Thunder Bay Area of Concern Beneficial Use Impairment Assessment Report

Fish Tumours or Other Deformities

Sample collection in Thunder Bay
Environment and Climate Change Canada

February 2017, revised October 2018

Table of Contents

Executive Summary

This report concludes that The Fish Tumours or Other Deformities BUI delisting criteria have been met; thus this BUI should be considered to be 'not impaired' and can be removed from the list of environmental issues facing the Thunder Bay Area of Concern (AOC).

Incidence of liver tumours in white suckers (*Catostomus commersoni*) from the Great Lakes are often increased by chemical contaminants, in particular polynuclear aromatic hydrocarbons (PAHs). Experts believe there is a state of impairment when the prevalence of liver tumours in sucker species is greater than 5% (Great Lakes Commission 2002 and Baumann 2010). In the late 1980s/early 1990s, the liver neoplasm prevalence (tumour rate) in white suckers sampled from Thunder Bay was 7.1% (Baumann et al. 1996), resulting in the Fish Tumours or Other Deformities impairment being identified in the Stage 1 Remedial Action Plan (RAP) report (Thunder Bay Remedial Action Plan Writing Team 1991), and the subsequent 2004 Stage 2 RAP report (Lake Superior Programs Office and the Thunder Bay Remedial Action Plan Team 2004).

In 2007, the analysis of 100 white suckers collected from Thunder Bay revealed a 2% tumour rate (Baumann 2010). This was not significantly different from no tumours found at the reference location 140 km to the east (Mountain Bay), and is well below the 5% threshold experts established for environmental impairment in sucker species. However, recognizing that the median age of the Thunder Bay samples collected in 2007 were relatively young (6 years old versus 11 years old for Mountain Bay), a follow-up survey was recommended (Baumann 2010). It is important to compare fish of the same age for this type of analysis. The age of the fish is positively correlated with tumor prevalence. This is not only because fish that have lived longer have usually been exposed to environmental contaminants longer, but also because there is a latent period between induction and tumor development. The document 'Data Analysis and Fish Tumor BUI Assessment for Lake Superior and the St. Clair River AOCs' by Paul C. Baumann (2010) is appended to this report.

Based on the recommendation of a follow-up survey, there was agreement between RAP agencies to identify this impaired beneficial use as 'requires further assessment' in the 2011 Thunder Bay RAP Update (Lakehead University 2012).

In 2013, the analysis of 100 white suckers from Thunder Bay (median age 9 years) revealed a tumour rate of 1%, with one fish having a 1mm diameter benign tumour. The 2% tumour rate in the 2007 sampled fish and the 1% tumour rate in the 2013 sampled fish are both well below the 5% threshold experts establish for environmental impairment.

The results from these two intensive surveys provide compelling evidence of improved fish health in the Thunder Bay Area of Concern, and clearly demonstrates that this BUI is not impaired within the Thunder Bay AOC.

Introduction

Thunder Bay was listed as an Area of Concern (AOC) under the Canada-U.S. Great Lakes Water Quality Agreement of 1987. Discharges of pollutants from local industry and municipal wastewater treatment plants were impairing water quality and ecosystem health, resulting in the identification of a number of beneficial use impairments.

When the Stage 1 Remedial Action Plan (RAP) was released in 1991, the Fish Tumours or Other Deformities BUI was identified due to an abnormal incidence of liver cancers in white suckers (*Catostomus commersoni*). Discharges of chlorinated organics from the pulp and paper industry in the area were identified as a possible contributor to the problem, along with polyaromatic hydrocarbons (PAHs) [1] from the Northern Wood Preservers site (MOE et al. 1991), where for over 60 years creosote was used or produced for wood treatment.

As presented in Baumann et al. (1996), white suckers sampled from the Thunder Bay AOC (specifically near the Kaministiquia River) during 1985-90 had a 7.1% tumour rate (n = 112). This was much higher than neighbouring reference sites in Black Bay (no tumours were identified, n=231) and Mountain Bay (2.4% tumour rate, n = 75) assessed during the same period. Noting that PAHs had been proven to induce liver cancer in fish, Baumann et al. (1996) believed that it was highly probable that the Thunder Bay's high fish tumour rate was the result of exposure to elevated PAH concentrations in the sediment of the intercity harbour at that time.

As documented in the Stage 2 RAP report, a number of initiatives took place from the 1990s to early 2000s that significantly improved water and sediment quality in Thunder Bay. These included upgrades to secondary wastewater treatment at the Abitibi-Consolidated newsprint mill (1995), the Cascades Fine Papers Group fine paper mill (1995, 1996-97) and the Bowater bleached kraft pulp and newsprint mill (1993-95). This coincided with stricter regulations through the provincial Municipal-Industrial Strategy for Abatement strategy (1993) and federal Pulp and Paper Effluent Regulations (1992). In 2003, the Northern Wood Preservers Alternative Remediation Concept (NOWPARC) project was completed that contained or removed, treated and reused 32,000 cubic metres of highly contaminated sediment, which included high levels of PAHs.

Citing the success of the process improvements by local industry and the completion of the NOWPARC project, the Stage 2 RAP report maintained the impaired status for the Fish Tumours or Other Deformities BUI, but with the caveat that it should no longer be considered to be impaired due to contaminant sources within the AOC. Further assessments of this impairment, however, were still needed for its eventual re-designation. Fish tumour studies were therefore

[1] Also called polycyclic (or polynuclear) aromatic hydrocarbons, PAHs are a group of chemicals released as a by-product of combustion. The production of creosote-treated products is also major source to the environment: www.ec.gc.ca/toxiques-toxics/default.asp?lang=En&n=9C252383-1

undertaken in 2007 and 2013. The methodology and results of these two surveys are presented within this report.

Fish Tumour Assessment and Discussion

Methodology – fieldwork

In 2007, the fieldwork was carried out by the Aquatic Contaminants Research Division of ECCC from September 26 to October 3. Sample collections focused on the industrial waterfront of Thunder Bay, particularly around the Kaministiquia River delta and mouth of the McIntyre River (Fig. 1). This area was selected because: 1) historically it has had some of the highest contaminant concentrations within the AOC; 2) it remains downstream of active industrial operations, including the pulp and paper mill on the Kaministiquia River; and 3) it is where the original 1985-90 fish tumour survey sampled, allowing for a historical comparison. The majority of fish were collected at the Kaministiquia River delta; a few additional fish were taken from two other sites in Thunder Bay along the city's waterfront to supplement the collection.

