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Temperature effects on spawning and fingerling production of channel catfish Ictalurus punctatus

Patrice Arnold Pawiroredjo
Louisiana State University and Agricultural and Mechanical College

TEMPERATURE EFFECTS ON SPAWNING AND FINGERLING PRODUCTION OF CHANNEL CATFISH *ICTALURUS PUNCTATUS*

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Patrice Arnold Pawiroredjo
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Abstract

The goal of this study was to develop several techniques that utilize the use of geothermal water that can contribute to increased profitability for commercial catfish producers. The primary objectives of this study were to: 1) determine the heating requirement using degree-days of channel catfish spawning 2) improve the efficiency of artificial spawning and, 3) evaluate the effectiveness of stocking outdoor ponds and pools with channel catfish fry.

The heating requirement for channel catfish spawning was determined to be between 99 and 129 degree days at the peak of spawning. There was no difference in degree-day values between spawns collected from heated ponds, and those collected from ambient ponds. There was also no difference in the weight and fertilization percentages between the egg masses collected before and after the start of natural spawning. In artificial spawning, female-female and male-female pairs showed no differences in the volume of unfertilized eggs collected, spawning latency, and neurulation in trials conducted before and after the start of natural spawning. Spawning behavior and egg release proved to be the most accurate way to determine the optimum time to manually strip female broodstock. Survival of fry before the start of regular spawning was greater in pools then in 0.04-ha ponds but that there was no difference in the stocking of sacfry or swim-up fry before or after the start of regular spawning in ponds or pools. The size, growth rate and number of fingerlings harvested are all affected by the survival percentage of fry stocked. Lower survival results in fewer fingerlings that grow faster and

larger. Survival was mostly affected by the date of stocking and the number of growing days the fingerlings remained in the ponds or pools.

These results support the use of geothermally heated ponds to increase the productivity of commercial catfish producers by providing better control over spawning, increased opportunities for artificial spawning, production of improved seedstock (including hybrids), and lengthened growing period for channel catfish fry. Future research needs to focus on improving these techniques and develop methods for efficient use of geothermal water in commercial catfish production

CHAPTER 1 - FOREWORD

Aquaculture is a diverse, loosely associated group of enterprises. More than 200 different cultured species of aquatic animals and plants are reported by the Food and Agriculture Organization (FAO) of the United Nations. The FAO describes aquaculture as the fastest-growing food producing industry worldwide, which accounts for close to 30% of global seafood production (FAO 2002). Aquaculture contributes significantly to global food production and represents over 4% of the world's total animal protein supply. Aquaculture has been utilized as a way to generate foreign exchange for developing countries that focus on the production of high value cash crops (Avault 1996). Economic development in impoverished rural societies and diversification for traditional farmers have also been successful effects of aquaculture.

In the early 1960's, in economically depressed areas of the southern United States, farmers transformed cropland into fish ponds to grow channel catfish *Ictalurus punctatus.* Forty years later the activities of those farmers have grown into a vertically integrated aquaculture industry. Channel catfish farming has become the most important aquaculture industry in the United States with harvests around 650 million ponds and sales totaling over $425 million annually (National Agricultural Statistics Service 2004). Each year Louisiana receives $35 million or more from catfish sales (LSU Agricultural Center 2003) which ranks the state fourth in the nation behind Mississippi, Alabama, and Arkansas. Although channel catfish can be considered an aquaculture success story, the industry faces several problems that could affect future development. For example, the number of producers in Louisiana declined from 116 in 2000 and to 89 in 2002, a 23% reduction. Production value also decreased from $47.9 million to $35.7 million, a 25%

decline over the same period (LSU Agricultural Center 2001, 2003). In 2001 the prices of channel catfish started to decline and in 2003, prices fell to their lowest level ($0.53 per pound) in almost a decade (Economic Research Service 2003). These record low prices occurred because of the arrival of the low end of the approximately five year price cycle (LSU Agricultural Center 2001) in combination with record import levels of catfish products and a slowing national economy (ERS 2003). Without strong prices, the catfish industry needs continued research and development to lower production costs and raise productivity to remain a viable agricultural enterprise.

The studies in this thesis address the application of geothermal water as a tool in channel catfish spawning and fingerling production. The main goal of this thesis research was to improve the control and productivity of channel catfish spawning and seedstock production. The specific objectives were to:

1) Further investigate early spawning of channel catfish in heated outdoor ponds.
2) Apply degree-days to describe the timing of channel catfish spawning.
3) Improve the efficiency of artificial spawning of broodstock conditioned in heated outdoor ponds.
4) Study the differences in spawning behavior between male-female and female-female broodstock pairs.
5) Evaluate the effectiveness of outdoor stocking of channel catfish fry before and during the normal spawning season.

Chapter two of this thesis provides relevant background information about the catfish industry. Chapter two also overviews the channel catfish industry and the different stages of production to which this research will be of significance, including the seasonal

spawning cycle, artificial spawning, hybridization, culture of early life stages and the concept of degree-days. Three research chapters (3-5), discuss specific experiments involving reproduction and rearing of catfish. Chapter three addresses the use of geothermal water to induce spawning prior to the natural spawning season of channel catfish. The use of degree-days to describe the timing of channel catfish spawning is also examined in this chapter. Chapter four focuses on the artificial spawning of channel catfish broodstock conditioned in heated outdoor ponds. These experiments compared the spawning results and behavioral differences of broodstock spawned as female-female pairs or male-female pairs. Chapter five addresses the stocking of outdoor ponds and pools with sacfry before the start of the regular spawning season. The variables in these trials were time of stocking, temperature at stocking and pond or pool size. Chapter six summarizes the findings of the experiments completed during this thesis research and links these results to practical applications in commercial catfish farming.

The results of this thesis have thus far yielded five published abstracts in conference proceedings (Table1). For consistency of presentation all chapters of this thesis have been prepared in the format of the *Journal of the World Aquaculture Society*. It is anticipated that Chapters 3, 4, and 5 will be submitted for publication in peer-reviewed journals.

Table 1.1. Conference presentations and abstracts based on research presented in this thesis.

Date	Title	Conference	Location
2003	Natural early spawning of channel catfish	Louisiana Chapter of the American Fisheries Society	Baton Rouge, Louisiana
2003	Continued development of natural and artificial early-season spawning of channel catfish	Aquaculture America 2003	Louisville, Kentucky
2003	Hot water and ammo cans: swimming in the spawning zone	Gulf Coast Reproductive Biology Meeting	New Orleans, Louisiana
2004	Heat requirements for channel catfish spawning	Annual meeting of the Institute of Biological Engineers	Fayetteville, Arkansas
2004	Degree-days: a management tool for spawning of channel catfish	Louisiana Chapter of the American Fisheries Society *	Baton Rouge, Louisiana
2005	Quantifying the thermal requirements of channel catfish spawning	Aquaculture America 2005 **	New Orleans, Louisiana

* Awarded second place in the Best Abstract category

** Abstract submitted for meeting in January, 2005

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CHAPTER 2 - INTRODUCTION

The channel catfish industry began in the early 1960's and has become the most important farmed commercial fish enterprise in the United States. Channel catfish *Ictalurus punctatus* are primarily farmed for human consumption and but also for recreational fisheries. Catfish live stages can be divided in five basic groups relevant to aquaculture: fry, fingerlings, stockers, food fish, and broodstock (Huner and Dupree 1984). In parallel with these stocks, catfish farming can be divided into a series of five different farming programs: 1) hatchery production of fry and fingerlings 2) stocker production; 3) food fish production; 4) broodstock production and 5) fee fish production. Initially, most commercial catfish growers were involved in a combination of the first four industry segments, although at present most commercial growers have specialized in either seedstock or food fish production. The production of fee fish for recreational fisheries is primarily done by state and federal hatcheries. The normal series of actions involved in the production of foodfish is as follows: 1) spawning of broodstock in the spring or early summer (April-May in southern United States), 2) stocking of fry in ponds in summer (May-July), 3) growth of fingerlings over the summer and winter, 4) stocking of fingerlings into grow-out ponds in early spring of year two, and 5) growth to market food size and harvest in late summer or fall of year two or in the winter or early spring of year three. The time from egg to human consumption can vary from 15 months to 48 months. (Huner and Dupree 1984) but is typically between 18 to 24 months (Lee 1981).

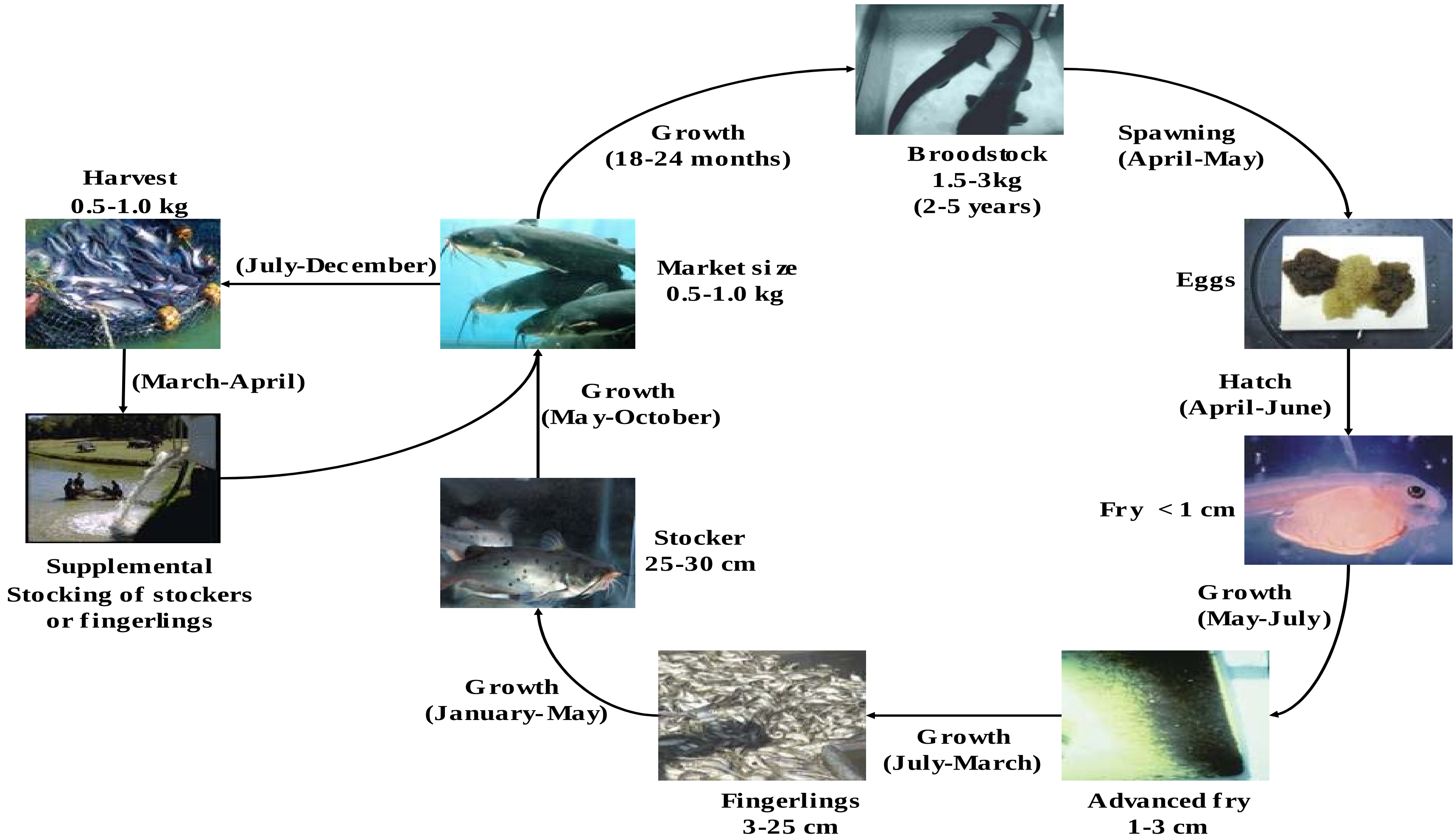

Figure 2-1: The lifecycle of cultured channel catfish

Food Fish Production

Two methods of production have been developed in the food fish segment of the catfish industry: 1) single-batch production, and 2) multiple-batch production (Tucker and Robinson 1990). In a single-batch production system, only one year class of fish is present in a pond at any one time. In one variation of the single-batch system, the entire crop is harvested at one time, whereas in the second variation, larger fish are selectively harvested two or three times until all fish are harvested or the pond is drained. In a multiple-batch production system different year classes are present in the pond. The larger fish are also selectively harvested but the ponds are never drained. Instead, the pond is re-stocked with fingerlings relative to the quantity harvested and this continuous harvesting and restocking cycle repeats itself several times. The single-batch system maximizes net production based on total yield (Tucker et al. 1994) yet reduced demands and unexpected inventory costs can reduce the profitability of this production system. The multiple-batch system provides a constant supply of harvestable fish, thus generating improved cash flow for commercial growers and reduced inventory costs (Engle and Pounds 1994). This continued product availability allows processing plants to remain open year round.

Seedstock Production

Channel catfish seedstock comprises three groups of fish, fry, fingerlings, and stockers, which are placed into grow-out ponds for food fish production. Fingerlings are juvenile fish between 3 cm and 20 cm in length. Stockers are intermediate fish between fingerlings and food fish that have an approximate length of 20 cm to 30 cm (Huner and Dupree 1984). Fingerling and stocker production starts with the rearing of recently

hatched fry. In the past, fry were produced by letting the egg mass hatch in the pond and rearing the fry in the same pond with removal of the broodstock. The fry could also be removed immediately after hatch and transported to a different rearing pond (Lee 1981). Currently, the egg masses are collected from the broodstock ponds and hatched indoors. Nursery ponds can be stocked with sacfry immediately after hatch or the sacfry can be reared to swim-up fry in troughs before being distributed to the nursery ponds (Busch 1985). Fingerlings between 10 and 18 cm can usually be produced in the 4 to 8 months between spawning in late spring and the stocking of grow-out ponds in winter or early spring. The production of stockers usually requires part of the second growing season (Lee 1981). Stocking density of fry has a significant effect on the growth rate of fry and thus on the production of fingerlings and stockers. Lower stocking densities of fry will result in larger fingerlings or even stockers whereas higher densities will produce smaller fingerlings (Thomas and Tucker 1985).

Natural Spawning

Spawning is the release of eggs by the female and subsequent fertilization by the male and is accompanied by specific behavioral patterns (Grizzle 1985). Channel catfish undergo a yearly cycle of gonad development and spawning. The timing of the reproductive cycle of most organisms seems to be a combination of endogenous rhythms and external environmental clues (Sumter 1990). Temperature is the primary environmental influence on channel catfish spawning (Davis et al.1986, Lang et al. 2003). In the southern United States the natural spawning season of channel catfish usually begins in late spring (April - May) and lasts through early summer (May – June). Spawning activity is reported to begin when minimum water temperatures remain

between 21 and 30ºC (Busch 1985; Huner and Dupree 1984) but spawning can drop sharply at temperatures below 21ºC or above 30 ºC (Lee 1981).

Three methods of propagation have been developed in the catfish industry: 1) the pond method; 2) the pen method, and 3) the aquarium method (Busch 1985; Tucker and Robinson 1990). The primary method of spawning channel catfish used by commercial growers is the semi-controlled pond method where natural spawning is accommodated. In the pond method, spawning ponds (usually 0.04 to 0.4 ha) are stocked with broodstock at male to female ratios between 1:1 and 1:4. Metal, plastic or wooden containers (35-50 L) that simulate natural spawning sites are placed in the ponds and the fish are allowed to spawn naturally. This method has proven practical and reliable, requires minimal time and skilled labor, and limited indoor facilities. In the pen and aquarium methods, one male is typically paired with one female. This pair will spawn naturally in a pen with a spawning container. In the aquarium method, a conditioned or ripe pair is induced to spawn via hormone injection. The latter two methods provide more control over the spawning process by enabling selection of specific individuals and longer separation of the sexes in holding ponds before placing them together, thus delaying spawning.

Early Life Stages and Development

Channel catfish produce a relatively large number of eggs (10,000 – 20,000 per female) that are fertilized by external union of the gametes. The fertilized eggs are adhesive and form a wide, flat mass. These egg masses typically become attached to the inside wall of the spawning container. Channel catfish females can produce between 6000-8000 eggs per kg of body weight. After the female deposits the eggs, the male assumes responsibility for their care and the female is driven away from the nest (Huner

and Dupree 1984). Eggs hatch after approximately 5 to 10 d at water temperatures between 21 and 28ºC, and temperatures below 21ºC and above 30 ºC should be avoided and can lead to mortality (Huner and Dupree 1984). Artificial spawning techniques have enabled the production of channel catfish fry before the start of natural spawning when ambient water temperatures are usually below 21ºC. The stocking of outdoor ponds and pools with fry before the start of regular spawning was investigated and the results are reported in chapter 5 of this thesis. An egg mass undergoing normal development will initially be yellow and progressively turn reddish as the circulatory system develops (Small et al. 2003). Unfertilized and dead eggs will be clear and eventually turn white and enlarge. Fertilized eggs are often treated in the hatchery with an iodine or formalin solution to minimize fungus infestation or bacterial decay. In commercial settings, channel catfish egg masses are hatched indoors in troughs or tanks. The water is usually agitated by mechanical paddle wheels or forced air to simulate the male parental care (Carmichael et al. 1993). Catfish sacfry are golden-pink in color and will remain on the bottom for ~5 d after hatch while they absorb their yolksac. During that period the fry do not feed and receive their nutritional needs from the yolk. After absorption of the yolksac, the fry darken in color and swim to the surface of the water looking for food (so called “swim-up fry”). Fry can be stocked immediately after hatch or grown in the hatchery for several days or weeks before they are stocked into ponds.

Genetic Improvement

Channel catfish culture is a relatively young industry and lags far behind terrestrial livestock industries in genetic research as well as in the application of fundamental principles of breeding (Tucker and Robinson 1990). The use of superior

domestic strains is the fastest way to improve productivity. Domestic strains are fish that are at least two generations removed from the wild (Tucker and Robinson 1990) and display improved production traits and are commonly referred to as “strains”. Breeding programs involving selection require several generations to produce results. Crossbreeding and hybridization (defined below) however, can result in superior offspring within one generation (Avault 1996). Several commonly used channel catfish strains include Harvest select, Norris, Auburn, Kansas and Rio Grande strains (Kelly 2004). In February 2001 the USDA National Warmwater Aquaculture Center (NWAC) in Stoneville, Mississippi, released the latest strain of Channel Catfish broodstock named NWAC 103. This strain was selected for faster growth and showed higher production than the Norris strain and the hybrid of the channel catfish female and blue catfish male although the meat yield of the channel by blue hybrid was higher than the NWAC 103 and Norris strain.

Selective Breeding

Selective breeding occurs when individuals or families that display a certain performance level for a specific trait are chosen in efforts to increase the mean occurrence of that trait in the next generation (Tave 1993). The goal in directional selection is to drive the population average towards a desired phenotypic level. Channel catfish populations have been subjected to mass selection and family selection (Tucker and Robinson 1990). Mass selection compares each individual to all others regardless of their relation to one another while in family selection, comparisons are based on the average family performance. Selection programs are expensive and involve intensive effort because they cover several generations and require diligent planning and thorough

record keeping. In addition, a control population must be maintained to measure the results of the selection process. Commercial fish growers should also be aware of the potential for unintentional selection while propagating their production stocks, which could lead to loss of genetic resources.

Crossbreeding

Crossbreeding is defined as the mating of broodstock from different strains within the same species. The goal of crossbreeding is to identify the combination of broodstocks that creates offspring with trait production levels superior than their parents (Tave 1993). Crossbreeding does not always result in better performing offspring and the cross between a male of strain A and a female of strain B can produce differing results than the cross of the female of strain A and the male of strain B. In eleven different crosses involving ten channel catfish strains, only six resulted in offspring that were superior to both parents (Dunham and Smitherman 1983). Crosses between different channel catfish strains provide a potential for genetic improvement and extensive research has focused on the comparing the performance of the hybrid between the channel catfish female and the blue catfish male to different strains of channel catfish.

Hybridization

Hybridization is defined in fishes as the crossing of broodstock from two different species. Similar to crossbreeding, a specific combination of species can result in offspring with higher productivity. Other than the channel catfish *Ictalurus punctatus*, several other catfish species have been cultured or have been evaluated for hybrid production. These species are: blue catfish *I. furcatus,* white catfish *Ameirus catus*, the brown bullhead *A. nebulosus,* yellow bullhead *A. natalis* and black bullhead *A. melas* and the flathead

catfish *Pylodictus olivaris* (Thomas and Tucker 1985). Around thirty hybrid crosses have been attempted between these seven catfish species, but only the cross between the channel catfish female and blue catfish male emerged as a feasible production alternative (Thomas and Tucker 1985). This hybrid is can be useful for growth in commercial aquaculture conditions because it exhibits improved growth rate, dress out percentage, disease resistance, and greater resistance to low dissolved oxygen concentrations compared to the channel catfish (Smitherman and Dunham 1985). The production of this hybrid however, has not been adopted on a large scale by commercial growers because of the natural reproductive barriers between species and the additional techniques required to overcome them. These techniques include the collection of unfertilized eggs from female channel catfish through artificial spawning and the collection of sperm from blue catfish by sacrificing the male.

Artificial Spawning

The aquarium spawning method of enables collection of unfertilized eggs. In traditional artificial spawning, male and female broodstock are paired in aquaria and females are induced to ovulate by hormone injection. Carp pituitary extract and human chorionic gonadotropin have been used to induce final oocyte maturation, and synthetic luteinizing hormone - releasing hormone (LH-RH) and gonadotropin releasing hormone have been used to induce ovulation in channel catfish (Bates and Tiersch 1998). Artificial spawning techniques provide more control over the timing of spawning, the selection of individual or multiple males used for fertilization, and enable the production of hybrid catfish. These techniques have not been widely adopted by the industry because they are labor and capital intensive. In efforts to reduce the cost per unit effort in artificial

spawning, the possibility of all-female and out-of-season artificial spawning has been investigated. Artificial spawning of females in groups (Bates and Tiersch 1998; Dunham et al. 1998; Lang 2001) and female-female pairs (Dunham et al. 1998) have been reported. All-female artificial spawning of channel catfish can more than double the number of potential egg producers and requires further investigation. Early out-of-season artificial spawning using geothermally heated ponds was successfully utilized to created catfish hybrids (Lang 2001).

Warm Water Ponds

The use of heated water from geothermal and industrial sources for aquaculture purposes has grown in the past two decades. In areas of the northwest United States geothermal water has been used to grow aquatic species. Geothermal energy is being used for fish farming in countries around the world and constitutes 11% of the global geothermal energy use. In the United States, 10% of total geothermal energy use is devoted to aquaculture (Fridliefsson 1998). Approximately thirty facilities in the western United States culture aquatic species including channel catfish, tilapia *Oreochromis spp*., striped bass *Morone saxitillis*, fresh water prawns *Macrobrachium rosenbergii,* and tropical ornamental fish by use of geothermal water (NREL 1998). In experiments at the Aquaculture Research Station (ARS) of the Louisiana State University Agricultural Center, geothermal water has been used to induce spawning in channel catfish broodstock, prior to the regular spawning season (Lang 2003). A 762-m deep well provides water at a temperature of 36°C which is used to heat twelve 0.1-ha ponds. The water is distributed by an automatic thermostatic control system (Hall et al. 2001) developed by the Department of Biological and Agricultural Engineering (BAE) of the

Louisiana State University Agricultural Center. Cooperative research between the BAE and the ARS has resulted in the development of an energy balance model for these geothermally heated earthen aquaculture ponds (Lamoureux 2003). A computer program was developed to solve this energy balance to predict pond temperatures and estimate the energy required to control pond. Because channel catfish spawning is affected by water temperatures, geothermal ponds could be a useful tool to gain better control over spawning in a commercial setting.

