Project Title: Evaluating novel methods for preventing Aeromonas-associated losses in Yellow Perch (Perca flavescens) using laboratory and field-based vaccination trials [Termination Report]

Total Funds Committed: $35,000

Initial Project Schedule: April 1, 2021-March 31, 2022 (Extended to December 31, 2022)

Current Project Year: April 1, 2021-August 31, 2022

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 within the North Central Region (NCR), yet few resources have been devoted to boosting their health on farms, a matter complicated by seemingly emergent YP-pathogenic bacteria (*Aeromonas* spp.). Towards improving farmed YP health, our team used an immersion vaccination approach (vaccine produced by Kennebec River Biosciences) that effectively protects young trout against *Aeromonas salmonicida* (Asal) to preliminarily assess protection in YP against NCR-*Aeromonas* strains under field and lab conditions. In lab, vaccinated and unvaccinated (control) YP were injected with Asal (low dose, LD; 3 mo vaccination; and high dose, HD, 4 mo vaccination; ~$4x10^6$ – ~$2x10^7$ bacteria/fish), after which growth, infection status, and mortality was assessed. Significant differences in growth, infection prevalence or mortality in vaccinated vs. unvaccinated fish were not observed; however, infection intensity tended to be lower in vaccinated dead HD-YP and, among fish surviving to experiment's end, Asal was detected exclusively in unvaccinated LD-YP, possibly indicating protective effects. Results from the field trials suggest a boost in growth in some vaccinated fish, lack of *Aeromonas* infections, and lower mortality when compared to unvaccinated fish. Study findings suggest this vaccination approach should be investigated further, as it has potential to improve farmed YP health and productivity in the NCR.

Technical Summary and Analysis

Objective 1. To assess the protective effectiveness of a new vaccination approach and preparation against *Aeromonas* infections in farm raised YP (Dr. R. Smith/W. West).

Two preliminary experimental field vaccination trials were conducted at Blue Iris Fish Farm, LLC (Black Creek, WI) and overseen by Dr. R. Smith and W. West (Table 1). In the first trial, fish received a single immersion vaccine treatment. This trial was completed utilizing out of season, non-uniform, hatched and feed-trained YP (~8.5 – 9.0 months, mo, of age; provided by Dr. Dong-Fang Deng, UW-Milwaukee). Immersion vaccination occurred in two tanks receiving recirculating pond water, whereas a third tank (n=300 fish/tank) was managed as an unvaccinated control (Table 1). Fish in tanks Control 1, Vaccinated 2 (V2) and Vaccinated 3 (V3) were approximately 12-months-old upon trial completion. Fish in V2 and V3 (60.6° F water temperature; 8.7 mg/L dissolved oxygen (DO); 89% oxygen saturation) were immersion vaccinated on 5-12-21 using the

30-minute immersion vaccination method without water flow per Dr. R. Smith's vaccination protocol (bivalent autogenous bacterin manufactured by Kennebec River Biosciences, Richmond, ME). Over the trial duration, length and weight measurements were periodically collected, condition factor calculated, veterinary health assessments made, and cumulative mortality in each tank assessed (Table 1). Of note, tank V2 experienced low water flow, which was corrected during the first half of the trial. Kidney tissues from all mortalities were inoculated onto trypticase soy agar for bacterial analyses, whereby personnel from Blue Iris Fish Farm collected and froze all dead fish until Dr. R. Smith's visits (fish were thawed ~12 hours prior to Dr. Smith's arrival).

In the second trial, ~300 YP (~3.5 mo old at study completion) in a Fry Control (FC) tank (housed inside a "hoop" house, feed trained/sorted just prior to 6-30-21) and ~550 YP in a Fry Vaccinated (FV) tank were enrolled in the study. Fish in FV (63.0°F water temperature; 11.4 mg/L DO; 128% oxygen saturation) were immersion vaccinated on 5-15-21 as described above, and then vaccinated a second time (i.e., "boosted") on 6-5-21 (74.1°F water temperature; 11.7 mg/L DO; 137% oxygen saturation). FV was comprised of males and females ungraded when they were hatched and feed trained in the tank. Data was collected as for Trial 1 (Table 1).

