Environment and Natural Resources Trust Fund
2009 Phase 2 Request for Proposals (RFP)

LCCMR ID: 075-C1

Project Title: Improving Emerging Fish Disease Surveillance in Minnesota

Total Project Budget: $ $80,101

Proposed Project Time Period for the Funding Requested: 1 year, July 2009 to June 2010

Other Non-State Funds: $ $0.00

Priority: C1. Aquatic and Terrestrial Invasive Species

First Name: Katharine **Last Name:** Pelican

Sponsoring Organization: U of M

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Region: Statewide

County Name:

City / Township:

Summary: Little is known about Heterosporosis, an emerging fish disease in Minnesota. Validating a molecular diagnostic test and performing a survey of high-risk waters will inform future management decisions and research.

Main Proposal: 1008-2-018-proposal-Emerging fish disease_Proposal_K Pelican09.doc

Project Budget: 1008-2-018-budget-Emerging fish disease_Budget_K Pelican09.xls

Qualifications: 1008-2-018-qualifications-Emerging fish disease_Proj Manager_K Pelican09.do

Map: 1008-2-018-maps-Emerging fish disease_Map_K Pelican09.pdf

Letter of Resolution:

PROJECT TITLE: Improving emerging fish disease surveillance in Minnesota.

I. PROJECT STATEMENT

Emerging fish diseases are an important factor threatening already at risk wild fish populations and sport fishing in Minnesota. This, in turn, is resulting in increased regulatory and management action by the Minnesota Department of Natural Resources (DNR), a significant drain on state resources. One emerging disease of importance is Heterosporosis, associated with the spread of the microsporidian parasite, *Heterosporis* sp., that damages the muscle tissue of susceptible fish hosts. This damage can be extensive, giving the filet a freezer-burn appearance and rendering the fish unfit for human consumption. First discovered in Catfish Lake (Villas County, WI) in 2000, Heterosporosis has since been found in lakes of Minnesota, Wisconsin, Michigan, and Ontario. Upon introduction, this parasite has been shown to infect up to 40% of fish in lakes. Susceptible species include a number of economically important fish populations including yellow perch, walleye, northern pike, cisco, rainbow trout, channel catfish, baitfish, largemouth bass, and koi.

Since its introduction to Minnesota, Heterosporosis has been a disease of concern for the DNR. A survey in 2002 revealed that heterosporosis was present in 8 of 24 lakes assessed (see attached map) making continued surveillance for this parasite a high priority for this agency. Currently, 19 lakes are positive including, Leech, Mille Lacs, Gull (Crow Wing), Winnibigoshish, and Vermillion. In addition, the DNR stopped using feeder fish in its fish hatcheries to minimize disease risk to its fish stocking program and prevent state-wide disease dissemination, resulting in increased per fish production costs. Thus, management of Heterosporosis is already a significant financial burden to the DNR, and these costs are likely to increase. However, little is known about the epidemiology of the disease including transmission between fish and bodies of water, treatment regimens, environmental influences on susceptibility, affects on individual physiology and population dynamics, and the risk of movement from wild populations to aquaculture. Understanding these mechanisms and developing tools to improve surveillance are a high priority for the DNR to effectively regulate this emerging parasitic disease in Minnesota waters.

This proposal requests preliminary funding to inform future research investigating mechanisms and control of *Heterosporis* transmission. The first critical step for this project will be the development of a sensitive and accurate diagnostic test for *Heterosporis* parasites. Current diagnostic tests involve visual inspection of fillets, with suspects confirmed by light microscopy. These methods are time consuming and unreliable. The parasites are microscopic, therefore only heavy infections of muscle tissue can be observed (freezer-burn appearance) with the naked eye. This technique likely leads to false negative diagnoses for low grade infections not exhibiting clinical signs. The objectives of this project are to: 1) develop, a quantitative polymerase chain reaction (qPCR) assay, which detects *Heterosporis* DNA, and can diagnose an infection of as few as one intact parasite; 2) determine tissue testing protocols to optimize the efficiency of this new diagnostic test; and 3) use the newly developed assay to conduct a state-wide survey of Minnesota's high risk waters using banked and submitted samples from ongoing DNR surveillance programs. This will provide, for the first time, an accurate diagnostic test for Heterosporosis, and a more complete understanding of the extent of this emerging disease in the state. This information, in turn, will be critical to developing efficient control strategies to prevent the spread of this disease in Minnesota fish populations.

