A Study of Tumors in Fish of Presque Isle Bay

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Sponsored by
Pennsylvania Department of Environmental Protection

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I. INTRODUCTION

A. Objective

The potential relationship between chemical pollution in bodies of water and the presence of neoplasms in aquatic organisms has for years caused concern about the quality of water as it relates to human health and wellbeing. As a result, analyses of bodies of water for the presence of chemicals and the use of aquatic species as sentinels of the potential effects of chemicals has become commonplace. One aspect of such analysis is examination of fish tissues by histopathology for the presence of neoplasia or hyperplastic foci in organs, especially the liver and skin.

The Pennsylvania Department of Environmental Protection enlisted the services of the Pennsylvania Department of Agriculture to conduct a cooperative study of several species of fish from Presque Isle Bay. The original plan was to capture one hundred fish representing bowfin, white suckers, brown bullheads and yellow bullheads. The results of a thorough pathologic examination along with analysis of bile for polycyclic aromatic hydrocarbons were to be evaluated as an aid in evaluating the overall condition of that aquatic system.

B. Subject Animals

The species of primary interest was the brown bullhead (*Ictalurus nebulosus*). Other common species inhabiting the waters of Presque Isle Bay include yellow bullheads (*Ictalurus natalis*), bowfin (*Amia calva*) and white suckers (*Catastomus commersoni*). The initial objective was to examine approximately one hundred fish of these species. Ultimately the season and weather conditions limited the collection to brown bullheads and bowfin.

C. Humane Considerations

All of the fish were caught by biologists from the Department of Environmental Protection who are skilled in the use of established electroshocking techniques. The fish were transferred to holding tanks with non-recirculating continuous flow lake water at the necropsy site. They were held for as little time as necessary prior to sedation and euthanasia. Every effort was made to minimize stress and exposure prior to sacrifice.

II. MATERIALS & METHODS

A. Site and Facilities

The fish which were collected by Departmental of Environmental Resources personnel were taken to the Presque Isle Canoe Livery (which is operated by Dave Parker, a concessionaire who was very helpful to us) where they were held in tanks containing non-recirculating continuous flow lake water until they were examined. Postmortem examinations were conducted at that location, outdoors. All the equipment which was needed was transported to the site from the Summerdale Diagnostic Laboratory. Necropsy boards were designed and built to assist in holding the fish during the procedure. Portable fluorescent lights were available in the event that inclement weather forced the work into a concrete building which was present on the site.

B. Personnel and Resources

The Study Director was Mark K Walter, DVM, MS. Hans Rothenbacher, DVM, PhD assisted Dr Walter in supervising postmortem examinations. Technical assistance was provided by staff from the Summerdale Diagnostic Laboratory: J Thomas Wampler, William Lagoda and Matthew Sweger. Donald Linville, PhD, of Mercyhurst College in Erie, Pennsylvania is a scientific collaborator with the sponsor of the study and contributed his technical services by performing necropsy examinations. Data recording and technical assistance was provided by Paula Teats and Regina Grimm, technicians, and Raymond Hasse, biologist, with the Department of Environmental Resources, for a portion of the study. Harry Leslie, Park Superintendant, was ever helpful, provided broad access to the park and helped with equipment at the necrosy site. Robert Wellington, a biologist with the Erie County Health Department, assisted with fish capture. Donna M Dambach, VMD collaborated in the interpretation of pathologic findings. Numerous members of the staff of the Summerdale Diagnostic Laboratory assisted in preparation of histologic slides under the direction of Elaine Breski. Lynda Champlin was extremely helpful throughout the study by ordering and organizing supplies. A group from US Fish and Wildlife Service in Columbus, Ohio was led by Paul Baumann, PhD and included Mark Ashbaugh and Jennifer Jones. They were present during the first day of necropsies. They assisted in the task and offered useful suggestions.

C. Locations for Sampling

Fish were collected from the following locations in Presque Isle Bay:

The Lagoons	The Point
Misery Bay	Sara's Cove
Thompson Bay	Cascade Creek.

Those locations associated with Presque Isle Bay are shown on the map in Appendix 1. A reference population of brown bullheads was collected from Eaton Reservoir which is located near Northeast, PA. A reference population of bowfin was not available. The identification of each fish and the site at which it was caught is shown in the table in Appendix 2.

D. Allocation of Fish to Study Populations

A group of ninety fish was captured from several specified sites within the area generally known as Presque Isle Bay and was designated as the Study Population. These included twenty-one bowfin (*Amia calva*) and sixty-nine brown bullheads (*Ictalurus nebulosus*). In addition ten brown bullheads were collected from Eaton Reservoir to serve as the Reference Population. Eaton Reservoir was nearby yet had sources of water which were unrelated to Presque Isle Bay and was generally believed to be free of pollution, especially industrially related contaminants such as polycyclic aromatic hydrocarbons. Collections occurred during the period June 6 through 8, 1995. The design of the study could not predict the numbers, species or sex of fish which might be harvested. Yellow bullheads and white suckers were originally intended to be included but these species were not among those fish actually collected. A similar reference population for bowfin from a non-polluted site was not available.

E. Collection of Specimens

Electrofishing and Pennsylvania Trapnets were used to collect fish in Presque Isle Bay. A 16 foot flat-bottom jon boat was outfitted with direct current electrofishing equipment. A 5000 watt generator in conjunction with a coffelt variable pulsator supplied a single boom Wisconsin ring. A light mounted on the bow facilitated fishing at night. Electrofishing runs were timed so that a catch per unit effort could be established to record

catch rates. Pennsylvania Trapnets were supplied by the Pennsylvania Fish and Boat Commission. Trapnets had a main lead which is 100 feet long attached perpendicular to the shore. Wing nets were attached to the sides at 45 degree angles to the main lead so that fish were funneled to the main lead and into the hoop section of the net. The fish swim through the hoop portion of the net to the pot, which is the holding area of the net, where they remain until they are lifted from the net. The Pennsylvania Trapnets used in this study were made of one-half inch nylon twine mesh. Fish were removed daily from each net. Unwanted species were returned to the bay.

F. Consideration of Sex and Age

The nature of the collection method did not allow selection by sex, age or any other criterion. The relative abundance of bowfin and brown bullheads is reflected in the numbers which were caught. Ten reference bullheads were planned as a necessary part of this study.

G. Holding Tanks

The facilities at the study site provided several holding tanks. Fish collected at various times of the day or night were placed in the tanks as soon as practical after being caught and protected from sunlight. Water was pumped continuously from the lake and passed through the tanks. A few fish became moribund or died. These were removed and necropsied as soon as possible to avoid postmortem deterioration.

H. Euthanasia

Finquel® (MS-222; tricaine methanesulfonate; Argent Chemical Laboratories) was used to induce sedation prior to sacrifice by severing the spinal cord. It was used at or above the concentration recommended by the manufacturer to assure efficacy.

I. Necropsy Procedure

Prior to the time of the study, the sponsor provided several fish as prototypes for selection of materials and methods of necropsy and tissue preparation. Procedures were devised, materials were assembled and data recording sheets were written so that the postmortem examinations could be conducted quickly, smoothly and efficiently.

Each fish was placed in dorsal recumbency and the body cavity was opened by a ventral midline incision. The gastrointestinal tract was removed in its entirety. The stomach and at least the proximal portion of the intestine was opened and examined. Other organs were removed systematically, examined, incised when indicated and were placed in formalin. Livers were weighed on a triple beam balance. Bile was removed from the gallbladder with a needle and syringe and placed in containers provided by the sponsor. The location and description of gross lesions were recorded.

J. Measurements and Specimens

1. Length and weight were recorded immediately prior to necropsy.
2. Sex was determined upon examination of the gonads.
3. Weight of liver was determined on a triple beam balance and was recorded on the necropsy record forms.
4. Age was determined by Michael Garthaus, Research Assistant for Bruce Tezlaff at Southern Illinois Cooperative Fisheries Research Laboratory at Southern Illinois University, a consultant to the Pennsylvania Department of Environmental Protection, using established techniques of measuring laminations in otoliths. Otoliths were removed upon trimming the wet tissues. They were shipped to the sponsor and subsequently to the contractor. Scales of bowfin and pectoral spines of bullheads were saved as potential alternate means of determining age.
5. Analysis of bile was conducted by the laboratories of the Department of Environmental Protection in Harrisburg. Samples were removed by aspiration from the gallbladders, placed in special vials provided by the sponsor and quickly chilled.

K. Preservation of Data and Specimens

Original record sheets from necropsy, tissue preparation and pathological examination have been retained. Formalin fixed wet tissues as well as paraffin blocks and original slides will be kept. Whenever possible, duplicate slides will be made to send to consultants or for any other purposes in order to preserve the particular slides upon which original interpretations were based.

L. Histological Preparations

Wet tissues preserved in 10% neutral buffered formalin were trimmed and placed in prenumbered plastic cassettes for processing through paraffin by conventional techniques. Descriptions of gross findings were available as an aid in selecting specific sites for microscopic examination. Additional findings were recorded when appropriate. After infiltration with paraffin in an automated tissue processor, the tissues were embedded into paraffin blocks. Microtomy was conducted with rotary microtomes set at 5 μm and floated on a water bath for mounting onto glass slides. Tissues were stained with Mayer's hematoxylin and eosin.

M. Missing Specimens or Data

Occasionally specimens or data are reported as missing, lost, unreported, unknown or with an interrogation mark. This study was planned so that lost data points and specimens would be minimal. Certain tissues, notably cranial kidney and corpuscle of Stannius, were difficult to identify. Normal thyroid can not be seen grossly and so the region of its expected presence is examined. A few samples of bile were lost at necropsy due to difficulties in handling. Human error, too, was a factor in failing to record a few weights and measures. In general, the integrity of these data is quite acceptable for a study of this nature.

N. Histological Interpretations

Slides from all animals were examined by Mark K Walter, DVM, MS, Dipl. ACVP and also by Donna M Dambach, VMD, Dipl. ACVP. The independent interpretations of each pathologist are displayed in parallel columns as Appendix 11_. These findings were examined for potential inconsistencies and additional studies were conducted to arrive at concensus. Variation of interpretations and detailed terminology among pathologists is expected. In order to avoid underreporting of potentially important findings, the result which was used in the analysis of particular tissue/lesion incidences was that which was considered the more serious (eg, benign *versus* malignant neoplasia; hyperplasia *versus* neoplasia; necrosis *versus* inflammation). Sometimes only a small amount of a tissue was present on the slide. If an individual pathologist felt that there was an insufficient amount for examination, it was described as not present. For the same animal the other pathologist may have felt comfortable with the

amount of tissue and described a lesion or no significant lesions. If either pathologist considered the tissue present, it was considered present for purposes of tabulation and statistics. The histopathologic findings of both pathologists were categorized, condensed and simplified so that the tables were managable. This was especially justified since the primary objective was to evaluate the occurrence of neoplasms yet certain non-neoplastic changes may have direct or indirect effects upon neoplasia.

O. Statistical Considerations

As a field study, there was little control which could be exercised upon the selection of animals which were examined. Statistical analysis of certain parameters of the study was conducted by Mr Rod Kime of the Pennsylvania Department of Environmental Protection.

III. RESULTS

A. Results: Bowfin

1. Characterization of the Population of Bowfin

Twenty-one bowfin were captured from the greater study area. Thirteen were males and 8 were females. This species does not reside in Eaton Reservoir so there was no reference population for comparison. The number of bowfin captured at each site was as follows:

Location	Males	Females	Total
Lagoons	0	1	1
Misery Bay	2	0	2
Thompson Bay	1	1	2
The Point	8	4	12
Sara's Cove	1	1	2
Cascade Creek	1	1	2
Eaton Reservoir	0	0	0
Total	13	8	21

The ages of bowfin are expressed in years, were quite uniform and were described as follows:

	All	Males	Females
Age Range (years)	3-10	3-10	3-10
Mean Age (years)	6.42	6.77	5.67

Length and weight were recorded and preserved in the individual animal data. Length, weight and especially age were useful to characterize the lesions in individual fish as well as the populations of which they were a part. Each fish was ranked for length and weight in decending order. Rank length and weight are shown on individual histopathology tables located Appendix 16. Rank length and weight were useful to estimate the approximate age of bowfin #013 and #072 at 7-10 years based upon the ages of fish two ranks higher and lower.

2. **Summary of Gross Observations in Bowfin**

Bowfin were generally in good physical condition. Four of 21 had no observable gross lesions. Two had injuries related to fish hooks. Scars and abrasions were present on various locations of the body

but were most often associated with the mouth. These were described in 11 fish. Intestinal parasites were the most frequent gross observations. For the purposes of this study, these are considered abnormalities although we recognize that cestode and nematode parasites are expected in wild populations. Fourteen bowfin had tapeworms in the stomach and/or intestines.

3. **Summary of Neoplastic Microscopic Observations in Bowfin**
A single, tiny neoplasm was reported in bowfin. It was a pancreatic ductal adenoma in #013. No significance, either to the individual fish or to the population, can be attributed to this finding.

4. **Summary of Non-Neoplastic Microscopic Observations in Bowfin**
Non-neoplastic lesions in bowfin occurred in each tissue except cranial kidney and corpuscle of Stannius. A summary of non-neoplastic lesions is presented in Appendix 3. A similar but more detailed table containing identification of individual fish with each lesion is presented in Appendix 4.

Melanomacrophage foci were described in liver, caudal kidney, ovary and spleen. These are normal structures associated with immunologic function in fish and which can vary greatly in their occurrence. They are included for completeness so that interested readers can evaluate the data for their own purposes. Dr Dambach offers a quantitative evaluation of their presence in the parallel histopathology tables for individual fish in Appendix 16. Similarly, interstitial hematopoietic cells are normal, although apparently less ubiquitous, feature of the caudal kidney.

Chronic inflammation was the most frequently described lesion and occurred in nearly every tissue. The cellular population of the inflammatory infiltrate variably included lymphocytes, plasmacytes and eosinophils. Granulomatous inflammation was occasionally reported, especially in liver, and was frequently associated with eosinophils and remnants of parasites. Acute inflammation was

rarely present.

Liver was the organ with the largest array of non-neoplastic lesions. Chronic inflammation in hepatic parenchyma consisted of aggregates variably composed of lymphocytes, plasmacytes and/or eosinophils. They occurred in ten males and five females. Clearing of hepatocellular cytoplasm is not specific to a particular kind of injury and may be related to momentary nutritional status, as these fish were held in tanks for varying periods prior to sacrifice. Presence or absence of glycogen could account for this mild change. Hemorrhage was described in the liver of only one male bowfin while hemosiderosis was present in five males and one female. Hemosiderosis could result from breakdown of hemoglobin deposits from hemorrhagic events or from catabolism of senescent or injured erythrocytes in the circulation.

