1-1-2012

A Study Design For The Assessment Of Fish Tumour Prevalence In The Lower St. Clair River

Liliya Baranova
Ryerson University

A STUDY DESIGN FOR THE ASSESSMENT OF FISH TUMOUR PREVALENCE IN THE LOWER ST. CLAIR RIVER

by

Liliya Baranova
Honours Bachelor of Science with Specialization in Biology with Minor in Statistics
University of Ottawa, Ottawa, Ontario
2010

A Professional Project Paper presented to Ryerson University
in partial fulfillment of the requirement for the degree of
Master of Applied Science
In the Program of Environmental Applied Science and Management

Toronto, Ontario, Canada, 2012

AUTHOR'S DECLARATION FOR ELECTRONIC SUBMISSION OF A PROJECT PAPER.

I hereby declare that I am the sole author of this project paper. This is a true copy of the project paper including any required final revisions, as accepted by my examiners.

I authorize Ryerson University to lend this project paper to other institutions or individuals for the purpose of scholarly research I further authorize Ryerson University to reproduce this project paper by photocopying or by other means, in total or in part, at the request of other institutions or individuals for the purpose of scholarly research.

I understand that my project paper may be made electronically available to the public

A study design for the assessment of fish tumour prevalence in the Lower St. Clair River

Liliya Baranova, 2012
Master of Applied Science
Environmental Applied Science and Management
Ryerson University

ABSTRACT

A conclusive fish tumour prevalence assessment has never been conducted in the lower part of the St. Clair River Area of Concern, despite possible re-contamination of the river and anecdotal evidence of fish abnormalities. This paper provides a study design for a comprehensive fish tumour prevalence assessment of the Lower St. Clair River with special focus on Walpole Island First Nation and surrounding waters. Study details such as area of focus, sentinel species, suggested sampling locations, sample size, field protocols and statistical methods are identified. A brief guide for histopathological examination and interpretation is provided. An alternate method of sampling location siting is suggested. This study design is intended to provide a guide and background reference for the implementation of a future full scale fish tumour assessment in the Lower St. Clair River.

ACKNOWLEDGEMENTS

First and foremost I would like to extend my sincere gratitude to my supervisors, Dr. Andrew Laursen and Dr. Ron Pushchak, who have provided me with invaluable guidance and support throughout the past two years. We have encountered great challenges together, and you have always encouraged me to persevere, in the completion of this project, despite that. I am especially thankful for the patience and understanding that you have shown me, for I know well that it must not have been easy at times. I feel very lucky to have been guided through this process by two such excellent academic figures and human beings. Thank you.

Special thanks to Dr. Lynda McCarthy, who prompted me to address necessary aspects of my work and think critically at just the right time. This feat would have taken me much longer to complete if not for you.

Thank you to the Walpole Island First Nation Heritage Center for welcoming our questions and proposals, and for their assistance in establishing potential research collaboration.

My sincere thanks to Nick Mandrak and Jason Barnucz, at Fisheries and Oceans Canada, for providing information and data about previous fish surveys of the St. Clair River. Thank you to Dr. J.S. Lumsden at Guelph University for answering my infinite questions and providing key advice about fish histopathology and preparation for laboratory examination.

I am very grateful to Maral Yazdandoost, my teaching assistant for the Vertebrate Histology course at the University of Toronto in Scarborough. You've helped me immensely in acquiring knowledge of this complex subject, and opened up my interests to a new academic field.

Thank you also to my fellow graduate student colleagues, in particular Annette Maher and Dan Beare, who were always there for me in all our academic endeavours, and soon became lifelong friends.

And last, but most definitely not least, thank you to my parents, brother, grandparents and friends for encouraging me to undertake this feat, listening to all my whining, supporting me, and criticizing me just when I needed it. I could not have gotten this far without you.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1.0 INTRODUCTION

Pollution of aquatic systems has been a topic of serious concern for the past several decades due frequent discharges of industrial, agricultural and domestic waste into the environment. Fish, and other aquatic organisms, are often exposed to contaminated water as a result of these discharges. The effects of this pollution are widespread and affect entire ecosystems, especially when there are multiple contaminants which do not break down easily and are potentially biologically active. In fish, water pollution can cause individual biological changes on a cellular level, potentially leading to tumours and other abnormalities (Bernet et al., 1999). On a greater scale, water pollution can affect the entire population structure and species diversity (Sheenan, 1984) of fish and other aquatic organisms (Borowitzka, 1972; Camargo and Alonso, 2006; Livingstone, 2001).

This paper describes the process of designing a comprehensive survey of a fish population to determine if prevalence of tumours and abnormalities is above background rates. It focuses on the lower St. Clair River, with specific relevance to waters surrounding Walpole Island First Nation (WIFN). A survey undertaking such as the one proposed is necessary in the Lower St. Clair River region because there currently exists no complete, recent data on the prevalence of fish tumours in this Area of Concern (AOC), despite the concern of local residents and fishermen. The purpose of this paper is to establish guidelines for conducting such as survey, defining sampling and assessment criteria, and establishing a rationale for selection of reference and sampling locations. Protocol for effective sampling fish in the Lower St. Clair River, and guidance for histopathological interpretation is obtained from a literature review of similar fish tumour prevalence assessments in other AOCs. The ultimate intent of such a study design is to provide necessary background information for a full scale study, assessing fish tumour prevalence, to be implemented in the future.

1.1 Study Area Background

The St. Clair River is a bi-national portion of the Great Lakes Seaway system, separating Michigan, U.S and Ontario, Canada. The river flows 64km from the tip of Lake Huron to the mouth of Lake St. Clair. It is an important channel for shipping and industry and supports several sizeable communities along its shores. At the southern end, the river forms a large delta with many channels and wetlands where it meets the lake. This delta is a transitional environment between the river and the lake and provides important habitat for many species. The river above the delta is a fairly straight and uniform channel with only two islands, Stag Island and Fawn Island, and now has mostly artificial shoreline (Environment Canada, 1991). Because of this it has relatively high flows, with an average flow velocity reaching 3.2 km/hr (Manny et al., 1988).

Forty-five species of fish have been found in the St. Clair River, including the wetland areas (Manny et al. 1988). Many of these fish spend the majority of their lives in the river, with white suckers *(Catostomus commersonii)*, common carp *(Cyprinus carpio)* and perch (*Percidae* family) being the most abundant (Edwards et al., 2006). Some of the other species use macrophyte rich areas of the river for spawning, nursery and feeding; alewife *(Alosa pseudoharengus)*; rainbow smelt *(Osmerus mordax)*; gizzard shad *(Dorosoma cepedianum)* and rock bass *(Ambloplites rupestris, Ambloplites constellatus)* are the most common of these (Mandrak and Crossman, 1992). The St. Clair River is also sustains impressive populations of several important sport fish including northern pike *(Esox Lucius)*, muskellunge *(Esox masquinongy)*, walleye *(Sander vitreus)*, yellow perch *(Perca flavescens)*, smallmouth bass *(Micropterus dolomieu)*; and largemouth bass *(Micropterus salmoides)* (Manny et al., 1988).

Since 1985, the St. Clair River has been designated an Area of Concern (AOC) under the Canada – United States Great Lakes Water Quality Agreement, which defines it as a site in the Great Lakes system where environmental quality is significantly degraded and beneficial uses are impaired (International Joint Commission, 1991). The borders of this AOC contain the majority of the river, spanning from the Blue Water Bridge, near Sarnia, to the southern tip of Seaway Island, west to St. John's Marsh and east to include the north shore of Mitchell's Bay on Lake St. Clair in Ontario, including the delta channels of Walpole Island, and the main river's immediate drainage basin consisting of five sub-watersheds (Figure 1) (Environment Canada and Ontario Ministry of Environment, 2011).

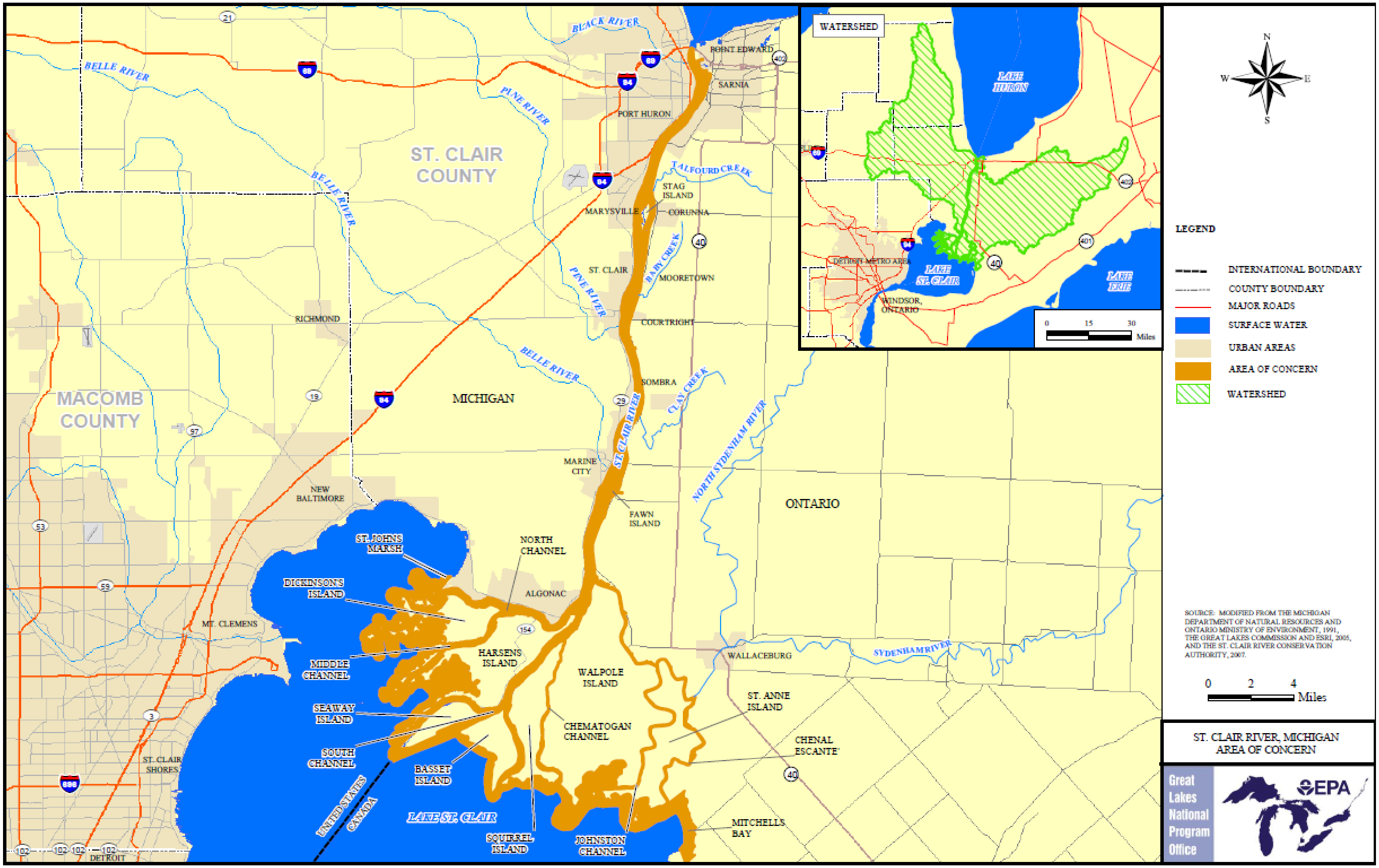


Figure 1. St. Clair River AOC boundaries. (U.S. Environmental Protection Agency, 1994)

Walpole Island, at the southernmost end of the AOC, consists of six individual islands, each of which is separated by a series of channels, part of the St. Clair River Delta. It has been occupied by the Walpole Island First Nation (WIFN) for thousands of years. This land has one of the most diverse ecosystems in the Great Lakes drainage basin, including one of the richest wetlands. The area is so abundant with natural resources that many First Nations citizens still support their families through hunting, fishing, trapping and guiding activities, with recreational tourism as their primary industry (Smith, 2002). In 2008, a new water treatment facility was constructed on the island to serve its 2,200 inhabitants (Aboriginal Affairs and Northern Development Canada, 2005). Despite the significant upgrade in the level of water treatment provided to the island, the water is still sourced directly from the St. Clair River (Figure 2). Hence, this vulnerable population still has the potential to be exposed to contaminants in their drinking water.

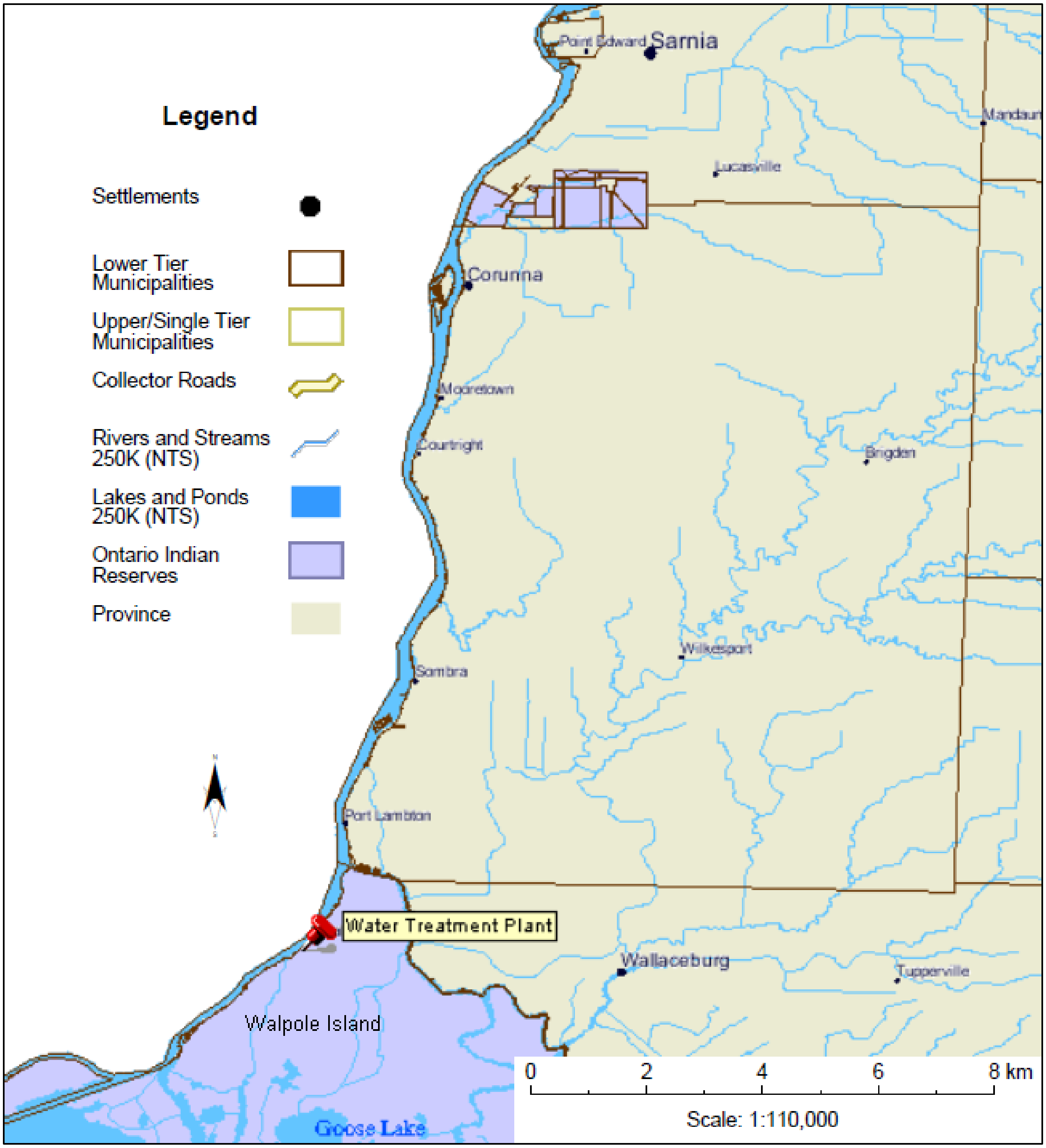


Figure 2. Location of Walpole Island Water Treatment Plant.

The original Remedial Action Plan (RAP) set up to deal with the status of the St. Clair River AOC identified fourteen Beneficial Use Impairments (BUIs), defined as "a change in the chemical, physical or biological integrity of the Great Lakes System sufficient to cause any impairment of the following:

1) restrictions on fish and wildlife consumption

2) tainting of fish and wildlife flavour;

3) degradation of fish wildlife populations;

4) fish tumours or other deformities;

5) bird or animal deformities or reproduction problems;

6) degradation of benthos;

7) restrictions on dredging activities;

8) eutrophication or undesirable algae;

9) restrictions on drinking water consumption, or taste and odour problems;

10) beach closings;

11) degradation of aesthetics;

12) added costs to agriculture or industry;

13) degradation of phytoplankton and zooplankton populations; and

14) loss of fish and wildlife habitat" (CRIC Delisting Working Group [CRIC], 2011).

These BUIs were largely a result of the contamination present in the St. Clair River at that time. The sediments were heavily affected by nutrient loading, and elevated concentrations of metals and organic compounds (ENVIRON International Corporation, 2009).

These conditions stemmed from prolonged periods of industrial development in and around the City of Sarnia, and along the eastern shore of the river. In the 1940s, several petrochemical facilities emerged in this area in support of the war effort. Sediment impairment linked to this development was detected some decades later, as impacts on benthic communities were noted by scientists at the mouth of the river (Ontario Ministry of Environment, 1979). From this point onwards, there have been intermittent efforts to restore the ecological health of the affected waterway, and by 1990 the extent of the most heavily impacted area was considered to be greatly reduced (ENVIRON International Corporation, 2009). Acknowledging the improvements made through the Remedial Action Plan since the time of its inception, the status of four of the ten BUIs has been re-designated. The status of the "tainting of fish and wildlife flavour" BUI has been changed to "not impaired", while the other three, including the fish tumours or other deformities BUI, now display a status of "requiring further assessment" (Environment Canada and Ontario Ministry of Environment, 2011).

However, the effect of contaminant discharge to the river continues to be of great concern. Currently, the primary sources of contaminants originate from a complex of twenty seven industrial facilities on the Canadian side and six in the United States. Other sources of contaminants include ten municipal point sources and associated non-point sources such as agricultural runoff (Environment Canada and Ontario Ministry of Environment, 2011).

1.2 Historical Fish Tumours and Abnormalities

Fish abnormalities were first noted in the scientific literature as far back as 1925 (Osburn, 1925). However, a scientific link between the presence of fish tumours and aquatic contaminants was not drawn until several decades later. In 1964, Dawe et al. reported the first benign liver tumours discovered in a population of wild fish (white suckers) from Deep Creek Lake, MD, which they presumed to be the result of chemical contaminants in the aquatic environment. In 1977, McCain reported biliary tumours in white perch *(Morone americana)* collected from the tributaries of Chesapeake Bay, MD and several more incidences of liver tumours in English sole *(Parophrys vetulus)* from Puget Sound, WA. Subsequently, incidences of malignant liver tumours were discovered by Black (1984) in sauger *(Sander canadensis)* and walleye from Torch Lake, MI as well as by Smith et al. (1979) in tomcod *(Microgadus tomcod)* from the Hudson River, NY.

The first Canadian fish tumour study in the Great Lakes was published by Sonstegard in 1977, linking environmental carcinogens to superficial tumours in white suckers. The trend of liver tumours in brown bullhead *(Ameiurus nebulosus)* from the Great Lakes drainage basin was first reported by Baumann et al. (1982) in the Black River, Ohio. Since then, an elevated prevalence of fish tumours has been demonstrated in 15 species from approximately 50 polluted sites in Great Lakes bays and tributaries, both in Canadian and United States waters (Baumann et al. 1996; Baumann 1998; Harshbarger, 1991). Table 1 provides a summary of historical tumour prevalence rates from polluted waters of the Great Lakes drainage basin. Common contaminants in these studies included heavy metals, organochlorines (e.g. PCBs, DDT) andaromatic compounds (e.g. PAHs). The vast majority of the sentinel species used in these studies is brown bullhead, due to their elevated susceptibility to exposure by contaminants concentrated in the sediments (Harshbarger, 1991). Although liver tumours in suckers have been less frequently documented in the Great Lakes, elevated prevalence of several types of tumours have been found in white suckers from Lake Ontario (Hayes et al., 1990).

Since then various laboratory exposure and field studies have confirmed that liver lesions in wild fish are induced by chemical contaminants such as metals, PCBs and PAHs (Baumann et al. 1996; Baumann and Okihiro 2000). Field studies have correlated a dramatic decline in tumour incidence with a decline in sediment contamination (Baumann and Harshbarger, 1995) (Table 1) and have shown that fish exposed to elevated contaminant concentrations in the wild had significantly higher liver tumour prevalence than those that were not (Vogelbein et al. 1990; Baumann and Okihiro, 2000). Thus liver tumours are consistent markers of chemical contaminant exposure.

