

A Multivalent Oral Vaccine for Treatment of Bacterial Coldwater Disease

Targeted Research/Industry Development/Extension Area or Emerging Opportunities/Issues being addressed:
Theme B: Fish Health Sessions – 1. Disease Management & Medication Use – b. Bacterial Coldwater Disease

Chairperson: Casey Wright, Ph.D., Medgene
Co-Investigator(s): Luke Oliver, Ph.D., University of Idaho
Extension Liaison: Myron Kebus, D.V.M, M.S.
Industry Liaison: Luke Fredrickson, Houdek
Funding Request: \$148,387.50
Duration: 1/01/26-12/31/26 (One year)

Objectives:

1. Develop a novel subunit vaccine against the bacterial coldwater disease (BCWD) pathogen *Flavobacterium psychrophilum*.
2. Assess the serological response to the BCWD vaccine in vaccinated fish by serological assay.
3. Assess the efficacy of the BCWD vaccine at preventing disease in *F. psychrophilum*-challenged rainbow trout.

Deliverables:

1. Stable, scalable production of two or more antigenic proteins from *F. psychrophilum*.
2. Quantified serological responses to the multivalent BCWD vaccine in vaccinated trout for two feeding regimens at four dose levels.
3. A multivalent BCWD vaccine with the ability to confer immunological protection against BCWD.

Proposed Budget

Institution/Company	Principal Investigator(s)	Objective(s)	Year 1	Total
Medgene (VST LLC dba Medgene)	Casey Wright, Ph.D.	1, 2, 3	\$148,387.50	\$148,387.50
Totals			\$148,387.50	\$148,387.50

Project Summary

Bacterial coldwater disease (BCWD), caused by *Flavobacterium psychrophilum*, is one of the most important diseases leading to losses of farmed rainbow trout (*Oncorhynchus mykiss*), an industry valued at more than \$200M. The disease has led to significant economic losses, accounting for 70% of total mortalities. Typically, BCWD-infected fish would be treated with antibiotics, but this raises issues due to antibiotic resistance and stringent regulatory controls on antibiotic treatments. To counter these losses, new technologies to prevent disease, including vaccines, are necessary to reduce costs to farmers and continue to grow the industry. There are currently no commercially approved vaccines to prevent BCWD in the U.S. Medgene intends to develop a novel subunit vaccine that can be incorporated into the fishes' feed. By utilizing our innovative vaccine-development platform to rapidly produce vaccine products for changing and emerging diseases, we can quickly produce vaccines against BCWD for implementation in fish trials. This project specifically targets the NCRAC Research Priority "**Theme B: Fish Health Sessions – 1. Disease Management & Medication Use – b. Bacterial Coldwater Disease.**" With the development of a stable, safe, and effective vaccine for trout feed, Medgene has the potential to significantly decrease economic losses for the trout farmer.

Justification

Valued at more than \$200M, rainbow trout (*O. mykiss*) are one of the most valuable species of farm-raised finfish in the U.S. (Gula 2023). One of the most devastating diseases affecting farmed rainbow trout is BCWD caused by the pathogenic bacterium *F. psychrophilum*. This disease can lead to losses of 70% on farms, leading to significant economic losses to farmers and the trout industry (Gula 2023). BCWD, also referred to as peduncle disease and low-temperature disease in the U.S. and as rainbow trout fry syndrome in Europe, is characterized by a range of clinical signs in adult fish, including large open lesions in the tail area, lethargy, poor appetite, and spinal deformities (LaFrentz and Cain 2004). It is a septicemic disease of salmonids, and the causative bacterial pathogen is thought to be ubiquitous in freshwater. Outbreaks are typically triggered at water temperatures of 39-50°F, although severe disease has also been reported at 59°F (Rucker et al. 1954). Mortality varies according to the age of the fish, with rates of up to 90% for young fish in hatcheries (Barnes and Brown 2011; Nilsen et al. 2011). The disease spreads easily by horizontal transmission, and prevention is challenging, being restricted to reducing the incidence of risk factors such as stress and skin damage, which promote transmission, and removing sick, dying, or dead fish from commercial facilities. There are no approved drugs to treat BCWD, although antibiotics may be used. Antibiotics are used only for the treatment of active bacterial infections and not for prevention, and their use on farms and in food fish is limited by regulatory agencies due to factors contributing to antibiotic resistance (Marana et al. 2022). Consequently, BCWD has become one of the most common and economically important bacterial diseases in farmed trout and salmon. Genomic selection techniques have shown positive results in improving BCWD resistance in trout (Vallejo et al. 2021), but simpler and more cost-effective measures are likely required to achieve the long-term resilience that is needed in the aquaculture industry. Vaccines may offer an alternative means of establishing BCWD resistance in farmed salmonids. Currently, there are no approved and commercially available vaccines to prevent BCWD in the U.S., and when used, administration of vaccines by injection can be difficult, increases handling stress to the fish, and is both time and cost inefficient for producers. Additional options for administration include oral vaccines, which offer a number of key advantages for producers, including their low cost of administration, relative safety of administration versus injected vaccines, easy adaptability for administration to fish at different stages in their life cycle in commercial production, and the stress-free nature of administration for the fish, which do not need to be individually handled to be vaccinated (Bøgwald and Dalmo 2021). In this application, we propose to develop a multivalent subunit vaccine against BCWD, i.e., a vaccine comprising multiple recombinant protein antigens from the pathogen *F. psychrophilum* combined with an established adjuvant, and to test its ability to induce the production of corresponding antibodies and provide protection from mortalities when orally administered to rainbow trout by incorporation into feed pellets. The multivalent nature of this vaccine will prevent the development of resistance that might otherwise occur through mutation and selection of the pathogen. If successful, the vaccine would have the potential to be used in other BCWD-susceptible fish species with economic importance in commercial aquaculture, such as farmed salmon. In addition, there is the potential that this or a similar vaccine may provide a level of protection to fish susceptible to other *Flavobacterium* species, such as those causing columnaris disease. This expanded application of the vaccine is an area of interest to Medgene but is beyond the scope of the current project, which prioritizes assessing immunogenicity and dosing in farmed trout due to the relative economic importance of this species both in the North Central region and the U.S. as a whole.

Related Current and Previous Work

In 2008, Dr. Kenneth Cain and colleagues at the University of Idaho reported data on their newly developed vaccine against BCWD that was based on a live, attenuated strain of *F. psychrophilum* (LaFrentz et al. 2008). The vaccine was shown to result in significant protection against the virulent parent strain of *F. psychrophilum* when rainbow trout were vaccinated by intraperitoneal (IP) injection or by immersion, and vaccinated fish exhibited elevated titers of *F. psychrophilum*-specific antibodies. A subsequent study published in 2018 tested an enhanced version of the vaccine and showed that the protective effect extended to challenges with nine virulent *F. psychrophilum* isolates from several different fish species, with relative percent survival ranging from 51% to 72%. While these studies produced encouraging data that support the efficacy of live, attenuated vaccines, scalability and ease of administration remain as barriers to implementation.

Oral vaccination has significant practical advantages over other routes of administration, as noted above, but it is also a highly effective means of stimulating both mucosal and systemic immune responses in fish (Xue et al. 2013; Mutoloki et al. 2015; Chen et al. 2015). Vaccine antigens are thought to be primarily taken up by enterocytes in the lining of the gut (Joosten et al. 1997; Chen et al. 2015), with gut-associated lymphoid tissue (GALT) mediating an immune response. The use of feed pellets as a means of delivering a vaccine to the gut of rainbow trout has been tested with a DNA vaccine, which elicited production of neutralizing antibodies against the infectious pancreatic necrosis virus (IPNV) and strong protection in a challenge study (Ballesteros et al. 2014). An immune response to an IPNV DNA vaccine in rainbow trout, together with a significant protective effect of immunization, has also been shown (de las Heras et al. 2010). These studies used vaccine–alginate microspheres mixed with feed pellets, with the microspheres intended to provide a degree of protection to the DNA against degradation in the gut. This protection is an important consideration, and a number of different approaches have been tested, including oil adjuvants that allow the vaccine to be applied as a coating on feed pellets. Chen et al. showed that alginate-encapsulated IPNV antigens mixed with an oil mixture and applied to feed pellets were effective as a vaccine boost one year after IP vaccination of Atlantic salmon (*Salmo salar*), with increased levels of IPNV antibodies detected in encapsulated-IPNV-boosted fish at seven weeks post-boost (Chen et al. 2014). Tobar et al. tested a *Piscirickettsia salmonis* whole-cell vaccine formulated with a bioadhesive cationic polysaccharide and mixed with oil to coat feed (Tobar et al. 2011). Their experiments in Atlantic salmon showed that the oral vaccine elicited both a local and a systemic immune response and protected fish against a lethal challenge with *P. salmonis* regardless of whether the oral vaccine was used for primary immunization or as a booster for an injected vaccine (Tobar et al. 2011). Other tested approaches have omitted the use of oil as a mixing agent for coating feed and protecting the antigen. Xue et al., for example, showed that antibodies against the Grass Carp Reovirus protein VP6 could be detected in grass carp (*Ctenopharyngodon idella*) that were fed a freeze-dried powder of silkworm pupae that expressed the antigen (Xue et al. 2013). However, based on the results of prior published work in this area, the degree of protection offered by an oil-containing adjuvant is likely to be beneficial for oral vaccination of fish with protein antigens, and this consideration coupled with the practicality of these adjuvants with regard to preparing vaccine-coated feed is the basis for our approach to vaccine preparation for the present project. We will use a commercially available adjuvant, Montanide™ GR 01, manufactured by Seppic, to formulate our vaccine. Montanide™ GR 01 is a water-in-oil adjuvant that is designed to protect vaccine antigens against the gastric environment through a protective matrix that allows the antigens to be released into the intestine, where they can then interact with immune cells. The palatability and safety of Montanide GR have been confirmed by Seppic in salmon and tilapia.

