Louis Bay up to Fond du Lac dam) or Superior Bay and St. Louis Bay (combined; Hoffman et al. 2010, Blazer et al. 2014) (Figure 3). This is because the carbon (C) or nitrogen (N) stable isotope composition, ${}^{13}C$: ${}^{12}C$ or ${}^{15}N$: ${}^{14}N$ (denoted as $\delta^{13}C$ or $\delta^{15}N$, respectively), of soft tissues in a fish reflects that fish's diet. The average difference between the C and N stable isotope composition of a consumer and its recent diet is +0.4 $\& \delta^{13}C$ and +3.4 $\& \delta^{15}N$ for whole organisms and muscle tissue (Vander Zanden and Rasmussen 2001). Thus, where the stable isotope composition of prey differs among locations, often owing to underlying biogeochemical differences among those locations (as between Lake Superior and the St. Louis River), it can be used to characterize fish movement or site fidelity (Hoffman 2016).

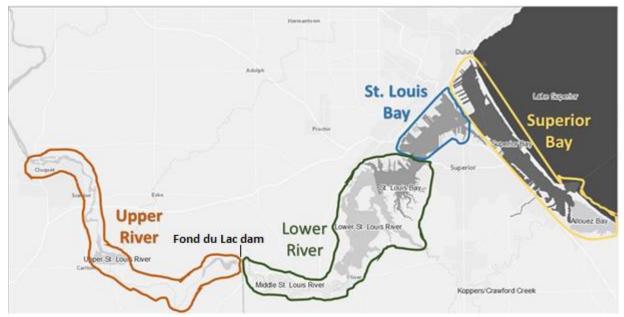


Figure 3. River zones: Lake Superior, Superior Bay, St. Louis Bay, Lower River, and Upper River.

3. Fish Sampling and Analysis of White Sucker 2011 through 2015

Adult white sucker were sampled during the spawning period May 3-23, 2011, May 29-30, 2013 and May 6-8, 2015. A total of 200 fish were collected in 2011, 172 in 2013 and 250 in 2015. Throughout the study suckers were collected within the river zones: Superior Bay, St. Louis Bay, Lower Lower River (upstream of St. Louis Bay and to Fond du Lac Dam), and Upper River (upstream of Fond du Lac Dam to near Cloquet, MN;Figure 3). Fish were collected by seine, trap-nets, backpack and boat electroshocking. Individuals > 250 mm in length were targeted in order to ensure they were 3 years of age or older. Fish were euthanized with FinquelTM (Argent Chemical Laboratories, Inc., Redmond, WA), weighed and measured. A necropsy-based assessment was completed on all fish collected to document grossly visible abnormalities. A variety of raised lesions were observed on body surfaces, fins and lips (Figure 4).

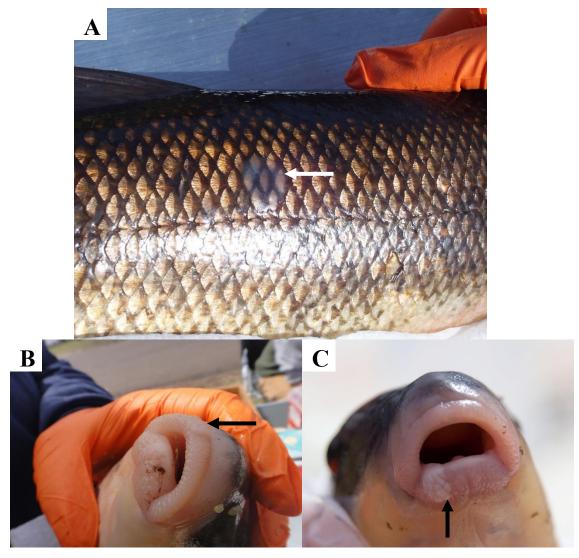


Figure 4. External skin and lip lesions observed on white sucker captured in the St. Louis River Area of Concern. A. Slightly raised mucoid lesion (arrow) on the lateral body surface. B and C. Raised lip lesions.

Pieces of any observable abnormalities were preserved in Z-fix (Anatech LtD, Battle Creek, MI) for subsequent histological analyses. At least five pieces of liver were placed in Z-fix for subsequent microscopic analyses. A section of dorsal muscle (approximately 1 square centimeter) was removed from the mid-body area above the lateral line for stable isotope analyses. Otoliths were removed from the inner ear for aging. In 2011 as part of a separate Great Lakes Restoration Initiative project blood samples as well as pieces of liver preserved in RNAlater solution (ThermoFisher Scientific Inc., Middletown, VA) for molecular analyses were collected from a subset of white sucker captured in St. Louis Bay. Reproductive

hormones and vitellogenin were measured in the plasma of these fish as previously described (Blazer et al. 2014a). Water and sediment chemical analyses were also available from 2011 sampling sites (Lee et al. 2012). In 2015, skin from five normal fish and five fish with raised skin abnormalities was wrapped in foil and frozen. Liver tissue was also collected and frozen. Once samples from individual fish were diagnosed microscopically, 20 normal livers and 20 livers with abnormalities were analyzed by AXYS Analytical Services LTD for PCB congeners, dioxins and furans (AXYS, 2011a, 2011b, 2011c).

Age was determined by reading the annual rings of the lapillus otoliths. Lapillus otoliths were prepared using a modification of the multiple-stage process described by Koch and Quist (2007). The caps of plastic 2.0-mL flat-top microcentrifuge tubes (Fisher Scientific, Pittsburgh, Pennsylvania) were filled with modeling clay and the tapered ends removed to create a cylinder. Single lapilli were placed into the clay such that the "thumb" of the otoliths was embedded into the clay. The vial was filled using the Epoxicure brand of resin and hardener (Buehler Inc., Lake Bluff, Illinois) and allowed to harden. The plastic case was removed and the otolith sectioned at 7.6 mm thickness using an Isomet low speed saw (Buehler Inc., Lake Bluff, Illinois). Sections were read under transmitted light using a stereo microscope. Region-specific median ages were estimated and regional differences were statistically analyzed for 609 white suckers (2011-2015 combined) using the Kruskal-Wallis test because the age data were not normally distributed.

The fixed tissue samples were trimmed into cassettes, routinely processed and embedded into paraffin. Blocks were sectioned at 6 µm and routinely stained with hematoxylin and eosin (H&E). Tissue sections of skin were examined microscopically for hyperplastic and neoplastic lesions as previously described (Blazer et al. 2007; 2009a; 2013). Non-neoplastic proliferative, presumptive preneoplastic and neoplastic changes in the liver (Blazer et al. 2006; 2009b; 2014) were documented. All histology slides were examined by two pathologists (Blazer and Walsh) and a subset was reviewed by Dr. John Fournie (retired U.S. Environmental Protection Agency) for quality assurance.

For stable isotope analysis, dorsal muscle tissue samples were dried (55 °C for 24 h), ground, and 0.7 mg packed into a tin capsule. Samples were analyzed using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (University of California-Davis Stable Isotope Facility). Stable isotope ratios were reported in δ notation in which δX : $\delta X = (R_{sample}/R_{standard} - 1) \times 10^3$, where X is the C or N stable isotope, *R* is the ratio of heavy:light stable isotopes, and Vienna Pee Dee Belemnite and air are the standards for δ^{13} C and δ^{15} N, respectively. The analytical error, the mean standard deviation (SD) of replicate laboratory reference material, was ± 0.1 ‰ for both δ^{13} C and δ^{15} N. The δ^{13} C values were corrected for lipid content because the molar C:N varied among individuals (range 3.1–6.7), indicating variable lipid content; we used the mass balance correction from Hoffman et al. (2015). 619 of the 622 fish collected were analyzed (one sample from each of St. Louis Bay, the Lower River, and the Upper River were not successfully analyzed).

To determine habitat usage based on stable isotope analysis, a C and N stable isotope mixing model was used to quantify the percentage of a white sucker's recent (1-2 years) diet that was derived from each of three areas: Lake Superior (reference), Superior Bay and St. Louis Bay combined, and the Lower River (above St. Louis Bay) (Figure 3). A proportional contribution (based on "source" isotopic signatures) to the fish's isotopic signature from each of the three geographic areas, or "sources" was calculated using the mixing model, which assumes mass balance (i.e. the proportions must add to 1; Phillips and Gregg 2001). As such, a fish with an isotopic signature that was exactly intermediate between the three sources would have a contribution of 0.33 from each area (i.e. it acquired one-third, or 33 % of its recent diet from each zone). To do so, the methods described in Blazer et al. (2014) were followed. In brief, we ran a mixing model for each white sucker sampled below Fond du Lac Dam (n = 465; the remaining 154 fish were sampled above the Fond du Lac Dam and presumed to be land-locked) and individual fish δ^{13} C and δ^{15} N values were fit to the model by constraining the model results (proportions) to between 0 and 1. Source stable isotope ratios for putative resident white sucker were as follows (Blazer et al. 2014): Lower River δ^{13} C -34.0% (SD ±1.9%), δ^{15} N 8.6% (SD ±1.3%); Superior Bay and St. Louis Bay δ^{13} C -23.5% (SD ±1.0%), δ^{15} N 12.0% (SD ±1.0%); and Lake Superior δ^{13} C -16.0% (SD ±1.0%),

 δ^{15} N 5.0% (SD ±1.0%). Where required, the mean absolute fit value for δ^{15} N was 0.6 % (maximum = 5.9%, n = 50), and for δ^{13} C was 1.8% (maximum = 3.1%, n = 3).

Logistic regression was used to test relationships between biological variables, habitat usage (i.e., amount of diet derived from a certain area based on stable isotope signatures), and prevalence of either skin or live neoplasia. The factors considered in both logistic regression models were sampling year, age, sex, % Lower River contributing to diet and % Lake Superior contributing to the diet (Blazer et al. 2014). The Lower River variable was included because this is the portion of the system in which two Superfund sites are located. The Lake Superior variable was included because a lake versus estuary distinction might be significant given the many sources of contamination to the SLRAOC. Sampling year was not a significant factor in either model, therefore, the data were re-analyzed excluding this variable. An α -level of 0.05 was used to indicate significance. A lack of significance for the habitat variables indicated that there was not a relationship between the habitat from which the white sucker had fed over the past one- to two-years and the prevalence of neoplasia.

Because age was a significant factor and habitat factors (% Lower River, % Lake Superior) were not significant for both neoplasia types, a logistic regression with two factors - age and habitat was used to test whether tumor prevalence varied between the SLRAOC below (pooling Superior Bay, St. Louis Bay, and the Lower River regions; n = 455) and above Fond du Lac dam (Upper River; n = 154).

