U.S. Fish & Wildlife Service

Tumor Prevalence in Brown Bullheads *(Ameiurus nebulosus)* from the South River, Anne Arundel County, Maryland

CBFO-C05-04

December 2005

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December 2005

ABSTRACT

The South River is a tributary of the Chesapeake Bay flowing near Annapolis, Maryland. In March 2005, brown bullheads were collected in a fyke net set about 1.25 km downriver of the Route 50 Bridge. A total of 30 brown bullheads (*Ameiurus nebulosus*) $\geq$ 260 mm were randomly selected for analysis, placed in coolers and transported live to the U.S. Fish and Wildlife Chesapeake Bay Field Office. The fish were held in aerated site water and necropsied over the next two days. A gross examination was performed of the external organs, focusing on raised skin lesions and the appearance of the barbels. For all fish, livers were excised, weighed, cut into sections and preserved in 10% buffered neutral formalin. Sixteen fish had raised skin lesions, which were excised along with adjacent tissues, decalcified, and preserved similarly. Tissues were processed and histopathological examinations were performed. The objective was to determine the prevalence of liver and skin tumors and preneoplastic lesions. We reported a 20% (6 of 30) prevalence of liver tumors, split evenly between hepatocellular carcinomas and cholangiocarcinomas. All sixteen fish with the raised skin lesions were diagnosed with skin tumors (53% prevalence). Thirteen of these cases were invasive squamous carcinomas and three were non-invasive epidermal papillomas. Liver tumor prevalence was significantly ($p=0.01$, Fisher's Exact Test) higher than that observed previously in collections from the Tuckahoe River (MD), considered a reference area (prevalence = 4% (5 of 117)). The liver tumor prevalence in South River bullheads also exceeded the 5% criterion suggested as indicative of highly contaminated areas. Skin tumor prevalence was significantly different between locations (South River 16/30 = 53%, Tuckahoe: 1/117 = 1%, $p<0.001$). The skin tumor prevalence in South River bullheads was about four times the 12% suggested criterion for highly contaminated areas. Barbel abnormalities were not significantly different (South: 3/30 = 10%, Tuckahoe: 3/87 = 3%, $p=0.17$).

The South River ranks first in skin tumor prevalence (53%) and second in liver tumor prevalence (20%) among the Chesapeake Bay locations where bullhead surveys have been conducted. In brown bullheads, both liver and skin tumors have been associated with exposure to carcinogens, with the most persuasive linkage to polynuclear aromatic hydrocarbons (PAHs) in sediments. The mean total PAH concentration reported in 29 sediments from the South River, 2.2 ppm, however, was similar to the mean of 1.8 ppm measured in 1996 at the Tuckahoe River collection site. Thus, the findings in the South River contrast with those in other Chesapeake Bay tributaries, where elevated tumor prevalence coincided with high sediment PAH concentrations. At present, we have insufficient evidence to implicate a particular chemical class as a major contributor to the tumors. We recommend a followup survey that includes tumor prevalence and analysis of biomarkers such as biliary PAH metabolites and DNA adducts to evaluate PAHs as a primary agent. Surveys of other western shore tributaries, such as the Severn and Rhode Rivers, would be useful for determining the extent of the tumor problem.

ACKNOWLEDGMENTS

We appreciate the assistance of Steve Minkkinen, Mike Mangold, Tina McCrobie, and Clif Tipton of the Maryland Fishery Resources Office in the collection of the fish. Peter McGowan of the Chesapeake Bay Field Office helped with the analysis of sediment data. Leslie Gerlich, prepared the maps. Paul Baumann of the U.S. Geological Survey reviewed a draft of the report and provided useful comments. This project was jointly funded by the Chesapeake Bay Field Office and the South River Federation. We thank Drew Koslow of the South River Federation for his assistance.
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PREFACE

This report documents the health status of brown bullheads (*Ameiurus nebulosus*) collected from the South River, Anne Arundel County, Maryland. It compares liver and skin tumor prevalence in brown bullheads from the South River with data from the Tuckahoe River, used as a reference area in three previous brown bullhead tumor surveys. Study design, implementation, and reporting were completed by staff of the Fish and Wildlife Service's Chesapeake Bay Field Office. Funding for the project was provided by the Service and by the South River Federation. Histological determinations were conducted by Dr. John Harshbarger of 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.

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INTRODUCTION

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. Environmental managers in the Great Lakes have used the presence of 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.

For liver tumors, the strongest evidence for chemical etiology exists for polynuclear aromatic hydrocarbons (PAHs) in sediments (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 (Metcalfe *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.

Skin tumors in brown bullhead have been induced by repeatedly painting the skin with sediment extracts containing high PAH concentrations (Black *et al.* 1985). In a summary of studies conducted in the Great Lakes, Baumann *et al.* (1996) reported that higher oral and cutaneous tumor prevalence occurred in PAH-contaminated tributaries compared with reference sites. 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. 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. 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.

Biomarkers are physiological, biochemical, or histological changes used as indicators of chemical exposure, chemical effects, or both. Because fish rapidly metabolize PAHs (Krahn *et al.* 1986), researchers have used biomarkers rather than tissue concentrations as indicators of exposure and response. The presence of PAH-like metabolites in bile 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), PAHs and nitro-PAH compounds, forming adducts with DNA. These adducts can be an early stage in carcinogenesis (Reichert *et al.*

1998). Pinkney *et al.* (2004a) reported a 50-68% liver tumor prevalence and high concentrations of liver DNA adducts (with a strong DRZ signature) in brown bullheads from the Anacostia River, Washington, DC. They concluded that it was likely that PAHs played a major role in the development of the tumors.

This report describes a spring 2005 survey of tumor prevalence in brown bullheads from the South River, a tributary of the Chesapeake Bay, located in Anne Arundel County (Figures 1, 2). The watershed has become more developed as the County experienced a 65% increase in population between 1970 and 2000 (Anne Arundel County Government 2003). Tumor prevalence was compared with data from the Tuckahoe River, used as a reference site in three studies conducted between 1996 and 2001 (Pinkney *et al.* 2001, 2004 a,b). In addition, we evaluate available sediment PAH data for evidence of a possible association with tumor prevalence.

OBJECTIVES

We designed this study with the following objectives:

1. Determine liver tumor, skin tumor, and barbel abnormality prevalence in bullheads collected from the South River;
2. Compare the prevalence with a database for the Tuckahoe River, used as a reference site in three previous studies, and with Baumann's suggested criteria,
3. Evaluate existing sediment contaminant data with literature for indications of chemical causation, and
4. Provide recommendations for further research.

MATERIALS AND METHODS

Site Selection and Sampling

The U.S. Fish and Wildlife Service (USFWS), Chesapeake Bay Field Office is developing a database of tumor and biomarker information for the Bay tributaries. These data are useful as indicators of habitat quality, for compiling evidence of the effects of chemical stressors, and for monitoring the success of restoration. Initial studies have focused on rivers in and near major metropolitan areas – Washington, DC and Baltimore, MD – as well as the tidal Potomac River (Pinkney *et al.* 2001, 2004a, b). The South and Severn Rivers (Figure 1) are of particular interest because changes within these watersheds are occurring that may adversely affect habitat quality.

A fyke net was set by the USFWS Maryland Fishery Resources Office on the South River on March 21, 2005 and harvested two days later. The net was located about 1.25 km downriver of the Route 50 Bridge, extending out from the western shore of the River (38.96882 EN, 76.59978 EW, Figure 2). The fyke net was set to collect yellow perch (*Perca flavescens*) for hatchery broodstock and to monitor numbers and sizes of the population. Water quality parameters on the collection date were: Temperature - 9EC; dissolved oxygen - 8.23 mg/L, conductivity - 14.15 msiemens; salinity - 8.2 ppt. Because previous sets had yielded large numbers of brown bullheads in addition to the yellow perch, the Principal Investigator was invited to accompany the crew and retain a sample of bullheads for a tumor survey. A group of 30 brown bullheads (total lengths: $\geq$ 260 mm) was obtained. Fish that appeared to be of sufficient size were randomly selected (without regard to gross appearance), measured, and placed in coolers containing site water. The coolers were transported to the USFWS, Chesapeake Bay Field Office and held under aeration until necropsies were conducted over the next two days. Three additional fish (assigned numbers 05STH13-15) were collected because they had readily apparent mouth lesions. Since they were not randomly sampled, they were not used as part of the survey data. Instead, they were photographed, treated identically with the other fish (including necropsy, tissue preparation, and histopathology), and the photographs and photomicrographs were used as particularly vivid examples of the various tumor types.