Medgene is a South Dakota-based company that specializes in the development and commercialization of new vaccines, including prescription vaccines, tailored to the needs of U.S. agriculture. To date, the company has shipped millions of doses of vaccines produced using its proprietary production platforms. Current prescription vaccine formulations produced by Medgene include custom and off-the-shelf vaccines against swine influenza, senecavirus A, bovine coronavirus, and highly pathogenic avian influenza (H5N1). The company has also produced vaccines against foreign animal diseases other than H5N1, including the Rift Valley fever virus, African horse sickness, classical swine fever, and Nipah/Hendra virus. These vaccines have been produced using Medgene's proprietary, USDA-licensed baculovirus platform for antigen production. Medgene has also developed a bacterial platform for antigen expression, which will be used in the proposed work. This project will leverage the company's experience, its vaccine production resources, and its close relationship with the feed manufacturer Houdek to produce and test a novel BCWD vaccine for aquaculture. Additionally, vaccines produced on the licensed platform are not required to obtain an Experimental Use Permit from the USDA Animal and Plant Health Inspection Service (APHIS), decreasing the time needed to perform the proposed studies and to make them available to producers.

Statement Regarding Duplication of Research

Searches of the Current Research Information System (CRIS or REEport), the National Sea Grant Office Funding page, and NOAA Office of Aquaculture Funding Opportunities page with the keywords “coldwater,” “vaccine,” and “psychrophilum” yielded 16 results in the USDA CRIS, corresponding to 16 funded external and internal research projects. Review of the topics and scope of these funded projects did not identify any potential overlap or duplication of research when compared with the scope and activities of the project proposed in the present application.

Anticipated Benefits

The proposed project will provide the foundation for the development and rigorous testing of a novel BCWD vaccine for trout that can be easily implemented in trout farming operations in a cost-effective manner, encouraging adoption across the industry. The anticipated benefits include:

- A significant advance in the ability to protect farmed trout (and, hopefully, other farmed salmonids) against BCWD.
- Improvement in the health of farmed trout through reduced morbidity and mortality in those exposed to *F. psychrophilum* present in fresh water.
- Reduction in economic losses due to BCWD across the trout farming industry.
- Greater resilience of the trout farming industry and downstream customers to the threat of BCWD outbreaks.

The results of this project and subsequent field studies will be communicated to aquaculture industry stakeholders through peer-reviewed publications where appropriate, through articles in industry publications (e.g., Journal of Aquatic Animal Health, Transactions of the American Fisheries Society, Aquaculture, Aquaculture North America), and through active engagement with the aquaculture and fisheries communities via virtual and in-person workshops (as part of our proposed extension activities) to raise awareness of oral vaccination as a cost-effective, practical approach to BCWD control and of specific approaches to implementing oral vaccination in aquaculture operations. In addition to our proposed workshops, members of our team will participate in industry conventions and workshops together with presentation of educational and training seminars. For the potential benefits of this project to be realized, it is essential that the results are translated into a commercially available vaccine. Medgene has an experienced sales and marketing team to support the anticipated outreach activities and the commercialization of the research for the benefit of U.S. trout farmers.

Objective(s)

Objective 1: Develop a novel subunit vaccine against the BCWD pathogen *F. psychrophilum*. To achieve this objective, Medgene will develop multiple vaccine constructs encoding full-length proteins for use as subunit antigens in a multivalent vaccine. The proteins will be produced by our scalable recombinant bacterial platform and adjuvanted with the Montanide GR 01 adjuvant for oral administration via coated fish feed.

Objective 2: Assess the serological response to the BCWD vaccine in vaccinated fish by serological assay. To provide initial *in vivo* validation of the proposed BCWD vaccine, Medgene will evaluate the serum from vaccinated fish. Two feeding regimens with four dose levels for the BCWD vaccine will be evaluated, and the serological response to vaccination will be assessed.

Objective 3: Assess the efficacy of the BCWD vaccine at preventing disease in *F. psychrophilum*-challenged rainbow trout. To achieve this objective, Medgene will perform a challenge experiment in rainbow trout vaccinated with the proposed BCWD vaccine. Fish will be vaccinated at the dose level determined most effective in Objective 2 and exposed to the pathogen to assess the ability of the vaccine to confer immunological protection against BCWD.

Deliverables(s)

Objective 1 Deliverable: Stable, scalable production of two or more antigenic proteins from *F. psychrophilum* (GroEL, SecG, DnaK, Arginyl-tRNA synthetase, and/or the alpha chain of ATP synthase) by Medgene’s recombinant bacterial platform in quantities suitable for multivalent vaccine formulation (≥ 20 antigen units/mL) and testing in Objective 2.

Objective 2 Deliverable: Quantified serological responses to the multivalent BCWD vaccine in vaccinated trout for two feeding regimens at four dose levels.

Objective 3 Deliverable: A multivalent BCWD vaccine with the ability to confer immunological protection against BCWD.

Procedures

Approach:

Medgene is a leader in the new field of prescription vaccines, specializing in developing vaccines based on protein subunit antigens combined with proprietary adjuvants for use in animals. We have partnered with the USDA Center for Veterinary Biologics (USDA–CVB) to develop and leverage guidelines that permit the production and immediate availability of highly targeted vaccine products to address rapidly changing or newly emerging diseases in the U.S. Medgene has developed a USDA–CVB-approved platform for prescription vaccine production. This product comprises a standardized manufacturing method, a vector backbone, an adjuvant, and administration parameters (minimum age, maximum antigen level, dose size, and route of administration). All vaccines are produced using a standardized, proven-safe manufacturing process regardless of the vaccine target produced, and new vaccines can be produced within a matter of weeks from conception to field use.

Our platform can be used to manufacture prescription vaccines by using different genes of interest to generate antigens without the need for additional licensing. This enables us to rapidly respond to the needs of producers by providing specific vaccines prescribed to at-risk animals. As an immunological service provider, Medgene monitors the production environment for new pathogens, continuously updates its pathogen sequence library and vaccine construct bank to rapidly counter production health risks, and serologically monitors vaccinated animals for an antibody response. We provide these immunological services to our ISPRIME® community of livestock producers and their veterinarians. We have incorporated significant bioinformatics into our program for predicting protein structures, mapping the geographical distribution of specific variants of targets, developing methods for enhancing protein production, predicting the feasibility of antigen protein stocks made using our platform, and predicting epitopes for target antigens. The proposed vaccine will be produced using our bacterial platform.

Objective 1: Develop a multivalent vaccine that targets *F. psychrophilum*, the causative agent of BCWD.

Generating a BCWD Vaccine (Medgene): Medgene’s bacterial platform for prescription vaccine production will be used to produce novel vaccine constructs designed to drive the expression of multiple known immunogenic proteins from *F. psychrophilum* (Sudheesh et al. 2007b). Constructs encoding full-length proteins for the 60-kDa chaperonin GroEL, the protein translocase subunit SecE (63% identical to Thermolysin), the chaperone protein dnaK (identical to 70-kDa hsp), arginyl-tRNA synthetase, and the alpha chain of ATP synthase will be used in these studies. A modified (codon-optimized) sequence will be sent to Integrated DNA Technologies, Inc. (IDT; Coralville, IA) for synthesis. The modifications introduced for vaccine production include *Xba*I and *Not*I restriction sites for cloning purposes and sequences encoding a C-terminal TEV protease site followed by a His tag and stop codon. The sequence also contains PCR priming sites that will be used to perform quality control analysis during vaccine production. The vaccine construct will be produced by subcloning the coding sequence into pMGL-fabV vector (a derivative of the recombinant expression vector pET). The resulting Master Sequence will be validated by PCR sequencing and used to transfect competent bacteria for antigen production. *Escherichia coli* carrying the expression vector will be grown in either a shake flask or stirred glass fermenter. Recombinant protein production will be induced for two to five hours using isopropyl β-D-1-thiogalactopyranoside (IPTG) addition after the culture reaches appropriate levels. Once induction is complete, cells will be pelleted via centrifugation to remove spent media, resuspended in a denaturing buffer containing urea, and disrupted using a microfluidizer. The released recombinant protein will be separated from cellular debris via tangential flow filtration using an appropriately sized hollow fiber. Finally, the sterile recombinant protein will be refolded using diafiltration to reduce the urea levels. All Medgene serials will be adjuvanted with the Seppic Montanide GR 01 proprietary adjuvant. Actual serial formulation records, including lot numbers, will be available upon request. Vaccines will be stored at 2-8°C (35-46°F) until use. The work described for Objective 1 will be conducted by the Principal Investigator, Dr. Casey Wright, and a vaccine production team comprising a Senior Scientist, Scientist, Clinical Scientist, Constructs Scientist, and Production Technician, at Medgene’s Brookings, SD facility, with an allocated budget of \$78,687.50.

Expected Results and Alternative Approaches: We expect that the approach outlined above will produce antigenic proteins. The process of screening Master Sequences to verify a 100% match between the original amino acid sequence of the U.S. isolate(s) and that of the recombinant protein will continue until the required Master Sequence

is identified for scaled-up production. The experience of Medgene’s production team with this established process will help to ensure that this objective is achieved, resulting in the production of a validated vaccine for testing in Objective 2. If antigen production is lower than expected, we will consider a purification step to increase the concentration of the antigenic protein.

Objective 2: Assess the immunogenic response to the BCWD vaccine in vaccinated trout by serological assay.

