

Field Manual for Assessing Internal and External Anomalies in Brown Bullhead (*Ameiurus nebulosus*)



Sean Rafferty¹

Jim Grazio²

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*

Pennsylvania Sea Grant, part of the National Sea Grant Program, is a partnership of Penn State, the Commonwealth of Pennsylvania, and the National Oceanic and Atmospheric Administration. This publication is available in alternative media upon request. Penn State is committed to affirmative action, equal opportunity, and the diversity of its workforce. U.Ed. EBO 07-267. Published in December 2006.

ACKNOWLEDGEMENTS

The development of this document, *A Field Manual for Assessing Internal and External Anomalies in Brown Bullhead (Ameiurus nebulosus)*, has been a joint effort of Pennsylvania Sea Grant (PASG) and the Pennsylvania Department of Environmental Protection (DEP). The manual was made possible through funding awarded by the United States Environmental Protection Agency's Great Lakes National Program Office (USEPA GLNPO).

We gratefully acknowledge and appreciate the time that a number of individuals took to review and contribute their expertise to the manual. Reviewers included: Dr. Paul Baumann, United States Geological Survey (USGS); Dr. Vicki Blazer, USGS; Steve Smith, USGS; Dr. Fred Pinkney, United States Fish and Wildlife Service (USFWS); Dr. Robert Light, Penn State Behrend and PASG; Eric Obert, PASG; Chuck Murray, Pennsylvania Fish and Boat Commission (PFBC); Bob Wellington, Gannon University; Doug Ebert, Erie County Department of Health; Lori Boughton, DEP; Scott Dudzic, DEP; Erin Stuart, DEP; and Rich Neville, DEP.

TABLE OF CONTENTS

1.0	Introduction	1
1.1	Background Information	1
1.2	Purpose of the Manual	1
1.3	Relationship to Other Field Guides and Manuals	1
2.0	Recommended Equipment and Supplies	2
2.1	Field Collections	2
2.2	Processing Fish	2
2.3	Additional Safety Equipment	2
3.0	Anatomy	3
3.1	External Anatomy	3
3.2	Internal Anatomy	4
4.0	Field Collection	5
4.1	Collection Permit Requirements	5
4.2	Collection Methods	5
4.3	Electrofishing Safety	5
4.4	Holding Methods	5
5.0	Processing Fish	6
5.1	Work Area	6
5.2	Recording Data in the Field	6
5.2.1	Standard Data	6
5.3	Gross Visual Observations: Lesions and Anomalies	7
5.4	External and Internal Necropsy	14
5.4.1	Euthanizing	14
5.4.2	Raised Epidermal Lesion Biopsy	15
5.4.3	Raised Mouth Lesion Biopsy	15
5.4.4	Liver Biopsy	15
5.5	Bullhead Aging	17
5.5.1	Otolith Removal	18
5.5.2	Spine Removal	19
6.0	Post-Processing Procedures	20
6.1	Data Verification	20
6.2	Clean-up	20
6.3	Shipping Samples	20
7.0	Fish Health Data Sheet	21
8.0	References	22

FIGURES

Figure 1:	External Anatomy	3
Figure 2:	Internal Anatomy	4
Figure 3:	Holding Tank	5
Figure 4:	Outdoor Work Area Setup	6
Figure 5:	Total Length Measurement	6
Figure 6a:	Raised Black Skin Lesion (mild)	8
Figure 6b:	Raised Black Skin Lesions (moderate)	8
Figure 6c:	Raised Black Skin Lesions (severe)	8
Figure 6d:	Raised Yellow Skin Lesions (severe)	8
Figure 7a:	Raised Mouth Lesion (mild)	9
Figure 7b:	Raised Mouth Lesion (mild)	9
Figure 7c:	Raised Mouth Lesion (moderate)	9
Figure 7d:	Raised Mouth Lesions (severe)	9
Figure 8a:	Shortened Barbel (mild)	9
Figure 8b:	Multiple Shortened Barbels (moderate)	10
Figure 8c:	Missing Barbel (mild)	10
Figure 8d:	Deformed Barbel (mild)	10
Figure 9a:	Black Pigmentation (mild)	10
Figure 9b:	Black Pigmentation (moderate)	10
Figure 9c:	Black Pigmentation (severe)	11
Figure 9d:	Yellow Pigmentation (mild)	11
Figure 10a:	Fin Erosion (severe)	11
Figure 10b:	Fin and Body Erosion (severe)	11
Figure 11a:	Mouth Ulcer (mild)	12
Figure 11b:	Epidermal Ulcer (mild)	12
Figure 11c:	Epidermal Ulcer (moderate)	12
Figure 11d:	Epidermal Ulcers (severe)	12
Figure 12a:	Scarring	12
Figure 12b:	Left Pectoral Spine Wound (moderate)	13
Figure 12c:	Wound (severe)	13
Figure 12d:	Infected Wound (severe)	13
Figure 13a:	Normal Eye	13
Figure 13b:	Cloudy Right Eye (moderate)	13
Figure 13c:	Discolored Right Eye (mild)	14
Figure 13d:	Missing Right Eye (moderate)	14
Figure 14:	Raised Epidermal Lesion	15
Figure 15:	Raised Mouth Lesion	15
Figure 16a:	Exposed Liver	15
Figure 16b:	Exposed Organs	16
Figure 17a:	Normal Liver	16
Figure 17b:	Granular, Discolored Liver	16
Figure 17c:	Liver Lesion	16
Figure 17d:	Pale Liver with Heavy Parasite Burden	16
Figure 18:	Liver with Attached Gallbladder	17
Figure 19:	Liver Biopsy	17

FIGURES CNTD.

Figure 20a:	Mid-Dorsal Head Incision	18
Figure 20b:	Separated Head Cavity	18
Figure 20c:	Left Auditory Capsule	19
Figure 20d:	Right Auditory Capsule	19
Figure 20e:	Otolith	19
Figure 21:	Pectoral and Dorsal Spines	19

1.0 INTRODUCTION

1.1 Background Information

Annex 2 of the Great Lakes Water Quality Agreement (GLWQA) between the United States and Canada called for the identification of the most severely degraded geographical Areas of Concern (AOCs) within the Great Lakes basin and the development of Remedial Action Plans (RAPs) to restore impaired beneficial uses within these areas. Annex 2 specifically lists fourteen discrete Beneficial Use Impairments (BUIs) for AOCs, including “fish tumors or other deformities.” As defined by International Joint Commission (1991) guidelines, the fish tumors or other deformities BUI occurs:

“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 pre-neoplastic liver tumors in bullheads or suckers.”

The fish tumors or other deformities BUI has been identified in 14 of the 31 AOCs located within or partially within the United States. In U.S. AOCs, this BUI is most often related to the brown bullhead catfish (*Ameiurus nebulosus*). Therefore, the ability to accurately and consistently identify tumors or other deformities in brown bullhead is critical for proper assessment and monitoring of the status of this BUI.

1.2 Purpose of the Manual

This field guide is the result of decades of experience with brown bullhead in Presque Isle Bay, located along the southern shore of Lake Erie in northwestern Pennsylvania. The fish tumors or other deformities BUI was listed as impaired in Presque Isle Bay based on a high incidence rate of tumorous growths and other lesions in the bay’s brown

bullhead population. Extensive investigation of this BUI has occurred since Presque Isle Bay was designated as the 43rd and final AOC in 1991, and active monitoring has continued since Presque Isle Bay became the first U.S. AOC to attain the “Recovery Stage” designation in 2002. One of the most important lessons learned in the course of the work in Presque Isle Bay was the need for a clear, consistent, and standardized approach for assessing and tracking this BUI over time. This 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;
- recommend standard operating procedures for the necropsy of brown bullhead.

