DEVELOPMENT OF BAITFISH, GOLDFISH, AND ORNAMENTAL FISH HATCHERY METHODS

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PROJECT OBJECTIVES

1. Develop goldfish and ornamental fish hatchery methods that result in cost-effective methods to de-stick adhered goldfish and ballyhoo eggs from spawning substrate within 24 hours of deposition.

    a. Determine the composition of the egg matrix of goldfish and ballyhoo.
    b. Identify the compounds that will de-stick goldfish and ballyhoo eggs from spawning substrate.
    c. Perform embryo viability and hatch assays to determine toxicity thresholds for selected de-sticking agents.

2. Develop baitfish hatchery methods that result in cost-effective methods to maximize egg collection within 1 to 2 days of fertilization of fathead minnows.

    a. Determine the optimal nest site to male fathead minnow ratio that will maximize egg number and collection efficiency (egg per unit of substrate).
    b. Determine the effect of pond water temperature, dissolved oxygen, and depth on fathead minnow egg production in earthen ponds.

ANTICIPATED BENEFITS

Removal of eggs from spawning substrate will enable producers to hatch eggs in a controlled environment. They will be able to obtain more accurate counts of the number of fry that are stocked into ponds. Increased production costs have severely affected the profitability of bait, feeder and forage fish farming. New hatchery methods have the potential to reduce production costs and keep farms profitable.

PROGRESS AND PRINCIPAL ACCOMPLISHMENTS

Objective 1. *Develop goldfish and ornamental fish hatchery methods that result in cost-effective methods to de-stick adhered goldfish and ballyhoo eggs from spawning substrate within 24 hours of deposition.*

Subobjective 1. *To determine the composition of the egg matrix of goldfish and ballyhoo.*

Louisiana State University

Glycoproteins are large molecules composed of proteins and sugars that are attached to the outer membranes of fish eggs. Glycoproteins are also responsible for the "sticky" nature of eggs developed after fertilization, allowing eggs to adhere to a substrate and preventing river and tidal currents from dislodging eggs (Riehl and Patzner 1998). In aquaculture settings, adhesive eggs prevent a speedy harvest of eggs and increase labor. The purpose of this sub-objective is to characterize the nature of goldfish (*Cyprinus carpio*) and ballyhoo (*Hemiramphus brasiliensis*) egg membrane glycoproteins to better identify appropriate de-sticking compounds.

Goldfish eggs were received from the University of Arkansas at Pine Bluff (UAPB). Isolation of glycoproteins was first attempted using a protocol derived from Mansour et al. 2009. The protocol was created with the assistance of Dr. Ted Gauthier and Tamara Chouljenko of the Louisiana State University (LSU) H.D. Wilson Laboratories Protein Center. The protocol called for removal of the egg membrane using forceps in an isotonic saline solution seated in ice. Egg membranes were then ground in a container that was seated in ice to release the glycoproteins. The saline solution was removed and frozen at -4 degrees F for later analysis and the ground egg membranes were transferred to a new container. The egg membranes were then incubated in a urea and bicarbonate solution for six hours at 39 degrees F. The solution was centrifuged to pellet the egg membranes and the supernatant was tested using SDS-PAGE gel electrophoresis for the presence of proteins. Low concentrations of proteins were detected and were determined insufficient to proceed with glycoprotein staining. A new protocol was developed from Scapigliati et al. 1995 with the assistance of Dr. Gauthier and Mrs. Chouljenko. The new protocol called for the chorion removal over ice and 15 minute incubation in a solution containing Tris-HCl buffer, NaCl, EDTA, PMSF, and Triton X-100 at 39 degrees F. The egg membranes were transferred to a solution similar to the one listed previously, but with added ßME, where it was homogenized. The mixture was centrifuged to pellet the chorions and the supernatant was collected.

The Mansour protocol produced four protein bands at the highest concentration of 20 µL of sample (Fig. 1, lanes 1-3). Bands were detected at approximately 125, 105, 70, and 22 kDa. Lanes 5-7 (Fig 1.) represent a lower concentration of 5 µL of sample. Lanes 8-10 (Fig. 1) represent the saline solution in which the egg membranes were homogenized. The fourth lane (Fig. 1) represents the Precision Plus Protein Standard from Biorad. Proteins isolated using the Scapigliati protocol have been measured at total protein concentrations (mean ± SD) of 1,775 ± 62.21, 454.12 ± 41.37, and 1,426.22 ± 573.87 mg/L. These samples have been submitted to the H. D. Wilson protein lab for electrophoresis analysis and glycoprotein staining.

