

**Tumor Prevalence in Brown Bullheads (*Ameiurus nebulosus*) from  
Darby Creek, John Heinz National Wildlife Refuge at  
Tinicum, Philadelphia, PA**

CBFO-C04-03 and PAFO-C04-02

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## ABSTRACT

The John Heinz National Wildlife Refuge at Tinicum (JHNWRT) is located along Darby Creek near its confluence with the Delaware River, south of Philadelphia, Pennsylvania (USA). The Refuge hosts the largest freshwater tidal marsh in Pennsylvania, about 142 hectares. A 3.2 km reach of Lower Darby Creek was listed as an Environmental Protection Agency Superfund site in 2001 due to a history of contamination and documented sources including Folcroft and Clearview Landfills. In June 2003, for an assessment of habitat quality, we collected 31 brown bullheads (*Ameiurus nebulosus*)  $\geq$  260 mm total length, from a 1.6 km stretch of Darby Creek near the middle of the Superfund area. The objective was to determine the prevalence of liver and skin tumors, preneoplastic lesions, and barbel abnormalities. We reported a 26% (8 of 31) prevalence of liver tumors, primarily hepatocellular carcinomas. Total PAH concentrations in sediments within the sampling area were documented by an EPA contractor to average 48.9 ppm (maximum: 64.8 ppm). Liver tumor prevalence was significantly ( $p < 0.001$ , chi-square test) higher than that observed in an area of the Tuckahoe River (MD), used as a reference area (prevalence = 4% (5 of 117)). It also exceeded the 5% criterion suggested as indicative of highly contaminated areas. Skin tumor prevalence was not significantly different between locations (Darby: 2/31 = 6%, Tuckahoe: 1/117 = 1%,  $p = 0.21$ ), and was less than the 12% suggested criterion. Barbel abnormalities were not significantly different (Darby: 3/31 = 10%, Tuckahoe: 3/87 = 3%,  $p = 0.38$ ). In view of the high liver tumor prevalence and high concentrations of PAHs, we recommend a followup study, including analysis of biomarkers such as biliary PAH metabolites and DNA adducts. Future tumor surveys are recommended for monitoring the effects of active or passive remediation.

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## PREFACE

This report documents the health status of brown bullheads (*Ameiurus nebulosus*) inhabiting the surface water and sediment of Darby Creek, the primary freshwater tidal creek of the John Heinz National Wildlife Refuge at Tinicum, Philadelphia, Pennsylvania. This section of Darby Creek was included on EPA's National Priority List as a Superfund Site on June 14, 2001. This study investigates the liver and skin tumor prevalence in brown bullheads from Darby Creek and compares the results to other those from an uncontaminated area. Study design, implementation, and reporting were completed by staff of the Fish and Wildlife Service's Chesapeake Bay and Pennsylvania Field Offices. All funding for the project was provided by the Service. Histological determinations were conducted by Dr. John Harshbarger at George Washington University Medical Center. The Service requests that no part of this report be taken out of context, and if reproduced, the document should appear in its entirety. The use of trade names in this report is solely for identification purposes and does not constitute an endorsement by the Federal government.

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## INTRODUCTION

### *The Refuge and its Resources*

The John Heinz National Wildlife Refuge at Tinicum (JHNWRT, Figures 1a, 1b, and 1c) was established by Congress in 1972 to preserve diverse fish and wildlife habitat for natural and educational purposes, and is located near the confluence of Darby Creek and the Delaware River, just south of Philadelphia, Pennsylvania. Darby Creek, Cobbs Creek, Muckinipattis Creek, Thoroughfare Creek, and Hermesprota Creek are the major streams that form the Refuge watershed, with Darby Creek being the primary watercourse that essentially bisects the Refuge. The Refuge hosts the largest freshwater tidal marsh in Pennsylvania, approximating nearly 350 acres (142 hectares). Annually, over 100,000 people visit the Refuge to hike, bicycle, canoe, fish, bird watch, photograph nature, receive environmental education, and participate in other outdoor activities.

The diversity of habitat at the Refuge provides the food, cover, and nesting requirements for a variety of wildlife. The tidal marsh historically contained vigorous stands of wild rice. More recently, the marsh is characterized by dense stands of spatter-dock, cattail, phragmites, and countless combinations of associated wetland plant species. The values of this wetland type to birds are magnified in the case of the Refuge marshes because of their strategic location on the Atlantic Flyway. Delaware Bay represents a major interchange on the Atlantic Flyway, and the Refuge is used extensively by migrating birds. Over 280 species of birds have been observed in the Refuge, and the 145-acre impoundment attracts large concentrations of wintering waterfowl. Over 40 species of fish have been documented at the Refuge, including anadromous and catadromous species such as the American shad, white perch, blueback herring, alewife, gizzard shad, and American eel. Almost 30 species of amphibians and reptiles have been reported, including several listed as rare or threatened by the Commonwealth of Pennsylvania. The large snapping turtles that inhabit the Refuge were harvested commercially in the past. In addition, three plant species listed as “proposed rare” by the Commonwealth of Pennsylvania occur in the refuge, and a Pennsylvania “tentatively undermined” rare spike-rush has also been observed there (U.S. Environmental Protection Agency (EPA) and U.S. Fish and Wildlife Service (FWS) 1986).

### *Folcroft Landfill and Annex*

In 1980, Congress authorized the Department of Interior (Public Law 96-315) to purchase the 62-acre Folcroft Landfill and Annex to increase the size of the John Heinz NWR (formerly Tinicum National Environmental Center). The Folcroft Landfill was formerly privately owned by Mr. Wilbur C. Henderson and was permitted from approximately 1959 until 1974 to accept municipal, demolition, and hospital wastes. Because of the alleged hazardous wastes contained at the landfills, Congress directed EPA, in coordination and consultation with the FWS, to investigate potential environmental health hazards resulting from the Folcroft Landfill and to develop alternative recommendations about how such hazards, if any, might best be addressed to ensure the protection of the Refuge and the general public (EPA and FWS 1986).

Over the years of investigation and reporting, many types of wastes have been documented in the Folcroft Landfill and Annex. Investigations have documented elevated soil concentrations of acenaphthene, anthracene, benzo(a)anthracene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, benzo(g,h,i)perylene, chrysene, fluoranthene, dibenzofuran, fluorine, indeno(1,2,3-cd)pyrene, naphthalene, 2-methyl naphthalene, phenanthrene, pyrene, antimony, copper, lead, magnesium, mercury, selenium, silver, vanadium, and zinc (EPA 1989). Folcroft Landfill leachate was acutely toxic to aquatic organisms in bioassays and determined to be a source of aluminum, cyanide, chromium, copper, and nickel (EPA 1989). Groundwater sampling results from 1988 thru 1996 revealed that the following constituents were present in ground water: trans-1,2-dichloroethene; vinyl chloride; 1,2-dichloroethene; 1,1-dichloroethene; 1,1-dichloroethane; 2,2-dichloroethane; chloroform; benzene; trichloroethene; cis-1,2-dichloroethene; chlorobenzene; xylene; arsenic; chromium; copper; iron; lead; manganese; mercury; nickel; and, zinc. Annual groundwater sampling performed by the Service from 1988 thru 2001 showed that groundwater regulatory criteria were exceeded for 1,2-dichloroethene, benzene, chlorobenzene, trichloroethene, vinyl chloride, arsenic, cadmium, chromium, iron, lead, manganese, nickel, and selenium (FWS 2001).

### *Darby Creek*

In 1984, the FWS collected brown bullheads (*Ameiurus nebulosus*) from Darby Creek and submitted them to Dr. Hans Rothenbacher at Penn State University for histological examination. Several bullheads were diagnosed with “hemorrhagic erosive dermatitis” and “fatty livers,” both of which may have resulted from exposure to hazardous substances. This finding, combined with the fact that chlordane and polychlorinated biphenyl (PCB) concentrations in Darby Creek fish were approaching the FDA consumption advisory action level, prompted the Commonwealth of Pennsylvania to issue a public health advisory limiting fish consumption in Darby Creek (EPA and FWS 1986). Commonwealth consumption advisories currently in effect for Darby Creek include: 1) do not eat American eels; 2) 1 meal/month of white perch, flathead catfish, striped bass, and carp; and 3) 6 meals/year of channel catfish. These formal advisories are for PCBs and include the Delaware River mainstem and all tidal tributaries in the southeasternmost three counties in Pennsylvania (Pennsylvania Fish and Boat Commission, 2004).

