

Project Title: Improving Fish Health in the NCR by Integrating Extension with the Development of Alternative Disease Prevention Methods [Termination Report]

Total Funds Committed: \$601,387

Initial Project Schedule: September 1, 2021-August 31,2022 [Extended to August 31, 2024]

Current Project Year: September 1, 2022-August 31, 2023

Participants: Thomas Loch (Michigan State University); Nicholas Phelps (University of Minnesota); Myron Kebus (Michigan State University)

Extension Liaison: Matthew Smith YSI/Xylem Analytics

Industry Liaison: Dan Vogler, Harrietta Hills Trout Farm

Project Objectives

Objective #1- Develop practical and usable fish health applications for producers and fish health professionals through farm visits, trainings, and the creation of pragmatic resources by NCR fish health veterinarians.

Objective #2- Determine, for the first time, the predominating flavobacterial variants driving economic losses in the NCR trout industry.

Objective #3- Evaluate the effectiveness of newly developed vaccines in preventing losses caused by regionally predominating flavobacteria under laboratory and field conditions.

Project Summary

Arming producers with practical means of improving farmed fish health in the NCR was identified as a priority need during the 2020 NCR Aquaculture Roundtable Sessions. This is achievable in short term with off-the-shelf tools previously developed by NCRAC-funded projects and regional synergistic efforts. We will address the knowledge- action gap by building upon existing capacity in the NCR to deploy a multifaceted and sustainable fish health Extension program designed to significantly improve professional capacity, on-farm best-management-practices (BMPs), and real-time responses to fish health challenges. Simultaneously and complimentary, we will create and integrate solutions into the Extension program for flavobacteria, (i.e., causes of bacterial cold-water and columnaris disease, etc.), one of the most pressing fish health issues in the NCR and globally. Indeed, recent research determined flavobacteria causes more losses than all other pathogens combined in Michigan state hatcheries. Surveys for flavobacteria will be conducted during NCR farm visits to isolate, characterize, and identify the most NCR-problematic variants. With this information, targeted and immediately deployable vaccine preparations will be developed and evaluated under laboratory and on-farm conditions. The proposed study addresses many 2021 NCRAC priority thematic areas and, if funded, would arm producers with actionable strategies to immediately improve fish health.

Anticipated Benefits

One of the primary audiences and benefactors of this study will be trout farmers in the NCR. Many trout producers lose a significant number of fish, often early on in growth, for unexplained reasons more years than not. Due to our experience and preliminary testing, the bacterium *Flavobacterium psychrophilum* is likely directly contributing to these losses. As a benefit of this work, producers will know, for the first time, how and what variants of *F. psychrophilum* are contributing to these losses, what the sources of these infections may be, and possibly most importantly, how to proactively protect their fish – through use of a region-specific vaccine. Upon study completion, the regionally prevalent bacterial variants from which the BCWD vaccines were derived will be

maintained and be available to producers under veterinary and fish health professional oversight for cost-effective autogenous vaccine production. This indeed is a model being widely used across the USA and in compliance with USDA-APHIS Center for Veterinary Biologics, with growing success. In addition, this research approach will be broadly applicable, in particular to other regions of the U.S. with significant trout production (e.g. northwest and southeast), where the needs are abundantly clear. An important additional benefit of developing and capitalizing on BCWD preventative (i.e., vaccine) measures is reduced reliance on expensive antibiotics that yielded mixed success. In addition to avoiding inconsistent treatment outcomes that are costly, reduced (or eliminated) antibiotic use will benefit producers serving customers who view antibiotic use in a negative light. A no less important benefit to NCR trout farmers will be the additional fish health data, thereby highlighting additional and possibly previously unrecognized production impediments that can be addressed via newly developed and implemented BMPs and/or prioritized as future NCR research targets.

Another major benefit of this study; all producers in the region will have access to the regional Extension veterinarian for assistance, a resource that many in the NCR have never been able to benefit from. Some producers in the region will have an actual aquaculture veterinarian on their farm for the first time. This gives the producer the opportunity to discuss limiting stress on the animals, which will undoubtedly lead help the producer with a host of biological or system design questions. All NCR farmers and veterinary students will also benefit if they decide to participate in any workshop trainings or if they utilize the published content developed by the Extension veterinarian (e.g., BMP documents). Similarly, a graduate student partially funded by this project will gain tremendous research, outreach, and scholarly expertise in working closely with NCR producers, the proposed project team, and the Extension veterinarian. Multiple undergraduate interns will likewise benefit from involvement in the proposed project.

