REPORT DRAFT ON PROJECT PROGRESS
Covering August 2008 through August 2009

Genetic Detection and Geographic Analysis of Great Lakes Fish Infection by Viral Hemorrhagic Septicemia

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I. Research Plan:

The project objective is to investigate the emerging viral hemorrhagic septicemia (VHS) disease, a new strain of rhabdovirus that broke out in Lake Erie and other Great Lakes regions during the spring seasons of 2006 and 2007, causing massive fish die-offs that threaten fisheries, economic development, tourism, and negatively affecting public health perception. This research merges the powers of Geographic Information Systems (GIS) and Molecular Genetic Technology to understand and combat this new disease outbreak in the Great Lakes region. University researchers are collaborating with federal and state agencies and other universities to (1) develop key genetic tools for rapid and accurate detection of the virus in infected fish, (2) analyze fish population susceptibility and disease resistance, and (3) profile the disease dynamics through geographic and population mapping. The research product is being presented through geographic tools that integrate ecological, genetic, and environmental health data in a user-friendly format that is easily web-accessible by researchers, agencies, educators, policy-makers, and the general public. Broader impacts include that improved natural resources and water

quality enhance quality of life, and can help promote tourism, enhance fisheries and water recreation, and reduce treatment costs for drinking water. Lake Erie's sport fishing alone, which is the largest recreational fishery in the Great Lakes, has been estimated to generate nearly $1 billion in economic value annually, making this research well worth the investment. In addition to the fishery, the coastal wetlands provide essential habitat for some 300 species of birds moving along the Atlantic and Mississippi flyways, which may also be affected by fish mortality caused by VHS. The current tourism revenues and potential revenues in the future depend upon Lake Erie's water quality safeguarded for recreation, public health, and commerce.

II. Team Personnel and their Roles:

A. **Erin Crawford**, Technician- assisted in the development and optimization of primers and internal standards for fish housekeeping gene beta-actin, VHSv all strains, and VHSv genotype IVb, successfully PCR amplified RNA from several fish species: white perch, smallmouth bass, and round goby, attended the workshop for Great Lakes managers at the Lake Erie Center, and presented VHS project presentations at the International Association of Great Lakes Research.

B. **Lindsey Pierce**, Ph.D. student (Dr. Stepien)- worked under the direction of Erin Crawford in Dr. Willey's lab to develop and optimize primers and internal standards for fish housekeeping gene beta-actin, VHSv all strains, and VHSv genotype IVb, successfully extracted, reverse transcribed, and PCR amplified RNA from several fish species: white perch, smallmouth bass, and round goby, presented VHS project presentations at Sigma Xi, Midwest Fish and Wildlife, workshop for Lake Erie managers,Walleye and Yellow Perch Task Group, Great Lakes Fishery Commission, International Association for Great Lakes Research, USDA, constructed VHS link on the Lake Erie Center website displaying project background, objectives, goals, current progress, and future considerations (http://www.utoledo.edu/as/lec/research/glgl/VHS/VHS_main.html), and participated in a VHS interview featured in Great Lakes Echo, June 18, 2009 (http://www.utoledo.edu/as/lec/research/glgl/VHS/VHS_main.html)

C. **Douglas Murphy**, Technician- working on DNA microsatellite discrimination among test strains of yellow perch, assisting graduate student Lindsey Pierce with IACUC project paperwork.

D. **Dr. Carol Stepien-** attended and developed contacts with other VHS researchers at the International Conference on Aquatic Invasive Species in Montreal, Canada in May 2009, and at the American Fisheries Society Annual conference in Ottawa, Canada in August 2008, and planned, organized, and hosted the International Association for Great Lakes Research at the University of Toledo with a special session: VHS in the Great Lakes: Impacts and Outlooks.

E. **Dr. James Willey**- attended and developed contacts with other VHS researchers at the Annual Meeting for the ODA and IAGLR conferences, presented VHS project presentations at the ODA Annual meeting, developed

the molecular biology test, supervised lab work, and holds the patents for the StaRT-PCR process being used.

