Fish Tumors Related to Great Lakes Areas of Concern Conference Proceedings

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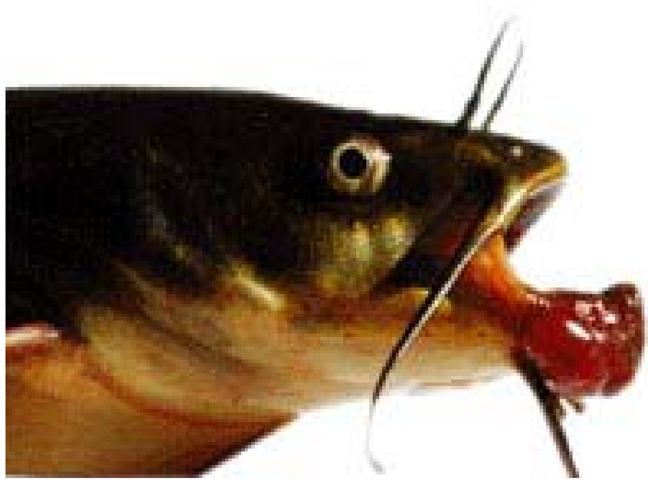

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Erie, Pennsylvania

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Preface

The Fish Tumors Related to Great Lakes Areas of Concern Conference Proceedings was compiled with the intention of capturing the thoughts of the conference held in Erie, Pennsylvania from January 21-22, 2003; as well as to provide information on fish tumors as they relate to the beneficial-use impairment in Areas of Concerns. Two working subcommittees, monitoring and histopathology have been formed as an outcome of the conference. They are being chaired by Paul Baumann (monitoring) and Vicki Blazer (histopathology), and are preparing standardized criteria for this beneficial-use impairment to be used in all Areas of Concern addressing this use impairment. The resulting work of the subcommittees will be presented at a follow up conference on August 18-19, 2003, at Penn State Erie. The August conference attendees will attempt to complete their recommendations of standardized criteria for fish tumors and deformities and submit a concept paper to the International Joint Commission at its September meeting in Ann Arbor, Michigan.

A special thanks is extended to all the speakers at the conference, including Dr. Paul Baumann, Dr. Vicki Blazer, Kelly Burch, Dr. John (Jack) Fournie, Dr. John Gannon, Dr. John Harshbarger, Chuck Murray, Dr. Fred Pinkney, Roger Thoma, and Bob Wellington, and also to the U.S. Environmental Protection Agency and the Pennsylvania Department of Environmental Protection for providing funding for the conference. We would also like to extend our thanks to Gannon University for hosting the conference.

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Introduction – Conference Objectives

The International Joint Commission (IJC) characterized fish tumors and other deformities as one of 14 beneficial-use impairments to be used by Areas of Concern as criteria for the listing and delisting process. An Area of Concern is considered to have a fish tumor and deformity impairment if the following criteria are observed.

1) An intestinal or liver tumor prevalence of ≥ 5 to 7% occurs in common native nearshore species of benthic dwelling fish (brown bullhead), walleye, perch or salmonid species offshore. Samples must consist of 30 fish, each of which is 250 mm or greater in length. Tumors are defined as neoplasms of either intestinal, bile duct, or liver cells as determined by histopathology
2) A prevalence of lip tumors ≥ 8-10% or overall external tumors ≥ 13-15% in white sucker and brown bullhead. Tumors are defined as papillomas or other neoplasms as determined by histopathology. Samples must consist of at least 30 fish, each of which is 250 mm in length or greater.
3) A Deformities, Erosion, Lesions & Tumors (DELTs) external anatomy index of > 0.5% occurs. (Baumann; LaMP, 2000)

Tumors in various fish species, predominately brown bullheads, in Presque Isle Bay and other Areas of Concern have concerned researchers and citizens since the creation of Areas of Concern on the Great Lakes in 1984. In order to refine and coordinate the standardization of protocols currently being used to evaluate this beneficial-use impairment, staff of Pennsylvania Sea Grant, the Pennsylvania Department of Environmental Protection (DEP), and the U.S. Environmental Protection Agency (EPA) worked together to coordinate a conference addressing fish tumors in Areas of Concern.

On January 21–22, 2003, Pennsylvania Sea Grant, the DEP, and the EPA co-sponsored the first Area of Concern Conference on Fish Tumors, held at Gannon University in Erie, Pennsylvania. This conference brought together more than 40 researchers, fishery and wildlife biologists, pathologists, and agency representatives. The goal of the conference was to share information, concerning fish tumors and deformities, from American and Canadian Areas of Concern, and to refine and coordinate the standardization of protocols currently being used to evaluate this beneficial-use impairment. Organizers wanted to develop criteria for this beneficial-use impairment based on the recommendations of the participants. During the two-day conference several panel discussions were held in order to address concerns in relation to establishing criteria for analyzing fish tumors and deformities. Many questions and concerns were left unresolved; however, the conference was the first step in establishing a functioning network of scientists to collaborate on research issues concerning fish tumors and deformities, and develop standardized criteria for the analysis of this beneficial-use impairment.

Speakers and conference participants helped set the stage for many of the panel discussions by submitting preconference questions on several topics that required the need for standardization. Presenters and participants proposed the following questions and concerns regarding this beneficial-use impairment.

The need for histology of internal and external lesions:

- How much liver or what parts of the liver should be sampled?
- How many similar external lesions should be sampled?
- Should "normal" skin sections be sampled?
- Should internal organs, aside from the liver, be sampled for deformities?

How tumors are diagnosed; standardization of criteria used in naming deformities:

- Criteria for cellular alteration vs. hepatoma vs. hepatocellular carcinoma?
- Criteria for bile duct cell neoplasms vs. cellular proliferation?
- Criteria for papillomas vs. carcinomas?
- Diagnosis of pigmented skin lesions?

The relationship of age to tumor occurrence, including:

- Minimal age restrictions to allow for exposure and latent periods?
- Balancing numbers needed vs. accuracy in deciding to use single age comparisons?
- Looking at how neoplasms of differing stages relate to age, do they all follow the same pattern?
- Use of spines vs. otoliths for aging; how accurate are spines for older ages?
- Is there a source for bullheads of known age? If not, who can culture them?

Statistical considerations:

- Occurrence of lesions vs. counts of lesions?
- Use of most advanced lesion vs. using combined stages?
- Is a neoplasm index possible using counts, stages, and/or areas?
- Level of probability needed?

It is the hope of conference organizers that the work and collaboration that began at the conference will lead to the development of standardized criteria concerning fish tumors and deformities.

Background Information

The International Joint Commission (IJC) was formed in 1909, comprised of American and Canadian officials, to assist these governments in finding solutions to the problems facing the waters bordering the United States and Canada, and to manage and protect these waters for the benefit of today's citizens and future generations.

In 1987, the U.S. and Canadian governments signed a protocol promising to report on the progress associated with the improvement of Areas of Concern and requiring the IJC to review Remedial Action Plans (RAPs). Areas of Concern are described as geographic areas, within the Great Lakes Basin, that fail to meet the general or specific objectives of the agreement where such failure has caused or is likely to cause impairment of beneficial use of the area's ability to support aquatic life.

Remedial Action Plans are being developed and implemented at the 42 current Areas of Concern. The objective of the RAP is to restore the beneficial uses, as identified in Annex 2 of the Great Lakes Water Quality Agreement (GLWQA). The mechanisms responsible for the loss of ecological integrity in Areas of Concern are identified in the first step of the RAP development process. Plans of action are then designed to rejuvenate these areas to levels that meet government and public expectations. The restorative measures use an ecosystem approach which considers not only land, air, and water degradation; but also the loss or restriction of human uses in the Great Lakes Basin.

The focus of the conference was fish tumors and other deformities in relationship to Areas of Concern. Tumors can be defined as a swelling on some part of the body; whereas, the swelling or lump represents an abnormal growth of new tissue and the new tissue differs in appearance from the surrounding tissue. Tumors and deformities affecting the brown bullhead (indicator species for this beneficial-use impairment) are hypothesized to be the result of viruses, parasitic invasion, hybridization, and contaminated sediment.

Brown bullheads are commonly affected with epidermal neoplasms of the mouth and skin, where neoplasms are defined as an abnormal tissue that grows by cellular proliferation to form a distinct mass of tissue that may be benign or malignant. The neoplasms are diagnosed as either papillomas (benign) or carcinomas (malignant) and occur singly or in multiples varying in size from several millimeters to several centimeters. When taking a tissue sample of a skin tumor, the entire deformity along with a portion of the underlying tissue should be removed. Tumors in brown bullheads normally originate from two cell types: lightly pigmented neoplasms are usually composed of neoplastic epithelial cells, and black to dark brown growths are usually composed of neoplastic pigment cells. Brown bullheads have exhibited variably sized irregular areas of superficial, dark brown to black pigmentation known as melanosis.

In brown bullheads, lesions include healing wounds and ulcers; excessive scar tissue accumulations around old injuries, especially in the mouth; abrasions from sampling gear; bacterial infection; epidermal hyperplasia; and injuries from pectoral and dorsal spines.

Neoplasia of hepatocytes (liver cells) is termed hepatic cell adenoma (non-invasive, benign) or hepatic cell carcinoma (invasive, malignant). Neoplasia of the bile duct is diagnosed as cholagioma (benign) or cholangiocarcinoma (malignant). Grossly, cholangiomas may appear as white or cream-colored foci or nodules that may be several centimeters in diameter. Early stage neoplasms of hepatocellular origin may be similar to the bile duct tumors in gross appearance, and more advanced tumors may appear as white, gray, cream-colored, or reddish-tan masses bulging from or as nodules within the liver tissue.

Conference Summary

The purpose of the fish tumor conference was to bring researchers and agency staff together to develop standardized criteria for the analysis of fish tumors and other deformities in Areas of Concern. The objectives were to determine what is currently being done in the analysis of fish tumors and deformities, and develop a uniform system for this beneficial-use impairment.

History

The Great Lakes Water Quality Agreement was first signed in 1972, in which the United States and Canada agreed to restore and preserve the chemical, physical, and biological integrity of the Great Lakes Basin ecosystem. In 1978, a new agreement was reached, in which both countries pledged a commitment to rid the Great Lakes of persistent toxic substances (substances that remain in the environment for long periods of time, poisoning food sources for animals and humans). In 1987, a Protocol was signed by both governments, promising to report on restorative progress and calling on the International Joint Commission to review Remedial Action Plans proposed by the 42 Areas of Concern. The mission of the Remedial Action Plans is to restore beneficial uses as identified in Annex 2 of the Great Lakes Water Quality Agreement, in degraded areas within the basin.

Current Knowledge

A fish tumor or deformity impairment occurs when incidence rates of tumors and/or deformities exceeds the specified rate at un-impacted control sites or when data verifies the presence of neoplastic or preneoplastic liver tumors. Un-impacted sites are areas where there is a lack of industrial or municipal pollutant discharges located upstream or in the immediate area where neighboring land uses have not disrupted ecosystem function. Bullheads and suckers are considered inshore fish species and are not known to extensively migrate; therefore, the health of these species can be used to assess the impacts of localized aquatic environments on the health of fish species.

The purpose in assessing fish tumors and deformities is to use these as an indicator of environmental degradation of the aquatic ecosystem and a measure of health impairment to fish populations. Tumors are defined as heritably altered, independent (meaning functions outside host), relatively atypical tissue growths. Tumors can be induced genetically, virally and chemically. Deformities are defined as twisted, missing, forked, or bulging body parts including deformed fins, barbels, abdomen, or skeleton. Deformities are caused by several factors, including: environmental degradation (e.g. chemical contaminants), rapid temperature change during early development, viruses, bacteria, and parasites. Lesions are open sores, exposed tissue, and/or prominent bloody areas.

Currently, 16 of the 42 (Collingwood Harbor was delisted in 1994) Areas of Concern have impairment of beneficial uses due to the presence of fish tumors and other deformities. Those included are: Ashtabula River, Ohio; Black River, Ohio; Buffalo River, New York; Cuyahoga River, Ohio; Detroit River, Michigan; Grand Calumet River, Indiana; Maumee River, Ohio; Milwaukee Estuary, Wisconsin; Niagara River, New York; Presque Isle Bay, Pennsylvania;

Rouge River, Michigan; Sheboygan River, Wisconsin; St. Louis River and Bay, Minnesota and Michigan; St. Mary's River, Michigan; Thunder Bay, Ontario; and Jackson Bay, Ontario.

Future Focus:

- Standardize the fish tumor and other deformities sampling protocol for Great Lakes' Areas of Concern.
    - Establish minimum criteria: sample size, age, length, gender, year, etc.
    - Which organs are to be analyzed for tumors and other deformities
    - The number of slides needed for histopath analysis of samples
    - Data needs for the proposed database
    - How many and what sites should be sampled within an Area of Concern
    - DELT versus histopathological analysis

- The frequency at which fish are sampled within an Area of Concern needs to be determined

- The use of otolith or spine analysis to age fish

- Bullhead migratory patterns (are they resident or do they migrate)

- Standardization of fish tumor and deformity criteria

- Development of a list of causes for fish tumors and other deformities

- Comprise a chart that depicts the various tumor types

- Investigate the possibility of a central data repository for Great Lakes Areas of Concern

- Establish task forces and committees to ensure that all concerns in regard to fish tumors and other deformities are addressed and to investigate funding for the assessment of beneficial-use impairments in Areas of Concern

Presentations – Keynote Address

Kelly Burch

Mr. Burch began the address by thanking Pennsylvania Sea Grant and the U.S Environmental Protection Agency for providing the funding for the Fish Tumor Conference. He then provided an overview of the history of Presque Isle Bay, as it relates to fish tumors. Presque Isle Bay was designated as an Area of Concern in 1991, representing the last Area of Concern to be listed. Out of the 43 Areas of Concern, Presque Isle Bay is the only to be listed because of community involvement as opposed to government recommendation, and was designated because of the presence of tumor-containing fish and contaminated sediment.

Following Presque Isle Bay's new designation, a Remedial Action Plan was developed to restore and manage the health of the bay. In 1992, a tumor study was conducted in Presque Isle Bay and the results determined that: 64% of bullheads contained external tumors and 22% contained liver tumors. In 1992 and 1993, bullheads were tagged to determine migratory behavior, population estimates, and identification for recapture. The Department of Environmental Protection determined that in order to eliminate the problem facing the health of Presque Isle Bay, both point and nonpoint sources of pollution needed to be eliminated prior to any consideration of dredging activity. Since its designation as an Area of Concern, more than $100 million has been spent building more efficient sewage treatment facilities and eliminating 60-65 sewage overflows, which affected Presque Isle Bay.

One of the concerns facing researchers involved with restoring Presque Isle Bay was the development of a uniform protocol for determining the age of effected bullheads; use of otoliths versus the use spines? In order to restore and manage a designated Area of Concern, goals and targets need to be set for each beneficial-use impairment at hand. Several of the Canadian Areas of Concerns have developed such protocols, and the American Areas of Concern are now beginning to adopt the use of goals and targets.

Mr. Burch concluded the keynote address with several quotes, including: "if it cannot be measured it cannot be managed," "Great Programs, Great People, Great Lakes," and "Thanks for making the Lakes Great!"

Presentations – Session I: Historical Overview

Dr. John Harshbarger - Overview of Fish Tumor History and Epidemology

Dr. Harshbarger opened his presentation by clarifying the following terms: tumor, neoplasm, toxin, and hyperplasia. Tumor and neoplasm are interchangeable in current medical usage. A tumor or neoplasm, is a heritably altered (mutated), relatively independent (autonomous), relatively atypical (dysplastic) growth of tissue of no use and often detrimental to the host. In other words a neoplasm is a population of abnormal cells that continue to proliferate after mutation is no longer present. Tumors that are growing by simple expansion are often benign while tumors that are invading and destroying host tissue are cancers. Since cancers can arise in benign tumors one should not become complacent about benign tumors. Causes of the heritable abnormality include certain chemicals, ionizing radiation, ultraviolet radiation, and certain viruses. There is no minimum threshold level for the oncogenic mutagen.

Toxin is derived from the Latin word *toxicum* meaning poison. Poison kills cells via the production of free radicals that interfere with intracellular mechanisms: thus, toxin causes the cessation of cellular-proliferation in contrast to neoplastic transformation, which enhances cellular proliferation. Toxins have a minimum threshold.

Hyperplasia is the unscheduled proliferation of normal cells and is often accompanied by organ hypertrophy. Examples include: 1) Kidney donors have compensatory hyperplasia and hypertrophy of the retained kidney; 2) Overeaters have nutritional hyperplasia of adipose tissue to store the excess calories; 3) Hypertension induces functional hyperplasia and hypertrophy of cardiac muscle; 4) Sunburn releases toxic free radicals leading to regenerative hyperplasia to replace the dead skin cells; 5) Iodine deficiency biofeedback causes endocrine hyperplasia of the thyroid tissue with goiter formation.

Following the clarification of these terms, Dr. Harshbarger briefly outlined the history of the role of carcinogens in tumors and milestones in the use of fish environmental sentinels.

- 1775: Sir Percival Potts reported that boys used as chimney sweeps developed scrotal cancer.
- 1850: Fish neoplasms first documented in North America.
- 1900: The carcinogenicity of coal tar (chimney soot) was experimentally confirmed.
- 1930: Benzo(a)pyrene was the first pure carcinogen isolated from coal tar.
- 1940: Skin papillomas were discovered on brown bullheads in industrially polluted Delaware and Schuylkill Rivers in Philadelphia.
- 1956: Evidence that a herpesvirus can cause cancer was discovered in northern leopard frogs.
- 1957: Oral papillomas were found on the lips of white croakers feeding at a California sewage outfall while white croakers feeding in relatively pristine water were tumor free.

- 1962: The carcinogenicity of aflatoxin, a common, potent human carcinogen produced by fungi, was discovered when hatchery rainbow trout developed panzootic liver cancer following the global introduction of mold contaminated pelleted trout chow.
- 1963: White suckers in a polluted waterway had oral papilloma and liver cancer.
- 1964: Zebrafish were exposed to diethylnitrosamine in the first experimental carcinogen study with small fish.
- 1965: The Registry of Tumors in Lower Animals was started.
- 1970: The 1940's report of skin cancer was confirmed and liver cancer was discovered in brown bullhead catfish sampled every 10 miles in the Delaware River between Trenton, NJ and Philadelphia, PA.
- 1972: Neoplasms reported in several fish species in the polluted Fox River west of Chicago compared to almost none in the same species from pristine Canadian lakes.
- 1977: Liver cancer reported in English sole in a polluted tributary of Puget Sound.
- 1978: Tomcod liver cancer discovered in lower Hudson River, NY heavily polluted by PCB's and PAH's.
- 1979: Paul Baumann found skin and liver cancer in brown bullheads at a coking plant outfall in the Black River, Ohio.
- 1981: Neoplasms reported in fish species from the Buffalo River, Buffalo NY.
- 1981: Liver cancer reported in sauger and walleye from Torch Lake MI contaminated by copper mine tailings and chemicals used to extract copper.
- 1982: CNN ran a series of reports on fish tumors associated with chemicals dumped into Torch Lake (Michigan), the coking plant on the Black River and the lower Hudson R. There was a huge unexpected worldwide response.
- 1983: Congressional hearing held in concern of fish cancer prevalence where human cancer was also high.
- 1985: Winter flounder liver cancer reported from Deer Island sewage outfall, Boston Harbor.
- 1985: Skin painting of extracts of sediment from Black River, OH and Buffalo River, NY produced cancer on brown bullheads and mice.
- 1987: Bowfin liver cancer reported from Detroit River, MI.
- 1987: White perch liver tumors reported from Chesapeake Bay.
- 1988: Oyster toadfish pancreas and liver cancer reported from York River, VA near a refinery.
- 1988: Oral papillomas and liver neoplasms in white sucker reported from polluted sites on Lake Ontario
- 1990: Mummichog liver cancer reported from creosote polluted Elizabeth River, VA.
- 1991: Experimental trophic transfer of carcinogens to winter flounder fed contaminated blue musssels
- 1991: Brown bullhead liver cancer reported in Cuyahoga River, Cleveland, OH.
- 1995: Brown bullhead liver cancer in Black River, OH drops sharply after coking plant closes and PAH's plummet.
- 1995: Oral papilloma reported in white sucker from St Lawrence River, PQ Canada
- 1998: Lake whitefish liver cancer reported in St Lawrence River, PQ Canada.
- 2001: Brown bullhead liver and skin cancer reported in the Potomac River, and Anacostia River

This incomplete list shows the importance of fish liver and skin neoplasms as sentinels for environmental carcinogens. Dr. Harshbarger discussed epizootic tumors in other organ systems as well including the hematopoietic system, pigment and nerve cell neoplasm, excretory system, etc., according to the predominate species and site of their occurrences. The number of epizootic fish, amphibian, reptile, and mollusk neoplasms has increased steadily from a combined total of 18 in 1954 to 145 in 1994.

Several examples were given linking pollutants and chemical contaminants to tumors and deformities in fish species associated with affected environments. These included mid-western frogs with polydactyly (Fig 1) and sea lampreys in the Great Lakes and tributaries with teratoid anomalies (Fig 2). In Orange County, California, oral papillomas in white suckers near the sewage outfall (Fig 3) declined to zero following the renovation of the sewage facilities.

Dr. Harshbarger was an expert witness at a trial concerning Millstone nuclear power plant in Connecticut and its involvement in the massive discharge of carcinogenic compounds into the surrounding aquatic ecosystem. Nuclear power plant operators add huge volumes of chemical oxygen scavengers such as hydrazine to the cooling water to prevent internal corrosion. The principle compounds used are carcinogenic and associated with carcinogenic contaminants; therefore, the large number of nuclear power plants around the great lakes are a likely source of environmental carcinogens and fish in the vicinity of the discharges should be monitored for liver cancer.

Dr. Harshbarger was asked by the IJC to put together a report relating contaminants and fish tumor occurrence. It was his suggestion that all sources of point source pollution should be documented and the chemicals that were being put into the environment should be noted. Bioassays of fish containing tumors and deformities could be carried out to determine if the chemical causing these deformities could be linked to point source pollution. He then concluded by stating: "the companies or persons responsible for putting these harmful pollutants into the environment should be responsible for their actions, and if they were not, penalties should be administered." Selected data from that report are included here.

Dr. John Harshbarger's Power Point Presentation

Figure 1: Frog With Polydactyly

Figure 2: Histopath of a Teratoid Anomaly Found in Sea Lampreys

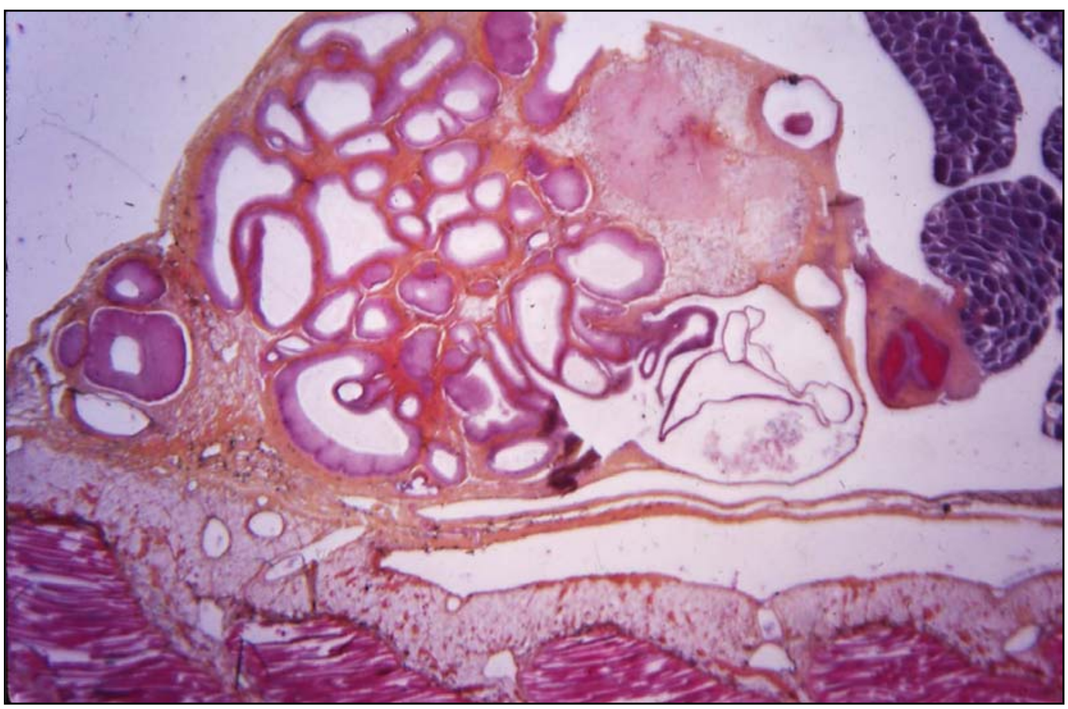

Figure 3: Oral Papillomas on a White Sucker

Toward a Transboundary Monitoring Network:
A Continual Binational Exploration

Vol. 2

Proceedings of a Workshop Convened by the International Joint Commission, U.S.A. and Canada

And

Relata Assembled During the Editing Process

Bruce L. Bandurski, Peter T. Haug, and Andrew L. Hamilton
Editors

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Table II
Fish Used in Experimental Carcinogenesis

Atheriniformes	
Atherinidae	
Menidia beryllina	Inland silverside
Cyprinodontiformes	
Aplocheilidae	
Rivulus marmoratus	Rivulus
Cyprinodontidae	
Cyprinodon variegatus	Sheepshead minnow
Fundulus grandis	Gulf killifish
Oryziidae	
Oryzias latipes	Medaka
Poeciliidae	
Poecilia formosa	Amazon molly
Poecilia reticulata	Guppy
Poeciliopsis lucida	Topminnow
Poeciliopsis monica	Topminnow
Xiphophorus helleri	Green swordtail
Xiphophorus maculatus	Southern platyfish
Xiphophorus montezumae	--
Xiphophorus xiphidium	--
Xiphophorus variatus	Variable platyfish
Cypriniformes	
Cyprinidae	
Danio rerio	Zebra danio
Pimephales promelas	Fathead minnow
Rhodeus aceletus	Bitterling
Gasterosteiformes	
Gasterosteidae	
Gasterosteus aculeatus	Threespine stickleback
Salmoniformes	
Salmonidae	
Oncorhynchus nerka	Sockeye salmon
Salmo gairdneri	Rainbow trout
Salmo trutta	Brown trout
Salvelinus fontinalis	Brook trout
Siluriformes	
Ictaluridae	
Ictalurus nebulosus	Brown bullhead
Ictalurus punctatus	Channel catfish

Table III
Chemicals Used in Experimental Fish Carcinogenesis

Aflatoxins	
AFB$_1$	Aflatoxin B$_1$
AFG$_1$	Aflatoxin G$_1$
AFM$_1$	Aflatoxin M$_1$
AFQ$_1$	Aflatoxin Q$_1$
AFL	Aflatoxicol
AFL^1	Aflatoxicol
Sterigma	Sterigmatocystin
VCN	Versiculorin A
Aromatic Amines	
AAT	O-aminoazotoluene
DAB	4-dimethylaminoazobenzene
AAF	2-acetylaminofluorene
Nitrosamines	
DMN	Dimethylnitrosamine
DEN	Diethylnitrosamine
DNNM	2,6-dimethylnitrosomorpholine
DNP	N-dinitrosopiperazine
NM	N-nitrosomorpholine
NPYR	N-nitrosopyrrolidine
MNU	N-methyl-N-nitrosourea
MNNG	N-methyl-N'-nitro-N-nitrosoguanidine
Polycyclic Aromatic Hydrocarbons	
Anthracene	Anthracene
BP	Benzo[a]pyrene
DMBA	7,12-dimethylbenz[a]anthracene
MC	3-methylcholanthrene
Others	
EMS	Ethyl methanesulfonate
MAMA	Methylazoxymethanol acetate
BRKN	Bracken
CPFA	Cyclopropanol fatty acids
	Sterculic acid
	Malvalic acid
DDT	Dichlorodiphenyltrichloroethane
DEPH	Di-2-ethylhexyl phthalate
EDB	Ethylene dibromide (1,2-dibromoethane)
NP	Nifurpirinol

Relevance of Fish Cancer to Human Cancer

Common Basis
Many human cancers are believed to be due to altered activity of 20 or so host growth factor genes or oncogenes. Data has been published for similar oncogenes in fish and in various invertebrates down to the primitive level of sponges. Thus, many types of cancer appear to have a common basis throughout phylogeny.

Common Metabolism
Most carcinogens act indirectly, that is, they are not carcinogenic themselves, but when they are metabolized for excretion, usually by the liver, reactive, proximate, carcinogenic intermediate compounds are created. Fish utilize metabolic pathways similar to mammals in the process.

Common Results
Experimentally, mammalian carcinogens are also carcinogenic for fish and the liver is the primary target organ for most chemicals in both cases.

Carcinogen Bioassay

Pure test material can be microinjected directly into Mt. Shasta strain rainbow trout ova at a rate of 200 ova/hr/person. This combines a well-known sensitive fish having a 20-year record of carcinogenecitity studies with the most sensitive stage (embryo). It uses the least amount of chemical in a closed route of exposure for maximum safety and minimum by-product for disposal. In lieu of injection, ova can be bathed in the test chemical for 15 minutes. Liver tumors begin appearing in three months and 12 months is the usual post exposure period.

Also, the ova bathing exposure can be utilized with small fish species that appear especially suitable for carcinogen bioassay. Medaka appears to be the best small fish species for bioassay, but several others are also promising, including guppy, rivulus, platyfish/swordtail hybrid, zebra danio, topminnow and Amazon molly. Small fish have the advantage that a sagittail section of the entire fish will fit on a single micro slide for expeditious examination of all tissues. A second advantage is that liver tumors begin appearing in seven weeks; therefore, six months is a suitable post treatment period.

Advantages of Fish Bioassay

(1) Miniscule amount of test chemical for safer handling and disposal.

(2) High sensitivity, equivalent or better than rodents and significantly, this based on a single short exposure.

(3) Six months to one-year experimental period versus two years for rodents.

(4) All or none response. No tumor in controls, as often happens in rodent experiments requiring statistical evaluation to significant difference.

(5) Cost: Approximately $20,000/test versus $500,000 to $1,500,000 for rodents.

(6) No sentimental lobby groups protesting cruelty to fish.

(7) Useful to test carcinogenicity of chemical mixtures in concentrated effluent, sediment extracts and extracts of smokestack filtrate. Can also be used to bioassay bile extracts of wild fish and liver equivalents of wild invertebrates for carcinogenic reactive metabolic intermediates.

(8) Fish are real world organisms, i.e., they are part of the natural ecology rather than being inbred laboratory animals.

Conclusions

One necessary step to clean up the environment is to eliminate the input of noxious chemicals. Eventually, as shown by declining DDT levels in the Great Lakes, microbial and other types of degradation will gradually reduce residues. The only way to stop input of noxious chemicals into the environment is to register every outfall and smokestack, test the output regularly for noxious chemicals, penalize owners for non-compliance and make owners fully responsible for resulting detrimental effects.

It is proposed that chronic fish bioassays of effluent are an efficacious method to detect carcinogens and teratogens in outfall effluent concentrates and in smokestack filtrates. Rodent bioassays of effluent are too costly and time consuming for broad chronic carcinogenic screening, but positive results of fish bioassay could be funneled to rodent tests for corroboration if fish results were challenged (so far rodents have been little used for bioassays of chemical mixtures). In addition to testing effluent, the chronic fish bioassay is useful in: (1) testing bile from wild fish or extracts from liver equivalents of wild invertebrates to determine presence of carcinogens in water ways and (2) testing new or untested existing chemicals to prevent or eliminate exposures to unsuspected carcinogens in common usage.

Dr. John Gannon – Fish Tumor Listing/ Delisting Criteria

Dr. Gannon began his presentation by discussing how the problems facing the health of fish species arose and what needs to be done to restore fish health. The appearance of fish tumors and other deformities are believed to have appeared with the onset of the industrial revolution. In order to restore fish health, sources of pollution must be eliminated and aquatic habitats must be restored through human intervention (i.e. dredging and excavation) and/or natural recovery.

Dr. Gannon followed the fish health issues by outlining the history of the binational management policy. In 1972, the Great Lakes Water Quality Agreement (GLWQA) was developed in order to decrease phosphorus concentrations in the hope of preventing eutrophication. The GLWQA was revised in 1978 to include toxic substances. Areas containing toxic substances were separated into two classifications: Class A – severely polluted and Class B – moderately polluted. A protocol was designed in 1987, which included the designation of Areas of Concern and the development of Remedial Action Plans in order to restore these areas.

In 1988, listing and delisting criteria were developed in order to restore the 43 Areas of Concern (currently there are 42 Areas of Concern – Collingwood Harbor in Canada has been delisted). These criteria are known as beneficial-use impairments and are related to both human activity and ecosystem impacts. There are currently 14 beneficial-use impairments:

1) Restrictions on drinking water consumption, or taste and odor problems
2) Beach closings
3) Degradation of aesthetics
4) Added costs to agriculture of industry
5) Restrictions on fish and wildlife consumption
6) Tainting of fish and wildlife flavor
7) Restrictions on dredging
8) Eutrophication or undesirable algae
9) Degradation of phytoplankton and zooplankton populations
10) Degradation of benthos
11) Degradation of fish and wildlife populations
12) Loss of fish and wildlife habitat
13) Bird or animal deformities or reproduction problems
14) Fish tumors or other deformities

Dr. Gannon went into detail discussing the beneficial-use impairment: fish tumors or other deformities. When bullhead liver tumors exceed 2% or 3.5% in suckers, it is listed as a beneficial-use impairment, and when rates drop below these levels it is delisted. Dr. Gannon proposed the question, “does this value need to be updated to reflect current trends or new scientific evidence?”

Dr. Gannon concluded his presentation by proposing challenges and opportunities that need to be addressed as they relate to the science linkage and science-management linkage.

Science linkage

- Cause and effect links between fish tumors and environmental contaminants
- Population and ecosystem response to remediation (i.e. changes in biodiversity)
- Habitat creation, restoration, and protection of soft sediments or "soft engineering" of hard substrate (alternatives to rock or steel rip-rap)

Science-Management linkage

- Monitoring, assessment and evaluation component
- Guidance on Area of Concern fish tumor abnormality studies based on case studies in the Black River and other areas
- Refine listing/delisting criteria for fish tumors

Dr. John Gannon's Power Point Presentation

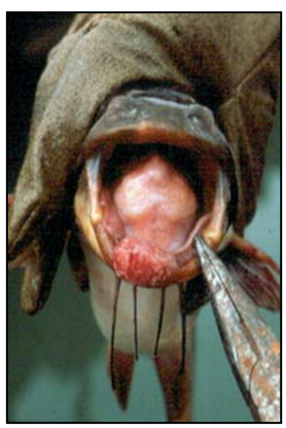
The Fish Tumor Listing / Delisting Criterion

Its History and Prognosis for the Future in Linking Science and Management in the Great Lakes Areas of Concern

By Dr. John Gannon
Great Lakes Regional Office
International Joint Commission
Windsor, Ontario

Fish Health Problems:

Just One Expression of the Loss of Ecosystem Integrity



How Did We Get Into This Mess?

How Do We Get Out of This Mess?

- Eliminate Sources of Pollution
- Remediate and Restore Habitat Through
  - Human Intervention
  - Natural Recovery

History of Binational Resource Management Policy Response

1972 – Great Lakes Water Quality Agreement (GLWQA): Focus on Eutrophication of Phosphorus Control.

1978 – GLWQA: emphasis on Toxic Substances
- Class A Areas of Concern (Severely polluted)
- Class B Areas of Concern (Moderately polluted)

1987 – GLWQA Revision by Protocol
- 42 Areas of Concern
- The "How Clean is Clean?" Debate
- Evolution of Listing/De-listing Criteria

2001 – Recognition of "Area of Recovery"

14 Beneficial Use Impairments

- Restrictions on drinking water consumption, or taste and odor problems
- Beach closings
- Degradation of aesthetics
- Added cost to agriculture or industry
- Restrictions on fish and wildlife consumption
- Tainting of fish and wildlife flavor
- Restrictions on Dredging
- Eutrophication or undesirable algae
- Degradation of phytoplankton and zooplankton populations
- Degradation of benthos
- Degradation of fish and wildlife populations
- Loss of fish and wildlife habitat
- Bird or animal deformities or reproduction problems
- Fish tumors or other deformities

Fish Tumors or Other Deformities

Listing Criteria

When the incidence of neoplastic or pre-neoplastic liver tumors exceeds 2% in bullheads or 3.5% in suckers.

De-Listing Criteria

When the incidence of neoplastic or pre-neoplastic liver tumors in bottom-dwelling fishes does not exceed 2% in bullheads or 3.5% in suckers.

Dr. Paul Baumann - History Lessons: Two Decades of Field Data and Its Ability to Assess Changes in Fish Pathology in the Black River

Since tumor prevalence is a good measure of whether an ecosystem is improving or not, there is a need to match historic data with more recent data to establish trends. While historic databases have much valuable information, there are also inconsistencies in what data were often recorded, and these need to be recognized. When analyzing bullheads from ages 3 (age of maturation) and up, several assumptions were often made:

1. Tumor rates are independent of age, or variation is stable across sample years and sample sites.

2. Hepatic and biliary tumors have the same trends or causes.

3. Presence and absence of the most advanced lesions (cancers or at least neoplasms) is the best measure for liver pathology.

Studies associated with the Black River, Ohio have shown that these assumptions may be incorrect. A comparison between age 3 and age 4 fish in a 1992 study showed that age 4 fish possessed higher rates of hepatocellular and cholangiocellular carcinomas and higher rates of hepatocellular neoplasms, indicating that the tumor rates are dependent on age. Also the ratio of neoplasms to cancers is about equal in age 3 and 4 fish for hepatocellular (liver) tumors, but not for cholangiocellular (bile duct) tumors. Thus these types of tumors have different patterns and may have different causes.

A comparison between fish containing various types of lesions may cause information to be lost on trends and causes. For example: fish A has hepatic cancer, biliary cancer, and hepatic neoplasms, fish B only has biliary cancer, and fish C has hepatic neoplasms, biliary neoplasms, and hepatic altered foci. In many data sets, fish A and fish B would be classified equally as having cancer, and fish C would be classified as having a neoplasm. Obviously this represents only a fraction of the information available.

A series of studies of the Black River spanning the period from 1980 to 1990 provides an example of how a historic database can be used to look at trends in fish health in an Area of Concern. The earliest studies (1980 and 1981) only collected grossly visible liver tumors for histopathology. In 1982, 1987, 1992, 1994, and 1998 all livers (regardless of the presence of gross lesions) were collected for histopathology. Results from the studies in which all livers were examined revealed that examining only grossly visible lesions underestimated the actual tumor prevalence.

After the coking plant, the principal point source for carcinogenic PAH, closed in 1983, the prevalence of liver cancer declined over the next four years as PAH levels in surface sediment declined. Then the contaminated portion of the river received remedial dredging in 1990. This dredging, done with an open clam shell dredge and a barge, re-suspended heavily contaminated sediments which had been buried, resulting in a much higher liver cancer prevalence two and three years later. However by eight years after the dredging the cancer prevalence of mature fish had declined to about 7%.

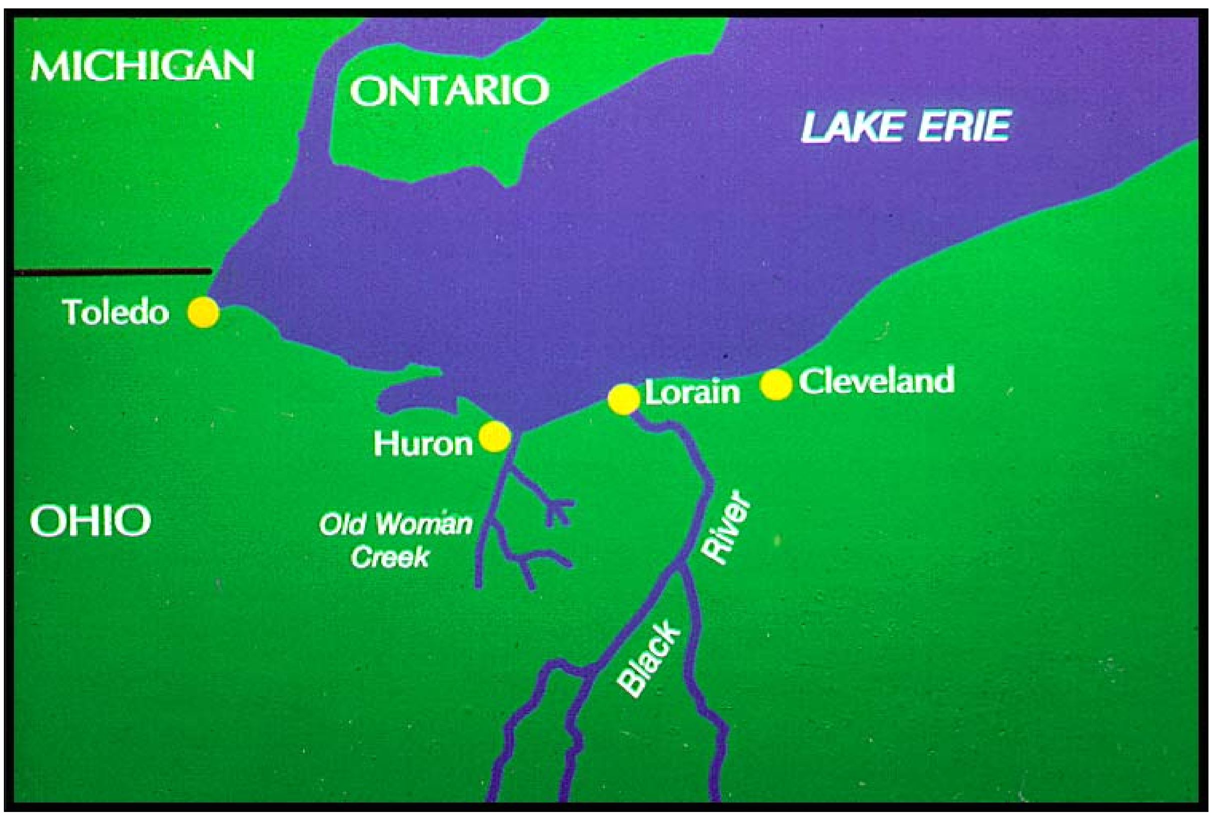
We can get a better feeling for cause and effect by looking at age-specific fish around the time of the dredging. Fish that were age one at the time of the dredging and captured at age 3 (having grown for two years after exposure) had a 27% liver cancer prevalence and a 19% neoplasm prevalence. Fish that were young-of-the-year when the dredging occurred and were captured at age 3 (having grown for three years after exposure) had an even higher cancer prevalence of 41% and a neoplasms prevalence of 20%. In sharp contrast, fish hatched a year after the dredging and captured at age 3 had no cancer or neoplasms, only a 15% prevalence of altered foci.

Similarly, a smaller sampling targeting age 3 fish in 1994 allows us to use age-specific comparisons to look at fish health recovery following the closure of the coke plant and the completion of the dredging. Fish hatched the year after the coke plant closed and the year following the completion of the dredging were both captured at age 3. The total cancer and neoplasm prevalence of the fish hatched following the coke plant closure is about 20% while that of the fish following the end of the dredging is zero. Similarly, the prevalence of foci of cellular alteration is much higher in the fish hatched after the coke plant closure. This would argue that these fish were still being exposed to higher PAH levels than were those hatched after the dredging.

Dr. Paul Baumann's Power Point Presentation

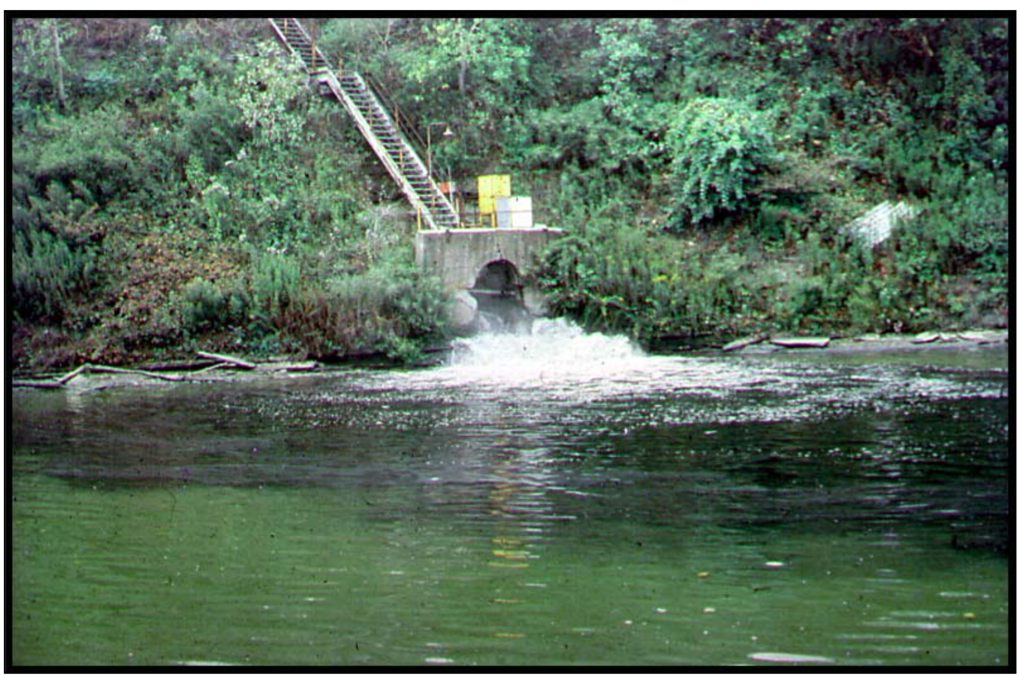
History Lessons:

Two Decades of Field Data
And its' Ability to Assess
Changes in Fish Pathology
In the Black River



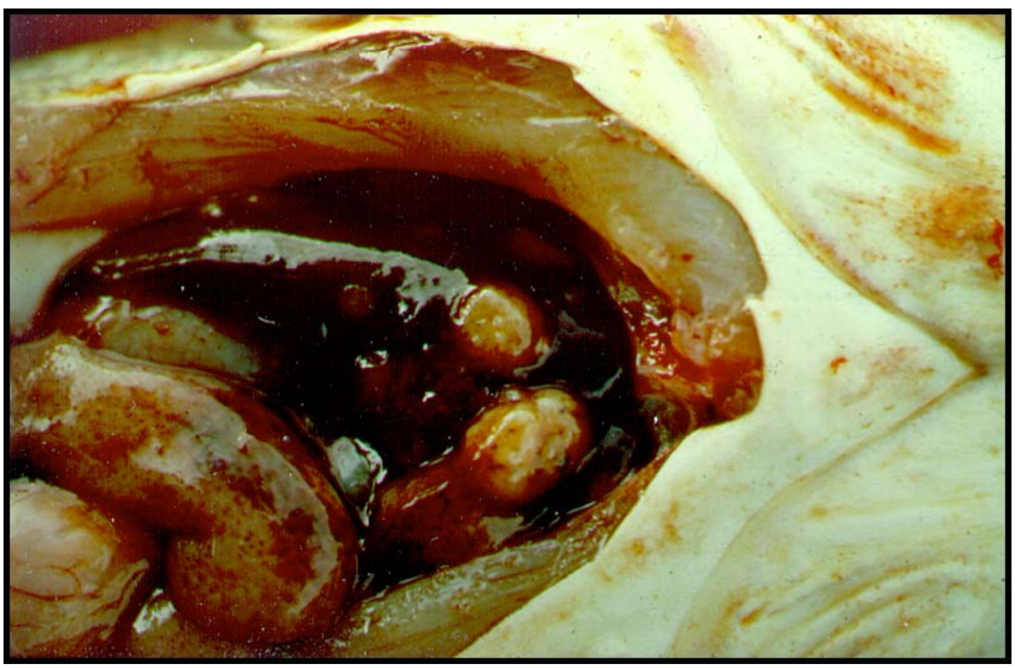
USX Facility

Coke Plant Outfall



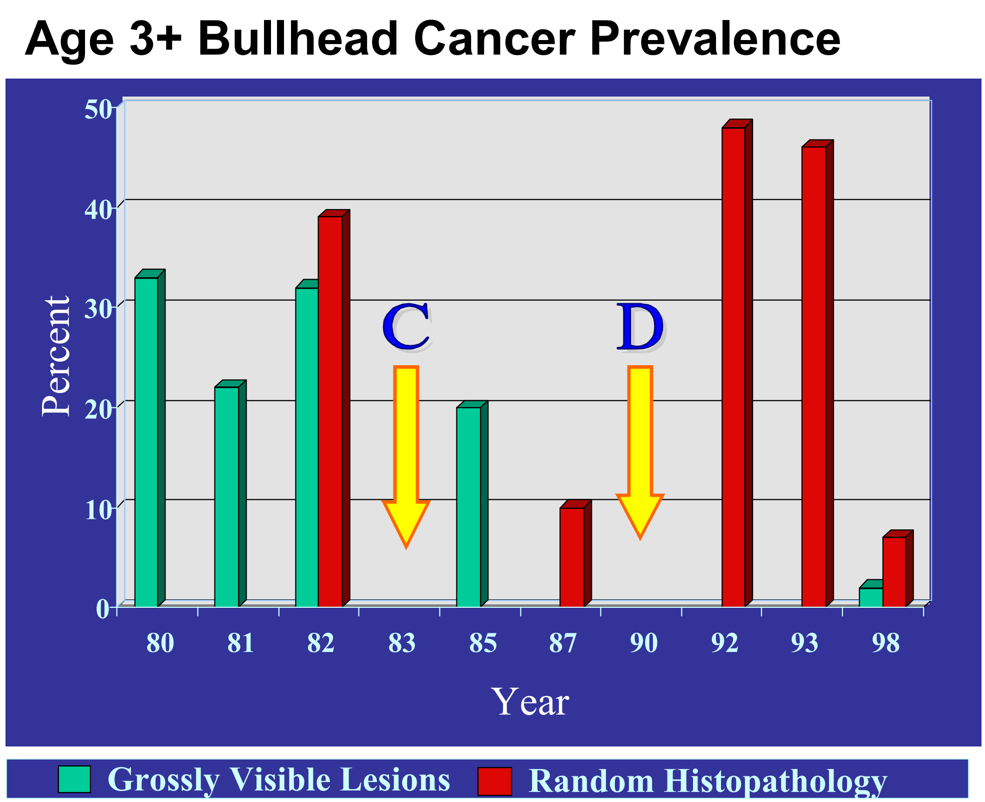
External Abnormalities and Tumors

Internal Pathology



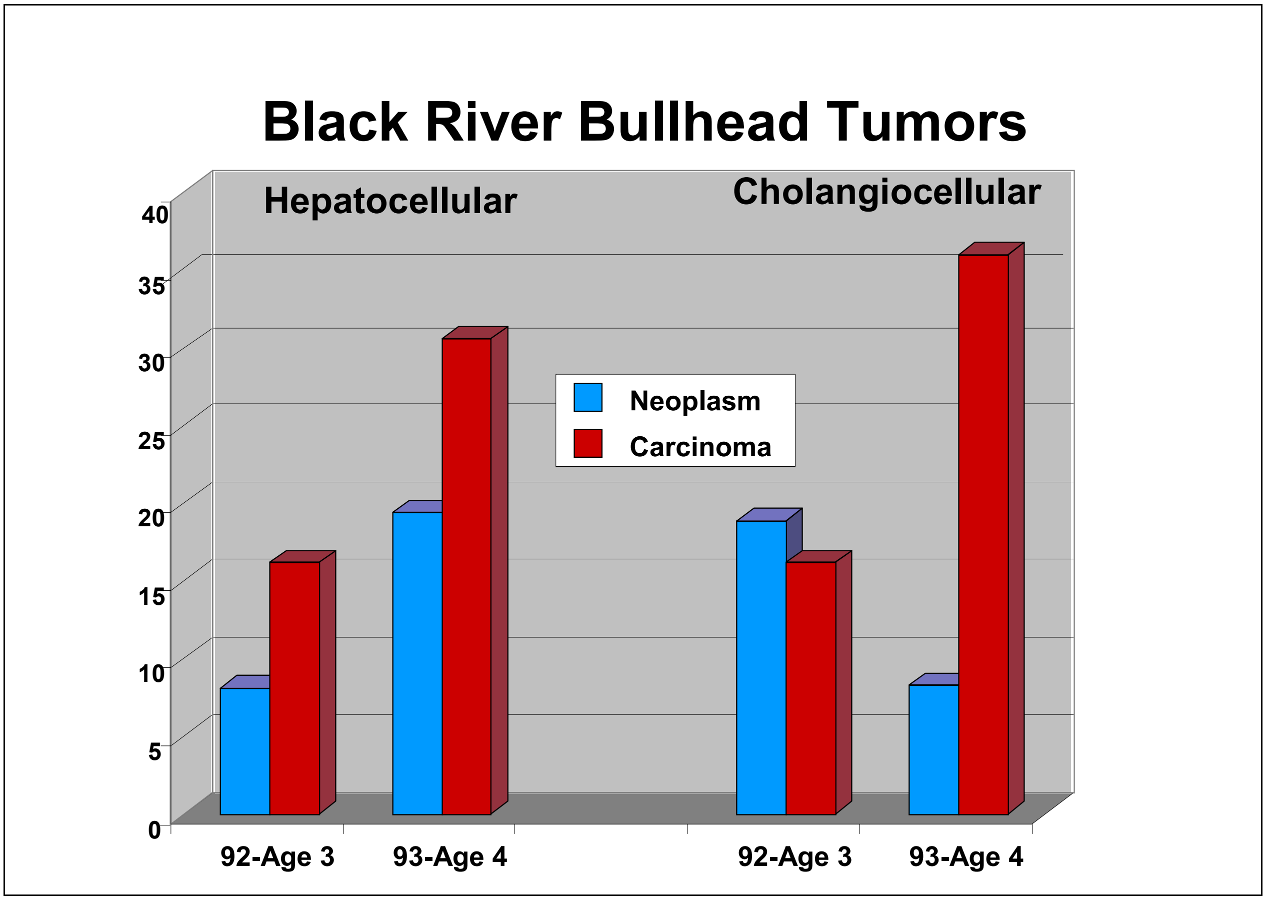
Black River Timeline

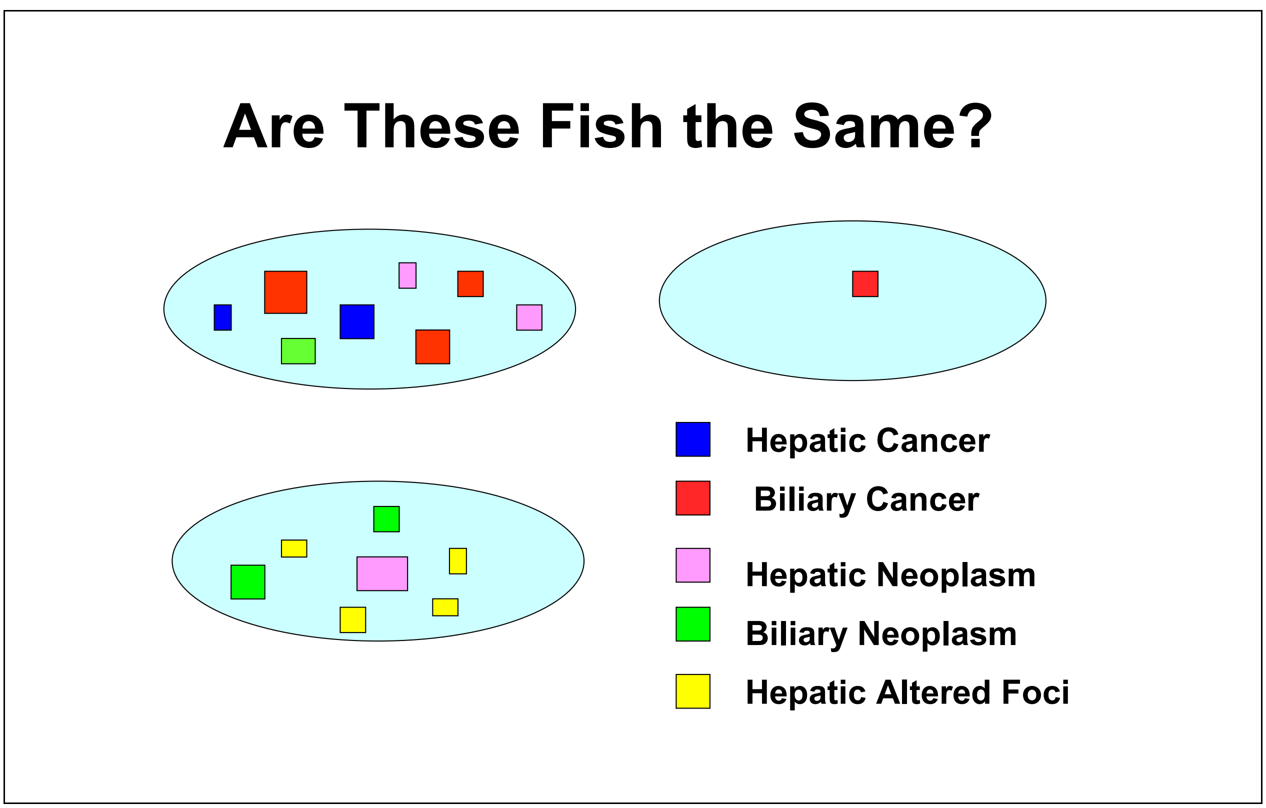
- 1980 Select (94)
- 1981 Select (175)
- 1982 All
- 1983 Coking Stops
- 1984
- 1985 Select (57)
- 1986
- 1987 All (80)
- 1990 Dredging
- 1992 All (93)
- 1993 All (99)
- 1994 All (44)
- 1995
- 1996
- 1997
- 1998 All (44)



