



## REPRODUCTION AND LARVAL REARING OF FRESHWATER ORNAMENTAL AND MARINE BAITFISH

### Reporting Period

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### PROJECT OBJECTIVES

1. Develop improved technologies for spawning and larval rearing of pinfish.
  - a. Evaluate efficacy of catfish pituitary extract on spawning induction of pinfish.
  - b. Evaluate dosing of catfish pituitary extract on spawning induction of pinfish.
  - c. Compare human chorionic gonadotropin and catfish pituitary extract on the spawning induction of pinfish.
  - d. Evaluate commercial rotifer enrichments and their effects on larval survival and growth.
  - e. Evaluate larval feeding regimes employing copepods and rotifers and their effects on larval survival and growth in pinfish.
  - f. Evaluate the effects of stocking density on survival and growth of larval pinfish.
2. Develop improved technologies for spawning and larval rearing of goggle eye.
  - a. Evaluate the efficacy of Ovaprim on spawning induction of goggle eye.
  - b. Evaluate larval feeding regimes employing copepods and rotifers and their effects on larval survival and growth.
  - c. Evaluate the effects of stocking density on survival and growth of larval goggle eye.
3. Evaluate spawning substrate preference for captive ballyhoo.
4. Develop improved technologies for egg hatching and larval rearing of *Fundulus grandis* and *Fundulus seminolis*
  - a. Evaluate air incubation of *Fundulus* eggs.

- b. Identify a replacement of live feeds for *Fundulus*.
  - c. Determine relationship between larval density and performance in *Fundulus*.
5. Develop improved technologies for spawning and larval rearing of Bala shark
- a. Improve Bala shark broodstock maturation.
  - b. Develop technologies for induced spawning of Bala shark.
  - c. Develop improved technologies for larval rearing of Bala shark.
  - d. Design water treatment technologies for commercial larval rearing of Bala shark.
6. Publication, extension, and dissemination of results.

## **ANTICIPATED BENEFITS**

Baitfish culture has long been dominated by production of freshwater species. Culture of marine baitfish is a logical progression for the region and offers enterprise diversification and increased marketing opportunities. Pinfish, *Lagodon rhomboides*, will be induced to spawn with both HCG and catfish pituitary hormone. At the termination of the project, research results will provide knowledge about specific methods for induced spawning using an FDA approved hormone (HCG). Additionally, the results may provide the impetus for a potential INAD expansion for catfish pituitary extract. A larval feeding regime that includes the identification of optimal live feed organisms with proper enrichments will be characterized for pinfish. Goggle eye, *Selar crumenophthalmus*, will be spawned using previously established methods. Optimal stocking density and larval feeding regimes, including live feed and enrichment selection, will be defined. The spawning substrate preference of ballyhoo *Hemiramphus* sp. will be investigated. Research with Gulf killifish, *Fundulus grandis*, and Seminole killifish, *Fundulus seminolis*, will address the development of protocols for air incubation of eggs which will optimize fry production, survival, and growth. This data will help to establish future recommendations to producers about the optimal methods of incubating eggs within a humid environment to delay hatch and better coordinate stocking of larger numbers of *Fundulus* fry. Feeding and density trials will identify efficient culture methods to produce *Fundulus* juveniles.

## **PROGRESS AND PRINCIPAL ACCOMPLISHMENTS**

**Objective 1.** *Develop improved technologies for spawning and larval rearing of pinfish.*

**Sub-objectives 1 a)** *evaluate efficacy of catfish pituitary extract on spawning induction of pinfish, 1 b) evaluate dosing of catfish pituitary extract on spawning induction of pinfish, and 1 c) compare human chorionic gonadotropin and catfish pituitary extract on the spawning induction of pinfish.*

### **University of Florida Indian River Research and Extension Center**

Mature pinfish (approximate means: 214 g, 216 mm) originally collected from the Indian River Lagoon in Sebastian, Florida were used in two experiments to examine the efficacy of varying bolus doses of channel catfish pituitary extract (CCPE) on the induced spawning of pinfish as

well as the efficacy of priming/resolving injections vs. bolus injections of CCPE. During each experiment, Ovaprim® administered in a bolus dose to females at 0.5 mL/kg (males at 0.25 mL/kg) was used as a positive control (found to be efficacious on spawning induction for pinfish by DiMaggio et al. 2013) rather than HCG as originally proposed. During the dose efficacy experiment, females with vitellogenic oocytes received one of four possible bolus dosages (5, 10, 20, or 40 mg/kg injected into the coelomic cavity) of CCPE centrifuged and dissolved in saline solution; males of each treatment received half the corresponding female dose to ensure spermiation. Fish were paired at a density of 1 female:1 male and stocked into separate 1,600 L spawning tanks within recirculating aquaculture systems (RAS) for each treatment. Each of the four tanks in each RAS was equipped with an external egg collector to collect floating and sinking eggs. Two experimental RAS (eight total spawning tanks) were used for each of the spawning trials. For the dose efficacy study, treatments were distributed among trials and randomized among tanks to yield a total of 6 replicate brood pairs for each CCPE bolus dose and 8 replicate brood pairs for the Ovaprim® treatment. After completion of the bolus dosing experiments for each species, a random dose was chosen to use during the priming and resolving doses experiment. For this experiment, two CCPE treatments were evaluated as well as an Ovaprim® control treatment. Females received CCPE dosage of 10 mg/kg split into two priming:resolving injections given as either the following two treatments: 20% priming and 80% resolving (20:80) or 50% priming and 50% resolving (50:50). Priming and resolving injections were given 24 hours apart. The injections for the bolus CCPE treatment as well as the Ovaprim® treatment were given at the same time as the resolving doses of the other two CCPE treatments. Males of each CCPE treatment received a bolus injection of 5 mg/kg CCPE at the same time as the resolving injections. Between the priming and resolving injections of each trial, fish selected for treatments were held individually by treatment pairs in 8, 85 L aquaria within a single RAS located inside the greenhouse. After resolving injections, fish were stocked into the same spawning tanks used in the bolus dosing experiments and according to the same stocking protocol. Each of the three treatments was randomized among three tanks of each system. Three trials were conducted to yield a total of 6 replicates for each CCPE and Ovaprim® treatment.

Egg collectors were monitored daily at 24, 48, and 72 hours after stocking. Floating and sinking eggs of each spawn collected were enumerated volumetrically after allowing the eggs to settle within small graduated cylinders and quantified based on small volumetric subsamples (0.5 ml). Fertilization success, egg morphometrics, and percentage of eggs containing a single oil droplet were quantified from subsamples of floating and sinking eggs. Subsamples of floating eggs were stocked into 1 L containers with 55 µm screen-bottoms which were then floated (~750 mL water capacity in each container) in a temperature controlled water bath filled with sterilized natural seawater. Three replicate containers were stocked to examine both hatching percentage and survival to first feeding (3 days post hatch [DPH]). At the time of hatch (approximately 24-36 hours after spawning), live larvae which fully emerged from eggs were counted in each replicate container for determination of hatching percentage and a subsample of larvae from each replicate container were sampled and photographed for larval morphometric analysis. At the conclusion of each trial, 72 hours after stocking, all fish were removed from spawning tanks. Females were also biopsied again to obtain vitellogenic oocytes for diameter measurements to detect pre and post-injection changes in vitellogenic oocyte diameters.

CCPE failed to induce spawning or ovulation at any administered bolus dose as well as priming:resolving ratios for a 10 mg/kg total CCPE dose. Multiple spawns were obtained from the positive control Ovaprim® treatment in each experiment (suggesting no environmental spawning inhibition or negative inherent condition among brood) and the quality of those spawns were high and parameters within the ranges reported by DiMaggio et al. (2013). We also investigated the same bolus doses on pigfish (*Orthopristis chrysoptera*) with the same batch of CCPE and observed successful spawning induction at all doses testes, suggesting adequate LH content of the CCPE batch used. The lack of spawning induction in pinfish suggests possible pinfish gonadotropin receptor incompatibility to channel catfish LH.