We compared the pathology of South River bullheads with an existing database of 117 brown bullheads, collected from one area of the Tuckahoe River in 1996, 1998, 2000, and 2001. This site was 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 used.

Laboratory Procedures

Bullheads 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. External lesions, mostly associated with the mouth, were described, sketched in some cases and selectively photographed. Barbels were observed and those determined to be shortened, clubbed (stubbed), or missing were pooled into a single category of “abnormal”. Internal organs were exposed by a longitudinal, ventral abdominal incision and the liver was excised. Livers were weighed and the hepatosomatic index (HSI) was calculated as the liver weight divided by the body weight. Four blocks of hepatic tissue cut from each liver by scalpel were placed in a numbered cassette and submerged in a dedicated bottle containing 10% formalin fixative. As most external lesions were too hard to cut by scalpel due to underlying bone, intact lesions with adjacent tissue were cut from the fish with a bone cutter. After fixation, bone was decalcified with formic acid, the softened tissues were cut and tissue blocks placed in cassettes.

Cassettes containing the fixed liver and skin tissues were shipped to a processing laboratory where they were dehydrated, infiltrated with paraffin and embedded in paraffin blocks. Paraffin blocks were sectioned with a microtome at 4-5 microns. Tissue sections were mounted on glass microscope slides, deparaffinized, stained by hematoxalin and eosin, coverslipped, cleaned and labeled (Luna 1968). Finished slides were returned for pathological evaluation. Procedures for Tuckahoe bullheads were identical to those used for the South River.

Slides were individually examined with a research photomicroscope. Lesions consisting of aberrant masses of heritably altered, proliferating cells exhibiting a degree of autonomy and anaplasia were diagnosed as neoplasms (see Appendix A for a glossary of pathological terms). All skin neoplasms were epidermally derived. Non-invasive epidermal neoplasms exhibiting a papillary growth pattern were diagnosed as epidermal papilloma. Epidermal neoplasms that had digested the basement membrane and infiltrated connective tissue, often following squamous metaplasia, were diagnosed as squamous carcinoma.

Incipient liver neoplasms consisting of focal, uncompressive, tinctorially altered, hepatocellular populations without pattern or cytologic atypia were diagnosed as foci of hepatocellular alteration. More advanced, fully committed hepatocellular neoplasms with a compressive, non-invasive perimeter along with possible cytologic and pattern atypia were diagnosed as hepatocellular adenoma. Invasive hepatocellular neoplams with cytologic atypia were diagnosed as hepatocellular carcinoma. Masses of poorly formed ducts invading normal liver were diagnosed as cholangiocarcinoma. Non-invasive masses were diagnosed as cholangiomas.

Brown bullheads were aged using pectoral spines with a modification of the methods described in Baumann *et al.* (1990), Marzolf (1955), and Scholl (1968). Spines were cleaned of excess tissue, decalcified with 5% aqueous nitric acid, rinsed in distilled water, and stored in 50% isopropyl alcohol. A 6-10 mm portion of the spine, anterior of the basal groove, was used for sectioning after mounting in a paraffin medium using a microtome. These sections were mounted on slides and aged with confirmation by an independent scientist.

Data Analysis

Histopathological data were summarized as the prevalence of the various types of lesions among the collections of bullheads. The South and Tuckahoe Rivers are more than 100 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.* (2005) 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 in both rivers was compared using two-tailed Fisher's Exact Tests (Sokal and Rohlf 1981), with a critical p value of 0.05. Because the risk of liver neoplasms is known to increase with age (Moore and Myers 1994), two sets of analyses were performed—one with all fish and one with four and five year olds (South: n=25; Tuckahoe: n=41). Biological data for the collections (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 South River bullheads were much larger than the Tuckahoe bullheads. Although a 260 mm minimum total length was used for both collections, the smallest South River bullhead was 272 mm. The median length (312 mm) of the South River bullheads was significantly greater than the Tuckahoe bullheads (267 mm, $p<0.001$, Mann-Whitney test, Table 1). The South River bullheads also had significantly greater median weight (368 g) than those from the Tuckahoe (237 g, $p<0.001$, Table 1). Condition factors, however, were similar. There were statistically significant differences in HSI, although the means were quite close (0.017 for South River, 0.019 for Tuckahoe).

The ages of the South River bullheads ranged from three to six, with the bulk of the distribution between age-4 (15 or 50%) and age-5 (10 or 33%). Seventy four of the 117 Tuckahoe fish were aged, with a distribution as follows: age-2 (n=6), age-3 (n=27), age-4 (n=30), and age-5 (n=11). Using the age-4 and -5 subsets for both rivers, the South River fish were still significantly longer and heavier than the Tuckahoe fish (Table 1).

Pathology

Gross examination revealed that three of the 30 South River bullheads had abnormal (shortened, clubbed, or missing) barbels. The prevalence of abnormalities on one or more barbels was therefore 10% in the South River bullheads compared with 3 of 87 (3%) examined for barbel abnormalities in the Tuckahoe collections. Using a two-tailed Fisher's Exact Test, this difference was not statistically significant ($p=0.17$, Table 2). A spreadsheet containing all biological and pathological data is provided as Appendix B.

Upon gross examination of the South River bullheads, sixteen individuals had oral lesions that were preserved for histopathology. Fish 05STH015 (Figure 3a) had large reddish-pink raised lesions on the upper and lower lip. This individual was not part of the random sample and was chosen as a particularly dramatic example of this type of lesion (later diagnosed as a squamous carcinoma). A less dramatic case (05STH011, also a squamous carcinoma) is shown as Figure 3b. Three cases were categorized as epidermal papilloma, a non-invasive skin tumor. Invasion is a defining characteristic for cancer and 13 skin neoplasm cases were diagnosed as squamous carcinomas (SC) of oral mucosa (see Figures 3-8). The prevalence of squamous carcinomas in the South River bullheads (13 of 30 = 43%) was statistically significant when compared with the 0 of 117 (0%) prevalence in the Tuckahoe collections ($p<0.001$). The prevalence of skin tumors of either type (16 of 30 or 53%) in the South River bullheads was significantly greater than the 1 of 117 (1%) prevalence in the Tuckahoe River fish ($p<0.001$, Table 2). The South River four and five year old fish had a significantly higher prevalence of squamous carcinomas (11 of 25 =44%) and skin tumors (14 of 25=56%) than the age-4 and -5 Tuckahoe fish which had no skin tumors of either type (Table 3; $p<0.001$ for both comparisons).

No visible liver lesions were observed upon gross examination of either the South or Tuckahoe River fish. Three of the South River liver neoplasm (10% of total) cases were diagnosed as hepatocellular carcinoma (Figure 9) and three as cholangiocarcinoma (Figure 10; 10% of total). Thus the prevalence of liver tumors (of either type) was 6 of 30 or 20%. One individual (05STH016) had a hepatocellular adenoma (Figure 11), a hepatocellular carcinoma, and a cholangiocarcinoma. A spreadsheet showing the lesions for each fish is provided in Appendix B.

There was a significantly higher prevalence of liver tumors (6 of 30 or 20%) in the South River bullheads compared with the entire population of Tuckahoe bullheads (5 of 117 or 4%, p=0.01; Table 2). When only the four and five year olds were compared, however, the difference was not statistically significant – South River: 5 of 25=20%; Tuckahoe River: 2 of 41 = 5% (p=0.09, Table 3).