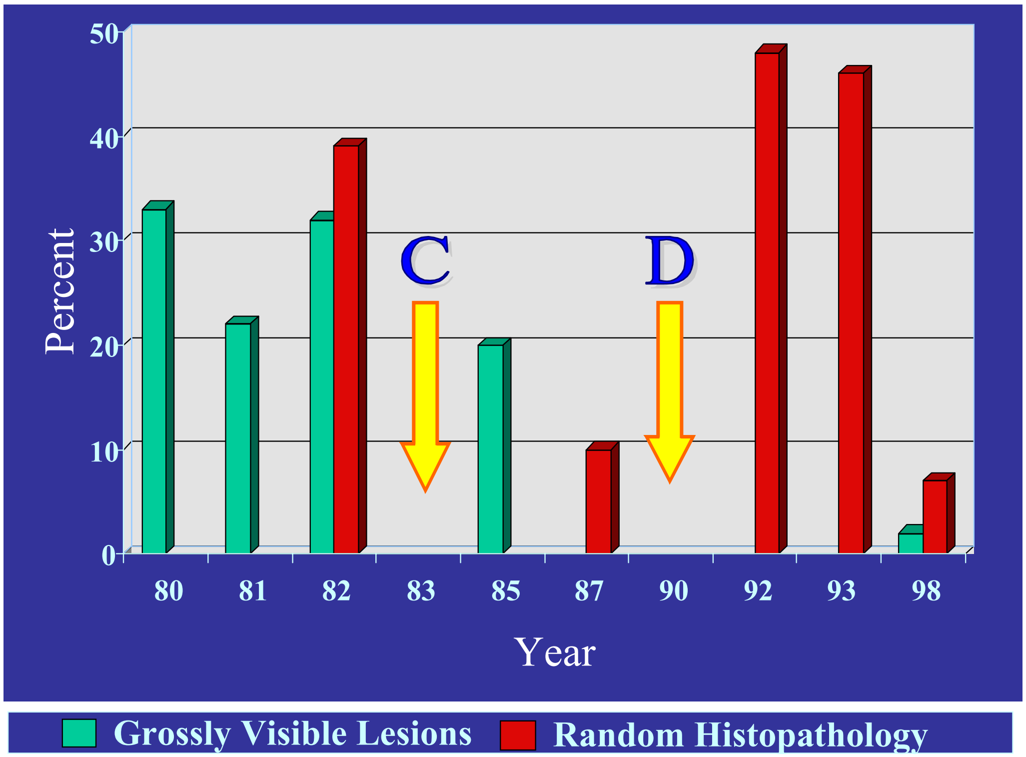
Assumptions: Age 3+ Cancer Plot

- Tumor rate is independent of age, or the age mix is stable across years or sites
- Hepatic and biliary tumors have the same trends or causes
- Presence or absence of the most advanced lesions is the best measure of liver pathology

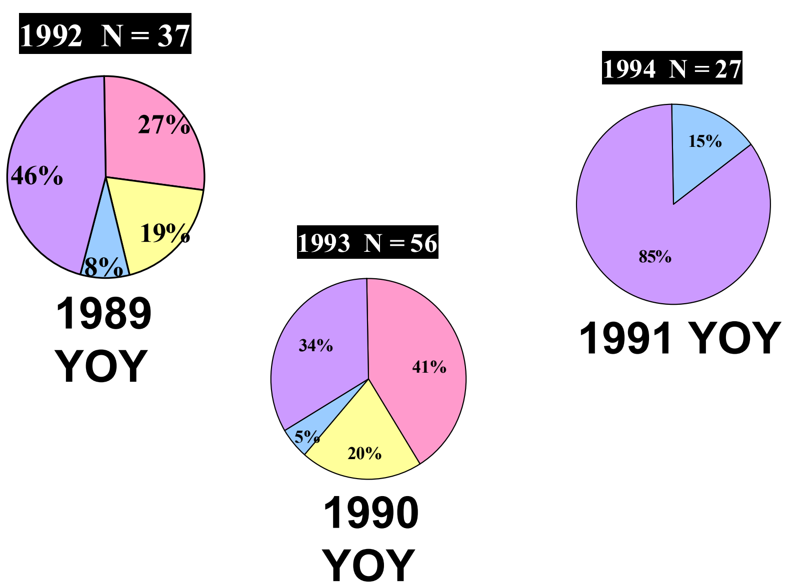

Black River Bullhead Tumors
Hepatocellular
Cholangiocellular
0
5
10
15
20
25
30
35
40
Neoplasm
Carcinoma
92-Age 3
93-Age 4
92-Age 3
93-Age 4


Are These Fish the Same?
Hepatic Cancer
Biliary Cancer
Hepatic Neoplasm
Biliary Neoplasm
Hepatic Altered Foci

Age 3+ Bullhead Cancer Prevalence



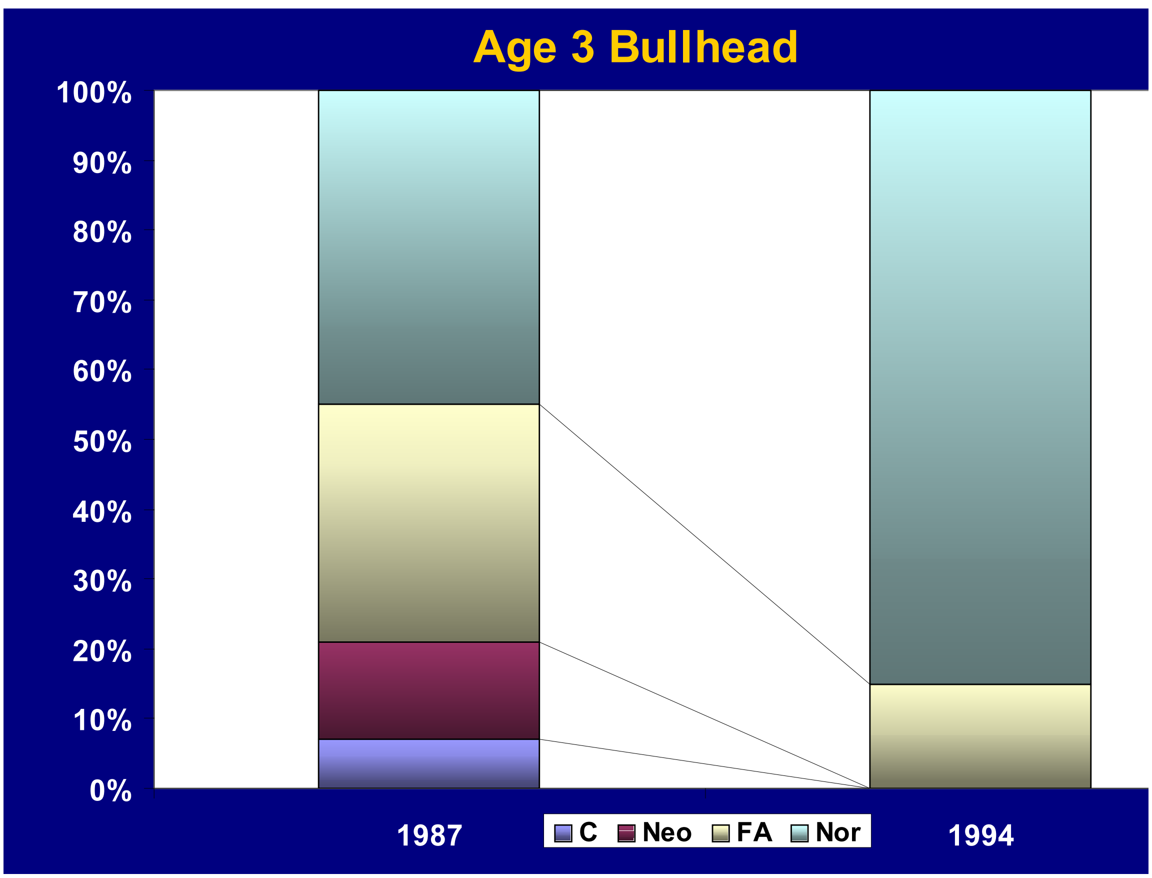
Neoplasms in Age 3 Fish

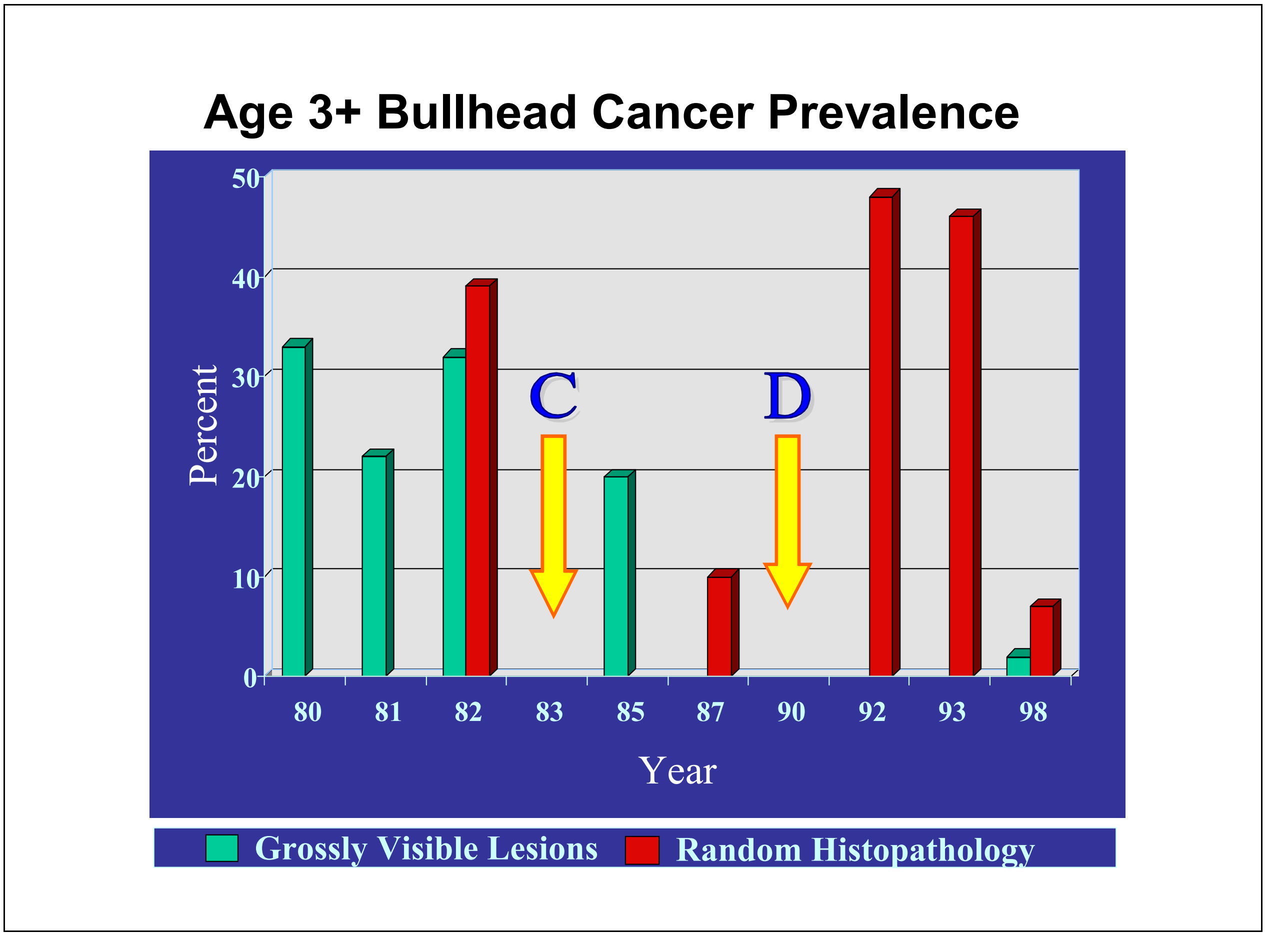


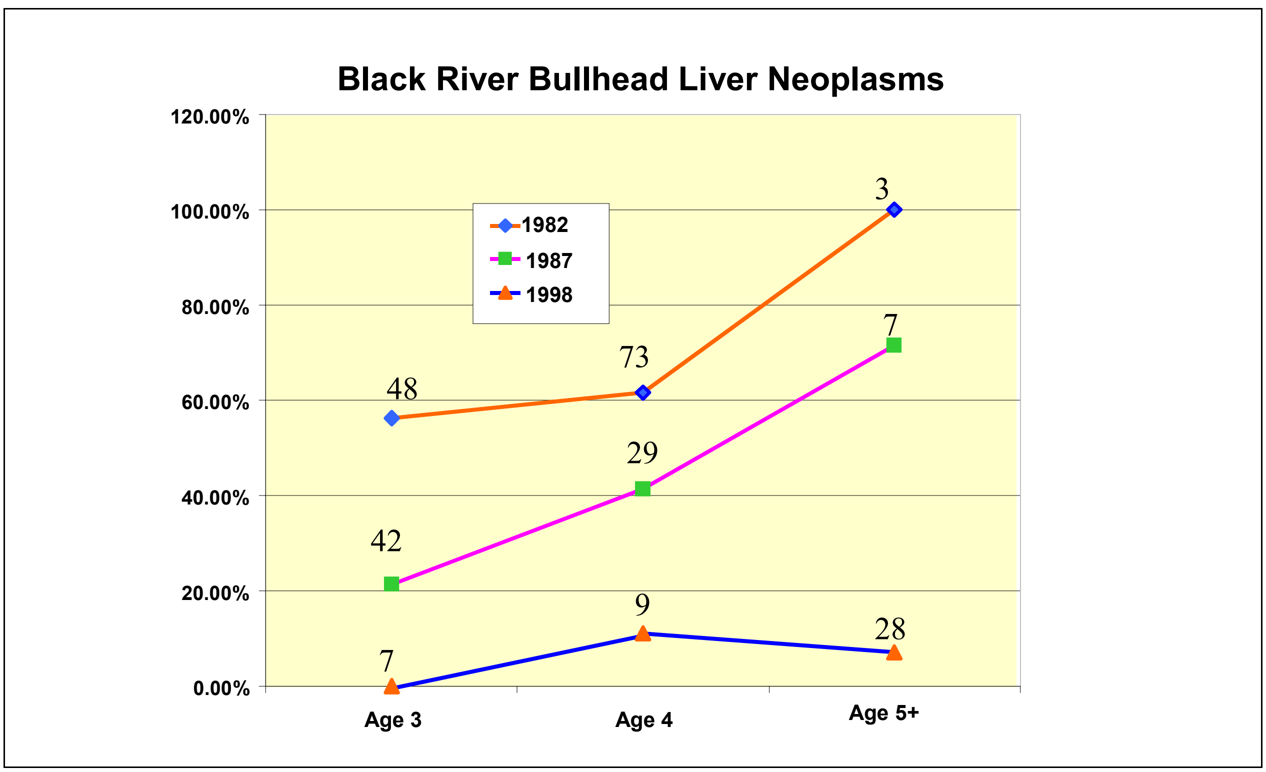
Source Closure vs. Remediation

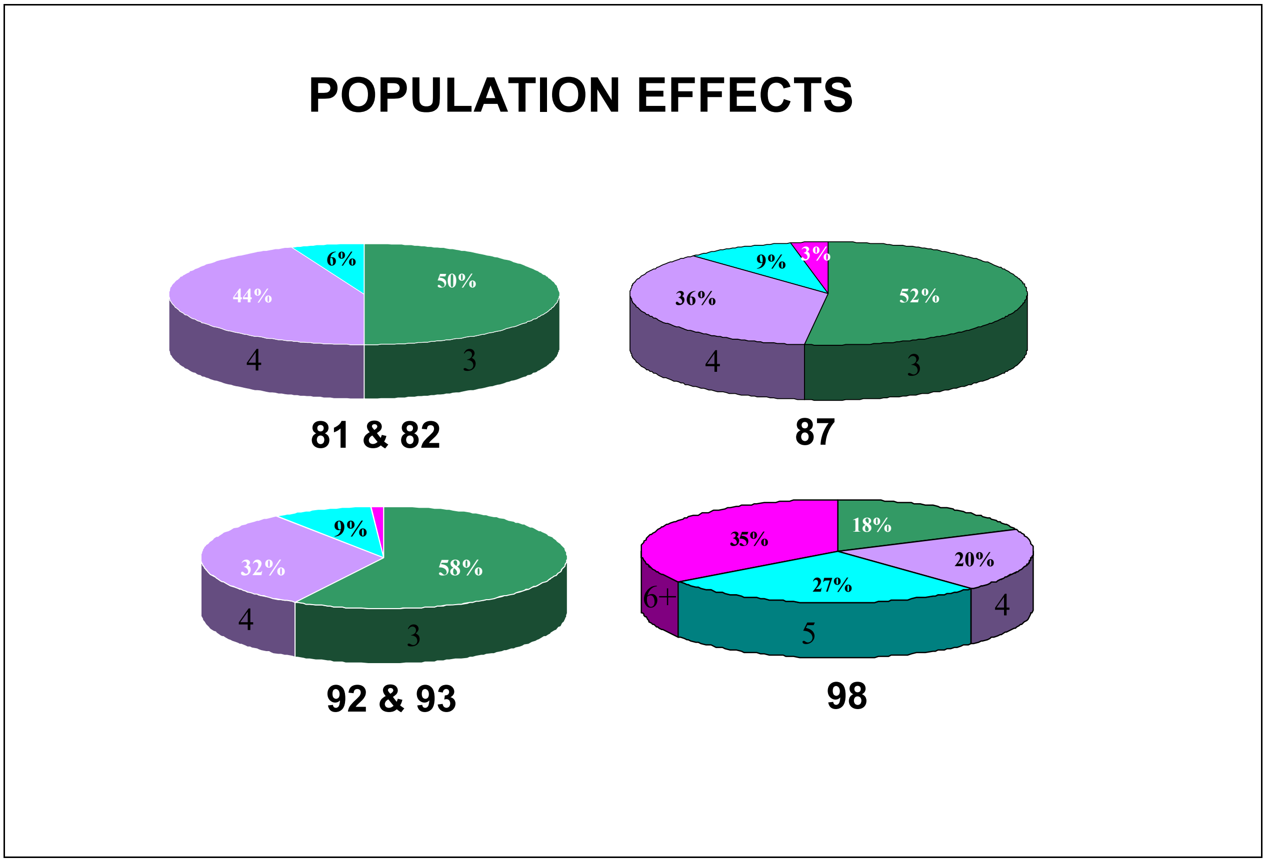
- 1983 Coking Stops
- 1984 Fish Hatch
- 1985 Fish Age 1
- 1986 Fish Age 2
- 1987 Age 3 study

- 1990 Dredging
- 1991 Fish Hatch
- 1992 Fish Age 1
- 1993 Fish Age 2
- 1994 Age 3 study




Age 3+ Bullhead Cancer Prevalence
Percent
50
40
30
20
10
0
C
D
80
81
82
83
85
87
90
92
93
98
Year
Grossly Visible Lesions
Random Histopathology


Black River Bullhead Liver Neoplasms
120.00%
100.00%
80.00%
60.00%
40.00%
20.00%
0.00%
1982
1987
1998
3
7
73
48
29
42
9
28
7
Age 3
Age 4
Age 5+


POPULATION EFFECTS
6%
44%
50%
4
3
81 & 82
9%
3%
36%
52%
4
3
87
9%
32%
58%
4
3
92 & 93
35%
18%
20%
27%
6+
4
5
98

Dr. Paul Baumann – Detroit River Tumor Data: Problems in Comparing Different Studies

Dr. Baumann discussed the possibilities of comparing fish tumor rates from different studies conducted in the same area. The three studies used were: Maccubin and Ersing 1985-87, Leadley et al. 1996, and USGS 2000. Criteria used to assess for tumor survey comparisons included: diagnostic criteria (comparable), comparable ages, comparable locations, and sufficient sample sizes.

Diagnostic differences can attribute to varying tumor results; therefore, leading to skewed results. The Maccubin and Ersing study included foci of altered hepatocytes as tumors, whereas the Leadley study did not include foci of altered hepatocytes as tumors. These differences lead to different tumor rates between the two studies.

The Maccubin and Ersing study compared 1-7 year old bullheads, the Leadley study compared 3-4 year old bullheads (ages were not accurate because length of fish was used to determine the age of fish), and the USGS study compared 3-9 year old bullheads. The percentage of fish younger than age 4 was much greater for the 85-87 survey; thus, since tumor rates increase with age, that study would be expected to have a lower "average" prevalence. In determining the age of fishes, spinal analysis allows fish between 1-4 years of age to be differentiated, but older fish are difficult to differentiate because they lose their growth rings (creating a lower age estimate). Otolith analysis is more useful across a broader range of ages because otolith rings are more distinct.

Specific sampling locations were different in the various studies. This is especially a problem for the larger Areas of Concern because of the point source discharges and other influences at specific sites sampled. Because bullheads are relatively localized, they will tend to reflect the contaminant conditions in their immediate area. If different areas are sampled by different research teams, as was the case in the Detroit River, then results will vary.

Samples sizes that are too small can also lead to results that are not comparable. In performing fieldwork it is sometimes difficult to obtain a large sample size; however, if the number of fish sampled is too small to have statistically valid results, then the sampling effort may be largely wasted.

Dr. Paul Baumann's Power Point Presentation

Detroit River Tumor Data

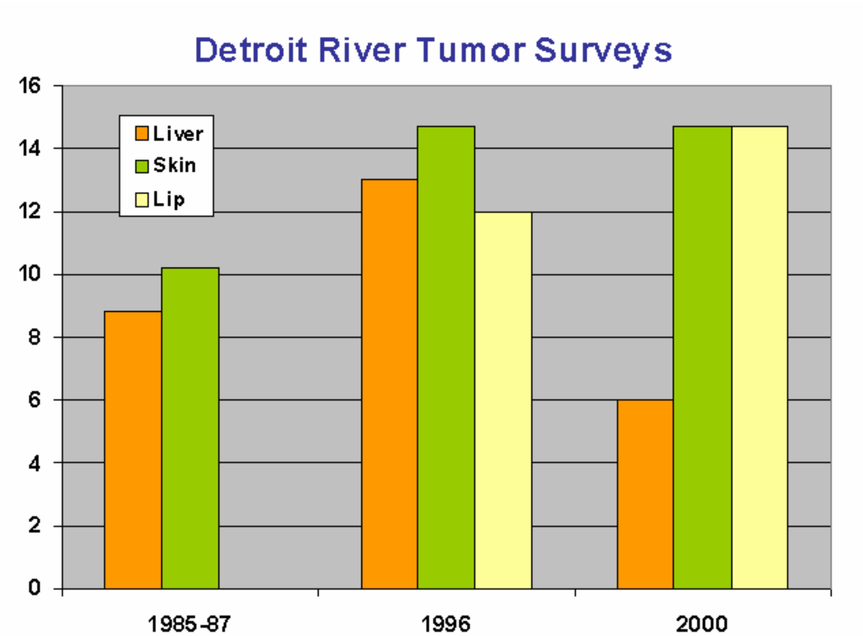
Problems in Comparing Different Studies

Detroit River Studies

- **Maccubin & Ersing (1985 – 87)**
- **N = 306 Liver**
- **N = 449 Skin**

- **Leadley et al. (1996)**
- **N = 75 Liver and Skin**

- **USGS (2000)**
- **N = 34 Liver and Skin**



Criteria for Tumor Survey Comparison

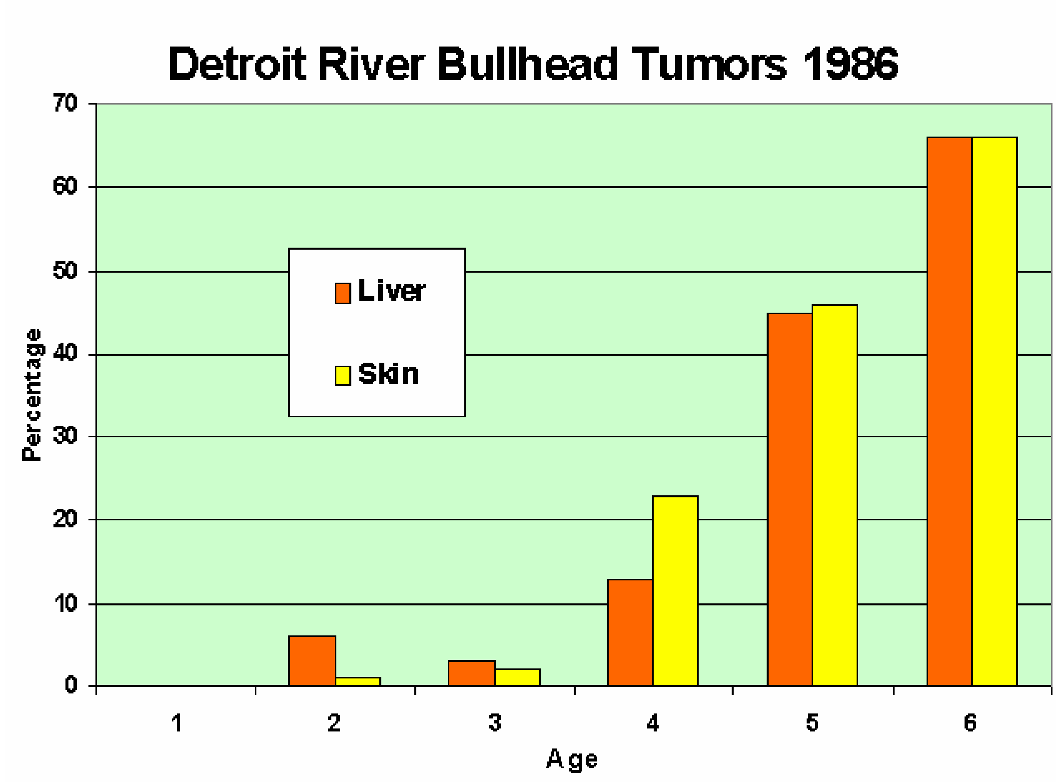
- **Are diagnostic criteria comparable**
- **Are ages sampled comparable**
- **Are locations sampled comparable**
- **Are sample sizes sufficient**

Diagnostic Differences

- **Maccubin and Ersing (1985 – 87)**
    - **Included "Foci of Altered hepatocytes" as tumors**
- **Leadley et al. (1996) and USGS (2000)**
    - **Did not include Altered Foci as tumors**

Age Distribution Sampled

- **Maccubin and Ersing (1985 – 87)**
    - **Ages 1 - 7**
- **Leadley et al. (1996)**
    - **Ages 3 – 4 (estimated)**
- **USGS (2000)**
    - **Ages 3 - 9**

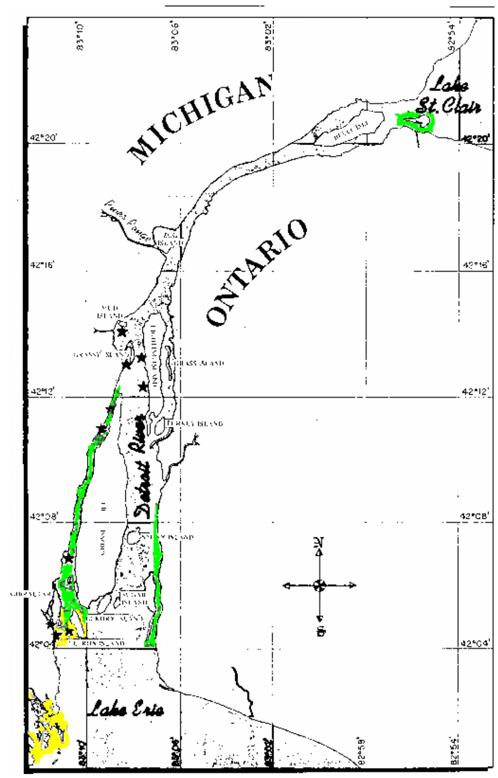


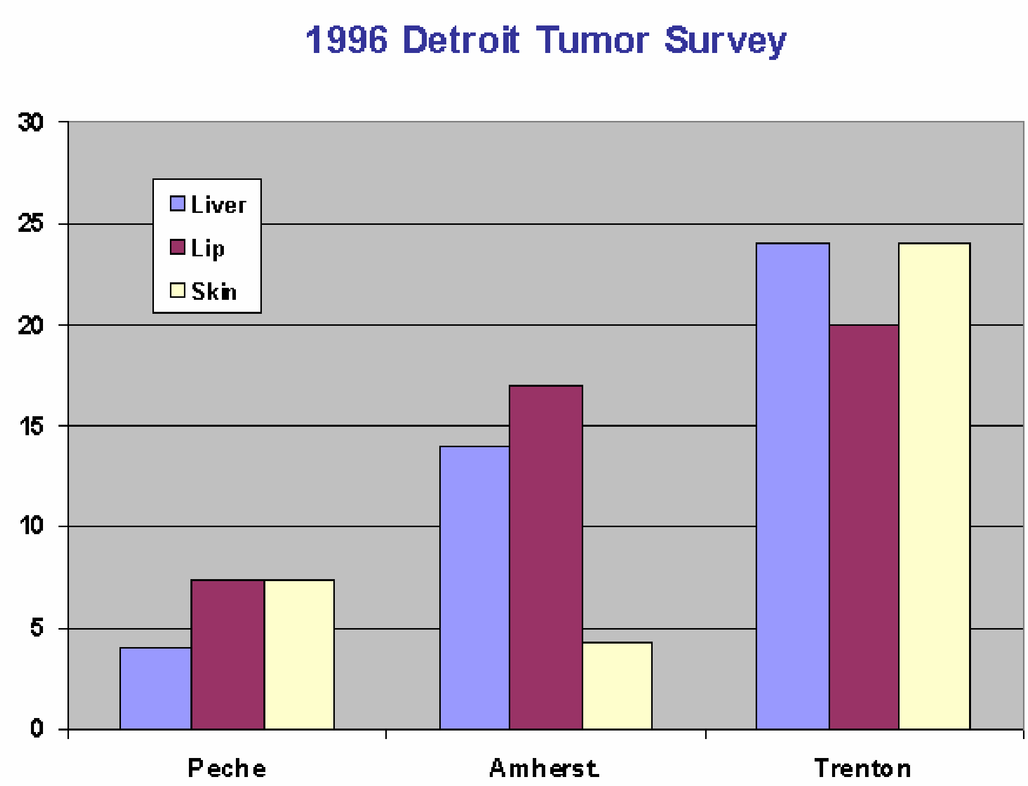
Relative Age Abundance

	<Age 4 Liver	<Age 4 Skin
1985-87	32%	50%
2000	9%	9%

Field Sampling Location

- Maccubin & Ersing (1985 – 87) ★
  - Trenton Channel
  - Northern and Southern Islands
- Leadley et al. (1996) (green)
  - Trenton Channel
  - Amherstburg Channel
  - Peche Island
- USGS (2000) (yellow)
  - Southern Islands
  - Huron River Mouth





Criteria for Tumor Survey Comparison

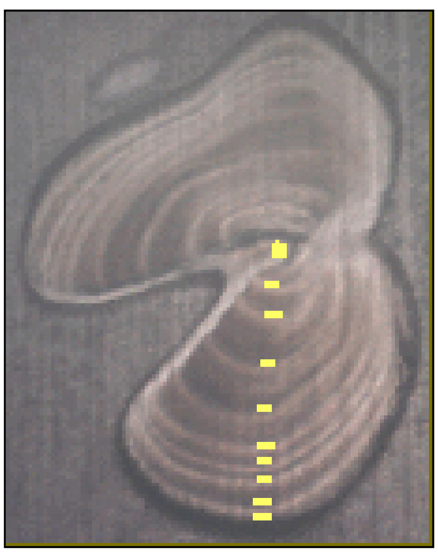
- **Are Diagnostic Criteria Comparable**
  - *** Leadley et al. (1996) and USGS (2000)**
- **Are Ages Sampled Comparable**
  - *** Maccubin & Ersing (1985 – 87) and USGS (2000)**
- **Are Locations Sampled Comparable**
  - *** Leadley et al. (1985 – 87) and USGS (2000)**
- **Are Sample Sizes Sufficient**
  - *** Maccubin & Ersing (1985 – 87)**

Panel Discussion – Historical Overview

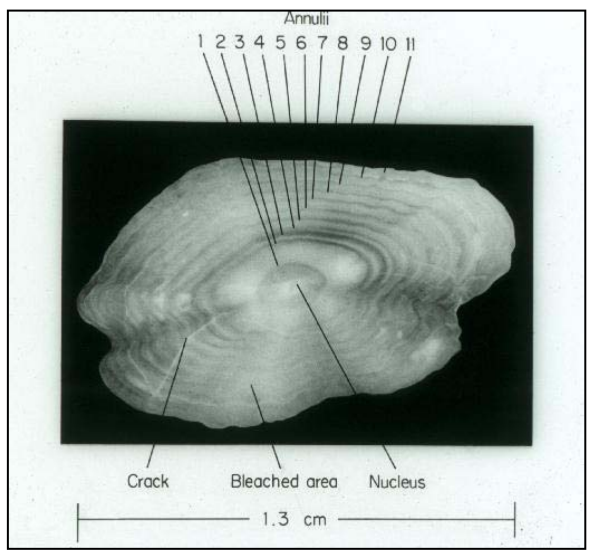
Paul Baumann, John Gannon, John Harshbarger, and Eric Obert

Question:
What is known about the life history of brown bullheads (e.g. spawning habitat, typical growth rates, and terrestrial range)?
Answer: (Eric Obert)
Spawning typically occurs in vegetated, shallow aquatic areas. Bullheads retreat into lagoons and ponds at Presque Isle Bay for spawning. In the spring of 1991 and 1992 fish were tagged at Presque Isle Bay, most of which were found in 3-4 feet of water with heavy vegetation.

Question:
What is spine and/or otolith analysis?
Answer: (Paul Baumann)
As fish grow, they develop rings in the spine and otolith. The ring development is dependent on various factors, including: temperature variation and growth rate. The difficulty with spinal aging is that the interior expands and erodes early rings as the fish grows. Growth rates also decrease as the fish ages; therefore, the rings are closer together, making them difficult to decipher from one another. Otoliths have more distinct rings, but false rings can occur due to local environmental conditions (e.g. warm water discharges). Another way to estimate age is by measuring length (e.g. 250 mm is equivalent to a 3-year-old brown bullhead); however, this method may be inaccurate.



Growth rings of the spine
(<http://www.1854.org>)



Growth rings of the otolith
(http://www.iphc.washington.edu/staff/joan/otolith>)

Question:
How are bullheads collected?
Answer: (Eric Obert)
An electroshocking boat is used in water areas less than 6 ft in depth; or trap nets (100-200 ft long) attached to shore.

Question:
Do bullheads migrate in river habitats or do they stay in resident areas?
Answer: (John Gannon)
There is not a clear-cut answer, but bullheads appear to stay close to home. Relative to lake fish, bullheads are more likely to be a resident species. It is difficult to address this through research because tagging is not effective in polluted areas (tags open the fish to infection).

Question:
Where did the tagging study occur?
Answer: (Eric Obert)
The tagging studies took place in Presque Isle Bay. Bird banding tags were used to mark a total of 2000 bullheads in Presque Isle Bay and were attached to the operculum of the fish. A total of 49 recaptured fish showed that only 2 fish had migrated to the outer area of the bay and one migrated from the outer bay to the inner bay. The majority stayed within the bay. Tags were returned up to 7 years later. Bullheads appear to grow rapidly until age 3 and then growth slows. Tagged bullheads recaptured from Presque Isle Bay only grew about 5 mm/yr. Bullheads are found near shore in Presque Isle Bay between April and June, but are difficult to catch in the summer because they move to deeper, cooler water.

Question:
In the Black River study, when the coking plant closed and tumor rates dropped, was there a decrease in PAH's?
Answer: (Paul Baumann)
UV light causes short-chained PAHs to break down but long-chained PAHs are stable. In the Black River Study surface sediment samples, PAH's declined and were covered over with agricultural based silt. Before dredging takes place a core sample should be taken to determine what the concentrations of toxins are at the dredging depth.

Question:
Are the proposed criteria (5% liver, 12% skin tumors) in Paul's paper finalized?
Answer:
No, the suggested criteria serve only as a guideline to think about.

Question:
Why do bullheads exhibit more tumors than other fish?
Answer: (John Harshbarger)
Bullheads exhibiting tumors are usually found in polluted sites; reference sites have much lower levels of tumors. It is not really known why bullheads exhibit more tumors.

Question:
Can young-of-year fish data be used to target remediation (i.e. target spawning areas to decrease tumor rates)?
Answer: (Paul Baumann)
If spawning and nursery areas could be identified that would be useful in targeting areas of remediation.

Question:
What is the rationale in using monitoring/recovery as mitigation?
Answer: (John Gannon and Paul Baumann)
There are gaps in programs because management agencies have very limited funding. Funding is needed to follow-up on the effectiveness of dredging. It is important to convey to the public and politicians that "clean-up" dollars are being well spent. Should be codified in regulatory programs (i.e. 10% of remediation costs should go to long-term monitoring or effectiveness studies). Shows change in age structure, and how remediation alters the ecosystem. Provides feedback on techniques (i.e. in the Black River, the method of remediation is very important when you compare the effects of shutting down the coking plant versus dredging). There should also be an emphasis on what type of dredging you use (i.e. suction, etc.) to minimize contamination escape. Mark Meyers at NOAA (Seattle lab) conducted a long-term monitoring project. He found that when the site was capped for remediation, tumor rates decreased.

Question:
Regarding nuclear power plants, does the list of discharge chemicals apply to conventional power plants as well?
Answer: (John Harshbarger)
In Millstone, CT, the nuclear plant takes in 4 billion gallons of water/day and returns it to Long Island Sound. There is not a presence of life at the outfall because of the heat and toxicity of the discharged water. There is some concern that nuclear plants leak radioactivity (e.g. strontium or cesium 137). Some plants also use hydrazine to reduce corrosion from oxygen in seawater intake systems. Hydrazine breaks down into methyl-nitrosamine, both of which are potent carcinogens. Cooling towers limit water discharge to the environment, which is a better modification.

Question:
Is there less use of hydrazine in the Great Lakes because it's a fresh water system?
Answer: (Bob Wellington)
A power plant in Erie, PA for many years pumped about 100 million gallons of cooling water per day. Some hydrazine was used on a regular basis; however, the plant shut down sometime around 1991, and its discharge permit was cancelled in 1993.

Question:
Is sediment data from Areas of Concern useful for determining beneficial-use impairments without bioaccumulation and histopathology studies?
Answer: (Paul Baumann)
The relationship of PAH to liver tumor rate is not a 1:1 correlation. Sediments can be used as a proxy based on concentration of contaminants, but there is a lot of variability with the

sediment/tumor rate relationship. PAHs attach to fine particles, so there may be a spotty distribution. Sometimes highly contaminated sediment may be in areas used frequently by fish, and other times it may be in areas avoided by fish because of low oxygen or unsuitable habitat. This means that sediment concentrations are not the best predictor of fish pathology.

Question:
In Canadian areas of concern fish tumors have been found in walleye. Would walleye be an effective sentinel species?
Answer: (John Harshbarger)
The Torch Lake (Michigan) study is the only one we are aware of that looked at walleye and found a significant number of liver tumors, indicating walleye may be a possibility; however, benthic fish species in polluted areas are normally correlated with fish tumors.

Question:
In the St. Lawrence Seaway, the rates of altered foci and hepatomas increase with age, but because they are not aggressive tumors, should they be included as tumors?
Answer: (John Harshbarger)
Altered foci are transformed cells, but not all progress to carcinomas. In Ashley's study of rainbow trout, the progression of liver tumors is shown. Bauman said that five years after remediation, there was a significant decrease in cancer rate, but an increase in foci rate. Tumor cells were initiated, but not promoted.

Question:
Are there other benthic species (e.g. sculpins and gobies) that could be used to measure fish tumors?
Answer: (Paul Baumann)
These are localized species, but there is a lack of background data. They are a possibility.

Question:
The key listing criteria appears to be the biggest impediment to the momentum in the Area of Concern delisting process. Tumor rates of 3.5% and 2% are not feasible even in low impact areas. Is it unrealistic based on Great Lakes and atmospheric deposition.
Answer: (Bob Wellington)
A 2002 study on 47 brown bullheads from the Presque Isle Bay areas revealed a liver tumor rate of only about 2.1% (only 1 out of 47 exhibited liver tumors).

Question:
Are criteria for delisting firm? Are many sites listed?
Answer: (John Gannon)
No, the criteria are guidelines and are not based on good science. Almost all Areas of Concern have sediment contamination; therefore, it is expected that fish tumors are a good proxy for beneficial-use impairments.

Question:
How would we change the criteria, if needed?
Answer: (John Gannon)
Either the binational or U.S. committee could propose alterations to the IJC.

Question:
Is there a standard protocol for listing fish tumors as a BUI (i.e. age cutoff for 2%, number of samples, sample size)? Are there any criteria for other types of tumors and deformities?
Answer: (Paul Baumann)
The science is limited; therefore, there are not protocols available. That could be a recommendation from this conference. As for the number of bullheads, there are areas where none are found (e.g. the Rouge River and Indiana Harbor, which have habitat or contamination extremes). In the Detroit River, the ecosystem is showing signs of recovery; however, there are hotspots of contamination. The biodiversity is increasing, but it's difficult to find bullheads. Ecosystem improvements also decrease available habitat for bullheads.

Question:
Are epidermal lesions due to contact exposures opposed to ingestion of sediments, which generally leads to liver tumors?
Answer: (John Harshbarger)
Non-benthic fish are less likely to develop mouth tumors, so the difference is not in metabolism, but habitat requirements of littoral and pelagic versus benthic fish.

Question:
Are there set criteria for tumors, (i.e. if there is funding given to restore a site but the 2% benchmark is not met, how do you address public perception)?
Answer: (Paul Baumann)
We can use this conference as a steppingstone to future meetings to establish diagnostics/criteria. Tumors are objective and of the available criteria for other beneficial-use impairment, fish tumors and other deformities are more readily assigned a number.

Question:
Why are we focusing on the bullhead?
Answer: (Paul Baumann)
There is a problem with perception, (i.e. who cares about the bullhead)? Although they are not a sport fish, they do have a tie-in to human carcinogens and the concern about cancer is widespread. The brown bullhead has value as an indicator species because it is pollution tolerant. We need to pick a species that can survive in contaminated sediments. Bullheads are a sport fish in St. Lawrence and Quebec, and people consume it readily.

Question:
How does Presque Isle Bay compare to other Areas of Concern?
Answer: (John Gannon and John Harshbarger)
There would not have been a celebration surrounding the delisting to an Area of Recovery without the active interest and action by the local community. Of the 43 AOCs, only one (Collingwood Harbor) has been delisted. Erie's combined sewer overflow program progress is

very effective. Heavy industry and power plants have been shut down, leading to the decline of industrial pollutants and there has been an improvement in infrastructure.

Presentations – Session II: Histopathology Diagnosis and Criteria

Dr. John (Jack) Fournie - Importance of Specific Diagnostic Criteria for Non-Neoplastic and Neoplastic Lesions in Fishes

Dr. Fournie discussed the importance of specific diagnostic criteria when identifying neoplastic and non-neoplastic lesions in fishes. Standardization of nomenclature and consensus on diagnostic criteria are necessary to assists pathologists evaluating lesions from laboratory and field studies, achieve uniformity in diagnosis, allow for relevant communication of results, permit comparisons of results among different studies, and ensure a historical database. The histopath images of altered foci, adenomas, carcinomas, cholangiomas, and cholagiocarcinomas can be seen in Dr. Fournie's power point presentation.

Dr. Fournie focused on liver and bile duct tumors. The hepatocellular lesions Dr. Fournie discussed were foci of cellular alteration, hepatocellular adenoma, and hepatocellular carcinoma. The biliary lesions he discussed were cholangioma and cholangiocarcinoma.

Four categories of altered foci can be recognized in sections stained with hematoxylin and eosin, based on the tinctorial characteristics of the hepatocyte cytoplasm (basophilic, eosinophilic, clear, and vacuolated cell foci). The margins of foci are distinct; however, the hepatic tubules are arranged in a relatively normal pattern and merge imperceptibly with the surrounding parenchyma. The histopathology of altered foci can be seen in images #1 - #4.

Hepatocellular adenomas are usually well demarcated by compression of the adjacent parenchyma, exhibit altered staining properties and growth pattern, and have a larger degree of cellular atypia. The histopathology of an adenoma can be seen in image #5.

Hepatocellular carcinomas may be small or large lesions and usually exhibit distinct, irregular borders with neoplastic cells invading the adjacent parenchyma. Cellular pleomorphism and nuclear atypia are key features of hepatocellular carcinomas and there is usually an increase in the number of miotic figures and some tumor giant cell formation. The carcinomas may be poorly or well differentiated. Histopathology of carcinomas can be seen in images #6-9.

Cholangiomas consists of clusters of well-differentiated bile ducts that form an expansive mass with discrete borders. These lesions are differentiated from bile duct hyperplasia, which consists of scattered, well-differentiated bile ducts. Histopathology of a cholangioma can be seen in image #10.

Cholangiocarcinomas consist of atypical proliferating bile ducts that are often admixed with abundant proliferating stroma. These lesions display invasive growth into the surrounding parenchyma. Proliferating bile ducts are usually irregularly shaped and the neoplastic epithelium is pleomorphic with numerous mitotic figures. Histopathology of a cholangiocarcinoma can be seen in images #11 and #12.

Dr. John (Jack) Fournie's Power Point Presentation

Importance of Specific Diagnostic Criteria for Non-neoplastic and Neoplastic Lesions in Fishes

John W. Fournie

U.S. Environmental Protection Agency
National Health and Environmental Effects Research Laboratory
Gulf Ecology Division
Gulf Breeze, Florida

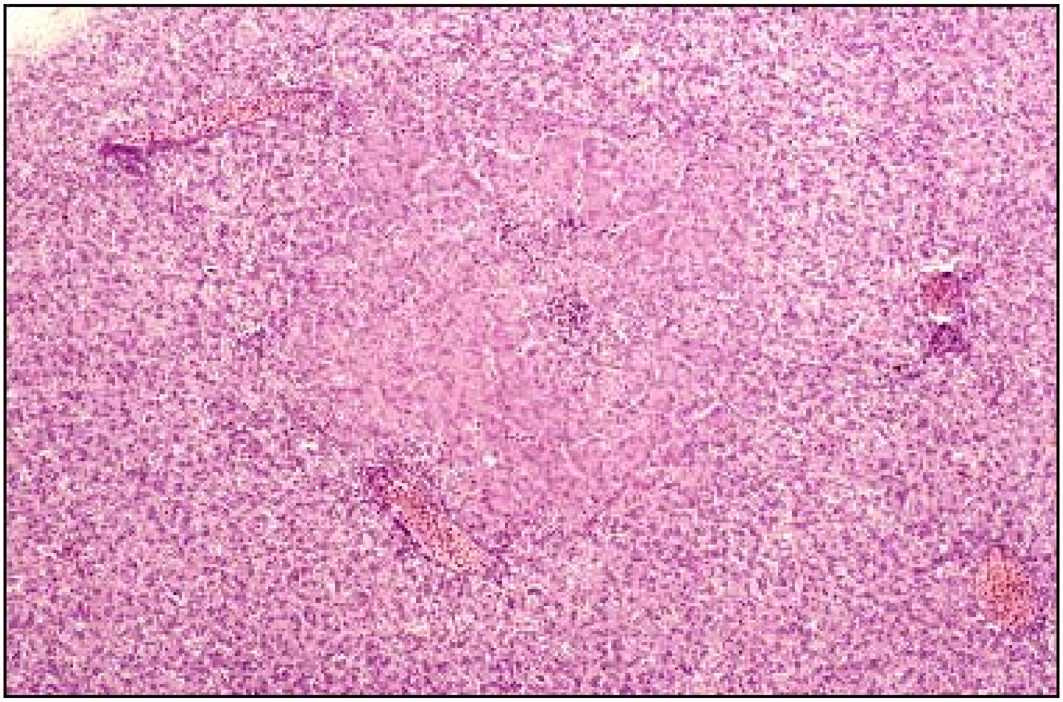
Standardization of Nomenclature and Consensus On Diagnostic Criteria are Necessary to:

- **Assist pathologists evaluating fish lesions from laboratory and field studies**
- **Achieve uniformity of diagnosis**
- **Allow for meaningful communication of results**
- **Permit comparison of results among studies**
- **Insure a useful historic database**

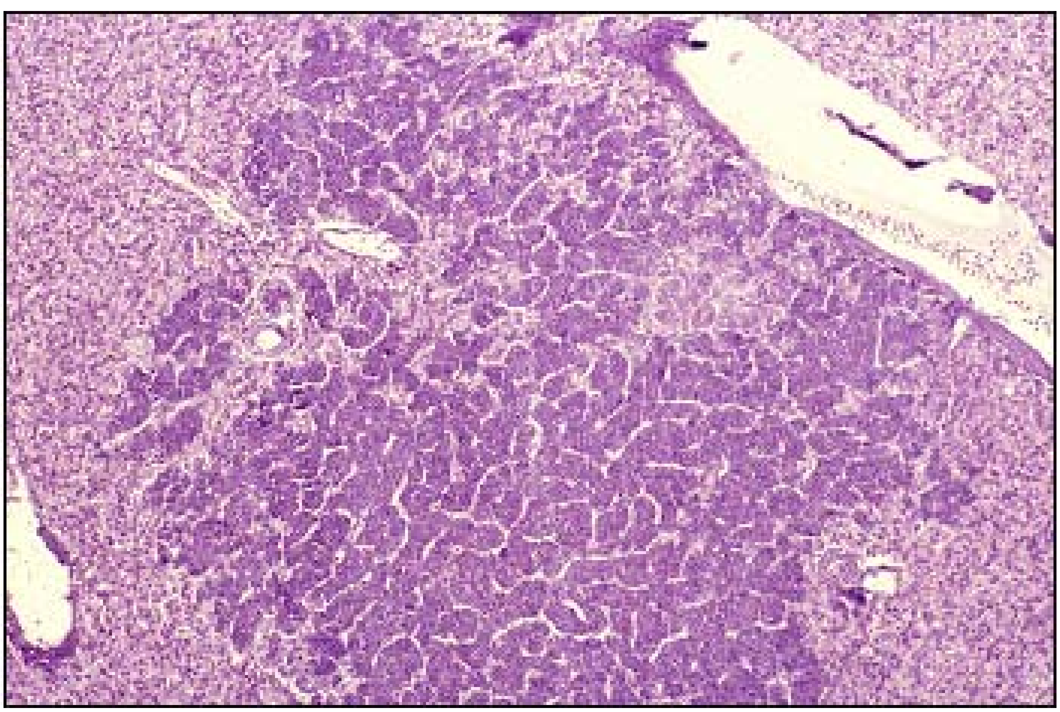
Foci of Cellular Alteration

- **Four categories of altered foci can be recognized in sections stained with hematoxylin and eosin based on the tinctorial characteristics of the hepatocyte cytoplasm**
    - **Basophilic, Eosinophilic, Clear and Vacuolated Cell Foci**
- **Margins of foci are distinct but the hepatic tubules are arranged in a relatively normal pattern and merge imperceptibly with the surrounding parenchyma**

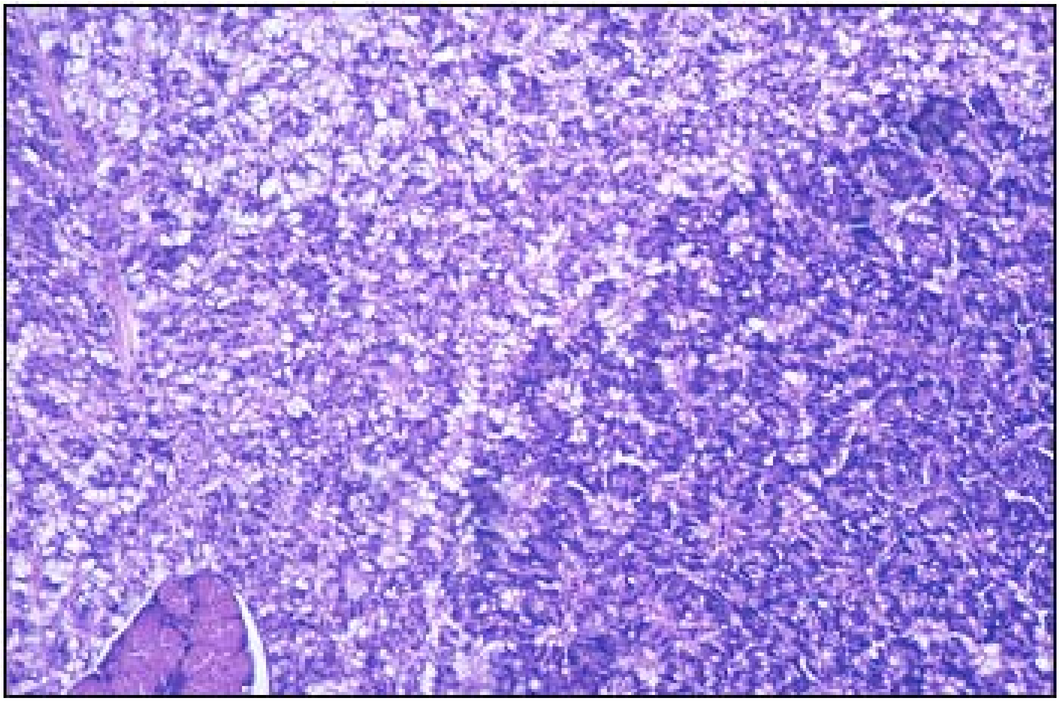
Eosinophilic Focus (Image #1)



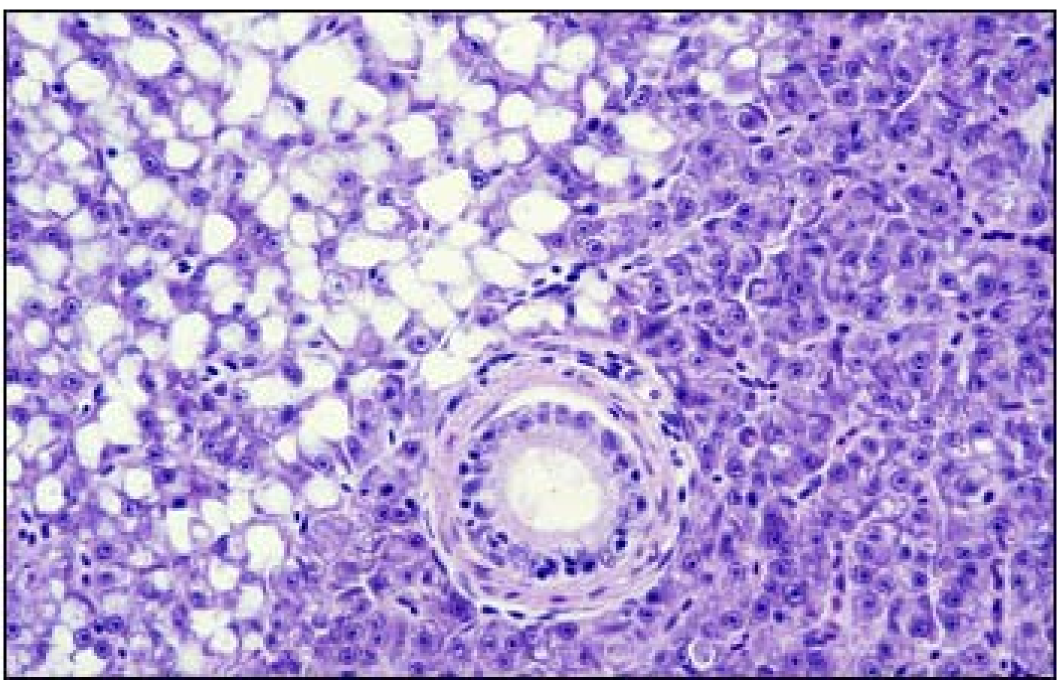
Basophilic Focus (Image #2)



