**California Sea Grant College Program**
**Progress/Completion Report**

## Project Information

**Year** _____ **NOAA Grant No.:** NA10OAR4170060

**Number** R/AQ-133 **Start Date:** 3/1/2012 **Completion Date:** 2/28/2015

**Title** Development of Sustainable Tuna Aquaculture in the United States Using Yellowfin Tuna as a Model

## Project Leader

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## Project Leader

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**Project Hypothesis**

Yellowfin tuna eggs or larvae can be consistently cultured to a juvenile stage with at least 3% survival.

**Project Goals and Objectives**

The primary goal of this project is to promote the development of environmentally responsible tuna aquaculture by improving tuna larval culture success, which is the bottleneck to mass production. In measurable terms, our goal is to increase the survival of eggs or larvae cultured to a juvenile stage from

**Briefly describe project methodology**

Objective 1 - quantify YFT egg and larval quality throughout one or more spawning seasons. Egg and larval quality characteristics were measured for 12 spawn events in Year 1. The number of spawn events was less than planned because the fish stopped spawning from 7/08/12 to 8/27/2012, which was unanticipated. Standard measures were made by both HSWRI and IATTC staff at Achotines. Egg quality characteristics that were quantified included egg volume, oil globule number and volume, symmetry of cell cleavage, and hatching success rate. Larval quality was assessed by measuring size at hatch, survival to first feeding, and yolk sac volume. Egg and larval measures were made for 20 individuals directly under a dissecting microscope. Hatching success rates were determined by placing 10 eggs into each of ten 1L beakers filled to 800 ml with sterile seawater and maintained in a 26°C to 28°C water bath. The percent hatch was quantified at 26 hr from stocking. Survival to first feeding was measured 48 to 72 hrs post hatch by stocking 10 larvae into each of ten 1L beakers and then counting survivors at 48 and 72 hr.

Objective 2 - characterize the microbial communities associated with rearing YFT and test appropriate methods to mitigate pathogenic bacteria that may be found. To achieve this objective, we applied standard bacterial plating methods to qualitatively determine bacterial loads associated with the eggs, rearing units and live feeds at Achotines and HSWRI as well as shipment bags pre and post shipment. Marine agar was used as a general screening tool and TCBS agar was used to test for Vibrio spp. Samples were diluted as appropriate to allow quantification of colony forming units (CFUs).

Objective 3 - determine if air shipping has an effect on larval culture success beyond first feeding. To achieve this objective HSWRI staff compared the culture success of eggs and larvae that underwent shipping simulations with those that did not at Achotines. The evaluation period was extended from the typical T0 (when the box is opened) to T14dph. The simulation treatment groups included both eggs and larvae in separate boxes. For each treatment there were two replicates. Endpoint measures included growth and survival.

Objective 4 – characterize the incidence of swim bladder inflation through ontogeny under various typical culture conditions and test appropriate methods to improve swim bladder inflation as necessary. Within this objective we attempted to document the timing and incidence of swim bladder inflation as larvae developed under various typical culture conditions. These culture conditions included both production and experimental-scale. Samples of 20 larvae were collected daily from replicate rearing units from 2-7 dph and examined under a microscope. The presence-absence of inflated swim bladders was recorded for each larva.

Objective 5- apply traditional and novel nutritional approaches to maximize health and survival of YFT larvae. For this objective we reared two tanks of larvae from the same cohort at the Achotines laboratory and focused on the quality and quantity of live prey during culture from 0 to 14 dph. Food consumption levels and larval growth were measured daily by subsampling 10 larvae per tank. The larvae were euthanized with MS-222 and notochord length was measured to the nearest millimeter. After lengths were taken, individual food items were counted in the gut of each larva.

Objective 6 - involve one or more graduate students in these studies. We achieved this objective. See progress section for details.

Objective 7- disseminate the information broadly through the media, internet, newsletters, reports, conference presentations, and scientific publications. See output section for details.

**Describe progress and accomplishments toward meeting goals and objectives.**

During the first year of this project, HSWRI staff visited the Achotines Laboratory twice - once in May (Visit 1) and again in July (Visit 2). Unfortunately, the YFT broodstock stopped spawning from July through August, so little was accomplished during Visit 2. We were able to ship larvae to San Diego as planned during Visit 1 but no other shipments were made due to the interrupted spawning. Spawning resumed to full capacity in the fall, so we anticipate being able to resume as scheduled in 2013. Due to the unanticipated delay, we will be requesting a no-cost extension to the project.

Objective 1 - quantify YFT egg and larval quality throughout one or more spawning seasons.

Egg and larval quality was assessed starting in May of 2012. During Visit 1 HSWRI staff trained IATTC staff on HSWRI egg and larval quality protocols so that measures could be taken on spawning events throughout the year as originally proposed. We analyzed 12 spawning events from May through November 2012 (Table 1). Hatching rates ranged from 16 to 81% (67.3 ± 12.7) and survival to first feeding (2 dph) ranged from 28 to 90% (62.8 ± 16.2).

