IMPROVING REPRODUCTIVE EFFICIENCY OF CULTURED FINFISH

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PROJECT OBJECTIVES

1. Improve broodfish management protocols for increased reproductive efficiency through:
    a. Developing pre-selection methods of potential broodfish to be included in the broodstock population.
    b. Improving conditioning and preparation of broodfish.
    c. Final identification of broodstock for spawning.
2. Improve spawning protocols to increase reproductive efficiency through:
    a. Managing spawning conditions.
    b. Improving the collection and handling of fertilized eggs.

ANTICIPATED BENEFITS

Captive-bred finfish rarely experience all aspects of natural spawning conditions, and thus dependence on natural reproduction is often unreliable. Consequently, reproductive efficiency is often less than desired, frequently requiring creative management or compensatory protocols to overcome the failure to reproduce spontaneously and at full potential. This project will improve reproductive efficiency of commercially cultured finfish of immediate importance to the Southern Region. Management protocols will be established that address reproductive bottlenecks and result in improved protocols that increase reproductive efficiency for the target species and have the potential for use with other similarly cultured finfish species.

PROGRESS AND PRINCIPAL ACCOMPLISHMENTS

Objective 1. *Improve broodfish management protocols for increased reproductive efficiency.*

Sub-Objective 1a. *Develop pre-selection methods of potential broodfish to be included in the broodstock population.*

USDA-ARS Catfish Genetics Research Unit

Channel catfish farming is the largest sector of the U.S. aquaculture industry. Production of catfish fry relies on random spawning of mature male and female catfish in brood ponds. Spawning success is highly variable, but research and farm data indicate that on average about 40% of females produce a spawn. Little is known about males' contribution to spawning, but most farmers stock a ratio of 1:1 or 2:1 female to male broodfish. In addition to spawning incidence, spawning time (early to late in the year) is important to farmers since early spawning allows earlier stocking of fry and production of larger fingerlings by the end of the first growing season. The inability to identify parentage of pond-spawns has hindered determination of factors influencing spawning in catfish. However, the advent of molecular marker techniques for parentage determination allows evaluation of factors influencing spawning in pond-spawned catfish. Understanding factors influencing spawning could lead to development of improved fish and management techniques for more efficient reproduction of farm-raised catfish.

One hundred channel catfish spawns were collected from eight commercial catfish farms in the spring of 2006 as part of a project to establish a diverse population of catfish for selection of an improved catfish line. Full-sib families were maintained in separate tanks until fish were large enough to be individually tagged (> 4 inches), and then tagged fish were reared communally in ponds. Fish were fed a 32% protein commercial catfish diet throughout the study. The largest 5 to 9 females and 3 to 7 males from each family (~ 800 females and 500 males) were selected during the fall of 2007 to be used as future broodfish and blood samples were collected from each fish for DNA marker analysis. Each broodfish was scored for a series of highly polymorphic microsatellite loci and inheritance patterns at these loci were used to determine parentage of spawns. Broodfish were weighed and blood samples were drawn for determination of estrogen and testosterone levels in females and testosterone levels in males in the late winter (February to March) of 2008 and 2009. Ultrasound images of maximum cross-sectional area of ovaries were also

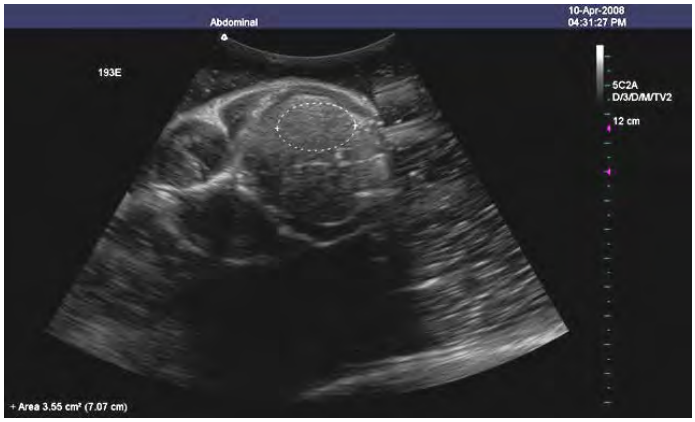


Figure 1. Ultrasound image of catfish ovary.

recorded for females at this time. Following sampling, broodfish were stocked into 0.25-acre ponds at a 2:1 female to male ratio in the spring of 2008 (2-year-old broodfish, 24 ponds) and again in 2009 (3-year-old broodfish, 20 ponds). Eight spawning cans were placed in each pond in early April and checked for spawns through the end of August each year. Data were analysed to determine relationships among spawning incidence and time; and broodfish weight, farm-of-origin, family-of-origin, spawning pond, hormone levels, and estimated ovary size.

In March of 2010, 4-year-old broodfish were categorized based on their previous spawning history: spawning males, spawning females, non-spawning males and non-spawning females. Two, 0.1-acre ponds were stocked with each possible combination of males and females (previously spawning males with previously spawning females, previously spawning males with previously non-spawning females, previously non-spawning males with previously spawning females, and previously non-spawning males with previously non-spawning females) to determine if spawning history was predictive of future spawning success for either gender. Broodfish were stocked at 1.5:1 female to male ratio and six spawning cans were placed in each pond in early April and checked for spawns through the end of August. Data were analyzed to determine effects of previous spawning history and farm of origin on spawning incidence and farm of origin on spawning time.

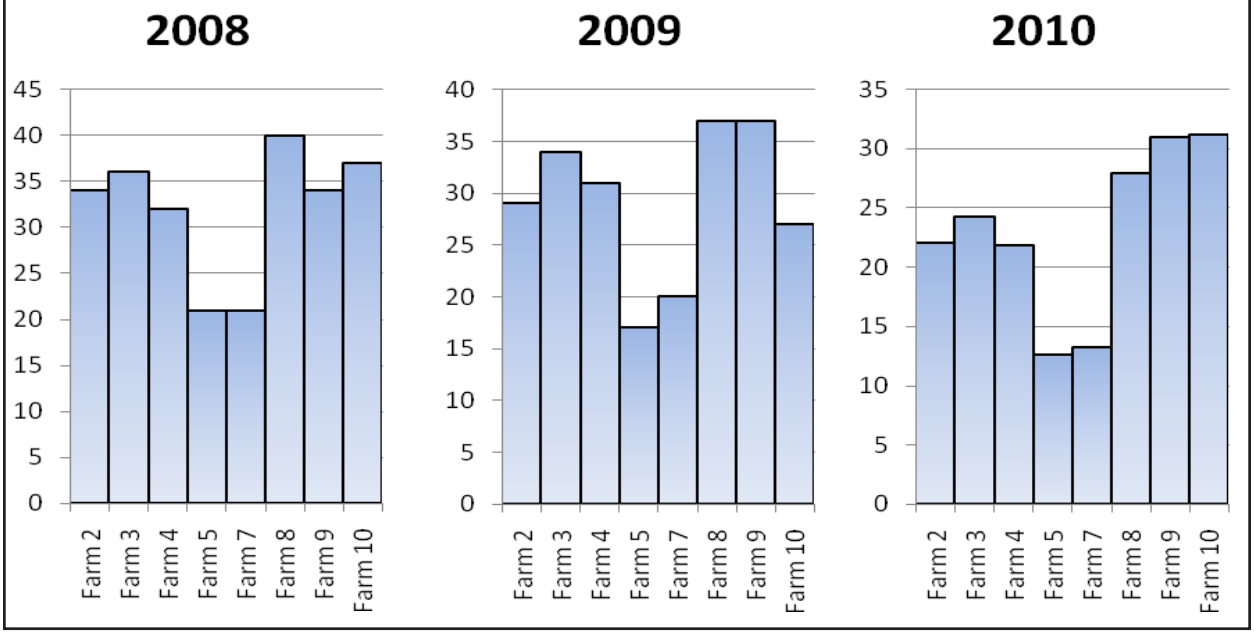
Spawns were collected over a 103-day period in 2008, a 98-day period in 2009, and a 75-day period in 2010; however, over 60% of the spawns were collected within 35 days of the first spawn each year. Spawning percentages were 27.4%, 48.3%, and 60.3% for 2-, 3-, and 4-year-old females, respectively; and 25.7%, 37.7%, and 46.6% for 2-, 3-, and 4-year-old males, respectively. Due to frequent multiple spawning by males, over 60% of spawns were attributed to fewer than 15% of the males each year.

