

Project ID SN-74:

Emerging Contaminants as Threats to Upper Mississippi Walleye

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Abstract

In the summer of 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 42 sites and included fish samples from four species, including walleye and smallmouth bass, as well as water and sediment samples from each location. 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. These effects include the feminization of male fish, which has been linked to hemaphroditism 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 potentially 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. 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. At four sites identified as "hotspots" in the previous study, and two sites with no indications of feminization, male walleye will be collected during two field seasons. All fish will be processed for plasma vitellogenin and histopathological observations. In addition, caged fathead minnows will be deployed at all six sites to confirm the presence and/or absence of endocrine disrupting compounds in the vicinity of walleye collections. In addition, analysis of plasma and tissues of caged fathead minnow will allow us to link field exposure result to a large body of existing toxicological literature. Results from this study will (1) confirm the presence of "hotspots" of endocrine disruption in the Mississippi River, (2) assess the effects of exposure to endocrine disrupting compounds in walleye, and (3) assess the effects of long-term endocrine disruption on the genetic health of exposed fish populations.

Background

Minnesota is in the unique position of being home to the source and first 650 miles of the Mississippi River, the largest North American river. Previous studies have demonstrated that this river receives many suspected and confirmed endocrine disrupting compounds (EDCs) through point source discharges such as municipal wastewater treatment plants (Barber et al. 2000; Barber et al. In press) and non-point source pollution. These EDCs include steroidal hormones such as estrone (E1) and estradiol (E2), synthetic hormones such as ethinylestradiol (EE2), alkylphenolic surfactants (APEs), personal care products, and pharmaceuticals (Kolpin et al. 2002). Exposure to EDCs in the Upper Mississippi River has been shown to result in feminizing effects on male fish, including the presence of plasma vitellogenin (VTG) in male fish (Folmar et al. 1996; 2001; Barber et al. In press), behavioral changes (Schoenfuss et al. 2002; Bistodeau et al. 2006), reduced size of reproductive organs and increased mortality in larval fishes (Bistodeau et al. 2006). In severe circumstances, EDC exposure has been linked to

histopathological changes and even intersex, a condition in which male fish develop female ovarian tissues within their testis (Jobling et al. 1998; Lee & Blazer 2005). Several studies of the effects of EDC on fishes have included study sites in the Mississippi River in Minnesota (Goodbred et al. 1996; Folmar et al. 1996; 2001; Barber et al. 2000; Kolpin et al. 2002; Bistodeau et al. 2006). Unfortunately, none of these studies surveyed a significant portion of the river or attempted to link biomarkers of endocrine disruption to overall fish health, reproductive ability, or genetic health of the exposed fishes.

In recent years, advancements in our understanding of the effects of pollutants on genetic diversity (Barata et al. 2002; Tatara et al. 2002; Theodorakis 2002; Theodorakis et al. 2006) raise particular concerns for the overall health of fish populations in the Mississippi River. Genetic diversity is the foundation that allows populations to persist and adapt to ever-changing environments. Pollutants can alter genetic diversity within and between wild populations if they affect the survival and reproduction of individuals (Theodorakis et al. 2006). Several scenarios related to the exposure to EDCs can affect the genetic structure of fish populations (Theodorakis et al. 2003; Whitehead et al. 2003). (1) EDCs may increase mutation rates in a population if these compounds have mutagenic effects. (2) If reduced EDC sensitivity increases reproductive fitness, EDCs may cause selective genetic change in the population. (3) If EDC exposed fishes reproduce at reduced rates, exposed populations may become bottlenecked or "sink-like" in having more immigrant and fewer emigrant fishes.

Hypotheses and Rationale

Regarding the effects of EDCs on fish populations in the Mississippi River, we propose the following hypotheses:

Hypothesis (1): Walleye exposed to EDCs at several known "hotspots" will exhibit signs of endocrine disruption including plasma vitellogenin induction in male fishes and histopathological changes to their metabolic and reproductive organs not seen at reference sites.

Rationale. The "hotspot" sites identified in our 2006 reconnaissance study show patterns consistent with the presence of EDCs. Preliminary results of water and sediment analysis corroborate these findings. Walleye, a long lived species near the apex of the aquatic food chain is likely exposed to these EDCs and should exhibit signs of exposure consistent with adverse effects found in our previous reconnaissance study and consistent with effects documented in the published literature at comparable point-source discharge sites.

Hypothesis (2): Caged fathead minnows will express biomarkers of acute EDC exposure at "hotspot" sites but not at reference sites.