F. **Dr. Jonathan Bossenbroek-** conducted GIS research on VHSv outbreaks and its spread, is relating these data to gravity models and transport vectors, serving on Ph.D. committee of L. Pierce, and attended IAGLR VHS session.

III. Collaborations:

A. **ODA, Animal Disease Diagnostic Laboratory**- collaboration to compare newly developed StaRT-PCR to cell culture to test accuracy, reliability, and speed, and to detect active replicating VHS in infected fish tissues
B. **Michigan State University**- future collaboration in an immersion trial using muskellunge and yellow perch to compare quantification abilities of newly developed StaRT-PCR to current cell culture methodology, and comparison of StaRT-PCR to cell culture to detect active replicating VHS in infected fish tissues.
C. **Cornell University**- received positive sample of kidney and spleen tissues from an infected smallmouth bass fish.
D. **Ohio DNR, Michigan DNR, and USGS**- collaboration to collect infected and non-infected fish tissues.
E. **University of Wisconsin, Milwaukee-** Collaborator in susceptibility of VHSv for yellow perch aquaculture strains.

IV. Impacts to Date:

(1) We conducted a fishery genetic workshop for Lake Erie fishery managers on Friday, November 21, 2008 at the University of Toledo's Lake Erie Center. This meeting featured presentations on our VHS work to date, website updates, future management goals, and featured feedback.
(2) We created a new VHS research project link on the Lake Erie Center website. This page describes background information, current methodology, current projects, current progress, and future considerations.
http://www.utoledo.edu/as/lec/research/glgl/VHS/VHS_main.html
(3) We presented our project to the Ohio Department of Agriculture (ODA) where we presented our VHS research plan, rapid test design, and its implementation. We engaged in a question and answer session to determine the areas that the ODA is most concerned about. During this meeting, we established collaboration with the ODA, Animal Disease Diagnostic Laboratory, specifically with Dr. Yan Zhang to test our StaRT-PCR method with the current "gold-standard" cell culture methodology at the facility.
(4) Dr. Stepien attended and developed contacts with other VHS researchers at the International Conference on Aquatic Invasive Species in Montreal, Canada in May 2009. She also did the same in August 2008 at the American Fisheries Society Annual conference in Ottawa, Canada.
(5) We planned, organized, and hosted the International Association for Great Lakes Research at the University of Toledo via a special session hosted by Dr. Carol Stepien: VHS in the Great Lakes: Impacts and Outlooks. This session examined the occurrence

and spread of Viral Hemorrhagic Septicemia (VHS) in the Great Lakes region and its current and projected impacts on fisheries and aquaculture. There were 10 presentations from leading researchers from federal agencies and universities with topics including VHS background information, newly developed diagnostic and detection methods, and prevention strategies. A special question and answer session was held at the end of the session where we exchanged knowledge with other researchers. From this conference, we gained collaborations with Cornell University and Michigan State University, and
(6) We developed plans to lead a special VHS issue for the Journal of Great Lakes Research, which Dr. Stepien plans to organize and edit.
(7) We also developed plans to conduct a challenge test experiment to test our method with Dr. Mohamed Faisal of Michigan State University.

