Reproduction of channel x channel, channel x blue, and blue x blue catfish as influenced by morphological characteristics and behavior

by

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Abstract

Although hybrid catfish that are produced from channel female (*Ictalurus punctatus*) and blue male (*Ictulurus furcatus*) display superior characteristics for aquaculture, hybridization successes in natural environments are low and not completely understood compared to reproduction of channel catfish. Five experimental runs were conducted under hatchery conditions to determine relationships between external appearances, body metrics, and the ratios of body metrics of channel catfish and blue catfish brooders and reproductive successes (spawning rate, 1st spawning time, number of eggs per kg female, % viable eggs, % hatching rate, % swim-up fry, and number of fry per kg female). The study was also designed to identify spawning behaviors occurring at 1, 3, 6, and 12 h prior to spawning and differences in percentages of brooders that exhibited behaviors, frequency/h/fish, and time duration of each behavior between the channel and blue brooders that spawned or did not spawn. Correlations of body metrics, behaviors, and spawning successes were also determined. Information obtained was used to determine criteria of good broodfish for reproduction and give possible explanations for the differences in spawning successes between channel x channel (C X C) and channel x blue (C X B) and (B X B). All female brooders were injected with carp pituitary (10 mg kg^{-1}) while no injections were given to males. External coloration, body metrics and their ratios were measured before randomly stocking them into aquarium system. Camera system was installed to record spawning behaviors during experimental period.

Selection of the channel brooders can be a factor affecting the spawning successes in some cases. The results from this study showed the broodfish that spawned and did not spawn exhibited some differences in terms of body coloration, body metrics and the ratios of body metrics. Channel female spawners had smaller ratios of body length-to-width and length-to-girth than the ratios of the non-spawners. Channel males that spawned had a smaller length-to-head width ratio compared to the channel males that did not spawn. In addition, the differences in body ratios also correlated with differences in spawning successes. Channel females having the ratios of length-to-width less than 5.5 and length-to-girth less than 1.9 spawned more successfully than those with the larger values. The females in this study with the similar sizes (46.5 – 62.9 cm) having the larger measures of body girths (≥ 28 cm) and widths (≥ 9 cm) had higher spawning rates than those with smaller girth and width measures. Likewise, the males spawned better when they had smaller ratios of length-to-head width (≤ 5.5) or dark “underside” coloration of head.

Spawning behaviors between brooders that spawned and those that did not spawn significantly differed. The percentages of channel males that spawned exhibited dancing and curling behaviors statistically higher than those of the non-spawners from 6 to 1h before the first eggs were observed. Additionally, the males that spawned danced and curled females more frequently than the male non-spawners. Similarly, spawning channel females had a greater proportion of the females displaying dancing behavior than the females that did not spawn. The frequencies of rubbing, dancing and air releasing behaviors of the spawners were more than those of the non-spawners.

In this study, the ovulation rate of channel females induced with carp pituitary was similar as they paired with channel males (74.2%) or blue males (70.0%). Hormone-induced blue females paired with blue males (B X B) also resulted in 69.2% of the females releasing eggs. However, the percentage of eggs masses ovulated that fertilized by C X C was significantly higher than that from C X B and B X B. Overall, 91.3% of eggs masses from C X C were fertilized while the values for C X B and B X B were 14.3% and 33.3%, respectively. The viability per egg mass of C X C, C X B, and B X B was not different and ranged from 49.2 to 77.4%. The results suggest that although carp pituitary was effective in inducing females to ovulate in many cases, the males may have not fertilized them and quality of the males may be an issue that caused the differences in fertilization rate. Behavior of male blue and channel catfish were similar; however, for spawners and non-spawners, there were differences in the percentage of males showing a given behavior and frequencies/h between behaviors. Overall, spawning successes of channel catfish can be improved based upon morphological characteristics and low natural hybridization might be issues of blue male catfish.

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INTRODUCTION

Culturing catfish (mostly channel catfish, *Ictalurus punctatus* and hybrids of channel female x blue male *I. furcatus*) is one of the largest aquaculture industries in the Unites States producing about 100 thousand metric tons in 2011(USDA 2012a). Total water surface area used for catfish production in 2012 in the three main producing states Alabama, Arkansas, and Mississippi was 78.3 thousand acres (USDA 2012b).

The channel x blue hybrid catfish has the potential to make a major contribution to the catfish industry being superior to either parent in several traits. The hybrid has better disease resistance (Smitherman et al. 1996), greater survival rate (Dunham et al. 1983), increased seine catchability (Dunham and Argue 1998), faster growth (Dunham and Brummett 1999), better feed conversion rates (Smitherman et al. 1996), and higher dress-out percentage (Chappell 1979) than those for channel catfish. When hybrid catfish are compared to blue catfish, they have significantly higher dress-out and fillet percentages (Argue et al. 2003), faster growth, higher survival rates of fingerlings and brood fish, and better production (Dunham et al. 2008). Jiang et al. (2008) also found that survival rate of hybrid catfish stocked in earthen ponds averaged 92.4% while the mean of blue catfish was 88.1%.

Overall, channel catfish accounts for more than 90% of the total acreage in catfish production. Only 7.3% of total water surface areas were used for channel x blue hybrid production in 2009 (USDA 2011). The C x B hybrid is not used widely because of the lack of fingerlings. In an open pond setting where only channel females are stocked with blue males, the rate of hybridization is low (Silverstein et al.1999). Natural spawning in pens is also limited, ranging from 0 to 20% (Tave and Smitherman 1982; Tieman 1995) and averaging 15% (Masser and Dunham 1998; Dunham et al. 2000). Exogenous hormone inducement to stimulate a spawn has been attempted where brooders are held in pens within a pond or held in a hatchery in tanks or aquaria (Tave and Smitherman 1982; Dunham et al. 2000). Differences in courtship patterns may be part of the reason that channel and blue catfish do not readily hybridize. Generally, hybridization between two different species is not common in the natural environment because of incompatibility of spawning behavior (Hubbs 1955; Tave and Smitherman 1982), habitat and seasonal barriers, and ethological isolation, such as courting conspecific females and chasing other females away, and approaching and entering only conspecifics males' nests (Keenleyside 1966).

However, hybridization does happen in natural environments in conditions of crowded breeding areas, great inequality in number of heterospecific individuals in spawning areas, and contacts of two species populations with geographical isolation for a long time (Hubbs 1955). This process does occur naturally in some fish species with more than a hundred hybrids identified (Smith 1992). Thompson (1935) reported that within certain groups of fishes hybrids were common and ranged a few to as high as 10% of a population. Keenleyside (1966) stated that the family of sunfish *Lepomis* commonly hybridized in nature in some localities making up to 75% or more of sunfish populations. Bettles et al. (2005) found that about 50% of genotyped fish were hybrids of cutthroat

(*Oncorhynchus clarkii*) and rainbow trout (*Oncorhynchus mykiss*) as they were sampled from thirteen sympatric populations (28 to 39 fish per population) on Vancouver Island in 2002 to 2003. DNA of fish was extracted and analyzed to determine population hybridization dynamics. Aspinwall and McPhail (1994) found that 5.7 – 14.1% of the combined gene pools of extensive hybridization in Stave Lake, British Columbia were engaged in by peamouth (*Mylocheilus caurinus*) and redside shiner (*Richardsonius balteatus*). Natural hybridization is also found among freshwater species especially in the Cyprinidae family like *Cyprinella clutrensis* and *Cyprinella venusta* in some localities of southwestern North America (Broughton et al. 2011), in *Nothonotus* darters (Keck and Near, 2010), black basses and sunfishes (*Micropterus salmoides*, *Micropterus floridanus*, *Lepomis gulosus*, or *Lepomis macrochirus*) (Keenleyside 1966; Bolnick and Near 2005), minnows and chubs (Aboim et al. 2010), and *Oncorhynchus clarkii* and *Oncorhynchus mykiss* (Docker et al. 2003; Bettles et al. 2005).

Hybridization has been commonly used in aquaculture to increase growth rate, improve flesh quality (Childers and Bennett 1961), disease resistance, and environmental tolerances (Bartley et al. 1997). Hooe at al. (1986) reported that hybrids between white crappies (*Pomoxis annularis*) and black crappies (*P. nigromaculatus*) had a low recruitment but similar growth rate to their parents; so, they can be an alternative to stock in small impoundments. Hybrids of black drum (*Pogonias cromis*) and red drum (*Sciaenops ocellatus*) showed a faster growth rate than their parents (Henderson-Arzapalo and Maciorowski 1994). Hybrids of Nile and blue tilapia (*Oreochromis niloticus x O. aureus*) exhibit superior growth of females, tolerance to cold and salinity, and fertile condition (Wohlfarth 1999) or predominate male offspring characteristics between *O. mossambicus x O. hornorum* (Krasnai 1987).

Brood selection is a factor influencing spawning success. Fish often show secondary breeding characteristics during the spawning season and their prominence may be important in mate selection and spawning success, and perhaps natural hybridization. Some fish actively choose mating partners based on pronounced secondary characteristics to ensure that spawning can occur. Atlantic salmon (*Salmo salar)* males with dominances of body size, cardiac-somatic index, and relative size occupy and mate females more frequently than subordinate males (Jarvi 1990). Female gobies (*Eviota prasina*) tend to select males with longer dorsal fins as their mates (Karino and Arai 2006). Siamese fighting females (*Beta splendens*) choose winners from male vs. male fighting with which to mate (Herb et al. 2003). Female bullies (*Gobiomorphus breviceps*) with poorer physical conditions had fewer mate inspections and lower quality mates than females with better physical conditions (Poulin 1994).

In aquaculture, brood catfish females are often selected by hatchery personnel based on secondary sexual characteristics, such as well-rounded, soft palpable abdomens while males with wide and muscular heads and grayish mottling of jaw and abdomens are selected (Tucker and Robinson 1990; Kelly 2004). How much these secondary sexual characteristics are actually important in mate selection and spawning success by the fish is not clear. Neu (1995) evaluated male channel catfish that differed in head width and ratio of head width to body length and found that channel males that spawned had statistically wider heads than those of channel males that did not spawn. Phelps et al. (2011) found that under hatchery conditions females categorized as good condition have higher spawning successes than those classified as fair or poor condition. In addition, percent of viable eggs from females listed as good or fair is significantly higher than "poor" females. Body metrics and the ratios of body metrics are also criteria for assessing selecting catfish brooders. Phelps et al (2011) reported that the ratio of length-to-girth and length-to-width of channel female spawners was less than those of channel female non-spawners. They also found that channel females that have a ratio of length-to-girth of less than 1.8 or length-to-width of larger than 5.5 spawned earlier than those in high value categories. Whether fish with distinct secondary sexual characteristics are more likely to hybridize is unknown.

Courtship behaviors that occur prior to spawning often differ by species. Spawning behaviors of two species of white suckers (*Catostomus commersoni*) and long-nose suckers (*C. catostomus*) were described by Dion et al. (1993). The authors described courtship behaviors like nuzzling, fin erection, vibration between females and males of spawned white suckers or chasing between females and males, fin erection, gamete release of spawned long-nose suckers. Inter-specific courtship was also observed between two species. In the case white female sucker remained close, the male longnose suckers would start nuzzling. If the female failed to initiate a chase, the males would give up courtship and moved off. In the case of male white suckers and female longnose suckers, the males also nuzzled the female and then to begin a chase and showed fin erection and vibration. This was followed by gamete release. A male angelfish (*Chaetodontopulus mesoleucus*) approaches a female and would stimulate her to mutual courtship by showing his caudal fin, fully spreading all fins, and vibrating his caudal fin to head of the female (Hioki and Suzuki 1995). Male paleback darters (*Etheostoma pallididorsum*) and butterflyfish (*Chaetodon trifascialis*) usually snuggle and push their snouts against abdomen or genital papilla of females to stimulate spawning (Johnston 1995; Yabuta and Kawashima 1997). Males and females of butterflyfish and angelfish often swim up to the water surface and gametes are released after the pair moves forward to the water surface and returns to bottom of experimental aquarium (Hioki and Suzuki 1995; Yabuta and Kaswashima 1997).

Ictalurid catfish are pair spawners often with an extended courtship behavior before actual spawning. Lenz (1947) and Clemens and Sneed (1957) described the spawning of channel catfish noting several behavioral patterns associated with courtship. Spawning behaviors such as biting, hazing, swim-up, gulping, and running were described by Clemens and Sneed (1957). Bullhead catfish (*Ictalurus,* Ictaluridae) also showed similar activities. Mouth opening, undulating, holding, nipping, and butting were spawning behaviors described by Wallace (1969) and Blumer (1982). An important behavior that occurs prior to spawning is where males or females use their tails to cover or twist around the mating partners during the 10 to 15 seconds before they release sexual gametes (Clemens and Sneed 1957). Anselme et al. (2008) described several spawning behaviors of African catfish (*Heterobranchus longifilis*) in their study including swimming together, air-gaping, and agonistic behaviors. Clarias catfish often swim up to the water surface for swallowing the air, move around tanks, chase, and nudge each other (Thakur 1976).

The objectives of this study were to determine if there were relations between morphological characteristics (external appearance, body metrics, the ratios of body metrics) of channel or blue male brooders and spawning successes such as spawning rate, relative fecundity, the pawning time, and egg and fry characteristics. The same criteria were also used to assess the channel females. A second objective of the study was to determine if behaviors of C X C pairs that spawned successfully differed from non-spawners, and likewise for C X B that spawned or did not spawn. Occurrence, frequencies, and time duration of each spawning behavior were analyzed to determine what behavioral characteristics might affect spawning successes. A third objective was to determine if channel and blue males when paired with channel females behaved similarly for those that spawned successfully and whether female behavior was altered. Finally, the research also gives explanations about low success of hybrid production and recommendations on how to improve based on the catfishes' morphological characteristics and behaviors.

LITERATURE REVIEW

Natural Spawning of Channel Catfish

Channel catfish (*Ictulurus punctatus*, Rafinesque 1818) are native species in temperate environments in the eastern United States, southern Canada, and northern Mexico. Channel catfish usually reach sexually maturity in two or three years (Tucker and Robinson 1990). This species often prefers to spawn in isolated or semi-dark places. Channel males build nests in holes of pond banks, hollow logs, and rocks in natural environment (Rubec 1979; Wellborn 1988). Channel catfish usually spawn from February to August in natural environments, depending on weather conditions and locations (Lenz 1947; Wellborn 1988; Wolters 1993) at water temperature ranging from 21 to 30^{0}C (Meyer at al. 1973). Water temperature is an important factor affecting the spawning of channel catfish. Optimal water temperature for catfish reproduction is around 27 ^{0}C (Lenz 1947; Wellborn 1988; Wolters 1993). Under Texas hatchery conditions, water temperature for catfish spawning is approximately 21 to 27 ^{0}C (Wyatt et al. 2006).

In aquaculture, spawning containers made of metal, ceramic, fiberglass, or plastic materials are placed in ponds as spawning nests. The containers should be periodically checked every few days in the spawning season for the presence of eggs. Channel females prefer to breed in containers made of metal rather than plastic containers (Busch 1983; Steeby 1987).

Open pond spawning is the most common method used for commercial scale spawning of channel catfish (Tucker and Robinson 1990). Spawning containers should be placed into the ponds as spawning nests several days before spawning and checked periodically (Busch 1983; Steeby 1987, Tucker and Robinson 1990). Channel females lay adhesive eggs in the spawning containers that remain on the bottom of containers until they hatch or are removed (Canfield 1947, Tucker 1996). Overall, the spawning rate of brooders used with the open pond method for channel catfish is approximately 30 to 50% (Brauhn 1971; Bondari 1983; Wolters 1993). In this method, spawning successes are similar in ponds stocked at male to female ratios of 1:1, 1:2, 1:3, and 1:4 (Bondari 1983). This method is appropriate for producing large amount of fry on a commercial scale (Wolters 1993).

In addition to the open pond method, pen and aquarium spawning methods are used to reproduce channel catfish, but require more control and effort to obtain egg masses. Pen method is quite similar to the pond method (Graham 1999) except individual fish are selected for spawning and are confined in wire mesh pens placed next to the pond bank. The pens are made of ½ to 2 inch mesh material so that water can flow through them. Females may be allowed to spawn naturally or may be injected with hormone for inducing ovulation. This method allows selecting breeding of stock and controlling time of spawning, but this required the addition of pen construction and brooder selection (Hunter and Dupree 1984; Tucker and Robinson 1990; Masser and Dunham 1998; Graham 1999). Spawning success rates of channel catfish induced with human chorionic gonadotropin (HCG) in the pen method was 88% (Tave and Smitherman 1982) or 67% if they were injected with Ovaprim (Guedie et al. 1992) or carp pituitary in aquarium system (Dunham et al. 2000).

Spawning of channel brooders in the aquaria needs careful selection of broodstock and the use of hormones for success (Graham 1999). They have been induced to spawn by using several kinds of hormones including HCG, extracted pituitary, or LHRH-a (Sneed and Clemens 1960; Brauhn 1971; Dunham et al. 2000). This method brings the possibility of manual stripping of eggs, of crosses with more than one male, production of hybrids, and ploidy manipulation, but it is time consuming, complex, and relatively expensive (Bates and Tiersch 1998). The successful rates for spawning of channel catfish stocked as pairs or in groups in rectangular 120-L fiberglass tanks were 22 to 41% for paired trial and 58% for grouped trial (Bates and Tiersch 1998). Dunham et al. (2000) also reported that channels placed in aquaria reached a spawning rate of 67% while it is about 57% when females and males were kept in tanks (Brauhn 1971).