Two trials were conducted to examine the effects of commercial rotifer enrichments on growth and survival of larval pinfish. Eggs were collected from two volitional spawns from brood held in a 2,000 L re-circulating system. Eggs were incubated in seawater (~32 g/L) at 77.6 degrees F. At 1 days post hatch (dph), larvae were stocked into replicate tanks (14.75 L) at 100 larvae/L for each of the treatments (Non-enrichment, OriGo, AlgaMac 3050, and DHA Protein Selco) in each trial and fed rotifers twice daily beginning at 3 dph throughout 11 dph. Rotifer enrichment procedures adhered to manufactures' recommendations. Tanks were flushed with continuous flow through seawater (~32 g/L; 73.4 degrees F) with a minimum daily water exchange of 200%. Tanks were also inoculated daily with microalgae (*T-iso*) at approximately 200,000 cells/mL. Larvae were sampled at 6 and 11 dph and photographed for growth measurements (notochord length (NL)). Survival and percent swim bladder inflation were determined from all larvae harvested at 11 dph.

Pinfish larvae fed rotifers enriched with OriGo had higher survival and growth at 11 dph. Larvae fed rotifers enriched with DHA Protein Selco had higher swim bladder inflation rates (Table 1). Additional trials will be conducted to confirm these results.

<b>Table 1. Mean notochord length (NL) of larval pinfish fed different enrichments via rotifers. Survival and swim bladder inflation as a percentage of 11 DPH.</b>						
Run	Enrichment	NL (µm) at Stocking	NL (µm) at 6 DPH	NL (µm) at 11 DPH	Survival (%)	Swim Bladder inflation (%)
1	Non-enriched	281.07 ± 1.09	288.54 ± 3.56	349.42 ± 6.07	12.78 ± 3.29	16.99 ± 1.20
	Ori-Go	281.07 ± 1.10	287.27 ± 4.32	369.46 ± 6.89	17.36 ± 7.53	14.79 ± 4.26
	Algamac 3050	281.07 ± 1.11	285.79 ± 4.77	344.78 ± 7.21	9.97 ± 1.42	2.27 ± 2.27
	DHA Protein					
	Selco	281.07 ± 1.12	305.41 ± 4.85	364.95 ± 7.19	14.81 ± 0.17	18.93 ± 1.07
2	Non-enriched	283.12 ± 1.65	294.67 ± 2.50	352.72 ± 8.63	0.86 ± 0.11	22.86 ± 19.48
	Ori-Go	283.12 ± 1.66	292.52 ± 2.01	341.52 ± 9.103	3.66 ± 2.69	17.92 ± 16.29
	Alagamac 3050	283.12 ± 1.67	296.71 ± 3.03	349.22 ± 14.44	0.57 ± 0.21	27.27± 27.27
	DHA Protein					
	Selco	283.12 ± 1.68	294.18 ± 2.61	355.29 ± 6.06	1.72 ± 0.54	25.51 ± 13.69
Mean	Non-enriched	282.10 ± 0.99	293.02 ± 2.08	350.36 ± 4.95	3.84 ± 2.05	21.18 ± 13.49
	Ori-Go	282.10 ± 0.10	291.21 ± 1.86	356.63 ± 5.80	7.09 ± 3.31	16.87 ± 10.38
	Algamac 3050	282.10 ± 0.10	292.88 ± 2.65	345.67 ± 6.39	2.92 ± 1.57	14.77 ± 13.30
	DHA Protein					
	Selco	282.10 ± 0.10	296.99 ± 2.35	359.58 ± 4.64	4.99 ± 2.18	23.6 3± 9.53

**Sub-objective 1e.** *Evaluate larval feeding regimes employing copepods and rotifers and their effects on larval survival and growth in pinfish.*

One trial of this experiment has been conducted with unclear results. One or two more trials will be conducted during January-May 2014.

**Sub-objective 1f.** *Evaluate the effects of stocking density on survival and growth of larval pinfish.*

One trial of this experiment has been conducted with unclear results. One or two more trials will be conducted during January-May 2014.

**Objective 2.** *Develop improved technologies for spawning and larval rearing of goggle eye.*

Goggle eye broodstock were difficult to acquire and even gentle handling caused disease outbreaks and mortality to occur. We have been unsuccessful keeping broodfish alive in our tanks. To compensate we started collaborative research with University of Miami researchers who had acclimated broodstock in large volume flow-through tanks. From this population we have acquired eggs and conducted two trials with larval goggle eye. We have evaluated larval feeding treatments including rotifers enriched with Ori-go, the copepod *Pseudodiaptomus pelagicus*, the copepod *Parvocalanus* sp., and a combination of *Parvocalanus* sp. and enriched rotifers. In both trials, larvae displayed feeding success and looked great on day 5 post-hatch, then on day 6 post-hatch all larvae had died in all treatments. Water quality was ideal in all tanks and the mortality occurred on the same day post-hatch.

Potential explanations include the live food organisms were not digested properly, the live food organisms did not have the proper nutritional quantities or qualities, or 6 days post-hatch there was a change in development or a developmental stage that required nutrients not provided or some physical parameter not provided in order to survive.

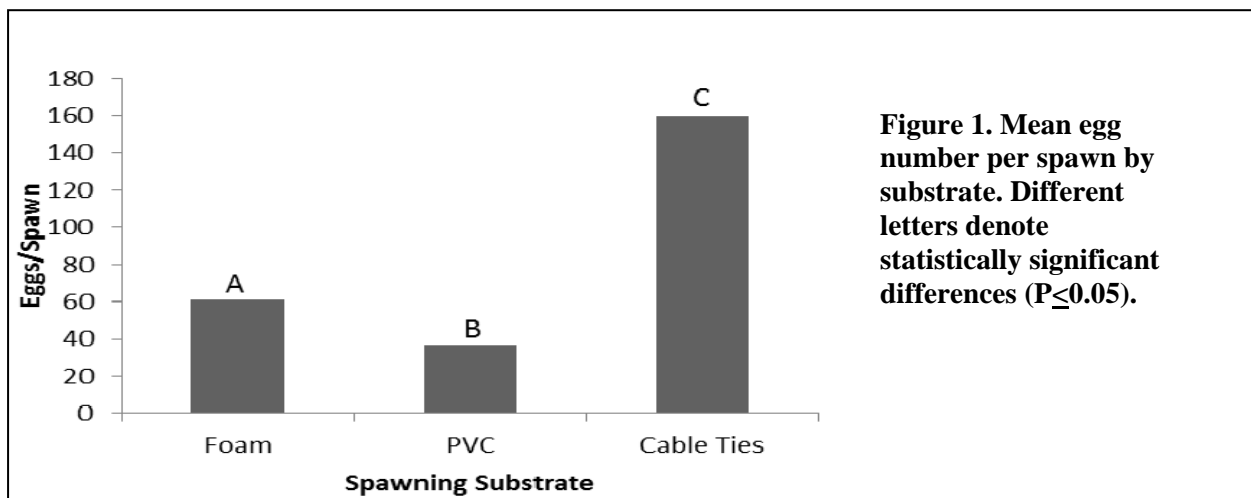
The population of brood goggle eye should provide spawns in spring 2014 to allow for further investigation. We will investigate a mixed copepod and rotifer diet, different lighting intensities, different stocking densities and different feeding rates to hopefully allow for growth and survival past day 6 post-hatch.

**Objective 3.** *Evaluate spawning substrate preference for captive ballyhoo.*

Ballyhoo are a high valued marine baitfish commonly used by anglers to target multiple species of pelagic game fish. In the wild, ballyhoo attach their adhesive eggs to marine substrate (*Sargassum*, sea grasses, flotsam etc.). Broodfish were collected from the wild and have been cultured in tanks for over two years at University of Florida Indian River Research and Education Center. Volitional spawning was observed in tanks and eggs were collected from filters and substrate placed within the tanks. Little is known about captive spawning and substrate preference.

