Evaluating novel methods for preventing *Aeromonas*-associated losses in Yellow Perch (*Perca flavescens*) using laboratory and field-based vaccination trials (*NCRAC Out of Cycle Proposal*)

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

Chairperson:

Thomas P. Loch Phone: 517-884-2019
Michigan State University – Aquatic Animal Health Laboratory Fax: 517-432-2310

Depts. of Fisheries & Wildlife, Pathobiology & Diagnostic Invest. Email: lochthom@cvm.msu.edu

1129 Farm Lane, East Lansing, MI 48824

Co-Principal Investigators:

Robert K. Smith DVM Phone: 715-948-2971 Clayton Veterinary Care Fax: 517-948-2999

123 S. Hwy 63 Box 99 Email: clayvet1@amerytel.net

Clayton WI 54004

Matthew A. Smith Phone: 740-956-5049
The Ohio State University Fax: 740-852-0744

College of Food, Agricultural and Environmental Sciences

Email: smith.11460@osu.edu

OSU Extension, 217 Elm St., London, OH 43140

Industry Liaison:

Mr. William M. West, Blue Iris Fish Farm, LLC

Extension Liaison:

Matthew A. Smith, The Ohio State University

Funding Request: \$35,000

Duration: 1 year (04/01/2021 - 03/31/2022)

Objectives:

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

Deliverables:

- 1. Report results from field trials and laboratory challenge study in a scientific journal
- 2. Report results from field and laboratory challenge study at scientific and producer association meetings
- 3. Documentation of vaccination procedures
- 4. Farm field day with the cooperating producer depending on the progress of COVID-19

Proposed Budget:

Institution	PI(s)	Objectives	Year 1	Total
Michigan State University	Thomas P. Loch	2	\$18,000	\$18,000
Ohio State University	Matthew A. Smith	1-2	\$1,500	\$1,500
Clayton Veterinary Care	Robert K. Smith	1	\$15,500	\$15,500
		Totals	\$35,000	\$35,000

Non-funded Collaborators:

Facility	Collaborators
Blue Iris Fish Farm, Black Creek, WI	Mr. William M. West
University of Wisconsin-Milwaukee	Dr. Dong-Fang Deng

Project Summary

Yellow Perch (*Perca flavescens*; YP) is a priority species within the North Central Region (NCR), and yet few resources have been devoted to improving their health on farms, a matter complicated by the apparent emergence of several YP-pathogenic *Aeromonas* spp. across the upper Midwest. Dr. Smith recently developed a novel approach for **immersion** vaccinating young (<1-week-old) salmonids against *Aeromonas salmonicida*, without handling the fry (i.e., no handling stress). Using this method, vaccinated fish showed robust protective immunity exceeding 5 years, translating to farm survivability boosts of 40% pre-vaccination to >95% post-vaccination. Using this promising approach, our team proposes to extend this method to effectively vaccinate YP against predominating *Aeromonas* strains affecting YP across the NCR. The bivalent bacterin (i.e., vaccine) will be produced by Kennebec River Biosciences through United States Department of Agriculture (USDA) approved means and administered to YP under laboratory conditions. Vaccinated and control (i.e., mock-vaccinated) YP will then be challenged with an *A. salmonicida* strain previously recovered from an NCR YP mortality event and protective effects determined. Likewise, the protective effects of this vaccine and procedure will be assessed under farm conditions under multiple scenarios (e.g., multiple densities) in conjunction with pre- and post-treatment veterinary health assessments.

Justification

YP have been a high priority farmed fish species in the Great Lakes region and the NCR of the USA for quite some time (Malison 2000), where it continues to be a species garnering high demand. Regrettably, a multitude of factors continue to hamper fish health and subsequent YP farm productivity in the NCR, including diseases caused by microbial fish pathogens. In fact, disease research on vaccines was noted as a priority in 2020 NCR Aquaculture Roundtable Sessions (Columbus, OH), specifically in the percids discussion section. Over the last 20+ years, Co-PI Dr. R. Smith has been investigating disease outbreaks that have occurred in farmed YP stocks across the NCR (e.g., WI, MN, MI, SD, MO) and determined that *A. salmonicida*, causative agent of furunculosis (Cipriano and Bullock 2001), has been at the root of many of these mortality events. Similarly, PI Loch's group isolated this same bacterium and confirmed its role in a YP mortality event in a MI facility in 2007 (Diamanka *et al.* 2013). Concerningly, this bacterium is typically recognized as a pathogen of salmonids, meaning much of the research towards preventing and controlling this disease has not included percids, leaving YP farmers with limited prevention and treatment options at best. To this end, our assembled project team proposes to utilize a potentially revolutionary and immediately deployable and promising method (please see previous work conducted by Dr. Smith below) to vaccinate farmed YP against the predominating *Aeromonas* strains driving losses across the NCR. This project is needed to provide scientific data beyond anecdotal evidence and provide a major boost to the aquaculture industry.

Related Current and Previous Work

Successful vaccination against multiple bacterial pathogens has been achieved by a novel method of low stress and neutral environmental practices. Both humoral and amnestic responses have been successful by this method. *A. salmonicida* bacterins have been used multiple times, in multiple ways; however, Dr. Smith has found his method to yield positive results consistently. For example, he has vaccinated salmonids as early as 7 days post-hatch and YP up to 16 months old, both of which have led to antibody production sufficient to prevent disease. Of note, we are unaware of any published literature on vaccine development for use in farmed YP in general, nor against *A. salmonicida* in this species in specific, leaving perch farmers facing a substantial knowledge gap.