Because of the long history of contamination and documented sources of contamination to Darby Creek (Folcroft Landfill and Annex, Clearview Landfill, Industrial Drive Properties, Sunoco Oil Darby Creek Tank Farm, former Delaware County Sewage Treatment Plant, former Delaware County Incinerator), EPA proposed the Creek and its major sources of contamination for inclusion on the National Priorities List as a Superfund hazardous waste site on May 11, 2000. Although the site, referred to as the Lower Darby Creek Area, was known to have many suspected sources of contamination (Federal Register 2000), only three source areas (Folcroft Landfill, Folcroft Annex, and Clearview Landfill) and approximately 3.2 km of Darby Creek were included in the final listing on June 14, 2001 (Federal Register 2001).



Polycyclic aromatic hydrocarbons (PAHs) have been documented for many years at varying concentrations on and adjacent to the JHNWRT and in Darby Creek. Refuge areas previously identified with elevated PAH concentrations include the soil on Folcroft Landfill and Annex (EPA 1989), sediments in Darby Creek (EPA 1989 and EPA 1998), and the site of the Cuasano Environmental Education Center (PMC Environmental, 2001). Off-site areas known to have elevated PAHs include the upstream Clearview Landfill soils and leachate (EPA and FWS 1986), many potential sources in the highly urban setting surrounding the upstream portions of Darby Creek, Cobbs Creek, and Naylor's Run (EPA and FWS 1986; EPA 1989, EPA 1998) and parcels adjoining the refuge and Darby Creek to the south of Folcroft Landfill (PMC 1999). Soil and sediment total PAH concentrations in Darby Creek and the above-mentioned sites are generally well above background concentrations.

EPA awarded a contract to TetraTech/Black & Veatch to conduct Remedial Investigation/Feasibility Study (RI/FS) activities for Clearview Landfill and Darby Creek (Tetra Tech/Black&Veatch 2001). Another Principal Responsible Party (PRP) group is conducting formal RI assessment activities on Folcroft Landfill. Quantitative sampling in Darby Creek to date has revealed that total PAHs in sediments are clearly elevated. Darby Creek sediments were collected from several areas over which we collected bullheads for this investigation. Mean concentration across the area from which we collected bullheads (Samples Numbers 25 thru 30; n=6) was 48.9 ppm (range: 36.3 to 64.8 ppm, TetraTech 2004).

### *Tumors*

The prevalence of tumors in wild fish has been used as an indicator of environmental quality in saltwater (Malins *et al.* 1987; Vogelbein *et al.* 1990; Myers *et al.* 1994) and freshwater (Baumann *et al.* 1991, 1996, Smith *et al.* 1994) ecosystems. The strongest evidence for chemical etiology exists for polynuclear aromatic hydrocarbons (PAHs) in sediments, which have been implicated in the development of liver tumors (e.g., Baumann *et al.* 1991; Malins *et al.* 1987; Vogelbein *et al.* 1990). A cause and effect relationship between PAHs and liver tumors or preneoplastic lesions in fish has been established by experimental studies (Metcalf *et al.* 1988; Hawkins *et al.* 1990). Further evidence linking PAHs in sediments with liver tumors was developed by Baumann and Harshbarger (1998) from surveys conducted in the 1980s and 1990s with bottom-feeding brown bullheads (*Ameiurus nebulosus*) in the Black River, Ohio. They observed that liver tumor prevalence increased and decreased according to changes in sediment PAH concentrations.

Because fish rapidly metabolize PAHs (Krahn *et al.* 1986), researchers have used biomarkers rather than tissue concentrations to demonstrate exposure and response to these chemicals. Exposure is best determined by the presence of PAH-like metabolites in bile which indicates recent exposure on the order of days (Collier and Varanasi 1991). The presence of a diagonal radioactive zone (DRZ) on chromatograms of liver DNA results from polycyclic aromatic compounds (PACs) forming adducts with DNA. These adducts can be an early stage in the development of cancer (Reichert *et al.* 1998). In the Anacostia River (Washington, DC), Pinkney *et al.* (2004a) reported a 50% to 68% liver tumor prevalence in brown bullheads. They

noted sediment PAH concentrations in the 15 to 30 ppm range in the collection area, and high concentrations of PAC-DNA adducts in liver and PAH metabolites in bile. The authors cited these observations as a weight of evidence implicating PAHs as a contributor to the high tumor prevalence.

Skin tumors in brown bullhead have been induced by repeatedly painting the skin with sediment extracts containing high PAH concentrations (Black *et al.* 1985). Grizzle *et al.* (1984) observed an increased prevalence of papillomas in black bullhead (*Ameiurus melas*) exposed to chlorinated wastewater effluent, and prevalence decreased when chlorination was decreased. In a summary of studies conducted in the Laurentian Great Lakes, Baumann *et al.* (1996) reported that higher oral and cutaneous tumor prevalence occurred in PAH-contaminated tributaries compared with reference sites. Poulet *et al.* (1994), however, noted the occurrence of orocutaneous tumors in 94 brown bullheads collected from 17 locations (both contaminated and uncontaminated) in New York State. They found that the distribution of lesions did not suggest a strict correlation with chemical carcinogens. Spitsbergen and Wolfe (1995) stated that, in addition to PAHs, nitrosamines may contribute to both skin and liver tumor development in brown bullhead. Bunton (2000) concluded that, although skin tumors in brown bullhead are associated with bottom-dwelling and feeding and contact with contaminated sediments, other factors may also be involved.

Environmental managers in the Great Lakes have used the presence of tumors (especially liver tumors) as a criterion for identifying and prioritizing contaminated areas or Areas of Concern. Baumann *et al.* (1996) summarized tumor data from the Great Lakes and reported that, in brown bullheads, liver tumor prevalence exceeding about 9% and skin tumor prevalence exceeding about 20% were nearly always observed in chemically contaminated areas. Baumann (2002) suggested criteria of about 5% liver tumors and 12% skin tumors for distinguishing highly contaminated Great Lakes Areas of Concern from less contaminated Areas of Recovery.

Horness *et al.* (1998) applied regression analysis to a data base of West Coast tumor surveys. They proposed 2.8 ppm total PAHs (confidence limit of 0.011-5.5 ppm; all sediment concentrations as dry weight) as a threshold sediment concentration for an increased prevalence of hepatic lesions in bottom-dwelling English sole (*Pleuronectes vetulus*). No similar statistical analyses have been performed with brown bullheads. The Anacostia bullheads had a dramatic liver tumor response (50-68%) when exposed to sediment PAHs in the 15 to 30 ppm range (Pinkney *et al.* 2004a). In contrast, in Back River (near Baltimore, MD) where a transect through the collection area averaged  $6.5 \pm 6.4$  ppm total PAHs, liver tumor prevalence was only 8% (Pinkney *et al.* 2004b). Whereas considerably more data are needed to establish a threshold sediment PAH concentration for tumor response in bullheads, these data may provide a rough outline. It is possible, however, that other chemicals may also be playing a role in these systems.

## OBJECTIVES

Since exposure to sediment PAHs at the concentrations reported in Darby Creek has been implicated in increased tumor prevalence in bullheads (Pinkney *et al.* 2004a and others), we designed this preliminary evaluation with the following objectives:

1. Determine existing liver tumor, skin tumor, and barbel abnormality prevalence in Darby Creek;
2. Compare the prevalence with those from an unimpaired freshwater tidal ecosystem and Baumann's suggested criteria, and,
3. If the tumor prevalence is significantly elevated, evaluate existing contaminant data to make a determination of potential contaminant-related causation and recommendations for further action.