Basophilic Focus (Image #3)



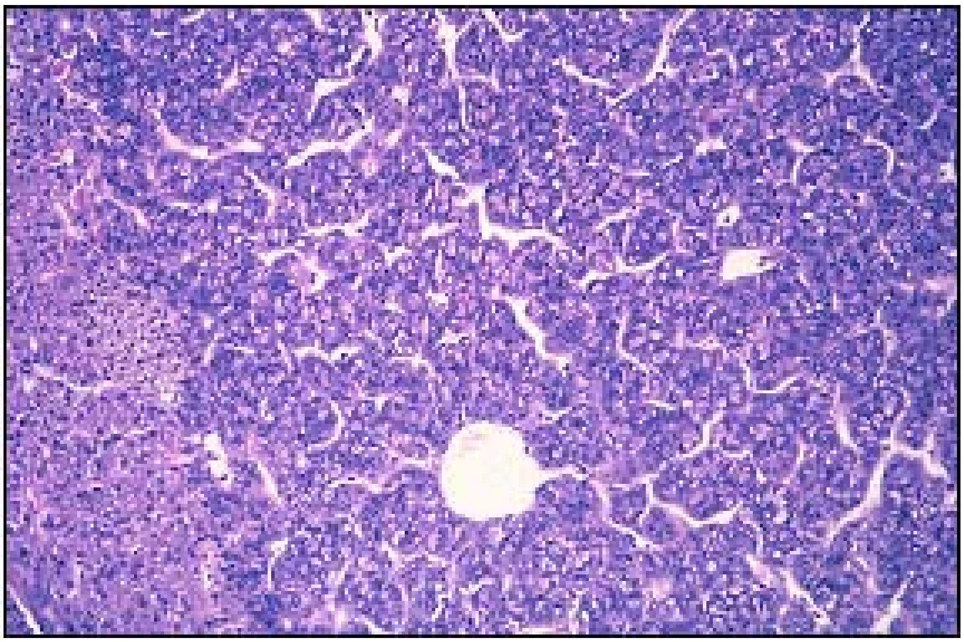
Vacuolated Foci Alteration (Image #4)



Hepatocellular Adenoma

- Adenomas are usually well demarcated by compression of the adjacent parenchyma
- Usually exhibit altered staining properties and growth pattern
- Have a greater degree of cellular atypia

Hepatocellular Adenoma (Image #5)



Hepatocellular Carcinoma

- May be small or large lesions

- Usually exhibit distinct, irregular borders with neoplastic cells invading the adjacent parenchyma

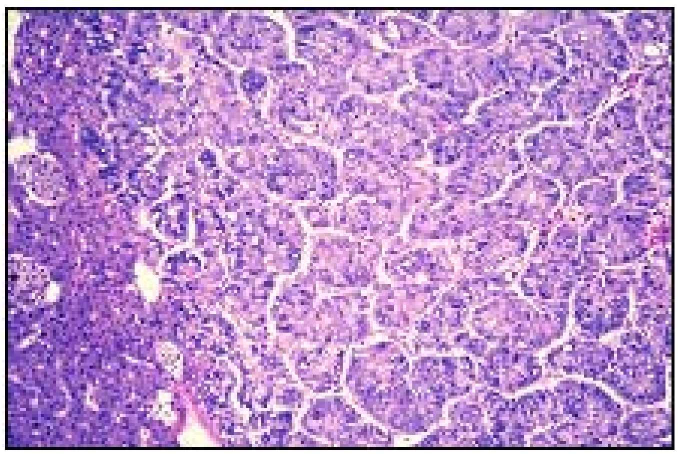
- Cellular pleomorphism and nuclear atypia are key features

- Usually an increase in the number of mitotic figures and some tumor giant cell formation

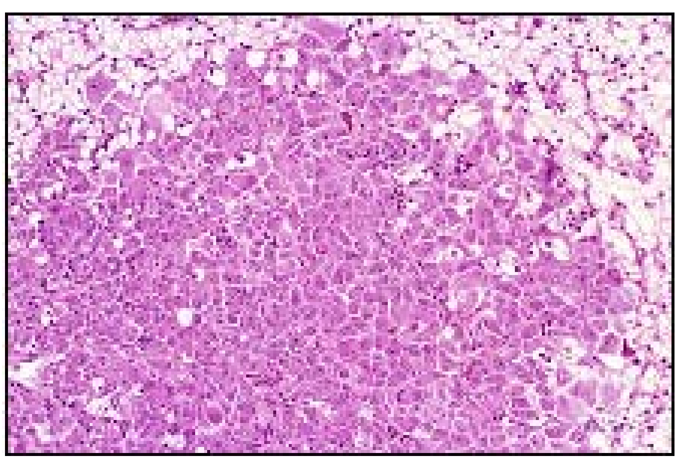
- May be well or poorly differentiated

Gross Hepatocellular Tumor

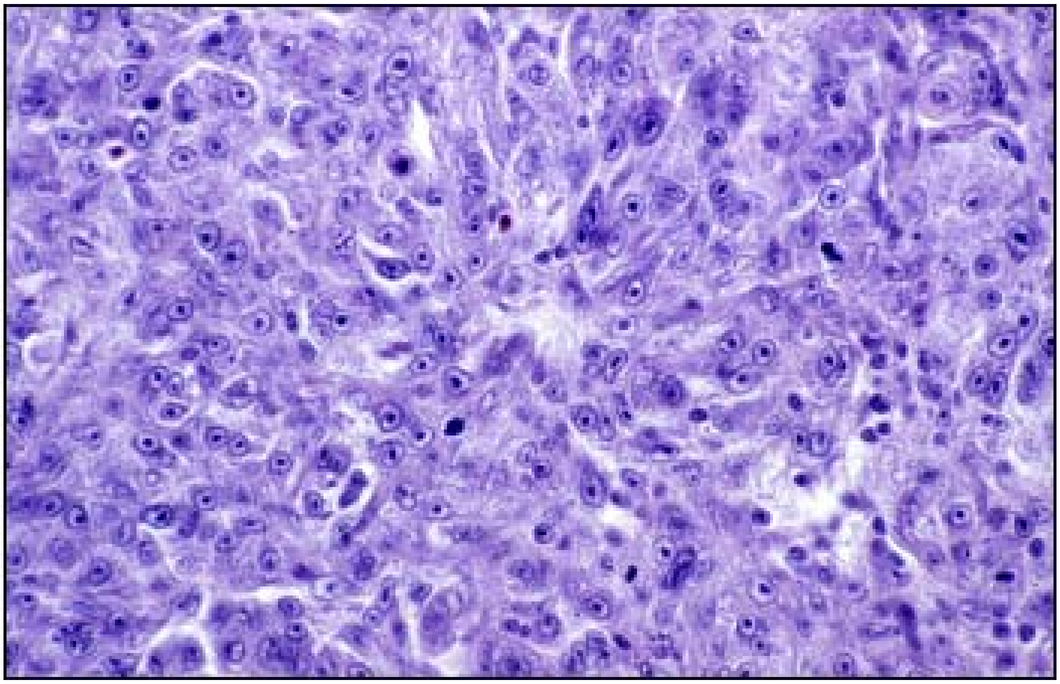
Well-Differentiated Hepatocellular Carcinoma (Image #6)



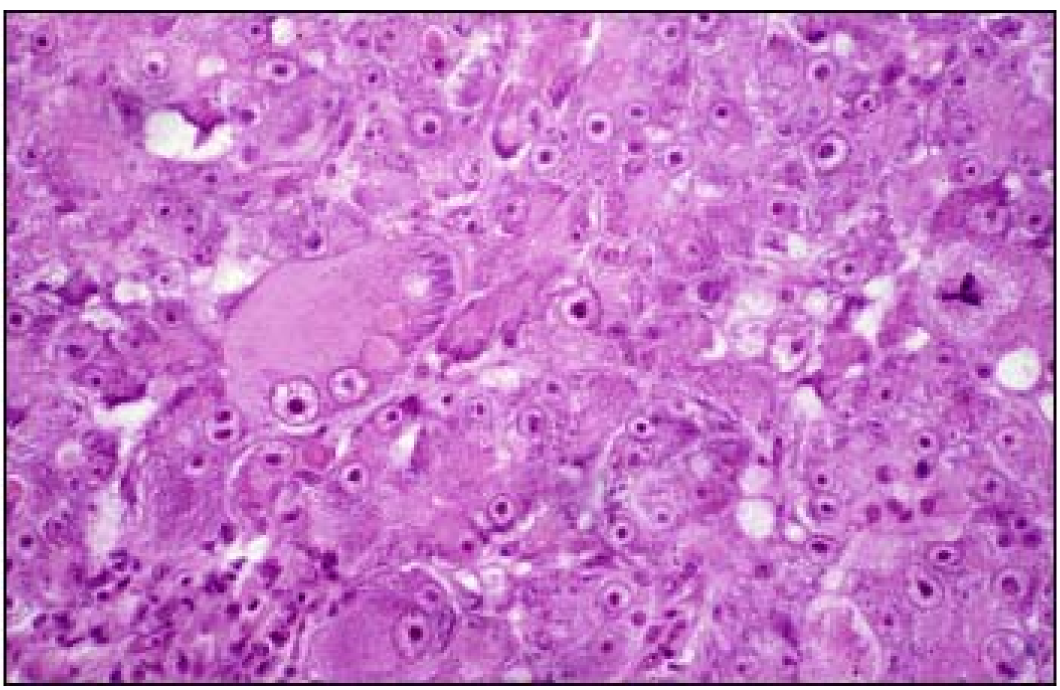
Well-Differentiated Hepatocellular Carcinoma (Image #7)



Poorly-Differentiated Hepatocellular Carcinoma (Image #8)



Poorly Differentiated Hepatocellular Carcinoma (Image #9)

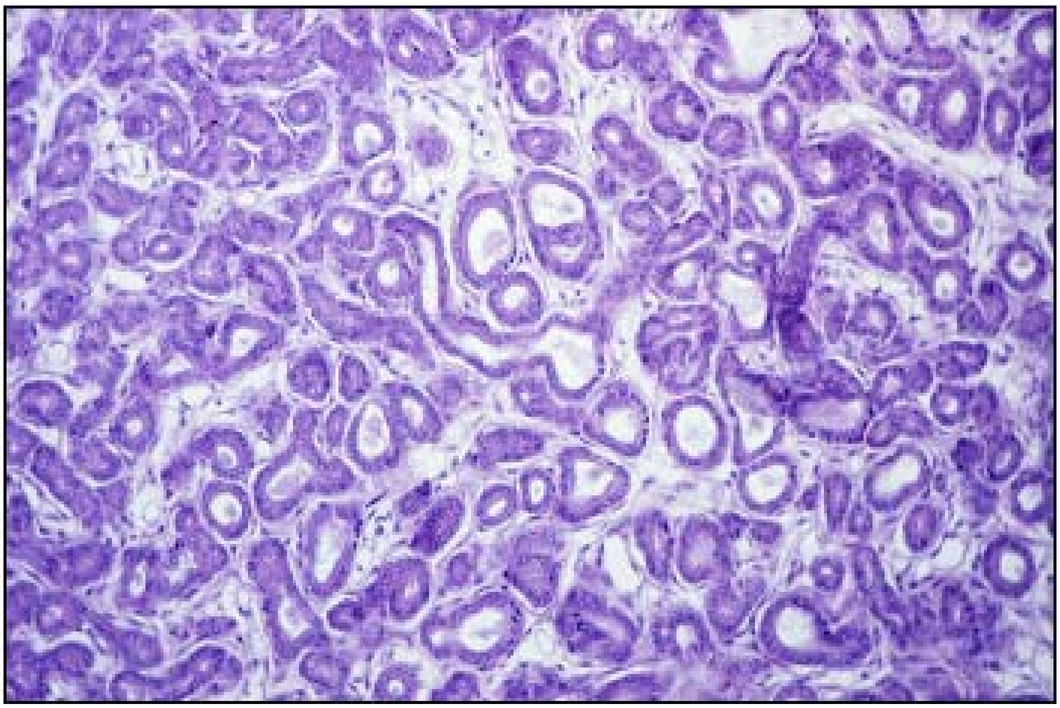


Cholangioma

- Consists of clusters of well-differentiated bile ducts that form an expansive mass with discrete borders

- This lesion is differentiated from bile duct hyperplasia, which consists of scattered, well-differentiated bile ducts

Cholangioma (Image #10)



Cholangiocarcinoma

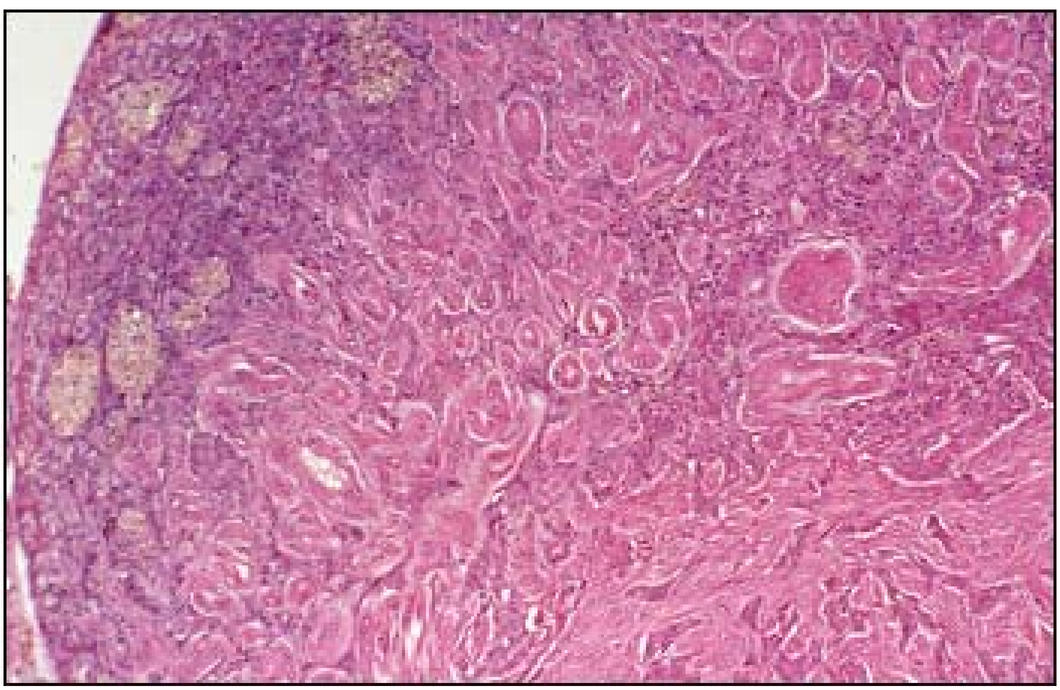
- Consists of atypical proliferating bile ducts often admixed with abundant proliferating stroma

- These lesions display invasive growth into the surrounding parenchyma

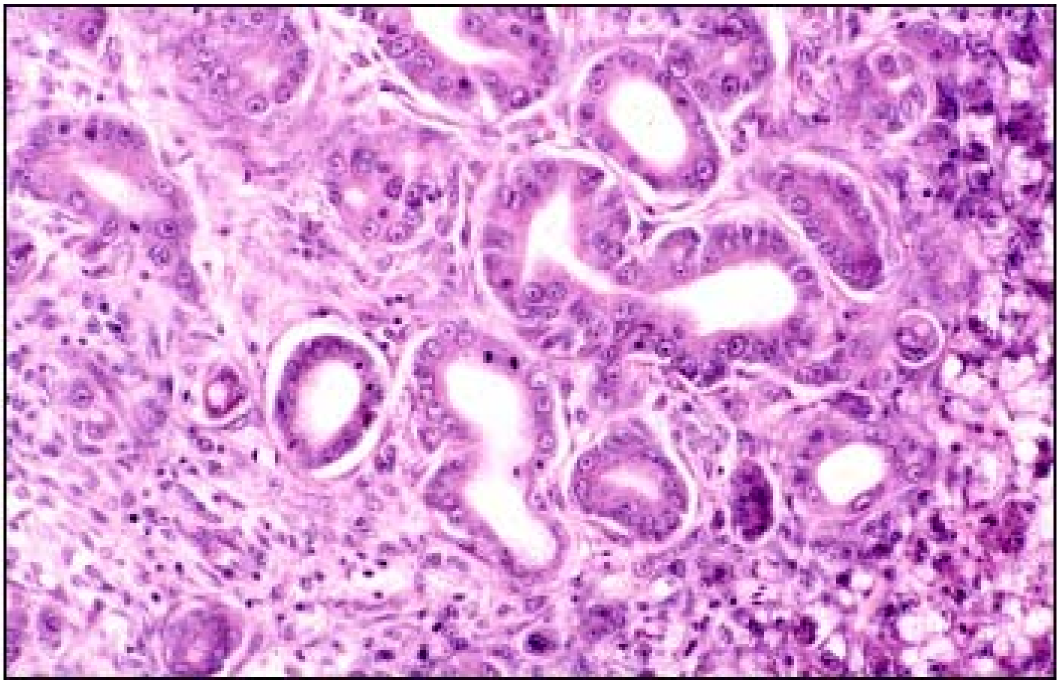
- Proliferating bile ducts are usually irregularly shaped and the neoplastic epithelium is pleomorphic with numerous mitotic figures

Gross Photograph of a Cholangiocarcinoma

Cholangiocarcinoma (Image #11)



Cholangiocarcinoma (Image #12)



Dr. Vicki Blazer - Skin Lesions and Other Miscellaneous Histological Findings as Indicators of Environmental Stress

Dr. Blazer discussed skin lesions, macrophage aggregates as indicators of contaminant exposure and common reproductive endpoints. Skin lesions often attract public concern and in the Great Lakes, raised lesions are commonly reported to be papillomas, squamous cell carcinomas or melanomas. **Raised lesions cannot be diagnosed by gross observation; therefore, histology is needed to make a more accurate diagnosis**. If funding is not immediately available for histopath analysis, lesion samples can be fixed in buffered formalin and stored for future analysis.

The advantages of histology include: changes at a cellular level can be evaluated, fixed tissue can be stored indefinitely, and paraffin embedded blocks can be re-cut and analyzed later using new techniques. Histopath was performed on samples from Menhaden in the Chesapeake Bay and it was determined that the skin lesions were caused by an inflammatory response to a fungal disease. It was also found in another sample that myxosporidian spores were the cause of raised lesions. These examples were used to point out that without knowing the cause of lesions and the environmental factors involved, it is hard to make a definite diagnosis. Raised lesions are not necessarily always tumors or neoplasia.

Macrophage aggregates (similar to mammalian lymph nodes) accumulate foreign and cellular matter, recycle and/or store iron, accumulate exogenous and endogenous waste products, and increase in number and size with exposure to environmental stressors. Variables affecting macrophage aggregates include age, diet and infectious disease. Macrophage aggregates are early indicators of adverse effects of exposure to environmental stressors, may be useful in remediation, and can be quantified by image analysis. The tissue containing aggregates can be quantified by calculating density ($\#/mm^2$) and mean size (area in mm^2), which in turn can be used to determine the percent of tissue occupied by macrophage aggregates. A study concerning the remediation of Lake Champlain showed that macrophage aggregate occurrences decreased following the upgrade of sewage plant: therefore, providing evidence that measuring aggregate occurrence is an effective means to evaluate the restoration of fish health.

Common reproductive histopathological endpoints include: sex verification, developmental stage, percent atretic (degenerating) eggs, identification of intersex species (indicator of endocrine disruption), presence of tumors and parasites, focal areas of necrosis, and other abnormalities such as change in egg size, yolk development and sertoli cell proliferation.

A study was performed in response to the decline of the yellow perch population and shift of yellow perch age class (lack of young fish) in Lake Michigan. Yellow perch were collected for three seasons in both Lake Michigan and Lake Mendota. There were greater PCB burdens, smaller gonads in relation to fish size, female vitellogenin (yolk development) levels were lower, and testicular tumors were more prevalent in Lake Michigan. During the spawning season in Lake Michigan it was determined that 83% of males were spent while 0% of females were spent and only 50% of females sampled had developing eggs. It was concluded that there was disconnect between sexual development in males and females at the Lake Michigan sample site.

Dr. Vicki Blazer's Power Point Presentation

Skin Lesions, Gonadal History And Macrophage Aggregates

Vicki S. Blazer
National Fish Health Branch
USGS Leetown Science Center

Skin Lesions

- Variety of skin lesions have attracted public attention and concern
- Great Lakes: Raised lesions often reported to be papillomas, squamous cell carcinomas or melanomas
- Chesapeake Bay and other estuarine sites: raised lesions and ulcerative lesions

Raised Mouth Lesions – Probable Papillomas

Raised Black Lesions – Possible Melanomas

Cause of External Lesions

- Type and/or cause CANNOT be diagnosed by gross observation only

- Always a good idea to take pieces of abnormal tissue and fix in buffered formalin or some other fixative

Histopathological Assessment Advantages

- Evaluate changes at the cellular level
  - * indicative of early effects/damage
  - * can be diagnostic

- Store fixed tissue for long periods of time

- Paraffin-embedded blocks can be stored and recut

“Tumor” Findings
Mississippi Drainage BEST

- 14 fish were rated as having “tumors” by field personnel
    * 10/14 – no tissue taken (lost data)
    * 3/14 – parasite-induced proliferative inflammation
    * 1/14 – actual papilloma

Chesapeake Bay Skin Lesions
Menhaden

Ulcerative lesions reported to be the typical “*Pfiesteria*” lesion

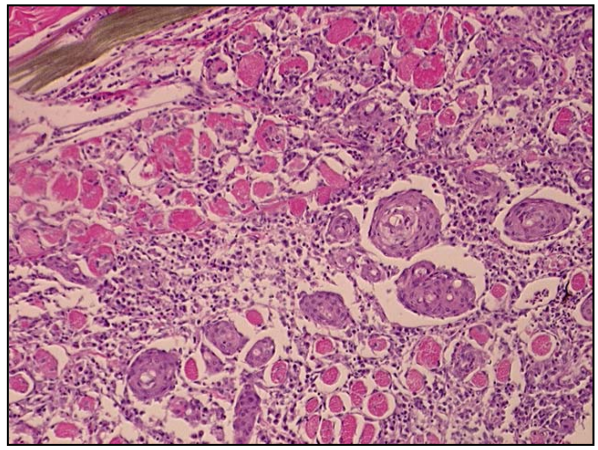
Believed by some to be the precursor to ulcerative lesions.

Ulcerative Menhaden Lesions

- Cause: *Aphanomyces invadans*
    * Based on isolation from lesions; reproduction of identical lesions with fungal zoospores; PCR results

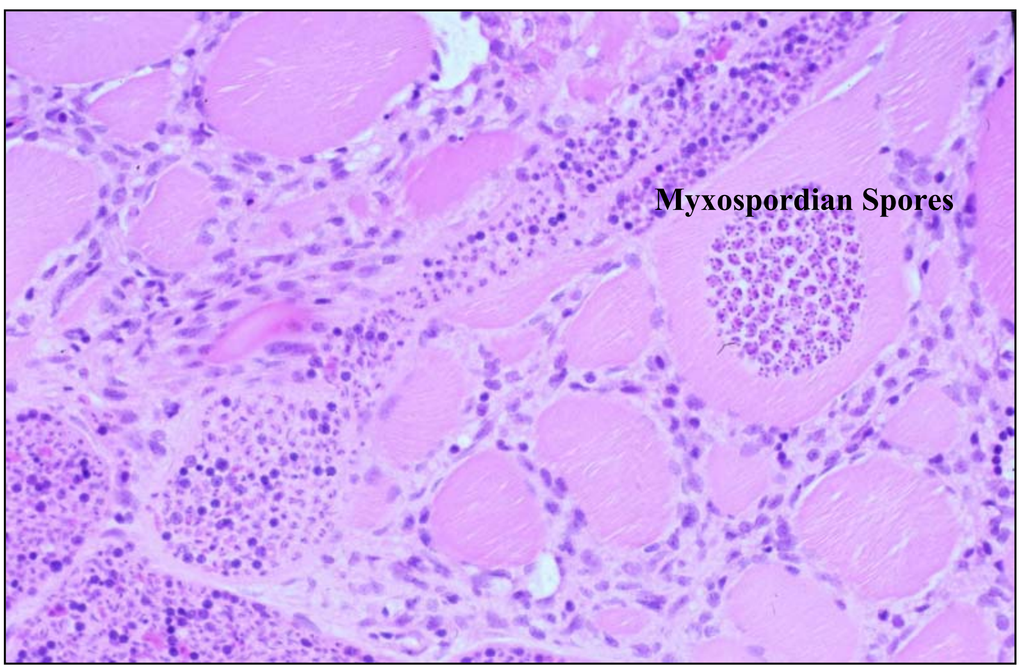
- Understanding environmental influences on fish host and pathogen

Histopathology of Ulcerative Lesions



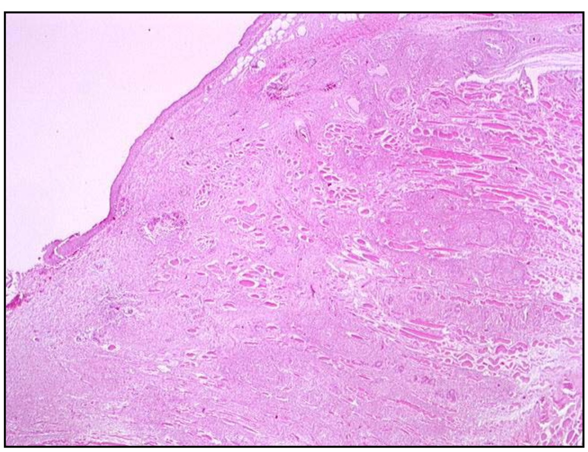
Consistency in the histopathological presentation

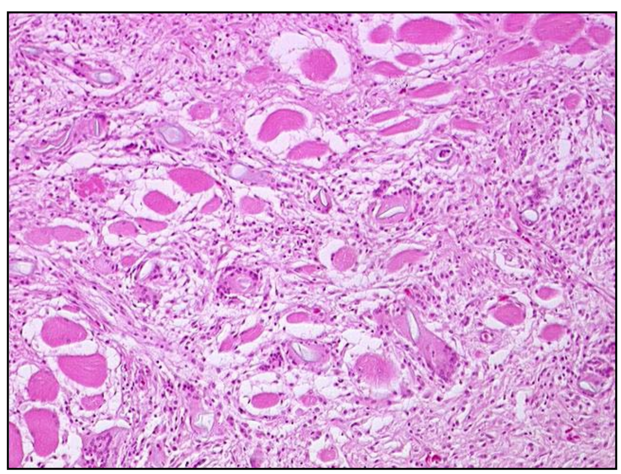
Raised Lesions on Young Fish *Kudoa* Infections



Bullheads
Raised Lesions and Barbel Formations

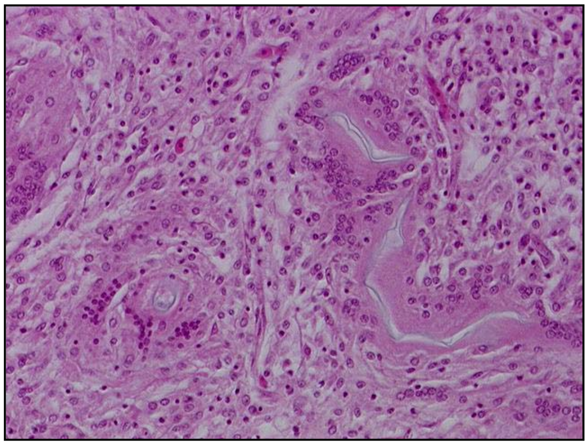
Histology of Raised Black Lesions

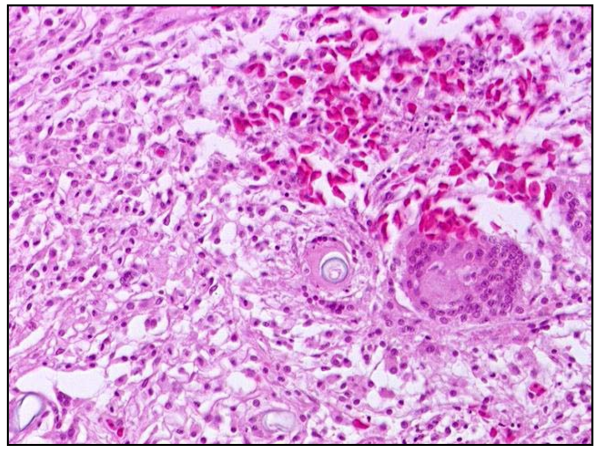




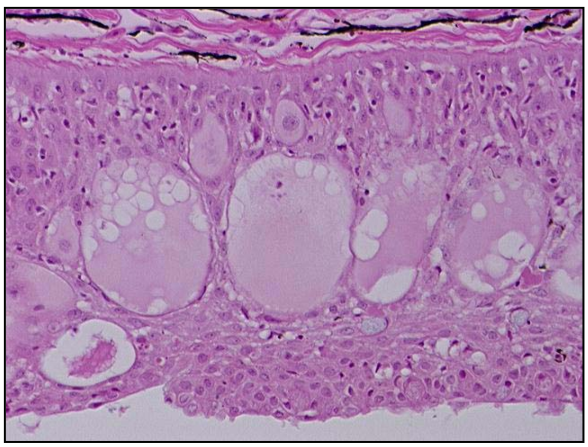
Ichthyophonous sp.

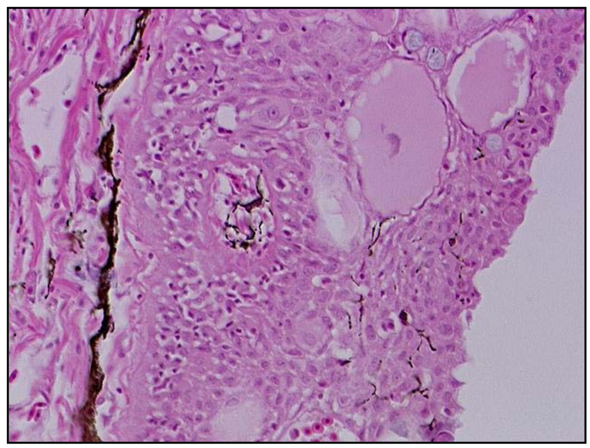
Raised Lesions on Bullhead





Melanistic Spots





Normal Skin **Black Area**

Macrophage Aggregate Parameters as Indicators of Environmental Contamination In Lake Erie Tributaries

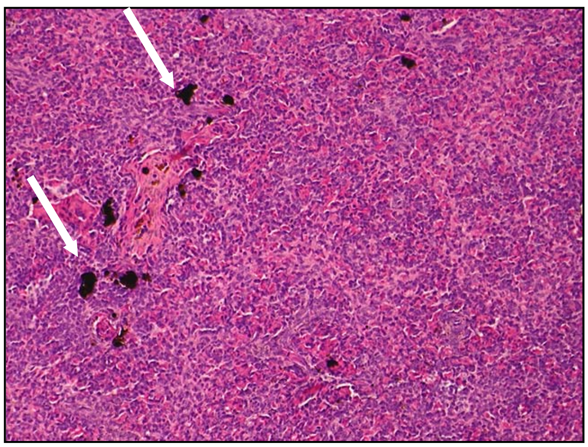
Macrophage Aggregates

- Similar to mammalian lymph nodes: accumulate foreign and cellular matter, recycle and/or store iron, and accumulate exogenous and endogenous waste products

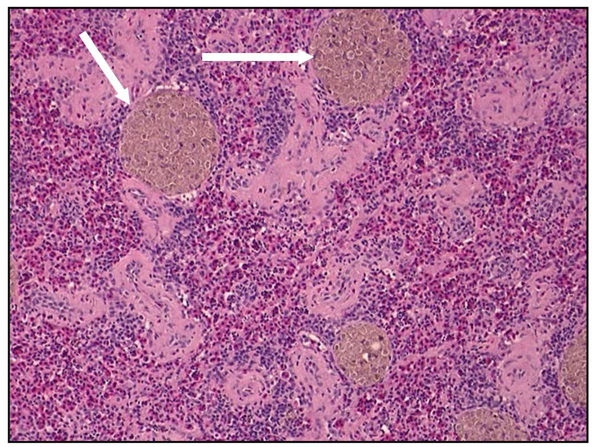
- Increase in size & number with exposure to environmental stressors

- Variables such as age, diet, infectious disease

Macrophage Aggregates
Morphology



Trout



Yellow Perch

Macrophage Aggregates as
Indicators of Contaminant Exposure

- Well over 60 studies indicating an increase in MA number, size and/or hemosiderin content at contaminated versus reference sites or lab studies

Field Studies
* Pulp Mill Effluent
* PAHs
* Sewage Sludge
* Mercury
* PCBs
* Crude Oil

Laboratory Studies
* Chromium
* Sewage Sludge
* Arsenic

Macrophage Aggregates as Indicators of Contaminant Exposure

- **Advantage: earlier indicator of contaminant effects than tumor and other gross lesions**

- **May be useful in evaluating remediation**

Macrophage Aggregate Parameters

- **In spleen, head kidney and liver can be rated on a scale of 0 - 4**

- **Use image analysis:**
  - **Density - #/mm^2**
  - **Mean Size – Area in μm**
  - **Percent of tissue occupied by MA**

Comparison of Macrophage Aggregates in Bullheads from Ashtabula River and Lake Erie 1990

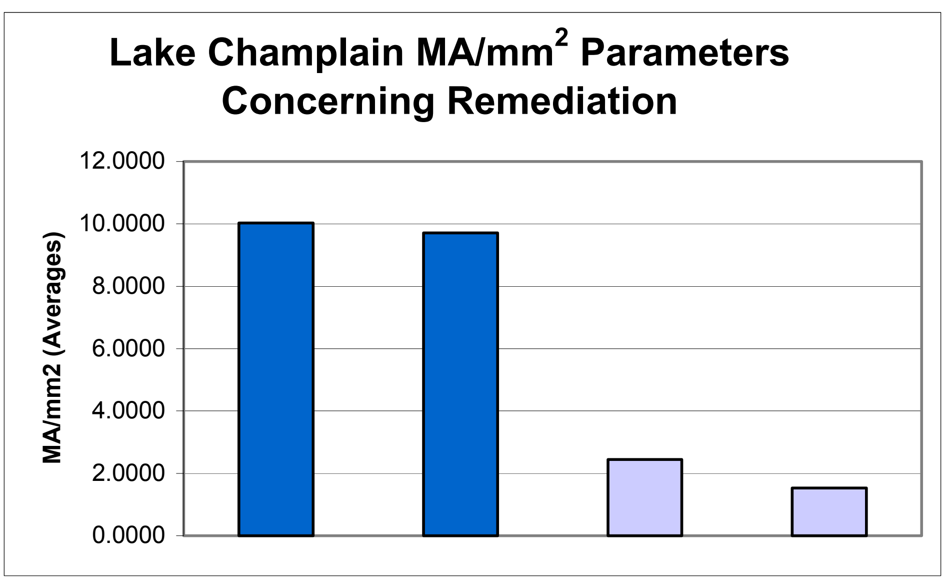
Paramater	River	Breakwater	Harbor
N	**14**	**19**	**28**
Age	**5.1 ± 1.2**	**4.8**±1.1	5.0±1.0
MA#	17.8±7.2 [a]	14.4±10.9 [a,b]	10.9±9.0 [b]
MA size	990±296 [a]	640±302 [b]	705±271 [b]
% Tis.Occ	1.8±0.8 [a]	1.1±0.9 [a,b]	0.8±0.6 [b]
Preneoplastic Foci %	9.2	6.1	4.1
Liver Neoplasia (%)	14.3	2.2	0

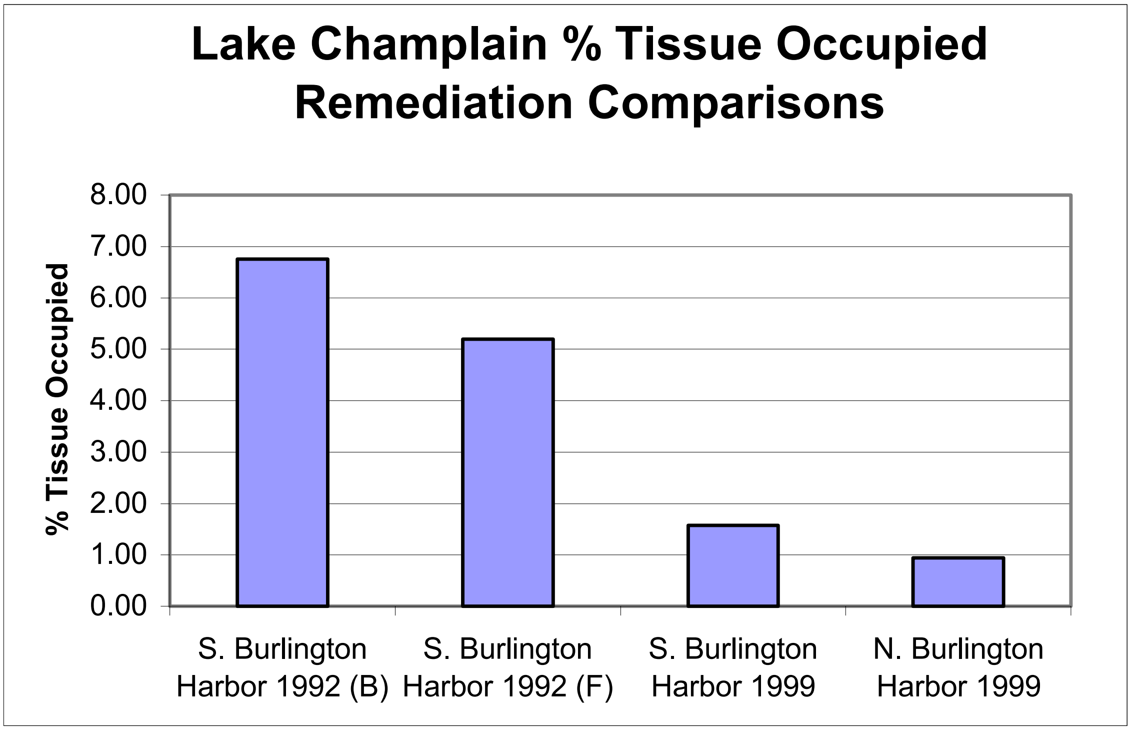
Comparison of Age 3 and Age 4 Bullheads
1990

Paramater	River	Harbor
Age	**3.5**±0.6	3.6±0.5
MA #	16.8±6.6[a]	6.2±4.9[b]
% Tis.Occ.	1.6±0.8 [a]	0.4±0.3 [b]
Lip papilloma	0	0
Liver tumors	0	0

Lake Champlain: Evidence of MA Effectiveness

- **Sewage plant runoff and PCBs**
- **1992: Collect rock bass from a number of sites around the lake**
- **1994: Remediated (upgraded sewage treatment, etc.)**
- **1999: Collection for Burlington Harbor area**
  - *** compared only 4 year olds**





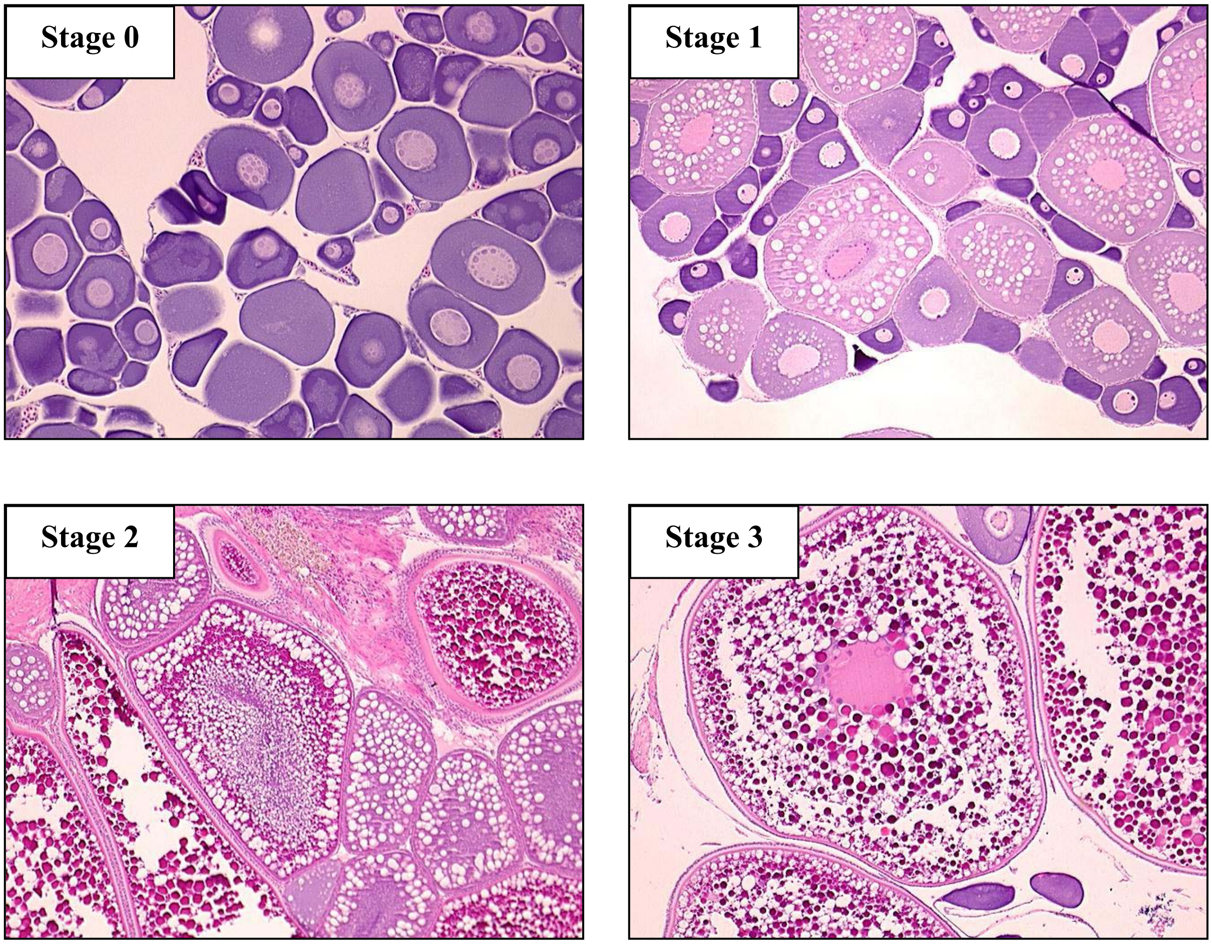
Common Reproductive Histopathological Endpoints

- **Sex verification**
- **Developmental stage**
- **Percent atretic eggs**
- **Identification of intersex**
- **Presence and identification of:**
  - **Parasites**
  - **Tumors**

Other Histological Changes: Reproductive System

- **Focal area of necrosis**
- **Other abnormalities**
  - **Ceroid/lipofuscin/oxidized lipid accumulation**
  - **Seretoli Cell Proliferation**
  - **Egg size**
  - **Yolk development**

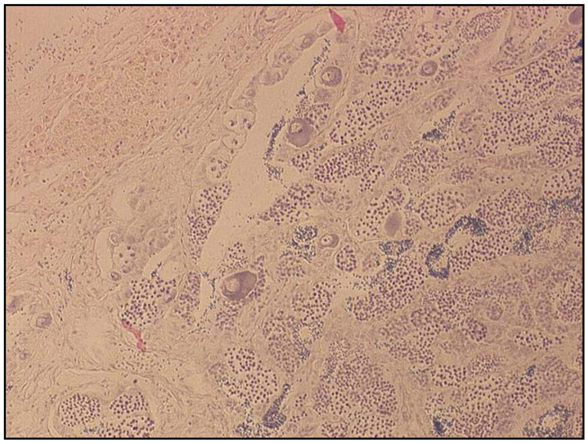
Ovarian Stages



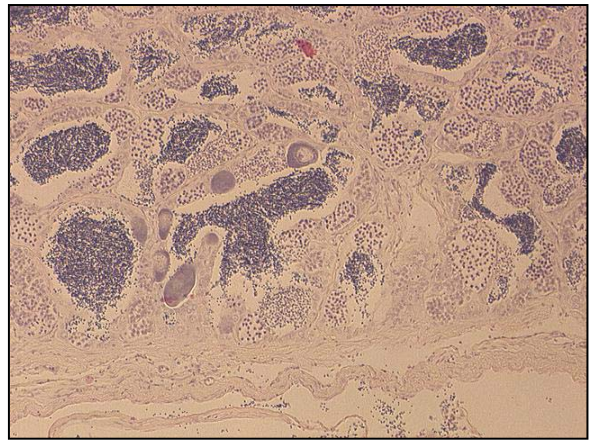
Intersex in Fish

- Hermaphroditism and sex reversal are normal in some fish species
- Most teleosts are gonochorists – distinct and separate male and female sexes
- Sex reversal and/or intersex can be induced by exposure to natural and synthetic hormones and/or armotase

Intersex



Testes with very few mature sperm



Testes with many mature sperm

Lake Michigan Yellow Perch

- **Series of weak year classes since 1991**
  - * **limited recruitment to the adult population**
- **Shift in age-class structure**
  - * **lack of young fish**
- **Overall decline of the yellow perch population**
- **New harvest restrictions – both sport and commercial**

Summary of Results

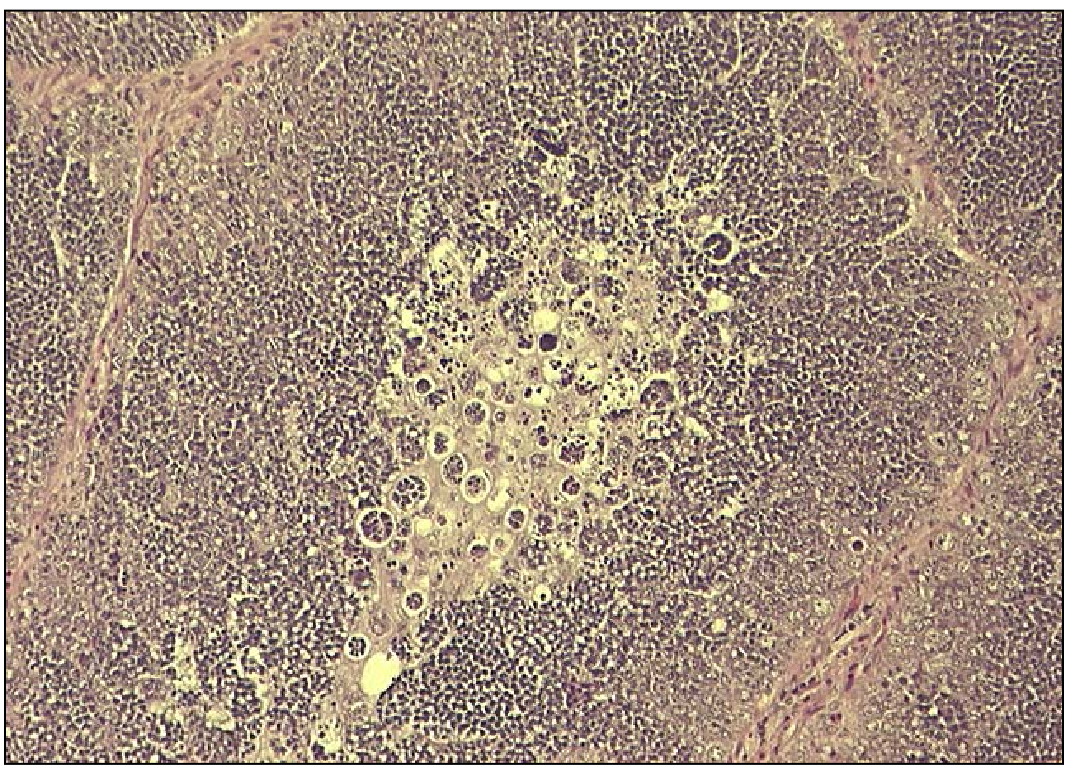
- **Greater PCB burden**
- **Greater hepatic EROD activity in the spring**
- **Smaller GSI in winter and fall**
- **Female blood vitellogenin levels lower**
- **High incidence of testicular tumors**
- **Presence of other gonadal normalities higher**

Testicular Tumors (Germ Cell)
Lake Michigan Yellow Perch

	Winter 1996	Spring 1997	Fall 1997
Tumors	**3**	**5**	**3**
(year class)	**(1yc88;2yc89)**	**(all yc88)**	**(all yc 88)**
	1 early	**2 early**	**1 early**
	(yc89)	**(yc89;85)**	**(yc89)**
Prevalence	**31%**	**27%**	**25%**

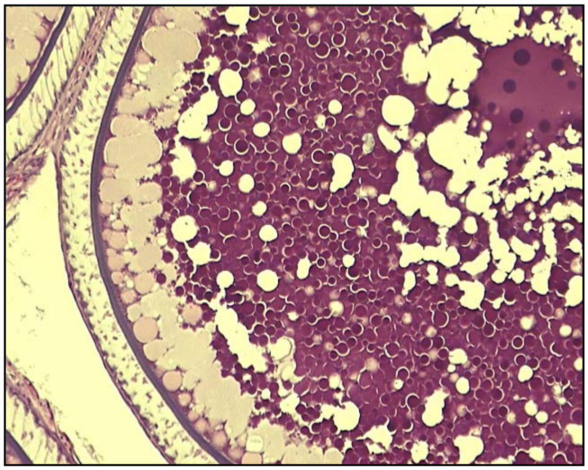
Testicular Abnormalities

Lake Michigan Yellow Perch



Comparison of Yellow Perch Females

Year Class 90 – Winter Collection



Lake Mendota **Lake Michigan**

Stage 3

Gonad Stages in Yellow Perch

From Lake Mendota

	Fall	Winter	Spring
Male	**3 (100%)**	**4 (100%)**	**4 (9%)** **5 (91%)**
Female	**2 (100%)**	**3 (93%)** **1 (7%)**	**3 (8%)** **4 (46%)** **5 (46%)**

From Lake Michigan

	Fall	Winter	Spring
Male	**1 (56%)** **2 (44%)**	**1 (23%)** **2 (54%)** **3 (23%**	**4 (17%)** **5 (83%)**
Female	**2 (100%)**	**1 (39%)** **2 (5%)** **3 (56%)**	**3 (50%)** **4 (50%)**

Dr. John Harshbarger – Selected Fish Tumor Histology

Dr Harshbarger presented slides of various types of fish tumors that were not included by earlier speakers. Nephroblastoma, a kidney cancer, was emphasized because the human equivalent (Wilm's tumor) is a major solid cancer in children harboring paired mutant recessive Wt suppressor genes. The Wt gene locus is also present in fish. Kidneys are formed by epithelio-mesenchymal interactions. In the embryo, kidney development proceeds from the paired growth of anterior-posterior (pro-, meso- and meta-) nephric ducts. The mesonephric portion of the ducts becomes the mesenephric kidney of fish. The mesonephric duct sends out buds that induce adjacent mesenchyme to form nephrogenic blastema from which the glomerulus (filter apparatus), Bowmans capsule, neck segment and proximal convoluted tubule differentiate. The proximal convoluted tubule joins the distal convoluted tubule arising from the mesonephric bud and emptying into the nephric duct or ureter via collecting ducts. Tumors that arise from the epithelial cells of the convoluted tubules are adenocarcinomas while tumors arising from the nephrogenic blastema are nephroblastomas. Grossly, nephroblastomas in fish are dorsal, nodular, retroperitoneal masses (Fig 1 showing the cut surface). Histologically, nephroblastomas are caracatures of the kidney. They consist of undifferentiated renal blastema, poorly formed glomeruli, and poorly formed renal tubules (Fig 2). They are usually devoid of hematopoietic tissue found in the interstitium of normal fish kidney (Fig 3). Some nephroblastomas produce aberrant tissues such as cartilage and muscle. Spontaneous and chemically induced nephroblastoma are better known in fish than renal adenocarcinoma.

Epidermal papillomas have been reported on white sucker from numerous sites in the Great Lakes watershed. The tumors are raised, often cauliflower like and can occur anywhere on the body with a predilection for the lips (Fig 4). Many of the sites are obviously polluted with anthropogenic chemicals consistent with a chemical etiology for the tumors; however, other sites where white sucker papillomas are abundant, such as the mouth of tributaries along the north shore of Lake Superior, are relatively uncontaminated except for the use of larval lampricides, especially trifluoromethyl-4-nitrophenol (TFN). This supports studies suggesting that larval lampricides such as TFN are carcinogenic. Monitoring of sentinel fish exposed to lampricides for skin and liver neoplasms should be intensified and experimental exposures conducted in the laboratory.

Peripheral nerve sheath cell neoplasms are frequently encountered on the surface of fish as well as internally. Peripheral nerves consist of fascicles of axons individually insulated with a myelin sheath or endoneurium, deposited by Schwann cells of neural crest origin. Each fascicle is sheathed in a perineurium of Schwann cells and fibrocytes. The nerve trunk is made up of fascicles sheathed in an epineurium of Schwann cells, fibrocytes, and other cell types. Peripheral nerve sheath cell tumors occur singly or as multiple nodules along a nerve similar to neurofibromatosis in humans. They consist of spindle cells, often with palisades of nuclei and the tumors are often associated with nerves and forming caricatures of nerves (Fig 5). They are histologically variable due in part to whether the tumor arose in the endo-, peri- or epineurium, the variable presence of fibrocytes and the potential of Schwanns cell for variable expression such as to occasionally produce pigment. Non-invasive tumors are often diagnosed as schwannoma or neurofibroma. Invasive tumors are often diagnosed as Malignant Peripheral Nerve Sheath Tumors (MPNST) (Fig 6).

Carp and goldfish naturally hybridize in the Great Lakes and it is not uncommon to find a hybrid with a visceral mass originating from the gonad. Similar masses can originate in cultured fancy carp, which are also hybrids (Fig 7). The masses are usually encapsulated and seldom invade organs other than the gonad. Residual normal appearing gonad might be present but it has been reported that these fish are sterile. Histologically, a mass can vary with the plane of section and can contain both germinal and stromal elements, but the later usually predominates. Germinal elements can represent either gender, regardless of the gender of any normal appearing gonad tissues present. Non-gonadal tissues, such as muscle and nerve, have been reported. Diagnoses often reflect the composition of the section that was examined and thus may not be representative of the entire mass. Diagnoses have included Sertoli cell tumor, granulosa-theca cell tumor, gonadal tumor, germinoma or dysgerminoma, gonadoblastoma and teratoma. While most people have considered these masses to be neoplasms and alternative speculation has been offered that at least some of these masses arise as abortive attempts at gonad development in hybrid animals with prohibitively mismatched genes from the two parents. If that speculation prevails, these so called hybrid carp x goldfish gonadal tumors are developmental or teratoid anomalies that sometimes give rise to a neoplasm.

Hematopoietic neoplasia is epizootic in northern pike and muskellunge in some rivers and lakes of the Great Lakes drainage area. Pike lymphoma was first reported from that area in 1898, well before the use of synthetic organic chemicals. It is also epizootic in some populations of northern pike in Ireland and in the Baltic Sea. Lesions arise anywhere in the skin as laterally expanding raised patches and as fleshy lesions that are often ulcerated (Fig 8). Lesions consist of sheets of uniform cells (Fig 9) that invade the underlying muscle and ultimately metastasize to viscera and other organs. Maturity of the tumor cells varies little within a population of fish but can vary considerable between populations. The cell of origin is a tissue histiocyte and the tumor is a True Histiocytic Lymphoma. The disease can be transmitted through fish to fish contact and by cell free extract. Virus-like C-type particles bud from tumor cell plasma membranes and reverse transcriptase has been reported. These observations indicate that the lymphoma of northern pike and muskellunge has a retroviral etiology.

Fibrous connective tissue tumors are illustrated grossly by a whitefish fibrosarcoma (Fig 10) and a coho salmon fibrolipoma (Fig 11). A brown bullhead melanoma is an example of a pigment cell tumor (Fig 12).

The above examples indicate that neoplasms common to fish in the Great Lakes region arise from a spectrum of cell types in a variety of species. Many of the neoplasms have a chemical etiology but some are viral and others have a genetics basis. Since neoplasms arise from cells, it is of critical importance to diagnose tumors at the cellular level using histology. Even then it is sometimes difficult to accurately interpret the origin of a lesion and predicting its behavior.