Substrate preference experiments were conducted with two populations of *H. balao* (21 and 22 fish), maintained in 6000 L tanks at a salinity of 35 g/L and temperature of 67.1 to 85.3 degrees F. Three substrates (foam, PVC, and plastic cable ties) were placed within the tanks and left undisturbed for a minimum of 18 hours. Substrates were subsequently removed and spawned eggs were enumerated for each substrate material. Additionally, the tank bottom, bag filter, and submerged airlines, were also monitored daily for any egg deposition. Substrate position was rotated daily to eliminate any positional bias within the tank. Spawned eggs were quantified five times per week (Monday-Friday) and water quality was monitored daily.

A total of 266 spawning events were recorded from ballyhoo cultured in both experimental tanks from January 23, 2012 through August 7, 2012, with 142,822 eggs collected. Analysis of substrate preference revealed cable ties were the preferred spawning substrate with a mean of 160 eggs collected from each spawning event (Figure 1).



**Objective 4.** *Develop improved technologies for egg hatching and larval rearing of Fundulus grandis and Fundulus seminolis.*

**Sub-objective 4a.** *Evaluate air incubation of Fundulus eggs.*

### **Louisiana State University**

For Gulf killifish and some related coastal species, spawning events are timed to semilunar tidal cycles where embryos are deposited at the high water mark of marsh grasses during spring tide and are exposed to air once the tide recesses. During this period, commonly referred to as terrestrial or air incubation, embryogenesis occurs at an accelerated rate compared to incubation in typical aquatic conditions. Air incubation appears to be a common occurrence in wild Gulf killifish. Females are known to lay their eggs among the marsh grass during maximum high tides where they develop fully exposed to the humid air when the tide recedes. The eggs then hatch when they are flooded by the next maximum high tide, approximately 13-15 days later. This situation can be replicated in an aquaculture setting. Air incubation encourages all of the eggs to hatch at the same time yielding uniform sized larvae and subsequently uniform adult minnows. This reduces the likelihood of larger older minnows eating the newly hatched larvae. In addition,

air incubation provides the opportunity for easy transport of eggs to grow out facilities or other locations.

## Year 1

Embryos were manually removed from the spawning substrate material and dead and pigmented embryos were discarded. Live embryos were quantified and treatments consisted of approximately 1,300 embryos sandwiched between two pieces of polyurethane hobby foam in triplicate for each respective temperature treatment. A solution of saline water (7.6 g/L) was mixed using artificial sea salts and was used to moisten the foam. Embryos and hobby foam were then covered with plastic to prevent desiccation while in the incubation chambers. Incubation chambers were set to nominal values of 68, 73, 79, and 86 degrees F with adjustable thermostats.

Time required for embryos to progress through five stages of development was recorded to determine the rate of embryogenesis. Staging was based upon descriptions detailed for the mummichog. Twelve embryos were randomly selected from each temperature-treatment triplicate to determine stage of development. If more than 75% of embryos were at a target stage, treatments were sampled for heart rate and ammonia, urea, and lactate concentrations. Embryos began terrestrial incubation for this study at stage 15. Stage 35 marked the stage at which embryos attain the ability to hatch when placed in an aqueous medium and therefore the transition into delayed hatch. Replicates were sampled in 48-hour delayed hatch intervals after reaching stage 35 until embryos could no longer be sampled due to mortalities. Embryos were sampled at 48-hour intervals for heart rate, morphometric parameters at hatch, and ammonia, urea, lactate and ATP concentrations.

Temperature did not have a significant influence on percent of viable embryos at stage 25. Percent of viable embryos were  $59 \pm 2\%$  at 68 degrees F,  $62 \pm 3\%$  at 73 degrees F,  $58 \pm 8\%$  at 79 degrees F, and  $75 \pm 1\%$  at 86 degrees F. Temperature had a significant effect on the period of time that delayed hatch embryos remained viable. Embryos began to hatch spontaneously on the substrate beginning at 96 delayed hatch hours in the 79 degrees F and 86 degrees F, but did not hatch on the substrate in the 73 degrees F and 68 degrees F treatments. The longest extent of delayed hatch was 320 hours post stage 35 for the 68 degrees F treatment, followed by 272, 224, and 176 hours for 73, 79, and 86 degrees F treatments, respectively. Hours of delayed hatch was significantly related to the total length (TL) of the embryo upon hatch. Size at hatch (TL) and body cavity area were not significantly related to temperature.

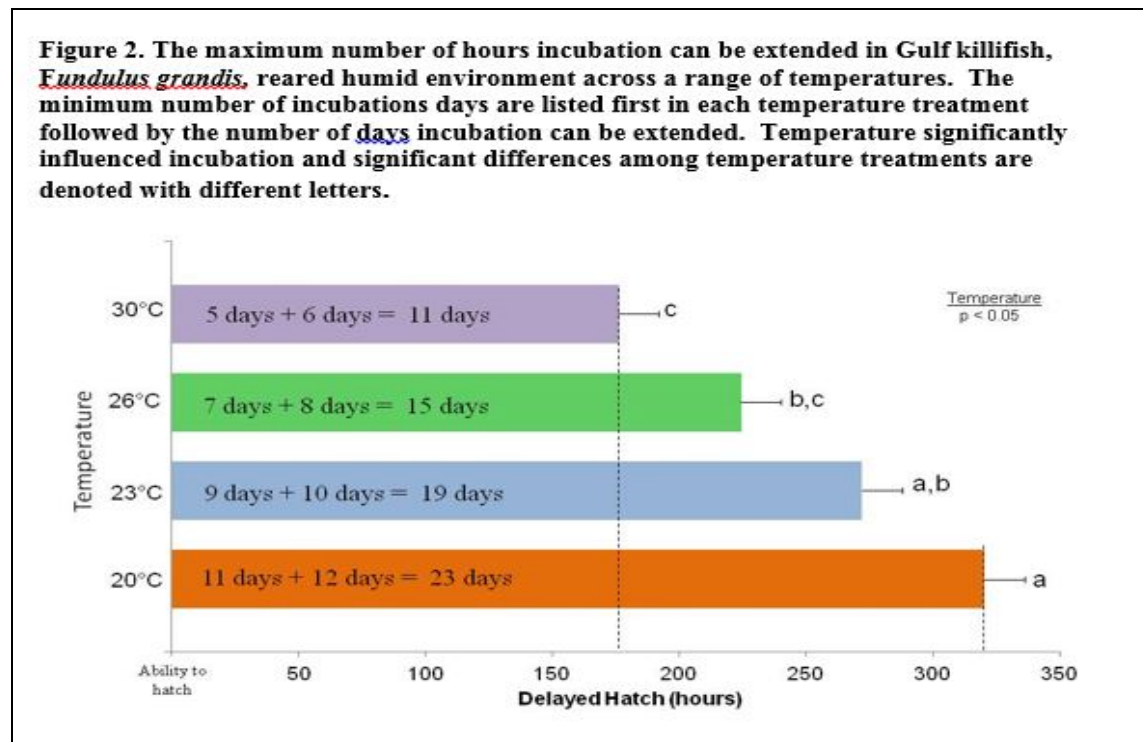
An accelerated rate of embryogenesis was observed during air incubation relative to aquatic incubation of this species. Temperature associated stresses were also observed in addition to stresses caused by air incubation. Embryogenesis for the 86 degrees F treatment was relatively brief compared to lower temperatures and first hatch occurred at 96 delayed hatch hours, although embryo viability began to decrease upon the initiation of delayed hatch and high urea concentrations were observed with delayed hatch. Temperature can likely be modified during incubation to custom delay or accelerate embryo development based on the specific need of the culturist to time the hatching of different batches of eggs.

## Year 2

Incubators were constructed from small dormitory style refrigerators and each was fitted with an external thermostat. Incubation temperatures were set at 68, 73, 79, and 86 degrees F. Sheets of synthetic foam (Expanded Polystyrene) or soft hobby foam were soaked in clean saline water at a salinity of 10 g/L. These foam sheets were wet to the touch and not overly saturated or dripping with water. Sheets of foam were placed in a shallow plastic storage container. Newly fertilized Gulf killifish eggs were placed in a monolayer across the foam and gently covered with another moist foam sheet of the same size. The lids on the containers were secure but did not form an airtight seal. Temperature data loggers were placed in each incubator to record humidity and temperature for the duration of incubations.