Before spawning, channel catfish males use their fins to remove debris from the nest site. Females are attracted by the male's pheromones to spawn in the nests (Rubec 1979). A few hours prior to spawning, the channel catfish females use their pelvic and pectoral fins to sweep the area where eggs are to be deposited and also stimulate the channel males. The male wraps his caudal fin around the head of the female and the female wraps her tail around the male as part of the courtship for about 5 seconds. At the same time, both the male's and female's bodies quiver for a few seconds and eggs and sperm are released (Clemens and Sneed 1957). Fecundity of channel females is around 6,600 – 8,800 eggs kg^{-1}. The females only spawn once a year while males can spawn several times, especially under hatchery conditions (Legendre et al. 1996).

Eggs of channel catfish are released in a glycoprotein matrix. This matrix can be dissolved by using 1.5% sodium sulfite solution (Lenz 1947; Isaac and Fries 1991). The eggs hatch 5 to 10 days after spawning (Wolters 1993) depending upon water temperature. By the 4^{th} or 5^{th} day, the eyes have become pigmented (Canfield 1947). Average hatching rate of channel catfish is about 67% for the pen spawning method (Silverstein et al. 1999) and 52.7% for the pond spawning method (Small and Wolters 2003). Hatched fry can survive 2 to 5 days after hatch by utilizing the large yolk reserve (Wellborn 1988). By approximately 3 days after hatch, mouth parts are functional and the fish will accept a formulated feed (Tucker and Robinson 1990).

Natural Spawning of Blue Catfish

Blue catfish (*Ictalurus furcatus*) is the largest species of the family Ictaluridae in the United States and can live over 20 years and exceed 45 kg and 165 cm. They have similar physical characteristics to channel catfish (Rubec 1979; Graham 1999). However, blue catfish have a smaller head and a nearly straight anal fin when compared to channel catfish (Graham 1999).

Blue catfish are native to the Mississippi, Missouri, and Ohio River basins and Gulf Coast streams of the central and southern United States, southern Mexico, northern Guatemala, and have been introduced into other states along the Gulf, Atlantic, and Pacific slopes including Washington, Oregon, California, Arizona, Colorado, Maryland, Virginia, South Carolina, and Florida (Graham 1999). They are most common in large reservoirs, main channels of rivers, backwaters, and strong flowing water (Smith 1979; Burr and Warren 1986).

Blue catfish have several desirable characteristics for aquaculture. They have a higher dressing percentage than channel catfish (Dunham et al. 1983a). Chappell (1979) reported that blues were more easily seined than channel catfish. However, these fish are not popular in aquaculture industry of the United States since they take longer to mature, have high food conversion ratios (FCR), a greater disease susceptibility, and poorer spawning success in captive conditions (Graham 1999).

Maturation of blue catfish in southern parts of their range is earlier than northern parts (Graham 1999). Blue catfish become sexually mature at the age of 4 to 5 years and at total length of 590 mm for females and 410 – 490 mm for males in Louisiana (Perry and Carver 1973) while Graham (1999) reported that blue catfish overall reach sexual maturity at age 6-7. Hale and Timmons (1988) found that 50% of the blue females were mature when 661 mm in length and 100% were mature when 722 mm in Kentucky Lake, Kentucky – Tennessee. They typically spawn during April to June in Louisiana (Perry and Carver 1973). In Kansas reservoirs, Goeckler et al. (2003) found that the spawning season for blue catfish was from June to July at water temperatures of 21 to 24 ^{0}C. Blue catfish were reported to produce 900 – 1,350 eggs kg^{-1} (Graham 1999).

Generally, the spawning habits of this species are similar to those of channel catfish (Pflieger 1997), but blue catfish were more sensitive to surrounding environment rather than channel catfish (Lenz 1947). They are substrate spawners and usually build nests in places where the water currents are not too strong to affect egg deposit on. Each egg mass may contain 40 – 50 thousand eggs (Graham 1999). Pflieger (1997) found that eggs of this species hatched after 7 – 8 days at the temperature 21 – 24 ^{0}C. Hatching success of blues was estimated at 90% and was higher than channels (Tave and Smitherman 1982).

Hybridization of Fish in Natural Environment

Hybridization is the mating of two different species resulting in viable offspring (Bartley et al. 1997). This process occurs successfully when there is cross fertilization of sperm and eggs from species that spawn at the same time and in similar habitats (Hubbs 1955). In aquaculture, hybrids are intentionally made to obtain animals that contain desired qualities of both parents (Masser and Dunham 1998). However, this process can be a threat to biodiversity and ecological balance (Perry et al. 2002).

Generally, two different species in the natural environments rarely mate with each other because of spawning behavioral incompatibility, environment and other factors Hubbs 1955; Tave and Smitherman 1982). However, hybridization does occur naturally in some fish species with more than a hundred hybrids identified (Hubbs 1955; Smith 1992). External fertilization, changes in living habitat quality and quantity, breakdown of behavioral isolating mechanisms, and competition for restricted breeding habitat are considered as the main factors leading to the hybridization of fish species in natural environments (Hubbs 1955; Herrington et al. 2008). Hybridization is easier to occur in nature if species have the same reproductive modes and spawning behaviors especially for external fertilization. In addition, captive conditions in aquarium also help the fish have closer contact and break spawning behavioral isolation and limit the spawning habitat which results in hybridization between two species. Hybridization between longnose and alligator gars in a 4.54 m^3 aquarium is a good example of natural hybridization of two species in captive condition (Herrington et al. 2008).

Broughton et al. (2011) reported that natural hybridization is common among freshwater species especially in the Cyprinidae family. Hybrids were recorded between *Cyprinella clutrensis* and *C. venusta* in some localities of southwestern North America (Broughton et al. 2011), in *Nothonotus* darters (Keck and Near 2010) and other minnows and chubs (Aboim et al. 2010). Natural hybrids are also found in the Centrarchidae family including black basses (*Micropterus salmoides* and *Micropterus floridanus*) and sunfishes (*Lepomis gulosus* and *Lepomis macrochirus*) (Bolnick and Near 2005).

Hybridization is commonly used in aquaculture to increase growth rate, improve flesh quality, disease resistance, and environmental tolerances (Bartley et al. 1997). Hooe at al. (1986) reported that hybrids between white crappies (*Pomoxis annularis*) and black crappies (*P. nigromaculatus*) had a low recruitment and similar growth rate to their parents; so they can be an alternative to stock in small impoundments. Hybrids of black drum (*Pogonias cromis*) and red drum (*Sciaenops ocellatus*) also showed a faster growth rate than their parents (Henderson-Arzapalo and Maciorowski 1994). Hybrids of Nile and blue tilapia (*Oreochromis niloticus x O. aureus*) exhibit superior growth of males, tolerance to cold and salinity, and fertile condition (Wohlfarth 1999) or predominate male offspring characteristics between *O. mossambicus x O. hornorum* (Krasnai 1987). Hybridization of channel female and blue male results in heterosis with higher fillet percent, faster growth, better feed conversion, tolerance of low oxygen and diseases (Chappell 1979; Dunham et al. 1983b; Dunham et al. 1987; Argue and Dunham 2003).

According to Masser and Dunham (1998), studies on catfish hybridization in North America have been conducted for more than thirty years, but only the hybrid of channel female (*Ictalurus punctatus*) and blue male (*Ictalurus furcatus*) exhibit dominant characteristics exceeding their parents' genetic characteristics. Several methods for producing the hybrid catfish including open-pond spawning, pen spawning, and induced spawning with artificial fertilization have been used (Tave and Smitherman 1982; Dunham et al. 2000). However, open-pond spawning does not consistently produce hybrids because spawns of channel females and blue males seldom happen in the natural environment (Dunham and Smitherman 1987).

Confinement of channel females and blue males in pens without hormone injection rarely results in successful spawning. Tave and Smitherman (1982) obtained no spawns from nine pairings of channel x blue pairs when placed in outdoor pens. Confinement in pens combined with hormone induced spawning has resulted in hybridization. Typically, blue males are placed in the pens a day or two days before stocking the channel females. The selected females usually reveal good spawning characteristics, such as soft belly and red and swollen genital papilla. The females are usually injected with HCG, LHRH-a, or carp pituitary (Tave and Smitherman 1982; Dunham et al. 2000). After approximately 72 hours, the females spawn in the pen. Tave and Smitherman (1982) reported the spawning success of channel female x blue male held in pens was 40% but success rates in later studies ranged from 0 to 20%, averaging 15% (Tieman 1995; Dunham et al. 2000). Generally, hormone induced spawning with artificial fertilization is an important method to produce hybrid seed with successful ovulation rate of 80% compared to 15% of pen method (Dunham et al. 2000). To artificially fertilize the eggs, male blue catfish are sacrificed to remove the testes from mesentery. Sperm is obtained by squeezing the testes (Masser and Dunham 1998; Dunham et al. 2000). Normally, 100 ml of eggs can be fertilized by 0.5 g testes. One male can be used to fertilize for three to five females, depending on male size and quality. With this method, brood females are given the first injection in the evening when they are seined from the ponds. The spawning rate of the brooders can be affected by holding time in containers. The longer the brooders are kept in the containers, the lower the number of fish spawned (Masser and Dunham 1998). The second injection can be given to female 12 hours after the first injection. At the temperature 26.7 to 27.8 ^{0}C, the females ovulate after thirty-six hours from the first injection and the eggs are obtained by hand stripping. Successful rate of artificial spawning method can reach 67 to 100% (Masser and Dunham 1998). The number of eggs per kg female from hormone-induced but naturally released and fertilized using the pen spawning method can be higher than when females are manually stripped. Dunham et al. (2000) obtained 7,950 ± 1,027 eggs/kg from hormone induced natural egg releases in pens and 3,488 ± 972 eggs/kg when females were manually stripped to obtain eggs. He also found that the hatching rate of naturally fertilized eggs from pens was nearly eight times higher than hatching rate of hand-stripped fish.

Spawning Behaviors

Sexual selection has been a major factor in evolutionary biology (Real 1990). It is believed that animals actively select mating partners since this process affects survival rates of offspring (Parker 1983; Real 1990). Females tend to mate with males that have outstanding external appearance and territories with protection and food for offspring (Weigmann et al. 1992). Female gobies (*Eviota prasina*) tend to select males with longer dorsal fins as their mates (Karino and Arai 2006). Siamese fighting females (*Beta splendens*) choose winners from male vs. male fighting with which to mate (Herb et al. 2003). Care-giving from parents is also an important behavior appearing to benefit offspring in many ways such as protecting the offspring from predators (Blumer 1982). This reproductive behavior can improve survival rate of the next generation (Blumer 1982; Jennings 1997; Abe and Munehara 2005; Karino and Arai 2006). Clutton –Brook (1991) reported that parental care increased survival rate of offspring and reproductive success of the parents. However, this behavior also requires energy and time consumption from parents in order to perform caring activities. Parental cares were found in black basses (*Micropterus spp*), goby (*Eviota prasina*) or maternal cares were seen in the sculpin (*Radulinopsis taranetzi*). In addition, the spawning or reproductive behaviors also played vital roles in processes of recruitment, growth, and mortality in the Centrachidae family of fish (Jennings 1997). Spawning behaviors are considered as signals to attract attention of mating partners in bullhead fish (*Ictulurus,* Ictaluridae), so that spawning can occur (Wallace 1969).

Different species express spawning behaviors in different ways. A male angelfish (*Chaetodontopulus mesoleucus*) approaches a female and stimulates her to mutual courtship by showing his caudal fin, fully spreading all fins, and vibrating his caudal fin around the head of the female. The male also circles the female and displays his lateral body to the female (Hioki and Suzuki 1995). Male paleback darters (*Etheostoma pallididorsum*) and butterflyfish (*Chaetodon trifascialis*) usually snuggle and push their snouts against the belly or genital papilla of females to stimulate spawning (Johnston

1995; Yabuta and Kawashima 1997). Males and females of butterflyfish and angelfish often swim up to water surface and gametes are released after the pair moves forward to the water surface and returns to bottom of experimental aquarium (Hioki and Suzuki, 1995; Yabuta and Kaswashima 1997). Johnston (1995) also demonstrated that males of paleback darters (*Etheostoma pallididorsum*) showed aggressive attitudes to other males as a spawning behavior. This spawning behavior was also found in butterflyfish (*Chaetodon trifascialis*) when they chase conspecific intruders away from their territories (Yabuta and Kaswashima 1997). Darter males erect dorsal fins to encounter other males and occasionally bump the side of other intruders. These fish also change to bright orange colors except the dorsal area. Females of this species were brown mottled during spawning. There was no elaborate courting display between males and females of this species. The males pursue the females as they moved about tanks and mounted the back of the females with caudal peduncle curved down and to the side of female (Johnston 1995). Reproductive behaviors of Siamese fighting fish include several interactions of courting behavior, gill cover erection and construction of bubble nests (Herb et al. 2003). Males of goby (*E. prasina*) approach female by moving its pectoral fins and erecting dorsal fin, and lead females to the nest. They spend more time for fanning eggs by moving their pectoral and other fins within their nest and sometimes peck their eggs with mouth (Karino and Arai 2006).

Sound production accompanies reproductive behaviors of many species (Myberg et al. 1986). Sounds are produced by adult haddock (*Melanogrammus aeglefinus*) when male and female are held together. No sounds are produced as they are kept in isolated condition. Frequencies of hums that produced by adult haddock range 31 to 66 Hz for sounds from spawning. This is considered as a reproductive behavior as well as a means of communication of this species (Bremner et al. 2002). Myrberg et al. (1986) reported that females of damselfish *Pomacentrus partitus* use the courtship sounds to locate males nest sites during spawning seasons.

Time and frequencies of courtship and spawning behaviors were recorded in some species. A courtship behavior of *Chaetodon trifascialis* was recorded as male visits females who live in his territory and swim together for a short time (frequencies 9.3 times per 20 minutes) and stay together for 20.4 sec. per visit in day time (Yabuta and Kawashima1997). Males of *Beta splendens* that win in male fighting spend similar time duration to court eavesdropping females (who gathered information from interactions of male and male fighting) and naïve females (who did not witness male and male fighting) while the losers in the fighting spend more time to courtship the naïve females rather than eavesdropping females (Herb et al. 2003).

Monitoring systems have been developed for measuring and observing fish behaviors. Doi and Miyake (2000) used successive three-dimensional shape modeling to analyze the fish behaviors from recorded images. Some of characteristics of fish, including body shape, skeleton, head, tail, gravity center, direction, and so on, are recorded by this system for reconstructing fish behaviors. A two-dimensional multi-tracking system to measure schooling behaviors in fish was also applied in laboratory (Decourt et al. 2009). Kane et al. (2004) designed a video analysis system combined with specific software to quantify fish behavioral stress in environment. Abe and Munehara (2005) used Panasonic video camera to record spawning behavior of sculpin fish. Garcia et al. (2008) observed *Austrolebias reicherti* in aquarium that were recorded by a video player. Spawning behaviors of males and females of this species were taped as lateral display, sigmoid display, invitation to dive, and invitation to follow (males) as well as diving acceptance, and display of females.

Mating Behaviors in Catfish

Anselme et al. (2008) monitored behaviors of African catfish (*Heterobranchus longifilis*) as they were kept in 1000-L aquaria at water temperature of 26^{0}C. Swimming, air-gaping, and agonistic behaviors were recorded during the experimental period. Generally, the activities of catfish occurred more frequently in nighttime than daytime. Frequency of agonistic behaviors decreased in groups with more fish. Understanding behavioral plasticity may provide more benefits for aquaculture of this species. Thakur (1976) placed adult *Clarias batrachus* in plastic pools or aquaria for observing spawning behaviors. The fish were injected with Indian carp pituitary and kept in 26 to 29^{0}C water. After 16 to 20 hours, the spawning behaviors of catfish were recorded. Both male and female just settled at the bottom in shadowed areas and swam up to the surface for gulping air. They also moved around, chased, and nudged each other. Snapping activity, the sound that is produced by the jaws of the fish, was also found in pre-spawning period of this species. The female usually leads the spawning activities and attracted the male to take part in the process. For mating activity, the male often used its tail to embrace the female's snout and would stay motionless for 5 to 10 seconds. After releasing eggs, the females rested at the bottom while the male continued to move around and waited for the next run.

Spawning behaviors of catfish in the genus *Ictalurus* have been described by Clemens and Sneed (1957), Wallace (1969), and Blumer (1982). Clemens and Sneed (1957) described some of behaviors like biting, hazing, or swimming in circles that were seen during their experiment when channel females were induced by pituitary hormone injection and placed in aquaria with non-injected channel males. Generally, the channel males were described as being the aggressor, attacking the females to keep her at certain sites on the bottom. The authors concluded that the activity of swimming up to gulp air at the water surface shows that the male or female was not ready for spawning and should be taken out of the aquaria. Another behavior also described in the Clemens and Sneed (1957) study was "running" activities when females used their pelvic and pectoral fins to fan against the bottom of aquaria for laying eggs. Wallace (1969) observed similar activities in black bullhead catfish (*Ameriurus melas*). Gravid bullhead females usually excavated nests by side to side fanning of anal fin and up and down fanning of pelvic fin. Females occasionally pushed gravel or pebbles out of location of their nests. This activity of bullhead females seems to increase the males' interests in them. The males of black bullhead catfish did not participate in building the nests. This was also similar for yellow bullhead catfish (*Ictalurus natalis*) when the females excavated the nests in pre-spawning period. The males of yellow bullhead catfish were reported to take part in building the nests after the nests were nearly completed. In contrast, channel males usually prepared nests and attracted the females to come and spawn in the nests under natural conditions (Lenz 1947).