Dr. John Harshbarger's Slide Presentation

Figure 1: Gross Nephroblastoma

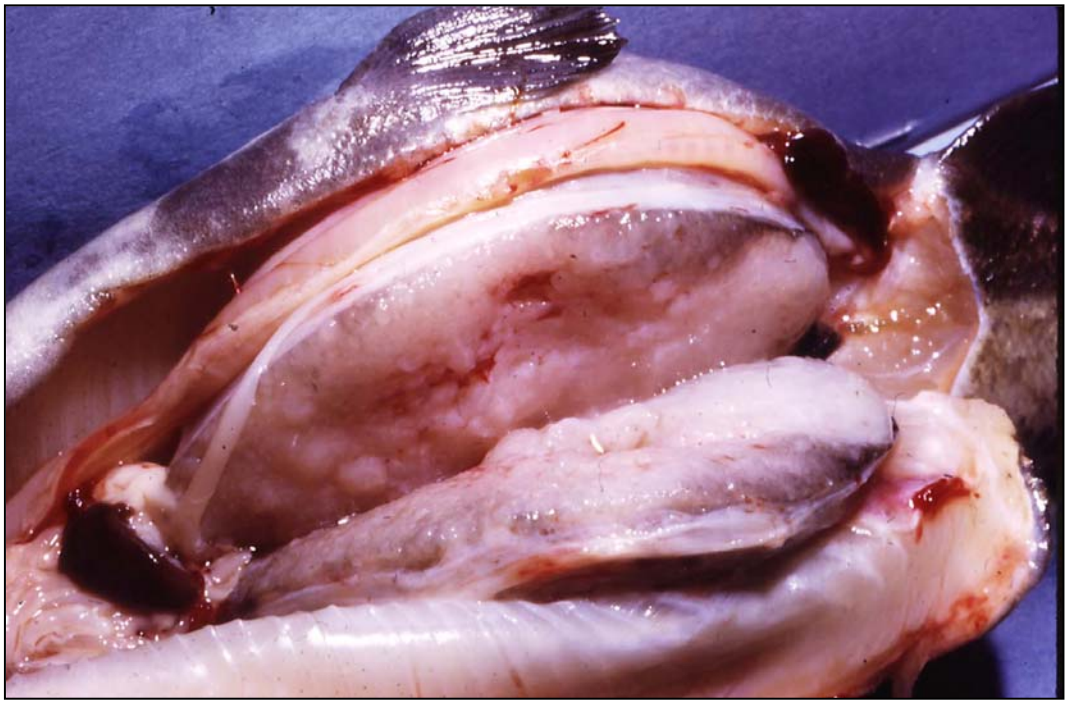


Figure 2: Histopathology of a Nephroblastoma

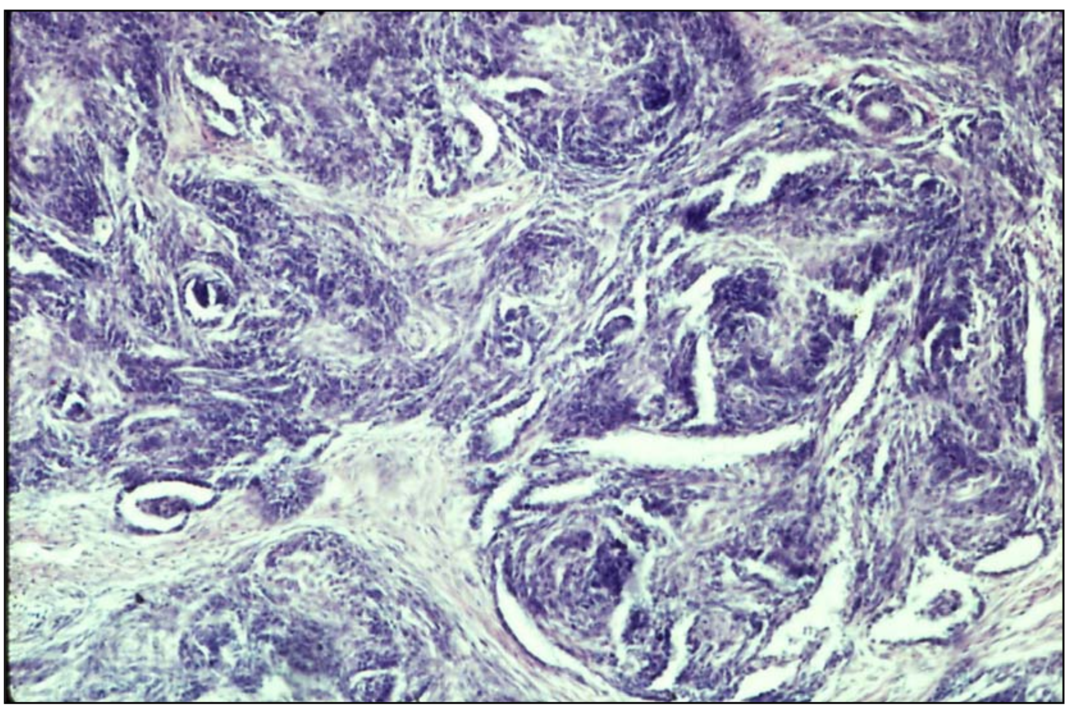


Figure 3: Histopathology of Normal Kidney Tissue in Fish

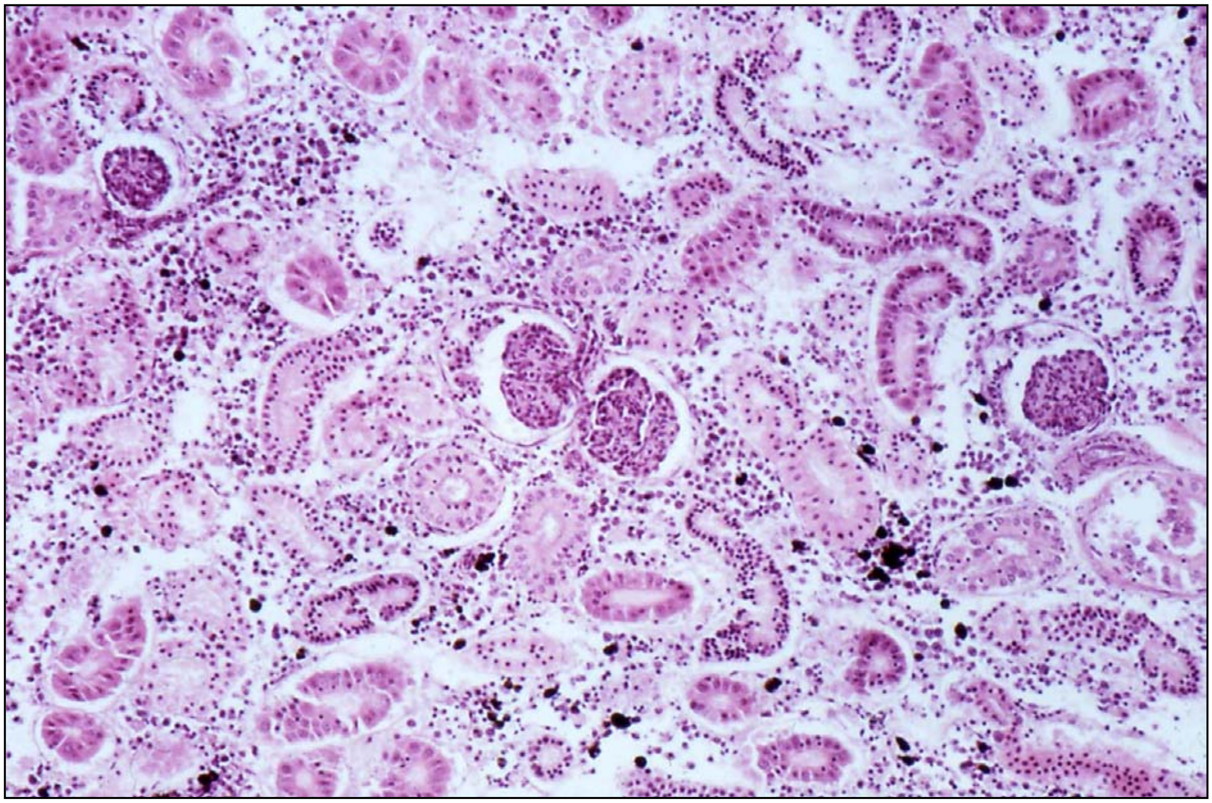


Figure 4: Gross Lip Papilloma on a White Sucker

Figure 5: Histopathology of a Peripheral Nerve Sheath Cell Neoplasm in a Chinook Salmon

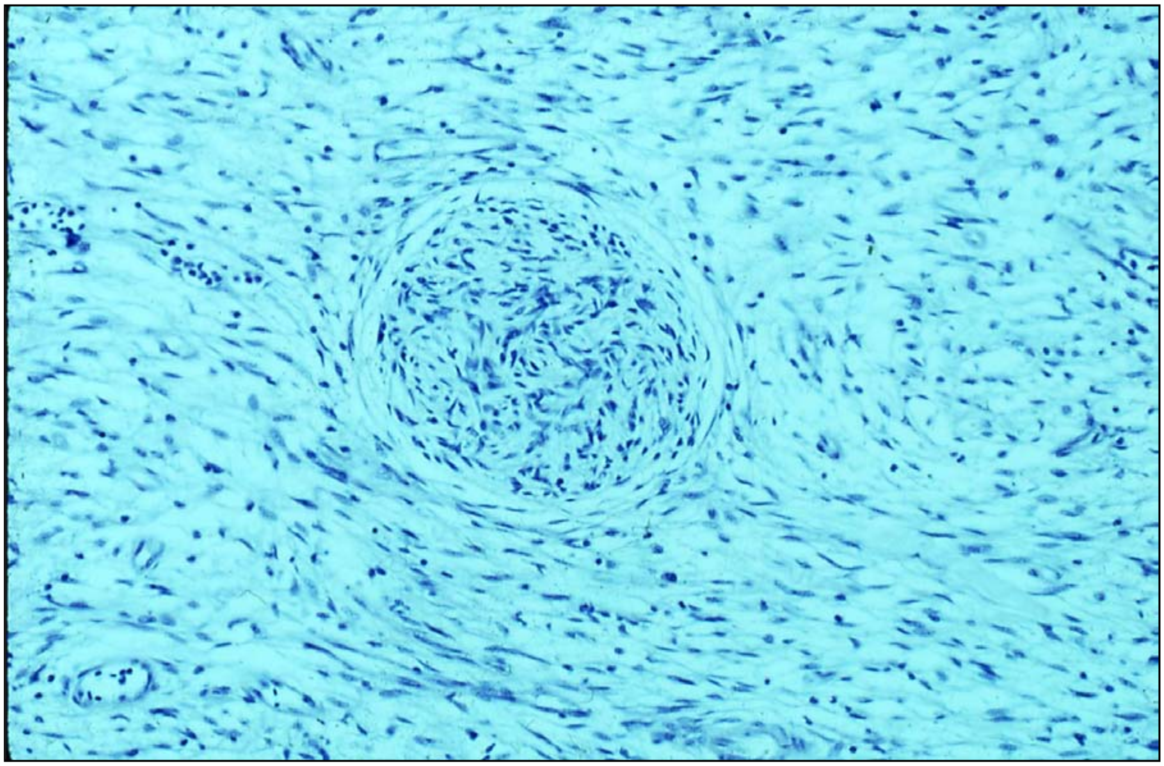


Figure 6: Histopathology of a Malignant Peripheral Nerve Sheath Tumor (MPNST) in a Coho Salmon

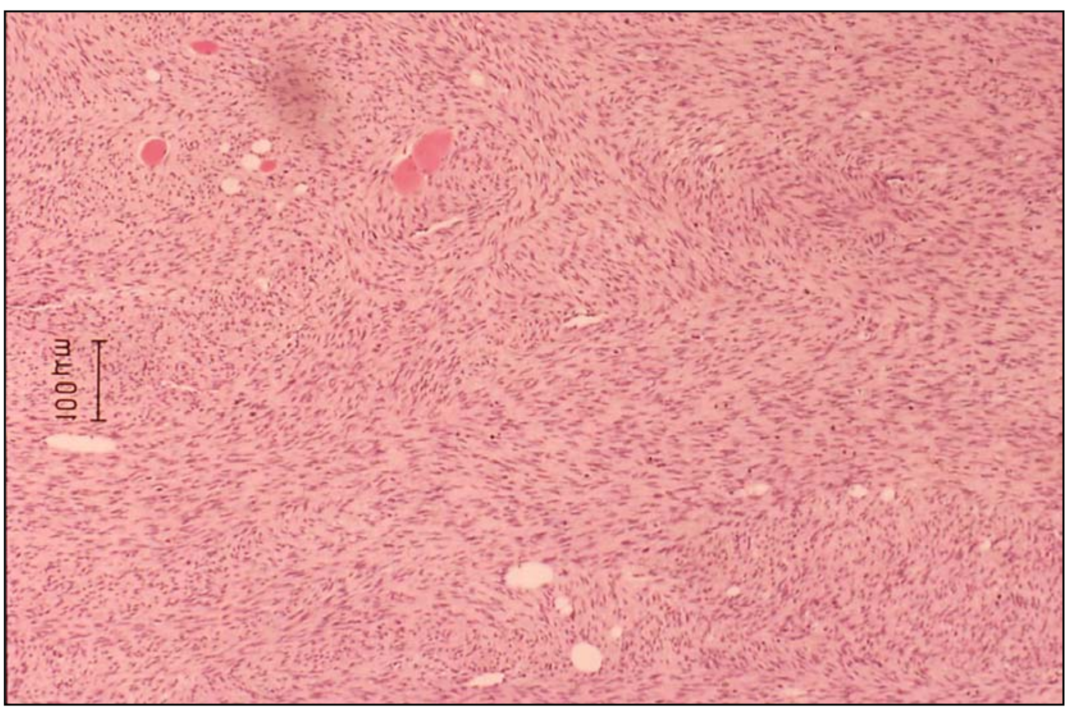


Figure 7: Gross Gonadal Tumor in a Carp

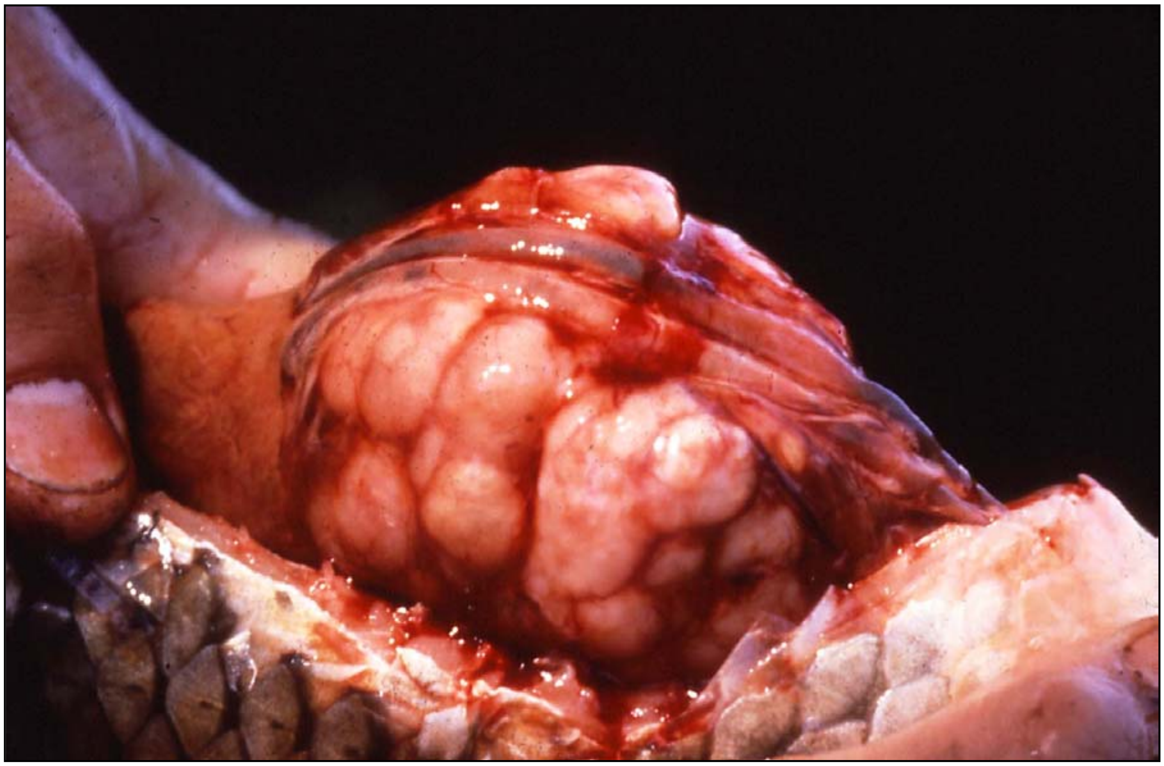


Figure 8: Lymphoma on a Northern Pike

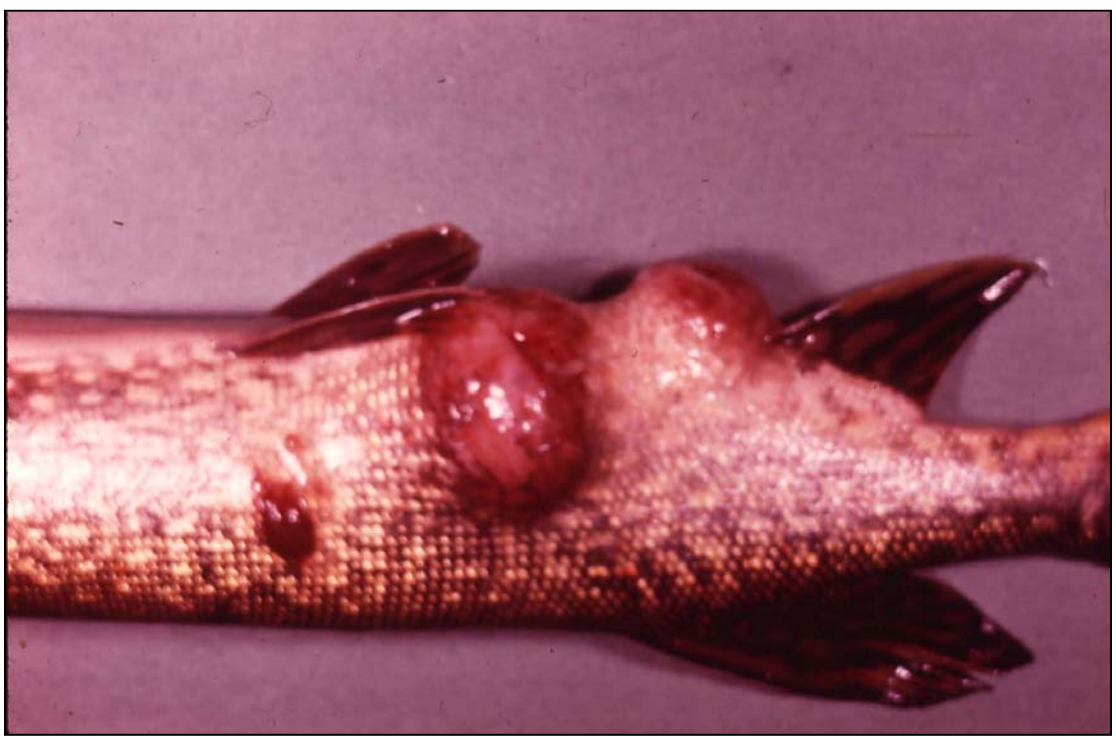


Figure 9: Histopathology of a Lymphoma on a Northern Pike

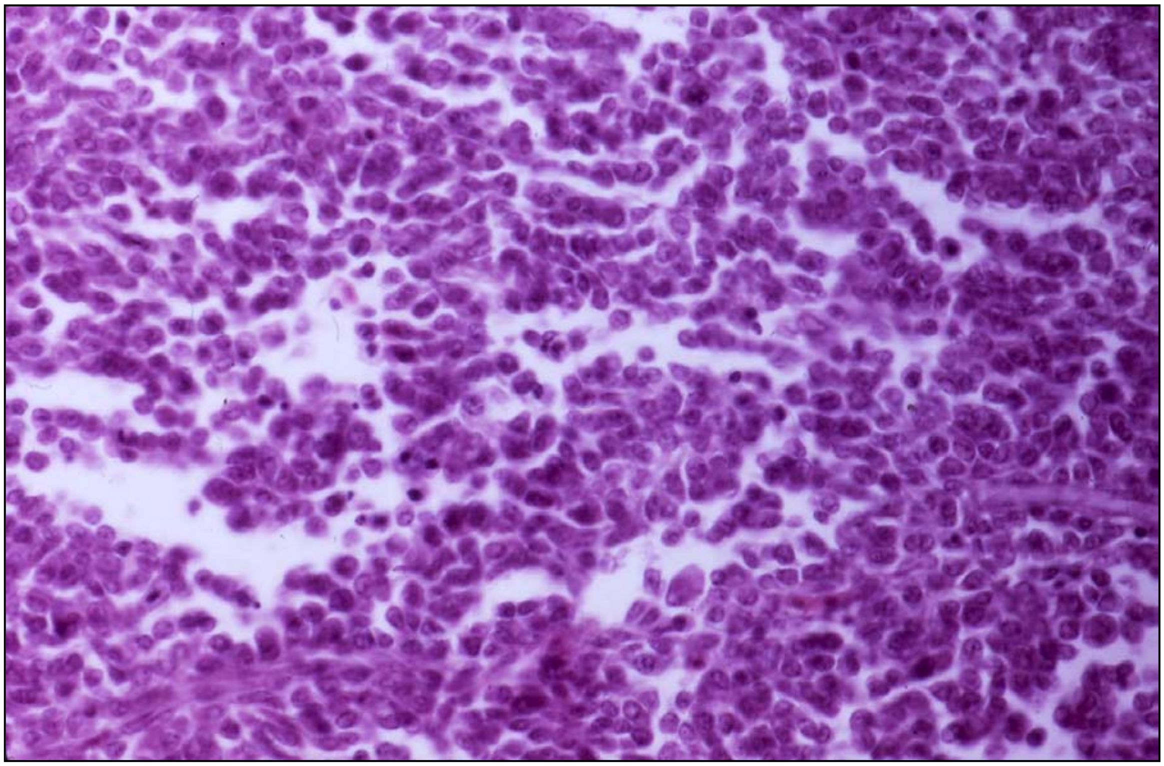


Figure 10: Gross Fibrosarcoma in a Whitefish

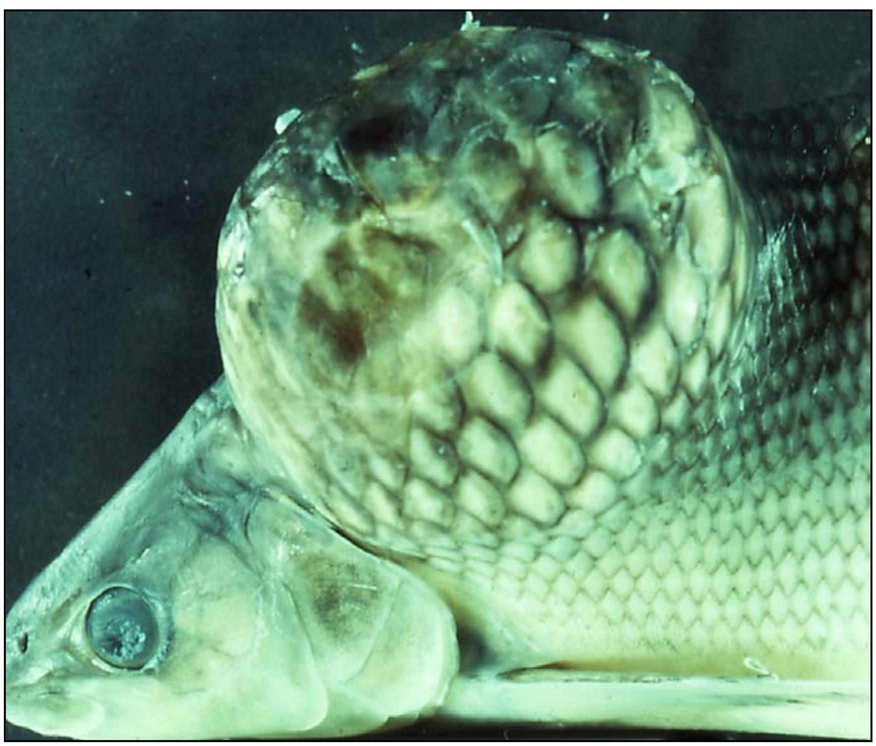


Figure 11: Gross Fibrolipoma in a Coho Salmon

Figure 12: Gross Melanoma on a Brown Bullhead

Dr. Fred Pinkney - Tumor Prevalence and Biomarkers of Exposure and Response in Brown Bullheads

Dr. Pinkney discussed the relationship between tumor prevalence and biomarkers of exposure and response in brown bullheads. Biomarkers are defined as physiological, biochemical, or histological changes that are used as indicators of chemical exposure and/or effects. The presentation focused on bullhead studies in the Chesapeake Bay watershed, experience with biomarkers, and future research ideas. Objectives for the USFWS include: use of bullheads as an indicator of habitat quality, develop weight of evidence for specific classes of chemicals and recommend remedial action, and monitor the recovery of ecosystems.

In 1992, a study was conducted in regard to fish tumor prevalence in three tributaries of the Potomac River (Neabsco Creek, Marumsco Creek and Farm Creek). Tumor analysis was conducted on fish 280 mm and greater, histopath of liver and skin lesions was performed, and sediment was tested for PAHs and metals. The results suggested that Neabsco Creek had the highest percentage of liver and skin tumors. The results in this study provided a stepping-stone to the 1996 Tidal Potomac Watershed Survey. The objectives of this survey were to determine tumor prevalence at several tidal Potomac River locations including Neabsco Creek and the Anacostia River, and to examine possible associations between tumor prevalence and indicators of contaminant exposure. Contaminants in the sediment and tissues of the bullheads were analyzed and biomarkers were used to link contaminants to tumor prevalence.

Dr. Pinkney outlined the relationship of biomarkers of PAH exposure and emphasized that biomarkers can be used to analyze the effects of specific PAHs. Bile metabolites are needed because PAHs are rapidly metabolized and do not accumulate in the tissue of bullheads, they provide evidence of recent exposure (days), display seasonal differences, and give insight to the feeding status of bullheads. Ethoxyresorufin-O-deethylase (EROD) is a biomarker for the exposure of bullheads to a variety of compounds, including: PAHs, PCBs, PCDDs, PCDFs, and persistent and non-persistent chemicals. EROD can be used for several species of fish and varies according to sex, season, temperature, reproductive activity and diet.

Results of the 1996 Tidal Potomac Watershed Survey included: in the Anacostia River, 55% of bullheads exhibited liver tumors and 23% had skin tumors; bile metabolites were twice the reference in the fall, and 10 times in the spring; EROD was one-and-a-half times the reference in the fall, and twice the reference in the spring. From the regression analysis, it was suggested that age, hepatosomatic index (HSI), and bile metabolites were significant risk factors for liver and skin tumors. Hepatosomatic index (HSI) is the proportion of liver weight to total body weight, expressed as a percent. Enlarged livers can occur in fish exposed to trace metals and organic pollutants.

Following the onset of restoration efforts in the Anacostia River, a study was conducted from 2000 to 2001 in which tumors and biomarkers were compared in two age classes, and statistical associations between biomarkers and tumors were examined. Two size classes were examined (150-225 mm and $\geq$260 mm), sediment data from a related study were used, tissue organochlorines and PCBs were analyzed in large fish only, and bile metabolites and DNA adducts were analyzed. DNA adducts are simply explained as bulky material that attaches to DNA; the advantages of using DNA adducts are: they represent early steps in the cancer process, increase with increased exposure, and are diagnostic for specific chemicals. The limitation on the use of DNA adducts include: differences between species and the occurrence of DNA repair.

Results of the 2000-01 Anacostia-Tuckahoe Survey included: large bullheads had 50-68% prevalence of liver tumors; 10-17% prevalence of tumors in small fish; there were similar concentrations of DNA adducts among large and small Anacostia bullheads; DNA adducts were approximately 16-28 times greater in the Anacostia River; bile benzo(a)pyrene levels were 10-40 times higher in the Anacostia River. The logistic regression analysis suggested that sex, HSI, length, bile metabolites, tissue PCB, and tissue DDE are all significant risk factors for liver tumors, and HSI is a significant risk factor for skin tumors.

Along with tumor studies, an Anacostia bullhead movement study was conducted in 2000-01. Fish were implemented with ultrasonic transmitters and their movements were tracked for three seasons. The results suggested that none of the bullheads left the river, bullheads had a linear home range of 0.5 km in the summer, 1.5 km in the fall-winter and 2.1 km in the spring, and adult bullheads are resident.

Dr. Pinkney concluded his presentation by addressing the current lines of thinking in relation to biomarkers, DNA adducts and assays, and by suggesting future research areas including: comet assay versus histopathology in young and old fish of the Anacostia River, tumor prevalence in Potomac River areas near the Anacostia River, and the relationships between tumors and population structure. Comet assay or single cell gel (SCG) electrophoresis is a rapid and very sensitive fluorescent microscopic method to examine DNA damage and repair at the individual cell level, and has critically important applications in fields of toxicology.

Dr. Fred Pinkney's Power Point Presentation

Tumor Prevalence and Biomarkers of Exposure and Response in Brown Bullheads

Fred Pinkney
U.S. Fish and Wildlife Service
Chesapeake Bay Field Office
Annapolis, MD
January 2003

Acknowledgements

- **Funding: U.S. Fish and Wildlife Service, D.C. Dept. of Health, Anacostia Watershed Toxics Alliance**
- **Collaborators: John Harshbarger (GWU), Mark Melancon (PWRC), Bill Reichert (NOAA), Eric May, Roman Jesien and Pete Sakaris (UMES)**
- **Assistance: Peter McGowan and Beth McGee**

Biomarker:

Physiological, biochemical or histological change used as an indicator of chemical exposure and/ or effects.

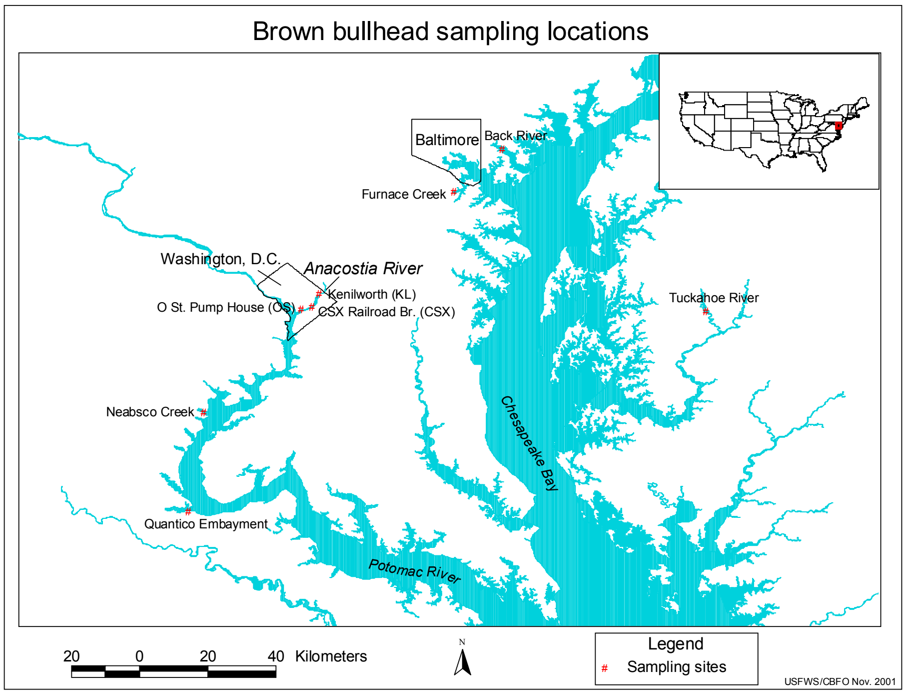
* **Cytochrome P450**
* **Bile Metabolites**
* **DNA adducts**

Overview

- **Bullhead surveys in Chesapeake Bay watershed**
- **Experience with biomarkers**
- **Future directions**

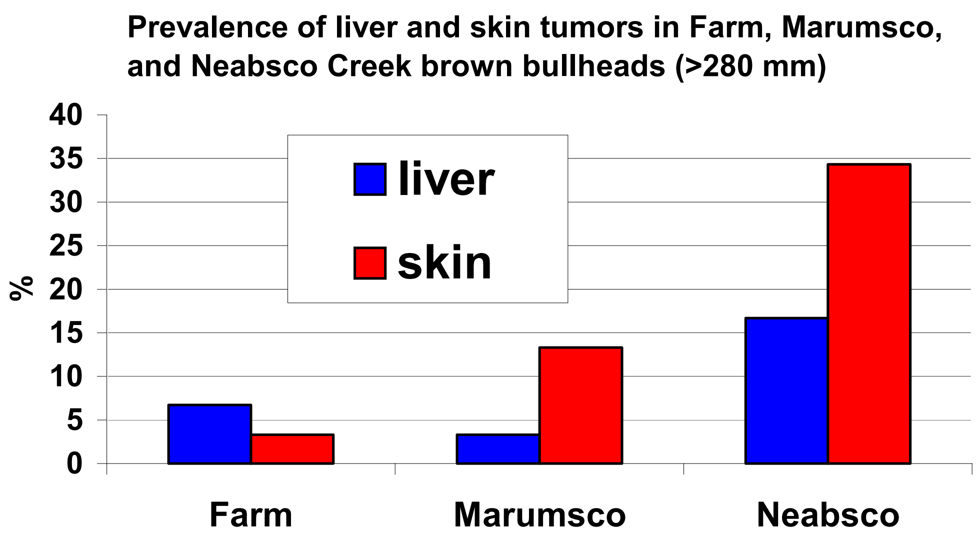
USFWS Objectives

- Protect trust resources-anadromous fish, migratory birds, endangered species and their habitats
- Use bullheads as an indicator of habitat quality
- Develop weight of evidence for specific classes of chemicals and recommend remedial action
- Monitor recovery of ecosystem



1992 – Neabsco Creek

- Tidal tributary near Featherstone National Wildlife Refuge
- Tumor analysis: 30 fish (280mm +) - Farm, Neabsco, Marumsco Creeks (within 4km)
- Histopathology – all livers and visible skin lesions
- Sediment: PAHs and metals
- Pinkney et al. 1995. USFWS, CBFO-C95-02



Objectives

1996 Tidal Potomac Watershed Survey

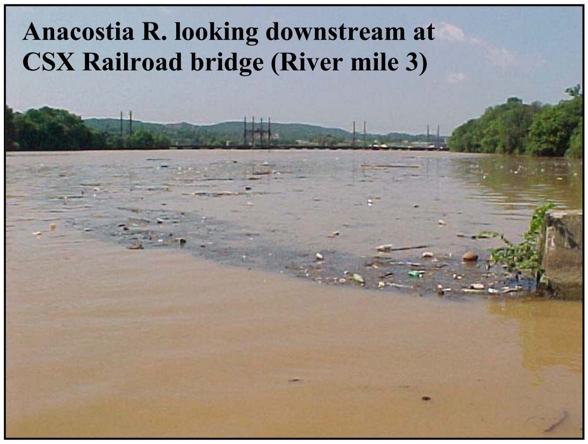
- Determine tumor prevalence at several tidal Potomac R. locations including the Neabsco Creek and the Anacostia River
- Examine possible associations between tumor prevalence and indicators of contaminant exposure
- Pinkney et al. 2001. Env. Tox. Chem. 20: 1196 - 1205

Design

- 4 locations: 30 fish (260mm +), Anacostia River spring and fall
- Sediment: PAHs, PCBs, and Organochlorines
- Tissues: PCBs, Organochlorines
- Cytochrome P450 as EROD
- Bile: Fluorescent metabolites
- Logistic Regression

Anacostia River

- 8 mile freshwater tidal tributary of Potomac through poorer sections of Washington, DC
- Chesapeake Bay Region of Concern
- Fish advisory – no consumption of bottom feeding fish due to PCBs and chlordane
- Focus on economic redevelopment and river restoration



Relationship of Biomarkers of PAH Exposure

Benzo(a)pyrene → Bile Metabolites

↓ CYPIA as EROD

Diol epoxide

↓

Repair → Mutations → Oncogene activation → Tumor Formation

Bile PAH Metabolites

- Needed because PAHs are rapidly metabolized and do not accumulate in muscle tissues
- Provides evidence of recent exposure (days)
- Seasonal differences
- Feeding status
- Critical exposure may have been years before collection

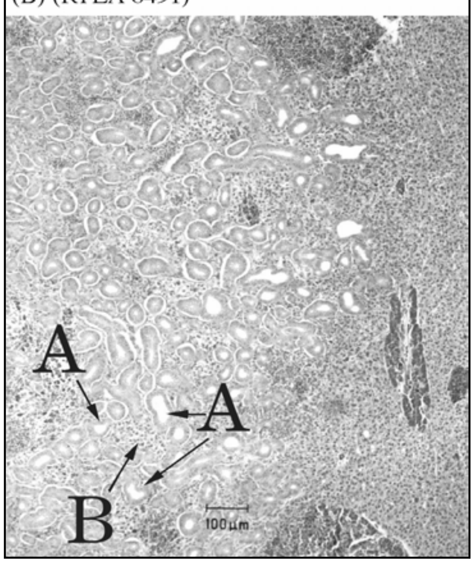
EROD

- Biomarker for exposure to wide range of compounds: PAHs, PCBs, PCDDs and PCDFs
- Persistent and non-persistent chemicals
- Many species
- Sex, Season, Temperature
- Reproductive activity and Diet
- Linkage with toxicity still being studied
- Critical exposure much earlier

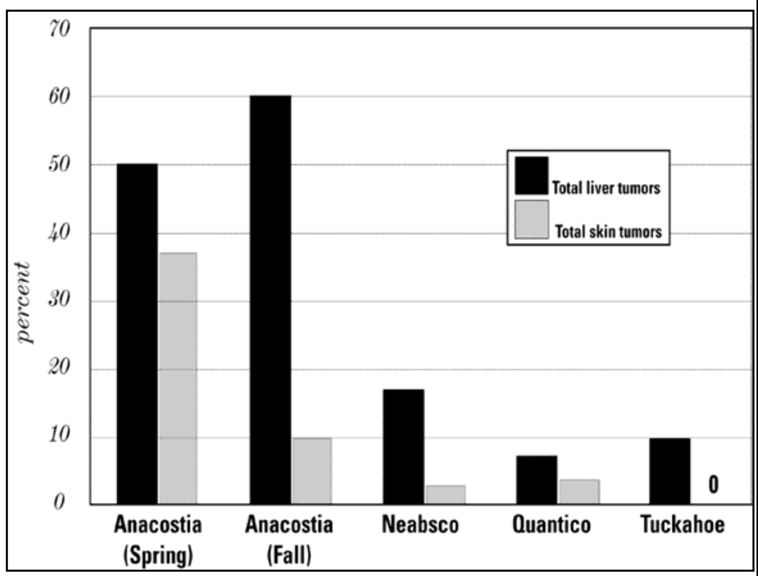
Results:

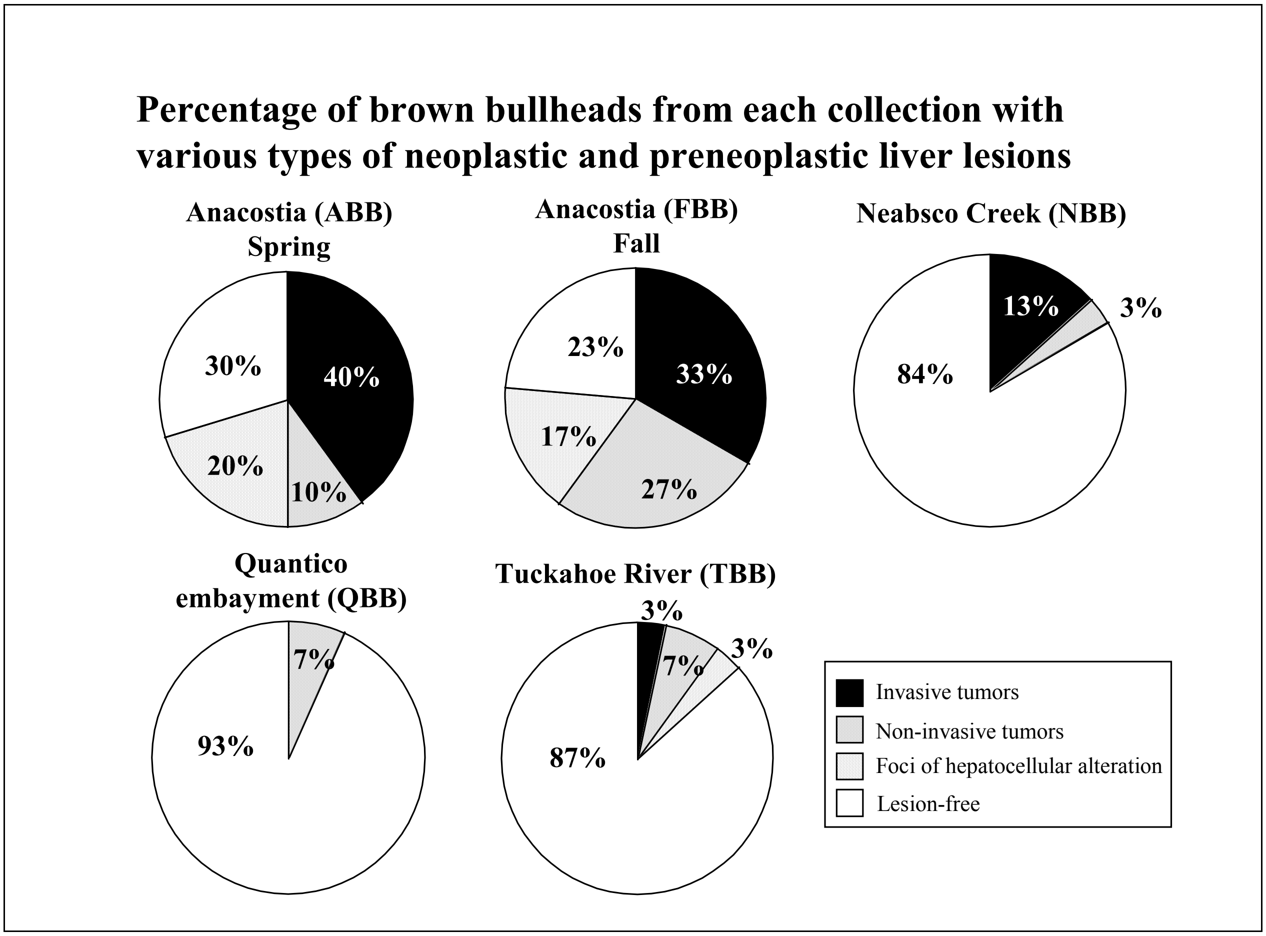
1996 Tidal Potomac Watershed Survey

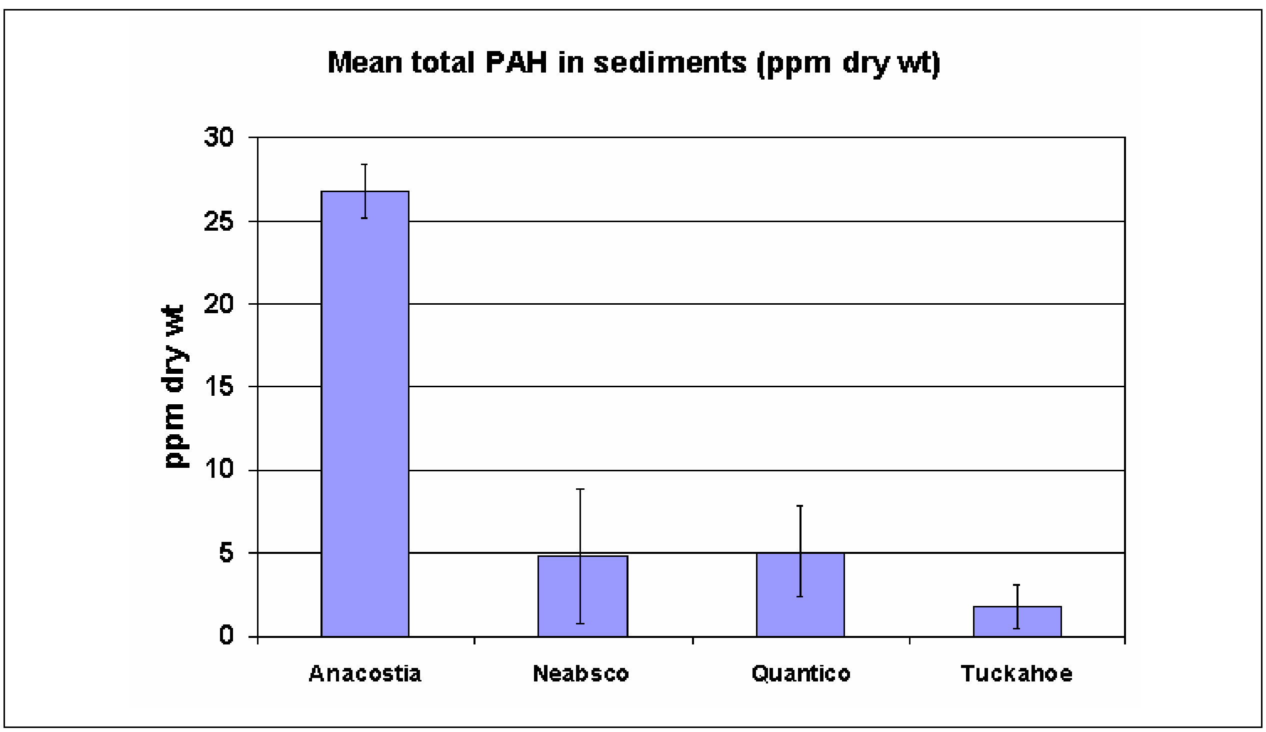
Figure 6. Cholangiocarcinoma: neoplastic bile ducts (A) entrapping normal liver tissue (B) (RTLA 6491)

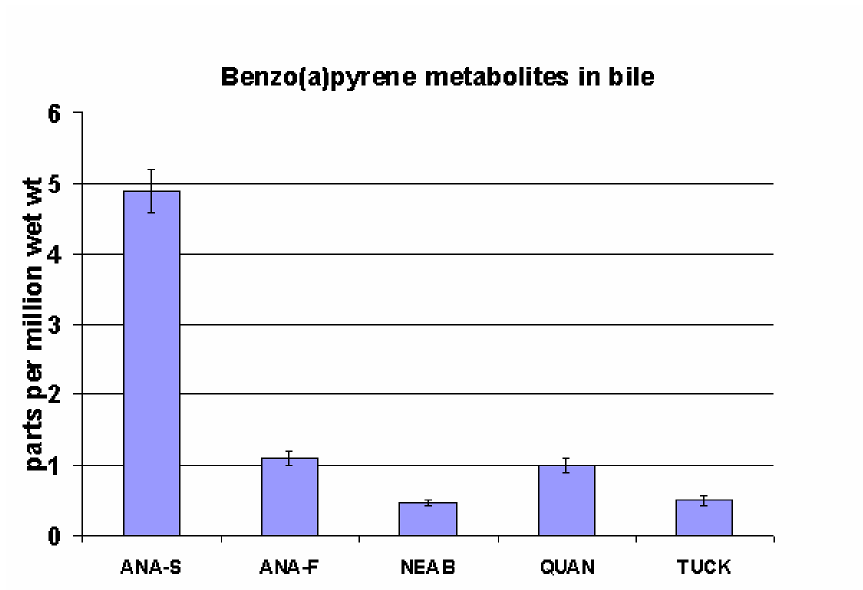


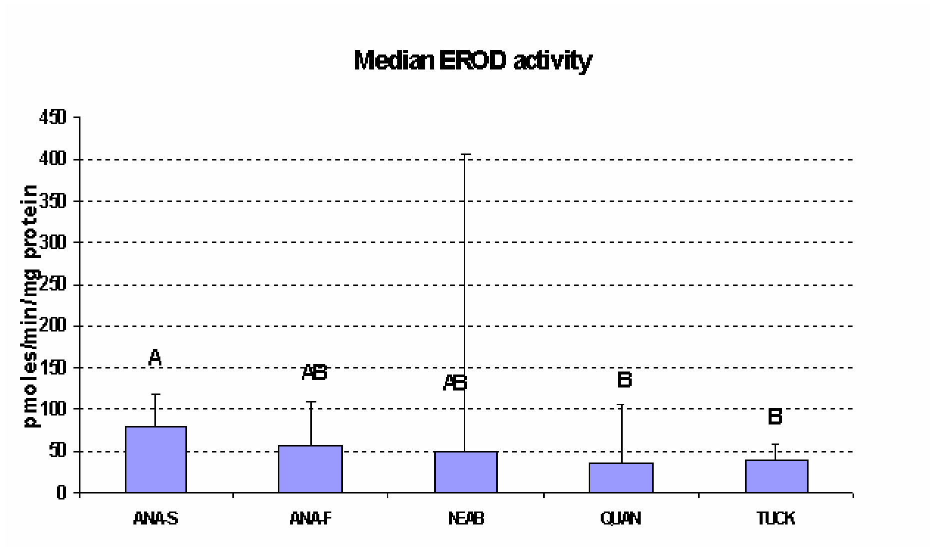
1996 Tidal Potomac Watershed Survey




Percentage of brown bullheads from each collection with various types of neoplastic and preneoplastic liver lesions
Anacostia (ABB) Spring
40%
10%
20%
30%
Anacostia (FBB) Fall
33%
27%
17%
23%
Neabsco Creek (NBB)
13%
3%
84%
Quantico embayment (QBB)
7%
93%
Tuckahoe River (TBB)
3%
7%
3%
87%
Invasive tumors
Non-invasive tumors
Foci of hepatocellular alteration
Lesion-free


Mean total PAH in sediments (ppm dry wt)
ppm dry wt
30
25
20
15
10
5
0
Anacostia
Neabsco
Quantico
Tuckahoe





Key Findings

1996 Tidal Potomac Watershed Survey

- **Anacostia: avg 55% liver and 23% skin**
- **Bile Metabolites: Fall 2X reference and Spring 10X reference**
- **EROD: Fall 1.5X reference and Spring 2X reference**
- **Regression: age, HSI, bile metabolites are significant risk factors for liver and skin tumors; sex for liver only**

Objectives

2000 – 01 Anacostia -Tuckahoe Survey

- **In depth study of Anacostia**
- **Compare tumors and biomarkers in two age classes**
- **Examine statistical associations between biomarkers and tumors**

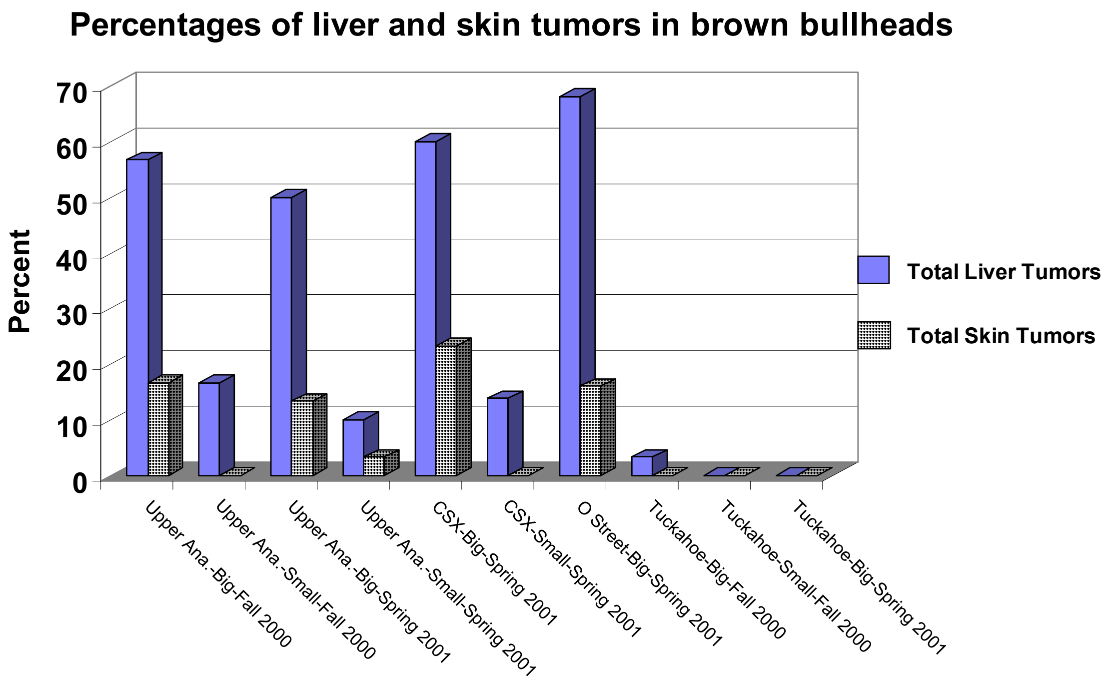
Design

2000 – 01 Anacostia -Tuckahoe Survey

- **30 fish/site, 2 size classes: 260mm + and 150-225mm; 3 Anacostia locations**
- **Spring and fall sampling**
- **Sediment data from related studies**
- **Tissue organochlorines/ PCBs (large fish only)**
- **Bile metabolites**
- **DNA adducts**
- **Pinkney et al. 2002. USFWS, CBFO-C02-07**

DNA Adducts

- **Advantages**
  - **Early step in cancer process**
  - **Increases with increased exposure**
  - **Diagnostic for specific chemicals**
- **Limitations**
  - **Species differences**
  - **DNA repair**



