



## REQUEST FOR PRE-PROPOSALS

*Please Copy and Distribute to All Interested Parties*

The USDA-NIFA Southern Regional Aquaculture Center solicits response from qualified multi-state teams interested in participating in the regional project:

### **FIELD-TESTING OF A RAPID ASSAY TO DETECT THE MARINE PARASITE *AMYLOODINIUM OCELLATUM* IN COMMERCIAL AQUACULTURE FACILITIES**

SRAC's Board of Directors has authorized up to \$100,000 for a 1-year project for detecting the marine parasite *Amyloodinium ocellatum* in commercial aquaculture facilities. This project will be developed using the "competitive proposal method" where a team of multi-state scientists, having demonstrated records of expertise in the subject, complete a single pre-proposal that addresses all project objectives. One proposal will be selected for funding based on review by a committee of scientists not involved in any of the proposals that are submitted.

#### **Background**

*Amyloodinium ocellatum* is an obligate marine parasite that has far-reaching deleterious impacts on marine and brackish water aquaculture production. As US producers attempt to increase and intensify marine fish aquaculture in recirculating aquaculture systems and even pond systems, *A. ocellatum* often serves as a key hindrance to consistently profitable production. The parasite has a life cycle with three stages: the trophont (adult feeding stage), the tomont (the reproductive stage), and the dinospores (free-swimming infective stages) similar to those seen in the common freshwater parasite Ich (*Ichthyophthirius multifiliis*). The trophont is found on the gills and skin of host fish where it holds fast to the fish's mucosal epithelium. The dividing tomont produces up to 256 infective dinospores and is the only stage shown susceptible to drug/chemical treatments to-date. As the life cycle can be completed in 3-6 days, parasite loads and mortalities mount rapidly, often before the presence of the parasite is suspected. Current diagnostic methods relying on microscopic identification of trophonts on skin or gills often allows the parasite to spread beyond control before treatments (copper sulfate, chloroquine, etc) begin. A rapid, sensitive, and affordable loop-mediated isothermal amplification method (LAMP) assay has been recently developed which may aid in early detection and improved control of *A. ocellatum* outbreaks in marine aquaculture facilities and can be incorporated into weekly water quality monitoring efforts when conditions are appropriate for disease development. However, field-testing and implementation procedures are needed to transition this technology from bench science to commercial application.

#### **Objectives**

- 1) Evaluate the developed LAMP assay in commercial aquaculture facilities, assessing:
  - a. time to diagnosis,
  - b. sensitivity as compared to standard microscopic evaluation,
  - c. cost,
  - d. disease incidence/production outcomes, and
  - e. false positives and negatives using positive and negative controls.
- 2) Develop a standard operating procedure (SOP) for *A. ocellatum* surveillance utilizing the LAMP assay and incorporating findings from Objective 1

## Experimental Approach

End users of the standard operating procedure developed in this project may include producers, aquarium operators, fish health managers, and researchers. Emphasis will be given to approaches that provide thorough, rigorous evaluation of the assay under conditions of commercial production. Proposals will be evaluated on the basis of the project team's research experience, productivity, complimentary expertise, as well as the availability of appropriate facilities and other resources required to implement the research. Surveillance should be carried out in marine aquaculture production facilities by standard microscopic means and comparisons made utilizing the LAMP assay employed at differing intervals for preventative diagnosis. Treatment regimens should continue previous standard practice when *A. ocellatum* is detected. Data regarding sensitivity, accuracy, labor associated with testing, and impacts on disease incidence and production parameters should be gathered and used to compile a SOP. Proposals leveraging existing resources and infrastructure will be looked upon favorably.

## How to Respond

Pre-proposals must address all objectives. Preference will be given to pre-proposals that show a high degree of collaboration and coordination among participants. To meet the criterion for a regional project, the pre-proposal must include collaboration from scientists in two or more 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).

The pre-proposal must include a one page vita for each participant and a proposed budget for each participating institution or organization. Pre-proposals, vitae, and budgets that are not in the proper format will not be considered. The *Guidelines for Writing a SRAC Pre-proposal (Comprehensive)* contains an example of a pre-proposal. Contact Kristen Thompson at 662-686-3269 for any assistance.

Send an electronic copy of the pre-proposal in Word format to Jimmy Avery, SRAC Director as an email attachment (jimmy.avery@msstate.edu) **by August 7, 2016**. Proposals received after that date will not be considered.