Degree-days

Degree-days are a unit that combines temperature and time to enable the calculation and measurement of the total heat requirement of a variety of processes. A degree-day is defined as an average of 1°C above a certain threshold temperature for one day.Degree-day units are employed by heating, ventilation and air conditioning engineers to estimate the fuel requirement to heat or cool buildings based on the daily ambient temperatures. Meteorologists and engineers refer to this unit as heating degree-days if the mean daily temperature is below 18°C and cooling degree- days if it is above 18°C (CPC 2003). Degree-days can also be used to measure the progression of an organism from one point in their life cycle to another. The development of most ectothermic organisms follows a time scale which is temperature dependent (Allen 1975). As temperature increases, development time decreases until the temperature becomes too high and development slows (Wilson and Barnett 1983).

The degree-day unit provides a common reference for biological developmental processes. Each life stage of an organism can have a specific total heat requirement. The accumulated degree-days from a starting point can help predict when a developmental

stage will be reached. The heat requirements for the developmental stages of plants, insect pests and other animals are sometimes referred to as growing degree-days. The degree-day concept has been used to describe the growth and development of plants such as corn *Zea mais* (Roth and Yocum 1997; Nielsen et al. 2002), cotton *Gossypium hirsutum* (De Tar et al. 1997), and insect pest such as the strawberry root weevil *Otiorhynchus ovatus* (Umbel and Fisher 2000) and an apple moth *Lacanobia subjuncta* (Doerr et al. 2002). Degree-days or similar units have been used to describe egg hatching in salmonids (Jobling 1996), the growth-temperature relationship of several salmonid species, channel catfish, tiger muskellunge *Esox masquinongy* X *E. lucius* and blue tilapia *Oreochromis aureus* (Soderberg 1992), and the reproductive heat requirements of common carp *Cyprinus carpio* Horvath 1986; Rothbard and Yaron 1995) and grass carp *Ctenopharyngodon idella* (Zonneveld 1983).

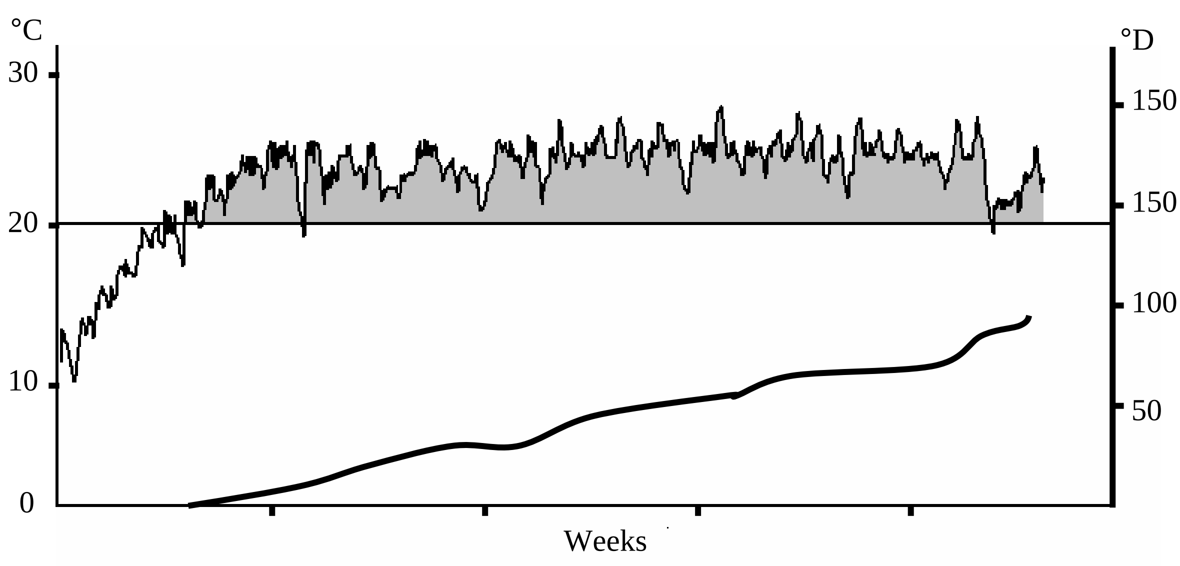

Figure 2-2: Illustration of degree day calculations. Degree days are the summation of the difference between the mean pond temperature and the threshold temperature for one day. In this example 21°C is used as a threshold to calculate degree-days over a 6-wk period.

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CHAPTER 3 - DEGREE-DAYS AS A TOOL TO DETERMINE THE HEATING REQUIREMENT FOR CHANNEL CATFISH SPAWNING

The reproductive cycle of channel catfish *Ictalurus punctatus*, is primarily influenced by environmental temperature (Davis et al.1986, Lang et al. 2003). In the southern United States, channel catfish spawn naturally in the spring and early summer (late April to June). Reports on the range of water temperature required for spawning vary between 21 and 30 ºC (Table 3-1). Spawning activity can decrease rapidly at temperatures below 21ºC or above 30ºC (Huner and Dupree 1984), and can increase again when temperatures recover within the permissive range. Because natural spawning is dependent on weather conditions, individual growers and the industry as a whole have little control over channel catfish spawning and the production of seedstock. Controlled water temperatures and hormone treatments can be used to induce spawning and provide an alternative to natural spawning (Kelly and Kohler 1996). Controlled spawning offers considerable benefits for future development of the catfish industry because it increases spawning predictability and provides opportunities for genetic improvement through selective breeding and hybridization of production lines. Out-of-season spawning facilitates genetic selection and improvement and potentially allows for a constant supply of fry and fingerlings (Blythe et al. 1994) which could double or triple annual crops of fry in rearing ponds (Kohler et al. 1994).

Studies addressing natural and out-of-season spawning of channel catfish report a combination of temperature and time as the main variables in controlling spawning. Channel catfish have been observed to spawn two wk prior to the regular season when they are held in small (< 0.04 ha), shallow ponds with relatively warmer water than larger broodstock ponds (0.4 -1.0 ha) (Lee 1981, Huner and Dupree 1984). Channel catfish

were spawned out of season (August, November) using a combination of water temperature manipulation and injection of carp pituitary (Brauhn 1971). The fish were maintained at 17 ± 2ºC for 109 d followed by an increase in water temperature to 24ºC over 7 d where they were maintained for 7 d. The females were injected with macerated fresh carp pituitary at 20.8 g/ml of 0.15 Molar of MaCl and paired with a male in compartments with water temperatures of 26ºC. Fish were also induced to spawn out of season (November through January) in indoor recirculating systems by holding them at 17 ± 2°C for 6, 9 or 15 months followed by 3 months at 25 to 28°C (Kelly and Kohler 1996). Half of the fish received luiteinizing hormone-releasing hormone analog (LH-RHa) (100 mg/kg) and human chorionic gonadotropin (HCG) (1000 IU/kg) injections to induce ovulation.

Table 3-1: Selected references on the spawning temperature of channel catfish

Citation	Temperature range (ºC)	Type of spawning
Brauhn 1971	24 -26	Natural and Artificial spawning
Lee 1981	21-30	Natural spawning
Busch 1985	21-29	Natural spawning
Davis et al. 1986	24-26	Natural spawning
MacKenzie et al. 1989	27.5	Natural spawning
Bates and Tiersch 1998	> 21	Induced spawning
Lang 2001	27	Induced spawning
Lang et al. 2003	27	Natural spawning

Out of season (March to April) spawning of channel catfish was achieved in geothermally heated outdoor ponds through temperature manipulation (Lang et al. 2003a). Ponds were heated from ambient temperatures (13 - 17ºC) at a rate of 2ºC per day to 24 to 30ºC. Spawning was advanced by 20 to 62 d and occurred at 12 to 30 d following ambient water temperatures being raised to within the spawning range (24°C - 30 °C) and 21-30 d after the start of initial heating (Lang et al. 2003a). Broodstock were also conditioned to spawn in geothermally heated ponds and then artificially spawned indoor in male-female pairs or groups of 2 to 8 females (Lang et al. 2003b, Bates and Tiersch 1998). These studies indicate that temperature is a dominant variable influencing the timing of channel catfish spawning. In all of these studies, spawning was manipulated by incubating the fish at, above, or below a certain temperature for a minimum time period. The combination of temperature and time appears to be critical in determining when spawning will occur. The variables of temperature and time are combined in the degree-day concept, which creates a consistent parameter that can be used to compare channel catfish spawning studies involving temperature and time.

The degree-day concept has been used to describe different developmental processes in several fish species. For example, for larval development, the product of time (d) required to reach a specific developmental stage and the temperature in °C above a biological threshold was relatively constant (Hempel 1979). In this case, the threshold refers to the temperature below which development is negligible. Hatching for several coldwater fish species has been described using degree-days (°D). The degree-day value required for eggs to hatch in Atlantic salmon *Salmo salar* is 500°D; for brown trout

Salmo trutta is 490°D; for rainbow trout *Oncorhynchus mykiss* 375°D, and for Arctic charr *Salvelinus alpinus* is 450°D (Jobling 1996). The relationship between temperature and growth in fish has also been described in units similar to degree-days. The United States Fish and Wildlife Service adapted the monthly thermal unit (MTU), originally developed by Haskell (1959), to describe the growth-temperature relationship of several salmonid species (Soderberg 1992). The MTU is defined as 1°C above 0°C for one month. In this scenario, 0°C is the minimum threshold temperature above which growth occurs. Assuming an average month of 30 days, 1 MTU would generally equal 30 degree-days. The MTU requirements for each cm of growth have been described for channel catfish, blue tilapia *Oreochromis aureus*, brook trout *Salvelinus fontinalis*, rainbow trout, lake trout *Salvelinus namaycush*, Atlantic salmon, and tiger muskellunge *Esox masquinongy* X *E. lucius* (Soderberg 1992).

Temperature also has been shown to play a major role in the timing of ovulation in common carp *Cyprinus carpio* (Horvath 1986) and other fish species. The reproductive heat requirements of common carp and grass carp *Ctenopharyngodon idella* have been described using degree-days. The optimal interval between consecutive ovulations for common carp was reported as 2000°D above 17°C (Horvath 1986). In Israel however, common carp were reported to require an accumulation of 1000 to 1200°D above 15°C to complete ovarian development (Rothbard and Yaron 1995). Grass carp, were reported to require 1350 to 1450°D above 15°C to complete ovarian development (Zonneveld 1983). The degree-day concept has received criticism because it does not always correctly describe the relationship between developmental times and temperature (Jobling 1996), because the actual relationship between time and temperature in embryonic development

processes is an exponential function (Howell et al. 1998). However, the degree-day concept has proven accurate over the limited temperature ranges experienced during egg development and is still useful in practical hatchery management (Jobling 1996).

The goal of this study was to evaluate the utility of the degree-day concept for the determination of the heating requirement of channel catfish spawning in naturally heated and geothermally heated ponds. Accordingly, the objectives were to: 1) use degree-days to quantify and describe the heat requirement of channel catfish spawning; 2) quantify and compare the heat requirements for channel catfish spawning in heated ponds before the natural spawning season and in ambient temperature ponds during the natural spawning season; 3) compare egg mass weight, percent fertilization, and percent hatch of the spawns collected before the natural spawning season in heated ponds to spawns collected during the natural spawning season in ambient temperature ponds, and 4) provide degree-day guidelines and terminology that can be used by different groups when reporting channel catfish spawning studies.

Methods

Broodstock

In the winter (December-February) of 2002, 2003, and 2004, twelve 0.04-ha ponds at the Aquaculture Research Station (ARS) of the Louisiana State University Agricultural Center were stocked with channel catfish broodstock. In 2002, the ponds were stocked with broodstock from two different populations. Six ponds were stocked with females from a research population (LSU) maintained at the ARS. The remaining ponds were stocked with Gold Kist strain D (GKD) females obtained from Harvest Select Farms (formerly Gold Kist Farms) (Iverness, Mississippi). All males were from LSU

broodstock. In the 2002 spawning season, the ponds were grouped into three sets of four ponds. Each group involved two ponds with LSU females and two ponds with GKD females (Figure 3-1).

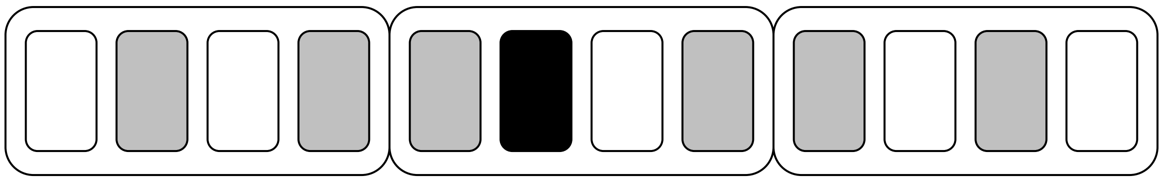

Figure 3-1: Stocking of LSU and Gold Kist strain D (GKD) broodstock channel catfish in grouped warm water ponds for the 2002 spawning season. The GKD pond received 18 females (black). The LSU X LSU ponds received 12 females and 5 males (grey), while the GKD X LSU ponds received 18 females and 5 males (white).

In 2003 and 2004, all the broodstock were obtained from Haring's Pride Catfish (Wisner, Louisiana) and were identified as Louisiana commercial catfish (LCC) and the ponds were also heated in groups of four. In one of the four ponds of each heating group, the male and female broodstock were separated by placing the males in cages. The purpose of this separation was to avoid pond spawning by preventing normal interactions between males and females. These ponds are referred to as "separated ponds." The broodstock in these ponds were used for artificial spawning in the hatchery (Chapter 4). The dimensions of the cages were 1.22 m X 1.83 m X 0.61 m with a total volume of 1.35 m^3. Stocking of ten males per cage provided an average of 135 L of space per male. The ponds with the free-swimming males are referred to as "mixed ponds." The ponds were also grouped in three sets of four. Each heating group involved one separated pond and three mixed ponds (Figure 3-2).

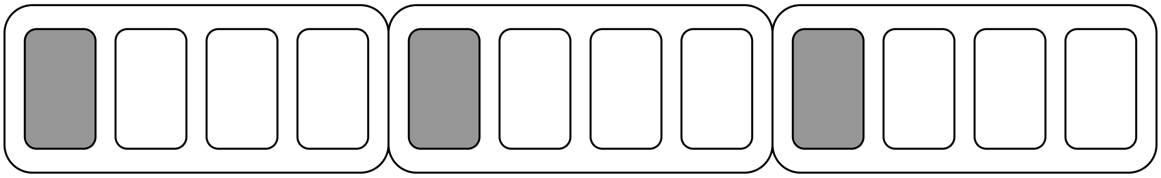

Figure 3-2: Stocking of Louisiana commercial channel catfish (LCC) in warm water ponds for the 2003 and 2004 spawning seasons. In 2003 all ponds received 16 females. The mixed ponds received 5 males (white) and the separated ponds received 10 males (grey). In 2004 all ponds received 20 females and 10 males.

Pond Heating

Ponds were heated by the addition of 36ºC water from a 760-M deep geothermal well (30° 22' 13.803" N, 91° 11' 12.827" W; World Geodetic System 1984). Ponds were heated at a rate of 2ºC per day from ambient temperatures to the target spawning temperature of 27°C (Lang et al. 2003, Hall et al. 2001). The temperatures were maintained at 27 ± 1°C until the end of the heating period or until water temperature in the unheated ponds reached these temperatures and spawns were collected. Water was delivered to the ponds through 10-cm PVC ball-valves (Model LB308, Hayward Industrial Products, Elizabeth, New Jersey). The valves were controlled by an automatic thermostatic system developed by the Department of Biological and Agricultural Engineering at LSU (Hall et al. 2001). Water temperatures were recorded with data loggers (Campbell Scientific CR-23X, Campbell Scientific Inc., North Logan, Utah) that were an integral part of the control system and separate individual data loggers (HOBO data logger, Onset Computer Corporation, Pocasett, Massachusetts) that were submerged 1 m below the water surface in the middle of each pond (Figure 3-3).

Spawning Containers

Channel catfish are cavity spawners and normally spawn in any natural cavity available to them. In the experimental ponds, these cavities were provided in the form of metal spawning containers. In 2002, four spawning containers were placed in each pond; in 2003 all ponds received six spawning containers (Figure-3-3). The spawning containers were checked for egg masses every 3 to 4 days beginning when water temperatures reached 25 to 27°C.

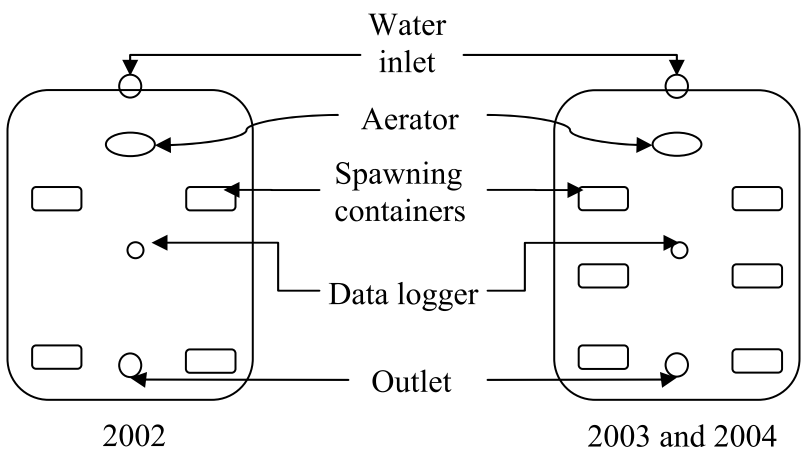

Figure 3-3: Placement of spawning containers in ponds in 2002, 2003, and 2004 spawning seasons.

Collection of Egg Masses

Egg masses were manually loosened and gently removed from the spawning containers and each was placed into a 4-L container with water from the pond (22 - 30°C) which was aerated with a portable aerator (Bubble Box™ B-11, Marine Metal Products, Clearwater, Florida). The egg masses were transported to the hatchery within 30 min of removal from the pond and were acclimated (15-30 min) to hatchery water temperatures

(26 - 27 °C), weighed, and held in a 10% Iodine solution (Argentyne, Argent Chemical Laboratories, Redmond, Washington) for 10 min.

The variables that were analyzed for the comparison of spawning before and during the natural spawning season were weight of egg masses collected, fertilization percentage of the eggs and the hatching percentage of the egg masses. The number of eggs per mass was estimated by counting the number of eggs in three randomly selected 5-10 g samples from the egg mass and dividing them by the weight of the samples to yield an estimate of the number of eggs per gram. The total weight of the egg mass was divided by the average number of eggs per gram calculated from the three selected samples. Fertilization percentage of the egg masses was determined by counting the number of unfertilized eggs in the three samples and dividing by the total number of eggs in the samples. Fertilized eggs were identified by determining the eggs that reached neurulation. Neurulation is the stage in embryonic development when the neural tube is formed which is visible as a line across the embryo when illuminated with a light source. The egg masses were divided into 250 g sections and were placed in mesh baskets and suspended in 80-L tanks in a recirculating system outfitted with a bubble washed bead filter (Aquaculture Systems Technologies, LLC, New Orleans, Louisiana). Percent hatch was calculated by determining the total number of sacfry divided by the total number of eggs estimated in each 250 g section of the egg mass. The number of sacfry was determined volumetrically (Tucker 1985). The water displacement of a known number of fry was determined in a graduated cylinder. The total number of fry was calculated by determining the water displacement of the entire batch of fry and dividing that by the water displacement of the sample.

Degree-day Calculations and Terminology

Degree-days were calculated from pond temperature measurements during the spawning season. Celsius degree-days (°D) were calculated for 254 spawns collected before and during the natural spawning season between 2000 and 2004. The three threshold temperatures that were used as the lower limit above which degree-day values were accumulated were 18, 21, and 24°C. These threshold temperatures were selected based on scenarios that describe the conditions where channel catfish spawning is used. These conditions and thresholds were outlined in three definitions for each scenario, as follows:

1) Biological definition

This designation is intended to describe the biological time and temperature requirements for channel catfish spawning. The threshold temperature selected for this definition was 18°C to ensure that the starting point of the degree-day accumulation was below the previously reported minimum spawning temperatures for channel catfish. Two studies (Kelly and Kohler 1996; Brauhn 1971) reported prevention of spawning by maintaining broodstock at 17°C during the spawning season.

2) Commercial definition

This is intended to be used as a tool to predict a reliable starting point for natural spawning for commercial producers. The threshold temperature selected for the second definition was 21°C and is the previously reported lower temperature limit for channel catfish spawning and generally used as a guideline by commercial producers (Huner and Dupree 1984; Avault 1996)

3) Research definition

This definition is derived from previous experiments conducted at the ARS and has become part of the standard method of operation for out-of-season spawning experiments for that laboratory group (Lang et al. 2003). The threshold temperature selected for this definition was 24°C which was the median temperature of the spawning range in these experiments (21 - 27 °C).

Three spawning parameters were created to permit consistent comparison of the numerical data among different temperature thresholds. The parameters are spawning onset, median spawning, and spawning conclusion. "Spawning onset" were defined as the number of degree-days accumulated when 10% of the spawns had been collected. The number of degree-days accumulated when 50% of the total spawns had been collected was designated as "median spawning", and "spawning conclusion" identified the degree-day value accumulated when 90% of total spawns had been collected. These three spawning parameters were used to compare the three previously described definitions. Celsius degree-days were calculated by summation of the daily difference between the mean daily temperature and the established threshold temperature:

$$\sum \text{(mean daily temperature (°C) – threshold temperature (°C))} \times \text{1day} = \text{Celsius °D.}$$

The temperature and spawning data used for these calculations were collected over a 5-year span (2000 - 2004) at the ARS (Lang 2001). The start dates of these temperature measurements were between January 1 and February 27 and varied among the years these data sets were collected. Degree-days were calculated from the earliest available measurement below the threshold temperature to minimize the risk of missing degree-

days accumulated before measurements started. The degree-day values were calculated using the macros function of the Microsoft® Excel spreadsheet software (Appendix 1). The spawns between 2000 and 2003 came from ponds that were heated from ambient water temperatures at a rate of 2°C per day to 27°C and were maintained at that temperature until the start of the next heating trial. In 2004, the heated ponds were maintained at three different temperatures (Figure 3-4).

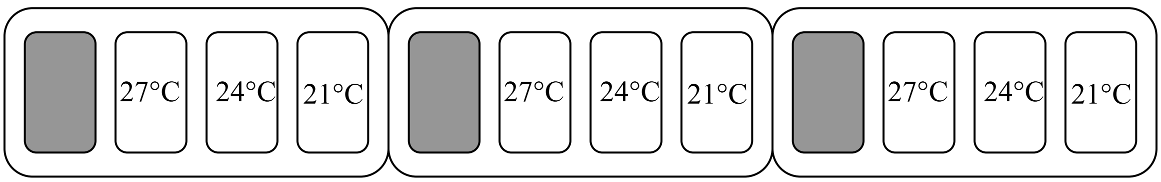

Figure 3-4: Temperature regime in heated mixed ponds during the 2004 spawning season. The mixed ponds (white) received 20 females and 10 males and were maintained at three different target temperatures. The separated ponds (grey) received 20 females and 10 males in cages. All mixed ponds had an additional 10 females and 10 males in cages and the separated ponds also had 10 females in cages.

Degree-day values for spawns collected from these ponds were calculated using a specific threshold for a pond with a specific temperature regime. Degree-day values for spawns collected from ponds heated to 21°C, 24°C and 27°C were calculated using the 18°C, 21°C and 24°C thresholds. All ponds were stocked with between 16 and 20 females. Ponds from 1999 to 2002 had four spawning containers (sites) available per pond, and in 2003 and 2004 the ponds had six spawning containers. It is possible that all females could have been ready to spawn at the same time but were prevented from doing so because of the limited number of spawning sites. Accordingly, degree-days were calculated for the first four spawns from 2000 to 2002 and the first six spawns in 2003 and 2004 to minimize the effect of spawning site limitations on the degree-day values.

These data points were referred to as degree-day values with “no spawning site limitations”. When all the 254 spawns were included in data analysis this data set is referred to as degree-day values “with spawning site limitations.”

