Development of Standardized Criteria for the Assessment of Brown Bullhead Lesions and Deformities in Areas of Concern Conference Proceedings

Cosponsored by:

Pennsylvania Sea Grant

United States Environmental Protection Agency Great Lakes National Program Office

and

Pennsylvania Department of Environmental Protection

February 14-15, 2006
Erie, PA

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PREFACE

The *Development of Standardized Criteria for the Assessment of Brown Bullhead Lesions and Deformities in Areas of Concern Conference Proceedings* was compiled with the intention of capturing the thoughts of the conference held in Erie, Pennsylvania on February 14-15, 2006. This two-day workshop was the third in a series focused on the fish tumors or other deformities beneficial-use impairment and studies of the Presque Isle Bay Area of Concern (AOC).

Participants had the opportunity to discuss and finalize standardized protocols for assessing liver and external lesions on brown bullhead, evaluate reference lesion rate data for Lake Erie, and review proposed delisting targets for the Presque Isle Bay AOC. The results of this conference and the previous two were presented at the 2006 International Association of Great Lakes Research (IAGLR) Conference. Also, the recommendations of the conference participants will be incorporated into a concept paper outlining standardized criteria for evaluating this beneficial-use impairment, which will be submitted to the Environmental Protection Agency (EPA) and International Joint Commission (IJC) in the hope that these criteria will be adopted by all AOCs attempting to restore this use impairment.

Special thanks is extended to all the speakers at the conference, including Eric Obert (Pennsylvania Sea Grant), Lori Boughton (Pennsylvania Department of Environmental Protection), Dr. Fred Pinkney (US Fish and Wildlife Service), Scott Brown (Environment Canada), Steve Smith (US Geological Survey), Dr. Paul Baumann (US Geological Survey), Bob Wellington (Gannon University), Colleen Wellington (Pennsylvania Sea Grant), Dr. Vicki Blazer (US Geological Survey), Sean Rafferty (Pennsylvania Sea Grant), Dr. Dave Hunnicutt (Penn State Behrend), Jim Grazio (Pennsylvania Department of Environmental Protection), Dr. Mike Rutter (Penn State Behrend), and Dr. Mike Millard (US Fish and Wildlife Service); and also to Dr. Thomas Wortman (Penn State Behrend) for facilitating and the United States Environmental Protection Agency Great Lakes National Program Office (EPA GLNPO) for providing funding for the conference.

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INTRODUCTION

On February 14-15, 2006, Pennsylvania Sea Grant, the Pennsylvania Department of Environmental Protection, and the U.S. Environmental Protection Agency Great Lakes National Program Office (GLNPO) co-sponsored the *Development of Standardized Criteria for the Assessment of Brown Bullhead Lesions and Deformities in Areas of Concern Conference*, held at the Stull Interpretive Center on Presque Isle State Park in Erie, Pennsylvania. This conference was a continuation of the previous Fish Tumors Related to Great Lakes Areas of Concern (AOC) conferences. However, participants at this conference specifically intended to develop reference rates and determine appropriate delisting targets for Lake Erie AOCs.

The goal of the conference was to discuss and finalize standardized protocols for assessing liver and external lesions on brown bullhead, evaluate reference lesion rate data for Lake Erie, and review proposed delisting targets for the Presque Isle Bay AOC.

The conference was conducted in a workshop format with the purpose of encouraging broad participation from attendees. The conference included several facilitated discussions in which attendees had the opportunity to collectively answer several key questions related to reference sites, reference rate data, and delisting targets. As a result of the discussions, the participants helped establish recommendations to consistently identify Lake Erie reference sites and determine appropriate delisting targets for the fish tumors or other deformities beneficial use impairment (BUI) in Lake Erie AOCs. These recommendations will be presented, in the form of a concept paper, to the Environmental Protection Agency (EPA) and International Joint Commission (IJC) with the hope that the recommendations will be approved and adopted by all AOCs.

SESSION ONE: RESEARCH PRESENTATIONS

DR. FRED PINKNEY

Tumor prevalence in brown bullhead from the South River, Anne Arundel County Maryland

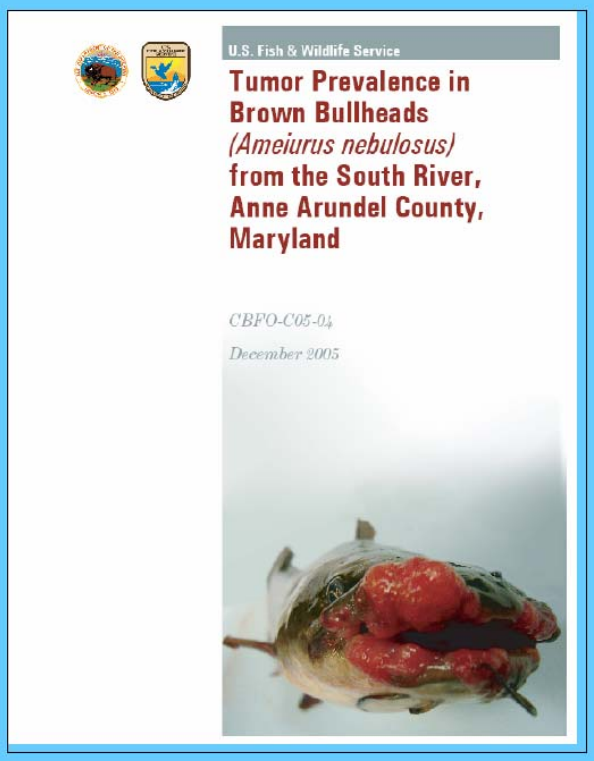
Alfred E. Pinkney[1] and John C. Harshbarger[2]

[1]U.S. Fish and Wildlife Service, Chesapeake Bay Field Office, 177 Admiral Cochrane Drive, Annapolis, MD 21401 [2]George Washington University Medical Center, 2300 I Street, NW, Washington, DC 20037

Abstract: In March 2005, brown bullhead were collected in the South River from a fyke net set about 1.25 km downriver of the Route 50 Bridge. A total of 30 brown bullhead (*Ameiurus nebulosus*) $\geq$ 260 mm were randomly selected for analysis, placed in coolers and transported live to the U.S. Fish and Wildlife Chesapeake Bay Field Office. The fish were held in aerated site water and necropsied over the next two days. A gross examination was performed on the external organs, focusing on raised skin lesions and the appearance of the barbels. For all fish, livers were excised, weighed, cut into sections and preserved in 10% buffered neutral formalin. Sixteen fish had raised skin lesions, which were excised along with adjacent tissues, decalcified, and preserved similarly. Tissues were processed and histopathological examinations were performed. All fish were aged using spines. The objective was to determine the prevalence of liver and skin tumors and preneoplastic lesions. We reported a 20% (6 of 30) prevalence of liver tumors, split evenly between hepatocellular carcinomas and cholangiocarcinomas. All sixteen fish with the raised skin lesions were diagnosed with skin tumors (53% prevalence). Thirteen of these cases were invasive squamous carcinomas and three were non-invasive epidermal papillomas. Liver tumor prevalence was significantly ($p=0.01$, Fisher's Exact Test) higher than that observed previously in collections from the Tuckahoe River (MD), considered a reference area (prevalence = 4% (5 of 117)). The liver tumor prevalence in South River bullhead also exceeded the 5% criterion suggested as indicative of highly contaminated areas. Skin tumor prevalence was significantly different between locations (South River 16/30 = 53%, Tuckahoe: 1/117 = 1%, $p<0.001$). The skin tumor prevalence in South River bullhead was about four times the 12% suggested criterion for highly contaminated areas.

The South River ranks first in skin tumor prevalence (53%) and second in liver tumor prevalence (20%) among the Chesapeake Bay locations where bullhead surveys have been conducted. In brown bullhead, both liver and skin tumors have been associated with exposure to carcinogens, with the most persuasive linkage to polynuclear aromatic hydrocarbons (PAHs) in sediments. The mean total PAH concentration reported in 29 sediments from the South River, 2.2 ppm, however, was similar to the mean of 1.8 ppm measured in 1996 at the Tuckahoe River collection site. Thus, the findings in the South River contrast with those in other Chesapeake Bay tributaries, where elevated tumor prevalence coincided with high sediment PAH concentrations. At present, we have insufficient evidence to implicate a particular chemical class as a major contributor to the tumors. We recommend a follow-up survey that includes tumor prevalence and analysis of biomarkers such as biliary PAH metabolites and DNA adducts to evaluate PAHs as a primary agent. Surveys of other western shore tributaries, such as the Severn and Rhode Rivers, would be useful for determining the extent of the tumor problem.

Presentation:



Tumor Prevalence in Brown Bullheads (*Ameiurus nebulosus*) from the South River, Anne Arundel County, Maryland

CBFO-C05-04

Alfred E. Pinkney[1]
John C. Harshbarger[2]

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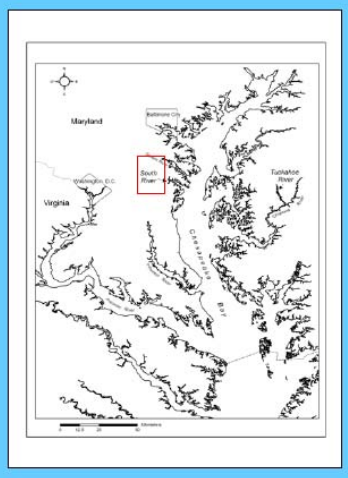
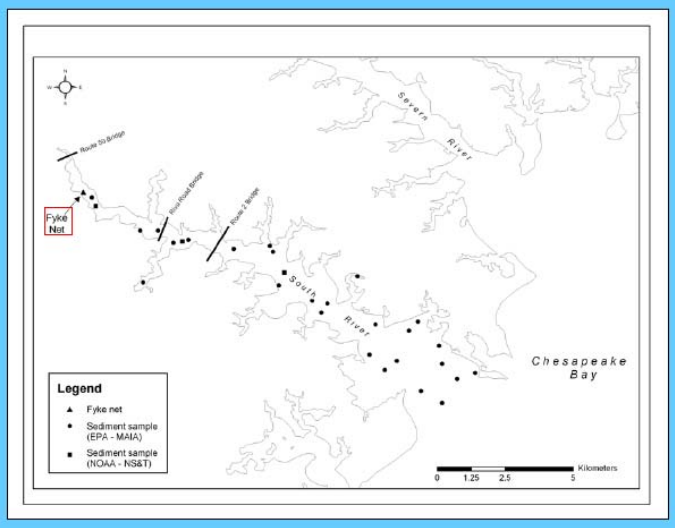
Washington, DC 20037

December 2005

Funding provided by the South River Federation
and the U.S. Fish and Wildlife Service
Reports and fact sheets can be downloaded from the CBFO
web site: http://www.fws.gov/chesapeakebay





The South River—looking north with Route 50 Bridge in distance

Background

- Brown bullhead – sediment dwelling catfish with a small home range, develops skin and liver tumors in response to carcinogen exposure—strongest link with polynuclear aromatic hydrocarbons (PAHs)
- Tumor surveys – data base for Great Lakes (Baumann, USGS) and Chesapeake Bay (Pinkney, USFWS)

Objectives

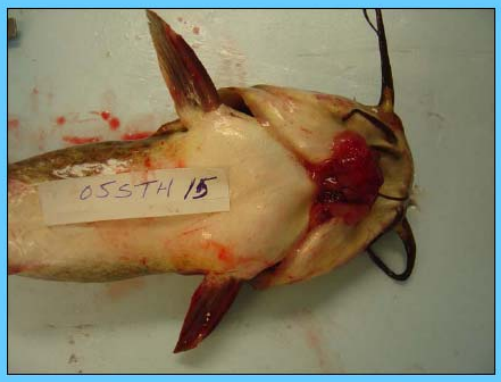
- Determine liver tumor, skin tumor, and barbel abnormality prevalence in South River brown bullheads
- Compare the prevalence with a database for the Tuckahoe River, used as a reference site in three previous studies and with criteria
- Evaluate existing sediment contaminant data for possible stressors
- Provide recommendations for further research.

Sampling: March 2005

- Randomly sampled 30 fish > 260 mm
- Kept live in aerated coolers and examined at USFWS laboratory
- Photographed, tissues prepared for histopathological examination

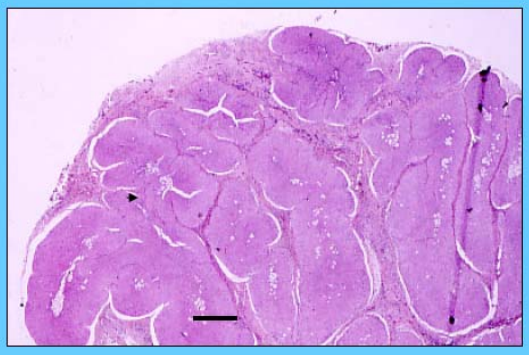
Examples of skin tumors

Fish 015, lesion diagnosed as squamous carcinoma, an invasive skin tumor



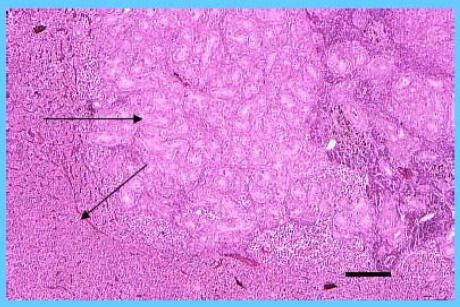
Underside of fish 015

Fish 011 – squamous carcinoma

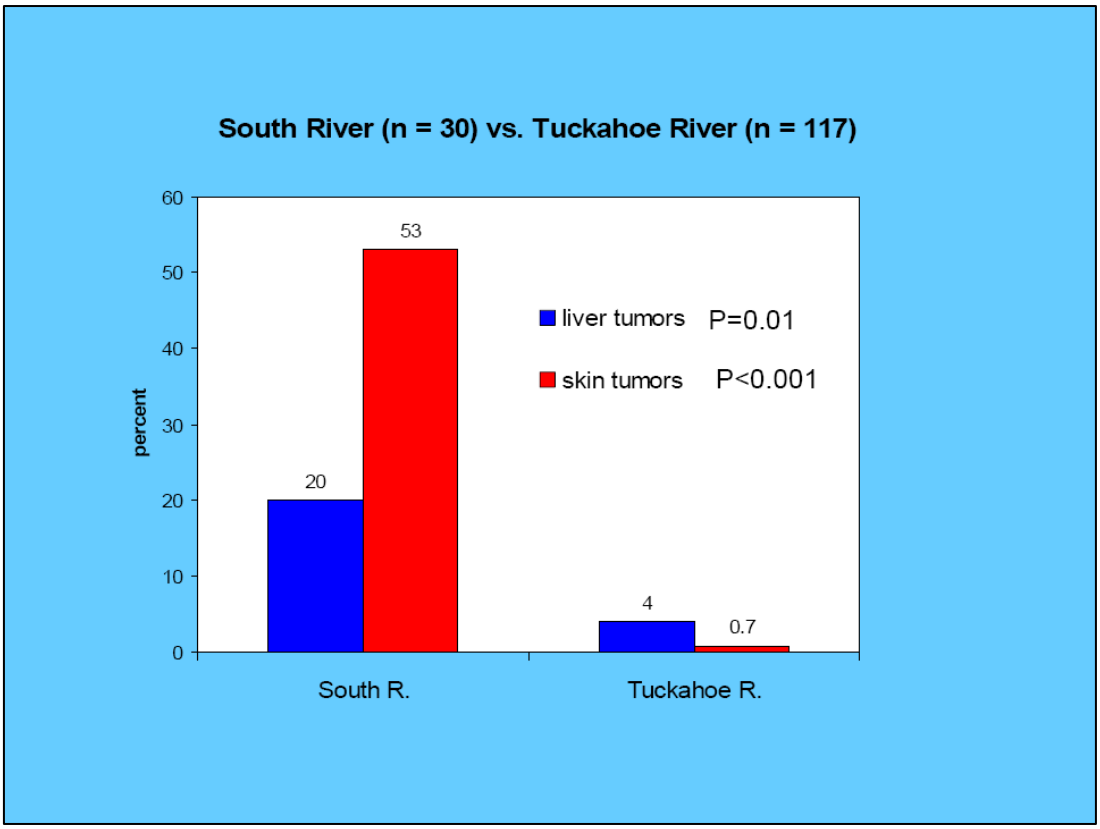


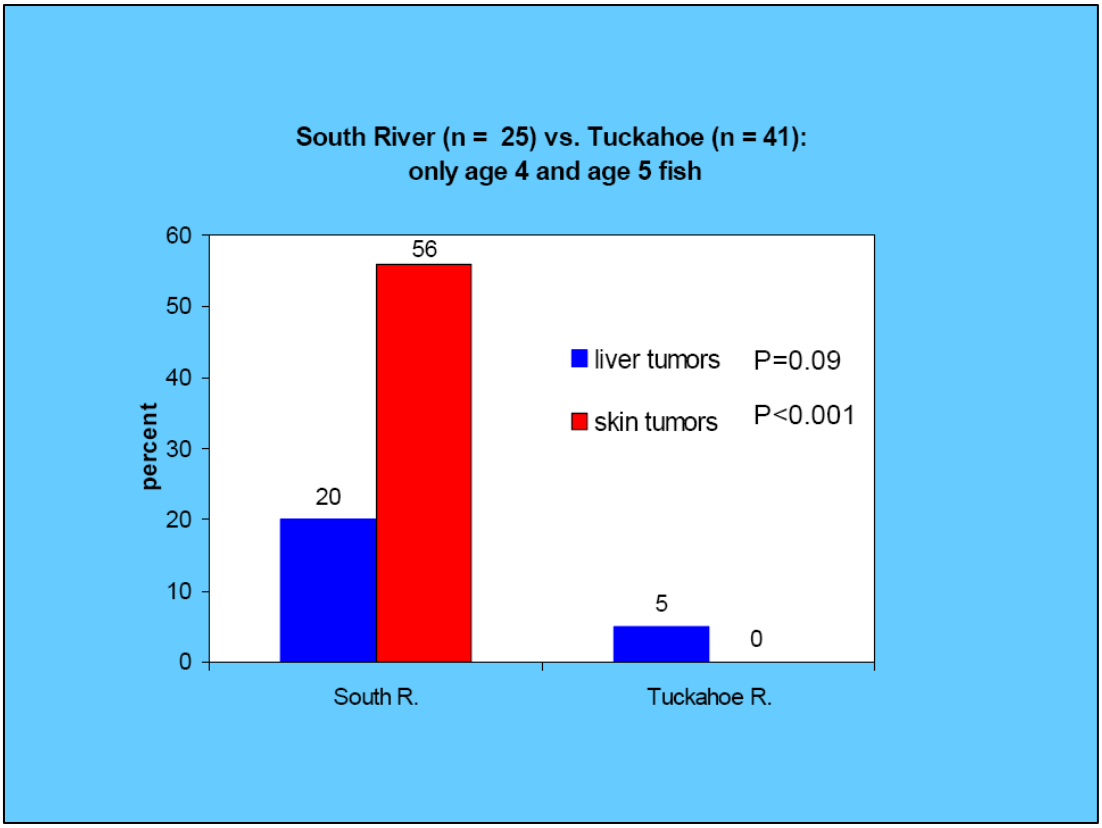
Squamous carcinoma of lip with exophytic papillary pattern. Strands of tumor (triangle) interdigitate with fibrovascular connective tissue (fish 014). Bar = 75 µm.

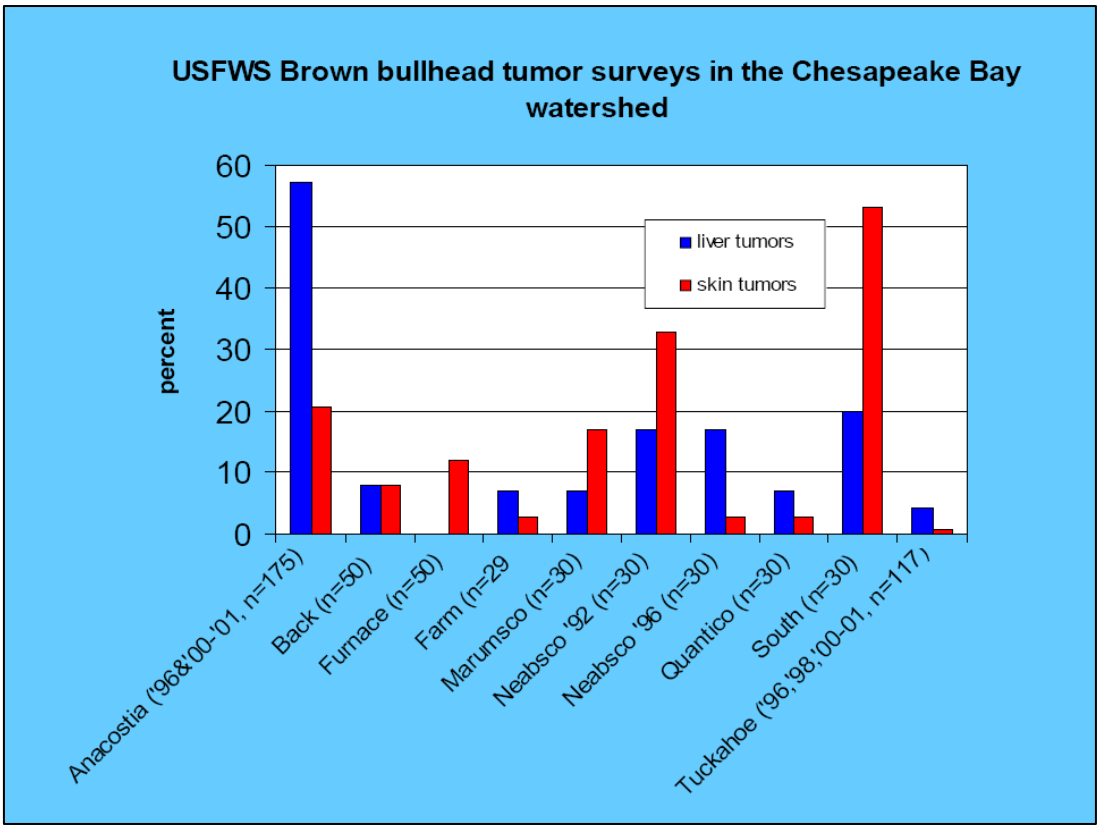
Liver tumor example

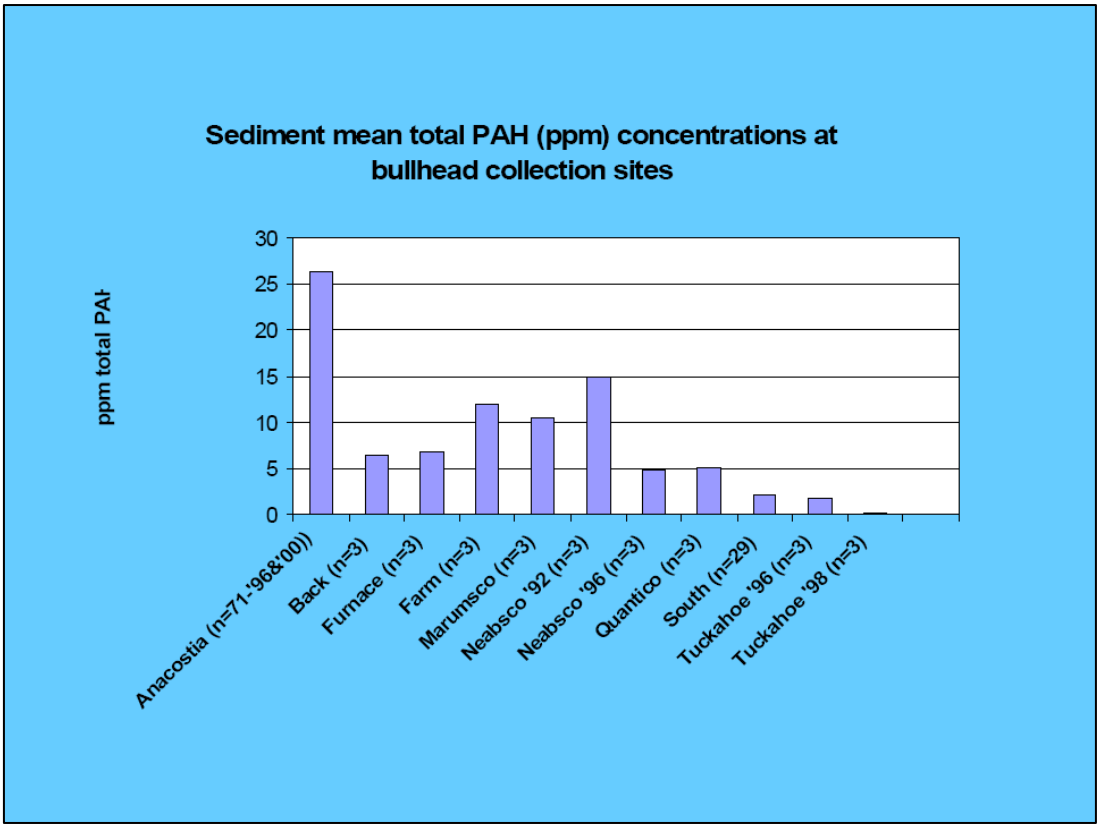


Cholangiocarcinoma. Irregular mass of poorly formed bile ducts (3 o'clock arrow) invading normal liver (7 o'clock arrow) (fish 016). Bar = 190 µm.


South River (n = 30) vs. Tuckahoe River (n = 117)
percent
60
50
40
30
20
10
0
53
20
4
0.7
liver tumors P=0.01
skin tumors P<0.001
South R.
Tuckahoe R.


South River (n = 25) vs. Tuckahoe (n = 41):
only age 4 and age 5 fish
percent
60
50
40
30
20
10
0
56
20
5
0
liver tumors P=0.09
skin tumors P<0.001
South R.
Tuckahoe R.


USFWS Brown bullhead tumor surveys in the Chesapeake Bay watershed
percent
60
50
40
30
20
10
0
liver tumors
skin tumors
Anacostia ('96&'00-'01, n=175)
Back (n=50)
Furnace (n=50)
Farm (n=29
Marumsco (n=30)
Neabsco '92 (n=30)
Neabsco '96 (n=30)
Quantico (n=30)
South (n=30)
Tuckahoe ('96,'98,'00-01, n=117)


Sediment mean total PAH (ppm) concentrations at bullhead collection sites
ppm total PAH
30
25
20
15
10
5
0
Anacostia (n=71-'96&'00))
Back (n=3)
Furnace (n=3)
Farm (n=3)
Marumsco (n=3)
Neabsco '92 (n=3)
Neabsco '96 (n=3)
Quantico (n=3)
South (n=29)
Tuckahoe '96 (n=3)
Tuckahoe '98 (n=3)

Discussion

- Anacostia and Neabsco had high sediment PAHs – consistent with literature
- Anacostia – biomarkers of PAH exposure (bile metabolites) and response (DNA adducts)
- South – sediments have similar total PAH concentration as Tuckahoe
  - **Doesn't fit pattern—needs further investigation**

Questions

- Are the bullheads exposed to carcinogens in the South River or are they moving between several Bay tributaries?
- Is the South River unique or do other western shore tributaries such as the Rhode River and Severn River show a similar pattern in tumor rates?
- What data are needed to rule out PAHs as major contributors to the tumors?
- Are there other chemicals that should be investigated?

Key Elements for Followup Study

- Severn, Rhode, South, Tuckahoe, additional reference site?
- 30 fish each >260 mm + aging
- Histopathology, bile PAH metabolites, DNA adducts
- Tissue analysis?
- Compare across sites
- Logistic regression to identify risk factors

SCOTT BROWN

Overview of fish and wildlife health effects and exposure studies in Canadian AOCs

Scott Brown[1]

[1]Environment Canada, 867 Lakeshore Rd., P.O. Box 5050, Burlington, Ontario L7R 4A6

Abstract: Environment Canada has undertaken studies in Canadian AOCs to measure present concentration of chemicals of established concern, to assess previously unmeasured chemicals in the aquatic environment that could be associated with environmental health outcomes, and to determine the current state of fish and wildlife health. In addition to measuring persistent contaminants such as POPs, novel ways to measure less persistent chemicals and determine environmental exposure are evaluated. Physiological and reproductive effect endpoints have been chosen in fish and wildlife that are fundamental to the functional health of individuals and populations. Evaluated health effects in fish, snapping turtles, birds, and mink involving specific endpoints that relate to populations and individual health include wildlife population trends, tests for measuring fish and wildlife reproductive success, tests for status of endocrine systems, tests for assessing components of liver function, tests for measuring immune function, and tests for other health effects such as prevalence of tumors and surficial anomalies, enzymes and other plasma constituents which have diagnostic value. So far, the focus for health effect evaluation has been on AOCs in western Lake Erie and Lake Ontario.

Presentation: No slide presentation was provided.

STEPHEN SMITH

Sediment, invertebrate, and fish health parameters from 1998-2000 collections at Lake Erie AOCs

Stephen B. Smith[1]

[1]USGS, National Center, 12201 Sunrise Valley Dr., Reston, VA 20192

Abstract: Studies completed during the Lake Erie Ecological Investigations (LEEI) from 1998-2000 included collection of sediments, invertebrates and fish for community analyses, and brown bullhead for complete fish health analysis. Grain size, metals (TE), and organic chemicals from the Lake Erie Areas of Concern (AOC) and reference sites were compared during the LEEI collections. Fish and invertebrate communities from these same AOCs and reference sites were also compared. Brown bullhead external anomalies at the collection sites from 1998-2000 were compared to similar sites collected in 1986-87. A reference site showed increased prevalence of external anomalies and several other sites show fewer anomalies between the two time periods. External anomalies compared to age showed that as age increases (age 3, ages 4/5, and ages 6/7) so did the prevalence of external anomalies. Endocrine biomarkers of plasma collected from the brown bullhead found four sites (Black River upstream, Cleveland Harbor, Presque Isle Bay, and the Buffalo River) with concentrations of vitellogenin in males significantly higher than 0.0 mg/mL, the concentration considered normal for males.

Presentation:

Sediment, Invertebrate, and Fish Health Parameters from 1998-2000 Collections at Lake Erie AOC's

By
Stephen B. Smith, Dora P. Reader and
Paul. C. Baumann
U.S. Geological Survey
Reston, VA; Ann Arbor, MI; and Columbus, OH

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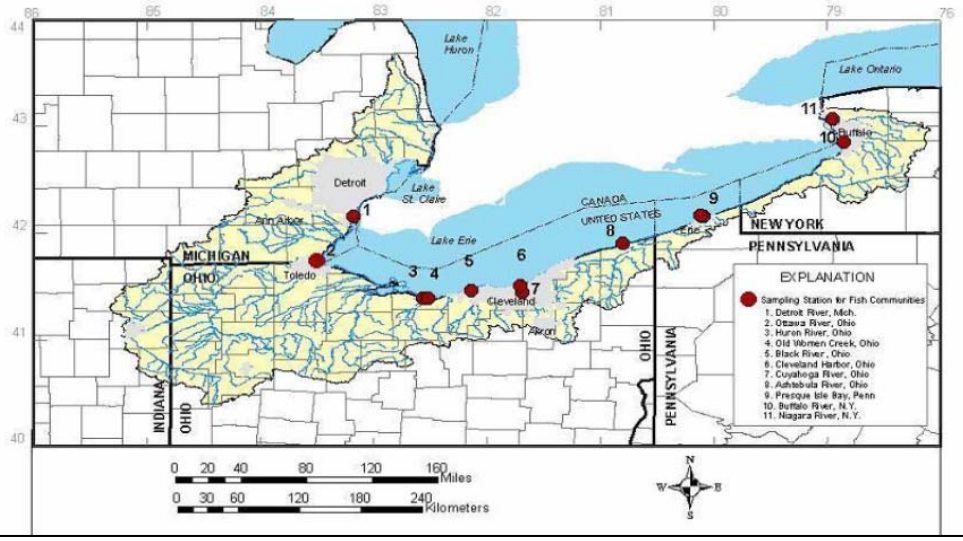
Lake Erie Ecological Investigations

LEEI Sites



Lake Erie Ecological Investigations (LEEI)

- Sediment Residue
- Invertebrate Communities
- Fish Health
  - External Anomalies
  - Endocrine Biomarkers
- Fish Communities

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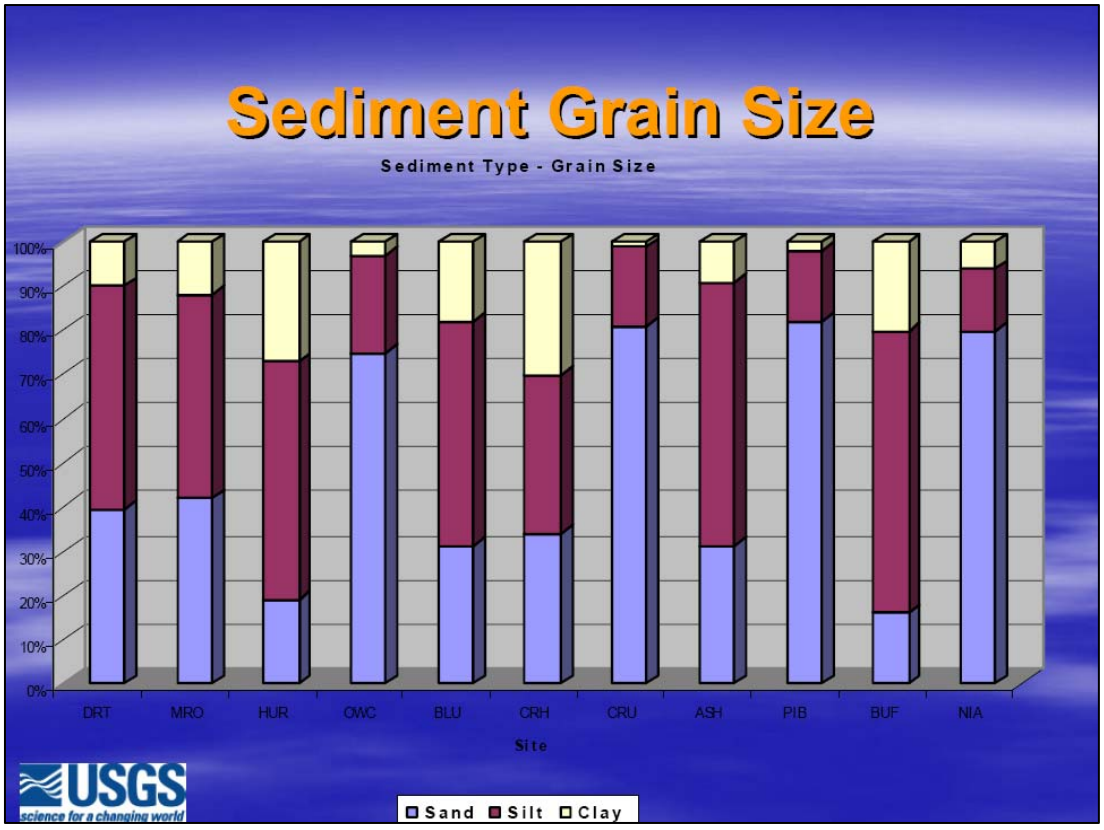
Sediment Grain Size



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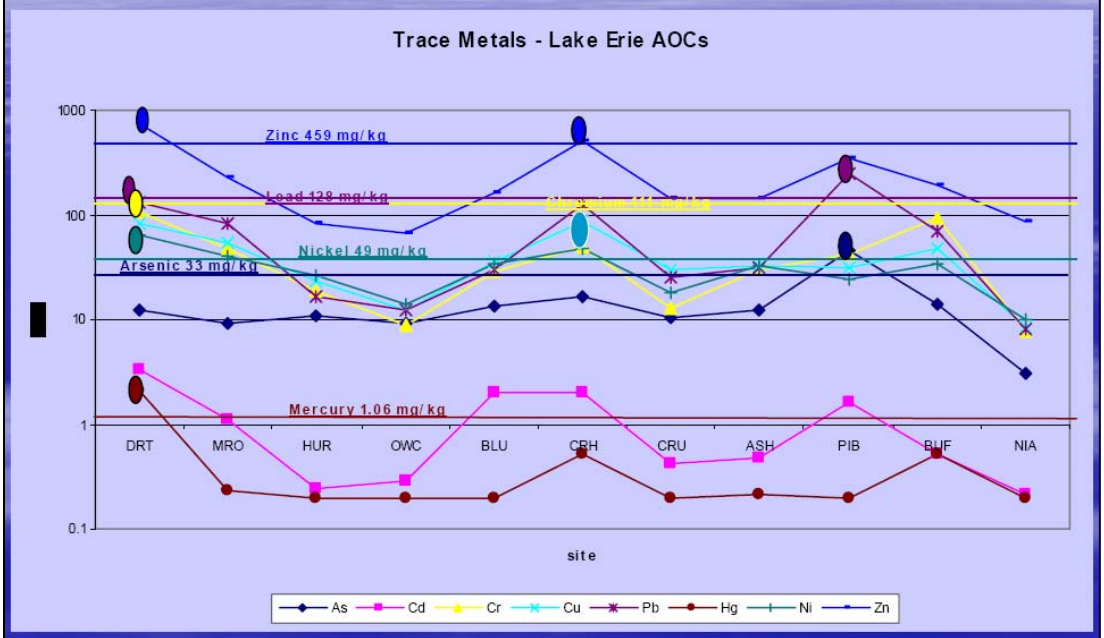
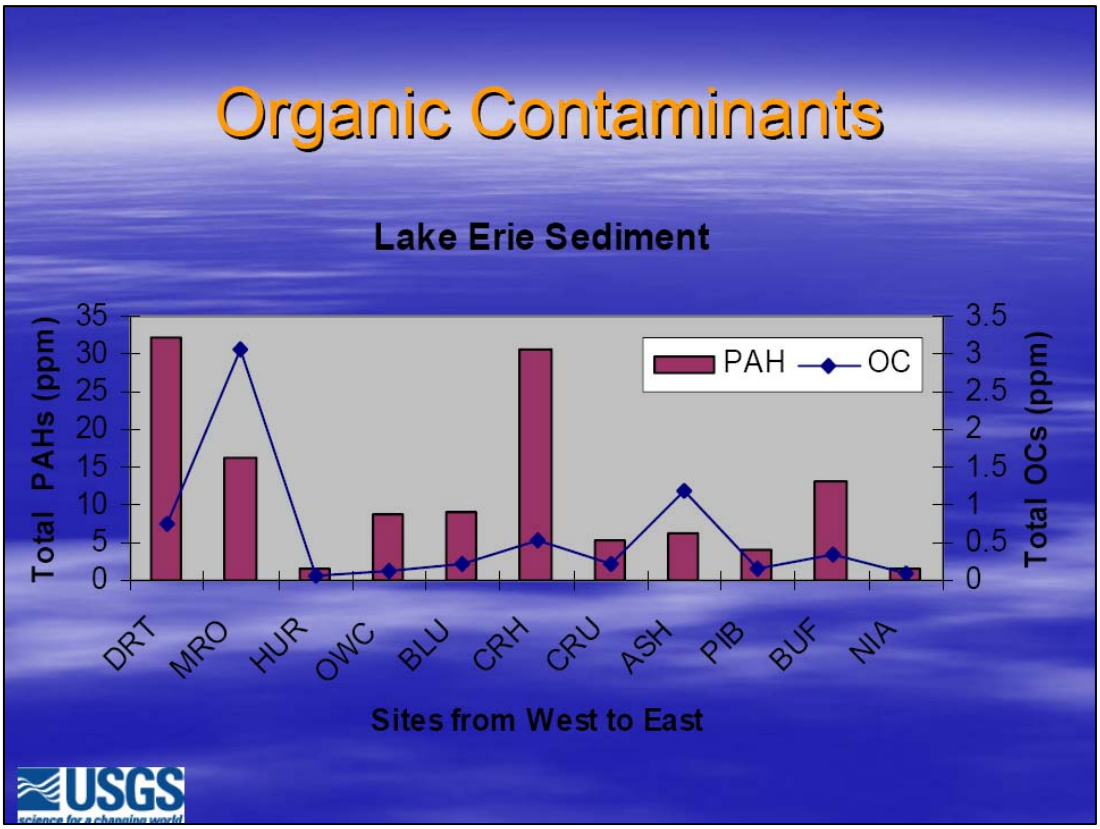
Total Metals at LEEI Sampling Sites