## MATERIALS AND METHODS

### *Site Selection and Sampling*

A group of 31 brown bullheads (total lengths:  $\geq 260$  mm) was collected from Lower Darby Creek (Figure 1) on June 9-11, 2003. The upper limit of sampling was about 200 meters upstream of the Refuge's canoe launch (39.89505 EN, 75.25670EW), the northernmost portion of the JHNWRT. The lower limit was at the rock weir (39.88591 EN, 75.26473EW), across from Folcroft Landfill. This stretch of the creek is approximately 1.6 km and approximated the middle of the 3.2 kilometers of Darby Creek listed as the Superfund site. A few fish were collected by baited hook and line, but most were obtained by boat electroshocking. The electroshocking unit consisted of a 4.9 meter jon boat equipped with a 3000-watt John Deere gasoline generator. AC current was passed through a 2000-watt capacity Coffelt conversion box and transmitted to the water with various metal dropper electrodes. Electroshocking was most successful on the last few hours of outgoing tides where visibility reached three to four feet. All fish were netted and held in the boat in plastic tubs containing site water until transferred to the Refuge holding facility. The holding facility consisted of a temperature controlled room, where fish were held in 47-liter plastic coolers with aeration for no longer than 48 hours. Fish were transported with aeration to the Chesapeake Bay Field Office where necropsies were conducted.

A total of 117 fish were collected from the Tuckahoe River, on the Eastern Shore of Maryland over a total of 4 collections conducted in 1996, 1998, 2000, and 2001. These fish were used as a reference location for studies of the tidal Potomac River (Pinkney *et al.* 2001), Anacostia River, Washington, DC (Pinkney *et al.* 2004a), and two tributaries near Baltimore (Pinkney *et al.* 2004b). Otter trawling was used to collect the Tuckahoe bullheads, which school in a 3-5 meter deep bend in the river (38.8674EN, 75.9352EW; Figure 1). In these studies, a minimum length of 260 mm was also required.

### *Laboratory Procedures*

Darby Creek bullheads were maintained for one to two days in coolers with aerated collection-site water. They were measured for total length, weighed to the nearest gram, euthanized by severing the spinal cord, and necropsied. Condition factor,  $K = (\text{wt (g)} \times 10^5) / \text{length (mm)}^3$ , was calculated. Gross lesions were noted and the liver was excised and weighed so that the hepatosomatic index (HSI=liver wt/body wt) could be determined. Sex was noted. Livers and skin areas with visible lesions were preserved in 10% buffered formalin and transported to George Washington University Medical Center (Washington, DC) for histopathological examination and tumor diagnosis. The tissues were processed according to standard histological procedures, sectioned at 6  $\mu$ m, and stained with hematoxylin and eosin (Luna 1968). Four tissue blocks were sectioned from each liver and two from each skin lesion. At least two sets of slides were prepared from each tissue block for histopathological examination. All materials from the study were maintained and case reports will be entered into the Registry of Tumors in Lower Animals. Procedures for Tuckahoe bullheads were identical to those used for Darby Creek.

### *Data Analysis*

Histopathological data were summarized as the prevalence of the various types of lesions among the collections of bullheads. A glossary of terms is provided in Appendix A. Lower Darby Creek and the Tuckahoe River are in different watersheds and more than 150 km apart, so that crossover of fish from the two rivers is highly unlikely. In tidal freshwater rivers, bullheads may be highly localized. Sakaris *et al.* (2002) estimated that the linear home range of bullheads in the tidal Anacostia River, Washington, DC ranged from 0.5 to 2.1 km, depending on the season.

The prevalence of liver and skin lesions and barbel abnormalities between the two locations were compared using two-tailed, chi-square analysis (Sokal and Rohlf 1981), with a critical  $p$  value of 0.05. Biological data including the length, weight, condition factor, and HSI were compared using  $t$ -tests, with log-transformation if necessary to meet the assumptions for parametric statistics. If these assumptions could not be satisfied, a Mann-Whitney U test was used to compare the median values for the two groups. A  $p$  value of 0.05 was used.

## **RESULTS**

### *Biological Data*

In general, the Darby Creek bullheads were much larger than the Tuckahoe bullheads. Although a 260 mm minimum total length was used for both collections, the smallest Darby Creek bullhead was 285 mm. The median length (335 mm) of the Darby Creek bullheads was significantly greater than the Tuckahoe bullheads (267 mm,  $p < 0.001$ , Mann-Whitney test, Table 1). The Darby Creek bullheads also had significantly greater median weight (525 g) than Tuckahoe (237 g,  $p < 0.001$ , Table 1). The greater weight was reflected in the median condition factor (1.43 - Darby, 1.24 - Tuckahoe,  $p < 0.001$ ).

Mean HSI was also significantly greater in the Darby Creek bullheads ( $0.025 \pm 0.006$ ) than the Tuckahoe bullheads ( $0.019 \pm 0.003$ , ANOVA,  $p < 0.001$ ). Aging was not attempted on the Darby Creek bullheads, although the spines have been archived. Thus it is not possible to compare ages among the collections.

### *Pathology*

Gross examination revealed that three of the 31 Darby Creek bullheads had abnormal (either shortened, clubbed, or missing) barbels. The prevalence of abnormalities on one or more barbels was therefore 10% in the Darby Creek bullheads compared with 3 of 87 examined for barbel abnormalities in the Tuckahoe collections (3%). Using a two-tailed chi-square test, this difference is not statistically significant ( $p = 0.38$ , Table 2).

Upon gross examination of the Darby Creek bullheads, two individuals had oral lesions that were preserved for histopathology. One fish (DCB04) had two visible lesions—one on the upper dental ridge and one on the lower. These were 1-2 cm pale pink nodules. DCB11 had a nodular mass of similar size on the upper inside lip, two on the dental plate and one on the lower lip. These are described as polycentric, exophytic (growing outward from an epithelial surface), papillary skin neoplasms originating from the proliferation, piling and pegging of oral epithelium. Tumor pegs, rimmed by a basal cell layer of columnar cells, were bordered by a collagenous basement membrane derived from interdigitating, fibrovascular, dermal papillae. Tumor cells had become squamate (flattened) in the center of some pegs. Some tumor cells had breached the basement membrane and were invading the adjacent connective tissue. Invasion is a defining characteristic for cancer and both skin neoplasm cases were diagnosed as squamous cell carcinoma (SC) of oral mucosa (Figures 2 and 3, see Appendix A for abbreviations and glossary). The prevalence of squamous carcinomas in the Darby Creek bullheads (2 of 31 = 6%) was not statistically significant when compared with the 0 of 117 (0%) prevalence in the Tuckahoe collections ( $p = 0.059$ ) or with the 1 of 117 (1%) prevalence of skin tumors (either invasive or non-invasive,  $p = 0.21$ , Table 2).

No visible liver lesions were observed upon gross examination. Eight of the 31 (26%) Darby Creek bullheads had neoplasms originating within the liver parenchyma: six from hepatocytes and two from closely related bile duct cells sometimes called cholangiocytes. Five were hepatocellular carcinomas (HC) (Figure 4 and 5), one was a cholangiocarcinoma (CC) (Figure 6), one was a hepatocellular adenoma (HA) and one was a cholangioma (C). The six tumors interpreted as carcinomas had features consistent with invasive cancerous behavior. The lesions, diagnosed as C and HA were neoplasms that had not yet progressed to the invasive (cancerous) stage.

Three Darby Creek bullheads had more than one lesion. DCB-04 had 5 Foci of Hepatocellular Alteration (FHA - the precursor lesion) one HA, one HC, and a SC. DCB-11 had two SC's and DCB-29 had one FHA and one HA.

Based on histopathological examinations, there were significantly higher prevalences of hepatocellular carcinomas (HC), total liver cancers (TLC, including invasive hepatocellular or bile duct tumors), and total liver tumors (TLT, either invasive or non-invasive hepatocellular or bile duct tumors) in Darby Creek bullheads. For example, HC prevalence was 5 of 31 (16%) in Darby Creek bullheads compared with 0 of 117 (0%) in Tuckahoe bullheads (chi-square,  $p < 0.001$ , Table 2). TLT prevalence was 8 of 31 (26% in Darby Creek vs. 5 of 117 (4%) in the Tuckahoe River ( $p < 0.001$ ). A spreadsheet containing all biological and pathological data is provided as Appendix B.

## DISCUSSION

### *Tumors*

The 26% liver tumor prevalence in the Darby Creek bullheads raises concern that these fish are responding to exposure to carcinogens in the sediments and/or water column. This prevalence is about five times the 5% suggested by Baumann (2002) as a criterion for distinguishing between highly contaminated and less contaminated areas. In contrast, the 6% prevalence of skin tumors in the Darby Creek bullheads is less than the 12% criterion suggested by Baumann (2002).