DISCUSSION

Tumor Prevalence and Contaminant Exposure

The 20% liver tumor prevalence in the South River bullheads raises concern that these fish are responding to exposure to carcinogens in the sediments and/or water column. This prevalence is four times the 5% suggested by Baumann (2002) as a criterion for distinguishing between highly contaminated and less contaminated areas. Compared with other Chesapeake Bay tributaries, the 20% liver tumor prevalence is second only to the Anacostia River (55% and 58%, Table 4). The 53% prevalence of skin tumors in the South River bullheads is more than four times the 12% criterion suggested by Baumann (2002). This prevalence is the highest reported in a Chesapeake Bay tributary (Table 4). In reviewing the tumor data in the Chesapeake tributaries (Table 4), the South River most closely resembled the 1992 Neabsco Creek data (17% liver tumors, 33% skin tumors) It is interesting that a repeat sampling of the Neabsco in 1996 resulted in 17% liver tumors but only 3% skin tumors. Analysis of the Chesapeake Bay database indicates a greater variability in skin tumors compared with liver tumors from repeat sampling of the same location (e.g., Back River, Table 4).

Sex can be an important factor in liver tumor prevalence in brown bullheads with females at higher risk than males (Pinkney *et al.* 2001, 2004a; Baumann *et al.* 1990). In the South River, 3 of 11 females (27%) and 3 of 19 males (16%) had liver tumors. This difference was not statistically significant (p=0.64, Fisher's Exact Test). For skin tumors, Poulet *et al*. (1994) reported no significant sex difference in prevalence in brown bullheads. In the South River, the prevalence was 8 of 11 females (73%) vs. 8 of 19 males (42%, p=0.14).

The South River flows approximately 20 kilometers from north of Route 50 to its mouth in the Chesapeake Bay (Figures 1 and 2). Located in Anne Arundel County, Maryland, the South River watershed is approximately 142 square kilometers (Center for Watershed Protection 2002). The major land uses are as follows: residential (30%), vacant (27%), open space/recreational (14%), and agriculture (13%). The percentage of impervious cover was estimated at 14%. According to the Center for Watershed Protection (2002);

imperviousness can be used as an indicator of stream quality, with significant degradation at levels of 10% or greater. MDE (2004) included the tidal South River on its 303(d) list of impaired waters. It is listed for nutrients, sediments, and toxics (PCBs in fish tissue). There is currently an advisory urging the public not to consume more than fifty 8-ounce meals of spot and thirty seven 8-ounce meals of white perch because of PCB contamination. Such advisories are in place for many of the tributaries of the Chesapeake Bay.

The most extensive sediment chemistry survey of the South River was conducted as part of the Mid-Atlantic Integrated Assessment study (U.S. Environmental Protection Agency 2000). Twenty six surface sediment samples were collected in August 1997 (Figure 2). Mean concentrations of total PAHs, and carcinogenic PAHs (sum of the following compounds: benzo[a]pyrene, benz[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[ghi]perylene, chrysene, dibenzo[a,h]anthracene, and indeno[1,2,3-c,d]pyrene) were 1.7 ppm and 0.60 ppm, respectively. In 2002, three samples were collected by the National Oceanic and Atmospheric Administration (I. Hartwell, pers.comm.). These samples had somewhat higher total PAH concentrations of 3.734, 8.967, and 7.570 ppm. When the data sets are merged, the average total and carcinogenic PAH concentrations for the South River are 2.2 ppm and 0.72 ppm, respectively (all sediment concentrations as dry weight, Table 4).

These mean PAH concentrations are low compared with concentrations measured in other Bay tributaries where bullhead tumor surveys were performed (Table 4). The 2.2 ppm total PAH concentration in the South River is about 12 times lower than the mean concentration measured in the Anacostia River sediments collected near three bullhead collection sites in 1996 and 2000 (Pinkney *et al.* 2004a). Many of the other bullhead sampling areas were in the 5 to 15 ppm range. Thus, in contrast to previous surveys in the Anacostia/tidal Potomac and Baltimore areas, PAHs in South River sediments were not high compared with the reference location. For example, a nearly equal mean total PAH concentration of 1.8 ppm was measured in the 1996 transect in the Tuckahoe River.

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) 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. Relying on comparisons with other tributaries, the South River data do not fit the pattern in which highly elevated tumor prevalence is associated with elevated PAH concentrations. In the absence of biomarker data, especially DNA adducts, there is insufficient evidence to link the tumor prevalence with specific contaminants or chemical classes.

Barbel Abnormalities

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%. While there have been associations between barbel abnormalities and contaminants (including PAHs), there is no evidence of a mechanistic linkage between barbel abnormalities and neoplasia.

There is no suggested criterion for barbel abnormalities. The 10% prevalence in the South River bullheads was not statistically different from the 3% observed in the Tuckahoe River bullheads. 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 the South River, despite the large size of the fish and the high prevalence of liver and skin tumors, only a low to moderate prevalence of barbel abnormalities was documented.

HSI and Condition Factor

In several bullhead surveys, HSI has increased along with increases in liver tumors or preneoplastic liver lesions. The overall mean HSI for the Tuckahoe bullheads (0.019) is based on four collections where mean HSI was 0.015, 0.016, 0.021, and 0.021. The prevalence of non-neoplastic or neoplastic liver lesions for these four collections were 0%, 5%, 13%, and 23%, respectively. Higher lesion frequency with higher HSI was also 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.

The mean HSI in the South River bullheads (0.017 units) was nearly equal to the 0.019 units in the Tuckahoe collections, and less than the 0.025 and 0.023 units in Anacostia bullheads (Pinkney *et al.* 2001), that had a greater than 50% liver tumor prevalence. Thus, the South River bullheads are not consistent with the general pattern where collections with higher HSIs have higher tumor prevalence.

Mean condition factors were similar in the South (1.22) and Tuckahoe (1.24) Rivers. These values were somewhat less than the mean 1.30 value reported by Sinnott 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 mean K values of 1.20-1.31 in bullheads from the Anacostia River, which overlaps with the mean K values from the present study.

CONCLUSIONS AND RECOMMENDATIONS

We report a 20% prevalence of liver tumors and a 53% prevalence of skin tumors in $\geq$260 mm brown bullheads from the South River. Both of these are indicative of a contaminated habitat; in that they are four times the Baumann (2002) criteria for distinguishing highly contaminated Areas of Concern from less contaminated Areas of Recovery. The South River ranks first in skin tumor prevalence and second in liver tumor prevalence among the Chesapeake Bay locations where bullhead surveys have been conducted.

A more comprehensive study would include simultaneous sampling of 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 do not have sufficient evidence for linkage with specific chemical classes. Chemical analysis of other classes of carcinogens in sediment and water appears warranted.

The following areas of uncertainty are stated in terms of research questions:

1) Are the South River bullheads collected in the spring fyke nets from a population that is restricted to the South River or are they entering other systems? The only available tracking data from the tidal Anacostia indicates a home range on the order of 0.5 to 2.1 km for adults (Sakaris *et al.* 2005). Specific movement data on bullheads from western shore Bay tributaries such as the South or Severn Rivers is lacking. A movement study coordinated with sediment sampling could also document whether bullheads frequent areas with high concentrations of contaminants. We presume that the home range of younger bullheads (which would be the critical stage for starting the cancer process) is less than that for adults but no such tracking studies have been done.

2) What is the prevalence of tumors in other western shore Bay tributaries such as the Severn and Rhode Rivers? Such data may shed light on whether the South is truly a “hot spot” for tumors or representative of a broader problem.

3) Despite the sediment data, might PAHs still play a major role in these tumors? At present, relying on sediment data, we state that there is insufficient evidence to link the tumors with any chemical class. A tumor study including biomarker analysis would be needed to evaluate the concentrations of PAH bile metabolites (an indicator of recent exposure) and liver DNA adducts (shown to be an early

stage in carcinogenesis). Without these types of biomarker analyses, we cannot rule out PAHs as a contributor.

4) What other chemicals may play a role in the tumors? Among the other chemical classes that have been linked with tumors in fish are nitrosamines (Bunton 1996), which can be formed in waters containing raw sewage (Yordy and Alexander 1981). Spitsbergen and Wolfe (1995) reported detecting these compounds in sediments and suggested that they may contribute to both skin and liver tumor development in brown bullhead. No data are available for this class of chemicals in waters or sediments of the Bay tributaries.

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TABLES

Table 1. Biological data for brown bullhead from the South and Tuckahoe Rivers[a].

