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Ecotoxicological Investigation Of Three Brown Bullhead (*Ameiurus nebulosus*) Populations In The Detroit River

by

Todd A. Leadley

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Abstract

Three populations of brown bullheads (*Ameiurus nebulosus*) and associated sediments were sampled from the Detroit River, and examined for concentrations of chlorinated hydrocarbons, polychlorinated biphenyls, pesticides and polyaromatic hydrocarbons. Bullheads were further examined for external oral and dermal lesions and internal hepatic histopathology. Chemical analysis revealed that Trenton Channel sediments, as well as the resident bullhead population, had higher concentrations of chemical contaminants compared with two other sites (Amherstburg Channel and Peche Island) in the river. Results also indicate a close association between sediment contaminant concentrations and the incidence of oral/dermal and biliary lesions in the brown bullheads. Trenton Channel bullheads had a higher prevalence of external abnormalities such as lip and skin lesions, stub barbels and fin erosion as well as a higher prevalence of cholangiocarcinomas, cholangiomas and other biliary lesions.

HPLC analysis of polyaromatic hydrocarbon (PAH) metabolite concentrations in the bile of brown bullheads confined to cages in the river for a 16 day exposure period revealed significant spatial and temporal heterogeneity with respect to PAH exposure. Results suggest sediments and stormwater runoff events are important sources of chemical exposure to bullhead populations in the Trenton Channel.

The results of this investigation indicate that feral brown bullhead populations in the Detroit River are stressed by chemical exposure present in the river and contributes further evidence for a cause-effect relationship with respect to contaminant exposure and organism health in aquatic ecosystems.

Key words: Brown bullheads, Tumors, PCB's, PAH's, Bile metabolites, Detroit River.

To my family

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General Introduction

In 1978, the Great Lakes Water Quality Agreement (GLWQA) between Canada-United States was revised and expanded to address toxic contaminant issues in the Great Lakes Basin. An amendment of the agreement in 1987 focused on restoring and maintaining the chemical, physical and biological integrity of the Great Lakes Basin ecosystem. This approach was promoted through the development and implementation of RAPs or remedial action plans, whose purpose was to identify and fully characterize areas of contamination in the Great Lakes Basin, and to provide remedial options for restoration (Hartig *et al.* 1994, State of the Great Lakes 1995).

Currently in the Great Lakes watershed, there are 43 specific geographical areas identified as significantly "impaired" and failing to meet the objectives of the GLWQA. The International Joint Commission (IJC) has termed these locations, Areas of Concern (AOCs). The majority of these AOCs are located, not surprisingly, near large industrial complexes and heavily populated urban areas, where human encroachment, degradation of habitat and where toxic contaminants typically originate.

For an area to be officially recognized (listed) as an AOC, evidence of impairment to ecological health, habitat, human health and human use must be demonstrated. The IJC Great Lakes Water Quality Board has adopted 14 impairment guidelines, used to recommend listing or delisting an area as an AOC. Impairments such as, degradation of benthos, restrictions on fish consumption, loss of fish and wildlife habitat and animal deformities are some examples on which an AOC characterization is based (Focus, 1991).

One particular guideline used in the recommendation procedure is the incident rate of neoplastic or pre-neoplastic tumors in brown bullheads (*Ameiurus nebulosus* Lesueur) and

white suckers (*Catastomus commersoni* Lacépède), which are ubiquitous in the Great Lakes basin. This guideline was developed based on the prevalence of these two fish species to exhibit an increased incidence of neoplastic disease (i.e. epidermal and hepatic tumors) in areas of significant chemical contamination compared to unaffected control sites. The relationship between chemical contaminants in the environment and the occurrence of fish tumors is not, however a proven cause-effect association based on direct experimental evidence, but an association based on convincing but circumstantial information.

Initial reports of fish neoplastic diseases date back to the turn of the century (Harshbarger and Clark, 1990). These early observations were particularly evident in inbred ornamental species and in cultured salmonids (Post 1987). The first report to suggest that exposure to chemical contaminants in the environment may be a possible cause for the occurrence of the feral fish tumors, can be traced to Lucke and Schlumberger in the early 1940s. The authors describe an elevated incidence of epidermal papillomas in a localized brown bullhead *(Ameiurus nebulosus)* population, collected from the contaminated Delaware and Schuylkill rivers in Philadelphia (Sindermann, 1979). The area remains heavily industrialized today and a survey, more than 40 years later, revealed that epidermal papillomas are still present in the brown bullhead population (Anon, 1988, cited in Baumann, 1992).

Interestingly, it was not until more than 20 years after the Lucke and Schlumberger report, that the first liver neoplasms in feral fish were noted (Dawe *et al.* 1964, cited in Harshbarger and Clark 1990). These hepatic lesions were observed in brown bullhead and white suckers in Deep Creek Lake, Maryland, and once again it was suggested that chemical contaminants might have played a role in the occurrence of these fish tumors.

Since the first published report, an increasing number of fish tumor epizootics have been recorded in areas known to be chemically contaminated (Harshbarger and Clark 1990). Even more questionable is the increased incidence of hepatic neoplasms in both marine and freshwater benthic fish species, from geographically restricted areas such as isolated contaminated rivers where contact with other infected populations is not possible (Stich *et al.* 1976, Malins *et al.* 1984, Black 1992, Smith *et al.* 1994).

Historically, the increased rate of fish tumor incidences and epizootics corresponds well with the increase in world wide industrial activity (Harshbarger and Clark, 1990). Over the last century, and particularly since World War II, there has been a tremendous increase in production of synthetic chemicals for human use. Each year, approximately 1000 new chemicals are manufactured and released into the market place (Harte *et al.* 1991), with little or no information as to their potential environmental effects. The direct or indirect environmental fates of many of these compounds are the streams, lakes and oceans of the earth, where a large number of the chemicals remain as persistent cytotoxic and genotoxic contaminants. As a result, many affected areas have shown a steady increase in biota contaminant concentrations and concurrently, toxic effects resulting from this exposure. For example, egg shell thinning in eagles and ospreys was observed following consumption of DDT contaminated fish. (Toxic Chemicals in the Great Lakes and Associated Effects, Harte *et al.* 1991).

Given the large and diverse number of contaminants in the Great Lakes, linking the occurrence of fish neoplastic diseases to a specific compound is difficult, if not impossible. The occurrence of natural carcinogens (e.g. natural pesticides) and the potential synergistic effects of the many synthetic chemical compounds in the environment creates an even more complicated scenario with respect to clearly establishing a cause-effect relationship (Kurelec *et al.* 1989, Ames and Gold 1990, Balch

et al. 1995). It is now well known that many feral fish tumors have natural etiologies. For example, polychlorinated biphenyls (PCB) and dichlorodiphenyltrichloroethane (DDT) were once implicated in gonadol tumors in common carp X goldfish hybrids (*Cyprinus carpio* X *Carassius auratus*) (Sonstegard 1977). The observed gonadal tumors which are geographically widespread, are now thought to be the result of a genetic susceptibility in the hybrids. Similarly, lymphoma in northern pike (*Esox lucius* L.) and muskellunge (*Esox masquinongy* Mitch.), and some papillomas observed in common carp, have been demonstrated to have viral etiologies (Baumann 1992).

As a result of the uncertainty surrounding tumor epizootics, the use of fish tumors as early warning signals of environmental degradation should be considered cautiously. Fish neoplasms as bioindicators of ecosystem health will only be useful if chemical contaminants have a clearly defined role in their etiology. There is accumulating evidence, however, of a link between sediment-bound contaminants and the occurrence of neoplastic diseases (tumors) in benthic-feeding fishes. Earlier studies conducted in Puget Sound, Washington, consistently revealed positive correlations between the prevalence of hepatic neoplasms in English sole (*Parophrys vetulus*) and the concentration of PAHs in the sediments (Malins *et al.* 1984). PAHs are a ubiquitos group of environmentally persistent carcinogenic compounds that largely originate from incomplete combustion of fossil fuels. Comparisons made to fish in less contaminated sites, within Puget Sound, revealed no evidence of hepatic neoplasms or foci of cellular alteration (Malins *et al.* 1985)

As previously described, freshwater fish appear to be no less prone to neoplastic disease than their marine counterparts. The Great Lakes Basin, a watershed well known for its major urban and industrial activity, is also no stranger to contaminated aquatic ecosystems. Numerous fish surveys throughout the basin, particularly over the past two

decades, have documented many cases of elevated tumor incidence from benign external papillomas to malignant hepatic carcinomas, especially in species such as white sucker (*Catostomus commersoni*) and brown bullheads (*Ameiurus nebulosus*). In most cases, these tumor epizooitcs were observed in environments where sediment profiles are heavily burdened with chemical pollutants, most notably PAHs. Similar to the English sole in Puget Sound, the research in freshwater systems suggests a linkage between the incidence of tumors and exposure to elevated concentrations of PAHs in the sediments (Black 1983, Malins *et al.* 1984, Harshbarger and Clark 1990, Smith *et al.* 1989, Maccubbin and Ersing 1991, and Baumann *et al.* 1991).

Research has clearly shown that fish readily accumulate many of the chemicals in the environment and possess the necessary enzyme systems (e.g. aryl hydrocarbon hydroxylase (AHH)) to actively metabolize a large number of these chemicals to biologically-reactive compounds, for example Benzo(a)Pyrene diol-epoxides (Varanasi and Gmur 1980, Tan and Melius 1986, Stegemen et al. 1987, Lin *et al.* 1994). Laboratory studies have demonstrated the cytotoxic and genotoxic potential of many of these metabolites, most notably, polynuclear aromatic hydrocarbons (Sikka *et al.* 1990, Alguacil et al. 1991, Ali et al 1993, Balch *et al.* 1995). Further support for this chemical-tumor association are laboratory studies which employed contaminated sediment samples from the field to induce tumor formation in fish. Black (1983) successfully induced papilloma formation in brown bullheads by skin painting the fish with contaminated Buffalo River sediment extract. Metcalfe *et al.* (1990), also induced tumor formation (hepatocellular carcinomas) in rainbow trout fry (*Oncorhynchus mykiss*) following microinjections of contaminated Hamilton Harbour sediment extract into the yolk sacs.

Mounting evidence from the field and lab studies, although still inferential, strongly suggest that certain fish species, when exposed to carcinogenic compounds in aquatic

ecosystems, are susceptible to neoplastic disease. The ultimate hurdle still remains to define a clear cause-effect relationship, where specific chemical exposure and subsequent injury can be proven. This difficulty confounds regulatory officials who must make policy decisions without a proven cause and effect. "The present system of regulation does not generally make provisions for applying the weight of evidence approach relating injury to specific causes" (Reports of GLWQB, IJC 1993). Clearly this statement accentuates the obvious need to unravel the multifactorial etiologies of tumor induction in feral fish. Although both governments currently recognize the occurrence of hepatic tumors in brown bullhead and white sucker as an accepted AOC guideline, future remedial options must ultimately be based on hard facts providing sound evidence linking injury, in this case, fish neoplasia, to specific causes. This will empower regulatory officials at all levels to make appropriate decisions with minimum uncertainty (Focus, International Joint Commission 1991, GLWQB, IJC 1993).

Introduction

The Detroit River is the southern most link in the Huron - Erie corridor of the Laurentian Great Lakes. This short, but economically important channel is home to more than 5 million people in nearly 100 surrounding communities. The river basin, particularly the lower western border, is one of the most heavily industrialized areas in the world. Major industries include steel mills, petroleum refineries, electrical power generating plants, and manufactures of chemicals, automotive parts, salts and plastics (UGLCCS 1988).

The Detroit River has long been recognized as a contaminated waterway, and is designated as one the 43 Areas Of Concern (AOC) by the International Joint Commission. Tremendous volumes of industrial wastewater discharge, sewer overflow, and urban and agricultural runoff over the years, have resulted in severe contamination of the fine sediments of the river. It is estimated that the City of Detroit discharges about 3.2 billion liters of wastewater every day into the river (Detroit River RAP report 1991).

Additional chemical contaminant burden to the Detroit River arrives from numerous tributaries, such as the Ecorse River, Monguagon Creek, and Conners Creek in Michigan and Little River, Turkey Creek, and River Canard in Ontario. The Rouge River, one of the larger tributaries of the Detroit River, is also a recognized AOC. This small river drains an intensively industrialized and urbanized basin and is a significant source a wide variety of chemical contaminants to the Detroit River (UGLCCS 1988).

In a survey of the soft sediments of the Detroit River, Fallon and Horvath (1985) confirmed the presence of 43 US EPA priority pollutants in the sediments. Elevated levels of PCBs, PAHs, chlorinated pesticides, trace metals, and numerous other chlorinated compounds have all been confirmed to exist in the river sediments (Kaiser *et al.* 1985, Furlong *et al.* 1988).

Despite past and present pollution problems, the Detroit River ecosystem still supports remarkably diverse and abundant fish and wildlife populations (Manny and Kenaga 1991). Close examination of the ecological communities in areas of the river where significant contamination has occurred, provides clear signs of degradation, particularly in the diversity and abundance of the benthic communities (Beake Consultants Report 1993).

Studies investigating the frequency of tumors in the Detroit River fish community have revealed that the incidences of epidermal and hepatic lesions in benthic fish species, such as the brown bullhead, are elevated (Maccubbin and Ersing 1991). The authors did not speculate on a possible etiology except to note that the fish had been exposed to carcinogens in the sediments. The frequency of tumors found in fish populations of the Detroit River were similar to that observed in studies of other industrially-polluted waterways.

Maccubbin *et al.* (1991) later investigated the potential mutagenicity of Detroit River sediments. Of the 30 sediment stations sampled in the lower Detroit River, more than half contained organic chemicals that could be metabolically activated to mutagenic compounds. Further evidence of potentially hazardous compounds in the Detroit River sediments was confirmed in a recent study by Ali *et al.* (1993). Employing a brown bullhead dorsal muscle cell line, and sediments collected from various sites within the river, Ali *et al.* (1993) demonstrated an increase in cell DNA repair in response to various dilutions of sediment chemical extracts. These two investigations clearly demonstrate the potential genotoxicity of sediment-bound contaminants within the Detroit River. Although the studies suggest a chemical etiology for fish tumor induction, the data were not sufficient to support the conclusion that field exposures are sufficient to cause genotoxic stress in natural populations. Contaminant exposures in the field, such as through food or water, are important issues that need to be addressed to discern the ambiguous link between exposure and subsequent injury of aquatic organisms in the field.

Maccubbin and Ersing's (1991) Detroit River tumor survey revealed a 4.5% incidence of fibroma (skin) tumors and a 10% incidence of liver tumors in Walleye (*Stizostedion vitreum*). These results are interesting if we consider that adult walleye are typically not benthic feeding fish like the brown bullhead, and exposure to chemical contaminants via sediment contact appears quite unlikely. Baumann *et al.* (1991) investigating walleye and brown bullhead tumor frequencies in a number of Lake Erie tributaries, reported a significant frequency of neoplastic disease in brown bullheads from Lake Munuscong, one of the least contaminated sites investigated. Although the sediment extracts from the lake were recorded as slightly mutagenic, levels of potential carcinogens were lower in this lake then all others examined. These two examples emphasize the complexities involved in addressing tumor etiologies, where the occurrence of some fish tumor epizootics remain unexplained.

Significant correlations between concentrations in the sediment and the incidence of fish lesions (Malins *et al.* 1984) does not rule out other potential factors, viral or other, that may have implications with regard to fish tumor induction. Carcinogensis is a process that often depends upon the effects of many chemical compounds that act as initiators, promoters, and cocarcinogens (Balch *et al.* 1995).

It is well established that fish, such as brown bullheads, readily accumulate many of the chemicals found in the environment (Krahn *et al.* 1984). Many of the accumulated chemicals, such as the PAHs, are metabolized primarily in the liver by hepatic microsomes into mutagenic compounds, such as B(a)P diol-epoxides (Tan and Melius 1986, Sikka *et al.* 1990, Lin *et al.* 1994). These biologically-reactive metabolites in turn form covalent bonds with macromolecules of the cell, such as proteins and DNA (i.e. DNA adducts). Unstable DNA adducts can result in single-strand breaks in the DNA molecule, which may ultimately contribute to the eventual transformation of the cell (Shugart 1988). This is basically the underlying rational of how chemicals, such as benzo(a)pyrene, act as genotoxic agents. Whether this is the underlying process of tumor induction transpiring in the field remains to be confirmed.

The philopatric nature of brown bullheads combined with their tolerance of poor water quality condition, and their omnipresence in the Great Lakes Basin, makes them ideal aquatic biological monitors. The first objective of this study was to determine the relationship between chemical body burdens in Detroit River brown bullheads and the sediments to which the fish are exposed. The second objective was to assess the spatial distribution of external and internal lesions in feral brown bullhead populations as related to their chemical exposure. This was followed by the final aim of the study, to test the relative importance of brown bullhead exposure to sediments in the formation of DNA reactive bile metabolites.