Tumor prevalence is known to increase with age (Baumann *et al.* 1990, Baumann 2002). Because the Darby Creek bullheads were not aged, it is necessary to make inferences based on length, which can be misleading. However, in view of the large difference in median length (335 vs. 267 mm), it is safe to assume that the Darby Creek bullheads were somewhat older than those from the Tuckahoe (which were in the 3-5 year range, Pinkney *et al.* 2001, 2004a, b). In an ideal study, statistical comparisons would only be made between same aged fish. Unfortunately, cost limitations in the design of this study made it necessary to rely on the existing Tuckahoe database for a reference and did not allow for aging of the Darby Creek bullheads. Nevertheless, the liver tumor prevalence of 26% is a clear indicator that fish inhabiting this area are responding to carcinogen exposure and that the area could be classified as contaminated.

### *Barbel abnormalities*

There is no suggested criterion for barbel abnormalities. The 10% prevalence we observed in bullheads from Darby Creek was not statistically different from the 3% observed in the Tuckahoe River bullheads. Other researchers have reported barbel abnormality prevalences of about 5% in cleaner areas compared with greater than 40% in contaminated areas. Smith *et al.* (1994) stated that the presence of abnormal barbels was correlated with the presence of elevated PAHs in sediments. They reported an abnormal barbel prevalence of about 45% and 70% in fish from the contaminated Black and Cuyahoga Rivers, respectively, compared with about 5% from the cleaner Huron River. Steyermark *et al.* (1999) reported a nearly 40% prevalence of abnormal barbels in brown bullheads from the industrialized Schuylkill River (Philadelphia) vs. about 5% in a New Jersey pond not affected by industry. In the highly contaminated Anacostia River (Washington, DC) Pinkney *et al.* (2004a) reported barbel abnormalities of 23%, 47%, 53%, and 56% in four collections in 2000-2001. In two tributaries near Baltimore, MD with industrial and

sewage treatment plant inputs, Pinkney *et al.* (2004b) reported barbel abnormalities of 10% and 28%.

Smith *et al.* (1994) stated that barbel abnormalities are more frequently observed in larger fish (those approaching or greater than 300 mm total length). In Darby Creek, despite the large size of the fish and the high prevalence of liver tumors, only a low to moderate prevalence of barbel abnormalities has been documented.

#### *Linkage between Tumors and PAHs in the Sediments*

In the absence of biomarker data, especially PAC-DNA adduct work, it is not possible to link the tumor prevalence with specific contaminants. However, the detection of total PAHs as high as 124.7 ppm suggests that exposure to PAHs is contributing to the elevated tumor prevalence. To evaluate the significance of the Darby Creek PAH sediment data on a local basis, we compared that data to a regional Delaware River sediment data set (Academy of Natural Sciences of Philadelphia (ANSP) 1996). The ANSP data are a compilation of sediment samples collected along a 40 kilometer stretch of the Delaware River near Philadelphia, mostly collected in support of maintenance dredging projects. The maximum concentration of total PAHs found in the Delaware River was 40.1 ppm with a mean concentration among 71 samples of 5.2 ppm. The maximum Darby Creek PAH concentration of 124.7 ppm and the average of 48.9 ppm along the 1.6 kilometer stretch of our sampling area (TetraTech 2004) is far in excess of typical Delaware River sediment concentrations. Consistent with the home range of 0.5-2.1 km estimated by Sakaris *et al.* (2002) for brown bullheads in a tidal freshwater river, it is likely that contaminant exposure in Darby Creek, rather than the Delaware River is being reflected with these data.

#### *HSI and Condition Factor*

The mean HSI in the Darby Creek bullheads (0.025 units) was similar to those reported previously (0.025 and 0.023) in Anacostia bullheads (Pinkney *et al.* 2001) which had a greater than 50% liver tumor prevalence. The overall HSI for the Tuckahoe bullheads (0.019) is based on collections where HSI has ranged from 0.015 to 0.021. Within the Tuckahoe collections, no liver lesions were reported when HSI was 0.015 whereas the highest liver lesion prevalence (23% - primarily foci of hepatocellular alteration) was reported when HSI was 0.021.

A similar pattern (higher lesion frequency with higher HSI) was reported in a survey of four Great Lakes sites by Baumann *et al.* (1991) who determined mean HSIs of 0.026 for bullheads from the Cuyahoga River and 0.026 for those from Munuscong Lake, both sites where bullheads had liver tumors. Baumann *et al.* (1991) did not detect liver tumors in bullheads from the Fox River (HSI=0.022) or Menominee River (HSI=0.020). Higher HSI values were also reported by Arcand-Hoy and Metcalfe (1999) in collections from two Great Lakes contaminated sites (0.024 and 0.030) compared to a control site (0.020). Fabacher and Baumann (1985) reported HSI values of 0.047 for male and 0.057 for female bullheads from the Black River compared with 0.017 to 0.027 in fish from uncontaminated locations. Pinkney *et al.* (2001, 2004a), using

logistic regression, identified HSI as a significant risk factor for both preneoplastic and neoplastic liver lesions in brown bullheads.

Based on these data, the Darby Creek bullheads have HSIs in the range that has been associated with tumors and preneoplastic lesions whereas the Tuckahoe fish are in the control range. Sex and seasonal effects on liver enlargement, however, must be taken into consideration (Fabacher and Baumann 1985).

The median K of 1.43 in the Darby Creek bullheads exceeds the mean 1.30 value reported by Sinott and Ringler (1987) for bullheads in a highly productive eutrophic lake. The variability in K between systems is shown by a study of brown bullheads in PCB-contaminated and uncontaminated sections of the Hudson River (Kim *et al.* 1989). The mean K values in fish from the contaminated and clean sections of the River were 1.30 and 1.50, respectively. Pinkney *et al.* (2001, 2004a) reported median K values of 1.20-1.31 in bullheads from the Anacostia River, which overlaps with the median K value of 1.24 from the Tuckahoe. Previously, Pinkney *et al.* (2004b) reported a nearly equally high median K value of 1.41 in Furnace Creek, a tidal tributary of the Patapsco River which receives inputs from many industrial facilities. Thus, the 1.43 median K in Darby Creek suggests that the fish are robust, probably due to high food availability.

## CONCLUSIONS AND RECOMMENDATIONS

We report a 26% prevalence of liver tumors and a 6% prevalence of skin tumors in  $\geq 260$  mm brown bullheads from Lower Darby Creek. The liver tumor prevalence is indicative of a contaminated habitat; it is more than five times the Baumann (2002) criteria for distinguishing highly contaminated Areas of Concern from less contaminated Areas of Recovery.

A more comprehensive study would include a reference area with similar-aged fish, and would utilize biomarkers to document exposure (including tissue chemistry and bile PAH metabolites), and response (DNA adducts). At this point, we suspect but cannot prove a linkage with PAHs based on the elevated concentrations detected in sediments in the collection area, the body of literature linking PAHs with tumors in bullheads, and the movement data. Brown bullhead tumor surveys should be used as a tool to monitor the recovery of the ecosystem, whether the remedy is active or passive.

A focused effort should be undertaken to identify and eliminate the source of highly elevated PAHs in Darby Creek. EPA (1984) identified 19 significant disposal or fill sites adjacent to Darby Creek from 1953 – 1983, including many sites that should still be considered significant potential sources of PAHs to Darby Creek (12 acres of formerly open industrial impoundments on the Darby Creek Tank Farm and a 13.5 acre commercial dump/auto repair/junkyard operation near 84<sup>th</sup> Street). Both of these sites are in the vicinity of the highest PAH sediment concentration found in the recent RI data (124.9 ppm total PAHs).



We note that although there was a large 190,000 gallon crude oil pipeline spill within the JHNWRT 145-acre freshwater impoundment in 2001, no oil reached Darby Creek. Extensive sediment sampling immediately after the spill indicated that the oil, and the residual PAHs were contained in an approximate 3-acre area of the freshwater impoundment (Entrix 2000). Groundwater sampling showed very low levels of contamination and should not be considered a viable pathway to Darby Creek. Therefore, it would be inappropriate to link any adverse effects observed in this investigation to the Sunoco crude oil pipeline spill.

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## **TABLES**

Table 1. Biological data for the Darby Creek and Tuckahoe River brown bullheads<sup>a</sup>.