II. DESCRIPTION OF PROJECT RESULTS

Result 1: Validation of a quantitative PCR assay to detect *Heterosporis* sp. in fish
Budget: $8,900 (PCR supplies)
Methods: Primer sets and probes will be developed using Beacon Designer, based on mismatches between the *Heterosporis* sp. DNA sequence and related species. The selection of the best primer/probe combination will be determined based on sensitivity, accuracy, specificity, and precision results. An Eppendorf MasterCycler machine (provided by University of Minnesota Veterinary Diagnostic Laboratory) will be used for all PCR development. Positive control samples will be provided by the MN DNR from a recent Lake Superior diagnostic case involving a commercially caught cisco. Negative control samples will be obtained from unsusceptible fish, confirmed by light microscopy.
Deliverable Outcome: A new, more accurate, sensitive, and specific quantitative diagnostic test for the detection of *Heterosporis* sp. that could be used as a diagnostic test and in future research.
Completion Date: November 2009

Result 2: Determination of optimal sampling methods and tissue selection for *Heterosporis* sp in fish
Budget: $40,400 (aquaculture equipment, PCR supplies)
Methods: Although *Heterosporis* is known to target muscle tissue, immature microsporidians of related species have been found in the blood stream indicating that internal organs (i.e. kidney, spleen, liver) might have a higher likelihood of concentrating the DNA compared to muscle tissue. To determine optimal sampling methodology, yellow perch (Infected; n = 15) will be experimentally infected with Heterosporis spores and compared to un-infected fish (Control; n = 15). Fish will be held in fish tanks designed to isolate fish populations for disease research and modified for this use in cooperation with the University of Minnesota Department of Fisheries, Wildlife, and Conservation Biology Aquatics Laboratory. Two months following exposure to Heterosporis spores, fish will be humanely euthanized, and matched tissue (three sections of muscle (dorsal, central, and caudal) kidney, spleen, liver) tested from Infected and Control fish. Tissue will be homogenized and three replicate tests will be performed on each sample. Results will be necessary to develop optimal testing protocols for *Heterosporis* in fish, and also will provide preliminary information on internal host parasite spread.
Deliverable Outcome: Optimal sampling methods and tissue selection for *Heterosporis* diagnosis in fish.
Completion Date: February 2010

Result 3: Minnesota-wide survey for Heterosporosis
Budget: $8,700 (PCR supplies, sample storage supplies)
Methods: The veterinary diagnostic laboratory performs routine diagnostic tests for the Minnesota DNR on aquaculture, broodfish and wild populations. This service has resulted in a bank of fish tissue samples from across the state. These samples will be re-tested for Heterosporosis using the new assay. In addition, samples submitted throughout the year from the DNR will be tested.
Deliverable Outcome: Two important pieces of information will be determined: 1) the sensitivity of the test in comparison to traditional testing methods (since some of the banked samples will have had the traditional test run); and 2) an initial screen of Heterosporosis incidence throughout the state.
Completion Date: May 2010

III. PROJECT STRATEGY AND TIMELINE

A. Project Partners

1. Katey Pelican, DVM, PhD; Wildlife Physiologist, Department of Veterinary Population Medicine, University of Minnesota. Role: PhD advisor and Project Manager.
2. Mr. Nicholas Phelps: Fish Pathologist, Role: Assistant Project Manager and PhD student.
3. Dr. Peter Sorensen; Professor, Department of Fisheries, Wildlife, and Conservation Biology, University of Minnesota. Role: Supervise aquatic laboratory facility.
4. Ms. Ling Shen; Minnesota Department of Natural Resources. Role: Provide fish samples for qPCR validation and state survey.

B. Project Impact

This project will provide, for the first time, a sensitive, accurate diagnostic test for an emerging fish pathogen that threatens Minnesota waters. This test will be a critical tool for controlling the spread of Heterosporosis in the state. Data generated through this project will form the foundation of future research to improve understanding of Heterosporosis transmission and control in fish, and its impact on fish populations, a critical step for developing informed management of this disease in Minnesota.