II. Summary of Findings

Overall, in the three years of monitoring a total of 622 white sucker (309 females and 313 males) were examined. Morphometric and age comparisons among years, sex and sites are presented in Table 1. Fish ranged in age from 2 to 25 years. The mean age of fish collected in 2013 was somewhat lower than those collected in 2011 and 2015.

| | 9 | 0 1 1 | | T (1 () | XXX • 1 • () | |
|------------------|-------|-------------|---------------|------------------|----------------------|--|
| | Sex | Sample size | Age (years) | Length (mm) | Weight (gm) | |
| All sites (2011) | F | 94 | 7.9 ± 2.1 | 427.1 ± 46.7 | 923.4 ± 321.1 | |
| | Μ | 106 | 8.5 ± 2.7 | 408.4 ± 48.5 | 736.0 ± 185.8 | |
| Site Compar | rison | | | | | |
| Superior Bay | | 50 | 7.3 ± 2.3 | 395.8 ± 65.1 | 678.9 ± 209.1 | |
| St. Louis Bay | | 50 | 8.6 ± 2.5 | 412.2 ± 36.5 | 756.9 ± 212.8 | |
| Lower River | | 50 | 8.8 ± 2.8 | 430.0 ± 42.5 | 959.6 ± 334.6 | |
| Upper River | | 50 | 8.1 ± 1.9 | 430.8 ± 36.2 | 900.9 ± 231.5 | |
| | | | | | | |
| All sites (2013) | F | 90 | 6.6 ± 2.5 | 413.2 ± 58.0 | 749.2 ± 246.7 | |
| | Μ | 82 | 6.4 ± 3.2 | 379.9 ± 42.7 | 561.8 ± 177.9 | |
| Site Compar | rison | | | | | |
| Superior Bay | | 89 | 6.0 ± 1.9 | 380.8 ± 37.1 | 584.9 ± 189.7 | |
| Lower River | | 30 | 8.8 ± 4.3 | 440.3 ± 40.5 | 864.2 ± 233.2 | |
| Upper River | | 53 | 5.9 ± 2.4 | 404.6 ± 60.1 | 670.1 ± 239.5 | |
| | | | | | | |
| All sites (2015) | F | 125 | 7.6 ± 2.8 | 432.4 ± 49.6 | 974.3 ± 409.0 | |
| | Μ | 125 | 7.2 ± 2.5 | 399.9 ± 33.5 | 724.0 ± 188.4 | |
| Site Compar | rison | | | | | |
| Superior Bay | | 37 | 7.4 ± 1.8 | 397.7 ± 41.4 | 924.8 ± 484.1 | |
| St. Louis Bay | | 87 | 8.1 ± 2.8 | 417.4 ± 39.4 | 874.5 ± 348.1 | |
| Lower River | | 75 | 7.4 ± 2.6 | 422.1 ± 38.6 | 864.8 ± 267.1 | |
| Upper River | | 51 | 6.2 ± 2.5 | 396.5 ± 53.6 | 728.0 ± 278.8 | |

Table 1. Morphometric and age characteristics (mean \pm standard deviation) of whitesucker collected within the St. Louis River Area of Concern in 2011 - 2015

External raised lesions included raised reddened lesions, papillomatous lesions on the lips and slightly raised mucoid lesions on the body surface and fins (Figure 4).

Microscopically, the majority of the mucoid lesions were hyperplasia of epidermal cells. Raised lip lesions and some of the body and fins were papillomas or benign neoplasms of the epidermis (Figure 5).

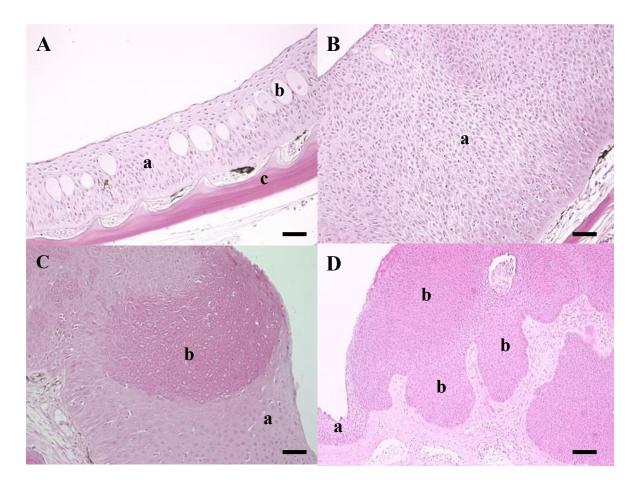


Figure 5. Microscopic pathology of raised skin lesions of white sucker captured within the St. Louis Area of Concern. A. Normal skin illustrating the epidermal epithelial cells (a) and club or alarm cells (b) within the epidermis and a scale (c) within the dermis of the skin. Scale bar equals 50 μ m. B. Microscopic appearance of a raised mucoid area with a proliferation of epithelial cells and a thickening of the epidermis (a). Scale bar equals 50 μ m. C. A raised mucoid area in with proliferating epithelial cells (a), some of which stain darker, are enlarged and have enlarged nuclei (b). Scale bar equals 50 μ m. D. A papilloma with a greatly thickened epidermis in relation to normal skin (a) with pegs of proliferating cells (b). Scale bar equals 120 μ m. Hematoxylin and eosin stain.

Liver lesions were primarily observed microscopically (other than the presence of parasites which could be seen grossly) and included inflammation and necrosis caused by helminth (cestode) parasites within the hepatic parenchyma, foci of cellular alteration, inflammation and fibrosis of bile ducts, the presence of a myxozoan parasite within the bile ducts and bile duct proliferation. Neoplastic lesions of the liver (Figure 6) were primarily bile duct neoplasms (cholangioma, cholangiocarcinoma) although hepatocellular adenomas were occasionally observed.

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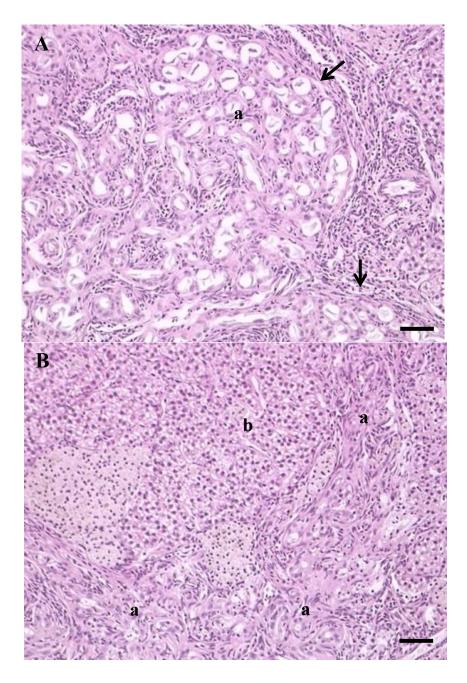


Figure 6. Neoplastic lesions observed in the liver of white sucker collected within the St. Louis River Area of Concern. A. Cholangioma with proliferating bile ducts (a) showing an encapsulation (arrows). B. Cholangiocarcinoma with proliferating ductal elements (a) extending into normal liver tissue (b). Scale bars equal 50 µm. Hematoxylin and eosin stain.

A. Summary 2011

The results of the 2011 white sucker collection have been published (Blazer et al. 2014). Briefly, 31% of the 200 white suckers collected in 2011 had some type of raised orocutaneous lesions, however only 4.5% had neoplastic lesions of the skin. All of the skin neoplasms were papillomas and were observed in fish 4 years and older (Appendix 1). The prevalence varied among sites with 0% of the fish collected in Superior Bay to 12% of those collected in St. Louis Bay (Table 2).

The prevalence of liver neoplasms observed in 2011 was 4.5%. All observed neoplasms were of bile duct origin and were observed in fish 6 years and older (Appendix 1). White sucker collected in the Lower River had a slightly higher prevalence (6.0%) than the other sites (4.0%) which were all the same (Table 2).

B. Summary 2013

In 2013, 172 white sucker were collected in three areas (Superior Bay, Lower River and Upper River) of the SLRAOC. Of the 11 raised lesions observed, six (3.5%) were papillomas (Table 2), the remaining five were hyperplastic lesions. Neoplastic orocutaneous lesions were observed in fish age 7 or older (Appendix 1). The lowest prevalence was observed in white sucker collected in Superior Bay (1.1%), the Upper River had an intermediate prevalence (3.8%), while those collected in the Lower River had the highest prevalence (10.0%).

The liver neoplasms were observed in 5.8% of the white sucker collected in 2013. One fish had both a cholangiocarcinoma and a hepatic cell adenoma, all others had only bile duct tumors. Liver neoplasms were only observed in white sucker age-7 and older (Appendix 1). There was a difference among sites with white sucker collected in the Upper River (1.9%) and Superior Bay (4.5%) having a lower prevalence than those collected in the Lower River (16.7%).

C. Summary 2015

In 2015, a total of 250 white suckers were collected within the four areas of the SLRAOC. Orocutaneous neoplasms were observed in 5.2% and all were papillomas observed in fish 6 years of age and older (Appendix 1). Fish collected in the Upper River had the lowest prevalence (2.0%), while those from the Lower River had the highest prevalence (8.0%).

