# Trust Fund 2009 Work Program

**Date of Report:** May 6, 2009
**Date of Next Progress Report:** December 31, 2009
**Date of Work Program Approval:**
**Project Completion Date:** June 30, 2010

**I. PROJECT TITLE**: Improving Emerging Fish Disease Surveillance in Minnesota


**Project Manager**: Katharine Pelican, DVM, PhD
**Affiliation:** College of Veterinary Medicine, University of Minnesota
**Mailing Address:** 136B Andrew Boss Laboratory, 1354 Eckles Ave
**City / State / Zip:** St. Paul, MN 55108
**Telephone Number:** 612-625-8561
**E-mail Address:** pelicank@umn.edu
**FAX Number:** (612) 624-4906
**Web Site Address:**


**Location:** The majority of the project will take place on the University of Minnesota, St. Paul campus, in the Veterinary Diagnostic Laboratory and aquaculture facility. Fish collected for surveillance testing have already been acquired and banked from throughout the state during 2008 through a relationship with the Minnesota Department of Natural Resources (see map).

| | | | |
|---|---|---|---|
| **Total Trust Fund Project Budget:** | **Trust Fund Appropriation** | **$** | **80,000** |
| | **Minus Amount Spent:** | **$** | **0** |
| | **Equal Balance:** | **$** | **80,000** |

**Legal Citation: M.L. 2009, Chp.143, Sec.2, Subd. 6c**

**Appropriation Language:**
$80,000 is from the trust fund to the Board of Regents of the University of Minnesota to assess mechanisms and control of the transmission of Heterosporosis, an emerging fish disease in Minnesota, to assist in future management decisions and research.

## II. PROJECT SUMMARY AND RESULTS:

Over the last decade, a parasitic disease, Heterosporosis, has spread to infect 20 water bodies in Minnesota. This microscopic parasite targets the muscle tissue of economically important fish species in Minnesota, rendering the fillet unpalatable. Current disease detection methodology relies on visual examination of muscle tissue, a highly insensitive test. The inability to diagnose early stages of disease, in turn, limits understanding of disease transmission and control. Thus, a high priority for managing the spread of this disease in Minnesota is to develop a sensitive test for early diagnosis of the disease.

Anticipated Results:

**Study 1) Validation of a quantitative PCR assay to detect *Heterosporis* sp. in fish.** The developed assay will provide a more specific, sensitive, and rapid diagnostic test for *Heterosporis* than current methods.

**Study 2) Determination of optimal sampling methods and tissue selection for *Heterosporis* sp. in fish.** We hypothesize that immature heterosporis spores are present in the blood stream, where organs that filter blood might have a higher likelihood of concentrating the DNA compared to muscle tissue. Therefore, targeting these organs would result in a more specific and sensitive test. These results will also provide preliminary information on internal host parasite spread.

**Study 3): Minnesota-wide survey for Heterosporosis.** This study will take advantage of banked and submitted tissue to perform a preliminary state-wide survey for heterosporosis. This preliminary opportunistic screen will provide, for the first time, accurate information about the extent and severity of Heterosporosis infections in Minnesota waters.

## III. PROGRESS SUMMARY AS OF

## IV. OUTLINE OF PROJECT RESULTS*:*

**Result 1:** Validation of a quantitative PCR assay to detect *Heterosporis* sp. in fish.

**Description:** We will validate a quantitative PCR assay that satisfies four areas 1) Sensitive – the assay will have a sensitivity comparable to other qPCR assays, about 10 copies per reaction. 2) Specific – the assay will be specific for Heterosporis DNA and not detect related species. 3) Accurate – the assay will be have a PCR efficiency between 90 – 110% and correlation of the standard curve greater than 0.9. 4) Precise – the assay will be provide the similar results with samples tested over three days, and by two different laboratories. The Minnesota Department of Natural Resources will provide positive and negative control samples. The validation will be completed July – November 2009.

