Second Fish Tumors Related to Great Lakes Areas of Concern Conference Proceedings

Cosponsored by:

PA Department of Environmental Protection
U.S. Environmental Protection Agency GLNPO
&
Pennsylvania Sea Grant

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Erie, Pennsylvania

Compiled by: Sean Rafferty
Pennsylvania Sea Grant
Penn State Erie

Preface

The Second Fish Tumors Related to Great Lakes Areas of Concern Conference Proceedings was compiled with the intention of capturing the thoughts of the conference held in Erie, Pennsylvania from January 21-22, 2003. Conference attendees had the opportunity to participate in break out sessions related to monitoring protocols for assessing the fish tumors or other deformities beneficial-use impairment in Areas of Concern or methodology for histopathological studies in Areas of Concern. The monitoring sessions were chaired by Dr. Paul Baumann and the histopathology sessions were chaired by Dr. Vicki Blazer; both of the United States Geological Survey (USGS). The recommendations, developed by the conference participants, were presented by Paul Baumann to the International Joint Commission at a September 18, 2003 meeting in Ann Arbor, Michigan. A concept paper outlining, in detail, the recommendations for standardized criteria for evaluating this beneficial-use impairment will be submitted to the International Joint Commission in the hope that these criteria will be adopted by all Areas of Concern attempting to restore this use impairment.

A special thanks is extended to all the speakers at the conference, including Eric Obert, Lori Boughton, Dr. Paul Baumann, and Dr. Vicki Blazer, as well as key experts Dr. John Harshbarger, Dr. Fred Pinkney, and Dr. Jack Fournie, and also to the Pennsylvania Department of Environmental Protection and the United States Environmental Protection Agency Great Lakes National Program Office (GLNPO) for providing funding for the conference.

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Contacts:
For additional information on fish tumor concerns, conferences, or on follow-up activities, please contact:

Eric C. Obert
Extension Director for Lake Erie
Pennsylvania Sea Grant
Penn State Behrend
Erie, PA 16563
(814) 898–6453
eco1@psu.edu

Lori Boughton
Chief, Office of the Great Lakes
PA Department of Environmental Protection
230 Chestnut Street
Meadville, PA 16335
(814) 332-6816
lboughton@state.pa.us

Introduction

On August 18-19, 2003, 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 Second Fish Tumors Related to Great Lakes Areas of Concern Conference, held at Penn State Behrend in Erie, Pennsylvania. This conference was a follow up to the January 2003 Fish Tumors Related to Great Lakes Areas of Concern Conference, which brought together more than 40 researchers, fishery and wildlife biologists, pathologists, and agency representatives with the goal of sharing information, concerning fish tumors and deformities from American and Canadian Areas of Concern, and to refine and coordinate the standardization of protocols currently being used to evaluate the fish tumors or other deformities beneficial-use impairment.

The purpose of the second conference was to refine and coordinate the standardization of protocols for assessing this beneficial-use impairment, which were highlighted by the fish tumor taskforce subcommittees.

The conference was conducted in a workshop format with the purpose of encouraging broad participation from attendees. The attendees had the option of attending break-out sessions related to the refinement of monitoring criteria or histopathological criteria. A panel of nationally recognized experts in the study of fish tumors and pathology were recruited to speak and interact with the participants at this conference**.** The monitoring sessions were chaired by Dr. Paul Baumann and the histopathology break-out sessions were chaired by Dr. Vicki Blazer.

As a result of the break-out sessions, the participants helped refine their recommendations of standardized criteria for assessing the fish tumors or other deformities beneficial-use impairment in Areas of Concern. These refined protocols will be presented, in the form of a concept paper, to the International Joint Commission with the hope that the recommendations will be approved by the International Joint Commission and adopted by all Areas of Concern.

Presentations: Overview of Fish Tumors and Deformities Beneficial-Use Impairment

Eric Obert – Welcome and Why Are We Here?

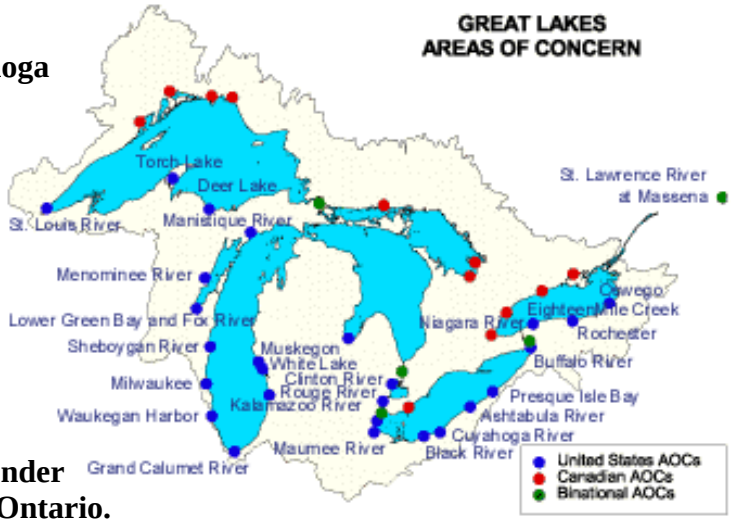
Welcome to the Second Fish Tumor Conference for Developing Standardized Criteria for AOCs

Why Are We Here?

The International Joint Commission (IJC) characterized fish tumors and other deformities as one of 14 beneficial-use impairments to be used by Areas of Concern as criteria for the listing and delisting process.

16 of the 42 Areas of Concern have impairment of beneficial uses due to the presence of fish tumors and other deformities.

Those included are: Ashtabula River, Ohio; Black River, Ohio; Buffalo River, New York; Cuyahoga River, Ohio; Detroit River, Michigan; Grand Calumet River, Indiana; Maumee River, Ohio; Milwaukee Estuary, Wisconsin; Niagara River, New York; Presque Isle Bay, Pennsylvania; Rouge River, Michigan; Sheboygan River, Wisconsin; St. Louis River and Bay, Minnesota and Michigan; St. Mary's River, Michigan; Thunder Bay, Ontario; and Jackson Bay, Ontario.



Results of January 2003 Fish Tumor Conference

- **Established Fish Tumor Taskforce of recognized experts**
- **Developed conference proceedings**
- **Solicited stakeholder input**
- **Developed histopathology and monitoring committees**

Conference Purpose?

- **Discuss subcommittee reports**
- **Define final standardized criteria for histopathology and monitoring plans**
- **Deliver criteria recommendations to the International Joint Commission**

Conference Outcomes?

