



# **Producing Hybrid Catfish Fry:**

## **Workshop Manual**

**USDA – ARS Catfish  
Genetics Research Unit**

and the

**Mississippi State University  
National Warmwater  
Aquaculture Center**

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# Acknowledgments

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The workshop manual is organized into sections summarizing established techniques for hormone-induced spawning of catfish and highlights factors the authors consider important for production of hybrid catfish fry. Topic sections include text information, literature citations, a vendor/supplier list, forms, and copies of slide presentations used to highlight important points. Photographs have been included to illustrate important points and tables summarize important information. Supplementary materials in the form of SRAC publications have also been included.

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- # 190 - Production of Hybrid Catfish
- SRAC 17<sup>th</sup> Annual Progress Report 'Improving Reproductive Efficiency to Produce Channel x Blue Hybrid Catfish Fry' pp 61-76

# Introduction

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## Production of Eggs and Fingerlings

Production of hybrids between the channel catfish (*Ictalurus punctatus*) and the blue catfish (*Ictalurus furcatus*) was reported as early as 1966 (Giudice). Research to develop and refine methods for producing hybrids and to evaluate their performance for economically important traits has continued until present. Although researchers from several state and federal agencies have conducted research with hybrids, much of the research on hybrids has been done at Auburn University. The hybrid generally performs better than either parent species for several important production traits including survival, growth, disease resistance, and carcass yield (Giudice 1966, Yant et al. 1975, Dunham et al. 1983, Dunham et al. 1987, Dunham et al. 1990, Ramboux 1990, Wolters et al. 1996, Dunham and Argue 1998, Dunham and Brummett 1999, Chatakondi et al. 2000, Bosworth et al. 2004, Li et al. 2004). The hybrid produced by crossing a female channel catfish and blue male catfish is the mostly commonly produced hybrid and tends to be easier to produce and performs better than the blue catfish female x channel catfish male hybrid (Dunham et al. 1982). Therefore, throughout this manual the term hybrid refers to the channel catfish female x blue catfish male hybrid.

The primary constraint to commercial production of the hybrid has been the lack of reliable, cost-effective methods for producing large quantities of fry needed for commercial catfish farming. However, continued problems with production of channel catfish, primarily related to diseases, coupled with refinements of techniques for producing hybrids have spurred renewed interest in use of hybrids for commercial production.

Traditional pond-spawning, which is effectively used to produce channel catfish fry, is ineffective for consistent, large-scale production of hybrid fry because of reproductive barriers (behavioral, physiological etc.) that prevent spawning between blue and channel catfish. Although there have been reports of successful spawning in brood ponds stocked with male blue catfish and female channel catfish, the occurrence of pond-spawning is very rare and not reliable for commercial hatchery production. Unless some future development dramatically improves the spawning rate in traditional brood ponds, production of hybrid fry will depend on the use of hormones to induce ovulation (final maturation and release of eggs) in females, manual 'stripping' of eggs, and manual fertilization of the eggs with blue catfish sperm (Tave and Smitherman 1982, Dunham et al. 2000).

Hormone-induced spawning is a commonly used technique for production of fry of many fish species and a variety of compounds (common carp pituitary extract - CCP, lutenizing hormone releasing hormone analog – LHRHa, human chorionic gonadotropin) have been successfully used for inducing ovulation in catfish. These compounds initiate the chain of events that lead to ovulation and release of eggs. The two most commonly used, and presumably most effective, compounds for inducing ovulation in channel catfish are CCP and LHRHa. Therefore the methods section of this manual will focus on the use of these two compounds.

## **Considerations in Using Hybrids in Growout Ponds**

While the hybrid has several advantages in disease resistance, growth, and ease of catch, experienced hybrid producers report that there will have to be adjustments to the typical channel catfish production system to successfully raise hybrids. Due to the smaller size of the hybrid's head compared to a channel catfish, harvesting can be difficult if traditional mesh sizes are used (especially when using grading nets with larger than 1-inch mesh). Release of gilled fish often results in subsequent *Columnaris* infections, therefore complete harvest of all fish that have been caught is recommended. This may require increased flexibility on the part of the processor.

Hybrids are also reported to congregate nearer the water surface than channel catfish when needing aeration. Also, if improved growth and survival is realized, additional aeration will be required. Most hybrid growers recommend 3-4 hp of paddlewheel aeration per acre.

The spines of the hybrid are sharp and can be difficult to collapse compared with channel catfish. Workers should take special care when handling the hybrids. If possible contact a producer that has already grown hybrids and consult with them on other aspects.

### **This Workshop**

The goal of this workshop is to present practical information and hands-on experience related to hormone-induced spawning and production of hybrid catfish fry. However, hormone-induced spawning of catfish is still part art and part science. The information supplied in the workshop provides basic information on techniques used for production of hybrid fry, but experience is the best way to learn how to produce hybrids. Start small, get a feel for the process and gain some experience, and alter the protocols and techniques as you see fit. Even people with substantial experience have variable results in producing hybrid fry, so don't be discouraged if your early results are less than what you expected. Also remember that the content emphasized in the workshop is based on the authors experience and knowledge. Other people may use variations in techniques and have different opinions on which issues are more or less important than those highlighted in this manual. You would be well served to gather information, advice, and opinions on the production of hybrid fry from as many sources as possible. We hope you find the workshop to be informative and enjoyable.

# Producing Hybrid Catfish Fry

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Hormone-induced production of hybrid catfish involves injecting female channel catfish with compounds to induce ovulation, determining the occurrence of ovulation, manually stripping eggs from females, and then fertilizing the eggs with blue catfish sperm. After fertilization the eggs can be hatched using procedures used to hatch channel catfish eggs.

Strategies for hormone-induced production of hybrids can be classified into two main categories: pair-spawning and group-spawning. In pair-spawning, a mature male and female channel catfish are placed in a tank together and the female is injected with hormone (sometimes the male is also injected but injection of males is probably not necessary). Typically a large aquarium or a tank with a window is used so the fish can be observed after injection. When the fish are observed engaging in spawning behavior (e.g. clenching – male and female lay head to tail with slight spasmodic contractions of the body and/or the presence of eggs in the tank) the female is removed and tranquilized. The female is then stripped of eggs and the eggs are fertilized with blue catfish sperm. Originally it was thought that the presence of the male and the behavioral interaction between the male and female were required for the ovulation process to proceed efficiently (Dupree et al. 1969) and pair-spawning was a commonly used method. However, research at Auburn University demonstrated that groups of females could be injected and held in tanks and ovulation would occur with or without male catfish being present (Dunham et al. 1998). Group-spawning allows larger numbers of females to be spawned in a more efficient manner and lends itself more readily to large scale production of hybrid fry than does pair-spawning. Therefore the techniques described in the manual are related to group-spawning of channel catfish females to produce hybrid fry.

Important factors for successful production of hybrid fry include: good broodstock quality, proper calculation and administration of hormone dosage, proper testes collection and sperm preparation, accurate determination of the time of ovulation in females, good stripping and fertilization techniques, and aggressive egg treatment. A general overview of the process followed by detailed sections on each of these topics follow.

## Overview

This overview provides a summary of the group-spawning techniques used to produce hybrid catfish. It is difficult to spawn more than 50-60 fish per day with a 4- to 5-person crew. Split the fish into two groups of 25-30 fish each, with one group 'scheduled' to spawn in the morning and one group in the afternoon. Even though you may schedule the fish to spawn at a certain time, it rarely works out that way, but at least with two groups of fish scheduled to spawn at different times the workload will be spread out. Seine and select female catfish for spawning just after lunch early in the season when the water is cool, as the weather warms seine fish earlier in the day when the water is cooler to minimize stress. Select good quality females and transport them to your tank facility.

Hormone-induced spawning of catfish is generally a 3-day process. Both CCP and LHRHa typically require a dual injection process with an initial dose given the first day, a second dose given the second day, and the fish are spawned on the third day. Use the same injection schedule for CCP and LHRHa, specific information for CCP and LHRHa is discussed later. Give the first injection in the afternoon the day females are seined. You can inject females as you take them off the transport tank or you can put females in tanks in the morning and then come back and catch them and inject them in the afternoon. Weigh the females and inject with proper hormone dose (dosage is based on female weight so you will need to individually weigh the fish). Give the second injection about 9 to 10 a.m. the next day and females typically ovulate 20–28 hours later, although the time between the second injection and ovulation varies. Hold females in tank water temperatures of 24-27 ° (76-80 °F). On the third day, collect and prepare testes from blue males early in the morning, check to see if females have ovulated, and begin stripping eggs from females and fertilize eggs with blue catfish sperm.

- **Day 1** - Seine, select, and transport females to tanks. Weigh and inject females with first injection. Blue males can be harvested and put in tanks on either Day 1 or Day 2, whichever is convenient.
- **Day 2** - Weigh and give females second injection.
- **Day 3** – Kill blue males, collect testes, and prepare sperm. Check females for ovulation, strip ovulated females, and fertilize eggs with blue catfish sperm.

### **Broodstock Care/selection**

There are ongoing research projects to determine the effects of diet, strain of fish, and other broodstock management issues on the production of hybrid fry but results are not conclusive (Ligeon 1993, SRAC 17<sup>th</sup> Annual Progress Report 'Improving Reproductive Efficiency to Produce Channel x Blue Hybrid Catfish Fry' pp 61-76, included at back of manual). Therefore the same broodfish care and husbandry guidelines (feeding, water quality etc.) used to prepare catfish for pond spawning can be used to prepare catfish for hormone-induced spawning. One potential change between management of broodfish for pond vs. hormone-induced spawning is that higher stocking densities could probably be used and the proportion of males could be decreased if fish are only being used for hormone-induced spawning. However, using stocking densities as high as those used for food fish production or stocking only females in brood ponds intended for hormone-induced spawning is not suggested since the presence of male channel catfish in the ponds may play a beneficial role in the reproductive development of females. Information on broodfish management is available in SRAC publications included at the back of this manual: # 1802 (Channel Catfish Broodfish Management) and # 1803 (Channel Catfish Broodfish and Hatchery Management).



*Blue catfish male.*

Channel catfish females used for production of hybrid catfish fry should be 3 years old or older. It is generally believed that younger, smaller channel catfish females spawn later in the year and

therefore it makes sense to use older, larger females for hybrid production early in the spawning season and younger females later in the season. Preferences for age/size of females used for hybrid production vary. A 5-8 pound fish may be the ideal size. Smaller fish don't produce many eggs per fish while stripping large numbers of big females can lead to fatigue.

Perhaps the easiest way to determine when your female broodfish are reproductively ready for hormone-induced spawning is to put a few spawning cans in the pond and when you start to collect spawns you can assume some portion of the females are ready to spawn. Spawning activity in other ponds on your farm or on a neighboring farm is another good indicator that some of your fish are ready to spawn.

Stop feeding about 3 days before selecting females for spawning since one of best indicators of their suitability for spawning is a soft, full abdomen indicative of large, well developed ovaries (egg sacs). If the fish have been recently fed it is difficult to tell if the fullness of the abdomen is due to feed in the stomach or large ovaries. If the fish have been fed by mistake, wait a few days until the feed has cleared and then check them again. To select fish, seine the fish and hold females by the tail with their head hanging down and keep females that have a full, swollen belly. Another indicator of readiness for spawning is a red, swollen vent. However, we generally base our decisions on choosing a female for hybrid production on how full the belly looks since it is a rapid method to screen a larger number of fish. Fish with flat or only slightly swollen bellies are generally poor candidates for spawning and should not be used. The same pond can be screened again at a later date and some of the fish that were not ready the first time may be good candidates later in the spawning season. If you don't find enough good quality females to meet your needs, you should reduce the number of fish you plan on spawning or check another pond for better quality fish. It is a waste of money to inject poor quality females with hormone.

It is important to minimize stress during the selection procedure, avoid low oxygen and if the weather is warm try to seine in the mornings when the water is cooler. Don't pull the fish up and leave them concentrated in the seine for extended periods of time. Handle the fish quickly but carefully and load them onto transport tanks with adequate aeration, use pure oxygen when possible. Avoid rough handling, since it could result in damage to the ovary and lead to bleeding and clotting inside the ovary. Blood clots in the ovary make timing of ovulation and stripping females more difficult and will have a negative effect on spawning results.

Injecting females prior to spawning activity in ponds or after the major spawning activity has past may reduce hybrid production. It seems logical that you would have better results with hormone-induced spawning if you are injecting fish when they are in prime spawning condition. However, because most hatcheries are committed to channel catfish fry production, it sometimes is necessary to attempt hybrid production before or after the normal peak of pond-spawning. Remember that hybrid production results will most likely be best when the majority of females are ready to spawn naturally. If you are injecting females taken after the peak spawning season, it is especially important to check for signs of ovulation/over-ripeness at the time of selection. Some females may have already ovulated while in the pond and are not good candidates for injection.

There has been some success using warm water wells to increase pond temperatures and accelerate the maturation process so that hybrid production can begin earlier than would be possible based on natural temperature cycles. However, the flow from warm water aquifers is generally limited and having a significant effect on the water temperature requires use of fairly small ponds. Research at LSU has suggested that 100 degree days above 21° C (~ 70° F) are required to bring catfish into spawning condition, that is equivalent to 25 days of 25° C (78° F) pond temperature: 100 degree days = [(25° C – 21° C) x 25 days] (see SRAC 17<sup>th</sup> Annual Progress Report). Hybrid striped bass producers frequently prolong their spawning season by holding white bass in cool water to maintain fish in spawning condition well past the normal spawning season. A similar approach might be useful for delaying spawning in catfish. However, any approach to accelerate or delay spawning by environmental manipulation will generally have additional costs associated with it.

Blue catfish males generally are not sexually mature until they are 4-5 years old (Graham 1999). Blue catfish can be sexed in the same manner as channel catfish, males have a pronounced genital papilla and the opening is rounded and females have a recessed papilla and the opening is slit like. Blue catfish males ‘generally’ have well developed testes during the same time frame that female channel catfish are ready to spawn. However, testes development varies widely among individuals and is difficult to predict an individual’s testes development (and therefore its usefulness for hybrid production) based on the fish’s external appearance or size. Using current methods, the status of testes development is not known until after the male has been killed and the testes have been surgically removed. We suggest harvesting about twice the number of males you think you will need for a round of spawning to insure sufficient testes/sperm for hybrid production. As a general rule of thumb, one blue male with well-developed testes will fertilize eggs from about 8 average sized channel catfish females, so use 4-5 males with adequately developed testes to fertilize eggs from 32-40 females. Masser and Dunham (1998, SRAC # 190, Production of Hybrid Catfish) suggest a lower ratio of 1 male for 3-5 females, or based on testes weight they recommend 0.5 g of testes be used to fertilize 100 ml of eggs (~ 100 grams or slightly less than a quarter pound of eggs). Of course the ratio of blue males to channel females required depends on the size and development of the testes and quality of the sperm and the quantity of eggs produced by each female. It is important to use sufficient males/sperm to insure high fertility, but at the same time the cost and availability of blue catfish males requires that some attention be given to efficient use of blue catfish males. Development of a quick assay to identify the developmental status of blue catfish testes in live fish would be beneficial. Techniques for preparing sperm and checking sperm quality are described later in this manual. Unused males can be held in tanks and used for the next hybrid production cycle or returned to ponds.

### **Hormone Dosage and Administration**

The two most commonly used compounds for inducing ovulation (final maturation and release of the eggs) in female channel catfish are common carp pituitary extract (CCP) and lutenizing hormone releasing hormone analog (LHRHa). Both compounds are effective and the opinions on which is best for hybrid production vary among users. We have used both successfully and both have advantages and disadvantages. LHRHa is a synthetic product and therefore it is a purified, consistent product. However, early in the spawning season the effective dose for

LHRHa tends to be fairly high and therefore the cost is increased. In addition, the standard INAD (Investigational New Animal Drug) available from the U.S. Fish and Wildlife Service for LHRHa has a maximum limit of 100 ug/kg of female body weight, which is generally considered to be lower than the effective dose early in the spawning season. An INAD is required for use of either LHRHa or CCP (details related to INAD requirements are included later in the manual). Experience suggests that the time from injection to ovulation varies more among LHRH-injected fish than for CCP-injected fish. This can be an advantage or a disadvantage, it tends to increase the time you spend checking fish for ovulation but by spreading the timeframe for ovulation, you may be more accurate in determining ovulation for each individual fish. Additional information on reproductive biology and hormone-induced spawning of fish is available in SRAC publications included at the back of this manual: # 421 (Introduction to Hormone-Induced Spawning of Fish), # 422 (Capturing, Handling, Transporting, Injecting and Holding Brood Fish for Induced Spawning), # 424 (Hormonal Control of Reproduction in Fish for Induced Spawning), # 425 (Hormone Preparation, Dosage Calculation, and Injection Techniques for Induced Spawning of Fish), # 426 (Techniques for Taking and Fertilizing the Spawn of Fish).

### *Using CCP*

CCP is dried, ground pituitary collected from carp. This organ lies directly beneath the brain. The main source of CCP in the U.S. is Stoller Fisheries in Iowa (see contact info in the appendix). Other vendors do sell CCP (for example Argent Laboratories), but most other vendors are supplied by Stoller Fisheries. The pituitary gland produces and stores gonadotropin hormones that play a key role in triggering ovulation. CCP is effective for inducing ovulation in a wide variety of fish species. Because it is collected from fish in an unpurified form, it is possible that the potency of CCP may vary from lot to lot. However, poor spawning results due to variation in CCP potency among lots have not been experienced. CCP appears to be more effective for inducing ovulation in catfish early in the spawning season than LHRH and CCP induced fish tend to have a more synchronus ovulation time than LHRHa fish.

The effective dosage for CCP induced ovulation of catfish is an initial (or priming) dose of 0.91 mg/lb (2 mg/kg) female body weight followed by a final (or resolving) dose of 3.64 mg/lb (8 mg/kg) female body weight. The timing of injection varies some among users, but typically the initial injection is given on day 1, the second injection is given 12-18 hours later after the first, and the fish ovulate 20-30 hours later. The time to ovulation varies among individual fish and therefore determining when ovulation has occurred is important for successful results (see next section). We typically give the initial injection sometime during the afternoon of the first day, give the second injection around 9 a.m. the next morning and then start checking for signs of ovulation the next morning around 7 a.m. However, these times are simply guidelines, the time between the last injection and ovulation varies among individuals, and it also tends to be longer early and shorter later in the spawning season, and longer at cooler holding water temperatures than at warmer temperatures.

CCP is typically sold in a dry powder form in 1 gram vials, it can be safely stored in the dry form in the dark at room temperatures for long periods (months - years). One gram of CCP is enough to inject about 220 lbs of female catfish. Prior to using CCP you should mix the powder with a physiological saline solution, use phosphate buffered saline (PBS). Contact information for

vendors that sell premixed sterile PBS are listed in the appendix along with a recipe for PBS if you'd like to make your own. Mix the entire 1 gram vial of CCP at one time which eliminates the need to weigh and measure small amounts of CCP powder. If lesser amounts are needed, a fairly sensitive scale (one that measures to 0.01 g) will be needed.

Give CCP dosage in approximately 0.5 to 1.5 cc total volume. In order to make determining the injection volume straight forward, mix the concentrations so that 0.1 cc of CCP mixture is injected per lb of female body weight (a 5 lb female gets 0.5 cc and a 10 pound fish gets 1.0 cc). To do this, add the 1 gram of CCP powder to a tube containing 27.5 cc (=27.5 ml) of PBS = 36.4 mg CCP/cc, shake it up, and let it sit in the refrigerator for 1-2 hours. This lets some of the particulate matter settle out. The particulate can cause problems by plugging your syringe needle (use a needle with a 18 or 20 gauge needle, smaller needles will plug) and also seems to increase the incidence of infections at the injection site. After the tube has sat for 1-2 hours most of the large particles will settle out, then use a syringe to pull 5.5 cc of the solution off the top and transfer this to a new tube and add 16.5 cc of fresh PBS to the 5.5 cc to get a total volume of 22 cc at a concentration of 9.1 mg CCP/cc. Label this tube as Day 1 injection (this is the solution used for the first injection). Shake up the original tube (36.4 mg/cc) and let it settle out again. Use a syringe and needle to pull off all the fluid you can, transfer to a new tube, and record the volume. It's OK if you get a little of the particulate, but try to avoid a lot of it. There is 22 cc of solution in the tube but you will probably only be able to draw out 19-20 cc. After you get what you can out, add enough fresh PBS back to the particulate so you can shake the tube and draw off enough to get a total of 22 cc. If you got 19 cc the first time, you want to get another 3 cc off the second time so the total is 22 cc. This solution is 36.4 mg CCP per cc, label it Day 2 injection (it is 4 times as concentrated as the Day 1 injection). Inject the females on the first day with the Day 1 injection solution at 0.1 cc/lb of body weight (= 0.91 mg CCP/lb) and inject fish on the second day with the Day 2 injection solution at 0.1 cc/lb of body weight (=3.64 mg CCP/lb). The mixed CCP can be stored in the refrigerator after mixing for a couple days or frozen. Reports indicate that freezing does not affect its potency, but avoid repeated freeze-thaw cycles.

The most commonly used injection site is the depression just behind the pelvic fin (see photo). Tranquilize (100 ppm MS-222 or 40 ppm AQUI-S) females when injecting, it is probably less stressful on the fish and prevents a syringe from being thrown into your eye by a thrashing fish. Hold the fish by the tail with the head down and the fish can be quickly injected behind the pelvic fin. Use a 3 or 5 cc syringe with an 18 or 20 gauge needle for injecting CCP. A 3 or 5 cc syringe allows injection of fairly accurate amounts and will inject 30-50 lbs of fish. Using a needle with smaller diameter than 20 gauge with CCP can result in frequent plugging. Move the fish that have been injected into another tank or section of the tank so you can keep them separate from the fish that still need to be injected.

### ***Using LHRHa.***

Use the same time schedule and technique to inject LHRHa. LHRHa is available from Syndel in Vancouver, British Columbia, Canada (see appendix for contact information). Other vendors sell LHRHa, but Syndel is the participating vendor listed on the INAD. LHRHa is sold in 1, 5, or 25 mg vials in a dry form. 5 mg is enough to inject ~ 110 lbs of female catfish at 45 ug/lb.

The maximum LHRH dosage allowed on the standard INAD is 45 ug/lb of female body weight (100 ug/kg) and the total dose is split into a weaker initial and stronger final dose as with CCP.

Inject  $\leq 1$ ml of hormone solution and so mix the concentration of hormone accordingly. Use a syringe to add 5 cc PBS to the vial and dissolve the powdered LHRHa, sometimes the powder will be stuck around the top of the vial so be sure to get it all dissolved. After it is dissolved, draw out the 5 cc, be careful because when you added it, there was probably some backpressure created. Add the 5 cc containing the 5 mg LHRHa to a clean tube and then add 8.8 cc more of PBS, now you have 13.8 cc of  $\sim 364$  ug/cc solution (label this Day 2 injection). Mix this solution and use a syringe to draw off 2.8 cc of this solution and put it in another tube, add 8.2 cc of PBS to this tube to give a total volume of 11.0 cc of  $\sim 91$  ug/cc (label this Day 1 injection). You should have 11.0 cc of Day 1 injection and 11.0 cc of Day 2 injection. Inject females on day 1 with the Day 1 solution at 0.1 cc per lb (= 9.1ug/lb). A 5 lb fish gets 0.5 cc and 10 lb fish gets 1.0cc. Inject females on day 2 with the Day 2 solution at 0.1 cc per lb (= 36.4 ug/lb). A 5 lb fish gets 0.5 cc and 10 lb fish gets 1.0cc.

### Testes Collection and Sperm Preparation

Improper handling and storage of testes/sperm can lead to poor or zero fertilization. As indicated earlier, it is difficult to estimate testes development from the external appearance of a blue male catfish so have approximately 2 times the number of blue males you think you will need seined up and ready for testes collection. Most people kill the blue catfish by a percussive blow to the head, this allows the fish to be eaten without concern about residual tranquilizer. In addition, there is a concern that if the fish are anesthetized, some of the anesthetic might get into the sperm preparation and negatively impact sperm motility. After the blue male has been killed, dry off the fishes skin well with a towel. It is very important to make sure that you do not get fresh water on the testes or in your sperm preparation because the sperm is activated by exposure to fresh water. After activation, the sperm only retains its motility and its ability to fertilize eggs for about 1 minute. Therefore, you need to make sure exposure to fresh water does not activate the sperm until you have mixed it with the eggs. Prior to fertilization the testes/sperm are kept in Hanks Buffered Salt Solution (HBSS), a solution that mimics the ionic concentration of the fish's body fluids and keeps the sperm in an inactive, but live state. If you activate the sperm during testes collection/preparation you can do everything else in the spawning process correctly and get little or no fertilization.

Place the male on its back and make an incision along the midline of the body cavity to expose the abdominal cavity.



*Blue catfish male with abdomen opened for testes removal.*



*Good quality testes, feathery projections white in color.*

The testes are paired organs running from the vent toward the anterior part of the body cavity and lay along the upper wall of the body cavity. Well developed catfish testes are white and feathery in appearance, poorly developed testes are brownish pink and not as large. Remove the testes with a sharp knife, scalpel or by gently teasing them out with your fingers. The fish will bleed during this procedure and you can use a dry paper towel to blot up the blood. The blood should have ionic concentration similar to the testes so it should not activate the sperm, but try to minimize the amount of blood collected with the testes. Place the testes (or pieces of testes) in a container containing HBSS (the recipe for HBSS is included in the appendix, this recipe works well for me, other recipes are available and should be adequate). Use the disposable containers with lids available from Rubbermaid, Tupperware etc. They are cheap and the lid prevents water from being splashed into your HBSS. Rinse the testes in HBSS by moving it around in the solution to wash away blood and use your fingers or tweezers to remove blood clots. After the testes has been rinsed, move it to a container with about 1 cup (about 250 cc or ml) of HBSS. Place the testes in a metal sieve (commonly used for food preparation and available at Wal-Mart etc.) press the testes against the strainer with your thumb and fingers, this crushes the testes and releases the sperm into the HBSS (see photo). Continue crushing the testes until they are flat and most of the sperm have been released. Save the solution, it contains the sperm, The sperm solution will have a pinkish-white appearance. You can discard the tissue remaining in the sieve after crushing. Transfer the solution to a clean, waterproof container, either plastic or glass will work, and store the container on ice or in the refrigerator. Store the sperm solution on ice. Continue collecting testes from males until you feel you have sufficient sperm for your spawning requirements. Sperm solution from 4 or 5 blue males with decent testes development is sufficient to fertilize eggs from 35-40 fish, others may recommend a lower number of males per female. Pool the sperm from 4-5 fish into your sperm solution container (Use 1 liter bottles which is about equivalent to 1 quart, just make sure the container is clean and dry!). Then increase the volume of the sperm solution to about 1.0-1.5 liters by adding more HBSS, not water. If you don't like the metric system, you can dilute the sperm solution to about 1 quart. Then estimate about how many spawns you think you will get, usually 75-90% of good quality females will ovulate, so if you injected 45-50 fish you will typically get 35-45 spawns and you can get an estimate of



*Rinsing testes in HBSS and removing blood clots prior to crushing testes for sperm solution preparation.*



*Testes ready to be crushed and sieved through screen.*



*Sperm solution being poured into tube and, stored on ice. Use 1 tube per spawn.*

how much sperm to add to each spawn at fertilization. For example if you have 1 quart of sperm solution and think you will get 40 spawns, use 1/40 of a quart of sperm solution for each spawn. If you need more sperm you can collect additional testes and prepare it as you need it, but have sperm solution made up and split into tubes (1 tube per spawn) the morning before spawning so you can focus on spawning. If females produce fewer spawns than expected you can use more sperm per spawn or you can store excess sperm solution refrigerated or on ice for at least 1 day with good results. See Christensen and Tiersch (1996) for information on catfish sperm refrigeration.

It is a good idea to check the motility of the sperm to insure you have good quality sperm prior to using it. The easiest method to check sperm quality is to examine it with a microscope. It does not require an expensive microscope, and you can probably borrow one from a local high school or veterinary clinic if you don't have access to one. Place a drop of the sperm solution on a microscope slide and add a slipcover, start with the low power objective and focus, then move up to a higher power. Under higher power the sperm should appear as small oval shaped dots and you may see a string like tail projecting from them. At this point the sperm should not be moving, you may see some movement of the fluid under the scope, this is called Brownian movement, and is simply the fluid under the cover slip moving. Don't worry if you see some sperm drifting across the slide as long as they are all drifting in the same direction. Then put a drop of water on the slide, then add a small drop of the sperm solution and add a cover slip. Now when you look in the microscope you should see the sperm moving quickly in many different directions. This is an indicator that the sperm quality is good and you prepared the sperm solution correctly. Not all the sperm will be motile, but if it looks like about 30-50% of them are moving, consider that to be good quality. Look fairly quickly, because the sperm will stop moving after about 1 minute. If there was a delay between adding the water and when you looked for motility and you don't see any movement, try another slide and look more quickly. If you still don't see any motile sperms you have problems and will likely have poor fertilization. You will need to check your HBSS preparation and make sure it is correct. Although motility may vary some from male to male and declines some during storage, if you have no motility it is generally an indicator that your HBSS was incorrectly made and the sperm were activated during collection and preparation.



*Checking sperm motility.*

Acquiring large numbers of blue catfish males can be problematic since currently few people have large supplies of mature blue catfish available, but some producers are starting to grow blue catfish so availability should become less of an issue in the near future. Little information is available on the performance of hybrids produced by different blue catfish strains and we don't have any recommendation on what strain of blue catfish should be used. At this point, just finding blue catfish males can be a challenge. Could sperm from wild caught blue catfish be used to make hybrids? If collected correctly, sperm from wild-caught blue males could be used to produce hybrids. It is not known whether the performance of hybrids produced from wild-caught blue males would be as good as hybrids produced from domesticated blue catfish, but it is an interesting question. It would probably be best to base hybrid production on using

males from a domesticated line of blue catfish, but if none are available wild-caught blue might be the only available option. It would be fairly easy for a hand-grabber or commercial fisherman to collect testes from blue male catfish and place it in a container of HBSS and put it on ice until you could prepare it.

### Timing Ovulation

Proper timing of ovulation is critical to successful hybrid production. At ovulation, the eggs are released from the ovarian wall and if they are not removed the egg quality will begin to degrade. Therefore the key to successful strip-spawning of catfish (and other fish species) is to accurately determine when the female has ovulated. If you attempt to strip the female prior to ovulation, the eggs are not released and the yield of eggs and quality of eggs will be poor, if you wait until too long after ovulation, the egg yield may be good but the quality will be poor and fertilization will be low. In addition, catfish do not ovulate as synchronously as some fish species, meaning catfish do not ovulate and release all their eggs at one time. Therefore it can be difficult to determine if a fish is just starting to ovulate and you should wait until stripping her eggs or the process has proceeded to the point that the female is ready to be stripped. The best way to get good at determining when a female has ovulated and is ready to strip is through experience.

The easiest method to determine if ovulation has occurred in most fish species, including catfish, is to see if gentle pressure to the abdomen results in the flowing of eggs from the vent. Another method that has been used to help to determine if at least some portion of the female catfish in a group-spawning scenario have ovulated is to place a 4-6 foot long piece of 4 inch PVC in the bottom of the tank or vat downstream of the water flow. Tie a string and cork to the pipe so you can pull it up and occasionally take a look at it. On the morning you expect the fish to spawn, start looking at the pipe a 2-3 hours before you expect ovulation and examine it about every 30 minutes. At ovulation females will release some eggs into the water and some of the eggs will stick to the pipe. When you start to see eggs accumulating on the pipe it is a good indicator that you need to start checking the females for ovulation. To check females, gently crowd them and catch them with a dip-net. If you are right-handed, grasp the fish just ahead of the tail with your left-hand and hold her with her belly up so you can see the vent clearly. Use your right hand to gently squeeze the female's abdomen. Place the thumb on one side of her abdomen and forefinger on the other side and gently squeeze thumb and finger together as the hand moves down the abdomen towards the vent. Do this 3 or 4 times and if eggs are flowing from the vent the fish is ready for stripping. The amount of pressure required to get eggs to flow varies from fish to fish and relating the amount of pressure needed and how well the eggs flowed with a properly timed and stripped female can only really be learned through experience. Do not sedate fish when



*Pipe placed in tank with injected females.*



*Checking a female for egg flow.*

checking for ovulation. The fish that are not ready for spawning are put into another section of the raceway to be checked again about 2 hours later. You can sedate fish prior to checking them for ovulation but it slows the process and is probably not necessary. If you are having a difficult time deciding whether to strip or not or if the fish keeps contracting its muscles and won't relax, sedate it so you can get a better look.

### Stripping and Fertilization

The fish that are determined to be ready for stripping are sedated by placing them in a container of aerated water with 100 ppm MS-222 (0.38 gram per gallon) or 40 ppm AQUI-S (0.15 mls per gallon). When the fish is sedated, remove it from the anesthetic, dip it in fresh water to wash off the tranquilizer, and then dry the fish off with a towel. When the fish has been dried, grasp the fish at the base of the tail with your left hand and place the head of the fish in the crook of your right elbow with the fish belly down. The fishes head should be slightly elevated so gravity will help the eggs flow towards the vent. Reach under the fish with your right hand and put your thumb on one side of the belly and fingers on the other side just ahead of the pelvic fins and gently squeeze as you slide your hand back toward the vent. Eggs should begin to flow out of the vent, continue this 'milking' process and move further up the abdomen as eggs begin to empty out of the rear portion of the ovary. Continue the process until the eggs stop flowing and/or the ovaries appear to be empty. When you strip a female a good indicator of proper timing is smooth flowing, yellowish green eggs, with few clumps or blood clots. A fish that does not flow well and has lots of egg clumps is usually a sign that ovulation was just starting or incomplete. Although putting these fish back in the tank and stripping them again later is an option, success with this practice has been poor. A fish that flows freely but the eggs come out somewhat stuck together or 'ropey' with a dull color and some whiteness is a good indicator that the eggs were ovulated earlier and have begun to degrade. Although some of these eggs may be viable, fertility is typically low and hatch is poor.

Catch the eggs in a gallon plastic bucket available at most home supply or retail stores. Any container can be used, but it is important to remember that catfish eggs will stick to the container after fertilization if some type of lubricant is not put on the container surface. Various lubricants can be used (vegetable shortening, valve grease) but we find that non-stick cooking spray works well and is easy to use. Spray a small amount in the bucket and wiped it around and remove excess with a paper towel. Although some feel that cooking oil might inhibit fertilization by coating the



*Female catfish being dried off prior to egg collection.*



*Coat egg bucket with no-stick cooking spray, then add ~ 1 inch HBSS.*



*Stripping female.*

eggs, there is no evidence that this occurs. After lubricating the bucket, put about 0.75-1.0 inch of HBSS in the bucket so the eggs go into the HBSS as they are stripped. After stripping the fish, weigh the eggs to get an estimate of the egg number. If there are more than 0.75 lb (about 350 grams) of eggs, split them into another bucket and treat it as 2 spawns. You can make egg masses larger than 0.75 lb in the buckets but they get very thick and tend to have poor hatch because of reduced water flow and failure of treatments getting to eggs in the interior of a thick egg mass. If there is a lot of blood or egg clumps, rinse the eggs with HBSS and remove any large egg clumps or blood clots since these provide substrate for bacteria and fungal growth on eggs during hatching.

Use a fair amount of HBSS for egg washing. HBSS is cheap (~ 0.20 cents per gallon for the chemicals). For the sperm preparation, use HBSS mixed with distilled water, but for egg wash HBSS just use hatchery water (just be sure not to use a chlorinated source) and mix it up in 25-gallon batches in a large plastic garbage can. The first time filling the can with water, carefully measure out 25 gallons (or whatever volume you chose) and then mark the water level on the garbage can. This will allow you to fill the can to that mark and know it is 25 gallons. To make HBSS for egg washes, fill the can to the mark and then add the proper amount of chemicals and be sure to stir it well so the chemicals mix. You can drop an airstone in the water and that should provide adequate mixing and aerate the HBSS. Weigh out the chemicals for several batches of HBSS and store them in Ziploc<sup>®</sup> bags so all you have to do is add the water, then dump in the bag of pre-weighed chemicals and mix. Washing out the blood and removing egg clumps and other tissue improves fertility and hatch percentage. After the eggs have been rinsed, pour off the HBSS and the eggs are ready to be fertilized.



*Egg rinsing using HBSS.*



*Egg mass ready to move.*

You can prepare the sperm solution and pour it into tubes so that each tube can be used to fertilize one bucket of eggs. Store the sperm solution tubes on ice in a cooler so they are readily available. Add one tube of sperm solution to the eggs and quickly stir it around, then add about 1 inch of hatchery water and stir again quickly. At this point the sperm are activated and the eggs are fertilized. After 1-2 minutes, fill the bucket the rest of the way with water and the eggs now begin to water harden and stick together. At this point, set the bucket under a valve supplying hatchery water and just let the water overflow. Use enough flow to get good water exchange but don't have the flow so high that the eggs are being rolled around in the bucket. Have several buckets of eggs being water hardened at one time so you don't have to do anything with the eggs and can focus on spawning fish. When there is a break you can check the eggs and if they are stuck in a mass you can move them to the hatching troughs and hatch them as you would channel catfish.

