Improving fish health in the NCR by integrating extension with the development of alternative disease prevention methods

Theme A (Aquacult. Prod., TRA A-4: Fish Health), Theme C (Extension/Education, TEA C-1: Producer Education)

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Extension Liaison:
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Funding Request: $601,387

Duration: 2 years (09/01/2021 - 08/31/2023)

Objectives:
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.

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

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

Deliverables:
1. Publish fish health survey results in an Extension document.
2. Deliver fish health survey results and knowledge during farm visits in NCR.
3. Create site-specific and generalizable BMPs for producers.
4. Conduct workshops for farmers, veterinary students, and veterinarians.
5. Create low-cost fish health kits along with videos and prints describing how materials are used.
6. Generate and deploy vaccine preparations for use against a bacterium responsible for one of the most damaging diseases of coldwater fish in the USA.
7. Determine primary transmission sources for flavobacterial diseases on NCR farms and interrupt these sources through targeted and newly developed best management practices.
8. Cryo-bank of bacterial isolates and samples (maintained at no charge to NCRAC by *PI* Loch) available upon request for aquatic veterinarians, fish health laboratories, and researchers to use in NCR farm disease prevention and control plans.
9. Provide education, training, and outreach experience for undergraduate students, graduate students, and a post-doctoral researcher involved in the proposed research.
10. Publications in peer-reviewed scientific journals (including Journal of Extension) and presentations at multiple professional conferences and stakeholder meetings.

Proposed Budget:

Institution	PI(s)	Objectives	Year 1	Year 2	Total
Michigan State University	Thomas Loch	1-3	$262,585.98	$280,111.48	$542.697.46
Ohio State University	Matthew Smith	1	$0	$0	$0

University of Minnesota	Nicholas Phelps	1	$29,005	$29,685	$58,690
		Totals	**$291,591**	**$309,796**	**$601,387**

Non-funded Collaborators:

Facility	**Collaborators**
Harrietta Hills Trout Farm	Mr. Dan Vogler
Crystal Lake Fisheries, Inc.	Mr. Marvin Emerson
Superior Fresh	Dr. Steven Summerfelt
WI Dept. of Agriculture, Trace, and Consumer Protection	Dr. Myron Kebus

Project Summary

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

Justification

Infectious disease as an impediment to aquaculture productivity in the USA and the NCR.
According to the USDA National Agriculture Statistics Service (2020; ISSN 1949-1948), **>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. Although data for each fish species in each state in the North Central Region (NCR) is unavailable, it is abundantly clear that infectious diseases of fish are also a significant impediment to aquaculture productivity, as relayed in the 2020 NCR Aquaculture Roundtable Sessions (Columbus, OH). Despite such challenges and based on preliminary explorations in a 2020 North Central Regional Aquaculture Center (NCRAC) funded project aiming to evaluate the effectiveness of previously funded NCRAC research (led by Virginia Tech, co-PI Smith, and colleagues from Purdue University), the bulk of previous fish health studies appear to have focused on drugs (including Investigational New Animal Drugs) for use in aquaculture (i.e., reactive tools for use after disease outbreaks have already begun) as opposed to the alternative approach of empowering producers to prevent disease outbreaks before they ever occur (i.e., an ounce of prevention is worth a pound of cure). For producers to reap the benefits of proactive and disease-preventative approaches, however, they must have access to available fish health expertise, be equipped with a set of best management practices (BMPs) that are customized for the NCR and its variety of farm conditions, and have immediately implementable tools that reduce or altogether eliminate both known and unknown fish disease problems they face. Indeed, more thoroughly understanding and preventing infectious diseases are key to improving industry productivity in the NCR.

Improving fish health and productivity on coldwater farms in the NCR.
Despite coldwater farms comprising 22% of NCRAC farm food fish production (79 reported farms) and rainbow trout (*Oncorhynchus mykiss*) representing the most valuable cultured fish species in the NCR ($8.8. million farm-gate for food fish trout reported; 29% of the NCR industry by value; USDA 2019), **only four (out of >112) NCRAC-funded projects have specifically focused on improving salmonid productivity and profitability in this region, all of which were conducted prior to 1999**. There are unique challenges that can only be addressed on a regional level, and as such, not all trout research should be conducted in the western region of the U.S. This lack of emphasis on trout has not been lost on NCR aquaculture industry members, as was made abundantly clear in the 2020 NCR Aquaculture Roundtable Sessions (Columbus, Ohio), particularly in the salmonid and flow-through/semi-recirculation system discussion sections. Specifically, the Listening Session Assessment from NCRAC states “Practical Fish Health Applications” and “Fish Health and Disease Management” as specific focal points for the NCR industry, including Salmonids. These industry-voiced needs are at the core of the research-outreach study proposed below.

Bacterial coldwater disease: a significant challenge to coldwater fish farming.
Bacterial coldwater disease (BCWD), caused by *Flavobacterium psychrophilum*, is a critical disease issue affecting farmed trout and salmon, causing tens of millions of dollars in annual economic losses globally in the form of direct mortality, costly treatments that are partially effective at best, poor fish growth, and damage to the fillets (Figure 1A-E; reviewed in Loch and Faisal 2017; AVMA 2020). Partially owing to its efficient transmission from infected

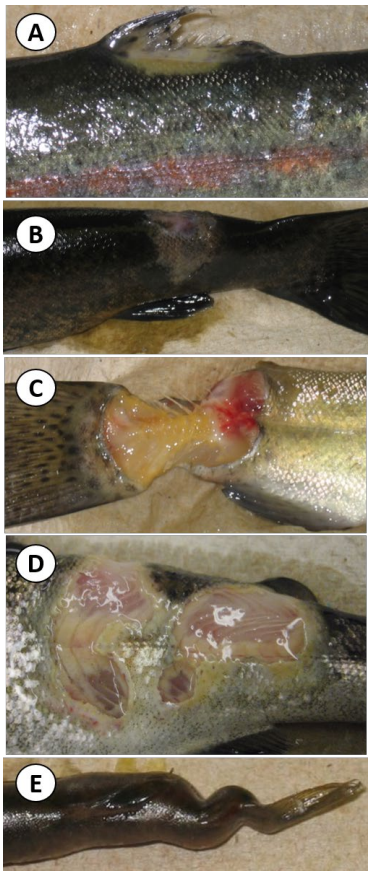


Figure 1. Selected external signs of disease in rainbow trout (*O. mykiss*) infected with *F. psychrophilum*, ranging from fin erosion (A) to complete fin loss and skin ulcers (B) to severe ulceration into the underlying muscle/fillet (C-D) to spinal curvature (i.e., scoliosis) in survivors (E). Note the yellow-tinged discoloration of the lesions in A-D that is characteristic of diseases caused by yellow-pigmented flavobacteria.

broodstock to offspring via reproductive fluids and within eggs (Brown et al. 1997), this bacterium is believed to occur everywhere salmon and trout are raised (Nematollahi et al. 2003), continues to be a leading cause of disease in salmonid farms across the USA, and thus has been identified as a priority pathogen in the 2020-2024 USDA - Agricultural Research Service (USDA-ARS) National Aquaculture Action Plan. Compounding losses caused by *F. psychrophilum*, only two antibiotics (i.e., Terramycin®, oxytetracycline; and Aquaflor®, florfenicol) are US-FDA approved to treat BCWD in food fish (www.fda.gov/cvm) and mounting resistance to both drugs have been reported in the USA (Van Vliet et al. 2017; AVMA 2020), leading to frequent treatment inconsistencies and occasional outright treatment failures. Of further concern, effective BCWD vaccines are not commercially available for use in the USA, a need that has also been deemed a research priority in the USDA-ARS National Aquaculture Action Plan.

Although rigorous studies to determine the incidence of BCWD in each NCR state are lacking, *F. psychrophilum* is one of the most important diseases negatively affecting trout and salmon productivity in the region. In Michigan, for example, multi-year surveillance studies revealed *F. psychrophilum* and its close relatives cause more losses in state fish hatcheries than all other pathogens combined (Faisal and Hnath 2005; Faisal et al. 2013; Van Vliet et al. 2015). Likewise, trout farmers, natural resource agencies, and aquatic veterinarians in Michigan, Wisconsin, and Missouri (see attached letters of support), as well as members of the Great Lakes Fishery Commission – Great Lakes Fish Health Committee (see attached letter of support) consider BCWD to be a top fish health concern in their respective states/region. Compounding the issue, not all diagnostic laboratories utilize culture media/diagnostic tests that allow for the detection of *F. psychrophilum* and other fish-pathogenic flavobacteria, which have specialized growth requirements and therefore often go undetected and/or misdiagnosed, leading to an underestimation of their role in disease-associated losses. Similarly, many trout farms and hatcheries have become accustomed to sub-optimal eye-up and hatch out rates, along with elevated mortality at the sac- and swim-up fry stages (i.e., "we always use lose X per year at this stage, it's normal"). Based upon our groups recent and ongoing USDA-NIFA funded studies, *F. psychrophilum* and other closely related flavobacteria are either directly causing or exacerbating such losses (see preliminary data below).

A need for integrated Extension and collaborative industry-guided research to tackle leading causes of fish disease in the NCR.

Our proposed research team is fully aware that fish health needs are not unique to the salmonid industry in the NCR, but rather across farmed fish species (from cold to cool to warm-water species), non-fish species (e.g. shrimp), farms (flow through to recirculating aquaculture systems to ponds), and NCR states. Nevertheless, given: a) the importance of trout farming in the NCR; b) the growing interest in Atlantic salmon (*Salmo salar*) farming; c) a lack of NCRAC funding (and funding applications) devoted to improving salmonid farm productivity since 1999; d) the widespread incidence and economic damage caused by *F. psychrophilum* in salmonid farms in the USA at large and in the NCR in specific; e) that one or more *Flavobacterium* spp. are important sources of disease (e.g., columnaris disease, caused by *Flavobacterium columnare*; bacterial gill disease, caused by *F. branchiophilum*, etc.) in all farmed freshwater fish species; and f) the experience and related ongoing studies of our proposed research team not only in advancing NCR fish health in general, but also generating practical tools for preventing and controlling flavobacterial fish diseases, we propose to educate and train producers, veterinarians, and veterinary students, while simultaneously arming NCR farmers with immediately deployable tools to effectively prevent and control one of the most problematic groups of fish pathogens in our region. To meet these priority industry-

identified needs, we have assembled a collaborative and multidisciplinary team of experts from industry, Extension, research, and combinations therein, to work in concert with one another to synergistically deliver on industry needs (Figure 2) every step of the way. The project team is well positioned to make immediate progress on the proposed objectives and complete the entire scope of work within the two-year project period. Although we recognize that we have requested a significant amount of the funds available in the 2020 NCRAC funding cycle for this ambitious project, we are quite optimistic that **should our proposed multidisciplinary study be funded, a maximal return on investment will be made for farmers in the NCR and far exceed that of multiple projects working independently towards improving fish health in the region**.

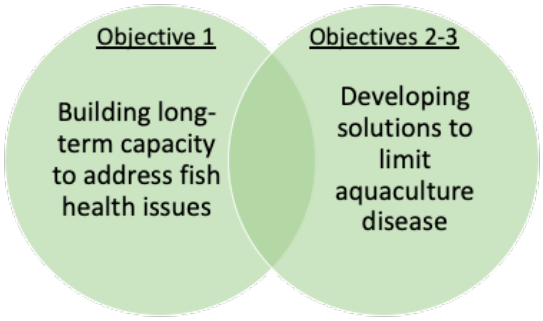


Figure 2. Overview of the strategic approach to improved fish health in the North Central Region.

Related Current and Previous Work

Preliminary Data & Resources Supporting the Proposed Extension and Research Activities.

Previous/ongoing fish health Extension activities within the NCR.

The need for accessible fish health resources in the NCR has been recognized for quite some time, but unfortunately this gap remains and thus is the rationale behind Objectives 1-3. In terms of the Extension activities that are largely proposed under Objective 1, the most relevant efforts previously conducted in the NCR were spearheaded in Wisconsin (and funded by NCRAC in 2008). The State of Wisconsin's Department of Agriculture, Trade and Consumer Protection (Dr. Myron Kebus) and University of Wisconsin-Stevens Point (UW-SP; Dr. Chris Hartleb) created free online fish health modules with topics on: 1) Introductory and Practices, 2) Risk Management and Biosecurity, 3) Water Quality Management, Monitoring and Disease Prevention, 4) Fish Health Inspections, 5) Veterinary Health Assessments, and 6) Cases Studies. This information for farmers, in addition to the modules created for veterinarians, are fantastic resources that can be viewed at any time. These modules can be found on the UW-SP Northern Aquaculture Demonstration Facility website under *resources*. The distinction between this previous work and what is proposed herein is to create a boots-on-the-ground aquaculture-trained veterinarian with region-wide Extension duties. In essence, to bring the modules created by Wisconsin to life through farm visits (complete with on farm fish health assessment, survey data, and sample collection), workshops, and the creation of additional tangible resources to help producers be better equipped for managing aquatic animal health on their farm. Thus, the proposed project would complement and uplift, rather than duplicate, the resources created by Drs. Kebus and Hartleb (see letter of support from Dr. Kebus).

Co-PI's Smith and Phelps also led a previously funded project entitled "*Comprehensive Training and Outreach Program to Expand Development of NCR Aquaculture,"* in which veterinarians, fish health professionals, and Extension collaborated to develop farmer workshops in several Midwest states. Working with various state associations, fish health was repeatedly identified as a need and thus a focus for the project. Water samples and fish were available for producers to obtain hands-on experience testing various water quality parameters and conducting an evaluation of the health of the available fish. The information that was generated and delivered was also recorded and placed on NCRAC's Vimeo channel. However, no farm visits were conducted as part of this project and a dedicated region-wide veterinarian was not provided. Here again, our proposed work would complement the work conducted through the comprehensive training and outreach program, as we understand producers have limited time to scour the Internet for assistance during a fish health emergency. It is optimal that producers will utilize the already available information and the information created from this project to support **proactiveness**; however, we all realize that it is necessary to be **reactive** when problems arise. For the duration of this project, and hopefully beyond should additional funding be secured, an aquaculture veterinarian will be there (phone, email, Zoom, or in-person) to support Midwest farmers working on being **proactive**, along with the times when being **reactive** is

necessary. Additional training materials (e.g., fact sheets, online modules, videos, etc.) produced as part of the proposed project will build from this previously developed material and will include input from several producers and aspiring fish health professionals to ensure they are easily understandable to those without extensive training in the subject matter being presented.

Previous/ongoing research on flavobacterial fish diseases in the NCR.
Although published reports of regionwide surveillance for *F. psychrophilum* in the NCR do not exist, extensive flavobacterial surveillance has and continues to be undertaken in some NCR states. For instance in Michigan, several multiyear studies have uncovered how prevalent multiple fish-pathogenic *Flavobacterium* spp. are in both feral and captive-reared salmonid broodstock, whereby annual prevalence can exceed >90% in some affected stocks (Loch et al. 2013; Loch and Faisal 2015; Van Vliet et al. 2015; Faisal et al. 2016). Moreover, additional studies have revealed that *Flavobacterium* spp. are commonly transmitted along with reproductive fluids and eggs in Michigan salmonids and are tied to previously unappreciated early life stage mortality (Loch and Faisal 2016a; Loch and Faisal 2016b; Loch and Faisal 2018). In addition to clarifying their previously under-recognized role in disease outbreaks, these studies generated materials (e.g., bacterial isolates recovered from infected fish) that were further analyzed in recently completed and ongoing USDA-NIFA funded studies (by PI Loch's group) to determine if certain *F. psychrophilum* "strains" (i.e., genetic variants) were more problematic (i.e., more widespread and/or most frequently associated with disease outbreaks) than others, thereby representing important targets to emphasize in future disease prevention and control plans. Using these *F. psychrophilum* isolates, as well as additional isolates opportunistically recovered from other US states, it became clear that some strains were indeed repetitively associated with disease outbreaks in farmed salmonids and appeared to be at the forefront of BCWD-associated losses across the USA, including in some NCR states (Van Vliet et al. 2016; Knupp et al. 2019; Sebastião et al. 2020). Unfortunately, without regionwide flavobacterial surveillance, the *F. psychrophilum* strains acting as the primary drivers behind the BCWD-associated losses in the NCR (see Letters of Support) remain all but unknown. *Why should NCR producers care about this knowledge gap? Because a growing body of evidence suggests that until BCWD prevention and control methods account for the tremendous variation present within this trout and salmon pathogen, their effectiveness will be equally variable and inconsistent.*

The need for an effective and immediately deployable BCWD vaccine: past & ongoing efforts.
BCWD continues to drive losses that can reach up to 70% in affected farmed salmonid stocks (Nematollahi et al. 2003) largely as a result of poor antibiotic treatment outcomes (Bruun et al. 2000; Van Vliet et al. 2017), partially effective egg disinfection techniques (Kumagai et al. 1998), and possibly most important, the lack of commercially available vaccines. Many studies have attempted to generate effective BCWD vaccines with little success (reviewed in Gomez et al. 2014). However, PI-Loch's team has been collaborating with Dr. Ken Cain's group (U of Idaho) to evaluate the effectiveness of a new BCWD vaccine (Ma et al. 2019; Bruce et al. 2020) that uses a live *F. psychrophilum* strain that was modified in the lab so as not to cause disease in vaccinated fish. Other promising ongoing studies (funded by Wisconsin Sea Grant and led by Drs. McBride, Sepulveda Villet, and Hunnicutt, for example) are currently working on ways to generate mutations in closely related *Flavobacterium* spp. (e.g., *F. columnare*, cause of columnaris disease) in the lab with an eye towards vaccine development and are in the proof-of-concept stage (https://www.seagrant.wisc.edu/news/secretion-system-is-key-to-understanding-columnaris-disease). Although promising, some have safety concerns when using "live attenuated" vaccines, including bacterial reversion to a disease-causing form and the spreading of antibiotic resistance genes, to name a few. These concerns, as well as the lengthy times it takes to get such vaccines licensed by FDA, reinforce the need for multiple and more immediately deployable approaches.