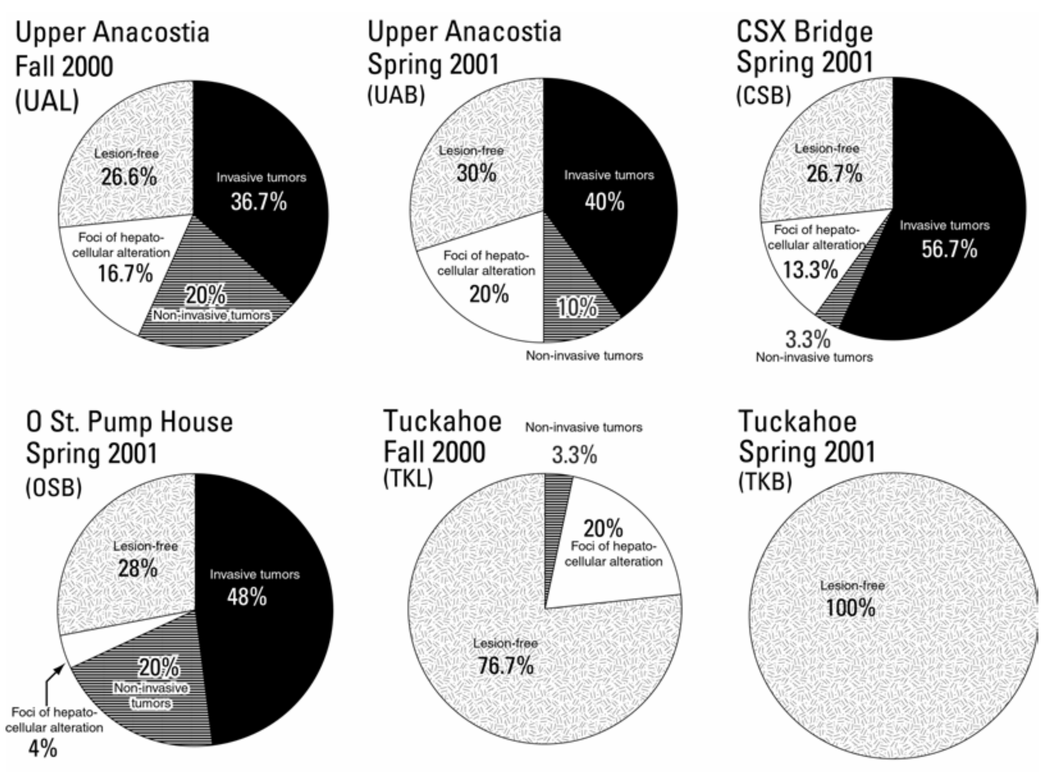
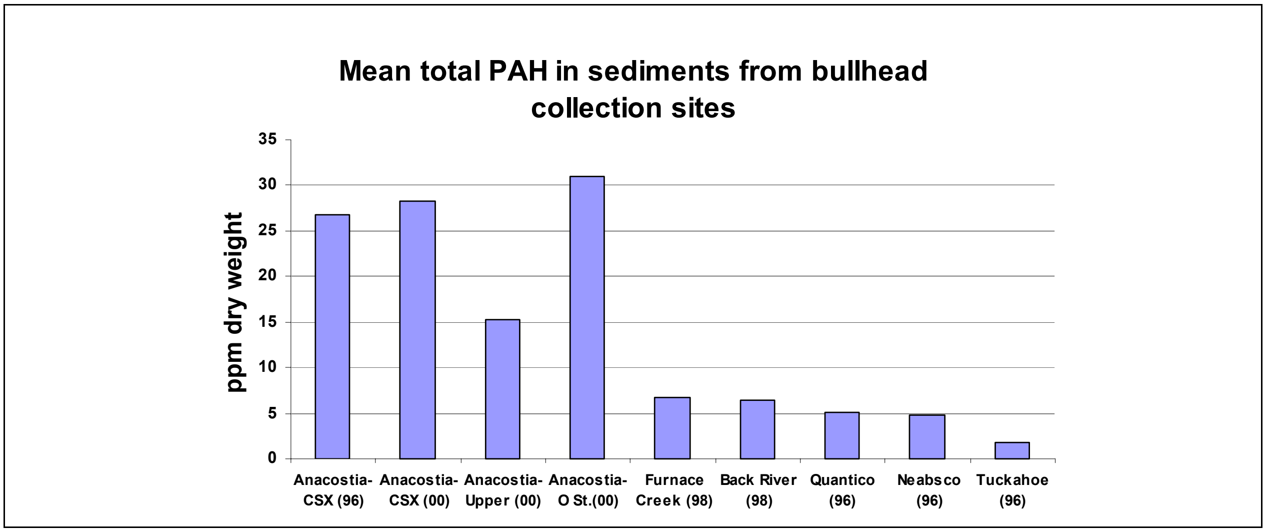
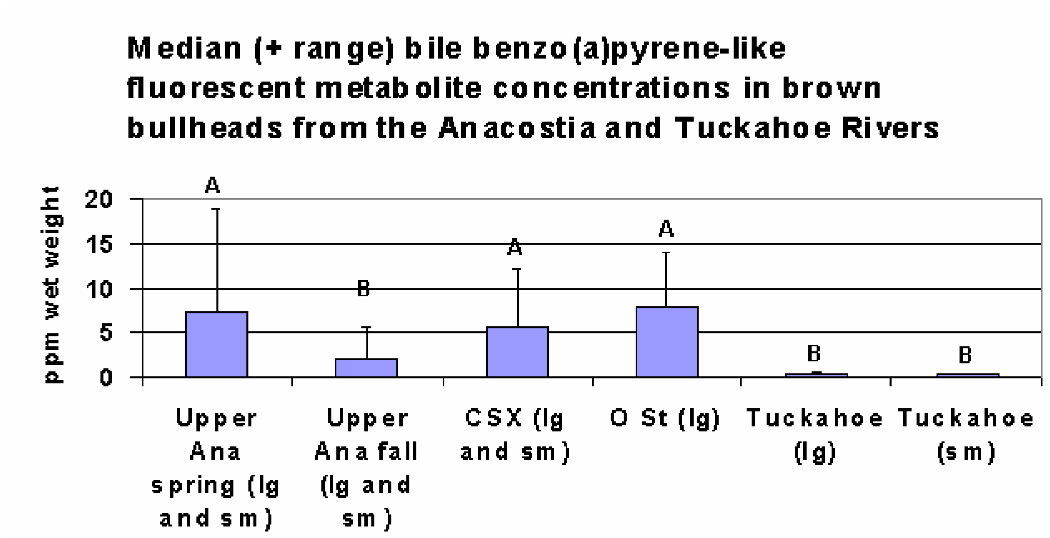
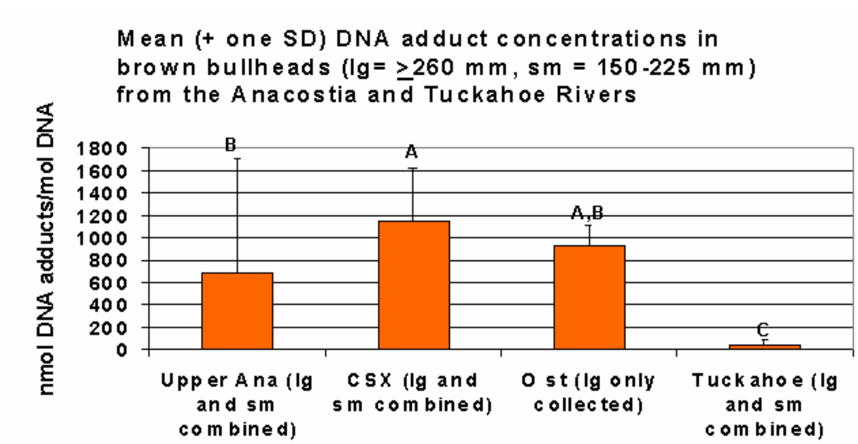
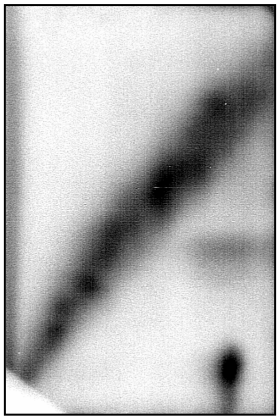


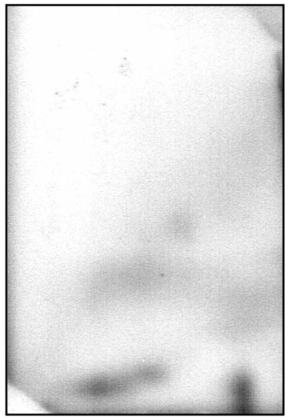
Figure 3: Percentages of 260 mm+ brown bullheads from the Anacostia and Tuckahoe Rivers with various types of neoplastic and preneoplastic liver lesions

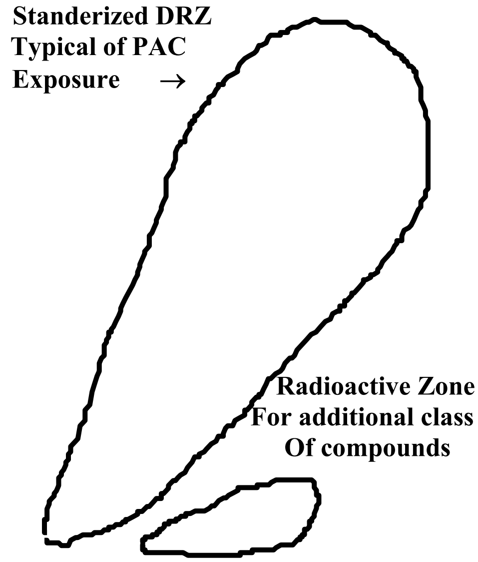

Mean total PAH in sediments from bullhead collection sites
ppm dry weight
35
30
25
20
15
10
5
0
Anacostia- CSX (96)
Anacostia- CSX (00)
Anacostia- Upper (00)
Anacostia- O St.(00)
Furnace Creek (98)
Back River (98)
Quantico (96)
Neabsco (96)
Tuckahoe (96)


Median (+ range) bile benzo(a)pyrene-like fluorescent metabolite concentrations in brown bullheads from the Anacostia and Tuckahoe Rivers
ppm wet weight
20
15
10
5
0
A
B
A
A
B
B
Upper Ana spring (lg and sm)
Upper Ana fall (lg and sm)
CSX (lg and sm)
O St (lg)
Tuckahoe (lg)
Tuckahoe (sm)


Mean (+ one SD) DNA adduct concentrations in brown bullheads (lg= >260 mm, sm = 150-225 mm) from the Anacostia and Tuckahoe Rivers
nmol DNA adducts/mol DNA
1800
1600
1400
1200
1000
800
600
400
200
0
B
A
A,B
C
Upper Ana (lg and sm combined)
CSX (lg and sm combined)
O st (lg only collected)
Tuckahoe (lg and sm combined)







Representative chromatograms of DNA adducts in livers of brown bullheads from the Anacostia and Tuckahoe rivers and schematic. (DRZ = diagonal radioactive zone; PAC = polycyclic aromatic)

Key Findings

2000 – 01 Anacostia – Tuckahoe Survey

- **50 – 69% liver tumor prevalence in large (3+) Anacostia bullheads**
- **10 – 17% prevalence in small Anacostia fish (these age classes at great risk for tumors)**
- **Similar concentrations of DNA adducts in large and small Anacostia fish**
- **16 – 28 times greater in Anacostia vs. Tuckahoe**
- **Bile benzo(a)pyrene: 10-40X reference**
- **Logistic regression**
    * **Liver tumors: sex, HSI, age, length, bile metabolites, tissue PCB and DDE are significant risk factors**
    * **Skin tumors: HSI**

USFWS Objectives

Use Bullheads as an indicator of habitat quality

P.C. Sakaris, R.V Jesien,
A.E. Pinkney. 2002
USFWS, CBFO - C0

Seasonal movement patterns, home ranges, and habitat use of the brown bullhead in the Anacostia River

2000 – 2001 Anacostia Bullhead Movement Study

- Ultrasonic transmitters in three batches of 10 – 18 bullheads
- 3 Seasons: ~ 80- 90 days per season
- None detected leaving the river
- Linear home range: 0.5km in summer, 1.5km from fall to winter and 2.1km in spring
- More movement in period leading to spawning
- Bottom line – adults are resident

Current Thinking

- Use biomarkers showing genotoxicity with younger fish to estimate critical period
- DNA adducts advantageous in specificity for chemical classes but time consuming, expensive
- Comet assay and other gentic assays as indicators of response – non-destructive, quicker? Cheaper? Closer to endpoint?
- Calibrate against histopathology

Research Areas

- Comet assay vs. histopathology in younger and older fish from Anacostia and Tuckahoe
- Tumor prevalence in Potomac River areas near the Anacostia
- Relationship between tumors and population structure

Panel Discussion – Histology Diagnosis and Criteria

Vicki Blazer, John (Jack) Fournie, John Harshbarger, and Alfred Pinkney

Question:
Bullheads tend to have a higher bile concentration in the spring. Are they more active in the fall because the water is warmer or in the spring because of variation in water parameters?
Answer: (Alfred Pinkney)
Yes, bullheads are more active and metabolism is increased in the spring, and they feed more actively on "things that are dirty" in the spring because there is more runoff and the bullheads situate near the outfalls.

Question:
Should the diagnostic criteria be standardized?
Answer: (Jack Fournie and Vicki Blazer)
Standardization of nomenclature and well defined diagnostic criteria are an essential aid to pathologists who evaluate fish studies because they promote diagnostic uniformity, allow for meaningful communication of results, permit comparisons of findings among studies, and encourage the formation of a useful historic database.

Question:
Can you make errors on identifying skin lesions or tumors if you do not send them for histology?
Answer: (Vicki Blazer)
Yes, it is very likely errors will occur. If the lesions are not examined microscopically there is absolutely no credibility in "diagnosing" the lesion. All you can do is describe the appearance and speculate on the cause, i.e., infectious versus mechanical injury versus neoplasia.

Question:
Do the criteria for Areas of Concern require a link to contaminated sediments? Does it make any difference to beneficial-use impairments?
Answer: (Vicki Blazer)
It is not always possible to make a direct link between certain sediment contaminants and some effects on fish health for many reasons. As Paul pointed out, underlying, heavily contaminated sediments may be covered with silt, etc. There may be hot spots that are missed during sediment sampling. It is important to have both sediment contaminant information and biological effects. Tumor production may require an initiator and a promoter, one of which we may not understand or measure for. It may be the metabolites of certain contaminants that cause problems; it may be a particular mixture of chemicals rather than high levels of one, and in some cases there may actually be fewer lesions at a contaminated site. Many of the grossly visible foci in the liver and other organs are actually parasitic and many of these internal parasites have intermediate hosts such as snails, benthic worms, etc., which sediment contaminants may wipe out.

Question:
Are bullheads more susceptible to fungal pathogens?

Answer: (Vicki Blazer)
There are certainly species differences in susceptibility to all infectious agents: fungal, viral, bacterial and parasitic. We do not know whether bullheads would be more susceptible to a particular fungal pathogen, than say bass. Some fungal and bacterial diseases are more likely to occur in contaminated sites because the immune system is already suppressed. We know many environmental contaminants are immunosuppressive and this is used as an earlier warning of potential problems.

Question:
Is there an available list in regard to the causes of fish tumors?
Answer: (Vicki Blazer and Jack Fournie)
Many chemicals have been shown to be carcinogenic in laboratory studies using a number of small fish species; however, most of the epizootics of neoplasia in wild fishes have been associated with PAHs. It is difficult to distinguish between the classes of compounds causing tumors, but it is known that multiple carcinogenic compounds are involved.

Question:
What does the IJC want from us? Are we developing a plan?
Answer: (Eric Obert)
We are not under any immediate pressure; however, we need to develop monitoring guidelines. We have six months to develop a monitoring plan for Presque Isle Bay.

Question:
Is the primary concern what the public sees on the fish?
Answer:
We spend $50 million to clean contaminated sediments, but then an angler pulls up a cancerous fish and he or she wonders where the money went and what other causes are involved.

Question:
There is a lot of evidence that fungal-induced growths occur in older fish. Is there a pollutant/immune system link that increases susceptibility to cancer? Is it age related?
Answer: (Vicki Blazer)
Actually it is not true that fungal lesions occur in older fish. All ages are susceptible to fungal pathogens. In most cases, but not all, fungi are opportunistic pathogens that will colonize damaged skin. There is good evidence that immune suppression is often associated with increased incidence of infectious disease. There is some evidence, although not much in fish, that suppression of the activity of certain cells, natural killer or cytotoxic cells, and certain soluble mediators may be associated with increased cancer incidence. We have added immune function assays to the suite of measurements we are doing in the Ashtabula study to start to address this question.

Question:
Is there a possibility of hybridization between yellow and brown bullheads in Presque Isle bay? Would they be more susceptible to tumors?
Answer: (John Harshbarger and Roger Thoma)
We don't know. Hybrid vigor does occur, but some hybrids have a higher risk of tumors. In

Ohio there are two areas with hybrids, Portage Lake and the Ohio River. In both populations there is little evidence of external tumors. When we counted anal fin rays, and looked at shape and coloration, OSU confirmed that there were no genetic differences. In the Cuyahoga River, 30% of the brown bullheads had tumors and there was less evidence of external lesions on yellow bullheads.

Question:
If macrophage aggregates are linked to PAHs, can different age classes be assessed?
Answer: (Vicki Blazer and Jack Fournie)
Yes, different age classes can and should be assessed. We know that macrophage aggregates increase with age so it is very important to have the age data. For instance, if you compare macrophage aggregates between sites and you have 6-9 year old fish at the reference site and 2-3 year old fish at the contaminated site you may not see a difference; whereas, if you compared fish of the same age there would be a large difference. Macrophage aggregates are a more cumulative indicator and particularly in migratory fish, the number of aggregates may have no relationship to the site they are collected from because the insult could have happened earlier.

Question:
Are there differences in macrophages in different organs?
Answer: (Vicki Blazer)
Yes, the spleen seems to be the best indicator, but there is no historic data available for comparison in the Great Lakes. Only liver samples have been taken.

Question:
Do macrophage aggregates change in testes? Can they be used as an environmental indicator, especially endocrine disruptors?
Answer: (Vicki Blazer)
There have been some reports of macrophage aggregates in testes and that there are more at contaminated sites than reference sites. However, there has been no detailed studies of the actual type of cells involved, i.e., are these pigmented cell accumulations really macrophage aggregates or not, the pigmented cell accumulations could most certainly be a good indicator of injury to the reproductive organs.

Question:
Do parasites promote tumors by causing damage, which increases susceptibility?
Answer: (Jack Fournie and John Harshbarger)
There is some good evidence for correlation between the presence of a parasite and bile duct cancer in humans, a nematode infection, and esophageal sarcomas in dogs.   Good evidence exists for fish to indicate a relationship between parasitic infection and tumor promotion; however, there is a recent report indicating a possible association between intestinal neoplasms and a nematode infection in zebrafish.

Question:
Parasites may cause cell regeneration. Could they produce mutated products?
Answer:
It's possible, but then parasites would be found embedded in the tumor.

Panel Discussion – Histopath Recommendations

Vicki Blazer, Jack Fournie, and John Harshbarger

Question:
What is the most important thing that is being overlooked (missing) in histopath analysis (e.g. standardization, reporting criteria)?
Answer: (Vicki Blazer)
The established criteria available for liver lesions need to be followed. Multitiered approach to sampling, observe external lesions and sample subset, then decide whether or not the complete histopath is needed. Standardization in terms of what people consider cancer, neoplasia, or preneoplastic lesions, and the reporting criteria need serious consideration. In reporting findings, not only does terminology need to be standardized but the age, sample size, and time of year needs to be included and considered.

Question:
Should the number of blocks per liver sample be standardized?
Answer: (Vicki Blazer and Jack Fournie)
There is not a current standard because it depends on the size of the fish, the question being asked, and the statistical rigor needed. The liver size varies with the size of fish and species and so the number of blocks needed in a small fish may be different than needed in a larger fish. It is important to remember you take 5-6, 1cm pieces, which are processed, embedded in paraffin blocks, and then sectioned to produce 6μm thick, slide-mounted tissue sections. For consistency, there probably should be some standardization based on the size of the liver.

Question:
Why is the whole liver not examined?
Answer: (Vicki Blazer and John Harshbarger)
Large livers cannot be fixed whole because the fixative will not penetrate the entire organ. The rationale for slicing liver into 1 cm blocks is that it is not realistic to collect whole livers from multiple fish in field samples.

Question:
Should we expand tumor analysis to organs other than the liver?
Answer: (Jack Fournie)
If the goal is to identify internal neoplastic lesions associated with contaminants, focus on livers. Generally, you do not see contaminant-associated neoplasms in any other organs.

Question:
Concerning the focus on Great Lakes with the brown bullhead as an indicator species, is pathology of liver samples the best/only place we should focus?
Answer: (Vicki Blazer and Jack Fournie)
If abnormalities are present in other organs, and fish are already being dissected, some of the tissues from other organs can be fixed for later analysis. It may be important to take gonadal

samples to look at reproduction/recruitment issues. If the goal is to assess changes in contaminated areas, focus on those organs where the most evidence will be visible. It is easy to remove organs from bullheads; it may be useful to look at the GI tract. Collection of other tissues would be consistent with Annex 12 of GLWQA. A frozen fish tissue bank is available for retrospective monitoring of emerging chemicals. Spleen samples would be very useful to compare Areas of Concern.

Question:
How can external lesions be sampled in endangered/threatened species without causing harm?
Answer: (Vicki Blazer)
Sampling increases susceptibility to fungal pathogens and bacteria, so remove a small piece and use disinfectant on surface. Can clip pieces of gill arch with minimal impact.

Question:
The competitive bid process may affect outcome of studies, i.e., type of pathologist hired, how could this be avoided?
Answer: (Jack Fournie)
Look for collaborations before preparing a proposal. They will have a more vested interest in participating and you can select an individual who has the credentials. Contracting someone that says they can do the histopath does not mean they are qualified to perform thorough histopathological evaluations.

Question:
Do digital photographs of histopath provide sufficient quality for a second opinion?
Answer: (Vicki Blazer)
It would be best to use slides for a second opinion, but it is helpful to include a photograph of the gross lesion.

Question:
How can bias be removed from liver sampling in order to calculate percent incidence of tumors?
Answer: (Vicki Blazer)
Bias can be removed by collecting random, statistically significant samples opposed to only fish with abnormal livers.

Question:
Would it be useful to follow National Water Quality Assessment Data Warehouse (NAWQA) protocol or is the Presque Isle Bay database sufficient?
Answer: (Chuck Murray)
The database can be altered to reflect new needs and can be linked with histopath findings.

Question:
Is it necessary to verify all samples when a voucher specimen of a tumor in a particular fish species is present?
Answer: (Vicki Blazer and Jack Fournie)
Yes, it is necessary to verify. As we recently saw in a study Paul and I did in bullheads from some Cape Cod ponds, raised lesions that were listed as papillomas by field personnel ranged

from actual papillomas to a squamous cell carcinoma to parasite infections.

Question:
What is the future of fish histopathology?
Answer: (Jack Fournie and Vicki Blazer)
There is only a small group of fish histopathology experts. Few students have a specific interest in pathology and for those who are interested, there are not any programs available that provide thorough training in fish anatomical pathology. There is a recognized need for increased knowledge of fish histopathology and while it is true there may be only a small group of experts, there are a number of courses that are trying to train interested students and increase the number of qualified people. The FVS Conservation Training Center offers a weeklong coursed of lectures and slide observation and the AquaVet II course (for veterinary students, etc.) in Woods Hole also has fish pathology training. Many of us teach informal courses to our students; however, course work is not sufficient, it takes a lot of experience.

Question:
Should there be a distinction between benign and malignant tumors?
Answer: (Jack Fournie)
Benign and malignant are clinical terms and should not really be used in reference to fish neoplasms. It is important to determine if a lesion is non-neoplastic or neoplastic and then to accurately assign a specific diagnosis.

Question:
From the perspective of management are there other things that should be looked at as indicators of tumor growth?
Answer: (Jack Fournie and John Harshbarger)
Foci alterations may be precursors to neoplasms (adenomas and carcinomas) but should not be considered neoplasms; however, both types are associated with contaminants and can provide useful information.

Question:
How expensive is the histopath analysis of liver tumors?
Answer: (Vicki Blazer)
Private contractors may charge $6 - $12 per liver slide, but if a collaborator is used, i.e., USGS it may only cost $5 per liver slide.

Presentations – Session III: Monitoring for AOCs

Roger Thoma – Using the IBI and DELT Metric to Evaluate Sediment Impacts in Great Lakes AOCs

Roger Thoma discussed the use of IBI and DELTs in bullheads as a means of evaluating sediment impacts in Great Lakes Areas of Concern. DELTs are classified as deformities, erosions, lesions and tumors; whereas, IBI is the index of biotic integrity. In assessing DELTs and IBI in Lake Erie Areas of Concern it was difficult to establish reference criteria because most sites were impacted by external factors; therefore, least impacted areas in Ohio were used as a baseline. From the regression analysis it was concluded that there was not a correlation between DELTs and IBI in Lake Erie, which was expected because of the range of metrics response to environmental factors. Box and whisker plots, which are a more descriptive analysis, suggested that Presque Isle Bay could be delisted according to the Ohio standards for percent allowance of DELTs (0.5%).

Approximately 70% of lacustuary sites (Areas of Concern and non-Areas of Concern) were above the Ohio standards for percent allowance of tumors. Site-specific regression assessment of the Black River suggests a decline in DELTs between 1982 and 2002, but the results are not statistically significant, and box and whisker plots suggest the same downward trend. The Black River is the only Ohio area of concern in which remediation action is taking place. Roger Thoma also performed GIS assessments that were site specific in relation to DELTs, tumor rates, and IBI.

In concluding, it was suggested that: Ohio areas of Lake Erie have no reference conditions; therefore, a significant relationship between IBI and tumor rates in Ohio lacustuarys cannot be demonstrated. There is not a significant relationship between DELT and Area of Concern conditions in Ohio lacustuarys, and a significant relationship between tumors, DELT, IBI, or Area of Concern designation in Ohio lacustuarys cannot be established. However, IBI and DELTs can be used to assess trends in individual Areas of Concern and Presque Isle can potentially be delisted for anomaly impairments.

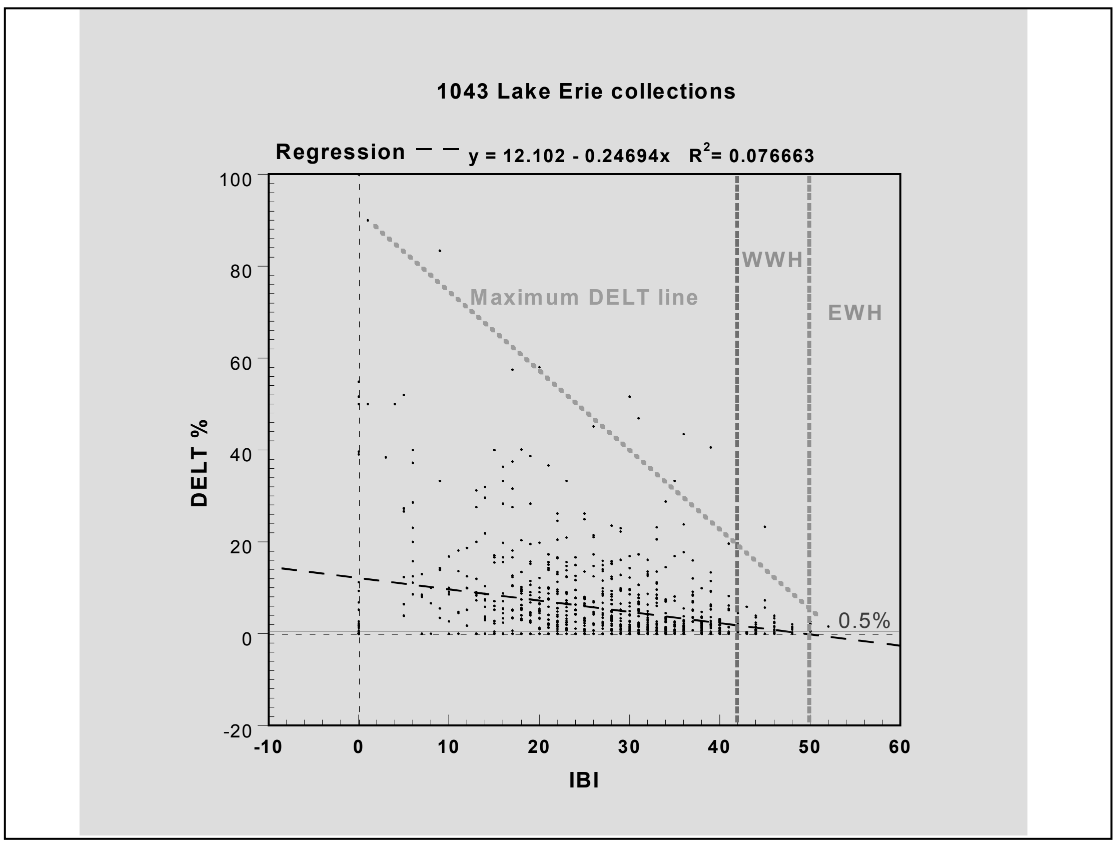
Mr. Roger Thoma's Power Point Presentation

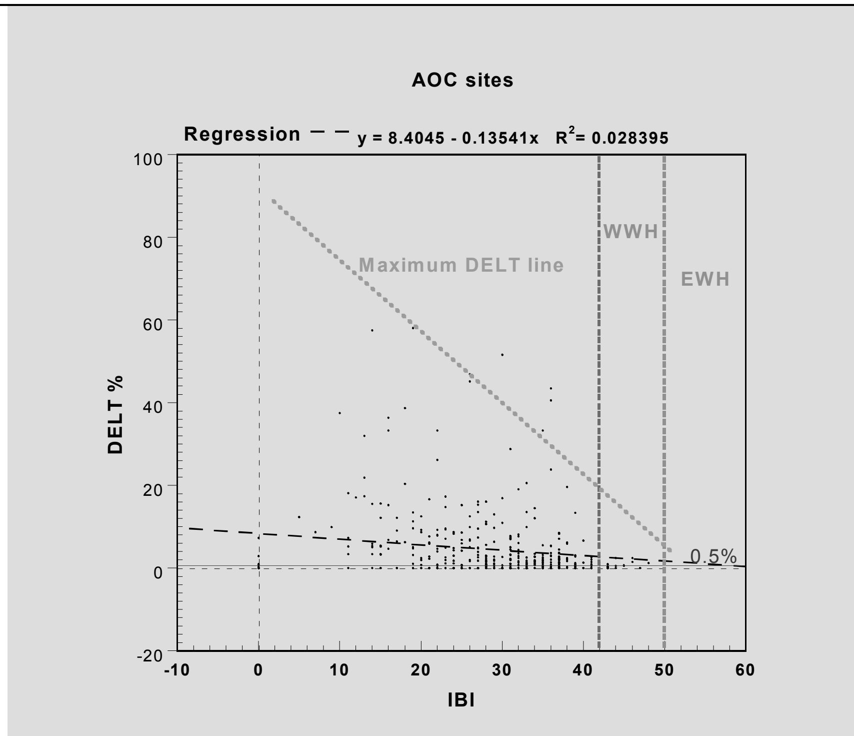
Ohio EPA

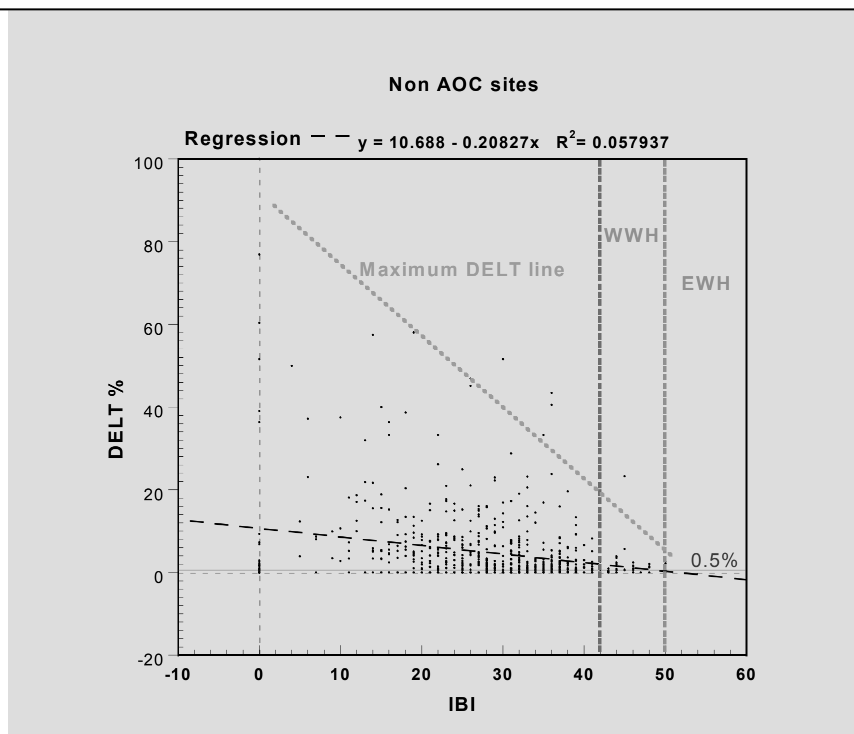
Roger F. Thoma

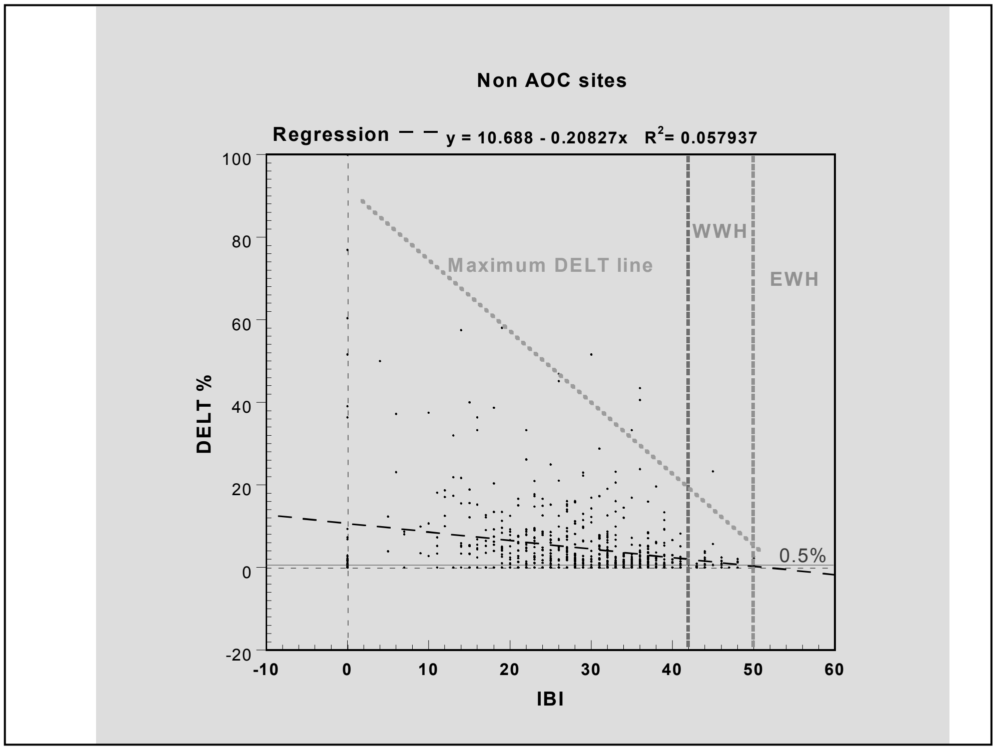
Using of the IBI and DELT Metric To Evaluate Sediment Impacts in Great Lakes AOC

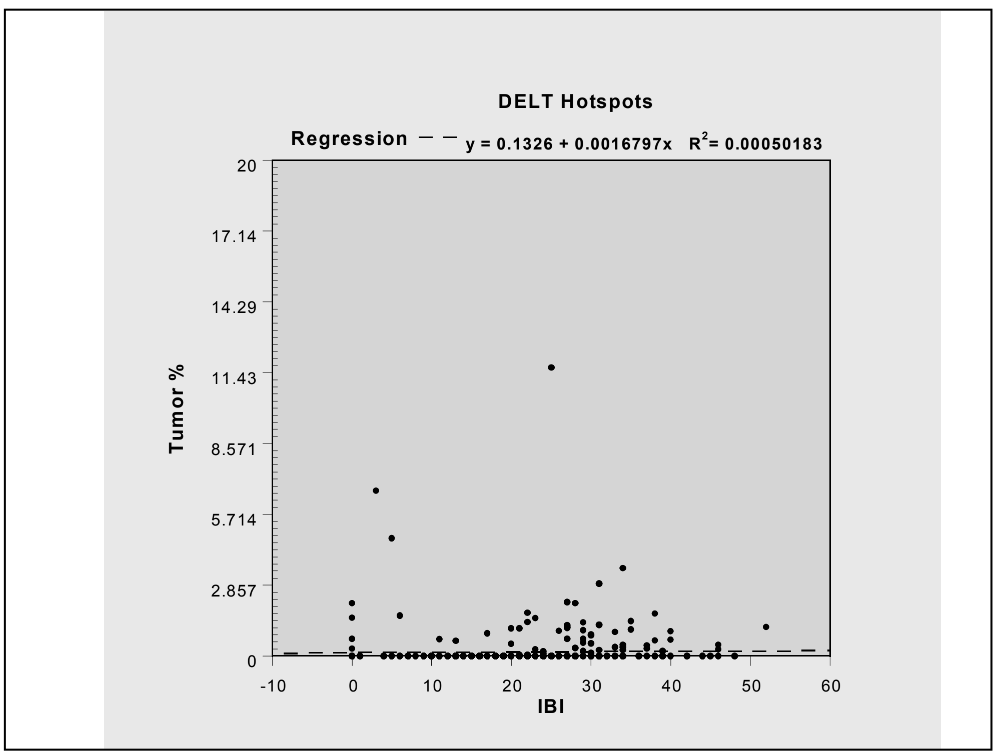
Regression Analysis

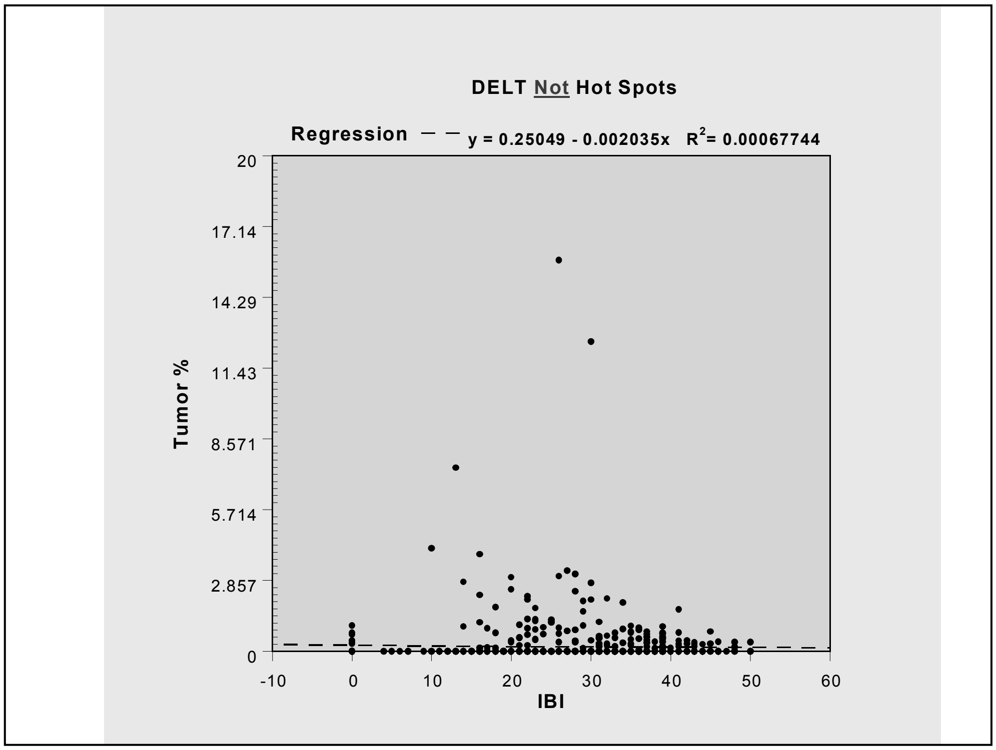



AOC sites
Regression
y = 8.4045 - 0.13541x R2= 0.028395
100
80
60
40
20
0
-20
-10
0
10
20
30
40
50
60
DELT %
IBI
Maximum DELT line
WWH
EWH
0.5%

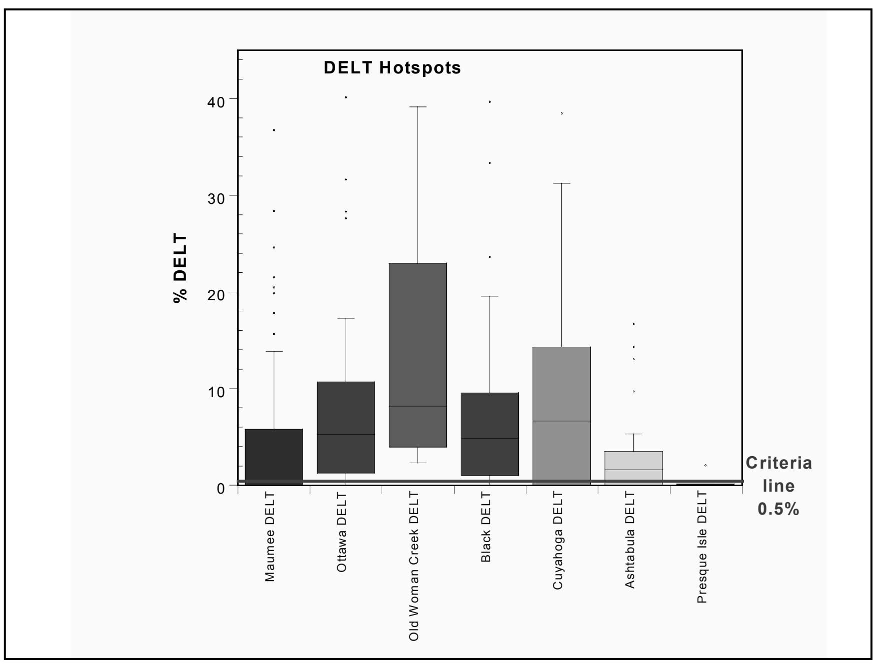

Non AOC sites
Regression
y = 10.688 - 0.20827x R2= 0.057937
100
80
60
40
20
0
-20
-10
0
10
20
30
40
50
60
DELT %
IBI
Maximum DELT line
WWH
EWH
0.5%

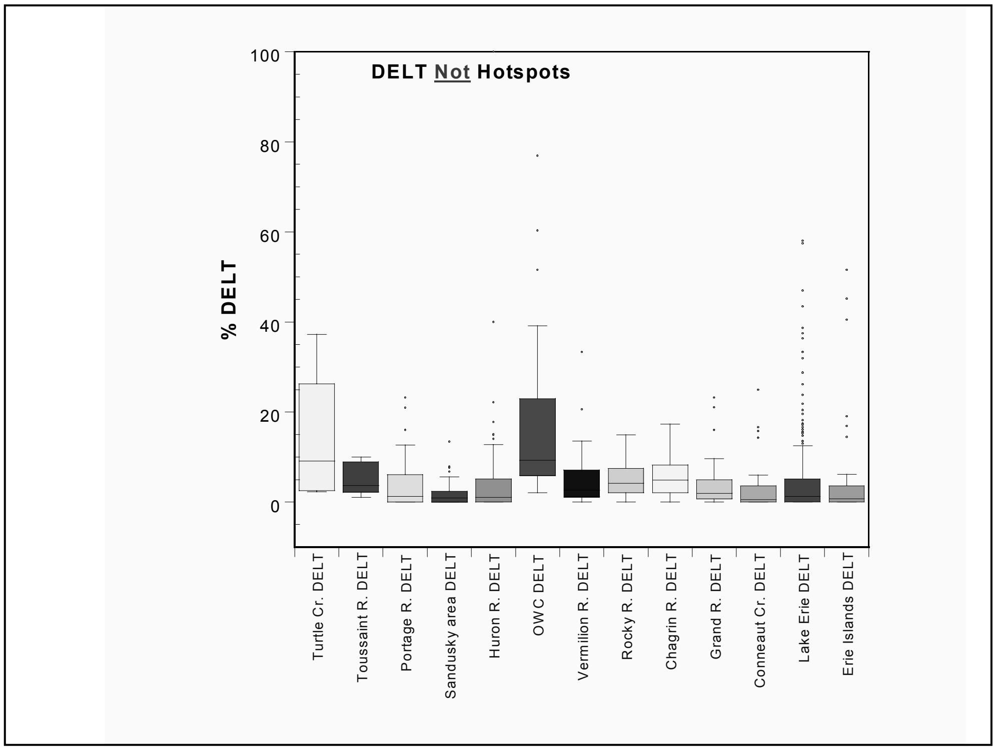

Non AOC sites
Regression — — y = 10.688 - 0.20827x R2= 0.057937
DELT %
IBI
Maximum DELT line
WWH
EWH
0.5%

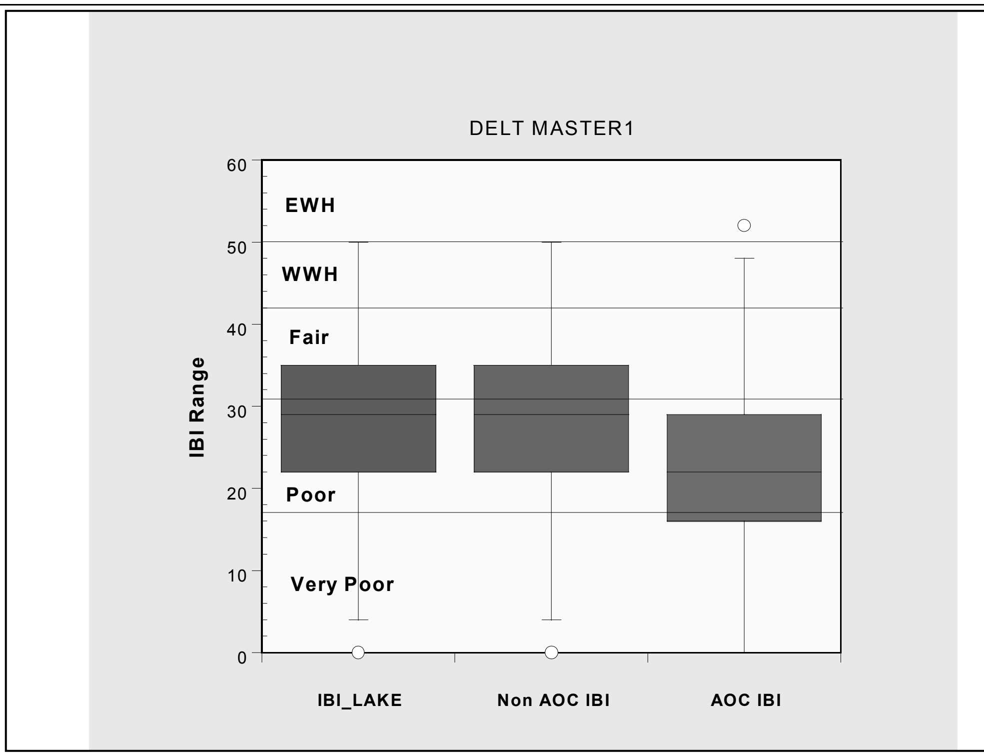

DELT Hotspots
Regression — — y = 0.1326 + 0.0016797x R2= 0.00050183
Tumor %
IBI



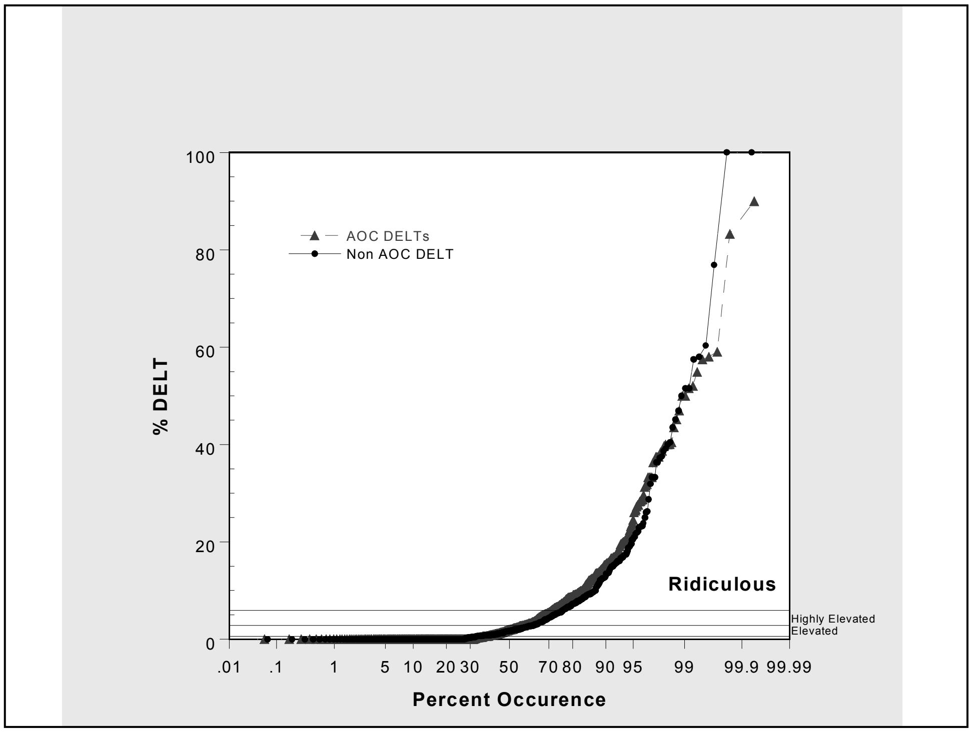
Box & Whisker Plots

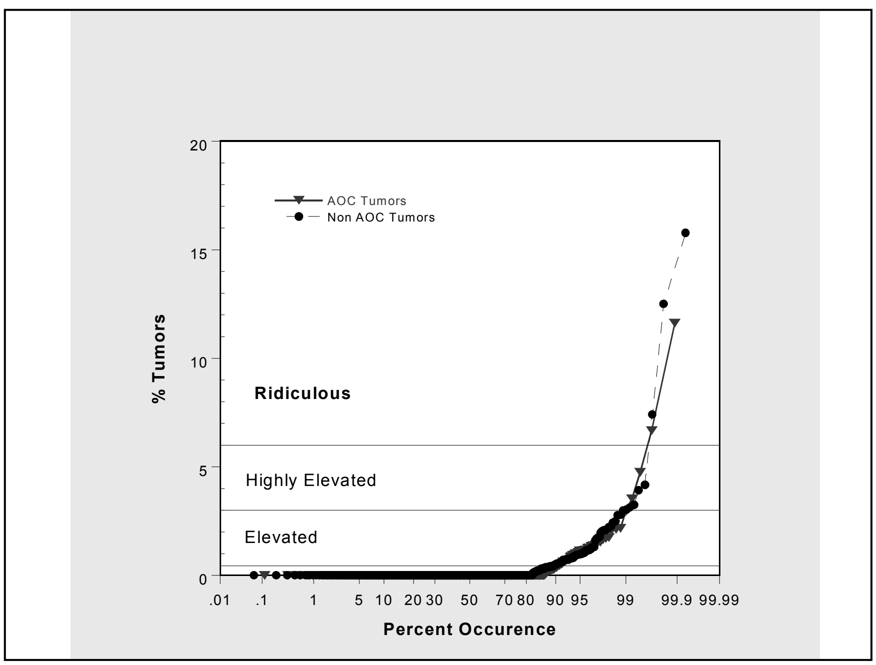


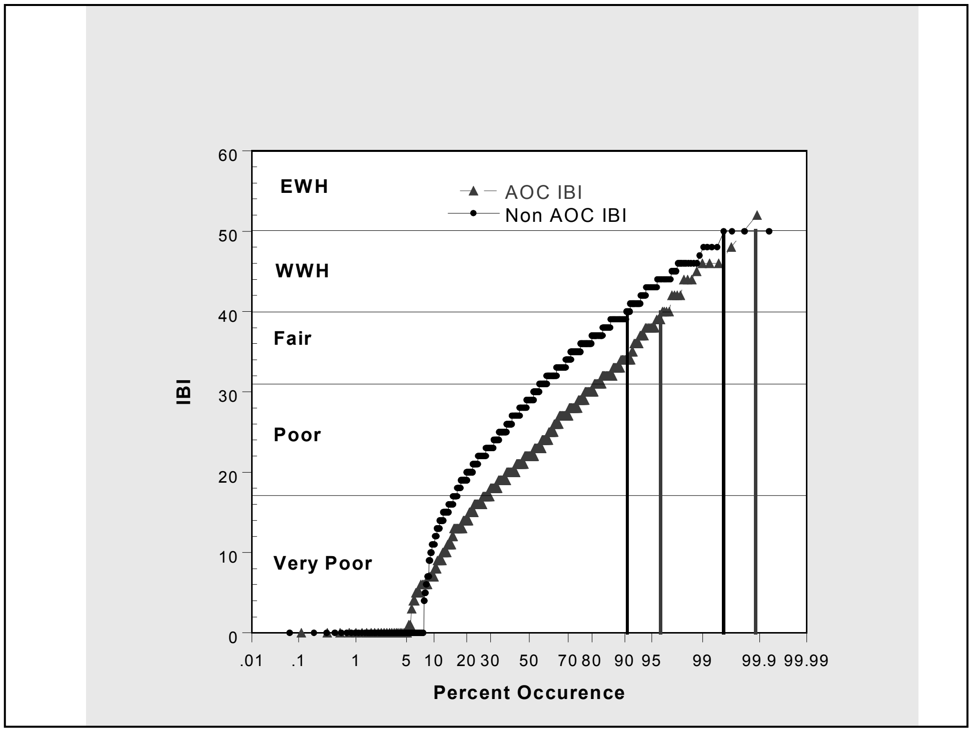

DELT Not Hotspots
% DELT
100
80
60
40
20
0
Turtle Cr. DELT
Toussaint R. DELT
Portage R. DELT
Sandusky area DELT
Huron R. DELT
OWC DELT
Vermilion R. DELT
Rocky R. DELT
Chagrin R. DELT
Grand R. DELT
Conneaut Cr. DELT
Lake Erie DELT
Erie Islands DELT


DELT MASTER1
IBI Range
60
50
40
30
20
10
0
EWH
WWH
Fair
Poor
Very Poor
IBI_LAKE
Non AOC IBI
AOC IBI

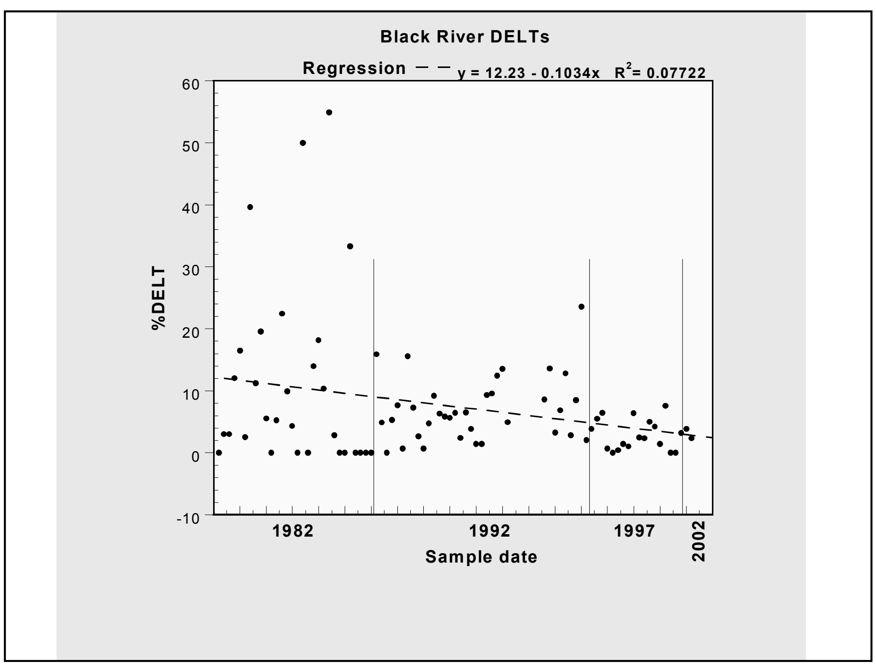
Probability of Occurrence

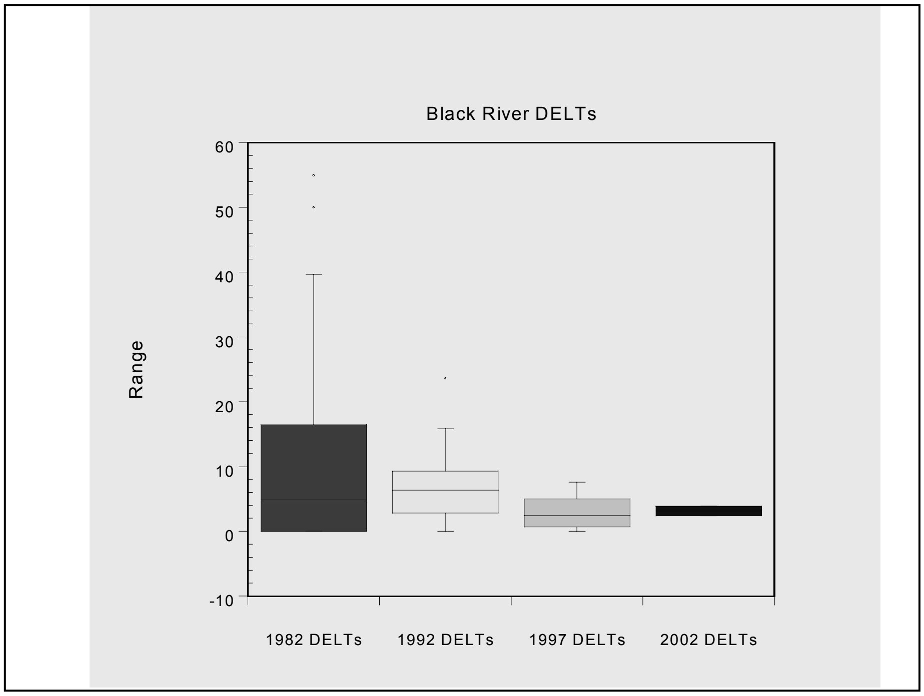




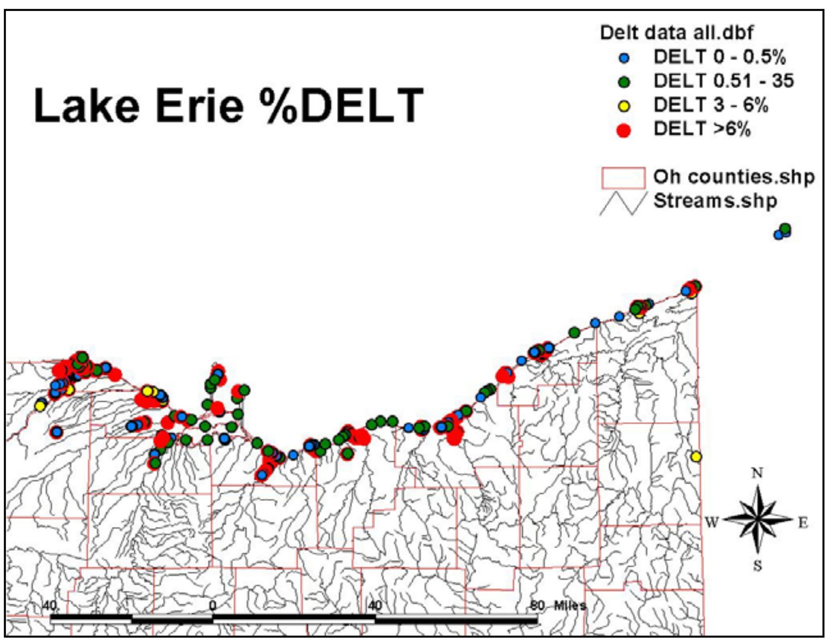


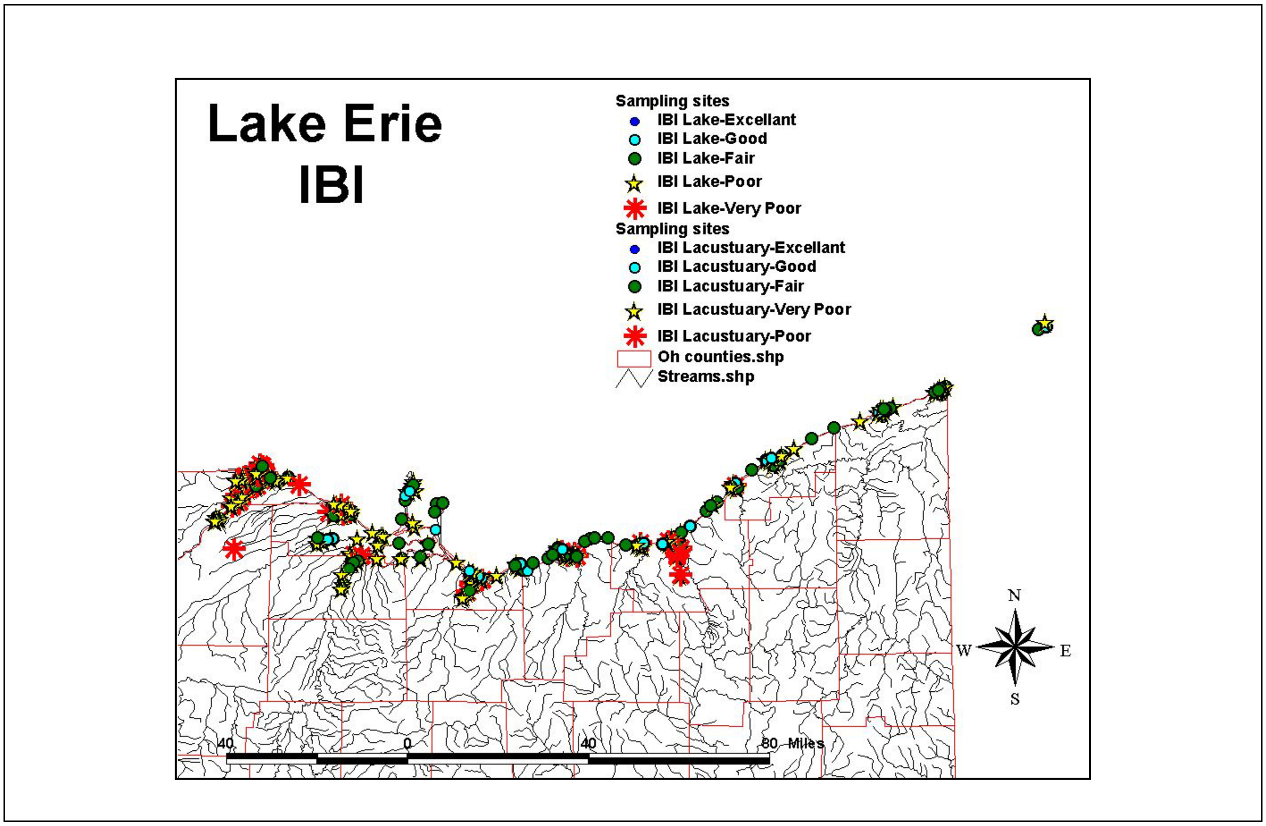
Site Specific Assessment: Black River Lacustuary