Vaccine Administration (Houdek): A multivalent BCWD vaccine will be formulated and orally administered to trout with two feeding regimens and four different dose levels to identify dose(s) and feeding regimen(s) that produce an appropriate immunologic response in trout. The four tested antigen dose levels will be 25, 50, 100, and 200 µg of antigen per dose (Serials 1-4, respectively). The vaccine will be tumble-coated onto the feed and then drawn into the pellet by vacuum. Coated feed pellets will be stored at 2-8°C (35-46°F) until used for the vaccination trials in fish. A negative control group will be fed pellets with no antigen coating. A positive control group will be intraperitoneally vaccinated for comparison with the serological response elicited by the orally administered multivalent vaccine. Fish will be vaccinated at one of four dose levels for the oral vaccine by two different feeding regimens (Table 1). Under feeding regimen 1, trout will be fed coated feed for five days, uncoated feed for five days, then coated feed for five days as the prime vaccination. Under feeding regimen 2, trout will be fed coated feed for ten days as the prime vaccination. As a boost vaccination, both groups will receive a five-day feeding of coated feed 28 days after the start of the prime vaccination. Each treatment group will be housed separately in a single tank with a minimum of 27 fish per tank. Fish will be fed at a rate of approximately 4% wet weight, with adjustment after each sampling to ensure proper dosage. Serial numbers and serial information for each vaccine will be recorded in the Vaccination Record.

Table 1. Treatment groups for Objective 2.

Group	Feeding Regimen	Serial/Dose
A	Uncoated Feed – Negative Control	N/A
B	Uncoated Feed – Positive Control	IP vaccination
C	Regimen 1	Serial 1, 25 µg
D	Regimen 1	Serial 2, 50 µg
E	Regimen 1	Serial 3, 100 µg
F	Regimen 1	Serial 4, 200 µg
G	Regimen 2	Serial 1, 25 µg
H	Regimen 2	Serial 2, 50 µg
I	Regimen 2	Serial 3, 100 µg
J	Regimen 2	Serial 4, 200 µg

Blood Collection (Houdek): To assess the response to vaccination, whole blood will be collected in serum separator tubes (SST) on days 39, 53, and 67 per the schedule of events (Table 2). Collection tubes will be labeled with the

Table 2. Schedule of events in Objective 2.

Study Day	Study Activity Feeding Regimen 1 and Positive Control	Study Activity Feeding Regimen 2
Day 0	Twenty fish blood collection (10 ml SST) for pre-vaccination controls	N/A
Day 1	IP vaccination Dose One of positive control fish	N/A
Days 1-5	Feeding oral vaccine to all dose levels	Feeding oral vaccine to all dose levels
Days 6-10	N/A	Feeding oral vaccine to all dose levels
Days 11-15	Feeding oral vaccine to all dose levels	N/A
Day 28	Vaccination: IP Dose Two for the positive control fish	N/A
Days 28-32	Feeding oral vaccine to all dose levels	Feeding oral vaccine to all dose levels
Day 39	Blood collection (10 ml SST) from three replicates per treatment	Blood collection (10 ml SST) from three fish per treatment
Day 53	Blood collection (10 ml SST) from three replicates per treatment	Blood collection (10 ml SST) from three fish per treatment
Day 67	Blood collection (10 ml SST) from three replicates per treatment	Blood collection (10 ml SST) from three fish per treatment

fish ID number. After collection, the samples will be placed on ice packs in a cooler until delivery to Medgene. The sample collection will be documented on the sample collection record. Samples will be centrifuged,

serum collected and frozen, and samples will then be shipped to the University of Idaho for analysis. A budget of \$34,700 has been allocated for the production of vaccine-coated feed, vaccination, and blood sampling by Houdek.

Serological Assay (University of Idaho): Briefly, enzyme-linked immunosorbent assays (ELISAs) will be performed using serum samples obtained from treatment groups following immunization to identify anti-*F. psychrophilum* antibodies. The assay will be performed as described by LaFrentz et al. (LaFrentz et al. 2002) at the University of Idaho’s Aquatic Animal Lab and the Fish Health/Immunology lab by Dr. Oliver. A budget of \$10,000 has been allocated to the University of Idaho for this serological analysis.

Statistical Analysis: Antibody titer data will be summarized using descriptive statistics (mean, standard deviation). The data will be analyzed using the Kruskal–Wallis test to identify significant differences among the groups. Post-

hoc pairwise comparisons will be performed with a non-parametric test (e.g., the Dunn test with correction for multiple comparisons) to identify the treatment groups exhibiting significant differences ($\alpha = 0.05$).

Expected Results and Alternative Approaches: The methods proposed are well established, and we do not anticipate technical challenges with the serological assay. The number of fish per treatment group/replicate is sufficient to meet the primary goal of detecting a neutralizing response to infections by the virus. We expect that more than one dose level/feeding regimen combination will result in a robust immune response.

Objective 3: Assess the efficacy of the BCWD vaccine at preventing disease in *F. psychrophilum*-challenged rainbow trout.

Challenge (University of Idaho): Twenty-five pathogen-free, healthy, juvenile rainbow trout will be fed with feed coated with the multivalent vaccine following the feeding regimen established in Objective 2 that produced the highest antibody titers. The challenge will be performed in triplicate (N=75). Twenty-five additional trout will be fed uncoated feed and will serve as non-vaccinated controls and 25 non-vaccinated fish will be injected IP with sterile phosphate buffered saline (PBS) to serve as the mock infected control (Table 3). Each group will be housed separately and observed for approximately eight weeks after vaccination at which time blood from a random subset of fish in each group will be drawn to assess serum antibody titers. At eight weeks after initial vaccination, vaccinated treatment and non-vaccinated control fish will be challenged with a virulent strain of *F. psychrophilum* (CSF-259-93) via intramuscular (IM) injection following a standard challenge method (Sudheesh and Cain 2016; Bruce et al. 2020) and observed for 28 days. Mortalities will be monitored at least twice daily and promptly removed from the tanks. To confirm infection, re-isolation of *F. psychrophilum* will be attempted by sampling 20% of the daily mortalities and streaking kidney, liver, and spleen samples on tryptone yeast extract salts (TYES) agar and incubating plates at 15°C (59°F) for 96 h.

Analysis: Presumptive identification of isolates as *F. psychrophilum* will be based on colony color (yellow) and morphology (convex with smooth morphology or convex with a thin spreading margin). Cumulative percent mortality (CPM) and relative percent survival (RPS) will be plotted with differences determined using one-way ANOVA and Tukey HSD post hoc test for determining group differences ($\alpha = 0.05$). A budget of \$15,000 has been allocated to the University of Idaho for this challenge study.

Expected results and alternative approaches: We expect vaccinated fish will have a statistically significant higher rate of survival as compared to fish not offered the vaccinated feed or fish intraperitoneally injected with PBS ($P \leq 0.05$). This study will also demonstrate the efficacy of the regimen and dose level determined most effective in Objective 2.

Table 3. Treatment groups for Objective 3.

Group	Treatment	Number
A	Coated Feed – Treatment Group 1	25
B	Coated Feed – Treatment Group 2	25
C	Coated Feed – Treatment Group 3	25
D	Uncoated Feed – Negative Control	25
E	IP injected – Negative Control	25

Outreach and Evaluation Plan

Our target audience includes fish producers, industry stakeholders, Federal and State fisheries personnel, and aquatic veterinarians. As an immunological services provider, Medgene has invested in the concept of prescription vaccines and in developing an approach to animal health that utilizes the community of producers and aquatic veterinarians who care for fish. Our outreach objectives include raising awareness about the importance of fish health, educating veterinarians and trout farmers about vaccine benefits, and encouraging vaccine adoption. To support a community-driven approach, Medgene has implemented the ISPRIME® service and is continually building a network of customers who use the service to access prescription vaccines that more effectively target the diseases affecting their animals. The ISPRIME® community is an important resource that will support the commercialization of our proposed vaccine, enabling us to inform stakeholders about the availability and benefits of the vaccine, including its potential to reduce the financial impact of BCWD on producers. Our outreach efforts will also include a significant educational component to train aquaculture industry members on the appropriate use of our oral vaccine. The use of a feed for oral administration of the vaccine will be new to the industry, necessitating clear communication of information on important factors relating to the receipt, storage, and use of the vaccine. Training seminars will be developed to help inform users on how best to incorporate our fed vaccine into their current aquaculture practice. Additionally, to raise awareness of the availability of the product, our ISPRIME® team will engage with the aquaculture industry by attending industry conventions/workshops. As part of our evaluation plan, we will measure the impact of our outreach through workshops, assessing knowledge and behavior changes while collecting feedback on improving the distribution and implementation of the vaccine.

To aid in the dissemination of results from this study, we will conduct a series of virtual and in-person meetings and

workshops. Two virtual meetings will be conducted following the conclusion of our proposed challenge study (Objective 3). The first will be data centered, focused on the results and their applicability to the aquaculture industry. For this meeting we will invite a range of stakeholders from fish producers, industry partners, government fisheries representatives, and aquatic veterinarians from across the U.S. with interest in fish health. The second meeting will focus on gaining producer input to guide the development of our larger in-person conference/workshop. We intend to gain a better understanding of the most pressing issues of fish health currently affecting the aquaculture and fisheries industries and use this information to put together a succinct and informative selection of topics and industry partners in fish health (such as Merck and fish vaccine producers) and those producing solutions for BCWD to present at our workshop. This workshop will be made available to the entire NCRAC region and held at South Dakota State University (SDSU) in Brookings, SD.