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# Care of Hybrid Catfish Egg Masses

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One aspect of hybrid production that requires strict attention to detail is treatment of eggs for bacteria and fungus. Catfish hatcheries typically have some sort of egg treatment protocol in place, but it is even more important to treat hybrid eggs aggressively. The main reason for a good egg treatment protocol is that even with the best technique it is difficult to produce hybrid egg masses with the levels of fertility commonly observed in pond-spawned channel catfish eggs. The unfertilized eggs provide excellent substrate for growth of bacteria and fungus which then spreads to the fertilized eggs and by the time fry are ready to hatch the majority of the fry are dead and hatch rate is greatly reduced.

Occasionally you will produce hybrid spawns with nearly 100% fertility and these egg masses hatch very well, but you may also get some with very low fertility (10-20%) that produce very few fry. Aggressive egg treatment may help in situations where you have 50–80% fertility. Without treatment, the hatch in a spawn with 50% fertility will be greatly reduced. Even with aggressive treatment, hatch results can be much lower than fertility rates.

Improving hybrid egg survival requires good husbandry practices and continuous attention to detail. Optimal health of the developing fry is best achieved with a good environment, reduced handling, and aggressive egg treatments. Unlike pond-spawned catfish, you can control the size and timing of the spawn. It is important not to make your egg masses too thick or treatments will not be as effective. It is also important to start treatments 8-12 hours post fertilization and possibly avoid treatment with any compound at 40-46 hours post fertilization window. Research suggests that this time frame may be a sensitive point for the developing embryos. The following recommendations are guidelines, and you find other treatment protocols to be effective. Regardless, hybrid catfish eggs require an aggressive treatment protocol for successful fry production.

## **Factors Leading to Dead Eggs and Disease**

Strip-spawning can often result in egg masses with a large number of unfertilized or dead eggs. In the hatchery, over-handling, overcrowding, and adverse environmental factors, such as high temperatures and poor water quality, also result in egg stress and death. Unfertilized and dead eggs are the primary target of disease-causing pathogens and provide a starting point for diseases to spread. Even in the most sanitary of hatcheries, pathogens are present. Once a disease outbreak has begun, it can quickly get out of hand. Prevention should be the first goal of a good hatchery disease management plan.

### ***Dead eggs***

Dead eggs need to be managed to prevent massive disease outbreaks. Live eggs should appear transparent and progress from a pale yellow color to an orange–red color. Dead eggs are often difficult to observe during the first 1-2 days after spawning. By the third day, dead eggs typically appear opaque and colorless. Some dead eggs will also be enlarged. When dead eggs

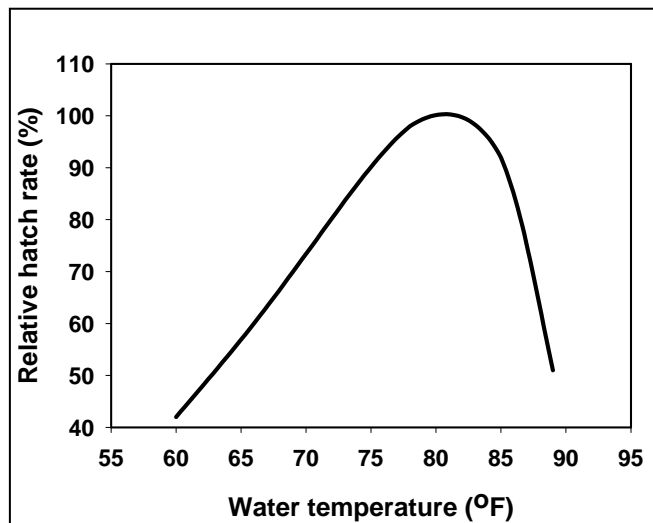
are observed, they can be manually removed to prevent infection. When removing dead eggs, care must be taken not to damage good eggs.

### ***Overcrowding***

Many factors affect the maximum loading rate a hatchery can sustain. Generally, two large egg masses (approx. 1 kg (2 lb) each) or three small egg masses (approx. 0.5 kg (1 lb) each) can be incubated in a single hatching basket 20 cm (8 in) wide x 41 cm (16 in) long x 10 cm (4 in) deep). Egg masses that overlap substantially are subject to poor water circulation, reduced egg survival, and the direct transfer of diseases between egg masses.

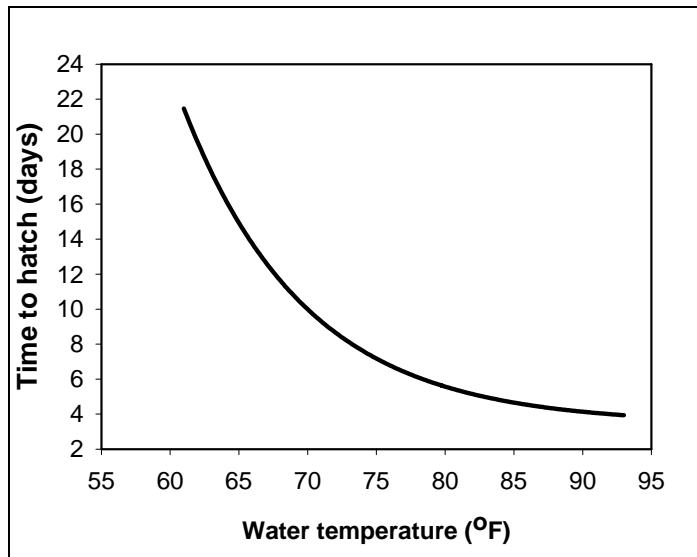
### ***Temperature***

Temperature is an important environmental factor affecting fry development, hatch rates, and disease susceptibility. The optimal temperature range for incubating catfish eggs is between 26–28°C (78-82° F). At temperatures above and below this range, egg death and prevalence of disease increases, reducing hatch rates (Figure 1).



**Figure 1. Effect of water temperature on catfish hatch rates.**

The time it takes for catfish eggs to hatch also depends on water temperature. Catfish typically spawn in the spring, when water temperatures are between 21 – 29° C (70-84° F). At these temperatures, the time to hatch is between 5 and 10 days (Figure 2).



**Figure 2. Effect of water temperature on time to hatch catfish eggs.**

### ***Water hardness***

Water hardness plays an important role in catfish fry development. Low calcium levels in hatchery water can increase egg death and reduce hatch rates. Hatch rates from eggs incubated in water with less than 10 ppm calcium-hardness during the first 24 hours after spawning are reduced by as much as 70%. Low calcium-hardness during later stages of development can cause up to a 25% reduction in hatch rates. For this reason, it is important to maintain adequate water hardness in the hatchery. It is recommended that a minimum calcium-hardness of 20 ppm be maintained, especially during the first 24 hours after spawning.

### **Disease Causing Organisms**

Bacterial and fungal infections are the primary threats when incubating catfish eggs. Generally, bacterial infections occur when hatchery water temperatures are above 28° C (82° F) and when egg masses are overcrowded. Bacterial egg rot appears as a milky-white patch in the egg mass. This patch of bacteria will contain dead and deteriorating eggs, and is often seen on the underside and in the middle of the egg mass. Anytime milky-white patches are observed on the egg mass, care should be taken in the removal of the bad spot and surrounding dead eggs.

Fungus is more prevalent at lower temperatures, usually 26° C (78° F) and below, and rapidly attacks infertile and dead eggs. Fungal infections are easy to spot, appearing as white or brown cotton-like growths made of many small filaments. If left untreated, these filaments can invade and kill adjacent healthy eggs, expanding to cover the entire egg mass and potentially every egg mass in the hatching trough. Mechanical removal of dead and infected eggs can be time consuming, but is beneficial. Chemical control of fungal infections is quite effective. Regular disinfection of eggs with chemical disinfectants is commonplace in most commercial catfish hatcheries.

## **Chemical Disinfection**

The use of drugs and chemicals for disinfecting eggs in aquaculture facilities is regulated by various federal and state agencies. Treatments must be effective, safe, and cost efficient. The United States Food and Drug Administration (FDA) is responsible for ensuring the safety and effectiveness of aquaculture drugs, including those chemicals used to treat diseases of fish eggs. There are presently four options for the legal use of chemotherapeutants in the United States: (1) the chemical has been approved by the FDA; (2) the chemical is the subject of an Investigational New Animal Drug (INAD) exemption; (3) the chemical has been determined by the FDA to be of low regulatory priority; and (4) the chemical is not low regulatory priority, but regulatory action has been deferred pending the outcome of ongoing research. More information on obtaining and using drugs and chemicals for aquaculture purposes can be acquired through the FDA's Center for Veterinary Medicine (CVM; <http://www.fda.gov/cvm/index/aquaculture/aqualibtoc.htm>), which regulates the manufacturing, distribution, and use of animal drugs.

Several factors must be considered when developing a chemical treatment strategy for managing egg disease. Biological, environmental, and physical factors all play a role in the effectiveness of the treatment. Each hatchery is unique in the way it's built, the source and quality of water, the capacity of the facility, and the management. It is highly recommended that visits be made to existing hatcheries to discuss their disease management strategies. The following discussion of chemical treatment methods is meant to be a guideline for hatchery managers and is based on research, experience, and personal communications with hatchery managers and extension specialists.

### ***Biological factors***

The primary biological factor to consider is the age of the developing eggs. Many studies have been conducted to determine the effects of treating eggs at various stages of development. In general, the egg is a very tough protective environment for the developing fry. When using the correct concentration, length, and frequency of treatment, the developing fry is protected up to the time it hatches from the egg. As such, treatments should be continued until the "eyed" egg stage, the point in time when eye pigmentation (black eye spots) can be observed without magnification. Newly hatched fry are vulnerable to chemical disinfectants.

The best hybrid hatch rates have been achieved by treating three times daily with formalin; however, research has suggested that treatments should not be conducted 40-46 hours post-fertilization, since this stage of development may be chemically sensitive. If trying to salvage severely diseased egg masses with more frequent or prolonged treatments, an isolated incubation trough should be used.

### ***Environmental factors***

Temperature and water quality not only affect the development and survival of developing catfish fry, but they can have a significant impact on the effectiveness of the chemical. Toxicity of some chemical treatments is directly proportional to temperature; as temperature increases so

does the toxicity of the chemical. On the other hand, some chemicals work better at higher temperatures than at lower temperatures. Fortunately, for the few chemicals that are FDA-approved or of low-regulatory priority for use in disinfecting catfish eggs, temperature does not appear to have a significant impact on effectiveness or toxicity.

Organic load in the water system can also impact the effectiveness of chemical treatments. High concentrations of organics in hatchery water systems should be avoided. Organics provide a food source for pathogens and may increase diseases in the hatchery. High levels of organics can also reduce the effectiveness of chemical disinfectants such as formalin and hydrogen peroxide.

### *Physical factors*

Water flow rates and volume are the two biggest factors affecting how chemical treatments will be administered and can greatly impact the effectiveness of the treatment. When determining chemical concentrations for egg disinfection, the volume of water being treated must be precisely known. If eggs are to be treated as a bath in the hatching troughs, the time to completely turnover the volume should be factored into the decision of how long to treat the eggs, as part of the total time exposed to the disinfectant solution.

Turning off the water for bath treatments can be a substantial risk, with millions of eggs being lost in the event water flow is not restored. As an alternative, a flush treatment can be conducted in which the chemical is added to the trough with continuous water flow. The time it takes for one complete water exchange will dictate whether chemical concentrations must be increased or decreased.

Most recommendations for disinfecting catfish eggs suggest that the eggs be exposed to the treatment for 15 minutes at a given concentration. During flush treatments, faster water turnover (higher flow rates) must be compensated for by increasing the chemical concentration. Slower turnover rates provide for longer contact time between the egg mass and the chemical solution and require a reduction in the concentration of chemical used.

There are many opinions as to how long and how often eggs should be treated. Treatment durations that are too short and infrequent will not sufficiently kill the disease-causing pathogen, but treatment durations that are too long or too frequent may be toxic to the developing fry. In both cases, fry survival will be unacceptably low. A good hatchery manager will use the guidelines as a starting point and adjust treatment methods accordingly.

### *Disinfectants*

**Formalin.** Formalin is an FDA-approved aquaculture drug for the control of fungi on all fish eggs. Formalin products for egg disinfection can currently be purchased under the trade names Formalin-F (Natchez Animal Supply Co.), Paracide-F (Argent Laboratories, Inc.), and Parasite-S (Western Chemical, Inc.). The maximum approved treatment concentration for use in disinfecting catfish eggs is 2000 ppm for 15 minutes as a flush treatment. Under typical hatchery

conditions with an average of one complete water exchange every 45-60 minutes, 2000 ppm can be toxic to channel catfish eggs.

In most hatcheries, fungus can be controlled by treating with 100 ppm formalin for 15 minutes as a bath treatment. Turn the water off during treatment, but leave the paddles turning or air flowing from airstones. Flush completely with fresh water when treatment time has elapsed. As a flush treatment, concentrations between 100 and 400 ppm formalin have been used successfully at temperatures ranging from 24-30°C (75-86°F). Hatch rates tend to improve when formalin treatments are administered twice daily. Recommended formalin treatments are presented in Table 1.

**Hydrogen peroxide.** Hydrogen peroxide is currently an aquaculture drug of low regulatory priority according to the FDA. It is expected that hydrogen peroxide will eventually be approved by the FDA as a new animal drug, and that the label will include the treatment of catfish eggs. As a drug of low regulatory priority, permitted use of hydrogen peroxide includes the control of fungi on all life stages of fish, including eggs, at concentrations of 250-500 ppm on an active ingredient basis (100% hydrogen peroxide). Hydrogen peroxide is extremely caustic in its concentrated form and can be purchased as 3, 35, and 50% solutions. The most practical concentration for use as a chemical disinfectant is the 35% solution.

**Table 1. Recommended volumes of formalin and hydrogen peroxide for use as flush treatments to disinfect hybrid catfish eggs in a hatching trough containing 100 gallons of water.**

Water flow (GPM)	Milliliters (fluid ounces)	
	Formalin (37% formaldehyde solution)	Hydrogen peroxide (35% solution)
1.0	10 (0.3)	40 (1.4)
2.0	30 (1.0)	75 (2.5)
3.0	50 (1.7)	110 (3.7)
4.0	70 (2.4)	150 (5.1)
5.0	90 (3.0)	190 (6.4)
6.0	110 (3.7)	225 (7.6)

\* Chemical volumes are provided as starting points and may require adjustment for unique hatchery conditions.

\*\* Recommended treatment frequency is 3 times per day for formalin and 1 time per day for hydrogen peroxide.

\*\*\* *DO NOT treat eggs that are hatching.*

The effectiveness of hydrogen peroxide appears to be impacted by temperature, and may be the result of increased toxicity at higher temperatures. When hatchery water temperature is 26°C (78°F), a daily, 15-minute bath in a solution of 250 ppm active hydrogen peroxide (715 ppm of 35% hydrogen peroxide) is as effective as formalin at disinfecting eggs and improving hatch rates. It is important to note, however, that twice as much hydrogen peroxide at this temperature is toxic to the eggs. At cooler temperatures, toxicity is reduced and higher concentrations of hydrogen peroxide are more effective. Recommended hydrogen peroxide treatments are presented in Table 1.

**Povidone iodine.** Povidone iodine is also an aquaculture drug of low regulatory priority according to the FDA. Permitted use of povidone iodine compounds includes the disinfection of catfish eggs in a solution of 100 ppm for 10 minutes. Daily iodine treatments are not as effective as daily formalin treatments for controlling fungal infections. Povidone iodine is, however, a very good preliminary disinfectant when transferring eggs from the pond to the hatchery. A 10 minute bath in a 100 ppm iodine solution prior to adding new egg masses to communal incubating troughs can substantially reduce the transfer of pathogens from the pond to the hatchery and may improve hatch rates by as much as 10% when used in coordination with a daily disinfectant scheme of either formalin or hydrogen peroxide.

**Copper sulfate.** Copper sulfate currently falls under the FDA label of investigational new animal drug (INAD), and regulatory action has been deferred pending the outcome of ongoing research. Compounds in the INAD category are used under an investigational new animal drug exemption administered by the FDA/CVM to allow for the purchase, shipment, and use of unapproved new animal drugs for collection of effectiveness and safety data that will support a decision for drug approval. Data in support of copper sulfate as an egg disinfectant is currently being collected for use in the drug approval process. Preliminary data suggests that copper sulfate is a very effective disinfectant for controlling fungal infections of catfish eggs.

As once or twice daily 15-minute bath treatments, copper sulfate concentrations of 2.5-10 ppm appear effective in reducing disease and improving hatch rates. Higher levels of copper sulfate have been found to reduce hatch rate, suggesting a toxic effect. When adding copper sulfate to the hatching trough, crystalline copper sulfate should be mixed in a 5-gallon bucket of hatchery water and added as a solution. Copper sulfate is not recommended for use in aluminum troughs since it reacts with the aluminum and causes the trough surface to become pitted.

## **Summary**

### ***General recommendations***

Hybrid catfish eggs require greater attention to detail and aggressive treatment for bacteria and fungus. While it's clear that many factors can be attributed to poor hatch rates, knowing the optimal conditions for handling and hatching catfish eggs and following good hatchery practices will improve fry survival. Some recommendations include:

1. Avoid unnecessary handling of eggs.
2. Disinfect egg masses with povidone iodine prior to placement in a communal incubation trough.
3. Maintain hatchery water temperatures between 26–28° C (78-82° F) for optimal hatching success.
4. Avoid overcrowding of egg masses in troughs.
5. Maintain adequate water hardness in the hatchery to improve fry survival.
6. Be familiar with the laws regulating chemical use for disinfecting catfish eggs.
7. Treat eggs aggressively with an approved chemical disinfectant to manage diseases and improve hatch rates.

### ***Timing of Treatments***

1. Start treatments: 8-12 hours post fertilization.
2. Do not treat with any compound in the 40- to 46-hour post fertilization window. Applications of formalin during this period have been linked to egg loss.
3. Treat egg masses 3 times per day:
  - Early morning (6-7 a.m.)
  - Noon
  - Evening (6-7 p.m.)
4. Stop treatments when eggs begin hatching. Dip those masses that are not yet hatched if the delay is expected to be several hours.

### ***Treatment Regimes***

1. Static treatment: 15 minute bath.
  - Option 1: Three treatments per day with 100 ppm of formalin (37% formaldehyde).
  - Option 2: Three treatments per day with 100 ppm povidone iodine (1%).
  - Option 3: Three treatments per day with 2.5 ppm copper sulfate.
2. Flowing water treatment: In 100-gallon troughs with 3 gallons per minute of flow.
  - Option 1: Three treatments per day with 50 ml of formalin (37% formaldehyde).
  - Option 2: Three treatments per day with 50 ml of povidone iodine (1%).
  - Option 3: Two treatments per day with 50 ml of povidone iodine (1%) and one treatment per day with 10 grams of copper sulfate crystals. (Dissolve copper sulfate in 5 gallons of water and pour across trough.)

### **Additional Resources**

Hargreaves, J.A. and C.S. Tucker. 1999. Design and Construction of Degassing Units for Catfish Hatcheries. SRAC Publication No. 191. Southern Regional Aquaculture Center.

Small, B.C. 2004. Accounting for water temperature during hydrogen peroxide treatment of channel catfish eggs. *North American Journal of Aquaculture* 66:162-164.

Small, B.C., W.R. Wolters, and T.D. Bates. 2004. Identification of a calcium-critical period during channel catfish embryo development. *Journal of the World Aquaculture Society* 34:313-317

Small, B.C. and W.R. Wolters. 2003. Hydrogen peroxide treatment during egg incubation improves channel catfish hatching success. *North American Journal of Aquaculture* 65:314-317.

Small, B.C. and T.D. Bates. 2001. Effect of low-temperature incubation of channel catfish, *Ictalurus punctatus*, eggs on development, survival and growth. *Journal of the World Aquaculture Society* 32:49-54.

- Steeby, J.A. and J.L. Avery. 2005. Channel Catfish Broodfish Selection and Hatchery Management. SRAC Publication No. 1803. Southern Regional Aquaculture Center.
- Tucker, C.S. and J.A. Steeby. 1993. A practical calcium hardness criterion for channel catfish hatchery water supplies. *Journal of the World Aquaculture Society* 24:396-401.
- Tucker, C.S. and E.H. Robinson. 1990. Channel Catfish Farming Book. Van Nordstrand Reinhold: New York, New York.
- Tucker, C.S. and J.A. Hargreaves. 2004. *Biology and Culture of Channel Catfish*. Elsevier, Amsterdam, The Netherlands.
- Tucker, C.S. 1991. Water Quantity and Quality Requirements for Channel Catfish Hatcheries. SRAC Publication No. 461. Southern Regional Aquaculture Center.
- Walser, C.A. and R.P. Phelps. 1993. The use of formalin and iodine to control *Spaprolegnia* infections on channel catfish, *Ictalurus punctatus*, eggs. *Journal of Applied Aquaculture* 3:269-278.
- Wedemeyer, G.A. 2001. *Fish Hatchery Management*, 2<sup>nd</sup> edition. American Fisheries Society, Bethesda, Maryland.

# Appendix

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## LIST OF SUPPLIES AND VENDORS:

(Mention of any trade name or vendor does not imply endorsement by the USDA or Mississippi State University).

### Carp Pituitary Extract

#### Stoller Fisheries

P.O. Box B  
Spirit Lake, IA 51360, USA (Mailing address)  
1301 18th Street  
Spirit Lake, Iowa 51360, USA (Physical Address)  
800-831-5174  
712-336-1750  
fax 712-336-4681  
e-mail address: stollerfisheries@mchsi.com  
website: <http://www.sfishinc.com>

### LHRHa (cat # 13440)

#### Syndel International Inc.

9211 Shaughnessy Street  
Vancouver, British Columbia V6P 6R5, Canada  
800-831-5174  
712-336-1750  
fax 712-336-4681  
e-mail address: info@syndel.com  
website: <http://www.syndel.com>

### Lab Supplies

(Syringes/needles  
Glassware, Scalpels,  
Volumetric Flasks, Tubes  
General lab supplies)

#### A Daigger & Co. Inc.

620 Lakeview Parkway  
Vernon Hills, IL 60061  
800-621-7193  
fax 800-320-7200  
website: <http://www.daigger.com>

### Chemical supplies

(General Chemicals,  
Pre-made PBS)

#### Fisher Scientific

800-766-7000  
website: <http://www.fishersci.com>

#### Sigma Aldrich

800-325-3010  
website: <http://www.sigmaaldrich.com>

Tranquilizers and some of the items listed above (syringes, needles etc.) can be purchased from an aquaculture supply store. Spawning buckets, testes strainers, no-stick oil, towels etc. can be purchased from many retailers.

**RECIPES:****Hanks Buffered Salt Solution (HBSS) for English units (gallons)**

<b>Ingredient</b>	<b>1 gal</b>	<b>5 gal</b>	<b>25 gal</b>
<b>Sodium chloride (NaCl)</b> You can use uniodized salt from the grocery store, make sure you keep it dry.	30.05 g	150.25 g	751.25 g
<b>D-Glucose (dextrose)</b> Buy anhydrous (no water), Fisher Scientific sells a reagent grade (Catalog # S734181) 1 kg (1000 g or ~ 2.25 lbs) for ~ \$12.15	3.79 g	18.95 g	94.75 g
<b>Potassium chloride (KCl)</b> Fisher Scientific sells a reagent grade (Catalog # S773751), 500 g for ~ \$7.85	1.52 g	7.60 g	38.0 g
<b>Sodium bicarbonate (NaHCO<sub>3</sub>)</b> Fisher Scientific sells a reagent grade (Catalog # S78284), 500 g for ~ \$5.65 You can also use baking soda.	1.32 g	6.6 g	33.0 g
<b>Potassium phosphate (anhydrous) (KH<sub>2</sub>PO<sub>4</sub>)</b> Fisher Scientific sells a reagent grade (Catalog # S801462), 100 g for ~ \$8.50	0.23 g	1.15 g	5.75 g
<b>Sodium phosphate (dibasic anhydrous) (Na<sub>2</sub>HPO<sub>4</sub>)</b> Fisher Scientific sells a reagent grade (Catalog # S75218), 100 g for ~ \$9.45	0.19 g	0.95 g	4.75 g

To mix HBSS for testes preparation, use distilled or deionized water. A good alternative is bottled water that is commonly sold in stores, most brands of bottled water go through a series of filters and ozonation so they are basically distilled. Any brand bottled water should work. Using sterile water for testes preparation is not really necessary, but if you want you can sterilize it by boiling in a glass bottle with the lid loosened and place in a pot of boiling water. Be sure to cool it before using.

To mix HBSS for egg rinsing, carefully measure out the volume by adding a gallon or liter at a time to a large plastic garbage can and then mark the volume. After that, just fill the can to that level and add the chemicals for the known volume. Use well water for egg rinse HBSS. Unless the ionic concentration of your well water is really strange it should be fine for the egg rinse HBSS. Be sure HBSS is mixed well, and it has adequate oxygen, and don't use a chlorinated water source.

**RECIPES:****Hanks Buffered Salt Solution (HBSS) for metric units (liters)**

<b>Ingredient</b>	<b>1 liter</b>	<b>10 liter</b>	<b>100 liter</b>
<b>Sodium chloride (NaCl)</b> You can use uniodized salt from the grocery store, make sure you keep it dry.	7.94 g	79.4 g	794 g
<b>D-Glucose (dextrose)</b> Buy anhydrous (no water), Fisher Scientific sells a reagent grade (Catalog # S734181) 1 kg (1000 g or ~ 2.25 lbs) for ~ \$12.15	1.0 g	10.0 g	100.0 g
<b>Potassium chloride (KCl)</b> Fisher Scientific sells a reagent grade (Catalog # S773751), 500 g for ~ \$7.85	0.4 g	4.0 g	40.0 g
<b>Sodium bicarbonate (NaHCO<sub>3</sub>)</b> Fisher Scientific sells a reagent grade (Catalog # S78284), 500 g for ~ \$5.65 You can also use baking soda = sodium bicarbonate.	0.35 g	3.5 g	35.0 g
<b>Potassium phosphate (anhydrous) (KH<sub>2</sub>PO<sub>4</sub>)</b> Fisher Scientific sells a reagent grade (Catalog # S801462), 100 g for ~ \$8.50	0.06 g	0.6 g	6.0 g
<b>Sodium phosphate (dibasic anhydrous) (Na<sub>2</sub>HPO<sub>4</sub>)</b> Fisher Scientific sells a reagent grade (Catalog # S75218), 100 g for ~ \$9.45	0.05 g	0.5 g	5.0 g

To mix HBSS for testes preparation, use distilled or deionized water. A good alternative is bottled water that is commonly sold in stores, most brands of bottled water go through a series of filters and ozonation so they are basically distilled. Any brand bottled water should work. If you use water that comes in a 1 liter or 500 ml bottle it is already measured out. 1 liter = 1000 ml (two 500 ml bottles = 1 liter). Using sterile water for testes preparation is not really necessary, but if you want you can sterilize it by boiling in a glass bottle with the lid loosened and place in a pot of boiling water. Be sure to cool it before using.

To mix HBSS for egg rinsing, carefully measure out the volume by adding a gallon or liter at a time to a large plastic garbage can and then mark the volume. After that, just fill the can to that level and add the chemicals for the known volume. Use well water for egg rinse HBSS. Unless the ionic concentration of your well water is really strange it should be fine for the egg rinse HBSS. Be sure HBSS is mixed well, and it has adequate oxygen, and don't use a chlorinated water source.

**RECIPES:**

**PHOSPHATE BUFFERED SALINE (PBS)**

PBS is used to mix the powdered carp pituitary extract and LHRHa for injection. Any physiological saline (ionic makeup similar to body fluids) would work, but PBS is commonly used and can be purchased already made from many vendors. Although a recipe for PBS is included, it is recommended that pre-made PBS be purchased. It will save you time and you won't have to worry if you mixed it correctly.

Use distilled or bottled water to make PBS or dilute purchased PBS if necessary. Since you are going to inject this in the fish you would probably have fewer problems with infections if you sterilize the water as suggested earlier.

Fisher Scientific sells a 1X (1X means it is ready to use as is) PBS (cat # BP2438-4) which is 4 liters for \$80.75. This is the smallest amount of 1X PBS that they sell, and is enough to inject about 20,000 lbs of fish.

Fisher Scientific also sells a 10X (10X means it needs to be diluted 1 to 10 prior to use, so you add 1 part 10X PBS to 9 parts water to give PBS ready to use) (cat # BP399-500) for 500 ml (500 cc) for \$22.12. This will make 5000 ml (5000 cc) of ready to use PBS which is enough to inject about 25,000 lbs of fish. Therefore, it is suggested to buy the 10X and dilute it.

Fisher Scientific also sells a powder that you add 10 liters of water to make 10 L of ready to use PBS for \$32.04 (catalog # BP661-10).

Sigma Aldrich sells a similar liquid 500 ml, 10X PBS for \$22.70 (catalog # D1283). Sigma Aldrich also sells a powder, you add water, to make 10 L of PBS for \$9.90 Catalog # D5773).

An internet search will turn up lots of other vendors of PBS.

If you want to make your own PBS you can use the following recipe.

<b>Phosphate Buffered Saline*</b>	1 liter	1 gallon
Salt (NaCl)	8 g	30.3 g
Potassium Chloride (KCl)	0.2 g	0.76 g
Sodium phosphate (Na <sub>2</sub> HPO <sub>4</sub> )	1.44 g	5.45 g
Potassium phosphate (KH <sub>2</sub> PO <sub>4</sub> )	0.24 g	0.91
Adjust pH to 7.4 with dilute hydrochloric acid		
Autoclave to sterilize		

\* Same chemicals are listed for HBSS so you can use vendors listed for HBSS.

## INVESTIGATIONAL NEW ANIMAL DRUG (INAD) PERMIT

- An INAD permit is required for use of LHRH or Carp Pituitary (CCP) if you intend to sell fish for human consumption.
- Issued by U.S. Fish and Wildlife Service. Intent is to collect data to support FDA approval of drug.
- Cost is \$400.00 per INAD.
- Maximum dose allowed for LHRH is 100 ug/kg (45.5 ug/lb.) and 10 ug/kg (4.5 ug/lb) for CCP.
- Required forms (copies on following pages)
  - Study design worksheet
  - Receipt of drug report form
  - Drug inventory form
  - Results report form
- There is a 30 day withholding period before you can sell broodfish that have been injected with either CCP or LHRH.
- Monitor required – person reviews forms and sends to U.S.F.W.S.
- Results include # of females injected, # of females that release eggs, percent eyed eggs, and percent hatch.
- Requires some paperwork but most of the data collected will be useful to you.
- INAD is not designed to prevent you from using the hormones but is intended to help collect data so that these compounds can be approved for commercial use.

Contact person for INAD information:

Bonnie Johnson\*  
US Fish & Wildlife Service  
Aquatic Animal Drug Approval Partnership Program  
4050 Bridger Canyon Rd  
Bozeman, MT 59715  
406-587-9265 ext. 105  
406-582-0242 fax  
<http://fisheries.fws.gov/aadap/>

\* Bonnie is very helpful and the turnaround time on getting an INAD is quick, about 2 days.

## Common Carp Pituitary Clinical Field Trials

### CCP-W: Worksheet for Designing Study Numbers - Version 4

### Common Carp Pituitary INAD 8391

#### **INSTRUCTIONS**

1. Investigator must fill out Form CCP-W for each trial conducted under this INAD **before** actual use of Common Carp Pituitary. The Investigator is responsible that Form CCP-W is completed accurately.
2. Investigator should keep the original on file, and fax a copy to the Study Monitor for review.
3. After review, the Study Monitor will fax a copy to the Bozeman NIO for assignment of the Study Number.
4. The Bozeman NIO will review the worksheet, and then fax the assigned trial Study Number to both the Investigator and Study Monitor, at which time the trial may be initiated.
5. **Note:** Both Investigator and Study Monitor should sign and date Form CCP-W.

#### **SITE INFORMATION**

Facility			
Address			
Investigator			
Reporting Individual (if not Investigator)			
Phone		Fax	

#### **FISH CULTURE AND DRUG TREATMENT INFORMATION**

Fish species to be treated			
Average fish size (in)		Average fish weight (gm)	
Number of treated males		Number of treated females	
Number of control males		Number of control females	
Anticipated date treatment will be initiated			
Intended CCP dosage (mg/kg)	Female	Male	Estimated total amount of drug for proposed treatments (mg)
Number of injections	Female	Male	Injection interval (hrs or days)
Drug manufacturer			Drug lot number

## Worksheet for Designing Study Protocols - Version 4

**STUDY DESIGN:** Describe in detail the purpose of the clinical trial. For example you might compare dosage, or treated fish compared to untreated fish. Study design must be carefully focused and lend itself to rigorous evaluation. If more space is required to describe study details, title additional page(s) "Study Design" and attach them to this Worksheet.

Study designed by \_\_\_\_\_

### DISPOSITION OF TREATED FISH (Human Food Safety Considerations):

\_\_\_\_\_ Estimated time (days, months) from last treatment day to first possible harvest for human consumption

Investigator should initial here to indicate awareness that fish disposition must be in compliance with FDA-mandated withdrawal times as described in Section XV of the Study Protocol.

### WORKER SAFETY CONSIDERATIONS:

Investigator should initial here to indicate that all personnel handling drug have read Material Safety Data Sheet for Common Carp Pituitary and have been provided protective equipment, in good working condition, as described in the MSDS.

**Date Prepared:** \_\_\_\_\_ **Investigator:** \_\_\_\_\_

**Date Reviewed:** \_\_\_\_\_ **Study Monitor:** \_\_\_\_\_

## FORM CCP-1. Report on Receipt of Drug - Guide for Reporting Investigational New Animal Drug Shipments for Poikilothermic Food Animals

### INSTRUCTIONS

1. Investigator must fill out Form CCP-1 **immediately** upon receipt of CCP.
2. Investigator should keep the original on file, and send one copy to the Study Monitor for review.
3. Within 10 days of receipt, the Study Monitor should send a copy to the Bozeman NIO.
4. **Note:** Both Investigator and Study Monitor should sign and date Form CCP-1.

*The sponsor, U.S. Fish and Wildlife Service, submits a notice of claimed investigational exemption for the shipment or delivery of a new animal drug under the provisions of Section 512 of the Federal Food, Drug, and Cosmetics Act. The following information is submitted in triplicate:*

Name of Drug	<b>CCP</b>	INAD Number	<b>8391</b>
Proposed Use of Drug	To induce gamete maturation in a variety of fish species.		
Date of CVM Authorization Letter	April 15, 1996		
<b>Date of Drug Receipt</b>		<b>Amount of Drug Received</b>	
<b>Drug Lot Number</b>		<b>Study Worksheet Number</b>	
<b>Name of Investigator</b>			
<b>Address of Investigator</b>			
<b>Location of Trial</b>			
Pivotal Study (yes/no)		Non-pivotal Study (yes/no)	
<b>Approximate Number of Treated Animals</b>		<b>Approximate Number of Control Animals</b>	
<b>Number of Animals Used Previously<sup>1</sup></b>			
Study Protocol Number	8391		
<b>Approximate dates of trial (start/end)</b>			
<b>Species, Size, and Type of Animals</b>			
Maximum daily dose and duration	25 mg/Kg body weight		
Methods(s) of Administration	Injection		
Withdrawal Period	72 hrs for wild stock; 30 days for domestic (non-wild) broodstock.		

<sup>1</sup> To be filled out by the NIO

**Date Prepared:** \_\_\_\_\_ **Investigator:** \_\_\_\_\_

**Date Reviewed:** \_\_\_\_\_ **Study Monitor:** \_\_\_\_\_

**Date Reviewed:** \_\_\_\_\_ **Sponsor:** \_\_\_\_\_



## Common Carp Pituitary Clinical Field Trials

### CCP-3: Results Report Form - Version 4

### Common Carp Pituitary INAD 8391

**INSTRUCTIONS**

1. Investigator must fill out Form CCP-3 no later than 10 days after completion of the study period. Study Number must be recorded on all pages of Form CCP-3. Attach lab reports and other information.
2. If Common Carp Pituitary was not used under the assigned Study Number, fill out only the Site Information portion on this page, and skip to the end of page 3 and fill out only the "Negative Report" section.
3. Investigator should keep the original on file, and send a copy to the Study Monitor. Within 10 days of receipt, the Study Monitor should send a copy to the Bozeman NIO for inclusion in the permanent file.
4. **Note:** Both Investigator and Study Monitor should sign and date Form CCP-3.

**SITE INFORMATION**

Facility	
Reporting Individual	

**FISH CULTURE AND DRUG TREATMENT INFORMATION**

Drug lot number		Total amount drug used (mg)	
Fish species treated		Water temperature (°F)	
Drug dosage male (mg/kg body wt)		Drug dosage female (mg/kg body wt)	
Average fish weight (gm)		Average fish length (in)	
Number of treated males		Number of treated females	
Number of control males		Number of control females	
Treatment dates			
Number of injections/males		Number of injections/females	
Injection interval (hrs or days)		Treatment method (IP or IM injection)	
Spawning/evaluation interval (time from treatment until spawning)		Spawning/evaluation date	

### Hormone Results Record - Version 3

**INSTRUCTIONS**

1. Green females are those fish that have not ovulated or released their eggs, green males are those fish that are not actively spermiating.
2. Motility Score based on a scale of 0 - 4 (see Study Protocol Section VI).
3. Use additional copies of this form for additional treatment days.
4. Please attach additional documentation to further describe treatment procedures/evaluation.