Statistical Analysis

A binomial data set was created by comparing the degree-day value for each spawn to a range of degree day values at 25°D intervals between 0 and 500 °D. This data set was analyzed using the Proc Logistic function of SAS system (Statistical Analysis Software system version 9 for windows®; SAS Institute Inc., Cary, North Carolina). The results of this analysis yield the slope and intercept of functions that allow calculations of spawning probabilities for each threshold. The Proc Freq function was used to create frequency tables displaying the percentage, and frequency of spawns at 25 degree-day intervals. All other data was analyzed using the Proc Mixed function of SAS for analysis of variance. Differences were considered to be significant at $\underline{P} \leq 0.05$. The variables used to compare the spawns collected before the start of the spawning season to the spawns collected after the start of regular spawning season were egg mass weight, percent fertilization and percent hatch.

Results

Degree-days

The average degree-day values for the data with spawning site limitations were 250 ± 76 for the 18°C threshold, 127 ± 51 for the 21°C threshold, and 45 ± 27 for the 24°C threshold, and for the data without spawning site limitations the average values were 214 ± 56 for the 18°C threshold, 103 ± 35 for the 21°C threshold, and 34 ± 19 for the 24°C threshold. The cumulative percentage of spawns at different degree-day values

above the three thresholds were calculated for the data with spawning site limitations and for the data without spawning site limitations (Figure 3-5). The values for the onset of spawning, median spawning and the conclusion of spawning at the three threshold temperatures for both data sets (Table 3-1 and 3-2) were determined through interpolation of the values produced with the frequency tables (Appendix 1). In 2004, 18 spawns were collected from three ponds (the first 6 spawns per pond) that were maintained at three target temperatures (21, 24 and 27 °C). Degree-day values were calculated above the18°C, 21°C and 24°C thresholds for spawns collected from the ponds with the target temperature of 21°C, 24°C and 27°C (Table 3-5). All three ponds with different temperature regimes produced a constant value of 98 ± 4 °D above the 21°C threshold.

The degree-day values at onset and median spawning were 16% greater and at the conclusion of spawning 21% greater for the data collected with spawning site limitations compared to spawns collected with no spawning site limitations. This means that the difference in onset, median and concluding of spawning using the 18°C threshold was between 10 and 50°D. This difference is between 10 and 42°D for the 21°C threshold, and between 3 and 22°D for the 24°C threshold. The degree-day value at which channel catfish will spawn with a 10% probability was 7% greater at the 18°C threshold, 6% greater at the 21°C threshold and not different at the 24°C threshold when compared between data with and without spawning site limitations. The same comparisons done for the 50% spawning probability were 13% greater at the 18°C threshold, 16% greater at the 21°C threshold and 14% at the 24°C threshold, and for the 90% spawning probability they were 16% greater at the 18°C threshold, 23% greater at the 21°C threshold and 20% at the 24°C threshold.

The average degree-day value for the spawns collected from heated ponds with no spawning limitations (n = 136) were 207 ± 60 for the 18°C threshold, 104 ± 34 for the 21°C threshold and 36 ± 17 for the 24°C threshold. The spawns with no spawning limitations collected from ambient temperature ponds (n = 45) had an average degree-day value of 214 ± 43 for the 18°C threshold, 92 ± 34 for the 21°C threshold and 25 ± 21 for the 24°C threshold. There was no significant difference in the degree-day values between spawns collected from heated ponds and those collected from ambient temperature ponds using spawns with no spawning site limitations, calculated using the 18°C threshold (P = 0.38), the 21°C threshold (P = 0.52) and the 24°C threshold (P = 0.32). There were also no significant differences among spawns collected from heated and ambient temperature ponds using spawns collected with spawning site limitations, calculated using the 18°C threshold (P = 0.60), the 21°C threshold (P = 0.59) and the 24°C threshold (P = 0.54).

There was no significant difference among the years (2000-2004) in degree-day values calculated for spawns above the 18°C threshold (P = 0.61), the 21°C threshold (P = 0.94) and the 24°C threshold (P = 0.61) for spawns collected with no spawning limitations. There was also no significant difference for the 18°C threshold (P = 0.87), the 21°C threshold (P = 0.74) and the 24°C threshold (P = 0.57) in the same analysis done for spawns collected with spawning limitations.

Early Spawning

The data for this experiment were collected during the 2002 and 2003 early spawning and natural spawning periods. The ponds were heated in three groups of four. While one group was being heated, the second group served as a control group. In 2002 the heating of the first pond group began on February 17. Heating of the second group

began on April 8 and the last group on May 18. The first early season spawns were recorded on March 15, the first regular season spawns on April 18 and spawning continued until June 8. During this period, the GK X LSU ponds produced 25 egg masses and the LSU X LSU ponds produced 41 egg masses. A total of 66 spawns were recorded, of which 32% (21) occurred before natural spawning was observed in ambient (unheated) control ponds (Figure 3-6). In 2002 the spawning season was extended by 34 days. In 2003 the ponds were heated starting on February 11 and the second group on March 31. The first early spawns were recorded on March 10, the first natural spawn on April 23 and spawning continued to June 25. A total of 83 spawns were recorded during the 2003 spawning season of which 51% (42) occurred before natural spawning. In 2003 the spawning season was advanced by 44 days.

No natural spawns were recorded in ponds with males in cages or in the all-female ponds. During the 2002 and 2003 spawning seasons, data were collected from a total of 111 spawns of which 58 spawns were designated as early spawns and 53 as natural-season spawns. There was no significant difference ($P = 0.08$) in the average weight of the egg masses for early spawns (979 ± 458 g) and for regular season spawns (846 ± 391 g). The average fertilization percentage for the early collected egg masses was 87 ± 7% while for the egg masses collected during the regular spawning season it was 88 ± 6%. There was no significant difference ($P = 0.85$) in fertilization percentage. However there was a significant difference ($P = < 0.001$) in hatching rate between early spawns (53 ± 25%) and natural-season spawns (31 ± 29%).

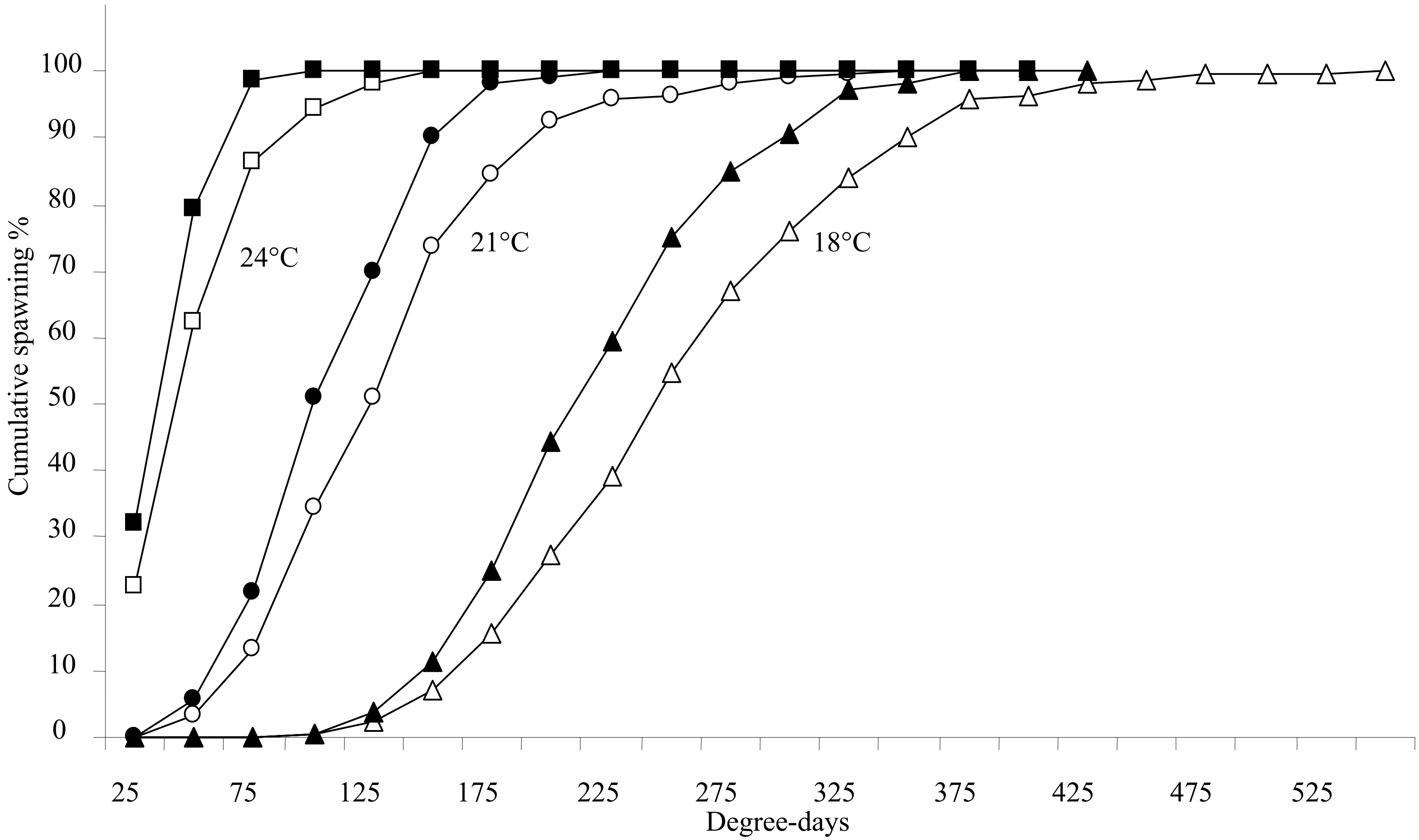

Figure 3-5: Cumulative percentage of channel catfish spawns at different totals of Celsius degree-days accumulated above three threshold temperatures (18ºC (triangles) ; 21ºC (circles) and 24ºC (squares)). The data points indicated by the white symbols calculated using the first 4 spawns per pond from 2000-2001 and the first six spawns per pond from 2002-2004. The data points indicated by the black symbols were calculated using all 254 spawns collected between 2000 and 2004.

Table 3-2: The number of degree-days at onset (10%), median (50%) and conclusion (90%) spawning accumulated above three threshold temperatures (18, 21, and 24 °C) using all 254 spawns collected between 2000 and 2004.

Threshold (°C)	Definition	Degree-days		
		Onset	Median	Conclusion
18	Biological	159	242	349
21	Commercial	67	124	192
24	Research	11	42	86

Table 3-3: The number of degree-days at onset (10%), median (50%) and conclusion (90%) spawning accumulated above three threshold temperatures (18, 21, and 24 °C). These values were determined for the first 4 spawns per pond in 2000 and 2001 and the first 6 spawns in 2002, 2003 and 2004 for a total of 153 of 254 spawns collected between 2000 and 2004.

Threshold (°C)	Definition	Degree-days		
		Onset	Median	Conclusion
18	Biological	149	209	296
21	Commercial	57	99	150
24	Research	8	36	64

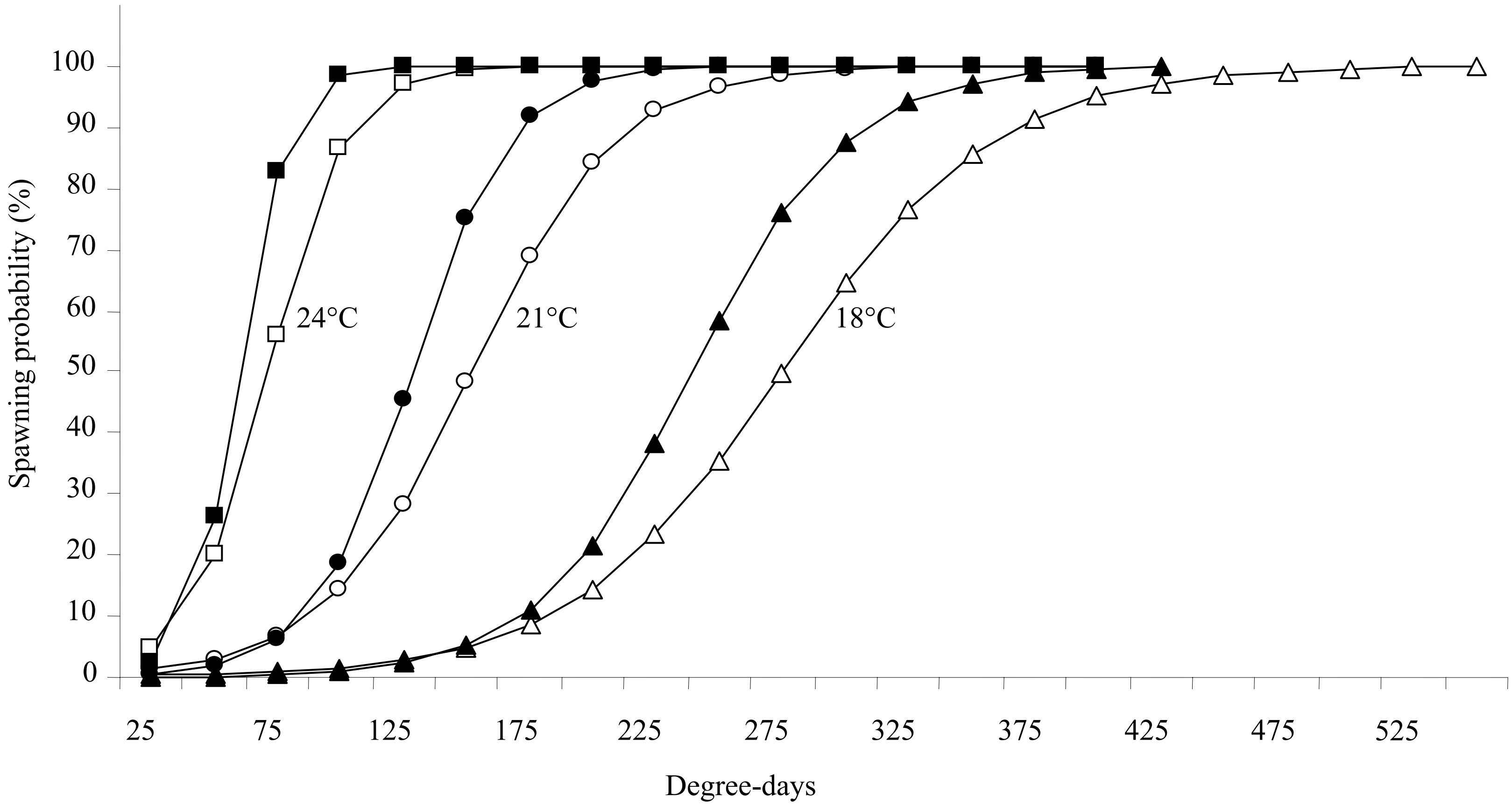

Figure 3-6: Spawning probability for channel catfish at different degree-days values accumulated above three different thresholds (18ºC (circles); 21ºC (triangles) and 24ºC (squares)). The data points indicated by black symbols were calculated using the first 4 spawns per pond from 2000-2001 and the first 6 spawns per pond from 2002-2004. The data points indicated by the white symbols were calculated using all the spawns per collected between 2000 and 2004.

Table 3-4: The degree-day values above three thresholds (18, 21, and 24 °C) at which there is a 10%, 50% or 90% probability of spawning occurring. These values were determined for the first 4 spawns per pond in 2000 and 2001 and the first 6 spawns in 2002, 2003 and 2004 to eliminate the effect of spawning site limitations.

Threshold (°C)	Spawning probability		
	10%	50%	90%
18	171	240	308
21	81	129	172
24	33	61	87

Table 3-5: The degree-day values above three thresholds (18, 21, and 24 °C) at which there is a 10%, 50% or 90% probability of spawning occurring. These values were determined for all spawns between 2000 and 2004 and did not account for spawning site limitations

Threshold (°C)	Spawning probability		
	10%	50%	90%
18	183	275	367
21	86	153	217
24	33	71	109

Table 3-6: The average degree day value for spawns above three thresholds from ponds maintained at different temperatures. Values sharing letters within columns are not significantly different.

Target temperature	Actual temperature	Threshold		
		18°C	21°C	24°C
21°C	23.1 ± 1.5°C	234[a]	95[a]	8[a]
24°C	23.1 ± 2.6°C	203[ab]	98[a]	22[b]
27°C	24.6 ± 3.0°C	184[b]	102[a]	41[c]

Table 3-7: Number of spawns and mean (±SD) of egg mass weight, fertilization rate, and hatching rate of catfish spawned before and during the natural spawning season in 2002 and 2003. Values sharing letters within rows are not significantly different.

Year		Number of egg masses collected		Egg mass weight (kg)		Percent fertilization		Percent hatch (%)	
		Early	Natural	Early	Natural	Early	Natural	Early	Natural
2002	162	21	45	1.2 ± 0.6	1.0 ± 0.4	91 ± 7	88 ± 6	51 ± 24[a]	18 ± 16[b]
2003	144	42	41	0.9 ± 0.3	0.6 ± 0.2	86 ± 6	88 ± 6	55 ± 26[a]	46 ± 33[a]
Total	306	63	86	1.0 ± 0.5	0.8 ± 0.4	87 ± 7	88 ± 6	53 ± 25[a]	31 ± 29[b]

Discussion

Degree-day values at onset, median and conclusion of spawning were an average of 18% lower for spawns collected without spawning site limitations compared to those with spawning site limitations. This could be explained by the lag period that occurs between the first 4-6 spawns, and the rest of the spawns collected in a pond. All females could be ready to spawn at the same time, but there may not be enough spawning sites and males available. Once all spawning sites in a pond are occupied by male-female pairs, it takes 2-3 d for the eggs to be removed and an additional number of d for another spawning pair to occupy that site and complete the spawning interactions. This could increase the degree-day values in situations with spawning site limitations and could inaccurately extend the range of degree-day values for channel catfish spawning. Future studies involving degree-day calculations for spawning should be aware of the effect of spawning site limitations on the accumulation of degree-days and the possible distortion of the collected data. This discussion will focus on the data derived from the first 4 to 6 spawns collected from the ponds.

The timing of the reproductive cycle in channel catfish is influenced by water temperature (Davis et al. 1986). Channel catfish spawning starts when minimum water temperatures remain above 21°C for several d (Busch 1985). Channel catfish broodstock held in three ponds heated and maintained at different temperature regimes, all produced spawns ($n = 16$) at 98 ± 4 degree-days above the 21°C threshold. The degree-day values calculated for the other two thresholds (18 and 24 °C) were not as consistent and were significantly different among the three ponds. This indicates that 21°C was the threshold temperature that yielded consistent degree-day values for channel catfish spawning. This

temperature (21°C) has been reported in the literature as the minimum temperature required for spawning of channel catfish. Thus, the commercial definition results in the most constant degree-day values compared to the biological and research definitions.

The degree-day terminology used in this study was intended to be used by different user groups when reporting channel catfish spawning studies. The three degree-day definitions (biological, commercial and research) provide basic reference points which can be used to compare results from future studies and past data about channel catfish spawning. This is an important step towards standardizing research findings concerning catfish reproduction. Regular monitoring of water temperatures provides a general indication when channel catfish will spawn barring unexpected weather events. Degree-days combine temperature and time into a unit that can be used to provide more accurate and consistent predictions of catfish spawning. The results of these degree-day calculations present confidence intervals that can serve as guide for the timing of catfish spawning.

Channel catfish producers could use the degree-day values and spawning probabilities of the commercial definition as targets to predict the spawning readiness of their broodstock based on water temperature and weather data. The commercial definition yielded 57 degree-days for the onset, and 99 degree-days for the median spawning point of spawning. The spawning probability results were 81 degree-days for the 10% spawning probability and 129 degree-days for the 50% spawning probability. According to these numbers spawning will begin after the accumulation of between 57 and 81 degree-days and that most broodstock will spawn between 99 and 129 degree-days.

There were no significant differences among years in the degree-day values calculated for the onset, median and conclusion of spawning above the three different threshold temperatures (18, 21, and 24 °C) in the years from 2000 to 2004. There was also no significant difference in the degree-day values between spawns collected from heated ponds, and those collected from ambient temperature ponds. This indicates that the heat requirement for channel catfish spawning is a consistent value above a certain threshold. There was no significant difference in weight and fertilization percentages between the egg masses collected before and during the natural spawning season. There was however, a significant difference in hatching rate between egg masses collected from early spawns and natural spawns. The difference in hatch rate was attributed to water quality problems in the recirculating hatchery systems. There were several instances when the total ammonia nitrogen levels in the hatching systems exceeded 5 mg/L. The poor water quality occurred episodically from the beginning to the middle of the natural spawning season. This was due to the lag period between the increased ammonia production in the system and the sufficient nitrification capacity of the biofilter. The increase in ammonia production was created by a sudden in increase in the number of egg masses collected during the peak of the spawning season.

Determining the heating requirement for channel catfish spawning is a step towards the commercial-scale production of hybrid catfish. With pond heating commercial producers can schedule the optimum time to transport the females conditioned in outdoor ponds to indoor facilities to induce final oocyte maturation. The extension of the channel catfish spawning season can contribute to increased and more cost-efficient production of hybrid catfish. Reliable heating requirements can also help

increase the efficiency of extending the spawning season through geothermal heating of ponds. Female broodstock can be heated to specific temperatures for specific time periods to synchronize batches of females for artificial spawning at a favorable time. Knowledge of the correct heating requirements necessary for channel catfish to spawn, will also improve the ability to efficiently manage costly geothermal water resources or other methods of pond heating. Extension of the spawning season could lead to increased fry and fingerling production. This greater fry production capacity could be an advantage for specialized fingerling producers. Future studies need to focus on the application and fine-tuning of these definitions and degree-day values and establish the lower threshold temperature for channel catfish. This true lower threshold can only be established if broodstock in ponds are consistently held below that temperature. The detailed degree-day data on channel catfish spawning could become the base to develop a user-friendly chart or computer program that predicts the spawning probability of channel catfish in a geographic region based on climate data. This chart or program would enhance the predictability and control of channel catfish spawning and simplify planning for fingerling producers and hatchery managers when used with climatic or weather data.

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CHAPTER 4 - ARTIFICIAL SPAWNING OF CHANNEL CATFISH IN FEMALE-FEMALE AND MIXED-SEX PAIRS

An agricultural enterprise requires economically available seedstock in sufficient quantities to develop into an industry (Thomas and Tucker 1985). Commercial catfish producers and researchers have developed three methods of spawning channel catfish *Ictalurus punctatus* for the production of seedstock: 1) the pond method; 2) the pen method, and 3) the aquarium method (Busch 1985; Lee 1991). In the pond method, broodstock at varied ratios of females and males (e.g., 1:1, 2:1, or 3:2) are placed in a pond, and provided with containers that function as spawning sites where the fish spawn naturally. The number of broodstock in the pen and aquarium methods is reduced to single male-female pairs. In the pen method male-female pairs are allowed to spawn naturally in an enclosure within a pond supplied with a spawning container. In the aquarium method a conditioned male-female pair is induced to spawn via hormone injection and the unfertilized eggs are manually collected from females. Although the latter two methods provide for selection of specific broodstock pairs, the primary method of seedstock production in the channel catfish industry is the pond method (Lee 1981). This method has proven practical and reliable and requires minimal time, labor and facilities compared to the pen and aquarium methods. However, it provides limited control of specific crosses, thus limiting the possibilities for genetic improvement.

Genetic improvement through selective breeding and hybridization has the potential to increase catfish production in the near future. Crossbreeding is defined as the mating of two different strains from the same species and hybridization as the mating of two different species (Avault 1996). Crossbreeding and hybridization can each result in improved phenotypic performance within one generation (Avault 1996). The aquarium method or artificial spawning allows collection of unfertilized eggs that can be fertilized

with sperm from multiple males or from blue catfish, *Ictalurus furcatus* (Bates and Tiersch 1998). The hybrid of the channel catfish female and the blue catfish male is considered to be superior in commercial aquaculture conditions because it exhibits improved growth rate, higher dress-out percentage, increased disease resistance, and greater resistance to low dissolved oxygen concentrations compared to the channel catfish (Smitherman and Dunham 1985).