Spawning incidence was influenced by fish weight and spawning pond; but farm-of-origin, family-of-origin, plasma estrogen and testosterone, and ultrasound estimates of ovary size were not predictive of spawning incidence. As 2-year-old fish, spawning females (1.8 lbs) were larger than non-spawning females (1.6 lbs), but there was no difference in weight of spawning and non-spawning 3-year-old females (3.2 lbs). Spawning males and non-spawning males were not different for weight as 2-year-olds (2.1 lbs), but spawning males were larger than non-spawning males as 3-year-olds (3.5 lbs and 3.0 lbs, respectively) and 4-year-olds (4.7 lbs and 4.1 lbs, respectively). Previous spawning incidence was not

predictive of future spawning incidence for males or females. There was no difference in spawning percentage of 4-year-old fish for the various stocking combinations based on previous spawning success.

Our data suggest variation in female spawning date has a genetic component. Average spawning date of females from the same two farms-of-origin (farms 5 and 7) was significantly earlier than other farms by 9 days in both 2008 and 2009, and 12 days in 2010 (Figure 2). Farm-of-origin and family-of-origin were significant predictors of female spawning date; combined these factors accounted for 26% and 16% of variation in female spawning date in 2008 and 2009, respectively. There was a positive correlation among spawning date for individual females across years, and for mean spawning date of full-sib sisters across years for the 2008 and 2009 data. There were insufficient numbers of females per family in 2010 to provide accurate estimates for effects of family-of-origin on female spawning date or correlation of female spawning date across years. Analysis and interpretation of male spawning date was precluded due to the high proportion of males that spawned multiple times over each spawning season. We also observed multiple spawning by female channel catfish. Approximately 20% of spawning females produced more than one egg mass during a spawning season each year. These were not interrupted spawning events as the egg masses were collected greater than 7 days apart and typically the second egg mass was collected 20 to 50 days after the first egg mass. Most multiple-spawning females produced 2 separate egg masses, although 3 females produced 3 separate egg masses. To our knowledge, this is the first report of female channel catfish producing multiple egg masses over a spawning season. Plasma hormone levels and ultrasound estimates of ovary size were not predictive of female spawning date for 2008 and 2009.

Figure 2. Mean spawning day for female channel catfish from eight commercial farms in 2008, 2009, and 2010.



Spawning incidence of females appears to be primarily under environmental control suggesting that future work should focus on identification of environmental factors that influence spawning incidence. Management of environmental factors to promote spawning would reduce the number of broodfish needed and reduce costs. A relatively small proportion of males did the majority of spawning, indicating that the number of males typically used by farmers (1:1 or 1:2 male to female ratio) is probably excessive. Reducing the number of males could reduce broodfish costs substantially but it will be important to determine how few males can be stocked without reducing spawning incidence. Selection for early spawning date appears to be feasible and could allow farmers to stock fry earlier and potentially produce larger fingerlings at the end of the first growing season.

Results at a glance...

- *Timing of female spawning (early or late season, for example) has a genetic component that can be exploited in breeding programs to expand the spawning season. On the other hand, the incidence of females spawning is primarily under environmental control, suggesting that spawning success can be improved by identifying and managing appropriate environmental factors affecting spawning success.*

University of Arkansas at Pine Bluff and USDA-ARS Stuttgart National Aquaculture Research Center

Some female white bass do not respond to changes in the duration of the reproductive cycle (i.e. compression, shifting, or expansion). Females may fail to spawn after being included in groups that are projected to spawn when seed stock is required. Ultrasonography has the potential to guide decisions regarding which females to include in a production cycle. Quantification of characteristics of the images collected with an ultrasound machine could be used to determine which females will be most likely to spawn following photothermal manipulation. This would reduce the number of female white bass necessary to hold under controlled photothermal regimes for use in hybrid striped bass seed stock production.

A Tela-Vet portable ultrasound system (Classic Medical, Tequesta, FL) equipped with a 5-8 MHz linear transducer was used for this study. During 2010-2011 we captured digital images from 170 fish cataloging more than 6000 digital images. We have begun the process of collecting information regarding ovary characteristics (diameter, cross sectional area, perimeter) using image analysis software (Image-Pro Plus Version 4.5.1.22, Media Cybernetics, Inc., Silver Spring, Maryland). Our initial efforts have included developing a standardized assessment for determining cross sectional surface area. Multiple technicians are involved with this process. Quality control steps include the evaluating agreement between data collected from different personnel. The consistency of the 'rule-set' for interpreting the image and defining the perimeter of each ovary is evaluated for each worker evaluating images. To further optimize training we have collected ultrasound images from three white bass that were then sacrificed. The animals were then frozen and cross sections of the peritoneal cavity were exposed. Photographs of the cross section of the fish are being compared to data gathered using ultrasound. Finally, during April-May, 2011, 24 wild female white bass were also collected during their spawning migration in Caney Bayou, a tributary of the Arkansas River. Nine of these fish had mature ovaries and the remaining fish

were in varying post-spawning stages. Ultrasound images were captured from each of these fish. Ovaries of each fish were removed and length and diameter measurements were recorded so that a cross sectional area of the ovary was determined. The average cross-sectional area determined for the nine gravid fish was 1078 ± 74 mm^2 (average ± standard error). Cross sectional areas were also determined using the image analysis program. Mean cross sectional area determined using ultrasound and image analysis was 966 ± 104 mm^2.

A standardized approach to collecting images has been developed. During 2010 and 2011 images were collected over 16 different dates from female white bass. Fish were anesthetized and held upright, submerged in a holding tank. Images were collected from the body region between the posterior insertion of the pelvic fin and the anterior insertion of the anal fin. Whether or not the female responded to hormone injection, and the time to ovulation (if spawning occurred) was recorded. Multiple images of cross sections of the peritoneal cavity were captured. Length and weight of each fish was recorded and age data were gathered from hatchery passive integrated transponder (PIT) tag records. The female white bass were held for about 24 hours before initial inspection for ovulation. The eggs were visually inspected to determine readiness for spawning. Eggs were then expressed from 'ripe' females into plastic containers. Fish not ready for spawning initially were inspected at several intervals over a 24-hour period. The total mass of eggs expressed was recorded for the females screened using ultrasound imaging. Three small samples of eggs (0.1-0.2 g) were weighed. The number of eggs in each of these samples was tallied to determine the number of eggs/g. Female fecundity (eggs/kg fish weight) was then determined by multiplying eggs/g by the total mass of eggs expressed. The digital images of these gonads are being currently being examined. Depth, power, gain, frequency and decibel settings and the quality of each image collected by the of the ultrasound system are being summarized. This procedure does have the potential to guide hatchery decisions and improve reproductive efficiency during production of hybrid striped bass seed stock.

No new images were captured in 2012. We continue to assess images to determine ovary characteristics (diameter, cross sectional area, perimeter) using image analysis software.

Texas A&M University-Corpus Christi and Auburn University

Steroid analysis was conducted for channel catfish and blue catfish. The objective was to determine if the steroid analysis can be used as a predictor for sexual maturity and ripeness of the fish or as a predictor for future reproductive performance. Steroids were also measured as an indicator of the relative effectiveness of different diets supplemented with vitamin C when fed to broodfish.

Blood and serum samples were collected during three periods corresponding to spring (March-prior to visual spawning activity), early summer (June-during spawning activity), and after spawning (July). Additional studies included exposure studies of blue and channel catfish to vitamin C treatments, and establishment of baseline data for immature 2-3 year-old blue catfish males. Serum samples were immediately frozen and stored at -112 degrees F until processed. Samples were purified, concentrated, and analyzed by high-performance liquid chromatography (HPLC). Eight steroids were targeted: estradiol (E2), 11-ketotestosterone (11-KT), 11β-hydroxyandrostenedione (11β-OHA), 11β-hydroxytestosterone (11β-HT), 17, 20β-dihydroxypregn-4-en-3-one (17, 20β-P), estrone (E1), testosterone (T), and 17α-hydroxyprogesterone (17-OHP).