With future processed samples the resulting bands from SDS-PAGE will be excised and purified using C-18 columns. These glycoproteins will then be analyzed for composition using mass spectroscopy.

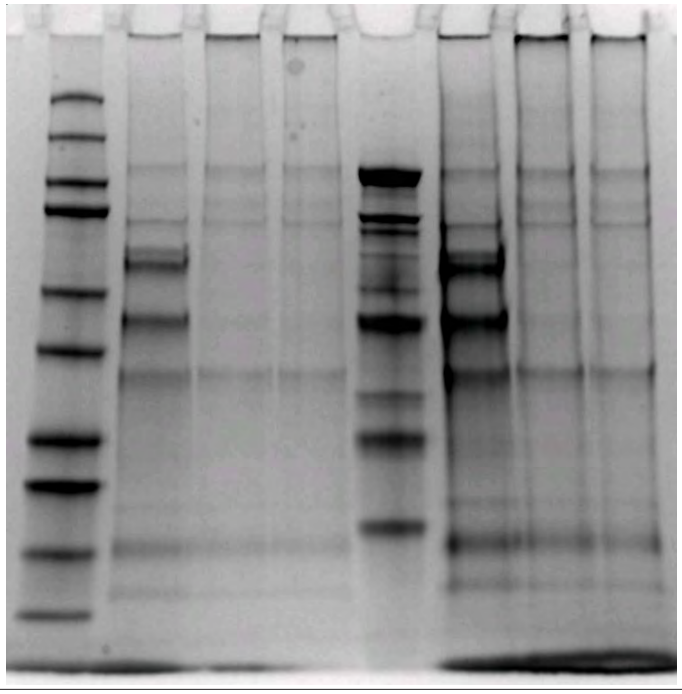

Figure 1. SDS-PAGE electrophoresis of protein samples derived from the Mansour protocol.

The current and future results of this portion of the project are ongoing and will develop as we continue to extract and characterize these proteins.

We received frozen ballyhoo embryos from Florida State University in September 2011, however, did not initially extract chorion proteins as we had to modify our extraction protocols from Mansour et al. (2009, Animal Reproduction Science 114:256-268) to Scapigliati et al. (1995, Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology 112:169-175). The Scapigliati protocol resulted in greater amounts of egg shell proteins when compared to the Mansour method and as a result we continued protein extractions with the Scapigliati protocol. We utilized Gulf killifish egg shells as surrogate for method development due to the availability of eggs at our home institution.

Glycoprotein isolation from extracted egg shell proteins was performed using a Thermo Scientific Pierce Glycoprotein Isolation Kit (model # 89804). This method uses lectins con canavilin to bind ligands with specific classes of glycoprotiens from a sample with several types of proteins. After several extractions with this kit and modifying the extraction protocol, the use of this isolation method was discontinued due to a significant loss of glycoprotein in comparison to glycoprotein staining. Glycoprotein staining was used in subsequent examinations to prepare and isolate for mass spectrometry.

The first large band from the egg shell protein samples was extracted and purified in December 2011. This protein was submitted for mass spectroscopy and subsequent comparison analysis against a protein library. To accomplish this we used Mascot, search engine software that uses mass spectrometry data to identify proteins from sequence databases. This software was developed by Matrix Science Ltd. and is available on: (*www.matrixscience.com*). The two databases used for this search were SwissProt and the National Center for Biotechnology Information. Mascot software indicated that the

first band in our gel had an 84% similarity to vitellogenin, a yolk precursor protein from *Fundulus heterclitus*. This is a sister species to the Gulf killifish and indicates that our first protein band was contamination from the yolk or embryo and not a protein that originates in the chorion.

New batches of koi and Gulf killifish egg shells were extracted using the Scapigliati protocol in the spring and summer of 2012 yield low and/or no proteins on gels. An updated protocol was obtained from Scapigliati. The updated protocol utilizes an S-TNE buffer with Urea rather than the previous Triton X-100, which is more efficient at extracting a greater number of proteins in comparison to Triton. In order to increase the quality of this initial egg shell homogenization and protein extraction method we investigated several urea concentrations and homogenization methods.