Portal areas and biliary tracts were similarly affected with chronic inflammation as well as portal fibrosis, and changes within the bile ducts, including inflammation of bile ducts (cholangitis), vaculoation of ductal epithelium and hyperplasia. In the gallbladder, chronic inflammation of a similar nature was the only lesion described and it occurred in the fish which also had chronic inflammation in the liver.

Focal hepatocellular alteration was present in five fish without predilection for sex. In mammals and in fish these foci are considered to be reversible preneoplastic changes [1,2]. Since there were no hepatocellular neoplasms in this study, it remains unknown whether they retain this association in bowfin.

Caudal kidney had only one lesion of interest and potential significance. Hyaline droplet accumulation occurred in eight males and only two females. Its significance in bowfin is unknown. Those fish which exhibited this change came from various locations within Presque Isle Bay. Protozoan parasites were discovered in tubular epithelial cells of one female bowfin (#088). These might be related to the myxosporidian parasites of the genera *Mitrospora*

and *Sphaerospora*. [3] The pathologic significance of these parasites in bowfin is uncertain.

Chronic inflammation was described in the heart of every bowfin. This was nearly always related to the adventitia rather than to the myocardium. Never was it of sufficient intensity to be considered of importance to the health of the individual. These could be a population of cells which is normal in this location. [4]

Stomach, intestine and gas bladder also had notable amounts of chronic inflammation. Parasites in the gastrointestinal tract accounted for substantial inflammation. Protozoan organisms were observed either alone or in conjunction with other parasites in the intestinal tracts of most of the bowfin. No further attempt was made to identify these organisms. Eosinophils in the inflammatory population lend credibility to that coincidence. Males had a slightly greater incidence of chronic inflammation in the skin and mouth/lip/barbel. This might be attributable to fighting.

Changes in muscle fibers included rarefaction of the sarcoplasm, myolysis and necrosis. This occurred in five fish and was not associated with inflammation. This could be the result of the stress associated with capture.

Various other changes were reported and were enumerated in Appendices 3 and 4. They occurred in small numbers, usually were of low severity and had no importance to the life and health of the fish.

5. **Analysis of Polycyclic Aromatic Hydrocarbons in Bile of Bowfin**

Concentrations of naphthalene, phenanthrene and benzo-α-pyrene in bile were determined for each of 21 bowfin captured in this study. Individual values are presented in Appendix 11. Mean concentrations were:

Naphthalene	60474 µg/kg of bile
Phenanthrene	14650 µg/kg of bile
Benzo-α-pyrene	1121 µg/kg of bile.

Since there was no reference population of bowfin and the numbers of fish caught at each location within Presque Isle Bay varied so greatly, no further attempt was made to analyze these data. Twelve of 21 bowfin were caught at Point - Sara's Cove and perhaps constitute sufficient numbers for evaluation of these compounds at this location. For purposes of comparison, mean concentrations for bowfin collected at all locations are displayed in Table 5 along with similar values for brown bullheads.

B. Results: Brown Bullheads

1. **Characterization of the Population of Brown Bullheads**

Seventy-nine brown bullheads were captured for the study. Ten were taken from Eaton Reservoir, an impoundment located five miles south of Northeast, PA. The water source for this reservoir is relatively free of industrial pollution and is not related to that of Presque Isle Bay. Fish taken from the reservoir served as a reference population for comparison with those bullheads taken from Presque Isle Bay. Bowfin do not reside in Eaton Reservoir.

Study data were examined to characterize the populations of fish. Of particular interest was the distribution by age, sex and location. Tables 1 and 2 display the numbers of fish caught at each location by sex and also the age of fish caught at each location expressed as the range and the mean. As was the case with bowfin, brown bullheads seemed to be similarly distributed by age and by sex regardless of the location from which they were taken. It therefore seems reasonable to combine sexes when age and location are not factors under consideration.

TABLE 1 NUMBER OF FISH, BY SPECIES AND SEX, CAUGHT AT VARIOUS LOCATIONS				
	Bowfin		Brown Bullheads	
Location	Males	Females	Males	Females
Lagoons	0	1	10	10
Misery Bay	2	0	0	0
Thompson Bay	1	1	7	12
The Point	8	4	4	5
Sara's Cove	1	1	11	6
Cascade Creek	1	1	3	1
Eaton Reservoir	0	0	5	5
Total	13	8	40	39

TABLE 2
RANGE AND MEAN AGE, BY SEX, OF BROWN BULLHEADS CAUGHT AT VARIOUS LOCATIONS

Location		Total	Males	Females
Lagoons	Range	3-15	3-15	3-15
	Mean	7.8	7.4	8.2
Misery Bay	Range	-	-	-
	Mean			
Thompson Bay	Range	4-14	4-14	4-14
	Mean	8.6	7.6	9.1
The Point	Range	4-16	4-15	4-16
	Mean	10.8	10.3	11.2
Sara's Cove	Range	4-16	4-16	4-16
	Mean	8.1	8.6	7.3
Cascade Creek	Range	4-17	4-17	16
	Mean	11.0	9.3	16.0
Eaton Reservoir	Range	5-17	6-17	5-16
	Mean	10.6	10.8	10.4
Composite of Bay Locations	Range	3-17	3-17	3-16
	Mean	8.7	8.3	9.0
All Locations	Range	3-17	3-17	3-16
	Mean	8.9	8.6	9.2

2. Summary of Gross Observations in Brown Bullheads

A wide variety of gross observations was made at the time of necropsy and reported on the individual animal data sheets in Appendix 16. These were classified into eleven categories and are shown below in Table 3. There were eight remaining lesions or observations which were each found in only one fish. They were considered insignificant and were not included with the others in the Table 3.

It was the impression of the study team that the brown bullheads from the Presque Isle Bay study population were thin and in generally poor body condition. Only three, however, were recorded as such in the necropsy data. The number was probably understated. Healed or active wounds in the skin and around the mouth and lips occurred very frequently. Their cause could not be determined. Because sport fishing is so common in this area it can be speculated that propellers and fish hooks might be involved. Skin lesions, other than wounds, were classified as variations in pigmentation, usually melanotic, and as proliferative. Such findings appeared to be more common in the study population than in the reference population.

Appendix 5 is a listing of individual brown bullheads which were afflicted by each type of gross lesion as summarized in Table 3.

TABLE 3 INCIDENCE OF CATEGORIZED GROSS OBSERVATIONS IN BROWN BULLHEADS		
Categorized Gross Observations	Study Population N=69	Reference Population N=10
No gross lesions	3 4%	0
Poor body condition	3 4%	0
Healed wounds and skin lesions	33 48%	6 60%
Active or recent wounds and skin lesions	20 29%	2 20%
Lips and mouth abrasions, ulcers, growths	28 41%	5 50%
Parasites in liver	16 23%	1 10%
Parasites in gastrointestinal tract	4 6%	0
Pigmented skin lesions	**28 41%**	**1 10%**
Proliferative skin lesions	**26 38%**	**1 10%**
Liver masses	5 7%	1 10%
Liver pale and/or mottled	4 6%	0
Eye lesions	5 5%	3 30%

3. **Summary of Neoplastic Microscopic Observations in Brown Bullheads**

Neoplastic microscopic lesions were limited to four tissues:

liver
skin of the body
oral mucosa of the mouth and lip, and the skin of the barbels (recorded as Mouth/Lip/Barbel)
testis.

Both benign and malignant neoplasms were present in each tissue.

Table 4 displays these findings for the Study Population and for the Reference Population of brown bullheads as the number of fish which had at least one of each neoplasm as well as the percentage of the population. Percentages were rounded to the nearest integer for simplicity. Appendices 6 and 7 are similar tables which display, respectively, the identities of individual fish which had each type of neoplasm and the distribution by sex within the study and reference populations.

TABLE 4
SUMMARY OF NEOPLASTIC FINDINGS IN BROWN BULLHEADS

Organ	Lesion	Study Population N=69	Reference Population N=10
Liver	Biliary adenoma	1 1%	0
	Biliary carcinoma	7 10%	2 20%
Skin	Papilloma	3 4%	0
	Squamous cell carcinoma	2 3%	0
	Melanoma	1 1%	0
	Melanosarcoma	1 1%	0
Mouth/Lip/Barbel	Papilloma	15 22%	0
	Squamous cell carcinoma	6 9%	0
Testis	Seminoma	11 16%	0
	Adenoma of tubular lining cells	2 3%	0
	Carcinoma of tubular lining cells	1 1%	1 10%

4. **Summary of Non-Neoplastic Microscopic Observations in Brown Bullheads**

Non-neoplastic lesions in brown bullheads occurred in every tissue

although very few were reported in gallbladder, corpuscle of Stannius or thyroid. A summary of non-neoplastic observations is presented in Appendix 8. A similar but more detailed table containing identification of individual fish with each lesion is presented in Appendix 9.

Structures homologous with melanomacrophage foci in bowfin were described as hemosiderosis (by Dr Walter) and as pigment-laden macrophage aggregates (by Dr Dambach) in brown bullheads. The material stains with Prussian Blue and therefore contains iron. These structures were present in liver, cranial kidney, caudal kidney and spleen with noteworthy frequency, and to a much lesser extent in heart, stomach, urinary bladder, ovary and intestine. These are normal structures associated with immunologic function in fish and which can vary greatly in their occurrence. They are included here for completeness so that interested readers can evaluate the data for their own purposes. Dr Dambach offers a quantitative evaluation of their presence in the parallel histopathology tables of Appendix 16.

Inflammation was described in nearly every organ. Chronic inflammation consisted of lymphocytes, plasma cells and eosinophils in variable proportion. Eosinophils were prominent when parasites were present. Granulomatous inflammation was frequently associated with, and generally attributable to, the presence of parasites in bile ducts or blood vessels. Injury to the structure frequently resulted in its necrosis, sometimes to the extent that its prior existence was surmised based upon location.

Parasites were mostly cestodes and nematodes with a few arthropods and protozoa and possibly some trematodes. A detailed description of the effects of specific parasites was beyond the scope of this study. The microscopic appearance of nematodes and cestodes was compatible with those observed at necropsy in the gastrointestinal tract. Some of the flatworms had microscopic characteristics of flukes but flukes were not observed at necropsy. Nematodes had a propensity to migrate into blood vessels and were

found in numerous tissues, especially those associated with the gastrointestinal tract. Arthropods and/or their eggs were seen mostly in the gas bladder but were also distinctly present within blood vessels of several tissues. Small organisms, presumably protozoa, were found in the bile ducts of one individual (#014).

Liver was the organ with the widest array of non-neoplastic lesions. Chronic inflammation in the hepatic parenchyma consisted of aggregates variably composed of lymphocytes, plasmacytes and/or eosinophils. They occurred in three males and three females. Clearing of hepatocellular cytoplasm is not specific to a particular kind of injury and may be related to momentary nutritional status, as these fish were held in tanks for varying periods without food prior to sacrifice. Presence or absence of glycogen could account for this mild change.

Portal areas and biliary tracts were similarly affected with chronic inflammation as well as portal fibrosis, and changes within the bile ducts, including inflammation of bile ducts (cholangitis) and hyperplasia. Hyperplasia of bile ducts occurred in 7 male and 4 female brown bullheads. Four of 7 males and 1 of 4 females also had concurrent carcinoma of bile ducts (see Appendix 9b).

It is of particular interest that hyperplasia of bile ducts occurred largely in very old fish as did neoplasms in liver, skin and mouth/lip/barbel. Following is a summary of biliary hyperplasia:

	All	Males	Females
Age Range (years)	7-17	7-17	10-16
Mean Age (years)	12.8	12.4	13.5

Of these 11 fish, all except three were observed also to have parasites in the lumina of bile ducts. A single male (#014) had what appeared to be protozoan parasites, possibly coccidia, in epithelial cells of bile ducts. Among mammals, rabbits also contract biliary

coccidiosis caused by *Eimeria stiedae*.

Focal hepatocellular alteration was present in 32 fish without predilection for sex (18 males and 14 females). In mammals and in fish these foci are considered to be reversible preneoplastic changes. [1,2] Since there were no hepatocellular neoplasms in this study, it remains unknown whether they retain this association in brown bullheads.

Caudal kidney is the functional excretory organ of fish. In brown bullheads there was noteworthy occurrence of hyaline droplet accumulation in renal epithelial cells of both sexes in nearly a third of the animals examined. Hyaline droplets may be an indicator of injury to renal tubules or represent benign accumulation of certain types of metabolites in those cells. Their significance in these brown bullheads has not been determined. Accumulation of hyaline droplets in renal epithelial cells has not been observed as an effect of treatment with potential toxicants in controlled studies using species of fish common for laboratory studies. (M J Wolfe, personal communication).

The heart of nearly every brown bullhead was afflicted with an inflammatory reaction of some sort: chronic inflammation NOS, epicarditis, myocarditis, adventititis or granulomatous inflammation. These lesions were always of a low degree of severity and were present without regard to sex or population. The cellular infiltrate was composed of lymphocytes, plasma cells and sometimes histiocytes. In most cases the epicardium was involved but a few fish had lesions in the adventitia and the myocardium. Granulomatous inflammation was recognized in two fish and one could speculate that this might have been associated with migration of a parasite. One might also speculate that, since these cellular infiltrates were ubiquitous near the heart that they might constitute a normal reservoir of lymphoid tissue in fish. [3]

Stomach and intestines displayed a similar picture of widespread granulomatous inflammation in the submucosa with easily

recognized parasites in the lumina of the organs (gross observations in the case of stomach) as well as inside blood vessels. A variety of incidental findings in small numbers was also likely to be a result of parasite infestation, *eg*, vasculitis, thrombosis, serositis, edema and hemorrhage. Fish from the reference population were not without their share of these lesions but it was beyond the scope of this study to attempt to demonstrate a difference in incidence or severity of non-neoplastic lesions between study and reference populations.

The gas bladders of brown bullheads were infested by very interesting parasites. In addition to parasites (usually nematodes) and their associated inflammation in the vasculature near the gas bladder, there was evidence of arthropods in the lumen of the organ. Often only chitinous brown eggs were seen in the gas bladder. This suggests that the gas bladder is the natural site of infestation of this parasite. It is also surmized that arthrodpods within vessels are migrating to or from the gas bladder. Lesions likely to be associated with the parasites included mucosal metaplasia, hyperplasia and pigmentation. It is of interest that only one fish of the reference population had distinct evidence of parasitism in the gas bladder while at least 47 of the study population were so affected.

