GUIDELINES FOR WRITING A SRAC PRE-PROPOSAL
Individual Objective Method
Updated November 4, 2020

General Instructions:

Type the project proposal double-spaced using any standard 12 pt. typeface using the guidelines below. The completed proposal should contain the following elements:

1) A cover sheet with project summary
2) The project narrative
3) Vita for each participating scientist
4) Budgets pages consisting of budgets for each institution and an overall budget page for the entire project.

Send an electronic copy (Word) of your proposal to the SRAC Director as email attachment to jimmy.avery@msstate.edu.

Proposal Format:

1) Cover Sheet with Project Summary (Page 1)

   This page should include the following: a) title of the project; b) the name, institution, address, phone number, and email address of the lead scientist; c) a list of cooperating scientists (if appropriate) and their corresponding institutions; and d) a Project Summary of 250 words or less. The Project Summary must be self-contained and describe the approach to meeting the project objective.

2) Project Narrative (start on new page)

   The Project Narrative should not exceed 12 double-spaced pages. The Narrative should contain the following items:

   a) Objectives: Restate the project objective as stated in the Request for Pre-Proposals.

   b) Procedures: The procedures or methodology to be applied to the proposed effort should be explicitly stated and directly linked to the project objective. This section should include but not necessarily be limited to a description of the proposed investigations and/or experiments; techniques to be employed, including their feasibility; kinds of results expected; means by which data will be analyzed or interpreted; pitfalls which might be encountered; and limitations to proposed procedures. Also see the description of

desired project components under "Experimental Approach" in the *Request for Pre-proposals* for additional considerations that you should address in this section.

c) Institutional Units Involved: Pre-proposals can be submitted by individual institutions or as a collaboration of multiple institutions. When multiple institutions are involved, identify each institution and define the roles and responsibilities of each unit of the project team and point out the nature of collaboration. Institutions should be from states or territories in the Southern Region (Alabama, Arkansas, Florida, Georgia, Kentucky, Louisiana, Mississippi, North Carolina, Oklahoma, Puerto Rico, South Carolina, Tennessee, Texas, U.S. Virgin Islands, and Virginia).

d) Project Timetable: The proposal should outline all important phases as a function of time, year by year, for the entire project, including periods beyond the grant funding period.

e) Logic Model: All pre-proposals must include a Logic Model that clarifies the linkages between investments and activities, outputs, and expected outcomes of the funded project. This addition is intended to help rationalize a project and facilitate stronger focus and reporting on expected outcomes or notable accomplishments. If you have submitted either a USDA AFRI or US EPA grant recently, you already have experience in Logic Model development. The USDA NIFA Generic Logic Model can be accessed at: https://nifa.usda.gov/resource/generic-logic-model-nifa-reporting
Logic Model information and training is available at the University of Wisconsin Division of Extension Logic Model Information and Training website (https://fyi.extension.wisc.edu/programdevelopment/logic-models).

f) Duplication of Research Statement: To address the important issues of potential duplication of research, ensuring efficient and effective use of federal funds, and to facilitate new knowledge beyond the current state of science, all proposals must include a statement that indicates that relevant federal aquaculture-related research databases were accessed and reviewed. This is to ensure that the proposed project does not duplicate any previously funded research and that the proposed work is original research.

Therefore, proposals should include a statement stating that "…relevant federal databases that include funded aquaculture-related research were accessed and reviewed including: the USDA Research, Education, and Economics Information System (REEIS); national and state Sea Grant programs and funded project databases; the National Sea Grant Library, and the USDA Regional Aquaculture Centers' websites. These databases were accessed to search for and review any projects that are related to or the same as the research project proposed herein does not duplicate any previously funded projects found in these databases, and that the proposed work is original research."