	DRT	MRO	HUR	OWC	BLU	GRH	CRU	ASH	PIB	BUF	NIA
[in mg / kg]											
Al	7820	13500	12100	4350	10400	13900	3890	11800	5000	12600	4130
As	12.4	8.99	10.8	9	13.4	16.4	10.6	12.5	46.1	13.9	2.89
B	5	11.3	15	5	5	5	5	5	5	5	5
Ba	113	114	66.7	28.5	61.5	113	37.8	94.9	175	133	24.7
Be	0.89	0.97	0.86	0.39	0.93	1.24	0.44	0.94	0.59	0.86	0.31
Cd	3.3	1.1	0.24	0.29	1.96	2.05	0.42	0.47	1.66	0.52	0.21
Cr	108	46.8	18.9	8.7	28.7	50.4	13.2	30.6	41.4	94.4	7.8
Cu	81.9	54.7	23.2	12.4	35.4	86	30.2	32.2	31	48.1	8.51
Fe	30900	21500	20800	12500	25500	34400	14200	29200	25000	27800	8020
Hg	2.11	0.226	0.1	0.1	0.1	0.534	0.1	0.213	0.1	0.528	0.1
Mg	15700	10800	8160	5660	4040	6940	2630	5130	2800	7960	15600
Mn	425	339	442	218	448	646	313	379	491	561	238
Mo	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Ni	63.8	40.4	26.5	14.2	33.4	48.3	18	32.5	24.3	33.8	10.1
Pb	130	82.8	16.3	12.5	29.5	124	25.5	30.8	248	71.2	8.09
Se	1.6	1.25	0.5	0.5	1.07	1.07	0.5	0.5	1.21	0.5	0.5
Sr	137	173	41.2	28.3	23	68.5	15.5	15.2	93	33.6	43.6
V	21.1	25.6	23.8	11.8	30.1	28.1	10.6	36.6	14	24.1	9.89

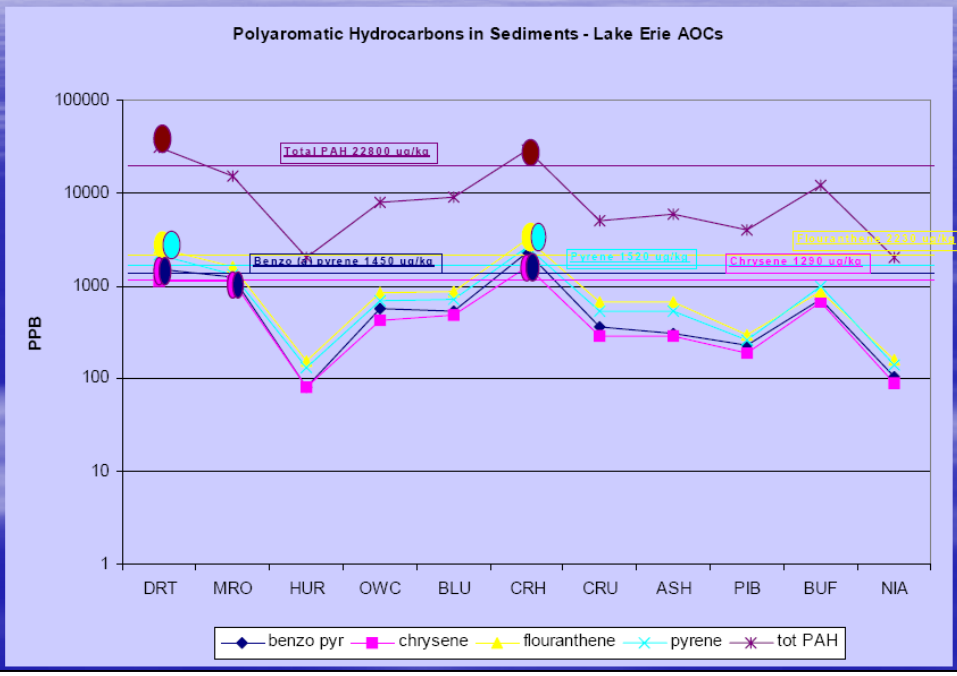
Trace metals - Sediment Quality Guidelines



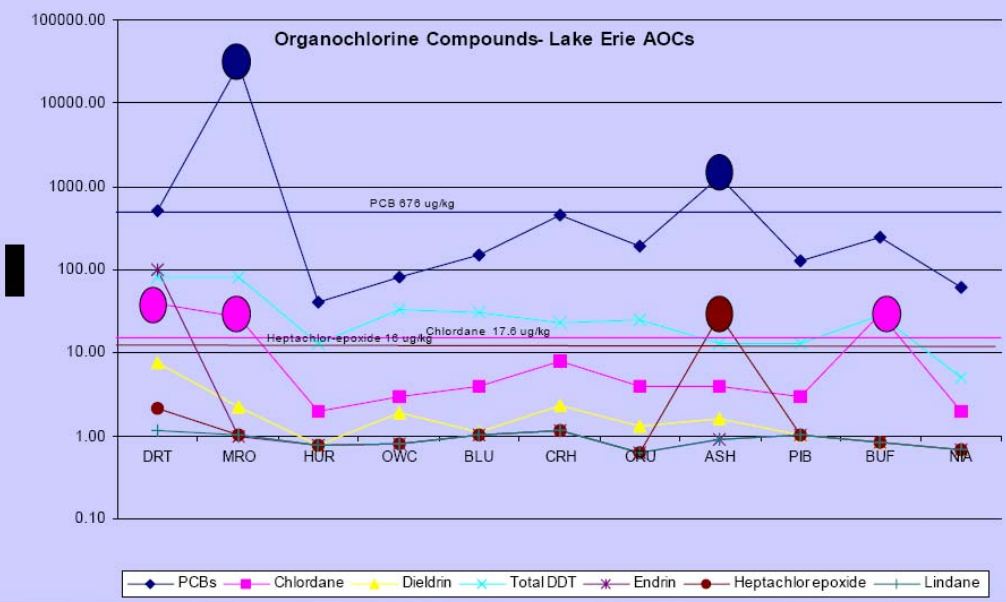
Organic Contaminants

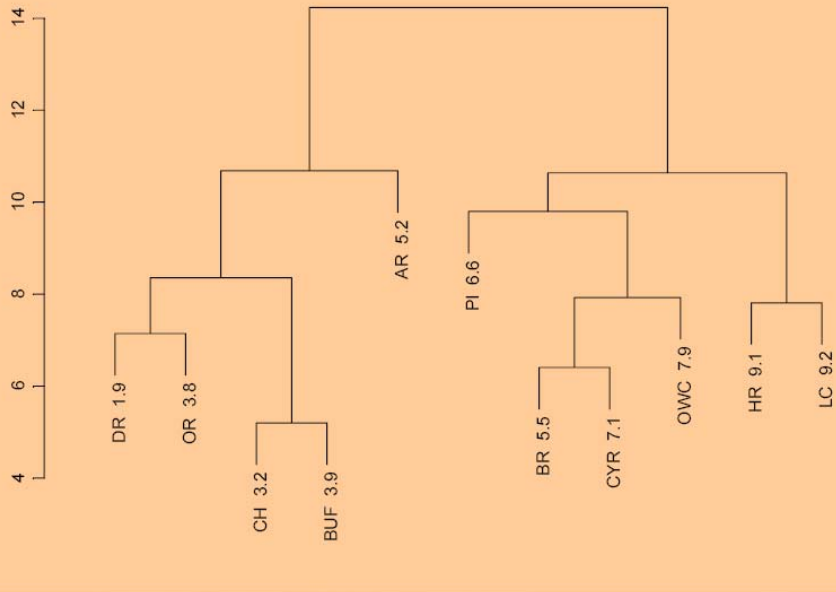


Polyaromatic Hydrocarbons (PAH) -Sediment Quality Guidelines



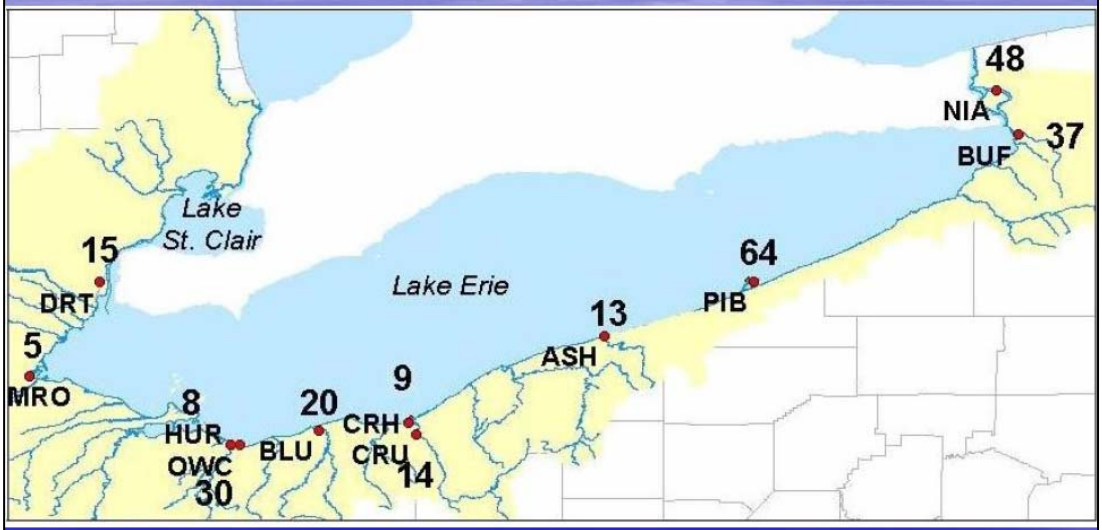
Organochlorine Contaminants (OCs) - Sediment Quality Guidelines





Cluster analysis of site ranks for 8 categories: PAHs, PCBs, pesticides, DDTs, As, Hg, Ni, Zn.

LEEI Invertebrate Taxa



Benthic Invertebrates

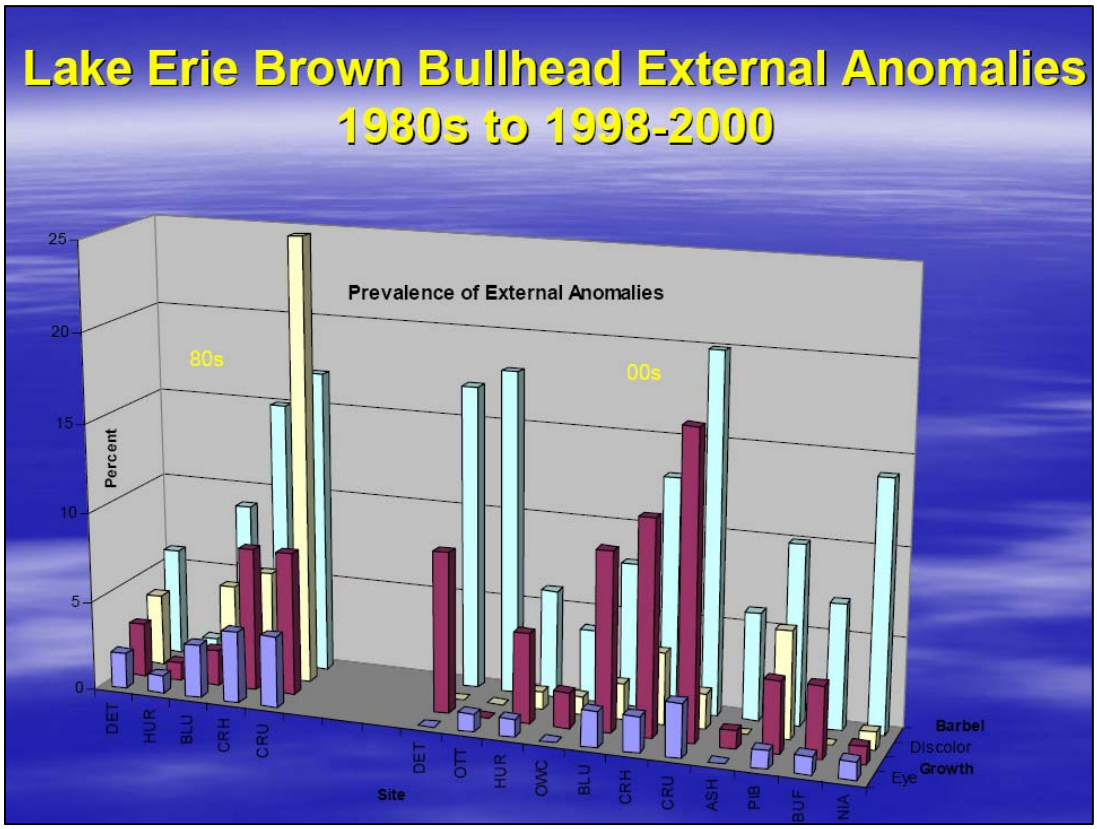
Location	Number of Individuals Present	Number of Taxa Present	Margalef Species Richness	Rich Rank	S-W Diversity Index	S-W Rank	% EPT
Detroit (1999)	643	15	2.17	9	1.54	8	0.00%
Ottawa (1999)	45	5	1.05	11	0.98	11	0.00%
Huron (1999)	19	8	2.38	8	1.88	5	0.00%
Old Woman (1999)	726	30	4.40	4	1.07	9	0.00%
Black River (1999)	126	20	3.93	5	2.09	3	0.00%
Cleveland Harbor (1999)	43	9	2.13	10	1.03	10	0.00%
Cuyahoga upstream (1999)	97	14	2.84	7	1.79	6	0.00%
Ashtabula (1999)*	44	13	3.17	6	1.76	7	0.00%
Presque Isle (1998)	1884	64	8.35	1	2.30	2	1.59%
Buffalo (1998)	2906	37	4.51	3	1.91	4	0.13%
Niagara (1998)	1766	48	6.29	2	2.56	1	0.11%

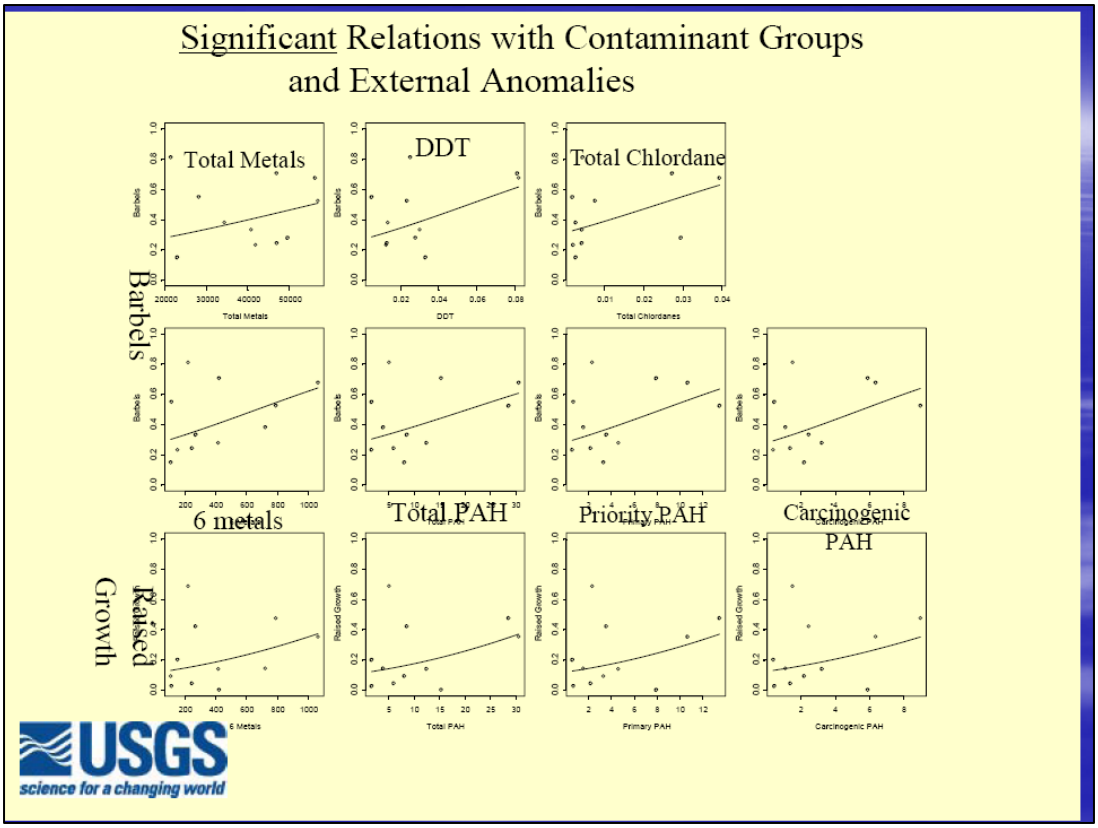
Huron River Benthic Invertebrates 1987 to 1999

80s to 90s

Location	Number of Taxa	Margalef Species Richness	S-W Diversity Index	Most Common Taxa At Site*
Huron (1987)	69	8.61	2.52	Naididae, Tubificidae, and Chironomidae
Huron (1999)	8	2.38	1.88	Tubificidae, Chironomidae, and Nematoda

FISH HEALTH
External Anomalies


Lake Erie Brown Bullhead External Anomalies
1980s to 1998-2000
Prevalence of External Anomalies
80s
00s
Percent
25
20
15
10
5
0
DET
HUR
BLU
CRH
CRU
DET
OTT
HUR
OWC
BLU
CRH
CRU
ASH
PIB
BUF
NIA
Site
Barbel
Discolor
Growth
Eye


Significant Relations with Contaminant Groups
and External Anomalies
Total Metals
DDT
Total Chlordane
Barbels
6 metals
Total PAH
Priority PAH
Carcinogenic PAH
Raised Growth
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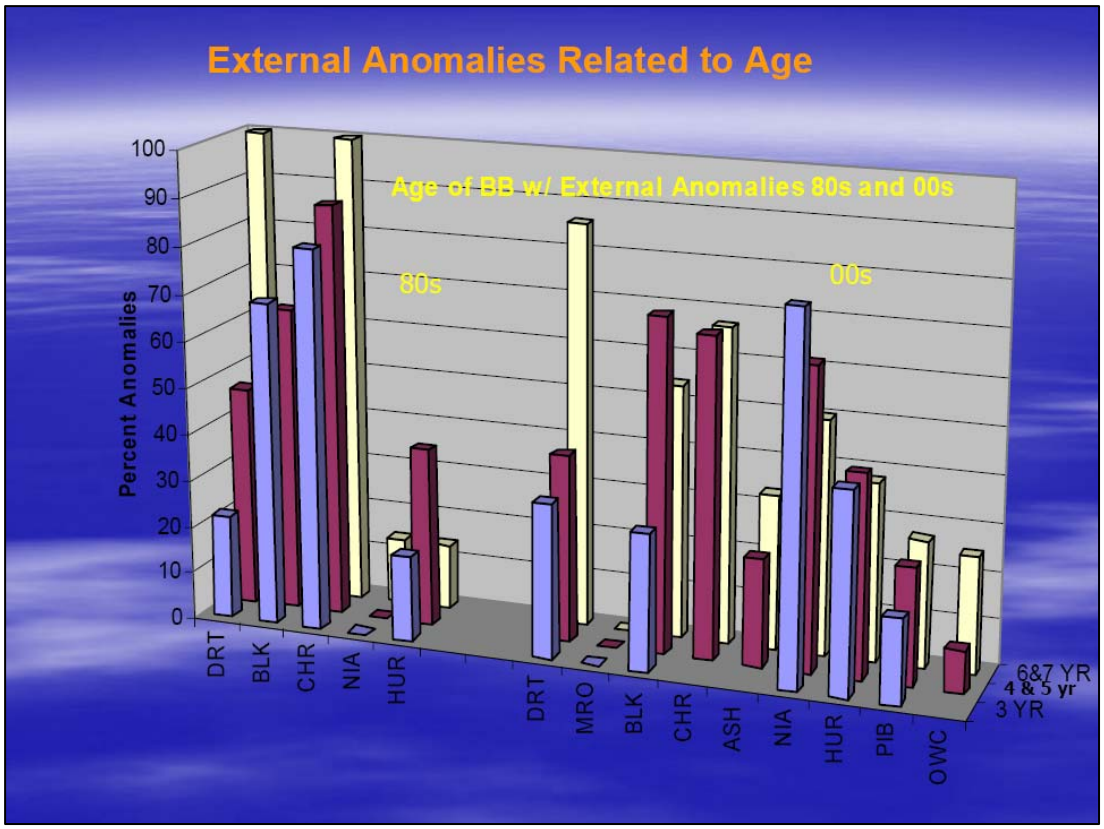

Pathology Indices

- Raised Growths
  - Oral and Body Growths
- Barbel Pathology
  - Shortened or knobbed

USGS

What is considered Impaired

- 2. A prevalence of raised growth on lips >10%, or of overall external raised growth on body and lips >15% in any of the mature benthic species.
- 3. A prevalence of barbel abnormalities (missing or deformed barbels) of >20% occurs in mature brown or black bullhead.

USGS science for a changing world

Percent for "clean" prevalence of neoplasitc external growths

	Skin/lip BB[1,2,3]	WS.
Erie	<15	<20
Huron	<15	<10
Michigan	<15	<10/20
Ontario	<15	<20
Superior	<15	<10

1. Baumann et al 1996, 2.Smith et al.1994, Smith et al.2004

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Delisting/Reference Conditions

- Fish Tumors and other Deformities
  - When the incidence rates of fish tumors or other deformities do not exceed rates of unimpacted control sites and when survey data confirm the absence of neoplastic or pre-neoplastic liver tumors in bullheads or suckers.

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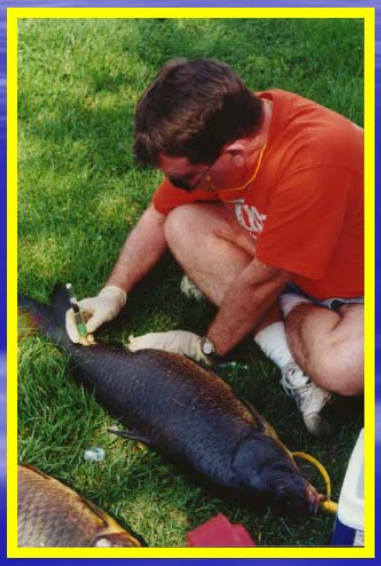
WE NEED REFERENCE CONDITIONS & SITES

Collection of Blood for Sex Steroid and Vitellogenin Analysis



Collecting blood from the caudal vein of a Largemouth bass

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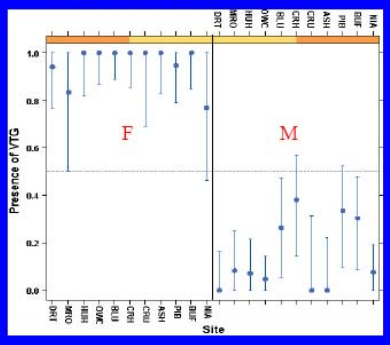
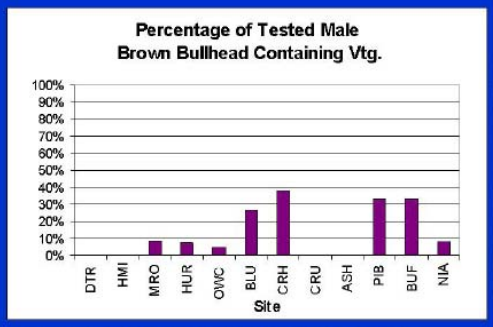
Vitellogenin in Female and Male Brown Bullhead Collected for LEEI in 1998-2000

Detection of Vitellogenin





LEEI 1998-2000: Vitellogenin (yolk protein) in males

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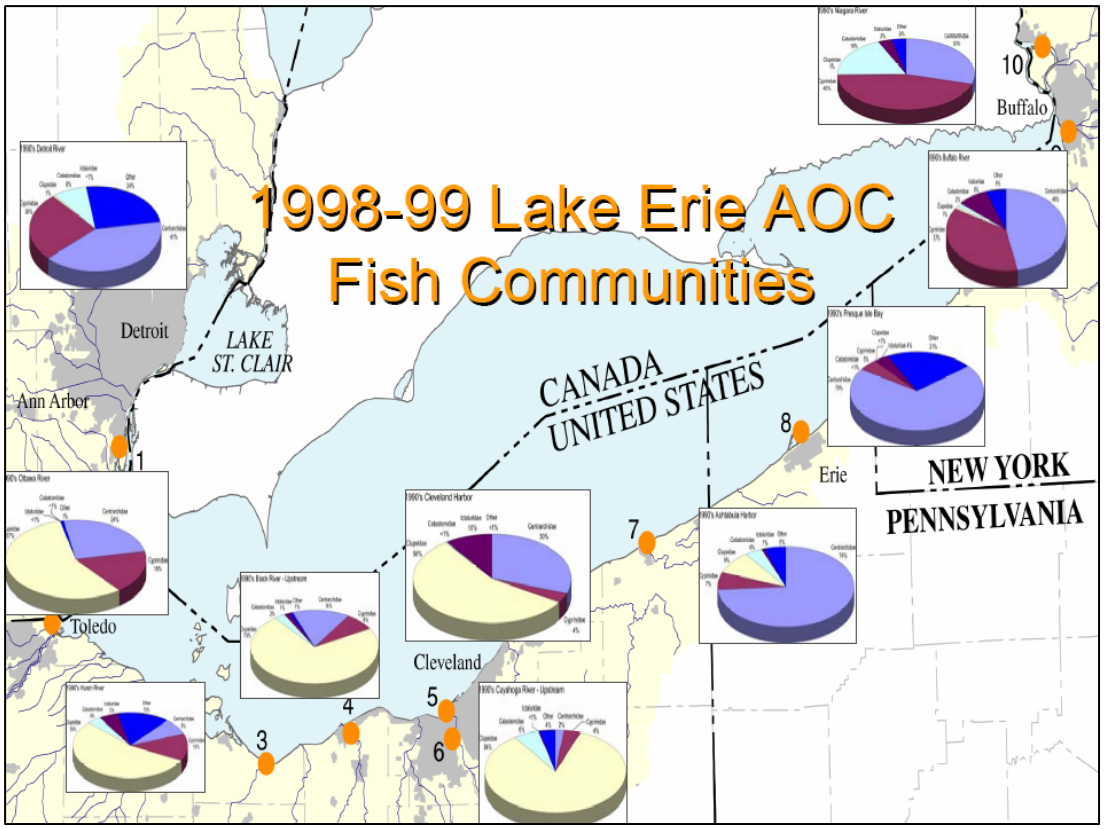


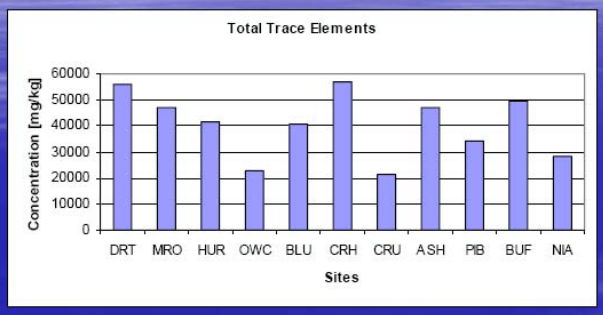
Lake Erie Ecological Investigations

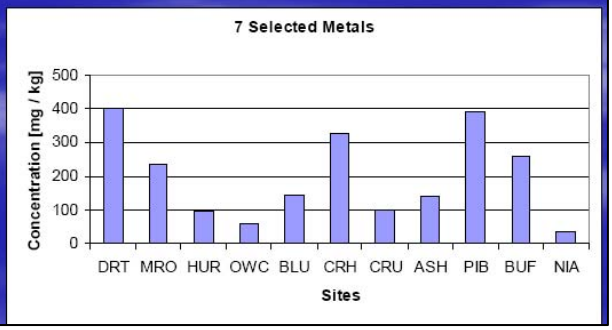
- Sediment Residue
- Invertebrate Communities
- Fish Health
  - External Anomalies
  - Endocrine Biomarkers
- Fish Communities
  - Some of the biological parameters seem related to the contaminants while others do not.

MOVE FORWARD TO DO INTERNATIONALY CONSISTANT BASIN WIDE MONITORING AT BOTH CONTAMINATED AND REFERENCE SITES

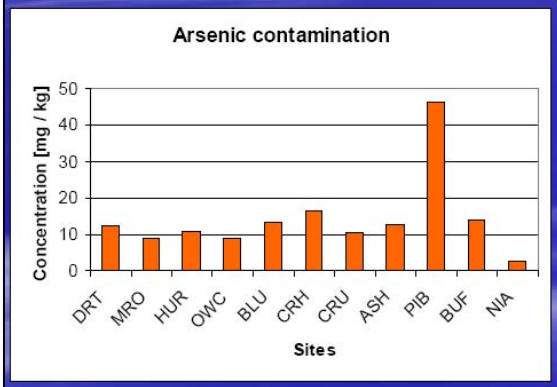
Field Guides

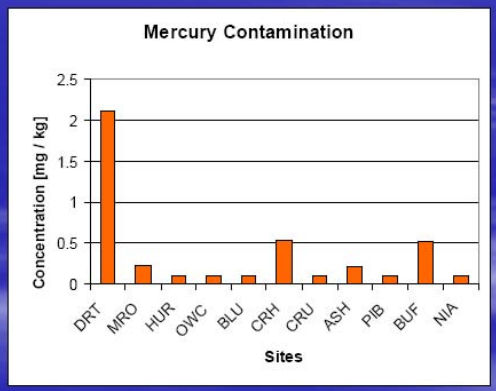
Total Metals and Seven Selected Trace Elements





Select Trace Metals





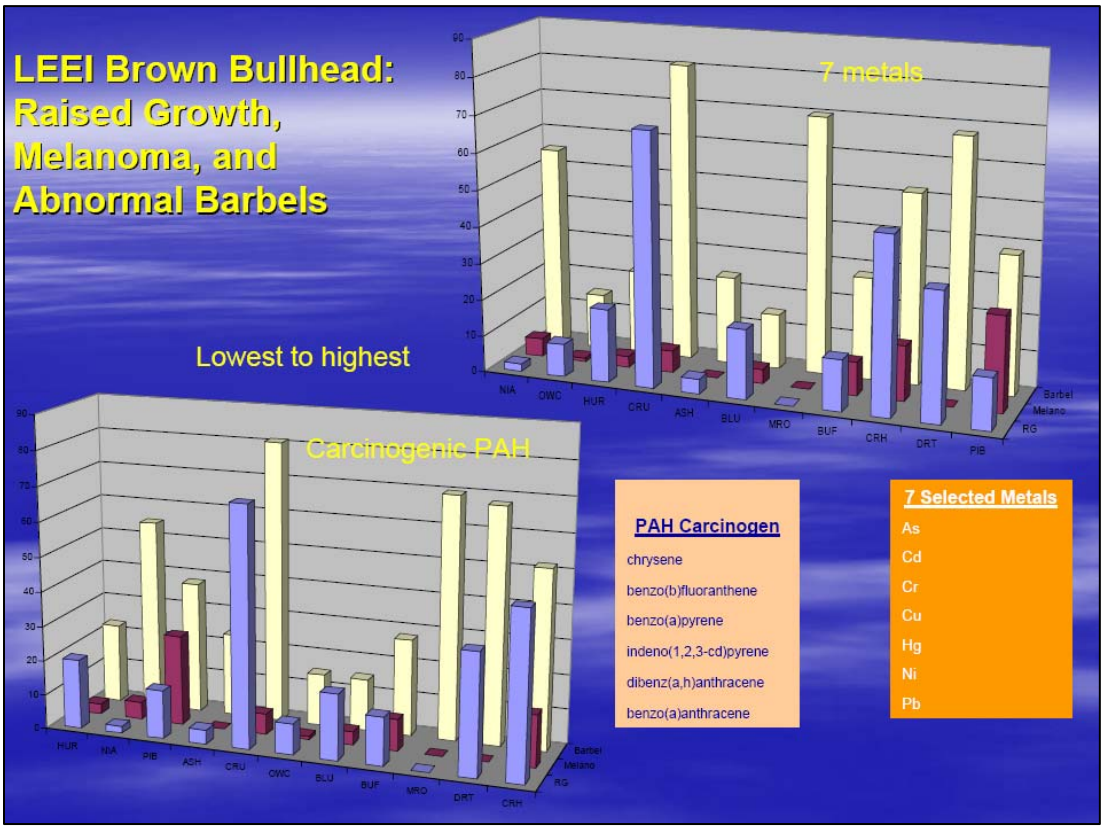
Sum of all Sediment Contaminants

SITES	Total PAHs	Total PCBs	Pest.*	Total DDTs	As	Hg	Ni	Zn	Sum of ranks
DRT	1	3	2	1	5	1	1	1	15
MRO	3	1	4	2	9	4	3	4	30
HUR	11	11	11	10	7	6	7	10	73
OWC	6	9	9	3	9	6	10	11	63
BLU	5	7	7	4	4	6	5	6	44
CRH	2	4	5	7	2	2	2	2	26
CRU	8	6	8	6	7	6	9	7	57
ASH	7	2	1	9	5	4	6	8	42
PIB	9	8	10	8	1	6	8	3	53
BUF	4	5	3	5	3	2	4	5	31
NIA	10	10	6	11	11	6	11	9	74

FISH HEALTH

- External Anomalies

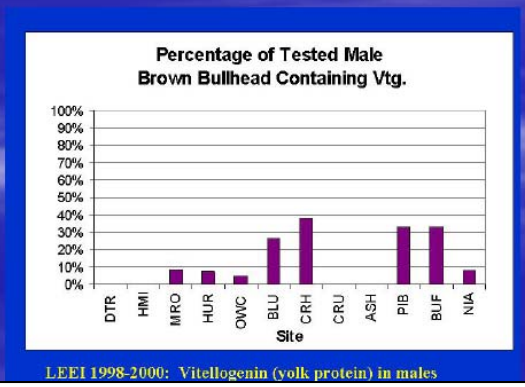
LEEI Brown Bullhead: Raised Growth, Melanoma, and Abnormal Barbels



What Prevalence Rate should be considered as Reference for Fish Tumor /Deformities for beneficial use.

- 1990 How Clean is Clean
  - Mac and Smith – 2%
    - Too low for realistic goal?
- Need to establish goal for each Lake?
  - or even areas within lake

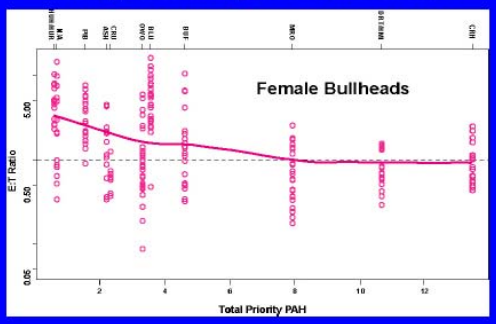
Male Brown Bullhead with Vitellogenin



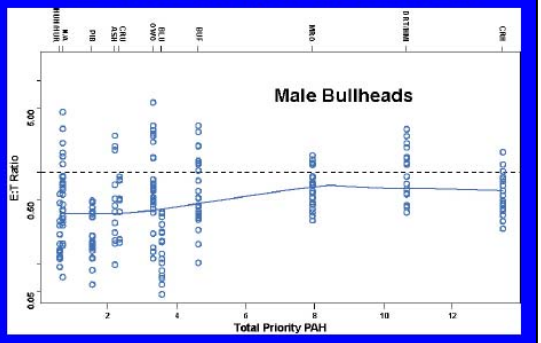
LEEI 1998-2000: Vitellogenin (yolk protein) in males

Female and Male Brown Bullhead E2:11Kt relation to PAHs

Priority PAHs as a predictor of E2:11KT



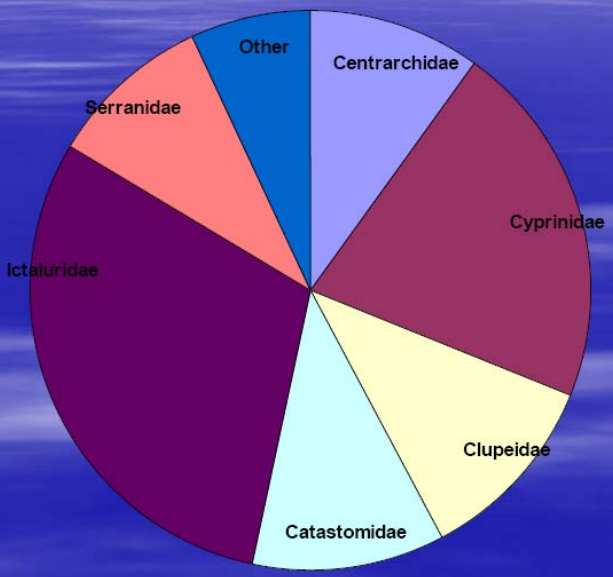
Priority PAHs as a predictor of E2:11KT



Lake Erie Ecological Investigations

- Sediment Residue
- Invertebrate Communities
- Fish Health
  - External and Internal Anomalies
  - Blood - Endocrine Biomarkers
  - Histopathology
- Fish Communities

Dominant Families Collected in LEEI 1998-1999



Lake Erie Ecological Investigations

- Sediment Residue
- Invertebrate Communities
- Fish Health
  - External and Internal Anomalies
  - Blood - Endocrine Biomarkers
  - Histopathology
- Fish Communities

BOB WELLINGTON

Distribution and migration of brown bullhead in Presque Isle Bay as related to the Great Lakes Water Quality Agreement – Area of Concern

Robert J. Wellington[1] and David J. Gustafson[1]

[1]Gannon University, 109 University Square, Erie, Pennsylvania 16541-0001

Abstract: In 1984, some brown bullhead (*Ameiurus nebulosus*) in the Presque Isle Bay/Thompson Bay areas at Erie, Pennsylvania, were noted to have external lesions. There was some concern whether the "tumors" were related to environmental contaminants. Studies were conducted by the United States Fish and Wildlife Service in an effort to clarify the matter. In its 1985 study, several bullhead were found to have various types of deformities/lesions and unusual patches of black pigment on their skin. Subsequent studies by others revealed a continuing pattern of lesions in bullhead. On January 30, 1991, the United States Department of State pursuant to Annex 2 of the Great Lakes Water Quality Agreement designated "Presque Isle Bay and the waters of Lake Erie in the immediate vicinity of Erie, Pennsylvania, as an Area of Concern under the terms of said agreement." Erie's Presque Isle Bay became the 43rd listed Area of Concern.

In summer of 2005, Gannon University investigated the deeper waters of Presque Isle Bay and the outer Erie Harbor in Lake Erie (July-October) to see if adult brown bullhead were present. Historically most of the bullhead sampling was done in the bay waters three meters deep or less. Electrofishing, which accounted for many of the captured fish, did not work in waters much over two meters deep. It was believed the brown bullhead stayed in Presque Isle Bay most if not for all their lives. If this were true, the observed lesion problem most likely would be due to something in the water or sediments of Presque Isle Bay. However, if bullhead migrated to Lake Erie after spawning, this would present an entirely different scenario. A question developed as to where the adult bullhead went after they left the shallow water spawning areas.