1.3 Relationship to Other Field Guides and Manuals

Several excellent field guides and recommended Standard Operating Procedures have been developed for field assessments of fish health, including, but not limited to:

- *Illustrated Field Guide for Assessing External and Internal Anomalies in Fish* (Smith et al., 2002).
- *Fish Health/Condition Assessment Procedures Part 1* (Goede, 1993).
- *Gross Signs of Tumors in Great Lakes Fish: A Manual for Field Biologist* (Black, n.d.).

While this guide is consistent with the existing literature, it was specifically written to facilitate the assessment and monitoring of tumors or other deformities in brown bullhead.

2.0 RECOMMENDED EQUIPMENT AND SUPPLIES

2.1 Field Collections

Collection Equipment

- √ Electrofishing boat and associated gear
- √ Hoop or trapnets
- √ Seines
- √ Large tubs or live wells
- √ Aerators
- √ Dip nets
- √ Polarized sunglasses

Field Safety

- √ Personal Flotation Devices (one for each person onboard)
- √ Throwable Flotation Device
- √ Visual and auditory distress signaling devices (e.g. flares, whistles, horn, etc.)
- √ Marine radio and/or cell phone
- √ Fire extinguisher
- √ Anchor with sufficient line
- √ Rubber linesman's gloves
- √ Heavy duty work gloves
- √ Rubber boots/waders
- √ Automated External Defibrillator (AED) kit
- √ First aid kit
- √ Ear protection
- √ Additional boater safety equipment required by your jurisdiction

2.2 Processing Fish

Data Collection and Recording

- √ Field data sheets (see *Section 7.0*)
- √ Chain-of-custody and sample submission sheets
- √ High quality digital camera
- √ Mechanical pencils and/or pens
- √ Permanent markers
- √ Index cards
- √ Masking tape
- √ Clipboard

- √ Field guide
- √ Material safety data sheets for all chemicals

Fish Health Assessment

- √ Fish measuring board
- √ Balance (minimum 1 kg capacity)
- √ Anesthetic (e.g., MS-222)
- √ Dissecting trays
- √ Dissecting kit, including
 - Scalpel with disposable blades
 - Forceps (fine tipped)
 - Small scissors
 - Bone cutters or heavy duty kitchen shears
 - Bone saw or hacksaw (for otolith removal)
 - Pliers (for pectoral spine removal)
- √ 125 or 250 ml polyethylene containers for tissue preservation
- √ 10% neutral-buffered formalin for tissue preservation
- √ Scale envelopes or cryovials
- √ Coolers

Cleanup/Miscellaneous

- √ Garbage bags
- √ Paper towels
- √ Deionized rinse water or 80% ethanol, water solution
- √ Duct tape