Be sure the facility name is written here: \_\_\_\_\_

		TREATED FISH - Females						CONTROL FISH - Females					
Date Treated	Date Evaluated	# of Fish	Number Ripe	Number Green	% Ripe	% Eye-Up	% Hatch	Number of Fish	Number Ripe	Number Green	% Ripe	% Eye-up	% Hatch

		TREATED FISH - Males						CONTROL FISH - Males					
Date Treated	Date Evaluated	# of Fish	Number Ripe	Number Green	% Ripe	Milt/ fish (mL)	Motility Score	# of Fish	Number Ripe	Number Green	% Ripe	Milt/ fish (mL)	Motility Score

**RESULTS:** Describe in detail treatment results. Was treatment successful? If treatment did not appear to be successful, explain why not? Were there any mitigating environmental conditions that may have impacted treatment results? Were there any deviations from the Study Protocol? Attach pathology reports; Both Pre-and Post-Treatment.

**Toxicity observations:** Report any apparent drug toxicity including a description of unusual fish behavior.

**OBSERVED WITHDRAWAL PERIOD OF TREATED FISH:**

Observed withdrawal period: \_\_\_\_\_ no withdrawal period \_\_\_\_\_ 72 hours \_\_\_\_\_ 30 days

Estimated number of days between last treatment and first availability of fish for \_\_\_\_\_ human consumption (ensure this time period meets the withdrawal period).

**NEGATIVE REPORT** Common Carp Pituitary was not used at this facility under this Study Number during the reporting period. (Investigator should initial for negative reports as soon as the Study Number is known to be no longer needed or valid.)

**Date Prepared:** \_\_\_\_\_ **Investigator:** \_\_\_\_\_

**Date Reviewed:** \_\_\_\_\_ **Study Monitor:** \_\_\_\_\_

# Luteinizing Hormone-Releasing Hormone Analog Clinical Field Trials

## LHRHa: Worksheet for Designing Study Numbers - Version 4 LHRHa INAD 8061

**INSTRUCTIONS**

1. Investigator must fill out Form LHRHa-W for each trial conducted under this INAD **before** actual use of Luteinizing Hormone-Releasing Hormone analog. The Investigator is responsible that Form LHRHa-W is completed accurately.
2. Investigator should keep the original on file, and fax a copy to the Study Monitor for review.
3. After review, the Study Monitor will fax a copy to the Bozeman NIO for assignment of the Study Number.
4. The Bozeman NIO will review the worksheet, and then fax the assigned trial Study Number to both the Investigator and Study Monitor, at which time the trial may be initiated.
5. **Note:** Both Investigator and Study Monitor should sign and date Form LHRHa-W.

**SITE INFORMATION**

Facility			
Address			
Investigator			
Reporting Individual (if not Investigator)			
Phone		Fax	

**FISH CULTURE AND DRUG TREATMENT INFORMATION**

Fish species to be treated					
Average fish size (in)				Average fish weight (gm)	
Number of treated males				Number of treated females	
Number of control males				Number of control females	
Anticipated date treatment will be initiated				Estimated total amount of drug for proposed treatments (mg)	
Intended LHRHa dosage (ug/kg)		Female		Male	Method(s) of administration (Injection or Pellet implant)
Number of injections		Female		Male	Injection interval (hrs or days)
Drug manufacturer				Drug lot number	

## Worksheet for Designing Study Protocols - Version 4

**STUDY DESIGN:** Describe in detail the purpose of the clinical trial. For example you might compare dosage, or treated fish compared to untreated fish. Study design must be carefully focused and lend itself to rigorous evaluation. If more space is required to describe study details, title additional page(s) "Study Design" and attach them to this Worksheet.

Study designed by \_\_\_\_\_

### DISPOSITION OF TREATED FISH (Human Food Safety Considerations):

\_\_\_\_\_ Estimated time (days, months) from last treatment day to first possible harvest for human consumption

Investigator should initial here to indicate awareness that fish disposition must be in compliance with FDA-mandated withdrawal times as described in Section XV of the Study Protocol.

### WORKER SAFETY CONSIDERATIONS:

Investigator should initial here to indicate that all personnel handling drug have read Material Safety Data Sheet for Luteinizing Hormone-Releasing Hormone analog and have been provided protective equipment, in good working condition, as described in the MSDS.

Date Prepared: \_\_\_\_\_ Investigator: \_\_\_\_\_

Date Reviewed: \_\_\_\_\_ Study Monitor: \_\_\_\_\_

# FORM LHRHa-1. Report on Receipt of Drug - Guide for Reporting Investigational New Animal Drug Shipments for Poikilothermic Food Animals

## INSTRUCTIONS

1. Investigator must fill out Form LHRHa-1 **immediately** upon receipt of LHRHa.
2. Investigator should keep the original on file, and send one copy to the Study Monitor for review.
3. Within 10 days of receipt, the Study Monitor should send a copy to the Bozeman NIO.
4. **Note:** Both Investigator and Study Monitor should sign and date Form LHRHa-1.

*The sponsor, U.S. Fish and Wildlife Service, submits a notice of claimed investigational exemption for the shipment or delivery of a new animal drug under the provisions of Section 512 of the Federal Food, Drug, and Cosmetics Act. The following information is submitted in triplicate:*

Name of Drug	<b>LHRHa</b>	INAD Number	<b>8061</b>
Proposed Use of Drug	To induce gamete maturation in a variety of fish species.		
Date of CVM Authorization Letter	August 15, 2003		
<b>Date of Drug Receipt</b>		<b>Amount of Drug Received</b>	
<b>Drug Lot Number</b>		<b>Study Worksheet Number</b>	
<b>Name of Investigator</b>			
<b>Address of Investigator</b>			
<b>Location of Trial</b>			
Pivotal Study (yes/no)		Non-pivotal Study (yes/no)	
<b>Approximate Number of Treated Animals</b>		<b>Approximate Number of Control Animals</b>	
<b>Number of Animals Used Previously<sup>1</sup></b>			
Study Protocol Number	8061		
<b>Approximate dates of trial (start/end)</b>			
<b>Species, Size, and Type of Animals</b>			
Maximum daily dose and duration	100 ug/Kg body weight		
Methods(s) of Administration	Injection or pellet implant		
Withdrawal Period	10 days for injection; No release of fish treated with pellet implant.		

<sup>1</sup> To be filled out by the NIO

**Date Prepared:** \_\_\_\_\_ **Investigator:** \_\_\_\_\_

**Date Reviewed:** \_\_\_\_\_ **Study Monitor:** \_\_\_\_\_

**Date Reviewed:** \_\_\_\_\_ **Sponsor:** \_\_\_\_\_

# LHRHa Clinical Field Trials

## LHRHa-2: Drug Inventory Form - Version 4 Luteinizing Hormone-Releasing Hormone Analog INAD 8061

### INSTRUCTIONS

1. Investigator should initiate a new form LHRHa-2 **immediately** upon receipt of each shipment of Luteinizing Hormone-Releasing Hormone analog.
2. Form LHRHa-2 should be updated whenever drug is used, transferred, or discarded.
3. Investigator should save all copies of this form until the end of the calendar year, at which time they should maintain all originals on file and send one copy of the completed form(s) to their Study Monitor. Within 10 days of receipt, the Study Monitor will ensure accuracy and send a copy to the Bozeman NIO for inclusion in the permanent file.
4. **Note:** Both Investigator and Study Monitor should sign and date Form LHRHa-2.

Qty of LHRHa from \_\_\_\_\_ Facility \_\_\_\_\_ Reporting individual \_\_\_\_\_  
previous page (mg)

Date	Amount of new LHRHa received (mg)	Lot number of LHRHa received	Study Number	Amount of LHRHa used in treatment (mg)	LHRHa transferred (mg)	LHRHa discarded (mg)	LHRHa remaining on hand (mg)	Inventory by (Initials)
	xxxx	xxxx						
	xxxx	xxxx						
	xxxx	xxxx						
	xxxx	xxxx						
	xxxx	xxxx						
	xxxx	xxxx						
	xxxx	xxxx						
	xxxx	xxxx						
	xxxx	xxxx						
	xxxx	xxxx						
	xxxx	xxxx						
	xxxx	xxxx						
	xxxx	xxxx						
	xxxx	xxxx						
	xxxx	xxxx						
	xxxx	xxxx						
	xxxx	xxxx						

Date Prepared: \_\_\_\_\_ Investigator: \_\_\_\_\_

Date Reviewed: \_\_\_\_\_ Study Monitor: \_\_\_\_\_

## Luteinizing Hormone-Releasing Hormone Analog Clinical Field Trials

### LHRHa-3: Results Report Form - Version 4

### Luteinizing Hormone-Releasing Hormone Analog INAD 8061

#### **INSTRUCTIONS**

1. Investigator must fill out Form LHRHa-3 no later than 10 days after completion of the study period. Study Number must be recorded on all pages of Form LHRHa-3. Attach lab reports and other information.
2. If Luteinizing Hormone-Releasing Hormone analog was not used under the assigned Study Number, fill out only the Site Information portion on this page, and skip to the end of page 3 and fill out only the "Negative Report" section.
3. Investigator should keep the original on file, and send a copy to the Study Monitor. Within 10 days of receipt, the Study Monitor should send a copy to the Bozeman NIO for inclusion in the permanent file.
4. **Note:** Both Investigator and Study Monitor should sign and date Form LHRHa-3.

#### **SITE INFORMATION**

Facility	
Reporting Individual	

#### **FISH CULTURE AND DRUG TREATMENT INFORMATION**

Drug lot number		Total amount drug used (mg)	
Fish species treated		Water temperature (°F)	
Drug dosage male (ug/kg body wt)		Drug dosage female (ug/kg body wt)	
Average fish weight (gm)		Average fish length (in)	
Number of treated males		Number of treated females	
Number of control males		Number of control females	
Treatment dates			
Treatment method (injection or pellet implant)		Injection interval (hrs or days)	
Number of injections/males		Number of injections/females	
Spawning/evaluation interval (time from treatment until spawning)		Spawning/evaluation date	

### Hormone Results Record - Version 4

**INSTRUCTIONS**

1. Green females are those fish that have not ovulated or released their eggs, green males are those fish that are not actively spermiating.
2. Motility Score based on a scale of 0 - 4 (see Study Protocol Section VI).
3. Use additional copies of this form for additional treatment days.

Be sure the facility name is written here: \_\_\_\_\_

		TREATED FISH - Females						CONTROL FISH - Females					
Date Treated	Date Evaluated	# of Fish	Number Ripe	Number Green	% Ripe	% Eye-Up	% Hatch	Number of Fish	Number Ripe	Number Green	% Ripe	% Eye-up	% Hatch

		TREATED FISH - Males						CONTROL FISH - Males					
Date Treated	Date Evaluated	# of Fish	Number Ripe	Number Green	% Ripe	Milt/ fish (mL)	Motility Score	# of Fish	Number Ripe	Number Green	% Ripe	Milt/ fish (mL)	Motility Score

**RESULTS:** Describe in detail treatment results. Was treatment successful? If treatment did not appear to be successful, explain why not? Were there any mitigating environmental conditions that may have impacted treatment results? Were there any deviations from the Study Protocol? Attach pathology reports; Both Pre-and Post-Treatment.

**Toxicity observations:** Report any apparent drug toxicity including a description of unusual fish behavior.

**OBSERVED WITHDRAWAL PERIOD OF TREATED FISH:**

Observed withdrawal period : \_\_\_\_\_ no withdrawal period      \_\_\_\_\_ 10 days      \_\_\_\_\_ no release

Estimated number of days between last treatment and first availability of fish \_\_\_\_\_ for human consumption (ensure this time period meets the withdrawal period).

\_\_\_\_\_ **NEGATIVE REPORT** Luteinizing Hormone-Releasing Hormone Analog was not used at this facility under this Study Number during the reporting period. (Investigator should initial for negative reports as soon as the Study Number is known to be no longer needed or valid.)

Date Prepared: \_\_\_\_\_ Investigator: \_\_\_\_\_

Date Reviewed: \_\_\_\_\_ Study Monitor: \_\_\_\_\_

## NWAC Workshop: Production of Channel x Blue Catfish hybrid fry



Thad Cochran National Warmwater  
Aquaculture Center,  
Stoneville, MS  
May 25, 2005

### Induced Spawning of Catfish

- Two main methods:
  - 1) pair-spawning.
  - 2) group-spawning.
- Pair-spawning:
  - channel male and female put in a tank.
  - female injected with hormones.
  - females observed, when begin to lay eggs, female removed, sedated, stripped of eggs.
- Group-spawning:
  - group of channel females, with or without males, put in a large tank.
  - females injected with hormones, checked for release of eggs periodically.
  - females releasing eggs are stripped, other females are checked again later.
- Group-spawning better for commercial production.
- Tank water temperatures should be 76-82 F, good water quality, D.O., minimal disturbance.

### Overview- Group Spawning of Catfish

- A 3 day process.
- Day 1
  - Seine/select channel females, transport to tanks.
  - Weigh/inject females with 1st hormone dose (afternoon).
- Day 2
  - Weigh/inject females with 2nd hormone dose (9 to 10 AM). If splitting into two groups then do one group ~ 9-10 AM and other group ~ 1 PM.
  - Blue catfish males can be seined and put in tanks either day 1 or 2.
- Day 3
  - Kill blue males, remove testes, prepare sperm.
  - Check for ovulating females, sedate and strip ovulated females, fertilize (blue catfish sperm).
- 50 – 60 females/day with a 3-5 person crew. With > 30 fish, split time of 2<sup>nd</sup> injection, one group in morning, 1 group in afternoon to spread work load.

### Important Factors

- Good quality broodfish.
- Proper hormone dosage/timing.
- Proper preparation of testes/sperm.
- Good prediction of ovulation in females.
- Good egg stripping/fertilization technique.
- Aggressive egg treatment.

### Broodfish selection – channel catfish females

- Channel females  $\geq 3$  years or older.
- Broodfish management (feeding etc.) similar to pond-spawning.
- Higher stocking densities, fewer males than pond-spawning?
- Start when pond spawning starts on your or neighbors farm.
- No feed for ~ 3 days before spawning, full belly main method used to select female.
- To select, hold fish by tail, head down, keep fish with full, soft, swollen belly.
- Also look for swollen, red vent.
- Late in season check if they release eggs at seining (ovulated in pond), if so they are poor candidates.
- Don't inject poor quality females.
- Handle fish quickly but carefully.
- Minimize stress (low D.O., crowding, hot temperatures etc.).
- Transport to spawning tanks.



Channel catfish female, swollen belly, good candidate for injection.

### Broodfish selection – blue catfish males

- Blue males should be  $\geq 4$  years old.
- 'Generally' males have developed testes when females are ready.
- Difficult to predict testes development. Have ~ 2 times the males you need.
- Return unused males to ponds or use for next spawning cycle.
- Well developed testes: large, white, feathery.
- Kill males by blow to the head.
- Cut open belly, remove testes
  - located at the roof of the body cavity and run forward from the vent.
- Place testes in HBSS. DO NOT PUT IN FRESH WATER
- Rinse away blood with HBSS and remove blood clots and other tissue.
- Testes/sperm can be stored refrigerated 1-2 days in HBSS.
- Process testes by crushing/mashing to release sperm into HBSS.



Blue catfish male with abdomen opened for testes removal.



Good quality testes, feathery projections white in color.



Testes ready to be crushed and sieved through screen.



Crushing testes and sieving in HBSS to make sperm solution.

### Blue catfish males – testes preparation

- Crush testes through screen in HBSS.
- Pool sperm from 4-5 males and bring to a volume of 1.5 to 2 liters (~1 to 1.5 quarts) with HBSS.
- 4-5 ‘decent’ males for 35-40 spawns, others suggest 4-5 males for 15-20 spawns.
- Can split sperm solution in tubes, 1 tube per spawn (~ 3/4lb of eggs).
- Sperm activated (become motile) and fertilize eggs when released into fresh water.
- Motility lasts ~ 60 seconds, after sperm is no good.
- Keep in HBSS before use, similar salt content to body fluids.
- Activation of sperm before mixing with eggs will give poor fertilization.
- Check sperm motility with a basic microscope.
  - Add drop of water to a microscope slide.
  - Add small drop of sperm, put cover slip over the drop.
  - Look for movement, should be motile 30 – 60 seconds.
  - Microscope from school or veterinarian.



Sperm in HBSS, stored on ice.



Checking sperm motility, sperm is activated by water, when you add water you should see motile sperm.



Sperm solution being poured into tubes, stored on ice, and use 1 tube per spawn.

### Mixing and dosage: CCP

- CCP vendor: Stoller Fisheries.
- Sold as dry powder in 1 gram = (1000 mg) vials.
- Store powder in dark at room temperature.
- Inject  $\leq 1$ ml of hormone solution, mix accordingly.
- Mix 1 gram CCP in 27.5cc (1cc = 1ml) of sterile saline solution (such as PBS). Shake and let sit in refrigerator for 1-2 hours.
- A syringe or pipette can be used to measure volume.
- Concentration = 36.4 mg CCP/cc.
- Take 5.5cc of this initial solution (avoid particulate at bottom of tube), add 5.5cc to another tube with 16.5 cc of PBS, Gives 22cc of 9.1 mg CCP/cc, use for the 1<sup>st</sup> injection.
- Take off remaining solution from initial tube, measure volume, and transfer to a new tube (avoid particulate).
- There should be 22cc in the initial tube, but you will only get 18-19 cc. Add a little more PBS to the tube and shake, let settle and then add to what you just collected.
- Want a total of 22cc: if you got 19cc first time, add enough to get 3 more cc (19cc + 3cc = 22cc). Tube 3 = 22 cc of 36.4 mg/cc for Day 2 injection.
- Cost ~ \$0.90/lb female.

### CCP-continued

- Label concentrations on tube, store in refrigerator. Excess can be frozen, avoid repeated freeze thaws.
- Use 3 or 5 cc syringe with 18 or 20 ga. needle, inject sedated females behind pelvic fin.
- 0.91mg CCP/lb (2mg/kg) 1<sup>st</sup> injection, and 3.6 mg CCP/lb (8g/kg) 2<sup>nd</sup> injection.
- Mixed as described, 1 gram CCP give 22cc of Day 1 and 2 solutions, enough for ~ 220 lbs of females.
- Mixed as described, both 1<sup>st</sup> and 2<sup>nd</sup> injection are given at a 0.1cc/lb of body weight.
  - 5 lb female = 0.5cc Day 1 and 0.5cc Day 2 solution.
  - 10 lb female = 1.0cc of Day 1 and 1.0cc Day 2 solution.
- Following chart lists injection volumes based on fish weight. The volume injected/lb is the same on day 1 and day 2.
- To mix less CCP, need a sensitive scale.
- SRAC publication # 425 has equations for calculating dosage and volume if you want to use different injection volumes.

## Mixing CCP

**Step 1.** Add 1 g CCP and 27.5 cc of PBS to Tube 1. Mix and store in refrigerator for 1-2 hours. Concentration in Tube 1 is 36.4 mg CCP/cc.

Tube 1. 27.5 cc of 36.4 mg CCP/cc.



**Step 2.** Take 5.5cc from Tube 1, put in Tube 2 and then add 16.5 cc PBS to Tube 2. Label as Day 1 injection. Inject females with 0.1 cc/lb with Day 1 solution.



Tube 2. Day 1 Injection, 22 cc of 9.1 mg/cc.

**Step 3.** Transfer remaining solution from Tube 1 to Tube 3 (avoid particulate). Should be 22cc, but will get less ~ 17-19cc. Add enough additional PBS to Tube 1, mix, and remove to Tube 3 until you get a total volume of 22 cc in Tube 3.

For example: 19cc from Tube 1 to Tube 3 first time, add ~ 4cc PBS to Tube 1, mix, and pull off 3cc from Tube 1 add to Tube 3: total = 22 cc. Label as Day 2 injection. Inject females with 0.1 cc/lb with Day 2 solution.



Tube 3. Day 2 Injection, 22 cc of 36.4 mg/cc

## CCP Dosage Table

1 gram of CCP injects ~ 220 lbs of females. Mixed as suggested, the same volume will be injected at both injections. 1<sup>st</sup> injection is 0.91 mg/lb (2 mg/kg), 2<sup>nd</sup> injection is 3.64 mg/lb (8mg/kg).

Female weight (lbs)	Injection volume (cc)	Female weight (lbs)	Injection volume (cc)
3	0.30	8.0	0.80
3.5	0.35	8.5	0.85
4.0	0.40	9.0	0.90
4.5	0.45	9.5	0.95
5.0	0.50	10.0	1.00
5.5	0.55	10.5	1.05
6.0	0.60	11.0	1.10
6.5	0.65	11.5	1.15
7.0	0.70	12.0	1.20
7.5	0.75	12.5	1.25



Injecting female behind pelvic fin.



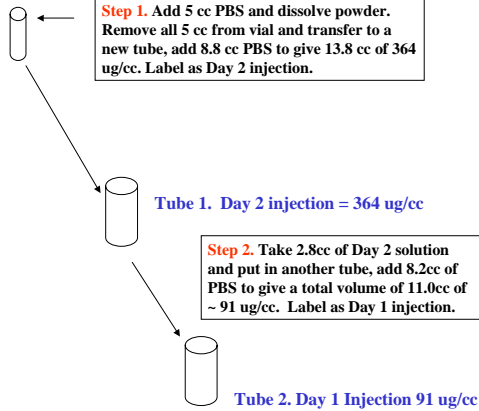
Close-up of injecting female.

## Mixing hormones/determining dosage: LHRH

- LHRHa vendor is Syndel.
- Dry powder: 1, 5, or 25 mg (1 mg = 1000 ug).
- Store powder in dark at room temperature.
- Mix with PBS, can be stored in refrigerator temporarily or frozen.
- Maximum U.S. FWS INAD dosage is 45 ug/lb, use maximum dosage.
- 5 mg (5000ug) injects ~ 110 lbs of females, cost is about \$0.90/lb female at this dosage.
- Inject  $\leq$  1ml of hormone, mix accordingly.
- Add 5cc PBS to 5 mg vial, dissolve all powder.
- Take 5cc of solution from vial (be careful of backpressure), add the 5cc to a clean tube, and add 8.8cc of PBS to the 5cc; = 13.8cc of ~ 364 ug/cc solution (label as Day 2 injection).
- Take 2.8cc of Day 2 solution, add to a new tube, add 8.2cc PBS to the 2.8cc (label as Day 1 solution) = 11.0cc of ~ 91 ug/cc.
- Mixed as described, 1<sup>st</sup> and 2<sup>nd</sup> injections given at 0.1 cc/lb female.
  - 5 lb female = 0.5cc of Day 1 and 0.5cc Day 2 solution.
  - 10 lb female = 1.0cc of Day 1 and 1.0cc Day 2 solution.

### Mixing LHRH - 5 mg vial

5 mg vial of LHRH from vendor.



You should have 11.0 cc of Day 1 solution and 11.0 cc of Day 2 solution. Inject at 0.1 cc/lb of female.

### LHRH Dosage Table

5 mg of LHRH will inject ~ 110 lbs of female broodfish. If mixed as suggested, the same volume will be injected at both injections. 1st injection gives ~ 9.1 ug LHRH/lb (20 ug/kg), 2<sup>nd</sup> injection ~ 36.4 ug LHRH/lb (80 ug/kg).

Female weight (lbs)	Injection volume (cc)	Female weight (lbs)	Injection volume (cc)
3	0.30	8.0	0.80
3.5	0.35	8.5	0.85
4.0	0.40	9.0	0.90
4.5	0.45	9.5	0.95
5.0	0.50	10.0	1.00
5.5	0.55	10.5	1.05
6.0	0.60	11.0	1.10
6.5	0.65	11.5	1.15
7.0	0.70	12.0	1.20
7.5	0.75	12.5	1.25

### Checking Females for Ovulation

- Look for egg release ~ 20 hours after 2<sup>nd</sup> injection at water temperature ~ 76-82 F.
- Time to ovulation varies.
- May be longer if water is colder or early in spawning season or shorter if water is warmer or later in spawning season.
- Put piece of 3-4" PVC in tank with fish. Look at pipe occasionally, released eggs will stick to the pipe, then start checking females for egg release.
- To check for egg release, gently crowd fish and catch with a dip-net.
- Roll fish on her back, apply pressure with thumb and forefinger just ahead of the vent, move back toward the vent.
- If eggs flow 'fairly' freely sedate fish, strip eggs.
- If eggs don't flow freely, check fish again 2-3 hours later.



Pipe placed in tank with injected females, check pipe for eggs as an indicator that females are starting to ovulate and release eggs.



Checking a female for egg flow to determine if she is ready to strip.



Another view of checking a female for egg flow to determine if she is ready to strip.

### Stripping Channel Catfish Females

- Sedate fish identified as ready (ovulated) with MS-222 (100 ppm) or Aqui-S (40 ppm).
- Rinse with hatchery water, then dry with a towel.
- If right handed, grasp just forward of tail with left hand, lay head of fish across right forearm, belly down, reach underneath fish with right hand.
- Place thumb and forefinger on opposite sides of belly, just ahead of pelvic fin, apply pressure as moving hand back toward the vent. Eggs should flow out fairly freely.
- Continue this milking motion, moving up farther as eggs empty from back of ovary.
- Collect eggs in a container (bucket etc.).
- Spray container with non-stick cooking spray, wipe out spray with paper towel, add ~ 1" of HBSS to bucket.
- Split eggs into 2 buckets if more than ~ 350 to 400 grams (about ¾ lb) to keep egg mass from being too thick.



Coat egg bucket with no-stick cooking spray, then add ~ 1 inch HBSS.



Female catfish being dried off prior to egg collection.



Female being stripped, eggs collected in HBSS in bucket.

### Fertilization

- Good eggs flow smoothly, are greenish-yellow, free of blood clots and clumps.
- Rinse eggs with HBSS to remove blood.
- Pick out the large clumps/clots.
- Pour off HBSS, add sperm solution (I use about 25 ml of sperm solution to ¾ lb of eggs).
- Stir eggs/sperm, add 1-2 inches of hatchery water, activates sperm and fertilization takes place.
- After 2-3 minutes fill bucket with hatchery water.
- Fresh water causes egg to swell and stick, continue to put new water on eggs until they stick together.
- Eggs need oxygen, so do not leave eggs in static water for long periods.
- After egg mass sticks (10-30 minutes) move to hatching troughs.
- Some eggs don't stick, usually not good quality, but may get some hatch. Put unsticky eggs in hatching basket lined with window screen.



Large volume of HBSS used for egg rinsing, made using well water.



Egg mass ready to move to hatching trough.

### INAD (Investigational New Animal Drug)

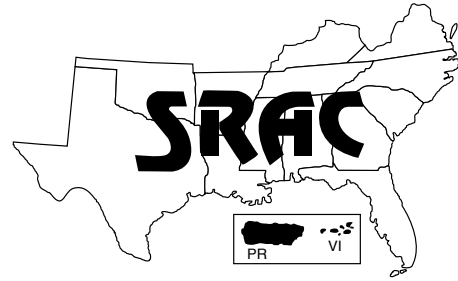
- **INAD required for use of LHRH or Carp Pituitary (CCP). Cost is \$400.00 per INAD.**
- **Issued by U.S. Fish and Wildlife Service.**
- **Maximum total dose is 45.5 ug/lb (100 ug/kg) for LHRH and 4.5 mg/lb (10 mg/kg) for CCP.**
- **Required forms (included in Appendix )**
  - Study design worksheet.
  - Receipt of drug report form.
  - Drug inventory form
  - Results report form
- **Monitor required – person that review forms and sends to U.S. F&W Service.**
- **Results include # of females injected, # of females that release eggs, % eyed eggs, and % hatch.**
- **Some paperwork but information is useful.**
- **Contact for INAD information is:**

Bonnie Johnson  
US Fish & Wildlife Service, Aquatic Animal Drug Approval Partnership Program  
4050 Bridger Canyon Rd  
Bozeman, MT 59715  
406-587-9265 ext. 105  
406-582-0242 fax  
<http://fisheries.fws.gov/aadap/>

### Egg Treatments

- **Start treatments 6-8 h post hatch**
- **Do not treat with any compound in the 40-46 h post fertilization window.**
- **Static treatment regime: 15 minute bath**
  - 3 formalin treatments per day at 100 ppm,
  - 3 iodine treatments per day with 100 ppm
  - 3 treatments per day with 2.5 ppm coppersulfate.
- **Flowing water treatment regime: Treatments in troughs with 3 gal/min flow (100 gallon trough).**
  - **Formalin (37% formaldehyde) 50 ml (3 times per day)**
  - **Povidone Iodine (1%) 50 ml (3 times per day) and may substitute one copper sulfate treatment in place of one iodine treatment copper Sulfate crystal 10 g (dissolve in 5 gal H<sub>2</sub>O pour across trough).**
  - **We have combined 2 copper and 2 iodine treatments daily with no apparent detrimental effects: copper early AM and early evening, iodine at noon and late PM.**

## Southern Regional Aquaculture Center



July 1998

# Production of Hybrid Catfish

Michael Masser and Rex Dunham\*

The mating or crossing of two different species is a process called hybridization, with the offspring known as hybrids. Probably the best recognized animal hybrid is the mule, which is a cross between a female horse and a male donkey. Hybrids can have some characteristics of both parents. Breeding hybrids with selected or favored characteristics of each parent is one of the goals of animal husbandry. When a hybrid has characteristics superior to both parents it is said to have hybrid vigor or positive heterosis which, of course, is the ultimate breeding goal.

Hybrids between different species of North American catfish (ictalurids) have been researched for more than 30 years. Of all these interspecific catfish hybrids (crosses between two distinct species) only one hybrid has characteristics that would favor commercial application. That hybrid is the channel catfish (*Ictalurus punctatus*) x blue catfish (*I. furcatus*) hybrid (denoted as the CxB hybrid). More specifically, it is the hybrid produced by crossing the female channel catfish with the male blue catfish. It is important

to note that the reciprocal cross, crossing the male channel catfish with the female blue catfish, does not have the same superior production characteristics of the CxB hybrid.

Research on CxB hybrids has demonstrated that they exhibit many commercially desirable characteristics. Compared to most commercially cultured strains of channel catfish, the CxB hybrid exhibits superior characteristics for the following traits:

- faster growth;
- better feed conversion;
- tolerance of low oxygen;
- increased resistance to many diseases;
- tolerance to crowded growth conditions in ponds;
- uniformity in size and shape;
- higher dressout percentages;
- increased harvestability by seining; and
- increased vulnerability to angling.

The simultaneous improvement of so many traits in a single line of catfish has not been possible through traditional genetic improvement programs, and there are no other examples of a single mating that has produced

improvement in so many commercially important traits.

The problem with commercializing CxB hybrids has been the inconsistency of seed production. The gametes (sperm and eggs) are compatible but the two species seldom mate with one another because of behavioral incompatibility, preferences in spawning environments, or some other factor(s). However, recent advances in artificial spawning and fertilization techniques have resulted in improved seed production. Another important development has been the use of different strains of channel catfish and blue catfish to make the hybrid. The crossing of different parental strains has produced genotypically distinct CxB hybrids with even more superior production characteristics. A genotype refers to the actual genes or genetic makeup that produces a trait.

### Genotype-environment interactions

The genotype-environment interaction is defined as the way the value of a genotype changes relative to other genotypes when the environment changes. In other words, the best genetic type for one set of environmental conditions may not be the best genetic

\*Extension Fisheries Specialist, The Texas A&M University System; and Professor, Auburn University, Alabama.

type for another set of environmental conditions, or the advantage of the particular genotype may increase or decrease in a second environment. In general, the genetic advantage of the CxB hybrid relative to channel catfish or blue catfish can increase or decrease depending on the environment in which they are grown.

### Appearance of hybrid

The channel catfish has a gentle slope from the tip of the snout to the base of the dorsal fin/spine and is spotted. It has high-set eyes; long, thick lateral barbels (whiskers); and a rounded anal fin with 24 to 26 fin rays. The blue catfish has a steep slope from the tip of the snout to the base of the dorsal fin, giving it the appearance of a "hump." It has no spots (except the Rio Grande strain); the eyes are set lower than the channel; it has short, light colored, thin lateral barbels and a straight or squared anal fin with 30 to 36 fin rays (Fig. 1).

The CxB hybrid looks much more like the male blue catfish parent than the channel catfish. The hybrid has a steep slope from the tip of the snout to the base of the dorsal fin, so it has the "humped" appearance. It has few or no spots (unless the Rio Grande blue is used in the cross); the eyes are set low; the barbels are intermediate and it has a straight anal fin with an intermediate number of fin rays (usually 28).

### Production characteristics of CxB hybrids

#### Growth and feed conversion

Early experiments demonstrated an approximately 20 percent improvement in growth of CxB hybrids over commonly cultured strains of channel catfish. However, recent research using selected parental strains of channel catfish and blue catfish have shown that growth of the CxB hybrid can be twice as fast as commercial strains of channel catfish, depend-

ing on environmental conditions. In general, CxB hybrids will demonstrate a 15 to 25 percent improvement in production over improved strains of channel catfish. This increased growth is due to a combination of increased food consumption and improved feed conversion efficiency.

In general, the CxB hybrid grows faster than the channel catfish, at both the fingerling and food-fish phases, when stocking density increases or when there are mixed size populations in the pond, as is the case in the multiple stocking and harvesting systems (multiple-batch system) currently used in most commercial catfish operations. Apparently the CxB hybrid is less affected by high density pond culture conditions than channel catfish. It is important to note the key words **pond culture**, as research has **not** shown that hybrids have any growth advantage under high density cage, tank, or raceway culture conditions. Actually, the growth of CxB hybrids is generally slower under cage, tank, or raceway culture conditions, which appears to be caused by a behavioral problem produced by the extremely high densities or confinement of these systems.

CxB hybrids have even more superiority over channel catfish under pond culture conditions at higher stocking densities. Fingerling CxB hybrids out-perform channel catfish fingerlings at all densities when grown to food-size fish. If fry are stocked at low densities (less than 60,000 per acre), the CxB hybrid grows at the same rate as channel catfish fingerlings until they reach a length of approximately 6 inches; after that the CxB hybrid grows faster. Generally, the CxB hybrid displays its superior growth in the second year and this growth is even more pronounced at densities of 7,000 to 22,000 per acre. Experiments comparing channel catfish to CxB hybrids suggests that the hybrids grow faster because they start feeding earlier

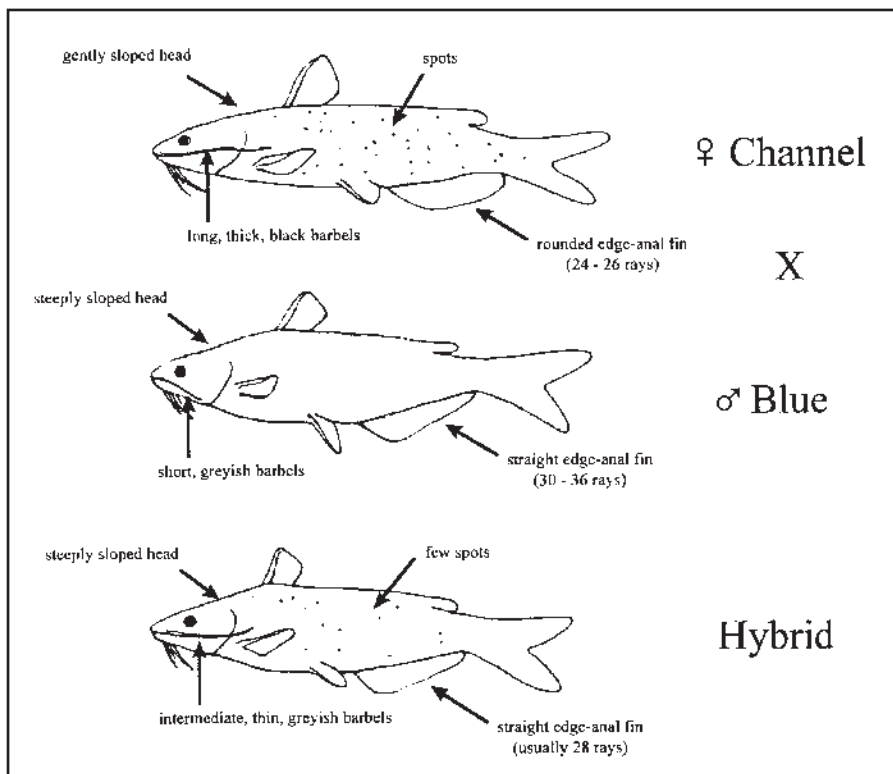


Figure 1. External characteristics of channel catfish, blue catfish and hybrid catfish.

in the spring. Growth rates in the summer and winter are approximately the same as in channel catfish.

Research has shown that feed conversion in CxB hybrids averages 10 to 15 percent better than channel catfish.

### Effects of parental strain

The parent strain or line of channel catfish and blue catfish affects the performance of the CxB hybrid. Growth of the CxB hybrid is affected by the genotype of the parents. In some experiments the CxB hybrid did not grow as fast as selected strains of channel catfish or blue catfish. However, in these cases the channel catfish or blue catfish was not the same strain used to make the hybrid. When CxB hybrids are produced by crossing superior strains of channel and blue catfish, these hybrids out-perform the parental strains for growth in open ponds. The strain of the parents also affects characteristics such as dressout percentage, body composition, seinability, angling vulnerability, and tolerance to low oxygen.

### Uniformity

The uniformity of growth and body shape of hybrids is superior to channel catfish, but not always superior to blue catfish. Dressout percentage and fillet percentage are generally higher for the CxB hybrid compared to channel catfish. However, some selected strains of channel catfish can have higher carcass yields than the hybrid.

CxB hybrid fingerlings produced at high densities (200,000 per acre) are not particularly uniform, but fingerlings produced at the lower densities have very uniform growth.