Table 1. Summary of historical fish tumour prevalence rates from surveys conducted in North American polluted waters.

Author	Type of tumour	Location	Year	Fish Age / Size	Impacted Site Prevalence	Reference Site Prevalence
Harshbarger et al., 1984	skin and/or liver neoplasms	Black River, Lorain, OH			33.0%	
Smith et al., 1994	neoplastic liver lesions	Black River, OH	1986-1987	>250 mm	35.0%	
		Cuyahoga River, Cleveland, OH	1986-1987	>250 mm	21.0%	
		Huron River, OH	1986-1987	>250 mm		1.0%
[a]Baumann and Harshbarger, 1995	hepatocellular carcinoma and cholangiocarcinoma	Black River, OH	1982	3 yrs	31.2%	
				4 yrs	41.1%	
			1987	3 yrs	2.1%	
				4 yrs	6.9%	
[b]Baumann and Harshbarger, 1998	hepatocellular carcinoma and cholangiocarcinoma	Black River, OH	1993	3 yrs	41.0%	
			1994	3 yrs	0.0%	
Leadley et al., 1998	cholangiocarcinoma	Trenton Channel, Detroit River			20.0%	
		Peche Island, Detroit River				4.0%
Pinkney et al., 2001	liver tumour	Anacostia River, Potomac River		>260 mm	55.0%	
		Neabsco Creek, Potomac River		>260 mm	17.0%	
		Quantico embayment, Potomac River		>260 mm	7.0%	
		Tuckahoe River, MD		>260 mm		10.0%

[a] Fish exposed during 1982 coke plant operation; fish not hatched until after closure of coke plant in 1987.

[b] Age 3 fish exposed by re-suspension of contaminated sediments during dredging in 1990; age 4 fish not hatched until after 1990 dredging.

A more recent fish tumour BUI assessment for the Lower Great Lakes, conducted on behalf of Environment Canada, reported more conservative tumour rates in Great Lakes AOCs (Table 2) (Baumann, 2010). Perhaps this is due to more stringent definitions of which tumours were quantified or perhaps due to improved aquatic conditions in affected areas since the 1990's. This study also produced a background tumour prevalence estimate at relatively unpolluted reference sites throughout the Great Lakes system, which was used to determine significant differences at the AOC locations. A database of 1150 sampled fish from urbanized and non-urbanized reference locations was used to determine a 2% Impairment Criterion against which AOC tumour prevalence rates could be compared (Baumann, 2010).

Table 2. Summary of current tumour prevalence rates in Great Lakes AOCs with sample sizes, ages, gender percentage, neoplasm numbers and prevalence, and significant differences (S), or not (N), from the impairment criterion. (Baumann, 2010)

Exposed Site	Sample Size	Median Age	% Female	Neoplasm # (%)	P Value	Significance
Wheatley Harbour	100	7	47%	4 (4%)	0.27	N
Niagara River	101	5	50%	3 (3%)	0.47	N
Hamilton Harbour	200	8	48%	11 (5.5%)	0.013	S
Toronto and Region	213	7	45%	8 (3.8%)	0.14	N
Bay of Quinte	100	5	42%	4 (4%)	0.27	N
St Lawrence River	100	5	46%	2 (2%)	1	N

Age and gender are biometric factors which may also influence the prevalence of liver tumours in fish. Several studies of tumour prevalence have shown that incidence of neoplasia in brown bullhead from polluted waters is higher in sexually mature fish older than three years of age (Hinton, 1989). This is partly an effect of longer exposure to the environmental contaminant, but also potentially a result of a latent period between exposure and tumour development (Baumann, 2010). For this reason, it is common for studies to have a minimum size requirement for sampled fish (Rafferty and Grazio, 2006).

Gender is a less influential factor in tumour development; however, there is still some indication that female fish are more likely to develop liver neoplasms than males of the same age (Baumann, 1992). A database of brown bullhead tumour prevalence data for Chesapeake Bay has shown that females had a significantly greater co-variance with liver neoplasms than did males (Pinkney et al., 2009). Therefore, it is best to ensure that equal proportions of male and female fish are collected in the field.

1. 3 Fish Tumour Definition

The Great Lakes Water Quality Agreement definition of the “fish tumour or deformity” beneficial use impairment requires the “presence of neoplastic or pre-neoplastic liver tumours” (International Joint Commission, 1991); however, it does not provide a clear explanation as to what this means. This element is especially pertinent when referring to pre-neoplastic tumours, where a variety of different studies have given their own definition to what is considered to be a true neoplasm. For this reason, it is essential to define tumours as they are to be considered throughout the study design.

The terms tumour, neoplasm and cancer are often used interchangeably. Generally, the term cancer denotes a fast spreading disease which has the capacity to spread (metastasize) throughout the body, ultimately killing the host (Black, 1984). Not all tumours are cancers, and for this reason it is important set a clear definition of the term “neoplasm”. The terms neoplasia (the disease process) and neoplasm (a tumor), include both the malignant and benign forms of the disease (Black, 1984). A typical neoplasm is characterized by independent growth of abnormal cells, which may proliferate to exert pressure on surrounding cells or invade healthy tissues, thereby causing destruction of cells and organs and interrupting normal physiological function (Black, 1984). There is a wide range of neoplasm growth potentials in fish, as in other animals such as humans. Very slow growing and localized neoplasms are referred to as benign adenomas. Those that invade the host's tissues and are malignant carcinomas, which are more threatening to the overall wellbeing of the individual than the former (Black, 1984).

The liver is a frequent site of tumours in fish, as it is the main organ responsible for detoxification. It is the function of liver enzymes to convert chemicals in the environment to polar metabolites, which are highly reactive and interact with critical components of the cell to initiate the cancer process (Fabacher and Baumann 1985). Fish like bullhead and suckers are often affliced by external lesions, such as lip papillomas. However, these are not as closely linked to chemical contaminant exposure. Certain types of papillomas have been demonstrated conclusively to be caused by a viral infection (Baumann and Okihiro 2000). Additionally, it should be noted that papilloma prevalence does not necessarily indicate an increased prevalence of liver tumours (Baumann et al., 1987).

1.4 Fish Tumours and Abnormalities in the St. Clair River

The St. Clair River Remedial Action Plan (RAP) reported that external tumours or skin lesions in fish such as walleye were cause for concern among local fishermen (Mayne, 2003). Additionally, the WIFN Heritage Center has expressed personal concern about fish collected from nearby waters displaying grossly visible deformities and lesions (R. Pushchak, Personal communication, Jan. 2011). Despite this public concern, there have been very few scientific studies assessing the extent of the problem, and prevalence data for fish tumours in the St. Clair River are scarce in the primary literature.

A study assessing sediment toxicity downstream of the Sarnia industrial complexes found several incidences of liver tumours and many more incidences of precancerous tissue changes in caged fish exposed to the effluent stream (ENVIRON International Corporation, 2009). However, this study provides little insight into sediment toxicity and fish exposure in areas further downstream. In 1999, a more comprehensive study assessing liver tumours was conducted, where liver samples from 63 fish representing 17 species from different trophic levels were evaluated using histopathologic criteria. No true tumours were found in any of the liver samples examined, and only one fish showed precancerous tissue changes of the type observed in some fish species from locations where liver cancers occur (Hayes, 2002). Many of the fish used in this study may have been too young to develop true tumours, and many different species were used, rendering any prevalence estimates unusable for comparison with other datasets (Hayes, 2002). Although this study did not indicate an elevated prevalence of fish tumours, it is not conducted in such a way as to provide a result of sufficient confidence to delist the "fish tumours and deformities" BUI. In 2006, Environment Canada's National Water Research Institute (EC-NWRI) began collecting redhorse suckers from the St. Clair River in order to undertake a comprehensive evaluation of the fish tumours and deformities BUI (Hayman, 2009). However, the results from this study are inaccessible at this time.

Of the few studies which have assessed this issue, all have been somewhat inconclusive in their results and have not provided substantial evidence to the BUI Delisting committee to merit a change in the current status of "requiring further assessment" to "impaired" or "not impaired".

1.5 Reason for Concern

Frequent occurrence of tumours and other abnormalities in fish populations raise concern for overall ecosystem integrity and its impact on human health. Dating as far back as the beginning of the archaeological record, hunting and fishing has played a central role in human nutrition. Fish have been, and continue to be, a major protein component of the human diet all over the world. This factor is of particular significance to Aboriginal and First Nations communities, which rely heavily on local fisheries for sustenance and livelihood. These populations are particularly vulnerable to any disruptions in the ecosystem, as it could potentially result in the scarcity of a culturally important nutritional source and jeopardize drinking water quality. Understandably, Aboriginal and First Nations populations are especially concerned about the potential risks associated with consuming fish with abnormalities, or even fish which appear normal but are obtained from the same source. Although several studies which have evaluated the effects of ingestion of fish from contaminated sites indicate that risk to human health is low (Hartig and Zarull, 1992; Pflugh et al., 1999; Urban et al., 2009), no studies of that nature have been conducted for the lower St. Clair River. Additionally, a high prevalence of diseased fish could imply that concentrations of chemical contaminants are too high for drinking water consumption from those locations. These issues are of great importance to the people who live in areas impacted by chemical contamination, therefore it is imperative to investigate whether prevalence of fish abnormalities is unusually elevated in these areas, and whether there exists reason for concern regarding drinking water safety and fish consumption.

In addition to concerns for human health, it is important to consider the ecological integrity of the aquatic community and its potential implications on the fisheries industry. There have been few monitoring programs assessing the effects of fish tumours on overall health, longevity and behaviour of the individual fish as well as overall population dynamics. Of the few studies carried out on this topic, data suggest that tumoured fish often die prematurely, and that survivors frequently show symptoms of other diseases (Sonstegard, 1977). Hence, it is important that a standardized dataset of fish tumour prevalence be established in order for fisheries managers to be able to proceed accordingly.

As a result of a growing number of scientific studies indicating an elevated prevalence of fish tumours in polluted waters, “fish tumours and deformities” were designated as Beneficial Use Impairment (BUI) criteria used to determine Areas of Concern (AOC) in Annex 2 of the 1987 Protocol Amending the Great Lakes Water Quality Agreement. In this agreement, the beneficial use of the ecosystem in question is considered impaired “when the incidence rates of fish tumours or other deformities exceed rates at un-impacted control sites...” (International Joint Commission, 1991).

1.6 Study Justification

Monitoring of sediment contaminant concentrations has been ongoing in the St. Clair River since the 1950’s, and it has been well established that the sediments downstream of industrial complexes were contaminated with a variety of heavy metals (e.g., mercury, copper, and lead), PCBs (polychlorinated biphenyls), PAHs (polycyclic aromatic hydrocarbons), volatile hydrocarbons (e.g. trichlororethene and perchloroethylene), semi-volatiles (e.g. hexachlorobutadiene — HCBD) and a number of chlorinated benzenes and chlorinated compounds such as hexachlorobenzene (HCB) and octachlorostyrene (OCS) (Oliver and Pugsley, 1986) (Table 3).

Table 3. St. Clair River surficial sediment contaminant concentrations, 1990 (Ontario Ministry of the Environment and Energy, 1993).

Contaminant	Concentration
Cadmium (µg/g)	0.28 ± 0.35
Chromium (µg/g)	11.7 ± 2.7
Copper (µg/g)	26.2 ± 16.9
Iron (µg/g)	8362 ± 2083
Lead (µg/g)	62.7 ± 152
Mercury (µg/g)	4.92 ± 3.47
Zinc(µg/g)	71.2 ± 51.5
Solvent Extractables (µg/g)	902 ± 412
total PCBs (ηg/g)	86 ± 289
Hexachlorobenzene (ηg/g)	1562 ± 5233
Octachlorostyrene (ηg/g)	349 ± 732

As a result of this extensive contamination, the benthic community was considered to be impaired within a reach of the river extending as much as 50km downstream of Sarnia (Thornley, 1985). The contaminated sediment in the St. Clair River acted as the primary reason for its AOC status designation in 1985.

Since then, several efforts have taken place to improve the ecological health of the region. The chlor-alkali plant located in the upper part of the St. Clair River was shut down in 1970 and many industrial and municipal facilities have been upgraded to meet more stringent government demands on discharge control (Richman and Milani, 2009). Later, three zones in the river were prioritized by the St. Clair River Remedial Action Plan (RAP) for further study, which identified mercury and octachlorostyrene as the main contaminants of concern (Figure 3). In 2004, portions of Zone 1 were remediated through dredging (ENVIRON International Corporation, 2009). As a result of this remediation, by 1990, the area of "extensive" degradation had been reduced to an 8.3km stretch within Zones 2 and 3 (Pope, 1993), hereafter referred to as the Area of Investigation (AOI).

Despite these efforts, surface sediment contaminant data collected by the Ontario Ministry of Environment (MOE) and Environment Canada (EC) in the AOI from 1990 to 2008 indicate that levels of mercury and several other chlorinated organic compounds vastly exceed

Provincial Sediment Quality Guidelines (SQG) and reference conditions (Richman and Milani, 2010), along with subsurface sediment showing concentrations of mercury up to five times the surface concentrations and spanning a much greater distribution area (ENVIRON International Corporation, 2009).

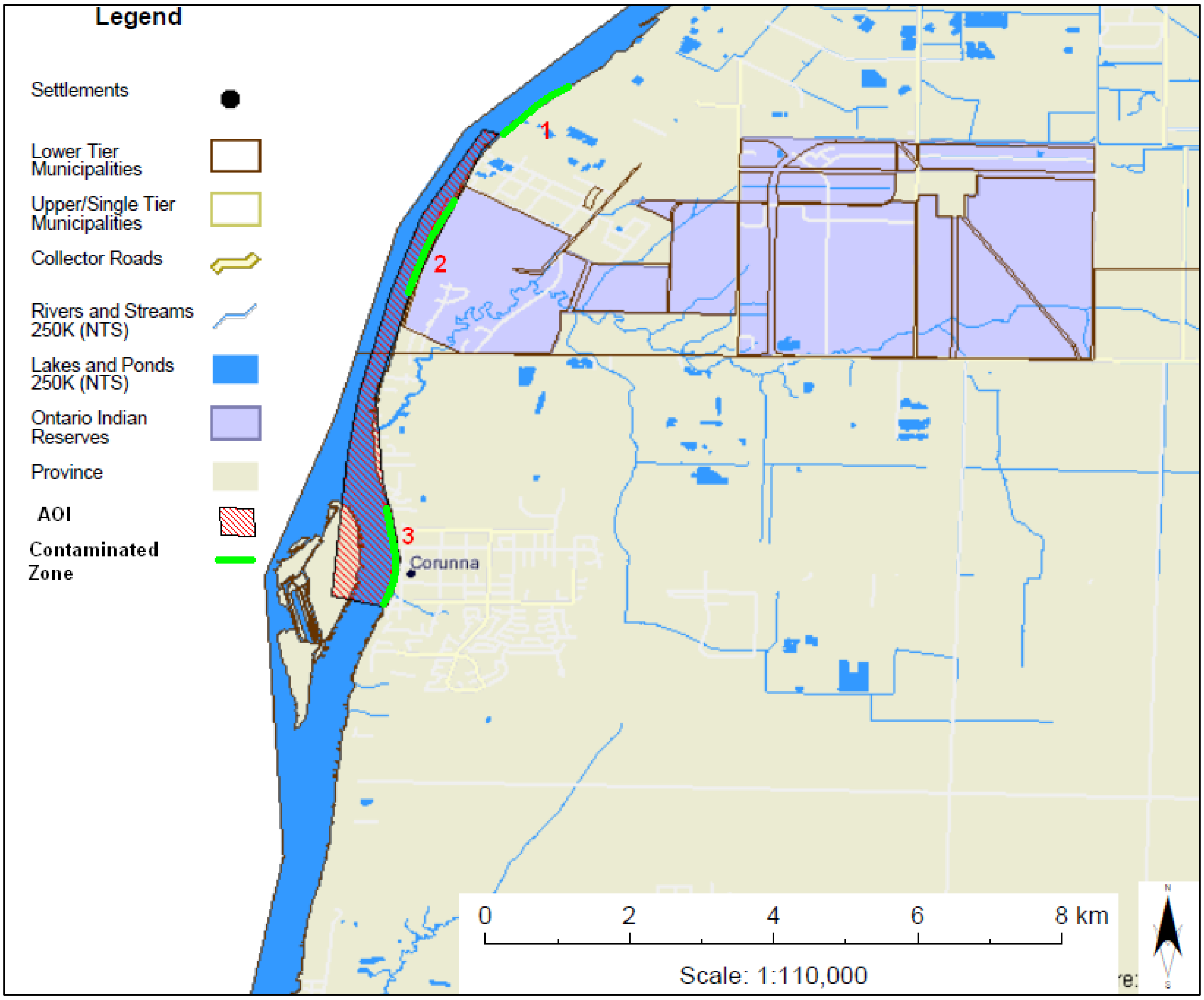


Figure 3. St. Clair River Zones and Area of Investigation (AOI) identified by the COA Framework. (ENVIRON International Corporation, 2009)

Although the source of contamination has been reduced over the past few decades, subsurface sediment can continue to act as a secondary source of contaminants and become bioavailable over time (Salomons et al., 1987). It is not uncommon for sediment redistribution to occur naturally in dynamic waterways such as the St. Clair River, potentially causing contaminants to be dispersed far from the original sediment source (Reible and Savant-Malhiet, 1993). Factors such as vessel traffic and ice scour, both probable events in the area, present a high risk of contaminant redistribution in the St. Clair River (ENVIRON International Corporation, 2009). It is also possible that new contaminants being added to the St. Clair River are adding to contaminants remaining from previous discharges (Richman and Milani, 2010). Although all point sources of discharge have been eliminated over the past decade (Bi-National Public Advisory Council, n.d.), several non-point sources of discharge remain, such as municipal and agricultural runoff, and railroads (Bi-National Public Advisory Council, n.d.).

The Canadian RAP Implementation Committee (CRIC) had taken these concerns into consideration during the application of the Canada-Ontario Decision-Making Framework for the Assessment of Great Lakes Contaminated Sediment (COA Framework), acknowledging areas of uncertainty due to: “1) the presence and effect of chemicals in sediment that were not evaluated by the report; 2) past and future changes in the spatial distributions of chemicals in sediment; 3) interpolation of chemical concentrations in un-sampled areas; and 4) limited ability of mercury SQGs to predict adverse effects in benthic invertebrates and fish” (CRIC Delisting Working Group, 2011).

It is, perhaps, for this reason that historical data show contaminant concentrations in fish tissue to have declined since 1978, but remaining stable since the mid-1980s, consistent with the sediment contamination data described above. This information suggests that, despite remediation efforts, contaminated sediments continue to be bioavailable in the St. Clair River (Richman and Milani, 2010). Species-specific analysis of fish tissue contamination indicate the highest levels of total contaminants in the tissue of northern pike and redhorse sucker, respectively, and intermediate levels of risk for carp, freshwater drum, white sucker and yellow perch, respectively (ENVIRON International Corporation, 2009).

Given the variable nature of aquatic contaminants, it is probable that fish tumor prevalence may change through time as well, particularly when point sources are being added or eliminated from a system, or when remediation has been undertaken, as is the case with the St. Clair River (Baumann et al., 2000). Since routine tumor surveys have not been conducted in the St. Clair River, the limited tumour prevalence data pertaining to this waterway may be outdated and no longer valid. For this reason it is important to assess the concentrations of contaminants in fish tissue and possible cellular changes over more recent time scales, since changes to contaminant inputs may have occurred more recently.

In addition to re-assessing the state of tumour prevalence in areas of the AOC which have received prior attention, it is important to survey fish tumour prevalence in areas which have never been assessed before, such as the lower part of the river, including areas adjacent to Walpole Island. The limited quantity of fish tumour prevalence data for the St. Clair River clearly outlines an information gap in this area. This is possibly due to the assumption that contaminants have not been dispersed to this section of the river, and therefore fish tumours should not exceed reference levels. However, as per the previous discussion, it is clear that this may not be the case.

It is especially important to have an accurate understanding of the status of ecological and health risk to this vulnerable population, whose cultural practices and livelihood depend largely on their ecosystem. The municipal water intake for Walpole Island First Nation is located within this area, giving potential impacts even greater significance.

1.7 Survey Design Methodology

1.7.1 Objectives

There are several key steps in the process of designing any ecological survey. The first, and most important of these, is to clearly identify the question which needs to be answered by the survey. This step is vital to deciding how the survey will be designed and which tools will be used in the process. The major question of this survey is to determine whether the prevalence of fish with tumours, from the lower St. Clair River, exceeds that of background rates. This question immediately spells out that a prevalence survey should be used in this case, as opposed to an incidence survey, as we are interested in the number of affected fish at a single point in time as a proportion of the total population at risk at that time, not the number of new cases over a period of time (Cameron, 2002).