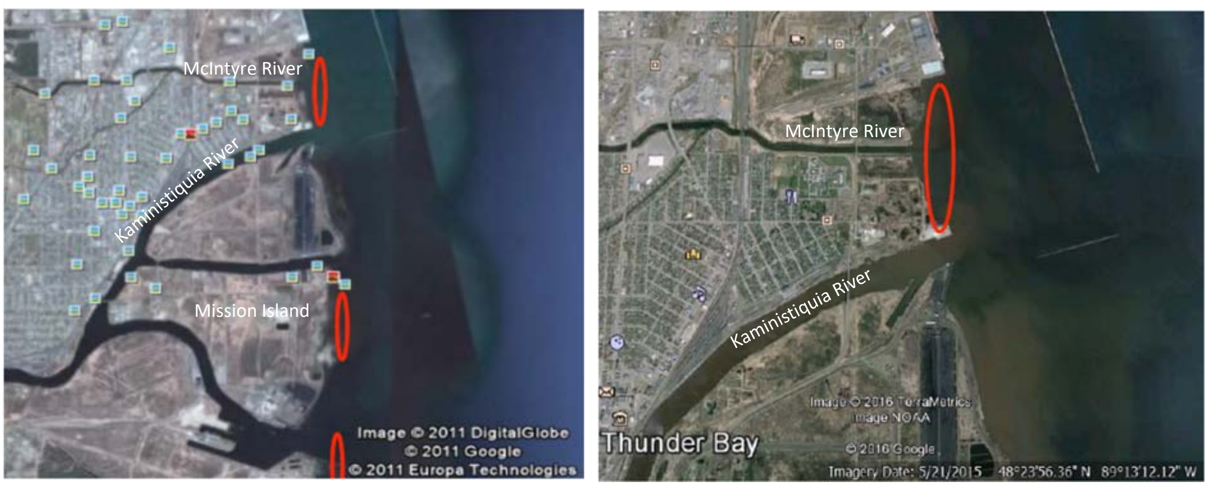


Figure 1: Sampling areas in the Thunder Bay Area of Concern for the 2007 (left) and 2013 (right) fish tumour surveys.

In 2013 the fieldwork was again carried out by the Aquatic Contaminants Research Division of ECCC (from August 20 to 23) and remained focused on the industrial waterfront around the Kaministiquia River delta, as was the 2007 survey.

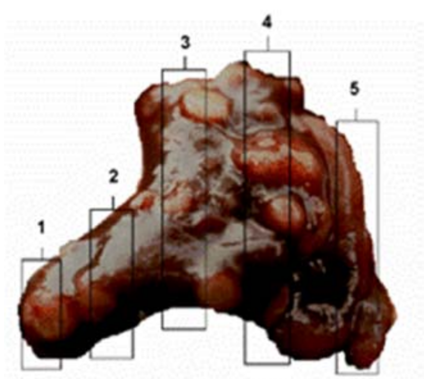
For a high-confident assessment, a minimum sample size of 100 adult fish per survey is required (Baumann 2010). This requirement was achieved in both the 2007 and 2013 surveys, with 100 white suckers collected by overnight gill net sets for each survey. Recognizing the median age of the 2007 samples was relatively young (6 years old versus 11 years old for the Mountain Bay reference site); the field team targeted larger white suckers in the 2013 survey and released the smaller fish. It is important to compare fish of the same age for this type of analysis. The age of the fish is positively correlated with tumor prevalence. This is not only because fish that have lived longer have usually been exposed to environmental contaminants longer, but also because there is a latent period between induction and tumor development.

Following capture, the fish were placed into a live well for transport to the shore. Fish were anaesthetized in a clove oil bath and then sacrificed using standard operating procedures (animal use protocol 0816 and 1312 respectively). Their physical state was assessed using a visual examination of physical abnormalities (any external deformities or lesions were noted), and general measurements were taken, including gender, fork length and body weight. The operculae – hard, bony flaps covering and protecting the gills – were collected for aging in the laboratory, and the subsequent results aged the 2013 fish from 3+ to 17 years old, with a median age of 11. Appendix A and B provide the statistics on the age, gender, length, and body weight of all the fish collected in 2007 and 2013, respectively.

In addition, the livers were removed from each fish and separated into sections for histological evaluation, fixed in Davidson's Fixative and later transferred and stored in 70% ethanol one to four weeks after collection.

Methodology – lab work

At the ECCC laboratory in Burlington, the samples were further processed. Five liver sections from each fish were distributed on ten slides (two slides per sample). Figure 2 provides an illustrated example of what is done, showing a brown bullhead liver. Each slide contained three to five serial or step-sections from the same paraffin block. In both the 2007 and 2013 surveys, staff with ECCC's Aquatic Contaminants Research Division first conducted a preliminary examination of slides to confirm good sections, staining and mounting.



McMaster, 2016

Figure 2: Sample histology slide (each liver is eventually divided into five samples)

The 2007 samples were assessed by the Department of Fisheries and Oceans' lab in Winnipeg, MB (Brad Park), and the 2013 samples were sent to the British Columbia Ministry of Agriculture (Dr. Gary Marty). In both surveys, the histological evaluations (i.e., liver tumour analyses) followed the *Blazer Protocol*, a process developed by experts with the U.S. Geological Survey and U.S. Environmental Protection Agency for the systematic identification of liver tumours in brown bullhead (Blazer et al. 2007). The protocol has become the standard approach for identifying fish tumours and determining tumour rates in the Canadian Great Lakes Areas of Concern, including Jackfish Bay, St. Marys River, St. Clair River, St. Lawrence River, the Bay of Quinte, Hamilton Harbour, and Toronto Harbour in addition to Thunder Bay.

There are different kinds of liver abnormalities, including putatively preneoplastic lesions, foci of cellular alteration; neoplastic hepatocellular lesions; non-neoplastic biliary lesions; and neoplastic biliary lesions (McMaster 2016). As outlined in Baumann (2010), some of these liver abnormalities are not conclusively linked to environmental contaminants, and some may not progress into actual liver tumours (i.e., *pre*-neoplastic lesions may never become neoplastic lesions). For this reason, only neoplastic lesions are used for evaluating the Fish Tumours or Other Deformities BUI in Areas of Concern.

Therefore, to ensure the analysis and diagnosis of liver samples can produce a high-degree of confidence that detected tumours are potentially linked to chemical contaminants in the environment, the 2007 and 2013 surveys examined the following abnormalities (McMaster 2016):

- Neoplastic Hepatocellular Lesions: Hepatocellular Adenoma and Hepatocellular Carcinoma, which are cancers in the liver; and
- Neoplastic Biliary Lesions – Cholangioma and Cholangiocarcinoma, which are cancers in the bile duct of livers in fish.

As outlined in Marty and Snyman (2015), staff at the B.C. Ministry of Agriculture and the Department of Fisheries and Oceans conducted a blind examination, meaning no information was known about the fish other than the species, year, and province where the fish were collected.

For each liver sample, every section was systematically scanned using the 4× objective lens (low power), and then a single section was systematically scanned using the 10× objective lens (medium power). When needed, higher magnification (20× and 40×) was used. See Figure 3.