All fish			
	South River (n=30)	Tuckahoe River (n=117)	Statistics[b]
Length (mm)	312 (272-334) A	267 (260-298) B	Mann-Whitney (M-W), p<0.001
Weight (g)	368 (236-474) A	237 (197-337) B	M-W, p<0.001
Liver weight (g)	6.18 ± 1.61	4.52±0.91	Not compared
Condition Factor (K)	1.22±0.06 A	1.24 ± 0.08 A	t-test, log-transformed[c] p=0.21
Hepatosomatic Index (HSI)	0.017±0.003 A	0.019±0.003 B	t-test, p=0.007
Sex	19 M, 11 F	81M, 27F, 9?	Not compared
Age	4.4±0.7 (n=29)	3.6±0.8 (n=74)	Not compared
Age-4 and age-5 fish			
	South River (n=25)	Tuckahoe River (n=41)	
Length (mm)	313 ± 13 A	272 ± 8 B	t-test, log-transformed[c] p<0.001
Weight (g)	378 ± 51 A	252 ± 26 B	t-test, log-transformed[c] p<0.001
Liver weight (g)	6.24 ± 1.66	4.66±1.15	Not compared
Condition Factor (K)	1.22±0.06 A	1.25± 0.09 A	t-test, p=0.17
Hepatosomatic Index (HSI)	0.018 (0.010-0.028) A	0.016(0.010–0.023)B	Mann-Whitney, p=0.04
Sex	17 M, 8 F	30 M, 10 F, 1 ?	Not compared
Age	age 4: 15; age 5: 10	Age 4: 30; age 5: 11	Not compared

[a] Mean ± one standard deviation (SD) or median with range in parentheses.
[b] Groups with different letters are significantly different at p<0.05
[c] Log-transformed data used for t test, untransformed means and SD reported.

Table 2. Summary of lesion data and statistical comparisons (two-tailed Fisher's Exact Test)-all fish.

Lesion[a]	South R. (n=30)	Tuckahoe R. (n=117)	P value
Focus of hepatocellular alteration	4 (13%)	8 (7%)	0.27
Hepatocellular adenoma	1 (3%)	2 (2%)	0.50
Hepatocellular carcinoma	3 (10%)	0 (0%)	0.008
Cholangioma	0 (0%)	2 (2%)	1.0
Cholangiocarcinoma	3 (10%)	1 (1%)	0.03
Total liver cancers	5 (17%)	1 (1%)	0.001
Total liver tumors	6 (20%)	5 (4%)	0.01
Total liver lesions	9 (30%)	13 (11%)	0.02
Epidermal papilloma	3 (10%)	1 (1%)	0.03
Squamous carcinoma	13 (43%)	0 (0%)	<0.001
Total skin tumors	16 (53%)	1 (1%)	<0.001
Altered barbels	3 (10%)	3 (3%)[b]	0.17

[a] see Appendix A for glossary of pathological terms.
[b] n=87 (barbels not examined in 1996 sampling of 30 fish)

Table 3. Summary of lesion data and statistical comparisons using only age 4 and age 5 brown bullheads (two-tailed Fisher's Exact Test).

Lesion[a]	South R. (n=25)[b]	Tuckahoe R. (n=41)[c]	P value
Focus of hepatocellular alteration	4 (16%)	3 (7%)	0.41
Hepatocellular adenoma	1 (4%)	0 (0%)	0.38
Hepatocellular carcinoma	2 (8%)	0 (0%)	0.14
Cholangioma	0 (0%)	1 (2%)	1.0
Cholangiocarcinoma	3 (12%)	1 (2%)	0.15
Total liver cancers	4 (16%)	1 (2%)	0.06
Total liver tumors	5 (20%)	2 (5%)	0.09
Total liver lesions	8 (32%)	5 (12%)	0.06
Epidermal papilloma	3 (12%)	0 (0%)	0.05
Squamous carcinoma	11 (44%)	0 (0%)	<0.001
Total skin tumors	14 (56%)	0 (0%)	<0.001
Altered barbels	2 (8%)	3 (10%)[d]	1.0

[a] see Appendix A for glossary of pathological terms.
[b] South River: age 4: n=15, age 5: n=10
[c] Tuckahoe River: age 4: n=30, age 5: n=11
[d] n=29 (barbels only examined in 2000 and 2001 collections)

Table 4. Tumor and sediment PAH data for brown bullhead surveys within the Chesapeake Bay watershed[a].

Location/date of fish collection	Subwater-shed	Total liver tumors	Total skin tumors	Total PAHs (ppm)	Carcinogenic PAHs[b] (ppm)	Reference
Anacostia, 1996	Potomac	33/60=55%	14/60=23%	26.8±1.6 (n=3)	9.0±0.2 (n=3)	Pinkney *et al.* (2001)
Anacostia, 2000-2001	Potomac	67/115=58%	20/115=17%	26.4±17.2 (n=68)[c]	13.7±8.3 (n=68)[c]	Pinkney *et al.* (2004a)
Back River, 1998	Back	4/50=8%	4/50=8%	6.5±6.4 (n=3)	2.4±2.4 (n=3)	Pinkney *et al.* (2004b)
Back River, not stated	Back	Not reported	19/42=45%	not sampled	not sampled	Bunton (2000)
Furnace Creek, 1998	Patapsco	0/50=0%	6/50=12%	6.8±3.1 (n=3)	3.3±1.6 (n=3)	Pinkney *et al.*(2004b)
Farm Creek, 1992	Potomac	2/29=7%	1/29=3%	12.0±1.7 (n=3)	0.34±0.04 (n=3)	Pinkney *et al.* (1995)
Marumsco Creek, 1992	Potomac	2/30=7%	5/30=17%	10.5±1.3 (n=3)	0.63±0.34 (n=3)	Pinkney *et al.* (1995)
Neabsco Creek, 1992	Potomac	5/30=17%	10/30=33%	14.9±9.3 (n=3)	1.4±1.2 (n=3)	Pinkney *et al.* (1995)
Neabsco Creek, 1996	Potomac	5/30=17%	1/30=3%	4.8±4.0 (n=3)	1.4±1.3 (n=3)	Pinkney *et al.* (2001)
Quantico embay., 1996	Potomac	2/30=7%	1/30=3%	5.1±2.7 (n=3)	1.2±0.6 (n=3)	Pinkney *et al.* (2001)
South River , 2005	South	6/30=20%	16/30=53%	2.2 ± 2.7 (n=29)[d]	0.72 ± 0.88 (n=29)[c]	Present study
Tuckahoe River, 1996	Tuckahoe	3/30=10%	0/30=0%	1.8 ± 1.3 (n=3)	0.48 ± 0.71 (n=3)	Pinkney *et al.* (2001)
Tuckahoe River, 1998	Tuckahoe	1/39=3%	0/39=0%	0.19 ± 0.22 (n=3)	0.056 ± 0.080(n=3)	Pinkney *et al.* (2004b)
Tuckahoe River, ‘00-01	Tuckahoe	1/48=2%	0/48=0%	Not sampled	not sampled	Pinkney *et al.* (2004a)

[a] Tumor surveys used a minimum length of 260 mm except Pinkney *et al.* (1995) (280 mm) and Bunton (2000) (160-300 mm)

[b] Sum of benzo[a]pyrene, benz[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[ghi]perylene, chrysene, dibenzo[ah]anthracene, and indeno[1,2,3-c,d]pyrene

[c] Sediment data from Velinksy and Ashley (2001), samples collected in 2000, only sites within 1 km of fish collection sites are shown.

[d] Sediments collected in 1997 by EPA MAIA program and 2002 by NOAA (I. Hartwell, pers. comm..)