Myron Kebus, D.V.M., M.S. (Assistant Professor, College of Veterinary Medicine, Michigan State University) will act as the Extension Liaison for this project to disseminate its results to trout farmers and the broader aquaculture industry and engage stakeholders in providing feedback on the continued development of the BCWD vaccine and the needs of the industry. Dr. Kebus is a graduate of the University of Wisconsin-Madison School of Veterinary Medicine (DVM) and holds a master's degree in Aquaculture/Veterinary Science from the University of Wisconsin-Madison. He founded the Wisconsin Aquatic Veterinary Service to provide veterinary services to fish farms and other clients. Dr. Kebus has conducted research into stress in rainbow trout and was Wisconsin's State Fish Health Veterinarian from 1999-2023, directing the state's Fish Health Program. He is also a past president of the American Association of Fish Veterinarians and is currently their liaison to the American Veterinary Medical Association (AVMA). He was the chair of the Aquatic Veterinary Medicine Committee of the AVMA and served as a liaison for the Association's Environmental Issues Committee. Currently, he works closely with fish farmers and veterinarians to improve fish health in the North Central Region by integrating extension with the development of alternative disease prevention methods, such as vaccination, best management practices, and on-farm training. He joined MSU in 2022 as an Assistant Professor of Health Programs for the Department of Pathobiology and Diagnostic Investigation. His position received initial funding from the USDA-NIFA North Central Regional Aquaculture Center (NCRAC)-funded project titled "Improving fish health in the NCR by integrating extension with the development of alternative disease prevention methods." His extensive experience with fish health in commercial aquaculture and his connections in the aquaculture industry will be leveraged in the outreach portion of the proposed project. The PI, Dr. Casey Wright, will work with Dr. Kebus to develop outreach materials, plan outreach activities, and gain actionable insights.

Luke Fredrickson (Houdek) will serve as our Industry Liaison for this project. Mr. Frederickson, through his industry connections, will help develop our messaging around the benefits and best practices of use of the BCWD vaccine. He will also work with PI Dr. Wright and Extension Liaison Dr. Kebus to develop outreach webinars and producer workshops to disseminate the results of this study to industry partners and producers and any other interested parties. Mr. Fredrickson is a 2008 graduate from the University of Minnesota, earning his Bachelor of Science degree in Fisheries and Wildlife Management. During this time, he completed an internship serving as a Pathology Lab Assistant at the Minnesota Department of Natural Resources Pathology Lab, giving him the ability to visit many State and private hatcheries and connect with those raising the fish. After graduation, Mr. Fredrickson took the position of Agriculturalist, Biologist, and Hatchery Specialist at Oswald Fisheries in Bruce, SD where he assisted in producing many species of baitfish and gamefish. It was during this time that he was introduced to Dr. Bill Gibbons at Houdek (at the time Prairie AquaTech) and became their Aquaculture Research Manager. At Houdek, he has performed over 100 fish and shrimp feeding and research trials, assisting in the development of Houdek's proprietary feed ingredients. Mr. Fredrickson's extensive expertise in performing fish trials and industry connections will be valuable in developing outreach materials and workshops to industry, veterinary, and governmental partners.

Intellectual Property

Medgene's vaccines are produced using the company's USDA-licensed platforms, and Medgene will retain the exclusive right to pursue patent protection for immunogenic compositions including the specific antigens or combination of antigens tested in this project. Intellectual property protection through patents will support continued investment by Medgene in the commercialization of this vaccine in order to make it available to the aquaculture community. Making a vaccine available to aquaculture producers requires the resources of a company to support manufacturing, marketing, sales, and distribution. No vaccine (other than autogenous vaccines, which are site restricted) currently in use by producers has been made available through a non-commercial route. Companies like Medgene play an essential role in supporting the aquaculture and agriculture industries through commercial products

to support animal health. Medgene routinely engages with the agricultural industry, governmental personnel, and veterinarians to understand needs and communicate advances in vaccines. Medgene’s position as holder of the IP related to the proposed oral vaccine will not limit access. In addition, IP protection encourages continued investment in vaccine production in the U.S. Moreover, obtaining a license to market the vaccine for fish would support the development of vaccines for other diseases affecting farmed fish. This demonstrates broader impact of the project beyond BCWD. Medgene can quickly generate and provide prescription vaccines for other fish diseases once a species extension has been approved by the CVB. The proof of concept provided by demonstrating a protective immune response to the vaccine in this proposed study would justify Medgene’s further investment in the additional work necessary to obtain USDA Product and Establishment licenses to market the vaccine as non-prescription product.

The product will be priced at an affordable level that will ensure economic benefits for trout producers by reducing the impacts of BCWD on their operations. The cost to produce the vaccine is quite low, keeping the cost to producers low and thereby encouraging widespread use. Medgene is a successful but relatively small company. Non-dilutive funding can therefore play an important role in facilitating early-stage development of vaccines by supporting the allocation of company resources to these projects and bringing together research teams that combine industry and academic researchers to address specific needs that have remained unaddressed by large agriscience companies.

Logic Model

Situation: BCWD outbreaks in commercial trout farms cause high morbidity, mortality, and economic losses to trout producers and downstream commercial operations.

Goal: Resilience to infection by the BCWD pathogen *F. psychrophilum* in farmed trout.

Objective: Develop a cost-effective, orally administered multivalent subunit vaccine against *F. psychrophilum*.

Logic Model						
INPUTS	OUTPUTS			OUTCOMES		
	Activities	Deliverables	Knowledge Gain	Short-term	Medium-term	Long-term
<p><u>People</u></p> <p>Medgene staff Houdek staff University of Idaho staff</p> <p><u>Resources</u></p> <p>Medgene's bacterial vaccine-production platform Houdek's feed production facility and equipment Houdek's aquaculture research facility (Brookings, SD) University of Idaho's Fish Health/Immunology lab Medgene's Business Development, Sales, and Marketing operations Houdek's Feed Production Facility (Volga, SD)</p>	<p><u>Topics</u></p> <p>Vaccine efficacy evaluation Dose regimen evaluation Dose evaluation Production process refinement Antigen production scale-up Coated feed production scale-up Education</p> <p><u>Delivery</u></p> <p>Coordination of vaccination, blood sampling, and serological analysis Statistical analysis of data Outreach to stakeholders, e.g., via workshops/conference, participating in industry conventions/workshops</p>	<p><u>Products</u></p> <p>Quantified serological responses to the multivalent BCWD vaccine in vaccinated trout for two feeding regimens at four dose levels Customer discovery/outreach feedback summaries Marketing recommendations Educational and training materials on oral vaccination for BCWD Enrollment of aquaculture stakeholders in ISPRIME</p>	<p><u>Technical knowledge</u></p> <p>Immunogenic dose(s) and dose regimen(s) for a challenge study to evaluate vaccine efficacy Understanding of the temporal response to oral vaccination in trout Defined feed coating conditions for successful incorporation of the vaccine into feed pellets</p> <p><u>Commercial knowledge</u></p> <p>Insight into customers’ pain points in relation to BCWD, current control measures, and implementing routine oral vaccination</p>	<p><u>Research</u></p> <p><i>In vivo</i> data on immunogenicity and dosing and vaccine efficacy evaluated via a challenge study</p> <p><u>Industry</u></p> <p>Customer discovery information on farm’s BCWD-related costs, pain points, and vaccine awareness Formal engagement with producers, industry stakeholders, government personnel, and aquatic vets through virtual and in-person workshops disseminating study outcomes and topics relevant to fish health Additional engagement with</p>	<p><u>Research</u></p> <p>BCWD field studies in trout to evaluate protective efficacy (with morbidity and mortality endpoints)</p>	<p><u>Industry</u></p> <p>Significantly reduced rates of BCWD-related morbidity in U.S. farmed trout Significantly reduced rates of BCWD-related mortality in U.S. farmed trout Increased profitability of the U.S. trout farming industry</p> <p><u>Research</u></p> <p>Additional oral vaccines against diseases with economic impacts on U.S. commercial aquaculture operations, based on the BCWD oral vaccine</p>

	<p>Extending ISPRIME to aquaculture producers and veterinarians</p> <p>Drafting training materials on oral vaccine administration for aquaculture personnel</p>			<p>potential users of the vaccine through ISPRIME, facilitating ongoing communication regarding vaccine benefits, study outcomes, implementation, and availability to industry stakeholders</p>	<p>model and in other fish species and against other pathogens</p>
--	---	--	--	---	--

Facilities

Medgene has a 13,500 sq ft dedicated R&D facility at Brookings, SD, for the design and construction of the recombinant constructs to produce the antigens. This facility includes specialized labs that are critical to this project. The **Construct Laboratory Suite** is primarily used for co-transfection of pre-master seed constructs, laying down of pre-master seed, and PCR techniques. Our **Construct Lab** is used for DNA activities in preparation of pre-master seed constructs. The **Process Development Suite** has the **Process Development Lab and the Bacterial Lab**, which are both Biosafety Level 2 labs for R&D, process development, and autogenous work with food poisoning-causing bacteria with Cooked Meat Media. This suite also includes the **Analytical Lab**, where *in vitro* assay development and validation and DNA activities for Quality Control of Master Seeds are carried out. Equipment in this suite includes biosafety cabinets, incubators, and plate readers. The **Production Facility** consists of the **Quality Control Testing Lab**. Equipment includes centrifuges, Automated Western Blot machines, incubators, autoclaves, laminar flow hoods, and freezers/refrigerators. Other equipment includes walk-in coolers for the storage of in-process antigen and working seed lots, completed products in bulk and final containers, and raw materials. Preparation of chemicals, reagents, and released supplies to be used in biologics production and storage of seeds/cells and final product also take place in this facility. The facility is equipped for bacterial production, antigen fluid processing, working seed production, and cell culture maintenance for production. In addition to the larger pieces of equipment such as ultra-low freezers, benchtop centrifuges benchtop shaker / incubators, bioreactor controllers, wave bioreactors, and glass bioreactors, Medgene owns small lab instruments and tools, including but not limited to micropipettes, pipettes, balances, stir plates, vortex mixers, etc. that will be used to complete the proposed work. Medgene also has office space with desks, chairs, computers, printers, scanners, etc., for laboratory staff. Office amenities include high-speed wireless internet, janitorial resources/support, copiers/printers, and access to breakroom and conference rooms.