The egg shell proteins extracted using 3M urea were different in molecular weight when compared to samples from the previous year. Figure 1a shows lane 1 as a molecular marker, lane 2 as the proteins from a 3M urea extraction, and lane 3 as proteins extracted from the previous year. When stained for glycoproteins the new samples did not indicate that any of the extracted proteins where glycoproteins in comparison to previously extracted samples. It is possible that the previous protein samples were contaminated with remnants of yolk proteins or embryos as seen in the mass spectroscopy results.

Work on protein extraction and isolation continues in samples of both goldfish and killifish egg shells with a majority of the work centered on protocol refinement for glycoprotein isolation. We have ballyhoo eggs frozen for future use, but due to the small numbers of eggs we plan to work with these egg shells only after the goldfish and killifish egg glycoproteins are successfully extracted and characterized.

University of Arkansas at Pine Bluff

Newly laid goldfish eggs were manually removed from spawning substrate using tweezers. Eggs shells were removed and glycoproteins were extracted following the protocol developed by the LSU researchers. Extracted glycoproteins were sent to LSU for isolation and identification.

Subobjective 2. *Identify the compounds that will de-stick goldfish and ballyhoo eggs from spawning substrate.*

University of Arkansas at Pine Bluff

The inability of producers to remove goldfish eggs from spawning substrate does not allow for the producer to accurately count the number of fry that are stocked into a pond. Mats are currently placed into ponds where eggs hatch. The number of eggs per mat is usually a guess and the number of fry hatched and produced in the pond is often an inaccurate estimate of the true population. De-sticking eggs from spawning material is also a bottleneck for the commercial efficiency for ballyhoo.

Another bottleneck with ballyhoo is the hatching rates of the eggs have been low. Removal of ballyhoo eggs from the spawning substrate would enable producers to efficiently collect and hatch eggs. By hatching eggs in a controlled environment, hatching rates in goldfish and ballyhoo may also be increased.

Spawning mats containing freshly laid (<12 h) goldfish eggs were obtained from a local fish farm.

Mats were cut into 3 × 3 inch squares and placed into 1 pint beakers containing one of the following concentrations of a selected solution: sodium sulfite, 1.5%, 2.0%, and 3.0%; tannic acid 75%, and urea 3%, 4%, 6%, and 8%; control in hatchery water; fresh squeezed pineapple juice 1%, 3%, 5%, and 10%; bromelain 1%, 3%, 5%, and 10%; papaya 1%, 3%, 5%, and 10%; papain 1%, 3%, 5%, and 10%; 4M urea; lithium chloride 1 mg/L, 3 mg/L, 5 mg/L, and 10 mg/L; cadavarine 1 mg/L, 3 mg/L, and 5 mg/L; lysozyme; acetone; sodium bicarbonate; sodium hydroxide; ammonium chloride; ethylene glycol mono-butyl ether; propolyene glycol n-butyl ether; citric acid; and alcalase, 20 mL/L, 40 mL/L, and 80 mL/L. Eggs were placed into treatment solutions for 2 minutes and removed. Loose eggs and eggs still attached to the substrate were counted and the percentage of eggs removed was calculated. The eggs were then placed into a tank and allowed to hatch. Newly hatched fry were counted and the percent survival was calculated.

Significant removal of eggs was only accomplished with the alcalase enzyme. The 20 mL/L alcalase treatment removed 10% of the eggs from the mat, the 40 mL/L treatment removed 78% and the 80 mL/L treatment removed 82% of the eggs in 2 minutes. To increase the number of eggs removed the alcalase was mixed in a 3% salt solution. Removal of eggs exposed to 20 mL/L alcalase with 3% salt was 36%, for 40 mL/L was 99%, and for the 80 mL/L was 100%.

Survival of the eggs to hatch was low with less than 2% surviving. This may have been due to the fact that the eggs were not placed into hatching jars but laid in a single layer on the bottom of an aquarium. Eggs did succumb to fungal infections. Once the egg membrane compounds are identified, other potential compounds will be tested and eggs will be hatched in a hatching jar.