Embryo viability and ability to hatch at treatment temperatures was monitored once daily. A sample of embryos from each temperature treatment was placed in water to observe if they hatched and determine the minimum number of incubation days required at each temperature treatment. If larvae hatched within five hours of immersion, they were preserved in 10% buffered formalin for morphometric analysis. Throughout incubation, egg mortalities were monitored to determine the maximum number of incubation days allowed for viable embryos to be extended.

The earliest or minimum number of incubation days required for hatch occurred at a temperature of 86 degrees F at 5 days (Figure 2). At this high temperature the maximum number of days allowable for viable hatch is approximately 11 days. Past 11 days at 86 degrees F the embryos utilize all of their yolk volume and expire. At the lowest treatment temperature (68 degrees F) the minimum number of days required to obtain hatch is 10 days, while the maximum number of incubation days is approximately 23 days.

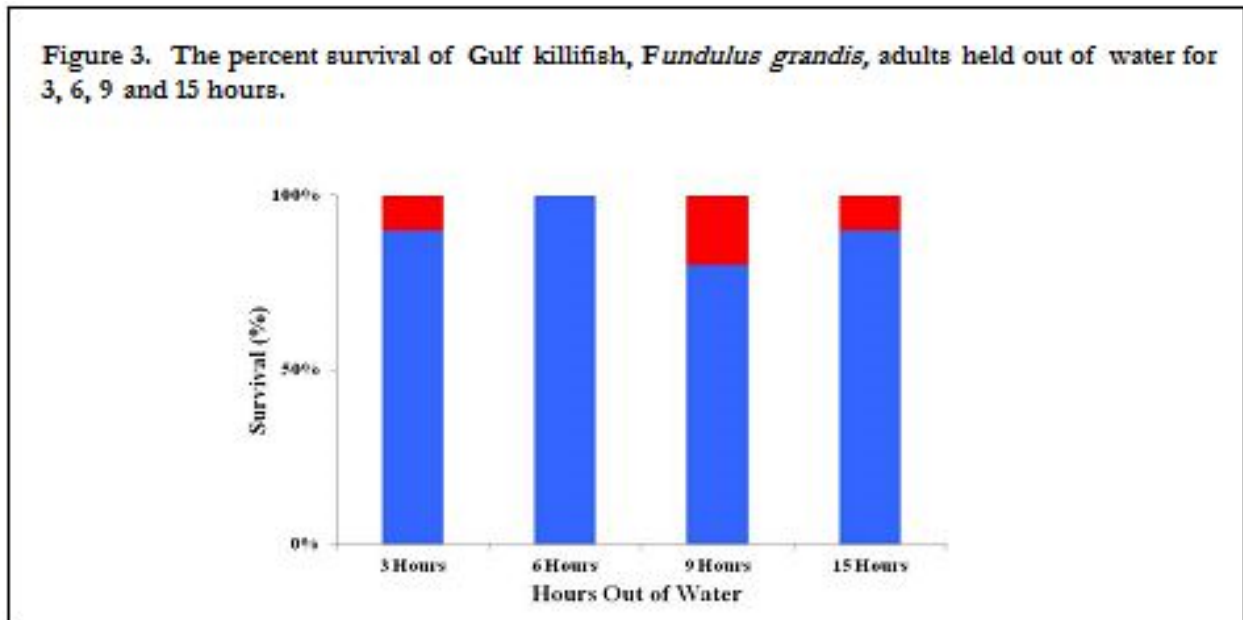




During periods of stranding many fish species will undergo a buildup of metabolites that can lead to their demise. In order for the Gulf killifish to survive it must respire while managing the accumulation of toxic metabolites that it usually removes through the gills. We have previously demonstrated that eggs can be held in moist environments and have been contacted by many stakeholders due to their interest in transporting small numbers of these baitfish out-of-water.

For this study Gulf killifish were wrapped in moist cheesecloth, placed inside a plastic container and then stranding periods of 0, 3, 6, 9, and 15 hours. Respirometry was used to measure standard metabolic rate in fish during an aquatic recovery period immediately following stranding. Remaining survivors were sampled for plasma and gill tissue. Plasma samples were used in assays to determine urea, ammonia, and lactic acid concentrations. Urea and ammonia are nitrogenous wastes that build up in plasma as a result of protein utilization. Lactic acid is produced when undergoing anaerobic (lacking in oxygen) conditions. All of these metabolites can normally be processed at the gills but if not they may prove fatal when high concentrations occur in the blood.

In many species, terrestrial stranding proves lethal relatively quickly, possibly due to critical increases in high concentrations of lactate, urea, and ammonia. Survival was independent of stranding which highlights the remarkable ability of the Gulf killifish to withstand extended periods of terrestrial stranding (Figure 3). Surviving fish were sampled after terrestrial ammonia and urea may indicate that the metabolites are being processed in alternative ways throughout stranding.



Respirometry data showed a significant decrease over time, which indicates that the fish undergo metabolic changes dependent on stranding. It is possible that an accumulation of mucus on the gills prevents them from drying and this would be reflected in the recovery because it may take time to remove the mucus and begin respiring normally. This data may also indicate a change in

heart rate known as bradycardia that would be used to slow down the respiration and buildup of metabolites. It is clear that both Gulf killifish embryos and adults possess the rare ability to sustain terrestrial stranding. Culturists can take advantage of these unique attributes with both fry production and the transport of embryos and adults.

**Sub-objective 4b.** *Identify a replacement of live feeds for Fundulus.*

### **University of Florida Indian Research and Education Center**

Use of a microparticulate diet saves time, space, and labor associated with live feeds, eliminates the potential of disease introduction from the live feeds, and ultimately should reduce the cost of production of juvenile fish. The microparticulate microbound diet used was previously proven to be an effective and complete substitute for live *Artemia* nauplii in the culture of two species of crustaceans, *Macrobrachium rosenbergii* and *Litopenaeus vannamei*, and zebrafish (*Danio rerio*). The diet served as a partial *Artemia* replacement for 20-days post-hatch pinfish larvae and is being tested with larvae of several different marine fishes.

#### Year 1

Larval Gulf killifish were cultured in 15L circular fiberglass tanks with flow-through water providing an exchange of approximately 2 tank volumes/day. Upon hatching, 50 larvae were randomly stocked into a tank and randomly assigned one of three diet treatments: microbound microparticulate diet exclusively for 15 days (MICRO), *Artemia* nauplii exclusively for 15 days (ART), or *Artemia* nauplii for 5 days followed by a mix of *Artemia* and microparticulate diet for 5 days followed by the microparticulate diet exclusively for the remaining 5 days (MIX). There were 5 replicates assigned for each treatment. The microparticulate diet was developed by L. R. D'Abramo at Mississippi State University. On a dry weight basis, the proximate composition of the microparticulate diet was 46.1% crude protein and 37.4% crude lipid. The microparticulate diet was stored frozen at -4 degrees F. Prior to every feeding, a portion of the microparticulate diet was removed from storage and added to a small volume of culture water. This was done to prevent it from clumping and floating, and to achieve a homogeneous particle size. Larvae were fed the microparticulate diet in excess twice daily.

The proximate composition of *Artemia* nauplii on a dry weight basis was 53.8% crude protein and 16.2% crude lipid. *Artemia* cysts were disinfected prior to hatching by exposing to a 2% hypochlorite solution for 10 minutes and aerated. *Artemia* cysts were disinfected and hatched daily at a salinity of 3 g/L. After harvesting, the concentration of hatched *Artemia* was determined by counting a subsample so each treatment received the same amount of *Artemia*. Larvae were fed in excess twice daily. Before feeding, uneaten microparticulate diet and dead *Artemia* were removed from the bottom of each tank. Excess uneaten live *Artemia* were removed from the surface of the water with a fine-mesh net.

At 0, 5, 10, and 15 days post-hatch, five larvae were removed from each tank. Photographs of larvae were taken using a stereo microscope outfitted with a digital camera to measure total length (TL) of each larva.

