Evaluation of Liver and Skin Tumor Prevalence in Fish from the Delaware Estuary Watershed

Proposed by

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**Project Title:** Evaluation of Liver and Skin Tumor Prevalence in Fish from the Delaware Estuary Watershed  

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**INTRODUCTION**

Tumor surveys in bottom-dwelling fish have been used widely as an indicator of contamination and a method for monitoring the success of cleanup actions (e.g., Baumann et al. 1996, Baumann and Harshbarger 1998, Myers et al. 1994). In freshwater ecosystems, the brown bullhead (Ameiurus nebulosus) has been shown to develop skin and liver tumors in response to contaminant exposure, with the most persuasive linkage for areas with polynuclear aromatic hydrocarbon (PAH)-contaminated sediments (Baumann and Harshbarger 1998). Baumann et al. (1996) suggested that, in brown bullheads, liver tumor prevalence above 9% and skin tumor prevalence above 20% were nearly always observed in chemically contaminated areas. Recently, Baumann (2002) refined these estimates and stated that liver tumor prevalence above about 5% and skin tumor prevalence above 12% could be used to distinguish between highly contaminated and less contaminated areas in the Great Lakes. The U.S. Fish and Wildlife Service, Chesapeake Bay Field Office (CBFO) has developed a database on tumors in bullheads from the tidal freshwater areas of the Chesapeake Bay watershed (Pinkney et al. 1995, 2001, 2002).

Brown bullheads have been captured in waters with salinity as high as 15 parts per thousand (Boyer 1995) but they are primarily a freshwater species and can be difficult to locate in estuarine waters. The mummichog, Fundulus heteroclitus, has been used in tumor surveys in estuaries of the U.S. east coast. Vogelbein et al. (1990) reported a 33% prevalence of liver tumors in mummichogs collected near the Atlantic Wood Treating Superfund Site on the Elizabeth River, Virginia, where sediments are contaminated with creosote. A sediment sample measured in the study at the site with the 33% tumor prevalence contained 2200 ppm total PAHs. In a pilot study, Harshbarger (2003) diagnosed hepatocellular carcinomas in 30% of mummichogs collected in Hershey Run, adjacent to the Koppers Wood Treating Superfund Site, near Newport, Delaware. In Hershey Run, total PAH concentrations as high as 13,300 ppm has been reported (Woodward Clyde 1996). If a size minimum of 7.0 cm is applied, the Harshbarger (2003) prevalence is increased to 43%. This report is attached as an appendix to this proposal.

Here we propose an approach for an initial tumor survey of selected sites in the Delaware Estuary. We identify areas as possible sites of interest due to sediment contamination with PAHs and other compounds. We propose to conduct sampling at or near the two Delaware Estuarine Research Reserve sites - Blackbird Creek and the Lower St. Jones River - to establish baselines for long-term monitoring of these areas.

**MATERIALS AND METHODS**
Sites of Interest

Area 1: The Christina River is a tidal freshwater system that flows into the Delaware through a highly industrialized watershed, containing eight federal and several state Superfund sites. For example, the Koppers Superfund site is a former wood treating facility that released PAHs and metals into Hershey Run, and wetlands adjacent to White Clay Creek and the Christina. Junkyards with automobile parts can be observed along the banks of some stretches of the river. Throughout the watershed, sediments are contaminated with polychlorinated biphenyls (PCBs), polynuclear aromatic hydrocarbons (PAHs), pesticides, and toxic metals (Olinger 1997). A fish tissue advisory is in place (R. Greene, Delaware Department of Natural Resources and Environmental Control (DNREC), pers. comm.). Olinger (1997) reported the occurrence of sediment toxicity and measured carcinogenic PAHs in the 20 ppm (wet weight) range using immunoassay screening procedures. Limited sampling conducted as part of the Koppers investigation, indicated that a Christina River sediment sample collected near the confluence of White Clay Creek contained a total PAH concentration of 23.530 ppm dry weight (Woodward-Clyde 1996). One location in Hershey Run had a total PAH concentration of 13,300 ppm. This section of the Christina River (mid-Christina River) was listed on the Delaware 1998 303(d) list of impaired waters due to PCBs and nutrients.

Mummichogs were collected from Hershey Run for the Harshbarger (2003) study and have been collected from the tidal Christina (Woodward-Clyde 1996). Brown bullheads and mummichogs are included in the species lists for the tidal Christina River compiled by Woodward-Clyde Associates (1992).