	<b>Lower Darby Creek</b> (n=31)	<b>Tuckahoe River</b> (n=117)	<b>Statistics<sup>b</sup></b>
<b>Length (mm)</b>	335 (285-365) A	267 (260-298) B	Kruskal-Wallis (K-W);p<0.001
<b>Weight (g)</b>	525 (344-731) A	237 (197-337) A	K-W, p<0.001
<b>Liver weight (g)</b>	12.83±3.66	4.52±0.91	Not compared
<b>Condition Factor (K)</b>	1.43 (1.15-1.72) A	1.24 (1.04-1.43) A	K-W;p<0.001
<b>Hepatosomatic Index (HSI)</b>	0.025± 0.006A	0.019±0.003 B	ANOVA;p<0.001 <sup>c</sup>
<b>Sex</b>	15M,13F, 3?	81M, 27F, 9?	

<sup>a</sup> Mean ± one standard deviation (SD) or median with range in parentheses.

<sup>b</sup> Groups with different letters are significantly different at p<0.05 using Tukey's test (ANOVA) or Dunn's method (K-W).

<sup>c</sup> Log-transformed data used in ANOVA, untransformed means and SD reported.

Table 2. Summary of lesion data and statistical comparisons (two-tailed chi-square test with Yates correction for continuity).

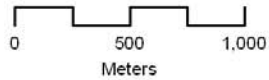
<b>Lesion<sup>a</sup></b>	<b>Lower Darby Creek (n=31)</b>	<b>Tuckahoe River (n=117)</b>	<b>P value</b>
<b>Focus of hepatocellular alteration (FHA)</b>	2 (6%)	8 (7%)	0.74
<b>Hepatocellular adenoma (HA)</b>	2 (6%)	2 (2%)	0.41
<b>Hepatocellular carcinoma (HC)</b>	5 (16%)	0 (0%)	<0.001
<b>Cholangioma (C)</b>	1 (3%)	2 (2%)	0.85
<b>Cholangiocarcinoma (CC)</b>	1 (3%)	1 (1%)	0.89
<b>Total liver cancers (TLC)</b>	6 (19%)	1 (1%)	<0.001
<b>Total liver tumors (TLT)</b>	7 (22%)	5 (4%)	0.003
<b>Total liver lesions (TLL)</b>	8 (26%)	13 (11%)	0.073
<b>Epithelial papilloma (EP)</b>	0 (0%)	1 (1%)	0.47
<b>Squamous carcinoma (SC)</b>	2 (6%)	0 (0%)	0.059
<b>Total skin tumors (TST)</b>	2 (6%)	1 (1%)	0.21
<b>Altered barbels</b>	3 (10%)	3 (3%) <sup>b</sup>	0.38

<sup>a</sup> see Appendix A for glossary of pathological terms.

<sup>b</sup> n=87 (barbels not examined in 1996 sampling of 30 fish)



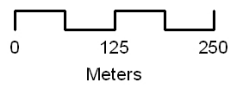
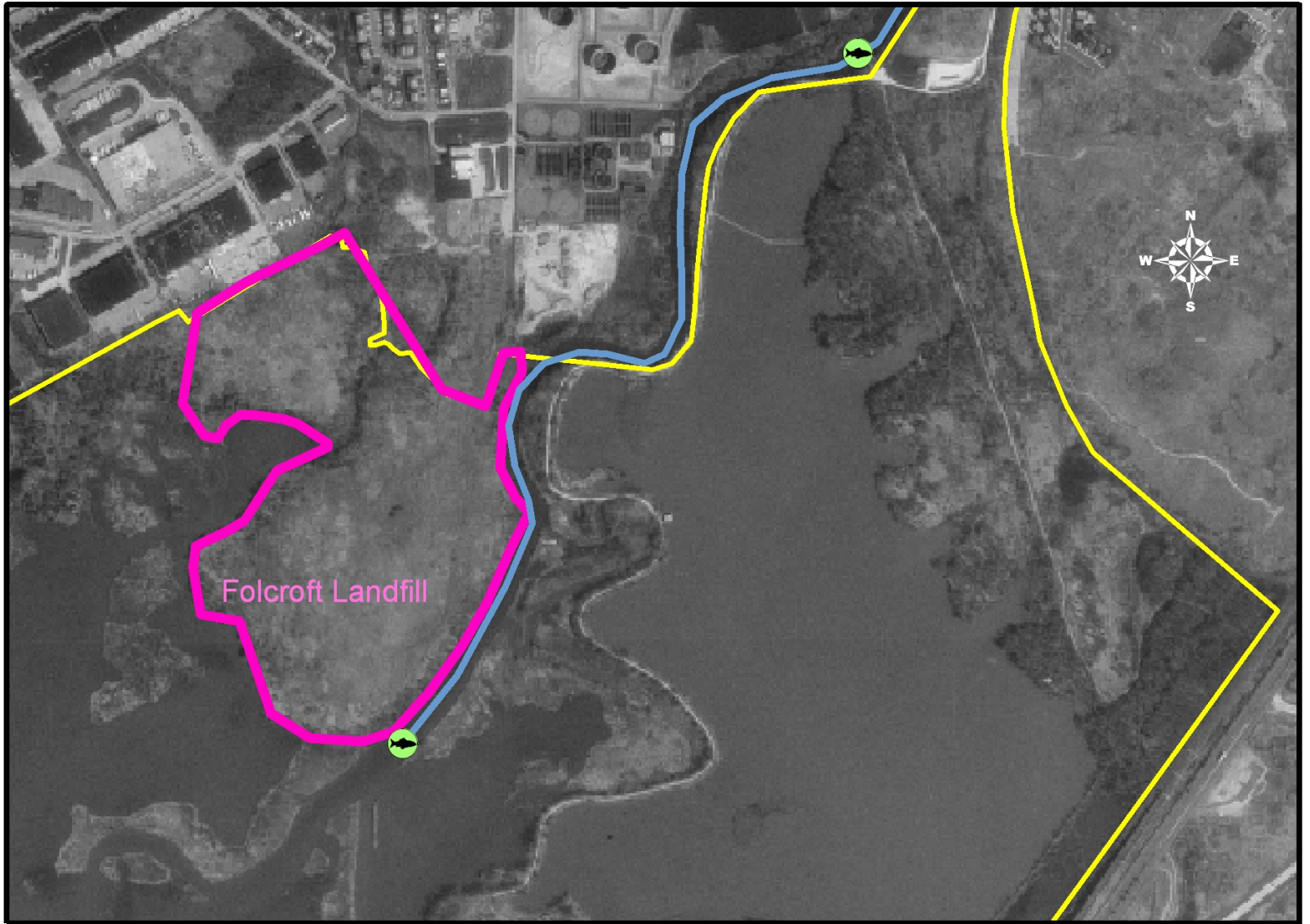
## **FIGURES**