In the summer 2005 investigation, Gannon University used gill nets, wooden catfish traps, "metal" fish traps, set hooks, and an otter trawl to attempt to capture bullhead in the deeper waters. The collectors were also evaluating the capture methods to see which would be the most efficient if future studies were to be conducted. The gill nets captured more adult bullhead than any of the other devices. The wooden catfish traps, and the metal traps, as they were deployed at the time, were relatively ineffective in capturing bullhead. An adult brown bullhead was captured in Lake Erie in the otter trawl. The adult bullhead were evaluated for lesions or other obvious external anomalies, tagged on their opercula, and released. These tagged fish may be recaptured in the future.

Aside from the goals of the study, the investigators were also on the lookout for any new exotic species of fish (such as Ruffe) that might show up as an incidental catch. No "new" exotic species of fish were captured during this study period. White perch and round goby (introduced species), which have been observed for several previous years were rather common compared to some native species. No-young-of-the-year or adult Rudd (a recently identified invasive species in the bay) were captured during the sampling period.

Presentation:

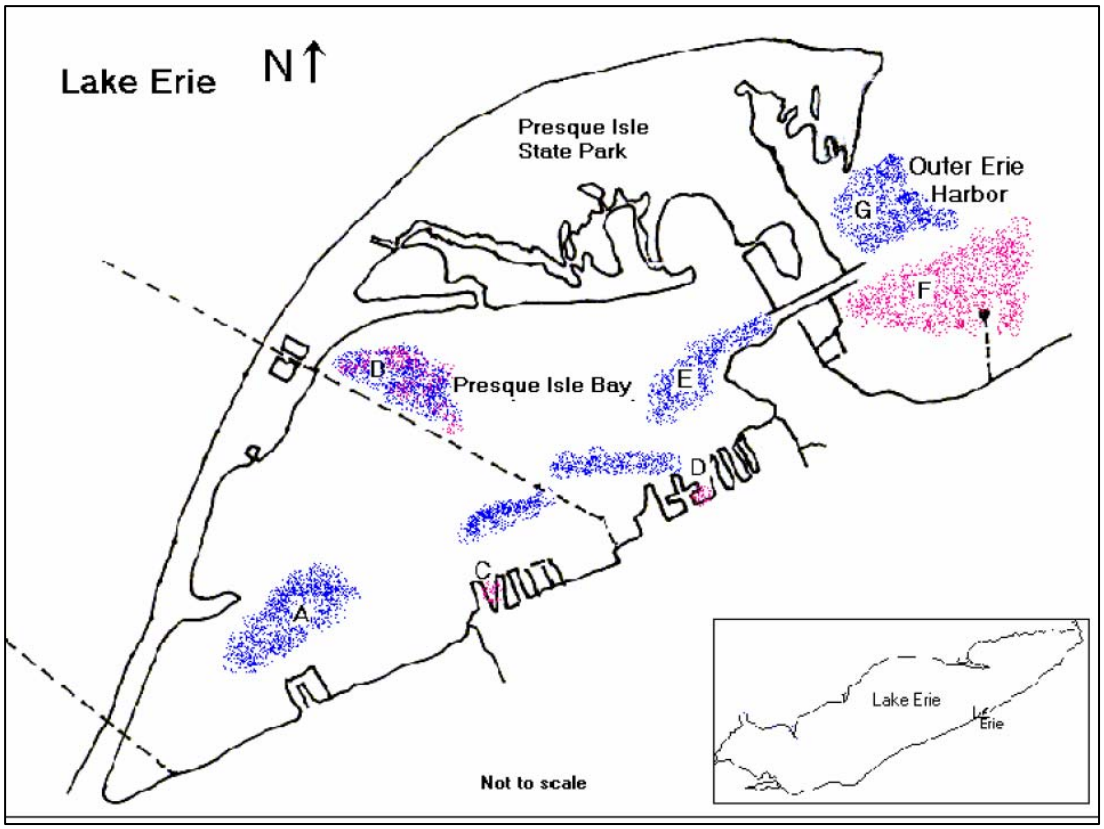
Distribution and Migration of Brown Bullheads in Presque Isle Bay as Related to the Great Lakes Water Quality Agreement – Area of Concern

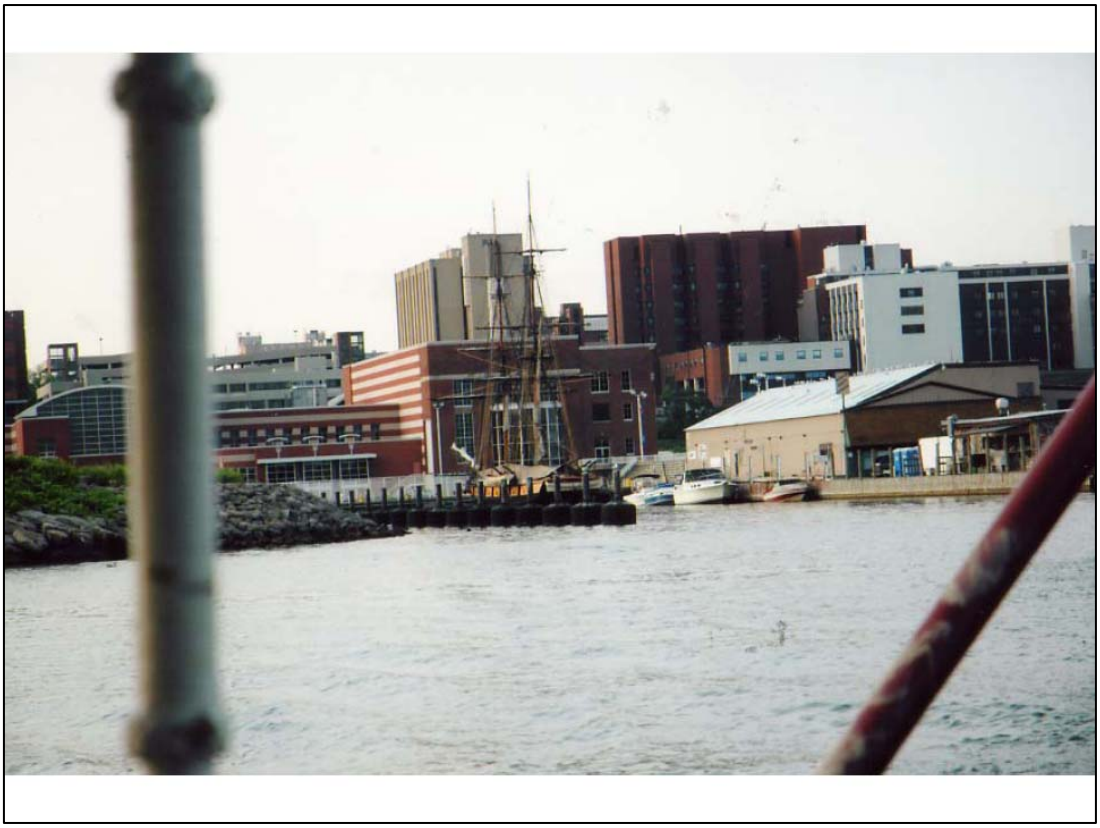
Presque Isle Bay/Lake Erie Study
2005

Dr. David J. Gustafson Ph.D.
Chairperson and Distinguished Professor of Biology
Principal Investigator

Robert J. Wellington RES
Adjunct Professor of Biology

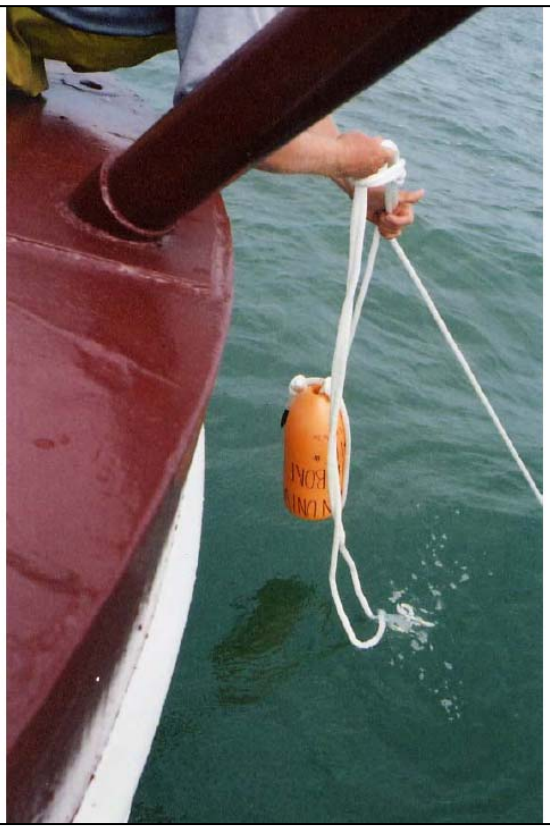
Biology Department
Gannon University
Erie, PA 16541


Lake Erie
N
Presque Isle
State Park
Outer Erie
Harbor
G
F
D
Presque Isle Bay
E
D
C
A
Lake Erie
Erie
Not to scale




ICE


URBAN



COLLEEN WELLINGTON

The effects of sedimentary pollution on the young-of-year population of Ameiurus nebulous (brown bullhead) in Presque Isle Bay

Colleen Wellington[1]

[1]Pennsylvania Sea Grant, Tom Ridge Environmental Center, 301 Peninsula Dr., Suite 3, Erie, PA 16505

Abstract: *Ameiurus nebulous* (brown bullhead) have been an important indicator species in Presque Isle Bay (PIB) – their tumor rates are both part of the cause for listing PIB as an Area of Concern (AOC) and for upgrading it to the first Great Lakes Area of Recovery. Recently, however, there has been concern that the contaminants causing tumors may also be affecting *A. nebulous* reproduction. The purpose of this study was first to determine the status of the young-of-year (YOY) population of *A. nebulous* in PIB, especially in comparison to surrounding areas. Once a problem was recognized, the focus shifted to determining whether pollution was the cause. Based on theories regarding the existence of morphological *A. nebulous* that are actually *A. nebulous / A. natalis* (black bullhead) hybrids, I hypothesized that pollution is not responsible for the YOY disappearance in PIB. This part of the experiment was tested through exposing YOY bullhead and toxicologically similar eggs to bay and control sediments and food. Measurements included: survival, growth, behavior, and skin abnormalities. The results indicate no statistical significance between treatments, thus supporting the hypothesis. However, more research needs to be done, both on the effects of pollution and other possible causes such as hybridization.

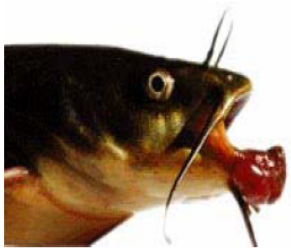
Presentation:

The Effects of Sedimentary Pollution on the Young-of-Year Population of *Ameiurus nebulous* (brown bullhead) in Presque Isle Bay

Colleen Wellington

Rational: Area of Concern

- International Joint Commission's criteria listing Presque Isle Bay as an AOC
  - Fish Tumors/Deformities (Bullheads)
  - Restrictions on Dredging
  - Reproductive Problems???



Reason for Reproduction Concern

- Bullhead population changes
  - **1972:** 240,000 (Based on standing crop estimate)
  - **1992:** 31,715
  - **1999:** 30,950
- In 1999 Dr. Mark Pyron only found 3 young-of-year (YOY) in the PIB area.
- Bullheads are good parents who guard their young

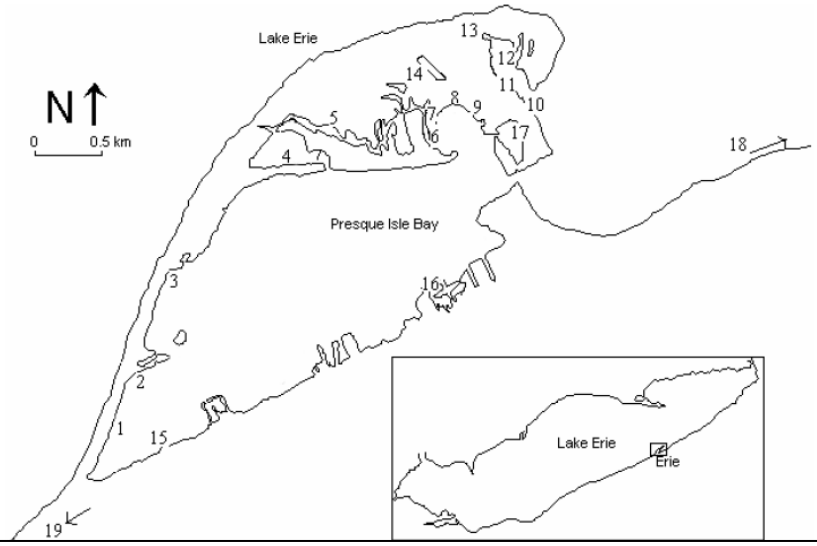
Purpose and Hypothesis

- **First:** determine the status of bullhead reproduction in PIB
  - I predicted to find a scarcity of YOY
- **Second:** determine effects of pollution (from sediments and food chain) on YOY bullheads and toxicologically similar eggs
  - Based on hybridization evidence I hypothesized pollution would not have an effect (eggs from hybrids infertile)

Sampling Methods: Collection

- Used combination of 5 methods to locate and capture YOY bullheads: trapping (64 days), seining (20ft seine), dip netting, shocking (boat and backpack), and observing (combined 120+ hours)

Sampling Points



Methods: Pollution Effects on Eggs

- Fathead minnow eggs – toxicologically and morphologically similar to bullheads
- Measured on hatch/die basis
- 12 Glass Jars (2 eggs in each):
  - 3 Elk Creek Water
  - 3 Elk Creek Mud and Water
  - 3 Bay Water
  - 3 Bay Mud and Water

Methods: Effects of Sediment Pollution on YOY Bullheads

- Same conditions and replications as with egg study (12 aquariums, 2 fish each)
- Measured: **survival**, growth, behavior, skin abnormalities

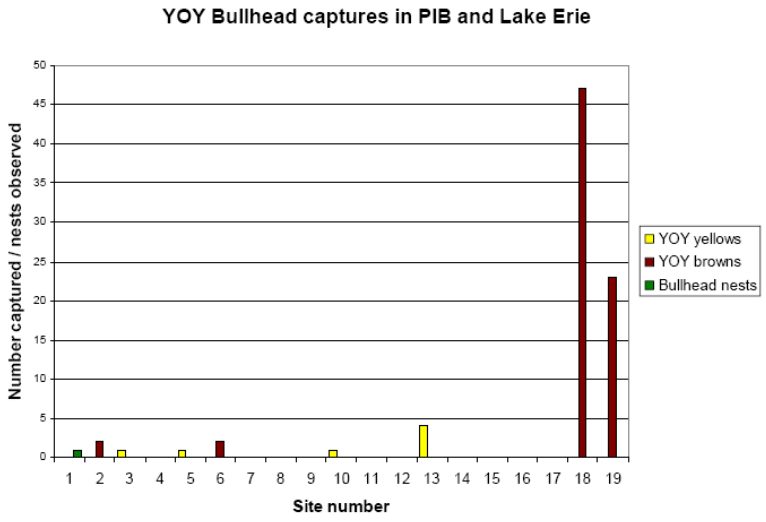
Methods: Effects of Bioaccumulation on YOY

- 6 Aquariums (2 fish each): Fish in 3 fed shrimp pellets, others fed chironimids and zebra mussels
- Measured: **survival**, growth, behavior, skin abnormalities

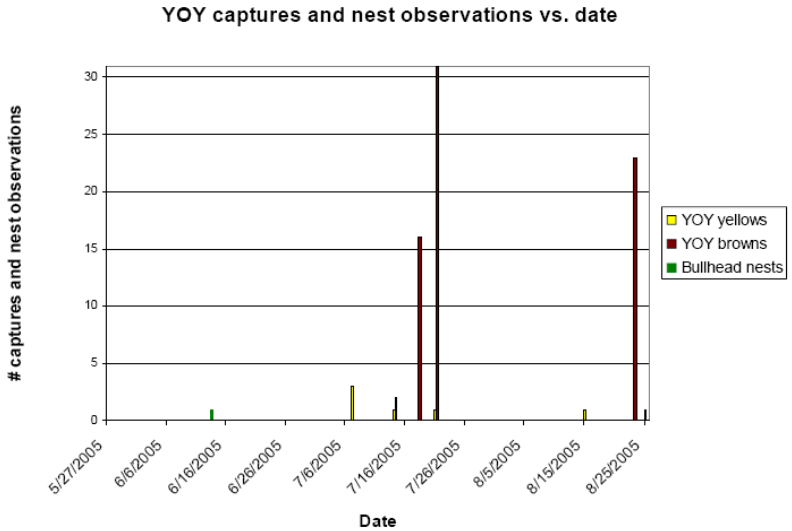
Results: Sampling for YOY

- **Browns in Bay:** 4 (2 in AOC)
- **Yellows in Bay:** 6 (1 in AOC)
- **Browns in Sixteenmile:** 2-400
- **Browns in Elk Creek:** 50+

Distribution between sites

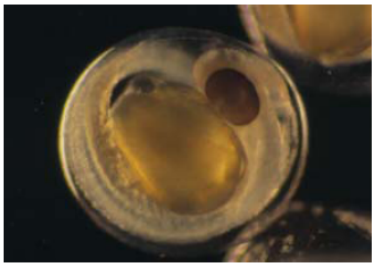


Distribution over time



Results: Pollution Effects on Eggs

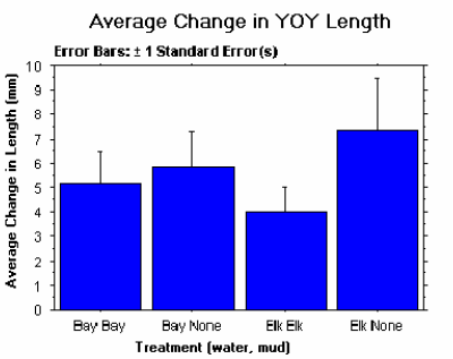
- 100% hatched within 8 days
- No significant differences in time of hatching

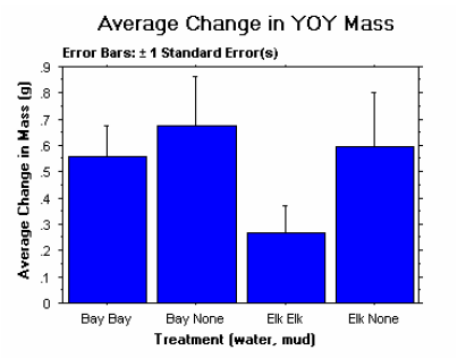


Results: Effects of Sediment Pollution on YOY

- **Survival:** no statistical effect (p=0.4158)
- **Behavior:** no behavioral differences
- **Skin Abnormalities:** none apparent

Results: Effects of Sediment Pollution on YOY Growth

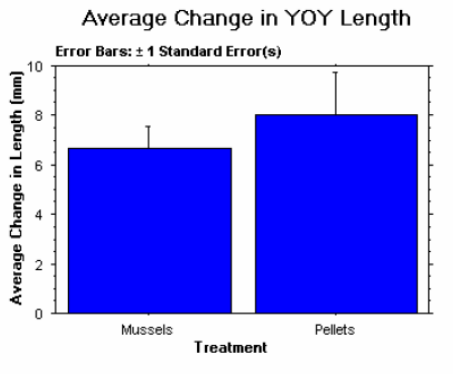


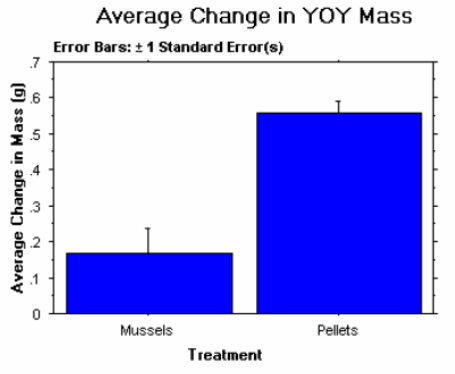


Results: Effects of Bay Food on YOY

- **Survival:** 100% survival
- **Behavior:** no observed differences
- **Skin Abnormablities:** none observed

Results: Effects of Bay Food on YOY Growth





Conclusions

- There is a problem with Bullhead reproduction/survival in PIB
- Egg study does not rule out possibility that *bullhead* eggs unable to survive in Bay sediments (need better setup)
- No evidence that sediments affect YOY survival
- Food effects on YOY inconclusive

Further Experimentation

- Look at **inland lakes** for YOY
- Effects of **pollution on bullhead eggs** (since they are not available at this time of year)
- Test **possibilities besides pollution**:
  - predation (gobies, blue gills etc.), and other ecosystem interactions

Future Experimentation (Cont.)

- Continue **hybridization** work
  - to confirm that it is occurring and is responsible for the tumors and/or reproductive problem
- Use **transmitters**
  - to see if Bay bullheads spawn in streams
  - and if they move to deep water (could pick up pollutants there – wouldn't be good indicator for the Bay)

Acknowledgements

- Eric Obert
- Scott Wissinger
- Rory Hanczar
- Robert Wellington
- Bob Zawadzki
- Chuck Murray
- Donald Wolfgong
- Doug Ebert
- Jim Grazio
- John Arway

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SESSION TWO: STANDARDIZED CRITERIA DEVELOPMENT

DR. VICKI BLAZER

The histopathology subcommittee progress report

Vicki S. Blazer[1], John W. Fournie[2], Jeffrey C. Wolf[3] and Marilyn J. Wolfe[3]

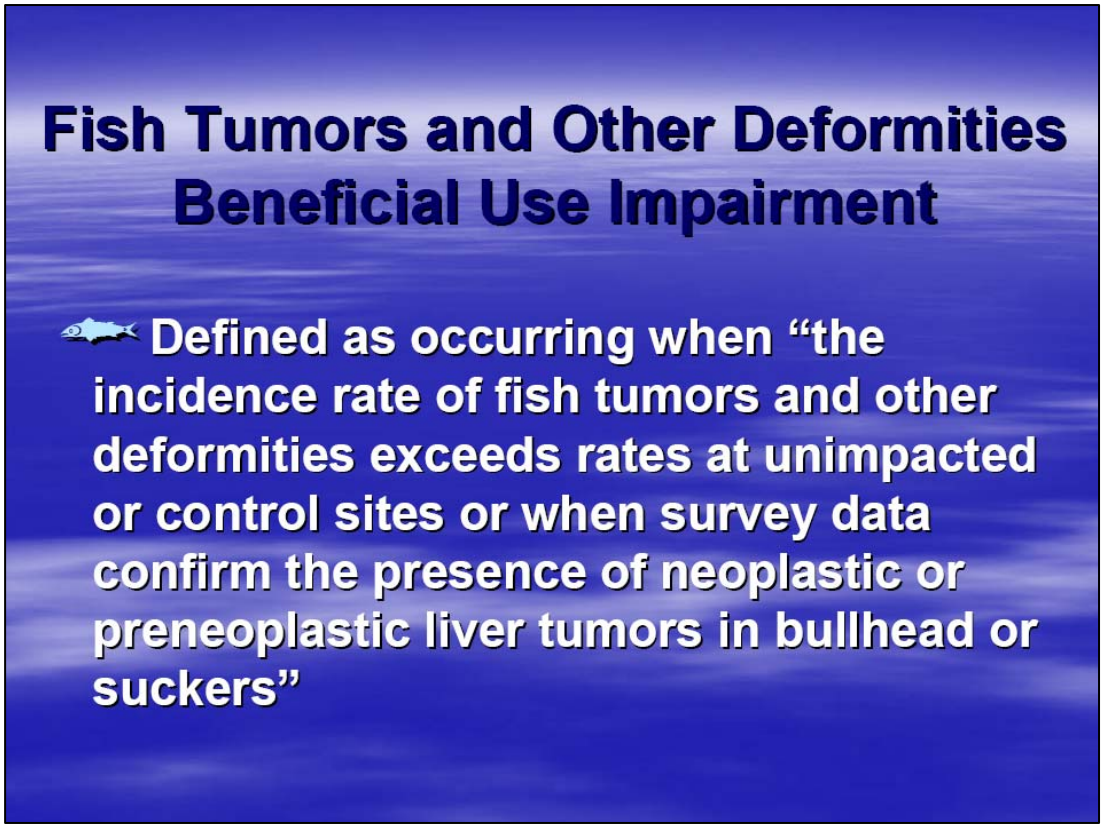
[1]National Fish Health Research Laboratory, U.S. Geological Survey, 11649 Leetown Road, Kearneysville, WV 25430 [2]U.S. Environmental Protection Agency, Gulf Ecology Division, 1 Sabine Island Drive, Gulf Breeze, FL 32561 [3]The Registry of Tumors in Lower Animals, 22900 Shaw Road, Suite 107, Sterling, VA 20166

Abstract: One of the beneficial use impairments at numerous AOCs is "fish tumors or other deformities." An impairment occurs when the prevalence of fish tumors or other deformities exceeds those at unimpacted or control sites or when survey data confirm the presence of neoplastic or preneoplastic liver lesions in bullhead or white sucker *Catostomus commersonii*. Numerous surveys have been conducted over the years assessing neoplasia in these fishes, both liver and skin tumors. However, a major problem in comparing the results has been a lack of consistent criteria for evaluating histological changes in bullhead livers. As individual AOCs develop and implement remedial action plans, realistic and attainable delisting targets need to be specified. For this to occur and be consistent from site to site there must be standardization of the criteria being used to evaluate specific impairments. Hence, the Histopathology Subcommittee was charged with developing specific diagnostic criteria for non-neoplastic and neoplastic proliferative hepatocellular and biliary lesions. A manuscript was submitted and accepted to the *Diseases of Aquatic Organisms* journal describing the non-neoplastic proliferative lesions bile duct proliferation, a proliferative inflammatory response to a cestode parasite, and foci of cellular alteration. The foci are the only non-neoplastic lesions considered pre-neoplastic. Neoplastic lesions described include hepatic adenomas, hepatic carcinomas, cholangioma, and cholangiocarcinoma.

In addition to the journal article on liver lesions, a Pathology Manual illustrating gross and microscopic proliferative lesions of both liver and skin is under production. Non-neoplastic skin (and barbel) lesions include melanistic areas, epithelial hyperplasia, and inflammatory responses. Neoplastic skin lesions include papilloma, squamous cell carcinoma, and melanoma.

We recognize that the number of sections examined may influence the prevalence of lesions observed and research is needed to determine the appropriate number of sections.

Presentation:



Issues with this BUI in Terms of Delisting

- No definition of "fish tumors and other deformities"
- No definition of "preneoplastic"
- No definition of "unimpacted or control areas"

Confounding Issues Terminology

- Historically there have been various definitions of cancer/tumor/neoplasia
- Some papers have differentiated between "neoplasia" and "cancer"
- In some studies hepatocellular neoplasia ranged from small foci of altered foci to hepatocellular carcinoma
- In other studies only carcinomas were considered neoplasms

Presque Isle Bay
Report published in 2001

- Suggests the liver tumor rate decreased from 22% in 1992 to 0% in 1999
- Livers were removed from a subsample
- "examined for histological evidence of tumors as biliary carcinoma" – no pictures, no description of lesions

Confounding Issues

- Methodology
  - Criteria for the microscopic diagnosis of liver and skin neoplasia
  - Number of sections of liver that should be examined

Subcommittee Assignments

1) Produce peer-reviewed publications for the diagnostic criteria of proliferative liver and skin lesions of bullhead
2) Make recommendations on the terminology that should be used for neoplastic and preneoplastic
3) Make recommendations on methodology considerations

Diagnostic Criteria

- Journal article :

  "Diagnostic Criteria for Proliferative Hepatic Lesions in Brown Bullhead" by V. Blazer, J. Fournie, J. Wolf and M. Wolfe

  Reviewed by M. Myers and J. Hawkins

  Manuscript accepted by Diseases of Aquatic Organisms

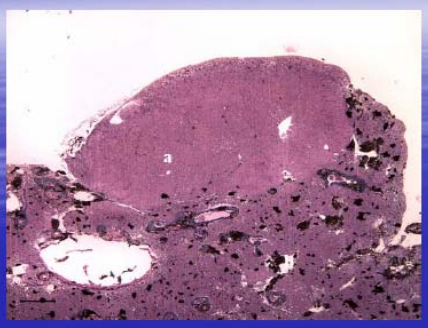
Diagnostic Criteria

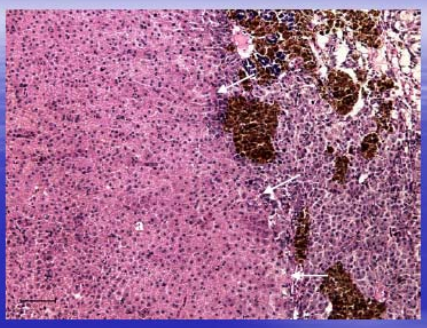
- Manual
  - "Diagnostic Criteria for Proliferative Skin and Liver Lesions in Brown Bullhead" printed by PA Sea Grant
  - More examples of each lesion; match histological appearance with gross pictures

Terminology
Neoplasia

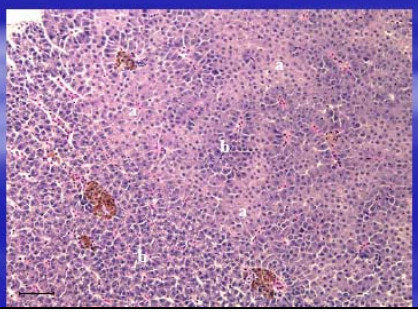
- Neoplasia – both benign and malignant proliferative growths
  - Hepatocellular adenomas and hepatocellular carcinomas
  - Cholangiomas and cholangiocarciomas

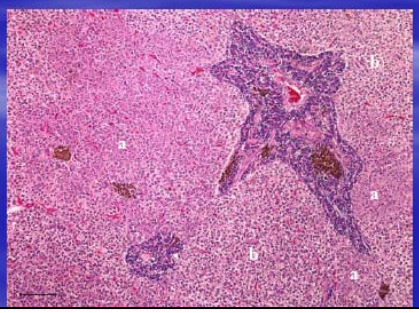
Hepatocellular Adenoma





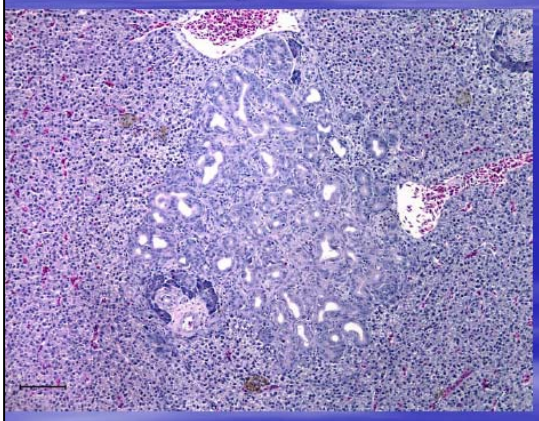
Hepatocellular Carcinoma



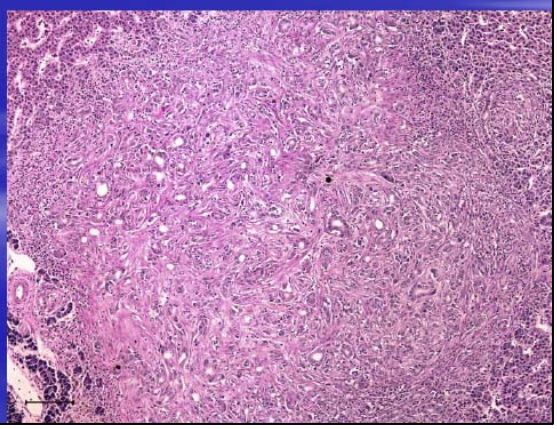


Neoplasia of Bile Ducts

Cholangioma



Cholangiocarcinoma

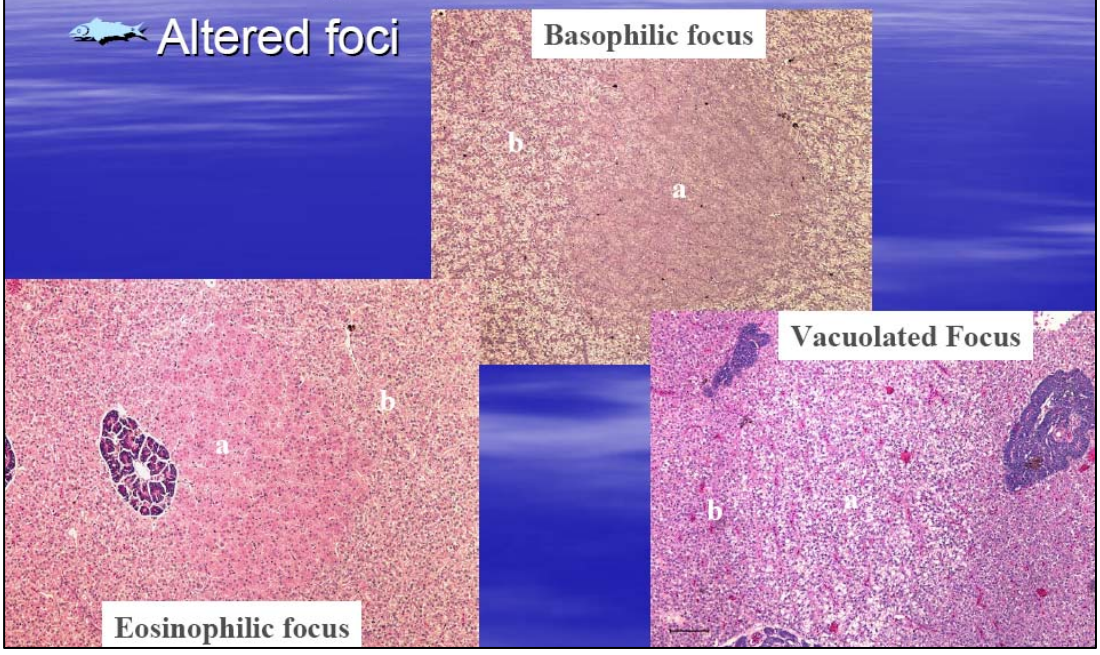


Putative Preneoplastic Changes

- Foci of cellular alterations are associated with chemical exposures
- In studies with other fish species some have been found to be preneoplastic
- There have not been good exposure studies with bullhead to document which changes actually are preneoplastic

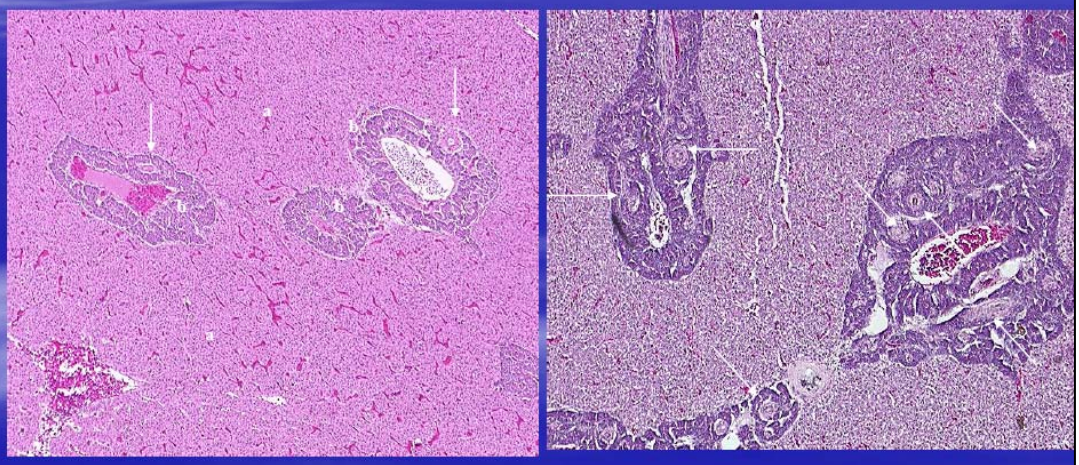
Putative Preneoplastic Hepatocellular Changes

- Altered foci



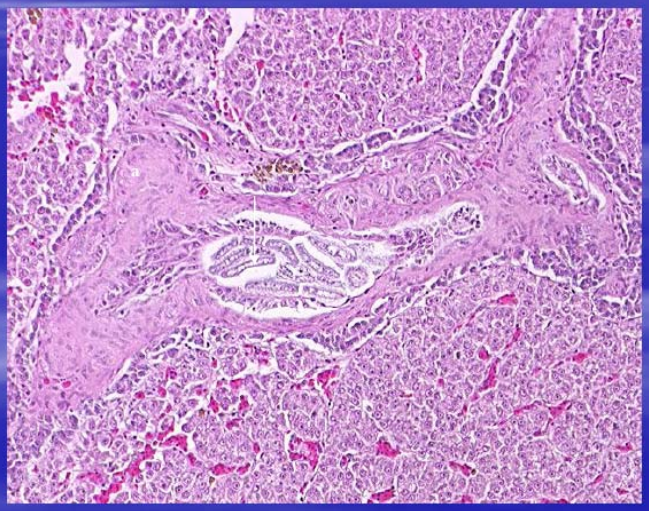
Proliferative Biliary Lesions

- Bile duct hyperplasia



Proliferative Biliary Lesions

- Parasites, Contaminants, Both



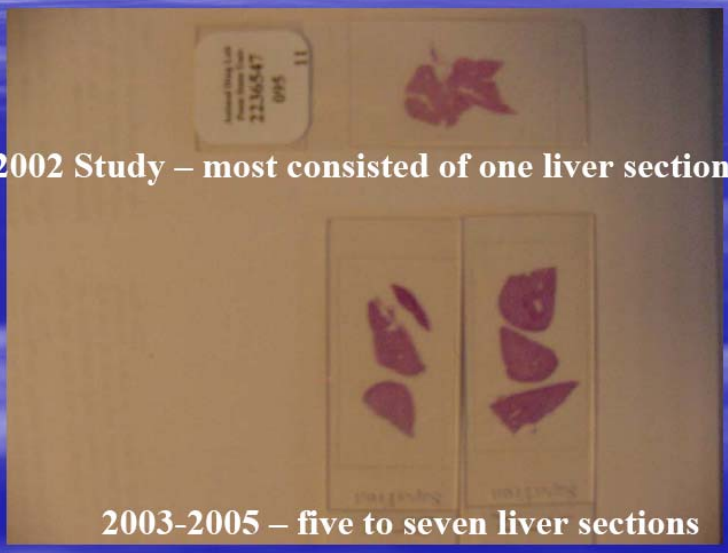
Conclusions/Recommendations

- Should be consistent in what we term neoplasia
  - Hepatocellular adenoma, hepatocellular carcinoma
  - Cholangioma, cholangiocarcinoma
  - Papilloma, squamous cell carcinoma, melanoma
- Experimental work should be done to determine which if any altered foci, bile duct hyperplasia, or epidermal hyperplasia are preneoplastic in bullhead
  - At this time altered foci are the only lesions we would consider putatively preneoplastic

Recommendations

- The number of sections examined may certainly influence prevalence
- Need experimental evaluation of how many sections are required for statistical certainty that neoplasia is or isn't present

Number of Sections



Recommendations

- Suggest that there be some oversight or quality control in terms of diagnoses used for regulatory purposes:
  - Perhaps a panel of pathologists
  - At least a “second opinion”