FIGURES

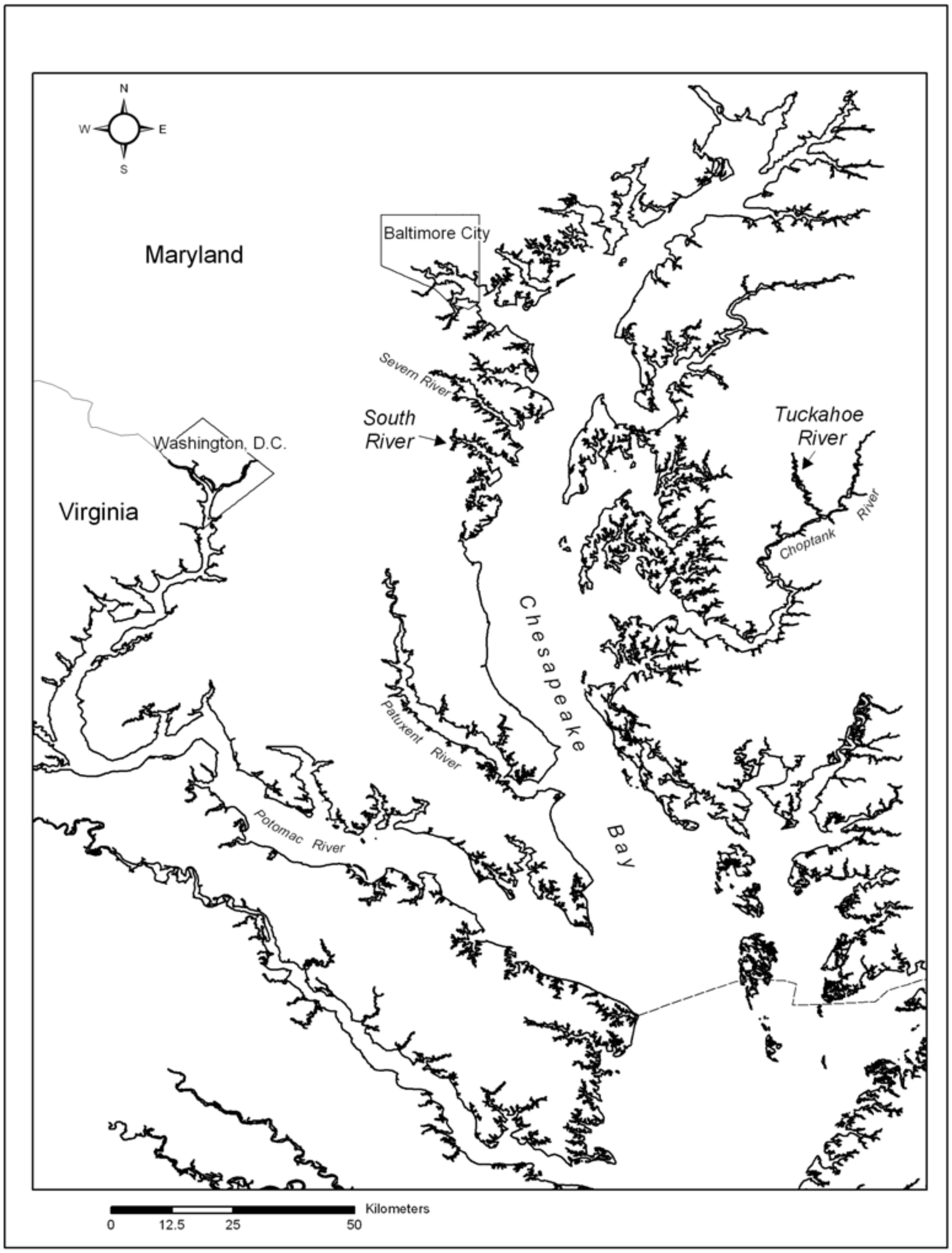

Figure 1. General map showing the South and Tuckahoe Rivers.

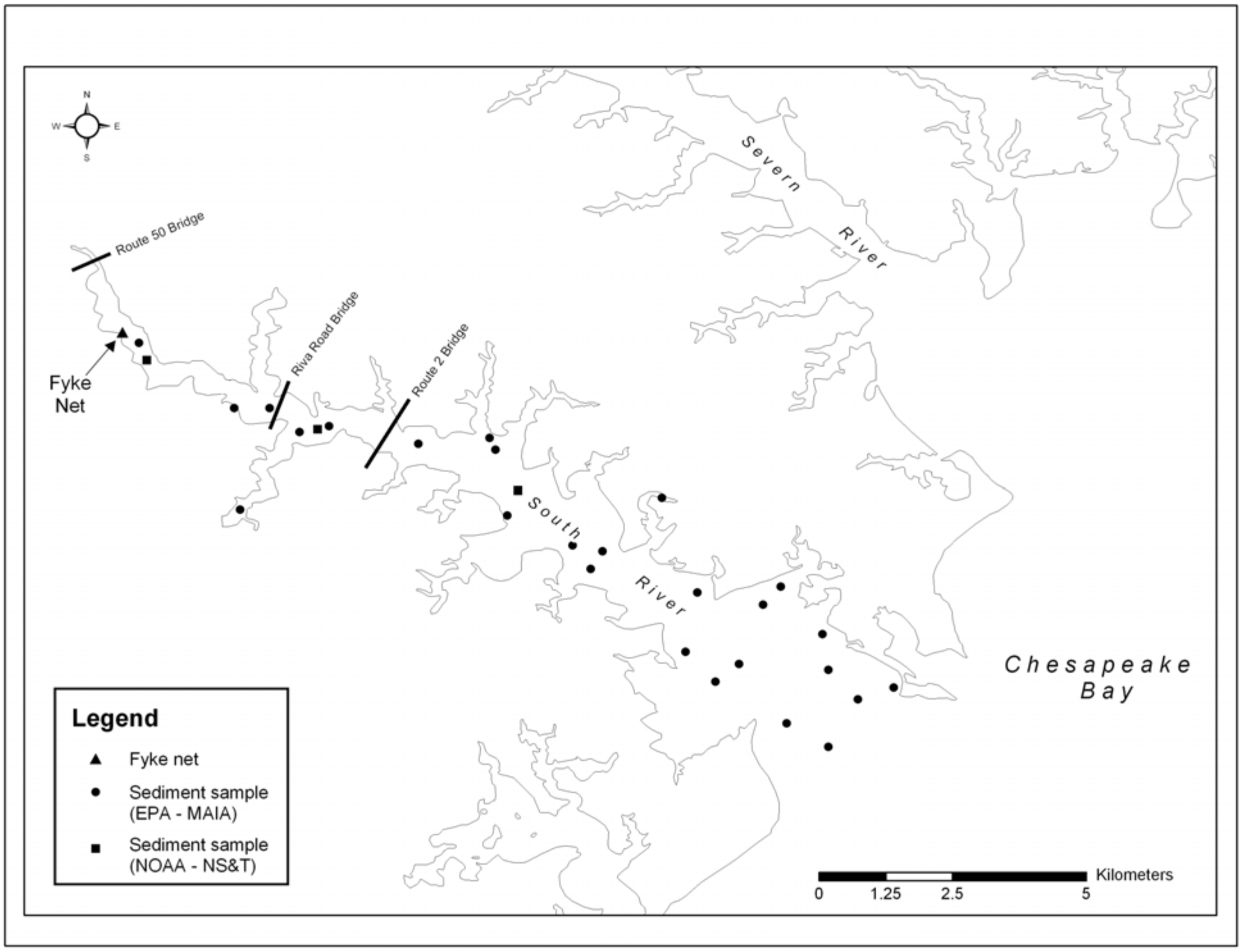

Figure 2. Map of the South River showing fyke net location and the locations of sediment samples collected by the EPA Mid-Atlantic Integrated Assessment (MAIA) program and the NOAA National Status and Trends (NS&T) program.