In summary of Field Trial 1 and after > three mo post-vaccination, more rapid average percent weight gain [= (final average weight on 8-25-21 minus average weight on 6-30-21)/average weight on 6-30-21)x 100] was evident in one tank of vaccinated fish compared to the non-vaccinated fish (i.e., average weight of control fish increased by 48.6% from 6-30-21 to 8-25-21 compared to 48.2% average weight gains in V2 and 96.0% weight gains in V3; based on 20 fish sample sizes/treatment; as calculated from Table 1). A similar trend in average percent increase in length was also observed, with 14.0% in control YP vs. 12.5% in V2 and 33.1% in V3. Condition factors (i.e., KTLs) were highest in the vaccinated fish, whereas fat content was lower in vaccinated fish (Table 1). Additionally, a slight decrease in mortality was observed in the vaccinated tanks at Trial 1 completion (2.0-4.3% in V2 and V3) when compared to unvaccinated fish (4.7% cumulative mortality); however, some mortality in vaccinated fish resulted from the reduced water flows.

Field Trial 2 results revealed larger increases in average length in vaccinated YP, whereby average YP length increased by 73.9% in unvaccinated YP fry from 6-30-21 to 8-25-21 vs. 78.5% over the same duration in vaccinated YP (as calculated from Table 1). However, average weight increased by 570% in unvaccinated YP fry from 6-30-21 to 8-25-21 vs. 525.9% over the same duration in vaccinated YP, though issues with heavy algal growth/varying oxygen levels occurred in the "hoop" house. Nevertheless, cumulative mortality in unvaccinated YP was more than double that of vaccinated YP (6.7% vs. 2.6%) at trial completion (Table 1). Bacteriological analyses on fish that died in unvaccinated and vaccinated groups in both field trials revealed a lack of any bacterial infections in vaccinated fish, whereas multiple bacteria (not *A. salmonicida*) were detected in all unvaccinated fish, including *A. veronii* (identified by the WI Veterinary Diagnostic Laboratory).

A third field trial was performed on another YP farm. However, inconsistencies in feeding, "within-trial" pond modifications, and mixing of fish between vaccinated and control fish precluded robust assessments; thus, this trial was excluded from any further analyses.

Objective 2. To assess the protective effectiveness of a new vaccination approach and preparation against *Aeromonas* infections in YP under controlled laboratory conditions (Dr. M. Shavalier/Dr. T. Loch). For the laboratory phase of this study (which required >$25K in additional funds provided by other sources from PD-Loch), YP (provided by Dr. Deng; average 36.6g, 14.8cm at arrival) were divided and maintained in two ~1500L recirculating tanks. Both tanks received 1-2 daily water changes (ranging from 25-50% each) to maintain water quality. Levels of ammonia, nitrite and nitrate were monitored in both tanks using a commercial kit (Aquarium

Pharmaceuticals, Inc.). Average water temperatures were 19.8-20.2°C and DO was >7.0 mg/L. Fish were fed a commercial diet (3.0mm extruded sinking; Skretting, Tooele, UT) at a reduced rate of ~0.5% BW/day given the unplanned larger fish sizes relative to available holding aquaria.

Fish were vaccinated or unvaccinated (but handled/treated identically) by immersion (n = 188 per treatment). Feed was withheld on the day of vaccination. Briefly, holding tanks were drained to 150 gallons each (the maximum volume recommended for vaccine use) and the vaccine or "mock" vaccine (comprised of Tryptic Soy Broth, 30g/L) was added directly to respective fish tanks. Fish behavior and DO were monitored (DO decreased by <10% in both tanks). After one-hour, residual vaccine/mock vaccine was removed via multiple water changes. Following vaccination, fish were held and monitored for 90 days as described above and prior to bacterial challenge studies.