Hormones have been used to induce spawning in several species of catfish. Human chorionic gonadotropin (HCG) has been used to induce ovarian maturation and spawning in Chinese catfish (*Clarias fuscus*) (Young et al. 1989) and Asian catfish (*Clarias macrophelus*) (Mollah and Tan 1983). The combination of synthetic leuteinizing hormone releasing hormone (LH-RH) and the dopamine receptor antagonist, Pimozide was also successful in achieving spawning of *Clarias macrophelus* (Tan-Fermin et al. 1996) and *Clarias batrachus* (Manickam and Joy 1989). Injections of carp pituitary extract, HCG, LH-RH and gonadotropin-releasing hormone have been used to induce final oocyte maturation and ovulation in channel catfish (Bates and Tiersch 1998, Busch and Steeby 1990). Synthetic LH-RH and pimozide was also successfully used in cage spawning of channel catfish and produced higher ovulation rate in the beginning of spawning season than only LH-RH injection (Silverstein et al. 1999).

These artificial spawning techniques are necessary for the collection of unfertilized eggs that can be fertilized with sperm from specific males or males from a different species of catfish. These breeding practices can lead to genetic improvement trough cross breeding and hybridization of channel catfish. These techniques have not been widely adopted by commercial producers because they are labor and capital intensive. The efficiency of artificial spawning must be improved to be generally adopted

by the catfish industry. Several previous studies have investigated ways to improve the efficiency of artificial spawning. Spawning of channel catfish females without males has been studied in pairs (Dunham et al. 1998) and in groups of 2 to 7 females (Bates and Tiersch 1998) and 15 females (Dunham et al. 1998; Lang 2001). Artificially spawning channel catfish in female-female (F-F) pairs could lead to increased egg production compared to male-female (M-F) pairs by doubling the number of females by, thus making more efficient use of indoor tank space.

In their natural habitat, channel catfish are cavity spawners with sites varying from undercut banks and burrows, to hollow logs (Tucker and Robinson 1990). Typically the male prepares the spawning site and attracts the female. Channel catfish will not spawn without a suitable cavity unless induced by hormone injection (Grizzle 1985). During spawning, the fish assume a head-to-tail position and wrap their caudal fins around each other while irregularly vibrating their bodies until egg and sperm release occurs (Busch 1985). Not all eggs are released at the same time and egg deposition can extend over 4 to 12 hours (Busch 1985). The eggs are typically released in batches of several hundred. It is not clear if the male releases sperm before or after egg release. These spawning behaviors have been reliably observed when previously conditioned broodstock were paired in laboratory tanks for artificial spawning (Busch 1985; Lang 2001).

The goal of this study was to improve the efficiency of artificial spawning of channel catfish broodstock. Extension of the spawning season by heating earthen ponds could improve the efficiency of artificial spawning by increasing the numbers of conditioned females available. Conditioning of males and females in the same ponds,

while preventing spawning by keeping the males in cages could make more efficient use of limited heated pond space by conditioning larger numbers of broodstock of both sexes. The objectives of this study were to: 1) evaluate conditioning of female broodstock in ponds with males in cages before the start of the regular spawning season; 2) evaluate the differences in spawning success, spawning latency and percent fertilization between M-F and F-F pairs; 3) study the differences in spawning behavior between M-F and F-F pairs, and 4) evaluate artificial spawning using M-F and F-F pairs before and during the regular spawning season. Spawning latency is defined as the time between hormone injection and the release of unfertilized eggs and was used to compare final oocyte maturation between the treatments. Fertilization was determined by evaluating the percent of embryos per sample that reached neurulation.

Methods

Broodstock preparation

In the winter of 2002 and 2003, twelve 0.04-ha ponds at the Aquaculture Research Station (ARS) of the Louisiana State University Agricultural Center were stocked with channel catfish broodstock (for details see Chapter 3). In 2002, six ponds were stocked with females from a research population maintained at the ARS (LSU) and six with Gold Kist strain D (GKD) females obtained from Harvest Select Farms (formerly Gold Kist Farms) (Iverness, Mississippi). The average size of these broodstock was 3.1 ± 0.5 kg. Eleven ponds received LSU males and one pond received females only. In 2003, all the broodstock were Louisiana commercial catfish (LCC) obtained from Haring's Pride Catfish (Wisner, Louisiana). The average size of these broodstock was 2.1 ± 0.4 kg. In three ponds the males were placed in cages to prevent normal spawning interactions between males and females. The cage dimensions were 1.22 m X 1.83 m X

0.61 m with a total volume of 1.35 m^3 per cage. The cages were constructed at the ARS (Whaley 2002). Stocking of ten males per cage provided an average volume of 135-L per male.

Pond heating

The ponds were heated in three groups of four by the addition of 36ºC water from a 760-m deep geothermal well (Lang et al. 2003, Hall et al. 2001) located at the ARS (30° 22' 13.803" N, 91° 11' 12.827" W). In 2003 each heating group included three mixed-sex ponds and one all-female pond with the males in cages. The pond heating rate was 2 ºC per day from ambient temperature to the target spawning temperature of 27 ± 1°C until the end of the heating period or until ambient water temperature in unheated ponds reached the temperature of heated ponds.

Artificial spawning

At the end of each heating period, fish in one of the four ponds were collected by seining and the conditioned broodstock were transported indoors. In 2002, the broodstock for the first and the third spawning trials were obtained from a mixed-sex pond. The broodstock used in the second trial were from an all-female pond. In 2003, all of the broodstock were conditioned in all-female ponds with males in cages. After moving the broodstock indoors, the lengths and weights were measured and ovulation was induced by a single intraperitoneal injection of synthetic leuteinizing hormone-releasing hormone (LH-RHa) at a dose of 100 µg/kg. After injection, the fish were placed in 80-L aquarium tanks as M-F or F-F pairs. Fish of similar size were paired (Busch 1985) to minimize the potential for injury due to aggressive spawning behavior. The fish were monitored at 2-h intervals until ovulation occurred. When the female released eggs in the tank, she was removed from the tank and anesthetized using tricaine methanesulfonate (MS-222;

Argent Chemical Laboratories, Redmond, Washington) at a concentration of 250 mg/L. Females that did not release eggs in the tank were checked for ovulation based on the level of spawning activity of the pair. The eggs were manually stripped into plastic containers coated with silicone grease (Dow Corning, Midland, Michigan) containing Hanks' balanced salt solution (HBSS) at ~290 mOsm (Bates and Tiersch 1998). The plastic bowls were coated to prevent the unfertilized eggs from sticking to the bowl and the HBSS prevented the activation of the eggs. The eggs (200-300) were fertilized with refrigerated channel catfish sperm to determine egg quality. Cryopreserved or refrigerated sperm of blue catfish (Harvest Select strain G, Iverness MS) was used to fertilize the remainder of the eggs for the production of hybrid catfish. The volume of eggs collected from an ovulating female, spawning latency and fertilization percentage were determined. Spawning latency was defined as the time (h) between injection and ovulation (Bates and Tiersch 1998). Fertilization percentage was determined by counting the number of embryos in samples of 200-300 eggs that reached neurulation (Lang 2001), 27 ± 3h after fertilization at water temperatures of 26 ± 2°C.

Spawning behavior index

In the artificial spawning trials of 2002, we observed that the F-F pairs displayed spawning behavior similar to that of the M-F pairs. To further investigate the spawning behavior of F-F pairs, a channel catfish spawning behavior index was developed (Table 4-1). This index lists the normally observed stages in male-female spawning. The broodstock pairs were monitored every 2 h until the start of ovulation. During these observations, the spawning behavior of the pairs was recorded. During the last artificial trial in 2002 and the three artificial trials in 2003 the differences were recorded based on incidence and stage of spawning behavior observed.

Table 4-1: Behavioral index for spawning of channel catfish

Stage		Description
0	(inactive)	Both fish laying on the bottom; some swimming
1	(beginning spawning behavior)	Active swimming; biting or evidence of biting; nudging one another
2	(active spawning behavior)	Assumption of head-to-tail position
3	(intense spawning behavior)	Vibrating of body in head-to-tail position; no egg release
4	(start of spawning)	Eggs released

Statistical Analysis

The data on the length and weight of the broodstock, and the latency of spawning, volume of unfertilized eggs collected, neurulation rates, and the number of spawns were analyzed using the Proc Mixed function of the SAS system (Statistical Analysis System Software version 9 for Windows®; SAS Institute Inc., Cary, North Carolina). In 2003 the experimental arrangement was a 2 X 3 factorial design. In this design, sex (M-F and F-F pair) and time were the main effects. The time periods during which the spawning trials were conducted were early (prior to the natural spawning season), middle (at the beginning of the natural spawning season), and late (at the end of the natural spawning season). Spawning latency, volume of eggs collected and neurulation rates were analyzed per female. The number of normal spawns and stripped spawns were analyzed per female and per pair. The spawning behavior data were analyzed per pair of broodstock using a one way non parametric method “Proc Npar1way.” Differences were considered to be significant at $P \leq 0.05$.

Results

Three artificial spawning experiments were conducted in 2002 and in 2003. The dates of the artificial spawning trials in 2002 were March 25, April 26, and June 3. In 2003 the trial dates were March 31, May 5, and June 30. The trials conducted in March of 2002 and 2003 were before the onset of natural spawning in unheated ponds. The trials in April and May corresponded with the beginning of the natural spawning season and the trials in June with the end of the spawning season. The broodstock used in the first and second trials in 2002 and 2003 were conditioned before the start of the natural spawning season. During all three heating periods in 2003, 10 males were placed in cages in the all-female ponds at the start of the conditioning period. From each of these cages, 5 males were recovered when the fish were transported indoors.

The three artificial spawning trials in 2002 yielded 13 spawns and three trials in 2003 yielded 30 spawns (Table 4-2). The first trial included five M-F pairs producing three spawns and three F-F pairs producing one spawn. During the second trial, a broken air line resulted in the loss of 7 of the 16 females before injection, thus the trial only had four F-F pairs that produced 7 spawns. The June 3 artificial spawning in 2002 produced two F-F spawns of poor egg quality. The eggs were white, sticky and had low (1%) fertilization. All three trials in 2003 consisted of four M-F and four F-F pairs. During the 2003 spawning season, unfertilized eggs were manually collected from 30 of 36 females. Every M-F pair spawned in all three trials. The four F-F pairs in the first and third trial produced five spawns each. In the second trial the four F-F pairs produced eight spawns. The broodstock used in the third trial also remained in the all-female pond for 12 weeks after natural spawning had begun. Attempts to maintain water temperatures below 24ºC were unsuccessful, but water temperatures were maintained 4 °C below ambient at 27ºC

by addition of well water at 21ºC. These females did have swollen abdomens and 9 of the 12 females spawned.

Table 4-2: Spawns produced by male-female and female-female pairs during six artificial spawning trials in 2002 and 2003.

Year	Trial	M-F pairs	M-F spawns	F-F pairs	F- F spawns	Total spawns
2002	1	5	3	3	1	4
	2	0	0	4	7	7
	3	3	0	5	2	2
2003	1	4	4	4	4	8
	2	4	4	4	8	12
	3	4	4	4	5	9
Totals		20	15	24	27	42

All broodstock used in this experiment were of similar length (*P* = 0.17) across all treatments. There was, however, a difference (*P* = 0.03) in the weight of the females used in this experiment. The females were of similar weight between M-F and F-F pairs (*P* = 0.51) and among the different time periods (*P* = 0.06). The females used in the late F-F pairs were larger (*P* = 0.02) than those used in the early F-F pairs. There was no significant difference in egg volume (*P* = 0.52) and neurulation (*P* = 0.22) between time periods and between F-F and M-F pairs (Table 4-3). Latency of females in the F-F pairs was not different from that of the females in M-F pairs (*P* = 0.07). Latency for the early spawning trials was greater (*P* < 0.001) than that for the middle and late spawning periods. There was no difference (*P* = 0.35) in neurulation among the eggs collected at different time periods and fertilized with refrigerated catfish sperm (Table 4-2) and no difference (*P* = 0.05) in neurulation among eggs collected from M-F and F-F pairs.

Table 4-3: Average volume of eggs collected, latency per spawn and neurulation of eggs for male-female and female-female pairs during spawning trials 2003.

Pairs	Volume (ml)	Latency (h)	Neurulation (%)
Male-Female (n = 12)	247 ± 84^{a}	50 ± 13^{a}	69 ± 17^{a}
Female-Female (n = 12)	230 ± 104^{a}	47 ± 14^{a}	47 ± 34^{a}

Table 4-4: Average volume of eggs collected, latency per spawn and neurulation of eggs for the early, middle and late spawning trials during the 2003 season.

Pairs	Volume (ml)	Latency (h)	Neurulation (%)
Early (n = 8)	228 ± 121^{a}	65 ± 10^{a}	59 ± 18^{a}
Middle (n = 8)	265 ± 58^{a}	45 ± 8^{b}	55 ± 36^{a}
Late (n = 8)	221 ± 99^{a}	40 ± 7^{b}	53 ± 34^{a}

There was no significant difference between the F-F and M-F pairs in the number of stripped spawns per pair ($P = 0.24$) or per female ($P = 0.12$). There was also no significant difference between the F-F and M-F pairs in the number of normal spawns per pair ($P = 0.21$). However there was a significant difference ($P = 0.03$) in the number of tank spawns per female. The average number of tank spawns per female was 0.25 for F-F pairs and 0.83 for M-F pairs (Table 4-5). There was no significant difference between the F-F and M-F pairs in the number of stripped spawns per female during the early ($P =$ 0.21) middle ($P = 0.67$), and late ($P = 0.89$) spawning trials and also no significant difference in the number of stripped spawns per pair during the early ($P = 0.76$), middle ($P = 0.15$), and late ($P = 0.67$) spawning trials (Table 4-6).

Table 4-5: Average spawning behavior of broodstock pairs and average spawning per female and per pair for male-female and female-female pairs during artificial spawning trials in 2003. Normal spawning was defined as releasing of eggs in the tank and strip spawning as manual collection of eggs. Each included four female-female pairs and four male female pairs.

		Male-female pair trials				Female-female pair trials			
	Parameter	1	2	3	Totals	1	2	3	Totals
Pairs	Spawning behavior	4.00	4.00	3.50	3.80	2.25	3.00	2.75	2.67
Per pair	Tank spawning	1.00	1.00	0.50	0.83	0.25	0.75	0.50	0.50
	Strip spawning	1.00	1.00	1.00	1.00	1.00	2.00	1.25	1.50
Per female	Tank spawning	1.00	1.00	0.50	0.83	0.13	0.38	0.25	0.25
	Strip spawning	1.00	1.00	1.00	1.00	0.5	1.00	0.63	0.75

Table 4-6: Spawns produced by female-female and male-female pairs during six artificial spawning trials in 2002 and 2003. The trials conducted in March of 2002 and 2003 (early) were before the onset of natural spawning in unheated ponds. The trials on April 26, 2002 and May 5, 2003 (middle) correspond with the middle of natural spawning season and the trials in June of both years (late) with the end of the spawning season

			Female-male pairs			Female-female pairs		
Year	Date	Time period	Spawns	Spawns per female	Spawns per pair	Spawns	Spawns per female	Spawns per pair
2002	03/25	Early	3	0.60	0.60	1	0.17	0.33
	04/26	Middle	--	--	--	7	0.88	1.75
	06/03	Late	0	0.00	0.00	2	0.20	0.40
2003	03/31	Early	4	1.00	1.00	4	0.50	1.00
	05/05	Middle	4	1.00	1.00	8	1.00	2.00
	06/30	Late	4	1.00	1.00	5	0.63	1.25
Total			15	0.72	0.75	27	0.59	1.17

Table 4-7: Active spawning behavior, spawning stage, and spawning events observed from F-F and M-F pairs during artificial spawning trials in 2003. Spawning events are recorded as tank spawning and stripped spawning. Normal spawning was defined as the release of eggs in the tank as would occur during natural spawning and stripped spawning as manual collection of eggs.

	Male-female pairs				Female-female pairs			
Trial	Behavior stage	Tank spawning	Females stripped	Fertilization (%)	Behavior stage	Tank spawning	Females stripped	Fertilization (%)
1	4	1	1	41	3	0	0	–, –
	4	1	1	36	2	0	1	–, 0
	4	1	1	74	0	1	1	–, 62
	4	1	1	84	4	1	2	66, 70
2	4	1	1	90	4	1	2	0, 75
	4	1	1	73	4	1	2	48, 93
	4	1	1	79	4	1	2	0, 80
	4	1	1	83	0	0	2	8, 63
3	4	1	1	67	4	1	1	–, 87
	3	0	1	70	4	1	2	0, 45
	3	0	1	90	2	0	1	–, 0
	4	1	1	70	1	0	1	–, 50

The behavioral observations made during the 2003 spawning season revealed a significant difference (*P* = 0.02) in the level of spawning behavior between the F-F and M-F pairs. All 12 M-F pairs displayed a spawning level of at least 3 (intense spawning behavior). In 10 of the 12 M-F pairs, the females started releasing eggs in the tank indicating the start of natural spawning and eggs were manually collected from all females. The F-F pairs displayed a less uniform spawning pattern. In 7 of the 12 pairs intense spawning behavior (level 3 or above) was observed. The remaining 5 pairs showed active spawning behavior (level 2) in 2 pairs, and the other 3 displayed little or no spawning behavior (levels 1 or 0). The spawning behavior of all pairs was consistent with previously described catfish spawning (Busch 1985). Eggs were released in the tank by 7 of the 12 F-F pairs. Six of those 7 pairs displayed intense spawning behavior. Unfertilized eggs were manually collected from 13 of the 14 females (the seven F-F pairs) that displayed intense spawning behavior. Eggs were also collected from 2 of 4 females in the two F-F pairs that displayed active spawning behavior, and 4 of the 6 females in the three F-F pairs that showed no spawning behavior (Table 4-7).

Discussion

Improved efficiency of artificial spawning of channel catfish broodstock is needed to enable the commercial-scale production of the hybrid of the channel catfish female and the blue catfish male. Increasing the number of female broodstock that are artificially spawned in a single spawning season can reduce the cost per unit effort of artificial spawning in space limited hatchery facilities. This cost reduction could make commercial production of hybrid catfish more economically feasible. The artificial spawning of females in groups using carp pituitary extract (CPE) (Dunham et al. 1998) and LH-RHa (Bates and Tiersch 1998), and all-female pairs using CPE (Dunham et al 1998)

are techniques that could yield increased efficiency for artificial spawning (Silverstein et al. 1999).Other techniques include the extension of the spawning season by heating ponds with geothermal water (Lang et al. 2003a) and the synchronized conditioning of broodstock in geothermally heated all-female ponds for artificial spawning (Lang et al. 2003b). These methods have provided conditioned broodstock as early as two months prior to naturally conditioned broodstock. The present study combined the use of geothermal water to advance the spawning season, the conditioning of broodstock in all-female ponds with males in cages, and the artificial spawning of these conditioned females in female-female pairs using LH-RHa as the ovulation inducing agent.

In 2002 and 2003 the first artificial spawning trials were conducted 3-4 weeks before the start of natural spawning. The use of geothermal water to condition broodstock could also provide the producer with more control over the timing of spawning, and the ability to create ideal spawning conditions before and during the regular spawning season. In the 2002 and 2003 spawning seasons no spawning activity was recorded and no spawns were collected from the all-female ponds with the males in cages. In 2003, the females conditioned in all-female ponds with the males in cages produced eggs in 100% of the females in M-F pairs and 71% of the females in F-F pairs. All M-F pairs and 91% of the F-F pairs produced eggs.

The survival of 50 % of the males in the cages can be attributed to the aggressive behavior shown towards each other, evidenced by the presence of bite marks on the fish. Feeding the males in the cages proved to be difficult at times because the tops of the cages were even with the water level and the males could not access the feed and in future studies, the cages should be elevated. This could also have attributed to the loss of

half the males in the cages. Similar cages can also be used to condition blue catfish males and channel catfish females in the same pond space for hybrid production.

The M-F and F-F pairs were not significantly different in the volume of unfertilized eggs collected, spawning latency, and neurulation. There was also no significant difference between the F-F and M-F pairs in the number of stripped spawns per pair or per female. In 2003 however, the average number of spawns per pair for F-F pairs was 1.50 compared to 1.0 for M-F pairs. This indicates that males could be eliminated from indoor artificial spawning activities, thus creating more hatchery space for females and potentially increasing the hybrid production per unit effort. Although there was no significant difference in neurulation between F-F and M-F pairs although, there were five F-F spawns with 1% neurulation compared to no M-F spawns below 36%. The eggs collected from these F-F spawns were partly or completely white, slimy and did not form cohesive egg masses indicating that they were of poor quality and could have been collected before or after the optimal timing of ovulation. Ovulation could be detected in 83% of the M-F pairs by the voluntary release of eggs in the tank, however in F-F pairs only 50% of the pairs released eggs in the tank and there was no significant difference between the F-F and M-F pairs in the number of tank spawns (egg release) per pair. As long as there were two females per tank, ovulation could be determined by the level of spawning behavior and the presence of eggs in the tank. The number of eggs released by females in M-F pairs tallied 100 whereas the number in the F-F pairs was typically less than 20 eggs. Egg release in M-F pairs was followed by continued tank spawning if the female was not removed. The F-F pairs did not continue tank spawning even if intense spawning behavior was previously observed. There was however, a significant difference in the number of normal spawns per female which indicates that

ovulation could not be determined for all the females in F-F pairs. As soon as one female was removed from the F-F pair in the spawning tank, the remaining female did not release any eggs and thus could not be identified as ovulating. Future studies should return a stripped female back to the tank or replace her with another female that was conditioned to spawn and was injected with an ovulation-inducing agent.

The level of spawning behavior in the M-F pairs was significantly higher than in the F-F pairs which indicates that spawning behavior is a more accurate way of predicting ovulation in M-F pairs than in F-F pairs. Several females in F-F pairs had swollen abdomens and displayed active or intense spawning behavior (stages 2 and 3), but did not produce eggs. Although spawning behavior does provide some indication of when to collect unfertilized eggs from F-F pairs, the tanks need to be monitored for floating eggs, and if possible the females can be removed and handled periodically to estimate when to collect eggs. The behavior of female-female spawning pairs was not reported in the previous study (Dunham et al. 1998) involving F-F paired spawning.

There was no significant difference in egg volume, spawning latency, and neurulation among time periods. There was no significant difference between the F-F and M-F pairs in the number of stripped spawns per female and the number of stripped spawns per pair for trials conducted during the different time periods. This indicates that channel catfish can be artificially spawned in M-F and F-F pairs, before the start of the regular spawning season and also early and late in the regular spawning season. The final artificial spawning trial in 2002 produced a significantly lower number of spawns compared to the other trials. The broodstock that were induced to spawn during that trial had remained in the pond for seven weeks after natural spawning had started and were perhaps undergoing atresis. Attempts were made to delay the reproductive readiness of

these broodstock by maintaining the water temperature in that pond below 24 °C by the addition of well water at 21°C. The pond water temperatures remained consistently at 27°C to 29 °C. When the broodstock were moved indoors for artificial spawning the females did not have swollen abdomens.

The combination of spawning behavior and egg release proved to be the most accurate way to determine the optimum time to manually strip female broodstock without stressful handling of the fish. The pairing of females in artificial spawning increases the number of females that can be stripped in limited hatchery space. Careful monitoring of spawning behavior of the female pairs increases the accuracy of stripping the females at the right time to collect unfertilized eggs. Replacing females after one of the pair has been stripped could help improve the collection of eggs from the remaining female by providing a partner for further behavioral interaction that can be observed. Preliminary studies with ultrasonography (our unpublished data) have potential for determination of the timing for manual stripping and collection of unfertilized eggs from channel catfish females. This technique has been used to determine ovarian maturation in striped bass (*Morone saxatilis*) (Blythe et al. 1994) and Atlantic halibut (*Hippoglossus hippoglossus*) (Martin-Robichaud and Rommens 2001). The ability to determine the size of the ovary and oocyte at ovulation could improve the ability to collect eggs at the optimal time during artificial spawning. Techniques for sorting salmonids by sex as they swam by a stationary ultrasound transducer (Reimers et al. 1987) could serve as an example for the development of ultrasound techniques that can detrmine the spawning readiness of female broodstock in female pairs and group spawning in channel catfish.