Histology and Pathology Criteria

- **Criteria for the standardized assessment of skin and liver lesions**
- **Plan for archiving tissue for historical record and comparison needs**

Monitoring Recommendations

- **Standardized protocols for sampling and monitoring fish tumor assessments**
- **List of research needs**

Develop Concept Paper for IJC

- **Consensus of Experts**

Sustain Taskforce and Efforts

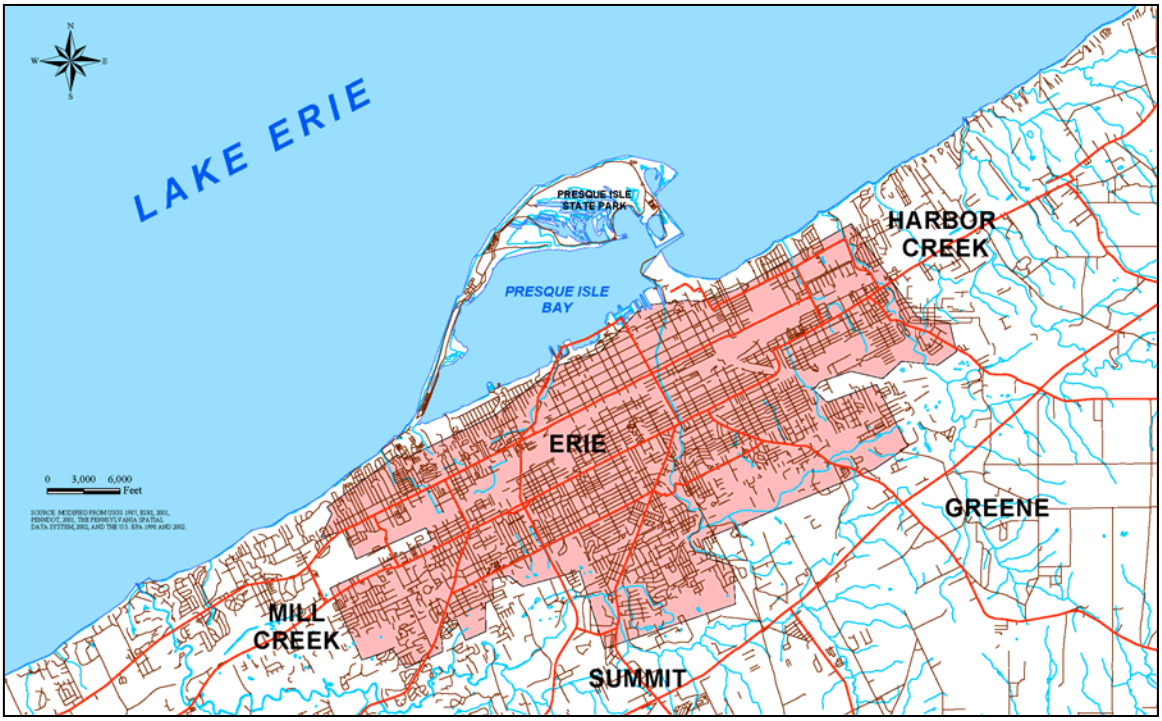
- **Maintain taskforce networks**
- **Develop an advisory panel for Area of Concern needs**

Lori Boughton – RAP and LaMP Update

Presque Isle Bay

Thank you to Eric Obert for his contributions and efforts in assessing bullheads in Presque Isle Bay

Presque Isle Bay Area of Concern



Beneficial-Use Impairments

Restrictions on Dredging

Fish Tumors or Other Deformities

The Bay's Brown Bullheads

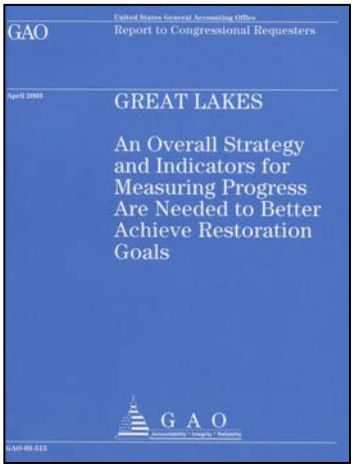
The Good …….

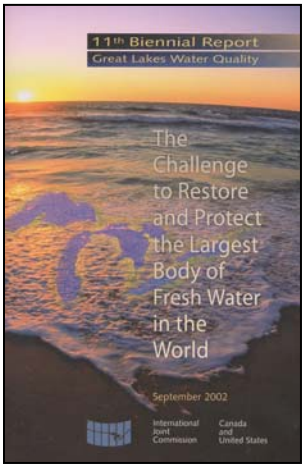
The Ugly …….

The Bad ……..

Area of Recovery: Monitoring and Setting Restoration Targets

Great Lakes Commission
des Grands Lacs





Presque Isle Bay: The Focus of the Great Lakes

Bullhead Tumor Surveys

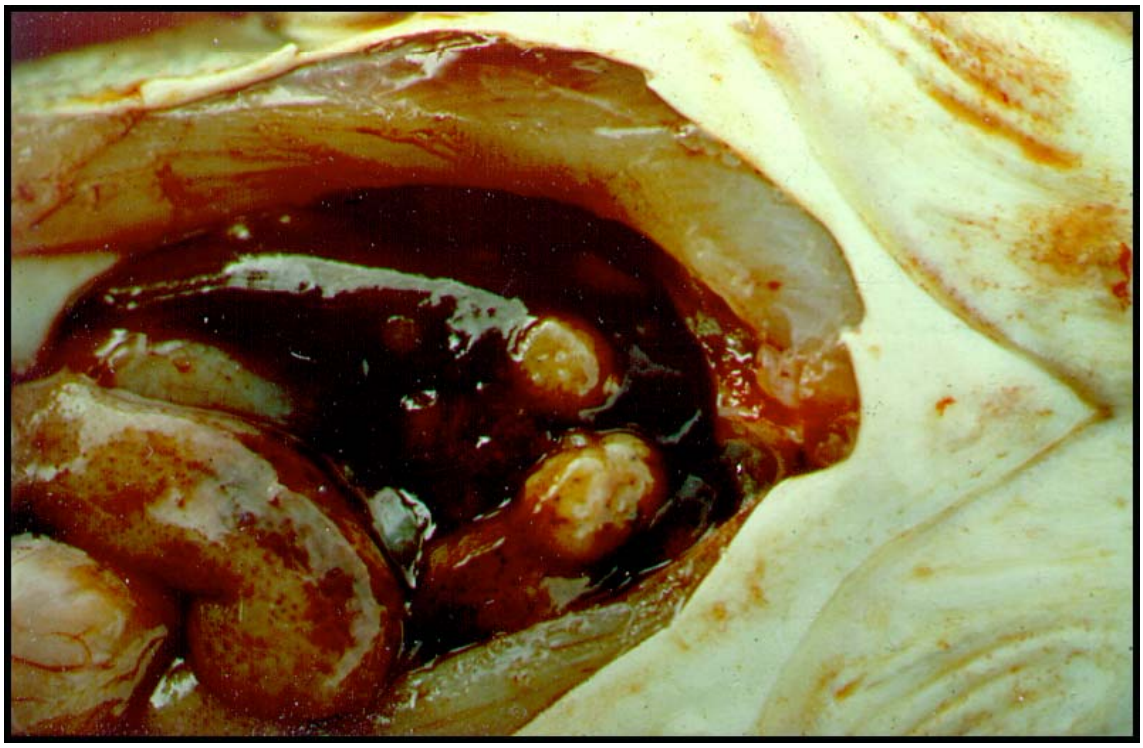
Methodology Questions:

Discussion and

Recommendations

External Abnormalities and Tumors

Internal Pathology



Histopathology Questions

- How should the liver be sampled?