Materials and Methods

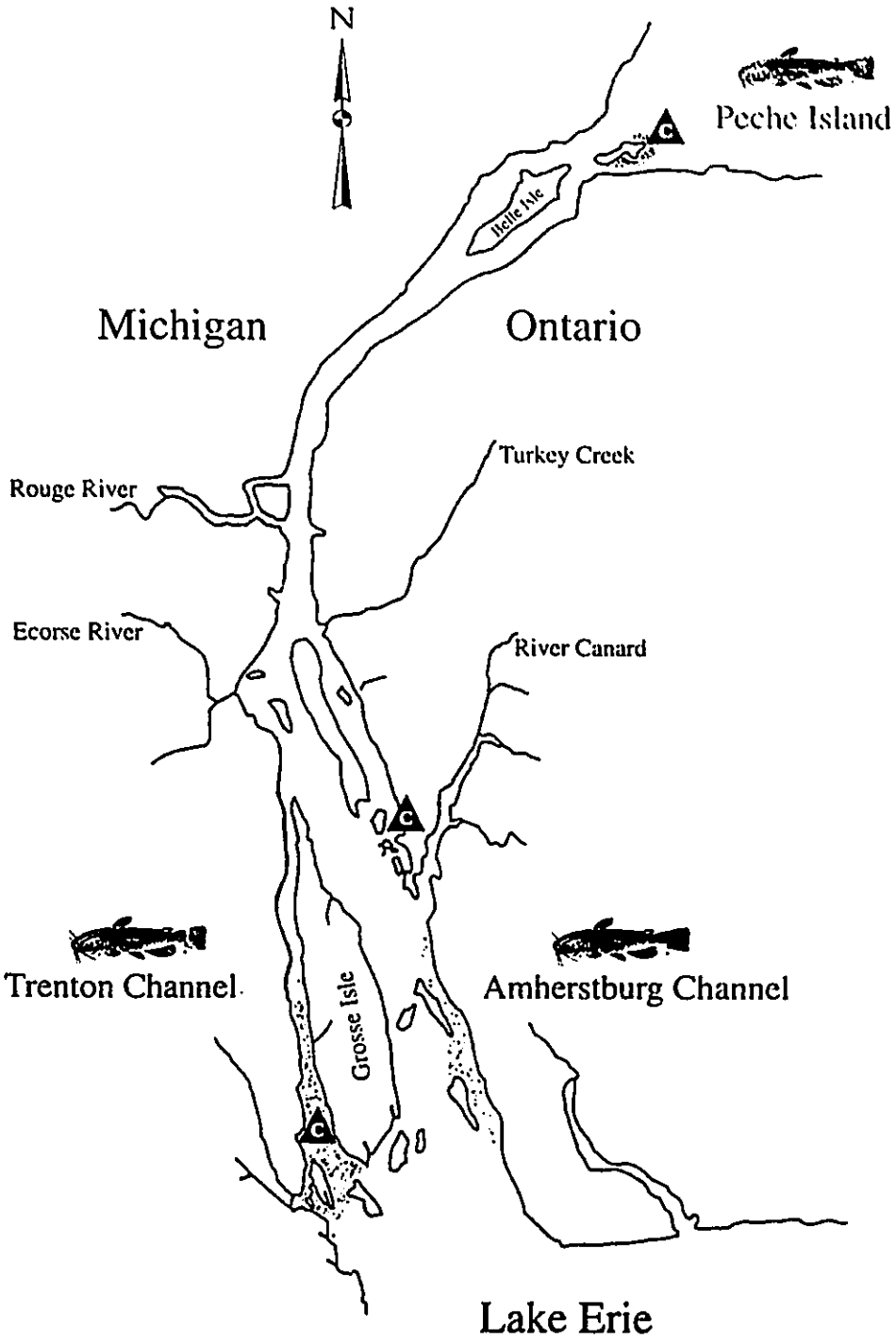
Study Area

Brown bullheads and sediments were sampled at three locations in the Detroit River (Figure 3.1): the Trenton Channel (42° 04.020 N, 83° 11.020 W), the Amherstburg Channel (42° 06.000 N, 83° 06.058 W) and upstream reference site offshore of Peche Island (42° 20.039 N, 82° 55.040 W). The western shore of the river (Trenton Channel), is as an area with a wide range of high contaminant loading (Furlong *et al.* 1988). The Amherstburg Channel, on the eastern shoreline of the river, receives chemical inputs from both the city of Windsor, Turkey Creek, River Canard and Fighting Island upstream. There have been very few toxicological investigations on this side of the river although Fighting Island has been previously identified as an area with elevated chemical contaminant levels in herring gulls (Weseloh *et al.* 1990). Peche Island, upstream at the headwaters of the Detroit River, was used as a reference site.

Sediment Collection and Analysis:

Composite sediment samples (N=3) were collected at each of the three sites using a Petite Ponar sediment grab. Samples were immediately transferred to hexane rinsed amber glass jars and stored at 4° c until analyzed. In the laboratory, the sediment samples were homogenized and portioned into 5 subsamples of 20g each for chemical analysis. The samples were mixed with anhydrous Na_2SO_4 and soxhlet extracted with 300 mL acetone:hexane (1:1) mixture for 16 hours. Each subsample was concentrated to 50 mL and passed through an anhydrous Na_2SO_4 column, and then further concentrated to 5 mL. The subsamples were then composited for further clean-up using Florisil. Two fractions were recovered from the Florisil column, the first fraction was obtained by eluting the

Figure 3.1 Detroit River and associated tributaries. Shaded sections designate brown bullhead collection areas. The dark triangles represent the cage locations for the brown bullhead exposure study (see Plate 3.1).


N
Peche Island
Belle Isle
Michigan
Ontario
Turkey Creek
Rouge River
Ecorse River
River Canard
Trenton Channel
Grosse Isle
Amherstburg Channel
Lake Erie

column with 100% hexane. This fraction contained a mixture of PCB's, pesticides, and other chlorinated compounds. The second fraction (containing PNAH's) was obtained by eluting the Florisil column with dichloromethane (DCM):Hexane (1:1). The first fraction was analyzed using GC-ECD techniques, and the second fraction was analyzed using GC-MSD (Lazar *et al.* 1992) as described further on.

Contaminant Analysis

Following the removal of the bullhead livers, a section of dorsal muscle from each fish was also removed and wrapped in hexane rinsed aluminum foil. Samples were then quickly frozen until thawed for contaminant analysis.

All glassware used was washed with soap and water and placed in a drying oven (35° c) for approximately 12 hrs. Prior to use, all glassware was rinsed three times with acetone, petroleum ether and hexane. Tissue samples (5 g) were homogenized in 20g of anhydrous Na_2SO_4 using a glass mortar and pestle. The free flowing powder obtained was poured into a 2cm x 35cm long glass column (with Teflon stopcock)which had been previously plugged with glass wool and filled with 30 mL of 50% Dichloromethane (DCM) / Petroleum Ether (v:v) and 2 cm of anhydrous Na_2SO_4. Another 10 g Na_2SO_4 was mixed into the mortar (to remove all residue of the sample) and then added to the top of the column. The mortar and pestle was rinsed three times with a total volume of 30 mL of 50% DCM / Petroleum Ether and these rinses were transferred to the column. After one hour (to soak sample in the solvent mixture) the stopcock was opened and the sample was eluted with another 250 mL of 50% DCM / Petroleum Ether. The eluate was collected in a 500 mL round bottom flask with a steady drip of 5-10 mL/min, then rotoevaporated (Buchi-Rotaevaporator) to approximately 5 mLs and transferred to a 50 mL centrifuge

tube (with round glass stopper), made up to 25 mL with Petroleum Ether, mixed and allowed to stand until the Na_2SO_4 settled out.

Lipid determination was made by pipeting 2 mL of the above extract into a preweighed small glass beaker. The solvent was evaporated to dryness in a fume hood and the beaker was placed into a drying oven at 105°c for one hour, removed from the oven, allowed to sit for an additional hour in a desiccator and reweighed. The difference in weight represented the weight of the lipid. Percent lipid was calculated as follows:

%Lipid = WE/wt x Vt/Ve x 100, where

WE = weight of lipid

wt = total weight of sample extracted

vt = total volume of extract (25 mL)

Ve = volume of extract used for lipid determination (23 mL)

The remaining 23 mL of extract (after 2-4 mL of octanol was added) was rotoevaporated to 1.5 - 2 mL and transferred to a glass column (1cm x 35cm with Teflon stopcock, plugged with DCM-rinsed glass wool), filled with 6 g of Florisil and approximately 2 cm of Na_2SO_4 in hexane. The 500 mL flask containing the sample was rinsed three times with Hexane and this was transferred to the column. In total, 50 mL of Hexane was passed through the Florisil column to obtain fraction I (steady drip of 5 mL/min). When the hexane reached the top of the Na_2SO_4, the stop cock was turned off, the sample flask which contained fraction one was replaced with another 250 mL flask, and the column was eluted with 50 mL of 15% DCM / Hexane (v:v) and fraction II was collected. Each fraction was evaporated to a final volume of 2 mL.

Compounds contained in fraction I were analyzed with a 5890 Hewlett-Packard GC/ECD (Ni^{63}) equipped with an HP-7673A Autosampler, an HP-3396A Integrator and a 30m x 0.25mm i.d. J.& W. Durabond DB-5 capillary column with a 0.25 μm film thickness, using a splitless injection mode. Helium carrier gas passes at 30 cm/sec. through the column and the make up gas flow (5% Methane in Argon) was 38 mL/min. The oven temperature was programmed to start at 100°c. After 1 min. temperature was increased at 3° c/min to 270°c and maintained at that temperature for 7 minutes. Injection temperature was 250°c and the detector temperature is 300°c. Injection volume was 2 μL and the running time is 65 minutes.

Analysis for fraction II compounds (e.g. PNAH's) was conducted using a Hewlett-Packard 5890/5970 GC/MSD equipped with an HP-7673A Autosampler, an Durabond DB-5 30m x 0.25 mm i.d. fused silica column with a 0.25 μm film thickness with 1μL splitless injection. Oven temperature was programmed at 80° c for 1 minute then 20° c/min up to 200° c maintained for 2 minutes, then 5° c/min up to 280° c and held for 10 minutes. Injection temperature was at 250°c. Detection: Electron Impact (EI) and Selected Ion Monitoring (SIM). The dwell time was 50-75 msec/ion.

Standards were obtained from the Canadian Wildlife Service (organochlorines) and the Environmental Protection Agency (PAH's). Every tenth sample was a reference sample (gull egg) obtained from CWS. Using the above techniques, detection levels for OCCs were (0.05 μg/Kg) and for PNAHs (0.20 mg/kg).

Assessment of External Condition and Histopathological Examination of Liver

Upon collection using both hook and line and electrofishing techniques, the bullheads were immediately examined for any external abnormalities (e.g., dermal lesions, stubbed

barbels). The fish then were weighed, measured and sacrificed in the field by severing the spinal cord. The livers were quickly removed, weighed, sectioned with a razor blade and fixed in Bouin's solution.

Depending on size, livers were divided into four or six tissue blocks. Each sample was embedded in paraffin and 5μm sections were stained with hemotoxalyn and eosin in preparation for viewing. Each tissue block was examined by selecting tissue samples at six predetermined depth locations (200 μm). Tissues collected for microscopic examination were obtained in a systematic manner to ensure that the histopathological survey was similar with respect to intensity and location for each liver.

Liver anomalies were identified and categorized as altered cells, pre-neoplastic or neoplastic lesions originating from either hepatic or cholangiolar tissue. Lesion identification and classification were based primarily on characteristics described by Baumann *et al.* (1990) and Maccubbin *et al.* (1990) and to a lesser extent on criteria outlined by Hayes *et al.* (1990), Hendricks *et al.* (1984) and Myers et al. (1987). Lesion incidence was based on absence or presence and did not identify the frequency of specific lesions within individual livers. Statistical differences ($p \leq 0.05$) in lesion incidence among collection sites was determined by Chi-square tests.

Hepatosomatic Index

Prior to sectioning, each bullhead liver was weighed and the liver weight was divided by the body weight to obtain a hepatosomatic Index (i.e. relative liver weight).This is a technique used to estimate liver enlargement. It has been suggested that the relative liver weight is a useful biological indicator of exposure to chemical contaminants (Fabacher and Baumann, 1985). The hepatosomatic indexes of the brown bullheads were compared

among the three different populations sampled in the river. Data were analyzed by two way ANOVA. Fishers Least Significant Difference (LSD) was used to determine mean differences in which the class variable included all combinations of weight, collection site and sex.

Exposure Study

For the purpose of the exposure study, uncontaminated brown bullheads (*Ameiurus nebulosus*) were purchased from an aquaculture facility ensuring limited previous exposure to environmental contaminants, particularly PNAH's. These brown bullheads (approximate size 15-20 cm) were placed in two types of cages at three locations (Plate 3.1, 3.2, and 3.3). within the Detroit River for an intended 32-day exposure period. Bullhead cages were constructed out of pine lumber and galvanized concrete matting (2.54 cm x 1.27cm, gage). Cage design consisted of two types (Figure 3.2). The first type, (referred to as top cages) were cylindrical in shape and designed to constrain the fish in the water column i.e., denying fish access to the sediments. The second type of cage (referred to as bottom cages), was designed with a flat bottom covered with large gauge screening. These bottom cages were then pressed into the sediment (approx. 15 cm or greater) at each site. This allowed the bullheads ample access to the sediments. The cages were secured to the bottom with angle iron and a backup concrete anchor for each cage. All cages were built with two solid walls on each end. Once the cages were submersed, these solid ends were oriented parallel to the flow of the river. This design and orientation prevents rapid fouling of the cage screening, thereby allowing an unobstructed exchange of water through the cage during the study period. Previous experience with cage studies in the Detroit River have shown that screens quickly become choked with

Plate 3.1 Satellite image of Detroit River and surrounding area. Letters designate cage locations used for the brown bullhead exposure study. A: Peche Island. B: Turkey Island. C: Trenton Channel. Note the dark plume entering Lake Erie from the lower western border of the river (NASA image SL3-83-152).

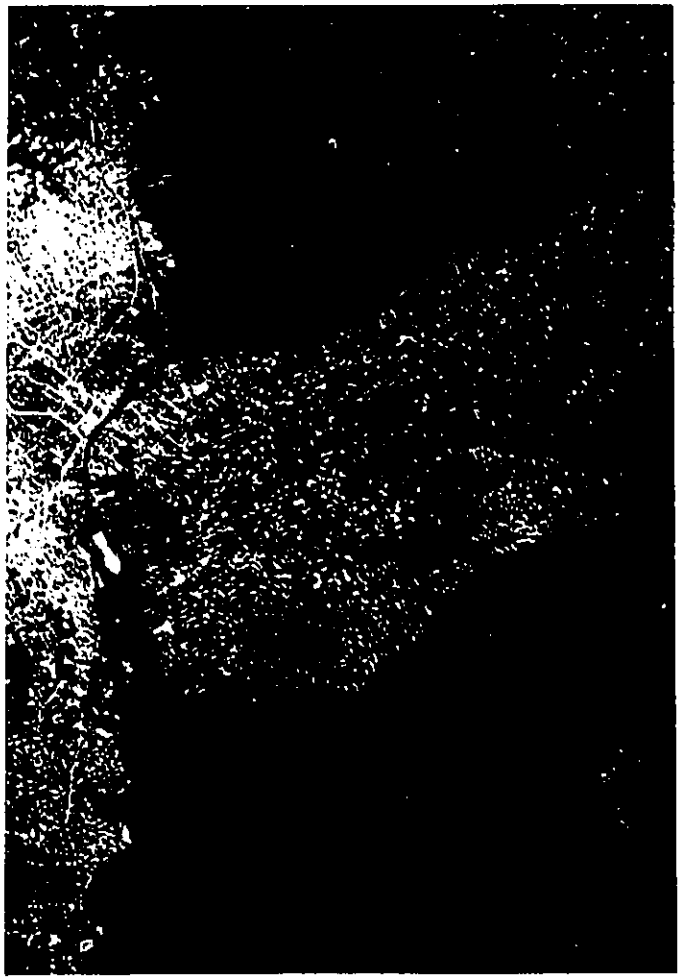


Plate 3.2 **(Top)**: Aerial photograph of Peche Island facing south on the Detroit River. **A**: shows location of brown bullhead cages.

(Bottom): Aerial photograph of the Turkey Island area, facing north on the Detroit River. **B**: shows location of the bullhead cages. Large land mass in the background is Fighting Island.


