

2012

Atrazine Influence on Northern Pike Sperm Motility and Viability in Minnesota

Andy Stevens

Minnesota State University, Mankato

Paul Pallardy

Minnesota State University, Mankato

Follow this and additional works at: <http://cornerstone.lib.mnsu.edu/jur>

 Part of the [Aquaculture and Fisheries Commons](#)

Recommended Citation

Stevens, Andy and Pallardy, Paul (2012) "Atrazine Influence on Northern Pike Sperm Motility and Viability in Minnesota," *Journal of Undergraduate Research at Minnesota State University, Mankato*: Vol. 12, Article 12.

Available at: <http://cornerstone.lib.mnsu.edu/jur/vol12/iss1/12>

This Article is brought to you for free and open access by the Undergraduate Research Center at Cornerstone: A Collection of Scholarly and Creative Works for Minnesota State University, Mankato. It has been accepted for inclusion in Journal of Undergraduate Research at Minnesota State University, Mankato by an authorized administrator of Cornerstone: A Collection of Scholarly and Creative Works for Minnesota State University, Mankato.

Student Agreement:

I am submitting my research article to be published in the JUR (The Journal of Undergraduate Research at Minnesota State University, Mankato), an electronic journal of the Minnesota State University Undergraduate Research Center.

I/We certify have followed the accepted standards of scientific, creative, and academic honesty and ethics.

I understand that my article submission will be blind-reviewed by faculty reviewers who will recommend acceptance for publication; acceptance with revisions; or reject for publication.

I understand that as author, I retain the right to present any part of the research in any form in other publications.

The JUR has the right to reproduce and reprint published submissions for instructional or promotional purposes.

For complete details, see [*Journal of Undergraduate Research at Minnesota State University, Mankato policies page*](#).

Mentor Agreement:

I have reviewed the submission, and I support its inclusion in the JUR (The Journal of Undergraduate Research at Minnesota State University, Mankato). I understand that I will be acknowledged as the faculty mentor for the student author(s). To the best of my knowledge, the student has followed the accepted standards of scientific, creative, and academic honesty and ethics.

ATRAZINE INFLUENCE ON NORTHERN PIKE SPERM MOTILITY AND VIABILITY IN MINNESOTA

Andy Stevens and Paul Pallardy
Environmental Science Program

and

Shannon J. Fisher (*Faculty Mentor*)
Water Resources Center

ABSTRACT -- Concern has been noted by fish hatchery biologists with the Department of Natural Resources that southern Minnesota northern pike *Esox lucius* populations have exhibited reduced hatching rates, a trend not occurring with northern Minnesota pike populations. The chemical atrazine is a frequently used herbicide in Minnesota and has been found to cause gonadal dysgenesis and reproductive development issues in amphibians and fish. Sperm, length, and age data were collected from northern pike in four Minnesota lakes. Water samples were obtained from two of the lakes to test for atrazine. Test results indicated atrazine concentrations $<0.05 \mu\text{g/L}$ which was lower than the detectable limit. Sperm concentrations ($\#/mL$) were not significantly different among lakes ($P=0.302$). Sperm motility data were significantly different among lakes ($P<0.001$). A pairwise multiple comparison revealed several noteworthy sperm motility differences; however, due to lack of atrazine detection, we cannot connect these observations to that chemical. Six different multiple and single linear regression analyses were completed to assess sperm concentration and motility based on pike age and length. No significant correlations were detected ($P>0.10$ and $r^2 < 0.06$ for all regressions). Sperm motility reductions in northern pike could be related to declining populations and warrants further research.

Introduction

Northern pike *Esox lucius* populations in southern Minnesota have declined over the past few decades. A concern noted by hatchery staff is an observed low hatching percentage among southern Minnesota pike populations from 1991 to 2011, where the average hatch rate was 43.2% - an observation not noted in northern Minnesota lakes where hatch rates are consistently much higher (Bruce Pittman, personal communications MN DNR). Northern pike are an important game species and declines are a concern of anglers. In addition, the species provides predatory balance to many lake ecosystems.