In a similar broadly beneficial context, Extension and research outputs (e.g., NCR specific and generalizable farm BMPs, fish health related manuals/videos/documents, training opportunities, etc.) will be provided open access through www.NCRAC.org and any videos created will be through NCRAC's Vimeo channel. All online educational materials will be developed in a manner to be easily understood by the target audience (e.g. producers, and/or veterinarian and vet student). Workshops will be cost recovery only; increasing the opportunity for quality turnout. We will engage the State aquaculture association and universities' College of Veterinary Medicine, if there is one in the state, to increase awareness of our presence for our audience. Research findings will be published in peer-reviewed scientific journals (forecast at least three-four research manuscripts), and at least one publication to the Journal of Extension showcasing NCR Extension activities, all of which will be of an applied nature towards boosting aquaculture productivity and fish health.

Project Progress

A major accomplishment during the current reporting period was a successfully completed faculty search at the Michigan State University (MSU)-College of Veterinary Medicine and subsequent hiring of the first ever NCR-Regional Aquaculture Outreach Veterinarian. Dr. Myron Kebus officially began in Nov. 2022, and has not only played a primary role in our substantial progress under Objs. 1 and 2, but also has had an immediate positive impact (per producers) on fish health and production in the NCR aquaculture industry. Notably, the initial investment to support an aquaculture outreach veterinarian by USDA-NIFA NCRAC was leveraged to generate future support for Dr. Kebus beyond this project, thereby creating a long-term resource to enhance fish health and fish production in the NCR and beyond.

Objective 1

Our team designed and conducted a qualitative 30 question survey from ~April to July 2023 that focused on categories of general farm description, farm specific fish health concerns, desired fish health needs, disease prevention/diagnosis/treatment, and also allowed for open ended discussion. The survey protocol was reviewed by the MSU IRB, and an approved consent letter was provided to all participants prior to survey. Invitations were sent via email to contacts at 71 fish farms in 11/12 NCR states, with 24 agreeing to participate. The survey was conducted in-person and by phone. Although the survey was designed to be completed in 30 minutes, participants generally took up to 75 minutes to share input on fish health needs and what would most benefit them. Examples of survey focus included whether participants had fish veterinarians, conducted regulatory Fish Health Inspections, had written biosecurity plans, vaccinated, had disinfection protocols, used Veterinary Feed Directives for antibiotic use, and what types of fish health outreach efforts they would prefer (e.g., consult, workshops, online courses, fact sheets). A range of initial notable trends were apparent, including differences in fish health issues and fish health professional use by fish species raised, state, and fish rearing system (i.e., pond vs. RAS vs. flow through), among others. Although analyses are ongoing, initial survey results indicated that 65% of participants conducted regulatory Fish Health Inspections, 44% had fish veterinarians, 26% had written biosecurity plans, 11% vaccinate, 47% have disinfection protocols, and 4% use Veterinary Feed Directives for antibiotic use. It appears that voluntary non-regulatory production medicine related activities are relatively low in the NCR, highlighting a key point of intervention for improving fish health and production in the future.

Towards specifically improving fish health and productivity of the NCR aquaculture industry, Dr. Kebus, in collaboration with the rest of our team, undertook substantial efforts to visit a range of fish farms across the NCR. In total thus far, Dr. Kebus has visited 18 fish farms and 10 state fish hatcheries in 7 NCR states, with some facilities having been visited multiple times (n=31 facility visits completed). During these visits, Dr. Kebus has examined and assessed fish health management practices, performed gross and microscopic examinations on fish, and discussed options and resources in the region to improve fish health. Dr. Kebus has also conducted 108 consults with producers and veterinarians, including 47 with producers from 8 NCR states on critical fish disease issues occurring on their farms. Based upon industry feedback, these visits and consults have been tremendously well-received, notably improving or altogether solving a range of fish health and aquaculture production issues in the NCR. A challenge in delivering direct consultation to fish farmers is the travel and expense in getting to farms in this large region of the country. Telehealth in the form of virtual (e.g., Zoom), email, or phone is a potential alternative.