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CHAPTER 5 - REARING OF CHANNEL CATFISH FRY BEFORE AND DURING THE NATURAL SPAWNING SEASON IN OUTDOOR PONDS AND POOLS

The acquisition of seedstock is required for any aquaculture enterprise. The production of channel catfish seedstock involves several juvenile stages between the fry and adult stages. These juvenile stages are termed: fry (< 1 cm), advanced fry (1-3 cm), fingerlings (3-25 cm) and stockers (25-30 cm) (Lee 1981). Yolk absorption is normally completed in sacfry within 4 to 5 d after hatching (26-27 °C), at which time the fry begin feeding and swimming at the surface. These actively swimming and foraging fry are termed swim-up fry (Huner and Dupree 1984). In the fingerling production process, fry can be placed directly into ponds or raised in hatcheries for several d before stocking in ponds (Busch 1985). The rearing of fry in hatcheries for 7 to 14 d before placing them into nursery ponds is a common production method (Tucker and Steeby 1993; Weirich et al. 2001). Some commercial producers consider the rearing of fry in hatcheries as crucial to avoiding high mortality (Murai 1979), and most usually grow fry to swim-up stage and feed them for several d before stocking them into nursery ponds (Tucker and Steeby 1993).

Studies on the methods of fingerling production from fry have yielded varied results. The average survival rate of fry reared and fed in troughs for 15 to 25 d before stocking (75%) and that of fry stocked directly into ponds but protected and fed for 7 d (75%) were higher than the survival rates of sacfry stocked directly into ponds and fed but not protected (43%) and the survival rate of fry stocked directly into ponds but not fed or protected (40%) (Snow 1962). Fingerlings produced from fry reared in a hatchery for 7 d had significantly lower mortality than fry that were stocked immediately after swim-up (Tidwell et al. 1995). Although the level of care and protection provided to

newly hatched fry seems to affect their survival, hatchery rearing of fry consumes time, energy (Dupree and Huner 1984), and labor. Swim-up fry require six or more feedings per day and uneaten feed and other debris must be removed from hatchery troughs several times per day (Tucker and Robinson 1991). Hatchery rearing of fry also increases the risk of losses to disease and power failure (Dupree and Huner 1984).In an attempt to reduce the costs of production, labor, and the risk of hatchery operations, catfish producers have implemented the stocking of sacfry into nursery ponds 2-3 d after hatch (Weirich 2001). Currently the stocking of sacfry directly into nursery ponds is being used by 70-80% of catfish producers in Louisiana (personal communication, C. Greg Lutz, Extension Specialist, Louisiana State University Agricultural Center).

During the 1980's, the catfish industry underwent a dramatic intensification. This change in the production scale made vertical separation of production segments possible. Several catfish producers concentrated their efforts exclusively on fingerling production while the majority of producers decided to focus on the grow-out (fingerling to food fish) segment of the industry. In the four most important catfish producing states (Mississippi, Alabama, Arkansas and Louisiana which account for 96% of the total catfish production in the US), only 13% of catfish producers operate a hatchery and 30% produce fingerlings from fry (USDA 2003). Currently only 3 out of an estimated 45 commercial catfish producers in Louisiana operate a hatchery that produces their own seedstock (personal communication, C. Greg Lutz). The specialization in fingerling production has increased interest in ways to improve and maximize fingerling production. Channel catfish have been induced to spawn 20 to 62 d before the natural spawning season in geothermally heated ponds (Lang et al. 2003). Early spawning has created the

opportunity for the production and stocking of catfish fry before the regular spawning season. The early production and stocking of catfish fry would lengthen the growing season for the sacfry and could result in larger fingerlings for growers at the start of the growing season. Larger fingerlings could also reach harvestable size faster and potentially reach market size within one growing season. Larger fingerlings gain weight faster than smaller fingerlings when comparing absolute weights (Carmichael 1994). Consequently, the stocking of larger fingerlings in a multiple-batch production system could lead to a higher yield of market-size fish during a 2 yr growing period.

The goal of this study was to evaluate the effectiveness of stocking outdoor ponds and pools with channel catfish fry. The fry were produced before and after the start of natural spawning by using geothermally heated ponds. The specific objectives were to: 1) compare the survival and growth rate of fry stocked before the start of the natural spawning season to those stocked during the natural spawning season; 2) determine the difference in survival and growth of fry stocked as sacfry compared with those stocked as swim-up fry, and 3) determine the difference in survival and growth rate among fry reared in 0.16-ha and 0.04-ha ponds, and 0.001-ha pools. The pools had a volume of 12000-L and a depth of 1.2 m. This study will provide information for producers with the ability to conduct out-of-season spawning, on survival and growth rates of fry stocked at different ages and in different size ponds before the start of natural spawning.

Methods

In 2002 and 2003 channel catfish broodstock were induced to spawn before the natural spawning season by heating 0.04-ha ponds. Water temperatures were raised by addition of geothermal water to ponds (Hall et al. 2001). Egg masses were collected from

spawning containers in ponds and hatched indoors by forced air incubation (Lutz and Tiersch 1994). Spawns were collected from early March until early June in both years. Fry hatched from these eggs were stocked in ponds and outdoor pools before and during the natural spawning season. Fish in all ponds were fed 45% protein fry starter feed to once a day after stocking.

Ponds and pools

Ponds and pools of three different sizes were used in this experiment. The depth of the ponds and pools averaged 120 cm. The surface area of the ponds were 0.16-ha and 0.04-ha. The surface area of the pools was 0.001-ha. The pool bottoms were covered with a ~30 cm layer of Mississippi river soil to simulate pond conditions. In 2002, the ponds were stocked at the densities of 500,000 fry/ha or 375,000 fry/ha, and in 2003 all ponds were stocked at a density of 500,000 fry/ha. The water temperatures followed the natural thermal conditions. At a density of 500,000 fry/ha the 0.16-ha ponds were stocked with 80,000 fry, the 0.04-ha ponds with 16,000 fry, and the pools with 520 fry. Water temperatures were recorded at the time of stocking and were monitored with individual data loggers (HOBO data logger; Onset Computer Corporation, Pocasett, Massachusetts) for 2.5 months. The pools were filled with water from an 8.6-ha lake that had an established algal bloom. The ponds where filled with well water and fertilized with liquid organic fertilizer (11:37:0) (N:P:K) at an equivalent rate of 4.5 L per ha and cottonseed meal at 140 kg per ha. The ponds were fertilized with a combination of liquid fertilizer, cottonseed meal and catfish starter feed for a week after filling. The ponds were treated with a mixture of diesel fuel and motor oil at a ratio of 10:1 at a rate of 30 L per ha, 2 d before stocking and 4 d after stocking to control predatory insects.

Stocking of ponds and pools

In 2002, spawning limitations prevented the production of sufficient quantities of fry to stock the 0.16-ha ponds simultaneously. Each pond received two or more batches of fry at separate intervals (Figure 5-1). In 2003, egg production capacity was increased by placing six instead of four spawning cans in the broodstock ponds. Also in 2003, primarily 0.04-ha ponds were stocked instead of 0.16-ha ponds. These changes enabled the stocking of one or more ponds at a time at more regular intervals as eggs were collected from ambient temperature ponds (Figure 5-2). Fry were stocked before and after the start of the natural spawning season. All fry stocked before April 18, 2002 and before April 15, 2003 were designated as early-stocked fry. These dates corresponded with the collection of eggs from unheated ponds the second year. The ponds were stocked with sacfry or swim-up fry. Fry with yolksacs were designated as sacfry and were released 2 to 3 d after hatching. Swim-up fry, which had absorbed the yolksac, were released 7 to 10 d after hatching. In 2003, one 0.16-ha pond was stocked before the start of the natural spawning season. All other ponds were stocked after the start of the natural spawning season and all fry stocked in the two 0.16-ha ponds were swim-up fry. All 0.04-ha ponds were stocked before the start of the natural spawning season, of which four were stocked with sacfry and the other four with swim-up fry (Figure 5-2). Ten of the 22 pools were stocked before the natural spawning season, and the remaining 12 pools were stocked after the start of the regular spawning season. One pool was stocked with swim-up fry and 21 pools were stocked with sacfry. All pools were stocked with 520 fry each and 2 to 4 pools were stocked simultaneously (Figure 5-3).

The number of fry was determined volumetrically (Tucker and Robinson 1990). Three samples of 100 fry from each egg mass were counted and weighed. The total number of fry was estimated by dividing the total weight of fry from a hatched egg mass by the average weight of three samples of 100 fry:

Number of fry = (Total fry weight / Average weight of 100 fry) *100 fry

Sampling and harvest

In 2003 the ponds and pools were sampled by cast net (mesh size) once during the grow-out period to estimate the growth rate of the fry. The 0.04-ha ponds were sampled an average of 155 ± 18 d after stocking and the pools were sampled 140 ± 40 d after stocking. The lengths and weights of 100 fingerlings from the ponds and 50 fingerlings from the pools were measured. The effectiveness of the cast net sampling method was verified in 2002 by comparing the average weight and length of four samples of fingerlings collected immediately before harvest, with the length and weight of the fingerlings at harvest. Four samples of 50 fingerlings were collected by cast net between 8 d and 22 d before harvest from each of the four 0.04-ha ponds in 2002. The fingerlings were harvested by seining the ponds three times, followed by complete draining of the ponds and collection of the remaining fingerlings by hand. At harvest the total number of fingerlings in the ponds and pools was estimated and lengths and weights of a sample of 100 fingerlings were measured. The total number of fingerlings in ponds and pools was estimated by dividing the total weight of fingerlings harvested by the average weight of the five fingerling samples:

Total number of fingerlings = (Total weight harvested / Average weight of samples) X Number of fingerlings in sample

Figure 5-1. Dates when ponds were stocked, number of fry, and the corresponding average daily water temperatures in 2002. The grey circles indicate stocking before the start of regular spawning and black circles indicate stocking after the start of natural spawning.

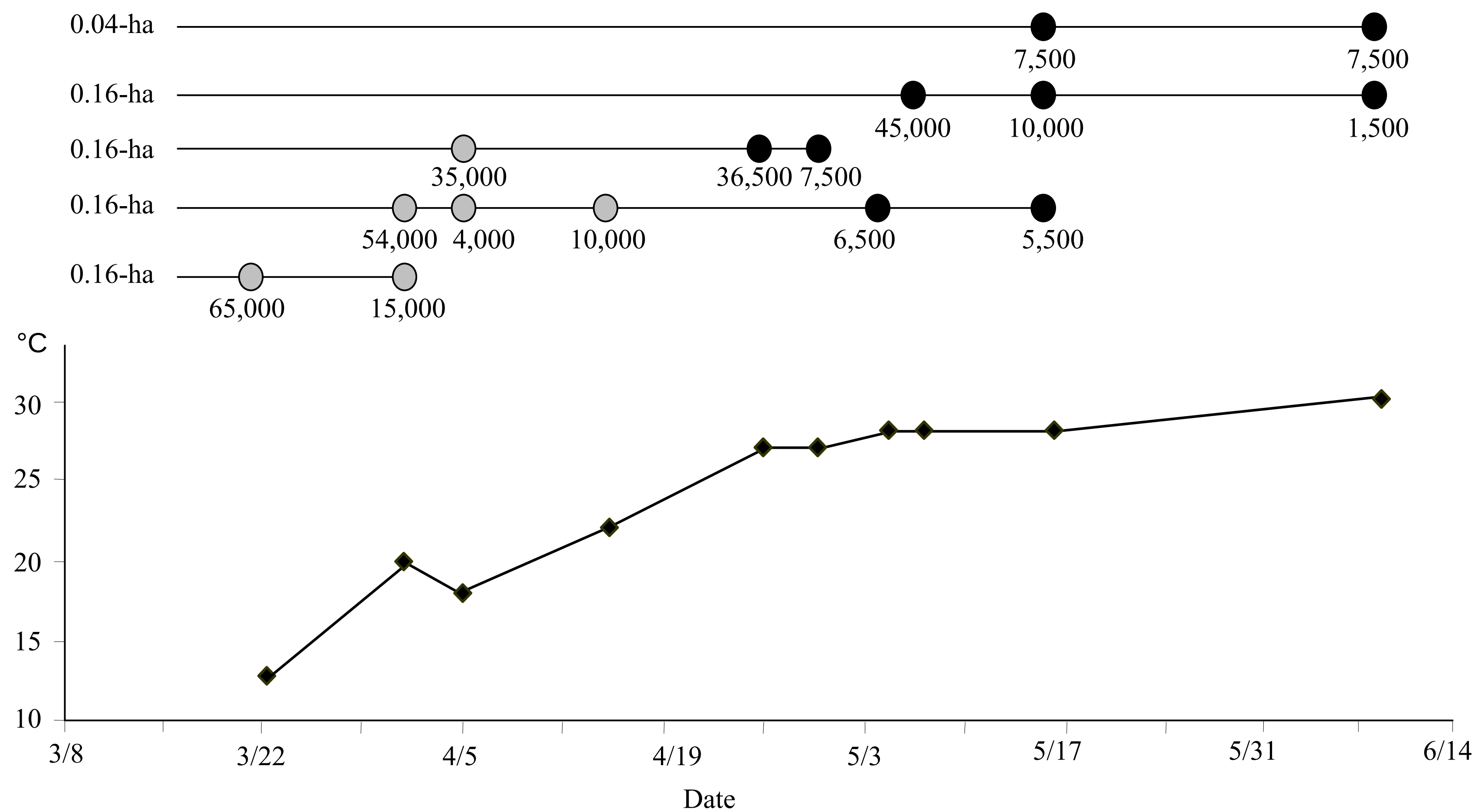


Figure 5-2. Dates when ponds were stocked, number of fry and the corresponding average daily water temperatures in 2003. The grey circles indicate stocking before the start of regular spawning and black circles indicate stocking after the start of natural spawning.

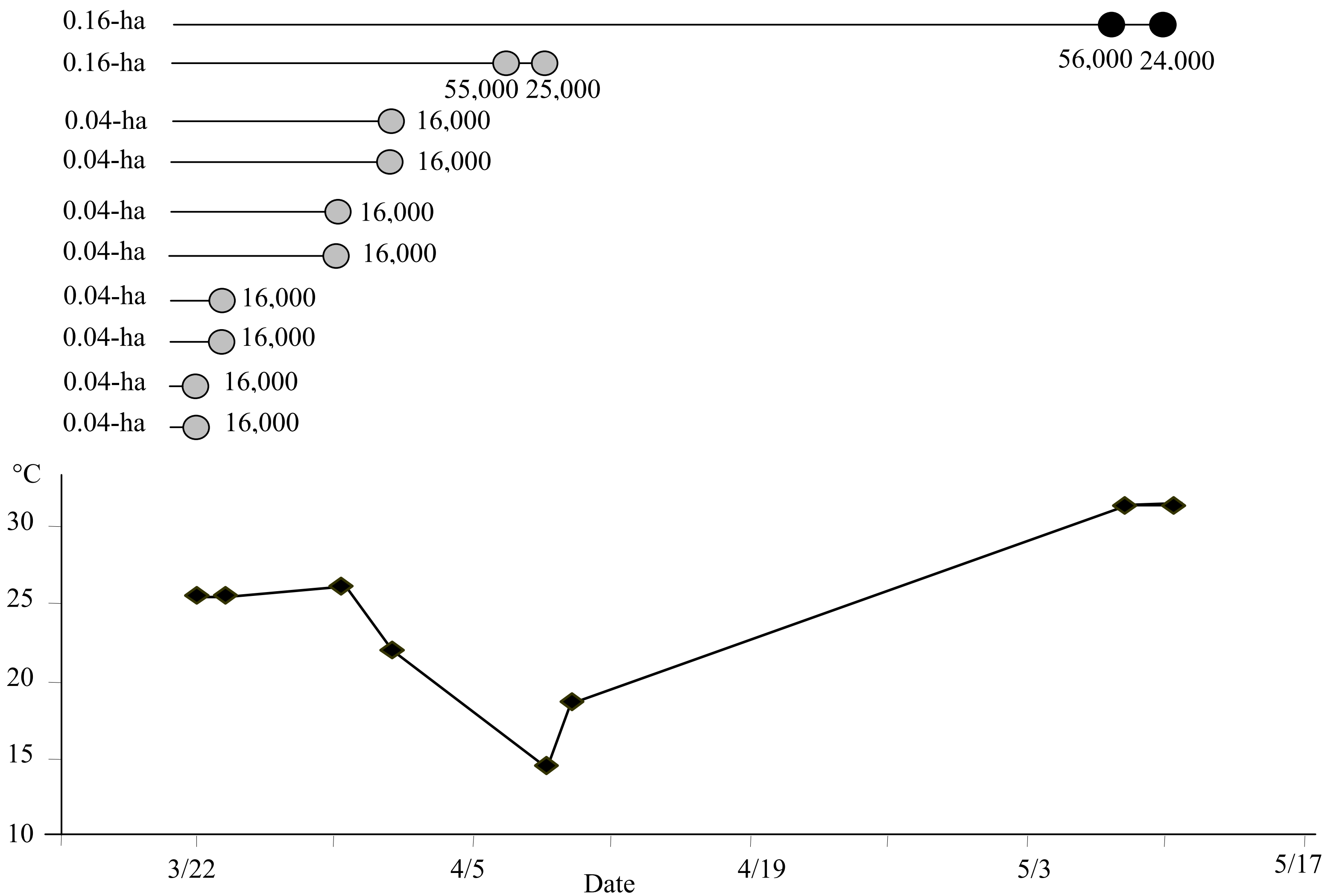
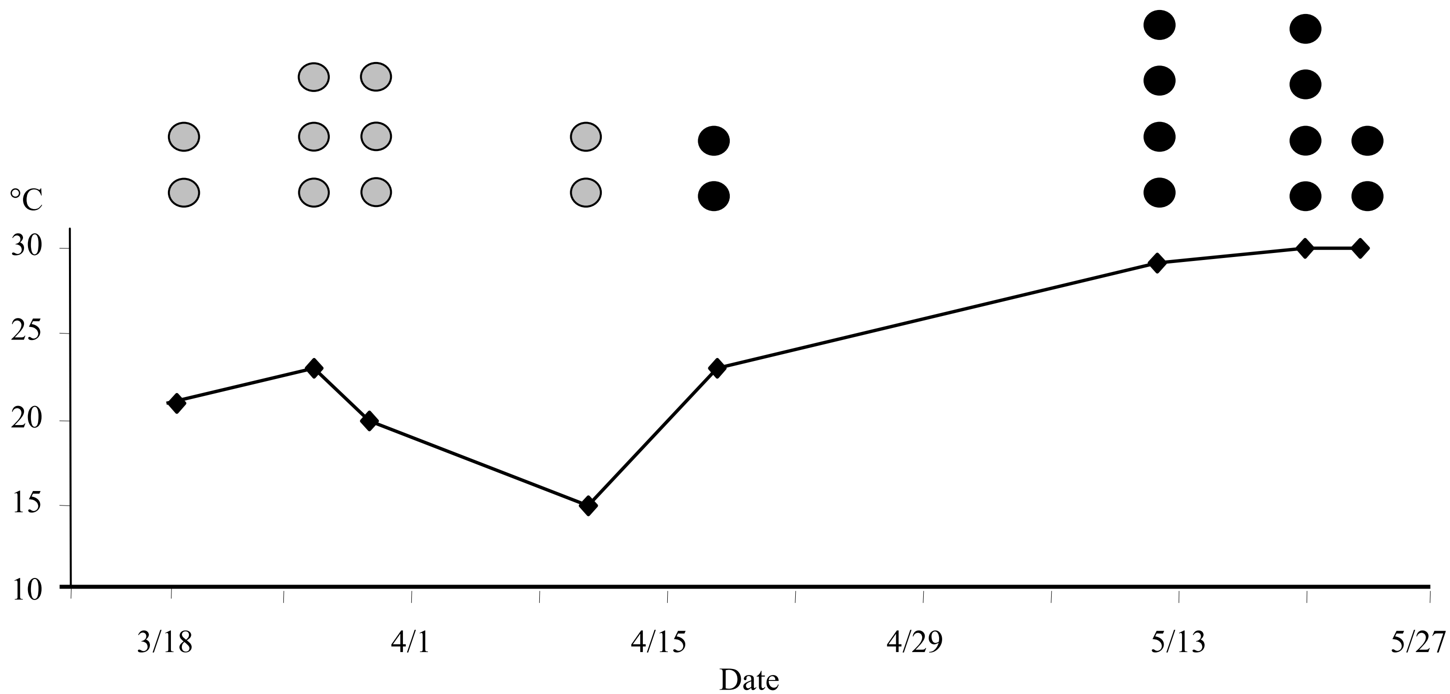


Figure 5-3: Dates when pools were stocked and the corresponding average daily water temperatures in 2003. The grey circles indicate stocking before the start of regular spawning and each circle represents one pool. Each pool was stocked with 520 fry.



The two parameters used to evaluate fingerling production in the ponds and pools were survival percentage of the fry stocked, and the average daily growth rate of these fry until the harvest as fingerlings.

Survival % = (Number of fingerlings harvested / Number of fry stocked) X 100%

Growth rate = (Average weight of fingerlings / Number of growing days)

Treatment groups

The time of stocking, age of the fry, and size of the ponds were used to create different treatment groups (Table 5-4). The ponds were stocked before (B) and after (A) the start of the natural spawning season. The ponds and pools of three different sizes that received fry were 0.16-ha ponds or large ponds (L), 0.04-ha ponds or medium ponds (M), and pools (P). The two types of fry that were distributed among the ponds were sacfry (SA) and swim-up fry (SW). Ponds that were stocked both before and after the start on natural spawning were labeled as before and after (BA) ponds and ponds that received both sacfry and swim-up fry were named mixed fry (MF) ponds. The combination of these three variables formed the different treatment groups. The acronym for a treatment group first identifies the size and type of pond or pool (L, M, or P) followed by the time of stocking (B, A, or BA). The final letters indicate the type of fry (SA, SW, or MF) placed in the pond. Accordingly a 0.16-ha pond (L) that was stocked before the start of regular spawning (B) with sacfry (SA) is identified as LBSA. The five ponds stocked in 2002 were grouped as follows: LBMF (1); LAMF (1); LBAMF (2); MASW (1). In 2003 the 32 ponds and pools that were stocked were grouped as: LBSW (1); LASW (1); MBSA (4); MBSW (4); PBSW (1); PBSA (9); PASA (12).

Table 5-1. The treatment groups for fingerling production were determined by pond size, age of fry and time of stocking. Sacfry (SA) and swim-up fry (SW) were distributed among 0.16-ha ponds (L), 0.04-ha ponds (M), and pools (P), before (B) and after (A) the start of the regular spawning season. Ponds that received sacfry and swim-up fry were labeled mixed fry (MF). Ponds that received fry before and after the start of regular spawning were labeled (BA) for time of stocking. Fingerlings were produced in every 0.16-ha and 0.04-ha pond stocked, and in 7 of 9 PBSA pools, 8 of 12 PASA pools and one PBSW pool. The numbers in parenthesis indicate the year and the number of ponds in a specific treatment.

