

Removal Recommendation

Fish Tumors or Other Deformities Beneficial Use Impairment

St. Marys River Area of Concern

Issue

The Michigan Department of Environment, Great Lakes, and Energy (EGLE), Water Resources Division, Areas of Concern (AOC) program recommends removal of the Fish Tumors or Other Deformities Beneficial Use Impairment (BUI) from the U.S. side of the St. Marys River AOC. This recommendation is being made with the support of EGLE technical staff, U.S. Fish and Wildlife Service (FWS) technical staff, U.S. Environmental Protection Agency (EPA) Great Lakes National Program Office (GLNPO) staff, and the St. Marys River Binational Public Advisory Council (BPAC), in accordance with the process and criteria set forth in the *Guidance for Delisting Michigan's Great Lakes Areas of Concern* (Michigan Department of Natural Resources [MDNR], 2018).

Background

The St. Marys River is a binational AOC, shared jointly between the U.S. and Canada. To date, 7 out of 10 BUIs have been restored on the U.S. side of the St. Marys River AOC: Degradation of Aesthetics, in January 2014; Bird or Animal Deformities or Reproductive Problems, in March 2014; Beach Closings, in July 2016; Eutrophication or Undesirable Algae, in December 2016; Restrictions on Dredging Activities, in November 2017; Degradation of Fish and Wildlife Populations, in September 2019; and Loss of Fish and Wildlife Habitat, in September 2019. This removal recommendation pertains only to the Fish Tumors or Other Deformities BUI. Two other beneficial uses remain impaired on the U.S. side of the AOC: Restrictions on Fish and Wildlife Consumption and Degradation of Benthos.

Tumors can be either internal or external, and can be caused by physical, chemical, or biological factors. Presence of some types of tumors does not necessarily indicate causation by legacy contaminants. Similarly, deformities may be caused by several factors, including genetic mutation or viral infection. It cannot be assumed that a deformity is necessarily indicative of environmental contamination. However, there is scientific evidence of a cause-and-effect relationship between polycyclic aromatic hydrocarbon (PAH) exposure and liver cancer in wild fish, especially in bottom feeders (Baumann, 1995). The existence of this direct connection between PAHs and liver cancer provides the rationale for Michigan's restoration criteria for this BUI, as well as

the recent assessment work that was conducted to evaluate its current status. Also, because of that connection, in 1991 the International Joint Commission published the following guidance for when to remove the BUI: “When the incidence rates of fish tumors or other deformities do not exceed rates at unimpacted control sites and when survey data confirm the absence of neoplastic or preneoplastic liver tumors in bullheads or suckers” (IJC, 1991).

PAHs are a class of organic compounds that are known to be carcinogenic, persistent in the environment, and are associated with industrial uses/combustion, spills and processing of fossil fuels/petroleum products. PAHs have been shown to be present in St. Marys River sediments associated with the former Consumers Energy Manufactured Gas Plant site, the Algoma Boat Slip, the Federal Water Lot, the Bellevue Marine Park, and areas east of Bellevue Marine Park.

According to the 1992 Stage 1 Remedial Action Plan (RAP) for the St. Marys River AOC, prepared jointly by the Ontario Ministry of the Environment and the MDNR, the rationale for including the Fish Tumors or Other Deformities BUI in the list of original impairments was the discovery of a “relatively high incidence” of liver tumors in brown bullheads from Munuscong Bay, and an “abnormal incidence” of liver tumors in white suckers collected along the Ontario shoreline, in a study conducted by Baumann et al. (1991), although a specific tumor prevalence rate was not reported. One of the findings from the study concluded, “(w)hen only randomly selected fish subjected to histopathology are considered, about one third of the Munuscong Lake brown bullhead...had either neoplastic or preneoplastic liver lesions” (Baumann, 1991). In that study, only brown bullhead longer than 200 mm in length were examined to ensure that the fish were at least two years old. As a result of that work, the authors of the Stage 1 RAP determined the status of the BUI to be, “(i)mpaired due to the incidence of liver tumors in brown bullheads from Munuscong Bay” (OMOE, 1992).

On the Canadian side of the AOC, monitoring to assess the status of this BUI has occurred multiple times from the late 1980s through 2023, looking for liver tumors in white suckers. According to Ontario Ministry of Natural Resources fisheries biologist Jason Ritchie (pers. comm., 2026), brown bullhead are less prevalent in Canadian waters, hence the focus on white suckers. Both species occupy a similar ecological niche as bottom feeders. During the various collection efforts during that time, liver tumor rates were found to range between 6 percent (6%) in 2015 and 10.9 percent

(10.9%) in combined survey results from 2021 and 2023, with variability in sampling locations in Ontario waters (Chambers, 2025).

Restoration Criteria

In April of 2023, following a process involving BPAC, EGLE AOC staff and management, GLNPO staff, and FWS technical staff, local restoration criteria for the Fish Tumors BUI were established for the St. Marys River AOC. Leading up to that decision, overall discussion by BPAC members and EGLE regarding the change emphasized that this would be a more scientifically rigorous demonstration that the BUI has improved, as compared with the statewide criteria in effect at the time. There was also agreement that the revised restoration criteria would be more in accordance with the Canadian redesignation criteria for this BUI.

Fish tumors, both internal and external, may have causes that are difficult, if not impossible to determine. Similarly, physical deformities can be caused by several factors, which may or may not include chemical contaminants, which is the connection of primary interest. Due to this uncertainty, the restoration criteria for this BUI focus exclusively on cancerous liver tumors, the presence of which can be said with a high degree of certainty points to exposure to chemical contaminants (especially PAHs) in the aquatic environment (Baumann, 1995). Specifically, histopathology laboratory technicians examine hepatic (liver) tissue for the presence of neoplastic lesions, due to their link to the presence of PAHs.

The restoration criteria now in effect for the Fish Tumors or Other Deformities BUI on the U.S. side of the St. Marys River AOC reads as follows in its entirety:

“The BUI will be considered restored when:

A study of at least 100 resident benthic fish (e.g. brown bullhead OR white suckers), with a minimum age of 3 years, collected from at least two sites within the AOC, indicates a cancerous liver tumor rate of 5 percent (5%) or less.”

As mentioned previously, the study that was the original basis for designating this beneficial use as impaired in the St. Marys River AOC found liver tumors in brown bullhead in Michigan waters (Baumann, 1991). Therefore, EGLE chose to conduct follow up work on the same species, since they are readily found in Michigan waters.

The 5% target is based on the background prevalence of neoplastic and preneoplastic liver lesions previously found in brown bullhead from AOCs and non-AOC comparison locations in the Great Lakes region (Blazer, 2009 and Baumann, 2010).

Summary of Remedial Actions

Remedial actions on the U.S. side of the St. Marys River include a Great Lakes Legacy Act sediment cleanup at the former Consumers Energy Manufactured Gas Plant site near downtown Sault Ste. Marie (Figure 1). The project was completed in 2011 and included the removal of 26,000 cubic yards of sediment contaminated primarily with PAHs. In total, the cleanup cost about \$4 million, \$2.4 million from the Great Lakes Legacy Act program and \$1.6 from Consumers Energy. Post remedial dredge sediment samples were collected as the project progressed. Analytical results from each location were assessed to determine whether additional remedial work was required. Finally, a residual sand cover averaging about nine inches thick was placed over dredged areas where total PAHs remained over a predetermined concentration (CH2M Hill, 2012).

Additionally, on the U.S. side of the AOC, there was an \$8 million sediment remediation project completed in 2007 in Tannery Bay, adjacent to the former Cannelton Industries tannery facility, which closed in the 1950s (Figure 1). The target contaminants for this effort were metals, primarily chromium and mercury, as opposed to PAHs, which are not generally associated with tannery operations. Funding for that project included approximately \$4.8 million from the Great Lakes Legacy Act program, \$2.6 million from the property owner, and \$600,000 from the Clean Michigan Initiative. In all, about 39,000 cubic yards of contaminated sediment were removed. Based on results from confirmation sampling at the site, one sediment sample exceeded the site-specific criteria for both chromium and mercury. That area was re-dredged and a confirmatory sample was collected at the same location, prior to placement of a sand cover (Weston Solutions, 2007). Those two projects comprise the full list of known contaminated sediment sites on the U.S. side of the AOC.

On the Canadian side of the AOC, sediment surveys between the 1990s and the 2010s in Algoma Steel's boat slip revealed elevated concentrations of PAHs, metals, petroleum hydrocarbons, oil, and grease (Derickx, 2024). To address the contaminants, sediment remediation efforts removed a cumulative approximate total of 30,000 cubic meters of

sediment from the boat slip during four dredging events that occurred in 1995, 2006, 2017, and 2019 (Chambers, et al., 2025).

Sediment characterization has occurred at Bellevue Marine Park, the Transport Canada federal water lot, and areas East of Bellevue Marine Park. Findings are that contaminants at these locations are largely at depth, under layers of relatively clean and stable sediments, and are currently planned for monitored natural recovery. Additional remedial dredging is not anticipated, but the areas are subject to requirements under the St. Marys River Area of Concern Dredging and In-Water Administrative Controls Guidance Document to guard against unintended future exposure of buried contaminants (Integral, 2022).

Monitoring

More than ten years following the most recent sediment remediation, most of the brown bullhead population on the US side of the AOC would have developed without exposure to contaminated sediments, assuming our understanding of those areas was comprehensive. Therefore, fish tumor studies should allow for accurate assessment of remedial efforts and their anticipated impact on ecosystem health.

In 2023, EGLE contracted with the Great Lakes Environmental Center (GLEC) to perform an assessment of the Fish Tumors or other Deformities BUI on the U.S. side of the St. Marys River AOC. That spring, GLEC personnel performed electroshocking and fish collection at four out of five identified locations, spaced geographically throughout the AOC, chosen using recommendations from and with the approval of BPAC members (Figure 1). Attempts to collect brown bullheads at the former Manufactured Gas Plant site were unsuccessful, resulting in zero specimens from that site. Based on feedback from the field crew, there didn't seem to be suitable habitat at that location, which may be influenced by heavy boat traffic and industrial activity in the area, so the lack of brown bullheads was not surprising. Poor weather conditions influenced the decision not to sample at Lower Raber Bay. Fortunately, successful collections at the remaining sites resulted in attainment of the target number of samples. It was determined that the lack of specimens from these two areas did not adversely affect the results of the study and was not necessarily an indication of ongoing environmental degradation.

Brown bullhead aged three and older were targeted for collection, using the approximate length of 250 mm as a surrogate in the field, until the otolith and/or pectoral fin spines of each fish could be examined and ages more definitively determined in a lab setting. Justification for the three-year age class is that animals of that age would have developed after remedial activities were complete, while allowing sufficient exposure time to environmental conditions such that tumors may be likely to develop if PAHs remained prevalent in the system (Rafferty, 2009). Otoliths are calcium carbonate structures located near the brain of most fish that assist with balance and hearing. They develop annual growth rings due to a slower winter metabolism versus faster warm weather growth rates, and can be counted to determine age (Luell, 2020). Similarly, fin spines develop growth rings that can also be counted to determine age, but since this method is more difficult and potentially less accurate, fin spines were used as a backup method, in case otoliths were lost or damaged. Otoliths can be challenging to locate and harvest from the animal, due to their size, location and coloration, as compared with surrounding tissues. Otoliths and pectoral fin spines were collected from each animal in the field for subsequent laboratory age evaluations to confirm that each fish was at least three years old, and results were recorded (GLEC, 2024).

Livers were removed from each fish after no more than five minutes of being euthanized, to ensure the best possible tissue quality for histopathological evaluations. Animals that were dead for longer than five minutes were not assessed. Following removal, livers were cut into ~5 mm thick strips, yielding a minimum of five liver sections per fish. Those samples were preserved and placed in sample jars, then shipped to Experimental Pathology Laboratories, Inc. (EPL) for processing to determine the presence or absence of cancerous liver tumors. EPL evaluated the liver sections using brightfield microscopy for liver lesions and other distinguishing characteristics. Results were recorded. See Appendix A for GLEC's complete report on the work conducted to assess the current status of the BUI. Laboratory results can be found in the pathology report in Appendix C.

Results

A total of 113 brown bullhead were collected from the St. Marys River AOC in May of 2023, as follows: 68 from Baie de Wassai, 41 from Munuscong Bay, and 4 from Waiska Bay. No fish were caught at the former Consumers Energy Manufactured Gas Plant site.

The Lower Raber Bay site was not attempted due to adverse weather conditions. Table 1 provides a breakdown of the total catch by location, size, and gender.

Table 1. Number, Sex, and Total Length Range of Fish Collected at Each Location

Collection Location	Number of Males Collected >3 yrs of age	Number of Females Collected >3 yrs of age	Range in Total Length (mm)	Number of Cancerous Liver Tumors
Baie de Wassai	36	32	225 - 330	0
Munuscong Bay	20	21	260 - 353	0
Waiska Bay	1	3	238 - 259	0
Consumers MGP	-	-	-	-
Lower Raber Bay	-	-	-	-
TOTAL	57	56	225 - 353	0

Out of the 113 animals examined in the study, 93 were able to be aged in the GLEC lab. For the remaining 20 specimens, ages could not be definitively determined because otoliths and fin spines disintegrated during sample preparation and processing, or otoliths were not collected due to a technician error and the corresponding fin spine samples disintegrated during cleaning.

In total, 108 brown bullheads collected were at least 250 mm long. Five fish were under 250 mm, but three of them (225 mm, 240 mm, and 243 mm) were determined to be at least three years old, using otoliths or fin spines. Of the 20 fish that could not be aged, 18 were at least 250 mm in length, so those are assumed to be at least three years old. Just two that were not aged were under 250 mm (238 mm and 245 mm). Based on the known ages and size structure of the collected population, it is assumed that all 113 animals were at least three years old. See Appendix B.

No neoplastic cancerous tumors were found among the liver samples from the 113 brown bullheads collected from the U.S. side of the St. Marys River AOC. All 113 fish were either shown to be at least three years old or were assumed to be at least three years old based on their length, as compared with the length of the shortest fish determined to be at least three when looking at otoliths and/or fin spines. One abnormal growth, determined to be a benign hepatocellular adenoma, a non-cancerous tumor, was found in the liver of a female from Baie de Wassai. See Appendix C.

Analysis

In summary, zero cancerous liver tumors were found in 113 brown bullhead aged at least three years, collected from three sites in the St. Marys River AOC. One benign liver tumor was discovered in one animal. While it was not possible to definitively determine the age of 20 specimens, the length of those fish indicate they were likely to be at least three years old. For comparison, it's worth noting that in the original fish tumors study conducted in the St. Marys River that provided the initial basis for including this BUI, the target size for brown bullhead was only 200 mm in length, assuming an age of at least two years, as opposed to this study's target of 250 mm and three years of age.

Despite the lack of cancerous tumors present in the fish collected in 2023, we sought to take a statistically robust and defensible approach to data analysis. On behalf of EGLE, FWS engaged with Michael Rutter, PhD, at Rutter Statistical Consulting and Professor of Statistics at Penn State Erie, The Behrend College, to analyze the data. Dr. Rutter has previous experience with statistical analyses of brown bullhead cancerous liver tumors in Great Lakes Areas of Concern, including the Presque Isle Bay AOC (Rutter, 2010), the Niagara River AOC (Rutter, 2015), and the Buffalo River AOC (Rutter, 2024). His report, attached as Appendix D, concludes:

"All statistical analyses conducted in this final report indicate the tumor incidence in the sampled area of the St. Marys River AOC is very low. When it is assumed that tumor incidence is not a function of age or length, there is a 99.1% probability that the tumor incidence is less than 5%. If tumor incidence is assumed to increase as a function of age or length, the probability of the tumor incidence rate being less than 5% is 91.0% for an average length bullhead and 80.3% for an average age bullhead. When the effect of the age or length covariate is not forced to increase, the probability of tumor incidence being less than 5% increases to 96.2% for length, 94.8% for age. However, the model with no covariate best fits the observed data. When the St. Marys River data is compared to a simulated data set with no tumors, the 90% TOST interval is (-0.8%, 3.1%), indicating the observed data and simulated no tumor data are statistically equivalent." (Rutter, 2025)

It should be acknowledged that white suckers have been collected to assess the status of this BUI in Canadian waters of the St. Marys River AOC (Mahmood et al. 2014). Like

brown bullhead, white suckers are also bottom feeders, representing not only a very similar ecological niche, but also a very comparable exposure pathway for river contaminants. In at least three distinct collection efforts on the Canadian side, cancerous liver tumor rates have been consistently found above 5% in animals at least three years old.

It is frequently assumed that fish do not observe the international boundary between the U.S. and Canada that runs through the St. Marys River. We are not aware of any existing brown bullhead telemetry research or other work aimed at tracking the movement of this species in this system. Anecdotal reports from the Ontario Ministry of Natural Resources indicate that brown bullhead are harder to find in Canadian waters, which is one reason the BUI is assessed using white suckers on that side.

While both brown bullhead and white suckers are ideal indicator species to evaluate the presence of certain contaminants, brown bullhead have a smaller home range (Sakaris et al. 2005, Doherty et al. 2010). Movements of brown bullhead were similar throughout the year with fish having a mean linear home range of 2.1 km (Sakaris et al. 2005), based on research conducted in the Anacostia River in Washington, D.C. In comparison, Doherty et al. (2010) reported white suckers have a mean linear home range of 2.6 km in the summer which expands to 9.2 km in the fall and winter, with some fish traveling upwards of 40 km, based on findings from the Saint John River in New Brunswick, Canada. The smaller bullhead home range suggests they may be an indicator of more localized conditions.

There is not an obvious explanation for the difference in findings between studies of ecologically similar trophic level species on opposite sides of the geopolitical boundary. The most recent assessment of white suckers on the Canadian side revealed a combined liver tumor rate of 10.9 percent (10.9%) in fish collected during 2021 and 2023 (Chambers et al, 2025). Previous investigations of white suckers in Canada revealed liver tumor rates of 10.6 percent (10.6%) in 2009 and 6 percent (6%) in 2015 (Chambers et al, 2018). It is known that PAHs were more prevalent in sediments due to the particular industrial operations discharging to the St. Marys River in Canadian waters than on the U.S. side.

Perhaps the larger home range of white suckers provides for greater exposure to contaminants. There may be other ecological differences between the two sides that we

don't fully understand, such as habitat quality, preferred food sources, or other factors. Those variables could potentially explain why brown bullhead are not only less prevalent in Canadian waters, but also why they respond with fewer biological effects from contaminants on the U.S. side.

Conclusion

In 2023, 113 brown bullheads were collected from three sites on the U.S. side of the St. Marys River AOC. The majority of the fish were aged and determined to be at least three years old. Livers from each of the fish were preserved and examined by a qualified laboratory. No neoplastic cancerous liver tumors were found and just one non-cancerous growth was discovered. Therefore, the restoration criteria for the Fish Tumors or Other Deformities BUI, requiring a study of at least 100 resident benthic fish with a minimum age of 3 years, collected from at least two sites within the AOC indicating a cancerous liver tumor rate of 5% or less have been met on the U.S. side of the AOC.

Recommendation

Results of the study were reviewed by EGLE technical and AOC program staff, EPA GLNPO staff, and FWS technical staff. The results were shared and discussed with the St. Marys River BPAC, which provided a letter of support for this action. Based on review of the data and with support from BPAC, EGLE recommends removal of the Fish Tumors or Other Deformities Beneficial Use Impairment from the U.S. side of the St. Marys River Area of Concern.

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St. Marys River
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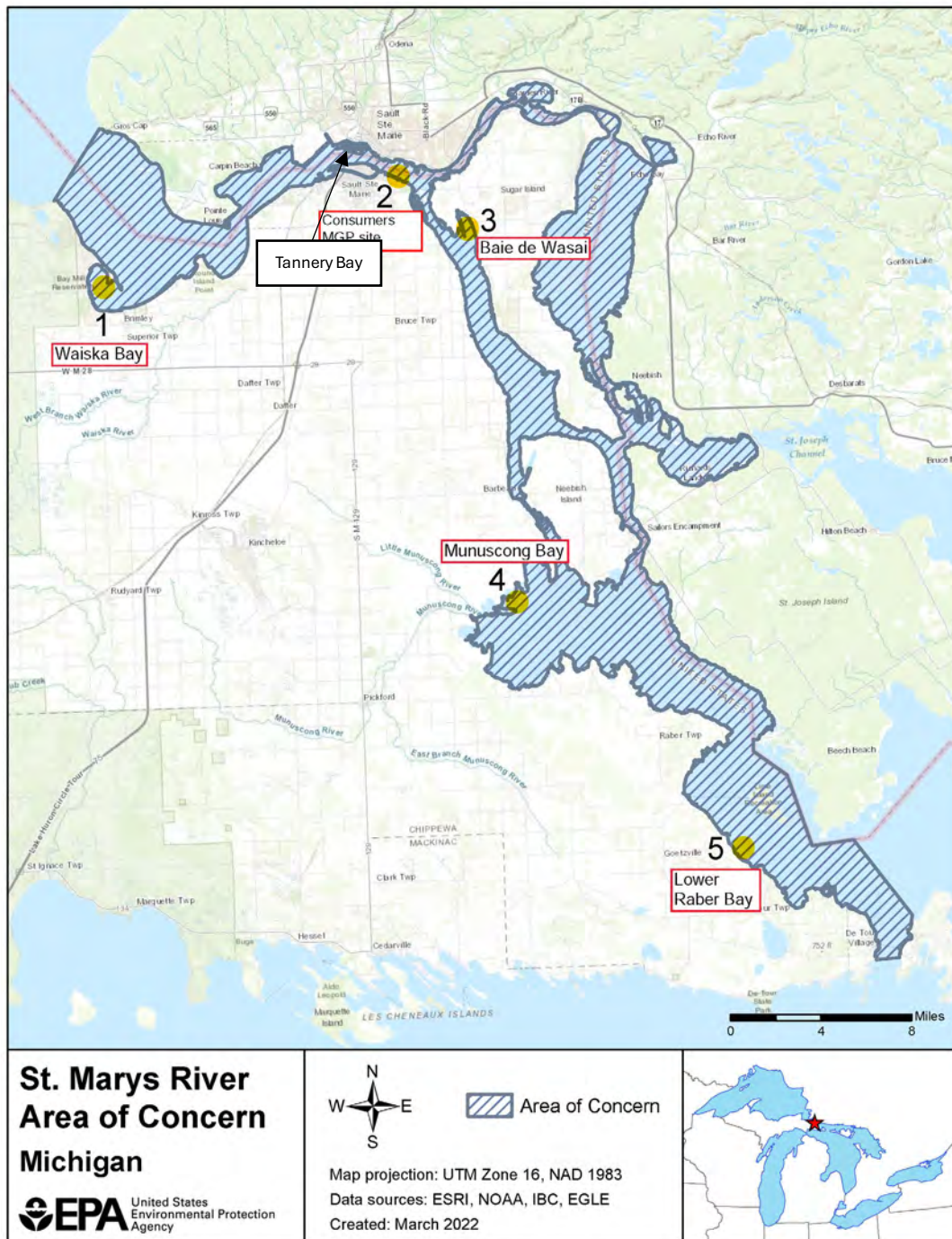


Figure 1. Targeted Brown Bullhead Sampling Locations in the St. Marys River. Fish were collected at locations 1 (Waiska Bay), 3 (Baie de Wasai), and 4 (Munuscong Bay); no fish were collected at locations 2 (near Consumers MGP Site) or 5 (Lower Raber Bay).

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Appendices

- A- Great Lakes Environmental Center, Inc., 2024 final narrative report
- B- Great Lakes Environmental Center, Inc., 2024 final report:
 - Length, Weight, and Age Data for Brown Bullhead Collected from the St. Marys River AOC. Fish Were Aged by Examining Otoliths or Pectoral Fin Spines, or Estimated by Age-Length Key
- C- Experimental Pathology Laboratories Pathology Report, 2023
- D- St. Marys River Area of Concern Tumor BUI Statistical Analysis Final Report, 2025
- E- Letter of Support from BPAC

APPENDIX A

Great Lakes Environmental Center, Inc., 2024 final report:

Histological Study of Brown Bullhead (*Ameiurus nebulosus*) Collected in the St. Marys River Area of Concern

**Histological Study of Brown Bullhead (*Ameiurus nebulosus*)
Collected in the St. Marys River Area of Concern**

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GLEC's Final Report for EGLE WA 23-04
Histological Study of Brown Bullhead – St. Marys River

April 27, 2024

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APPENDIX A - Length, Weight, and Age Data for Brown Bullhead Collected from the St. Marys River AOC. Fish Were Aged by Examining Otoliths or Pectoral Fin Spines, or Estimated by Age-Length Key (ALK)

Appendix B - Histological Study of Brown Bullhead (*Ameiurus Nebulosus*) Collected in the St. Marys River Area of Concern: Pathology Report

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INTRODUCTION AND BACKGROUND

Areas of Concern (AOCs) are defined by the U.S.-Canada Great Lakes Water Quality Agreement as "geographic areas designated by the Parties where significant impairment of beneficial uses has occurred as a result of human activities at the local level." The St. Marys River AOC is one of the 31 U.S.-based AOCs across the Great Lakes and was designated an AOC under the 1987 US-Canada Great Lakes Water Quality Agreement. The St. Marys River AOC includes the area of the river that extends from Whitefish Bay between Point Iroquois, Michigan, and Gros Cap, Ontario, downstream to Quebec Bay, Ontario – Humbug Point, Ontario, in the St. Joseph Channel, and De Tour Passage, Michigan and Ontario.