All of the liver neoplasms (4.0%) observed were of bile duct origin, both cholangiomas and cholangiocarcinomas and were observed in fish 5 years and older. Superior Bay (0%) and Upper River (2.0%) sites had lower prevalence than the Lower River (6.7%) and St. Louis Bay (4.6%) (Table 2).

| | External O | bservations | Microsc | Microscopic Liver Lesions | | | |
|---------------|-----------------------|--------------|---------------|---------------------------|-----------|--|--|
| Site | External | Orocutaneous | Bile duct | Altered | Hepatic | | |
| | Raised Lesions | Neoplasms | Proliferation | Foci | Neoplasms | | |
| 2011 | | | | | | | |
| Superior Bay | 10.0 | 0 | 52.0 | 8.0 | 4.0 | | |
| St. Louis Bay | 44.0 | 12.0 | 40.0 | 6.0 | 4.0 | | |
| Lower River | 32.0 | 4.0 | 58.0 | 4.0 | 6.0 | | |
| Upper River | 38.0 | 2.0 | 46.0 | 0 | 4.0 | | |
| All sites | 31.0 | 4.5 | 49.0 | 3.5 | 4.5 | | |
| 2013 | | | | | | | |
| Superior Bay | 2.2 | 1.1 | 42.7 | 4.5 | 4.5 | | |
| Lower River | 10.0 | 10.0 | 73.5 | 3.3 | 16.7 | | |
| Upper River | 11.3 | 3.8 | 45.3 | 1.9 | 1.9 | | |
| All sites | 6.4 | 3.5 | 48.8 | 3.5 | 5.8 | | |
| 2015 | | | | | | | |
| Superior Bay | 10.8 | 5.4 | 48.6 | 2.7 | 0.0 | | |
| St. Louis Bay | 16.1 | 4.6 | 55.2 | 5.7 | 4.6 | | |
| Lower River | 14.7 | 8.0 | 64.0 | 0.0 | 6.7 | | |
| Upper River | 7.8 | 2.0 | 33.3 | 2.0 | 2.0 | | |
| All sites | 13.2 | 5.2 | 53.2 | 2.8 | 4.0 | | |

Table 2. Prevalence (percentage) of white sucker collected from the St. Louis Area ofConcern with selected microscopic lesions 2011 - 2015

D. Summary of Neoplastic Lesions 2011-2015

A total of 622 white sucker were surveyed from 2011 through 2015. 106 (17.0%) had raised orocutaneous lesions, however only 27 (4.3%) of these were neoplasms, all of which were papillomas. A total of 4.7% of the white suckers had liver neoplasms. In general, liver and skin neoplasms were highest in the Lower River and lowest in the Upper River (Table 3).

| within the St. Louis Area of Concern 2011 through 2015 | | | | | | | |
|--|--------|---------------|----------------|----------------|--|--|--|
| Site | Sample | Age (years) | Skin Neoplasm | Liver neoplasm | | | |
| | size | | # Observed (%) | # Observed (%) | | | |
| Superior Bay | 176 | 6.7±2.1 | 3 (1.7%) | 6 (3.4%) | | | |
| St. Louis Bay | 137 | 8.3±2.7 | 10 (7.3%) | 6 (4.4%) | | | |
| Lower River | 155 | 8.1±3.2 | 11 (7.1%) | 13 (8.4%) | | | |
| Upper River | 154 | 6.8 ± 2.5 | 3 (1.9%) | 4 (2.6%) | | | |
| All Sites | 622 | 7.4±2.7 | 27 (4.3%) | 29 (4.7%) | | | |

Table 3. Summary of age (mean, ±standard deviation) neoplasm prevalence at siteswithin the St. Louis Area of Concern 2011 through 2015

There were significant age differences between the river zones, as well (Kruskal-Wallis test statistic = 43.9, df = 3, p<0.001). The median age was highest in St. Louis Bay (8 years), lowest in Superior Bay (6 years), and the same between the Lower and Upper River (7 years). Thus, the zones with the lowest neoplasm prevalence (Superior Bay, Upper River) also had the youngest fish sampled.

E. Summary of Fish Habitat Usage 2011-2015

The δ^{13} C and δ^{15} N values of fish tissue samples spanned the range of values expected for fish moving between the SLRAOC below Fond du Lac Dam and Lake Superior (Figure 7). This included a few white sucker that had a stable isotope composition indicating exclusive reliance on one of three habitats – Lake Superior, Superior Bay and St. Louis Bay, and the Lower River (Figure 3). Most white sucker, however, had a stable isotope composition indicating they were feeding in a mix of zones. Within each of the regions sampled, white sucker exhibited a broad range of stable isotope ratios (Figure 7), indicating that the location where the white sucker was captured during the spawning run was not representative of the longer-term habitat from which the white sucker was feeding.

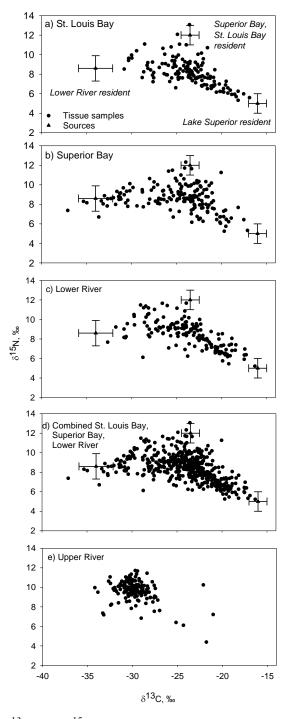


Figure 7. Distribution of $\delta^{13}C$ and $\delta^{15}N$ values in muscle samples of white sucker collected at the sampling areas and below Fond du Lac dam (where white sucker are migratory) combined. The source stable isotope ratios with error bars (±1 standard deviation) for putative resident white sucker are shown.

Most of the white sucker sampled below Fond du Lac Dam had a diet that was at least partially dependent on river areas within the SLRAOC, implying some recent exposure to SLRAOC

sediment contaminants. For the three areas, 17.2% of white sucker obtained >50% of their diet from the Lower River, 18.9% of white sucker obtained >50% of their diet from Superior Bay and St. Louis Bay, and 26.2% of white sucker obtained >50% of their diet from Lake Superior (Figure 8).

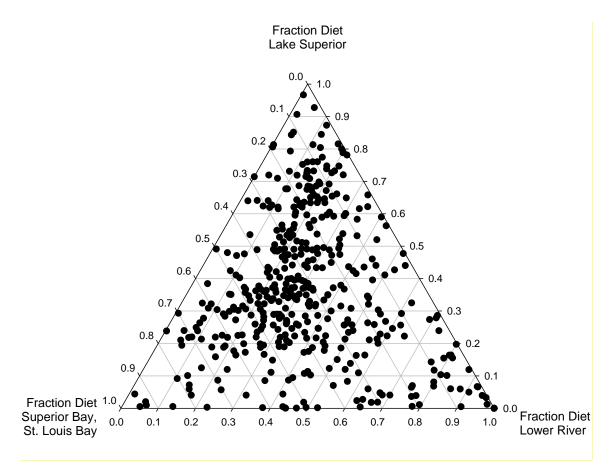


Figure 8. Diet estimates from the stable isotope mixing model for white sucker captured below Fond du Lac Dam, combined 2011, 2013 and 2015. Each point represents the estimate for an individual fish; white suckers in the upper triangle have a diet nearly 100% based on Lake Superior, those in the lower left triangle nearly 100% based on Superior Bay and St. Louis Bay, and those in the lower right triangle nearly 100% based on the Lower river.

We did find that habitat use and age were confounded; older white sucker had higher diet fractions from Lake Superior than younger white sucker (Figure 9).

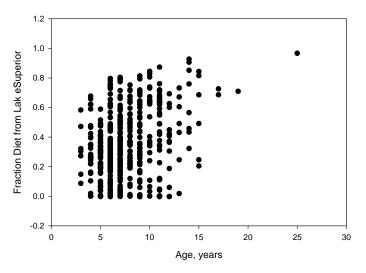


Figure 9. Fraction of diet from Lake Superior with respect to fish age, combined white sucker captured below Fond du Lac dam 2011-2015

F. Relationship Between Neoplasia Prevalence and Fish Habitat Usage 2011-2015

Sex and age were significantly associated with skin neoplasia prevalence, whereas neither sampling year nor either habitat variable (% Lake, % Lower River) were significant (Table 4). Excluding sampling year as a factor yielded a similar result (Table 5). With increasing age, white sucker were significantly more likely to have skin neoplasia, and female white sucker less likely than males. Because the diet contribution from Lake Superior also increased with age (Fig. 9), indicating age and habitat use was confounded, we calculated the average diet fraction among white sucker ages 5-11 to compare fish with and without skin neoplasia. This was an age range in which neoplasia were present but before we captured fish that demonstrated very high (>0.90) Lake Superior diet fraction (Fig. 9). Among fish ages 5-11, diet fraction was similar among white sucker with and without skin neoplasia, indicating there was not an apparent habitat effect. For fish with skin neoplasia (n = 15), the average diet fractions were 0.28 for the Upper River, 0.38 for the Lower River, and 0.35 for Lake Superior, whereas they were 0.31, 0.33 and 0.35, respectively, for white sucker without skin neoplasia (n = 365).

| Parameter | Estimate (±SE) | Z | p-value |
|---------------|----------------|-------|---------|
| Constant | 4.08 (0.99) | 4.13 | 0.000 |
| Year (2011) | 0.35 (0.52) | 0.68 | 0.496 |
| Year (2013) | 0.40 (0.64) | 0.62 | 0.533 |
| Sex (F) | 1.36 (0.57) | 2.38 | 0.017 |
| Age | -0.25 (0.07) | -3.55 | 0.000 |
| % Lake | 0.80 (1.24) | 0.65 | 0.517 |
| % Lower River | 0.18 (1.46) | 0.12 | 0.904 |

Table 4. Logistic regression model of skin neoplasia prevalence, including parameter estimates, Z scores, and associated p-values (p < 0.05 in bold). The full model p = 0.002.

Table 5. Logistic regression model of skin neoplasia prevalence excluding sampling year as a factor, including parameter estimates, Z scores, and associated p-values (p < 0.05 in bold). The full model p = 0.001.

| Parameter | Estimate (±SE) | Z | p-value |
|---------------|----------------|-------|---------|
| Constant | 4.37 (0.92) | 4.77 | 0.000 |
| Sex (F) | 1.34 (0.57) | 2.34 | 0.019 |
| Age | -0.24 (0.07) | -3.40 | 0.000 |
| % Lake | 0.66 (1.20) | 0.55 | 0.581 |
| % Lower River | -0.04 (1.37) | -0.03 | 0.975 |

Only age was significantly associated with liver neoplasia prevalence (Table 6). Excluding sampling year as a factor yielded a similar result (Table 7). As with skin neoplasia, older white sucker were significantly more likely to have liver neoplasia than younger white sucker. As before, average diet fraction was calculated among young white sucker ages 5-11 with and without liver neoplasia. Among fish with age estimates, diet fraction was similar among white sucker with and without liver neoplasia. For fish with neoplasia (n = 17), the average diet fractions were 0.29 for the Upper River, 0.34 for the Lower River, and 0.37 for Lake Superior, whereas they were 0.31, 0.33 and 0.35, respectively, for white sucker without liver neoplasia (n = 363).

| Parameter | Estimate (±SE) | Z | p-value |
|---------------|----------------|-------|---------|
| Constant | 4.48 (1.00) | 4.49 | 0.000 |
| Year (2011) | 0.34 (0.57) | 0.59 | 0.553 |
| Year (2013) | -0.81 (0.51) | -1.6 | 0.110 |
| Sex (F) | 0.44 (0.46) | 0.95 | 0.341 |
| Age | -0.22 (0.07) | -3.11 | 0.002 |
| % Lake | 0.18 (1.18) | 0.15 | 0.881 |
| % Lower River | 0.36 (1.38) | 0.26 | 0.795 |

Table 6. Logistic regression model of liver neoplasia prevalence, including parameter estimates, Z scores, and associated p-values (p < 0.05 in bold). The full model p = 0.019.