Data for testes of males are still under review. Hyperplasia and neoplasia were initially described. Colleagues with more specific experience with ictalurid testes have been consulted but concensus has not yet been reached.

Skeletal muscle had a few incidences of chronic and granulomatous inflammation associated, in most cases, with parasites. Degenerative changes were present in muscle tissue of fifteen brown bullheads. These changes were generally of low severity and had no characteristics of chronicity. They could be related to the process of being captured by electroshocking. Also, this could be a manifestation of capture myopathy in fish, a process which is recognized in wild mammals.

The mouth/lip/barbel consists of bone and cartilage covered by modified skin. The changes seen in the skin of the body are similar to those seen on the mouth/lip/barbel. Parasites and associated chronic or granulomatous inflammation were seen in these tissues as was the case in nearly every other tissue. Hyperplasia, usually of the epidermis but also of alarm cells and pigment-producing cells, is a response to injury of some sort. The associated presence of fibrosis, erosion, ulceration, edema or hemorrhage suggest that physical injury may be important. Presque Isle Bay is used heavily by sport fisherman and so hook and propeller injuries are suspect. Patchy areas of hyperpigmentation, epidermal spongiosis, hyperplasia of alarm cells and mucus metaplasia are more suggestive of a response to environmental irritation. In mouth/lip/barbel there may be an overestimation of the incidence of epidermal hyperplasia. Thickened epidermis , especially on the lips, is probably, in most cases, within physiologically normal limits.

Gallbladder, cranial kidney, corpuscle of Stannius, thyroid, urinary bladder and spleen had only occasional lesions mostly of a mild inflammatory nature and of no significance to the health of individuals or the population.

5. **Analysis of Polycyclic Aromatic Hydrocarbons in Bile of Brown Bullheads**

A summary of the results of analysis of bile for three polycyclic aromatic hydrocarbons are displayed in Table 5. The results of those analyses by individual fish are in Appendix 12.

TABLE 5 MEAN CONCENTRATION OF POLYCYCLIC AROMATIC HYDROCARBONS IN BILE OF BROWN BULLHEADS, BY LOCATION			
Location	Naphthalene µg/kg	Phenanthrene µg/kg	Benzo-α-pyrene µg/kg
Lagoons	202100	65791	328
Thompson Bay	184735	40762	443
Cascade Creek	189609	98874	321
Sara's Cove	135404	40423	1219
Point Sara's Cove	137448	27795	1510
Eaton Reservoir	154808	14171	486
Bowfin from all locations	60427	18355	1152

Statistical analyses were conducted at the Pennsylvania Department of Environmental Protection. A summary was provided but technical details were not. Only correlation analysis was conducted. The reference population from Eaton Resevoir was not included because it is not appropriate to include "controls" in that type of test. The statistician's interpretation follows:

- There was no relationship between phenanthrene and age, sex and weight.
- There was no relationship between naphthalene and age, sex and weight.
- As a group there was a significant but low correlation (r^2=0.14) between benzo-α-pyrene and length. This group correlation was due to the males that have r^2=0.29. The females showed no correlation with benzo-α-pyrene. The correlation of length and benzo-α-pyrene in males was complicated by constant variance. Correlation analysis assumes that the variability in benzo-α-pyrene is constant over the entire range of lengths. The variability of benzo-α-

pyrene decreases, however, with increasing length. In other words, as the bullheads get larger they tend to accumulate benzo-α-pyrene more uniformly.

- In brown bullheads there is no correlation among naphthalene, phenanthrene and benzo-α-pyrene regardless of sex.

Based upon mean values, brown bullheads from Eaton Reservoir had lower concentrations of phenanthrene than at any other site. Mean concentrations of naphthalene and benzo-α-pyrene from brown bullheads in Eaton Reservoir were at the median ranking of their respective sites. Stated differently, among all of the collection sites, from fish of Eaton Reservoir had concentrations of phenanthrene and benzo-α-pyrene in bile which were approximately midway between the highest and lowest mean values which were measured. It was expected that concentrations of these three compounds would be lowest in Eaton Reservoir.

Appendix 13 separates by location of capture the values for all of the polycyclic aromatic hydrocarbons as well as the age of each fish.

Bile was analyzed (*ie*, there were no missing samples of bile) for each brown bullhead which had one or more external or internal neoplasms. Appendix 14 lists all 30 such brown bullheads along with the rank value, from highest to lowest, of the concentration of the specific hydrocarbon found in the bile of that fish. Ranking the values offers an easy way to quickly assess in relative terms the amount of contaminant compared to other fish in the study.

Examination of the data offer only some weak suggestions about the relationship of three polycyclic aromatic hydrocarbon contaminants to the sites and the fish of this study.

Eaton Reservoir was expected to yield the lowest concentrations because there is no apparent source of industrial pollution. The brown bullheads taken there were only slightly less contaminated

that those from Presque Isle Bay. That population was also of similar age to those of Presque Isle Bay. Correlation analysis indicated that larger, older fish accumulated greater amounts of these hydrocarbons and they did more uniformly. It also seems that each of the three compounds is found independently.

The rank numbers in the various tables fail to suggest in any profound way that the lowest rank integers (highest relative concentration) are more numerous than any others. In the tables, the bold rank numbers represent the quartile of all brown bullheads with the highest contaminant concentrations. In Appendix 15a we see that two fish with hepatic tumors (they were, in fact, malignant) indeed had the highest concentrations of naphthalene and phenanthrene. The paucity of bold numbers on that table and its companions fails to offer convincing evidence that these potentially oncogenic agents are actually causing cancer.

Appendix 16 ranks brown bullheads based upon the concentration of the three hydrocarbons and indicates the location from which they came. Naphthalene seems to be the leading contaminant in the Lagoons and Thompson Bay while benzo-α-pyrene is noteworthy in Sara's Cove and the Point - Sara's Cove. There did not seem to be any site of predelection for phenanthrene. These were subjective evaluations which should be tested with appropriate statistical procedures.

IV. DISCUSSION

A. Effect of capture methods upon the study population

Both electrofishing and Pennsylvania Trapnets select for larger fish, allowing smaller and presumably younger fish to escape. Individuals netting electrically stunned fish from the front of the boat tend to net larger fish because large fish are easier to see and most people have a natural tendency to target larger rather than smaller fish. In addition, larger body surface area causes more electrical charge to be drawn upon larger fish and therefore more effective stunning and immobilization. It is reported by the Project Coordinator that small fish can be observed

swimming through an electrical field that is effectively catching large fish. Trapnets may select larger fish based upon the size of the mesh and the particular location in which they are set. Trapnets set near spawning grounds in the spring will select for larger, mature fish. Electrofishing at night selects larger fish which are more easily seen compared with smaller ones. Electrofishing at night is more effective in terms of catch per unit effort. Bullheads are typically caught at a much greater rate at night compared with daytime fishing (Eric Obert, personal communication).

B. Effect of Age Upon Oncogenic Effects in a Population

Under controlled conditions of study, chemical substances are evaluated for their oncogenic potential by evaluating two fundamental parameters. When compared with controls, test animals may develop neoplasia at an earlier age, at a greater incidence, or both. There are, of course, numerous assumptions of proper experimental design which are taken for granted. Those assumptions are not necessarily applicable when evaluating wild populations. It is also a fundamental theory that aged populations have an ever increasing likelihood of acquiring spontaneous neoplasia. Both species of fish in this study are of advanced age and are considered to be likely to possess an increasing rate of neoplasia. Studies of this nature seldom have reliable data to support the age distribution of individuals. If age is considered as a factor in development of neoplasia, age must otherwise be inferred from body weight and length measurements. Rate of growth based upon the availability of dietary nutrients would also be a significant factor. In this study age was well documented and was unavailable in only 3 fish (two bowfin, one brown bullhead). Age must be considered an important factor in future studies.

C. Discussion: Bowfin

Only a single, small, benign neoplasm was found in one bowfin. It was a female which was estimated by its size to be 7-10 years old. (Otoliths were not available for aging this fish.) This is the upper end of the age of the population and must therefore be considered a very old fish. A benign neoplasm in a very old fish under such condiditons can not be considered evidence of an environmental effect. Five bowfin had foci of hepatocellular alteration. In mammals this is a reversible preneoplastic change which is often related to hepatocellular tumors. Since there were

no hepatocellular neoplasms in bowfin, even in this population of old fish, it is uncertain whether their significance is similar to that in mammals. Bowfin with altered hepatocellular foci had a mean age of approximately 8.5 years (range 6 to 10 years).

Bowfin had a very high incidence of gastrointestinal parasitism. Only one failed to have such a report. Three-fourths of the fish had chronic inflammation in the intestines which was probably attributable to the parasites. There was scarcely any evidence of other lesions, such as were found in brown bullheads, which were due to migrations of parasites. The general nutritional condition of bowfin in Presque Isle Bay was adequate.

Half of the caudal kidneys (8 males and 2 females) had accumulations of hyaline droplets in the epithelial cells of the renal tubules. The significance of this finding in fish is unknown. In rats, such accumulations have been caused by hydrocarbons in unleaded gasoline. They have also been shown to contain lysozyme in rats which had certain sarcomas elsewhere in their bodies. Hyaline droplets have not been associated with an effect of treatment in controlled toxicological studies using other species of fish (M J Wolfe, personal communication).

D. Discussion: Brown Bullheads

Brown Bullheads were captured from six locations in Presque Isle Bay. They were distributed equally by sex. Ages ranged from 3 to 17 years with a mean age of 9 years. Males and females were similar and there was no obvious difference based upon age or location (statistical analysis was not attempted.) Brown bullheads from the bay were in generally poor physical condition. They were thin and had little reserves of internal body fat. Reference fish from the reservoir had normal body condition. Wounds and skin lesions were present on the body and mouth of numerous fish including reference fish. Thirty-eight percent of brown bullheads from the study population in Presque Isle Bay had *grossly observable* proliferative skin lesions compared with 10% in the reference population. Similarly, 41% and 10%, repectively, had pigmented skin lesions (Table 3).

The principal neoplastic microscopic findings in brown bullheads were:

- Biliary adenoma and carcinomas in liver;

- Papillomas and carcinomas in the skin of the body and around the head and mouth;
- Testicular seminomas, adenomas and carcinomas.

Refer to Table 4 and Appendix 7 for details of the distribution. Except, of course, for testicular changes there was no convincing predilection on account of sex. It was noteworthy, however, that nearly all of these changes occurred in very old fish. Table 6 emphasises this observation as well as the paucity of neoplastic findings in the reference population. It was considered that the population of fish captured from Presque Isle Bay might be biased toward older fish on account of the methods of capture. The Project Coordinator indicates, however, that several attempts have been made in previous years to collect young brown bullheads. Each time he has been unable to find substantial numbers of young fish (Eric Obert, personal communication). It was beyond the scope of this study to evaluate the structure of the population in either the study site or the reference site. The data of Table 2 suggest, however, that the brown bullheads from Eaton Reservoir were of comparable age to, or perhaps slightly older than, to those of Presque Isle Bay.

TABLE 6
SUMMARY OF NEOPLASTIC OBSERVATIONS IN BROWN BULLHEADS. AGES, IN YEARS, OF FISH WITH NEOPLASMS.

		Study Population			Reference Population		
Organ	Lesion	N=	Range of ages	Mean age	N=	Range of ages	Mean age
Liver	Biliary adenoma	1	13	13			
	Biliary carcinoma	7	9-16	12.7	2	15-16	15.5
Skin	Papilloma	3	12-15	13.3			
	Squamous cell carcinoma	2	14-15	14.5			
	Melanoma	1	12	12			
	Melano-sarcoma	1	16	16			
Mouth/ Lip/ Barbel	Papilloma	15	10-16	12.6			
	Squamous cell carcinoma	6	10-16	13.6			
Gonad (Testis)	Seminoma	11	4-15	8.5			
	Adenoma of tubular lining cells	2	9-13	11			
	Carcinoma of tubular lining cells	1	15	15	1	15	15

Appendix 10 contains a listing of the neoplastic observations and selected non-neoplastic observations in brown bullheads. These are lesions which were noteworthy because of their incidence or because of their pathologic nature and significance.

V. CONCLUSIONS

The populations of bowfin and brown bullhead in this study were aged but could have been skewed toward older fish on account of the method of capture.

The population of bowfin examined in this study was healthy and aged yet there was no indication of neoplasia which could be attributable to an adverse effect of the environment in Presque Isle Bay.

Brown bullheads had neoplasms in the liver, the skin of the body, the skin and mucosa associated with the mouth, lip and barbels, and the testes. The liver tumor rate was 10% and the external tumor rate was 28% for fish from Presque Isle Bay. Liver tumors originated only from bile ducts. Hepatocellular neoplasms were not observed. This was an aged population of fish in poor body condition with a heavy parasite burden. Neoplasms which occurred in the liver, on the skin of the body and on the skin and mucosa in the region of the head occurred in fish which were much older than the mean age of the population.

There was no substantial indication that brown bullheads which had relatively higher concentrations of naphthalene, phenanthrene or benzo-α-pyrene had any propensity toward the development of neoplasms. The fish with the highest concentrations of naphthalene were notably found in the Lagoons and in Thomspon Bay. Similarly, fish with the highest concentrations of benzo-α-pyrene were found in Sara's Cove and Point-Sara's Cove.

The data suggest that there is a higher incidence of certain types of neoplasia and hyperplasia in brown bullheads from Presque Isle Bay than in the reference population. The study was unable to demonstrate an

association with certain polycyclic aromatic hydrocarbons in bile.