PI Loch's research team has substantial research experience with *A. salmonicida* (Loch and Faisal 2010b, Diamanka *et al.* 2013, Diamanka *et al.* 2014) and other *Aeromonas* spp. (Loch and Faisal 2010a, Loch *et al.* 2012) causing disease in fish. Also, PI Loch's research team has several ongoing studies (funded by USDA-NIFA and the Great Lakes Fishery Commission) towards developing vaccines against economically important diseases affecting farmed and hatchery reared fishes; however, the proposed research activities will benefit from, rather than duplicate, these ongoing efforts.

OSU Extension and the University of Minnesota recently created Extension programming activities in the Midwest. However, there were no farm tours accompanying those workshops and trainings activities. Additionally, our work did not incorporate the potential for vaccine use in fish. Our proposed novel work would allow OSU Extension to work with the veterinarians and cooperating farmers to create an on-farm Extension deliverable regarding vaccine usage in aquaculture.

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: yellow perch, *Aeromonas*, vaccine, 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 will be a multitude of benefits to the industry if the proposed project were to be funded. First and foremost, YP farmers within the NCR will be a primary benefactor of the proposed study. It is likely that perch farmers will be able to treat both young of the year fry (potential long-term immunity) and older fish with immersion vaccination technology. Vaccination will likely be more successful, and affordable, for fry due to lower labor requirements and necessary equipment. An immersion vaccine is also likely to be less stressful on the animals, as smaller fish can be handled more easily by the farmer or veterinarian. Vaccine administration via immersion instead of injection is also easier from a technical standpoint. Vaccinating the fish expediently is vital as this is an increased cost the farmers would incur. However, our goal is to develop a vaccine and vaccination process that leads to healthier fish which do not get sick from *Aeromonas*, can reach market size sooner, and be more marketable. If the vaccine is successful and the vaccination process is expedient, the farmers can work with Extension to evaluate whether or not increased health and survival of the perch outweigh the increased costs of vaccination on their particular farm. This technique may be cost effective and can be administered by a licensed fish veterinarian or by the farmer under direction of the veterinarian. More importantly, this technique is not unique to perch. It is believed that similar approaches will be initiated with other species – note that this approach has already proven effective for salmonids.

Due to the global COVID-19 pandemic, we also believe it's worth noting the drastic increase in discussions regarding human vaccination. Polls now show over six in ten Americans intend to be vaccinated as soon at a vaccine is available. Although there will always be some who have concerns regarding giving anything to an animal that may later be consumed, we believe that the inevitable success of these COVID-19 vaccines will assist producers with elevator speeches and marketing campaigns as they can discuss the comparison of agriculture vaccines and COVID-19 vaccines. Vaccines are a scientific approach to being proactive and limiting the chance for an infection to occur. A significant biological benefit to the successful vaccine technique must be accompanied by social license, and Extension can provide benefits to producers by making the comparison of COVID-19 vaccines and agriculture vaccines.

Objectives

The overarching goal of the proposed study is to increase the productivity of YP culture in the NCR by improving fish health using an alternative disease prevention method. To this end, the objective of this project is to establish and carry out protocols for the vaccination of YP to impart immunity against problematic strains of *Aeromonas* (particularly *A. salmonicida*), thereby increasing overall health, growth, and survival. In specific, we propose:

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

Procedures

Dr. Smith was one of Wisconsin's first private fish veterinarians, working in the field of aquaculture for over 20 years. He currently devotes about 60% of his time to providing fish veterinary services and is currently licensed in three states (e.g., Wisconsin, Minnesota and Missouri). Dr. Smith is working on a two-phased approach to dealing with fish disease. His new and alternative **immersion** vaccination protocols have shown to impart long-term immunity to fish in the fry stage or immediate disease treatment to older fish. Some of this work has already been done by Dr. Smith with respect to brook trout. Additional work cited by Dr. Myron Kebus, Wisconsin Veterinarian

with Department of Agriculture, Trade and Consumer Protection (DATCP), shows increased production with respect to tilapia once fish health issues have been addressed. In these cases, vaccines have already been prepared and have shown to work in the field. Currently, Dr. Smith is working to have a vaccine made to address several strains of *Aeromonas* that appear to be increasing in prevalence in perch populations in the Great Lakes region.

Obj. 1- To assess the protective effectiveness of a new vaccination approach and preparation against *Aeromonas* infections in farm raised YP (Dr. Smith). Four to five month old feed trained YP (to be obtained in collaboration with Dr. Dong-Fang Deng, University of Wisconsin-Milwaukee) and one week old YP hatched and feed trained at Blue Iris Fish Farm are proposed to be immersion vaccinated, per methods developed by Dr. Smith, in tanks to be monitored for at least three months at Blue Iris Fish Farm located in Black Creek, WI. The vaccine will be an autogenous bivalent bacterin supplied by Dr. Smith. The vaccine will be produced by Kennebec River Biosciences as a USDA approved vaccine, under strict guidelines followed by Kennebec and Dr. Smith starting at culture through actual vaccine production. This bacterin will be used to produce antibodies against several strains of *A. salmonicida* that have been recovered from yellow perch over the past three years by the Wisconsin Veterinary Diagnostic Lab (Madison, WI) and Kennebec River Biosciences (Richmond, ME).