**Figure 1a**  
**John Heinz National Wildlife Refuge**

**Legend**

-  Refuge Boundary Line
-  Darby Creek
-  Landfill Boundary
-  Fish Sample Reach



**Figure 1b**  
**John Heinz National Wildlife Refuge**

**Legend**



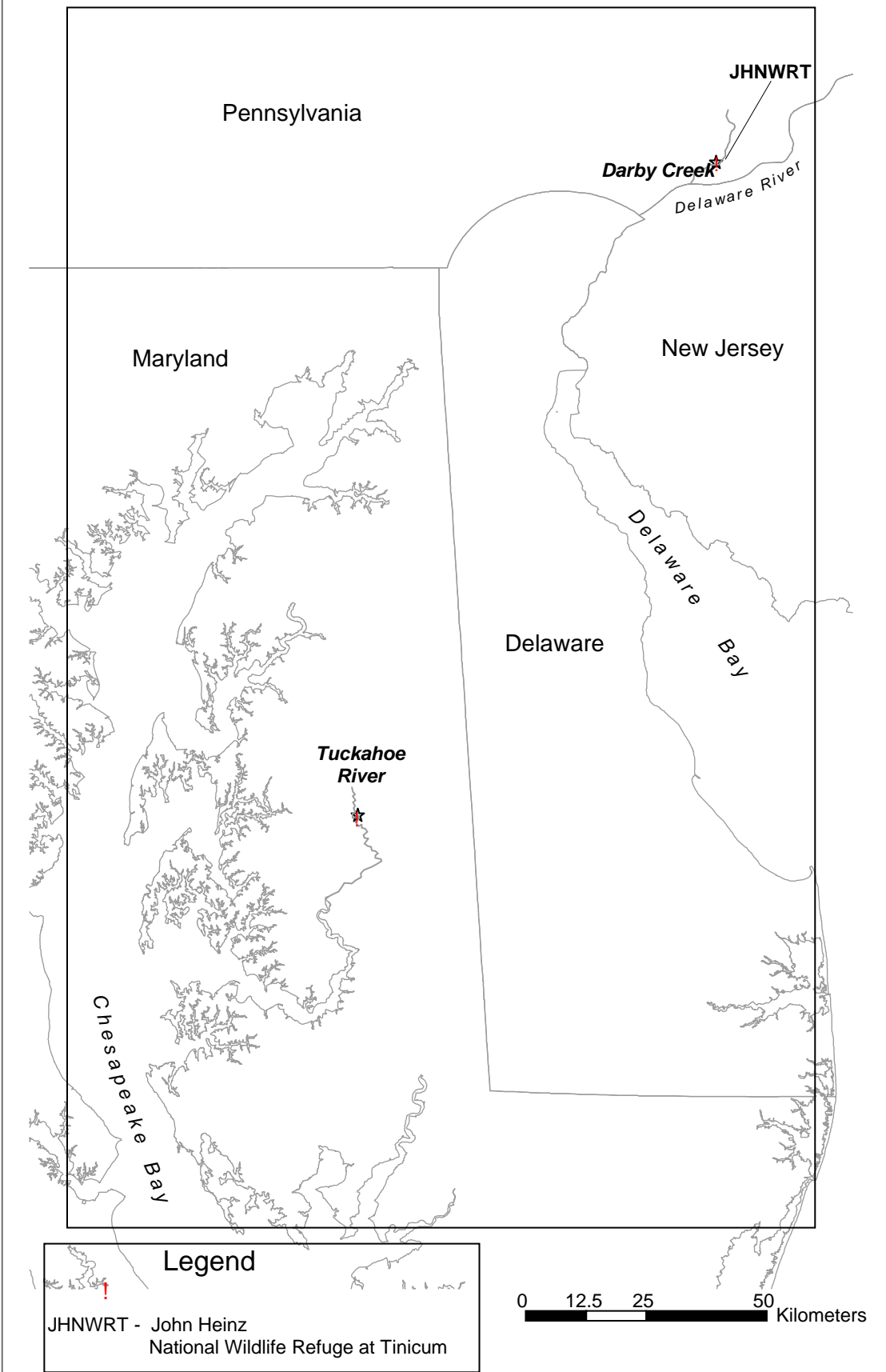
-  Refuge Boundary Line
-  Darby Creek
-  Landfill Boundary
-  Fish Sample Reach

Figure 1c. Darby Creek and Tuckahoe River.



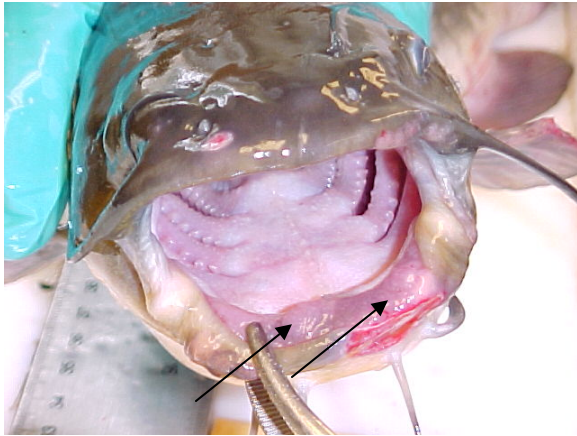


Figure 2a. Photograph of DCB-11 later diagnosed with squamous cell carcinomas on the lower dental ridge.

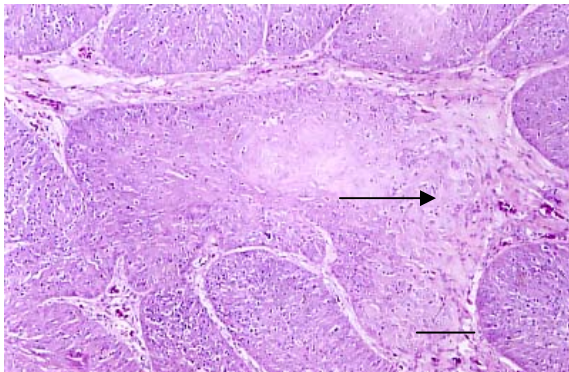


Figure 2b. Squamous cell carcinoma (DCB-11). Cells from tip of one peg in the array (arrow) have breached the basement membrane and are invading the connecting tissue. Bar = 83  $\mu$ m.

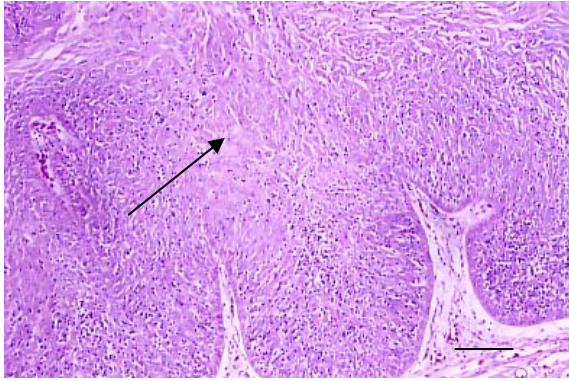


Figure 3. Squamous cell carcinoma (DCB-04). A distinct basal cell layer rims the pegs of tumor but the collagenous basement membrane between the basal layer and the connective tissue is not visible at this magnification. Squamous cells are within the tumor (arrow). Invasion is not shown in this view. Bar = 83  $\mu$ m.

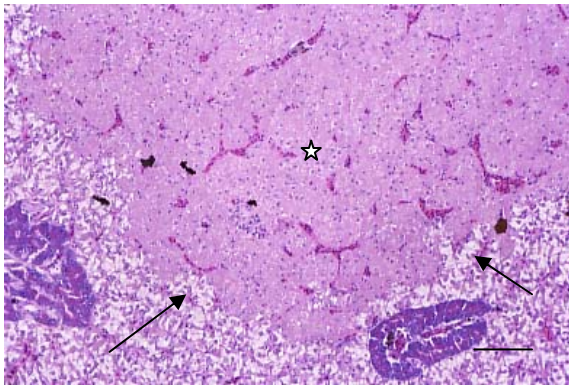


Figure 4. Hepatocellular carcinoma (DCB-20). Mass of tumor cells (star) is invading and replacing normal liver rather than just expanding and compressing it. Arrows point to the scalloped leading edge of the tumor invading normal liver. Bar = 83  $\mu$ m.

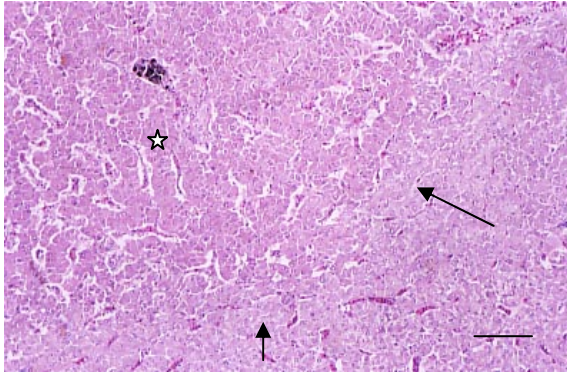


Figure 5. Hepatocellular carcinoma (DCB-02). This mass of tumor cells (star) has a more distinct trabecular pattern than the tumor shown in Figure 4. Arrows point to the scalloped leading edge of the tumor invading normal liver. Bar = 83  $\mu\text{m}$ .

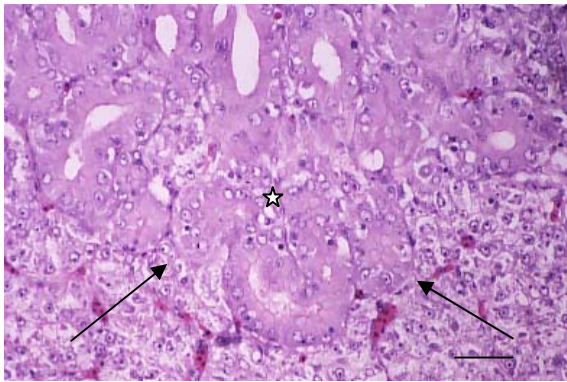


Figure 6. Cholangiocarcinoma (DCB-10). Cancer cells are forming a ductal neoplasm (star). Arrows point to the scalloped leading edge of the tumor invading adjacent normal liver. Bar = 33  $\mu\text{m}$ .