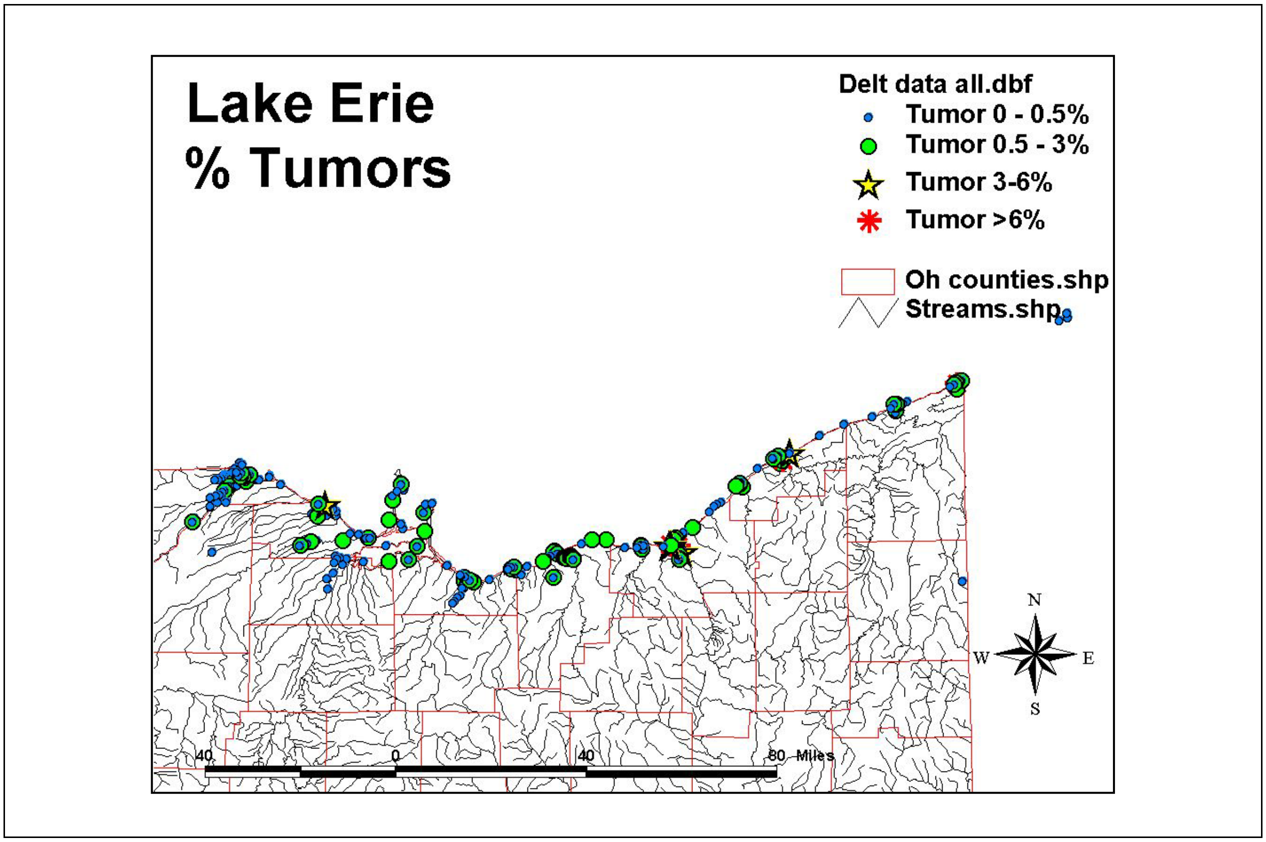




GIS Assessment




Lake Erie
IBI
Sampling sites
IBI Lake-Excellant
IBI Lake-Good
IBI Lake-Fair
IBI Lake-Poor
IBI Lake-Very Poor
Sampling sites
IBI Lacustuary-Excellant
IBI Lacustuary-Good
IBI Lacustuary-Fair
IBI Lacustuary-Very Poor
IBI Lacustuary-Poor
Oh counties.shp
Streams.shp
N
W
E
S
40
0
40
80 Miles


Lake Erie
% Tumors
Delt data all.dbf
Tumor 0 - 0.5%
Tumor 0.5 - 3%
Tumor 3-6%
Tumor >6%
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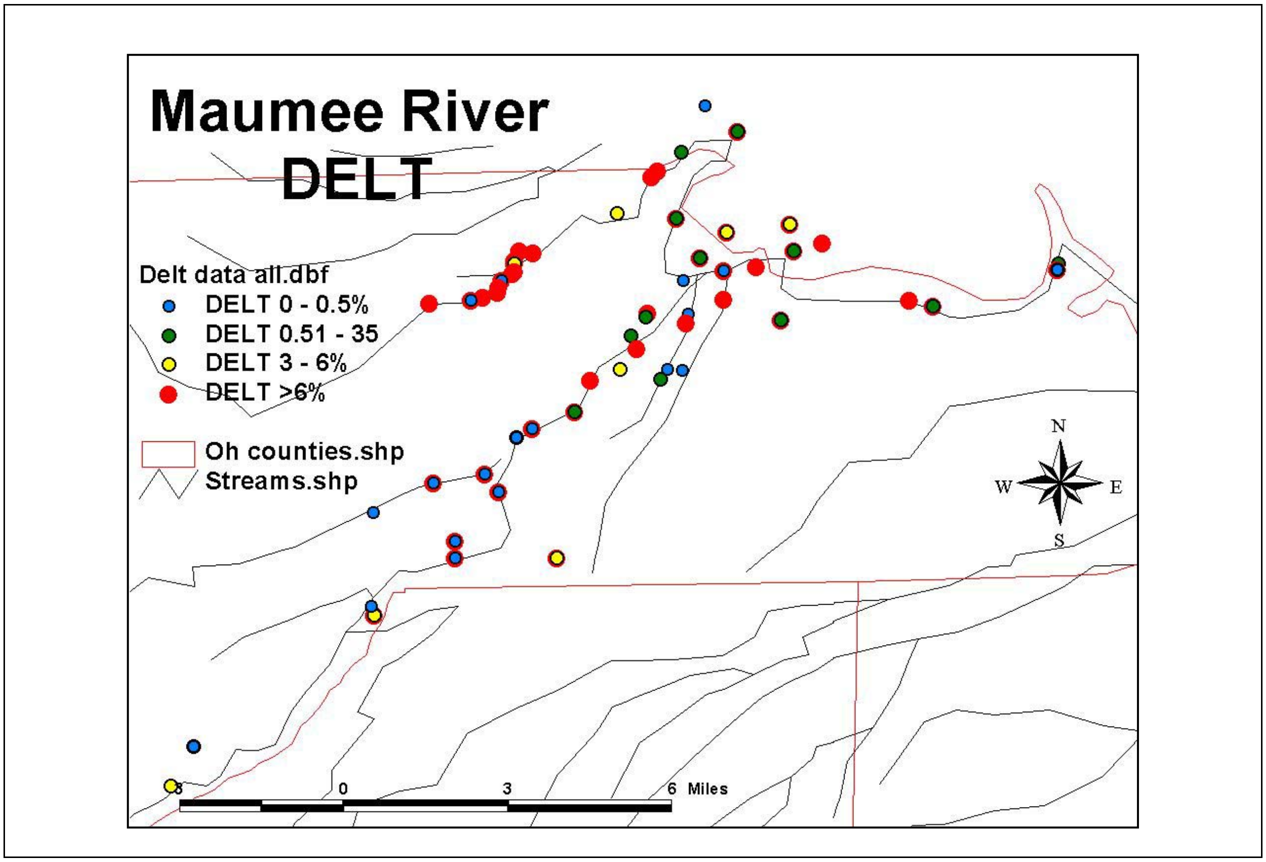
Streams.shp
N
W
E
S
40
0
40
80 Miles


Maumee River
DELT
Delt data all.dbf
DELT 0 - 0.5%
DELT 0.51 - 35
DELT 3 - 6%
DELT >6%
Oh counties.shp
Streams.shp
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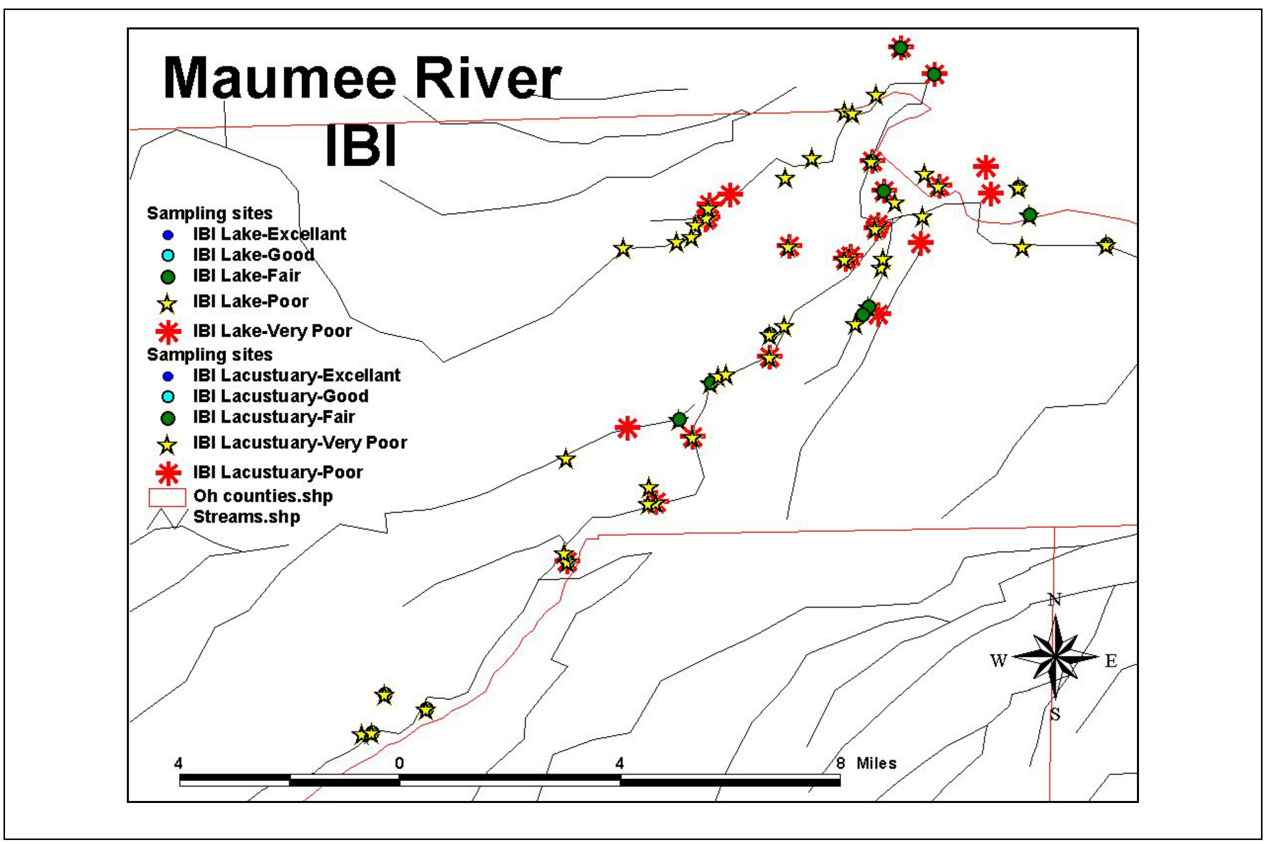
S
3
0
3
6 Miles


Maumee River
IBI
Sampling sites
IBI Lake-Excellant
IBI Lake-Good
IBI Lake-Fair
IBI Lake-Poor
IBI Lake-Very Poor
Sampling sites
IBI Lacustuary-Excellant
IBI Lacustuary-Good
IBI Lacustuary-Fair
IBI Lacustuary-Very Poor
IBI Lacustuary-Poor
Oh counties.shp
Streams.shp
N
W
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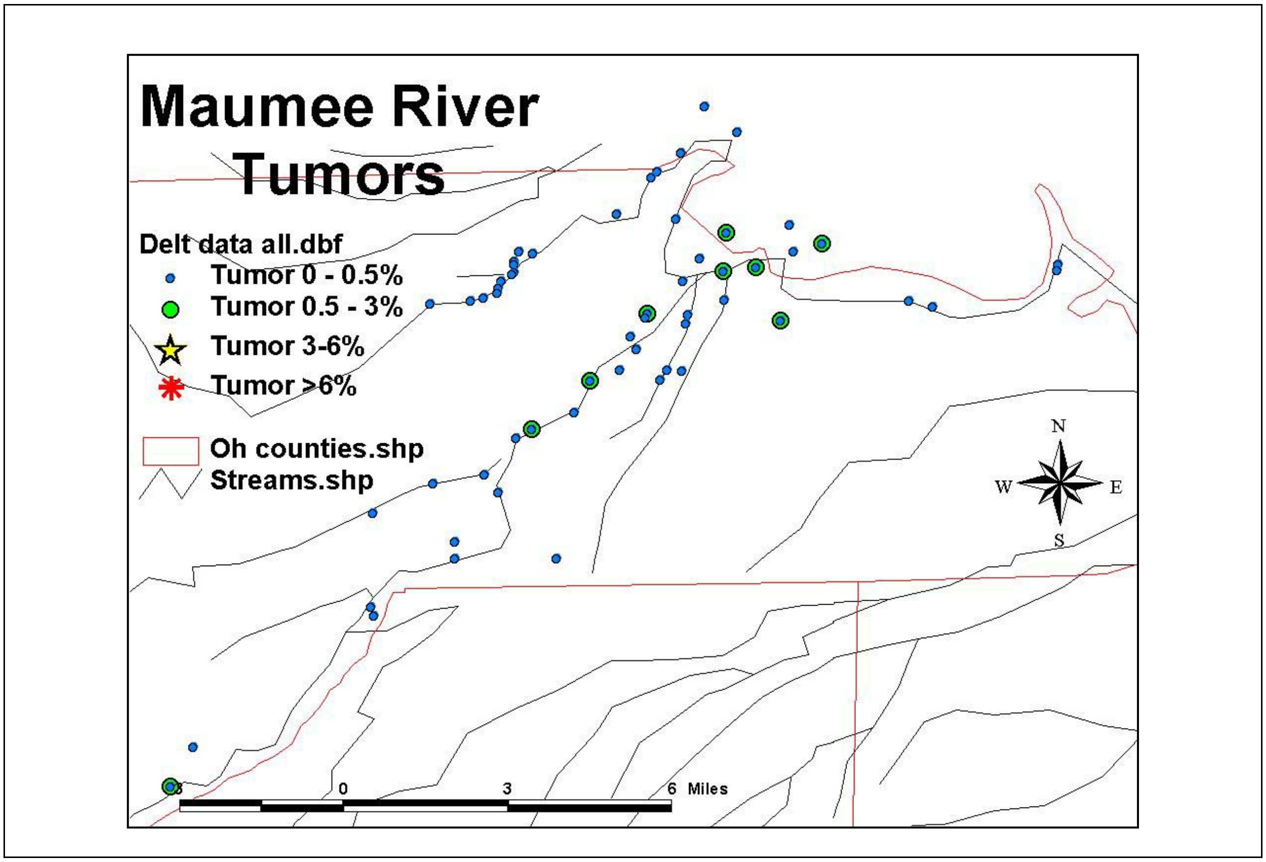
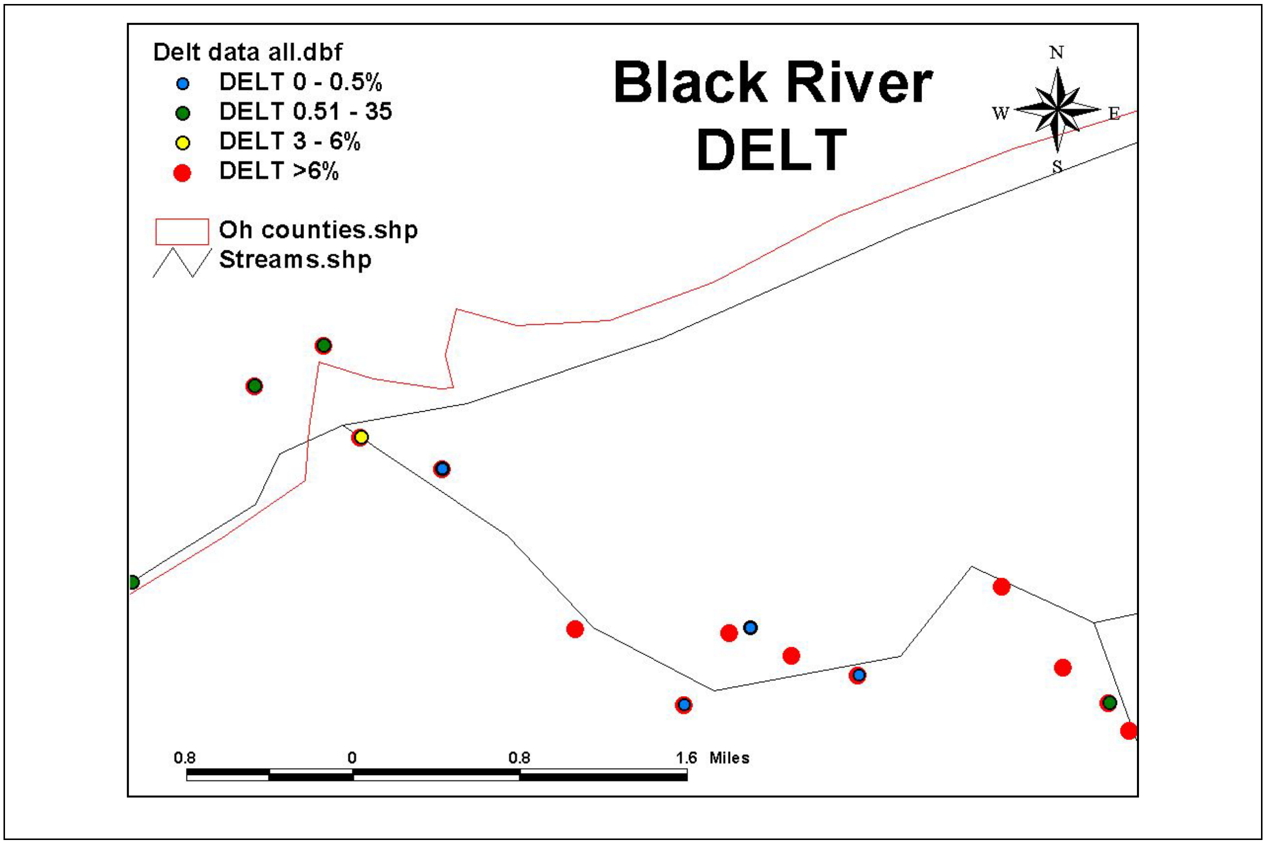
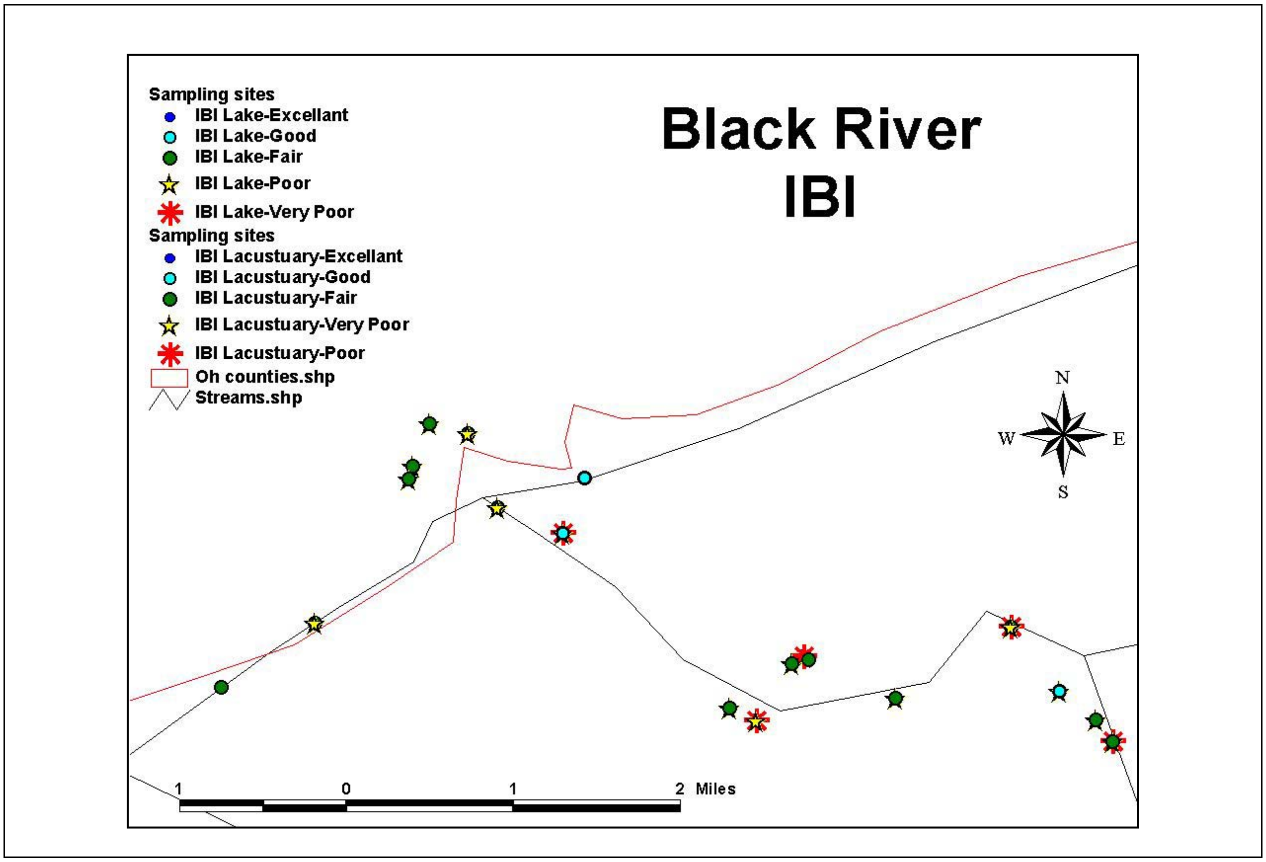
S
4
0
4
8 Miles

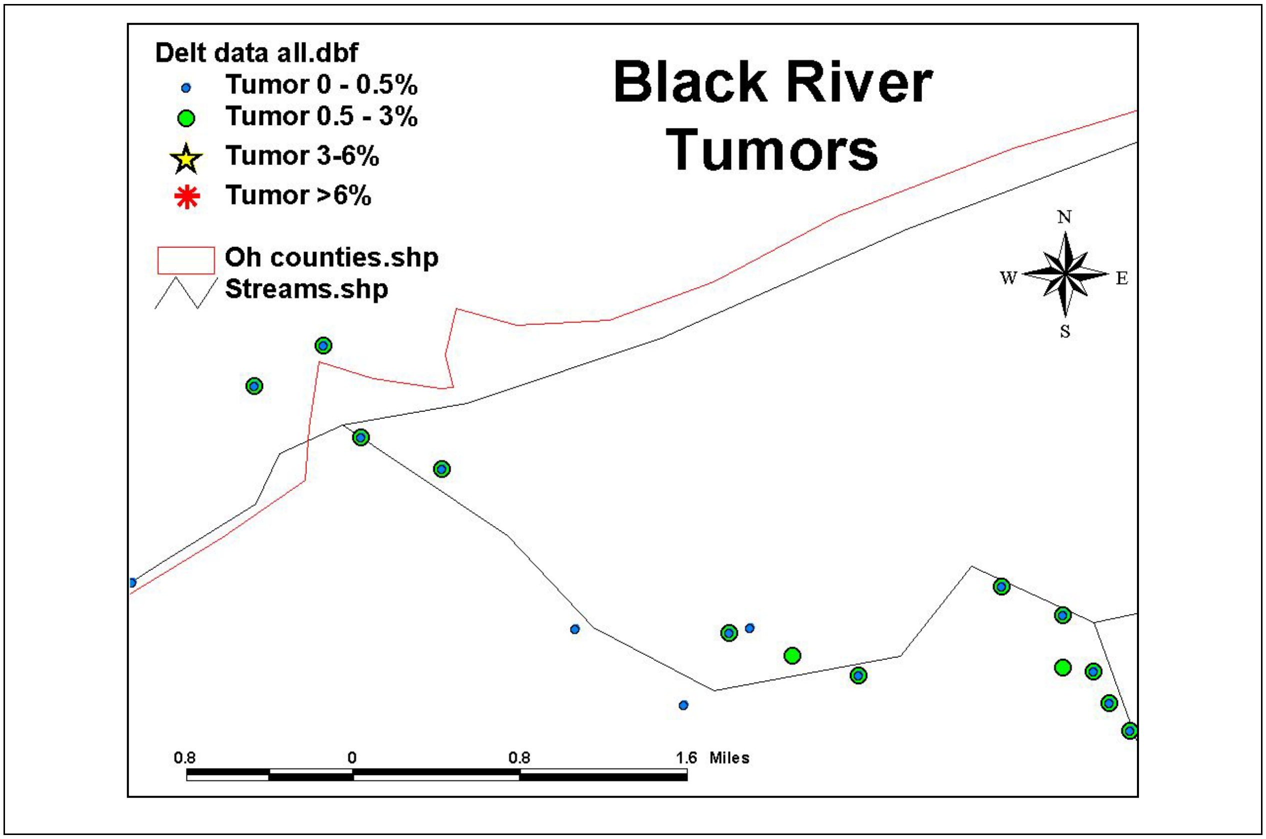


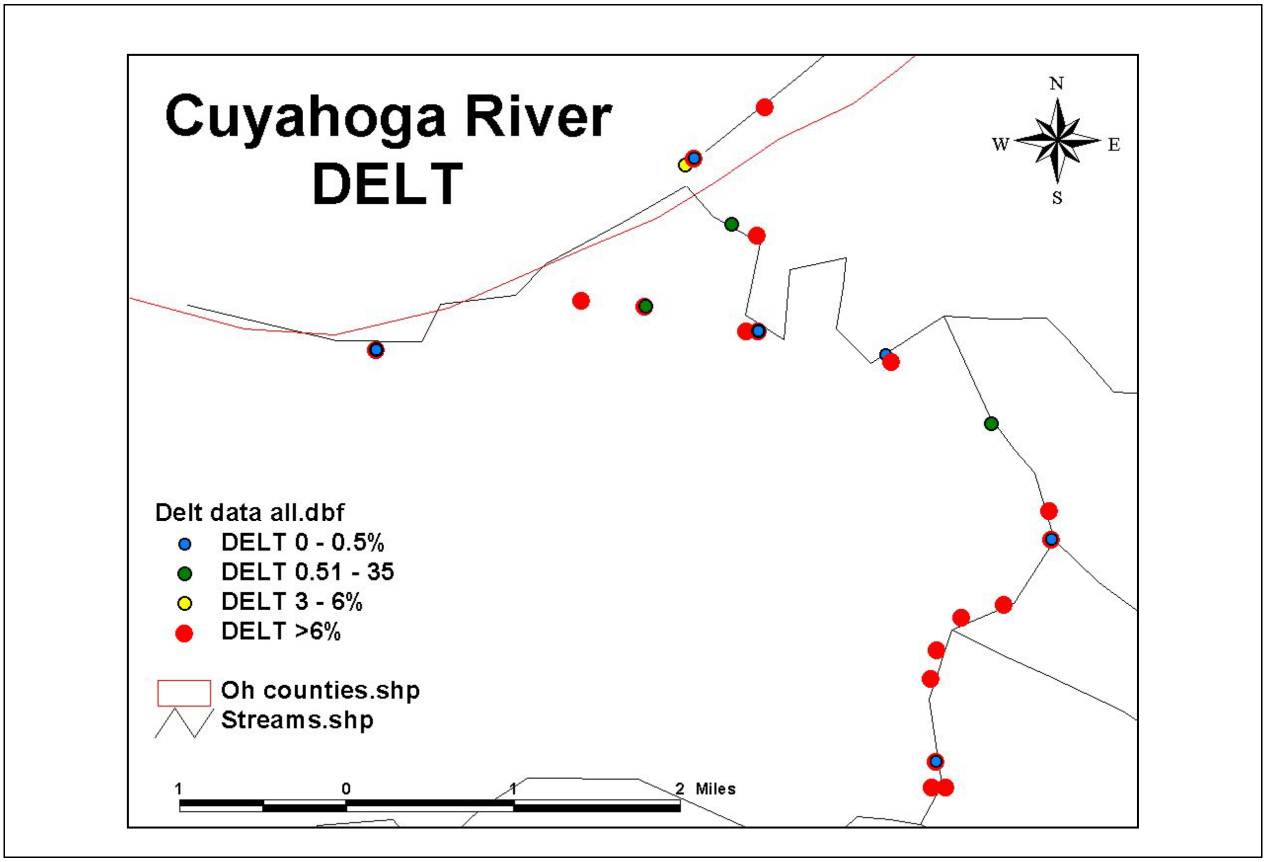
Maumee River
Tumors
Delt data all.dbf
Tumor 0 - 0.5%
Tumor 0.5 - 3%
Tumor 3-6%
Tumor >6%
Oh counties.shp
Streams.shp
N
W
E
S
3
0
3
6 Miles

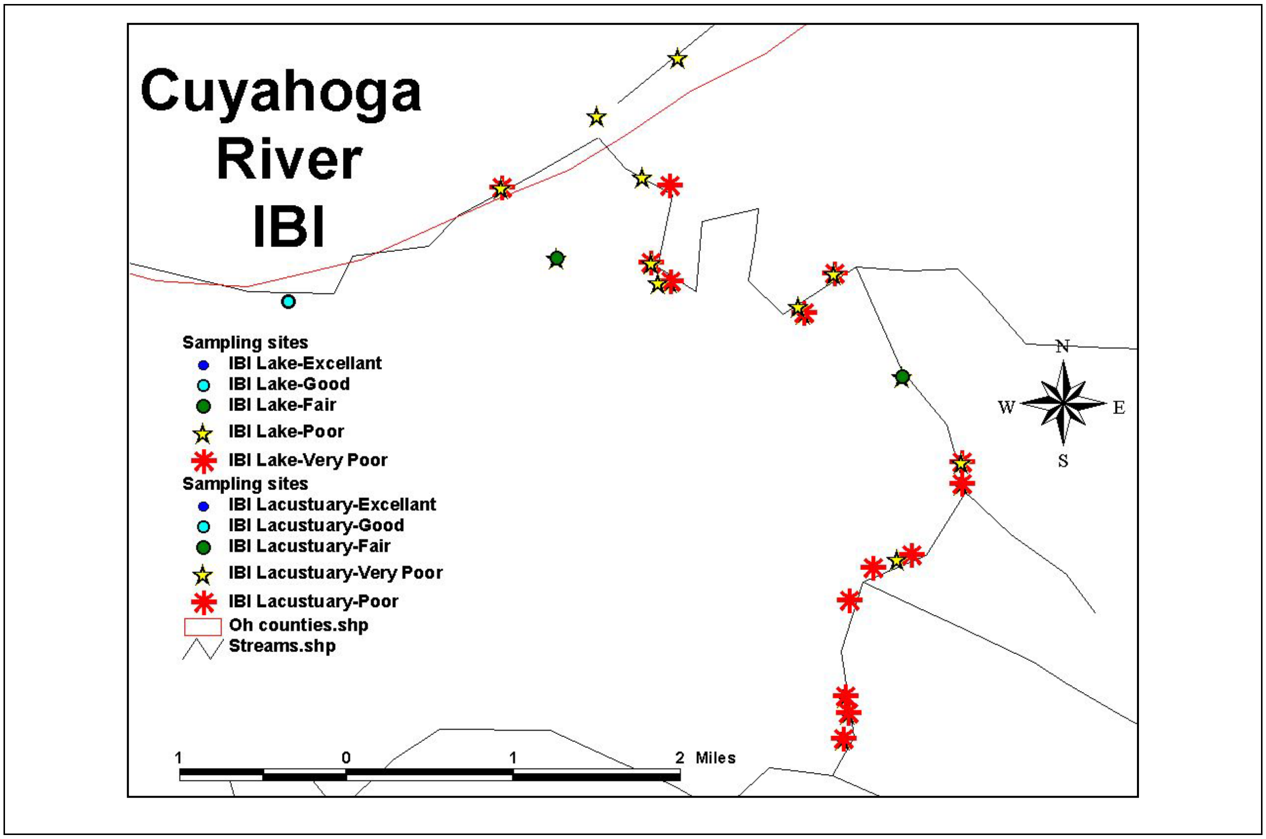


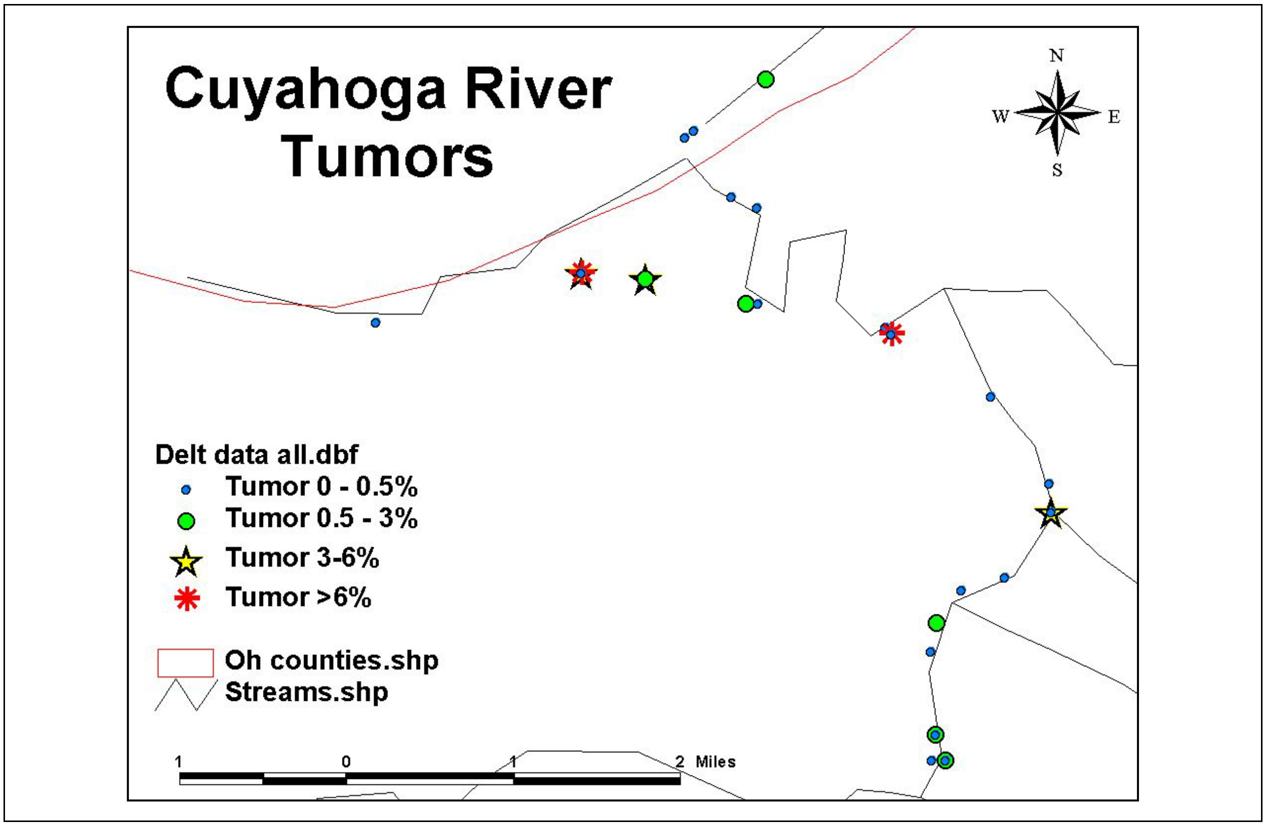
Delt data all.dbf
DELT 0 - 0.5%
DELT 0.51 - 35
DELT 3 - 6%
DELT >6%
Oh counties.shp
Streams.shp
Black River
DELT
N
W
E
S
0.8
0
0.8
1.6 Miles

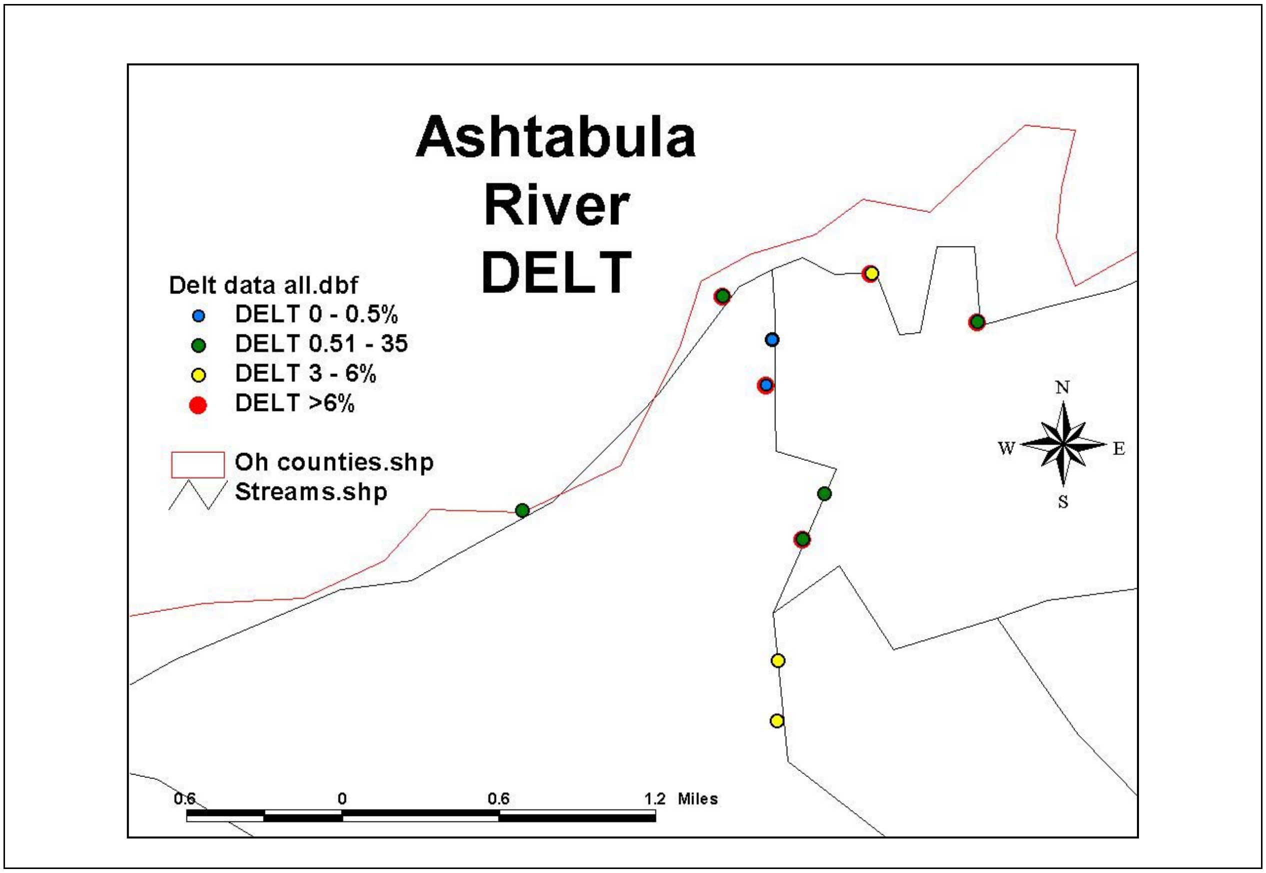

Black River
IBI
Sampling sites
IBI Lake-Excellant
IBI Lake-Good
IBI Lake-Fair
IBI Lake-Poor
IBI Lake-Very Poor
Sampling sites
IBI Lacustuary-Excellant
IBI Lacustuary-Good
IBI Lacustuary-Fair
IBI Lacustuary-Very Poor
IBI Lacustuary-Poor
Oh counties.shp
Streams.shp
N
W
E
S
1
0
1
2 Miles

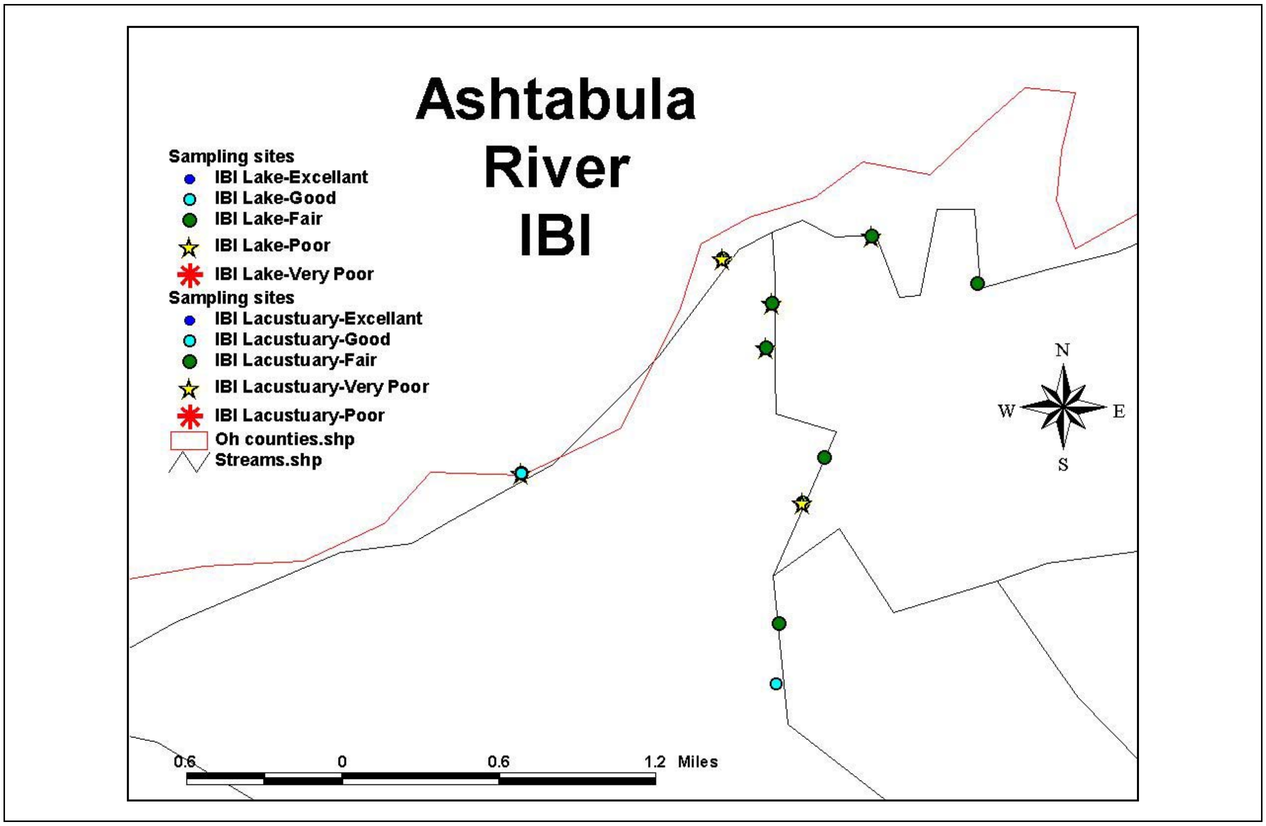

Black River
Tumors
Delt data all.dbf
Tumor 0 - 0.5%
Tumor 0.5 - 3%
Tumor 3-6%
Tumor >6%
Oh counties.shp
Streams.shp
N
W
E
S
0.8
0
0.8
1.6 Miles

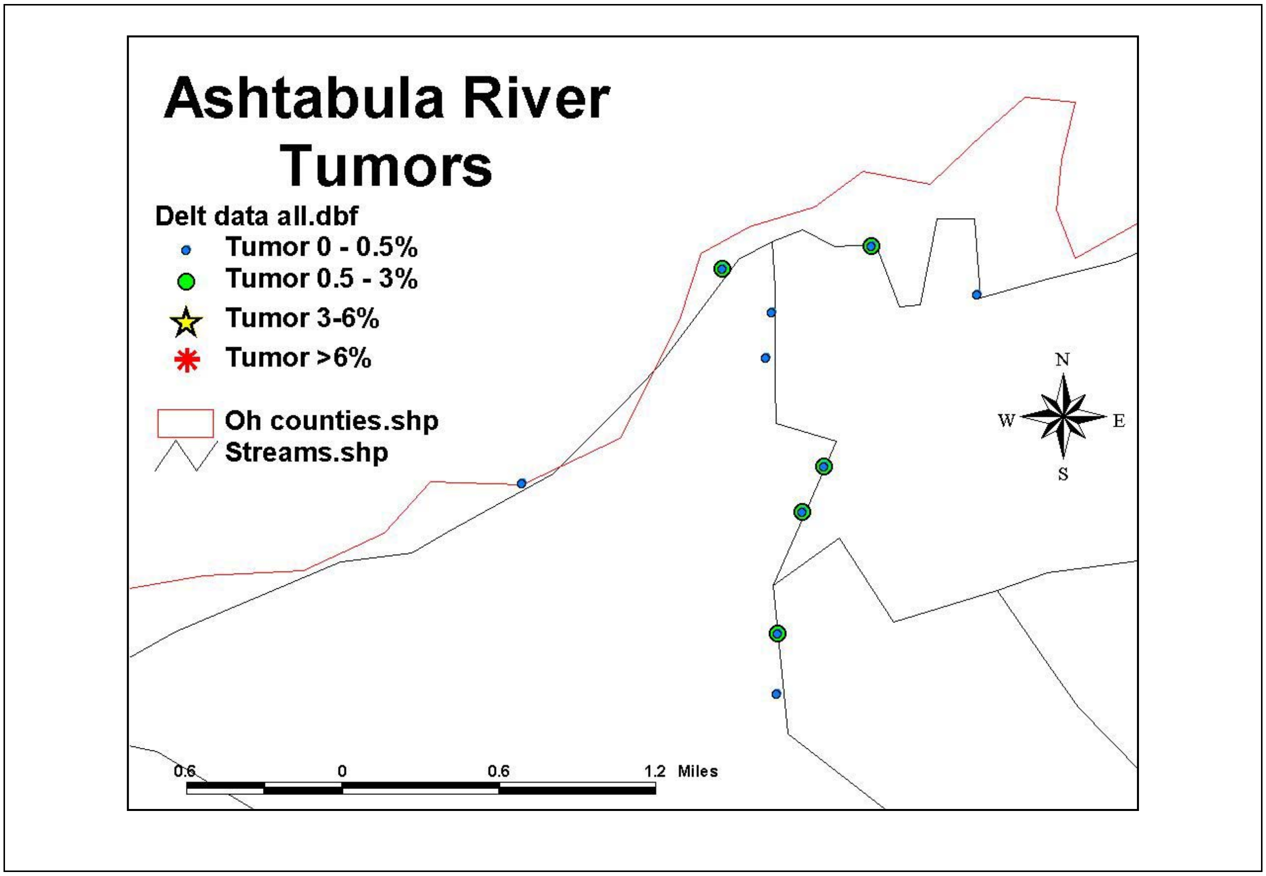

Cuyahoga River
DELT
N
W
E
S
Delt data all.dbf
DELT 0 - 0.5%
DELT 0.51 - 35
DELT 3 - 6%
DELT >6%
Oh counties.shp
Streams.shp
1
0
1
2 Miles


Cuyahoga
River
IBI
N
W
E
S
Sampling sites
IBI Lake-Excellant
IBI Lake-Good
IBI Lake-Fair
IBI Lake-Poor
IBI Lake-Very Poor
Sampling sites
IBI Lacustuary-Excellant
IBI Lacustuary-Good
IBI Lacustuary-Fair
IBI Lacustuary-Very Poor
IBI Lacustuary-Poor
Oh counties.shp
Streams.shp
1
0
1
2 Miles


Cuyahoga River
Tumors
N
W
E
S
Delt data all.dbf
Tumor 0 - 0.5%
Tumor 0.5 - 3%
Tumor 3-6%
Tumor >6%
Oh counties.shp
Streams.shp
1
0
1
2 Miles


Ashtabula
River
DELT
Delt data all.dbf
DELT 0 - 0.5%
DELT 0.51 - 35
DELT 3 - 6%
DELT >6%
Oh counties.shp
Streams.shp
N
W
E
S
0.6
0
0.6
1.2 Miles


Ashtabula
River
IBI
Sampling sites
IBI Lake-Excellant
IBI Lake-Good
IBI Lake-Fair
IBI Lake-Poor
IBI Lake-Very Poor
Sampling sites
IBI Lacustuary-Excellant
IBI Lacustuary-Good
IBI Lacustuary-Fair
IBI Lacustuary-Very Poor
IBI Lacustuary-Poor
Oh counties.shp
Streams.shp
N
W
E
S
0.6
0
0.6
1.2 Miles


Ashtabula River
Tumors
Delt data all.dbf
Tumor 0 - 0.5%
Tumor 0.5 - 3%
Tumor 3-6%
Tumor >6%
Oh counties.shp
Streams.shp
N
W
E
S
0.6
0
0.6
1.2 Miles

Summary

- **Ohio areas of Lake Erie have no reference conditions**
- **No significant relationship can be demonstrated between IBI and tumor rates in Ohio lacustarys**
- **No significant relationship can be demonstrated between DELT and AOC condition in Ohio lacustarys**
- **IBI and DELT can be used to assess trends in individual AOCs**
- **Presque Isle can potentially be de-listed for anomaly impairments**

Dr. Paul Baumann - Use of External Anomaly Prevalence Index (EAPI) to Determine the Health of Fish in Lake Erie Areas of Concern

Dr. Baumann (in replacement of Steve Smith) presented the "Use of the External Anomaly Prevalence Index (EAPI) to Determine the Health of Fish in Lake Erie Areas of Concern." When using EAPI, the focus falls on four external anomalies: raised growths, barbel abnormality, focal discoloration and eye problems. Advantages of using EAPI include: it is easy and quick to conduct in the field, and gross observation can be used as a cost effective monitoring tool; however, values for predicting impacted areas may be altered as additional data are analyzed.

Fish population studies and sediment analysis in various areas of Lake Erie were carried out in the 1980s and again from 1998 to 2000. Results from identical sites suggested an increase in gizzard shad in 1998-2000; however, there is no evidence that this increase is correlated to the presence of contaminants. It is thought that environmental variables (winter lake temperatures – lack of freeze) may be responsible for the increased gizzard shad population. Other results in the 1998-2000 study, in comparison to the 1980s study, included: same or lower concentrations of sediment contaminants in Lake Erie Areas of Concern, higher contaminant concentrations at reference sites, an overall higher incidence of anomalies in brown bullheads, lower catch per effort (CPE), and a change in dominant species in fish communities. At the Ottawa River site there was actually a decline in raised growth (RG) and focal discoloration (FD) levels related to increased concentrations of polychlorinated biphenyls in the sediments. At the same time there was a higher incidence of barbel abnormalities. A significant regression showing an increase in incidence of external anomalies (particularly barbels and raised growths) may be related to metals, organochlorines (OCs) and PAHs. The results suggest that the use of EAPI and/or tumor prevalence will help to determine fish impairment.

Near-shore benthic fish, e.g. brown bullhead, black bullhead, white sucker and redhorse species, can be used to assess impairment based on comparisons of rates of occurrence of internal tumors or related external anomalies at sites of interest. Interpretations of impairments in mature (> 3 years old) near-shore benthic fish, include:

- histopathologically verified internal tumor prevalence (liver or bile duct) of >5%;
- prevalence of raised growth on lips >10%;
- prevalence of raised growth on body and lips > 15%;
- in mature brown or black bullhead - prevalence of barbel abnormalities (stubbed, deformed or missing) of > 20%.

The presentation concluded by emphasizing the possibility of using EAPI as part of a common index for collaborative US-Canadian monitoring at Great Lakes Areas of Concern as an indicator of ecosystem health.