2.3 Additional Safety Equipment

Protective Apparel

- √ Latex or nitrile gloves
- √ Aprons
- √ Raingear
- √ Safety glasses

3.0 ANATOMY

3.1 External Anatomy

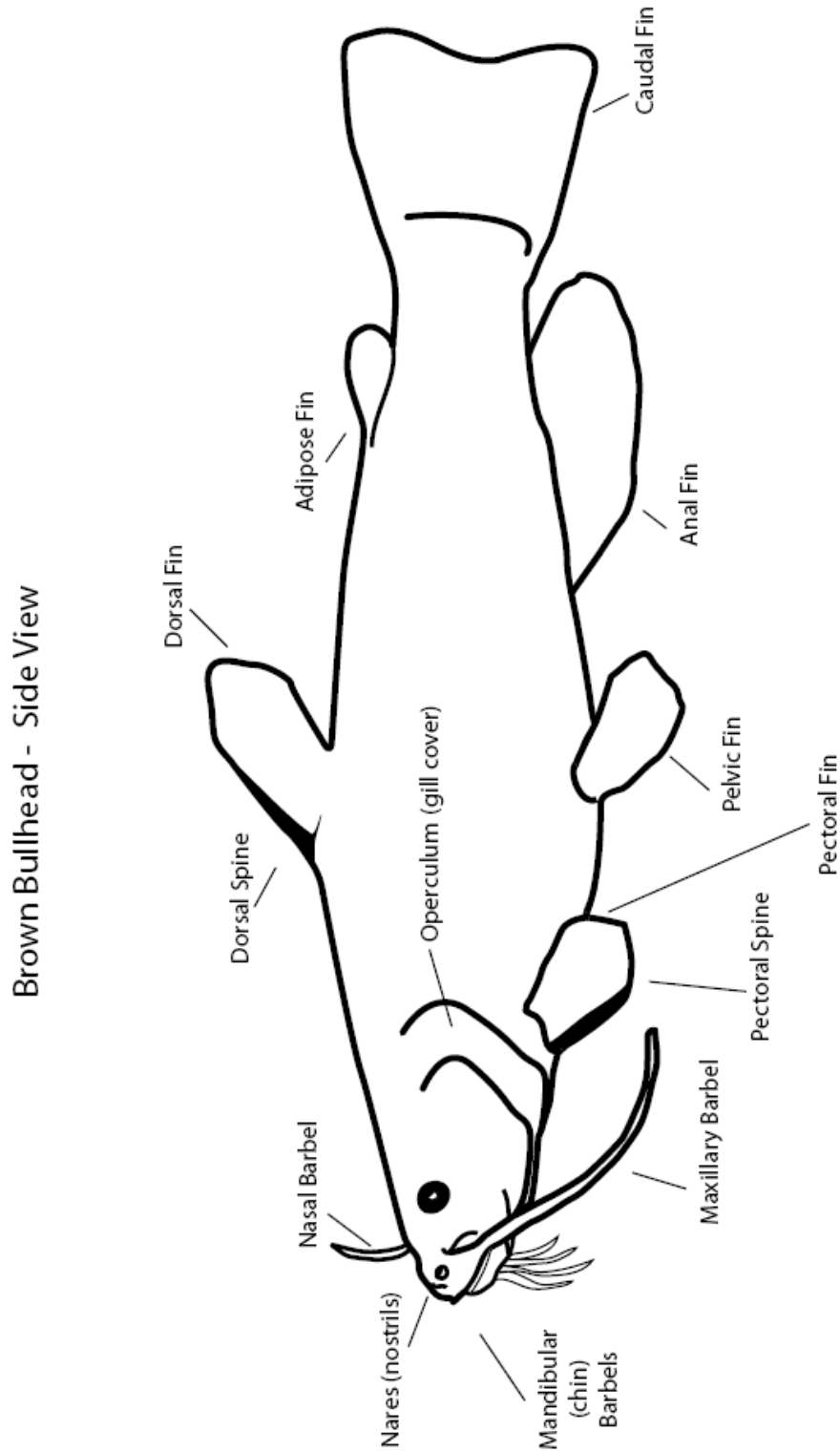


Figure 1: External Anatomy

3.2 Internal Anatomy

Brown Bullhead Internal Anatomy Diagram

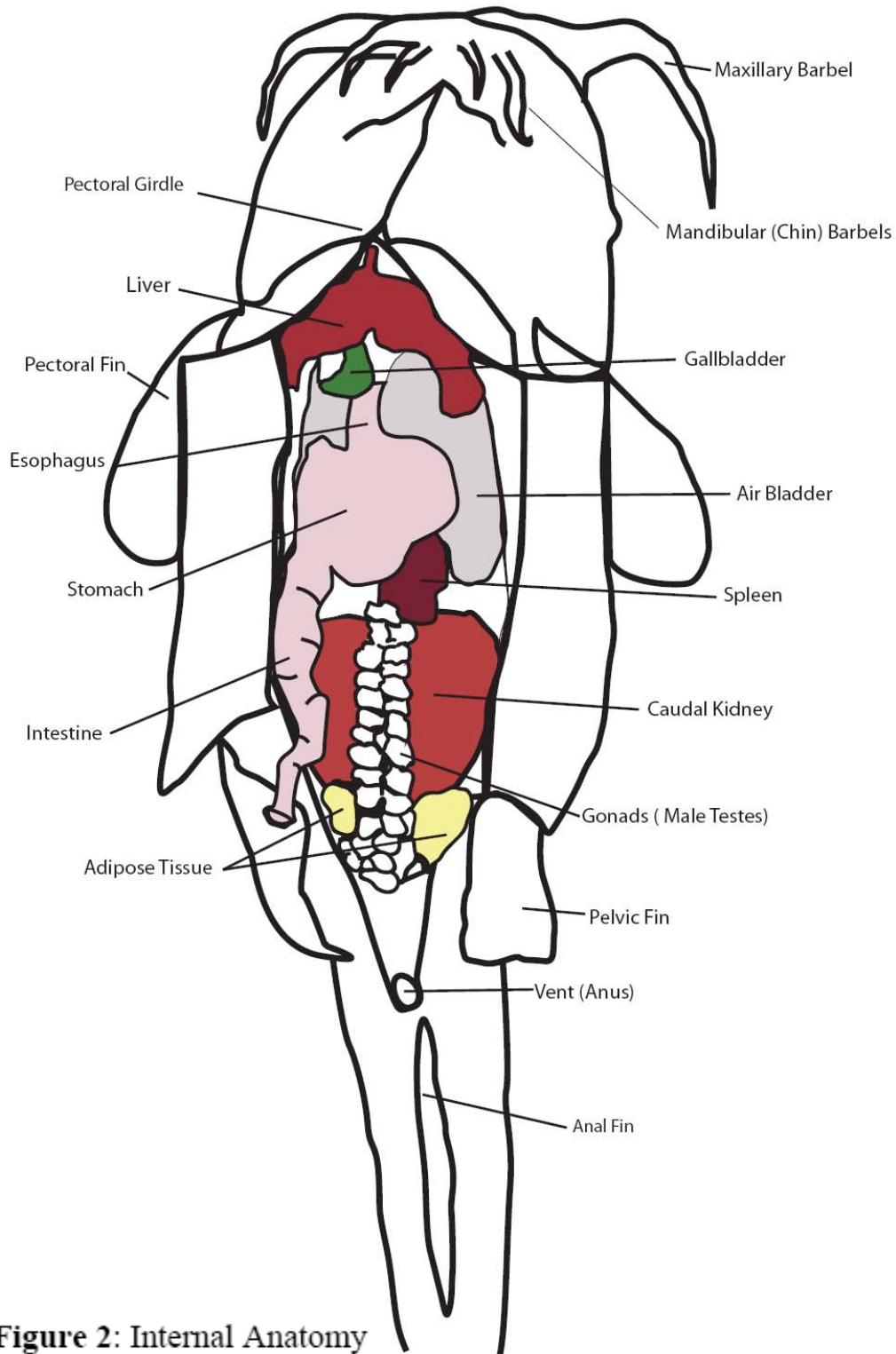


Figure 2: Internal Anatomy

4.0 FIELD COLLECTION

4.1 Collection Permit Requirements

In most jurisdictions, a Scientific Collector's Permit or the equivalent must be held by one or more of the field sampling crew. The principal investigator should consult with the appropriate state or provincial fishery agency to ensure that all required permits and permissions are secured prior to the commencement of any field collections.

4.2 Collection Methods

Bullhead are readily collected using standard field sampling gear such as electrofishing boats, trapnets, hoop nets, and seines. Collection gear should be selected in accordance with the goals of the study. Collections using shallow-water gear (e.g., electrofishing) should be conducted during an "index period" between April and June. During this period, bullhead tend to migrate into near-shore habitat to spawn, making them easier to collect.

4.3 Electrofishing Safety

**Important Safety Note:* Prior to boat electrofishing, be sure to check that you have all the necessary and required safety equipment. A list of recommended equipment can be reviewed in Section 2.0. Operators of electrofishing equipment should be properly trained and certified to use such equipment, and hold current cardiopulmonary resuscitation (CPR) and first aid certifications.

4.4 Holding Methods

Fish should be held in a well-aerated holding tank until processed. A flow-through system is preferable, although mechanical aerators may also be used. Refer to *Figure 3* for sample tank setup. Note that overcrowding

can lead to injury and the spread of disease. *Fish that die while being held in the holding tanks should not be grossly assessed or necropsied because of the possible occurrence of postmortem lesions.* Necropsy, in the context of this manual, refers to a postmortem examination. Biopsy, conversely, refers to the removal of tissue for diagnostic purposes.



Figure 3: Holding Tank

5.0 PROCESSING FISH

All brown bullhead collected should be grossly assessed for anomalies prior to necropsy. Other incidentally captured *Ameiurus* spp. may also be examined if consistent with the study goals. A representative subsample of brown bullhead should be necropsied for histopathological analysis of selected tissues. It is important to randomly subsample fish for this purpose as opposed to purposively selecting individuals which appear healthy or unhealthy in order to avoid biasing tumor rate estimates. Detailed instructions for processing bullhead are provided in *Sections 5.1-5.5*

5.1 Work Area

Indoor laboratories provide an ideal workspace for processing fish; however, assessments are often performed outdoors at the field collection sites. In the latter case, it is important to have a large enough surface to complete all tasks. Tasks may include:

- obtaining fish lengths and weights;
- gross observation of anomalies;
- necropsy and biopsy of fish;
- otolith removal for aging;
- photography;
- data recording.



Figure 4: Outdoor Work Area Setup.

A table-sized work area should suffice. A plastic cover (e.g. large garbage bags) will

help to keep body fluids of the fish from staining the table. Refer to *Figure 4* for a sample outdoor work area setup.

5.2. Recording Data in the Field

A standardized field data sheet such as the *Fish Health Data Sheet* included in *Section 7.0* will facilitate the consistent recording of data in the field. The designated data recorder should be properly trained to record data and complete each *Fish Health Data Sheet* in its entirety.

5.2.1 Standard Data

The following data, at a minimum, should be recorded on the *Fish Health Data Sheet*:

- Unique identification number. One simple approach is to use a sequential numbering system for each year of the study. For example, the first fish sampled in 2006 would receive the identification number 2006-0001, the next 2006-0002, and so on.
- Collection and processing dates.
- Field observers present.
- Collection location.
- Capture gear.
- Total length, in millimeters. Total length is measured as the length from the snout to the farthest point of the slightly compressed caudal fin. Refer to *Figure 5* for total length measurement detail.



Figure 5: Total Length Measurement.

- Weight, in grams.
- Sex and aging technique (for necropsied specimens).

5.3 Gross Visual Observations: Lesions and Anomalies

Following the recording of standard data, fish should be assessed for the presence of any grossly observable external anomalies (i.e. lesions and deformities). In general:

- record any notes taken in the field and use the diagrams included on the *Fish Health Data Sheet* (Sec. 7.0) to document the locations of anomalies;
- photograph anomalies whenever possible and include the fish identification number in the photograph by placing a numbered index card or scale envelope next to the fish.

A variety of anomalies have been described for brown bullhead, including:

- barbel abnormalities;
- raised skin lesions;
- raised mouth lesions;
- abnormal pigmentation (typically yellow and black);
- fin erosion;
- ulcers;
- scar/wounds;
- eye abnormalities.

These anomalies may reflect varying causes ranging from chemical exposure to infectious disease. All of these anomalies provide information about the general condition of the subject population. However, for the purpose of assessing the fish tumors or other deformities BUI, we prefer to focus on raised skin and mouth (lip) lesions, and liver lesions. Grossly observable raised skin and mouth lesions correlate well with the presence of histologically verified neoplasms (i.e., true “tumors”) in Presque Isle Bay and

therefore directly address the “tumor” portion of the IJC’s listing guidelines for this BUI. There is also experimental evidence that skin lesions can be induced in brown bullhead by the application of chemically contaminated sediment to the epidermis (Black, 1983).