An alternative method that avoids these pitfalls, **can be deployed immediately** when following FDA Center for Veterinary Biologics guidelines, and could be **commercially available immediately following experimental trials** with veterinary oversight is the use of "whole killed vaccine" preparations. Indeed, PI-Loch is currently involved in a USDA-NIFA funded collaborative study (Grant # 2019-70007-30372) evaluating this approach for developing a vaccine against columnaris disease in multiple fish species. Although some studies have tested this approach for protecting fish against BCWD with varying effectiveness, experiments failed to account for the substantial *F. psychrophilum* strain diversity within the USA that was unknown at the time and uncovered only recently (see Objective 2 below). In this context, a research group in the United Kingdom recently characterized hundreds of *F. psychrophilum* strains from Europe (Ngo et al. 2017) and, after identifying the predominating disease causing strains, tested an experimental whole-killed vaccine specifically targeting those strains, leading to very promising protective effects in vaccinated fish (Hoare et al. 2017). Importantly, this vaccine was administered via immersion to

2 - 5g (0.004 - 0.01 lb.) fish, making this a practical and promising means of vaccinating young fish when they are most vulnerable to BCWD. Unfortunately, it is impossible to extrapolate those results to the USA and the NCR, as >90% of the *F. psychrophilum* strains in the USA are distinct from those of Europe (Van Vliet et al. 2016; Knupp et al. 2019). Nevertheless, these promising results, along with our findings that predominating strains are the source for most of the BCWD outbreaks elsewhere in the USA (Van Vliet et al. 2016; Knupp et al. 2019), provide strong justification for attempting a similar approach here in the NCR.

Statement Regarding Duplication of Research

The following terms were searched in the USDA Current Research Information System (CRIS), the National Sea Grant Office Funding page, and the NOAA Office of Aquaculture Funding Opportunities Page without any research duplication being discovered: *Flavobacterium*, biosecurity, aquaculture, fish health assessment, and fish disease. Rather, this search revealed that our proposed project will build from, rather than duplicate, any previous and ongoing research activity.

Anticipated Benefits

There are a substantial number of benefits Industry will receive should the proposed work be funded. One of the primary audiences and benefactors of the proposed study will be trout farmers in the NCR. Indeed, many trout producers lose a significant number of fish, often early on in growth, for unexplained reasons more years than not. Due to our experience and preliminary testing by PI-Loch's group, *F. psychrophilum* is likely directly contributing to these losses, as has been determined in NCR states where in depth studies have been undertaken (Faisal et al. 2011; Loch et al. 2013; Van Vliet et al. 2015; Loch and Faisal 2018). As a benefit of this work, producers will know, for the first time, how and what variants of *F. psychrophilum* are contributing to these losses, what the sources of these infections may be, and possibly most importantly, how to proactively protect their fish – through use of a region-specific vaccine. Upon study completion, the regionally prevalent bacterial strains from which the BCWD vaccines were derived will be maintained (by PI Loch and at no charge to NCRAC) and be available to producers under veterinary and fish health professional oversight (e.g., Kennebec River Biosciences, Aquatactics, etc.) for cost-effective autogenous vaccine production. This indeed is a model being widely used across the USA and in compliance with USDA-APHIS Center for Veterinary Biologics, with growing success. In addition, this research approach will be broadly applicable, in particular to other regions of the U.S. with significant trout production (e.g. northwest and southeast), where the needs are abundantly clear. An important additional benefit of developing and capitalizing on BCWD preventative (i.e., vaccine) measures is reduced reliance on reactive and expensive antibiotics that are increasingly being hampered by growing reports of antibiotic resistance. In addition to avoiding inconsistent treatment outcomes that are costly, reduced (or eliminated) antibiotic use will be concurrently beneficial for producers serving customers who view antibiotic use in a negative light. A no less important benefit to NCR trout farmers will be the additional fish health data (i.e., in addition to flavobacteria; Objective 2; all data will be kept confidential to each trout farm and anonymized for publication/outreach), thereby highlighting additional and possibly previously unrecognized production impediments that can be addressed via newly developed and implemented BMPs (Objective 1) and/or prioritized as future NCR research targets.

In addition to benefits to NCR trout farmers, all producers in the region will have access to the regional Extension veterinarian for assistance, a resource that many in the NCR have never been able to benefit from. At the same time, an aquaculture veterinarian will benefit from receiving additional hands-on experience here in the NCR, hopefully increasing trust between producers and researchers/Extension. Some producers in the region will have an actual aquaculture veterinarian on their farm for the first time. This gives the producer the opportunity to discuss limiting stress on the animals, which will undoubtedly lead help the producer with a host of biological or system design questions. All NCR farmers and veterinary students will also benefit if they decide to participate in any workshop trainings or if they utilize the published content developed by the Extension veterinarian (e.g., BMP documents). Similarly, a post-doctoral researcher partially funded by the requested funds will gain tremendous research, outreach, and scholarly expertise in working closely with NCR producers, the proposed project team, and the Extension veterinarian. Multiple undergraduate interns at MSU will likewise benefit from involvement in the proposed project. Additionally, we believe that veterinary students, current veterinarians, and College of Veterinary Medicine programs will find interest in our farm tours and BMPs materials (videos and prints) that are proposed.

In a similar broadly beneficial context, Extension and research outputs (e.g., NCR specific and generalizable farm BMPs, fish health related manuals/videos/documents, training opportunities, etc.) will be provided open access

through www.NCRAC.org and any videos created will be through NCRAC's Vimeo channel. All online educational materials will be developed in a manner to be easily understood by the target audience (e.g. producers, and/or veterinarian and vet student). Workshops will be cost recovery only; increasing the opportunity for quality turnout. We will engage the State aquaculture association and universities' College of Veterinary Medicine, if there is one in the state, to increase awareness of our presence for our audience. Research findings will be published in peer-reviewed scientific journals (forecast at least three-four research manuscripts), and at least one publication to the *Journal of Extension* showcasing NCR Extension activities. Of note, all *Journal of Extension* publications are open access, increasing the opportunity for this work to be available to a broader audience. A more thorough overview of the anticipated benefits of the proposed project can be found in the Logic Model.

Objectives

Fish health has long been identified as a high priority issue that must be addressed to improve aquaculture production in the NCR. This was reiterated during multiple species-focused discussions at the 2020 NCR Aquaculture Roundtable Sessions - the inspiration for this proposal is a direct result of those conversations. Consequently, the overall goal for this proposed project is to address industry-identified fish health needs in the NCR by 1) building long term producer and professional fish health capacity, and 2) developing immediately deployable innovative solutions to production limiting diseases. Our approach is informed by previous NCRAC and USDA-funded research by the project team members that has identified strategic opportunities to help solve the region's fish health issues. To that end, this ambitious project will employ a unique and multifaceted approach (Figure 3) that incorporates the creation of a first-of-its-kind veterinarian outreach program in the NCR, with vaccine evaluation for bacterial coldwater disease, one of the region's most consequential fish diseases. More specifically, we will:

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.
2. Determine, for the first time, the predominating flavobacterial variants driving economic losses in the NCR trout industry.
3. Evaluate the effectiveness of newly developed vaccines in preventing losses caused by regionally predominating flavobacteria under laboratory and field conditions.

Deliverables

1. Publish fish health survey results in an Extension document.
2. Deliver fish health survey results and knowledge during farm visits in NCR.
3. Create site-specific and generalizable BMPs for producers.
4. Conduct workshops for farmers, veterinary students, and veterinarians.
5. Create low-cost fish health kit along with videos and prints describing how material are used.
6. Generate and deploy vaccine preparations for use against a bacterium responsible for one of the most damaging diseases of coldwater fish in the USA.
7. Determine primary transmission sources for flavobacterial diseases on NCR farms and interrupt these sources through targeted and newly developed best management practices.
8. Cryo-bank of bacterial isolates and samples (maintained at no charge to NCRAC by PI Loch) available upon request for aquatic veterinarians, fish health laboratories, and researchers to use in NCR farm disease prevention and control plans.
9. Provide education, training, and outreach experience for undergraduate students, graduate students, and a post-doctoral researcher involved in the proposed research.
10. Publications in peer-reviewed scientific journals (including Journal of Extension) and presentations at multiple professional conferences and stakeholder meetings.

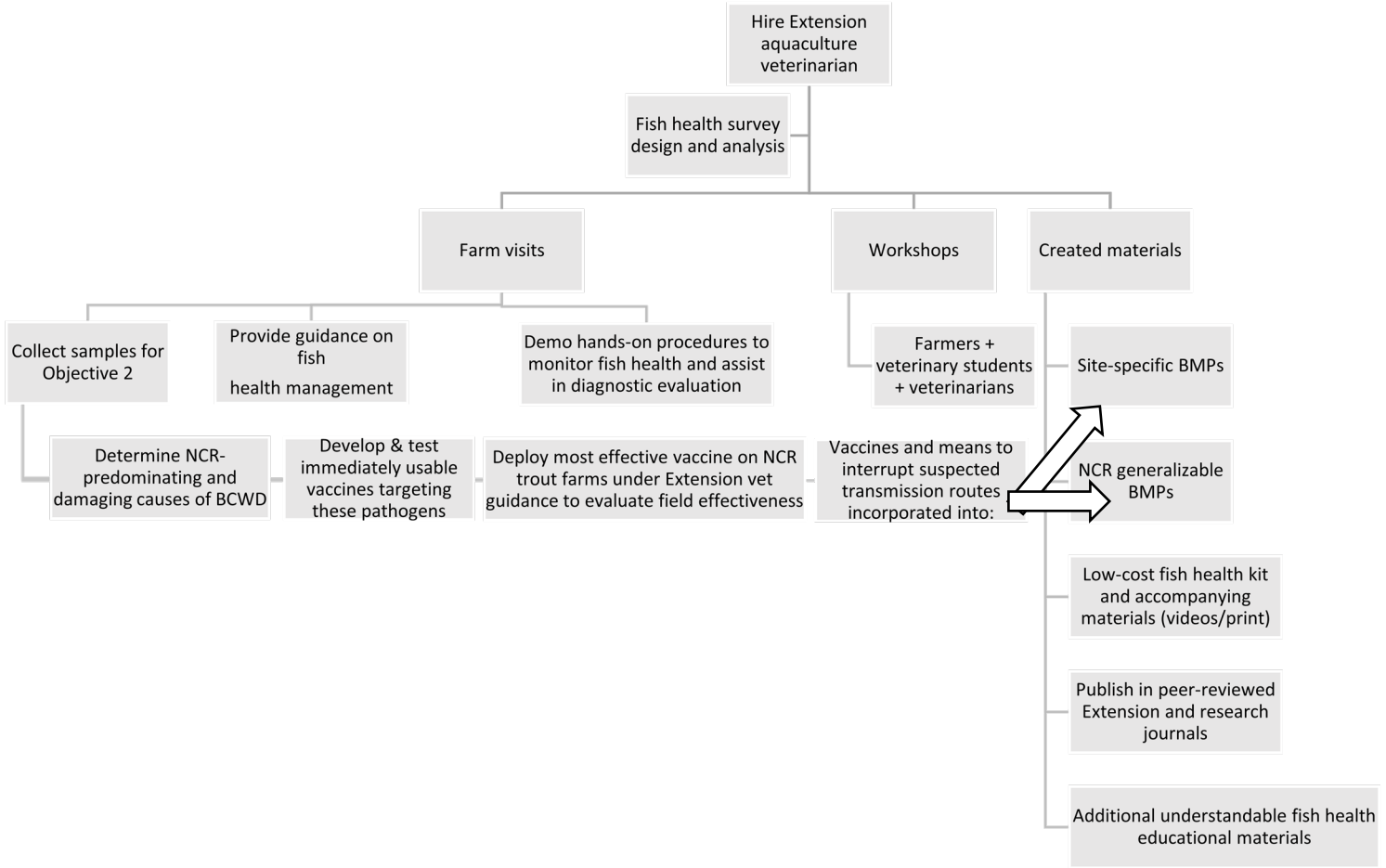


Figure 3. Overview depicting the sequence of events and integration of the proposed synergistic project activities.

Procedures

Objective 1. To 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 (*To be fulfilled by Smith, Phelps, and Loch*)

One of the biggest challenges facing the growth and success of economically and environmentally sustainable aquaculture are infectious diseases. Requests for hands-on fish health training and water quality education from numerous NCR aquaculture associations and university groups led to the funding and recent completion of a NCRAC project managed by Co-PIs Smith and Phelps, focused on these objectives. The project, *Comprehensive Training and Outreach Program to Expand Development of NCR Aquaculture*, was successful (i.e., 330+ workshop participants, 15+ hours of video content created, and very positive attendee evaluations); however, the fish health resources available to producers (including guides for developing fish health best management practices, BMPs), and the number of trained fish health professionals in the NCR remains limited. The need to address these limitations was also emphasized during discussions at the 2020 NCR Aquaculture Roundtable Listening Session, which highlighted a continuing need for additional fish health support within the region. To address these industry-driven needs, the following tasks are proposed:

Activities Proposed & Sequence of Events:

Task 1: Design, conduct, and analyze an NCR-wide fish health survey in concert with a newly hired Extension Aquaculture Veterinarian (*To be fulfilled by Smith and Phelps*)**.**

A veterinarian with a strong background in aquatic animal health will be funded to lead a region-wide action plan to address fish health needs - a first for the NCR - with coordinated support from existing fish health and outreach professionals. The first task of the newly hired veterinarian will be to conduct a region-wide fish health survey. Although previous research and experience of the project team have identified initial priorities, we will conduct a thorough survey to identify the most pressing fish health concerns for NCR aquaculture producers. In addition, the survey will identify knowledge gaps and opportunities for intervention that will guide the development of NCR-specific fish health BMPs, outreach needs and future research directions. We will employ the Delphi approach (Bunting 2008), a multi-stage iterative survey design, to solicit feedback from NCR fish health stakeholders (i.e., aquaculture producers, veterinarians, academic researchers, and state agency personnel). Through various NCR aquaculture listservs and professional networks of the project team, we will invite stakeholders via email to participate in the survey. The online-based survey will be conducted in two rounds, first asking respondents to identify high-priority diseases with production limiting or regulatory impacts to the NCR aquaculture industry. The survey participants will then be asked to rank all diseases identified in round one, assign confidence scores, and provide a justification for their decisions. Demographic data, such as stakeholder type (i.e., producer, veterinarian, etc.), location (i.e., state), species produced, fish health expertise (i.e., 1-5), etc. will allow the project team to evaluate the needs and knowledge based on the diversity of stakeholder perspectives within the NCR. The surveys will be completed within the first four months of the project (preparation will be conducted to ensure a quick start) to help inform the development of BMPs in Objective 1. The survey instrument and data storage will be approved by the University of Minnesota's Institutional Review Board to ensure compliance with data privacy protocols. These surveys will not only focus activities within this objective, but also serve as the foundation of continued fish health monitoring within the region and likely focus future NCR research needs.

Task 2: Provide Extension veterinary expertise to aquaculture producers in the NCR, leading to rapid responses to fish health needs, Best Management Practices for NCR farms, fish health related manuals/videos/documents, and training opportunities (*To be fulfilled by Smith, Phelps, and Loch*).

Once the survey is developed, conducted, and analyzed, the veterinarian will develop a program to help address the stated needs of the industry. The PIs of this proposal will advise the veterinarian on which farmers/Extension personnel to contact in order to set up programming in their state, how the veterinarian can best refine their programs, and how to do so efficiently and complimentary with existing capacity. Through our contacts with farmers and our desired impacts on the industry, we know the veterinarian will conduct at least twelve farm visits (ideally in each NCR state), create practical farm- and NCR-specific BMPs for fish health (complimenting information developed by Drs. Kebus and Hartleb), deliver training on the application of these BMPs, and additional training for aspiring fish health professionals throughout the region. A key component to Objective 1 will be the Extension veterinarian's availability to the industry.

On-site visits to farms in each of the 12 NCR states will be to directly address on-site fish health concerns, provide guidance on fish health management, collect samples for Objective 2, and demonstrate hands-on procedures that can be used by producers to help monitor fish health and assist in diagnostic evaluation. In coordination with our industry liaisons and local Extension specialists, farm selection will be prioritized to represent the diversity of fish species and production systems in the NCR, and when possible, additional effort will be made to visit salmonid systems to support Objective 2. The feasibility of visiting a substantial number of farms throughout the Midwest is high given that there is an Extension veterinarian dedicated to the project. The site-specific BMPs will be developed for each farm we visit, which will then guide the creation of region-specific BMP templates. The fish health BMP templates will address disease prevention, biosecurity, health monitoring, and action plans for disease outbreaks and will build from those that have been previously developed (e.g., https://www.ncrac.org/files/biblio/FS115Biosecurity.pdf). The BMP templates will then be widely disseminated to NCR producers, with assistance available from the project team to answer questions and review draft plans prior to implementation. Templates will be hosted on www.NCRAC.org.