Rationale. A seven day exposure of caged fathead minnow will be sufficient to induce vitellogenin in male fathead minnows in the presence of estrogenic EDCs. Caging of fathead minnows serves two purposes, first it confirms the presence of EDCs in the area in which walleye are sampled and second, it allows us to draw on a large body of laboratory research studies to increase the interpretive power of our experimental results with walleye.

Hypothesis (3): Walleye populations at "hotspot" sites will exhibit altered genetic diversity when compared with walleye from reference sites.

Rationale. Previously published studies have raised the specter that prolonged exposure to pollutants can alter the genetic diversity of an exposed population. By comparing walleye genetic diversity between "hotspot" sites and reference sites, we will be able to determine if walleye populations differ in their genetic structure in a pattern that is consistent with the

presence of EDCs. The use of walleye is preferred over other species of fishes, as a large body of research literature and genetic information is available for walleye in Minnesota.

Objective and Scope

We selected six sites based on our previous reconnaissance study of the Minnesota portion of the Mississippi River. Fish populations at four of these sites suggested the presence of EDCs as manifested in the induction of vitellogenin in male carp, redhorse, and smallmouth bass ("hotspot" sites). The remaining two sites appeared to be free of EDCs and will serve as reference sites. At all sites we will collect up to 60 walleye over a two field season period. All walleye will be analyzed for plasma vitellogenin concentrations and histopathology of livers and gonads. Using DNA-based genetic markers, we will assess patterns of genetic diversity within and between walleye populations collected at all sites. We will also cage male fathead minnows at each site for seven days and analyze the same endpoints after the exposure. Results of this study will improve our understanding of the effects of EDCs on fish reproductive health and genetic diversity of fish populations.

Approach

The followings sections are divided based on the previously established three components of the study. It is important to note that the collaborators have extensive experience with all employed methodology and when appropriate detailed methodology will be omitted in favor of references to published studies by the authors of this proposal.

Site Selection and Field Collection (see map following proposal)

The six sites selected for this study were part of the 43 site reconnaissance survey conducted jointly by St. Cloud State University and the US Geological Survey in 2006. Four sites, including downstream of Grand Rapids, downstream of St. Cloud, downstream of St. Paul and in Lake Pepin produced male fish with high plasma concentrations of vitellogenin, an indicator of acute exposure to EDCs. In addition, preliminary water and sediment chemistry indicates the presence of several classes of EDCs including alkylphenolic surfactants at these sites. These data are also consistent with previous, smaller scale reconnaissance studies conducted between 1990 and 2005 (Goodbred et al. 1996; Barber et al. 2000; Kolpin et al. 2002). As a result these four sites were classified as "hotspot" sites for this study based on the historic presence of EDCs and the observed biological effects on fish collected from these sites. An additional two sites free of signs of EDC presence or effects were selected as reference sites for the study (upstream of Grand Rapids, upstream of St. Cloud).

Field sites will be sampled on multiple occasions in spring and summer of both years of the study to ensure an adequate sample size of up to 60 walleye from each field site. Collection will principally occur via electro-shocking, a method we used effectively in the same river segments during the 2006 reconnaissance study. All walleye collected will be deeply anesthetized before a blood sample is drawn. Fish will then be sacrificed with an overdose of anesthetic, measured and weighed and dissected for livers and reproductive organs. Each organ will be weighed and representative sections (cranial, middle, posterior) will be collected and placed in labeled histocassettes. A fin clip will be stored in ethanol for DNA analysis.

Fathead minnow caging will occur during the first field season and will follow protocols established by the US EPA and employed in 2006 on the Ohio River (Adam Bialis, US EPA Cincinnati, pers. comm.). Briefly, two stainless steel cages, each containing 30 male fathead minnows from the SCSU Aquatic Toxicology Laboratory fathead minnow culture will be

anchored securely in a low-current stretch of the river just downstream of the point-source influent. An additional 30 fish will be randomly removed from the culture at the same time and their blood and tissues samples will serve as baseline controls for the study. Cage locations will be hidden to avoid attention and will be marked using GPS data. Cages will be visually inspected daily to ensure their continued operation. After seven days, fathead minnows will be removed from the two cages and processed as described above for walleye.

Laboratory Assessment of Fish Health

Blood samples from walleye and caged fathead minnows will be stored in a heparinized vacutainer on ice until they can be centrifuged (within 8 hours of collection). Three aliquotes of plasma from each centrifuged whole blood sample will be collected and stored in separate -80°C freezer at the Aquatic Toxicology Laboratory at St. Cloud State University. Plasma vitellogenin analysis will follow established protocols outlined in Bistodeau et al. (2006).