We treat barbel deformities, which may include nodules or other raised lesions of the barbel, separately from raised mouth and skin lesions. While some investigators have found an association between the presence of barbel deformities and contaminated sediment (Smith et al., 1994; Steyermark et al., 1999; Yang et al., 2003), the association is inconsistent (Poulet et al., 1994) and a mechanistic linkage between barbel abnormalities and neoplasia has not been established (Pinkney and Harshbarger, 2005). In addition, trematode parasites have been associated with raised barbel and head lesions in brown bullhead and these lesions look very similar to true neoplasia upon gross observation (Blazer et al., in press). Therefore, we prefer to treat barbel anomalies and other abnormalities as “other deformities” under the IJC definition.

It is also important to recognize that lesions and deformities occur in varying degrees from mild to severe. For the purpose of calculating incidence rates, the simple occurrence of a particular anomaly, regardless of its severity, is sufficient to classify the specimen as possessing the anomaly in question. Still, while admittedly a subjective measurement, we have found it useful to record the severity of the lesion as well to help understand trends in the study population over time. We use the following scale for this purpose:

- 0: No visible anomaly
- 1: Mild Condition
- 2: Moderate Condition
- 3: Severe Condition

Raised Skin Lesions

In the field, it is appropriate to classify raised tissue as raised lesions rather than as tumors. Tumors (i.e. neoplastic growths) are abnormal growths of tissue that can only be confirmed by histopathological analysis. Raised skin lesions are commonly black in color; however, normal skin-colored, yellow, pink, and white raised lesions have been noted. Field biologists should feel any focal discoloration to determine whether or not it is a raised lesion. Refer to *Figures 6a-d* for examples of raised-skin lesions.

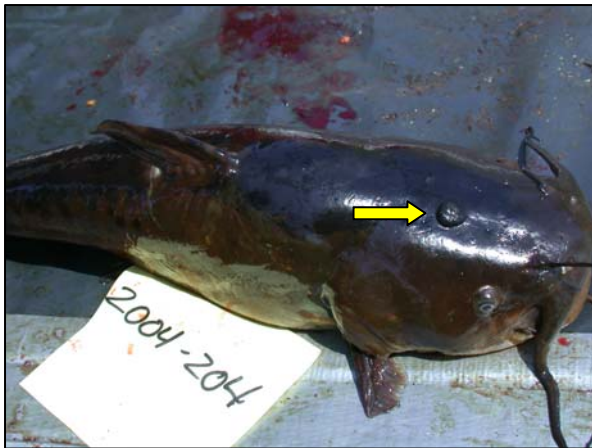


Figure 6a: Raised Black Skin Lesion (mild)

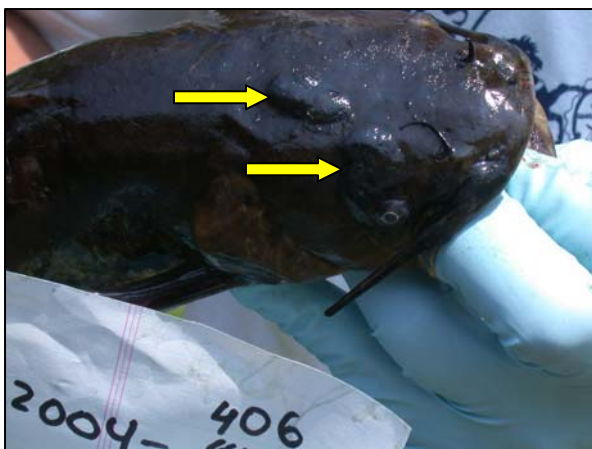


Figure 6b: Raised Black Skin Lesions (moderate)

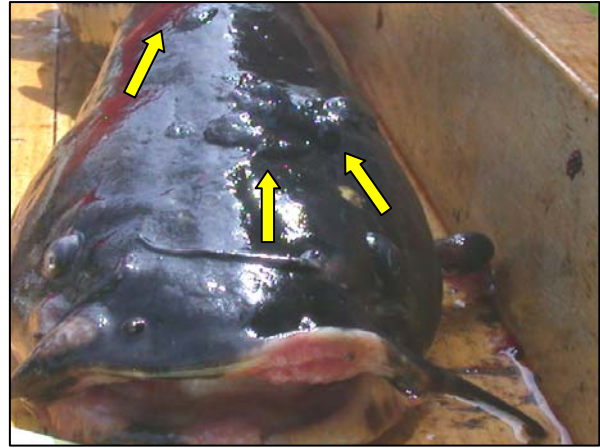


Figure 6c: Raised Black Skin Lesions (severe)

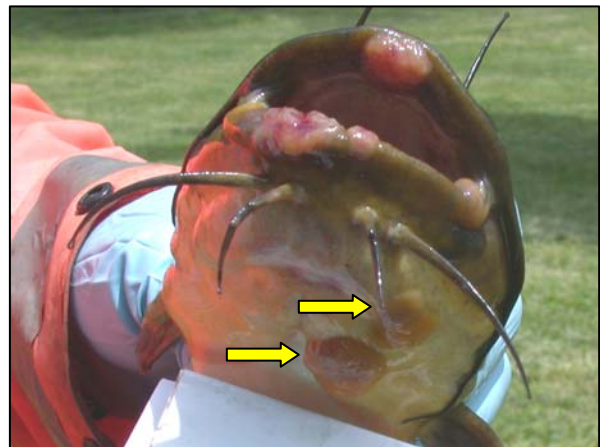


Figure 6d: Raised Yellow Skin Lesions (severe)

Raised Mouth Lesions

Raised mouth (lip) lesions are commonly bright to light-red or creamy/white colored. Again, the field biologist should feel the oral tissue for the presence of raised lesions. With experience, lesions are readily distinguished from normal tissue. Refer to *Figures 7a-d* for examples of raised-mouth lesions.



Figure 7a: Raised Mouth Lesion (mild)

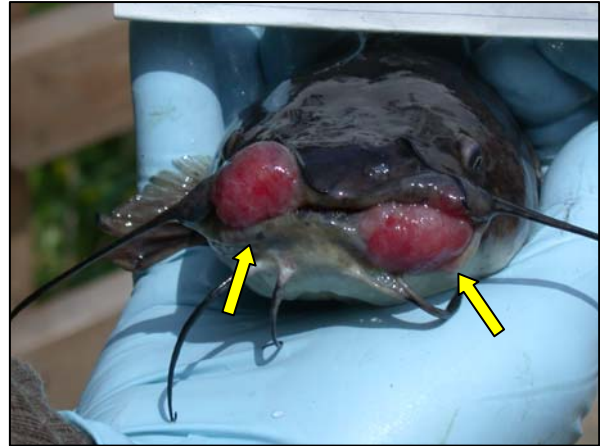


Figure 7d: Raised Mouth Lesions (severe)



Figure 7b: Raised Mouth Lesion (mild)



Figure 7c: Raised Mouth Lesion (moderate)

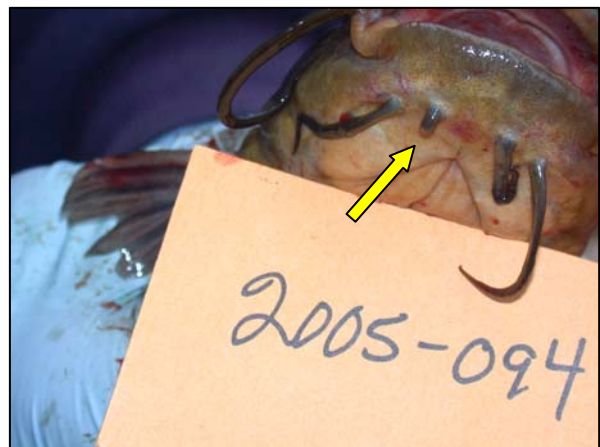


Figure 8a: Shortened Barbel (mild)

Barbel Abnormalities

Bullheads have eight whisker-like barbels, four on the chin (mandibular), two on the upper jaw (maxillary), and two near the nares. Common barbel anomalies include shortened barbels, missing barbels, and/or deformed (clubbed, forked, or stubbed) barbels. Refer to *Figures 8a-d* for barbel anomalies.