Dr. Paul Baumann's Power Point Presentation

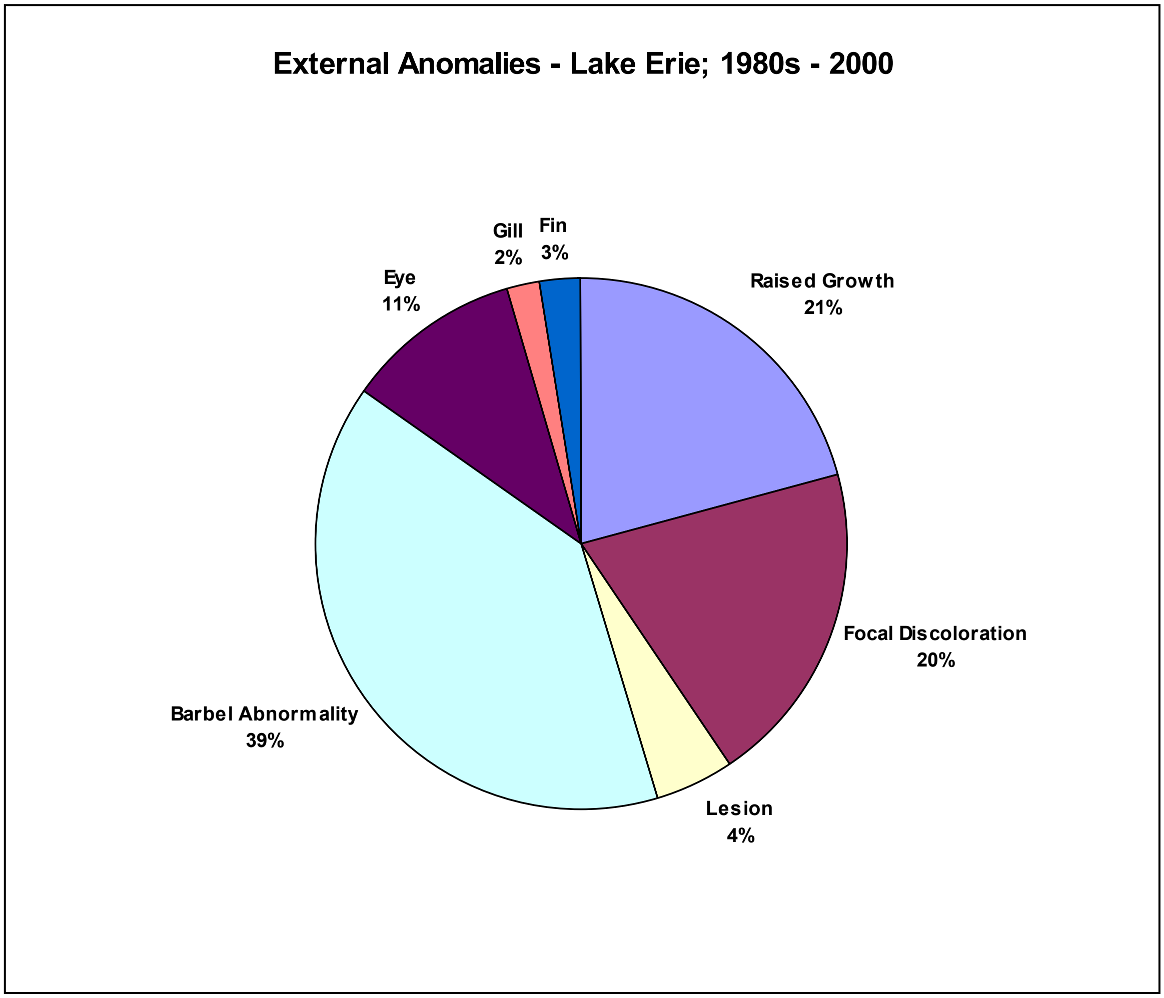
Use of External Anomaly Prevalence Index (EAPI) to Determine the Health of Fish in Lake Erie Areas of Concern

Stephen B. Smith and
Paul C. Baumann

US Geological Survey – Reston, VA
Columbus, OH

What is EAPI?

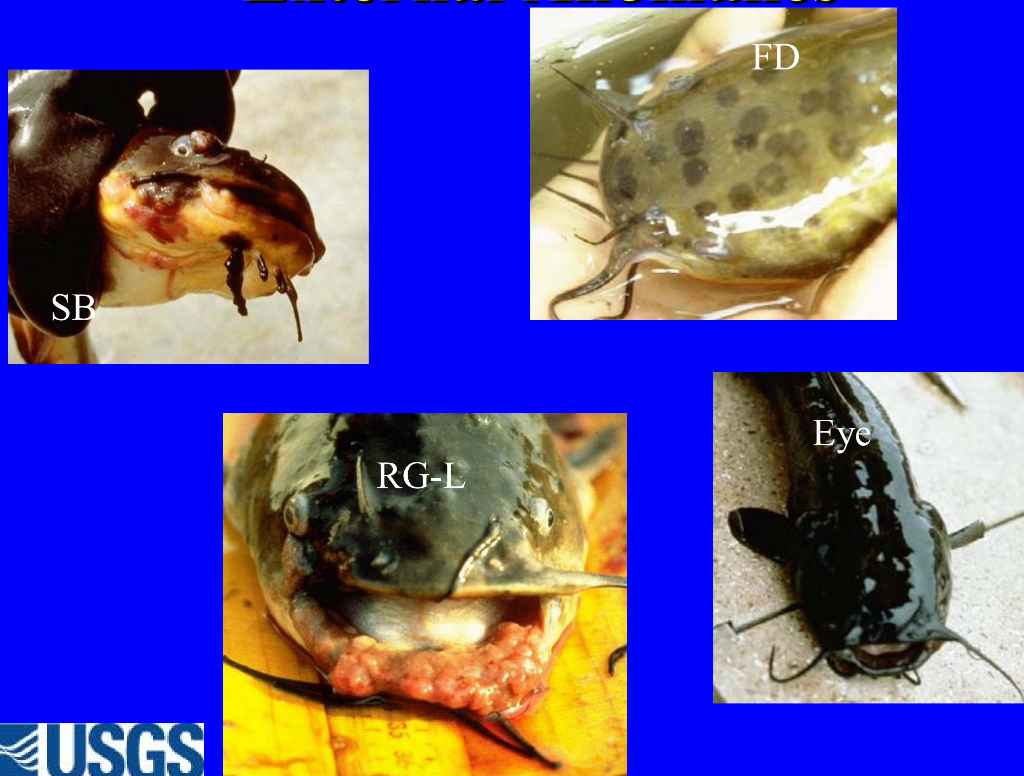
- **State of the Lakes Ecosystem Conference (SOLEC)**
  - * **Revision of Bio-indicator 101**
  - * **Use of bottom dwelling species**
    - **e.g. brown bullhead and white sucker**
  - * **DELT vs. EAPI**
    - **saw several differences of DELT indicators were not prevalent**
      - * **Deformities, fin erosion and "tumors"**



Why Brown Bullheads?

- **Brown Bullhead or White Sucker**
  - **International Joint Commission – bio-indicator species of choice**
  - **In near-shore areas of many Great Lakes**
  - **Relatively abundant**
  - **Bottom Feeder**
  - **Tolerant**

External Anomalies



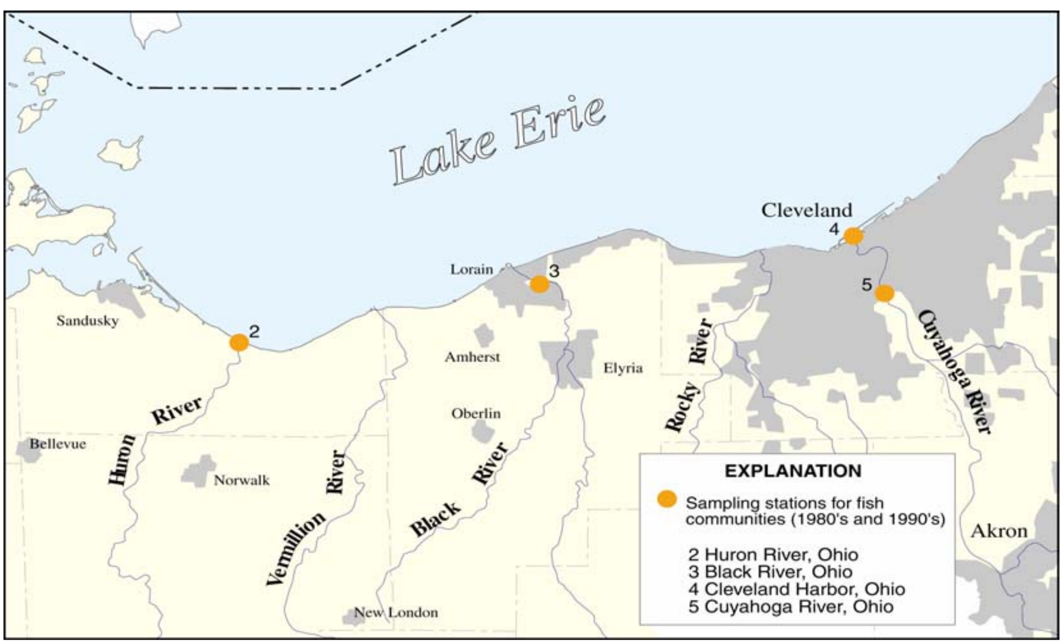
Lake Erie
Ecological Investigation

- Re-evaluate AOCs in Lake Erie
    * Same sites that same group of researchers studied in 1980s
    * Fish Communities
    * Invertebrate Communities
    * Residue Analysis - Sediments
    * Brown Bullhead
        - Necropsy
        - Biomarkers
    * Ecological Risk Assessment

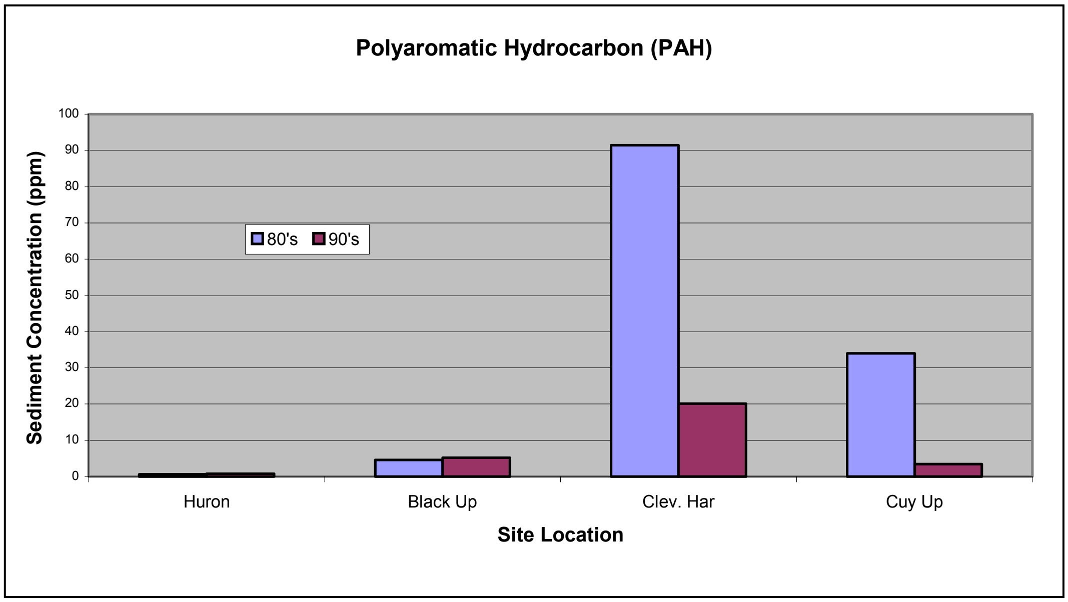
Lake Erie Sampling Sites –1990's

USGS science for a changing world

Sites – 1980s



Polyaromatic Hydrocarbon (PAH) In Sediments 80s to 98-00



Sites Commonly Sampled 1980s and 98-00'

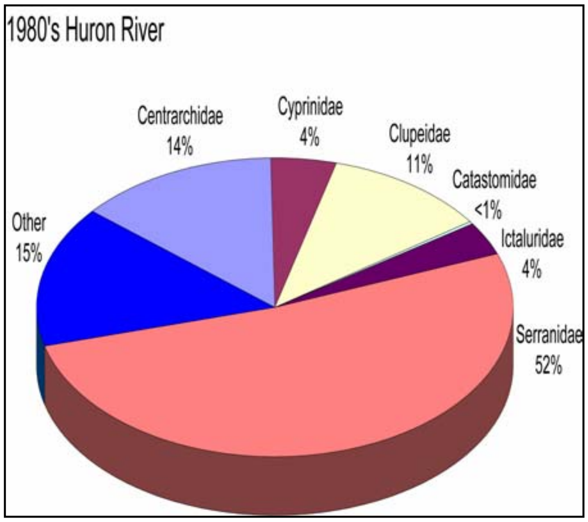
1980's CPE

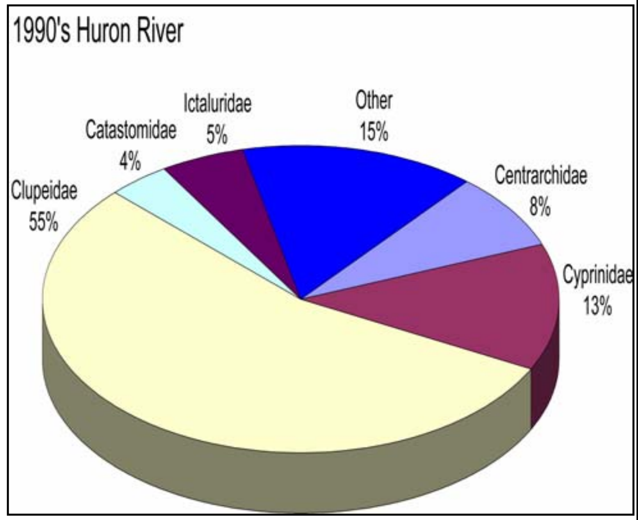
- White perch	1010
- Emerald shiner	924
- Gizzard shad	700
- Freshwater drum	415
- Brown bullhead	269
- White Bass	223
- Orange spotted sunfish	119
- Pumpkinseed	115
- White Crappie	109
- Spot tail shiner	79

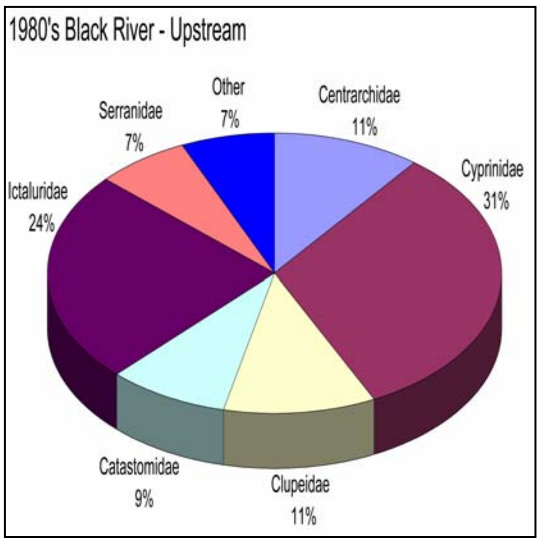
1998-00 CPE

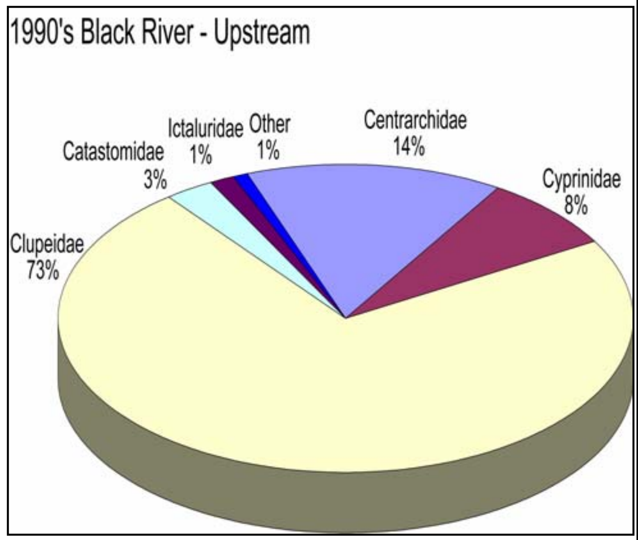
- Gizzard shad	357
- Pumpkinseed	82
- Brown bullhead	28
- Carp	21
- White sucker	10
- Largemouth bass	9
- Bluegill	7
- Freshwater drum	6
- Goldfish	4
- Trout-perch	3

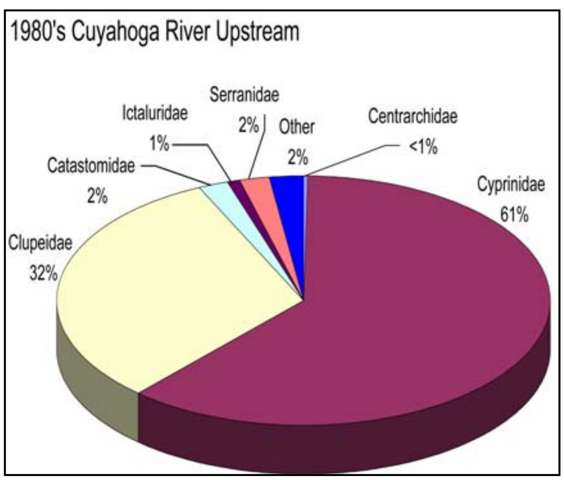
80's vs. 90's Lake Erie AOC Fish Communities

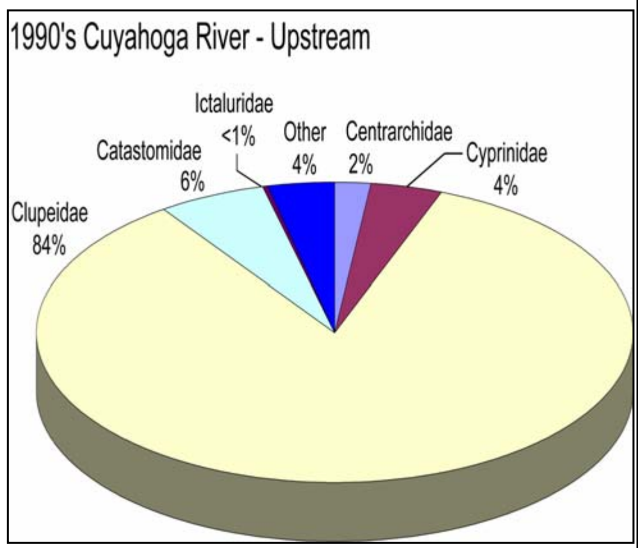

1980's Huron River
Centrarchidae 14%
Cyprinidae 4%
Clupeidae 11%
Catastomidae <1%
Ictaluridae 4%
Serranidae 52%
Other 15%

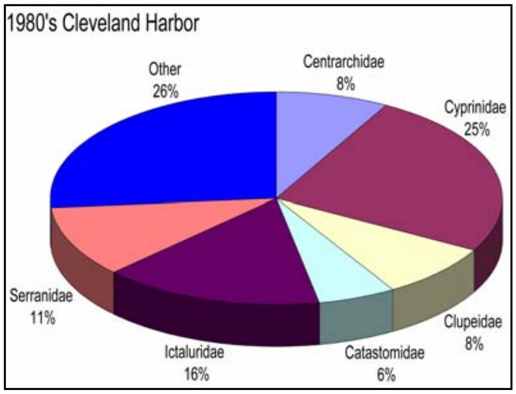

1990's Huron River
Ictaluridae 5%
Other 15%
Catastomidae 4%
Clupeidae 55%
Centrarchidae 8%
Cyprinidae 13%

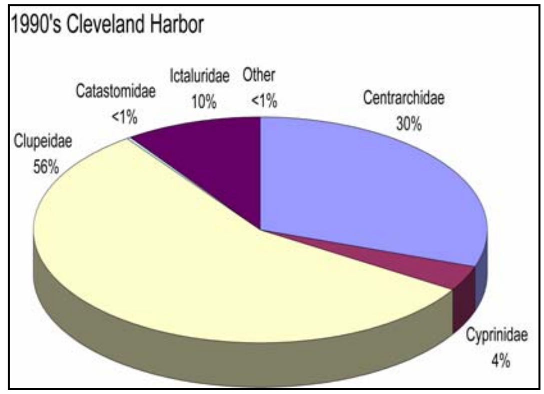

1980's Black River - Upstream
Other 7%
Serranidae 7%
Centrarchidae 11%
Cyprinidae 31%
Ictaluridae 24%
Catastomidae 9%
Clupeidae 11%

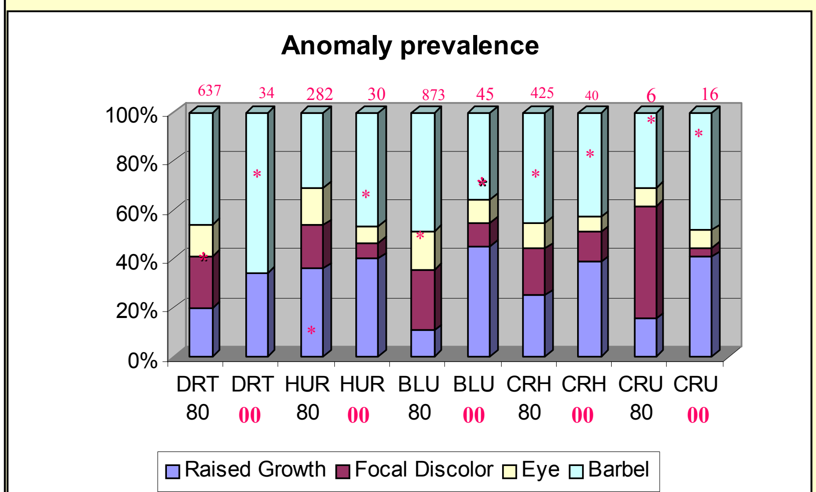

1990's Black River - Upstream
Ictaluridae 1%
Other 1%
Centrarchidae 14%
Catastomidae 3%
Cyprinidae 8%
Clupeidae 73%


1980's Cuyahoga River Upstream
Serranidae 2%
Ictaluridae 1%
Other 2%
Centrarchidae <1%
Catastomidae 2%
Cyprinidae 61%
Clupeidae 32%


1990's Cuyahoga River - Upstream
Ictaluridae <1%
Other 4%
Centrarchidae 2%
Catastomidae 6%
Cyprinidae 4%
Clupeidae 84%

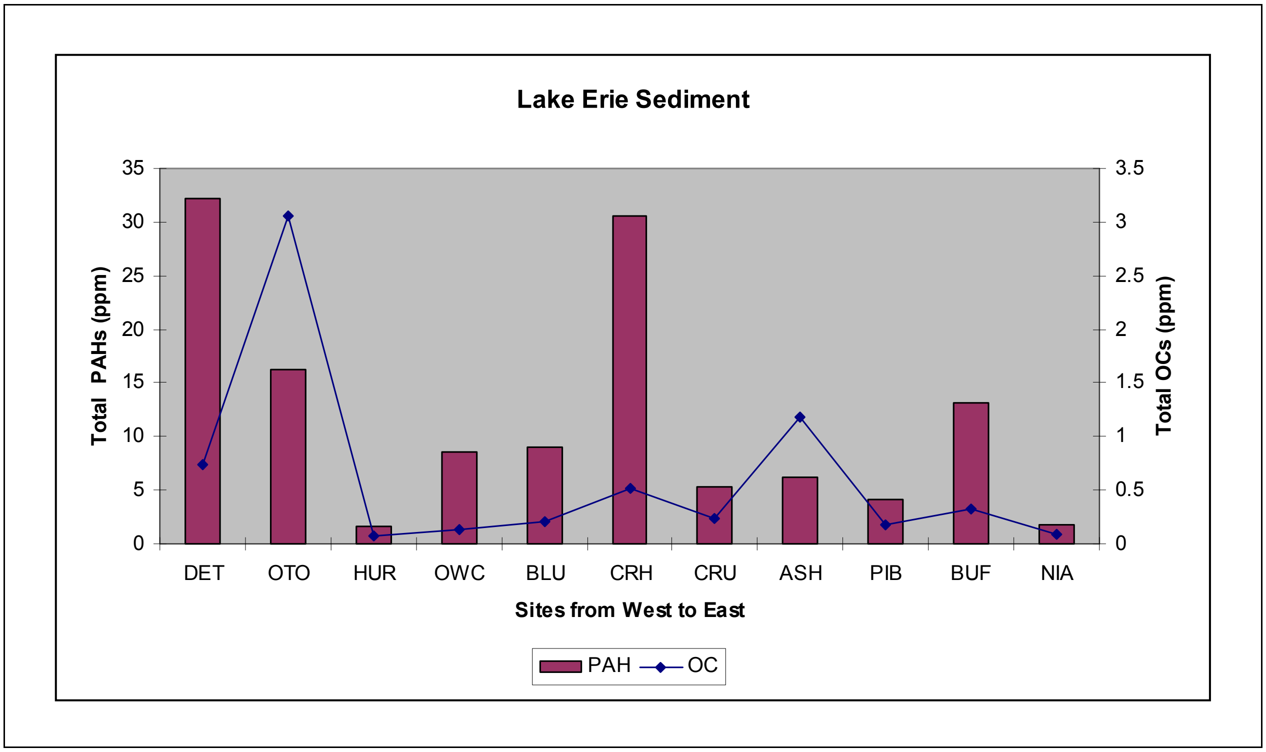


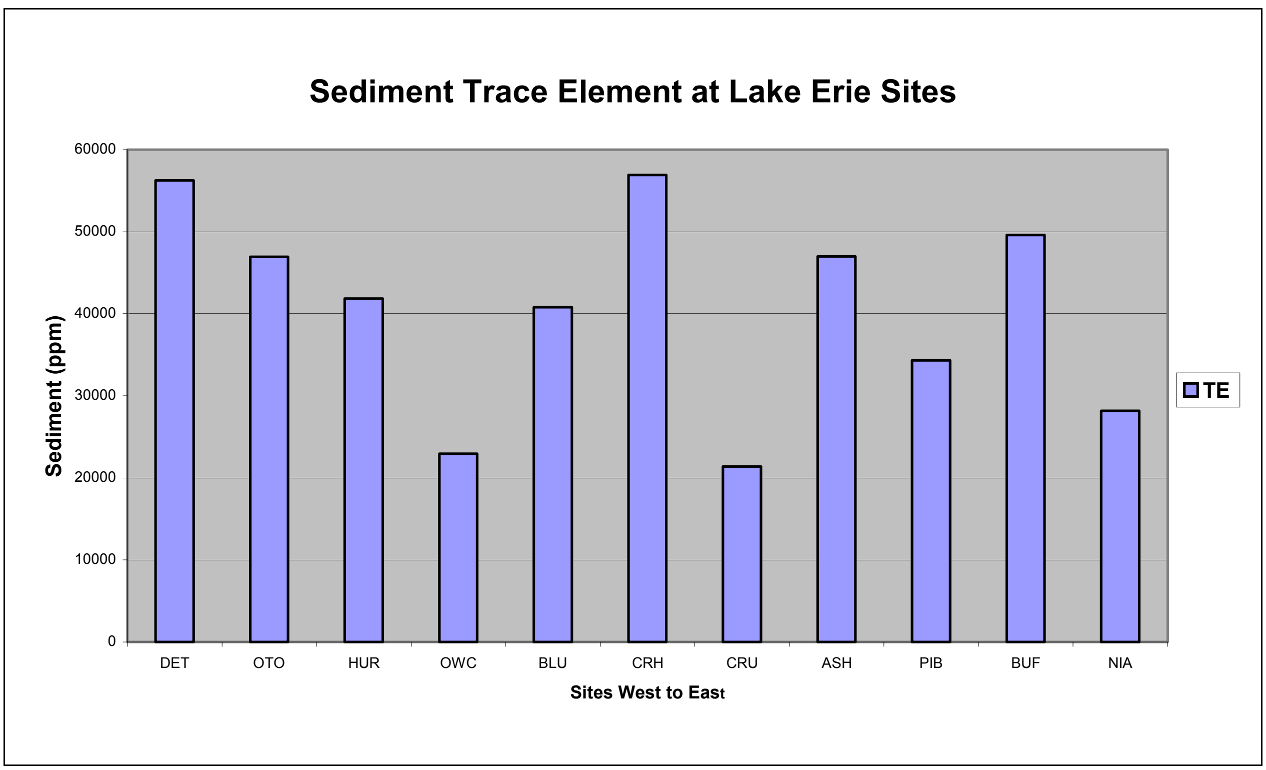


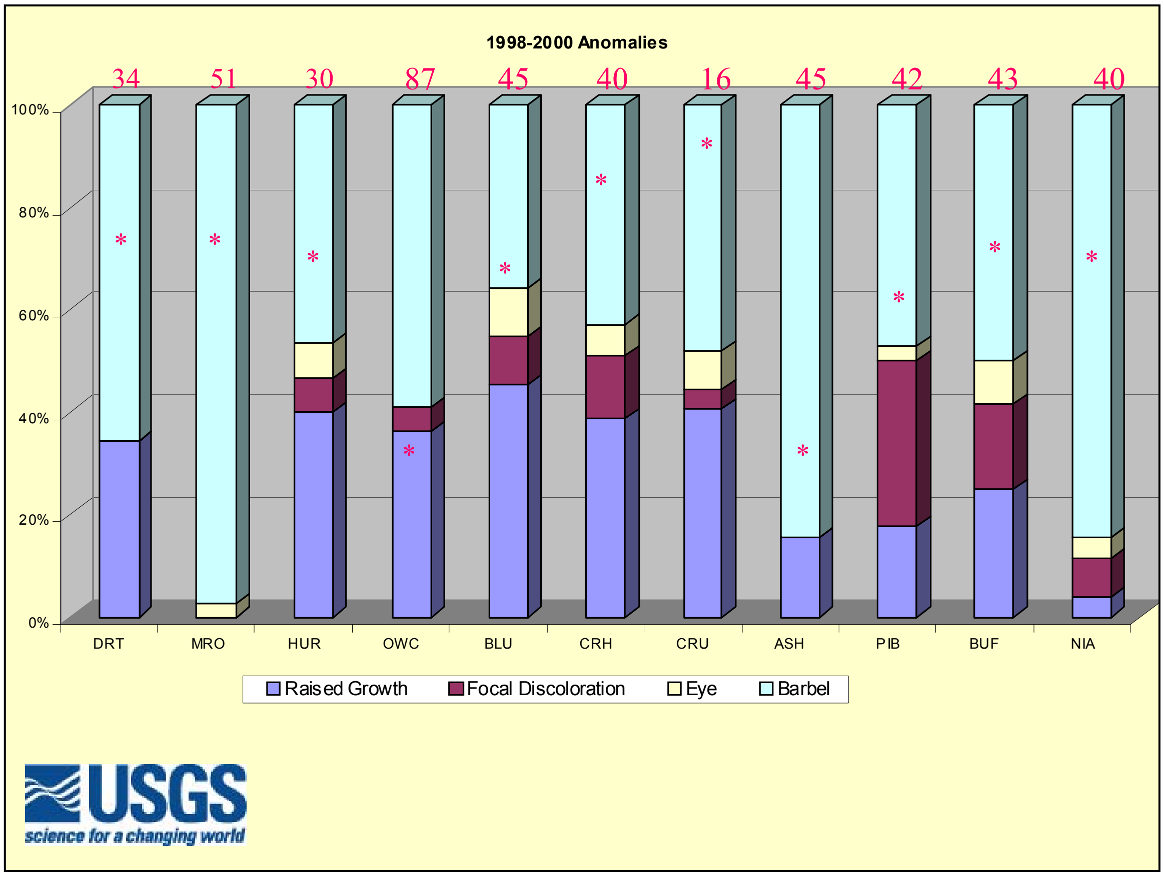


Lake Erie Contaminant Groups
Sediments 1998 – 2000 (PAH/OCs PPB – Metals-PPM)

Site	PAH Total	PAH priority	PAH carcino	OC Total	PCB Total	DDx Total	Chlordane Total	Metals Total	Metals 6 selected
DRT	31	10.7	6.3	0.72	0.51	0.082	0.039	56,268	1,064
MRO	15	7.9	5.9	3.05	2.93	0.081	0.027	46,932	423
HUR	2	0.6	0.4	0.06	0.04	0.013	0.002	41,830	153
OWC	8	3.3	2.2	0.12	0.08	0.033	0.003	22,941	108
BLU	9	3.6	2.4	0.20	0.15	0.030	0.004	40,794	271
CRH	29	13.5	9.0	0.51	0.46	0.023	0.008	56,932	791
CRU	5	2.3	1.5	0.22	0.19	0.025	0.004	21,378	222
ASH	6	2.2	1.4	1.18	1.03	0.013	0.004	46,971	247
PIB	4	1.5	1.1	0.16	0.13	0.013	0.003	34,329	722
BUF	12	4.6	3.2	0.32	0.25	0.028	0.029	49,579	417
NIA	2	0.7	0.4	0.08	0.06	0.005	0.002	28,154	113


Lake Erie Sediment
Total PAHs (ppm)
Total OCs (ppm)
35
30
25
20
15
10
5
0
3.5
3
2.5
2
1.5
1
0.5
0
DET
OTO
HUR
OWC
BLU
CRH
CRU
ASH
PIB
BUF
NIA
Sites from West to East
PAH
OC


Sediment Trace Element at Lake Erie Sites
Sediment (ppm)
60000
50000
40000
30000
20000
10000
0
DET
OTO
HUR
OWC
BLU
CRH
CRU
ASH
PIB
BUF
NIA
Sites West to East
TE



Conclusions I

- **EAPI developed for SOLEC for external anomalies: Indicator 101**

- **LEEI – 1998-2000**

Compared to 80s
*** NOW – Same or lower concentrations of sediment contaminants**
*** Higher incidence of anomalies in BB**
*** CPE and dominance in fish communities has shifted**

Now:
*** Increase in sediment OCs and decline in RG and FD at selected sites**
*** 31% of testest indicate significant regression of increased contaminants and increase in incidence of external anomalies – particulary barbells and raised growths**

How do theses conclusions make the

EAPI meaningful?

- **As part of the Monitoring "toolbox"**

- **Fish Health Component**
  - **Necropsy not necessary on all fish, only those with external anomaly**

- **EAPI and/or use of Tumor Prevalence**
  - **will help to determine impairment**

Impairment based on Comparison

Of Rates of Occurrence Of Internal Tumors or Related External Anomalies at Sites of Interest

Use of Mature (> 3yrs old) Near Shore Benthic Fish

Brown bullhead, Black bullhead, White sucker and Redhorse species

Interpretation of Impairment

Mature Near-shore Benthic Fish

- **Histopathologically Verified Internal Tumor Prevalence (liver or bile duct) of > 5%**

- **Prevalence of Raised Growth on Lip > 10%**

- **Prevalence of Raised Growth on body and lips > 15%**

Mature Brown or Black bullheads

- **Prevalence of Barbel abnormalities (stubbed, deformed or missing) of > 20%**

Conclusions II

- EAPI: part of monitoring effort for evaluating the state of the ecosystem and de-listing goals

Multi-tiered approach

- **Fish and invertebrate communities**
- **Organism**
  - **Fish Health**
    - **Endocrine biomarkers**
    - **Immune biomarkers**
    - **External Anomalies**
  - **Tissue**
    - **Histopathology**

Relate to contaminants and ecological risk assessment

Conclusions III

- Use of EAPI as part of a common index for collaborative US-Canadian monitoring at Great Lakes Areas of Concern as an indicator of: Ecosystem Health

Chuck Murray – Presque Isle Bay Database as a Model for AOCs

Chuck Murray discussed the development of the Presque Isle Bay database in order to manage data in regard to fish tumors, which could be used as a model for other Areas of Concern. Prior to the development of the database, data were tallied manually, making the process time consuming and error prone. The database was developed using Microsoft Access 2000 and was designed to include the following information: reference number (year and fish #), date, location and/or area, species of fish, length, weight, age, sex, notes and comments, collectors, tag information, health (barbells, tumors, pigmentation), digital information, and histological information. Advantages in using the database include: summaries can be obtained with the click of a button, counting errors can be eliminated, "what if" scenarios can be quickly assessed (i.e. data for fish of different age classifications), raw data can be easily shared among researchers, and digital images can be used to validate what was seen in the field.

A severity index (score) was developed for analyzing DELTs in the database. The severity score is a semi-quantitative application of physiological anomalies, and the score ranges from 0 to 3 in which: 0 = clean, 1 = mild, 2 = moderate and 3 = severe. The DELTs included in the database are skin lesions, mouth lesions, lesions/wounds, yellow pigmentation, black pigmentation, barbels, scars, eyes, and ulcers.

In a 2002 bullhead assessment, four sites were analyzed for tumors and other deformities (Presque Isle Bay, Canadohta Lake, Elk Creek, and Sugar Lake). Mouth and skin lesion rates were highest in Presque Isle Bay for both fish above 200 mm and above 250 mm; however, the severity of the lesions varied from site to site and among the two length classes of bullheads. These variations are an example of how the database can be used to assess "what if" scenarios.

The data within the database will be available on the Internet and the hope is to develop a DELT database to include all Areas of Concern. In order to achieve this goal, field names will have to be standardized and what is or is not comparable among Areas of Concern will need to be determined. Mr. Murray concluded his presentation by giving an example of how the database works.

Chuck Murray's Power Point Presentation

Presque Isle Bay
Area of Concern

Data Management

Chuck Murray
Pennsylvania Fish and Boat Commission

Brown Bullhead
Delt Survey

1985 – 2002

5000 + Observations

- **Gross visual observations**
- **6 different sampling sites**
- **~1/2 tagged**
- **Sub-sample of histopathologies**
- **2 primary species**
  - *** others can be incorporated**

Historical Data Analysis

- **Data tallied manually**
- **Time consuming**
- **Prone to error**

Microsoft Access 2000

- **Unique Reference number** **20030001-20039999**
- **Date**
- **Location, Area**
- **Species**
- **Length, Weight, Age, Sex**
- **Collectors**
- **Tag information**
- **Health (barbels, tumors, pigmentation)**
- **Digital images**
- **Notes/ Comments**
- **Histopathological summary**

Access 2000

- **Summaries with the "click" of a button**
- **Elimination of counting errors**
- **Ability to quickly assess "what if" scenarios**
- **Easily share "raw" data with other researchers**
- **Digital images to validate what was seen in the field**
- **Fairly large file (300 MB)**

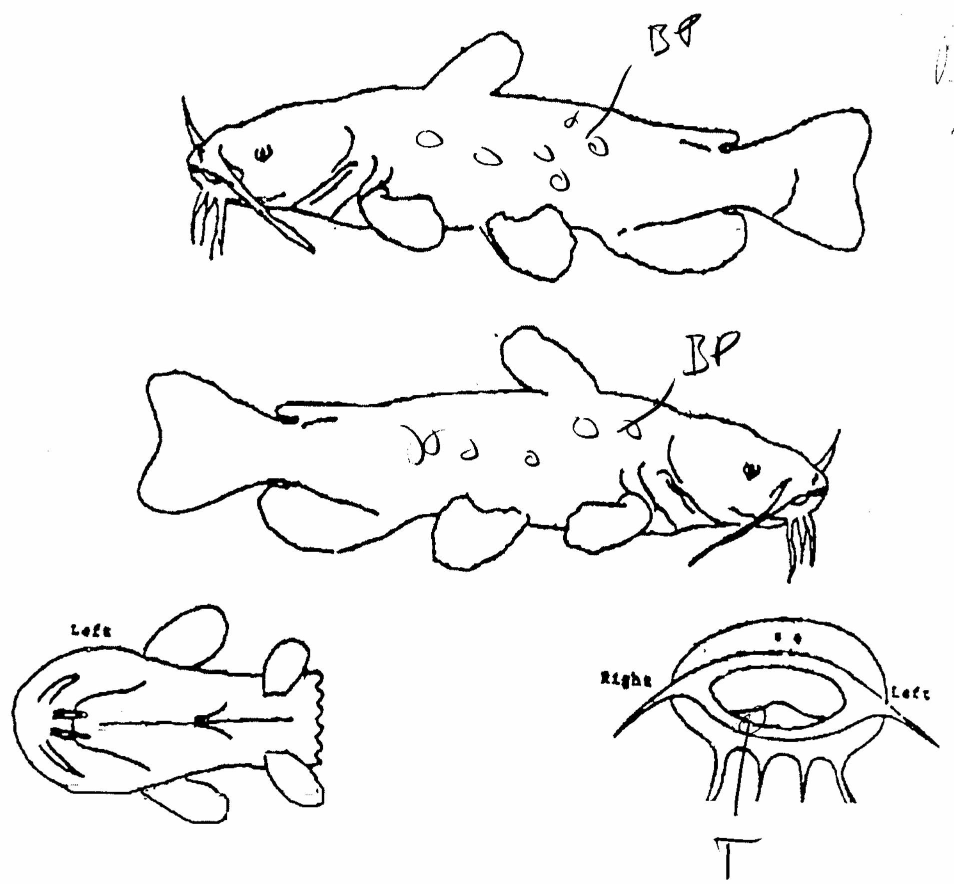
DELTS

- **Skin Lesions**
- **Mouth Lesions**
- **Lesions/ Wounds**
- **Yellow Pigmentation**
- **Black Pigmentation**
- **Barbels**
- **Scars**
- **Eyes**
- **Ulcers**

Pre-2002 Field Data Sheet

BIOINDICATOR SURVEY-FISH DATA RECORD

Species BBH | Fish No. 2387
Collection Date 4/15/99 Time: | Length 360 (cm)
Collection Site Lagoon | Weight 640 (grams)
Capture Tags No. | Collection Method



2002 Data Form

Presque Isle Bay Fish Tumor Study
Fish Health Data Sheet

Reference Number 2002- **Date:** / / **Time:** : **Field Observers**

Location ☐Eaton Reservoir ☐Lake LeBoeuf ☐Lake Pleasant ☐ Edinboro Lake

☐ Presque Isle Bay:____________________ ☐Other______________________________

Capture Gear		Species		Tagging Information
Trapnet	☐	Brown Bullhead	☐	
Electrofishing	☐	Yellow Bullhead	☐	Tag Number Recapture Date / /
Angling	☐	Carp	☐	
Other	☐	Other__________		Recapture Location

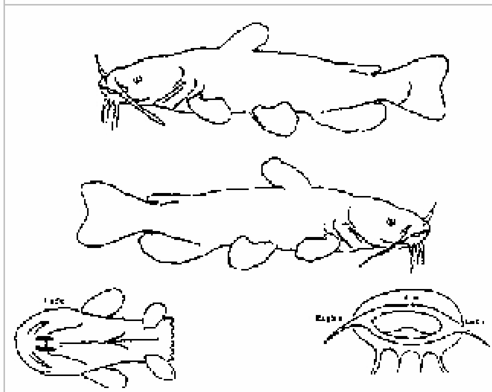
Sex **M** **F** **Length (mm)** **Weight (grams)**

Aging Technique: Spines ☐ Otoliths ☐ Other ☐ Age:

Fish Health Information

Clean ? **Yes** ☐ **No** ☐ **(Note Physical Condition Below)**

	*Severity Score	
Barbels	0 1 2 3	Barbels-Notes
Skin Tumor	0 1 2 3	Skin Tumor-Notes
Mouth Tumor	0 1 2 3	Mouth Tumor-Notes
Pigmentation (Yellow)	0 1 2 3	Yellow Pigmentation-Notes
Pigmentation (Black)	0 1 2 3	Black Pigmentation-Notes
Lesions	0 1 2 3	Lesion-Notes
Ulcers	0 1 2 3	Ulcer-Notes
Scars	0 1 2 3	Scars-Notes
Eyes	0 1 2 3	Eyes-Notes
Histopathology	Yes No	Histopathology-Notes
Tissue Chemistry	Yes ☐ Whole Fish ☐ No ☐ Fillet ☐	Tissue-Notes



Pictures: Yes ☐ No ☐ File #________________

Notes:

* 0 No visible maladies
* 1 Mild Condition
* 2 Moderate
* 3 Severe

Bullhead Tumor Database

- Initiated a "Severity Score"
    - Semi-quantitative application of physiological anomalies
        - * 0 = clean
        - * 1 = mild
        - * 2 = moderate
        - * 3 = severe
- Standardization

Assigning a Severity Score

- Barbel 0
- Black Pigment 0
- Mouth Lesion 0

- Fish is "clean"

Reference number
20020231

- Barbel 1
- Black Pigment 2
- Mouth Lesion 3

- This fish died in the holding tank

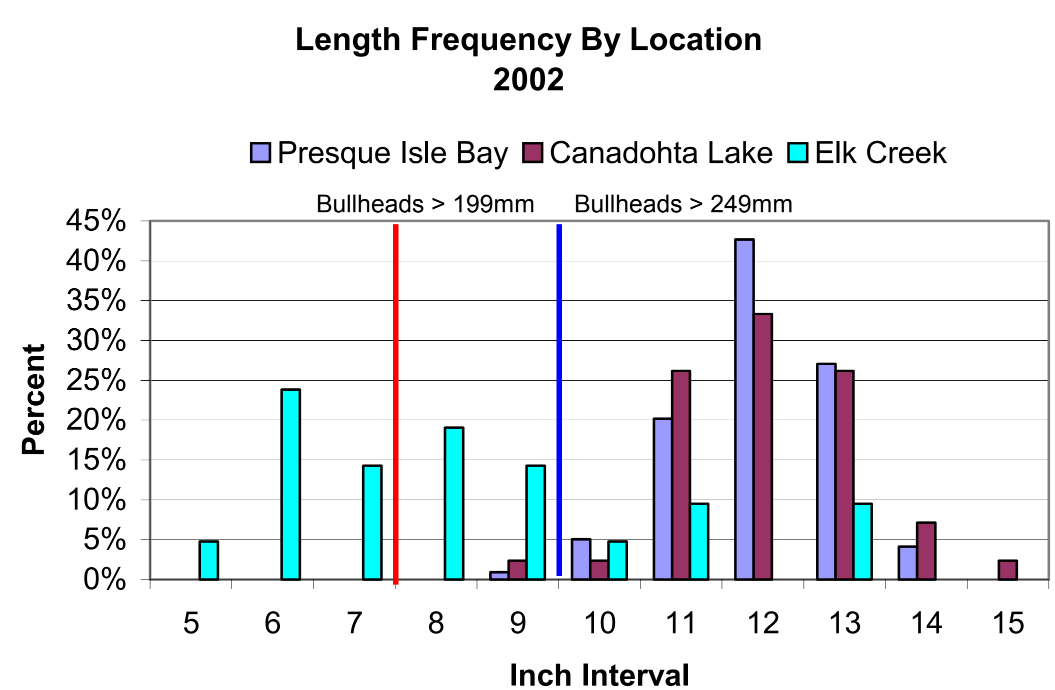
Reference number
20020153

2002 Bullhead Assessment

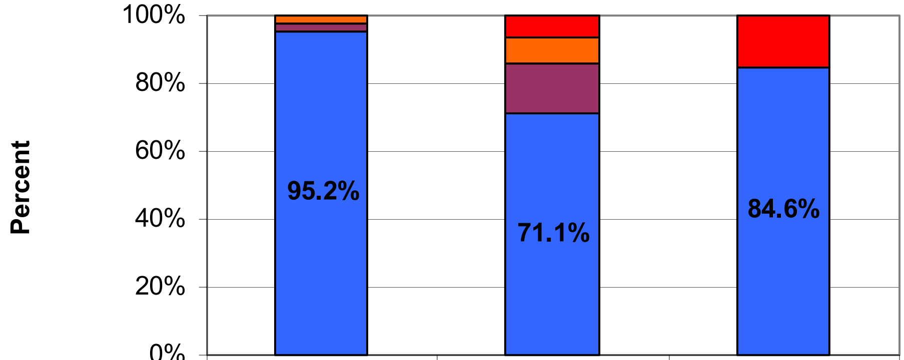
- 4 Sites
  * Presque Isle Bay
  * Canadohta Lake
  * Elk Creek
  * Sugar Lake

Brown Bullhead Sample Size – 2002

Location	Total Sampled	>199mm	>249mm
Presque Isle Bay	222	218	216
Canadohta Lake	42	42	41
Elk Creek	21	13	5
Sugar Lake	3	3	3



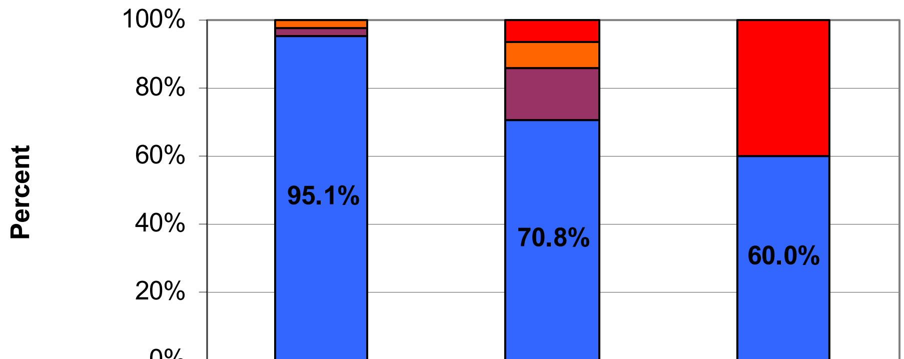
Brown Bullhead Mouth Lesion Rate
2002
(200 mm+)



Location	Canadohta Lake	Presque Isle Bay	Elk Creek
Severe	0.00%	6.42%	15.38%
Moderate	2.38%	7.80%	0.00%
Mild	2.38%	14.68%	0.00%
Clean	95.24%	71.10%	84.62%

Location

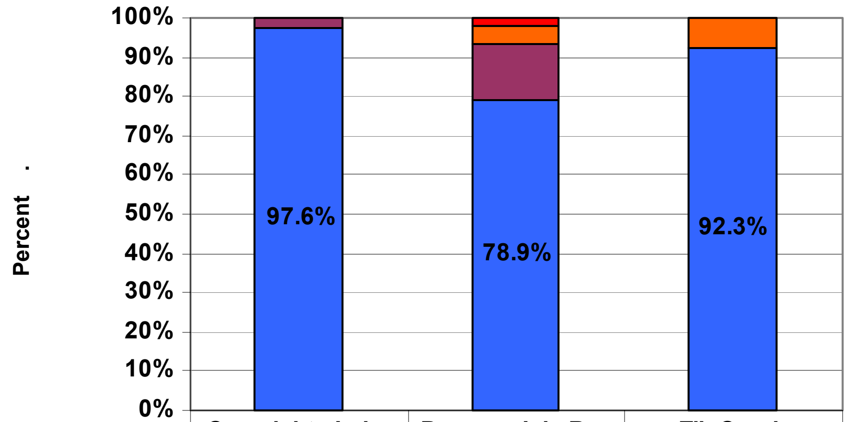
Brown Bullhead Mouth Lesion Rate
2002
250+ mm



Location	Canadohta Lake	Presque Isle Bay	Elk Creek
Severe	0.00%	6.48%	40.00%
Moderate	2.44%	7.87%	0.00%
Mild	2.44%	14.81%	0.00%
Clean	95.12%	70.83%	60.00%

Location

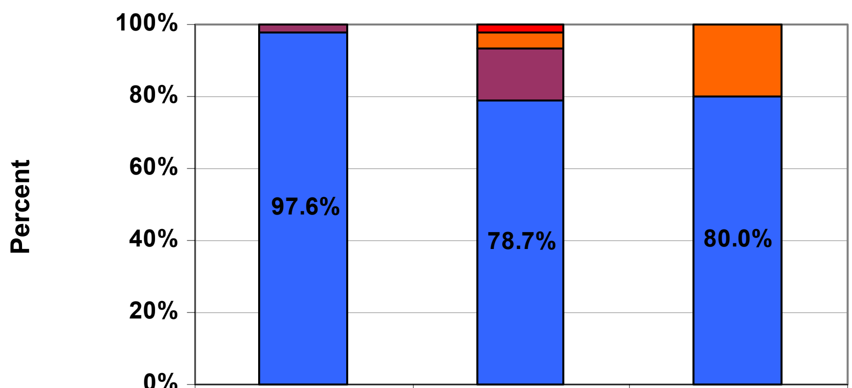
Brown Bullhead Skin Lesion Rate by Location
2002
(200 mm +)



	Canadohta Lake	Presque Isle Bay	Elk Creek
Severe	0.00%	2.29%	0.00%
Moderate	0.00%	4.13%	7.69%
Mild	2.38%	14.68%	0.00%
Clean	97.62%	78.90%	92.31%

Location

Brown Bullhead Skin Lesion Rate by Location
2002
250+ mm



	Canadohta Lake	Presque Isle Bay	Elk Creek
Severe	0.00%	2.31%	0.00%
Moderate	0.00%	4.17%	20.00%
Mild	2.44%	14.81%	0.00%
Clean	97.56%	78.70%	80.00%

Location

Sharing Data Among Areas of Concern

- **Data will be available on the Net**
- **AOC Delt Database**
- **Standardize the Field Names**
- **Identify what is not “comparable” in other AOCs or investigations**

Bob Wellington - Presque Isle Bay Monitoring Plan for Area of Recovery Stage

Bob Wellington discussed the Presque Isle Bay fish tumor investigations that were conducted from 1992 to 2002. The purpose of the investigations was to assess beneficial-use impairments (fish tumor rates and other deformities) in the hope of delisting Presque Isle Bay as an Area of Concern, plan for long-term monitoring (10-year minimum) of tumor rates of brown bullheads in Presque Isle Bay, and to conduct monitoring of bullheads in relatively unimpacted reference sites. The target population was brown bullheads (*Ameiurus nebulosis*) but yellow bullheads (*A. natalis*) were incidentally collected. Secondary targets could include killifish, largemouth bass, and carp. Fish were collected by electro-fishing and trap netting with trap netting being the most effective method. Data were collected from bullheads greater than 200 mm in length, and otolith and spines were collected from almost every fish in order to perform aging analysis.

Bullheads were collected between April and June (index period) and the hope is to conduct annual observations of gross tumors through 2012, and histopathological analysis in 2003 and 2004 and every three years thereafter. Gross observations and histopathological analysis is proposed in reference lakes (Canadohta and Sugar) in 2003 and 2004, and every three years thereafter. Tagging efforts have been carried out in Presque Isle Bay dating back to 1992. The purpose of these tagging efforts are to characterize migration patterns (validate Presque Isle residency), trace the health of individual fish over time, conduct population estimates, and validate ages of fish. Tagging studies have shown most migration of bullheads to occur within Presque Isle Bay itself; however, there have been documented occurrences of migration between Lake Erie and Presque Isle Bay. All the data collected from the bullhead studies are to be incorporated into the database developed by Chuck Murray, who is with the Pennsylvania Fish and Boat Commission.

Results from 1999 to 2000 studies suggest an increase in lesion rates (skin and mouth) of Presque Isle Bay brown bullheads greater than 199mm and greater than 250mm. Orocutaneous lesion rates have declined in relation to results obtained in 1992 studies. On a positive note, liver tumors appear to be staying at approximately 2% occurrence. Results of a 2002 assessment conducted in Canadohta Lake, Sugar Lake, Elk Creek, and Presque Isle Bay suggest orocutaneous lesion rates are highest in Elk Creek, but skin lesion rates are the highest in Presque Isle Bay.

Mr. Wellington concluded by proposing future investigations including: looking at other indicator species (e.g. killifish, carp, and bass), development of accurate ageing analysis, genetics (Does hybridization occur in Presque Isle Bay bullheads?), and additional sediment and water sampling for metals and/or organics. Mr. Wellington also mentioned that historically Presque Isle Bay had black bullheads, but in recent years after having looked at thousands of bullheads, there has not been a single black bullhead identified.