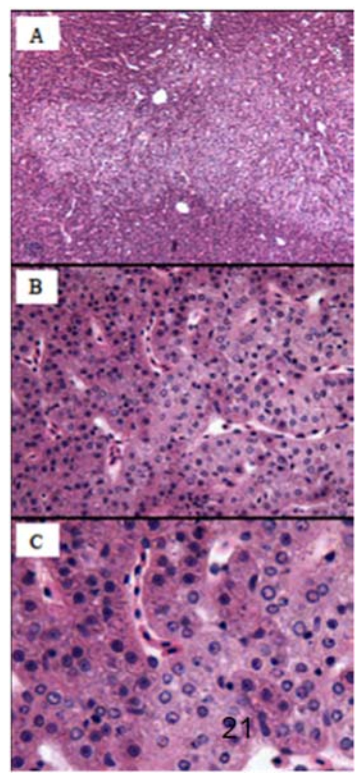


Figure 3: Same liver sample at increasing magnification

For neoplasia, the number of foci per liver was recorded. Other lesions such as foci of cellular alteration were scored on a relative scale as none (0), mild/small amounts (1), moderate (2), or severe/abundant (3).

Fish Tumour Results

Of the 100 white suckers sampled in 2007, two fish were found to have liver tumors. One smaller male had a Hepatocellular carcinoma (cancer in the liver) and one larger female had a Cholangiocarcinoma (cancer in the bile duct). Mountain Bay was sampled as the reference location in 2007, and none of those reference fish had liver tumours.

In a review of the 2007 findings, Baumann (2010) stressed the influence of age on tumour incidence. The age of the fish is positively correlated with tumor prevalence. This is not only because fish that have lived longer have usually been exposed to environmental contaminants longer, but also because there is a latent period between induction and tumor development.

The median age of the Thunder Bay white suckers collected in 2007 was 5 years younger than the reference location. An additional survey of 100 fish was recommended, using a length cut-off to reduce younger age groups (Baumann, 2010). Such a survey emphasizing older fish would add certainty to the decision on the status of this beneficial use impairment.

A follow-up sampling campaign was completed in August 2013. Of the 100 white suckers collected, the histological evaluation by Dr. Gary Marty found one fish that had a 1mm diameter cholangioma. Cholangiomas are well-demarcated and well differentiated, and are composed of variable differentiation towards biliary preductular epithelial cells, bile ductular epithelial cells, and supporting stroma (Marty and Snyman 2015). Dr. Marty specifically identified it as a benign tumour of the biliary tract that could be the result of toxin exposure. None of the other 99 livers had tumours.

As discussed, larger fish were sampled during the 2013 sampling program with the average age of white sucker being 9 years old, significantly older that the 2007 sample. Marty and Snyman (2015) conclude from the 2013 samples that: “the lack of neoplasia and the low prevalence of preneoplastic foci and lesions associated with toxin exposure provide evidence that chronic toxin exposure is not significantly affecting liver morphology in Thunder Bay fish populations”.

Conclusion

This report documents the efforts of two intensive fish tumour surveys examining white suckers in the Thunder Bay AOC.

The Great Lakes Commission (2002) and Baumann (2010) recommend a liver tumour rate of 5% in sucker species as the threshold for environmental impairment. Assuming adequate sample size and appropriate indicator species are collected, anything lower should be considered as evidence of no impairment.

In the Thunder Bay AOC, the liver tumour rate of white suckers in 2007 was determined to be 2%, and the liver tumour rate of white suckers in 2013 was found to be 1%. Both are well below the 5% threshold experts establish for environmental impairment and are comparable to the Mountain Bay non-AOC reference location.

Based on this, it is determined that the delisting criteria for the Fish Tumours or Other Deformities BUI have been met, and the BUI no longer applies to the AOC.

References

Baumann, P.C. 2010. Data Analysis and Fish Tumor BUI Assessment for Lake Superior and the St. Clair River AOCs.

Baumann, P.C., I. Smith, C. Metcalfe. 1996. Linkages between Chemical Contaminants and Tumors in Benthic Great Lakes Fish. Journal of Great Lakes Research. 22(2): 131-152.

Blazer, V., J. Fournie, J. Wolf, M. Wolfe. 2007. Manual for the Microscopic Diagnosis of Proliferative Liver and Skin Lesions in the Brown Bullhead (Ameiurus nebulosus). U.S. Geological Survey, U.S. Environmental Protection Agency, and Experimental Pathology Laboratories Inc.

Great Lakes Commission. 2002. An Overview of U.S. Great Lakes Areas of Concern. Under cooperative agreement with the U.S. EPA. Page 101.

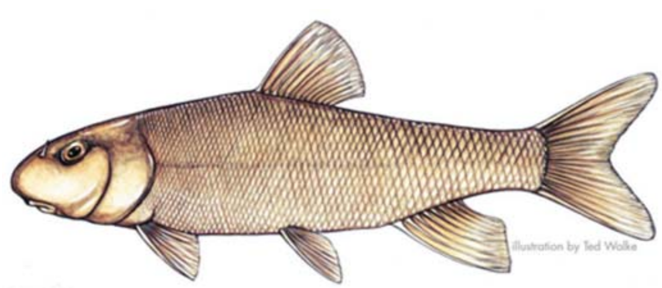
Lake Superior Programs Office and the Thunder Bay Remedial Action Plan Team. 2004. Stage 2: Remedial Strategies for Ecosystem Restoration.

Lakehead University. 2012. Thunder Bay Remedial Action Plan Update 2009-2012.

Marty, G. and H. Snyman. 2015. Final Histopathology Report. Case #: KW405-13-1397 W14-5132. British Columbia Ministry of Agriculture – Animal Health Centre.

McMaster, M. 2016. Liver Tumor Incidence in White Sucker from the Thunder Bay AOC-Presentation to the Thunder Bay PAC June 1, 2016.

Thunder Bay Remedial Action Plan Writing Team. 1991. Stage 1 Report: Environmental Conditions and Problem Identification.



Appendix A: White Suckers collected in 2007

Statistics on age, gender, fork length and total body weight

	Fish ID #	Length (cm)	Weight (g)	Age (yrs)*	Gender
1	AOC2007-81	43.7	1078	6	F
2	AOC2007-84	41.7	1166	5	F
3	AOC2007-86	46.2	1514	7	F
4	AOC2007-90	44.7	1339	7	F
5	AOC2007-91	44.8	1510	7	F
6	AOC2007-93	47.4	1530	8	F
7	AOC2007-T140	40.1	963		F
8	AOC2007-T142	41.9	1104	5	F
9	AOC2007-T144	45.8	1533	7	F
10	AOC2007-T147	43.9	1374	5	F
11	AOC2007-97	42.5	1257	5	F
12	AOC2007-100	48.0	1569	9	F
13	AOC2007-T149	42.9	1166	5	F
14	AOC2007-T154	47.2	1505	11	F
15	AOC2007-T157	44.8	1332	4	F
16	AOC2007-T160	42.9	1160		F
17	AOC2007-T163	45.7	1562	6	F
18	AOC2007-103	50.4	1868	9	F
19	AOC2007-T164	44.5	1225	7	F
20	AOC2007-T165	48.7	1850	9	F
21	AOC2007-108	53.0	2146	5	F
22	AOC2007-109	47.6	1575	6	F
23	AOC2007-111	43.6	1340	4	F
24	AOC2007-112	43.2	1254	5	F
25	AOC2007-113	41.3	1037	4	F
26	AOC2007-115	46.6	1439	5	F
27	AOC2007-116	43.3	1026		F
28	AOC2007-117	42.9	1230	4	F
29	AOC2007-118	48.9	1544	9	F
30	AOC2007-119	45.8	1464	6	F
31	AOC2007-120	46.9	1559	5	F
32	AOC2007-T173	44.5	1349	6	F
33	AOC2007-T174	52.5	1829	10	F
34	AOC2007-T177	45.0	1310	4	F
35	AOC2007-T179	43.5	1222	5	F
36	AOC2007-121	50.4	2277	10	F
37	AOC2007-122	47.5	1602	4	F
38	AOC2007-123	48.1	1616	5	F