Histological preparation will be conducted in the SCSU Aquatic Toxicology laboratory following procedures outlined in Bistodeau et al. (2006) and Barber et al. (In press). Briefly, liver and gonads from walleye and fathead minnows will be dehydrated and embedded in paraffin using a Leica Automated Tissue processor. After sectioning of the tissue blocks at 5-7µm, sections will be stained in a Autostainer, using a standard H&E staining protocol (Barber et al. In press) before being coverslipped. Histological examination will be conducted by an experienced histologist (HLS) and will use a previously established numerical matrix for liver and gonad histology.

Analysis for Population-Level Genetic Effects

Molecular genetic data

We will produce genetic fingerprints of 60 individuals per sampling site using eight microsatellite DNA loci we developed specifically for walleye (Eldridge et al. 2002; Borer et al. 1999). We will extract DNA from fin tissue collected from each individual using the simple extraction procedure described in Miller and Kapuscinski (1996) or using a commercial kit (Qiagen). Microsatellite DNA loci will be amplified by polymerase chain reaction (PCR) and resulting products will be submitted to the Advanced Genetic Analysis Center (UMN – St. Paul) for electrophoretic analysis to resolve alleles, the basis for the DNA fingerprints. Using this core facility saves on equipment costing more than \$100,000 with thousands in annual supplies and service fees. The Miller laboratory has evaluated these microsatellite loci on over 2000 walleye from Minnesota and has data for 12 populations.

We will also explore the use of additional genetic marker types for characterizing genetic diversity of walleye populations. A recently developed marker type, AFLP, has increasingly been applied to fish populations, including studies of genetic impacts of pollutants (Whitehead et al. 2003; Bagley et al. 2001). AFLP has not been applied to walleye, to our knowledge. We will apply the procedures of Whitehead et al. (2003) to a test set of walleye DNA samples. If proven effective, we will apply AFLP to our complete sample set to increase the number of genetic markers, and hence genomic coverage, of our genetic data set.

Data analysis and signatures of genetic alteration

The individual DNA fingerprints provide the basis for analyzing genetic variation at the individual level, and when combined for samples, at the population level. Comparison of multiple control and polluted sites will allow us to determine if genetic trends are consistently associated with pollutant exposures. The signature of pollutant-induced genetic change may take different forms depending on the underlying mechanisms (Theodorakis 2003; Whitehead et al. 2003).

Pollutants may cause changes in frequencies of alleles linked closely to genes under selection

by the pollutant. In this case, specific alleles should have consistently higher or lower frequencies in contaminated versus control sites. We might also detect an association of specific alleles with affected or unaffected individuals within populations, as indicated by histological tests. Pollutants may also induce increased heritable mutations. If this is so, polluted sites should consistently have higher frequencies of rare alleles than control sites. Finally, pollutants can induce population bottlenecks, resulting in reduced genetic variation. This may be of particular concern for endocrine disruptors because genetic bottlenecks can be induced by both reductions in population size and increased variance in reproductive success among individuals. If bottlenecks are induced, polluted sites should show reduced genetic diversity compared to controls. Pollutants may also affect patterns of genetic diversity by affecting migration and gene flow. We will examine patterns of genetic diversity among individuals to determine if polluted sites may be acting as sinks for migrants (i.e., more fish move into contaminated areas than move out of them), as was found for a sunfish species in a pulp-mill effluent site (Theodorakis et al. 2006). Although the impacts on genetic diversity can be varied depending on the mechanism, any of these results should be of concern. Alterations of genetic diversity can serve as early bioindicators of other effects such as changes in community structure and population dynamics may affect the growth, sustainability, and probability of extinction of populations (Theodorakis 2003, and references therein).

Many free software packages are available to analyze various aspects of genetic variation. We will test for differences in within population diversity based on several measures of genetic variation (e.g., heterozygosity, allelic richness) as implemented in Fstat (Goudet 2001). The software Genepop (Raymond and Rousset 1995) will be used to test for Hardy-Weinberg equilibrium within populations and for differentiation among populations. If genetic differences exist among partially-isolated local populations, the software Structure (Pritchard 2000) can be used to detect recent migrants among the populations. Bottleneck (based on Luikart and Cornuet 1998) can determine if allele frequency distributions are indicative of recent and severe population bottleneck events.

Anticipated Challenges

Field studies are prone to producing complex data sets due to the variability of the biological data collected. Walleye at each field site are likely to move considerable distances and the exposure history of each fish will naturally be unknown. However, field sites were selected on a large spatial grid to ensure limited overlap of populations. In addition, dams in St. Cloud and below the Twin Cities at Hastings will prevent the movement of walleye between the field sites. Of particular interest will be the upstream and downstream St. Cloud sites, which are in close spatial proximity, but where a dam limits the upstream movement of walleye.