| | | |
|---|---|---|
| **Summary Budget Information for Result 1:** | **Trust Fund Budget:** | **$ 11,000** |
| | **Amount Spent:** | **$ 0** |
| | **Balance:** | **$ 11,000** |

**Result 2:** Determination of optimal sampling methods and tissue selection for *Heterosporis* sp. in fish.

**Description:** To determine optimal sampling methodology, yellow perch (Infected; n = 20) will be experimentally infected with Heterosporis spores per os and compared to un-infected fish (Control; n = 20). Two months following exposure to *Heterosporis* spores, fish will be humanely euthanized, and matched tissue (muscle (three

sections: dorsal, central, and caudal) kidney, spleen, liver, blood) collected and tested from Infected and Control fish. Tissues from each fish will be homogenized separately and three replicate PCR tests will be performed on each homogenized sample. The tissue that results in the most specific and sensitive identification of infected fish over control fish will be identified. Optimization of sampling methods will be completed November 2009 – January 2010.

To prevent the risk of contaminating the public water supply and wild fish while maintaing the infected fish, a safe biosecure facility must be developed. An aquatic system of 6 50-gal aquaria will be constructed in the Veterinary Diagnostic Laboratory. The system will operate as either a static of recirculating system, depending on the presence of pathogens. An ultra violet filter and individual aerators will be used to maintain water quality. All potentially infected water and fish will be disinfected in the VDL tissue digester. The tanks will be subsequently be disinfected with a 10% bleach solution to prevent contamination of future studies. This laboratory will be a first at the University of Minnesota and provide the necessary space for future fish disease investigations. Laboratory development will be completed June – September 2009.

| | | |
|---|---|---|
| **Summary Budget Information for Result 2:** | **Trust Fund Budget:** | **$ 38,000** |
| | **Amount Spent:** | **$ 0** |
| | **Balance:** | **$ 38,000** |

**Result 3:** Minnesota-wide survey for Heterosporosis.

**Description:** The heterosporosis quantitative PCR test developed, validated, and optimized in studies 1 and 2 will be used to test fish samples submitted to the state veterinary diagnostic laboratory for viral hemorrhagic septicemia. These samples will include banked tissue from years 2007 - 2008 as well as all samples submitted during the study year. Samples will be pooled by year, lake, and species to provide an initial determination of the location, timing, and species involved across the state. For example, if Gull Lake was sampled in 2007 and 2008, samples will be pooled for each species for each year and tested. Due to the possibility of false-positive results, all positive samples from previously negative lakes or species will undergo DNA sequencing to confirm. Testing of the banked samples will be completed February – May 2010.

The results of the studies will be presented at the annual American Fisheries Society – Fish Health Section meeting. In addition, a report will be provided to the DNR for management purposes.

| | | |
|---|---|---|
| **Summary Budget Information for Result 3:** | **Trust Fund Budget:** | **$ 31,000** |
| | **Amount Spent:** | **$ 0** |
| | **Balance:** | **$ 31,000** |

| Deliverables | Completion Date | Budget |
|---|---|---|
| **Result 1.** *Validation of a quantitative PCR assay to detect* Heterosporis *sp. in fish.* | Dec 2009 | $11,000 |
| **Result 2.** *Development of a fish disease research facility.* | March 2010 | $30,000 |
| **Result 2.** *Infect yellow perch with* Heterosporis *spores.* | June 2010 | $3,000 |
| **Result 2.** Determination of optimal sampling methods and tissue selection for *Heterosporis* sp. in fish. | Dec 2010 | $5,000 |
| **Result 3.** *Minnesota-wide survey for Heterosporosis.* | May 2010 | $29,300 |
| **Result 3.** *Present research at scientific conference and provide a report to the Minnesota DNR* | June 2010 | $1,700 |