C. Time

Completion of the validation and survey will take 12 months. Funds are requested to renovate existing aquaculture facilities at the University of Minnesota for emerging disease research. This equipment then will be available for future research, including planned follow-up Heterosporis transmission studies. Funds also are requested for the supplies necessary to develop and run the PCR test. In addition, funding is requested for one month of salary and fringe for the Project Manager ($8,801), for a student assistant for animal care and sample management throughout the year ($10,800), and for travel to present results at a scientific conference ($2,500).

D. Long-Term Strategy

The proposed project will provide preliminary data to seek further funding for a long-term research program on Heterosporosis at the University of Minnesota investigating mechanisms of transmission and control, environmental influences, and evaluating risk of spread between wild and farm-raised fish.

Project Budget

INSTRUCTIONS AND TEMPLATE (1 PAGE LIMIT)

(One page limit, single-sided, 10 pt. font minimum Retain the bold text and remove all instructions typed in italics. Add or delete rows as is necessary. If a category is not applicable you may write "N/A" , leave it blank, or delete the row.)

IV. TOTAL PROJECT REQUEST BUDGET

BUDGET ITEM *(See list of Eligible & Non-Eligible Costs, p. 17)*	**AMOUNT**	**% FTE**
Personnel: *Who is getting paid to do what and what is the % of full-time employment for each position? List out by position.*		
Katharine Pelican (Project Manager on a 9 month appointment) - 8% effort	6,749	8%
Fringe 30.4%	$ 2,052	%
Contracts: *With whom and for what? List out by item.*	$ -	
Student assistant to manage sample collections and animal care (900 hrs x $12/hour)	$ 10,800	
	$ -	
Equipment/Tools: *6 aquaculture tanks with individual water filtration systems, nets, fish, other misc auatic lab supplies*	$ 31,700	
Acquisition (Including Easements): *List # of acres and who will hold title (e.g., DNR, Non-profit)*	$ -	
Restoration: *List # of acres.*	$ -	
Other: *Laboratory supplies (media, pipette tips, PCR supplies)VDL tests*	$ 26,300	
Travel (1 conference for presenting results, local travel to coordinate with DNR sample collections)	$2,500	
TOTAL PROJECT BUDGET REQUEST TO LCCMR	$ 80,101	

V. OTHER FUNDS

SOURCE OF FUNDS	**AMOUNT**	**Status**
Remaining $ From Previous Trust Fund Appropriation (if applicable): *How much Trust Fund money remains not spent or legally obligated from any previous Trust Fund appropriation for any directly related project of the proposing project, project manager, or project organization? Specify the appropriation.*	$ -	*Unspent or Not Legally Obligated*
Other Non-State $ Being Leveraged During Project Period: *What additional non-state cash $ will be spent on the project during the funding period? For each individual sum, list out the source of the funds, the amount, and indicate whether the funds are secured or pending approval.*	$ -	*Secured or Pending*
Other State $ Being Spent During Project Period: *What additional state cash $ (e.g. bonding, other grants) will be spent on the project during the funding period? For each individual sum, list out the source of the funds, the amount, and indicate whether the funds are secured or pending approval.*	$ -	*Secured or Pending*
In-kind Services During Project Period: *What in-kind services will be provided during the funding period? List type of service(s) and estimated value. In-kind services listed should be specific to the project.*	$ -	
Past Spending: *List money spent or to be spent on this specific project, cash and/or in-kind, for 2-year timeframe prior to July 1, 2009*	$ -	

Katharine Pelican Qualifications and Organization Description

Education:

University of Minnesota, Saint Paul, MN	D.V.M.	1993-1997	Veterinary Medicine
University of Maryland, College Park, MD	Ph.D.	1997-2002	Wildlife Physiology
Smithsonian's National Zoological Park	Fellowship	2002-2007	NIH Career Development Award