Since the matrix of the egg shells is still unknown, we tested different types of spawning substrate to determine if eggs could be removed more easily from a smooth substrate versus the grass-shaped spawning mats typically used. Spawning mats were constructed of geoweb material, strips of which were connected to make a 2 ft × 3 ft mat, and from corrugated PVC board that was weighted down with wire frames. Spawning mats were placed into commercial goldfish spawning vats and left overnight. Spawning mats containing freshly laid (<12 h) goldfish eggs were cut into 3 × 3 inch squares and placed into 1 pint beakers containing one of the following concentrations of a selected solution: sodium sulfite, 1.5%, 2.0%, and 3.0%; tannic acid 75%, and urea 3%, 4%, 6%, and 8%; control in hatchery water; fresh squeezed pineapple juice 1%, 3%, 5%, and 10%; bromelain 1%, 3%, 5%, and 10%; papaya 1%, 3%, 5%, and 10%; papain 1%, 3%, 5%, and 10%; 4M urea; lithium chloride 1 mg/L, 3 mg/L, 5 mg/L, and 10 mg/L; cadavarine 1 mg/L, 3 mg/L, and 5 mg/L; lysozyme; acetone; sodium bicarbonate; sodium hydroxide; ammonium chloride; ethylene glycol mono-butyl ether; propolyene glycol n-butyl ether; citric acid; and alcalase, 20 mL/L, 40 mL/L, and 80 mL/L. Eggs were placed into treatment solutions for 2 minutes and removed. Loose eggs and eggs still attached to the substrate were counted and the percentage of eggs removed was calculated. The eggs were then placed into a tank and allowed to hatch. Newly hatched fry were counted and the percent survival was calculated.

No significant removal of eggs was accomplished with any of the treatments tested.

University of Florida

Ballyhoo were collected from the wild and held in quarantine for 21 days. They were treated with five one hour formalin baths (100 mg/L) on alternating days and were fed mysid shrimp soaked in an antibiotic for 10 days. Thereafter, they were fed a combination of mysid shrimp, krill, and pelleted feed.

Ballyhoo maintained in tanks readily spawned onto substrate constructed of PVC and plastic zip ties during late May and early June. However, only small batches of eggs were spawned daily. Three compounds at three concentrations were exposed to egg samples in triplicate. These included sodium sulfite 1.5, 3.0, 6.0%; trypsin 0.05%, 0.25%, 0.5%; and alcalase 20, 40, 80 mL/L of water each for 15 minutes each. Additionally, samples of unexposed eggs were collected to determine the composition of the egg membrane and associated microfibrils which serve to adhere the eggs to substrate.

None of these compounds has been successful at the tested concentrations and exposure time in breaking up the microfibrils which adhere the eggs to substrate and to each other. Continued evaluation of other concentrations, exposure times, and compounds will be conducted when more eggs are naturally spawned. All eggs including those unexposed, and exposed to the various chemicals are stored in a -112 degrees F freezer. The composition of the egg matrix of ballyhoo eggs will be identified in the next few months.

Adult ballyhoo were collected from the wild and held in quarantine for 21 days. They were treated with five one hour formalin baths (100 mg/L) on alternating days and were fed mysid shrimp soaked in an antibiotic for 10 days. Thereafter, they were fed a combination of mysid shimp, krill, and pelleted feed.

Two populations of ballyhoo were maintained in tanks for one year. Ballyhoo spawned repeatedly onto substrate provided in the tanks which was constructed of PVC and plastic zip ties. Eggs were gently removed from the substrate. Egg masses consisting of 25 to 100 eggs were added to beakers containing solutions of saltwater and one of three compounds each at various concentrations. Each combination of compound and concentration was replicated three to five times. A small stir bar was added to the beakers to gently move the egg masses. Treatment compounds and concentrations included sodium sulfite 1.5%, 2.0%, 3.5%, sodium sulfite 1.5%, 3.0%, 6.0%; trypsin 0.05%, 0.25%, 0.5%; trypsin 1.0%, 1.5%, 2.0%, and alcalase 20, 40, 80 mL/L of water, each with an exposure time of 15 minutes. Additionally, samples of unexposed eggs were collected to determine the composition of the chorion and associated microfibrils which serve to adhere the eggs to substrate and to each other.