Bob Wellington's Power Point Presentation

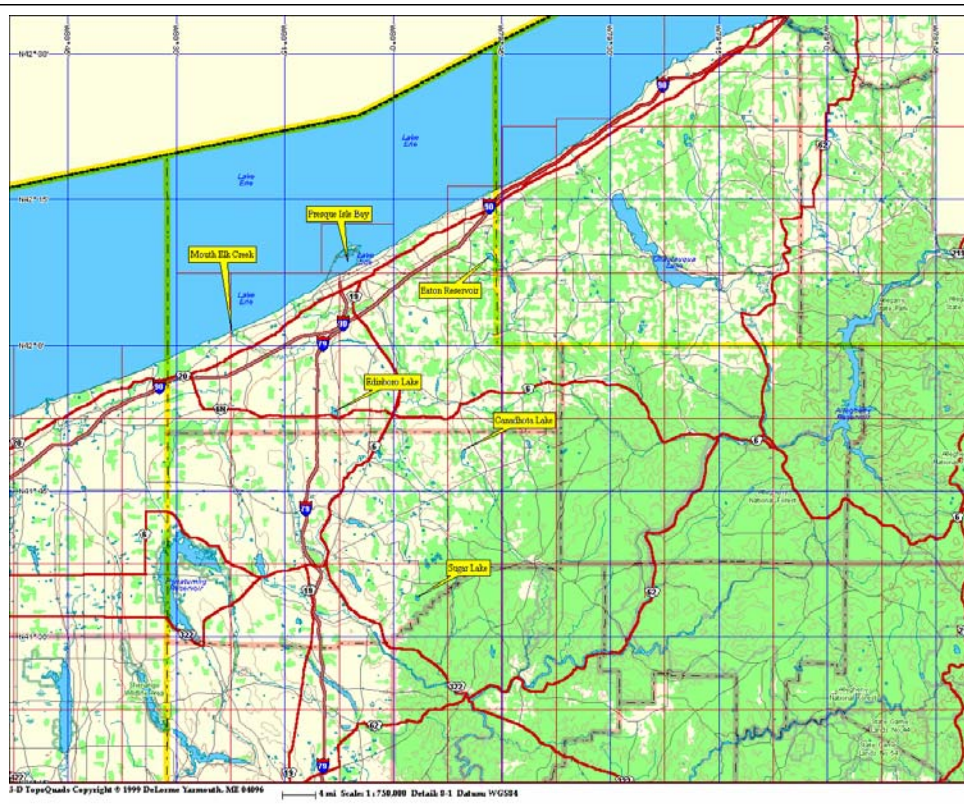
Presque Isle Bay
Fish Tumor Investigations
1992 – 2002

Principle Investigators

Pennsylvania DEP	**Jim Grazio**
Pennsylvania Sea Grant	**Eric Obert**
Erie County Health Department	**Bob Wellington**
Pennsylvania Fish and Boat Commission	**Chuck Murray**

Logistical Support

- **Pennsylvania DCNR**
- **Education**
- **Federal Government**
- **Private Citizens/ Sportsman Groups**
- **Presque Isle State Park Staff**
- **Schools/ Universities**



Purpose

- Assess BUI (tumor rates) with the goal of de-listing Presque Isle Bay as an AOC
- Plan for Long-term monitoring (10 year minimum) of tumor rates of brown bullhead in Presque Isle Bay
- Concurrent monitoring of bullheads in relatively unimpacted reference sites

Target Population

- Brown bullhead (*Ameiurus nebulosis*) and incidentally collected yellow bullheads (*Ameiurus natalis*)
- Secondary targets may be other species such as killifish, largemouth bass and carp

Carp with Bile Duct Carcinoma

Carp with External Deformities

Bullhead Sampling Location

Collection Methods

- Electrofishing
- Gill/ Trap netting
- Angling

Minimum Size of Bullheads

- Generally > 200mm
- Otoliths/ spines collected from randomly selected individuals
    * Goal - ~30 fish/site/year

Minimum Sample Size

- 30 randomly sampled bullhead for liver tumors
- Sub-sample any external neoplastic lesions noted on these 30 fish
- 200 individual for gross visual observation

Sampling Frequency

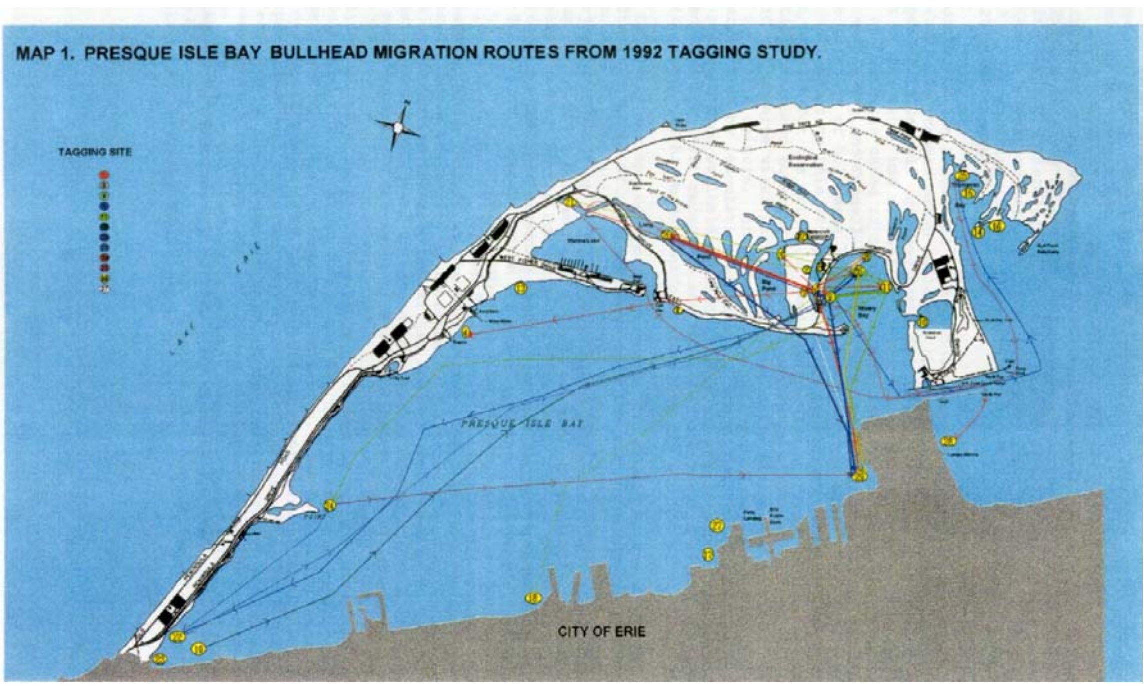
- Presque Isle Bay: Annual observations of gross tumors through 2012
- Histopathological analysis annually 2003, 2004 and every three years thereafter
- Reference Lakes: gross observation of tumors and histopathology in 2003, 2004 and thereafter as resources allow

Index Period

Spring: April – June

Tagging

- **Tagging has been conducted since 1992**
- **Grossly observed bullheads will be tagged for future reference**
- **Objectives**
    * **Migration patterns (validate PIB residency)**
    * **Ability to trace the health of individual fish over time**
    * **Population estimates**
    * **Age validation**
- **Need to address tag retention**



Obert et al.

Data Management

- **Numerical and diagrammatic data initially recorded on a standardized field data sheet**
- **Digital photograph of each specimen to the degree practical**
- **Data entered into electronic database by qualified staff**
- **Database validation**

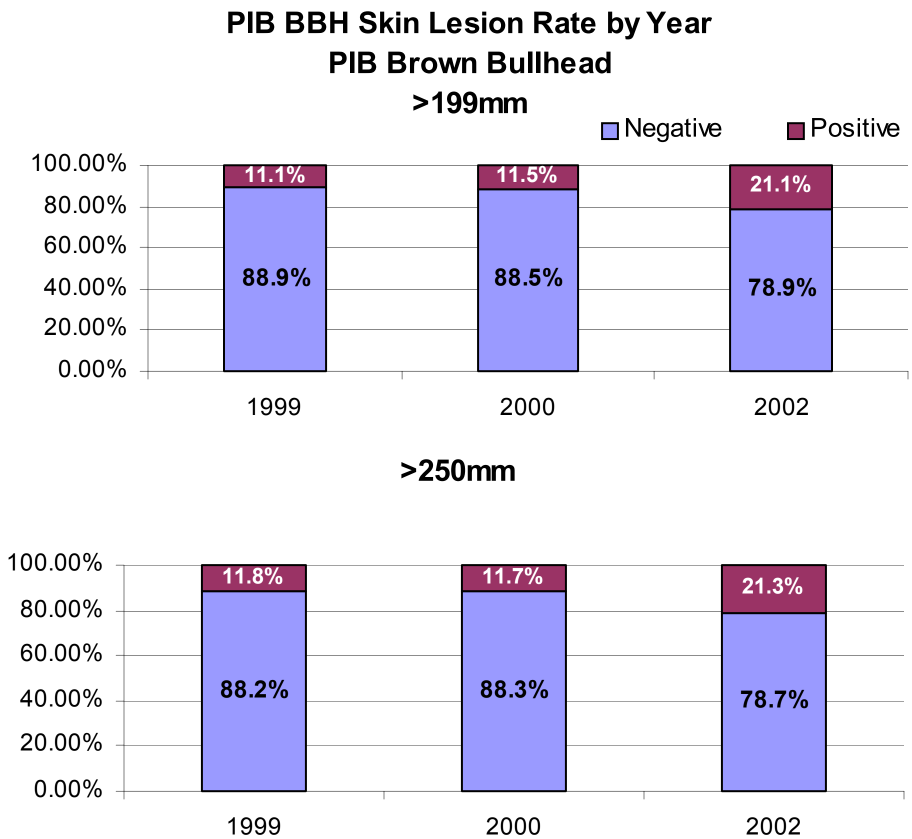
Other Species

- Carp as indicator species

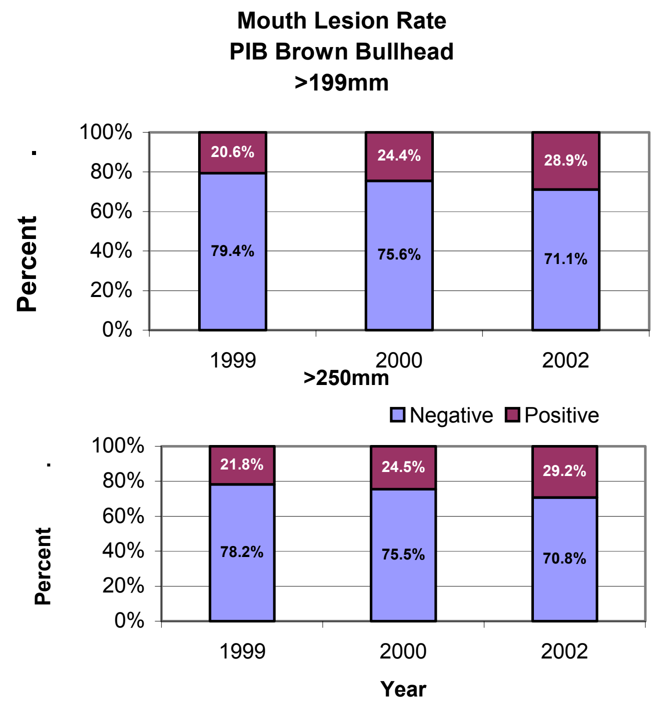
- Estimated 10 – 15% deformed
  * Liver tumor
  * Spinal deformities
  * Missing eyes
  * Lesions

Presque Isle "Tumor" Rates Over Time

1992 – Present



(Results based on gross observations opposed to histopathological analysis)



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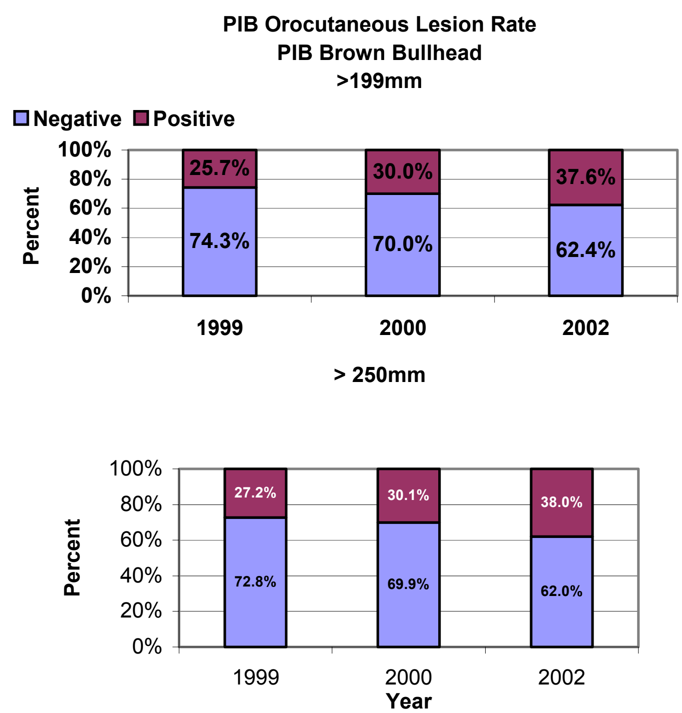
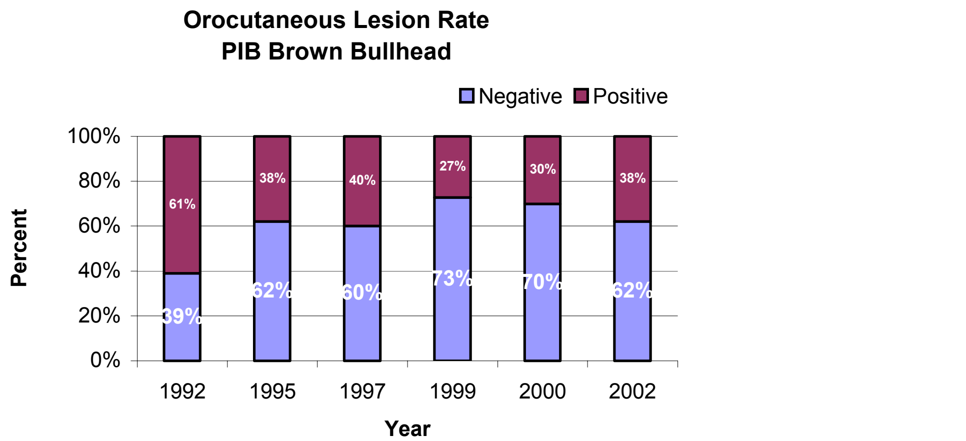


(Results based on gross observations opposed to histopathological analysis)



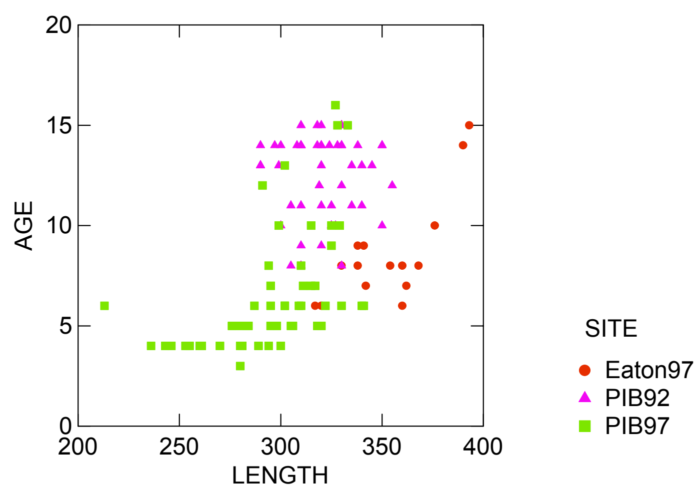
(Results based on gross observations opposed to histopathological analysis - 1995 Data taken from the Department of Agriculture and 1997 data was taken from Penn State Data)

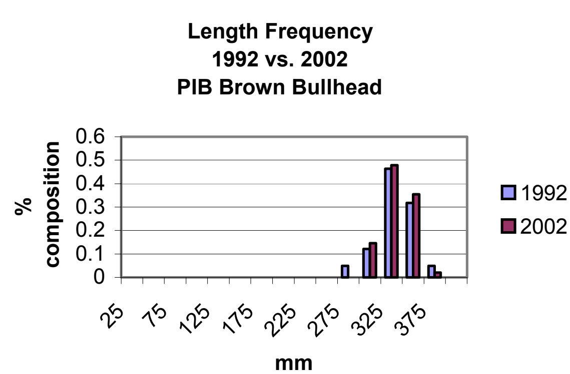
Comparing Lesion Rates Among Areas and Years

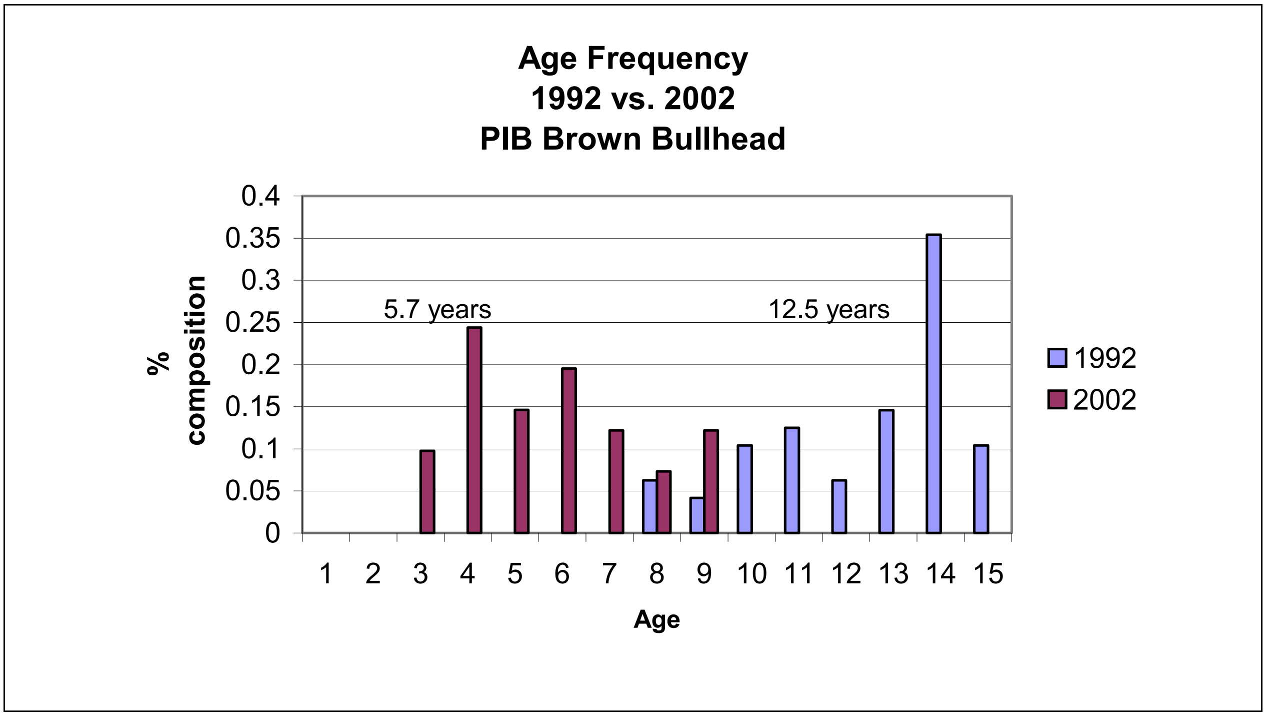
Eaton 1997 = No tumors
PIB 1992 = High Tumor Rate
PIB 1997 = Lower Tumor Rate

- **Eaton Reservoir tended to have larger, older fish but no lesions were found.**

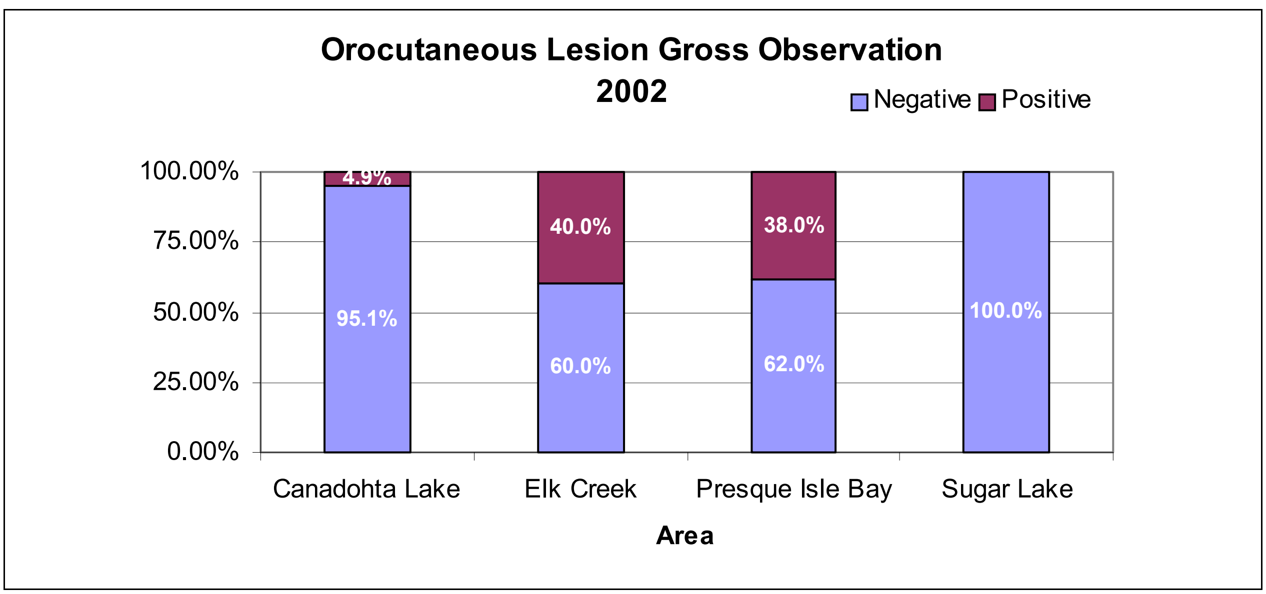
- **PIB fish exhibited lower lesion rates when the population was composed of smaller, younger fish.**

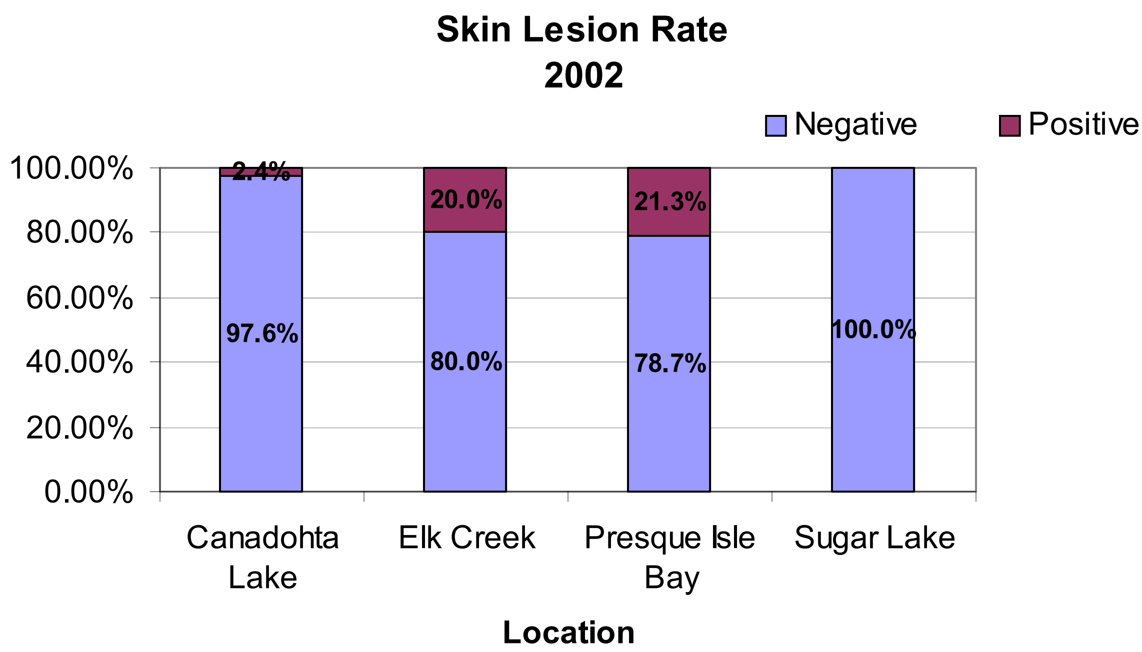




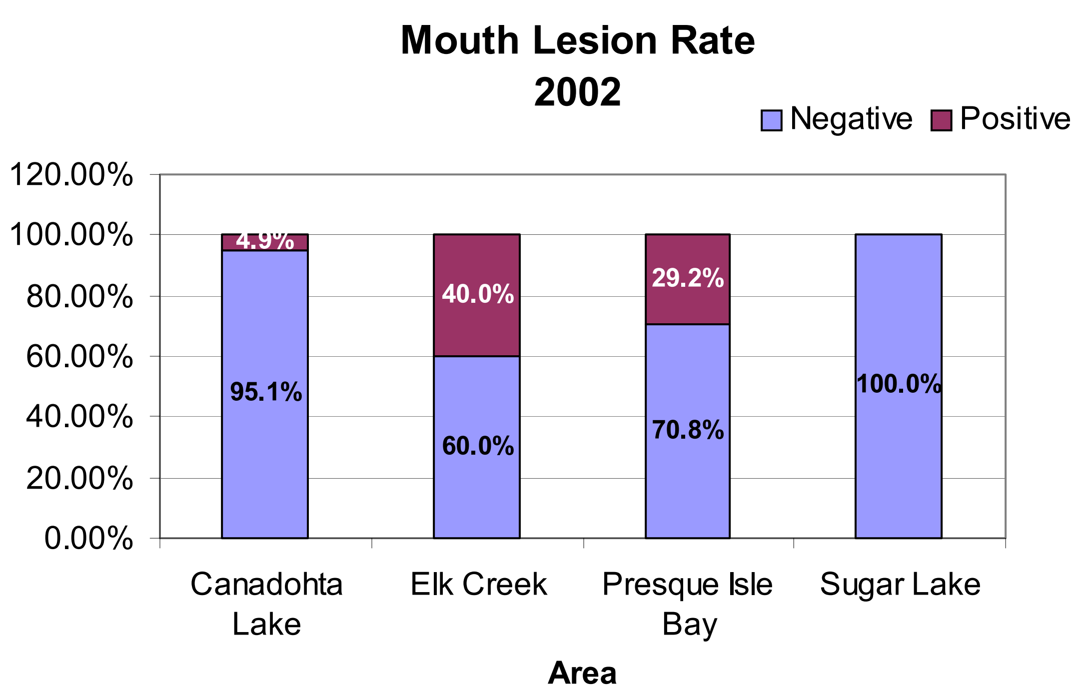


2002 Assessment
Lesion Rates Among Different Sampling Sites





(Results based on gross observations opposed to histopathological analysis)



(Results based on gross observations opposed to histopathological analysis)

Unknown Inputs from Lake Erie

Blue-green Algae Bloom in Presque Isle Bay

Future Investigations

- **Look at other indicator species**
  - **Killifish, Carp and Bass**
- **Accurate Aging**
- **Genetics**
  - **Hybrids**
- **Additional sediment/ water sampling for metals and/ or organics**

Panel Discussion – Monitoring Recommendations

Paul Baumann, Chuck Murray, Roger Thoma, and Bob Wellington

Question:
How frequently should fish sampling take place?
Answer: (Paul Baumann and Roger Thoma)
If there are not any special events (e.g. dredging), samples should be taken every three years; otherwise, fish samples should be collected before and after special events. Ohio EPA uses a five-year rotation, depending on litigation pressures. The duration of the study is more important than frequency of sampling. Presque Isle Bay monitoring has been an annual effort.

Question:
Will the formal EPA plan have an annual monitoring component?
Answer: (Bob Wellington)
For Presque Isle Bay it is proposed that studies will take place in 2003 and 2004, and then every three years until 2012. This could speed up the delisting process.

Question:
How many locations should be sampled within Presque Isle Bay, or if fish are known to be residents, can sampling efforts be concentrated in one location?
Answer: (Chuck Murray and Bob Wellington)
Sampling sites are limited to where the optimal conditions occur for bullheads; water quality and shoreline structures impact site identification. PA Fish and Boat Commission trap nets remain in a site annually, so this could be used for consistent data collection. The actual number of bullheads collected may not be as important as sex/age ratios.

Question:
What would be a good surrogate species be if brown bullheads were not present in an Area of Concern?
Answer: (Roger Thoma)
In Ohio some sites are so polluted that the emphasis is restoring fish populations to the area rather than quantifying tumors/DELT's.

Question:
Are there any studies on sediment characteristics of urban runoff?
Answer: (Roger Thoma)
In Ohio, runoff is a problem but there are not enough catchments or settling basins to collect sediment samples and determine effects.

Question:
How are you addressing Total Maximum Daily Load Programs (TMDLs) in Ohio?
Answer: (Roger Thoma)
A TMDL is a written, quantitative assessment of water quality problems in a water body and

contributing sources of pollution. Basinwide assessments are being used to address these issues. Communities are urged to control urban runoff. In middle Cuyahoga, it was recommended all low-head dams be removed to reach TMDL.

Question:
How do you ensure a random sample when sites and collection methods vary?
Answer: (Paul Baumann)
The difficulty is having enough fish to select a random sample. In most cases, the first 30 fish at a proper age caught are sampled. In a large system, where tumor rates vary across sites, the sites need to be treated as separate locations. Statistical analysis of sample size needed for tumor assessment should be conducted. The problem with sampling without replacement is that older fish are being removed and this could lead to a decrease in the population/recruitment, resulting in the skewing of results related to tumor occurrence.

Question:
Is there a reference site for Presque Isle Bay?
Answer: (Bob Wellington)
Could use Canadohta Lake, which is a relatively clean site containing healthy fish. Skin tumors are prevalent across the lake, but Presque Isle Bay and the Black River may be the best reference sites for liver tumors because of the relationship between remediation and changes in health (reduction in tumors).

Question:
Are bullheads with tumors a public health concern?
Answer: (John Harshbarger)
Feeding studies with rats were recommended; however, there were never any published experiments. Fish do metabolize PAHs in the liver; thus, they rarely show up in the filets. Based on this information, there is a low risk of human health impacts if filets are prepared correctly. Contaminants like PCBs and mercury are present in edible tissues, but these contaminants are not carcinogens.

Additional Comments/Concerns:

There is not a documented increase in tumor frequency in closely related species that hybridize, such as the yellow and brown bullhead. Likely to be the same with pumpkinseeds and blue gills.

A sampling protocol needs to be developed regarding Areas of Concern and fish tumors across the Great Lakes Basin. Minimum criteria need to be established, including: age, length, gender, season, and year. Possibly a working group could be formed to draft criteria.

Conference attendees are primarily Lake Erie and St. Lawrence experts, representatives from other Areas of Concern need to be contacted in order to establish criteria for a basinwide sampling/monitoring program.

White suckers have different morphology (scales), are susceptible to viruses that differ from diseases/cancers affecting bullheads, and have a greater range than bullheads. However, suckers do return to same site for spawning every year; therefore, they are a plausible indicator species. Carp are useful, but liver is not a discrete organ making difficult to isolate. Many species are omnivores, and feeding strategies may affect tumor occurrence. However, carp suffer from many deformities making them an excellent study organism. Carp also hybridize with goldfish- is there an impact on tumor rates?

A list of alternative species for beneficial-use impairment assessments needs to be developed.

Tumor surveys are useful in order to determine fish health, but other chemical analyses play a role in fish consumption advisories in Ontario. In Ohio, carp and catfish are listed as “Do not Eat.” Might want to link tumor monitoring with contaminant assays to determine if there is a link. A 1991 study showed fish with tumors had lower contaminant levels than fish without tumors.

The rationale used for listing and delisting an Area of Concern is the primary goal or focus of fish tumor studies. Focus on developing simple protocol to align science and management requirements.

DELT data dilutes rate of brown bullhead tumors because all species with deformities are used rather than a single species. This results in a need for lower criteria (0.5%) to reflect multiple-species aspect. DELT is a blunt instrument making it difficult to suggest statistical relationships.

Great Lakes Areas of Concern and Fish Tumors or Other Deformities

Remedial Action Plans (RAPs) originated in 1985 upon the recommendation of the International Joint commission. In 1987, the recommendations were made a permanent component of the Canadian-United States Great Lakes Water Quality Act, under Annex 2, with the goal of restoring beneficial uses in Great Lakes basin Areas of Concern. Currently there are 42 Areas of Concern within the United States and Canadian Great Lakes drainage basin, with as many as 43 Areas of Concern existing in the past (Collingwood Harbour was de-listed in 1994); all of which were listed according to the impairment of any one of the 14 beneficial uses as listed in Annex 2 of the GLWQA.

Currently, 16 of the 42 Areas of Concern are affected by the presence of the beneficial use impairment: fish tumors and other deformities, while four others are under assessment. The United States Areas of Concern with this beneficial-use impairment are St Louis Bay/River, Sheboygan River, Milwaukee Estuary, Grand Calumet River, Rouge River, Maumee River, Black River, Cuyahoga River, Ashtabula River, Presque Isle Bay and the Buffalo River. Canadian Areas of Concern with the presence of fish tumor or other deformities include Thunder Bay and Jackfish Bay, and binational Areas of Concern with this beneficial-use impairment are St. Marys River, Detroit River and the Niagara River (New York). The four Areas of Concern under assessment for the presence of fish tumors or other deformities are Peninsula Harbour, Bay of Quinte, Metro Toronto, and the St. Clair River.

The **Ashtabula River** Area of Concern, located in northeastern Ohio, has a drainage basin of 137 mi^2 and flows into the central basin of Lake Erie. The rivers' sediments have become severely contaminated due to the unregulated discharges and mismanagement of hazardous waste between the 1940s and 1970s. The Ashtabula River was designated as an Area of Concern due to the identification of six of the 14 beneficial-use impairments (restrictions on fish and wildlife consumption; degradation of fish and wildlife populations; fish tumors or other deformities; degradation of benthos; restrictions on dredging activities; and loss of fish and wildlife habitat). The impairments are caused by toxic substances (e.g. PCBs, heavy metals, and chlorinated organic compounds), sedimentation, cultural eutrophication (e.g. nutrients), and habitat modifications (e.g. marina construction and commercial shipping). The sources of these contaminants include bottom sediments, municipal and industrial discharges, commercial development, hazardous waste disposal sites, combined sewer outflows (CSOs), coal handling facilities, and rail yards.

The **Black River** Area of Concern, located in north-central Ohio, has a drainage basin of 467 mi^2 and is the only Ohio Area of Concern that includes an entire watershed. The Black River discharges into Lake Erie at the Port of Loraine. Land use within this Area of Concern includes agricultural (51%), rural (38%), urban residential (7%), and industrial (1%). Although contaminant loadings from point sources such as factories and wastewater treatment plants have been significantly reduced in the Black River, land disturbances associated with high residential growth rate and intensive agricultural practices are a problem. Considerable disruption of the riparian zone along the Black River has provided easy access for nonpoint source pollution (runoff) to enter the Black River. The Black River is faced with the task of restoring 10 of the 14 beneficial uses (restrictions on fish and wildlife consumption; degradation of fish and wildlife populations; fish tumors or other deformities; degradation of benthos; restrictions on dredging activities; eutrophication or undesirable algae; restrictions on drinking water consumption, or taste and odor; beach closings, degradation of aesthetics; and loss of fish and wildlife habitat).

The **Buffalo River** Area of Concern, located in the city of Buffalo in western New York, flows from the east and discharges into Lake Erie. The remedial actions in the Buffalo River are focused on six areas; stream water quality monitoring, river bottom sediments, inactive hazardous waste sites, municipal and industrial wastewater treatment facilities, combined sewer overflows, and fish and wildlife habitat. Chemical contamination, physical disturbances to the river bottom and shoreline, PCBs, chlordane, PAHs, navigational dredging, low dissolved oxygen levels, and DDT are all known causes of the five beneficial-use impairments (restrictions on fish and wildlife consumption; fish tumors or other deformities; degradation of benthos; restrictions on dredging activities; and loss of fish and wildlife) in the Buffalo River Area of Concern.

The **Cuyahoga River** Area of Concern, located in northeast Ohio, has a drainage basin of 813 mi^2 and discharges into Lake Erie. In 1936, a spark from a blowtorch ignited floating debris and oil on the Cuyahoga River, and several more fires erupted before June 1969, when a river fire gained national attention due to an article in Time magazine. Through the RAP process, 10 of 14 use impairments (restrictions on fish and wildlife consumption; degradation of fish and wildlife populations; fish tumors or other deformities; degradation of benthos; restrictions on dredging activities; eutrophication or undesirable algae; beach closings; degradation of aesthetics; degradation of phytoplankton and zooplankton populations; and, loss of fish and wildlife habitat) have been identified in the Cuyahoga River. Causes of these problems include cultural euthrophication (e.g. nutrients), toxic substances (e.g. PCBs and heavy metals), bacterial contamination, habitat modification, and sedimentation. The sources of these contaminants include municipal and industrial discharge, bank erosion, commercial/residential development, atmospheric deposition, hazardous waste disposal sites, urban storm water runoff, CSOs, and wastewater treatment plant bypasses.

The **Grand Calumet River** Area of Concern, located in heavily industrialized Indiana cities of Gary, East Chicago, and Hammond, discharges one billion gallons of water into Lake Michigan each day through the Indiana Harbor and shipyard. To date, approximately 90% of the Grand Calumet River's flow originates as municipal and industrial effluent, cooling and process water, and storm water overflows. The Grand Calumet River was designated as an Area of Concern because all 14 of the beneficial uses are considered impaired. Contamination from PCBs, PAHs, and heavy metals such as mercury, cadmium, chromium, and lead has played a key role in the impairment of the beneficial uses. Also contributing to the impairment of beneficial uses are high fecal coliform bacteria levels, biochemical oxygen demand (BOD), suspended solids, oil, and grease.

The **Maumee River** Area of Concern, located between the Bowling Green water intake and Lake Erie in Ohio, has the largest drainage area of any Great Lakes river, encompassing 3,942 miles of stream. Originally, the Maumee River was designated as an Area of Concern due to the large problem of agricultural runoff, but upon further investigation it was discovered that old dumps or contaminated industrial sites, CSOs, and disposal of dredged material have influenced the problems facing the Maumee River. The Maumee River is affected by 10 of the 14 use impairments (restrictions on fish and wildlife consumption; degradation of fish and wildlife populations; fish tumors or other deformities; degradation of benthos, restrictions on dredging activities, euthrophication or undesirable algae; restrictions on drinking water consumption, or taste and odor; beach closings; degradation of aesthetics; and, loss of fish and wildlife habitat). These impairments are caused by toxic substances (e.g. PCBs and heavy metals), habitat modification (channel development), bacterial euthrophication (e.g. nutrient enrichment), and landfill leachate.

The **Milwaukee Estuary** Area of Concern, located in Wisconsin, has a drainage basin of 22 mi^2 and discharges into Lake Michigan. The drainage basin includes lands that drain directly into the Milwaukee Estuary via storm sewers and combined sewer systems, and the drainage area contributes large amounts of pollutants associated with urban runoff. Through the RAP process, 11 of 14 use impairments (restrictions on fish and wildlife consumption; degradation of fish and wildlife populations; fish tumors or other deformities; bird or animal deformities or reproductive problems; degradation of benthos; restrictions on dredging activities; eutrophication or undesirable algae; beach closings; degradation of aesthetics; degradation of phytoplankton and zooplankton populations; and loss of fish and wildlife habitat) have been identified in the Milwaukee Estuary Area of Concern.

The **Presque Isle Bay** Area of Concern, located in northwestern Pennsylvania on the southern shore of Lake Erie, has a watershed comprised of urban and industrial areas with the city of Erie and Millcreek Township. Presque Isle Bay was designated as an Area of Concern in 1991, becoming the forty-third and last Area of Concern to be designated. Presque Isle Bay is a relatively closed system with a flushing time of approximately 2.5 years, causing the bay to suffer from the accumulation and degradation of wastes discharged by point and nonpoint pollution sources. Through the RAP process two of the 14 use impairments have been identified in the Presque Isle Bay Area of Concern (fish tumors or other deformities, and restrictions on dredging activities). The presence of fish tumors and other deformities may be a result of elevated levels of nitrosamines and PAHs in the sediments. The majority of the PAHs in the sediment is of pyrogenic origin (incomplete combustion of organic matter, generally fossil fuels), and is derived from airborne particulate from combustion sources, runoff from roadways via CSOs, and from historic and current industrial practices.

The **Rouge River** Area of Concern, located in the oldest and most heavily populated and industrialized area in southeastern Michigan, has a drainage basin of 438 mi^2 that includes more than 400 lakes and ponds. The priorities in restoring the Rouge River Area of Concern are focused on the elimination of CSOs, storm sewer overflows (SSOs), nonpoint source pollution control, industrial discharge pretreatment, peak storm water discharge reductions, and contaminated site restoration. The Rouge River was listed as an Area of Concern due to occurrence of 11 of the 14 use impairments (restrictions on fish and wildlife consumption; tainting of fish and wildlife flavor; degradation of fish and wildlife populations; fish tumors or other deformities; bird or animal deformities or reproductive problems; degradation of benthos; restrictions on dredging activities; eutrophication or undesirable algae; beach closings; degradation of aesthetics; and loss of fish and wildlife habitat).

The **Sheboygan River** Area of Concern, encompassing the lower Sheboygan River downstream from the Sheboygan Falls Dam in Michigan, drains into Lake Michigan and serves as a “sink” for pollutants carried from three watersheds. Pollutants in the area that are receiving attention include: suspended solids, fecal coliform bacteria, phosphorus, nitrogen, PCBs, PAHs, and heavy metals. The Sheboygan River is affected by eight of the 14 use impairments (restrictions on fish and wildlife consumption; degradation of fish and wildlife populations; fish tumors or other deformities; bird or animal deformities or reproductive problems; degradation of benthos; restrictions on dredging activities; eutrophication or undesirable algae; and degradation of phytoplankton and zooplankton populations).

The **St. Louis River** Area of Concern, encompassing the 39 miles of the St. Louis River below Cloquet, Minnesota, has a drainage basin of 3,634 mi^2 and discharges into Lake Superior. The St. Louis River Area of Concern was designated due to the occurrence of 9 of the 14 use impairments (restrictions on fish and wildlife consumption; degradation of fish and wildlife populations; fish tumors or other deformities; degradation of benthos; restrictions on dredging activities; eutrophication or undesirable algae; beach closings; degradation of aesthetics; and, loss of fish and wildlife habitat). The impairments are associated with physical loss and degradation of habitat, and pollution and toxicity. Pollution sources include contaminated sediments, abandoned hazardous waste sites, poorly designed or leaky landfills, airborne deposition, industrial discharges, chemical spills, improperly processed waste, and surface runoff.

The **Niagara River** Area of Concern, located in Erie and Niagara counties in western New York, discharges into Lake Ontario. Municipal and industrial discharges, along with waste disposal sites have served as sources of contaminants to the Niagara River in the past. Habitat degradation and the health of aquatic life in the Niagara River Area of Concern have been impaired by the presence of PCBs, mirex, chlordane, dioxin, dibenzofuran, hexachlorocyclo-hexane, PAHs, pesticides, and through fish migration from Lake Ontario. The sediments contaminated with metals and cyanides from the Niagara River area of concern prevent the dredging of this area and disposal of its sediments into Lake Ontario. The Niagara River Area of Concern identifies the occurrence of five of the 14 use impairments (restrictions on fish and wildlife consumption; fish tumors or other deformities; degradation of benthos; restrictions on dredging activities; and loss of fish and wildlife habitat.

The **Detroit River** Area of Concern is a 31.5 mi international connecting channel between Lake St. Clair and Lake Erie. The Detroit River Area of Concern is affected by eight of the 14 use impairments (restrictions on fish and wildlife consumption; fish tumors or other deformities; degradation of benthos; restrictions on dredging activities; restrictions on drinking water, and taste and odor problems; beach closures, degradation of aesthetics; and loss of fish and wildlife habitat). The impairments are a result of urban and industrial development in the watershed, bacteria, PCBs, PAHs, metals, and oils and greases. Major sources of contaminants in the Detroit River Area of Concern include CSOs, municipal and industrial discharges, storm water runoff, and tributaries of the Detroit River.

The **St. Marys River** is a 69.5 mi connecting channel linking Lake Huron and Lake Superior. The St. Marys River Area of Concern is affected by nine of the 14 use impairments (restrictions on fish and wildlife consumption; degradation of fish and wildlife populations; fish tumors or other deformities; degradation of benthos; restrictions on dredging activities; eutrophication with undesirable algae; beach closures; degradation of aesthetics; and loss of fish and wildlife habitat). Contaminants contributing to the impairments include oils and greases, suspended solids, metals, phenols, ammonia, bacteria, and PAHs. Sources of contaminants in the St. Marys River Area of Concern include water pollution control plants, paper mills, wastewater treatment plants, tributaries, CSOs, by-pass events, loss of wetlands and rapids habitats due to urban/industrial development, and operation of navigational structures.

The **Thunder Bay** Area of Concern, located on the north shore of Lake Superior, is one of the largest grain handling ports in the world. Water quality problems primarily arise from discharges from the forest product industry (i.e. pulp and paper, and wood preservation), industrial and municipal point sources, atmospheric deposition, agricultural runoff, and in-place pollutants. Through the RAP process, 10 of the 14 use impairments have been identified in the Thunder Bay Area of Concern (restrictions on fish and wildlife consumption; degradation of fish and wildlife populations; fish tumors or other deformities; degradation of benthos; restrictions on dredging activities; beach closures; degradation of aesthetics; added costs to agriculture and industry; degradation of phytoplankton and zooplankton communities; and loss of fish and wildlife habitat).

The **Jackfish Bay** Area of Concern, located along the north shore of Lake Superior, has been affected by various sources of contaminants including wastewater discharges from pulp mills, nonpoint sources (e.g. atmospheric deposition), in-place sediment contamination, and spills. Currently, the Jackfish Bay Area of Concern is designated as an Area of Concern due the impairment of six of the 14 beneficial uses (degradation of fish and wildlife populations; fish tumors or other deformities; degradation of benthos; restrictions on dredging activities; degradation of aesthetics; and loss of fish and wildlife habitat).

References

1854 Authority.
<http://www.1854.org/page60.html>

Baumann, Paul, Victor Cairns, Bill Kurey, Lauren Lambert, Ian Smith and Roger Thoma. "*Lake Erie Lakewide Management Plan (LaMP) Technical Report Series No.6: Fish Tumors or Other Deformities.*" January 2000.
<http://www.epa.gov/glnpo/lakeerie/buia/lamp6.pdf>

Binational Remedial Action Plans (RAPs). © 1999, Environment Canada.
<http://www.on.ec.gc.ca/water/greatlakes/raps/>

Botulism in Lake Erie. Workshop Proceedings, 28 February 2002, Buffalo, New York.

Comet Assay Interest Group
<http://www.cometassay.com/>

Fish: Methods and Primary Results.
<http://www.snr.uvm.edu/bbay/fish/fishmethods.html>

International Joint Commission.
<http://www.ijc.org/>

Neoplasms of Brown Bullheads (*Amerius nebulosus*).
<http://www.glfc.org/tumor/tumor2.htm>

Obert, Eric C. *Presque Isle Bay Brown Bullhead Study*. Pennsylvania Department of Environmental Protection, 1992-1993.

Ohio EPA: Division of Surface Water.
<http://www.epa.state.oh.us/dsw/tmdl/>

The Otolith Page.
<http://www.iphc.washington.edu/staff/otolith.htm>

Pennsylvania Sea Grant.
<http://www.pserie.psu.edu/seagrant/tumor.html>

Pinkney, Alfred E., John C. Harshbarger, Eric B. May and Mark J. Melancon. "*Tumor Prevalence and Biomarkers of Exposure and Response in Brown Bullheads (Ameiurus nebulosus) from the Tidal Potomac River Watershed.*"
<http://www.fws.gov/r5cbfo/TUMOR.PDF>

US Environmental Protection Agency: Area of Concern (AoCs) On-Line.
<http://www.epa.gov/glnpo/aoc/>

Speaker Biographies

Paul Baumann, Ph.D.
Paul Baumann received a Ph.D. in Zoology from the University of Wisconsin at Madison in 1975. For the past 25 years he has been the leader of a federal field research station at Ohio State University and has held an adjunct professor position at OSU in Natural Resources, Zoology, and Environmental Science. The field station is currently part of the Leetown Science Center in the U.S. Geological Survey. His research has focused on carcinogens (particularly PAHs) and other genotoxins in sediment, and their effects on native fish, especially tumors in brown bullhead. Recent studies have included research on external lesions and blood cell genetic damage.

Vicki Blazer, Ph.D.
Vicki Blazer received her Ph.D from the Department of Fisheries, Aquaculture and Pathology at the University of Rhode Island, Kingston, RI, in 1982, and went on to complete a two-year postdoctoral position at the College of Veterinary Medicine, University of Georgia, Athens, GA. She then moved to the School of Forest Resources, University of Georgia, where she was the assistant leader, Georgia Cooperative Fish and Wildlife Research Unit, from 1984 – 1992. Vicki has been a research fishery biologist/fish pathologist with the National Fish Health Research Laboratory, U.S. Geological Survey, Leetown, WV, since 1992. Her research interests include development of immunological and histological indicators of contaminant exposure, development of histological reproductive biomarkers for assessing endocrine disruption, whirling disease research, *Mycobacteria* infections in striped bass and *Aphanomyces* infections in menhaden.

Kelly Burch
Kelly Burch is the director of the Pennsylvania Department of Environmental Protection's (DEP) regional office in Meadville. The office serves 12 counties in northwest Pennsylvania and maintains primary responsibility for permitting and enforcement actions in the areas of air quality, solid waste, water quality, water supply, brown fields, and the extraction of oil and gas.

Prior to his appointment as regional director in 2001, Kelly served for six years as DEP's Chief of the Office of the Great Lakes. His responsibilities included development of a Remedial Action Plan for Presque Isle Bay and representing the Commonwealth on the IJC's Water Quality Board, the Binational Executive Committee, and the Lake Erie Lakewide Management Plan.

John (Jack) Fournie, Ph.D.
Jack Fournie is a fish pathologist at the U.S. Environmental Protection Agency, Gulf Ecology Division, in Gulf Breeze, Florida. He obtained B.A. and M.S. degrees in Biology from St. Louis University in 1974 and 1979. Dr. Fournie received a Ph.D. from the University of Mississippi and Gulf Coast Laboratory in 1985 where his research emphasized fish parasitology and tumor pathology. He was a postdoctoral fellow at the laboratory from 1985-1986, conducting research in small fish carcinogenesis.

Dr. Fournie has attained international recognition as a fish parsitologist for his work on coccidian parasites in fishes. Jack's more recent work has expanded into tumor pathology of fish and the use of small fish as carcinogenesis models. He has published numerous papers on various aspects of neoplastic diseases of fish and his research on the International Pancreatic Cancer Study Group in 1988 at Verona, Italy. Dr. Fournie has authored chapters on neoplasms of the exocrine pancreas and cardiovascular system and co-authored a chapter on neoplasms of bone cartilage and the soft tissues.

John Gannon, Ph.D.
Dr. John Gannon is a limnologist and fisheries research biologist. He received his Ph.D. in Zoology at the University of Wisconsin. He has broad interests in aquatic ecology, fisheries, and water pollution biology, and special interests in Great Lakes limnology, zooplankton ecology, water quality protection, and environmental communications. He has authored over 80 papers on the limnology and fisheries biology of the Great Lakes and inland waters of the Great Lakes basin and on field-oriented approaches to environmental education.

John has worked on the Great Lakes all of his career he was resident scientist for six years at the University of Michigan Biological Station near the Straits of Mackinac where he taught summer and winter courses and conducted research on lakes Michigan, Huron, St. Clair, and northern Michigan inland lakes with emphasis on zooplankton ecology and eutrophication problems. Then he spent six years as associate director of the State University Research Center at Oswego (NY) where he was involved in teaching and research on Lake Ontario with emphasis on toxic substances problems. He joined the International Joint Commission's Great Lakes Regional Office in Windsor, Ontario in 1983 and was appointed Assistant Director in 1984 and Acting Director in 1987. In addition to administrative duties, John was responsible for oversight of the Great Lakes International Surveillance Plan (GLISP).

From 1987 to 2002 John was a science coordinator (often also acting center director) at the Great Lakes Science Center of the U.S. Geological Survey's Biological Resources Discipline (formerly U.S. Fish and Wildlife Service and National Biological Survey) in Ann Arbor, Michigan where he managed over 90 staff conducting research on fishes and other biological resources of the Great Lakes.

In fall 2002, John returned to the International Joint Commission's Great Lakes Regional Office in the capacity of senior scientist. He continues to have broad research interests in invasive species, habitat restoration, emerging toxic substances, and the linkages between science, management, and policy

John Harshbarger, Ph.D.
John C. Harshbarger grew up on a dairy farm in Augusta County Virginia. He received a B.A. degree from Bridgewater College in 1957, a M.S. degree from Virginia Polytechnic Institute in 1959, and a Ph.D. from Rutgers University in 1962. From 1962–1964 he had a National Science Foundation Post Doctoral Fellowship with the U.S. Department of Agriculture at Beltsville, MD. From 1964–1967 he was a research pathobiologist on the Irvine campus of the University of California. From 1967–1995 he directed The Registry of Tumors in Lower Animals at the Smithsonian Institution, and from 1995–2001 he continued to direct the The Registry of Tumors

in Lower Animals at the George Washington University Medical Center as professor of pathology. He is currently doing research in environmental pathology at the GWU Medical Center.

Chuck Murray
Chuck Murray has been employed as a fisheries biologist with the Pennsylvania Fish and Boat Commission's Lake Erie Research Unit Since 1993. He received his B.S. in Biology from Penn State Erie in 1985. His primary duties are fish population assessment in Pennsylvania waters of Lake Erie, including sport-fishing assessment, forage fish community structure, and interagency coldwater fisheries management in Lake Erie. He was previously employed as a faculty research assistant with the University of Maryland's Horn Point Environmental Laboratory as an environmental chemist, documenting the effects of acid precipitation on native brook trout streams.

Eric Obert
Eric received his B.S. in Environmental Resource Management from Penn State University in 1973 and attended graduate classes at Clarion University 1984 to 85.

Currently, Eric serves as Extension Director and Coastal Environmental Quality Specialist for Pennsylvania Sea Grant. Past experience includes 25 years of combined experience with the Pennsylvania Department of Environmental Resources, Division of Water Quality and the Pennsylvania Fish & Boat Commission's Fisheries Management Section. His specialties include stream and lake ecology, fisheries ecology, limnology, with special expertise related to the Area of Concern designation of Presque Isle Bay.

Eric is responsible for the development, implementation and ongoing evaluation of a coastal environmental quality extension program relating to the Lake Erie watershed and Delaware Estuary, including, but not limited to: (1) identification and addressing of specific issues related to coastal environmental quality; (2) development of conferences, workshops, seminars targeting environmental practitioners and other members of the community; (3) appropriate educational outreach programs targeting teachers; (4) participation as an active member of the Sea Grant Assembly of Extension Leaders; and (5) writing and editing relevant material for articles, news releases, newsletters, fact sheets, and other publications.

Eric serves on a number of boards and commissions, including: Great Lakes Fishery Commission's Lake Erie Advisory Committee; Presque Isle Partnership Executive Board; Presque Isle Partnership Research Sub-Committee Chairman; Pennsylvania Lake Erie Watershed Association Board; and Presque Isle State Park Advisory Board

Fred Pinkney, Ph.D.
Fred Pinkney received his Ph.D. in Marine-Estuarine-Environmental Science from the University of Maryland in 1988. Fred is the Aquatic Contaminants Leader for the U.S. Fish and Wildlife Service's Environmental Contaminants Program. He has worked in the Environmental Contaminants Program at the U.S. Fish and Wildlife Service, Chesapeake Bay Field Office in Annapolis, MD for the past 10 years. His professional interests are focused on the use of fish tumors as an environmental indicator, fish biotelemetry, the investigation of frog abnormalities

on National Wildlife Refuges, the non-target effects of pesticides, and ecological risk assessments. Fred worked with several environmental consulting firms before taking a position with the U.S. Fish and Wildlife Service. He received a B.S. in Zoology from the University of Michigan in 1976, an M.S. in Environmental Health Sciences from New York University in 1982.

Roger Thoma
Roger Thoma is a fisheries biologist with the Ohio Environmental Protection Agency and has been working on Lake Erie and its' Ohio Areas of Concern since 1986. Roger received his B.S. degree from Ohio State University in Fisheries Management in 1975. His research involved the development of an index of biotic integrity (IBI) and deformity (DELT) index for Ohio's Areas of Concern. Roger is also an adjunct assistant professor at Ohio State and is a curator of crayfish collections.

Robert (Bob) Wellington
Bob Wellington is a lifetime Erie resident who has always lived within walking distance of Presque Isle Bay. He has been interested in the environment since childhood, and at the age of 14 bought his own rowboat to use for hunting and fishing on Presque Isle Bay. Bob received a B.S. degree in zoology from Penn State University in 1965 and in 1972 his M.A.T. from the University of Alaska (Fairbanks) in teaching science.

Bob Started his career in 1966 as a water pollution control specialist for the PA Department of Health in the Meadville region. He later took a position as an aquatic biologist with the Pennsylvania Fish Commission in charge of research on Lake Erie. In 1970, he worked as a water pollution control specialist with the Erie County Department of Health and in 1971, attended the University of Alaska to get his masters degree. He eventually returned to Erie and in 1975 assumed the position as an aquatic biologist with the Erie County Department of Health. Bob has over 35 years of experience in studying the changing ecosystem of Presque Isle Bay and Lake Erie. A considerable part of his professional career has been focused on fish health and contaminant studies. He has been studying the problem of tumors in brown bullheads in Presque Isle Bay since 1984.

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