**APPENDIX A**

**GLOSSARY OF PATHOLOGICAL TERMINOLOGY**



## GLOSSARY OF PATHOLOGICAL TERMINOLOGY

### 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.

### Other Terms

**Metaplasia**: Change in the type of cells in a tissue to a form which is not normal for that tissue.

**Squamous**: Scaly or platelike.

**Exophytic**: a neoplasm or lesion that grows outward from an epithelial surface.

## **APPENDIX B**

### **SPREADSHEETS WITH BIOLOGICAL AND PATHOLOGICAL DATA**

1996, 1998, 2000, 2001  
Tumor Data  
(Tuckahoe)

Sample	Method	FHA	HA	HC	C	CC	TLC	TLT	TLL	EP	SC	TST	AB	Tot. Length (mm)	Wt (g)	Liver wt (g)	Condition factor	H.S.I.	Age	Sex
TBB01 -11/13/96	trawl	0	0	0	0	0	0	0	0	0	0	0	N/M	277	247	4.80	1.16	0.019	4	F
TBB02		0	0	0	0	0	0	0	0	0	0	0	N/M	272	235	4.25	1.17	0.018	NA	F
TBB03		0	0	0	0	0	0	0	0	0	0	0	N/M	273	251	4.70	1.23	0.019	4	M
TBB04		0	0	0	0	0	0	0	0	0	0	0	N/M	277	269	6.10	1.27	0.023	4	F
TBB05		0	0	0	0	0	0	0	0	0	0	0	N/M	271	246	4.30	1.24	0.017	4	F
TBB06		0	0	0	0	1	1	1	1	0	0	0	N/M	273	241	5.85	1.18	0.024	4	F
TBB07		0	0	0	0	0	0	0	0	0	0	0	N/M	267	230	4.95	1.21	0.022	3	F
TBB08		0	0	0	0	0	0	0	0	0	0	0	N/M	265	230	4.60	1.24	0.020	NA	M
TBB09		0	0	0	0	0	0	0	0	0	0	0	N/M	263	225	4.85	1.24	0.022	3	F
TBB10		0	0	0	0	0	0	0	0	0	0	0	N/M	265	208	3.25	1.12	0.016	NA	M
TBB11		0	0	0	0	0	0	0	0	0	0	0	N/M	261	231	5.45	1.30	0.024	3	F
TBB12		0	0	0	0	0	0	0	0	0	0	0	N/M	265	205	4.40	1.10	0.021	4	F
TBB13		0	0	0	0	0	0	0	0	0	0	0	N/M	265	236	5.25	1.27	0.022	3	M
TBB14		0	0	0	0	0	0	0	0	0	0	0	N/M	267	241	4.35	1.27	0.018	4	M
TBB15		1	0	0	0	0	0	0	1	0	0	0	N/M	266	236	6.20	1.25	0.026	3	M
TBB16		0	0	0	0	0	0	0	0	0	0	0	N/M	265	215	4.20	1.16	0.020	3	F
TBB17		0	0	0	0	0	0	0	0	0	0	0	N/M	274	233	4.70	1.13	0.020	4	F
TBB18		0	0	0	0	0	0	0	0	0	0	0	N/M	262	214	4.00	1.19	0.019	3	M
TBB19		0	0	0	0	0	0	0	0	0	0	0	N/M	271	220	6.15	1.11	0.028	4	M
TBB20		0	0	0	0	0	0	0	0	0	0	0	N/M	271	234	3.95	1.18	0.017	NA	M
TBB21		0	0	0	0	0	0	0	0	0	0	0	N/M	271	270	6.35	1.36	0.024	4	M
TBB22		0	1	0	0	0	0	1	1	0	0	0	N/M	263	210	4.30	1.15	0.020	3	F
TBB23		0	0	0	1	0	0	1	1	0	0	0	N/M	265	263	6.20	1.41	0.024	3	M
TBB24		0	0	0	0	0	0	0	0	0	0	0	N/M	264	233	5.15	1.27	0.022	3	F
TBB25		0	0	0	0	0	0	0	0	0	0	0	N/M	262	197	4.10	1.10	0.021	3	M
TBB26		0	0	0	0	0	0	0	0	0	0	0	N/M	270	251	4.40	1.28	0.018	4	M
TBB27		0	0	0	0	0	0	0	0	0	0	0	N/M	267	270	4.85	1.42	0.018	4	F
TBB28		0	0	0	0	0	0	0	0	0	0	0	N/M	262	224	4.05	1.25	0.018	3	M
TBB29		0	0	0	0	0	0	0	0	0	0	0	N/M	261	237	5.10	1.33	0.022	3	F
TBB30		0	0	0	0	0	0	0	0	0	0	0	N/M	270	228	5.30	1.16	0.023	3	F
TCB01 10/14/98	trawl	0	0	0	0	0	0	0	0	0	0	0	0	260	217	3.45	1.23	0.016		M
TCB02		1	0	0	0	0	0	0	1	0	0	0	0	270	239	4	1.21	0.017		M
TCB03		0	0	0	0	0	0	0	0	0	0	0	0	260	224	4.20	1.27	0.019		M
TCB04		0	0	0	0	0	0	0	0	0	0	0	0	272	223	3.60	1.11	0.016		M
TCB05		0	0	0	0	0	0	0	0	0	0	0	0	265	215	3.35	1.16	0.016		M
TCB06		0	0	0	0	0	0	0	0	0	0	0	0	265	234	4	1.26	0.017		M
TCB07		0	0	0	0	0	0	0	0	0	0	0	0	261	223	4.35	1.25	0.020		M
TCB08		0	0	0	0	0	0	0	0	0	0	0	0	267	232	3.80	1.22	0.016		M
TCB09		0	0	0	0	0	0	0	0	0	0	0	0	263	233	3.9	1.28	0.017		M
TCB10		0	0	0	0	0	0	0	0	0	0	0	0	280	274	4.15	1.25	0.015		M
TCB11		0	0	0	0	0	0	0	0	0	0	0	0	264	225	3.5	1.22	0.016		M
TCB12		0	0	0	0	0	0	0	0	0	0	0	0	271	257	3.85	1.29	0.015		M
TCB13		0	0	0	0	0	0	0	0	0	0	0	0	270	249	3.75	1.27	0.015		M
TCB14		0	0	0	0	0	0	0	0	0	0	0	0	289	288	4.7	1.19	0.016		M
TCB15		0	0	0	0	0	0	0	0	0	0	0	0	263	236	3.90	1.30	0.017		M
TCB16		0	0	0	0	0	0	0	0	0	0	0	0	263	220	3.70	1.21	0.017		M
TCB17		0	0	0	0	0	0	0	0	0	0	0	0	270	242	3.45	1.23	0.014		
TCB18		0	0	0	0	0	0	0	0	0	0	0	0	265	242	4.10	1.30	0.017		M
TCB19		0	0	0	0	0	0	0	0	0	0	0	0	262	220	3.9	1.22	0.018		M
TCB20		0	0	0	0	0	0	0	0	0	0	0	0	275	266	4	1.28	0.015		F
TCB21		0	0	0	0	0	0	0	0	0	0	0	0	278	265	3.35	1.23	0.013		
TCB22		0	0	0	0	0	0	0	0	0	0	0	0	267	217	4.75	1.14	0.022		M
TCB23		0	0	0	0	0	0	0	0	0	0	0	0	287	290	4.50	1.23	0.016		M

