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Cover Sheet Form

LCCMR Proposal 2007

Project Title: Threat of Emerging Contaminants to Upper Mississippi Walleye

Total Project Budget: $ 97,000

Proposed Project Length: July 2007 - June 2009

Other Funds: $ 0

Project Manager: First Name: Heiko Last Name: Schoenfuss

Sponsoring Organization: St. Cloud State University

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Location: County: City/Township: Other: Upper Mississippi Watershed

I. PROJECT SUMMARY AND RESULTS

We will assess whether the genetic diversity of walleye in the Upper Mississippi is negatively impacted by emerging contaminants at pollution "hotspots" where we previously identified feminized male fish.

Innovative _____ Outcomes _____ Statewide/Regional _____

Knowledge Base _____Partnership Leverage _____ **Total** _____

Serial # 74

Project Title: Threat of Emerging Contaminants to Upper Mississippi Walleye

II. DESCRIPTION OF PROJECT RESULTS

Rationale. In 2006 St. Cloud State University in collaboration with the US Geological Survey conducted a study of fish health in the Upper Mississippi River from Lake Itasca to the Iowa border. Our study sampled 43 sites and included fish samples from four species, including walleye and smallmouth bass, as well as water and sediment samples from each location. This survey of fish health in the context of emerging contaminants, especially endocrine disruptors and pharmaceuticals, represents the largest such effort in North America to date.

Our results indicate that there are several "hotspots" where fish health in the Mississippi River is impaired in a fashion that is consistent with the effects of emerging contaminants (see map). These effects include the feminization of male fish, which has been linked to intersex (hemaphrodism) and reduced reproductive ability in male fish. The long-term health of Minnesota fish populations may be at risk due to the impacts of these emerging contaminants on fish health. This is especially true since recent genetic research has demonstrated that long-term fish exposure to treated wastewater effluents can alter the genetic structure of fish populations and potentially result in genetic bottlenecks that can cripple a population. Fish populations that may appear healthy could in fact be approaching a critical deficit in genetic diversity to overcome subsequent environmental challenges. This is a crisis that is not obvious to the naked eye until it is too late.

We propose a combined field and laboratory approach that would for the first time, link the occurrence of emerging contaminants in the Mississippi River to feminization in male fish and explore the possibility of reduced genetic diversity in populations of walleye. The study is designed to make use of existing data and the broad expertise of our collaborative group, including an aquatic toxicologist (HLS), a fish geneticist (LMM), and aquatic ecologist (MLJ). Results of this study would allow resource managers and agencies to focus resources on particularly vulnerable populations of walleye and could be extrapolated to fish populations in all Minnesota waters.

Result 1 - Field Collection of Feminized Fish **Budget: $** *19,000*

We will confirm the feminizining effects of Mississippi River waters on walleye and fathead minnows at four contamination "hotspots" identified in our extensive existing data set. We will also identify two reference sites where fish and water samples showed no indication of the presence of emerging contaminants. All six sites will be re-sampled for walleye (up to 60 fish) and fathead minnows to generate a larger fish sample than was possible in our previous study (we will be able to add archived samples from the previous study). In addition we will cage male fathead minnows at each sites for seven days, an US EPA recommended length of time. The caging of fathead minnows will serve two purposes, first, it will confirm the presence of emerging contaminants at the identified hotspot sites before the commencement of the costly genetic analysis, and second, it will allow us to link our field studies to an existing large body of laboratory research on the effects of emerging contaminants on fish. All fish collected will be assessed for their reproductive health (Result 2) and genetic diversity (Result 3).

Result 2 - Laboratory Assessment of Fish Health **Budget:** *$29,000*

We will (1) confirm the presence of emerging contaminants at the field site and (2) link the findings in the wild caught walleye to more defined laboratory endpoints in the fathead minnow. The SCSU Aquatic Toxicology Laboratory is well equipped to analyze the reproductive health of all captured and caged fish. We have extensive expertise in documenting the likely effects of fish exposure to emerging contaminants in a timely and cost efficient manner. All fish captured and caged will undergo a histopathological analysis of the reproductive organs to test for the occurrence of hemaphrodism, a blood plasma analysis for the female egg yolk protein in male

fish (a bioindicator of acute exposure to emerging contaminants), and would be measured for morphometric endpoints. The inclusion of caged fathead minnows will provide a linkage between relevant field and laboratory data. This unique study design would greatly increase the interpretive power by establishing a cause and effect relationship between emerging contaminants and fish samples instead of merely allowing for correlations to be made.

Result 3 - Emerging Contaminant Population-Level Genetic Effects **Budget: $** *49,000*

Using DNA-based genetic markers, we will assess patterns of genetic diversity within and between walleye and fathead minnow populations collected at the contaminated "hotspot" sites and the uncontaminated reference sites. Evaluation of multiple sites along the length of the river will determine if consistent patterns emerge in relation to pollution levels. These assessments will determine if the documented biological effects on individual fish are also translating to genetic and ecological effects at the population level, possibly threatening the genetic integrity and persistence of fish populations. The AquaGen Laboratory at the University of Minnesota is a well established molecular genetic laboratory focusing specifically on fish population genetics and has a long history of analyzing population level genetic diversity in game fish.