Culture Unit	Sacfry (SA)		Swim-up fry (SW)		Mixed fry (MF)		
	Before (B)	After (A)	Before (B)	After (A)	Before (B)	After (A)	Before and After (BA)
0.16-ha (L)	----	----	2003-1 (LBSW)	2003-1 (LASW)	2002-1 (LBMF)	2002-1 (LAMF)	2002-2 (LBAMF)
0.04-ha (M)	2003-4 (MBSA)	----	2003-4 (MBSW)	2002-1 (MASW)	----	----	----
Pool (P)	2003-9 (PBSA)	2003-12 (PASA)	2003-1 (PBSW)	----	----	----	----

Statistical analysis

The complete data set included 11 treatment groups distributed over 37 ponds and pools: LBMF (1); LAMF (1); LBAMF (2); MASW (1); LBSW (1); LASW (1); MBSA (4); MBSW (4); PBSW (1); PBSA (9) and PASA (12). Regression analyses were performed to determine the influence of selected variables on the experimental results. The data collected when the ponds were harvested were: 1) number of fingerlings, 2) percent survival, 3) average weight of fingerlings, and 4) average growth rate of fingerlings. Variability in these data could be a function of: 1) date of stocking, 2) number of growing d, 3) size of the culture unit, 4) age of fry, 5) number of fry stocked, and 6) percent survival. The maximum R^2 improvement (MAXR) method of the regression procedure in SAS was used to determine the contribution of each treatment to the variability in the data. In this procedure, the regression analysis begins by finding the one-variable model producing the highest R^2. The process continues by adding the variable that yields the greatest increase in R^2 to the model. The model with the largest R^2 and all significant components was selected as the final model that best describes the influence of the selected variables on the experimental results. All effects were considered significant at $P \leq 0.05$.

The effects of a variety of variables on fingerling yield were investigated using four potential regression models (Table 5-4). The first model investigated the effects of the date of stocking, number of growing d, size of culture unit, age of fry, percent survival and the number of fry stocked on the number of fingerlings harvested. The effect of the date of stocking, the number of growing d, the size of the culture unit, and the age of the fry on percent survival was determined in the second analysis. In the third analysis, the

Table 5-2. Variables that could have contributed to differences in harvest yield among treatments. An "X" means that the corresponding treatment was included in a regression model to determine the contribution of that treatment to the variability in the data.

Parameters	Date of stocking	Growing d	Culture unit	Age of fry	Fry stocked	Percent survival
Number of fingerlings	X	X	X	X	X	X
Percent Survival	X	X	X	X	X	--
Weight of fingerlings	X	X	X	X	--	X
Growth of fingerlings	X	X	X	X	X	X

average weight of fingerlings harvested was correlated to the number of fry stocked, the date of stocking, the number of growing d, percent survival, size of the culture unit and the age of the fry stocked. The fourth model examined the effects of the number of fry stocked, the date of stocking, the number of growing d, percent survival, the size of the culture unit and the age of the fry on the average growth of fingerlings.

The data were also analyzed using the Proc Mixed function of the SAS system (Statistical Analysis Software system version 9 for Windows®; SAS Institute Inc., Cary, North Carolina). The data were arranged to determine differences in survival rate and growth rate of the fish harvested based on pond sizes, time of stocking, and the age of the fry stocked. In the Proc Mixed analysis, differences were considered significant at $P \leq 0.05$. Data from treatments that contained only one data point (one pond) are reported here, but were not included in the Proc Mixed analysis. These treatments were: LBMF; LAMF; MASW; LBSW; LASW and PBSW. The LBAMF treatment also was removed from the data set used in the analysis of variance (Proc Mixed) because two of the three variables (time of stocking and age of fry) that were used to determine treatment groups overlapped. The data set used for the analysis of variance included 29 ponds and pools in the MBSA; MBSW; PBSA and PASA treatments.

Results

The first model investigated what selected variables influenced the number of fingerlings harvested. Most variability in the number of fingerlings harvested can be attributed to the survival rate of the fry stocked. Survival was the only variable with a significant influence ($P < 0.0001$) and had a high positive correlation ($R^2 = 0.99$) with the number of fingerlings harvested. The variability in the number of fingerlings harvested is

a function of the survival rate of fry stocked. The second model investigated the influence of selected variables on the survival of fry. Date of stocking ($P = 0.005$) and number of growing days ($P = 0.003$) had significant effects on the survival of fry to the fingerling stage. The survival of fry stocked is function of the date of stocking and the number of growing days. This model of two variables has a positive correlation ($R^2 = 0.42$) with the survival of fry. In the third analysis the variability in the average weight of fingerlings harvested was investigated. The average weight of fingerlings harvested was significantly affected by the survival of fry in the ponds ($P = 0.01$) and the size of the culture unit ($P = 0.02$) although the correlation in this model was weak ($R^2 = 0.28$). The fourth model examined the effects of the variables on the average growth of fingerlings. The average growth of fingerlings significantly effected by survival ($P = 0.005$), date of stocking ($P = 0.03$) and the number of growing days ($P = 0.08$). This model however also had a weak correlation ($R^2 = 0.33$).

In 2002 and 2003, six 0.16-ha ponds, nine 0.04-ha and 22 pools were stocked with fry before and after the start of the natural spawning season. In 2002, three 0.16-ha ponds were stocked at 500,000 fry/ha, one 0.16-ha pond and one 0.04-ha pond were stocked at 375,000 fry/ha. In 2002 the first ponds were stocked on March 22 and stocking continued to May 16, with two late batches of fry stocked on June 8 (Table 5-1). The fry stocked in 2002 remained in the ponds between 159 and 172 growing d. In 2002 all 0.16-ha ponds were stocked with a mixture of sacfry and swim-up fry. The first 0.16-ha pond in 2002 was stocked before the start of natural spawning, the fourth 0.16-ha pond was stocked after, and the second and third 0.16-ha ponds were stocked before and after the start of natural spawning. The only 0.04-ha pond in 2002 was stocked with swim-up fry after the

Table 5-3: Survival percentage, growth rate and fingerling weight of fry stocked in four 0.16-ha and one 0.04-ha ponds before and after the start of the natural spawning season in 2002. The 5 ponds were distributed over 4 treatment groups. The treatment groups were determined by the combination of the pond size, age of fry and time of stocking. The first letter of the label specifies the size of the pond (0.16-ha ponds (L) or 0.04-ha ponds (M)), the second letter indicates the time of stocking (before (B) or after (A) the start of natural spawning), and the last 2 letters indicate the age of the fry stocked (sacfry (SA) or swim-up fry (SW) of a combination of both (MF)).

	LBMF (n = 1)	LBAMF (n = 2)	LAMF (n = 1)	MASW (n = 1)
Survival (%)	8	21 ± 4	52	10
Growth (g/d)	0.23	0.16 ± 0	0.09	0.16
Fingerling weight (g)	41.1	22.2 ± 3.4	15.8	27.7

start of regular spawning. The data collected in 2002 (Table 5-6) could not be analyzed because of the lack of replication within the treatments, which led to the stocking of 0.04-ha ponds and pools in addition to 0.16-ha ponds in 2003.

From March 18 to May 30, 2003, two 0.16-ha ponds, eight 0.04-ha ponds and 22 pools were stocked at a density of 500,000 fry/ha (Tables 5-2 and 5-3). Sixteen of the 22 pools (73%) and all 0.16-ha and 0.04-ha ponds produced fingerlings. The fry in the remaining six pools were lost at an early stage of the experiment. Blooms of macrophytes and filamentous algae in three pools prevented the fry from feeding at the water surface. Two additional pools were excluded from the analysis because of the accidental stocking of larger fish in the same pools, and another pool was lost to a broken air line. The fry distributed between the 0.16-ha ponds in 2003 remained in the ponds for 86 to 112 d at the original density of 500,000 fry/ha. These fingerlings where harvested and redistributed to the same ponds at a density of 12,500 fingerlings/ha. The growing period for fry stocked in the 0.04-ha ponds was between 168 d and 304 d, and the growing period for fry stocked in the pools was between 270 d and 350 d.

Table 5-4: The average number of growing period of the fry stocked in ponds and pools in 2002 and 2003.

	Growing d		
	0.16-ha ponds	0.04-ha ponds	pools
2002	192 ± 11 (n=4)	186 (n=1)	none
2003	225 ± 21 (n=2)	286 ± 16 (n=8)	318 ± 25 (n=16)

Survival

The survival rate in the 0.16-ha pond stocked with swim-up fry before natural spawning (LBSW) was 8% compared to 13% survival in the 0.16-ha pond stocked with swim-up fry after the start of natural spawning (LASW). All eight 0.04-ha ponds were stocked before the start of the regular spawning season. Survival in 0.04-ha ponds stocked with sacfry (MBSA) was 9 ± 4% and for those stocked with swim-up fry (MBSW) survival was 11 ± 10%. There was no significant difference ($P = 0.94$) in survival rate between sacfry and swim-up fry that were stocked in 0.04-ha ponds before natural spawning season began (MBSA vs. MBSW). The survival of sacfry was 44 ± 22% before the start of natural spawning (PBSA) and 57 ± 35% after the start of natural spawning (PASA). Survival in the one 0.04-ha pond stocked with swim-up fry (PBSW) was 24%. There was no significant difference ($P = 0.15$) in survival rate between sacfry that were stocked before or after the start of natural spawning in pools (PBSA vs. PASA). The survival rate of sacfry stocked in 0.04-ha ponds, before the start of the natural spawning season (MBSA) was significantly lower ($P = 0.03$) than that of sacfry stocked in pools before the start of natural spawning (PBSA).

Growth

The growth rate of fry in the LBSW pond was 0.09 g/d and in the LASW pond the growth rate was 0.17 g/d. The growth rate of fry in the 0.04-ha ponds was 0.15 ± 0.07 g/d for the MBSA treatment and 0.18 ± 0.18 g/d for fry in the MBSW treatment. There was no significant difference ($P = 0.74$) in growth rate between sacfry and swim-up fry that were stocked in 0.04-ha ponds, before the start of natural spawning (MBSA vs. MBSW).

In the pools the growth rate was 0.14 ± 0.07 g/d for the PBSA and 0.18 ± 0.14 g/d for the PASA treatment. There was no significant difference ($P = 0.53$) in growth rate between sacfry that were stocked before or after the start of natural spawning in pools (PBSA vs. PASA). Survival of the fry showed a weak negative correlation with the growth rate to fingerling size ($r = -0.36$).

Table 5-5: Survival, growth and fingerling weight of sacfry (SA) and swim-up fry (SW) stocked before (B) and after (A) the start of the 2003 spawning season in 0.04-ha (M) ponds and pools (P).

	MBSA (n = 4)	MBSW (n = 4)	PBSA (n = 8)	PASA (n = 8)
Survival (%)	9 ± 4	11 ± 10	44 ± 22	57 ± 35
Growth (g/d)	0.15 ± 0.07	0.18 ± 0.18	0.14 ± 0.07	0.18 ± 0.14
Fingerling weight (g)	43.7 ± 22.7	53.3 ± 52.8	63.5 ± 31.4	53.6 ± 38.9

Discussion

The goal of this study was to evaluate the effectiveness of stocking ponds and outdoor pools with channel catfish fry prior to the start of the regular spawning season. There were no significant differences in growth rate in 0.04-ha ponds stocked before the start of the natural spawning season with sacfry and swim-up fry. There also was no significant difference between growth rates of fish in pools stocked with sacfry before and after the start of regular spawning. The regression analysis confirmed that survival greatly affects the fingerling yield at harvest. Survival of the fry stocked is positively correlated to the date of stocking and the number of growing days. However this

correlation is not very strong (R^2 = 0.42) and stocking fry before the start of natural spawning can still be an effective production practice.

Size of the ponds

The survival rate in pools stocked with sacfry before the start of regular spawning was significantly (P = 0.01) greater (44 ± 22%) than survival in 0.04-ha ponds stocked with sacfry before the start of regular spawning (9 ± 4%). The regression analysis showed that the size of the ponds did not have a significant influence on the survival of fry (P = 0.99).This great variability in survival rates could be attributed to the differences in food abundance and predation in the different culture units. The higher probability of finding food and better predator control in the pools could have led to higher survival in pools. The larvae of most fish species are poor swimmers and they are limited to searching a relatively small volume of water for prey, thus areas of high prey density are of vital importance to the survival of fish larvae through the first feeding phase (Jobling 1995). Elimination of predator populations is essential to the survival of catfish fry (Weirich et al. 2001). Predator populations were easier to control in the pools because of the ease of application, and the more complete coverage of the water surface, by the oil solution used to kill insect predators. The pools had no occurrences of the predatory green sunfish *Lepomis cyanellus* which were encountered in most ponds. The pools had a noticeably denser algal bloom at the time of stocking compared with the 0.04-ha and 0.16-ha ponds. Catfish ponds usually have a relatively low abundance of phytoplankton in the months of March and April compared with the regular production season because of lower water temperatures and reduced solar radiation (Couch 1998). The ponds had no standing algal bloom at the time of stocking. The presence of natural food and predators for catfish fry

in outdoor ponds before the start of natural spawning was not determined during this study. These factors could have a significant effect on the survival of early-stocked fry. Future studies should focus on the quantity and quality of natural food and the presence of predatory insects in recently filled ponds before the start of natural spawning. This information can help develop best management practices that could increase the survival and growth of fry released in ponds before natural spawning.

<u>Time of stocking</u>

There was no significant difference (P = 0.40) in survival between pools stocked with sacfry before regular spawning (44 ± 22%) and pools stocked with sacfry after the start of regular spawning (57 ± 35%). The regression analysis however, did identify the date of stocking as a significant effect on the survival of fry. The large variability in survival rates before and after the start of natural spawning could have led to Type 2 statistical error, such that actual differences in survival could not be detected. An important factor that could have led to the large variability in survival is water temperature. The average daily temperature of ponds at the time of stocking before the natural spawning season was 21.3°C. Ponds stocked after the start of the regular spawning season had average daily water temperatures of 28.3°C at the time of stocking. Although water temperatures at stocking were higher after the start of natural spawning than before, there was no correlation (R^2 = 0.28) between fry survival rates and the temperature of the ponds when the fry were stocked. Water temperature can influence the survival and growth of recently stocked fry because it has a direct effect on yolk conversion efficiency (Howell et al. 1998; Small et al. 2001). The average daily pond water temperature during the time the ponds were stocked in 2003 (March 18 - May 22) was 20.9 ± 2.8 °C (minimum –

maximum; 14.2 – 25.3°C) before the start of natural spawning and 25.8 ± 1.7 °C (minimum – maximum; 22.9 – 28.3°C) after the start of natural spawning. The average pond water temperature before the start of natural spawning was 5 °C lower than after the spawning season. This change in temperature can cause changes in the relative timing of organogenesis and influence the development of the body and musculature which, may affect larval feeding ability (Howell et al. 1998). The average lower water temperature of 18.4 ± 2.6 °C (14.2 – 21.8 °C) in the four weeks prior to the start of natural spawning also could have negatively affected the establishment of a sufficient algal bloom to provide nutrition for the early-stocked fry. Future studies should focus on the development of fertilization protocols for the establishment of an algal bloom at temperatures between 10 and 20 °C. A well-established algal bloom before the start of natural spawning could benefit the survival of fry stocked at that time.

Age of the fry

The 0.04-ha ponds showed no significant difference (P = 0.78) in survival between sacfry (9 ± 4%) in the MBSA treatments (n = 4) and swim-up fry (11 ± 10%) in the MBSW (n = 4) treatments. Regression analysis showed no significant effect (P = 0.34) of the age of fry on the survival rates of fry stocked in ponds or pools. There was also no difference in survival between 0.04-ha ponds stocked with swim-up fry or sacfry before the start of regular spawning. These results agree with previous studies that report that the age at which fry were stocked had no effect on survival and growth to fingerlings (Tidwell 1995, Weirich et al. 2001). Survival of fry before the start of regular spawning was greater in pools then in 0.04-ha ponds but that there was no difference in the stocking of sacfry or swim-up fry before or after the start of regular spawning in similar conditioned

ponds or pools. The size, growth rate and number of fingerlings harvested are affected by the survival percentage of fry stocked. Lower survival results in less fingerlings that grow faster and larger. Survival was mostly affected by the date of stocking and the number of growing d the fingerlings remained in the ponds or pools. There correlation between survival and date of stocking ($R^2 = 0.39$) indicates that percent survival increases with a later date of stocking but the effect is not very strong, indicating that early-stocking can still be a viable option for producers.

The earliest pond spawning reported was 60 d before the start of regular spawning (Lang et al. 2003). Assuming the regular spawning season also lasts 60 d (April 15 to June 15), fingerling production could thus theoretically be doubled. The ability to have better control over spawning will allow improved planning and scheduling which can lead to a steady product and cash flow for commercial producers. The extended spawning season is important to producers of hybrid catfish fingerlings. The additional costs incurred from artificial spawning to produce hybrids could be reduced if the corresponding fingerling yield per year could be doubled. The similar survival of sacfry before and after the start of the regular spawning season could lead to different production protocols and greater production by producers of channel catfish and hybrid fingerlings. Future research needs to be focused on the availability of natural food for channel catfish fry in outdoor ponds before the start of natural spawning. A better understanding of the presence of natural food in the ponds between February and April could lead to improved fertilization protocols for the distribution of fry in outdoor ponds before natural spawning. This could in turn lead to higher survival and growth rates for fry produced before the natural spawning season and reared in outdoor ponds, thus

creating opportunities for greater productivity of catfish fingerling producers. The extension of the channel catfish spawning season is a necessary step towards genetic improvement in the catfish industry. The additional costs incurred in the production of genetically superior seedstock such as catfish hybrids can only be recovered if they can be produced on a commercial-scale. These studies on the commercial scale spawning and fingerling production of channel catfish before the natural spawning season will develop techniques that can be used for the production genetically improved catfish in the future.

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CHAPTER 6 - SUMMARY AND CONCLUSIONS

Artificial spawning of channel catfish *Ictalurus punctatus* can lead to genetic improvement by the selective breeding of broodstock that display superior traits and by hybridization of catfish species. Artificial spawning techniques for catfish have been developed for research purposes, but have not been commercially implemented across the industry. The main reasons for the limited implementation of artificial spawning techniques is because they are capital intensive, time consuming, labor intensive, and complicated in comparison to pond spawning (Lang 2001). The studies in this thesis evaluated methods and techniques that could improve the efficiency of artificial spawning.

A longer spawning season can lead to improved artificial spawning efficiency because it increases the production capacity of commercial producers. The use of geothermal water (36°C) to heat ponds has advanced the catfish spawning season by as many as 60 d (Lang 2003). In ponds heated from ambient temperatures at a rate of 2°C per day and maintained between 24°C and 30°C, spawning occurred between 14 d and 21 d after the start of heating, including the 7 to 8 d required to heat ponds to within the spawning temperature range. An additional 14 to 21 d were required for the collection of 10 spawns from 18 to 20 females per pond, amounting to a maximum of 42 d per heating per group.

Knowledge of the heat requirement for channel catfish spawning could lead to optimal use of costly heat sources such as geothermal water. If the amount of heating required for catfish to spawn is known, producers could manage geothermal water use to generate that amount of heating over a pre-determined time period. Chapter 3 introduced

the use of degree-days to quantify the heat requirement for channel catfish spawning before and after the start of natural spawning, when using geothermal water to induce spawning. Control of pond temperatures and knowledge of the heat requirement for spawning enables the producer to plan spawning periods independent of weather conditions. For example, assuming an average of 30 to 40 d per spawning period, and a start date of January 15, three spawning periods could be completed before the start of natural spawning. Controlled pond temperatures also permit uninterrupted spawning during the natural spawning season when cold fronts disrupt spawning with sudden drops in pond temperatures. The planning and the number of spawning periods also applies to artificial spawning where female broodstock can be conditioned in all-female ponds or female ponds with males in cages. The female broodstock ponds can be controlled at temperatures between 21°C and 27°C for specific time periods. This allows for the synchronization of groups of females for artificial spawning at a desired time. Artificial spawning before the start of natural spawning increases the capacity to produce unfertilized eggs for the production of hybrid catfish.

Once female broodstock have been conditioned to spawn outdoors in heated or ambient temperature ponds, they are transported indoors to induce final oocyte maturation by injection of synthetic leuteinizing hormone-releasing hormone (LH-RHa). Traditionally the females are paired with males in spawning tanks, but if the males are eliminated to increase the egg production capacity, the broodstock are spawned as female-female pairs or as female groups. The intensity of the spawning interactions between a male-female pair can serve as an indicator of how close to ovulation the female is. Female group spawning eliminates the normal spawning interactions, thus

increasing the chance to miss ovulating females between periods of observation. More labor would be required to observe the female groups at shorter time intervals, and the amount of handling to check females for ovulation and ensuing stress would also be increased. In Chapter 4, spawning interactions were observed and quantified in female-female pairs induced to ovulate by injection of LH-RHa after being conditioned in heated outdoor ponds. There was no significant difference in fertilization rate or egg volume between eggs collected from male-female and female-female pairs. The number of stripped spawns per pair or per female was also not significantly different between the F-F and M-F pairs, however the level of spawning behavior was more consistent and assisted identification of ovulation in the M-F pairs compared to the F-F pairs.

Advancing the channel catfish spawning season by approximately two months creates the situation of producing channel catfish before the natural spawning season. The environmental conditions at that time are different from those experienced during the natural spawning season. The producer would incur additional cost when rearing the fry in hatcheries until the natural spawning season. The stocking of ponds with channel catfish fry 3 to 4 weeks before the regular spawning season was investigated in Chapter 5. There was no significant difference in survival and growth rates between fry stocked before and during the natural spawning seasons. To our knowledge this is the first report of stocking sacfry before the start of the natural spawning season. The ability to stock channel catfish fry before the natural spawning season with no ill effects on growth and survival rates, could provide a longer growing season for these fry. An extended growing season can lead to larger fingerlings with a higher market value.

The heating of the ponds adds cost to the overall cost of fingerling production. The heating costs involved in the previously discussed early spawning experiments provide a perspective on the possibility for commercial application of spawning and fingerling production before the natural spawning season. The Department of Biological and Agricultural Engineering at LSU conducted several studies on the energy balance of the experimental geothermal ponds at the Aquaculture Research Station of the Louisiana State University Agricultural Center and created a computer model (PHATR) that estimates the energy requirements needed to heat these ponds (Lamoureux 2003). PHATR estimates were used for the calculations for Chapter 6. These estimates were calculated for the conditions in January in southern Louisiana which can be regarded as a worst-case scenario. The average dimensions of the earthen geothermal pond were 10 m by 30 m with an average depth of 1.3 m. The average volume of water per pond was around 400 m^3. Under the conditions in January of an average year, the geothermal well would be operated between 10 and 12 hours per d. One theoretical heating trial in January required 9 d to increase the pond temperature from 10°C to 27°C. Spawning would occur between 7 and 21 d after the start of heating and an additional 14 to 21 d would be required for the collection of 10 egg masses from 18 to 20 females per pond. Thus the average hypothetical heating trial would last about 30 d. The pump that distributes the geothermal water to the ponds has a 30 kW capacity. Conservative estimates based on preliminary experiments indicate that at least 50% of the water at the Aquaculture Research Station (ARS) is not utilized directly for pond heating. The remaining portion of the water pumped by the well is directed into a 8.6-ha lake to relieve pressure on the water distribution system. The maximum total flow rate delivered from the pump to the

ponds would be 40 X 10^{-3} m^3/s. The actual volume of water pumped from the well could exceed 80 X 10^{-3} m^3/s. Thus the maximum flow rates required to heat one of the 400-m^3 ponds from 10.5°C to 27°C over a 9 d period and maintain the pond temperature at 27°C by addition of 36°C water, would be 6.6 X 10^{-3} m^3/s. The number of ponds that could be heated and maintained at 27°C at one time based on the flow rate restrictions equals 80 X 10^{-3} m^3/s divided by 6.6 X 10^{-3} m^3 or 12 ponds.

Table 6-1: Time of operation and associated costs for the geothermal well at the ARS required for pond heating before the start of natural spawning during an average year.