Relevant aquaculture-related research databases include:

USDA REEIS: http://www.reeis.usda.gov/

USDA Regional Aquaculture Centers:

- Northeastern Regional Aquaculture Center: http://www.nrac.umd.edu/
- Southern Regional Aquaculture Center: http://www.srac.msstate.edu/
- North Central Regional Aquaculture Center: www.ncrac.org
- Western Regional Aquaculture Center: http://www.fish.washington.edu/wrac
- Center for Tropical and Subtropical Aquaculture: http://www.ctsa.org

NOAA/Sea Grant Projects:

- Search Projects: http://seagrant.noaa.gov/WhatWeDo/SearchProjects.aspx
- State Programs: http://www.seagrant.noaa.gov/other/programsdirectors.html
- National Sea Grant College Program: http://www.seagrant.noaa.gov
- National Sea Grant Library: http://nsgd.gso.uri.edu

Also, you may also want to consider searching Google Scholar for information relevant to the subject of interest: http://scholar.google.com

3) Vitae

Include a one-page vita for each participating scientist. Use the attached format.

4) Budget pages

A one-page budget proposal for the project should be prepared. If there are multiple institutions, the overall budget must be followed by separate budgets for each participating institution. Use the Budget pages provided. **NOTE:** Indirect costs and tuition remission are not allowed. Contracts are awarded on a 12-month basis so there is no carryover from year to year for those on contract. Accountability of expenditures and distribution of funds to participants will be the responsibility of each participating institution.

Salaries of 100% hard-funded faculty with a 12-month appointment who will be serving as principal and co-investigators are not allowed and should be considered as institutional contributions. Salary is allowed for soft-funded positions (faculty, Post-docs, research associates, GRAs, etc) and summer salary for PIs or CO-PIs on 9-month appointments.

Budget Justifications

Equipment Purchases. Equipment purchases will be allowed when 1) the project is a high priority to industry development or problem-solving, 2) the equipment is essential for the success and accomplishment of specific research objectives, 3) the equipment has a useful life beyond the project for long-term benefits for the institution's capacity for research as well as contributions to industry development, and 4) the equipment is of a specialized nature and would not normally be expected to be in University inventory. SRAC grant funds for research and extension projects may not be used for office equipment and furnishings, air-conditioning, standard computers, or other general purpose equipment. A separate page for this justification should be attached to the budget.

Travel: All proposals must contain strong justification for any travel outside of the region or not directly required for field work or project team collaboration. The travel should be directly linked to the funded project and deemed supportive of the specific objectives of the regional project. This justification should be included in the budget summary. The use of electronic communication tools other than travel is encouraged when feasible to reduce travel costs and time.

Attending a national conference to "Present findings", "Gather background information", or "To meet with other project participants" will have to pass additional scrutiny in today's audit-sensitive environment. Certainly, making scientific presentations on SRAC funded projects at national meetings is encouraged. However, it can hardly be justified early in the project when data is still incomplete or when multiple project participants want to present on the same subject. If an investigator is using SRAC funds, then there should be an accepted abstract or agenda item based on work generated by SRAC funding. Travel will be reimbursed for only one presenter.

VITA (centered at top)

(skip one line)
Name
Address
Phone
Fax
E-mail
(skip one line)

EDUCATION
(skip one line)
B.S. (year, major, institution,)
M.S. (year, major, institution,)
Ph.D. (year, major, institution,)
(skip one line)

EMPLOYMENT
(skip one line)
List each position held on a separate line from most recent to oldest
(skip one line)

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS
(skip one line)
List each organization on a separate line
(skip one line)

SELECTED PUBLICATIONS
(skip one line)
List several recent publications (from most recent to oldest) relevant to the subject area of the project. Skip one line between each entry.

Proposed Budget for the Regional Project:
TITLE OF THE REGIONAL PROJECT

Institution:				Year 1	Year 2	Year 3	Total
Scientist name:							
Salaries and Wages	CSREES-FUNDED WORK MONTHS						
1. No. Of Senior Personnel	Calendar	Academic	Summer				
a. ____ (Co)-PD(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							
Fringe Benefits (If charged as Direct Costs)							
Total Salaries, Wages, and Fringe Benefits							
Nonexpendable Equipment (Attach justification. List items and dollar amounts for each item.)							
Materials and Supplies							
Travel (Attach justification.)							
Publication Costs/Page Charges							
All Other Direct Costs							
Total Direct Costs							
Total Amount of This Request							