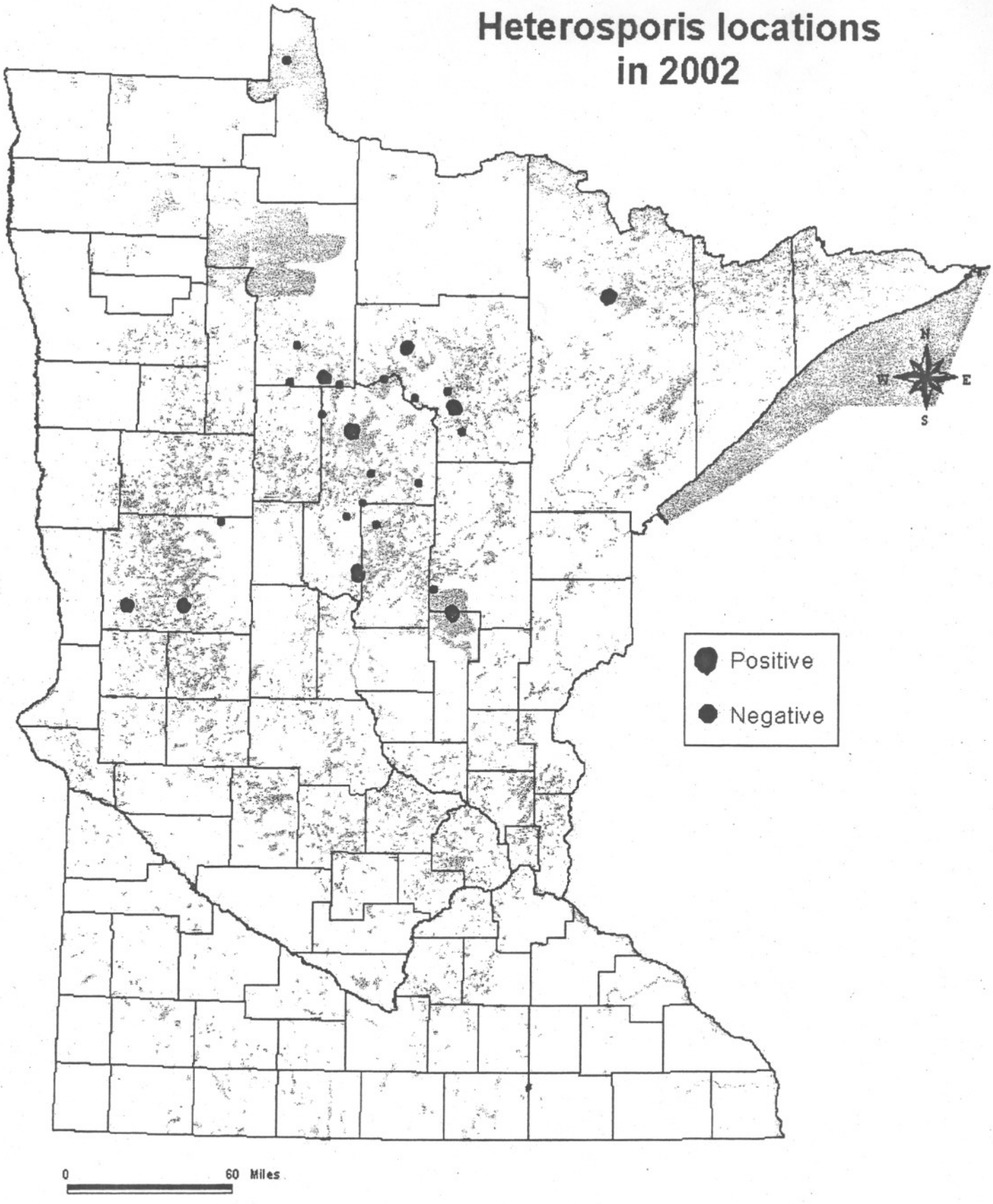
Professional Experience

1997-2002 Post-doctoral Fellow, Department of Reproductive Sciences, National Zoological Park
2002-date Research Fellow, National Institutes of Health Fellowship, Special Emphasis Research Career Award, National Zoological Park
2002-date Guest Researcher, The Thailand Zoological Parks Organization and the Thailand Department of National Parks, Wildlife and Plant Conservation
2004-date Captive Breeding Consultant and Researcher, Iberian Lynx Captive Breeding Program, Spain.
2006-date Leader, Smithsonian Amphibian Working Group, Smithsonian Institution, Washington, DC
2007-date Adjunct Professor, University of Minnesota School of Public Health, Minneapolis, MN
2007 Head, Department of Environmental Change and Species Survival, Center for Species Survival, Smithsonian's National Zoological Park, Washington, DC
2007-date Vertebrate Working Group Coordinator, Smithsonian Institution Global Earth Observatories, Smithsonian Institution,
2007-date Assistant Professor of Ecosystem Health, Department of Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota, St. Paul, MN
2008 –date PhD advisor to Nicholas Phelps, M.S., Fish Pathologist, Veterinary Diagnostic Laboratory.