Studies have shown that atrazine causes gonadal development problems in leopard frogs *Rana pipiens*. Frogs exposed to atrazine commonly experienced gonadal dysgenesis and sex reversal (Hayes et al. 2003). Atrazine can also reduce reproduction and cause tissue abnormalities in fathead minnows *Pimephales promelas* (Tillet et al. 2010). Atrazine is a frequently detected herbicide in agricultural area streams (Tillet et al. 2010) and can impact fish gamete health (Hayes et al. 2003).

Southern Minnesota has higher concentrations of atrazine in its streams than northern Minnesota (Figure 1); however, little is known about atrazine impacts in surface waters. Atrazine is almost non-volatile and its half-life in neutral condition is about 200 days but varies from 4-57 weeks, depending on various environmental factors like pH, moisture content, temperature and microbial activity (Ghosh 2006). At 25 deg C, a 5 mg/l solution of fulvic acid (naturally occurs in soils and most surface waters) resulted in half-lives of 34, 174, 398 and 742 days at pHs of 2.9, 4.5, 6.0 and 7.0, respectively (Spectrum Labs). Southern Minnesota surface waters tend to be pH neutral or slightly basic; therefore, atrazine should persist longer and have the potential to cause greater impacts (e.g., lower pike reproduction rates) due to extended exposure.

The objectives of this project were to 1) assess sperm motility and density between atrazine-exposed and atrazine-free northern pike populations and 2) review sperm traits as a function of northern pike length and age. To achieve our objectives, we attempted to determine if northern pike sperm concentrations and motility were different among several potentially atrazine-impaired populations. We anticipated that sperm concentration and motility would decrease with increasing atrazine levels. We also evaluated if pike sperm concentrations and motility were influenced by male age or length, as bigger and older fish may have been exposed to atrazine for longer time periods.

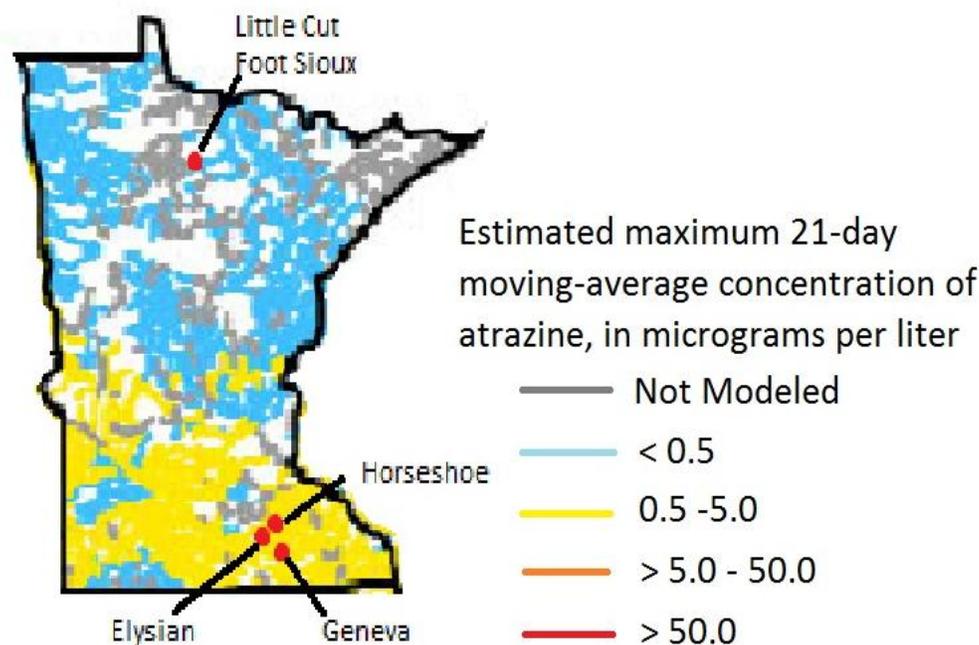


Figure 1. An estimated 21-day moving-average concentration of atrazine ($\mu\text{g/L}$) in Minnesota streams. Red markers indicate the four lakes at which pike samples were taken. The model statistically relates atrazine concentrations in streams to watershed characteristics determined from data sources with national coverage. The model was developed using standard statistical techniques. Work is currently underway to extend the WARP model approach to other pesticides (USGS 2007).