B

Plate 3.3. **(Top)**: Aerial photograph of Trenton Channel site. photograph is oriented southwest.

(Bottom): Close up of cage location. Note the plume running adjacent to the Island. (C) denotes area where bullhead cages were deployed.

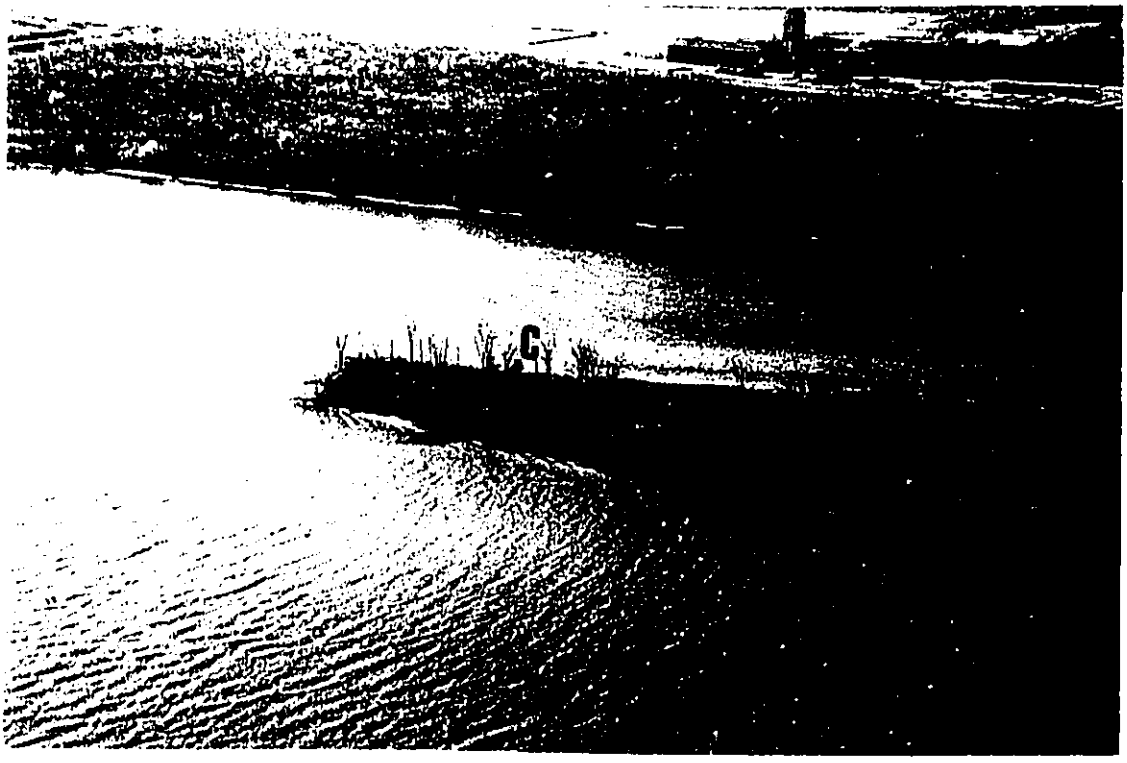


Figure 3.2. Line drawing depicting design and orientation of brown bullhead cages (top and bottom) used in the Detroit River field exposure study.

macrophytes and other drifting debris which eventually reduce water quality within the cage.

The bottom cages were larger than the top cages. This was done to maximize sediment surface area. The top cages were approximately equivalent to a 300-L aquarium and the bottom cages were approximately the size of an 800-litre aquarium with an exposed sediment surface area equal to 1.27 m^2.

In order to alleviate any additional stress on the fish, the empty cages were deployed 2 weeks prior to the start of the experiment to allow the surface of the cages to "condition", i.e. establish some algae growth and colonization of the sediment. This also allowed ample time for the sediments in the area to settle following the disturbance of deploying the cages. At each site, two cages were deployed, a top cage and a bottom cage. The cages were placed at a depth of no less than 1 meter of water. During this time, the brown bullheads were quarantined and carefully monitored in two 400 L aquaria in the laboratory, in order to make sure the fish were in good health and well fed prior to placing them in the field.

At the start of the exposure study, 17 fish (mean standard length = 150 mm) were placed in each top cage and 17 fish in each bottom cage at all three sites. This density provided enough fish for a 32 day exposure study allowing for some mortality and to minimize stress from overcrowding. The study was initiated August 10 1994 (day 0), and continued only for 16 days. Three fish from each cage were sampled on day 2, day 4, day 8, day 16. Basic water quality parameters (dissolved oxygen, temperature, pH, conductivity and redox) were also measured using Hydrolab II datasonde on all sampling days.

Fish were removed with a nylon net through a door on the side of the cage. The fish were retrieved using Scuba or snorkeling which allowed removal of fish without disturbing the cages from their anchor. This also reduced the stress on the remaining fish. Mortality during the study was quite high by day 8 and the experiment was terminated on day 16 at all three sites.

Upon retrieval, fish were sacrificed in the field by cervical dislocation and were then weighed and measured for standard length. A small ventral incision was made on each fish and bile was removed from the gall bladder using a 1cc sterile syringe (27½ gauge). The bile was immediately transferred to 5.0 mL disposable cryogenic vials and promptly frozen on dry ice in the field. All bile samples were subsequently stored at -40°c in the lab for later bile FAC analysis. The gastrointestinal tract of each fish was removed for gut content identification. The fish were then wrapped in hexane rinsed foil and frozen for organic chemical contaminant analysis.

Due to the small size of the brown bullheads used in this study, each fish was homogenized in a stainless steel blender. The blender was washed with Alconox and rinsed well with R.O. water. The blender was then rinsed 3 times with acetone, followed by petroleum ether, and hexane (solvents used were pesticide grade). The resulting homogenized samples were then wrapped in hexane rinsed foil and frozen until analyzed. These samples were later analyzed for organochlorinated hydrocarbons, pesticides and PCBs (method described previously).

Concentrations of fluorescent aromatic compounds (FACs) contained in unhydrolized bile were determined by HPLC with fluorescence detection as described by Krahn *et al.* (1986c). FACs were analyzed with a Waters model 600E HPLC with a Waters 470 scanning fluorescence detector. The injection port was a Waters U6K injector unit with a

2mL sample loop. Peak integration was performed with "Baseline 810" (v.3) software by dynamic Solutions (Millipore). A reverse phase column (0.46 cm x 25 cm (ID) stainless steel 5µm C18 column, Supelco) was used to separate out metabolites. The guard column (C18 Novopak (4 µm)) was replaced regularly in response to increased system pressure. The mobile phases were 0.5% acetic acid in water (solvent A) and methanol (solvent B), and the elution program was: 100% solvent A to 100% solvent B in 15 minutes, using an exponential gradient; 7 minutes at 100% B; 3 minutes to return to 100% A, linear gradient; and 10 minutes of solvent A for re-equilibration of column. Flow rate was 1.0 mL/min, except during re-equilibration, when it was increased to 1.5 mL/min for a period 10 minutes. Total fluorescence was measured at excitation/emission wavelength pairs specific for phenanthrene (256/380 nm) and BaP (380/430 nm). Integrated peak areas for samples measured at the phenanthrene wavelength pairs were referenced against the peak area generated by injection of 2.5 ng phenanthrene in 5 µL of methanol, and peak areas of samples measured at the BaP wavelength pairs were referenced against the peak area generated by injection of 1.8 ng BaP in 5 µL of methanol. Concentrations of total fluorescent bile metabolites were quantitated in units of pg of BaP or phenanthrene equivalents per µL of bile.

The HPLC instrumentation and operating conditions used in this study were:

a) Controller System:	Waters 600E Controller
b) Injection Port:	Waters U6K Injector with 2mL sample loop.
c) Detectors:	Waters 470 Programmable Fluorescence Detector.
d) Chromatography Data Software:	Dynamics Solutions Inc., Baseline 810 (v.3.3).
e) Guard Column:	Waters #15220 - C ^{18} Novopak, 4 µm
f) Column:	Supelco #5-8229 - Supelco, LC-PAH, 5 µm, 25 cm. x 4.6 cm stainless steel column

g) Solvent Program:

Time	Flow (mL/min)	**Solvent A** Acetic acid & %Water	**Solvent B** %Methanol
Initial	1.0	100	0
15 min	1.0	0	100
22 min	1.0	0	100
25 min	1.5	100	0
33 min	1.0	100	0
35 min	1.0	100	0

Data was analyzed by two way ANOVA. Fishers Least Significant Difference (LSD) was used to determine mean differences in which the class variable included all combinations of cage site and cage position.

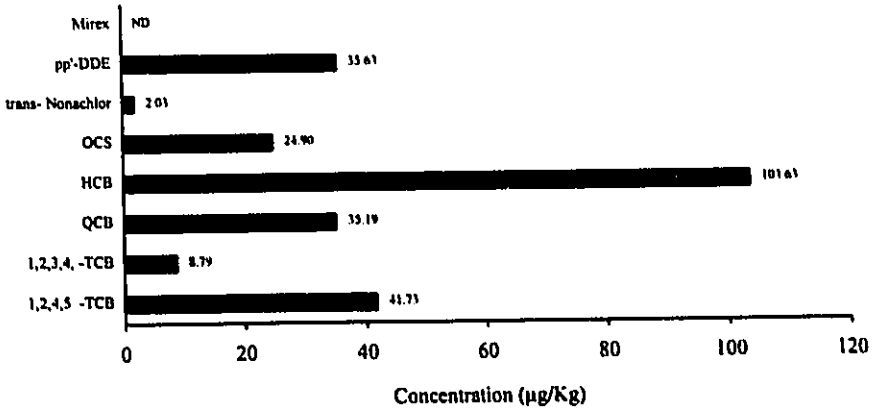
Results

The concentrations of PCBs (e.g. Aroclor 1254:1260: Trenton Channel = 12074.24 μg/Kg, Amherstburg = 531.96 μg/Kg, and Peche Island = 223.45 μg/Kg) and OCCs (e.g. HCB: Trenton Channel = 651.30 μg/Kg, Amherstburg Channel = 103.63, and Peche Island = 134.40) in the sediment samples collected from Trenton Channel, Amherstburg Channel and Peche Island are summarized in Figure 4.1. Figure 4.2 is a summary of the concentrations of 16 PNAHs for the same sediment samples. Trenton Channel sediments contained higher levels of both OCCs, PCBs and PNAHs when compared with sediment extracts from Peche Island and the Amherstburg Channel. The total concentration of the 16 PNAHs in Trenton Channel was 12 and 60 times greater then concentrations recorded at Amherstburg and Peche Island respectively. PCB Aroclor 1254:1260 sediment concentrations showed similar differences with concentrations 23 and 54 times greater in Trenton Channel as compared to Peche Island and Amherstburg Channel respectively. Furthermore, the Trenton Channel sediments had the lowest organic carbon content, and thus had a lower capacity to bind organic contaminants than sediments from the other two sites. Chemical concentrations in the Trenton Channel sediments were similar to those reported by Furlong *et al.* (1988).

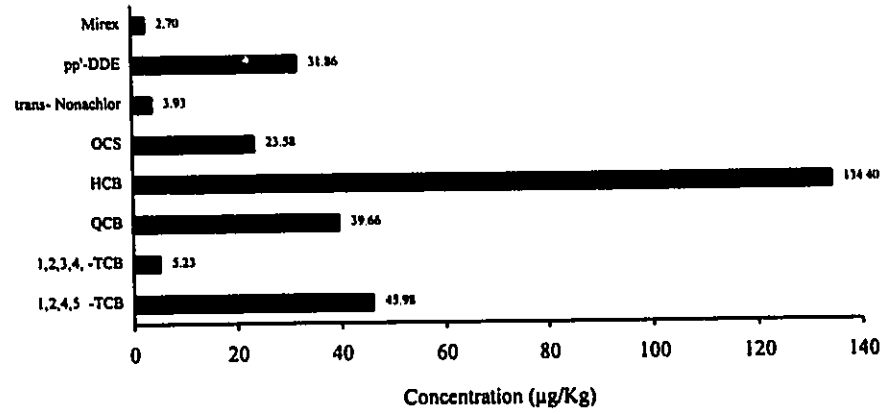
Contaminant concentrations in the dorsal muscle of the brown bullheads collected from the three sites (Figure 4.3), revealed that PCB concentrations in Trenton Channel brown bullheads were higher than concentrations observed from Peche Island or the Amherstburg Channel populations. Toxic non-ortho substituted PCB's (congeners 77, 126 and 169) were also detected in the bullhead muscle samples, with the highest concentrations again detected in fish collected from the Trenton Channel.

Figure 4.1 Organochlorinated hydrocarbons, pesticides and PCB mean chemical contaminant concentrations (μg/Kg, dry weight basis) in sediments collected from Peche Island, Amherstburg Channel, and Trenton Channel (Detroit River).

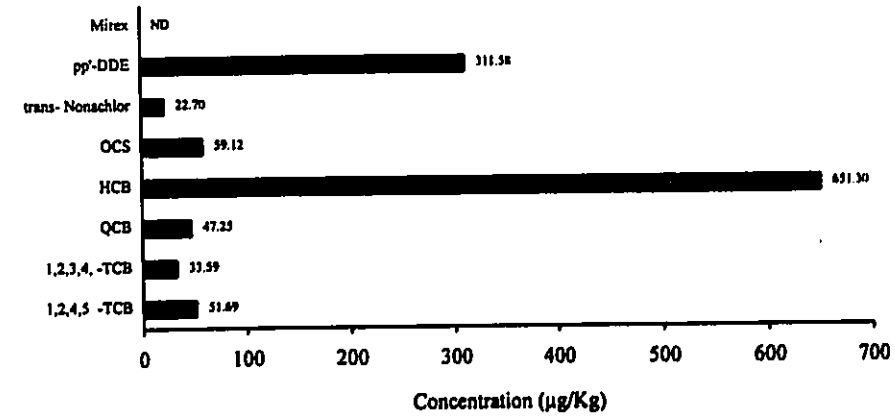
Amherstburg Channel



Peche Island



Trenton Channel



Aroclor 1254:1260

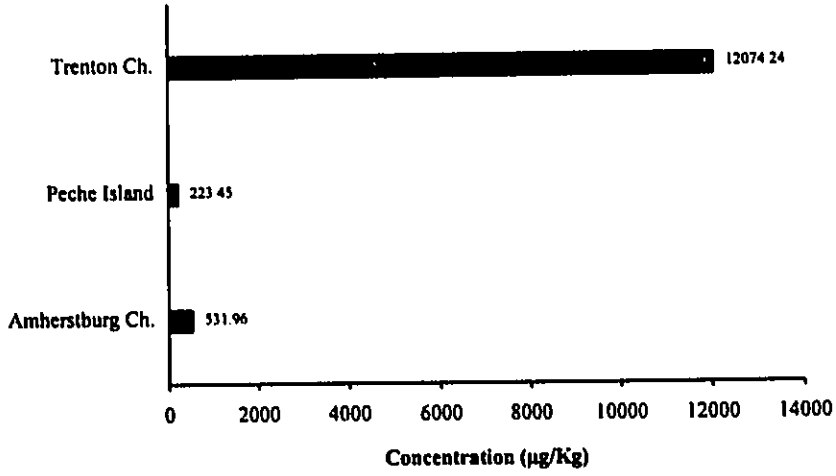


Figure 4.2 Mean concentration (mg/Kg, dry weight) of 16 PAHs identified in sediments collected from Trenton Channel, Amherstburg Channel, and Peche Island, Detroit River.

