Executive Summary

Improving Fish Health in the NCR by Integrating Extension with the Development of Alternative Disease Prevention Methods
Per USDA, >28 million US-farmed trout died due to disease in 2019 vs 47 million trout that were sold that same year. Relatedly, producers in the North Central Region (NCR) relayed needs for available fish health expertise to better prevent and control disease. To help NCR producers solve these challenges, our team first leveraged USDA-NIFA NCRAC funds from this project into long-term investment by the College of Veterinary Medicine at Michigan State University to create a regional aquaculture outreach veterinary position that has continued beyond this project into the long-term. This regional aquaculture veterinarian continues to be a highly accessible and beneficial resource for NCR producers. Simultaneously, we paired our industry survey efforts with regionally widespread clinical and flavobacterial recovery methods to create experimental vaccines against bacterial coldwater disease (BCWD), a top disease impediment to trout and salmon production in the USA. Promisingly and despite being exposed to high concentrations of the microbe causing BCWD, not a single vaccinated rainbow trout died from BCWD in a series of multiple month laboratory experiments (in contrast to mock-vaccinated fish that experienced severe disease and mortality). With these tools, we anticipate equipping producers with a means to effectively prevent BCWD in the short/medium term. Per industry feedback, this project has substantially benefited NCR farmed fish health.

Termination Report

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

Total Funds Committed: $291,591 (Year 1), $309,796 (Year 2)
Initial Project Schedule: September 1, 2021-August 31,2022 [Extended to August 31, 2025]
Current Project Year: September 1, 2024-August 31, 2025
Participants: Thomas Loch, Michigan State University; Nisha Shrestha, Michigan State University; Nicholas Phelps, University of Minnesota; Myron Kebus, Michigan State University; Matthew A. Smith, YSI/Xylem Analytics
Extension Liaison: Myron Kebus, Michigan State University
Industry Liaison: Dan Vogler, Harrietta Hills Trout Farm

Relevance: According to the USDA National Agriculture Statistics Service (2020) >28 million US-farmed trout were lost due to bacterial and/or parasitic diseases in 2019 compared to 47.2 million trout that were sold that same year, illustrating the substantial disease-induced losses facing US trout producers. In the same vein, producers in the North Central Region continue to relay a need for available fish health expertise to more effectively prevent and control disease-associated losses.

Response: To help NCR producers solve these challenges, our team first leveraged USDA-NIFA NCRAC funds from this project into long-term investment by the College of Veterinary Medicine at Michigan State University to create a regional aquaculture outreach veterinary

position that has continued beyond this project into the long-term. This regional aquaculture veterinarian continues to be a highly accessible and beneficial resource for NCR producers. Simultaneously, we paired our industry survey efforts with regionally widespread clinical and flavobacterial recovery methods to create experimental vaccines against bacterial coldwater disease (BCWD), a top disease impediment to trout and salmon production in the USA. Promisingly and despite being exposed to high concentrations of the microbe causing BCWD, not a single vaccinated rainbow trout died from BCWD in a series of multiple month laboratory experiments (in contrast to mock-vaccinated fish that experienced severe disease and mortality). With these tools, we anticipate equipping producers with a means to effectively prevent BCWD in the short/medium term. Per industry feedback, this project has substantially benefited NCR farmed fish health.

Technical Summary:

Obj. 1: Develop practical and usable fish health applications for producers and fish health professionals

During this USDA-NIFA NCRAC funded project, we completed a qualitative semi-structured interview on fish health with North Central Region (NCR) aquaculture producers (representing 24 farms in six NCR states; MI, OH, IN, WI, MN, MO). Although 65% of interviewed farmers participate in regulatory fish health inspections and 44% have a relationship with a fish veterinarian, only 26% have written biosecurity plans, 11% vaccinate, 47% have disinfection protocols, and 4% use Veterinary Feed Directives (VFDs) for antibiotics. Health inspections are a legal requirement for interstate movement of some fish species, and appears to be a leading reason NCR farmers seek veterinary expertise. Many fish farmers under-utilize veterinary services, presenting an opportunity for improved veterinary engagement focused on production and health of farmed fish. Our results provide insight into the needs and perspectives of NCR fish farmers, and full details are published (Kebus et al. 2025).

We learned that use of best management practices (BMPs) to support NCR fish health is inconsistent. To empower producers to implement effective BMPs, we developed a first of its kind online interactive tool for aquaculture producers using the RShiny platform. A producer can interact with the online tool by answering questions about their farm practices (species, system type, activities, planning, training, monitoring, etc.) and receive a report that highlights opportunities for improvement, summarizes areas of importance, and provides additional resources. Beta testing is underway (expected public dissemination in 2026).

