Project Title: Evaluating novel methods for preventing *Aeromonas*-associated losses in Yellow Perch (*Perca flavescens*) using laboratory and field-based vaccination trials [Progress Report]
Total Funds Committed: $35,000
Initial Project Schedule: April 1, 2021-March 31, 2022 [Extended to June 30, 2022]
Current Project Year: April 1, 2021-August 31, 2021
Participants: Thomas P. Loch (Michigan State University), Robert K. Smith (Clayton Veterinary Care, MI
Extension Liaison: Matthew Smith, The Ohio State University
Industry Liaison: Willian M. West, Blue Iris Fish Farm, LLC, WI

Project Objective

1. To assess the protective effectiveness of a new vaccination approach and preparation against *Aeromonas* infections in farm raised Yellow Perch.
2. To assess the protective effectiveness of a new vaccination approach and preparation against *Aeromonas* infections in Yellow Perch under controlled laboratory conditions.

Project Summary

Yellow Perch (*Perca flavescens*; YP) is a priority species within the North Central Region (NCR), yet few resources have been devoted to improving their health on farms, a matter complicated by the apparent emergence of several YP-pathogenic Aeromonas spp. across the upper Midwest. Dr. Smith recently developed a novel approach for immersion vaccinating young salmonids against *Aeromonas salmonicida*, resulting in robust protective immunity and farm survivability boosts of 40% pre-vaccination to >95% post-vaccination. Our team extended this method to vaccinate YP against predominating Aeromonas strains affecting YP across the NCR. The vaccine was produced by Kennebec River Biosciences through United States Department of Agriculture (USDA) approved means and administered to YP under laboratory conditions. Vaccinated and control (i.e., mock-vaccinated) YP were then challenged with an *A. salmonicida* strain previously recovered from a NCR YP mortality event and protective effects determined. Likewise, the protective effects of this vaccine and procedure were assessed under farm conditions in conjunction with pre- and post-treatment veterinary health assessments.

Anticipated Benefits

YP farmers within the NCR will benefit from the ability to treat young fry and potentially older fish with this immersion vaccination technology. An immersion vaccine is not only less stressful on the animals (allowing for smaller fish to be handled more easily) but also technically easier for the farmer or veterinarian to administer. A successful vaccine will lead to healthier fish which do not get sick from Aeromonas, can reach market size sooner, and be more marketable. Individual farmers will have the ability to work with Extension to evaluate whether or not the increased health and survival of the perch outweigh the costs of vaccination. It is believed that similar approaches will be initiated with other species as well.

Objective 1.— The Blue Iris humoral trial (utilizing out of season, non-uniform, hatched YP provided by Dr. Dong-Fang Deng, UW-Milwaukee) was performed by immersion vaccinating two tanks of 300 fish each. A third tank was managed as an unvaccinated control. After a 3-month study period, an increase in growth (weight) was evident in the vaccinated fish compared

to the non-vaccinated fish (the average weight of vaccinated fish was approximately 15% greater than that of the control group). Ktls (used to calculate grow rates) were highest in the vaccinated fish while the fat content was lower in the vaccinated fish. Additionally, a slight decrease in mortality was observed in the vaccinated tanks (3.2% versus 4.7%), understanding most of the mortality in vaccinated fish came from a tank of which water flow was of issue. Bacteriological analyses on fish that died in both control and vaccinated groups revealed a lack of any bacterial infections in vaccinated fish, whereas multiple bacteria (not A. salmonicida) were detected in all negative control fish.

The Blue Iris amnestic trial (using Blue Iris YP fry) showed similar results, whereby vaccinated fish weighed approximately 30% more than the non-vaccinated controls and had slightly greater total length (almost 9% longer) by the time they reached 3 months of age.