The caging of fathead minnows is also problematic and requires great care to avoid failure. This research team has conducted combined laboratory and field studies, including exposure of fathead minnows at field sites for almost 10 years and has the necessary expertise to complete the task.

Anticipated Results and Future Directions

The data collected in this study will allow us to test the three hypotheses stated previously. If hypothesis (1) is not corroborated, but hypothesis (2) is, results would indicate that walleye are less sensitive to EDC exposure than most species of fish, as fathead minnows are generally considered relatively hardy. We would then proceed with a different species of fish (i.e., smallmouth bass) which is known to be sensitive to EDCs, but which has less published genetic

data available. In contrast, if hypothesis (1) is corroborated, but hypothesis (2) is not, the results would suggest that walleye are indeed very sensitive to EDC exposure at concentrations that may be too low to induce signs of acute EDC exposure in male fathead minnows. If hypothesis (3) is corroborated, our results would indicate that long-term EDC exposure can alter the genetic structure of an exposed fish population. This finding would clearly warrant further investigation. If hypothesis (3) is rejected, but it is found that "hotspot" sites are population "sinks", a linkage to population level reproductive effects of EDCs could be established in subsequent studies. If hypothesis (3) is rejected and no asymmetrical gene flow into or out of the "hotspot" is noted, the data provide assurance of the limited effect of EDCs on the overall population genetic structure of Minnesota walleye populations.

Product

The proposed study will result in several tangible products. First, a final report will be furnished describing in detail the results of each aspect of the conducted study. Second, an annual report after the first year of the study will describe progress and preliminary findings and will note any deviations from the proposed plan of study. Third, as data sets become available, presentations in Minnesota and nation-wide will be delivered describing study results at scientific conferences. Fourth, manuscripts based on the results of this study will be prepared and submitted to appropriate peer-reviewed journals. As an indirect benefit, the genetic data will add to Dr. Miller's database of genetic marker information that he applies to fisheries management and enforcement questions under his contract with the MN DNR.

References

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Timetable

Task	2007		2008				2009	
	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2
Field work	X			X	X			
Caging fathead minnows	X							
Vitellogenin analysis		X			X	X		
Histological analysis		X	X		X	X	X	
Genetic Analysis		X	X	X	X	X	X	
Annual Report				X				
Data Analysis				X			X	X
Final Report								X

Budget and Invoicing

Funds requested from LCCMR:

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

Invoicing: We expect to invoice based on time expended by Schoenfuss and Miller, and on progress of fieldwork (walleye collection, caging fathead minnows - year 1; collecting walleye - year 2) and laboratory analysis at St. Cloud State University (vitellogenin and histology in year 1 and year 2) and the University of Minnesota (genetic analysis year 1 and year 2 fish samples).