(Example: Individual Objective Method)

Project Title: Development of Management Options against Epidemic *Aeromonas hydrophila*
Objective 2: Evaluate the effect of specific disinfectant(s) on the abundance of the epidemic *A. hydrophila.*

Lead Scientist: Cova Arias
Department of Fisheries and Allied Aquacultures
Auburn University
203 Swingle Hall. Auburn, AL 36849
(Phone) 334 844 9215, (E-mail) ariascr@auburn.edu

Project Summary:

Comparison of disinfection protocols to inactivate *Aeromonas hydrophila* in seines.

Since 2009, a new strain of *Aeromonas hydrophila* has caused a devastating epidemic of motile aeromonas septicemia in catfish grown in West Alabama. To date, efforts to control this epidemic have failed and the disease is now widespread in Alabama. Based on a recent study, the use of commercial seining companies appears to be a risk factor for this new form of the disease. Although disinfecting protocols for seines and farm-related equipment are well known, they are not always implemented at the farms as part of their best management practices protocols. This project will identify the best disinfecting protocol that inactivates the epidemic strain of *A. hydrophila* in seines. In addition to examining the sensitivity of *A. hydrophila* planktonic cells to the disinfecting treatments, we will also examine the efficacy of those on biofilm developed on seines.

PROJECT NARRATIVE

Introduction

The Gram negative bacterium *Aeromonas hydrophila* is one of the causative agents of Motile Aeromonas Septicemia (MAS) in fish. Considered an opportunistic pathogen, *A. hydrophila* can affect a variety of freshwater fish species around the world (Austin and Austin 1999). This pathogen is ubiquitous in the aquatic environment although MAS typically occurs when predisposing stressors unbalance the host-pathogen equilibrium in favor of the bacterium. However, sporadic examples of *A. hydrophila* as a primary pathogen have also been reported in the literature (Plumb 1999). Since 2009, catfish producers in Alabama have lost more than 7.5 million pounds of market size catfish due to an epidemic of MAS that started in the heart of the catfish-producing region in West Alabama. The causative agent of this epidemic was quickly isolated and identified as *A. hydrophila* and further characterized as a unique strain with higher virulence towards catfish than other previously studied *A. hydrophila* strains (Hemstreet 2010). Despite joint efforts between USDA, Auburn University and Mississippi State University scientists, new cases continue to arise every summer. Currently, no preventive or palliative measures are effective against this disease.

In a recent survey, Dr. Bebak (private consultant for the Alabama Catfish Farmers Association) identified several environmental factors and management practices linked to an increased risk of disease prevalence in farms. Results from the study showed that farms that used commercial seines to harvest their fish suffered more *A. hydrophila* outbreaks than those who used their own seining equipment. This practice is likely to increase the contagion risk between farms if seines are not properly disinfected in between ponds.

Disinfection protocols for seines and other farm-related equipment have been previously proposed to increase the biosecurity of fish farms (Sadler and Goodwin 2007) but they are not frequently used in commercial catfish farm operations. In addition, the resistance of *A. hydrophila* (including the epidemic strain) to standard disinfectants is unknown. This project aims at providing valuable information regarding the best disinfection protocols to inactivate the epidemic strain of *A. hydrophila* in seines and other farm-related equipment.

Our group has been investigating the attachment and colonization of *A. hydrophila* to seines. We have shown that A. hydrophila not only is able to attach to seine materials but can also develop into biofilm. Biofilm is produced when bacterial cells attached to a physical support surround themselves with a matrix of organic compounds that protects them against adverse conditions including desiccation and disinfection agents. Therefore, the proposed experimental design will evaluate the effects of disinfectants on both planktonic *A. hydrophila* cells (cells in water or attached to seines but without the protective cover) and *A. hydrophila* biofilm.

Objectives

Objective 2. Evaluate the effect of specific disinfectant(s) on the abundance of the epidemic *A. hydrophila*.