Additionally, during the current reporting period, our team gave 22 presentations (please see list below) on this NCRAC-funded research to >625 attendees at local, regional, national, and international professional meetings, including 2 by Nisha Shrestha, the MSU PhD student working on this project, one of which she won “Best Student Presentation” award. Moreover, our team has conducted 8 workshops and presentations for veterinarians and veterinary students, including 6 full days of training (note, our goal in the proposal was 2 one-day trainings).

Objective 2

To more effectively prevent and control bacterial coldwater disease (BCWD)-associated losses, efforts to isolate, identify, and characterize the predominating *F. psychrophilum* variants in NCR trout and salmon facilities were undertaken. In this reporting period, moribund salmonids from seven facilities in seven NCR states e.g., Michigan (MI), Ohio (OH), Iowa (IA), Wisconsin (WI), Minnesota (MN), Missouri (MO), and South Dakota (SD) have been collected, clinically examined, and bacteriologically analyzed. Examined fish (n=161) presented with a range of gross disease signs consistent with BCWD, including fin erosion, external ulceration, exophthalmia,

visceral hemorrhage and/or pallor, and splenic swelling and enlargement. Cultures for flavobacterial isolation (n=651) were prepared on FPM-A medium and inoculated with fresh tissues from the gills, brain, kidney, spleen, and representative external lesions. As a result, 183 yellow-pigmented bacterial isolates were recovered, 121 of which were identified as *F. psychrophilum* via *F. psychrophilum*-specific endpoint polymerase chain reaction (PCR) analyses. Overall, *F. psychrophilum* was detected in ~33% of the examined fish and from six of the seven facilities. As additional facilities in the NCR are being sampled, genotyping via multi locus sequence typing is underway. Likewise, molecular serotyping has been completed for a subset of the recovered *F. psychrophilum* isolates, revealing some interesting trends, including host species to serotype associations and that some facilities were affected by a single molecular serotype and others by two or more serotypes. After identifying the predominating *F. psychrophilum* variants in the sampled NCR facilities, lab and field-based experiments will test the protective efficacy of various autogenous bacteria preparations, with the aim of producing efficacious, site-specific bacteria's capable of enhancing fish health and productivity throughout the region (Obj. 3). The findings from the study are expected to be of interest not only to fish health specialists but also to stakeholders in the aquaculture sector seeking effective strategies to mitigate the impacts of BCWD in the NCR.

Objective 3

To begin during the upcoming reporting period.

Outreach Overview

This USDA-NIFA NCRAC funded project has outreach intertwined throughout. Indeed, we have undertaken substantial efforts to visit a multitude of fish farms across the NCR.

Thus far, Dr. Kebus has visited 18 fish farms and 10 state fish hatcheries in 7 NCR states, with some facilities having been visited multiple times (n=31 facility visits completed). During these visits, Dr. Kebus has examined and assessed fish health management practices, performed gross and microscopic examinations on fish, and discussed options and resources in the region to improve fish health. Dr. Kebus has also conducted 108 consults with producers and veterinarians, including 47 with producers from 8 NCR states on critical fish disease issues occurring on their farms. Based upon industry feedback, these visits and consults have been tremendously well-received, notably improving or altogether solving a range of fish health and aquaculture production issues in the NCR.

Our team also gave 22 presentations on this NCRAC-funded research to a multitude of stakeholder groups at local, regional, national, and international professional meetings. We also conducted 8 training workshops and presentations for veterinarians and veterinary students, including 6 full days of training (note, our goal in the proposal was 2 one-day trainings).

Moving forward and once vaccine (i.e., bacterin) preparations have been tested, the materials for preparing those will be made available for those who would like to use them and per USDA-APHIS Center for Veterinary Biologics. Initial results from the on-farm flavobacteria sampling have also been communicated to facility personnel, and as further genetic and sero- typing information is available, those too will be relayed to the respective producers.