- How should external lesions be sampled?

- Should melanistic skin areas be sampled?

- Should other organs be sampled?

How should the liver be sampled?

- Take any grossly visible lesions.

- Take a piece of tissue from each end and the middle of the liver.

- If the liver is large, take five pieces.

- Make sure preservative is sufficient for the size of the sample.

How should external lesions be sampled?

- Take one to three samples if lesions are very similar.

- Take any lesion which is different.

- Count and measure the remaining lesions (or count by size groups).

Should melanistic skin areas be sampled?

- Yes, include adjacent normal tissue
- Sample one or two, more if raised

Should other organs be sampled?

- Gonads?
- No other organs for neoplasms

Non-Exposure Variables

- Age

- Gender

- Season of capture

- Other species

Age Considerations

- Does tumor prevalence vary with age?

- Can length be used as an age surrogate?

- Do biliary and hepatic tumors have the same pattern?

- Should spines or otoliths be used for aging?

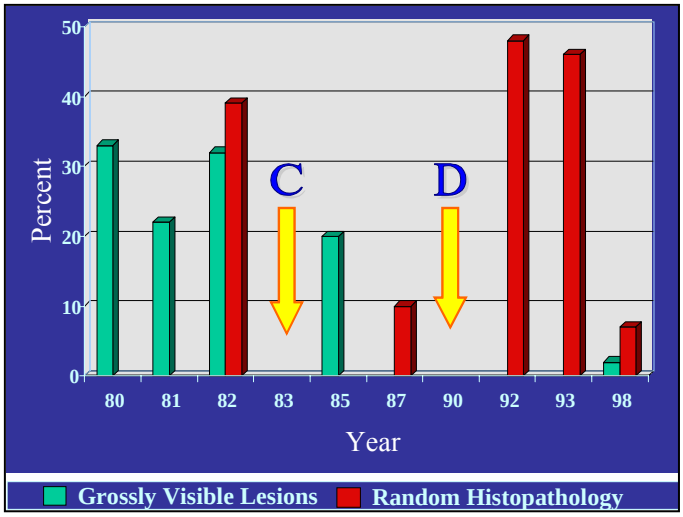
Does tumor prevalence vary with age?

- **Latent period causes young age groups to have no neoplasms even if exposed.**

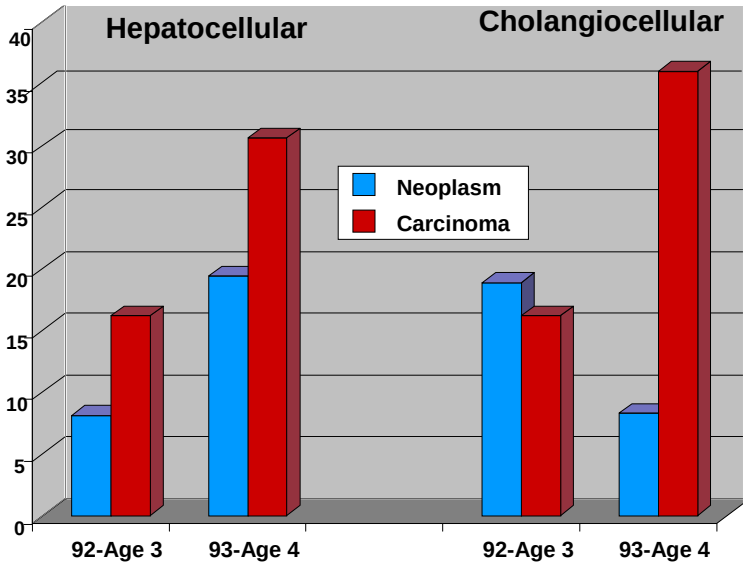
- **Both total neoplasm and cancer prevalence increase with age.**

- **The tumor prevalence of populations with differing age structures cannot be compared.**