As a result of industrial and municipal discharges, sediment in the St. Marys River AOC was contaminated with various toxic chemicals, suspended solids, metals, and polycyclic aromatic hydrocarbons (PAHs). Along with industrial and municipal discharges, pollutants in the AOC also came from tannery operations, a steel mill, wastewater treatment discharges, combined sewer overflows, and various nonpoint sources.

An investigation conducted in the late 1990s by the U.S. Fish and Wildlife Service (U.S. FWS) indicated that the incidence of liver tumors in Brown bullhead (*Ameiurus nebulosus*) from St. Marys River's Munuscong Bay was higher than would be expected for a control site (Smith, Portt, and Rokosh, 1990, as cited in EC et al., 2002). An explanation for the cause of the tumors could not be determined at that time. In addition, White suckers (*Catostomus commersonii*) sampled on the Canadian side of the St. Marys River exhibited liver tumor prevalence in excess of 99%, likely associated with exposure to chemical contaminants, such as PAHs in contaminated sediments (OMOE and MDNR, 1992; EC et al., 2002).

The U.S. Environmental Protection Agency (U.S. EPA) is working cooperatively with other federal and state agencies to restore AOCs in the Great Lakes basin. Successful cleanup and restoration in a Great Lakes AOC is predicated on the removal of Beneficial Use Impairments (BUIs), which are designations given by the International Joint Commission (IJC) representing different types of significant environmental degradation. As cleanup work is completed, and monitoring demonstrates enough environmental health improvements, BUIs can be removed. For the U.S. side of the St. Marys River AOC, the accepted Fish Tumors BUI restoration criterion is stated as follows: "The BUI will be considered restored when: a study of at least 100 resident benthic fish (e.g., Brown bullhead or White suckers), with a minimum age of three years, collected from at least two sites within the AOC, indicates a cancerous liver tumor rate of 5% or less." This threshold does not include the Canadian side of the river and it is a combined percentage rate for all sampling locations, not a percentage rate per sampling location.

Michigan's Department of Environment, Great lakes, and Energy (EGLE) contracted with Great Lakes Environmental Center, Inc. (GLEC) to collect adult (age 3+) Brown bullhead from two to five lacustrine areas of the St. Marys River AOC to determine if there was potential to remove the St. Marys River Fish Tumors BUI. Livers from the collected organisms were extracted, preserved, and analyzed for neoplastic (cancerous) tumors, and the results of the survey were compared to previous studies to determine, in part, whether cleanup and restoration efforts have been successful. The results of GLEC's study are presented in this report and directly address whether the restoration criterion for the St. Marys River Fish Tumors BUI has been met.

Sampling and Analysis Methods

The field sampling, fish handling and processing, otolith removal, liver biopsy, and liver pathology procedures implemented for this study followed those outlined in the study's Quality Assurance Project Plan (QAPP) (GLEC 2023). Specific details regarding study procedures are provided in the following sections.

Sampling

A total of five St. Marys River locations were targeted for sampling (Figure 1). Each location has different industrial legacies and therefore varying concentrations of PAHs, the contaminant of concern in this study correlated to fish liver tumors. GLEC collected Brown bullheads on May 15, 16, 17, 18, 23, and 24, 2023 from three of the five targeted sampling locations including Baie de Wassai (BDW), Munuscong Bay (MB), and Waiska Bay (WB). GLEC attempted to collect Brown bullheads at the Consumers MGP location but was unsuccessful. The Lower Raber Bay location was not sampled due to poor weather conditions and fishing success at the other four locations.

Brown bullheads were collected using boat mounted pulsed DC electrofishing gear. Brown bullheads were netted, placed in a live well and subsequently measured (total length). Fish greater than 250 mm in total length (age three and older; Bauman et al. 2008) were targeted for this study; however, fish between 225 mm and 250 mm were retained to confirm their age of at least three years. All other fish were released unharmed at the site of collection.

Handling and Processing

Once collected, fish were held in a well-aerated holding tank containing ambient water and transported from the collection location to a trailer deployed at the boat launch that served as an indoor workspace for processing fish. All Brown bullhead collected and meeting the 250 mm total length requirement were processed. Additionally, five fish less than 250 mm in total length were processed. Fish were euthanized individually using tricaine methanesulfonate (MS222). To prevent interference with histopathologic processing of the liver, any fish that died prior to euthanasia were discarded. However, fish that were alive and incapable of movement were processed. Following the recording of standard data (e.g., collection and processing dates, field observers present, collection location, capture gear, total length in millimeters, weight in grams, and sex), each fish was assessed for the presence of any grossly observable external anomalies (i.e., lesions and deformities). Details of the processing of Brown bullheads followed Sections 5.1-5.5 of Blazer et al. (2007).

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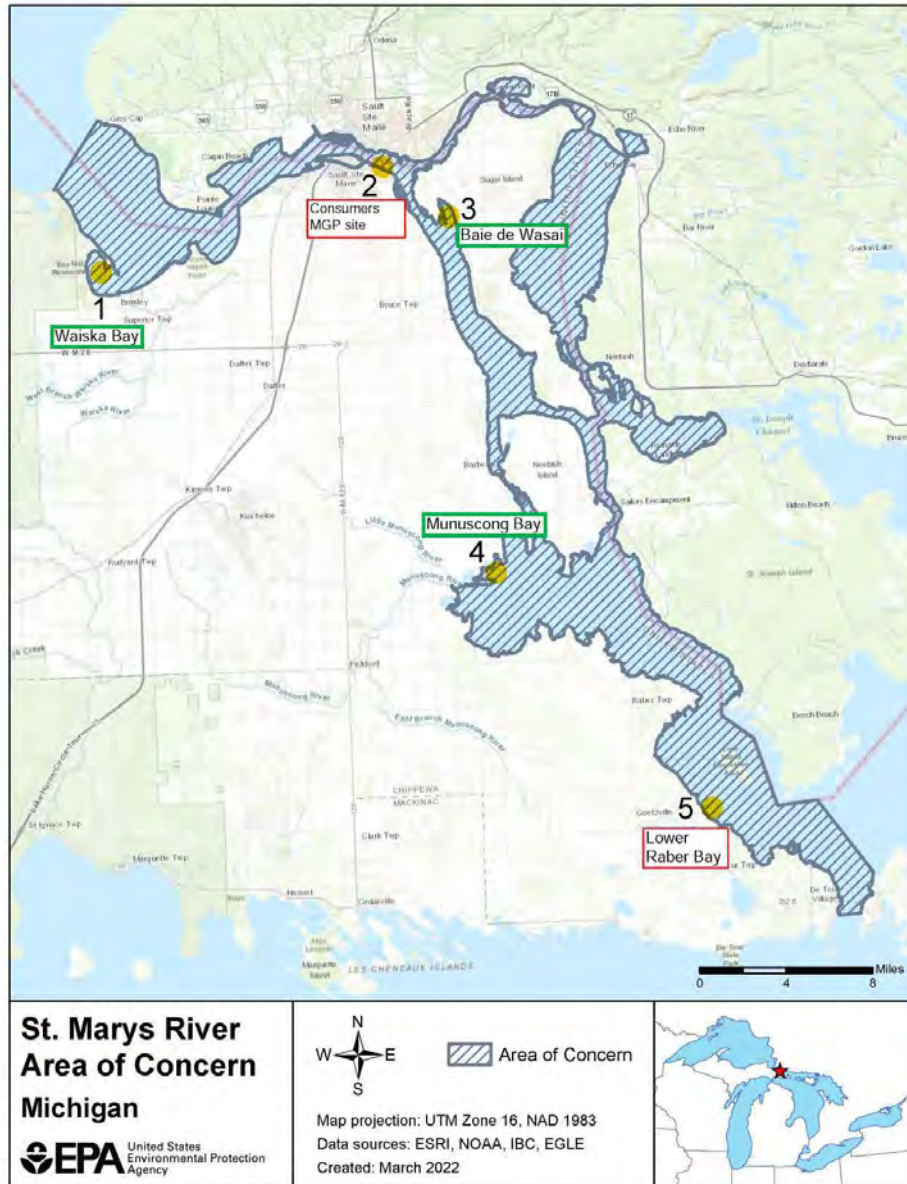


Figure 1. Targeted Brown Bullhead Sampling Locations in the St. Marys River. Fish Were Collected at the Three Locations Outlined in Green; No Fish were Collected at the Two Locations Outlined in Red.

Otolith Removal and Liver Biopsy

GLEC field crews followed the procedures for fish necropsy, otolith and pectoral fin spine removal, and liver sampling for histopathology assessments as described in Rafferty and Grazio (2006). Livers were extracted from each Brown bullhead after no more than five minutes of being euthanized to ensure high tissue quality for histopathology; animals that were dead for longer than five minutes before dissection were not used for histology assessments. Once removed, livers were cut into ~5mm thick strips for a minimum of five liver sections per animal. These pieces representing the entire liver were placed in a single labeled 125 mL polyethylene sample jar supplied by Experimental Pathology Laboratories, Inc. (EPL). Livers were preserved with 10% neutral buffered formalin and packed into a cooler without ice. Liver samples were subsequently shipped to EPL in Sterling, Virginia for histopathologic processing to determine the presence or absence of neoplastic (cancerous) tumors.

Otoliths and pectoral fin spines (as a backup sample in case otoliths were damaged during sectioning or lost) were also collected from each Brown bullhead in the field. Otoliths and pectoral fin spines were placed in a labeled sample envelope and returned to GLEC's Traverse City, Michigan laboratory for subsequent aging to confirm that the animals were at least three years of age. No preservation was required for these samples.

Liver Pathology

Samples of fixed liver tissue from collected Brown bullhead were received by EPL for histopathologic processing and evaluation. Once at EPL, liver tissues were trimmed as needed so that each standard tissue cassette contained one to two liver pieces. Livers were processed routinely for paraffin embedding using an automated tissue processor. Two step sections (each 4-6 microns thick) were microtomed from each block at intervals of approximately 250 microns, and each section was placed on a separate glass slide, which resulted in a total of six slides per fish. Sections were stained with hematoxylin and eosin (H&E), and covered with glass cover slips using a commercial mounting medium.

The pathologist evaluated the histologic sections by brightfield microscopy for proliferative and non-proliferative liver lesions, and other distinguishing characteristics (e.g., degree of hepatocyte basophilia). Diagnostic criteria and terminology used for this assessment generally followed Blazer et al. (2007), with minor modifications. Severity grading of non-neoplastic and pre-neoplastic findings was performed according to the following scale: “-” = not remarkable, Grade 1 = minimal, Grade 2 = mild, Grade 3 = moderate, and Grade 4 = severe. Tumors and parasites were not graded for severity but were instead recorded as Present (P). Findings were recorded into an electronic pathology data reporting system.

Results

Collection

GLEC collected a total of 113 Brown bullhead from the St. Marys River AOC in 2023. The majority of fish were collected from Baie de Wassai (BDW), followed by Munuscong Bay (MB). Only four Brown bullhead were collected from Waiska Bay (WB), and no fish were collected from the other two targeted locations. A total of 57 males and 56 females were collected, and

retained fish ranged from 225 mm to 353 mm in total length. A summary of the number of individuals, sex, and total length of Brown bullheads collected at each location is presented in Table 1.

Table 1. Number, Sex, and Total Length Range of Fish Collected at Each Location

Collection Location	Number of Males Collected	Number of Females Collected	Range in Total Length (mm)
Baie de Wassai (BDW)	36	32	225 - 330
Munuscong Bay (MB)	20	21	260 - 353
Waika Bay (WB)	1	3	238 - 259
Consumers MGP	-	-	-
Lower Raber Bay	-	-	-
TOTAL	57	56	225 - 353

Electrofishing, which tends to bias toward larger individuals, was the only type of sampling gear used for this survey. Greater visibility of larger fish makes them more likely to be netted when electrofishing, and smaller fish require a stronger electric field to stun/capture compared to larger fish. However, the electrofishing equipment settings for this survey were set at a frequency of 60 Hz. This frequency may bias towards smaller fish versus using a lower frequency setting which may have allowed for the collection of larger (and presumably older) fish. Additionally, if trap nets had been used, larger and older Brown bullheads may have been collected. Regardless of potential sampling bias associated with this survey, previous Brown bullhead total length data (O'Connor et al. 2024) indicate that the Brown bullhead collected during this survey are representative of the St. Marys River Brown bullhead population.

Ageing

The age of each fish was determined by examining otoliths or pectoral fin spines, following the procedures of Stevenson and Campana (1992). Of the 113 Brown bullhead retained in this study, 93 were aged. Reasons for not aging the other 20 fish include: 1) otoliths and spine disintegrated when cleaning and 2) otoliths were not collected due to a technician error and corresponding spine samples disintegrated when cleaning. The age results, including the reasons why some samples could not be aged, are tabulated in Appendix A.

A scatterplot depicting the relationship between Brown bullhead total length and weight is provided in Figure 2. Unexpectedly, there was a significant positive correlation between the total length and weight of Brown bullhead collected from the St. Marys River AOC. The Brown bullhead total length/weight relationship observed during this study is similar to that observed by O'Connor et al. (2024).

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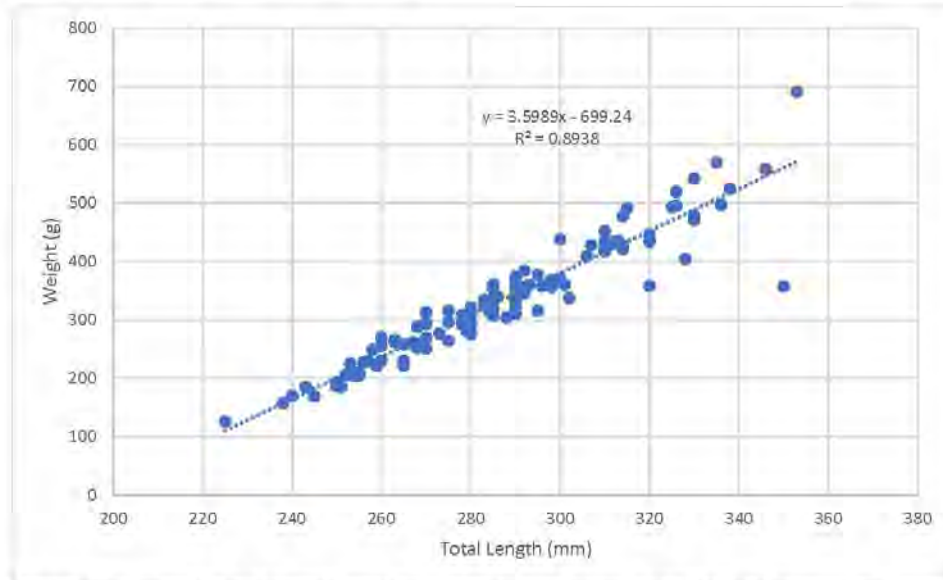


Figure 2. Scatter Plot of Length vs. Weight for Brown Bullhead Collected from the St. Marys River.

A box plot depicting age (for the 93 fish aged with otoliths or pectoral fin spines) versus total length for St. Marys River Brown bullhead is provided in Figure 3. Figure 4 provides the same St. Marys River data compared to the age versus mean length relationship presented in Carlander (1969). The age-length data showed that Brown bullheads collected in the St. Marys River AOC in 2023 grew faster than those highlighted in Carlander (1969).

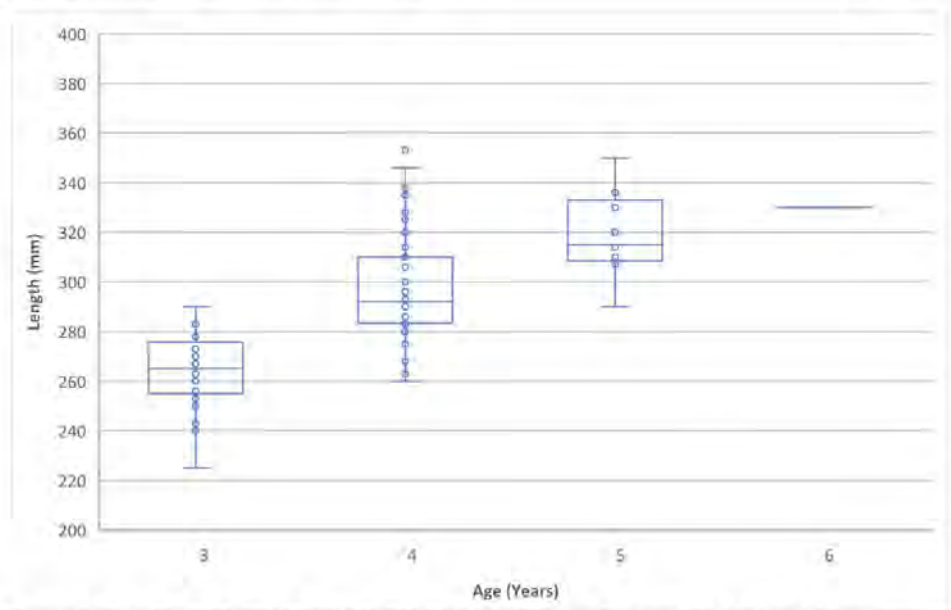


Figure 3. Box Plot of Age (for Fish Aged with Otoliths or Pectoral Fin Spines) Versus Length for Brown Bullhead Collected from the St. Marys River

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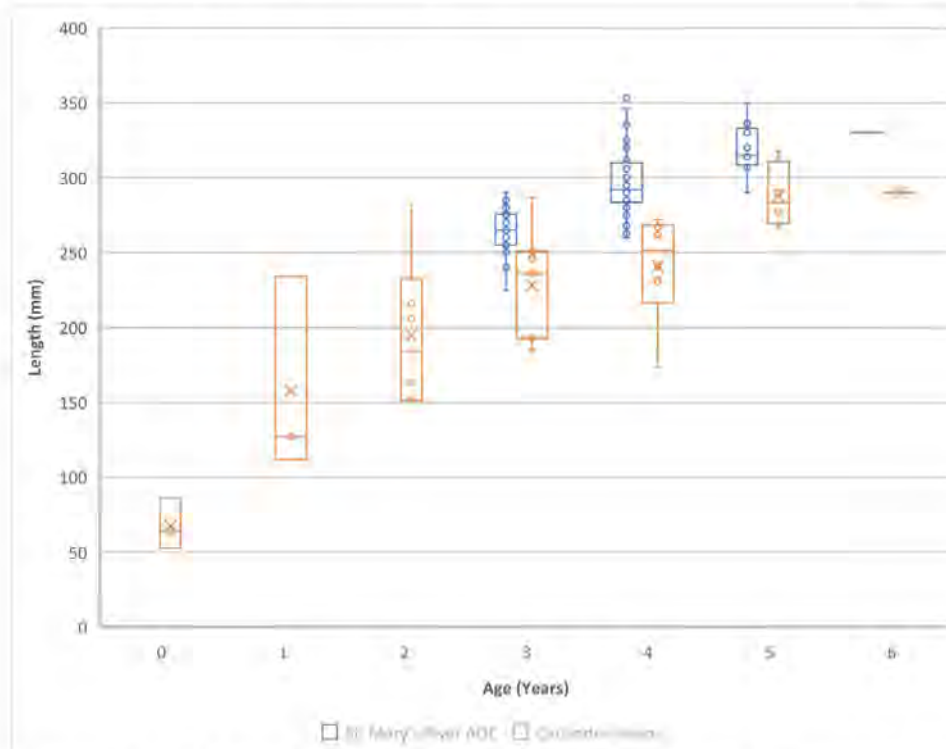


Figure 4. Box Plot of Age Versus Length Relationship Between Brown Bullhead Collected from the St. Marys River Versus Carlander (1969)

A forward age-length key (ALK) was developed from the age-length data of the 93 aged Brown bullheads. A tabulation of the number of age 3-6 fish in 10 length bins, and the age probabilities in each length bin, is presented in Table 2. The ALK was then applied to estimate the age of the 20 unaged Brown bullhead. These age estimates are included in the tabulation in Appendix A.

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Table 2. Number of Age 3-6 Fish in 10 Length Bins, and the Age Probabilities in Each Length Bin

Total Length Bin (range in mm)	Number of Aged Fish in Bin	Number of Age i Fish in Length Bin j				Probability of Age i Given Length Bin j			
		age 3	age 4	age 5	age 6	age 3	age 4	age 5	age 6
225 - 250	4	4	0	0	0	1	0	0	0
251 - 260	10	9	1	0	0	0.9	0.1	0	0
261 - 270	12	7	5	0	0	0.58	0.42	0	0
271 - 280	13	7	6	0	0	0.54	0.46	0	0
281 - 290	17	3	13	1	0	0.18	0.76	0.06	0
291 - 300	12	0	12	0	0	0	1	0	0
301 - 310	7	0	5	2	0	0	0.71	0.29	0
311 - 320	6	0	3	3	0	0	0.5	0.5	0
321 - 330	6	0	4	1	1	0	0.67	0.17	0.17
331 - 355	6	0	4	2	0	0	0.67	0.33	0

Liver Pathology

No cancerous tumors were found in the 113 liver samples from age 3+ Brown bullheads collected from the St. Marys River AOC. A summary of the neoplastic liver lesions reported by EPL is presented in Table 3. The sole neoplasm observed in this study was a benign hepatocellular adenoma in the liver of a female collected at Baie de Wassai (BDW), Animal No. BDW0027. Probably not coincidentally, the liver of this female additionally contained numerous eosinophilic altered foci (graded as severe). The adenoma resembled an eosinophilic focus to some degree, except the margins of this ovoid mass were better circumscribed, and there was slight compression of the neighboring non-neoplastic hepatic parenchyma. The results of the histology study of Brown bullhead livers collected from the St. Marys River AOC is presented in Appendix B.

Table 3. Prevalence of Neoplastic Liver Lesions in Male and Female Brown Bullheads by Collection Location

Sex	Males			Females		
Sample Location	BDW	MB	WB	BDW	MB	WB
Number Examined	36	20	1	32	21	3
Hepatocellular adenomas	0	0	0	1 (3) ^a	0	0
Total Tumors	0	0	0	1 (3) ^a	0	0

^a Number of fish affected, with percentage of fish affected in parenthesis

Summary and Conclusion

The accepted Fish Tumors BUI restoration criterion for the St. Marys River AOC is stated as follows: "The BUI will be considered restored when: a study of at least 100 resident benthic fish (e.g., Brown bullhead or White suckers), with a minimum age of three years, collected from at least two sites within the AOC, indicates a cancerous liver tumor rate of 5% or less." This

threshold does not include the Canadian side of the river and it is a combined percentage rate for all sampling locations, not a percentage rate per sampling location.

In 2023, a total of 113 Brown bullhead were collected from three locations within the St. Marys River AOC for the Fish Tumors BUI assessment. Fish ranged from 225 mm to 353 mm in total length and were between three and six years of age. Although one neoplasm was observed in the liver of a female collected at Baie de Wassai, it was a benign hepatocellular adenoma. No cancerous tumors were found in the 113 liver samples submitted to EPL. Therefore, we conclude that the restoration criterion for the St. Marys River Fish Tumors BUI has been met.