None of these compounds has been successful at the tested concentrations and exposure time in breaking up the microfibrils which adhere the eggs to substrate and to each other. Additional evaluation of different concentrations, exposure times, and compounds is warranted.

All eggs including those unexposed and exposed to the various compounds and concentrations were stored in a -112 degrees F freezer until shipped on dry ice to LSU for analyses of composition and change in composition following exposure to the various compounds.

Subobjective 3. *Perform embryo viability and hatch assays to determine toxicity thresholds for selected de-sticking agents.*

This objective will be completed once the egg membrane compounds have been identified.

Objective 2. *Develop baitfish hatchery methods that result in cost-effective methods to maximize egg collection within 1-2 days of fertilization of fathead minnows.*

Subobjective 2a. *Develop baitfish hatchery methods that result in cost-effective methods to maximize egg collection within 1-2 days of fertilization of fathead minnows.*

University of Arkansas at Pine Bluff

A new hatchery method for fathead minnow is based on the collection of eggs from brood ponds for indoor hatching. Egg collection is costly, given the required spawning substrate and labor. The purpose of this study was to improve the efficiency of egg collection by evaluating resulting egg production from four different ratios of substrate area to male fish.

Approximately 121 rosy red fathead minnows (200 g total per pool; average weight per fish of 1.65 g) were stocked into each of 16, 35.5 ft^2, 3-ft deep, outdoor plastic pools in June 2011. A male:female sex ratio of 1:2 was determined by visual inspection of 100 fish. Treatments consisted of 1, 2, 3, or 4 sections of 3 in-wide textured plastic geoweb material (Envirogrid® EGA20), each with an underside surface area of ~ 4.4 ft^2, resulting in substrate area to male fathead minnow ratios of 0.01, 0.02, 0.03, and 0.04. Water from a reservoir replenished by a shallow well was added to each pool at a rate of 6 L/min, and constant aeration was provided using a low pressure blower. Fish were fed once daily at 3% of initial body weight with extruded commercial catfish feed (32% protein). Temperature was recorded every four hours and dissolved oxygen concentration and mortalities were recorded twice daily. The number of nests and eggs were determined every two to three days and eggs were removed with 1.5% sodium sulfite. Eggs were collected a total of eight times. Mean temperature was 85 degrees F, close to the upper limit for reproduction. Less than half of the males were actively nesting at any one time and there was no difference in the number of nests collected between treatments (Table 1).

Table 1. Mean (SEM) number of nests for each collection event, mean (SEM) number of eggs per male per day, and number of eggs per kg of fish per day. Means within a row with different letters were significantly different (P < 0.05).

	Substrate Area (m2): Male			
	0.01	0.02	0.03	0.04
Mean (SEM) nests/collection	9.8 ± 4.3	9.7 ± 3.7	10.9 ± 3.2	9.2 ± 4.1
Mean (SEM) eggs·male-1·day-1	31 ± 23ab	37 ± 25a	36 ± 17ab	26 ± 16b
Mean eggs·kg fish-1·day-1	6,115ab	7,366a	7,130ab	5,231b

Fathead minnows are traditionally propagated using the spawning-rearing pond method, where brood fish are stocked, spawning substrate is added, and resulting young are reared together with the adults. Unfortunately, using this method, diseases are passed from adults to the young, there is little control over fish density, and undesirable fish such as mosquitofish may be transferred together with brooders. A new hatchery method to overcome the limitations of the spawning-rearing pond method involves collection of fathead minnow eggs from brood ponds, indoor hatching of eggs, and stocking of resulting fry at known densities. However, spawning substrate and labor for egg collection are costly. A research study was designed to compare the numbers of fathead minnow eggs that could be collected when brood fish were supplied with differing amounts of spawning substrate. If the quantity of supplied substrate can be reduced without decreasing egg numbers, this will reduce the costs of this method.