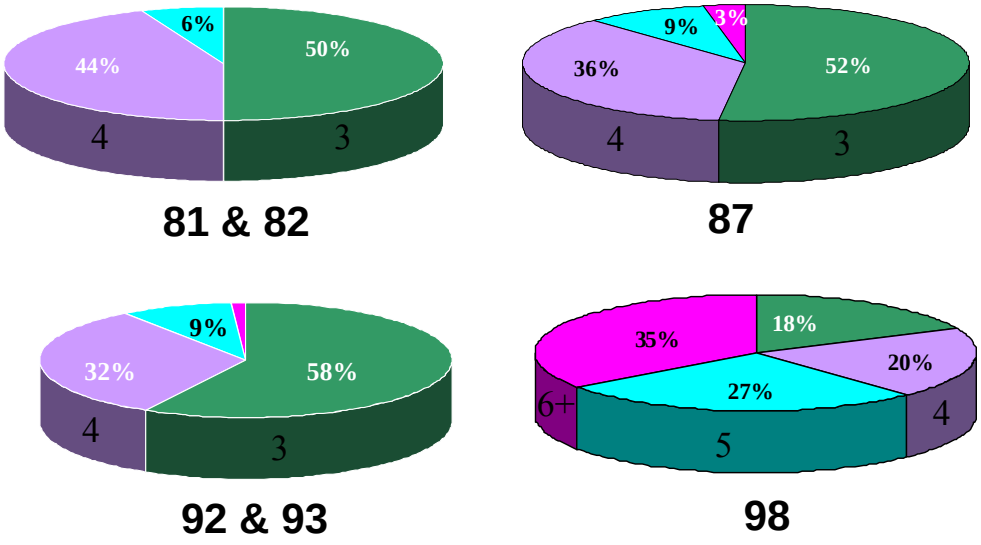
Age 3+ Bullhead Cancer Prevalence



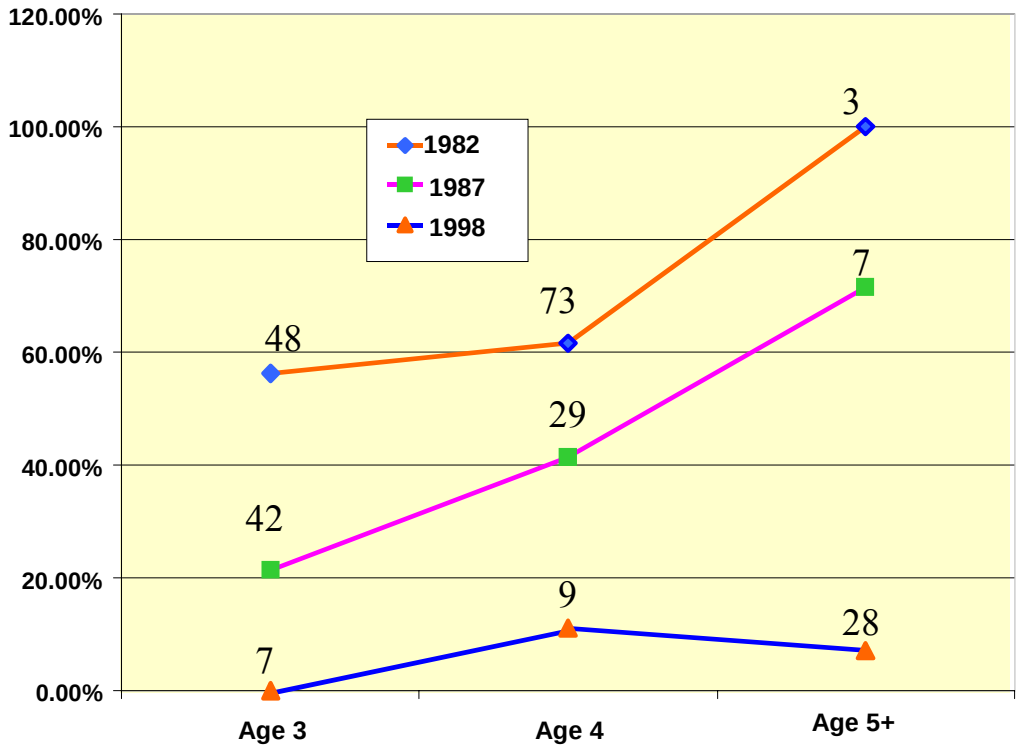
Black River Bullhead Tumors



Population Effects



Black River Bullhead Liver Neoplasms



Can length be used as an age surrogate?

- **Yes, but only for the younger ages.**
- **Use length in the field to eliminate ages too young to display neoplasms.**
- **250mm is a good approximation for bullhead at least 3 years old.**
- **Adjust length criteria for each system.**

Do biliary and hepatic tumors have the same pattern?

- **No, pattern versus age may be different.**
- **More research needed.**

Should spines or otoliths be used for aging?

- **Otoliths may be more accurate.**
- **Spine ages dominate the older data.**
- **More research needed.**

Statistical Considerations

- **Should ages be grouped?**
- **What level of probability is needed?**
- **How often should monitoring occur?**
- **Can reference sites and dates be combined?**

Should ages be grouped?

- **Age by age comparisons are best if numbers are available.**
- **Group ages by 2s, 3s, or 4s if numbers are needed for statistical analysis.**
- **More older ages might need to be grouped due to smaller numbers in the catch.**

What level of probability is needed?

- At p=0.05, chances are 1 in 20 of being wrong.

- From each location you would need:
- **50 fish for a 10% difference (5% vs 15%)**
- **140 fish for a 5% difference (5% vs 10%)**

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

Can reference sites and dates be combined?

- Yes, all tumor surveys are since 1960, so none are excluded by date.

- Lakewide background rates by definition should include averages of multiple sites.

- Lake-specific reference data are best.

- Inland lake data can provide a goal to shoot for unless genetics are different.

Vicki Blazer – Histopathology Subcommittee

Histopathology Subcommittee

V. Blazer
J. Fournie
J. Harshbarger
J. Wolf
A. Hayes
P. Bowser

Assignment

- Histopathological criteria for the description of:
Liver Lesions
Skin Lesions
Other organs?

- With an emphasis on neoplastic lesions

Primary Point

Neoplastic lesions (tumors) CAN NOT be diagnosed grossly – histopathology is required

Tumor versus Neoplasia

- <u>Tumor</u> – a swelling or raised area
To many it means cancer/neoplasia

- <u>Neoplasia</u> – abnormal cellular proliferation
Can be benign or malignant

Liver Lesions: Proliferative Hepatocellular

- ***Non-neoplastic***
  - **Foci of cellular alterations**

- ***Neoplastic***
  - **Hepatocellular adenoma**
  - **Hepatocellular carcinoma**

Liver Lesions: Proliferative Biliary

- ***Non-neoplastic***
  - **Bile duct hyperplasia**

- ***Neoplastic***
  - **Cholangioma**
  - **Cholangiocarcinoma**

Liver Lesions: Non-Proliferative

- **Inflammatory**

- **Pigmented cell accumulations (macrophage aggregates)**

- **Necrosis**

- **Cellular Infiltrations**

- **Parasites**

Raised Skin Lesions: Proliferative

- *Non-neoplastic* may or may not appear raised grossly
Hyperplasia of epidermal cells, alarm substance cells, pigment cells

- *Neoplastic*
Epithelial
Papilloma
Carcinoma

Pigment
Melanoma

Raised Skin Lesions: Non-Proliferative

- *Inflammatory*
Acute and chronic

- *Parasitic*
Helminth
Protozoa

Non-Raised Skin Lesions

- Melanistic spots – melanosis

- Red spots – hemorrhage, congestion, inflammation

- Ulcerations

- Deep erosions

Products

- Document on liver and skin lesion classifications

*** Text with illustrations specifically from brown bullhead**

*** Attempt to match pictures of gross lesions with the histology illustrations**

*** Peer-reviewed**

Questions

- Should other species that will/are being used in monitoring programs in the Great Lakes be included?

- Should we produce slide sets with examples of the lesions?

Difficulties

- Areas where the "monitoring" and the "histopathology" assignments overlap

How many pieces of liver are necessary?

Do pieces of all external lesions that appear similar grossly need to be examined histopathologically?

Do we need to take every melanistic spot?

Should normal skin be routinely sampled?

Research Needs

- How many pieces (and then sections) of liver and what parts are necessary to find proliferative or neoplastic lesions that are not visible grossly?

Small liver = 50 cm^3 pieces: Take 4-5 1 cm^3 pieces to process.

Pathologist is examining only a 5-6 μm thick slice of each

Research Needs

- If there are multiple melanoma- or papilloma-appearing lesions on one fish are they all likely to be the same thing?

- Similarly, if at one site there are many fish which have similar-appearing lesions are they likely to be the same on every fish?

Research Needs

- Melanistic spots

What causes them?