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APPENDIX B

Great Lakes Environmental Center, Inc., 2024 final report:

Length, Weight, and Age Data for Brown Bullhead Collected from the St. Marys River AOC. Fish Were Aged by Examining Otoliths or Pectoral Fin Spines, or Estimated by Age-Length Key (ALK)

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#	Species	Sample Identification	Collection Date	Process Date	Length (mm)	Weight (g)	Liver Preservative	Sex	# Otoliths collected	# Spines collected	Age	Aged by...	Comments ... (*)
1	Brown Bullhead	20230515WB0001	5/15/23	5/15/23	238	157	Buffered 10% Formalin	F	2	4	-	-	Otoliths & spine disintegrated when cleaning and are unable to be aged.
2	Brown Bullhead	20230515WB0002	5/15/23	5/15/23	250	186	Buffered 10% Formalin	F	4	4	-	-	Otoliths & spine disintegrated when cleaning and are unable to be aged.
3	Brown Bullhead	20230515WB0003	5/15/23	5/15/23	259	221	Buffered 10% Formalin	F	4	4	-	-	Otoliths & spine disintegrated when cleaning and are unable to be aged.
4	Brown Bullhead	20230515WB0004	5/15/23	5/15/23	261	185	Buffered 10% Formalin	M	2	4	-	-	Otoliths & spine disintegrated when cleaning and are unable to be aged.
5	Brown Bullhead	20230516MB0005	5/16/23	5/16/23	313	435	Buffered 10% Formalin	F	2	4	-	-	Otoliths & spine disintegrated when cleaning and are unable to be aged.
6	Brown Bullhead	20230516MB0006	5/16/23	5/16/23	312	434	Buffered 10% Formalin	F	2	4	-	-	Otoliths & spine disintegrated when cleaning and are unable to be aged.
7	Brown Bullhead	20230516MB0007	5/16/23	5/16/23	330	471	Buffered 10% Formalin	F	2	4	-	-	Otoliths & spine disintegrated when cleaning and are unable to be aged.
8	Brown Bullhead	20230516MB0008	5/16/23	5/16/23	310	418	Buffered 10% Formalin	F	2	4	-	-	Otoliths & spine disintegrated when cleaning and are unable to be aged.
9	Brown Bullhead	20230516MB0009	5/16/23	5/16/23	314	421	Buffered 10% Formalin	M	2	4	-	-	Otoliths & spine disintegrated when cleaning and are unable to be aged.

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#	Species	Sample Identification	Collection Date	Process Date	Length (mm)	Weight (g)	Liver Preservative	Sex	# Otoliths collected	# Spines collected	Age	Aged by...	Comments ... (*)
10	Brown Bullhead	20230516MB0010	5/16/23	5/16/23	280	275	Buffered 10% Formalin	F	2	4	-	-	Otoliths & spine disintegrated when cleaning and are unable to be aged.
11	Brown Bullhead	20230516MB0011	5/16/23	5/16/23	320	446	Buffered 10% Formalin	M	2	4	-	-	Otoliths & spine disintegrated when cleaning and are unable to be aged.
12	Brown Bullhead	20230517BDW0012	5/17/23	5/17/23	302	337	Buffered 10% Formalin	M	0*	4	-	-	*Otoliths were not collected due to technician error. Technician was corrected. Spine disintegrated when cleaning and was unable to be aged.
13	Brown Bullhead	20230517BDW0013	5/17/23	5/17/23	278	292	Buffered 10% Formalin	M	0*	4	-	-	*Otoliths were not collected due to technician error. Technician was corrected. Spine disintegrated when cleaning and was unable to be aged.
14	Brown Bullhead	20230517BDW0014	5/17/23	5/17/23	268	254	Buffered 10% Formalin	F	0*	4	-	-	*Otoliths were not collected due to technician error. Technician was corrected. Spine disintegrated when cleaning and was unable to be aged.
15	Brown Bullhead	20230517BDW0015	5/17/23	5/17/23	290	310	Buffered 10% Formalin	M	2	4	-	-	Otoliths & spine disintegrated when cleaning and are unable to be aged.

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16	Brown Bullhead	20230517BDW0016	5/17/23	5/17/23	270	269	Buffered 10% Formalin	M	2	4	-	-	Otoliths & spine disintegrated when cleaning and are unable to be aged.
17	Brown Bullhead	20230517BDW0017	5/17/23	5/17/23	252	206	Buffered 10% Formalin	M	0*	2	-	-	*No otoliths collected. Spine disintegrated when cleaning and was unable to be aged.
18	Brown Bullhead	20230517BDW0018	5/17/23	5/17/23	245	169	Buffered 10% Formalin	F	2	4	-	-	Otoliths & spine disintegrated when cleaning and are unable to be aged.
19	Brown Bullhead	20230517BDW0019	5/17/23	5/17/23	265	230	Buffered 10% Formalin	M	2	4	-	-	Otoliths & spine disintegrated when cleaning and are unable to be aged.
20	Brown Bullhead	20230517BDW0020	5/17/23	5/17/23	258	249	Buffered 10% Formalin	F	2	4	-	-	Otoliths & spine disintegrated when cleaning and are unable to be aged.
21	Brown Bullhead	20230517BDW0021	5/17/23	5/17/23	263	266	Buffered 10% Formalin	F	2	1	3+	Otolith	
22	Brown Bullhead	20230517BDW0022	5/17/23	5/17/23	268	252	Buffered 10% Formalin	M	1	1	4+	Otolith	
23	Brown Bullhead	20230517BDW0023	5/17/23	5/17/23	255	210	Buffered 10% Formalin	M	1	1	3+	Otolith	
24	Brown Bullhead	20230517BDW0024	5/17/23	5/17/23	254	203	Buffered 10% Formalin	F	2	1	3+	Otolith	
25	Brown Bullhead	20230517BDW0025	5/17/23	5/17/23	255	209	Buffered 10% Formalin	M	1*	1	3+	Otolith	*Data sheet said 2 otoliths were collected. Envelope only had 1 otolith in it.

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#	Species	Sample Identification	Collection Date	Process Date	Length (mm)	Weight (g)	Liver Preservative	Sex	# Otoliths collected	# Spines collected	Age	Aged by...	Comments ... (*)
26	Brown Bullhead	20230517BDW0026	5/17/23	5/17/23	250	193	Buffered 10% Formalin	F	2	1	3+	Otolith	
27	Brown Bullhead	20230517BDW0027	5/17/23	5/17/23	260	255	Buffered 10% Formalin	F	2	1	3+	Otolith	
28	Brown Bullhead	20230517BDW0028	5/17/23	5/17/23	253	225	Buffered 10% Formalin	F	1	1	3+	Otolith	
29	Brown Bullhead	20230517BDW0029	5/17/23	5/17/23	243	185	Buffered 10% Formalin	F	0	2*	3	Spine	*One spine was broken at removal. Used other spine to age.
30	Brown Bullhead	20230517BDW0030	5/17/23	5/17/23	256	227	Buffered 10% Formalin	F	1	1	3+	Otolith	
31	Brown Bullhead	20230517BDW0031	5/17/23	5/17/23	280	282	Buffered 10% Formalin	M	1	1	4+	Otolith	
32	Brown Bullhead	20230517BDW0032	5/17/23	5/17/23	270	251	Buffered 10% Formalin	M	2	1	3+	Otolith	
33	Brown Bullhead	20230517BDW0033	5/17/23	5/17/23	278	308	Buffered 10% Formalin	F	2	1	3+	Otolith	
34	Brown Bullhead	20230517BDW0034	5/17/23	5/17/23	263	261	Buffered 10% Formalin	F	1*	1	4+	Otolith	*Data sheet said 2 otoliths were collected. Envelope only had 1 otolith in it.
35	Brown Bullhead	20230517BDW0035	5/17/23	5/17/23	240	170	Buffered 10% Formalin	F	0	2	3	Spine	
36	Brown Bullhead	20230517BDW0036	5/17/23	5/17/23	267	261	Buffered 10% Formalin	M	1	1	3+	Otolith	

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#	Species	Sample Identification	Collection Date	Process Date	Length (mm)	Weight (g)	Liver Preservative	Sex	# Otoliths collected	# Spines collected	Age	Aged by...	Comments ... (*)
37	Brown Bullhead	20230517BDW0037	5/17/23	5/17/23	260	230	Buffered 10% Formalin	F	1	1	3+	Otolith	
38	Brown Bullhead	20230517BDW0038	5/17/23	5/17/23	265	221	Buffered 10% Formalin	F	2	1	3+	Otolith	
38	Brown Bullhead	20230517BDW0039	5/17/23	5/17/23	301	360	Buffered 10% Formalin	M	2	1	4+	Otolith	
40	Brown Bullhead	20230518MB0040	5/18/23	5/18/23	290	357	Buffered 10% Formalin	F	2	1	3+	Otolith	
41	Brown Bullhead	20230518MB0041	5/18/23	5/18/23	290	324	Buffered 10% Formalin	M	0	2	4+	Spine	
42	Brown Bullhead	20230518MB0042	5/18/23	5/18/23	310	417	Buffered 10% Formalin	M	2	1	4+	Otolith	
43	Brown Bullhead	20230518MB0043	5/18/23	5/18/23	320	434	Buffered 10% Formalin	M	2	1	4+	Otolith	
44	Brown Bullhead	20230518MB0044	5/18/23	5/18/23	350	357	Buffered 10% Formalin	M	1	1	5+	Otolith	
45	Brown Bullhead	20230518MB0045	5/18/23	5/18/23	314	477	Buffered 10% Formalin	F	2	1	4+	Otolith	
46	Brown Bullhead	20230518MB0046	5/18/23	5/18/23	298	356	Buffered 10% Formalin	M	2	1	4+	Otolith	
47	Brown Bullhead	20230518MB0047	5/18/23	5/18/23	275	264	Buffered 10% Formalin	M	2	2	3+	Otolith	
48	Brown Bullhead	20230518MB0048	5/18/23	5/18/23	275	315	Buffered 10% Formalin	F	2	1	4+	Otolith	

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#	Species	Sample Identification	Collection Date	Process Date	Length (mm)	Weight (g)	Liver Preservative	Sex	# Otoliths collected	# Spines collected	Age	Aged by...	Comments ... (*)
49	Brown Bullhead	20230518MB0049	5/18/23	5/18/23	292	356	Buffered 10% Formalin	F	2	1	4+	Otolith	
50	Brown Bullhead	20230518MB0050	5/18/23	5/18/23	280	299	Buffered 10% Formalin	M	2	1	3+	Otolith	
51	Brown Bullhead	20230518MB0051	5/18/23	5/18/23	267	260	Buffered 10% Formalin	M	2	1	3+	Otolith	
52	Brown Bullhead	20230518MB0052	5/18/23	5/18/23	295	377	Buffered 10% Formalin	F	2	1	4+	Otolith	
53	Brown Bullhead	20230518MB0053	5/18/23	5/18/23	312	431	Buffered 10% Formalin	M	2	1	4+	Otolith	
54	Brown Bullhead	20230518MB0054	5/18/23	5/18/23	285	307	Buffered 10% Formalin	M	2	1	3+	Otolith	
55	Brown Bullhead	20230518MB0055	5/18/23	5/18/23	285	361	Buffered 10% Formalin	F	2	1	4	Otolith	
56	Brown Bullhead	20230518MB0056	5/18/23	5/18/23	338	524	Buffered 10% Formalin	M	2	1	4+	Otolith	
57	Brown Bullhead	20230518MB0057	5/18/23	5/18/23	283	334	Buffered 10% Formalin	F	1	1	3+	Otolith	
58	Brown Bullhead	20230518MB0058	5/18/23	5/18/23	310	435	Buffered 10% Formalin	M	2	1	4	Otolith	
59	Brown Bullhead	20230523MB0059	5/23/23	5/23/23	292	384	Buffered 10% Formalin	F	2	1	4+	Otolith	
60	Brown Bullhead	20230523MB0060	5/23/23	5/23/23	314	427	Buffered 10% Formalin	M	1*	1	5+	Otolith	*Data sheet said 2 otoliths were collected. Envelope only had 1 otolith in it.

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#	Species	Sample Identification	Collection Date	Process Date	Length (mm)	Weight (g)	Liver Preservative	Sex	# Otoliths collected	# Spines collected	Age	Aged by...	Comments ... (*)
61	Brown Bullhead	20230523MB0061	5/23/23	5/23/23	260	270	Buffered 10% Formalin	F	1	1	4	Otolith	
62	Brown Bullhead	20230523MB0062	5/23/23	5/23/23	310	452	Buffered 10% Formalin	F	2	1	5	Otolith	
63	Brown Bullhead	20230523MB0063	5/23/23	5/23/23	306	409	Buffered 10% Formalin	F	1	1	4+	Otolith	
64	Brown Bullhead	20230523MB0064	5/23/23	5/23/23	346	558	Buffered 10% Formalin	M	1	1	4+	Otolith	
65	Brown Bullhead	20230523MB0065	5/23/23	5/23/23	315	491	Buffered 10% Formalin	F	1	1	5+	Otolith	
66	Brown Bullhead	20230523MB0066	5/23/23	5/23/23	270	293	Buffered 10% Formalin	F	1	1	4+	Otolith	
67	Brown Bullhead	20230523MB0067	5/23/23	5/23/23	320	358	Buffered 10% Formalin	M	2	1	5+	Otolith	
68	Brown Bullhead	20230523MB0068	5/23/23	5/23/23	307	428	Buffered 10% Formalin	M	1*	1	5+	Otolith	*Data sheet said 2 otoliths were collected. Envelope only had 1 otolith in it.
69	Brown Bullhead	20230523MB0069	5/23/23	5/23/23	335	570	Buffered 10% Formalin	F	2	1	4+	Otolith	
70	Brown Bullhead	20230523MB0070	5/23/23	5/23/23	325	493	Buffered 10% Formalin	M	2	1	4+	Otolith	
71	Brown Bullhead	20230523MB0071	5/23/23	5/23/23	336	497	Buffered 10% Formalin	M	2	1	5	Otolith	
72	Brown Bullhead	20230523MB0072	5/23/23	5/23/23	353	691	Buffered 10% Formalin	F	2	1	4+	Otolith	

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#	Species	Sample Identification	Collection Date	Process Date	Length (mm)	Weight (g)	Liver Preservative	Sex	# Otoliths collected	# Spines collected	Age	Aged by...	Comments... (*)
73	Brown Bullhead	20230523MB0073	5/23/23	5/23/23	270	313	Buffered 10% Formalin	F	2	1	3+	Otolith	
74	Brown Bullhead	20230524BDW0074	5/24/23	5/24/23	265	258	Buffered 10% Formalin	F	2	1	3+	Otolith	
75	Brown Bullhead	20230524BDW0075	5/24/23	5/24/23	326	495	Buffered 10% Formalin	M	2	1	4+	Otolith	
76	Brown Bullhead	20230524BDW0076	5/24/23	5/24/23	280	316	Buffered 10% Formalin	M	1	1	4+	Otolith	
77	Brown Bullhead	20230524BDW0077	5/24/23	5/24/23	290	342	Buffered 10% Formalin	M	2	1	4+	Otolith	
78	Brown Bullhead	20230524BDW0078	5/24/23	5/24/23	288	304	Buffered 10% Formalin	F	2	1	4+	Otolith	
79	Brown Bullhead	20230524BDW0079	5/24/23	5/24/23	300	370	Buffered 10% Formalin	M	2	1	4+	Otolith	
80	Brown Bullhead	20230524BDW0080	5/24/23	5/24/23	255	204	Buffered 10% Formalin	M	1	1	3+	Otolith	
81	Brown Bullhead	20230524BDW0081	5/24/23	5/24/23	330	478	Buffered 10% Formalin	F	1	1	6	Otolith	
82	Brown Bullhead	20230524BDW0082	5/24/23	5/24/23	330	542	Buffered 10% Formalin	F	1*	1*	5+	Spine	*Data sheet said 1 otolith was collected. No otolith found in envelope. *Spine broke at removal, was still able to be aged

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#	Species	Sample Identification	Collection Date	Process Date	Length (mm)	Weight (g)	Liver Preservative	Sex	# Otoliths collected	# Spines collected	Age	Aged by...	Comments ... (*)
83	Brown Bullhead	20230524BDW0083	5/24/23	5/24/23	286	339	Buffered 10% Formalin	M	1*	1*	4	Otolith	*Possibly a different otolith was collected, might be the lapilli or asteriscii, instead of the sagittae otolith. Was still able to read age. *Spine was broken at removal.
84	Brown Bullhead	20230524BDW0084	5/24/23	5/24/23	310	423	Buffered 10% Formalin	M	2	1*	4+	Otolith	*Spine was broken at removal.
85	Brown Bullhead	20230524BDW0085	5/24/23	5/24/23	225	126	Buffered 10% Formalin	M	1	1	3+	Otolith	
86	Brown Bullhead	20230524BDW0086	5/24/23	5/24/23	328	404	Buffered 10% Formalin	F	2*	1*	4	Otolith	*Both otoliths broke while sanding, was still able to read age. *Spine also was aged 4yrs.
87	Brown Bullhead	20230524BDW0087	5/24/23	5/24/23	284	317	Buffered 10% Formalin	M	2	1	4+	Otolith	
88	Brown Bullhead	20230524BDW0088	5/24/23	5/24/23	290	335	Buffered 10% Formalin	F	2	1	4+	Otolith	
89	Brown Bullhead	20230524BDW0089	5/24/23	5/24/23	296	357	Buffered 10% Formalin	F	2	1	4+	Otolith	
90	Brown Bullhead	20230524BDW0090	5/24/23	5/24/23	279	281	Buffered 10% Formalin	M	1	1	3+	Otolith	
91	Brown Bullhead	20230524BDW0091	5/24/23	5/24/23	280	311	Buffered 10% Formalin	M	2	1	4	Otolith	

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#	Species	Sample Identification	Collection Date	Process Date	Length (mm)	Weight (g)	Liver Preservative	Sex	# Otoliths collected	# Spines collected	Age	Aged by...	Comments ... (*)
92	Brown Bullhead	20230524BDW0092	5/24/23	5/24/23	273	277	Buffered 10% Formalin	M	1	1	3+	Otolith	
93	Brown Bullhead	20230524BDW0093	5/24/23	5/24/23	293	360	Buffered 10% Formalin	M	2	1	4	Otolith	
94	Brown Bullhead	20230524BDW0094	5/24/23	5/24/23	283	325	Buffered 10% Formalin	F	2	1	4+	Otolith	
95	Brown Bullhead	20230524BDW0095	5/24/23	5/24/23	298	368	Buffered 10% Formalin	M	2	1	4+	Otolith	
96	Brown Bullhead	20230524BDW0096	5/24/23	5/24/23	275	296	Buffered 10% Formalin	M	2	1	3+	Otolith	
97	Brown Bullhead	20230524BDW0097	5/24/23	5/24/23	257	229	Buffered 10% Formalin	F	1	1	3+	Otolith	
98	Brown Bullhead	20230524BDW0098	5/24/23	5/24/23	280	294	Buffered 10% Formalin	M	0	2	3+	Spine	
99	Brown Bullhead	20230524BDW0099	5/24/23	5/24/23	295	315	Buffered 10% Formalin	M	2	1	4+	Otolith	
100	Brown Bullhead	20230524BDW0100	5/24/23	5/24/23	300	438	Buffered 10% Formalin	F	1	1	4+	Otolith	
101	Brown Bullhead	20230524BDW0101	5/24/23	5/24/23	326	519	Buffered 10% Formalin	M	2	1	4+	Otolith	
102	Brown Bullhead	20230524BDW0102	5/24/23	5/24/23	285	318	Buffered 10% Formalin	F	1*	1	4+	Otolith	*Data sheet said 1.5 otoliths collected, only 1 otolith was found in envelope. The 0.5 was just a broken piece of bone.

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#	Species	Sample Identification	Collection Date	Process Date	Length (mm)	Weight (g)	Liver Preservative	Sex	# Otoliths collected	# Spines collected	Age	Aged by...	Comments ... (*)
103	Brown Bullhead	20230524BDW0103	5/24/23	5/24/23	292	345	Buffered 10% Formalin	M	1	1	4+	Otolith	
104	Brown Bullhead	20230524BDW0104	5/24/23	5/24/23	285	352	Buffered 10% Formalin	F	2	1	4+	Otolith	
105	Brown Bullhead	20230524BDW0105	5/24/23	5/24/23	268	288	Buffered 10% Formalin	F	2	1*	4+	Otolith	*No spine was found in envelope
106	Brown Bullhead	20230524BDW0106	5/24/23	5/24/23	300	367	Buffered 10% Formalin	M	2	1	4+	Otolith	
107	Brown Bullhead	20230524BDW0107	5/24/23	5/24/23	290	374	Buffered 10% Formalin	M	1	1	4+	Otolith	
108	Brown Bullhead	20230524BDW0108	5/24/23	5/24/23	285	333	Buffered 10% Formalin	M	1*	1	4+	Otolith	*Data sheet said 2 otoliths were collected. Envelope only had 1 otolith in it
109	Brown Bullhead	20230524BDW0109	5/24/23	5/24/23	290	359	Buffered 10% Formalin	F	0*	1*	5	Spine	*Data sheet said 1 otolith was collected, no otolith was found in envelope. *Spine was broken at removal
110	Brown Bullhead	20230524BDW0110	5/24/23	5/24/23	270	296	Buffered 10% Formalin	F	1*	1	4	Otolith	*Otolith cracked at removal. Was still able to age with it.
111	Brown Bullhead	20230524BDW0111	5/24/23	5/24/23	290	361	Buffered 10% Formalin	F	2*	1	4	Otolith	*One otolith broke while sanding, still able to read age. Verified age using other otolith

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#	Species	Sample Identification	Collection Date	Process Date	Length (mm)	Weight (g)	Liver Preservative	Sex	# Otoliths collected	# Spines collected	Age	Aged by...	Comments... (*)
112	Brown Bullhead	20230524BDW0112	5/24/23	5/24/23	280	321	Buffered 10% Formalin	F	2	1	4	Otolith	
113	Brown Bullhead	20230524BDW0113	5/24/23	5/24/23	280	283	Buffered 10% Formalin	M	2	1	4	Otolith	

APPENDIX C

Experimental Pathology Laboratories, Inc., 2023 final report

Histological Study of Brown Bullhead (*Ameiurus nebulosus*) Collected in the St. Marys
River Area of Concern Pathology Report

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Experimental Pathology Laboratories, Inc.

GREAT LAKES ENVIRONMENTAL CENTER, INC.
GLEC WORK ASSIGNMENT NUMBER 23-04
EPL PROJECT NUMBER D54-001

HISTOLOGICAL STUDY OF BROWN BULLHEAD (*AMEIURUS NEBULOSUS*)
COLLECTED IN THE ST. MARY'S RIVER AREA OF CONCERN

PATHOLOGY REPORT

Submitted by:

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Traverse City, MI 48686

October 24, 2023

FINAL REPORT



Experimental Pathology Laboratories, Inc.

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PATHOLOGY NARRATIVE



Experimental Pathology Laboratories, Inc.

GREAT LAKES ENVIRONMENTAL CENTER, INC.
GLEC WORK ASSIGNMENT NUMBER 23-04
EPL PROJECT NUMBER D54-001

HISTOLOGICAL STUDY OF BROWN BULLHEAD (*AMEIURUS NEBULOSUS*)
COLLECTED IN THE ST. MARY'S RIVER AREA OF CONCERN

PATHOLOGY NARRATIVE

INTRODUCTION

As described by the United States Environmental Protection Agency (USEPA, 2023), the St. Mary's River Area of Concern (AOC) is one of the 31 U.S.-based AOCs across the Great Lakes and was designated an AOC under the 1987 Great Lakes Water Quality Agreement. As a result of industrial and municipal discharges, sediment was contaminated with various toxic chemicals, suspended solids, metals, and polycyclic aromatic hydrocarbons (PAHs). Along with industrial and municipal discharges, pollutants in the AOC also came from tannery operations, a steel mill, wastewater treatment discharges, combined sewer overflows, and various nonpoint sources. An investigation conducted in the late 1990's by the U.S. Fish and Wildlife Service (USFWS) indicated that the incidence of liver tumors in Brown bullheads from Munuscong Bay was higher than would be expected for a control site.

Successful interim cleanup and restoration work involves the removal of Beneficial Use Impairments (BUIs). BUIs are designations given by the International Joint Commission representing different types of significant environmental degradation. As cleanup work is completed, and monitoring demonstrates enough environmental health improvements, BUIs can be removed.

The purpose of the current study was to conduct histopathological evaluation of livers collected from adult (age 3+) brown bullhead (*Ameiurus nebulosus*) in a minimum of two (targeting five) lacustrine areas of the river, to determine the presence of tumors.

The sampling locations, and the number and sex of fish collected at each location are presented in Text Table 1.



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Text Table 1: Sampling Locations, and the Number and Sex of Fish Collected at Each Location		
Collection Site	Number of Males Sampled	Number of Females Sampled
Baie de Wassai (BDW)	36	32
Munuscong Bay (MB)	20	21
Waiska Bay (WB)	1	3
TOTAL	57	56

METHODS

Samples of fixed liver tissue from 113 fish, generally consisting of five samples per fish, were received by Experimental Pathology Laboratories, Inc. (EPL®), Sterling, Virginia, for histopathologic processing and evaluation. At EPL, liver tissues were trimmed as needed, so that each standard tissue cassette contained 1-2 liver pieces. Livers were processed routinely for paraffin embedding using an automated tissue processor. Two step sections (each 4-6 microns thick) were microtomed from each block at intervals of approximately 250 microns, and each section was placed on a separate glass slide, which resulted in a total of six slides per fish. Sections were stained with hematoxylin and eosin (H&E), and covered with glass cover slips using a commercial mounting medium.