Area 2: The industrialized Delaware City area of the Delaware River is impacted by releases from several operating and former facilities. There are reports of repeated National Pollutant Discharge Elimination System (NPDES) violations from the Motiva Refinery and several Superfund sites (Standard Chlorine, Tybouts Corner) are located in the nearby Red Lion Creek subwatershed. NOAA (2001) reported a total sediment PAH concentration of 12.6 ppm in a sample collected several miles downriver of the refinery outfall. A fish tissue advisory is in place for this section of the Delaware River (R. Greene, DNREC, pers. comm.). Red Lion Creek contains a mixture of contaminants in sediments including chlorinated benzenes from the Metachem (Standard Chlorine) Superfund site and PCBs from unknown sources. This creek is also covered by a fish advisory.

Brown bullheads were observed in small numbers in Red Lion Creek but may be difficult to obtain in sufficient numbers for surveys. Mummichogs are likely to be available from both Red Lion Creek and the areas near the Motiva facility.

Area 3: One of the two Delaware National Estuarine Research Reserve sites is the Lower St. Jones River Reserve. The St. Jones River watershed drains a portion of the coastal plain in central Kent County, DE, including the city of Dover, the surrounding suburbs, industrial areas, agricultural areas and Dover Air Force Base (NERR web site: http://inlet.geol.sc.edu/DEI/st-jones-river.html). The river is less than pristine; four National
Priority List Superfund sites, including the former Dover Gas Light facility that released PAHs, are in the watershed. In 1993, DNREC issued a fish tissue consumption advisory based on PCB concerns for catfish, carp, white perch, and largemouth bass (DNREC 1999). The upper St. Jones River is included on the 303(d) list for nutrients, pathogens, low dissolved oxygen, and PCBs. The Lower St. Jones River is listed on the state's 1998 303(d) list of impaired waters due to PCBs, nutrients, and low dissolved oxygen.

Brown bullheads have been identified in preliminary trapping efforts in the St. Jones River near the Route 13 Bridge. It is possible that electroshocking would produce sufficient numbers in areas near Dover, Delaware. It is likely that mummichogs could be easily collected, especially from the lower section of the river. Their abundance at the Reserve was documented on the web site: http://www.dnrec.state.de.us/dnrec2000/divisions/soil/dnerr/tmp1023300816.htm.

Area 4: The other Delaware National Estuarine Research Reserve is Blackbird Creek. Blackbird Creek is believed to be relatively unimpacted from local sources of industrial contaminants (D. Carter, DNREC, pers. comm.), although there is concern about non-point source pollution from silviculture and agricultural runoff (DNREC 1999). Preliminary surveys (conducted last fall) and salinity data suggest that mummichogs would be the appropriate species for this river.

Brown bullheads are listed on the Reserve web site http://inlet.geol.sc.edu/DEL/blackbird-creek.html as being found in the freshwater areas.

Sampling Design

We propose to conduct a tumor survey in the two industrial areas (the Christina River and the Delaware City area of the Delaware River) and the two NOAA Estuarine Research Reserve (Blackbird Creek and St. Jones River) sites in Delaware. The proposed study would be the first systematic fish tumor survey in Delaware and should give DNREC a useful environmental indicator for watershed monitoring. The proposed areas and sites are listed in Table 1.

We propose to use the mummichog in an initial survey of these areas. Mummichogs are known to be highly localized (Whitehead 1995) with summer home range of 36 meters in tidal creeks and somewhat more extensive movements in fall and winter. They have considerable contact with sediments, feeding on bottom-dwelling invertebrates. Advantages of the mummichog in tumor surveys are that they are often easy to obtain large numbers at low cost and they have been shown to develop liver tumors when exposed to high concentrations of PAHs (Vogelbein et al. 1990, Harshbarger 2003). Their response to lower PAH concentrations and other sediment-bound carcinogens is not yet known. Based on available data, we believe that mummichogs will be available from all six of the proposed collection sites (Table 1).

The number of collection sites depends on the availability of funding (see below). Based on conversations with DNREC, we propose to work together with DNREC personnel to obtain samples. One approach would be to collect mummichogs from two sites in each of the industrial areas and one site in each of the Estuarine Reserves. Thus, for the Christina area, the possible sites would be (1) Hershey Run near the Koppers site, and (2) either Churchman's Marsh or the
Christina River near White Clay Creek. For the Delaware City Area, the possible sites would be (1) Red Lion Creek, and (2) the near shore area near the Motiva refinery (Table 1).