Equal weights (0.44 lb) of rosy red fathead minnow brooders (3.6 lbs per 1,000) were stocked into each of 16, 800-gallon outdoor pools. The sex ratio of the broodfish was 2 females to 1 male. As male fathead minnows use the undersides of submerged materials as nest sites, sections of 3-inch deep textured plastic geoweb material (Envirogrid® EGA20) were supplied as spawning substrate. Geoweb material was added to pools at four different rates (four replicates per treatment); 13, 26, 40, or 53 inches of under-surface area of substrate per male fathead minnow. Water from a reservoir replenished by shallow wells was added to each pool at a rate of 1.6 gpm, and constant aeration was provided using a low pressure blower. Fish were fed once daily at 3% of initial body weight with extruded commercial catfish feed (32% protein). Temperature was recorded every four hours and dissolved oxygen concentration and mortalities were recorded twice daily. The number of nests and eggs on spawning substrates were determined every two to three days and eggs were removed with 1.5% sodium sulfite. Eggs were collected a total of eight times. Average water temperature was 85 degrees F, close to the upper limit for reproduction.

Total egg numbers (estimated from egg volume) per collection event did not differ among the three lowest substrate area to male ratios. Egg numbers for the treatment with 53 square inches of substrate per male was less than for the middle two rates, and equal to that of 13 square inches of substrate per male. Based on nest numbers, less than half of the males were actively nesting at any one time.

Providing too little spawning substrate for fathead minnows encourages males to select other surfaces in a brood pond as nesting sites, such as sticks, vegetation, and aerators, and would reduce egg numbers that could be collected. Providing too much substrate increases costs. Results from this study show that providing only 13 square inches of spawning substrate per male fathead minnow did not reduce egg numbers that could be collected, and suggest that even lower amounts of substrate area could be supplied with no decrease in egg numbers.

Subobjective 2b. *To determine the effect of pond water temperature, dissolved oxygen, and depth on fathead minnow egg production in earthen ponds.*

University of Arkansas at Pine Bluff

Placing spawning substrates within brood ponds in favorable locations for egg deposition should improve egg harvest and reduce the cost per million fry. This study was designed to examine nest location and egg number on spawning substrates in relation to depth, temperature and early morning dissolved oxygen (DO).

Two 0.04-ac earthen ponds were fertilized and each stocked with 46.85 lbs (1.9 ± 0.6 g/fish) of adult rosy red fathead minnows with a visually determined male:female sex ratio of 7:10. The fish were fed once daily to satiation with extruded commercial catfish feed (32% protein). Spawning substrate consisted of three, 11-ft^2 sections of 3 in-wide Envirogrid® EGA20 geocell per pond, each suspended between a floating pipe at the surface and a weighed pipe at a depth of 3.3 ft.

Thermographs recorded temperature every 4 h at 10-in intervals from the surface to a depth of 3.3 ft, and DO was recorded at 10-cm intervals every morning. The number of nests and eggs were determined every two days and eggs were removed with 1.5% sodium sulfite and collected nine times. Temperature, DO and other water quality parameters were similar in both ponds and within acceptable ranges for this species. Cumulatively, the number of eggs was greatest on rows 2 and 4. Depth, temperature, DO, and date all had interactive effects on the number of nests per day and the total number of eggs produced per day. Data analysis is continuing.

For a new hatchery method for fathead minnows to be cost-effective, egg collection efficiency should be optimized. Placing fathead minnow spawning substrates within brood ponds in favorable locations for egg deposition should improve egg harvest and reduce the cost per million fry. This study was designed to examine nest location and egg number on spawning substrates in relation to depth, temperature and early morning dissolved oxygen (DO).

Two, 0.1-acre earthen ponds were fertilized and each stocked with 46.8 lbs of 4.2 lbs per 1,000 adult rosy red fathead minnows. The sex ratio was 7 males to 10 females. Fish were fed once daily to satiation with extruded commercial catfish feed (32% protein). Three, 10.8 square foot sections of 3-inch-deep Envirogrid® EGA20 geoweb were placed into each pond as spawning substrate. Each honeycomb-like section of geoweb was suspended between a floating pipe at the surface and a weighed pipe at a depth of 3.3 ft. Thermographs recorded temperature every 4 hours at 10-inch intervals from the surface to a depth of 3.3 ft, and DO was recorded at 10-inch intervals every morning. The number of nests and eggs at 5-inch depth intervals (corresponding to each set of horizontal cells of the geoweb material) on each substrate were determined every two days for a total of nine collection events. Eggs were removed with 1.5% sodium sulfite.