Are they contaminant related?

Do they go away?

Do they progress to something more serious?

Research Needs

- Do we need to sample normal skin from all fish?

If so, from where?

How many pieces?

What do any differences mean?

Research Needs

- Papillomas and other skin tumors

*** Is the cause important?**

*** Viral versus contaminant exposure**

*** Should polymerase chain reaction (PCR), *in situ* hybridization, or some other molecular method be routinely used on these neoplastic skin lesions to determine if there is a viral etiology?**

Presque Isle Bay Area of Concern Monitoring Status

In 1991, Presque Isle Bay in Erie, Pennsylvania was listed as an Area of Concern because of impairment of two of the 14 beneficial-use impairments, as listed in the Great Lakes Water Quality Agreement. These impaired beneficial uses, Restrictions on Dredging and Fish Tumors or Other Deformities, are thought to be related to sediment contaminated with Polycyclic Aromatic Hydrocarbons (PAHs). In 2002, following several years of brown bullhead tumor incidence research showing declining liver tumor rates, Presque Isle Bay became the first Area of Concern to achieve the Recovery Stage designation in 2002. The Recovery Stage designation recognizes the progress done to restore the beneficial-use impairments and states that monitoring rather than active remediation will be conducted to achieve restoration.

With the change in focus brought about by the Recovery Stage designation, monitoring plans for Presque Isle Bay's beneficial-use impairments are required to be developed to show how the continued improvement of the Area of Concern will be measured. A key component of these plans is specifying realistic and reachable delisting targets and collecting the data necessary to demonstrate that the beneficial use is no longer impaired. In line with this requirement, the Presque Isle Bay Advisory Council (PAC) has developed a recovery plan for Presque Isle Bay, which includes monitoring the health of the bullhead population and sediment quality in the bay for the next 10 years. To achieve this goal, the PAC's Fish Tumor Subcommittee has developed a monitoring plan that includes gross and histopathological examination of the bullhead population for a prescribed number of years, and the methodology for the studies. The figure shown below illustrates the data sheet used by the Presque Isle Bay Area of Concern research team to monitor the fish tumors or other deformities beneficial-use impairment, in hope that data collected will lead to the delisting of this beneficial-use impairment.

Figure 1: Presque Isle Bay Monitoring Data Sheet

2003 Presque Isle Bay Fish Lesion Study
Fish Health Data Sheet

Reference Number: 2003-_____ Collection Date: ____/____/2003 Process Date: ____/____/2003 Time: ___:___ Field Observers_____

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

☐ Presque Isle Bay:_____ ☐Other_____

Capture Gear		Species	
Trapnet	☐	Brown Bullhead	☐
Electrofishing	☐	Yellow Bullhead	☐
Angling	☐	Carp	☐
Other	☐	Other_____	

Tagging Information: Tag Number_____ Recapture Date____/____/____ Recapture Location_____

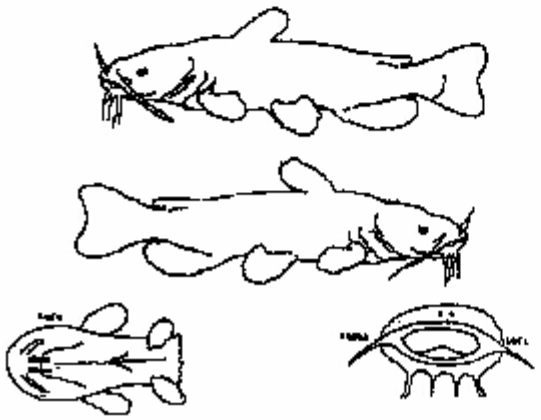
Sex: M F Length (mm)_____ Weight (grams)_____

Aging Technique: Spines ☐ Otoliths ☐ Other ☐_____ Age:_____

Fish Health Information **(DELTS)**

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

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



Pictures: Yes ☐ No ☐ File #_____

Notes:

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

Break-Out/ Discussion Sessions

Two break-out sessions were conducted during the conference in order to allow the attendees to ask questions, and provide comments on monitoring and histopathological methodologies. The following sections are transcriptions of the break out sessions.

Monitoring Break-Out Session

A. HOW SHOULD THE LIVER BE SAMPLED?

General Discussion Summary:

- Recommend, from a practical standpoint, to sample in the spring because this is when the bullheads are most abundant and easiest to capture.
- Need a random sample; therefore, necropsy the first x number of fish. Do we want to recommend a number of gross observations that should be made?
- Go with current recommendations on minimum fish length to sample. However, consider an age study to correlate better size and age for a specific Area of Concern.
- Typically, you want to sample bullheads that are at least age 3. Run an age length correlation to come up with the appropriate minimum length. Want a 90% probability that the number chosen is okay.
- Establish a standard labeling system for all of your samples.
- For Areas of Concern, the liver is the only organ needed for delisting purposes. Possibly, part of the concept paper report can suggest other analyses and the organs needed.
- Remove the entire liver and gallbladder; poke holes in the liver to allow formalin in, and assign number (label properly).
- It is better to remove the gallbladder where it attaches to the liver to prevent puncturing the gallbladder; bile from the gallbladder, can contaminate the liver tissue, causing it to be unusable.
- We should do a workshop to describe the methodologies for removing the gallbladder opposed to producing a field manual or including in the concept paper.
- The gallbladder is not needed in samples for histopathology.
- Is there degradation of the liver with cutting of gallbladder? Yes, if you want to do Ethoxyresorufin-O-deethylase (EROD) analysis. If the gallbladder is punctured, run the liver under water before putting it into fixative.
- Gross lesions of the liver are rarely seen; therefore, completely slice through the liver using a microtome blade
- The protocol for gross observations needs to be developed separately from the liver sampling protocol.
- Use histopathology working group suggestions for sampling liver – 5 sections not adjacent. One sample should be of any noticeable lesions. Cuts should be 5-7 mm. Improves fixation of tissue. Need good, sharp blades. Do not mash the liver down. Use disposable microtone blade. Process cuts on a flat board.
- Is calculating Hepatosomatic index (HSI) useful (weighing liver etc)? Yes, because the liver concentrates compounds for elimination purposes and reacts depending on what it is making soluble.

- We may want to have an Appendix in the concept paper, stating recommendations on protocols like weighing the liver, more fish for gross observations, etc.
- Do not put the whole liver into fixative. Cut the liver into pieces for better fixation of the tissue.
- Use 10% formalin as fixative (approximately 10 to 1 fixative to tissue sample ratio).
- It is always a good idea to change fixative after 12 hours (Use buffered solution for change).