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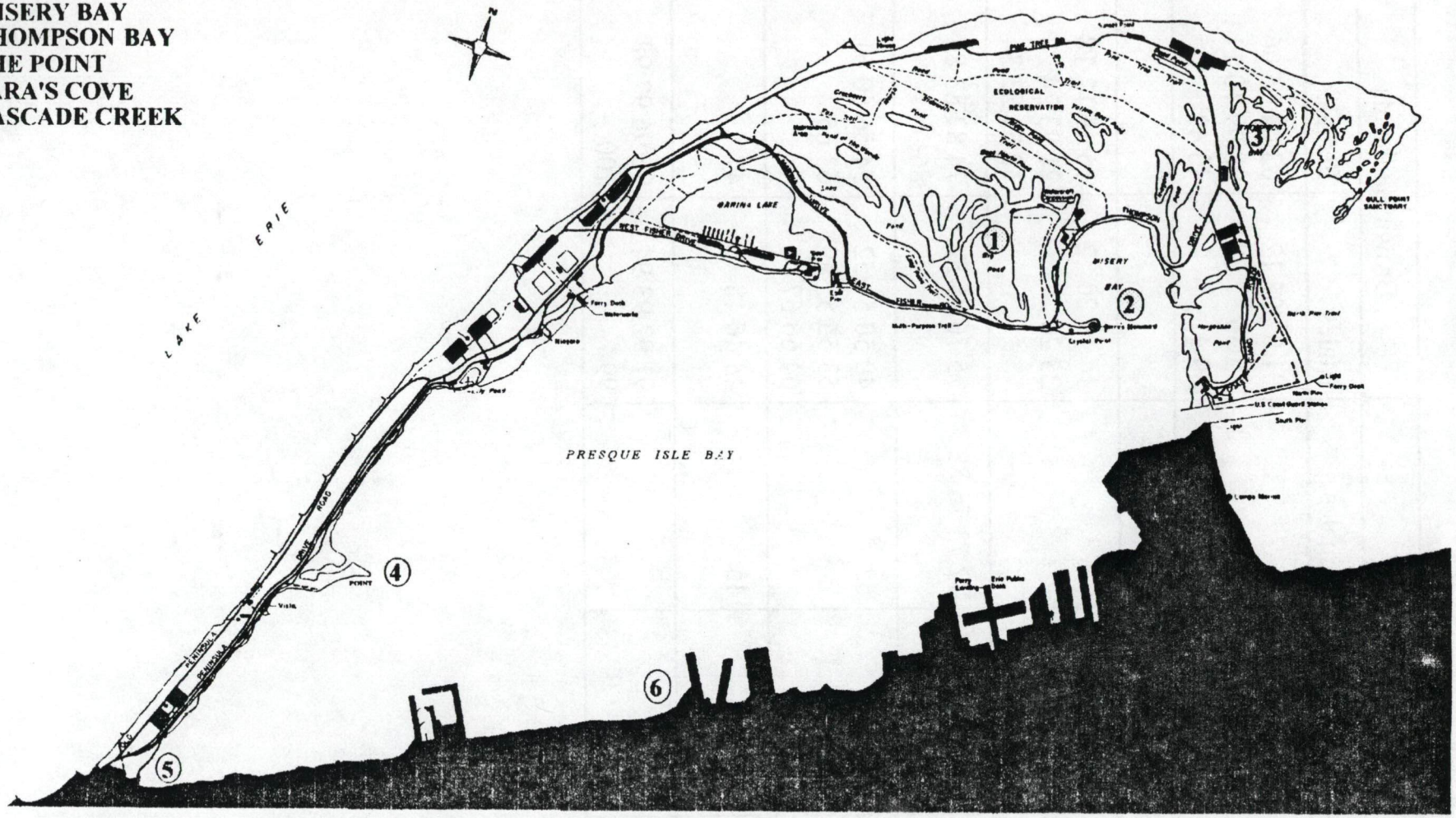
MAP 1. FISH COLLECTION SITES FOR THE 1996 HISTOLOGY STUDY, PRESQUE ISLE BAY.

1. LAGOONS
2. MISERY BAY
3. THOMPSON BAY
4. THE POINT
5. SARA'S COVE
6. CASCADE CREEK



Appendix 1. Map of the Study Area.

Appendix 2: Identification of Fish, by Species and Sex, Caught at Various Locations. List of individual fish by number.

	Bowfin		Brown Bullheads	
Location	Males	Females	Males	Females
Lagoons		13	01 02 07 12 35 36 37 38 39 41	03 04 05 06 08 09 10 11 40 42
Misery Bay	89 90			
Thompson Bay	32	29	14 19 20 22 25 26 27	15 16 17 18 21 23 24 28 30 31 33 34
The Point	68 69 70 71 77 79 80 81	72 73 78 88	75 76 85 87	74 82 83 84 86
Sara's Cove	58	62	49 50 51 52 53 55 57 59 64 66 67	54 56 60 61 63 65
Cascade Creek	43	45	44 46 48	47
Eaton Reservoir			91 92 93 94 99	95 96 97 98 100

Appendix 3: Summary of Non-Neoplastic Microscopic Observations in **Bowfin**.

Tissue	Lesion or Observation	Male N=13	Female N=8	Total N=21
Gallbladder	Chronic inflammation	4	5	9
Liver	Melanomacrophage foci	13	8	21
	Hemosiderosis	5	1	6
	Hyperplasia of bile ducts	4	2	6
	Chronic inflammation; lymphocytic aggregates	10	5	15
	Chronic (peri)portal inflammation	3	4	7
	Portal fibrosis	0	1	1
	Inflammation of bile ducts	1	0	1
	Vacuolation of bile ducts	2	0	2
	Focal/multifocal granulomatous inflammation ± eosinophils, parasites	1	2	3
	Hemorrhage	1	0	1
	Focal hepatocellular alteration	3	2	5
	Clearing of hepatocellular cytoplasm	2	1	3
Cranial kidney	(None)			
Corpuscle of Stannius	(None)			
Caudal kidney	Melanomacrophage foci	11	7	18
	Hyaline droplet accumulation	8	2	10
	Chronic inflammation	2	0	2
	Interstitial hemopoietic cells	11	6	17
	Tubular necrosis	1	0	1
	Tubular casts	1	0	1
	Protozoan parasites in tubules	0	1	1

Tissue	Lesion or Observation	Male N=13	Female N=8	Total N=21
Heart	Chronic inflammation	13	8	21
Thyroid	Chronic inflammation	2	0	2
Stomach	Chronic inflammation	8	8	16
	Acute inflammation	1	0	1
	Edema	1	0	1
	Parasites in lumen	3	3	6
	Hemorrhage	3	0	3
Gas bladder	Chronic inflammation	13	5	18
	Metaplasia/hyperplasia of goblet cells	6	2	8
	Myolysis	1	0	1
	Edema	0	1	1
Urinary bladder	Chronic inflammation	0	3	3
	Hypertrophy of mesothelial cells	3	2	5
	Hyperplasia	1	0	1
Gonad	Melanomacrophage foci	0	1	1
	Chronic inflammation	0	4	4
	Hemosiderosis	1	0	1
	Cyst	1	0	1
Spleen	Hemosiderosis	5	3	8
	Melanomacrophage foci	8	3	11
Intestine	Chronic inflammation	7	8	15
	Parasites	12	8	20
	Hemorrhage	1	0	1
	Hemosiderosis	1	1	2

Tissue	Lesion or Observation	Male N=13	Female N=8	Total N=21
Skeletal muscle	Myofiber rarefaction, myolysis or necrosis	3	2	5
Skin	Chronic inflammation	6	4	10
	Hyperplasia	2	0	2
Mouth/Lip/ Barbel	Chronic inflammation	9	4	13
	Ulceration	2	0	2
	Parasite	0	1	1
Non-protocol tissues:				
Pancreas	Chronic inflammation	2	0	2
Mesentery and gastrointestinal serosa	Chronic inflammation	2	1	3

Appendix 4a: Non-Neoplastic Observations in **Bowfin**.
Detail of microscopic findings with identification of individual fish.

Gallbladder					
Lesion or Observation	Individual Males	Individual Females	Male N=13	Female N=8	Total N=21
Tissue not present	32 70 80	13 73	3	2	5
No significant lesions	68 69 77 79 89 90	62	6	1	7
Chronic inflammation	43 58 71 81	29 45 72 78 88	4	5	9

Appendix 4b: Non-Neoplastic Observations in **Bowfin**
Detail of microscopic findings with identification of individual fish

Liver					
Lesion or Observation	Individual Males	Individual Females	Male N=13	Female N=8	Total N=21
Tissue not present			0	0	0
No significant lesions			0	0	0
Melanomacrophage foci	32 43 58 68 69 70 71 77 79 80 81 89 90	13 29 45 62 72 73 78 88	13	8	21
Hemosiderosis	32 77 81 89 90	13	5	1	6
Hyperplasia of bile ducts	32 58 81 90	13 78	4	2	6
Chronic inflammation; lymphocytic aggregates	43 58 69 70 71 77 80 81 89 90	29 45 62 72 88	10	5	15
Chronic (peri)portal inflammation	32 43 77	13 29 45 73	3	4	7
Portal fibrosis		72	0	1	1
Inflammation of bile ducts	80		1	0	1
Vacuolation of bile ducts	32 58		2	0	2
Focal/MF granulomatous inflammation ± eosinophils, parasites	77	29 73	1	2	3
Hemorrhage	77		1	0	1
Focal hepatocellular alteration	79 89 90	13 62	3	2	5
Clearing of hepatocellular cytoplasm	43 68	29	2	1	3

Appendix 4c: Non-Neoplastic Observations in **Bowfin**
Detail of microscopic findings with identification of individual fish

Cranial kidney					
Lesion or Observation	Individual Males	Individual Females	Male N=13	Female N=8	Total N=21
Tissue not present	32 43 58 68 69 70 71 77 79 80 89 90	13 29 45 62 72 73 88	12	7	19
No significant lesions	81	78	1	1	2

Appendix 4d: Non-Neoplastic Observations in **Bowfin**
Detail of microscopic findings with identification of individual fish

Corpuscle of Stannius					
Lesion or Observation	Individual Males	Individual Females	Male N=13	Female N=8	Total N=21
Tissue not present	32 43 58 69 70 71 77 79 80 89 90	13 29 45 62 72 73 78 88	11	8	19
No significant lesions	68 81		2	0	2

Appendix 4e: Non-Neoplastic Observations in **Bowfin**
Detail of microscopic findings with identification of individual fish

Caudal kidney					
Lesion or Observation	Individual Males	Individual Females	Male N=13	Female N=8	Total N=21
Tissue not present	81	45	1	1	2
No significant lesions			0	0	0
Melanomacrophage foci	32 43 58 68 69 70 71 79 80 89 90	13 29 62 72 73 78 88	11	7	18
Hyaline droplet accumulation	32 43 58 68 77 80 89 90	72 88	8	2	10
Chronic inflammation	32 77		2	0	2
Interstitial hemopoietic cells	32 58 68 69 70 71 77 79 80 89 90	13 29 62 72 73 78	11	6	17
Tubular necrosis	58		1	0	1
Tubular casts	71		1	0	1
Protozoan parasites in tubules		88	0	1	1

Appendix 4f: Non-Neoplastic Observations in **Bowfin**
Detail of microscopic findings with identification of individual fish

Heart					
Lesion or Observation	Individual Males	Individual Females	Male N=13	Female N=8	Total N=21
Tissue not present			0	0	0
No significant lesions			0	0	0
Chronic inflammation	32 43 58 68 69 70 71 77 79 80 81 89 90	13 29 45 62 72 73 78 88	13	8	21

Appendix 4g: Non-Neoplastic Observations in **Bowfin**
Detail of microscopic findings with identification of individual fish

Thyroid					
Lesion or Observation	Individual Males	Individual Females	Male N=13	Female N=8	Total N=21
Tissue not present	43 68 69 70 71 79 80 81	13 29 62 72 78 88	8	6	14
No significant lesions	77 89 90	45 73	3	2	5
Chronic inflammation	32 58		2	0	2

Tissue	Lesion or Observation	Male N=40	Female N=39	Total N=79
	Serositis	3	1	4
	Fibrosis	1	2	3
	Thrombosis	2	5	7
	Hemorrhage	0	1	1
	Edema	0	3	3
	Lymphangiectasia	0	4	4
	Vasculopathy	2	2	4
Skeletal muscle	Chronic inflammation	1	1	2
	Granulomatous inflammation ± parasites, necrosis	2	0	2
	Degeneration / myolysis	9	6	15
	Atrophy	1	0	1
	Parasites	1	0	1
	Hemorrhage	0	1	1
	Fatty infiltration	0	1	1
Skin	Chronic inflammation	3	3	6
	Granulomatous inflammation ± parasites, necrosis	1	0	1
	Fibrosis	2	1	3
	Epidermal hyperplasia	12	10	22
	Hyperpigmentation	15	9	24
	Hyperplasia of alarm cells	1	2	3
	Epidermal spongiosis	1	1	2

Tissue	Lesion or Observation	Male N=40	Female N=39	Total N=79
	Parasite	1	0	1
	Edema	1	0	1
	Erosion / ulceration	1	2	3
Mouth/Lip/Barbel	Chronic inflammation	6	7	13
	Acute inflammation	0	1	1
	Granulomatous inflammation ± parasites, necrosis	8	2	10
	Fibrosis	1	2	3
	Epidermal hyperplasia	36	32	68
	Hyperpigmentation	2	1	3
	Parasite	1	2	3
	Edema	0	1	1
	Erosion / ulceration	2	6	8
	Hemorrhage	4	3	7
	Mucus metaplasia	0	1	1

Apendix 9a: Non-Neoplastic Microscopic Observations in **Brown Bullheads**.
Detail of microscopic findings with listing of individual fish.

Gallbladder					
Lesion or Observation	Individual Males	Individual Females	Male N=40	Female N=39	Total N=79
Tissue not present	14 25 **93**	04 10 16 24 30 74 **97**	3	7	10
No significant lesions	02 07 12 19 20 22 26 27 35 36 37 38 39 41 44 46 48 49 50 51 52 53 55 57 59 64 66 67 75 76 85 87 **91 92 94 99**	03 05 06 08 09 11 15 17 18 21 23 28 31 3334 40 42 47 54 56 60 61 63 65 82 83 84 86 **95 96 98 100**	36	32	68
Chronic inflammation	01		1	0	1

Bold numbers are identification of fish from the Reference Population.

Apendix 9b: Non-Neoplastic Microscopic Observations in **Brown Bullheads**.
Detail of microscopic findings with listing of individual fish.