	January	February	March	April	Season
Weeks of heating	4	4	4	2	14
Average pumping time (h/wk)	12	10	8	6	9
Average pumping cost ($/wk)	176	147	118	88	139
Total pumping cost ($)	704	588	472	176	1940

The total amount of energy for 12 h would be 360 kWh. The average price of electricity in Louisiana in August 2004 was $ 0.07 per kWh (DOE). The daily cost of running the well would amount to $25.2 per day for January. Heating of the ponds for the early spawning season can start as early as January 1 and last until April 15 for a total of 98 d or enough time to complete the heating of 3 groups of ponds. The total pumping costs for a season would total $1940 (Table 6-1). The current configuration at the ARS allows for the heating of four ponds at once which means a maximum of 12 ponds per season could be heated at $162 per pond. The flow rate produced by the well allows 12 ponds to be heated at once. If the total pump capacity was used to heat ponds, a total of

36 ponds could be heated per season at a cost of $54 per pond. This assumes no measure being taken to prevent evaporative and convective heat loss. Covering the ponds could provide a cost effective means to reduce heating costs. The revenue from fry production per pond can be calculated and compared to the costs of production. The assumptions used for the revenue calculations per pond were: 20 to 30 females per pond, 50 % spawning rate for females, 5000 eggs per kg bodyweight per female, average weight of 2 kg per female, 50% fertilization rate, 50% hatch rate, and $0.02 per fry. The revenue per pond of 20 females would amount to $600. Assuming the heating costs of $54 per pond this would leave net revenue of $546 per pond. By extending the spawning season, the current hatchery facilities could process the fry production of 36 ponds over 14 weeks compared to only 12 ponds over 10 weeks. Heating of the ponds could allow for revenue of $19650 compared to $7200 for 12 ponds during the regular spawning season. The revenue would increase with the production of hybrid catfish fry that have an expected sale price of $0.03 per fry.

Diesel fuel is an alternative energy source that could be used to distribute geothermal water on a farm. The comparison of the cost of diesel fuel and electricity requires knowledge of the fuel consumption of pumps with similar capacity. The pump used in this case is a 53 kW diesel pump (CD 150M Godwin Pumps, Bridgeport, New Jersey) that consumes 14.4 L of fuel per hour of operation. The average price of diesel fuel in the gulf south region of the United States was $ 0.48 per L in August 2004 (DOE). The total pumping costs for a season would total $3,432 if the pump was powered by electricity and $6,388 if it was powered by diesel fuel (Table 6-2). The use of diesel fuel

instead of electricity to power pumps for geothermal water distribution is almost twice (1.86 times) as costly.

Determination of the total amount of energy required per heating trial includes the energy requirements for increasing the pond temperatures to 27°C (54×10^{10} J) and maintaining it there (7.6×10^{10}J) for 40 days and equals 61.6×10^{10} J (17.1×10^{4} kWh) per pond in January. Energy is usually a costly commodity and should be used as efficiently as possible to be commercially viable. The average household energy use in the USA is 3×10^{4} kWh per year. The amount of energy required to heat one pond during January could provide the energy requirement for at least 5 average households for a year.

Methods need to be developed for more efficient use of the geothermal energy. Methods that could reduce energy losses from the pond include greenhouse covers for the ponds, installation of a windbreak and the use of a thermal pump to store excess energy (Lamoureux 2003). Another option could be the recirculation or multiple use of geothermal water. Geothermal water exiting ponds at lower temperatures can be redirected to other broodstock ponds where the females accumulate degree-days at slower rates and will reach spawning condition at a later time than the females in the initially heated ponds. Future research should focus on the use of the pond overflow to condition broodstock at lower water temperatures, determine the lower temperature threshold for channel catfish spawning, and accumulation of degree-days below the spawning threshold. Additional research is required to commercialize the production of hybrid catfish.

Table 6-2: Comparison of costs incurred for pumping of geothermal water using identical 53 kW pumps powered by electricity and diesel fuel for pond heating before the start of natural spawning during an average year.

	Electricity					Diesel fuel				
	January	February	March	April	Season	January	February	March	April	Season
Weeks of heating	4	4	4	2	14	4	4	4	2	14
Average pumping time (h/wk)	12	10	8	6	9	12	10	8	6	9
Average pumping cost ($/wk)	312	260	208	156	245	581	484	387	290	456
Total pumping cost ($)	1248	1040	832	312	3432	2324	1936	1548	580	6388

The extension of the spawning season and the availability of more females for egg production could help accelerate research efforts and commercial application. These techniques also allow improved planning of resource use and labor on a commercial farm because of the ability to schedule catfish spawning in advance. Additional benefits of these techniques include the stabilized production of seedstock and continuity in the production of foodfish, which all lead to increased overall production capacity for commercial catfish producers.

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APPENDIX A – STANDARD OPERATING PROCEDURES

The standard operating procedures (SOP) included below originated from previous research conducted at the Louisiana State University Agricultural Center Aquaculture Research Station. SOP's one through eight have been adapted from Lang 2001 and Bates 1997. SOP's nine through thirteen have been adapted from protocols developed for research use at the Louisiana State University Agricultural Center Aquaculture by Dr. Charles R. Weirich and Dr. Robert P. Romaire in 2000.

Lang, R. P. 2001. Induction of early out-of-season spawning in channel catfish *Ictalurus punctatus*. Masters thesis. Louisiana State University

Bates, M.C. 1997. Production of transgenic channel catfish. Dissertation, Louisiana State University.

SOP-1. Hanks' balanced salt solution (HBSS)

Hanks' balanced salt solution is used as a collection medium for the eggs during artificial spawning. The HBSS should be prepared with an osmolality of 290 to 300 mOsmol/Kg to prevent activation of the eggs.

Table A-1. Ingredients for Hanks' balanced salt solution

Ingredient	g/L	Molarity
NaCl	8.0	0.14
KCl	0.4	0.005
$CaCl_2 \cdot 2H_2O$	0.16	0.001
$MgSO_4 \cdot 7H_2O$	0.20	0.001
Na_2HPO_4	0.06	0.0004
KH_2PO_4	0.06	0.0004
$NaHCO_3$	0.35	0.004
$C_6H_{12}O_6$ (glucose)	1.00	0.006

SOP-2. Hydration and injection of synthetic leuteinizing-hormone releasing-hormone (LH-RH).

D-Ala6DesGly10 LH-RH-ethylamide (synthetic leuteinizing-hormone releasing-hormone) in freeze dried powder form is used to induce ovulation in female channel catfish.

1) Dissolve the LH-RH powder using distilled deionized bacteriostatic water to yield a final concentration of 1mg / mL.

2) Administer the hormone by intraperitoneal injection behind the pectoral fin at a dose of 100 μg LH-RH / Kg bodyweight.

SOP-3. Artificial tank spawning method

Induce artificial spawning by injecting female channel catfish with 100 μg per kg bodyweight and pairing them with males and females of similar size. Do not inject the males.

1) Anesthetize the female with MS-222 and measure weight and length before injection with the hormone.

2) Clip barbells for the identification of fish and place her in a spawning tank. Revive the fish by flushing the gills with aerated water. Inject all females before processing males.

3) Males should be anesthetized, weighed and measured, and placed in a spawning tank with a female of similar weight and length while sedated.

SOP-4. Spawning behavior observation.

The lights in the laboratory remain off during the spawning trials. Use a hand-held light when checking on the fish. After injection of LH-RH monitor the fish every 2 h and record spawning behavior observations according to the spawning behavior chart (Table A-2). Spawning usually begins 24-36 h after injection but can occur at any time and may take up to four days. Fish may spawn during the day or night.

Table A-2: Behavioral index for spawning of channel catfish

Stage		Description
0	(inactive)	Both fish laying on the bottom; some swimming
1	(beginning spawning behavior)	Active swimming; biting or evidence of biting; nudging one another
2	(active spawning behavior)	Assumption of head-to-tail position
3	(intense spawning behavior)	Vibrating of body in head-to-tail position; no egg release
4	(start of spawning)	Eggs released

SOP-5. Hand-stripping of eggs from female channel catfish

1) Anesthetize the fish with MS-222. Try to minimize release of eggs by holding the fish so the genital port faces upward during transport.

1) Remove fish from anesthetic, and gently pat the fish dry using paper towels to avoid activation of the eggs with water.

2) Rinse hands with HBSS. Hold the caudal peduncle of the fish with the weaker arm and support the body of the fish with the fore arm of the stronger arm. Apply gentle pressure upwards and to the oviduct. At no time should excessive pressure be applied as this can damage the ovaries and obstruct the oviduct.

4) Collect eggs into a greased bowl containing 5cm of HBSS. The bowl is greased to prevent the eggs from sticking to the bowl and the HBSS prevents activation of the eggs by contamination with water. Eggs should remain covered with HBSS at all times. If blood appears when stripping eggs, remove the liquid and replace with fresh HBSS. Remove any blood clots and ovarian tissue from the bowl.

5) When stripping is complete, revive the fish and note the color, size, and shape of the eggs.

SOP-6. Collection and incubation of channel catfish egg masses from pond spawning containers.

1) Turn off the aerator and disconnect it fro the power box.

2) Carefully locate the spawning container and kick it to encourage the male to leave the container. Males guarding the nest and may get trapped in the container and can damage the egg mass.

3) Lift container such that the opening faces upward. Slowly drain the water from the container pivoting it over a knee for support. Periodically look inside the container to locate the mass.

4) Gently scrape the mass from the wall of the container. Place the mass into a 4-L cooler with pond water cooler and close the lid as sunlight can damage the eggs. Use a portable Bubble-box aerator (Bubble Box ™ B-11, Marine Metal Products, Clearwater, Florida) to aerate the water in the cooler.

5) Transport the egg mass to the hatchery within 30 min of collection and acclimated them for 15 min to hatchery water temperatures.

6) Weigh the egg mass and disinfect it by dipping in a 10% Iodine solution (Argentyne, Argent Chemical Laboratories, Redmond, Washington) for 10 min.

7) Break the mass into 250 g portions and incubate them in aerated recirculating system containing water at 27 °C. Plastic baskets can be used to suspend the mass above the airstone.

8) Quantify the number of eggs present by counting the number of eggs in 3 weighed samples of the mass.

9) Percent fertilization is estimated by examining 6 random portions of the mass for unfertilized eggs.

SOP-7. Estimation of fertilization success of eggs collected through artificial spawning

1) Pour approximately 5-mL of eggs into a 450-mL greased beaker to form a monolayer of eggs.

2) Add 0.5-mL of sperm to the eggs and activate the sperm by adding a layer of tank water to cover the eggs.

3) Wait 5 to 10 min to add more water and allow the eggs to harden and form an egg mass.

4) Place mass in a piece of plastic pipe with holes drilled in the side to allow water circulation. The holes and one end of the pipe should be covered with mosquito screen. These plastic incubation units should be placed in a basket that is suspended off the bottom of the hatching trough.

5) At 28 h following activation examine the mass for the presence of unfertilized eggs. They will appear white or clear and are often swollen. Count the number of unfertilized eggs.

6) Count the number of neurulated eggs in the same sample. The neurulated eggs can be identified by the formation of the neural tube. The neural tube becomes visible when illuminating the egg monolayer with a hand held light from the bottom of the plastic container. Estimate the percentage of fertilization based on the number of unfertilized eggs and neurulated eggs.

SOP-8. Determination of sex in channel catfish broodstock

The urogenital morphology is the most reliable method of determining the sex of channel catfish.

1) Lay the fish on its back and locate the anal pore which is the largest opening and closest to the anal fin.

2) Insert a plastic micropipette tip or an object of similar dimensions into the posterior portion of the papillary structure. It will enter at a 45 degree angle. This opening is the urinary pore.

3) Determine if an opening exists between this opening and the anal pore. If an opening exists that allows penetration of the probe at either 45 or 90 degrees it is the genital pore and the fish is a female. Males will lack this third opening.

SOP-9. Preparation of ponds for the rearing of fry

1) Drain the pond completely. If puddles remain, add household bleach to kill predaceous fish and insects. Use between 1 and 2 gallons of bleach per 0.04-ha pond depending on size of puddles. Let chlorine remain in pond for 24 hours later.

2) Fill the pond with well water. While flooding pond add about 200 mL liquid inorganic fertilizer (11-37-0) every other day until phytoplankton bloom develops or until fry are stocked. Add 8 kg of cotton seed meal at filling, and thereafter add 4.5 kg of catfish starter, floating pellets, or cottonseed meal per pond every other day for 2 to 3 weeks as organic fertilizer to promote zooplankton production.

SOP-10. Control of predacious insects

1) Apply 4 to 6 L of a mixture of diesel fuel and motor oil (10:1), two days before placing the fry in the pond. Apply the mixture along the margin of the upwind side of the pond. Turn the aerator off before and during the application. Wait 24-hrs before stocking.

2) Add another 4 to 6 L of the diesel fuel and motor oil mixture 3 to 4 d after stocking to prevent the return of predaceous insects. Repeat the diesel: motor oil application two more times at 3 to 4 day intervals. The ponds should be stocked with fry within 2 weeks after they were filled to prevent significant insect predation.

SOP-11. Acclimation and stocking of fry

The ponds should be stocked a rate of 500,000 fry/ha preferably in the morning or afternoon.

1) Place the fry in a container with proper aeration and temperature and acclimate them by gradually adding pond water to the container. The rate of temperature change should not exceed 2°C per 15 min until the temperatures of pond and container are within 1°C. Failure to acclimate the fry slowly and properly will result in high fry mortality

2) When the fry are acclimated, release them along the pond margin.

SOP-12. Feeding of recently stocked fry

1) Add 2 kg of catfish starter feed per pond immediately after stocking once daily until the fry swim up. The starter feed is serving as organic fertilizer.

2) Continue feeding after the application of the diesel fuel motor oil mixture. Add feed where the oil slick has dissipated and mix the feed with water before application so that it doesn't float and become contaminated with oil.

SOP-13. Feeding of fingerlings

1) Observe the ponds daily for a month after stocking to see if the fry are swimming up to feed. Add catfish starter feed for the first 5 days after swim-up fry are first observed.

2) Gradually mix the catfish starter with 36% protein feed (BB) pellets in a 1:1 ratio. Switch to the complete pellet diet at 2 weeks after first feeding.

3) After 3 weeks of feeding on the BB pellets, start feeding the slightly larger pellet (35% protein) and eventually a 32% protein pellet as fingerlings increase in size.

APPENDIX B - ESTIMATES OF GEOTHERMAL WATER USE

At the Louisiana State University Agriculture Center (LSUAC) Aquaculture Research Station (ARS), the out-of-season spawning of Channel Catfish (*Ictalurus punctatus*) is a central research theme. Early-season catfish spawning is induced by heating the water in spawning ponds to optimum spawning temperatures (21-27 ºC) before they naturally occur in the late spring and early summer months. The ponds are heated by addition of geothermal water (36 ºC) from a 700-m deep well that produces a flow rate of 2400-L per minute. The pump that distributes the geothermal water to the ponds has a 30 kW capacity. The well usually runs between ten to sixteen hours per day from mid February to mid April or a total of 60 d. The total pumping costs at 600 to 960 h per season at $ 0.07 per kWh amounts to between $1260 and $2016 per season. The economic feasibility of out-of-season catfish spawning will depend for a large part on the efficiency of pond heating. This study investigated the efficiency of the use of geothermal water at the LSUAC.

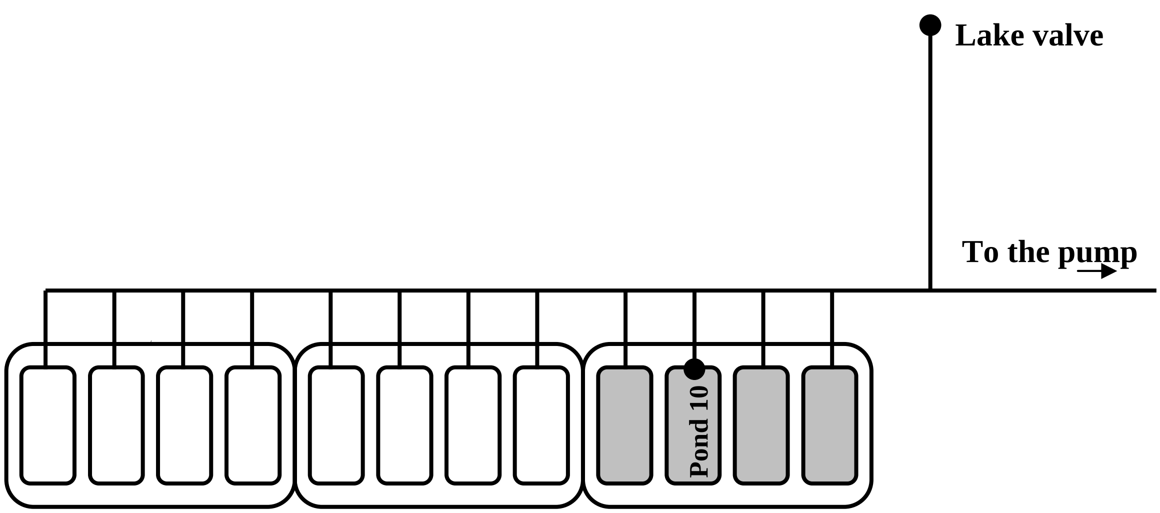


Figure B-1. Schematic of the warm water ponds water distribution system

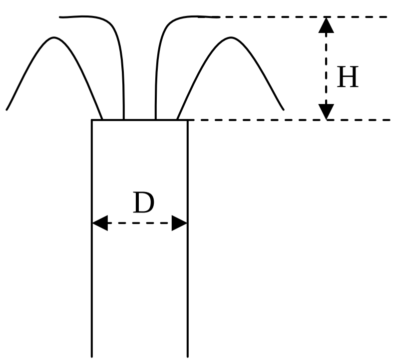
The study intended to determine the quantity of the geothermal water pumped from the well that is actually being used to heat the ponds in certain situations. In the current situation, a certain amount of the water distributed by the pump of the geothermal well is directed into a lake to relieve pressure on the water distribution system of the ponds. These situations are defined as a typical night at the beginning of the early spawning season or “early” night (02/25/02) and a typical night at the end of the early spawning season or “late” night (03/20/02). The flow rate at the lake valve was estimated using the flow rate measured at the pond and Bernoulli’s equation.

Equation 1:

$Z1 + V1^2/2g = Z2 + V2^2/2g$ (Bernoulli’s equation)

Z = elevation V = water velocity

The flow rate of geothermal water entering the pond was estimated using the vertical trajectory method. The flow rate entering one pond with all the valves to all other ponds closed and the valve to the lake open was 15.7 m^3 per min.



D = 14.4 cm H = 27.5 cm

Equation 2: $Q = 5.01D^{1.99}*H^{1.35}$

$Q = 5.01*5.75^{1.99}*11^{1.35}$

Figure B-2. Illustration of the vertical trajectory method used to determine the flow rate at the valve of pond 10

To use Bernoulli's equation, the flow rate (Q) was converted to water velocity (V)

Equation 3: Q = V*A   A = 167.4 cm^2 Q = 15.7 m^3 per min.

The water velocity at the pond was 15.6m/s

Another variable that were measured in order to use Bernoulli's equation was the difference in elevation between the valve at the pond and the valve at the lake. The valve at the lake measured a relative elevation of 39 cm compared to the elevation of the pond which measured 110 cm thus a difference in elevation of 71 cm. Using equation 1 the water velocity at the lake valve was 15.1 m/s. Using equation 3 the flow rate at the lake was calculated to be 15.2 m^3/min.

Table B-1. Time the valve was open and the quantity of water delivered for the valve at the lake and the reference valve at pond 10.

	Ponds		Lake	
	Time (min)	Volume (m^3)	Time (min)	Volume (m^3)
Early (2/25/02)	83	1306	945	14360
Late (3/20/02)	46	723	600	9120

On the early night the valve at the pond was open for 83 min and on the late night the valve was open for 46 min. The amount of water being distributed to the lake on a early night is 14364 m^3 and 9120 m^3 on a late night and the amount of water that being distributed to one pond on a early night is 1306 m^3 and 723 m^3 for a late night

(Table B-1). The ponds are heated in groups of four so the total amount of water being distributed to the ponds on a early night would be 5224 m^3 and 2892 m^3 on a late night. The total amount of water being pumped on an early night amounts to 19590 m^3 and 12012 m^3 on a late night. These numbers indicate that around 75% of the water being distributed by the pump of the geothermal well is not being used to heat ponds but is dumped into the lake. One possible method to reduce this waste of valuable geothermal water is the use of reservoir ponds that can serve as temporary storage areas for unused geothermal water. More efficient use of geothermal water can improve future research involving the use of geothermal water for channel catfish spawning and be a more realistic model of the commercial application of this technology.

APPENDIX C – UNANALYZED DATA

Table C-1. Data for channel catfish egg masses collected from heated and ambient temperature ponds in 2002 and 2003 (Chapter 3).

Date	Pond number	Number of egg mass	Weight (g)	Number of Eggs	Percent Fertilization	Number of Fry	Percent hatch
Early spawning 2002							
03/15/02	11	1	900	20000	95	8000	40
	10	1	200	1500	65	1300	87
	9	1	1600	30000	90	23000	77
	9	2	930	9000	85	5900	66
03/18/02	12	1	1100	28000	95	12800	46
	11	2	580	9300	95	8600	92
	11	3	680	17900	95	11000	61
	10	2	520	8900	95	6600	74
	9	3	1270	35800	95	16500	46
	9	4	1660	24500	95	---	---
03/21/02	12	2	1430	47000	95	21000	45
	10	3	1360	22700	95	6600	29
	9	5	1680	36200	95	7600	21
03/24/02	11	4	1400	37000	90	20300	55
	11	5	850	21500	95	11770	55
	10	4	2280	32000	90	17500	55
03/27/02	10	5	490	8600	95	1800	21
	9	6	2690	38000	90	5000	13
04/12/02	8	1	900	13900	85	1400	10
04/15/02	8	2	1580	35000	90	23000	66
Natural spawning 2002							
04/18/02	8	3	1580	44000	90	9000	20

	8	4	810	22000	90	3600	16
	4	1	1570	49000	85	1900	4
	4	2	1350	39000	95	6500	17
04/22/02	8	5	960	25800	95	---	---
	8	6	1550	38500	90	500	1
	8	7	780	23500	80	4500	19
	7	1	910	21000	90	270	1
	5	1	1120	26500	95	140	1
	4	3	760	23500	85	1100	5
04/26/02	8	8	770	45500	90	12600	28
	8	9	1250	29500	95	8900	30
	7	2	1200	27000	95	5600	21
	5	2	760	20000	85	8000	40
	4	4	900	20500	85	9500	46
	3	1	320	9500	85	630	7
04/30/02	8	10	1150	29000	90	1200	4
	8	11	1390	30500	95	---	---
	7	3	55	29000	75	500	2
	5	3	1100	29000	80	---	---
	5	4	1260	---	95	---	---
	4	5	1400	36500	85	---	---
	3	2	570	14500	75	---	---
	3	3	1200	34000	85	---	---
05/03/02	5	5	1980	45000	85	2800	6
	4	6	870	16000	95	9000	56
	4	7	1000	24000	95	2500	10
	4	8	800	17500	80	---	---
	3	4	1280	30500	95	2500	8
05/07/02	3	5	40	900	70	---	---
	3	6	240	7000	80	---	---
	3	7	680	19000	85	8500	45

	3	8	750	29000	90	5000	17
	4	9	1560	47000	85	2500	5
	4	10	1150	31000	85	6000	19
	7	4	940	20000	80	---	---
05/10/02	7	5	1080	28000	80	---	---
05/14/02	3	9	910	21500	70	80	0
05/22/02	1	1	1400	42500	90	10000	24
05/25/02	4	11	380	11000	95	2500	23
	3	10	600	14700	85	7500	51
05/28/02	1	2	1110	34000	85	---	---
05/31/02	2	1	970	21500	80	---	---
06/05/02	3	11	700	9000	85	---	---
06/08/02	5	6	750	17000	90	---	---
Early spawning 2003							
03/10/03	9	1	1660	22000	90	11500	52
	9	2	1275	27000	90	22400	83
03/13/03	10	1	1450	23400	80	22300	95
	9	3	1060	22400	80	15500	69
03/17/03	12	1	610	20100	90	18400	92
	12	2	400	6200	80	1240	20
	12	3	400	10000	90	10000	100
	10	2	970	26100	90	22100	85
	10	3	600	16000	90	2400	15
	9	4	630	16000	95	4200	26
	9	5	975	25000	90	18200	73
03/20/03	12	4	1760	10600	70	7400	70
	12	5	550	15200	85	9800	64
	10	4	1070	14600	85	8600	59
	10	5	730	19100	90	11500	60
	9	6	1210	15400	85	7500	49

	9	7	775	14000	85	400	3
	9	8	1270	20300	85	16000	79
	9	9	875	21600	85	8700	40
	9	10	1110	26700	90	12700	48
03/25/03	12	6	620	11900	70	8100	68
	10	6	830	25500	90	23300	91
	10	7	640	21000	95	19000	90
	10	8	910	20000	90	6900	35
03/28/03	12	7	775	20700	85	7520	36
	12	8	1300	16200	90	7700	48
	9	11	600	28400	85	25300	89
	9	12	685	18800	85	13100	70
	8	1	40	---	---	---	---
04/02/03	12	9	820	26000	85	10700	41
04/08/03	12	10	1010	16800	80	10700	64
	12	11	860	18200	90	10200	56
	8	2	500	7650	85	3100	41
04/10/03	8	3	2960	47000	75		
	8	4	590	14000	85	6000	43
04/15/03	8	5	595	12200	90	8000	66
	8	6	750	17000	90	10600	62
	8	7	---	---	---	14350	68
	7	1	915	23200	85	6800	38
04/18/03	12	12	50	1100	95	---	---
	8	8	775	19000	80	7200	19
	7	2	1130	18000	85	3500	53
	7	3	1240	23900	85	1050	---
	7	4	830	1900	75	1000	---
	6	1	520	10300	80	2050	---
Natural spawning 2003							

04/23/03	8	9	715	13600	75	9100	67
	8	10	485	13000	85	1500	12
	7	5	365	10300	90	9400	91
	6	2	350	6930	95	6750	97
	6	3	460	12400	85	6200	50
	4	1	1000	28000	95	3500	13
	4	2	835	27000	85	13100	49
	4	3	---	---	---	---	---
	3	1	250	6000	85	2700	45
04/28/03	7	6	550	---	85	---	---
	7	7	800	---	85	---	---
	6	4	715	20000	95	4300	22
	4	3	930		80	---	---
	4	4	860	24000	90	1100	5
	3	2	745	20000	90	600	3
	3	3	710	---	90	15350	---
05/02/03	12	13	555	---	85	---	---
	6	5	720	---	85	---	---
	6	6	945	16400	80	15100	92
	6	7	475	12700	90	500	4
	4	5	345	---	90	---	---
	4	6	535	14700	80	5800	39
	4	7	385	10000	85	2200	22
	4	8	690	15900	90	5800	36
	3	4	600	18800	95	17600	94
	3	5	1080	30100	80	29000	96
	3	6	630	18500	95	10000	54
	3	7	690	18800	90	2400	13
05/07/03	7	8	770	---	---	---	---
	3	8	590	---	85	---	---
	3	9	740	---	75	---	---
	3	10	730	20000	85	11500	58

05/12/03	4	9	550	18000	90	7600	42
	4	10	660	17200	85	2800	16
	3	11	630	13800	80	---	---
06/06/03	8	11	---	---	---	---	---
	3	11	---	---	---	---	---
06/18/03	8	12	---	---	---	---	---
	8	13	---	---	---	---	---
	8	14	---	---	---	---	---
	3	12	---	---	---	---	---

Table C-2. Degree-day values calculated for channel catfish spawns collected from heated and ambient temperature ponds during 2000 and 2004 above three threshold temperatures (Chapter3).