Dr. Smith or another veterinarian trained by Dr. Smith will perform a VHA every six weeks, with an additional one performed during vaccination (either just before or just after). A VHA has both subjective and objective parameters that help measure the current health/condition (and growth) of the fish, which is well-documented through research performed by Ron W. Goede and Dr. Myron Kebus, for example. This procedure has been employed in several prior studies of which Dr. Smith was asked to be part of, including an on-going walleye (WAE) study with the Great Lakes tribes, a past Steelhead production study, Wisconsin Department of Natural Resources, and a past/on-going WAE pond growth study for St. Croix tribal.

Each group of vaccinated fish will be raised separate from a control group (i.e., mock-vaccinated), and raised in identical fashion (e.g., same rearing density, age, water temperature, oxygen content, percent of biomass feeding, etc.). Each control group will have an initial VHA plus corresponding VHAs performed in conjunction of the test tanks. During the project, three VHAs will be performed per tank. Each VHA requires a total of twenty fish to be sacrificed. A summary worksheet will be completed for each VHA and a full summary will be prepared compiling all data obtained.

Obj. 2- To assess the protective effectiveness of a new vaccination approach and preparation against Aeromonas infections in YP under controlled laboratory conditions (Dr. Loch). Concurrent with the field studies, vaccination experiments under controlled laboratory conditions will be undertaken in the Research and Containment Facility of the Michigan State University – Aquatic Animal Health Laboratory. YP for vaccination experiments will be procured (from Dr. Deng as noted above) and maintained in flow-through polyvinyl chloride (PVC) tanks supplied with dechlorinated pathogen-free water and fed ad lib. (photoperiod of 12 hours of light). Upon reaching 4 months of age, yellow perch will be immersion vaccinated as above using the autogenous bivalent bacterin supplied by Dr. Smith. Eight weeks post immunization, YP will be challenged with an A. salmonicida subsp. salmonicida isolate that was previously recovered and identified from a yellow perch mortality event. Treatment groups will include vaccinated A. salmonicida challenged (n = 20 fish, in triplicate), vaccinated mockchallenged (immersion in 0.65% saline, n = 20 fish, in triplicate), and unvaccinated (n = 20 fish, in triplicate). Fish will be monitored daily for 30 days; moribund fish will be euthanized with an overdose of sodium bicarbonatebuffered tricaine methanesulphonate (MS-222; 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 percent mortality (CPM) and relative percent survival (RPS) of all replicates will be calculated (RPS = [1-(% mortality of vaccinated fish / % mortality of non-vaccinated fish)] x 100. Analysis of CPM, RPS, and growth of YP over the study period will be analyzed by one-way ANOVA and pairwise comparisons will be made using Tukey's test.

Project Deliverables:

- 1. Report results from field trials and laboratory challenge study in a scientific journal
- 2. Report results from field and laboratory challenge study at scientific and producer association meetings
- 3. Documentation of vaccination procedures
- 4. Farm field day with the cooperating producer depending on the progress of COVID-19

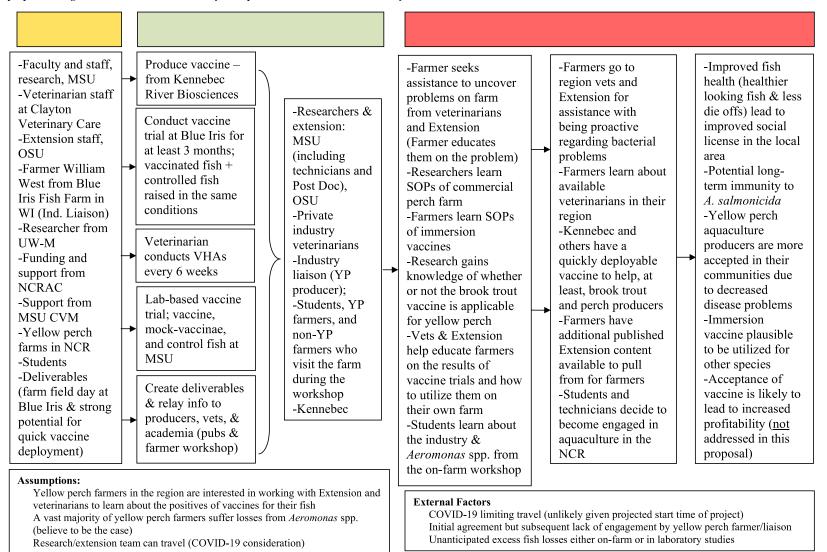
Evaluation and Outreach

YP are coveted food and sport fish in the NCR, but commercial harvests frequently cannot match demand. Indeed, recent severe limitations in commercial harvests from areas previously rich in YP (e.g., Lake Michigan, Green Bay, and Lake Erie) led to little or no availability for restaurant menus in spring of 2020, for example. Thus, although YP has always been a priority species for NCRAC, interest in YP commercial aquaculture is growing. Paradoxically, few resources have been dedicated towards development of YP disease prevention and control. To address this gap of resources facing NCR YP producers, the proposed research will test the effectiveness of a promising novel immersion-based vaccination approach for protecting YP against Aeromonas infections. Importantly, should this vaccine be as effective as it has been to date in earlier trials conducted by Dr. Smith, this vaccine could be deployed immediately within the NCR when following FDA Center for Veterinary Biologics guidelines, and could be commercially available immediately following experimental trials with veterinary oversight. Importantly, the proposed research will also provide undergraduate students and a post-doctoral researcher at MSU with hands-on research, training, and opportunities to develop as a scientific scholar. In addition to outreach revolving around YP vaccination and fish health procedures between the proposed research team and the producers at the farms involved in this study, we also propose to host a field day at Blue Iris Fish Farm in collaboration with NCRAC. Invitees will include producers and high school and college students interested in aquaculture and/or fish health and veterinary medicine, who will receive a hands-on day of training in contemporary YP culture methods, fish health basics, and an introduction to novel methods for prevention of fish disease outbreaks. Additionally, the team will attempt to publish our work in a peer reviewed scientific journal, in producer association journals (e.g., Wisconsin's CREEL and Ohio's newsletters), and at scientific and producer conferences if travel is allowed due to COVID-19.