Other Funds and Partners

Project Partners

Heiko L. Schoenfuss – Project Leader St. Cloud State University

Matthew L. Julius – Aquatic Ecologist, St. Cloud State University

Loren M. Miller – Population Geneticist, AquaGen, University of Minnesota

Other Funds being Spent during the Project Period 0

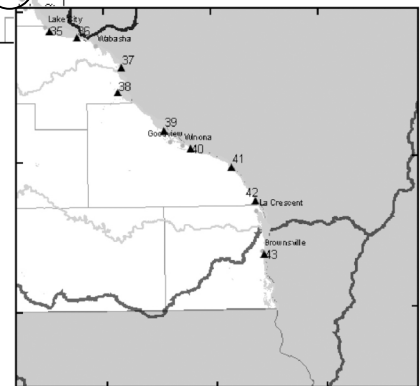
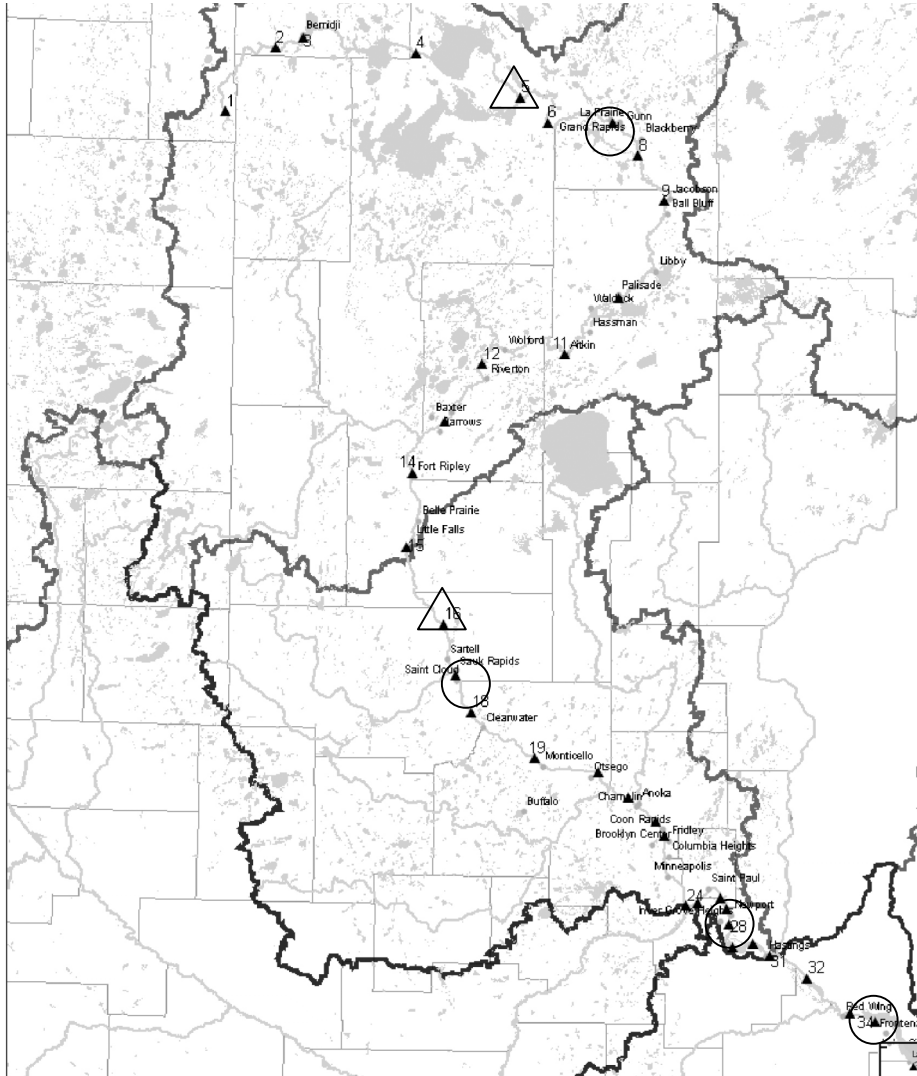
Past Spending \$120,000 for longitudinal study of Mississippi River at 42 sites from Lake Itasca to Iowa border.

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 and sediment would have to be conducted, more than doubling the overall cost of the project.

Curricula Vitae for the three investigators follows the map of field sites.

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Research Addendum*

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"

CURRICULUM VITA

Heiko Lars Schoenfuss

Associate Professor of Anatomy

&

Director, Aquatic Toxicology Laboratory

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St. Cloud State University, St. Cloud, MN 56301; hschoenfuss@stcloudstate.edu

Education

Ph.D., Museum of Natural Science, Louisiana State University, Baton Rouge, Louisiana, 1997
(Zoology). Dissertation Topic: Metamorphosis of the Hawaiian Stream Goby
Sicyopterus stimpsoni: A Structural, Functional, and Behavioral Analysis

M.S., School of Veterinary Medicine, Louisiana State University, Baton Rouge, Louisiana, 1997
(Veterinary Anatomy). Thesis Topic: Laryngeal Anatomy and Mechanisms of Sound
Production in the Bowhead Whale (*Balaena mysticetus*). Advisor: Dr. D. J. Hillmann.

B.S., University of Bayreuth, Germany, 1991 (Biology).

Employment

Associate Professor of Anatomy, Department of Biological Sciences, St. Cloud State University,
August 2005 – Present.

Assistant Professor of Anatomy, Department of Biological Sciences, St. Cloud State University,
August 27, 2001 – July 2005.

Research Associate, Department of Fisheries & Wildlife, University of Minnesota (1999-2001)

Instructor, Department of Biological Sciences, Southeastern Louisiana University (1995-97).

Selected Publications (out of 21)

Barber, L.B., K.E. Lee, D. Swackhamer and **H.L. Schoenfuss**. In press. Response of Male
Fathead Minnows Exposed to Wastewater Treatment Plant Effluent, Effluent Treated
with XAD8 Resin, and an Environmentally Relevant Mixture of Alkylphenol Compounds.
Aquatic Toxicology.

Bistodeau, T.J., L.B. Barber, S.E. Bartell, R.A. Cediell, K.J. Grove, J. Klaustermeier, J.C.
Woodard, 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.