Although some producers report they have access to trained aquaculture veterinarians and fish health professionals, many producers do not have sufficient access to satisfy their needs. Our proposed work aims to fill this gap by empowering producers and training professionals, while creating a region-wide Extension-minded veterinarian available for at least two years. Producers need practical fish health trainings and tools that require a relatively small financial/time investment but yield a significant ROI. For example, our new aquatic animal veterinarian will create a low-cost (<$500) fish health kit with videos/manuals on how to utilize each piece of the kit (containing, for example, a microscope with cell phone adapter, dissection tools, microscopy materials, etc.), why it is necessary for NCR operations, where to find the items, and how to analyze the results that the producer obtains. The project team will purchase enough supplies for two of these kits to be available for farm visits and workshops; although it is not financially feasible to purchase these for all farmers who will engage with our veterinarian over the two-year period. In addition, on-farm trainings, workshops (at least four, each in a different NCR state), and other Extension programming will be conducted during this project.

Task 3: Provide on-farm training and workshops for veterinarians, veterinary students, and NCR producers (*To be fulfilled by Smith, Phelps, and Loch*)**.**

The veterinarian will coordinate with veterinary schools in the region (to include MSU, OSU, and UMN) to conduct farm tours so that veterinary students will learn more about the practical aspects of fish farms in the NCR. The PIs will work with the MSU, OSU, and UMN veterinary schools and utilize our connections to make sure this happens. Two of the four workshops will have an additional day just for veterinarians, veterinary students, and other aspiring fish health professionals so that they can obtain practical hands-on skills towards building more fish health capacity in the NCR. A charter bus will be rented to take the students to the farm and back, giving students additional opportunities to chat with any farmers, veterinarians/fish health professionals, and Extension. This event (led by the Ohio Aquaculture Association) was very successful when it was conducted in 2018 in Ohio.

Sustainability of the Extension veterinarian

The Extension veterinarian (housed at MSU) will be dedicated to our proposed work over the next two years and PI Loch and Co-PIs Smith and Phelps will facilitate and support this veterinarian throughout Objective 1. This will afford the PI/Co-PIs time to work together to develop additional regionally important fish health proposals to further support the veterinarian and regionally important research. Co-PI Smith has already been in discussion with Ohio Sea Grant about creating a fish health Extension-focused proposal for the next round of National Sea Grant RFAs, if our work is applicable to the call. Ohio Sea Grant Director Chris Winslow is very supportive of this work. Additionally, OSU Extension, Ohio Aquaculture Association, Ohio Division of Wildlife, and Ohio Department of Agriculture met in the summer of 2019 to start the discussion about the need for fish health expertise in the area. While COVID-19 has undoubtedly caused budgets to be upended, we will reinvigorate this group to discuss how we can retain the expertise that is gained through funding of this project. Please note the letter of support from the Interim Director of Extension at OSU. The reality is that few hard-funded positions are being created in our region. What is imperative is to first show extreme value to farmers, agencies, and the university. Then, over time, a strong case can be made to develop this valuable resource into the budget of the university, instead of relying solely on extramural funds. *In this context and since being notified of funding, PI Loch and Co-PIs Smith and Phelps all initiated discussions with their respective university administrations, all of whom expressed interest in potentially extending the Extension Veterinarian position for up to three years past the two years funded by this proposal.*

Excitingly and as of October 2021, the College of Veterinary Medicine at Michigan State University has committed to providing funding to maintain the Aquaculture Extension Veterinarian position beyond the two years of the current project in the form of a third year as a fixed term faculty, then in years 4 and 5 as a tenure stream faculty position. Thus, this initial investment by NCRAC has been leveraged into a long-term resource to the aquaculture industry within the NCR. <u>It is important to note that the objectives and deliverables of the proposed project will not change as a result of this longer term commitment to this Extension Aquaculture Veterinarian position.</u>

Objective 2. To determine, for the first time, the predominating flavobacterial variants driving economic losses in the NCR trout industry (*To be fulfilled by Smith, Phelps, and Loch*)

PI-Loch's recent and ongoing USDA-NIFA funded research has made advances toward improved BCWD control by uncovering why current BCWD prevention and control methods continue to be unsatisfactory. Findings to date suggest that a primary factor behind continued BCWD prevention and control inconsistencies may be the overwhelming diversity of *F. psychrophilum* strains (i.e., variants) causing disease outbreaks in the USA. In this context, >500 *F. psychrophilum* isolates from 23 US states were recently analyzed by PI Loch's team, revealing the existence of >100 strains, >95 of which are unique to the USA (Figure 4; Van Vliet et al. 2016; Knupp et al. 2019; Sebastião et al. 2020; Li et al. in preparation). **Why does this diversity matter?** <u>Similar to the need for repeated annual iterations of vaccines to protect humans against the flu, it appears current BCWD vaccine failures may, in part, relate to the tremendous variation in *F. psychrophilum* strains that are responsible for BCWD outbreaks in the USA and yet to date, have essentially been treated as one "strain".</u>

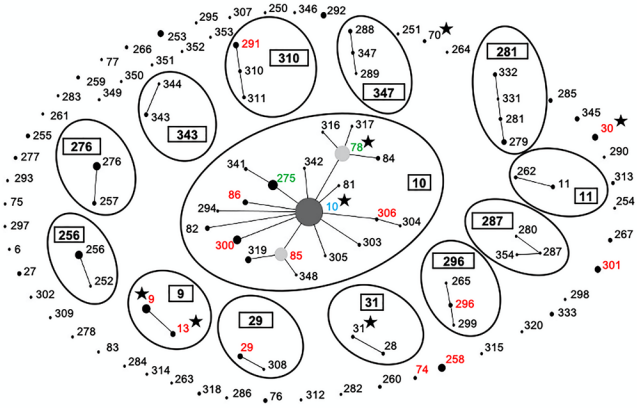


Figure 4. eBURST diagram of 507 US *Flavobacterium psychrophilum* isolates genotyped via multilocus sequence typing, which fell into 103 sequence types (STs). STs colored in black were recovered from 1 US state, whereas those colored in red, green, or blue were recovered in 2-4, 5-9, or >10 US states, respectively. Stars denote STs (e.g., STs 9, 10, 13, 30, 31, 70, and 78) that have also been recovered outside the USA. Clonal complexes (CCs) are encircled and the founding ST for a CC is depicted as a number within a rectangle. The founding ST, or founder, is the ST with the highest number of single-locus variants (SLVs).

Although much progress has been made in clarifying this *F. psychrophilum* diversity across the USA, such data for most NCR states is all but non-existent. Out of the ~500 *F. psychrophilum* isolates that have been analyzed by PI-Loch's group (Van Vliet et al. 2016, Knupp et al. 2019, Sebastião et al. 2020, Li et al. in preparation), only 15 isolates originated from NCR states (e.g., WI=7, SD=4, MN=3, IN=1) with the exception of MI (n=112). However, it is important to note that all analyzed NCR isolates originated from state or federal trout and salmon facilities, leaving knowledge as to the variants driving BCWD losses in private NCR trout and salmon farms unknown.

Similarly, a significant challenge facing efficacious *F. psychrophilum* vaccine development is the serological diversity of this species (Gomez et al. 2014). Unfortunately, large scale analyses of US *F. psychrophilum* serotypes have never been conducted and rather have been limited to one to a handful of isolates using a variety of methods. In the same context, the serotypes of *F. psychrophilum* isolates causing disease in NCR trout farms is completely unknown, a shortcoming of significant importance when aiming to develop effective vaccines. To fill these knowledge gaps and arm NCR producers with new and effective tools to prevent BCWD (Objective 3), the following tasks are proposed:

<u>Activities Proposed & Sequence of Events:</u>

Task 4: Disease surveillance, flavobacterial isolation and identification from NCR farms (*To be fulfilled by Smith, Phelps, and Loch*)**.**

In concert with onsite visits to farms in each of the 12 NCR states (Objective 1; year 1), samples for flavobacterial isolation will be collected by the Extension veterinarian, targeting multiple life stages (e.g., eggs, fry, fingerlings, and broodstock, when present) and moribund fish (when present). Collected samples will either be shipped overnight or driven (when logistically possible) to PI-Loch's laboratory. Using optimized and routinely employed flavobacterial culture and identification techniques (Loch et al. 2013; Van Vliet et al. 2015; Knupp et al. 2019), flavobacteria will be isolated, identified, and cryopreserved for molecular analyses (Tasks 5 and 6) and vaccine experiments (Objective 3). Although flavobacteria are being emphasized under Objectives 2 and 3, full clinical examinations, fish health assessments, and thorough diagnostic evaluation (e.g., bacteriological, virological, and parasitological examination) will be performed on all fish as regularly performed in PI-Loch's lab for the state of Michigan (AFS-FHS 2014). Thus, not only will farmers be alerted to the presence of harmful flavobacteria, but also will be made aware of all other findings (in collaboration with the Extension veterinarian) that can be used to broadly improve fish health on each respective farm (and will be invaluable for guiding future similar efforts at tackling top fish health challenges in the NCR).

Task 5: Genotyping of F. psychrophilum isolates recovered from the NCR via multilocus sequence typing (MLST) (*To be fulfilled by Loch*)**.**

Bacterial isolates PCR-confirmed as *F. psychrophilum* (Task 4) will be genotyped via MLST and phylogenetically analyzed as routinely performed by our research group (Van Vliet et al. 2016; Knupp et al. 2019; Sebastião et al. 2020; Li et al. in preparation). In brief, near complete sequences of seven housekeeping genes (*trpB*, *gyrB*, *dnaK*, *fumC*, *murG*, *tuf*, and *atpA*) (Nicolas et al. 2008) will be PCR-amplified from each *F. psychrophilum* isolate, PCR products electrophoresed and viewed under UV transillumination, and then amplicons will be purified (via ExoSAP-IT) and submitted for sequencing at the Genomics Technology Support Facility (MSU). Based on our previous experience and through the sampling of multiple life stages and moribund fish, we are forecasting that 10-20 *F. psychrophilum* isolates will be genotyped from each facility. In the unlikely instance that fewer *F. psychrophilum* isolates are recovered than anticipated, our research team will capitalize upon connections with regional colleagues and enroll isolates from other facilities in the NCR into our study. Importantly, we will also be MLST-typing multiple single colony forming units (cfus) from individual fish, as our previous work (Van Vliet et al. 2016; Knupp et al. 2019) and those of others (Avendaño-Herrera et al. 2020) have revealed that multiple sero-/geno-variants can be present in a single fish.

***Task 6: Serotyping of F. psychrophilum isolates recovered from the NCR via multiplex* PCR** (*To be fulfilled by Loch*)**.**

Using the *F. psychrophilum* multiplex PCR (mPCR) assay developed by Rochat et al. (2017) that has been optimized in PI-Loch's lab, cryopreserved *F. psychrophilum* isolates recovered from NCR farms (Task 4) will be molecularly serotyped. In brief, genomic DNA (gDNA) from each isolate will be extracted using a routinely used commercial kit/protocol, DNA quantified, and then assayed using an optimized protocol based upon Rochat et al. (2017). Amplified PCR products will be electrophoresed, viewed under UV transillumination, and each isolate assigned to its respective mPCR serotype (Rochat et al. 2017).

Upon completion of all tasks under Objective 2, we will have determined, for the first time, the contemporarily predominating *F. psychrophilum* strains that are impeding NCR trout farm productivity, while simultaneously uncovering some of the sources of infection and transmission routes across the NCR, thus informing biosecurity and fish health management plans (Obj ective1) to subsequently disrupt them. As the most damaging *F. psychrophilum* variants are identified, so too will their serotype (i.e., how a fish's immune system views when mounting an immune response to their invasion) be determined so that generated vaccine preparations (Objective 3) are built to target them.

Objective 3. Evaluate the effectiveness of newly developed vaccines in preventing losses caused by regionally predominating flavobacteria under laboratory and field conditions (*To be fulfilled by Smith, Phelps, and Loch*)

Recent research conducted in the United Kingdom clearly shows the promise that whole-killed vaccine preparations hold for protecting farmed salmonids against BCWD, provided that these preparations account for the genetic and sero- diversity of *F. psychrophilum* present within the region of use (Hoare et al. 2017; Ngo et al. 2017; Hoare et al. 2019a; Hoare et al. 2019b). After identifying the predominating *F. psychrophilum* killing fish in the NCR (Objective 2), we will utilize straightforward lab techniques to test the BCWD-protective effects of immediately deployable killed vaccine preparations under lab and field conditions. To this end, the following research is proposed:

Activities Proposed & Sequence of Experiments:

Task 7: Evaluate the protective effects of vaccination with whole-killed F. psychrophilum variants in rainbow trout challenged with NCR-predominating causes of BCWD (*To be fulfilled by Loch)***.***

Sub-task 7a: Selection of predominating F. psychrophilum variants for use in vaccine preparations, determination of growth kinetics, and preparation of formalin inactivated bacteria. The three NCR-predominating *F. psychrophilum* genetic and/or sero-variants (Objective 2) will be selected for use in vaccine preparations and laboratory challenge experiments. As PI-Loch's ongoing USDA-NIFA funded research has revealed that some *F. psychrophilum* variants vary in growth kinetics and to ensure that vaccine preparations are derived from cultures in a logarithmic phase of growth, *in vitro* growth kinetic experiments will be performed for each of the three strains as previously described (Loch and Faisal 2014). Vaccine preparations will be completed by growing each of the three selected *F. psychrophilum* isolates in gently stirring tryptone yeast extract salts (TYES) broth until reaching a logarithmic phase of growth as routinely done in PI-Loch's lab, harvested, and formalin inactivated per routine protocol (Hoare et al. 2017). Inactivated cells will then be washed, concentrations adjusted to an optical density corresponding to ~ 10^9 colony forming units (cfu)/mL, and inactivation confirmed via bacterial culture. Upon completion, three monovalent (i.e., single isolate) vaccine preparations and one trivalent (i.e., all three isolates combined in equal parts) will be ready for use in vaccine efficacy experiments.

Sub-task 7b: Origin of fish for challenge studies. The laboratory vaccination and challenge experiments will be performed in rainbow trout, a primary species affected by BCWD that our research team also has extensive experience in hatching and maintaining in the laboratory. Industry standard eyed rainbow trout eggs will be obtained from a commercial vendor and maintained in flow-through egg stacks/heath trays supplied with dechlorinated pathogen-free (11 ºC±1; ~52 ºF) until hatching at the Michigan State University Research and Containment Facility as routinely performed. Just prior to exogenous feeding, alevins will be transferred to flow-through PVC tanks supplied with dechlorinated pathogen-free (11 ºC±1; ~52 ºF) and then fed *ad lib.* (photoperiod of 12 hours of light) upon feeding commencement.

Sub-task 7c: Assessing the protective efficacy of whole-killed F. psychrophilum vaccine preparations under controlled laboratory conditions. Upon reaching ~3g (0.006 lb.), multiple groups of rainbow trout (n=20, in triplicate; housed in 37.8L, 10 gallon flow through aquaria) will be immersion vaccinated in a solution containing one of the four vaccine preparations (Task 7a; 3 monovalent, 1 trivalent) at a 1:10 ratio vaccine solution to tank water (i.e., ~10^8 cfu/mL), or will be sham-vaccinated in tank water alone (i.e., unvaccinated controls). It should be noted, however, that we will conduct several small pilot experiments using similarly aged rainbow trout from PI-Loch's ongoing studies to initially evaluate vaccine immersion concentration and duration and will modify if needed. At ~300 degree days (Hoare et al. 2017), triplicate groups of fish will either be booster vaccinated using one of the four vaccine preparations in the same fashion, sham booster vaccinated (i.e., vaccinated only once), or sham vaccinated a second time (i.e., unvaccinated controls). At ~600 degree days, treatment groups will be immersion challenged with one of the three selected *F. psychrophilum* strains as previously described (Hoare et al. 2017) and routinely performed in PI-Loch's lab and then returned to their respective flow-through aquaria. Replicate groups vaccinated with the trivalent vaccine preparation will be similarly immersion challenged with one of each of the three *F. psychrophilum* strains to similarly assess the protective effects against each of the variants alone. Fish will be monitored daily for 30 days (subject to extension based upon course of mortality); terminally moribund fish will be euthanized via carbonate buffered tricaine methanesulfonate (MS-222) at a concentration of 250 mg/L, and mortalities/euthanized fish subsequently necropsied, clinically examined, and bacteriologically analyzed. Gross pathological lesions will also be scored and means/standard deviations calculated. The cumulative % mortality (CPM) and relative % survival (RPS) of all replicates will be calculated: RPS = [1-(% mortality of vaccinated fish / % mortality of non-vaccinated fish)] x 100. Analysis of CPM and RPS of challenge studies will be analyzed by one-way ANOVA and pairwise comparisons will be made using Tukey's test.