Although the vaccines developed for this project will be species specific (i.e., protection of YP from A. *salmonicida*), the protocols to be used for this project are not species specific. It is important for the aquaculture industry to recognize the importance of this work as it relates to many species currently under culture in the NCR as there are more than fifty species culture in the region.

EVALUATING NOVEL METHODS FOR PREVENTING Aeromonas-ASSOCIATED LOSSES IN YELLOW PERCH (Perca flavescens) USING LABORATORY AND FIELD-BASED VACCINATION TRIALS

Goal: Evaluate a novel trout immersion vaccine for potential use with yellow perch. Objectives: 1. To assess the protective effectiveness of a new vaccination approach and preparation against Aeromonas infections in farm raised yellow perch. 2. To assess the potential effectiveness of a new vaccination approach and preparation against Aeromonas infections in yellow perch under controlled laboratory conditions.



Facilities

Dr. Loch's laboratory is comprised of resources ideal for the proposed research, with dedicated spaces for virology, bacteriology, general microbiology, holding of live fish, *in vivo* experimental challenge systems, clinical examination, necropsies, histopathology, molecular microbiology, serology, and microscopic examinations. The lab includes 232 sq-m (2,500 sq-ft) of wet laboratory space for the housing of aquatic animals within a 9,290 sq-m (100,000 sq-ft) state-of-the-art quarantine facility, which is under the MSU-College of Veterinary Medicine and provides 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 in the facility, including pumps, motors, fans, air conditioning, heating, refrigeration, and water levels. The facility enables optimal long- and short-term maintenance for aquatic animals during experiments, equipped with filtered 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 oxygen treated, passed through a series of sand filters and activated carbon filters, and passed through UV sterilizers.

The primary farm to be used for the field assessment protocols will be Blue Iris Fish Farm in Black Creek, WI. This facility is an outdoor perch production facility but much of the culture used at Blue Iris is in pond-side tanks. Blue Iris will have available twelve production tanks, six each of low profile 76 cm (30 in) depth for fry culture, and six grow-out tanks (approximate 127 cm [50 in] working depth). Additional outdoor facilities can be made available depending on the number of duplicates and controls deemed needed.

References

- Cipriano, R.C. & Bullock, G.L. (2001) Furunculosis and other diseases caused by *Aeromonas salmonicida*. In: *Fish Disease Leaflet*. US Fish and Wildlife Service, USGS, Kearneysville, WV.
- Diamanka, A., Loch, T.P., Cipriano, R.C. & Faisal, M. (2013) Polyphasic characterization of *Aeromonas* salmonicida isolates recovered from salmonid and non-salmonid fish. *Journal of Fish Diseases*, **36**, 949-963
- Diamanka, A., Loch, T.P., Cipriano, R.C., Winters, A.D. & Faisal, M. (2014) Infection of sea lamprey with an unusual strain of *Aeromonas salmonicida*. *Journal of Wildlife Diseases*, **50**, 159-170.
- Loch, T.P. & Faisal, M. (2010a) Infection of lake whitefish (*Coregonus clupeaformis*) with motile *Aeromonas* spp. in the Laurentian Great Lakes. *Journal of Great Lakes Research*, **36**, 6-12.
- Loch, T.P. & Faisal, M. (2010b) Isolation of *Aeromonas salmonicida* subspecies salmonicida from lake whitefish (*Coregonus clupeaformis*) inhabiting lakes Michigan and Huron. *Journal of Great Lakes Research*, **36**, 13-17.
- Loch, T.P., Scribner, K., Tempelman, R., Whelan, G. & Faisal, M. (2012) Bacterial infections of Chinook salmon, *Oncorhynchus tshawytscha* (Walbaum), returning to gamete collecting weirs in Michigan. *Journal of Fish Diseases*, **35**, 39-50.
- Malison, J.A. (2000) A white paper on the status and needs of yellow perch aquaculture in the North Central Region. In: *NCRAC White Paper*, pp. 1-17. North Central Regional Aquaculture Center Publications Office, Iowa State University, Ames, Iowa.

Project Leaders

State	Name/Institution	Area of Specialization
Michigan	Thomas P. Loch, Michigan State University	Fish health, flavobacterial diseases, vaccine development
Wisconsin	Robert K. Smith, DVM Clayton Veterinary Care	Private veterinarian working in the field of Aquaculture
Ohio	Matthew A. Smith, The Ohio State University	Aquaculture Extension/water quality