III. TOTAL PROJECT REQUEST BUDGET

Staff or Contract Services: $ 73,000

Schoenfuss, Project Leader – 1 Month/year 100% time (incl. fringe) to supervise field study and lab analysis. ($15,635 +37% fringe) **$** 21,420

Graduate Students, St. Cloud State University – 9 months/year 50% time to conduct laboratory assessment of fish health and assist in field study **$** 19,000

Loren M. Miller, Population Geneticist – University of Minnesota – 3 months/year to conduct genetic analysis. ($21,789 +33% fringe) **$** 28,980

Undergraduate Assistant, U of MN (10 weeks/year 50% time) **$** 3,600

Equipment: $ 24,000

Field supplies $1,800; expendable SCSU lab supplies $ 4,980; field site travel $ 800; expendable AquaGen Lab supplies $ 15,920; field site travel $ 500.

TOTAL BUDGET: $ ***97,000***

IV. OTHER FUNDS & PARTNERS

A. Project Partners

Heiko L. Schoenfuss – Project Leader St. Cloud State University
Loren M. Miller – Population Geneticist, AquaGen, University of Minnesota
Matthew L. Julius – Aquatic Ecologist, St. Cloud State University

B. Other Funds being Spent during the Project Period *0*

C. Past Spending $120,000 for longitudinal study of Mississippi River at 43 sites from Lake Itasca to Iowa border.

D. Time Our recently completed longitudinal study of the Mississippi River provides the most exhaustive data set to date on the health of fish in the Mississippi River in correlation with the presence of emerging contaminants in the water and sediment. The transient nature of this information in the context of future studies requires any approach that uses this data set to occur in the very near future, i.e., the next two years. Beyond such time, a complete re-sampling of water ands sediment would have to be conducted, more then doubling the overall cost of the project.

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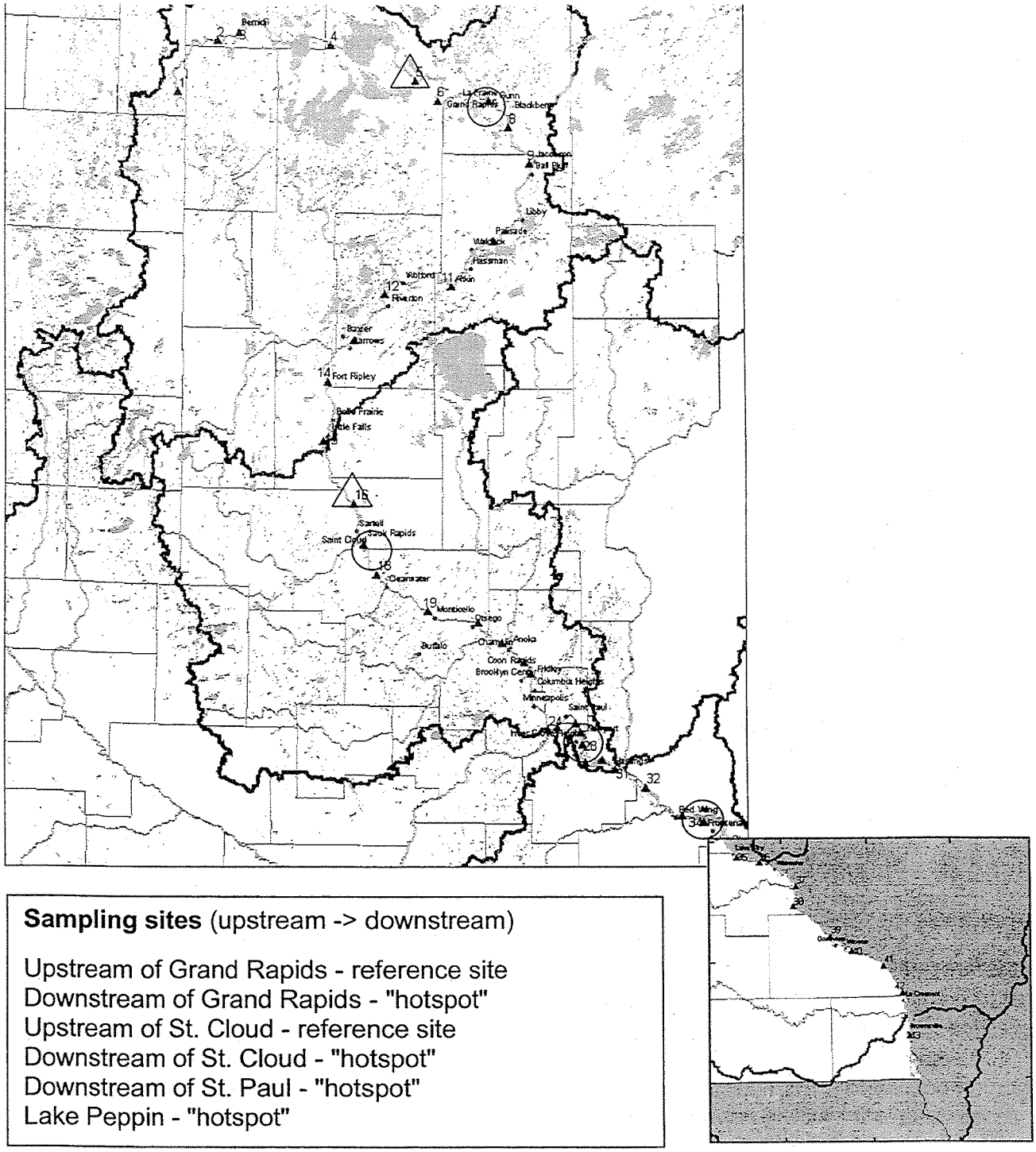
Map of the Upper Mississippi River. 43 Field sites from previous summers study are marked with numbers. Circled sites (o) indicate four "hotspots" where feminized fish were collected in 2006. Triangles indicate (Δ) appropriate reference sites. All six sites (4 hotspots, 2 reference sites) are proposed for sampling of walleye and fathead minnows, as well as fathead minnow caging.