Task 8: Evaluate the protective effectiveness of the most efficacious BCWD vaccine preparation under NCR-field conditions (*To be fulfilled by Smith, Phelps, and Loch*)**.**

The BCWD vaccine preparation generating the highest RPS (i.e., most protective) under Objective 2 will be selected for field trials at an originating NCR farm during the final phase of the project under the guidance and oversight of our licensed Extension veterinarian (Obj. 1) and in compliance with USDA-APHIS Center for Veterinary Biologics. At least three production groups (i.e., tanks or raceways) of rainbow trout (size comparable to

Objective 2) will be immersion vaccinated per the process determined to be most effective under Objective 2, and at least three matching production groups will be mock vaccinated in an identical fashion. Of note at least one NCR trout facility that experiences BCWD-losses is interested in participating in these field trials. Depending on interest in participation by additional NCR trout farms, we have further capacity to conduct identical field trails at one additional NCR trout farm. In the unlikely event that such interest does not exist, field trials will be conducted at a Michigan DNR hatchery raising rainbow trout with a history of BCWD (please see letter of support). Production parameters (e.g., growth, fish quality) and CPM will be compared between treatments, and RPS in vaccinated groups farmed under field conditions will be calculated. Additionally, at two points in the rearing cycle (to be determined based upon historical issues with BCWD and results from lab experiments under Obj. 2), 60 fish will be collected and shipped live to PI-Loch's laboratory for clinical examination, health evaluation, and diagnostic testing to evaluate multiple health parameters (e.g., visceral fat index, condition factor, blood parameters, infection status) between vaccinated and unvaccinated groups. Upon completion of on-farm vaccination trials, the efficacy and practicality of these new and immediately deployable vaccine preparations will be determined and be immediately available for use in concert with newly developed NCR BMPs (Objective 1).

Data Management Plan

Data sharing, protection, and public access
Data access within each lab is to be determined on a laboratory basis by the PI/co-PIs according to policies determined university requirements and policies. The PI and all co-PIs agree to share all data among the participants. Raw data will be maintained for a minimum of five years and will be available to NCRAC upon request.

1. Expected data types
There are multiple types of data that will be produced in the proposed study:

a) Fish health survey data- All survey data will be managed according to the UMN Institutional Review Board requirements using the Box secure data management platform. Responses to the survey will be anonymous and include non-identifiable demographic data, as well as attitudes, perceptions and opinions related to fish health in the NCR. This data will be made available on the Data Repository at the UMN (DRUM) with accompanying report and/or publication.
b) During the creation of videos, fact sheets, on-farm trainings materials, and peer-reviewed Extension publications, all information will remain on our institutional computers which are protected by either fingerprint or password. Writings and videos will also be stored on our respective institutional cloud servers (e.g., Box and OneDrive). Computers are also continuously backed up by our respective institutions. Once created and published, videos and fact sheets will be housed on NCRAC's website and peer-reviewed publications will be housed on the respective Journal's website and possibly in print. Videos will be housed on NCRAC's website and Vimeo channel to ensure consistent archives for later use by Extension professionals.
c) Disease surveillance and associated culture results, management modification data, and experimental vaccine data- This data will be maintained in a non-digital form via lab books, and in a digital form stored electronically in PI Loch's laboratory's shared drive that is maintained by Michigan State University (MSU) Information Technologies, which is backed up around the clock. The final data product will be thoroughly documented by publications in reputable scientific periodicals, progress and completion reports, meetings and presentations. Of note, all aquaculture facilities will be anonymized in any publications.
d) *F. psychrophilum* isolate and DNA library- All recovered flavobacterial isolates will be given corresponding labels that contain the sample ID/case number, date cryopreserved, and freezer location number. Accompanying metadata also includes fish species, type of lesions, date of isolation, etc. Multiple copies of the isolates are located in PI Loch's lab in multiple freezers. Frozen DNA extracts are also maintained in PI Loch's -20 °C freezers. In addition to hard copies in lab books, all associated metadata is maintained in a digital form PI Loch's laboratory's shared drive.
e) Genotyping and serotyping data- This data will be maintained in a non-digital form via lab books, and in a digital form stored electronically in PI Loch's laboratory on the backed-up data storage system. The final data product will be thoroughly documented by publications in reputable scientific periodicals, progress and completion reports, meetings and presentations.
f) Teaching materials- data generated in the proposed study will also be incorporated into didactic course sessions and undergraduate and graduate student learning opportunities. As described above, these materials will be primarily stored electronically in PI Loch's backed up storage system. However, hard

copies in the forms of printed posters and printed power point lectures for student materials will also be maintained.

2. Data format
All sequence data will be maintained in the standard format (i.e., in .fasta files) and will also be deposited into the NCBI GenBank database according to standard protocol upon acceptance for publication. The remaining data will be available in peer-reviewed publications and accompanying metadata supplied in accompanying supplemental tables. Likewise, after publication, data and supplemental material will be shared upon request.

3. Data storage and preservation
MSU's Information Technology Services include managing the laboratory's shared drive which is part of a mega storage disc of MSU, which is backed up each night and provides long-term archiving.

4. Data sharing, protection and public access.
SHORT TERM: The data product will be updated monthly. The date of the update will be included in the data file and will be part of the data file name. Versions of the data product that have been revised due to errors / updates (other than new data) will be retained in an archive system. A revision history document will describe revisions made. Daily and monthly backups of the data files will be retained at the shared drive of MSU.
LONG TERM: Our intent is that the long-term high-quality final data product generated by this project will be available for use by the research and policy communities in perpetuity. The raw supporting data will be available in perpetuity as well, for use by researchers to build upon as new flavobacteria are described.
The final data product will be released in the form of progress and final reports and manuscripts submitted for publications at study conclusion.

5. Roles and responsibilities
PI Loch will be responsible for ensuring all project investigators are compliant with the Data Management Plan. In the event of personnel departure, PI Loch will identify an appropriate co-PI to assume responsibility for data management for that data type.

Project Deliverables, Outreach and Evaluation Plan

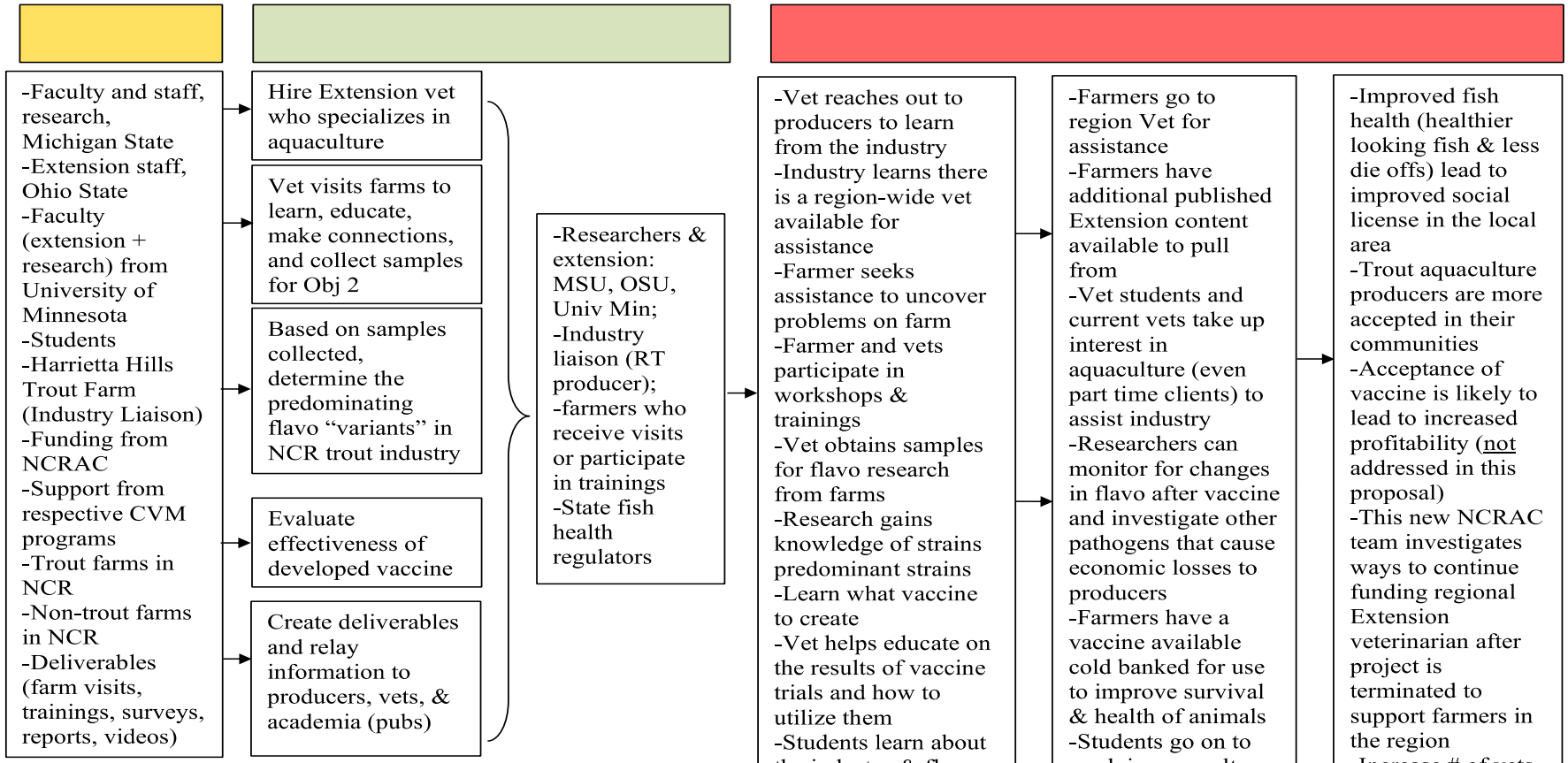
A significant amount of Extension, outreach, and education is built into this project and addressed in the *Procedures* section of Objective 1. Together with the PIs, the Extension veterinarian dedicated to this project will lead workshops, visit farms (trout and non-trout), educate on the importance of infectious disease (including *Flavobacterium* spp.) in aquaculture production, develop site-specific and generic BMPs, and create written and video deliverables for farmers and aspiring fish health professionals. During farm visits, the veterinarian (and in many cases at least one PI) will further educate producers on fish health BMPs, train farmers to be able to conduct some simple but extremely useful diagnostic techniques including conducting wet-mounts and fish health assessments, create biosecurity plans for specific farms and templates for others, and coordinate the collection of samples for our proposed work. Development of educational materials for wider dissemination (e.g., fact sheets, online modules, videos, etc.) will include input from several producers and aspiring fish health professionals to ensure they are easily understandable to those without extensive training in the subject matter being presented. Other deliverables include a fact sheet describing region-specific fish health problems, biosecurity templates and how farms can implement them, and a list of current aquatic veterinarians in each of the NCR states. It is important that producers understand the available veterinarians and veterinarians know the producers in their area. A fact sheet specific for flavobacterial prevention and control in the NCR ("what every NCR producer should know about flavobacteria") will also be produced. Likewise, should the new BCWD vaccine preparations prove effective (which we strongly believe will be the case), not only will the protocols be made widely available (via publications, presentations, and dissemination to fish health labs), but the recovered and preserved *F. psychrophilum* strains will be maintained and made available for laboratories (e.g., Aquatactics, Kennebec River Biosciences, etc.) to commercially disseminate vaccine preparations to farms under veterinary guidance. Other important deliverables include providing a post-doctoral researcher and undergraduate interns at MSU with hands-on research, training, and opportunities to develop as scientific scholars, as well as hands-on and on-farm training opportunities for veterinary students from MSU, OSU and UMN. Selected aspiring aquatic animal-focused veterinary students from each institution will also participate in the development and on-site review of BMPs and attend NCRAC or local aquaculture annual conference to network with industry leaders. A *Journal of Extension* article will also be

generated and submitted, highlighting the importance of region-specific inter and transdisciplinary approach to address farmer problems in a world where Extension FTE's continue to decrease. Research results generated as part of Objective 2 and 3 will be submitted to a peer-reviewed journal as well.

As part of Extension's mandated university annual reporting, the Extension veterinarian will report to NCRAC numbers of contacts made and the method they were made (e.g. phone calls, personal visits, Zoom, etc.) as well as preventative vs. reactive cases and support. Number of participants at workshops and other training events will be recorded, as well as number of copies of printed materials that are dispersed to the region. Lastly, while video views and publication downloads build over time (long after the termination of our proposed work), the work created and posted to NCRAC.org in year one will have analytics which will be generated for the final report.

IMPROVING FISH HEALTH IN THE NCR BY INTEGRATING EXTENSION WITH THE DEVELOPMENT OF ALTERNATIVE DISEASE PREVENTION METHODS

Goal: Integrate a region-wide Extension veterinarian with research on prevalent strains of bacteria to improve farm health and success. Objectives: 1. Develop practical/usable fish health applications for producers & fish health professionals through farm visits, trainings, & the creation of pragmatic resources by NCR fish health veterinarians. 2. Determine the predominating flavobacterial strains driving economic losses in the NCR trout industry. 3. Evaluate the effectiveness of newly developed vaccines in preventing losses causes by regionally predominating flavobacteria under lab & field conditions.



Assumptions:

R. trout farmers in the region are interested in working with Extension to learn about predominant strains of flavobacterial "variants" negatively impacting their farm

Farmers utilize the Extension veterinarian as they should

Research/extension team can travel (COVID-19 consideration)

External Factors

COVID-19 limiting travel (unlikely given projected start time of project)

Initial agreement but subsequent lack of engagement by r. trout farmers. Will address by incorporating state/fed agencies raising trout if necessary.

Facilities

MSU. PI-Loch is the Principal Investigator of the Michigan State University – Aquatic Animal Health Laboratory (MSU-AAHL), which is equipped to conduct many phases of research, including dedicated spaces for tissue culture, virology, bacteriology, parasitology, housing and maintenance of live fish, *in vivo* experimental challenge systems, clinical examination, histopathology, molecular and serological analyses, and more. Of note, these BSL-2 certified spaces include 2,500 sq. ft. of wet laboratory space for the housing of aquatic animals within the MSU-Research Containment Facility (MSU-URCF), a 100,000 ft^2 state-of-the-art facility within the MSU-College of Veterinary Medicine built to assist researchers by providing a safe and efficient environment in which to conduct studies without danger to the researchers or releases into the environment. A centralized computer system monitors and logs all the critical systems and back-up generators in the facility, including pumps, motors, fans, air conditioning, heating, refrigeration, and water levels. Any irregularities cause researchers and maintenance personnel to be immediately notified. The facility enables optimal long- and short-term maintenance for aquatic animals during experiments and is equipped with filtered, chilled water supplies to all aquatic animal spaces, and ultraviolet (UV) sterilizers, compressed air sources, and biosafety barriers in individual tank areas. Water is supplied to the building from a well network that is injected with pure oxygen, and gases are equilibrated to 100% saturation by an aeration tower. Wet lab areas are sub-divided by heavy plastic curtains that can be arranged in multiple configurations to accommodate various experiments while providing additional biological separation. PVC and glass holding tanks of various sizes and shapes can be set up for either recirculating or flow-through water exposure for aquatic organisms ranging from larval fish and invertebrates to adult spawning trout.

OSU: PI Smith maintains data and all potentially sensitive information on a password-protected/finger-scanned computer that is backed up into OSU's cloud, which is fully recoverable in the event of system failure.

UMN: Minimal facilities will be needed at the University of Minnesota; however, there is the extensive capacity to conduct and support fish health-related research and outreach, and survey design. This includes a 10,000 sq-ft biosecure fish holding facility, a top-tier veterinary diagnostic laboratory, numerous faculty, staff and students with expertise in aquaculture, fish health, biosecurity, infectious disease, and veterinary medicine and network of regional Extension professionals. In addition, faculty are well supported in the development, production, and dissemination of online educational modules and videos (please see data management plan); all of which will be housed on NCRAC's website and Vimeo channel to ensure consistent archives for later use by Extension professionals.