	GANIZATION AND ADDRESS				USDA AWARD N	o. Year 1: Object	ctives: 1&2	
Michigan State University Hannah Administration Building			Duration Proposed	Duration Proposed	Non-Federal Proposed Cost-	Non-federal Cost-Sharing/		
426 Auditorium Rd, Room 2			Months: 12	Months:	Sharing/	Matching Funds		
East Lansing, MI 48824.2600 PROJECT DIRECTOR(S)			Funds Requested by	Funds Approved by CSREES	Matching Funds (If required)	Approved by CSREES		
	omas Loch	ı			Proposer	(If different)		(If Different)
A.	Salaries and Wages	CSREES FL	JNDED WORK	MONTHS				
	No. of Senior Personnel	Calendar	Academic	Summer				
	a1_ (Co)-PD(s)	0	0	0				
	b Senior Associates							
	2. No. of Other Personnel (Non-Faculty) a. 1 Research Associates-Postdoctorates	2	0	0	\$8,418.35			
	b Other Professionals							
	c Paraprofessionals							
	d Graduate Students							
	e1_ Prebaccalaureate Students				\$1,296.19			
	f Secretarial-Clerical							
	g Technical, Shop and Other				\$9,714.54			
	Total Salaries and Wages							
B.	Fringe Benefits (If charged as Direct Costs) Total Salaries, Wages, and Fringe Benefits (A p	lue B)			\$2,140.18			
					\$11,854.72			
D.	Nonexpendable Equipment (Attach supporting data for each item.)	a. List items	and dollar	amounts				
E. Materials and Supplies				\$4,496.53				
F.	Travel							
G. Publication Costs/Page Charges								
H. Computer (ADPE) Costs								
l. 	Student Assistance/Support (Scholarships/fellowsheducation, etc. Attach list of items and dollar amou			ost of				
J.	All Other Direct Costs (In budget narrative, list item provide supporting data for each item.)	ns and dolla	r amounts a	and	\$1,648.75			
K.	Total Direct Costs (C through I)				\$18,000.00			
L.	F&A/Indirect Costs. (If applicable, specify rate(s) activity. Where both are involved, identify itemized				\$0			
М.	Total Direct and F&A/Indirect Costs (J plus K)				\$18,000.00	_		
N.	Other			П	15,500.00			
0.	Total Amount of This Request				\$33,500.00			
	Carryover (If Applicable) Federa			Non-Federa		Total \$		
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ų.	Cost Sharing/Matching (Breakdown of total am Cash (both Applicant and Third Party)			<i>'</i> 			Leave Blank	
	Non-Cash Contributions (both Applicant and	Third Party)						
_	NAME AND TITLE (Type or print)		SI	GNATURE	(required for revis	ed budget only)		DATE
	oject Director Omas Loch							
Au	thorized Organizational Representative							
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ORGANIZATION AND ADDRESS		USDA AWARD N	o. Year 1: Object	ctives: 1&2			
The Ohio State University Madison County Extension			Duration Proposed	Duration Proposed	Non-Federal Proposed Cost-	Non-federal Cost-Sharing/	
217 Elm St., London, OH 43140				Months: 12	Months:	Sharing/ Matching Funds	Matching Funds Approved by
PROJECT DIRECTOR(S) Matthew A. Smith				Funds Requested by Proposer	Funds Approved by CSREES (If different)	(If required)	CSREES (If Different)
A. Salaries and Wages	CSREES F	UNDED WORK	MONTHS				
1. No. of Senior Personnel	Calendar	Academic	Summer				
a1_ (Co)-PD(s)			0				
b Senior Associates							
 No. of Other Personnel (Non-Facul 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 plue B)						
D. Nonexpendable Equipment (Attach supporting for each item.)	g data. List item	s and dollar	amounts				
E. Materials and Supplies			\$600				
F. Travel			\$900				
G. Publication Costs/Page Charges							
H. Computer (ADPE) Costs							
Student Assistance/Support (Scholarships/fel education, etc. Attach list of items and dollar attach)			ost of				
 J. All Other Direct Costs (In budget narrative, lis provide supporting data for each item.) 	items and dolla	ar amounts a	and				
K. Total Direct Costs (C through I)							
L. F&A/Indirect Costs. (If applicable, specify ra activity. Where both are involved, identify item							
M. Total Direct and F&A/Indirect Costs (J plus		•	<u> </u>	\$1,500			
N. Other				¢4 500			
O. Total Amount of This Request			🗆	\$1,500			
P. Carryover (If Applicable) Fe	deral Funds: \$	l	Non-Federa	al funds: \$	Total \$		
Q. Cost Sharing/Matching (Breakdown of total Cash (both Applicant and Third Party)						Leave Blank	
Non-Cash Contributions (both Applicant	and Third Party	,					
			(required for revis	ed budget only)		DATE	
Project Director Matthew A. Smith							
Authorized Organizational Representative							
	-						

Budget Explanation for Michigan State University (Thomas P. Loch)

Objective 1&2

A. Salaries and Wages: \$9,714.54

Year 1: \$9,714.54

- Salary costs for post-doctoral research associate Dr. Megan Shavalier are to perform tasks
 contributing to this proposed project; e.g., vaccine and immunization experiments,
 maintenance and challenges of live fish, isolation of bacteria, biochemical and molecular
 confirmatory testing.
- Hourly wages are for an undergraduate student research assistant to provide support during the academic year for this project; e.g., daily live fish care, sample processing and extractions, and assistance with laboratory maintenance and assays.
- B. Fringe Benefits: \$2,140.18

Costs for fringe benefits are requested in the amount of \$2,140.18 for health insurance benefits for research associate Dr. Shavalier.

E. Materials and Supplies: \$4,496.53

Items	Year 1	Total
bacteriological culture supplies for growing and reisolating		
the bacterium	\$935.70	\$935.70
biochemical reagents for identification	\$645.00	\$645.00
chemicals and supplies for molecular confirmation	\$720.30	\$720.30
microbiological supplies for experiments	\$539.00	\$539.00
needles and syringes for vaccinations and infection challenges	\$65.00	\$65.00
necropsy supplies	\$146.28	\$146.28
disposable laboratory consumables (e.g., pipette tips, gloves,		
petri dishes, sample collection tube, syringes, transfer		
pipettes, scalpels)	\$1,390.25	\$1,390.25
fish food	\$55.00	\$55.00
Total	\$4,496.53	\$4,496.53

F. Travel (Domestic):

Year 1: \$0

J. Other Direct Costs:

Year 1: Fish Housing Facility Rental - Rental fees for fish holding space at the MSU-Research Containment Facility are requested to house fish to carry out the proposed live fish experiments. (\$1,648.75).