1.7.2 Target Population

Secondly, the target population needs to be defined. In the case of this study design, it is the total population of fish in the lower St. Clair River. The definition of the target population needs to be detailed enough to explicitly identify which sampling units will be considered (U.S. Environmental Protection Agency, n.d.). Hence, at this stage, it is necessary to identify exactly which areas of the lower St. Clair River are included in the survey design, and which species of fish will be sampled.

1.7.3 Survey Design

Survey sampling is intended to characterize the entire population of interest; therefore, all members of the target population should have a known chance of being included in the sample. The survey design determines which process is used for site selection where a sample of fish will be taken. The most common survey design is simple random selection (SRS), which assumes all members of the target population have an equal chance of being selected, and therefore simple randomization procedures should ensure a representative sample (U.S. Environmental Protection Agency, n.d.). However, fish are often not evenly distributed within a watershed; therefore, it is unreasonable to assume that a simple random selection survey design would provide a truly representative sample of the target population. Other, more complex, survey design methods have been developed for the purposes of aquatic resource research. The US EPA suggests using Generalized Random Tessellation Stratification (GRTS) for fish population surveys. This method provides spatial balance of the sample across the resource. It can also allow sampling to focus on special study areas within study-wide design (U.S. Environmental Protection Agency, n.d.). However, with a more complex and detailed survey design, comes the necessity for more in depth information about the target population. The sampling frame is the information about the individuals in the target population. In the context of this study design, a sampling frame would be a census of the fish species of interest within the boundaries of the St. Clair River AOC. Ideally, fish population data complete with GIS coverage data would be necessary to create a spatially balanced sampling strategy which is truly randomized and representative of the entire target population (U.S. Environmental Protection Agency, n.d.). However, it is not always possible to obtain such information for all watersheds, and thus, a simplified sampling strategy must be used.

1.7.4 Site Selection

The goal for site selection in a survey is to obtain a sample which is most representative of the target population. This can be attained through two basic alternatives: authoritative selection and statistical design. Statistical design for a project such as this would involve a survey strategy like GRTS, described above, where the target population is divided spatially and sampling sites are selected based on weights of proportionally balanced segments (U.S. Environmental Protection Agency, n.d.). Authoritative design, on the other hand, allows the researcher to select sites using judgement and bias, based on knowledge sources other than statistical, to achieve a specific objective (Olsen, n.d.). In this project, a combined approach of authoritative and statistical site selection is used. Several areas of interest are to be selected within the target population and a suggestion is made to sample at random using an SRS survey design.

1.7.5 Sample Size

The next step is to determine how many fish in the population need to be sampled in order to calculate tumour prevalence with a satisfactory degree of certainty. Since the aim of this study design is to determine whether fish tumour rates at impacted sites differ significantly from those in fish from non-impacted sites, the required sample size depends on the variance in tumour rates between the sites. If the estimated difference in tumour rates is small between impacted and non-impacted sites, a larger sample size will be required to detect a true difference in tumour rates. However, if there is a dramatic difference in these two rates, even a small sample size can be effective in providing statistical verification of this disparity (Bernet et al., 1999).

A true estimate of variance is difficult to obtain in individual studies. For this reason, prevalence rates from previous studies of a similar nature may be used to calculate the variance for the sample size calculation (Bernet et al., 1999). In areas such as the St. Clair River, few tumour prevalence studies have been conducted historically, and therefore there is little tumour prevalence information for this area. Tumour prevalence rates in other polluted waters in the Great Lakes Drainage Basin may provide a comparable estimate of variance to determine sample size for the St. Clair River. Obviously, there are many factors of a tumour prevalence study which may be different from the St. Clair River, leading to prevalence estimates which are not exactly relevant. However, even an approximate prevalence statistic is sufficient for the purposes of sample size estimation (Cameron, 2002).

1.7.6 Indicators and Response Design

The indicator to be measured is the characteristic of interest in the target population. In this case, it is the presence or absence of tumours in fish. In order for this indicator to be useful in a prevalence study it is necessary to determine exactly which indicator is to be assessed, including definitions of types of tumours, and how to the presence or absence of said tumours will be used to establish overall prevalence.

The response design determines how the indicator will be measured. This includes details such as which time period within a year will be chosen to collect samples and which methods will be used to assess the indicator. In the case of fish ecology the time of year when data collection is conducted is especially relevant to sample yield and ease of sampling particular species (Bonar et al., 2000).

Different methods can be used to assess the effects of aquatic pollution on fish. Histopathology is an effective biomarker for assessing internal abnormalities in fish, such as tumours, and is closely linked to aquatic contamination (Sindermann, 1979; Bucke et al., 1996). Histological examination allows the detection of internal changes in animals at a cellular level, possibly caused by environmental degradation, prior to the death of the animal and drastic changes to population dynamics (Johnson et al., 1993). Therefore, histological examination is the preferred tool in the response design for evaluating the prevalence of fish tumours.

2.0 METHODS

2.1 Target population

2.1.1 Species selection

An effective sentinel species, for the assessment of pollution effects, must be: 1) abundant in the area of interest and reference locations; 2) exposed to the targeted disturbance; and 3) have life history characteristics that are measurable (Environment Canada, 2005). In accordance with these characteristics, the IJC delisting guidelines define the absence of a Beneficial Use Impairment when “the incidence rates of fish tumours or other deformities do not exceed rates at un-impacted control sites or when survey data confirm the absence of neoplastic or pre-neoplastic liver lesions in bullheads or suckers” (International Joint Commission, 1991). Hence, bullheads and suckers must be the sentinel species of focus in this fish tumour prevalence study. Most likely for these reasons, the vast majority of studies assessing fish tumour prevalence in the Great Lakes have used brown bullhead as their sentinel species (Bowser et al., 1991; Brown et al., 1973; Brown and Sinclair, 1977; Pinkney et al., 2001; 2004; Spitsbergen and Wolfe, 1995). Bullheads are bottom dwelling species that are in prolonged and direct contact with the sediments during cold weather (Loeb, 1964). They also have a very limited home range, maximizing contaminant exposure in areas of interest (Millard et al., 2009; Sakaris et al., 2005) and making them an ideal indicator fish for environmental stressors (Baumann and Okirhiro, 2000). The brown bullhead has value as an indicator species because it has a high pollution tolerance, which is very important since some less tolerant species die from contaminant exposure prior to tumour development. Hence, a good sentinel species is one that can survive in contaminated sediments. Unfortunately, brown bullheads are not abundant in the St. Clair River, eliminating them as a possible sentinel species for this study design. Surveys of fish populations in the St. Clair River by DFO, in 2004, and MNR, in 1994,did not find any bullhead present in the main channel of the river (Edwards et al., 2006; Maclennan and Hyatt, 1996) However several species of suckers are plentiful in these waters (Edwards et al., 2006).

White suckers are a larger fish, with scales, which are commonly used in environmental monitoring (Gagnon et al., 1994; Lalonde et al., 1999; Munkittrick et al., 1991; Servos et al., 1992; Vajda et al., 2008). However, they are considered a less effective indicator species due to their morphology and life history. The presence of scales makes the analysis of external lesions somewhat problematic (Baumann et al., 1996). They also have a much greater home range than bullhead (Munkittrick et al., 2002), and have been known to migrate across long distances for spawning and embryo incubation (Geen et al., 1966; Rainey and Webster, 1942). This sort of subject movement introduces uncertainty into pollution assessment studies because it is possible for the individual to have been affected by multiple exposure environments (Environment Canada, 2005). Additionally, they are more susceptible to viruses than bullhead, which may be misleading in preliminary judgement of tumour prevalence (Baumann et al., 1996). However, during the cold weather months, white suckers have limited activity, residing primarily in slow, downstream-flowing backwaters which are correlated with high-discharge events, therefore increasing their exposure (Brown et al., 2001).

Shorthead redhorse suckers (*Moxostoma macrolepidotum*) have also been used previously in pollution studies (Munkittrick et al., 1991) and have many of the same life history characteristics as white suckers (Reid, 2009). However, they are not as abundant in the St. Clair River as white suckers (Edwards et al., 2006), and therefore would require more effort to sample. Thus, white suckers are the preferred sentinel species in this study design.

2.1.2 Survey Area

The geographic scope of the study area includes a section of the Lower St. Clair River from the southernmost tip of Stag Island to the fork in the main channel of the St. Clair River adjacent to the western side of Walpole Island (Figure 4). This segment spans the entirety of the Lower St. Clair River south of the AOI prioritized in sediment contamination assessments, and has never been surveyed for fish tumour prevalence to date. Since part of the focus of this study design is on Walpole Island, the upper portion of the channels diverging from the main part of the St. Clair River is also included in the survey area.

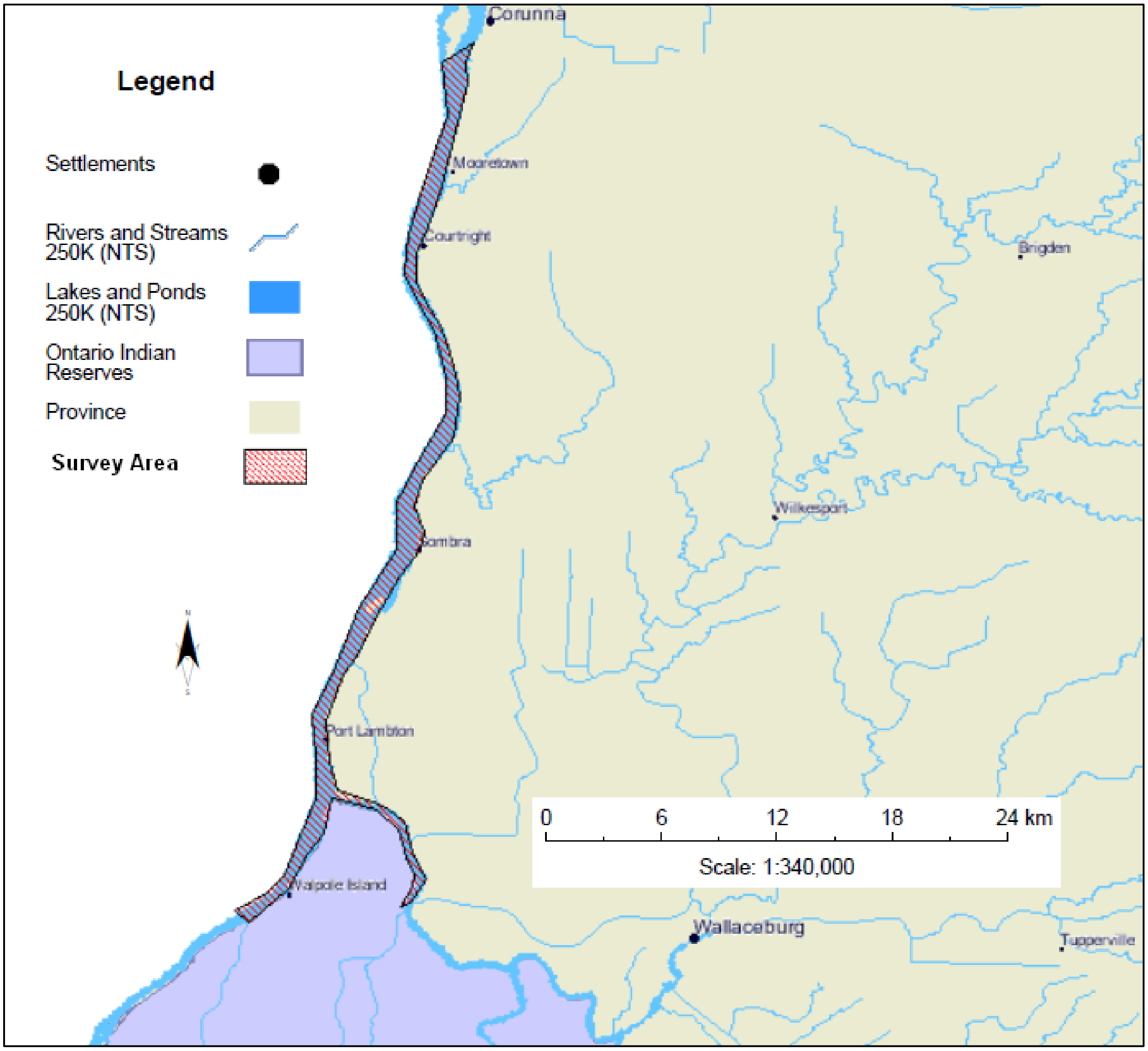


Figure 4. Geographic extent of survey area.

2.1.3 Reference Area

Un-impacted reference sites by definition of the Great Lakes Water Quality Agreement (GLWQA) should be areas where industrial or municipal pollutant discharges are not located upstream or in the immediate vicinity and the surrounding land use patterns have not disrupted ecosystem function (Baumann, 2000). Hence, the location of these reference sites must be selected outside the AOC, and should provide a background tumour prevalence rate which can be used to compare the tumour prevalence rates in contaminated areas.

In order to be an effective reference site, the same species of fish, white suckers, must be available for sampling as those chosen for sampling within the AOC. Although it is preferable to select reference sites which are similar in their limnological and geomorpholgical characteristics to the St. Clair River, this is very difficult to do since the St. Clair River is a broad, fast flowing river whose shores have been heavily manipulated. Thus, a practical equivalent of the St. Clair River which has relatively unpolluted waters is not readily available as a reference for this study.

There are many smaller watersheds in the area of Lambton County, which satisfy the requirements of relatively un-polluted aquatic conditions (St. Clair Region Conservation Authority, 2008). For instance, Black Creek and Bear Creek (Figure 5) are being used in current DFO studies as reference sites by the St. Clair AOC to compare Indices of Biotic Integrity (IBI) (J. Barnucz, personal communication, Dec. 2011). However, due to the reduced scale and geomorphological differences in these watersheds, the inhabitant fish communities consist of warm water species (St. Clair Region Conservation Authority, 2008). White suckers, on the other hand, are cold water species that are not abundant in these watersheds (Ontario Ministry of Natural Resources, 1994). Therefore, it is not possible to use these watersheds as a reference area for this study design.

Lake Huron, albeit not a river, has a fish community similar to the St. Clair River. White suckers are abundant in the lake (Scott and Crossman, 1973). This Great Lake also meets the GLWQA standards for an appropriate reference site, not having any significant industrial or municipal pollutant discharges located upstream (Lake Huron Centre for Coastal Conservation, 2004). Environment Canada used Lake Huron as a reference site, and reported an un-elevated fish tumour prevalence rate of 1% in an assessment of Great Lakes toxicity (Baumann, 2010). This background tumour prevalence statistic acts as a good indicator of appropriate reference conditions in the lake. Therefore, it provides the most suitable reference site for fish tumour assessment in this study. In order to most closely simulate limnological and geomorphological characteristics of the St. Clair River, survey sampling should be carried out in near shore environments (Bonar et al., 2000).

To supplement fish tumour prevalence data gathered in the Lake Huron reference area, it is also possible to compare collected data against a previously derived database of reference fish tumour prevalence rates from the Great Lakes. Baumann (2010) provides a comprehensive reference dataset for brown bullhead tumour prevalence in various Great Lakes locations (including urban non-point sites). A 2% liver tumour prevalence rate was obtained from examination of 700 pristine reference site fish and 450 urban reference site fish (Baumann, 2010). Although this dataset provides a prevalence rate for bullhead tumours only, it is still comparable to prevalence rates for suckers as these have been shown to be similar within polluted locations (Hayes et al., 1990), and are used interchangeably in the definition of the "Fish Tumours and Deformities" BUI (International Joint Commission, 1991).

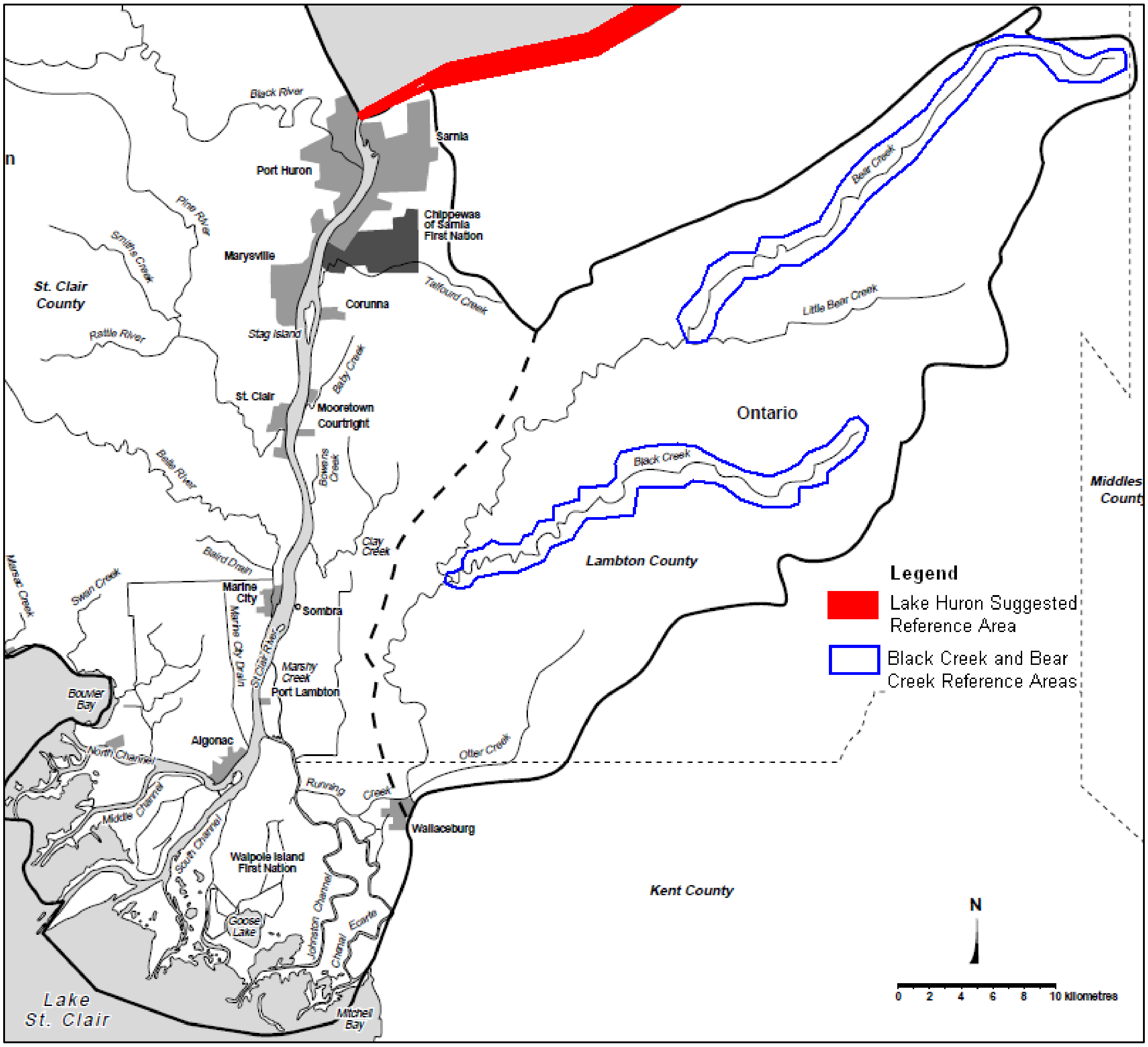


Figure 5. Lake Huron suggested reference area and considered Black Creek and Bear Creek reference areas.

2.2 Survey Design

A sampling frame for the St. Clair AOC should consist of fish population data within the boundaries of the study area. It is known which species are abundant in the study area, and relative abundance estimates have been calculated by DFO (Edwards et al., 2006). However, spatial distribution data for species of interest are not available for the St. Clair River AOC (N. Mandrak, personal communication, Apr. 2012).

In 2004, DFO conducted a boat electrofishing survey of the fish assemblages in the St. Clair River. Summary catch, species breakdown and sampling effort data are available from this study. These data can be used to calculate relative abundance of fish in the St. Clair AOC, thus providing an approximate sampling frame for the study area.

Because spatial distribution data are not available for this region, the application of a GRTS survey design, albeit preferable, is not possible in this case. With the given informational data constrictions, an SRS design would be best applied. Simple random sampling for the entire survey area is not practically feasible, since it is a very large area. Therefore, it is best to select several sampling sites based on authoritative criteria, and conduct simple random sampling with higher sample sizes within those sites.

Such authoritative selection of sampling sites is often implemented in fish tumour surveys. Commonly, four or five sampling sites are selected; usually three of the sites are located within the boundaries of the survey area (impacted areas), and one or two sites are selected outside the survey area to serve as a reference (Arcand-Hoy and Metcalfe, 1999; Baumann, 1998; Baumann and Harshbarger, 1995; Brown et al., 1973).