IV. Outputs to Date:

Our continuing project objectives are to develop, test, and implement new molecular genetic and Geographic Information Systems (GIS) tools to rapidly and accurately detect active, replicating Viral Hemorrhagic Septicemia virus (VHSv) infection in fish and its disease pathways. Our project goals are to: (1) develop key molecular genetic tools for rapid and accurate detection of VHSv in infected fish, (2) optimize and compare this Standardized-PCR-based test (StaRT-PCR) for detecting VHS fish infection to the "gold-standard" method of cell culture and with alternate PCR methods, and (3) track the virus's geographic and environmental spread.
Our progress to date includes:
(1) development and optimization of primers for housekeeping gene beta-actin and all variant strains of VHSv and VHS genotype IVb. To date, a beta-actin primer, VHS all strains primer, and a VHS genotype IVb primer have been chosen for use in future experiments. Internal standards for VHS gene N have been developed and optimized. Sensitivity testing has proven a lower detection threshold of <10 molecules/reaction. We successfully extracted, reverse transcribed, and PCR amplified RNA from several fish species: white perch, smallmouth bass, and round goby. Other species collected for future extraction include: yellow perch, freshwater drum, and muskellunge. RNA has been quantified and proves superior for diagnostic testing. Validation of primers is currently underway with a known positive control,
(2) collaboration with the Ohio Dept. of Agriculture to test our StaRT-PCR procedure against their cell culture results,
(3) new and positive outbreaks of VHS have been incorporated into a GIS database; the spread model is under development, currently aggregating data necessary to construct and parameterize the model; the port-to-port shipping module data has been acquired from U.S Army Corp of Engineers; and contact has been made with Robert Haas of the Michigan DNR to form the boating/bait module.
(4) collaboration with Dr. Faisal of Michigan State Univ. to engage with us in VHSv challenge tests using muskellunge and yellow perch, to facilitate testing the effectiveness of our StaRT-PCR test. We are working on the experimental design with Dr. Faisal.

Workshop and conference dissemination output includes a fishery genetic workshop for Lake Erie managers on November 21, 2008 at the Lake Erie Center, which featured presentations on our VHS work, and engaged in efforts to coordinate future

plans. We led and coordinated a contributed research paper session on VHSv at the International Association for Great Lakes Research annual conference held at the University of Toledo on May 18-22, 2009. Other progress includes website development and updates (http://www.utoledo.edu/as/lec/research/glgl/VHS/VHS_main.html), VHS project research presentations (Sigma Xi, Midwest Fish and Wildlife, Walleye and Yellow Perch Task Group, Great Lakes Fishery Commission, International Association for Great Lakes Research, USDA), and a special VHS interview featured in Great Lakes Echo, June 18, 2009 (http://greatlakesecho.org/2009/06/18/great-lakes-fish-hatcheries-could-benefit-from-new-tests-for-a-deadly-virus/).

VI. Target Audience:

We conducted a fishery genetic workshop for Lake Erie fishery managers on Friday, November 21, 2008 at the University of Toledo's Lake Erie Center. This meeting featured presentations on our VHS work to date, website updates, future management goals, and feedback. This workshop was created to assess current progress on the VHS project and understand long-term goals of lake fishery managers. Additionally, we created a new VHS research project link on the Lake Erie Center website (http://www.utoledo.edu/as/lec/research/glgl/VHS/VHS_main.html) which displays background information, current methodology, current projects, current progress, and future considerations. This website was constructed to reach the general public for awareness, and the scientific community in order to not repeat any experimental designs. The VHS team has presented project presentations at several meetings (Sigma Xi, Midwest Fish and Wildlife, workshop for Great Lakes managers, Ohio Department of Agriculture Annual Meeting, Walleye and Yellow Perch Task Group, Great Lakes Fishery Commission, International Association for Great Lakes Research, USDA) to increase awareness of current DNA diagnostic detection methods and collaborations with other federal agencies and universities. Finally we planned, organized, and hosted the International Association for Great Lakes Research at the University of Toledo on May 18-22, 2009 with a special research paper session on VHSv. This special session was designed to create awareness of current diagnostic testing and detection methods, a comparison of current experimental methodologies, and to create collaborations with other federal agencies and universities.

VII. Publications, Newsletters, and Abstracts to Date:

Sepulveda-Villet, O.J., Ford, A.M., Williams, J.D., Stepien, C.A. 2009. Population genetic diversity and phylogeographic divergence patterns of yellow perch (Perca flavescens). Journal of Great Lakes Research. 35(2): 107-119.