SEAN RAFFERTY

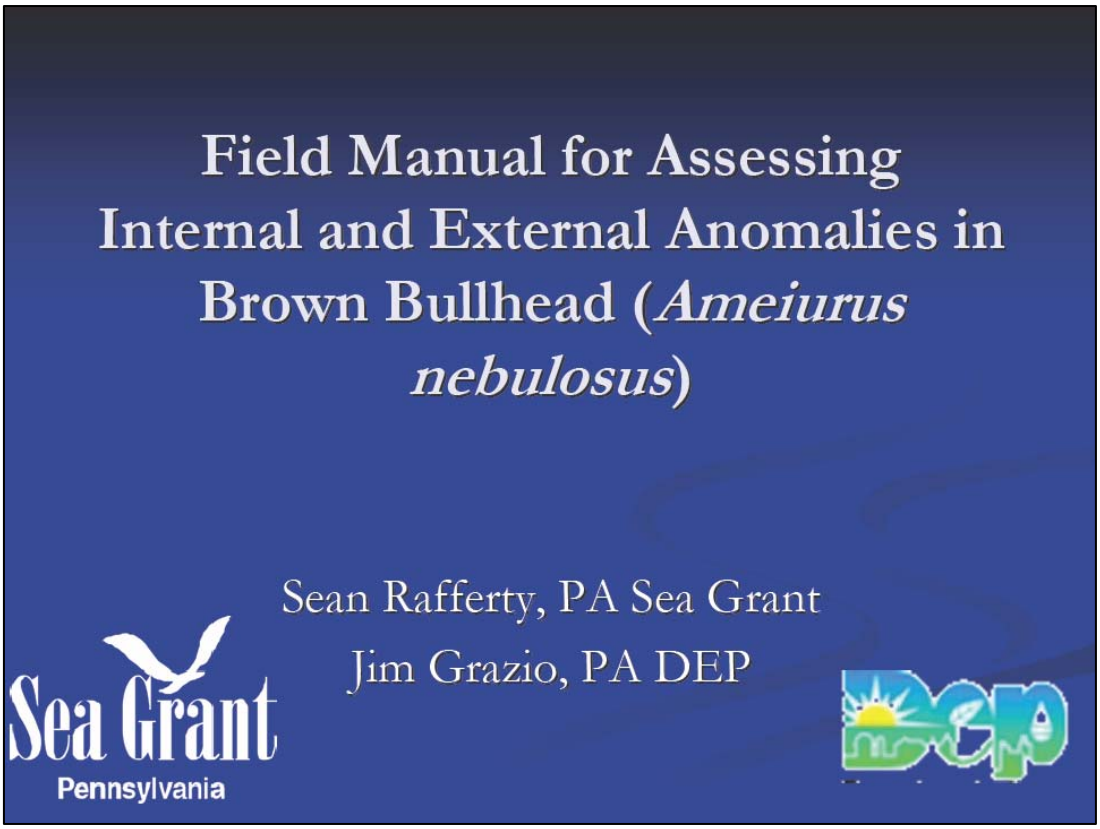
Standardized field procedures for assessing internal and external anomalies in brown bullhead (Ameiurus nebulosus)

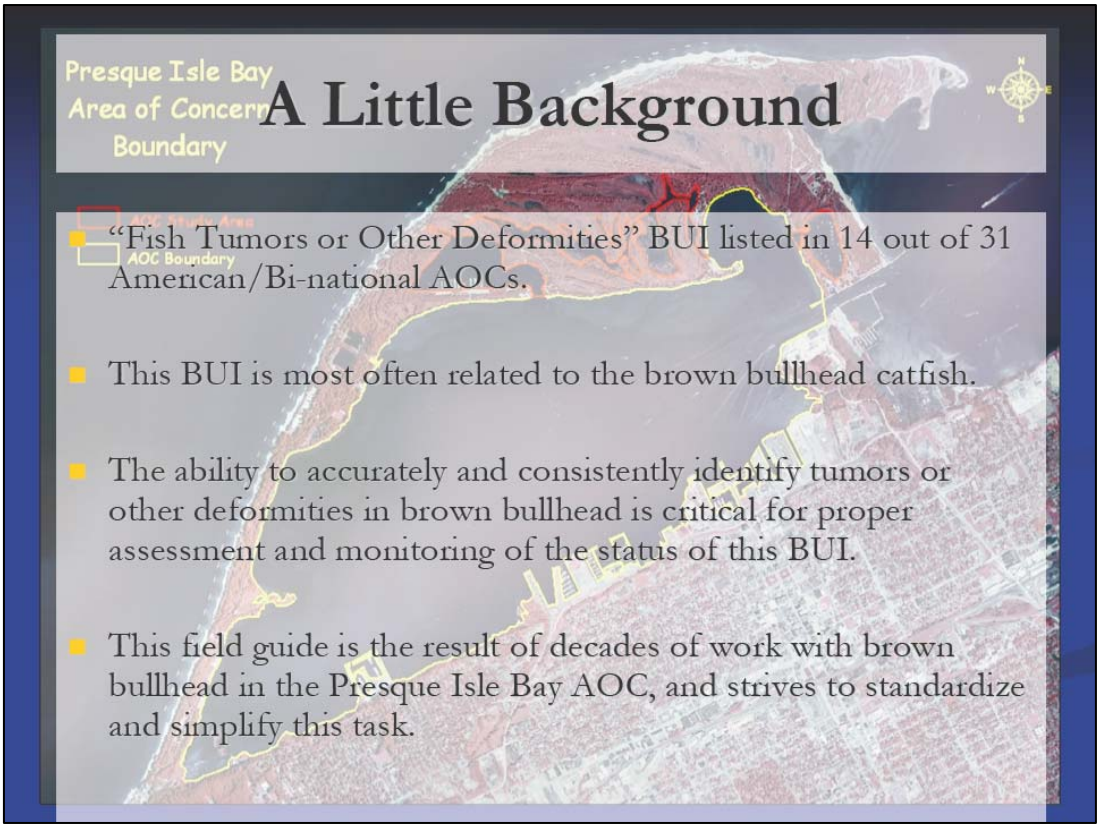
Sean Rafferty[1] and Jim Grazio[2]

[1]Pennsylvania Sea Grant, Penn State Behrend, Tom Ridge Environmental Center, 301 Peninsula Dr., Suite 3, Erie, PA 16505 [2]Pennsylvania Department of Environmental Protection, Tom Ridge Environmental Center, 301 Peninsula Dr., Suite 4, Erie, PA 16505

Abstract: For nearly two decades, the brown bullhead (*Ameiurus nebulosus*) has served as an indicator species for assessing the "fish tumors or other deformities" beneficial use impairment in Presque Isle Bay, Erie, PA. To address this beneficial use impairment (BUI) it is necessary to accurately and consistently characterize lesions and other deformities. To simplify the task of assessing fish tumors and other deformities in Areas of Concern (AOCs), the Pennsylvania Department of Environmental Protection and Pennsylvania Sea Grant staff developed a field guide that clearly explains, illustrates, and standardizes the criteria and methodology for assessing brown bullhead health. The guide was developed for field biologists to: improve the consistency of assessing, documenting, and monitoring the fish tumors or other deformities BUI in Great Lakes AOCs; and recommend standard operating procedures for the necropsy of brown bullhead. The field guide differs from previously produced fish health assessment guides in that it is specific to the health of brown bullhead.

Presentation:





Purpose of the Guide

For field biologists, to:

- aid in the consistency of identifying lesions and deformities during the gross observation of brown bullheads; and
- recommend standard operating procedures for the collection, necropsy, and preservation of brown bullhead tissue in the field.

Relationship to Other Guides

- Several excellent field guides and recommended Standard Operating Procedures have been developed for field assessments of fish health.
- This guide is intended to remain consistent with existing literature.
- However, the present guide was specifically written to facilitate the assessment and monitoring of "tumors or other deformities", in bullhead.

Breaking it Down

- Introduction
- Recommend Equipment
- Anatomy
- Field Collection
- Processing Fish
- Post Processing Procedures
- Appendix

1.0 Introduction

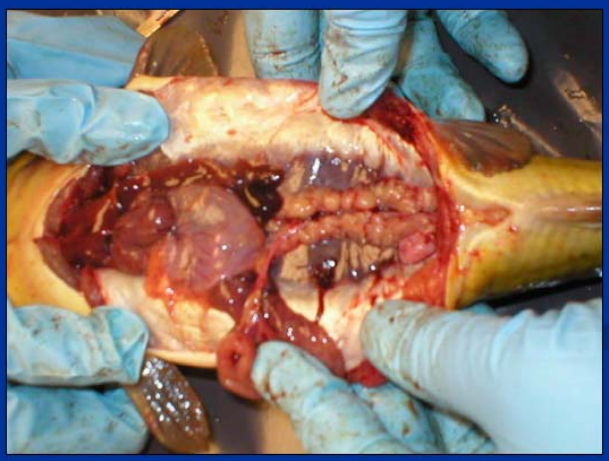
- Background Information
- Purpose of the Manual
- Relationship to Other Field Manuals

2.0 Recommended Equipment

- Field Collections
- Processing Fish
- Safety Equipment

3.0 Anatomy

- External Anatomy
- Internal Anatomy



4.0 Field Collection

- Permit Requirments
- Collection Methods
- Holding Methods

5.0 Processing Fish

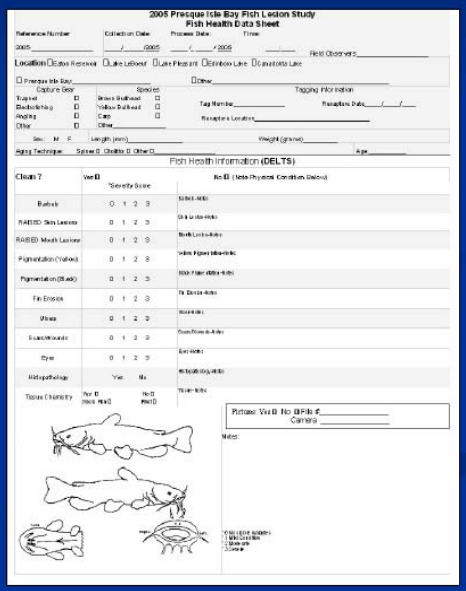
- Work Area
- Gross Visual Observations
- External and Internal Necropsy
- Spine and Otolith Removal

6.0 Post Processing Procedures

- Data Verification
- Clean Up
- Shipping Samples

7.0 Appendix

- Fish Health Data Sheet
- Acknowledgements
- References


2005 Presque Isle Bay Fish Lesion Study
Fish Health Data Sheet

FACILITATED DISCUSSION: *Comments and feedback on the manuals*

Facilitated by: Dr. Thomas Wortman

***Comments*:**

Pathology Manual:

- How important is it to include liver sections that may or may not develop into tumors?
- How do you measure severity?
    - This could be added to the manual, but would be a qualitative component.
    - A semi-qualitative scale is already being used, but caution must be used because everyone assesses severity differently.
- How confident can we be that the altered foci are due to environmental problems?
    - It was expressed that no other causes aside from contaminant exposure were known to cause altered foci.

Field Biologists Manual:

General comments and issues were addressed during the facilitated discussion. Specific comments and suggestions were written and submitted on an individual basis.

Section 1: Introduction

- Is the purpose clear? YES.

Section 2: Recommended Equipment

- Is there anything missing from the list? Anything not needed on list? NO.
- Safety Equipment (Any comments? Suggestions?)
    - Expand electrofishing safety equipment.
    - Gloves should be worn to avoid being stabbed by the fish.

Section 3: Drawing

- Is this clear? YES.

Section 4: Field Collection

- Should include issues related to use and abuse of animal subjects.
- Add a section on trolling.

Section 5: Processing

- Add more information regarding safety
- Is rating severity of anomalies a key component or should it be removed?
    - Keep severity rankings for records.
    - Will this info be used? How much does it matter?
    - Size could be used as a severity index, but this takes a lot of time. Is it worth it?
    - No, the main thing we're looking at is if there is an increase or decrease over the years.
    - What is severity? Size? Number? Grossness? Right now we go by number. Should we go by size? Would changing the rating affect use of historical data? NO.

SESSION THREE: LESION REFERENCE RATES FOR LAKE ERIE

DR. PAUL BAUMANN

Using historical data

Dr. Paul Baumann[1]

[1]USGS/Ohio State University, 473B Koffman Hall, 2021 Coffrey Rd., Columbus, OH 43210

Abstract: In the process of delisting Areas of Concern (AOCs) with the fish tumor beneficial use impairment (BUI), it becomes important to know whether historical data sets, both at AOCs and at reference locations, can be used to compare with results from more recent surveys. Over three times as many fish were collected at AOCs compared with reference sites during the period from 1980-1999. However, since 2000, the numbers have been approximately equivalent. In order for historic data to be comparable, the diagnostic criteria must be comparable, ages must be available and comparable, and locations sampled must be specific enough to determine comparability. If we examine three surveys of the Detroit River made in 1985-87 (Maccubin and Ersing), 1996 (Leadley et al.) and 2000 (USGS), some of these conditions are not met. Only the 1985-86 study included altered foci in the category of neoplasms, and did not distinguish between these designations. Since altered foci may or may not progress and are no longer considered neoplasms, this inflates the tumor rate in this paper. Similarly the 1985-86 paper was the only one not to use age or size criteria. Thus they sampled a large number of fish of age 3 or less, but only supplied ages for a portion of the fish used in the study. Because neoplasm prevalence increases with age, this paper reported a lower neoplasm rate for the population by skewing the age distribution toward younger fish. Finally, all three surveys took place on differing portions of the Detroit River complex.

In an attempt to use historical data to gauge a background tumor prevalence in Lake Erie, I created two location groupings using surveys having sufficient diagnostic and age criteria. Those four locations having the lowest tumor prevalence were grouped as "Reference," while five others with slightly higher prevalence were grouped as "Borderline Degraded." Over 50% of age 3 fish and over 60% of age 4 and 5 fish had neoplasms in the Black River in 1982. Neoplasm prevalence in slightly less-polluted AOCs (Detroit and Cuyahoga) averaged 7.1% for age 3 and 18.3% for ages 4 and 5. The Borderline Degraded group had a 2% and 6.7% neoplasm incidence for ages 3 and 4 and 5, respectively. Age 3 fish from the Reference group had no neoplasms, while ages 4 and 5 had a 1.5% neoplasm prevalence. This would suggest that background tumor prevalence in Lake Erie might be around 0.5% for age 3 fish and 2% for ages 4 and 5. However, more reference location data is needed to establish meaningful numbers.

Presentation:

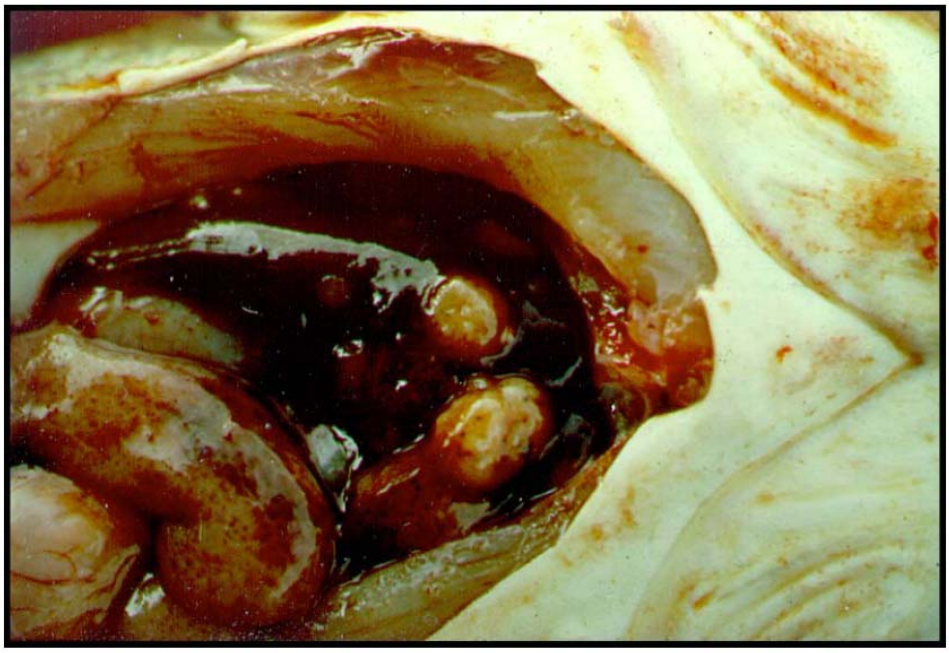
USING HISTORICAL DATA

COMPARABILITY

REFERENCE CRITERIA

USGS

Liver Neoplasms

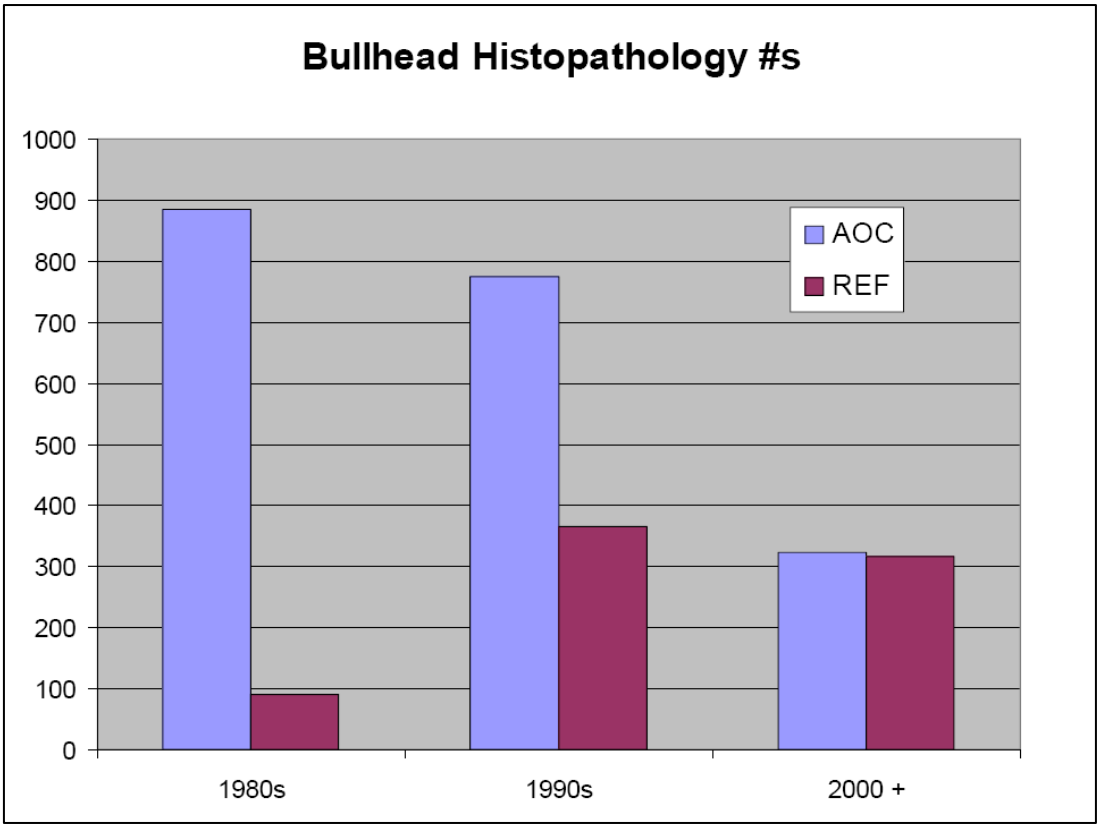


USGS

Historical Surveys 1980s-Present

- **AOCs**
- Black River
- Detroit River
- Buffalo River
- Hamilton Har. (80s)
- Ashtabula (90s +)
- Presque Isle (90s +)

- **REFERENCE**
- Old Woman Creek
- Huron River
- Long Point (80s)
- Presque Isle Ref (90s)
- Conneaut Creek (00 +)



Criteria for T umor Survey Comparisons

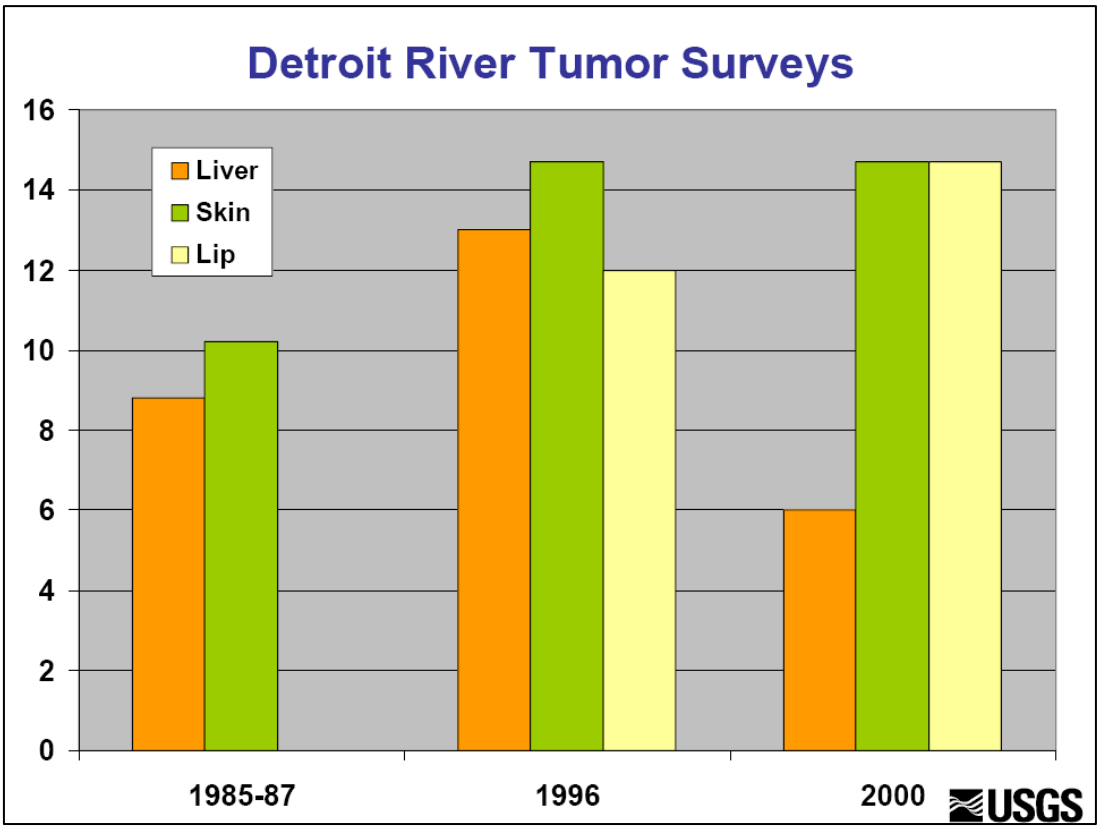
- Are Diagnostic Criteria Comparable
- Are Ages Sampled Comparable
- Are Locations Sampled Comparable
- Are Sample Sizes Sufficient

USGS

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

USGS



Criteria for Tumor Survey Comparisons

- Are Diagnostic Criteria Comparable
- Are Ages Sampled Comparable
- Are Locations Sampled Comparable
- Are Sample Sizes Sufficient

USGS

Diagnostic Differences

- Maccubin & Ersing 1985-87
  - "Foci of Altered Hepatocytes" included as Tumors
- Leadley et al. 1996 & USGS 2000
  - Foci of Altered Hepatocytes not included as Tumors

Criteria for Tumor Survey Comparisons

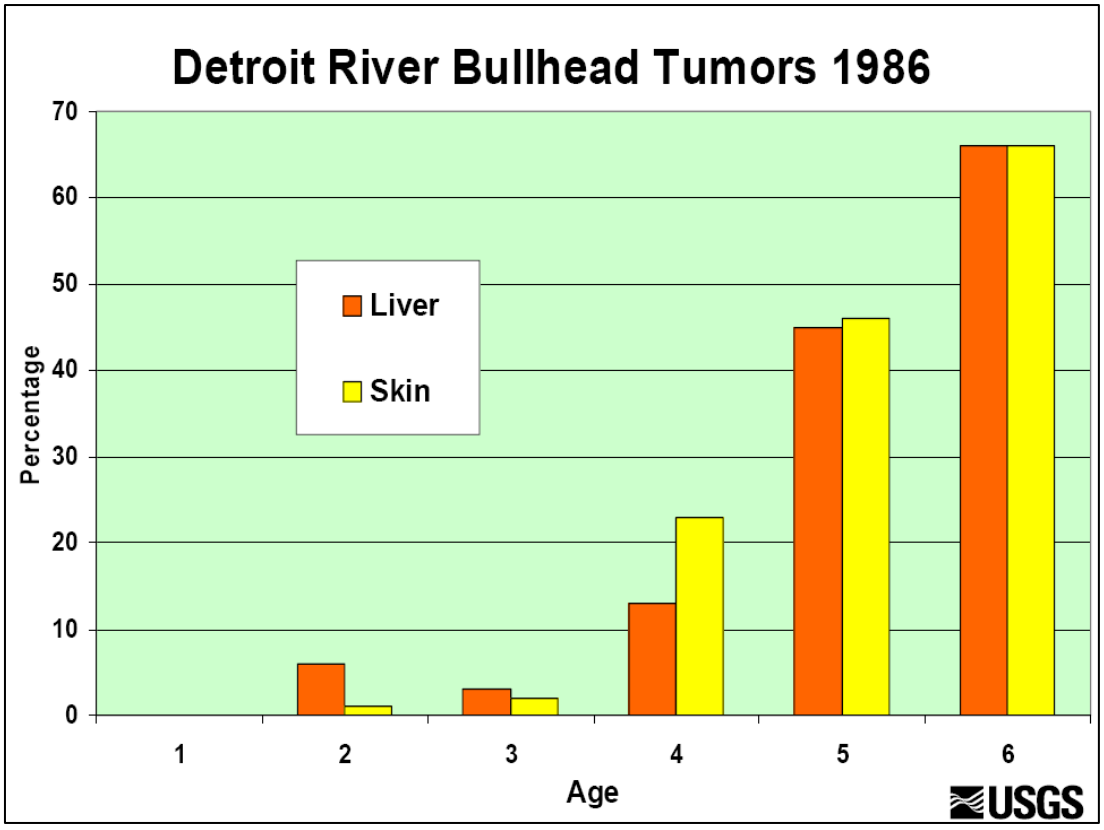
- Are Diagnostic Criteria Comparable
- Are Ages Sampled Comparable
- Are Locations Sampled Comparable
- Are Sample Sizes Sufficient

USGS

Age Distribution Sampled

- Maccubin & Ersing 1985-87
  - Ages 1-7
- Leadley et al. 1996
  - Ages 3-4 (Estimated)
- USGS 2000
  - Ages 3-9

USGS



Relative Age Abundance

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

USGS

Criteria for Tumor Survey Comparisons

- Are Diagnostic Criteria Comparable
- Are Ages Sampled Comparable
- Are Locations Sampled Comparable
- Are Sample Sizes Sufficient

USGS

Field Sampling Location

- Maccubin & Ersing 1985-87
  - Trenton Channel
  - Southern & Northern Islands
- Leadley et al. 1996
  - Trenton Channel
  - Amherstburg Channel
  - Peche Island
- USGS 2000
  - Southern Islands
  - Huron River Mouth

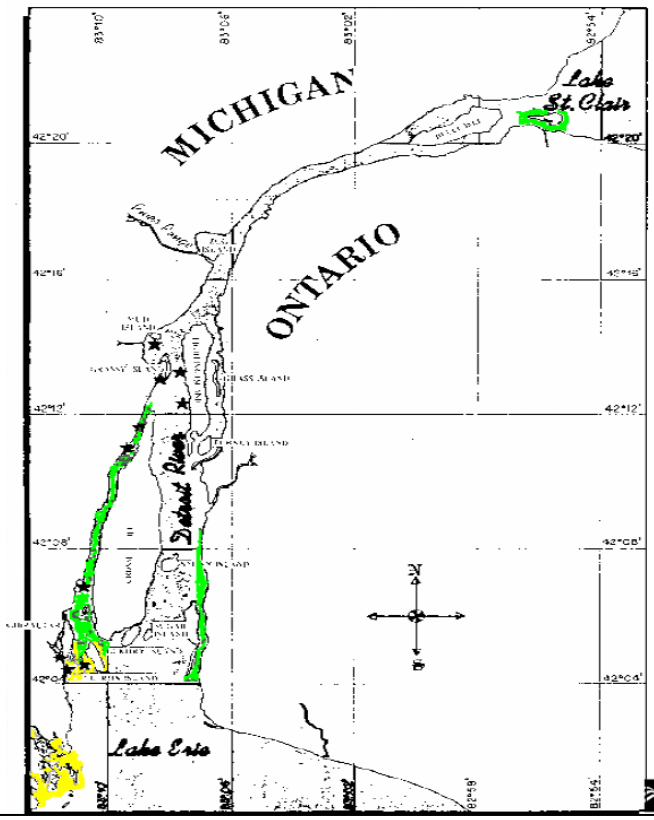
USGS

Detroit River Field Samples

1996

2000

85-86 Stars



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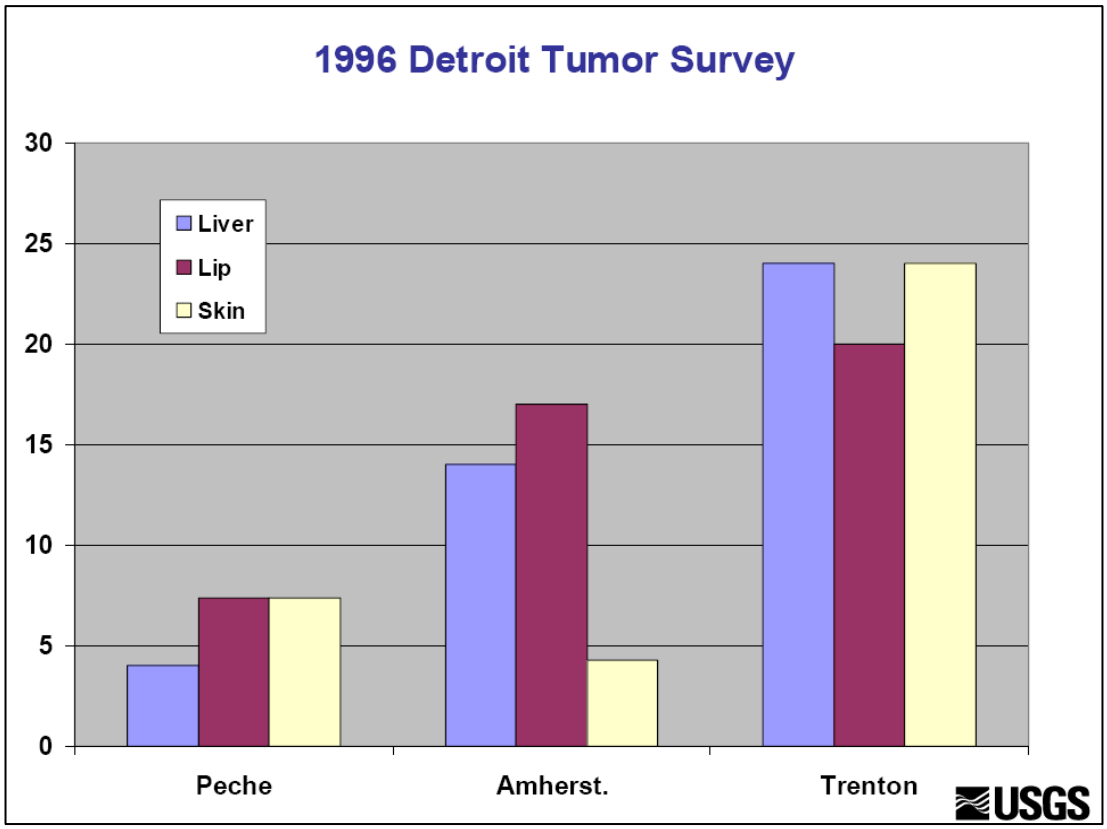


Criteria Comparisons

- Are Diagnostic Criteria Comparable
  - Leadley et al. & USGS
- Are Ages Sampled Comparable
  - Maccubin & Ersing & USGS
- Are Locations Sampled Comparable
  - Leadley et al. & USGS
- Are Sample Sizes Sufficient
  - Maccubin and Ersing

USGS

Designing Reference Criteria

- **Age Comparisons**
  - Use Age 3 alone
  - Group older ages by 2s
- **Combine Reference Sites**
  - Seeking background rate
  - Lake-wide criteria

USGS

River Groupings

- **Borderline Degraded:**
  - Old Woman Creek 1992 & 1993
  - Huron River 1998
  - Niagara River 1998
  - Ashtabula River 2000
- **Reference:**
  - Old Woman Creek 1984
  - Menominee River 1984
  - Huron River 1986 & 1987
  - Presque Isle Bay 1998

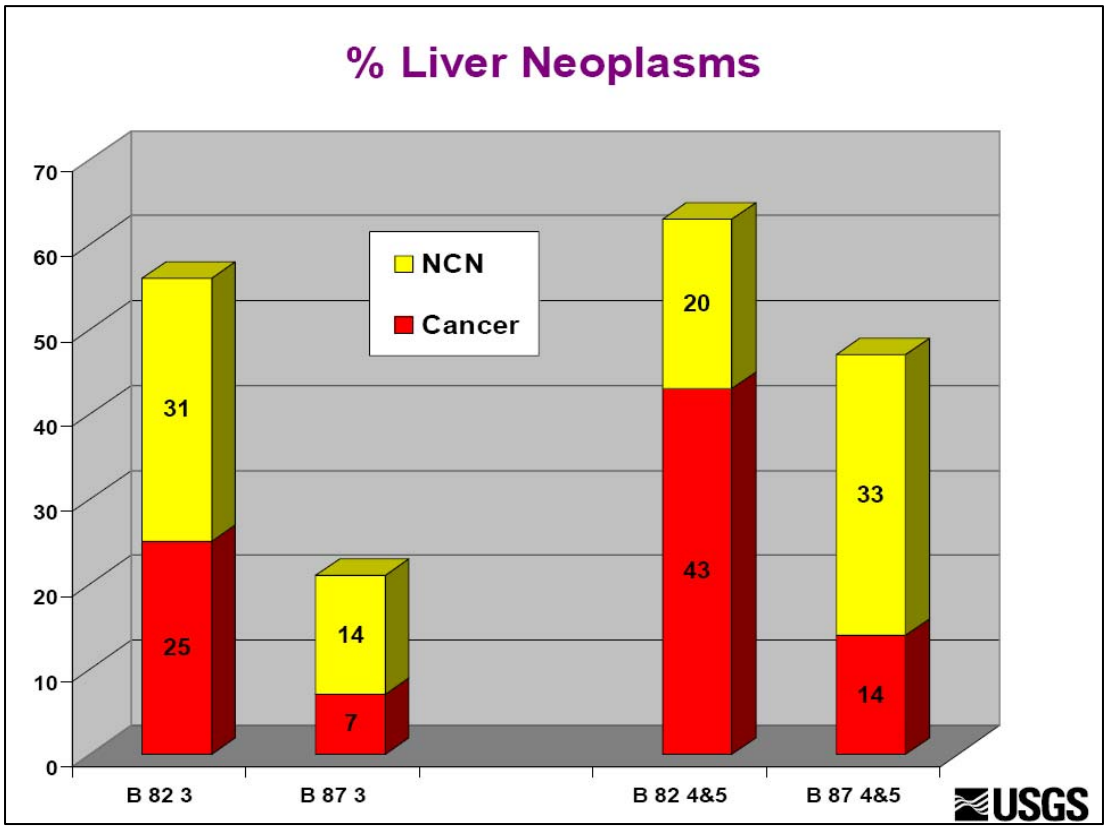
USGS

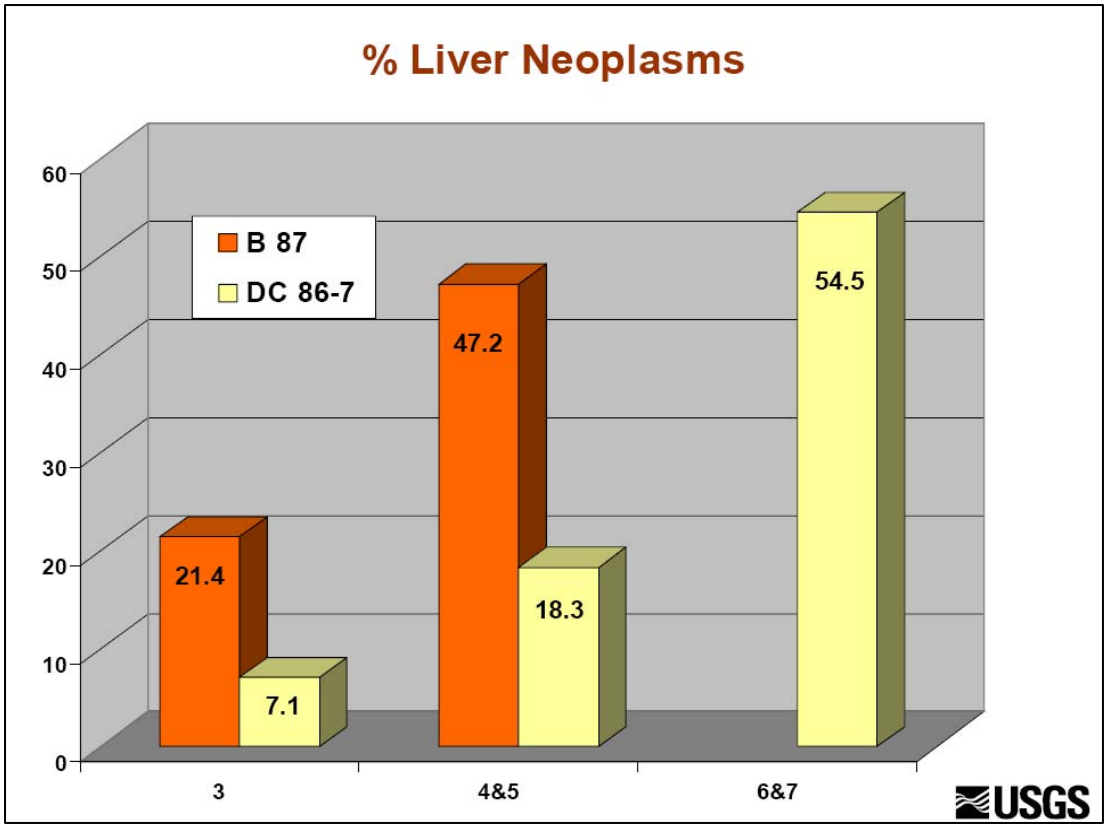
River System Comparisons

- Black 82 vs Black 87
- Black 87 vs Cuy/Det 86-7
- Cuy/Det 86-7 vs Borderline Degraded (BD)
- BD vs Reference

Sample Sizes by Age

- Age 3: N= 41-98
- Ages 4 & 5: N= 36-90
- Ages 6 & 7: N=11-18


% Liver Neoplasms
70
60
50
40
30
20
10
0
NCN
Cancer
31
25
14
7
20
43
33
14
B 82 3
B 87 3
B 82 4&5
B 87 4&5
USGS


% Liver Neoplasms
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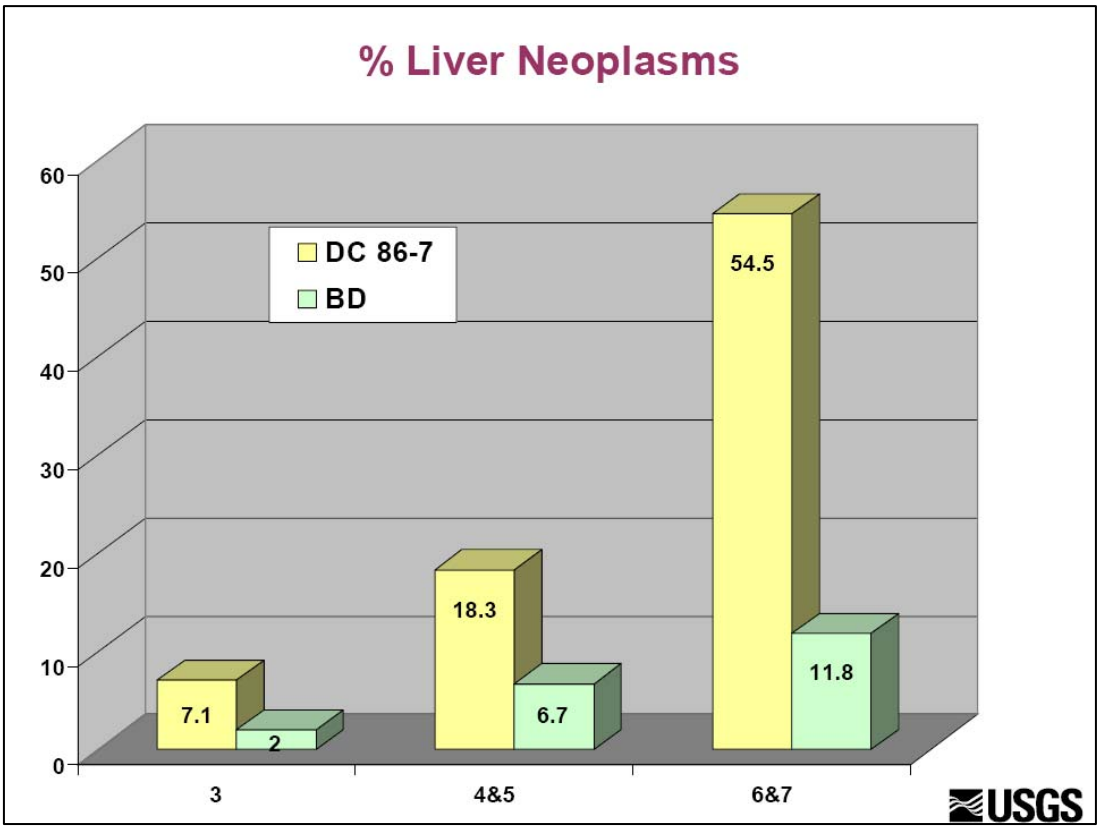
18.3
54.5
3
4&5
6&7
USGS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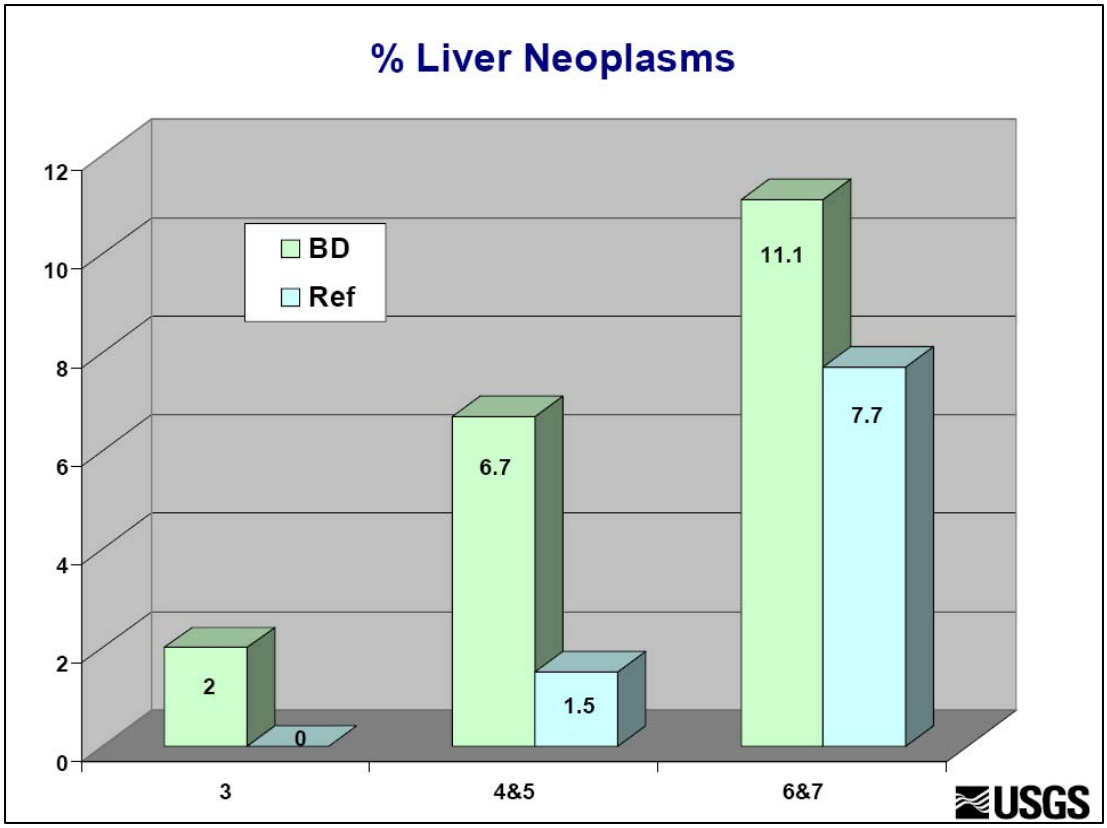
USGS


% Liver Neoplasms
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8
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BD
Ref
2
0
6.7
1.5
11.1
7.7
3
4&5
6&7
USGS

Can reference sites and dates be combined?