Two separate lab experiments were performed (one at 3-months post-vaccination [low challenge dose, LD], ~$4x10^6$ colony forming units (cfu) of bacteria per fish; and one at 4-months [high challenge dose, HD], ~$2x10^7$ cfu per fish) to evaluate the protectiveness of this vaccine against *Aeromonas salmonicida* subsp. *salmonicida* (Asal) previously recovered from a YP mortality event. Due fish age/size, challenges occurred via intracoelomic injection, as deemed necessary via multiple pilot experiments (data not shown). The challenge bacteria (Asal) was revived from frozen cryostock onto Trypticase Soy agar, incubated/purity verified, and subcultured into tryptic soy broth for fish challenges. Inoculum was prepared by pelleting the bacterial cells via centrifugation, after which bacteria were resuspended in sterile saline to an optical density representing the target challenge dose, which was then intracoelomically injected into fish (200uL per fish), with negative control fish receiving the same volume of sterile saline.

Both the LD and HD experiments had identical treatment groups (Fig. 1) as follows:
- Vaccinated + Asal injection
- Vaccinated + saline injection
- Unvaccinated + Asal injection
- Unvaccinated + saline injection

Each treatment group consisted of four replicates with 12 (LD) or 11 (HD) fish per replicate tank (Fig. 1). Fish were held in static, 42L tanks receiving constant supplemental sponge filter aeration. Water temperature (average of 18.5-19.2°C across treatment groups) and quality were monitored daily and maintained at acceptable levels via twice daily water changes (20-50%/day). Mortalities were removed from the tanks daily, and necropsies immediately performed. Weight and length of the fish were recorded, as were any noticeable external or internal abnormalities. Bacterial cultures were taken from the kidney of each fish to screen for Asal. After ~3-weeks, surviving YP were humanely euthanized (via tricaine methane sulfonate, 250 mg/L, buffered with sodium bicarbonate, 500 mg/L), and necropsy performed/samples collected as detailed previously.

Throughout both experiments and as observed in farm trials, the vaccinated group had slightly higher growth rates than unvaccinated YP. From their arrival to the start of the first challenge study, the unvaccinated fish grew from an average of 36.3g to 58.1 (160% increase in weight), while the vaccinated fish grew from an average of 37.0g to 65.2g (176% increase in weight). From this point to the end of the second challenge study, the unvaccinated fish experienced relatively higher growth rates so that the ultimate weight gain differences between the two groups was not as pronounced (183% total gain for unvaccinated, 186% total gain for vaccinated); however, vaccinated gained more overall weight than unvaccinated fish. Significant differences (via one-way ANOVA analyses) in percent survival between vaccinated and unvaccinated fish were not observed (Fig. 2); in the low dose challenge, a one-way ANOVA (alpha = 0.05) resulted in a P-

value of 0.97, while in the high dose challenge for the same test resulted in a P-value of 0.15, both of which indicate no statistical difference in percent survival by treatment.

When examining Asal infection status in the LD experiment, Asal was detected in 50% of dead unvaccinated fish, and identically in 50% of dead vaccinated fish. Recovered Asal intensities were comparable between both vaccinated and unvaccinated fish. However, when examining surviving fish, Asal was exclusively detected in unvaccinated fish, albeit at a low prevalence.

In the HD experiment, Asal was recovered from 87% (13/15) of dead unvaccinated fish vs. 85% (17/20) of dead vaccinated fish, and was not recovered from any of the surviving fish (vaccinated or unvaccinated) at the experiment's end. Interestingly, Asal infection intensities were always high (i.e., colonies too numerous to count) in dead unvaccinated 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 of the vaccine. In both experiments, Asal was recovered from 76% of unvaccinated fish (at mainly high loads) and from 77% of vaccinated fish (with a higher percentage of fish having lower bacterial loads; Fig. 3).