Whole blood samples yielded inconsistent extraction results compared to sera samples. Fish fed diets supplemented with vitamin C had significantly higher overall steroid concentrations relative to fish fed a control diet. Several trends were evident from comparing female blue versus channel catfish. Estradiol and testosterone were more strongly expressed and accumulated in blue catfish compared to channel catfish, whereas estrone was found in higher concentration in channel catfish post-spawn (June). Male catfish had a larger number of steroids represented in the samples than females. Blue catfish males had higher total steroid content than channel catfish during pre- and spawning periods. 11-Ketotestosterone was highest in pre-spawning for both catfish species and decreased over the study period. T was below detection threshold for most of the study period in both species, peaking in channel catfsh post-spawn. 17α-Hydroxy-progesterone concentrations peaked during spawning period for both channel and blue catfish. 11bP was not present in male catfish of either species. Steroids of female blue and channel catfish show several expected observations. Peaks in estradiol and testosterone occurred during spawning, whereas 17, 20β-dihydroxypregn-4-en-3-one was most abundant after this period.

Species differences were observed for steroid levels in both and female fish. This may help explain the differences in the success of open pond spawning and for induced spawning observed between these two species.

Sub-Objective 1b. *Improve conditioning and preparation of broodfish.*

University of Arkansas at Pine Bluff, University of Tennessee, Texas A&M University

To achieve maximum Atlantic croaker production efficiency, larvae must be available throughout the year. In addition, nothing is known of nutritional requirements for Atlantic croaker broodfish, which must be known to ensure quality spawns. Four studies were undertaken to resolve these problems. Study 1 examined the feasibility of conditioning and inducing Atlantic croaker to spawn during the spring/summer utilizing 90- or 120-day abbreviated conditioning cycles and hormones. Due to failure to spawn in suitable quantities during study 1, study 2 was conducted to determine the feasibility of delayed spawning through photoperiod and temperature manipulation. Study 3 examined the effects of dietary lipid source and inclusion rate on reproductive performance of Atlantic croaker. Study 4 examined the effects of fish meal replacement and alternative protein sources, as well as dietary lipid percentage and protein source interactions on reproductive performance of Atlantic croaker.

Study 1: Accelerated spawning cycles

The biology of Atlantic croaker dictates they spawn during the autumn months, but baitfish production relies on availability of small fish throughout the year, especially during spring and summer. Atlantic croaker broodstock were spawned in November, 2009, and then maintained under static winter conditions. Broodstock were measured (mean = 11.9 inches), gender determined (male:female ratios of 3:5 or 4:4) and fish were stocked into eight experimental tanks, in two systems on March 1, 2010. Each system underwent either 90- or 120-day abbreviated cycles that condensed annual photoperiod/water temperatures into the experimental duration. Treatments ended during autumn conditions optimal for spawning of Atlantic croaker (10 hours light/14 hours dark, water temperature 66 degrees F). The broodfish were then injected with a 75-microgram salmon

gonadotropin-releasing hormone analogue implant (sGnRHa; Ovaplant®). Fish were allowed to spawn within the tanks and eggs were collected. No spawning occurred during the 90-day cycle treatment and only two small spawns were collected from fish in the 120-day cycle. In the 120-day cycle, a 4,300 egg spawn and a 2,700 egg spawn were collected 4 and 6 days after implantation, respectively. Fertilization was less than 5% for both spawns, and eggs were atypically small and discolored. Egg incubation was not attempted due to poor egg quality. Two plausible explanations for low reproductive output are 1) annual cycles compressed to 90 or 120 days were too short a duration to allow adequate deposition of nutrients to gametes; and 2) spawning twice in a 6-month period does not allow proper physiological preparation through environmental cues. Abbreviated 90- or 120-day cycles are ineffective for out-of-season conditioning and spawning in Atlantic croaker that have previously spawned.

Study 2: Effects of delayed spawning

Due to the poor reproductive success experienced during study 1, a second study was conducted that examined the effects of delayed spawning on the reproduction of Atlantic croaker. Atlantic croaker broodstock were *not* spawned during their natural spawning season in November of 2010, and instead maintained under static summer conditions of 82-86 degrees F and a photoperiod of 15 hours light and 9 hours dark. Histological examination revealed approximately 80% of females developed immature ova during this period, but only 10% demonstrated advanced stage ova development. In February, 2010, the un-spawned broodstock were measured (mean = 12.8 inches), gender determined (male:female ratios of 3:5 or 4:4), and stocked into eight tanks in two systems. Each system underwent a 90-day cooling period with a reduction in photoperiod simulating natural autumn conditions. After the water temperature and photoperiod reached conditions conducive to spawning for Atlantic croakers, the fish in one system were injected with Ovaprim® (sGnRHa and a dopamine inhibitor; 0.23 cc/pound), followed by a second injection 2 days later. The fish in the second system received a single 75 µg sGnRHa implant (Ovaplant®), and all fish were allowed to spawn within the tanks. No spawning occurred from fish administered the implant although egg hydration occurred and several females died due to over-hydration of eggs. All males receiving the 75-µg implant did not express milt upon application of pressure to the abdomen. Although spawning was irregular among the tanks administered the aqueous Ovaprim® injections, 1.1 million eggs were produced. The mean number of eggs produced per tank was 275,530 with fertilization rates ranging from 42 to 88%. At the conclusion of spawning, all males receiving the injection expressed milt upon application of abdominal pressure. Although both treatments resulted in egg production by females, only the injection treatments resulted in viable spawns. The limiting factor of successful spawning appears to be milt production by the males. Only males receiving the Ovaprim® injections successfully produced milt, indicating that a dopamine blocker is required for males to produce milt outside on the natural spawning season. The results of this study indicate Atlantic croaker can be spawned out-of-season for year round production.

Results at a glance...

- *Atlantic croaker can be spawned out-of-season for year-round production. Hormone implants (sGnRHa) improved spawning success, egg production, fecundity, and synchronized spawning events for commercial production. Only males receiving sGnRHa and a dopamine inhibitor successfully produced milt, indicating that a dopamine blocker is required for males to produce milt outside of the natural spawning season.*

Study 3: Lipids in broodfish diets

No information is currently known on the nutritional requirements of Atlantic croaker broodstock. Broodstock nutrition is vital to producing good quality eggs in sufficient quantities to support commercial production while keeping costs down. Atlantic croaker broodstock (4 males:4 females) were stocked into each of 12 tanks in three experimental systems in September, 2010. Four experimental diets were formulated and manufactured to contain 45% protein and either 6 or 10% lipid. Lipids sources and contents were 10% menhaden fish oil, 6% menhaden fish oil, 10% poultry fat, or 10% soybean oil. In November 2010, all fish received a single 75 microgram sGnRHa implant (Ovaplant®), and all fish were allowed to spawn naturally within the tanks. Fish fed the 6 and 10% fish oil diets produced more spawns per tank (3.0-3.7 per tank), a greater percentage of floating eggs (64.3-73.2%), larger egg diameters, greater number of eggs produced (879,320-1,470,215 eggs), greater fertilization rates (37.5-65.3%), and better hatching rates (15.3-29.3%) than fish fed the 10% soybean oil or poultry fat diets. While fish fed the 10% soybean oil or poultry fat diets did produce eggs, fertilization rates were extremely poor (< 4.9%) and no egg hatching occurred. While it appears that fish oil cannot be entirely removed from broodstock diets, it can at least be reduced as the 6% fish oil diet produced more spawns, more eggs, and greater fertilization and hatch rates than the 10% fish oil diet. This study provides the first step in determining the nutritional requirements of Atlantic croaker to support commercialized production, and it provides a good basal diet for producers wanting to undertake Atlantic croaker production immediately.

Results at a glance...