Table 7. Logistic regression model of liver neoplasia prevalence excluding sampling year as a factor, including parameter estimates, Z scores, and associated p-values (p < 0.05 in bold). The full model p = 0.027.

| | Estimate | | |
|---------------|--------------|-------|---------|
| Parameter | (±SE) | Z | p-value |
| Constant | 4.44 (0.90) | 4.92 | 0.000 |
| Sex (F) | 0.40 (0.46) | 0.87 | 0.385 |
| Age | -0.20 (0.07) | -2.98 | 0.003 |
| % Lake | -0.05 (1.17) | -0.04 | 0.969 |
| % Lower River | -0.09 (1.35) | -0.07 | 0.946 |

Because habitat usage (i.e., % Lake, % Lower River) was not a significant factor for neoplasia prevalence in fish captured in the SLRAOC below the Fond du Lac Dam, neoplasia prevalence was compared between fish captured throughout the area below the Fond du Lac Dam (Lower River, St. Louis Bay, Superior Bay) and fish captured above the Fond du Lac Dam (Upper River). Although both areas are within the AOC boundaries, sediment contaminants are less prevalent above Fond du Lac dam than below the dam. Where age was a significant factor for both skin and liver neoplasia prevalence, the SLRAOC area was not (Tables 8 and 9). Thus, there was no significant difference in neoplasia prevalence between the regions above and below Fond du Lac dam.

Table 8. Logistic regression model of skin neoplasia prevalence with both fish age and SLRAOC area (Superior Bay, St. Louis Bay, and Lower River combined versus Upper River) as factors, with parameter estimates, Z scores, and associated p-values (p < 0.05 in bold). The full model p = 0.002,

| Parameter | Estimate (±SE) | Z | p-value |
|-----------------|----------------|-------|---------|
| Constant | -5.01 (0.68) | -7.36 | 0.000 |
| Age | 0.19 (0.06) | -3.31 | 0.001 |
| Area (Combined) | 0.54 (0.55) | 0.98 | 0.330 |

Table 9. Logistic regression model of liver neoplasia prevalence with both fish age and SLRAOC area (Superior Bay, St. Louis Bay, and Lower River pooled versus Upper River) as factors, with parameter estimates, Z scores, and associated p-values (p < 0.05 in bold). The full model p < 0.001.

| Parameter | Estimate (±SE) | Z | p-value |
|-----------------|----------------|-------|---------|
| Constant | -5.29 (0.70) | -7.58 | 0.000 |
| Age | 0.22 (0.06) | 3.83 | 0.000 |
| Area (Combined) | 0.45 (0.56) | 0.81 | 0.417 |

G. Comparison to Other Areas of Concern

Although not required by the SLRAOC BUI removal strategy, comparing prevalence results from the SLRAOC to other Great Lakes AOCs provides a basin-wide context. Based on the binary logistic regression results (Tables 8, 9), St. Louis River white sucker have a skin neoplasia prevalence of 2.5% at age-7, and a liver neoplasia prevalence of 2.3% age age-7. In comparison, the liver and skin neoplasia prevalence reported by Rutter (2010) for age-7 brown bullhead from the least-impacted potential reference site (Long Point Inner Bay) for Presque Isle Bay AOC was 1.2% and 6.4%, respectively. Using identical methods to this study, white sucker from two other Wisconsin AOCs (Sheboygan River and Milwaukee Estuary) and the Kewaunee River as a "least impacted site" were sampled in 2011 through 2013 (Blazer et al. In Press). The prevalence of skin tumors was higher (p < 0.0001) at the Kewaunee River (21.0%) than at the SLRAOC (4.3%), while there was no significant difference (p = 0.5570) between the sites with respect to liver neoplasm prevalence (Table 10). Both Kewaunee and St. Louis River had significantly lower liver tumor prevalence when compared to the Sheboygan and Milwaukee AOCs (Table 10). Interestingly, at both the Milwaukee and Sheboygan numerous large external body surface tumors were observed and many of these were squamous cell carcinomas (malignant) which were not observed at St. Louis River or Kewaunee. Liver neoplasms at Milwaukee and Sheboygan included hepatocellular carcinomas not observed in white suckers from the SLRAOC.

| SILE | | | | |
|-----------------|-------------|---------------|----------------|-----------------|
| Area of Concern | Sample size | Age | Skin Neoplasms | Liver neoplasms |
| Milwaukee | 200 | 10.2 ± 3.0 | 48.0 % | 15.0 % |
| Sheboygan | 192 | 10.2 ± 4.4 | 32.6 % | 8.3 % |
| St. Louis River | 622 | 7.4 ± 2.7 | 4.3 % | 4.7% |
| Reference | | | | |
| Kewaunee | 200 | 8.9 ± 3.0 | 21.0 % | 3.5% |
| Reference | 0 | | | |

Table 10. Comparison of neoplasm prevalence at WI/MN Areas of Concern and a reference site

H. Potential Risk Factors

PCBs, Dioxins and Furans

In 2015, pieces of liver and skin were analyzed for PCB congeners, as well as dioxins and furans. It was initially thought that a comparison of chemical concentrations in tissues with and without tumors or potentially preneoplastic changes may provide some evidence for their role in the initiation or tumor promotion. However, the low number of individuals with actual neoplasms necessitated analyzing tissue from white suckers captured in different locations. The full data set is presented in Appendix 2. A number of flags were placed on the data by the contract laboratory: B = analyte found in associated blank and concentration in sample was less than 10X the blank concentration; J = concentration less than lowest calibration equivalent; R = peak detected but did not meet quantification criteria; and U = not detected at the reporting limit. Values with any of these flags were not used in comparisons presented below.

The PCB tissue concentrations were generally lower in the Upper River and Superior Bay, which may be a function of location or age. The mean liver PCB concentrations was significantly lower (p = 0.0109) in fish collected in the Upper River when compared to fish collected in St. Louis Bay. Because of this difference among SLRAOC river areas and the variability (Tables 11 and 12), there were not sufficient sample sizes of tumor and normal

fish to actually compare the relationship between PCB tissue concentrations and neoplasm presence.

| Fish # | 238 | 301 | 302 | 201 | 353 | 240 | 244 | 264 | 221 | 376 |
|------------------------|---------|---------|---------|---------|--------|---------|------------------|---------|---------|--------|
| Age | 5 | 7 | 8 | 6 | 4 | 12 | 15 | 9 | 13 | 9 |
| Type Skin | Normal | Normal | Normal | Normal | Normal | Raised | Pap ³ | Mucoid | Рар | Mucoid |
| Site ¹ | StL Bay | LR | LR | Sup Bay | UR | StL Bay | StL Bay | StL Bay | Sup Bay | UR |
| Total PCB ² | 362,420 | 229,998 | 230,255 | 48,927 | 2,751 | 172,822 | 270,298 | 43,415 | 60,003 | 3,875 |
| Total Tetra-dioxins | 1.01 | 0.13 | 0.39 | 0.18 | BD^4 | 0.28 | 0.19 | BD | 0.33 | BD |
| Total Penta-dioxins | 0.73 | 0.17 | 0.32 | 0.14 | BD | BD | BD | BD | 0.09 | BD |
| Total Hexa-dioxins | 2.14 | 0.14 | 0.31 | 0.17 | BD | 0.38 | 0.19 | BD | BD | BD |
| Total Hepta-dioxins | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD |
| Total Tetra-furans | 5.53 | 0.87 | 1.56 | 0.82 | 0.13 | 1.24 | 1.16 | 0.34 | 0.71 | 0.14 |
| Total Penta-furans | 3.29 | 0.36 | 0.91 | 0.42 | 0.13 | 0.93 | 0.52 | BD | 0.72 | BD |
| Total Hexa-furans | 5.05 | 0.41 | 1.17 | 0.25 | 0.07 | 1.32 | 0.63 | 0.23 | 0.39 | BD |
| Total Hepta-furans | 1.42 | 0.13 | 0.11 | 0.09 | BD | 0.60 | 0.43 | BD | 0.10 | BD |

Table 11. Total PCB, Dioxin and Furan concentrations in skin of white suckercollected within the St. Louis River Area of Concern 2015

¹Sites are St. Louis Bay (StL Bay), Superior Bay (Sup Bay), lower river (LR) and upper river (UR).

²Total PCBs, total dioxin groups and total furan groups are in pg/g tissue wet weight.

³Papilloma (Pap)

BD = below detection or any flagged values.

| Fish # | 257 4 | 263 4 | 271 5 | 289 | 210 | 212 4 | 230 6 | 360 4 | 361 4 | 369 4 |
|----------------------------------|-------------|-------------|----------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
| Age Liver | 4 Normal | 4 Normal | Normal | 6 Normal | 6 Normal | 4 Normal | o Normal | 4 Normal | 4 Normal | 4 Normal |
| Site ¹ | StL Bay | StL Bay | StL Bay | StL Bay | Sup Bay | Sup Bay | Sup Bay | UR | UR | UR |
| Total PCB ² | 225,204 | 27,191 | 329,296 | 35,144 | 8,008 | 43,735 | 256,257 | 2,345 | 3,717 | 7,443 |
| Total Tetra-dioxins | BD^4 | BD | BD | BD | BD | BD | 0.65 | BD | BD | BD |
| Total Penta-dioxins | BD | 0.37 | 1.07 | BD |
| Total Hexa-dioxins | 0.65 | BD | 1.18 | 0.20 | BD | 0.28 | 1.04 | BD | BD | BD |
| Total Hepta-dioxins | 1.14 | 0.50 | 3.12 | BD | 2.06 | 1.66 | 1.49 | BD | BD | BD |
| Total Tetra-furans | 1.64 | 0.74 | 3.96 | BD | 1.62 | 0.77 | 3.39 | 0.16 | BD | BD |
| Total Penta-furans | 1.66 | BD | BD | 0.58 | BD | BD | 0.75 | 0.16 | BD | 0.34 |
| Total Hexa-furans | 1.87 | BD | BD | 0.72 | 2.05 | 2.27 | BD | BD | BD | BD |
| Total Hepta-furans | BD | BD | 1.44 | BD | 1.21 | 1.03 | 1.10 | BD | BD | BD |
| | | | | | | | | | | |
| Fish # | 240 | 283 | 405 | 324 | <i>438</i> | 442 | 445 | 449 | 353 | 368 |
| Age | 12 | 7 | 5 | 11 | 5 | 12 | 6 | 6 | 4 | 9 |
| Liver Lesio ³ | СО | CC | CC | bd prol | CC | СО | CC | CC | CC | bd prol |
| Site | StL Bay | StL Bay | StL Bay | LR | LR | LR | LR | LR | UR | UR |
| Total PCB | 111,131 | 316,501 | 263,907 | 149,703 | 338,520 | 1,187,477 | 67,077 | 91,922 | 7,144 | 6,576 |
| Total Tetra-dioxins ³ | BD | 1.06 | 0.56 | 0.47 | 0.78 | 1.25 | BD | BD | BD | 0.14 |
| Total Penta-dioxins | 0.32 | BD | 0.62 | 0.71 | 0.55 | 2.14 | 0.70 | 0.40 | BD | 0.49 |
| Total Hexa-dioxins | BD | BD | 0.61 | 0.41 | 1.08 | 3.94 | BD | 0.22 | BD | 0.22 |
| Total Hepta-dioxins | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD |
| Total Tetra-furans ⁶ | 0.94 | 1.50 | 2.01 | 1.42 | 1.97 | 3.83 | 2.01 | 1.75 | BD | 0.56 |
| Total Penta-furans | 0.35 | BD | 1.03 | 1.80 | 1.67 | 3.19 | 1.05 | 0.25 | BD | 0.81 |
| Total Hexa-furans | 1.19 | 1.33 | 1.36 | 1.11 | 4.32 | 8.23 | BD | 0.60 | BD | 0.15 |
| Total Hepta-furans | 0.47 | 0.31 | 0.86 | 0.34 | 0.39 | 2.80 | BD | 0.25 | BD | BD |

Table 12. Total PCB, Dioxin and Furan concentrations in liver of white suckercollected within the St. Louis River Area of Concern 2015

¹Sites are St. Louis Bay (StL Bay), Superior Bay (Sup Bay), lower river (LR) and upper river (UR).