There were significant differences in total length of larvae among diet treatments at 5, 10, and 15 days post-hatch (Table 2). ART larvae were the largest during the 15-day experiment. Survival among the treatments was significantly different. The MIX diet larvae had no mortalities during the experimental period. The growth of larvae in the MICRO and MIX treatments were 71.5% and 83.9%, respectively, of that of the MICRO treatment after 15 days. However, the feeding schedule used in this experiment most likely affected growth in the treatments which received microparticulate diet because live *Artemia* nauplii were available in the water column for a longer period of time than the microparticulate diets. *Artemia* nauplii were present in the appropriate tanks at the next feeding. If the microparticulate diet would have been available in the water column for a longer period of time, the larvae may have been able to increase consumption, thereby increasing growth. If the feeding of the microparticulate diet had been split into more feedings or placed in an automatic feeder, the results may differ. Feeding schedules need additional investigation.

**Table 2. Mean TL ± SE of larvae at 0,5,10, and 15 days post hatch (dph) and mean survival at the conclusion of the study. Within a row, different letters denote significant differences in TL and survival (Pd ± 0.05).**

	MICRO	ART	MIX
Total length (mm)			
0 dph	8.36 ± 0.09 z	8.36 ± 0.07 z	8.39 ± 0.08 z
5 dph	9.17 ± 0.06 y	9.77 ± 0.34 yz	10.29 ± 0.019 z
10 dph	10.00 ± 0.12 y	13.80 ± 0.20 z	13.29 ± 0.24 z
15 dph	11.58 ± 0.16 x	16.20 ± 0.17 z	13.59 ± 0.15 y
Survival %	95.20 ± 0.02 y	99.20 ± 0.01 z	100.00 ± 0.00 z

## Year 2

Larval Gulf killifish are characterized as precocial larvae with well developed mouths and eyes upon hatch. Previous work with *Fundulus* spp. Indicates that species within this genus can accept a powdered or microparticulate diet upon first feeding. Currently the default strategy in rearing killifish is to provide the larvae with *Artemia* nauplii because few studies are available to indicate the performance of powdered or microparticulate diets at this early-life stage. The ability to avoid or at least reduce the use of *Artemia* in the culture of *Fundulus* spp. has the potential to reduce cost, simplify labor, and reduce pathogen transfer.

This study was designed to compare larval growth and survival of larval Gulf killifish fed *Artemia* nauplii, a microparticulate diet, and a third treatment group consisting of a combination of these two diets. Embryos were harvested from spawning mats at the LSU AgCenter Aquaculture Research Station and shipped to the UF Indian River Research and Education Center where they were subsequently hatched after approximately 12 days of incubation. Five replicates of each treatment were stocked at a density of 5 larvae per liter at a salinity of 7.5 g/L. Larvae were fed twice daily (9am and 3pm) equivalent amounts by volume of either *Artemia* or microparticulate diet. At 5, 10, and 15 days post hatch (DPH) survival was determined as well as a subsample of the larvae from each replicate tank was photographed for morphometric analysis.

Standard length (SL) was determined from digital images captured at the UF Indian River Research and Education Center and sent to the LSU AgCenter Aquaculture Research Station.

Mean SL among the three treatments did not differ at 5 and 10 dph. At 15 dph the dry feed treatment SL was significantly smaller (REGWQ post hoc) (Table 3). Using a two-way ANOVA, time and treatment was significant while interaction (time\*treatment) was not. Mean survival among the *Artemia*, Dry, and Mixed feeding groups was 89.6, 87.7, and 93.8%, respectively. Using an arcsin square-root transformation and Tukey-Kramer post-hoc, the mixed feeding group had significantly higher survival. While SL of larvae between the *Artemia* and Mixed feeding groups was not different there was a potential benefit seen from increased survival. Although a microparticulate feed resulted in reduced length compared to the other groups at 15 dph the similar survival indicates that an artificial diet would work under culture conditions for Gulf killifish in a recirculating system. Two additional trials have been conducted but data has not been analyzed yet.

**Table 3. Mean SL  $\pm$  SE of larvae at 5,10 and 15 days post hatch (dph) and mean survival at 15 dph for Gulf killifish, *Fundulus grandis*, fed a microparticulate diet (MICRO), Artemia (ART), or a mixture (MIX) of the two diets from first feeding. Within a row different letters denote significant in SL and survival.**

	MICRO	ART	MIX
Standard length (mm)			
5 dph	5.4 $\pm$ 0.13 z	5.9 $\pm$ 0.20 z	5.7 $\pm$ 0.12 z
10 dph	5.9 $\pm$ 0.10 z	6.1 $\pm$ 0.18 z	6.0 $\pm$ 0.13 z
15 dph	6.6 $\pm$ 0.08 z	7.4 $\pm$ 0.14 y	7.1 $\pm$ 0.14 y
Survival %	87.7 $\pm$ 0.8 z	89.6 $\pm$ 2.0 z	93.8 $\pm$ 0.7 y

**Sub-objective 4c. Determine relationship between larval density and performance in *Fundulus*.**

There is little information available on *Fundulus* spp. culture in recirculation systems. Previous research with this species group has been pond based, where larvae are placed in fertilized ponds and allowed to feed on natural zooplankton. Densities of killifish fry and juveniles were estimated by weight within a specific pond area. Our research seeks to investigate growth performance and survival of larvae and juveniles within recirculation systems. Compared to the traditional systems, the ability to culture *Fundulus* spp. fry at high densities with a control over the culture environment in recirculation systems will enable aquaculturists to raise and market more fish per unit volume of water. Fry rearing utilizing recirculation capabilities will further increase the numbers of juveniles for grow-out phase within a production system and hence the numbers of adults and broodstock.

An 8 week study was conducted in four separate recirculating systems with newly hatched Gulf killifish. Salinity in all four systems was maintained between 9.5-10 g/L with synthetic marine salt. Each system consisted of eight 50-L aquaria, four aquaria were stocked at 7 larvae per liter, and the remaining four were stocked at 18 larvae per liter to represent high and low larval stocking densities. Larvae were sampled at 0, 1, 2, 7, 10, 14, and 28 days post hatch for dry

weight. Survival, wet weight, and length from each density treatment was determined at the end of the 8 week study.

Both mean length and weight were significantly different between the two larval rearing densities at the end of the 8 week study period (Table 4). Larvae reared at 18 per liter were significantly smaller than larvae reared at 7 per liter with the lower density having twice the survival. Dry weights of larvae from hatch to 28 days post hatch at regular intervals indicated that the lower density had greater weight gain beginning between 14 and 21 days post hatch.

**Table 4. Mean SL and weight ( $\pm$ SE) of Gulf killifish, *Fundulus grandis*, juveniles initially stocked at 7 and 18 per liter and reared from hatch to eight weeks. Within a row, different letter denote significant differences in SL, weight, and survival.**

	7 larvae/L	18 larvae/L
Standard length (mm)	22.4 $\pm$ 0.48 z	17.5 $\pm$ 0.43 y
Wet weight (g)	0.15 $\pm$ 0.03 z	0.07 $\pm$ 0.01 y
Survival (%)	63.5 z	30.2 y

Based on the results of the density study completed at 7 and 18 larvae per liter, Gulf killifish larvae were stocked in triplicate 40-L tanks within a large joined recirculating system at densities of 2, 5, 8, and 11 larvae per liter. The salinity of the system was maintained at 10 g/L using synthetic marine salt. Each tank was fed a commercially available feed that was ground and sieved with a 500-um mesh (40% crude protein, 9% crude fat, 4% crude fiber; Burriss Mill and Feed, Franklinton, Louisiana). Individuals were fed daily at 10% body weight divided into three feeding times, 9 am, 12 noon and 3 pm.

Quantity of feed given to the fry was adjusted biweekly according to body weight of the killifish. The wet weight (nearest 0.0001 g), and SL (nearest 0.1 mm) was determined from a sample of individuals ( $n = 20$ ), while survival was determined every four weeks for this 16 week study.

