U.S. Fish & Wildlife Service

# Evaluation of Tumor Prevalence in Mummichogs *(Fundulus heteroclitus)* from the Delaware Estuary Watershed

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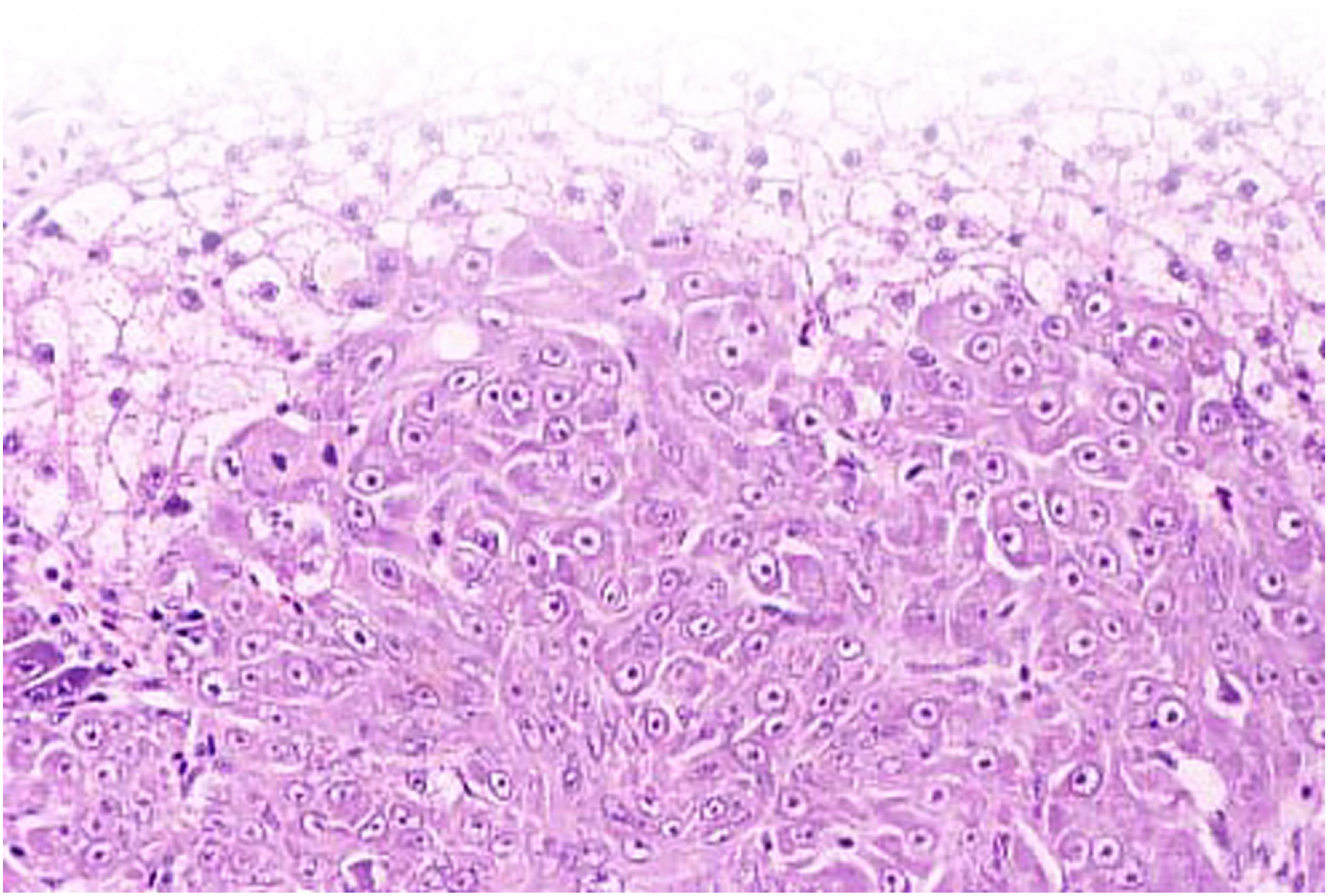

**Evaluation of Tumor Prevalence in Mummichogs *(Fundulus heteroclitus)* from the Delaware Estuary Watershed**
**Final Report**

**CBFO-C04-04**

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**ABSTRACT**

Environmental scientists and managers often use tumor surveys with freshwater, estuarine, and marine fish as indicators of habitat quality. In 2002-2004, we collected mummichogs (*Fundulus heteroclitus*, $\geq$ 70 mm total length, n = 21-30 per location) from four areas within the Delaware Estuary watershed. Necropsies were performed and livers and raised skin lesions were examined histopathologically for tumors and preneoplastic lesions. Area 1 was a portion of the Christina River watershed, where one location in Hershey Run was sampled in 2002 and 2003. Two samples were collected in 2003 in the newly restored Newport Marsh along the Christina River. In Area 2, the effluent channel of the Motiva oil refinery on the Delaware River, one location was sampled in 2004. In Area 3, the St. Jones River, one location near the Delaware National Estuarine Research Reserve (DNERR) was sampled in 2003. In Area 4, Blackbird Creek, one location near the DNERR was sampled in 2003. No skin tumors were diagnosed in any of the mummichogs. No liver tumors were diagnosed in the St. Jones River, Motiva, Newport Marsh Upriver, or Newport Marsh Downriver collections. In the Blackbird Creek collection, one of 30 fish had a liver with foci of hepatocellular alteration (FHA, a preneoplastic lesion) and one was diagnosed with hepatocellular carcinoma (HC; 3% prevalence for each lesion). The prevalence of HC was dramatically higher in the Hershey Run mummichogs in 2002 (7 of 21, 33%) and 2003 (3 of 29, 10%) compared to the other locations. In 2002, 5 of 21 (24%) Hershey Run mummichogs had livers with FHA whereas, in 2003, only 1 of 29 (3%) had FHA. The prevalence of HC and FHA was significantly higher in the Hershey Run mummichogs (2002 and 2003 collections pooled) compared with the pooled other collections (Fisher's Exact Test, p = 0.001 for FHA, p<0.001 for HC).

Because high sediment PAH concentrations have been associated with liver tumors in mummichogs, we searched the literature for such data from the fish collection locations. No data were available for Motiva or Newport Marsh. Data were identified for the St. Jones River about one km upriver and Blackbird Creek about five km downriver of the fish collection locations. Total PAH concentrations were 10.976 ppm and 1.486 ppm, respectively. For Hershey Run, which is located adjacent to the Koppers Superfund Site where creosote was released, one sample close to the collection site had a total PAH concentration of 13,300 ppm and others were above 1000 ppm. Although data are limited, in this study Hershey Run had both the highest sediment PAHs and the highest lesion prevalence. These findings are consistent with Vogelbein *et al.* (1990), who reported a 33% HC prevalence in mummichogs collected from the Elizabeth River adjacent to a Superfund site that released creosote (2200 ppm total PAH in sediment at collection site).

We conclude that preneoplastic lesions and tumors in mummichog livers were associated with the presence of highly contaminated sediment containing PAHs derived from creosote. Mummichogs collected from the areas not heavily contaminated with PAHs had few or no liver tumors or preneoplastic lesions.

An increased prevalence of tumors or other deformities is used as an indicator of Beneficial Use Impairment in the monitoring and evaluation of Great Lakes Areas of Concern. In the Chesapeake Bay, tumor surveys have been used to highlight contaminant impacts in two Regions

of Concern, the Elizabeth and Anacostia Rivers, and monitor progress. For the Delaware Estuary, we recommend developing a database so that tumors and preneoplastic lesions can be used effectively as a monitoring tool. This would involve surveys with brown bullheads and/or mummichogs, depending on salinity and availability. For each species, there is a need to develop a database containing age-specific and sex-specific tumor prevalence at reference and contaminated sites. Studies that include tumor prevalence, biomarker analyses (such as DNA adducts and PAH metabolites in bile), and sediment/tissue chemistry can be used to provide a weight-of-evidence for specific chemical classes.

## ACKNOWLEDGMENTS

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## INTRODUCTION

Environmental scientists conduct tumor surveys in bottom-dwelling fish to serve as an indicator of habitat quality and a tool for monitoring the success of cleanup actions (e.g., Baumann *et al.* 1996, Baumann and Harshbarger 1998, Myers *et al.* 1994). In North American freshwater ecosystems, the brown bullhead (*Ameiurus nebulosus*) has been shown to develop skin and liver tumors in response to contaminant exposure, with the most persuasive linkage for areas with polynuclear aromatic hydrocarbon (PAH)-contaminated sediments (Baumann and Harshbarger 1998). Recently, Baumann (2002) stated that liver tumor prevalence above about 5% and skin tumor prevalence above 12% could be used to distinguish between highly contaminated and less contaminated areas in the Great Lakes. The U.S. Fish and Wildlife Service, Chesapeake Bay Field Office (CBFO) has developed a database on tumors in brown bullheads from the tidal freshwater areas of the Chesapeake Bay watershed (Pinkney *et al.* 1995, 2001, 2004a, b).

Although brown bullheads have been captured in waters with salinity as high as 15 parts per thousand (Boyer 1995), they are primarily a freshwater species and can be difficult to locate in estuarine waters. In the higher salinity waters of the Elizabeth River, a Chesapeake Bay Region of Concern, the mummichog, *Fundulus heteroclitus*, has been used in tumor surveys (Vogelbein *et al.* 1990, Vogelbein and Zwerner 2000, Vogelbein and Unger 2003). Vogelbein *et al.* (1990) reported a 33% prevalence of liver tumors in mummichogs collected near the Atlantic Wood Treating Superfund Site on the Elizabeth River, Virginia, where sediments are contaminated with creosote. A sediment sample measured near the fish collection area contained 2200 parts per million (ppm) total PAHs (dry weight). Mummichogs are a suitable species for such surveys because they: 1) are highly localized with summer home range of 36 meters in tidal creeks and somewhat more extensive movements in fall and winter; 2) have considerable contact with sediments, feeding on bottom-dwelling invertebrates; 3) tolerate a wide range of salinity (Whitehead 1995); and 4) are easy to collect and transport. Mummichogs are currently used by the Elizabeth River Project as an indicator of the quality of estuarine habitats (Elizabeth River Project 2002).