Research has shown that the body weight and length of the CxB hybrid are more uniform than that of channel catfish. Body shape is also more uniform in CxB hybrids than in channel catfish. This

should increase dressout percentage for processing plants using automatic processing equipment, because it allows the equipment to make more precise cuts and achieve maximum carcass yield. However, this uniformity can be a problem, as fish that are too uniform will not utilize all the processing lines simultaneously, possibly reducing processing plant efficiency. There has been some concern that automated processing machines would have to be reset to accommodate the hybrid's body shape. However, recent observations have shown that the hybrid can be processed by the same equipment used for channel catfish without any adjustments.

### Survival and disease resistance

Many disease problems in the channel catfish industry could be reduced or alleviated by the culture of CxB hybrids. Survival of the CxB hybrid has proven superior to channel catfish in all growth phases. In 20 years of research at Auburn University, fingerling production survival has averaged 85 percent and food-fish production survival has averaged 90 percent.

Although the CxB hybrid is not totally resistant to disease, it is more resistant than channel catfish to columnaris (*Flexibacter columnaris*), enteric septicemia of catfish (*Edwardsiella ictaluri*), aeromonas (*Aeromonas hydrophila*), Ich (*Ichthyophthirius multifiliis*), and channel catfish virus.

### Other commercially important traits

One of the most important traits of the CxB hybrid is its seinability. The hybrid is generally two to three times easier to catch by seining than channel catfish. This makes the hybrid better suited for all open-pond culture systems, particularly where seining of large channel catfish is problematic and ponds are seldom completely drained for harvest. Hybrids in hill ponds are also easier to trap than channel catfish, and more susceptible to angling. In fact, the

CxB hybrid is about twice as easy to catch by hook-and-line as channel catfish, a trait that has important implications for fee-fishing and recreational fisheries.

### Potential commercial problems

Seines and grading socks in current use do not work well with CxB hybrids. The hybrid has a small head and sharp spines inherited from the paternal blue catfish. This trait means that when hybrids are selectively graded by traditional equipment, they tend to gill themselves in the netting. Obviously, this creates a handling and stress problem. Alternative grading systems or seining procedures need to be developed for the hybrid. In the case of a single-crop system, this problem is solved by using a smaller mesh seine than normally used for channel catfish.

### Production expectations

Research and commercial trials have shown that CxB hybrids stocked in May or early June at 100,000 fry per acre (in Alabama, Mississippi) can yield 7,000 to 10,000 pounds of fingerlings per acre by late October. If these fingerlings (7+ inches) are stocked in a single-crop system at 3,000 fish per acre in the spring, then 5,500 pounds of marketable fish should be ready for harvest in the fall. If stocked at 5,000 fingerlings per acre, then 9,000 pounds of marketable fish should be ready for harvest in the fall. This production level requires near satiation feeding and adequate aeration to sustain the fish.

### Spawning methods

#### Open-pond spawning

Open-pond spawning is not a consistent way to produce CxB hybrids. Usually, no spawns occur, but there have been rare instances where up to 33 percent of the female channels have spawned. In many cases where spawns have been reported in

open ponds, it appears that the adults were not sexed properly (i.e., only female channels and male blues were stocked) and either channels spawned with channels or blues spawned with blues. No hybrids were actually produced. Therefore, the open-pond spawning of channel catfish with blue catfish to produce CxB hybrids cannot be recommended.

### Pen spawning

Pen spawning is a more consistent way of producing CxB hybrids. Pens should be similar to those used in traditional channel catfish spawning. Spawning pens are constructed next to the bank of the pond, using treated lumber driven into the pond bottom and plastic mesh or plastic coated wire mesh for sides. The mesh should allow for adequate water circulation (1/2- to 2-inch). Most spawning pens have dimensions of 4 x 6 or 4 x 8 feet. Spawning containers must be large enough to accommodate the size of the male blue catfish.

Male blue catfish and female channel catfish are individually paired in pens when the water temperature at the depth of the spawning container is between 75 and 82° F at sunrise. Male blue catfish should be placed in the pens a day or two before the female channel catfish. Female channel catfish chosen should show the classic signs of readiness for spawning, including a soft, distended belly and, preferably, a genital opening that is red and swollen. Female channel catfish are injected with human chorionic gonadotropin (HCG) at 1,100 to 1,800 IU/kg, lutenizing hormone (LHRH) at 100 mg/kg, or carp pituitary extract (CPE) at 2 mg/kg. **At the time this publication was written, not all of these hormones were approved by FDA for spawning fish. Please check with your Extension fisheries/aquaculture specialist for current registrations before using these hormones.**

If matings occur, spawns will usually be found 72 hours after the female is introduced into the pen. In rare cases, spawning has occurred up to a week after the female is introduced into the pen. Pen spawning success has been as high as 100 percent, but usually results in 0 to 20 percent success. Average spawning success over 14 years of continuous research at Auburn University is approximately 15 percent. Therefore, pen spawning is not considered a dependable method of producing CxB hybrid fry.

### Artificial spawning

Artificial spawning and fertilization can virtually guarantee production of CxB hybrid fry every year if properly conducted. The success of artificial spawning of female channel catfish should be 67 to 100 percent. The economics of this approach are still being examined. The following protocol has been the most successful in producing CxB hybrids.

Female channel catfish broodstock should be stocked at no more than 1,500 pounds/acre and fed a commercially manufactured 32 percent protein diet at 3 percent of body weight 3 days a week during summer and fall. Starting in January and continuing through spawning, the female broodstock should be fed a 48 percent protein brood fish feed that contains 60 percent fish meal twice a week, and liver (chicken, beef or pork) once a week. Females are spawned most successfully when water temperature is between 75 and 82° F at a depth of 2 to 3 feet at sunrise. Females selected for injection should have soft, distended bellies and, preferably, red, swollen genital openings. Selected females are placed in holding vats. Water flow and aeration in the vats should maintain total ammonia near 0 mg/l and dissolved oxygen above 6 mg/l. If possible, water temperature should be slowly increased to 80 to 82° F in the vats after the first injection.

First injections (called the “priming dose”) should be made in the evening of the same day the female brood fish are seined from the broodstock ponds. The longer fish are held in vats, the lower the number that will spawn, the poorer the egg quality, and the lower the number of fry that will hatch. It is essential to get an accurate weight of each female in kilograms so that precise doses of hormone can be calculated. Carp pituitary extract (CPE) has been the most consistently successful hormone for spawning channel catfish. The first injection of CPE at 2 mg/kg of female body weight should be administered intraperitoneally (into the body cavity under the base of one of the pelvic fins) between 6 and 8 pm. After the injection, the female should be placed in a separate vat for injected females. This process is repeated 12 hours after the first injection, with a second dose of 8 mg/kg of body weight (called the “resolving dose”). If water temperature in the brood fish pond has reached 82° F at sunrise prior to seining the fish, then the resolving dose should be reduced to 4 mg/kg of body weight.

Thirty-six hours after the first injection, the female channel catfish should be ready to ovulate and hand stripped if they have been held at 80 to 82° F. Just before stripping the females, the male blue catfish must be sacrificed and their testes removed. Males are usually euthanized by bludgeoning them across the head. The male blues must be surgically opened with an incision from the anal opening to about three-fourths of the way to the head along the belly. The testes are removed by gently cutting them from the mesentery connective tissue. Try to minimize bleeding, as this will obscure the view of the testes and make removal difficult (Fig. 2). Remove only the white portion of the testes. Gently dry the testes until all blood and moisture have been removed. This prevents activation of the sperm as the testes are macerated for

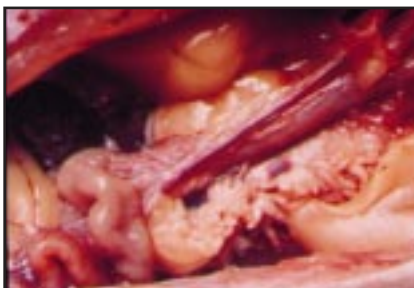


Figure 2. Testes of male blue catfish.

release of the sperm. Finally, weigh the testes with an accurate electronic gram scale.

Approximately 0.5 g of testes is needed for each 100 ml of eggs.

Females are removed from the tanks and placed in an anesthetizing solution of MS-222 at a concentration of 250 mg/l. When the fish are immobilized but the gills are still slowly moving, remove the female from the anesthetic solution and quickly rinse her with clean water to remove any remaining anesthetic. Carefully towel dry the female and wrap the towel around her head and upper body. Again, this is to remove moisture that might cause premature activation of the eggs and sperm. The female is held head up and tail down with the genital opening over a bowl lightly coated with vegetable shortening during the stripping process. Feel the belly region to locate the roll (mass) of eggs in each ovary. Gently but firmly press the belly with strokes beginning at the top of one ovary (one side at a time) and ending at the genital opening (Fig. 3). When it becomes difficult to get eggs to flow out of one ovary, begin stripping the other ovary and alternately strip each ovary as needed. The eggs are stripped into the dry, lubricated bowl. When no more eggs can be stripped or blood begins to come out of the genital opening, then stripping is completed and the female should be returned to a vat for recovery.

After the eggs have been stripped the male testes should be macerated and the sperm squeezed out of the testes and over the eggs at the rate of 0.5 g of testes per 100 ml of eggs. As a general rule, it takes one male to fertilize three to five females, depending on the size and quality of the male. Well oxygenated water is added to the egg and sperm mixture and the eggs are allowed to "harden" for 45 minutes with gentle agitation. Gentle agitation allows maximum contact between eggs and sperm. Water on the eggs should be exchanged every 15 minutes. Then the eggs are placed in a traditional catfish egg hatching trough. After this point the egg and fry are handled like channel catfish eggs.



Figure 3. Hand stripping of eggs from female channel catfish.

### Hybrid fertility

Most first generation (F1) CxB hybrids can spawn and produce a second generation (F2) of hybrids, or they can be crossed with a pure strain of channels or blues to produce "backcross" hybrids. However, the performance of these F2 and backcross hybrids is inferior to the F1 hybrids. Therefore, production of fry from CxB hybrid parents is not recommended.

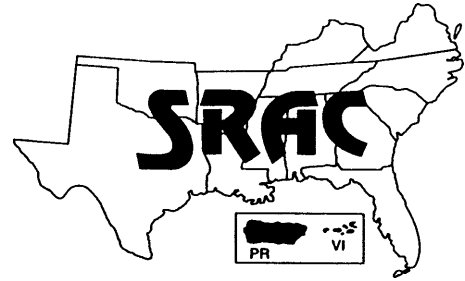
### Conclusion

Obviously, there is more expense involved in producing CxB hybrid catfish than channel catfish (produced by traditional pond spawning methods). The best estimate for this increased cost is approximately  $1/2$  cent per inch. In other words, where channel catfish purchased in commercial quantities presently cost about  $1\frac{1}{2}$  cents per inch, the CxB hybrids will cost 2 cents per inch. This additional cost should be recovered through the improved feed conversion efficiency, higher survival, and higher capture rates (seinability).

Use of the CxB hybrid in the catfish industry could improve farm productivity and profitability by reducing the incidence of disease, increasing total production (pounds/acre), improving seinability, and increasing dressout yield. In limited field studies, the CxB hybrid, even with the increased hatchery costs, could increase farm profitability by as much as 10 percent. The major impediments to the rapid expansion of hybrid production are the current lack of blue catfish brood stock, since virtually all of the catfish industry is based on channel catfish, and the need for improved spawning techniques.

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**Southern  
Regional  
Aquaculture  
Center**



November 1991

# Introduction to Hormone-Induced Spawning of Fish

R.W. Rottmann, J.V. Shireman, and F.A. Chapman\*

The demand for fish for food, recreation, and ornamental aquariums is steadily increasing. Natural fish populations have declined during the last several decades because of environmental degradation and over-fishing. This has resulted in an increased effort in the development of techniques for hatchery production of fish. Traditional aquaculture species such as trout, catfish, common carp, golden shiner, and goldfish reach sexual maturity and spawn in hatcheries or ponds, when conditions are appropriate.

However, a number of fish species that have or potentially have great economic significance for aquaculture do not reproduce spontaneously in captivity. Many of these fish spawn in environments that are nearly impossible to simulate in a hatchery. Hormone-induced spawning is the only reliable method to induce reproduction in these fishes.

Hormone-induced spawning of fish has been used for almost 60 years. Surprisingly, the same procedures, with only minor modifications, have been used to spawn an entire range of fishes from the ancient sturgeon and paddlefish to carp, catfish, salmon, sea bass,

redfish, snook, and mullet. In addition to breeding other desirable fish species, induced spawning can be used to:

- produce hybrids that are different from the parent species;
- produce sterile polyploid fish (for example, sterile triploid grass carp for aquatic weed control);
- synchronize reproduction of large numbers of fish for simultaneous spawning, thereby simplifying production and marketing of the fish;
- produce fry outside the normal spawning season for maximum hatchery production and to provide fish when the price and market demand is greatest; and
- maximize survival of fry under controlled hatchery conditions.

## Proper fish handling

The physical injury and physiological stress of capturing, handling, transporting, injecting, and holding brood fish can have a greater detrimental effect on spawning success than almost any other factor. Fish must be handled carefully and optimum water conditions must be maintained to minimize stress. The importance of proper handling and water quality cannot be overemphasized. Female brood fish ready for spawn-

ing are in a particularly delicate condition. When female fish are stressed or injured, they may undergo rapid physiological changes that can result in the breakdown (resorption) of eggs in the ovary. Crowding, dissolved oxygen depletion, rapid changes in temperature, and osmotic imbalance are well known causes of stress and must be avoided. Suboptimum conditions, while not immediately lethal, may stress brood fish, resulting in delayed mortality or failure to spawn. Reducing stress and injury to brood fish can greatly increase the success of hormone-induced spawning.

## Determine sexual maturity

The external appearance of brood fish has long been used to assess the stage of sexual development. In some species, males change in appearance during the spawning season. These physical changes make it relatively easy to identify sexually mature males. However, secondary female sex characteristics such as plumpness of the abdomen and redness of the vent are extremely subjective and can be misleading. Sampling the eggs and sperm of the brood fish eliminates the guesswork in determining the stage of sexual development.

Milt can usually be stripped from males of most species when they

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are ready for spawning by applying gentle pressure to the abdomen between the pelvic fins and the vent. Sperm viability usually can be determined by observing motility with a microscope.

Several methods are available to determine the developmental stage of the eggs in the fish's ovary. The diameter and appearance of the egg and the position of the nucleus in the egg are visual indicators of development. The steroid assay procedure determines the physiological response of the eggs to hormones. Both require that an egg sample be collected. The ovary can be sampled with either a rigid or flexible tube (catheter). An egg sample can also be taken by making a small incision along the belly or side of the fish. This technique is commonly used for sturgeon and paddlefish. An understanding of sperm viability and egg stage development will greatly improve the success of hormone-induced spawning of fish.

### Control of reproduction

Reproduction in fishes is regulated by external environmental factors that trigger internal mechanisms. The final event of the reproductive cycle, the release of eggs and sperm resulting in spawning, can be controlled by either placing the fish in an appropriate environment or by changing the fish's internal regulating factors with injected hormones or other substances. The internal mechanisms that regulate spawning are similar for most fishes. The external environmental factors that control reproduction, however, vary considerably among species.

For this reason, more is known about the internal regulatory mechanism of fish reproduction than the specific environmental requirements for spawning each species. Environmental factors that have been shown to play a significant role in the reproductive cycle are:

- photoperiod;
- water temperature;
- water quality (e.g., dissolved oxygen, pH, hardness, salinity, alkalinity);
- flooding and water current;
- tides and cycles of the moon;
- weather cycles (e.g., atmospheric pressure, rainfall);
- spawning substrate (e.g., aquatic plants, sticks, gravel, spawning caverns);
- nutrition;
- disease and parasites; and
- presence of other fish.

These factors do not function independently of each other, but are interrelated.

The internal mechanism that regulates the process of reproduction in fish is the brain-hypothalamus-pituitary-gonad chain (Figure 1). Hormone-induced spawning techniques influence this sequential mechanism at several levels, by either promoting or inhibiting the process. The primary substances used for hormone-induced spawning have been: (1) pituitary extracts and (2) purified gonadotropin to stimulate the ovaries

and testes; (3) LHRH analogs (LHRHa) alone or in combination with (4) dopamine blockers which enhance the potency of LHRHa to stimulate the pituitary; or (5) steroids to stimulate the gametes directly. The appropriate hormone preparation should be selected on the basis of the species to be spawned and the availability of the hormones.

### Preparation of hormones

The hormones must be mixed and stored properly to prevent contamination and preserve potency. The proper dosage must be calculated for the brood fish, and the optimum injection schedule must be used for best results. To calculate the proper dosage, (1) the recommended dose, (2) approximate weight of the brood fish, and (3) the desired volume of the injection must be determined. The quantity of hormone to be injected can then be calculated from the weight of each individual brood fish and the appropriate injection schedule.

### Taking the spawn

The eggs and milt of fish can be taken by several different methods: (1) tank spawning; (2) hand stripping, and (3) surgically removing the eggs. The method of choice depends on the fish species,

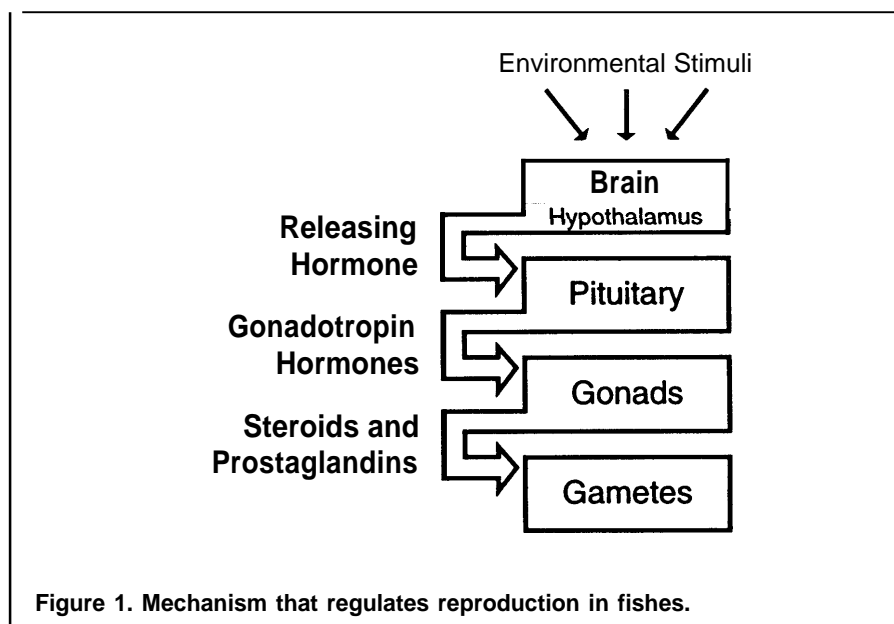


Figure 1. Mechanism that regulates reproduction in fishes.

hatchery facilities, experience and skill of the hatchery staff, and the desired manipulations of eggs, sperm, or fertilized eggs.

Tank spawning is the simplest method for obtaining a hormone-induced spawn. Brood fish of both sexes are placed together in the spawning tank following injection(s). The female ovulates when she is physiologically ready. The males stimulate the female to release the eggs and fertilize the spawn.

Hand stripping is commonly used for taking the spawn of many species of fish. Brood fish are separated by sex prior to hormone injection to prevent spawning in the holding tank. It is important to determine the exact time of ovulation when hand stripping. In many species, egg quality can deteriorate rapidly if the eggs are not taken shortly after ovulation. For most species, ovulation can best be verified by checking the female to determine when eggs flow freely from the vent. To strip the eggs, the fish is held slightly on her side, tail down; gentle hand pressure is applied to the abdomen, moving toward the vent. The stream of eggs is directed into a clean, dry bowl positioned so that water from the fish does not drip onto the eggs. It is important to insure that no water comes in contact with the eggs until after the milt is added and mixed. Water activates the sperm and also causes the opening through which the sperm enters the egg to close.

Because the internal anatomy of fish vary greatly, hand stripping may be difficult in some species. Sturgeon and paddlefish have no ovarian sac; the eggs are released into the abdominal cavity during ovulation. The best method for taking the spawn of these species is to surgically remove the eggs. For delicate species that seldom survive the rigors of hand stripping, humanely killing them and surgically removing the eggs may be the best option. In addition, more eggs can usually be obtained by this method than by hand stripping.

### **Fertilizing the spawn**

The eggs obtained by hand stripping or surgical removal are usually fertilized with fresh milt. Males are captured, wiped off, and held belly down over the bowl containing the eggs. The portion of the abdomen posterior to the pelvic fins is gently massaged to extrude the milt onto the eggs. Milt can be collected from males and stored up to three weeks prior to stripping eggs.

Ovulated eggs of many species such as white bass, sturgeon, paddlefish, and common carp become sticky after water is added. During natural spawning, this stickiness causes the eggs to become attached to rocks, sticks, or aquatic plants. Catfish eggs are connected by a sticky matrix that holds the eggs together in a mass. In the hatchery, this stickiness causes problems during incubation. Silt-clay, Fuller's earth, or bentonite

suspension, urea and salt solution, and tannic acid solution are preparations commonly used to deactivate the sticky layer of fish eggs. In addition, the gelatinous matrix of catfish egg masses can be dissolved with sodium sulfite so the eggs can be incubated in hatching jars.

Induced hatchery spawning of fish requires a continuous series of decisions, any of which if improperly made, can diminish or completely obliterate the success of the project. There are many ways to fail at each step and only a very few that are productive. Consistent performance requires strict attention to detail.

### **Additional SRAC fact sheets on induced spawning**

*SRAC 422 Capturing, Handling, Transporting, Injecting, and Holding Brood Fish for Induced Spawning*

*SRAC 423 Determining Sexual Maturity of Broodstock for Induced Spawning of Fish*

*SRAC 424 Hormonal Control of Reproduction in Fish for Induced Spawning*

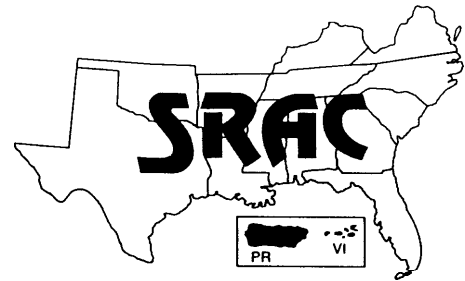
*SRAC 425 Hormone Preparation, Dosage Calculation, and Injection Techniques for Induced Spawning of Fish*

*SRAC 426 Techniques for Taking and Fertilizing the Spawn of Fish*

*SRAC 427 Induction and Verification of Triploidy in Fish*

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## Southern Regional Aquaculture Center



November 1991

# Capturing, Handling, Transporting, Injecting and Holding Brood Fish for Induced Spawning

R.W. Rottmann, J.V. Shireman, and F.A. Chapman\*

The physical injury and physiological stress of capturing, handling, transporting, injecting and holding brood fish can have a greater detrimental effect on spawning success than almost any other factor. Fish must be handled carefully and optimum water conditions must be maintained to minimize stress. The importance of proper handling and water quality cannot be overemphasized. Female brood fish ready for spawning are in a particularly delicate condition. When female fish are stressed or injured, they may undergo rapid physiological changes that can result in the break-down (resorption) of the eggs in the ovary. Fluctuations in temperature and low dissolved oxygen can hasten the resorption of eggs. Sub-optimum conditions, while not immediately lethal, may stress brood fish, resulting in delayed mortality or failure to spawn.

### Capture of brood fish

*Always check with your state conservation department to determine legal capture methods and obtain proper permits.*

#### Haul seine

Haul seines are effective for fishing large areas to collect brood fish. While this collection method is probably the most versatile and popular, the area to be seined

must be free of bottom snags or obstacles. Mesh size is dependent on fish size, and seine depth determines the depth of water that can be fished. Bag seines with extra lead weight are usually more effective than straight seines for brood fish capture.

Brood fish raised in ponds are usually captured by haul seine. Partial draining of the pond can simplify capture. Water level should not be lowered during the heat of the day. The temperature of shallow water increases rapidly, stressing the fish. Dissolved oxygen should be checked frequently, before and during draining. If the dissolved oxygen drops below 4 mg/L while draining brood fish ponds, stop draining, refill and aerate. If additional brood fish are still in the pond after seining, the pond should be flushed with fresh water to counteract the effects of disturbing the bottom sediments on reduced dissolved oxygen and the release of hydrogen sulfide and other toxic chemicals.

#### Dip net

Dip nets can be an effective capture method when brood fish are concentrated on the spawning grounds or in tailwater areas. This method inflicts minimal damage to fish. Brood fish raised in tanks are usually captured with dip nets. A crowding net should be used in large tanks to simplify capture. Dip nets are also used when trans-

ferring fish from seines, trap nets, hauling tanks, etc. Knitted small-mesh dip nets are recommended for handling brood fish to minimize scale loss and injury.

#### Gill net

Both stationary and drift gill nets are effective for brood fish capture. They allow a large area of water to be fished to determine migratory routes and areas of brood fish concentration. However, these nets often cause physical damage and stress. Mortality may be appreciable, but losses can be reduced by checking the nets and removing fish every 15 to 30 minutes.

#### Trap net

Trap nets such as pound nets, fyke nets and hoop nets have also been used to capture brood fish during the spawning migration. However, entrapment gear is usually limited in its application because of site selection, manpower requirements, mobility, and equipment expense.

#### Electrofishing

When brood fish are concentrated on the spawning grounds or in tailwater areas below dams, electrofishing is an efficient method of capture. Fish collected in this manner usually will not struggle vigorously and, in most instances, are immobile for 1 to 3 minutes during the critical pickup and initial

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transportation period. Various types of electrofishing gear can be adapted to widely differing habitats. Electrofishing, however, is restricted in all states and a special collecting permit is required.

### Angling

Mature fish may often be taken by hook and line in tailwater areas or where fish are concentrated and vulnerable to fishing pressure. For many fish species, this may be the only option for commercial fish farmers.

### Handling brood fish

Handling of brood fish should be kept to an absolute minimum. Gentleness when handling fish is of utmost importance to prevent physical injury and physiological stress. Damage to the slime (mucus) layer, scales, and skin of the fish can result in infection. It also causes excessive uptake of water by freshwater fish or loss of water from marine species (osmotic stress). Knitted fine-mesh dip nets are recommended for handling fish to minimize injury and scale loss; do not use knotted dip nets. Minimize the number of times the fish are lifted from the water, and work as quickly as possible when transferring fish. Time spent with the fish out of the water during handling can mean the difference between a good spawn, no spawn, or death.

### Transporting brood fish

Fish crowded in a transport tank can rapidly become stressed due to physical injury, deteriorating water quality, rapid changes in water temperature, and osmotic imbalance. Handling tanks in capture boats and transport trucks should be large enough to allow complete freedom of movement to the brood fish and have no sharp corners or edges that might injure the fish. Hauling tanks are usually aerated with oxygen (bottled or liquid) with air stones, electrical agitators (12-volt), or both. A high level of dissolved oxygen is crucial for rapid recovery of the brood fish from the oxygen debt incurred

during capture and handling. Oxygen bottled or liquid is recommended for reviving fish immediately following capture. For long hauls, water agitators should be used in addition to oxygen to drive off the carbon dioxide that accumulates in the water. The combination of aerators also provides a backup in case of system failure. Water in small containers can warm quickly, resulting in temperature shock. Warm water also reduces available oxygen and increases the metabolic rate of the fish, adding further physiological stress. Capture and transport brood fish during cool evening or early morning hours to minimize stress. Ice may be added to the water during hauling to prevent an increase in water temperature. Salt (0.3 -1.0 percent) may be used in the transport water to minimize osmotic stress and infection. Anesthetics have also been used successfully during transport of fish.

### Injecting brood fish

Females that have eggs in a sufficiently advanced stage of development for successful hormone-induced spawning (See *Determining Sexual Maturity of Broodstock for Induced Spawning of Fish*) should be injected as soon as possible. Any delay in injecting the brood fish greatly diminishes the chance for a successful spawn. While injecting the fish, every effort must be made to minimize stress and injury. It is unnecessary to remove the fish from the water when giving injections. Brood fish are usually captured and gently restrained in a net for injections. Avoid squeezing or forcefully holding the fish. Fish may be anesthetized with MS-222 if necessary. The fish may struggle less if a cloth is placed over its head. Underwater injections while the fish is stationary or swimming slowly are sometimes used for large, delicate species of fish because it eliminates the stress of forcible restraint.

### Holding brood fish at the hatchery

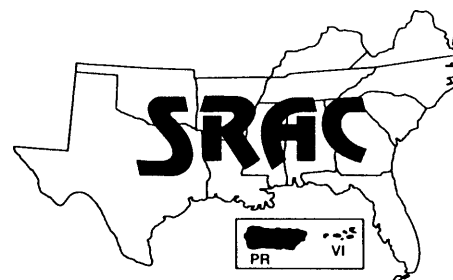
Environmental factors in the brood fish holding tank such as dissolved oxygen, water temperature and absence of disturbance to the fish following hormone injections are believed to play an important role for successful induced spawning. The handling stress and the physiological processes of final maturation of eggs and sperm increase the oxygen demand of the brood fish. High temperature accelerates egg maturation, resulting in an even greater oxygen demand by the fish. Elevated temperature will also increase the rate of development of disease organisms. However, if the temperature is too low, spawning will be delayed or in many cases completely inhibited.

Holding tanks should be large enough to allow complete freedom of movement to the brood fish. Round tanks or tanks with rounded corners are preferable because they minimize injury to the fish. Holding tanks should be covered to provide shading that will help quiet excitable species and prevent the fish from jumping to their death.

### Conclusions

Brood fish must be handled carefully to minimize physical injury and stress. Speed and gentleness during fish capture and handling are of utmost importance. Crowding, dissolved oxygen depletion, rapid changes in temperature, and osmotic imbalance are well known causes of stress and must be avoided when transporting fish. Females that have eggs in a sufficiently advanced stage of development for successful hormone-induced spawning should be injected as soon as possible. Any delay greatly diminishes the chance for a successful spawn. Dissolved oxygen content of the water, proper temperature, and absence of disturbance to the fish following hormone injection(s) are believed to play an important role for successful induced spawning.

## Southern Regional Aquaculture Center



1 November 1991

# Hormonal Control of Reproduction in Fish for Induced Spawning

R.W. Rottmann, J.V. Shireman, and F.A. Chapman\*

Reproduction in fishes is regulated by external environmental factors that trigger internal mechanisms into action. The final event of the reproductive cycle, the release of eggs and sperm resulting in spawning, can be controlled by either placing the fish in an appropriate environment or by changing the fish's internal regulating factors with injected hormones or other substances. The internal mechanisms that regulate spawning are similar for most fishes. The external environmental factors that control reproduction, however, vary considerably among species. For this reason, more is known about the internal regulatory mechanism of fish reproduction than the specific environmental requirements for spawning each species.

Many fish spawn in environments that are nearly impossible to simulate in a hatchery. Hormone-induced spawning is the only reliable method to induce reproduction in these fishes. Hormone-induced spawning of fish has been used for almost 60 years. Surprisingly, the same procedures, with only minor modifications, have been used to spawn an entire

range of fishes from the ancient sturgeon and paddlefish to carp, catfish, salmon, sea bass, redfish, snook, and mullet.

## Reproductive control mechanisms

Environmental factors that have been shown to play a significant role in the reproductive cycle are:

- photoperiod;
- water temperature;
- water quality (e.g., dissolved oxygen, pH, hardness, salinity, alkalinity);
- flooding and water current;
- tides and cycles of the moon;
- weather cycles (e.g., atmospheric pressure, rainfall);
- spawning substrate (e.g., aquatic plants, sticks, gravel, spawning mats, spawning caverns);
- nutrition;
- disease and parasites; and
- presence of other fish.

These factors do not function independently of one another, but are interrelated. While proper environmental conditions stimulate the reproductive process, unsuitable conditions can override any attempt at induced spawning.

The internal mechanism that regulates the process of reproduction in fish is the **brain-hypothalamus-pituitary-gonad** chain (Figure 1). This mechanism is complex, and additional scientific information is continually being added. The following is a simplified explanation.

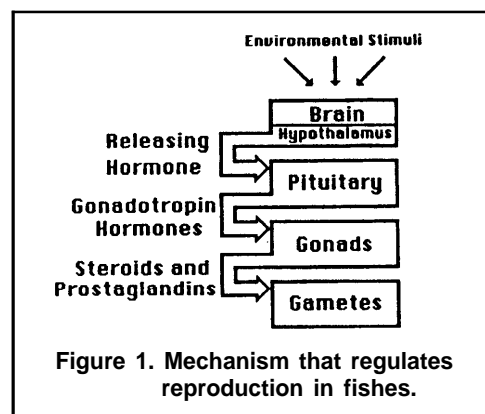


Figure 1. Mechanism that regulates reproduction in fishes.

Environmental stimuli are received and translated by the **brain**. Stimuli of reproductive importance are routed to a portion of the brain called the **hypothalamus**. The hypothalamus produces go-

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**gonadotropin releasing hormone (GnRH)** and also gonadotropin release inhibiting factors. Experimental results suggest that **dopamine** is a substance that inhibits the release of gonadotropin.

Gonadotropin releasing hormone (GnRH) is thought to stimulate the **pituitary**, a small gland located beneath the brain, to produce and release **gonadotropin hormones (GtH)**. Studies of induced ovulation of many fishes using injected pituitary extract indicate that an increased blood GtH is a prerequisite for ovulation.

Gonadotropin hormones (GtH) act on the ovaries and testes (**gonads**). **Steroids and prostaglandins** appear to be the local ovarian mediators of GtH action causing release of the eggs. Elevated blood levels of GtH trigger two distinct ovarian processes: 1) final maturation of the egg, which appears to be stimulated by steroids (e.g., progesterone) that are produced by the follicle, and 2) rupture of the follicle (ovulation), which evidently is stimulated by prostaglandins. Steroids also appear to induce spermiation in the male.

### Hormones for induced spawning

Hormone-induced spawning techniques influence this sequential mechanism at several levels, by either promoting or inhibiting the process. The primary substances used for hormone-induced spawning have been:

- **pituitary extracts** and
- **purified gonadotropins** to stimulate the ovaries and testes;
- **LHRH analogs (LHRHa)** alone or in combination with
- **dopamine blockers** which enhance the potency of LHRHa to stimulate the pituitary; or
- **steroids** to stimulate the gametes directly.

The appropriate hormone preparation should be selected on the basis of the species to be spawned and the availability of the hor-

mones. Many variables impact the ability of injected hormones to induce spawning, including: 1) condition of the fish; 2) stage of sexual maturity; 3) size of the fish; 4) previous spawning history, 5) water temperature; and 6) season of the year.

### Pituitary extract

The pituitary gland produces and stores gonadotropin hormones (GtH), which play a decisive role in ovulation and spermiation. Injected pituitary material bypasses the brain-pituitary link, acting directly on the ovaries and testes, providing the surge in blood GtH levels that normally precedes spawning (Figure 2).

just prior to spawning. This is a problem when adult fish are scarce.

Fresh pituitary glands should be used immediately or preserved by either freezing or acetone-drying. Glands can simply be placed in a sterile vial or plastic bag and stored in a freezer until needed. To acetone-dry, the glands are immediately placed in a vial with acetone. After collecting the required number, the acetone in which the glands were placed is drained off and replaced with fresh acetone. The acetone is again changed 8 to 12 hours later. After 24 hours in acetone, the glands are air dried on a paper towel. The dried pituitaries are then stored in a sealed

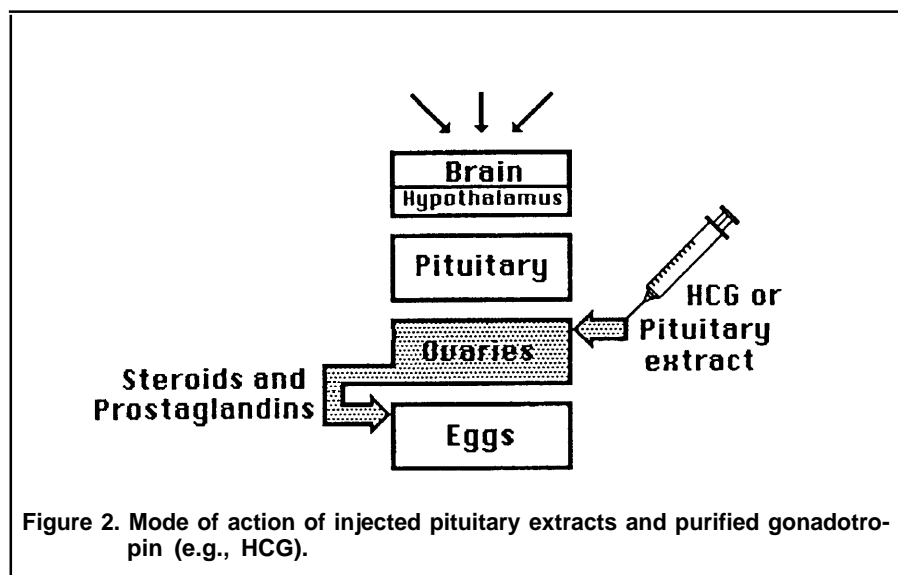


Figure 2. Mode of action of injected pituitary extracts and purified gonadotropin (e.g., HCG).

### Fresh pituitaries

The first material used for hormone-induced spawning was pituitary glands collected from fish of the species to be spawned. This method is still widely practiced today. To gain access to the pituitary, the top of the skull is removed with a saw or knife. When the brain is removed from the skull, the pituitary remains connected to the brain in some species, or more commonly, the gland is left behind on the base of the skull. Unfortunately, brood fish must be killed to obtain the pituitaries, because hormone content is greatest in sexually mature fish

clean vial at room temperature or in a desiccator. They can be stored in this manner for 5 to 8 years.

