**SCOPE OF WORK**

**January 24, 2014**

**Project Title:** Milwaukee Estuary Area of Concern Fish Tumor Analysis

**Project Manager:** Vicky Blazer & Dr. Patricia Mazik, PhD.

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**Project Location:** Samples will be collected in the Root River

**Total Budget:** $59,036

**Project Description and Background:**

In mid-1980’s, the Milwaukee River and Estuary were designated an Area of Concern (AOC) because of water quality and habitat problems associated with the historical discharge of pollutants into the AOC. The Milwaukee River and Estuary AOC includes the lower 5 km of the Milwaukee River; the lower 4.8 km of the Menomonee River; the lower 4 km of the Kinnickinnic River; the inner and outer Harbor and the nearshore waters of Lake Michigan. Pollutants of concern, both conventional and toxic, have been identified as suspended solids, fecal coliform bacteria, phosphorus, nitrogen, polychlorinated biphenyls (PCBs), Polynuclear Aromatic Hydrocarbons (PAHs) and heavy metals. The high levels of nutrients, solids and toxics entering the river had caused a series of problems including nuisance algal blooms, fish consumption advisories and contaminated sediments. The pollutant discharges also were suspected of contributing to the degradation of wildlife, fish, benthos and plankton populations and the reduction in fish and wildlife habitat.

The “Fish Tumors and Other Deformities” Beneficial Use Impairment (BUI) is present in Milwaukee River AOC and an investigation in 2013 suggests that the tumor incident rate is above stated the stated goal (5%). Understanding the extant tumor rate in an appropriate reference to the Milwaukee AOC is a priority in determining whether the fish tumor BUI should be removed once sufficient remediation has occurred.

This project will determine the extant rate of tumors or other deformities found in fish in an area that is not classified as an AOC and compare this rate to the rate found in the Milwaukee River AOC in 2013. The Milwaukee Technical Advisory Group has agreed that the Root River is an appropriate reference site. White sucker *(Catostomus commersoni)* is the species that will be sampled. Choice of the appropriate indicator fish species to sample was determined by IJC guidance and likelihood of reaching the intended sample size. Both bullheads *(Ameiurus sp.)* and suckers (*Catostomus sp.)* are specifically mentioned in the IJC 1991 BUI definition and have demonstrated increased tumor rates in association with contaminants. The density of brown bullhead is low in tributaries of Lake Michigan, therefore white suckers will be targeted for sampling. However, any brown bullhead captured will be sampled.

Intensive sampling results suggest that tumor incidence rates in the Milwaukee AOC are be above target rates. Therefore, white sucker collection at an appropriate reference site (Root River Estuary) will be conducted.

**Justification:**

Historic industrial pollution within the AOC has led to contamination of fish, waterfowl and other wildlife. The fish tumors or deformities have been associated with chemical contaminants. This project does not seek to correct any habitat impairment, but rather to assess if a beneficial use impairment persists in the Milwaukee River AOC.

**Project Goals:**

• Determine tumor incidence rate in the Root River Estuary for comparison to the fish tumor incidence rate in the Milwaukee AOC.