* age undetermined where missing

All Fish (100)

median length	44.5
median weight	1273.0
median age	6.0

Females (49)

median length	45.8
median weight	1464.0
median age	6.0

Males (51)

median length	43.5
median weight	1192.0
median age	6.0

Notes:

Fish ID # AOC2007-137 (line 48) was found to have a Cholangiocarcinoma, which is a cancer in the bile duct of the liver.

Fish ID # AOC2007-95 (line 58) was found to have a Hepatocellular carcinoma, which is a cancer in the liver.

	Fish ID #	Length (cm)	Weight (g)	Age (yrs)*	Gender
39	AOC2007-124	49.0	1665	8	F
40	AOC2007-125	44.4	1237	11	F
41	AOC2007-127	46.4	1341	14	F
42	AOC2007-128	51.0	1723	9	F
43	AOC2007-129	46.4	1621	11	F
44	AOC2007-130	42.6	1037	5	F
45	AOC2007-131	47.0	1244	5	F
46	AOC2007-135	51.6	1725	9	F
47	AOC2007-136	45.8	1530	7	F
48	**AOC2007-137**	50.8	1941	14	F
49	AOC2007-138	49.9	1497	9	F
50	AOC2007-82	46.7	1316	12	M
51	AOC2007-83	39.9	929	4	M
52	AOC2007-85	42.4	1086	4	M
53	AOC2007-87	43.4	1175	4	M
54	AOC2007-88	42.6	1065	5	M
55	AOC2007-89	44.8	1321	8	M
56	AOC2007-92	46.0	1288	7	M
57	AOC2007-94	45.1	1233	6	M
58	**AOC2007-95**	41.3	972	5	M
59	AOC2007-96	39.7	1114	5	M
60	AOC2007-T141	45.5	1274	6	M
61	AOC2007-T143	41.2	1137	6	M
62	AOC2007-T145	43.0	1258	10	M
63	AOC2007-T146	40.9	1044	4	M
64	AOC2007-T148	42.6	1182	5	M
65	AOC2007-98	43.9	1129	7	M
66	AOC2007-99	41.0	952	5	M
67	AOC2007-101	39.0	909	3	M
68	AOC2007-T150	42.4	1189	5	M
69	AOC2007-T151	40.6	1023	6	M
70	AOC2007-T152	44.9	1218	9	M
71	AOC2007-T153	47.0	1501	10	M
72	AOC2007-T155	46.0	1443	5	M
73	AOC2007-T156	42.0	1056	7	M
74	AOC2007-T158	44.0	1218	5	M
75	AOC2007-T159	41.4	1165	5	M
76	AOC2007-T161	43.9	1099	5	M
77	AOC2007-T162	45.0	1279		M
78	AOC2007-102	43.8	1252	8	M
79	AOC2007-104	42.5	1125	7	M
80	AOC2007-105	45.3	1342	9	M
81	AOC2007-106	42.9	1087	6	M
82	AOC2007-107	46.5	1346	8	M
83	AOC2007-110	43.5	1183	5	M
84	AOC2007-114	44.5	1317	6	M

	Fish ID #	Length (cm)	Weight (g)	Age (yrs)*	Gender
85	AOC2007-T166	46.0	1401	8	M
86	AOC2007-T167	43.4	1125	5	M
87	AOC2007-T168	45.8	1365	11	M
88	AOC2007-T169	44.5	1192	6	M
89	AOC2007-T170	43.5	1250	6	M
90	AOC2007-T171	41.7	1127	5	M
91	AOC2007-T172	41.1	1097	7	M
92	AOC2007-T175	42.1	1128	9	M
93	AOC2007-T176	46.0	1498	9	M
94	AOC2007-T178	43.5	1272	7	M
95	AOC2007-T180	46.0	1516	9	M
96	AOC2007-126	43.6	1202	10	M
97	AOC2007-132	45.9	1310	6	M
98	AOC2007-133	45.1	1313	15	M
99	AOC2007-134	47.8	1458	5	M
100	AOC2007-139	41.0	884	11	M

Appendix B: White Suckers collected in 2013

Statistics on age, gender, fork length and total body weight

	Fish ID #	Length (cm)	Weight (g)	Age (yrs)	Gender
1	152	34.0	554.5	3+	F
2	112	42.7	1189.1	8	F
3	113	35.8	611.3	6+	F
4	115	43.0	1073.8	7+	F
5	117	36.8	798.2	4+	F
6	120	44.3	1326.7	6	F
7	121	53.2	2097.8	11	F
8	122	32.9	481.6	3+	F
9	123	35.0	551.6	3+	F
10	124	33.7	554.4	3+	F
11	125	45.8	1308.9	9	F
12	128	36.9	662.0	5+	F
13	129	47.8	1469.9	13	F
14	130	44.8	1423.2	8	F
15	133	44.1	1182.5	8	F
16	134	48.5	1705.1	11	F
17	135	41.7	1104.2	5+	F
18	137	50.7	1812.1	15	F
19	139	46.0	1389.6	7	F
20	**140**	46.6	1351.0	10	F
21	141	47.1	1543.7	9	F
22	151	33.1	507.0	4	F
23	153	52.2	1988.1	10	F
24	154	52.8	1995.2	12	F
25	155	49.9	1904.0	13	F
26	158	54.3	2145.2	15	F
27	161	52.0	1788.4	10	F
28	163	45.9	1443.6	7+	F
29	164	45.0	1253.5	5+	F
30	165	47.2	1428.0	8+	F
31	167	42.5	1074.5	7	F
32	169	51.5	1943.2	12	F
33	170	54.2	1987.6	17	F
34	171	51.0	1877.4	12	F
35	173	45.2	1346.0	6+	F
36	175	44.8	1198.7	6+	F

All Fish (100)

median length	44.7
median weight	1318.4
median age	11.0

Females (49)

median length	45.0
median weight	1326.7
median age	10.5

Males (51)

median length	44.6
median weight	1310.0
median age	11.0

Notes:

Fish ID # 140 (line 20) was found to have a 1-mm-diameter Cholangioma, which is a benign tumour of the biliary tract.