### Commercial pituitary extracts

Common carp pituitary or salmon pituitary extracts, available commercially, are widely used for induced spawning. These are crude acetone-dried powdered whole pituitaries. As with fresh pituitaries, these preparations also contain the pituitary tissue and hormones unrelated to reproduction, in addition to GtH. In general, the closer the donor species is related to the recipient fish, the greater the chance of successful induced

spawning. Therefore, carp, goldfish, Chinese carps, catfish, etc., are more likely to spawn successfully when injected with pituitary extracts from carp. Salmon, trout, etc., are more likely to spawn successfully when salmon pituitary is used. However, both are effective on a wide variety of fish species.

There is always uncertainty about the hormone potency of pituitary material. Hormone content necessary for spawning is greatest in sexually mature fish just prior to spawning and lowest in immature fish and mature fish after spawning. The potency of pituitary material can also be destroyed by improper collection, processing, or storage.

### Purified gonadotropin

To better quantify the hormone injected, purified gonadotropin hormones are frequently used.

Human Chorionic Gonadotropin (HCG) is the most common purified gonadotropin hormone used for induced spawning. In fish, the injected gonadotropin mimics the natural GtH produced by the fish's pituitary. Just as is the case with pituitary extracts, purified hormones such as HCG bypass the brain-pituitary link, acting directly on the ovaries and testes (Figure 2). HCG has been used to spawn fish such as striped bass, white bass, red drum, catfish, and mullet.

### HCG + pituitary extract

HCG, however, is not effective on all species. HCG has been used in combination with common carp pituitary extract; for some species, the combination has shown to have improved potency than either preparation used alone. The two hormones can be prepared and injected separately, or the HCG solution can be used when mixing the pituitary extract.

### Luteinizing hormone-releasing hormones

Injections of mammalian Luteinizing Hormone-Releasing Hormone (LHRH) have been used experi-

mentally to mimic the fish's GnRH. However, a comparatively large dose and frequent injections were required. Recently, synthetic LHRH analogs, referred to as LHRHa or GnRH<sub>a</sub>, have been manufactured. These hormones last longer in the fish's system and have potent stimulator effects on ovulation and spermiation in fishes. Therefore, only one or two small doses are needed to induce spawning. LHRHa stimulates the fish's own pituitary to produce and release the GtH necessary for spawning (Figure 3). LHRHa has been used to induce ovulation in a wide range of fishes. One of the synthetic analogs that has been used successfully is **Des-GLY<sup>10</sup>, [D-Ala<sup>6</sup>]-LH-RH Ethylamide**.

hibiting the binding of dopamine. Experimental results indicate that the use of dopamine blockers prevents this negative feedback, enhancing the effectiveness of LHRHa for these species (Figure 4).

Because of the tremendous variety of aquarium species and their individual spawning requirements, as compared to food and sport fish, development of hatchery spawning technology has been more difficult. Many ornamental species have had to be imported from wild populations. The use of LHRHa with dopamine blockers has helped change this situation.

Haloperidol {4-[4-(4-chlorophenyl)-4-hydroxy-piperidino]-4'-fluorobutyrophenone} has been used recently as a dopamine blocker in ornamental fishes and tested experimentally for food and sport fish production.

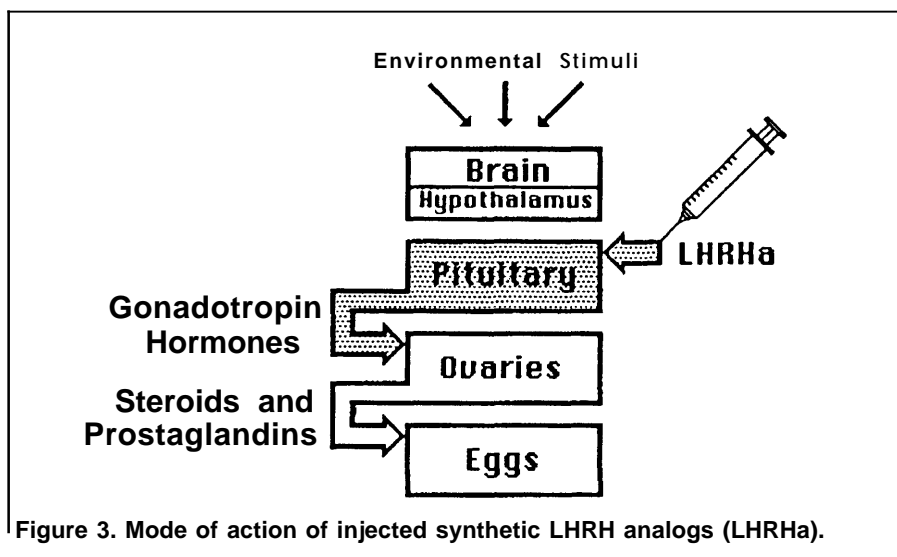


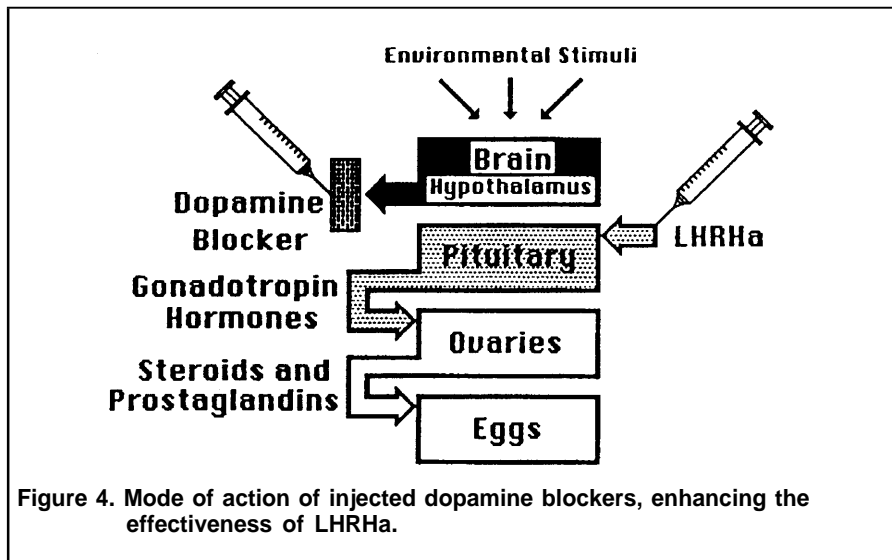
Figure 3. Mode of action of injected synthetic LHRH analogs (LHRHa).

### LHRHa + dopamine blockers

Although LHRHa has not been shown to be species specific, some fish do not respond to injections of LHRHa alone (e.g., goldfish, red-tailed black shark, rainbow shark). Dopamine inhibits the release of hormones from the pituitary, effectively blocking the pituitary's positive response to injected LHRHa. There is a family of drugs that act as dopamine blockers, either by preventing the release or by in-

### Steroids

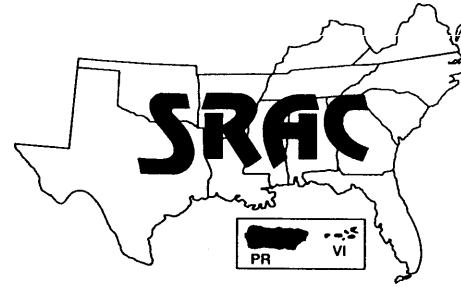
Several steroids (e.g., progesterone and testosterone) have been tried experimentally for inducing maturation, ovulation and spermiation in fishes. However, there appears to be little indication of widespread importance of these substances for hormone-induced spawning.



## Conclusions

Reproduction in fishes is regulated by both internal mechanisms within the fish and external environmental factors. The environmental factors trigger the internal mechanisms into action. The internal mechanism that controls the process of reproduction in fish is the brain-hypothalamus-pituitary-gonad chain. Hormone-induced spawning techniques influence this sequential mechanism at several levels, by either promoting or inhibiting the process. The primary substances used for hormone-induced spawning are: (1) pituitary extracts and purified gonadotropin to stimulate the ovaries and testes; or (2) LHRH analogs (LHRHa) alone or in combination with dopamine blockers which enhance the potency of LHRHa to stimulate the pituitary.

## Southern Regional Aquaculture Center



November 1991

# Hormone Preparation, Dosage Calculation, and Injection Techniques for Induced Spawning of Fish

R.W. Rottmann, J.V. Shireman, and F.A. Chapman\*

Induced spawning of fish often requires hormone injections. Preparations used for hormone-induced spawning must be mixed properly, and the correct amount must be used to be effective. This publication describes common techniques used to calculate the hormone concentration and dose, prepare hormone solutions, and inject the fish for induced spawning. To calculate the proper dosage, 1) the recommended dose, 2) approximate weight of the brood fish, and 3) desired volume of the injection must be determined. The quantity of hormone to be injected can then be calculated from the weight of each individual brood fish and appropriate injection schedule.

The hormones must be mixed and stored properly to prevent contamination and preserve potency. Always use sterile syringes, needles, vials, and utensils when mixing, injecting, or storing hormones. Non-sterile items should be boiled in water for at least 10 minutes. Water or saline solution (0.7 percent NaCl) should be boiled before mixing with the hormone for injections. If the hormone is to be stored after mixing, it is recommended that bacteriostatic water or bacteriostatic physiological saline, which contains antibacterial ingredients, be used. Using sterile procedures,

**At this time the Food and Drug Administration has failed to clear any hormones for use as spawning aids in food fish. Check with your state aquaculture specialist for the latest information on legal implications.**

hormones that have been mixed with bacteriostatic water or saline can be stored in a freezer for several years without loss of potency. However, bacterial contamination or improper storage can quickly destroy the potency of the hormone. All hormone preparations must be properly labeled to avoid confusion. The volume of hormone should be divided into small (1-2 cc) plastic vials before storing in the freezer so that only the required amount need be defrosted, saving the potency of the remaining vials.

### ■ Recommended dose

To determine the amount of hormone to be injected, first check the literature for the recommended dose and injection schedule for the fish species. Hormone doses are given in units of: 1) weight such as kilogram (kg), gram (g), and microgram ( $\mu\text{g}$ ); 2) volume such as cubic centimeter (cc) or milliliter (ml) and microliter ( $\mu\text{l}$ ); or 3) biological activity such as International Units (IU). Recommended hormone doses vary considerably for different species of fish and even from different

hatcheries spawning the same species. Table 1 provides doses for various hormones used for induced spawning. These values represent a sample of a number of studies conducted by many workers. The actual dose used may be tailored for specific conditions encountered at your hatchery. It is usually better to slightly over-estimate the dose. This is especially true when using pituitary material because potency can vary. When first evaluating a particular dose and schedule, it is best to try it on a small number of fish to determine its effectiveness.

### ■ Weight of brood fish

When calculating the hormone dose, it is necessary to determine the weight of the fish. Individual fish can be weighed in a container of water using a hanging scale or platform scale. However, this method may be impractical for large species. It is preferable to estimate the weight of the fish rather than inflicting damage by weighing. A length-weight relationship for the species of fish can often be obtained from the literature or can be calculated from fish that have died, fish not used for spawning, or fish that have already spawned. The length of brood fish can be determined with the fish under water in the holding tank using a measuring stick or tape. The approximate weight is calculated, averaged with the other brood fish, and rounded up to the next figure.

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**Table 1. Commonly used hormone concentrations in weight and International Units (IU) per fish body weight, time interval in hours (h) between injections, and percentage of total dose in each injection.**

Hormone	Total dose	Interval	Percentage
Pituitary	4-8 mg/kg	6-24 h	20% - 80% 33% - 67%
HCG	300-1,800 IU/kg	0 h 24 h	100% 33% - 67%
HCG + Pituitary	430-2,300 IU/kg 5-9 mg/kg	6-24 h 6-24 h	10% - 90% + 100%
HCG & Pituitary Mixture	60-1,000 IU/kg + 2.5-12 mg/kg	6-24 h	20% - 80% 50% - 50%
LHRHa	5-100 µg/kg	0 h 6-18 h (48 -72 h for trout)	100% 20% - 80% 50% - 50%
LHRHa+	5-100 µg/kg	6-18 h	20% - 80% 50% - 50% +
Haloperidol	0.1-1 mg/kg		100%

### ■ Volume of injection

The volume of the injection is usually measured in cubic centimeters (cc) which are identical in volume to milliliters (ml). Injections should be small enough to avoid injuring the fish, yet large enough to be accurately measured. The total volume of injections for large species such as striped bass, sturgeon, paddlefish or grass carp should be no more than 1-2 cc. If the volume is greater than 1 cc, it should be split and injected in different locations. Small ornamental aquarium fish should be injected with no more than 0.1-0.2 cc.

### Preparation of hormone solutions

To calculate the concentration of hormone to be mixed, the recommended dose (Table 1), multiplied by the approximate weight of individual brood fish, is divided by the desired volume of the injection.

$$\text{Hormone Concentration} = \frac{\text{Recommended Dose} \times \text{Fish Weight}}{\text{Desired Injection Volume}}$$

#### 1) Pituitary extract

Fresh or dried pituitary material is usually mixed with sterile or boiled water or saline (0.7 percent NaCl)

approximately 1 hour before injection. When mixing pituitary material that is to be stored in a freezer, bacteriostatic water or bacteriostatic physiological saline is recommended to minimize contamination.

A mortar and pestle or hand tissue grinder is used to pulverize the dried pituitary material. The liquid is added a little at a time, mixing thoroughly to produce a uniform suspension. The tissue residue should be allowed to settle to the bottom of the vials while on ice or in a refrigerator, because once in solution, the hormone has a relatively short shelf life at room temperatures. If the preparation is to be stored in a freezer, the tissue residue will settle to the bottom during freezing. Only the liquid above the tissue residue is injected. Dried pituitary extract is usually injected into the muscle at 4-8 milligrams/kilogram (mg/kg) of fish body weight.

For example, if the recommended dose of pituitary extract is 4 mg/kg, the fish weigh approximately 6 kg, and the desired volume of injection is 1 cc. Then the concentration of hormone is equal to 4 mg/kg, multiplied by 6 kg, divided by 1 cc. In this case, 24 mg/cc.

$$\text{Hormone Concentration} = \frac{4 \text{ mg/kg} \times 6 \text{ kg}}{1 \text{ cc}} = 24 \text{ mg/cc}$$

### ■ Mixing hormone to be injected immediately

If the reconstituted hormone is to be used immediately, the total quantity of hormone required for all brood fish to be injected is calculated and mixed together. The quantity of hormone is then dispensed by volume according to individual brood fish weight. The quantity of hormone to be weighed is determined by multiplying the recommended dose by the total weight of brood fish.

$$\text{Total Weight of Hormone} = \frac{\text{Recommended Dose} \times \text{Total Weight of Fish}}$$

How many milligrams (mg) of hormone are needed to spawn ten fish with an average weight of 6 kg, if the recommended dose is 4 mg/kg? The total fish weight is 10 fish x 6 kg or 60 kg. Then the total weight of hormone is equal to 4 mg/kg, multiplied by 60 kg, or in this case, 240 mg.

$$\text{Total Weight of Hormone} = 4 \text{ mg/kg} \times 60 \text{ kg} = 240 \text{ mg}$$

It is advisable to add an additional 10 to 15 percent to the total for loss or wastage while mixing and filling syringes.

$$\text{Additional 10\%} = 240 \text{ mg} \times 0.1 = 24 \text{ mg}$$

$$\text{Total Weight of Hormone} = 240 \text{ mg} + 24 \text{ mg} = 264 \text{ mg}$$

What volume of liquid solvent should be mixed with the hormone to obtain the desired concentration? The volume of liquid solvent required is equal to the total weight of hormone (264 mg) divided by the desired hormone concentration (24 mg/cc) or in this case, 11 cc.

$$\text{Volume of Liquid} = \frac{\text{Total Weight of Hormone} = 264 \text{ mg}}{\text{Hormone Concentration} = 24 \text{ mg/cc}} = 11 \text{ cc}$$

### ■ Mixing hormone to be stored

If the reconstituted hormone is to be stored in a freezer, the weight of

hormone to be mixed with a vial of bacteriostatic saline or water is calculated by multiplying the hormone concentration calculated in the example above (24 mg/cc) by volume of the vial (30 cc); 720 mg of hormone are then weighed and mixed in a 30 cc vial.

$$\frac{\text{Quantity of Hormone}}{\text{Hormone Concentration} \times \text{Volume}}$$

$$\frac{\text{Quantity of Hormone}}{24 \text{ mg/cc} \times 30 \text{ cc}} = 720 \text{ mg}$$

The quantity of hormone should be divided into small (1-2 cc) plastic vials before storing in the freezer so that only the required amount need be defrosted, saving the potency of the remaining vials. The hormone preparations should be properly labeled according to hormone type, concentration and date mixed to avoid confusion.

## 2) Human chorionic gonadotropin (HCG)

HCG is measured not by weight but biological activity called International Units (IU). It is usually available in sterile vials containing 5,000 or 10,000 IU. The unopened vial of hormone should be stored in a refrigerator at 35-45°F (2-7°C). HCG is mixed with bacteriostatic water, usually supplied with the hormone. The hormone solution should either be used immediately or divided into small volumes and kept in a freezer, or potency may be reduced. Intramuscular injections (in the muscle) of 300 to 1,800 IU of HCG per kg of fish body weight are usually recommended.

What volume of liquid solvent should be added to a vial of HCG? Again, the concentration of hormone to be mixed, must be calculated from the recommended dose (Table 1), the approximate weight of the individual brood fish, and the desired volume of the injection.

$$\frac{\text{Hormone Concentration}}{\text{Recommended Dose} \times \text{Fish Weight}} = \text{Desired Injection Volume}$$

For example, if you have a 10,000 IU vial of HCG, the recommended dose is 400 IU/kg, the fish weigh approximately 1 kg, and the de-

sired volume of injection is 0.2 cc. Then the desired concentration of hormone is equal to 400 IU/kg, multiplied by 1 kg, divided by 0.2 cc, in this case, 2,000 IU/cc.

$$\frac{\text{Hormone Concentration} = 400 \text{ IU/kg} / 1 \text{ kg} = 2,000 \text{ IU/cc}}{0.2 \text{ cc}}$$

What volume of water must be mixed with the 10,000 IU vial to get the desired concentration? The required volume of liquid is equal to the amount of hormone in the vial (10,000 IU), divided by the desired concentration (2,000 IU/CC). This equals 5 cc.

$$\frac{\text{Volume of Liquid} = \frac{\text{Amount of Hormone} = 10,000 \text{ IU}}{\text{Hormone Concentration} = 2,000 \text{ IU/cc}}}{= 5 \text{ cc}}$$

## 3) Luteinizing hormone-releasing hormone analog (LHRHa)

One of the synthetic LHRH analogs that has been used successfully is Des-GLY<sup>10</sup>[D-Ala<sup>6</sup>]-LH-RH Ethylamide. LHRHa is available in a pre-weighed quantity in a sterile vial. The unopened vial of hormone should be stored in a freezer. The hormone should be mixed with bacteriostatic water and either used immediately or divided into small volumes and kept in a freezer. LHRHa is usually injected in the muscle at 5 to 10 micrograms/kilogram (µg/kg) of fish weight. However, doses as high as 100 µg/kg and as low as 1 µg/kg have been successful.

What volume of liquid solvent must be added to a vial containing LHRHa to obtain the desired concentration of the hormone? Again, the concentration of hormone to be mixed is calculated from the recommended dose (Table 1), the approximate weight of the individual brood fish, and the desired volume of the injection.

$$\frac{\text{Hormone Concentration} = \text{Recommended Dose} \times \text{Fish Weight}}{\text{Desired Injection Volume}}$$

For example, if you have a 1 mg (1,000 µg) vial of hormone, the recommended dosage is 10 µg/kg,

the fish weigh approximately 50 g each, and the desired volume of injection is 0.1 cc. Then the desired concentration of hormone is equal to 10 µg/kg, multiplied by 50 g, which is converted to 0.05 kg, divided by 0.1 cc (5 µg/cc).

$$\frac{\text{Hormone Concentration} = 10 \text{ µg/kg} \times 0.05 \text{ kg} = 5 \text{ µg/cc}}{0.1 \text{ cc}}$$

The required volume of liquid is equal to the weight of hormone in the vial, divided by the desired concentration. This equals 200 cc.

$$\frac{\text{Volume of Liquid} = \frac{\text{Weight Of Hormone} = 1,000 \text{ µg}}{\text{Hormone Concentration} = 5 \text{ µg/cc}}}{= 200 \text{ cc}}$$

Rather than mixing such a large volume of hormone, three dilutions are made. First draw 10 cc of bacteriostatic water in a syringe and then mix the liquid with the vial of hormone. Nine cc of this mixture are then placed in sterile 1-cc vials and labeled (100 µg/cc).

The remaining 1 cc of hormone is mixed with 9 cc of bacteriostatic water. Nine cc of this mixture are then placed in sterile 1-cc vials and labeled (10 µg/cc).

This time, the remaining 1 cc of hormone is mixed with 1 cc of bacteriostatic water to obtain the desired concentration of 5 µg/cc. This mixture is placed in sterile 1-cc vials and labeled (5 µg/cc). This dilution is used to inject the fish. The remaining vials of 10 µg/cc and 100 µg/cc concentration are diluted as needed.

## 4) Haloperidol

Haloperidol {4-[4-(4-chlorophenyl)-4-hydroxy-piperidino] -4'-fluorobutyrophenone} powder should be mixed with bacteriostatic water and dissolved in solution by acidifying with lactic acid. Once in solution, haloperidol can be used immediately or divided into small volumes and kept in a freezer.

For example, if the recommended dose of haloperidol is 0.5 mg/kg, the fish weigh approximately 10 kg, and the desired volume of injection is 1 cc. Then the concentration

of hormone is equal to 1 mg/kg, multiplied by 5 kg, divided by 1 cc. In this case, 5 mg/cc.

$$\frac{\text{Hormone Concentration} = 0.5 \text{ mg/kg} \times 10 \text{ kg} = 5 \text{ mg/cc}}{1 \text{ cc}}$$

The weight of hormone to be mixed with a vial of bacteriostatic water is calculated by multiplying the hormone concentration (5 mg/cc) by the volume of the vial (30 cc); 150 mg of haloperidol are then weighed and mixed in a 30 cc vial of bacteriostatic water.

$$\text{Quantity of Hormone} = \text{Hormone Concentration} \times \text{Volume}$$

$$\text{Quantity of Hormone} = 5 \text{ mg/cc} \times 30 \text{ cc} = 150 \text{ mg}$$

Lactic acid is gradually added to adjust the pH to 3.0 to 3.6. Use pH paper to determine the end point or add lactic acid, drop by drop, until all the haloperidol is in solution and the mixture is clear, not cloudy. The haloperidol solution is injected in the muscle at 0.1 to 1 mg/kg of fish body weight. The dopamine blocker is usually administered with the first of two injections of LHRHa.

### Quantity of hormone injected

The total volume of hormone to be injected can be calculated by multiplying the recommended dose by the weight of individual fish and then dividing by concentration of the hormone mixture prepared.

$$\frac{\text{Volume of Injection} = \text{Recommended Dosage} \times \text{Fish Weight}}{\text{Hormone Concentration}}$$

#### 1) Example (large fish)

For example, if the recommended dose is 4 mg/kg, the individual fish weighs approximately 9 kg, and the concentration of hormone is 30 mg/cc. Then the fish is injected with 1.2 cc,

$$\frac{\text{Volume of Injection} = 4 \text{ mg/kg} \times 9 \text{ kg} = 1.2 \text{ cc}}{30 \text{ mg/cc}}$$

#### 2) Example (small fish)

For example, if the recommended dose is 10 µg/kg, individual fish weighs 80 g or 0.08 kg, and the hormone concentra-

tion is 5 µg/cc. Then the volume of hormone injected is equal to 0.16 cc.

$$\frac{\text{Volume of Injection} = 10 \text{ @kg} \times 0.08 \text{ kg} = 0.16 \text{ cc}}{5 \text{ µg/cc}}$$

### Hormone injection schedules

The number of injections required, is dependent on the response of each species to the selected hormone. Some ovulate following a single injection of the total dose. However, multiple injections are usually more successful.

For pituitary extracts or purified gonadotropins, first an injection of 10 to 33 percent volume of the total dose is administered, followed by a final or resolving injection. A rule-of-thumb is do not make the initial dose too large or the resolving dose too small.

Two injections of LHRHa, either 20 percent followed by 80 percent or 50 percent initial and 50 percent final, will usually give a better spawning response than a single injection of the same total dose. The dopamine blocker (e.g., haloperidol) is usually injected at the same time as the first LHRHa injection.

The time interval between multiple injections is usually 48 to 72 hours for cold-water species (e.g., trout), 12 to 18 hours for warm-water species (e.g., sturgeon, paddlefish, striped bass, white bass, and grass carp) and 6 hours for tropical species (e.g., red-tailed black shark and rainbow shark). Males are usually given only a single injection when the female is given the resolving dose.

### Injecting brood fish

The hormone is injected into the fish with a sterile syringe and hypodermic needle. Large food fish or sport fish should be injected with no more than a total of 2 cc of solution. No more than 1 cc should be administered at any one injection site. A 19-23 gauge needle may be used for these larger species. Small ornamental

fish species should be injected with no more than 0.2 cc of material using a 26-30 gauge needle.

Hormones are either injected into the muscle (intramuscular) or the body cavity (intrapertitoneal). Intramuscular injections are usually preferred because they result in a more constant delivery of hormone and there is less chance of injuring the fish. The preferred site for an intramuscular injection is into the thick muscle of the back. For scaled fish, the needle is inserted directly behind or along side the dorsal or back fin, where there are no scales. For intraperitoneal injections, the needle is inserted at the base of a pelvic fin, where there are no scales, and the mixture is injected into the body cavity (Figure 1).

### Conclusions

In this publication we have described common techniques to prepare the hormone mixture, calculate the hormone concentration to be mixed, determine the amount of hormone to be injected, and inject the hormone into the fish for induced spawning. The concentration of hormone to be mixed is calculated from the recommended dose, approximate weight of the brood fish, and the desired volume of the injection. Quantity of hormone to be injected can then be calculated from the estimated weight of each individual brood fish. Always use sterile syringes, needles, vials, and utensils when mixing, injecting, or storing hormones.

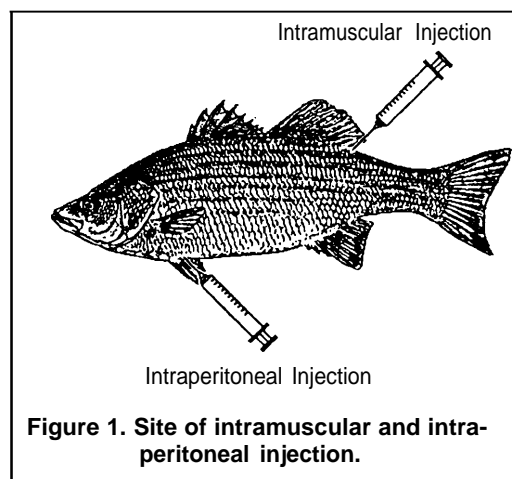
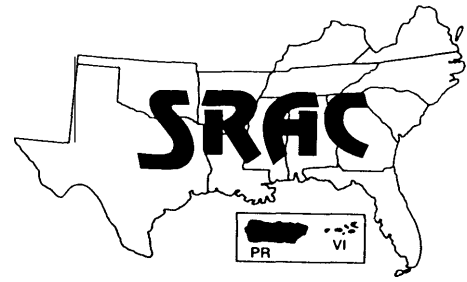


Figure 1. Site of intramuscular and intraperitoneal injection.

**Southern  
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# Techniques for Taking and Fertilizing the Spawn of Fish

R.W. Rottmann, J.V. Shireman, and F.A. Chapman\*

Following hormone injection, the eggs and milt of fish can be taken by several different methods:

- tank spawning,
- hand stripping, and
- surgically removing the eggs.

The method of choice depends on the fish species, hatchery facilities, experience and skill of the hatchery staff, and the desired manipulations of eggs, sperm or fertilized eggs.

## Taking the eggs

Ovulation is the final phase of normal egg development. The time between the final or resolving dose of hormone and ovulation is referred to as the latency period. This is usually dependent on the species of fish, water temperature, and hormone preparation used. It is especially important to know the latency period when hand stripping or surgically removing the eggs. Check the literature for the latency period for the fish species you are spawning.

During ovulation, the connection between the female fish and the eggs in the ovary is eliminated. In warm-water fishes, egg quality can deteriorate rapidly if eggs are not taken shortly after ovulation; they become "overripe" and can no longer be fertilized. In general, the eggs of tropical and sub-tropical species of fish become overripe more quickly than those that spawn at cooler water temperatures. The eggs of cold-water species remain viable for several days after ovulation. Table 1 presents the reported maximum period between ovulation and the deteriora-

tion of egg quality for some species of fish commonly spawned by hormone injection.

## Tank spawning

Tank spawning is the simplest method for obtaining a hatchery spawn. Brood fish of both sexes are placed together in the spawning tank following injection(s). Brood fish should not be disturbed and subdued lighting is recommended. The female ovulates when she is physiologically ready. The males stimulate the female to

**Table 1. The maximum period between ovulation and deterioration of the egg quality for various species of fish.**

Bighead carp ( <i>Hypophthalmichthys nobilis</i> )	50 to 80 minutes
Common carp ( <i>Cyprinus carpio</i> )	50 to 80 minutes
Grass carp ( <i>Ctenopharyngodon idella</i> )	30 to 45 minutes
Rainbow trout ( <i>Salmo gairdneri</i> )	7 days
Red-tailed black shark ( <i>Labeo bicolor</i> )	15 to 30 minutes
Snook ( <i>Centropomus</i> sp.)	15 to 30 minutes
Striped bass ( <i>Morone saxatilis</i> )	15 to 30 minutes
Sturgeon ( <i>Acipenser</i> sp.)	2 hours
White bass ( <i>Morone chrysops</i> )	30 to 45 minutes

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release the eggs and fertilize the spawn.

Better fertilization occurs if males are accustomed to the tank, and have been injected with a preparatory dose of hormone several days prior to and again at the same time as the female. Males can be used for several tank spawns, week after week, until their milt flow diminishes. Unless the males are aggressive toward each other, it is advisable to put two or three males for each female in a tank to ensure fertilization.

If the spawning tank is of sufficient size, more than one female may be spawned in the same tank. The presence of other individuals may help stimulate fish that are mass spawners. However, too many breeders in a small tank might be disruptive to the spawning process.

A round tank is advantageous for species with non-sticky, floating or semi-buoyant eggs that spawn in a river or estuary. The circular flow simulates the current in which these fish naturally spawn. The vigorous swimming action of the female in the swift water current is believed to assist in emptying the ovaries. The eggs are carried with the drain water from the spawning tank to a screened collector (Figure 1). Eggs are then transferred to an incubator.

Nest breeders and substrate spawners can also be tank spawned if suitable nesting sites or spawning material are provided. When tank spawning species that scatter sticky eggs, it is advisable to place spawning mats or brushes on the bottom of the tank. The eggs will attach to the substrate. Brood fish are removed from the tank after spawning, unless they provide parental care to the eggs. Fertilized eggs are usually incubated in the spawning tank.

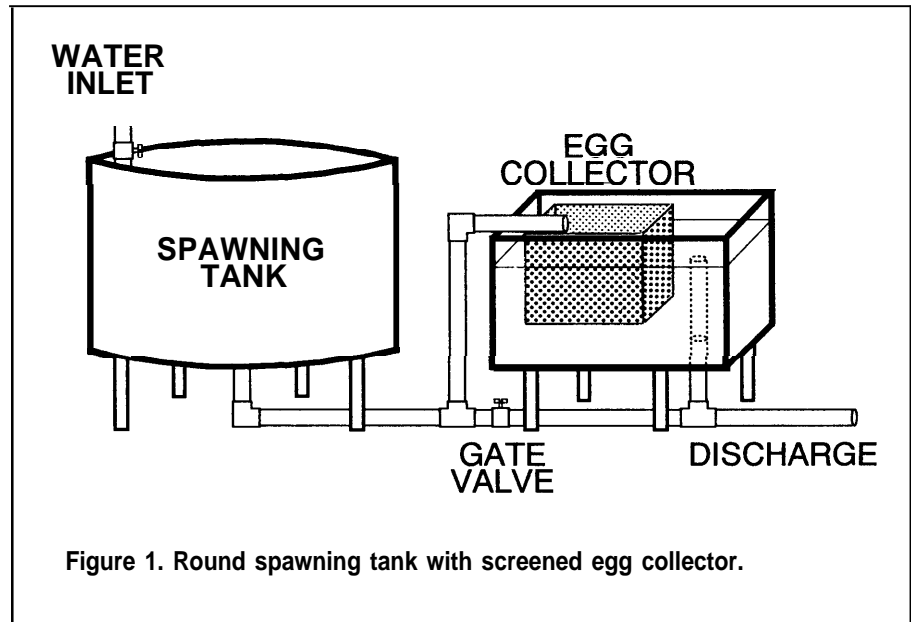


Figure 1. Round spawning tank with screened egg collector.

Tank spawning has both advantages and disadvantages. Advantages are:

- skill on the part of the hatchery staff in predicting the exact time of ovulation or checking females to verify ovulation is unnecessary;
- rapid deterioration of eggs in the ovary after ovulation is not a problem;
- there is less potential of injury to the brood fish because it is unnecessary to check and strip the fish; and
- less labor is required.

Disadvantages are:

- a screened egg collector or suitable spawning substrate is required;
- dirt and debris may be mixed with the eggs, potentially causing problems during incubation;
- some females may not release all their eggs;
- it is more difficult to accurately estimate the number of eggs;

- the surface area of substrate in tanks is insufficient for large species that release large quantities of sticky eggs. The eggs clump together, resulting in fungus problems and poor hatch; and
- this method cannot be used if induction of polyploidy or other manipulations of eggs, sperm, or fertilized eggs are desired.

## Hand stripping

Hand stripping is commonly used for taking the spawn of many species of fish. Brood fish are separated by sex prior to hormone injection to prevent spawning in the holding-tank.

It is important to determine the exact time of ovulation when hand stripping. However, the eggs of cold-water species may remain viable for several days after ovulation; for example, in trout, eggs are usually stripped within 3 to 4 days. The eggs of some species such as striped bass and white bass progressively clear or become transparent as they near ovulation.

This visual cue is used by hatchery workers to estimate the approximate time of ovulation. An egg sample is taken by carefully inserting a tube (catheter) into the urogenital opening and examining the sample under a microscope (See SRAC Publication No. 423, *Determining Sexual Maturity of Broodstock for Induced Spawning of Fish*). Eggs taken more than 15 hours before ovulation cannot be accurately staged using this method.

For most species, ovulation can best be verified by checking the female to determine when eggs flow freely from the vent. At least one hour prior to the earliest anticipated time of spawning, female fish are captured and the process of checking to verify ovulation is initiated. Tropical species are usually checked every 45 minutes until ovulation is verified, temperate water species are usually checked every hour. It is not necessary to take the fish out of the water to verify ovulation. The fish is turned belly up and gentle finger pressure is applied to the abdomen starting at the pectoral fins, moving slowly toward the vent. Do not try to squeeze or force the eggs from the fish; this will only injure the female. Frequent or rough handling of females retards ovulation, reduces spawning success and increases fish mortality.

If only a few eggs flow from the vent when slight pressure is applied, partial ovulation has occurred; the fish should be released and checked again later. Attempting to hand strip a female fish that has only partially ovulated will result in few mature eggs and physical damage to the ovaries, preventing a complete spawn. When eggs flow freely from the vent, complete ovulation has occurred. The hatchery worker quickly plugs the flow of eggs by placing a thumb over the vent. Brood fish may be anesthetized with MS-222 for stripping, if necessary, after ovulation is verified.

It is important to insure that no water comes in contact with the

eggs until after the milt is added and mixed. Water and slime from the vent and tail area of the female fish are dried with a towel. Water activates the sperm and also causes the opening through which the sperm enters the egg (micro-pyle) to close. For many fish, this closure takes place within only 45 to 60 seconds.

To strip the eggs, the fish is held slightly on her side, tail down; gentle hand pressure is applied to the abdomen, moving toward the vent (Figure 2). The stream of eggs is directed into a clean, dry bowl positioned so that water from the fish does not drip onto the eggs. The head of small fish can be held by one hand while the eggs are stripped with the other. A cloth glove may be worn to help hold the fish while stripping. Larger species are either wrapped in a towel and held by one or more hatchery workers while another strips the eggs, or the fish maybe restrained on a padded table or stretcher for stripping.

Good quality eggs usually flow readily from the genital opening of the female and have little ovarian fluid. If the ovarian fluid is watery or milky and many of the eggs are cloudy white, this indicates poor quality eggs.

## Surgically removing the eggs

Because the internal anatomy of fish varies greatly, hand stripping may be difficult in some species. Sturgeon and paddlefish have no ovarian sac; the eggs are released into the abdominal cavity during ovulation. The best method for taking the spawn in many of these species is to surgically remove the eggs. The first indication of ovulation for sturgeon and paddlefish is the appearance of several eggs stuck to the sides or bottom of the tank. The brood fish are usually left undisturbed for an additional 1 to 2 hours, depending on the size of the female (small females 1 hour and large females 2 hours), to insure complete ovulation.