N. Other. \$15,500 total Contract to Dr. Robert K. Smith for services to the project. Provide labor and supervision on extra label vaccination (Dr. Smith); 12 Veterinary Health Assessments performed by Dr. Smith (or Dr. Andrew Martin), assisted by Certified Animal Technician, computation of values, clerical labor (in house CVC); 30 hours of labor for travel (3 trips to BlueIris Fish Farm)- \$8,300.

Materials and Supplies

Items	Year 1	Total
Procurement of Autogenous bacterin through Kennebec River		
Biosciences, bacterial isolations, bacterial Identifications,		
transfer media and mailers	\$2500.00	\$2500.00
Autogenous Bivalent bacterin – 9 liters @\$325/liter	\$2925.00	\$2925.00
Use of YSI Oxygen meter, model 20, microscope usage,		
detail timers, weigh scales etc.		
Equipment usage provided by CVC	\$350.00	\$350.00
Necropsy supplies for 240 fish (VHAs)	\$400.00	\$400.00
VHA supplies such as MS-222, towels, micro slides/slips,		
miscellaneous, etc.	\$125.00	\$125.00
Total	\$6300.00	\$6300.00

Travel (Domestic):

Year 1: \$900

- 3 trips to BlueIris Fish Farm (500 miles each roundtrip) vehicle usage @\$.50/mile (\$750) -each trip meals (\$50) for 2-3 employees X 3 trips (\$150) -actual travel labor above in salaries/wages

Budget Explanation for Ohio State University (Matthew A. Smith)

Objectives 1 & 2

A. SALARIES AND WAGES:

Salaries and wages: \$0

Other Professionals (A&P Staff) PI/PD: Smith is the OSU PI on the project; however, Smith is not requesting any salary for his engagement. Either Smith or another OSU Extension Educator will travel to Wisconsin to participate in the on-farm workshop at Blue Iris Fish Farm.

B. FRINGE BENEFITS:

Fringe: \$0

• Fringe rate for FY2021 for A&P staff is 31.5%.

E. MATERIALS AND SUPPLIES:

Items	Year 1	Year 1 Total
Office materials for on-farm workshop.		
Total	\$600	\$600

F. TRAVEL (DOMESTIC):

Travel: \$900

• Transportation for OSU Extension person travel to Blue Iris Fish Farm to participate in the on-farm workshop in order to assist with dissemination of results.

Total requested funds for the 1-year project: \$1,500

Summary Budget for Year <u>1 of 1</u>

		7		
	Objective #	Michigan State University (Thomas Loch)	Ohio State University (Matt Smith)	Project TOTAL
Salaries, Wages, and Fringe Benefits	1, 2	\$11,854.72	\$0	\$20,154.72
Nonexpendable Equipment	-	\$0	\$0	\$0
Materials and Supplies	1, 2	\$4,496.53	\$0	\$10,796.53
Travel	1	\$0	\$900.00	\$1,800.00
Other	1, 2	\$15,500.00		
All Other Direct Costs	2	\$1,648.75	\$600.00	\$2,248.75
TOTAL		\$33,500.00	\$1,500.00	\$35,000.00

Schedule for Completion of Objectives

Objectives & Tasks		Year 1						
	M	M	J	S	N	J	M	
	A	J	Α	О	D	F	Α	
Objective 1								
To assess the protective effectiveness of a new vaccination approach and preparation against Aeromonas infections in farm raised Yellow Perch (Dr. Smith)	X	X	X	X	X	X	X	
Objective 2								
To assess the protective effectiveness of a new vaccination approach and preparation against Aeromonas infections in Yellow Perch under controlled laboratory conditions (Dr. Loch).	X	X	X	X	X	X	X	

List of Principal Investigators per Institution

Michigan State University

Loch, Thomas P.

Ohio State University

Smith, Matthew A.

Clayton Veterinary Care

Smith, Robert K.

VITA

Thomas P. Loch, Assistant ProfessorPhone: (517) 884-2019Michigan State University – Aquatic Animal Health LaboratoryFax: (517) 432-23101129 Farm Lane, Room 342, East Lansing, MI 48824Email: 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.
- **Loch, T. P.**, and M. Faisal. 2016. Flavobacteria isolated from the milt of feral Chinook Salmon *Oncorhynchus tshawytscha* of the Great Lakes. North American Journal of Aquaculture 78(1):25–33.
- 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

Robert K. Smith D.V.M.