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Project Leaders

State	Name/Institution	Area of Specialization
Michigan	Thomas Loch, Michigan State University	Fish health, flavobacterial diseases, vaccine development
Minnesota	Nick Phelps, University of Minnesota	Fish health, diagnostics, aquatic invasive species
Ohio	Matthew A. Smith, The Ohio State University	Aquaculture Extension/water quality

Budget

ORGANIZATION AND ADDRESS Michigan State University Hannah Administration Building 426 Auditorium Rd, Room 2 East Lansing, MI 48824.2600 PROJECT DIRECTOR(S) Thomas Loch				**USDA AWARD NO.** Year 1: Objectives: 2&3			
				Duration Proposed Months: 12 **Funds Requested by Proposer**	Duration Proposed Months: ____ **Funds Approved by CSREES** (If different)	Non-Federal Proposed Cost-Sharing/ Matching Funds (If required)	Non-federal Cost-Sharing/ Matching Funds Approved by CSREES (If Different)
A. Salaries and Wages 1. No. of Senior Personnel	**CSREES FUNDED WORK MONTHS**						
	Calendar	Academic	Summer				
a. _1_ (Co)-PI(s)	0	0	1	$9,400.06			
b. ___ Senior Associates							
2. No. of Other Personnel (Non-Faculty)	6	0	0	$25,458.34			
a. _1_ Research Associates-Postdoctorates . . . b. _1__ Other Professionals	12			$100,00.00			
c. ___ Paraprofessionals							
d. ___ Graduate Students							
e. _2_ Prebaccalaureate Students				$22,301.50			
f. ___ Secretarial-Clerical							
g. ___ Technical, Shop and Other							
Total Salaries and Wages				$157,159.90			
B. Fringe Benefits (If charged as Direct Costs)				$42,219.97			
C. Total Salaries, Wages, and Fringe Benefits (A plus B)				$199,379.87			
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for each item.)							
E. Materials and Supplies				$42,496.11			
F. Travel				$13,500.00			
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.)				$7,210.00			
K. Total Direct Costs (C through I)				$262,585.98			
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)				$262,585.98			
N. Other							
O. Total Amount of This Request				$262,585.98			
P. Carryover -- (If Applicable) . . . . . **Federal Funds: $**				**Non-Federal funds: $**		**Total $**	
Q. Cost Sharing/Matching (Breakdown of total amounts shown in line O) Cash (both Applicant and Third Party) Non-Cash Contributions (both Applicant and Third Party)						Leave Blank	
NAME AND TITLE (Type or print)				**SIGNATURE** (required for revised budget only)			**DATE**
Project Director							

Thomas Loch							
Authorized Organizational Representative Craig O'Neill							
ORGANIZATION AND ADDRESS Michigan State University Hannah Administration Building 426 Auditorium Rd, Room 2 East Lansing, MI 48824.2600				**USDA AWARD NO.** Year 2: Objectives:1&3			
				Duration Proposed Months: 12 **Funds Requested by Proposer**	Duration Proposed Months: ____ **Funds Approved by CSREES** (If different)	Non-Federal Proposed Cost-Sharing/ Matching Funds (If required)	Non-federal Cost-Sharing/ Matching Funds Approved by CSREES (If Different)
PROJECT DIRECTOR(S) Thomas Loch							
A. Salaries and Wages 1. No. of Senior Personnel	**CSREES FUNDED WORK MONTHS**						
	Calendar	Academic	Summer				
a. _1_ (Co)-PI(s)	0	0	1	$9,588.06			
b. ___ Senior Associates							
2. No. of Other Personnel (Non-Faculty)	6	0	0	$25,967.50			
a. _1_ Research Associates-Postdoctorates . . .							
b. _1__ Other Professionals	12			$103,000			
c. ___ Paraprofessionals							
d. ___ Graduate Students							
e. _2_ Prebaccalaureate Students				$22,747.53			
f. ___ Secretarial-Clerical							
g. ___ Technical, Shop and Other							
Total Salaries and Wages				$161,303.09			
B. Fringe Benefits (If charged as Direct Costs)				$43,499.20			
C. Total Salaries, Wages, and Fringe Benefits (A plus B)				$204,802.29			
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for each item.)							
E. Materials and Supplies				$50,164.19			
F. Travel				$9,500.00			
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.)				$15,645.00			
K. Total Direct Costs (C through I)				$280,111.48			
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)				$280,111.48			
N. Other							
O. Total Amount of This Request				$280,111.48			
P. Carryover -- (If Applicable) . . . . . **Federal Funds: $**				**Non-Federal funds: $**		**Total $**	
Q. Cost Sharing/Matching (Breakdown of total amounts shown in line O) Cash (both Applicant and Third Party) Non-Cash Contributions (both Applicant and Third Party)						Leave Blank	

NAME AND TITLE (Type or print)	SIGNATURE (required for revised budget only)	DATE
Project Director Thomas Loch		
Authorized Organizational Representative Craig O'Neill		

ORGANIZATION AND ADDRESS
Michigan State University
Hannah Administration Building
426 Auditorium Rd, Room 2
East Lansing, MI 48824.2600

PROJECT DIRECTOR(S)
Thomas Loch

USDA AWARD NO. Year 1&2: Objectives:1-3

	CSREES FUNDED WORK MONTHS			Duration Proposed Months: 12 **Funds Requested by Proposer**	Duration Proposed Months: ____ **Funds Approved by CSREES** (If different)	Non-Federal Proposed Cost-Sharing/ Matching Funds (If required)	Non-federal Cost-Sharing/ Matching Funds Approved by CSREES (If Different)
A. Salaries and Wages 1. No. of Senior Personnel	Calendar	Academic	Summer				
a. _1_ (Co)-PI(s)	0	0	2	$18,988.12			
b. ___ Senior Associates							
2. No. of Other Personnel (Non-Faculty)	12	0	0	$51,425.84			
a. _1_ Research Associates-Postdoctorates . . .							
b. _1__ Other Professionals	24			$203,000.00			
c. ___ Paraprofessionals							
d. ___ Graduate Students.....							
e. _2_ Prebaccalaureate Students.....				$45,049.03			
f. ___ Secretarial-Clerical							
g. ___ Technical, Shop and Other							
Total Salaries and Wages.....				$318,462.99			
B. Fringe Benefits (If charged as Direct Costs)				$85,719.17			
C. Total Salaries, Wages, and Fringe Benefits (A plus B).....				$404,182.16			
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for each item.)							
E. Materials and Supplies				$92,660.30			
F. Travel				$23,000			
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.)				$22,855.00			
K. Total Direct Costs (C through I).....				$542,697.46			
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)..... .				$542,697.46			
N. Other.....							
O. Total Amount of This Request.....				$542,697.46			

P. Carryover -- (If Applicable) . . . . . **Federal Funds: $** **Non-Federal funds: $** **Total $**

Q. Cost Sharing/Matching (Breakdown of total amounts shown in line O) Cash (both Applicant and Third Party)	Leave Blank	

Non-Cash Contributions (both Applicant and Third Party)		

NAME AND TITLE (Type or print)	**SIGNATURE** (required for revised budget only)	**DATE**
Project Director Thomas Loch		
Authorized Organizational Representative Craig O'Neill		

Budget Explanation for Michigan State University
(Thomas Loch)

Objective 1

A. Salaries and Wages: $0

Year 1: $100,000.00

- An Extension aquaculture veterinarian would be hired through Michigan State University to be dedicated to this project for 100% FTE (12 months) for year 1. Time is requested for objective 1, which includes leading the development of many of the deliverables (survey administration and interpretation, region-wide farm visits, on-farm trainings, creation of BMPs, work closely with PIs, veterinarian student farm tour, creation of farmer/ veterinarian workshops, etc.)

Year 2: $103,000.00

- An Extension aquaculture veterinarian would be hired through Michigan State University to be dedicated to this project for 100% FTE (12 months) for year 2. Time is requested for objective 1, which includes leading the development of many of the deliverables (region-wide farm visits, on-farm trainings, creation of BMPs, work closely with PIs, assist with objectives 2 and 3, veterinarian student farm tour, creation of farmer/ veterinarian workshops, etc.)

B. Fringe Benefits: $63,945.00

$63,945.00

E. Materials and Supplies: $8,263.00

Items	Year 1	Year 2	Total
Printing materials and other office supplies for workshops/farm visits (including lamination of materials for use by farmers/veterinarians around water)	$500	$500	$1,000
Fish dissection and blood/tissue collection materials (e.g. syringes, scalpels, trays, low cost fish health kits, etc.)	$4,263	$2,000	$7,263
Total	$4,763	$2,500	$8,263

F. Travel (Domestic): $14,000

Year 1: $8,000

- Transportation for Extension aquaculture veterinarian to travel to farms throughout the Midwest (rainbow trout farms and non-rainbow trout farms) and to objective 1 related workshops, on-site trainings and delivery of created deliverables, and veterinary student farm tours throughout the Midwest. Farms will be identified during the project. **$1200 also included for travel for Extension liaison co-PI Smith for participation in bus tours of these facilities.**

- Transportation to participate in regional state aquaculture association conferences (e.g., OAA, MAA, MNAA, IAAI.). Associations that the veterinarian will participate in will be based on requests and needs from the associations. Researchers will not attend the same meetings, thereby ensuring that results are disseminated as widely as possible.

Year 2: $6,000

- Transportation for Extension aquaculture veterinarian to travel to farms throughout the

Midwest (rainbow trout farms and non-rainbow trout farms) and to objective 1 related workshops, on-site trainings and delivery of created deliverables, and veterinary student farm tours throughout the Midwest. Farms will be identified during the project**. $1200 also included for travel for Extension liaison co-PI Smith for participation in bus tours of these facilities.**

- Transportation to participate in regional state aquaculture association conferences (e.g., OAA, MAA, MNAA, IAAI.). Associations that the veterinarian will participate in will be based on requests and needs from the associations. Researchers will not attend the same meetings, thereby ensuring that results are disseminated as widely as possible.

- Less travel is needed in year 2 as the Extension veterinarian will travel more in year 1 to simultaneously collect samples required for objectives 2 and 3. Associations that the veterinarian will participate in will be based on requests and needs from the associations.

I. Student Assistance/Support: $0
   Year 1: $0
   Year 2: $0

J. Other Direct Costs: $4,000.00
   Year 1: $2,000.00
   - Funds are requested to rent a charter bus in year 1 to transport students to/from an established fish farm in Ohio to learn about the farm, their BMPs, biosecurity practices, and interact with the farmer, Extension, and participating veterinarians. Rates are approximately $6 per mile, and specific mileage will depend on farms that will be identified during the project. Previous rentals in Ohio have been approximately $1,500, and $500 is requested for snacks and drinks for participants.

   Year 2: $2,000.00
   - Funds are requested to rent a charter bus in year 2 to transport students to/from an established fish farm in Ohio to learn about the farm, their BMPs, biosecurity practices, and interact with the farmer, Extension, and participating veterinarians. Rates are approximately $6 per mile, and specific mileage will depend on farms that will be identified during the project. Previous rentals in Ohio have been approximately $1,500, and $500 is requested for snacks and drinks for participants.

L. Indirect Costs: $0
   Year 1: $0
   Year 2: $0

Objective 2

A. Salaries and Wages: $51,443.91
   Year 1: $51,443.91
   - Salary costs include commitment for PI Dr. Thomas Loch in the amount $8,460 (90% of 1 summer month) for mentoring the undergraduate students and post-doctoral research associate funded by this project, and in report writing, overall project coordination between institutions, completion of experiments, and data analyses and publication.
   - Salary costs in the amount of $22,913 for 45% time for research associate Dr. Megan Shavalier are to perform tasks contributing to this proposed project; e.g., vaccine preparations, immunization experiments, hatching and maintenance of rainbow trout, live fish challenges, multilocus sequence typing, molecular serotyping and confirmatory testing.
   - Hourly wages ($10.10/hr) are for two undergraduate student research assistants to provide support for this project (15hr/wk during academic year, 29hr/wk during breaks); e.g., daily

live fish care, sample processing and extractions, and assistance with laboratory maintenance and assays.

Year 2: $0

B. Fringe Benefits: $9,647.97

Costs for fringe benefits are requested at 7.65% in the amounts of $647.20 for summer tax for PI Loch and $840.20 for summer tax for undergraduate research assistants; and $8,160.57 for health insurance benefits for research associate Dr. Shavalier (35.616%).

E. Materials and Supplies: $32,159.80

Items	Year 1	Year 2	Total
laboratory reagents, chemicals and supplies for MLST typing	$7,938.00	$0	$7,938.00
molecular serotyping	$1,620.00	$0	$1,620.00
bacteriological chemicals and reagents for flavobacterial culture and detection	$2,639.70	$0	$2,639.70
other PCR reagents and chemicals	$1,296.00	$0	$1,296.00
preparation of formalin-killed bacterins	$0	$0	$0
egg disinfection reagents	$427.50	$0	$427.50
chemicals and reagents for experiments	$1,858.60	$0	$1,858.60
clinical examination, necropsy, and microbiological supplies	$10,800.00	$0	$10,800.00
glassware	$225.00	$0	$225.00
disposable laboratory consumables (e.g., pipette tips, gloves, petri dishes, sample collection tube, syringes, transfer pipettes, scalpels)	$2,475.00	$0	$2,475.00
optical density meter	$0	$0	$0
equipment maintenance and calibration	$2,880.00	$0	$2,880.00
fish maintenance supplies	$0	$0	$0
Total	$32,159.80	$0	$32,159.80

F. Travel (Domestic):

Year 1: $2,900.00

- $1,500 is requested for domestic travel for the PI and the post-doctoral research associate to present findings at professional conferences and meetings over the course of the two-year project, including the Eastern Fish Health Workshop and the American Fisheries Society – Fish Health Section annual meeting. The PI/co-PIs and post-doc will not attend the same meetings, thereby ensuring that results are disseminated as widely as possible.
- $1,400 is requested to visit a subset (number of trips to be determined) of the aquaculture facilities project (one person per visit).

Year 2: $0

I. Student Assistance/Support

Year 1: $0

Year 2: $0

J. Other Direct Costs:

Year 1: $1,800.00

- Funds are requested to ship live fish from aquaculture facilities.

Year 2: $0

L. Indirect Costs:

Year 1: $0

Year 2: $0

Objective 3

A. Salaries and Wages: $64,019.08

Year 1: $5,715.99

- Salary costs include time commitment for PI Dr. Thomas Loch in the amount $940 (10% of 1 summer month) for mentoring the undergraduate students and post-doctoral research associate funded by this project, and in report writing, overall project coordination between institutions, completion of experiments, and data analyses and publication.
- Salary costs in the amount of $2,546 for 5% time for research associate Dr. Megan Shavalier are to perform tasks contributing to this proposed project; e.g., vaccine preparations, immunization experiments, hatching and maintenance of rainbow trout, live fish challenges, multilocus sequence typing, molecular serotyping and confirmatory testing.
- Hourly wages ($10.10/hr) are for two undergraduate student research assistants to provide support for this project (15hr/wk during academic year, 29hr/wk during breaks); e.g., daily live fish care, sample processing and extractions, and assistance with laboratory maintenance and assays.

Year 2: $58,303.09

- Salary costs include time commitment for PI Dr. Thomas Loch in the amount of $9,588 (1 summer month) for mentoring the undergraduate students and post-doctoral research associate funded by this project, and in report writing, overall project coordination between institutions, completion of experiments, and data analyses and publication.
- Salary costs in the amount of $25,968 for 50% time for research associate Dr. Megan Shavalier are to perform tasks contributing to this proposed project; e.g., vaccine preparations, immunization experiments, hatching and maintenance of rainbow trout, live fish challenges, multilocus sequence typing, molecular serotyping and confirmatory testing.
- Hourly wages ($10.33/hr) are for two undergraduate student research assistants to provide support for this project (15hr/wk during academic year, 29hr/wk during breaks); e.g., daily live fish care, sample processing and extractions, and assistance with laboratory maintenance and assays.

B. Fringe Benefits: $12,126.20

Costs for fringe benefits are requested at 7.65% in the amounts of $805.40 for summer taxes for PI Loch and $1,045.59 for summer taxes for undergraduate research assistants; and $10,275.21 for health insurance benefits for research associate Dr. Shavalier (35.616% in Y1 and 36.078% in Y2).

E. Materials and Supplies: $52,237.50

Items	Year 1	Year 2	Total
laboratory reagents, chemicals and supplies for MLST typing	$882.00	0	$882.00
molecular serotyping	$180.00	0	$180.00
bacteriological chemicals and reagents for flavobacterial culture and detection	$293.30	$5,784.00	$6,077.30
other PCR reagents and chemicals	$144.00	$4,000.00	$4,144.00
preparation of formalin-killed bacterins	$0.00	$440.00	$440.00
egg disinfection reagents	$47.50	$500.00	$547.50
chemicals and reagents for experiments	$206.51	$5,530.19	$5,736.70
clinical examination, necropsy, and microbiological supplies	$1,200.00	$15,000.00	$16,200.00
glassware	$25.00	$1,700.00	$1,725.00
disposable laboratory consumables (e.g., pipette tips, gloves, petri dishes, sample collection tube, syringes, transfer pipettes, scalpels)	$275.00	$6,410.00	$6,685.00
optical density meter	$0.00	$2,600.00	$2,600.00

equipment maintenance and calibration	$320.00	$3,200.00	$3,520.00
fish maintenance supplies	$1,500.00	$2,000.00	$3,500.00
Total	$5,073.31	$47,164.19	$52,237.50

F. Travel (Domestic): $6,100.00

Year 1: $2,600.00

- $2,600 is requested to visit a subset (number of trips to be determined) of the aquaculture facilities project (one person per visit).

Year 2: $3,500.00

- $1,500 is requested for domestic travel for the PI and the post-doctoral research associate to present findings at professional conferences and meetings over the course of the two-year project, including the Eastern Fish Health Workshop and the American Fisheries Society – Fish Health Section annual meeting. The PI/co-PIs and post-doc will not attend the same meetings, thereby ensuring that results are disseminated as widely as possible.
- $2,000 is requested to visit a subset (number of trips to be determined) of the aquaculture facilities project (one person per visit).

I. Student Assistance/Support

Year 1: $0

Year 2: $0

J. Other Direct Costs: $17,055.00

Year 1: $3,410.00

- Rental fees for fish holding space at the MSU-Research Containment Facility are requested for 3 months during Year 1 to hatch/raise fish, immunize fish, and to carry out the proposed live fish experiments.

Year 2: $13,645.00

- Rental fees for fish holding space at the MSU-Research Containment Facility are requested for 12 months during Year 2 to hatch/raise fish, immunize fish, and to carry out the proposed live fish experiments.

