REPRODUCTION AND LARVAL REARING
OF FRESHWATER ORNAMENTAL AND
MARINE BAITFISH

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Mississippi State University ......................... Louis D’Abramo

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.
   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 sp. 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. These 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**

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

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-iio) 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.

**Table 1. Mean notochord length (NL) of larval pinfish fed different enrichments via rotifers. Survival and swim bladder inflation as a percentage at 11 DPH.**

<table>
<thead>
<tr>
<th>Run</th>
<th>Enrichment</th>
<th>NL (μm) at Stocking</th>
<th>NL (μm) at 6 DPH</th>
<th>NL (μm) at 11 DPH</th>
<th>Survival (%)</th>
<th>Swim Bladder inflation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Non-enriched</td>
<td>281.07 ± 1.09</td>
<td>288.54 ± 3.56</td>
<td>349.42 ± 6.07</td>
<td>12.78 ± 3.29</td>
<td>16.99 ± 1.20</td>
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<td></td>
<td>Ori-Go</td>
<td>281.07 ± 1.10</td>
<td>287.27 ± 4.32</td>
<td>369.46 ± 6.89</td>
<td>17.36 ± 7.53</td>
<td>14.79 ± 4.26</td>
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<td></td>
<td>Algamac 3050</td>
<td>281.07 ± 1.11</td>
<td>285.79 ± 4.77</td>
<td>344.78 ± 7.21</td>
<td>9.97 ± 1.42</td>
<td>2.27 ± 2.27</td>
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<tr>
<td></td>
<td>DHA Protein Selco</td>
<td>281.07 ± 1.12</td>
<td>305.41 ± 4.85</td>
<td>364.95 ± 7.19</td>
<td>14.81 ± 0.17</td>
<td>18.93 ± 1.07</td>
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<tr>
<td>2</td>
<td>Non-enriched</td>
<td>283.12 ± 1.65</td>
<td>294.67 ± 2.50</td>
<td>352.72 ± 8.63</td>
<td>0.86 ± 0.11</td>
<td>22.86 ± 19.48</td>
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<td>283.12 ± 1.66</td>
<td>292.52 ± 2.01</td>
<td>341.53 ± 9.103</td>
<td>3.66 ± 2.69</td>
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<td>296.71 ± 3.03</td>
<td>349.22 ± 14.44</td>
<td>0.57 ± 0.21</td>
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<td>DHA Protein Selco</td>
<td>283.12 ± 1.68</td>
<td>294.18 ± 2.61</td>
<td>355.29 ± 6.06</td>
<td>1.72 ± 0.54</td>
<td>25.51 ± 13.69</td>
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<tr>
<td>Mean</td>
<td>Non-enriched</td>
<td>282.10 ± 0.99</td>
<td>293.02 ± 2.08</td>
<td>350.36 ± 4.95</td>
<td>3.84 ± 2.05</td>
<td>21.18 ± 13.49</td>
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<tr>
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<td>Ori-Go</td>
<td>282.10 ± 0.10</td>
<td>291.21 ± 1.86</td>
<td>356.63 ± 5.80</td>
<td>7.09 ± 3.31</td>
<td>16.87 ± 10.38</td>
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<td>Algamac 3050</td>
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<td>292.88 ± 2.65</td>
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<td>DHA Protein Selco</td>
<td>282.10 ± 0.10</td>
<td>296.99 ± 2.35</td>
<td>359.58 ± 4.64</td>
<td>4.99 ± 2.18</td>
<td>23.63 ± 9.53</td>
</tr>
</tbody>
</table>

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

Objective 2 will be addressed in year 3.
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).

![Figure 1. Mean egg number per spawn by substrate. Different letters denote statistically significant differences (P≤0.05).](image)

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 sp. 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 recedes. 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 ± 2% at 68 degrees F, 62 ± 3% at 73 degrees F, 58 ± 8% at 79 degrees F, and 75 ± 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...
Reproduction and Larval Rearing of Freshwater Ornamental and Marine Baitfish

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 (Figure2). At this high temperature

![Figure 2. The maximum number of hours incubation can be extended in Gulf killifish, Fundulus grandis, reared humid environment across a range of temperatures. The minimum number of incubations days are listed first in each temperature treatment followed by the number of days incubation can be extended. Temperature significantly influenced incubation and significant differences among temperature treatments are denoted with different letters.](image)
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 kept in a temperature controlled incubation chamber. Surviving fish were sampled after terrestrial 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 and can cause things like cramping while jogging. 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). Under normal conditions the fish would filter waste through the gills but absence of a significant increase in plasma concentrations of

![Figure 3. The percent survival of Gulf killifish, Fundulus grandis, adults held out of water for 3, 6, 9 and 15 hours.](image-url)
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 bradychardia that would be used to slow down the respiration and build up 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

Results at a glance...

This was the first study to show that microparticulate diets can be used to culture Fundulus larvae. Survival of fish fed the microparticulate diet alone from 0 to 15-days post-hatch was 95%.
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.