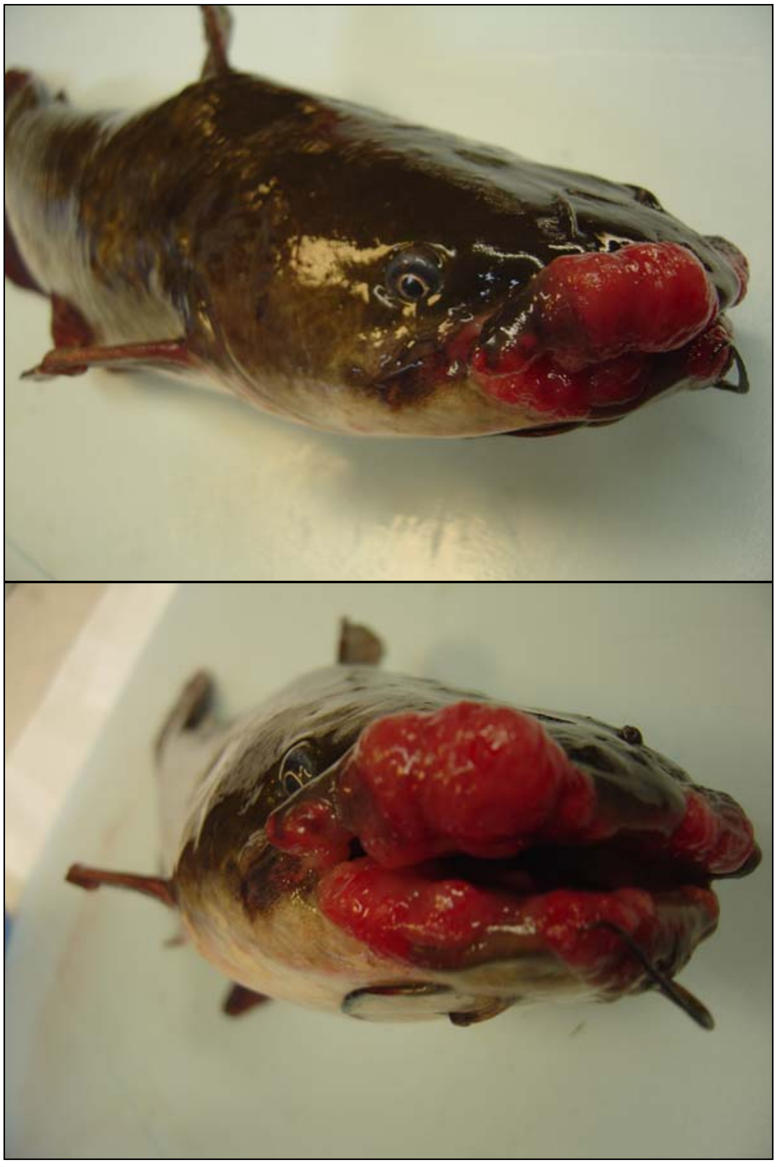

Figure 3a. Photographs of 05STH13, later diagnosed with squamous cell carcinomas.

Figure 3b. Photograph of 05STH11, later diagnosed with squamous cell carcinomas.

Figure 4. Squamous cell carcinoma. A papillary mass of neoplastic epidermal cells protrudes in a semicircle from one side of the barbel (05STH023). Bar = 300 µm.

Figure 5. Squamous cell carcinoma of lip with exophytic papillary pattern. Strands of tumor (triangle) interdigitate with fibrovascular connective tissue (05STH014). Bar = 75 µm.

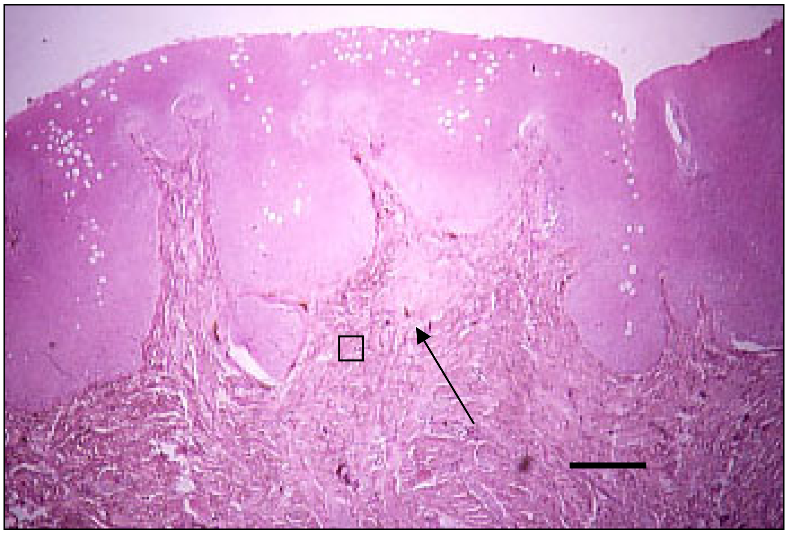

Figure 6. Squamous cell carcinoma of lip. Endophytic papillary pattern. Tumor cells at the tip of the central peg (arrow) have broken through the basement membrane and are invading the underlying dermis (square) (05STH017). Bar = 190 µm.

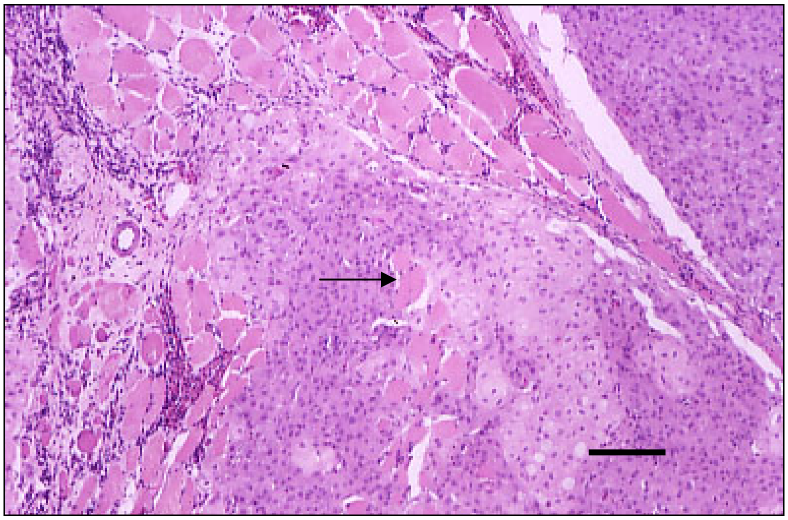

Figure 7. Squamous cell carcinoma of lip. Solid pattern of undifferentiated, squamous (flattened) cells invading muscle. Muscle trapped within the advancing cancer (arrow) (05STH015). Bar = 75 µm.

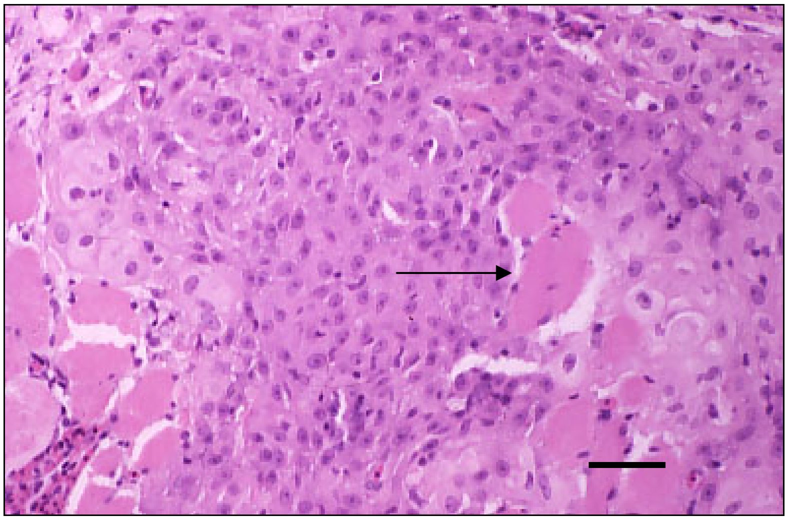

Figure 8. Squamous cell carcinoma of lip showing trapped muscle (arrow) at higher power (05STH015). Bar = 30 µm.

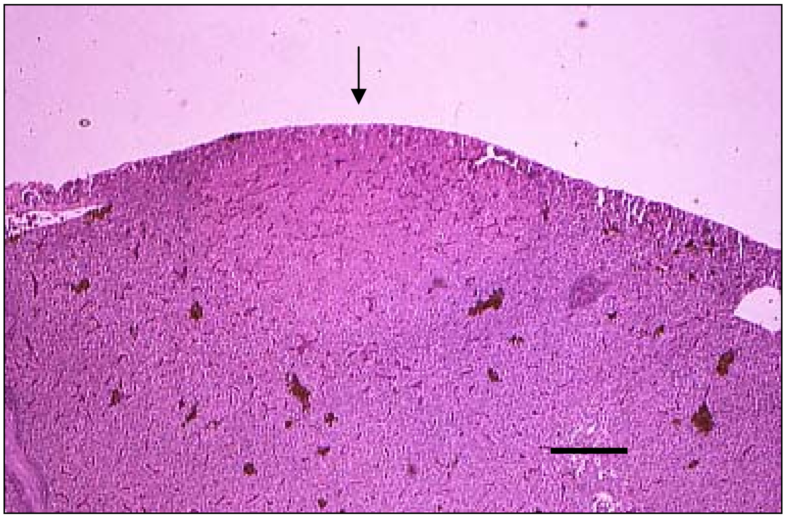

Figure 9. Hepatocellular carcinoma. A protruding mass with a jagged edge indicative of invasion (arrow). (05STH016). Bar = 190 µm.