After two weeks of stocking, fry stocked at 5/L and 11/L had attained a significantly greater mean weight compared to individuals stocked at 2/L and 8/L. After six weeks in culture, fish stocked at 8/L had the highest weight, although not statistically different from the 11/L. From week 10 to the completion of the study (week 16), the fry stocked at 11/L had the highest mean weight and hence ended with the highest mean weight. There was a negative relationship between stocking density and survival, a majority of which could be attributed to cannibalism. The onset of cannibalism was observed between weeks 6 and 8 of the study and progressed until the completion of the 16 week study. Removal of cannibals was not conducted so the study results show a severe impact of cannibalism at densities of 5, 8, and 11 fish per liter.

These results indicate that optimum stocking densities of Gulf killifish in recirculation systems may be below 5 per liter after 6 to 8 weeks of growth, coinciding with significant increases in the incidence of cannibalism (Table 5). Although the lowest density (2 fish/L) had the lowest growth, it had the highest survival (slightly above 82%) at the end of the 16 week study period.

One possible solution would be to decrease rearing densities as the fish progress from larvae to juveniles.

	2 larvae/L	5 larvae/L	8 larvae/L	11 larvae/L
Final weight (g)	0.33 $\pm$ 0.13 z	0.51 $\pm$ 0.01 y	1.16 $\pm$ 0.11 x	1.43 $\pm$ 0.10 w
SGR	1.68	2.06	2.79	2.98
Survival (%)	82.8	28.3	10.7	6.7

**Sub objective 4d.** *Determine relationship between salinity and performance in Fundulus.*

### Louisiana State University

Salinity is a major factor in growth rates of Gulf killifish juveniles. This species has been observed to survive in waters ranging from freshwater (0 ppt) to nearly twice seawater (70 ppt). Culture conditions in both brackish water ponds and recirculation systems have the potential to vary widely in salinity and these conditions can impact growth performance. A series of investigations were initiated to determine the influence of salinity on growth and survival in Gulf killifish and specific ion manipulations and the influence on growth, survival, and osmoregulation.

Juvenile Gulf killifish were obtained from a population cultured at the LSU Aquaculture Research Station and held in a single recirculating tank with aeration and a salinity of 7 ppt. Prior to the current study fish were fed a commercial starter diet containing approximately 52% protein and 14% lipid. Groups of juvenile fish were grown in triplicate at four different salinities; 0.5, 5.0, 8.0, and 12.0 ppt. No acclimation occurred during transfer from 7 ppt to other salinities, so the fish were considered directly transferred for purposes of molecular work. Each of the four systems was randomly stocked in triplicate with fish at a mean mass of 0.50 $\pm$ 0.01 g. After initial stocking, wet mass and total length were measured for a random sample of 20 individuals per replicate every 14 days. Survival, condition factor, and specific growth rate was calculated at the completion of the 12 week study. Fish were fed a 32% protein and 4% lipid diet at a rate of 4% of body weight per day divided into morning and afternoon feedings with the amount fed adjusted following biweekly growth sampling.

Data from biweekly sampling is detailed in Figure 4. Growth is reported as mean change in g per fish sampled. Weight gain and SGR were significantly lower than all other treatment levels in the 0.5 ppt salinity while the 12.0 ppt treatment had significantly higher values in these parameters than the 5.0 ppt treatment (Table 6). Survival was lowest in fish reared at 0.5 ppt and salinity was found to have a significant effect on this parameter. Final condition factor (Kn) was significantly lower in the 0.5 ppt treatment, indicating that fish from this salinity had lower body mass per unit length compared to the experimental population.

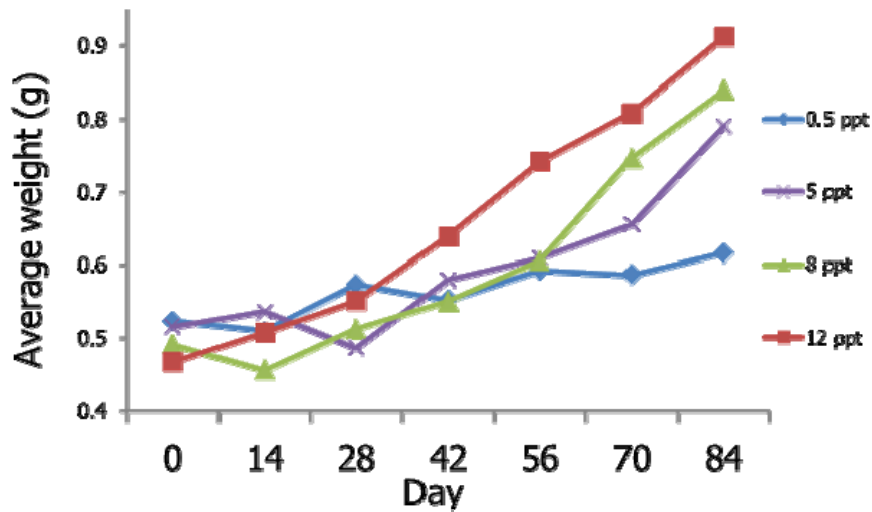


Figure 4. Growth of juvenile Gulf killifish, *Fundulus grandis*, stocked at one fish per L and fed a 32% protein, 4% lipid diet for 12 weeks.

Table 6. Growth, survival, and condition factor (*Kn*) data for Gulf killifish, *Fundulus grandis*, fed 32% protein and 4% lipid over a 12-week trial at different salinities. Letters denote statistical significance across parameters (REGWQ;  $P < 0.05$ ).

	Nominal salinity (ppt)			
	0.5	5.0	8.0	12.0
Initial weight (g)	0.53±0.02 <sup>a</sup>	0.51±0.02 <sup>a</sup>	0.49±0.02 <sup>a</sup>	0.46±0.02 <sup>a</sup>
Final Weight (g)	0.62±0.03 <sup>b</sup>	0.79±0.05 <sup>ab</sup>	0.84±0.05 <sup>a</sup>	0.91±0.07 <sup>a</sup>
Weight gain (g)	0.09±0.03 <sup>c</sup>	0.28±0.03 <sup>b</sup>	0.35±0.04 <sup>ab</sup>	0.45±0.03 <sup>a</sup>
SGR (%/day)	0.18±0.06 <sup>c</sup>	0.51 ±0.04 <sup>b</sup>	0.63±0.07 <sup>ab</sup>	0.81±0.02 <sup>a</sup>
Survival (%)	59.3	86.5	96.3	89.7
Initial <i>Kn</i>	1.053 <sup>a</sup>	1.010 <sup>a</sup>	0.999 <sup>a</sup>	0.989 <sup>a</sup>
Final <i>Kn</i>	0.976 <sup>b</sup>	1.091 <sup>a</sup>	1.096 <sup>a</sup>	1.104 <sup>a</sup>

Three separate four week trials were conducted exposing newly hatched Gulf killifish to varying concentrations of potassium ( $K^+$ ), calcium ( $Ca^{2+}$ ), and magnesium ( $Mg^{2+}$ ). Trials consisted of four treatment groups, each in quadruplicate. The concentrations of these ions were supplemented to range from values found in municipal water to those found in marine mix salt at

10 ppt salinity. K<sup>+</sup> supplementation was maintained at 51.3 and 80.6 mg/L K<sup>+</sup>, while one system was maintained with crystal salt and no K<sup>+</sup> supplementation (12 mg/L K<sup>+</sup>) and the fourth system maintained using a standard marine mix salt (114.7 mg/L K<sup>+</sup>). Treatment groups for the Ca<sup>2+</sup> trial consisted of 8.0, 43.2, 60.0, and 82.8 mg/L Ca<sup>2+</sup>. Treatment groups for the Mg<sup>2+</sup> trial consisted of 1.2, 65.6, 122.9, and 251.9 mg/L Mg<sup>2+</sup>. All treatments were maintained at a salinity of 9.5-10 ppt using crystal salt (99.6% NaCl) and potassium in the Ca<sup>2+</sup> and Mg<sup>2+</sup> trials was supplemented using KCl to approximately 90 mg/L K<sup>+</sup> due to the results of the first trial. Samples were collected across time for dry weight, whole body ion composition, Na<sup>+</sup>/K<sup>+</sup>-ATPase activity, and immunohistology. At the conclusion of each trial final weight and survival were determined.