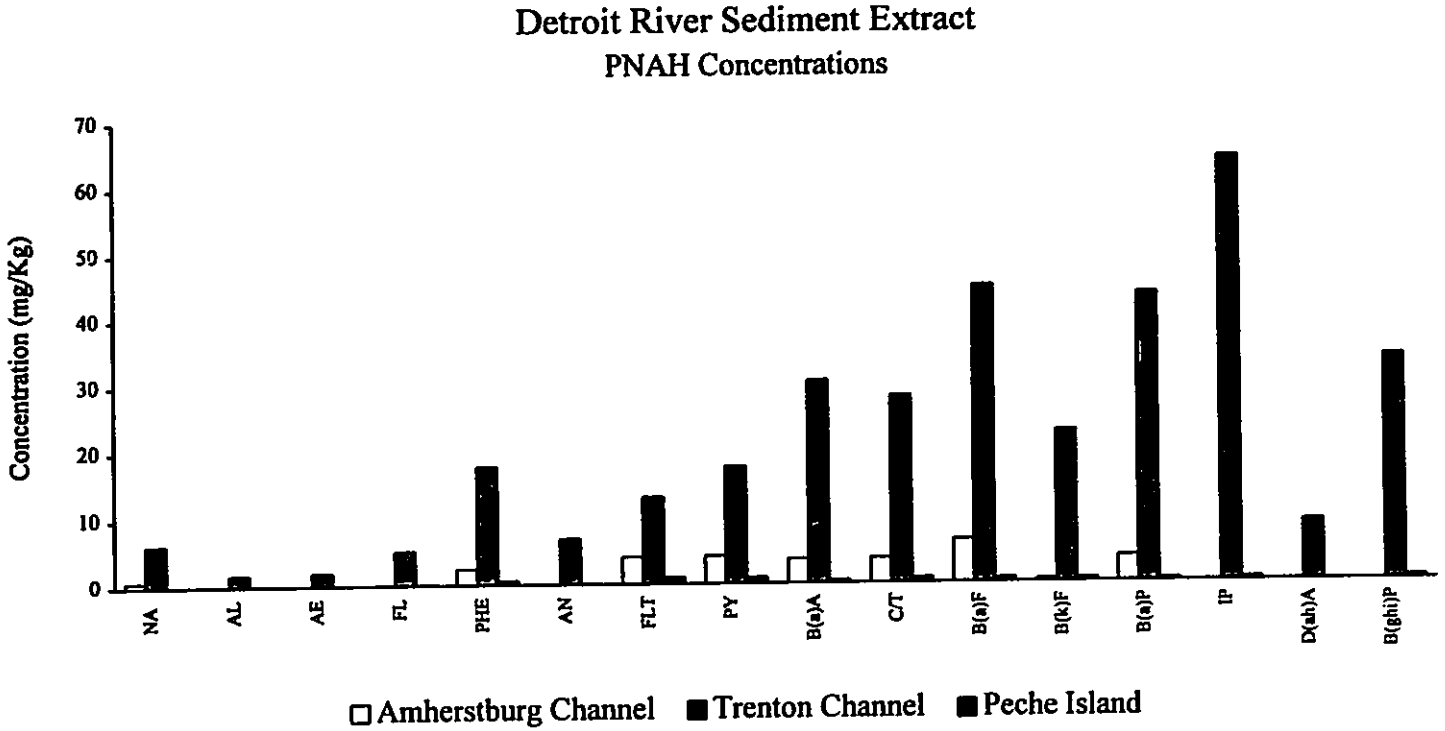
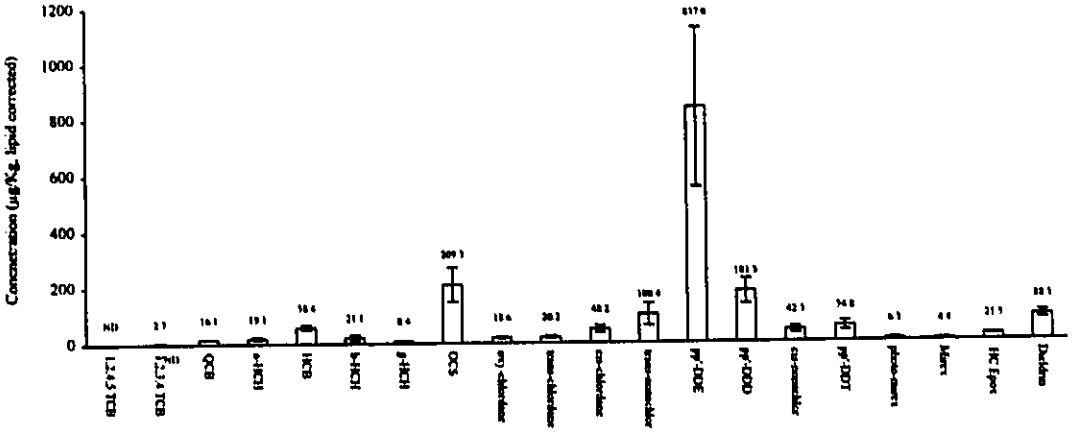
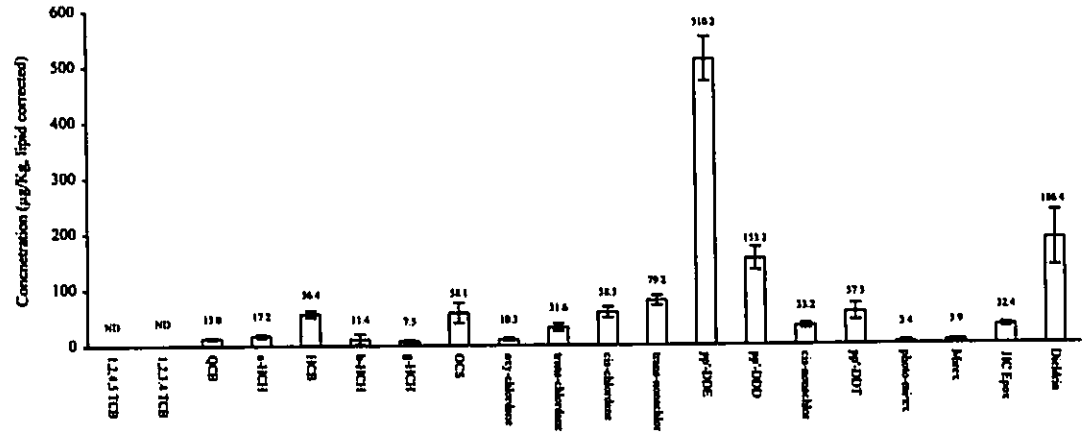

Detroit River Sediment Extract
PNAH Concentrations
Concentration (mg/Kg)
70
60
50
40
30
20
10
0
NA
AL
AE
FL
PHE
AN
FLT
PY
B(a)A
C/T
B(a)F
B(k)F
B(a)P
IP
D(ah)A
B(ghi)P
Amherstburg Channel
Trenton Channel
Peche Island

Figure 4.3 Bar charts representing organochlorinated hydrocarbon, pesticide, and PCB concentrations (µg/Kg, corrected for lipid) in brown bullhead (*Ameiurus nebulosus*) dorsal muscle tissue. Note change of scale in the Y axis.

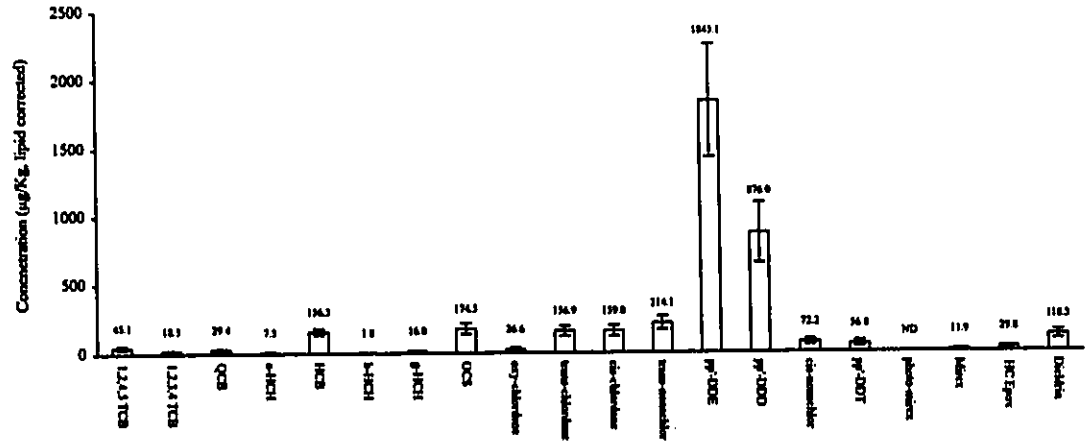
Peche Island Brown bullheads



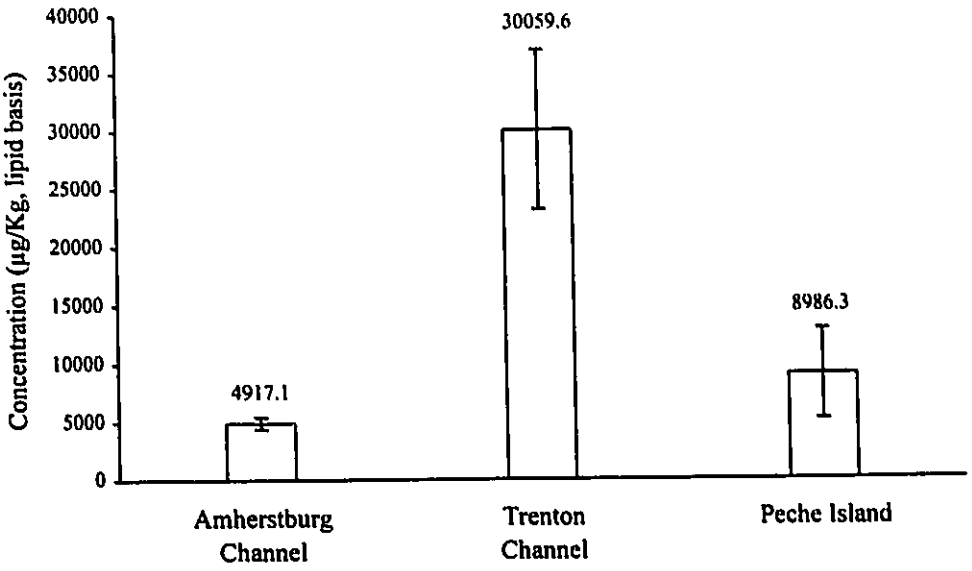
Amherstburg Channel Brown bullheads



Trenton Channel Brown bullheads



Brown bullhead Aroclor 1254:1260 Concentration



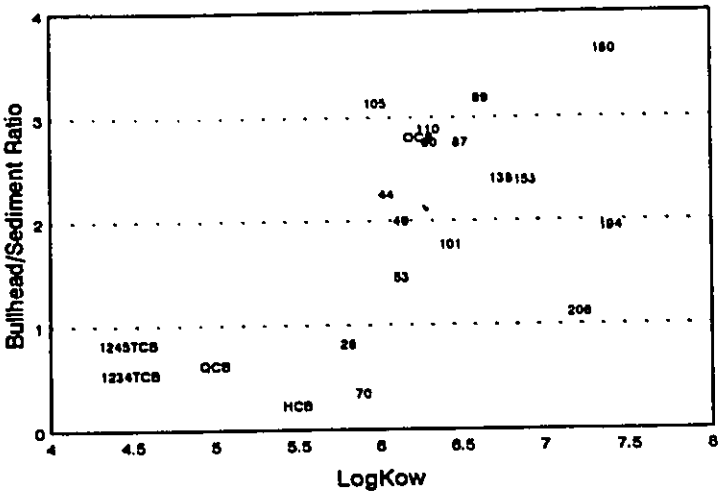
As a result of the rapid rate of metabolism of aromatic hydrocarbons in fish (Steward *et al.* 1990), concentrations of parent PAHs (e.g. Benzo (a) pyrene) in the bullhead muscle tissue, were found in only trace amounts, with most concentrations below detection (< 0.20 mg/Kg). This has been observed in other studies, were standard analysis for parent PNAHs were found in very low concentrations in fish tissue, even when the sediments were heavily contaminated (Baumann *et al.* 1982).

To assess the exposure dynamics of various sediment-associated organic contaminants, Bio-Sediment Accumulation Factor (BSAF's), were calculated for all three sites (Figure 4.4). In order to investigate the relationship of the BSAF with the hydrophobicity of the different chemical contaminants, the BSAF is plotted against the Log K_{ow} of the compound (Baudo *et al.* 1990). For all chemicals regardless of their hydrophobicity, equilibrium partitioning models predict that the BSAF should approximate 1. At all three sites, observed BSAF values were significantly greater than 1 for compounds with log K_{ow}>6. Also note that the measured BSAF's differed from site to site. The lower BSAF values found at Trenton Channel indicate these bullheads have contaminant body burdens which corresponds to the sediment concentrations found within the channel. In contrast, the brown bullheads at Peche Island were more contaminated than would be predicted from sediment concentrations at that site. It is speculated that Peche Island bullheads probably leave the shallow bays of the island during winter ice cover, and move into the river, and thus encounter higher contaminant concentrations.

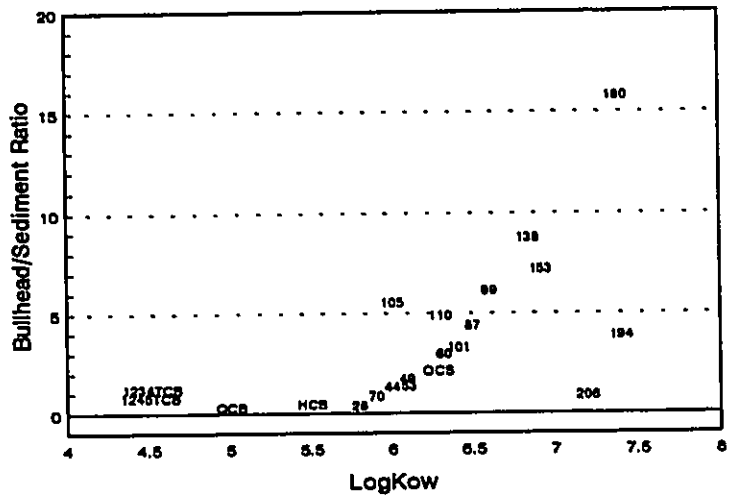
Previous studies have revealed a relationship between the age of brown bullheads and the occurrence of hepatocholangiolar tumors (Baumann *et al.* 1990, Baumann *et al.* 1991). Pectoral spines for bullhead aging were not available, so brown bullhead ages in this study were estimated from length-age relationships determined for other bullhead populations (Sinnott and Ringler 1987, Scott and Crossman 1977). There was no

Figure 4.4 BioSediment Accumulation Factors (BSAFs) for Trenton Channel, Amherstburg Channel and Peche Island. Numbers represent PCB congeners (IUPAC).

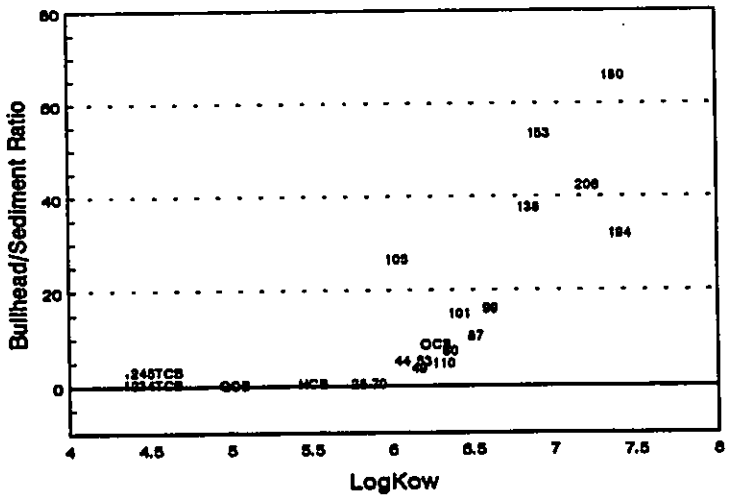
Trenton Channel BSAF



Amherstburg BSAF



Peche Island BSAF

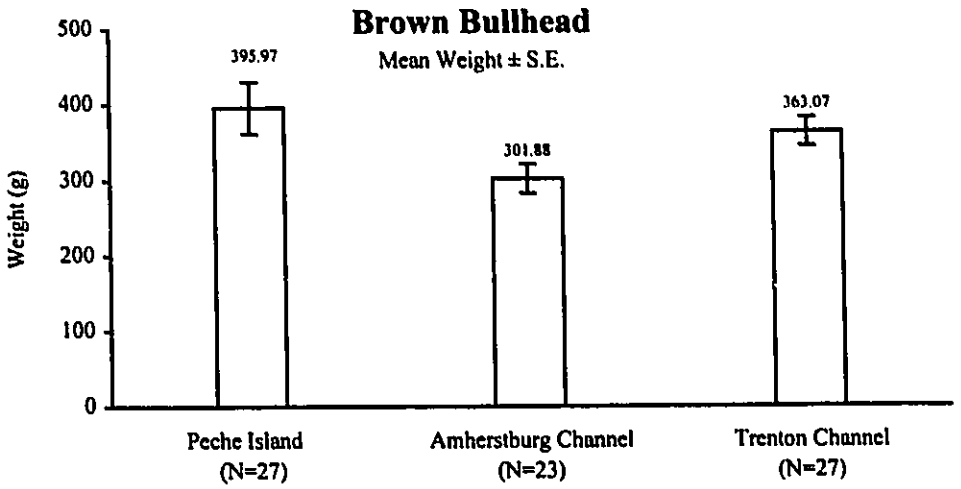


significant difference in standard length between the three bullhead populations (Kruskal-Wallis non parametric method in lieu of one way ANOVA, $p = .073$) and fish from all three sites were concluded to be in the 3-4 year age class (Figure 4.5).