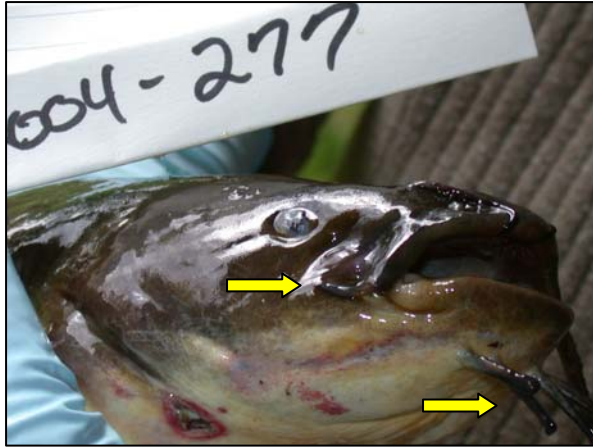


Figure 8b: Multiple Shortened Barbels (moderate)



Figure 8c: Missing Barbel (mild)



Figure 8d: Deformed Barbel (mild)

Pigmentation

Brown bullhead can exhibit irregular areas of black and/or yellow pigmentation on the body and mouth, with black pigmentation being more common. This abnormal pigmentation may or may not progress to neoplasia; however, any areas of irregular pigmentation should be noted. Refer to *Figures 9a-d* for examples of pigmentation.



Figure 9a: Black Pigmentation (mild)



Figure 9b: Black Pigmentation (moderate)



Figure 9c: Black Pigmentation (severe)

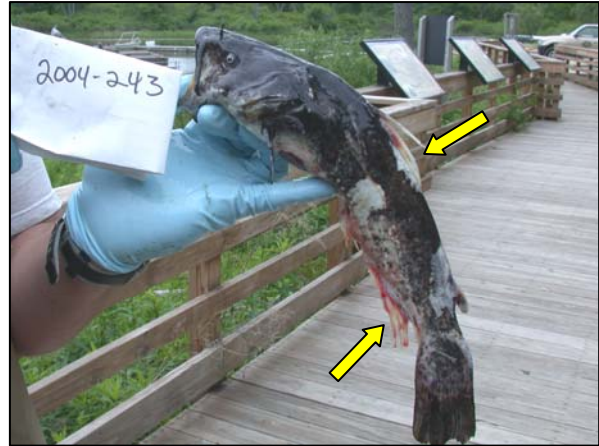


Figure 10a: Fin Erosion (severe)



Figure 9d: Yellow Pigmentation (mild)



Figure 10b: Fin and Body Erosion (severe)

Fin Erosion

Fin erosions are characterized by the loss of soft, inter-ray fin tissue and active hemorrhaging may be present in moderate-severe cases. In extremely severe cases, erosions can include the body tissue as seen in Figure 10b. Refer to *Figures 10a-b* for examples of fin erosion.

Ulcers

An ulcer is characterized by the perforation of all layers of the skin that may lead to tissue necrosis. Refer to *Figures 11a-d* for examples of ulcers.

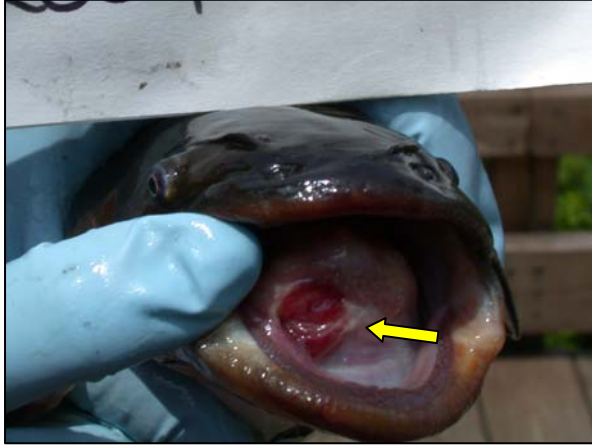


Figure 11a: Mouth Ulcer (mild)

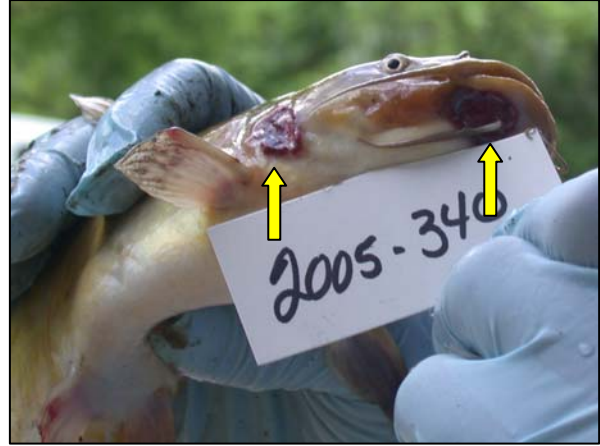


Figure 11d: Epidermal Ulcers (severe)

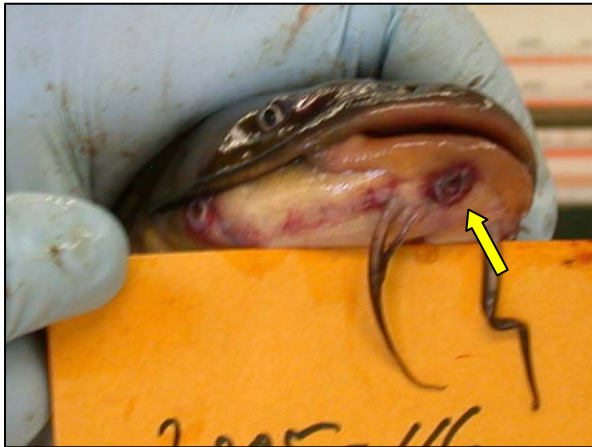


Figure 11b: Epidermal Ulcer (mild)



Figure 11c: Epidermal Ulcer (moderate)



Figure 12a: Scarring

Scars/Wounds

Scarring and wounds may either be the result of interactions with other fish and anglers or an artifact of being held in the holding tank prior to processing. Refer to *Figures 12a-d* for examples of scars and wounds.

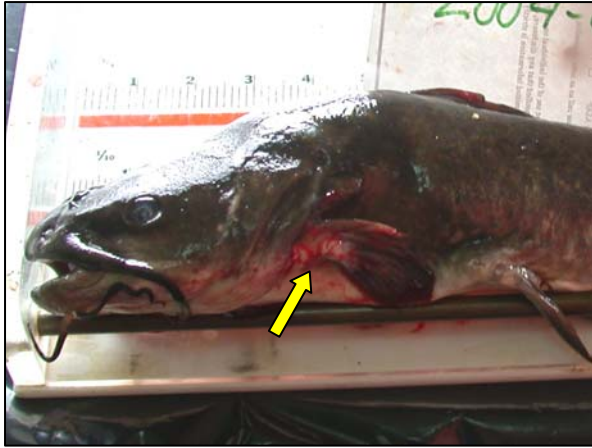


Figure 12b: Left Pectoral Spine Wound (moderate)



Figure 12c: Wound (severe)



Figure 12d: Infected Wound (severe)

Eye Abnormalities

Abnormalities of the eye commonly include cloudiness of the eye(s), including cataracts; discoloration; and/or missing eyes. Refer to *Figures 13b-d* for examples of eye anomalies.