Houdek Feed Production Facilities and Resources, Brookings, SD, is the manufacturer of ME-PRO® plant-based protein for the aqua feed (aquaculture) industry. In 2019, the company began production of its signature aquatic and terrestrial feed ingredient brand, ME-PRO, at its Volga, South Dakota manufacturing facility. ME-PRO has since made a significant impact in the global aquaculture industry, winning international awards in addition to its inclusion in fish and shrimp diets worldwide. For this project, Houdek will serve as the feed site where trout will be fed the oral vaccine as per the study design (Objective 2 - Vaccine Administration). The site has several large tanks for research and development and is routinely used for fish feed projects (Fig. 1). Joe Kline, DVM oversees all fish health-related aspects of trials performed at the facility. Houdek also has a large grower network and novel feed strategic partnerships and offers product development and go-to-market IP and branding services. Luke Fredrickson at Houdek will serve as our industry liaison.



Figure 1. Houdek’s aquaculture tanks for research use.

The Aquatic Animal Lab (AAL) at University of Idaho (UI) provides space for fish rearing and pathogen challenge for testing of vaccines and other fish disease management tools. The AAL is supplied with temperature-controlled

(6-26°C [43-79°F]) flow through dechlorinated Specific Pathogen Free (SPF) municipal water. This facility has four isolated challenge and experiment rooms that allow small and large fish replicated immunization and challenge (48 replicated 19L [5 gal] tanks) experiments. In addition, tanks, troughs, and egg incubation systems are available to rear fish at all life stages and rooms are equipped with independent photoperiod manipulation capabilities. This facility is critical to projects in the aquatic animal health field and allows conduct of disease challenges with sufficient replications to ensure statistically justifiable results. All water exiting the UI AAL is directly discharged to the Moscow water treatment plant, where complete disinfection occurs. The Fish Health/Immunology lab is fully equipped for molecular genetics, bacteriology, virology, immunology, and diagnostic studies. Further work on experimental vaccines occurs in this facility, which is equipped for vaccine production as needed. For this project, Dr. Luke Oliver will test serum samples to assess the responses of fish to vaccination.

References

- Ballesteros, N. A., S. Rodriguez Saint-Jean, and S. I. Perez-Prieto. 2014. Food pellets as an effective delivery method for a DNA vaccine against infectious pancreatic necrosis virus in rainbow trout (*Oncorhynchus mykiss*, Walbaum). *Fish Shellfish Immunol* 37(2):220–8.
- Barnes, M. E., and M. L. Brown. 2011. A review of *Flavobacterium psychrophilum* biology, clinical signs, and bacterial cold water disease prevention and treatment. *Open Fish Science Journal* 4:40.
- Bøgwald, J., and R. A. Dalmo. 2021. Protection of teleost fish against infectious diseases through oral administration of vaccines: update 2021. *International Journal of Molecular Sciences* 22(20):10932.
- Bruce, T. J., J. Ma, C. Knupp, T. P. Loch, M. Faisal, and K. D. Cain. 2020. Cross-protection of a live-attenuated *Flavobacterium psychrophilum* immersion vaccine against novel *Flavobacterium* spp. and *Chryseobacterium* spp. strains. *Journal of Fish Diseases* 43(8):915–928.
- Chen, L., Ø. Evensen, and S. Mutoloki. 2015. IPNV Antigen Uptake and Distribution in Atlantic Salmon Following Oral Administration. *Viruses* 7(5):2507–17.
- Chen, L., G. Klaric, S. Wadsworth, S. Jayasinghe, T. Y. Kuo, Ø. Evensen, and S. Mutoloki. 2014. Augmentation of the antibody response of Atlantic salmon by oral administration of alginate-encapsulated IPNV antigens. *PLoS One* 9(10):e109337.
- Gula, L. T. 2023. Aquaculture Research Aims to Reduce Rainbow Trout Losses in U.S. Hatcheries.
- de las Heras, A. I., S. Rodríguez Saint-Jean, and S. I. Pérez-Prieto. 2010. Immunogenic and protective effects of an oral DNA vaccine against infectious pancreatic necrosis virus in fish. *Fish Shellfish Immunol* 28(4):562–70.
- Joosten, P. H., M. Y. Engelsma, M. D. van der Zee, and J. H. Rombout. 1997. Induction of oral tolerance in carp (*Cyprinus carpio* L.) after feeding protein antigens. *Vet Immunol Immunopathol* 60(1–2):187–96.
- LaFrentz, B. R., and K. D. Cain. 2004. Bacterial Coldwater Disease An Extension Bulletin for the Western Regional Aquaculture Center (WRAC).
- LaFrentz, B. R., S. E. LaPatra, D. R. Call, and K. D. Cain. 2008. Isolation of rifampicin resistant *Flavobacterium psychrophilum* strains and their potential as live attenuated vaccine candidates. *Vaccine* 26(44):5582–5589.
- LaFrentz, B. R., S. E. LaPatra, G. R. Jones, J. L. Congleton, B. Sun, and K. D. Cain. 2002. Characterization of serum and mucosal antibody responses and relative per cent survival in rainbow trout, *Oncorhynchus mykiss* (Walbaum), following immunization and challenge with *Flavobacterium psychrophilum*. *Journal of Fish Diseases* 25(12):703–713.
- Marana, M. H., I. Dalsgaard, P. W. Kania, A. Mohamed, J. Hannibal, and K. Buchmann. 2022. *Flavobacterium psychrophilum*: Response of Vaccinated Large Rainbow Trout to Different Strains. *Biology* 11(12):1701.
- Mutoloki, S., H. M. Munang'andu, and Ø. Evensen. 2015. Oral Vaccination of Fish - Antigen Preparations, Uptake, and Immune Induction. *Front Immunol* 6:519.
- Nilsen, H., A. B. Olsen, Ø. Vaagnes, H. Hellberg, K. Bottolfsen, H. Skjelstad, and D. J. Colquhoun. 2011. Systemic *Flavobacterium psychrophilum* infection in rainbow trout, *Oncorhynchus mykiss* (Walbaum), farmed in fresh and brackish water in Norway. *Journal of Fish Diseases* 34(5).
- Rucker, R. R., B. J. Earp, and E. J. Ordal. 1954. Infectious diseases of Pacific salmon. *Transactions of the American Fisheries Society* 83(1):297–312.
- Sudheesh, P., B. LaFrentz, D. Call, W. Siems, S. LaPatra, G. Wiens, and K. Cain. 2007a. Identification of potential vaccine target antigens by immunoproteomic analysis of a virulent and a non-virulent strain of the fish pathogen *Flavobacterium psychrophilum*. *Diseases of aquatic organisms* 74:37–47.
- Sudheesh, P. S., and K. D. Cain. 2016. Optimization of efficacy of a live attenuated *Flavobacterium psychrophilum* immersion vaccine. *Fish Shellfish Immunol* 56:169–180.

Sudheesh, P. S., B. R. LaFrentz, D. R. Call, W. F. Siems, S. E. LaPatra, G. D. Wiens, and K. D. Cain. 2007b. Identification of potential vaccine target antigens by immunoproteomic analysis of a virulent and a non-virulent strain of the fish pathogen *Flavobacterium psychrophilum*. *Diseases of aquatic organisms* 74(1):37–47.

Tobar, J. A., S. Jerez, M. Caruffo, C. Bravo, F. Contreras, S. A. Bucarey, and M. Harel. 2011. Oral vaccination of Atlantic salmon (*Salmo salar*) against salmonid rickettsial septicaemia. *Vaccine* 29(12):2336–2340.

Vallejo, R. L., H. Cheng, B. O. Fragomeni, G. Gao, R. M. O. Silva, K. E. Martin, J. P. Evenhuis, G. D. Wiens, T. D. Leeds, and Y. Palti. 2021. The accuracy of genomic predictions for bacterial cold water disease resistance remains higher than the pedigree-based model one generation after model training in a commercial rainbow trout breeding population. *Aquaculture* 545:737164.

Xue, R., L. Liu, G. Cao, S. Xu, J. Li, Y. Zou, H. Chen, and C. Gong. 2013. Oral vaccination of BacFish-vp6 against grass carp reovirus evoking antibody response in grass carp. *Fish Shellfish Immunol* 34(1):348–55.