**Result Completion Date:**

**Result Status as of:** December 31, 2009.

**Result Status as of:** June 30, 2010.

**Result Status as of:** December 31, 2010.

**Final Report Summary:** June 30, 2011.

## V. TOTAL TRUST FUND PROJECT BUDGET:

**Personnel**: $19,601. Funds will be used for one month of salary and fringe for the Project Manager ($8,801). In addition, funding is needed to support a student assistant for fish care (2 hours/day x 365 days) and sample management (3.5 hours/week) throughout the year (900 hrs x $12/hr; $10,800).
**Equipment/Tools/Supplies**: $31,354. Funds will be used to develop an aquatic research laboratory at the University of Minnesota Veterinary Diagnostic Laboratory for emerging fish disease research. For general bio-secure precautions ($5,000), several items will be purchased, including equipment for hand washing, foot dipping and clothing changes to maintain quarantine between diseased and clean fish populations. Funds will also be used for system design and management ($26,650), including six 50-gal tanks, shelving and support structures, ultraviolet filtration systems to prevent disease movement between tanks, individual tank aeration, water quality maintenance supplies, and fish handling tools, including the purchase of required nets and cleaning supplies.
**Travel:** $2,500. Funds will be used to cover travel (airline tickets and per diem) to one conference for PhD student, Nicholas Phelps to present results and discuss

findings with other fish health professionals at the American Fisheries Society – Fish Health Section annual meeting, location to be determined. In addition, travel expenses are needed to cover local travel costs (vehicle and fuel) to and from the DNR to maintain the active collaboration. Funds are also needed for Nicholas Phelps to travel to field collection sites to advise on appropriate sample collection and storage techniques for submitted tissues.

**Other:** $26,454. Other funds will be used for laboratory supplies, sample collection and storage supplies. Laboratory supplies and costs include PCR primers and probe, reagents, pipette tips, gloves, instrument maintenance, computer software, DNA extraction kit, microcentrifuge tubes, and PCR plates. Sample collection and storage supplies include sample boxes, sample vials, alcohol, coolers, and ice packs.

**TOTAL TRUST FUND PROJECT BUDGET: $80,000**

**Explanation of Capital Expenditures Greater Than $3,500:** To prevent the movement of experimental pathogens from the laboratory to the environment, existing space must be renovated to meet the unique bio-safety requirements of an aquatic health research laboratory at the Veterinary Diagnostic Laboratory. The laboratory and equipment set up will be a permanent addition to the research infrastructure at the University and used to do equivalent fish disease research in the future. The Principal Investigator will work with the Co-PIs of the project to ensure that this facility is accessible and useful for future fish disease research efforts at the University of Minnesota.

## VI. PROJECT STRATEGY:

**A. Project Partners:** Nicholas Phelps, Veterinary Diagnostic Laboratory, University of Minnesota. Dr. Peter Sorenson, Department of Fisheries, Wildlife, and Conservation Biology, University of Minnesota. Ling Shen, Ecological Resources, Minnesota Department of Natural Resources. Project partners will not directly receive any funds from the appropriation.

**B. Project Impact and Long-term Strategy:** *Heteropsoris* sp. is an emerging parasite infecting many economically important and popular game fishes in Minnesota. The current diagnostic methods are not robust enough to succesfully control for this disease. The qPCR assay developed in this study will be used to opportunistically survey the State for *Heterosporis*, which for the first time, will provide managers with the necessary information to better control the spread of this disease between lakes. This will also decrease the time needed to perform diagnostic inspecions and reduce future laboratory costs for the State. Furthermore, this study is the first part of a larger PhD project by co-investigator Nicholas Phelps. These results will inform his research on *Heterosporis* classification, transmission, treatment, and host suseptibility.

**C. Other Funds Proposed to be Spent during the Project Period:** $0

**D. Spending HIstory:** $0

**VII. DISSEMINATION**: Findings from this research will be immediately provided to fisheries managers at the Minnesota Department of Natural Resources to inform fish disease control strategies in the state. In addition, findings will be presented to the scientific community at a research conference and a peer-reviewed publication and a report will be provided to the Minnesota DNR detailing all relevant results from the study.