²Total PCBs, total dioxin groups and total furan groups are in pg/g tissue wet weight

 $^{3}CO =$ cholangioma; CC = cholangiocarcinoma; bd prol = bile duct proliferation.

 ${}^{4}\text{BD}$ = below detection or any flagged values.

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Low concentration of dioxins and furans were measured in skin and liver tissue. There did not appear to be any patterns among sites or between fish with and without neoplasms. The number of flagged results made statistical comparison difficult. For skin samples, if the flagged results were removed, there was no significant difference between the two groups.

Biological Agents

Neoplasia is most often a multifactorial disease which may involve initiators and promoters of abnormal cell proliferation. In other animals, including humans, viruses and parasites have been widely recognized as risk factors for carcinogenesis. Chronic infections of Hepatitis B (Asia and developing countries) and C (United States) viruses are one of the most common risk factors for hepatocellular carcinoma. During liver transcriptome analyses of fishes at Great Lakes AOCs, including the St. Louis River (Hahn et al. 2016), a novel Hepatitis B virus was identified in white sucker (Hahn et al. 2015). Although the presence of the virus was not correlated with observed liver tumors or other lesions, more research is necessary to determine any possible role it may play in carcinogenesis in fish.

Major risk factors for cholangiocarcinoma in humans are the trematodes *Opisthorchis* and *Clonorchis*, primarily in East Asia and Eastern Europe where uncooked fish are part of the diet. The adult worms reside in the bile ducts and mechanical damage, oxidative DNA damage and excretory/secretory products of the parasites that induce cell proliferation have all been linked with the carcinogenesis (Sripa et al. 2005).

III. Findings and Conclusions

The overall prevalence of both skin and liver neoplasms was below 5% in white suckers collected throughout the SLRAOC. For white sucker, a benthic consumer, diet is a substantial pathway for exposure to sediment contaminants in the SLRAOC. Neither the relative amount of diet obtained from Lake Superior (the reference location) nor the Lower River, however, was a significant factor associated with tumor prevalence. That is, increasing feeding in the SLRAOC (versus Lake Superior) was not associated with an increased prevalence of neoplasia. The results should be interpreted with due caution, however, as stable isotope ratios are a mid-term (1-2 years of exposure via diet) biomarker of habitat use. In contrast, age and sex were significant

factors associated with skin neoplasia, and age was a significant factor for liver neoplasia. Also, we found that age and diet source were confounded in the white sucker captured below Fond du Lac dam, where they are potentially migratory; for these white sucker, the fraction of Lake Superior diet increased with age. Examining only white sucker ages 5-11 to reduce this confounding relationship, we found diet fractions by regions were similar among white sucker with and without skin or liver neoplasia. Further, there was not a significant difference in skin or liver neoplasia prevalence between migratory white sucker captured below the dam and land-locked white sucker captured above Fond du Lac Dam.

Interestingly, there were differences among the various areas sampled. The tumor prevalence for white sucker captured in Superior Bay and the Upper River was lower than for white sucker captured in St. Louis Bay and the Lower River (Table 3). Consistent with the logistic regression model results, these regional differences in tumor prevalence were also associated with age differences, such that those regions in which white sucker had higher tumor prevalence were also those regions in which older white sucker were captured. An age-effect notwithstanding, the higher neoplasm rates together with the higher PCBs concentrations in tissues of white sucker captured in these two areas of the SLRAOC do suggest a role for contaminants in carcinogenesis. Ultimately, the causal relationships between ontogenetic development, age, contaminant exposure, and the presence of other initiators or promoters is not well-understood. While PCBs may play a role, other co-occurring compounds may also be important as either initiators or promoters. Additionally, it is possible white sucker are exposed to initiators of proliferative responses early in life and either annual sporadic exposure during migrations to spawning habitat or continued exposure in feeding habitats may eventually induce actual neoplasia.

A formal comparison to neoplasia prevalence from other AOCs is not required to meet the BUI removal target. Moreover, comparisons to other AOCs are challenged by our lack of understanding of the variables most relevant to initiating or promoting neoplasia. This knowledge gap notwithstanding, we found that prevalence of contaminant-related internal and external tumors and deformities were similar to or less than found in resident, benthic fish species sampled from unimpaired areas elsewhere in the Great Lakes Basin (Mountain Bay, Long Point Inner Bay, Kewaunee River). Age is a variable that can be considered. Controlling

for age, neoplasia prevalence in age-7 white sucker from the SLRAOC was low (<2.5%), which was also similar to or less than neoplasia prevalence at sites where the data are available (Rutter 2010).

References

- AXYS. January 2011a. AXYS Method MLA-017: Analytical Method for the Determination of Polychlorinated Dibenzodioxins and Dibenzofurans". AXYS Analytical Services Ltd., Sidney, BC, AXYS Method MLA-017, Rev 20.09. <u>http://www.axysanalytical.com</u>.
- AXYS. June 2011b. Analytical Method for the Determination of: Polybrominated Diphenyl Ethers, Polychlorinated Biphenyl Congeners, Chlorinated Pesticides, Technical Toxaphene, Toxaphene Congeners/Parlars and Polychlorinated Dibenzodioxins and Dibenzofurans Using Co-extraction Techniques". AXYS Analytical Services Ltd., Sidney, BC. AXYS Method MLA-013, Rev 09.05. <u>http://www.axysanalytical.com</u>.
- AXYS. October 2011c. Analytical Method for the Determination of 209 PCB Congeners by EPA Method 1668A, EPA Method 1668C or EPA Method CBC01.2". AXYS Analytical Services Ltd., Sidney, BC. AXYS Method MLA-010, Rev 11.06. http://www.axysanalytical.com.
- Baumann, P.C., I.R. Smith and C.D. Metcalfe. 1996. Linkages between chemical contaminants and tumors in benthic Great Lakes fish. Journal of Great Lakes Research 22:131-152.
- Blazer, V.S., J.W. Fournie, J.C. Wolf and M.J. Wolfe. 2006. Diagnostic criteria for proliferative liver lesions in the brown bullhead (*Ameiurus nebulosus*). Diseases of Aquatic Organisms 72:19-30.
- Blazer, V.S., J.W. Fournie, J.C. Wolf and M.J. Wolfe. 2007. Manual for the microscopic diagnosis of proliferative liver and skin lesions in the brown bullhead (*Ameiurus nebulosus*). Available at <u>http://seagrant.psu.edu/publications/technicaldocs/</u>histofieldmanual.pdf
- Blazer, V. S., J. Hoffman, H. L. Walsh, R. P. Braham, C. Hahn, P. Collins, Z. Jorgenson and T. Ledder. 2014. Health of white sucker within the St. Louis river Area of Concern associated with habitat usage as assessed using stable isotopes. Ecotoxicology, 23:236-251.
- Blazer, V.S., P.M. Mazik, L.R. Iwanowicz, R. Braham, C. Hahn, H.L. Walsh and A. Sperry. 2014. Monitoring of wild fish health at selected sites in the Great Lakes basin – methods and preliminary results. U.S. Geological Survey Open-File Report 2014-1027.
- Blazer, V.S., S.D. Rafferty, P.C. Baumman, S.B. Smith and E.C. Obert. 2009a. Assessment of the "tumors or other deformities" beneficial use impairment in brown bullhead: I. Orocutaneous tumors. Journal of Great Lakes Research 35:517-526.
- Blazer, V.S., S.D. Rafferty, P.C. Baumman, S.B. Smith and E.C. Obert. 2009b. Assessment of the "tumors and other deformities" beneficial use impairment in brown bullhead: II. Liver tumors. Journal of Great Lakes Research 35:527-537.
- Blazer, V.S., H.L. Walsh, R.P. Braham, C.M. Hahn, P. Mazik and P.B. McIntyre. 2016. Tumours in white suckers from Lake Michigan tributaries: pathology and prevalence. Journal of Fish Diseases: In Press