- Yes, all tumor surveys are since 1970.
- Lake-wide background rates should include multiple sites.
- Need more reference data!!

USGS

Preliminary Criteria: Bullhead Liver Neoplasms

Age Groups:	**3**	**4 & 5**	**6 & 7**
Designation			
OLD	**5% (for 3+)**		
AOR	**2%**	**5%**	**10%**
DEL	**0.5%**	**2%**	**5%**

USGS

Statistical Considerations

- **What level of probability (what # of fish) are needed?**

•At p=0.05, you need 140 fish from each site to see a 5% difference (5% vs 10%)

•At p=0.1, chances are 1 in 10 of being wrong, but less numbers are needed.

USGS

DR. DAVE HUNNICUTT

Gene introgression among catfish

Dave Hunnicutt[1], Margaret Voss[1], and John Cingolani[2]

[1]School of Science, Penn State Erie, The Behrend College, 5091 Station Rd, Erie, PA 16563 [2]School of Forest Resources, Penn State University, 222 Forest Resource Building, University Park, PA 16802

***Abstract*:** Brown bullhead (*Ameiurus nebulosus*) are known to naturally hybridize with closely related black (*Ameiurus melas)* and yellow bullhead (*Ameiurus natalis)* species. The presence of hybrid specimens in studies designed to link sediment contamination with skin carcinogenesis may obscure results. This may in turn influence evaluations of aquatic ecosystem health. Thus, there is a need for a reliable method to detect hybrid bullhead from tissue samples collected in the field. Specimens from each bullhead species were identified using taxonomic keys and descriptions of morphological characteristics. The morphological identifications were compared with a molecular identification technique using nucleotide sequences from a 437 bp region of the mtDNA control region. We found evidence for a single *A. melas* x *A. nebulosus* hybrid out of 11 fish identified as having *A. nebulosus* morphology from Presque Isle Bay, Erie PA. We suggest that future studies linking tumor incidence rates to changes in sediment contaminant load also account for the degree of hybridization within the bioindicator population.

***Presentation*:**

Gene Introgression Among Catfish

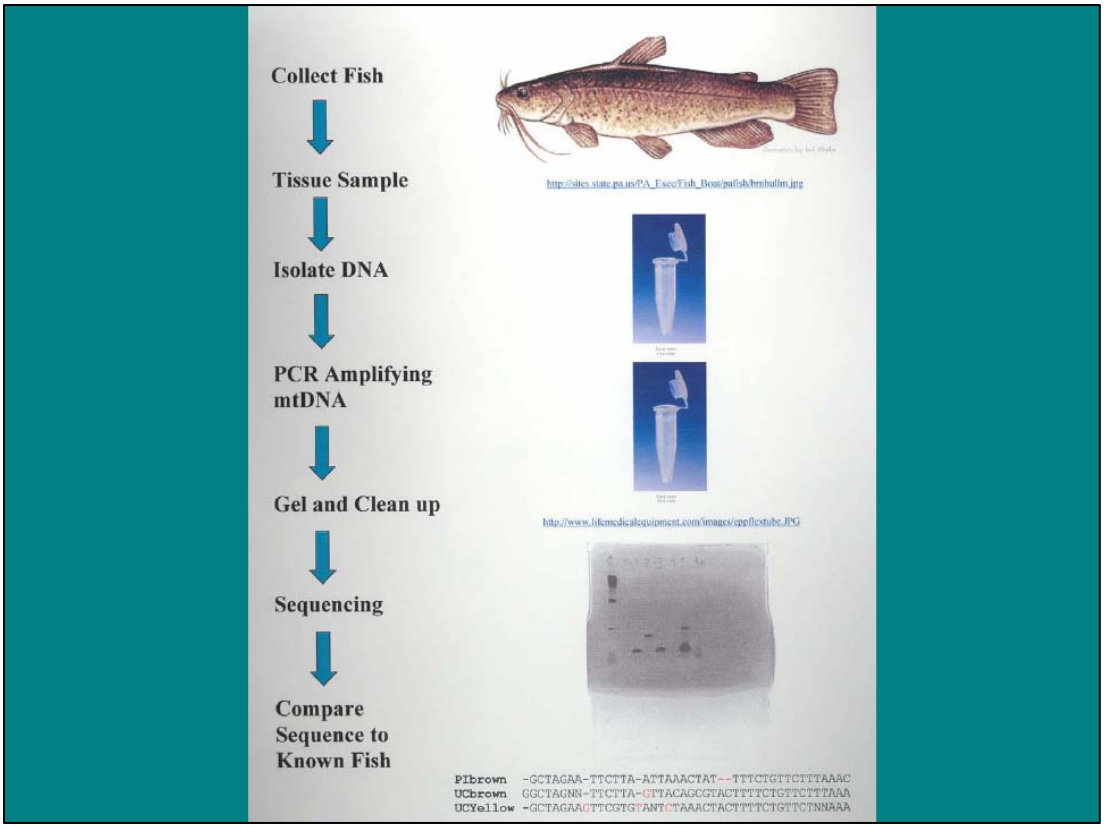
D. Hunnicutt, J. Cingolani, and M. Voss
Penn State Erie, The Behrend College

Brown Bullhead Genetics

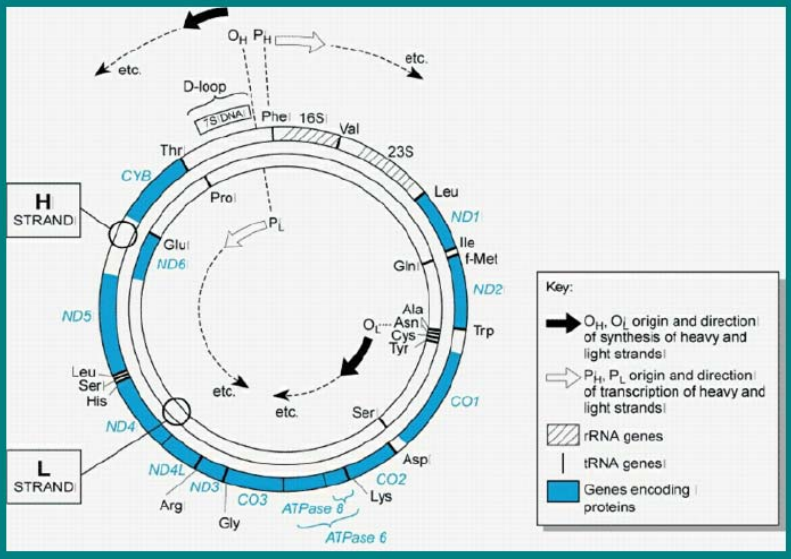
- Brown, black, and yellow bullheads have co-existed in PIB
- These species have been known to hybridize
- Are the brown bullheads in Presque Isle Bay hybrids?

Molecular Approach

- Isolate DNA from tissue samples of *A. nebulosus, A. melas,* and *A. natalis*
- PCR amplify
- Sequence
- Compare sequences obtained from:
  - PI browns
  - Non-PI browns
  - Blacks
  - Yellows
- Compare sequence to morphology



Mt genome

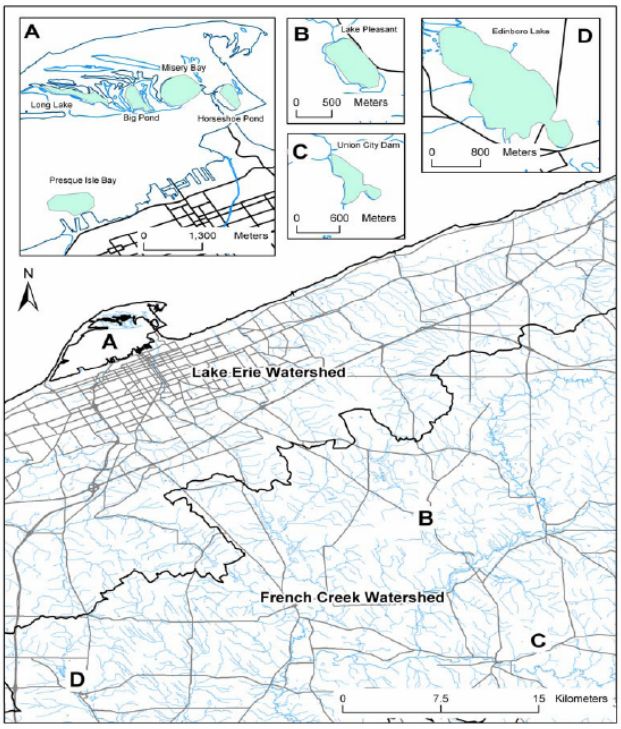


Why mtDNA D-loop?

- Commonly used for species and strain comparisons
- Universal primers available
- D-loop highly variable
  - Allows for distinguishing among close relatives
- Shows direction of hybridization events
  - Maternally inherited

Sources of Fish

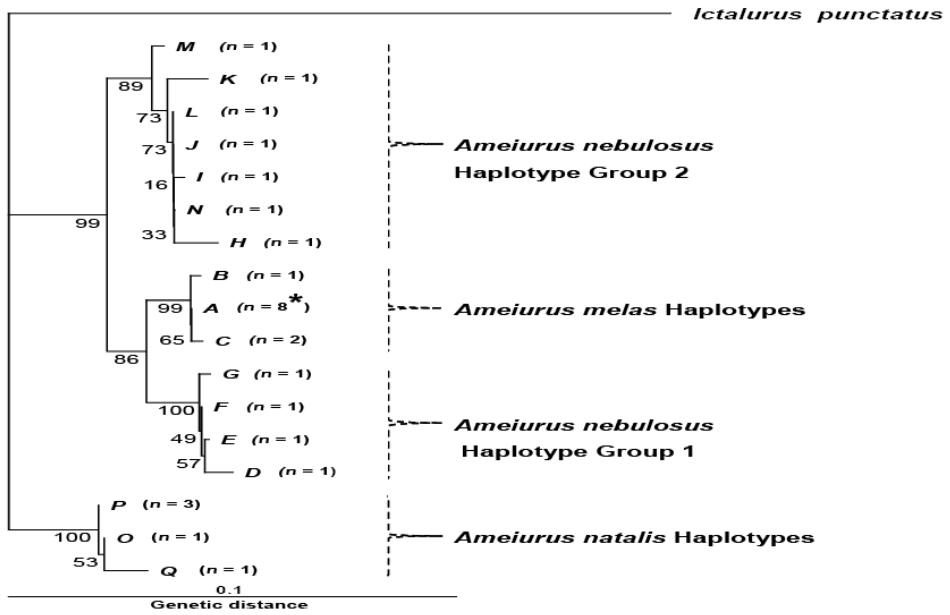
- Sea Grant staff obtained brown and yellow bullheads from
  - Presque Isle Bay
  - Lake Pleasant
  - Union City Dam
  - Edinboro Lake
- Black bullheads obtained from Wisconsin DNR and private farmers in Illinois



Results in General

- Black and yellow bullheads showed little variability
- Brown bullheads showed considerable variability
- One set of browns grouped closer to black bullheads than to the other browns
- One brown bullhead had mtDNA from a black bullhead

mtDNA-based Tree



Position Differences Between Groups

	29	44	85	92	93	97	99	128	133	164	202	204	206	221	226	240	246	271	279	321	323	325	395
Channel	C	C	C	T	C	T	T	C	T	A	G	C	T	T	C	A	C	T	C	C	A	T	T
Yellow	C	C	C	C	C	G	T	C	T	T	G	T	G	T	C	A	C	A	T	C	A	A	G
Brown 2	C	T	T	C	T	A	A	C	T	A	G	A	G	C	T	C	T	T	C	C	C	T	G
Brown 1	C	T	T	T	C	A	A	C	C	A	G	A	A	T	C	T	T	T	T	C	A	C	A
Black	T	T	T	C	T	A	A	T	C	A	A	A	A	T	C	T	T	T	C	T	A	C	A

Conclusions

- mtDNA suggests the possibility of brown-black hybridization
  - Some brown bullheads may have black bullhead mothers
- The pure browns have a *Sca* I site that is missing from the potential hybrids and the blacks
  - This should allow a “quick” check for hybrids
- Microsatellite analysis would be a good confirmation of these results

Acknowledgements

- PA Sea Grant
- Bob Wellington
- James Thompson at WI DNR
- Bruce Scott at IL Natural Heritage

JIM GRAZIO

Data from 2004-05 reference studies and inland lakes

Jim Grazio[1]

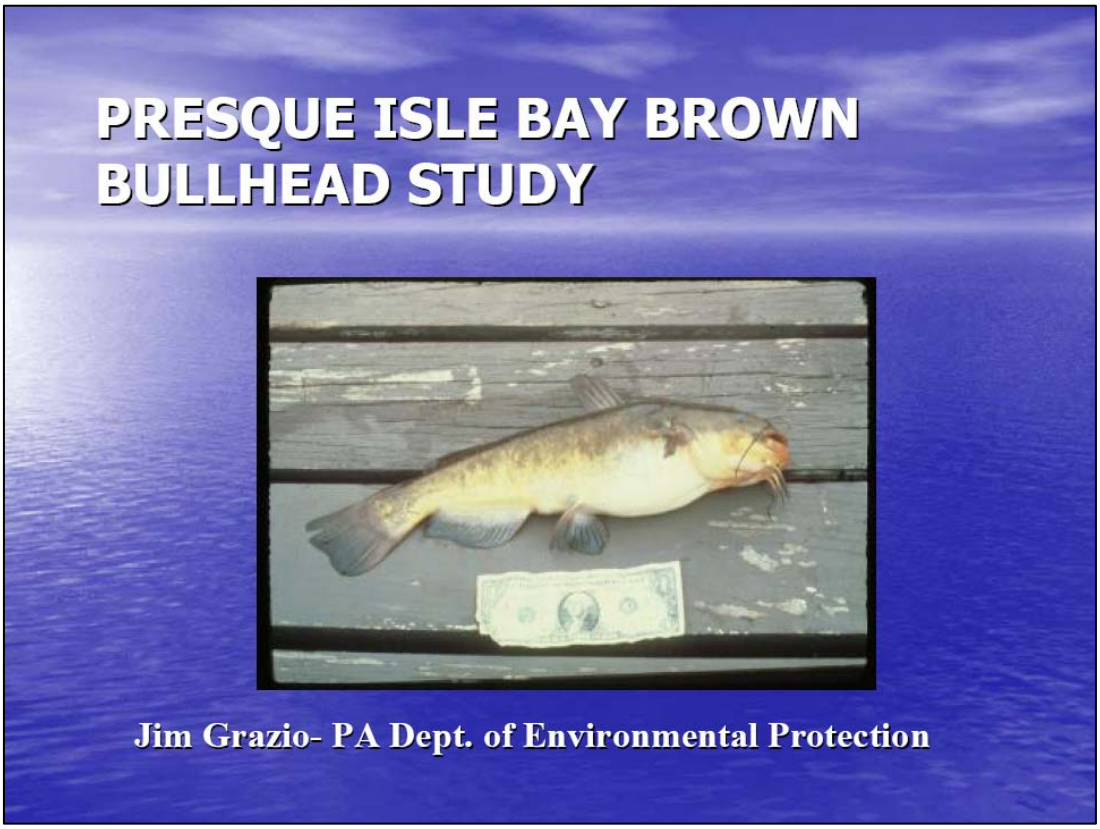
[1]Pennsylvania Department of Environmental Protection, Tom Ridge Environmental Center, 301 Peninsula Dr., Suite 4, Erie, PA 16505

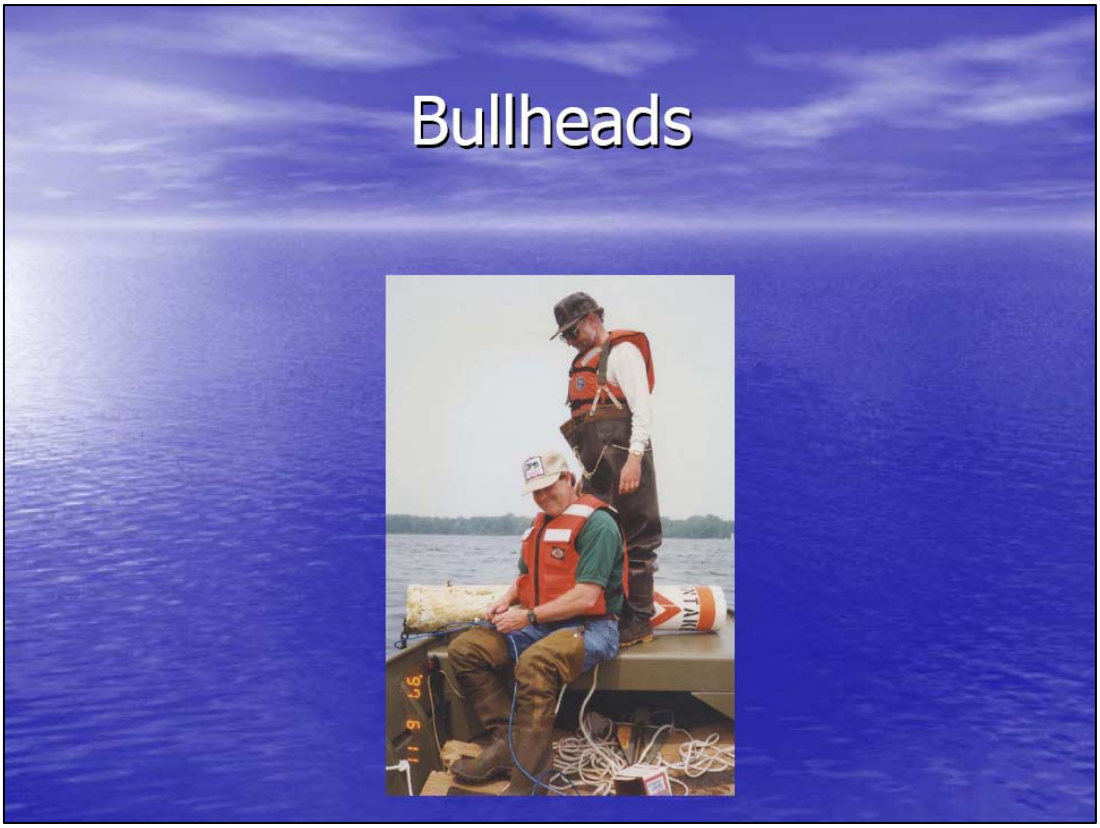
Abstract: Presque Isle Bay (PIB) was deemed to be in a "Recovery Stage" in 2002. The "fish tumors or other deformities" beneficial use impairment (BUI) has been monitored annually in Presque Isle Bay since this point to verify that the trend of decreasing liver and orocutaneous neoplasia in the bay's brown bullhead population has remained stable during the recovery period. The Pennsylvania Department of Environmental Protection also initiated an investigation of brown bullhead neoplasia rates in three inland and five Lake Erie reference sites for the purpose of establishing appropriate delisting targets for this BUI. Gross visual observation data through 2005 and histopathology results through 2004 (i.e., all available data) were reported at the workshop.

Grossly observable raised orocutaneous lesion rates from PIB were compared with rates from the various reference sites. Rates in PIB brown bullhead > 249 mm total length ranged from 38.1% in 2002 to 25.6% in 2005 based on sample sizes ranging from N=215 to N=176, respectively. Sample sizes of brown bullhead >249 mm from reference sites were smaller and displayed more inter-annual variability, ranging from N=1 to N=113 in an inland site (Eaton Reservoir) and N=5 (Old Woman Creek, 2004) to N=47 (Long Point Inner Bay, 2005) in Lake Erie reference sites. In order to reduce variability and increase sample sizes, data from the monitoring period were pooled into three categories: PIB, Inland Reference Site, and Lake Erie Reference Site. Mean grossly observable raised orocutaneous lesion rates were found to be 29.6%, 18.4%, and 11.7%, respectively.

Since neoplasia rates in brown bullhead are known to be positively correlated with specimen age, histopathology results must be considered in this context. The incidence of histopathologically-verified liver neoplasms in brown bullhead from Presque Isle Bay increased from 2.9% in 2002 to 6.0% in 2003 to 19.5% in 2004 based on random subsamples of N=34, 50, and 46, respectively. However, the mean age of the necropsied brown bullhead (based on otolith ages) during this period also increased from 6.1 years in 2002 to 7.0 years in 2003 to 8.3 years in 2004. Liver neoplasia rates from bullhead collected from inland sites ranged from 0% during the 2002-2004 inland lake monitoring period in one site (Canadohta Lake) to 0%, 10.0%, and 16.7% during the same period in another site (Eaton Reservoir—a drinking water supply for the Borough on North East). However, the mean ages of bullhead sampled at Eaton Reservoir were the oldest of any site examined, ranging from 11.6 to 13.0 years. Histopathology results for the Lake Erie sites were incomplete as of the date of the workshop. The incidence of liver neoplasia from one Lake Erie reference site was reported as 40.0% in 2004, but qualified as based on a very small sample size (N=5). The incidence of orocutaneous neoplasms in PIB brown bullhead was 26.5% in 2002, 26.0% in 2003, and 37.0% in 2004. Rates in inland reference lakes were considerably lower, ranging from 0-5.9% in Canadohta Lake (mean brown bullhead age 5.4 years) in a given year to 0-16.7% in Eaton Reservoir (mean age 12.3 years).

***Presentation*:**





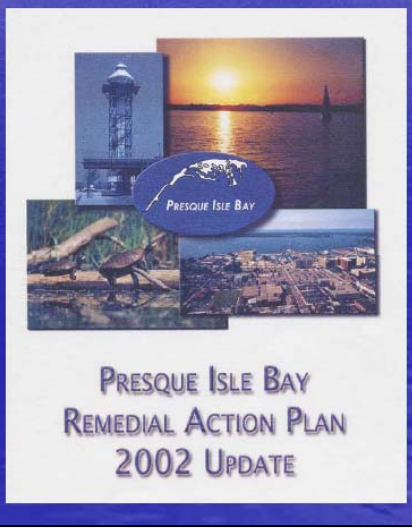
Bullheads

Great Lakes Areas of Concern

- Great Lakes Water Quality Agreement
- Presque Isle Bay AOC
  - 43rd and final AOC
  - Only AOC to be designated based on citizen petition
  - Triggered development of a Remedial Action Plan (RAP)
  - 2 Beneficial Use Impairments:
    - Contaminated Sediments
    - Fish Tumors and other Deformities

2003 Recovery Stage Designation

- First Great Lakes AOC to attain "Recovery Stage" status
- DEP focus shifted from problem identification/remediation to monitoring
- Initiative to identify appropriate delisting targets



IJC Fish Tumor BUI Criteria

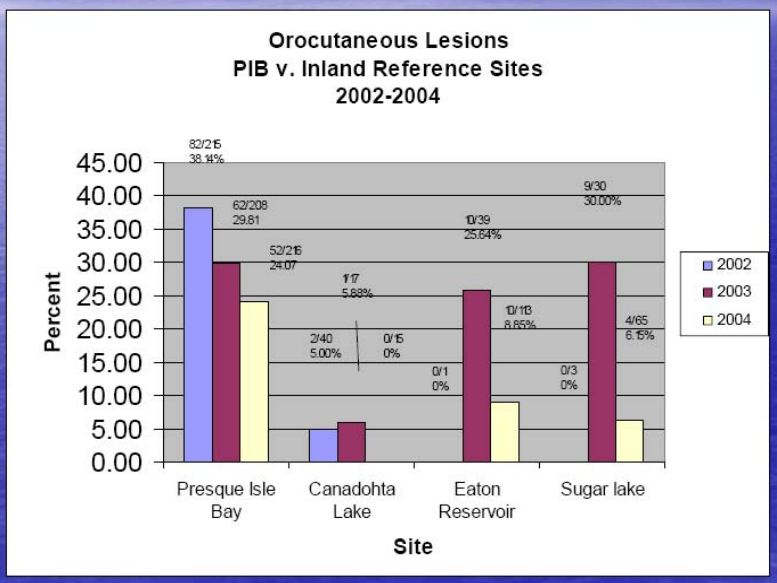
- **LISTING GUIDELINE**
  - When the incidence rates of fish tumors or other deformities exceed rates at unimpacted control sites or when survey data confirm the presence of neoplastic or preneoplastic liver tumors in bullheads or suckers.
- **DELISTING GUIDELINE**
  - When the incidence rates of fish tumors or other deformities do not exceed rates at unimpacted control sites and when survey data confirm the absence of neoplastic or preneoplastic liver tumors in bullheads or suckers.

Initial focus on inland reference lakes

- 3 Primary Inland PA Reference Sites
  - PIB v.
    - Canadohta Lake
    - Eaton Reservoir
    - Sugar Lake

Gross Observations!

Orocutaneous Lesions-Inland



2004-2005 Lake Erie Reference Study

- How clean is clean?
  - Baseline brown bullhead tumor rate in Great Lakes is > 0%!
  - Inland Lakes may not be appropriate reference sites

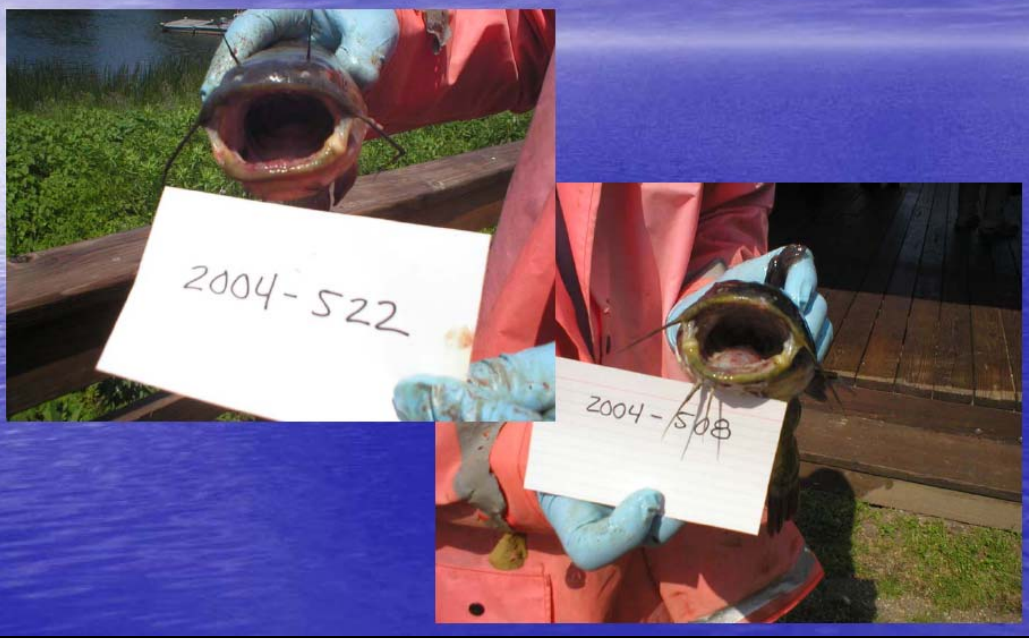
Dunkirk Harbor, New York

Huron River, Ohio

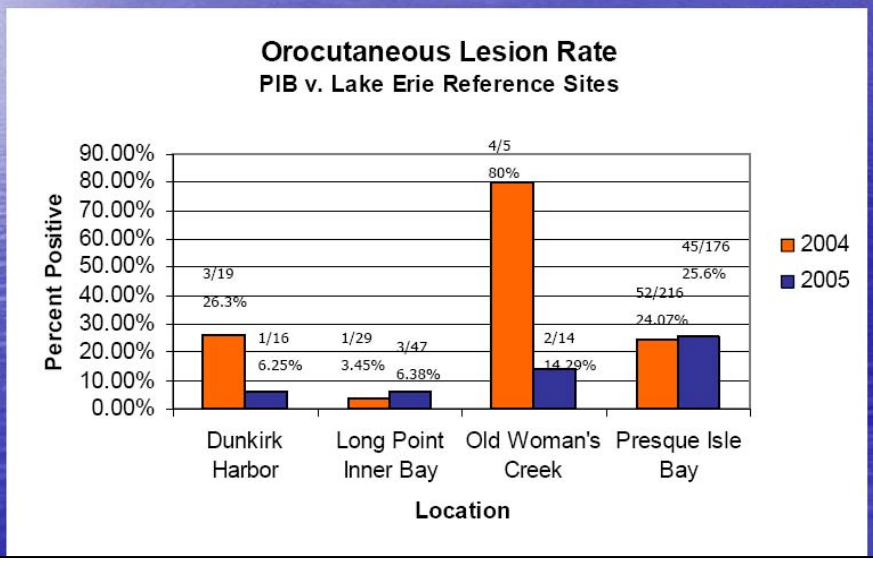
Old Woman Creek, Ohio

Long Point, Ontario

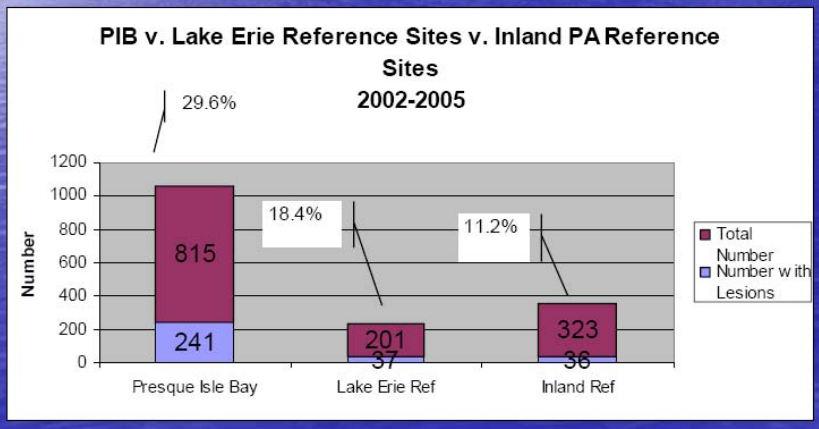
2004 OWC Fish



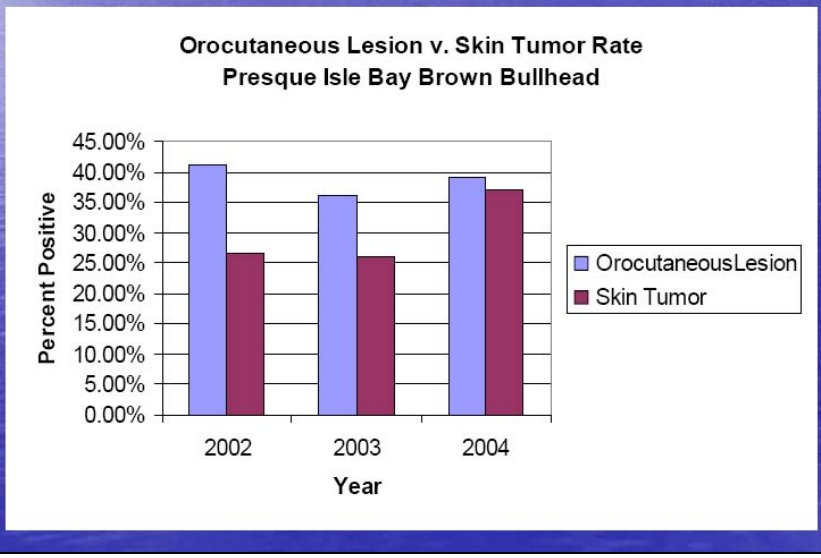
Lake Erie Reference Site Lesion Rates



Pooled Orocutaneous Lesion Rates 2002-2005



Comparison of Gross Observations to Histopathology Results on Necropsied bullhead form PIB