Principal Accomplishments

Objective 1: To assess the protective effectiveness of a new vaccination approach and preparation against *Aeromonas* infections in farm raised YP.

- Two field trials on a Midwest YP farm led to the following conclusions by Dr. R. Smith, who oversaw these trials:
    - Vaccination has a huge advantage over feed additives when only a portion of the fish eat the medicated feed and avoids potential antibiotic resistance issues
    - Fish of the vaccinated tanks looked better
    - Fish of the vaccinated tanks were more aggressive
    - Fish of the vaccinated tanks were more active
    - Reduced mortality in vaccinated groups
    - Vaccinated fish grew better

- These same two trials and previous experience with YP vaccination led to the following observations/conclusions by the study Industry Liaison (Mr. W. West), who was integrally involved in field trial completion:
    - Vaccination after failure of salt treatments ceased all mortality in adult YP in a grow-out system
    - Reduction in stress seems to result in better health; however, vaccination seems to help even if stressful conditions (e.g., high rearing densities, poor water quality) are present.
    - Vaccination led to reduction in mortality, much higher percent reaching fillet weight, more robust fish that stay on feed
    - Ease of application- this immersion application can easily treat 100,000 fry per treatment in 20 to 30 minutes.
    - Time of life cycle for application, i.e., the grower has an option in almost any stage of the perch life when to vaccinate. However, to obtain longer immunity, vaccinating when at the fry stage (twice) may be more beneficial, especially depending upon long-term goals (e.g., food fish, broodstock)

- Desirable for shorter term immunity (grow-out), assuming ~10 month grow-out
- Desirable for long term immunity (broodstock)
- Reasonable cost- Cost of vaccination is about $300 per dose (one dose can treat 75 to 120 pounds of fish)

Objective 2: To assess the protective effectiveness of a new vaccination approach and preparation against *Aeromonas* infections in YP under controlled laboratory conditions.

- In these preliminary experiments, a new vaccination approach against *Aeromonas* infections in YP under controlled laboratory conditions was readily accomplished, with no observable negative effects on vaccinated fish. Thus, one important accomplishment was generation of preliminary data indicating this vaccination preparation and approach is safe and readily accomplished under controlled laboratory conditions.
- These preliminary lab-based experiments did not reveal a significant difference in survival between vaccinated and unvaccinated fish. However, a slight positive effect on growth of vaccinated fish compared to unvaccinated fish was observed, as were possible reductions in infection intensities in some vaccinated fish. Our study team suggests that had these experiments been carried out in smaller/younger and more size-uniform YP (as originally planned, but that became impossible due to funding/study timing), which would have allowed for a more natural Asal exposure route (e.g., immersion) and other parameters more in line with field conditions (i.e., full feeding rates, rearing densities, water temperatures), any protective/growth effects may have been more pronounced. Thus, in addition to providing preliminary data that suggest potential positive effects of the evaluated vaccination approach/preparation, this out-of-cycle study has provided rationale for further and larger scale experiments with a more natural exposure route and younger YP to be undertaken in the future.
- This out-of-cycle USDA-NIFA NCRAC-funded study led to the development of an intracelomic injection-based experimental challenge model for Asal in YP. Likewise, pilot data towards the development of an immersion-based Asal challenge model was generated herein. These are tools that will be useful for, among others, future YP vaccine evaluation studies.
- Under Objective 2, important accomplishments also included the hands-on research, training, and mentor/mentee opportunities to develop as scientific scholars and researchers for one undergraduate student, one Doctor of Veterinary Medicine student, and one post-doctoral researcher at Michigan State University. These opportunities provided by USDA-NIFA NCRAC were invaluable ways of training the next generation of scientists and veterinarians in boosting the health of farmed fishes now and into the future.

Impacts

- This USDA-NIFA NCRAC-funded study generated and reported data on an experimental approach for vaccinating YP against apparently emerging *Aeromonas* strains that are causing disease and losses in NCR YP farms. Although further research is needed, field trials suggest

there may be a boost in growth/vigor and a reduction in mortality in vaccinated YP, as well as potential reduction in risk of infection by *Aeromonas* spp.