L. Indirect Costs:

Year 1: $0

Year 2: $0

ORGANIZATION AND ADDRESS The Ohio State University 217 Elm St. London, OH 43140 PROJECT DIRECTOR(S) Matthew A. Smith				**USDA AWARD NO.** Year 1: Objective 1			
				Duration Proposed Months: __ **Funds Requested by Proposer**	Duration Proposed Months: ____ **Funds Approved by CSREES** (If different)	Non-Federal Proposed Cost-Sharing/ Matching Funds (If required)	Non-federal Cost-Sharing/ Matching Funds Approved by CSREES (If Different)
A. Salaries and Wages 1. No. of Senior Personnel	**CSREES FUNDED WORK MONTHS**						
	Calendar	Academic	Summer				
Q. ___ (Co)-PI(s)							
b. ___ Senior Associates							
2. No. of Other Personnel (Non-Faculty)							
a. ___ Research Associates-Postdoctorates . . .							
b. ___ Other Professionals							
c. ___ Paraprofessionals							
d. ___ Graduate Students.....							
e. ___ Prebaccalaureate Students.....							
f. ___ Secretarial-Clerical							
g. ___ Technical, Shop and Other							
Total Salaries and Wages..... □							
B. Fringe Benefits (If charged as Direct Costs)							
C. Total Salaries, Wages, and Fringe Benefits (A plus B)..... □							
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for each item.)							
E. Materials and Supplies							
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.)							
K. Total Direct Costs (C through I)..... □							
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.)							
M. Total Direct and F&A/Indirect Costs (J plus K)..... . □							
N. Other..... □							
O. Total Amount of This Request..... □				$0			

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) .....□		
Non-Cash Contributions (both Applicant and Third Party) .....□		

NAME AND TITLE (Type or print)	**SIGNATURE** (required for revised budget only)	**DATE**
Project Director		

Authorized Organizational Representative		

ORGANIZATION AND ADDRESS The Ohio State University 217 Elm St. London, OH, 43140 PROJECT DIRECTOR(S) Matthew A. Smith	**USDA AWARD NO.** Year 2 : Objective 1			
	Duration Proposed Months: __ **Funds Requested by Proposer**	Duration Proposed Months: ____ **Funds Approved by CSREES** (If different)	Non-Federal Proposed Cost-Sharing/ Matching Funds (If required)	Non-federal Cost-Sharing/ Matching Funds Approved by CSREES (If Different)

	CSREES FUNDED WORK MONTHS						
	Calendar	Academic	Summer				
A. Salaries and Wages 1. No. of Senior Personnel **Q.** ___ (Co)-PI(s)							
b. ___ Senior Associates							
2. No. of Other Personnel (Non-Faculty) a. __ Research Associates-Postdoctorates . . .							
b. ___ Other Professionals							
c. ___ Paraprofessionals							
d. ___ Graduate Students							
e. ___ Prebaccalaureate Students							
f. ___ Secretarial-Clerical							
g. ___ Technical, Shop and Other							
Total Salaries and Wages							
B. Fringe Benefits (If charged as Direct Costs)							
C. Total Salaries, Wages, and Fringe Benefits (A plus B)							
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for each item.)							
E. Materials and Supplies							
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.)							
K. Total Direct Costs (C through I)							
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.)							
M. Total Direct and F&A/Indirect Costs (J plus K)							
N. Other							
O. Total Amount of This Request				$0			

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)		
Non-Cash Contributions (both Applicant and Third Party)		

NAME AND TITLE (Type or print)	**SIGNATURE** (required for revised budget only)	**DATE**

Project Director		
Authorized Organizational Representative		

ORGANIZATION AND ADDRESS
The Ohio State University
217 Elm St.
London, OH, 43140

PROJECT DIRECTOR(S)
Matthew A. Smith

USDA AWARD NO. Year 1 & 2: Objective 1

	CSREES FUNDED WORK MONTHS			Duration Proposed Months: __ **Funds Requested by Proposer**	Duration Proposed Months: ____ **Funds Approved by CSREES** (If different)	Non-Federal Proposed Cost-Sharing/ Matching Funds (If required)	Non-federal Cost-Sharing/ Matching Funds Approved by CSREES (If Different)
A. Salaries and Wages 1. No. of Senior Personnel	Calendar	Academic	Summer				
Q. ___ (Co)-PI(s)							
b. ___ Senior Associates							
2. No. of Other Personnel (Non-Faculty)							
a. __ Research Associates-Postdoctorates . . .							
b. ___ Other Professionals							
c. ___ Paraprofessionals							
d. __ Graduate Students							
e. ___ Prebaccalaureate Students							
f. ___ Secretarial-Clerical							
g. ___ Technical, Shop and Other							
Total Salaries and Wages							
B. Fringe Benefits (If charged as Direct Costs)							
C. Total Salaries, Wages, and Fringe Benefits (A plus B)							
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for each item.)							
E. Materials and Supplies							
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.)							
K. Total Direct Costs (C through I)							
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.)							
M. Total Direct and F&A/Indirect Costs (J plus K)							
N. Other							
O. Total Amount of This Request				$0			

P. Carryover – (If Applicable) . . . . . **Federal Funds: $** **Non-Federal funds: $** **Total $**

Q. Cost Sharing/Matching (Breakdown of total amounts shown in line O) Cash (both Applicant and Third Party)	Leave Blank	

Non-Cash Contributions (both Applicant and Third Party)		
NAME AND TITLE (Type or print)	**SIGNATURE** (required for revised budget only)	**DATE**
Project Director		
Authorized Organizational Representative		

Budget Explanation for Ohio State University

(Matthew Smith)

No funding is requested (but please see travel budget under MSU/PI Loch, as $1,200 in travel is budgeted for Co-PI Smith in both year 1 and year 2 for involvement and attendance of bus farm tours).

NAME AND TITLE (Type or print)	SIGNATURE (required for revised budget only)	DATE
Project Director Thomas Loch		
Authorized Organizational Representative Craig O'Neill		

ORGANIZATION AND ADDRESS	USDA AWARD NO. Year 1: Objectives: 1-3			
University of Minnesota Skok Hall 135 2003 Upper Bufford Circle St. Paul, MN 55108	Duration Proposed Months: 12 **Funds Requested by Proposer**	Duration Proposed Months: ____ **Funds Approved by CSREES** (If different)	Non-Federal Proposed Cost-Sharing/ Matching Funds (If required)	Non-federal Cost-Sharing/ Matching Funds Approved by CSREES (If Different)
PROJECT DIRECTOR(S) Nicholas Phelps				

A. Salaries and Wages	CSREES FUNDED WORK MONTHS						
1. No. of Senior Personnel	Calendar	Academic	Summer				
a. _1_ (Co)-PD(s)	1.2	0	0	$13,000			
b. ___ Senior Associates							
2. No. of Other Personnel (Non-Faculty)							
a. __ Research Associates-Postdoctorates . . .							
b. ___ Other Professionals							
c. ___ Paraprofessionals							
d. ___ Graduate Students							
e. __ Prebaccalaureate Students				$2,260			
f. ___ Secretarial-Clerical							
g. ___ Technical, Shop and Other							
Total Salaries and Wages				$15,260			
B. Fringe Benefits (If charged as Direct Costs)				$4,745			
C. Total Salaries, Wages, and Fringe Benefits (A plus B)				$20,005			
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for each item.)							
E. Materials and Supplies				$3,500			
F. Travel				$3,000			
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.)				$2,500			
K. Total Direct Costs (C through I)				$29,005			
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)				$29,005			
N. Other							
O. Total Amount of This Request				$29,005			

P. Carryover -- (If Applicable) . . . . . **Federal Funds: $** **Non-Federal funds: $** **Total $**

Q. Cost Sharing/Matching (Breakdown of total amounts shown in line O)		
Cash (both Applicant and Third Party)	Leave Blank	

Non-Cash Contributions (both Applicant and Third Party)		

NAME AND TITLE (Type or print)	**SIGNATURE** (required for revised budget only)	**DATE**
Project Director		
Authorized Organizational Representative		

ORGANIZATION AND ADDRESS
University of Minnesota
Skok Hall 135
2003 Upper Bufford Circle
St. Paul, MN 55108

PROJECT DIRECTOR(S)
Nicholas Phelps

USDA AWARD NO. Year 2: Objectives:1-3

	CSREES FUNDED WORK MONTHS			Duration Proposed Months: 12 **Funds Requested by Proposer**	Duration Proposed Months: ____ **Funds Approved by CSREES** (If different)	Non-Federal Proposed Cost-Sharing/ Matching Funds (If required)	Non-federal Cost-Sharing/ Matching Funds Approved by CSREES (If Different)
A. Salaries and Wages 1. No. of Senior Personnel	Calendar	Academic	Summer				
a. _1_ (Co)-PD(s)	1.2	0	0	$13,390			
b. ___ Senior Associates							
2. No. of Other Personnel (Non-Faculty)							
a. __ Research Associates-Postdoctorates . . .							
b. ___ Other Professionals							
c. ___ Paraprofessionals							
d. ___ Graduate Students.....							
e. _1_ Prebaccalaureate Students.....				$2,408			
f. ___ Secretarial-Clerical.....							
g. ___ Technical, Shop and Other.....							
Total Salaries and Wages.....				$15,798			
B. Fringe Benefits (If charged as Direct Costs)				$4,887			
C. Total Salaries, Wages, and Fringe Benefits (A plus B).....				$20,685			
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for each item.)							
E. Materials and Supplies				$2,500			
F. Travel				$4,000			
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.)				$2,500			
K. Total Direct Costs (C through I).....				$29,685			
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).....				$29,685			
N. Other.....							
O. Total Amount of This Request.....				$29,685			

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)		
Non-Cash Contributions (both Applicant and Third Party)		

NAME AND TITLE (Type or print)	**SIGNATURE** (required for revised budget only)	**DATE**
Project Director		
Authorized Organizational Representative		

ORGANIZATION AND ADDRESS
University of Minnesota
Skok Hall 135
2003 Upper Bufford Circle
St. Paul, MN 55108

PROJECT DIRECTOR(S)
Nicholas Phelps

USDA AWARD NO. Year 1&2: Objectives:1-3

	CSREES FUNDED WORK MONTHS			Duration Proposed Months: 12 **Funds Requested by Proposer**	Duration Proposed Months: ____ **Funds Approved by CSREES** (If different)	Non-Federal Proposed Cost-Sharing/ Matching Funds (If required)	Non-federal Cost-Sharing/ Matching Funds Approved by CSREES (If Different)
A. Salaries and Wages 1. No. of Senior Personnel	Calendar	Academic	Summer				
a. _1_ (Co)-PD(s)	2.4	0	0	$26,260			
b. ___ Senior Associates							
2. No. of Other Personnel (Non-Faculty)							
a. __ Research Associates-Postdoctorates . . .							
b. ___ Other Professionals							
c. ___ Paraprofessionals							
d. ___ Graduate Students							
e. _1_ Prebaccalaureate Students				$4,668			
f. ___ Secretarial-Clerical							
g. ___ Technical, Shop and Other							
Total Salaries and Wages				$31,058			
B. Fringe Benefits (If charged as Direct Costs)				$9,632			
C. Total Salaries, Wages, and Fringe Benefits (A plus B)				$40,690			
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for each item.)							
E. Materials and Supplies				$6,000			
F. Travel				$7,000			
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.)				$5,000			
K. Total Direct Costs (C through I)				$58,690			
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)				$58,690			
N. Other							
O. Total Amount of This Request				$58,690			

P. Carryover -- (If Applicable) . . . . . **Federal Funds: $** **Non-Federal funds: $** **Total $**		
Q. Cost Sharing/Matching (Breakdown of total amounts shown in line O) Cash (both Applicant and Third Party) ..... Non-Cash Contributions (both Applicant and Third Party)	Leave Blank	

NAME AND TITLE (Type or print)	**SIGNATURE** (required for revised budget only)	**DATE**
Project Director		
Authorized Organizational Representative		

Budget Explanation for University of Minnesota
(Nick Phelps)

Objective 1

A. **Salaries and Wages**: $31,058

Year 1: $15,260

- Salary costs include 10% effort during Year 1 for Co-PI Dr. Nicholas Phelps. Dr. Phelps will be primarily contributing to Objective 1, leading the creation and implementation of the fish health stakeholder survey, and ensure the integration of survey results into the project plan. Dr. Phelps will participate in team meetings, report writing and manuscript preparation throughout the project. In addition, 150 hours of undergraduate student support is included at $15 per hour (no fringe required).

Year 2: $15,798

- Salary costs include 10% effort during Year 2 for Co-PI Dr. Nicholas Phelps. Dr. Phelps will be primarily contributing to Objective 1, leading the creation and implementation of the fish health stakeholder survey, and ensure the integration of survey results into the project plan. Dr. Phelps will participate in team meetings, report writing and manuscript preparation throughout the project. In addition, 160 hours of undergraduate student support is included at $15 per hour (no fringe required).

B. Fringe Benefits: $9,632

Year 1: $4,745

- This will support the University of Minnesota faculty fringe rate of 36.5% for Dr. Phelps.

Year 2: $4,887

- This will support the University of Minnesota faculty fringe rate of 36.5% for Dr. Phelps.

E. Materials and Supplies: $6,000

Items	Year 1	Year 2	Total
Diagnostic materials for farm visits and workshops including slides, cover slips, dissection tools, slide preps of pathogens, dissection trays, gloves, water quality testing kits, fish, mobile microscope with camera, etc.	$3,000	$2,000	$5,000
Office materials for preparation of laminated reference documents for farm visits and workshops including fact sheets, pictures of common pathogens, FHA documents and keys, etc.	$500	$500	$1,000
Total	$3,500	$2,500	$6,000

F. Travel (Domestic): $7,000

Year 1: $3,000

- This funding will support two on-site visits and workshop attendance by Dr. Phelps during Year 1. The specific locations are to be determined, however we estimate $780 for lodging (6 nights, $130/night per room), $495 for per diem (9 days, $55/full day) and $1,725 for mileage (3,025 miles, $0.57/mile).

Year 2: $4,000

- This funding will support four on-site visits and workshop attendance by Dr. Phelps during Year 2. The specific locations are to be determined, however we estimate $1,300 for lodging (10 nights,

$130/night per room), $825 for per diem (15 days, $55/full day) and $1,875 for mileage (3,290 miles, $0.57/mile).

I. Student Assistance/Support: $0

Year 1: $0
Year 2: $0

J. Other Direct Costs: $5,000

Year 1: $2,500

- To cover video and online educational module support from internal (UMN) or external contract service providers. This will cover recording, editing, and digital ownership for at least 2 videos (~$1,250 per 5 min video), which will be disseminated openly and widely. Video content will be generated by the project team.

Year 2: $2,500

- To cover video and online educational module support from internal (UMN) or external contract service providers. This will cover recording, editing, and digital ownership for at least 2 videos (~$1,250 per 5 min video), which will be disseminated openly and widely. Video content will be generated by the project team.

L. Indirect Costs: $0

Year 1: $0
Year 2: $0

Budget Summary

YEAR 1

	MSU (Loch)	OSU (Smith)	UMinn (Phelps)
Salaries & Wages	$157,160	$0	$15,260
Fringe Benefits	$42,219.97	$0	$4,745
Total Salaries, Wages, and Fringe Benefits	$199,379.87	$0	$20,005
Nonexpendable equipment	$0	$0	$0
Materials and Supplies	$42,496.11	$0	$3,500
Travel	$13,500.00	$0	$3,000
All Other Direct Costs	$7,210.00	$0	$2,500
Totals	$262,585.98	$0	$29,005

YEAR 2

	MSU (Loch)	OSU (Smith)	UMinn (Phelps)
Salaries & Wages	$161,303.09	$0	$15,798
Fringe Benefits	$43,499.20	$0	$4,887
Total Salaries, Wages, and Fringe Benefits	$204,802.29	$0	$20,685
Nonexpendable equipment	$0	$0	$0
Materials and Supplies	$50,164.19	$0	$2.500
Travel	$9,500.00	$0	$4,000
All Other Direct Costs	$15,645	$0	$2,500
Totals	$280,111.48	$0	$29,685

YEAR 1 & 2

	MSU (Loch)	OSU (Smith)	UMinn (Phelps)
Salaries & Wages	$318,462.99	$0	$31,058
Fringe Benefits	$85,719.17	$0	$9,632
Total Salaries, Wages, and Fringe Benefits	$404,182.16	$0	$40,690
Nonexpendable equipment	$0	$0	$0
Materials and Supplies	$92,660.30	$0	$6,000
Travel	$23,000.00	$0	$7,000
All Other Direct Costs	$22,855.00	$0	$5,000
Totals	$542,697.46	$0	$58,690

Schedule for Completion of Objectives

Objectives & Tasks	Year 1						Year 2					
	S O	N D	J F	M A	M J	J A	S O	N D	J F	M A	M J	J A
Objective 1												
Survey creation, distribution, and analysis												
Farm visits by Extension veterinarian												
Creation of site-specific BMPs												
Creation of generalizable BMPs												
Creation of farmer field kit and videos/manuals												
Workshops in coordination with farms/CVMs												
Veterinarian student/veterinarian farm tours												
Objective 2												
Disease surveillance, flavobacterial isolation and identification from NCR farms.												
Genotyping of Fp isolates recovered from the NCR via multilocus sequence typing (MLST).												
Serotyping of Fp isolates recovered from the NCR via multiplex PCR.												
Objective 3												
Evaluate the protective effects of vaccination with whole-killed F. psychrophilum variants in rainbow trout challenged with NCR-predominating causes of BCWD.												
Evaluate the protective effectiveness of the most efficacious BCWD vaccine preparation under NCR-field conditions.												