Methodology

Collection of samples

Male northern pike, 3-6 years of age and 415-855 mm total length, were captured by trap nets from Lake Geneva, Horseshoe Lake, Lake Elysian, and Little Cut Foot Sioux, which is part of Lake Winnibigoshish, during the spawning season (mid-March-Early April) of 2012. Up to 20 fish were collected from each lake; sample size depended on availability of fish. Semen was stripped from un-anesthetized fish by applying abdominal pressure and was collected with a syringe. Care was taken to avoid contamination of the semen sample with urine. The semen was then immediately stored in vials on ice for six hours and then in a refrigerator for another 17 hours before being analyzed. These storing times were used to standardize holding time lengths based on the varying travel distances across the state to obtain samples. Fish length measurements were also obtained using a measuring board, and scales were taken from the middle back of the fish and stored in a scale envelope. These scales were obtained to determine age. Water samples were taken from Lakes Geneva and Horseshoe and screened for atrazine content at Minnesota Valley Testing Laboratories in New Ulm, Minnesota.

Analysis of sperm samples

Each slide of sperm was prepared by mixing one μL of semen in one ml of distilled water, which was used to activate the sperm. One μL of this mixture was then extracted and placed on the haemocytometer with a cover slip placed over the droplet. Sperm samples were viewed at 40x objective power on an Improved Neubauer haemocytometer. Five of the 25 individual sections of the haemocytometer grid were analyzed. The five sections selected started in the top left and preceded downward in a diagonal fashion to the bottom right section being used as the ending point. Motility counts were determined by individual sperm movement: any sperm appearing to move or vibrate was deemed motile. Total sperm counts were obtained by manually counting individual sperm on photomicrographs of each section. Total sperm concentration and motility counts were obtained by adding the totals of the five sections and multiplying by the appropriate dilution factor.

Statistical analyses were completed with Sigma Plot 11.2 using the following tests: Shapiro-Wilk for normality, ANOVA, Kruskal-Wallis, Dunn's multiple comparison, and linear regressions. Regression comparing sperm motility and density as functions of northern pike length and age were completed. The presence of no significant relationships in the overall regressions was used as an indicator to suggest that all sperm samples, regardless of age and length, could be used to calculate means and standard errors for the ANOVA comparison among lakes. Significance was accepted at $P < 0.05$.

Analysis of scale samples

Scale samples were analyzed using a microfiche reader to count annuli, thereby determining age based off the growth season the sample was obtained from. The edge of the scale is counted as the slow growth period, thus an annuli, for fish collected in the spring because the season's growth has not yet started. These data was linearly regressed against both total sperm concentration and motility values.

Results

Sperm Concentration and Motility as Function of Age and Length

Multiple and single regressions of sperm concentration and motility, based on both age and length, revealed no significant relationships for all samples combined. All regressions had $P > 0.10$ and $r^2 < 0.06$ (Figures 2 and 3). Therefore analysis of the means among populations could be completed.