The pathologist evaluated the histologic sections by brightfield microscopy for proliferative and non-proliferative liver lesions, and other distinguishing characteristics (e.g., degree of hepatocyte basophilia). Diagnostic criteria and terminology used for this assessment generally followed Blazer et al., 2007, with minor modifications. Severity grading of non-neoplastic and pre-neoplastic findings was performed according to the following scale: “-“ = not remarkable, Grade 1 = minimal, Grade 2 = mild, Grade 3 = moderate, Grade 4 = severe. Tumors and parasites were not graded for severity but were instead recorded as Present (P). Findings were recorded into an electronic



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pathology data reporting system and tabulated in the accompanying individual animal and summary tables.

RESULTS AND DISCUSSION

Summarized **neoplastic liver lesions** are presented in Text Table 2.

The sole neoplasm observed in this study was a benign hepatocellular adenoma in the liver of a BDW female, Animal No. BDW0027. Probably not coincidentally, the liver of this female additionally contained numerous eosinophilic altered foci (graded as severe). The adenoma resembled an eosinophilic focus to some degree, except the margins of this ovoid mass were better circumscribed, and there was slight compression of the neighboring non-neoplastic hepatic parenchyma.

Table 2. Prevalence of Neoplastic Liver Lesions in Males and Females by Collection Site						
Sex	Males			Females		
Site	BDW	MB	WB	BDW	MB	WB
Number Examined	36	20	1	32	21	3
Hepatocellular adenomas	0	0	0	1 (3) ^a	0	0
Total Tumors	0	0	0	1 (3)^a	0	0

^a Number of fish affected, with percentage of fish affected in parenthesis.

Summarized **non-neoplastic proliferative** findings are presented in Text Table 3.

Findings in this category included various types of altered hepatocellular foci (minimal to severe), cholangiofibrosis (minimal to mild), and a single occurrence of atypical bile duct hyperplasia (mild). The common feature of lesions in this category is non-neoplastic proliferation of hepatocellular or biliary epithelial tissue.

The most common type of altered focus in this study was eosinophilic, in which the hepatocytes tended to be larger than those of the surrounding unaffected liver, in addition to the relative tinctorial difference. Excluding WB females, which consisted of only three fish, the highest prevalence of eosinophilic foci (22%) was in BDW males. Foci were graded as severe in three fish of that group, which meant that multiple

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variably-sized foci were scattered throughout the examined liver sections. It is suspected that at least certain forms of altered foci may represent pre-neoplastic lesions.

Cholangiofibrosis was identified in small numbers of male and female fish in each of the BDW and MB groups, with the greatest prevalence (20%) occurring in MB males. This finding, which is not described specifically in the Blazer et al., 2007 manual, has been observed periodically in bullhead catfish collected in survey studies (personal experience of the pathologist). Cholangiofibrosis is characterized by the proliferation of immature-looking (albeit completely formed) bile ducts and ductules, which are encased entirely in dense proliferating fibrous connective tissue. Although these lesions bear some resemblance to cholangiocarcinoma, they tend to be more well-circumscribed, and expand the biliary tree without invading the hepatic parenchyma.

Table 3. Prevalence and Severity of Non-neoplastic Proliferative Lesions in Males and Females by Collection Site						
Sex	Males			Females		
Site	BDW	MB	WB	BDW	MB	WB
Number Examined	36	20	1	32	21	3
Altered focus, basophilic	1 (3) ^a	1 (5)	0	0	1 (5)	0
minimal	1 (3)	-	-	-	1 (5)	-
moderate	-	1 (5)	-	-	-	-
Altered focus, eosinophilic	8 (22)	0	0	1 (3)	2 (10)	1 (33)
minimal	1 (3)	-	-	-	-	-
mild	4 (11)	-	-	-	1 (5)	-
moderate	-	-	-	-	1 (5)	-
severe	3 (8)	-	-	1 (3)	-	1 (33)
Altered focus, clear cell	1 (3)	1 (5)	0	1 (3)	4 (19)	0
minimal	1 (3)	-	-	1 (3)	3 (14)	-
mild	-	-	-	-	1 (5)	-
moderate	-	1 (5)	-	-	-	-
Total Foci	10 (28)	2 (10)	0	2 (6)	7 (33)	1 (33)
Cholangiofibrosis	2 (6)	4 (20)	0	1 (3)	2 (10)	0
minimal	1 (3)	3 (15)	-	-	2 (10)	-
mild	1 (3)	1 (5)	-	1 (3)	-	-
Bile duct epithelium, atypical hyperplasia	0	0	0	0	1 (5)	0
mild	-	-	-	-	1 (5)	-

^a Number of fish affected, with percentage of fish affected in parenthesis.

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Summarized **non-proliferative** findings are presented in Text Table 4.

The most common types of non-neoplastic non-proliferative findings in this study included hepatocyte vacuolation (which generally indicates energy storage in the form of glycogen and/or lipid), and various types of parasites (myxozoans within bile ducts, nematodes, cestodes, and trematodes), accompanied by findings that were most likely related to the presence of live parasites, degenerating parasites, and/or parasite migration (e.g., peribiliary fibrosis, cholangiofibrosis, granulomatous inflammation, and mononuclear cell infiltrates).

Other histopathologic findings in this study tended to occur at low frequency, and/or were anticipated types of findings for wild-caught adult bullheads.

Table 4. Prevalence and Severity of Non-proliferative Lesions in Males and Females by Collection Site						
Sex	Males			Females		
Site	BDW	MB	WB	BDW	MB	WB
Number Examined	36	20	1	32	21	3
Hypertrophy, hepatocellular	0	0	0	2 (6) ^a	0	0
minimal	-	-	-	1 (3)	-	-
moderate	-	-	-	1 (3)	-	-
Vacuolation, hepatocyte	27 (75)	14 (70)	0	11 (34)	17 (81)	3 (100)
minimal	13 (36)	7 (35)	-	11 (34)	9 (43)	-
mild	12 (33)	7 (35)	-	-	7 (33)	3 (100)
moderate	2 (6)	-	-	-	1 (5)	-
Inflammation, granulomatous	32 (89)	18 (90)	0	25 (78)	17 (81)	0
minimal	17 (47)	11 (55)	-	18 (56)	9 (43)	-
mild	15 (42)	7 (35)	-	7 (22)	8 (38)	-
Infiltrate, mononuclear cell	20 (56)	8 (40)	0	5 (16)	2 (10)	0
minimal	18 (50)	8 (40)	-	5 (16)	2 (10)	-
mild	2 (6)	-	-	-	-	-
Fibrosis, peribiliary	29 (81)	14 (70)	1 (100)	25 (78)	21 (100)	2 (67)
minimal	12 (33)	8 (40)	1 (100)	8 (25)	5 (24)	2 (67)
mild	15 (42)	4 (20)	-	13 (41)	12 (57)	-
moderate	2 (6)	2 (10)	-	4 (13)	4 (19)	-
Fibrosis	4 (11)	3 (15)	0	1 (3)	0	0
minimal	4 (11)	2 (10)	-	1 (3)	-	-
mild	-	1 (5)	-	-	-	-

^a Number of fish affected, with percentage of fish affected in parenthesis.

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Table 4. Prevalence and Severity of Non-proliferative Lesions in Males and Females by Collection Site (continued)							
Sex		Males			Females		
Site		BDW	MB	WB	BDW	MB	WB
Number Examined		36	20	1	32	21	3
Fibrovascular proliferation, serosa		5 (14)	4 (20)	0	4 (13)	2 (10)	0
	minimal	4 (11)	1 (5)	-	3 (9)	1 (5)	-
	mild	1 (3)	2 (10)	-	1 (3)	1 (5)	-
	moderate	-	1 (5)	-	-	-	-
Necrosis		0	1 (5)	0	0	0	0
	minimal	-	1 (5)	-	-	-	-
Necrosis, single cell		0	0	0	6 (19) ^a	2 (10)	0
	minimal	-	-	-	6 (19)	1 (5)	-
	mild	-	-	-	-	1 (5)	-
Cystic degeneration		1 (3)	3 (15)	0	2 (6)	3 (14)	0
	minimal	1 (3)	3 (15)	-	2 (6)	2 (10)	-
	mild	-	-	-	-	1 (5)	-
Parasite, cestode		1 (3)	9 (45)	0	0	4 (19)	0
Parasite, myxozoan		18 (50)	15 (75)	1 (100)	15 (47)	17 (81)	2 (67)
Parasite, nematode		12 (33)	9 (45)	0	15 (47)	10 (48)	0
Parasite, trematode		21 (58)	4 (20)	1 (100)	29 (91)	10 (48)	1 (33)
Parasite, not otherwise specified (NOS)		3 (8)	3 (15)	0	1 (3)	5 (24)	0

^a Number of fish affected, with percentage of fish affected in parenthesis.

DocuSigned by:

Jeffrey C. Wolf



Signer Name: Jeffrey C. Wolf
Signing Reason: I approve this document
Signing Time: 24-Oct-2023 | 12:38:39 PM EDT
707A7E1D9AD542129743644019A0E0C9

JEFFREY C. WOLF, DVM, Diplomate, ACVP
Senior Pathologist

JCW/cb

St. Marys River
Fish Tumors or Other Deformities BUI
Removal Recommendation
September 2026



Experimental Pathology Laboratories, Inc.

Great Lakes Environmental Center, Inc.
GLEC Work Assignment Number 23-04

REFERENCES

- Blazer VS, Fournie JW, Wolf JC, Wolfe MJ. (2007) Manual for the microscopic diagnosis of proliferative liver and skin lesions in the brown bullhead (*Ameiurus nebulosus*). Pennsylvania Sea Grant. Available: seagrant.psu.edu/publications/technicaldocs/HistoFieldManual.pdf. (May 2008).
- USEPA. (2023) St. Mary's River AOC. Accessed 12 October, 2023, at <https://www.epa.gov/great-lakes-aocs/st-marys-river-aoc>.

STUDY REPORT: NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS

St. Marys River
Fish Tumors or Other Deformities BUI
Removal Recommendation
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Study Report

Page : 1 / 6
Study : 23-04

Test Item : N/A Sponsor : Great Lakes Environmental Center, Inc.
Test System : Fish, TERMINAL Created : 01-Aug-2023 12:34:19 EDT

Number of Animals with Microscopic Findings by Organ/Group/Sex, Grade 1..4
Status at Necropsy: K0 Combined, incl. Decedents

	Sex Groups	M		
		Baie de Wassai	Munuscong Bay	Waiksa Bay
	No. Animals	36	20	1
LIVER	LIVER			
	NO. EXAM.	36	20	1
	NAD	0	0	0
	Adenoma hepatocellular	0	0	0
	Altered focus, basophilic	1	1	0
	Grade: 1	1	0	0
	Grade: 3	0	1	0
	Altered focus, clear cell	1	1	0
	Grade: 1	1	0	0
	Grade: 2	0	0	0
	Grade: 3	0	1	0
	Altered focus, eosinophilic	8	0	0
	Grade: 1	1	0	0
	Grade: 2	4	0	0
	Grade: 3	0	0	0
	Grade: 4	3	0	0
	Bile duct epithelium, atypical hyperplasia	0	0	0
	Grade: 2	0	0	0
	Cholangiofibrosis	2	4	0
	Grade: 1	1	3	0
	Grade: 2	1	1	0
	Cystic degeneration	1	3	0
	Grade: 1	1	3	0
	Grade: 2	0	0	0
	Fibrosis	4	3	0
	Grade: 1	4	2	0
	Grade: 2	0	1	0

NO.EXAM. = Number of animals examined
NAD = Nothing Abnormal Discovered

CONFIDENTIAL

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Study Report

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Study : 23-04

Test Item : N/A Sponsor : Great Lakes Environmental Center, Inc.
Test System : Fish, TERMINAL Created : 01-Aug-2023 12:34:19 EDT

Number of Animals with Microscopic Findings by Organ/Group/Sex, Grade 1..4
Status at Necropsy: K0 Combined, incl. Decedents

	Sex Groups	M	M	M
		Baie de Wassai	Munuscong Bay	Waiksa Bay
	No. Animals	36	20	1
LIVER	Fibrosis, peribiliary	29	14	1
	Grade: 1	12	8	1
	Grade: 2	15	4	0
	Grade: 3	2	2	0
	Fibrovascular proliferation, serosa	5	4	0
	Grade: 1	4	1	0
	Grade: 2	1	2	0
	Grade: 3	0	1	0
	Hypertrophy hepatocellular	0	0	0
	Grade: 1	0	0	0
	Grade: 3	0	0	0
	Infiltrate, mononuclear cell	20	8	0
	Grade: 1	18	8	0
	Grade: 2	2	0	0
	Inflammation, granulomatous	32	18	0
	Grade: 1	17	11	0
	Grade: 2	15	7	0
	Necrosis	0	1	0
	Grade: 1	0	1	0
	Necrosis single cell	0	0	0
	Grade: 1	0	0	0
	Grade: 2	0	0	0
	Parasite, NOS	3	3	0
	Parasite, cestode	1	9	0
	Parasite, myxozoan	18	15	1
	Parasite, nematode	12	9	0
	Parasite, trematode	21	4	1
	Pigmented macrophage aggregates	16	17	0

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Study Report

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Test Item : N/A Sponsor : Great Lakes Environmental Center, Inc.
Test System : Fish, TERMINAL Created : 01-Aug-2023 12:34:19 EDT

Number of Animals with Microscopic Findings by Organ/Group/Sex, Grade 1..4
Status at Necropsy: K0 Combined, incl. Decedents

		Sex Groups		
		Baie de Wassai	Munuscong Bay	Waiska Bay
		No. Animals		
LIVER	Grade: 1	15	13	0
	Grade: 2	1	4	0
	Vacuolation, hepatocyte	27	14	0
	Grade: 1	13	7	0
	Grade: 2	12	7	0
	Grade: 3	2	0	0

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Study Report

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Test Item : N/A Sponsor : Great Lakes Environmental Center, Inc.
Test System : Fish, TERMINAL Created : 01-Aug-2023 12:34:19 EDT

Number of Animals with Microscopic Findings by Organ/Group/Sex, Grade 1..4
Status at Necropsy: K0 Combined, incl. Decedents

	Sex Groups	F		
		Baie de Wassai	Munuscong Bay	Waiksa Bay
	No. Animals	32	21	3
LIVER	LIVER			
	NO. EXAM.	32	21	3
	NAD	0	0	0
	Adenoma hepatocellular	1	0	0
	Altered focus, basophilic	0	1	0
	Grade: 1	0	1	0
	Grade: 3	0	0	0
	Altered focus, clear cell	1	4	0
	Grade: 1	1	3	0
	Grade: 2	0	1	0
	Grade: 3	0	0	0
	Altered focus, eosinophilic	1	2	1
	Grade: 1	0	0	0
	Grade: 2	0	1	0
	Grade: 3	0	1	0
	Grade: 4	1	0	1
	Bile duct epithelium, atypical hyperplasia	0	1	0
	Grade: 2	0	1	0
	Cholangiofibrosis	1	2	0
	Grade: 1	0	2	0
	Grade: 2	1	0	0
	Cystic degeneration	2	3	0
	Grade: 1	2	2	0
	Grade: 2	0	1	0
	Fibrosis	1	0	0
	Grade: 1	1	0	0
	Grade: 2	0	0	0

NO.EXAM. = Number of animals examined
NAD = Nothing Abnormal Discovered

CONFIDENTIAL

St. Marys River
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Study Report

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Test Item : N/A Sponsor : Great Lakes Environmental Center, Inc.
Test System : Fish, TERMINAL Created : 01-Aug-2023 12:34:19 EDT

Number of Animals with Microscopic Findings by Organ/Group/Sex, Grade 1..4
Status at Necropsy: K0 Combined, incl. Decedents

	Sex Groups	F	F	F
		Baie de Wassai	Munuscong Bay	Waika Bay
	No. Animals	32	21	3
LIVER	Fibrosis, peribiliary	25	21	2
	Grade: 1	8	5	2
	Grade: 2	13	12	0
	Grade: 3	4	4	0
	Fibrovascular proliferation, serosa	4	2	0
	Grade: 1	3	1	0
	Grade: 2	1	1	0
	Grade: 3	0	0	0
	Hypertrophy hepatocellular	2	0	0
	Grade: 1	1	0	0
	Grade: 3	1	0	0
	Infiltrate, mononuclear cell	5	2	0
	Grade: 1	5	2	0
	Grade: 2	0	0	0
	Inflammation, granulomatous	25	17	0
	Grade: 1	18	9	0
	Grade: 2	7	8	0
	Necrosis	0	0	0
	Grade: 1	0	0	0
	Necrosis single cell	6	2	0
	Grade: 1	6	1	0
	Grade: 2	0	1	0
	Parasite, NOS	1	5	0
	Parasite, cestode	0	4	0
	Parasite, myxozoan	15	17	2
	Parasite, nematode	15	10	0
	Parasite, trematode	29	10	1
	Pigmented macrophage aggregates	11	17	0

NO.EXAM. = Number of animals examined
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St. Marys River
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Study Report

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Test Item : N/A Sponsor : Great Lakes Environmental Center, Inc.
Test System : Fish, TERMINAL Created : 01-Aug-2023 12:34:19 EDT

Number of Animals with Microscopic Findings by Organ/Group/Sex, Grade 1..4
Status at Necropsy: K0 Combined, incl. Decedents

		Sex	F	F	F
		Groups	Baie de Wassai	Munuscong Bay	Waiska Bay
		No. Animals	32	21	3
LIVER	Grade: 1		11	7	0
	Grade: 2		0	10	0
	Vacuolation, hepatocyte		11	17	3
	Grade: 1		11	9	0
	Grade: 2		0	7	3
	Grade: 3		0	1	0

NO EXAM. = Number of animals examined
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ANIMAL SHEET REPORTS

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Animal Sheet Report

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Test Item : N/A Sponsor : Great Lakes Environmental Center, Inc.
Test System : Fish, TERMINAL Created : 01-Aug-2023 12:33:27 EDT

ANIMAL HEADING DATA All Protocol-Designated Animals

Dose Group: BDW / M

Animal Number	Sex	Defined and Final State of Necropsy	Test Days	First and Last Day under Test	Date of Necropsy
BDW0012	M	K0 / K0	64	15-Mar-2023/17-May-2023	17-May-2023
BDW0013	M	K0 / K0	64	15-Mar-2023/17-May-2023	17-May-2023
BDW0015	M	K0 / K0	64	15-Mar-2023/17-May-2023	17-May-2023
BDW0016	M	K0 / K0	64	15-Mar-2023/17-May-2023	17-May-2023
BDW0017	M	K0 / K0	64	15-Mar-2023/17-May-2023	17-May-2023
BDW0019	M	K0 / K0	64	15-Mar-2023/17-May-2023	17-May-2023
BDW0022	M	K0 / K0	64	15-Mar-2023/17-May-2023	17-May-2023
BDW0023	M	K0 / K0	64	15-Mar-2023/17-May-2023	17-May-2023
BDW0025	M	K0 / K0	64	15-Mar-2023/17-May-2023	17-May-2023
BDW0031	M	K0 / K0	64	15-Mar-2023/17-May-2023	17-May-2023
BDW0032	M	K0 / K0	64	15-Mar-2023/17-May-2023	17-May-2023
BDW0036	M	K0 / K0	64	15-Mar-2023/17-May-2023	17-May-2023
BDW0039	M	K0 / K0	64	15-Mar-2023/17-May-2023	17-May-2023
BDW0075	M	K0 / K0	71	15-Mar-2023/24-May-2023	24-May-2023
BDW0076	M	K0 / K0	71	15-Mar-2023/24-May-2023	24-May-2023
BDW0077	M	K0 / K0	71	15-Mar-2023/24-May-2023	24-May-2023
BDW0079	M	K0 / K0	71	15-Mar-2023/24-May-2023	24-May-2023
BDW0080	M	K0 / K0	71	15-Mar-2023/24-May-2023	24-May-2023
BDW0083	M	K0 / K0	71	15-Mar-2023/24-May-2023	24-May-2023
BDW0084	M	K0 / K0	71	15-Mar-2023/24-May-2023	24-May-2023
BDW0085	M	K0 / K0	71	15-Mar-2023/24-May-2023	24-May-2023
BDW0087	M	K0 / K0	71	15-Mar-2023/24-May-2023	24-May-2023
BDW0090	M	K0 / K0	71	15-Mar-2023/24-May-2023	24-May-2023
BDW0091	M	K0 / K0	71	15-Mar-2023/24-May-2023	24-May-2023
BDW0092	M	K0 / K0	71	15-Mar-2023/24-May-2023	24-May-2023
BDW0093	M	K0 / K0	71	15-Mar-2023/24-May-2023	24-May-2023
BDW0095	M	K0 / K0	71	15-Mar-2023/24-May-2023	24-May-2023
BDW0096	M	K0 / K0	71	15-Mar-2023/24-May-2023	24-May-2023

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Test Item : N/A Sponsor : Great Lakes Environmental Center, Inc.
Test System : Fish, TERMINAL Created : 01-Aug-2023 12:33:27 EDT

ANIMAL HEADING DATA All Protocol-Designated Animals

Dose Group: BDW / M

Animal Number	Sex	Defined and Final State of Necropsy	Test Days	First and Last Day under Test	Date of Necropsy
BDW0098	M	K0 / K0	71	15-Mar-2023/24-May-2023	24-May-2023
BDW0099	M	K0 / K0	71	15-Mar-2023/24-May-2023	24-May-2023
BDW0101	M	K0 / K0	71	15-Mar-2023/24-May-2023	24-May-2023
BDW0103	M	K0 / K0	71	15-Mar-2023/24-May-2023	24-May-2023
BDW0106	M	K0 / K0	71	15-Mar-2023/24-May-2023	24-May-2023
BDW0107	M	K0 / K0	71	15-Mar-2023/24-May-2023	24-May-2023
BDW0108	M	K0 / K0	71	15-Mar-2023/24-May-2023	24-May-2023
BDW0113	M	K0 / K0	71	15-Mar-2023/24-May-2023	24-May-2023

Dose Group: MB / M

Animal Number	Sex	Defined and Final State of Necropsy	Test Days	First and Last Day under Test	Date of Necropsy
MB0009	M	K0 / K0	63	15-Mar-2023/16-May-2023	16-May-2023
MB0011	M	K0 / K0	63	15-Mar-2023/16-May-2023	16-May-2023
MB0041	M	K0 / K0	65	15-Mar-2023/18-May-2023	18-May-2023
MB0042	M	K0 / K0	65	15-Mar-2023/18-May-2023	18-May-2023
MB0043	M	K0 / K0	65	15-Mar-2023/18-May-2023	18-May-2023
MB0044	M	K0 / K0	65	15-Mar-2023/18-May-2023	18-May-2023
MB0046	M	K0 / K0	65	15-Mar-2023/18-May-2023	18-May-2023
MB0047	M	K0 / K0	65	15-Mar-2023/18-May-2023	18-May-2023
MB0050	M	K0 / K0	65	15-Mar-2023/18-May-2023	18-May-2023
MB0051	M	K0 / K0	65	15-Mar-2023/18-May-2023	18-May-2023
MB0053	M	K0 / K0	65	15-Mar-2023/18-May-2023	18-May-2023
MB0054	M	K0 / K0	65	15-Mar-2023/18-May-2023	18-May-2023
MB0056	M	K0 / K0	65	15-Mar-2023/18-May-2023	18-May-2023
MB0058	M	K0 / K0	65	15-Mar-2023/18-May-2023	18-May-2023
MB0060	M	K0 / K0	70	15-Mar-2023/23-May-2023	23-May-2023
MB0064	M	K0 / K0	70	15-Mar-2023/23-May-2023	23-May-2023
MB0067	M	K0 / K0	70	15-Mar-2023/23-May-2023	23-May-2023

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Test Item : N/A Sponsor : Great Lakes Environmental Center, Inc.
Test System : Fish, TERMINAL Created : 01-Aug-2023 12:33:27 EDT

ANIMAL HEADING DATA All Protocol-Designated Animals

Dose Group: MB / M

Animal Number	Sex	Defined and Final State of Necropsy	Test Days	First and Last Day under Test	Date of Necropsy
MB0068	M	K0 / K0	70	15-Mar-2023/23-May-2023	23-May-2023
MB0070	M	K0 / K0	70	15-Mar-2023/23-May-2023	23-May-2023
MB0071	M	K0 / K0	70	15-Mar-2023/23-May-2023	23-May-2023

Dose Group: WB / M

Animal Number	Sex	Defined and Final State of Necropsy	Test Days	First and Last Day under Test	Date of Necropsy
WB0004	M	K0 / K0	62	15-Mar-2023/15-May-2023	15-May-2023