Liver					
Lesion or Observation	Individual Males	Individual Females	Male N=40	Female N=39	Total N=79
Tissue not present			0	0	0
No significant lesions	35	04	1	1	2
Chronic inflammation	02 14 27	31 33 34	3	3	6
Granulomatous inflammation ± parasites, necrosis	01 02 07 20 22 25 27 36 44 48 49 50 51 53 57 59 64 66 67 76 85 87 **92 93 99**	05 08 15 16 17 21 23 30 40 42 61 65 82 84 86 **95 96 97 98**	25	19	44
Hemosiderosis; pigment-laden macrophage aggregates	01 02 07 12 14 19 20 22 25 26 27 37 38 39 41 44 46 48 49 50 52 53 55 57 59 64 66 67 75 76 87 **91 92 93 94 99**	03 05 06 08 09 11 15 16 17 18 21 23 24 28 30 31 33 34 40 42 47 54 56 60 61 63 65 74 82 83 84 86 **95 96 97 98 100**	36	37	73
Hepatocellular alteration	01 02 07 14 25 27 38 46 48 49 53 75 76 85 **91 92 94 99**	03 06 16 17 30 40 63 65 82 83 86 **95 97 98**	18	14	32
Clearing of hepatocytes; tinctorially altered region	01 02 07 12 19 20 22 27 36 37 38 39 48 50 51 52 55 59 **92 93**	09 10 16 17 21 24	20	6	26
Hyperplasia of bile ducts	02 25 27 37 41 44 **93**	24 33 63 **98**	7	4	11

Lesion or Observation	Individual Males	Individual Females	Male N=40	Female N=39	Total N=79
Parasites in bile ducts	07 19 22 25 27 36 37 44 48 49 50 53 57 59 64 66 67 75 76 85 87 **91 92 93 94 99**	06 16 17 18 23 28 30 33 42 54 63 65 84 86 **95 97 98**	26	17	43
Biliary ectasia	14		1	0	1
Biliary coccidiosis	14		1	0	1
Fibrosis	36	05 24 28 31 33 34 82	1	7	8
Mineralized focus	41	18	1	1	2
Bacteremia	41		1	0	1
Amyloidosis	67		1	0	1
Hepatocellular necrosis		10 21 84	0	3	3
Hyperplasia of intima		33	0	1	1
Telangiectasis		**96 97**	0	2	2
Thrombosis	52	17	1	1	2

Bold numbers are identification of fish from the Reference Population.

Apendix 9c: Non-Neoplastic Microscopic Observations in **Brown Bullheads**.
Detail of microscopic findings with listing of individual fish.

Cranial kidney					
Lesion or Observation	Individual Males	Individual Females	Male N=40	Female N=39	Total N=79
Tissue not present	12 36		2	0	2
No significant lesions	20 04	10 21	2	2	4
Hemosiderosis; pigment-laden macrophage aggregates	01 02 07 14 19 22 25 26 27 35 37 38 39 41 44 46 48 49 50 51 52 53 55 57 59 64 66 67 75 76 85 87 **91 92 93 94 99**	03 05 06 08 11 15 16 17 18 23 24 28 30 31 33 34 40 42 47 54 56 60 61 63 65 74 82 83 84 86 **95 96 97 98 100**	37	35	72
Chronic inflammation		08 09	0	2	2
Granulomatous inflammation ± parasites, necrosis	27 44		2	0	2
Vacuolation of endocrine tissue	02		1	0	1
Hypertrophy of endocrine tissue	41 48		2	0	2

Bold numbers are identification of fish from the Reference Population.

Apendix 9d: Non-Neoplastic Microscopic Observations in **Brown Bullheads**.
Detail of microscopic findings with listing of individual fish.

Corpuscle of Stannius					
Lesion or Observation	Individual Males	Individual Females	Male N=40	Female N=39	Total N=79
Tissue not present	14 20 39 41 51 66 75 87 **93**	10 21 30 34 54 60 61 **96**	9	8	17
No significant lesions	01 02 07 12 19 22 25 26 27 35 36 37 38 44 46 48 49 50 52 53 55 57 59 64 67 76 85 **91 92 94 99**	03 05 06 08 09 11 15 16 17 18 23 24 28 31 33 40 42 47 56 65 74 82 83 84 86 **95 97 98 100**	31	29	60
Hyperplasia		04	0	1	1
Cystic		63	0	1	1

Bold numbers are identification of fish from the Reference Population.

Apendix 9e: Non-Neoplastic Microscopic Observations in **Brown Bullheads**.
Detail of microscopic findings with listing of individual fish.

Caudal kidney					
Lesion or Observation	Individual Males	Individual Females	Male N=40	Female N=39	Total N=79
Tissue not present			0	0	0
No significant lesions	12	04 21	1	2	3
Hemosiderosis; pigment-laden macrophage aggregates	01 02 07 14 19 25 26 27 35 36 38 39 41 44 46 48 49 50 51 52 53 55 57 59 64 66 67 75 76 85 87 **91 92 93 94 99**	03 05 06 08 09 10 11 15 16 17 18 23 24 28 30 31 33 34 40 42 47 54 56 60 61 63 65 74 82 83 84 86 **95 96 97 98 100**	36	37	71
Granulomatous inflammation ± parasites, necrosis	49 52	09 10	2	2	4
Hyaline droplet accumulation	14 20 22 37 38 41 50 51 59 67 85 87 **99**	03 06 10 16 28 30 33 40 56 60 86 **97**	13	12	25
Thrombosis	02		1	0	1
Vasculitis	02		1	0	1
Parasites in ducts	49	10	1	1	2
Interstitial bacterial colonies	**91 94**		2	0	2

Bold numbers are identification of fish from the Reference Population.

Apendix 9f: Non-Neoplastic Microscopic Observations in **Brown Bullheads**.
Detail of microscopic findings with listing of individual fish.

Heart					
Lesion or Observation	Individual Males	Individual Females	Male N=40	Female N=39	Total N=79
Tissue not present			0	0	0
No significant lesions			0	0	0
Chronic inflammation		15 16 17	0	3	3
Epicarditis	01 02 07 12 14 19 20 22 25 26 27 35 36 37 38 39 41 44 46 48 49 50 51 52 53 55 57 59 64 66 67 75 76 85 87 **91 92 93 94 99**	03 04 05 06 08 09 10 11 15 16 17 18 23 24 28 30 31 33 40 42 47 54 56 60 61 63 65 74 82 83 84 86 **95 96 97 98 100**	40	37	77
Myocarditis	02		1	0	1
Fibrosis	01	34	1	1	2
Hemosiderosis; pigment-laden macrophage aggregates	07		1	0	1
Osseous metaplasia	14		1	0	1
Adventititis	48 49 85	21 23 63 82	3	4	7
Granulonatous inflammation	85	33	1	1	2

Bold numbers are identification of fish from the Reference Population.

Apendix 9g: Non-Neoplastic Microscopic Observations in **Brown Bullheads**.
Detail of microscopic findings with listing of individual fish.

Thyroid					
Lesion or Observation	Individual Males	Individual Females	Male N=40	Female N=39	Total N=79
Tissue not present		54	0	1	1
No significant lesions	01 02 07 12 14 19 20 22 25 26 27 35 36 37 39 41 44 46 48 49 50 51 52 53 55 57 59 64 66 67 75 76 85 87 **91** **92 93 94 99**	03 04 05 06 08 09 10 11 15 16 17 18 21 24 28 30 31 33 34 40 42 47 56 60 61 65 74 83 84 86 **95 96** **97 98 100**	39	35	74
Chronic inflammation	38	23 63 82	1	3	4

Bold numbers are identification of fish from the Reference Population.

Apendix 9h: Non-Neoplastic Microscopic Observations in **Brown Bullheads**.
Detail of microscopic findings with listing of individual fish.

Stomach					
Lesion or Observation	Individual Males	Individual Females	Male N=40	Female N=39	Total N=79
Tissue not present			0	0	0
No significant lesions	01 02 07 12 22 25 38 39 46 49 51 55 66 67 75 76 87 **93**	04 05 06 08 10 42 54 83 **100**	18	9	27
Chronic inflammation		82	0	1	1
Granulomatous inflammation ± parasites, necrosis	20 27 35 36 37 41 44 48 50 53 57 59 85 **91 92 94 99**	09 11 15 16 17 18 21 23 28 30 31 33 34 40 47 63 65 74 84 86 **95 96 97**	17	23	40
Parasites in vessels	14 19 26 35 37 41 44 48 50 52 53 57 85 **91 92 94 99**	03 09 11 15 16 17 18 21 23 28 30 34 40 47 60 61 63 65 74 84 86 **95 96 97**	17	24	41
Hemorrhage	36		1	0	1
Edema	36	**98**	1	1	2
Lymphangiectasia	36	**98**	1	1	2
Hyperplasia of mucus cells	64		1	0	1
Thrombosis	**92**	17 18 23 28 30 31 34	1	7	8
Hemosiderosis; pigment-laden macrophage aggregates		24	0	1	1
Serositis		56	0	1	1

Lesion or Observation	Individual Males	Individual Females	Male N=40	Female N=39	Total N=79
Vasculitis		74	0	1	1

Apendix 9i: Non-Neoplastic Microscopic Observations in **Brown Bullheads**.
Detail of microscopic findings with listing of individual fish.

Gas bladder					
Lesion or Observation	Individual Males	Individual Females	Male N=40	Female N=39	Total N=79
Tissue not present			0	0	0
No significant lesions	14 19 20 26 35 36 50 **91 92 93 94**	09 10 11 15 18 21 23 24 54 83 84 86 **95 96 97 98**	11	16	27
Chronic inflammation	01 07 12 38 48	04 **100**	5	2	7
Granulomatous inflammation ± parasites, necrosis	02 07 22 25 27 37 38 39 41 44 46 48 49 51 52 53 55 57 59 64 66 67 75 76 85 87 **99**	03 05 06 08 16 17 28 30 31 33 34 40 42 47 56 60 61 63 65 74 82	27	21	48
Parasites in lumen, mucosa or vessels	01 27 37 38 46 48	04 56	6	2	8
Serositis	**99**		1	0	1
Mucosal metaplasia	27	40	1	1	2
Mucosal hyperplasia	27 37	30	2	1	3
Melanocytosis / pigmentation	01 02	03 04	2	2	4
Hemorrhage		56 **100**	0	2	2

Bold numbers are identification of fish from the Reference Population.

Apendix 9j: Non-Neoplastic Microscopic Observations in **Brown Bullheads**.
Detail of microscopic findings with listing of individual fish.

Urinary bladder					
Lesion or Observation	Individual Males	Individual Females	Male N=40	Female N=39	Total N=79
Tissue not present		03 05 11 28 61 63	0	6	6
No significant lesions	01 02 07 12 14 19 20 22 25 26 27 35 36 37 38 39 41 44 46 48 49 50 51 52 53 55 57 59 64 66 67 75 76 87 **91** **92 94 99**	04 06 09 10 16 18 21 23 24 30 31 33 34 40 42 47 54 56 60 65 74 83 86 **95** **96 97 98** **100**	38	28	66
Chronic inflammation		15	0	1	1
Metaplasia of mucus cells	**93**		1	0	1
Hyperplasia of mucus cells	85 **93**	15 82 84	2	3	5
Hemosiderosis; pigment-laden macrophage aggregates		08	0	1	1
Hemorrhage		17	0	1	1

Bold numbers are identification of fish from the Reference Population.

Apendix 9k: Non-Neoplastic Microscopic Observations in **Brown Bullheads**.
Detail of microscopic findings with listing of individual fish.

Gonad					
Lesion or Observation	Individual Males *	Individual Females	Male N=40	Female N=39	Total N=79
Tissue not present			0	0	0
No significant lesions	57	04 09 10 11 15 16 18 21 23 24 28 30 31 33 34 40 42 60 61 63 74 82 83 86 **97 98**	1	26	27
Chronic inflammation		03 65	0	2	2
Hemosiderosis; pigment-laden macrophage aggregates		03 47	0	2	2
Granulomatous inflammation ± parasites, necrosis		65 84	0	2	2
Atretic follicles		03 05 06 08 47 54 56 65 **95 100**	N/A	10	10
Atrophy		17	0	1	1
Hyperplasia of granulosa cells		17 54 56 **95 96 100**	N/A	6	6
Fibrosis		47	0	1	1
Hyperplasia/ hypertrophy of tubular lining cells	01 02 07 12 14 19 22 25 27 35 36 37 38 41 44 46 48 49 50 51 52 53 55 59 64 66 67 75 76 85 87 **91 92 93 94 99**		36	N/A	36

Lesion or Observation	Individual Males *	Individual Females	Male N=40	Female N=39	Total N=79
Vacuolation of tubular lining cells	02 12 14 19 25		5	N/A	5
Aspermagenesis	02 07 19 27 38 44 46 49 50 55 59 64 67 **94 99**		15	N/A	15

Bold numbers are identification of fish from the Reference Population.

*Data for males are still under review. Hyperplasia and neoplasia were initially described. Colleagues with more specific experience with ictalurid testes have been consulted but concensus has not yet been reached.

Apendix 9l: Non-Neoplastic Microscopic Observations in **Brown Bullheads**. Detail of microscopic findings with listing of individual fish.

Spleen					
Lesion or Observation	Individual Males	Individual Females	Male N=40	Female N=39	Total N=79
Tissue not present	20 46	08 34 42 61 **95**	2	5	7
No significant lesions			0	0	0
Chronic inflammation	48		1	0	1
Granulomatous inflammation ± parasites, necrosis	26		1	0	1
Hemosiderosis; pigment-laden macrophage aggregates	01 02 07 12 14 19 22 25 26 27 35 36 37 38 39 41 44 48 49 50 51 52 53 55 57 59 64 66 67 75 76 85 87 **91 92 93 94 99**	03 04 05 06 09 10 11 15 16 17 18 21 23 24 28 30 31 33 40 47 54 56 60 63 65 74 82 83 84 86 **96 97 98 100**	38	34	72
Bridging connective tissue; accentuated stroma	14	17 18	1	2	3
Cyst	48		1	0	1

Bold numbers are identification of fish from the Reference Population.

Apendix 9m: Non-Neoplastic Microscopic Observations in **Brown Bullheads**.
Detail of microscopic findings with listing of individual fish.

Intestine					
Lesion or Observation	Individual Males	Individual Females	Male N=40	Female N=39	Total N=79
Tissue not present			0	0	0
No significant lesions	12 14 46 52 53 55 75 87 **91**	16 21 40 42 56 60 61 83 84 86 **97** **100**	9	12	21
Chronic inflammation	02 25 44	05 74	3	2	5
Subacute inflammation	**99**	04	1	1	2
Granulomatous inflammation ± parasites, necrosis	01 26 27 35 36 41 48 49 50 51 85 **92**	03 09 30 31 33 34 47 74 **95 98**	12	10	22
Parasites in lumen	01 19 20 22 26 27 36 37 38 41 44 51 57 59 64 67 76 **94 99**	04 05 06 08 18 28 30 65 74 82 **95 98**	19	12	31
Parasites in vessels	26 36 37 41 44 48 49 51 66 85 **92**	09 11 30 33 34 47 54 63 82 **98**	11	10	21
Hemosiderosis; pigment-laden macrophage aggregates	02 07 20 25 26 27 35 39	03 15 23 24 28 30 31 33 34	8	9	17
Serositis	02 26 44	74	3	1	4
Fibrosis	25	05 23	1	2	3
Thrombosis	49 **99**	30 31 33 **95** **96**	2	5	7
Hemorrhage		05	0	1	1
Edema		08 09 47	0	3	3
Lymphangiectasia		08 09 17 47	0	4	4

Lesion or Observation	Individual Males	Individual Females	Male N=40	Female N=39	Total N=79
Vasculopathy	**93 99**	10 **96**	2	2	4

Bold numbers are identification of fish from the Reference Population.