The percent of bullheads expressing external gross abnormalities at each sampling site are shown in Figure 4.6. External gross observations were broken down into five general categories. Lip lesions (LL), stub barbels (STB), and body lesions (SKL) were recorded as observed (Plate 4.1). Deformities, including ocular lesions, missing barbels, missing pectoral spines and skeletal deformities were combined under this one category. Fin and tail erosion (Plate 4.2), a disease which has been associated with fish in polluted environments (Sindermann 1979, McCain *et al.* 1992), was recorded as independent observations. The clear (CL) category were fish observed to have no apparent anomalies in their external appearance (Plate 4.3). The Trenton Channel population had the greatest percent of abnormalities, and only 11% of this population were found to be devoid of lesions. In the Amherstburg Channel, 36% of the bullheads were free of external abnormalities and in the Peche Island population, 41% were observed to be free of gross abnormalities.

An hepatosomatic index (relative liver weight) was used to estimate liver enlargement in the feral bullheads at each site. Liver enlargement in brown bullheads has been used as an indicator of exposure to chemical contamination (Fabacher and Baumann, 1985). The mean index of female and male bullhead livers is shown in Table 4.1 and Figure 4.7. The results of the hepatosomatic index revealed a significant difference in relative liver weight of the bullheads among the three sites ($F_{[2,63]} = 9.523$, $p < 0.001$). Trenton Channel bullhead livers were significantly larger than Amherstburg Channel bullhead livers (Fisher's LSD, $p < 0.001$) and Peche Island bullhead livers (Fisher's LSD, $p = 0.024$).

Figure 4.5 Mean brown bullhead weight in (g) ± standard error and mean standard length in (mm) ± standard error.


Brown Bullhead
Mean Weight ± S.E.
Weight (g)
500
400
300
200
100
0
395.97
301.88
363.07
Peche Island
(N=27)
Amherstburg Channel
(N=23)
Trenton Channel
(N=27)

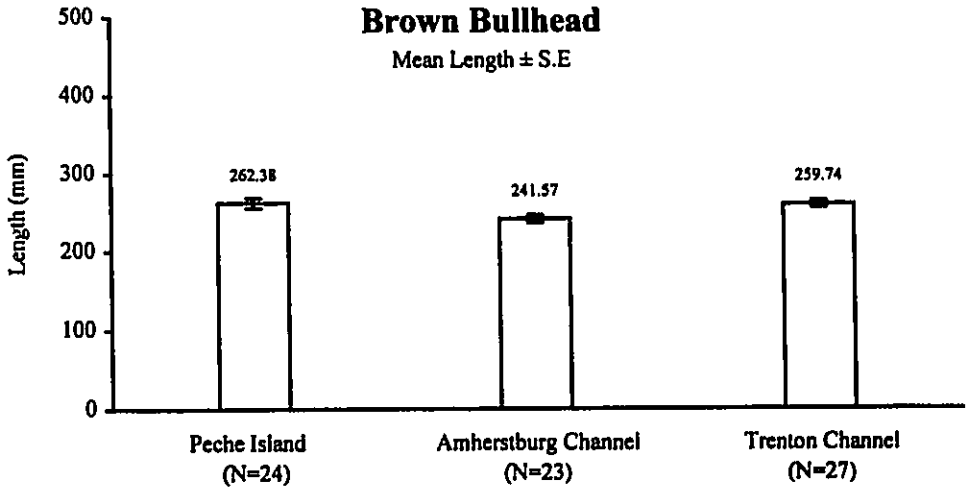
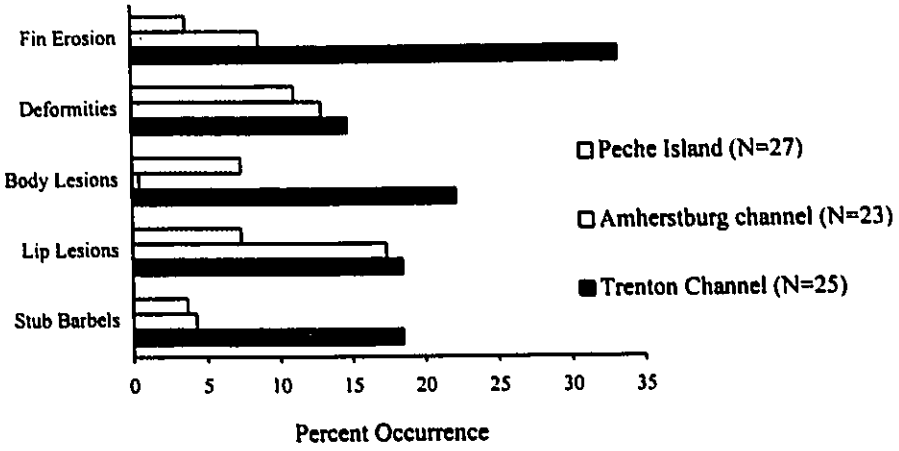
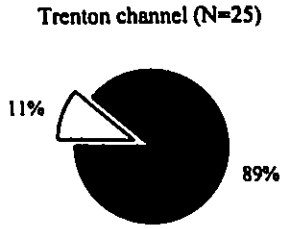
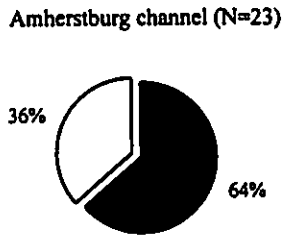

Brown Bullhead
Mean Length ± S.E
Length (mm)
500
400
300
200
100
0
262.38
241.57
259.74
Peche Island
(N=24)
Amherstburg Channel
(N=23)
Trenton Channel
(N=27)

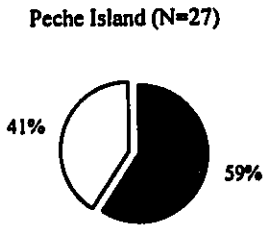
Figure 4.6 **(Top)**: Percent occurrence of external lesions observed on brown bullheads (*Ameiurus nebulosus*) collected from Peche Island, Amherstburg Channel and Trenton Channel, Detroit River.

(Bottom): Pie charts showing the percentage of brown bullheads (*Ameiurus nebulosus*) with external abnormalities compared to fish which displayed no obvious external abnormalities at each sampling location.


Fin Erosion
Deformities
Body Lesions
Lip Lesions
Stub Barbels
0
5
10
15
20
25
30
35
Percent Occurrence
Peche Island (N=27)
Amherstburg channel (N=23)
Trenton Channel (N=25)


Trenton channel (N=25)
11%
89%


Amherstburg channel (N=23)
36%
64%


Peche Island (N=27)
41%
59%

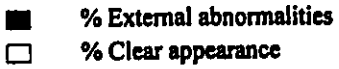

% External abnormalities
% Clear appearance

Plate 4.3 **(Top)**: Photograph of a healthy brown bullhead (*Ameiurus nebulosus*) raised in the laboratory.

(Bottom): Ventral view of a healthy brown bullhead. Note the absence of external lesions and ventral redness.

Plate 4.1 **(Top)**: Photograph of a Trenton Channel brown bullhead with extensive lip lesions and stub barbels.

(Bottom): Ventral view of a Trenton Channel brown bullhead. Note excessive ventral redness and lesions near the mouth.

Plate 4.2 **(Top)**: Photograph of a brown bullhead with extensive fin erosion and ulceration. This fish was captured in the Trenton Channel.

(Bottom): Brown bullhead with severe external lesions. Note body lesions as raised gray and red foci.

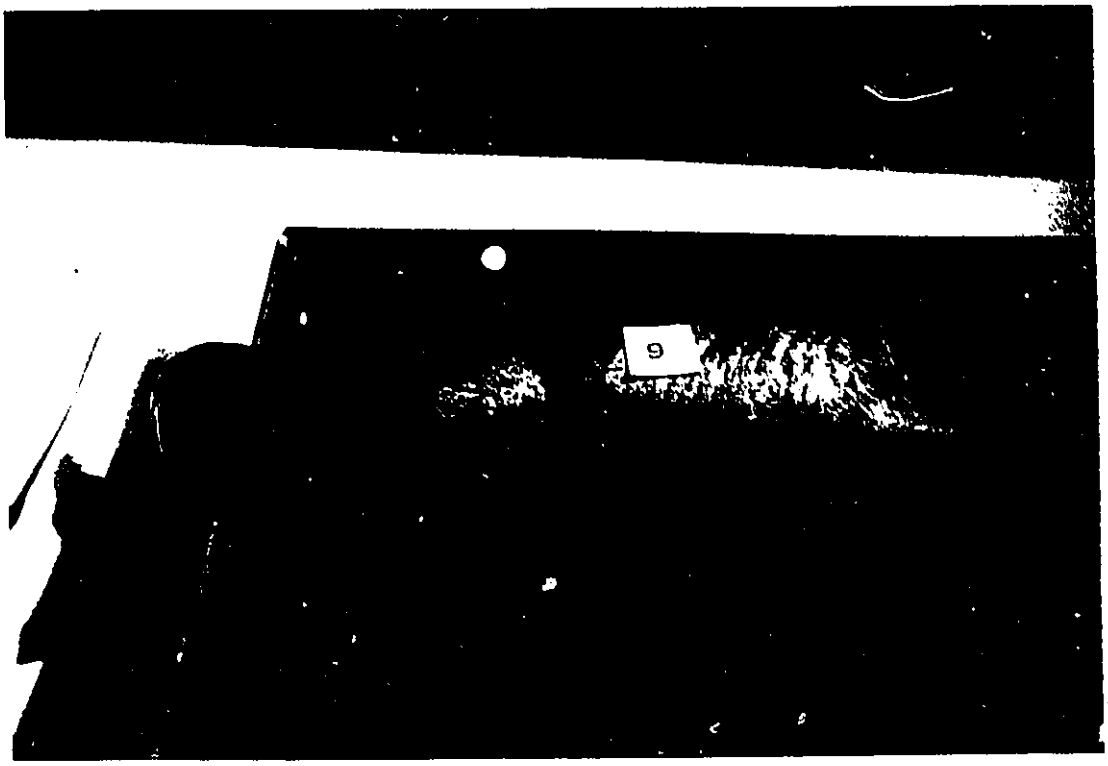

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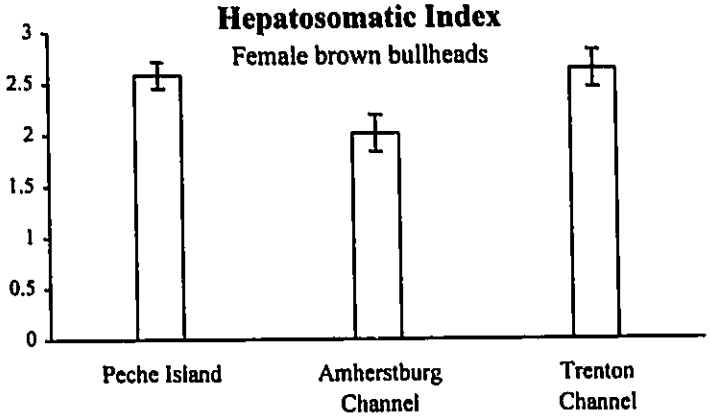
Table 4.1 Hepatosomatic Index of male and female brown bullheads (*Ameiurus nebulosus*) from Peche Island, Amherstburg Channel and Trenton Channel, Detroit River.

Figure 4.7 Hepatosomatic Index ± standard error, for both female and male brown bullheads.

Sampling Location	N	Females	N	Males
Peche Island	9	2.58 ± .13	13	1.68 ± .13
Amherstburg Channel	9	2.01 ± .18	13	1.46 ± .13
Trenton Channel	13	2.63 ± .18	14	2.32 ± .10



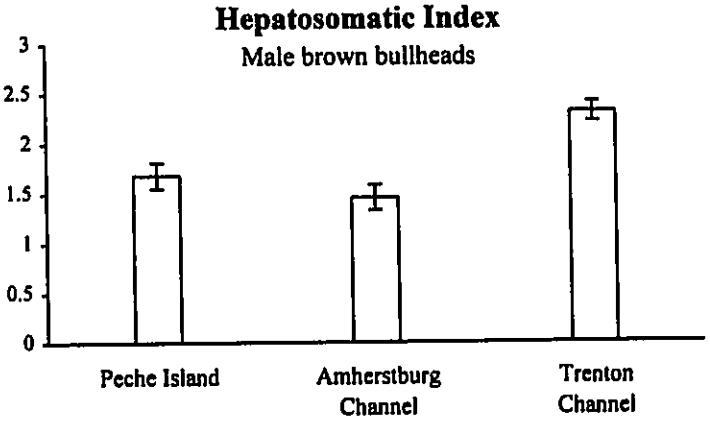


Table 4.2 Hepatic and Cholangiolar lesions frequency in brown bullheads collected from two contaminated sites in the Detroit River (Amherstburg Channel and Trenton Channel) and a less contaminated upstream reference site (Peche Island). Values in (brackets) are expressed as % incidence.

		Sample site:	Peche Is	Amherstburg C	Trenton Ch.
		Sample size:	27	23	25
Tissue	**Category**	**Lesion**			
Hepatic					
	A) Altered Foci	Eosinophilic	0 (0)	2 (9)	0 (0)
	B) Pre-neoplastic	Basiophilic	0 (0)	0 (0)	1 (4)
	Number of fish affected		0 (0)	2 (9)	1 (4)
Cholangiolar					
	A) Altered Structure	Cholangiohepatitis	7 (26)	5 (22)	7 (28)
		Fibrotic thickening of bile duct	2 (7)	13 (57)	14 (56)
		Cholangiofibrosis	1 (4)	7 (30)	6 (24)
		Hyperplastic bile ducts	2 (7)	11 (48)	5 (20)
		Mishappen bile ducts	2 (7)	11 (48)	5 (20)
		Anaplastic bile ducts	2 (7)	12 (52)	5 (20)
	Number of fish affected		11 (41)	16 (70)	19 (76)
	B) Pre-neoplastic	Cholangiocellular adenoma	0 (0)	0 (0)	1 (4)
	C) Neoplastic	Cholangiocellular carcinoma	1 (4)	3 (13)	5 (20)
	Number of fish affected		1 (4)	3 (14)	6 (56)
	Total number of affected fish		11 (41)	16 (70)	19 (76)

Chi Square analysis, significant @ p=0.05

Total number of fish affected with liver lesions

A) Peche Isl. vs. Amherstburg Ch. (p=.0036) significant

B) Peche Isl. vs. Trenton Ch. (p=.0036) significant

C) Amherstburg Ch. vs. Trenton Ch. (————) non significant

There was no significant difference in relative liver size between Peche Island and Amherstburg bullheads (Fisher's LSD, $p = 0.071$).

A comparison among males and females at all three sites show a significant difference between males and females at Peche Island (Fisher's LSD, $p = 0.006$) and in Amherstburg Channel (Fisher's LSD, $p = 0.022$). There was no difference detected between male and female relative liver weights in bullheads from the Trenton Channel (Fisher's LSD, $p = 0.280$). See appendix D for statistics results.

The histological assessment of the bullhead livers identified two types of lesions originating from hepatocytes and eight types of lesions arising from cholangiolar tissue. Table 2 summarizes lesion occurrence for each collection site. Cholangiocellular carcinomas were the only neoplastic lesions observed (Plate 4.4). The incidence of cholangiocellular carcinomas ranged from a high of 20% (5/25) in fish collected from the Trenton channel to a low of 4% (1/27) in fish from Peche island. Although not significantly different ($p > 0.05$), the data do suggest that this lesion is more prevalent in fish collected from the Trenton Channel. These lesions were characterized by the loss of cellular polarity, an increase in the nucleus to cytoplasm ratio, the proliferation of bile ducts which had lost the characteristic tubular architecture, compression of surrounding hepatocytes and the invasive growth of the lesion into the surrounding parenchyma. One additional fish collected from the Trenton Channel had a cholangiocellular adenoma (Plate 4.5). This proliferative lesion was similar to the cholangiocellular carcinomas, except the tubular architecture was more characteristic of normal tissue and the lesion was encapsulated in a thick band of fibrotic tissue. Cholangiocellular adenomas were not observed in fish from the Amherstburg or Peche Island sites.

Plate 4.4 (**Top**): Cholangiocarcinoma in a brown bullhead from Trenton Channel (40X).

(**Bottom**): Brown bullhead cholangiocarcinoma. Poorly differentiated cancer cells are spindled-shaped and do not form ducts (150x).