If the female sturgeon or paddlefish is to be saved, it is first anesthetized. The fish is temporarily placed in an aerated holding tank with MS-222. When opercular movement slows and the fish is unable to right herself when turned over, she is then placed belly-up on a stretcher. Two hoses are used to ventilate the gills during surgery. One hose delivers aerated hatchery water, and the other delivers water from a recirculating tank containing aerated water with MS-222. During surgery, one

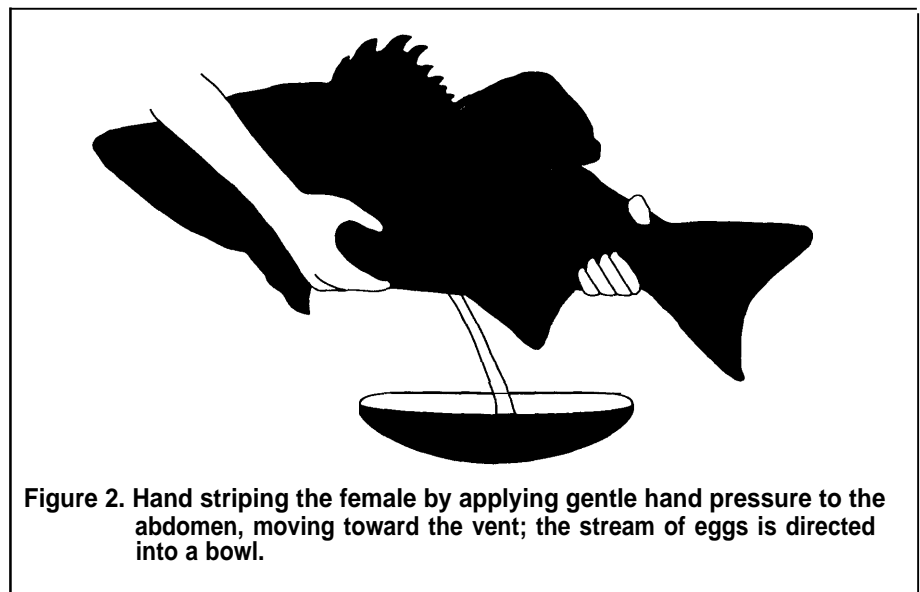
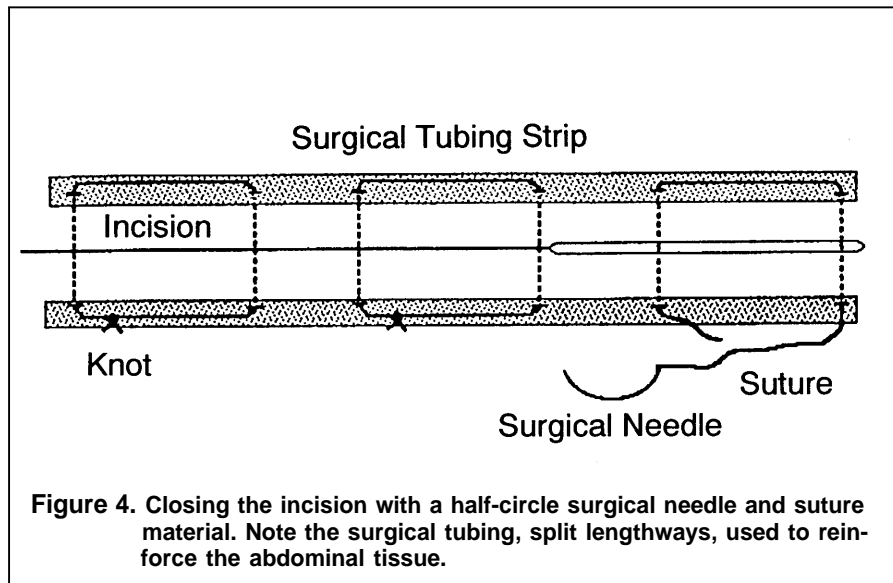


Figure 2. Hand stripping the female by applying gentle hand pressure to the abdomen, moving toward the vent; the stream of eggs is directed into a bowl.

of the two hoses is always in the fish's mouth, flushing the gills providing dissolved oxygen. The hoses are exchanged so that the fish remains anesthetized but opercular movement continues. Too much anesthetic will kill the fish. Before surgery, an antibiotic solution is applied to the abdomen. An incision is made along the midline of the abdomen, and the eggs are carefully removed with a spoon (Figure 3). To avoid injuring the internal organs, only the eggs easily accessible are taken. The incision is closed with a half-circle surgical needle and suture material; a length of surgical tubing, split lengthways, may be used to reinforce the abdominal tissue (Figure 4). The incision area is treated with an antibiotic before the fish is returned to the holding tank. A high level of dissolved oxygen is crucial for rapid recovery of the fish.

A greater quantity of eggs can be obtained by sacrificing the female sturgeon or paddlefish. If the female is to be sacrificed, it is: 1) held in a net and killed with a blow to the head; 2) hung from a hook; 3) the tail is cut off to bleed the fish, minimizing the contamination of the eggs with blood; 4) an incision is made in the abdomen starting at the vent; and 5)



**Figure 4.** Closing the incision with a half-circle surgical needle and suture material. Note the surgical tubing, split lengthways, used to reinforce the abdominal tissue.

a bowl is placed under the vent, directly below the incision. The eggs flow quickly from the abdomen, pulled by the force of gravity.

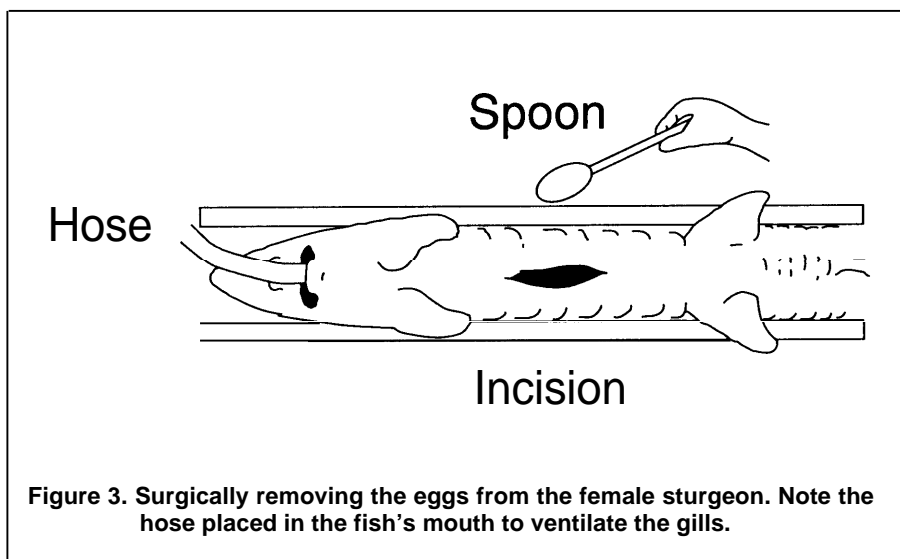
Not all the eggs will be free in the abdomen; some remain attached to the folds of the ovaries. These eggs are removed by hand and placed in separate bowls. The process of ovulation will be completed in the bowls. Within 15 to 20 minutes these eggs can be fertilized. Visible amounts of blood, which can inhibit fertilization, may be present with the eggs.

After fertilization, these eggs are processed and incubated separately.

For delicate species that seldom survive the rigors of hand stripping, humanely killing them and surgically removing the eggs may be the best option. In addition, more eggs can usually be obtained by this method than by hand stripping. Once ovulation has been verified (see section on "Hand stripping"), the brood fish is held in a net and administered a blow to the head to kill it. The eggs can then be removed from the fish by carefully cutting open the abdomen, pinching off the oviduct, and removing the ovaries individually. The total volume of eggs can then be gently squeezed out of the ovarian sac into a clean dry bowl to be fertilized.

### Fertilizing the spawn

Eggs are usually fertilized with fresh milt. Males are captured, wiped off, and held belly down over the bowl containing the eggs. The portion of the abdomen posterior to the pelvic fins is gently massaged to extrude the milt onto the eggs. The number of sperm in a volume of milt is extremely variable, ranging from millions to billions of sperm per milliliter.



**Figure 3.** Surgically removing the eggs from the female sturgeon. Note the hose placed in the fish's mouth to ventilate the gills.

Creamy-white milt contains more sperm per volume than grayish-white milt. When available, milt from two or more males is used to insure fertilization of the spawn. Individual males can be used to fertilize more than one spawn.

The fresh milt is spread over the eggs and thoroughly mixed by hand, plastic spatula or feather. Only then is water added to activate the sperm. The sperm remain active in water for a very short time (less than 1 minute to 5 minutes), depending on the species of fish and the temperature of the water. Water also results in closure of the micropyle of the egg in approximately the same amount of time. Water is added to only cover the eggs. Do not add too much water because the sperm may be too diluted to ensure fertilization of the eggs. To fertilize sticky eggs, see section on "Eliminating the stickiness from eggs."

The bowl containing the eggs and sperm should be gently rocked, swirled or stirred continuously for several minutes to insure uniform distribution of the sperm and to prevent the eggs from settling. Additional water is added as the eggs take up the water and enlarge (water harden). After several minutes, the fertilized eggs are transferred to the hatching apparatus. Fertilized eggs should not be exposed to direct sunlight. Subdued lighting should be used in the spawning and incubation area.

### **Storage of milt**

Milt can be collected from males and stored up to three weeks prior to stripping eggs. Males are captured and anesthetized, if necessary. The fish is then turned belly up, and the vent area dried by blotting with a towel. The area just behind the pelvic fins is gently massaged toward the vent to extrude the milt. The first few drops of milt are wiped away. The milt is collected by inserting a plastic tube attached to a syringe into the urogenital opening. Suction is applied while stripping to draw the

milt into the syringe. Care must be taken to insure that water, urine, intestinal contents, slime, or blood is not mixed with the milt. It is best to collect and store milt separately from each male to avoid contamination.

The milt is expelled into a sterile plastic bag, and antibiotic (e.g., 50 micrograms of dry streptomycin sulfate per milliliter of milt) may be added to control bacteria. The bag is filled with oxygen, sealed with a rubber band, and gently swirled to mix the antibiotic. Rough handling and shaking of milt may be detrimental to the sperm; mixing should be done slowly and gently.

Bags of milt should be laid flat to maximize the surface area of milt exposed to the oxygen and immediately stored on ice in a cooler or refrigerator. Do not freeze the milt as this will kill the sperm. Oxygen may be replaced and the milt should be gently swirled in the bag periodically to insure maximum aeration. Milt that has been contaminated with blood, slime, etc., will appear to have congealed and should be discarded. Milt has been held in this manner for up to three weeks; however, the quality of the stored milt may deteriorate with time.

The motility of stored sperm should be checked before it is used to fertilize eggs (See SRAC Publication No. 423, *Determining Sexual Maturity of Broodstock for Induced Spawning of Fish*). Milt with no or low motility should be discarded. When using stored milt to fertilize the spawn, it should be mixed with water first and then gently shaken for five seconds before being added to the bowl with the eggs. Remember, the sperm remain active in water for a very short period of time, so this must be done quickly.

### **Eliminating the stickiness from eggs**

Ovulated eggs of many species such as white bass, sturgeon, pad-

delfish, common carp, and channel catfish become sticky after coming in contact with water. During natural spawning, this stickiness causes the eggs to become attached to rocks, sticks, or aquatic plants. Catfish eggs are connected by a sticky matrix that holds the eggs together in a mass in the spawning cavern or container. In the hatchery, this stickiness causes problems during incubation.

Silt-clay, bentonite and Fuller's earth have been used to remove the stickiness from the eggs of many species of fish. Do not use diatomaceous earth because the sharp edges of the diatoms will damage the eggs. The dried material is added to hatchery water until a suspension is formed and a residue accumulates on the bottom of the container.

Paddlefish and sturgeon eggs are commonly treated with silt-clay as soon as the first few sticky eggs are noticed after fertilization, usually 1 to 4 minutes. The silt-clay suspension is added to the fertilized eggs at a ratio of 2 to 4 parts suspension to 1 part fertilized eggs. The mixture is gently stirred by hand. Any clumps of eggs on the side of the container are gently broken up. The suspension is poured off the eggs, and fresh suspension should be added every 10 minutes to maintain proper temperature and dissolved oxygen. Continue the process until the eggs do not stick to fingers or each other when removed from the suspension (minimum of 20 minutes).

Urea and salt solution has been used to remove the stickiness from common carp eggs. The addition of water to common carp eggs and milt will result in their sticking together in a clump within a few seconds. By using urea-salt solution instead of water, the spawn can be fertilized without the eggs sticking. A commonly used solution is prepared by dissolving 30 grams of urea and 40 grams of salt in 10 liters of hatchery water. The solution is added

to the eggs and sperm. The initial volume of solution added is approximately 25 percent of the volume of eggs. The mixture is gently stirred continuously with a feather, plastic spatula, or by hand. It has been observed that the motility of common carp sperm lasts much longer in the urea-salt solution (20 to 25 minutes) than in water (1 to 2 minutes). As the eggs water harden, additional solution is added. A portion of the solution with the dissolved sticky material is poured off at intervals and replaced. After about 1 to 1.5 hours the water hardening process is completed. The eggs are then transferred to a tannic acid solution (750 mg/L) for 5 seconds to eliminate any remaining stickiness. To remove the tannic acid, the eggs are thoroughly rinsed with fresh water.

The urea and salt solution has also been used to remove the stickiness from white bass eggs for the production of hybrid striped bass. A solution is prepared in a McDonald jar with 5 liters of hatchery water, 15 grams of urea and 20 grams of salt. The solution is aerated with a weighted air stone at the bottom of the jar until the chemicals are dissolved. A small amount of the solution is added to cover the eggs and sperm, fertilizing the spawn. The mixture is gently stirred. Four minutes after the solution is added to the egg-sperm mixture, 400 milliliters or less of fertilized eggs are placed in each jar with the solution. Air flow is adjusted to keep the eggs in suspension without rupturing them. After 6 minutes, the urea and salt solution is poured off the eggs, and 0.75 grams of tannic acid mixed in 5 liters of hatchery water (150 milligrams/liter) is added and aerated for an additional 6

minutes to eliminate any remaining stickiness. The water inlet valve to the jar is opened, flushing the tannic acid.

Tannic acid alone has also been used to remove the stickiness from white bass, sturgeon, and paddlefish eggs. A tannic acid solution of 150 milligrams/liter is often used for this purpose. This solution is prepared by adding 0.75 grams of tannic acid to 5 liters of hatchery water in a McDonald jar just prior to adding the eggs. The solution is aerated with a weighted air stone at the bottom of the jar. The fertilized eggs (1 minute after the water is added to activate the sperm) are placed in the jar. Aeration is adjusted to just keep the eggs in suspension. The air stone is removed after 10 to 12 minutes, and the water inlet valve is opened to the jar. Although not absolutely necessary, an excess quantity of milt appears to help reduce stickiness of the eggs.

When the alkalinity of the water is above 200 milligrams/liter, additional tannic acid maybe needed. However, an excessive amount of tannic acid can strengthen the egg shell, resulting in difficulties at hatch. For white bass eggs, an additional disadvantage of this procedure over the use of urea-salt is that some batches of eggs are extremely sticky, especially if only a limited quantity of milt is available. In addition, the resulting egg shell is opaque rather than clear, preventing microscopic examination of the developing embryo.

A sodium sulfite ( $\text{Na}_2\text{SO}_3$ ) solution can be used to dissolve the gelatinous matrix of catfish egg masses so they may be incubated in hatching jars, eliminating many problems associated with traditional paddle-wheel-trough incu-

ators. The solution is prepared by mixing 15 grams of sodium sulfite with 1 liter of hatchery water. If the water supply has low alkalinity, the pH must be adjusted back to that of the hatchery water supply using 10 percent hydrochloric acid (HCl). The egg mass is removed from the spawning container at least 24 hours after spawning and placed in a plastic pan. One liter of the sodium sulfite solution is added per 500 grams of eggs. The egg mass is gently kneaded and stirred until the gelatinous matrix is completely dissolved. The entire contents of the pan is poured into a hatching jar and the water flow is adjusted to gently tumble the eggs without washing them from the jar. No more than 1400 grams of eggs should be incubated in a seven-liter hatching jar. Dead eggs, white in color, float near the top of the egg mass and should be removed by siphon to prevent fungus problems.

## Conclusions

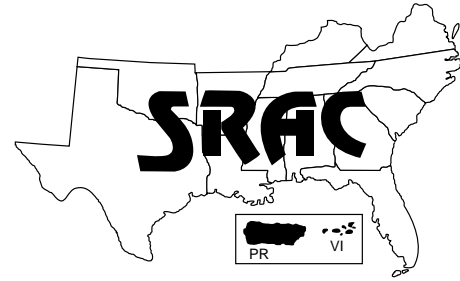
The spawn can be taken from fish by several different methods, including allowing the fish to spawn in the tank, hand stripping, and surgically removing the eggs. When hand stripping or surgically removing the eggs, either fresh or stored milt can be used to fertilize the spawn. Ovulated eggs of many species of fish have a sticky exterior. Several common preparations can be used to eliminate the sticky layer of fish eggs.

Induced hatchery spawning requires a continuous series of steps. A mistake during any phase of the process can diminish or completely obliterate the success of the project. Consistent performance requires strict attention to detail.



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## Southern Regional Aquaculture Center



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Revision

# Channel Catfish Broodfish Management

Anita M. Kelly\*

Broodfish management is an important aspect of channel catfish culture because having a reliable source of fingerlings is essential to making a profit. Successful management of broodfish includes selecting for the genetic qualities desired by the culturist and processor and then producing high quality fry. The type of breeding program chosen by the fingerling producer determines the population(s) from which the broodstocks are obtained. Broodfish must be selected and then managed for maximum reproductive output. The potential of fish with desirable genetic background is inconsequential if spawning success is poor.

## Selection

Broodfish should be selected for the visible characteristics and genetic traits that are desirable to the producer. Visually inspecting the fish is important whether you are obtaining fish from within an operation or purchasing from other producers. If broodfish are selected from within an operation, remember that male channel cat-

fish grow faster than females so that keeping only the largest fish may result in a disproportionate number of males. Also, avoid using large fish that are in foodfish ponds when drained. These fish are not necessarily fast growers, but may just be adept at avoiding the seine.

Broodfish purchased from other producers should be obtained during the late summer to early winter of the year before the spawning season when they will be used. This allows time for stocking at desirable densities and for recovery from the stress of seining, handling and transporting. Proper feed, feeding schedules and water quality will ensure maximum gonadal development before the spawning season. Though it is difficult to inspect individual broodfish on large farms, every effort should be made to select only healthy broodfish of the proper size with well-developed secondary sexual characteristics. Check the fish for suitable body conformation and freedom from sores or hemorrhages on the skin. Although channel catfish can mature sexually at 2 years of age, fish should be at least 3 years old and weigh at

least 3 pounds for reliable spawning. Channel catfish 4 to 6 years old and weighing 4 to 8 pounds are prime spawners. Older and larger fish produce fewer eggs per pound of body weight, are more difficult to handle, and may have difficulty entering certain spawning containers. Cull fish that weigh more than 10 pounds and replace them with younger, smaller fish.

Some traits particularly useful to channel catfish producers are not visible. These include disease resistance, fast growth, good feed conversion and high dress-out weight. These traits can be obtained only by selectively breeding for them. Breeding programs for channel catfish are not advanced, but some selective breeding has been done by individuals and research institutions. Several strains of channel catfish are available throughout the United States. A strain is usually named for the water body the fish were obtained from or the farm they inhabit. For example, the Rio Grande strain originated from the Rio Grande River in Texas. Strains differ in their growth rate; resistance to viral, bacterial and parasitic infections; dress-out percent-

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age; ability to escape seining; and time of spawning. In 1984, Auburn University published a circular entitled *Ancestry and Breeding of Catfish in the United States*. This circular lists more than 300 strains of channel, blue, white and flathead catfish, as well as bullheads. Many of the strains listed were not selected by the breeders for specific traits, but rather were subjected to random mating. A few strains have been selected for desirable traits. These include, but are not limited to, Harvest Select (formally Gold Kist), NWAC 103, Kansas and Rio Grande. This fact sheet does not recommend one strain over another, but describes the characteristics of some of the strains available. This list is far from comprehensive, but includes some strains for which research results have been published.

### **NWAC 103**

The newest line of catfish to be introduced is the USDA 103 (renamed NWAC 103 after public release in 2001). This strain was originally obtained from the U.S. Fish and Wildlife Service. These fish were selected for growth rate. Studies have demonstrated that the NWAC strain grows faster than the other channel catfish strains to which it has been compared. This strain also consumes more feed than the other strains, which may explain the rapid growth. NWAC 103 catfish are susceptible to ESC infection, but whether they are less susceptible than other strains still needs to be explored.

### **Harvest Select**

Since 1991, Harvest Select (formally Gold Kist) has been selecting catfish for improved feed conversion, processing yields, reproductive success and fingerling survival. This breeding program pairs males and females from different strains with individual

characteristics to achieve superior offspring. They are testing for strains that are resistant to ESC and *Columnaris* diseases.

### **Kansas**

The Kansas strain is perhaps the oldest domestic strain of catfish. It was bred for increased growth and disease resistance. However, this strain of fish is not sexually mature until 4 years of age, 1 to 2 years later than other strains. Although this fish does grow rapidly, it is difficult to spawn.

### **Rio Grande**

The Rio Grande strain demonstrates excellent dress-out percentages. These fish spawn later than other strains, but are sexually mature at 2 years of age. They also have poor growth and are susceptible to channel catfish virus, *Ichthyophthirius* and *Columnaris*.

### **Auburn**

The Auburn strain of channel catfish demonstrates moderate growth rates, but female produce fast-growing progeny when cross-bred with other strains. Albinism is common in this strain. These fish are difficult to seine and have excellent dress-out percentages.

### **Norris**

The Norris strain of channel catfish is known for its fast growth rate. Hybrids between the Norris and the blue catfish are fairly resistant to ESC, compared to other strains. Most of the USDA-103 comparison trials have been conducted with the Norris strain.

## **Breeding programs**

Some more progressive catfish farmers have established breeding programs. They realize that breeding programs are necessary to improve production and increase profit margins. Production efficiency cannot be optimized unless the biological potential of the fish

is optimized. Some types of breeding programs are discussed below.

### **Inbreeding**

Inbreeding is defined as the mating of related individuals. Very few studies have been done on inbreeding in channel catfish. However, these studies consistently demonstrated that inbreeding reduces growth, reproductive performance and survival, and increases the incidence of deformities.

Inbreeding does not always result in undesirable characteristics. Selection programs are mild forms of inbreeding. Inbreeding and its less desirable effects can be avoided by ensuring that broodfish replacements come from at least 50 random matings. Inbreeding may also be counteracted through crossbreeding.

### **Mass selection and family selection**

Selection occurs on a farm every time the catfish reproduce. Domestication is a form of selection. It is widely known that domesticated strains of channel catfish grow faster than wild catfish—an average of 3 percent. Selection programs are successful only if the genetic component responsible for the improvement is passed from parent to offspring.

There are two types of selection programs—mass selection and family selection. Mass selection evaluates the performance of all individuals regardless of parentage. Family selection evaluates the performance of families, and whole families are selected or culled. With family selection, specific pairs of broodfish are mated and the progeny are reared separately.

### **Crossbreeding**

Crossbreeding can be used to increase productivity. For a cross-

breeding program, two strains with good qualities are identified and the females from one strain are mated with the males from the other strain. The object is to obtain offspring with the desirable qualities of both parental strains. This method does not guarantee that the offspring will possess all of the qualities desired; some crosses will produce superior hybrids, while others will not. Crossbreeding can increase disease resistance, cause earlier spawning in crossbred adults, and increase spawning rates (more females spawning) and fecundity (more eggs per female). However, the positive aspects of crossbreeding decrease with age. To date, the USDA 103 strain has not been crossbred with other channel catfish strains.

### **Hybridization**

Crossing two different species, for example the channel catfish with the blue catfish, is called hybridization. Approximately 30 hybrid crosses, using seven species of catfish, have been evaluated. Many of these hybrid crosses were difficult to produce, or the crosses resulted in high proportions of abnormal fry. One hybrid cross is promising—the channel catfish female and the blue catfish male. These hybrids grow faster than either parental line, are more disease resistant, more uniform in size, more tolerant of low dissolved oxygen and easier to capture by seining. The major obstacle to hybrid production is that sufficient numbers of fingerlings cannot be produced for commercial application. Researchers are studying ways to improve the number of fingerlings produced. One drawback to the hybrid is the small head size. Many farmers complain that the head of the hybrid is so small the fish get caught in the seine and must be freed manually. This could be remedied by using smaller mesh seines when harvesting hybrids.

## **Comparisons of Breeding Programs**

The three breeding programs discussed differ in their effectiveness. Crossbreeding frequently improves performance. Hybridization has produced only one promising hybrid from 30 different crosses. The easiest and most effective breeding program is mass selection, which improved performance in all strains tested. The actual performance or value of the fish from a selection program is not known unless controlled experiments are conducted.

### **Guidelines for an On-Farm Breeding Program**

Selection for certain traits such as growth, color, or age at reproductive maturity occurs whenever broodstock are chosen from a population. If the choice of stock is made according to a plan, progress can be made in improving the performance of future generations. If little thought is given to the choice of broodstock, the culturist may unintentionally select for undesirable traits. Farmers unwilling to develop even a simple breeding program should realize that imprudent selection of broodstock could decrease productivity and profits. A little extra effort and common sense can help prevent this. The minimum guidelines for a catfish breeding program are:

1. Choose broodstock from domesticated strains. Wild fish are unreliable spawners in captivity and the fingerlings may be susceptible to disease or grow slowly under culture conditions.
2. Select broodfish from stocks that are known to perform well under commercial culture conditions. This will be difficult as few field trials have been done.
3. Do not mistake large fish for fast growing fish, as large fish may be the fish capable of escaping the seine. Try to select broodfish from fish of known

age, but be aware that large fish selected from a pond containing a single year class could result in the selection of mostly siblings.

4. Prevent inbreeding by obtaining broodfish from as many different spawns as possible. Initial stocks should be obtained from several different ponds or, ideally, from unrelated stocks in different locations.
5. If replacement broodstock comes from fingerlings produced on the farm, they should come from at least 50 random matings. If this is not possible because the breeding population is too small (less than 75 to 100 breeding pairs), enrich bloodlines by adding unrelated stock as part of the broodfish replacement program.
6. Keep accurate records of spawning output, egg hatchability, fry survival, and growth rates of fingerlings and food-sized fish. If performance decreases over time it may be because of inbreeding or other problems associated with imprudent selection.

### **Gender determination**

Sex of broodfish should be determined so that females and males can be stocked into brood ponds at the desired ratios. The sex of sexually mature channel catfish in good condition is relatively easy to determine. The urogenital area is located ventrally, posterior to the anus and anterior to the anal fin (Fig. 1). The male releases sperm through an opening called the urogenital pore, a small, fleshy nipple on the genital papilla posterior to the anus. The female has two separate openings—an anterior genital pore for expulsion of eggs and a posterior urinary pore for release of urinary products. The two openings are located in a groove with surrounding tissue forming a distinguishable slit just below the anus.



Figure 1. The genital papilla region of the female (left) and the male (right) channel catfish. The female's region is slit shaped compared to the fleshy nipple appearance of the male.

Mature fish have secondary sexual characteristics that are also useful for selection. These characteristics are most evident near spawning time. The male has a broad, muscular head wider than the body, thickened lips, and often a grayish mottling on the underside of the jaw and abdomen (Fig. 2). The female's head is narrower than the body and she usually lacks the muscularization and pigmentation common in males. During the spawning season, a sexually mature female will have a well-rounded abdomen that

extends past the pelvic fin region into the genital area. If the abdomen is hard to the touch, it is probably filled with feed, whereas a soft, palpable abdomen indicates that the ovaries are well developed. Broodfish usually eat much less during the spawning season. Therefore, it is not difficult to tell if a female has well developed ovaries or a belly full of food.

### Care

A common management practice among many fingerling producers is to have separate ponds for

holding and spawning. Stocking densities in holding ponds are usually 2,000 to 3,000 lbs./acre. Spawning ponds need to be new ponds, newly renovated ponds, or former fingerling ponds that have been thoroughly dried out and exposed to air for several months after the sale of fingerlings in the winter and spring. Once the spawning ponds are refilled with water, broodfish from the holding pond are examined and introduced into the newly filled ponds. Several producers have noticed that when older ponds are used, spawning containers in those ponds may be left unattended by the males or spawns may consist of poor quality eggs. This phenomenon has been attributed to poor water quality and other unknown factors.

Stocking density in spawning ponds is usually 800 to 1,200 lbs./acre. Male-to-female sex ratio should be approximately 2:3. Broodfish are kept in the spawning pond until spawning ceases and then removed with a large-mesh seine and returned to holding ponds. Near the end of the spawning season, some producers add fry to spawning ponds containing broodfish to use the available space. Broodfish not captured during seining do not appear to cannibalize the stocked fry. Once the spawning season has ended, broodfish are routinely moved from spawning ponds into holding ponds.

### Water quality management

Water quality in the broodfish ponds must be maintained to ensure the survival of the broodfish and the production of large numbers of good quality eggs. Excess nutrients from feed is the main reason for a decline in water quality. Poor quality water (which contains low dissolved oxygen levels and high levels of ammonia, nitrites and carbon dioxide) stresses the fish, making them more vulnerable to diseases and parasites.

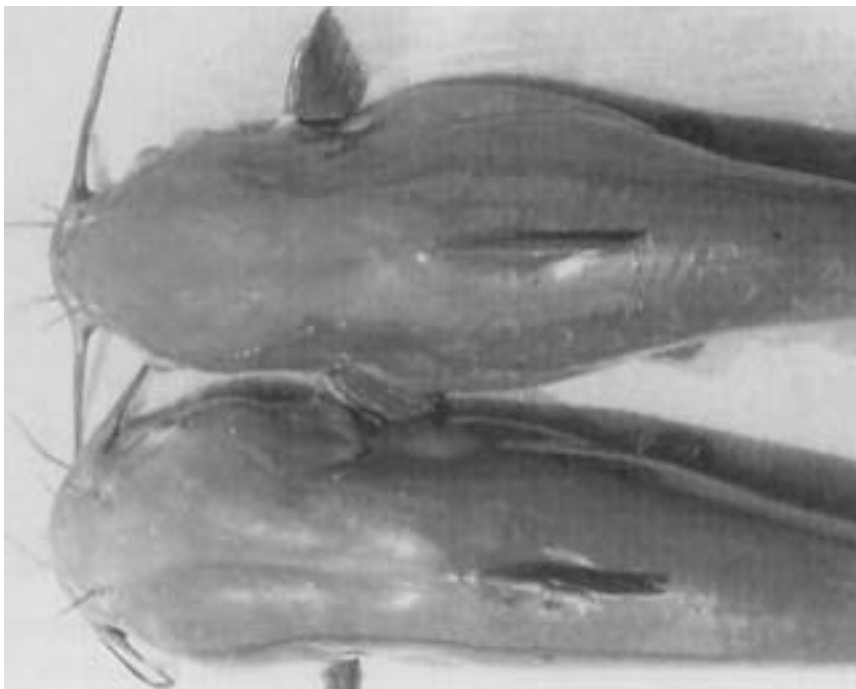


Figure 2. The male catfish (bottom) has a muscular head and the female (top) has a smaller, less muscular head.

Adding feed to the water also adds nitrogen and phosphate, nutrients that can increase phytoplankton production. The phytoplankton use oxygen at night, creating an even greater oxygen demand than a pond with fish alone.

Poor water quality should be corrected before the fish become diseased and die. Options include temporarily reducing feeding to limit the nutrients in the water, aerating to increase the amount of oxygen in the water, and flushing the pond with water from a well or reservoir.

Producers should have emergency aeration equipment available. Dissolved oxygen levels can decrease rapidly when large phytoplankton die-offs occur or when decaying feed and organic matter cause an excessive biological oxygen demand. Large, paddlewheel aerators are the most popular emergency aeration devices in channel catfish farming, although fountain aerators are also used. Flushing the pond with well water, which is often void of oxygen, requires that emergency aeration be used as well. Generally, the first sign of poor water quality is that the fish stop feeding or reduce the amount of feed they consume.

## Nutrition

Reproductive performance in broodfish is much more important than growth rate. Nonetheless, fish must have adequate food throughout the year, particularly during the period of egg formation and development. Underfed catfish have low reproductive success and poor egg quality. When both sexes are held in the same pond, an insufficient food supply can result in poor quality female broodfish because the larger, more aggressive males consume most of the limited ration. Broodfish are usually fed the same feed used for food fish grow out. Some catfish producers use a sinking feed because broodfish are more hesitant to feed at the surface. However, because brood-

fish feed slowly, sinking pellets may disintegrate before they can be consumed. When the water temperature is above 70 °F, a nutritionally complete feed of at least 32 percent crude protein is fed at about 2 percent of body weight daily. At water temperatures of 55 to 70 °F, a ration consisting of about 1 percent of the body weight is fed on alternate days. At water temperatures below 55 °F, about 0.5 percent of the body weight is fed once a week.

Forage fish are often stocked into broodfish ponds as a simple way of ensuring that ample food is consistently available during the egg production period. Mature fathead minnows, the most commonly used forage fish, are stocked in the late winter or early spring at 5 to 10 pounds per acre (1,000 to 2,000 fish/acre). Some structure, such as wooden pallets, may be added to the ponds to enhance minnow reproduction.

## Estimating the number of broodfish

Production goals determine the number of broodfish required to produce the desired number of fingerlings. If the fingerlings will be used on the farm the producer needs only enough to replace fish that will be harvested and sold in the following year. However, if the fingerlings will be sold to other producers, the annual production goal is based on the number of fingerlings needed to achieve a certain income. Produc-

tion, however, may be limited by the available pond space.

The number of pounds of female broodfish required to produce a specific number of fingerlings can be estimated based on assumptions of egg production, survival of eggs to fry in the hatchery, and survival of fry to fingerlings in the nursery.

Broodfish ponds ranging from 5 to 30 acres are commonly used in the southeastern U.S., with more manageable ponds ranging from 5 to 10 acres. While all the broodfish in the previous example could be stocked into one pond, that would be extremely risky. All of the broodfish and subsequent progeny could be lost if water quality deteriorates or a disease outbreak occurs. Stocking broodfish into several ponds is recommended.

## Spawning in ponds

Seasonal changes in water temperature control the reproductive cycle in channel catfish. Exposure to water temperatures below 50 °F for a month or more over the winter stimulates egg production. The subsequent slow rise in the average water temperature to 68 to 77 °F initiates spawning in the spring. The vast majority of channel catfish are spawned using the open pond method: Broodfish are held in ponds with spawning containers (such as milk or cream cans, metal barrels, nail kegs, tile, ammunition cans, plastic buckets or plastic containers, Fig. 3) and allowed to select their mates and spawn naturally. Most spawning

### To produce 1,000,000 (million) fingerlings:

If 70 percent of swim-up fry stocked in ponds survive to become fingerlings, then  $1,000,000/0.70 = 1,429,000$  swim-up fry are needed.

To produce this number of swim-up fry, if survival in the hatchery from egg to swim-up fry is 80 percent, then  $1,429,000/0.8 = 1,786,000$  eggs are required.

If 3,000 eggs are produced per pound of body weight, then  $1,786,000/3,000 = 596$  pounds of female broodfish are needed.



Figure 3. These plastic spawning cans, shaped like milk cans, are used in channel catfish culture.

containers have an internal volume of 20 gallons and an opening of 6 to 9 inches across. Spawning containers are not placed in the pond until the water temperature reaches 75 °F. The channel catfish spawning season in the U.S. can begin in early April and last until mid-July. The length of the season and the start of the season depend on water temperature. Once water temperature has reached 70 °F and remains at that temperature for at least 3 consecutive days, spawning begins. In the southern U.S. spawning season usually begins in late April; in the northern U.S. it does not begin until mid-May.

Spawning containers are placed in the ponds several days before the beginning of spawning season is anticipated. This gives the males time to clean and prepare them. Containers are placed along the pond bank in 2 to 3 feet of water at 10- to 30-foot intervals with the open end of the container toward the center of the pond. Containers are marked with a stake or float for convenient location when there is a need to check for egg masses.

Not all fish spawn at the same time so it is not necessary to have a spawning container for every pair of fish. The number of containers needed depends on whether the egg mass will remain in the pond and receive paternal care or will be removed to the hatchery. If egg masses are not immediately removed to the hatchery, more containers will be needed because each container will be occupied longer. Various ratios of containers to stocked broodfish pairs have been used, but 1:4 and 1:5 are common. Spawning may cease if water quality deteriorates or weather turns unseasonably warm. Spawning may resume if water temperature drops within 10 to 25 days of the onset of warm weather. Draining and replacing one-fourth to one-half of the pond water with cool, high quality well water may also cause spawning to resume.

Once spawning begins, containers should be inspected during the late morning of every third or fourth day to determine if eggs are present. If little or no spawning activity occurs, broodstock should be checked for parasites or dis-

ease. Feeding should always be continued. If the lack of spawning cannot be attributed to poor water quality or disease, then producers should consider moving the fish. Moving reluctant, healthy spawners into newly filled ponds or into existing brood ponds where spawning has been successful and is nearly complete can cause the transferred fish to resume spawning.

### Additional Sources:

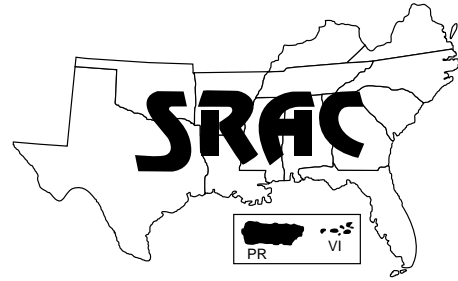
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**Southern  
Regional  
Aquaculture  
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# Channel Catfish Broodfish and Hatchery Management

Jim Steeby and Jimmy Avery\*

This is one of several SRAC publications on specific topics related to hatchery economics, water quality, broodfish selection, fry pond preparation and compounds used for egg treatment. This publication is a reference and planning guide for channel catfish hatchery managers. In addition to reading each publication in the series, managers should visit hatcheries in their areas to view construction and management techniques.