Opening mouth, undulating, holding, nipping, and butting have also been observed in bullheads (Wallace 1969, Blumer 1982). If a bullhead female accepts a male for mating, they remain very close together at the bottom of the aquarium. If the female is not ready, the male and female may fight (Wallace 1969). A channel female that is ready to spawn often using its tail to cover the male's head (Clemens and Sneed 1957). This was similar to the observation of Wallace (1969) with black, yellow and brown bullheads where a male twisted his caudal fin and caudal peduncle over female's snout and eyes for 10 to 15 seconds. Unlike channel catfish (Clemens and Sneed 1957), reproductive vents of a pair of black, yellow or brown bullheads are close together and the female does not lunge forward after depositing the eggs. Eggs and sperm from the female and male were released at the same time when the channel male and female were in position of head-to-tail wrapping each other (Clemens and Sneed 1957). If bullhead males were unacceptable or unready to spawn, bullhead females would lose their eggs in the aquarium (Wallace 1969). Convulsion was seen in brown bullhead males as they paired with brown females. However, this behavior was only found in yellow and black bullhead females as they were paired with conspecific males.

External Characteristics and Measurements and Spawning Results

Channel catfish brood quality is often classified by secondary characteristics of the fish. Generally, channel females should have rounded, soft abdomens, and red and swollen genital papilla while males should show well-developed sexual characteristics including broad, muscular heads, dark mottled pigmentation on the underside of the jaws and abdomens (Tucker and Robinson 1990; Kelly 2004). Phelps et al. (2011) classified catfish brooders as being in good, fair or poor condition and compared their spawning success. Those subjectively classified as good had 85.7% spawning success, fair at 72.0% and those classified as poor at 47.0%. Females categorized as good and fair had a greater percentage of viable eggs than poor females, but the egg hatching rates were similar from all brood categories. As subjective rankings can vary depending on the person classifying the fish, the authors evaluated the fish based on morphometric traits. The research showed that the average weight and the length to weight ratio of fish were not significantly different between the channel broodfish that spawned or did not spawn. However, the ratios of length to girth and length to width were different between two groups of spawners and non-spawners. In addition, if the ratio of length to width of channel catfish was less than 5.5, the spawning frequency was higher than groups without selection criteria (Phelps et al. 2011). Neu (1995) evaluated male channel catfish that differed in head width and ratio of head width to body length and found that channel males that spawned had statistically wider heads than those of channel males that did not spawn. Newman (1990) reported that channel females weighing 1.0 -1.5 kg had differences in gonosomatic index (GSI) from those females that weighed 1.5 to 2.0 kg. She found no correlations of body weight and relative fecundity (the number of egg per kg of body weight) or mean egg size categories. Blumer (1983) reported that no correlation between body length and weight of bullhead was found in his experiment. However, larger size bullhead males spawned earlier than smaller ones.

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METHODS & MATERIALS

The research was conducted at the E. W. Shell Fisheries Research Center, Auburn University, Auburn, Alabama, during 01st May to 10th June 2011. Five spawning trials were conducted in 100-L glass aquaria (38 cm x 45 cm x 60 cm) supplied with water from a common recirculating water system and biological filter. Water used in the experimental system was municipal, potable water that was stored and aerated a minimum of 24 h in outdoor 3 m^3 circular tanks before being pumping into the experimental recirculating system. The water quality of the recirculating system was tested at the beginning and end of each trial. Total ammonia nitrogen averaged 0.29 ± 0.07 ppm; pH 6.74 ± 0.06; total alkalinity 33.80 ± 11.03 ppm; and total hardness 46.90 ± 6.36 ppm. Each aquarium was aerated with a single air stone supplied from a blower. A three-phase, 240V heater (Process Technology Corp.) was used to control the water temperature of the system, maintaining a mean water temperatures of 29.23 ± 0.35^{0}C. Water temperature and dissolved oxygen concentration in each aquarium were measured twice a day, around 09.00-10.00 h and 17.00-18.00 h using YSI Model 55 DO meter. Total ammonia, hardness, alkalinity and nitrite concentration were checked at the beginning of each experimental trial by using aquatic test kits.

Fish behavior was recorded using video cameras and a digital recorder. Seaview Model SM-50-C video cameras were placed beside eight aquaria to continuously record the activities of the brooder pairs from right after stocking the male and female together to a minimum of 48-h post-stocking. Each camera provided a video of the entire aquarium. Cameras were linked to a H.264 8CH Digital Video Recorder (Zhuhai Raysharp Technology company) having a 500-gigabyte hard drive to store all the videos from the experimental periods. Color patterns and other morphological traits of each brooder were photographed prior to brood stocking into the aquaria using a Nikon D40X digital camera.

Experimental Broodstock

Three to four-year-old Marion strain channel brooders (*Ictalurus punctatus*) and seven-year-old Rio Grande blue brooders (*Ictalurus furcatus*) were held in 0.4-ha earthen ponds and fed a 36% protein, floating, commercial catfish diet before being used in this research. The channel females and males were maintained in separate ponds while the blue females and males were cared for in the same pond. A total of five experimental trials were conducted. Brooders for each trial were collected by seine in the morning just prior to the start of a trial. The blue and channel males were sorted based on several criteria including body coloration, muscular head width, and shape and coloration of papilla. Females were also selected based on body coloration, rounded and swollen abdomen, and papilla characteristics. The selected brooders were transported to the hatchery and kept separately in 2-m^3 circular tanks for several hours before the start of a trial.

External Appearances

Each brooder was photographed prior to stocking into the aquaria. Photographs of the females included: side of the body, abdomen, and papilla. Photographs of the males included: side of the body, under-head, upper head, and papilla. They were used to evaluate the relationships between external appearances and spawning success.

External Body Measurements

All brooders were weighed to nearest 1g before placing into the aquaria. Each female brooder's total body length, body girth and width were measured to nearest 1mm with a plastic measuring tape. Body length was measured from the top of the mouth to the end of caudal fin by putting the fish on wooden measuring board. Body girth was measured as the body circumference measured from the posterior base of the dorsal fin. Body width was measured placing the female perpendicular on a ruler and recording the body width at the intersection with the posterior base of the dorsal fin. Male body length was measured similar to that of females, from the top of the mouth to the end of caudal fin. Male head width was measured as the widest lateral width of the head when placed over a ruler.

Induced Spawning

Females were weighed and measured then randomly stocked in aquaria and induced to spawn using common carp pituitary at 10 mg kg^{-1} body weight divided in two intramuscular injections. The first injection at 2 mg kg^{-1} was given at 20.00 h at the same day brooders were collected from the holding pond. A second injection at 8 mg kg^{-1} was given 12 h later. Males did not receive any hormone injection and were stocked into the aquaria after the females had received their second injection. Each aquarium was randomly stocked with a pair of brooders to give one of three types of crosses including channel female x channel male, channel female x blue male, or blue female x blue male. Crosses made per trial are given in Table 4.

Aquaria Management

Fish remained paired for up to 72 h following the resolving injection. During that period they were allowed to spawn naturally. Where there was a spawn, the fish remained paired until no new spawning activity was observed, the male was actively guarding the eggs and would chase the female away from the egg mass. At that point the digital video recorder was turned off, the female removed and the male left to guard the egg mass. Approximately 24 h after the end of spawning the male was removed and the egg mass collected. Spawning success per trial was calculated by dividing the number of spawned fish obtained by the total number of pairs used in the treatment and multiplying by 100.

The egg mass was weighted to the nearest 0.1 g. Three subsamples were taken from each egg mass at different sites, weighed to the nearest 0.1 g and counted to determine the average number of eggs g^{-1}. Total number of eggs in the egg mass was calculated by multiplying average number per gram by total weight of the egg mass. The number of eggs kg^{-1} female was calculated by dividing the total eggs by the female weight. The matrix of each egg mass was removed using a 1.5% sodium sulfite solution before incubating each egg mass individually in MacDonald jars. After 6 hours of incubation, the percentage of viable eggs was determined classifying any white colored egg as non-viable and yellow to orange colored as viable. Three egg samples were randomly collected from different heights in the water column of the MacDonald jars. The number of non-viable eggs and total eggs were counted and the non-viable eggs expressed as a percentage. Similarly, hatching rate was computed by dividing number of hatched sac fry by number of viable eggs in the jar and multiplying by 100. Hatched sac fry were removed from the MacDonald jars to graduated cylinders for measuring the total volume of sac fry per jar. Three one-milliliter samples were taken from the cylinder to determine the average number of sac fry mL^{-1}. The quantity of hatched sac fry was determined by multiplying the average number of sac fry mL^{-1} by the total volume of sac fry.

After determining the total number of sac fry per spawn, each set of fry was transferred to separate aquaria and held until the fry had developed mouthparts and began to feed. At that time, they were fed a powdered commercial food every three hours during daylight hours. The quantity of feed depended on the number and requirements of the fry in each aquarium. Uneaten feed and dead fry were siphoned out every morning before providing new feed. Water temperature was monitored twice a day and held at 28.89 ± 0.49 °C using an electric heater. Total ammonia nitrogen, nitrite, hardness, and alkalinity were checked daily following the same procedures used for testing water quality in the spawning aquaria. The number of swim-up fry was determined by weighing the total sac fry per aquarium, subsampling to determine the number of fry g^{-1} then extrapolating the total number. The total number of swim-up fry was divided by the female weight to the total number live fry kg^{-1} female. The percent survival from sac fry to swim-up fry was determined by multiplying the number of live sac fry in each gram by the total number of hatched larvae and multiplying by 100.

Body Proportions, Still Photo and Video Evaluations

The total length, body width and girth, and weight of female blue and channel catfish were measured and divided into different categories to evaluate the relationships of these parameters to spawning success. Similarly, the spawning success of blue males and channel females and males was also evaluated based on total length, head width, and weight categories (Table 1).

The relationship of spawning success and body ratios was evaluated using the ratio categories given in Table 2. Length/weight, length/girth, and length/width ratios were computed by dividing the total length by the weight, body girth, and abdominal width, respectively. Female girth/width ratio was computed by dividing the body girth by the abdominal width. Female girth/weight, and width/weight ratios were also computed by dividing the body girth and width by total weight. Female/male weight ratio was computed by dividing the female weight by the male weight.

Photos of body (side view), abdomen and papilla were carefully examined to assess the conditions of the papilla, the body, abdominal coloration (Table 3) and the significance of those characteristics to spawning success. The body and abdomen coloration of the females were visually classified as three categories: dark, grey, and whitish grey. Females with large reddish prominent papilla were classified as good, pinkish prominent papilla as fair, and less prominent whitish papilla were classified as poor (Table 3).

The body coloration of the blue and channel males was classified as dark or grey. The coloration of the underside of the head of the males was classified as dark, grey or whitish grey. The papilla conditions were divided into two categories: a long and prominent papilla (good) or a whitish and inconspicuous papilla (fair) (Table 3).

Videos were examined to determine the spawning time. Behaviors during 1 h time blocks at 1, 3, 6 and 12 h before spawning were examined for the crosses of channel female x channel male (C x C), channel female x blue male (C x B), and blue female x blue male (B x B). Fish behavior of pairs that did not spawn was monitored using as the "1 h prior time" the mean 1 h before spawning for each cross that spawned. The total number of pairs made, pairs video taped, and pairs video taped that spawned per trial are given in Table 4.

Spawning behaviors monitored included rubbing, tail curling, tail dancing, air releasing, swimming up, and mouth widening. A rubbing activity was where male or female brooders used their pectoral fins or belly to rub the bottom of aquaria. They turn slightly from one side to the other side to clean certain sites on the bottom (Fig 1). The curling activity was where the male or female used its tail to cover head of the other while the other partner shows little or no participation (Fig 2). A dancing activity was classified as where both females and males wrap their caudal fin over the front portion of the head of its partner and quiver. The dance would take place over the aquaria bottom (Fig 3). Air bubble releasing consisted of air bubbles being released from gills or mouth of the brooders (Fig 4). A swimming-up activity consisted of where the male or female would swim up to the surface of the water and gulp air (Fig 5). The mouth widening activity was where the male or female would open its mouth widely as might be described as a yawn (Fig 6).

Behavior data for each pair were recorded as to: did it occur within the hour period being observed (yes/no), the frequencies of occurrence within the hour period being observed, and the duration of each behavior activity. Percent occurrence of a specific behavior was calculated by dividing the number of pairs that performed that activity by the total pairs watched during time period and fish combination and multiplying by 100.

Data Analysis

Data were analyzed by analysis of variance (ANOVA) and t-test to determine if there were differences in spawning characteristics as a function of fish cross. Such characteristics included mean response time to first egg release, the mean number of eggs per gram egg mass, the average number of eggs per kg female, the mean percentages of fertilization rate, mean hatching rate, and mean swimming up rate, and mean number of fry obtained per channel female. For each cross, differences in morphological traits and ratios, appearance, and behavior were analyzed as a function of whether a pair spawned or not. Crosses were compared as to whether behavior of fish that spawned was similar. Likewise, fish behavior of fish that did not spawn were compared as a function of fish cross. Linear regression was also used to test the correlations between factors of body metrics, eggs & fry characteristics, and spawning behaviors.

All data analyses were performed using the Statistical Analysis System 9.2 (TS2M2) developed by SAS Institute Inc. Cary, NC. USA.

TABLE 1. *The total length, body girth, body width or head width, and weight categories of channel males and females and blue males used in the evaluation of spawning success, time to 1st egg release, egg and fry characteristics in three combinations (C x C, C x B, and B x B).*

Fish type	Sex	Total length (cm)	Body Girth (cm)	Body/Head width (cm)	Weight (kg)
Channel	Female	≤ 50	≤ 28	≤ 9.0	≤ 1.5
		50 – 55	28 – 30	9.0 – 10	1.5 - 2.0
		≥ 55	≥ 30	≥ 10	≥ 2.0
	Male	≤ 55		≤ 10	≤ 1.5
		55 – 60		10– 11	1.5 - 2.0
		≥ 60		≥ 11	≥ 2.0
Blue	Male	≤ 55		≤ 10	≤ 1.5
		55 – 60		10– 11	1.5 - 2.0
		≥ 60		≥ 11	≥ 2.0

TABLE 2. *The ratios of body measurements of channel and blue males and channel females classified into different categories used to evaluate the spawning successes and time to 1st egg release, egg and fry characteristics in three crosses.*

	Ratio					
	Length/Weight	Length/Width	Width/Weight	Length/Girth	Girth/Width	Girth/Weight
Females	≤ 30	≤ 5.5	≤ 5.5	≤ 1.9	≤ 2.9	≤ 17
	30 – 35	5.5 – 6.0	5.5 – 6.0	1.9 – 2.0	2.9 – 3.0	17 – 20
	≥ 35	≥ 6.0	≥ 6.0	≥ 2.0	≥ 3.0	≥ 20
Males	≤ 30	≤ 5.5	≤ 5.5			
	30 – 35	5.5 – 6.0	5.5 – 6.0			
	≥ 35	≥ 6.0	≥ 6.0			

TABLE 3. *External appearance of blue males and channel males and females classified into different categories to assess the relationship to spawning success, time to 1st egg release and egg and fry characteristics during experimental period.*

Classification	Blue catfish		Channel catfish	
	Females	Males	Females	Males
Body coloration	Dark	Dark	Dark	Dark
	Grey	Grey	Grey	Grey
			Whitish	
Abdominal coloration or	Dark		Dark	Dark
underside head coloration	Grey		Grey	Grey
	Whitish grey		Whitish grey	Whitish grey
Papilla condition		Good	Good	Good
		Fair	Fair	Fair
			Poor	

TABLE 4. *The total pairs, the pairs spawned and did not spawned, the total pairs taped in each trial in three different crosses was listed in the table.*

	C x C				C x B				B x B			
Trial	Total pairs	No. pairs spawned	N. pairs taped	N. pairs spawned on tape	Total pairs	No. pairs spawned	N. pairs taped	N. pairs spawned on tape	Total pairs	No. pairs spawned	N. pairs taped	N. pairs spawned on tape
1st	4	4	4	4	3	0	3	0	0	0	0	0
2nd	4	2	4	2	4	0	4	0	4	0	0	0
3rd	6	6	4	4	4	1	3	1	3	1	0	0
4th	5	3	4	0	4	0	4	0	2	0	0	0
5th	12	8	0	0	5	1	4	1	4	2	4	2
Total	31	23	16	10	20	2	18	2	13	3	2	2

FIGURE 1. *Females channel catfish (outside) exhibited rubbing behavior by using her belly and pectoral fin to clean and rub certain site on the aquarium bottom.*

FIGURE 2. *Male channel catfish (inside) exhibited curling behavior by using his tail to cover the female's head although the female was not ready to participate in his activity.*

FIGURE 3. *Channel male (inside) and female (outside) showed dancing behavior. Both male and female wrap their caudal fins over the head of its partner.*

FIGURE 4. *Channel male (outside) showed air bubble release behavior. Air bubbles were released from gills of the channel males.*

FIGURE 5. *Channel female (above) exhibited swim-up behavior as she swam up to the surface of water and gulp the air.*

FIGURE 6. *Channel male (above) exhibited mouth widening behavior by widely opening his mouth as yawn activity.*

RESULTS

Environmental Setting

Overall, water temperature during the experimental period ranged from 28.0 to 31.0^{0}C, and averaged 29.5 ± 0.35^{0}C. Mean water temperatures in five experimental runs for the crosses of C X C, C X B, and B X B combinations were similar ($P > 0.05$) and averaged 29.49 ± 1.24, 29.53 ± 1.38, and 29.44 ± 1.33^{0}C, respectively (Table 5). However, water temperatures for C X C that spawned (n = 23) or did not spawn (n = 8) differed (28.53 ± 0.21 and 30.07 ± 2.13 respectively) but were not different for C X B that spawned and did not spawn (Table 6). Water temperatures were similar in the 1st and 2nd run but higher in the 3rd, 4th, and 5th run (Table 7, $P < 0.0001$). Average temperature was highest in the 4th run (31.91 ± 0.03^{0}C) and lowest in the 2nd run (28.25 ± 0.03^{0}C).