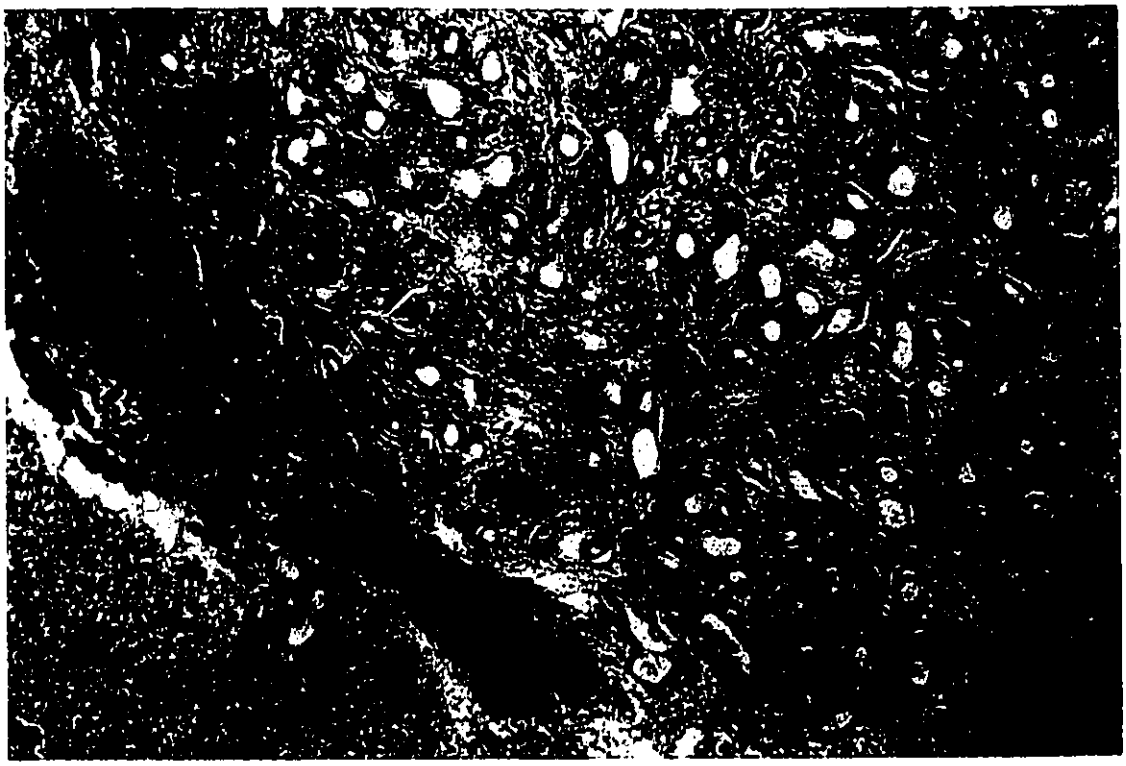
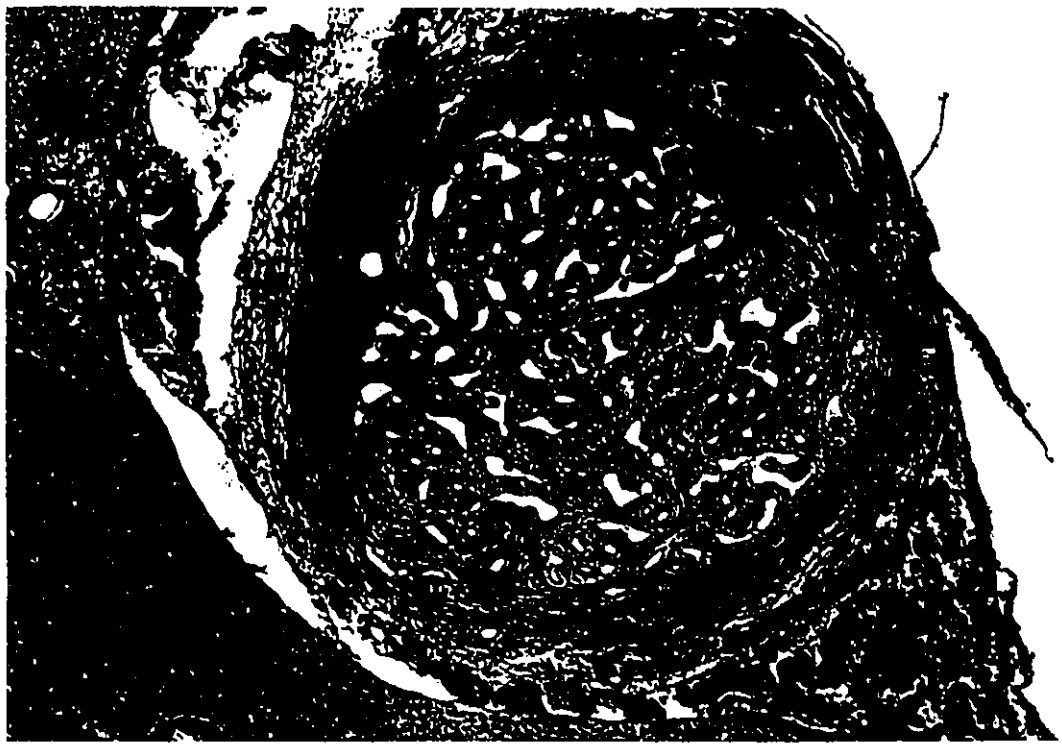


Plate 4.5 **(Top)**: Example of a cholangioma identified in a brown bullhead collected from Hamilton Harbour, Ontario (100x).

(Bottom): Brown bullhead cholangiofibrosis. Clusters of well differentiated biliary ductules with increased periductular fibrosis (100x).



Cholangiohepatitis and cholangiofibrosis lesions, similar to those observed in white suckers (*Catostomus commersoni*) by Hayes *et al.* (1990) were observed in fish from all three sites. Cholangiohepatitis was characterized by a chronic inflammatory condition confined primarily to the major intrahepatic portal tracts. The incidence of this lesion was similar (5 to 7 fish per site) for all three sample locations. Cholangiofibrosis were lesions composed of large encapsulating masses which encircled six or more normal appearing bile ductules (Plate 4.5). The incidence of this lesion was elevated at both the Trenton Channel (24%) and Amherstburg (41%) sites with respect to the lower incidence observed in fish from the less contaminated Peche island site (4%).

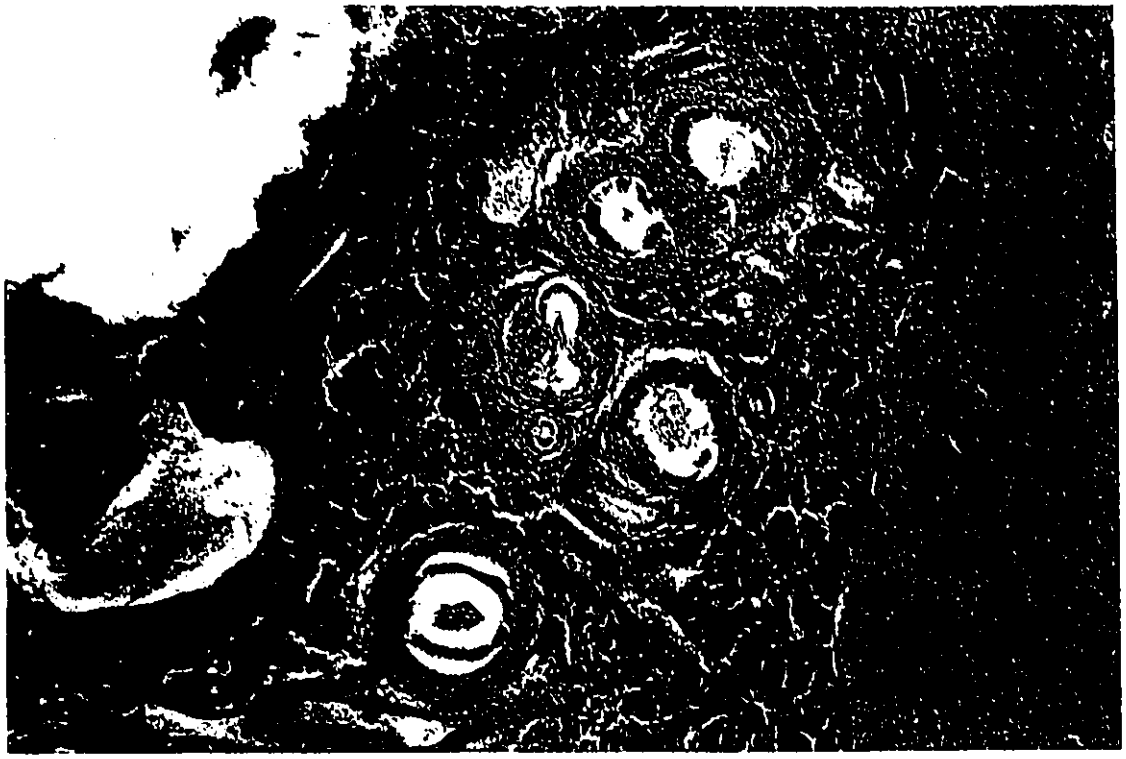
This investigation identified lesions of altered cholangiolar tissue has not been previously characterized in the published literature. These lesions are identified as: i) fibrotic thickening of normal appearing bile ducts (FBD), ii) foci of hyperplastic bile ducts (HBD), iii) misshappen bile ducts (MBD), and iv) anaplastic bile ducts (ABD).

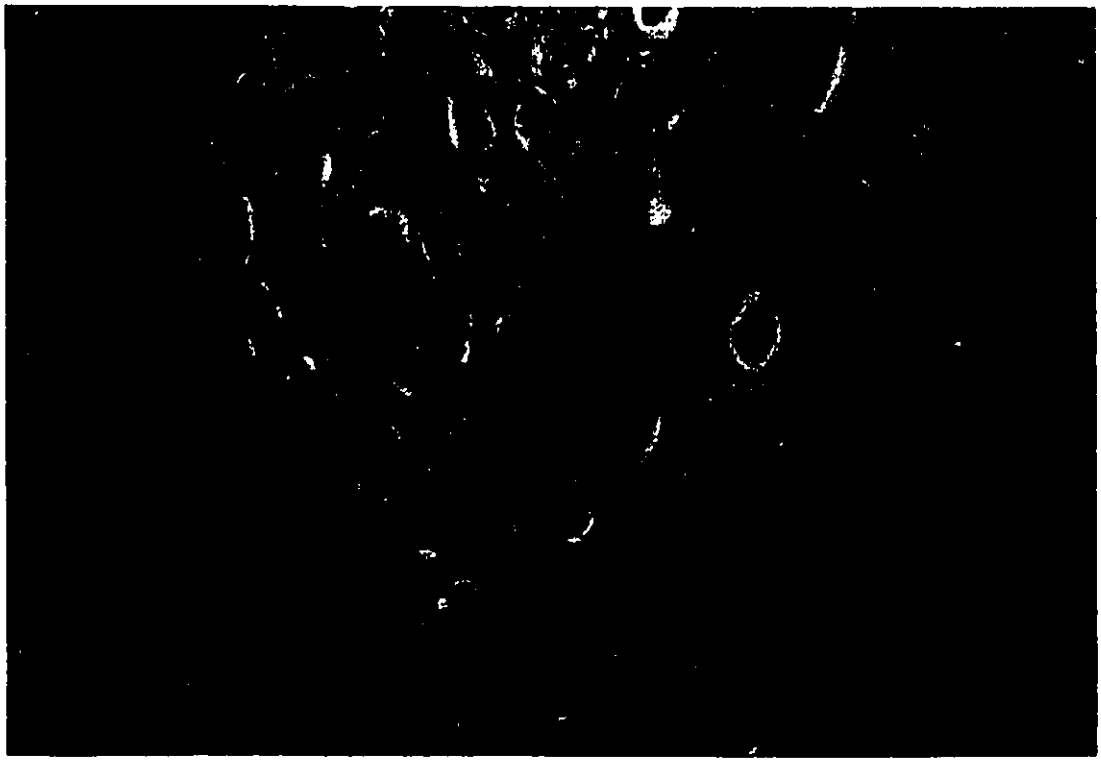
The most prominent characteristic of the fibrotic thickening of bile ducts (FBD) was an unusually thick wall of fibrotic tissue surrounding normal appearing ductular epithelial cells (Plate 4.6). The surrounding fibrotic wall was typically two to three times the thickness observed in normal surrounding bile ducts. This condition was found throughout the liver and does not appear to be related to the normal bile duct wall thickening that is often observed at locations close to the gall bladder.

The foci of hyperplastic bile ducts (HBD) were aggregates of small diameter bile ducts. Each foci generally contained less than twenty normal appearing bile ducts (Plate 4.6). These bile duct aggregates were not encapsulated in fibrotic tissue but were often found in close proximity to FBD lesions.

Plate 4.6 **(Top)**: Fibrotic thickening of bile ducts. Thick wall of fibrotic tissue surrounding normal appearing ductular epithelial cells (100x).

(Bottom): Foci of hyperplastic bile ducts (400x).





Misshapen bile duct foci (MBD) consisted of a proliferative aggregation of distorted bile ducts (Plate 4.7). The misshapen appearance was due primarily to a hyperplastic growth of the ductular epithelial cells which extended into the lumen of the duct. The epithelial lining was at times detached from the ductular wall and in other cases the proliferation of cells completely filled the lumen. The typical size of this foci was small and similar in diameter to the HBD foci. Both the HBD and MSD lesions were confined to the portal tract.

Foci of anaplastic bile ducts (ABD) were characterized by a proliferation of ductular epithelial cells scattered throughout an unorganized pattern of cell originating from what appears to be supportive tissue. The epithelial cells were generally arranged in groups of ten or less cells which formed a non-canuliculized ductular (circular) pattern. These ductular cells were surrounded by supportive tissue. Cells of the supportive tissue lacked polarity and were generally spindled-shaped. The foci were not encapsulated by fibrotic tissue. The shape of the foci was irregular with extensions protruding into surrounding hepatic parenchyma. The ABD lesion was typically small but was often two to three times the size of the HBD and MBD lesions. With the exception of one fish, all ABD lesions were always found in association with both HBD and MBD foci.

Eosinophilic and basophilic foci were the only hepatocytic lesions observed. The incidence of these lesions was low. The only pre-neoplastic lesion observed was a basophilic foci from a fish collected at the Trenton Channel site.

Exposure Study Results

As a result of high fish mortality, particularly at the Turkey and Peche Island locations, the exposure study was terminated at all three sites on day 16 and all remaining fish were

Plate 4.7 Misshapen bile dust foci consisting of an aggregation of distorted bile ducts.

removed from the cages. Examination of the gut content of each bullhead, revealed that the fish in the top cages consumed little food. This was observed at all three cage locations. The majority of the brown bullheads that had access to the sediments (bottom cages), did contain varying amounts of food in the gastrointestinal tract. Typical gut contents included fragments of aquatic insect larva (e.g., ceratipogonidae, chironomids), cladocerans (ostracods), gastropods (snails), filamentous algae, bits of aquatic plants and small sediment grains (sand). It was not expected that the fish would satiate themselves while constrained to the cages, but fish, that had access to the sediments, would search in the sediments for prey, not only increasing contaminant exposure through food, but also increasing their dermal exposure through regular sediment contact.

To estimate the bullheads exposure to PAHs at the various sites, concentrations of aromatic hydrocarbon metabolites were measured in the brown bullhead bile. Metabolite concentration was estimated by measuring fluorescent aromatic compounds (FACs), which has been shown to be a good indicator of recent exposure to PAHs (McCain *et al.* 1992). Metabolite concentrations (reported as ng/ml BaP equivalents) in the Trenton Channel caged brown bullheads were determined for the day 2, 4, 8, and day 16. Aromatic metabolite concentrations for Turkey Island and Peche Island bullheads were analyzed for day 8 samples only. On day 8 of the study, the fish from Peche and Turkey Islands sites still appeared healthy and bottom cage fish were still feeding according to gut content analysis.

HPLC analysis of bile FACs revealed no significant difference ($F_{[1,12]} = 0.055$, $p = 0.819$) in metabolite concentrations in fish from the top cages compared to fish confined to the bottom cages. As a result, day 8 fish samples from both top and bottom cages were pooled to represent a single sample from each site. Trenton Channel bullhead FAC concentrations (Day 8) were significantly greater than Peche Island

(Fisher's LSD, $p < 0.001$) and Amherstburg Channel (Fisher's LSD, $p = 0.003$) bullhead FACs. There was no significant difference between Peche Island and Amherstburg Channel FAC concentrations (Fisher's LSD, $p = .298$). The concentration of bile FACs in the Trenton Channel bullheads on Day 8 was approximately 5 times greater than concentrations found in Peche Island bullheads and 4 times greater than the Turkey Island bullheads (Table 4.3 and Figure 4.8). It was therefore concluded that the brown bullheads in the cages at Trenton Channel were more exposed to the high PAH concentrations of the Trenton Channel.