- Vaccine trials under laboratory conditions also suggest this vaccine/vaccination approach may hold promise for positive health effects and protection against *Aeromonas* infections. Collectively, these preliminary field and laboratory-based experiments/trials provide rationale for future studies to build upon these results and evaluate the positive effects of this experimental vaccine/approach in younger/smaller uniformly-sized YP in both lab and field settings.
- Despite the pandemic, the workshop and farm tour in Black Creek, Wisconsin at the Black Creek Town Hall and Blue Iris Fish Farm provided a wealth of education, training, and learning opportunities for producers, veterinarians, and Extension/outreach Specialists, particularly related to fish health and the potential benefits and opportunities surrounding the use of vaccines in farmed Yellow Perch production.
- This project provided substantial laboratory and research experiences for an undergraduate student majoring in Fisheries and Wildlife at Michigan State University (student has since graduated and applied to graduate school) during the YP laboratory experiment trails. Although not paid by this project, a Doctor of Veterinary Medicine student also gained substantial experience in aquatic animal husbandry and aquatic animal health research through his participation in this project. Likewise, the post-doctoral researcher and veterinarian (Dr. Megan Shavalier) that conducted the majority of research under Objective 2 continued to gain substantial mentorship and aquaculture research experience resulting from this NCRAC-funded study.

Recommended Follow-Up Activities

- Our team strongly recommends that future studies utilize younger/smaller and more uniformly-sized YP in both field and laboratory experiments. In the field, we posit that: a) pond-side tanks will provide a robust opportunity to subject vaccinated and similarly maintained unvaccinated YP to NCR-typical field conditions while still allowing for optimal monitoring and assessments of the fish therein, and thus recommend their use; and b) larger numbers of fish from each replicate be analyzed for VHAs/bacterial infections, especially if fish size variation becomes an issue. Likewise, we recommend that laboratory experiments continue to have ample tank replication (at least 4-6 tanks/treatment), but such experiments will benefit from: a) using younger/smaller fish that allow for more natural bacterial exposure routes (e.g., immersion); b) utilizing water temperatures/exchanges and feeding rates that are more routine in NCR-YP farms; and c) possibly evaluating the ability of this experimental vaccine/approach to control disease in already Asal-infected fish (based on field observations from Dr. R. Smith/W. West).
- Our team likewise recommends that the multipronged approach of utilizing both lab- and field-based experiments and following all protocols associated with the USDA-APHIS Center for Veterinary Biologics bacterin use be continued in any and all future related studies.

Papers/Presentations Presented (*, signifies presenter)

West WM, Smith R, Smith MA, Shavalier M, Deng D-F, Keleher B, Loch TP*. Evaluating novel methods for preventing *Aeromonas*-associated losses in Yellow Perch (*Perca flavescens*) using laboratory and field-based vaccination trial. Scheduled to be presented at the USDA-NIFA NCRAC 2023 Annual Program Meeting, Eau Claire Wisconsin, February 2023.

West WM, Smith R, Smith MA, Shavalier M, Deng D-F, Keleher B, Loch TP*. Evaluating novel methods for preventing *Aeromonas*-associated losses in Yellow Perch (*Perca flavescens*) using laboratory and field-based vaccination trial. Presented at the Virtual USDA-NIFA NCRAC 2022 Annual Program Meeting, March 2022.

Smith MA* and West WM. Welcome and Housekeeping. Vaccinating Yellow Perch Against Aero*monas salmonicida*: Lab, Farm, and Field Trials. presented during the USDA-NIFA NCRAC funded Workshop and Farm Tour entitled "Vaccinating Yellow Perch Against *Aeromonas salmonicida*: Lab, Farm, and Field Trials," Sept. 18, 2021, Black Creek, WI.