**METHODS**

**Field Methods**

Two hundred age 3+ white sucker will be collected within the Root River Estuary by electrofishing or fyke/trap nets during the spring 2014 spawning run. Fish collection will be conducted by the WDNR or associated contractors such as University of Wisconsin-Madison. Personnel from the USGS National Fish Health Laboratory (NFHRL), Kearneysville and West Virginia University (WVU), under the direction of Dr. Vicki Blazer will conduct collection of samples for the fish health assessments. Fish will be anesthetized, weighed and measured. A blood sample will be taken and two blood smears made which will be used for quantification of micronuclei as an indication of genotoxicity. The remaining blood will be stored on wet ice until centrifuged and plasma aliquoted into 1-3 cryovials. Samples will be stored at -80C until analyzed for plasma hormone and vitellogenin. A comprehensive necropsy based assessment (Goede and Barton 1990; Smith et al. 2002; Rafferty and Grazio 2007) will be completed on each fish and any abnormalities documented. Pieces of skin abnormalities (raised lesions, melanistic spots etc.) and raised areas on barbels (brown bullhead) will be removed and fixed. Liver will be removed and weighed for hepatosomatic index, gonads removed and weighed for gonadosomatic index. These necropsy-based and morphometric observations have been used in numerous monitoring programs and while not as sensitive as histopathological or molecular analyses do provide important information at the organism level (Goede and Barton 1990; Schmitt and Dethloff, eds 2000; Foumie et al. 1996). Liver will be cut into pieces 10-15 mm in size and at least 7 pieces placed in fixative. If there are areas of discoloration, raised areas, white spots etc. pieces will include the abnormalities as well as some normal tissue. In addition, pieces of spleen, kidney, gill, gonad and thyroid from the first 20 fish (and any other that appear abnormal) at each site will be fixed for histopathology. Fixed tissue will be maintained at room temperature and transported to the NFHRL. Pieces of liver, spleen and anterior kidney will be placed in RNAlater for molecular analyses. Otoliths will be removed and age will be subsequently estimated.

**Laboratory Analyses**

Histology slide preparations will be completed in the Histology Laboratory at the U.S. Geological Survey's National Fish Health Research Laboratory, Kearneysville, WV. The histology laboratory is equipped with all the necessary equipment and is operated under the direction of a board certified histotechnician. The slides will be processed by a technician employed by West Virginia University and working at the National Fish Health Research Laboratory. Standard operating procedures for the necropsy procedure and the slide preparations will be followed.

The fixed tissue samples will be examined for any abnormalities at the microscopic level. Numerous histopathological changes have been used as biomarkers of exposure and/or environmental stress (Myers and Fournie 2002; Stentiford et al. 2003; Au 2004; Lyons et al

2004). These include proliferative, preneoplastic and neoplastic changes in the skin and liver (Blazer et al. 2007; Blazer et al. 2009 a,b). Neoplastic changes in the skin include papillomas, squamous cell carcinomas and melanomas and within the liver include hepatic cell adenomas and carcinomas as well as the biliary cholangiomas and cholangiocarcinomas. In addition, potential preneoplastic changes such as hyperplasia in the skin, bile duct hyperplasia and altered foci in the liver will be documented. Nonneoplastic changes such as the accumulation of ceroid/lipofuscin or macrophage aggregates (Fournie et al. 2001; Raldua et al. 2008), inflammatory changes, presence of parasites and other abnormalities (Wolf and Wolfe 2005) will also be documented.

**Data Management and Analysis**

Five to eight pieces of each liver will be cut in using a standardized protocol. Pieces of any skin abnormalities will be examined. Microscopic changes will be assessed by Vicki S. Blazer, PhD. Morphometric measurements and field observations are entered onto a field data sheet and each fish is given a unique identifier in the field, which is maintained throughout the project. All data will be entered into a database. Prevalence of individual observation/abnormalities will be determined.

**Total Timeline for Project:**

March 1, 2014 to June 30, 2015

**Milestones:**

April-June 2014 Conduct field sampling in the Root River Estuary (Milwaukee AOC reference site)

June-December 2014 Preparation of the histopathology slides, assessment of microscopic pathology and initial data reporting of grossly observed lesions, morphometric data and preliminary tumor data to the WDNR

March 2015 Report on Tumor prevalence and associated database to the WDNR

December 2015 Complete preparation of journal article integrating tumor and other histologic observations and stable isotope data

**Project Budget:**

Salaries: **$39,204**

PhD Graduate stipend and benefits (3 months) $ 4,962

Fringe (8.7%) $ 432

Histology technician salary (6 months) $27,048

Fringe (25%) $ 6,762

Supplies: **$6,000**

Field supplies

Fixative, syringes, needles, tubes/containers etc. $2,000.00

Histology analyses

Mounting media, blades, slides, stains, coverslips, etc. $4,000.00

Travel: **$1,650**

Travel to field sites for 3 people

 Hotel 4 nights at $100/night $1200

 Per diem $50/day for 3 people/6days $ 450

Total per trip to field site $1,650

Direct Costs $46,854

Indirect costs (26% Overhead) $12,182

Total $**59,036**

**References**

Au, D.W.T. 2004. The application of histo-cytopathological biomarkers in marine pollution monitoring: a review. Marine Pollution Bulletin 48:817-834.