Project Leaders

State	Participant and Institution(s)	Specialization(s)
South Dakota	Casey Wright, Ph.D.; Medgene (VST LLC)	Vaccine development
Idaho	Luke Oliver, Ph.D.; University of Idaho	Aquaculture, fish immunology, disease management

Budget

ORGANIZATION AND ADDRESS University Medgene (VST LLC dba Medgene) Address 1006 32nd Avenue Ste 104 City, State, ZIP Brookings, South Dakota, 57006-4711				USDA AWARD NO. Year 1: Objective 1, 2, 3			
PROJECT DIRECTOR(S) PI Name Casey Wright				Duration Proposed Months: 12	Duration Proposed Months: ____	Non-Federal Proposed Cost-Sharing/ Matching Funds (If required)	Non-federal Cost-Sharing/ Matching Funds Approved by CSREES (If Different)
A. Salaries and Wages				Funds Requested by Proposer	Funds Approved by CSREES (If different)		
1. No. of Senior Personnel							
CSREES FUNDED WORK MONTHS							
Calendar Academic Summer							
a. <u> 1 </u> (Co)-PD(s)				0.5	-	-	\$6,166.67
b. <u> </u> Senior Associates							
2. No. of Other Personnel (Non-Faculty)							
a. <u> </u> Research Associates-Postdoctorates . . .							
b. <u> 5 </u> Other Professionals				4.25			\$28,020.83
c. <u> </u> Paraprofessionals							
d. <u> </u> Graduate Students							
e. <u> </u> Prebaccalaureate Students							
f. <u> </u> Secretarial-Clerical							
g. <u> </u> Technical, Shop and Other							
Total Salaries and Wages <input type="checkbox"/>				\$34,187.50			
B. Fringe Benefits (If charged as Direct Costs)				\$0			
C. Total Salaries, Wages, and Fringe Benefits (A plus B) <input type="checkbox"/>				\$34,187.50			
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for each item.)				\$5,000.00			
E. Materials and Supplies				\$39,500.00			
F. Travel							
G. Publication Costs/Page Charges							
H. Computer (ADPE) Costs							
I. Student Assistance/Support (Scholarships/fellowships, stipends/tuition, cost of education, etc. Attach list of items and dollar amounts for each item.)							
J. All Other Direct Costs (In budget narrative, list items and dollar amounts and provide supporting data for each item.)				\$69,700.00			
K. Total Direct Costs (C through I) <input type="checkbox"/>				\$148,387.50			
L. F&A/Indirect Costs. (If applicable, specify rate(s) and base(s) for on/off campus activity. Where both are involved, identify itemized costs in on/off campus bases.)				\$0			
M. Total Direct and F&A/Indirect Costs (J plus K) <input type="checkbox"/>				\$148,387.50			
N. Other <input type="checkbox"/>							
O. Total Amount of This Request <input type="checkbox"/>				\$148,387.50			
P. Carryover -- (If Applicable)				Federal Funds: \$	Non-Federal funds: \$	Total \$	
Q. Cost Sharing/Matching (Breakdown of total amounts shown in line O)						Leave Blank	
Cash (both Applicant and Third Party) <input type="checkbox"/>							
Non-Cash Contributions (both Applicant and Third Party) <input type="checkbox"/>							
NAME AND TITLE (Type or print)				SIGNATURE (required for revised budget only)			DATE
Project Director							
Authorized Organizational Representative							
Signature (for optional use)							

Budget Explanation per Institution

A. Salary, Wages and Fringe Benefits (total \$34,187.50)

Key Personnel (total \$6,166.67)

Casey Wright, PhD, Principal Investigator and Bacteriology Development Manager, Medgene Labs – Dr. Wright is an experienced molecular biologist who currently leads Medgene Labs' development of the Bacterial Antigen Platform for licensure as a vaccine platform. His past experience includes diagnostic immunoassay development, antigen purification, and vector design at Inanovate, Inc., where as Director of Content production, he was responsible for the design and qualification of equipment for the startup of an antigen production facility. Dr. Wright therefore has both the scientific expertise in vaccine development and the project management experience necessary to successfully direct the proposed project. He will be responsible for overseeing both the production of the construct and vaccine preparation for testing in trout and will coordinate the work of the Medgene Labs team and the CRO responsible for fish testing. A total of 0.5 calendar month of salary support is requested for this role (\$148,000 annual salary x 0.5 month = \$6,166.67)

Other Personnel (total \$28,020.83)

The following personnel will be involved in generating and validating the vaccine construct (Objective 1) and for preparing the vaccine for the fish study in Objective 2.

Scientist – 1.0 calendar month of effort (\$70,000 annual salary x 1.0 month = **\$5,833.33**)

Senior Scientist – 1.0 calendar month of effort (\$113,000 annual salary x 1.0 month = **\$9,416.67**)

Clinical Scientist – 0.25 calendar months of effort (\$85,000 annual salary x 0.25 months = **\$1,770.83**)

Production Technician – 1.0 calendar month of effort (\$60,000 annual salary x 1.0 month = **\$5,000.00**)

Constructs Scientist – 1.0 calendar month of effort (\$72,000 annual salary x 1.0 month = **\$6,000.00**)

B. Nonexpendable Equipment (total \$5,000.00)

High Shear Mixer: \$5,000.00 is requested for a High Shear mixer that is needed for the adjuvant process.

C. Materials and Supplies (total \$39,500.00)

Lab Supplies: \$15,000.00 is requested for culture flasks and plates, assay plates, Jess assay cartridges, separation columns, gloves, pipette tips, serological pipettes, and wave bags.

Reagents: \$24,500.00 is requested for reagents used in construct generation, validation, and target antigen production, including restriction enzymes, serum, cells, adjuvant, buffers, polymerases, antibodies, and cell culture media for vaccine production, etc.

D. Travel

None requested

E. All Other Direct Costs (total \$48,600.00)

Houdek Feed Production Facilities and Resources (total \$34,700.00)

Our CRO Houdek will maintain the fish, produce the feed (both uncoated and coated), and conduct the vaccination of the fish and collection of blood samples for use in the serum neutralization assay. The following is the cost breakdown for these services:

- Fish Housing and Husbandry: \$22,700.00 is requested for maintaining the fish during the study.
- Fish Study Execution: \$12,000. In more detail, which is \$9,100 for ingredients/raw materials and \$2,500 for labor. They will need to produce 4,800 lbs. of feed. Of this total, 800 lbs. is the regular control diet to feed the fish in the 90-day pretrial to bring them to the appropriate size for trial, and to feed all the tanks during the trial when they are not being fed the treatment feeds. The remaining 4,000 lbs. is the amount of feed that will be required to meet the minimum batch size in the vacuum coater to coat the treatment diets for 20 separate days of coating.

- The total labor estimate to produce and coat all the feeds for the duration of the study is 54 hours.

University of Idaho (total \$25,000.00)

Dr Luke Oliver will assess the response of the fish to vaccination (Objective 2) and perform the challenge study (Objective 3). He will develop and test serum samples from the fish. These assays will be performed at the Aquatic Animal Lab (AAL), and the Fish Health/Immunology lab at the University of Idaho. Dr Oliver has significant expertise in Aquaculture Vaccine Development, Fish Health/Diseases, and Fish Immunology/Pathology. Our request will cover all expenses related to these assays and challenge studies.

Promotion and Facility Rental for Virtual Meetings and Conference (total \$10,000)

Funds are requested to prepare promotional materials (\$5,000) for the virtual meetings and the on-site conference to be held at SDSU. Additional funds are requested for the rental of a conference room and to provide a meal during the conference (\$5,000)

Extension Liaison – Myron Kebus, D.V.M., M.S.

Medgene will pay the costs associated with the Extension Liaison’s work under a budget and scope of work to be agreed with Michigan State University.

Industry Liaison – Luke Fredrickson

Medgene will pay the costs associated with the Industry Liaison’s work under a budget and scope of work to be agreed with Houdek.

Total: \$148,387.50

Budget Summary

YEAR 1

	Medgene (Casey Wright)
Salaries & Wages	\$34,187.50
Fringe Benefits	\$0
Total Salaries, Wages, and Fringe Benefits	\$34,187.50
Nonexpendable Equipment	\$5,000.00
Materials and Supplies	\$39,500.00
Travel	\$0
All Other Direct Costs	\$69,700.00
Totals	\$148,387.50

Schedule for Completion of Objectives

Start date: 1/01/2026

Completion date: 12/31/2026

Objectives and Tasks	Year 1					
	J-F	M-A	M-J	J-A	S-O	N-D
Objective 1						
Construct and Master Seed generation						
Antigen production and vaccine formulation						
Quality control assays						
Objective 2						
Feed preparation, vaccination, sampling						
ELISA, data analysis						
Objective 3						
<i>F. psychrophilum</i> challenge						
Sample and data analysis						
Outreach and Evaluation						
Dissemination of results, outreach activities						

Participating Institutions and Principal Investigators

Medgene – Casey Wright, Ph.D.

University of Idaho – Luke Oliver, Ph.D.

Curriculum Vitae

VITA

Name: Casey Wright, Ph.D.
Address: VST, LLC dba Medgene Labs
1006 32nd Avenue, Suite 104
Brookings, SD 57006-4728

Phone: 605-697-2600
Fax:
E-mail: caseyw@medgenelabs.com

EDUCATION

B.S.	South Dakota State University, Brookings, SD	1998	Animal Science
M.S.	South Dakota State University, Brookings, SD	2000	Animal Science
Ph.D.	Colorado State University, Fort Collins, CO	2005	Biomedical Sciences

POSITIONS

2022 - Present	Analytical Development Manager, Medgene Labs, Brookings, SD
2020 - 2022	Vice President of Assay Development, Inanovate, Inc, Sioux Falls, SD
2019 - 2020	Director of Content Production, Inanovate, Inc, Sioux Falls, SD
2017 - 2019	Scientist, Alumend, LLC, Sioux Falls SD
2012 - 2017	Research Scientist, Novartis/Elanco Animal Health, Inc., USA, Larchwood, IA
2011 - 2012	Assistant Scientist, Sanford Research/USD, Sioux Falls, SD
2005 - 2011	Postdoctoral Fellow, Sanford Research/USD, Sioux Falls, SD

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

Not Applicable

SELECTED PUBLICATIONS

Not Applicable

VITA

Name: Luke Oliver, Ph.D.
Address: University of Idaho
875 Perimeter Drive MS1136
Moscow, ID 83844-1136

Phone: 208-997-8170
Fax:
Email: loliver@uidaho.edu

EDUCATION

B.S.	University of Maine, Orono, ME	2011	Environmental Science
M.S.	Kentucky State University, Frankfort, KY	2016	Aquaculture
Ph.D.	University of Idaho, Moscow, ID	2022	Aquaculture

POSITIONS

2022 - Present	Postdoctoral Fellow, University of Idaho, Moscow, ID
2019 – 2022	Research Assistant, University of Idaho, Moscow, ID
2017 – 2019	Aquaculture Research Specialist, University of Idaho, Moscow, ID
2015 – 2016	Fish Biologist, Ash Meadows Fish Conservation Facility, Amargosa, NV
2012 – 2015	Aquaculture Technician, Thoroughbred Shrimp Company, Frankfort, KY
2012 – 2015	Research Assistant, Kentucky State University, Frankfort, KY

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

American Aquaculture Association
Idaho Aquaculture Association
American Fisheries Society (Fish Culture Section and Fish Health Section)

SELECTED PUBLICATIONS

Oliver L. P., T. J. Bruce, J. Ma, E. M. Jones, and K. D. Cain. 2023. Development of a monoclonal antibody specific to burbot (*Lota lota*) IgM and optimization of an ELISA to measure anti-*Aeromonas* sp. antibody titers following pathogen challenge. *Fish & Shellfish Immunology* 137:108775.