We conducted a mummichog tumor survey at selected locations within the Delaware Estuary watershed. Our objective was to evaluate the prevalence of skin and liver tumors in mummichogs from locations where polynuclear aromatic hydrocarbon (PAH) contamination is suspected and from the two Delaware National Estuaries Research Reserves (DNERRs), where long term monitoring is being conducted. This study is the first systematic fish tumor survey in Delaware and is intended to provide environmental managers with a meaningful indicator for watershed monitoring.

## MATERIALS AND METHODS

### *Sampling Locations*

We sampled within four general areas: Christina River and tributaries, Motiva effluent channel to the Delaware River, St. Jones River, and Blackbird Creek. The sampling locations within each area are shown in Figure 1 a, b, c (see Table 1 for latitude and longitude). Locations were chosen based on proximity to a suspected PAH source area, a DNERR, or representative tidal marsh and availability of mummichogs. A general description of each area is provided below including the rationale for the specific sampling locations and sediment PAH data retrieved from the literature. Photographs of the sampling locations are provided in Appendix A.

Area 1: Christina River watershed

The Christina River is a tidal freshwater system that flows into the Delaware through a highly industrialized watershed, containing eight federal and several state Superfund sites. For example, the Koppers Superfund site is a former wood treating facility that released PAHs and metals into Hershey Run, and wetlands adjacent to White Clay Creek and the Christina. Throughout the watershed, sediments are contaminated with polychlorinated biphenyls (PCBs), polynuclear aromatic hydrocarbons (PAHs), pesticides, and toxic metals (Olinger 1997). The public is urged not consume any finfish from the tidal Christina River because of PCBs and dieldrin and from tidal White Clay Creek due to PCBs (DNREC 2004). Olinger (1997) reported the occurrence of sediment toxicity and measured carcinogenic PAHs in the 20 ppm (wet weight) range using immunoassay screening procedures. Sampling conducted as part of the Koppers investigation, indicated that a Christina River sediment sample collected near the confluence of White Clay Creek contained a total PAH concentration of 23.5 ppm and one location in Hershey Run had a total PAH concentration of 13,300 ppm (Woodward-Clyde 1996).

We selected Hershey Run as a sampling location because it is known to be highly contaminated with PAHs from spillage of creosote. We selected two locations within Newport Marsh, a newly restored tidal marsh, in an attempt to determine tumor prevalence in a representative marsh within the watershed. In a restoration effort started in 2002 and completed in 2003, tidal flows were restored to the 44-acre marsh by removing a dike between the river and the marsh. Further restoration efforts included the removal of rubbish and old tires from decades of use as an illegal dumping ground (Connectiv Power Delivery 2002). No sediment chemistry data were identified for Newport Marsh.

Mummichogs were collected from Hershey Run, within 100 meters upstream and downstream of the Railroad Bridge, adjacent to the Koppers Superfund Site on August 9, 2002 and October 1, 2003. The collections were labeled as Hershey 02 (HR02, n=21) and Hershey 03 (HR03, n=29). During collection, an oil sheen arose from the sediments. Two collections of mummichogs were made at Newport Marsh on October 2, 2003 and designated as Newport Marsh Upriver (NMU, n=29) and Newport Marsh Downriver (NMD, n=30, Figure1a).

Area 2: Motiva Effluent Channel

The industrialized Delaware City area of the Delaware River is impacted by releases from several operating and former facilities. Several Superfund sites (Standard Chlorine, Tybouts Corner) are located in the nearby Red Lion Creek subwatershed. A fish consumption advisory is in place for the Delaware River north of the Chesapeake and Delaware Canal (including the effluent channel), which advises the public not to eat finfish due to PCBs, dioxin, mercury, and chlorinated pesticides (DNREC 2004). There are reports of repeated National Pollutant Discharge Elimination System (NPDES) violations from the Motiva Refinery (DNREC 2000), including an unpermitted discharge of lubricant oil into surface water of the effluent channel leading to the Delaware River.

We selected the Motiva effluent channel as a sampling area because of the history of releases of petroleum products. Sediment data have been collected (L. Hall, University of Maryland, Wye Research and Education Center, pers. comm.) but are not available because of a legal case. We collected mummichogs from the Motiva effluent channel (Figure 1a) on June 7 and 17, 2004. The sample was designated as Motiva (MOT, n=30). There were visible oil sheens and oily odors in the intertidal marsh areas where the fish were collected.

Area 3: Lower St. Jones River Estuarine Reserve

One of the two DNERR sites is the Lower St. Jones River Reserve. The St. Jones River watershed drains a portion of the coastal plain in central Kent County, DE, including the city of Dover, the surrounding suburbs, industrial areas, agricultural areas and Dover Air Force Base (NERR web site: http://inlet.geol.sc.edu/DEL/st_jones_river.html). The river is less than pristine; four National Priority List Superfund sites, including the former Dover Gas Light facility that released PAHs, are in the watershed. DNREC (2004) advises the public not to eat more than two 8-ounce meals of finfish per year from the St. Jones River due to PCBs and dioxin contamination. The abundance of mummichogs at the Reserve was documented on the web site: http://www.dnrec.state.de.us/dnrec2000/divisions/soil/dnerr/tmp1023300816.htm.

We chose the St. Jones River as part of the study objective of obtaining tumor data within or near the DNERRs. A collection of 27 mummichogs (SJ) were obtained through efforts on June 17, June 23, and July 31, 2003 from an area of the St. Jones River about 200 meters downstream of the Route 10 Bridge, approximately 3 km upriver of the Reserve boundary. The National Oceanic and Atmospheric Administration (NOAA) collected and analyzed a sediment sample (NOAA 89), located about 1 km upriver (Hartwell *et al.* 2001, Figure 1b). Total PAHs were reported to be 10.976 ppm.

Area 4: Upper Blackbird Creek Estuarine Reserve

Upper Blackbird Creek is believed to be relatively unimpacted from local sources of industrial contaminants (D. Carter, DNREC, pers. comm.), although there is concern about non-point source pollution from silviculture and agricultural runoff (DNREC 1999). Blackbird Creek is not listed by DNREC (2004) as having any fish consumption advisories.

A total of 30 mummichogs (designated as BC) were collected from a side channel of Blackbird Creek on June 30 and August 1, 2003, in a largely agricultural subwatershed. The sampling location is about 3 km downriver of the Reserve boundary. NOAA) collected and analyzed a sediment sample (NOAA 85), located about 5 km upriver of the fish collection location (Hartwell *et al.* 2001, Figure 1c). Total PAHs were reported to be 1.486 ppm.

*Collection Procedures*

Our sampling goal was to collect 30 adult individuals at each location. The minimum length of 70 mm was established by balancing the recommended 75 mm minimum of Vogelbein *et al.* (1990) with the limited availability of larger fish. Mummichogs were collected with a 10-foot or 25-foot beach seine, often aided by using a second 25-foot seine as a block net. Fish were measured for total length, kept alive in aerated site water, and transported to the Chesapeake Bay Field Office, where necropsies were conducted within 24 hours. Water quality parameters (temperature, pH, conductivity, salinity, and dissolved oxygen) were recorded at the time of collection (Appendix B).

*Laboratory Procedures*

Fish were measured for total length, weighed, euthanized by severing the spinal cord, and necropsied. Condition factor [$K = (wt\ (g) \times 10^5) / length\ (mm)^3$] was determined. Visible lesions were noted and examples photographed. The viscera were opened and livers were excised and weighed so that the hepatosomatic index (HSI=liver wt/body wt) could be calculated. The liver, cut in 0.5-cm wide slides, and skin pieces with raised lesions were stored in 10% buffered neutral formalin and transported to the George Washington University Medical Center (Washington, DC) for histopathological examination and tumor diagnosis. The tissues were embedded, sectioned at 4 um, and stained with hematoxylin and eosin (Luna 1968). Tissue blocks were prepared from each liver and from each skin lesion. A glossary of histopathological terms is provided as Appendix C.

*Data Analysis*

Histopathological data were summarized as the prevalence of the various types of lesions among the collections of mummichogs. A glossary of terms is provided in Appendix B. The significance of differences in the prevalence of lesions between pairs of sampling locations was determined using a series of two-tailed chi-square tests (Sokal and Rohlf 1981), with Yates Correction for continuity, using a critical *p* value of 0.05. In cases where there was less than a minimum expected count of 5, Fisher's Exact test was used (Jandel Corp. 1995). Pooling was used in the analysis to reduce the number of comparisons and, therefore, the likelihood of Type I errors (false positives). Biological data including the length, weight, condition factor, and HSI were compared using analysis of variance, with log-transformation if necessary to meet the assumptions for parametric statistics. If these assumptions could not be satisfied, a Kruskal-Wallis test was used to compare the median values of the collections. Tukey's multiple comparison test (ANOVA) or Dunn's method (Kruskal-Wallis) were used to identify statistical significance between collections with a significance level of 0.05.

## RESULTS

*Biological Data*

Among the seven collections of mummichogs, there were significant differences in length, weight, condition factor, and HSI (Table 1). For example, the median length of the MOT mummichogs (82 mm) was significantly greater than the median length of the HR02 mummichogs (74 mm; Kruskal-Wallis test, p=0.017, Dunn's method, p<0.05). There were significant differences in both weight and condition factor among the collections. The most robust (highest K) was in the MOT (mean: 1.39) and HR03 (mean: 1.37) collections. These collections had significantly higher mean K than the SJ (1.26), HR02 (1.23), and BC (1.23) collections (ANOVA, p<0.001; Tukey's test, p<0.05). HSI differences were apparent, with the median values at HR03 (0.038), NMU (0.038), and NMD (0.036) significantly higher than the medians at MOT (0.022), SJ (0.018), and BC (0.017) (Kruskal-Wallis test, p<0.001, Dunn's method, p<0.05).