Blob, R.W., K.M. Wright, M. Becker, T. Maie, T.J. Iverson, M.L. Julius and **H.L. Schoenfuss**. In
press. Ontogenetic change in novel function: waterfall climbing in adult Hawaiian gobiid
fishes. *Journal of Zoology*, London.

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environments: effects of locomotor style and substrate texture on the waterfall climbing
performance of Hawaiian gobiid fishes. *Journal of Zoology*, London 268: 315-324.

Schoenfuss, H.L. and R.W. Blob. 2003. Kinematics of waterfall climbing in Hawaiian
freshwater fishes (Gobiidae): vertical propulsion at the aquatic-terrestrial interface.
Journal of Zoology 261:191-205.

Schoenfuss, H.L. 2003. The need for novel approaches in assessing the biological impact of biologically active compounds. *In: Pharmaceuticals and Endocrine Disrupting Chemicals In Water*, R. Masters, Minneapolis, MN, March 19-21, 2003, pp. 103-121.

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 17 β -estradiol on nest holding ability and sperm quality in fathead minnows. *Water Resources Update* 120: 49-55.

Levitt, J.T., **H.L. Schoenfuss** and I.R. Adelman. 2001. Possible effects of endocrine disrupting compounds on walleye, *Stizostedion vitreum*, near the Metro Sewage Treatment Plant, Saint Paul, MN. *In: Pharmaceuticals and Endocrine Disrupting Chemicals in Water*, D. Guth (ed.), Minneapolis, MN, October 9-11, 2001, pp. 191-202.

Selected External Funding Awarded

2007: Minnesota Pollution Control Agency. "Occurrence and Persistence of Alkylphenols in Minnesota Wastewaters and Receiving Streams. (B) Biological Implications" **Schoenfuss, H.L.**, L.B. Barber, K.E. Lee. \$43,000 for one year.

2006: Minnesota Pollution Control Agency. "Integrated Chemical and Biological Study to Define the Occurrence of Intersexuality in Minnesota Fish within the Mississippi River: Fish Population Health". **Schoenfuss, H.L.**, L.B. Barber and K.E. Lee. \$16,000.

2005: US Environmental Protection Agency – Science to Achieve Results (STAR). Developing Rapid Assessment Tools to Evaluate the Biological Effects of Complex and Biologically Active Chemical Mixtures. **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.**, L.B. Barber and M.L. Julius. \$63,000 for two years.

2004: Minnesota Pollution Control Agency. "Determining the Endocrine Disrupting Effects of Alkylphenols (APs) on the Reproductive Competence of Fishes in Biologically Relevant Laboratory and Field Studies". **Schoenfuss, H.L.**, Lee, Barber. \$50,000.

2003: Sea Grant College Program. "Assessing whether exposure to low levels of estrogens whose actions mimic that of effluent from a Great Lakes sewage plant treatment plants poses a threat to fish reproductive health". Sorensen, P.W. and **H.L. Schoenfuss**..

2000: National Sea Grant Program. "Assessing the Validity of Vitellogenin as a Biomarker of Endocrine Disruption in Populations of Fish". Swackhamer, D. L., P. W. Sorensen, I. R. Adelman, and **H. L. Schoenfuss**. \$300,000 for two years.

1999: USGS Water Resource Center Grant. "Assessing the effects of endocrine disrupters (EDCs) from a St. Paul sewage treatment plant on sperm viability and testicular development in fish: Adding a new dimension to an existing project". Sorensen P.W., **H.L. Schoenfuss**, I.R. Adelman and D. Swackhamer. \$30,000 for two years.

CURRICULUM VITA

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EDUCATION:

B.S. 1993 Butler University
M.S. 1995 University of Michigan – Natural Resources (Aquatics)
Ph.D. 2000 University of Michigan – Natural Resources (Aquatics)
Postdoc JUN-NOV 2000 University of Michigan

PROFESSIONAL EXPERIENCE:

2005-present Associate Professor of Aquatic Biology, St. Cloud State University
2001-2005 Assistant Professor of Aquatic Biology, St. Cloud State University.
2000- 2001 Assistant Research Scientist, Center for Great Lakes and Aquatic Sciences, University of Michigan

FIELDS OF SPECIALIZATION:

Aquatic Ecology and Limnology, Systematics and Evolution, Diatom Taxonomy, Micropaleontology, Paleoecological Reconstruction, Environmental Toxicology

FIVE RELEVANT PUBLICATIONS:

- Julius, M.L., Blob, R., and Schoenfuss, H.L. 2005. The survival of *Sicyopterus stimpsoni*, an endemic amphidromous Hawaiian gobiid fish, relies on the hydrological cycles of streams: evidence from changes in algal composition of diet through growth stages. *Aquatic Ecology* 39: 473-484.
- Blob, R.W., Raj, R., Julius, M.L. and Schoenfuss, H.L. 2005. Functional diversity in extreme environments: effects of locomotor style and substrate texture on the waterfall climbing performance of Hawaiian gobiid fishes. *Journal of Zoology* 268: 315-324.
- Schoenfuss, H.L., M.L. Julius, and R. Blob. 2004. Colonization of a recent, volcanically formed freshwater habitat: an example of primary succession. *Ichthyological Exploration of Freshwaters*.15: 83-90.
- Julius, M.L., E.F. Stoermer, C.M. Taylor, and C.L. Schelske. 1998. Local extinction of *Stephanodiscus niagarae* Ehrenb. (Bacillariophyta) in the recent limnological record of Lake Ontario. *Journal of Phycology* 34: 766-771.
- Julius, M.L., E.F. Stoermer, S.M. Colman, and T.C. Moore. 1997. A preliminary investigation of siliceous microfossil succession in late Quaternary sediments from Lake Baikal, Siberia. *Journal of Paleolimnology* 18: 187-204.

FIVE OTHER PUBLICATIONS:

- Julius, M.L. 2003. Reflecting Phylogenetic Relations in Ecotoxicological Investigations of Algae. *In: Proceedings of the 3rd International Conference on Pharmaceuticals and Endocrine Disrupting Chemicals in Water*

*LCCMR 2007 -- (SN-74) Emerging Contaminants as Threats to Upper Mississippi Walleye
Research Addendum*

Stoermer, E.F. and M.L. Julius. 2003. Centric Diatoms. Chapter 11 In: Wehr, J.D. and Sheth, R.G. (eds.), *Freshwater Algae of North America: Classification and Ecology*. Academic Press, San Diego.

Tuji, A., A. Kawashima, M.L. Julius, and E.F. Stoermer. 2003. *Stephanodiscus akanensis* Sp. Nov., A new recent diatom from Lake Akan, Japan. *Bulletin of the National Science Museum, Tokyo*

Julius, M.L. and Y. Tanimura. 2001. Cladistic analysis of plicated *Thalassiosira* (Bacillariophyceae). *Phycologia* 40: 111-122.

Julius, M.L., G.F. Estabrook, M.B. Edlund, and E.F. Stoermer. 1997. Recognition of taxonomically significant clusters near the species level, using computationally intense methods, with examples from the *Stephanodiscus niagaraecomplex* (Bacillariophyta). *Journal of Phycology* 33: 1049-1054.

SYNERGISTIC ACTIVITIES:

Professional

Participant, 2002 Dissertations Symposium for the Advancement of Coastal, Estuarine and Great Lakes Sciences (DIACES), October 28- November 2, 2002, Copamarina Resort, Puerto Rico.

Participant, Environmental Fate of Alkylphenols sponsored by the United States Geological Survey, United States Environmental Protection Agency, and St. Cloud State University, February 25, Mounds View, MN.

Graduate Education

Currently serving as the chair for three masters students (*Micheal Curtin, Tiffany Kapushinsi, and Carolyn Gamble*), and serving on the committees of three others (1 Biology (*Kent Grove*), 1 Geography (*Chad Yost*), 1 Computer Sciences (*Yavda Seema*))

Undergraduate Education

Serve as the academic advisor for 50+ aquatic ecology and general biology majors. Undergraduate researchers supervised at SCSU: *Noel Krueger* 2002, *O'Neil Tedrow* 2002, *Erin Howard* 2003, *Zoe Howell* 2003, *Tiffany Kapushinski* 2003, *Meghan Reese* 2004, *Dennis Hansen* 2005, *Kristie Englehart* 2005, *Jessica Timperley* 2005, *Theresa Iverson* 2005, *Katie Kotschevar* 2005, *Eric Greene* 2005, *Elizabeth Kummer* 2005, *Cassie Kraetsch* 2006, *Alima Gikineh* 2006

CURRICULUM VITA

Loren Michael Miller

Dept. Fisheries, Wildlife & Conservation Biology
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Education

Ph.D. Fisheries, Univ. of Minnesota, 1996
M.S. Fisheries, Univ. of Minnesota, 1992
B.S. Biology, Univ. of Minnesota, 1986

Employment Record

Research Associate - Department of Fisheries, Wildlife, and Conservation Biology, University of Minnesota, St. Paul, MN, 1998-present.
Assistant Professor – Department of Biological Sciences, St. Cloud State University, St. Cloud, MN, 2003-2006.
Post-doctoral Research Associate/Fellow - Department of Veterinary PathoBiology, University of Minnesota, St. Paul, MN, 1996-98.
Graduate Research/Teaching Assistant - Department of Fisheries, Wildlife, and Conservation Biology, University of Minnesota, St. Paul, MN, 1989-96.
Biologist - Division of Fish and Wildlife, U.S. Department of Energy, Bonneville Power Administration, Portland, OR, 1991.