Table 1. Egg and larval quality results for YFT broodstock population held at Achotines.

Objective 2 - characterize the microbial communities associated with rearing YFT and test appropriate methods to mitigate pathogenic bacteria that may be found.

During both visits to Achotines during this reporting period, bacterial sampling was done on the live feeds area, larval culture tanks, and the water coming into the laboratory. Vibrio spp. counts were high for incoming water supplying culture tanks as well as within egg incubation tanks (Tables 2 and 3). During Visit 2, high counts were found in the larval culture tanks as well as in the live feeds.

Bacterial samples were also taken from the shipment bags pre and post shipment. Prior to shipment, the bacterial counts were 53 CFU/ml and they increased during shipment to 110 to 657 CFU/ml in the simulation bags and 1,200 to 75,000 CFU/ml in the actual shipment bags upon arrival at HSWRI's laboratory in San Diego, CA. At the end of 26 hr shipment, lower bacterial counts were measured in the simulation bags (Table 4), unlike the bags that were shipped, these bags were not agitated, which does have the potential for suspending flocculent and other debris where bacteria can be growing.

Table 2. Bacterial sampling (Vibrio spp.) at Achotines during Visit 1.

Table 3. Bacteria sampling (Vibrio spp.) at Achotines during Visit 2. TMTC denotes samples that had too many colonies to count.

Table 4. Bacteria sampling (Vibrio spp.) from the May shipment of YFT larvae from Achotines to San Diego, CA.

Objective 3 - determine if air shipping has an effect on larval culture success beyond first feeding

As mentioned above, only one shipment was sent from Achotines to HSWRI's laboratory in San Diego during this reporting period. Two cohorts were shipped (2012_0505 and 2012_0506) and the 2012_0505 cohort was used for the simulation trial. The shipment time from Panama to San Diego was 26 hrs from packing to opening the boxes. At both facilities water quality and survival parameters were measured (Table 5). Results showed variable survival of larvae in both the simulated bags (60.4 and 92.4%) and more consistent but lower survival among larvae that were shipped(36.8 and 37.3%).

The shipment simulation larvae were stocked into standard 1000 L rearing tanks at the Achotines laboratory and low mortality occurred the first day after stocking. The initial feeding response revealed four out of twenty larvae feeding on rotifers. However, there was significant mortality during the second and third day after stocking (3 and 4 dph) and no larvae survived after 4 dph.

At HSWRI, the shipped larvae were stocked into similar (1000 L) rearing tanks to those at the IATTC and we observed generally similar results. After shipping, no larvae were observed feeding but they did survive to 6 dph instead of only 4 dph. Presumably this was due to the difference in rearing temperatures. The water temperature in the IATTC tanks was 27.3 ± 0.6ºC (26 - 28ºC) and the water temperature in the experimental system at HSWRI was maintained between 25.6 ± 0.2ºC (25 - 26ºC).

Table 3. Water quality and survival data collected after shipment and simulated shipment on May 8, 2012. The yellow highlighted groups were the specific units compared for shipping verses simulation sequence.

Objective 4 – characterize the incidence of swim bladder inflation through ontogeny under various typical culture conditions and test appropriate methods to improve swim bladder inflation as necessary

One cohort (2012_0429) was sampled to determine swim bladder inflation through ontogeny. We sampled 20 larvae every day from 2 to14 dph. For this cohort, swim bladder inflation was low (1.1 %), and swim bladders were not observed during development until 6 dph.

Objective - 5 apply traditional and novel nutritional approaches to maximize health and survival of YFT larvae.

In Panama we successfully reared larvae to 14 dph from the 2012_0429 cohort and documented feeding and survival data under typical culture conditions. Larval survival from 0 to 14 dph was 9.9 and 24.5%. We observed an increase in feed intake (rotifers per larvae) with growth and increased feed densities (Figure 1), showing that as the larvae develop they become more efficient feeders. Even though Artemia was offered at 9 dph (4.53 ± 0.40 mm, notochord length), the YFT larvae consumed 1st instar Artemia at approximately 12 dph (6.35 ± 0.52 mm, notochord length). This was a few days later than what has been described in the literature (8 to 10 dph, 5.0 to 6.0 mm, notochord length) for YFT larvae reared at the Achotines laboratory. The differences in timing could be due to differences between cohorts and or ambient water temperatures at the time of the studies.

**PROJECT MODIFICATIONS:** Explain briefly any substantial modifications in research plans, including new directions pursued and ancillary research topics developed. Describe major problems encountered and how they were resolved.

During the two years of this project, HSWRI staff visited the Achotines Laboratory five times - once in May 2012 (Visit 1), July 2012 (Visit 2), June 2014 (Visit 3), August 2014 (Visit 4) and September 2014 (Visit 5). We were able to ship larvae to San Diego as planned in four of the five visits.

Objective 1 - quantify YFT egg and larval quality throughout one or more spawning seasons.