Dose Group: BDW / F

Animal Number	Sex	Defined and Final State of Necropsy	Test Days	First and Last Day under Test	Date of Necropsy
BDW0014	F	K0 / K0	64	15-Mar-2023/17-May-2023	17-May-2023
BDW0018	F	K0 / K0	64	15-Mar-2023/17-May-2023	17-May-2023
BDW0020	F	K0 / K0	64	15-Mar-2023/17-May-2023	17-May-2023
BDW0021	F	K0 / K0	64	15-Mar-2023/17-May-2023	17-May-2023
BDW0024	F	K0 / K0	64	15-Mar-2023/17-May-2023	17-May-2023
BDW0026	F	K0 / K0	64	15-Mar-2023/17-May-2023	17-May-2023
BDW0027	F	K0 / K0	64	15-Mar-2023/17-May-2023	17-May-2023
BDW0028	F	K0 / K0	64	15-Mar-2023/17-May-2023	17-May-2023
BDW0029	F	K0 / K0	64	15-Mar-2023/17-May-2023	17-May-2023
BDW0030	F	K0 / K0	64	15-Mar-2023/17-May-2023	17-May-2023
BDW0033	F	K0 / K0	64	15-Mar-2023/17-May-2023	17-May-2023
BDW0034	F	K0 / K0	64	15-Mar-2023/17-May-2023	17-May-2023
BDW0035	F	K0 / K0	64	15-Mar-2023/17-May-2023	17-May-2023
BDW0037	F	K0 / K0	64	15-Mar-2023/17-May-2023	17-May-2023
BDW0038	F	K0 / K0	64	15-Mar-2023/17-May-2023	17-May-2023
BDW0074	F	K0 / K0	71	15-Mar-2023/24-May-2023	24-May-2023
BDW0078	F	K0 / K0	71	15-Mar-2023/24-May-2023	24-May-2023

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St. Marys River
Fish Tumors or Other Deformities BUI
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Test Item : N/A Sponsor : Great Lakes Environmental Center, Inc.
Test System : Fish, TERMINAL Created : 01-Aug-2023 12:33:27 EDT

ANIMAL HEADING DATA All Protocol-Designated Animals

Dose Group: BDW / F

Animal Number	Sex	Defined and Final State of Necropsy	Test Days	First and Last Day under Test	Date of Necropsy
BDW0081	F	K0 / K0	71	15-Mar-2023/24-May-2023	24-May-2023
BDW0082	F	K0 / K0	71	15-Mar-2023/24-May-2023	24-May-2023
BDW0086	F	K0 / K0	71	15-Mar-2023/24-May-2023	24-May-2023
BDW0088	F	K0 / K0	71	15-Mar-2023/24-May-2023	24-May-2023
BDW0089	F	K0 / K0	71	15-Mar-2023/24-May-2023	24-May-2023
BDW0094	F	K0 / K0	71	15-Mar-2023/24-May-2023	24-May-2023
BDW0097	F	K0 / K0	71	15-Mar-2023/24-May-2023	24-May-2023
BDW0100	F	K0 / K0	71	15-Mar-2023/24-May-2023	24-May-2023
BDW0102	F	K0 / K0	71	15-Mar-2023/24-May-2023	24-May-2023
BDW0104	F	K0 / K0	71	15-Mar-2023/24-May-2023	24-May-2023
BDW0105	F	K0 / K0	71	15-Mar-2023/24-May-2023	24-May-2023
BDW0109	F	K0 / K0	71	15-Mar-2023/24-May-2023	24-May-2023
BDW0110	F	K0 / K0	71	15-Mar-2023/24-May-2023	24-May-2023
BDW0111	F	K0 / K0	71	15-Mar-2023/24-May-2023	24-May-2023
BDW0112	F	K0 / K0	71	15-Mar-2023/24-May-2023	24-May-2023

Dose Group: MB / F

Animal Number	Sex	Defined and Final State of Necropsy	Test Days	First and Last Day under Test	Date of Necropsy
MB0005	F	K0 / K0	63	15-Mar-2023/16-May-2023	16-May-2023
MB0006	F	K0 / K0	63	15-Mar-2023/16-May-2023	16-May-2023
MB0007	F	K0 / K0	63	15-Mar-2023/16-May-2023	16-May-2023
MB0008	F	K0 / K0	63	15-Mar-2023/16-May-2023	16-May-2023
MB0010	F	K0 / K0	63	15-Mar-2023/16-May-2023	16-May-2023
MB0040	F	K0 / K0	65	15-Mar-2023/18-May-2023	18-May-2023
MB0045	F	K0 / K0	65	15-Mar-2023/18-May-2023	18-May-2023
MB0048	F	K0 / K0	65	15-Mar-2023/18-May-2023	18-May-2023
MB0049	F	K0 / K0	65	15-Mar-2023/18-May-2023	18-May-2023
MB0052	F	K0 / K0	65	15-Mar-2023/18-May-2023	18-May-2023

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Test Item : N/A Sponsor : Great Lakes Environmental Center, Inc.
Test System : Fish, TERMINAL Created : 01-Aug-2023 12:33:27 EDT

ANIMAL HEADING DATA All Protocol-Designated Animals

Dose Group: MB / F

Animal Number	Sex	Defined and Final State of Necropsy	Test Days	First and Last Day under Test	Date of Necropsy
MB0055	F	K0 / K0	65	15-Mar-2023/18-May-2023	18-May-2023
MB0057	F	K0 / K0	65	15-Mar-2023/18-May-2023	18-May-2023
MB0059	F	K0 / K0	70	15-Mar-2023/23-May-2023	23-May-2023
MB0061	F	K0 / K0	70	15-Mar-2023/23-May-2023	23-May-2023
MB0062	F	K0 / K0	70	15-Mar-2023/23-May-2023	23-May-2023
MB0063	F	K0 / K0	70	15-Mar-2023/23-May-2023	23-May-2023
MB0065	F	K0 / K0	70	15-Mar-2023/23-May-2023	23-May-2023
MB0066	F	K0 / K0	70	15-Mar-2023/23-May-2023	23-May-2023
MB0069	F	K0 / K0	70	15-Mar-2023/23-May-2023	23-May-2023
MB0072	F	K0 / K0	70	15-Mar-2023/23-May-2023	23-May-2023
MB0073	F	K0 / K0	70	15-Mar-2023/23-May-2023	23-May-2023

Dose Group: WB / F

Animal Number	Sex	Defined and Final State of Necropsy	Test Days	First and Last Day under Test	Date of Necropsy
WB0001	F	K0 / K0	62	15-Mar-2023/15-May-2023	15-May-2023
WB0002	F	K0 / K0	62	15-Mar-2023/15-May-2023	15-May-2023
WB0003	F	K0 / K0	62	15-Mar-2023/15-May-2023	15-May-2023

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Test Item : N/A Sponsor : Great Lakes Environmental Center, Inc.
Test System : Fish, TERMINAL Created : 01-Aug-2023 12:33:27 EDT
ANIMAL NUMBER : BDW0012 SEX: Male DOSE GROUP: Baie de Wassai
Defined Sacrifice Group : K0
State at Necropsy : K0
Date of Necropsy : 17-May-2023
Days on Test : 64 (15-Mar-2023 - 17-May-2023)

* MICROSCOPIC FINDINGS

LIVER

- Fibrosis, peribiliary, grade 2
- Inflammation, granulomatous, grade 2
- Parasite, nematode, present only
- Parasite, trematode, present only
- Pigmented macrophage aggregates, grade 1
- Vacuolation, hepatocyte, grade 2

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER : BDW0013 SEX: Male DOSE GROUP: Baie de Wassai
Defined Sacrifice Group : K0
State at Necropsy : K0
Date of Necropsy : 17-May-2023
Days on Test : 64 (15-Mar-2023 - 17-May-2023)

* MICROSCOPIC FINDINGS

LIVER

- Fibrosis, peribiliary, grade 3
- Fibrovascular proliferation, serosa, grade 1
- Inflammation, granulomatous, grade 1
- Parasite, trematode, present only
- Vacuolation, hepatocyte, grade 1

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

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Test Item : N/A Sponsor : Great Lakes Environmental Center, Inc.
Test System : Fish, TERMINAL Created : 01-Aug-2023 12:33:27 EDT
ANIMAL NUMBER : BDW0015 SEX: Male DOSE GROUP: Baie de Wassai
Defined Sacrifice Group : K0
State at Necropsy : K0
Date of Necropsy : 17-May-2023
Days on Test : 64 (15-Mar-2023 - 17-May-2023)

* MICROSCOPIC FINDINGS

LIVER

- Fibrosis, grade 1
- Infiltrate, mononuclear cell, grade 1
- Inflammation, granulomatous, grade 1
- Parasite, myxozoan, present only
- Parasite, nematode, present only
- Parasite, trematode, present only
- Pigmented macrophage aggregates, grade 1
- Vacuolation, hepatocyte, grade 2

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER : BDW0016 SEX: Male DOSE GROUP: Baie de Wassai
Defined Sacrifice Group : K0
State at Necropsy : K0
Date of Necropsy : 17-May-2023
Days on Test : 64 (15-Mar-2023 - 17-May-2023)

* MICROSCOPIC FINDINGS

LIVER

- Inflammation, granulomatous, grade 1
- Parasite, NOS, present only
- Parasite, trematode, present only
- Pigmented macrophage aggregates, grade 1
- Vacuolation, hepatocyte, grade 2

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Test Item : N/A Sponsor : Great Lakes Environmental Center, Inc.
Test System : Fish, TERMINAL Created : 01-Aug-2023 12:33:27 EDT

Continued Animal: BDW0016

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER : BDW0017 SEX: Male DOSE GROUP: Baie de Wassai

Defined Sacrifice Group : K0

State at Necropsy : K0

Date of Necropsy : 17-May-2023

Days on Test : 64 (15-Mar-2023 - 17-May-2023)

* MICROSCOPIC FINDINGS

LIVER

- Inflammation, granulomatous, grade 1
- Parasite, trematode, present only
- Vacuolation, hepatocyte, grade 2

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER : BDW0019 SEX: Male DOSE GROUP: Baie de Wassai

Defined Sacrifice Group : K0

State at Necropsy : K0

Date of Necropsy : 17-May-2023

Days on Test : 64 (15-Mar-2023 - 17-May-2023)

* MICROSCOPIC FINDINGS

LIVER

- Fibrosis, peribiliary, grade 2
- Infiltrate, mononuclear cell, grade 1
- Inflammation, granulomatous, grade 1
- Parasite, myxozoan, present only
- Parasite, trematode, present only
- Vacuolation, hepatocyte, grade 1

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

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Test Item : N/A Sponsor : Great Lakes Environmental Center, Inc.
Test System : Fish, TERMINAL Created : 01-Aug-2023 12:33:27 EDT
ANIMAL NUMBER : BDW0022 SEX: Male DOSE GROUP: Baie de Wassai
Defined Sacrifice Group : K0
State at Necropsy : K0
Date of Necropsy : 17-May-2023
Days on Test : 64 (15-Mar-2023 - 17-May-2023)

* MICROSCOPIC FINDINGS

LIVER

- Fibrosis, peribiliary, grade 1
- Inflammation, granulomatous, grade 1
- Parasite, myxozoan, present only
- Parasite, nematode, present only
- Parasite, trematode, present only
- Vacuolation, hepatocyte, grade 1

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER : BDW0023 SEX: Male DOSE GROUP: Baie de Wassai
Defined Sacrifice Group : K0
State at Necropsy : K0
Date of Necropsy : 17-May-2023
Days on Test : 64 (15-Mar-2023 - 17-May-2023)

* MICROSCOPIC FINDINGS

LIVER

- Altered focus, clear cell, grade 1
Comment: Slide 1B, step 2
- Fibrosis, grade 1
- Fibrosis, peribiliary, grade 1
- Infiltrate, mononuclear cell, grade 1
- Inflammation, granulomatous, grade 1
- Vacuolation, hepatocyte, grade 1

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Test Item : N/A Sponsor : Great Lakes Environmental Center, Inc.
Test System : Fish, TERMINAL Created : 01-Aug-2023 12:33:27 EDT

Continued Animal: BDW0023

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER : BDW0025 SEX: Male DOSE GROUP: Baie de Wassai

Defined Sacrifice Group : K0

State at Necropsy : K0

Date of Necropsy : 17-May-2023

Days on Test : 64 (15-Mar-2023 - 17-May-2023)

* MICROSCOPIC FINDINGS

LIVER

- Fibrovascular proliferation, serosa, grade 1
- Infiltrate, mononuclear cell, grade 1
- Parasite, NOS, present only
- Parasite, trematode, present only
- Pigmented macrophage aggregates, grade 1
- Vacuolation, hepatocyte, grade 1

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER : BDW0031 SEX: Male DOSE GROUP: Baie de Wassai

Defined Sacrifice Group : K0

State at Necropsy : K0

Date of Necropsy : 17-May-2023

Days on Test : 64 (15-Mar-2023 - 17-May-2023)

* MICROSCOPIC FINDINGS

LIVER

- Altered focus, eosinophilic, grade 2
Comment: Slide 1B, steps 1 and 2
- Cystic degeneration, grade 1
- Fibrosis, grade 1
- Fibrosis, peribiliary, grade 1

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Test Item : N/A Sponsor : Great Lakes Environmental Center, Inc.
Test System : Fish, TERMINAL Created : 01-Aug-2023 12:33:27 EDT

Continued Animal: BDW0031

* MICROSCOPIC FINDINGS

LIVER

- Fibrosis, peribiliary, grade 1
- Infiltrate, mononuclear cell, grade 1
- Inflammation, granulomatous, grade 2
- Parasite, myxozoan, present only
- Parasite, nematode, present only
- Parasite, trematode, present only
- Pigmented macrophage aggregates, grade 1
- Vacuolation, hepatocyte, grade 2

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER : BDW0032 SEX: Male DOSE GROUP: Baie de Wassai

Defined Sacrifice Group : K0
State at Necropsy : K0
Date of Necropsy : 17-May-2023
Days on Test : 64 (15-Mar-2023 - 17-May-2023)

* MICROSCOPIC FINDINGS

LIVER

- Fibrosis, peribiliary, grade 1
- Fibrovascular proliferation, serosa, grade 2
- Inflammation, granulomatous, grade 1
- Parasite, trematode, present only
- Vacuolation, hepatocyte, grade 1

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER : BDW0036 SEX: Male DOSE GROUP: Baie de Wassai

Defined Sacrifice Group : K0
State at Necropsy : K0

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Test Item	: N/A	Sponsor	: Great Lakes Environmental Center, Inc.
Test System	: Fish, TERMINAL	Created	: 01-Aug-2023 12:33:27 EDT
ANIMAL NUMBER	: BDW0036 SEX: Male	Continued Animal: BDW0036 DOSE GROUP: Baie de Wassai	
Defined Sacrifice Group	: K0		
State at Necropsy	: K0		
Date of Necropsy	: 17-May-2023		
Days on Test	: 64 (15-Mar-2023 - 17-May-2023)		

* MICROSCOPIC FINDINGS

LIVER

- Altered focus, eosinophilic, grade 2
Comment: Slides 1, steps 1 and 2
- Infiltrate, mononuclear cell, grade 1
- Inflammation, granulomatous, grade 2

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER	: BDW0039 SEX: Male	DOSE GROUP: Baie de Wassai
Defined Sacrifice Group	: K0	
State at Necropsy	: K0	
Date of Necropsy	: 17-May-2023	
Days on Test	: 64 (15-Mar-2023 - 17-May-2023)	

* MICROSCOPIC FINDINGS

LIVER

- Altered focus, eosinophilic, grade 4
Comment: Numerous foci throughout liver
- Fibrosis, peribiliary, grade 2
- Infiltrate, mononuclear cell, grade 1
- Inflammation, granulomatous, grade 2
- Parasite, nematode, present only
- Pigmented macrophage aggregates, grade 1
- Vacuolation, hepatocyte, grade 1

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Test Item : N/A Sponsor : Great Lakes Environmental Center, Inc.
Test System : Fish, TERMINAL Created : 01-Aug-2023 12:33:27 EDT

Continued Animal: BDW0039

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER : BDW0075 SEX: Male DOSE GROUP: Baie de Wassai

Defined Sacrifice Group : K0

State at Necropsy : K0

Date of Necropsy : 24-May-2023

Days on Test : 71 (15-Mar-2023 - 24-May-2023)

* MICROSCOPIC FINDINGS

LIVER

- Altered focus, eosinophilic, grade 2
Comment: Slide 1A, step 1
- Cholangiofibrosis, grade 2
- Fibrosis, peribiliary, grade 3
- Infiltrate, mononuclear cell, grade 1
- Inflammation, granulomatous, grade 2
- Parasite, cestode, present only
- Parasite, myxozoan, present only
- Parasite, nematode, present only
- Pigmented macrophage aggregates, grade 1
- Vacuolation, hepatocyte, grade 1

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER : BDW0076 SEX: Male DOSE GROUP: Baie de Wassai

Defined Sacrifice Group : K0

State at Necropsy : K0

Date of Necropsy : 24-May-2023

Days on Test : 71 (15-Mar-2023 - 24-May-2023)

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Test Item : N/A Sponsor : Great Lakes Environmental Center, Inc.
Test System : Fish, TERMINAL Created : 01-Aug-2023 12:33:27 EDT

Continued Animal: BDW0076

* MICROSCOPIC FINDINGS

LIVER

- Altered focus, eosinophilic, grade 4
Comment: Numerous foci throughout liver
- Fibrovascular proliferation, serosa, grade 1
- Infiltrate, mononuclear cell, grade 1
- Inflammation, granulomatous, grade 1
- Pigmented macrophage aggregates, grade 1
- Vacuolation, hepatocyte, grade 2

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER : BDW0077 SEX: Male DOSE GROUP: Baie de Wassai

Defined Sacrifice Group : K0
State at Necropsy : K0
Date of Necropsy : 24-May-2023
Days on Test : 71 (15-Mar-2023 - 24-May-2023)

* MICROSCOPIC FINDINGS

LIVER

- Fibrosis, peribiliary, grade 2
- Inflammation, granulomatous, grade 2
- Parasite, myxozoan, present only
- Parasite, trematode, present only
- Pigmented macrophage aggregates, grade 1
- Vacuolation, hepatocyte, grade 2

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER : BDW0079 SEX: Male DOSE GROUP: Baie de Wassai

Defined Sacrifice Group : K0
State at Necropsy : K0

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Test Item	: N/A	Sponsor	: Great Lakes Environmental Center, Inc.
Test System	: Fish, TERMINAL	Created	: 01-Aug-2023 12:33:27 EDT
ANIMAL NUMBER	: BDW0079 SEX: Male	Continued Animal: BDW0079 DOSE GROUP: Baie de Wassai	
Defined Sacrifice Group	: K0		
State at Necropsy	: K0		
Date of Necropsy	: 24-May-2023		
Days on Test	: 71 (15-Mar-2023 - 24-May-2023)		

* MICROSCOPIC FINDINGS

LIVER

- Fibrosis, peribiliary, grade 1
- Inflammation, granulomatous, grade 2
- Parasite, myxozoan, present only
- Parasite, nematode, present only
- Parasite, trematode, present only
- Vacuolation, hepatocyte, grade 3

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER	: BDW0080 SEX: Male	DOSE GROUP: Baie de Wassai
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Defined Sacrifice Group	: K0
State at Necropsy	: K0
Date of Necropsy	: 24-May-2023
Days on Test	: 71 (15-Mar-2023 - 24-May-2023)

* MICROSCOPIC FINDINGS

LIVER

- Fibrosis, peribiliary, grade 1
- Infiltrate, mononuclear cell, grade 1
- Inflammation, granulomatous, grade 1
- Pigmented macrophage aggregates, grade 1
- Vacuolation, hepatocyte, grade 1

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

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Test Item	: N/A	Sponsor	: Great Lakes Environmental Center, Inc.
Test System	: Fish, TERMINAL	Created	: 01-Aug-2023 12:33:27 EDT
ANIMAL NUMBER	: BDW0083 SEX: Male	DOSE GROUP:	Baie de Wassai
Defined Sacrifice Group	: K0		
State at Necropsy	: K0		
Date of Necropsy	: 24-May-2023		
Days on Test	: 71 (15-Mar-2023 - 24-May-2023)		

* MICROSCOPIC FINDINGS

LIVER

- Cholangiofibrosis, grade 1
- Fibrosis, peribiliary, grade 1
- Infiltrate, mononuclear cell, grade 1
- Inflammation, granulomatous, grade 1
- Parasite, trematode, present only

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER	: BDW0084 SEX: Male	DOSE GROUP:	Baie de Wassai
Defined Sacrifice Group	: K0		
State at Necropsy	: K0		
Date of Necropsy	: 24-May-2023		
Days on Test	: 71 (15-Mar-2023 - 24-May-2023)		

* MICROSCOPIC FINDINGS

LIVER

- Fibrosis, peribiliary, grade 1
- Inflammation, granulomatous, grade 2
- Parasite, myxozoan, present only
- Parasite, trematode, present only
- Vacuolation, hepatocyte, grade 3

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER	: BDW0085 SEX: Male	DOSE GROUP:	Baie de Wassai
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Test Item : N/A Sponsor : Great Lakes Environmental Center, Inc.
Test System : Fish, TERMINAL Created : 01-Aug-2023 12:33:27 EDT

ANIMAL NUMBER : BDW0085 SEX: Male Continued Animal: BDW0085
DOSE GROUP: Baie de Wassai
Defined Sacrifice Group : K0
State at Necropsy : K0
Date of Necropsy : 24-May-2023
Days on Test : 71 (15-Mar-2023 - 24-May-2023)

* MICROSCOPIC FINDINGS

LIVER

- Fibrosis, peribiliary, grade 1
- Infiltrate, mononuclear cell, grade 2

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER : BDW0087 SEX: Male DOSE GROUP: Baie de Wassai

Defined Sacrifice Group : K0
State at Necropsy : K0
Date of Necropsy : 24-May-2023
Days on Test : 71 (15-Mar-2023 - 24-May-2023)

* MICROSCOPIC FINDINGS

LIVER

- Fibrosis, peribiliary, grade 2
- Infiltrate, mononuclear cell, grade 1
- Inflammation, granulomatous, grade 1
- Parasite, myxozoan, present only

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER : BDW0090 SEX: Male DOSE GROUP: Baie de Wassai

Defined Sacrifice Group : K0
State at Necropsy : K0
Date of Necropsy : 24-May-2023

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Test Item : N/A Sponsor : Great Lakes Environmental Center, Inc.
Test System : Fish, TERMINAL Created : 01-Aug-2023 12:33:27 EDT
Continued Animal: BDW0090
Days on Test : 71 (15-Mar-2023 - 24-May-2023)

* MICROSCOPIC FINDINGS

LIVER

- Fibrosis, peribiliary, grade 1
- Infiltrate, mononuclear cell, grade 1
- Inflammation, granulomatous, grade 2
- Vacuolation, hepatocyte, grade 1

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER : BDW0091 SEX: Male DOSE GROUP: Baie de Wassai

Defined Sacrifice Group : K0

State at Necropsy : K0

Date of Necropsy : 24-May-2023

Days on Test : 71 (15-Mar-2023 - 24-May-2023)

* MICROSCOPIC FINDINGS

LIVER

- Fibrosis, peribiliary, grade 2
- Fibrovascular proliferation, serosa, grade 1
- Inflammation, granulomatous, grade 2
- Parasite, myxozoan, present only
- Parasite, trematode, present only
- Pigmented macrophage aggregates, grade 1
- Vacuolation, hepatocyte, grade 2

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER : BDW0092 SEX: Male DOSE GROUP: Baie de Wassai

Defined Sacrifice Group : K0

State at Necropsy : K0

Date of Necropsy : 24-May-2023

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Test Item : N/A Sponsor : Great Lakes Environmental Center, Inc.
Test System : Fish, TERMINAL Created : 01-Aug-2023 12:33:27 EDT
Continued Animal: BDW0092
Days on Test : 71 (15-Mar-2023 - 24-May-2023)

* MICROSCOPIC FINDINGS

LIVER

- Fibrosis, peribiliary, grade 2
- Inflammation, granulomatous, grade 1

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER : BDW0093 SEX: Male DOSE GROUP: Baie de Wassai

Defined Sacrifice Group : K0
State at Necropsy : K0
Date of Necropsy : 24-May-2023
Days on Test : 71 (15-Mar-2023 - 24-May-2023)

* MICROSCOPIC FINDINGS

LIVER

- Altered focus, eosinophilic, grade 4
Comment: Multiple foci throughout liver
- Fibrosis, peribiliary, grade 2
- Parasite, myxozoan, present only
- Parasite, nematode, present only
- Vacuolation, hepatocyte, grade 1

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER : BDW0095 SEX: Male DOSE GROUP: Baie de Wassai

Defined Sacrifice Group : K0
State at Necropsy : K0
Date of Necropsy : 24-May-2023
Days on Test : 71 (15-Mar-2023 - 24-May-2023)

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Test Item : N/A Sponsor : Great Lakes Environmental Center, Inc.
Test System : Fish, TERMINAL Created : 01-Aug-2023 12:33:27 EDT

Continued Animal: BDW0095

* MICROSCOPIC FINDINGS

LIVER

- Fibrosis, peribiliary, grade 2
- Inflammation, granulomatous, grade 2
- Parasite, nematode, present only
- Vacuolation, hepatocyte, grade 1