B. HOW SHOULD EXTERNAL LESIONS BE SAMPLED?

General Discussion Summary:

- If there are a sufficient number of bullheads to analyze for liver tumors, there should be enough bullheads for gross observation. Given Presque Isle Bays history of skin lesions, it would be good to have a larger number of gross observations. However, rely on statistical significance.
- Ulcerated lesions are not a result of contamination; however, they could be a result of an immune deficiency problem that is caused by exposure to contamination. Include ulcerated lesions in gross observation but do not sample these lesions.
- If you think it is raised at all, take a tissue sample. This includes raised melanistic areas. However, if it is not raised do not take a sample.
- Only need to take a single section through a lesion approximately 5-7 mm in width. Be sure to include adjacent and underlying tissue in the sample. If the lesion is extremely large, take a section from one edge. If the lesion is very small (i.e., less than 5 mm), sample the entire lesion.
- Sample the largest lesion present and surrounding tissue by making a square cut around the tissue. Take 1-3 samples if the lesions are small ($\leq$ 0.5 cm is considered "small"). Any lesion > 0.5 cm is considered large.
- This 0.5 cm "standard" can vary in relation to the size of the fish. If the fish is small, a 0.5 cm lesion on the fish could be considered large in comparison to body size.
- We do not need to complicate the issue by categorizing lesions by size. A raised tumor is a raised tumor. Size does not matter.

C. SHOULD MELANISTIC SKIN AREAS BE SAMPLED?

General Discussion Summary:

- Melanistic spots are NOT raised bumps.
- We need to determine whether melanistic lesions are pigmented cell tumors or skin tumors that have darkened the pigment. In some fish, melanistic tumors have been associated with exposure to carcinogens.
- We could possibly sample 1-2 melanistic areas from each bullhead sampled for a period of two years and analyze the samples to determine the nature of these areas.

D. SHOULD OTHER ORGANS BE ROUTINELY SAMPLED?

General Discussion Summary:

- Group says NO! However, for special studies it may be necessary to collect other organs.

E. DOES TUMOR PREVALENCE VARY WITH AGE?

General Discussion Summary:

- There is a latent period between exposure to contaminants and tumor incidence; therefore, fish < 3 years old rarely display tumors.
- Both total neoplasm and cancer prevalence increase with age.
- The tumor prevalence of populations with differing age structures cannot be compared.

F. CAN LENGTH BE USED AS AN AGE SURROGATE?

General Discussion Summary:

- Length can only be used as a surrogate for younger-aged fish.
- Length should be used in the field to eliminate fish too young for lesion analysis (generally fish < 3 years old).
- It is widely accepted that fish 250 mm in length are usually at least 3 years old.
- The minimum length criteria should be adjusted for each system under analysis.

H. SHOULD SPINES AND OTOLITHS BE USED FOR AGING?

General DiscussionSummary:

- Otoliths are thought to be a better indicator of age. However, otoliths are more difficult to extract than spines. Possibly, some instruction on removal of spines and otoliths may be helpful. Spine analysis is relatively accurate up to age 5.
- Suggestion: take photos during the process or extracting otoliths so that other groups can learn how to do it correctly by studying the images.
- Using the right tools to remove otoliths is extremely important; otherwise, the otoliths could be damaged beyond use.
- Is there a Web site on otolith aging?
- Recommend using otoliths as the method for aging fish population
- There needs to be more discussion in order to pin down exactly which method this group is going to recommend: otolith or spine analysis.
- It is critical to age the otoliths so that you do have an accurate mean age of the fish sample.
- Over 200 fish were sampled in Presque Isle Bay this year, and both otoliths and spines were taken from the majority of fish sampled; therefore, this will provide us with a good comparison between the two methods and the ability to validate each method.
- Presque Isle Bay has been doing this analysis for several years; may be able to validate size and age based on past data.
- Presque Isle Bay may be the only Area of Concern that uses otoliths; therefore, we may want to sample both spines and otoliths for a few more years.
- The University of Georgia has studies on factors affecting the accuracy of otolith aging (e.g. warm water exposure) that we may want to take a look at.
- The 3 and 4 year old bullheads in the Great Lakes are almost always 250 mm or larger based on aging techniques
- Older data rely on 5 and 6+ year old fish for comparison purposes.

I. SHOULD AGES BE GROUPED?

General Discussion Summary:

- Age by age comparisons are best if sufficient numbers of bullheads are available.
- If there are not sufficient numbers of bullheads for statistical analysis then group by ages. This will depend on the sample as far as what groupings are made.

J. WHAT LEVEL OF PROBABILITY IS NEEDED?

More consultation is necessary on this topic.

General Discussion Summary:

- Need a sample of 280 fish to get at least a 5% confidence level.
- The probability would depend on the size of the population.
- These are chi-square analyses and do not necessarily depend on the population.
- The level of probability is very important when setting delisting targets. A fair amount of fish or series of years that are close together are needed in order to reach higher confidence levels. Eventually, we will say the delisting target has a plus or minus. There needs to be sufficient numbers to have at least $p = 0.05$. At $p = 0.05$ a sample of 280 fish is needed.
- The sample number is not dependent on the total number of fish in the system.

K. CAN REFERENCE SITES AND DATES BE COMBINED?

General Discussion Summary:

- Is there some background data on tumor rates for bullheads considering all the data that have been collected on them?
- We should bring together all the data we do have, including lesion/tumor rates and age data.
- There are a variety of bullhead studies that discuss "background/reference" levels. These reports should be pulled together and analyzed.
- In order to be accurate in comparing sites, the populations need to be comparable.
- The Great Lakes have a limited availability for reference sites; therefore, if we sample other areas that are not impacted, should the tumor rate be zero at those sites?
- A population with sufficiently older fish is needed for comparison purposes. Liver tumor rates should be near zero in reference sites.
- You absolutely need to have reference sites, and the tumor rates should be zero in order to be comparable.
- The Great Lakes are not pristine anymore; therefore, we need reference sites to tell us what the "background" rate should be, thus distinguishing whether an Area of Concern's local population has a higher tumor rate than "Great Lakes background/reference sites."
- A background rate for Lake Erie is needed, which is probably not going to be zero. We could possibly look at inland lakes and probably get something lower; thus, setting a goal for the Great Lakes.

- We may need to do more reference site studies around Lake Erie to determine what the background rate should be. We need to find reference sites on Lake Erie and put a reference base together.
- Primary brown bullhead habitat is typically located within embayments around Lake Erie, and these happen to be the areas that are impaired. Would it be possible to look at South Bass Island pond for the presence of bullheads?
- Recommend that the data from existing reference sites in Lake Erie and possibly even the Great Lakes be combined to determine a background rate. We could select locations in the Great Lakes that have established brown bullhead populations, but are not designated as Areas of Concern. (Good question for the Lake Erie LaMP)

Histopathology Break-Out Session

A. HOW SHOULD THE LIVER BE SAMPLED?

General Discussion Summary:

- Take five sections of the liver approximately 5-7 mm thick opposed to sampling the whole liver.
- Be sure to take a section from any grossly visible lesions.
- Be precise and make cuts (suggested: #22 blade) delicately to avoid damage of the cells.
- Sections of the liver should be preserved in the field with 10% buffered formalin (suggested: change preservative every 12 – 24 hrs if possible).
- Sections of liver should be preserved in a 10:1 ratio (formalin:liver).
- Slices used for histopathological analysis should be 4-6 µm thick.