Figure 13a: Normal Eye



Figure 13b: Cloudy Right Eye (moderate)

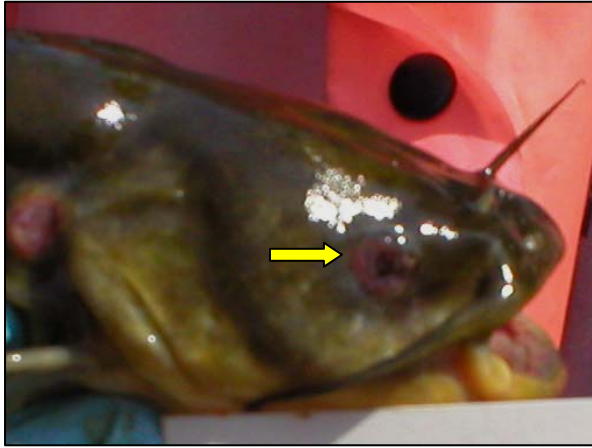


Figure 13c: Discolored Right Eye (mild)



Figure 13d: Missing Right Eye (moderate)

5.4 External and Internal Necropsy

A minimum of 30-50 brown bullhead should be randomly selected for histopathology from each sampling location, if possible. A larger subsample will increase the statistical power of the study.

In order to ensure that only adult specimens are being sampled, all fish selected for necropsy should be a minimum of 250 mm in total length. In general, brown bullhead longer than 250 mm tend to be sexually mature, 3+ year-old fish. Note those specimens selected for necropsy and histopathological analysis on the field data sheets.

For purposes of assessing the fish tumors or other deformities BUI:

- Internally, only livers from brown bullhead need to be assessed for the presence of tumors; however, additional organs may be sampled for other purposes.
- Externally, only raised skin and mouth (lip) lesions need to be sampled. Melanistic spots or other pigmentation, if not raised, should be noted but not sampled.

Prior to the biopsy of epidermal lesions, mouth lesions, or the liver:

- label a 125-250 mL polyethylene bottle with the fish identification number and processing date;
- fill the bottle approximately two-thirds of the way with 10% neutral-buffered formalin (or maintain a 10:1 fixative: tissue ratio);
- document the location and severity of the lesion;
- photograph the lesion(s).

All lesions sampled for an individual fish (i.e. raised skin, mouth, and liver) may be placed in the same polyethylene bottle provided that a 10:1 ratio of fixative to tissue is maintained to ensure proper preservation.

Outlined below are the procedures for sampling raised external lesions and internal lesions in brown bullhead.

5.4.1 Euthanizing

All fish must be humanely euthanized prior to necropsy using anesthesia or other humane methods. MS-222/Finquel®, carbon dioxide, and clove oil are commonly used anesthetics. Refer to *Prince and Powell, 2000*; *Jennings and Looney, 1998*; *Diewert et al., 1995*; and *Peake, 1998* for a review of

this topic. If chemicals are used, be sure to follow associated safety procedures, and properly dispose of chemicals and fish tissue.

5.4.2 Raised Epidermal Lesion Biopsy

Remove the raised lesion using a sharp scalpel, and place in the bottle containing buffered formalin. Each raised skin lesion sample should contain:

- a section of the raised lesion;
- adjacent normal tissue;
- underlying tissue.

The sample of surrounding normal tissue will assist the histopathologist in assessing neoplasia. Refer to *Figure 14* for raised external lesion sampling diagram.

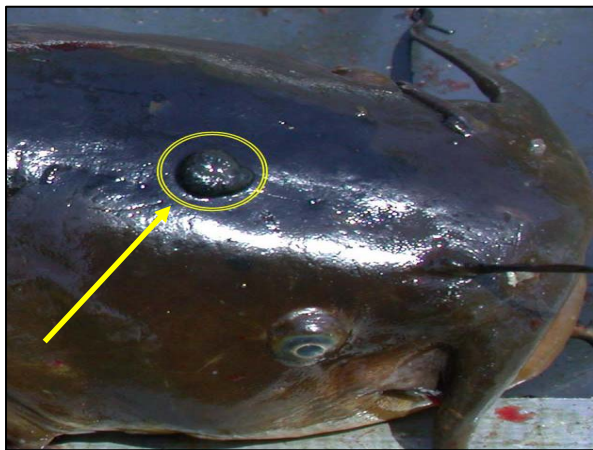


Figure 14: Raised Epidermal Lesion

5.4.3 Raised Mouth Lesion Biopsy

Remove mouth lesions using bone cutters or heavy scissors. Each raised mouth lesion sample should include adjacent and underlying normal tissue as suggested above. Refer to *Figure 15* for mouth lesion sampling diagram.

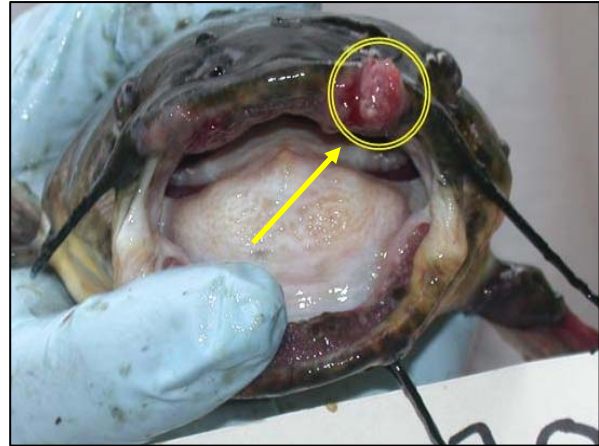


Figure 15: Raised Mouth Lesion

5.4.4 Liver Biopsy

Lesions in the liver are not always apparent to the naked eye; therefore, all livers in necropsied specimens should be assessed via histopathology for the presence of tumors.

To expose the liver (*Figure 16a*), open the abdominal cavity of the fish by making a mid-ventral incision along the abdomen from the vent to the pectoral girdle using scissors. Next, cut along both sides of the pectoral girdle to expand the thoracic cavity to facilitate organ removal (*Figure 16b*). Be sure not to puncture the internal organs while cutting; applying upward pressure while making your incision will help prevent the internal organs from being ruptured.

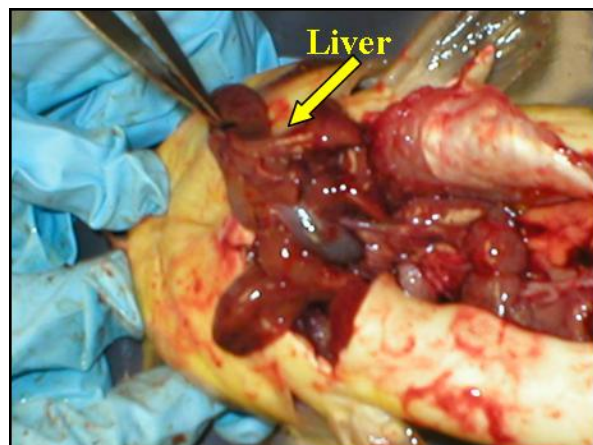


Figure 16a: Exposed Liver

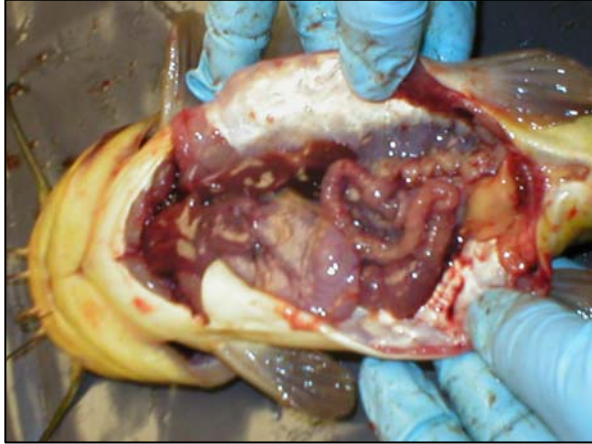


Figure 16b: Exposed Organs



Figure 17b: Granular, Discolored Liver

Prior to biopsy of liver tissue, document any grossly observable anomalies of the liver such as raised lesions, presence of parasites, and areas of general or focal discoloration (e.g. paleness or spots). A normal liver will appear light to dark-red with no discoloration, lesions, or parasites (*Figure 17a*). Refer to *Figures 17b-d* for examples of liver anomalies