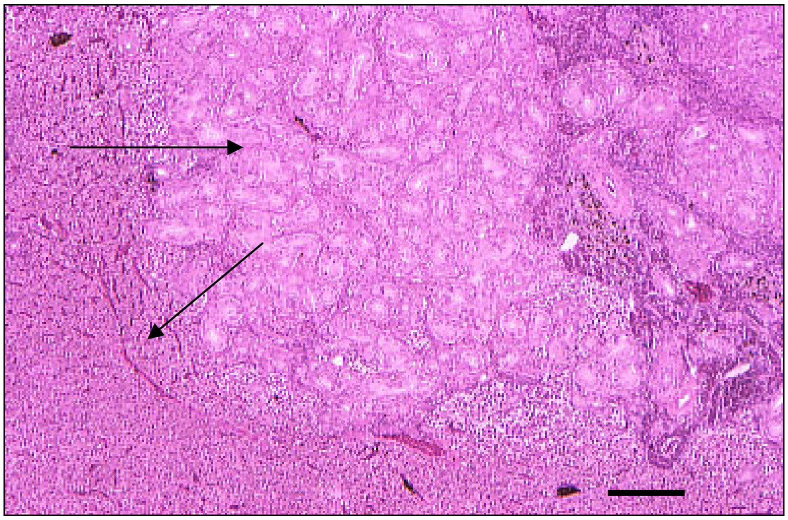

Figure 10. Cholangiocarcinoma. Irregular mass of poorly formed bile ducts (3 o'clock arrow) invading normal liver (7 o'clock arrow). (05STH016). Bar = 190 µm.

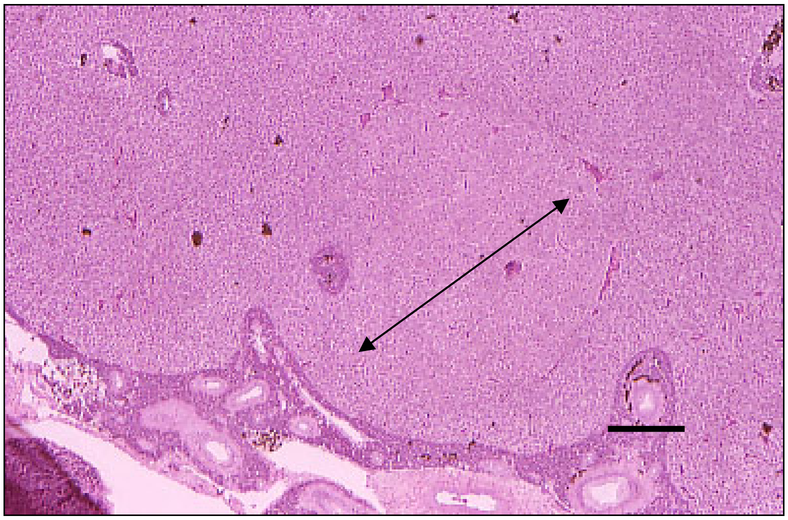

Figure 11. Hepatocellular adenoma. Discrete, slightly eosinophilic mass of neoplastic cells (double arrow) displacing normal liver (05STH016). Bar = 500 µm

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

Anaplastic: Characterized by, composed of, or being cells which have reverted to a relatively undifferentiated state

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

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

Squamous: Scaly or platelike.

APPENDIX B

SPREADSHEETS WITH BIOLOGICAL AND PATHOLOGICAL DATA

	Legend
FHA	Foci of hepatocellular alteration (often a precancerous condition)
HA	Hepatocellular adenoma (a non-invasive liver tumor)
HC	Hepatocellular carcinoma (an invasive liver tumor)
C	Cholangioma (a non-invasive bile duct tumor)
CC	Cholangiocarcinoma (an invasive bile duct tumor)
TLC	Total liver or bile cancer (all fish with either HC or CC)
TLT	Total liver tumors (all fish with either HA, HC, C, or CC)
TLL	Total liver lesions (all fish with either FHA, HA, HC, C or CC)
MSL	Most serious liver lesion (used to construct pie charts)
EP	Epidermal papilloma (a non-invasive skin tumor)
SC	Squamous carcinoma (an invasive skin tumor)
TST	Total skin tumors (all fish with either EP or SC)
AB	Shortened, clubbed, or missing barbel(s)
H.S.I.	Hepatosomatic index (liver wt/body wt)
K	Condition factor (wt x 100,000 divided by length cubed)

sample ID	FHA	HA	HC	C	CC	TLT	TLC	TLL	MSL	EP	SC	TST	AB		Tot. Length	Weight	Liver wt	Condition factor	H.S.I.	Age	Sex
3/23/05 FYKE																					
05STH1	0	0	0	0	1	1	1	1	CC	0	0	0	1		330	472	7.45	1.31	0.016	4	M
05STH2	0	0	0	0	0	0	0	0		0	0	0	0		305	384	5.4	1.35	0.014	4	M
05STH3	0	0	0	0	0	0	0	0		0	0	0	0		334	462	7.6	1.24	0.016	4	M
05STH4	0	0	0	0	0	0	0	0		0	1	1	0		314	360	5.7	1.16	0.016	4	M
05STH5	0	0	0	0	0	0	0	0		0	1	1	0		315	401	9.15	1.28	0.023	5	F
05STH6	0	0	0	0	0	0	0	0		0	1	1	0		315	388	6.95	1.24	0.018	NA	F
05STH7	0	0	0	0	0	0	0	1		0	0	0	0		281	271	3.25	1.22	0.012	4	M
05STH8	0	0	0	0	0	0	0	0		0	1	1	0		327	426	7.9	1.22	0.019	6	M
05STH9	0	0	0	0	0	0	0	0		0	1	1	0		306	355	7.15	1.24	0.020	4	M
05STH10	0	0	0	0	0	0	0	0		1	0	1	0		310	334	5.05	1.12	0.015	5	M
05STH11	0	0	0	0	0	0	0	0		0	1	1	0		327	387	6.85	1.11	0.018	4	F
05STH12	0	0	0	0	0	0	0	0		0	0	0	0		320	380	7.35	1.16	0.019	5	M
05STH16	0	1	1	0	0	1	1	1	HC	0	1	1	0		332	436	NA	1.19	NA	4	M
05STH17	0	0	0	0	1	1	1	1	CC	1	0	1	0		306	359	8.2	1.25	0.023	5	F
05STH18	1	0	0	0	0	0	0	1	FHA	0	0	0	0		309	336	5.3	1.14	0.016	4	M
05STH19	0	0	0	0	0	0	0	0		0	0	0	1		313	392	5.7	1.28	0.015	6	F
05STH20	1	0	1	0	0	1	1	1	HC	0	0	0	1		337	474	7.6	1.24	0.016	5	M
05STH21	0	0	1	0	0	1	1	1	HC	0	0	0	0		272	236	4.5	1.17	0.019	3	F
05STH22	0	0	0	0	0	0	0	0		0	0	0	0		297	311	5.7	1.19	0.018	5	F
05STH23	0	0	0	0	0	0	0	0		0	1	1	0		320	377	7.2	1.15	0.019	5	F
05STH24	0	0	0	0	0	0	0	0		0	0	0	0		308	357	5.65	1.22	0.016	4	M
05STH25	0	0	0	0	0	0	0	0		0	1	1	0		312	359	4.55	1.18	0.013	5	F
05STH26	0	0	0	0	0	0	0	0		0	0	0	0		301	345	4.8	1.27	0.014	4	M
05STH27	0	0	0	0	0	0	0	0		0	1	1	0		307	354	4.65	1.22	0.013	4	M
05STH28	0	0	0	0	0	0	0	0		0	1	1	0		305	355	5.8	1.25	0.016	4	M
05STH29	1	0	0	0	0	0	0	1	FHA	0	0	0	0		298	327	5.15	1.24	0.016	5	M
05STH30	1	0	0	0	0	0	0	1	FHA	0	1	1	0		313	396	5.7	1.29	0.014	4	M
05STH31	0	0	0	0	1	1	0	0	CC	0	1	1	0		330	452	10.25	1.26	0.023	5	F
05STH32	0	0	0	0	0	0	0	0		0	0	0	0		289	282	4.6	1.17	0.016	3	M
05STH33	0	0	0	0	0	0	0	0		1	0	1	0		318	413	4.15	1.28	0.010	4	F
MIN														**avg**	312	373	6.18	1.22	0.017	4.414	11F
MAX														**std**	15	57	1.61	0.06	0.003	0.733	19 M
MEAN														**min**	272	236	3.25	1.11	0.010	3.000	
SD														**max**	337	474	10.25	1.35	0.023	6.000	
sum	4	1	3	0	3	6	5	9		3	13	16	3							AGE 3	N=2
%(/30*100)	13.3	3.3	10	0	10	20	16.7	30		10	43.3	53.3	10.0							AGE 4	N=15
																				AGE 5	N=10
																				AGE 6	N=2
																				AGE ?	N=1