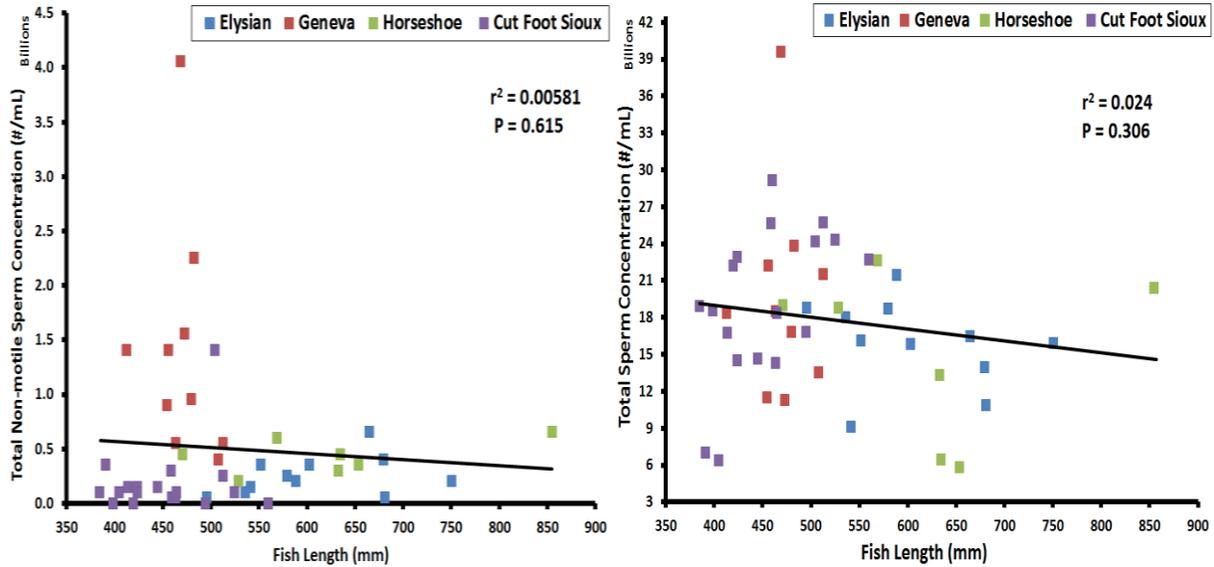


Figure 2. Scatter plots and results from single linear regressions of northern pike non-motile sperm concentrations and total sperm concentrations (billion/mL) as a function of fish length (mm). Color codes indicate lake origin for each data point and regression lines, along with r^2 and P-values, are noted for each test.

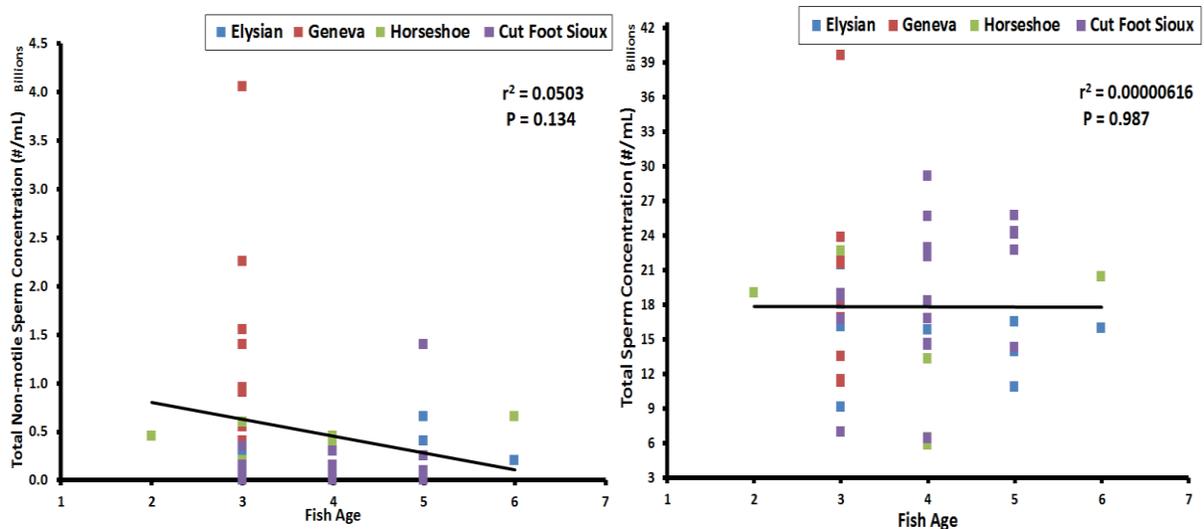


Figure 3. Scatter plots and results from single linear regressions of northern pike total sperm concentrations and non-motile sperm concentrations (billion/mL) as a function of fish age (years). Color codes indicate lake origin for each data point and regression lines, along with r^2 and P-values, are noted for each test.