In addition to the publications listed below, invaluable training and mentorship for the PhD student (Nisha Shrestha) working directly on this research has been made possible by this USDA-NIFA NCRAC funded project. Notably, Nisha won a "Best Student Presentation" award for the presentation she gave on her initial findings for this project during the American Fisheries Society - Fish Health Section summer seminar series (presentation recorded and available here; https://www.youtube.com/watch?v=pg_1_MLcvb0). Likewise, a plethora of training and

mentorship in flavobacteriological techniques, aquatic animal health research, and outreach was provided as a result of this project for two graduate students working on synergistic research projects in the MSU - Aquatic Animal Health Laboratory, as well as one MSU DVM student and five MSU undergraduate students.

Another substantial output to date was the creation of a long-term aquaculture outreach veterinarian for the North Central Region, which was enabled by the initial investment made by USDA-NIFA NCRAC. Over the past year while serving as the regional aquaculture outreach veterinarian, Dr. Kebus has helped multiple producers address a range of fish health concerns on their farms, with exceptionally positive feedback from industry. Similarly and as we have actively presented our findings and discussed this research at aquaculture association meetings, scientific conferences (including Aquaculture America), and other stakeholder meetings, we have been met with consistently positive feedback from industry and interest in participating. We believe this regional approach to fish veterinary medicine could become a model for other regions in the USA, whereby a regional outreach veterinarian works closely with local veterinarians and fish health professionals, producers, and others to boost farmed aquatic animal health and aquaculture productivity and profitability.

The genotypic data resulting from our ongoing *Flavobacterium psychrophilum* multilocus sequence typing (MLST) efforts have been sent to the central *F. psychrophilum* MLST repository (quality curated by Dr. P. Nicolas, INRA, France) and once completed, will also be an invaluable output for researchers and others study the epidemiology of bacterial coldwater disease here in the USA and abroad.

Targeted Audiences

The primary audience for this USDA-NIFA NCRAC funded project are fish farmers/producers in the North Central Region; the fish farming industry at large (but especially trout/salmon producers); anyone interested/involved in raising fish in hatcheries/aquaculture facilities; aquatic veterinarians and aquatic animal health professionals; students aspiring to become producers, veterinarians, aquatic animal health professionals, and/or researchers; researchers; and fishery management agencies. The assembled team has strong ties to these audiences, and we have been actively capitalizing upon already existing and newly formed relationships and collaborations for two-way information exchange and numerous training and education opportunities as a result of this USDA-NIFA NCRAC funded project.

Outputs/Impacts

This USDA-NIFA NCRAC funded study continues to have sizeable impacts; a primary example is providing, for the first time, an accessible regional aquaculture veterinarian with decades of aquatic animal medicine experience for NCR producers to benefit from. Indeed, a range of fish health concerns were brought to our team by industry, leading to timely recommendations and also encouragement to simultaneously work with local veterinarians on any potential treatment options. Likewise, our visits to fish farms across the NCR led to timely findings that informed means by which producers could not only control current/ongoing fish health issues, but also better prevent them in the future (i.e., preventative medicine). Industry initiated consults with Dr. Kebus were frequent, resulting in discussions with farmers on possible causes (ranging from things such as early trout fry mortality to ammonia toxicity in tilapia) and workable solutions. Impacts also included discussions during consults and visits towards connecting farmers with the broader fish health and aquaculture resource networks in the region and beyond, as well as fulfilling producer requested farm specific training of staff on fish health procedures that meet their needs.

This NCRAC project is also having a growing and sizeable impact for veterinarians seeking additional training in aquatic animal medicine, as well as for veterinary students, graduate students, and undergraduate students interested in aquatic animal health. In addition to knowledge and training (both hands on and didactic) being shared during workshops and presentations, this USDA-NIFA funded research has afforded multiple veterinary and graduate students the invaluable immersive experience of visiting NCR fish farms (notably for these students, a first), as well as experience and training in interacting with fish farmers and participating in devising strategies to help solve "real-world" limitations to fish health and farm productivity. The impact these opportunities have had on the students involved in this project cannot be understated, and is directly solving an industry stated need of more knowledgeable and experienced fish health expertise that is available to industry now and in the future.