The concentrations of PAH metabolites, in the bile of the caged Trenton Channel brown bullheads for the full 16 day exposure period are summarized in Table 4.4 and Figure 4.9. Bile FAC concentrations increased significantly by Day 2 and peaked in concentration by Day 4. Following day 4 FAC concentrations leveled out in a similar pattern as the initial uptake. By Day 16, the FAC concentrations were similar to laboratory control fish. Interestingly, a thunderstorm throughout Detroit River area on Day 3 of the study had resulted in 80 mm of rain in a very short period of time. The resulting large volumes of runoff and turbulent waters causing sediment resuspension, may have affected the PNAH concentrations in both the top and bottom cages. The metabolite concentration in the Trenton Channel bullheads peaked following the rain event and subsequently returned to lower concentrations.

Bile FAC concentrations in the Trenton Channel feral population (Arkand *et al.* In preparation) were similar in concentration to the bullheads maintained in the laboratory aquaria (576.29 ng/mL B(a)P equivalents). The bullheads collected in that study were, however, captured in the lower Trenton Channel, southwest of Grosse Isle, where sediment concentrations of PAHs, PCBs and other chlorinated compounds are much lower (Kaiser *et al.* 1985).

Table 4.3 Concentration of bile FACs (ng/mL BaP equivalents) in caged brown bullheads at Trenton Channel, Turkey Island, and Peche Island. Samples were analyzed after 8 days of exposure in the field. Laboratory fish represent bullheads maintained in aquaria during the course of the study.

Figure 4.8 Bar chart depicting concentration of bile FACs (ng/mL BaP equivalents) in caged brown bullheads at Trenton Channel, Turkey Island, and Peche Island. Samples were analyzed after 8 days of exposure in the field. Laboratory fish represent bullheads maintained in aquaria during the course of the study.

Cage Location	N	Conc. (ng/mL BaP equivalents)	± Standard Error
Trenton Channel	8	3574.13	804.44
Turkey Island	8	963.56	161.72
Peche Island	8	631.86	120.86
Laboratory Fish	3	576.29	94.72

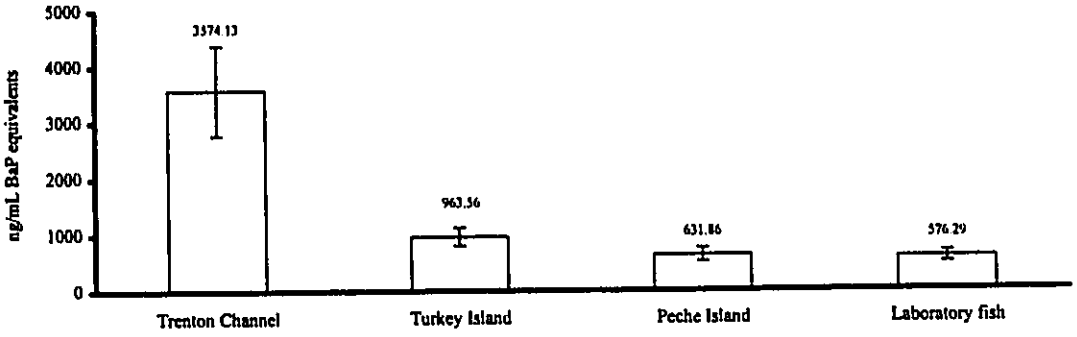
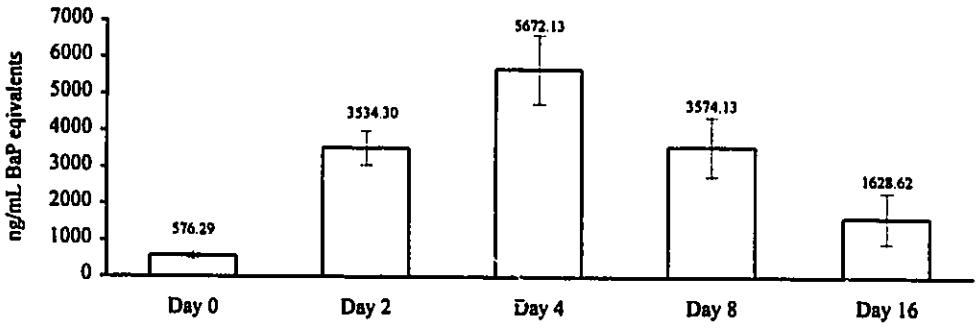


Table 4.4 Mean concentration of bile FACs (ng/mL B(a)P equivalents) in caged brown bullheads in the Trenton Channel.

Figure 4.9 Bar chart representing mean concentration (± standard error) of bile FACs (ng/mL B(a)P equivalents) in caged brown bullheads in the Trenton Channel.

Time	N	Mean Conc. Bile FACs (ng/mL BaP equivalents)	± Standard Error
Day 0	3	576.29	94.72
Day 2	6	3534.30	461.23
Day 4	6	5672.13	945.19
Day 8	6	3574.13	804.44
Day 16	7	1628.62	703.45



Discussion

Chemical analysis of surficial sediments of the Detroit River confirm the results of previous studies that reveal the sediments of the river contain a complex mixture of organic chemical compounds (Kaiser *et al.* 1985, Fallon and Horvath 1985, Furlong *et al.* 1988). This study also confirmed significant spatial heterogeneity in the distribution of those chemical contaminants (e.g. PCBs, PAHs, pesticides) within the river. The composition and concentrations of chemical contaminants in the sediments along the heavily industrialized Michigan shoreline were considerably more diverse and greater in concentration then sediment-associated contaminants sampled along the Ontario shoreline.

The chemical spatial distribution observed in the sediments was also evident in the chemical body burdens of the feral bullhead populations sampled from the river. Feral brown bullheads collected from the Trenton and the Amherstburg Channels had chemical body burdens similar in composition (e.g. PCBs, chlorinated hydrocarbons and pesticides) to the sediments from which they were captured. The amount of cross channel mixing in the lower Detroit River is negligible under most circumstances, and therefore it's assumed that the chemical body burdens observed result from contaminant exposure that originated from sources along the respective shorelines (Upper Great Lakes Connecting Channel Study, 1988). The chemical composition and concentrations of the sediments observed in the Trenton Channel were similar to other studies of contaminated waterways where incidences of fish lesions were found to be elevated (Poulton 1987, Black 1983, Hayes *et al.* 1990, Baumann *et al.* 1991).

It is important to emphasize that relative level of chemical concentrations observed in the sediment does not provide an adequate reflection of exposures observed in the fish.

Concentrations of DDE and HCB in sediments and fish provides a good example of the differential accumulation that occurs in the environment. Concentrations of DDE in the sediment samples at the three sites were low in comparison to HCB concentrations (figure 4.1). Concentrations of the same chemicals in the bullheads were contrary to what was observed in the sediments. The concentrations of DDE in the bullhead tissues were significantly greater than concentrations of HCB (figure 4.3). This illustrates why monitoring sediment concentrations alone does not reflect actual exposures observed in the field.

The equilibrium partitioning theory predicts that bio-sediment accumulation factor (BSAF) ratios should approximate a value of one, when equifugacity is achieved among compartments (Connolly and Pederson 1988, Mackay 1989). Results from our BSAF analysis clearly demonstrates that this was not the case. Ratios greater than one for compounds approximately log K_{ow} 6 or higher occurred at all three sampling sites and is strongly suggestive of trophic accumulation (biomagnification). Hebert and Haffner (1991) concluded that habitat partitioning was a significant factor regulating contaminant levels found in forage fish and that benthic-feeding fish had the highest exposures. Similar to the benthivore in their study, brown bullheads are also primarily benthic feeders consuming a wide range of aquatic organisms. These animals will forage deep in the soft sediments in search of food, and ingest quantities of organic detritus along with prey items. As a result of this feeding behavior and a characteristic habit of remaining motionless on the bottom during periods of inactivity, these animals increase their overall exposure to contaminated sediments. As a consequence, not only are contaminant body burdens elevated, but dermal exposure is also increased and might be related to the high frequency of external lesions, such the elevated occurrence of fin erosion and lip papillomas (i.e. areas in frequent contact with the sediment) observed in Trenton Channel bullheads.

A review of pollution-associated diseases by Sindermann (1979), supports an association between the occurrence of fin erosion and other external lesions in benthic fish species from areas where sediments are heavily contaminated. The frequency of fin erosion and body lesions observed in Trenton Channel bullheads was greater than frequencies observed in bullheads from the less-contaminated Amherstburg Channel and Peche Island. Only 11% of the bullheads sampled from the Trenton Channel were free of any obvious external abnormalities. In contrast, 36% and 41% of the bullheads from the Amherstburg Channel and Peche Island respectively were free of external abnormalities. Sherwood and Mearns (1977) suggested that dermal contact with contaminated sediments can remove the protective mucus layer of the fish, thereby increasing epithelial tissue exposure to cytotoxic chemicals. External observations in this study would support this conclusion.

The high BSAF ratios observed at Peche Island, the least contaminated site, suggests that the resident bullhead population is more contaminated then would be predicted from the relatively low chemical concentrations in the sediment. Since numerous samples of sediment were analyzed from the general vicinity of where the fish were captured to rule out spatial heterogeneity, we can only conclude that these animals are not as philopatric (site specific) as previously thought. Exposure may be taking place outside of the Peche Island area and, perhaps, concentrations might reflect Lake St. Clair sediments as opposed to the Peche Island bays.

Many of the chemical compounds identified in the sediments (e.g. PAHs) are known cytotoxicants, mutagens and/or carcinogens (Verschueren 1983). Earlier *in vitro* studies investigated the potential hazards of exposure to these chemical compounds associated with Detroit River sediments. Maccubbin *et al.* (1991) tested Detroit River sediments for

mutagenicity using the Ames test and demonstrated that with metabolic activation, 16 out 31 stations sampled along the lower western shoreline of the river showed mutagenic activity. More recently, Ali *et al.* (1993) also investigated the toxicity of sediment extracts, from the same three areas in the Detroit River as this study, using a brown bullhead dorsal muscle cell line. The results confirmed the cytotoxic and genotoxic potential of Trenton Channel sediments. Based on these results, they predicted higher cytotoxic and genotoxic stress in Trenton Channel fish exposed to these sediments, which was confirmed in this field study.

Studies have shown that fish readily accumulate PAHs from the environment and rapidly convert these compounds to reactive electrophilic metabolites, such as dihydrodiols and phenols, which are capable of producing DNA adducts in fish tissue (Tan and Melius 1986, Dunn *et al.* 1987, Maccubbin *et al.* 1988, Steward *et al.* 1990, Sikka *et al.* 1990). The covalent interaction of electrophilic intermediates with DNA is believed to be the initiating step of PAH-induced carcinogenesis (Grimmer 1983, Stegman and Lech 1991). As a result of this rapid aromatic hydrocarbon metabolism in fish, measuring tissue burdens (e.g. muscle tissue) of PAHs does not provide a realistic indication of environmental exposure to these compounds (Malins *et al.* 1984, Maccubbin *et al.* 1988). Aromatic hydrocarbon concentrations in the bullhead muscle tissue sampled in this study were found in trace amounts with most concentrations below detection, even though sediment concentrations were considerably elevated (Trenton Channel, total PAH concentration, Σ 346 mg/kg, dry weight). Studies have demonstrated that a large percentage of aromatic hydrocarbon metabolites are concentrated in the bile of the exposed fish (Steward *et al.* 1990). Considerable advancements have been made in detecting aromatic hydrocarbons in fish following the research of Krahn *et al.* (1984). She and her coworkers developed a procedure using HPLC fluorescent detection for measuring PAH metabolite concentrations in the bile of fish. This technique has since

been successfully used to demonstrate exposure of fish to aromatic hydrocarbons, both in the laboratory and the field (Malins *et al.* 1985, Maccubbin *et al.* 1988, Collier and Varanasi 1991).

Results from this investigation of PAH bioavailibility to bullhead populations in the Detroit River, confirm that brown bullheads rapidly accumulate and metabolize the PNAHs present in the river. Bile FAC concentrations in caged brown bullheads from the Trenton Channel were significantly greater ($p \leq .05$) than concentrations measured in bullheads confined to cages in the Amherstburg Channel or Peche Island. These results clearly demonstrate that the Trenton Channel is a significant exposure source of aromatic hydrocarbons to the brown bullhead population inhabiting this area of the river.

There was no observable difference in bile FAC concentrations between bullheads confined to the water column and those with access to the sediments. The importance of sediments as a primary route of PAH exposure to the brown bullheads in this exposure study is somewhat questionable. It is possible that exposure is primarily determined by runoff from surrounding areas and heavily PAH-contaminated tributaries (e.g. Rouge River). The rapid rise in FAC concentrations following the storm event suggests that the Trenton Channel bullhead population is subjected to significant pulses of aromatic hydrocarbons following runoff events.

Arkand *et al.* (in preparation) reported bile FAC concentrations in the Trenton Channel feral bullhead population that were in the range of bile FAC concentrations of the laboratory bullheads in this study (576.29 ng/mL bile, B(a)P equivalents). A possible reason for the unexpected low FAC concentrations in feral populations is the uneven distribution of contaminated sediments in the Detroit River, and differences in sampling times and runoff events. It is possible that the feral bullhead populations in the river

remain highly induced (e.g. Aryl hydrocarbon hydroxylase), as a result of intermittent exposure to elevated levels of aromatic hydrocarbons and PCBs, especially nonortho PCBs. In response to these pulse events of increased PAH exposure, the brown bullheads can rapidly metabolize and excrete these compounds quickly returning to a steady state level, as seen in the low FAC concentrations reported by Arkand's *et al.* (in prep) investigation. The pattern of Trenton Channel bullhead bile FAC concentrations (rapid increase and decline) observed during the 16 day exposure study, lends support to this hypothesis.

Balch *et al.* (1996) observed an association between DNA adduct formation in brown bullheads and sediment PAH concentrations in Hamilton Harbour, Ontario. The concentration of PAHs in the Hamilton Harbour sediment was 137 mg/kg (dry weight), whereas Trenton Channel sediment was 346 mg/kg. It is quite possible that the pre-neoplastic and neoplastic lesions observed in the bullheads from the Trenton Channel are also a result of exposure to these elevated concentrations. Sediment contaminant concentrations and body burdens at the Trenton Channel site compared with Peche Island support the tumorigenic trend observed from the histopathological survey.

Although the incidences were lower, hepatic neoplasms were also observed in fish collected from the Amherstburg Channel and Peche Island sites. The occurrence of these lesions may be related to increased contaminant exposure as a result of fish movement to other areas in the river. Fallon and Horvath (1985) reported high PAH concentrations in sediment cores collected in an area of deposition adjacent to the Amherstburg Channel, north of Bois Blanc Island, Ontario. Bullheads in this study were collected nearby and it's quite feasible that these fish may have spent time in that area. The source of contaminants to these sediments has not been determined. Since bullheads are known to be philopatric, a more likely explanation for the occurrence of these lesions is the prescence of other

agents in addition to PAHs that result in tumor formation. Exposure to other contaminants such as metals, have also been positively linked to the occurrence of various neoplasms in other species of fish (Malins *et al.* 1984, Black 1984).

Interestingly, the incidence of non-neoplastic cholangiolar disorders (cholangiofibrosis, bile duct thickening, and hyperplastic, mishappen and anaplastic bile ducts) was the highest at the Amherstburg Channel site. It has been suggested by Baumann *et al.* (1990), that some cholangiofibrosis lesions may represent toxic hyperplasia or even early neoplasia in brown bullheads. The bile duct thickening, and the foci exhibiting hyperplastic, mishappen, or anaplastic bile duct aggregates are thought to be precursors to cholangiofibrosis or more aggressive neoplastic lesions. Considering the relatively low chemical concentrations of the Amherstburg Channel site, combined with the low cytotoxic and genotoxic nature of the sediment extracts (Ali *et al.* 1993) a chemical etiology for the occurrence of these lesions is difficult to postulate. The Amherstburg Channel is downstream from tributaries which are intermittent sources of PCBs, metals and other contaminants. The area also receives significant runoff from agricultural land which predominates on the Ontario shoreline, and this runoff contains high levels of nutrients and numerous pesticides (Upper Great Lakes Connecting Channel Study 1988) which could potentially pose a cytotoxic threat. These lesions could be a response to chemical assault which predisposes the foci to becoming neoplastic under, as yet, undefined conditions.