Blazer, V.S. 2002. Histological assessment of gonadal tissue in wild fishes. Fish Physiology and Biochemistry 26:85-101.

Blazer, V.S., J.W. Fournie, J.C. Wolf and M.J. Wolfe. 2006. Diagnostic criteria for proliferative liver lesions in the Brown Bullhead *(Ameiurus nebulosus).*Diseases of Aquatic Organisms 72:19-30.

Blazer, V.S., J.W. Fournie, J.C. Wolf and M.J. Wolfe. 2007. Manual for the microscopic diagnosis of proliferative liver and skin lesions in the brown bullhead *(Ameiurus nebulosus).Available* at <http://seagrant.psu.edu/publications/technicaldocs/>histofieldmanual.pdf

Blazer, V.S., S.D. Rafferty, P.C. Baumman, S.B. Smith and E.C. Obert. 2009a. Assessment of the "tumors or other deformities" beneficial use impairment in brown bullhead: I. Orocutaneous tumors. Journal of Great Lakes Research 35:517-526.

Blazer, V.S., S.D. Rafferty, P.C. Baumman, S.B. Smith and E.C. Obert. 2009b. Assessment of the "tumors and other deformities" beneficial use impairment in brown bullhead: II. Livertumors. Journal of Great Lakes Research 35:527-537.

Fournie, J.W., J.K. Summers, L.A. Courtney, V.D. Engle and V.S. Blazer. 2001. Utility of splenic macrophage aggregates as an indicator of fish exposure to degraded environments. Journal of Aquatic Animal Health 13:105-116.

Fournie, J.W., J.K. Summers and S.B. Weisberg. 1996. Prevalence of Gross Pathological Abnormalities in Estuarine Fishes. Transactions of the American Fisheries Society 125:581-590.

Goede, R.W. and B.A. Barton. 1990. Organismic indices and an autopsy-based assessment as indicators of health and condition offish. In: *Biological Indicators ofStress in Fish.* American Fisheries Society Symposium 8, Adams, S.M. (ed.), American Fisheries Society, Bethesda, MD pp. 93-108.

Lyons, B.P., G.D. Stentiford, M. Green, J. Bignell, K. Bateman, S.W. Feist, F. Goodsir, W.J. Reynolds and J.E. Thain. 2004. DNA adduct analysis and histopathological biomarkers in European flounder *(Platichthys j/esus)* sampled from UK estuaries. Mutation Research Fundamental and Molecular Mechanisms ofMutagenesis 552:177-186.

Myers, M.S. and J.W. Fournie. 2002. Histopathological Biomarkers as Integrators of Anthropogenic and Environmental Stressors. pp. 221-287 *in* Biological Indicators of Aquatic Ecosystem Stress.

Rafferty S.D., V.S. Blazer, A.E. Pinkney, J.L. Grazio, E.C. Obert and L.Boughton. 2009. A historic perspective on the "fish tumors or other deformities" beneficial use impairment at Great Lakes Areas of Concern. Journal of Great Lake Research 35:496-506.

Raldua, D., F. Padro, M. Sole, E. Eljarrat, D. Barcelo, M.C. Riva and C. Barata. 2008. First evidence ofpolybrominated diphenyl ether (flame retardants) effects in feral barbel from the Ebro River basin (NE, Spain). Chemosphere 73:56-64.

Schmitt, C.J. and G.M. Dethloff, eds. 2000. Biomonitoring of Environmental Status and Trends (BEST) Program: Selected Methods for Monitoring Chemical Contaminants and Their Effects in Aquatic Ecosystems. U.S. Geological Survey, Biological Resources Division, Columbia (MO). Information and Technology Report USGS/BRD-2000-0005. 81 pp.

Stentiford, G.D., M. Longshaw, B.P. Lyons, G. Jones, M. Green and S.W. Feist. 2003. Histopathological biomarkers in estuarine fish species for the assessment of biological effects of contaminants. Marine Environmental Research 55: 137-159.