With a goal of improving fish health in the NCR, our team visited fish farms and provided consultations for producers (e.g., fish health management, examination of fish, fish health options and resources in the region). Dr. Kebus did 315 virtual and in-person consults with producers and veterinarians. 183 consults (10 NCR states) focused specifically on active fish health issues, and industry relayed these consults improved a range of NCR fish health and production issues.

Our team gave **64** presentations at local, regional, national, and international professional meetings; led 7 one-day workshops, 4 half-day workshops, and 3 five-day workshops, and gave 26 presentations for veterinarians and veterinary students (*note, our goal in the proposal*

was 2 one-day workshops). Notably, NCRAC funds were further leveraged into a long-term investment by the College of Veterinary Medicine (Michigan State University) to indefinitely support an aquaculture outreach veterinary position that continues to positively impact fish health in the NCR.

Obj. 2: Determine the predominating flavobacterial variants in the NCR
With the goal of improving bacterial coldwater disease (BCWD) prevention and control, our team isolated and characterized *Flavobacterium psychrophilum* (*Fp*) variants from the NCR. Moribund salmonids (n=267) were analyzed from 12 facilities in 9 NCR states (MN, MI, OH, WI, IN, IL, IA, MO, SD). Examined fish showed varying disease signs consistent with BCWD, and >340 yellow-pigmented bacteria were recovered, with 194 molecularly confirmed as *Fp*.

Genotyping of representative *Fp* isolates (n=109) via multi locus sequence (MLST) revealed 13 variants (i.e., sequence types, STs), 7 of which were novel (Fig 1). Other recovered *Fp* STs have been detected elsewhere in the USA or only previously reported abroad (Fig 1). Most NCR isolates belonged to a common and widespread Clonal Complex (CC), CC-ST10; however, there was notable *Fp* ST diversity in the region between and also within facilities.

A subset of the *Fp* isolates (n=149) were molecularly analyzed to determine serogroup (SG). Notably, all five known *Fp* SGs were detected within the NCR, with marked variation across states and facilities. Isolates recovered from some NCR states belonged to a single SG, but in other states, multiple SGs were detected in a single facility. Some facilities are battling *Fp* variants similar to other regions of the USA, whereas some facilities face BCWD losses caused by unique regional or farm variants.

Obj. 3: Evaluate the effectiveness of experimental vaccines in preventing losses caused by regionally predominating flavobacteria
Four batches of rainbow trout (RBT; *Oncorhynchus mykiss*) eggs (commercial vendor) were hatched and reared under quarantine in PI-Loch's lab. All eggs were disinfected with iodine upon receipt, and maintained in flow-thru egg stacks until hatching. Alevins were transferred to flow-thru tanks and fed by hand or belt feeder (up to ~2% body weight/day). All four groups were assessed for *Fp* infection (no detections).

Fp variants (n=11) were selected based on recovery location (6 NCR states), genotype (8 STs), and molecular SGs (4) and used for growth kinetics experiments, with the goal of determining when all variants reach a logarithmic growth phase. Although variation occurred, a common time range was identified.

Prior to vaccine development, the disease/mortality-causing potential of ten diverse NCR *Fp* variants was assessed in ~3-6 month RBT. Fish (n=10/tank, 3x/variant) were adipose fin-clipped, exposed to a variant via immersion, and monitored in aerated, flow-through glass aquaria. All terminally moribund and dead fish were analyzed, and at 27 days post exposure, surviving fish were euthanized and likewise analyzed. Recovered yellow-pigmented bacteria were phenotypically characterized, and representative isolates molecularly analyzed to confirm *Fp.* Cumulative Percent Mortality (CPM) and *Fp* infection status were recorded for each

variant.

Disease signs, mortality, and *Fp* infection were not detected in fish exposed to a control or to 3 of the *Fp* variants; however, fish exposed to the other 7 variants developed signs of BCWD, with mean CPM ranging from 3.3 ± 1.6% to 63.3 ± 3.7%. *Fp* was recovered from all dead fish, and from a subset of surviving fish (mean infection prevalence 9.5 – 23.8%). Based on these results, 3 virulent *Fp* variants (representing 3 SGs, 2 STs, and 3 NCR states) were selected for *in vivo* vaccine experiments.

Of note, the health-screening of the third group of fish (intended for vaccination studies) revealed clinical nephrocalcinosis (NC); elevated CO_2 in source well water was identified as a contributing factor. After remediation, new fish were hatched and raised for vaccination. While doing so, our team took the opportunity to study the potential interactions between NC, *Fp*, and vaccination through a series of originally unanticipated experiments. NC-afflicted RBT were exposed to two of the above identified NCR *Fp* variants, and CPM/disease/infection status determined as above. *Fp* infection, BCWD signs, and mortality (CPM from 34.8 ± 0.057% to 46.7 ± 0.1%) suggested NC status altered *Fp* virulence in RBT.