- Hahn, C.M., L.R. Iwanowicz, R.S. Cornman, C.M. Conway, J.R. Winton and V.S. Blazer. 2015. Characterization of a novel Hepadnavirus in white sucker (*Catostomus commersonii*) from the Great Lakes region of the USA. Journal of Virology 89:11801-11811.
- Hahn, C.M., L.R. Iwanowicz, R.S. Corman, P.M. Mazik and V.S. Blazer. 2016. Transcriptome discovery in non-model wild fish species for the development of quanitative transcript abundance assays. Comparative Biochemistry and Physiology Part D: Genomics and Proteomics 20:27-40.
- Hoffman, J.C. 2016. Tracing origin, migration and other movements of fish using stable isotopes, pp. 169-196 *In* Morais, P., and Daverat, F. (eds.), An Introduction to Fish Migration, CRC Press.
- Hoffman, J.C., A.M. Cotter, G.S. Peterson, and J.R. Kelly. 2010. Using stable isotope mixing in a Great Lakes coastal tributary to determine food web linkages in young fishes. Estuaries and Coasts. 33(6):1391-1405.
- Hoffman, J.C., M.E. Sierszen, and A.M. Cotter. 2015. Fish tissue lipid-C:N relationships for correcting δ^{13} C values and estimating lipid content in aquatic food web studies. Rapid Communications in Mass Spectrometry 29:2069-2077.
- Koch, J. D., and M. C. Quist. 2007. A technique for preparing fin rays and spines for age and growth analysis. North American Journal of Fisheries Management 27:782-784.
- Lee, K.E., S.K. Langer, M.A. Menheer, W.T. Foreman, E.T. Furlong and S.G. Smith. 2012. Chemicals of emerging concern in water and bottom sediment in Great Lakes areas of concern, 2010 to 2011 – Collection methods, analysesmethods, quality assurance and data. U.S. Geological Survey Data Series 723, 26 p.
- Mahmood, M., E.A. Blukacz-Richards, P.C. Baumann, M. McMaster, M. Hossain and G.B. Arhonditsis. 2014. A Bayesian methodological framework for setting fish tumor occurrence delisting criteria: A case study in St. Marys River Area of Concern. Journal of Great Lakes Research 40:88-101.
- Minnesota Pollution Control Agency. St. Louis River Area of Concern Implementation Framework: Roadmap to Delisting (Remedial Action Plan Update), By LimnoTech. St. Paul, Minnesota. July 15, 2013. (<u>http://www.pca.state.mn.us/index.php/view-document.html?gid=19677</u>)
- Wisconsin Department of Natural Resources and Minnesota Pollution Control Agency and Wisconsin Department of Natural Resources. St. Louis River Area of Concern Remedial Action Plan Annual Update. 2015. http://dnr.wi.gov/topic/greatlakes/st.louis.html
- Rafferty S.D., V.S. Blazer, A.E. Pinkney, J.L. Grazio, E.C. Obert and L. Boughton. 2009. A historic perspective on the "fish tumors or other deformities" beneficial use impairment at Great Lakes areas of concern. Journal of Great Lake Research 35:496-506.

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- Rutter, M.A. 2010. A statistical approach for establishing tumor incidence delisting criteria in areas of concern: a case study. Journal of Great Lakes Research 36:646-655.
- Vander Zanden, M.J., and J.B. Rasmussen. 2001. Variation in δ^{15} N and δ^{13} C trophic fractionation: implications for aquatic food web studies. Limnology and Oceanography 46: 2061–2066.

Appendix 2- Public input materials

- 1. GovDelivery bulletin
- 2. MPCA Press Release
- 3. Stakeholder invite email
- 4. Comment Form
- 5. Fact Sheet

WDNR GovDelivery bulletin, September 21, 2018 (3,381 recipients):

Research confirming that white sucker fish in the St. Louis River Area of Concern have a low rate of fish tumors and deformities is leading to a request by the Wisconsin Department of Natural Resources and Minnesota Pollution Control Agency to remove an impairment.

Fish were sampled in 2011, 2013 and 2015 and research was conducted to determine if fish tumors and deformities were more common in the St. Louis River Area of Concern. Multiple lines of evidence verified that the tumor incidence rate was not significantly different between the river and Lake Superior. Further, the St. Louis River AOC tumor rates were lower than other similarly studied AOC and non-AOC sites in the Great Lakes. The age and gender of the fish were found to be more important factors for fish tumor development.

Because of these findings, the Fish Tumors and Deformities Beneficial Use Impairment is proposed for removal. The removal recommendation and study are available <u>online</u> for public review and comment from September 21 through October 12, 2018.

To learn more about the proposal and to provide comments, please see the following documents:

- BUI Removal Recommendation for Fish Tumors and Other Deformities
- <u>Public Comment Form</u>
- <u>Fact Sheet</u>.

Comments can be submitted to Matt Steiger, Wisconsin DNR AOC Coordinator, at <u>Matthew.Steiger@wisconsin.gov</u> until 5:00 pm October 12, 2018.

Paper copies of the draft document are also available for review in the Superior Public Library at 1530 Tower Ave. Superior, WI and the Duluth Public Library at 520 W. Superior St. Duluth, MN. Public comment forms are included and formatted for mailing to Matt Steiger, Wisconsin DNR, 1701 N. 4th St, Superior, WI 54880. Comment sheets may also be scanned and emailed to Steiger.

Written and electronic comments sent or post-marked before 5:00 pm on October 12, 2018, will be considered. A final removal recommendation will be submitted to the U.S. Environmental Protection Agency after public input has been reviewed and considered.

General questions about the removal proposal may be directed to Matt Steiger at 715-395-6904 or Barb Huberty, Minnesota Pollution Control Agency AOC Coordinator, at 218-302-6630.

The St. Louis River Area of Concern will have seven remaining Beneficial Use Impairments to address before the entire Area of Concern can be delisted. The St. Louis River Area of Concern is one of forty-three Areas of Concern designated in 1987 as the most highly contaminated areas in the Great Lakes.

This work was included in the St. Louis River Area of Concern Remedial Action Plan and was funded primarily by the federal Great Lakes Restoration Initiative. The primary partners

involved in this work included the Environmental Protection Agency, Wisconsin Department of Natural Resources, Minnesota Department of Natural Resources, Minnesota Pollution Control Agency, and the Fond du Lac Band of Lake Superior Chippewa.

MPCA Press release:

Having trouble viewing this message? View it as a webpage.



For release: Sept. 26, 2018 Contact: Steve Mikkelson, 218-316-3887

Open for comment: Proposal to remove the fish impairment from the St Louis River Area of Concern

Recent research in the St. Louis River estuary shows that white sucker fish have a low rate of fish tumors and deformities. Previous observations led authorities to believe the fish were at risk, and to consider the estuary "impaired." (A body of water is considered "impaired" if it fails to meet one or more water quality standards.) However, in response to the recent research, the Wisconsin Department of Natural Resources (WDNR) and Minnesota Pollution Control Agency (MPCA) are requesting that the St. Louis River no longer be listed as impaired for fish tumors and deformities.

Fish sampled in 2011, 2013, and 2015 were studied to determine if fish tumors and deformities were more common in the St. Louis River Area of Concern (AOC) than elsewhere. (The St. Louis River AOC is one of 43 such areas designated by the EPA in 1987 as the most highly contaminated in the Great Lakes.) The research showed that the occurrence of tumors in white sucker fish was not significantly different between the river and Lake Superior, and lower than in other sites in the Great Lakes. The age and gender of the fish were found to be more important factors for fish tumor development.

Because of these findings, the MPCA and the WDNR proposed that the fish tumors and deformities impairment be removed for the St. Louis River AOC. The removal proposal and study are available on the <u>WDNR website</u> for review. See a fact sheet and comment form at: <u>http://dnr.wi.gov/topic/greatlakes/st.louis.html</u>

Submit comments to Matt Steiger, Wisconsin DNR, AOC Coordinator, 1701 N 4th St, Superior, WI 54880 or at <u>Matthew.Steiger@wisconsin.gov</u> by 5:00 p.m., Oct. 12, 2018.

Paper copies of the draft document are available for review at the Superior Public Library at 1530 Tower Ave., and the Duluth Public Library at 520 W. Superior St. Paper comment sheets are included and pre-formatted for mailing.

A final removal recommendation will be submitted to the U. S. EPA after public input has been reviewed and considered. General questions about the removal proposal can be directed to Matt Steiger at 715-395-6904, or Barb Huberty, MPCA AOC Coordinator, at 218-302-6630 or by email at <u>barbara.huberty@state.mn.us</u>.

The St. Louis River AOC will have seven remaining impairments to address before the entire St Louis River AOC can be delisted. Some species of fish in the river still contain high levels of pollutants such as mercury and polychlorinated biphenyls or PCBs.

This work was included in the St. Louis River AOC Remedial Action Plan and funded primarily by the federal Great Lakes Restoration Initiative. The primary partners involved in this work included the U.S. EPA, WDNR, Minnesota Department of Natural Resources, MPCA, and the Fond du Lac band of Lake Superior Chippewa.

Broadcast version

Recent research in the Saint Louis River estuary shows that white sucker fish have a low rate of fish tumors and deformities. Previous observations led authorities to believe the fish were at risk, and to consider the estuary "impaired." A body of water is considered "impaired" if it fails to meet one or more water quality standards.

However, in response to the recent research, the Wisconsin Department of Natural Resources and Minnesota Pollution Control Agency are requesting that the St. Louis River no longer be listed as impaired for fish tumors and deformities. The public is invited to comment on this proposal through October 12. More information is available on the Wisconsin DNR's website.

###

The mission of the MPCA is to protect and improve the environment and human health.

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Steiger, Matthew B - DNR

| From: Sent: | Steiger, Matthew B - DNR Friday, September 21, 2018 1:18 PM |
|----------------|---|
| то: | Steiger, Matthew B - DNR |
| | Steller, Matthew B - DNK St. Louis River Area of Concern Public input opportunity: Draft Fish Tumors and Deformities |
| Subject: | impairment removal open until October 12, 2018 |

Importance: High

Good afternoon St. Louis River Area of Concern Partners and Stakeholders,

The St. Louis River Area of Concern agencies are pleased to announce a public input opportunity on the recommendation to remove the Fish Tumors and Other Deformities impairment.

The removal recommendation and study are **available online for public review and comment from September 21 through October 12, 2018.**

The Fish Tumors and Deformities impairment removal proposal, comment form and fact sheet can be found at: <u>http://dnr.wi.gov/topic/greatlakes/st.louis.html</u>

Comments can be submitted to Matt Steiger, Wisconsin DNR, AOC Coordinator, at <u>Matthew.Steiger@wisconsin.gov</u> until 5:00 pm October 12, 2018.

Paper copies of the draft document are available for review at the Superior Public Library (1530 Tower Ave.) and the Duluth Public Library (520 W Superior St.). Paper comment sheets are included and pre-formatted for mailing to Matt Steiger, WIDNR, 1701 N 4th St, Superior WI 54880. Comment sheets may also be scanned and emailed to Steiger.

Written and electronic comments sent or post-marked before 5:00 pm on October 12, 2018 will be considered. A final removal recommendation will be submitted to the U. S. Environmental Protection Agency after public input has been reviewed and considered. General questions about the removal proposal can be directed to Matt Steiger at (715) 395-6904 or Barb Huberty, Minnesota Pollution Control Agency AOC Coordinator, at (218) 302-6630.

The St. Louis River AOC will have seven remaining Beneficial Use Impairments to address before the entire St Louis River AOC can be delisted. It is one of 43 such areas designated in 1987 as the most highly contaminated areas in the Great Lakes.

This work was included in the St. Louis River AOC Remedial Action Plan and was funded primarily by the federal Great Lakes Restoration Initiative. The primary partners involved in this work included the Environmental Protection Agency, Wisconsin Department of Natural Resources, Minnesota Department of Natural Resources, Minnesota Pollution Control Agency and the Fond du Lac band of Lake Superior Chippewa.

Please share this announcement and input opportunity.