Clayton Veterinary Care (owner/staff veterinarian since 9/1982) 123 S. Hwy 63 Box 99 Clayton WI 54004 Phone: 715-948-2971 Fax: 517-948-2999

Email: clayvet1@amerytel.net

EDUCATION

University of Missouri- Columbia, School of Veterinary Medicine, 1978-1982 Doctor of Veterinary Medicine

University of Wisconsin-River Falls, 1975-1978

Interests: Biochemistry/Animal Science

Current Licensure: Doctor of Veterinary Medicine (in good standing)

State of Wisconsin, 1982-present State of Missouri, 1982-present State of Minnesota, 2001-present

USDA – APHIS: American Veterinary Accreditation (level II)

State of Wisconsin, 1982-present State of Missouri, 2010-present

Wisconsin Department of Agriculture (DATCP): Wisconsin Certified Veterinarian of Aquaculture, 1998-present **Lead speaker or workshop leader:**

Bemidji State University (guest lecture) 3/2018. "Veterinary Medicine in Aquaculture" -Bemidji, MN NADF 'boot camp' FHA workshop 3/2017, 6/2017

-Bayfield, WI

-Lac du Flambeau, WI

Minnesota Veterinary Medical Association convention 2/2010. "Aquatic Veterinary Medicine" -St. Paul, MN Missouri Aquaculture Association Biosecurity Workshop 2/2008 -Jefferson City, MO

University of Missouri-Columbia. 8/2008 "Aquaculture in Veterinary Medicine"

-Columbia, MO, School of Veterinary Medicine

20th Great Lakes Regional Conference 10/2007

"Biosecurity on a commercial farm in Wisconsin"

-Lac du Flambeau, WI, Native American Fish and Wildlife Society

University of Wisconsin-Extension, 9/2007 Biosecurity Outline Workshop

- -Wisconsin Aquaculture Association
- -Stevens Point, WI

University of Wisconsin-Extension 6/2007 WIDATCP's Aquaculture Field Day NADF, Bayfield, WI

Wisconsin 2001-2002 Effluent Research Grant

-Administrative veterinarian, WAA/DATCP grant

Assisted instructing for DATCP's fish health medicine certificate program

- -2005, 2006, 2007 practical "wet lab"
- -Madison, WI, Dodgeville, WI

WAA conventions (speaker) 2001, 2006, 2016, 2018, 2020

- -Marshfield, Fond du Lac, Eau Claire WI
- "The Creel", WAA publication multiple articles 2000,2004,2014,2016,2019

Professional Organization Memberships:

American Veterinary Medical Association, AVMA Aquaculture Veterinarians-North Central Region (WAVMA), Wisconsin Veterinary Medical Association, NWVMA, British Columbia Aquaculture Veterinarian Association, AFS-Fish Health Section, NAA, World Aquaculture Society, WAA, MNAA, MOAA

VITA

Matthew A. Smith

Phone: 740.852.0975 217 Elm Street Fax: 740.852.0744

London, OH 43140 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 –
- 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 MA and Stone NM. 2017. Split Ponds Effectively Overwinter Golden Shiners. Journal of the World Aquaculture Society. 48(5): 760-769.

van Senten J, Smith MA, and Engle CR. 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 MA, Brehm M, and Lynch Jr W. 2019. Evaluation of alternative pond management systems to enhance production in Ohio. Ohio Aquaculture Association Summer Newsletter.

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

Engle CR, Phelps NBD, Quagrainie K, Smith MA, Weeks C, and Zajicek P. 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 MA, Phelps NBD, and Primus A. 2018. Comprehensive outreach and training program to expand development of north central region aquaculture. OSU South Centers Connections Newsletter Achievements Edition. Winter. 4.

Smith MA. 2017. Temperature effects on growth and metabolism of fishes. Buckeye Aquafarming. 2(2) 5-6.

July 3, 2020

Dr. Joseph Morris - Director USDA North Central Regional Aquaculture Center Iowa State University 339 Science II Ames, IA 50011-322 Onto Aquaculation

Sociation

Dear Dr. Morris:

I'm pleased to write this letter in support of a project submitted to the USDA NIFA North Central Regional Aquaculture Center Out-of-Cycle, Rapid Response program by Drs. T. Loch and R. Smith titled "Evaluating Novel Methods for Preventing Aeromonas-associated Losses in Yellow Perch using Laboratory and Field-based Vaccination Trials".

Aquaculture in the North Central Region (NCR) is challenged to operate profitability, with pond culture systems being the primary, profitable culture system used. Pond culture presents considerable challenges should a disease outbreak occur given whole pond treatments are expensive, sick fish often do not eat, and new regulations on therapeutic use make treatment to often be delayed. The proposal proposes to validate a novel, immersion vaccination approach to address recent outbreaks of what appears to be new, problematic *Aeromonas* strains recently affecting yellow perch in the upper NCR. These new strains may also be in Ohio as in the last month, several Ohio yellow perch farms have experienced unexplained mortality but with characteristic signs of *Aeromonas* sp. I anticipate these outbreaks will become more widespread in the immediate future. If successful, this novel vaccination approach could allow quick, mass vaccination of fry prior to their use in ponds.

While this proposal addresses a disease issue with yellow perch, validating this approach can only benefit other species cultured in the NCR as the technology should easily be applied to other species such as walleye, largemouth bass etc. This approach could become a powerful tool in our fish health toolbox, a toolbox that quite honestly has few tools in it. I urge the USDA-NIFA program to fund this project.

Sincerely,

Bill Lynch

William Lynch
Millcreek Perch Farm, LLC
President, Ohio Aquaculture Association

Silver Moon Springs Fish Hatchery



Tim Winkel
Silver Moon Springs Trout Farm, LLC
Elton, WI 54430

NCRAC Out of Cycle Proposal

June 29, 2020

NCRAC Proposal Reviews,

This letter is to provide support for the NCRAC proposal entitled: **Evaluating novel methods for preventing** *Aeromonas***-associated losses in Yellow Perch (***Perca flavescens***) using laboratory and field-based vaccination trials.** While it may seem odd that a trout farm owner would heartily recommend studies of this nature this is actually something of significance to the aquaculture industry.