	Fish ID #	Length (cm)	Weight (g)	Age (yrs)	Gender
37	177	38.0	763.7	4+	F
38	178	39.7	917.0	8	F
39	181	48.6	1516.5	13	F
40	185	50.8	1809.7	15	F
41	188	48.9	1825.1	11	F
42	191	44.4	1240.4	8+	F
43	193	43.5	1276.0	7	F
44	196	41.7	963.4	6+	F
45	199	36.9	746.7	7+	F
46	201	37.0	857.5	6+	F
47	202	37.1	809.4	5	F
48	207	53.7	2159.1	12	F
49	210	46.8	1941.1	15	F
50	111	46.4	1073.9	15	M
51	114	33.6	532.6	5+	M
52	116	36.7	776.2	6+	M
53	118	40.5	906.0	5+	M
54	119	35.8	665.3	4+	M
55	126	38.7	804.5	4+	M
56	127	41.6	1044.0	7+	M
57	131	47.0	1432.4	10	M
58	132	46.4	1475.5	13	M
59	136	48.4	1522.1	8	M
60	138	44.7	1401.4	15	M
61	142	44.3	1434.3	11	M
62	143	46.4	1345.0	14	M
63	144	45.9	1479.5	11	M
64	145	43.4	1179.5	7	M
65	146	48.2	1610.8	13	M
66	147	44.7	1266.0	13	M
67	148	44.6	1574.3	14	M
68	149	45.5	1429.6	13	M
69	150	41.9	1076.3	6+	M
70	156	46.5	1477.0	15	M
71	157	46.7	1438.5	11	M
72	159	43.4	1196.9	7	M
73	160	41.3	1156.2	5	M
74	162	44.0	1212.9	11	M
75	166	45.5	1371.0	11	M
76	168	46.0	1285.1	14	M
77	172	44.0	1232.7	7	M
78	174	44.3	1233.1	5	M
79	176	45.5	1466.8	11	M
80	179	41.9	1213.0	7	M
81	180	43.0	1254.0	7+	M
82	182	42.4	1156.0	6	M

	Fish ID #	Length (cm)	Weight (g)	Age (yrs)	Gender
83	183	47.1	1645.3	11	M
84	184	48.3	1722.0	12	M
85	186	46.6	1506.3	14	M
86	187	46.8	1489.8	11	M
87	189	45.3	1393.1	12	M
88	190	38.1	904.7	8+	M
89	192	44.5	1426.0	16	M
90	194	44.7	1347.1	11	M
91	195	44.9	1426.8	11	M
92	197	44.6	1284.4	15	M
93	198	46.5	1630.3	10	M
94	200	37.9	786.8	5+	M
95	203	43.6	1310.0	15	M
96	204	45.7	1474.8	11+	M
97	205	44.5	1360.5	13	M
98	206	44.0	1309.6	7+	M
99	208	41.8	1121.3	7+	M
100	209	46.0	1283.0	14	M

Appendix C: Data Analysis and Fish Tumor BUI Assessment for Lake Superior and the St. Clair River AOCs

Paul C. Baumann, PhD.

Submitted to: Environment Canada

March 31, 2010

Updated September 2018

Introduction and History:

Tumor epizootics in fish were first linked to environmental contaminants in the sixties (Dawe et al., 1964). In the seventies the first study was published implicating environmental carcinogens as part of the etiology of papillomas in white suckers in the Great Lakes (Sonstegard, 1977). In the 1980s the first liver cancer epizootic in brown bullhead from the Great Lakes drainage basin was reported in the Black River, Ohio (Baumann, et al., 1982). Research since that time has demonstrated an elevated tumor prevalence in brown bullhead and white sucker populations from a variety of urbanized areas in bays and tributaries of the Great Lakes in both Canadian and United States waters (Baumann et al. 1996). Concern over these discoveries resulted in fish tumors being designated as a Beneficial Use Impairment (BUI) used to determine Areas of Concern (AOC) in Annex 2 of the 1987 Protocol Amending the Great Lakes Water Quality Agreement. The IJC delisting guidelines from 1991 state that this Beneficial Use may be deemed to be Not Impaired "when the incidence rates of fish tumors or other deformities do not exceed rates at unimpacted control sites or when survey data confirm the absence of neoplastic or preneoplastic liver tumors in bullheads or suckers" (International Joint Commission, 1991). Details about the actual methodology used to establish this criterion were not spelled out, and as time has passed the understanding of what comprises accurate methodology in fish tumor surveys has changed (Blazer et al. 2006).

This report deals with those Areas of Concern with fish tumor BUIs located in the Lake Superior basin (Thunder Bay AOC and Jackfish Bay AOC) and the St. Clair River. All three of these locations were far enough north to make collecting sufficient brown bullhead (*Ameiurus nebulosus*) to use as a sentinel species impractical. Thus all locations used a locally abundant catostomid (member of the sucker family) for determining tumor incidence. Fish surveys at both of the two Lake Superior locations used the white sucker (*Catostomus commersoni*) and the fish survey of the St. Clair River used the shorthead redhorse (*Moxostoma macrolepidotum)*. Two of these AOCs and the reference location of Mountain Bay (located on northern Lake Superior) had been documented with both external (lip and body combined) and liver tumors in white suckers during studies carried out by Ian Smith and others from 1985-90 (Table 1). The range of white sucker liver tumor neoplasm incidence from studies of that era indicated that the Thunder Bay and Jackfish Bay locations were elevated compared to the norm (Baumann et al. 1996). However external neoplasm percentages did not appear to be as elevated, since such neoplasms occurred in the early and mid 1980s in close to 40% of the white suckers sampled from Hamilton Harbour (Cairns and Fitzsimons 1988 and Smith et al. 1989).

Table 1. Neoplasm prevalence in white suckers documented from studies carried out from 1985-90 (Baumann et al. 1996), including sample size for external tumors (E) and for liver tumors (L).

Location	Thunder Bay	Jackfish Bay	Mountain Bay
Sample Size	**E=199; L=112**	**E=300; L=194**	**E=304; L=75**
External Neoplasm %	**2.5%**	**7.6%**	**3.6%**
Liver Neoplasm %	**7.1%**	**7.2%**	**2.6%**

Methodology:

A sample size of one hundred white sucker (Catostomus commersoni) from the Jackfish Bay and Thunder Bay Areas of Concern and the Mountain Bay reference location as well as 100 shorthead redhorse ((*Moxostoma macrolepidotum*) from the St. Clair River Area of Concern and the corresponding Lake Huron reference location at the head of the St. Clair River were collected. The Lake Superior sites were sampled by overnight gill net or hoop net sets, the St. Clair River exposed site was sampled using a Smith Root electrofishing boat and the Lake Huron reference site was sampled by overnight trap nets set by Purdy Fisheries. Following capture, fish were placed into a live well for transportation to the sampling site. Fish were anaesthetized in a clove oil bath (~0.05% + ~0.025% ethanol to aid emulsification), then were sacrificed using standard operating procedures, and their physical state was assessed using a visual examination of physical abnormalities. Fork length (mm) and weight (g) were measured and operculae were collected for aging. The liver was removed and separated into sections for histology (Blazer et al., 2007) and were stored in Davidson's Fixative and transferred to 70% ethanol 1-4 weeks after collection.