## Broodfish selection and care

Selecting good broodfish is essential to the success of any catfish hatchery operation. Following are broodfish sources in order of preference.

1. Fish from a registered or recognized improved line (SRAC Publication 1802)
2. Fish from an established hatchery
3. Fish of known, equal age selected from food fish ponds

4. Fish obtained from a commercial fisherman

Producers surveyed (USDA-APHIS 2004) reported that about 14 percent of broodfish are lost annually to fighting, disease and spawning stress. It will be necessary to supplement broodfish every 2 to 3 years. Be sure to select the best-growing fish from a group that are of equal age. If selected by size, fish will need to be sexed to obtain a proper male-to-female ratio of 1:1 or 2:3.

If all female broodfish spawned in a timely manner, it is estimated that as few as 600 pounds (272 kg) would be needed to produce 1 million fry (SRAC Publication 1802). However, data from USDA-NASS reports, combined with USDA-APHIS producer surveys, suggest that spawning success across the industry is 30 to 40 percent. Therefore, most farms maintain two to three times the minimum weight of broodfish to ensure proper numbers of eggs. Assuming a 70 percent hatch, a 70 percent survival to swim-up fry stage, a 50 percent spawning rate, and 3,000 eggs per pound (6,615

per kg) of female, a hatchery should maintain 1,350 to 2,000 pounds (612 to 907 kg) of female broodfish to produce 1 million fry.

The total weight of broodfish in ponds (males and females) should not exceed 1,200 pounds per acre (1,344 kg/ha). Broodfish can be transported and sorted just before the anticipated spawning period if proper fish handling techniques are used. Broodfish should not be crowded or stressed.

Broodfish should be sorted and moved annually to evaluate their condition and numbers. Large numbers of broodfish can be quickly sexed and evaluated by placing a small, flatbottom boat between two empty grading nets in a suitable area adjacent to the pond levee (see SRAC publication 1802 for instructions on determining the sex of broodfish). A small number of fish are placed in the bottom of the boat using a loading net. Two workers seated opposite one another, with the fish between them, select fish, determine the sex, and place the fish into the two grading nets. Designate a net on one side for males and the opposite side for

\*Mississippi State University, National Warmwater Aquaculture Center, Stoneville, Mississippi.

females. This is repeated until all fish are sexed or the grading nets need to be emptied.

Many growers retire broodfish at a given size or age, but this decision should be based on the spawning success of the group. Fish larger than 10 pounds (4.5 kg) may spawn well but produce fewer eggs per pound. If production declines and a cause is not evident, consider replacing broodfish groups with less than a 30 percent spawning success.

### Brood pond preparation

Each spring, broodfish should be moved to ponds that will have excellent water quality. To this end, a new or rebuilt pond is best for broodfish. If the pond has been in use for more than 2 years it should be completely dried to ensure good levels of dissolved oxygen when refilled.

In brood ponds it is important to eliminate aquatic weeds because they can severely restrict spawning, feeding and fish movement. In commercial catfish ponds with hardness and alkalinity greater than 50 mg/L, applying nitrogen has produced the best results with only minimal addition of phosphorus required. As soon as the pond is filled, apply 20 pounds of nitrogen per acre (22.4 kg/ha), or 40 pounds per acre (44.8 kg/ha) as urea. Add a few grass carp per acre where state regulations allow. Additional nitrogen should be added at the rate of 2 to 3 pounds per acre (2.2 to 3.3 kg/ha) every other day until a dense green color develops in the water column. An alternate strategy is to apply 200 to 300 pounds per acre (224 to 336 kg/ha) of commercial catfish feed or cottonseed meal as soon as the pond is filled and feed 10 to 20 pounds per acre (11.2 to 22.4 kg/ha) daily until a bloom develops. To test the density of the bloom, extend your cupped hand into the water. If your hand is still visible when your elbow meets the water line, the bloom is not dense enough.

In regions where alkalinity is less than 50 mg/L, a high-phosphate

fertilizer such as 13-30-0 can be applied at the rate of 2 to 4 pounds per acre (2.2 to 4.4 kg/ha) on alternate days for 8 to 14 days.

Dissolved oxygen in the brood pond should remain above 5 mg/L at all times for successful catfish spawning. Brood ponds where spawning fails or stops should be examined for water quality, weeds and disease. If no cause is evident, moving the broodfish to another pond is often the best solution. Double stocking a current brood pond that has or is spawning well is often successful. Handle broodfish with extra care when moving them during the spawning period.

### Spawning containers

Channel catfish are “cavity spawners” and require a chamber into which they can deposit eggs. Eggs adhere to one another and form a large sponge-like mass or matrix when the spawning process is completed (Fig. 1). Spawning cavities can be fabricated from objects such as ammunition cans, aluminum milk cans, plastic buckets or barrels. The opening should be 5 to 7 inches (12.7 to 17.8 cm) in diameter so larger fish can enter. Container volume should be at least 10 gallons (38 L),

with 15 to 20 gallons (57 to 76 L) being optimal.

If eggs are transferred from the spawning containers to the hatchery two to three times each week, a ratio of one container for every three to four female brooders is adequate. For ease of collection and best spawning success, containers should be placed in water 2 to 3 feet (61 to 91 cm) deep around the pond perimeter, and spaced 6 to 7 feet (2 m) apart. There is less spawning at depths below 3 feet (1 m) because of low dissolved oxygen in the morning at lower depths.

The spawning season lasts several weeks. At peak spawning times it is not likely that more than 20 percent of females will spawn during a 48-hour period.

### Removing eggs to the hatchery

Eggs must arrive at the hatchery in excellent condition. Containers such as coolers, mesh-lined fish baskets, and metal or plastic tubs can be used to transport eggs to the hatchery (Fig. 2). Placing a slightly inflated tire tube around the upper edge of the container will keep it



Figure 1. Channel catfish egg mass.



Figure 2. Collecting egg masses using a cooler with a tire tube float.

floating upright as it is towed around the pond. Dissolved oxygen in the container should remain near 5 mg/L and water temperature should be near that of the spawning pond until eggs arrive at the hatchery. Tubs or coolers should be partially drained and fresh pond water added at regular intervals during collection to maintain the proper level of dissolved oxygen. Never leave eggs unattended in strong sunlight or where temperature may climb rapidly. Remember that eggs can be killed or damaged by poor water quality, but may appear normal for 48 hours or longer.

To transport eggs to the hatchery, place them in a fry transport tank supplied with oxygen bubbled from airstones. If the water temperature in the transport container is more than 5 to 7 °F (2 to 3 °C) different from the hatchery water source, eggs should be water tempered for 15 to 20 minutes.

Upon arrival at the hatchery, treat the eggs with an iodine-based disinfectant. While dip treatments may save on chemical costs, it is better to put the eggs into hatching baskets at the proper rate and then

make a trough treatment. Dip treatments must be well monitored and the eggs moved without delay to avoid over-treatment. Eggs are easily damaged if workers become distracted during a dip treatment and leave eggs in the treatment too long. Remember that dissolved oxygen is critical at all points, including during the disinfecting process. Using an airstone during this process can help relieve stress on eggs.

### Hatchery water and troughs

Both well and surface water are used successfully in catfish hatcheries. The important water parameters are reviewed in SRAC Publication 461. If the hatchery uses groundwater, it should be degassed before entering the hatchery (SRAC Publication 191). With surface waters, degassing is not necessary. However, water should be filtered through rapid sand filters similar to those used for swimming pools. Sand filtration removes sediment that adheres to eggs and screens out insects. Sand filters should be backwashed regularly to avoid low

water flows to troughs. Backwash filters at least twice a day when the hatchery is in full use.

The suggested water volume for a hatchery is 2 to 3 gallons (7.6 to 11.4 L) per minute per 100 gallons (380 L) of water volume in the hatchery. The calcium concentration in the water during the first 24 hours of egg development is critical to successful egg hatch (Small et al. 2004). If calcium hardness in the source water is less than 10 mg/L, add calcium chloride continuously using a peristaltic pump or a drip system. Either method should create a calcium concentration of 25 to 50 mg/L. Granular calcium chloride is approximately 77 percent calcium by weight. As a liquid, the calcium is 38 percent of the calcium chloride solution weight. Be sure to check the operation of the pump or drip system and measure the hardness of the incoming water at least once a day or whenever large numbers of troughs are brought on or off line.

A standard hatching trough is 8 feet long, 20 inches wide, and 10 inches deep (2.4 x 0.51 x 0.25 m), with 8 inches (20.3 cm) of water depth. It holds 100 gallons (380 L) of water. A trough of this volume will adequately hatch 20 to 25 pounds (9 to 11.3 kg) of eggs. Troughs are usually mounted on wooden or metal frames at a height of 30 inches (76 cm). Six to eight baskets made of 1/4-inch (6.3-mm) mesh-coated wire, 3 to 4 inches (7.6 to 10.1 cm) deep and 7 to 9 inches (17.8 to 22.9 cm) wide, are placed in the troughs. Hatching trough water is circulated with paddles or airstones (Fig. 3). Paddles are usually 2 to 3 inches (5 to 7.6 cm) wide and 6 to 7 inches (15.2 to 17.8 cm) long, and rotate at 20 to 25 rpm. Paddles made from high-density polyethylene plastic (Fig. 4) are recommended for safety because they will slip on the drive shaft when strong resistance is met. Airstones, an alternative to paddles, are effective when placed down the centerline of the hatching trough with egg masses on each side of the bubble stream. If airstones are used, you can prevent fungal and bacterial problems by



Figure 3. Hatching trough with metal paddles.



Figure 4. High-density polyethylene plastic hatching paddle.

making certain eggs are not stocked at too great a density.

Disinfect troughs between batches with household bleach (5.25%). Mix 1 cup (250 ml) of bleach with 1 gallon (3.8 L) of water. Add a little liquid dish soap to the solution if desired. Use rubber gloves and proper ventilation when cleaning troughs. Do not let this solution come in contact with eggs or fry.

### Estimating trough requirements

A single hatching trough will usually handle four to five weekly rotations of eggs, for an output of 200,000 eggs per week. Therefore, a single trough can usually yield 800,000 yolk-sac fry per season. After yolk-sac fry are transferred to rearing troughs or tanks, remove egg shells and dead fry from hatching troughs with a small mesh net or siphon hose.

Newly hatched yolk-sac fry can be transferred by siphoning them into a bucket using a  $\frac{1}{2}$ -inch-diameter (1.3-cm) clear plastic tube. Newly hatched yolk-sac fry average 1,200 to 1,500 per fluid ounce (40 to 50/ml) and are placed in rearing troughs at the rate of approximately 150,000 per 100 gallons (380 L) of water. The rearing troughs are usually equipped with an agitator or three or four 8-inch (20.3-cm) airstones for aeration and water movement. Airstones should be slightly elevated from the tank bottom by placing rubber or plastic bands around each end. This prevents yolk-sac fry from being pinched under the stones. A suggested hatchery layout diagram is shown in Figure 7. A hatchery with 40 to 50 troughs can produce 20 to 30 million fry per season.

### Egg hatching and treatments

Viable eggs are transparent, starting as a pale yellow and becoming darker yellow and finally orange-red. Dead eggs are opaque and usually enlarged. Water temperature in hatching troughs should be 78 to 82 °F (25 to 28 °C) (Table 1). Many hatchery managers believe they have the best success at 77 to 79 °F (25 to 27 °C). Depending on the stage at which eggs enter the hatchery, 5 to 6 days are usually required for hatching.

Hatching eggs will feel slippery; otherwise, masses should have a wet latex or rubber texture. External bacterial infections make eggs feel slippery to the touch. Eggs with “hairy patches” that appear white or brownish are infected with fungus. Small areas of fungus can be removed by hand, but keeping dead eggs clean with routine anti-fungal treatments is recommended. Catfish eggs can be treated with compounds such as hydrogen peroxide, iodine, formaldehyde, copper sulfate and common salt (see Table 2 and other SRAC publications).

Egg masses should not lie on top of each other in the hatching basket. Overcrowding causes problems that cannot be solved with egg treatments. Cloudy or bad-smelling water indicates severe egg degra-

Table 1. Egg development at 78 to 80 °F (25 to 28 °C).

Day	Egg appearance
1	Eggs pale, nearly white
2	Eggs dark yellow
3	Bloody streak appears
4	Embryo more golden with shape
5	Eyes visible—embryo moves frequently
6	Embryo complete—hatching
7	Yolk-sac fry

**Table 2. Egg treatments.**

All treatments are toxic to yolk-sac fry and should be discontinued as eggs become well eyed to eliminate the chance of killing eggs that may be hatching.

Compound	Method	Rate (for 100-gal. trough)
Hydrogen peroxide (35%)	Trough - 3 GPM flow*	110 ml (1-2 times per day)
Povidone iodine (1%)	Trough - 3 GPM flow*	50 ml (1-2 times per day)
Formalin (37% formaldehyde)	Trough - 3 GPM flow*	50 ml (1-2 times per day)
Copper sulfate crystal	Trough - 3 GPM flow*	10 g (dissolve in 5 gals. water, pour across trough 1-2 times per day)
Salt (NaCl)	Dip	1 lb. (dissolve in 5 gals. water, dip for 5 min. once a day as needed)

\*Water is not stopped for treatment but runs continuously.

dation and high bacterial counts in egg troughs.

### Yolk-sac fry

At hatching, the fry with attached yolk sacs will be orange-yellow with a black eye spot clearly visible (Fig. 5). At this stage, healthy fry will group tightly together near tank edges or in other areas with low current. Single fry or fry that appear whitish are frequently not viable. It is not uncommon to have a few fry (50 to 100) in each tank with this appearance. If more than

2 to 3 percent of fry are in this condition, problems with egg quality and water quality should be examined with the help of the state or regional aquaculture specialist.

### Hatchery feeds

High quality fry feeds (#00 size), usually 48 to 50% protein, can be purchased in 50-pound bags. Catfish, trout and salmon starter feeds yield good fry growth in the first days post swim-up. In most cases one bag is sufficient for each

1 million fry. Live artemia and dried krill have also been fed to swim-up fry in hatcheries. These feeds are very expensive and are recommended only as supplements to a full nutrient diet, as in prepared starter feeds. Until fry have turned dark and begun to rise to the top of the trough, no feeds are necessary, as the yolk sac has not been fully consumed. Depending on individual hatchery needs and equipment, most hatcheries keep fry 2 to 4 days post swim-up (Fig. 6) before stocking them into prepared ponds. Fry have been stocked at swim-up and even as yolk-sac fry with good success, but most managers prefer to stock larger fry that have been fed for 2 to 4 days.

Store bagged hatchery feed on pallets in a dry area. Open bags of feed should be kept in plastic garbage cans with lids to prevent moisture from getting to the feed and causing mold growth.

### Fry losses

Most fry losses are the result of poor handling in the egg stage, gas saturation in well water, or channel catfish virus (CCV). Consult your state or regional aquaculture specialist if your swim-up or yolk-sac fry losses are more than 2 to 3 percent of the total hatched in a given day.

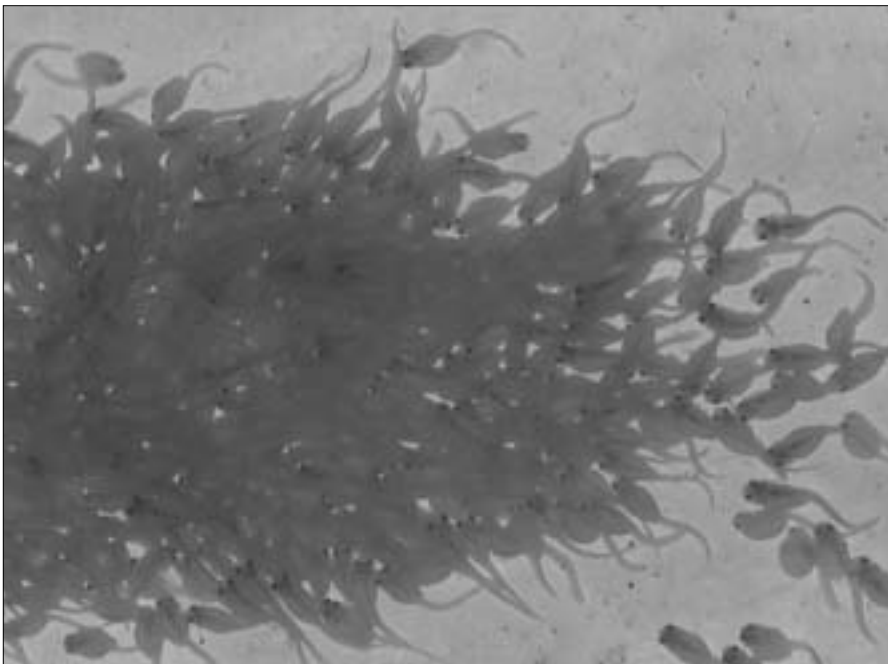


Figure 5. Yolk-sac fry at hatching.

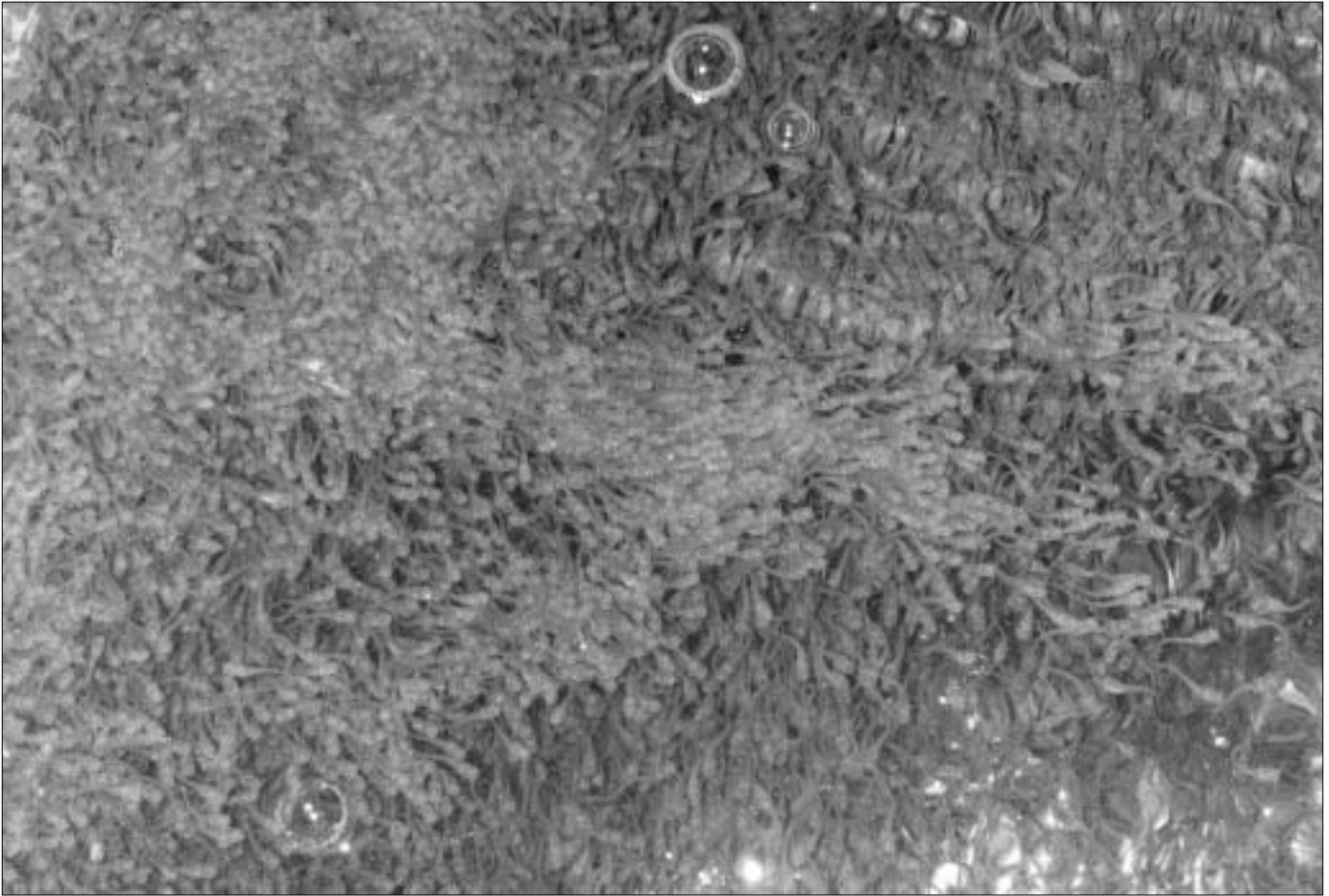


Figure 6. Swim-up fry 5 to 6 days post hatch.

### **Fry enumeration**

Fry stocked or sold from the hatchery should be enumerated by weight or volume. Scales capable of weighing 50 to 200 fry (0.1-g accuracy) or small volumetric cylinders (10 ml with 0.1-ml graduations) are best for estimating fry counts. Counting a total of 150 to 200 fry per trough and finding their weight or volume displacement is sufficient for most purposes. Larger platform scales accurate to 0.1 pound or 0.05 kg, or graduated beakers with 100-ml graduations, are required for final total fry estimates.

### **Fry transport tanks**

Unless fry are to be transported more than 20 miles from the hatchery, a single-wall, uninsulated,

metal tank equipped with airstones is sufficient. Fry transport tanks can be constructed or purchased. Most commercial tanks are pulled behind a truck as a small trailer. These tanks usually hold 200 to 300 gallons (760 to 1,140 L) of water and have a V-shaped bottom, which slopes to a 2-inch (5-cm) drain equipped with a valve or gate and an attachment for a length of hose to reach the pond. Oxygen is released through airstones lying on the tank bottom. Bottled oxygen is preferred for transport because agitators operate at the tank surface, some distance from the fry, and are easily clogged. Fry density in the transport tank should not exceed 0.5 pound per gallon (60 g/L). Vaccines can be added to the transport water. Follow label

directions carefully and maintain proper dissolved oxygen concentrations during the vaccination process. Salt may also be used during fry transport to reduce stress and coagulation of fry from transfer and enumeration. A concentration of 500 mg/L of common salt can be added when the transport tank is completely filled and the fry are on board. Because salt is very corrosive to metal surfaces, rinse the transport tank with water after fry are stocked into the receiving pond.

Fry are usually off-loaded when water temperature in the receiving ponds is close to that of the hatchery. This may be mid-day in the early portion of the season and early morning on hotter days. Tempering fry for differences in water quality is always recom-

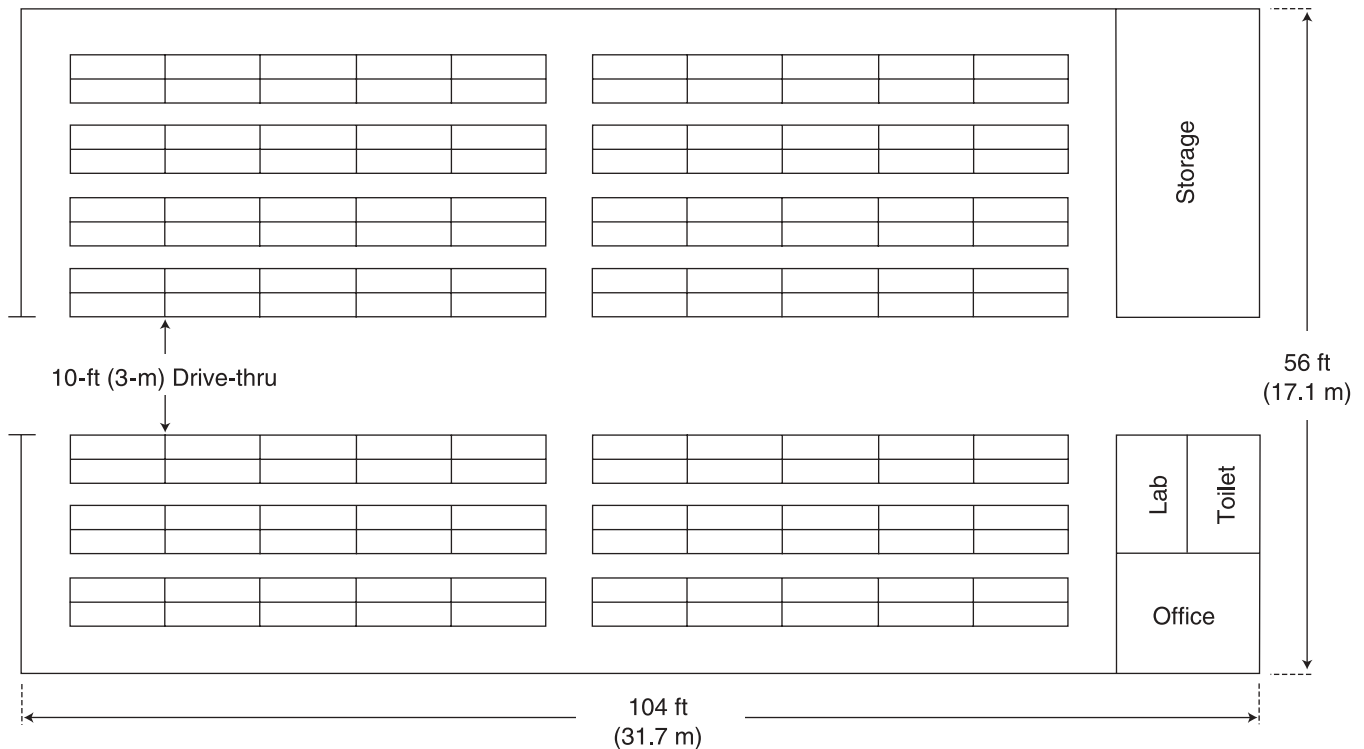


Figure 7. Layout for a medium-size hatchery.

mended and requires only 15 to 20 minutes. Buckets of pond water and/or a small pump discharging the receiving pond water can be used to rinse fry remaining in the corners and low areas of the transport tank.

### General recommendations

Channel catfish hatcheries have many different layouts. They can be totally enclosed or largely open, depending on the site and the preferences of the operator. Closed buildings usually have large exhaust fans to create air flow. Open buildings may require shading with plastic tarps to keep out direct sunlight. There should be vehicle access throughout the building, if possible, to make loading and unloading easy (Fig. 7). Most hatcheries are metal frame, metal wall buildings on concrete slabs. Plumbing can be placed in the slab, hung from ceiling supports, or attached to the trough stands. Main water lines should be

2-inch (5-cm) piping with  $\frac{3}{4}$ -inch (1.9-cm) pipe leading to the individual troughs. Most often the terminal outlet is a hose-bib releasing water 2 to 3 inches (5 to 7.6 cm) above the trough water surface. Drains are usually  $1\frac{1}{2}$ -inch (3.8-cm) pipe standing up 8 to 10 inches (20.3 to 25.4 cm) in the trough end opposite the water inflow. Window screen is usually attached to this standpipe to keep hatched fry from escaping the trough. Most hatcheries are plumbed largely with PVC pipe and galvanized metal pipe.

Hatcheries are labor-intensive operations that require a great deal of attention during the 2- to 3-month hatching season. Most operations require two or three seasonal employees in addition to a manager and two or three full-time assistants. The size of the operation and the size of fingerlings to be grown affect the hatchery labor budget.

If electric power fails there can be a total loss of the hatchery inventory in less than 30 minutes. Stand-by generators run by propane or diesel are necessary insurance against power failure. Even generators wired to start automatically in the case of power loss may fail, so check systems weekly. Consider having a back-up alarm system that notifies personnel in case of power failure. All personnel should know how to start generators (if they are manually operated) and who to contact in an emergency. There should be enough stand-by power to operate all systems, including the pump or well.

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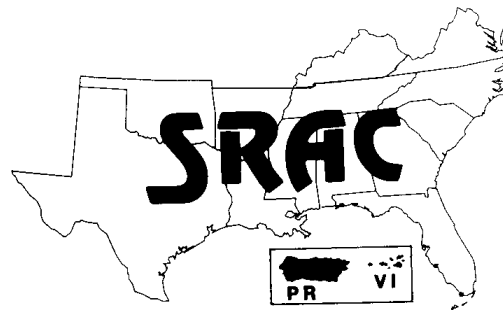
SRAC fact sheets are reviewed annually by the Publications, Videos and Computer Software Steering Committee. Fact sheets are revised as new knowledge becomes available. Fact sheets that have not been revised are considered to reflect the current state of knowledge.



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SOUTHERN  
REGIONAL  
AQUACULTURE  
CENTER



SEVENTEENTH ANNUAL PROGRESS REPORT

For the Period Through August 31, 2004

December, 2004

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In cooperation with the U.S. Department of Agriculture, Cooperative  
State Research, Education, & Extension Service

## **IMPROVING REPRODUCTIVE EFFICIENCY TO PRODUCE CHANNEL × BLUE HYBRID CATFISH FRY**

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### **Reporting Period**

March 1, 2004 - August 31, 2004

<b>Funding Level</b>	Year 1 .....	\$118,390
	Year 2 .....	\$111,610
	Year 3 .....	\$123,000
	Year 4 .....	\$123,000
	Total .....	\$476,000

<b>Participants</b>	Auburn University (Lead Institution) .....	Rex Dunham, Allen Davis, Ron Phelps
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	Mississippi State University .....	Lou D'Abramo
	University of Memphis .....	Charles Lessman, Bill Simco
	USDA/ARS .....	Brian Bosworth, Brian Small

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## **PROJECT OBJECTIVES**

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1. Develop brood stock selection and management protocols to optimize channel × blue hybrid embryo production.
  - a. Determine minimum cold period required and rates and patterns of application of thermal changes to promote synchronous gonadal development and spawning.
  - b. Improve hybrid embryo production by determining the best nutritional regime to maximize fecundity and hatch rate from induced channel catfish females and blue catfish males.
  - c. Improve hybrid embryo production via genetic enhancement.
  
2. Develop induced spawning techniques and management strategies to optimize gamete collection and storage.
  - a. Develop procedures to predict ovulation of channel catfish.
  - b. Conduct pivotal protocol studies for determining dosage rates and timing of application of luteinizing hormone releasing hormone (LHRHa), carp pituitary

- extract and catfish pituitary extract to maximize ovulation, hatch rate and fry production.
    - c. Improve hybrid embryo production via pheromonal manipulation of channel catfish males and blue catfish males for improved ovulation, spermiation, egg quality, hatch and fry production.
    - d. Develop extended refrigerated storage and cryopreservation of sperm.
  - 3. Develop techniques to identify, assess and improve gamete quality.
    - a. Develop criteria for standardizing and classifying egg quality prior to injection and after manual stripping and describe the morphological and physiological condition of channel catfish eggs including evaluation of morphological changes of oocytes during oocyte maturation in female catfish.
    - b. Determine the profile of estradiol hormone from serum plasma of 2-year-old female channel catfish over a 12-month period, determine changes in oocyte maturation during vitellogenesis and identify the different cathepsins that are responsible for vitellogenin degradation and oocyte maturation in female catfish.
    - c. Develop in vitro assays to evaluate sperm quality and evaluate their predictive ability in relation to fertilization and hatch.
  - 4. Develop economically viable standardized hatchery procedures and fertilization protocols to optimize hatching rate of hybrid embryos.
    - a. Determine optimal sperm (fresh, frozen and refrigerated)-to-egg ratios for fertilization and hatch.
    - b. Determine the effects of commonly used therapeutics on hatching success.

## **PROGRESS AND PRINCIPAL ACCOMPLISHMENTS**

**Objective 1.** *Develop brood stock selection and management protocols to optimize channel × blue hybrid embryo production.*

**Objective 1a.** *Determine minimum cold period required and rates and patterns of application of thermal changes to promote synchronous gonadal development and spawning.*

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**Louisiana State University and University of Memphis.** Water temperature is the primary environmental factor affecting the spawning of channel catfish, *Ictalurus punctatus*. Spawning begins when water temperatures consistently remain above 21EC at some locations such as Louisiana and west Mississippi. The spawning season at the Aquaculture Research Station of the Louisiana State University

Agricultural Center was lengthened by heating ponds through addition of geothermal water (36EC). This study attempted to use degree-days (ED) to describe and quantify the total heat requirement for channel catfish to initiate spawning, which should also indicate the same requirement to initiate artificial spawning to produce hybrid embryos. Degree days were calculated for 153

spawns between 1999 and 2004. Ponds from 1999 to 2002 had four available spawning sites (cans), and in 2003 and 2004 the ponds had six sites. Degree-days needed to obtain the first four (1999-2002) or six (2003-2004) spawns were calculated to prevent spawning site limitations effects on the degree-day values.

In 2004, three heated ponds were maintained at three different temperatures. Degree-day values were calculated for 18 spawns using three threshold temperatures as the starting point to calculate the degree-days (Table 1). The 21EC threshold yielded a constant value of  $98 \pm 4$  ED for the heat requirement of channel catfish to initiate spawning.

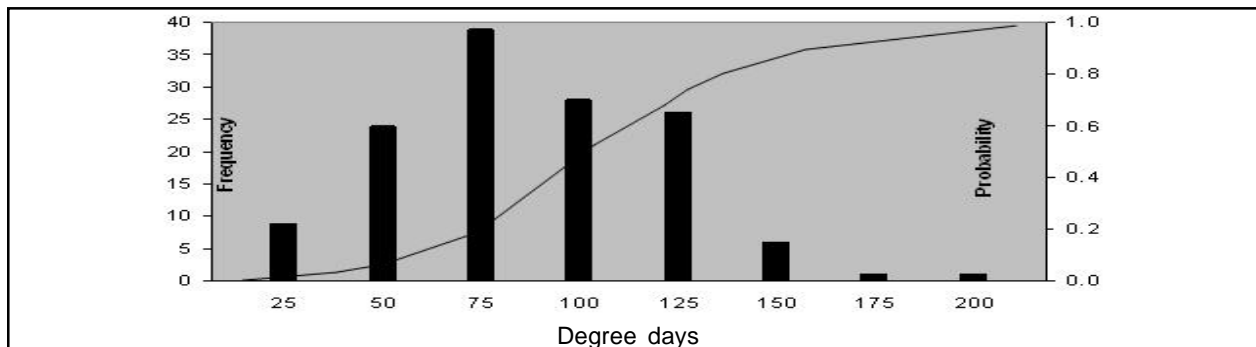
Degree-days were also calculated using the 21EC threshold for 135 spawns collected during the early

spawning and regular spawning periods between 1999 and 2003. The average ED value above the 21EC threshold was  $97 \pm 33$  ED. Spawning probabilities and frequency of spawns were plotted against ED values (Figure 1). The probability that a fish will spawn after 100 ED was 50% and increased to 93% after 150ED. Fifty percent of spawns occur between 75ED and 125 ED and ninety percent between 50 ED and 150 ED. These results concur with the literature that 21EC is the minimal water temperature needed to initiate the reproductive process in channel catfish.

Additionally, ED values above 21EC may be useful as a management tool to predict channel catfish spawning times in heated ponds, and the correct time to initiate artificial spawning for hybrid embryo production.

**Table 1. The average degree day value for spawns above three thresholds from ponds maintained at different temperatures. Values in the same row followed by the same letter do not differ significantly ( $P < 0.05$ ).**

Target temperature	Actual temperature	Threshold		
		18EC	21EC	24EC
21EC	$23.1 \pm 1.5$ EC	234a	95a	8a
24EC	$23.1 \pm 2.6$ EC	203ab	98a	22b
27EC	$24.6 \pm 3.0$ EC	184b	102a	41c



**Figure 1. Spawning probabilities and spawning frequency at different degree day values above the 21EC threshold.**

**Objective 1b.** *Improve hybrid embryo production by determining the best nutritional regime to maximize fecundity and hatch rate from induced channel catfish females and blue catfish males.*

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**Auburn University.** Female brood stock were maintained in 0.1-acre ponds at a density of 1,300 pounds/acre. Fish were offered a commercial floating feed (32% protein) diet three times a week at 1.5% of their body weight. Water temperature and dissolved oxygen were measured daily in the early morning and after sunset. Floating vertical pump aerators were used when oxygen concentrations dropped below 2 mg/L. Ammonia and nitrite concentrations were estimated twice weekly. Hardness and alkalinity were measured at the beginning and at the end of the experiment. Three months prior to the onset of spawning, four dietary treatments were assigned to four replicate ponds each containing three strains of catfish. A factorial 2 × 2 design was used to evaluate feed quality and feeding rate. The two test diets were: 32% typical practical catfish feeds, and 42% high fish meal practical catfish feeds, and the feed was offered either three or six times a week, to apparent satiation. Females were spawned in three periods (early, middle and late spawning periods), and egg mass, egg diameter, fertilization rate at 48 hours, and biochemical analy-

ses, were recorded as indicators of egg quality. Fish strains used were selected based on previous spawning productions, such as differences in performance related to feed quality and feeding rate.

Statistical analyses of the spawning data is currently under way to determine the influence of the various dietary treatments as well as the influence of strain, age, sex and spawning period. All statistical analyses were performed using SAS version 8.2 software. The effects of feeding rate and feed quality on fish performance, fecundity and egg quality, related to strain and spawning period are being evaluated. Preliminary analysis indicates nutrition affected hybrid embryo production. Definitive recommendations cannot be made because of apparent genotype × nutrition, age, egg mass position and tank size effects on hatch rate and fry/kg, and analysis is ongoing to account for these factors. Once the spawning data is analyzed selected egg samples representing good and bad quality spawns will be selected and further analyzed for biochemical composition.