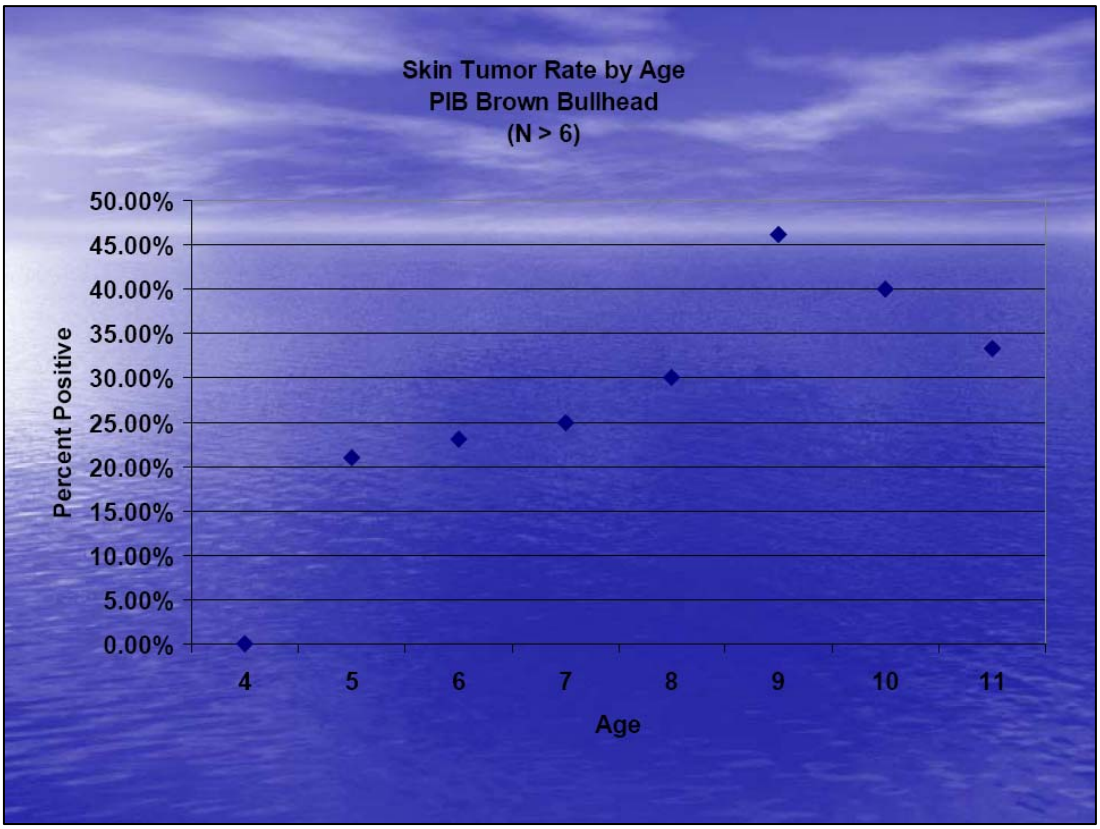


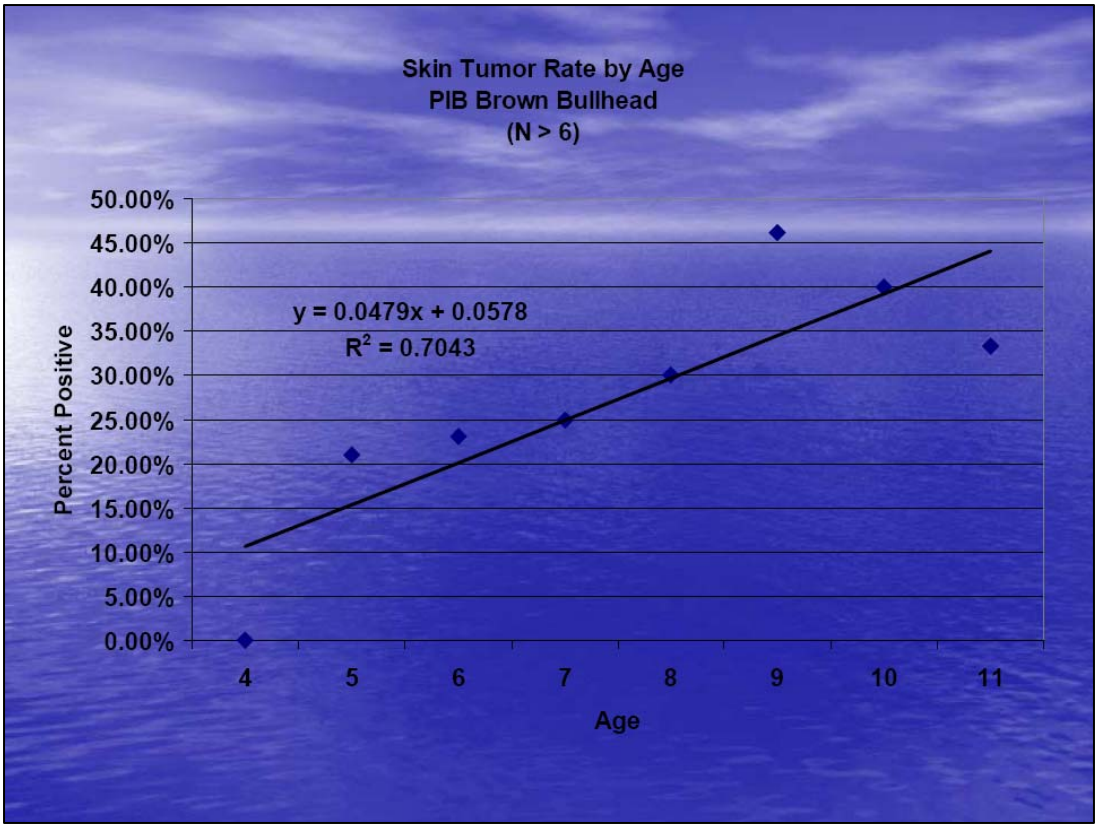
Conclusions

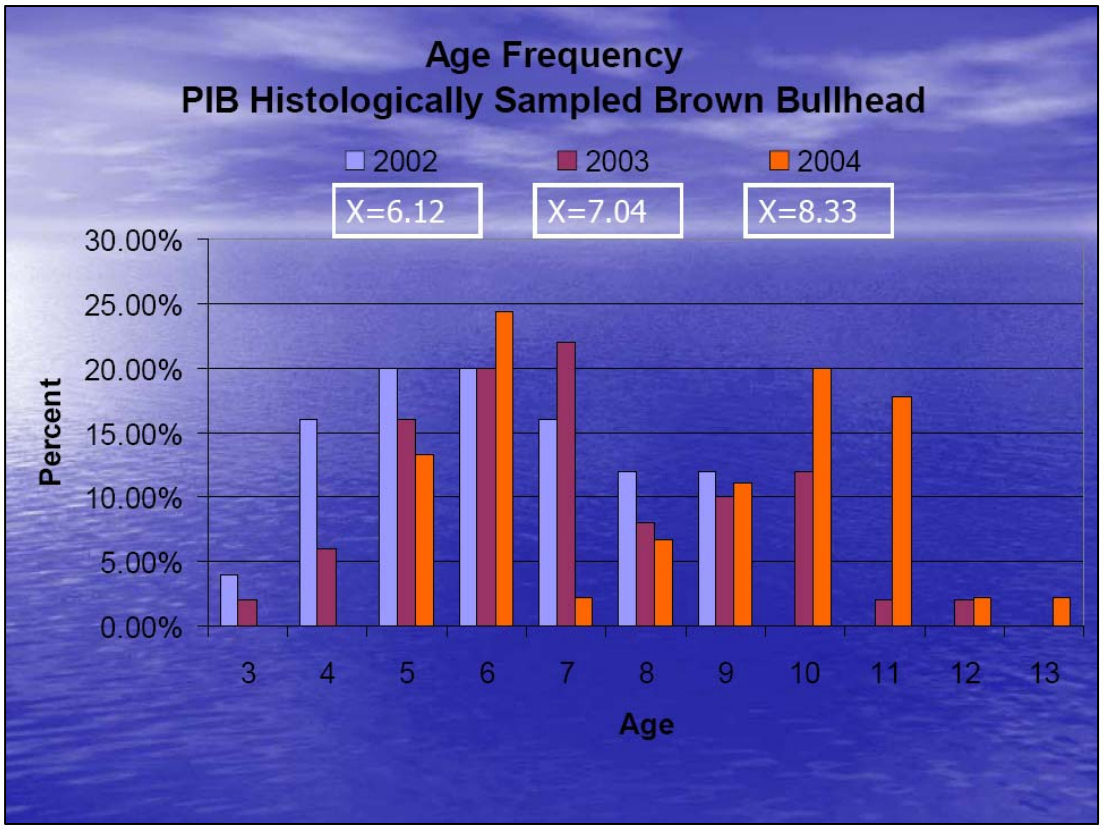
- Inland Reference Sites:
  - 323 Brown Bullhead were collected from 2002 through 2004 from 3 lakes
  - Gross External Lesion Rates ranged from 0% to 30% in any given year
  - Some Sample Sizes were very small
    - Canadohta Lake averaged 4.17%
    - Eaton Reservoir averaged 13.07%
    - Sugar lake averaged 13.27%
  - When combined, the grossly observable external lesion rate in inland lakes was 11.15%

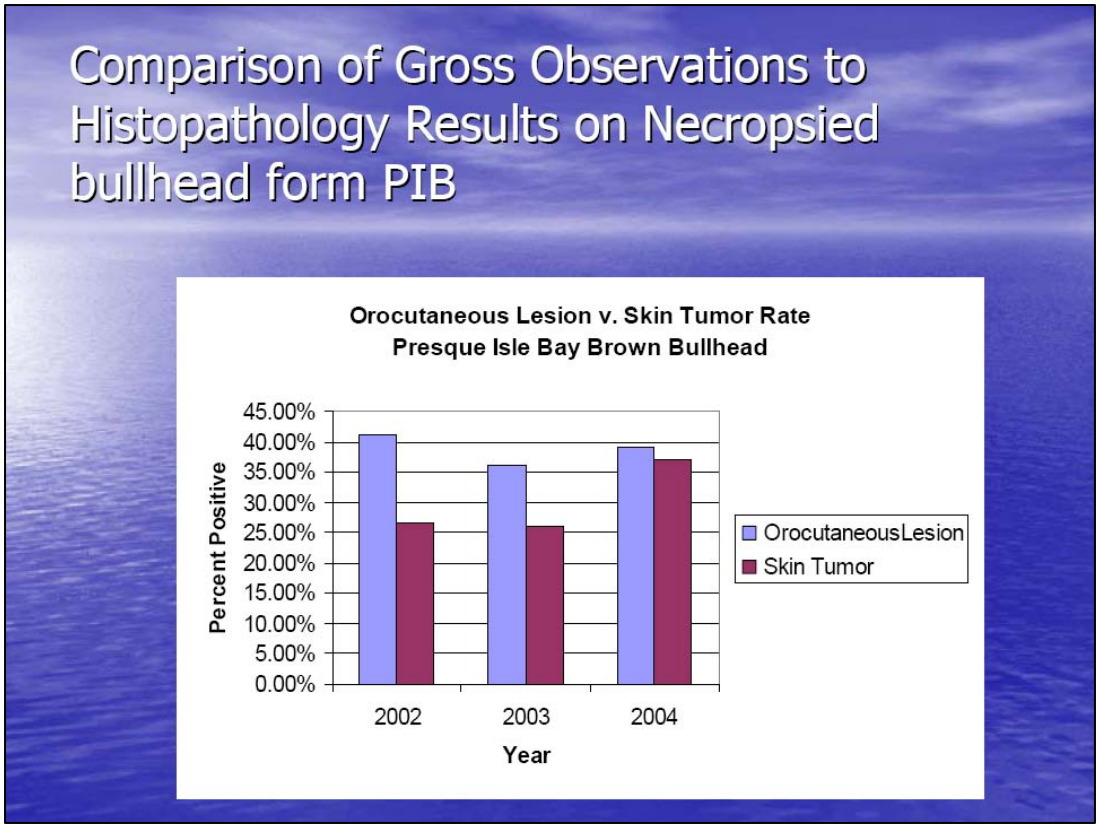
Conclusions

- Lake Erie Reference Sites:
  - 201 Brown Bullhead were collected from 2002 through 2005 from 5 reference sites (Elk Creek, PA; Dunkirk, NY; Old Woman's Creek, OH; Sandusky OH; Long Point, Ontario)
  - Gross External Lesion Rates ranged from 6.25% to 80% in any given year
  - Some Sample Sizes were very small-Notably Elk Cr. And OWC
    - Elk Creek averaged 38.5%
    - Dunkirk Harbor averaged 17.14%
    - OWC averaged 31.58%
    - Long Point averaged 5.26%
    - Sandusky Bay averaged 27.59% (Sampled in 2005 Only)
  - When combined, the grossly observable external lesion rate in Lake Erie reference sited was 18.41%

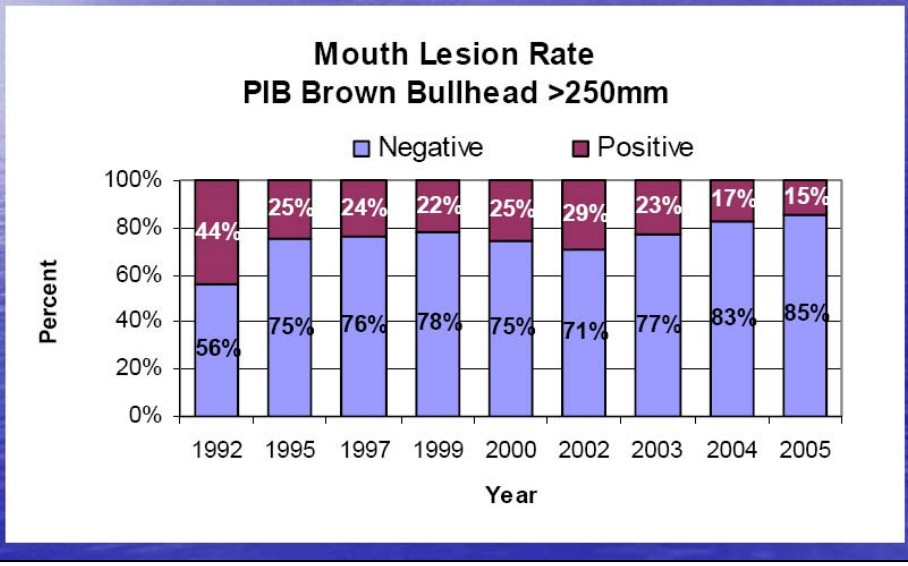

Skin Tumor Rate by Age
PIB Brown Bullhead
(N > 6)
Percent Positive
50.00%
45.00%
40.00%
35.00%
30.00%
25.00%
20.00%
15.00%
10.00%
5.00%
0.00%
4
5
6
7
8
9
10
11
Age


Skin Tumor Rate by Age
PIB Brown Bullhead
(N > 6)
y = 0.0479x + 0.0578
R2 = 0.7043
Percent Positive
50.00%
45.00%
40.00%
35.00%
30.00%
25.00%
20.00%
15.00%
10.00%
5.00%
0.00%
4
5
6
7
8
9
10
11
Age

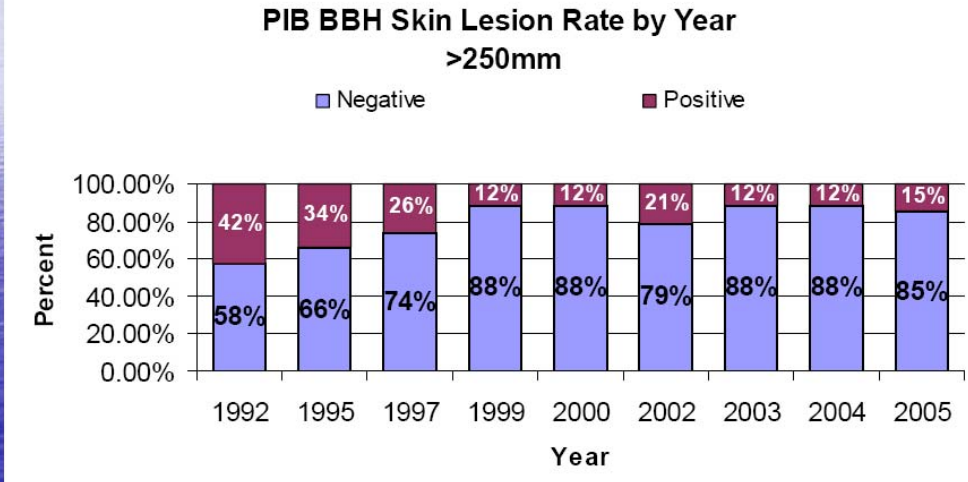

Age Frequency
PIB Histologically Sampled Brown Bullhead
2002
2003
2004
X=6.12
X=7.04
X=8.33
30.00%
25.00%
20.00%
15.00%
10.00%
5.00%
0.00%
Percent
3
4
5
6
7
8
9
10
11
12
13
Age


Comparison of Gross Observations to Histopathology Results on Necropsied bullhead form PIB
Orocutaneous Lesion v. Skin Tumor Rate
Presque Isle Bay Brown Bullhead
45.00%
40.00%
35.00%
30.00%
25.00%
20.00%
15.00%
10.00%
5.00%
0.00%
Percent Positive
2002
2003
2004
Year
OrocutaneousLesion
Skin Tumor

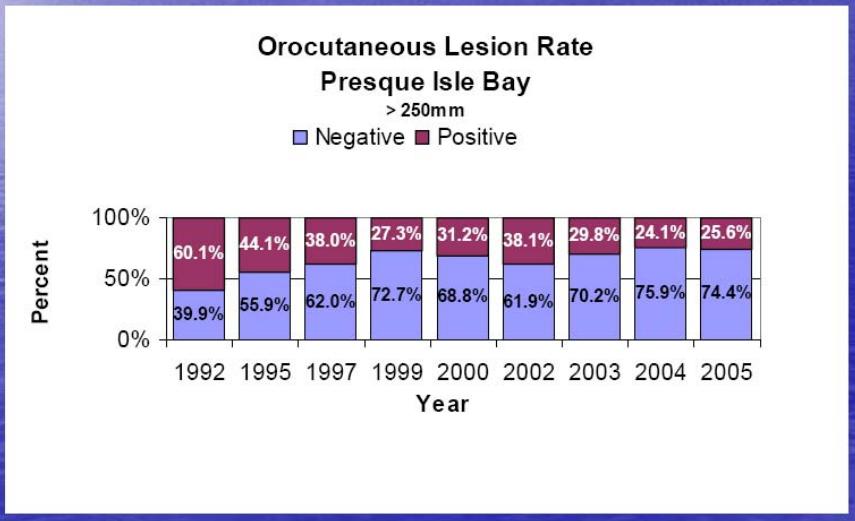
PIB Mouth Lesion Trends



PIB Skin Lesion Trends



PIB Orocutaneous Lesion Trends



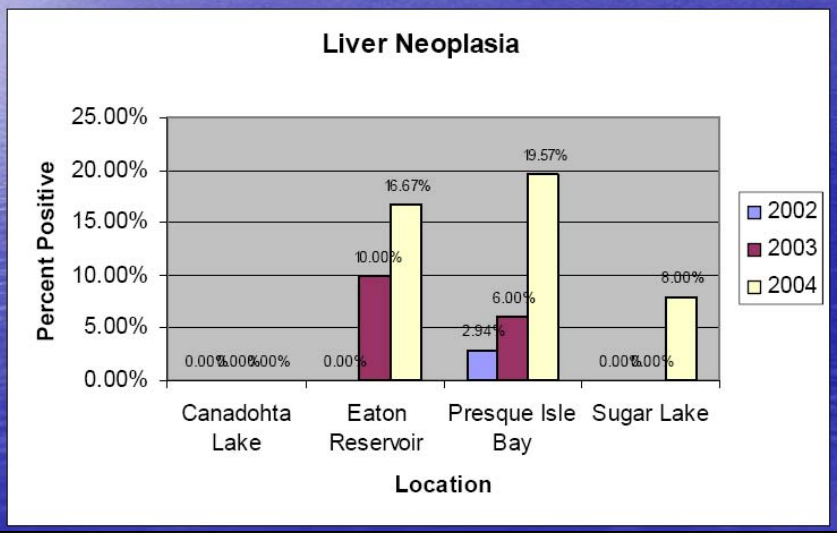
Conclusions

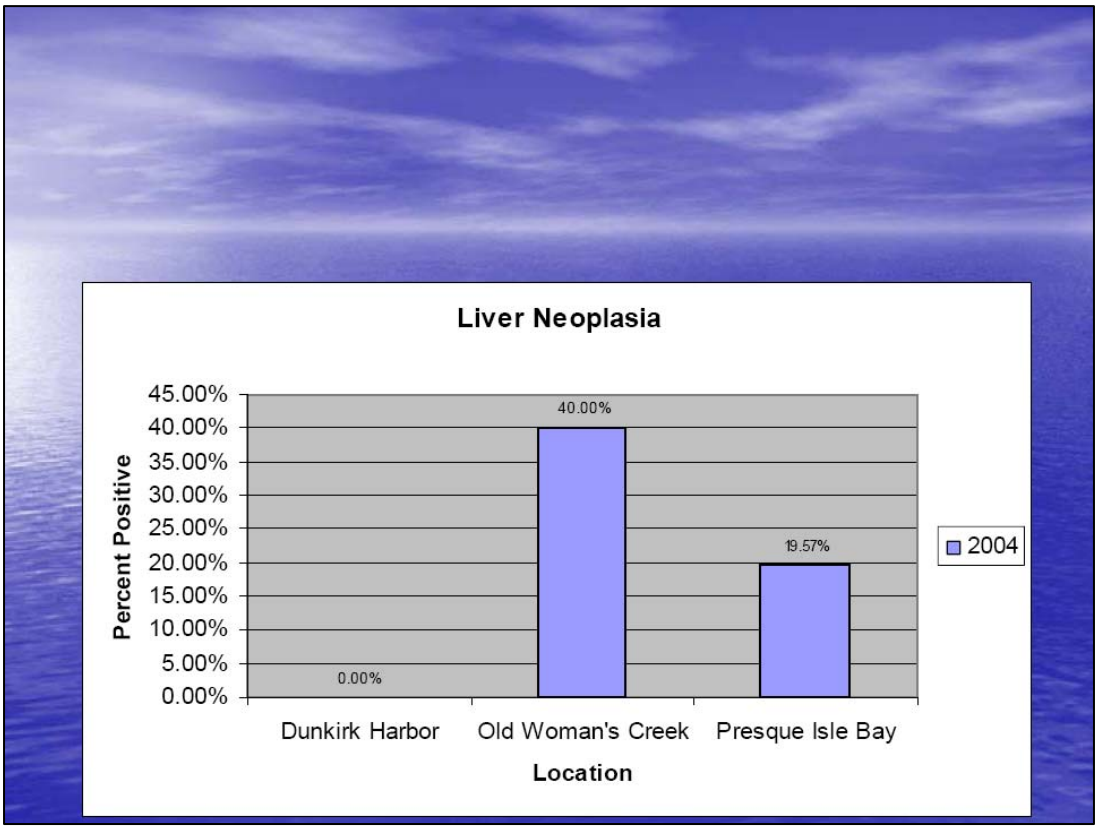
- Presque Isle Bay Grossly Observable Lesion Rates during the 2002-2005 Monitoring Period:
  - 815 Brown Bullhead were collected from 2002 through 2005 from Presque Isle Bay (in addition to roughly 3400 previously collected!)
  - Gross External Lesion Rates ranged from 24.07% to 38.14% in any given year
  - During the Recovery Stage, the average grossly observable external lesion rate in Presque Isle Bay was 29.57%
  - Specimen age must be considered when setting delisting targets

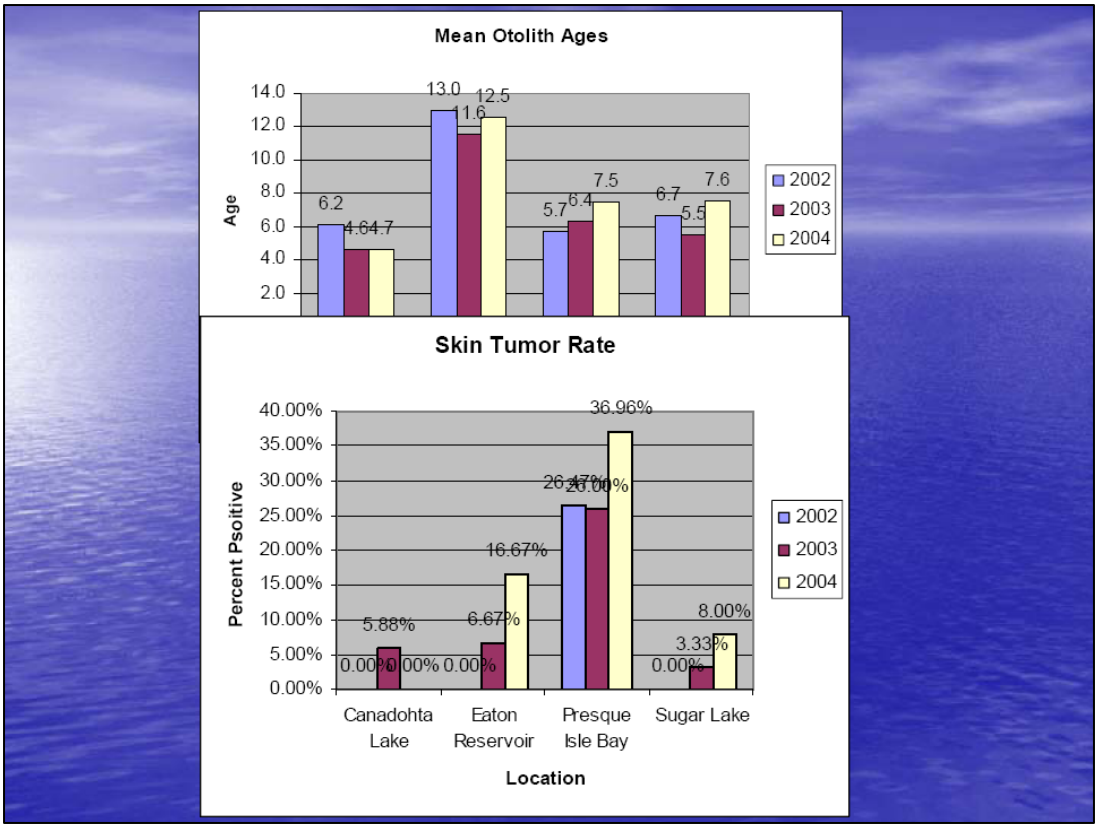
Acknowledgements

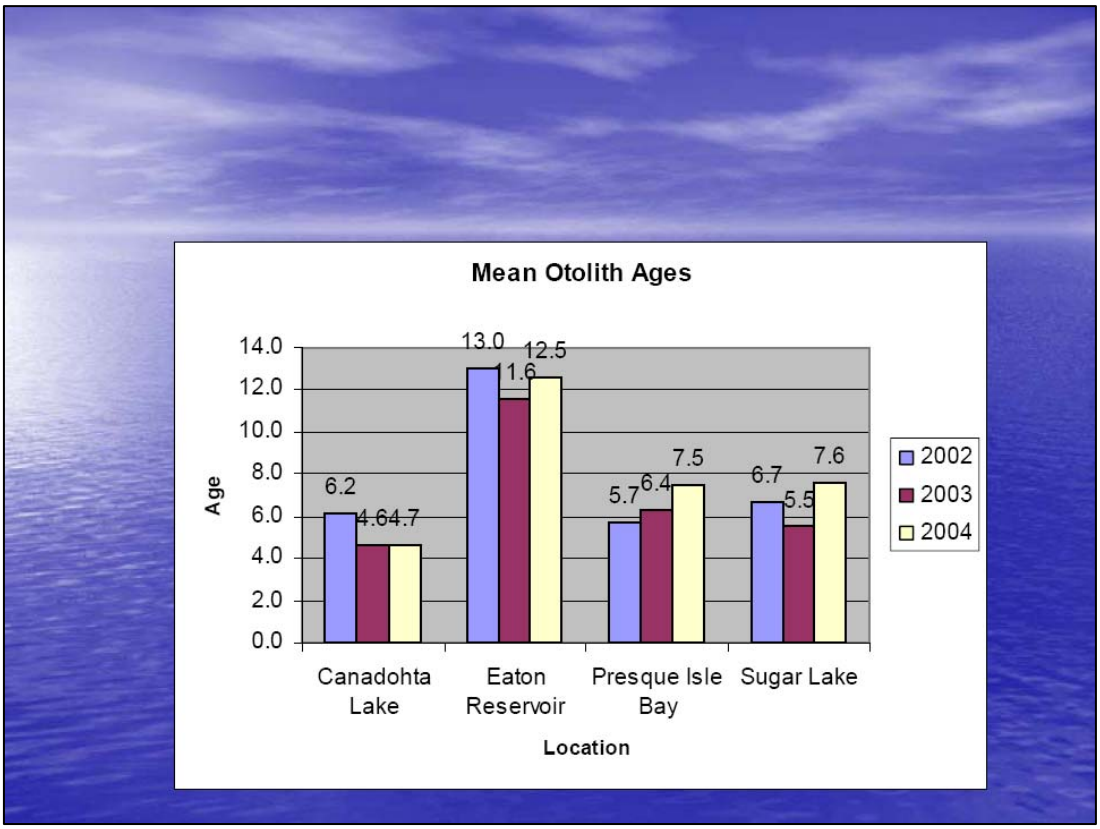
- Heartfelt thanks to Judy Taylor for going above and beyond the call of duty (once again) to get all of our bullhead field data into the computer
- Special thanks to Chuck Murray for spending much time with little notice to help get it back out
- And sincere thanks to Sean Rafferty, Bob Wellington, Judy Taylor (again!) and many others for their invaluable assistance in the collection of ***1339*** brown bullhead since 2002.

Histopathology Results




Liver Neoplasia
Percent Positive
45.00%
40.00%
35.00%
30.00%
25.00%
20.00%
15.00%
10.00%
5.00%
0.00%
40.00%
19.57%
0.00%
2004
Dunkirk Harbor
Old Woman's Creek
Presque Isle Bay
Location


Mean Otolith Ages
Age
14.0
12.0
10.0
8.0
6.0
4.0
2.0
6.2
4.6
4.7
13.0
11.6
12.5
5.7
6.4
7.5
6.7
5.5
7.6
2002
2003
2004
Skin Tumor Rate
Percent Psoitive
40.00%
35.00%
30.00%
25.00%
20.00%
15.00%
10.00%
5.00%
0.00%
5.88%
0.00%
0.00%
6.67%
16.67%
0.00%
26.47%
26.00%
36.96%
3.33%
8.00%
2002
2003
2004
Canadohta Lake
Eaton Reservoir
Presque Isle Bay
Sugar Lake
Location


Mean Otolith Ages
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
Age
6.2
4.6
4.7
13.0
11.6
12.5
5.7
6.4
7.5
6.7
5.5
7.6
2002
2003
2004
Canadohta Lake
Eaton Reservoir
Presque Isle Bay
Sugar Lake
Location

DR. VICKI BLAZER

Presque Isle Bay brown bullhead study microscopic findings

Vicki S. Blazer[1]

[1]National Fish Health Research Laboratory, U.S. Geological Survey, 11649 Leetown Road, Kearneysville, WV 25430

Abstract: We have examined brown bullhead from Presque Isle Bay, other Areas of Concern, and selected reference sites around Lake Erie since 1998. The following prevalence of preneoplastic and neoplastic lesions has been documented at Presque Isle Bay:

Year	Sample Size	Altered Foci	Liver Neoplasia	Skin Neoplasia
1998	42	19.0	7.1	No sample
2002	34	17.6	2.9	23.5
2003	51	25.5	5.9	27.5
2004	47	19.0	19.0	36.0
2005	46	17.4	2.2	23.9

Foci of cellular alteration, a preneoplastic lesion has not changed significantly over the eight-year period. Liver neoplasia has fluctuated with a high in 2004. The prevalence of skin neoplasia was also highest in 2004. There have been some differences among years in terms of site of collection within the bay that may partially explain these differences. Age has also been recognized as an important factor in neoplasia incidence. In 2004 the mean age was 8.3 and no 3 or 4 year olds were examined. In 2005 the mean age was 6.2 and there were a few 3 and 4 year olds. The majority of liver tumors are of bile duct origin. A myxosporidian parasite, within the bile duct lumens, is observed and appears to have increased over the years. In 1998 very few parasites were noted and bile duct proliferation was minimal (9/42 or 21.4%) while in 2005 there was a higher prevalence of bullhead with the parasite and more parasites were noted in those infected. Concurrently, much higher prevalence of bile duct proliferation (24/46 or 52.2%) was observed. This raises the question of a possible role for the parasite in proliferation and perhaps neoplastic changes.

***Presentation*:**

Presque Isle Bay
Percent of Fish with Specific Lesions

Year	Total # of fish	Altered Foci	Liver Neoplasia	Skin Neoplasia
1998	42	19.0	7.1	No samples
2002	34	17.6	2.9	23.5
2003	51	25.5	5.9	27.5
2004	47	19.0	19.0	36.0
2005	46	17.4	2.2	23.9

Collection Locations

- 2002 - Lagoons (34)
- 2003 - Lagoons (51)
- 2004 - Lagoons (7) and Graveyard Pond (40)
- 2005 – Lagoons (23), Misery Bay (14), Sara's Cove (6)

Collection Sites/Age

- 2004 – mean age 8.3 – no 3 or 4 year olds
- 2005 – mean age 6.2 – a few 3 and 4 year olds

- Same problem with reference sites
- 2004 – Old Woman Creek – mean age 3.6
  – 73.3% were 2 yrs with a few old fish
- 2005 – Old Woman Creek – mean age 3.5
  – 86 % were 3 yrs with a few old fish

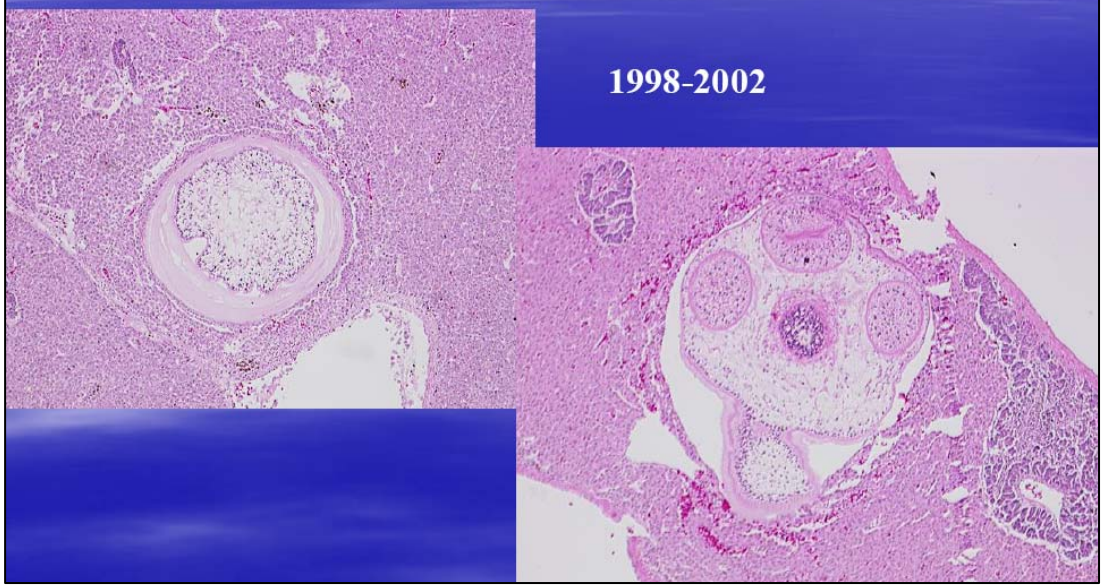
Changes Over Time Parasites

- From 1998 to 2005 there has been a significant increase in liver parasite loads and the response to them
- Bile duct myxosporidian parasite
- Helminth parasites, primarily immature cestodes, within the liver parenchyma

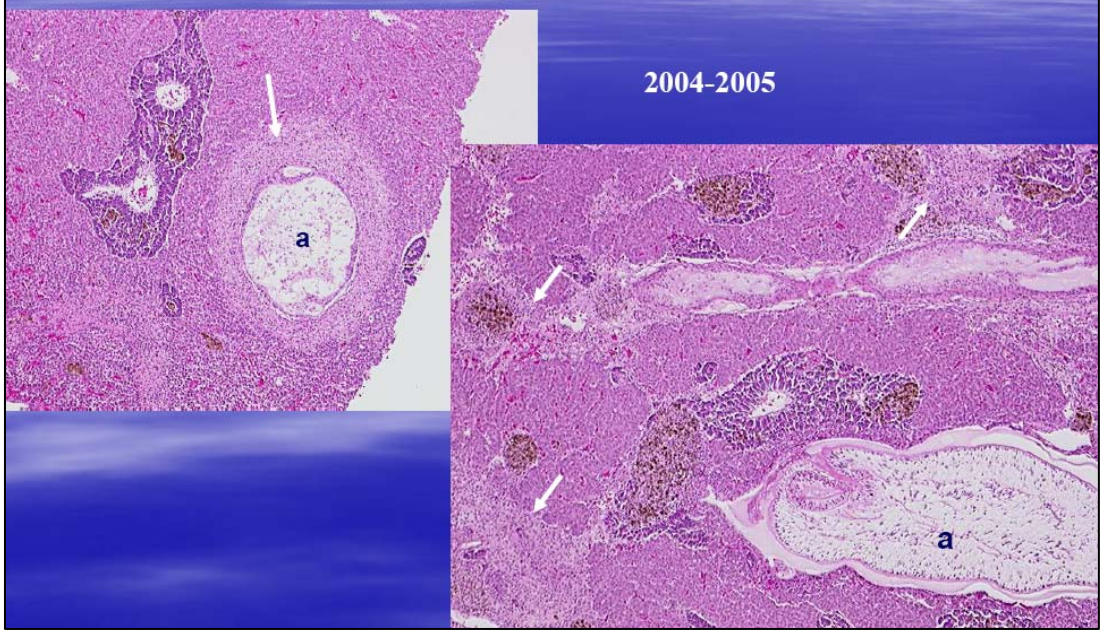
Cestode Infections

- Increased prevalence
  - 5/42 or 11.9% in 1998
  - 34/46 or 73.9% in 2005
- More severe and proliferative inflammatory reaction

Helminth Parasites



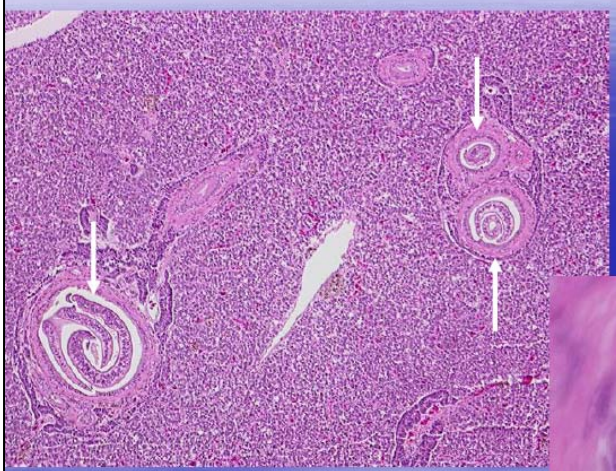
Helminth Parasites



Bile Duct Myxosporidian

- In 1998 very few parasites were noted and bile duct proliferation was minimal
  - 9/42 or 21.4%
- In 2005 there was a higher prevalence of bullheads with the parasite and more noted in those infected; a much higher prevalence of bile duct proliferation
  - 24/46 or 52.2%

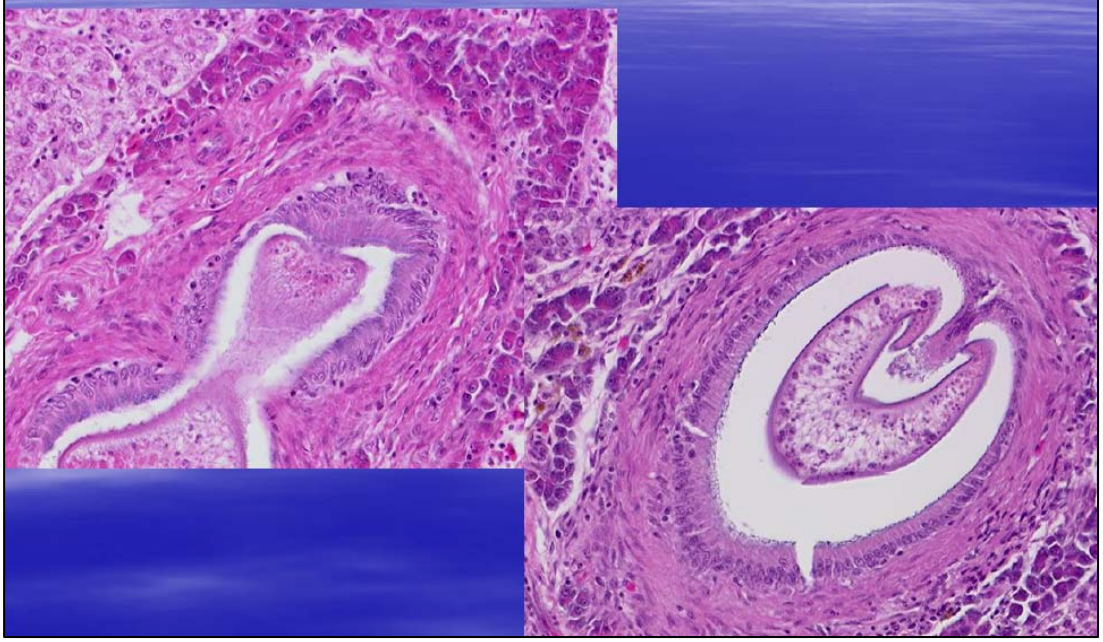
Myxosporidian Parasite



2005

Myxidium sp.?