Research Activities

Loren Miller applies genetic principles and techniques to questions of fisheries management and ecology. He is currently conducting numerous studies using molecular genetic markers to assess genetic population structure, to identify species and their hybrids, and to determine the source population or parentage of individuals. His "species list" includes: walleye, northern pike, muskies, rainbow trout, brook trout, yellow perch, sculpins, and fathead minnows. He is also concerned with applying genetic principles to the management of hatchery programs. Loren is the primary fisheries geneticist for the MN Dept. of Natural Resources through University contracts.

Selected Publications

Johnston, T.A., L.M. **Miller**, D.M. Whittle, S.B. Brown, M.D. Wiegand, A.R. Kapuscinski, and W.C. Leggett. 2005. Effects of maternally-transferred organochlorine contaminants on early life survival in a freshwater fish. *Environmental Toxicology and Chemistry* 24:2594-2602.
Linda Laikre, L., L.M. **Miller**, A. Palmé, S. Palm, A.R. Kapuscinski, G. Thoreson, and N. Ryman. 2005. Spatial genetic structure of northern pike (*Esox lucius*) in the Baltic Sea. *Molecular Ecology* 14:1955-1964.
Miller, L.M., T. Close and A.R. Kapuscinski. 2004. Lower fitness of hatchery and hybrid rainbow trout compared to naturalized populations in Lake Superior tributaries. *Molecular Ecology* 13:3779-3388.
Miller, L.M., A.R. Kapuscinski and W. Senanan. 2004. A biosafety approach to addressing risks posed by aquaculture escapees. *In* M.V.Gupta, D.M. Bartley, B.O. Acosta (eds.) Use of

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Genetically Improved and Alien Species for Aquaculture and Conservation of Aquatic Biodiversity in Africa. World Fish Center Conference Proceedings 68.

Senanan W., A.R. Kapuscinski, U. Na-Nakorn and L.M. **Miller**. 2004. Genetic impacts of hybrid catfish (*Clarius macrocephalus* x *C. gariepinus*) farming on native populations in central Thailand. *Aquaculture* 235:167-184.

Miller, L.M. 2003. Microsatellite DNA loci reveal genetic structure of yellow perch in Lake Michigan. *Transactions of the American Fisheries Society* 132:503-513.

Miller, L.M., and A.R. Kapuscinski. 2003. Genetic guidelines for hatchery supplementation programs. Pages 329-355 in E.M. Hallerman, ed *Population Genetics: Principles and Practices for Fisheries Scientists*. American Fisheries Society, Bethesda, MD.

Ardren, W.R., L.M. **Miller**, J.A. Kime, and M.A. Kvitrud. 2002. Microsatellites for fathead minnows (*Pimephales promelas*). *Molecular Ecology Notes* 2:226-227.

Eldridge, W. H., M.D. Bacigalupi, I.R. Adelman, L.M. **Miller**, and A. R. Kapuscinski. 2002. Determination of relative survival of two stocked walleye populations and resident natural-origin fish by microsatellite DNA parentage assignment. *Canadian Journal of Fisheries and Aquatic Sciences* 59:282-290.

Miller, L.M., L. Kallemeyn, and W. Senanan. 2001. Spawning site and natal site fidelity by northern pike in a large lake: mark-recapture and genetic evidence. *Transactions of the American Fisheries Society* 130:307-316.

Miller, L.M. 2000. Classifying genealogical origins in hybrid populations using dominant markers. *J. Heredity* 91:46-49.

Borer, S, L.M. **Miller**, and A.R. Kapuscinski. 1999. Microsatellites in walleye *Stizostedion vitreum*. *Molecular Ecology* 8:36-37.

Miller, L.M. and A.R. Kapuscinski. 1997. Historical analysis of genetic variation reveals low effective population size in a northern pike (*Esox lucius*) population. *Genetics* 147:1249-1258.

Miller, L.M. and A.R. Kapuscinski. 1996. Microsatellite DNA markers reveal new levels of genetic variation in northern pike. *Transactions of the American Fisheries Society* 125:671-677.