In a survey used to collect fish for the assessment of the "Tainting of Fish and Wildlife" BUI, researchers collected samples at the following six locations of the St. Clair River AOC: Suncor, Stag Island, Talfourd Creek, Cathcart Park, Fawn Island, and above Port Lambton. The reference sites for this study were Bluewater Bridge and Sarnia Bay (Myllyoja and Johnson, 1995). Sampling locations were established above, near, and below the main industrial point sources. In this study design, the focus lies in the Lower St. Clair River, therefore fixed sampling sites should be located at the northern boundary of the survey area, Fawn Island, and the WIFN Water Treatment Plant. Other sampling locations would be sited based on consultation with

WIFN fishermen. Edwards et al. (2006) suggests that effective sampling sites in the St. Clair River should be 1km in shoreline length, and sampled in two runs of 500m each; an upstream and a downstream location.

The Walpole Island First Nation Heritage Center has provided anecdotal evidence of fish with grossly visible tumours collected by local fishermen in various areas of the Lower St. Clair River. As a result of the expressed concern, sampling sites within the survey area were to be selected based on consultation with fishermen from the Walpole Island First Nation. Verbal permission was received from the WIFN Heritage Center to consult local fishermen. A questionnaire was produced for the purposes of this consultation, and received preliminary approval of the Ryerson University Research Ethics Board (Appendix A).

In March, 2012 Dr. Andrew Laursen and Liliya Baranova presented the prepared questionnaire and an update of research progress to the WIFN Heritage Committee at the WIFN Heritage Center. The committee's concerns were addressed and suggestions were recorded for future action. A verbal consensus was reached for an official agreement (Memorandum of Understanding [MOU]) to be drafted by the WIFN Heritage Center, allowing Ryerson University to collect information from members of the WIFN community. However, no MOU has been drafted, and collection of such information has not been permitted to date. Therefore, the questionnaires were never distributed to local fishermen, and no information was collected on proposed sampling sites near Walpole Island.

2.2.1 Sample Size

For statistical purposes, the prevalence rate of tumours is a dichotomous variable, since a tumour can only either be present or absent, and no continuum exists in between the two responses. Thus, a sample size calculation for a dichotomous variable only requires an estimation of the variable in the control group and experimental group, and a decision of power and significance used for the experiment (Dell et al., 2002). An estimation of background tumour prevalence rates obtained from the literature can be used as the value of the variable in the control group while literature-derived tumour prevalence rates from similar AOC studies can be used as an estimate of the value of the experimental variable. Generally, the smaller the estimated difference between the control variable and the predicted experimental variable, the higher the sample size needed to detect a significant difference (Dell et al., 2002).

With these variables in mind, sample size is calculated using the following equation:

$$n = C\,\frac{p_c q_c + p_e q_e}{d^2} + \frac{2}{d} + 2 \quad \text{Equation 1 (Fleiss, 1981)}$$

Where: n is the sample size required; p_c represents the value estimated for the control group; $p_e$represents the estimated value for the experimental group; $q_c = 1 - p_c$; $q_e = 1 - p_e$ and $d = \|P_c - P_e\|$. C is a constant that depends on the values chosen for the significance level (α) and power (β). A significance level of α=0.05 is commonly used in environmental studies, warranting that the probability of observing a tumour in a case where no tumour is present is no greater than 5%. A statistical power 1-β=0.8 is chosen to ensure that the chance of detecting a statistically significant difference is at least 80% (Dell et al., 2002).

The value for p_c can be obtained from Baumann's (2010) database of tumour prevalence rates at reference locations. Hence, $p_c = 0.02$. p_e can be calculated as the average of recently quantified tumour prevalence rates at AOC locations in the Great Lakes (Table 2) (Baumann, 2010). Therefore, $p_e = 0.04$. Logically, $q_c = 0.08$, $q_e = 0.06$ and $d = 0.02$. Finally, $C = 7.85$ when α=0.05 and 1-β=0.8.

$$n = 7.85\,\frac{0.02 \times 0.08 + 0.04 \times 0.06}{0.02^2} + \frac{2}{0.02} + 2 = 180.5$$

Therefore, at least 180 fish should be sampled in each group (AOC and reference) in order to have an 80% chance of detecting a significant difference in tumour prevalence between the two groups, with 5% confidence.

2.3 Data Collection

Before going out in the field and collecting data, it is important to identify exactly which data need to be collected, and how they will be recorded. The following data parameters should be recorded in the field for all fish captured belonging to the species of interest (white suckers): 1) site, 2) field number (transect #, upstream/downstream), 3) date/time, 4) start latitude, 5) start longitude, 6) narrative locality description, 7) duration, 8) electrofishing settings, 9) fish length, 10) weight, 11) sex, 12) observed external abnormalities, 13) DELT index score, and 14) liver weight. The following parameters should be recorded for lab for samples selected for further examination: 1) age, 2) number of liver sections submitted, 3) presence or absence of a particular lesion, and 4) characterization of tumour (neoplastic / non-neoplastic).

Several factors need to be considered when planning data collection in the field. The season which sampling takes place may influence the pathological conditions of fish (Bettross and Willis 1988, Guy and Willis, 1991). This is potentially caused by the influence of temperature on the biological activity of the contaminant, the immune system response of the fish to the causative agent, or hormonal variations in disease susceptibility (Bernet et al., 1999). It is recommended to sample fish in the fall, as this is the season when fish tumours are most visible, and CPUE for many species is highest at this time (Pope and Willis, 1996). It is also recommended that all sampling be conducted within the same season (unless used for comparison purposes), since some species migrate during the life-cycle and have quick flight reactions caused by pollution events, which can affect the distribution of diseased fish within a geographical region (Triebskorn et al., 1997).

The age of all sampled fish should be recorded, since it has been shown that tumours are significantly more frequent in older fish (Baumann et al., 1990). Age determination is done by reading of scales, otoliths or interpercular bones. However, these are complex techniques which are best carried out by experts in a lab setting. In order to simplify the task of ageing fish, it is recommended to sample fish of a standard size (Bernet et al., 1999). Baumann et al. (1990) suggest fish of size less than 250 mm total length should be discarded to exclude fish of age 2 or less from the sample, since true neoplasia reported by Baumann et al. (1987) occurred in less than 2% of the livers of 2 year-old fish.

There is also evidence that females are more prone to pollution-related tumours than males of the same age. Gender effects on brook trout, attributed to endogenous estrogens, have been reported by Nunez et al. (1989) and Cooke and Hinton (1999), and similar increased prevalence of neoplasia has been found in bullhead (Baumann et al., 1990; Pinkney et al., 2001; 2004). These factors should be accounted for in statistical analysis of neoplasm prevalence data and attempts should be made to ensure balanced samples of both female and male fish collected in the field.

In order to ensure comparability of samples, data collection should occur in the same manner at all sites. Furthermore, it is best for histopathological examination to be conducted by the same person for all samples, due to the subjective nature of the histopathological diagnoses.

2.3.1 Field Methods

Methods for sampling fish are well established in the scientific literature. A good sampling method needs to be efficient within the habitat and season the sampling takes place, and must be suitable for catching the species of fish being investigated (Portt et al., 2006). In addition, it is very important that fish are collected alive with the least amount of physical damage (U.S. Environmental Protection Agency, 1994). Electrofishing and various types of trap netting meet these criteria (Blazer et al., 2009; Baumann et al., 1990; Metcalfe and Arcand-Hoy, 1999); however electrofishing is preferable where possible because it reduces chance of fish injury caused by fish fighting (U.S. Environmental Protection Agency, 1994).

Electrofishing is an active fishing method which establishes an electric field in the water, which in turn stuns and temporarily immobilizes fish, allowing them to be captured (Portt, 2006). It can be used for a wide range of habitats and species, and can be conducted using a boat electrofishing apparatus or backpack electrofishing apparatus. This method is effective for capturing larger fish, and is most efficient for fish, like white suckers, which do not startle easily and swim towards the electric current (Mahon et al., 1979). Mortality rates for this method are low, but spinal injury can occur, especially among larger fish.

Habitat parameters should be taken into consideration when deciding on sampling details. Conductivity of the water, which can vary due to salinity and temperature, influences the efficiency with which an electrofishing apparatus works. Visibility at the time of sampling is also critical to the efficiency of the fish collection, since even stunned fish may not be caught if they are not visible. Hence, times of day which provide greater Secchi depth and areas of relatively clear water are preferred in this process (McInerny and Cross, 2000).

Boat electrofishing is necessary for large, non-wadeable areas, such as the St. Clair River. Typically, an electrofishing boat moves through the water in “runs” with the operator controlling the settings of the electrical current, and assistants collect shocked fish which accumulate behind the boat with dipnets. DFO (Edwards et al., 2006) and OMNR (Maclennan and Hyatt, 1996) surveys of fish in the St. Clair River collected samples by electrofishing 500m runs for approximately 500s, using settings of 1000V, 5.5 -8 amps, 30-60Hz at 40-60%. The sampling was carried out during daylight hours to minimize the influence of diurnal effects on fish movement and increase visibility. These methods are in accordance with literature recommendations for fish sampling in large rivers, and can be applied in this study.

Once fish are captured, they should be kept alive, in a live well or other kind of tank, until processing. Prior to external examination of captured fish, fish must be sacrificed using humane methods that minimize trauma to the tissues. This should be done to each fish individually to curtail post-mortem tissue changes, which may confuse histopathological analyses (U.S. Environmental Protection Agency, 1994). The EPA suggests that an overdose of anaesthetic is best for this purpose.

Fish length and weight should be measured immediately after fish death. These data are used to calculate a Condition Factor giving some indication of the overall state of fish wellbeing (Baumann et al., 1990; Blazer et al., 2009; Pinkey et al., 2004). Pectoral spines, scales, or otoliths should be excised from the carcass to determine the age of the fish. These structures display a ringed pattern which increases as the animal ages, thus allowing an estimate of fish age to be made (Blouin and Hall, 1990). The external body surface of the fish should be carefully examined, and any abnormalities such as papillomas, discoloration and stubbed barbels should be noted as these are indicators of skin tumours (melanomas) which are common in fish from contaminated areas (Baumann et al., 1990).

External abnormalities are often characterized using the DELT index (deformities, eroded fins, lesions and tumours) (Baumann et al., 2000; Rafferty and Grazio, 2006; Smith et al., 2002). This index was developed as a measure for the Index of Biological Integrity (IBI), therefore it is intended to look at the entire fish community, including all species and age groups, which lessens its discriminatory power in distinguishing amongst levels of contaminant exposure of individual fish (Environment Canada and U.S. Environmental Protection Agency, 2001). However, a more species focused index is only now being developed based on historical fish abnormality data. In the meantime, DELT remains the index of choice for assessment of external abnormalities in tumour studies. This index is used in combination with histological analysis to assess the state of the BUI. A DELT external anomaly index exceeding 0.5% of the total population is indicative of beneficial use impairment. External anomalies should be excised and stored in a fixative or frozen for future microbiological examination.

Following external inspection, a ventral incision should be made exposing internal organs for examination and allowing the excision of tissue samples for later histopathological examination. The liver should be located, removed, weighed, and then grossly examined for the presence of any abnormalities such as swelling or nodules. Any abnormalities should be excised along with a sample of the normal tissues. One to six small pieces (approximately 1cm) of the liver should be taken from each animal (Figure 6) and placed in labelled containers with fixative (10% neutral buffered formalin) (Blazer et al., 2009). Labelling should reflect whether any external anomalies or liver abnormalities were observed in the individual the sample came from. The weight of the fish and weight of the corresponding liver can later be used to calculate the Hepatosomatic Index (HSI); the proportion of liver weight to total body weight, expressed as a percentage (Blazer et al., 2009). Enlarged livers can occur in fish exposed to pollutants, and this index can be indicative of liver tumour presence (Rafferty, 2003).

After sampling has been concluded, fish carcasses and liver samples should be delivered to a histopathology laboratory as soon as possible (within a 24 hour period) in order to avoid tissue degradation. Fish histopathologists will examine provided liver samples for presence of tumours or precancerous cells. It is possible for one section of a liver to exhibit more than one type of lesion or abnormality. In this case, all observations should be recorded; however, the most serious or progressed lesion should be considered to be the definitive diagnosis to be used in data analysis (U.S. Environmental Protection Agency, 1994). Microbiological examination of external abnormalities can also be requested to determine if anomalies are of viral/bacterial origin or potentially cancerous. Histopathology examination services are provided by Animal Health Laboratories, University of Guelph. Fish aging services are provided by North Shore Environmental, Thunder Bay, Ontario (J.S. Lumsden, personal communication, Mar. 2011).

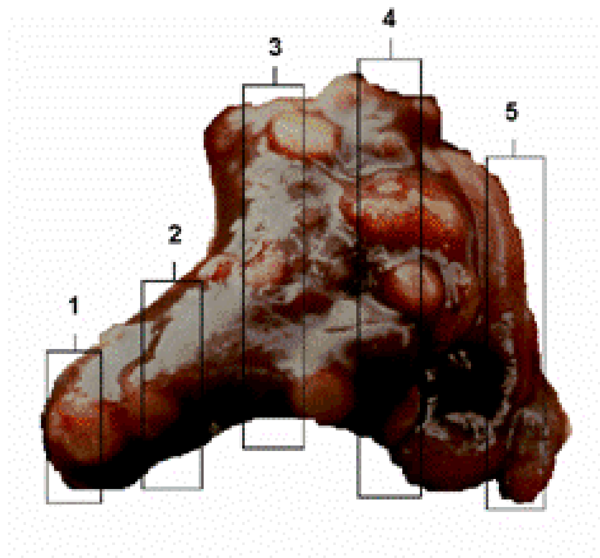


Figure 6. Example of sectioning of the liver pieces to be sampled and placed in fixative in the field (Blazer et al., 2007).

2.4 Laboratory Methods

The following section is intended to provide guidance to the reader on how to examine and interpret various histopathological indicators appearing in the livers of white suckers. Any deviation from normal histology of the liver may indicate the presence or development of a tumour. The different manifestations of liver histopathology in white suckers are described, with comparison drawn to normal histology of the liver. Not all of the histopathological conditions described below necessarily implicate the presence of a true tumour, and therefore not all conditions should be used in the quantification of tumour prevalence. However, all conditions are described in detail in order to provide greater certainty of identification by comparison.

2.4.1 Specimen Processing

The number of sections microscopically examined from the liver pieces may influence the probability of observing the presence of a neoplasm if one does exist. However, there have not been any studies done to date that concluded what is the most reliable number of sections needed from white sucker livers in order to accurately diagnose a neoplasm. Generally, it is suggested that the larger the liver, the greater the number of sections per piece of liver need to be examined. Blazer et al. (2007) suggests that from the five or more pieces collected in the field, six to ten sections should be prepared for microscopic examination.

Prior to sectioning, liver pieces should go through a fixation process that preserves the tissue through dehydration (Profet et al. 1992). Fixed tissue should then be embedded with paraffin wax, which hardens the specimen allowing sectioning, and easy storage and handling (Profet et al. 1992). Specimens should then be sectioned at a thickness of 4-6 µm using a microtome, and then placed on a microscope slide ready for staining (Luna, 1992). Cassettes can then be stained with hematoxylin and eosin to produce the basophilic and eosinophilic characteristics (Luna 1992).

2.4.2 Normal histology

Normal histology and histopathology of bullhead livers is well documented; however, there is less specific information available pertaining to histopathology of white suckers. A healthy liver in white suckers is attached by mesentery to the stomach and intestine and is much longer and thinner than the bullhead liver. Like bullhead, white sucker livers are composed of hepatic tubules, but bile ducts are sparse and not prominent (Figure 7A). Blood vessels are commonly surrounded by pancreatic tissue and bile ducts may be observed in these areas (Figure 7B). Normal hepatocytes can be surrounded by a varying quantity of vacuoles, lipid and glycogen storage. Macrophage aggregates are also commonly found in the spleen, kidney and liver of fish (Figure 8A) (Blazer et al., 2007).

2.4.3 Neoplasm Pathology in White Suckers

The detection of small neoplasms and large embedded tumours is easier in white suckers, as the surface area of the liver is much larger than in bullhead, and large tumours are often visible without slicing the liver. Most hepatocellular and cholangiolar neoplasms occur on the posterior, ventral lobes of the liver, but cholangiomas have been found on the anterior dorsal lobe and on the dorsal surface adjacent to the gall bladder (Hayes et al., 1990).

2.4.4 Non-proliferative Lesions

Some non-proliferative liver lesions include macrophage aggregates, which have been shown to increase in size and number with age and in response to environmental stress. Hence, in young fish, and/or fish from reference sites, macrophage aggregates may not be observable or they may be present at a low density within the liver or hepatic pancreatic tissue, while at impacted sites a higher density may be observed. Also, helminth parasites are commonly observed within liver tissue of fish collected in polluted areas. These may be the most commonly observed, grossly visible lesions. They may appear as irregularly-shaped, elongate or rounded, pale, raised areas. However, raised pale areas may also be tumors and are easily confused (Figure 8B) (Blazer et al., 2007).

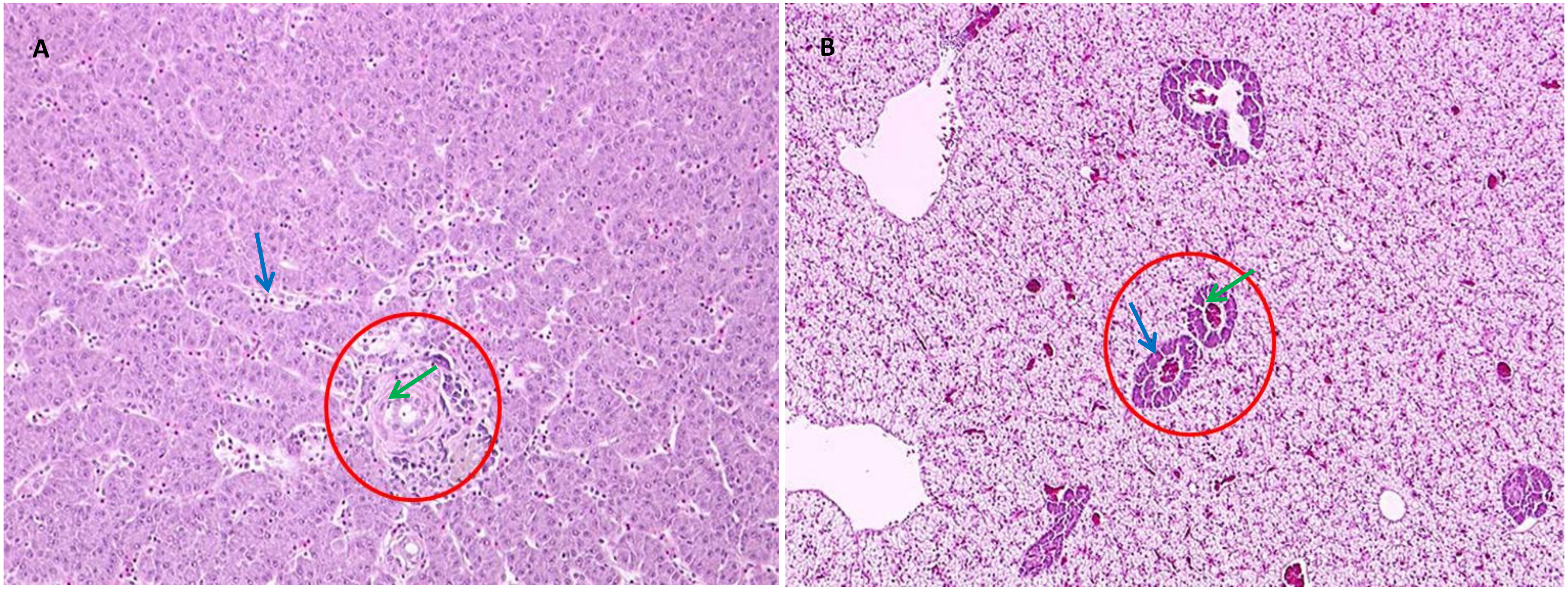


Figure 7. Healthy fish liver tissue. A) Hepatic tubules (blue) and sparsely distributed bile ducts (green); B) blood vessels (blue) surrounded by pancreatic tissue (green) and bile ducts within (Magnification not reported) (Blazer et al., 2007).