- *These studies provide a good basal diet for producers wanting to undertake Atlantic croaker production. Fish oil cannot be entirely removed from broodstock diets, but it can be reduced to an inclusion rate of 6%, or possibly lower.*

Study 4: Protein in broodfish diets

Broodstock nutrition is vital to producing good quality eggs in sufficient quantities to support commercial production. Now that some information is known on lipid sources and inclusion rates to sustain Atlantic croaker production, more information is needed on the protein requirements for broodstock. Proteins and free amino acids (FAA) are important energy sources and structural elements for embryo and larval development. This study examined the effects of fish meal replacement and alternative protein sources, as well as dietary lipid percentage and protein source interactions on reproductive performance of Atlantic croaker. Atlantic croaker broodstock (2 males: 4 females) were stocked into each of 12 tanks in three experimental systems in October, 2011. Four experimental diets were formulated and manufactured to contain 45% protein and either 6 or 10% lipid. Lipid concentrations were either 10% or 6% menhaden fish oil. Protein sources included combinations of menhaden fish meal, poultry by-product meal, meat, bone and blood meal, and soybean meal. All experimental diets proved to be un-palatable to Atlantic croaker, likely due to the reduced total fish content (12% fishmeal and 6 or 10% fish oil) of the diets compared to diets used in study 3 (30% fishmeal and 6 or 10% fish oil). Feed consumption was less than 0.2% of body weight/day, and the broodstock in all dietary treatments rapidly lost weight. Disease and parasite related mortalities quickly escalated resulting in a 47% loss of total broodstock numbers despite treatment efforts with antibiotics and Paracide and Dimilin. No reproductive response was induced through the use of sGnRH and domperidone. Little information

on protein requirements for reproduction can be ascertained from the study, other than certain protein sources or reduced fish meal content can lead to palatability issues in Atlantic croaker broodstock.

University of Florida

Ornamental fish production, like all of aquaculture, relies heavily on the successful spawning, hatching, and survival of larval fish. The complexity of this industry, which produces hundreds of species of fish, makes reproduction a critical challenge to improve efficiency. Very little information is available to formulate science-based recommendations for producers and their suppliers.

Research has shown that inclusion of highly unsaturated fatty acids (HUFA) in fish diets can lead to increases in hatch and survival of larval fish. To provide HUFAs in the diet to ornamental fish, current practices rely heavily on feeding a wide variety of foods, often at an extremely high cost which include frozen feeds (e.g. ground beef heart, adult *Artemia*, blood worms, shrimp, and squid). Formulation of an artificial diet which provides proper levels of HUFAs, and is designed to feed smaller broodstock typical to the ornamental industry, could lead to considerable savings in production costs and increased performance of broodstock.

Redtail black sharks, *Epalzeorhynchos bicolor*, are popular ornamental freshwater fish that have been in production in Florida since the late 1980s. This fish represents a variety of similar species which are spawned in hatcheries using induced spawning procedures. Annual sales of redtail black sharks from Florida farms is estimated to be in excess of 1,000,000/year, with a farm-gate value of $0.25 per fish. Mono sebae, *Monodactylus sebae*, are an ornamental fish species commonly captured from the wild along the western coast of Africa. In recent years their popularity in the ornamental industry has prompted interest in the development of culture methods. No information is available on their nutritional requirements.

Producers identified broodstock nutrition to be a major bottleneck in commercialization and thus served as the impetus for this experiment. These studies were designed to evaluate the effects of altering the fatty acid profiles of diets fed to brood fish on egg and larval quality in these two commercially important ornamental fishes.

Mono Sebae

Brood mono sebae were stocked into three independent systems and held for one year. Spawning was induced by increasing the salinity by 5 g/L every three days until a salinity of 25 g/L was attained. When a salinity of 25 g/L and 75 degrees F was attained, natural volitional spawning initiated. Eggs were collected in an air lift floating egg collector. Fish were fed the various experimental diets daily to apparent satiation. The three formulated diets included an ornamental fish industry standard formulation, a diet fortified with docosahexaenoic acid (DHA) and a diet fortified with DHA plus arachadonic acid (ARA). The formulation of the DHA and DHA+ARA diets was altered by adding commercially available algal additives Algamac 3050 Flake and Algamac-ARA to increase the n-3 and n-6 fatty acid contents, respectively.

The effects of feeding the experimental diets on spawning performance was measured by quantifying the number of spawns, egg quantity, fertilization percent, hatch percent, egg morphology (egg diameter, oil droplet diameter), larval survival at 24 and 48 hours post-hatch, larval morphology (oil droplet diameter, yolk volume, and notochord length) at hatch, and larval morphology (notochord length) at 24 and 48 hours post-hatch using standard methods. The fatty acid composition of the eggs was determined using standard methods.

A total of 49, 33, and 67 spawning events were recorded over the 88-day experimental period for the control, DHA, and DHA+ARA diets, respectively. The total number of eggs spawned was 758,282 for the control, 521,211, and 1,260,255 for the DHA+ARA diets. The mean total egg production per female was 2160 for the control, 1959 for the DHA diet, and 2813 for the DHA+ARA diet. The mean floating egg percent fertilization were all greater than 93.6% and the mean sinking egg percent fertilization ranged from 70.6 to 88.4%. Mean hatching percentage was 57.4% for the control diet, 55.2% for the DHA diet, and 47.5% for the DHA+ARA diet. The mean 24 hour survival was 62.0% for the control diet, 42.8% for the DHA diet, and 46.4% for the DHA+ARA diet with the control diet being significantly greater than the DHA and DHA+ARA diets. The mean 48-hour survival was 45.3% for the control diet, 30.5% for the DHA diet, and 30.3% for the DHA+ARA diet with the control diet being significantly greater that the DHA and DHA+ARA diets.

The mean egg and oil globule diameters were significantly different among diets with those in the DHA+ARA diet being smaller. At hatch, mean notochord length and oil droplet diameter were significantly different among diets with the DHA+ARA diet being smaller, however, the yolk volume was not significantly different among diets.

Results at a glance...

- *Mono sebae broodfish fed increased diets fortified with DHA and ARA produced the greatest number of eggs and greatest number of spawns. Redtail black shark larval survival at 2-days post hatch was significantly lower for the control diet which indicates a possible benefit from incorporation of DHA and ARA in brood diets.*

At 24 hours post-hatch, the notochord length was significantly different among treatments with the DHA+ARA diet being significantly smaller. At 48 hours post-hatch, the notochord length was significantly different among treatments with the DHA+ARA being significantly smaller than the control but not different from the DHA diet.

Redtail Black Sharks

Seven female broodfish were stocked into each of nine 1,000-L concrete tanks in a greenhouse at the University of Florida Tropical Aquaculture Laboratory. Each experimental diet was fed to fish in three tanks. Females were individually weighed at the beginning of the experiment and feed portions were weighed for each feeding. All female fish were fed the brood diets for 27 days at 5% body weight divided into a morning and evening feeding. Males were kept in a tenth tank on the same system and fed the control diet at 5% of their body weight per day. On day 28 the fish were not fed and all female fish were administered Ovaprim injections at a dosage of 1 mL/kg body weight. The injections were divided into a 10% priming dose at midnight and a 90% resolving dose at 6 a.m. Female fish were injected with a 20-minute interval between each tank to allow for the timing of spawning and egg sampling.

Fish were hand-stripped when ovulation occurred using a dry method, mixing eggs and sperm in a bowl and then adding water to initiate fertilization. A combined sample of non-fertilized eggs was taken from females in each tank and placed in a -112 degree F freezer until fatty acid analysis. Fertilization percent was determined by examining a subsample of eggs at the onset of gastrulation, and hatching percent was based on a random subsample taken from spawns from each tank. Survival of larvae was based on a 50 fry sample at day 2 (fully developed gut and functional mouth) and a 100 fry sample at day 30 following standard feeding protocol.

Redtail Black Sharks represent several cyprinid species in commercial production and therefore were a good model candidate for this study. However, due the length of time required to fully mature eggs in their gonad and the seasonality of maturation, we were limited on the number of replications in this trial. Added to the problem were early failures of all treatment eggs to hatch due to elevated water temperatures in the hatchery. Fatty acid profiles of the eggs were determined for both trial but the percent hatch and larval survival data was only for one spawning event in year 2.

Percent fertilization ranged from 89-95%, percent hatch ranged from 82-87.3%, 2-day post-hatch survival ranged from 44-97.3%, and 30-day survival ranged from 42-73.3%. Percent fertilization, percent hatch, percent survival and percent deform at hatch, 2 and 30 days post-hatch were not significantly different among fish fed the different diets. The only statistically significant difference was survival at 2 days post-hatch, which was significantly lowest for eggs for fish fed the control diet. Egg diameters at the onset of the blastomere were not significantly different.