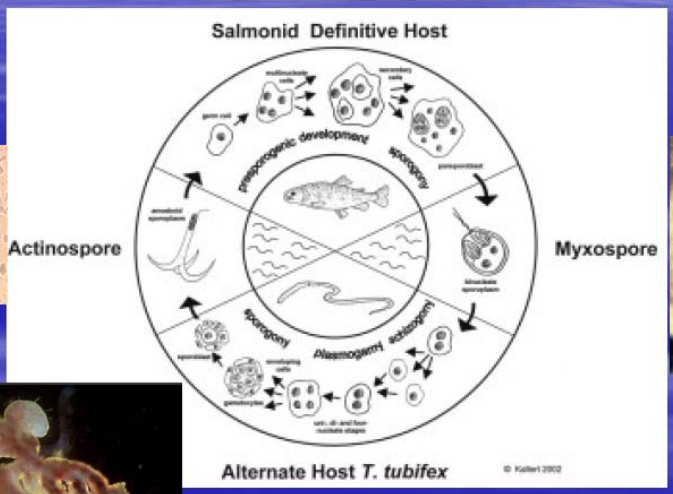
Damage by Myxosporidian Bile Duct Parasite

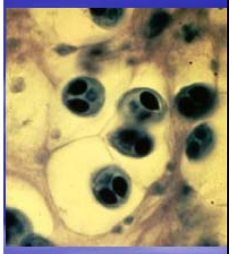


Myxosporean Life Cycles

- Most have not been elucidated
- Most probably have complex life cycles that include a fish host, an invertebrate intermediate host (oligochaetes, polychaetes) and two different types of spores

Whirling Disease Life Cycle





Bile Duct Parasite

- Raises the questions:
  - Has an improved benthic environment or increased nutrient loads allowed for increased numbers of the intermediate host and hence increasing prevalence of infection?
  - Are the fish living longer and infection rate increases with age?
  - Could recent biliary neoplasia be related to parasite damage?

Parasites and Neoplasia

- Generalized cell hyperplasia or cellular proliferation is recognized as a causative factor in human liver cancer
- Recognized that carcinogenesis, especially the initiation and promotion stages, may include interactions between a variety of agents – infectious and chemical

Further Research

- Experimental studies on the interaction of parasites, particularly the bile duct myxosporidia and chemical carcinogens in the initiation of bile duct neoplasia

DR. MICHAEL RUTTER

Bayesian analysis of Presque Isle Bay brown bullhead data

Michael A. Rutter[1]

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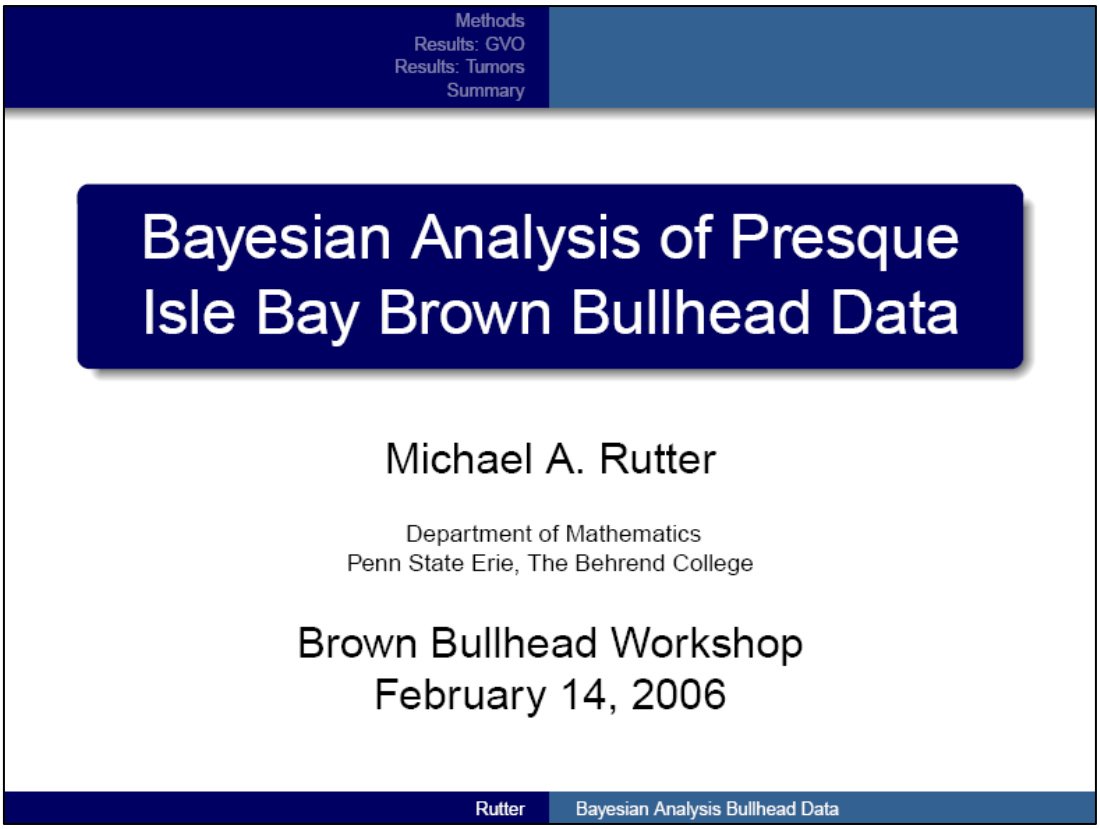
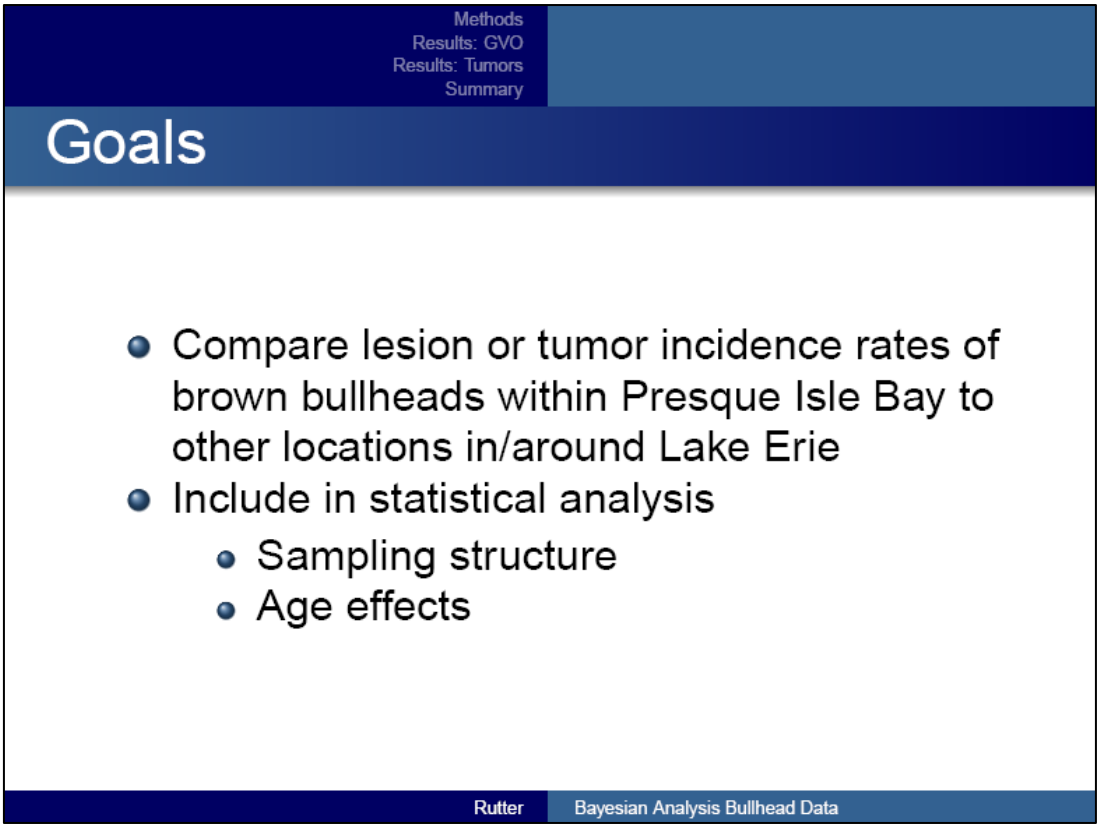
Abstract: Brown bullhead were sampled at sites in Presque Isle Bay (PIB), Lake Erie, and inland lakes. In order to better analyze the liver tumor and skin tumor and lesion data collected, the age or length of the brown bullhead must be accounted for. These covariates were included by using logistic regression to measure incidence rates as a function of age or length. Bayesian statistical techniques were also used to compare incidence rates between areas, and random effects were included to account for multiple sampling locations and dates. Skin lesion rates, based on gross visual observations, were shown to be significantly higher in non-PIB Lake Erie sites than in inland lake sites when fish length was included as a covariate. A similar analysis showed that liver lesion rates in brown bullhead sampled in PIB and Lake Erie sites were virtually identical. Due to data limitations, liver and skin tumor rates could only be compared between PIB and inland lake sites, not other Lake Erie sites. Liver (neoplastic) and skin (orocutaneous) tumor incidence rates were found to be significantly higher in PIB sites than in inland lake sites when age was included as a covariate. Preneoplastic liver tumors were not found to be statistically different between inland lake sites and PIB sites. Point estimates for the probability of an age seven brown bullhead, the average-aged fish in the samples, of having a skin or liver tumor were also given.

***Presentation*:**





Brown Bullhead Data

- Brown bullhead were sampled in three areas
  - Inland sites
  - "Reference" Lake Erie sites
  - Presque Isle Bay sites
- Only fish longer than 250mm are used in analysis
- Fish collected from 1999 to 2005

Brown Bullhead Data continued

- Fish were examined via gross visual observation (GVO) for lesions, then weighed, and measured
- In addition, a subset of these fish were aged and histological tumor information obtained
  - Neoplastic liver tumors (tissue and bile duct)
  - Preneoplastic liver tumors
  - Orocutaneous skin/mouth tumors

Inland Sites

Location	Sampling Year(s)
Canadohta Lake	'02,'03,'04,
Eaton Reservoir	'02,'03,'04,
Sugar Lake	'02,'03,'04

Lake Erie Sites

Location	Sampling Year(s)
Dunkirk Harbor	'04,'05
Elk Creek	'02,'03
Long Point Inner Bay	'04,'05
Old Woman's Creek	'04,'05
Sandusky Bay	'05

Presque Isle Bay Sites

Location	Sampling Year(s)
Lagoons	'99,'00,'*02*,'*03*,'*04*,'05
Sara's Cove	'99,'00,'02,'04,'05
Misery Bay	'99,'00,'04,'05
Duck Pond	'02
Graveyard Pond	'*04*
Horseshoe Pond	'05

Estimating Incidence Rates

- The goal is to estimate lesion or tumor incidence rates as a function of location and possibly a covariate
  - Length
  - Weight
  - Age (if available)
    - Otolith
    - Spine
- Logistic Regression is a standard approach since we have a binomial response

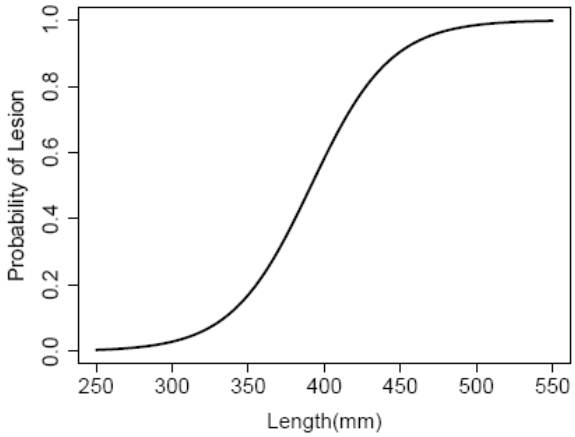
Logistic Regression

The probability (P) of a brown bullhead having a lesion or tumor is given by

$$P = \frac{e^{f(x)}}{1 + e^{f(x)}}$$

where $f(x)$ is a linear function of the covariates of interest

Logistic Regression Continued



Testing Different Areas

To test to see if lesion/tumor incidence rates in two areas are different, let

$$f(x) = \beta_1 x_1 + \beta_2 x_2$$

where x_1 and x_2 are indicator variables corresponding to the sampling areas

Adding Covariates

To allow the lesion/tumor incidence rate to vary as a function of a covariate, length for example, let

$$f(x) = \beta_1 x_1 + \beta_2 x_2 + \beta_l l$$

where l is the length of the brown bullhead

Accounting for Sampling Design

- Most statistical techniques assume all locations have an equal chance of being sampled
- Many fish assessments sample from fixed locations
- Failing to account for fixed sampling locations underestimates variance components
- Sampling date is also an important consideration

Random effects model

Fixed sampling locations can be accounted for by adding a random effect to the model

$$f(x) = \beta_1 x_1 + \beta_2 x_2 + \tau_i + \beta_l l$$

where τ is normally distributed with a mean of zero and variance σ_τ^2 and i indicates each sampling location/year combination

Bayesian Estimation Framework

A Bayesian approach was used to estimate model parameters

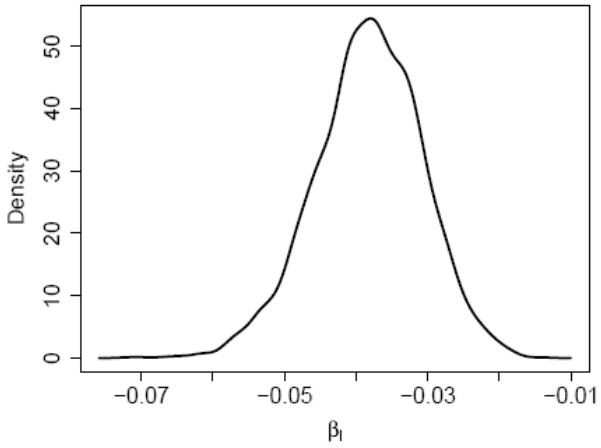
- Easy to compare tumor incidence rates between areas
- Prediction intervals for tumor incidence rates

Compare Lake Erie vs. Inland Sites

- Compare GVO lesion incidence rates on brown bullhead collected at inland lake sites to those collected in "reference" Lake Erie sites
- Allowed lesion incidence rates to vary as a function of brown bullhead length
- Similar results are seen if weight is used as the covariate

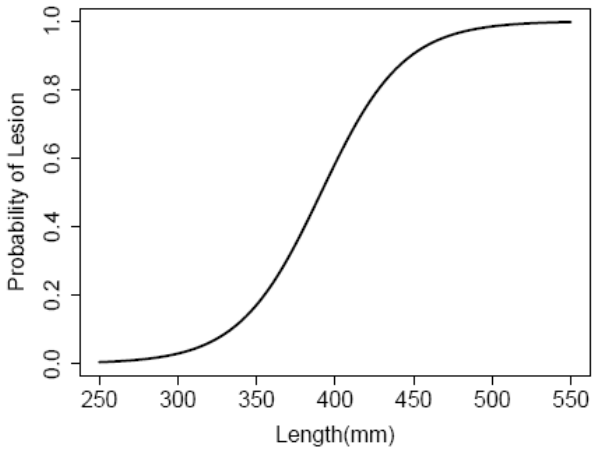
Effect of Length

95% Prediction Interval for β_l: [-0.053,-0.025]



Methods
Results: GVO
Results: Tumors
Summary
Inland vs. Reference Sites
PIB vs. Reference Sites

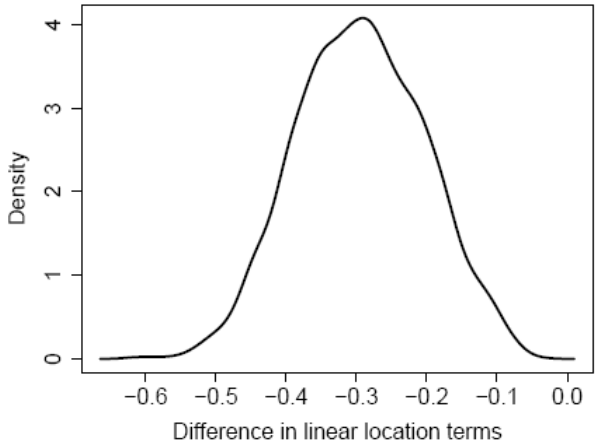
Effect of Length



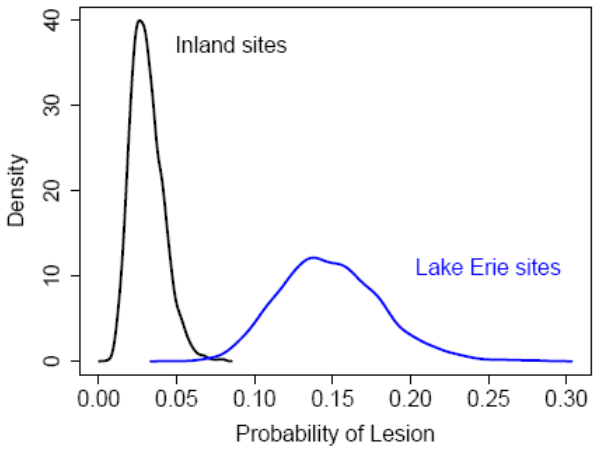
Rutter
Bayesian Analysis Bullhead Data

Difference in Linear Location Terms

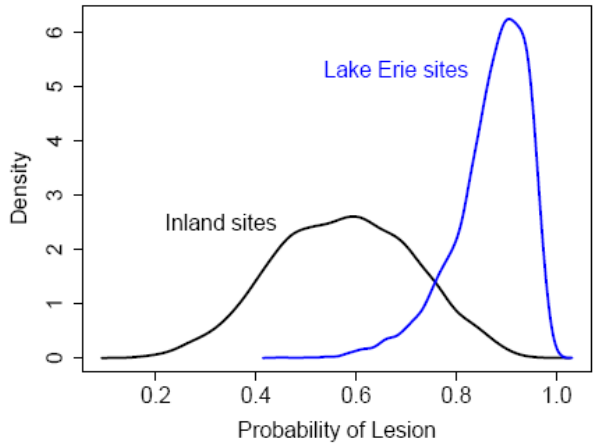
95% Prediction Interval for $\beta_{IL} - \beta_R$: [-0.211,-0.778]



GVO Incidence Rates 300mm



GVO Incidence Rates 400mm



Conclusions

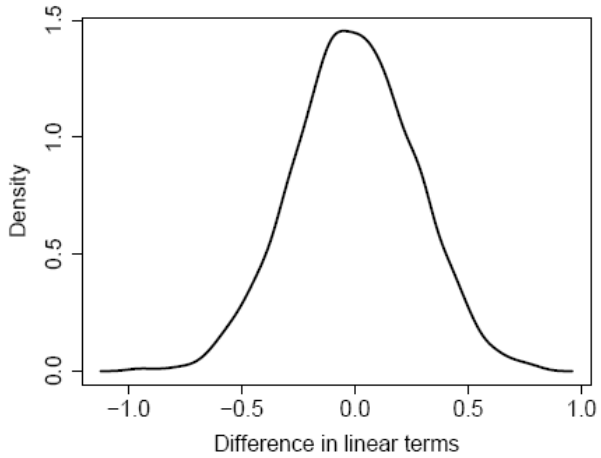
- GVO lesion incidence rates are a function of brown bullhead length
- Lesion incidence rates for inland sites are significantly lower than in "reference" Lake Erie sites
- Only compare Lake Erie data to Presque Isle Bay data

Presque Isle Bay vs. "Reference" Sites

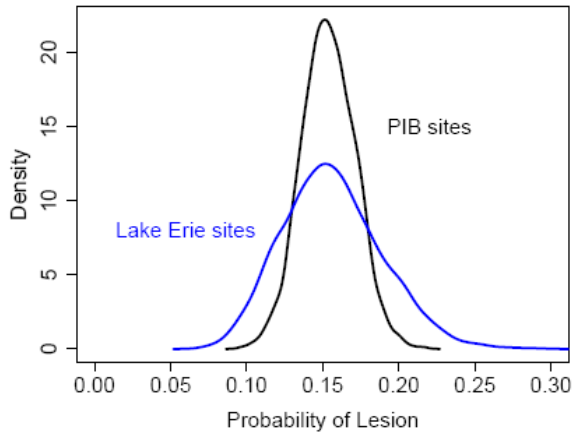
- Compare GVO lesion incidence rates on brown bullhead collected at Presque Isle Bay sites to those collected in "reference" Lake Erie sites
- Allowed lesion incidence rates to vary as a function of brown bullhead length
- Similar results are seen if weight is used as the covariate

Difference in Linear Location Terms

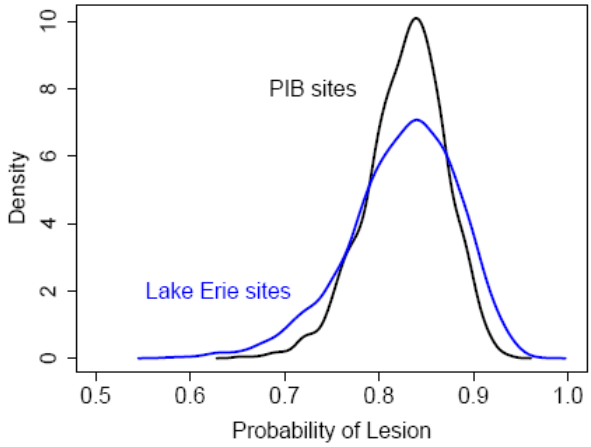
95% Prediction Interval for $\beta_{PIB} - \beta_R$: [-0.528,0.508]



GVO Incidence Rates 300mm



GVO Incidence Rates 400mm



Result Summary

- GVO lesion incidence rates in Presque Isle Bay and "reference" Lake Erie sites are similar
- Point estimates for GVO lesion rate on a 300mm brown bullhead
  - Presque Isle Bay: 15.4%
  - Lake Erie: 15.6%

Tumors and Age

- Goal was to repeat logistic regression analysis using tumors and the covariate age
- Limited "reference" Lake Erie data
  - 26 Observations
  - Few fish had tumors (0-2)
- Define "reference" as inland and non-PIB Lake Erie sites

Tumor Analysis Results: Details

- Examine difference between "reference" and PIB sites via linear location term
- Examine the effect of age on tumor incidence rates
- Compare tumor incidence rates between areas for
  - Age 7 (average age)
  - Age 15

Types of Tumors

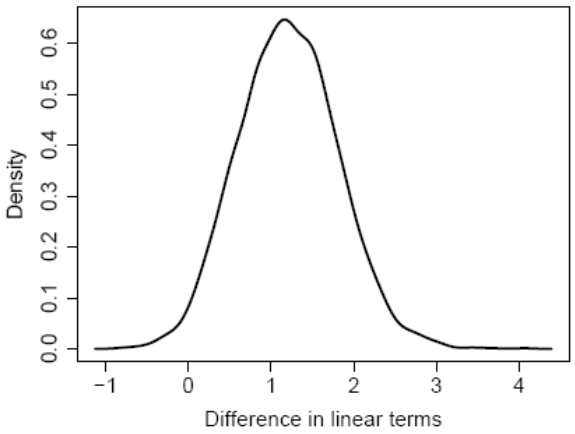
- Neoplastic liver tumors
  - Tissue (hepatic) neoplasia
  - Bile duct neoplasia
- Preneoplastic liver tumors
- Orocutaneous skin/mouth tumors

Sample sizes

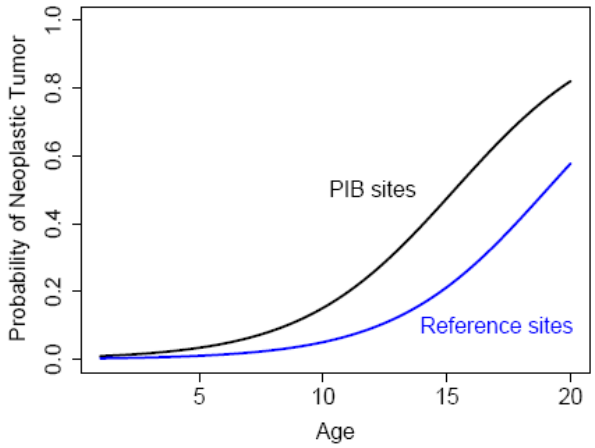
- "Reference" sites
  - 194 brown bullhead
  - 12 Sites
- Presque Isle Bay sites
  - 128 Brown Bullhead
  - 4 Sites

Difference in Linear Location Terms

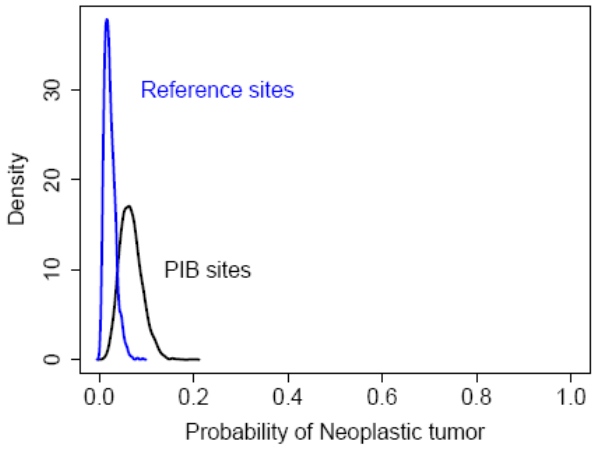
Neoplastic tumors:
95% Prediction
Interval for $\beta_{PIB} - \beta_R$
[0.081,2.389]



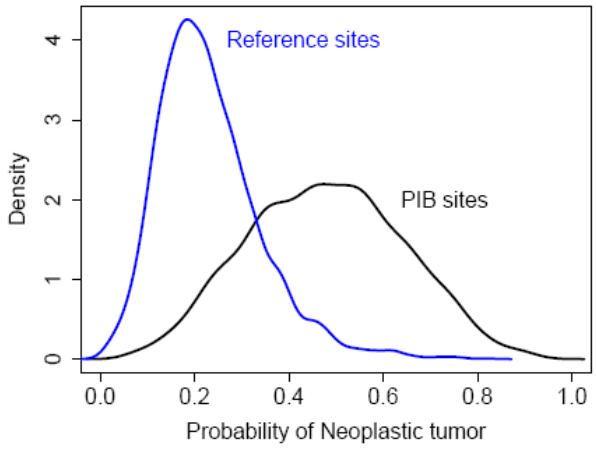
Neoplastic Effect of Age



Neoplastic Tumor Rates: Age 7

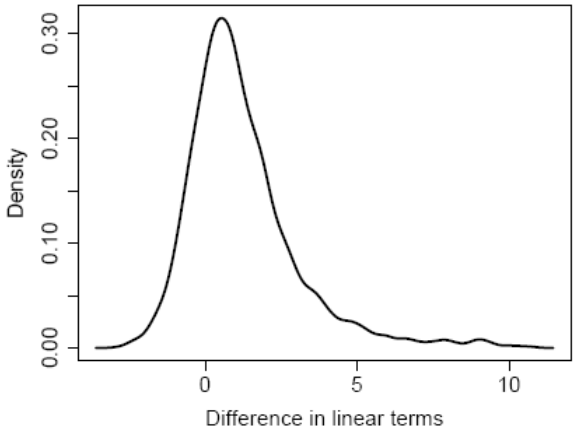


Neoplastic Tumor Rates: Age 15



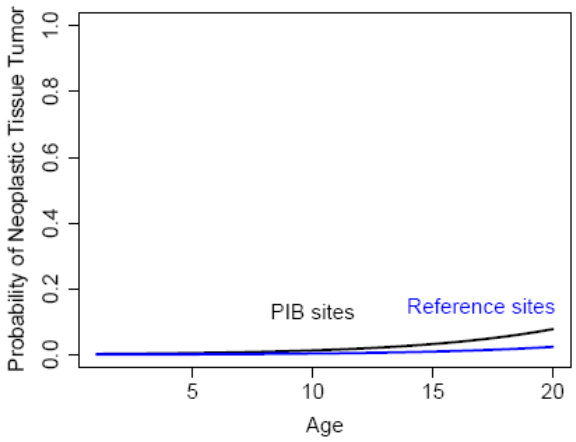
Difference in Linear Location Terms

Neoplastic tissue tumors:
95% Prediction Interval for $\beta_{PIB} - \beta_R$
[-1.313,6.203]

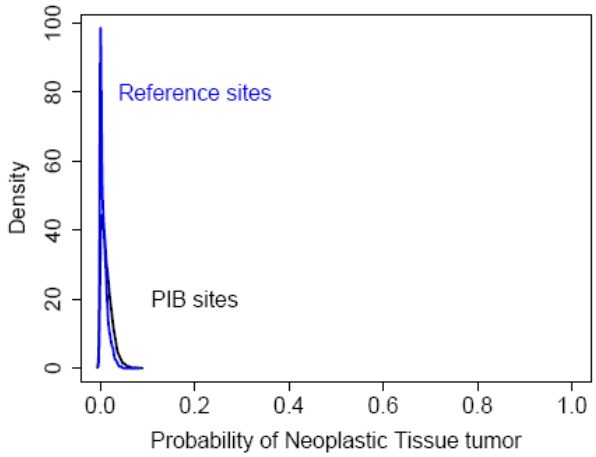


Effect of Age

Neoplastic tissue tumors:
95% Prediction Interval for β_L
[-0.183,0.006]

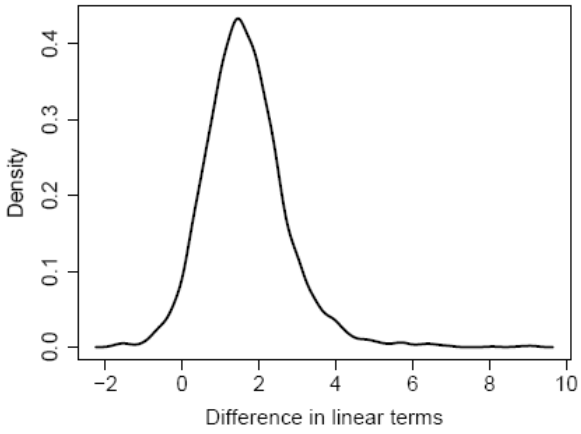


Tissue Tumor Rates: Age 7

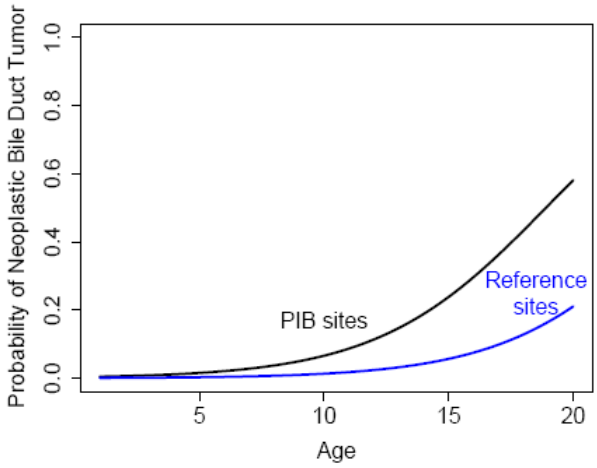


Difference in Linear Location Terms

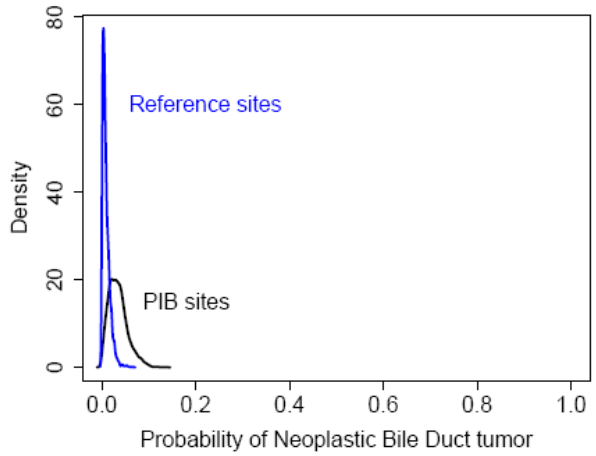
Neoplastic bile duct tumors:
95% Prediction Interval for $\beta_{PIB} - \beta_R$
[-0.189,3.992]



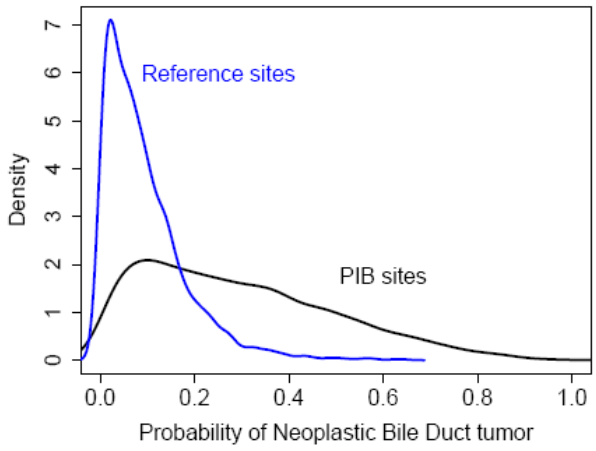
Bile Duct Effect of Age



Bile Duct Tumor Rates: Age 7

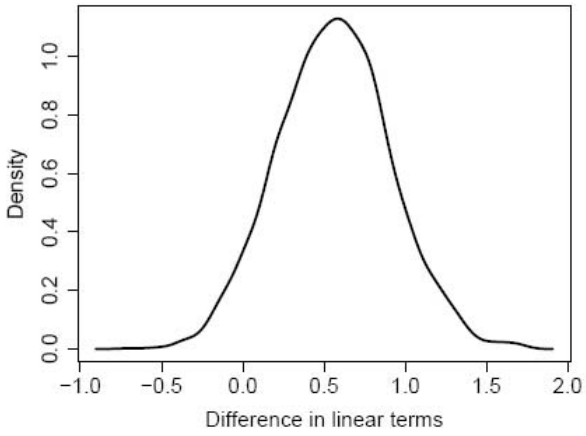


Bile Duct Tumor Rates: Age 15

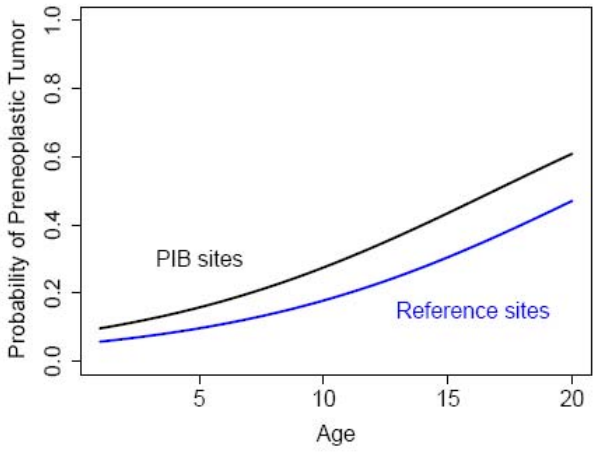


Difference in Linear Location Terms

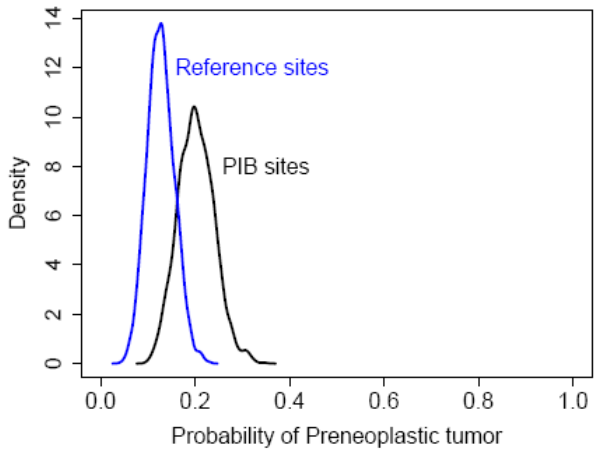
Preneoplastic tumors:
95% Prediction Interval for $\beta_{PIB} - \beta_R$
[-0.118,1.258]



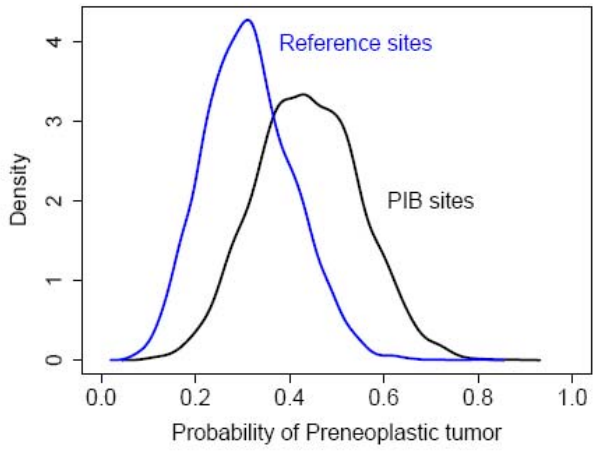
Preneoplastic Effect of Age



Preneoplastic Tumor Rates: Age 7

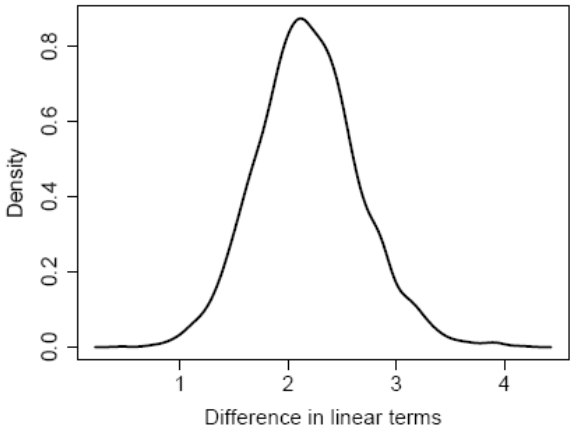


Preneoplastic Tumor Rates: Age 15

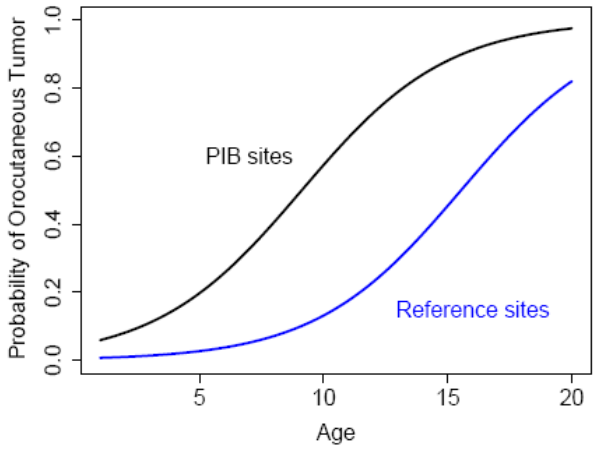


Difference in Linear Location Terms

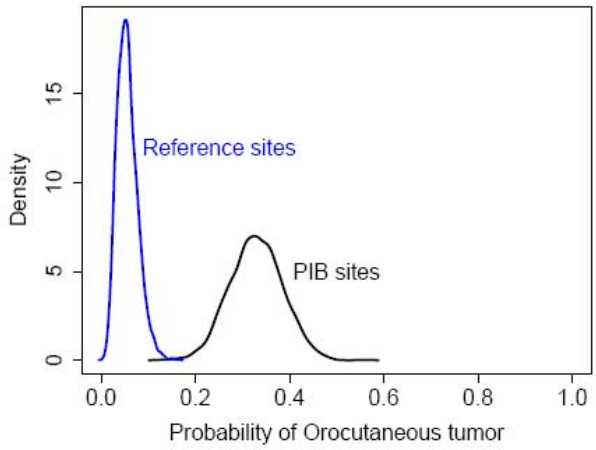
Orocutaneous tumors:
95% Prediction Interval for $\beta_{PIB} - \beta_R$
[1.298,3.187]



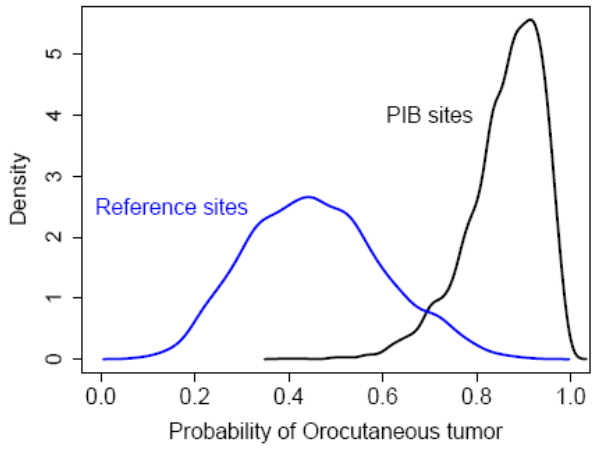
Orocutaneous Effect of Age



Orocutaneous Tumor Rates: Age 7



Orocutaneous Tumor Rates: Age 15



Summary

- Lesion incidence rates on brown bullhead can be modeled as a function of length (or weight)
- Brown bullhead sampled at Inland lake sites have a lower GVO lesion incidence rate than "reference" Lake Erie sites
- Brown bullhead sampled at Presque Isle Bay sites have similar GVO lesion incidence rates to those collected at "reference" Lake Erie sites

Methods
Results: GVO
Results: Tumors
Summary

Summary

- Most tumor incidence rates are a function of age (hepatic neoplasia is exception)
- Neoplastic and Orocutaneous tumor rates higher in PIB sites than "reference" sites
- Preneoplastic tumor rates are statistically similar among PIB and "reference" sites
- Hepatic and bile duct neoplastic tumor rates are also statistically similar

PIB Tumor Rate Point Estimates

For age 7 brown bullhead

- Neoplastic: 6.7
- Preneoplastic: 20.2
- Orocutaneous: 32.9

Further Research

- Include more Lake Erie "reference" site tumor data
- Compare tumor incidence rates between PIB and Lake Erie "reference" sites
- Create a "tumor-at-age" model to measure year effects

FACILITATED DISCUSSION: *What reference lesion rate should be used for Presque Isle Bay and should inland lakes be used as a reference*

Facilitated by: Dr. Thomas Wortman

***Comments*:**

Define reference site:

- IJC suggests comparing tumor rates at AOCs to rates found at "unimpacted control sites." This is unrealistic; therefore, we are really looking for least-impacted sites.
- You can choose sampling sites which you believe are reference sites, but you need to base it on the fish (with the lowest tumor rates) even if you do not know what the cause of the tumors are.
- Are we talking about least impacted, unimpacted, background, non-AOC, non-AOC unimpacted, or several references (gradation)?
    - The notion of using non-AOC areas as references is bad because there are some sites that are contaminated; however, are not listed.
    - The Canadian perspective is to use least impacted sites (e.g. possibly pick four sites with the lowest tumor rates based on histopathology).
    - Because we do not know what the factor(s) causing the tumors are, we cannot necessarily just go by the types and amounts of pollutants in a certain area.
    - The IJC definition is simply a guideline; we should not lock ourselves to that definition.
    - What if this is a lakewide problem rather than an AOC problem?
- Perhaps we should determine what reference sites to use based on a combination of the sediment chemistry, and the external and internal tumor rates (we need to choose criteria to use, or base it on the best information available).
- Our methodology and reference areas will constantly change; should we change to adapt or do we need to find a stopping place? There is pressure to delist AOCs, and claiming we might never have an answer is not good; there needs to be a balance. Also, if we change things up too much future data may not be comparable with historical data.
- Keep in mind we have to take into account where we can find bullhead, we need to have some knowledge of the population (some may have higher mortality rates or different age distributions), and it is also important to know that we have the same species of fish (e.g. fish in Long Point look different from Presque Isle Bay fish – there maybe a hybridization issue).
- The stressor or causative agent should not be present in the reference site; however, we do not know for sure what the causative agents are.
- Going back to the list of what should be included in the definition of a reference site:
    - We only want to look at non-AOCs.
    - What about AOCs that do not have the fish tumors or other deformities BUI listed, can we use those? Probably not, because many of them have simply not had an assessment of fish tumors or other deformities BUI.
    - We can take "unimpacted AOC" off the list.
    - Remove "gradient of references" because if there are reference sites like this you should be using them anyway.
    - Take "unimpacted" off the list because it is not realistic.