I am the current owner of Silver Moon Springs Trout Farm in northern Wisconsin. Typically, trout farms are based around natural water sources and can only be as biosecure as open farm situations allow. That means from time to time we are exposed to various trout diseases. In the past several years we have been working with Dr. Bob Smith who has initiated vaccinations of trout as a method of controlling disease. The results seem to have imparted both temporal and long-term immunity to typical trout diseases. The results vary depending on the age of the fish treated but it seems as though the earlier the treatment, the longer the immunity and the carryover seems to be in terms of years. The results of vaccination are several-fold including better feed uptake for increased growth and significant increase in survival. The techniques which are outlined in the proposal have resulted in a significant cost savings for my operation.

Sincerely,

Tim Winkel
Silver Moon Springs Trout Farm

To whom it may concern

I have spent over 30 years in the restaurant business, first as a Chef and then as a restaurateur. This has given me the eyes to look at our food system from a unique viewpoint. It also instilled a discipline in regards to feasibility so we can live better and grow more healthy options for our family.

After 25 years, my wife and I returned to New Ulm, MN where I grew up. We purchased a warehouse and began building a unique location that houses many different showcases to educate how we can live and grow our own food in a sustainable manner.

We use the bottom floor of the warehouse to house our fully functioning aquaculture center. We have a Yellow Perch Hatchery with thousands of fish in various stages of life. This includes 2 fully mature broodstock groups, from different genetic regions as well as several other groups and ages of fish. Our lab has the ability to isolate and study small groups of yellow perch for various scientific results. We also have a 4,000 square foot grow out facility that currently houses several groups of fish being produced for both the food fish market and pond stocking.

During this time challenges have arisen and we think that now is the time for collaboration.

We have discovered multiple strains of *Aeromonas*. We now feel we can study ways to control this bacteria so that fish especially in containment can live and grow in a more stress free and healthy way. We need partners in this endeavor and think that with the production of a bivalent vaccine we can move into the study phase to maximise results.

We cannot stress enough that ONLY through comprehensive collaboration can we overcome these challenges. We strongly support this project for funding NCRAC Out of Cycle "Rapid Response" Proposal

Project Title: Evaluating novel methods for preventing *Aeromonas*-associated losses in Yellow Perch using laboratory and field-based trials.

Please do not hesitate to contact us if more information is required.

Topher Jacobson PepperBoy Farms

3 Ju Science II Ames, loxar 5001 (-322) 513-204-5200 FAX 513-204-2005 http://m.rac.mrem.ustaic.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): Wi	liam M. West					
Title of ProjectEvaluating	novel methods for preventing Aero	omonas-associated losses in Yellow				
Perch (Perca flavescens) using laboratory and field-based vaccination trials.						
Project Duration: April 1, 2021	- March 31, 2022					
The conditions and terms of th	e offer being made to you are outlin	ned below:				
Position (Extension or Industry	:Industr_y					
		nd the industry that will allow for efficient yledge and new technology between the PI and				
Appointment offered by:	Jester	Sept. 4, 2020				
	Project Chair	Date				
Offer approved by:						
	NCRAC Director	Date				
		itions, and I agree to these terms and accept uent written agreement signed by both partic				
Liaison Signature: //	ligh my lilest	9/25/20				
		Date				
Please return this letter by:	Emailto the Project	t Chair.				

Revised February 2017

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): Matthew A. Smith

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

Project Duration: 04/01/2021 - 03/31/2022

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

Position (Extension or Industry): Extension

Primary Duties/Activities of Liaison: Assist with the deliverables of the vaccine project. Assist with the on-farm workshop at Blue Iris Fish Farm.

Appointment offered by:	Thomas Loch Project Chair	12/04/2020_ 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 Suith 12/04/2020

Date

Please return this letter by (date): 12/06/2020 to the Project Chair

Checklist for Submission of Full Proposals

X	Format manuscripts for 22×28 cm $(8\frac{1}{2} \times 11$ inch).	
<u>X</u>	Number all pages sequentially.	
<u>X</u>	Use 10-12 font; Times New Roman. Do not justify right margins.	
<u>X</u>	Format headings appropriately.	
<u>X</u>	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).	
<u>X</u>	Define all abbreviations the first time they are used.	
<u>X</u>	Express ratios by using a slant line (e.g. mg/L).	
<u>X</u>	Scientific names should accompany common names in the title and when they are first mentioned in the abstract and the text. Authority for scientific names need not accompany the genus and species unless needed for clarity.	
<u>X</u>	Spell out one to ten unless followed by a unit of measurement (e.g. four fish, 4kg , 14fish). Do not begin a sentence with numeral. Use $1,000$ instead of 1000 ; 0.13 instead of $.13$; and % instead of percent.	
<u>X</u>	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).	
<u>X</u>	Include signed Letters of Intent for identified Extension and Industry Liaisons.	
<u>X</u>	ned Authorized Organization Representative (AOR) form from each funded PI's institution are welcomed but not nired at this time.	
<u>X</u>	Include the required three (3) Letters of Support from Industry members who are not directly involved in the proposed project.	
<u>X</u>	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 Institution and Principal Investigators, Curriculum Vitae for Principal Investigators (PIs).	
<u>X</u>	Provide names of three possible reviewers who will not have a Conflict of Interest	
<u>X</u>	All identified co-PIs have been provided a final draft of the full proposal.	
<u>X</u>	Submit proposal (including all required documentation) in single MS Word document.	
If the N	ICRAC Administrative Office cannot verify inclusion of any element, the Full Proposal will not be accepted.	
9	December 10, 2020	
Princip	al Investigator Signature Date	

in