Sub-Objective 1c. *Final identification of broodstock for spawning.*

USDA-ARS Catfish Genetics Research Unit

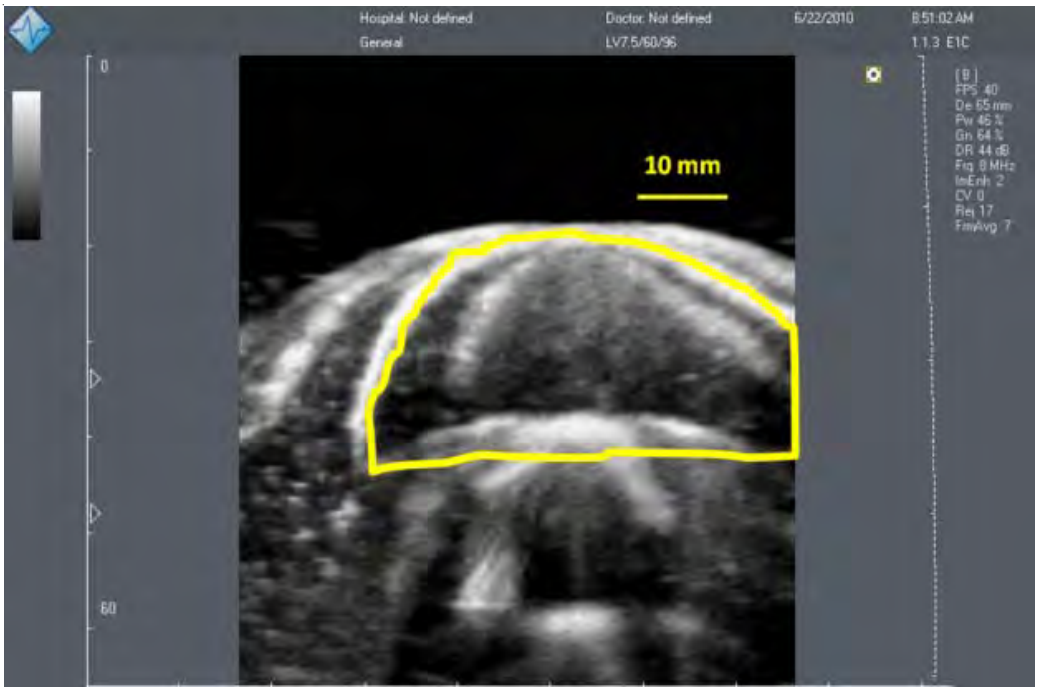
Results of studies in Objective 1a showed that ultrasound is not accurate for predicting spawning success of female channel catfish. Therefore, this objective was not addressed, given the labor and time required with no evidence indicating a successful outcome.

University of Arkansas at Pine Bluff and USDA-Stuttgart National Aquaculture Research Center

Often, injection of white bass females with hormone does not result in spawning during the next 24 to 48 hours. Females may fail to spawn at all, or they may spawn later than 48 hours, rendering a spawning effort less successful. Ultrasonography has the potential to guide decisions during the spawning process. Quantifying characteristics of the ultrasound images collected with an ultrasound machine could be used to determine which females will be most likely to spawn in a fixed period following hormone injection. This would substantially improve the efficiency of hybrid striped bass seed stock production.

Standardized imaging techniques for female white bass were developed in the first year of the project. A database of over 6,000 ultrasound images and corresponding indices of reproductive success has been developed and images continue to be assessed using image analysis software. Image analysis software (Image-Pro Plus Version 4.5.1.22, Media Cybernetics, Inc., Silver Spring, Maryland) is being used to determine several morphometric measures (diameter, cross sectional area, perimeter) to characterize ovaries of fish as they approach ovulation. Statistical models that include categorical variables such as ovulating/non-ovulating and continuous responses such as fecundity, as well as fertilization success, are being used to determine if variation in ovary images predicts higher or lower reproductive output.

Figure 3. Ultrasound image of pre-spawning white bass female collected during June 2010 spawning activities. Outline indicates area containing the ovary.



Objective 2. *Improve spawning protocols to increase reproductive efficiency*

Sub-Objective 2a. *Manage spawning conditions*

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Domestication of striped bass and white bass allows greater control over the reproductive cycle and spawning conditions. Domestication also allows choices related to age, size, and the duration of the reproductive photothermal period. The choices made may affect the success of induced spawning efforts. For example, choosing older or larger individuals might affect fertilization or hatching rates, or size of individuals at hatch or at yolk absorption. Quantifying the importance of these factors should lead to improvements in hatchery efficiency during production of hybrid striped bass.

A combination of 3-, 4-, and 5-year-old white bass were subjected to a 12-month photothermal regime. During the 12-month period, fish were fed a 45% protein diet twice daily to satiation. At the end of the 12-month period, fish were induced to spawn with hormone injections. Weights and lengths of females were determined prior to hormone injection. Fish were injected with 330 IU HCG per kg body weight. The eggs were treated with tannic acid and povidine, and maintained in McDonald hatching jars at 66 to 75 degrees F. During year 1, egg development ceased after approximately 19 hours.

It appears that povidine treatment of moronid eggs is lethal. During the second year, hatching percentages were determined. On the day of hatch, approximately 40 larvae from each cross were preserved in 4% buffered formalin. At 5 days post-hatch, approximately 40 larvae from each cross were preserved in 4% buffered formalin. Preserved larvae were photographed individually and larval total length was determined. The effect of female age on length at hatch was examined using an analysis of covariance with female weight utilized as the continuous covariate. The same statistical approach was used to examine the effect of female age on length at 5 days post-hatch.

This study was repeated during spring year 3 of the project. The procedures for year 3 were similar to those of year 2. Brood stock were subjected to a 12-month photothermal regime, fed a 45% protein diet twice daily to satiation, and induced to spawn with injections of 330 IU HCG per kg body weight. The eggs were treated with tannic acid and maintained in McDonald hatching jars at 70 degrees F. Larvae were sampled at hatch and at 5 dph, preserved in 4% buffered formalin, and later individually photographed. Lengths of larvae were determined. Statistical analyses were conducted as before, with the effects of female age and weight on length at hatch and length at 5 days post-hatch examined using analyses of covariance.

The study was repeated a third time during summer of year 3. The procedures for the summer year 3 study were similar to those of the previous two studies. Brood stock were subjected to a 12-month photothermal regime, fed a 45% protein diet twice daily to satiation, and induced to spawn with injections of 330 IU HCG per kg body weight. The eggs were treated with tannic acid and maintained in McDonald hatching jars at 70 degrees F. Larvae were sampled at hatch and at 5 dph, preserved in 4% buffered formalin, and later individually photographed. Lengths of larvae were determined. Statistical analysis consisted of examination of the effect of age on length at hatch and length at 4 days post-hatch. Age was not a factor in the third study because all the fish were age 2.

Altogether, three 3-year-old, thirteen 4-year-old, and two 5-year-old females were used during the year 2 study. Female weights averaged 614 ± 146 g (mean ± SD) and ranged from 400 to 890 g. Fertilization rates averaged 6 ± 6% and ranged from 0% to 25%. Hatch rates were fairly low (< 10%) for most crosses. A total of 11 females had enough hatching to collect adequate sample sizes for length at hatch estimates. Larvae from one of those 11 females all died before 5 days post-hatch. Length at hatch averaged 2.40 ± 0.27 mm TL. Length at 5 days post-hatch averaged 3.07 ± 0.31 mm. Neither female weight nor female age significantly influenced length at hatch (Table 1). Likewise, neither female weight nor female age significantly influenced length at 5 days post-hatch (Table 1).

During the spring year 3 study, six 2-year-old, nine 4-year-old, and three 6-year-old females were used. Female weights averaged 581 ± 170 g and ranged from 370 to 1090 g. Hatch rates varied considerably during the study. Length at hatch averaged 2.66 ± 0.15 mm TL. Length at 5 days post-hatch averaged 3.80 ± 0.21 mm TL. In this study, female weight and age both significantly influenced length at hatch (Table 2), while female age alone significantly influenced length at 5 days post-hatch (Table 2). The relationship between length at hatch and female weight was positive, suggesting that heavier females produced slightly larger larvae at hatch, but we note that this relationship was no longer significant at 5 days post-hatch. At hatch and at 5 days post-hatch, larvae from 2-year-old females were larger than larvae from 4-year-old or 6-year-old females. The magnitude of the difference in length between larvae from 2-year-old females and 4 or 6-year-old females was a few hundredths of a millimeter at hatch, but was 0.3 mm at 5 days post-hatch.