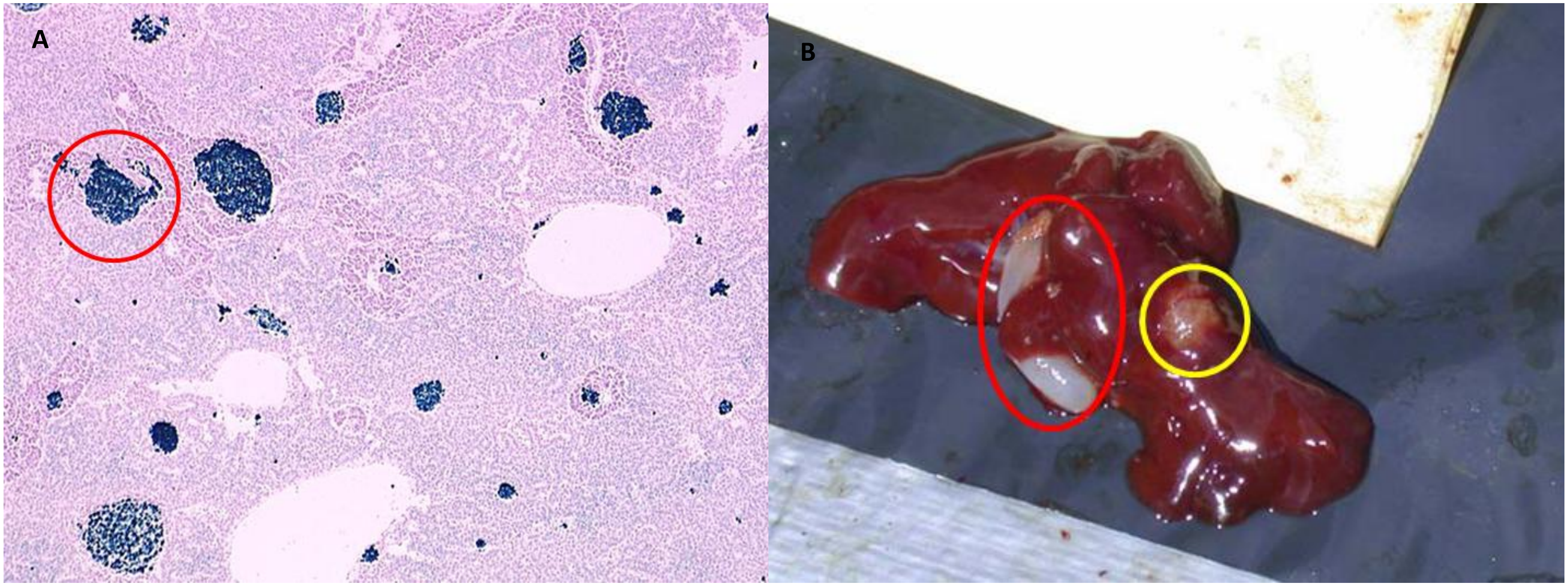


Figure 8. Examples of non-neoplastic liver lesions. A) proliferated macrophage aggregates; B) helminth parasite (red) and possible tumour (yellow) (Magnification not reported) (Blazer et al., 2007).

2.4.5 Pre-neoplastic Lesions:

Prior to the formation of a liver neoplasm, a pre-neoplastic lesion occurs, which is marked by the presence of foci of cellular alteration (AHF). These are morphological groupings of cells which display different characteristics from the normal surrounding cells. Four categories of foci of cellular alteration are recognized in fish livers stained with hematoxylin and eosin (red and blue colour respectively). These differences in staining are a result of accumulations of specific components within the cells. Cells within eosinophilic foci are rich in smooth endoplasmic reticulum (Figure 9A); cells within basophilic foci are rich in RNA (Figure9B); clear cell foci contain abundant glycogen; and foci of vacuolated cells contain lipid (Figure 9C). The margins of these foci are generally distinct, but the hepatic tubules are arranged in a relatively normal pattern. These foci merge imperceptibly with the surrounding parenchyma, and little to no compression is observed (Blazer et al., 2007).

Not all foci of alteration advance into true neoplasia (Baumann and Okihiro 2000; Bunton, 1996; Hinton et al., 1988). The true neoplasms that do from these foci are derived from either liver cells (hepatocellular) or bile duct cells (cholangiolar) (Blazer et al., 2007). Due to the uncertainties concerning progression of foci of cellular alteration it is suggested that pre-neoplastic lesions not be used as an actual impairment criterion (Baumann, 2010).

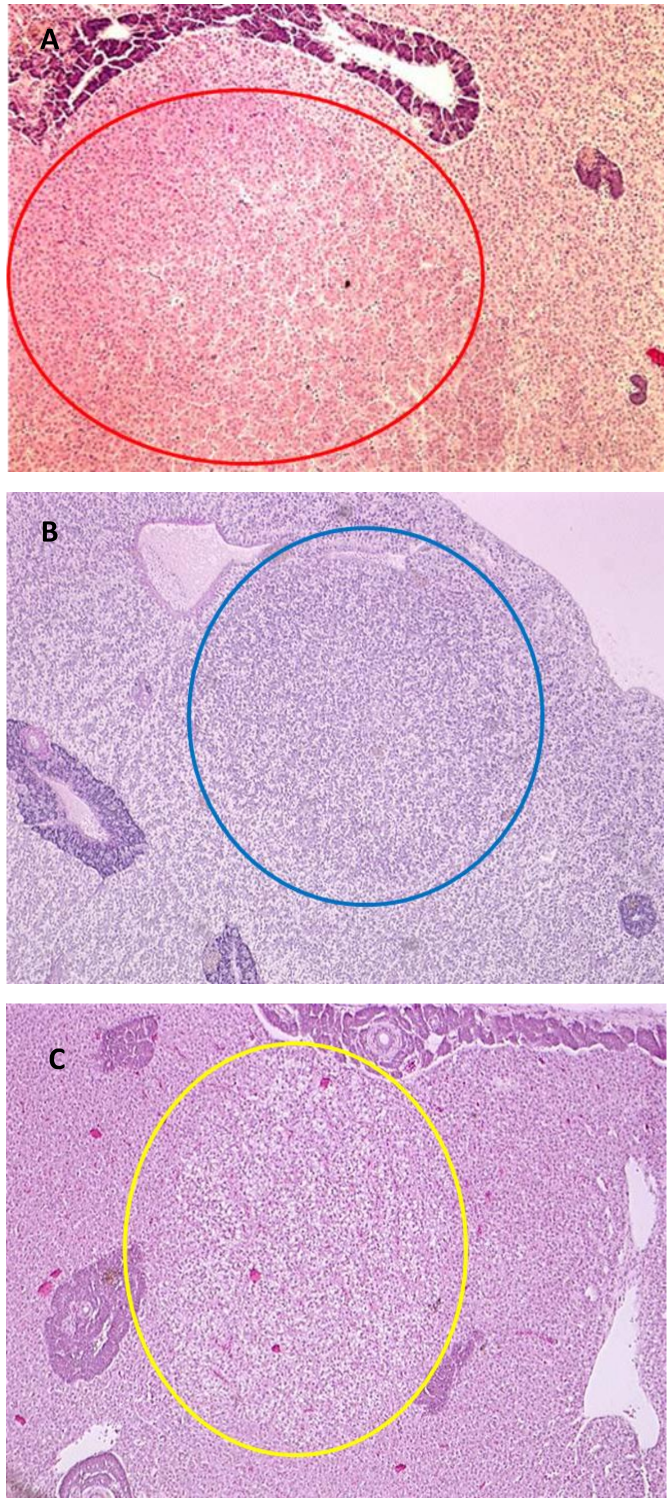


Figure 9. Categories of Foci of cellular alteration (AHF). A) Eosinophilic foci; B) basophilic foci; C) Vacuolated / clear cell foci (Magnification not reported) (Blazer et al., 2007).

2.4.6 Neoplastic Hepatocellular Lesions

There are two types of neoplastic hepatocellular lesions: adenomas and carcinomas. Adenomas are discrete lesions which generally have a distinct border (Figure 10A). The cells may exhibit altered staining properties and hence appear more eosinophilic, basophilic or vacuolated compared to the surrounding tissue. Mitotic figures are rarely observed. Macrophage aggregates, pancreatic tissue and other structures are often missing or sparse within the neoplastic lesion. These lesions also cause compression of the adjacent parenchymal cells. Carcinomas, on the other hand, are malignant hepatic neoplasms, which are often diffusely spread throughout the hepatic parenchyma and may be distinct foci with irregular borders (Figure 10B). These neoplastic cells invade the adjacent parenchyma features and an increase in the number of mitotic figures can be observed (Blazer et al., 2007).

2.4.7 Neoplastic Biliary Lesions

There are two types of neoplastic biliary lesions; cholangiomas and cholangiocarcinomas. Cholangiomas are benign tumors of bile ducts within the liver (Figure 11A). These are clusters of bile ducts which are well differentiated and often have a discrete border between the nodule and surrounding hepatic parenchyma. Many of the bile ducts may be irregularly-shaped and dilated. Cholangiocarcinomas are malignant tumors of bile ducts, which invade into the surrounding parenchyma (Figure 11B). The edges of a cholangiocarcinoma are often not well defined, and they have variable size and shape (Blazer et al., 2007).

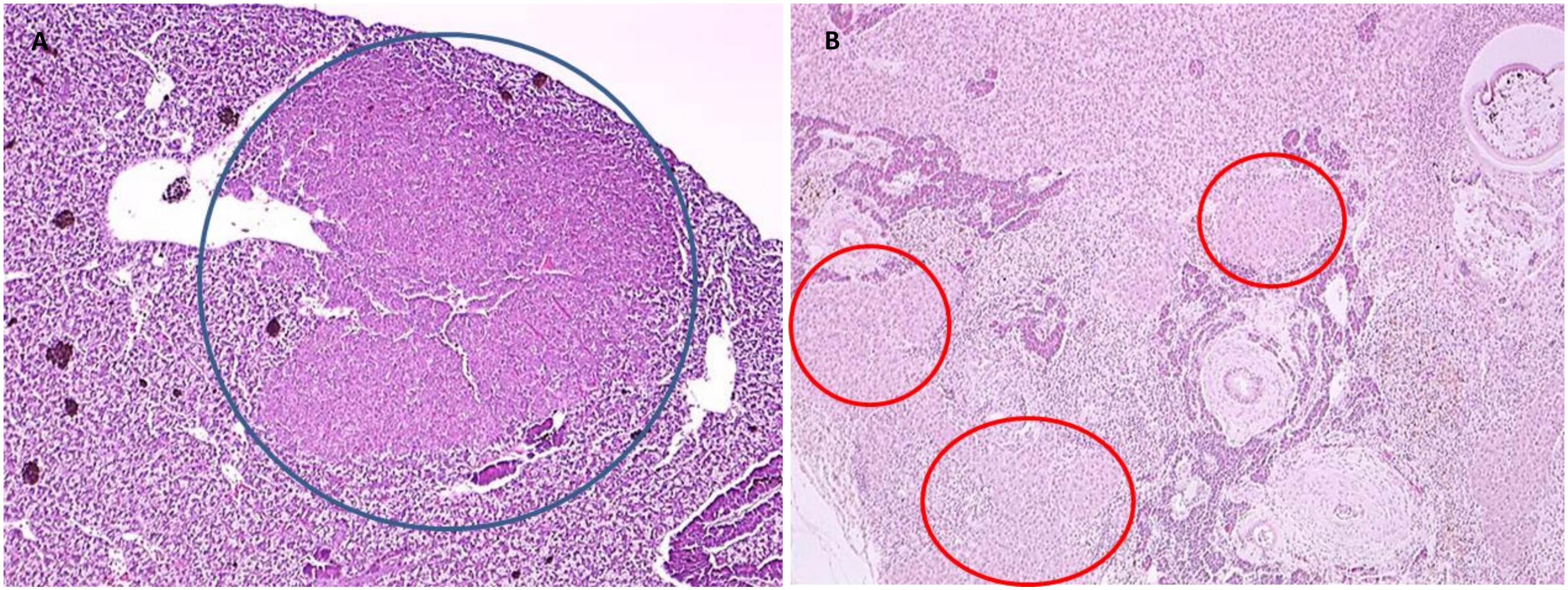


Figure 10. Neoplastic hepatocellular lesions. A) Hepatocellular adenoma; B) Hepatocellular carcinoma (Magnification not reported) (Blazer et al., 2007).

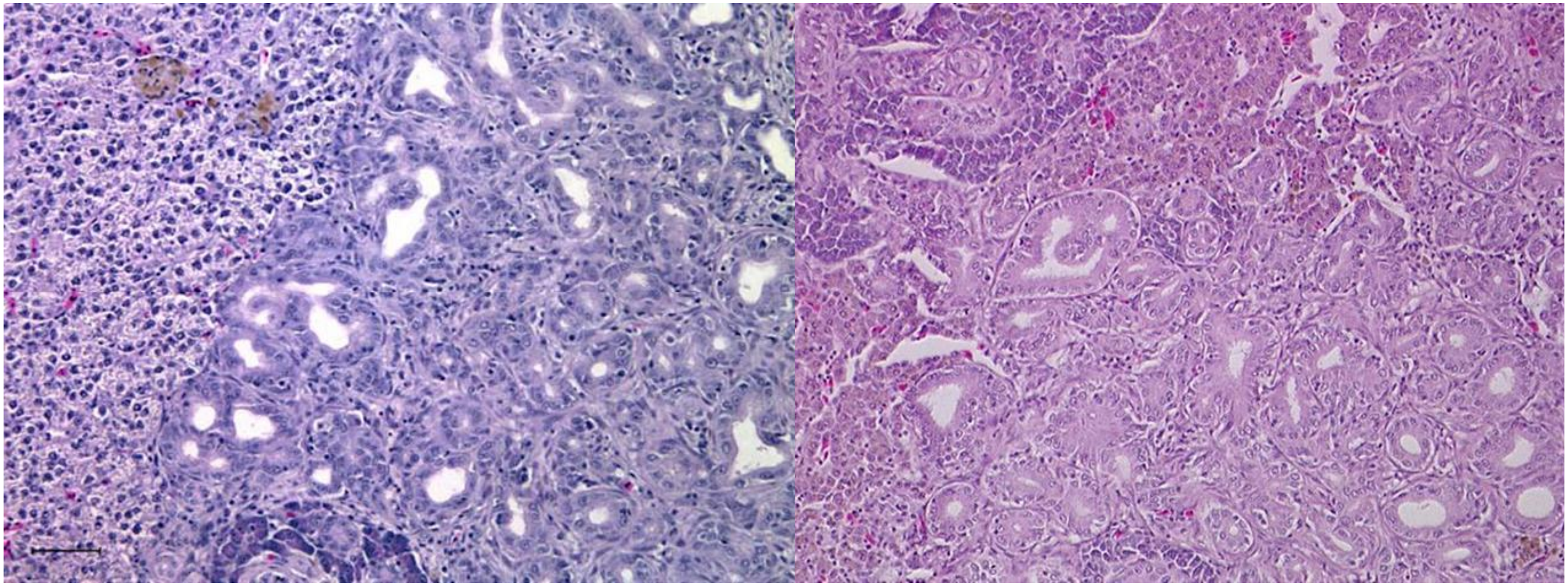


Figure 11. Neoplastic biliary lesions. A) Cholangioma; B) Cholangiocarcinoma (Magnification not reported) (Blazer et al., 2007).

2.4.8 Non-neoplastic Lesions

Hepatobiliary disease, where a proliferation of inter-hepatic bile ducts occurs, has been described by Hayes et al. (1990) in white suckers from Lake Ontario. These lesions included either cholangiohepatitis, characterized by excessive growth of biliary epithelium and an associated inflammatory reaction surrounding the bile ducts, or cholangiofibrosis, characterized by proliferation of cholangioles (fine terminal elements of the bile duct system), accompanied by the excessive formation of fibrous tissue (fibroplasia), atrophy of hepatocytes and enlargement of macrophages (Figure 12). Severity of occurrences of cholangiohepatitis and cholangiofibrosis coincided in fish with hepatic neoplasms from polluted regions of Lake Ontario. The most severe cases of these conditions displayed clustering of small hyperplastic ducts adjacent to the large ducts, surrounded by inflammatory cells and a ring of fibrotic tissue. Hepatocytes adjacent to the affected areas were not compressed and appeared unaffected. Due to the infrequent occurrence of this condition, etiology is not entirely established, but it is presumed that it may be related to parasite infiltration. Although hepatobiliary disease is not considered a true neoplasm, it is a potential precursor to the condition (Hayes et al., 1990; Hayes and Laws, 1991).

For the purposes of prevalence calculation, only true neoplasms will be considered as evidence of tumour presence. True neoplasms are defined as neoplastic hepatocellular or cholangiolar lesions. The observation of hepatocellular adenoma, hepatocellular carcinoma, cholangioma or cholangiocarcinoma should be recorded with a “1” for present or “0” for absent. All other types of lesions should be noted as observations but not considered in the prevalence calculation due to inconclusive evidence of true tumour formation. Pre-neoplastic hepatocellular lesions should be noted with description of the type of foci of cellular alteration and extent of progression. The non-proliferative lesions such as accumulation of lymphocytes/leucocytes and macrophages should be recorded as “inflammation” or “excess MAs” respectively. Non-neoplastic lesions should be recorded as “cholangiohepatitis” or “cholangiofibrosis”.

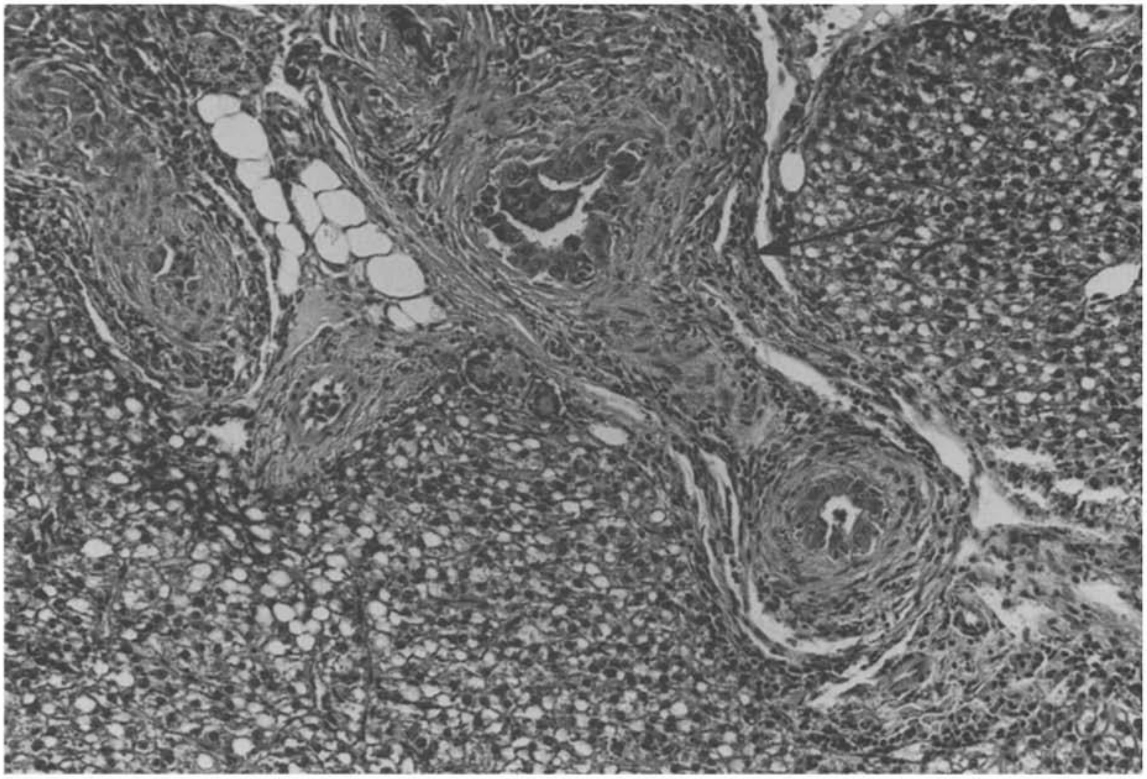


Figure 12. Cholangiohepatitis in the liver of a white sucker from Lake Ontario, with visible inflammatory reaction surrounding hyperplastic bile ducts and increased fibrous connective tissue (arrow) around the bile ducts (H & E section, magnification X 100). (Hayes et al., 1990).

2.5 Statistical Analysis

First and foremost, a statistical hypothesis needs to be clearly identified in order for any statistical analysis to be carried out. The null hypothesis (H_o) is that the prevalence of fish tumours in the Lower St. Clair River does not differ significantly from that at reference locations. The alternate hypothesis (H_1) is that the prevalence of fish tumours in the Lower St. Clair River differs significantly from that at reference locations.

To estimate tumour prevalence it is necessary to divide the total number of fish sampled which display tumours by the total number of fish sampled. Assuming that sampling is carried out in a representative fashion, this prevalence estimate should be proportional to the absolute abundance of fish in the area sampled.

In choosing a statistical test to compare prevalence rates between the AOC and reference locations, there are several key questions about the nature of the data collected which need to be answered. It is necessary to decide if the data follow a Gaussian distribution in order to determine whether a parametric or non-parametric test should be used (Motulsky, 1995). This is a difficult factor to determine in environmental studies since the distribution of natural data is not easily predictable and often does not follow a normal distribution. However, the risk of assuming that a dataset is parametric when in reality it isn't, is not so troubling when sample sizes are large. The central limit theorem ensures that parametric tests work well with large samples even if the population is non-Gaussian (Motulsky, 1995). Since sample sizes selected in this study design are reasonably large, parametric tests should be suitable.