*Pathology*

Gross examination revealed that many of the fish had small cream-colored raised lesions on the skin, often at the base of the pectoral, dorsal, and caudal fins. Some live cream-colored worms could be excised from these lesions upon gross examination. Eleven such skin lesions, from NMU fish submitted for histopathological examinations, were all determined to be parasitic worms.

The only fish with a grossly visible liver lesion suspected to be a tumor was HR0217, from Hershey Run in 2002. It had a large gray mass on one side of the organ. Based on histopathological examination, this was later diagnosed as a granuloma, a mass of tissue formed as an inflammatory reaction to a parasitic worm infestation (remnants of which were observed in the liver and adjacent heart tissue). Many fish were observed to have worms (believed to be nematodes) in the intestines and liver (Appendix A). Except for these infestations and the granuloma, there were no grossly visible liver lesions. Liver coloration was noted on the necropsy data sheets and varied from coffee-colored to deep red.

There were no histopathological liver lesions in the following collections: MOT, NMD, NMU, and SJ. We diagnosed foci of hepatocellular alteration (FHA) and hepatocellular carcinomas (HC) in mummichogs from each of the other three collections (HR02, HR03, and BC; Figure 2, see Appendix B for a spreadsheet listing individual fish). Eleven HR02 specimens had hepatocellular neoplasms and/or precursor neoplasms as follows. Two mummichogs (HR0223 and HR0227) had a FHA (tinctorially altered population without dysplasia) and nine (HR0201, 0204, 0213, 0214, 0216, 0217, 0218, 0220, 2024) had a small well-differentiated HC. Three of the nine (HR0201, 0216, and 0220) also had a FHA. One specimen (HR0201) with a liver neoplasm also had six clusters of necrotic cells (single cell necrosis), similar in appearance to spongiosis hepatis reported in rodents (Bannasch *et al.* 1981; Bannasch and Zerban 1986), illustrated in medaka (Hinton *et al.* 1984), and described in sheepshead minnow (Couch and Courtney 1991) experimentally exposed to chemicals.

For the HR03 mummichogs, three had HC and one fish had an incipient neoplasm in the form of a clear cell FHA. Two of the HCs were well differentiated and one was anaplastic. The anaplastic HC was a mass of unpolarized, basopholic, pleomorphic cancer cells each with a prominant bull's eye nucleus. The advancing mass was invading and replacing normal liver along the interface (Figure 3). The mass was rapidly growing as indicated by up to six mitotic figures per high power microscopic field (Figure 4).

Fisher's Exact tests compared the prevalence of FHA, HC, and total liver lesions (TLL; *i.e.* having either an FHA or HC) between the collections with no lesions (MOT, SJ, NMU, and NMD) and the BC collection (one FHA, one HC, two TLL). Since there were no significant differences ($p>0.05$), these collections were pooled (Table 2). The mummichogs collected from Hershey Run (HR02 and HR03) had much higher lesion prevalence (Fig. 2) than those from any of the other locations. After testing HR02 vs. HR03 (two-tailed, chi-square), it was determined that the two years could be pooled for FHA and HC but not for TLL. There was a significantly higher FHA prevalence in the pooled Hershey Run collections vs. the pooled other locations (12% vs. 0.7%; Fisher's Exact Test, $p=0.001$). There was also a significantly higher HC prevalence in mummichogs from Hershey Run vs. the other locations (20% vs. 0.7%, Fisher's, $p<0.001$). TLL prevalence in both the HR02 and HR03 collections was also significantly greater than that in mummichogs from the other locations (Table 2).

## DISCUSSION

Mummichogs from Hershey Run had a dramatically higher prevalence of liver tumors (10% and 33%) than those from the other Delaware Estuary locations (0-3%). The high HC prevalence near a Superfund Site that released creosote was consistent with Vogelbein *et al.* (1990), who reported a 33% prevalence of HC in mummichogs near the Atlantic Wood Superfund Site in the Elizabeth River.

Vogelbein and Unger (2003) stated that there was a clear positive association between PAH contamination and liver pathology, specifically FHA and/or tumors (either hepatocellular carcinoma or hepatocellular adenoma). They collected 60 mummichogs from each of 12 sites in the Elizabeth River watershed. At each site, two sediment samples were analyzed for 18 PAH compounds. Tumors were reported at four locations, with a prevalence of 1.7% (site SBB2), 1.7% (SBD3), 8.3% (SBB1), and 10.0% (EBB2). Three of these (SBD3, SBB1, and EBB2) had the highest FHA prevalence (60-68%), whereas the fourth (SBB2) had an FHA prevalence of 11.7%. Total PAHs in the sediments with >60% FHA ranged from 52.4 ppm to 490.8 ppm, while that at SBB2 was 13.6 ppm. At the lower end of the range, SBD4 had no tumors, 8.3% FHA and a total PAH concentration of 0.207 ppm. Previously, Vogelbein *et al.* (1990) did not diagnose any tumors or preneoplastic lesions in mummichogs collected from a site (Scuffletown Creek) where sediments contained 61 ppm total PAHs.

Environmental managers often develop guidance values for sediment contaminants associated with adverse biological effects. In an evaluation of West Coast tumor studies, Horness *et al.* (1998) used regression analysis to propose 2.8 ppm total PAH as a threshold sediment concentration, above which an increased prevalence of hepatic lesions occurred. No suggested tumor or lesion thresholds have been proposed for either freshwater or estuarine fish. The ongoing efforts in the Elizabeth River may ultimately yield a database sufficient to conduct such analyses for mummichogs. The work of Vogelbein and Unger (2003) suggests that a positive relationship exists but they did not conduct a statistical analysis. The present study cannot contribute to such a database because it lacked co-located sediment data for most sites. Only Hershey Run appears to be well-characterized with respect to sediment PAH concentrations. If possible, future tumor surveys should include collection of at least three sediment samples with analysis for the full suite of PAHs, both parent and alkylated.

Vogelbein and Zwerner (2000) classified study sites on a 1 to 4 scale as follows: *1-not a problem* - FHA (their term is AHF) <5% and no neoplasms; *2- borderline* – FHA: 5-20%, neoplasms 0%; *3 -a problem*: FHA 20-30%, neoplasms <5%; and *4 – severe problem* - FHA > 30%, neoplasms >5%. Based on this classification, Hershey Run 02 would rank as a severe problem based on the neoplasm prevalence of 33% with FHA of 24%. Hershey Run 03 would probably still rank as a severe problem, based on the 10% neoplasm prevalence, even though FHA was only 3%. Blackbird Creek would most likely be classified as borderline.

There was a large difference in the HC prevalence in Hershey Run between 2002 (33%) and 2003 (10%). Coincidentally, these collections were very different in sex ratio, with 2002 consisting of 4 males and 17 females and 2003 with 24 males and 5 females. Tumors occurred only in females in 2002 and only in males in 2003. Cooke and Hinton (1999) stated that there is often a higher prevalence of hepatocellular neoplasia in females, although they do not cite data for mummichogs. Pinkney *et al.* (2004a) found that female brown bullheads had nearly double the tumor prevalence of males (95% vs. 50%) and identified sex as a significant risk factor, using logistic regression. In the present study, however, it is unlikely that the lower tumor prevalence in 2003 reflects a difference in susceptibility between males and females, since all three of the tumors occurred in males.

In brown bullheads, several studies have reported higher HSI in fish from contaminated areas and a statistical association between HSI and tumors (Pinkney *et al.* 2001, 2004a). Because parasitic worms were observed frequently and to varying degrees in the liver and intestines, we suspect that differences in HSI among the collections of mummichogs reflected varying degrees of parasitic infestation rather than responses to carcinogens. Similarly, condition factor may also be affected by parasitic loads.

## RECOMMENDATION

An increased prevalence of tumors or other deformities is used as an indicator of Beneficial Use Impairment in the designation and monitoring of Great Lakes Areas of Concern. In the Chesapeake Bay, tumor surveys have been used to highlight contaminant impacts in two Regions of Concern, the Elizabeth River and the Anacostia River, and target areas for remediation. In the Delaware Estuary, we recommend developing a database so that tumors and deformities can be used in a similar manner as in the Great Lakes. This would involve surveys with brown bullheads and/or mummichogs, depending on the salinity of the habitat. For each species, there is a need to develop a database containing age-specific and sex-specific tumor prevalence at reference and contaminated sites. Studies that include tumor prevalence; biomarker analyses such as DNA adducts and bile metabolites; and sediment/tissue chemistry can be used to provide a weight-of-evidence for specific chemical classes (see Pinkney *et al.* 2001, 2004a).