B. HOW SHOULD EXTERNAL LESIONS BE SAMPLED?

General Discussion Summary:

- Raised Lesions: take a sectional sample of all lesions including adjacent normal tissue and underlying tissue.
- Orocutaneous lesions: should be de-calcified (remove bone) before histopathological analysis.
- Melanosis (black spots): if is not raised it should not be sampled; there is no evidence that the black spots are cancerous or are caused by contaminants.
- Ulcers: there is no need to sample (not directly caused by chemical contaminants).
- Yellow spots ("yellow jelly bumps"): we should take a few samples to determine what they are.

C. SHOULD OTHER ORAGANS BE SAMPLED?

General Discussion Summary:

- Only the liver and skin should be sampled for delisting purposes in the 16 Areas of Concern affected by the fish tumor and other deformities beneficial-use impairment.
- Other organs can be sampled for additional research purposes.

Standardized Criteria Recommendations

Monitoring and histopathological sampling recommendations were made by attendees at the conference. The recommendations are summarized in the following sections.

Monitoring Recommendations

A. DOES TUMOR PREVALENCE VARY WITH AGE?

Recommendation:
Latent period between contaminant exposure and tumor occurrence leads to young age groups not displaying neoplasms. Both total neoplasm and cancer prevalence increase with age. The tumor prevalence of a population with differing age structures cannot be compared. Smaller age groupings or similar ages should be used for comparison purposes.

B. CAN LENGTH BE USED AS AN AGE SURROGATE?

Recommendation:
Use length in the field to eliminate ages too young to display neoplasms; 250mm is a good approximation for bullhead at least 3 years old. *Length frequency should not be used as a surrogate for age* because bullheads age 3 and older tend to add growth in length very slowly; therefore, overlap in ages could occur with fish the same length. Thus, all fish sampled should be aged.

C. SHOULD SPINES AND OTOLITHS BE USED FOR AGING?

Recommendation:
General consensus is to use the otoliths for aging because they are widely accepted to be the most accurate.

D. SHOULD AGES BE GROUPED?

Recommendation:
Age by age comparison is best if sufficient numbers are available. Group ages if greater numbers are needed for statistical analysis.

E. WHAT LEVEL OF PROBABILITY IS NEEDED?

More consultation is necessary on this topic.

F. CAN REFERENCE SITES AND DATES BE COMBINED?

Further research is necessary on this topic.

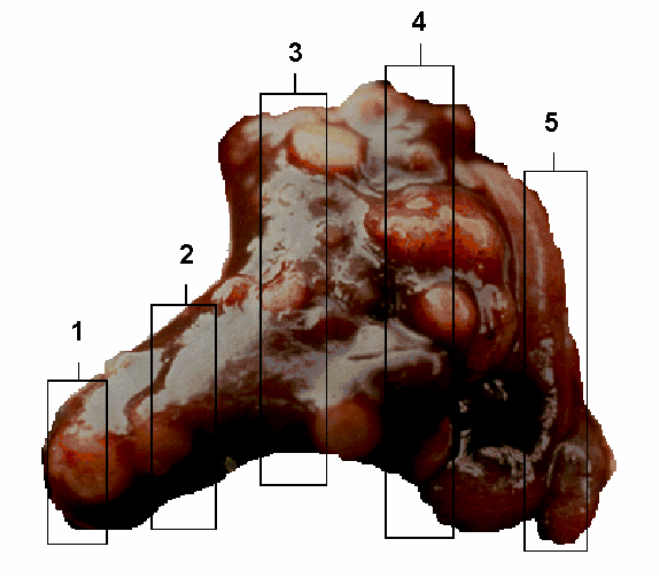
Histopathology Recommendations

A. HOW SHOULD THE LIVER BE SAMPLED?

Recommendation:

Protocol for Sampling Liver Tissue:

1. Fish should be randomly selected for necropsy; eliminate bias of sampling by avoiding intentionally taking unhealthy looking fish.
2. Remove gallbladder and weigh liver.
3. Using a sharp blade make five cross sectional cuts 5-7mm in thickness working from anterior to posterior. See diagram. If a grossly observable lesion is present on the liver be sure to include it in one of the cross sections.
4. The amount of preservative should be a 10:1 ratio of fixative to tissue, using 10% buffered formalin.

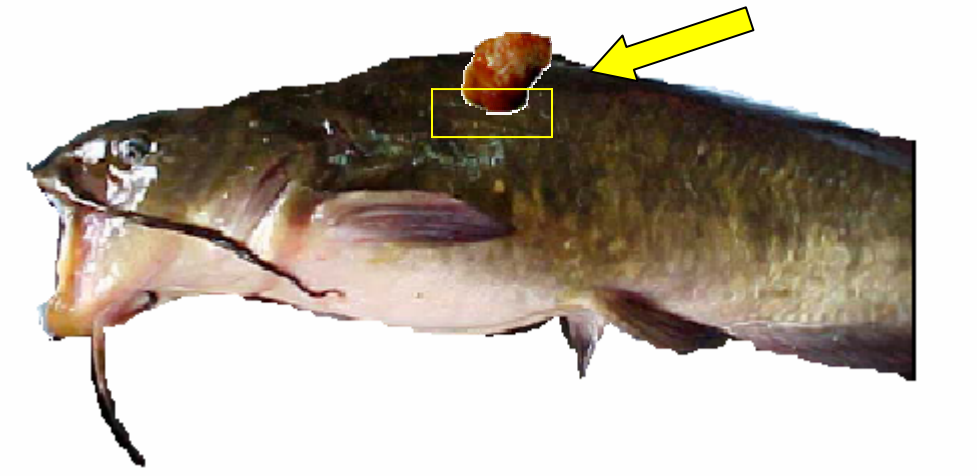


B. HOW SHOULD EXTERNAL LESIONS BE SAMPLED?

Recommendation:

Protocol for sampling of external raised lesions:

1. Document location of lesion(s) and severity.
2. Take a 5-7mm cut of each external raised lesion including margin of adjacent and underlying normal tissue. See diagram.
3. Do not routinely sample ulcerated lesions.
4. Do not routinely sample non-raised melanistic spots.