Next, it is necessary to decide whether a paired or unpaired test should be used. Paired tests are used when values represent repeated measurements on the same subject or measurements on matched subjects (Motulsky, 1995). Since, in our study design, all data will be independent and non-matched, an unpaired test should be used.

With these criteria in mind, suitable statistical tests for the comparison of two unpaired groups are the Fisher's Exact Test and the Chi-square Test. The Fisher's test always gives the exact P-value and is considered more accurate, but is more complex to compute. The Chi-square test is much simpler to compute, but is only capable of providing an approximate P-value and is not very effective when numbers compared are small. Many statistical applications use the Yates' continuity correction to improve approximation of the Chi-square test; however, this correction has a tendency to be overestimate P-values unless sample sizes are high (Motulsky, 1995). Since it is known that numbers compared in this study design are quite low (i.e. expected prevalence 1 to 10%), a Fisher's exact test is recommended.

3.0 DISCUSSION

3.1 Study Constraints

The originally envisioned project was to be a study design with proof of concept, acting as a preliminary assessment of fish tumours in the Lower St. Clair River. This idea was based on interaction of several Ryerson University faculty members with the Walpole Island First Nation Heritage Center, where it was revealed that an assortment of frozen fish, collected by WIFN community members in nearby areas of the St. Clair River, with visible abnormalities was being stored by the Heritage Center. Concern was expressed on behalf of members of the Heritage Center about these fish, and it was proposed that the collection be used as a tool of assessment of fish tumours in the Lower St. Clair River. It was verbally agreed that the collected fish would be used for histopathology purposes, to determine the nature and extent of observed abnormalities. Attempts were made by faculty to procure a written agreement of cooperation for this project, including permission to transport the fish from Walpole Island and their use in histopathological examination. However, after several attempts, no agreement was reached and with the WIFN Heritage Center.

Following another attempt to engage the WIFN Heritage Center in a written agreement, information was received that the fish collection, which was previously agreed to be used as a proof of concept in designing the study, had been discarded. After this unfortunate turn of events, alternative methods of data collection were proposed, such as hiring fishermen from the community to collect fish in nearby waters. However, after requesting contact information of local fishermen from the Heritage Center, no response was received.

The final attempt to collect data related to fish tumours from the WIFN community entailed producing a survey to be administered to local fishermen. The survey would ask fishermen to report on catches which included fish with visible abnormalities or suspected tumours; including identification of the approximate locations these fish were caught. This survey was originally produced as a questionnaire to be distributed to fishermen identified by the WIFN Heritage Center (Appendix A). It was reviewed and received preliminary approval of the Ryerson University Research Ethics Board, and was sent to committee members of the WIFN Heritage Center for review, along with a detailed description of the proposed research and responses to concerns on their behalf. A meeting was scheduled with the WIFN Heritage Center committee on February 20, 2012. Dr. Andrew Laursen and Liliya Baranova attended the meeting, presented the proposed research, elaborated on the content of the provided questionnaire, and responded to the committee's concerns.

Main points of concern on behalf of committee members included skepticism about the success of the proposed questionnaire. It was suggested that mailed questionnaires would not produce a satisfying response rate. A recommendation was made to collect information by conducting informal interviews with members of the community, on WIFN territory, during a public community event. Potential strategies to increase response rate included posters with images of fish with abnormalities and the accompaniment of liaison from the WIFN Heritage Center. Suggestions on the content of the survey included asking community members to identify areas which they would like to be assessed for fish tumour prevalence, such as areas of natural or community significance (e.g. Walpole Island Water Treatment Plant). Concern was expressed about data ownership rights, distribution and availability of the findings. The committee members were reassured that all data collection would be done with WIFN best interests in mind and a copy of the data would be made available to the WIFN Heritage Center. As well, an opportunity to review the final work and make suggestions would be given to the WIFN Heritage Center committee upon request.

The discussion ended with an understanding that an agreement specifying the terms of the project and responsibilities of the project would be drafted in the coming weeks. However, after several attempts to ascertain the draft progress of the agreement, previously agreed upon timelines passed and no agreement was received. At this point, it was decided that interaction with the WIFN Heritage Center would not be an effective means of collecting data for the study design in the time period allotted for its completion, and the previous plans were discarded.

The failure of attempts to reach an agreement with the WIFN Heritage Center likely stems from many complex reasons, outlined by centuries of poor relations between First Nations and organizations outside the First Nations community. This became particularly evident by the level of concern and the types of questions posed regarding issues such as protection of information rights, access and control of publications, etc. In addition to this, it seems some level of discouragement has been reached by the community with regards to environmental research. Some discontent was expressed about the St. Clair River AOC Delisting process. One WIFN council member stated that government officials sampled “only 100 fish and concluded that there was no beneficial use impairment”. Hence, the wariness of the WIFN Heritage Center committee to partake in yet another research project involving their community is understandable. Unfortunately, this resistance to efforts made to conduct thorough research, which may lead to the production of conclusive results, makes it extremely difficult to move forward with such a project and produce any level of meaningful findings.

3.2 Alternative Methods

Since anecdotal information from WIFN fishermen, describing locations of historical catches with visible abnormalities, was unavailable, alternative methods for determining preferred sampling areas were hypothesized. It was hypothesized that macro-invertebrate index data might be used to determine areas likely to have elevated prevalence of fish tumours.

Macro-invertebrate communities are a common environmental indicator used in the assessment of degradation of aquatic environments. Metrics such as taxa richness and ratio of tolerant to intolerant species are used to assess the relative health of a given system. Macro-invertebrates are abundant in a variety of aquatic environments, and have limited mobility making them suitable assessors of site specific impacts (Barbour et al., 1999).

Several indices using benthic macro-invertebrates have been developed to assess aquatic conditions, such as the Invertebrate Community Index (ICI) (DeShon, 1995), Rapid Bioassessment Protocols (RBPs) (Shackleford, 1988; Plafkin et al., 1989; Barbour et al., 1992), and the benthic Index of Biological Integrity (IBI) (Kerans and Karr, 1994; Fore et al., 1996). These indices are broadly applicable to different geographic areas, making them useful for comparison of conditions (Barbour et al., 1995).

Common metrics utilized in the computation of these indices include measures of taxa richness, composition measures, tolerance/intolerance measures, feeding guilds, and trophic dynamics (Barbour et al., 1999). Taxa richness reflects the diversity of distinct taxa within a given macro-invertebrate community (Resh et al., 1995). The number of Ephemeroptera, Plecoptera, and Trichoptera (EPT), which are known to be sensitive to pollution (Bazata, 2005), is a commonly used richness measure. The lack of diversity, rarity, or absence of taxa in these insect orders is indicative of polluted waters. Reference areas with relatively un-polluted waters generally display an EPT index value of 10 or higher, while polluted sites often have and EPT Index below 6 (Masterson and Bannerman, 1994). Composition measures usually quantify the relative abundance of key taxa, such as %EPT, and provide information on the diversity of the assemblage. In this metric, a high level of redundancy in composition is indicative of the dominance of pollution tolerant organisms, and hence a lowered diversity (Barbour et al., 1999). Tolerance/intolerance measures act as similar composition metrics, which compare the numbers of tolerant to intolerant taxa. The Hilsenhoff Biotic Index (HBI) (Hilsenhoff, 1987; 1988) is a commonly used example of this metric. Feeding guilds and trophic dynamics measure the relative abundance of functional feeding groups and their roles in the trophic cascade. Balanced trophic interactions and feeding guilds are necessary for maintaining a healthy macro-invertebrate community, and thus this metric is effective at identifying stressed conditions (Barbour et al., 1999).

Given the broad applicability of macro-invertebrate indices in the assessment of aquatic conditions, and the relative ease of data collection and analysis, databases of macro-invertebrate metrics already exist for many watersheds, including the St. Clair River AOC (CRIC Delisting Working Group, 2011), and are one of the first steps in evaluating the biotic integrity of aquatic systems. For this reason, macro-invertebrate indices are a possible suitable indicator to be correlated with fish tumour prevalence. EPT Taxa Richness is the most commonly used metric found in studies which also quantified fish tumour metrics, and is therefore chosen for this analysis.

To assess whether macro-invertebrate indices and fish tumours are correlated, a preliminary assessment of studies providing this data was conducted

Tissue concentration of contaminants data were collected from several studies, which recorded levels of cadmium (Cd), chromium (Cr), copper (Cu), mercury (Hg), zinc (Zn), dichlorodiphenyltrichloroethane (DDT), and polychlorinated biphenyls (PCBs) (Table 4).

Table 4. EPT Index and concentration of chemical contaminants in fish tissue data collected from various studies.

Source	Location	EPT Taxa	Cd	Cr	Cu	Hg	Zn	DDT	PCBs
Masterson & Bannerman, 1994	Lincoln	6	0.04	0.20	1.24	0.06	40.00	0.20	5.75
	Oak	15	0.00	0.00	2.10	0.08	69.00	0.23	0.27
	Reference	24	0.00	0.00	1.30	0.14	51.00	0.00	0.00
Caldwell, 1992	Beetree Creek	39	0.10	0.25	0.62	0.09			
	High Shoals Creek	32	0.10	0.25	0.67	0.08			
	North Harper Creek	43	0.10	0.25	1.60	0.12			
	Dutchmans Creek	24	0.10	0.25	0.34	0.24			
	New Hope River	29	0.10	0.25	0.36	0.11			
	Suck Creek	21	0.10	0.25	0.50	0.15			
	Limestone Creek	1	0.10	0.25	0.41	0.24			
	W.P. Brice Creek	13	0.10	0.25	0.49	0.28			
Bazata, 2005	Unknown	15	0.06	0.10		0.05	65.80	0.01	
Ohio State EPA, 2003	Unknown	0						0.03	0.91
Ohio State EPA, 1993	Little Beaver Creek 1	5							0.86
	Little Beaver Creek 2	1							1.55
	Big Beaver Creek	7							0.41

These metrics, along with measurements of lesion frequency in fish, are found commonly in regional studies assessing water quality and biological integrity in local watersheds. Most of these studies use the same macro-invertebrate metrics, EPT Taxa richness being the most common, allowing comparison of data between studies. Correlation analysis was carried out between all datasets of chemical contaminants and EPT Taxa Richness. Despite greater data availability for heavy metal concentrations in fish tissue, there is little evidence, in the literature, that metal contamination in aquatic environments is directly linked to tumour development in fish (Cormier et al., 2002; Hinton, 1993). However, there is some indication that metal contamination may be associated with increased rates of deformities and fin erosion (Eisler, 2000; Hinton, 1993; U.S. Environmental Protection Agency, 1985; 1987).

On the other hand, it is well established that concentrations of organochlorine and aromatic compounds, such as PCBs, in fish tissue are correlated with the prevalence of lesions in fish (Greenfield et al., 2008; Johnson et al. 1993; Malins et al., 1985; Triebskorn et al., 2008). Therefore, a correlation between fish tumours and macro-invertebrate metrics (i.e. EPT Taxa Richness) may be drawn if one or both of the following hypotheses is true: 1) EPT Taxa Richness is inversely correlated with the concentration of organochlorine or aromatic compounds (PCBs, DDTs, PAHs, etc.) in fish tissue; and 2) EPT Taxa Richness is correlated negatively with the prevalence of lesions in fish.

Figure 13 displays that the concentration of PCBs in fish tissue is inversely correlated with EPT Taxa Richness, in accordance with Hypothesis 1 ($p<0.05$).

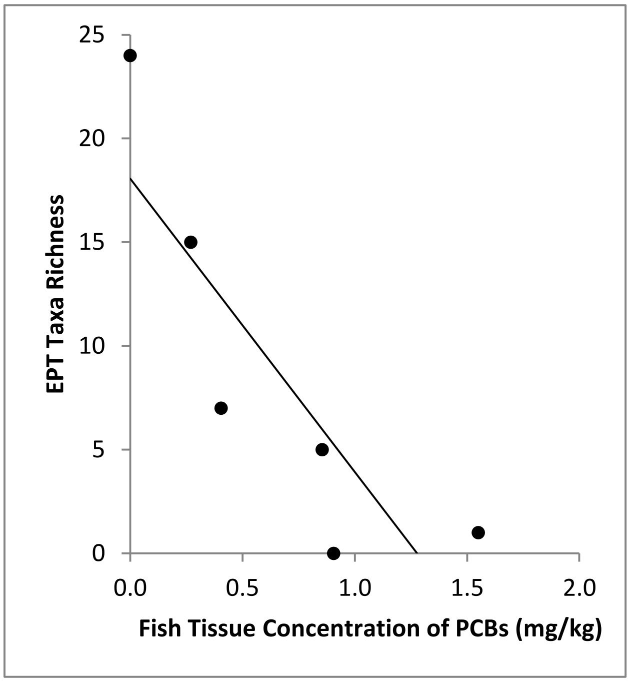


Figure 13. Correlation of concentration of PCBs in fish tissue with EPT Taxa Richness. Line of best fit: $y = -14.149x + 18.066$; R= 0.852; $p<0.05$.

However, no significant correlation is found between concentrations of DDT in fish tissue and EPT Taxa Richness ($p>0.05$) (Figure 14).

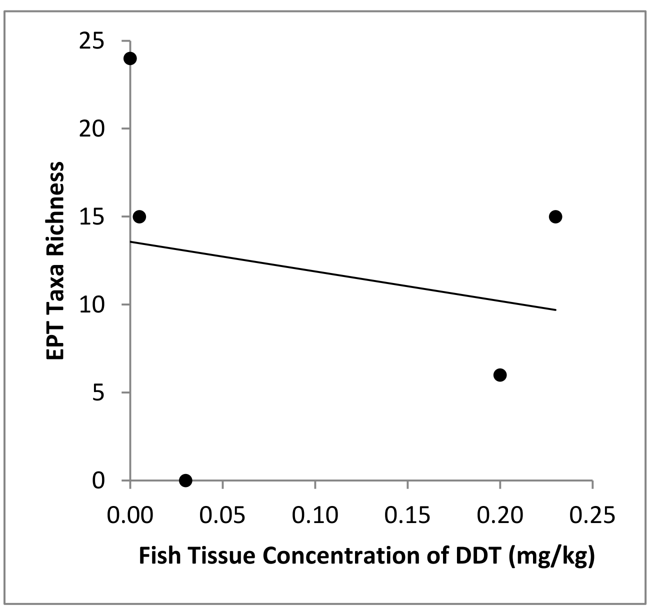


Figure 14. Correlation of concentration of DDT in fish tissue with EPT Taxa Richness. Line of best fit: $y = -16.904x + 13.572$; R= 0.206; $p>0.05$.

Concurrently, the concentration of Hg in fish tissue, along with all other metals listed above, does not display any significant correlation with EPT Taxa Richness (p>0.05) (Figure 15).

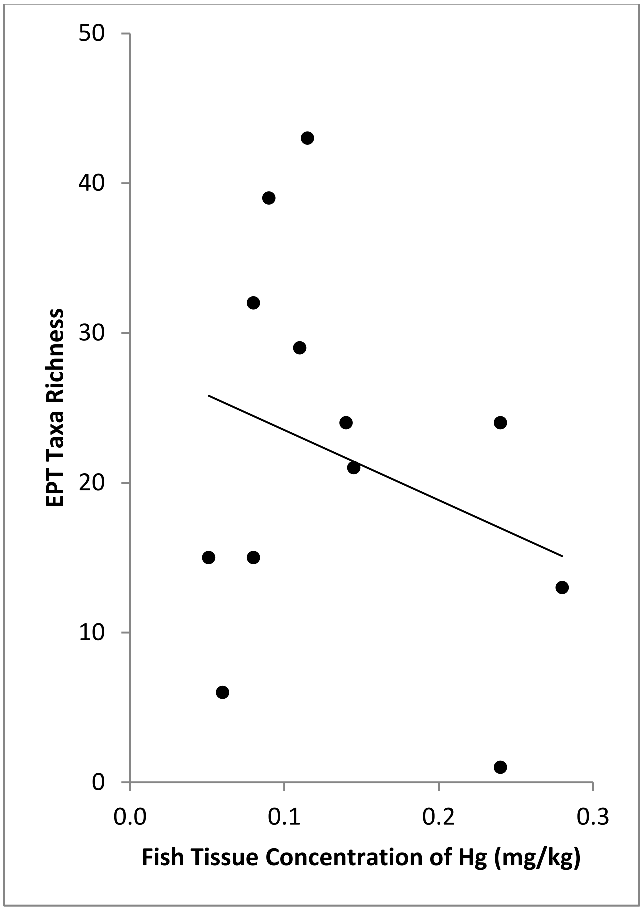


Figure 15. Correlation of concentration of Hg in fish tissue with EPT Taxa Richness. Line of best fit: y = -46.716x + 28.183; R= 0.284; p>0.05.

Data for prevalence of lesions in fish collected alongside EPT Taxa Richness metrics were not commonly found in studies, therefore the dataset used for correlation analysis is quite limited (Table 5).

Table 5. EPT Index and prevalence of lesions in fish data collected from various studies.

Citation	Location	EPT Index Score	% Lesions
Pinto et al., 2010	Upstream	4	29.0%
	Downstream	3	35.0%
Leroy et al., 2004	Site 1	3	1.0%
	Site 3	8	0.6%
Philadelphia Water Department, 2005	Site TF396	0	4.4%
	Site TF500	0	3.6%
	Site TF620	0	4.5%
	Site TF827	0	5.7%
	Site TF975	0	8.8%
	Site TF1120	0	9.0%
	Site FC1310	9	7.0%

There is no significant correlation displayed between EPT Taxa Richness and prevalence of lesions (Figure 16), as proven by the lack of a strong negative slope and very low correlation coefficients ($p>0.05$).

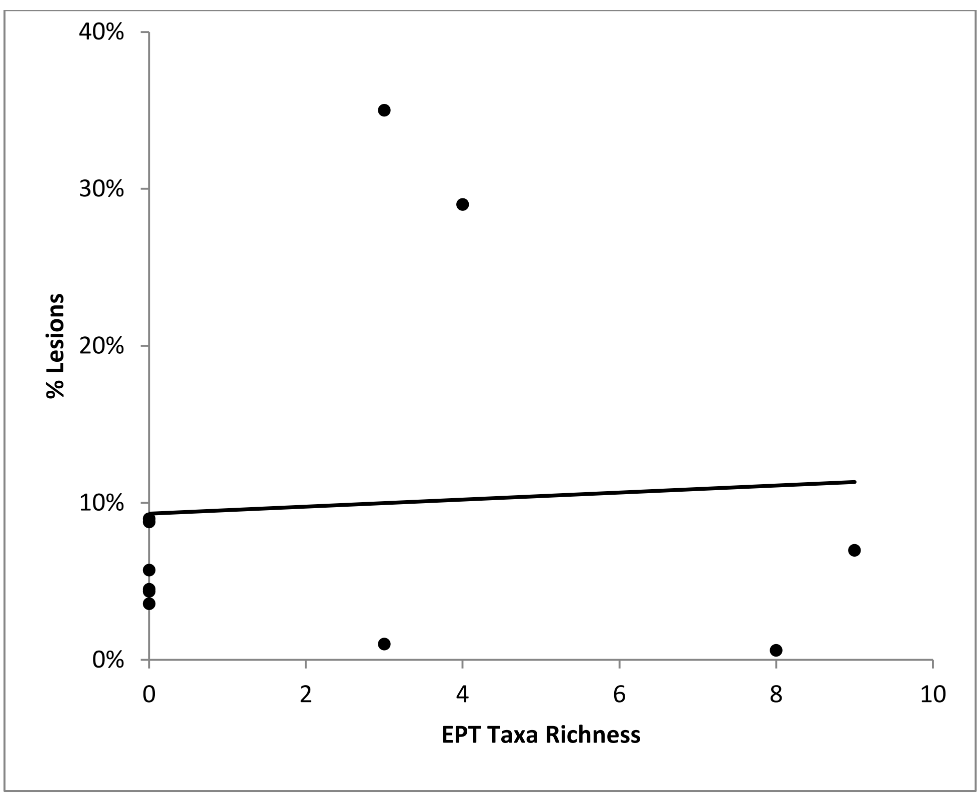


Figure 16. Relationship between EPT Taxa Richness and prevalence of lesions in fish. Line of best fit: $y = 0.0022x + 0.0931$; $R = 0.066$; $p>0.05$.

Hence, this preliminary analysis does not provide sufficient evidence of a correlation between fish tumours and macro-invertebrate indices to implement this idea as a method of determining preferred sampling locations. Although this analysis is somewhat supportive of the hypotheses, the data available are quite limited because there is very little overlap in the reporting of EPT Taxa Richness and concentration of contaminants, and lesions in fish tissue. Hence, the statistical power of the analysis is low. The correlations shown in Figures 13-16 are not entirely conclusive, and do not provide sufficient justification for use of the proposed method in the siting of sampling locations for a study. It is possible that the lack of strong correlation between some variables is due to differences in metric measurement between studies and inaccuracies of matching locations (since none of the referenced studies intended the two indicators to be compared). It would be useful to further explore the correlation between macro-invertebrate data, fish tissue contaminant concentrations and prevalence of lesions by means of targeted data collection. Such a study design could produce dramatically different results, and has the potential to influence justification methods for sampling site selection in future tumour prevalence studies.