**VIII. REPORTING REQUIREMENTS: Periodic work program progress reports will be submitted not later than December 30, 2009. A final work program report and associated products will be submitted between June 30, 2011 as requested by the LCCMR.**

**IX. RESEARCH PROJECTS: n/a**

**Attachment A: Budget Detail for 2009 Projects - Summary and a Budget page for each partner (if applicable)**

**Project Title:** Improving emerging fish disease surveillance in Minnesota

**Project Manager Name:** Katharine Pelican, DVM, PhD

**Trust Fund Appropriation: $** 80,000

| 2009 Trust Fund Budget | Result 1 Budget: | Amount Spent *(29 May 09)* | Balance *(29 May 09)* | Result 2 Budget: | Amount Spent *(29 May 09)* | Balance *(29 May 09)* | Result 3 Budget: | Amount Spent *(29 May 09)* | Balance *(29 May 09)* | TOTAL BUDGET | TOTAL BALANCE |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Validation of a quantitative PCR assay to detect Heterosporis sp. in fish | | | Determination of optimal sampling methods and tissue selection for Heterosporis sp. in fish | | | Minnesota-wide survey for Heterosporosis | | | | |
| **BUDGET ITEM** | | | | | | | | | | | |
| **PERSONNEL: wages and benefits** *(List individual names, amount budgeted and %FTE; add rows as needed)* | Katherine Pelican, 8%: $2200 | 0 | 2,200 | Katherine Pelican, 8%: $4400 | 0 | 4,400 | Katherine Pelican, 8%: $2201 | 0 | 2,201 | 8,801 | 8,801 |
| **PERSONNEL: wages and benefits** *(List individual names, amount budgeted and %FTE; add rows as needed)* | Student Assistant: $2700 | 0 | 2,700 | Student Assistant: $5400 | 0 | 5,400 | Student Assistant: $2700 | 0 | 2,700 | 10,800 | 10,800 |
| **Non-capital Equipment / Tools** *(what equipment? Give a general description and cost)* | 0 | 0 | 0 | 6 50-gal aquaculture tanks with UV filter and aeration, misc construction supplies to support tanks: $20,000. Laboratory tools (scalpels, forceps, scissors) for infective tissue handling and feeding: $250. Dissecting scope for visual inspection: $500. | 0 | 20,750 | 0 | 0 | 0 | 20,750 | 20,750 |
| **Printing** | 0 | 0 | 0 | 0 | 0 | 0 | Publication for DNR summarizing results: $200 | 0 | 200 | 200 | 200 |
| **Supplies** *(list specific categories)* | PCR reagents (primers, probe, master mix), centrifuge tubes, PCR plates, gloves, disinfectant: $6100 | 0 | 6,100 | Supplies for biosecurity (foot bath, disinfectant, gloves, rubber boots, lab coats), nets, and buckets: $5916. Fish food: $200. PCR reagents and supplies: $689. | 0 | 6,605 | PCR reagents and supplies: $23649 | | 23,649 | 36,354 | 36,354 |
| **Travel expenses in Minnesota** | 0 | 0 | 0 | Travel to collect fish: $250 | 0 | 250 | Travel to field sites to assist in sample collection, if needed: $750 | 0 | 750 | 1,000 | 1,000 |
| **Travel outside Minnesota** *(where?, for what purpose?)* | 0 | 0 | 0 | 0 | 0 | 0 | Travel to AFS-FHS Annual Conference to present findings: $1500 | 0 | 1,500 | 1,500 | 1,500 |
| **Other** *(Describe the activity and cost) be specific* | 0 | 0 | 0 | Positive (yellow perch) and negative (goldfish) fish: $595 | 0 | 595 | 0 | 0 | 0 | 595 | 595 |
| **COLUMN TOTAL** | **$11,000** | **$0** | **$11,000** | **$38,000** | **$0** | **$38,000** | **$31,000** | **$0** | **$31,000** | **$80,000** | **$80,000** |