Objective 2.— For the laboratory phase of this project, YP (provided by Dr. Deng) were vaccinated or mock-vaccinated (i.e., negative control treatment) by immersion (n = 188 per treatment) following the same protocol used in Objective 1. Two separate challenge experiments were performed (one at 3-months post-vaccination [low challenge dose, LD] and one at 4-months [high challenge dose, HD]) to evaluate the protectiveness of this vaccine approach against a strain of *Aeromonas salmonicida* subsp. salmonicida (Asal) previously recovered from a YP mortality event. Due to the age and size of the fish, challenges occurred via intracoelomic injection, as deemed necessary via pilot experiments. Throughout both challenge experiments and as observed in the on-farm fish, the vaccinated group had slightly higher growth rates (~6%) than mock-vaccinated YP, but significant differences (via initial one-way ANOVA) in percent survival between vaccinated and mock-vaccinated fish were not observed. When examining Asal infection status in the LD experiment, Asal was detected in 50% of dead mock-vaccinated fish, and identically in 50% of vaccinated fish. However, Asal was exclusively detected in mock-vaccinated fish that survived until the end of the LD experiment, albeit at a low prevalence. In the HD experiment, Asal was recovered from ~87% of dead, mock-vaccinated fish vs. 85% of vaccinated fish. Interestingly, Asal infection intensities were always high (i.e., too numerous to count) in dead mock-vaccinated fish, whereas ~35% of dead, Asal positive vaccinated fish showed very low to moderate bacterial loads (e.g.,1-~50 colony forming units), possibly indicating a protective effect. Future experiments should focus on more natural challenge routes (e.g., immersion) and in younger/smaller fish to further explore this potential growth boosting/protective effect.

Outreach Overview

A vaccination workshop was hosted at Black Creek Town Hall and Blue Iris Fish Farm (Sept. 2021), where Mr. Matt Smith (The Ohio State University Extension), Dr. Tom Loch (Michigan State University), and Dr. Bob Smith (Clayton Veterinary Care) spoke to NCR aquaculture industry representatives and veterinarians about fish health in general, the goals of the project, and initial project results. The workshop was held in Black Creek, Wisconsin. Additionally, Mr. Bill West gave a presentation on producer perspectives on YP vaccination, and Mr. Bill Kelleher (Kennebec River Biosciences) presented on the use of bacterins and vaccines in aquaculture. The presentations were recorded, and Extension Liaison Smith is pulling together the presentations and pictures to post on the NCRAC website. Mr. West then gave all attendees an extensive tour of his farm, with an emphasis on fish health matters.

Additional results will be made available through publication in scientific journals and presentations at regional conferences and meetings. Our team has several dedicated talks in a fish health session during the 2022 North Central Aquaculture Conference in Eau Claire, WI.

Target Audiences

Our targeted audiences are the YP farmers and the veterinary professionals who work with them. The success of this vaccination protocol would provide farmers and veterinarians a novel tool in producing healthier and more marketable YP. Additionally, we envision our project supporting future vaccine work for other regionally-important species.

Deliverables (Outputs)

Our team held a workshop in Black Creek, Wisconsin at Black Creek Town Hall and Blue Iris Fish Farm. Attendees included farmers, veterinarians (state and private) and Extension/outreach Specialists. The local town hall provided an avenue to deliver the necessary information in PowerPoint to the audience, while still affording the attendees the opportunity to tour Bill West's fish farm. This was the first visit to Blue Iris Fish farm for most attendees, where he described the facilities and best practices. Attendees not only received vaccination education at the town hall, but they were able to learn about a relatively new pond-based production system (pond-side tank culture) on a commercial scale. Additionally, printed educational materials by NCRAC were made available to attendees to take home with them.

Outcomes/Impacts

The findings of this study have demonstrated that the *Aeromonas* vaccine developed for salmonids can be used safely in yellow perch. Although further study is needed to determine the level of protection afforded to vaccinated fish, early evidence suggests an unintended but positive side effect is a boost to YP growth and "robustness," as well as a potential tool for reducing bacterial loads in vaccinated fish.

Impacts Summary

Relevance. — Yellow perch are an important farmed fish in the NCR, yet few resources are available to improve their health on farms, a matter complicated by the apparent emergence of YP-pathogenic *Aeromonas* spp. across the upper Midwest

*Respons*e. — Herein and for the first time, we have evaluated the protective efficacy of a novel vaccine preparation against *A. salmonicida* in YP under farm and laboratory conditions. We also discussed the potential of this technology with veterinarians and NCR producers during a workshop.

Results. — Thus far, we have showed that use of this vaccine has the potential to boost YP growth (as determined under lab and field conditions), and may provide some level of protection against *A. salmonicida* infections. The potential of this technology warrants further study and is a viable option that farmers should discuss with their veterinarians when making their fish health plans.

Recap. — This vaccine may result in increased growth and survivability compared to non-vaccinated control fish; however, additional laboratory challenges using younger/smaller fish are needed to determine true protection levels.