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# FIGURES

Figure 1. Maps of the mummichog collection areas. b) St. Jones River; c) Blackbird Creek. NOAA 85 and 89 are sediment sample locations from Hartwell et al. (2001)

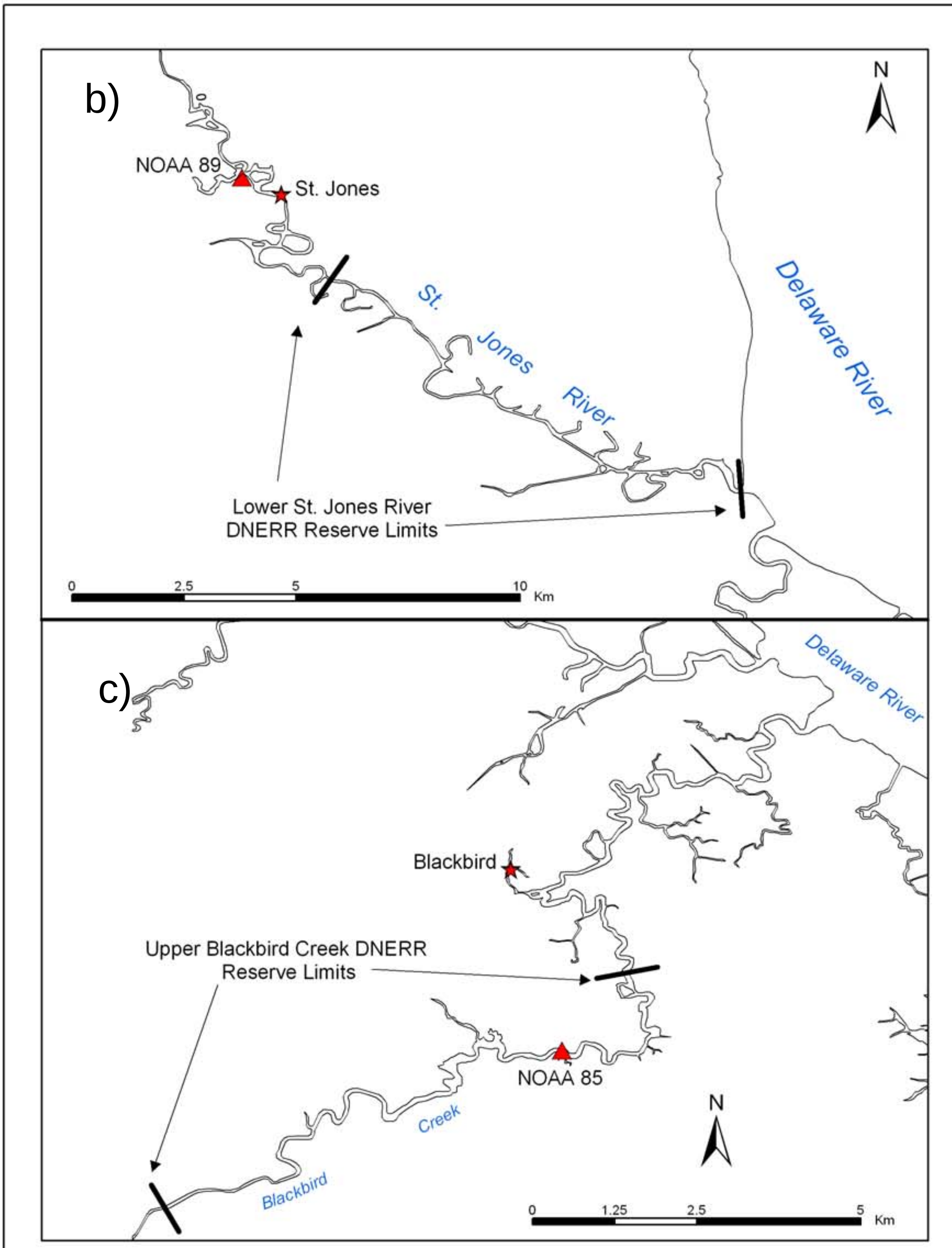
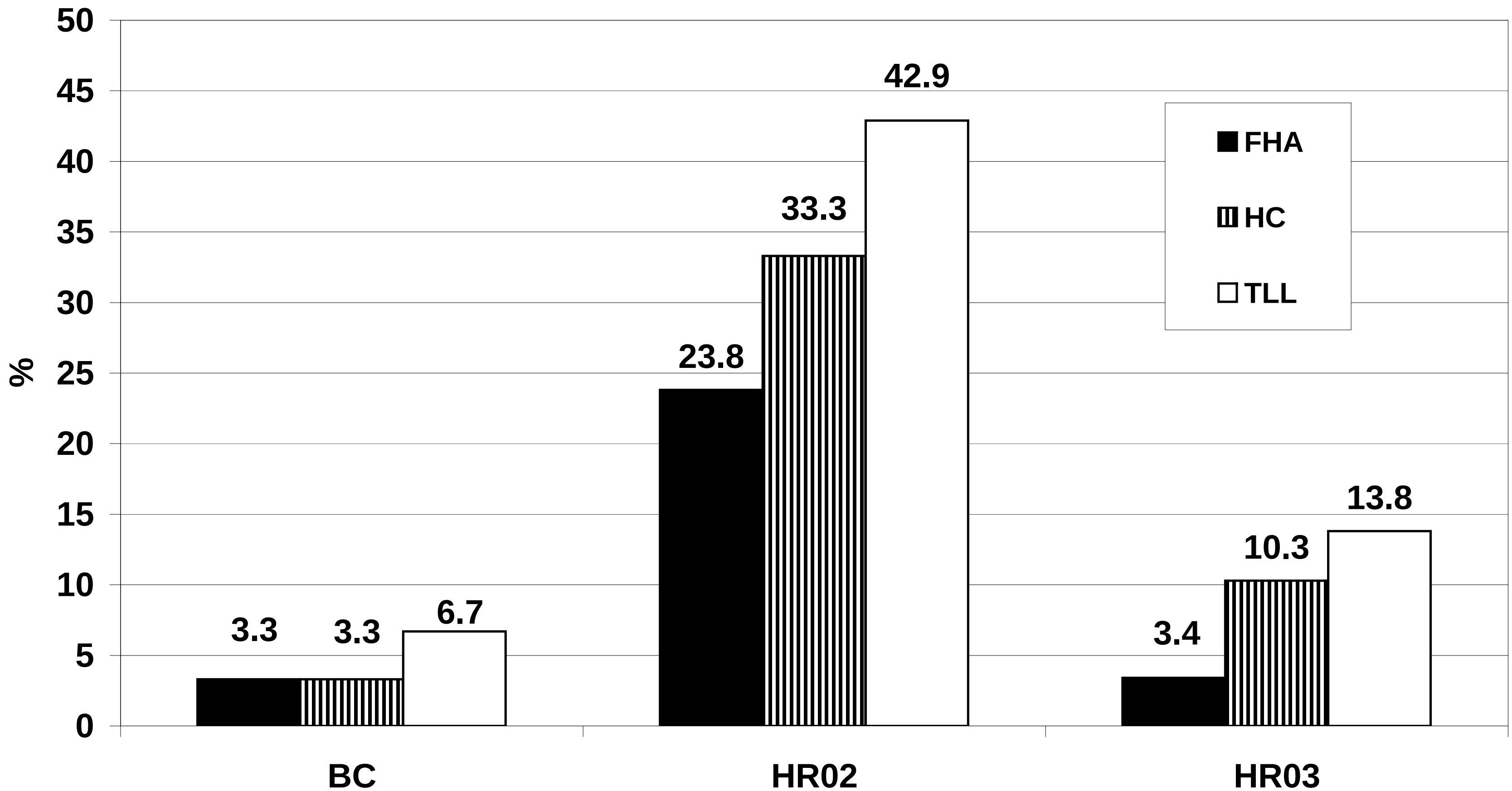


**Figure 2. Prevalence of liver lesions (FHA=foci of hepatocellular alteration,HC=hepatocellular carcinoma, TLL=a fish with either FHA or HC) in mummichogs from Blackbird Creek (BC), Hershey Run 2002 (HR02) or 2003 (HR03)**



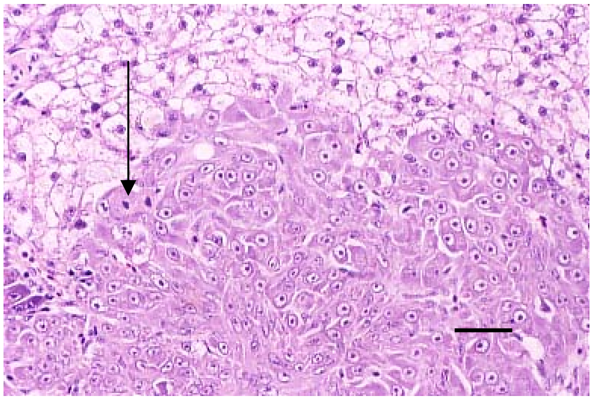


Figure 3. HR03-19 (Hershey Run 2003): Sculptured leading edge of hepatocellular carcinoma (dark cells) invading and destroying normal liver tissue. Note the enlarged bull's eye nuclei in the cancer cells. Arrow points to a mitotic figure in telophase.
Bar = 16 μm