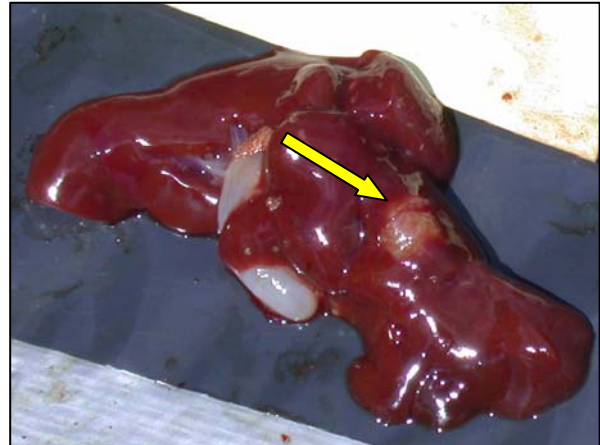


Figure 17c: Liver Lesion



Figure 17a: Normal Liver

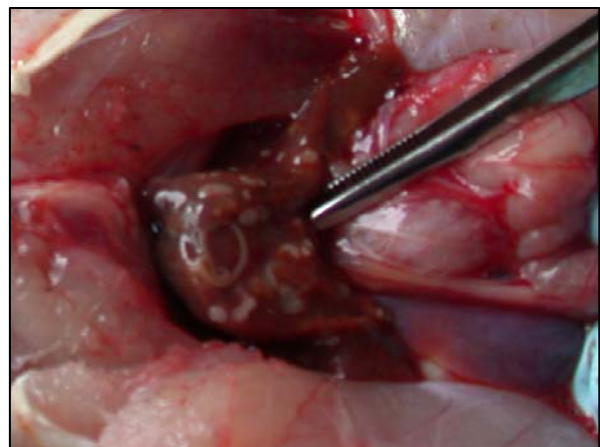


Figure 17d: Pale Liver with Heavy Parasite Burden

Processing the liver for histopathology:

- Sever the hepatic vein at the anterior end of the liver and carefully remove the liver from the body cavity by cutting away the connective tissue (see *Figure 16a* for liver location), making sure not to rupture the gallbladder.
- Once the liver is removed from the body, the gallbladder should be separated by suspending it from the liver, clamping the connecting common bile duct with forceps, and severing the duct (see *Figure 18* for gallbladder location). This is to ensure that the gallbladder is not ruptured during liver biopsy. *The gallbladder contains bile, which may be contaminated with toxins that can foul the liver sample and/or cause the deterioration of the surrounding liver tissue.* If the gallbladder is ruptured, quickly rinse the liver with water to remove any bile. Also, personnel should avoid direct contact of bile with their skin.
- Following the removal of the gallbladder, cut the liver into five sections approximately 5-7 mm wide each using sharp scissors or a scalpel. This is to ensure proper preservation of the liver tissue by the fixative. Be sure to include any grossly observable nodules in the histopathology sample. Refer to *Figure 19* for liver biopsy diagram.
- Place all liver sections in a labeled container filled with 10% buffered formalin (maintain 10:1 fixative to tissue ratio).



Figure 18: Liver with Attached Gallbladder

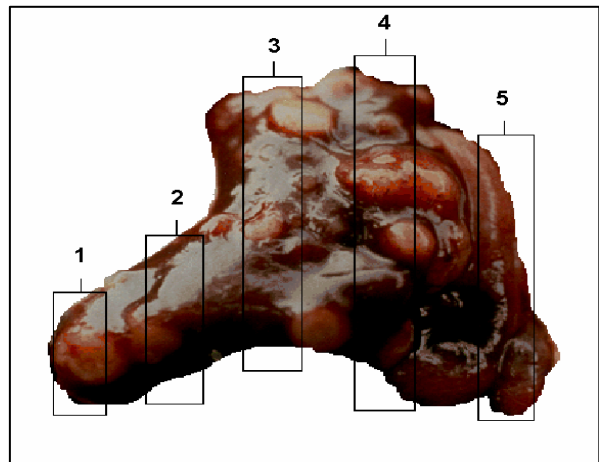


Figure 19: Liver Biopsy

5.5 Bullhead Aging

Bullhead age is highly and positively correlated with the incidence of tumors in brown bullhead. Therefore, it is critical to assess the age of each necropsied specimen. Length can be used in the field to exclude immature specimens. However, length should NOT be used as a surrogate for age because older bullhead tend to add growth in length very slowly, resulting in an overlap of ages with fish the same length. Thus, all fish necropsied must be aged. Because bullhead lack scales, alternative aging tissue must be used. Otoliths (ear stones) and pectoral spines are typically used for this purpose.

We recommend that otoliths be used for aging in place of spines. It has been documented that otolith aging is more accurate than spine aging (Erickson, 1983). Both otoliths of the fish should be collected for aging analysis, if possible, to provide the aging technician with redundant tissue. In the event no otoliths can be located, pectoral spines may be removed for aging; however, it has been our experience that spine derived ages will underestimate the age of specimens older than 6-7 years. Otoliths or spines for an individual fish should be placed in a scale envelope or cryovial labeled with the fish's identification number. This section outlines procedures for both otolith and spine sampling.

5.5.1 Otolith Removal

Otoliths are calcified sensory organs that play a role in hearing and balance. As fish grow, the otoliths do as well, leaving “growth rings” which can be used to age the fish. This is similar to counting the growth rings of a tree or scale annuli. Otoliths are located in the auditory capsule within the skull and can be difficult to locate if dislodged during fish processing. Therefore, care should be taken to avoid damaging the head area while subduing the specimen or during necropsy.

Brown bullhead have two otoliths which are bilaterally symmetrical in appearance and location. To remove the otoliths: make a mid-dorsal incision from the tip of the snout to the rear margin of the operculum (gill cover) using a bone saw or hack saw (*Figure 20a*);

- following the cut, spread apart the head cavity (*Figure 20b*);
- locate the auditory capsule on each side (located between the eye and operculum) and the otoliths (*Figures 20c-d*);

- remove the otoliths with forceps and place in a labeled scale envelope or cryovial. Be sure that you properly identify the otolith; oftentimes there are bone fragments or nerve tissue that resemble otoliths (*Figure 20e*).



Figure 20a: Mid-Dorsal Head Incision



Figure 20b: Separated Head Cavity



Figure 20c: Left Auditory Capsule



Figure 20d: Right Auditory Capsule



Figure 20e: Otolith

5.5.2 Spine Removal

Brown bullhead have three spines:

- Left pectoral spine (attached to left pectoral fin).
- Right pectoral spine (attached to right pectoral fin).
- Dorsal spine (attached to dorsal fin).

Pectoral spines may be sampled for aging purposes in the event that otoliths cannot be obtained from the specimen. If pectoral spines are used for aging purposes, both spines should be removed in order to provide redundant aging tissue. Refer to *Figure 21* for spine locations.