Histological Evaluation:

The tissues were processed at the Freshwater Institute, in Winnipeg, Manitoba. Prior to processing, the tissues were trimmed into an appropriate number of sub-samples (1 to 7) based on the original size of the sample. A small slice of tissue, between adjacent sub-samples, was removed and discarded. The sub-samples were processed in a routine ethanol/toluene series and individually embedded in paraffin blocks. The embedded tissues were sectioned at 4 – 6 microns and one slide, each with three tissue sections, was prepared from each block. The slides were stained with Harris hematoxylin and eosin.

Slides were examined with a Zeiss Photomicroscope III with Plan lenses and an Olympus Q-Color 3 digital camera. Images were captured from the system using QCapture Suite (Q-Imaging Corp.) software (Version 2.70.0 for Windows) at 2082 x 1542 pixel resolution. Brightness/contrast adjustments were performed in Adobe Photoshop 6.0 for Windows (Adobe Systems, Inc., San Jose, CA).

The data was presented with a 1 indicating the presence of a particular lesion and 0 indicating the absence of the indicated lesion. The proliferative lesions of the white sucker and the shorthead redhorse liver were categorized as non-neoplastic or neoplastic as described by Blazer et al., (2007).

The non-neoplastic hepatocellular lesions included the 4 types of foci of cellular alteration based on tinctorial characteristics of the hepatocyte cytoplasm. The non-neoplastic biliary lesion included only bile duct hyperplasia.

The neo-plastic hepatocellular lesions included hepatocellular adenoma and hepatocellular carcinoma. The neoplastic biliary lesions included cholangioma and cholangiocarcinoma.

In addition, the presence of non-proliferative liver lesions was noted. Small accumulations of lymphocytes/leucocytes were recorded as “Inflammation”, melanomacrophage aggregates in numbers in excess of the norm were recorded as “Excess MA’s”, and focal areas (minor) of necrosis were noted as “Necrosis”.

The visible presence of any parasite(s) in each liver was noted (“Parasites”), as were granulomata (”Granuloma”), which were generally associated with parasites. Although many livers had minor areas of blood congestion (increased blood vessel size and blood flow to an area), those with excessive areas were noted (“Congestion”). Instances of cholangiofibrosis (Baumann et al. 1990) were reported (“Cholangiofibrosis”). This was largely composed of large encapsulating masses that encircled six or more normal-appearing bile ductules. Under “Other Lesions” minor biliary fibrosis and other anomalies were reported.

Types of Lesions:

The use of external lesions including lip papillomas as a criteria related to carcinogen exposure is not recommended. Epidermal papillomas affecting white suckers come in several morphologically distinct varieties, some of which are known to regress under laboratory conditions (Smith and Zajdlik, 1987). Subsequently certain types of papilloma were demonstrated conclusively to be caused by a retrovirus using cell-free transmission experiments (Premdas and Metcalfe, 1996). These same authors believed that the etiologies of such tumors in wild fish would be multifactoral, with induction and progression of a virally induced lesion being influenced by environmental factors. It is our current inability to tease apart the interaction of contaminants and virus infection that prevents us from confidently using external lesions as BUI evaluation criteria. However, it is highly probable that liver lesions in white suckers from the Great Lakes are caused by chemical contaminants (Baumann et al. 1996). In particular polynuclear aromatic hydrocarbons (PAHs) have been proven to induce liver cancer in fish, and other compounds may also be carcinogenic to this species (Balch et al. 1995). Also no liver cancer in any species of fish has ever been diagnosed with a viral etiology (Dr. John Harshbarger, Director of the Tumor Registry in Lower Animals, Smithsonian Institution, Washington, DC, personal communication).

The original wording of the 'Fish Tumors or Other Deformities' BUI included the occurrence of "neoplastic or preneoplastic liver tumors in brown bullhead or suckers". However, no specifics were given for the definition of preneoplastic lesions. Foci of cellular alteration, depending upon morphological and staining characteristics, can be classified as basophilic, eosinophilic, vacuolated, and clear cell. Basophilic foci have been reported to advance to hepatocellular carcinoma in several species of fish (Blazer et al. 2006). However not all basophilic foci advance (Hinton et al. 1988), and the number of fish with basophilic foci from the two Lake Superior AOCs and one reference site only varied from 2% to 4%. There is no definitive evidence that other types of altered foci progress to neoplasia (Bunton, 1996). No studies on progression of any foci of cellular alteration have been performed on suckers or bullhead. Liver tumors in fish are, with rare stem cell exceptions, derived from either liver cells (hepatocellular) or bile duct cells (cholangiocellular). No non-neoplastic cholangiocellular changes, such as bile duct hyperplasia and cholangiocellular fibrosus, have been experimentally demonstrated as progressing to tumors. Such proliferation of bile duct epithelial cells has been demonstrated following laboratory carcinogen exposure in a number of species (Blazer et al. 2009). Similarly, such lesions have been reported along with tumors in wild populations from contaminated locations (Blazer et al. 2009). However, at least in bullhead, a myxozoan parasite has also been implicated in bile duct proliferation and fibrosus (Baumann et al. 2008). Because of the uncertainties concerning progression of both foci of cellular alteration (hepatic) and cholangiocellular proliferation and fibrosus (biliary), it is best that none of these preneoplastic lesions be used as an actual delisting criterion.

Age and Gender:

Two variables which might influence tumor prevalence are the age of the fish and fish gender. Age has long been recognized as being positively correlated with tumor prevalence (Baumann, 1992). This is not only because fish that have lived longer have usually been exposed to environmental contaminants longer, but also because there is a latent period between induction and tumor development. For instance the prevalence of spontaneous neoplasms in medaka

(*Oryzias latipes*) of ages 1 through 5 was greatest in females of age 4 and 5 and males of age 5 (Masahito et al. 1989). This same positive correlation between age and tumor prevalence has also been noted in wild populations of several species exposed to contaminants. English sole from contaminated locations in Puget Sound had a nearly 40% increased probability for having a hepatic neoplasm with each additional year lived (Rhodes et al. 1987). Similarly bullhead from the Potomac River also had an increased risk of hepatic carcinomas with age (3.5 times greater per year) (Pinkney et al. 2001). Brown bullhead from the Black River, Ohio were found to have a significantly ($p<0.05$) higher prevalence of biliary liver cancers at ages 4 and 5 (35.5%) than at ages 2 and 3 (18.4%) (Baumann et al. 1990). Blazer (2009) also reported an increasing prevalence of liver tumors with age in bullhead from Presque Isle Bay, particularly at ages 8 and older. Furthermore Slooff (1983) found that of 7,209 bream necropsied in Europe, all fish with grossly visible tumors were age 7 or older. White sucker have also shown this age and neoplasm link. In samples from five locations in the St. Lawrence Basin lip neoplasms occurred almost exclusively in fish >350mm (length being an age surrogate) (Mikaelian et al. 2000). Thus it is important to consider age when comparing neoplasm prevalence among populations.