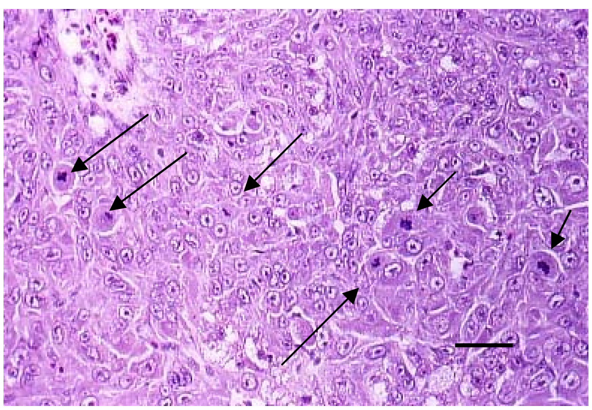
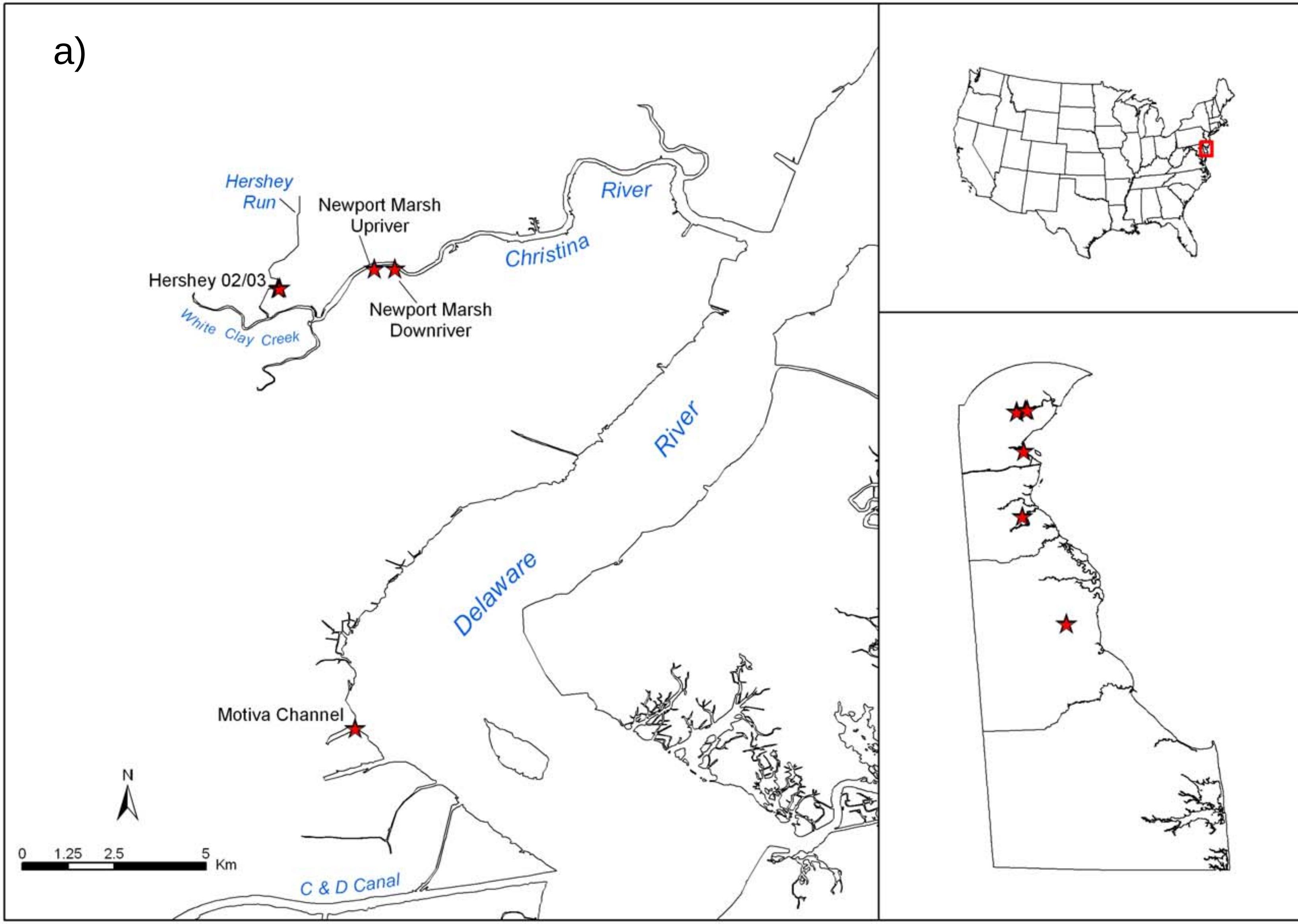


Figure 4. HR03-19 (Hershey Run 2003): Rapid cancer growth rate indicated by at least six mitotic figures (arrows). Bar = 9 μm

Figure 1. Maps of the mummichog collection areas. a) Christina River and Motiva Channel



# TABLES

Table 1. Biological data for the six collections of mummichogs[a:] BC: Blackbird Creek, HR02: Hershey Run 2002, HR03: Hershey Run 2003, MOT: Motiva, NMD: Newport Marsh Downriver, NMU: Newport Marsh Upriver, SJ: St. Jones.

| | **BC (n=30)** | **HR02 (n=21)** | **HR03 (n=29)** | **MOT (n=30)** | **NMD (n=30)** | **NMU (n=29)** | **SJ (n=27)** | **Statistics[b]** |
|---|---|---|---|---|---|---|---|---|
| **Collection date(s)** | 6/30; 8/1/03 | 8/9/02 | 10/1/03 | 6/7; 6/17/04 | 10/2/03 | 10/2/03 | 6/17, 6/23, 7/31/03 | |
| **Latitude** | 39.41918 | 39.70517 | 39.70528 | 39.59725 | 39.70998 | 39.71006 | 39.12585 | |
| **Longitude** | 75.61522 | 75.63132 | 75.63086 | 75.61239 | 75.60261 | 75.60674 | 75.61522 | |
| **Length (mm)** | 78 (70,90) A,B | 74 (70,95) B | 74 (71, 97) A,B | 82 (70,112) A | 75 (70,93) A,B | 75 (70,90) A,B | 76 (70, 91) A,B | K-W (p=0.017) |
| **Weight (g)** | 5.9 (4.0-9.0) B | 5.0 (3.8-10.6) B | 5.4 (4.3-13.1) A,B | 7.7 (4.4-21.0) A | 5.6 (3.8-11.4) B | 5.8 (4.0-9.2) B | 5.1 (3.8-10.0) B | K-W (p<0.001) |
| **Liver weight (g)** | 0.10±0.04 | ND[c] | 0.28±0.17 | 0.20±0.13 | 0.23±0.10 | 0.24±0.07 | 0.11±0.06 | Not compared |
| **Condition Factor (K)** | 1.23±0.08 C | 1.23±0.14 B | 1.37±0.14 A | 1.39±0.11 A | 1.32±0.11 A,B | 1.33±0.11A,B | 1.26±0.12 B | ANOVA (p<0.001) |
| **Hepatosomatic Index (HSI)** | 0.017 (0.007, 0.032) B | ND[c] | 0.038 (0.019, 0.089) A | 0.022 (0.005, 0.041) B | 0.036 (0.018, 0.061) A | 0.038 (0.023, 0.067) A | 0.018 (0.008, 0.043) B | K-W (p<0.001) |
| **Sex** | 17M, 13F | 4M, 17F | 24M, 5F | 16M, 14F | 17M, 8F, 5? | 19M, 1F, 9? | 19M, 8F | |

[a] Mean ± one standard deviation (SD) or median with range in parentheses.
[b] Groups with different letters are significantly different at p<0.05 using Tukey's test (ANOVA) or Dunn's method (K-W).
[c] Not determined; liver weights were not measured in the HR02 fish.

Table 2. Summary of lesion data and statistical comparisons (Fisher's Exact Test).

| **Lesion[a]** | **BC,MOT, NMD, NMU, SJ (pooled)** | **Hershey Run (pooled)** | **P value** |
|---|---|---|---|
| **Focus of hepatocellular alteration (FHA)** | 1/145 (0.7%) | 6/50 (12%) | 0.001 |
| **Hepatocellular carcinoma (HC)** | 1/145 (0.7%) | 10/50 (20%) | <0.001 |
| | **BC,MOT, NMD,NMU, SJ (pooled)** | **Hershey Run (02)** | |
| **Total liver lesions (TLL)** | 2/145 (1.4%) | 9/21 (43%) | <0.001 |
| | **BC,MOT, NMD,NMU, SJ (pooled)** | **Hershey Run (03)** | |
| **Total liver lesions (TLL)** | 2/145 (1.4%) | 4/30 (13.3%) | 0.008 |

[a] see Appendix C for glossary of pathological terminology

# APPENDIX A

## Photographs of Sampling Locations and Evidence of Parasitic Infestation


Upper Blackbird Creek sampling area


Motiva Channel sampling area


St. Jones River sampling area
—both shorelines

# Hershey Run mummichog sampling area

# Mummichog collection at Hershey Run:8/9/02

# Oily sheen at Hershey Run sampling area

## Male mummichog with belly enlarged due to parasitic worms

# Mummichog (NMD22) with infestation of parasitic worms

# APPENDIX B

## Spreadsheets of Biological and Pathological Data

| Site name | Waterbody | Lat/long | Date(s) | number | FHA | % | HC | % | TLL | % |
|---|---|---|---|---|---|---|---|---|---|---|
| Blackbird (BC) | Blackbird Creek | 39.41918 | 6/30/2003 | 30 | 1 | 3.3 | 1 | 3.3 | 2 | 6.7 |
| | | 75.61522 | | | | | | | | |
| | | | | | | | | | | |
| Hershey 02 (HR02) | Hershey Run | 39.70517 | 8/9/2002 | 21 | 5 | 23.8 | 7 | 33.3 | 9 | 42.9 |
| | | 75.63132 | | | | | | | | |
| | | | | | | | | | | |
| Hershey 03 (HR03) | Hershey Run | 39.70528 | 10/1/2003 | 29 | 1 | 3.4 | 3 | 10.3 | 4 | 13.8 |
| | | 75.63086 | | | | | | | | |
| | | | | | | | | | | |
| Newport Marsh Upriver (NMU) | Christina River | 39.71006 | 10/2/2003 | 30 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | 75.60764 | | | | | | | | |
| | | | | | | | | | | |
| Newport Marsh Downriver (NMD) | Christina River | 39.70998 | 10/2/2003 | 29 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | 75.60261 | | | | | | | | |
| | | | | | | | | | | |
| Motiva (MOT) | Motiva Effluent Channel | 39.59725 | 6/7/2004 | 30 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | 75.61239 | 6/17/2004 | | | | | | | |
| | | | | | | | | | | |
| St. Jones (SJ) | St. Jones River | 39.12585 | 6/17/2003 | 27 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | 75.49482 | 8/1/2003 | | | | | | | |