West WM, Smith R, Smith MA, Shavalier MA, Deng D-F, Keleher W, Loch TP*. Vaccinating Yellow Perch Against *Aeromonas salmonicida*: An Introduction to the Project & Fish Health Overview, presented during the USDA-NIFA NCRAC funded Workshop and Farm Tour entitled "Vaccinating Yellow Perch Against *Aeromonas salmonicida*: Lab, Farm, and Field Trials," Sept. 18, 2021, Black Creek, WI.

Smith R*. Vaccinating Yellow Perch Against *Aeromonas salmonicida*: History of the Aeromonas salmonicida Vaccination Project and how it Came About, presented during the USDA-NIFA NCRAC funded Workshop and Farm Tour entitled "Vaccinating Yellow Perch Against *Aeromonas salmonicida*: Lab, Farm, and Field Trials," Sept. 18, 2021, Black Creek, WI.

Smith R*. Vaccinating Yellow Perch Against *Aeromonas salmonicida*: Recent Data Derived from Trials Involving Vaccinated Yellow Perch, presented during the USDA-NIFA NCRAC funded Workshop and Farm Tour entitled "Vaccinating Yellow Perch Against *Aeromonas salmonicida*: Lab, Farm, and Field Trials," Sept. 18, 2021, Black Creek, WI.

Keleher W*. Vaccinating Yellow Perch Against *Aeromonas salmonicida*: The Use of Bacterins and Vaccines in Aquaculture, presented during the USDA-NIFA NCRAC funded Workshop and Farm Tour entitled "Vaccinating Yellow Perch Against *Aeromonas salmonicida*: Lab, Farm, and Field Trials," Sept. 18, 2021, Black Creek, WI.

West WM*. Vaccinating Yellow Perch Against *Aeromonas salmonicida*: A Producers Perspective on Yellow Perch Vaccination, presented during the USDA-NIFA NCRAC funded Workshop and Farm Tour entitled "Vaccinating Yellow Perch Against *Aeromonas salmonicida*: Lab, Farm, and Field Trials," Sept. 18, 2021, Black Creek, WI

Johnston AE, Knupp CK, Harrison CE, Shavalier MA, Lennox S, Van Deuren M, Loch TP*. MSU-AAHL Research Updates, Invited virtual presentation at the Great Lakes Fishery Commission - Great Lakes Fish Health Committee Meeting, Aug. 2021.

Tank (date assessments made)	Range in length (mm) of fish (average)	Range in weight (g) of fish (average)	KTL.	Modified KTL.	"VHA" score	Gills "VHA" score	Fat "VHA" score	Total percent mortality (out of 300 fish)
Experimental Trial 1 (Yellow Perch, immersion vaccinated 1x, ~12 months-old at study completion)								
Starter (5-15-21)	94-192 (140.8)	9.3-81.8 (32.2)	1.15	0.229	23.5	4.2	3.1	-
Control 1 (6-30-21)	138-227 (169.1)	25.5-146.7 (61.5)	1.27	0.364	18.3	3.3	4.6	1.6
Vaccinated 2 (6-30-21)	138-214 (175.6)	27.05-125.51 (70.3)	1.30	0.401	21.8	4.2	4.6	2.0
Vaccinated 3 (6-30-21)	139-220 (151.0)	26.92-106.6 (55.3)	1.61	0.366	25.1	4.6	4.5	0.7
Control 1 (8-25-21)	154-234 (192.9)	33.1-177.1 (91.4)	1.27	0.474	26.3	3.4	4.4	4.7
Vaccinated 2 (8-25-21)	162-264 (197.6)	60.9-225.2 (104.2)	1.35	0.528	28.1	3.8	4.1	4.3
Vaccinated 3 (8-25-21)	162-249 (201.0)	47.3-224.9 (108.4)	1.33	0.539	27.8	3.7	4.2	2.0
Experimental Trial 2 (Yellow Perch, immersion vaccinated 2x, ~3.5 months-old at study completion)								
Fry Control (6-30-21)	51-71 (58.9)	1.11-3.3 (2.0)	0.99	0.0345	23.5	3.4	3.5	-
Fry Vaccinated (6-30-21)	50-71 (60.9)	1.2-4.0 (2.7)	1.20	0.0443	22.9	3.2	3.3	-
Fry Control (8-25-21)	72-124 (102.4)	3.5-27.2 (13.4)	1.25	0.131	26.6	3.2	3.8	6.7
Fry Vaccinated (8-25-21)	58-125 (108.7)	2.1-28.7 (16.9)	1.31	0.155	27.1	3.1	3.7	2.6