Although ongoing, another notable outcome of this USDA-NIFA NCRAC funded study is the substantial active regional surveillance program for *Flavobacterium psychrophilum* that, to our knowledge, is the largest ever of its kind in the USA. The extensive number of flavobacterial isolates that have been recovered, identified, typed, and cryopreserved will not only serve as an invaluable resource in more effectively preventing bacterial coldwater disease (i.e., are being used to guide vaccine preparations in the next reporting period), but also is unveiling crucial data on the epidemiology of this devastating salmonid pathogen.

Recommended Follow-Up Activities

This USDA-NIFA NCRAC funded study is ongoing; future study recommendations will be given upon project completion.

Publications, Manuscripts, Workshops, and Conferences

Presentations-Oral

- n=22 during current reporting period; # Signifies Presenter
- Shrestha N, Kebus M, Shavalier MA, Loch TP#. Michigan State University - Aquatic Animal Health Laboratory Research Updates. Invited Presentation at the Great Lakes Fishery Commission - Great Lakes Fish Health Committee Meeting, Grand Rapids, MI, August 2023.
- Kebus M#, Loch TP, Smith MA, Phelps NB, Vogler D, Emerson M, Summerfelt S. Improving fish health in the NCR by integrating extension with the development of alternative disease prevention methods. Invited Presentation at the Great Lakes Fish Health Committee Annual Meeting, Grand Rapids, MI, August 2023.
- Knupp CK, Lennox SMG, Call D, Soto E, Shavalier MA, Shrestha N, Faisal M, & Loch TP#. Flavobacteria: An Emerging and Resurging Threat to Fish Health Worldwide. Invited Virtual Seminar for the Egyptian Aquatic Health Association Monthly Seminar series, Cairo, Egypt, August 2023.
- Shrestha N#, Kebus M, Lennox S, Shavalier M, Knupp C, Pilarski F, Smith M, Phelps N, Loch TP. Investigating the Geno- and Serotypic Diversity of *Flavobacterium psychrophilum* Infecting Captive-Reared Salmonids of the North Central Region of the USA. Presented at the Michigan Dept. of Natural Resources Fish Production Section Meeting, East Lansing, MI, August 2023.
- Loch TP, Kebus M#, Smith MA, Phelps NB, Vogler D, Emerson M, Summerfelt S. Improving Fish Health in the NCR by Integrating Outreach with the Development of Alternative Disease Prevention Methods. Presented at the Michigan Dept. of Natural Resources Fish Production Section Meeting, East Lansing, MI, August 2023.
- Phelps NB#, Loch TP, Kebus M, Smith MA. Prioritizing Fish Health Research and Outreach Activities in the North Central Region, USA. Presented at the Annual American Fisheries Society – Fish Health Section, Burlington, VT, July 2023.
- Shrestha N#, Kebus M, Lennox S, Shavalier M, Knupp C, Pilarski F, Smith M, Phelps N, Loch T. Investigating the Geno- and Serotypic Diversity of *Flavobacterium psychrophilum* Infecting Captive-Reared

Salmonids of the North Central Region of the USA. Presented at the American Fisheries Society – Fish Health Section Virtual Summer Seminar Series, July 2023. Best Student Presentation Award.