These studies indicate that the high concentrations of divalent ions present in marine mix salts may not be necessary, and in the case of calcium may be detrimental to the survival and growth of Gulf killifish larvae. At a salinity of 10 ppt obtained solely from the addition of NaCl, Gulf killifish larvae can be cultured in waters modified by the addition of chemicals, i.e., potassium chloride, magnesium chloride, and calcium chloride, in concentrations that provided ions for optimum survival and growth (Table 7).

**Table 7. Composition of critical ions (potassium, magnesium, and calcium) in freshwater and various saline mixtures. The addition of just rock salt (NaCl) to increase ‘salinity’ results in possible ion deficiencies. The column on the far right represents recommended minimum values of these ions for larval Gulf killifish, *Fundulus grandis*, culture.**

	Pond (freshwater)	Municipal (freshwater)	Municipal + NaCl	Municipal + synthetic sea salt	Recommended composition for larvae
Salinity (ppt)	-	0.1	9.8	9.8	9.5
K <sup>+</sup> (mg/L)	3.4	1.5	12.7	113	90
Mg <sup>2+</sup> (mg/L)	21.6	0.1	1	286	70
Ca <sup>2+</sup> (mg/L)	29.8	3	13.2	93.6	15

**Objective 5.** *Develop improved technologies for spawning and larval rearing of Bala shark*

**Sub-objective 5a.** *Improve Bala shark broodstock maturation.*

Monitoring of the development of viable broodstock was continued. Handling techniques for the broodstock were refined to include the use of floating 30L storage bins as vessels for transporting broodstock from ponds during harvest, allowing the fish to recover for three days post-harvest before any other handling was undertaken and sedating the fish with a low dose of Metomidate prior to tranquilizing for egg biopsy procedures and application of spawning agents. Tranquilizer was held in a 5 gallon bucket lined with a plastic fish bag to prevent bruising of the fish on the sides of the bucket. In February 2013 the first mature eggs were detected although the percentage of females with mature eggs was very low. Fish were restocked into outdoor ponds in



April 2013 and sampling was conducted every two weeks until sufficient numbers of broodstock with mature eggs to begin trials were detected.

**Sub-objective 5b.** *Develop technologies for induced spawning of Bala shark.*

**University of Florida Tropical Aquaculture Laboratory**

Year 1

Bala sharks are a high value and popular freshwater ornamental species but are only available from farms in Asia. Bala sharks have presented unique challenges in broodstock development, spawning techniques, and larval rearing for the U.S. ornamental aquaculture industry.

Bala sharks (2 g mean weight) were purchased from a local importer. Fish were stocked directly into two outdoor ponds. Pond water temperature was 84.2 degrees F. Six fish were sampled for dissection and histological examination of gonadal development. Fish were removed from the ponds in October and placed in recirculating water tank systems in a heated greenhouse. Gonadal samples were taken once a month since May 2011 to determine gonad maturation. At each sampling, twelve fish were anesthetized, weighed and measured, and an attempt was made to express sperm or extract eggs. For males, sperm maturation is determined by manually expressing sperm. For female sexual maturation, we are looking for fish that appear to be “fat.” When a fish is suspected as a female, a small catheter tube is inserted into the genital opening in an attempt to extract eggs. Subsamples of fish were used to determine the maturational stage on December 23, 2011. Mean weight of the fish was 26.5 g and mean length was 14.1 cm. To date, no eggs have been collected and no viable sperm has been expressed. The fish were returned to open ponds in April 2012.

Year 2

Monitoring of the development of viable broodstock was conducted. In May 2012, 50% of the Bala sharks were returned to open ponds and the remaining fish were retained for conditioning indoors in a recirculating water tank system.

Samples have been taken once a month throughout the year to determine sexual maturation and growth. The first eggs in females were detected in February 2012. The mean weight was 31.1 g and mean length was 14.4 cm. The first sexually mature males were detected April 2012. By July 2012, most of the fish were exhibiting gonadal development, and although the males are producing viable sperm, to date none of the females have produced mature eggs.

Year 3

Three trials were conducted. Comparisons were made in the effectiveness of Ovaprim, Chorulon, Carp Pituitary Extract (CPE) and Channel Catfish Pituitary Extract (CCPE) as agents to induce ovulation in Bala sharks. Methods of application of the agents were also compared between intramuscular injection (IM), intracoelomic injection (IC) and a technique developed at UF TAL of applying the agent directly into the ovary through a catheter to address treatments to

fish in which injections are not viable (OL). Fish eggs were biopsied to select only those fish with mature eggs. Mature eggs were defined as those 1.3mm in diameter in which the macronucleus had migrated from the center of the egg. Selected fish were segregated into groups of three females and three males for each treatment. Injected fish received a 10% priming dose of the assigned agent and a 90% resolving dose 6 hours later. Fish receiving the ovarian catheter treatment were given the entire dose at once with half going into each ovary. At the conclusion of the first trial, dosages on the ovulation inducing agents were adjusted on the less effective treatments and a second trial was conducted using six females and six males for each treatment. Once an effective treatment regime was determined, a trial was conducted to determine the viability of inducing volitional spawning. Six 55 gallon plastic drums had central drains plumbed into the bottoms with external standpipes through which the water and eggs would flow into 30L storage bins with 300 micron screen-covered openings as egg collectors. Water was supplied in a circular flow to the top of each drum. Fish were given IC injections of Ovaprim at a rate of 1mL/K of fish weight. Fish received a 10% priming dose and a 90% resolving dose 6 hours later. Three pairs were placed into each drum. Egg collectors were checked 12h, 18h and 36h post-resolving dose and broodstock were removed and evaluated after 36 hours to determine whether ovulation had occurred in the fish that did not release eggs.

Refined handling techniques developed during the study resulted in no adult fish being lost during the spawning trials due to handling stress or injection. Bala sharks receiving an IM injection series with Carp Pituitary Extract at a rate of 5mg/K of fish weight had the best spawning rate (100%). Ovaprim IC injections at a rate of 1mL/K of fish weight had an ovulation rate of 83%. Channel Catfish Pituitary Extract IM injections at a rate of 5mg/K of fish weight were 83% effective in inducing ovulation. Bala sharks did not respond to Chorulon with any application method. The non-injection (OL) application was unsuccessful in inducing ovulation with any agent. Volitional spawning was shown to be a non-viable method for reproducing Bala sharks with only two of the ten females that had ovulated releasing eggs.

**Subobjective 5c.** *Develop improved technologies for larval rearing of Bala shark.*

Year 2

Over 50 older Bala sharks were acquired which were capable of production of viable eggs. Mature eggs were first noted in June 2012 and spawning trials were begun at that time. The fish were successfully induced to ovulate in July 2012. A series of trials have been conducted to determine the optimum water quality parameters for hatching the eggs. Eggs were placed in hatching jars with water hardness ranging from 34 to 170 ppm, total alkalinity ranging from 34 to 68 ppm and pH ranging from 6.5 to 8.0. Successful hatching of the eggs occurred in water that was 140 ppm hardness, 52 ppm alkalinity, and a pH of 8.0. Newly hatched fry were 4 mm in length and grew to 6 mm by day five at which time they were ready to feed on newly hatched *Artemia*. At 6 weeks old, the fry ranged from 1.5 to 2 cm.

In addition, several batches of eggs produced were frozen and shipped Louisiana State University to be used in subobjective 5d.

Year 3

A trial was conducted to examine the efficacy of three live feeds (*Artemia*, microworms and the salt-water rotifer *Colurella* acclimated to 5 g/L salinity to feed in fresh water) as a first feed for the indoor rearing of larval Bala sharks. Fifty one day old Bala shark fry were placed into each of 12 plastic aquariums containing 6 liters of water duplicating the water in which the fry were hatched. Four tanks were fed *Artemia* 24h post culture inoculation, four tanks were fed *Colurella* enriched with algae and four tanks were fed microworms from ongoing cultures. Survival of the fish through ten days of feeding was the parameter selected to assess each feed. In addition, 16,000 one week old Bala shark fry were stocked into each of two ponds to determine length of time to achieve commercially salable size (2.5 inches).