Dissolved oxygen (DO) also showed the same trend for the three combinations. The DO level averaged 5.61 ± 0.44 and was similar in the C X C, C X B, and B X B aquaria (Table 5). The DO concentrations were similar for C X C and C X B treatments that spawned or did not spawn (Table 6). Oxygen concentrations did differ over time. The mean level of oxygen in the 5th run was highest (6.22 ± 0.22 mg L^{-l}) and statistically higher than those in the other runs. The DO level measured in the 3rd run (5.19 ± 0.26 mg L^{-l}) was lowest and different from the 4th and 5th run (Table 7).

Spawning Success by Cross and Mean Metrics of Those That Did or Did Not Spawn

The mean percentages of females releasing eggs were similar (*P* = 0.93), 74.2 ± 44.48% for C X C, 70.0 ± 48.94% for C X B, and 69.2 ± 48.04% for B X B pairs, respectively (Table 8). The crosses differed in the mean percentages of fertilized egg masses (*P* < 0.0001). The mean percent of C X C egg masses that was fertilized (91.3 ± 28.81%) was nearly 6.4 and 2.7 times higher than the percentages of C X B and B X B egg masses fertilized. The mean percentages of C X B and B X B fertilized egg masses were not statistically different, 14.3 ± 36.31 and 33.3 ± 50.0, respectively. The mean percentages of eggs fertilized did not differ significantly (Table 8).

As shown in Table 9 there were no significant differences in mean body weight, girth, width, and length between channel females that spawned and did not spawn in the combinations of channel x channel and channel x blue. Mean body ratios of channel females that spawned and did not spawn were also not significantly different. However, there was significant differences in channel catfish (C X C) those that spawned or did not spawn based upon the ratio of body length-to-width (*P* = 0.03). The mean length-to-width ratios of channel females that spawned and did not spawn were 5.57 ± 0.44 and 6.15 ± 0.73, respectively. Differences in the ratio of body length-to-girth of spawners and non-spawners were more distinct (*P* = 0.0006). Average values of the ratio of body length-to-girth for channel spawners was 1.88 ± 0.01 and for non-spawners, 2.01 ± 0.08 (Table 10).

In most cases, mean body measurements and their ratios for channel or blue male used within a given cross did not differ between spawners and non-spawners. However, in the cross of channel female x channel males there was a difference between male spawners and non-spawners in mean body length-to-head width ratio. The mean value for males in pairs that spawned was 5.39 ± 0.27 while the value for non-spawners was 5.48 ± 0.43 (Table 11).

Spawning Success as a Function of Body Coloration and Papilla Development Categories

Spawning success was not related to the "side of body" color categories used for both males and females for the three crosses attempted (Table 12). Likewise, there were no differences in spawning success as related to female abdominal color category for any cross, or papilla development for either sex in any cross. There were differences in spawning success of male channel catfish by "underside of the head coloration" categories for males paired with channel females. Males in the dark category had a 100% spawning success that differed ($P < 0.05$) from males in the white category (53.9%). Underside of the head coloration for male blue catfish had no relationship to spawning success when paired with blue or channel females.

Spawning Success as a Function of Categories of Body Metrics and Their Ratios

Spawning success rates of the blue and channel males used in the three different combinations (C X C, C X B, and B X B) when evaluated based on categories of weight, length, width, or their ratios (Table 13) did not differ in the majority of categories, except within the category of head width-to-body weight ratio for channel males crossed with channel females. Generally, channel males with a ratio of head width-to-body weight larger than 5.5 had a significantly higher spawning rate (83.50%) than channel males with the ratios of less than 5.5 (42.85%). No category of body metrics or ratio of metrics for blue males was indicative of a higher spawning success rate.

Body metrics categories of channel females in the cross of channel females x blue males did not result in any subsets associated with significantly greater spawning success rates. No relationships were also seen for blue females in the B X B cross. However, in the cross of C X C, the categories of body width, and body girth, ratios had subsets that differed in spawning success rates. Channel females with girths larger than 28 cm had a spawning rate of 89.5%, significantly more (P = 0.03) than channel females having average girth measurements of less than 28 cm. A similar trend was seen on body width. There was a significant difference (P = 0.0091) in spawning rates where 82.6 % of channel females with the body widths larger than 9.0 cm spawned successfully while channel females with widths of less than 9.0 cm had a rate of 25.0% (Table 14).

Categories based on ratios of body metrics had subsets with significantly higher rates of spawning than other subsets in the same category in the C X C cross. For the category length-to-width ratio, 100% of the channel females having the ratio of less than 5.5 spawned, almost doubling the percent of females with the ratio of length-to-width larger than 5.5 (56.7%) (P = 0.0009). Likewise, spawning success rates differed (P = 0.018) for channel females (x channel males) having length-to-girth ratios less than 1.9 relative to those with a ratio of larger than 1.9. Spawning rates were 94.1% and 37.5%, respectively (Table 15).

Effects of Parental Cross on Time to Spawn, Fecundity, and Egg and Fry Quality

Generally, mean time duration of fish to spawn from C X C, C X B, and B X B was significantly different (P = 0.003, Table 16). Time from the resolving dose for C X C pairs that spawned (19.6 ± 0.85 hours) was earlier than C X B and B X B, 21.7 ± 1.41

and 22.2 ± 0.24 hours, respectively. Mean number of eggs g^{-1} of B X B of 23.5 ± 6.19 was statistically lower than the number of eggs g^{-1} of C X B and C X C pairs (P = 0.002). Fecundity of blue females crossed with blue males averaged 3,150 eggs kg^{-1} and was significantly lower than fecundity of C X B and C X C females (P = 0.001). However, the percentage of viable eggs, hatching rate, and the number of fry collected kg $female^{-1}$ did not differ. The percentage of sac fry that survived to swim-up was greater for B X B fry (92.8%) than for C X C (30.1%). The percentage of sac fry from C X B (80.1 %) was not significantly different from percentages of C X B and B X B crosses.

Effects of Brood External Appearance on Time to Spawn, Fecundity, and Egg and Fry Quality

The relationships between external appearances and spawning results of channel females x channel males are described in Table 17. Overall, there were few differences in time when spawning began, the number of eggs per gram, the number of eggs per kg female, percentages of viable eggs, hatching rate, fry survival to swim-up, or the number of fry per kg female that were associated with categories of body coloration, abdominal coloration, and papilla condition. Channel females classified as dark abdomen had 91.09% viable eggs, which was nearly two-times larger than the percentage from females having grey abdomens (P = 0.01). Average number of fry collected per kg female from channel females having poor papilla condition (293 ± 254) was significantly lower (P = 0.049) than the numbers from females classified as having fair and good papilla (2,787 ± 3,385 and 2,312 ± 3,032 fry, respectively).

Weight of channel females at different categories also gave some significant differences. The females weighing less than 1.5 kg had fewer eggs g^{-1} (31.8 ± 4.32 eggs g^{-1}) than females ranging from 1.5 to 2.0 kg (36.9 ± 5.02 eggs g^{-1}). This contributed to fish in this category having the greatest number of egg kg^{-1} female (P = 0.044) when compared to fish weighing less than 1.5 kg, or those that were larger than 2.0 kg. Channel females weighing less than 1.5 kg had a significantly higher mean fry survival rate from sac fry to swim up (56.0 ± 47.10%) than that of channel females weighing more than 2.0 kg (10.50 ± 6.00%). Mean survival to swim-up for fry from 1.5 to 2.0 kg females (25.83%) was not significantly different than that for fry from females weighing less than 1.5 kg or 2.0 kg or more.

The number of eggs g^{-1} also differed significantly within girth and width categories of channel females (Table 18). Females with girths less than 28 cm differed from those with girths of 28 to 30 cm in the number of eggs g^{-1}, (P = 0.04, 32.2 and 37.5 g^{-1}, respectively). Females with larger abdominal widths had a smaller number of eggs g^{-1} compared to females with smaller abdominal measures. The average number of eggs g^{-1} from females having the width of larger than 10 cm (32.0 ± 3.43 eggs g^{-1}) was significantly less (P = 0.011) than that from females with a width of 9.0 to 10 cm (37.5 ± 4.99 eggs g^{-1}).

A difference in number of eggs g^{-1} was also found in length-to-weight ratio between three categories of females (P = 0.028). The average number of eggs g^{-1} of egg mass from females having the ratio of length-to-weight from 30 – 35 was 37.6 ± 4.79 and was significantly distinct from the females with ratios of ≥ 35 or less than ≤ 30 (31.8 ±

4.32 and 32.6 ± 1.95, respectively). There were no significant differences between length-to-girth or length-to-width ratios and larvae and fry characteristics (Table 19).

Overall, external appearance of channel males classified into different categories did not give significant distinctions as to time of 1st eggs, number of eggs g^{-1}, number of egg per kg female, percentages of viable eggs, hatching rate, swim-up rate, and number of swim-up fry kg $female^{-1}$ (Table 20). However, the body measurements of channel males did give several differences aomg categories (P = 0.049, Table 21). Males weighing less than 2.0 kg had an average of 87.1% viable eggs, which was significantly more (P = 0.046) than that from males larger than 2.0 kg (35.8%). The percent of viable eggs from channel males with lengths of more than 60 cm was significantly lower (34.2%) than males with lengths less than 60 cm (87.5%). The ratios of body length-to-weight, length-to-width, and width-to-weight of channel males gave no significant differences between different categories in channel males x females (Table 22).

TABLE 5. *Average water temperature (^{0} C) and dissolved oxygen concentration (mg L^{-1}) of three crosses channel female x channel male (C X C), channel female x blue male (C X B), and blue female x blue male (B X B) during five experimental periods. Values in the same column for the same category but with different letters of x, y, and z are significantly different ($P < 0.05$).*

Combination	Temperature (^{0}C)	DO (mg L^{-1})
C X C	29.49 ± 1.24^x	5.66 ± 0.36^x
C X B	29.53 ± 1.38^x	5.49 ± 0.45^x
B X B	29.44 ± 1.33^x	5.69 ± 0.54^x

TABLE 6. *Mean water temperature (^{0} C) and dissolved oxygen concentration (mg L^{-1}) of channel female x channel male (C X C), and channel female x blue male (C X B) that spawned and did not spawn during five experimental periods. Values in the same column for the same category but with different letters of x, y, and z are significantly different ($P < 0.05$).*

	Temperature (^{0}C)	DO (mg L^{-1})
C X C Spawned	28.53 ± 0.21^x	5.24 ± 0.23^x
C X C Non-spawned	30.07 ± 2.13^y	5.18 ± 0.22^x
C X B Spawned	29.44 ± 0.79^{xy}	5.58 ± 0.63^x
C X B Non-spawned	28.48 ± 0.23^x	5.23 ± 0.23^x

TABLE 7. *Average water temperature (^{0} C) and dissolved oxygen concentration (mg L^{-1}) of each experimental run. Values in the same column for the same category but with different letters of x, y, and z are significantly different (P < 0.05).*

Run	Temperature (^{0}C)	DO (mg L^{-1})
1st	28.45 ± 0.03^{x}	5.40 ± 0.15^{xy}
2nd	28.25 ± 0.03^{x}	5.30 ± 0.23^{xy}
3rd	28.72 ± 0.09^{y}	5.19 ± 0.26^{x}
4th	31.91 ± 0.03^{z}	5.46 ± 0.27^{y}
5th	29.77 ± 0.41^{t}	6.22 ± 0.22^{z}

TABLE 8. *Percent of egg releases and fertilization from three distinct crosses C X C, C X B, and B X B. All females were injected with carp pituitary (10 mg Kg^{-1}) while males were not given any injection. Values in the same column for the same category but with different letters of x, y, and z are significantly different (P < 0.05).*

Cross	N observed	N released	% releasing eggs	N fertilized	% egg masses fertilized	% eggs fertilized
B X B	13	9	69.2^{x}	3	33.3^{x}	55.9^{x}
C X B	20	14	70.0^{x}	2	14.3^{x}	49.2^{x}
C X C	31	23	74.2^{x}	21	91.3^{y}	77.4^{x}

TABLE 9. *Mean metrics for body weight, length, girth and width for channel females given carp pituitary that spawned and did not spawn in two crosses (C X B, and C X C). All values are shown by means ± SDs. Values in the same column for the same category but with different letters of x, y, and z are significantly different (P < 0.05).*

Characteristics	C X B		C X C	
	N	Value	N	Value
Body weight (kg)				
Spawned	2	1.63 ± 0.10^x	23	1.63 ± 0.08^x
Did not spawn	18	1.59 ± 0.32^x	8	1.64 ± 0.13^x
Body length (cm)				
Spawned	2	53.20 ± 2.69^x	23	53.41 ± 3.94^x
Did not spawn	18	53.37 ± 3.07^x	8	54.25 ± 3.42^x
Girth (cm)				
Spawned	2	29.05 ± 2.62^x	23	28.50 ± 2.41^x
Did not spawn	18	27.93 ± 2.42^x	8	27.11 ± 2.44^x
Width (cm)				
Spawned	2	9.90 ± 0.28^x	23	9.64 ± 0.12^x
Did not spawn	18	9.67 ± 0.64^x	8	8.95 ± 0.47^x

TABLE 10. *The values of measurements of channel females given carp pituitary spawned and did not spawn in two crosses (C X B, and C X C). All values are shown by means ± SDs. Values in the same column for the same category but with different letters of x, y, and z are significantly different (P < 0.05).*

Characteristics	C X B		C X C	
	N	Value	N	Value
Length/Weight ratio				
Spawned	2	32.74 ± 0.52^{x}	23	33.96 ± 5.81^{x}
Did not spawn	18	34.68 ± 6.49^{x}	8	34.88 ± 8.71^{x}
Length/Girth ratio				
Spawned	2	1.84 ± 0.08^{x}	23	1.88 ± 0.01^{x}
Did not spawn	18	1.92 ± 0.11^{x}	8	2.01 ± 0.08^{y}
Length/Width ratio				
Spawned	2	5.38 ± 0.12^{x}	23	5.57 ± 0.44^{x}
Did not spawn	18	5.53 ± 0.22^{x}	8	6.15 ± 0.73^{y}
Girth/Width ratio				
Spawned	2	2.93 ± 0.14^{x}	23	2.96 ± 0.13^{x}
Did not spawn	18	2.89 ± 0.15^{x}	8	3.06 ± 0.25^{x}
Girth/Weight ratio				
Spawned	2	17.86 ± 0.43^{x}	23	18.06 ± 2.78^{x}
Did not spawn	18	18.01 ± 2.53^{x}	8	17.30 ± 3.69^{x}
Width/Weight ratio				
Spawned	2	6.10 ± 0.13^{x}	23	6.11 ± 1.00^{x}
Did not spawn	18	6.28 ± 1.15^{x}	8	5.62 ± 0.75^{x}

TABLE 11. *Means for measurements and their ratios of blue and channel males spawned and did not spawn in three crosses (B X B, C X B, and C X C). All values are means ± SDs. Values in the same column for the same category but with different letters of x, y, and z are significantly different (P < 0.05).*

Characteristics	B X B		C X B		C X C	
	N	Value	N	Value	N	Value
Body weight (kg)						
Spawned	3	1.71 ± 0.68^{x}	2	2.22 ± 0.49^{x}	23	1.72 ± 0.27^{x}
Did not spawn	10	1.51 ± 0.38^{x}	18	1.80 ± 0.44^{x}	8	1.91 ± 0.23^{x}
Body length (cm)						
Spawned	3	58.30 ± 6.66^{x}	2	60.85 ± 3.89^{x}	23	57.29 ± 3.12^{x}
Did not spawn	10	55.23 ± 4.10^{x}	18	57.91 ± 4.82^{x}	8	58.04 ± 2.20^{x}
Head width (cm)						
Spawned	3	9.93 ± 1.90^{x}	2	11.25 ± 1.39^{x}	23	10.64 ± 0.79^{x}
Did not spawn	10	9.21 ± 1.12^{x}	18	10.50 ± 1.94^{x}	8	10.65 ± 0.80^{x}
Length/Weight ratio						
Spawned	3	36.81 ± 10.86^{x}	2	27.92 ± 4.36^{x}	23	33.77 ± 4.33^{x}
Did not spawn	10	36.27 ± 7.43^{x}	18	31.09 ± 6.72^{x}	8	30.67 ± 3.22^{x}
Length/ Head width ratio						
Spawned	3	5.93 ± 0.39^{x}	2	5.41 ± 0.10^{x}	23	5.39 ± 0.27^{x}
Did not spawn	10	5.70 ± 0.47^{x}	18	5.59 ± 0.39^{x}	8	5.48 ± 0.43^{y}
Head Width/Weight ratio						
Spawned	3	58.30 ± 6.66^{x}	2	6.10 ± 0.13^{x}	23	6.11 ± 1.00^{x}
Did not spawn	10	55.23 ± 4.10^{x}	18	6.28 ± 1.15^{x}	8	5.62 ± 0.75^{x}

TABLE 12. *Percent spawning rate in three distinct crosses (C X C, C X B, and B X B) related to external appearance of males and females classified into different categories. Values in the same column for the same category but with different letters of x, y, and z are significantly different (P < 0.05).*

	B X B (%)				C X B (%)				C X C (%)			
	N	Female	N	Male	N	Female	N	Male	N	Female	N	Male
Body coloration												
Dark	3	66.70^{x}	4	25.00^{x}	5	20.00^{x}	8	0.00^{x}	17	70.59^{x}	14	78.57^{x}
Grey	6	16.67^{x}	7	28.57^{x}	12	8.33^{x}	12	16.67^{x}	10	80.00^{x}	17	70.59^{x}
White	-	-	-	-	3	0.00^{x}	-	-	4	25.00^{x}	-	-
Abdominal coloration (Female) and Underside head coloration (Male)												
Dark	-	-	3	66.60^{x}	7	14.28^{x}	5	0.00^{x}	13	84.61^{x}	6	100.0^{x}
Grey	-	-	4	0.00^{x}	6	16.67^{x}	4	0.00^{x}	8	62.50^{x}	12	83.30^{xy}
White	-	-	4	25.00^{x}	7	0.00^{x}	11	18.18^{x}	10	60.00^{x}	13	53.86^{y}
Papilla condition												
Good	2	50.00^{x}	-	-	9	11.10^{x}	6	0.00^{x}	13	76.92^{x}	11	72.73^{x}
Fair	7	28.57^{x}	-	-	3	33.30^{x}	14	16.67^{x}	10	80.00^{x}	20	75.00^{x}
Poor	0	-	-	-	8	0.00^{x}	-	-	8	37.50^{x}	-	-