List of Principal Investigators per Institution

Michigan State University
Loch, Thomas

Ohio State University
Smith, Matthew

University of Minnesota
Phelps, Nicholas

VITA

Thomas P. Loch, Assistant Professor
Michigan State University – Aquatic Animal Health Laboratory
1129 Farm Lane, Room 342, East Lansing, MI 48824

Phone: (517) 884-2019
Fax: (517) 432-2310
Email: lochthom@msu.edu

EDUCATION

B.S. (Michigan State University, 2002, Zoology with specialization in Aquarium Science)
M.S. (Michigan State University, 2007, Veterinary Pathology)
Ph.D. (Michigan State University, 2012, Veterinary Pathology/Microbiology)

POSITIONS

Assistant Professor, Aquatic Animal Health Laboratory, MSU, East Lansing, MI, USA, **2018-Present**
Post-Doctoral Research Associate, Aquatic Animal Health Laboratory, MSU, East Lansing, MI, USA, **2012-2018**
Research Assistant, Aquatic Animal Health Laboratory, MSU, East Lansing, MI, USA, **2004-2012**

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

American Fisheries Society - Fish Heath Section (AFS-FHS), Certified Fish Health Inspector, **2010-Present**
AFS-FHS (Vice Pres.; Pres. Elect; Pres.; Past Pres.), Elected President, **2018-2022**
Great Lakes Fishery Commission - Great Lakes Fish Health Committee (GLFC-GLFHC) Member, **2018-Present**
Michigan Aquaculture Advisory Committee Member, **2014 – Present**

SELECTED PEER-REVIEWED PUBLICATIONS (out of 55)

Bruce, T. J., J. Ma, C. Knupp, T. P. Loch, M. Faisal, and K. D. Cain. (2020) Cross-protection of a live attenuated coldwater disease immersion vaccine against virulent *Flavobacterium* spp. and *Chryseobacterium* spp. strains. Journal of Fish Diseases. *In Press*

Sebastião, F., T. P. Loch, C. K. Knupp, K. Mukkatira, T. Veek, C. Richey, M. Adkison, M. J. Griffin, and E. Soto. 2020. Multilocus sequence typing (MLST) analysis of California *Flavobacterium psychrophilum* reveals novel genotypes and predominance of CC-ST10 in California salmonid hatcheries. Aquaculture Research.

Sebastião, F., T. P. Loch, D. P. Marancik, M. J. Griffin, J. Maret, C. Richey, and E. Soto. 2019. Identification of *Chryseobacterium* spp. isolated from clinically affected fish in California, USA. Diseases of Aquatic Organisms. 136(3):227–234.

Ma, J., T. J. Bruce, P. S. Sudheesh, C. K. Knupp, T. P. Loch, M. Faisal, and K. D. Cain. 2019. Assessment of cross-protection to heterologous strains of *Flavobacterium psychrophilum* following vaccination with a live-attenuated coldwater disease immersion vaccine. Journal of Fish Diseases. 42(1):75–84.

Knupp, C.K., G.D. Wiens, M. Faisal, D.J. Call, K. Cain, P. Nicolas, D. Van Vliet, C. Yamashita, J. Ferguson, D. Meuninck, H-M. Hsu, B. Baker, L. Shen, and T. P. Loch. 2019. Large-scale analysis of *Flavobacterium psychrophilum* MLST genotypes recovered from North American salmonids indicates both newly identified and recurrent clonal complexes are associated with disease. Applied and Environmental Microbiology 85(6):e02305–18.

Loch, T. P., and M. Faisal. 2018. Flavobacteria colonizing the early life stages of hatchery-incubated Chinook salmon *Oncorhynchus tshawytscha* are markedly diverse. Journal of Fish Diseases 41(5):829–845.

LaFrentz, B. R., J. C. Garcia, G. C. Waldbieser, J. P. Evenhuis, T. P. Loch, M. R. Liles, F. S. Wong, and S. F. Chang. 2018. Identification of four distinct phylogenetic groups in *Flavobacterium columnare* with biological relevance. Frontiers in Microbiology 13(9):452.

Van Vliet, D., T. P. Loch, P. Smith, and M. Faisal. 2017. Antimicrobial susceptibilities of *Flavobacterium psychrophilum* isolates from the Great Lakes basin, Michigan, U.S.A. Microbial Drug Resistance 23(6):791–798.

Van Vliet, D., G. D. Wiens, T. P. Loch, P. Nicolas, and M. Faisal. 2016. Genetic diversity of *Flavobacterium psychrophilum* isolated from three *Oncorhynchus* spp. in the U.S.A. revealed by multilocus sequence typing. Applied and Environmental Microbiology 82(11):3246–55.

VITA

Matthew A. Smith
217 Elm Street
London, OH 43140

Phone: 740.852.0975
Fax: 740.852.0744
Email: smith.11460@osu.edu

Education

The Ohio State University
Doctor of Philosophy, projected graduation 2023
Department of Agricultural Communication, Education, and Leadership | Columbus, Ohio

University of Arkansas at Pine Bluff
Master of Science in Aquaculture & Fisheries, 2015
Department of Aquaculture & Fisheries | Pine Bluff, Arkansas

Auburn University
Bachelor of Science in Fisheries Management, 2012
Department of Fisheries & Allied Aquaculture | Auburn, Alabama

Positions

2019 – Present	Program Director, Aquaculture Extension, The Ohio State University, Madison County Extension
2016 – 2019	Extension Aquaculture Specialist, The Ohio State University, Madison County Extension
2015 – 2016	Extension Fish Health Associate, University of Arkansas at Pine Bluff, Lonoke Fish Disease Diagnostics Laboratory

Scientific and Professional Organizations

- North Central Regional Aquaculture Center, *Chair of the Extension Technical Committee and Board member* (2018 – Current)
- North Central Regional Aquaculture Center, *Technical Committee member/Extension and Executive Committee member/Extension* (2016 – 2018)
- Ohio Aquaculture Association, *Active member and Ex-officio Board member* (member from 2016 – Current)
- United States Aquaculture Society, *member and Board Director* (member from 2012 – Current)
- World Aquaculture Society, *Student Board Director and Social Media Analysis Sub-Committee member* (member from 2012 – Current)

Published Journal Article and Editorial

Smith, M.A. and N.M. Stone. 2017. Split Ponds Effectively Overwinter Golden Shiners. Journal of the World Aquaculture Society. 48(5): 760-769.

van Senten, J, M.A. Smith, and C.R. Engle. 2020. Impacts of COVID-19 on U.S. aquaculture, aquaponics, and allied businesses. Journal of the World Aquaculture Society. 51(3). Invited editorial.

Extension Articles *(Partial list)*

Smith, M.A., M. Brehm, and W. Lynch Jr. 2019. Evaluation of alternative pond management systems to enhance production in Ohio. Ohio Aquaculture Association Summer Newsletter.

Smith, M. A. 2019. How much feed can a central Ohio pond assimilate before fish growth is negatively impacted? *Buckeye Aquafarming*. 3(1) 1-5.

Engle, C.R., N.B.D. Phelps, K. Quagrainie, M.A. Smith, C. Weeks, P. and Zajicek. 2019. Strengthening state aquaculture associations: results of NCRAC survey of aquaculture producers in the North Central Region. Indiana Aquaculture Association Newsletter. Spring. 6-7.

Smith, M.A., N.B.D. Phelps, and A. Primus. 2018. Comprehensive outreach and training program to expand development of north central region aquaculture. OSU South Centers Connections Newsletter Achievements Edition. Winter. 4.

Smith, M.A. 2017. Temperature effects on growth and metabolism of fishes. *Buckeye Aquafarming*. 2(2) 5-6.

VITA

Nicholas B. D. Phelps, Assistant Professor
Skok Hall 135
2003 Upper Bufford Circle, St. Paul, MN 55108

Phone: (612) 624-7450
Fax: N/A
Email: phelp083@umn.edu

EDUCATION

B.S. (Bemidji State University, 2005, Aquatic Biology)
M.S. (University of Arkansas at Pine Bluff, 2007, Fisheries and Aquaculture – Fish Health)
Ph.D. (University of Minnesota, 2012, Veterinary Medicine – Comparative Medicine and Pathology)

POSITIONS

Assistant Professor, Dept of Fisheries, Wildlife and Conservation Biology, UMN, St. Paul, MN, USA, **2016-Present**
Director, Minnesota Aquatic Invasive Species Research Center, UMN, St. Paul, MN, USA, **2016-Present**
Assistant Professor, Dept of Veterinary Population Medicine, UMN, St. Paul, MN, USA, **2013-2016**
Scientist, Minnesota Veterinary Diagnostic Laboratory, UMN, St. Paul, MN, USA, **2007-2013**
Research Assistant, Fish Health Laboratory, UAPB, Pine Bluff, AR, USA, **2005-2007**

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

American Fisheries Society - Fish Heath Section (AFS-FHS), multiple committee chair positions, **2005-Present**
Certified Fish Health Inspector, **2007-2019**

SELECTED PEER-REVIEWED PUBLICATIONS (out of 37)

Kanankege, K. S. T., N. B. D. Phelps, K. M. Errecaborde, J. Alvarez, J. B. Bender, S. J. Wells, A. M. Perez. 2020. Lessons learned from the stakeholder engagement and knowledge translation by application of spatial analytical tools on One Health problems. Frontiers in Veterinary Science. doi.org/10.3389/fvets.2020.00254.

Padhi, S., I. Tolo, M. McEachran, A. Primus, S. K. Mor, N. B. D. Phelps. 2019. Koi herpesvirus and carp edema virus: Infections and coinfections during mortality events of wild common carp in the United States. Journal of Fish Disease 42:1609-1621.

Phelps, N. B. D., I. Bueno, D. A. Poo-Munoz, S. J. Knowles, S. Massarani, R. Rettkowski, L. Shen, H. Rantala, P. L. F. Phelps, L. E. Escobar. 2019. Retrospective and predictive investigation of fish kills. Journal of Aquatic Animal Health 31:61-70.

Tomamichel, M. M., N. C. Hodgins, P. A. Venturelli, N. B. D. Phelps. 2018. The prevalence and potential fisheries consequences of *Heterosporis sutherlandae* in a Minnesota lake. PLoS One 13(6):e0199580.

Escobar, L. E., J. Escobar-Dodero, N. B. D. Phelps. 2018. Infectious disease in fish: Global risk of viral hemorrhagic septicemia virus. Reviews in Fish Biology and Fisheries 28:637-655.

Short, G., C. Yue, N. Anderson, C. Russell, N. B. D. Phelps. 2017. Consumer perceptions of aquaponics systems. HortTechnology 27:3 358-366.

Mor, S. K., N. B. D. Phelps, T. F. F. Ng, K. Subramaniam, A. Primus, A. G. Armien, R. McCann, C. Puzach, T. Waltzek. 2017. Genomic characterization of a novel calicivirus, FHMCV-2012, from baitfish in the USA. Archives of Virology doi:10.1007/s00705-017-3519-6.

Escobar, L. E., J. Escobar-Dodero, G. Kurath, M. E. Craft, N. B. D. Phelps. 2017. Potential distribution of the viral hemorrhagic septicemia virus (VHSV) in the Great Lakes region. Journal of Fish Diseases 40:11-28.

Phelps, N. B. D., S. K. Mor, A. Armién, K. Pelican, S. M. Goyal. 2015. Description of the microsporidian parasite, *Heterosporis sutherlandae* n. sp., infecting fish in the Great Lakes region, USA. PLOS One 10(8):e0132027.

Phelps, N.B. D., M. E. Craft, D. Travis, K. Pelican, S. M. Goyal. 2014. Risk-based management of viral hemorrhagic septicemia virus (VHSV-IVb) in Minnesota. North American Journal of Fisheries Management 34:373-379.

Phelps, N. B. D., S. K. Mor, A. G. Armien, W. Batts, A. E. Goodwin, L. Hopper, R. McCann, T. F. F. Ng, C. Puzach, T. B. Waltzek, E. Delwart, J. Winton, S. Goyal. 2014. Isolation and molecular characterization of a novel picornavirus from baitfish in the USA. PLOS One 9:e87593.

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): Mr. Dan Vogler

Title of Project: Improving fish health in the NCR by integrating extension with the development of alternative disease prevention methods

Project Duration: Sept. 1 2021 – Aug. 31 2023

<u>The conditions and terms of the offer being made to you are outlined below:</u>

Position (Extension or Industry): Industry Liaison

Primary Duties/Activities of Liaison: Communication between the Liaison and the PD/Co-PDs will facilitate results from the proposed study to be disseminated to the NCR industry and also provide an open channel for the transfer of newly developed technology and knowledge. Likewise and during the project, this channel will importantly be a two-way form of information exchange so industry can also provide input to the study team.

Appointment offered by:_____Dr. Thomas Loch_____10-3-2020_____
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.

October 29, 2020

Liaison Signature:_____

Date

Please return this letter by (date): __10-29-20_____ to the Project Chair

IOWA STATE UNIVERSITY
OF SCIENCE AND TECHNOLOGY

North Central Regional
Aquaculture Center
339 Science II
Ames, Iowa 50011-3221
515 294-5280
FAX 515 294-2995
http://ncrac.nrem.iastate.edu

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): Matthew A. Smith

Title of Project: Improving fish health across NCR facilities through integration of extension and the development of alternative disease prevention methods

Project Duration: September 1, 2021 – August 31, 2023

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

Position (Extension or Industry): Extension

Primary Duties/Activities of Liaison: Work with the researchers and IAC representative to ensure that the *Flavo* research is transferred to the industry in a way that is meaningful and impactful. Assist the DVM on the project with their Extension-focused deliverables, workshops, and meetings with farms in the Midwest.

Appointment offered by: Dr. Thomas Loch — May 12, 2021

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: Matthew Smith — **May 12, 2021**

Date

Please return this letter by: May 12, 2021 to the Project Chair.

Revised February 2017

Harrietta Hills
TROUT FARM
1681 South 7 ½ Road, Harrietta, MI 49638
Phone: (231) 389-2514 / Fax: (231) 389-2513
dan@harriettahills.com

Thomas P. Loch, MS, PhD
Michigan State University – Aquatic Animal Health Laboratory
1129 Farm Lane, 342 Food Safety and Toxicology Building
East Lansing, MI 48823

July 1, 2020

Dr. Loch

This letter is in support of your proposed NCRAC project "Improving fish health across NCR facilities through integration of extension and the development of alternative disease prevention methods".

Our farm is engaged in the production of rainbow trout from egg through food market size. Disease is a major production variable that can potentially impact our operation at any production stage with significant negative results. Although there is a considerable body of information about fish diseases, there is very little actual qualified and experienced assistance available to producers in the NCR. The development of an extension project that could put "experienced boots on the ground" could be very helpful to many producers and have an immediate positive impact on improving fish health and therefore the bottom line of many farms, including ours.

I believe that this type of project can be impactful in an immediate and significant way and assist a wide variety of producers in the NCR. We support this effort and hope that this project will be successful in receiving NCRAC funding.

Sincerely,

Dan Vogler
President

CRYSTAL LAKE
Fisheries, Inc.
Live Delivery of Rainbow Trout

417-683-2301
800-621-2301
Fax 417-683-6565
info@crystallakefisheries.com
www.crystallakefisheries.com

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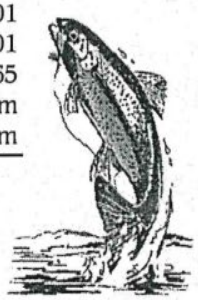


June 17, 2020

Review Team
North Central Regional Aquaculture Center
2020 Request for Proposal concerning CWD

Cold water disease has been and continues to be a problem for trout farmers in the North Central Regional Aquaculture Center region. There appears to be various strains of CWD in different regions in the US. Therefore it is needed to identify the strains in our region and develop strategies to address our NCRAC problems.

I support the proposal by Dr. Loch titled "Improving fish health across the NCR facilities through integration of extension and the development of alternative disease prevention methods."

I request this proposal be given serious consideration. This will assist trout farmers in the NCRAC region to address the issue of CWD.

Sincerely,

Marvin Emerson

July 1, 2020

Alexander Primus, DVM, PhD
Assistant Professor
University of Minnesota
Department of Veterinary Population Medicine
College of Veterinary Medicine
1365 Gortner Avenue
St. Paul, MN 55108

Re: letter of support for NCRAC proposal to improve fish health practices and help prevent disease caused by flavobacteria

Dear Dr. Primus,

I am writing to express Superior Fresh's support for the proposal titled "**Improving fish health across the NCR through integration of extension with the development of alternative disease prevention methods"** for the FY 2021 NCRAC Research and Outreach Funding Program.

I am the Chief Science Officer at Superior Fresh LLC. Superior Fresh LLC is an industry-leading aquaponics facility specializing in producing Atlantic salmon symbiotically with premium, USDA Organic certified leafy greens (lettuce, spinach, kale, etc.). Superior Fresh LLC is the first land-based Atlantic salmon producer in the USA, while also being the largest aquaponic producer in the USA, and possibly the largest in the world. The facility boasts nearly 6 acres of state-of-the-art glass greenhouse currently operating and another 7 acres of additional glass greenhouse under construction. Expansions in Wisconsin, to be completed in 2020, are expected to increase production to approximately 700 MT of Atlantic salmon and 3000 MT of leafy greens annually. Superior Fresh is making fresh food available to major markets across the Midwest, including Minneapolis, Milwaukee, and Chicago. Our sustainably raised fresh fish and leafy greens are nutritious and also available fresh in all four seasons. These combined qualities are not commonly found in food in the Midwest. Furthermore, our team is currently looking at other locations with plans to build much larger facilities that would be convenient to the population centers on the east and west coasts.