3.3 Next Steps

Although this study design, and the majority of other studies assessing fish tumour prevalence, focuses on species such as bullhead and suckers, several sources report that grossly evident abnormalities in walleye, an important sport fish, have often been observed in the St. Clair River AOC (Mayne, 2003). Walleye are a key species of interest for WIFN fishermen (Sands, 1997), and other fishing enthusiasts in the St. Clair River region. Although liver tumours in these species are poorly documented, and their suitability as a sentinel species is less than that of bullheads or suckers (Baumann, 2010), it may be beneficial to investigate the prevalence of liver tumours in walleye in order to address concerns expressed by the surrounding communities.

4.0 RESEARCH SUMMARY

The proposed study design, to assess prevalence of fish tumours in the Lower St. Clair River, is necessary because no comprehensive assessment of the “fish tumours and deformities” BUI has been conducted to date, despite possible contaminant redistribution and interaction since the last remediation and sediment assessment efforts. The Lower St. Clair River is the area of specific focus in this study design due to the presence of a vulnerable First Nations community, inhabiting Walpole Island (WIFN), whose water supply and fishery comes from the river. In addition to a lack of conclusive data on the “fish tumours and deformities” BUI, there has been anecdotal evidence of fish abnormalities observed by WIFN locals.

White suckers are selected as the sentinel species for sampling in this study design, due to their abundance in the St. Clair River and their life histories, which make them susceptible to tumours linked to sediment contamination. The geographic area of focus in this study design spans from the southernmost tip of Stag Island to the fork in the main channel of the St. Clair River adjacent to the western side of Walpole Island. A reference area is chosen along the south-eastern coast Lake Erie, due to evidence in the literature of un-elevated prevalence of fish tumours. As well, datasets of tumour prevalence rates from Great Lakes reference sites could be used as a control for prevalence rates within the AOC. Sampling sites within the AOC are not conclusively established due to constraints in obtaining data from the WIFN Heritage Center.

However, it is proposed that sampling locations be alternatively determined based on macro-invertebrate indicators, as well as chosen based on community and natural importance (e.g. Water Treatment Plant). The recommended sample size to ensure a representative sample is 180 fish in total from the AOC sites, and 180 fish from the reference sites in total. Collection of fish samples should preferably occur in the fall. Boat electrofishing is the recommended method for collection of fish. Fish should be externally examined and the liver should be removed and preserved in fixative solution. Parameters such as location of sample collection, external characteristics of the fish, DELT index and liver weight should all be recorded in the field. These preliminary parameters may be used to decide which samples should be submitted for histopatholgical analysis. Laboratory examination should produce the following data:

accurate estimate of age; presence or absence of a particular lesion; and characterization of tumour as neoplastic or non-neoplastic.

Only true neoplasms are to be considered as the presence of a tumour in the prevalence calculations. True neoplasms are defined as neoplastic hepatocellular or cholangiolar lesions. Non-neoplastic lesions should be recorded for informative purpose, but be excluded from calculation. Histological characteristics such as degree of invasiveness, compression of surrounding tissue and presence of mitotic apparatus should be used to differentiate between neoplastic hepatocellular adenomas/carcinomas and neoplastic biliary cholangiomas/cholangiocarcinomas.

It is suggested that a possible method for identification of preferred sampling sites in fish tumour studies could be carried out using macro-invertebrate indices as indicators. Although some datasets from the literature suggest that a correlation between macro-invertebrate indices and fish tumours may exist, differences between studies introduce uncertainty to this analysis. A more conclusive analysis of this relationship could be carried out with targeted macro-invertebrate data collection.

Future fish tumour research in the Lower St. Clair River could also benefit from collection of data on walleye, which have are an important sport fish for WIFN and local fishermen, and are anecdotally known to display abnormalities, but have rarely been the subject of fish tumour assessments in any Great Lakes studies.

APPENDIX A

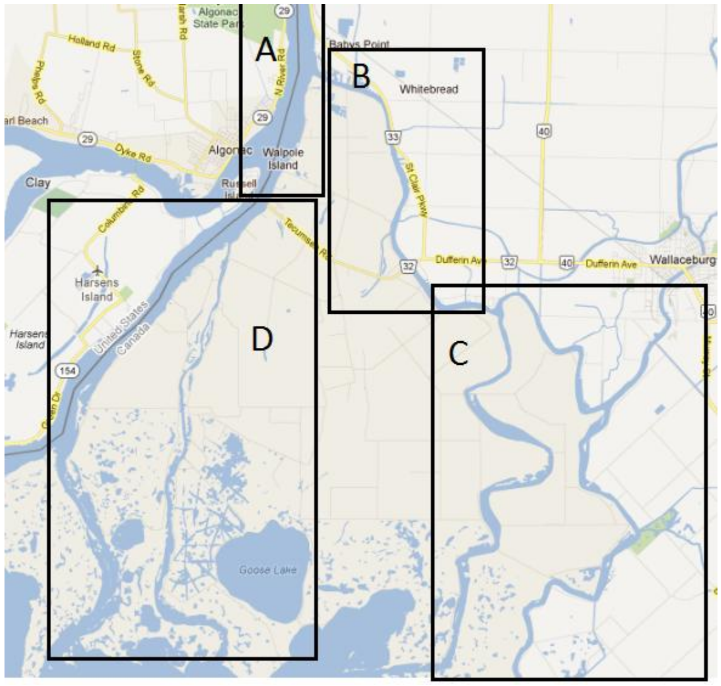
Fish Abnormalities Questionnaire

1. **In your experience as a fisherman, have any of the fish you have caught from the St. Clair River looked not normal?**

2. **Can you recall how many times you have seen something not normal in the fish you caught?**

3. **Can you remember when these fish were caught? What month or season? What year?**

4. **From the best your memory, please show us where you caught fish that looked not normal. There are maps of the St. Clair River and its channels on the following pages.**
   Please put an "X" in the area where you have caught a fish that looked not normal.
   If the location is not on the maps, please describe where you caught these fish by making a note below.


A
B
C
D
Whitebread
Babys Point
Algonac
Walpole Island
Clay
Harsens Island
Goose Lake
Dufferin Ave
Wallaceburg
Holland Rd
Dyke Rd
Columbine Rd
Tecumseh Rd
St Clair Pkwy

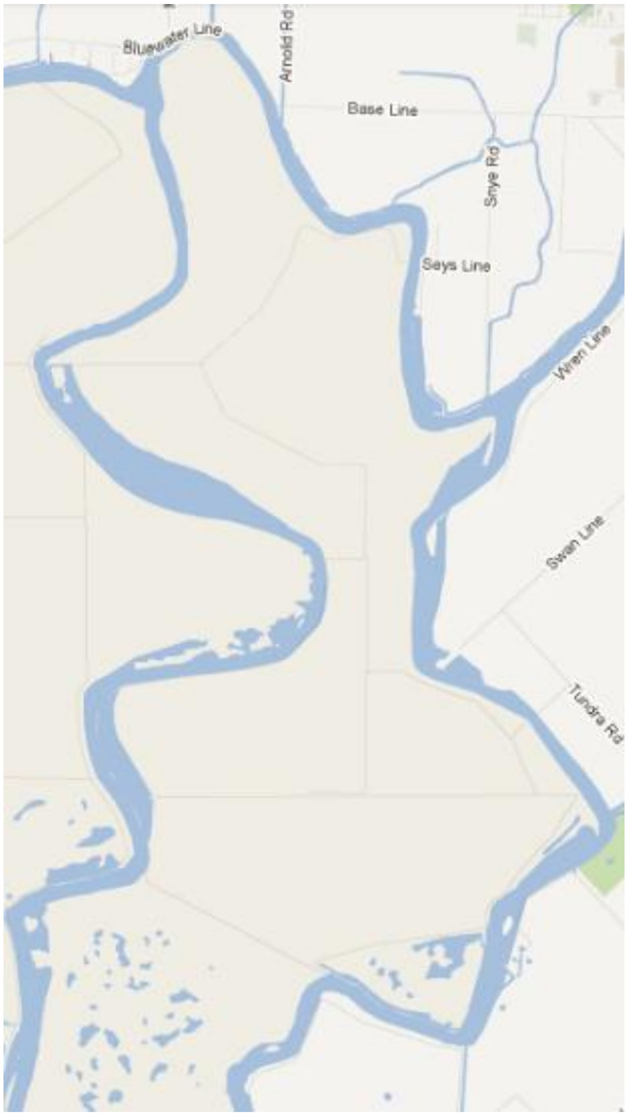
Map A:


St Clair Pkwy
Babys Point
N River Rd
Old River Rd
Riverside Dr
Island Ave
S Parkway Dr
Greenwood
River Rd
Sherwood Ln
United States
Canada
St Clair River Dr
Dan Shab Rd
Algonac - Walpole Island
Walpole Island
Austin Rd
N Russell Dr
S Russell Dr

Map B:


33
Whitebread
Whitebread Line
St Clair Pkwy
Langstaff Line
Payne Rd
Stewart Line
Bridge Rd
Dufferin Ave
32
Tecumseh Rd
Bluewater Line
Snye River Rd

Map C:


Bluewater Line
Arnold Rd
Base Line
Snye Rd
Seys Line
Wren Line
Swan Line
Tundra Rd

Map D:


154
Harsens Island
Lions Field
Green Dr
S Channel Dr
S Channel Dr
United States
Canada
River Rd
Squirrel Island Rd
Creek Rd S
Shawnee Rd
River Rd
River Rd

5. **Please circle the type of fish caught which looked not normal. If it is not listed below, please circle "Other" and describe what type of fish it was (if known).**

Minnow

2.5 inches

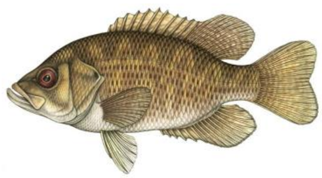
Bullhead/Catfish

14 inches

Sunfish

6–8 inches

Bass

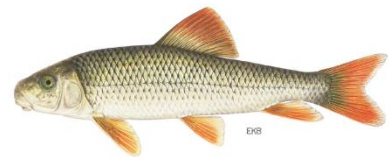


6-8 inches

Carp

12-25 inches

Sucker



20 inches

Pike

12-50 inches

Chub

8-10 inches

Darter

1.5-2.5 inches

Perch

6-12 inches

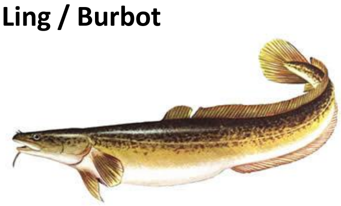
Stickleback

2 inches

Walleye

10-33 inches

Ling / Burbot



15 -22 inches

Smelt

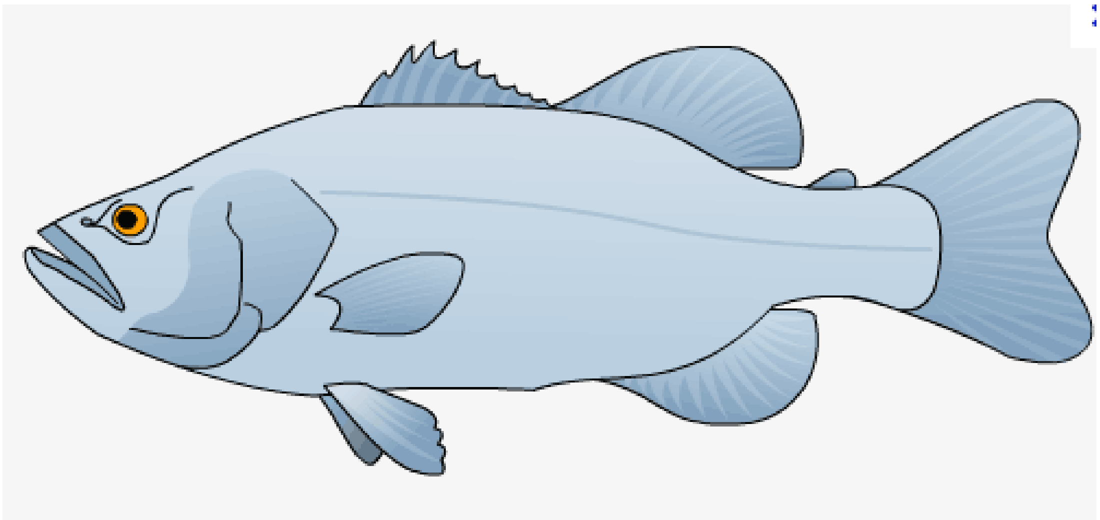
7 to 9 inches

Sturgeon

7–12 feet

Other (please describe):

6. **From what you can recall, please show us what part of the fish body looked not normal. Please mark an "X" on the area of the fish body where you saw something not normal.**



7. **Please describe what was not normal about the fish.**

   Some common examples include:
   growths - swelling, raised bumps, not normal colours
   wounds – damaged fins or barbells, gashes in the body, red, open or bloody sores
   deformities - curved spine, shortened body, missing or additional fins

REFERENCES

Aboriginal Affairs and Northern Development Canada. Walpole Island First Nation to Build $10 Million Water Treatment Facility. Wallaceburg, Ontario. November 25, 2005. Press Release.

Arcand-Hoy, L.D. and C.D.Metcalfe. 1999. Biomarkers of exposure of brown bullheads *(Ameirus nebulosus)* to contaminants in the lower Great Lakes, North America. Environ. Toxicol. Chem. 18, 740–749.

Barbour, M.T., J. Gerritsen, B.D. Snyder, and J.B. Stribling. 1999. Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates and Fish, Second Edition. EPA 841-B-99-002. U.S. Environmental Protection Agency; Office of Water; Washington, D.C.

Barbour, M.T., J.B. Stribling, and J.R. Karr. 1995. Multimetric approach for establishing biocriteria and measuring biological condition. In "Biological assessment and criteria: Tools for water resource planning and decision making" p 63-77. W.S. Davis and T.P. Simon Eds. Lewis Publishers, Boca Raton, Florida.

Barbour, M.T., J.L. Plafkin, B.P. Bradley, C.G. Graves, and R.W. Wisseman. 1992. Evaluation of EPA's rapid bioassessment benthic metrics: Metric redundancy and variability among reference stream sites. Environmental Toxicology and Chemistry 11(4):437-449.

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

Baumann, P.C. 1998. Epizootics of cancer in fish associated with genotoxins in sediment and water, Reviews in Mutation Research. Mutation Research. 411: 227-233.

Baumann, P.C. 1998. Epizootics of cancer in fish associated with genotoxins in sediment and water, Reviews in Mutation Research. Mutation Research. 411: 227-233.

Baumann, P.C. and J.C. Harshbarger. 1995. Decline in liver neoplasms in wild brown bullhead catfish after coking plant closes and environmental PAHs Plummet. Environmental Health Perspectives, Journal of the National Institute of Environmental Health Sciences. 103(2):168-170.

Baumann, P.C. and J.C. Harshbarger. 1998. Long term trends liver neoplasm epizootics of brown bullhead in the Black River, Ohio. Environmental Monitoring and Assessment, 53: 213 – 223.

Baumann, P.C. and M.S. Okihiro. 2000. Fish as a Cancer Model, In: The Laboratory Fish. G.K. Ostrander, ed. Academic Press, London. pp 591-616.

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., 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., 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., W.D. Smith, and W.K. Parland. 1987. Contaminants concentrations and tumor frequencies in brown bullhead from an industrialized river and a recreational lake. Trans. Amer. Fish. Soc. 116:79-86.

Bazata, K. 2005. Nebraska Stream Classification Using Fish, Macroinvertebrates, Habitat, And, Chemistry Evaluations From R-EMAP Data, 1997-2001. Nebraska Department of Environmental Quality, Surface Water Section, Water Quality Division. Web. Jun. 2012.

Bernet, D., H. Schmidt, W. Meier, P. Burkhardt-Holm, and T. Wahli. 1999. Histopathology in fish: proposal for a protocol to assess aquatic pollution. J. Fish Dis. 22: 25–34.

Bettross, E. A. and D. W. Willis. 1988. Seasonal patterns in sampling data for largemouth bass and bluegills in a northern Great Plains impoundment. Prairie Nat. 20: 193-202.

Bi-National Public Advisory Council (BPAC). Point Source and Non-Point Source Pollution. N.d. Web.

Black, J.J. 1983. Epidermal hyperplasia and neoplasia in brown bullheads (Ictalurus nebulosus) in response to repeated applications of a PAH containing extract of polluted river sediment. In Polynuclear Aromatic Hydrocarbons: Formation, Metabolism, and Measurement, M.W. Cooke and A.J. Dennis (eds). Columbus, OH, Battelle Press. pp. 99-112.

Black, J.J. 1984. Aquatic animal neoplasia as an indicator for carcinogenic hazards to man. In Hazard assessment of chemicals - current developments. Ed. J. Saxena. Academic Press Inc. New York. pp.181-232.

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 Department of Environmental Protection, Pennsylvania Sea Grant.

Blazer, V.S., S.D. Rafferty, P.C. Baumann, S.B. Smith, and E.C. Obert. 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.

Blouin, M.A. and G.R. Hall. 1990. Improved method for sectioning pectoral spines of catfish for age determination. J. Freshwater Eco. 5(4):489-490.

Bonar, S.A., Bolding, B.D., and M. Divens. 2000. Standard Fish Sampling Guidelines for Washington State Ponds and Lakes. Washington Department of Fish and Wildlife, Fish Program, Science Division, Inland Fisheries Investigation. Olympia, Washington. Web.

Borowitzka M.A. 1972. Intertidal algal species diversity and the effect of pollution. Australian Journal of Marine and Freshwater Research 23(2): 73 – 84.

Bowser, P.R., M.J. Wolfe, J. Reimer, and B.S. Shane. 1991. Epizootic papillomas in brown bullheads Ictalurus nebulosus from Silver Stream reservoir. New York. Dis aquat Org 11. 117-127.

Brown, C.R., N. Komar, S.B. Quick, R.A. Sethi, et al. Arbovirus infection increases with group size. Proc R Soc Lond B. 2001; 268:1833–1840.

Brown, E. and T. Sinclair. 1977. Chemical Pollutants in Relation to Diseases in Fish. Annals of the New York Academy of Sciences 298: 535-546.

Brown, E., J. Hazdra, L. Keith, I. Greenspan, J. Kwapinski and P. Beamer. 1973. Frequency of Fish Tumors Found in a Polluted Watershed as Compared to Nonpolluted Canadian Waters. Cancer Research 33; 189-198.

Bucke D., D. Vethaak, T. Lang and S. Mellergaard. 1996. Common diseases and parasites of fish in the North Atlantic: training guide for identification. ICES TIMES 19:1-27.

Bunton, T.E. 1996. Experimental chemical carcinogenesis in fish. Toxicol. Pathol. 4: 603-618.

Caldwell, W.S. 1992. Selected Water-Quality and biological Characteristics of Streams in some Forested Basins of North Carolina, 1985-1988. North Carolina Department of Environment, Health and Natural Resources.

Camargo, J.A. and A. Alonso. 2006. Ecological and toxicological effects of inorganic nitrogen pollution in aquatic ecosystems: A global assessment. Environment International 32(6): 831–849.

Cameron, A. 2002. Survey Toolbox for Aquatic Animal Diseases. A Practical Manual and Software Package. ACIAR Monograph No. 94, 375p.

Cooke J. and D. Hinton. 1999. Promotion by 17β-estradiol and β-hexachlorocyclohexane of hepatocellular tumors inmedaka, Oryzias latipes. Aquatic Toxicology , 45, 127–45.

Cormier S.M., S.B. Norton, G.W. Suter 2nd, D. Altfater, and B. Counts. 2002. Determining the causes of impairments in the Little Scioto River, Ohio, USA: part 2. Characterization of causes. Environ Toxicol Chem. 21(6):1125-37.

CRIC Delisting Working Group. St. Clair River Area Of Concern Revised Canadian Delisting Criteria, 2010. 2011.

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.

Dell, R.B., S. Holleran, and R. Ramakrishnan. 2002. Sample Size Determination. ILAR Journal 43 (4): 213.

DeShon, J.E. 1995. Development and application of the invertebrate community index (ICI). In Biological assessment and criteria: Tools for water resource planning and decision making. pp 217-243. W.S. Davis and T.P. Simon Eds. Lewis Publishers, Boca Raton, Florida.

Edwards, A., J. Barnucz, and N.E. Mandrak. 2006. Boat electrofishing survey of the fish assemblages in the St. Clair River, Ontario. Can. Manuscr. Rpt. Fish. Aquat. Sci. 2742: 57 p.

Eisler, R. 2000. Handbook of Chemical Risk Assessment. Vol 1. Lewis, Boca Raton, FL, USA.