TABLE 13. *Body measurements including body weight, body length, head width, and their ratios for blue and channel males and percent spawning success in three different combinations of C X C, C X B, and B X B. Values in the same column for the same category but with different letters of x, y, and z are significantly different (P < 0.05).*

Classification	B X B		C X B		C X C	
	N	%	N	%	N	%
Weight (kg)						
≤ 1.5	5	20.00^{x}	4	0.00^{x}	8	100.00^{x}
1.5 – 2.0	6	25.00^{x}	6	16.67^{x}	18	72.22^{x}
≥ 2.0	2	0.00^{x}	10	10.00^{x}	5	50.00^{x}
Length (cm)						
≤ 55	9	14.29^{x}	6	0.00^{x}	7	66.67^{x}
55 – 60	3	50.00^{x}	6	16.67^{x}	18	66.67^{x}
≥ 60	1	0.00^{x}	8	12.50^{x}	6	100.00^{x}
Head Width (cm)						
≤ 10	8	25.00^{x}	6	0.00^{x}	9	55.56^{x}
10 – 11	2	50.00^{x}	7	14.28^{x}	10	70.00^{x}
≥ 11	1	0.00^{x}	7	14.28^{x}	12	75.00^{x}
Length/ Weight ratio						
≤ 30	3	33.33^{x}	10	10.00^{x}	8	50.00^{x}
30 – 35	2	0.00^{x}	4	25.00^{x}	12	83.33^{x}
≥ 35	6	33.33^{x}	6	0.00^{x}	11	81.81^{x}
Length/ Head width ratio						
<5.5	4	25.00^{x}	10	20.00^{x}	16	75.00^{x}
≥ 5.5	7	28.57^{x}	10	0.00^{x}	15	60.00^{x}
Width/ Weight ratio						
≤ 5.5	3	33.33^{x}	11	9.00^{x}	7	42.85^{x}
5.5 – 6.0	4	25.00^{x}	5	20.00^{x}	8	75.50^{y}
≥ 6.0	4	25.00^{x}	4	0.00^{x}	16	87.50^{y}

TABLE 14. *Percent spawning success of channel females injected by carp pituitary at 10 mg per kg are compared at different body measurements including body weight, length, girth, and width in two combinations (C X B and C X C). Values in the same column for the same category but with different letters of* x, y, *and* z *are significantly different (P < 0.05).*

Classification	C X B		C X C	
	N	%	N	%
Weight (kg)				
≤ 1.5	2	0.00^{x}	8	75.00^{x}
1.5 – 2.0	11	16.67^{x}	18	72.22^{x}
≥ 2.0	7	0.00^{x}	5	80.00^{x}
Length (cm)				
≤ 50	4	0.00^{x}	6	66.67^{x}
50 –55	8	12.50^{x}	13	84.61^{x}
≥ 55	8	12.50^{x}	12	58.33^{x}
Girth (cm)				
≤ 28	8	12.50^{x}	12	50.00^{x}
28 – 30	8	0.00^{x}	13	84.60^{y}
≥ 30	4	25.00^{x}	6	100.00^{y}
Width (cm)				
≤ 9.0	4	0.00^{x}	8	25.00^{x}
9.0 –10	9	11.11^{x}	12	83.33^{y}
≥ 10	7	14.28^{x}	11	81.81^{y}

TABLE 15. *Percent spawning success of channel females injected by carp pituitary at 10 mg per kg are compared at different body measurement ratios in two combinations (C X B and C X C). Values in the same column for the same category but with different letters of x, y, and z are significantly different (P < 0.05).*

Classification	C X B		C X C	
	N	%	N	%
Length/ Weight ratio				
≤ 30	3	0.00^{x}	8	62.50^{x}
30 – 35	12	16.67^{x}	15	80.00^{x}
≥ 35	5	0.00^{x}	8	75.00^{x}
Length/Width ratio				
≤ 5.5	10	22.22^{x}	11	100.00^{x}
5.5 – 6.0	10	0.00^{x}	14	64.28^{y}
≥ 6.0	0	-	6	33.33^{y}
Length/Girth ratio				
≤ 1.9	10	20.00^{x}	17	94.12^{x}
1.9 – 2.0	8	0.00^{x}	6	66.67^{xy}
≥ 2.0	2	0.00^{x}	8	37.50^{y}
Girth/Width ratio				
≤ 2.9	10	10.00^{x}	8	87.50^{x}
2.9 – 3.0	6	0.00^{x}	14	78.57^{x}
≥ 3.0	4	25.00^{x}	9	55.55^{x}
Girth/weight ratio				
≤ 17	3	0.00^{x}	13	53.84^{x}
17 – 20	13	15.38^{x}	11	81.81^{x}
≥ 20	4	0.00^{x}	7	87.71^{x}
Width/weight ratio				
≤ 5.5	3	0.00^{x}	11	45.45^{x}
5.5 – 7.0	13	15.38^{x}	14	78.50^{x}
≥ 7.0	4	0.00^{x}	6	83.33^{x}

TABLE 16. *The relationships between different crosses and time to 1st spawning, and egg and fry characteristics. All values are showed as means ± SDs. Within column, different values of different categories superscripted by different letters (x, y, and z) are significantly different at* $P < 0.05$.

Cross	N	1st spawning time	No of eggs/g	Number of eggs/kg	% viable eggs	% hatched	% swim-up	No of fry /kg female
B X B	3	22.2 ± 0.24^{x}	23.5 ± 4.73^{x}	$3,156 \pm 1,453^{x}$	85.3 ± 10.35^{x}	46.1 ± 26.05^{x}	92.8 ± 17.2^{x}	$2,277 \pm 2,407^{x}$
C X B	2	21.7 ± 1.41^{xy}	37.3 ± 3.58^{y}	$10,112 \pm 892^{y}$	49.2 ± 37.59^{x}	44.0 ± 19.46^{x}	80.1 ± 15.13^{xy}	$3,223 \pm 3,263^{x}$
C X C	21	19.6 ± 0.85^{y}	35.0 ± 4.53^{xy}	$8,156 \pm 2,215^{y}$	77.4 ± 28.43^{x}	52.3 ± 30.66^{x}	30.1 ± 34.85^{y}	$2, 204 \pm 2,974^{x}$
P-value		0.003	0.002	0.0001	0.342	0.891	0.006	0.897

TABLE 17. *The relationships between appearance and time to 1st spawning, and egg and fry characteristics for channel females in channel x channel treatment injected by carp pituitary 10 mg Kg^{-1}. All values are showed as means ± SDs. Within column, different values of different categories superscripted by different letters (x, y, and z) are significantly different at* P < 0.05.

Criteria	N	1st spawning time	No of eggs/g	Number of eggs/kg	% viable eggs	% hatched	% swim-up	No of fry /kg female
Body coloration								
Dark	12	19.7 ± 1.23^{x}	34.6 ± 4.73^{x}	8,760 ± 2,228^{x}	80.0 ± 27.30^{x}	53.2 ± 32.14^{x}	18.0 ± 26.25^{x}	1,763 ± 2,811^{x}
Grey	18	19.6 ± 0.26^{x}	34.3 ± 3.58^{x}	7,102 ± 2,032^{x}	76.8 ± 31.60^{x}	50.5 ± 32.72^{x}	47.4 ± 42.18^{x}	2,945 ± 3,440^{x}
W. grey	1	19.2 ± 0.00^{x}	46.0 ± 0.00^{y}	9,371 ± 0,000^{x}	49.0 ± 00.00^{x}	58.0 ± 00.00^{x}	37.0 ± 00.00^{x}	1,574± 00.00^{x}
Abdominal coloration								
Dark	11	19.4 ± 0.70^{x}	33.9 ± 4.97^{x}	8,946 ± 1,893^{x}	91.1 ± 53.37^{x}	61.5 ± 24.70^{x}	31.2 ± 36.48^{x}	3,098 ± 3,578^{x}
Grey	5	20.2 ± 1.44^{x}	37.8 ± 4.92^{x}	6,863 ± 1,842^{x}	52.8 ± 31.68^{y}	38.4 ± 26.55^{x}	20.2 ± 16.40^{x}	672 ± 577^{x}
W. grey	5	19.5 ± 0.00^{x}	34.6 ± 4.16^{x}	7,710 ± 2,851^{x}	71.6 ± 40.52^{xy}	46.2 ± 44.46^{x}	37.6 ± 47.50^{x}	1,772 ± 2,552^{x}
Papilla condition								
Good	10	19.5 ± 0.90^{x}	33.7 ± 4.97^{x}	8,641 ± 2,568^{x}	83.3 ± 29.66^{x}	50.2 ± 34.60^{x}	29.2 ± 36.40^{x}	2,312 ± 3,032^{x}
Fair	8	19.7 ± 0.99^{x}	37.0 ± 4.99^{x}	8,228 ± 1,747^{x}	78.9 ± 15.61^{x}	56.4 ± 23.40^{x}	40.6 ± 36.78^{x}	2,787 ± 3,385^{x}
Poor	3	19.5 ± 0.00^{x}	34.0 ± 3.00^{x}	6,345 ± 1,609^{x}	53.3 ± 46.87^{x}	49.0 ± 45.17^{x}	5.0 ± 4.58^{x}	293 ± 254^{y}

TABLE 18. *The relationships between body measurement and time to 1st spawning and egg and fry characteristics for channel females in channel x channel treatment injected with carp pituitary 10 mg Kg^{-1}. All values are showed as means ± SDs. Within column, different values of different categories superscripted by different letters (x, y, and z) are significantly different at* $P < 0.05$.

Criteria	N	1st spawning time	No of eggs/g	Number of eggs/kg	% viable eggs	% hatched	% swim-up	No of fry/kg female
Weight (kg)								
≤ 1.5	5	-	31.8 ± 4.32^{x}	6,543 ± 2,371^{x}	74.6 ± 41.83^{x}	62.2 ± 37.53^{x}	56.0 ± 47.10^{x}	3,650 ± 3,977^{x}
1.5 – 2.0	12	19.7 ± 0.26^{x}	36.9 ± 5.02^{y}	8,939 ± 1,980^{y}	74.8 ± 27.27^{x}	42.2 ± 25.83^{x}	25.8 ± 30.20^{xy}	1,805 ± 2,869^{x}
≥ 2.0	4	19.6 ± 0.95^{x}	33.3 ± 1.50^{xy}	7,821 ± 1,975^{xy}	88.3 ± 10.87^{x}	70.8 ± 30.90^{x}	10.5 ± 6.00^{y}	1,596 ± 1,756^{x}
Length (cm)								
≤ 50	4	-	33.3 ± 3.30^{x}	5,598 ± 1,241^{x}	69.5 ± 46.47^{x}	57.8 ± 41.70^{x}	47.0 ± 49.25^{x}	2,215 ± 2,715^{x}
50 – 55	10	19.7 ± 0.96^{x}	36.4 ± 6.08^{x}	8,860 ± 2,130^{x}	82.8 ± 15.54^{x}	44.6 ± 24.44^{x}	30.3 ± 29.39^{x}	2,611 ± 3,044^{x}
≥ 55	7	19.4 ± 0.00^{x}	34.0 ± 3.21^{x}	8,611 ± 1,892^{x}	74.0 ± 33.70^{x}	60.4 ± 34.48^{x}	20.2 ± 34.95^{x}	2.661 ± 3,449^{x}

Girth (cm)								
≤ 28	6	18.9 ± 0.89^{x}	32.2 ± 3.70^{x}	6,890 ± 2,913^{x}	74.8 ± 41.96^{x}	55.6 ± 36.51^{x}	37.6 ± 47.55^{x}	1,772 ± 2,551^{x}
28 – 30	5	19.8 ± 0.91^{x}	37.5 ± 5.42^{y}	8,753 ± 2,027^{x}	73.1 ± 29.63^{x}	44.8 ± 29.14^{x}	30.4 ± 29.28^{x}	2,168 ± 3,037^{x}
≥ 30	10	19.7 ± 0.44^{x}	33.2 ± 2.23^{xy}	8,282 ± 1,700^{x}	86.5 ± 9.62^{x}	62.3 ± 30.70^{x}	23.3 ± 37.14^{x}	2,643 ± 3,628^{x}
Width (cm)								
≤ 9.0	2	-	36.0 ± 1.41^{xy}	6,144 ± 1,514^{x}	46.5 ± 65.70^{x}	63.6 ± 62.22^{x}	46.0 ± 65.05^{x}	2,980 ± 4,214^{x}
9.0 – 10	10	19.8 ± 0.92^{x}	37.5 ± 4.99^{x}	7,869 ± 2,477^{x}	73.3 ± 29.60^{x}	43.9 ± 29.24^{x}	30.7 ± 27.32^{x}	1,512 ± 1,676^{x}
≥ 10	9	19.4 ± 0.79^{x}	32.0 ± 3.43^{y}	8,921 ± 1,818^{x}	88.7 ± 8.55^{x}	63.7 ± 25.85^{x}	25.9 ± 39.69^{x}	2,820 ± 3,947^{x}

TABLE 19. *The relationships between body ratios and time to 1st spawning and egg and fry characteristics for channel females in channel x channel treatment injected by carp pituitary 10 mg Kg*$^{-1}$. *All values are showed as means ± SDs. Within column, different values of different categories superscripted by different letters (x, y, and z) are significantly different at* P < 0.05.

Criteria	N	1st spawning time	No of eggs/g	Number of eggs/kg	% viable eggs	% hatched	% swim-up	No of fry/kg female
Length/Weight ratio								
≤ 30	5	19.4 ± 0.00^{x}	32.6 ± 1.95^{x}	8,159 ± 1,870^{x}	86.0 ± 10.67^{x}	70.2 ± 26.78^{x}	28.0 ± 39.52^{x}	3,150 ± 3,793^{x}
30 – 35	11	19.6 ± 0.27^{x}	37.6 ± 4.79^{y}	8,886 ± 2,067^{x}	74.6 ± 28.59^{x}	39.8 ± 25.72^{x}	19.3 ± 20.99^{x}	1,118 ± 1,679^{x}
≥ 35	5	19.7 ± 0.26^{x}	31.8 ± 4.32^{x}	6,543 ± 2,371^{x}	74.6 ± 41.83^{x}	62.2 ± 37.53^{x}	56.0 ± 47.16^{x}	3,650 ± 3,977^{x}
Length/Girth ratio								
≤ 1.9	15	19.5 ± 0.85^{x}	34.8 ± 5.05^{x}	8,263 ± 2,226^{x}	85.7 ± 13.53^{x}	59.3 ± 25.90^{x}	37.2 ± 38.45^{x}	2,712 ± 3,293^{x}
1.9 – 2.0	4	20.4 ± 0.00^{x}	34.0 ± 1.63^{x}	7,847 ± 1,949^{x}	84.8 ± 10.60^{x}	52.8 ± 33.21^{x}	18.2 ± 11.10^{x}	1,405 ± 1,782^{x}
≥ 2.0	2	-	38.5 ± 3.12^{x}	7,966 ± 4,091^{x}	0.0 ± 0.00^{y}	0.0 ± 0.00^{x}	0.0 ± 0.00^{x}	0.00 ± 0.00^{x}

Length/Width ratio								
≤ 5.5	11	19.4 ± 0.57^{x}	33.6 ± 5.85^{x}	8,236 ± 2,232^{x}	87.0 ± 13.70^{x}	61.8 ± 22.23^{x}	36.6 ± 40.20^{x}	2,727 ± 3,584^{x}
5.5 – 6.0	9	20.1 ± 1.33^{x}	36.4 ± 0.32^{x}	8,400 ± 2,185^{x}	74.2 ± 29.80^{x}	46.7 ± 35.29^{x}	25.4 ± 28.47^{x}	1,811 ± 2,212^{x}
≥ 6.0	1	-	37.0 ± 0.00^{x}	5,073 ± 0.00^{x}	0.0 ± 0.00^{x}	0.0 ± 0.00^{x}	0.0 ± 0.00^{x}	0.00 ± 0.00^{x}
Girth/Width ratio								
≤ 2.9	7	19.3 ± 0.70^{x}	31.0 ± 4.43^{y}	8,414 ± 2,857^{x}	78.3 ± 35.23^{x}	61.9 ± 32.43^{x}	43.0 ± 46.54^{x}	3,352 ± 4,217^{x}
2.9 – 3.0	11	19.7 ± 0.98^{x}	37.3 ± 4.17^{x}	8,048 ± 1,978^{x}	73.5 ± 28.20^{x}	49.5 ± 27.53^{x}	20.2 ± 20.16^{x}	1,480 ± 1,934^{x}
≥ 3.0	3	20.4 ± 0.00^{x}	36.0 ± 1.00^{x}	7,945 ± 2,156^{x}	89.0 ± 7.80^{x}	41.0 ± 44.00^{x}	36.3 ± 48.95^{x}	2,183 ± 3,284^{x}
Girth/Weight ratio								
≤ 17	7	-	33.9 ± 0.32^{xy}	8,308 ± 1,995^{x}	70.6 ± 33.36^{x}	53.1 ± 41.44^{x}	24.7 ± 34.11^{x}	2,335 ± 3,399^{x}
17 – 20	9	19.6 ± 0.95^{x}	37.7 ± 5.07^{x}	8,932 ± 2,036^{x}	81.6 ± 15.97^{x}	46.0 ± 23.24^{x}	19.8 ± 21.90^{x}	1,300 ± 1,815^{x}
≥ 20	5	19.7 ± 0.26^{x}	31.8 ± 4.32^{y}	6,543 ± 2,371^{x}	74.6 ± 41.80^{x}	62.2 ± 37.50^{x}	56.0 ± 47.16^{x}	3,650 ± 3,977^{x}
Width/Weight ratio								
≤ 5.5	5	-	34.6 ± 3.29^{x}	8,428 ± 2,185^{x}	70.6 ± 40.57^{x}	56.0 ± 41.44^{x}	8.4 ± 30.59^{y}	1,277 ± 1,680^{x}
5.5 – 6.0	11	19.6 ± 0.95^{x}	36.6 ± 5.16^{x}	8,764 ± 1,977^{x}	81.6 ± 14.30^{x}	46.0 ± 23.24^{x}	28.2 ± 47.16^{xy}	1,969 ± 2,944^{x}
≥ 6.0	5	19.7 ± 0.26^{x}	31.8 ± 4.32^{x}	6,543 ± 2,371^{x}	74.6 ± 41.80^{x}	62.2 ± 37.50^{x}	56.0 ± 41.44^{x}	3,650 ± 3,977^{x}

TABLE 20. *The relationships between body coloration and time to 1st spawning, and egg and fry characteristics for channel males in the channel x channel treatments. All values are showed as means ± SDs. Within column, different values of different categories showed by different letters (x, y, and z) are significantly different at* $P < 0.05$.