Gender related differences in tumor prevalence have been less consistently reported than age related differences, particularly in wild exposed populations. Several species of laboratory fish have been reported to have a higher prevalence of spontaneous tumors in females (Baumann 1992). However gender was not a significant factor in the prevalence of hepatic lesions in English sole from Puget Sound (Rhodes et al. 1987). Female brown bullhead from the Black River, Ohio had a significantly higher ($P<0.05$) incidence of hepatocellular carcinoma only, but not of any other neoplasms. A review of Great Lakes brown bullhead data taken at United States locations since 1991 reinforces the view that gender differences are not discernable. However, an analysis of the brown bullhead data base for Chesapeake Bay found that being female was a significant ($P<0.001$) positive co-variant for liver neoplasms (Pinkney et al. 2009). Gender equivalency among samples should be considered for comparative purposes.

Variability and Statistics:

Determining whether a fish has a tumor provides a "yes" or "no" answer (binary response) rather than a number. Thus contingency table analysis is required for statistical differentiation of population values. Such statistics will test whether two locations have meaningfully different results at some level of confidence. The level of confidence is determined by selecting a P value to indicate significance. The typical P value for biological studies is 0.05 (a 5% or one in twenty random chance of being wrong). Thus P values less than or equal to 0.05 would indicate a real difference between the tumor prevalence at the sites being compared.

There are two methods which are commonly used to compute a P value from a contingency table: Chi-square and Fisher's exact test. Fisher's exact test gives the exact P value, while the Chi-square test calculates an approximate P value (Graphpad Software 2009). Chi-square often works better with multiple rows and columns, but the data here only has two of each. Additionally, Fisher's exact test is supposed to perform better when the expected values are small, which is the case here. Thus Fisher's exact test was used to determine the P values when comparing tumor prevalence at AOC locations and reference sites. Statistical calculations were done using a QuickCalcs online calculator by GraphPad Software (Graphpad Software 2009). This software includes a statement acknowledging that the Fisher's test actually has three methods that can be used to compute the two-sided (two-tailed) P value. The software used here incorporated the method of summing small P values.

Results:

White sucker were collected at two AOCs on Lake Superior: Thunder Bay and Jackfish Bay. The same species was collected at Mountain Bay on Lake Superior, as a reference location. Shorthead redhorse were collected at the St. Clair AOC, and Lake Huron at the head of the St. Clair River was used as a reference site. Fish were captured using electrofishing, gill nets, and trap and hoop nets, some run by commercial fishermen. All locations had at least one hundred fish collected (Table 2). Liver sections from the Lake Superior fish averaged around four per individual, while sections from both redhorse populations averaged less (Table 2). Females comprised 48% to 55% of the Lake Superior white sucker collections and 41% to 42% of the St. Clair River and Lake Huron redhorse collections, making each group of reference and AOC locations comparable in gender. Ages varied from a median of 6 to a median of 11, and will be discussed within the individual AOC impairment conclusions sections. Neoplasms were rare at both AOC and reference locations (Table 3). None of the five locations sampled had a neoplasm prevalence that exceeded 2%. All three locations in Lake Superior had a smaller percentage of fish with neoplasms than they had in the late 1980s (Table 1). White suckers from Thunder Bay and Jackfish Bay had declined in liver neoplasm prevalence by over 5% and 7% respectively. This decrease at Jackfish Bay was statistically significant. None of the AOCs differed significantly from their respective reference locations in the proportion of the population found to have liver neoplasms.

Table 2. Sample size, age, gender proportion and number of liver sections taken from three northern AOCs and two reference locations in 2006 and 2007.

Location	Sample Size	Median Age	Percent Female	Sections/Liver
Thunder Bay	100	6	48.5%	4.6 (average)
Jackfish Bay	100	9	50%	3.75 (average)
Mountain Bay	100	11	55%	3.9 (average)
St Clair River	126	10	41%	2-4 (range)
Lake Huron	100	6	42%	2-4 (range)

Table 3. Upper Great Lake AOCs (Thunder and Jackfish Bays and the St. Clair River) and reference locations (Mountain Bay (Lake Superior) and Lake Huron) tumor prevalence, and the significance of differences between AOCs and reference sites (2006 and 2007).

Location	Sample Size	Neoplasm #	% Neoplasms	Significance
Thunder Bay	100	2	2%	None
Mountain Bay	100	0	0%	
Jackfish Bay	100	0	0%	None
Mountain Bay	100	0	0%	
St Clair River	126	0	0%	None
Lake Huron	100	1	1%	

Conclusions by AOC:

Thunder Bay AOC:

In the late 1980s Thunder Bay was determined to have a liver neoplasm prevalence of 7.1% (Table 1). That frequency of liver tumor would have been viewed as elevated, and helped to assign a fish tumor BUI to the AOC. However a sample of 100 white suckers revealed that in the 2007 population of white sucker in Thunder Bay the tumor prevalence had declined to 2%. This neoplasm occurrence is not significantly different from the white sucker reference location rate (Table 3), nor would it be significantly different from the brown bullhead reference neoplasm prevalence. The 2% prevalence is also not significantly different from the 1980's 7.1% prevalence at the P= 0.05 level usually accepted. However this may well be due to relatively low sample sizes, as the P level was 0.1. In other words, even with relatively limited data there is only a one in ten chance that the actual population neoplasm prevalence has not declined. However the median age of these fish is 5 years younger than the reference location and three years younger than Jackfish Bay. This is partially compensated for by the more numerous liver sections examined. An additional survey of 100 fish is recommended, using a length cut-off to reduce younger age groups. Such a survey emphasizing older fish would add certainty to the decision on the status of this Beneficial Use. **If the results of the additional fish survey indicate a tumor prevalence of less than 5%, then the status of this Beneficial Use should be changed to Not Impaired.**

Jackfish Bay AOC:

In the late 1980s Jackfish Bay had a liver neoplasm prevalence of 7.2% (Table 1). That frequency of liver tumor would have been viewed as elevated, and helped to assign a fish tumor BUI to the AOC. However a sample of 100 white suckers taken in 2006 did not reveal any liver neoplasms. This is, of course, not statistically different from the neoplasm prevalence at the Mountain Bay reference location. Furthermore Fisher's exact test demonstrates that the liver neoplasm prevalence in the 2006 sample was significantly lower ($p<0.01$) than in the sample from the 1980s. This verification of a lower tumor prevalence was helped by the robust size of the 1980s sample taken for liver pathology (n=194). Although the median age is two years younger than the Mountain Bay reference location, at 9 years of age this is not a deterrent to delisting. **The status of this Beneficial Use can now be considered to be Not Impaired**. No further monitoring specifically for tumors is needed.