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER : BDW0096 SEX: Male DOSE GROUP: Baie de Wassai

Defined Sacrifice Group : K0

State at Necropsy : K0

Date of Necropsy : 24-May-2023

Days on Test : 71 (15-Mar-2023 - 24-May-2023)

* MICROSCOPIC FINDINGS

LIVER

- Altered focus, eosinophilic, grade 2
Comment: Slide 1, step 2
- Fibrosis, peribiliary, grade 2
- Infiltrate, mononuclear cell, grade 1
- Parasite, myxozoan, present only
- Parasite, trematode, present only
- Pigmented macrophage aggregates, grade 1
- Vacuolation, hepatocyte, grade 2

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER : BDW0098 SEX: Male DOSE GROUP: Baie de Wassai

Defined Sacrifice Group : K0

State at Necropsy : K0

Date of Necropsy : 24-May-2023

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Test Item : N/A Sponsor : Great Lakes Environmental Center, Inc.
Test System : Fish, TERMINAL Created : 01-Aug-2023 12:33:27 EDT
Continued Animal: BDW0098
Days on Test : 71 (15-Mar-2023 - 24-May-2023)

* MICROSCOPIC FINDINGS

LIVER

- Infiltrate, mononuclear cell, grade 1
- Inflammation, granulomatous, grade 2
- Parasite, trematode, present only

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER : BDW0099 SEX: Male DOSE GROUP: Baie de Wassai

Defined Sacrifice Group : K0

State at Necropsy : K0

Date of Necropsy : 24-May-2023

Days on Test : 71 (15-Mar-2023 - 24-May-2023)

* MICROSCOPIC FINDINGS

LIVER

- Fibrosis, peribiliary, grade 1
- Infiltrate, mononuclear cell, grade 2
- Inflammation, granulomatous, grade 2
- Parasite, nematode, present only
- Pigmented macrophage aggregates, grade 2

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER : BDW0101 SEX: Male DOSE GROUP: Baie de Wassai

Defined Sacrifice Group : K0

State at Necropsy : K0

Date of Necropsy : 24-May-2023

Days on Test : 71 (15-Mar-2023 - 24-May-2023)

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Test Item : N/A Sponsor : Great Lakes Environmental Center, Inc.
Test System : Fish, TERMINAL Created : 01-Aug-2023 12:33:27 EDT

Continued Animal: BDW0101

* MICROSCOPIC FINDINGS

LIVER

- Altered focus, basophilic, grade 1
Comment: Slide 1A, step 2
- Fibrosis, peribiliary, grade 2
- Inflammation, granulomatous, grade 1
- Parasite, myxozoan, present only
- Parasite, trematode, present only
- Vacuolation, hepatocyte, grade 2

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER : BDW0103 SEX: Male DOSE GROUP: Baie de Wassai

Defined Sacrifice Group : K0
State at Necropsy : K0
Date of Necropsy : 24-May-2023
Days on Test : 71 (15-Mar-2023 - 24-May-2023)

* MICROSCOPIC FINDINGS

LIVER

- Fibrosis, peribiliary, grade 2
- Inflammation, granulomatous, grade 2
- Parasite, NOS, present only
- Parasite, myxozoan, present only
- Parasite, trematode, present only
- Vacuolation, hepatocyte, grade 1

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER : BDW0106 SEX: Male DOSE GROUP: Baie de Wassai

Defined Sacrifice Group : K0
State at Necropsy : K0

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Test Item	: N/A	Sponsor	: Great Lakes Environmental Center, Inc.
Test System	: Fish, TERMINAL	Created	: 01-Aug-2023 12:33:27 EDT
ANIMAL NUMBER	: BDW0106 SEX: Male	Continued Animal: BDW0106 DOSE GROUP: Baie de Wassai	
Defined Sacrifice Group	: K0		
State at Necropsy	: K0		
Date of Necropsy	: 24-May-2023		
Days on Test	: 71 (15-Mar-2023 - 24-May-2023)		

* MICROSCOPIC FINDINGS

LIVER

- Fibrosis, peribiliary, grade 2
- Infiltrate, mononuclear cell, grade 1
- Inflammation, granulomatous, grade 2
- Parasite, myxozoan, present only
- Parasite, nematode, present only
- Parasite, trematode, present only
- Pigmented macrophage aggregates, grade 1
- Vacuolation, hepatocyte, grade 2

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER	: BDW0107 SEX: Male	DOSE GROUP: Baie de Wassai
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Defined Sacrifice Group	: K0
State at Necropsy	: K0
Date of Necropsy	: 24-May-2023
Days on Test	: 71 (15-Mar-2023 - 24-May-2023)

* MICROSCOPIC FINDINGS

LIVER

- Fibrosis, peribiliary, grade 2
- Infiltrate, mononuclear cell, grade 1
- Inflammation, granulomatous, grade 1
- Parasite, myxozoan, present only
- Parasite, nematode, present only

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Test Item : N/A Sponsor : Great Lakes Environmental Center, Inc.
Test System : Fish, TERMINAL Created : 01-Aug-2023 12:33:27 EDT

Continued Animal: BDW0107

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER : BDW0108 SEX: Male DOSE GROUP: Baie de Wassai

Defined Sacrifice Group : K0

State at Necropsy : K0

Date of Necropsy : 24-May-2023

Days on Test : 71 (15-Mar-2023 - 24-May-2023)

* MICROSCOPIC FINDINGS

LIVER

- Altered focus, eosinophilic, grade 1
Comment: Slide 1B, step 1
- Fibrosis, peribiliary, grade 1
- Infiltrate, mononuclear cell, grade 1
- Inflammation, granulomatous, grade 1
- Parasite, myxozoan, present only
- Pigmented macrophage aggregates, grade 1
- Vacuolation, hepatocyte, grade 2

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER : BDW0113 SEX: Male DOSE GROUP: Baie de Wassai

Defined Sacrifice Group : K0

State at Necropsy : K0

Date of Necropsy : 24-May-2023

Days on Test : 71 (15-Mar-2023 - 24-May-2023)

* MICROSCOPIC FINDINGS

LIVER

- Fibrosis, grade 1
- Fibrosis, peribiliary, grade 2
- Inflammation, granulomatous, grade 1

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Test Item : N/A Sponsor : Great Lakes Environmental Center, Inc.
Test System : Fish, TERMINAL Created : 01-Aug-2023 12:33:27 EDT

Continued Animal: BDW0113

* MICROSCOPIC FINDINGS

LIVER

- Inflammation, granulomatous, grade 1
- Parasite, myxozoan, present only
- Parasite, trematode, present only
- Pigmented macrophage aggregates, grade 1

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER : MB0009 SEX: Male DOSE GROUP: Munuscong Bay

Defined Sacrifice Group : K0

State at Necropsy : K0

Date of Necropsy : 16-May-2023

Days on Test : 63 (15-Mar-2023 - 16-May-2023)

* MICROSCOPIC FINDINGS

LIVER

Comment: Parasite eggs in pancreas step 2

- Inflammation, granulomatous, grade 2
- Parasite, nematode, present only
- Parasite, trematode, present only
- Pigmented macrophage aggregates, grade 1

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER : MB0011 SEX: Male DOSE GROUP: Munuscong Bay

Defined Sacrifice Group : K0

State at Necropsy : K0

Date of Necropsy : 16-May-2023

Days on Test : 63 (15-Mar-2023 - 16-May-2023)

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Test Item : N/A Sponsor : Great Lakes Environmental Center, Inc.
Test System : Fish, TERMINAL Created : 01-Aug-2023 12:33:27 EDT
Continued Animal: MB0011

* MICROSCOPIC FINDINGS

LIVER

- Fibrosis, peribiliary, grade 1
- Inflammation, granulomatous, grade 2
- Parasite, cestode, present only
- Parasite, myxozoan, present only
- Pigmented macrophage aggregates, grade 1

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER : MB0041 SEX: Male DOSE GROUP: Munuscong Bay

Defined Sacrifice Group : K0
State at Necropsy : K0
Date of Necropsy : 18-May-2023
Days on Test : 65 (15-Mar-2023 - 18-May-2023)

* MICROSCOPIC FINDINGS

LIVER

- Fibrosis, peribiliary, grade 2
- Inflammation, granulomatous, grade 2
- Parasite, myxozoan, present only
- Pigmented macrophage aggregates, grade 2
- Vacuolation, hepatocyte, grade 1

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER : MB0042 SEX: Male DOSE GROUP: Munuscong Bay

Defined Sacrifice Group : K0
State at Necropsy : K0
Date of Necropsy : 18-May-2023
Days on Test : 65 (15-Mar-2023 - 18-May-2023)

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Test Item : N/A	Sponsor : Great Lakes Environmental Center, Inc.
Test System : Fish, TERMINAL	Created : 01-Aug-2023 12:33:27 EDT
Continued Animal: MB0042	

* MICROSCOPIC FINDINGS

LIVER

- Cholangiofibrosis, grade 1
- Comment: Step 2
- Fibrosis, peribiliary, grade 1
- Infiltrate, mononuclear cell, grade 1
- Inflammation, granulomatous, grade 1
- Parasite, myxozoan, present only
- Parasite, nematode, present only
- Pigmented macrophage aggregates, grade 1
- Vacuolation, hepatocyte, grade 1

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER : MB0043	SEX: Male	DOSE GROUP: Munuscong Bay
Defined Sacrifice Group : K0		
State at Necropsy : K0		
Date of Necropsy : 18-May-2023		
Days on Test : 65 (15-Mar-2023 - 18-May-2023)		

* MICROSCOPIC FINDINGS

LIVER

- Cholangiofibrosis, grade 2
- Fibrosis, peribiliary, grade 2
- Parasite, NOS, present only
- Parasite, myxozoan, present only
- Pigmented macrophage aggregates, grade 1
- Vacuolation, hepatocyte, grade 1

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER : MB0044	SEX: Male	DOSE GROUP: Munuscong Bay
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Test System : Fish, TERMINAL Created : 01-Aug-2023 12:33:27 EDT
ANIMAL NUMBER : MB0044 SEX: Male Continued Animal: MB0044
DOSE GROUP: Munuscong Bay
Defined Sacrifice Group : K0
State at Necropsy : K0
Date of Necropsy : 18-May-2023
Days on Test : 65 (15-Mar-2023 - 18-May-2023)

* MICROSCOPIC FINDINGS

LIVER

- Fibrosis, peribiliary, grade 2
- Inflammation, granulomatous, grade 1
- Necrosis, grade 1
- Parasite, myxozoan, present only
- Parasite, trematode, present only
- Pigmented macrophage aggregates, grade 1
- Vacuolation, hepatocyte, grade 1

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER : MB0046 SEX: Male DOSE GROUP: Munuscong Bay
Defined Sacrifice Group : K0
State at Necropsy : K0
Date of Necropsy : 18-May-2023
Days on Test : 65 (15-Mar-2023 - 18-May-2023)

* MICROSCOPIC FINDINGS

LIVER

- Fibrosis, grade 1
- Infiltrate, mononuclear cell, grade 1
- Inflammation, granulomatous, grade 1
- Parasite, cestode, present only

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

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Test Item : N/A Sponsor : Great Lakes Environmental Center, Inc.
Test System : Fish, TERMINAL Created : 01-Aug-2023 12:33:27 EDT
ANIMAL NUMBER : MB0047 SEX: Male DOSE GROUP: Munuscong Bay
Defined Sacrifice Group : K0
State at Necropsy : K0
Date of Necropsy : 18-May-2023
Days on Test : 65 (15-Mar-2023 - 18-May-2023)

* MICROSCOPIC FINDINGS

LIVER

- Fibrosis, peribiliary, grade 1
- Infiltrate, mononuclear cell, grade 1
- Parasite, cestode, present only
- Pigmented macrophage aggregates, grade 1

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER : MB0050 SEX: Male DOSE GROUP: Munuscong Bay
Defined Sacrifice Group : K0
State at Necropsy : K0
Date of Necropsy : 18-May-2023
Days on Test : 65 (15-Mar-2023 - 18-May-2023)

* MICROSCOPIC FINDINGS

LIVER

- Inflammation, granulomatous, grade 1
- Parasite, cestode, present only
- Vacuolation, hepatocyte, grade 1

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER : MB0051 SEX: Male DOSE GROUP: Munuscong Bay
Defined Sacrifice Group : K0
State at Necropsy : K0
Date of Necropsy : 18-May-2023

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Test Item : N/A Sponsor : Great Lakes Environmental Center, Inc.
Test System : Fish, TERMINAL Created : 01-Aug-2023 12:33:27 EDT
Days on Test : 65 (15-Mar-2023 - 18-May-2023) Continued Animal: MB0051

* MICROSCOPIC FINDINGS

LIVER

- Infiltrate, mononuclear cell, grade 1
- Inflammation, granulomatous, grade 1
- Parasite, cestode, present only
- Parasite, myxozoan, present only
- Parasite, nematode, present only
- Vacuolation, hepatocyte, grade 2

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER : MB0053 SEX: Male DOSE GROUP: Munuscong Bay

Defined Sacrifice Group : K0

State at Necropsy : K0

Date of Necropsy : 18-May-2023

Days on Test : 65 (15-Mar-2023 - 18-May-2023)

* MICROSCOPIC FINDINGS

LIVER

- Altered focus, basophilic, grade 3
Comment: Slide 1A, steps 1 and 2
- Cystic degeneration, grade 1
- Fibrovascular proliferation, serosa, grade 1
- Inflammation, granulomatous, grade 1
- Parasite, myxozoan, present only
- Parasite, nematode, present only
- Parasite, trematode, present only
- Pigmented macrophage aggregates, grade 1
- Vacuolation, hepatocyte, grade 2

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

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Test Item : N/A Sponsor : Great Lakes Environmental Center, Inc.
Test System : Fish, TERMINAL Created : 01-Aug-2023 12:33:27 EDT
ANIMAL NUMBER : MB0054 SEX: Male DOSE GROUP: Munuscong Bay
Defined Sacrifice Group : K0
State at Necropsy : K0
Date of Necropsy : 18-May-2023
Days on Test : 65 (15-Mar-2023 - 18-May-2023)

* MICROSCOPIC FINDINGS

LIVER

- Fibrosis, grade 1
- Infiltrate, mononuclear cell, grade 1
- Inflammation, granulomatous, grade 1
- Parasite, myxozoan, present only
- Pigmented macrophage aggregates, grade 1
- Vacuolation, hepatocyte, grade 2

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER : MB0056 SEX: Male DOSE GROUP: Munuscong Bay
Defined Sacrifice Group : K0
State at Necropsy : K0
Date of Necropsy : 18-May-2023
Days on Test : 65 (15-Mar-2023 - 18-May-2023)

* MICROSCOPIC FINDINGS

LIVER

- Cholangiofibrosis, grade 1
- Fibrosis, peribiliary, grade 1
- Inflammation, granulomatous, grade 2
- Parasite, cestode, present only
- Parasite, nematode, present only
- Pigmented macrophage aggregates, grade 2

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

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Test Item : N/A Sponsor : Great Lakes Environmental Center, Inc.
Test System : Fish, TERMINAL Created : 01-Aug-2023 12:33:27 EDT
ANIMAL NUMBER : MB0058 SEX: Male DOSE GROUP: Munuscong Bay
Defined Sacrifice Group : K0
State at Necropsy : K0
Date of Necropsy : 18-May-2023
Days on Test : 65 (15-Mar-2023 - 18-May-2023)

* MICROSCOPIC FINDINGS

LIVER

- Altered focus, clear cell, grade 3
Comment: Slide 1A, steps 1 and 2
- Cholangiofibrosis, grade 1
- Fibrosis, peribiliary, grade 3
- Infiltrate, mononuclear cell, grade 1
- Inflammation, granulomatous, grade 1
- Parasite, NOS, present only
- Parasite, myxozoan, present only
- Pigmented macrophage aggregates, grade 1
- Vacuolation, hepatocyte, grade 2

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER : MB0060 SEX: Male DOSE GROUP: Munuscong Bay
Defined Sacrifice Group : K0
State at Necropsy : K0
Date of Necropsy : 23-May-2023
Days on Test : 70 (15-Mar-2023 - 23-May-2023)

* MICROSCOPIC FINDINGS

LIVER

- Fibrosis, peribiliary, grade 1
- Inflammation, granulomatous, grade 1
- Parasite, NOS, present only
- Parasite, cestode, present only

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Test Item : N/A Sponsor : Great Lakes Environmental Center, Inc.
Test System : Fish, TERMINAL Created : 01-Aug-2023 12:33:27 EDT
Continued Animal: MB0060

* MICROSCOPIC FINDINGS

LIVER

- Parasite, cestode, present only
- Parasite, myxozoan, present only
- Parasite, nematode, present only
- Pigmented macrophage aggregates, grade 1
- Vacuolation, hepatocyte, grade 2

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER : MB0064 SEX: Male DOSE GROUP: Munuscong Bay

Defined Sacrifice Group : K0
State at Necropsy : K0
Date of Necropsy : 23-May-2023
Days on Test : 70 (15-Mar-2023 - 23-May-2023)

* MICROSCOPIC FINDINGS

LIVER

- Cystic degeneration, grade 1
- Fibrosis, peribiliary, grade 1
- Fibrovascular proliferation, serosa, grade 3
- Infiltrate, mononuclear cell, grade 1
- Inflammation, granulomatous, grade 2
- Parasite, cestode, present only
- Parasite, myxozoan, present only
- Pigmented macrophage aggregates, grade 1
- Vacuolation, hepatocyte, grade 1

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER : MB0067 SEX: Male DOSE GROUP: Munuscong Bay

Defined Sacrifice Group : K0

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Test Item : N/A Sponsor : Great Lakes Environmental Center, Inc.
Test System : Fish, TERMINAL Created : 01-Aug-2023 12:33:27 EDT
ANIMAL NUMBER : MB0067 SEX: Male Continued Animal: MB0067
DOSE GROUP: Munuscong Bay
Defined Sacrifice Group : K0
State at Necropsy : K0
Date of Necropsy : 23-May-2023
Days on Test : 70 (15-Mar-2023 - 23-May-2023)

* MICROSCOPIC FINDINGS

LIVER

- Fibrosis, peribiliary, grade 3
- Fibrovascular proliferation, serosa, grade 2
- Inflammation, granulomatous, grade 2
- Parasite, cestode, present only
- Parasite, myxozoan, present only
- Parasite, nematode, present only
- Pigmented macrophage aggregates, grade 1

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER : MB0068 SEX: Male DOSE GROUP: Munuscong Bay
Defined Sacrifice Group : K0
State at Necropsy : K0
Date of Necropsy : 23-May-2023
Days on Test : 70 (15-Mar-2023 - 23-May-2023)

* MICROSCOPIC FINDINGS

LIVER

- Cystic degeneration, grade 1
- Fibrosis, grade 2
- Fibrosis, peribiliary, grade 1
- Inflammation, granulomatous, grade 1
- Parasite, myxozoan, present only
- Pigmented macrophage aggregates, grade 1

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Test Item : N/A Sponsor : Great Lakes Environmental Center, Inc.
Test System : Fish, TERMINAL Created : 01-Aug-2023 12:33:27 EDT
Continued Animal: MB0068

* MICROSCOPIC FINDINGS

LIVER

- Vacuolation, hepatocyte, grade 2

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER : MB0070 SEX: Male DOSE GROUP: Munuscong Bay

Defined Sacrifice Group : K0

State at Necropsy : K0

Date of Necropsy : 23-May-2023

Days on Test : 70 (15-Mar-2023 - 23-May-2023)

* MICROSCOPIC FINDINGS

LIVER

- Fibrosis, peribiliary, grade 1

- Infiltrate, mononuclear cell, grade 1

- Inflammation, granulomatous, grade 1

- Parasite, myxozoan, present only

- Parasite, nematode, present only

- Parasite, trematode, present only

- Pigmented macrophage aggregates, grade 2

- Vacuolation, hepatocyte, grade 2

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER : MB0071 SEX: Male DOSE GROUP: Munuscong Bay

Defined Sacrifice Group : K0

State at Necropsy : K0

Date of Necropsy : 23-May-2023

Days on Test : 70 (15-Mar-2023 - 23-May-2023)

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Test Item : N/A Sponsor : Great Lakes Environmental Center, Inc.
Test System : Fish, TERMINAL Created : 01-Aug-2023 12:33:27 EDT
Continued Animal: MB0071

* MICROSCOPIC FINDINGS

LIVER

- Fibrosis, peribiliary, grade 2
- Fibrovascular proliferation, serosa, grade 2
- Inflammation, granulomatous, grade 2
- Parasite, myxozoan, present only
- Parasite, nematode, present only
- Pigmented macrophage aggregates, grade 2
- Vacuolation, hepatocyte, grade 1

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER : WB0004 SEX: Male DOSE GROUP: Waika Bay
Defined Sacrifice Group : K0
State at Necropsy : K0
Date of Necropsy : 15-May-2023
Days on Test : 62 (15-Mar-2023 - 15-May-2023)

* MICROSCOPIC FINDINGS

LIVER

- Fibrosis, peribiliary, grade 1
- Parasite, myxozoan, present only
- Parasite, trematode, present only

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER : BDW0014 SEX: Female DOSE GROUP: Baie de Wassai
Defined Sacrifice Group : K0
State at Necropsy : K0
Date of Necropsy : 17-May-2023
Days on Test : 64 (15-Mar-2023 - 17-May-2023)

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Test Item : N/A Sponsor : Great Lakes Environmental Center, Inc.
Test System : Fish, TERMINAL Created : 01-Aug-2023 12:33:27 EDT

Continued Animal: BDW0014

* MICROSCOPIC FINDINGS

LIVER

- Fibrosis, peribiliary, grade 1
- Necrosis: single cell, grade 1
- Parasite, trematode, present only
- Vacuolation, hepatocyte, grade 1

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER : BDW0018 SEX: Female DOSE GROUP: Baie de Wassai

Defined Sacrifice Group : K0

State at Necropsy : K0

Date of Necropsy : 17-May-2023

Days on Test : 64 (15-Mar-2023 - 17-May-2023)

* MICROSCOPIC FINDINGS

LIVER

- Fibrosis, peribiliary, grade 2
- Inflammation, granulomatous, grade 2
- Parasite, nematode, present only
- Parasite, trematode, present only

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER : BDW0020 SEX: Female DOSE GROUP: Baie de Wassai

Defined Sacrifice Group : K0

State at Necropsy : K0

Date of Necropsy : 17-May-2023

Days on Test : 64 (15-Mar-2023 - 17-May-2023)

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Test Item : N/A Sponsor : Great Lakes Environmental Center, Inc.
Test System : Fish, TERMINAL Created : 01-Aug-2023 12:33:27 EDT

Continued Animal: BDW0020

* MICROSCOPIC FINDINGS

LIVER

- Fibrosis, peribiliary, grade 1
- Fibrovascular proliferation, serosa, grade 2
- Inflammation, granulomatous, grade 1
- Parasite, myxozoan, present only
- Parasite, trematode, present only
- Pigmented macrophage aggregates, grade 1

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER : BDW0021 SEX: Female DOSE GROUP: Baie de Wassai

Defined Sacrifice Group : K0
State at Necropsy : K0
Date of Necropsy : 17-May-2023
Days on Test : 64 (15-Mar-2023 - 17-May-2023)

* MICROSCOPIC FINDINGS

LIVER

- Cystic degeneration, grade 1
- Fibrosis, peribiliary, grade 1
- Fibrovascular proliferation, serosa, grade 1
- Necrosis: single cell, grade 1
- Parasite, myxozoan, present only
- Parasite, trematode, present only
- Vacuolation, hepatocyte, grade 1

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER : BDW0024 SEX: Female DOSE GROUP: Baie de Wassai

Defined Sacrifice Group : K0
State at Necropsy : K0

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Test System	: Fish, TERMINAL	Created	: 01-Aug-2023 12:33:27 EDT
ANIMAL NUMBER	: BDW0024 SEX: Female	Continued Animal: BDW0024 DOSE GROUP: Baie de Wassai	
Defined Sacrifice Group	: K0		
State at Necropsy	: K0		
Date of Necropsy	: 17-May-2023		
Days on Test	: 64 (15-Mar-2023 - 17-May-2023)		

* MICROSCOPIC FINDINGS

LIVER

- Inflammation, granulomatous, grade 1
- Parasite, nematode, present only
- Parasite, trematode, present only

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER	: BDW0026 SEX: Female	DOSE GROUP: Baie de Wassai
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Defined Sacrifice Group	: K0
State at Necropsy	: K0
Date of Necropsy	: 17-May-2023
Days on Test	: 64 (15-Mar-2023 - 17-May-2023)