If requested, we can provide a separate proposal for investigating tumors in brown bullheads. The advantage of bullheads is that there is an extensive data base on tumor prevalence in both the Chesapeake Bay and Great Lakes watersheds. Thus, we would be able to compare the prevalence in the Delaware Estuary against proposed criteria of 5% liver tumors and 12% skin tumors proposed by Baumann (2002) to distinguish highly contaminated Areas of Concern vs. less contaminated Areas of Recovery. Of the four identified areas, we believe that the most likely locations for brown bullheads in sufficient size and number would be the St. Jones River near Dover and sections of the Christina River. The disadvantage of the bullhead would be the expense and difficulty in collecting sufficient numbers of fish of appropriate size (≥250 mm).

Objectives

1. To evaluate the prevalence of skin and liver tumors in mummichogs from locations in the Delaware River watershed where polynuclear aromatic hydrocarbon (PAH) and other organic chemical contamination is suspected and from the two Delaware National Estuaries Research Reserves;
2. To evaluate the response of mummichogs in areas of extreme (Hershey Run) and less extreme (Churchman’s Marsh) sediment contamination;
3. To provide environmental managers with a monitoring tool to evaluate habitat quality and monitor changes in loadings and/or cleanup actions.

Methods/Procedures:

We will collect thirty mummichogs (≥ 70 mm) from each of the six sites. With the assistance of DNREC, fish will be collected either with minnow traps, seining, or electroshocking. Sampling efforts will be coordinated through David Carter and Bob Scarborough of DNREC. Fish would be held in collection site water at one of the Estuarine Reserve facilities and transported live to the Chesapeake Bay Field Office for necropsy.

Fish will be measured, weighed, euthanized, and necropsied. Visible lesions will be noted and examples will be photographed. Livers will be excised and weighed and visible skin lesions will be dissected. The organs will be preserved in 10% buffered neutral formalin, embedded, sectioned at 4 um, and stained with hematoxylin and eosin. Dr. John Harshbarger of George Washington University Medical Center will perform the histopathology and diagnose the presence of tumors and preneoplastic lesions. A statistical comparison of lesion prevalence will be performed using an extension of Fisher’s Exact Test (Stokes et al. 1995).

Dissemination of Results

Results will be presented as an FWS scientific report including a description of the habitats, compilation of existing contaminant and water quality data, statistical comparison of tumor prevalence, and interpretation of findings. A fact sheet will be prepared that can be placed on
FWS and DNREC web pages. The report will be distributed to appropriate state agencies, interagency groups working in the watershed, and to the public. An effort will be made to publish study results in a peer-reviewed journal. This may be dependent on the availability of relevant sediment quality data from the collection sites so that inferences can be made on the degree of contaminant exposure. If requested, FWS will collect and analyze sediment samples from transects at each collection site. Sediments would be analyzed for PAHs (including alkylated compounds), PCBs, organochlorine pesticides, grain size, and total organic carbon. FWS contract laboratories would be used and quality control/quality assurance would be provided by the FWS' Patuxent Analytical Control Facility.

Relevance to Environmental Managers:

The results of the study would be useful in helping to determine the suitability of the mummichog as a biological monitoring tool. The proposed study would serve as a 2003 baseline; a similar survey could be repeated several years hence as a tool to monitor habitat quality. Tumor rates in bottom-dwelling fish have been used as such an indicator in the Great Lakes and Puget Sound for the identification and monitoring of areas of concern (e.g., Hartig et al. 1990). Development of a fish tumor indicator would partially address the need identified in the Delaware Estuary Program's Comprehensive Conservation and Management Plan for data on the acute and chronic effects of contaminated sediments on aquatic biota.

Project Time Period: June 2003 - March 2004

June - July 2003: Fish collection and necropsy

July - November 2003: Tissue processing and histopathology

December 2003 - January 2004: Preparation of draft report

Final report to be provided 30 days after receipt of all comments

Table 1. Proposed sampling sites

<table>
<thead>
<tr>
<th>Area</th>
<th>Site 1</th>
<th>Site 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Christina Hershey</td>
<td>Hershey Run near Koppers</td>
<td>Churchman's Marsh or Christina</td>
</tr>
<tr>
<td>Churchman's</td>
<td></td>
<td>near White Clay Creek</td>
</tr>
<tr>
<td>Delaware City</td>
<td>near Motiva facility</td>
<td>Red Lion Creek</td>
</tr>
<tr>
<td>St. Jones</td>
<td>Lower St. Jones- Reserve</td>
<td></td>
</tr>
<tr>
<td>Blackbird Creek</td>
<td>Blackbird Creek - Reserve</td>
<td></td>
</tr>
</tbody>
</table>