Results at a glance...

This study was the first evaluation of diets to be used for culturing Seminole killifish larvae. A 95% survival was achieved in larvae fed the MICRO diet exclusively for 15 days. While the survival was statistically significantly less than the survival in the MIX and ART treatments, survival was very high and can be considered a success in the larval culture of Seminole killifish. This study demonstrated that Seminole killifish larvae can be cultured exclusively on a microparticulate diet from 0 to 15 dph.

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).

<table>
<thead>
<tr>
<th></th>
<th>MICRO</th>
<th>ART</th>
<th>MIX</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 dph</td>
<td>8.36 ± 0.09 z</td>
<td>8.36 ± 0.07 z</td>
<td>8.39 ± 0.09 z</td>
</tr>
<tr>
<td>5 dph</td>
<td>9.17 ± 0.06 y</td>
<td>9.77 ± 0.34 yz</td>
<td>10.29 ± 0.09 z</td>
</tr>
<tr>
<td>10 dph</td>
<td>10.00 ± 0.12 y</td>
<td>13.80 ± 0.20 z</td>
<td>13.29 ± 0.24 z</td>
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<tr>
<td>15 dph</td>
<td>11.58 ± 0.16 x</td>
<td>16.20 ± 0.17 z</td>
<td>13.59 ± 0.15 y</td>
</tr>
<tr>
<td>Survival (%)</td>
<td>95.20 ± 0.02 y</td>
<td>99.20 ± 0.01 z</td>
<td>100.00 ± 0.00 z</td>
</tr>
</tbody>
</table>
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 ± 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 letter denote significant differences in SL and survival. |
|-----------------|-----------------|-----------------|
|                 | MICRO           | ART             | MIX             |
| Standard length (mm) |                 |                 |                 |
| 5 DPH           | 5.4 ± 0.13 z    | 5.9 ± 0.20 z    | 5.7 ± 0.12 z    |
| 10 DPH          | 5.9 ± 0.10 z    | 6.1 ± 0.18 z    | 6.0 ± 0.13 z    |
| 15 DPH          | 6.6 ± 0.08 z    | 7.4 ± 0.14 y    | 7.1 ± 0.14 y    |
| Survival (%)    | 87.7 ± 0.8 z    | 89.6 ± 2.0 z    | 93.8 ± 0.7 y    |

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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 raise and market more fishes 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.

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; Burris Mill and Feed, Franklinton, Louisiana). Individuals were fed daily at 10% body weight divided into three (3) feeding times, 9 am, 12 noon and 3 pm.

<table>
<thead>
<tr>
<th>Table 4. Mean SL and weight (± 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.</th>
</tr>
</thead>
<tbody>
<tr>
<td>7 larvae/L</td>
</tr>
<tr>
<td>-------------</td>
</tr>
<tr>
<td><strong>Standard length (mm)</strong></td>
</tr>
<tr>
<td><strong>Wet weight (g)</strong></td>
</tr>
<tr>
<td><strong>Survival (%)</strong></td>
</tr>
</tbody>
</table>
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 the 6th and 8th week of the study and progressed till 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.

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.

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.

<table>
<thead>
<tr>
<th></th>
<th>2 larvae/L</th>
<th>5 larvae/L</th>
<th>8 larvae/L</th>
<th>11 larvae/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Final weight (g)</td>
<td>0.33 ± 0.13 z</td>
<td>0.51 ± 0.01 y</td>
<td>1.16 ± 0.11 x</td>
<td>1.43 ± 0.10 w</td>
</tr>
<tr>
<td>SGR</td>
<td>1.68</td>
<td>2.06</td>
<td>2.79</td>
<td>2.98</td>
</tr>
<tr>
<td>Survival (%)</td>
<td>82.8</td>
<td>28.3</td>
<td>10.7</td>
<td>6.7</td>
</tr>
</tbody>
</table>

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

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

Subobjective 5a will be addressed in year 3.
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 will be returned to open ponds in late March or early 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.

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

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. The fry are currently 6 weeks old and range 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.
**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 has also acquired supplies necessary for conducting analysis defining the organic and nitrogen loading for a variety of egg types. Since Bala Shark eggs are 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 has constructed and is evaluating 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 (BOD₅) readings. Each measurement was done in triplicate. Blank samples were also analyzed to ensure consistency. Averages (all samples) were: 0.72 mg/g BOD₅, 10.49% nitrogen and 67.86% proteins. A statistical analysis indicated that channel catfish BOD₅ is the most representative of the multi-species lot. Analysis of six other species, including bala sharks is continuing and should be completed by year’s end.

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 BOD₅ 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 has constructed a floating bead filter, a fine sand bed, and a moving bed reactor that will be subject to shock loading experiments in the upcoming months. Each filter holds 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.

**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.

**PUBLICATIONS AND PRESENTATIONS**

**Publications**


**Presentations**

Reproduction and Larval Rearing of Freshwater Ornamental and Marine Baitfish


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