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Tumor Data  
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Sample	Method	FHA	HA	HC	C	CC	TLC	TLT	TLL	EP	SC	TST	AB	Tot. Length (mm)	Wt (g)	Liver wt (g)	Condition factor	H.S.I.	Age	Sex
TCB24		0	0	0	0	0	0	0	0	0	0	0	0	292	337	5.6	1.35	0.017		M
TCB25		0	0	0	0	0	0	0	0	0	0	0	0	280	302	4.50	1.38	0.015		M
TCB26		0	0	0	0	0	0	0	0	0	0	0	0	262	212	3.50	1.18	0.017		M
TCB27		0	0	0	0	0	0	0	0	0	0	0	0	289	305	4.7	1.26	0.015		M
TCB28		0	0	0	0	0	0	0	0	0	0	0	0	273	242	3.8	1.19	0.016		
TCB29		0	0	0	0	0	0	0	0	0	0	0	0	275	272	4.65	1.31	0.017		M
TCB30		0	0	0	0	0	0	0	0	0	0	0	0	263	235	4.85	1.29	0.021		M
TCB31		0	0	0	0	0	0	0	0	0	0	0	0	272	236	4.40	1.17	0.019		
TCB32		0	0	0	0	0	0	0	0	0	0	0	0	270	236	3.75	1.20	0.016		M
TCB33		0	1	0	0	0	0	1	1	0	0	0	0	265	219	4.35	1.18	0.020		F
TCB34		0	0	0	0	0	0	0	0	0	0	0	0	262	241	4.70	1.34	0.020		F
TCB35		0	0	0	0	0	0	0	0	0	0	0	0	265	238	4.85	1.28	0.020		F
TCB36		0	0	0	0	0	0	0	0	0	0	0	0	262	212	2.7	1.18	0.013		
TCB37		0	0	0	0	0	0	0	0	0	0	0	0	269	227	3.45	1.17	0.015		
TCB38		0	0	0	0	0	0	0	0	0	0	0	0	286	288	5.10	1.23	0.018		
TCB39		0	0	0	0	0	0	0	0	0	0	0	0	265	254	4.00	1.36	0.016		
TKL 1 11/15/2000	trawl	1	0	0	0	0	0	0	1	0	0	0	0	267	253	5.33	1.33	0.0211	2	F
TKL 2		0	0	0	0	0	0	0	0	0	0	0	0	263	215	4.45	1.18	0.0207	3	M
TKL 3		1	0	0	0	0	0	0	1	0	0	0	0	285	330	6.94	1.43	0.0210	5	M
TKL 4		0	0	0	0	0	0	0	0	0	0	0	0	266	248	5.12	1.32	0.0206	3	M
TKL 5		0	0	0	0	0	0	0	0	0	0	0	0	277	266	4.93	1.25	0.0185	3	M
TKL 6		1	0	0	0	0	0	0	1	0	0	0	0	277	265	5.58	1.25	0.0211	4	M
TKL 7		0	0	0	0	0	0	0	0	0	0	0	0	286	276	5.43	1.18	0.0197	5	M
TKL 8		0	0	0	0	0	0	0	0	0	0	0	0	282	256	5.51	1.14	0.0215	4	M
TKL 9		0	0	0	0	0	0	0	0	0	0	0	0	263	235	4.75	1.29	0.0202	3	M
TKL 10		0	0	0	0	0	0	0	0	0	0	0	0	270	237	5.55	1.20	0.0234	2	M
TKL 11		0	0	0	0	0	0	0	0	0	0	0	0	267	231	4.14	1.21	0.0179	2	M
TKL 12		0	0	0	0	0	0	0	0	0	0	0	0	275	256	4.74	1.23	0.0185	2	M
TKL 13		0	0	0	0	0	0	0	0	0	0	0	0	270	212	4.03	1.08	0.0190	2	M
TKL 14		0	0	0	0	0	0	0	0	0	0	0	0	278	278	5.12	1.29	0.0184	4	M
TKL 15		0	0	0	0	0	0	0	0	0	0	0	0	274	254	5.57	1.23	0.0219	4	F
TKL 16		0	0	0	0	0	0	0	0	0	0	0	0	276	271	5.33	1.29	0.0197	3	M
TKL 17		0	0	0	0	0	0	0	0	0	0	0	0	278	258	4.73	1.20	0.0183	4	M
TKL 18		0	0	0	0	0	0	0	0	0	0	0	0	284	266	5.00	1.16	0.0188	3	M
TKL 19		0	0	0	0	0	0	0	0	0	0	0	0	265	233	4.62	1.25	0.0198	2	M
TKL 20		0	0	0	0	0	0	0	0	0	0	0	0	264	222	4.82	1.21	0.0217	3	F
TKL 21		0	0	0	0	0	0	0	0	0	0	0	0	274	255	7.02	1.24	0.0275	4	M
TKL 22		0	0	0	0	0	0	0	0	0	0	0	0	278	246	5.05	1.14	0.0205	3	M
TKL 23		0	0	0	0	0	0	0	0	0	0	0	0	274	246	5.07	1.20	0.0206	4	?
TKL 24		0	0	0	1	0	0	1	1	0	0	0	0	298	325	6.35	1.23	0.0195	5	M
TKL 25		1	0	0	0	0	0	0	1	1	0	1	0	272	259	6.29	1.29	0.0243	3	F
TKL 26		0	0	0	0	0	0	0	0	0	0	0	0	277	256	4.73	1.20	0.0185	4	M
TKL 27		0	0	0	0	0	0	0	0	0	0	0	0	273	211	3.86	1.04	0.0183	4	F
TKL 28		0	0	0	0	0	0	0	0	0	0	0	0	261	224	6.16	1.26	0.0275	3	M
TKL 29		1	0	0	0	0	0	0	1	0	0	0	0	262	222	5.20	1.23	0.0234	3	M
TKL 30		1	0	0	0	0	0	0	1	0	0	0	0	277	265	5.44	1.25	0.0205	4	M
TKB 1 6/25/2001	trawl	0	0	0	0	0	0	0	0	0	0	0	0	277	277	4.11	1.30	0.0148	4	M
TKB 2		0	0	0	0	0	0	0	0	0	0	0	0	267	230	3.29	1.21	0.0143	5	M
TKB 3		0	0	0	0	0	0	0	0	0	0	0	1	260	227	2.50	1.29	0.0110	5	M
TKB 4		0	0	0	0	0	0	0	0	0	0	0	0	268	240	2.60	1.25	0.0108	4	M
TKB 5		0	0	0	0	0	0	0	0	0	0	0	1	260	240	3.75	1.37	0.0156	5	M
TKB 6		0	0	0	0	0	0	0	0	0	0	0	0	260	227	3.37	1.29	0.0148	5	M
TKB 7		0	0	0	0	0	0	0	0	0	0	0	0	260	210	3.96	1.19	0.0189	3	F

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Sample	Method	FHA	HA	HC	C	CC	TLC	TLT	TLL	EP	SC	TST	AB	Tot. Length (mm)	Wt (g)	Liver wt (g)	Condition factor	H.S.I.	Age	Sex	
TKB 8		0	0	0	0	0	0	0	0	0	0	0	0	275	291	4.72	1.40	0.0162	5	M	
TKB 9		0	0	0	0	0	0	0	0	0	0	0	0	260	247	3.15	1.41	0.0128	5	M	
TKB 10		0	0	0	0	0	0	0	0	0	0	0	0	263	237	4.34	1.30	0.0183	5	M	
TKB 11		0	0	0	0	0	0	0	0	0	0	0	0	260	240	2.75	1.37	0.0115	4	M	
TKB 12		0	0	0	0	0	0	0	0	0	0	0	1	275	267	3.28	1.28	0.0123	4	M	
TKB 13		0	0	0	0	0	0	0	0	0	0	0	0	271	231	3.79	1.16	0.0164	4	M	
TKB 14		0	0	0	0	0	0	0	0	0	0	0	0	262	230	3.19	1.28	0.0139	4	M	
TKB 15		0	0	0	0	0	0	0	0	0	0	0	0	263	237	4.44	1.30	0.0187	5	M	
TKB 16		0	0	0	0	0	0	0	0	0	0	0	0	263	260	3.79	1.43	0.0146	3	M	
TKB 17		0	0	0	0	0	0	0	0	0	0	0	0	282	280	4.24	1.25	0.0151	4	M	
TKB 18		0	0	0	0	0	0	0	0	0	0	0	0	265	220	5.43	1.18	0.0247	4	F	
SUM		8	2	0	2	1	1	5	13	1	0	1	3								
%		6.838	1.7	0	1.71	0.85	0.85	4.3	11.1	0.9	0	0.85	3.45	avg	269.8	244	4.52	1.24	0.0185	3.6	
														std	8.0	26	0.91	0.08	0.0035	0.8	
														min	260	197	2.50	1.04	0.0108	2	
														max	298	337	7.02	1.43	0.0280	5	