Thank you,

Matt Steiger

St. Louis River Area of Concern Coordinator Office of Great Waters Lake Superior, Lake Michigan and Mississippi River Wisconsin Department of Natural Resources 1701 N 4th St. Superior, WI 54880 Phone: (715) 395-6904 <u>matthew.steiger@wisconsin.gov</u>

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COMMENT FORM

St. Louis River Area of Concern

Removing the Fish Tumors and Other Deformities Beneficial Use Impairment

Your feedback is very important to the Wisconsin Department of Natural Resources and Minnesota Pollution Control Agency. In the space below, please provide your comments regarding the proposal to remove the Fish Tumors and other Deformities impairment. You may fill out the form online and email it to <u>Matthew.Steiger@Wisconsin.gov</u> or complete this form and mail it to Matt Steiger (WDNR) at the mailing address on the back on or before 5:00pm <u>October 12, 2018</u>. You may attach additional pages if needed.

*To submit comments or petitions to the AOC agencies through the mail or email, you must state:

(1) Name and address

(2) The action you wish the AOC agencies to take, including specific references to the section of the draft BUI removal you believe should be changed.

(3) The reasons supporting your position, stated with sufficient specificity as to allow the AOC agencies to investigate the merits of the position.

Please print clearly:

*Name:

*Mailing address:

Comments:

Information about the St. Louis River AOC is available on the web at:

Wisconsin Department of Natural Resources website: <u>http://dnr.wi.gov/topic/greatlakes/st.louis.html</u>

Minnesota Pollution Control Agency website: https://www.pca.state.mn.us/waste/st-louis-river-area-concern-resources

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Matt Steiger St. Louis River AOC Coordinator Wisconsin Department of Natural Resources 1701 N 4th St. Superior, WI 54880

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If mailing, seal with tape

St. Louis River Area of Concern

Fish Tumors and Deformities Studies



The SLRAOC includes Wisconsin, Minnesota and portions of the Fond du Lac Reservation.

Want to learn more about the Fish Tumors and Deformities BUI? Check out the BUI removal package and full study online at: dnr.wi.gov/topic/ GreatLakes/st.louis.html

Do you eat local fish? Check out the fish consumption advice for a safe and healthy meal: <u>dnr.wi.gov/topic/</u> <u>fishing/consumption/</u>



September 2018

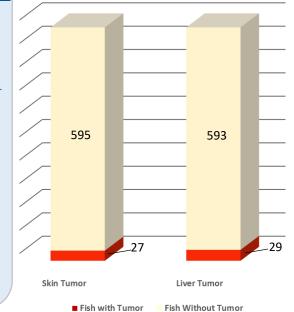
A study was undertaken to assess the Fish Tumors and Other Deformities Impairment in the St. Louis River.

Three rounds of sampling (2011, 2013, 2015) in the St. Louis River Area of Concern has shown the prevalence of tumors in white sucker is below 5% (Blazer et al., 2017).

622 white sucker were sampled in the river during their spring spawning run. The fish were analyzed for skin and liver tumors by researchers. The findings show that 4.3% of fish had a skin tumor and 4.7% of fish had a liver tumor. This is low compared to other areas in the Great Lakes.

The study also determined where the fish had been feeding. This information helped

St. Louis River AOC White Sucker Tumor Sampling 2011-2015





A proud fisherman at Barkers Island, Superior, WI

researchers to compare habitat usage of the river vs. Lake Superior and above the Fond du Lac dam. The study found no significant difference in tumor rates for fish that got more of their diet from the St. Louis River Bay and Harbor. This study found that the impairment removal target has been met.



Frequently Asked Questions

Q: What should I do if I catch a fish with an abnormality? **A:** If you observe a diseased fish or fish kill contact your local fisheries biologist or call the DNR TIP line. For more instructions visit: <u>dnr.wi.gov/topic/fishing/fishhealth/FishKillWhattoDo.html</u>

Q: Can I eat a fish that has an abnormality? A: Fish are susceptible to injuries, parasites and diseases. Some of these fish health issues can be mistaken for a tumor. It is recommended to exercise caution when consuming fish with abnormalities, but you may continue to enjoy catching and eating your catch as long as you fully cook them before eating.

Visit <u>dnr.wi.gov/topic/fishing/fishhealth/</u> <u>index.html</u> to learn about common fish diseases.

Appendix 3 Public Comments Received

- 1. Izaak Walton League comment
- 2. Agency Response to Izaak Walton League
- 3. City of Duluth Comment



W.J. MCCABE (DULUTH) CHAPTER IZAAK WALTON LEAGUE OF AMERICA

P. O. Box 3063. • DULUTH, MN 55803

Oct. 9, 2018

Matt Steiger WI DNR AOC Coordinator 1701 N. 4th St. Superior, WI 54880

Dear Mr. Steiger,

These comments are submitted to you on behalf of the MN Division and McCabe Chapter of the Izaak Walton League of America (Ikes). The Ikes are a longtime conservation group, established by hunters and fisherman, dedicated to the protection of our nation's soil, air, woods, waters, and wildlife. For several decades, the Ikes have been supporters and admirers of the broad collaborative efforts from both sides of the St. Louis River estuary, in their efforts to clean up the numerous sources of legacy pollution. The nearly heroic efforts to address the impairments have taken time and significant capital, but there is now some light at the end of the tunnel. So, thank you and everyone else that has been a part of this effort for all you've done.

We want to recognize the agencies and individuals involved in this study for their dedication, and for following good science. At this time, we feel that the science indicates the water quality has reached a point where it no longer causes chronic health issues in today's fish population.

The remediation efforts around the harbor and upstream in the estuary have addressed a number of contaminated sites, with the biggest sites to date containing huge quantities of wood waste. Impairments were primarily physical barriers to the establishment of healthy biological systems (though there were also lower levels of chemical contaminants).

However, the questions we raise today over the lifting of the fish impairment (tumor study) is one of timing and future accountability. With the largest Superfund site in the Great Lakes yet to be addressed near Gary, we now enter a phase where more serious levels of toxic contamination being exposed during the US Steel remediation could during that time compromise water quality through the liberation of fugitive contaminants, resulting in at least a potential health issue for aquatic organisms. With that in mind, we feel some consideration should be given to continuing the tumor study until "the dust has settled" so to speak, after all work at US Steel and the remaining remediation sites are completed. Our fear, whether justified or not, is that in the process of treating these sites, some level of contamination may inadvertently be re-dispersed in the water and surface sediment, again becoming a threat to fish health. We would prefer to side with caution, and continue the testing for tumors beyond completion of the US Steel remediation (using whatever timeline the research scientist feels is sufficient for tumors to reappear in the fish population).

Though late in doing so, the Ikes would also like to question the selection of comparative study sites. To say that the contamination level (tumors) in white suckers in the St. Louis River estuary are comparable to those in suckers in other Great Lakes sites, is not likely to instill much confidence. It's recognized that further down the Great Lakes, there is more contamination. So, bad in fact, that in Lake Erie the city of Toledo has to periodically shut off their drinking water supply, due to dangerous levels of toxic blue-green algae (stemming from excessively high levels of nutrients).

We feel it would have made more sense to compare fish tumors from upstream, not downstream. A comparison of tumor levels in the upper-levels of the St. Louis River watershed in one of the major tributary watersheds, such as the Cloquet or Savanna rivers, would have been preferable.

Finally, all too often once the remediation at a site is declared "complete", there is a loss of systematic follow-up to measure whether the project results actually met the short-term and long-term goals. Therefore, we would ask that during post-remediation of the AOC, the agencies involved periodically conduct a rigorous examination of all project areas to ensure that success has been as anticipated. This means going back to the field to re-measure the condition of the biotic community, look closely at the water quality, and measure the success or failure over time of the remediation efforts. Where there are shortcomings, this will call for additional actions to correct the problem(s).

Thank you for this opportunity to comment. We look forward to more successes in the estuary.

Sincerely,

Craig Sterle, President

Minnesota Division, IWLA

Rich Staffon, President W. J. McCabe Chapter, IWLA

State of Wisconsin DEPARTMENT OF NATURAL RESOURCES 1701 N 4th St. Superior WI 54880

Scott Walker, Governor Daniel L. Meyer, Secretary Telephone 608-266-2621 Toll Free 1-888-936-7463 TTY Access via relay - 711



November 7, 2018

Craig Sterle and Rich Staffon Izaak Walton League of America PO Box 3063 Duluth MN 55803

Subject: Response to BUI removal comments

Dear Mr. Sterle and Mr. Staffon:

Thank you for commenting on the Draft Fish Tumors and Deformities Beneficial Use Impairment (BUI) Removal Package. The Area of Concern (AOC) agencies and staff appreciate your support on continued improvements in the AOC.

The AOC program is focused on meeting the BUI target established by stakeholders, and the tumor study confirms that the target has been met. The study did use a few additional comparisons that were above and beyond the target to affirm the low incidence rate of tumors in the AOC. The tumor study collected 622 fish, of which 154 were collected by Fond du Lac Natural Resources above the dams (near Cloquet) and are referred to as "Upper River" habitat in the study. These fish were compared to estuary and migratory fish and there was no significant difference in tumor incidence between the regions above and below the Fond du Lac dam (Blazer et al., 2017 p. 21). The full study report was attached to the removal package as appendix 1.

The incidence rate in the AOC was not compared to any sites in Lake Erie, but as an additional line of evidence, the tumor rate was compared to AOC and non-AOC reference sites on Lake Michigan since a study there had taken place using identical methods:

Although not required by the SLRAOC BUI removal strategy, comparing prevalence results from the SLRAOC to other Great Lakes AOCs provides a basin-wide context. Based on the binary logistic regression results (Tables 8, 9), St. Louis River white sucker have a skin neoplasia prevalence of 2.5% at age-7, and a liver neoplasia prevalence of 2.3% age age-7. In comparison, the liver and skin neoplasia prevalence reported by Rutter (2010) for age-7 brown bullhead from the least-impacted potential reference site (Long Point Inner Bay) for Presque Isle Bay AOC was 1.2% and 6.4%, respectively. Using identical methods to this study, white sucker from two other Wisconsin AOCs (Sheboygan River and Milwaukee Estuary) and the Kewaunee River as a "least impacted site" were sampled in 2011 through 2013 (Blazer et al. In Press). The prevalence of skin tumors was higher (p < 0.0001) at the Kewaunee River (21.0%) than at the SLRAOC (4.3%), while there was no significant difference (p = 0.5570) between the sites with respect to liver neoplasm prevalence (Table 10). Both Kewaunee and St. Louis River had significantly lower liver tumor prevalence when compared to the Sheboygan and Milwaukee AOCs (Table 10). Interestingly, at both the Milwaukee and Sheboygan AOCs, numerous large external body surface tumors were observed and many of these were squamous cell carcinomas (malignant) which were not observed at St. Louis River or



Kewaunee. Liver neoplasms at Milwaukee and Sheboygan included hepatocellular carcinomas not observed in white suckers from the SLRAOC. (Blazer et al., 2017. p.22)

Remediating contaminated sites is a priority for the AOC program and the AOC agencies continue to perform this work under other listed BUIs (primarily restrictions on dredging). It is important to note that while contamination may be a factor in fish tumors, the tumor study was not designed to determine the cause of tumors, as this is extremely complex and environmental factors (e.g., viruses and parasites) are a known contributor to abnormal cell proliferation in other animals including humans. A description of contaminant testing is included in the full study, Blazer et al., 2017. Pages 23-27.