* MICROSCOPIC FINDINGS

LIVER

- Infiltrate, mononuclear cell, grade 1
- Inflammation, granulomatous, grade 1
- Parasite, nematode, present only
- Parasite, trematode, present only

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER	: BDW0027 SEX: Female	DOSE GROUP: Baie de Wassai
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Defined Sacrifice Group	: K0
State at Necropsy	: K0

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Test Item	: N/A	Sponsor	: Great Lakes Environmental Center, Inc.
Test System	: Fish, TERMINAL	Created	: 01-Aug-2023 12:33:27 EDT
ANIMAL NUMBER	: BDW0027 SEX: Female	Continued Animal: BDW0027 DOSE GROUP: Baie de Wassai	
Defined Sacrifice Group	: K0		
State at Necropsy	: K0		
Date of Necropsy	: 17-May-2023		
Days on Test	: 64 (15-Mar-2023 - 17-May-2023)		

* MICROSCOPIC FINDINGS

LIVER

- Adenoma hepatocellular (benign tumor)
Comment: Slide 1B, step 1
- Altered focus, eosinophilic, grade 4
Comment: Multiple foci throughout sections
- Fibrosis, peribiliary, grade 2
- Inflammation, granulomatous, grade 1
- Necrosis: single cell, grade 1
- Parasite, myxozoan, present only
- Parasite, trematode, present only
- Vacuolation, hepatocyte, grade 1

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER	: BDW0028 SEX: Female	DOSE GROUP: Baie de Wassai
Defined Sacrifice Group	: K0	
State at Necropsy	: K0	
Date of Necropsy	: 17-May-2023	
Days on Test	: 64 (15-Mar-2023 - 17-May-2023)	

* MICROSCOPIC FINDINGS

LIVER

- Vacuolation, hepatocyte, grade 1

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

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Test Item : N/A Sponsor : Great Lakes Environmental Center, Inc.
Test System : Fish, TERMINAL Created : 01-Aug-2023 12:33:27 EDT
ANIMAL NUMBER : BDW0029 SEX: Female DOSE GROUP: Baie de Wassai
Defined Sacrifice Group : K0
State at Necropsy : K0
Date of Necropsy : 17-May-2023
Days on Test : 64 (15-Mar-2023 - 17-May-2023)

* MICROSCOPIC FINDINGS

LIVER

- Fibrosis, peribiliary, grade 1
- Inflammation, granulomatous, grade 2
- Parasite, nematode, present only
- Parasite, trematode, present only
- Vacuolation, hepatocyte, grade 1

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER : BDW0030 SEX: Female DOSE GROUP: Baie de Wassai
Defined Sacrifice Group : K0
State at Necropsy : K0
Date of Necropsy : 17-May-2023
Days on Test : 64 (15-Mar-2023 - 17-May-2023)

* MICROSCOPIC FINDINGS

LIVER

- Fibrosis, grade 1
- Fibrosis, peribiliary, grade 2
- Inflammation, granulomatous, grade 1
- Parasite, myxozoan, present only
- Parasite, trematode, present only

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER : BDW0033 SEX: Female DOSE GROUP: Baie de Wassai

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Test Item	: N/A	Sponsor	: Great Lakes Environmental Center, Inc.
Test System	: Fish, TERMINAL	Created	: 01-Aug-2023 12:33:27 EDT
ANIMAL NUMBER	: BDW0033 SEX: Female	Continued Animal:	BDW0033
		DOSE GROUP:	Baie de Wassai
Defined Sacrifice Group	: K0		
State at Necropsy	: K0		
Date of Necropsy	: 17-May-2023		
Days on Test	: 64 (15-Mar-2023 - 17-May-2023)		

* MICROSCOPIC FINDINGS

LIVER

- Hypertrophy hepatocellular, grade 3
- Inflammation, granulomatous, grade 1
- Parasite, trematode, present only

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER	: BDW0034 SEX: Female	DOSE GROUP:	Baie de Wassai
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Defined Sacrifice Group	: K0
State at Necropsy	: K0
Date of Necropsy	: 17-May-2023
Days on Test	: 64 (15-Mar-2023 - 17-May-2023)

* MICROSCOPIC FINDINGS

LIVER

- Inflammation, granulomatous, grade 1
- Parasite, trematode, present only
- Pigmented macrophage aggregates, grade 1

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER	: BDW0035 SEX: Female	DOSE GROUP:	Baie de Wassai
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Defined Sacrifice Group	: K0
State at Necropsy	: K0
Date of Necropsy	: 17-May-2023

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Test Item : N/A Sponsor : Great Lakes Environmental Center, Inc.
Test System : Fish, TERMINAL Created : 01-Aug-2023 12:33:27 EDT
Continued Animal: BDW0035
Days on Test : 64 (15-Mar-2023 - 17-May-2023)

* MICROSCOPIC FINDINGS

LIVER

- Fibrosis, peribiliary, grade 2
- Infiltrate, mononuclear cell, grade 1
- Parasite, trematode, present only

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER : BDW0037 SEX: Female DOSE GROUP: Baie de Wassai

Defined Sacrifice Group : K0

State at Necropsy : K0

Date of Necropsy : 17-May-2023

Days on Test : 64 (15-Mar-2023 - 17-May-2023)

* MICROSCOPIC FINDINGS

LIVER

- Fibrosis, peribiliary, grade 2
- Fibrovascular proliferation, serosa, grade 1
- Parasite, trematode, present only

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER : BDW0038 SEX: Female DOSE GROUP: Baie de Wassai

Defined Sacrifice Group : K0

State at Necropsy : K0

Date of Necropsy : 17-May-2023

Days on Test : 64 (15-Mar-2023 - 17-May-2023)

* MICROSCOPIC FINDINGS

LIVER

- Fibrosis, peribiliary, grade 2
- Inflammation, granulomatous, grade 1

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Test Item : N/A Sponsor : Great Lakes Environmental Center, Inc.
Test System : Fish, TERMINAL Created : 01-Aug-2023 12:33:27 EDT

Continued Animal: BDW0038

* MICROSCOPIC FINDINGS

LIVER

- Inflammation, granulomatous, grade 1
- Parasite, trematode, present only

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER : BDW0074 SEX: Female DOSE GROUP: Baie de Wassai

Defined Sacrifice Group : K0
State at Necropsy : K0
Date of Necropsy : 24-May-2023
Days on Test : 71 (15-Mar-2023 - 24-May-2023)

* MICROSCOPIC FINDINGS

LIVER

- Fibrosis, peribiliary, grade 1
- Inflammation, granulomatous, grade 1
- Parasite, myxozoan, present only
- Parasite, nematode, present only
- Parasite, trematode, present only
- Pigmented macrophage aggregates, grade 1
- Vacuolation, hepatocyte, grade 1

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER : BDW0078 SEX: Female DOSE GROUP: Baie de Wassai

Defined Sacrifice Group : K0
State at Necropsy : K0
Date of Necropsy : 24-May-2023
Days on Test : 71 (15-Mar-2023 - 24-May-2023)

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Test Item : N/A Sponsor : Great Lakes Environmental Center, Inc.
Test System : Fish, TERMINAL Created : 01-Aug-2023 12:33:27 EDT

Continued Animal: BDW0078

* MICROSCOPIC FINDINGS

LIVER

- Fibrosis, peribiliary, grade 1
- Inflammation, granulomatous, grade 1
- Parasite, myxozoan, present only
- Parasite, nematode, present only
- Pigmented macrophage aggregates, grade 1

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER : BDW0081 SEX: Female DOSE GROUP: Baie de Wassai

Defined Sacrifice Group : K0
State at Necropsy : K0
Date of Necropsy : 24-May-2023
Days on Test : 71 (15-Mar-2023 - 24-May-2023)

* MICROSCOPIC FINDINGS

LIVER

- Fibrosis, peribiliary, grade 2
- Hypertrophy hepatocellular, grade 1
- Inflammation, granulomatous, grade 2
- Parasite, myxozoan, present only
- Pigmented macrophage aggregates, grade 1
- Vacuolation, hepatocyte, grade 1

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER : BDW0082 SEX: Female DOSE GROUP: Baie de Wassai

Defined Sacrifice Group : K0
State at Necropsy : K0
Date of Necropsy : 24-May-2023
Days on Test : 71 (15-Mar-2023 - 24-May-2023)

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Test Item : N/A Sponsor : Great Lakes Environmental Center, Inc.
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Continued Animal: BDW0082

* MICROSCOPIC FINDINGS

LIVER

- Fibrosis, peribiliary, grade 3
- Inflammation, granulomatous, grade 2
- Parasite, myxozoan, present only
- Parasite, nematode, present only
- Parasite, trematode, present only
- Pigmented macrophage aggregates, grade 1

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER : BDW0086 SEX: Female DOSE GROUP: Baie de Wassai

Defined Sacrifice Group : K0
State at Necropsy : K0
Date of Necropsy : 24-May-2023
Days on Test : 71 (15-Mar-2023 - 24-May-2023)

* MICROSCOPIC FINDINGS

LIVER

- Fibrosis, peribiliary, grade 2
- Fibrovascular proliferation, serosa, grade 1
- Inflammation, granulomatous, grade 1
- Parasite, myxozoan, present only
- Parasite, nematode, present only
- Parasite, trematode, present only
- Pigmented macrophage aggregates, grade 1

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER : BDW0088 SEX: Female DOSE GROUP: Baie de Wassai

Defined Sacrifice Group : K0
State at Necropsy : K0

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ANIMAL NUMBER	: BDW0088 SEX: Female	Continued Animal: BDW0088 DOSE GROUP: Baie de Wassai	
Defined Sacrifice Group	: K0		
State at Necropsy	: K0		
Date of Necropsy	: 24-May-2023		
Days on Test	: 71 (15-Mar-2023 - 24-May-2023)		

* MICROSCOPIC FINDINGS

LIVER

- Altered focus, clear cell, grade 1
Comment: Slide 1, step 1
- Fibrosis, peribiliary, grade 2
- Infiltrate, mononuclear cell, grade 1
- Inflammation, granulomatous, grade 1
- Parasite, myxozoan, present only
- Parasite, trematode, present only

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER	: BDW0089 SEX: Female	DOSE GROUP: Baie de Wassai
Defined Sacrifice Group	: K0	
State at Necropsy	: K0	
Date of Necropsy	: 24-May-2023	
Days on Test	: 71 (15-Mar-2023 - 24-May-2023)	

* MICROSCOPIC FINDINGS

LIVER

- Cholangiofibrosis, grade 2
- Fibrosis, peribiliary, grade 3
- Inflammation, granulomatous, grade 2
- Parasite, nematode, present only
- Parasite, trematode, present only

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Test Item : N/A Sponsor : Great Lakes Environmental Center, Inc.
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Continued Animal: BDW0089

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER : BDW0094 SEX: Female DOSE GROUP: Baie de Wassai

Defined Sacrifice Group : K0

State at Necropsy : K0

Date of Necropsy : 24-May-2023

Days on Test : 71 (15-Mar-2023 - 24-May-2023)

* MICROSCOPIC FINDINGS

LIVER

- Fibrosis, peribiliary, grade 2
- Infiltrate, mononuclear cell, grade 1
- Inflammation, granulomatous, grade 1
- Parasite, nematode, present only
- Parasite, trematode, present only

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER : BDW0097 SEX: Female DOSE GROUP: Baie de Wassai

Defined Sacrifice Group : K0

State at Necropsy : K0

Date of Necropsy : 24-May-2023

Days on Test : 71 (15-Mar-2023 - 24-May-2023)

* MICROSCOPIC FINDINGS

LIVER

- Inflammation, granulomatous, grade 1
- Parasite, nematode, present only
- Parasite, trematode, present only

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

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Test Item	: N/A	Sponsor	: Great Lakes Environmental Center, Inc.
Test System	: Fish, TERMINAL	Created	: 01-Aug-2023 12:33:27 EDT
ANIMAL NUMBER	: BDW0100 SEX: Female	DOSE GROUP:	Baie de Wassai
Defined Sacrifice Group	: K0		
State at Necropsy	: K0		
Date of Necropsy	: 24-May-2023		
Days on Test	: 71 (15-Mar-2023 - 24-May-2023)		

* MICROSCOPIC FINDINGS

LIVER

- Fibrosis, peribiliary, grade 2
- Inflammation, granulomatous, grade 2
- Necrosis: single cell, grade 1
- Parasite, myxozoan, present only
- Parasite, nematode, present only
- Parasite, trematode, present only
- Vacuolation, hepatocyte, grade 1

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER	: BDW0102 SEX: Female	DOSE GROUP:	Baie de Wassai
Defined Sacrifice Group	: K0		
State at Necropsy	: K0		
Date of Necropsy	: 24-May-2023		
Days on Test	: 71 (15-Mar-2023 - 24-May-2023)		

* MICROSCOPIC FINDINGS

LIVER

- Fibrosis, peribiliary, grade 2
- Inflammation, granulomatous, grade 1
- Parasite, nematode, present only
- Parasite, trematode, present only
- Pigmented macrophage aggregates, grade 1

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

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Test Item	: N/A	Sponsor	: Great Lakes Environmental Center, Inc.
Test System	: Fish, TERMINAL	Created	: 01-Aug-2023 12:33:27 EDT
ANIMAL NUMBER	: BDW0104 SEX: Female	DOSE GROUP:	Baie de Wassai
Defined Sacrifice Group	: K0		
State at Necropsy	: K0		
Date of Necropsy	: 24-May-2023		
Days on Test	: 71 (15-Mar-2023 - 24-May-2023)		

* MICROSCOPIC FINDINGS

LIVER

- Fibrosis, peribiliary, grade 3
- Inflammation, granulomatous, grade 2
- Parasite, myxozoan, present only
- Parasite, nematode, present only
- Parasite, trematode, present only
- Pigmented macrophage aggregates, grade 1
- Vacuolation, hepatocyte, grade 1

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER	: BDW0105 SEX: Female	DOSE GROUP:	Baie de Wassai
Defined Sacrifice Group	: K0		
State at Necropsy	: K0		
Date of Necropsy	: 24-May-2023		
Days on Test	: 71 (15-Mar-2023 - 24-May-2023)		

* MICROSCOPIC FINDINGS

LIVER

- Fibrosis, peribiliary, grade 1
- Necrosis: single cell, grade 1
- Parasite, trematode, present only
- Vacuolation, hepatocyte, grade 1

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

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Test Item	: N/A	Sponsor	: Great Lakes Environmental Center, Inc.
Test System	: Fish, TERMINAL	Created	: 01-Aug-2023 12:33:27 EDT
ANIMAL NUMBER	: BDW0109 SEX: Female	DOSE GROUP:	Baie de Wassai
Defined Sacrifice Group	: K0		
State at Necropsy	: K0		
Date of Necropsy	: 24-May-2023		
Days on Test	: 71 (15-Mar-2023 - 24-May-2023)		

* MICROSCOPIC FINDINGS

LIVER

- Fibrosis, peribiliary, grade 3
- Inflammation, granulomatous, grade 1
- Parasite, myxozoan, present only
- Parasite, trematode, present only
- Pigmented macrophage aggregates, grade 1

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER	: BDW0110 SEX: Female	DOSE GROUP:	Baie de Wassai
Defined Sacrifice Group	: K0		
State at Necropsy	: K0		
Date of Necropsy	: 24-May-2023		
Days on Test	: 71 (15-Mar-2023 - 24-May-2023)		

* MICROSCOPIC FINDINGS

LIVER

- Fibrosis, peribiliary, grade 2
- Necrosis: single cell, grade 1
- Parasite, NOS, present only
- Parasite, myxozoan, present only
- Parasite, trematode, present only

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER	: BDW0111 SEX: Female	DOSE GROUP:	Baie de Wassai
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Test Item : N/A Sponsor : Great Lakes Environmental Center, Inc.
Test System : Fish, TERMINAL Created : 01-Aug-2023 12:33:27 EDT
ANIMAL NUMBER : BDW0111 SEX: Female Continued Animal: BDW0111
DOSE GROUP: Baie de Wassai
Defined Sacrifice Group : K0
State at Necropsy : K0
Date of Necropsy : 24-May-2023
Days on Test : 71 (15-Mar-2023 - 24-May-2023)

* MICROSCOPIC FINDINGS

LIVER

- Cystic degeneration, grade 1
- Fibrosis, peribiliary, grade 1
- Infiltrate, mononuclear cell, grade 1
- Inflammation, granulomatous, grade 1
- Parasite, myxozoan, present only
- Parasite, nematode, present only
- Parasite, trematode, present only

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER : BDW0112 SEX: Female DOSE GROUP: Baie de Wassai

Defined Sacrifice Group : K0
State at Necropsy : K0
Date of Necropsy : 24-May-2023
Days on Test : 71 (15-Mar-2023 - 24-May-2023)

* MICROSCOPIC FINDINGS

LIVER

- Inflammation, granulomatous, grade 1
- Parasite, trematode, present only
- Pigmented macrophage aggregates, grade 1
- Vacuolation, hepatocyte, grade 1

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

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Test Item : N/A Sponsor : Great Lakes Environmental Center, Inc.
Test System : Fish, TERMINAL Created : 01-Aug-2023 12:33:27 EDT
ANIMAL NUMBER : MB0005 SEX: Female DOSE GROUP: Munuscong Bay
Defined Sacrifice Group : K0
State at Necropsy : K0
Date of Necropsy : 16-May-2023
Days on Test : 63 (15-Mar-2023 - 16-May-2023)

* MICROSCOPIC FINDINGS

LIVER

- Altered focus, clear cell, grade 1
 Comment: Slide 1B, step 1
- Fibrosis, peribiliary, grade 2
- Inflammation, granulomatous, grade 2
- Parasite, myxozoan, present only
- Parasite, nematode, present only
- Pigmented macrophage aggregates, grade 2
- Vacuolation, hepatocyte, grade 1

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER : MB0006 SEX: Female DOSE GROUP: Munuscong Bay
Defined Sacrifice Group : K0
State at Necropsy : K0
Date of Necropsy : 16-May-2023
Days on Test : 63 (15-Mar-2023 - 16-May-2023)

* MICROSCOPIC FINDINGS

LIVER

- Altered focus, eosinophilic, grade 2
 Comment: Slide 1, step 1
- Fibrosis, peribiliary, grade 2
- Inflammation, granulomatous, grade 1
- Parasite, myxozoan, present only
- Pigmented macrophage aggregates, grade 2

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Test Item : N/A Sponsor : Great Lakes Environmental Center, Inc.
Test System : Fish, TERMINAL Created : 01-Aug-2023 12:33:27 EDT
Continued Animal: MB0006

* MICROSCOPIC FINDINGS

LIVER

- Pigmented macrophage aggregates, grade 2
- Vacuolation, hepatocyte, grade 1

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER : MB0007 SEX: Female DOSE GROUP: Munuscong Bay

Defined Sacrifice Group : K0
State at Necropsy : K0
Date of Necropsy : 16-May-2023
Days on Test : 63 (15-Mar-2023 - 16-May-2023)

* MICROSCOPIC FINDINGS

LIVER

- Fibrosis, peribiliary, grade 2
- Inflammation, granulomatous, grade 2
- Parasite, myxozoan, present only
- Parasite, nematode, present only
- Parasite, trematode, present only
- Pigmented macrophage aggregates, grade 2

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER : MB0008 SEX: Female DOSE GROUP: Munuscong Bay

Defined Sacrifice Group : K0
State at Necropsy : K0
Date of Necropsy : 16-May-2023
Days on Test : 63 (15-Mar-2023 - 16-May-2023)

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Test Item : N/A Sponsor : Great Lakes Environmental Center, Inc.
Test System : Fish, TERMINAL Created : 01-Aug-2023 12:33:27 EDT
Continued Animal: MB0008

* MICROSCOPIC FINDINGS

LIVER

- Fibrosis, peribiliary, grade 1
- Inflammation, granulomatous, grade 2
- Parasite, NOS, present only
- Parasite, cestode, present only
- Parasite, myxozoan, present only
- Parasite, nematode, present only
- Pigmented macrophage aggregates, grade 2

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER : MB0010 SEX: Female DOSE GROUP: Munuscong Bay

Defined Sacrifice Group : K0
State at Necropsy : K0
Date of Necropsy : 16-May-2023
Days on Test : 63 (15-Mar-2023 - 16-May-2023)

* MICROSCOPIC FINDINGS

LIVER

- Altered focus, eosinophilic, grade 3
Comment: Slide 1A, step 2
- Fibrosis, peribiliary, grade 2
- Parasite, cestode, present only
- Pigmented macrophage aggregates, grade 1
- Vacuolation, hepatocyte, grade 1

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER : MB0040 SEX: Female DOSE GROUP: Munuscong Bay

Defined Sacrifice Group : K0
State at Necropsy : K0

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Test Item : N/A Sponsor : Great Lakes Environmental Center, Inc.
Test System : Fish, TERMINAL Created : 01-Aug-2023 12:33:27 EDT
ANIMAL NUMBER : MB0040 SEX: Female Continued Animal: MB0040
DOSE GROUP: Munuscong Bay
Defined Sacrifice Group : K0
State at Necropsy : K0
Date of Necropsy : 18-May-2023
Days on Test : 65 (15-Mar-2023 - 18-May-2023)

* MICROSCOPIC FINDINGS

LIVER

- Altered focus, clear cell, grade 1
Comment: Slide 1A, step 1
- Fibrosis, peribiliary, grade 2
- Inflammation, granulomatous, grade 1
- Parasite, myxozoan, present only
- Parasite, trematode, present only
- Pigmented macrophage aggregates, grade 2
- Vacuolation, hepatocyte, grade 1

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER : MB0045 SEX: Female DOSE GROUP: Munuscong Bay
Defined Sacrifice Group : K0
State at Necropsy : K0
Date of Necropsy : 18-May-2023
Days on Test : 65 (15-Mar-2023 - 18-May-2023)

* MICROSCOPIC FINDINGS

LIVER

- Cystic degeneration, grade 1
- Fibrosis, peribiliary, grade 2
- Inflammation, granulomatous, grade 1
- Parasite, myxozoan, present only
- Pigmented macrophage aggregates, grade 2

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Test Item : N/A Sponsor : Great Lakes Environmental Center, Inc.
Test System : Fish, TERMINAL Created : 01-Aug-2023 12:33:27 EDT
Continued Animal: MB0045

* MICROSCOPIC FINDINGS

LIVER

- Vacuolation, hepatocyte, grade 1

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER : MB0048 SEX: Female DOSE GROUP: Munuscong Bay

Defined Sacrifice Group : K0

State at Necropsy : K0

Date of Necropsy : 18-May-2023

Days on Test : 65 (15-Mar-2023 - 18-May-2023)

* MICROSCOPIC FINDINGS

LIVER

- Cystic degeneration, grade 1

- Fibrosis, peribiliary, grade 2

- Inflammation, granulomatous, grade 2

- Parasite, NOS, present only

- Parasite, myxozoan, present only

- Parasite, nematode, present only

- Parasite, trematode, present only

- Pigmented macrophage aggregates, grade 1

- Vacuolation, hepatocyte, grade 1

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER : MB0049 SEX: Female DOSE GROUP: Munuscong Bay

Defined Sacrifice Group : K0

State at Necropsy : K0

Date of Necropsy : 18-May-2023

Days on Test : 65 (15-Mar-2023 - 18-May-2023)

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Test Item : N/A Sponsor : Great Lakes Environmental Center, Inc.
Test System : Fish, TERMINAL Created : 01-Aug-2023 12:33:27 EDT
Continued Animal: MB0049

* MICROSCOPIC FINDINGS

LIVER

- Fibrosis, peribiliary, grade 2
- Infiltrate, mononuclear cell, grade 1
- Inflammation, granulomatous, grade 1
- Parasite, myxozoan, present only
- Parasite, trematode, present only
- Pigmented macrophage aggregates, grade 2
- Vacuolation, hepatocyte, grade 2

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER : MB0052 SEX: Female DOSE GROUP: Munuscong Bay

Defined Sacrifice Group : K0
State at Necropsy : K0
Date of Necropsy : 18-May-2023
Days on Test : 65 (15-Mar-2023 - 18-May-2023)

* MICROSCOPIC FINDINGS

LIVER

- Bile duct epithelium, atypical hyperplasia, grade 2
- Fibrosis, peribiliary, grade 3
- Infiltrate, mononuclear cell, grade 1
- Parasite, myxozoan, present only
- Parasite, nematode, present only
- Parasite, trematode, present only
- Pigmented macrophage aggregates, grade 1
- Vacuolation, hepatocyte, grade 2

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER : MB0055 SEX: Female DOSE GROUP: Munuscong Bay

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Test Item : N/A Sponsor : Great Lakes Environmental Center, Inc.
Test System : Fish, TERMINAL Created : 01-Aug-2023 12:33:27 EDT
ANIMAL NUMBER : MB0055 SEX: Female Continued Animal: MB0055
DOSE GROUP: Munuscong Bay
Defined Sacrifice Group : K0
State at Necropsy : K0
Date of Necropsy : 18-May-2023
Days on Test : 65 (15-Mar-2023 - 18-May-2023)