2000 Bullhead Study:
BLACKBIRD CREEK

| Sample | Method | FHA | HA | HC | C | CC | TLC | TLT | TLL | MSL | EP | SC | TST | | Length | Weight | Liver wt | K | H.S.I. | Sex |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Blackbird Creek** | | | | | | | | | | | | | | | (mm) | (g) | (g) | | | |
| **BC1 (6/30/03)** | **seine** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 84 | 8.30 | 0.15 | 1.40 | 0.0181 | M |
| **BC2** | | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | HC | 0 | 0 | 0 | | 85 | 7.50 | 0.10 | 1.22 | 0.0133 | M |
| **BC3** | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 85 | 7.95 | 0.10 | 1.29 | 0.0126 | M |
| **BC4** | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 78 | 6.05 | 0.05 | 1.27 | 0.0083 | M |
| **BC5** | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 71 | 4.45 | 0.05 | 1.24 | 0.0112 | M |
| **BC6** | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 77 | 5.60 | 0.10 | 1.23 | 0.0179 | M |
| **BC7** | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 83 | 7.55 | 0.05 | 1.32 | 0.0066 | M |
| **BC8** | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 77 | 5.00 | 0.05 | 1.10 | 0.0100 | M |
| **BC9** | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 80 | 6.90 | 0.15 | 1.35 | 0.0217 | F |
| **BC10** | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 90 | 9.05 | 0.15 | 1.24 | 0.0166 | F |
| **BC11** | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 72 | 4.10 | 0.10 | 1.10 | 0.0244 | F |
| **BC12** | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 81 | 7.05 | 0.10 | 1.33 | 0.0142 | M |
| **BC13** | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 75 | 5.30 | 0.10 | 1.26 | 0.0189 | M |
| **BC14** | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 80 | 6.25 | 0.20 | 1.22 | 0.0320 | F |
| **BC15** | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 84 | 7.45 | 0.15 | 1.26 | 0.0201 | F |
| **BC16** | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 73 | 4.60 | 0.10 | 1.18 | 0.0217 | F |
| **BC17** | | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | FHA | 0 | 0 | 0 | | 81 | 7.00 | 0.20 | 1.32 | 0.0286 | F |
| **BC18** | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 71 | 4.05 | 0.05 | 1.13 | 0.0123 | F |
| **BC19** | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 75 | 5.15 | 0.05 | 1.22 | 0.0097 | M |
| **BC20** | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 86 | 7.55 | 0.10 | 1.19 | 0.0132 | M |
| **BC21** | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 72 | 4.75 | 0.10 | 1.27 | 0.0211 | F |
| **BC22** | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 71 | 4.50 | 0.05 | 1.26 | 0.0111 | M |
| **BC23 (8/1/03)** | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 79 | 6.05 | 0.05 | 1.23 | 0.0083 | M |
| **BC24** | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 81 | 6.55 | 0.15 | 1.23 | 0.0229 | F |
| **BC25** | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 81 | 5.95 | 0.10 | 1.12 | 0.0168 | F |
| **BC26** | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 70 | 4.05 | 0.10 | 1.18 | 0.0247 | F |
| **BC27** | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 72 | 4.25 | 0.05 | 1.14 | 0.0118 | M |
| **BC28** | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 73 | 5.10 | 0.10 | 1.31 | 0.0196 | M |
| **BC29** | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 74 | 4.45 | 0.05 | 1.10 | 0.0112 | M |
| **BC30** | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 77 | 5.85 | 0.10 | 1.28 | 0.0171 | F |
| **SUM** | | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 2 | | 0 | 0 | 0 | **mean** | 77.9 | 5.95 | 0.10 | 1.23 | 0.0165 | |
| **Percent (%)** | | 3.33 | 0 | 3.33 | 0 | 0 | 0 | 3.33 | 6.67 | | 0 | 0 | 0 | **std** | 5.4 | 1.42 | 0.04 | 0.08 | 0.0063 | |
| **Min** | | | | | | | | | | | | | | **min** | 70 | 4.05 | 0.05 | 1.10 | 0.0066 | |
| **Max** | | | | | | | | | | | | | | **max** | 90 | 9.05 | 0.2 | 1.40 | 0.0320 | |
| **Mean** | | | | | | | | | | | | | | **median** | 77.5 | 5.9 | 0.1 | 1.24 | 0.0167 | 17M |
| **Stdev** | | | | | | | | | | | | | | **n** | 30 | 30 | 30 | 30 | 30 | 13F |
| **Median** | | | | | | | | | | | | | | | | | | | | |

2000 Bullhead Study:
ST. JONES

| Sample | Method | FHA | HA | HC | C | CC | TLC | TLT | TLL | MSL | EP | SC | TST | | Length | Weight | Liver wt | K | H.S.I. | Sex |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **St. Jones River** | | | | | | | | | | | | | | | **(mm)** | **(g)** | **(g)** | | | |
| **SJ1 (6/17/03)** | **seine** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 71 | 4.95 | 0.10 | 1.38 | 0.0202 | F |
| **SJ2** | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 72 | 4.55 | 0.10 | 1.22 | 0.0220 | F |
| **SJ3** | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 85 | 8.45 | 0.20 | 1.38 | 0.0237 | F |
| **SJ4** | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 78 | 7.15 | 0.20 | 1.51 | 0.0280 | M |
| **SJ5** | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 79 | 7.05 | 0.30 | 1.43 | 0.0426 | F |
| **SJ6** | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 90 | 9.50 | 0.15 | 1.30 | 0.0158 | M |
| **SJ7** | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 70 | 4.40 | 0.10 | 1.28 | 0.0227 | M |
| **SJ8** | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 70 | 4.15 | 0.05 | 1.21 | 0.0120 | M |
| **SJ9** | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 84 | 8.30 | 0.10 | 1.40 | 0.0120 | M |
| **SJ10** | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 73 | 5.65 | 0.10 | 1.45 | 0.0177 | M |
| **SJ11** | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 76 | 5.80 | 0.25 | 1.32 | 0.0431 | F |
| **SJ12** | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 83 | 7.95 | 0.10 | 1.39 | 0.0126 | M |
| **SJ13** | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 73 | 5.05 | 0.10 | 1.30 | 0.0198 | M |
| **SJ14** | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 70 | 3.75 | 0.10 | 1.09 | 0.0267 | M |
| **SJ15** | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 78 | 5.55 | 0.10 | 1.17 | 0.0180 | M |
| **SJ16** | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 76 | 4.90 | 0.15 | 1.12 | 0.0306 | F |
| **SJ17** | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 73 | 4.90 | 0.05 | 1.26 | 0.0102 | M |
| **SJ18** | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 71 | 4.55 | 0.05 | 1.27 | 0.0110 | M |
| **SJ19** | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 71 | 3.90 | 0.05 | 1.09 | 0.0128 | M |
| **SJ20** | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 78 | 5.60 | 0.05 | 1.18 | 0.0089 | M |
| **SJ21** | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 71 | 4.55 | 0.10 | 1.27 | 0.0220 | M |
| **SJ22** | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 77 | 5.85 | 0.05 | 1.28 | 0.0085 | M |
| **SJ23 (6/23/03)** | **seine** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 77 | 5.10 | 0.10 | 1.12 | 0.0196 | M |
| **SJ24** | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 91 | 10.00 | 0.15 | 1.33 | 0.0150 | M |
| **SJ25** | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 83 | 7.15 | 0.10 | 1.25 | 0.0140 | M |
| **SJ26 (7/31/03)** | **seine** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 76 | 4.60 | 0.10 | 1.05 | 0.0217 | F |
| **SJ27** | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 70 | 3.80 | 0.05 | 1.11 | 0.0132 | F |
| **SUM** | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | **mean** | 76.5 | 5.8 | 0.1 | 1.3 | 0.019 | |
| **Percent (%)** | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | **std** | 6.1 | 1.8 | 0.1 | 0.1 | 0.009 | |
| | | | | | | | | | | | | | | **min** | 70 | 3.75 | 0.05 | 1.048 | 0.009 | |
| | | | | | | | | | | | | | | **max** | 91 | 10 | 0.3 | 1.51 | 0.043 | |
| | | | | | | | | | | | | | | **median** | 76 | 5.1 | 0.1 | 1.27 | 0.018 | 19M |
| | | | | | | | | | | | | | | **n** | 27 | 27 | 27 | 27 | 27 | 8F |
| **Median** | | | | | | | | | | | | | | | | | | | | |

2000 Bullhead Study:
HERSHEY RUN 02

| Sample date | Method | FHA | HA | HC | C | CC | TLC | TLT | TLL | MSL | EP | SC | TST | | Length | Weight | Liver wt | K | H.S.I. | Sex |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Hershey Run** | | | | | | | | | | | | | | | **(mm)** | **(g)** | **(g)** | | | |
| **HR0201 (8/9/02)** | **Seine** | 1 | 0 | 1 | 0 | 0 | 1 | 1 | 1 | HC | 0 | 0 | 0 | | 72 | 4.15 | ND | 1.11 | ND | F |
| **HR0204** | | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 1 | HC | 0 | 0 | 0 | | 70 | 4.30 | | 1.25 | | F |
| **HR0205** | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | | 75 | 4.50 | | 1.07 | | M |
| **HR0209** | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | | 75 | 5.90 | | 1.40 | | F |
| **HR0211** | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | | 79 | 4.75 | | 0.96 | | F |
| **HR0212** | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | | 71 | 5.00 | | 1.40 | | F |
| **HR0213** | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | | 82 | 6.85 | | 1.24 | | F |
| **HR0214** | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | | 80 | 6.35 | | 1.24 | | F |
| **HR0215** | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | | 74 | 4.85 | | 1.20 | | F |
| **HR0216** | | 1 | 0 | 1 | 0 | 0 | 1 | 1 | 1 | HC | 0 | 0 | 0 | | 80 | 5.80 | | 1.13 | | F |
| **HR0217** | | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 1 | HC* | 0 | 0 | 0 | | 95 | 10.65 | | 1.24 | | F |
| **HR0218** | | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 1 | HC | 0 | 0 | 0 | | 71 | 5.05 | | 1.41 | | F |
| **HR0219** | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | | 86 | 7.40 | | 1.16 | | F |
| **HR0220** | | 1 | 0 | 1 | 0 | 0 | 1 | 1 | 1 | HC | 0 | 0 | 0 | | 73 | 6.15 | | 1.58 | | F |
| **HR0221** | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | | 70 | 4.65 | | 1.36 | | F |
| **HR0223** | | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | FHA | 0 | 0 | 0 | | 76 | 5.90 | | 1.34 | | M |
| **HR0224** | | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 1 | HC | 0 | 0 | 0 | | 70 | 4.00 | | 1.17 | | F |
| **HR0225** | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | | 74 | 4.75 | | 1.17 | | M |
| **HR0227** | | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | FHA | 0 | 0 | 0 | | 80 | 5.85 | | 1.14 | | F |
| **HR0228** | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | | 71 | 3.75 | | 1.05 | | M |
| **HR0230** | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | | 72 | 4.80 | | 1.29 | | F |
| **SUM** | | **5** | **0** | **7** | **0** | **0** | **7** | **7** | **9** | | **0** | **0** | **0** | **mean** | **76** | **5.50** | | **1.23** | | |
| **Percent (%)** | | **23.8** | **0.00** | **33.3** | **0.0** | **0.0** | **33.3** | **33.3** | **42.9** | | **0** | **0** | **0** | **std** | **6** | **1.52** | | **0.14** | | |
| | | | | | | | | | | | | | | **min** | **70** | **3.75** | | **0.96** | | |
| **ND: not determined** | | | | | | | | | | | | | | **max** | **95** | **10.65** | | **1.58** | | |
| | | | | | * also granuloma | | | | | | | | | **median** | **74** | **5.0** | | **1.24** | | 4M |
| | | | | | | | | | | | | | | **n** | **21** | **21** | | **21** | | 17F |

