PROJECT PROFILE

Synopsis

- **Grant Number:** 2010.1147
- **Project Title:** Emerging *Flavobacterium* spp. in the Great Lakes Basin: Identification and Assessment of Their Impacts on Fish Health
- **Grantee Organization:** Michigan State University
- **Project Team:** Mohamed Faisal[1,2], DVM, PhD; Thomas P. Loch[1], MS, PhD. [1] Department of Pathobiology and Diagnostic Investigation, College of Veterinary Medicine, Michigan State University; [2] Department of Fisheries and Wildlife, College of Agriculture and Natural Resources, Michigan State University.
- **Contact Person(s):** Dr. Mohamed Faisal (faisal@cvm.msu.edu)
- **Grant Amount:** *$220,674.00*
- **Time Frame:** 12/1/10 to 12/31/12
- **Focus Areas:** Fish Health

Brief Project Summary: Our previous studies have shown that *Flavobacterium* spp. threaten Great Lakes fish stocks. This study identified flavobacteria associated with fish mortality events and determined the potential routes they use to reach fish propagated in hatcheries. Surprisingly, multiple novel flavobacteria were isolated that were never described before, while others were similar to those reported from remote geographic areas (e.g., Antarctica). Evidence suggests that a few of these novel flavobacteria are vertically transmitted, a matter that requires proper management within hatcheries. Data produced from this GLFT-funded study provides the means to decrease flavobacterial transmission by selecting effective chemotherapeutants and biosecurity practices.

Project in Context

Over the last decade, studies performed in the laboratory of the PI demonstrated that *Flavobacterium* spp. have accounted for more fish mortality in the state of Michigan and its hatcheries than all other pathogens combined. Information on the biology of these bacteria, including how they produce disease and get access to susceptible fish in hatcheries, was unknown. The lack of knowledge regarding the true causes and sources of such disease-causing bacteria and efficacious ways to prevent and treat them in a hatchery setting can severely hinder fishery conservation efforts in the basin. Therefore, this study was designed in order to fill several of the knowledge gaps needed by fishery managers to prevent and control flavobacterial fish diseases

Goals of the Effort

The key research questions of this study include:

- What are the identities of these novel flavobacteria recovered from diseased fish/mortality events?
- Are these novel flavobacteria able to cause diseases to fish under controlled conditions?
- What is the role parental gametes may play in the transmission of flavobacterial infections in hatchery fish? Similarly, what roles do source water and hatchery practices play in the presence of flavobacteria within a hatchery setting?

- Identifying the factors produced by pathogenic flavobacteria when they are in contact with their fish host. This step is important on the long term for potential vaccine production.

Results & Significance

This study proved that multitudes of flavobacteria are associated with diseased wild, feral, and cultured fish stocks of the Great Lakes. While the "well-known" flavobacterial fish pathogens (e.g., *F. psychrophilum, F. columnare*) continue to plague Great Lakes fish stocks, this study demonstrated that many other *Flavobacterium* spp., as well another closely related genus of bacteria, *Chryseobacterium,* are also associated with fish mortality events. Many of these bacteria have either never been reported from the Great Lakes or have never before been described (i.e., novel species, such as *F. spartansii* sp. nov and *C. aahli* sp. nov). This is of major importance for fishery managers, as it means that the current method of diagnosing flavobacterial diseases in fish is vastly oversimplified and likely has implications for treatment outcomes in hatchery situations. Moreover, this study heightens the issue of the origin of these unusual flavobacteria; are they invasive pathogens brought here in today's globalization era, or have our traditional diagnostic and identification schemes misdiagnosed them? In this context, the results from this study will serve as a platform from which the role, source, and improved diagnosis of these unusual flavobacteria can be elucidated. In addition to *F. columnare* and *F. psychrophilum*, which continue to be negatively impact fish health in the Great Lakes, we have described the presence of over 60 distinct clusters of flavobacteria and chryseobacteria that were recovered from feral Chinook salmon, their eggs/reproductive fluids, their progeny at both the swim up fry and fingerling life stages, the waters that supply and run through hatchery systems, and tools used to clean hatchery rearing units. It is quite alarming that the many of these taxonomic clusters are genetically distinct from all described flavobacteria, many of which are likely novel bacterial species never before described. As a result, the impacts that these "less-typical" flavobacteria can have on the health of fish is not known. However, our studies have definitively shown that some of the flavobacterial isolates recovered from the various life stages of feral and hatchery propagated Chinook salmon, as well as hatchery waters systems, are nearly identical to those that were described as novel species early in this study and proved to be pathogenic to Great Lakes salmonids in laboratory challenges. The presence of such a multitude of fish-associated flavobacteria complicates the diagnosis and treatment of flavobacterial diseases and necessitates the revision of current disinfection protocols employed within Great Lakes fish hatcheries. Within hatcheries, special attention needs to be directed at ways to reduce or eliminate flavobacterial transmission, while also finding ways to lessen the likelihood of disease outbreaks. Our findings indicate that disinfecting hatchery pipelines and enclosures between rearing cycles is a viable way to reduce some flavobacterial reservoirs. Similarly, egg disinfection as currently adopted by the Great Lakes Fishery Commission/Fish Health Committee is not sufficient in eradicating all of the potentially pathogenic flavobacteria and chryseobacteria in and on eggs. Likewise, the persistence of flavobacteria and chryseobacteria on hatchery tools, as well as in hatchery water sources, dictates that improved eradication measures be adopted. This study has provided a more complete picture of the potentially problematic flavobacteria/chryseobacteria. It is now imperative to disentangle the truly pathogenic species from those that are innocuous and develop practical measures to combat them.