sample ID	FHA	HA	HC	C	CC	TLT	TLC	TLL	MSL	EP	SC	TST	AB		Tot. Length	Weight	Liver wt	Condition factor	H.S.I.	Age	Sex
3/23/05 FYKE																					
05STH1	0	0	0	0	1	1	1	1	CC	0	0	0	1		330	472	7.45	1.31	0.016	4	M
05STH2	0	0	0	0	0	0	0	0		0	0	0	0		305	384	5.4	1.35	0.014	4	M
05STH3	0	0	0	0	0	0	0	0		0	0	0	0		334	462	7.6	1.24	0.016	4	M
05STH4	0	0	0	0	0	0	0	0		0	1	1	0		314	360	5.7	1.16	0.016	4	M
05STH5	0	0	0	0	0	0	0	0		0	1	1	0		315	401	9.15	1.28	0.023	5	F
05STH7	0	0	0	0	0	0	0	0		0	0	0	0		281	271	3.25	1.22	0.012	4	M
05STH9	0	0	0	0	0	0	0	0		0	1	1	0		306	355	7.15	1.24	0.020	4	M
05STH10	0	0	0	0	0	0	0	0		1	0	1	0		310	334	5.05	1.12	0.015	5	M
05STH11	0	0	0	0	0	0	0	0		0	1	1	0		327	387	6.85	1.11	0.018	4	F
05STH12	0	0	0	0	0	0	0	0		0	0	0	0		320	380	7.35	1.16	0.019	5	M
05STH16	0	1	1	0	0	1	1	1	HC	0	1	1	0		332	436	NA	1.19	NA	4	M
05STH17	0	0	0	0	1	1	1	1	CC	1	0	1	0		306	359	8.2	1.25	0.023	5	F
05STH18	1	0	0	0	0	0	0	1	FHA	0	0	0	0		309	336	5.3	1.14	0.016	4	M
05STH20	1	0	1	0	0	1	1	1	HC	0	0	0	1		337	474	7.6	1.24	0.016	5	M
05STH22	0	0	0	0	0	0	0	0		0	0	0	0		297	311	5.7	1.19	0.018	5	F
05STH23	0	0	0	0	0	0	0	0		0	1	1	0		320	377	7.2	1.15	0.019	5	F
05STH24	0	0	0	0	0	0	0	0		0	0	0	0		308	357	5.65	1.22	0.016	4	M
05STH25	0	0	0	0	0	0	0	0		0	1	1	0		312	359	4.55	1.18	0.013	5	F
05STH26	0	0	0	0	0	0	0	0		0	0	0	0		301	345	4.8	1.27	0.014	4	M
05STH27	0	0	0	0	0	0	0	0		0	1	1	0		307	354	4.65	1.22	0.013	4	M
05STH28	0	0	0	0	0	0	0	0		0	1	1	0		305	355	5.8	1.25	0.016	4	M
05STH29	1	0	0	0	0	0	0	1	FHA	0	0	0	0		298	327	5.15	1.24	0.016	5	M
05STH30	1	0	0	0	0	0	0	1	FHA	0	1	1	0		313	396	5.7	1.29	0.014	4	M
05STH31	0	0	0	0	1	1	0	0	CC	0	1	1	0		330	452	10.25	1.26	0.023	5	F
05STH33	0	0	0	0	0	0	0	0		1	0	1	0		318	413	4.15	1.28	0.010	4	F
sum	4	1	2	0	3	5	4	7		3	11	14	2	**avg**	313	378	6.24	1.22	0.017	4.400	17M
% (/25*100)	16.0	4.0	8.0	0.0	12.0	20.0	16.0	28.0		12.0	##	56.0	8.0	**std**	13	51	1.66	0.06	0.003	0.500	8F
														min	281	271	3.25	1.11	0.010	4.000	
														max	337	474	10.25	1.35	0.023	5.000	
																				4 YRS=15	
																				5 YRS = 10	

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Sample	Method	FHA	HA	HC	C	CC	TLC	TLT	TLL	MSL	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	CC	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	FHA	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	HA	0	0	0	N/M		263	210	4.30	1.15	0.020	3	F
TBB23		0	0	0	1	0	0	1	1	C	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	FHA	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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Sample	Method	FHA	HA	HC	C	CC	TLC	TLT	TLL	MSL	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	HA	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	FHA	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	FHA	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	FHA	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	C	0	0	0	0		298	325	6.35	1.23	0.0195	5	M
TKL 25		1	0	0	0	0	0	0	1	FHA	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	FHA	0	0	0	0		262	222	5.20	1.23	0.0234	3	M
TKL 30		1	0	0	0	0	0	0	1	FHA	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

1996, 1998, 2000, 2001
Tuckahoe all ages

Sample	Method	FHA	HA	HC	C	CC	TLC	TLT	TLL	MSL	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**								
% (/117*100)		**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	
																				age 4	30	
																				age 3	27	
																				age 5	11	
																				age 2	6	

Tuckahoe 96,98,00,01 age4&5

Sample	Method	FHA	HA	HC	C	CC	TLC	TLT	TLL	MSL	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
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	CC	0	0	0	N/M		273	241	5.85	1.18	0.024	4	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
TBB14		0	0	0	0	0	0	0	0		0	0	0	N/M		267	241	4.35	1.27	0.018	4	M
TBB17		0	0	0	0	0	0	0	0		0	0	0	N/M		274	233	4.70	1.13	0.020	4	F
TBB19		0	0	0	0	0	0	0	0		0	0	0	N/M		271	220	6.15	1.11	0.028	4	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
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
TKL 3		1	0	0	0	0	0	0	1	FHA	0	0	0	0		285	330	6.94	1.43	0.0210	5	M
TKL 6		1	0	0	0	0	0	0	1	FHA	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 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 17		0	0	0	0	0	0	0	0		0	0	0	0		278	258	4.73	1.20	0.0183	4	M
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 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	C	0	0	0	0		298	325	6.35	1.23	0.0195	5	M
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 30		1	0	0	0	0	0	0	1	FHA	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 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 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		**3**	**0**	**0**	**1**	**1**	**1**	**2**	**5**		**0**	**0**	**0**	**3**								
%(/41*100)		**7.317**	**0**	**0**	**2.439**	**2.439**	**2.439**	**4.88**	**12.2**		**0**	**0**	**0**	**10.34**	**avg**	272.1	252	4.66	1.25	0.0185	4.3	10F
															std	8.2	26	1.15	0.09	0.0041	0.4	30M
															min	260	205	2.50	1.04	0.0108	4	1?
															max	298	330	7.02	1.43	0.0280	5	
																				age 4	30	
																				age 5	11	