2000 Bullhead Study:
HERSHEY RUN 03

| Sample date | Method | FHA | HA | HC | C | CC | TLC | TLT | TLL | MSL | EP | SC | TST | | Length | Weight | Liver wt | K | H.S.I. | Sex | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| HR03ppers (Hershey) | | | | | | | | | | | | | | | (mm) | (g) | (g) | | | | | |
| HR0301 (10/1/03) | Seine | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 73 | 5.25 | 0.10 | 1.35 | 0.0190 | M | | |
| HR0302 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 72 | 4.50 | 0.10 | 1.21 | 0.0222 | M | | |
| HR0303 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 80 | 7.30 | 0.30 | 1.43 | 0.0411 | M | | |
| HR0304 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 71 | 4.50 | 0.15 | 1.26 | 0.0333 | F | | |
| HR0305 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 85 | 7.60 | 0.20 | 1.24 | 0.0263 | F | | |
| HR0306 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 72 | 4.60 | 0.10 | 1.23 | 0.0217 | F | | |
| HR0307 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 81 | 6.60 | 0.35 | 1.24 | 0.0530 | F | | |
| HR0309 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 75 | 6.05 | 0.35 | 1.43 | 0.0579 | M | | |
| HR0310 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 74 | 5.25 | 0.20 | 1.30 | 0.0381 | M | | |
| HR0311 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 72 | 5.45 | 0.20 | 1.46 | 0.0367 | F | | |
| HR0312 | | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 1 | HC | 0 | 0 | 0 | | 90 | 10.75 | 0.45 | 1.47 | 0.0419 | M | | |
| HR0313 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 93 | 11.15 | 0.50 | 1.39 | 0.0448 | M | | |
| HR0314 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 71 | 5.00 | 0.20 | 1.40 | 0.0400 | M | | |
| HR0315 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 75 | 5.30 | 0.45 | 1.26 | 0.0849 | M | | |
| HR0316 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 71 | 4.35 | 0.20 | 1.22 | 0.0460 | M | | |
| HR0317 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 72 | 5.35 | 0.15 | 1.43 | 0.0280 | M | | |
| HR0318 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 74 | 4.30 | 0.10 | 1.06 | 0.0233 | M | | |
| HR0319 | | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 1 | HC | 0 | 0 | 0 | | 82 | 7.85 | 0.70 | 1.42 | 0.0892 | M | * LOOK FOR PHOTO | |
| HR0320 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 72 | 5.50 | 0.30 | 1.47 | 0.0545 | M | | |
| HR0321 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 88 | 10.25 | 0.40 | 1.50 | 0.0390 | M | | |
| HR0322 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 77 | 6.80 | 0.15 | 1.49 | 0.0221 | M | | |
| HR0323 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 72 | 4.95 | 0.15 | 1.33 | 0.0303 | M | | |
| HR0324 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 85 | 10.10 | 0.60 | 1.64 | 0.0594 | M | | |
| HR0325 | | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | FHA | 0 | 0 | 0 | | 97 | 13.10 | 0.55 | 1.44 | 0.0420 | M | | |
| HR0326 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 75 | 5.40 | 0.20 | 1.28 | 0.0370 | M | | |
| HR0327 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 73 | 5.15 | 0.10 | 1.32 | 0.0194 | M | | |
| HR0328 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 72 | 6.50 | 0.20 | 1.74 | 0.0308 | M | | |
| HR0329 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 81 | 7.50 | 0.45 | 1.41 | 0.0600 | M | | |
| HR0330 | | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 1 | HC | 0 | 0 | 0 | | 74 | 5.05 | 0.15 | 1.25 | 0.0297 | M | | |
| **SUM** | | **1** | **0** | **3** | **0** | **0** | **3** | **3** | **4** | | **0** | **0** | **0** | **mean** | **77.6** | **6.6** | **0.3** | **1.4** | **0.0404** | | | |
| **Percent (%)** | | **3.4** | **0** | **10.3** | **0** | **0** | **10.3** | **10.3** | **13.8** | | **0** | **0** | **0** | **std** | **7.3** | **2.3** | **0.2** | **0.1** | **0.0177** | | | |
| **Min** | | | | | | | | | | | | | | **min** | **71** | **4.3** | **0.1** | **1.061** | **0.01904762** | | | |
| **Max** | | | | | | | | | | | | | | **max** | **97** | **13.1** | **0.7** | **1.74** | **0.089172** | | | |
| **Mean** | | | | | | | | | | | | | | **median** | **74** | **5.45** | **0.2** | **1.39** | **0.038095** | **24M** | | |
| **Stdev** | | | | | | | | | | | | | | **n** | **29** | **29** | **29** | **29** | **29** | **5F** | | |
| **Median** | | | | | | | | | | | | | | | | | | | | | | |

2000 Bullhead Study:
NEWPORT MARSH DOWNRIVER

| Sample | Collection Date | FHA | HA | HC | C | CC | TLC | TLT | TLL | MSL | EP | SC | TST | | Length | Weight | Liver wt | K | H.S.I. | Sex |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Newport Marsh Down | 10/2/2003 | | | | | | | | | | | | | | (mm) | (g) | (g) | | | |
| NMD1 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 81 | 7.4 | 0.40 | 1.39 | 0.054 | M |
| NMD2 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 80 | 6.15 | 0.20 | 1.20 | 0.033 | M |
| NMD3 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 90 | 11.35 | 0.45 | 1.56 | 0.040 | ? |
| NMD4 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 86 | 8.75 | 0.40 | 1.38 | 0.046 | M |
| NMD5 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 79 | 5.75 | 0.20 | 1.17 | 0.035 | ? |
| NMD6 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 78 | 6.65 | 0.20 | 1.40 | 0.030 | M |
| NMD7 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 76 | 4.85 | 0.15 | 1.10 | 0.031 | M |
| NMD8 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 72 | 5.40 | 0.20 | 1.45 | 0.037 | M |
| NMD9 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 71 | 4.95 | 0.20 | 1.38 | 0.040 | M |
| NMD10 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 70 | 4.75 | 0.10 | 1.38 | 0.021 | M |
| NMD11 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 73 | 5.00 | 0.20 | 1.29 | 0.040 | M |
| NMD12 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 70 | 3.80 | 0.10 | 1.11 | 0.026 | M |
| NMD13 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 93 | 11.15 | 0.45 | 1.39 | 0.040 | F |
| NMD14 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 75 | 5.65 | 0.20 | 1.34 | 0.035 | F |
| NMD15 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 80 | 6.15 | 0.25 | 1.20 | 0.041 | F |
| NMD16 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 83 | 7.20 | 0.25 | 1.26 | 0.035 | M |
| NMD17 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 76 | 6.30 | 0.25 | 1.44 | 0.040 | ? |
| NMD18 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 80 | 6.80 | 0.30 | 1.33 | 0.044 | F |
| NMD19 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 75 | 6.10 | 0.25 | 1.45 | 0.041 | M |
| NMD20 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 70 | 4.40 | 0.15 | 1.28 | 0.034 | F |
| NMD21 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 70 | 4.25 | 0.10 | 1.24 | 0.024 | M |
| NMD22 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 72 | 5.45 | 0.10 | 1.46 | 0.018 | ? |
| NMD23 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 75 | 5.60 | 0.25 | 1.33 | 0.045 | F |
| NMD24 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 73 | 5.75 | 0.35 | 1.48 | 0.061 | M |
| NMD25 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 75 | 5.30 | 0.15 | 1.26 | 0.028 | M |
| NMD26 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 80 | 7.00 | 0.30 | 1.37 | 0.043 | F |
| NMD27 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 72 | 4.95 | 0.15 | 1.33 | 0.030 | M |
| NMD28 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 70 | 4.25 | 0.15 | 1.24 | 0.035 | F |
| NMD29 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 72 | 4.55 | 0.15 | 1.22 | 0.033 | M |
| NMD30 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 75 | 4.95 | 0.20 | 1.17 | 0.040 | ? |
| SUM | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | mean | 76.4 | 6.0 | 0.2 | 1.3 | 0.037 | |
| Percent (%) | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | std | 5.9 | 1.8 | 0.1 | 0.1 | 0.009 | |
| Min | | | | | | | | | | | | | | min | 70 | 3.8 | 0.1 | 1.105 | 0.018 | |
| Max | | | | | | | | | | | | | | max | 93 | 11.35 | 0.45 | 1.56 | 0.061 | 17M |
| Mean | | | | | | | | | | | | | | median | 75 | 5.625 | 0.2 | 1.33 | 0.036 | 8F |
| Stdev | | | | | | | | | | | | | | n | 30 | 30 | 30 | 30 | 30 | 5? |
| Median | | | | | | | | | | | | | | | | | | | | |