Bala shark fry survival was best with *Artemia* (mean return was 61.3%) followed by microworms (mean return was 60.5%). As the end of the experiment, the fry in ponds were still too small to sell (mean length = 1.7 inches).

**Subobjective 5d.** *Design water treatment technologies for commercial larval rearing of Bala shark.*

**Louisiana State University Agriculture Engineering, University of Florida  
Tropical Aquaculture Laboratory**

Eggs of fish are commonly collected and are incubated in a variety of hatching tanks and systems. There is no consistent design and most tanks and filters used are not capable of handling large inputs of ammonia and other compounds released from hatching and decaying egg masses. A design of a water treatment strategy appropriate for use in commercial larval production systems capable of handling shock loading of these compounds is necessary and this type of system has application for many species of fish.

Year 1

Water treatment components were designed which are capable of responding to shock loading of total ammonia nitrogen and organic matter when a proportion of the egg mass decays in a recirculating system. This effort has been divided into two steps.

The first step was to identify a surrogate to Bala shark eggs that would permit the research team to conduct shock loading response experiments in the LSU laboratory. The research team acquired supplies necessary for conducting analysis defining the organic and nitrogen loading for a variety of egg types. Since Bala Shark eggs were not yet available to the team, techniques for freeze drying eggs were developed utilizing trout egg masses. Student workers were trained to conduct the chemical analysis. Freeze dried egg matter from the trout eggs was then used in a preliminary chemical analysis.

The second step was to initiate the design of treatment components for evaluation. In support of this goal, visits between the LSU and University of Florida research teams were made to observe current breeding practice and establish system size. The LSU team constructed and evaluated

appropriately sized prototype floating bead, fluidized sand, and moving bed reactors. Formal testing of the units will be conducted once the waste characterization work is complete.

## Year 2

Eggs of Bala sharks, speckled trout (*Cynoscion nebulosus*), snapper (*Lutjanus campechanus*), tilapia (*Oreochromis niloticus*), and channel catfish (*Ictalurus punctatus*) were collected for comparative purposes, as a precursor for further Bala shark eggs studies. Eggs were weighed, freeze-dried, and powder-crushed to increase the surface area of each egg particle, thereby ensuring accurate biochemical oxygen demand (BOD<sub>5</sub>) readings. Each measurement was done in triplicate. Blank samples were also analyzed to ensure consistency. Averages (all samples) were: 0.72 mg/g BOD<sub>5</sub>, 10.49% nitrogen and 67.86% proteins. A statistical analysis indicated that channel catfish BOD<sub>5</sub> is the most representative of the multi-species lot.

A theoretical rationale was developed for evaluating the filters used to mitigate shock loading experienced during spawning events. A time dependent model was developed in the Stella™ modeling environment using Monod kinetics to simulate shock loading of ammonia-nitrogen and BOD<sub>5</sub> in recirculating aquaculture systems (RAS). The timely reduction of ammonia-nitrogen was found to be mostly governed by the half saturation constant. A literature review that defined half saturation nitrification values identified the fine fluidized bed as the best treatment option. However, the ability to remove heterotrophic growth might be a key issue that needs to be looked at, which could make a floating bead filter the best option. The research team constructed a floating bead filter, a fine sand bed, and a moving bed reactor that will be subject to shock loading experiments. Each filter held four liters of media.

The recirculation capabilities of small scale airlifts were investigated. Water delivery as a function of air input was determined by replicated studies for 1, 1.5, and 2 inch airlifts at a variety of lift to submergence ratios (S: L) – 2:1, 3:1, 4:1, and 5:1. Optimal S:L was determined to be 4:1. Water flow for these units is predictable by the relationship:

$$Q_w = 4.3 * Q_g$$

where  $Q_w$  is water flow (in gpm) and  $Q_g$  is the air input to airlift (in cfm). The relationship was constant across all pipe sizes tested. It is anticipated that these results will be used in the design generated by the research team in the next year of the project.

## Year 3

The research team investigated an assimilative strategy for the removal of ammonia from recirculating waters. An alternative to the nitrification process, this approach encourages the growth of bacteria on a carbon source that is nitrogen deficient. The bacteria then absorb ammonia, or nitrate, from the recirculating system's water. Some success with this strategy had been reported in the Florida aquaculture community using an ethanol drip system on waters that displayed low pH. The research investigated the use of a bed of a bioplastic, Polyhydroxyalkanoate or PHA, as the carbon source. The PHA beads also act as the biocarrier providing surface area for biofilm development. This material has been shown to be useful for

nitrate removal under both anaerobic and aerobic conditions. Removal of ammonia (total ammonia nitrogen or TAN) is quantified on a volumetric basis (VTR or volumetric TAN removal rate) allowing different bioreactors to be compared.

The PHA material that is available to the research team sinks, so the moving bed reactor and floating bead filter designs were not applicable to this strategy. An aerobic upflow packed bead bed has been shown to have a higher assimilative capacity than a fluidized bed for 3 mm PHA beads. Excessive abrasion was the likely cause of the poor fluidized bed performance. VTR values in excess of 350 gm-N/m<sup>3</sup>-day have been achieved with the upflow bead design. However, performance was not found to be consistent between different batches of PHA beads. Each batch of PHA has a potentially different composition as additives percentages for additives, such as plasticizer, is varied by the manufacturer. Residual nitrite concentrations (0.1-0.3 mg/L-N) have periodically appeared suggesting that partial denitrification or partial nitrification is occurring in the beds. Newer designs attempted to resolve this issue by improving the water distribution plates under the bead beds assuring even oxygen distribution.

The research team has concluded that PHA beds can function effectively to remove TAN from recirculating systems through assimilative processes when operated under aerobic conditions. The removal process is sensitive to oxygen levels, water distribution, and PHA composition. The process headlosses are low and compatible with airlift recirculation. The process is applicable to a variety of operational scales (i.e. adaptable to single tank or multiple tank clusters in a wide range of volumes). Current pricing of PHA beads (above \$5.50/kg wholesale) would suggest that the process is most applicable to smaller scale applications where the PHA cost may be offset by labor savings (when compared to ethanol drip systems).

## IMPACTS

- First report of repeated volitional spawning of ballyhoo in captivity and the optimal egg collection method has been defined.
- Gulf killifish embryos can be easily air incubated in moist foam and developmental times can be controlled by incubation temperature indicating the potential for coordinated hatching of multiple spawns at one time. This will increase efficiency and reduce production costs of hatcheries and growout facilities. Additionally, packaging and transportation of embryos on foam between producers is possible and increases efficiency.
- This project changed the amount of knowledge pertaining to several aspects of *Fundulus* sp. culture. For example, information from these investigations has been included in a recent SRAC fact sheet (Publication No. 1202), a locally produced Cocahoe Minnow Production Manual, and extension.org. This information will continue to be integrated into presentations and publications with the public and pertinent stakeholders.
- *Fundulus* spp. larvae can be cultured exclusively on a microparticulate diet from 0 to 15 days post-hatch and this will reduce the cost of hatchery production and reduce the likelihood of pathogen introduction from live feeds.

- Defined optimal dosages of HCG and Ovaprim for induced spawning of pinfish.
- Showed catfish pituitary extract does not induce spawning in pinfish following a single bolus dose but is efficacious for spawning induction in pigfish.
- Refined handling techniques have further reduced Bala shark broodstock post spawning mortalities which had been a significant deterrent to commercial production in Florida.
- Effective egg hatching and fry rearing techniques have been defined including optimal water quality parameters for hatching eggs, first feeds for indoor rearing and successful pond stocking of Bala shark fry.
- Four producers have acquired Bala shark broodstock including the study fish used in these trials. Two producers have successfully begun commercial production of Bala sharks in Florida.

## **PUBLICATIONS AND PRESENTATIONS**

### **Publications**

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