Next, a "pilot" vaccine experiment was undertaken with the NC-RBT. Two *Fp* variants were used to create two "killed" vaccines (i.e., bacterins). NC-RBT were immunized (via intracelomic injection) with a vaccine or mock-vaccine (sterile saline). After ~309 degree-days (dd), fish were booster-immunized via a second injection as before. At ~620 dd total, fish were adipose fin clipped and immersed in the corresponding live *Fp* variant. A group of mock vaccinated fish were exposed to each variant. Mortality occurred only in mock-vaccinated fish and *Fp* was not detected in vaccinated fish (in contrast to mean infection prevalence of 20 – 20.6% in mock-vaccinated fish). Marked differences in external lesion development were also obvious between treatments; active ulceration, swelling, and hemorrhage were evident at the fin clip site in mock-vaccinated fish (Fig 2a, c) compared to clip site healing in vaccinated fish (Fig 2b, d).

Further monovalent vaccine experiments began following histopathological screening of the 4th group of RBT to ensure freedom from clinical NC. Killed vaccines were produced from three geno-/serotypically diverse NCR *Fp* variants (Iso 4, 5, & 11). Three groups of fish (n=100 each) were intracelomically (ic) injected with 1 of 3 vaccines; control fish (n=150) were mock-vaccinated with sterile saline. After 300 dd, fish were booster- or mock-vaccinated using the same procedure as at time 0. After ~600 dd total and following adipose fin clip, vaccinated and mock-vaccinated fish were immersed in a respective *Fp* suspension, whereas negative control fish were immersed in tank water containing an equivalent volume of sterile saline. Fish (10 per tank, 3x by treatment), were observed/sampled/analyzed as above, and monitored for 27 days, at which time survivors were euthanized and sampled. CPM, relative percent survival (RPS), and *Fp* infection status were recorded for each treatment.

No mortality occurred in any *Fp*-exposed vaccinated fish, nor in any control fish (Fig 3). In contrast, mean CPM in mock-vaccinated, *Fp* exposed fish was 46.7% (iso-5), 63.3% (iso-4), and 80% (iso-11; Fig 3). Thus, RPS was 100% for fish vaccinated with each preparation. *Fp* was recovered from all mock-vaccinated dead fish, but not from any negative control fish.

Promisingly, *Fp* infection was detected in the surviving/euthanized, mock-vaccinated, *Fp* exposed fish at high prevalence (50% - 72.7%), but only in one vaccinated group and at low prevalence (1/30 fish; 3.3%).

To create a trivalent vaccine, the same three *Fp* variants were prepared as above and mixed in equal ratios. Vaccinated fish (220) were ic injected with the vaccine, whereas mock-vaccinated control fish (220) received sterile saline only. After 306 dd, fish received a second injection as before. After ~606 dd total, vaccinated and mock-vaccinated fish were immersed in an *Fp* suspension of each of the three *Fp* variants individually, whereas negative control fish were immersed in tank water containing an equivalent volume of sterile saline. Fish were observed/analyzed as above, monitored for 27 days, and survivors euthanized. CPM, RPS, and *Fp* infection status were recorded.

No mortality occurred in vaccinated *Fp* exposed fish, nor in any negative control fish (Fig 4). In contrast, mean CPM in mock-vaccinated, *Fp* exposed fish was 13.3% (iso-5), 73.3% (iso-4), and 93.3% (iso-11; Fig 4). Thus, RPS was 100% for fish vaccinated with the trivalent vaccine. *Fp* was recovered from all mock-vaccinated dead fish. Notably, while *Fp* infections were detected in the euthanized, mock-vaccinated *Fp* exposed fish (prevalence 65.4% - 100%), *Fp* was not detected in any vaccinated fish.

Results: Three objectives are being pursued in this project:

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.
   - Published papers on fish health issues in the NCR
     - https://avmajournals.avma.org/view/journals/javma/263/8/javma.25.01.0037.xml
     - https://avmajournals.avma.org/view/journals/javma/263/11/javma.25.06.0371.xml
   - Conducted fish health workshops for fish farmers
   - Conducted fish health workshops for aquaculture veterinarians
   - Conducted customized fish health workshops for fish farms that requested staff training
   - Provided on-farm fish health consults to address critical fish health problems
   - Provided phone consults for critical fish health problems
   - Conducted training of veterinary students on aquaculture veterinary medicine
   - Developed a MSU CVM website on Aquaculture Veterinary Outreach for Fish Farmers and Veterinarians https://cvm.msu.edu/research/faculty-research/infectious-disease/aquaculture-outreach
   - Developed innovative online and interactive tool that allows farmers to evaluate site-specific best management practices for fish health and identify resources to reduce risk.