Research has shown that fish from contaminated areas generally have a larger relative liver weight compared to fish from uncontaminated sites (Sherwood and Mearns 1977, Pierce *et al.* 1978, Fabacher and Baumann 1985, Bagnasco *et al.* 1991). Hepatosomatic index results in this study show a similar trend. Trenton Channel male bullhead livers were significantly larger ($p \leq 0.008$) compared with males sampled from the Amherstburg

Channel and Peche Island. Trenton Channel female bullheads were significant larger than Amherstburg females but not Peche Island female bullheads. The larger livers in Peche Island female bullheads may be due in part to a seasonal factor since these fish were collected over a 3 month period which included the breeding season. Since factors other than contaminants are known to cause increased liver size in fish (e.g. age, nutritional state or circulating reproductive hormones related to the reproductive cycle Fabacher and Baumann, 1985), a direct chemical association cannot be made based on relative liver size alone. Although, combined with the results from bullhead chemical body burdens, and histopathology, the trend towards larger livers in the Trenton Channel bullheads provides additional support that a chemical stress is affecting the bullhead population in that area.

Fabacher and Baumann (1985) have shown that concentrations of hepatic mixed-function oxidase (MFO) components in tumor bearing brown bullheads from a chemically contaminated river were not significantly different than bullheads from uncontaminated sites, although the bullhead livers from the contaminated site were significantly enlarged. The authors suggest bullhead acclimatization to the polluted environment results in an increased relative liver weight and subsequently increased total amounts of MFO components. Enlarged livers with greater than normal amounts of MFOs could result in increased elimination and activation of procarcinogens resulting in the formation of tumors and cancer. The elevated incidence of tumors in Trenton Channel bullheads in addition to the observed rapid increase and decrease in bile FACs following the runoff event supports this hypothesis.

Based on the chemical concentrations observed in the Trenton Channel sediments, and the results of benthic fish tumor surveys from similarly contaminated waterways (Black 1983) a greater frequency of tumors in the Trenton Channel bullheads might have been

expected than what was actually observed. Previous work by Baumann *et al.* (1990) has shown a positive correlation between the age of the bullheads and the frequency of tumors. Bullheads in this study were 3 to 4 years old, which allowed us to compare tumor frequencies among the three sites. Based on previous studies conducted in the lower Detroit river (Maccubbin and Ersing 1991), we would expect to find an increase in tumor frequency with age. Such a relationship would support the conclusion of a chemical etiology to explain tumor frequency in which a latent period between initiation and the eventual transformation of the cell (tumor development) would be expected to take months or even years (Baumann *et al.*, 1990, Stegman and Lech 1991). Temporal uncoupling of exposure and effect may explain the absence of hepatic lesions in the bullheads sampled, and the limited number of true malignant biliary lesions actually observed.

Conclusion

The result of this study confirms the earlier predictions of Ali *et al.* (1993), that of the three populations of brown bullheads sampled in the Detroit River, the population exposed to the cytotoxic and genotoxic sediments present in Trenton Channel displayed clear indications of cytological stress. Trenton Channel bullheads not only had elevated body burden concentrations of organic contaminants such as PCBs, chlorinated hydrocarbons and pesticides, but a greater frequency of biliary tumors, and a higher percent of external abnormalities (e.g. fin erosion, stub barbels and dermal lesions) in comparison to fish sampled from two less contaminated sites within the Detroit River.

The weight of evidence from numerous field and laboratory studies of tumor bearing fish in contaminated aquatic ecosystems supports a chemical relationship to explain the elevated frequency of tumors observed, particularly an association with aromatic hydrocarbon contamination of sediments (McCain *et al.* 1990, Gardner *et al.* 1989, Dunn *et al.* 1987, Baumann *et al.* 1987). All lines of evidence from this study provide additional support for a chemical etiology. An association of contaminant body burdens in the fish with sediments, combined with the observation of increased external and internal biliary lesions and increased relative liver weight corresponding to body burdens, provides strong evidence that Detroit River brown bullhead populations are stressed by chemical exposure. Both sediment and stormwater runoff events are important sources of chemical exposure that can significantly stress feral populations of brown bullheads in the Detroit River.

It is concluded that brown bullheads are excellent biological monitors that reflect both the spatial and temporal heterogeneity of chemical contaminants often observed in aquatic ecosystems. It is further concluded, that future field studies supported by *In vitro* dose

response assays can further resolve the cause-effect relationship of chemical stress in contaminated aquatic ecosystems.

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Appendix A: Organochlorinated hydrocarbons, pesticides and PCB chemical contaminant concentrations (µg/Kg, dry weight basis) in sediments collected from Peche Island, Amherstburg Channel, and Trenton Channel.

Concentration (µg/Kg) dry weight basis		Amherstburg Channel	Peche Island	Trenton Channel
% Organic Carbon		10.28	9.15	3.25
1,2,4,5 -TCB		41.7	46.0	51.7
1,2,3,4, -TCB		8.8	5.2	33.6
QCB		35.2	39.7	47.3
HCB		103.6	134.4	651.3
OCS		24.9	23.6	59.1
trans- Nonachlor		2.0	3.9	22.7
pp'-DDE		35.6	31.9	311.6
Mirex		ND	2.7	ND
PCB congeners	#31	ND	ND	249.9
	#28	15.5	2.1	128.4
	#52	32.3	13.9	401.9
	#49	23.1	4.4	223.5
	#44	44.9	15.8	215.9
	#42	9.2	ND	108.5
	#64	3.2	0.8	31.0
	#74	12.9	5.7	180.6
	#70	26.4	3.6	369.1
	#66/95	33.3	11.6	494.2
	#60	21.2	16.5	178.2
	#101	29.0	7.9	413.4
	#99	13.1	6.8	160.6
	#97	9.3	3.2	102.6
	#87	15.3	4.0	183.3
	#110	26.2	15.7	306.1
	#151	7.5	1.5	138.9
	#149	23.1	8.1	425.9
	#118	26.7	9.5	269.2
	#146	3.6	0.9	99.4
	#153	23.7	6.7	495.6
	#105	12.6	2.5	153.3
	#141	7.3	1.0	127.7
	#138	39.4	16.5	893.5
	#129	4.6	0.6	59.0
	#182/187	13.8	4.6	261.2
	#183	6.8	1.9	123.2
	#185	1.0	ND	23.8
	#174	27.8	ND	214.6
	#171	11.8	0.7	101.3
	#200	6.7	ND	51.6
	#172	2.2	ND	27.7
	#180	18.2	8.9	516.3
	#170/190	13.6	29.5	284.1
	#201	6.3	5.3	162.1
	#203	4.9	2.2	119.4
	#195	0.0	1.7	58.5
	#194	13.0	3.2	162.1
	#206	0.0	0.7	69.3
Arochlor 1254:1260		532.0	223.4	12074.2
Arochlor 1260		159.6	78.2	4529.1

Appendix B: 16 priority mean PAH concentrations (mg/Kg, dry weight) in sediment extracts from Trenton Channel, Amherstburg Channel, and Peche Island.

PNAHs	Amherstburg Channel	Trenton Channel	Peche Island
Napthalene	0.70	6.26	0.01
Acenapthylene	ND	1.65	0.03
Acenapthene	ND	1.95	0.03
Fluorene	ND	5.20	0.11
Phenanthrene	2.40	17.72	0.59
Anthracene	ND	6.85	0.10
Fluoranthene	4.15	13.01	0.97
Pyrene	4.25	17.53	0.85
Benzo(a)Anthracene	3.70	30.42	0.40
Chrysene/Triphenylene	3.80	28.08	0.66
Benzo(b)Fluoranthrene	6.45	44.75	0.52
Benzo(k)Fluoranthrene	0.35	22.60	0.41
Benzo(a)Pyrene	3.90	43.55	0.31
Indeno(1,2,3-cd)Pyrene	ND	64.14	0.39
A-Dibenzo(a,h)Anthracene	ND	8.88	ND
P-Benzo(g,h,i)Perylene	ND	33.87	0.42

ND: not detected

Appendix C: Organochlorinated hydrocarbon, pesticide, and PCB concentrations (µg/Kg, corrected for lipid) in brown bullhead (*Ameiurus nebulosus*) dorsal muscle tissue.

	Amhersiburg Ch. (N=10)		Trenton Ch. (N=12)		Peche Island (N=9)	
Compound	Mean	S.E.	Mean	S.E.	Mean	S.E.
1,2,4,5 TCB	ND	ND	45.1	13.1	ND	ND
1,2,3,4 TCB	ND	ND	18.5	4.8	2.7	2.7
QCB	13.0	1.9	29.4	4.6	16.1	0.8
a-HCH	17.2	3.3	7.5	3.6	19.1	5.1
HCB	56.4	6.1	156.3	21.6	58.4	8.8
b-HCH	11.4	8.5	1.8	1.1	21.1	10.6
g-HCH	7.5	2.8	16.0	1.6	8.4	1.9
OCS	58.1	18.1	174.5	38.4	209.3	61.1
oxy-chlordane	10.3	2.9	26.6	7.5	18.6	2.8
trans-chlordane	31.6	6.4	156.9	36.6	20.2	4.2
cis-chlordane	58.5	9.7	159.0	37.3	48.2	12.5
trans-nonachlor	79.2	8.5	214.1	48.4	100.4	40.4
pp'-DDE	510.2	39.7	1845.1	412.7	837.0	283.5
pp'-DDD	153.2	20.6	876.0	222.6	181.3	45.0
cis-nonachlor	33.2	4.5	72.2	18.7	42.3	11.2
pp'-DDT	57.5	15.1	56.8	21.9	54.8	15.4
photo-mirex	3.4	3.4	ND	ND	6.3	4.2
Mirex	5.9	1.5	11.9	3.1	4.4	2.0
HC Epox	32.4	3.4	29.8	5.0	21.5	1.9
Dieldrin	186.4	49.1	118.3	30.0	88.3	12.6
PCB congeners #28	7.4	2.1	110.2	20.0	2.7	1.3
#52	55.2	11.7	620.0	99.2	60.8	9.4
#49	37.3	8.0	468.2	70.7	23.0	4.4
#44	65.7	9.9	507.5	70.7	94.6	29.1
#42	3.6	2.4	235.6	41.8	1.7	1.7
#64	7.2	2.3	106.4	17.7	1.7	0.7
#74	49.3	18.0	577.9	92.9	34.9	9.6
#70	22.0	5.5	124.0	20.3	5.0	2.5
#66/95	108.9	23.8	1014.8	149.9	100.3	29.2
#60	64.2	16.9	517.3	80.0	137.5	55.5
#101	102.5	18.1	773.6	133.3	130.0	22.5
#99	84.4	14.8	534.4	100.6	121.7	24.9
#97	34.2	8.0	287.2	52.0	18.3	9.9
#87	72.5	12.7	527.8	96.8	47.1	17.1
#110	133.7	22.4	918.8	167.2	92.8	37.3
#151	93.4	11.8	587.6	110.1	120.0	64.0
#149	180.8	20.3	1129.9	233.6	213.4	131.3
#118	149.0	20.8	943.5	184.0	226.1	66.8
#146	62.6	6.5	298.5	59.2	109.5	54.2
#153	179.4	24.2	1237.5	300.7	372.3	91.9
#105	73.8	8.2	496.7	102.3	70.0	30.3
#141	73.8	8.4	475.1	104.7	137.2	69.7
#138	363.9	38.7	2224.4	510.1	658.7	286.6
#129	30.4	3.3	198.1	45.0	53.5	29.8
#182/187	262.0	34.3	1431.8	303.8	445.3	263.8
#183	80.2	19.0	410.0	100.9	117.1	52.0
#185	13.8	1.4	91.4	23.2	24.0	15.9
#174	104.3	11.1	691.2	157.9	162.2	111.8
#171	41.3	5.4	252.8	58.9	73.2	37.4
#200	15.6	3.1	105.6	23.9	25.4	15.2
#172	17.1	1.6	99.8	22.8	33.2	18.2
#180	301.4	31.2	1962.9	475.2	616.8	354.6
#170/190	131.0	14.0	1177.9	295.6	293.3	175.3
#201	89.3	9.1	501.2	110.8	165.2	98.9
#203	57.2	6.0	337.4	75.5	109.7	65.5
#195	29.8	4.1	163.0	33.5	50.1	30.1
#194	51.5	5.5	328.3	73.6	106.1	67.0
#206	19.4	2.1	81.7	14.1	31.2	13.8
Arochlor 1254:1260	4917.1	522.4	30059.6	6893.2	8986.3	3861.9
Arochlor 1260	2753.5	280.9	17218.8	4168.3	5104.5	3166.1
#77	9.1	2.6	60.9	13.3	17.1	10.2
#126	ND	ND	3.1	1.4	ND	ND
#169	ND	ND	0.6	0.3	0.1	0.1
#189	0.9	0.6	24.8	6.3	4.6	4.6

Appendix D: Statistical analysis

1.) Statistical analysis of bullhead bile FACs data using Analysis of Variance (ANOVA)

	SS	*df*	*MS*	*F*	*p*
Groups	2.017	3	.672	10.691	0.000
Error	1.069	17	0.063		

Groups represent log bile FAC Day 8 concentrations in bullheads from Trenton Channel, Turkey Island, Peche Island and laboratory bullheads.

Post Hoc Comparison
Fisher's Least - Significant Difference Test (LSD)
Matrix of Pairwise Comparison Probabilities

	Lab Fish	**Peche Island**	**Trenton Ch.**	**Turkey Island**
Lab Fish	1.000			
Peche Island	0.941	1.000		
Trenton Ch.	0.001	0.001	1.000	
Turkey Island	0.335	0.277	0.001	1.000

2.) Statistical analysis of bullhead bile FACs between sites and between top and bottom positions using Two Way Factorial Analysis of Variance (ANOVA)

	SS	*df*	*MS*	*F*	*p*
Groups	1.768	2	0.884	13.190	0.001
Top vs Bottom	0.004	1	0.004	0.055	0.819
Interaction Term	0.215	2	0.108	1.607	0.241
Error	0.804	12	0.067		

Groups represent log bile FAC Day 8 concentrations in bullheads from Trenton Channel, Turkey Island, Peche Island. Top and Bottom refer to cage position at the sites (i.e. Top = bullheads in the cages constrained to water column).

Post Hoc Comparison
Fisher's Least - Significant Difference Test (LSD)
Matrix of Pairwise Comparison Probabilities

	Peche Island	**Trenton Ch.**	**Turkey Island**
Peche Island	1.000		
Trenton Ch.	0.000	1.000	
Turkey Island	0.298	0.003	1.000

3.) Statistical analysis of brown bullhead relative liver weight (Hepatosomatic Index) using a Two Way Factorial Analysis of Variance (ANOVA)

	SS	*df*	*MS*	*F*	*p*
Sex	0.100	1	0.100	13.729	0.001
Site	0.138	2	0.069	9.523	0.001
Interaction Term	0.017	2	0.008	1.155	0.322
Error	0.458	63	0.007		

Sex represents comparison between male and female bullhead hepatosomatic indices. Site represent comparisons of hepatosomatic index from bullheads collected in Trenton Channel, Amherstburg Channel, and Peche Island.

Post Hoc Comparison (SEX)
Fisher's Least - Significant Difference Test (LSD)
Matrix of Pairwise Comparison Probabilities

	Females	**Males**
Female	1.000	
Male	0.001	1.000

Post Hoc Comparison (SITE)
Fisher's Least - Significant Difference Test (LSD)
Matrix of Pairwise Comparison Probabilities

	Turkey Island	**Peche Island**	**Trenton Ch.**
Turkey Island	1.000		
Peche Island	0.071	1.000	
Trenton Ch.	0.001	0.024	1.000

Post Hoc Comparison (SITE and SEX)
Fisher's Least - Significant Difference Test (LSD)
Matrix of Pairwise Comparison Probabilities

	Amherstburg Ch. (Females)	Peche Island (Females)	Trenton Ch. (Females)	Amherstburg Ch. (Males)	Peche Island (Males)	Trenton Ch. (Males)
Amherstburg Ch. (Females)	1.000					
Peche Island (Females)	0.142	1.000				
Trenton Ch. (Females)	0.031	0.558	1.000			
Amherstburg Ch. (Males)	0.022	0.000	0.000	1.000		
Peche Island (Males)	0.204	0.006	0.000	0.281	1.000	
Trenton Ch. (Males)	0.214	0.703	0.280	0.000	0.008	1.000

4.) Statistical analysis of standard bullhead lengths using Kruskal-Wallis One Way ANOVA. Non parametric method In-Lieu of One Way ANOVA
Dependent variable is Log standard lengths.
Grouping variable is sampling sites

Group	Count	Rank Sum
Amherstburg Channel	22	589.00
Trenton Channel	27	1065.50
Peche Island	19	691.50

Kruskal-Wallis Test Statistic = 5.238
Probability = 0.073 assuming a Chi-Square distribution with 2 degrees of freedom

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