During the summer year 3 study, seventeen 2-year-

Table 1. Output from statistical examinations of the effect of female weight and female age or dam on length at hatch and length at 5 days post hatch during the 2010 study (year 2).

Response variable	Effect	Type III SS	F	df	P	R^2
Length at Hatch (mm)	weight	0.071	0.94	1	0.332	0.008
	age	0.182	1.21	2	0.301	
Length at 5 dph (mm)	weight	0.109	1.10	3	0.295	0.005
	age	0.126	0.64	2	0.531	
Length at Hatch (mm)	dam	2.34	3.33	10	0.0004	0.098
Length at 5 dph (mm)	dam	0.710	0.79	9	0.622	0.027

Table 2. Output from statistical examinations of the effect of female weight and female age or dam on length at hatch and length at 5 days post hatch during the 2011 study (year 3).

Response variable	Effect	Type III SS	F	df	P	R^2
Length at Hatch (mm)	weight	1.671	93.05	1	<0.001	0.192
	age	0.266	7.41	2	<0.001	
Length at 5 dph (mm)	weight	0.001	0.00	1	0.986	0.058
	age	0.842	10.40	2	<0.001	
Length at Hatch (mm)	dam	3.811	19.45	15	<0.001	0.430
Length at 5 dph (mm)	dam	5.463	12.32	15	<0.001	0.334

old females were spawned. Female weights averaged 669 ± 124 g and ranged from 440 to 856 g. Length at hatch averaged 2.57 ± 0.13 mm TL. Length at 5 days post-hatch averaged 3.34 ± 0.21 mm TL. As in the spring year 3 study, the relationship between female weight and length at hatch was positive and significant (Table 3). However, the relationship between female weight and length at 5 dph was not significant. These results are also consistent with the spring year 3 study.

Table 3. Output from statistical examinations of the effect of female weight or dam on length at hatch and length at 5 days post hatch during the 2011 summer study (year 3). Note: all fish in this spawn were age 2.

Response variable	Effect	Type III SS	F	df	P	R^2
Length at Hatch (mm)	weight	.	7.62	1	0.006	0.026
Length at 5 dph (mm)	weight	.	0.00	1	0.944	0.001
Length at Hatch (mm)	dam	2.848	18.56	16	<0.001	0.518
Length at 5 dph (mm)	dam	1.710	4.02	12	<0.001	0.285

Earlier work suggested that there was a maternal effect influencing size at hatch and size at 5 days post-hatch. This earlier work was not designed to ascertain whether the maternal effect was genotypic or phenotypic. The results of our year 2 study point to a genotypic effect, since phenotype (i.e. age and weight of female) did not influence size at hatch or size at 5 days post-hatch. To further examine this possibility, we ran a one-way analysis of variance using female as the independent variable. The effect of female was statistically significant (Table 1) for length at hatch, but not for length at 5 days post-hatch. For example, larvae from females 3 and 11 were significantly larger than larvae from females 4 and 7. The results of the spring year 3 study also indicate a maternal effect, though it is less clear that the effect is genotypic, since age and weight significantly influenced length at hatch and age affected length at 5 days post-hatch. Statistical analysis indicated that females affected size at hatch and at 5 days post-hatch (Table 2). Larvae from two females in particular were larger at hatch than larvae from most other females. However, by 5 days post-hatch, larvae from three completely different females had caught and surpassed the average lengths of larvae from the two stand-out females identified by data collected at hatch. It appears that larvae from the latter three females were more efficient at utilizing endogenous energy reserves (available from yolk). This efficiency could conceivably be carried into latter life stages and might be a characteristic targeted by a selective breeding program. As in the spring year 3 study, statistical analysis indicated that females affected size at hatch and at 5 days post-hatch (Table 3).

The difference in results of the studies is noteworthy. In year 2, ages ranged from 3 to 5, but in spring year 3, ages ranged from 2 to 6. Hence, the age range examined was greater in the second study. Likewise, the weight range observed was greater in the spring year 3. The converse conclusions from the first two studies, regarding the importance of age and weight to length at hatch and length at 5 days post-hatch, could be due to the greater age and size ranges of the second study. The positive relation between female weight and larval characteristics supports earlier research on striped bass, which showed that larger females tended to produce larger larvae. However, our results indicate that younger females produce larger larvae at hatch. This result should be considered tentative. It is confounded by the fact that a few of the 2-year-old females from spring year 3 happened to be among the heaviest females in the study. To examine this observation further, we limited the data

analysis of larval length at 5 days post-hatch to females weighing between 450 and 650 g. This included five 2-year-old, three 4-year-old, and three 6-year-old females. Even when the weight range is reduced, we still observed that age significantly affected length at 5 days post-hatch, and that larvae from 2-year-old females were larger than larvae from 6-year-old females.

Several consistencies exist among the three studies. Regardless of whether female weight significantly affected length at hatch, in all three studies weight no longer significantly influenced larval length at 5 days post-hatch. The effect of female age on length at 5 days post-hatch could only be tested in two of the three studies. In only one of those two studies did female age significantly affect length at 5 days post-hatch. These facts taken together suggest that female weight and female age have limited influences on length at 5 days post-hatch. In all three studies, statistical analysis indicated that female had a significant effect on length at 5 days post-hatch. The preponderance of evidence suggests that the maternal effect on length at 5 days post-hatch is mostly genotypic.

If heritability of length at 5 days post-hatch is sufficient, then selection for this trait could increase the size of larvae and consequently, their gape. If gape is large enough, hybrid striped bass might be able to consume *Artemia* nauplii at first feeding, eliminating the current requirement for rotifers at first feeding. This might significantly change the economics of tank culture of fingerling hybrid striped bass and lead to year-round availability of fingerlings.

USDA-ARS Catfish Genetics Research Unit

Yearling USDA-403 fingerlings were randomly divided into two groups. Group one was fed to satiation and group two was fed half the amount fed to group one. Both groups were exposed to a compressed annual temperature cycle of 4 months at 79 degrees F and 2 months at 55 degrees F. Exposure to three complete temperature cycles was done over two calendar years and 30 females and 20 males from each group were stocked separately into two, 0.1-acre ponds with 10 spawning containers in April. Male fish fed to satiation weighed 2.2 lbs and females weighed 1.7 lbs. Male fish fed to half-satiation weighed 1.6 lbs and females weighed 1.3 lbs. Spawning cans were checked through the summer, however, there were no spawns produced from either group.

An experiment was designed to determine if fish exposed to extreme compressed cycles would spawn when they were 1 year old. One group of fish was USDA-103 and the other group was created from an industry pool and designated as Delta Select. Both groups of fish were grown 4 months at 79 degrees F then exposed to 55 degrees F for 1 month. A temperature cycle of 2 months at 79 degrees F followed by a month of cold temperature was repeated until the fish had been exposed to three cycles of cold and warm temperatures. The fish were then stocked into 0.1-acre ponds with spawning containers and the cans checked regularly through the summer. No spawns were produced in either group.

Another experiment was performed to determine if fish could be spawned after 18 months of alternating temperature cycles. Fish from an industry pool, designated as Delta Select, were raised in the hatchery at 79 degrees F until October 13, 2009. Four groups of 150 fish from an industry pool, mean weight 27.0 g, were stocked into each of four, 300-gallon tanks equipped with chillers. Another group of 300 fish was stocked into a 0.1-acre pond. Two tanks were fed to satiation and two tanks were fed to one-half satiation. Fish were exposed to three cycles of

2 months of 55 degree F water and three cycles of 79 degree F water. In early October, 2010, 30 females and 20 males from each group were stocked in each of two, 0.1-acre ponds with 10 spawning containers. Female fish from the fish fed to satiation weighed an average of 0.25 lbs and males weighed 0.51 lbs; female fish fed one-half-satiation weighed 0.13 lbs and male fish weighed 0.18 lbs. Female fish from the ponds weighed 0.74 lbs and males weighed 1.4 lbs. A sample of eight fish from each treatment were weighed, the gonads dissected and weighed and a blood sample taken. Gonadal development was reported as the gonadosomatic index (GSI). Fish fed to satiation were twice as heavy as those fed to half-satiation; however fish fed in the ponds were over twice as heavy as those fed in tanks. The GSI from both groups fed in tanks were larger than fish from the pond. Spawning cans were checked during October, however, no spawns occurred.