In response to your questions and comments regarding remediation sites, we have gathered information regarding remediation in the AOC and specifically the US Steel Superfund site.

Requirements that protect human health and the environment must be met during the design, construction, and post-construction phases of a remediation or restoration project in the St Louis River estuary. First, during the design phase, the environmental review and permitting requirements must be met before a project can proceed with implementation; this includes measures to control unwanted discharges and to avoid work during fish spawning periods. During the construction phase, the plans and specifications outline how contaminant redistribution is to be minimized and managed. Finally, after construction, AOC agencies will be completing post-construction sampling at the completed Area of Concern remediation sites to insure the as-built conditions are intact and to evaluate the success of restoration site recovery. The short-term risks associated with remedial construction are outweighed by the resulting long-term improvements in water quality.

As it relates specifically to the US Steel/Spirit Lake site, the 50% design documents for the sediment remediation include controls for minimizing and managing contaminant redistribution and resuspension, both during and after construction. During the remediation process, some areas will be dredged in the dry (i.e., coffer dams will be installed, after which the contained area will be dewatered, with treatment, and the sediment removed). Other dredged areas that are not dewatered will use controls such as booms and turbidity barriers. Some areas will be dredged to a set elevation, then capped with an engineered cap designed to protect the bioactive zone. Other areas will be dredged to remove all contamination exceeding remedial threshold levels and will have a 6-inch sand cover placed to manage any residuals. There will be water sampling, sediment sampling and imaging, and bathymetric surveys during remedy construction to ensure targets are being met and that conditions remain protective. Post-construction, U.S. Steel will be required to conduct long-term operation, maintenance, and monitoring activities to ensure the remedy is protective in both the short-term and long-term. This monitoring will be required, with 5-year reviews by EPA and MPCA, as long as contamination remains in place at the site. In summary, effective controls will be in place during the construction and post-construction stages to assess potential impacts to biota from the remediation. If monitoring results indicate the remedy is not performing as intended or is not protective of either human health or the environment, additional work can be required of the responsible party. That may include additional monitoring or additional remediation work, depending on the situation.

In addition to remedial monitoring, general fish health and population monitoring is part of the core duties of the natural resource management agencies that share jurisdiction in the St. Louis River AOC. Sampling the St. Louis River specifically for fish tumors is not anticipated in the future. Routine monitoring and communication with anglers occurs on a regular basis and will continue to inform fisheries managers of fish health in the river.

The AOC agencies are committed to cleaning up and restoring the St. Louis River AOC to meet BUI targets. We appreciate you taking the time to review and comment on the draft BUI removal package.

Sincerely, Ul 108 10

Matt Steiger Wisconsin DNR Area of Concern Coordinator (715) 395-6904

CC:

Barb Huberty Melissa Sjolund Rick Gitar Cherie Hagen Doug Wetzstine Pat Collins Heidi Bauman Erin Endsley

COMMENT FORM

St. Louis River Area of Concern

Removing the Fish Tumors and Other Deformities Beneficial Use Impairment

Your feedback is very important to the Wisconsin Department of Natural Resources and Minnesota Pollution Control Agency. In the space below, please provide your comments regarding the proposal to remove the Fish Tumors and other Deformities impairment. You may fill out the form online and email it to <u>Matthew.Steiger@Wisconsin.gov</u> or complete this form and mail it to Matt Steiger (WDNR) at the mailing address on the back on or before 5:00pm <u>October 12, 2018</u>. You may attach additional pages if needed.

*To submit comments or petitions to the AOC agencies through the mail or email, you must state:

(1) Name and address

(2) The action you wish the AOC agencies to take, including specific references to the section of the draft BUI removal you believe should be changed.

(3) The reasons supporting your position, stated with sufficient specificity as to allow the AOC agencies to investigate the merits of the position.

Please print clearly:

*Name: Diane Desotelle, Duluth Natural Resources Coordinator

*Mailing address: 411 W 1st St. Duluth, MN 55802

Comments:

The City of Duluth supports the removal of the fish tumor and other deformities beneficial use impairment on the St Louis River Area of Concern. We are pleased with the science driven approach to this work and excited to know that the SLRAOC has moved one step closer toward delisting. With that, we hope to continue to work closely with our partners on the river to remove the rest of the BUIs and to develop a means to monitor the restoration and remediation work being done so we can properly manage the area in the long term for the health and viability of this amazing resource. These plans must also include the importance of our communities surrounding the area to enjoy the resource in a sustainable fashion.

Appendix 4 – Letters of Support

- 1. St. Louis River Alliance
- 2. Mayor of Duluth
- 3. Mayor of Superior



St. Louis River Alliance 394 Lake Avenue S, Suite 405 Duluth, Minnesota 55802-2338 Phone: 218-733-9520

Working together to protect, restore, and enhance the St. Louis River

October 24, 2018

Matt Steiger, WDNR Cherie Hagen, WDNR SLRAOC Coordinators Wisconsin Department of Natural Resources 1701 North 4th Street Superior, WI 54880

Re: Support for Proposal to remove the St Louis River Area of Concern Fish Tumors and Deformities Impairment.

Dear Mr. Steiger,

On behalf of Board of Directors of the St. Louis River Alliance I am pleased to inform you that we have reviewed the information presented by your agency on September 16, 2018 and we are in agreement with the recommendation put forward by the Wisconsin Department of Natural Resources (WDNR), Minnesota Department of Natural Resources (MNDNR), Minnesota Pollution Control Agency (MPCA) and the Fond du Lac Band of Lake Superior Chippewa to request to the United States Environmental Protection Agency (USEPA) Great Lakes National Program Office's (GLNPO) to approve removal of the St. Louis River Area of Concern Fish Tumors and Deformities Impairment.

The Executive Board of Directors took formal action on this matter on October 24th, 2018 and passed a resolution supporting the removal of the Fish Tumors and Deformities Impairment in the St. Louis River Area of Concern.

As you know, the St. Louis River Alliance was actively involved in the development of the 2013 St. Louis River Remedial Action Plan and has been participating in the discussions of the specific actions that have been fully completed by the WDNR, the MPCA, and the MNDNR staff. Completion of this work and documentation that all actions have been taken is a tangible milestone for the delisting of the St. Louis River Area of Concern. This is a major accomplishment and we thank you for your work and commitment to this process.

We look forward to our continuing work together to remove the remaining 7 beneficial use impairments and to the eventual delisting of the St. Louis River Area of Concern.

Sincerely,

rist Detre

Kristi S Eilers Executive Director St. Louis River Alliance

City of Duluth Emily Larson, Mayor



411 West First Street * Room 403 * Duluth, MN 55802 218-730-5230 * Fax 218-730-5904 * Email: elarson@duluthmn.gov

Monday, November 5th, 2018

Matt Steiger, St Louis River Area of Concern Coordinator Wisconsin Department of Natural Resources 1701 North 4th Street Superior, WI 54880

Subject: City of Duluth Support to Remove the Fish Tumors and Deformities Beneficial Use Impairment

Dear Mr. Steiger,

On behalf of the City of Duluth, I am pleased to offer our support for removing the Fish Tumors and Deformities Beneficial Use Impairment for the St Louis River Area of Concern (SLRAOC). City staff reviewed the information provided during the public comment period and agree with the removal recommendation put forward by the SLRAOC's agency partners: the Wisconsin Department of Natural Resources, the Minnesota Pollution Control Agency, the Minnesota Department of Natural Resources, and the Fond du Lac Band of Lake Superior Chippewa.

The City of Duluth actively supports progress toward delisting the SLRAOC by 2025. Not only are the contaminant remediation and habitat restoration achievements important to us, but we are excited about the revitalization potential these achievements will support. We appreciate the efforts of all the SLRAOC local, state, and federal partners who are helping achieve these goals and thank you for your work.

We look forward to learning that the Environmental Protection Agency's Great Lakes National Program Office staff have approved this removal request. The City of Duluth will continue working with the SLRAOC staff as they continue their work to remove the seven remaining beneficial use impairments and ultimately delist the SLRAOC.

Sincere

Mayor Emily Larson

C: Diano Desotelle, City of Duluth Natural Resources Coordinator Barb Huberty, MPCA SLRAOC Coordinator Melissa Sjolund, MNDNR SLRAOC Coordinator Rick Gitar, Fond du Lac SLRAOC Coordinator



Office of the Mayor

Jim Paine, Mayor Rani Gill, Chief of Staff to the Mayor

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November 6, 2018

Matt Steiger, St Louis River Area of Concern Coordinator Wisconsin Department of Natural Resources 1701 North 4th Street Superior, WI 54880

RE: City of Superior Support to Remove the Fish Tumors and Deformities Beneficial Use Impairment

Dear Mr. Steiger:

On behalf of the City of Superior, I am pleased to offer our support for removing the Fish Tumors and Deformities Beneficial Use Impairment for the St Louis River Area of Concern (SLRAOC). We agree with the removal recommendation put forward by the SLRAOC's agency partners: the Wisconsin Department of Natural Resources, the Minnesota Pollution Control Agency, the Minnesota Department of Natural Resources, and the Fond du Lac Band of Lake Superior Chippewa.

The City of Superior has been actively involved in SLRAOC projects and supports the progress being made toward delisting the SLRAOC by 2025. We value the contaminant remediation and habitat restoration work and the potential for revitalization that these will bring. We appreciate the efforts of all the SLRAOC local, state, and federal partners who are helping achieve these goals and thank you for your work.

We look forward to learning that the Environmental Protection Agency's Great Lakes National Program Office has approved this removal request. The City of Superior will continue working with the SLRAOC staff as they continue their work to remove the seven remaining beneficial use impairments and ultimately delist the SLRAOC.

Sincerely,

Jim Paine Mayor

c: Barb Huberty, MPCA SLRAOC Coordinator Melissa Sjolund, MNDNR SLRAOC Coordinator Rick Gitar, Fond du Lac SLRAOC Coordinator