Sampling sites (upstream -> downstream)

Upstream of Grand Rapids - reference site
Downstream of Grand Rapids - "hotspot"
Upstream of St. Cloud - reference site
Downstream of St. Cloud - "hotspot"
Downstream of St. Paul - "hotspot"
Lake Peppin - "hotspot"

Project Manager Qualifications and Organization Description

Project Manager. Dr. Schoenfuss, an Associate Professor of Anatomy, has a well established history of research in aquatic toxicology. He directs the *Aquatic Toxicology Laboratory at St. Cloud State University* and focuses his research on the effects of emerging contaminants on aquatic organisms. His publication record includes recent publications in the leading journals of toxicology (see citations below). He is currently being funded by the US Environmental Protection Agency, the National Institute for Water Resources, and the MN Pollution Control Agency (see funding below). Dr. Schoenfuss collaborates closely with personnel in the US EPA (Duluth: G. Ankley, D. Martinovic; Chicago: E. Howe, A. Alwan; Cincinnatti: J. Lazorchack; North Carolina: M. Snyder), the USGS (Mounds View: K. Lee; Boulder: L. Barber; Denver: E. Furlong), and several academic institutions (U of Colorado, U of North Carolina, U of Minnesota). His research has been featured on KARE 11 news and has been incorporated into a *White Paper* delivered by the US Congressional Research Service. His research has also been integrated into current guidelines for nonylphenol established by the US EPA (2006).

Organization Description. St. Cloud State University is a comprehensive, Master's degree granting academic institution in the Minnesota State College and University System (MnSCU). The university enrolls over 16,000 students including approximately 30 students in the biological sciences, including toxicology. The *Aquatic Toxicology Laboratory* at St. Cloud State University is directed by Dr. Schoenfuss and employs 5 graduate and 15 undergraduate students in various projects assessing the health of MN fish populations and the threats posed by emerging contaminants. The laboratory facilities are freshly renovated and provide state of the art instrumentation for fish exposures and fish tissue analysis. In five structurally linked laboratories, we are able to (1) expose fathead minnows for prolonged period of times to ng/L concentrations of emerging contaminants; (2) conduct behavioral assays of exposed fishes; (3) analyze reproductive organs through histopathological investigations; (4) quantify feminizing compounds in the blood of fishes; and (5) measure water quality parameters. In addition, we are fully equipped for field sampling with an electroshocking boat.

Recent Publications

Bistodeau, T.J., L.B. Barber, K.E. Lee and H.L. **Schoenfuss**. 2006. Larval exposure to environmentally relevant mixtures of alkylphenolethoxylates reduces reproductive competence in male fathead minnows. *Aquatic Toxicology* 79: 268-277.

Schoenfuss, H.L. 2003. The need for novel approaches in assessing the biological impact of biologically active compounds. *In:* Proceedings of the *3rd International Conference on Pharmaceuticals and Endocrine Disrupting Chemicals In Water*, R. Masters (ed).

Schoenfuss, H.L., J.T. Levitt, G. Van Der Kraak and P.W. Sorensen. 2002. Ten week exposure to treated sewage effluent discharge has small, variable effects on reproductive behavior and sperm production in goldfish. *Environmental Toxicology & Chemistry* 21(10): 2185-2190.

Schoenfuss, H.L., Martinovic, D. and Sorensen, P.W. 2001. Effects of exposure to low levels of water-borne 17b-estradiol on nest holding ability and sperm quality in fathead minnows. *Water Resources Update* 120: 49-55.

Current Funding

US Environmental Protection Agency – Science to Achieve Results (STAR). **Schoenfuss**, H.L., L.B. Barber, D. Norris, M.L. Julius. $599,640 for 3 years.

National Institute for Water Resources. "Assessing the Ecotoxicology of Alkylphenol mixtures across the aquatic food chain". **Schoenfuss**, H.L. $63,000 for two years.

Minnesota Pollution Control Agency. "Determining the Endocrine Disrupting Effects of Alkylphenols (APs) . **Schoenfuss**, H.L., K.E. Lee, L.B. Barber. $63,000 for 1.5 years.