Sperm Motility and Concentration Comparison among Lakes

Due to limited availability of breeding pike in our sample lakes, sample sizes were limited to 7, 10, 11, and 18 individual males from Horseshoe, Geneva, Elysian and Cut Foot Sioux, respectively. Mean sperm concentration data among lakes were normally distributed ($P=0.216$) and therefore an ANOVA was used. No significant differences in mean sperm concentration were present among the four lakes ($P=0.302$; Figure 4). The percentages of sperm showing motility were not normally distributed and were assessed with a Kruskal-Wallis test. Mean motility proportions were found to be significantly different ($P<0.001$) and a multiple comparison indicated several groupings of significantly different observations (all $P<0.05$; Figure 5). For example, Cutfoot Sioux sperm had significantly greater motility than Horseshoe and Geneva, but was not significantly different than Elysian (Figure 5).

Atrazine Assessment

Atrazine concentrations were $<0.05 \mu\text{g/l}$ (below lab detection limit) for water samples collected at Geneva and Horseshoe Lakes. No samples were collected from Elysian and Lower Cut Foot Sioux due to no significant findings of the other two lakes and budget restrictions.

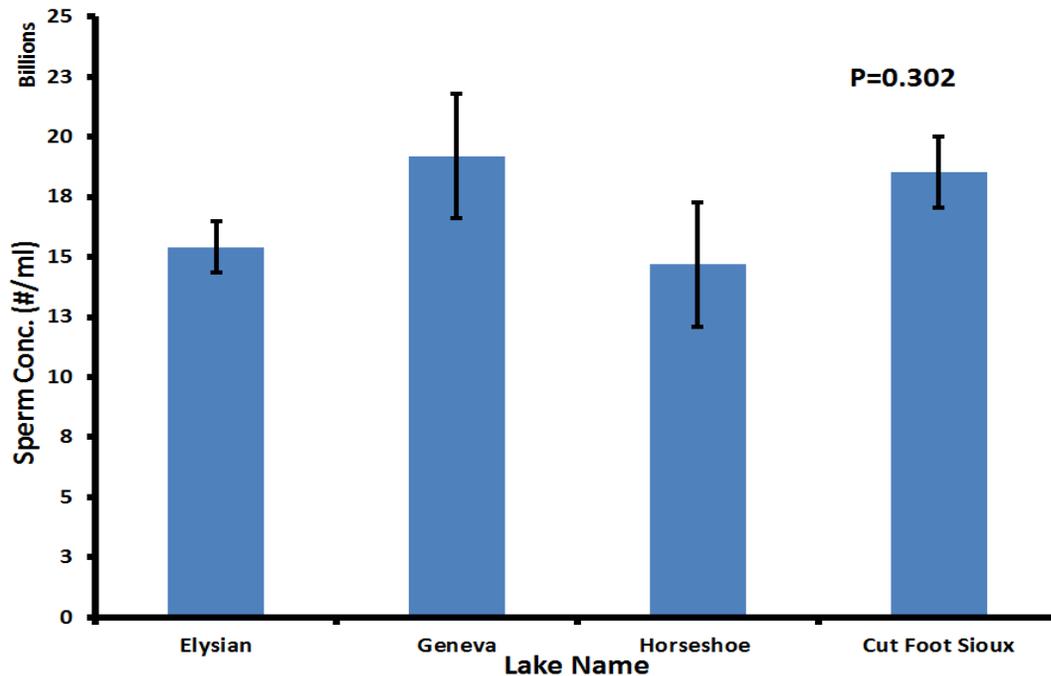


Figure 4. Mean northern pike sperm concentrations (billions/mL) among four sampled lakes in Minnesota during March 2012. Error bars represent \pm standard error and the P-value is noted.