The overall goal of this project is to provide the catfish producers with the best disinfecting protocol to eliminate *A. hydrophila* from seines and other equipment. To attain this goal, I propose the following subobjectives:

Subobjective 2a. Establishment of quantification methods to detect bacteria in seines.

This project will rely on the use of rapid and cost-efficient molecular tools to quantify the viable bacteria attached to seines after the different disinfection processes. First, these quantification methods will need to be adapted to work with seines. Since the protocols are very similar, I propose to evaluate the effect of the disinfection protocols in two other catfish pathogens *Edwardsiella ictaluri* and *Flavobacterium columnare* in addition to *A. hydrophila.*

Subobjective 2b. Testing disinfection protocols under laboratory conditions.

A myriad of disinfecting agents, and application times will be assayed to determine the best protocol. These assays will be conducted in the laboratory using artificial mesocosms in where known amounts of bacteria will be attached to the seines prior to disinfection.

Subobjective 2c. Field experiments at the E. W. Shell Fisheries Station, Auburn.

The three protocols showing the best results under laboratory conditions will be used in commercial size nets/seines in our experiment fisheries station to determine the feasibility of these methods under field conditions.

Procedures

Subobjective 2a. Establishment of quantification methods to detect bacteria in seines.

Based on our preliminary data, we have determined the best conditions to allow attachment of *A. hydrophila* cells to seines. Briefly, *A. hydrophila* epidemic strain ML09-119 will be cultured in Brain Heart Infusion (BHI) broth at 30°C for 18 h. Cells will be washed and resuspended in autoclaved pond water to a final concentration of ~10^4 colony forming units (CFU/ml). This concentration is well above the detection limit of the techniques used and will ensure an accurate determination of the reduction in cell numbers (if any) as a consequence of the different treatments. Commercial, twisted, polyethylene, meshed webbing (TPE) seines will be used across the experiments. Fragments of TPE seines will be cut into 1.5 in^2 pieces and dipped into the *A. hydrophila* suspension for 1 h. After exposure, bacterial DNA will be extracted from the TPE fragments using a commercial kit (PowerSoil-MoBio). During the first step of the extraction, beads and shaking will be used to release all bacterial cells from the net. The rest of the protocol will follow manufacturer's instruction.

To quantify the number of bacterial cell equivalents in the extracted DNA we will use quantitative PCR protocols. Specific primers against the epidemic strain ML09-119 have been developed by Dr. Liles' group (Auburn University, personal communication) and have already been tested in our laboratory. qPCR assays will be conducted following standard protocols in a ABI7700 thermocycler. In addition to detecting *A. hydrophila*, co-cultures of *A. hydrophila*, *E. ictaluri* and *F. columnare* will be used to test the disinfection power of the different protocols. qPCR protocols for *E. ictaluri* and *F. columnare* have been previously published and are currently used in our laboratory (Bilodeau et al. 2003, Panangala et al. 2007).

Subobjective 2b. Testing disinfection protocols under laboratory conditions.

Fragments of seines will be exposed to planktonic *A. hydrophila* alone or in combination with *E. ictaluri* and *F. columnare* cultures, as described above. After 30 minutes of exposure, the seines will be treated using the following methods (Sadler and Goodwin 2007):

- Ambient desiccation. Seines will be allowed to dry at room temperature in the dark for 30 min, 1 h, and 6 h.
- Ambient desiccation under sunlight. Seines will be allowed to dry at ambient temperature (that will be measured) under intense sunlight for 30 min, 1 h, and 6 h.
- Bleach. Seines will be immersed in 1000 ppm sodium hypochlorite for 30 min, 1 h, and 6 h. Seines will be rinsed in tap water and dried before further processing.
- Chlorine. Seines will be immersed in 6% calcium hypochlorite for 5 min, 30 min, and 1 h. Seines will be rinsed in tap water and dried before further processing.
- Brine. Seines will be immersed in 10% (w/v) sodium chloride for 30 min, 1 h, and 6 h. Seines will be dried before further processing.

In addition to these treatments, fragments of plastic and rubber (such as those used in boots or buckets) will be used as binding material for bacteria and treated with:

- Vikron® (as per instructions), rinsed with tap water and dried before testing.
- 200 ppm iodine, rinsed with tap water and dried before testing.