2. Determine, for the first time, the predominating flavobacteria variants driving economic losses in the NCR.
   - Collected and analyzed more than 250 moribund salmonids from 12 facilities located in 9 (MN, MI, OH, WI, IN, IL, IA, MO, SD) of the 12 states within the NCR.
   - Recovered >340 yellow-pigmented bacteria from the sampled fish, including 194 that were molecularly confirmed as *F. psychrophilum*.
   - Created a preserved cryobank of the recovered flavobacteria (being maintained in PI-

Loch's laboratory)

- Generated novel MLST-derived genotyping data for > 100 *F. psychrophilum* isolates that originated from nine different facilities in seven of the sampled nine NCR states.
- Generated novel molecular serogrouping data for > 140 *F. psychrophilum* isolates that originated from nine different facilities in seven of the sampled nine NCR states.
- Uncovered important epidemiological trends for *F. psychrophilum* within the NCR, including identifying widespread serogroups and genotypes, as well as those that were unique to some states and/or facilities.
- Generated a data-derived road-map for potentially "higher priority" *F. psychrophilum* variants within the NCR.

Dr. Loch with Students

3. Evaluate the effectiveness of experimental vaccines in preventing losses caused by regionally predominating flavobacteria under laboratory and field conditions.
   - Characterized the disease and mortality causing potential of ten NCR *F. psychrophilum* variants in rainbow trout via controlled *in vivo* laboratory experiments.
   - Developed and tested the protective efficacy of three newly developed experimental BCWD vaccine preparations (monovalent bacterins), derived from three distinct *F. psychrophilum* variants (representing different genotypes and serogroups), in rainbow trout. Notably, no mortality occurred in any *F. psychrophilum* exposed vaccinated fish throughout the experiments, in stark contrast to mock-vaccinated *F. psychrophilum* exposed fish, which experienced clinical BCWD and high mortality. Encouragingly, all three vaccines yielded 100% relative percent survival, and also substantially reduced the risk of *F. psychrophilum* infection in fish that were analyzed 27 days after exposure to the bacterium.
   - Developed and tested the protective efficacy of an experimental BCWD trivalent experimental vaccine in rainbow trout. Once again, no mortality occurred in any *F.*

psychrophilum exposed vaccinated fish throughout the experiments, in contrast to mock-vaccinated *F. psychrophilum* exposed fish, which experienced clinical BCWD and high mortality. This vaccine also yielded 100% relative percent survival, and *F. psychrophilum* infections were not detected in any vaccinated fish despite being prevalent in mock-vaccinated fish that were sampled at the same time.

- Generated pivotal protective efficacy data for four different vaccines experimental BCWD vaccines, which have now set the stage for additional testing and optimization. Notably, two of these vaccine preparations also provided protection against BCWD despite vaccinated fish suffering from a non-infectious co-morbidity (nephrocalcinosis).

Nisha Shrestha, the Michigan State University PhD student (Major Professor, Dr. T. Loch) who led the *Flavobacterium psychrophilum* characterization, virulence, and vaccine experiments in this USDA-NIFA NCRAC funded project, observes the experimental rainbow trout (*Oncorhynchus mykiss*) that she hatched from eggs.

Outcomes/Impacts

- This USDA-NIFA NCRAC funded study had sizeable impacts that enhanced fish health (a priority need voiced by producers in USDA-NIFA's North Central Region) and improved productivity in the aquaculture industry. To our knowledge and prior to this project, a Regional Aquaculture Center (RAC) had not invested heavily in supporting a region-wide Outreach Veterinarian with strong expertise in fish health and aquaculture and with a primary mission of being available and accessible to help producers solve and prevent recurrent and emerging fish health needs, including by working with already existing veterinary and fish health expertise in various NCR states. Importantly, the two-

year investment by NCRAC to do so in this project paid substantial dividends and was leveraged into numerous additional fish health resources that are and will continue to benefit fish producers into the future.