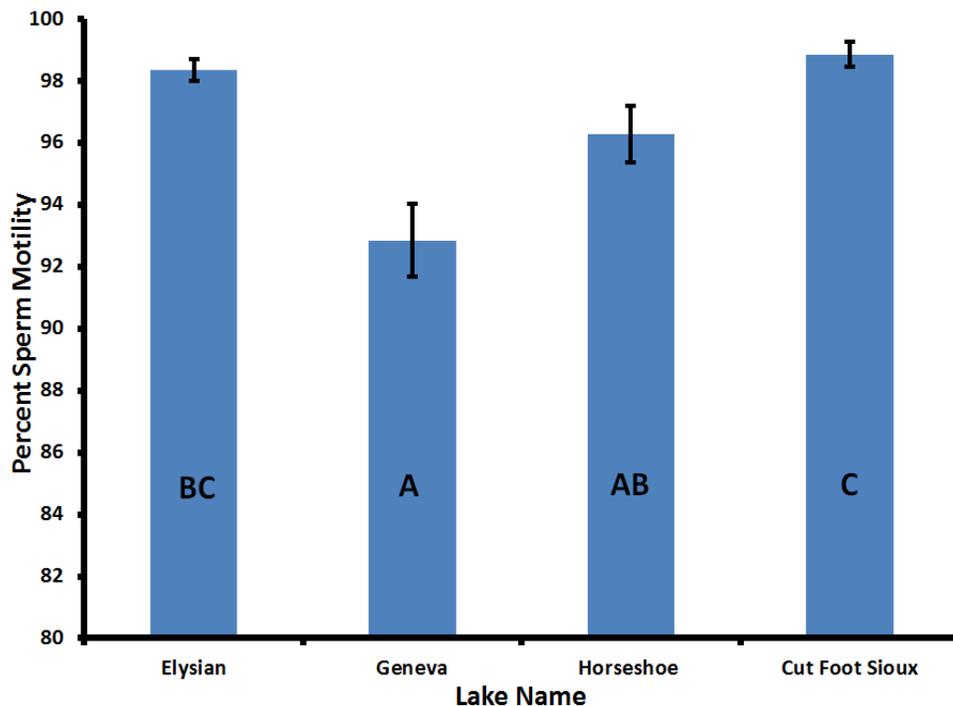


Figure 5. Percent sperm motility among four sampled lakes in Minnesota in March 2012. Lakes with different letter designations were significantly different ($P < 0.05$). Error bars represent \pm standard error and the P-value is noted.

Discussion

While we did not find any significant differences in sperm concentrations among the four pike populations, we do believe that our calculated sperm concentrations were determined correctly. The sperm concentrations we identified in this study were similar to muskellunge *Esox masquinongy* (Feng et al. 1996) – a closely related species. Of interest, our results do show a significant difference in sperm motility among our sample northern pike populations. However, our original intent to assess these values based on atrazine concentrations could not be completed due to undetectable atrazine levels.

Given significantly lower sperm motility in Geneva and Horseshoe Lakes, we still question the potential impact of atrazine; however, we did not have any historical information to suspect that atrazine concentrations would be detected – other than suspected issues from local scientists. Larval and juvenile fish growing in an environment containing atrazine could experience gonadal development abnormalities. In addition, the timing of our atrazine sample may not have been ideal. We sampled just before the annual application of atrazine and its concentration may have dropped substantially from the previous year; however, based on half-life, if it was present in appreciable quantities, detection should have been possible. Atrazine is applied as a pre-emergent herbicide in the late spring, thus concentrations may be detectable at other times of the year (Spectrum Laboratories).

Regardless of the cause, sperm motility reductions could impact hatching rates. For example, if a male northern pike with a sperm concentration of 18.2 billion sperm/mL experienced a 10% to 20% motility reduction, viable sperm reductions of 1.8 to 3.6 billion sperm/mL result. Pike, like many fishes, are broadcast spawners and rely on random contact between a viable sperm and an egg (unlike mammalian sperm that actively seeks out eggs). Therefore, even a slight reduction in sperm motility could impact the probability of successful fertilization and contribute to lower hatch rates in a natural environment.

Based on this argument, however, reduced hatching rates in the hatchery should not be impacted, as gametes are manually mixed together and contact odds are very high (even if motility is poor).

The identification of significantly lower sperm motility in some southern Minnesota northern pike populations raises some new and interesting questions about sperm quality and declining pike populations. Therefore, further research should be completed to assess gonadal health across pike populations, including a screening of water quality and tissue chemical presence in relation to controlled hatching experiments that may include crossing gametes from exposed and unexposed populations. Our findings that sperm count and motility don't appear to depend on fish age leads us to think that bio accumulation of pollutants that may impact motility is either not happening, or occurs very early in the species life history.