- Kebus M#, Loch TP, Smith MA, Phelps NB, Vogler D, Emerson M, Summerfelt S. Fish farming 101 for veterinarians; What veterinarians need to know about how fish are raised (on farms). Invited Presentation at the Annual Conference of the American Veterinary Medical Association Annual Conference, Denver, CO, July 2023.
- Knupp CK, Lennox SMG, Call D, Soto E, Ivan L, Brenden T, Shavaliar M, Shrestha N, Loch TP#. Prevention and Control of Bacterial Coldwater Disease: Perspectives from Michigan. Invited Virtual Presentation for USFWS National Hatchery System Guest Webinar, May 2023.
- Kebus M#, Loch TP, Smith MA, Phelps NB, Vogler D, Emerson M, Summerfelt S. Improving fish health in the NCR by integrating extension with the development of alternative disease prevention methods. Invited Presentation at the Virtual NOAA Sea Grant, Great Lakes Regional Aquaculture Call, May 2023.
- Kebus M#, Loch TP, Smith MA, Phelps NB, Vogler D, Emerson M, Summerfelt S. Improving fish health in the NCR by integrating extension with the development of alternative disease prevention methods. Invited Presentation at commercial fish farm, WI, May 2023.
- Kebus M#, Loch TP, Smith MA, Phelps NB, Vogler D, Emerson M, Summerfelt S. Improving fish health in the NCR by integrating extension with the development of alternative disease prevention methods. Invited Presentation at the Fish Health Selective Course, University of Wisconsin-Madison, School of Veterinary Medicine, Madison, WI, May 2023.
- Kebus M#, Loch TP, Smith MA, Phelps NB, Vogler D, Emerson M, Summerfelt S. Improving fish health in the NCR by integrating extension with the development of alternative disease prevention methods. Invited Presentation at the Aquatic Veterinary Medicine Clerkship, Michigan State University, College of Veterinary Medicine, Virtual Veterinary Nursing Seminar, East Lansing, MI, April 2023.
- Loch TP#, Kebus M#, Smith MA, Phelps NB, Vogler D, Emerson M, Summerfelt S. Improving Fish Health in the NCR by Integrating Outreach with the Development of Alternative Disease Prevention Methods. Invited Presentation at the US Trout Farmers Association Session, Aquaculture America, New Orleans, LA, February 2023.
- Loch TP#, Kebus M#, Smith MA, Phelps NB, Vogler D, Emerson M, Summerfelt S. Efforts to Advance Fish Farm Fish Health by the New Aquaculture Outreach Veterinarian in The North Central States. Invited Presentation at American Association of Fish Veterinarians Session, Aquaculture America, New Orleans, LA, February 2023.
- Loch TP#, Kebus M#, Smith MA, Phelps NB, Vogler D, Emerson M, Summerfelt S. Improving Fish Health in the NCR by Integrating Outreach with the Development of Alternative Disease Prevention Methods. Invited Presentation at the Wisconsin/Minnesota Aquaculture Conference, Eau Claire, WI, February 2023.
- Loch TP#, Kebus M#, Smith MA, Phelps NB, Vogler D, Emerson M, Summerfelt S. Improving Fish Health in the NCR by Integrating Outreach with the Development of Alternative Disease Prevention Methods. Invited Presentation at the Michigan Aquaculture Association Meeting, Clare, MI, February 2023.
- Kebus M#, Loch TP, Smith MA, Phelps NB, Vogler D, Emerson M, Summerfelt S. Improving fish health in the NCR by integrating extension with the development of alternative disease prevention methods. Invited Presentation at the Virtual Michigan State University, College of Veterinary Medicine, Veterinary Nursing Seminar, East Lansing, MI, February 2023.
- Kebus M#, Loch TP, Smith MA, Phelps NB, Vogler D, Emerson M, Summerfelt S. Improving fish health in the NCR by integrating extension with the development of alternative disease prevention methods. Invited Presentation at the Virtual Michigan State University, College of Veterinary Medicine, World Aquatic Veterinary Medicine Student Chapter Seminar, East Lansing, MI, January 2023.
- Kebus M#, Loch TP, Smith MA, Phelps NB, Vogler D, Emerson M, Summerfelt S. Improving fish health in the NCR by integrating extension with the development of alternative disease prevention methods. Invited

Presentation at the Virtual Michigan State University, College of Veterinary Medicine, Pathology Club Seminar, East Lansing, MI, January 2023.

- Kebus M#, Loch TP, Smith MA, Phelps NB, Vogler D, Emerson M, Summerfelt S. Improving fish health in the NCR by integrating extension with the development of alternative disease prevention methods. Invited Presentation at the Ohio Aquaculture Conference, Columbus, OH, January 2023.
- Kebus M#, Loch TP, Smith MA, Phelps NB, Vogler D, Emerson M, Summerfelt S. Improving fish health in the NCR by integrating extension with the development of alternative disease prevention methods. Invited Presentation at the Virtual University of Florida Aquaculture Graduate Student Seminar, Gainesville, FL, January 2023.