Jones E. M., L. P. Oliver, J. Ma, R. H. Leeuwis, V. Myrself, M. R. Arkoosh, J. P. Dietrich, C. M. Schuster, M. Hawkyard, A. K. Gamperl, and K. D. Cain. 2022. Production of a monoclonal antibody specific to sablefish (*Anoplopoma fimbria*) IgM and its application in ELISA, western blotting, and immunofluorescent staining. *Fish & Shellfish Immunology* 130:479-489.

Bruce T. J., J. Ma, E. M. Jones, B. M. Vuglar, L. P. Oliver, C. Knupp, T. P. Loch, and K. D. Cain. 2021. Assessment of *Flavobacterium psychrophilum*-associated mortality in Atlantic salmon (*Salmo salar*) and brook trout (*Salvelinus fontinalis*). *Journal of Fish Diseases* 44:645-653.

Bruce T. J., J. Ma, L. P. Oliver, E. M. Jones, B. R. LaFrentz, and K. D. Cain. 2020. Isolation and experimental challenge of cultured burbot (*Lota lota maculosa*) with *Flavobacterium columnare* and *Aeromonas* sp. isolates. *Journal of Fish Diseases* 43:839-851.

Ma J., T. J. Bruce, L. P. Oliver, and K. D. Cain. 2019. Co-infection of rainbow trout (*Oncorhynchus mykiss*) with infectious hematopoietic necrosis virus and *Flavobacterium psychrophilum*. *Journal of fish diseases* 42:1065-1076.

VITA

Name: Myron Kebus, DVM, M.S.
Address: 2318 Center Ave.
Madison, WI 53704-5627

Phone: 608-616-2133
Fax:
Email: kebusmyr@msu.edu

EDUCATION

B.S.	Michigan State University, Lansing, MI	1986 Biology
M.S.	University of Wisconsin-Madison, Madison, WI	1990 Veterinary-Aquaculture
D.V.M.	University of Wisconsin-Madison, School of Veterinary Medicine	1992 Veterinary Medicine

POSITIONS

Present	Diagnostic Investigation, College of Veterinary Medicine, Michigan State University.
2022 - Present	Subject Matter Expert, Secure Food Systems Team, University of Minnesota, College of Veterinary Medicine, VHS Risk Assessment Project.
Present	Director, Aquaculture Program, Division of Animal Health, Wisconsin Department of Agriculture, Trade & Consumer Protection.
Present	Honorary Fellow in Department of Pathobiological Sciences, University of Wisconsin-Madison – School of Veterinary Medicine,
Present	Adjunct Faculty at the School of Freshwater Sciences, University of Wisconsin – Milwaukee.

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

American Association of Fish Veterinarians
American Fisheries Society – Fish Health Section, Liaison to AVMA
American Veterinary Medical Association, Aquatic Veterinary Medicine Committee,
North Central Regional Aquaculture Center, Technical Committee
World Aquatic Veterinary Association

SELECTED PUBLICATIONS

Kebus, M., T. P. Loch, M. Smith, and N.B.D. Phelps. 2025. Opportunities for veterinary engagement to improve aquaculture production and the health of farmed fish in the North Central Region of the United States. *Journal of the American Veterinary Medical Association* 18:1-6.

Kebus, M. J., C. Walster. 2022. Legislation, Regulations, and Policies. Pages 211-217 *in* *Fundamentals of Aquatic Veterinary Medicine*, Wiley Blackwell Publishing, Chichester, West Sussex, UK.

Murray, K.N., T. S. Clark, M. J. Kebus, and M. L. Kent. 2021. Specific Pathogen Free - A review of strategies in agriculture, aquaculture, and laboratory mammals and how they inform new recommendations for laboratory zebrafish. *Research in Veterinary Science* 142:78-93.

Martinelli, L., O. Harris, M. T. Collins, and M. Kebus. 2020. Efficacy of a Modified Health Assessment Utilized on Two Genetically Distinct Stocks of Rainbow Trout. *Journal of Aquatic Animal Health* 32:59-64.

Karreman, G., K. Klotins, J. Bebak, L. Gustafson, A. Osborn, and M. Kebus. 2015. Aquatic Animal Biosecurity: A Case Study of Bioexclusion of Viral Hemorrhagic Septicemia Virus in an Atlantic Salmon Hatchery. *Journal of Applied Aquaculture* 27:299-317.

Kebus, M. J. and AVMA Committee on Environmental Issues. 2003. Waste management: aquaculture and fisheries. *Journal of the American Veterinary Medical Association* 223:56-57.

VITA

Name: Luke Fredrickson
Address: Houdek
705 32nd Ave S.
Brookings, SD 57006-7046

Phone: 605-692-1266
Fax:
Email: luke.fredrickson@houdeknature.com

EDUCATION

B.S. University of Minnesota, Minneapolis, MN 2008 Fisheries and Wildlife Management

POSITIONS

2015 - Present Aquaculture Research Manager, Prairie Aquatech (Houdek), Brookings, SD
2008 - 2015 Agriculturalist, Biologist, and Hatchery Specialist, Oswald Fisheries, Ellendale, MN
2006 - 2008 Pathology Lab Assistant, Minnesota Department of Natural Resources, Pathology Lab, St. Paul, MN

SELECTED PUBLICATIONS

White, B., L. Fredrickson, A. Fey, M. Boaventura, and S. Nates. Evaluating the effects of novel fermented plant protein on growth performance and feed utilization of juvenile freshwater and marine fish species. *Latin American and Caribbean Aquaculture* 2024. September 24-27, 2024. Medellín, Colombia.

Fredrickson, L., B. Modica, D. Adams, C. Kuball, L. Koutsos, B. White, and S. F. Nates. 2023. Evaluation of ME-PRO® and EnviroMeal Combinations as Partial and Complete Fish Meal Replacements in Diets for Juvenile Atlantic Salmon (*Salmo salar*) Reared in A Recirculating Aquaculture System. *Journal of Aquaculture, Marine Biology & Ecology* JAMBE-121.

White, B., L. Fredrickson, O. Araujo, N. Araujo, S. F. Nates. 2023. The Role of the Microbial Enhanced Protein ME-PRO® in Recirculation Aquaculture Systems (RAS) Using Precision Feeding. *Journal of Aquaculture, Marine Biology & Ecology* JAMBE-105.

McLean, E., L. Fredrikson, K. Alfrey, M. F. Tlusty, and F. T. Barrows. 2020. Growth, integrity, and consumer acceptance of largemouth bass, *Micropterus salmoides* (Lacépède, 1802), fed marine resource-free diets. *International Journal of Fisheries and Aquatic Studies* 8:365-369.

Letters of Support



Dr. Casey Wright
Medgene
1006 32nd Avenue
Brookings, SD, 57006

May, 5, 2025

Dr. Wright,

I would like to give my support for your North Central Regional Aquaculture Center proposal "A Multivalent Oral Vaccine for Treatment of Bacterial Coldwater Disease." As fish culturists with a focus on providing fish that live in a stress-free environment and produced without the use of antibiotics, we are very interested in your preventative vaccine technology, especially vaccines that can be cost effective and easy to administer.

At Hanilu Farms, we raise fish in a sustainable and antibiotic- and chemical-free manner, focusing on the preventative measures to maintain the health of the fish, rather than reactionary responses to disease. We converted to recirculating aquaculture systems in 2017 to ensure that our production systems are as environmentally sustainable as possible. Again, our goal is to reduce the environmental impact of our operation while producing high-quality, natural, and antibiotic-free fish. Vaccines, such as those provided by Medgene, especially those that can be produced in response to emerging diseases, are highly valued in our company. With sales of 1500 lbs per week of fish and maintaining 130,000 gallons of water, an easy to administer form of vaccination is highly desirable. With the number of fish we raise, it would be difficult to vaccinate all fish with traditional injectable vaccines, not to mention the large amount of handling stress this would place on our fish. A feed-based vaccination method would greatly enhance our ability to proactively prevent diseases in all our fish, ensuring a product that is healthy to the consumer, while reducing or removing reliance on antibiotics or other chemicals.

I am excited to learn more about your vaccines and wish you the best with your proposal. At Hanilu Farms, we look forward to your easy to administer and cost-effective vaccine technologies to prevent diseases on our farm.