- This USDA-NIFA NCRAC funded project enabled the creation of a first-of-its kind online interactive tool for aquaculture producers to evaluate site-specific best management practices for fish health. The tool will provide long-term impact beyond the life of the project, serving as a resource for fish farmers, extension specialists, and fish health professionals in the region. In addition, the underlying framework on which the tool is built can be leveraged to create similar interactive experiences for farmers across regions and production sectors.
- This USDA-NIFA NCRAC funded study had a 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 being shared during workshops and presentations (which far surpassed the number and scope we had originally proposed), this USDA-NIFA NCRAC funded research 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.
- This USDA-NIFA NCRAC project provided funds that were leveraged to create a Regional Aquaculture Veterinary Outreach position at MSU. Dr. Kebus has provided regional leadership and coordination of fish health efforts for fish farmers based on their requests for help. See MSU CVM website on Aquaculture Veterinary Outreach for Fish Farmers and Veterinarians https://cvm.msu.edu/research/faculty-research/infectious-disease/aquaculture-outreach
- This USDA-NIFA NCRAC funded study yielded new knowledge resulting from the multi-year regional surveillance for *Flavobacterium psychrophilum* (cause of bacterial coldwater disease) that, to our knowledge, is the largest ever of its kind in the USA. Indeed, the extensive library of flavobacterial isolates that have been recovered, identified, typed, and cryopreserved in this study is serving as an invaluable resource for enhancing fish health in the NCR (i.e., by guiding and being used in vaccine preparations against a top disease facing trout and salmon producers). Likewise, new knowledge generated in this USDA-NIFA NCRAC funded project has highlighted that some facilities within the NCR are battling *F. psychrophilum* variants that are similar or identical to those from other regions of the USA, whereas others face losses caused by different variants that are unique to the region, or in some cases, unique to the state/farm where they were recovered. Notably, this important information will allow fish health specialists and aquaculture stakeholders to boost fish health and productivity in the NCR, including through the development of vaccines that account for this variation (see below).
- This USDA-NIFA NCRAC funded project directly supported the development and laboratory testing of four experimental killed vaccines (i.e., bacterins) against bacterial coldwater disease (BCWD), a top disease impediment to trout and salmon production in

the USA. Promisingly and despite being experimentally exposed to high concentrations (> 100,000,000 bacteria/ml) of the bacterium causing this disease, not a single vaccinated rainbow trout died from BCWD in a series of three independent, multiple month laboratory experiments (in contrast to mock-vaccinated fish that were identically exposed and experienced severe disease and mortality). With this baseline of exceptionally promising data, we anticipate being able to equip trout and salmon producers with tools that effectively prevent BCWD in the short to medium term.

Targeted Audience

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.

Recommended Follow-Up Activities

- Additional studies to capitalize upon the success of the experimental BCWD vaccines developed herein, particularly in the vein of assessing multiple immunization routes, administration, and associated preparation procedures
- Equip NCR producers with additional information, knowledge, and access related to vaccination options for farmed fish
- Long-term sustainability of the interactive Best Management Practices tool developed herein
- Continued NCR producer access to veterinarians that are well-versed in aquatic animal health and fish farming

Partnerships

Mr. Marvin Emerson, Crystal Lake Fisheries, Missouri
Dr. Steven Summerfelt, Superior Fresh, LLC, Maryland