Communications

Dissertation

Loch TP. 2012. Identification of Novel Flavobacteria from Michigan and Assessment of Their Impacts on Fish Health. Ph.D. Dissertation, Department of Pathobiology & Diagnostic Investigation, Michigan State University.

Publications

- Faisal M, Loch TP, Fujimoto M, Woodiga S, Eissa A, Honeyfield D, Wolgamood M, Walker E, Marsh T. (2011): Characterization of Novel *Flavobacterium* spp. Involved in the Mortality of Coho Salmon (*Oncorhynchus kisutch*) in Their Early Life Stages. *Journal of Aquaculture Research and Development*. S2:1-8.
- Loch TP, M. Fujimoto, S. A. Woodiga, E. D. Walker, T. L. Marsh, Faisal M. Diversity of Fish-Associated Flavobacteria of Michigan. In press (to be published June, 2013), *Journal of Aquatic Animal Health.*
- Loch TP and Faisal M. *Flavobacterium spartansii* sp. nov., a pathogen of Great Lakes fishes, and emended descriptions of *Flavobacterium aquidurense* and *Flavobacterium araucananum.* Accepted, *Int. Journal of Systematic and Evolutionary Microbiology.*
- Loch TP and Faisal M. *Chryseobacterium aahli* sp. nov., isolated from 1 lake trout (*Salvelinus namaycush*) and brown trout (*Salmo trutta*) in Michigan, and emended descriptions of *Chryseobacterium ginsenosidimutans* and *Chryseobacterium gregarium.* Accepted, *Int. Journal of Systematic and Evolutionary Microbiology.*
- Loch TP and Faisal M. Emergence of *Chryseobacterium* spp. infections in Michigan fishes. Submitted to *Veterinary Microbiology.*
- Loch TP and Faisal M. Deciphering the biodiversity of fish-pathogenic *Flavobacterium* spp. recovered from Michigan, USA, using a polyphasic approach. Submitted to *Systematic and Applied Microbiology*.

Presentations

- Loch TP and Faisal M. Diversity of Fish-Associated Flavobacteria of the Laurentian Great Lakes. Presented at the 36th Annual Eastern Fish Health Workshop, Mt Pleasant, SC 2011.
- Loch TP and Faisal M. Bacterial diseases of fish in Michigan (emergent flavobacteria). St. Sault Marie, Aug. 2011. Great Lakes Fish Health Committee Meeting.
- Loch TP and Faisal M. *Flavobacterium spartani* sp. nov., a newly described pathogen of Great Lakes fishes. Presented at the 37th Annual Eastern Fish Health Workshop, Lake Placid, NY 2012**. Received the "Best Student Presentation Award"**
- Van Vliet D, Loch TP, Diamanka A, Faisal M. Prevalence of bacterial cold water disease within Michigan state fish hatcheries from 2005 to 2011, Annual Fish Health Section Meeting of the American Fishery Society, 2012, Lacrosse, WI.
- Loch TP, Xu W, Morrison JC, and Faisal M. Exposure of *Flavobacterium spartani* sp. nov. to host factors is associated with differential gene expression. Annual Fish Health Section Meeting of the American Fishery Society, 2012, Lacrosse, WI.
- Zokvic N, Loch TP, Van Vliet D, and Faisal M. Epizootiology of emergent flavobacteria in Great Lakes Chinook salmon (*Oncorhynchus tshawytscha*) and hatchery waterways. Presented at the MSU Undergraduate Research Forum, East Lansing, MI 2013. **Received first place in the Environmental Sciences category**