Classification	N	1st spawning time	Number of eggs/kg	% viable eggs	% hatched	% swim-up	No of fry/female
Body coloration							
Dark	9	20.1 ± 1.09^x	$7,680 \pm 2,145^x$	68.2 ± 39.87^x	48.22 ± 35.64^x	21.0 ± 29.91^x	$1,462 \pm 2,128^x$
Grey	12	19.3 ± 0.56^x	$8,512 \pm 2,291^x$	84.2 ± 13.81^x	55.50 ± 27.79^x	36.9 ± 37.85^x	$2,761 \pm 3,464^x$
Underside of head							
Dark	6	20.1 ± 1.33^x	$7,465 \pm 2,466^x$	72.5 ± 37.41^x	56.83 ± 35.70^x	31.7 ± 32.10^x	$2,544 \pm 2,420^x$
Grey	10	19.4 ± 0.80^x	$8,503 \pm 2,288^x$	80.0 ± 29.15^x	44.90 ± 25.15^x	28.3 ± 37.66^x	$1,784 \pm 3,079^x$
White	5	19.4 ± 0.15^x	$8,291 \pm 2,028^x$	77.8 ± 17.56^x	62.00 ± 37.79^x	31.8 ± 39.44^x	$2,639 \pm 3,835^x$
Papilla condition							
Good	8	19.0 ± 0.62^x	$9,724 \pm 1,458^x$	75.0 ± 31.49^x	48.50 ± 32.00^x	36.9 ± 42.00^x	$3,058 \pm 3,512^x$
Fair	13	20.0 ± 0.71^x	$7,190 \pm 2,071^x$	78.8 ± 27.56^x	54.77 ± 31.00^x	25.9 ± 30.66^x	$1,680 \pm 2,593^x$

TABLE 21. *The relationships between body measurements and time to 1st spawning, and egg and fry characteristics for channel males in the channel x channel treatments. All values are showed as means ± SDs. Within column, different values of different categories showed by different letters (x, y, and z) are significantly different at* $P < 0.05$.

Classification	N	1st spawning time	Number of eggs/kg	% viable eggs	% hatched	% swim-up	No of fry/kg female
Weight (kg)							
≤ 1.5	5	19.1 ± 1.15^x	8,960 ± 2,894^x	88.0 ± 11.34^x	47.8 ± 29.43^x	27.0 ± 48.02^x	2,456 ± 4,006^x
1.5 – 2.0	12	19.7 ± 0.87^x	7,914 ± 1,880^x	86.8 ± 9.23^x	64.0 ± 26.82^x	32.8 ± 33.90^x	2,654 ± 2,973^x
≥ 2.0	4	19.8 ± 0.81^x	7,875 ± 2,676^x	35.8 ± 45.18^y	23.3 ± 28.44^y	13.5 ± 17.59^x	541 ± 743^x
Length (cm)							
≤ 55	6	19.1 ± 1.15^x	8,908 ± 2,196^x	86.3 ± 10.22^x	49.9 ± 35.47^x	24.4 ± 35.53^x	2,039 ± 3,661^x
55 – 60	11	19.9 ± 0.90^x	7,887 ± 2,148^x	88.2 ± 9.86^x	59.3 ± 22.57^x	40.0 ± 37.94^x	2,913 ± 3,016^x
≥ 60	4	19.4 ± 0.21^x	7,764 ± 2,772^x	34.2 ± 42.61^y	36.7 ± 44.24^x	11.4 ± 17.55^x	505 ± 743^x
Width (cm)							
≤ 10	7	19.9 ± 0.00^x	8,353 ± 2,476^x	86.4 ± 10.35^x	52.8 ± 31.30^x	37.2 ± 47.82^x	2,471 ± 3,994^x
10 – 12	9	19.6 ± 0.89^x	8,581 ± 2,042^x	74.8 ± 31.70^x	52.1 ± 30.75^x	37.7 ± 38.08^x	2,688 ± 3,293^x
≥ 12	5		7,468 ± 2,419^x	74.1 ± 34.40^x	52.1 ± 35.19^x	15.3 ± 13.96^x	1,393 ± 1,790^x

TABLE 22. *The relationships between body ratios and time to 1st spawning, and egg and fry characteristics for channel males in the channel x channel treatments. All values are showed as means ± SDs. Within column, different values of different categories showed by different letters (x, y, and z) are significantly different at* $P < 0.05$.

Classification	N	1st spawning time	Number of eggs/kg	% viable eggs	% hatched	% swim-up	No of fry/ kg female
Length/Weight ratio							
≤ 30	4	20.3 ± 1.02^x	6,477 ± 1,962^x	51.5 ± 39.14^x	37.0 ± 26.69^x	18.8 ± 15.52^x	3,150 ± 3,793^x
30 – 35	9	19.4 ± 0.57^x	8,854 ± 1,786^x	78.56 ± 30.04^x	50.78 ± 32.99^x	38.1 ± 37.93^x	1,118 ± 1,679^x
≥ 35	8	19.2 ± 0.85^x	8,209 ± 2,546^x	88.9 ± 8.92^x	61.9 ± 30.20^x	26.3 ± 39.39^x	1,827 ± 3,188^x
Length/Width ratio							
<5.5	12	19.6 ± 1.02^x	8,025 ± 2,299^x	80.2 ± 27.03^x	50.1 ± 29.47^x	25.7 ± 29.10^x	2,163 ± 2.864^x
≥ 5.5	9	19.5 ± 0.34^x	8,329 ± 2,222^x	73.6 ± 31.35^x	55.4 ± 33.96^x	36.0 ± 42.30^x	2,259 ± 3,290^x
Width/Weight ratio							
≤ 5.5	3	19.2 ± 0.00^x	8,416 ± 2,996^x	78.3 ± 35.23^x	61.9 ± 32.43^x	43.0 ± 46.54^x	3,352 ± 4,217^x
5.5 – 6.0	5	19.9 ± 0.50^x	7,472 ± 1,599^x	73.5 ± 28.20^x	49.5 ± 27.53^x	20.2 ± 20.16^x	1,480 ± 1,934^x
≥ 6.0	13	19.5 ± 1.05^x	8,358 ± 2,361^x	89.0 ± 7.80^x	41.0 ± 44.00^x	36.3 ± 48.95^x	2,183 ± 3,284^x

BEHAVIORAL RESULTS

The results are presented as to the sex being considered for all behaviors except dancing, the activity observed, the species of male used, and whether or not the male spawned. The activity is described as what percent of the fish showed this activity at each time interval, how frequently/h it was observed, and what was the average duration of the activity. Dancing results are presented by the cross, the percentage of pairs exhibiting this behavior for those that spawned or did not spawn, and the frequency/h and the average duration.

Dancing behavior

Dancing behavior differed depending on the species of males paired with the channel females. This behavior was observed in 100% of C X C spawners from 6 to 1 h prior to spawning, and this was significantly higher than the percentage of C X C spawners dancing at 12 h (60%, P = 0.02, Table 23). Dancing was performed by 25% of the C X C non-spawners at all time intervals. At 6 to 1 h prior to spawning, 100 % of the C X C spawners exhibited the dancing behavior while 25% of the C X C non-spawners exhibited this behavior (P = 0.047).

Percentage of C X B spawners performed dancing behavior was similar from 6 to 1 h, averaged 66.67%. Dancing was not observed at 12 to 6 h prior to the mean spawning time in C X B non-spawners, and even though 20% of the pairs at 3 and 1 h exhibited this behavior. There were no statistical differences by time period ($P > 0.05$, Table 23). C X B spawners and non-spawners showed significant differences in dancing behavior at 3 h ($P = 0.0002$) with 100% of the spawners exhibiting this behavior while 20% of the non-spawners did. No differences were found between C X B spawners and non-spawners at the other times. Dancing was observed from 6 to 1 h prior to spawning in 100% of the C X C that spawned and the rate was similar ($P = 0.12$) to that seen in C X B that spawned (66.67%), (Table 23).

The average frequency (1.40 ±1.90 times/h) of dancing by C X C spawners at 12 h prior to spawning was significantly less than the average frequency (6.77 ± 1.27/h) observed from 6 to 1 h ($P = 0.027$). Dancing frequencies did not vary as a function of time interval for C X C non-spawners, C X B spawners or non- spawners. C X C spawners and non-spawners showed significant differences in the dancing frequency from 6 to 1 h. Average frequency/h/fish for C X C spawners was 6.77 ± 1.27 and was more than that of C X C non-spawners (1.08 ± 0.31), ($P = 0.002$, Table 23). No differences in the dancing activity were seen between C X B spawners or those that did not spawn at all time intervals. At 6 and 3 h the average frequency of dancing by C X C spawners was 6.65 ± 1.77 and was 6.5 times more than that of C X B spawners (Table 23).

Mean time durations of dancing activity by C X C spawners and non-spawners; and C X B spawners and non-spawners were not significantly different among time intervals. At 12 h prior to spawning duration of dancing activity by C X C that spawned was 32.75 ± 12.98 sec. and was significantly higher than the C X C non-spawners (14.00 ± 4.06 sec., $P = 0.039$). No significantly differences in time duration were exhibited by C

X C spawners and C X C non-spawners at other times. Average durations of dancing by C X B that spawned or did not spawn were similar, 25.02 and 25.84 sec., respectively at 3 and 1 h prior to spawning.

Rubbing Behavior

Males

Rubbing was observed 1 h before spawning in 30% of the channel males and 50% of the blue males that spawned with no significant differences between channel and blue males that spawned vs. those that did not. This behavior was not observed at any other time for the males that spawned. For non-spawners, rubbing was also seen at 12 and 6 h prior to spawning in 25% of channel males, and 20% of blue males at 3 and 1 h. No significant differences in frequency or duration of rubbing by channel males that spawned or did not spawn were found (Table 24). Mean rubbing frequency/h/fish for channel males that spawned was 0.60 ± 1.07 at 1 h prior to spawning and was similar to those of blue males that spawned and did not spawn (2.00 ± 2.83, 0.30 ± 0.67, respectively, $P > 0.05$).

Females

Rubbing activity was observed at 12 h in 20% of channel females that spawned when paired with channel males, but 93.33% of the females displayed this trait at 6 and 1 h ($P < 0.0001$, Table 25). Channel females did not spawn showed no difference in rubbing activity from 6 to 1 h ($P > 0.05$). Occurrence by channel females paired with channel males that spawned or did not spawn was not different at any time interval. The percent of females exhibiting rubbing was similar for females that spawned (75.0%) or did not (35%) when paired with blue males ($P > 0.05$, Table 25). Likewise, for C X B there were no differences in occurrences of rubbing as a function of time interval for spawners or non-spawners.

Rubbing frequency/h/fish for females that spawned when paired with channel males was 0.30 ± 0.67 at 12 h prior to spawning and significantly increased to 5.40 ± 3.66 at 3 h and 13.10 ± 6.19 at 1 h prior to spawning ($P = 0.0014$). Rubbing frequencies/h of C X C non-spawners, and C X B spawners and non-spawners averaged 0.83; 1.63; and 0.65, respectively. There was a significant difference in average frequency/h of females that spawned and did not spawned at 3 and 1 h ($P < 0.05$). Average rubbing frequency/h of C X C that spawned in the 3 and 1 h periods was 9.25 and was 15 times higher than that for the non-spawners. Females paired with blue males whether they spawned or not showed no difference in rubbing frequency at any time period. Rubbing frequency of C X C did not differ from that of C X B at 12 or 6 h prior to spawning. There were differences between females that spawned when paired with channel or blue males during 3 and 1 h periods ($P = 0.03$, Table 25).

There were no differences in rubbing duration between time intervals for females paired channel or blue males (Table 25). Rubbing duration did not differ for C X C that spawned or did not spawn. This was also true for C X B.

Curling Behavior

<u>Males</u>

Curling was a characteristic exhibited by males at all time intervals. Channel catfish males displayed this behavior at all time intervals whether or not they spawned. At 12 h prior to spawning 60% of the males that spawned exhibited this activity while 90 to 100% of males showed this activity at other times (P = 0.039, Table 26). Non-spawning males showed this activity at all time intervals with no difference between time intervals (Table 26). For the time periods of 6, 3, and 1 h before the time of spawning more channel males that spawned (93.33%) exhibited the curling behavior than did those male did not spawn (41.67%, P = 0.005). No significant differences in the occurrence of curling were found between channel male spawners (85.0%) and blue male spawners (75.0%) (P = 0.57, Table 26).

The frequencies of curling/h for channel males that spawned differed by time period (P = 0.0002, Table 26). Average frequency of this activity at 12 h before spawning was 2.90 ± 3.41 and was not different from the mean number at 6 h (7.80 ± 5.00), but significantly lower than mean frequencies at 3 and 1 h prior to spawning (15.00 ± 9.73 and 22.70 ± 14.47, respectively). Curling frequencies by channel spawners were significantly higher than those of channel non-spawners at 6, 3, and 1 h prior to spawning. Mean frequency/h of channel spawners at the three time intervals was 15.16 while the mean for channel non-spawners was 0.50 (P = 0.027). Curling frequencies/h/fish by channel males that spawned (13.53 ± 9.98) were similar to those of blue spawners (5.67 ± 4.54) at 12, 3, and 1 h prior to spawning (P = 0.28). A difference in frequency of this activity by channel and blue spawners was seen at 6 h. Frequency of curling by channel males that spawned was eight times as much as that of blue males.

Duration of this activity by channel male spawners was not different from channel males that did not spawn and averaged 9.36 sec. and 14.25 sec., respectively (P = 0.44, Table 26). The duration of blue male spawners did not differ from blue non-spawners at all time intervals. This was similar for channel and blue spawners as no differences in duration of curling activity were found at any time interval.

Females

Curling behavior of channel females paired with channel or blue males that spawned or did not spawn was not different between time intervals. At most observed times, the occurrence of the curling activity by female that spawned when paired with channel males did not differ from that of female non-spawners (Table 27). However, at 3 h prior to spawning, there was a significant increase in occurrence of this behavior as 70% of spawned channel females exhibited curling activity while 25% of channel females that did not spawn performed this activity. Curling activity was observed in 100% of channel female spawners paired with blue males and 13.33% channel female non-spawners paired with blue males at all time intervals (P < 0.0001, Table 27). Channel female spawners showed no significant differences between time intervals whether they were paired with channel or blue males.

Frequency of curling/h/fish for C X C that spawned differed at 12 and 1 h (0.90 ± 1.37 at 12 h and 4.00 ± 4.42 at 1 h, P = 0.043). No significant differences between time intervals were found in the frequency of curling/h for C X C pairs that did not spawn

(Table 27). Channel females paired with blue males that spawned or did not spawn showed similar frequencies/h of curling across the time periods. There were no significant differences in mean frequency/h/fish between channel female spawners or non-spawners when paired with channel or blue males. At 3 h prior to spawning, the frequency/h by C X C female spawners of 1.70 ± 1.44 was less than that of C X B female spawners (5.50 ±4.95).

The duration of curling at 3 h by females paired with channel males differed depending on if they spawned or not. The average duration for female spawners was 7.76 ± 4.16 sec. and was less than the 26.79 ± 13.03 sec. exhibited by female non-spawners ($P = 0.018$, Table 27). No differences were seen between spawners and non-spawners at other times. The duration at 12 h for channel females that spawned when paired with blue males was 18.77 ± 6.72 sec. and was less the mean duration by non-spawning females paired blues males (30.63 sec.) at the same time period. From 6 to 1 h prior to spawning, channel females paired with blue males that spawned or did not spawn showed similar curling duration. The duration of curling behavior by C X C spawners averaged 8.24 ± 1.28 sec. and was less than the mean duration of C X B spawners (15.33 ± 3.48 sec., Table 27).

Air Bubble Release Behavior

Males

Air bubbles were released by channel catfish males at all time intervals, whether or not they spawned, 90.0% and 75.0% respectively ($P = 0.22$), with no differences between time intervals (Table 28). There were no differences between blue spawners and non-spawners in this behavior. An average of 90% of the observed channel male spawners released air bubbles and 100% blue male spawners (Table 28).