    - Are background and least impacted the same thing? The attendees of the conference voted on this issue - "background (4 people)," "least impacted within the basin(13)," or "non-AOC (0)"
    - Remove non-AOC because no one voted for its inclusion.
- We are down to two models: the average rate of Lake Erie plus or minus the side effect (background - just take the average for the Lake) or baseline plus the side effect (least impacted - calculate the minimum side effect and the baseline is somewhere below that).
    - How would you calculate background reference rate? Randomly sample all sites in Lake Erie with bullhead; the background rate would be the mean rate of all those sites – this would involve going to many more sites other than AOCs
    - By definition, the closest you'll ever get to least impacted is a place with the lowest tumor rate.
    - Does background really mean the average or does it mean some number near zero? And is this really different from least impacted? Is base rate a better term than background? We can guess the base rate based on least-impacted sites, but we can never really estimate the base rate. The base rate would take more studies, more time, money, and we are trying to get to an answer - but if that is the best science, then money and time should not matter.
- Overwhelming opinion is that "least impacted" should be used to define reference sites.

Should inland lakes be used as a reference?

- No, because inland sites do not meet the criteria we discussed as being "in the same basin."
- Unanimous agreement.

What reference liver lesion rate should be used for PIB?

- Use the rate determined from the reference sites.
- But then we have to ask what is least impacted numerically speaking?
- We could use the Lake Erie reference sites we already have data for to determine a reference rate. The rate could be determined using Bayesian statistics and selecting for a specific age class.
    - But is this good enough? This data set only dates back to 2002.
    - If there is difference over time, then we are showing that the sites can improve, which indicates they are not least impacted
    - There is an issue with going back to earlier data, as has already been discussed (because of differing methodology)

SESSION FOUR: DELISTING TARGETS FOR THE FISH TUMORS OR OTHER DEFORMITIES BUI IN PRESQUE ISLE BAY

JIM GRAZIO

Presque Isle Bay brown bullhead study

Jim Grazio[1] and Eric Obert[2]

[1]Pennsylvania Department of Environmental Protection, Tom Ridge Environmental Center, 301 Peninsula Dr., Suite 4, Erie, PA 16505 [2]Pennsylvania Sea Grant, Penn State Behrend, Tom Ridge Environmental Center, 301 Peninsula Dr., Suite 3, Erie, PA 16505

Abstract: See abstract from Jim Grazio's presentation entitled *Data from 2004/05 reference studies and inland lakes* (page 109).

***Presentation*:**

PRESQUE ISLE BAY BROWN BULLHEAD STUDY

Eric Obert- PA Sea Grant

Jim Grazio- PA Dept. of Environmental Protection

Great Lakes Areas of Concern

- Great Lakes Water Quality Agreement
- Presque Isle Bay AOC
  - 43rd and final AOC
  - Only AOC to be designated based on citizen petition
  - Triggered development of a Remedial Action Plan (RAP)
  - 2 Beneficial Use Impairments:
    - Contaminated Sediments
    - Fish Tumors and other Deformities

TUMORED BULLHEAD FROM PRESQUE ISLE BAY

STILL NOT IMPRESSED?

EXAMPLES OF DIFFERENT TYPES OF TUMORS AND MELANOMAS




HISTOLOGY STUDIES 1991,1992,1995, 1997,1998




TUMOR AND MIGRATION STUDY IN 1992

FISH COLLECTED IN PENNSYLVANIA TRAPNETS

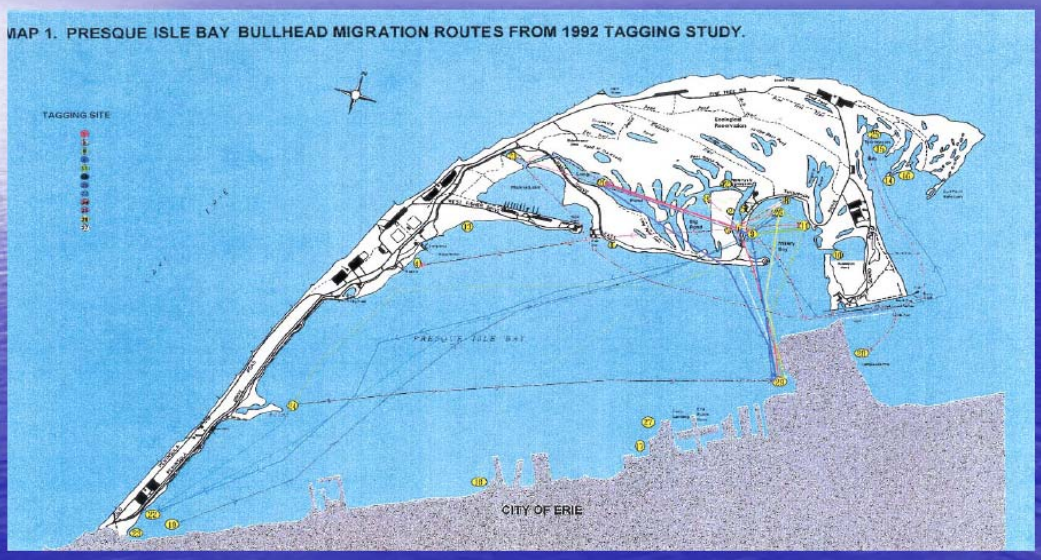
D.C. ELECTROFISHING BOAT

MEASUREMENTS AND TUMOR LOCATIONS ARE RECORDED FOR EACH FISH

TAGGING PROCEDURE

TAGGED FISH ARE RELEASED AT POINT OF CAPTURE

RESULTS OF 1992 MIGRATION STUDY



1992 HISTOLOGY AND TISSUE STUDY




1995 HISTOLOGY STUDY

1997 & 1998 Histology Studies

BILE EXTRACTION FOR PAH METABOLITE ANALYSES

SOURCES OF PAHS?

SEDIMENT SAMPLING FOR NITROSAMINES

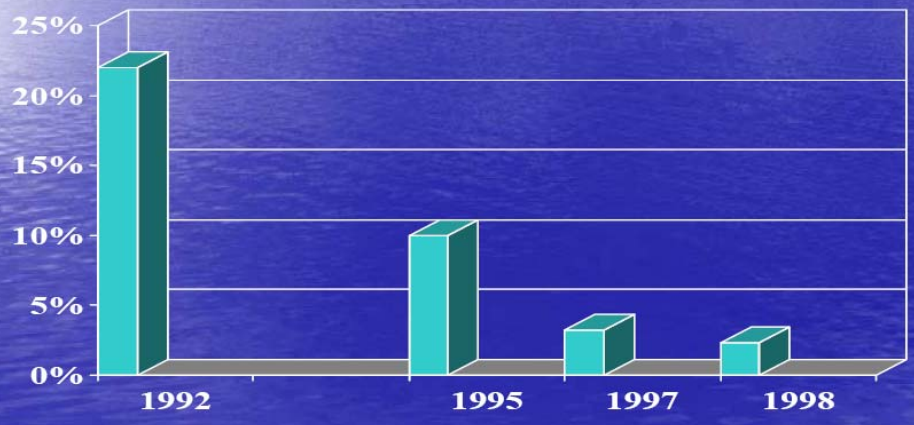
SOURCES OF NITROSAMINES?

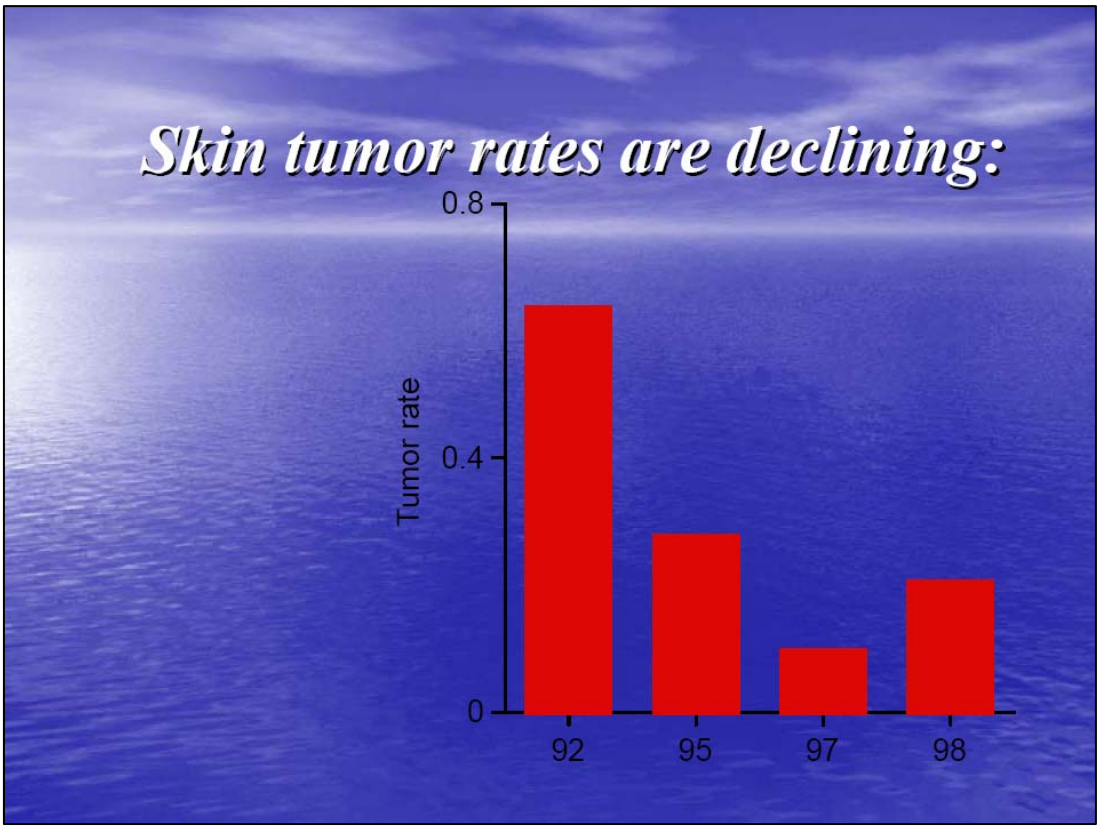
FISH TISSUE CONTAMINANT SAMPLING

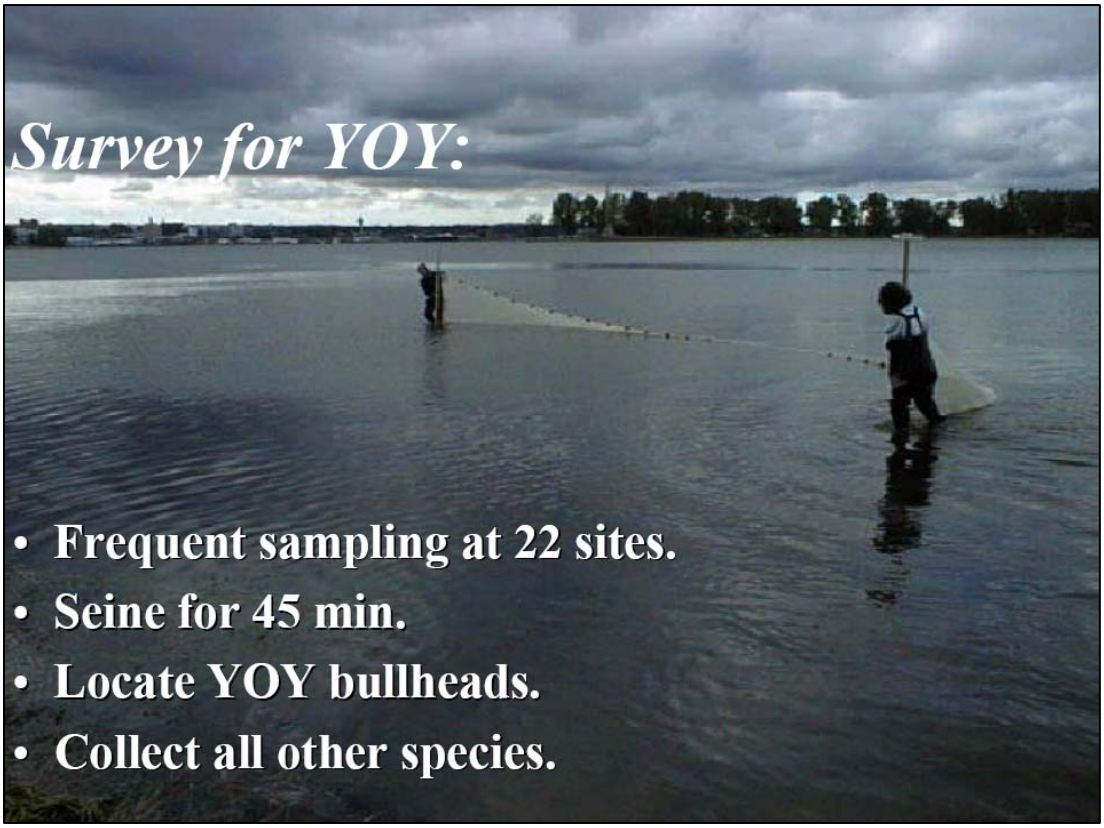
U.S. EPA MUDPUPPY

SEDIMENT CORES

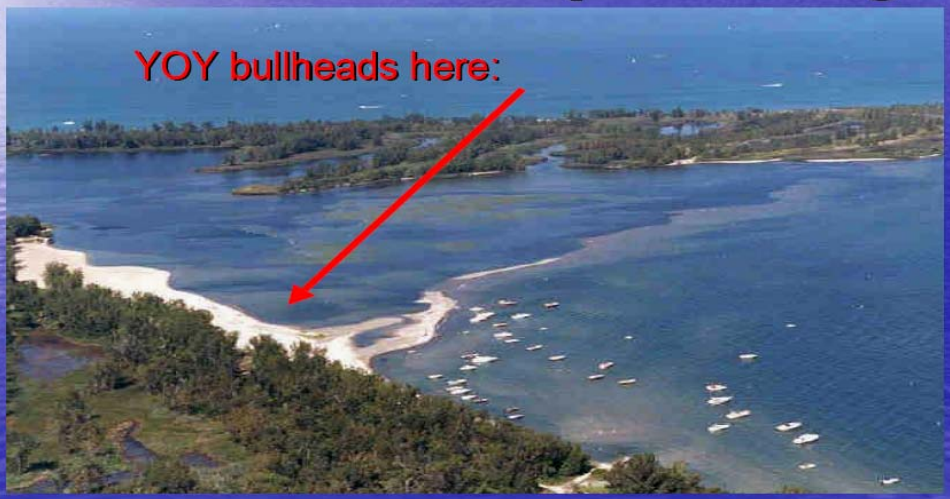
DECLINE IN LIVER TUMOR RATES SINCE 1992




Skin tumor rates are declining:
Tumor rate
0.8
0.4
0
92
95
97
98

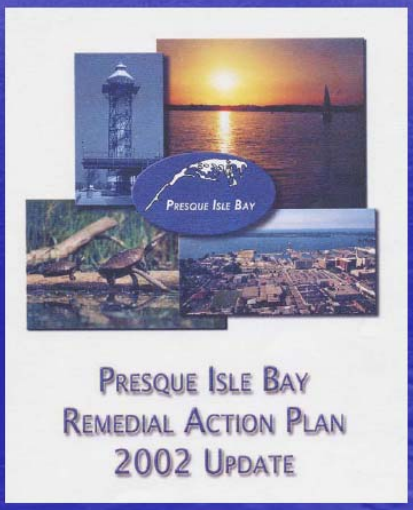

Survey for YOY:
• Frequent sampling at 22 sites.
• Seine for 45 min.
• Locate YOY bullheads.
• Collect all other species.

We observed bullhead adults guarding nests, bullheads are reproducing.



2003 Recovery Stage Designation

- First Great Lakes AOC to attain "Recovery Stage" status
- DEP focus shifted from problem identification/remediation to monitoring
- Initiative to identify appropriate delisting targets

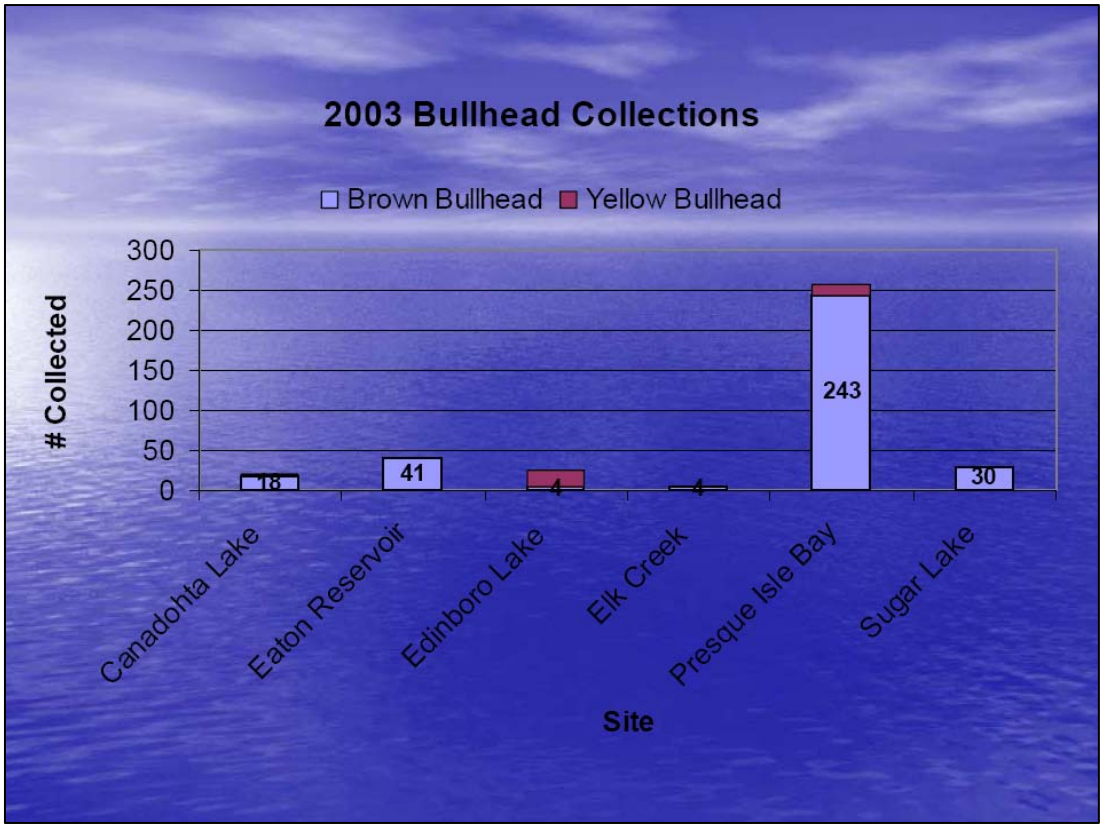


Monitoring Phase

- Monitoring to confirm long-term recovery
  - Fish & Sediment monitoring for 10 years
  - Develop appropriate delisting targets
  - Focus on watershed restoration

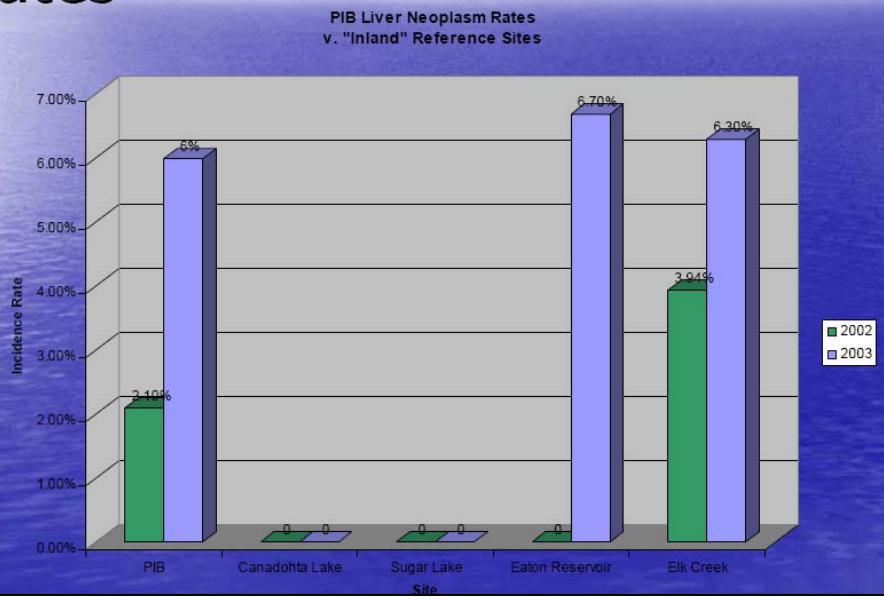
Focus on inland reference lakes

- 5 PA Reference Sites
  - PIB v.
    - Canadohta Lake
    - Eaton Reservoir
    - Edinboro Lake
    - Elk Creek
    - Canadohta Lake

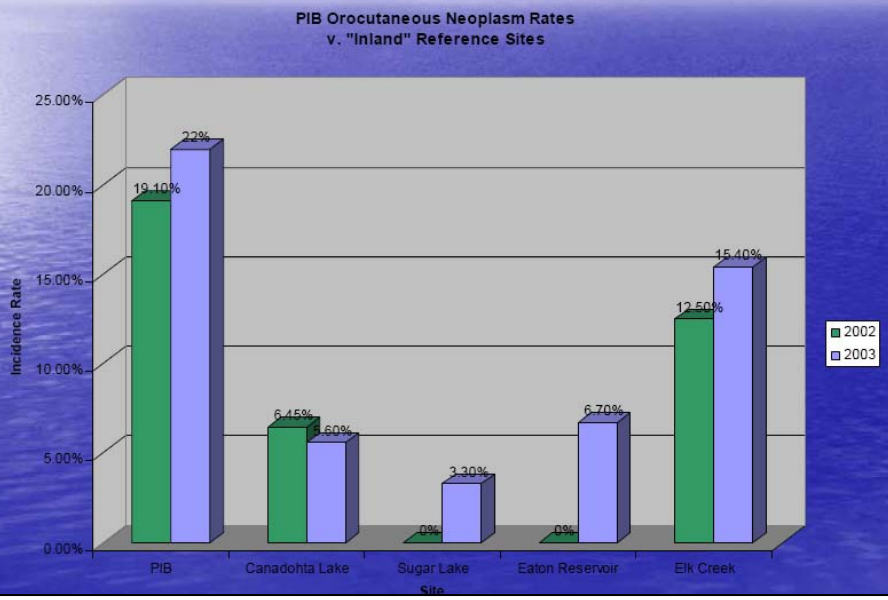


2002-2003 Results

Inland Reference Study results- 2002-2003 Liver Neoplasm Rates



Inland Reference Study results-2002-2003 Orocutaneous Neoplasm Rates



2004-2005 Lake Erie Reference Study

- How clean is clean?
  - Baseline brown bullhead tumor rate in Great Lakes is > 0%!
    - Past estimates of external tumor rates at "least impacted" GL reference sites ranged from "12-20%" on average
    - Liver tumor rates ranged from…." "
    - Hard data not available….

Dunkirk Harbor, New York

Huron River, Ohio

Old Woman Creek, Ohio

2004 OWC Fish

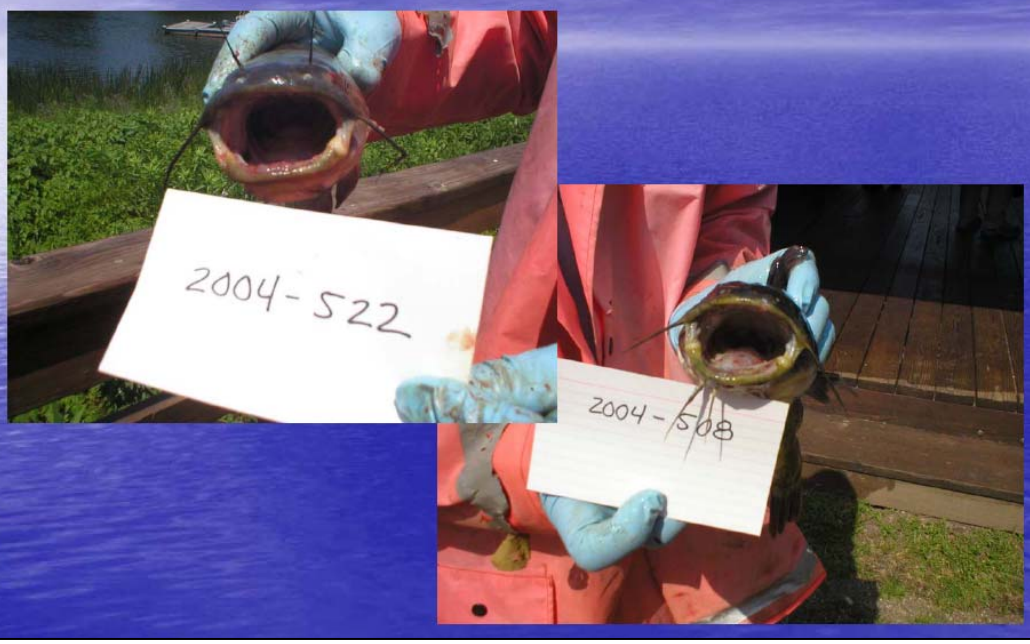
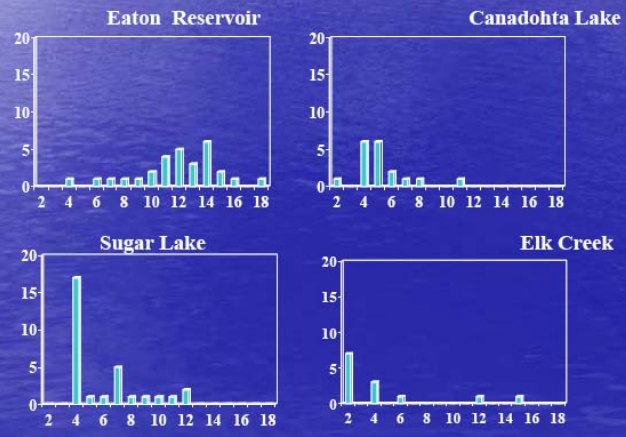


Figure 1. Age distribution of Bullheads Collected at Presque Isle Bay and Four Reference Sites in 2003

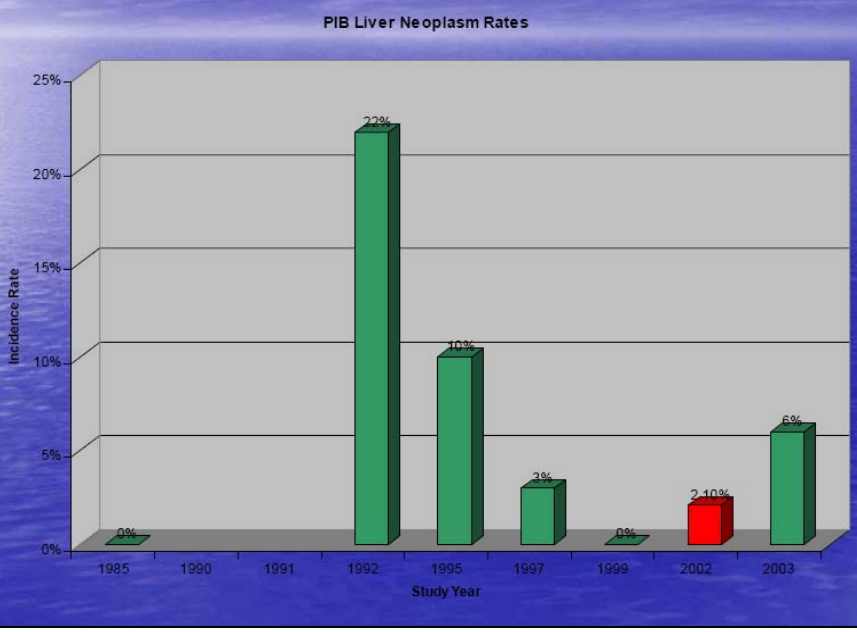
Conclusions: Aging is Hell!



Credit: V. Blazer, 2004

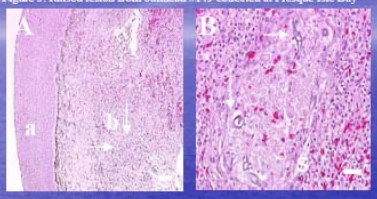
3

Gulf Ecology Div. "Value Added"

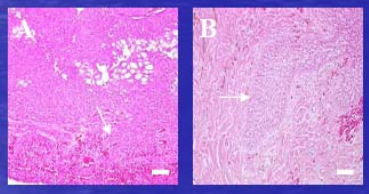


Gulf Ecology Div. "Value Added"

Figure 3. Raised lesion from bullhead #149 collected at Presque Isle Bay



A. Raised lesion consisting of hyperplastic epithelium (a) and chronic inflammatory myositis with hemorrhage (b). This inflammatory response ws a diffue, granulomatous response with giant cells (arrow). Scale bar equals 100um B. Higher magnification illustrating fungal hyphae (arrows). H&E stain, scale bar equals 40 um.



Credit:Blazer, Fournie, Wolfe, and Wolfe, 2004

A. Squamous cell carcinoma with invasion of neoplastic cells through the basement membrane (arrow). H&E stain. Scale bar equals 50 um. B. Pegs of neoplastic cells (arrow) can be observed extending into dense connective tissue and muscle. H&E stain. Scale bar equals 40 um.

FACILITATED DISCUSSION: *Are the proposed targets appropriate for PIB; and based upon the data, is the fish tumors or other deformities BUI impaired in Presque Isle Bay*

Facilitated by: Dr. Thomas Wortman

***Comments*:**

Reference rate for Presque Isle Bay:

- How many sites should be included in the collection of least impacted reference sites? As many as possible. Bottom line, we need a variety of bullhead from non-AOC Lake Erie sites.
- Two forces driving the reference rate: delist the Presque Isle Bay AOC site, or do something that all the AOCs around the Great Lakes could use (so they will not have to go through the same research and hardships). If we are just focusing on the Presque Isle Bay AOC we need to pressure people for funds.

Delisting criteria:

- What criteria was used to upgrade the Presque Isle Bay AOC to an AOC in recovery?
    - Decreasing trend of tumors: had 20% visual, 12% histopath, 5% liver tumor -these were the numbers we used.
    - The sediments coming in were presumably less contaminated than what was there and we are not dredging it should start getting better.
- Are the delisting criteria we are developing going to be the same for liver tumors and external tumors?
    - Liver tumors have a stronger indication of a contamination problem; it does not appear that we have the same confidence with external tumors.
- Should external tumors be given the same weight as liver tumors?
    - There is evidence that external tumors are related to contaminated sites, specifically to PAHs.
    - The public's perception is very important. If we delist the Presque Isle Bay AOC and people are still catching bullhead with tumors they will be concerned.
- Do we need to be concerned with both liver and skin tumors, or is one more telling than the other?
    - In the past we went by 12% external and 5% liver - decided the external rate could be higher for whatever reason.
- Should the rates for external lesions be determined by gross observations or by histopathology? Seems to be agreement that tumors should be confirmed by pathologist
- What about deformities? For the criteria we are mainly concerned with neoplasms
- Are there going to be different standards for every AOC - their references could be different? Should probably have the same standards for all Lake Erie AOC sites.
- Are the proposed delisting targets appropriate for Presque Isle Bay? We do not have proposed targets for delisting; as mentioned before we had voted on 20%, 12%, and 5% but these are probably going to change.
- Based upon the data, is the fish tumors or other deformities beneficial use impaired in Presque Isle Bay? Our data are too limited to answer this.

SESSION FIVE: WHAT ARE THE COMPONENTS OF A LONG-TERM MONITORING PLAN FOR PRESQUE ISLE BAY

FACILITATED DISCUSSION: *What is the recommended interval between sampling events, recommended sample size for gross observations, and recommended sample size for liver histopathology*

Facilitated by: Dr. Thomas Wortman

***Comments*:**

Recommended sample size for liver histopathology:

- If the sample sized is quadrupled, the confidence or prediction interval will be cut in half.
- Assessing the neoplastic tumor rates for age seven bullhead we would have to go from sampling 30 brown bullhead to 120 bullhead. You could either take more samples every year or group ages/years.
- For example, the number of fish needed to detect the difference between 5% and 10% would be roughly 2,000 fish based on previous experience.
    - If sampling 2,000 bullhead is not practical, how many should we sample? As many as we can (and accept error is involved)?
    - As many as you can get is a bad answer. A number must be set for the collectors and the limiting factor is how many bullhead the pathologist can process.
- In the past we used 5% as a reference rate for liver tumors; however, it appears that number is going to change. Currently, the reference rate seems to be at 7 or 8% but more data are needed. In other words, a number for delisting criteria should not be set before we have more reference site data and analysis; the delisting criteria will probably be small for liver tumors but a little higher for external tumors.
- How many liver sections should be assessed per liver sample? More research is needed to determine the number of sections.
- Can we lump together the liver and external rates so that there is only one rate, rather than several different standards?
    - If the cause of the tumors is not known we do not want to lump everything together.
    - Statistically, we would not be comfortable lumping things together.

Recommended sample size for gross observations:

- If we agree to collect 40 bullhead for liver tumor assessment, external samples from fish that display raised lesions should be taken and the rest should be labeled clean.
- Should we then continue to perform gross observations on fish we do not send for histopathology? No.
- Gross observations may not be a bad methodology; it is easer and cheaper to do than histopathology. Many agree that if this information is taken it should not be included in the central database.
- There is a strong correlation between gross observations and what the histopathology tells us, for analysis of that same tissue. We need a statistical analysis to continue this discussion.
    - The pathologists agree that we should continue to assess fish grossly. This is what people see and we would be losing a lot of data.

    - The gross observation data would have to be included in a separate database because it is not part of the listing/delisting criteria.
- Whatever you choose to sample, be sure that it is random.
- You can continue performing gross observations on bullhead that are left over or you have time to collect.
- The statistician will perform more analysis and get in contact with everyone.

Recommended interval between sampling events:

- The variability between sampling years needs to be assessed to determine how often sampling should occur.

FUTURE RESEARCH NEEDS:

DR. MIKE MILLARD

Proposed: Brown bullhead tagging and genetics study in Presque Isle Bay

Mike Millard[1]

[1] USFWS, NE Fishery Center, 308 Washington Ave., Lamar, PA 16848

Overview:

- Remote receivers would be set up along the channel connecting Presque Isle Bay to Lake Erie so that the movement of bullhead through the channel could be detected.
- The radio transmitter tags have a life span of approximately six months. We would tag 40-50 fish in late April-May and track their movement for the life of the tag.
- Fish from both the bay and lake would be tagged to determine if fish are moving in and out of the bay and lake.
- Each radio tag is individually coded so we could track specific fish.
- The range of tracking is heavily dependent on the conductivity of the water.
- If fish are found to be leaving the bay the question of where they go will still remain; however, the fish can be tracked using a hand-held receiver.
- Additional population research: genetics.
    - Assess the variability of the gene sequences.
    - Gene flow can be used to measure migration.

Comments:

- Why are we focusing on fish leaving the bay? What are our goals? Our goal is to determine if the bullhead are an indicator of the conditions in Presque Isle Bay opposed to Lake Erie. Are we interested in home range or if the fish are leaving the bay? We are interested in whether or not the fish are leaving the bay; however, home range would be interesting.
- Where should the bullhead be collected? Should they be sampled from a variety of locations?
    - If we sample fish from the "study area" (e.g. lagoons) we start to get into the AOC boundary issue. We should try to sample fish from the "contaminated" sites along the City of Erie's shoreline.
    - We should probably sample sites throughout the bay; however, only large fish should be sampled because of the surgery.
- We could be left with some uncertainty following the completion of the study; just because we do not detect any tagged fish leaving the bay does not suggest the fish do not leave.
- How long can the DNA samples be stored? For a long time (i.e. until we can get funding).

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