**Objective 1c.** *Improve hybrid embryo production via genetic enhancement.*

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**Auburn University.** The channel × blue catfish hybrid grows faster, has more efficient feed conversion, has a higher tolerance for low dissolved oxygen concentrations, and better survival compared to channel catfish. However, economic production of hybrid embryos is problematic. Some strains of channel catfish females or blue catfish males may have reproductive characteristics more suited for production of channel catfish female × blue catfish hybrid catfish embryos than others. AU channel catfish strains 1 through 5 consistently produced greater numbers of hybrid

fry than AU strains 6 through 10 for three consecutive years (Table 2). Additionally, AU-1 produced greater numbers of fry than AU-7 in a fourth year. Nutrition × genotype interactions were observed for hybrid fry produced/kg female body weight. Strain of male blue catfish affected hatching rate of hybrid embryos and sperm production. Genotype-environment interactions were also observed for sperm production. Utilization of genetic variation has the potential to double efficiency and productivity of hybrid embryo production.

**Table 2. Percentage of females ovulating, fecundity, and fry/kg for channel catfish female strains when injected with LHRHa and hybridized with blue catfish males in 2003.**

Channel catfish Female	% ovulation	fecundity (eggs/kg)	fry/kg
AU-1	100	11,047	1857
AU-2	75	7,133	2154
AU-3	100	11,997	1283
AU-4	82	6,545	1005
AU-5	100	8,790	858
AU-7	73	10,179	693
AU-8	100	9,122	625
AU-9	75	9,438	492
AU-6	90	7,814	395
103	80	7,425	257
AU-10	45	9,575	163

**Mississippi State University.** Groups of nine, 2-year old female channel catfish brood stock obtained from each of 4 different strains/sources were tagged and stocked into four 0.1-acre earthen ponds (36 fish per pond, 9 fish per strain) in April. Blood and egg samples were collected from twelve fish in each pond (3 fish/strain) every month for 11 and 9 months for blood and eggs, respectively. No

individual fish within a strain was subject to sampling more than once every four months. Plasma estradiol, plasma testosterone, cathepsins, protein content of eggs and egg size were measured. No noteworthy differences in the mean values of the physiological indices monitored were observed among the four strains during each month.

**Objective 2.** *Develop induced spawning techniques and management strategies to optimize gamete collection and storage*

**Objective 2a.** *Develop procedures to predict ovulation of channel catfish.*

**Auburn University.** Hybrid channel × blue catfish can be obtained by induced spawning and artificial fertilization but with variable results. A threshold degree of maturity must be reached before brood fish can be induced to spawn but selection of such fish can be very subjective. Temperature of the surrounding environment affects the rates of physiological processes in fish. Response time to applications of induced spawning hormones such as LHRHa is thought to be related to water temperature.

Female brood fish (Marion strain channel catfish) were given a subjective ranking of poor, fair or good as well as measurements of body weight, total body length, body width and girth were taken. Brooders were held at 24, 26, and 28EC in 60-gallon aquaria and injected with LHRHa at 20 Fg/kg as a preparatory injection followed 12 hours later with 100 Fg/kg. Fish were monitored hourly as ovulation approached, and the time of the first egg deposit and when approximately 100 eggs were found were recorded. Approximately half the

females were manually stripped soon after the first egg was observed and the other fish were stripped 4 to 6 hours after the first egg was observed. Eggs were artificially fertilized with blue catfish sperm and incubated. For each egg mass, the percentage of viable embryos at 24 hours after fertilization, the percent hatch, and percent survival at swim-up was determined.

The overall mean degree-hour response time (temperature in EC × time in hours to first egg release) was  $1,156 \pm 275$ . The mean degree hour response time was  $1,416 \pm 107$  at 24EC,  $1,228 \pm 211$  at 26EC and  $981 \pm 278$  at 28EC. The percentage of females that ovulated were 58, 62.5 and 87.5% at 24, 26, and 28EC, respectively. The majority of females which did ovulate did so between 58 to 64 hours at 24EC, 48 to 52 hours at 26EC and 24 to 40 hours at 28EC with the fish classified as “good” spawning sooner than the “poor” classification at all temperatures. When only the good quality females

were considered, the weight of eggs released/kg female varied by water temperature, At 24EC an average of  $70 \pm 60$  g were obtained/kg, at 26EC  $126 \pm 41$ , and at 28EC  $154 \pm 34$ . The number of eggs/g of eggs also varied by temperature,  $71 \pm 11$ ,  $53 \pm 6$ , and  $48 \pm 10$  at 24, 26 and 28EC respectively. Egg quality varied with how soon eggs were taken after the first egg was released. For females at 28EC, when eggs were taken within 2 hours of being observed the % viable embryos averaged  $76 \pm 13\%$  and the % hatch was  $31 \pm 16\%$ . When eggs were taken at 4 or more hours of being observed the % viable embryos averaged  $66 \pm 19\%$  and the % hatch was  $9.7 \pm 6.6\%$ . When a female was stripped within 2 hours after the first eggs were released, a lower weight and total number of eggs/kg ( $107.3 \pm 46.6$  and  $5,739.8 \pm 2174$ ) were obtained relative to fish stripped 4 or more hours after the first eggs were released ( $147.7 \pm 36$  and  $7,724 \pm 2120$ , respectively).

**Objective 2b.** *Conduct pivotal protocol studies for determining dosage rates and timing of application of luteinizing hormone releasing hormone (LHRHa), carp pituitary extract and catfish pituitary extract to maximize ovulation, hatch rate and fry production.*

**USDA-ARS.** The effectiveness of catfish pituitary extract, carp pituitary extract, and LHRHa for inducing spawning in female channel catfish and subsequent production of channel catfish × blue catfish hybrid fry was compared. Mature female catfish (3 to 5 years old) were injected with carp pituitary extract (n = 66), catfish pituitary extract (n = 51), or LHRHa (n = 58). Catfish pituitaries were collected in March and April at a commercial catfish processing plant from fish > 3 pounds, dried in acetone, and ground to a powder. Carp pituitary and LHRHa were purchased from commercial vendors (Stoller Fisheries, Spirit Lake, IA and Syndel International, Inc., Vancouver, BC, Canada, respectively). Injection regimes were 2 mg/kg female body weight (BW) initial injection and 8 mg/kg 20 hours

later for carp and catfish pituitary extract or 40 Fg/kg female BW initial injection followed by 80 Fg/kg 20 hours later for LHRHa. Females were checked for ovulation 24 hours following the final injection. Ovulating females were tranquilized and eggs were manually stripped into Hank's Balanced Salt Solution (HBSS). Eggs were weighed and then fertilized with blue catfish sperm. Blue catfish sperm was prepared by macerating testes from 4 to 5 blue catfish males and pooling the sperm in HBSS. Approximately 25 mL of sperm-solution was used to fertilize each 400 g sample of eggs. Egg masses were placed in hatching troughs following fertilization and percent viable embryos was determined at 48 hours post-fertilization. Fry numbers at hatch were estimated volumetrically. Data collected for

each treatment included: weight of females injected, percent of injected females that ovulated, fecundity (number of eggs/kg female body weight), percent viable embryos at 48 hours, fry/kg body weight of all females, fry/kg body weight of ovulated females, and total fry. Treatment means were compared by mixed model ANOVA.

There were no differences among treatments for

any of the variables measured (Table 3). Results demonstrate that catfish pituitary extract was as effective as carp pituitary extract or LHRHa for inducing ovulation in channel catfish females. Catfish pituitary is readily available from commercial catfish processing facilities, although regulatory issues associated with using it to induce spawning in fish are not known.

Females ovulated with LHRH flowed much easier and more completely, but it seemed their time-frame for ovulation was wider. The LHRH may have done better if a longer period of time would have been allowed for ovulation. The pituitary-treated fish seemed to ovulate more synchronously but never flowed as well as a good LHRH fish. This observation that CPE-treated fish ovulate more synchronously has been confirmed at Auburn University. Latency time for LHRH-treated fish is longer, and the observations observed at USDA are consistent with observations at other locations.

### Results at a glance...

★ Hatch rate of hybrid embryos is improved if LHRH-injected channel catfish females are stripped within 2 hours of first observation of egg release. Waiting longer will increase the number of eggs stripped, but this is more than offset by much lower hatch rate.

**Table 3. Comparison of catfish pituitary extract, carp pituitary extract, and LHRHa for inducing spawning in channel catfish females and production of channel catfish × blue catfish fry.**

Treatment	# of females injected	Mean weight of females (kg)	% females ovulating	Eggs/kg female BW	% viable embryos	Fry/kg BW all females	Fry/kg BE ovulated females	Total fry
Carp PE	66	2.9	71	6482	55.5	1348	1788	239,100
Catfish PE	51	2.8	68	6767	64.1	1128	1600	190,100
LHRHa	58	3.0	65	6482	66.3	1527	1999	254,100
Standard Error		0.2	8.4	720	8.6	344	350	

**Auburn University.** Luteinizing hormone releasing hormone analogue, LHRHa, injections were more effective than carp pituitary extract, gonadotropin hormone releasing hormone, GnRH, salmon GnRH and ovaprim injections for producing channel catfish female × blue catfish hybrid catfish embryos. LHRHa injections (one or two priming

injections and a resolving injection) ranging from 10/50 to 30/150 Fg/kg female body weight were compared. In general, the higher dosages of 20/100 and 30/150 were the most effective, but the dosage must be decreased as the spawning season progresses to maintain maximum effectiveness. At the end of the spawning season 10/50 is an

effective dosage. LHRHa implants were more effective than injections for producing channel catfish female × blue catfish hybrid catfish embryos. Implanted fish had a more variable time of ovulation, but females that ovulated up to 48 hours later than the average female gave high quality eggs, whereas late ovulating injected females give over ripened eggs. The advantage of the implants is greatest late in the spawning season. Fry/kg produced ranged from 200 to 3,000 for the various treatments.

**University of Memphis.** Channel catfish ovarian follicles were treated in vitro with 17 $\alpha$ ,

20 $\beta$ -dihydroxyprogesterone and human chorionic gonadotropin in vitro. Initial efforts have focused on screening for potentially effective hormones to influence oocyte maturation and ovulation. Evaluations have included various culture media, hormonal concentrations, and the timing of the application of hormones. Methods are being investigated to adequately evaluate the oocyte response to various treatments. Such findings will hopefully be applicable to the evaluation of gonadotropins used to induce spawning of eggs of high quality from channel catfish brood stock.

**Objective 2c.** *Improve hybrid embryo production via pheromonal manipulation of channel catfish males and blue catfish males for improved ovulation, spermiation, egg quality, hatch and fry production.*

**Auburn University.** Reducing handling and stress of channel catfish females may be key factors for effective production of channel catfish female × blue catfish hybrid catfish embryos. Females were either left free in tanks or confined in bags or aquaria. Confinement increased hybrid fry production and reduced labor involved in the production protocol. Exposure to the scent of conspecific males

sometimes increased and sometimes decreased hybrid fry production (Table 4). Method of exposure appears to have an effect. If water is introduced from separate tanks containing males is introduced, positive effects on hybrid fry production, whereas visual or actual contact appears to have negative effects (Table 5).

**Table 4. Mean eggs/kg female body weight (BW), hatching percentage, fry/kg female body weight and egg quality of channel catfish females (*Ictalurus punctatus*) exposed or not exposed to channel catfish male after injection with luteinizing hormone releasing hormone agonist, LHRHa when hybridized with blue catfish (*Ictalurus furcatus*) male (mean  $\pm$  SD) in 2001.**

Treatment	Spawning Percentage (N=24)	Egg/kg Female BW	Hatching Percentage	Fry/kg Female BW	Latency Time (hour)	Egg Quality
Unexposed	90 <sup>a</sup> $\pm$ 30	6,822 <sup>a</sup> $\pm$ 2,268	31.1 <sup>a</sup> $\pm$ 6.7	2,246 <sup>a</sup> $\pm$ 652	31 <sup>a</sup> $\pm$ 5	3.3 <sup>a</sup> $\pm$ 0.15
Exposed	100 <sup>a</sup> $\pm$ 0	7,358 <sup>a</sup> $\pm$ 1,756	40.5 <sup>b</sup> $\pm$ 1.6	3,031 <sup>b</sup> $\pm$ 1,028	30 <sup>a</sup> $\pm$ 5	3.7 <sup>b</sup> $\pm$ 0.11

<sup>a,b</sup> means followed by the same letter are not different ( $P > 0.05$ ) within each column.

**Table 5. Mean spawning percentage, egg/kg female body weight (BW), hatching percentage, fry/kg female body weight and latency time at 29°C for channel catfish (*Ictalurus punctatus*) females injected with luteinizing hormone releasing hormone agonist, LHRHa, with different exposures to channel catfish males (mean ± SD) in 2002.**

Treatment	Spawning Percentage (N=10)	Egg/kg Female BW (N=10)	Hatching Percentage (N=10)	Fry/kg Female BW (N=10)	Latency Time (hour) (N=10)
30 + 150 low male	80 <sup>a</sup> ± 42	9,368 <sup>a</sup> ± 1,519	14.4 <sup>a</sup> ± 0.64	1,351 <sup>a</sup> ± 219	31 <sup>a</sup> ± 0.10
- 150 no male	80 <sup>a</sup> ± 42	8,288 <sup>a</sup> ± 2,671	52.9 <sup>b</sup> ± 0.45	4,384 <sup>b</sup> ± 1413	31 <sup>a</sup> ± 0.10
30 + 150 high male	90 <sup>a</sup> ± 31	8,211 <sup>a</sup> ± 3,882	23.2 <sup>c</sup> ± 0.11	1,901 <sup>a</sup> ± 899	32 <sup>b</sup> ± 0.52

<sup>a,b</sup> means followed by the same letter are not different ( $P > 0.05$ ) within each column.

**Objective 2d.** *Develop extended refrigerated storage and cryopreservation of sperm.*

**Louisiana State University.** Knowledge of sperm concentration is essential for standardization of protocol for gamete cryopreservation and for optimizing fertilization in artificial spawning. Currently there is a lack of information regarding sperm concentration and how it relates to cryopreservation and fertilization in essentially all species including channel catfish. Practical methods for evaluation of sperm concentration in channel catfish are needed. The specific objectives of this study were to evaluate: 1) the use of a spectrophotometry in determining sperm concentrations; 2) sperm concentrations relative to gonad composition, and 3) optimal sperm concentration for fertilization during artificial spawning.

Channel catfish were seined from ponds during the months of April and May 2004. The males were killed and total lengths and weights were recorded. Testes were surgically removed and were suspended in calcium-free Hanks' balanced salt solution (C-F HBSS) at 290 mOsmol/kg. The testes were cleaned by removing adherent blood and tissue, separated into visually estimated anterior and posterior sections, weighed, and crushed in C-F HBSS (1g/20mL) to release sperm.

Sperm concentrations and motility estimates relative to gonad composition are summarized in Table 6. Sperm concentrations vary in relation to gonad composition.

**Table 6. Summary of sperm concentrations and motility from whole testis and posterior and anterior sections.**

	Concentration (/mL)	Total Concentration	Sperm / g Testis	Motility (%)
Intact	$1.73 \times 10^8 \pm 9.4 \times 10^7$ <sup>a</sup>	$1.78 \times 10^{10} \pm 2.0 \times 10^{10}$ <sup>a</sup>	$3.52 \times 10^9 \pm 1.89 \times 10^9$ <sup>a</sup>	35 ± 4.5 <sup>a</sup>
Posterior	$1.06 \times 10^7 \pm 2.7 \times 10^7$ <sup>b</sup>	$1.41 \times 10^8 \pm 2.37 \times 10^8$ <sup>b</sup>	$2.09 \times 10^8 \pm 5.4 \times 10^8$ <sup>b</sup>	23 ± 4.6 <sup>a,b</sup>
Anterior	$3.13 \times 10^8 \pm 1.18 \times 10^8$ <sup>c</sup>	$1.42 \times 10^{10} \pm 1.5 \times 10^{10}$ <sup>c</sup>	$5.74 \times 10^9 \pm 2.24 \times 10^9$ <sup>c</sup>	41 ± 4.6 <sup>b</sup>

<sup>a,b</sup> means in a column with different letters were significantly different ( $P < 0.05$ , n=21).

**Objective 3.** *Develop techniques to identify, assess and improve gamete quality.*

**Objective 3a.** *Develop criteria for standardizing and classifying egg quality prior to injection and after manual stripping and describe the morphological and physiological condition of channel catfish eggs including evaluation of morphological changes of oocytes during oocyte maturation in female catfish.*

**University of Memphis.** Initial images of catfish oocytes and embryos were made by automated transparency scanners. Automated transparency scanners imaged catfish oocytes and embryos during oocyte maturation and embryogenesis, respectively. This technology was developed for analysis of motility mutants in zebrafish (Computer-Aided-Screening, CAS) and is being adapted for analysis of catfish oocytes and embryos. Initial trials indicate that CAS may be used to follow catfish embryos throughout their 6 to 7 day period of development to hatching. The CAS system worked quite well in spite of the prolonged development time for catfish embryos (i.e., 6 to 7 days versus 2 days for zebrafish). Animations of time-lapse image stacks in ImageJ revealed a surprising amount of cell movement in cleavage stage embryos. Other details of embryonic development included gastrulation/epiboly, neurulation, initiation of motility and hatching. Arrested development and subsequent cytolysis of abnormal embryos could also be clearly documented, including the developmental events prior to arrest and death. Factors that require additional study include requirement for pathogen control of scanned embryos, density of scanned embryos in relation to fluid volume and peristaltic pump flow during the 6 days of development. Initial imaging of catfish oocytes suggested the feasibility of adapting this technique developed originally for zebrafish and called Computer-Aided Meiotic Maturation-Assay (CAMMA). Although fully-grown oocytes of catfish are significantly larger than those of zebrafish, the preliminary results indicate that CAMMA may be successfully used to follow oocyte maturation in the catfish system. Factors that require additional study include 1) best saline/medium formulation to support oocyte

viability and maturation in vitro, 2) relationship of oocyte clearing to cell cycle stages of the oocyte and 3) optimal hormone milieu to elicit oocyte maturation in vitro.

Currently, data is being extracted from stacks of scanned images from catfish oocytes treated with various media and hormones, and embryonic development is being analyzed. Catfish oocytes and ovary extracts will be screened for reaction with cell-cycle control protein antibodies (e.g., anti-cyclin B1) that may prove useful in studies of oocyte maturation in catfish.

**Louisiana State University.** Ultrasound is a non-invasive technique that has been used with female livestock to monitor follicular growth through ovulation. It has also been used as a tool for sex identification and carcass evaluation in several species of fish (e.g. Atlantic salmon, Atlantic halibut, striped bass, shovelnose sturgeon, and barfin flounder). The objectives of this study were to evaluate visibility of gonads at different life stages, ovarian development in strip spawned and non-spawning females, and oocyte diameter by use of ultrasound in channel catfish.

During February through June, 2004, channel catfish gonads were evaluated at three different life stages: fingerlings (under 1 pound), market-sized foodfish (1 to 2 pounds), and brood stock (more than 3 pounds). Fish were scanned using a linear ultrasound probe (3 to 10 MHz), and gonadal sex was verified by dissection. To evaluate ovarian development, twelve females were given injections of artificial luteinizing hormone-releasing hormone. Of these, five were strip spawned. Fish were scanned

daily to monitor gonadal development.

Gonads were correctly identified as testis or ovary for fingerlings (57%), food fish (90%), and brood stock (86%). Immature gonads were difficult to distinguish from surrounding tissues. Mature testes were partially visible, but we could not quantify their development due to lack of contrast with surrounding tissues. Unlike testes, mature ovaries were easily distinguished and their development quantified by measuring ovarian diameter, calculating the ratio of ovarian diameter to body wall diameter (OD:BD),

and measuring oocyte diameter. There were no significant differences ( $P < 0.05$ ) in ovarian diameter or in OD:BD between strip-spawned and non-spawning females (Table 7). Strip-spawned females had significantly larger ( $P < 0.01$ ) oocyte diameters than non-spawning females on days 3 and 4 after injection. The results indicate that ultrasonography could be a useful tool for monitoring ovarian development in channel catfish. This could be used in artificial spawning of large groups of females, such as in production of hybrids of channel catfish females and males of blue catfish.

**Table 7. Ovarian and oocyte development of strip-spawned (n = 5) and non-spawning (n = 7) females after hormone injection. Daily means for strip-spawned and non-spawning fish within each variable that share letters were not significantly different ( $P < 0.01$ ).**

Day	Ovarian Diameter (mm)		OD:BD*		Oocyte Diameter(mm)	
	Strip-spawned	Non-spawning	Strip-spawned	Non-spawning	Strip-spawned	Non-spawning
1	54.7 ± 5.8 <sup>a</sup>	54.2 ± 7.4 <sup>a</sup>	0.87 ± 0.03 <sup>a</sup>	0.84 ± 0.05 <sup>a</sup>	1.8 ± 0.4 <sup>a</sup>	1.8 ± 0.5 <sup>a</sup>
2	65.8 ± 5.1 <sup>a</sup>	50.4 ± 9.1 <sup>a</sup>	0.89 ± 0.03 <sup>a</sup>	0.85 ± 0.04 <sup>a</sup>	1.9 ± 0.4 <sup>a</sup>	1.9 ± 0.5 <sup>a</sup>
3	70.6 ± 6.6 <sup>a</sup>	63.9 ± 7.4 <sup>a</sup>	0.89 ± 0.03 <sup>a</sup>	0.88 ± 0.05 <sup>a</sup>	2.2 ± 0.5 <sup>a</sup>	1.8 ± 0.4 <sup>b</sup>
4	60.6 ± 0.0 <sup>a</sup>	64.3 ± 11.3 <sup>a</sup>	0.90 ± 0.00 <sup>a</sup>	0.87 ± 0.06 <sup>a</sup>	2.0 ± 0.4 <sup>a</sup>	1.8 ± 0.5 <sup>b</sup>

\*Ovarian diameter : Body wall diameter

**Objective 3b.** *Determine the profile of estradiol hormone from serum plasma of 2-year-old females of channel catfish over a 12-month period, determine changes in oocyte maturation during vitellogenesis and identify the different cathepsins that are responsible for vitellogenin degradation and oocyte maturation in female catfish.*

**Mississippi State University.** The catfish industry is hampered by a chronic inefficiency resulting from the low spawning success of female brood stock for the annual production of fingerlings. Current estimates of spawning success of females range from 20 to 30%. An understanding of the relationship of annual changes in physiological indices

during a reproductive cycle to oocyte maturation and successful spawning in channel catfish may contribute to an accurate prediction of successful spawns. The objective of this study was to evaluate the effects of plasma steroid concentrations (estradiol and testosterone), egg size and protein degradation by cathepsins D, L and B on in vivo

egg maturation in four strains of channel catfish.

Groups of nine, 2-year-old female channel catfish broodstock obtained from each of 4 different strains/sources were tagged and stocked into four 0.1-acre earthen ponds (36 fish per pond, 9 fish per strain) in April. Blood and egg samples were collected from twelve fish in each pond (3 fish/strain) every month for 11 and 9 months, respectively, for blood and eggs. No individual fish within a strain was subject to sampling more than once every four months.

For all strains, mean plasma estradiol concentrations ranged from 0.02 to 0.29 ng/mL from June through December, and increased dramatically in January, peaking in February (3.4 to 3.7 ng/mL), and remained above 1.00 ng/mL through May. Mean plasma testosterone concentrations increased from May through September (0.03 to 1.23 ng/mL), de-

creased in October, and then increased and remained at approximately 1 ng/mL through April. Mean activities of cathepsins D and L steadily increased beginning in October and were highest in March, whereas the activity of cathepsin B was variable from month to month. Mean protein content of eggs was highest in October (3.08 to 3.795) when eggs appeared and decreased to levels of 0.54 to 2.14% for the remainder of the year (November through April) when eggs were present. From October to November the mean egg size increased by approximately 40 %, to 1.0-1.4 mm, and remained at this size until May and June when size increased by approximately 75 to 100%.

This information should serve as a foundation to apply in the evaluation of the relative effectiveness of exogenous hormone treatments in increasing the spawning success of channel catfish for producing both intraspecific and interspecific embryos.

**Objective 3d.** *Develop in vitro assays to evaluate sperm quality and evaluate their predictive ability in relation to fertilization and hatch.*

**Louisiana State University.** Channel catfish were seined from ponds during the months of April and May 2004. The males were killed and total lengths and weights were recorded. Testes were surgically removed and were suspended in calcium-free Hanks' balanced salt solution (C-F HBSS) at 290 mOsmol/kg. The testes were cleaned by removing adherent blood and tissue, separated into visually estimated anterior and posterior sections, weighed, and crushed in C-F HBSS (1 g/20 mL) to release sperm. The sperm solutions were poured through a 100- $\mu$ m filter into a 50-mL conical tube. Sperm motility was estimated after activation with deion-

ized water and concentrations were calculated using duplicate hemacytometer counts. Optical density of the sperm solutions was measured using absorbance readings obtained by spectrophotometry (Spectronic 20 Genesys) at wavelengths of 400, 450, 500, 550 and 600 nm.

The most accurate absorbance readings for determining sperm concentrations from whole testis occurred at 500 nm ( $y = 2^{-9}x + 1.199$ ,  $R^2 = 0.531$ ). These results indicate that spectrophotometric assays can be used to determine sperm concentrations from crushed testis of channel catfish.

**Objective 4.** *Develop economically viable standardized hatchery procedures and fertilization protocols to optimize hatching rate of hybrid embryos.*

**Objective 4a.** *Determine optimal sperm (fresh, frozen and refrigerated)-to-egg ratios for fertilization and hatch.*

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**Louisiana State University.** Channel catfish were seined from ponds during the months of April and May 2004. The males were killed and total lengths and weights were recorded. Testes were surgically removed and were suspended in calcium-free Hanks' balanced salt solution (C-F HBSS) at 290 mOsmol/kg. The testes were cleaned by removing adherent blood and tissue, separated into visually estimated anterior and posterior sections, weighed, and crushed in C-F HBSS (1g: 20mL) to release sperm. The sperm solutions were poured through a 100-µm filter into a 50-mL conical tube. Sperm motility was estimated after activation with deionized water and concentrations were calculated using duplicate hemacytometer counts. The solutions were

diluted to contain  $1 \times 10^8$ ,  $1 \times 10^7$  and  $1 \times 10^6$  sperm cells / mL and were used for fertilization of during artificial spawning with eggs from two females and sperm from 3 males (0.5 mL / 400 eggs). The sperm concentration of  $1 \times 10^6$  yielded  $71 \pm 16\%$  fertilization for fresh sperm ( $3 \pm 5\%$  for thawed sperm);  $1 \times 10^7$  yielded  $88 \pm 9\%$  fertilization for fresh sperm ( $45 \pm 37\%$  for thawed), and  $1 \times 10^8$  yielded  $91 \pm 10\%$  fertilization for fresh sperm ( $48 \pm 55\%$  for thawed). The varied concentration of sperm used for artificial spawning yielded significant differences in fertilization ( $P < 0.05$ ) and there is a correlation between sperm concentration and fertilization.

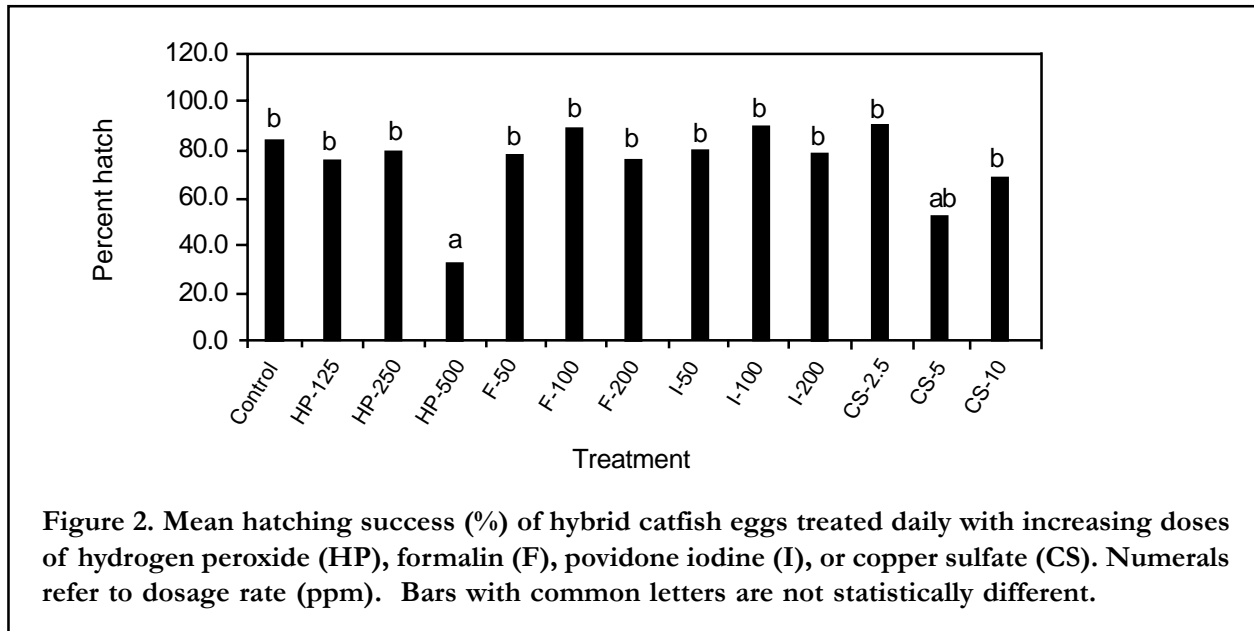
**Objective 4b.** *Determine the effects of commonly used therapeutics on hatching success.*

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**USDA-ARS.** The chemotherapeutic and respective concentration yielding the greatest hybrid hatching success was identified. Four hybrid catfish egg masses were each divided into thirteen equal sub-masses. Each sub-mass was subjected to once daily chemotherapeutic treatment as a 15-minute static bath until eyed. The treatments were as follows: (1) Control (no treatment), (2) 125 ppm hydrogen peroxide, (3) 250 ppm hydrogen peroxide, (4) 500 ppm hydrogen peroxide, (5) 50 ppm formalin, (6) 100 ppm formalin, (7) 200 ppm formalin, (8) 50 ppm povidone iodine, (9) 100 ppm povidone iodine, (10) 200 ppm povidone iodine, (11) 2.5 ppm copper sulfate, (12) 5 ppm copper sulfate, and (13) 10 ppm copper sulfate. Egg masses were allowed to hatch to completion within individual containers. When hatching was complete, the fry were siphoned

into a graduated cylinder and the volume of fry recorded. The total number of fry was calculated after determining the number of fry in 1 mL then multiplying times the total volume of fry collected. Hatching success was calculated as the percentage of eggs hatched.

Hatching success was high in the untreated controls (82.8%) and highly variable within treatments. Overall, hatching success was not significantly ( $P > 0.05$ ) improved with chemotherapeutic treatments; however, a tendency toward increased hatching success was observed among eggs treated with 100 ppm formalin (87.7%), 100 ppm iodine (88.1%), and 2.5 ppm copper sulfate (87.0%). A significant ( $P < 0.05$ ) decrease in percent hatch was observed in eggs treated with 500 ppm hydrogen peroxide (Figure 2).



The optimal treatment frequency for maximizing hybrid hatching success was determined. Formalin is the most common therapeutant used to treat catfish egg diseases, and formalin yielded one of the highest hatching success rates in the first experiment. For these reasons, formalin was chosen as the therapeutant for this experiment. Four trials were conducted with four egg masses per trial to determine the optimal frequency of formalin application for maximizing hatching success. Formalin treatments were administered 0, 2, 3, or 4 times daily as a 100 ppm static bath. Egg masses were allowed

to hatch to completion within individual containers. When hatching was complete, the fry were siphoned into a graduated cylinder and the volume of fry recorded. The total number of fry was calculated after determining the number of fry in 1 mL then multiplying times the total volume of fry collected. Hatching success was calculated as the percentage of eggs hatched.

The optimal frequency of formalin treatments was determined to be three times daily (Table 8).

**Table 8. Effect of daily formalin treatment frequency on hybrid hatching success.**

	Frequency of daily formalin treatments			
	0x	2x	3x	4x
Percent hatch	12.7 ± 4.5 <sup>a</sup>	31.4 ± 4.6 <sup>b</sup>	51.6 ± 3.6 <sup>c</sup>	33.7 ± 4.6 <sup>b</sup>

<sup>abc</sup>Means having different superscript are statistically different ( $P < 0.05$ ).

The effect of withholding formalin treatment during a putative sensitive developmental stage on hybrid hatching success was determined. A preliminary study was conducted to ascertain the developmental stage at which mortality most often occurs in hybrids. Briefly, hybrid eggs were collected throughout development, cleared in Stockard's solution and microscopically elevated for developmental differences indicative of egg mortality. At 28EC, mortality was observed between 42 and 46 hours post-fertilization. To determine the effect of withholding treatments during this potentially sensitive developmental period, formalin treatments (100 ppm) were administered three times daily such that treatments occurred at 42 hours post-fertilization (control) or were withheld from 42 to 44, 42 to 46, or 42 to 48 hours post-fertilization. Hatching success was calculated as previously described.

Formalin treatments administered at 42 hours post-fertilization significantly reduced ( $P < 0.05$ ) hatching success. Withholding treatments until 46 hours post-fertilization at 28EC yielded the greatest ( $P < 0.05$ ) percent hatch (Table 9).

### Results at a glance...

★ *The frequency of formalin treatments should be three per day to maximize hatch rate of hybrid embryos. Four treatments per day is excessive. At 28EC, hybrid embryos are chemically sensitive to formalin between 42 to 46 hours post-fertilization, and formalin treatments should be avoided during this period to maximize hatch rate.*

**Table 9. Effect of formalin treatments administered at 42 hours post-fertilization (control) or withheld from 42 to 44, 42 to 46, or 42 to 48 hours post-fertilization on hybrid hatching success at 28EC.**

	Time of formalin treatment (hours post- fertilization )			
	42 h	44 h	46 h	48 h
Percent hatch	19.6 ± 5.3 <sup>a</sup>	30.7 ± 11.0 <sup>b</sup>	58.3 ± 3.9 <sup>c</sup>	34.1 ± 8.5 <sup>b</sup>

<sup>abc</sup>Means having different superscript are statistically different ( $P < 0.05$ ).

### WORK PLANNED

Louisiana State University initiated, reported and plans to continue to evaluate ultrasound as a means to evaluate female gonadal development. University of Memphis initiated, reported and plans to

continue to evaluate various hormones in vitro for stimulating oocyte maturation. These experiments were not part of the original work plan, and are being conducted in addition to the original work planned.

## **IMPACTS**

Hatch rate of hybrid embryos is improved if channel catfish females are stripped within 2 hours of first observation of egg release. Spectrophotometric assays can be used to determine sperm concentrations from crushed testis of catfish. Utilization of this tool should result in more efficient use of sperm, and more consistent fertilization rates.

The frequency of formalin treatments should be 3X per day to maximize hatch rate of hybrid embryos and 4 treatments per day is excessive. At 28EC, hybrid embryos are chemically sensitive to formalin between 42-46 hours post-fertilization, and formalin treatments should be avoided during this period to maximize hatch rate.

## **PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED**

### **Doctoral Dissertations**

Kristanto, A. H. 2004. Evaluation of various factors to increase the efficiency of channel-blue hybrid catfish embryo production. PhD. Dissertation. Auburn University, AL.

### **Presentations**

Ballenger, J., A. Hutson, D. Beam, G. Umali, A. Kristanto, M. Trask, M. Templeton, A. Davis, H. Quintero, F. Wang and R. A. Dunham. 2005. Effect of genetics on channel-blue hybrid catfish embryo production. Special Session-Hybrid Catfish Reproduction. World Aquaculture Society Meeting. New Orleans, LA. January 2005.

Barrero, M. L., R. D'Abramo, A. M. Kelly, L. A. Hanson, B. C. Small. 2005. Plasma steroid, cathpsin activity and egg size and protein content during *in vivo* oocyte maturation in four strains of channel catfish broodstock. Special Session-Hybrid Catfish Reproduction. World Aquaculture Society Meeting. New Orleans, LA. January 2005.

Beam, D. A. Hutson, A. Kristanto, J. Ballenger, M. Templeton, G. Umali, F. Wang and R. A. Dunham. 2005. Effects of confinement in bags or aquaria and exposure to the scent of conspecific males on the production of channel-blue hybrid catfish embryos by channel catfish females. Special Session-Hybrid Catfish Reproduction. World Aquaculture Society Meeting. New Orleans, LA. January 2005.

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- the production of channel-blue hybrid catfish embryos. Special Session-Hybrid Catfish Reproduction. World Aquaculture Society Meeting. New Orleans, LA. January 2005.
- Pawiroredjo, P. A., N. Mandhani, S. G. Hall, and T. R. Tiersch. 2005. Quantifying the thermal requirements of catfish spawning. Special Session-Hybrid Catfish Reproduction. World Aquaculture Society Meeting. New Orleans, LA. January 2005.
- Phelps, R. P., R. Hasty, A. Pendetar, L. Linley and N. Papanikos. 2005. Effects of temperature and body characteristics on the induced spawning of channel catfish and the production of channel × blue catfish hybrid fry. Special Session-Hybrid Catfish Reproduction. World Aquaculture Society Meeting. New Orleans, LA. January 2005.
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