Each treatment will consist of five replicates and will be sequentially repeated a minimum of three times. All treatments will be repeated targeting cells in biofilm. For these experiments, seines will be exposed to the bacterial suspensions for 6 h prior to disinfection. The rest of the protocol will be the same as for planktonic cells.

Subobjective 2c. Field experiments at the E. W. Shell Fisheries Station, Auburn.

Based on the results from Objective 2b, the three most effective treatments will be tested in commercial size nets under pilot-field conditions. Because our fisheries station has not had any outbreaks caused by the *A. hydrophila* epidemic strain, the dipping of the nets will take place in an indoor, easy to disinfect facility but with net size and disinfectant volumes close to those required in farms. A minimum of 20 sub-samples per treated seine per experiment will be tested for the presence of *A. hydrophila*, *E. ictaluri*, and *F. columnare.* Treatments will be carried out in triplicate.

Data will be analyzed using a one-way or two-way ANOVA with Tukey's post hoc analysis to determine the significance of each treatment in the survival of *A. hydrophila* (and the other two

pathogens tested) in seines. The statistical analysis will be performed using the SAS Software 9.2 version (SAS Institute, Cary, N.C.).

Institutional Unit Involved

Department of Fisheries and Allied Aquacultures, Auburn University

Project Timetable

Year 1

1st Quarter	2nd Quarter	3rd Quarter	4th Quarter
Initial Planning: Detection protocols set up	Disinfection protocols: *A. hydrophila* (planktonic)	Disinfection protocols: *A. hydrophila+E. ictaluri* (planktonic)	Disinfection protocols: *A. hydrophila+F. columnare+E. ictaluri* (planktonic)

Year 2

1st Quarter	2nd Quarter	3rd Quarter	4th Quarter
Disinfection protocols: *A. hydrophila* (biofilm)	Disinfection protocols: *A. hydrophila+F. columnare+E. ictaluri* (biofilm)	Best performing protocols applied to commercial size seines	Data analysis & Final Report

Duplication of Research Statement

To the best of my knowledge there are no current projects investigating the optimal protocols to disinfect seine and farm-related equipment that have been exposed to the epidemic strain of *A. hydrophila*. A search of the relevant federal databases that include funded aquaculture-related research were accessed and reviewed including: the USDA Research, Education, and Economics Information System (REEIS); national and state Sea Grant programs and funded project databases; the National Sea Grant Library, the USDA Regional Aquaculture Centers' websites related to the keywords "Aeromonas hydrophila" and "disinfect*" revealed no current projects on the topic. These databases were accessed to search for and review any projects that are related to or the same as the research project proposed herein does not duplicate any previously funded projects found in these databases, and that the proposed work is original research.

References

Austin B., and D. A. Austin 1999. Bacterial fish pathogens: Disease of farmed and wild fish, Vol. Springer, New York, NY

Bilodeau A. L., G. C. Waldbieser, J. S. Terhune, D. J. Wise, and W. R. Wolters. 2003. A real-time polymerase chain reaction assay of the bacterium *Edwardsiella ictaluri* in channel catfish. Journal of Aquatic Animal Health 15:80-86.

Hemstreet, B. 2010. An update on *Aeromonas hydrophila* from a fish health specialist for summer 2010. Catfish Journal 24:4.

Panangala V. S., C. A. Shoemaker, and P. H. Klesius. 2007. Taqman real-time polymerase chain reaction assay for rapid detection of *Flavobacterium columnare*. Aquaculture Research 38:508-517.

Plumb J. A. 1999. Health maintainence and principal microbial diseases of cultured fish, Vol. Iowa State University Press, Ames, IA

Sadler J., and A. E. Goodwin. 2007. Disease prevention on fish farms. SRAC Publication No. 4703.