Peer-Reviewed Papers (Select of Pelican and Phelps):

1. Pukazhenthi, B.S., K.M. Pelican, D.E. Wildt and J.G. Howard. 1999. Sensitivity of domestic cat (*Felis catus*) sperm from normospermic versus teratospermic donors to cold-induced acrosomal damage. Biol Reprod 61:135-141.
2. Pukazhenthi, B.S., E. Noiles, K.M. Pelican, A.M. Donoghue, D.E. Wildt and J.G. Howard. 2000. Osmotic effects on feline spermatozoa from normospermic versus teratospermic donors. Cryobiology 40:139-150.
3. Pelican, K.M., M.A. Ottinger, J.L. Brown, D.E. Wildt and J.G. Howard. 2005. Short term ovarian suppression in the domestic cat using levonorgestrel versus antide. Gen Comp Endocrinol 144: 110-121.
4. Pelican, K.M., D.E. Wildt, B. Pukazhenthi and J.G. Howard. 2006. Ovarian control for assisted reproduction in the domestic cat and wild felids. Theriogenology 66 (1): 37-48.
5. Pelican, K.M., D.E. Wildt, and J.G. Howard. 2006. The GnRH agonist Lupron® (leuprolide acetate), prevents ovulation following gonadotropin stimulation in the clouded leopard (*Neofelis nebulosa*). Theriogenology 66: 1768-1777.
6. Pukazhenthi, B.S., D. Laroe, A.E. Crosier, L.M. Bush, R. Spindler, K. Pelican, M. Bush, J.G. Howard and D.E. Wildt. 2006. Challenges in cryopreservation of clouded leopard (*Neofelis nebulosa*) spermatozoa. Theriogenology 66: 1790-1796.
7. Pukazhenthi, B.S., K.M. Pelican, and D.E. Wildt. 2007. 'Appendix A: Genome Resource Banking.' Eds. C. Gascon, J. Collins, R. Moore, D. Church, J. McKay, and J. Mendelson. Amphibian Conservation Action Plan. The World Conservation Union (IUCN), Gland Switzerland. Pp. 38-39.
8. Pelican, K.M., M.A. Ottinger, D.E. Wildt and J.G. Howard. 2008. Ovarian suppression with the progestin levonorgestrel but not the GnRH antagonist antide induces a consistent response to gonadotropin stimulation in the domestic cat. Dom Animal Endocrinol 34: 160-175.
9. Phelps, N. B. D., and A. E. Goodwin. 2008. Vertical transmission of *Ovipleistophora ovariae* (Microspora) within the eggs of the Golden Shiner. Journal of Aquatic Animal Health 20:45-53.
10. Phelps, N. B. D., and A. E. Goodwin. 2007. Validation of a quantitative PCR diagnostic method for detection of the microsporidian *Ovipleistophora ovariae* in the cyprinid fish *Notemigonus crysoleucas*. Diseases of Aquatic Organisms 76: 215-221.
11. Phelps, N. B. D. 2007. Vertical transmission of *Ovipleistophora ovariae* within the eggs of golden shiners. M.S. Thesis, University of Arkansas – Pine Bluff, Pine Bluff, Arkansas.

Organization: Veterinary Diagnostic Laboratory, University of Minnesota, 1333 Gortner Ave, St. Paul, MN 51108
The Veterinary Diagnostic Laboratory (VDL) is an integral part of the College of Veterinary Medicine and serves as the state's only full service, accredited diagnostic facility for animal health and disease. The VDL utilizes standard operating procedures and appropriate controls. A contract is in place for preventive maintenance and calibration of electrical equipment, scales, and other laboratory equipment annually. The VDL has particular expertise in developing innovative assays for emerging diseases. Nicholas Phelps, the PhD student for this project, is the fish pathologist for the VDL and has published three papers on a related microsporidian, *Ovipleistophora ovariae.*


Heterosporis locations
in 2002
N
W
E
S
Positive
Negative
0
60 Miles
Minnesota Department of Natural Resources