* MICROSCOPIC FINDINGS

LIVER

- Altered focus, basophilic, grade 1
Comment: Slide 1A, step 2
- Fibrosis, peribiliary, grade 3
- Inflammation, granulomatous, grade 1
- Parasite, NOS, present only
- Parasite, myxozoan, present only
- Parasite, trematode, present only
- Pigmented macrophage aggregates, grade 1
- Vacuolation, hepatocyte, grade 2

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER : MB0057 SEX: Female DOSE GROUP: Munuscong Bay
Defined Sacrifice Group : K0
State at Necropsy : K0
Date of Necropsy : 18-May-2023
Days on Test : 65 (15-Mar-2023 - 18-May-2023)

* MICROSCOPIC FINDINGS

LIVER

- Fibrosis, peribiliary, grade 1
- Fibrovascular proliferation, serosa, grade 1
- Necrosis: single cell, grade 1
- Parasite, myxozoan, present only

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Test System : Fish, TERMINAL Created : 01-Aug-2023 12:33:27 EDT
Continued Animal: MB0057

* MICROSCOPIC FINDINGS

LIVER

- Parasite, trematode, present only
- Pigmented macrophage aggregates, grade 1
- Vacuolation, hepatocyte, grade 1

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER : MB0059 SEX: Female DOSE GROUP: Munuscong Bay

Defined Sacrifice Group : K0

State at Necropsy : K0

Date of Necropsy : 23-May-2023

Days on Test : 70 (15-Mar-2023 - 23-May-2023)

* MICROSCOPIC FINDINGS

LIVER

- Fibrosis, peribiliary, grade 1
- Inflammation, granulomatous, grade 2
- Parasite, nematode, present only
- Vacuolation, hepatocyte, grade 2

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER : MB0061 SEX: Female DOSE GROUP: Munuscong Bay

Defined Sacrifice Group : K0

State at Necropsy : K0

Date of Necropsy : 23-May-2023

Days on Test : 70 (15-Mar-2023 - 23-May-2023)

* MICROSCOPIC FINDINGS

LIVER

- Fibrosis, peribiliary, grade 2
- Inflammation, granulomatous, grade 1

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Test Item : N/A	Sponsor : Great Lakes Environmental Center, Inc.
Test System : Fish, TERMINAL	Created : 01-Aug-2023 12:33:27 EDT
Continued Animal: MB0061	

* MICROSCOPIC FINDINGS

LIVER

- Inflammation, granulomatous, grade 1
- Parasite, trematode, present only
- Vacuolation, hepatocyte, grade 1

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER : MB0062	SEX: Female	DOSE GROUP: Munuscong Bay
------------------------	-------------	---------------------------

Defined Sacrifice Group : K0

State at Necropsy : K0

Date of Necropsy : 23-May-2023

Days on Test : 70 (15-Mar-2023 - 23-May-2023)

* MICROSCOPIC FINDINGS

LIVER

- Cystic degeneration, grade 2
- Fibrosis, peribiliary, grade 2
- Inflammation, granulomatous, grade 1
- Parasite, myxozoan, present only
- Parasite, trematode, present only
- Pigmented macrophage aggregates, grade 2
- Vacuolation, hepatocyte, grade 1

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER : MB0063	SEX: Female	DOSE GROUP: Munuscong Bay
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Defined Sacrifice Group : K0

State at Necropsy : K0

Date of Necropsy : 23-May-2023

Days on Test : 70 (15-Mar-2023 - 23-May-2023)

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Test Item : N/A Sponsor : Great Lakes Environmental Center, Inc.
Test System : Fish, TERMINAL Created : 01-Aug-2023 12:33:27 EDT
Continued Animal: MB0063

* MICROSCOPIC FINDINGS

LIVER

- Altered focus, clear cell, grade 1
Comment: Slide 1B, steps 1 and 2
- Fibrosis, peribiliary, grade 1
- Fibrovascular proliferation, serosa, grade 2
- Inflammation, granulomatous, grade 2
- Parasite, cestode, present only
- Parasite, myxozoan, present only
- Parasite, nematode, present only
- Pigmented macrophage aggregates, grade 1

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER : MB0065 SEX: Female DOSE GROUP: Munuscong Bay

Defined Sacrifice Group : K0
State at Necropsy : K0
Date of Necropsy : 23-May-2023
Days on Test : 70 (15-Mar-2023 - 23-May-2023)

* MICROSCOPIC FINDINGS

LIVER

- Altered focus, clear cell, grade 2
Comment: Slide 1, step 2
- Fibrosis, peribiliary, grade 2
- Inflammation, granulomatous, grade 1
- Parasite, NOS, present only
- Parasite, myxozoan, present only
- Parasite, trematode, present only
- Pigmented macrophage aggregates, grade 1
- Vacuolation, hepatocyte, grade 2

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Test Item : N/A Sponsor : Great Lakes Environmental Center, Inc.
Test System : Fish, TERMINAL Created : 01-Aug-2023 12:33:27 EDT
Continued Animal: MB0065

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER : MB0066 SEX: Female DOSE GROUP: Munuscong Bay

Defined Sacrifice Group : K0
State at Necropsy : K0
Date of Necropsy : 23-May-2023
Days on Test : 70 (15-Mar-2023 - 23-May-2023)

* MICROSCOPIC FINDINGS

LIVER

- Fibrosis, peribiliary, grade 1
- Inflammation, granulomatous, grade 1
- Necrosis: single cell, grade 2
- Parasite, myxozoan, present only
- Parasite, nematode, present only

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER : MB0069 SEX: Female DOSE GROUP: Munuscong Bay

Defined Sacrifice Group : K0
State at Necropsy : K0
Date of Necropsy : 23-May-2023
Days on Test : 70 (15-Mar-2023 - 23-May-2023)

* MICROSCOPIC FINDINGS

LIVER

- Cholangiofibrosis, grade 1
- Fibrosis, peribiliary, grade 3
- Inflammation, granulomatous, grade 2
- Parasite, NOS, present only
- Parasite, cestode, present only
- Parasite, myxozoan, present only

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Continued Animal: MB0069

* MICROSCOPIC FINDINGS

LIVER

- Parasite, myxozoan, present only
- Parasite, nematode, present only
- Pigmented macrophage aggregates, grade 2
- Vacuolation, hepatocyte, grade 2

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER : MB0072 SEX: Female DOSE GROUP: Munuscong Bay

Defined Sacrifice Group : K0
State at Necropsy : K0
Date of Necropsy : 23-May-2023
Days on Test : 70 (15-Mar-2023 - 23-May-2023)

* MICROSCOPIC FINDINGS

LIVER

- Cholangiofibrosis, grade 1
- Fibrosis, peribiliary, grade 3
- Inflammation, granulomatous, grade 2
- Parasite, nematode, present only
- Pigmented macrophage aggregates, grade 2
- Vacuolation, hepatocyte, grade 2

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER : MB0073 SEX: Female DOSE GROUP: Munuscong Bay

Defined Sacrifice Group : K0
State at Necropsy : K0
Date of Necropsy : 23-May-2023
Days on Test : 70 (15-Mar-2023 - 23-May-2023)

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Test Item : N/A Sponsor : Great Lakes Environmental
Center, Inc.

Test System : Fish, TERMINAL Created : 01-Aug-2023 12:33:27 EDT

Continued Animal: MB0073

* MICROSCOPIC FINDINGS

LIVER

- Fibrosis, peribiliary, grade 2
- Parasite, myxozoan, present only
- Vacuolation, hepatocyte, grade 3

* ORGANS WITHOUT ABNORMALITIES

No organs without abnormalities noted

-

DOSE GROUP: Waiska Bay

SEX: Female

: WB0001

ANIMAL NUMBER

: K0

Defined Sacrifice Group

K0

:

State at Necropsy

15-May-2023

:

Date of Necropsy

Days on Test 62 (15-Mar-2023 - 15-May-2023)

:

* MICROSCOPIC FINDINGS

LIVER

- Altered focus, eosinophilic, grade 4

Comment: Multiple foci throughout the sections

- Fibrosis, peribiliary, grade 1

- Parasite, myxozoan, present only

- Parasite, trematode, present only

- Vacuolation, hepatocyte, grade 2

* ORGANS WITHOUT ABNORMALITIES

No organs without abnormalities noted

-

DOSE GROUP: Waiska Bay

SEX: Female

: WB0002

ANIMAL NUMBER

: K0

Defined Sacrifice Group

K0

:

State at Necropsy

15-May-2023

:

Date of Necropsy

Days on Test 62 (15-Mar-2023 - 15-May-2023)

:

* MICROSCOPIC FINDINGS

LIVER

- Fibrosis, peribiliary, grade 1

- Parasite, myxozoan, present only

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Test Item : N/A Sponsor : Great Lakes Environmental Center, Inc.
Test System : Fish, TERMINAL Created : 01-Aug-2023 12:33:27 EDT
Continued Animal: MB0073

* MICROSCOPIC FINDINGS

LIVER

- Fibrosis, peribiliary, grade 2
- Parasite, myxozoan, present only
- Vacuolation, hepatocyte, grade 3

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER : WB0001 SEX: Female DOSE GROUP: Waiska Bay
Defined Sacrifice Group : K0
State at Necropsy : K0
Date of Necropsy : 15-May-2023
Days on Test : 62 (15-Mar-2023 - 15-May-2023)

* MICROSCOPIC FINDINGS

LIVER

- Altered focus, eosinophilic, grade 4
Comment: Multiple foci throughout the sections
- Fibrosis, peribiliary, grade 1
- Parasite, myxozoan, present only
- Parasite, trematode, present only
- Vacuolation, hepatocyte, grade 2

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER : WB0002 SEX: Female DOSE GROUP: Waiska Bay
Defined Sacrifice Group : K0
State at Necropsy : K0
Date of Necropsy : 15-May-2023
Days on Test : 62 (15-Mar-2023 - 15-May-2023)

* MICROSCOPIC FINDINGS

LIVER

- Fibrosis, peribiliary, grade 1
- Parasite, myxozoan, present only

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Test Item : N/A Sponsor : Great Lakes Environmental Center, Inc.
Test System : Fish, TERMINAL Created : 01-Aug-2023 12:33:27 EDT
Continued Animal: WB0002

* MICROSCOPIC FINDINGS

LIVER

- Parasite, myxozoan, present only
- Vacuolation, hepatocyte, grade 2

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted

ANIMAL NUMBER : WB0003 SEX: Female DOSE GROUP: Waiska Bay
Defined Sacrifice Group : K0
State at Necropsy : K0
Date of Necropsy : 15-May-2023
Days on Test : 62 (15-Mar-2023 - 15-May-2023)

* MICROSCOPIC FINDINGS

LIVER

- Vacuolation, hepatocyte, grade 2

* ORGANS WITHOUT ABNORMALITIES

- No organs without abnormalities noted
-

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EXPLANATION OF CODES AND SYMBOLS

CODES AND SYMBOLS USED AT TABLE LEVEL:

ASR = Animal Sheet Reports (to include necropsy findings and microscopic findings)

CODES AND SYMBOLS USED AT ANIMAL LEVEL:

K0 = Terminal Sacrifice Group

K1 K9 = Interim Sacrifice Group 1 9

R1 R9 = Recovery / Post-Treatment Group 1 9

+ = Intercurrent Death / Sacrificed Moribund

SEVERITY GRADING SCALE

The severity of the non-neoplastic tissue lesions are graded as follows:

Grade 1 (1+): Minimal. This corresponds to a histopathologic change ranging from inconspicuous to barely noticeable but so minor, small, or infrequent as to warrant no more than the least assignable grade. For multifocal or diffusely-distributed lesions, this grade was used for processes where less than approximately 10% of the tissue in an average high-power field was involved. For focal or diffuse hyperplastic / hypoplastic / atrophic lesions, this grade was used when the affected structure or tissue had undergone a less than approximately 10% increase or decrease in volume.

Grade 2 (2+): Mild. This corresponds to a histopathologic change that is a noticeable but not a prominent feature of the tissue. For multifocal or diffusely-distributed lesions, this grade was used for processes where between approximately 10% and 25% of the tissue in an average high-power field was involved. For focal or diffuse hyperplastic / hypoplastic / atrophic lesions, this grade was used when the affected structure or tissue had undergone between an approximately 10% to 25% increase or decrease in volume.

Grade 3 (3+): Moderate. This corresponds to a histopathologic change that is a prominent but not a dominant feature of the tissue. For multifocal or diffusely-distributed lesions, this grade was used for processes where between approximately 25% and 50% of the tissue in an average high-power field was involved. For focal or diffuse hyperplastic / hypoplastic / atrophic lesions, this grade was used when the affected structure or tissue had undergone between an approximately 25% to 50% increase or decrease in volume.

Grade 4 (4+): Severe. This corresponds to a histopathologic change that is a dominant but not an overwhelming feature of the tissue. For multifocal or diffusely-distributed lesions, this grade was used for processes where approximately 50% or more of the tissue in an average high-power field was involved. For focal or diffuse hyperplastic / hypoplastic / atrophic lesions, this grade was used when the affected structure or tissue had undergone between an approximately 50% to 95% increase or decrease in volume.

APPENDIX D

Michael Rutter, PhD, 2025

St. Marys River Area of Concern (AOC) Tumor BUI Statistical Analysis Final Report

**St. Marys River Area of Concern (AOC) Tumor BUI Statistical
Analysis Final Report**

Prepared by
Michael A. Rutter, PhD

For:
Michigan Ecological Services Field Office
U.S. Fish and Wildlife Service
East Lansing, MI 48823

July 2025

Introduction

This is the final report of the statistical analysis of the St. Marys River Area of Concern (AOC) Brown Bullhead data collected during 15–24 May 2023 to assess the Fish Tumors or Other Deformities Beneficial Use Impairment (BUI). For the St. Marys River, the BUI will be considered restored when a study of at least 100 resident benthic fish (e.g., Brown bullhead or White suckers), with a minimum age of three years, collected from at least two sites within the AOC, indicates a cancerous liver tumor rate of 5% or less. This report utilizes methods similar to those described in Rutter (2010), although modifications were made due to the data set collected. For the Presque Isle (Erie, PA) AOC, sites were sampled in other Lake Erie locations to establish a reference tumor incidence for direct comparison. Other sites (Buffalo River, for example) used Long Point, Inner Bay (Lake Erie) as a reference site after being determined to be the Presque Isle reference site in Rutter (2010).

For the St. Marys River AOC no Brown Bullhead were sampled at a designated reference site. The St. Marys AOC Binational Public Advisory Council (BPAC) developed local criteria in 2023 to assess fish tumors prevalence and thresholds for BUI removal, which supersedes the statewide criteria in Michigan. Other AOCs in Michigan and ongoing studies do use reference locations. For example, collection of Brown Bullhead in 2024 in the Rouge River and Detroit River AOCs used a reference location in southeast Michigan. Given that the St. Marys AOC is a river along the northern Michigan and Ontario border, using previously collected reference data from Lake Erie is inappropriate. Therefore, an alternative logistic regression (Gelman et al. 2003) approach was utilized. Logistic regression is still appropriate as the response variable is binary (liver tumor present or liver tumor absent) and tumor incidence is believed to increase with age (or another co-variate) (Rutter 2010).

Methods

For Brown Bullhead, liver tumors were analyzed using logistic regression. All statistical analysis were conducted using R (R Core Team, 2024). The presence or absence of any liver tumor was the response variable while age, length, and weight were possible predictor variables. A unique factor of the data collected on the St. Marys River is the fact that only one out of the 113 fish collected was found to have a liver tumor. Typically, logistic regression is used to take into account covariates such as length or age in terms of tumor incidence. For the St. Marys River data set logistic regression can still be utilized, but since there is only one fish with a liver tumor, there is no need to determine which covariate best describes the data (i.e., all covariates-length, age, and weight all fit the data equally well in statistical terms). Since only some of the fish were aged via otolith, three analyses were conducted. The first used all 113 bullheads collected and used length as the covariate. The second analysis used age as the covariate, reducing the sample size to 93 observations. A third approach utilized a no co-variate model, essentially estimating the tumor incidence for any Brown Bullhead in the AOC regardless of age.

For each model, a point estimate of an average Brown Bullhead (in terms of age or length) was determined, as well as a 95% Bayesian prediction interval for the tumor incidence, using the R package “arm” (Gelman and Su, 2022). Since there are no reference data to compare the results to, predicted tumor incidence was compared to a reference rate of 5%, which often has been stated as a delisting target for fish tumors or other deformities BUI in the Great Lakes (e.g., Black River AOC in Ohio). While a modeling exercise using the Long Point, Inner Bay data from Rutter (2010) as a reference site would be inappropriate, it is useful to note that estimated liver tumor incidence at that site was 1.2% for

an average fish, with a 95% Bayesian prediction interval of 0.0% to 14.9%. Results from the St. Marys River analysis can be used to place these results in context.

To emulate the approach utilized in Rutter (2010), a reference data set similar in size (113 Brown Bullhead) can be simulated that contains all fish without tumors. To establish a baseline rate of equivalence, the St. Marys River data was compared to itself to determine uncertainty in the tumor instance via a 90% highest posterior density interval (HPDI). To test for equivalence between the St. Marys River AOC and the simulated reference site, a two one-sided test (TOST) was conducted using the 90% HPDI for the St. Marys River without age or length as a covariate, with an additional 5% tolerance added (see Rutter 2010 for details). The TOST procedure can be implemented by conducting two one-sided hypothesis tests (with a significance level α). The first test determines if the difference in tumor incidences is less than or equal to θ and the second determines if that the difference in tumor incidence is greater than or equal to $-\theta$, where θ is a predetermined tolerance level. A confidence interval-based approach to the TOST procedure is to construct a $(1 - 2\alpha)$ % confidence interval for the difference in the treatments (Berger and Hsu, 1996). If the 90% HPDI comparing the St. Marys River site to the simulated reference site is within the specified TOST reference interval, the sites would be considered to have statistically equivalent tumor incidence rates.

Results

The demographics of the St. Marys River data appear to be different from other AOCs sampled previously. The largest age Brown Bullhead observed in the St. Marys River was 6 years old, with a mean of 3.8 years. For context, the mean Long Point, Inner Bay Brown Bullhead (Lake Erie) was 7.0 years. The mean length of the sample was 287 mm. For a 287 mm long Brown Bullhead, the estimated tumor incidence using logistic regression was 0.8% with a 95% Bayesian prediction interval of 0.1% to 5.8%. For the logistic regression analysis using age, the tumor incidence for an age 4 (3.8 years rounded to nearest whole year) bullhead was 0.7% with a 95% Bayesian prediction interval of 0.1% to 7.5%. For the length-based analysis, there is a 96.2% probability the tumor incidence is lower than 5% for an “average” fish. With age as the covariate, there is a 94.8% probability the tumor incidence is lower than 5% for an “average” fish. For both analyses, the point estimate is well below 5%.

It is typically believed that tumor incidence in Brown Bullhead increases as a function of age, with older (thus larger) fish having higher tumor incidence. For the St. Marys River data, the fish with the observed tumor was slightly younger than the average in the St. Marys River sample (3 years vs. 3.8 years) and less than average length (260 mm vs. an average of 287 mm). When logistic regression is applied to a data set where the only tumor is less than the average age or length, tumor incidence is modeled to decrease as the fish gets older/longer. An alternative logistic regression approach would be to restrict the values of the coefficient corresponding to length or age to be greater than zero. This would only allow a model that says that tumor rates must increase as a function of age or length. When this approach to logistic regression is used, the estimated tumor incidence on average length bullhead is 1.3% with a 95% prediction interval of 0.2% to 8.8%. For the age analysis, the estimated tumor incidence on average bullhead (age 4) is 2.2% with a 95% prediction interval of 0.3% to 14.7%. Under these models, the probability of the tumor incidence for an average bullhead being less than 5% is 91% when using length, 80.3% when using length.

If it is assumed that tumor incidence is not a function of length or age, the data can be analyzed by a standard Bayesian approach to binomial data. Using this methodology, the point estimate of tumor

incidence is 1.3% with a 95% prediction interval of 0.0% to 3.4%. There is a 99.1% probability that the tumor incidence is less than 5%.

Table 1: Comparison of logistic regression models used to estimate tumor incidence. The model column indicates the covariate used, with an indicator if the model was forced to have the tumor incidence increase with the covariate.

Model	Point Estimate	95% Prediction Interval	Probability of < 5%
Length	0.8%	(0.1%, 5.8%)	96.2%
Age	0.7%	(0.1%, 7.5%)	94.8%
Length (Increasing)	1.3%	(0.2%, 8.8%)	91.0%
Age (Increasing)	2.2%	(0.3%, 14.7%)	80.3%
No covariate	1.3%	(0.0%, 3.4%)	99.1%

To apply the TOST approach used in Rutter (2010), a 90% HPDI for the St. Marys River without age or length as a covariate was compared to itself. To create the TOST tolerance interval, 5% was added to each end of the 90% HPDI, creating a tolerance interval of (-7.5%, 7.5%). The 90% TOST interval for comparing the St. Marys River site to the simulated baseline site is (-0.8%, 3.1%), well within the tolerance level. It can be concluded that the St. Marys River data is statistically equivalent to a simulated reference site with no tumors.

Conclusion

All statistical analyses conducted in this final report indicate the tumor incidence in the sampled area of the St. Marys River AOC is very low. When it is assumed that tumor incidence is not a function of age or length, there is a 99.1% probability that the tumor incidence is less than 5%. If tumor incidence is assumed to increase as a function of age or length, the probability of the tumor incidence rate being less than 5% is 91.0% for and average length bullhead and 80.3% for an average age bullhead. When the effect of the age or length covariate is not forced to increase, the probability of tumor incidence being less than 5% increases to 96.2% for length, 94.8% for age. However, the model with no covariate best fits the observed the data. When the St. Marys River data is compared to a simulated data set with no tumors, the 90% TOST interval is (-0.8%, 3.1%), indicating the observed data and simulated no tumor data are statistically equivalent.

References

- Berger, R.L., Hsu, J.C., 1996. Bioequivalence trials, intersection-union tests, and equivalence confidence sets. *Statistical Science* 11:283–319. DOI: 10.1214/ss/1032280304
- Gelman, Andrew, John B. Carlin, Hal S. Stern, and Donald B. Rubin. 2003. *Bayesian Data Analysis, Second Edition (Chapman & Hall/CRC Texts in Statistical Science)*. 2nd ed. Hardcover; Chapman; Hall/CRC.
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APPENDIX E

2026 Letter of Support from St. Marys River Binational Public Advisory Council for the Removal of the Fish Tumors or Other Deformities Beneficial Use Impairment from the US Side of the Area of Concern

St. Marys River
Fish Tumors or Other Deformities BUI
Removal Recommendation
September 2026

ST. MARYS RIVER

BINATIONAL PUBLIC ADVISORY COUNCIL



VIA E-Mail

July 20, 2026

John Riley, EGLE
Water Resources Division
525 W. Allegan Street
Lansing, MI 48909
Rileyj2@michigan.gov

Julie Carter, USEPA
Great Lakes National Program Office
77 West Jackson Blvd., G-9J
Chicago, IL 60604-3590
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RE: Fish Tumors BUI for St. Marys River Area of Concern, Sault Ste. Marie, Michigan

Dear John Riley and Julie Carter:

On behalf of the Binational Public Advisory Council (BPAC) for the St. Marys River Area of Concern (AOC), we are writing to convey agreement regarding the removal of the Fish Tumors Beneficial Use Impairment (BUI) from the Michigan side of the AOC.

The restoration criteria states that the BUI will be considered restored when:
"A study of at least 100 resident benthic fish (e.g. brown bullhead OR white suckers), with a minimum age of 3 years, collected from at least two sites within the AOC, indicates a cancerous liver tumor rate of 5% or less."

In June 2026, the Michigan Department of Environment, Great Lakes and Energy presented BPAC with a document titled Removal Recommendation Fish Tumors or Other Deformities Beneficial Use Impairment St. Marys River Area of Concern that explains the rationale for BUI removal supported by a survey of brown bullhead conducted by the Great Lakes Environmental Center (GLEC). Results of the survey indicated no observable tumors and statistical analyses confirmed that the survey meets the restoration criteria for a cancerous liver tumor rate of 5% or less.

St. Marys River
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Following the presentation by EGLE at the June 24, 2026 BPAC members discussed the results for the tumor survey and agreed that the results support the redesignation to “not impaired” status and that this BUI should be removed.

Sincerely,

Mike Ripley
Canadian BPAC Chair
mripleysault@gmail.com

Aubrey Macoux-LeDuc
U.S. Vice-Chair
AMaccoux-LeDuc@baymills.org

Cc: BPAC
RAP Coordinator lisa.derickx@algomau.ca