2000 Bullhead Study:
NEWPORT MARSH UPRIVER

| Sample | Method | FHA | HA | HC | C | CC | TLC | TLT | TLL | MSL | EP | SC | TST | | Length | Weight | Liver wt | K | H.S.I. | Sex |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Newport Marsh Up | | | | | | | | | | | | | | | (mm) | (g) | (g) | | | |
| NMU1 (10/2/03) | seine | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 75 | 6.45 | 0.40 | 1.53 | 0.062 | F |
| NMU2 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 75 | 5.40 | 0.20 | 1.28 | 0.037 | M |
| NMU3 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 73 | 5.50 | 0.30 | 1.41 | 0.055 | M |
| NMU4 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 78 | 5.85 | 0.20 | 1.23 | 0.034 | M |
| NMU5 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 77 | 5.50 | 0.15 | 1.20 | 0.027 | ? |
| NMU6 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 76 | 5.25 | 0.35 | 1.20 | 0.067 | M |
| NMU7 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 76 | 6.30 | 0.30 | 1.44 | 0.048 | M |
| NMU8 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 81 | 6.55 | 0.20 | 1.23 | 0.031 | M |
| NMU9 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 75 | 5.75 | 0.20 | 1.36 | 0.035 | M |
| NMU10 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 78 | 6.20 | 0.25 | 1.31 | 0.040 | ? |
| NMU11 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 75 | 5.85 | 0.25 | 1.39 | 0.043 | M |
| NMU12 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 80 | 6.05 | 0.30 | 1.18 | 0.050 | M |
| NMU13 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 71 | 5.55 | 0.25 | 1.55 | 0.045 | M |
| NMU14 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 71 | 3.95 | 0.15 | 1.10 | 0.038 | M |
| NMU15 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 74 | 5.35 | 0.25 | 1.32 | 0.047 | ? |
| NMU16 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 75 | 6.35 | 0.20 | 1.51 | 0.031 | M |
| NMU17 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 80 | 6.70 | 0.25 | 1.31 | 0.037 | ? |
| NMU18 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 73 | 5.25 | 0.20 | 1.35 | 0.038 | M |
| NMU19 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 82 | 6.80 | 0.25 | 1.23 | 0.037 | ? |
| NMU20 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 71 | 5.10 | 0.15 | 1.42 | 0.029 | M |
| NMU21 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 71 | 4.65 | 0.15 | 1.30 | 0.032 | M |
| NMU22 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 75 | 6.05 | 0.35 | 1.43 | 0.058 | M |
| NMU23 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 72 | 4.55 | 0.15 | 1.22 | 0.033 | M |
| NMU24 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 87 | 8.80 | 0.20 | 1.34 | 0.023 | ? |
| NMU25 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 75 | 6.05 | 0.25 | 1.43 | 0.041 | M |
| NMU26 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 70 | 4.55 | 0.15 | 1.33 | 0.033 | ? |
| NMU27 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 90 | 9.20 | 0.30 | 1.26 | 0.033 | M |
| NMU28 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 75 | 5.90 | 0.30 | 1.40 | 0.051 | ? |
| NMU29 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 72 | 4.75 | 0.25 | 1.27 | 0.053 | ? |
| SUM | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | mean | 76.0 | 5.87 | 0.24 | 1.33 | 0.041 | |
| Percent (%) | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | std | 4.7 | 1.11 | 0.07 | 0.11 | 0.011 | |
| Min | | | | | | | | | | | | | | min | 70 | 3.95 | 0.15 | 1.10 | 0.023 | |
| Max | | | | | | | | | | | | | | max | 90 | 9.2 | 0.40 | 1.55 | 0.067 | 19M |
| Mean | | | | | | | | | | | | | | median | 75 | 5.85 | 0.25 | 1.32 | 0.037975 | 1F |
| Stdev | | | | | | | | | | | | | | n | 29 | 29 | 29 | 29 | 29 | 10? |
| Median | | | | | | | | | | | | | | | | | | | | |

2000 Bullhead Study:
MOTIVA

| Sample | Method | FHA | HA | HC | C | CC | TLC | TLT | TLL | MSL | EP | SC | TST | | Length | Weight | Liver wt | K | H.S.I. | Sex |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Motiva** | | | | | | | | | | | | | | | **(mm)** | **(g)** | **(g)** | | | |
| **MO1 (6/7/04)** | **seine** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 74 | 5.85 | 0.10 | 1.44 | 0.017 | M |
| **MO2** | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 79 | 7.45 | 0.20 | 1.51 | 0.027 | F |
| **MO3** | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 87 | 9.3 | 0.20 | 1.41 | 0.022 | F |
| **MO4** | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 80 | 8.55 | 0.10 | 1.67 | 0.012 | M |
| **MO5** | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 71 | 5.1 | 0.10 | 1.42 | 0.020 | M |
| **MO6** | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 73 | 5.55 | 0.15 | 1.43 | 0.027 | F |
| **MO7** | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 76 | 5.7 | 0.05 | 1.30 | 0.009 | M |
| **MO8** | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 90 | 10.85 | 0.05 | 1.49 | 0.005 | M |
| **MO9** | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 70 | 5 | 0.10 | 1.38 | 0.021 | F |
| **MO10 (6/17/04)** | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 75 | 6 | 0.20 | 1.43 | 0.033 | F |
| **MO11** | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 86 | 10 | 0.40 | 1.55 | 0.041 | F |
| **MO12** | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 83 | 8 | 0.25 | 1.36 | 0.032 | M |
| **MO13** | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 70 | 4 | 0.10 | 1.27 | 0.023 | M |
| **MO14** | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 92 | 11 | 0.30 | 1.37 | 0.028 | F |
| **MO15** | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 96 | 12 | 0.30 | 1.37 | 0.025 | F |
| **MO16** | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 72 | 5 | 0.10 | 1.34 | 0.020 | M |
| **MO17** | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 112 | 21 | 0.60 | 1.49 | 0.029 | F |
| **MO18** | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 76 | 6 | 0.10 | 1.29 | 0.018 | M |
| **MO19** | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 104 | 14 | 0.35 | 1.21 | 0.026 | F |
| **MO20** | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 84 | 7 | 0.10 | 1.18 | 0.014 | M |
| **MO21** | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 80 | 7 | | 1.32 | | F |
| **MO22** | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 86 | 9 | 0.20 | 1.40 | 0.022 | M |
| **MO23** | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 78 | 7 | 0.15 | 1.37 | 0.023 | F |
| **MO24** | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 102 | 12 | 0.40 | 1.15 | 0.033 | F |
| **MO25** | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 89 | 11 | 0.20 | 1.51 | 0.019 | M |
| **MO26** | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 75 | 6 | 0.10 | 1.33 | 0.018 | M |
| **MO27** | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 80 | 7 | 0.20 | 1.29 | 0.030 | M |
| **MO28** | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 90 | 11 | 0.15 | 1.56 | 0.013 | M |
| **MO29** | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 110 | 19 | 0.40 | 1.42 | 0.021 | F |
| **SUM** | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 85 | 8.6 | 0.20 | 1.40 | 0.023 | M |
| **Percent (%)** | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | **mean** | **84.2** | **8.7** | **0.20** | **1.39** | **0.022** | |
| **Min** | | | | | | | | | | | | | | **std** | **11.5** | **4.0** | **0.13** | **0.12** | **0.008** | |
| **Max** | | | | | | | | | | | | | | **min** | **70** | **4.35** | **0.05** | **1.1496332** | **0.005** | |
| **Mean** | | | | | | | | | | | | | | **max** | **112** | **20.95** | **0.60** | **1.66992** | **0.041** | |
| **Stdev** | | | | | | | | | | | | | | **median** | **81.5** | **7.6** | **0.20** | **1.39** | **0.022** | **16M** |
| **Median** | | | | | | | | | | | | | | **n** | **30** | **30** | **29** | **30** | **29** | **14F** |

# APPENDIX C

## Glossary of Pathological Terminology

## Glossary of Pathological Terminology

**Neoplasm:** a tumor, either benign or malignant

**Anaplastic:** cancer cells that divide rapidly and bear little or no resemblance to normal cells.

**Granuloma:** a mass of granulation tissue formed in response to injury or inflammation

***Skin tumors***

**Epidermal Papilloma (EP):** The normal linear stratified squamous skin or lip epidermis is thickened due to an increase in cell number, resulting in a buckling pattern of intertwining epidermal pegs which interdigitate with fibrovascular stromal papillae. The basement membrane separating the basal layer of the pegs from the stroma is intact.

**Squamous Carcinoma (SC):** Consists of an epidermal papilloma that has undergone squamous metaplasia, often characterized by the presence of squamous pearls, and which has or appears about to breach the basement membrane and invade the adjacent connective tissue.

***Biliary tumors***

**Cholangiocarcinoma (CC):** A mass of poorly-formed bile ducts with significant increase in periductular fibrosis and an aggressive appearance with may include interdigitating with the normal liver. CCs are sometimes centrally necrotic.

**Cholangioma (C):** A cluster or small mass of well-differentiated bile ducts without increased periductular fibrosis and with a banal appearance.

***Hepatic tumors and pre-neoplastic lesions***

**Focus of Hepatocellular Alteration (FHA) (pre or incipient neoplasms):** a small, <1.0 mm chromophilic focus without cytologic or pattern atypia that blends into the cords of the normal liver. Believed to be in the neoplasm sequence but at a stage where they may still be reversible. Special stains would show reduced iron and glycogen.

**Hepatocellular Adenoma (HA):** A chromophilic lesion usually <1.5 mm with subtle cytologic and/or pattern atypia. Has a banal appearance.

**Hepatocellular Carcinoma (HC):** A lesion usually >1.5 mm with frank cytologic and pattern atypia. Appears to be replacing adjacent liver tissue