There was a significantly difference in release frequency/h by channel male spawners. The average frequency of air bubble release by channel males that spawned was 14.50 ± 14.57 times/h at 1h and was three times more that than the mean frequency at 12 h (P = 0.036). Non-spawning blue males showed a similar pattern with a mean frequency/h at 12 h of 2.30 ± 1.89 that was significantly less than the number at 1 h (5.60 ± 4.17). There were no differences in frequency of air releases by channel male spawners and non-spawners from 12 to 3 hours. Mean frequencies of spawners and non-spawers were 6.80 and 2.67, respectively. At 1 h prior to spawning, there was a significant difference in air release frequency between channel males that spawned (14.5 ± 14.5) or did not spawn (0.50 ± 0.63, $P < 0.05$). Air release by blue males that spawned or did not spawn had similar frequencies from 12h to 6 h, but at 3 to 1 h pre-spawning the frequency/h of this activity by blue male spawners averaged 24.25 that was significantly higher than that of blue male non-spawners 4.40 (P = 0.019). In a comparison of channel spawners and blue spawners, no difference in frequency/h was found (Table 28).

Duration of air releasing behavior of channel male spawners was not different from channel males did not spawn and averaged 1.08 for spawned channels and 1.0 sec., for non-spawning channels. Similarly, time duration of blue male spawners did not differ from blue non-spawners at all time intervals. Duration of air release by channel male spawners and blue spawners was similar.

Females

Air releasing behavior exhibited by channel females paired with channel males that spawned or did not spawn was not different between time intervals. This was also true for channel x blue that spawned or did not spawn. There were no differences in air bubble release by C X B spawners and non-spawners, 100% and 65%, respectively. Likewise, there was no difference in air bubble release by C X C spawners vs C X B spawners (Table 29).

There was a significant difference in frequency/h by female spawners paired with channel males at 1 h and other times (P = 0.025, Table 29). Average frequency of air release by spawned females at 1 h of 21.60 ± 13.0 times/h was more than the average frequency from 12 to 3 h (6.33 ± 2.90). No difference in air releasing activity by females non-spawners paired with channel males was found between time intervals (Table 29). Frequency of this activity by C X B spawners was similar at all time intervals. C X B non-spawners showed a difference in the frequency at 1 h (4.20 ± 3.94) and 12 h (1.20 ± 1.75). There were no differences in frequency of air releasing behavior by channel female spawners and non-spawners from 12 to 3 h, but the frequency at 1 h by female spawners of 21.60 ± 13.01 times/h differed from that of the non-spawners (3.25 ± 3.81). C X B spawners and non-spawners had similar frequencies at any time (P = 0.19, Table 29).

Duration of air releases by channel female spawners or non-spawners was not different at any time interval. The duration of releases by channel females paired with channel males that spawned or did not spawn averaged 1.04 sec. and 1.0 sec., respectively. Channel females paired with blue males that spawned or did not spawn showed similar times as that of channel females paired with channel males. There were no differences in air release duration between C X C and C X B female spawners.

Swim-up Behavior

<u>*Males*</u>

Swim-up behavior was shown by channel and blue males at all time intervals, whether they were spawners or non-spawners. From 12 to 1 h channel males that spawned exhibited this behavior as often as did non-spawning channel males, 77.5% and 81.3% respectively, (P = 0.71, Table 30). There were no significant differences in the occurrence of this behavior by blue males that spawned or did not spawn at all time intervals. The occurrence of this behavior was seen in 77.5% channel spawners and 87.5% blue males that spawned (P = 0.52, Table 30).

Frequencies/h/fish of behavior by channel male spawners, non-spawners, and spawned blue males were not significantly different between time intervals. However, the frequency of blue non-spawners at 12 h was 2.10 ± 1.85 and was distinct from the average of 7.27 ± 1.07 from 6 to 1 h prior to spawning by blue males ($P < 0.05$). Channel spawners and non-spawners showed no significant difference in frequency/h/fish overall (Table 30). From 12 to 3 h, average frequency/h/fish of blue spawners and non-spawners was similar, but at 1h the frequency by the spawners (32.0 ± 31.0) was significantly higher than that of blue non-spawers (7.50 ± 4.22). Generally, blue spawners had a higher frequency of swim-up activity than channel males spawned at 6 and 1 h prior to spawning. Average swim-up behavior frequency of channel male spawners was 3.4 ± 0.85 and was 22.0 ± 14.1for blue males spawned (P = 0.049, Table 30).

Average duration of the swim-up activity was 1.03 sec. for channel males that spawned and was similar to that of male channel non-spawners (1.0 sec.). The duration of swim-up behavior by blue spawners averaged 2.11 sec and did not differ from that of blue non-spawners (1.58 sec.). No difference in time duration of this activity by channel spawners and blue spawers was found.

Females

This behavior occurred similarly in C X C and C X B, spawners and non-spawners, at all time intervals. There were no significant differences in occurrence of swim-up behavior by channel females that spawned or did not spawn whether paired with channel males or blue males at any time.

Frequency/h/fish for channel female spawners or non-spawners paired channel males was not different between time intervals (Table 31). There was no difference of swim-up frequency/h by channel female spawners and non-spawners paired with channel males at any time ($P = 0.60$). Frequency/h by C X B female spawners at 1 h averaged 24.50 ± 34.65 and was significantly higher than frequencies at other times. There were no differences in swim-up frequency/h by channel female spawners and non-spawners when paired with blue males from 12 to 3 h ($P = 0.52$). At 1 h, there was a significant difference between channel spawners and non-spawners (24.50 ± 34.65 and 5.90 ± 7.53, respectively). Likewise, channel females that spawned when paired with blue males had a higher frequency of this activity at 1 h compared to female spawners paired with channel males (Table 31).

Duration of swim-up behavior of channel female spawners or non-spawners was not different at any time interval. The duration of channel females paired channel males spawned and did not spawned averaged 1.16 sec. and 1.0 sec. respectively. Durations seen with channel females paired with blue males that spawned or did not spawn (1.57 sec. for C X B spawners and 2.17 sec. for C X B non-spawners) were similar to channel females paired with channel males. There were no differences in the duration of swim-up behavior between C X C and C X B female spawners.

Swim-up activities by channel females spawners and non-spawners were similar in occurrence, frequency, and duration at all time intervals (Table 31).

Mouth Widening Behavior

Males

The mouth widening behavior occurred at 12 h prior to spawning in 30% of channel male spawners and was significantly less than the 83.33% seen from 6 to 1 h ($P = 0.045$, Table 32). Channel non-spawners or blue spawners or non-spawners did give similar occurrences of mouth widening behavior among time intervals. No significant differences in the occurrence of mouth widening by channel spawners and non-spawners between time intervals, blue males that spawned and did not spawn, and spawned channels and spawned blues were found at any time (Table 32).

Mean mouth widening frequency/h/fish for channels that spawned was 3.40 ± 1.66 and was similar to that of channel non-spawners (1.00 ± 0.54) and blue spawners

(3.25 ± 2.53) from 12 to 1 h prior to spawning. The frequency of this behavior by blue spawners did not differ from that of blue non-spawners at any time (*P* > 0.05).

Duration of this activity of channel male spawners was not different from channel males that did not spawn and averaged 1.00 sec. for spawned channels and non-spawning channels. The duration of mouth widening by blue male spawners did not differ from blue non-spawners at all time intervals (1.00 sec. for blues spawned or did not spawn). This was similar for channel and blue spawners as no significant differences in duration of mouth opening behavior were found at any time interval (Table 32).

Females

Mouth widening was observed in 100% of the spawning C X C females at 6 to 3 h prior and in 25% of C X C females that did not spawn (Table 33). No significant differences were seen at other times between C X C females that spawned or did not spawn. C X B females that spawned or did not spawn had similar occurrences at any time interval. Likewise, this behavior was observed in 100% of C X C spawners and 75% of C X B spawners with no differences between them at any time (*P* = 0.13, Table 33).

Frequency of this activity from spawned channel females paired with channel males was 0.80 ± 1.32 and significantly less than average frequency from other times (Table 33). The frequency/h/fish by channel females that spawned or did not spawn when they were paired channel males or blue males showed no significant differences at any time interval. The frequency of channel females that spawned when paired with channel males averaged 2.65 and was similar to that of channel females that spawned when paired with blue males (2.73) (*P* = 0.20, Table 33).

Duration of this activity from channel female spawners or non-spawners paired with channel or blue males was 1.0 sec. Thus, there was no differences in mouth widening duration by female spawners or non-spawners at any time interval. Channel female spawners or non-spawners as they paired channel males or blue males also showed similar duration of this behavior.

TABLE 23. *Occurrence (% fish), frequency (times/h), and duration (sec.) of dancing activity of blue males and channel males in two crosses (channel x channel, and channel x blue) that spawned and did not spawn were compared between four distinct time intervals (12, 6, 3, and 1 hour prior to spawning)*. Within the column, values with different letters of x, y, z were significant different at P < 0.05. Within row, values with different letters (a, b, c) were significantly different at P* < 0.05.

	Time interval prior to first spawning			
Characteristics	12h	6h	3h	1h
Occurrence (%)				
C X C Spawned	60.0 ± 51.64^{ax}	100.0 ± 0.00^{bx}	100.0 ± 0.00^{bx}	100.0 ± 0.00^{bx}
C X C Non-spawned	25.0 ± 50.00^{axy}	25.0 ± 50.00^{ay}	25.0 ± 50.00^{ay}	25.0 ± 50.00^{ay}
C X B – Spawned	0.0 ± 0.00^{ay}	50.0 ± 70.71^{axy}	100.0 ± 0.00^{ax}	50.0 ± 70.71^{axy}
C X B Non-spawned	0.0 ± 0.00^{ay}	0.0 ± 0.00^{ay}	20.0 ± 42.16^{ay}	20.0 ± 42.16^{ay}
Frequency (per hour)				
C X C Spawned	1.4 ± 1.90^{ax}	5.4 ± 2.46^{bx}	7.9 ± 2.88^{cx}	7.0 ± 2.10^{bcx}
C X C Non-spawned	0.5 ± 1.00^{ax}	1.0 ± 2.00^{ay}	1.0 ± 2.00^{ay}	1.3 ± 2.50^{ay}
C X B – Spawned	-	1.0 ± 1.41^{ay}	1.5 ± 0.71^{ay}	13.5 ± 1.91^{ax}
C X B Non-spawned	-	-	0.3 ± 0.67^{ay}	0.3 ± 0.67^{ay}
Duration (seconds)				
C X C Spawned	32.8 ± 12.98^{ax}	21.6 ± 9.86^{ax}	17.5 ± 6.53^{ax}	20.0 ± 8.51^{ax}
C X C Non-spawned	14.0 ± 4.06^{ay}	21.3 ± 3.54^{ax}	15.3 ± 3.30^{ax}	26.0 ± 5.66^{ax}
C X B – Spawned	-	29.5 ± 2.12^{ax}	30.7 ± 7.02^{ax}	19.4 ± 9.35^{ax}
C X B Non-spawned	-	-	30.7 ± 7.50^{ax}	21.0 ± 17.78^{ax}

* Non-spawners were observed at the time intervals based on the mean times for the spawners

TABLE 24. *Occurrence (% fish), frequency (times/h), and duration (sec.) of rubbing activity of blue males and channel males in two crosses (channel x channel, and channel x blue) that spawned and did not spawn were compared between four distinct time intervals (12, 6, 3, and 1 hour prior to spawning)*. Within the column, values with different letters of x, y, z were significant different at P < 0.05. Within row, values with different letters (a, b, c) were significantly different at P < 0.05.*

	Time interval prior to first spawning			
Characteristics	12h	6h	3h	1h
Occurrence (%)				
C X C Spawned	0.0 ± 0.00^{ax}	0.0 ± 0.00^{ax}	0.0 ± 0.00^{ax}	30.0 ± 48.30^{ax}
C X C Non-spawned	25.0 ± 50.00^{ax}	25.0 ± 50.00^{ax}	0.0 ± 0.00^{ax}	0.0 ± 0.00^{ax}
C X B Spawned	0.0 ± 0.00^{ax}	0.00 ± 0.00^{ax}	0.0 ± 0.00^{ax}	50.0 ± 70.71^{ax}
C X B Non-spawned	0.0 ± 0.00^{ax}	0.0 ± 0.00^{ax}	20.0 ± 42.16^{ax}	20.0 ± 42.16^{ax}
Frequency (per hour)				
C X C Spawned	-	-	-	0.6 ± 1.07^{x}
C X C Non-spawned	0.3 ± 0.31^{a}	0.3 ± 0.31^{a}	-	-
C X B Spawned	-	-	-	2.0 ± 2.83^{x}
C X B Non-spawned	-	-	0.2 ± 0.42^{a}	0.3 ± 0.67^{ax}
Duration (seconds)				
C X C Spawned	-	-	-	2.4 ± 1.34^{x}
C X C Non-spawned	5.0 ± 0.00^{a}	3.0 ± 0.00^{a}	-	-
C X B Spawned	-	-	-	5.3 ± 2.06^{xy}
C X B Non-spawned	-	-	10.5 ± 3.54^{a}	7.3 ± 0.35^{ay}

* Non-spawners were observed at the time intervals based on the mean times for the spawners

TABLE 25. *Occurrence (% fish), frequency (times/h), and duration (sec.) of rubbing activity of channel females in two crosses (channel x channel, and channel x blue) that spawned and did not spawn were compared between four distinct time intervals (12, 6, 3, and 1 hour prior to spawning)*. Within the column, values with different letters of x, y, z were significant different at $P < 0.05$. Within row, values with different letters (a, b, c) were significantly different at value $P < 0.05$.*

Characteristics	Time interval prior to first spawning			
	12h	6h	3h	1h
Occurrence (%)				
C X C Spawned	20.0 ± 42.16^{ax}	80.0 ± 42.16^{bx}	100.0 ± 0.00^{bx}	100.0 ± 0.00^{bx}
C X C Non-spawned	0.0 ± 0.00^{ax}	25.0 ± 50.00^{abxy}	50.0 ± 57.74^{abxy}	75.0 ± 50.00^{bxy}
C X B Spawned	50.0 ± 70.71^{ax}	100.0 ± 0.00^{axy}	50.0 ± 70.71^{axy}	100.0 ± 0.00^{axy}
C X B Non-spawned	20.0 ± 33.33^{ax}	30.0 ± 44.10^{ay}	50.0 ± 52.70^{ay}	40.0 ± 50.00^{ay}
Frequency (per hour)				
C X C Spawned	0.3 ± 0.67^{ax}	3.1 ± 2.68^{abx}	5.4 ± 3.66^{bx}	13.1 ± 6.19^{cx}
C X C Non-spawned	-	1.3 ± 1.56^{axy}	0.5 ± 0.63^{ay}	0.8 ± 0.69^{ay}
C X B Spawned	1.0 ± 1.41^{ax}	1.0 ± 0.00^{axy}	1.0 ± 1.41^{ay}	3.5 ± 0.71^{ay}
C X B Non-spawned	0.2 ± 0.42^{ax}	0.5 ± 0.97^{ay}	1.0 ± 1.33^{ay}	0.8 ± 1.14^{ay}
Duration (seconds)				
C X C Spawned	6.8 ± 1.06^{ax}	8.3 ± 3.99^{ax}	7.6 ± 3.27^{ax}	6.3 ± 2.60^{ax}
C X C Non-spawned	-	10.4 ± 0.00^{ax}	7.0 ± 1.41^{axy}	8.7 ± 4.73^{ax}
C X B Spawned	6.5 ± 0.00^{ax}	3.0 ± 2.83^{ax}	4.0 ± 0.00^{axy}	7.2 ± 0.65^{ax}
C X B Non-spawned	3.5 ± 3.54^{ax}	9.3 ± 9.71^{ax}	2.3 ± 1.48^{ay}	8.7 ± 5.93^{ax}

* Non-spawners were observed at the time intervals based on the mean times for the spawners

TABLE 26. *Occurrence (% fish), frequency (times/h), and duration (sec.) of curling activity of blue males and channel males in two crosses (channel x channel, and channel x blue) that spawned and did not spawn were compared between four distinct time intervals (12, 6, 3, and 1 hour prior to spawning)*. Within the column, values with different letters of x, y, z were significant different at* $P < 0.05$. *Within row, values with different letters (a, b, c) were significantly different at* $P < 0.05$.