References

- Feng, L., L. Liu, and K. Dabrowski. 1996. Characteristics of Muskellunge Spermatozoa I: Ultrastructure of Spermatozoa and Biochemical Composition of Semen. *Transactions of the American Fisheries Society* 125:187-195
- Hayes, T., K. Haston, M. Tsui, and others. 2003. Atrazine-Induced Hermaphroditism at 0.1 ppb in American Leopard Frogs (*Rana pipiens*): Laboratory and Field Evidence. *Environmental Health Perspectives* 111:568-575.
- Ghosh, K. Pranab, and Ligy Philip. 2006. Environmental Significance of Atrazine in Aqueous Systems and Its Removal by Biological Processes: An Overview: *Global NEST Journal* 8, No. 2:159-178.
- Spectrum Labs. (undated). Chemical Fact Sheet. <http://www.speclab.com/compound/c1912249.htm>
- Tillet, D., D.M. Papoulias, J.J. Whyte, and C.A. Richter. 2010. Atrazine reduces reproduction in fathead minnow (*Pimephales promelas*). *Aquatic Toxicology* 99:149-159.
- USGS Multimedia Gallery. 2007. WARP Estimates of Maximum 21-Day Moving-Average Atrazine Concentrations in Streams. http://gallery.usgs.gov/photos/05_24_2010_x1Tf83Ivu5_05_24_2010_0
- Wisc.edu. (undated). Determining the Concentration of Sperm with a Hemocytometer. http://www.ansci.wisc.edu/jjp1/ansci_repro/lab/procedures/hemocytometer/Hemocytometer%20use.html

Acknowledgements

We would like to thank the Undergraduate Research Center and Water Resources Center for funding and lab resources. In addition, we express our gratitude to Minnesota Department of Natural Resources fishery and hatchery staff in Grand Rapids and Waterville, particularly Bruce Pittman, T.J. Debates, Dale Logsdon, David Weitzel, Arne Danielson, and Sharon Kitterman for their input on the study and assistance in gathering samples. We also want to thank Minnesota State University, Mankato faculty members Beth Proctor, John Krenz, and Lois Anderson for assistance and advice. We are also grateful to Tony Kopari for his assistance with securing supplies and managing the URC funding.

Professional Biographies

Andy Stevens

Andy is currently a senior at Minnesota State University Mankato. Andy anticipates graduating in the spring of 2013 with a B.S in Environmental Science with a focus in aquatic ecology, and minors in biology and law enforcement. Andy is currently involved with the Water Resources Center as a work study intern and has been involved with them since summer of 2011. He has recently completed an internship working for the Streams Habitat Program with the Minnesota Department of Natural Resources. His involvement includes the National Biological Honor Society, American Fisheries Society, campus volunteer and Dean's list member. He will be presenting his research project at the Midwest Fish and Wildlife conference 2012 in Kansas this fall with Paul Pallardy Jr. Andy is planning on attending graduate school in the fall of 2013 and pursuing a career in either fisheries or environmental consulting.

Paul Pallardy Jr.

Paul is completing his final year at Minnesota State University, Mankato. Paul's anticipated graduation date is May 11, 2013 with a degree in Environmental Science with a focus in toxicology, minors in both Biology and Geography, and a GIS certificate. His research on this project was conducted during the 2011-2012 school year. For the past three summers Paul has held an intern position with Weaver Boos Environmental Consulting Firm in Chicago, Illinois. His experience with the firm has included water and soil sampling. Paul is looking to pursue a career in environmental consulting.

Dr. Shannon Fisher

Dr. Fisher received his B.S. (1994) from Northland College and his M.S. (1996) and Ph.D. (1999) from South Dakota State University. He was an Environmental Review Ecologist and Fisheries Biologist for the MN DNR for 5 years before starting with the Water Resources Center in April 2005. In addition to his WRC duties as executive director, he serves as the Executive Director for the Minnesota River Basin Joint Powers Board (MRB) and as a Professor of Water Resources.

In his collective capacity, Dr. Fisher provides administrative support to the WRC/MRB staff, leads a 38-county delegation of local officials, advises student researchers, maintains stakeholder relations, instructs courses, coordinates conferences, and lobbies policymakers. These activities focus on the WRC/MRB mission to collect and disseminate water quality, watershed, and aquatic ecology information – with an emphasis on engaging students and educating our regional community.