Although the October water temperature was warm enough to support spawning, no spawning occurred suggesting that age of the fish may be an important component of reproductive maturation and that there is a limit on the effectiveness of temperature cycles to advance spawning. Some maturational events may have been advanced suggested by the larger GSI in cycled female fish compared to pond raised fish. However, both female satiation fed and half-satiation fed fish had similar GSIs. In all three groups, males were heavier than females. Pond raised fish were heavier than both cycled groups and the group fed to satiation were about twice as heavy as fish fed the one-half satiation ration.

Fish from the pond raised group and the cycled fish fed to satiation were held through the winter in ponds and 10 males and ten females from each group were stocked in 0.1 acre ponds with spawning containers in April of the following spring. There were not enough fish from the one-half satiation group to attempt spawning in this group. Males in both groups were heavier than females and fish raised in ponds were heavier than the cycled fish. Only one spawn (10%) occurred in the pond with cycled fish and four spawns (40%) occurred in the pond raised fish.

Altering the temperature cycle was not effective in inducing spawning after 12 months or 18 months of age, in spite of their having experienced three cold cycles, the number of cold cycles thought to be necessary to induce precocious puberty. The cold cycles in the 12 month experiment was only one month in length compared to a 2 month cold exposure in previous experiments. Further, even fish exposed for 2 months and attempted to spawn in the fall failed to show any gonadal development or spawning. The only appreciable spawning (40%) occurred in pond raised fish when they were 2 years old and had been exposed to 2 winters in the pond. Fish exposed to 3 artificial (in tanks) cold periods and one winter in a pond only had 1 out of 10 fish spawning.

These data suggest that artificially inducing precocious puberty may be more difficult than originally thought. Regardless of the feeding or temperature regime male fish were heavier than females, which support earlier reports of differential growth in the sexes of channel catfish.

University of Arkansas at Pine Bluff, University of Tennessee, Texas A&M University

Atlantic croaker display asynchronous spawning with a prolonged spawning season, which limits the potential to reproduce this species on a scale capable of sustaining commercial culture. Therefore, a study was conducted to determine if 1) Atlantic croaker could be spawned naturally in captivity; 2) hormone

implants could induce spawning or improve fecundity; and 3) temperature, photoperiod, and hormone implants could synchronize spawning.

Atlantic croaker broodstock (average total length = 11.3 inches) were captured from Trinity Bay in August, 2009. Two males and three females were stocked into each of twelve, 300-gallon tanks in three recirculation systems with temperature/photoperiod controls. Natural photoperiod and temperature mimicked seasonal temperature fluctuations in Trinity Bay. Tanks were assigned to four treatments; 1) natural spawning (NAT); 2) pre-optimal temp (77 degrees F) hormone implant (PRE); 3) optimal temp (73 degrees F) hormone implant (OPT); or 4) post-optimal temp (70 degrees F) hormone implant (POST). Implants used were Ovaplant® 75-µg sGnRHa. Egg samples were taken for determination of egg diameter, fertilization rate, and hatch rate. Egg samples from each spawning event were placed into conical, 25-gallon hatching tanks to determine hatch rates at 27 to 30 hours.

Total egg production was 2.9 million from all treatments (36 females; 24 males). Parameter means were: water temperature at spawning, 67.8 degrees F; photoperiod at spawning, 10.1 hours daylight; eggs/spawn, 97,417; fertilization rate, 42%; hatch rate, 19%; and 3-day larval survival, 37%. The POST treatment produced the greatest quantity of eggs and spawns per tank. Spawning events were highly synchronized for hormone treatments compared to NAT. The shortest to longest latency occurred in the following order: 1) POST; 2) OPT; 3) PRE. The total egg per spawn was greater in the POST treatment than PRE or OPT. The quantity of eggs per spawn was greater from POST than from fish in PRE or OPT, while the quantity of eggs per spawn from NAT was not different from other treatments. Egg fertilization was greater in the NAT and POST treatments than for PRE or OPT. Overall fecundity for all treatments in the study (36 females) was 81,180 eggs per female. The mean fecundity for females in the POST treatment was greater than fecundity of the NAT, PRE, or OPT treatments.

The results of this study demonstrate that Atlantic croaker can be spawned passively in a captive environment, but 75-µg sGnRHa hormone implants used to actively induce maturation and spawning in Atlantic croaker can improve spawning success, egg production, fecundity, and synchronize spawning events for commercial production. Optimal spawning of captive Atlantic croaker occurs at a photoperiod consisting of 10 hour daylight/14 hour dark and a water temperature of 66 degrees F. A single 75-µg sGnRHa implant should be injected at 10 hours of daylight and water temperature of 68 to 70 degrees F in order to control, improve, and synchronize reproduction of Atlantic croaker.

Results at a glance...

- *Atlantic croaker can be spawned naturally in captivity, but a single 75-microgram sGnRHa implant injected at 10 hours of daylight and water temperature of 69 degrees F will control, improve, and synchronize reproduction of Atlantic croaker for commercial production.*

Sub-Objective 2b. *Improving the Collection and Handling of Eggs*

USDA-ARS-Catfish Genetics Research Unit

Channel catfish were first spawned in captivity nearly a century ago and the methods used have changed little. Egg masses are placed in hatchery troughs in baskets made with 0.25-inch-mesh hardware cloth or plastic screen, and are agitated with paddles located between the baskets. The paddles are attached to a shaft running the length of the hatchery trough, and either rotate 360 degrees or oscillate back and forth. Normally 10 to 12 spawns (roughly 18 lbs or 250,000 eggs) are held in each 100-gallon hatchery trough, with a water flow of 5 gallons per minute at 78 to 82 degrees F. This incubation system has proved functional, but it has limitations. If egg loading is increased, as normally happens during the peak of the spawning season when facilities are limited, water circulation between and through the spawns is greatly restricted resulting in a low dissolved oxygen concentration and dead eggs in the center of the spawns. Those areas may serve as foci for fungal and bacterial infection, greatly reducing the hatch rate in the entire trough. We believed that a new incubation system, one in which water (and oxygen) was more thoroughly and efficiently forced through the egg masses, would increase the efficiency of commercial catfish hatcheries.

The new incubator, called the "See-Saw" by collaborating farmers, utilizes an angle aluminum frame slightly smaller the standard hatchery troughs. Three baskets made with 0.25-inch PVC-coated hardware cloth contain the spawns and are held in place by the frame. The baskets have cross-partitions to evenly distribute the egg masses within the baskets, and hinged lids to hold the spawns in place during operation. Agitation is accomplished by raising and lowering the frame up and down through the water. A prototype of the new incubator underwent preliminary testing during the 2007 and 2008 spawning seasons. The first trial (2007) determined the appropriate cycle interval to be approximately 10 seconds. In the second trial (2008) the See-Saw was tested with twice the egg density as is recommended. Although a thoroughly replicated comparison with standard incubators was not conducted, the See-Saw operated flawlessly. Those preliminary studies were published and describe the construction and operation of the prototype incubator in more detail. With the initiation of this SRAC project, a non-funded cooperative agreement was initiated with Needmore Fisheries LLC, Glen Allen, Mississippi, to more thoroughly compare the See-Saw with conventional paddle-type incubators and to test and quantify several operational parameters. All studies reported here were conducted at that commercial hatchery using experimental incubators fabricated and operated by the hatchery employees.

Results at a glance...

- *A novel catfish egg incubator has been designed and tested on two commercial farms. More eggs can be incubated using less water exchange than with conventional incubators, while achieving increased survival to swim-out stage.*

Most of the first year (2009 spawning season) was used to design the system, purchase motors and material for fabrication, and preliminary stress-testing of the system without live eggs. Near the end of the spawning season the first comparative trial was conducted. Pairs of troughs (one control and one See-Saw, with four troughs for each treatment) were loaded with approximately 26 egg masses per trough (approximately 475,000 and 473,000 eggs per trough, respectively). Water quality was measured in the

water supply and in each trough daily. Sac fry were measured volumetrically and sub-sampled to determine total number, then transferred to rearing troughs. When the fry reached swim-up stage, they were measured volumetrically and sampled to determine total number before transfer to rearing ponds. Survival to swim-up stage averaged 54% in the See-Saw and 23% for the control troughs, a 2.3-fold difference.