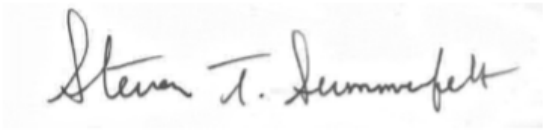
The importance of fish health to aquaculture operations cannot be overstated given the negative impacts that pathogens can have on production and fish welfare. Ensuring producers have a basic understanding of fish health and access to local fish health professionals is crucial for the success of the industry, as fish health is integral to the production process. Additionally, disease caused by flavobacteria are particularly challenging for the aquaculture industry due to their general prevalence and the lack of preventative options currently available in the US. For many salmonids, *Flavobacterium psychrophilum* can be particularly devastating and the development of preventative tools for this pathogen would likely be a great value to the industry as a whole. We hope that work addressing these issues continues to receive support and anticipate that this project would be a great contribution to aquaculture in the region.

W15506 Superior Fresh Drive
Hixton, WI 54635

715.984.2598
www.superiorfresh.com

We look forward to the opportunity of collaborating with the research team that has assembled this proposal, and if funded would be happy to contribute additional meaningful input to support the successful execution of the project.

Kind regards,



Steven Summerfelt, PhD, PE
Chief Science Officer

CC: Brandon Gottsacker, President

W15506 Superior Fresh Drive
Hixton, WI 54635

715.984.2598
www.superiorfresh.com

MICHIGAN AQUACULTURE ASSOCIATION
1681 South 7 ½ Road, Harrietta, MI 49638
info@michiganaquaculture.org

October 29, 2020

To Whom it may concern,

The Michigan Aquaculture Association fully supports the project titled: to "**Improving fish health in the NCR by integrating extension with the development of alternative disease prevention methods**"

Bacterial Coldwater Disease is a major issue to the US trout and salmon industry, causing innumerable mortalities, especially in the hatchery, but throughout production cycles. Improving management methods for Fp that can be immediately deployed in the industry would have an immediate and significant positive impact on the economics of salmonid aquaculture.

The possibility that the methods developed through this project will positively impact the immediate production success of salmonid farms and improve the bottom line, while simultaneously reducing reliance on antibiotics for treatment of sick fish is a double win for salmonid aquaculture.

We support this project and look forward to seeing the results deployed in the field.

Sincerely,

Dan Vogler
President

GRETCHEN WHITMER
GOVERNOR

STATE OF MICHIGAN
DEPARTMENT OF NATURAL RESOURCES
LANSING

DANIEL EICHINGER
DIRECTOR

October 29, 2020

Dr. Thomas P. Loch
Michigan State University – Aquatic Animal Health Laboratory
Depts. of Fisheries & Wildlife, Pathobiology & Diagnostic Investigation
1129 Farm Lane
East Lansing, Michigan 48823
lochthom@msu.edu

Dear Dr. Loch:

Re: Support for NCRAC Flavobacteria Proposal

The Michigan Department of Natural Resources (MDNR), Fisheries Division (Division) strongly supports your proposal entitled "Improving fish health in the NCR by integrating extension with the development of alternative disease prevention methods". This proposal has three key objectives to help control flavobacteria in fish hatcheries:

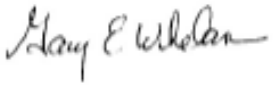
1) Develop practical and usable fish health applications for producers and fish health professionals through farm visits, trainings, and the creation of pragmatic resources by NCR fish health veterinarians;
2) Determine, for the first time, the predominating Flavobacterial strains driving economic losses in the NCR trout industry; and
3) Evaluate the effectiveness of newly developed vaccines in preventing losses caused by regionally predominating flavobacteria under laboratory and field conditions. The completion of work proposed for these objectives will improve fish rearing conditions and increase efficiency across the North Central region.

Flavobacterial infections are one of the most serious issues confronting aquaculture both in the North Central Region and in North America. These common bacteria affect a broad range of species and are one of the leading pathogens that kill fish both in hatcheries and wild situations. An examination of the Division's fish health cases over the last three years (2018-2020) shows that Flavobacterial infections were involved in 24 cases in 2018 (12 diagnostic and 12 inspection cases of a total of 55 diagnostic and 155 inspection cases), 49 cases in 2019 (15 diagnostic and 34 inspection cases of a total of 55 diagnostic and 142 inspection cases), and 17 cases thus far in 2020 (12 diagnostic and 5 inspection cases of a total of 27 diagnostic and 36 inspection cases). The 2020 case load is reduced from previous years due to production changes and reduced field inspections due to COVID19.

Most of these cases showed mortalities or reduced rearing efficiency from Flavobacterial infections and we consider this group of pathogens to be among the most problematic that we deal with in our system. As a result, we strongly support additional focused NCRAC research on these pathogens that will benefit all in our region.

We strongly support the proposed work on flavobacteria and request that NRCAC give this work strong consideration for funding. If you have any questions on this matter, please contact me.

Sincerely,



Gary E. Whelan
Program Manager
Fisheries Division
(517) 284-5840

Great Lakes Fishery Commission

October 19, 2020

Dr. Thomas P. Loch
Michigan State University - Aquatic Animal Health Laboratory
Dept. of Fisheries & Wildlife, College of Agriculture & Natural Resources
Dept. of Pathobiology & Diagnostic Investigation, College of Veterinary Medicine
1129 Farm Lane, Room 342, East Lansing, Michigan 48824

Dear Dr. Loch:

I am pleased to offer the Great Lakes Fishery Commission's enthusiastic support for your proposal titled *Improving fish health in the NCR by integrating extension with the development of alternative disease prevention methods*. This proposed outreach and research you and your collaborators seek to undertake is exactly the type of work needed to help fish producers and state hatchery operators deliver higher quality products with fewer losses to the market or to lakes and streams for recreational or rehabilitation purposes. Simply put, various *Flavobacterium* species can cause tremendous losses each year in private and state production settings. Your team's three pronged approach to work with producers to develop best management practices that will reduce the probability of spreading a pathogen through a facility; to identify the *Flabobacterium psychrophilum* strains that affect operations in the North Central Region (NCR); and to evaluate effectiveness of vaccines to combat these regional strains will be an incredibly important advance toward reducing production losses caused by bacterial coldwater disease and other related bacterial diseases. These vaccines also have the potential to improve wild fish health.

As a person who regularly interacts with state, federal, and provincial fish health professionals and hatchery managers, it is quite clear to me that various diseases caused by *Flavobacterium* species are a major concern across the entire NCR, having the potential to cause huge losses in production fish each year. Because these bacteria are widespread in coldwater hatchery settings and frequently cause extensive mortality outbreaks, combating these pathogens is a great and persistent concern of fishery management agencies throughout the Great Lakes basin and the NCR. In addition to their persistent and cryptic nature, research into the diversity of *Flavbacterium* strains demonstrates that this pathogen is remarkably diverse within a fairly restricted geographic area like the NCR. As a result, for the eventual goal of an effective vaccine to combat the pathogen, understanding the strain diversity within the region is a necessary first step.

I have every confidence that you and your collaborators have the necessary experience, laboratory facilities, and outreach capacity to achieve your goals. Completing this research and outreach will be a huge advancement in our ability to treat such persistent and widespread diseases like bacterial coldwater disease. I look forward to the successful funding of your proposal and to learning the outcomes of your research and outreach.

Sincerely,

John M. Dettmers

John M. Dettmers, Ph.D.
Fishery Management Director
Great Lakes Fishery Commission

THE OHIO STATE UNIVERSITY

College of Food, Agricultural, and Environmental Sciences
Ohio State University Extension

Office of the Interim Director
Jacqueline K. Wilkins

3 Agricultural Administration Building
2120 Fyffe Road
Columbus, Ohio 43210-1084

614-292-1842 Phone
614-688-3807 Fax

http://extension.osu.edu

October 28, 2020

Dr. Joseph Morris
Director, North Central Regional Aquaculture Center
Iowa State University
339 Science II
Ames, IA 50011-3221

Dear Dr. Morris,

I am very pleased to write a letter of support on behalf of OSU Extension in the College of Food, Agricultural, and Environmental Sciences at The Ohio State University for the proposal, *Improving Fish Health in the NCR by Integrating Extension with the Development of Alternative Disease Prevention Methods*. Matthew Smith, co-Principal Investigator (PI) of this proposal and Program Director for Aquaculture Extension in OSU Extension, approached the Department about the novelty of potentially creating an Extension aquaculture veterinarian position by initial funding through NCRAC. Smith discussed with us the plethora of veterinarians with an aquaculture familiarity in some Midwest states, yet many states remain barren. We believe this would complement Ohio State's efforts towards supporting aquaculture expansion in the Midwest. OSU Extension would be glad to house an Extension aquaculture veterinarian to support region-wide Extension activities along with the research proposed by Michigan State University and University of Minnesota in this proposal, with the understanding that this position will only be able to continue past the scheduled two-year grant period if additional extramural funding can be secured by Smith and/or other faculty or staff at Ohio State.

We thank you for your time and consideration and hope that reviewers and NCRAC understand our commitment to the Land Grant mission. Should you have any questions you may reach out to my office.

Sincerely,

Jacqueline Kirby Wilkins, Ph.D.
Interim Director, Ohio State University Extension
Director of Operations, Ohio State Extension

State of Wisconsin
Governor Tony Evers

Department of Agriculture, Trade and Consumer Protection
Secretary-designee Randy Romanski

October 28, 2020

Dr. Thomas P. Loch
Michigan State University-Aquatic Animal Health Laboratory
Depts. Of Fisheries & Wildlife, Pathology & Diagnostic Investigations
1129 Farm Lane, East Lansing, MI 48823

Dear Dr. Thomas Loch,

I write on behalf of the Wisconsin Department of Agriculture, Trade and Consumer Protection and myself in support of your grant proposal to NCRAC, **Improving fish health in the NCR by investigating extension with the development of alternative disease prevention methods**. I strongly support your grant proposal's focus on fish health and veterinary extension support to fish farmers. Your proposal would advance many of the main elements I have pioneered and worked on in my career as a fish veterinarian in the North Central Region (NCR). I also strongly agree that, **flavobacterial diseases are the major cause of mortalities on trout farms, and other fish species farms, in the NCR**, and your proposed efforts to reduce disease losses could greatly help fish farms in the NCR.

In 1993 I became the first private practice veterinarian in the NCR. I started by providing veterinary service to trout farms. This was the first time trout farmers were introduced to the veterinary medicine approach to fish health. My work focused on providing veterinary services ranging from issuing fish health certificates to production medicine investigations to increase farm profits. Your proposal would continue that effort on this theme and advance the efforts by increasing the level of veterinary fish health involvement on trout farms, and introduce more trout farms to fish veterinary services in the NCR.

In 1999 I began directing the first training program in the U.S. for veterinarians on fish farm veterinary medicine. It included hands-on training conducted at fish farms. Between 1999 and 2004 I conducted many workshops in Wisconsin, Minnesota, South Dakota, and Ohio. Veterinarians came from Iowa, Michigan, Nebraska and other NCR states to attend my workshops. So I heartily concur with your proposal's inclusion of expanding training including hands-on training for veterinarians, veterinary students, graduate students, fish farmers and other. Your efforts will provide great benefits to trout farmers.

My efforts resulted in developing a corps of private practice veterinarians in Wisconsin. As a result, more than fifteen years ago, Wisconsin eliminated the shortage of private practice fish veterinarians available to all fish farms including trout farms. Wisconsin continues to produce a surplus of fish veterinarians. Of course, this is in contrast to many states in the NCR where there

remains a shortage of fish veterinary services for fish farms. Your proposal would be a great help to NCR states that have a shortage of fish veterinary services. As you point out in your proposal, there is a great need for veterinary services, access to fish health professionals and fish diagnostic laboratories.

In an effort to broaden the reach of my training of fish veterinarians, in 2004, Dr. Michael Collins and I were awarded a USDA grant to develop a five module online course on fish farm veterinary medicine for veterinarians. **The Fish Health Medicine Certificate Program** has benefited over 400 veterinarians and veterinary students over the past 15 years. This program has been an important tool in supporting the training of veterinarians, especially where there is a shortage of fish veterinarians and fish health services in the NCR. I see that your proposed efforts would build on and utilize my course as one element in your effort to train more fish veterinarians in areas that your project will identify as suffering from a current shortage of fish veterinary services.

My next effort was to train fish farmers on how to work with fish veterinarians, and fish health professionals. Dr. Chris Hartleb worked with me to produce the NCRAC supported six module **Fish Health for Fish Producers** online course, which is a highly successful course that remains a strong component in fish farmer education in the U.S. It has trained producers on how to work with fish veterinarians, fish health professionals, and fish health laboratories. The course has been completed by over 700 participants, many of whom work for fish farms throughout the U.S. and the world. This is the course that was so kindly mentioned in your grant proposal. I am pleased to read that my work has been acknowledged and I support the efforts stated in your proposal to build on my work.

In recent years, my efforts have now focused on training veterinarians to do more production veterinary medicine; that is work that generates improvements in production and profitability of fish farms. I am pleased to see you propose to contribute and expand fish veterinary production medicine concepts to fish farmers and within the fish health infrastructure.

For the past 20 years I have consulted with fish farmers and veterinarians on an almost daily basis. Many have been from Wisconsin, but many are from other NCR states. I continue to see new challenges, but the fundamental issues of fish health are quite clear, yet remain only partially addressed. Many of the veterinarians and fish farmers who have completed my courses contact me for assistance on challenging fish veterinary issues, and fish farmers call with questions on how to address disease events on their farms. Your project would expand and provide more resources to support and advance efforts, such as mine, of providing consultation to fish farmers and veterinarians on fish health issues. Trout farmers would greatly benefit from a regional extension veterinarian, especially those in less served areas.

For the past 11 years, I have lead the University of Wisconsin-School of Veterinary Medicine's **Fish Health Selective Course**. It is a week-long hands-on training of a limited and select number of veterinary students. My training focuses on having the students conduct fish health procedures on commercial trout, natural resource trout hatchery, tilapia aquaponic, and clownfish farms in Southern Wisconsin. Your project proposes to use a similar approach to training fish farmers, veterinary students and veterinarians. I can say from experience that this is a very effective approach and if you select a veterinarian who understands how to successfully conduct such training, your project could also be very successful.

In my work with trout farms, I have for many years ranked **flavobacterial disease as the number one infectious disease category**. In my opinion, it contributes to more losses than any other group of infectious disease agents. Every trout farm has seen some level of trout losses due to flavobacterial diseases and I have seen many suffer catastrophic losses. I feel that you and your laboratory's efforts have made a major contribution to understanding flavobacterial diseases and your proposed efforts to address preventative medicine tools, including vaccines, would be a tremendous contribution to trout farming in the NCR.

I have had the pleasure to work with you, and all of your co-investigators over many years and I have confidence they can complete this project. I would be very interested and willing to work with you and your co-investigators on this project, which has the potential to advance the work that I have devoted much of my veterinary career to for over 25 years in the NCR.

If NCRAC has questions or they would like additional information from me I can be reached at myron.kebus@wisconsin.gov or 608-224-4876.

Sincerely,

Myron J. Kebus, M.S., DVM
Fish Health Veterinarian
Division of Animal Health
Wisconsin Department of Agriculture, Trade & Consumer Protection
2811 Agriculture Dr.
Madison, WI 53708

MICHIGAN STATE
UNIVERSITY

October 26, 2020

Dr. Joseph E. Morris, Director
North Central Regional Aquaculture Center
Iowa State University
339 Science II
Ames, IA 50011-3221

SUBJECT: Project entitled: "*Improving fish health in the NCR by integrating extension with the development of alternative disease prevention methods*"

Dear Dr. Morris:

As the Authorized Organizational Representative (AOR) I would like to inform you that Michigan State University (MSU) wishes to participate in the above referenced project as a subcontractor to Iowa State University. Dr. Thomas Loch will serve as the Principal Investigator of the subcontract and he has access to all of the necessary equipment, laboratory, and office space to successfully undertake this project.

The proposal has been reviewed and approved by the appropriate officials at Michigan State University and certified to its accuracy and completeness. Should this proposal be awarded, and a mutually acceptable agreement issued, MSU looks forward to working on this proposed work.

Sincerely,

Digitally signed by Craig O'Neill
DN: cn=Craig O'Neill, o=Michigan State University, ou=Manager - Office of Sponsored Programs AOR, email=oneillc@osp.msu.edu, c=US
Date: 2020.10.26 14:08:13 -04'00'

Craig O'Neill
Sponsored Programs Manager

KC

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ProposalTeam2@osp.msu.edu
http://www.osp.msu.edu

Checklist for Submission of Full Proposals

X Format manuscripts for 22 x 28 cm (8½ x 11 inch).

X Number *all* pages sequentially.

X Use 10-12 font; Times New Roman. Do not justify right margins.

X Format headings appropriately.

X Leave at least a 2.5-cm (1-inch) margin on all sides.

X Use metric units of measurement with English units in parenthesis, e.g. 2.54 cm (1 inch).

X Define all abbreviations the first time they are used.

X Express ratios by using a slant line (e.g. mg/L).

X 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.

X 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.

X 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).

X Include signed Letters of Intent for identified Extension and Industry Liaisons.

X Signed Authorized Organization Representative (AOR) form from each funded PI's institution are welcomed but not required at this time.

X Include the required three (3) Letters of Support from Industry members who are not directly involved in the proposed project.

X 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).

X Provide names of three possible reviewers who will not have a Conflict of Interest

X All identified co-PIs have been provided a final draft of the full proposal.

X 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.

Principal Investigator Signature  Date May 12, 2021