BUDGET

Histopathology: Dr. John Harshbarger, George Washington University Medical Center

Estimate based on 180 samples (6 locations x 30 fish/location) via purchase order from DNREC based on $100 per fish to Dr. Harshbarger
$14,000 ($4000 in kind)

**FWS charges**

- CBFO salary (collection and necropsy@ 10 days (2 biologists x 5 days@$560/day)) $4900 ($700 in kind)
- CBFO salary (data analysis and report) 7 days@$560/day $2800 ($1120 in kind)
- Supplies (traps, nets, jars, travel, reagents) $300
- Total FWS Direct Cost $8000
- Overhead (18%) $1440
- Total to FWS $9440
- Total Requested for Proposal (based on 180 fish estimate) $23,440

Note: Dr. Harshbarger would only be paid for the sites that are collected. For example, if due to collection problems, there are only 3 sites (90 fish), his fee would be $7000. The FWS charges would not change.

**REFERENCES**


Delaware Department of Natural Resources and Environmental Control (DNREC). 1999. Delaware National Estuarine Research Reserve Estuarine Profiles. DNREC, Dover, DE.


National Oceanographic and Atmospheric Administration (NOAA). 2001. Magnitude and extent of contaminated sediment and toxicity in Delaware Bay. NOS NCCOS CCMA 148, Silver Spring, MD.


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EDUCATION:
Ph.D., Marine-Estuarine-Environmental Science, University of Maryland, College Park, MD, 1988 (Research conducted at Chesapeake Biological Laboratory, Solomons, MD)
M.S., Environmental Health Sciences, New York University, Tuxedo, NY, 1982
B.S., Zoology, University of Michigan, Ann Arbor, MI, 1976

FIELDS OF COMPETENCE AND EXPERIENCE:
Aquatic toxicology, fish and invertebrate physiology and biochemistry, contaminant monitoring, ecological risk assessment, natural resource damage assessment.

EMPLOYMENT:
1993-present: Senior Environmental Contaminants Specialist/Team Leader, U.S. Fish and Wildlife Service, Chesapeake Bay Field Office, Annapolis, Maryland
1989-1993: Scientist, Versar, Inc., Columbia, Maryland
1986-1988: Graduate Research Assistant, Chesapeake Biological Laboratory, Solomons, Maryland
1983-1985: Research Assistant, Johns Hopkins University, Applied Physics Laboratory, Shady Side, Maryland

PRIMARY RESPONSIBILITIES:
Develops and conducts studies assessing the impacts of contaminants on fish and wildlife resources and their habitats; serves on the EPA Region III, Biological and Technical Assistance Group (BTAG), which reviews and advises EPA on ecological issues at hazardous waste sites.

SELECTED PUBLICATIONS:
(-20 peer-reviewed articles, 40 reports, 40 abstracts)
Pinkney et al. 1999. Ecological risk assessment--Quantico embayment, Marine Corps Combat Development Command - Quantico, Quantico, Virginia. CBFO-C99-03.
CURRICULUM VITAE

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Education:
1957 - B.A., Bridgewater College, Bridgewater, Virginia
1959 - M.S., Virginia Polytechnic Institute, Blacksburg, Virginia
1962 - Ph.D., Rutgers University, New Brunswick, New Jersey
1966-88 - Postgraduate pathobiology courses: Harvard Univ. Medical School, Georgetown Univ. Medical School and the NIH Graduate School

Employment:
1962-64 - National Science Foundation Postdoctoral Fellowship in Insect Pathology, Insect Pathology Laboratory, USDA, Beltsville, MD
1964-67 - Research Pathobiologist, University of California, Irvine, CA
1967-95 - Director, Registry of Tumors in Lower Animals, Smithsonian Institution, Washington, D.C.
1995- pres. Professor of Pathology and Director, Registry of Tumors in Lower Animals, George Washington University Medical Center, Washington, D.C.

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----- Forwarded by Chris Guy/CBFO/R5/FWS/DOI on 06/24/2003 02:20 PM -----

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cc:  
Subject: proposal--delaware mummichogs

(See attached file: delltumsurv2003.wpd)

gave to Chris