Table 1. Data resulting from two experimental vaccination field trials overseen by Dr. R. Smith in yellow perch (*Perca flavescens*; YP) conducted at Blue Iris Fish Farm, LLC, in 2021. In the first trial, fish in tanks Control 1, Vaccinated 2 (V2) and Vaccinated 3 (V3; n=300 fish/tank) were approximately 12-months-old upon completion of the study. Fish in V2 and V3 (60.6° F water temperature; 8.7 mg./L dissolved oxygen; 89% oxygen saturation) were immersion vaccinated on 5-12-21 using the 30-minute immersion vaccination method without water flow per Dr. R. Smith's vaccination protocol (bivalent autogenous bacterin manufactured by Kennebec River Biosciences, Richmond ME). In the second trial, YP (~3.5 months old at study completion) in the Fry Control (FC) tank contained ~300 fish (housed inside a "hoop" house, feed trained/sorted just prior to 6-30-21) and YP in the Fry Vaccinated (FV) tank containing ~550 fish were enrolled in the study. Fish in FV (63.0°F water temperature; 11.40 mg/L dissolved oxygen; 128% oxygen saturation) were immersion vaccinated on 5-15-21 using the 30-minute immersion vaccination method without water flow as described above and then vaccinated a second time (i.e., "boosted") on 6-5-21 (74.1°F water temperature; 11.7 mg/L dissolved oxygen; 137% oxygen saturation) in the same fashion. Note, FV was comprised of males and females ungraded when they were hatched and feed trained in the tank. In both field trials, "veterinary health assessments" (VHAs) were performed on the noted dates per established protocols, and cumulative percent mortality tracked. KTL, Condition Factor.