Figures and Charts-Improving Fish Health

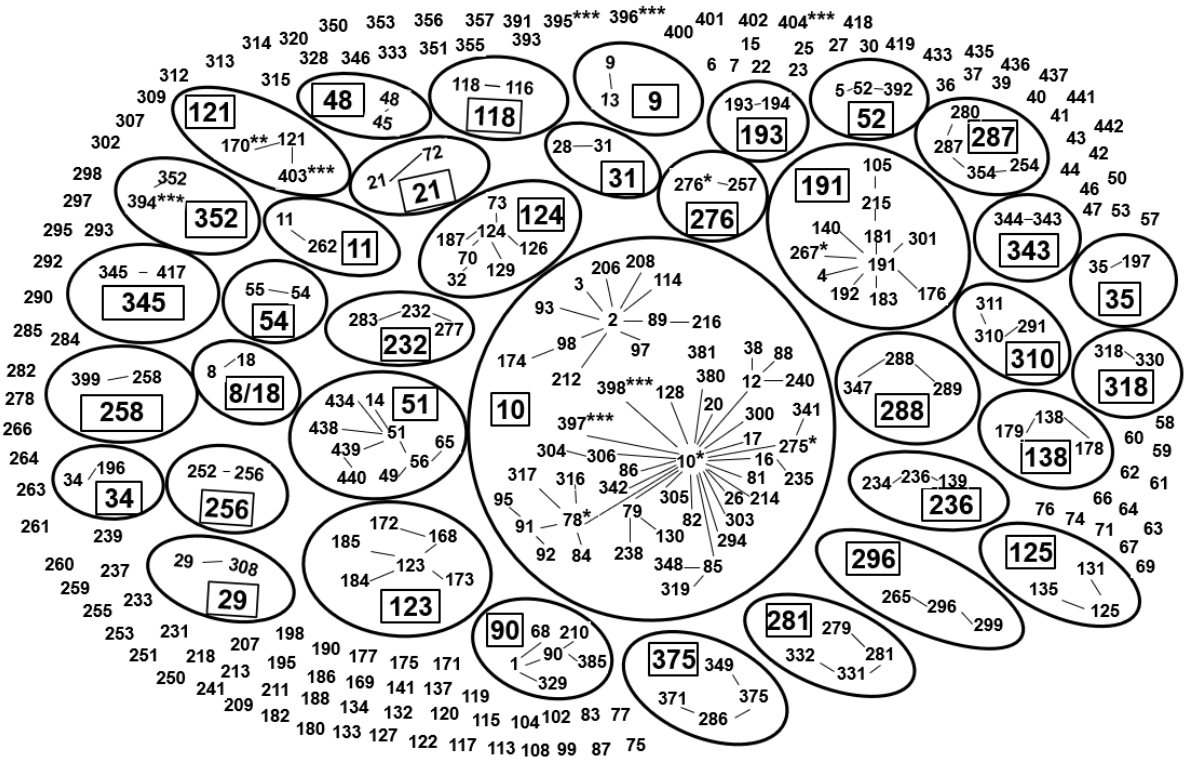

Figure 1. eBURST diagram depicting inferred genetic relatedness (as determined by multilocus sequence typing; MLST) of *Flavobacterium psychrophilum* isolates typed in the present study (n=109) compared to previously typed isolates in the pubMLST database (n=>1500). Asterisks (*) denote sequence types (ST) that include isolates recovered in this study but were previously described and reported in the USA. Double asterisks (**) denote STs recovered in this study for the first time in the USA, but previously reported in Europe. Triple asterisks (**) indicate newly identified STs that have not been reported previously. Clonal complexes (CC) are defined as groups of sequence types (ST) connected by single locus variant (SLV) links and are encircled. Each CC is named after the most likely founding ST (i.e., ST with the greatest number of SLVs). If multiple STs have the same number of SLVs within a CC, the CC is named after the ST with the largest number of isolates. If both STs have the same number of isolates, the CC is named after the earliest identified ST. The founding ST of each CC appears within a box.