VITA

Name: Cova R. Arias
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EDUCATION

B.S. (1993, Biochemistry, University of Valencia, Spain)
Ph.D. (1998, Microbiology, University of Valencia, Spain)

EMPLOYMENT

March, 2013 to present. Professor at the Department of Fisheries and Allied Aquacultures, Auburn University
January, 2007 to March, 2013. Associate Professor at the Department of Fisheries and Allied Aquacultures, Auburn University
April, 2002 to January 2007. Assistant Professor at the Department of Fisheries and Allied Aquacultures, Auburn University
January, 1999-March, 2002. Post-doctoral research associate at the Citrus Research and Education Center, University of Florida.

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

- Member, World Aquaculture Society, 2003 to present
- Member, American Society for Microbiology, 2000 to present,
- Member, American Fisheries Society, 2002 to present
- Member, Alabama Fisheries Association, 2002 to present

SELECTED PUBLICATIONS

Arias, C. R., S. LaFrentz, W. Cai, and O. Olivares-Fuster. 2012. Adaptive response to starvation in the fish pathogen *Flavobacterium columnare*: cell viability and ultrastructural changes. BMC Microbiology. 12: 266.

Bullard, S. A., H. Mohammed, and **C. R. Arias**. 2012. First record of the fish pathogen *Flavobacterium columnare* genomovar II from bluegill, *Lepomis macrochirus* (Rafinesque), with observations on associated lesions. Journal of Fish Diseases: 36: 477-451.

Arias, C. R., W. Cai, E. Peatman, and S. A. Bullard. 2012. Channel catfish x Blue catfish hybrid exhibits higher resistance to columnaris disease as compared to its parental species. Diseases of Aquatic Organisms. 100: 77-81.

Olivares-Fuster, O., S. A. Bullard, A. McElwain, M. J. Llosa, and **C. R. Arias**. 2011. Adhesion dynamics of *Flavobacterium columnare* to channel catfish (*Ictalurus punctatus*) and zebrafish (*Danio rerio*) after immersion challenge. Diseases of Aquatic Organisms. 96: 221-227.

Proposed Budget for the Regional Project:
COMPARISON OF DISINFECTION PROTOCOLS TO INACTIVATE *AEROMONAS HYDROPHILA* IN SEINES

Institution: Auburn University				Year 1	Year 2	Year 3	Total
Scientist Name: Cova Arias							
Salaries and Wages	CSREES-FUNDED WORK MONTHS						
	Calendar	Acad.	Summer				
1. No. of Senior Personnel							
a. **1** PD			0.5	4,366	4,366		8,732
b. Senior Associate							
2. No. of other personnel (non-faculty)							
a. Research Associates/Postdocs							
b. Other Professionals							
c. Paraprofesssionals							
d. 1 Graduate Students	12			17,000	17,000		34,000
e. Prebaccalaureate Students							
f. Secretarial-Clerical							
g. Technical, Shop and Other							
Total Salaries and Wages				21,366	21,366		42,732
Fringe Benefits (if charged as direct costs)				1,575	1,575		3,150
Total Salaries, Wages, and Fringe Benefits				22,941	22,941		45,882
Nonexpendable equipment							
Materials and Supplies				6,000	6,000		12,000
Travel							
Publication Costs					2000		2,000
All Other Direct Costs							
Total Direct Costs				28,941	30,941		59,882
Total Amount of This Request				28,941	30,941		59,882

Budget Justification Total:

A) Salaries

One month summer salary for PI	$ 8,732
Graduate research assistant	$34,000

B) Fringes

27.9% fringes on 1 month of PI summer salary ($8,731)	$ 2,436
2.10% fringes on 1 year of graduate research assistant salary ($17,000)	$ 714

C) Total Salary and Fringes **$45,882**

D) Expendable Supplies and Equipment:

Consumables and reagents are needed in the amount of $6,000/year to complete the project. These funds will be used to purchase bacteriological supplies such as culture media, plasticware, and molecular biology reagents used in the qPCR. In addition, these funds will be used to buy the disinfectants and seines needed for the study.

$12,000

G) Publication costs/Page charges:

We are requesting $2,000 for publishing research results in scientific journals as well as in extension publications.

$ 2,000

O) Total **$59,882**