Stepien, C.A., Murphy, D.J., Lohner, R.N., O.J. Sepulveda-Villet, O.J., and Haponski, A.E.. 2009. Signatures of vicariance, postglacial dispersal, and spawning philopatry: Population genetics and biogeography of the walleye Sander vitreus. Molecular Ecology. In Press.

NEWSLETTERS:

Coefield, S. 2009. Great Lakes fish hatcheries could benefit from new test for deadly VHS virus. Great Lakes Echo. Michigan State University. http://greatlakesecho.org/2009/06/18/great-lakes-fish-hatcheries-could-benefit-from-new-tests-for-a-deadly-virus/

ABSTRACTS:

Pierce, L.R., Crawford, E.L., Willey, J.C., Stepien, C.A. 2008. Developing a Rapid Molecular Assay with Internal Controls for Detecting the VHS Fish Virus. Sigma Xi Scientific Honorary Society Annual Conference, Toledo, OH. (best paper award to Pierce).

Stepien, C.A. 2008. Overview of VHS Molecular Biology Project and Results to the University of Gdansk, Poland and to the Russian Academy of Sciences, Moscow, Russia.

Pierce, L.R., Crawford, E.L., Willey, J.C., Bossenbroek J.M., Stepien, C.A. 2008. Genetic Detection and Geographic Analysis of Great Lakes Fish Infection by Viral Hemorrhagic Septicemia (VHS). Lake Erie Fisheries Genetics Workshop for Fishery Managers, Oregon, Ohio. November, 2008.

Willey, J.C., Crawford, E.L. 2008. Quality-Controlled RT-PCR Data that Support Development of New Diagnostics. Lake Erie Fisheries Genetics Workshop for Fishery Managers, Oregon, Ohio. November, 2008.

Pierce, L.R., Crawford, E.L., Willey, J.C., Stepien, C.A. 2008. Developing a Rapid Molecular Assay with Internal Controls for Detecting the VHS Fish Virus. Midwest Fish and Wildlife Conference, Columbus, Ohio. December, 2008.

Pierce, L.R., Crawford, E.L., Willey, J.C., Stepien, C.A. 2009. Developing a Rapid Molecular Assay with Internal Controls for Detecting the VHS Fish Virus. Ohio Fish and Wildlife Conference, Columbus, Ohio. February, 2009. (best poster award to Pierce).

Pierce, L.R., Crawford, E.L., Willey, J.C., Bossenbroek J.M., Stepien, C.A. 2009. Genetic Detection and Geographic Analysis of Great Lakes Fish Infection by Viral Hemorrhagic Septicemia (VHS). Walleye and Yellow Perch Task Group Meeting, Pickerel Creek, Ohio. March, 2009.

Stepien, C.A. and L.R. Pierce. 2009. Research on VHS fish virus. Great Lakes Fishery Commission annual Lake Committee Meeting conference. March, 2009.

Stepien, C.A. 2009. Overview of Rapid Genetic Test for VHS fish virus project results to date to US Congresswoman Marcy Kaptur and US Senator George Voinovich, March, 2009

Pierce, L.R., Crawford, E.L., Willey, J.C., Stepien, C.A. 2009. Developing a Rapid Molecular Assay with Internal Controls for Detecting the VHS Fish Virus. International Association for Great Lakes Research Annual Conference, Toledo, Ohio. May, 2009.

Crawford, E.L., Willey, J.C. 2009. Quality-Controlled RT-PCR Data that Support Development of New Diagnostics. International Association for Great Lakes Research Annual Conference, Toledo, Ohio. May, 2009.

Stepien, C.A. 2009. Introduction to the VHS Fish Virus Session. International Association for Great Lakes Research Annual Conference, Toledo, Ohio. May, 2009.

Stepien, C.A. 2009. Overview of Rapid Genetic Test for VHS fish virus project to US Congresswoman Marcy Kaptur and US Senator George Voinovich, June 2009

Stepien, C.A. 2009. Overview of Rapid Genetic Test for VHS fish virus project to the USDA, June 2009.