Fathead minnow egg production peaked at the third collection event (May 16), and then declined sharply, even though water temperatures at this time were similar to initial conditions in the ponds. Differences in temperature with depth were found in both ponds. In general, water temperature declined linearly with depth and was 9 degrees F cooler at a depth of 3.3 feet as compared to the surface. Overall, the greatest numbers of eggs were collected at depths of 10 and 20 inches. Fewer eggs were found at depths below 30 inches, except for collections on May 16 and 18, when relatively greater numbers of eggs were found at the bottom of substrates. On May 14 -16, a cold front caused surface waters of the ponds to be up to 7 to 8 degrees F colder than at 3.3 feet, albeit for a relatively short period, and this temperature inversion apparently caused fish to seek out this deeper, warmer water for nest sites. Early morning dissolved oxygen levels were also lower with depth, with typical early morning concentrations in the range of 0.1 to 0.2 mg/L at 3.3 feet. Despite these low levels, eggs were found at the bottom of the substrates early in the study, when egg production was greater overall. We observed that the ends of the substrates were favored as nest sites, suggesting that narrow vertical sections of geoweb might be best.

Results from this study confirmed that fathead minnows will spawn over a broad range of

temperatures, minimum dissolved oxygen levels, and water depths. No specific favorable locations (conditions) for substrate placement could be identified. As such, substrates can be placed in locations that are convenient for workers to collect.

IMPACTS

Identification of goldfish and ballyhoo egg membrane compounds will aid in the identification of substances that will break the adhesive bond between the egg and spawning substrate.

Desticking goldfish and ballyhoo eggs will improve production of goldfish and ballyhoo by enabling producers to utilize a smaller hatchery space. Current costs to run an indoor goldfish hatchery could save 90% of the costs per day.

PUBLICATIONS, MANUSCRIPTS OR PAPERS PRESENTED

Publications in Print

Weldon, D. B., N. Stone, and J. Sun. 2012. Effect of spawning substrate to male ratio on fathead minnow egg production. North American Journal of Aquaculture 74:419–423.

Stone, N., and D. Weldon. 2012. Potential fathead minnow spawning substrate for indoor egg incubation. Arkansas Aquafarming 29(1):1-2.

Presentations

Kelly, A. M., S, Kumaran, and N. Stone. 2011. A potential method of desticking goldfish eggs from spawning mats. Aquaculture America, San Diego, California.

Kelly, A. M., S, Kumaran, and N. Stone. A potential method of desticking goldfish eggs from spawning mats. Poster presentation. Annual Meeting of the Arkansas Bait and Ornamental Fish Growers Association. Lonoke, Arkansas.

Stone, N. 2012. The latest in new baitfish technology. UAPB Rural Life Conference, Pine Bluff, Arkansas.

Sun, J., C. Lee, and N. Stone. 2012. Effects of egg mimic number and sphere diameter on fathead minnow egg production. Poster presentation, Aquaculture America 2012, U.S. Aquaculture Society, Las Vegas, Nevada.

Sun, J., C. Lee, and N. Stone. 2012. Effects of egg mimic number and sphere diameter on fathead minnow egg production. Poster presentation, UAPB Aquaculture/Fisheries Field Day, Pine Bluff, Arkansas.

Weldon, D. B., and N. Stone. 2012. Effect of removal from substrate on fathead minnow egg hatching success. Poster presentation, UAPB Aquaculture/Fisheries Field Day, Pine Bluff, Arkansas.

Weldon, D. B., N. Stone, and J. Sun. 2012. Effect of spawning substrate to male ratio on fathead minnow *Pimephales promelas* egg production. Aquaculture America 2012, U.S. Aquaculture Society, Las Vegas, Nevada.

Weldon, D. B., N. Stone, J. Sun, and L. Xie. 2012. Effect of depth on fathead minnow *Pimephales promelas* egg production. Poster presentation, Aquaculture America 2012, U.S. Aquaculture Society, Las Vegas, Nevada.

Weldon, D. B., N. Stone, J. Sun, and L. Xie. 2012. Effect of temperature and dissolved oxygen on fathead minnow *Pimephales promelas* egg production in ponds. Poster presentation, Lonoke Aquaculture Workshop, Lonoke, Arkansas.

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