Apendix 9n: Non-Neoplastic Microscopic Observations in **Brown Bullheads**.
Detail of microscopic findings with listing of individual fish.

Skeletal muscle					
Lesion or Observation	Individual Males	Individual Females	Male N=40	Female N=39	Total N=79
Tissue not present			0	0	0
No significant lesions	01 07 12 14 19 25 26 27 35 36 37 38 46 50 51 53 55 64 66 67 75 76 85 **91 92 93 94 99**	03 05 06 08 09 11 15 16 17 21 24 28 30 31 33 34 40 42 54 56 60 61 74 82 83 84 86 **95 96 97 98 100**	28	32	60
Chronic inflammation	52	47	1	1	2
Granulomatous inflammation ± parasites, necrosis	44 48		2	0	2
Degeneration / myolysis	02 20 22 39 41 52 57 59 87	04 10 18 23 63 65	9	6	15
Atrophy	20		1	0	1
Parasites	49		1	0	1
Hemorrhage		18	0	1	1
Fatty infiltration		18	0	1	1

Bold numbers are identification of fish from the Reference Population.

Apendix 9o: Non-Neoplastic Microscopic Observations in **Brown Bullheads**.
Detail of microscopic findings with listing of individual fish.

Skin					
Lesion or Observation	Individual Males	Individual Females	Male N=40	Female N=39	Total N=79
Tissue not present			0	0	0
No significant lesions	12 19 20 22 26 27 35 36 41 51 52 59 67 75 **91** **92** **93**	04 09 10 11 15 17 18 33 34 54 56 60 61 65 74 **95** **96** **97** **100**	17	19	36
Chronic inflammation	01 55 87	03 08 47	3	3	6
Granulomatous inflammation ± parasites, necrosis	01		1	0	1
Fibrosis	25 **99**	**98**	2	1	3
Epidermal hyperplasia	01 02 14 25 37 38 48 49 50 53 55 66	06 08 16 23 24 40 42 47 82 86	12	10	22
Hyperpigmentation	07 25 37 38 39 44 46 49 50 53 57 64 76 85 87	05 16 30 31 63 83 84 86 **98**	15	9	24
Hyperplasia of alarm cells	07	21 23	1	2	3
Epidermal spongiosis	14	28	1	1	2
Parasite	48		1	0	1
Edema	87		1	0	1
Erosion / ulceration	**94**	03 **98**	1	2	3

Bold numbers are identification of fish from the Reference Population.

Apendix 9p: Non-Neoplastic Microscopic Observations in **Brown Bullheads**.
Detail of microscopic findings with listing of individual fish.

Mouth/Lip/Barbel					
Lesion or Observation	Individual Males	Individual Females	Male N=40	Female N=39	Total N=79
Tissue not present			0	0	0
No significant lesions	**91**	10 15 34	1	3	4
Chronic inflammation	01 02 20 75 85 **94**	03 05 24 40 42 74 82	6	7	13
Acute inflammation		**97**	0	1	1
Granulomatous inflammation ± parasites, necrosis	01 35 36 37 39 41 87 **93**	47 **95**	8	2	10
Fibrosis	**94**	60 63	1	2	3
Epidermal hyperplasia	02 07 12 14 19 20 22 26 27 35 36 37 38 41 44 46 48 49 50 51 52 53 55 57 59 64 66 67 75 76 85 87 **91 93 94 99**	04 05 06 08 09 11 16 17 18 21 24 28 30 31 33 42 47 54 56 61 63 65 74 82 83 84 86 **95 96 97 98 100**	36	32	68
Hyperpigmentation	27 44	31	2	1	3
Parasite	37	**95 100**	1	2	3
Edema		60	0	1	1
Erosion / ulceration	25 41	03 42 47 63 84 **97**	2	6	8
Hemorrhage	41 57 76 **94**	60 63 **97**	4	3	7
Mucus metaplasia		40	0	1	1

Bold numbers are identification of fish from the Reference Population.

Appendix 10a: Selected Neoplastic and Non-Neoplastic Observations in **Brown Bullheads**. Summary of incidences in Study and Reference Populations

Liver						
	Study Population			Reference Population		
Lesion or Observation	Male N=35	Female N=34	Total N=69	Male N=5	Female N=5	Total N=10
Biliary adenoma	1 3%	-	1 1%	-	-	-
Biliary carcinoma	6 17%	1 3%	7 10%	1 20%	1 20%	2 20%
Granulomatous inflammation ± parasites, necrosis	22 63%	15 44%	37 54%	3 60%	4 80%	7 70%
Hepatocellular alteration	14 40%	11 32%	25 36%	4 80%	3 60%	7 70%
Hyperplasia of bile ducts	6 17%	3 9%	9 13%	1 20%	1 20%	2 20%
Parasites in bile ducts	21 60%	14 41%	35 51%	5 100%	3 60%	8 80%

Appendix 10b: Selected Neoplastic and Non-Neoplastic Observations in **Brown Bullheads**. Summary of incidences in Study and Reference Populations

Caudal kidney						
	Study Population			Reference Population		
Lesion or Observation	Male N=35	Female N=34	Total N=69	Male N=5	Female N=5	Total N=10
Hyaline droplet accumulation	12 34%	11 32%	23 33%	1 20%	1 20%	2 20%

Appendix 10c: Selected Neoplastic and Non-Neoplastic Observations in **Brown Bullheads**. Summary of incidences in Study and Reference Populations

Stomach						
	Study Population			Reference Population		
Lesion or Observation	Male N=35	Female N=34	Total N=69	Male N=5	Female N=5	Total N=10
Granulomatous inflammation ± parasites, necrosis	13 37%	20 59%	33 43%	4 80%	3 60%	7 70%
Parasites in vessels	13 37%	21 62%	34 49%	4 80%	3 60%	7 70%

Appendix 10d: Selected Neoplastic and Non-Neoplastic Observations in **Brown Bullheads**. Summary of incidences in Study and Reference Populations

Gas bladder						
	Study Population			Reference Population		
Lesion or Observation	Male N=35	Female N=34	Total N=69	Male N=5	Female N=5	Total N=10
Chronic inflammation	5 14%	1 3%	6 9%	-	1 20%	1 10%
Granulomatous inflammation ± parasites, necrosis	26 74%	21 62%	47 68%	1 20%	-	1 10%
Parasites in lumen, mucosa or vessels	6 17%	2 6%	8 12%	-	-	-

Appendix 10e: Selected Neoplastic and Non-Neoplastic Observations in **Brown Bullheads**. Summary of incidences in Study and Reference Populations

Gonad (Testis) *						
	Study Population			Reference Population		
Lesion or Finding	Male N=35			Male N=5		
Seminoma	11 31%			-		
Adenoma of tubular lining cells	2 6%			-		
Carcinoma of tubular lining cells	1 3%			1 20%		
Hyperplasia/ hypertrophy of tubular lining cells	31 89%			5 100%		
Vacuolation of tubular lining cells	5 14%			-		

*Data for males are still under review. Hyperplasia and neoplasia were initially described. Colleagues with more specific experience with ictalurid testes have been consulted but concensus has not yet been reached.

Appendix 10f: Selected Neoplastic and Non-Neoplastic Observations in **Brown Bullheads**. Summary of incidences in Study and Reference Populations

Intestine						
	Study Population			Reference Population		
Lesion or Observation	Male N=35	Female N=34	Total N=69	Male N=5	Female N=5	Total N=10
Granulomatous inflammation ± parasites, necrosis	11 31%	8 24%	19 28%	1 20%	2 40%	3 30%
Parasites in lumen	17 49%	10 29%	27 39%	2 40%	2 40%	4 40%
Parasites in vessels	10 29%	9 26%	19 28%	1 20%	1 20%	2 20%

Appendix 10g: Selected Neoplastic and Non-Neoplastic Observations in **Brown Bullheads**. Summary of incidences in Study and Reference Populations

Skin						
	Study Population			Reference Population		
Lesion or Observation	Male N=35	Female N=34	Total N=69	Male N=5	Female N=5	Total N=10
Papilloma	3 9%	-	3 4%	-	-	-
Squamous cell carcinoma	-	2 6%	2 3%	-	-	-
Melanoma	1 3%	-	1 1%	-	-	-
Melanosarcoma	-	1 3%	1 1%	-	-	-
Epidermal hyperplasia	12 34%	10 29%	22 32%	-	-	-
Hyperpigmentation	15 43%	8 24%	23 33%	-	1 20%	1 10%
Hyperplasia of alarm cells	1 29%	2 6%	3 4%	-	-	-

Appendix 10h: Selected Neoplastic and Non-Neoplastic Observations in **Brown Bullheads**. Summary of incidences in Study and Reference Populations

Mouth/Lip/Barbel						
	Study Population			Reference Population		
Lesion or Observation	Male N=35	Female N=34	Total N=69	Male N=5	Female N=5	Total N=10
Papilloma	8 23%	7 21%	15 22%	-	-	-
Squamous cell carcinoma	3 9%	3 9%	6 9%	-	-	-
Epidermal hyperplasia	32 91%	27 79%	59 86%	4 80%	5 100%	9 90%
Hyperpigmentation	2 6%	1 3%	3 4%	-	-	-
Erosion / ulceration	2 6%	5 15%	7 10%	-	1 20%	1 10%

Appendix 11: Concentration of Polycyclic Aromatic Hydrocarbons in Bile of **Bowfin**

Location	Fish ID	Age	Naphthalene µg/kg	Phenanthrene µg/kg	Benzo-α-pyrene µg/kg
Lagoons	13	-	31958	5734	186
Thompson Bay	29	10	74533	7720	212
	32	8	199900	56644	959
Cascade Creek	43	7	95810	12193	221
	45	4	137587	42747	500
Sara's Cove	58	3	49972	14599	2089
	62	9	48650	13759	1145
Point Sara's Cove	68	8	32418	7960	4067
	69	9	49275	11804	4053
	70	4	37864	9680	899
	71	4	76408	23344	2732
	72	-	25171	8402	568
	73	4	43363	18215	707
	77	8	42448	15983	1164
	78	4	36691	4455	681
	79	10	44369	8673	605
	80	4	38558	5770	420
	81	10	39083	8493	987
	88	3	46010	86456	195
Misery Bay	89	7	49522	8818	470
	90	6	69378	14007	1339

Appendix 12a: Concentration of Polycyclic Aromatic Hydrocarbons in Bile of **Brown Bullheads.**

Location: Lagoons				
Fish ID	Age	Naphthalene µg/kg	Phenanthrene µg/kg	Benzo-α-pyrene µg/kg
01	13	89300	14238	327
02	15	206949	40630	385
03	4	279843	48184	520
04	7	-	-	-
05	12	424917	77139	924
06	15	314413	42853	576
07	7	-	-	-
08	3	133573	27999	359
09	4	32821	25687	792
10	4	-	-	-
11	4	356290	50711	821
12	4	62933	11115	230
35	3	224665	73771	2
36	4	98271	21709	6
37	12	106371	337000	146
38	4	133975	58620	1
39	3	163534	82120	32
40	15	214326	78076	157
41	9	437608	65462	67
42	14	155908	63136	228
		$\bar{x} = 202100$	$\bar{x} = 65791$	$\bar{x} = 328$

Appendix 12b: Concentration of Polycyclic Aromatic Hydrocarbons in Bile of **Brown Bullheads.**

Location: Thompson Bay				
Fish ID	Age	Naphthalene µg/kg	Phenanthrene µg/kg	Benzo-α-pyrene µg/kg
14	7	133884	35698	852
15	7	221285	48017	575
16	7	191830	26436	404
17	7	213648	49315	707
18	6	299758	45157	597
19	4	220598	45647	1025
20	4	100660	19542	412
21	4	237177	30997	346
22	7	126536	33545	583
23	11	146452	23919	359
24	12	200460	47654	718
25	14	209910	36272	624
26	4	119251	18140	394
27	13	181199	23688	276
28	14	272789	25453	8
30	12	311940	123142	329
31	8	136814	26550	59
33	10	171214	70127	145
34	11	135056	45188	9
		$\bar{x}$ = 184735	$\bar{x}$ = 40762	$\bar{x}$ = 443

Appendix 12c: Concentration of Polycyclic Aromatic Hydrocarbons in Bile of **Brown Bullheads.**

Location: Cascade Creek				
Fish ID	Age	Naphthalene µg/kg	Phenanthrene µg/kg	Benzo-α-pyrene µg/kg
44	17	184195	95532	323
46	4	164050	61807	11
47	16	127177	57955	34
48	7	283013	180202	915
		$\bar{x}$ = 189609	$\bar{x}$ = 98874	$\bar{x}$ = 321

Appendix 12d: Concentration of Polycyclic Aromatic Hydrocarbons in Bile of **Brown Bullheads.**

Location: Sara's Cove				
Fish ID	Age	Naphthalene µg/kg	Phenanthrene µg/kg	Benzo-α-pyrene µg/kg
49	16	149089	68083	159
50	16	168189	81964	144
51	4	130761	69813	89
52	7	91048	26128	559
53	13	36670	9638	85
54	4	106013	24768	474
55	4	115855	28298	919
56	4	-	-	-
57	12	166159	47836	1609
59	5	123628	32226	1251
60	5	149648	31796	1585
61	5	-	-	-
63	16	122120	25812	1112
64	5	121885	30113	973
65	10	114878	36764	2129
66	-	299708	52684	5975
67	4	-	-	-
		$\bar{x}$ = 135404	$\bar{x}$ = 40423	$\bar{x}$ = 1219

Appendix 12e: Concentration of Polycyclic Aromatic Hydrocarbons in Bile of **Brown Bullheads.**

Location: Point Sara's Cove				
Fish ID	Age	Naphthalene µg/kg	Phenanthrene µg/kg	Benzo-α-pyrene µg/kg
74	4	96853	23215	1930
75	4	273191	57243	6365
76	15	130174	41803	2081
82	16	174301	29488	935
83	11	126445	28688	669
84	14	94996	15145	362
85	10	100068	19192	351
85	11	158734	22967	627
87	12	82272	12415	269
		$\bar{x}$ = 137448	$\bar{x}$ = 27795	$\bar{x}$ = 1510

Appendix 12f: Concentration of Polycyclic Aromatic Hydrocarbons in Bile of **Brown Bullheads.**

Location: Eaton Reservoir				
Fish ID	Age	Naphthalene µg/kg	Phenanthrene µg/kg	Benzo-α-pyrene µg/kg
91	6	97647	10355	373
92	17	119911	12998	513
93	7	139090	12885	371
94	9	229733	17198	591
95	11	125616	12051	426
96	5	255727	20714	498
97	15	164844	14408	546
98	16	125774	12691	435
99	15	121397	9850	391
100	5	168336	18561	720
		$\bar{x}$ = 154808	$\bar{x}$ = 14171	$\bar{x}$ = 486

Appendix 13: Descending Rank of Concentration of Polycyclic Aromatic Hydrocarbons in Brown Bullheads Which Had At Least One Neoplasm
(N=79; 1 is highest, 79 is lowest; **Bold** = upper quartile). * For example, in Fish 06 the concentration of naphthalene was the 4th highest observed.