Egg and larval quality was assessed starting in May of 2012 and completed in September 2014. During Visit 1, HSWRI staff trained IATTC staff on HSWRI egg and larval quality protocols so that measures could be taken on spawning events throughout the year as originally proposed. We characterized 12 spawning events in 2012 from May through November (Table 1), and 18 spawning events in 2014 from March through September (Table 2). The number of oil globules was consistently one and cell cleavage was consistently normal. In 2012, hatching rates ranged from 16 to 81% (67.3 ± 12.7) and survival to first feeding (SFF=2 dph) ranged from 28 to 90% (62.8 ± 16.2). While in 2014 hatching rates ranged from 36 to 67% (50.8 ± 8.0) and survival to first feeding ranged from 19 to 69% (43.1 ± 10.8). At HSWRI, using the same exact methods we used for other marine fish species, we categorize hatching rates and SFF of 70% or greater as "high quality". We do this for every spawn of all species. This designation is used to select eggs to set up and also to benchmark overall egg quality if a problem should arise. In comparison, measures

**PROJECT OUTCOMES:** Briefly describe data, databases, physical collections, intellectual property, models, instruments, equipment, techniques, etc., developed as a result of this project and how they are being shared.

We demonstrated that eggs and larvae of YFT can be shipped to San Diego with high survival on the receiving end. The methods for achieving this success are clearly described. We demonstrated variable egg quality in YFT over two years of data, which we were able to compare with other marine species because we have a standard methodology. We demonstrated the importance of various disinfection processes in the culture of marine fish and identified several critical bacterial hotspots at Achotines. We did not successfully rear YFT to the juvenile stage during the study period even at Achotines, so we did not acheive our goal of 3%.

**IMPACTS OF PROJECT:** Briefly describe how this project has contributed to a discipline; to developing human resources; to developing physical, institutional or information resources; technology transfer; and society beyond science and technology. Please notify CASG of impacts that occur after your project ends; CASG may contact you after your project ends to learn about additional impacts that occur over time.

In this project we examined a variety of larval rearing approaches used in commercial marine aquaculture operations that can be applied to research laboratories like Achotines. Most significantly, we developed a list of recommendations for properly disinfecting larval rearing and live feeds areas; implementation of the recommendations is at the discretion of the specific laboratory.

**BENEFITS, COMMERCIALIZATION, AND APPLICATION OF PROJECT RESULTS:** Please list any companies, agencies, organizations or individuals who have used your proejct results, scientific/technical advice, etc., and provide names, emails and phone numbers. Briefly describe how results were used and quantify results and socioeconomic benefits, if possible.

The results reported here clearly identify the major areas of consideration for shipping eggs and larvae of any species of marine fish, especially those that are highly sensitive, like tunas. Methodologies were employed that can be repeated for other species in order to identify challenge areas. Key areas of consideration include egg quality, bacterial loading, and temperature and pressure differentials during shipping.

**ECONOMIC BENEFITS** generated by discovery, exploration and development of new, sustainable coastal, ocean and aquatic resources (i.e., aquaculture, marine natural products, foods, pharmaceuticals).

Issue-based **forecast capabilities** to predict the impacts of a single ecosystem stressor, developed and used for management (i.e., climate change, extreme natural events, pollution, invasive species, and land resource use).

**Tool, technologies** and **information services** developed (i.e., land cover data, benthic habitat maps, environmental sensitivity index maps, remove sensing, biosensors, AUVs, genetic markers, technical assistance, educational materials, curricula, training).

**Publications** (list in appropriate category below) Each listing should be a stand-alone bibliographic reference, including all authors' names. For each Publication type, specify title, authors, date and journal details, where appropriate (repeat headers as necessary).

**MEDIA COVERAGE: Select 'Yes' or 'No'. If yes, describe any radio, TV, web site, newspaper, magazine coverage your project has received. Send original clippings or photocopies to the Sea Grant Communications Office.**

**MEDIA NOTES: Brief description of the type media coverage your project has received.**

**COOPERATING ORGANIZATIONS: List those (e.g., county or state agencies, etc.) who provided financial, technical or other assistance to your project since its inception. Describe the nature of their cooperation.**

NOAA. Inter-American Tropical Tuna Commission

**INTERNATIONAL IMPLICATIONS: Does your project involve any colleagues overseas or have international implications?**

If successful, this project will have significant implications to the management and conservation of tunas worldwide.

**AWARDS: List any special awards or honors that you, or any co-project leaders, have received during the duration of this project.**

**KEYWORDS: List keywords that will be useful in indexing your project.**

**PATENTS: Please list any patents or patent licenses that have resulted from this project, and complete the patent statement form available on the web site.**

**NOTES: Please list any additional information in the notes area**

**FOR ALL STUDENTS SUPPORTED BY THIS GRANT, PLEASE LIST:**

**Graduation Date**
**Address**
**Address 2**
**City**
**Employer**