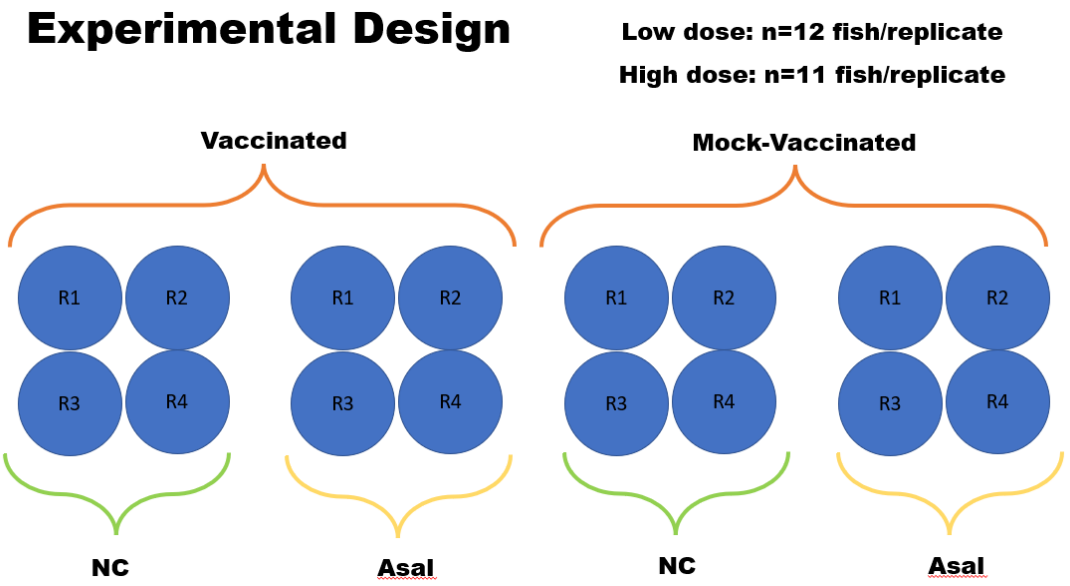

Figure 1. Experimental design for the laboratory-based yellow perch (*Perca flavescens*) vaccination experiments, which occurred in individual recirculating 42L aquaria. Feed-trained yellow perch (62g average weight, n=11-12 per tank; n=4 replicate tanks per treatment) received one of four treatments: immersion-vaccination ("vaccinated") or mock-vaccination ("mock-vaccinated) that were then intracoelomically-injected with *Aeromonas salmonicida* subsp. *salmonicida* ("Asal") or sterile saline (NC).

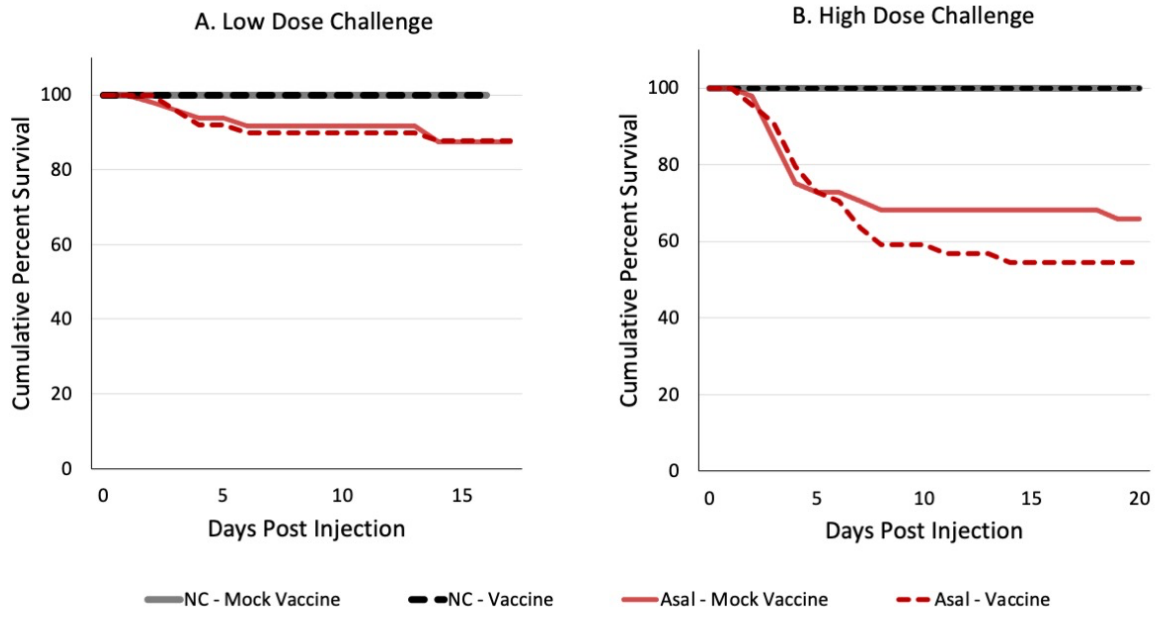

Figure 2. Average cumulative percent survival in yellow perch (*Perca flavescens*) that were either mock-vaccinated (mock-vaccine) or vaccinated (vaccine), maintained under quarantine conditions for three months (water temperature ~19-21°C), and then intracoelomically injected with either *Aeromonas salmonicida* subsp. *salmonicida* (Asal) or sterile saline (NC). Plot A represents the low dose study (~$4x10^6$ colony forming units of Asal per fish). Plot B represents the high dose study (~$2x10^7$ colony forming units of Asal per fish).