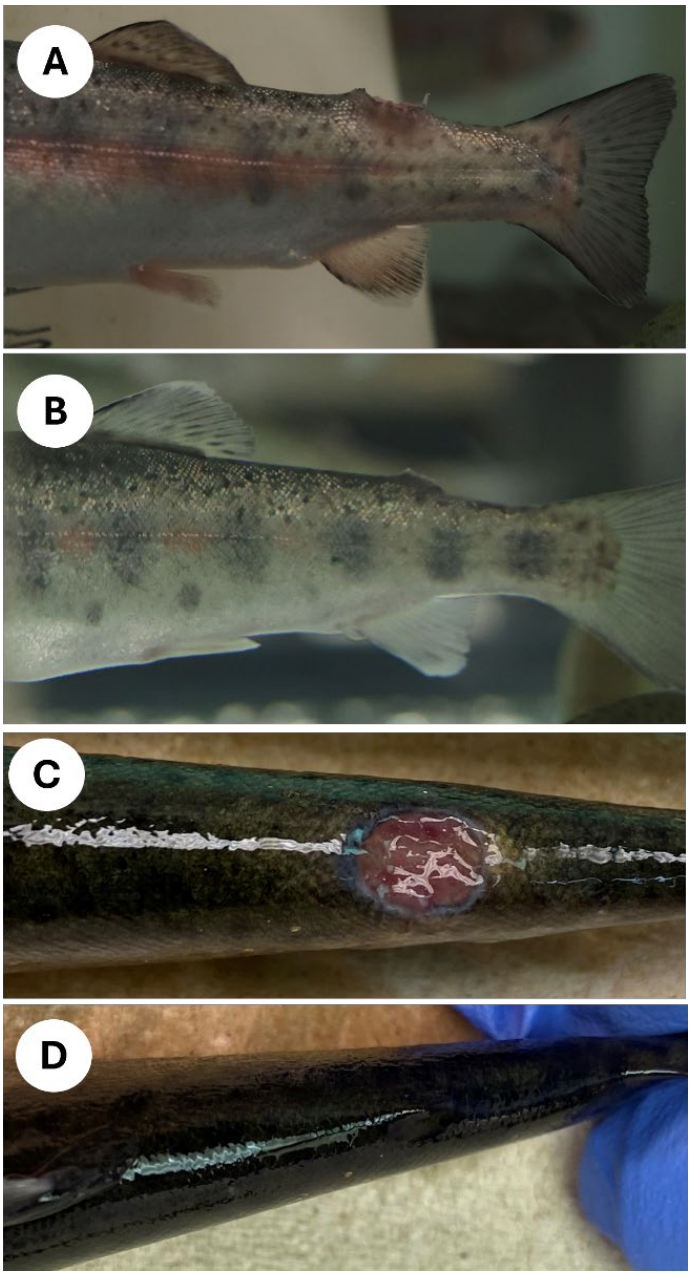

Figure 2. Images of live (A, B; 10 days post-bacterial exposure) and freshly euthanized (C, D; 26 days post-bacterial exposure) rainbow trout that were mock-vaccinated (A, C) or vaccinated (B, D) with one of our team's experimental killed *F. psychrophilum* vaccine preparations and subsequently exposed to live *F. psychrophilum* via bath exposure after having been adipose fin-clipped. Note the active ulceration, swelling, and hemorrhage at the fin clip site in mock-vaccinated fish (A, C) compared to the healing (B) and nearly completely healed (D) clip sites in vaccinated fish. Notably, *F. psychrophilum* was recovered from the internal organs and external lesions of some mock-vaccinated fish, but not from any fish that were vaccinated with these new preparations.

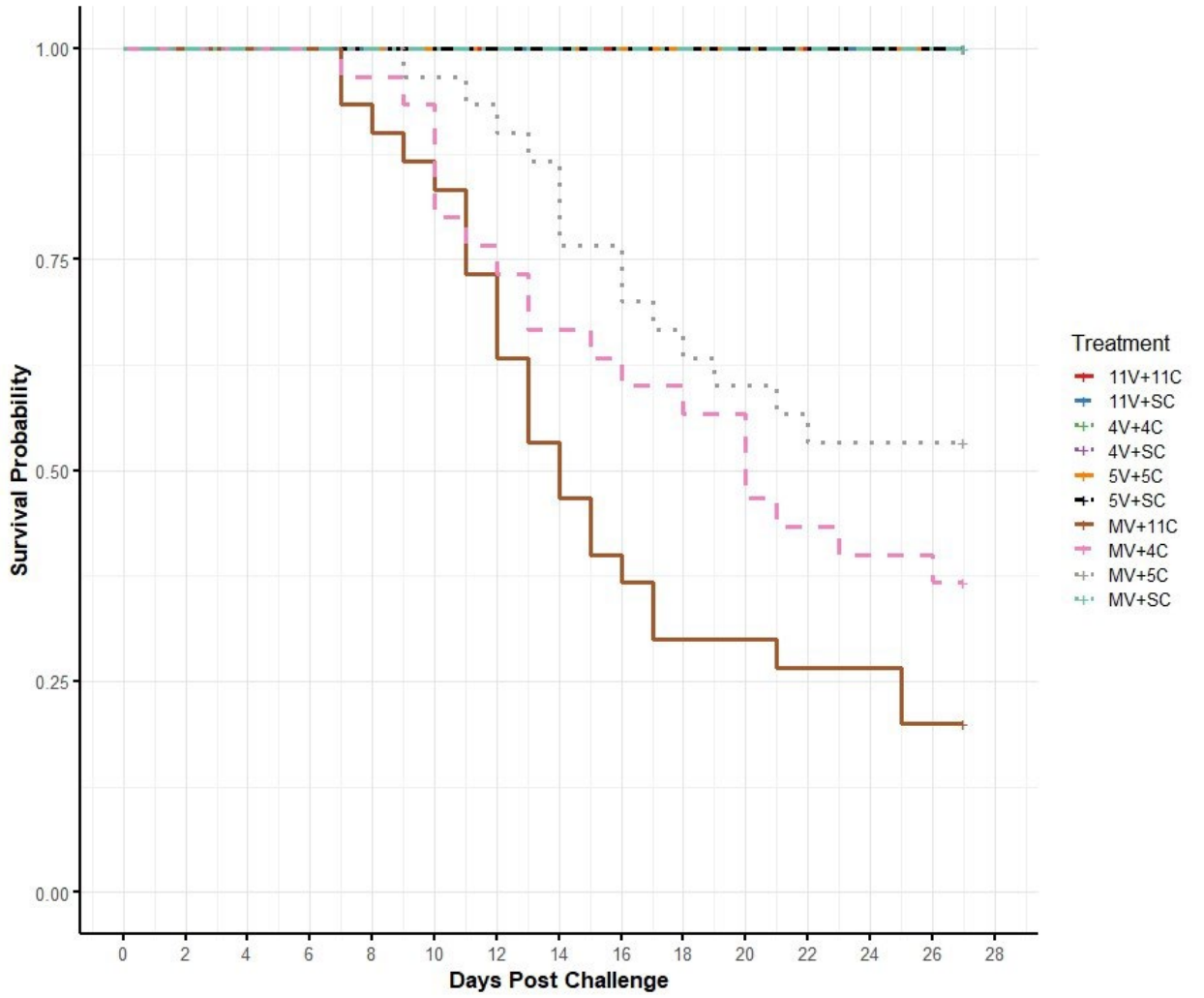

Figure 3. Kaplan-Meier survival curves of rainbow trout vaccinated (Monovalent vaccination) and challenged with *F. psychrophilum*. M= mock-vaccinated, V= vaccinated, C= *F. psychrophilum* challenged, S=0.65% saline solution. 4, 5 and 11 are numbers given to different strains of *F. psychrophilum*. Survival was monitored for 27 days post-challenge.