In Year 2 of the project (2010 spawning season) we measured the effect of egg loading density in See-Saw incubators on survival to hatch and swim-up. Further comparisons with the paddle-type incubators were not conducted. We loaded See-Saws (five troughs for each treatment) with approximately 15 lbs (220,000 eggs), 30 lbs (447,000 eggs), 45 lbs (670,000 eggs), and 60 lbs (893,000 eggs) of spawns. Water flow into the troughs averaged 2.1 gallons/

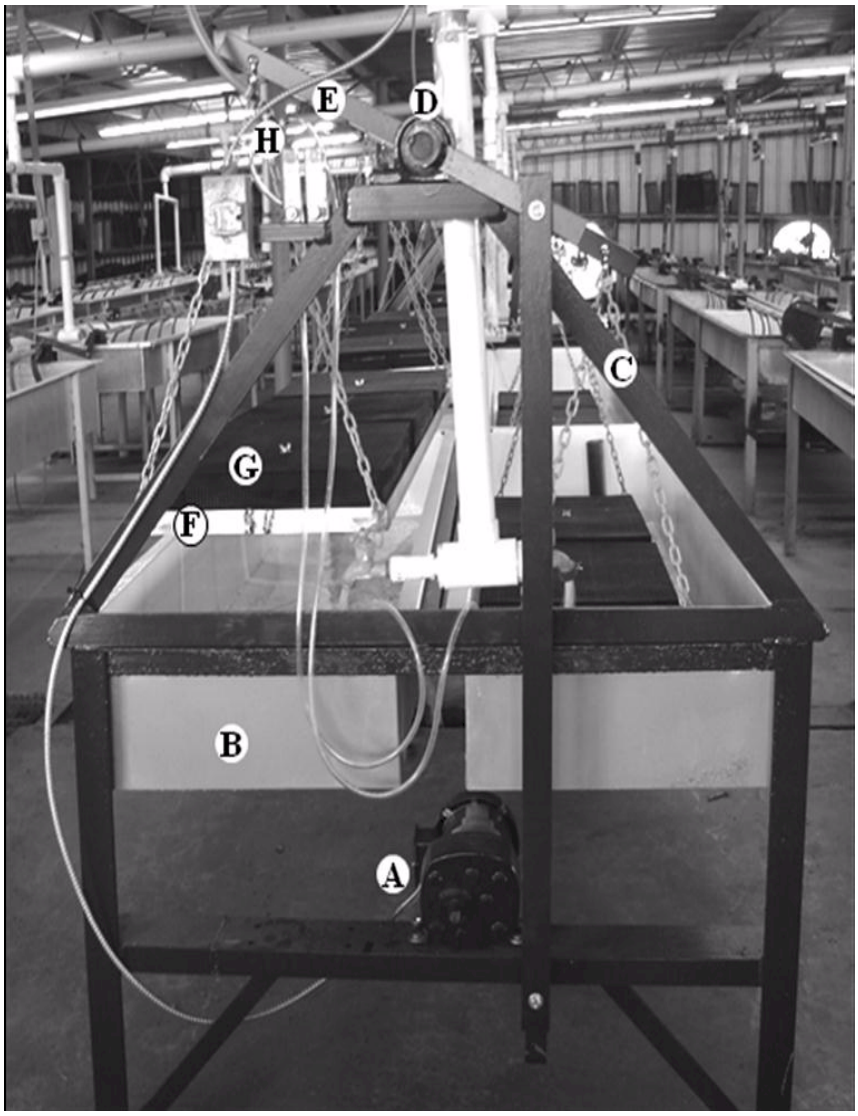

Figure 4. See-Saw incubator prior to loading eggs. Note that as the left rack is up in the air, the right rack is down in the water. Each rack contains three hatching baskets that are secured to the rack. The water supply for these two troughs is in the foreground and the drain is at the far end in each trough. The following components are labeled: (A) 6 rpm motor , (B) hatching trough, (C) angle-aluminum frame supporting the See-Saw, (D) steel shaft running the length of the troughs, (E) crossbars, (F) angle-aluminum rack that holds the baskets, (G) hatching baskets, and (H) oxygen supply used in Year 3 of this study.

minute, roughly half of the rate recommended for commercial hatcheries. The 15, 30, and 45 pound troughs produced an average of 132,700, 263,800, and 429,400 swim-up fry (survivals from egg of 60, 59, 64%, respectively, similar to values reported in commercial hatcheries). However, the 60-pound treatment produced only 417,200 swim-up fry (survival of 46%). The results of this year's study indicate that both hatchery space and water use would be maximized with See-Saw incubators loaded at the 45-pound rate.

In 2011 (Year 3 of the project) we examined the effect of oxygen supplementation on troughs loaded with 45 lbs of eggs. Fifteen troughs were incubated using no oxygen supplementation and had a mean oxygen saturation of 82.4%; 17 troughs were incubated using additional oxygen added through ceramic diffusers at an average rate of 0.12 liters/min resulting in an average oxygen saturation of 124.1%. Mean swim-up fry production overall was 462,363 fry/trough (10,327 fry/pound of eggs), for a survival from egg to swim-up of 71.2%. There were no significant differences between treatments, confirming that 45 lbs of eggs can be incubated per See-Saw trough without additional oxygen if the hatchery water supply is near air saturation.

We believe that even higher loading densities could be incubated using supplemental oxygen with no impact on hatch rate or survival to swim-up stage. Even without a pure oxygen supplement, the See-Saw incubator can incubate 3 to 4 times as many eggs as traditional paddle-type incubators using half the water, a tremendous savings in both floor space and energy use.

The use of this incubator across the commercial industry would result in considerable savings, particularly for those hatcheries that need to heat their well water. This incubator may have even greater application in the numerous state and federal hatcheries which are tasked with hatching a growing number of fish species. The See-Saw can reduce both the space and water flow needed to meet their channel catfish production quota, making those resources available for other priority species.

IMPACTS

Atlantic croaker display asynchronous spawning during a prolonged spawning season, which limits the potential to reproduce this species on a scale capable of sustaining commercial culture. This project has developed reliable hatchery methods to induce and synchronize Atlantic croaker spawning for production creating a new market for farm-raised marine baitfish. These methods could be implemented immediately at several hatcheries in the southern United States. At least two commercial redfish production facilities in Texas have acquired Atlantic croaker broodstock and started the first attempts at commercial production.

Delayed spawning combined with aqueous Ovaprim® injections results in successful spawning of Atlantic croaker out-of-season for year round production. This is a major breakthrough for a marine baitfish industry that relies upon the availability of specific sizes of bait throughout the year. Atlantic croaker is a high value marine baitfish species that can retail for more than $1 for a 2 to 5 inch fish. Current markets for this species rely upon wild-captured juveniles. During the off-season (April to September) Atlantic croaker are subject to limited availability which increases demand and price significantly. This project has provided a means to produce ideal-sized baitfish year round to meet consumer demand and create new markets.

This project has produced the first information on the dietary requirements for Atlantic croaker

broodstock in order to improve reproductive performance. While fish oil could not completely be eliminated from the diets to improve sustainability and reduce costs, production was improved by using lower inclusion rates (6% fish oil) than in diets previously used for Atlantic croaker. This will increase profitability over using higher lipid diets while still making moderate advances toward sustainability of the fish feed. The fish oil diets provide good basal diets for producers wanting to undertake Atlantic croaker production immediately while meeting or exceeding the reproductive performance of wild fish.

Over 100 million catfish eggs have been incubated thus far in on-farm trials. Next spring at least 16 four-trough see-saw units will be in commercial operation. Publication of blueprints and assembly instructions is planned. To speed transfer of the technology, a collaborating farmer is considering the manufacture and sale of single four-trough units so potential users can both test the unit in their hatchery and have a physical model to guide fabrication of additional units.

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