ENVIRON International Corporation. Final Project Report for Applying the COA Framework to the St. Clair River Area of Concern. 2009.

Environment Canada and Ontario Ministry of the Environment. St. Clair River Area of Concern, Canadian Section: Status of Beneficial Use Impairments, September 2010. 2011.

Environment Canada and U.S. Environmental Protection Agency. State of the Great Lakes. 2001. Web. Jun. 2012.

Environment Canada, Canadian National Environmental Effect Monitoring Office. Pulp and paper technical guidance for aquatic environmental effects monitoring. Gatineau, Quebec. 2005.

Environment Canada. Data Analysis and Fish Tumor BUI Assessment for the Lower Great Lakes and Interconnecting Waterways. Baumann, P.C. 2010.

Environment Canada. Toxic chemicals in the Great Lakes and associated effects: synopsis. 1991. Web. Feb. 2012.

Fabacher D.L. and P.C. Baumann. 1985. Enlarged livers and hepatic microsomal mixed-function oxidase components in tumor-bearing brown bullheads from a chemically contaminated river. Environ Toxicol Chem 4:703–710

Fleiss, J. L. 1981. Statistical Methods for Rates and Proportions. Wiley, New York.

Fore, L.S., J.R. Karr, and R.W. Wisseman. 1996. Assessing invertebrate responses to human activities: Evaluating alternative approaches. Journal of the North American Benthological Society 15(2):212-231.

Gagnon, M.M., J.J. Dodson, P.V.Hodson, G.Van Der Kraak, and J.H. Carey. 1994. Seasonal effects of bleached-kraft mill effluent on reproductive parameters of white sucker *(Catostomus commersoni)* populations of the St. Maurice River, Québec, Canada. Can. J. Fish. Aquat. Sci. 51: 337–347.

Geen, G.H., T.G. Northcote, G.F. Hartman, and C.C. Lindsey. 1966. Life histories of two species of catostomid fishes in the Sixteenmile Lake, British Columbia, with particular reference to inlet stream spawning. J. Fish. Res. Bd. Canada 23: 1761-1788.

Greenfield, B.K., S.J. Teh, J.R. Ross, J. Hunt, G. Zhang, J.A. Davis, G. Ichikawa, D. Crane, S.O. Hung, D. Deng, F. Teh, and P.G. Green. 2008. Contaminant Concentrations and Histopathological Effects in Sacramento Spittail *(Pogonichthys macrolepidotus)*. Arch Environ Contam Toxicol 55: 270-281.

Guy, C.S., and D.W. Willis. 1991. Evaluation of largemouth bass-yellow perch communities in small South Dakota impoundments. North American Journal of Fisheries Management 11:43–49.

Harshbarger, J.C. 1991. Introduction to Session on Pathology and Epizootiology. Environmental Health Perspectives 90: 5.

Harshbarger, J.C., L.J. Cullen, M.J. Calabrese, and P.M. Spero. 1984. Epidermal, Hepatocellular and Cholangiocellular Carcinomas in Brown Bullheads, *Ictalurus nebulosus*, from Industrially Polluted Black River, Ohio. Marine Environmental Research 14: 535-536.

Hartig J.H., and M.A. Zarull. 1992. Towards defining aquatic ecosystem health for the Great Lakes. Journal of Aquatic Ecosystem Stress and Recovery, 1 (2): 97-107.

Hartig, J. H., and G. Mikol. 1992. An operational definition of ''How clean is clean?'' for degraded areas in the Great Lakes. Journal of Environmental Engineering and Management 2:15–23.

Hayes, M.A., I.R. Smith, T.H. Rushmore, T.L. Crane, C. Thorn, T.E. Kocal and H.W. Ferguson. 1990. Pathogenesis of skin and liver neoplasms in white suckers from industrially polluted areas in Lake Ontario. Science of the Total Environ. 94: 105-123.

Hayes, T. 2002. St. Clair River fish liver tumour study. Environment Canada.

Hayes, W. J., and E. R. Laws Jr. (1991). Handbook of Pesticide Toxicology. Academic Press Inc. New York, pp. 816–22.

Hayman, P. 2009. St. Clair River Watershed Plan, December 2009. St. Clair Region Conservation Authority.

Hilsenhoff, W.L. 1987. An improved biotic index of organic stream pollution. Great Lakes Entomologist 20: 31-39.

Hilsenhoff, W.L. 1988. Rapid field assessment of organic pollution with a family level biotic index. Journal of the North American Benthological Society 7(1):65-68.

Hinton, D.E. 1993. Toxicologic histopathology of fishes: A systemic approach and overview. In "Pathobiology of Marine and Estuarine Organisms", Couch J.A., Fournie J.W., Eds. CRC, Boca Raton, FL, USA, pp 177-215.

Hinton, D.E., J.A. Couch, S.J. The, and L.A. Courtney. 1989. Cytological changes during the progression of neoplasia in selected fish species. Aquat. Toxicol. (Amst.). 11:77-112.

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

International Joint Commission. Report on Spills in the Great Lakes Basin with a Special Focus on the St. Clair–Detroit River Corridor. 2006. Web.

Johnson P.D., B.V. Dawson, S.J. Goldberg, and J.B. Ulreich. 1993. Cardiac teratogenesis of halogenated hydrocarbon-contaminated drinking water. J Am Coll Cardiol. 21:1466–1472.

Johnson, L. L., C.M. Stehr, O.P. Olson, M.S. Myers, S.M. Pierce, C.A. Wigren, B.B. McCain, and U. Varanasi. 1993. Chemical contaminants and hepatic lesions in winter flounder *(Pleuronectes americanus)* from the northeastern coast of the United States. Environmental Science and Technology, 27: 2759–2771.

Kerans, B.L. and J.R. Karr. 1994. A benthic index of biotic integrity (B-IBI) for rivers of the Tennessee Valley. Ecological Applications, 4:768-785.

Lake Erie Lakewide Management Plan (LaMP) Technical Report Series; Fish Tumors or Other Deformities. Baumann P., V. Cairns, B. Kurey, L. Lambert, I. Smith and R. Thoma. 2000.

Lake Huron Centre for Coastal Conservation. Nearshore Water Quality. 2004. Web. Feb. 2012.

Leadley, T. A., G. Balch, C. D. Metcalfe, R. Lazar, E. Mazak, J. Habowsky, and G. D. Haffner. 1998. Chemical accumulation and toxicological stress in three brown bullhead (*Ameiurus nebulosus*) populations of the Detroit River, Michigan, USA. Environmental Toxicology and Chemistry, 17: 1756–1766.

Livingstone, D.R. 2001. Contaminant-stimulated reactive oxygen species production and oxidative damage in aquatic organisms. Marine Pollution Bulletin 42(8): 656-666.

Loeb, H.A. 1964. Submergence of brown bullheads in bottom sediment. New York Fish and Game Journal 11(2):119-124.

Luna, L.G. 1992. Histopathologic Methods and Color Atlas of Special Stains and Tissue Artifacts. American Histolabs,Inc.

Maclennan, D. and R. Hyatt. 1996. Assessment of the fish community of the St. Clair River in 1994 associated with the Stage 1 RAP for this AOC. Ontario Ministry of Natural Resources, Lake St. Clair Fisheries Station. Draft Report, 33 pp.

Mahon, R., E.K. Balon, and D.L. Noakes. 1979. Distribution, community structure and production of fishes in the upper Speed River, Ontario: A preimpoundment study. Environ. Biol. Fish. 4: 2 19-244.

Malins, D.C., M.M. Krahn, M.S. Myers, L.D. Rhodes, D.W. Brown, C.A. Krone, B.B. McCain, and S-L. Chan. 1985. Toxic chemicals in sediments and biota from creosote-polluted harbor: Relationships with hepatic neoplasms and other hepatic lesions in English sole *(Parophrys vetulus)*. Carcinogenesis 6:1463-1469.

Mandrak, N. and E.J. Crossman. 1992. A Checklist of Ontario Freshwater Fishes. Royal Ontario Museum, Toronto, Ontario.

Manny, B.A., Edsall, T.A., and E. Jaworski. 1988. The Detroit River, Michigan: an Ecological Profile. U.S. Fish and Wildlife Service. U.S. Biological Report 85(7.17).

Masterson, J.P., and R.T Bannerman. 1994. Impacts of Stormwater Runoff on Urban Streams in Milwaukee County, Wisconsin. In National Symposium on Water Quality, American Water Resources Association.

Mayne, G. 2003. The St. Clair River Area of Concern Remedial Action Plan Progress Report. Environment Canada.

McCain, B.B., K.V. Pierce, S.R. Wellings, and B.S. Miller. 1977. Hepatomas in marine fish from an urban estuary. Bulletin of Environmental Contamination and Toxicology, 77, 1-2.

McInerny, M. C. and T. K. Cross. 2000. Effects of sampling time, intraspecific density, and environmental variables on electrofishing catch per effort of largemouth bass in Minnesota lakes. North American Journal of Fisheries Management 20:328-336.

Millard, M.J., D.R. Smith, E.C. Obert, J.L. Grazio, M.Bartron, C. Wellington, et al. 2009. Movement of Brown Bullheads in Presque Isle Bay, Lake Erie, Pennsylvania. J. Great Lakes Res. 35, 613–619.

Motulsky, H. Intuitive Biostatistics. 1995. Oxford University Press, New York, pp 386.

Munkittrick K.R., C.B. Portt, G.J. Van Der Kraak, I.R. Smith, and D.A. Rokosh. 1991. Impact of bleached kraft mill effluent on population characteristics, liverMFO activity, and serum steroid levels of a Lake Superior white sucker *(Catostomus commersoni)* population. Can. J. Fish. Aquat. Sci. 48:1371–1380.

Munkittrick K.R., S.C. Ribey, R. Prairie, S. Courtenay, C. Langlois, M. McMaster, S. Munger, A. Rosaasen, and G. Whitley. 2002. Development of a monitoring design for examining effects in wild fish associated with discharges from metal mines. Water Qual. Res. J. Canada 37(1).

Myers M.S., J.T. Landahl, M.K. Kahn, L.L. Johnson and B.B. McCain. 1990. Overview of studies on liver carcinogenesis in English sole from Puget Sound; Evidence for a xenobiotic chemical etiology I: Pathology and epizootiology. Sci. Total Environ. 94:33-50.

Myllyoja, R. and G. Johnson. 1995. St. Clair River Remedial Action Plan: Fish Tainting Evaluation. Texas Parks and Wildlife Department, Resource Protection Division. Status of Biotic Integrity, Water Quality, and Physical Habitat in Wadeable East Texas Streams. 2004. Web.

Nunez, O., J D. Hendricks, D. N. Arbogast, A.T. Fong, B.C. Lee, G.S. Bailey. 1989. Promotion of aflatoxin B, hepatocarcinogenesis in rainbow trout by 17b-estradiol. Aquat. Toxicol. 15: 289-302.

Ohio EPA, Division of Surface Water. Biological, Fish Tissue, and Sediment Study of the Ottawa River. OEPA Technical Report EAS/2003-1-2. 2003. Web.

Oliver, B.G., and C.W. Pugsley. 1986. Chlorinated contaminants in St. Clair River sediments. Water Poll. Res. J. Canada 21(3): 368–379.

Olsen, R. N.d. Monitoring Design: Component Details. U.S. Environmental Protection Agency, NHEERL Western Ecology Division.Web. March 15, 2012.

Ontario Ministry of Environment. St. Clair River Organics Study: Biological Surveys 1968 and 1977. Water Resources Assessment Unit, Southwestern Region. 1979.

Ontario Ministry of the Environment. The St. Clair River Area of Concern Environmental Conditions and Problem Definitions, Stage 1 Remedial Action Plan. 1992.

Ontario Ministry of the Environment and Energy (OMOEE). 1993. Data files and draft summary report on the 1990 water quality, sediment quality, and benthic macroinvertebrate surveys. Prepared by P. Kauss, Water Resources Branch, Toronto, Ontario.

Osburn, R.C. 1925. Black tumor of the catfish. Bull. Bur. Fish. 41:9-13.

Pflugh, K.K., L. Lurig, L.A. Von Hagen, and J. Burger. 1999. Urban anglers' perception of risk from contaminated fish. Science of The Total Environment, 228 (2–3): 203–218.

Philadelphia Water Department. Tookany/Tacony-Frankford Integrated Watershed Management Plan. 2005. Web. Jul. 2012.

Pinkney, A.E., J.C. Harshbarger, E.B. May, and M.J. Melancon. 2001. Tumor revalence 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, E.B. May, and W.L. Reichert. 2004. Tumor prevalence and biomarkers of exposure and response in brown bullhead *(Ameiurus nebulosus)* from the Anacostia River, Washington, DC and Tuckahoe River, Maryland, USA. Environ. Toxicol. Chem. 23, 638–647.

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.

Pinto, A.L., S. Varandas, A.M. Coimbra, J. Carrola, and A. Fontainhas-Fernandes. 2010. Mullet and gudgeon liver histopathology and macroinvertebrate indexes and metrics upstream and downstream from a wastewater treatment plant (Febros River – Portugal). Environ Monit Assess 169:569-585.

Plafkin, J.L., M.T. Barbour, K.D. Porter, S.K. Gross, and R.M. Hughes. 1989. Rapid bioassessment protocols for use in streams and rivers: Benthic macroinvertebrates and fish. U.S. Environmental Protection Agency, Office of Water Regulations and Standards.EPA 440-4-89-001.

Pope K.L. and D.W. Willis. 1996. Seasonal Influences on Freshwater Fisheries Sampling Data. Reviews in Fisheries Science 4(1): 57- 73.

Pope, R.J. 1993. An Assessment of 1990 St. Clair River Benthic-macroinvertebrate Communities Relative to Sediment Quality. Tarandus Associates Limited.

Portt, C.B., G.A. Coker, D.L. Ming, and R.G. Randall. 2006. A review of fish sampling methods commonly used in Canadian freshwater habitats. Can. Tech. Rep. Fish. Aquat. Sci. 2604 p.

Profet, E.B., B. Mills, J.B. Arrington and L.H. Sobin. 1992. Laboratory Methods in Histotechnology. Armed Forces Institute of Pathology, Registry of Pathology, Washington, DC.

Rafferty, S. 2003. Fish Tumors Related to Great Lakes Areas of Concern Conference Proceedings. Pennsylvania Sea Grant. Penn State Erie.

Rafferty, S. and J. Grazio. 2006. Field Manual for Assessing Internal and External Anomalies in Brown Bullhead *(Ameiurus nebulosus)*. Pennsylvania Department of Environmental Protection, Pennsylvania Sea Grant.

Rafferty, S.D. 2006. Development of Standardized Criteria for the Assessment of Brown Bullhead Lesions and Deformities in Areas of Concern conference proceedings. Pennsylvania Sea Grant.

Rainey, E.C., and D.A. Webster. 1942. The spring migration of the common white sucker, *Catostomus c. commersonnii (Lacepede)* in the Skaneateles Lake inlet, New York. Copeia 1942: 139-148.

Reible D.D. and S.A. Savant-Malhiet. 1993. Comparison of physical transport processes in noncohesive river sediments. J Environ Eng 119:90–102.

Reid S.M., N.E. Mandrak, L.M. Carl, and C.C. Wilson. 2009. Influence of dams and habitat condition on the distribution of redhorse *(Moxostoma)* species in the Grand River watershed, Ontario. Environmental Biology of Fishes 81: 111–125.

Resh, V.H., R.H. Norris, and M.T. Barbour. 1995. Design and implementation of rapid assessment approaches for water resource monitoring using benthic macroinvertebrates. Australian Journal of Ecology 20:108-121.

Richman, L. and D. Milani. 2009. Sediment contaminant concentrations in the St. Clair River 2006 and potential implications for sport fish tissue concentrations. Environment Canada and Ontario Ministry of the Environment.

Richman, L. and D. Milani. 2010. Temporal trends in near-shore sediment contaminant concentrations in the St. Clair River and potential long-term implications for fish tissue concentrations. Journal of Great Lakes Research 36: 722–735.

Sakaris, P.C., R.V. Jesien, and A.E. Pinkney. 2005. Brown bullhead, Ameiurus nebulosus, as an indicator species: seasonal movement patterns and home ranges within the Anacostia River, Washington, D.C. Trans. Amer. Fish. Soc. 134:1262- 1270.

Salomons, W., N.M. de Rooji, H. Herdick, and J. Bril. 1987. Sediment as a source for contaminants. Hydrobiologia. 149: 13-30.

Sands, K. Bkejwanong Walpole Island First Nation. 1997. Web. Jul. 2012.

Scott, W.B. and E.J. Crossman. 1973. Freshwater fishes of Canada. Bull. Fish. Res. Board Can. 184: 1-966.

Servos, M., J. Carey, M. Ferguson, G.Van Der Kraak, H. Ferguson, J. Parrott, K. Gorman, and R. Cowling. 1992. Impact of a modern bleached kraft mill with secondary treatment on white suckers. *Water Poll. Res. J. Can.* 27: 423-437.

Shackleford, B. 1988. Arkansas Department of Pollution Control and Ecology. Rapid Bioassessments of Lotic Macroinvertebrate Communities: Biocriteria Development.

Sheenan, P.J. 1984. Effects on Individuals and Populations. In “Effects of Pollution at the Ecosystem Level”. P. J. Sheehan, D. R. Miller, G. C. Butler and Ph. Bourdeau Eds. John Wiley & Sons Ltd.

Sindermann, C., L. Swanson, and G. Han. Oxygen depletion and the future: an evaluation. In Oxygen Depletion and Associated Benthic Mortalities in the New York Bight, 1976. NOAA Professional Paper 11. Rockville Md. December 1979.

Smith, C.E., T.H. Peck, R.J. Klauda, and J.B. McLaren. 1979. Hepatomas in Atlantic Tomcod Microgadus tomcod (Walbaum) Collected in the Hudson River estuary in New York. J. Fish Diseases. 2: 313-319.

Smith, R. 2002. Walpole Island First Nation Community Background. J. Aboriginal Economic Development , 3(1).

Smith, S.B., M.A. Blouin, and M.J. Mac. 1994. Ecological comparisons of Lake Erie tributaries with elevated incidence of fish tumors. Journal of Great Lakes Res., 20(4):701-716.

Sonstegard, R.A. (1977). Environmental carcinogenesis studies in fishes of the Great Lakes of North America. Annals of the New York Academy of Sciences 298, 261-269.

Spitsbergen, J.M., and M.J. Wolfe. 1995. The riddle of hepatic neoplasia in brown bullheads from relatively unpolluted waters in New York State. Toxicologic Pathology 23:716-725.

St. Clair Region Conservation Authority. Watershed Reportcard. 2008. Web. Feb.2012.

Thornley, S. 1985. Macrozoobenthos of the Detroit and St. Clair Rivers with comparisons to neighbouring waters. J. Great Lakes Res. 11 (3), 290–296.

Triebskorn R., I. Telcean, H. Casper, A. Farkas, C. Sandu, G. Stan, O. Colărescu, T.Dori, and H.R. Köhler. 2008. Monitoring Pollution in River Mureş, Romania, Part II: Metal accumulation and histopathology in fish. Environmental Monitoring and Assessment 141(1-3):177-188.

Triebskorn, R., H.R. Kohler, W. Honnen, S.M. Schramm Adams, and E. F. Muller. 1997. Induction of heat shock proteins, changes in liver ultra-structure, and alterations of fish behavior: Are these biomarkers related and are they useful to reflect the state of pollution in the field? Journal of Aquatic Ecosystem Stress and Recovery 6(1): 57-73.

U.S. Environmental Protection Agency, Great Lakes National Program Office. ARCS Assessment Guidance Document. EPA 905-B94-002. Chicago, Ill. 1994.

U.S. Environmental Protection Agency, Monitoring Design and Analysis Team, National Health and Environmental Effects Research Laboratory, Western Ecology Division. Aquatic Resource Monitoring. Corvallis, OR. N.d. Web. March 15, 2012.

U.S. Environmental Protection Agency, Office of Water. Ambient water quality criteria for copper – 1984. EPA 440/5-84-031. Final Report. 1985.

U.S. Environmental Protection Agency, Office of Water. Ambient water quality criteria for zinc – 1984. EPA 440/5-87-0003. Final Report. 1987.

Urban, J.D., J.A. Tachovsky, L.C. Haws, D.Wikoff Staskal, and M.A. Harris. 2009. Assessment of human health risks posed by consumption of fish from the Lower Passaic River, New Jersey. Science of the Total Environment. 408: p209–224.

Vajda A.M., L.B. Barber, J.L. Gray, E.M. Lopez, J.D. Woodling, and D.O. Norris. 2008. Reproductive disruption in fish downstream from an estrogenic wastewater effluent. Environmental Science & Technology 42(9):3407-3414.

Vogelbein, W.K., J.W. Fournie, P.A. Vanh Veld, and R.J. Huggett. 1990. Cancer Res. 50: 5978-5986.