Fish Number	Naphthalene	Phenanthrene	Benzo-α-pyrene
02	21	31	42
06	**4** *	29	27
12	71	70	55
16	23	45	39
20	62	57	38
23	38	51	47
25	**20**	33	24
26	57	60	40
27	25	52	53
28	**11**	49	70
33	27	**10**	60
35	**15**	**9**	72
36	64	55	71
37	60	**1**	59
40	**18**	28	58
41	**1**	**13**	64
46	32	**15**	68
47	47	**17**	66
48	**8**	**2**	**15**
49	37	**12**	57
50	29	**6**	61
53	72	73	63
66	**7**	**19**	**1**

Fish Number	Naphthalene	Phenanthrene	Benzo-α-pyrene
76	46	30	**4**
82	26	40	**12**
85	63	58	48
86	34	54	23
87	70	68	54
98	49	67	36
99	55	72	41

Appendix 14a: Descending Rank of Concentration of Polycyclic Aromatic Hydrocarbons in Brown Bullheads Which Had Benign or Malignant Neoplasms in the Liver. (N=79; 1 is highest; 79 is lowest; **Bold** = upper quartile). * For example, in Fish 20 the concentration of naphthalene was the 20th highest observed.

Fish Number	Naphthalene	Phenanthrene	Benzo-α-pyrene
25	**20 ***	33	24
28	**11**	49	70
37	60	**1**	59
41	**1**	**13**	64
50	29	**6**	61
53	72	73	63
87	70	68	54
98	49	67	36
99	55	72	41

Appendix 14b: Descending Rank of Concentration of Polycyclic Aromatic Hydrocarbons in Brown Bullheads Which Had Benign or Malignant Neoplasms in the Skin or the Mouth/Lip/Barbel. (N=79; 1 is highest; 79 is lowest; **Bold** = upper quartile). *For example, in Fish 04 the concentration of naphthalene was the 4th highest observed.

Fish Number	Naphthalene	Phenanthrene	Benzo-α-pyrene
02	21	31	42
06	**4 ***	29	27
16	23	45	39
23	38	51	47
27	25	52	53
28	**11**	49	70
33	27	**10**	60
37	60	**1**	59
47	47	**17**	66
48	**8**	**2**	**15**
49	37	**12**	57
50	29	**6**	61
53	72	73	63
66	**7**	**19**	**1**
82	26	40	**12**
85	63	58	48
86	34	54	23

Appendix 14c: Descending Rank of Concentration of Polycyclic Aromatic Hydrocarbons in Brown Bullheads Which Had Benign or Malignant Neoplasms in the Testis. (N=79; 1 is highest; 79 is lowest; **Bold** = upper quartile). *For example, in Fish 02 the concentration of naphthalene was the 21st highest observed.

Fish Number	Naphthalene	Phenanthrene	Benzo-α-pyrene
02	21 *	31	42
12	71	70	55
20	62	57	38
25	**20**	33	24
26	57	60	40
27	25	52	53
35	**15**	**9**	72
36	64	55	71
41	**1**	**13**	64
46	32	**15**	68
53	72	73	63
76	46	30	**4**
99	55	72	41

Appendix 15a: Descending Rank of Concentration of Naphthalene in the Bile of Brown Bullheads; Upper Two Quartiles (the 40 fish with the highest concentrations)

Rank	Fish ID	Age years	Location	Naphthalene µg/kg
1	41	9	Lagoons	437606
2	05	12	Lagoons	424917
3	11	4	Lagoons	356290
4	06	15	Lagoons	314413
5	30	12	Thompson Bay	311940
6	18	6	Thompson Bay	299758
7	66	-	Sara's Cove	299708
8	48	7	Cascade Creek	283013
9	03	4	Lagoons	279843
10	75	4	Point - Sara's Cove	273191
11	28	14	Thompson Bay	272789
12	96	5	Eaton Reservoir	255727
13	21	4	Thompson Bay	237177
14	94	9	Eaton Reservoir	229733
15	35	3	Lagoons	224665
16	15	7	Thompson Bay	221285
17	19	4	Thompson Bay	220598
18	40	15	Lagoons	214326
19	17	7	Thompson Bay	213648
20	25	14	Thompson Bay	209910
21	2	15	Lagoons	206949
22	24	12	Thompson Bay	200460
23	16	7	Thompson Bay	191830
24	44	17	Cascade Creek	184195

Rank	Fish ID	Age years	Location	Naphthalene µg/kg
25	27	13	Thompson Bay	181199
26	82	16	Point - Sara's Cove	174301
27	33	10	Thompson Bay	171214
28	100	5	Eaton Reservoir	168336
29	50	16	Sara's Cove	168189
30	57	12	Sara's Cove	166159
31	97	15	Eaton Reservoir	164844
32	46	4	Cascade Creek	164050
33	39	3	Lagoons	163534
34	86	11	Point - Sara's Cove	158734
35	42	14	Lagoons	155908
36	60	5	Sara's Cove	149648
37	49	16	Sara's Cove	149089
38	23	11	Thompson Bay	146452
39	93	7	Eaton Reservoir	139090
40	31	8	Thompson Bay	136814

Appendix 15b: Descending Rank of Concentration of Phenanthrene in the Bile of Brown Bullheads; Upper Two Quartiles (the 40 fish with the highest concentrations)

Rank	Fish ID	Age years	Location	Phenanthrene µg/kg
1	37	12	Lagoons	337000
2	48	7	Cascade Creek	180202
3	30	12	Thompson Bay	123142
4	44	17	Cascade Creek	95532
5	39	3	Lagoons	82120
6	50	16	Sara's Cove	81964
7	40	15	Lagoons	78076
8	5	12	Lagoons	77139
9	35	3	Lagoons	73771
10	33	10	Thompson Bay	70127
11	51	4	Sara's Cove	69813
12	49	16	Sara's Cove	68083
13	41	9	Lagoons	65462
14	42	14	Lagoons	63136
15	46	4	Cascade Creek	61807
16	38	4	Lagoons	58620
17	47	16	Cascade Creek	57955
18	75	4	Point - Sara's Cove	57243
19	66	-	Sara's Cove	52684
20	11	4	Lagoons	50711
21	17	7	Thompson Bay	49315
22	3	4	Lagoons	48148
23	15	7	Thompson Bay	48017
24	57	12	Sara's Cove	47836

Rank	Fish ID	Age years	Location	Phenanthrene µg/kg
25	24	12	Thompson Bay	47654
26	19	4	Thompson Bay	45647
27	34	11	Thompson Bay	45188
28	18	6	Thompson Bay	45157
29	6	15	Lagoons	42853
30	76	15	Point - Sara's Cove	41803
31	2	15	Lagoons	40630
32	65	10	Sara's Cove	36764
33	25	14	Thompson Bay	36272
34	14	7	Thompson Bay	36372
35	22	7	Thompson Bay	33545
36	59	5	Sara's Cove	32226
37	60	5	Sara's Cove	31796
38	21	4	Thompson Bay	30997
39	64	5	Sara's Cove	30113
40	82	16	Point - Sara's Cove	29488

Appendix 15c: Descending Rank of Concentration of Benzo-α-Pyrene in the Bile of Brown Bullheads; Upper Two Quartiles (the 40 fish with the highest concentrations)

Rank	Fish ID	Age years	Location	Benzo-α-Pyrene µg/kg
1	66	-	Sara's Cove	6975
2	75	4	Point - Sara's Cove	6365
3	65	10	Sara's Cove	2129
4	76	15	Point - Sara's Cove	2081
5	74	4	Point - Sara's Cove	1930
6	57	12	Sara's Cove	1609
7	60	5	Sara's Cove	1585
8	59	5	Sara's Cove	1251
9	63	16	Sara's Cove	1112
10	19	4	Thompson Bay	1025
11	64	5	Sara's Cove	973
12	82	16	Point - Sara's Cove	935
13	5	12	Lagoons	924
14	55	4	Sara's Cove	919
15	48	7	Cascade Creek	915
16	14	7	Thompson Bay	852
17	11	4	Lagoons	821
18	9	4	Lagoons	792
19	100	5	Eaton Reservoir	720
20	24	12	Thompson Bay	718
21	17	7	Thompson Bay	707
22	83	11	Point - Sara's Cove	669
23	86	11	Point - Sara's Cove	627
24	25	14	Thompson Bay	624

Rank	Fish ID	Age years	Location	Benzo-α-Pyrene µg/kg
25	18	6	Thompson Bay	597
26	94	9		591
27	6	15	Lagoons	576
28	15	7	Thompson Bay	575
29	22	7	Thompson Bay	563
30	52	7	Sara's Cove	559
31	97	15		546
32	3	4	Lagoons	520
33	92	17		513
34	96	5		498
35	54	4	Sara's Cove	474
36	98	16		435
37	95	11		426
38	20	4	Thompson Bay	412
39	16	7	Thompson Bay	404
40	26	4	Thompson Bay	394

Appendix 16: Concentration of Polycyclic Aromatic Hydrocarbons in the Bile of **Brown Bullheads.**

Fish ID	Age years	Location	Napthalene (µg/kg)	Phenanthrene (µg/kg)	Benzo-a-pyrene (µg/kg)
01	13	Lagoons	89300	14238	327
02	15	Lagoons	206949	40630	385
03	4	Lagoons	279843	48148	520
04	7	Lagoons	-	-	-
05	12	Lagoons	424917	77139	924
06	15	Lagoons	314413	42853	576
07	7	Lagoons	-	-	-
08	3	Lagoons	133573	27999	359
09	4	Lagoons	32821	25687	792
10	4	Lagoons	-	-	-
11	4	Lagoons	356290	50711	821
12	4	Lagoons	62933	11115	230
14	7	Thompson Bay	133884	35698	852
15	7	Thompson Bay	221285	48017	575
16	7	Thompson Bay	191830	26436	404
17	7	Thompson Bay	213648	49315	707
18	6	Thompson Bay	299758	45157	597
19	4	Thompson Bay	220598	45647	1025
20	4	Thompson Bay	100660	19542	412
21	4	Thompson Bay	237177	30997	346
22	7	Thompson Bay	125536	33545	563
23	11	Thompson Bay	146452	23919	359
24	12	Thompson Bay	200460	47654	718
25	14	Thompson Bay	209910	36272	624
26	4	Thompson Bay	119251	18140	394
27	13	Thompson Bay	181199	23688	276
28	14	Thompson Bay	272789	25453	8
30	12	Thompson Bay	311940	123142	329
31	8	Thompson Bay	136814	26550	59
33	10	Thompson Bay	171214	70127	145
34	11	Thompson Bay	135056	45188	9

Fish ID	Age years	Location	Napthalene (µg/kg)	Phenanthrene (µg/kg)	Benzo-a-pyrene (µg/kg)
35	3	Lagoons	224665	73771	2
36	4	Lagoons	98271	21709	6
37	12	Lagoons	106371	337000	146
38	4	Lagoons	133975	58620	1
39	3	Lagoons	163534	82120	32
40	15	Lagoons	214326	78076	157
41	9	Lagoons	437606	65462	67
42	14	Lagoons	155908	63136	228
44	17	Cascade Creek	184195	95532	323
46	4	Cascade Creek	164050	61807	11
47	16	Cascade Creek	127177	57955	34
48	7	Cascade Creek	283013	180202	915
49	16	Sara's Cove	149089	68083	159
50	16	Sara's Cove	168189	81964	144
51	4	Sara's Cove	130761	69813	89
52	7	Sara's Cove	91048	26128	559
53	13	Sara's Cove	36670	9638	85
54	4	Sara's Cove	106013	24768	474
55	4	Sara's Cove	115855	28298	919
56	4	Sara's Cove	-	-	-
57	12	Sara's Cove	166159	47836	1609
59	5	Sara's Cove	123628	32226	1251
60	5	Sara's Cove	149648	31796	1585
61	5	Sars's Cove	-	-	-
63	16	Sara's Cove	122120	25812	1112
64	5	Sara's Cove	121885	30113	973
65	10	Sara's Cove	114878	36764	2129
66	-	Sara's Cove	299708	52684	6975
67	4	Sara's Cove	-	-	-
74	4	Point-Sara's Cove	96853	23215	1930
75	4	Point-Sara's Cove	273191	57243	6365
76	15	Point-Sara's Cove	130174	41803	2081
82	16	Point-Sara's Cove	174301	29488	935

Fish ID	Age years	Location	Napthalene (µg/kg)	Phenanthrene (µg/kg)	Benzo-a-pyrene (µg/kg)
83	11	Point-Sara's Cove	126445	28688	669
84	14	Point-Sara's Cove	94996	15145	362
85	10	Point-Sara's Cove	100068	19192	351
86	11	Point-Sara's Cove	158734	22967	627
87	12	Point-Sara's Cove	82272	12415	269
91	6	Eaton Reservoir	97647	10355	373
92	17	Eaton Reservoir	119911	12998	513
93	7	Eaton Reservoir	139090	12885	371
94	9	Eaton Reservoir	229733	17198	591
95	11	Eaton Reservoir	125616	12051	426
96	5	Eaton Reservoir	255727	20714	498
97	15	Eaton Reservoir	164844	14408	546
98	16	Eaton Reservoir	125774	12691	435
99	15	Eaton Reservoir	121397	9850	391
100	5	Eaton Reservoir	168336	18561	720

Appendix 17

Tables of Histopathologic findings for individual fish, containing 100 pages.