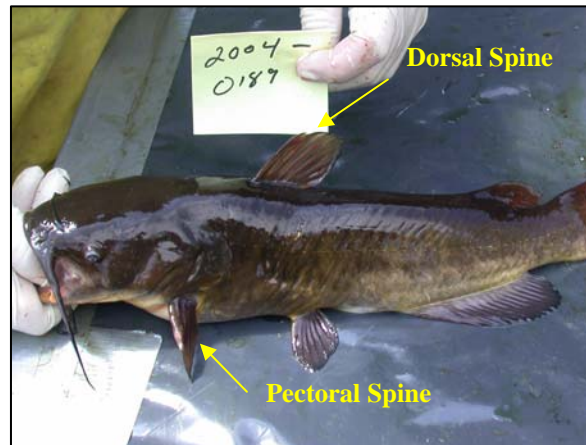


Figure 21: Pectoral and Dorsal Spines

6.0 POST-PROCESSING PROCEDURES

6.1 Data Verification

Following gross assessments and necropsy, all *Fish Health Data Forms* should be reviewed for completeness and accuracy. Be sure all applicable sections are properly completed and legible. Do not leave any sections blank. Photocopies of the verified field data sheets should be provided to all collaborating parties. Data may later be transferred to an electronic database for easy record keeping and analysis.

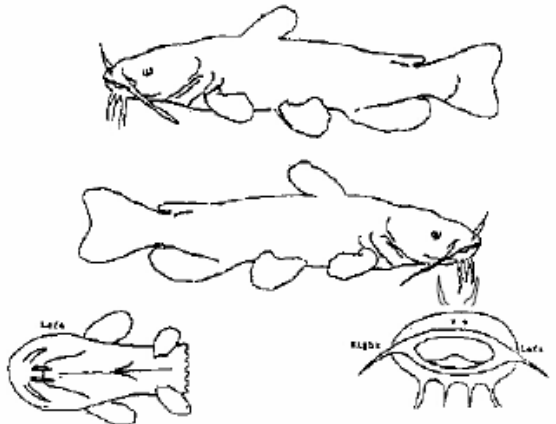
6.2 Clean-up

Following the collection and processing of fish, be sure to properly clean all equipment. It is recommended that all collection equipment (e.g. electrofishing gear, boat, waders, etc.) be thoroughly cleaned prior to use in another body of water. This will help prevent the transfer of aquatic invasive species. All processing equipment (e.g. scalpels, forceps, scale, measuring board, etc.) should be scrubbed and then rinsed using deionized water. Acetone or an ethanol-water solution will assist in removing dried blood from surgical instruments.

6.3 Shipping Samples


All preserved tissue samples should be shipped together to the laboratory along with chain-of-custody and sample submission forms, if appropriate. Be sure to check with the selected shipper for any shipping requirements or restrictions related to biological samples and your chosen fixative.

7.0 FISH HEALTH DATA SHEET

Fish Health Data Sheet					
Reference Number 2006-_____	Collection Date: ____/____/2006	Process Date: ____/____/2006	Time: ____:____	Field Observers _____	
Location _____					
Capture Gear Trapnet <input type="checkbox"/> Electrofishing <input type="checkbox"/> Angling <input type="checkbox"/> Other <input type="checkbox"/>		Species Brown Bullhead <input type="checkbox"/> Yellow Bullhead <input type="checkbox"/> Carp <input type="checkbox"/> Other _____		Tagging Information Tag Number _____ Recapture Date ____/____/____ Recapture Location _____	
Sex: M <input type="checkbox"/> F <input type="checkbox"/>	Total Length (mm) _____	Weight (grams) _____		Age: _____	
Aging Technique: Spines <input type="checkbox"/> Otoliths <input type="checkbox"/> Other <input type="checkbox"/>					
Fish Health Information					
Clean ?	Yes <input type="checkbox"/>	No <input type="checkbox"/> (Note Physical Condition Below)			
	*Severity Score				
Barbels	0 1 2 3	Barbels-Notes			
RAISED Skin Lesions	0 1 2 3	Skin Lesion-Notes			
RAISED Mouth Lesions	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			
Fin Erosion	0 1 2 3	Fin Erosion-Notes			
Ulcers	0 1 2 3	Ulcer-Notes			
Scars/Wounds	0 1 2 3	Scars/Wounds-Notes			
Eyes	0 1 2 3	Eyes-Notes			
Histopathology	Yes <input type="checkbox"/> No <input type="checkbox"/>	Histopathology-Notes			
Tissue Chemistry	Yes <input type="checkbox"/> Whole Fish <input type="checkbox"/> No <input type="checkbox"/> Fillet <input type="checkbox"/>	Tissue-Notes			
		Pictures: Yes <input type="checkbox"/> No <input type="checkbox"/> File # _____ Camera _____			
		Notes: * 0 No visible maladies * 1 Mild Condition * 2 Moderate * 3 Severe			

8.0 REFERENCES

- Black, John J. *Field and Laboratory Studies of Environmental Carcinogenesis in Niagara River Fish*. *Journal of Great Lakes Research* 9(2): 326-334, 1983.
- Black, John J. *Gross Signs of Tumors in Great Lakes Fish: A Manual for Field Biologist*. <http://www.glfrc.org/tumor/tumor1.htm>. n.d.
- Blazer, V.S., J.W. Fournie, J.C. Wolf, and M.J. Wolfe. *Manual for the Microscopic Diagnosis of Proliferative liver and Skin Lesions in the Brown Bullhead (Ameiurus nebulosus)*. In press.
- Diewert, Ron E., Angela M.J. Prince, Susan E. Low, Timothy J. Lissimore, and Scott G. Hinch. *Sodium Bicarbonate and Acetic Acid: An Effective Anesthetic for Field Use*. *North American Journal of Fisheries Management* 15:170-172, 1995.
- Erickson, C.M. *Age Determination of Manitoban Walleyes Using Otoliths, Dorsal Spines, and Scales*. *North American Journal of Fisheries Management* 3: 176-181, 1983.
- Goede, R. *Fish Health/Condition Assessment Procedures, Part 1*. Utah Division of Wildlife Resources. 31 pp., 1993.
- Jennings, Cecil A. and Gregory L. Looney. *Evaluation of Two Types of Anesthesia for Performing Surgery on Striped Bass*. *North American Journal of Fisheries Management* 18:187-190, 1998.
- Peake, S. *Sodium Bicarbonate and Clove Oil as Potential Anesthetics for Nonsalmonid Fishes*. *North American Journal of Fisheries Management* 18:919-924, 1998.
- Pinkney, A.E. and J.C. Harshbarger. *Tumor Prevalence in Brown Bullheads (Ameiurus nebulosus) from the South River, Anne Arundel County, Maryland*. U.S. Fish and Wildlife Service, Chesapeake Bay Field Office, Annapolis, MD. CBFO-C05-04, 2005.
- Poulet, F.M., M.J. Wolfe, and J.M. Spitsbergen. *Naturally occurring orocutaneous papillomas and carcinomas of brown bullheads (Ictalurus nebulosus) in New York State*. *Veterinary Pathology*. 31:8-18, 1994.
- Prince, Angela and Cynthia Powell. *Clove Oil as an Anesthetic for Invasive Field Procedures on Adult Rainbow Trout*. *North American Journal of Fisheries Management* 20: 1029-1032, 2000.
- Smith, S.B., M.A. Blouin, and M.J. Mac. *Ecological Comparisons of Lake Erie Tributaries with Elevated Incidence of Fish Tumors*. *Journal of Great Lakes Research* 20(4): 701-716, 1994.



Smith, S., A. Donahue, R. Lipkin, V. Blazer, C. Schmitt, and R. Goede. *Illustrated Field Guide for Assessing External and Internal Anomalies in Fish*. U.S. Geological Survey Information and Technology Report, 2002-0007, 46 pp., 2002.

Steyermark, A.C., J.R. Spotila, D. Gillette, and H. Isseroff. *Biomarkers indicate health problems in brown bullheads from the industrialized Schuylkill River, Philadelphia*. Transactions of the American Fisheries Society. 128:328-338, 1999.

Yang, X., P.S. Peterson, P.C. Baumann, and E.L.C Lin. *Fish Biliary PAH Metabolites Estimated by Fixed-wavelength Fluorescence as an Indicator of Environmental Exposure and Effects*. Journal of Great Lakes Research 29(1): 116-123, 2003.