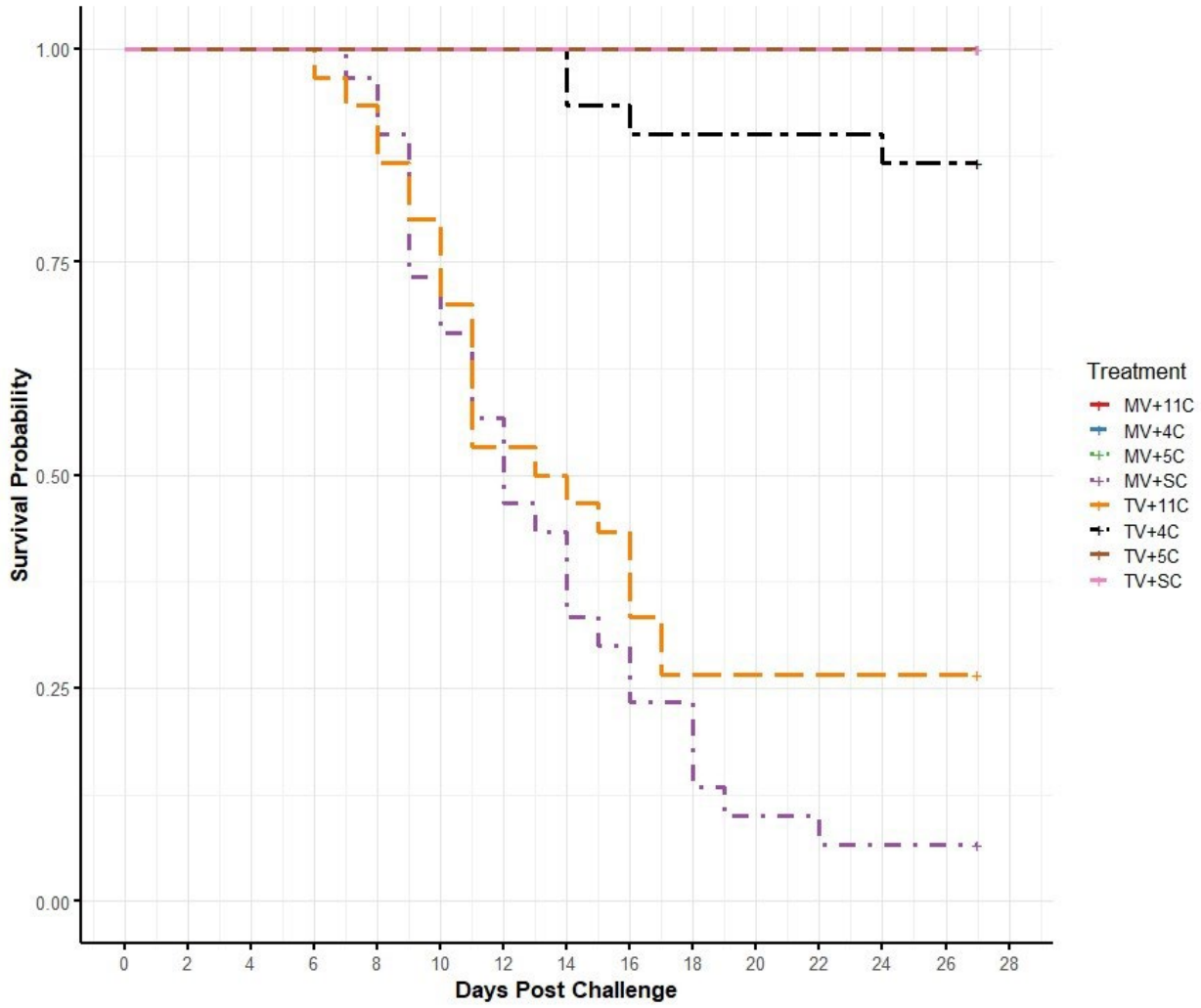

Figure 4. Kaplan-Meier survival curves of rainbow trout vaccinated (trivalent vaccine) and challenged with *F. psychrophilum*. M= mock, T= Trivalent, V= vaccinated, C= challenged, S= 0.65% saline solution. 4, 5 and 11 are numbers given to different strains of *F. psychrophilum*. Survival was monitored for 27 days post-challenge. Numbers 4, 5, and 11 are numbers given to different strains of *F. psychrophilum* used in this experiment.