Please refer to your previous version of this table.

Appendix 4h: Non-Neoplastic Observations in **Bowfin**
Detail of microscopic findings with identification of individual fish

Stomach					
Lesion or Observation	Individual Males	Individual Females	Male N=13	Female N=8	Total N=21
Tissue not present	70		1	0	1
No significant lesions	89		1	0	1
Chronic inflammation	32 43 58 68 69 71 80 81	13 29 45 62 72 73 78 88	8	8	16
Acute inflammation	79		1	0	1
Edema	80		1	0	1
Parasites in lumen	69 71 80	13 62 78	3	3	6
Hemorrhage	77 79 90		3	0	3

Appendix 4i: Non-Neoplastic Observations in **Bowfin**
Detail of microscopic findings with identification of individual fish

Gas bladder					
Lesion or Observation	Individual Males	Individual Females	Male N=13	Female N=8	Total N=21
Tissue not present			0	0	0
No significant lesions		62 72	0	2	2
Chronic inflammation	32 43 58 68 69 70 71 77 79 80 81 89 90	13 29 45 78 88	13	5	18
Metaplasia/hyperplasia of goblet cells	43 68 70 77 79 89	73 88	6	2	8
Myolysis	68		1	0	1
Edema		13	0	1	1

Appendix 4j: Non-Neoplastic Observations in **Bowfin**
Detail of microscopic findings with identification of individual fish

Urinary bladder					
Lesion or Observation	Individual Males	Individual Females	Male N=13	Female N=8	Total N=21
Tissue not present	32 69	78	2	1	3
No significant lesions	43 58 77 79 80 81 90	29 45 62 72	7	4	11
Chronic inflammation		13 73 88	0	3	3
Hypertrophy of mesothelial cells	68 70 71	73 88	3	2	5
Hyperplasia	89		1	0	1

Appendix 4k: Non-Neoplastic Observations in **Bowfin**
Detail of microscopic findings with identification of individual fish

Gonad					
Lesion or Observation	Individual Males	Individual Females	Male N=13	Female N=8	Total N=21
Tissue not present		88	0	1	1
No significant lesions	43 58 68 69 70 71 77 79 80 89 90	29 73	11	2	13
Melanomacrophage foci		13	0	1	1
Chronic inflammation		45 62 72 78	0	4	4
Hemosiderosis	32		1	0	1
Cyst	81		1	0	1

Appendix 4l: Non-Neoplastic Observations in **Bowfin**
Detail of microscopic findings with identification of individual fish

Spleen					
Lesion or Observation	Individual Males	Individual Females	Male N=13	Female N=8	Total N=21
Tissue not present	70		1	0	1
No significant lesions	77 80 90	45 78	3	2	5
Hemosiderosis	32 68 69 71 79	13 29 88	5	3	8
Melanomacrophage foci	43 58 68 69 71 79 81 89	62 72 73	8	3	11

Appendix 4m: Non-Neoplastic Observations in **Bowfin**
Detail of microscopic findings with identification of individual fish

Intestine					
Lesion or Observation	Individual Males	Individual Females	Male N=13	Female N=8	Total N=21
Tissue not present	70		1	0	1
No significant lesions			0	0	0
Chronic inflammation	43 58 68 69 71 80 89	13 29 45 62 72 73 78 88	7	8	15
Parasites (including protozoa)	32 43 58 68 69 71 77 79 80 81 89 90	13 29 45 62 72 73 78 88	12	8	20
Hemorrhage	77		1	0	1
Hemosiderosis	89	88	1	1	2

Appendix 4n: Non-Neoplastic Observations in **Bowfin**
Detail of microscopic findings with identification of individual fish

Skeletal muscle					
Lesion or Observation	Individual Males	Individual Females	Male N=13	Female N=8	Total N=21
Tissue not present			0	0	0
No significant lesions	43 58 68 69 70 71 77 79 81 90	13 29 72 73 78 88	10	6	16
Myofiber rarefaction, myolysis or necrosis	32 80 89	45 62	3	2	5

Appendix 4o: Non-Neoplastic Observations in **Bowfin**
Detail of microscopic findings with identification of individual fish

Skin					
Lesion or Observation	Individual Males	Individual Females	Male N=13	Female N=8	Total N=21
Tissue not present		62	0	1	1
No significant lesions	32 43 71 77 79 80 90	13 29 78	7	3	10
Chronic inflammation	58 68 69 70 81 89	45 72 73 88	6	4	10
Hyperplasia	69 81		2	0	2

Appendix 4p: Non-Neoplastic Observations in **Bowfin**
Detail of microscopic findings with identification of individual fish

Mouth/Lip/Barbel					
Lesion or Observation	Individual Males	Individual Females	Male N=13	Female N=8	Total N=21
Tissue not present			0	0	0
No significant lesions	58 68 69 80	29 62 72 78	4	4	8
Chronic inflammation	32 43 70 71 77 79 81 89 90	13 45 73 88	9	4	13
Ulceration	32 81		2	0	2
Parasite		73	0	1	1

Appendix 4q: Non-Neoplastic Observations in **Bowfin**
Detail of microscopic findings with identification of individual fish

Non-protocol tissue: Pancreas					
Lesion or Observation	Individual Males	Individual Females	Male N=13	Female N=8	Total N=21
Chronic inflammation	68 71		2	0	2

Appendix 4r: Non-Neoplastic Observations in **Bowfin**
Detail of microscopic findings with identification of individual fish

Non-protocol tissue: Mesentery and gastrointestinal serosa					
Lesion or Observation	Individual Males	Individual Females	Male N=13	Female N=8	Total N=21
Hemosiderosis	77		1	0	1
Chronic inflammation	77 80	62	2	1	3

Appendix 5: Detail Incidence of Gross Observations in **Brown Bullheads**.
List of individual fish.

Lesion or Observation	Study Population	Reference Population
NGL	04 10 35	
Poor body condition	03 09 54	
Healed wounds & skin lesions	02 03 07 12 15 17 18 19 20 22 23 24 33 37 38 41 42 46 49 51 57 59 61 64 65 74 75 76 82 84 85 86 87	91 92 93 95 98 100
Active/recent wounds & skin lesions	11 15 17 18 20 22 27 34 38 44 54 55 56 63 65 76 83 85 86 87	94 95
Lips and mouth abrasions, ulcers, growths	02 03 05 06 07 23 24 25 27 28 33 38 40 41 42 44 47 49 53 55 63 65 66 76 82 83 86 87	94 96 97 98 99
Parasites in liver	01 02 03 18 20 30 42 48 49 50 53 63 66 84 85 86	95
Parasites in GI tract	36 42 82 84	
Skin lesions pigmented	01 05 06 07 14 16 17 19 27 28 30 31 37 40 47 49 50 53 59 60 63 66 74 83 84 85 86 87	97
Skin lesions proliferative	01 02 06 07 16 17 23 24 25 28 31 37 40 41 44 47 48 52 57 60 66 75 82 83 84 87	99
Liver masses	01 03 37 39 87	97
Liver pale and/or mottled	26 27 41 49	
Eye lesions	18 31 41 65 86	92 97 98

Appendix 6: Detail of Neoplastic Observations in **Brown Bullheads**.
List of individual fish by number. There were no missing specified tissues from any of these fish.

Organ	Lesion	Study Population	Reference Population
Liver	Biliary adenoma	53	
	Biliary carcinoma	25 28 37 41 50 53 87	98 99
Skin	Papilloma	02 37 53	
	Squamous cell carcinoma	28 40	
	Melanoma	87	
	Melanosarcoma	47	
Mouth/ Lip/ Barbel	Papilloma	02 06 16 23 27 33 47 48 49 50 53 66 82 85 86	
	Squamous cell carcinoma	28 40 53 66 82 85	
Gonad (Testis)	Seminoma	02 12 20 25 26 27 35 36 46 76 53	
	Adenoma of tubular lining cells	27 41	
	Carcinoma of tubular lining cells	76	99

Appendix 7: Summary of Neoplastic Observations in **Brown Bullheads**.
There were no missing specified tissues from any of these fish.

		Study Population			Reference Population		
Organ	Lesion	Male	Female	Total	Male	Female	Total
Liver	Biliary adenoma	1	-	1	-	-	-
	Biliary carcinoma	6	1	7	1	1	2
Skin	Papilloma	3	-	3	-	-	-
	Squamous cell carcinoma	-	2	2	-	-	
	Melanoma	1	-	1	-	-	-
	Melano-sarcoma	-	1	1	-	-	-
Mouth/ Lip/ Barbel	Papilloma	8	7	15	-	-	-
	Squamous cell carcinoma	3	3	6	-	-	-
Gonad (Testis)	Seminoma	11	-	11	-	-	-
	Adenoma of tubular lining cells	2		2	-	-	-
	Carcinoma of tubular lining cells	1	-	1	1	-	1

Appendix 8: Summary of Non-Neoplastic Microscopic Observations in **Brown Bullheads.**

Tissue	Lesion or Observation	Male N=40	Female N=39	Total N=79
Gallbladder	Chronic inflammation	1	0	1
Liver	Chronic inflammation	3	3	6
	Granulomatous inflammation ± parasites, necrosis	25	19	44
	Hemosiderosis; pigment-laden macrophage aggregates	36	37	73
	Hepatocellular alteration	18	14	32
	Clearing of hepatocytes; tinctorially altered region	20	6	26
	Hyperplasia of bile ducts	7	4	11
	Parasites in bile ducts	26	17	43
	Biliary ectasia	1	0	1
	Biliary coccidiosis	1	0	1
	Fibrosis	1	7	8
	Mineralized focus	1	1	2
	Bacteremia	1	0	1
	Amyloidosis	1	0	1
	Hepatocellular necrosis	0	3	3
	Hyperplasia of intima	0	1	1
	Telangiectasis	0	2	2
	Thrombosis	1	1	2

Tissue	Lesion or Observation	Male N=40	Female N=39	Total N=79
Cranial kidney	Hemosiderosis; pigment-laden macrophage aggregates	37	35	72
	Chronic inflammation	0	2	2
	Granulomatous inflammation ± parasites, necrosis	2	0	2
	Vacuolation of endocrine tissue	1	0	1
	Hypertrophy of endocrine tissue	2	0	2
Corpuscle of Stannius	Hyperplasia	0	1	1
	Cystic	0	1	1
Caudal kidney	Hemosiderosis; pigment-laden macrophage aggregates	36	37	71
	Granulomatous inflammation ± parasites, necrosis	2	2	4
	Hyaline droplet accumulation	13	12	25
	Thrombosis	1	0	1
	Vasculitis	1	0	1
	Parasites in ducts	1	1	2
	Interstitial bacterial colonies	2	0	2
Heart	Chronic inflammation	0	3	3
	Epicarditis	40	37	77
	Myocarditis	1	0	1

Tissue	Lesion or Observation	Male N=40	Female N=39	Total N=79
	Fibrosis	1	1	2
	Hemosiderosis; pigment-laden macrophage aggregates	1	0	1
	Osseous metaplasia	1	0	1
	Adventititis	3	4	7
	Granulonatous inflammation	1	1	2
Thyroid	Chronic inflammation	1	3	4
Stomach	Chronic inflammation	0	1	1
	Granulomatous inflammation ± parasites, necrosis	17	23	40
	Parasites in vessels	17	24	41
	Hemorrhage	1	0	1
	Edema	1	1	2
	Lymphangiectasia	1	1	2
	Hyperplasia of mucus cells	1	0	1
	Thrombosis	1	7	8
	Hemosiderosis; pigment-laden macrophage aggregates	0	1	1
	Serositis	0	1	1
	Vasculitis	0	1	1
Gas bladder	Chronic inflammation	5	2	7

Tissue	Lesion or Observation	Male N=40	Female N=39	Total N=79
	Granulomatous inflammation ± parasites, necrosis	27	21	48
	Parasites in lumen, mucosa or vessels	6	2	8
	Serositis	1	0	1
	Mucosal metaplasia	1	1	2
	Mucosal hyperplasia	2	1	3
	Melanocytosis / pigmentation	2	2	4
	Hemorrhage	0	2	2
Urinary bladder	Chronic inflammation	0	1	1
	Metaplasia of mucus cells	1	0	1
	Hyperplasia of mucus cells	2	3	5
	Hemosiderosis; pigment-laden macrophage aggregates	0	1	1
	Hemorrhage	0	1	1
Gonad	Chronic inflammation	0	2	2
	Hemosiderosis; pigment-laden macrophage aggregates	0	2	2
	Granulomatous inflammation ± parasites, necrosis	0	2	2
	Atretic follicles	N/A	10	10
	Atrophy	0	1	1

Tissue	Lesion or Observation	Male N=40	Female N=39	Total N=79
	Hyperplasia of granulosa cells	N/A	6	6
	Fibrosis	0	1	1
	Hyperplasia/ hypertrophy of tubular lining cells	36	N/A	36
	Vacuolation of tubular lining cells	5	N/A	5
	Aspermagenesis	15	N/A	15
Spleen	Chronic inflammation	1	0	1
	Granulomatous inflammation ± parasites, necrosis	1	0	1
	Hemosiderosis; pigment-laden macrophage aggregates	38	34	72
	Bridging connective tissue; accentuated stroma	1	2	3
	Cyst	1	0	1
Intestine	Chronic inflammation	3	2	5
	Subacute inflammation	1	1	2
	Granulomatous inflammation ± parasites, necrosis	12	10	22
	Parasites in lumen	19	12	31
	Parasites in vessels	11	10	21
	Hemosiderosis; pigment-laden macrophage aggregates	8	9	17

Tissue	Lesion or Observation	Male N=40	Female N=39	Total N=79
	Serositis	3	1	4
	Fibrosis	1	2	3
	Thrombosis	2	5	7
	Hemorrhage	0	1	1
	Edema	0	3	3
	Lymphangiectasia	0	4	4
	Vasculopathy	2	2	4
Skeletal muscle	Chronic inflammation	1	1	2
	Granulomatous inflammation ± parasites, necrosis	2	0	2
	Degeneration / myolysis	9	6	15
	Atrophy	1	0	1
	Parasites	1	0	1
	Hemorrhage	0	1	1
	Fatty infiltration	0	1	1
Skin	Chronic inflammation	3	3	6
	Granulomatous inflammation ± parasites, necrosis	1	0	1
	Fibrosis	2	1	3
	Epidermal hyperplasia	12	10	22
	Hyperpigmentation	15	9	24
	Hyperplasia of alarm cells	1	2	3
	Epidermal spongiosis	1	1	2