Sincerely,



Leland Meador
Hanilu Farms
1481 W. County Road
1000 N
Cutler, IN 46920
ldmfarms@gmail.com



May 6th

Dr. Casey Wright
Analytical Development Manager
Medgene
1006 32nd Avenue
Brookings, SD, 57006

Dear Casey,

I strongly support Medgene's NCRAC grant proposal "A Multivalent Oral Vaccine for Treatment of Bacterial Coldwater Disease." The proposed work is of great interest to us and has the potential to address the critical need we have for an effective vaccine to treat bacterial coldwater disease in rainbow trout.

Rushing Waters Fisheries is a small farm in Wisconsin that produces premium quality rainbow trout. Our production facility is run by a passionate team that help to raise our fish using ecologically responsible aquaculture practices. Diseases can have a great impact on our facility and the ability of our team to help control fish loss. Current methods of treatment for bacterial infections, including bacterial coldwater disease, that are at our disposal include antibiotics. These antibiotic treatments are costly, and the FDA regulates the length of time necessary for drug clearance from the fish before they can be sold for human consumption. Antibiotic use is also a last resort mechanism as prophylactic treatment has been halted due to the risk of antibiotic resistance and goes against our ecologically responsible practices. Medgene's feed-based vaccination platform is ideal for our use as it will increase the efficiency of preventing diseases in our fish, is easy to administer, and reduces our reliance on costly and potentially environmentally unfriendly antibiotics

I look forward to hearing about the results of the proposal and especially the potential to efficiently control bacterial coldwater disease through oral vaccination of our trout.

Sincerely,

Peter Fritsch
Rushing Waters Fisheries
N301 County Road H
PO Box H
Palmyra, Wisconsin 53156
peter.fritsch@rushingwaters.net



N301 County Road H / Palmyra, WI 53156
<https://rushingwaters.net>
Phone: 262.495.2089



South Dakota Department of Game, Fish and Parks
19619 Trout Loop
Spearfish, SD 57783-8905
Phone: (605) 642-6302

April 28, 2025

To whom it may concern:

For the past 38 years I have been involved in the rearing of rainbow trout and other salmonids. I have an extensive history of published aquaculture research, with several of the over 200 articles involving fish health and disease. In particular, the following articles deal with Bacterial Coldwater Disease:

Barnes, M. E., and M. L. Brown. 2011. A review of *Flavobacterium psychrophilum* biology, clinical signs, and bacterial cold water disease prevention and treatment. *Open Fish Science Journal* 4:40-48.

Treft, C. E., M. E. Barnes, J. M. Voorhees, T. J. Martin, and B. L. Fletcher. 2017. Impacts of feeding three commercial trout starter diets to rainbow trout on Bacterial Coldwater Disease-induced mortality. *Journal of Marine Biology and Aquaculture* 3(2):1-5.

Martin, T. J., J. M. Voorhees, C. E. Treft, B. Fletcher, and M. E. Barnes. 2018. Effects of four commercial diets on rainbow trout *Oncorhynchus mykiss* growth, feeding efficiency, and mortality at a production hatchery with endemic bacterial coldwater disease. *Insights in Aquaculture and Biotechnology* 2:10.

Hawkins, M., N. Huysman, B. Hodges, C. Treft, and M. E. Barnes. 2023. Lack of effect of Biowish Multibio 3PS probiotic on bacterial cold-water disease induced mortality in rainbow trout. *Journal of Aquaculture and Fisheries* 7:074.

Based on fish culture experience, research, and administrative oversight, I thoroughly understand the fish health and economic issues arising from Bacterial Coldwater Disease. I also understand the practical issues with administering vaccines via injection, one-fish-at-a-time. Having a feed-based vaccine that is easy to administer and cost-effective would be a game-changer for trout production in both public and private fish hatcheries. As such, I strongly support Medgene's NCRAC grant proposal "A Multivalent Oral Vaccine for Treatment of Bacterial Cold-Water Disease".

Please contact me if you have any questions.

Michael E. Barnes, Ph.D.
Hatchery Program Administrator

North Central Regional Aquaculture Center

Liaison Letter of Intent

In accordance with the Guidelines for Extension Involvement in the North Central Regional Aquaculture Center (adopted in 1994), directives of the NCRAC Board of Directors and USDA-NIFA guidance, all NCRAC-funded projects must include an Extension Liaison that is funded to do extension and outreach activities associated with that project. NCRAC projects must also include an Industry Liaison who will serve as a contact between project PI(s) and the Industry.

Name (Appointed Liaison): Dr. Myron Kebus

Title of Project: A Multivalent Oral Vaccine for Treatment of Bacterial Coldwater Disease

Project Duration: 12 months

The conditions and terms of the offer being made to you are outlined below:

Position (Extension or Industry): Extension

Primary Duties/Activities of Liaison: The use of a feed for oral administration of the vaccine will be new to the industry, necessitating clear communication of information on important factors relating to the receipt, storage, and use of the vaccine. The Liaison will help develop training seminars, extension white papers and producer workshops to help inform users and veterinarians on how best to incorporate our fed vaccine into their current aquaculture practice. Additionally, to raise awareness of the availability of the product, the liaison will assist our ISPRIME® team with materials used to educate the aquaculture industry at conventions/workshops.

Medgene will be responsible for the funds to pay for the extension liaison work. Medgene will compensate Dr. Kebus at a rate of \$150/hr and reimburse all travel expenses. Additionally, Medgene will pay for all printing or publication costs associated to the outreach efforts of the extension liaison.

Appointment offered by: Casey Wright 9-12-2025
Project Chair Date

Offer approved by: _____
NCRAC Director Date

I have read and I understand the offer and its terms and conditions, and I agree to these terms and accept this offer. The terms of this offer may be modified only by subsequent written agreement signed by both parties.

Liaison Signature: Myron Kebus, DVM 9-18-2025
Date

Please return this letter by: 9-18-2025 to the Project Chair

North Central Regional Aquaculture Center

Liaison Letter of Intent

In accordance with the Guidelines for Extension Involvement in the North Central Regional Aquaculture Center (adopted in 1994), directives of the NCRAC Board of Directors and USDA-NIFA guidance, all NCRAC-funded projects must include an Extension Liaison that is funded to do extension and outreach activities associated with that project. NCRAC projects must also include an Industry Liaison who will serve as a contact between project PI(s) and the Industry.

Name (Appointed Liaison): Luke Fredrickson

Title of Project: A Multivalent Oral Vaccine for Treatment of Bacterial Coldwater Disease

Project Duration: 12 months

The conditions and terms of the offer being made to you are outlined below:

Position (Extension or Industry): Industry

Primary Duties/Activities of Liaison: The use of a feed for oral administration of the BCWD vaccine will be new to the industry, necessitating clear communication of information on important factors relating to the receipt, storage, and use of the vaccine. The Liaison will help develop training seminars and producer workshops to help inform users, producers, veterinarians, and Federal and State fisheries managers on how best to incorporate our fed vaccine into current aquaculture practices. Additionally, to raise awareness of the availability of the product, the liaison will assist our ISPRIME® team with materials used to educate the aquaculture industry at conventions/workshops.

Medgene will be responsible for the funds to pay for the industry liaison work. Medgene will compensate Mr. Fredrickson at a rate of \$150/hr and reimburse all travel expenses. Additionally, Medgene will pay for all printing or publication costs associated with the outreach efforts of the industry liaison.

Appointment offered by: Casey Wright 9-9-2025
Project Chair Date

Offer approved by: _____
NCRAC Director Date

I have read and I understand the offer and its terms and conditions, and I agree to these terms and accept this offer. The terms of this offer may be modified only by subsequent written agreement signed by both parties.

Liaison Signature: Luke Fredrickson 9-10-25
Date

Please return this letter by: 9-19-2025 **to the Project Chair**

Checklist for Submission of Full Proposals

Follow guidelines with the exception of the budget sheets.

- Format manuscripts for 22 x 28 cm (8½ x 11 inch).
- Number *all* pages sequentially.
- Use 10-12 font; Times New Roman. Do not justify right margins.
- Format headings appropriately.
- Leave at least a 2.5-cm (1-inch) margin on all sides.
- Use metric units of measurement with English units in parenthesis, e.g. 2.54 cm (1 inch).
- Define all abbreviations the first time they are used.
- Express ratios by using a slant line (e.g. mg/L).
- Scientific names should accompany common names in the title and when they are first mentioned in the abstract and in the text. Authority for scientific names need not accompany the genus and species unless needed for clarity.
- Spell out one to ten unless followed by a unit of measurement (e.g. four fish, 4 kg, 14 fish). Do not begin a sentence with a numeral. Use 1,000 instead of 1000; 0.13 instead of .13; and % instead of percent.
- Use the 24-hour clock for dial time: 0830, not 8:30 a.m. The calendar date should be day month year (7 August 1990).
- Include signed Letters of Intent for identified Extension and Industry Liaisons.
- Signed Authorized Organization Representative (AOR) form from each funded PI's institution are welcomed but not required at this time.
- Include the required three (3) Letters of Support from Industry members who are not directly involved in the proposed project.
- Assemble the full proposal in this order: Title Page, Project Summary, Justification, Related Current and Previous Work, Statement Regarding Duplication of Research, Anticipated Benefits, Objective(s), Deliverables, Procedures, Project Deliverables, Evaluation and Outreach (Logic Model included), Facilities, References, Project Leaders, Budget, Budget Explanation per Institution, Budget Summary, Schedule for Completion of Objectives. References, Participating Institutions and Principal Investigators, Curriculum Vitae for Principal Investigators (PIs).
- Provide names of three possible reviewers who will not have a Conflict of Interest
- All identified co-PIs have been provided a final draft of the full proposal.
- Submit proposal (including all required documentation) in single MS Word document.

If the NCRAC Administrative Office cannot verify inclusion of any element, the Full Proposal will not be accepted.



September 19, 2025

Principal Investigator Signature

Date