St. Clair River AOC:

This location was not listed among the older (1980s and early 1990s) studies demonstrating tumor epizootics (Baumann et al. 1996). Concerns for fish tumors might have been raised by the perception that external walleye lesions, probably with a viral etiology, seemed more common in the AOC population (Myllyoja and Johnson, 1995). However no tumors were seen in the shorthead redhorse samples taken in 2002, 2003, and 2006. Reference samples from Lake Huron had a 1% prevalence of tumors, which matches the prevalence in the brown bullhead reference data base. The male/female ratio was similar at the AOC and reference location, as were the number of sections taken per liver. The sample population from the St. Clair River was markedly older (4 years) than that from the reference site (Table 2). Although this should imply that the tumor prevalence would also be greater because of the older age (as discussed previously), in actuality, the tumor prevalence was not greater. **The status of this Beneficial Use can now be considered to be Not Impaired**. No further monitoring specifically for tumors is needed.

References

Balch, G.C., C.D. Metcfalfe, and S.Y. Huestis. 1995. Identification of potential fish carcinogens in sediment from Hamilton Harbour, Ontario, Canada. Envir. Tox. and Chem. 14:79-91.

Baumann, P.C., W.D. Smith, and M. Ribick. 1982. Polynuclear aromatic hydrocarbons (PAH) residue and hepatic tumor incidence in two populations of brown bullhead (Ictalurus nebulosus). In: Polynuclear Aromatic Hydrocarbons: physical and biological chemistry. Marcus Cook, Anthony J. Dennis, and Gerald Fisher, Eds. Batelle Press, Columbus, OH. PP. 93-102.

Baumann, P.C., J.C. Harshbarger, and K.J. Hartman. 1990. Relations of liver tumors to age structure of brown bullhead populations from two Lake Erie tributaries. Science of the Total Environment. 94:71-88.

Baumann, P.C. 1992. Methodological considerations for conducting tumor surveys of fishes. Journal of Aquatic Ecosystem Health, 1:33-39.

Baumann, P.C., I.R. Smith, and C.D. Metcalfe. 1996. Linkages between chemical contaminants and tumors in benthic Great Lakes fishes. J. Great Lakes Res. 22(2):131-152.

Baumann, P.C., D.R. LeBlanc, V.S. Blazer, J.R. Meier, S.T. Hurley, and Y. Kiryu. 2008. Prevalence of tumors in brown bullhead from three lakes in Southeastern Massachusetts, 2002. US Geological Survey, Scientific Investigations Report 2008-5198.

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

Blazer, V.S., J.W. Fournie., J.C. Wolfe, and M.J. Wolfe. 2007. Manual for the Microscopic Diagnosis of Proliferative Liver and Skin Lesions in the Brown Bullhead (Ameiurus nebulosus). Pennsylvania Sea Grant.

Blazer, V.S., Rafferty, S.D., Baumann, P.C., Smith, S.B., and Obert, E.C. 2009. Assessment of the "fish tumors or other deformities" beneficial use impairment in brown Bullhead (*Ameiurus nebulosus*): II. Liver neoplasia. J. Great Lakes Res. 35(4): 527-537.

Bunton, T. E. 1996. Experimental chemical carcinogenesis in fish. Toxicol. Pathol. 24:603-618.

Cairns, V.W. and J.D. Fitzsimons. 1988. The occurrence of epidermal papilloma and liver neoplasia in white suckers (*Catostomus commersoni*) from Lake Ontario. In Proc. 14th Ann.

Aquatic Tox. Wkshop, Nov. 1987, pp.151-152, Toronto, Ontario, Can. Nimi, A.J. and K.R. Solomon (eds). Tech. Rep. Fish. Aquat. Sci. No. 1607.

Dawe, C.J., M.F. Stanton, and F.J. Schwartz. 1964. Hepatic neoplasms in native bottom-feeding fish of Deep Creek Lake, Maryland. Cancer Research. 24:1194-1201.

Graphpad Software. 2009. QuickCalcs online calculators for scientists. Available at: http://www.graphad.com/quickcalcs/Contingency1.cfm

Hinton, D.E., J.A. Couch, S.J. The, and L.A. Courtney. 1988. Cytological changes during the progression of neoplasia in selected fish species. Aquat. Toxicol. (Amst.). 11:77-112.

International Joint Commission. 1991. Proposed listing/delisting guidelines for Great Lakes Areas of Concern. Focus on International Joint Commission Activities. Volume 14, Issue1, Insert.

Masahito, P., K. Aoki, N. Egami, T. Ishikawa, and H. Sugano. 1989. Life-span studies on spontaneous tumor development in the medaka (*Oryzias latipes*). Japan J. Cancer Res. 80:1058-1065.

Mikaelian, I., Y. de Lafontaine, P. Gagnon, C. Menard, Y. Richard, P. Dumont, L.Pellestier, Y. Mailhot, and D. Martineau. 2000. Prevalence of lip neopolasms of white sucker (*Catostomus commersoni*) in the St. Lawrence River basin. Can. J. Fish. Aquat. Sci. 57(S1):174-181.

Myllyoja, R. and G. Johnson. 1995. St. Clair Remedial Action Plan Fish Tainting Evaluation – Draft. Retrieved from: http://www.friendsofstclair.ca/pdf/FISH%20tainting%20evaluation_1995.pdf

Pinkney, A.E., J.C. Harshbarger, E.B. May, and M.J. Melancon. 2001. Tumor prevalence and biomarkers of exposure in brown bullheads (*Ameiurus nebulosus*) from the tidal Potomac River, USA, watershed. Environ. Toxicol. Chem. 20:1196-1205.

Pinkney, A.E., J.C. Harshbarger, and M.A. Rutter. 2009. Tumors in brown bullhead (*Ameiurus nebulosus*) in the Chesapeake Bay watershed: analysis of survey data – 1992 through 2006. J. Aquatic Animal Health. 21:71-81.

Premdas, P.D. and C.D. Metcalfe. 1996. Experimental transmission of epidermal lip papillomas in white sucker, *Catostomus commersoni*. Can.J. Fish. Aquat. Sci. 53(5):1018-1092.

Rafferty, S. and J. Grazio. 2006. Field Manual for Assessing Internal and External Anomalies in Brown Bullhead (Ameiurus nebulosus). Pennsylvania Sea Grant. U.Ed. EBO 07-267.

Rhodes, L.D., M.S. Myers, W.D. Gronlund, and B.B. McCain. 1987. Epizootic characteristics of hepatic and renal lesions in English sole, *Parophrys vetulus*, from Puget Sound. J. Fish Biol. 32:395-407.

Sloof, W. 1983. A study on the usefulness of feral fish as indicators for the presence of chemical carcinogens in Dutch surface waters. Aquat. Toxicol. 3:127-139.

Smith, I.R. and B.A. Zajdlik. 1987. Regression and development of epidermal papillomas affecting white suckers, *Catostomus commersoni* (Lacepede), from Lake Ontario, Canada. J. Fish. Dis. 10(6):487-494.

Smith, I.R., H.W. Ferguson, and M.A. Hayes. 1989. Histology and prevalence of epidermal papilloma epidemic in brown bullhead *Ictalurus nebulosus*, and white sucker *Catostomus commersoni* populations from Ontario, Canada. J. Fish Dis. 12:373-388.

Sonstegard, R.A. 1977. Environmental carcinogenesis studies in fishes of the Great Lakes of North America. Ann. N.Y. Acad. Sci. 298:261-269.