Year	Pond	Number of spawns	Threshold 18°C (°D)	Threshold 21°C (°D)	Threshold 24°C (°D)	Heating regime
2004	6	2	153	45	1	ambient
2004	6		153	45	1	ambient
2004	6	2	189	65	7	ambient
2004	6		189	65	7	ambient
2004	6	2	236	83	8	ambient
2004	6		236	83	8	ambient
2004	6	2	272	107	20	ambient
2004	6		272	107	20	ambient
2004	8	1	199	68	9	ambient
2004	8	2	217	77	9	ambient
2004	8		217	77	9	ambient
2004	2	1	294	151	55	heated
2004	2	3	316	164	59	heated
2004	2		316	164	59	heated
2004	2		316	164	59	heated

2004	2	1	362	189	68	heated
2004	3	1	197	87	21	heated
2004	3	1	221	102	28	heated
2004	3	2	283	140	42	heated
2004	3		283	140	42	heated
2004	3	3	298	148	44	heated
2004	3		298	148	44	heated
2004	3		298	148	44	heated
2004	4	3	314	132	21	heated
2004	4		314	132	21	heated
2004	4		314	132	21	heated
2004	10	2	225	128	54	heated
2004	10		225	128	54	heated
2004	10	3	253	148	65	heated
2004	10		253	148	65	heated
2004	10		253	148	65	heated
2004	10	4	308	182	81	heated
2004	10		308	182	81	heated
2004	10		308	182	81	heated
2004	10		308	182	81	heated
2004	11	1	134	61	14	heated
2004	11	2	185	88	19	heated
2004	11		185	88	19	heated
2004	11	2	215	107	25	heated
2004	11		215	107	25	heated
2004	12	1	171	63	3	heated
2004	12	1	192	73	3	heated
2004	12	2	231	93	7	heated
2004	12		231	93	7	heated
2004	12	1	253	107	11	heated
2004	12	2	280	118	13	heated

2004	12		280	118	13	heated
2004	12	1	306	135	22	heated
2004	12	2	329	149	27	heated
2004	12		329	149	27	heated
2003	3	1	175	53	5	ambient
2003	3	2	208	71	8	ambient
2003	3		208	71	8	ambient
2003	3	4	240	90	16	ambient
2003	3		240	90	16	ambient
2003	3		240	90	16	ambient
2003	3		240	90	16	ambient
2003	3	3	288	123	33	ambient
2003	3		288	123	33	ambient
2003	3		288	123	33	ambient
2003	3	1	336	157	52	ambient
2003	4	3	175	53	5	ambient
2003	4		175	53	5	ambient
2003	4		175	53	5	ambient
2003	4	2	208	71	8	ambient
2003	4		208	71	8	ambient
2003	4	4	240	90	16	ambient
2003	4		240	90	16	ambient
2003	4		240	90	16	ambient
2003	4		240	90	16	ambient
2003	4	2	336	157	52	ambient
2003	4		336	157	52	ambient
2003	6	1	189	75	29	heated
2003	6	2	233	103	43	heated
2003	6		233	103	43	heated
2003	6	1	267	123	49	heated
2003	6	3	300	143	57	heated

2003	6		300	143	57	heated
2003	6		300	143	57	heated
2003	7	1	169	63	25	heated
2003	7	3	199	84	37	heated
2003	7		199	84	37	heated
2003	7		199	84	37	heated
2003	7	1	244	114	51	heated
2003	7	2	278	133	57	heated
2003	7		278	133	57	heated
2003	7	1	358	186	83	heated
2003	8	1	141	49	17	heated
2003	8	1	211	86	23	heated
2003	8	2	224	93	25	heated
2003	8		224	93	25	heated
2003	8	3	273	127	43	heated
2003	8		273	127	43	heated
2003	8		273	127	43	heated
2003	8	1	302	147	54	heated
2003	8	1	347	177	69	heated
2003	9	2	172	99	32	heated
2003	9		172	99	32	heated
2003	9	1	198	115	39	heated
2003	9	2	231	137	49	heated
2003	9		231	137	49	heated
2003	9	5	263	156	57	heated
2003	9		263	156	57	heated
2003	9		263	156	57	heated
2003	9		263	156	57	heated
2003	9		263	156	57	heated
2003	9	1	291	164	57	heated
2003	10	1	157	78	23	heated

2003	10	2	191	100	33	heated
2003	10		191	100	33	heated
2003	10	2	226	123	44	heated
2003	10		226	123	44	heated
2003	10	3	244	129	44	heated
2003	10		244	129	44	heated
2003	10		244	129	44	heated
2003	12	3	228	134	49	heated
2003	12		228	134	49	heated
2003	12		228	134	49	heated
2003	12	2	263	157	60	heated
2003	12		263	157	60	heated
2003	12	1	282	164	60	heated
2003	12	2	292	165	60	heated
2003	12		292	165	60	heated
2003	12	1	325	177	60	heated
2003	12	1	382	209	69	heated
2003	12	1	485	269	89	heated
2002	3	1	264	138	57	ambient
2002	3	2	303	165	72	ambient
2002	3		303	165	72	ambient
2002	3	2	332	185	84	ambient
2002	3		332	185	84	ambient
2002	3	3	373	214	101	ambient
2002	3		373	214	101	ambient
2002	3		373	214	101	ambient
2002	3	1	430	250	116	ambient
2002	4	2	185	83	27	ambient
2002	4		185	83	27	ambient
2002	4	1	223	109	41	ambient
2002	4	1	261	135	55	ambient

2002	4	1	300	162	70	ambient
2002	4	3	329	182	81	ambient
2002	4		329	182	81	ambient
2002	4		329	182	81	ambient
2002	4	2	373	214	101	ambient
2002	4		373	214	101	ambient
2002	7	1	216	104	38	heated
2002	7	1	253	128	51	heated
2002	7	1	291	154	65	heated
2002	7	1	360	202	92	heated
2002	7	1	388	221	102	heated
2002	8	1	115	34	1	heated
2002	8	1	139	49	8	heated
2002	8	2	167	68	17	heated
2002	8		167	68	17	heated
2002	8	3	206	94	32	heated
2002	8		206	94	32	heated
2002	8		206	94	32	heated
2002	8	2	244	120	46	heated
2002	8		244	120	46	heated
2002	8	2	282	147	60	heated
2002	8		282	147	60	heated
2002	9	2	173	102	40	heated
2002	9		173	102	40	heated
2002	9	2	199	118	48	heated
2002	9		199	118	48	heated
2002	9	1	221	129	51	heated
2002	9	1	250	143	53	heated
2002	10	1	145	79	29	heated
2002	10	1	171	97	37	heated
2002	10	1	195	111	43	heated

2002	10	1	220	127	49	heated
2002	10	1	245	144	57	heated
2002	11	1	150	80	28	heated
2002	11	2	175	97	35	heated
2002	11		175	97	35	heated
2002	11	2	223	126	47	heated
2002	11		223	126	47	heated
2002	12	1	164	90	32	heated
2002	12	1	183	100	34	heated
2001	1	2	199	94	33	ambient
2001	1		199	94	33	ambient
2001	1	2	230	110	35	ambient
2001	1		230	110	35	ambient
2001	1	1	252	123	39	ambient
2001	1	2	275	137	45	ambient
2001	1		275	137	45	ambient
2001	5	1	204	114	46	heated
2001	5	1	229	129	52	heated
2001	5	2	256	148	62	heated
2001	5		256	148	62	heated
2001	5	1	305	182	80	heated
2001	5	2	331	198	88	heated
2001	5		331	198	88	heated
2001	9	2	101	57	17	heated
2001	9		101	57	17	heated
2001	9	3	142	83	28	heated
2001	9		142	83	28	heated
2001	9		142	83	28	heated
2001	9	2	203	123	47	heated
2001	9		203	123	47	heated
2001	9	2	370	227	92	heated

2001	9		370	227	92	heated
2001	9	2	432	268	112	heated
2001	9		432	268	112	heated
2001	9	1	459	287	121	heated
2001	9	1	487	302	126	heated
2001	9	1	561	343	141	heated
2000	2	2	223	85	29	heated
2000	2		223	85	29	heated
2000	2	2	255	108	42	heated
2000	2		255	108	42	heated
2000	3	1	130	48	17	heated
2000	3	2	219	109	51	heated
2000	3		219	109	51	heated
2000	3	3	267	140	64	heated
2000	3		267	140	64	heated
2000	3		267	140	64	heated
2000	3	1	343	173	75	heated
2000	3	1	367	188	81	heated
2000	4	1	166	47	8	heated
2000	4	1	190	63	15	heated
2000	4	1	352	168	63	heated
2000	7	1	130	48	17	heated
2000	7	2	157	65	25	heated
2000	7		157	65	25	heated
2000	7	2	188	87	39	heated
2000	7		188	87	39	heated
2000	10	1	119	71	28	heated
2000	10	1	148	90	38	heated
2000	10	2	202	126	57	heated
2000	10		202	126	57	heated
2000	10	3	231	146	68	heated

2000	10		231	146	68	heated
2000	10		231	146	68	heated
2000	10	1	240	152	71	heated
2000	10	1	272	172	79	heated
2000	10	1	299	190	87	heated
2000	10	2	435	281	134	heated
2000	10		435	281	134	heated
2000	11	1	80	44	17	heated
2000	11	1	103	59	22	heated
2000	11	2	129	75	29	heated
2000	11		129	75	29	heated

Table C-3. Data for channel catfish females used in six artificial spawning trials as male-female and female-female pairs during 2002 and 2003 (Chapter 4).

Female	Weight (kg)	Length (cm)	Latency (h)	Volume (ml)	Fertilization (%)	Pair	Trial	Year
CCF03F02	1.7	53	62.3	230	62	ff	early	2003
CCF03F03	1.8	59	75.5	40	40	ff	early	2003
CCF03F04	1.8	57	50.3	300	41	mf	early	2003
CCF03F05	2.2	61	53.0	400	36	mf	early	2003
CCF03F06	1.9	56	60.8	225	74	mf	early	2003
CCF03F07	2.2	61	66.5	150	84	ff	early	2003
CCF03F08	2.6	60	81.3	125	70	mf	early	2003
CCF03F12	1.6	52	66.5	350	66	ff	early	2003
CCF03F14	1.9	56	52.6	225	0	ff	middle	2003
CCF03F16	2.5	61	53.2	225	93	ff	middle	2003
CCF03F17	2.4	62	41.0	300	90	mf	middle	2003
CCF03F18	2.0	57	33.4	260	73	mf	middle	2003
CCF03F19	2.3	62	42.1	280	80	ff	middle	2003
CCF03F20	1.8	55	34.4	325	79	mf	middle	2003
CCF03F21	2.1	58	51.0	375	63	ff	middle	2003
CCF03F22	2.5	61	41.2	250	48	ff	middle	2003
CCF03F23	2.1	58	53.3	200	8	ff	middle	2003
CCF03F25	1.9	56	35.0	175	75	ff	middle	2003
CCF03F26	2.3	60	52.4	300	0	ff	middle	2003
CCF03F27	2.3	61	38.8	250	83	mf	middle	2003
CCF03F29	2.1	59	35.3	200	87	ff	late	2003
CCF03F31	2.9	66	49.5	375	0	ff	late	2003
CCF03F32	3.1	68	30.2	250	45	ff	late	2003
CCF03F34	2.1	58	35.1	250	67	mf	late	2003
CCF03F35	1.9	58	45.9	275	0	ff	late	2003
CCF03F36	1.9	55	45.3	40	70	mf	late	2003

CCF03F37	1.9	58	46.4	150	90	mf	late	2003
CCF03F38	2.8	67	35.6	300	50	ff	late	2003
CCF03F40	2.1	57	35.3	150	70	mf	late	2003
CCF02F01	3.7	63	38.9	400	85	mf	early	2002
CCF02F02	3.6	64	37.8	400	80	ff	early	2002
CCF02F04	3.1	65	33.6	200	70	mf	early	2002
CCF02F10	3.1	58	43.3	200	0	ff	early	2002
CCF02F12	3.5	67	40.2	350	18	ff	middle	2002
CCF02F14	3.7	66	41.6	265	40	ff	middle	2002
CCF02F15	3.3	62	42.5	150	80	ff	middle	2002
CCF02F16	3.3	63	49.3	250	0	ff	middle	2002
CCF02F17	3.7	66	34.8	380	75	ff	middle	2002
CCF02F18	2.6	58	31.4	60	35	ff	middle	2002
CCF02F20	2.9	64	54.9	110	0	ff	late	2002
CCF02F21	2.8	60	50.4	90	0	ff	late	2002

Table C-4. Data for spawning behavior of channel catfish broodstock in male-female and female-female pairs for first trial during the 2003 spawning season (Chapter 4).

	Trial 1 04/02/03							
Time after injection (h)	mf	mf	mf	mf	ff	ff	ff	ff
6	0	1	0	0	0	1	0	1
8	0	1	1	0	0	1	1	1
10	1	1	0	0	1	2	1	1
12	0	1	0	0	0	2	0	1
14	0	1	1	0	1	2	1	1
16	1	1	0	0	1	2	1	1

18	1	1	1	0	1	2	1	1
20	1	1	1	0	1	1	1	1
22	1	1	1	0	1	1	1	1
24	1	1	1	0	1	1	1	1
26	1	1	1	0	1	0	1	1
28	1	1	1	0	1	0	1	0
30	1	0	1	0	1	1	1	0
32	1	0	1	0	1	0	1	0
34	0	0	1	0	1	0	1	0
36	0	1	1	0	1	0	0	1
38	2	0	1	0	1	0	0	1
40	1	1	1	0	1	1	1	0
42	2	3	1	0	0	0	0	0
44	1	0	0	0	0	2	0	0
46	1	1	0	0	2	2	0	0
48	0	1	0	0	2	1	0	0
50	0	2	0	0	4	2	0	0
52	0	3	1	0		3	1	1
54	0	3	1	0		4	0	0
56	0	3	1	0			0	0
58	0	3	1	0			0	1
60	0	3	0	0			3	0
62	0	4	0	0			3	3
64	2		2	0			4	3
66	3		1	0				4
	Tank spawn 1 stripped spawns	Tank spawn 2 stripped spawns	Tank spawn 2 stripped spawns	No tank spawn 0 stripped spawns	Tank spawn 1 stripped spawn	Tank spawn 1 stripped spawn	Tank spawn 1 stripped spawn	Tank spawn 1 stripped spawn

Table C-5. Data for spawning behavior of channel catfish broodstock in male-female and female-female pairs for second trial during the 2003 spawning season (Chapter 4).

	Trial 2	5/12/2003						
Time after injection (h)	mf	mf	mf	mf	ff	ff	ff	ff
2	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0
12	0	1	1	0	0	0	0	0
14	0	0	0	0	0	0	0	0
16	2	3	0	0	0	1	0	1
18	1	0	1	1	0	0	0	1
20	0	1	0	1	0	0	0	0
22	0	0	0	0	0	0	0	1
24	1	1	2	0	1	1	0	1
26	0	0	1	1	0	3	0	3
28	1	1	0	0	0	4	1	4
30	3	0		3	1		2	0
32	4	1		2	3		2	0
34		4		3	4		4	2
36				4				3
38								3
40								4
	Tank spawn 2 stripped spawns	Tank spawn 2 stripped spawns	No tank spawn 2 stripped spawns	Tank spawn 2 stripped spawns	Tank spawn 1 stripped spawn	Tank spawn 1 stripped spawn	Tank spawn 1 stripped spawn	Tank spawn 1 stripped spawn

Table C-6. Data for spawning behavior of channel catfish broodstock in male-female and female-female pairs for third trial during the 2003 spawning season (Chapter 4).

Time after injection (h)	Trial 3			6/30/2003				
	ff	ff	ff	ff	mf	mf	mf	mf
2	0	1	0	0	1	0	0	1
4	0	1	0	0	1	0	0	1
6	1	0	0	1	0	0	0	1
8	1	0	0	0	1	1	0	1
10	0	0	0	1	0	1	0	1
12	1	0	1	1	0	0	1	1
14	0	1	1	1	1	1	1	1
16	1	1	1	1	1	1	1	1
18	2	1	1	1	2	1	2	2
20	1	1	1	1	2	1	2	2
22	1	1	1	1	1	1	1	2
24	1	1	1	1	1	2	2	2
26	1	1	1	2	0	2	2	2
28	1	1	3	2	0	2	1	2
30	0	1	4	2	2	0	2	2
32	1	1		2	2	2	2	2
34	1	1		2	2	2	2	2
36	1	1		2	4	2	4	2
38				2		2		2
40				2		3		3
42				2		3		3
44				2		2		3
46				4		3		3
	No tank spawn 1 stripped spawn	No tank spawn 1 stripped spawn	Tank spawn 2 stripped spawns	Tank spawn 1 stripped spawn	Tank spawn 1 stripped spawn	Tank spawn 1 stripped spawn	Tank spawn 1 stripped spawn	Tank spawn 1 stripped spawn

Table C-7. Data for channel catfish fry stocked in ponds and pools at two different stages of development, before and after the start of the natural spawning season and the yield of fingerlings harvested from these fry (Chapter 5).

Year	Pond size (ha)	Stocked	Harvested	Day stocked	Growing days	Survival (%)	Fingerling weight (g)	Growth (g/d)	Time of stocking	Type of fry
2002	0.16	80000	6400	81	181	8	41.1	0.223	B	MF
2002	0.16	80000	19200	90	207	24	24.6	0.136	BA	MF
2002	0.16	80000	14400	95	188	18	19.8	0.113	BA	MF
2002	0.16	57500	29900	107	191	52	15.8	0.088	A	MF
2003	0.16	80000	6400	99	240	8	21.7	0.091	B	SW
2003	0.16	80000	10400	105	213	13	35.0	0.167	A	SW
2003	0.04	16000	2080	85	268	13	17.0	0.063	B	SA
2003	0.04	16000	640	85	268	4	33.7	0.126	B	SW
2003	0.04	16000	960	84	271	6	30.9	0.114	B	SW
2003	0.04	16000	1760	84	295	11	132.3	0.448	B	SW
2003	0.04	16000	160	90	289	1	23.8	0.082	B	SW
2003	0.04	16000	1440	90	304	9	67.5	0.222	B	SA
2003	0.04	16000	1600	94	302	10	56.6	0.187	B	SA
2003	0.04	16000	3840	94	302	24	26.1	0.086	B	SW
2002	0.04	16000	1600	136	182	10	27.7	0.163	A	SW
2003	0.001	520	400	77	318	77	29	0.091	B	SA
2003	0.001	520	322	77	318	62	31.9	0.100	B	SA
2003	0.001	520	244	85	351	47	53.4	0.152	B	SA
2003	0.001	520	192	85	350	37	74.7	0.213	B	SA
2003	0.001	520	125	88	348	24	45.1	0.130	B	SW
2003	0.001	520	62	101	315	12	70	0.222	B	SA
2003	0.001	520	244	101	335	47	63.1	0.188	B	SA
2003	0.001	520	99	108	335	19	56.2	0.168	A	SA
2003	0.001	520	265	108	335	51	46.1	0.138	A	SA
2003	0.001	520	452	126	311	87	23	0.074	A	SA
2003	0.001	520	78	132	269	15	125.7	0.467	A	SA

2003	0.001	520	447	132	304	86	30.5	0.100	A	SA
2003	0.001	520	109	132	311	21	99.2	0.319	A	SA
2003	0.001	520	520	140	310	100	20	0.065	A	SA
2003	0.001	520	400.4	140	310	77	28.2	0.091	A	SA
2003	0.001	520	124.8	88	285	24	122.4	0.043	B	SA

VITA

Patrice Arnold Pawiroredjo was born in Paramaribo, Suriname, on December 13, 1978. He graduated from the Algemene Middelbare School, in 1996 earning his high school diploma. He attended the Anton de Kom Universiteit van Suriname from 1997 to 1998 where he majored in Agricultural Production. He transferred to Louisiana State University and Agricultural and Mechanical College, Baton Rouge, in 1998 and received the degree of Bachelor of Science in Wildlife and Fisheries in 2001. As an undergraduate he was a student worker at the Aquaculture Research Station. In 2001 he completed a research project through the Louisiana SeaGrant Undergraduate Research Opportunity Program titled "Development of a non-lethal detection method for *Streptococcus iniae* antibodies in *Tilapia* (*Oreochromis* spp.)" under the mentorship of Dr. John Hawke. He entered the master's program in the School of Renewable Natural Resources at Louisiana State University, Baton Rouge in 2001, and will receive the degree of Master of Science in Fisheries in December, 2004.