C. SHOULD MELANISTIC SKIN AREAS BE SAMPLED?

Recommendations:
Sample 1-2 raised melanistic areas from each fish for a period of two years to determine the nature of these areas.

D. SHOULD OTHER ORAGANS BE SAMPLED?

Recommendation:
For Area of Concern monitoring, evaluation, and delisting purposes only the liver is to be sampled.

Research Needs

Attendees of the conference discussed many areas in which further research was needed in order to complete their recommendations for the standardization of protocol for assessing the fish tumors or other deformities beneficial-use impairment in Areas of Concern. The following list highlights the research topics raised by the conference participants.

The hottest issue is determining a realistic background rate of liver and skin tumors for delisting purposes. Background rates are difficult to standardize because virtually all sites on the Great Lakes are thought to be impacted by anthropogenic sources of pollution. However, discussion among conference participants resulted in several alternatives for determining background rates. First, unimpacted inland lakes can be investigated to determine if background rates for liver tumors are near 0%. This could lead to the determination of what might be the ultimate achievable goal for tumor rates (how good is good?). Second, investigate potential reference sites in Lake Erie, which contain brown bullheads, but are not listed as an Area of Concern (e.g. embayments in the Western Basin Islands). Also, bullhead data from such new locations could be combined with data from historical reference sites, such as Old Woman Creek, to provide a lakewide tumor prevalence rate (as long as data meets the minimum agreed upon data quality standards). Tumor rates in brown bullheads found in a Lake Erie Area of Concern could then be compared to the Lake Erie background rate in order to delist that Area of Concern.

As stated in the monitoring recommendations section, otoliths are to be used for aging opposed to spines. Age comparison work conducted by the Penn State Cooperative Fisheries Laboratory (P. Kovinski) and Southern Illinois University Cooperative Fisheries Laboratory (B.Tezlaff) on bullheads from Presque Isle Bay and other Areas of Concern has shown otolith aging to be more accurate, especially at ages 5 and older. However, many past Great Lakes studies have used spines for age analysis. Research is needed to see if past studies utilizing spines can be compared to new studies using otoliths. The Presque Isle Bay research team has been collecting both otoliths and spines in order to age brown bullheads. It is proposed that the Presque Isle Bay Area of Concern data for brown bullhead ages, from both otoliths and spines, be compared to determine the degree of agreement between otolith and spine analysis and/or culture bullheads, in a controlled environment, to a known age (e.g. raising young of the year bullheads to age 8) and then compare otolith age and spine age to the known age to resolve the question concerning the two aging techniques. Information obtained from this study would verify whether old spine analysis data could be compared to future otolith data.

Participants at the conference determined that length should not be used as an age surrogate because bullheads age 3 and older tend to add growth in length very slowly; therefore, overlap in ages could occur with fish the same length. It is proposed that further aging research be conducted to determine the mean length of brown bullhead age 3, and use that length as a cutoff for sampling efforts.

Contaminants such as PAHs are often attributed to be the cause of tumors in brown bullheads. However, it is speculated that genetic differences among isolated populations of bullheads may contribute to a high tumor incidence in selected bullhead populations or that hybridization

between brown and black, or brown and yellow bullheads has created hybrid populations with a higher predisposition for tumors. Elevated gonadal tumor rates have been observed in hybrid carp and goldfish (Harshbarger, 2003). Research is currently being conducted on Presque Isle Bay bullheads to determine if the species sampled in this Area of Concern are brown, brown/black, or brown/yellow hybrids. Further research may be needed to determine if hybridization between bullhead species does lead to differential liver or skin tumor incidence in these populations.

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Attendees/ Contact List

Paul Baumann
Biological Resources Division, USGS
The Ohio State University
School of Natural Resources
473B Koffman Hall
2021 Coffrey Road
Columbus, OH 43210
(614) 469-5701
baumann.1@osu.edu

Lori Boughton
PA Department of Environmental Protection
230 Chestnut Street
Meadville, PA 16335
(814) 332-6816
lboughton@state.pa.us

Anne Danielski
PA Sea Grant
Penn State Erie
5091 Station Road
Erie, PA 16563-0504
(814) 898-6420
add118@psu.edu

Jim Grazio
PA Department of Environmental Protection
230 Chestnut Street
Meadville, PA 16335
(814) 332-6816
jagrazio@state.pa.us

John Harshbarger
George Washington University
Medical Center
Department of Pathology
2300 Eye Street, NW
Washington, DC 20037
(202) 994-3391
jcharshbarger@erols.com

Vicki Blazer
USGS, National Fish Health
Research Laboratory
1170 Leetown Road
Kearneysville, WV 25430
(304) 724-4434
vicki_blazer@usgs.gov

John Cingolani
PA Sea Grant
Penn State Erie
5091 Station Road
Erie, PA 16563-0504
(814) 898-6420
jkc140@psu.edu

Jack Fournie
U.S. EPA, National Health &
Environmental Effects Research Lab
Gulf Ecology Division
1 Sabine Island Drive
Gulf Breeze, FL 32561-5299
(850) 934-9272
fournie.john@epa.gov

Father Joseph Gregorek
Gannon University
Erie, PA 16541
(814) 871-7323

Dick Kubiak
2534 East 33rd Street
Erie, PA 16510
(814) 899-9676

Kelly Montgomery
International Joint Commission
100 Ouellette Ave., 8th Floor
Windsor, Ontario N9A 6T3
(519) 257-6723
(313) 226-2170 ext. 6723
montgomeryk@windsor.ijc.org

Eric Obert
PA Sea Grant
Penn State Erie
5091 Station Road
Erie, PA 16563-0504
(814) 898-6420
eco1@psu.edu

Sean Rafferty
PA Sea Grant
5091 Station Road
Erie, PA 16563-0504
(814) 898-6420
sdr138@psu.edu

Nick Schneider
International Joint Commission
Great Lakes Regional Office
100 Ouellette Avenue, 8th Floor
Windsor, Ontario N9A 6T3
nschneid@uoguelph.ca

Stanley Zagorski
Gannon University
Biology Department
Erie, PA 16541
(814) 871-7641
zagorski@gannon.edu

Chuck Murray
PA Fish and Boat Commission
Box 531
Farview, PA 16415
(814) 474-1515
chamurray@state.pa.us

Fred Pinkney
U.S. Fish and Wildlife Service
Chesapeake Bay Field Office
177 Admiral Cochrane Drive
Annapolis, MD 21401
(410) 573-4521
Fred_Pinkney@fws.gov

Jim Rutkowski
Strong Vincent High School
1330 West 8th Street
Erie, PA 16502
(814) 874-6522
jk.touch@verizon.net

Bob Wellington
Erie County Health Department
606 West 2nd Street
Erie, PA 16507
(814) 451-6772
c-rwelling@state.pa.us