	Time interval prior to first spawning			
Characteristics	12h	6h	3h	1h
Occurrence (%)				
C X C Spawned	60.0 ± 51.64^{ax}	100.0 ± 0.00^{bx}	90.0 ± 31.62^{abx}	90.0 ± 31.62^{abx}
C X C Non-spawned	25.0 ± 50.00^{axy}	50.0 ± 57.74^{ay}	50.0 ± 57.74^{ay}	25.0 ± 50.00^{ay}
C X B Spawned	50.0 ± 70.71^{axy}	100.0 ± 0.00^{axy}	100.0 ± 0.00^{axy}	50.0 ± 70.71^{axy}
C X B Non-spawned	10.0 ± 31.62^{ay}	30.0 ± 48.30^{ay}	20.0 ± 42.16^{ay}	40.0 ± 51.64^{ay}
Frequency (per hour)				
C X C Spawned	2.9 ± 3.41^{ax}	7.8 ± 5.00^{abx}	15.0 ± 9.73^{bx}	22.7 ± 14.47^{cx}
C X C Non-spawned	0.3 ± 0.31^{axy}	0.8 ± 0.44^{ay}	0.5 ± 0.38^{ay}	0.3 ± 0.31^{ay}
C X B Spawned	0.5 ± 0.71^{axy}	1.0 ± 0.00^{ay}	7.5 ± 9.02^{axy}	9.0 ± 12.73^{axy}
C X B Non-spawned	0.1 ± 0.32^{ay}	0.8 ± 1.62^{aby}	0.6 ± 1.58^{aby}	2.0 ± 3.30^{by}
Duration (seconds)				
C X C Spawned	10.3 ± 3.51^{ax}	11.8 ± 7.80^{ax}	8.6 ± 2.63^{ax}	6.7 ± 2.40^{ax}
C X C Non-spawned	10.0 ± 0.00^{ax}	31.5 ± 31.82^{ax}	10.5 ± 0.71^{ax}	5.0 ± 0.00^{ax}
C X B Spawned	6.0 ± 0.50^{ax}	6.5 ± 2.12^{ax}	14.7 ± 4.65^{ax}	7.7 ± 1.52^{ax}
C X B Non-spawned	10.0 ± 3.17^{ax}	12.9 ± 14.31^{ax}	14.6 ± 14.99^{ax}	9.7 ± 3.03^{ax}

* Non-spawners were observed at the time intervals based on the mean times for the spawners

TABLE 27. *Occurrence (% fish), frequency (times/h), and duration (sec.) of curling activity of channel females in two crosses (channel x channel, and channel x blue) that spawned and did not spawn were compared between four distinct time intervals (12, 6, 3, and 1 hour prior to spawning)*. Within the column, values with different letters of x, y, z were significant different at the P < 0.05. Within rows, values with different letters (a, b, c) were significantly different at P < 0.05.*

	Time interval prior to first spawning			
Characteristics	12h	6h	3h	1h
Occurrence (%)				
C X C Spawned	40.0 ± 51.64^{axy}	70.0 ± 48.30^{ax}	70.0 ± 48.30^{ax}	70.0 ± 48.30^{ax}
C X C Non-spawned	50.0 ± 57.74^{axy}	50.0 ± 57.74^{axy}	25.0 ± 50.00^{ay}	50.0 ± 57.74^{axy}
C X B – Spawned	100.0 ± 0.00^{ax}	100.0 ± 0.00^{ax}	100.0 ± 0.00^{ax}	100.0 ± 0.00^{ax}
C X B Non-spawned	20.0 ± 44.10^{ay}	$10.\ 0 \pm 33.33^{ay}$	-	10.0 ± 33.33^{ay}
Frequency (per hour)				
C X C Spawned	0.9 ± 1.37^{ax}	2.2 ± 2.74^{abx}	1.7 ± 1.44^{abx}	4.00 ± 4.42^{bx}
C X C Non-spawned	3.5 ± 4.12^{ax}	2.8 ± 1.69^{ax}	2.8 ± 3.44^{axy}	2.00 ± 0.75^{axy}
C X B – Spawned	1.0 ± 0.00^{ax}	1.5 ± 0.71^{axy}	5.5 ± 4.95^{ay}	4.00 ± 1.41^{axy}
C X B Non-spawned	0.2 ± 0.42^{ax}	0.1 ± 0.32^{ay}	-	0.80 ± 2.53^{ay}
Duration (seconds)				
C X C Spawned	6.8 ± 3.78^{ax}	8.6 ± 3.46^{ax}	7.7 ± 4.16^{ax}	9.8 ± 7.36^{ax}
C X C Non-spawned	11.0 ± 1.41^{axy}	13.6 ± 0.00^{ax}	26.8 ± 13.03^{ay}	18.4 ± 2.50^{ax}
C X B - Spawned	18.8 ± 6.72^{ay}	9.3 ± 3.22^{ax}	20.8 ± 9.55^{ay}	12.5 ± 3.54^{ax}
C X B Non-spawned	30.6 ± 3.17^{az}	7.0 ± 0.00^{ax}	-	5.0 ± 5.66^{ax}

* Non-spawners were observed at the time intervals based on the mean times for the spawners

TABLE 28. *Occurrence (% fish), frequency (times/h), and duration (sec.) of air releasing activity of blue males and channel males in two crosses (channel x channel, and channel x blue) that spawned and did not spawn were compared between four distinct time intervals (12, 6, 3, and 1 hour prior to spawning)*. Within the column, values with different letters of x, y, z were significant different at P < 0.05. Within rows, values with different letters (a, b, c) were significantly different at P < 0.05.*

Characteristics	Time interval prior to first spawning			
	12h	6h	3h	1h
Occurrence (%)				
C X C Spawned	90.0 ± 31.62^{ax}	90.0 ± 31.62^{ax}	80.0 ± 42.16^{ax}	100.0 ± 0.00^{ax}
C X C Non-spawned	75.0 ± 57.74^{ax}	50.0 ± 57.74^{ax}	100.0 ± 0.00^{ax}	75.0 ± 57.74^{ax}
C X B – Spawned	100.0 ± 0.00^{ax}	100.0 ± 0.00^{ax}	100.0 ± 0.00^{ax}	100.0 ± 0.00^{ax}
C X B Non-spawned	90.0 ± 31.62^{ax}	80.0 ± 42.16^{ax}	80.0 ± 42.16^{ax}	100.0 ± 0.00^{ax}
Frequency (per hour)				
C X C Spawned	4.7 ± 4.29^{ax}	7.1 ± 8.66^{abx}	9.3 ± 11.06^{abxy}	14.5 ± 14.57^{bxy}
C X C Non-spawned	3.5 ± 2.13^{ax}	1.3 ± 0.81^{ax}	3.3 ± 3.56^{ax}	0.5 ± 0.63^{ax}
C X B – Spawned	3.5 ± 3.54^{ax}	6.5 ± 6.36^{ax}	17.0 ± 1.41^{ay}	31.5 ± 31.82^{ay}
C X B Non-spawned	2.3 ± 1.89^{ax}	3.8 ± 2.78^{abx}	3.2 ± 2.90^{abx}	5.6 ± 4.17^{bx}
Duration (second)				
C X C Spawned	1.1 ± 0.15^{ax}	1.0 ± 0.07^{ax}	1.0 ± 0.04^{ax}	1.2 ± 0.36^{ax}
C X C Non-spawned	1.0 ± 0.00^{ax}	1.0 ± 0.00^{ax}	1.0 ± 0.00^{ax}	1.0 ± 0.00^{ax}
C X B – Spawned	1.0 ± 0.00^{ax}	1.0 ± 0.00^{ax}	1.0 ± 0.00^{ax}	1.9 ± 1.23^{ax}
C X B Non-spawned	1.0 ± 0.00^{ax}	2.9 ± 5.35^{ax}	1.0 ± 0.00^{ax}	1.0 ± 0.00^{ax}

* Non-spawners were observed at the time intervals based on the mean times for the spawners

TABLE 29. *Occurrence (% fish), frequency (times/h), and duration (sec.) of air-releasing activity of channel females in two crosses (channel x channel, and channel x blue) that spawned and did not spawn were compared between four distinct time intervals (12, 6, 3, and 1 hour prior to spawning)*. Within the column, values with different letters of x, y, z were significant different at the P < 0.05. Within rows, values with different letters (a, b, c) were significantly different at P < 0.05.*

	Time interval prior to first spawning			
Characteristics	12h	6h	3h	1h
Occurrence (%)				
C X C Spawned	80.0 ± 42.16^{ax}	90.0 ± 31.62^{ax}	90.0 ± 31.62^{ax}	100.0 ± 0.00^{ax}
C X C Non-spawned	75.0 ± 50.00^{ax}	75.0 ± 50.00^{ax}	75.0 ± 0.00^{ax}	75.0 ± 57.74^{ax}
C X B – Spawned	100.0 ± 0.00^{ax}	100.0 ± 0.00^{ax}	100.0 ± 0.00^{ax}	100.0 ± 0.00^{ax}
C X B Non-spawned	40.0 ± 50.00^{ax}	70.0 ± 50.00^{ax}	70.0 ± 42.16^{ax}	80.0 ± 44.16^{ax}
Frequency (per hour)				
C X C Spawned	3.5 ± 2.68^{ax}	6.2 ± 7.60^{ax}	9.3 ± 9.91^{ax}	21.6 ± 13.01^{bx}
C X C Non-spawned	4.3 ± 4.81^{ax}	3.0 ± 1.50^{ax}	2.8 ± 3.44^{axy}	3.3 ± 3.81^{axy}
C X B – Spawned	1.5 ± 0.71^{ax}	2.0 ± 0.00^{abx}	9.5 ± 7.78^{abxy}	14.0 ± 4.24^{bxy}
C X B Non-spawned	1.2 ± 1.75^{ax}	2.1 ± 1.79^{abx}	1.6 ± 1.51^{ay}	4.2 ± 3.94^{by}
Duration (seconds)				
C X C Spawned	1.0 ± 0.00^{ax}	1.0 ± 0.07^{ax}	1.1 ± 0.20^{ax}	1.1 ± 0.16^{ax}
C X C Non-spawned	1.0 ± 0.00^{ax}	1.0 ± 0.00^{ax}	1.0 ± 0.00^{ax}	1.0 ± 1.00^{ax}
C X B – Spawned	1.0 ± 0.00^{ax}	1.0 ± 0.00^{ax}	1.0 ± 0.00^{ax}	1.9 ± 1.25^{ax}
C X B Non-spawned	1.0 ± 0.00^{ax}	1.0 ± 0.00^{ax}	1.0 ± 0.00^{ax}	1.0 ± 0.00^{ax}

* Non-spawners were observed at the time intervals based on the mean times for the spawners

TABLE 30. *Occurrence (% fish), frequency (times/h), and duration (sec.) of swim-up activity of blue males and channel males in two crosses (channel x channel, and channel x blue) that spawned and did not spawn were compared between four distinct time intervals (12, 6, 3, and 1 hour prior to spawning)*. Within the column, values with different letters of x, y, z were significant different at* P < 0.05. *Within rows, values with different letters (a, b, c) were significantly different at P < 0.05.*

	Time interval prior to first spawning			
Characteristics	12h	6h	3h	1h
Occurrence (%)				
C X C Spawned	70.0 ± 48.30^{ax}	70.0 ± 48.30^{ax}	70.0 ± 48.30^{ax}	100.0 ± 0.00^{ax}
C X C Non-spawned	100.0 ± 0.00^{ax}	75.0 ± 50.00^{ax}	75.0 ± 50.00^{ax}	75.0 ± 50.00^{ax}
C X B – Spawned	50.0 ± 70.71^{ax}	100.0 ± 0.00^{ax}	100.0 ± 0.00^{ax}	100.0 ± 0.00^{ax}
C X B Non-spawned	70.0 ± 48.30^{ax}	90.0 ± 31.62^{ax}	8.00 ± 42.16^{ax}	100.0 ± 0.00^{ax}
Frequency (per hour)				
C X C Spawned	3.2 ± 3.39^{ax}	2.8 ± 3.15^{ax}	2.4 ± 2.41^{ax}	4.0 ± 2.62^{ax}
C X C Non-spawned	4.0 ± 4.25^{ax}	1.5 ± 1.83^{ax}	3.5 ± 4.38^{axy}	1.0 ± 0.75^{ax}
C X B – Spawned	3.5 ± 4.95^{ax}	12.0 ± 15.56^{ay}	9.5 ± 7.78^{axy}	32.0 ± 31.00^{ay}
C X B Non-spawned	2.1 ± 1.85^{ax}	6.1 ± 4.70^{bxy}	8.2 ± 5.57^{by}	7.5 ± 4.22^{bx}
Duration (seconds)				
C X C Spawned	1.0 ± 0.00^{ax}	1.0 ± 0.00^{ax}	1.0 ± 0.00^{ax}	1.1 ± 0.25^{ax}
C X C Non-spawned	1.0 ± 0.00^{ax}	1.0 ± 0.00^{ax}	1.0 ± 0.00^{ax}	1.0 ± 0.00^{ax}
C X B – Spawned	1.0 ± 0.00^{ax}	1.0 ± 0.00^{ax}	1.0 ± 0.00^{ax}	5.4 ± 6.26^{ax}
C X B Non-spawned	1.0 ± 0.00^{ax}	2.6 ± 4.88^{ax}	1.7 ± 1.91^{ax}	1.0 ± 0.00^{ax}

* Non-spawners were observed at the time intervals based on the mean times for the spawners

TABLE 31. *Occurrence (% fish), frequency (times/h), and duration (sec.) of swimming-up activity of channel females in two crosses (channel x channel, and channel x blue) that spawned and did not spawn were compared between four distinct time intervals (12, 6, 3, and 1 hour prior to spawning)*. Within the column, values with different letters of x, y, z were significantly different at the P < 0.05. Within rows, values with different letters (a, b, c) were significantly different at P < 0.05.*

Characteristics	Time interval prior to first spawning			
	12h	6h	3h	1h
Occurrence (%)				
C X C Spawned	60.0 ± 51.64^{ax}	80.0 ± 42.16^{ax}	80.0 ± 48.30^{ax}	70.0 ± 48.30^{ax}
C X C Non-spawned	50.0 ± 57.74^{ax}	75.0 ± 50.00^{ax}	75.0 ± 50.00^{ax}	75.0 ± 50.00^{ax}
C X B – Spawned	100.0 ± 0.00^{ax}	50.0 ± 70.71^{ax}	100.0 ± 0.00^{ax}	50.0 ± 70.71^{ax}
C X B Non-spawned	80.0 ± 42.16^{ax}	80.0 ± 42.16^{ax}	80.0 ± 42.16^{ax}	80.0 ± 42.16^{ax}
Frequency (per hour)				
C X C Spawned	2.1 ± 4.25^{ax}	3.3 ± 2.58^{ax}	3.1 ± 2.56^{ax}	3.3 ± 3.74^{ax}
C X C Non-spawned	4.0 ± 4.50^{ax}	2.0 ± 1.25^{ax}	1.8 ± 2.19^{ax}	2.8 ± 2.56^{ax}
C X B – Spawned	3.0 ± 2.82^{ax}	3.0 ± 4.24^{ax}	2.0 ± 1.41^{ax}	24.5 ± 34.65^{by}
C X B Non-spawned	2.0 ± 2.36^{ax}	3.8 ± 4.39^{abx}	3.6 ± 4.74^{abx}	5.9 ± 7.53^{bx}
Duration (seconds)				
C X C Spawned	1.0 ± 0.00^{ax}	1.0 ± 0.00^{ax}	1.0 ± 0.00^{ax}	1.6 ± 1.43^{ax}
C X C Non-spawned	1.0 ± 0.00^{ax}	1.0 ± 0.00^{ax}	1.0 ± 0.00^{ax}	1.0 ± 1.00^{ax}
C X B – Spawned	1.0 ± 0.00^{ax}	1.0 ± 0.00^{ax}	1.0 ± 0.00^{ax}	3.3 ± 0.00^{ax}
C X B Non-spawned	1.5 ± 1.39^{ax}	5.2 ± 1.95^{ax}	1.0 ± 0.00^{ax}	1.0 ± 0.00^{ax}

* Non-spawners were observed at the time intervals based on the mean times for the spawners

TABLE 32. *Occurrence (% fish), frequency (times/h), and duration (sec.) of mouth widening activity of blue males and channel males in two crosses (channel x channel, and channel x blue) that spawned and did not spawn were compared between four distinct time intervals (12, 6, 3, and 1 hour prior to spawning)*. Within the column, values with different letters of x, y, z were significantly different at the P < 0.05. Within rows, values with different letters (a, b, c) were significantly different at P < 0.05.*

	Time interval prior to first spawning			
Characteristics	12h	6h	3h	1h
Occurrence (%)				
C X C Spawned	30.0 ± 48.30^{ax}	80.0 ± 42.16^{bx}	80.0 ± 42.16^{bx}	90.0 ± 31.62^{bx}
C X C Non-spawned	25.0 ± 50.00^{ax}	50.0 ± 57.74^{ax}	50.0 ± 57.74^{ax}	50.0 ± 57.74^{ax}
C X B - Spawned	100.0 ± 0.00^{ax}	50.0 ± 70.71^{ax}	100.0 ± 0.00^{ax}	100.0 ± 0.00^{ax}
C X B Non-spawned	50.0 ± 52.70^{ax}	80.0 ± 42.16^{ax}	80.0 ± 42.16^{ax}	80.0 ± 42.16^{ax}
Frequency (per hour)				
C X C Spawned	1.2 ± 2.09^{ax}	3.1 ± 3.92^{ax}	5.0 ± 5.70^{ax}	4.3 ± 6.07^{ax}
C X C Non-spawned	0.5 ± 0.13^{ax}	0.8 ± 0.44^{ax}	1.8 ± 1.44^{ax}	1.0 ± 0.75^{ax}
C X B - Spawned	1.5 ± 0.71^{ax}	2.0 ± 2.83^{ax}	2.5 ± 0.71^{ax}	7.0 ± 2.83^{ax}
C X B Non-spawned	2.7 ± 3.69^{ax}	3.8 ± 3.71^{ax}	4.7 ± 6.11^{ax}	3.5 ± 3.21^{ax}
Duration (seconds)				
C X C Spawned	1.0 ± 0.00^{ax}	1.0 ± 0.00^{ax}	1.0 ± 0.00^{ax}	1.0 ± 0.00^{ax}
C X C Non-spawned	1.0 ± 0.00^{ax}	1.0 ± 0.00^{ax}	1.0 ± 0.00^{ax}	1.0 ± 0.00^{ax}
C X B - Spawned	1.0 ± 0.00^{ax}	1.0 ± 0.00^{ax}	1.0 ± 0.00^{ax}	1.0 ± 0.00^{ax}
C X B Non-spawned	1.0 ± 0.00^{ax}	1.0 ± 0.00^{ax}	2.5 ± 4.11^{ax}	1.0 ± 0.00^{ax}

* Non-spawners were observed at the time intervals based on the mean times for the spawners

TABLE 33. *Occurrence (% fish), frequency (times/h), and duration (sec.) of mouth widening activity of channel females in two crosses (channel x channel, and channel x blue) that spawned and did not spawn were compared between four distinct time intervals (12, 6, 3, and 1 hour prior to spawning)*. Within the column, values with different letters of x, y, z were significant different at the* $P < 0.05$. *Within rows, values with different letters (a, b, c) were significantly different at* $P < 0.05$.

	Time interval prior to first spawning			
Characteristics	12h	6h	3h	1h
Occurrence (%)				
C X C Spawned	100.0 ± 0.00^{ax}	100.0 ± 0.00^{ax}	100.0 ± 0.00^{ax}	100.0 ± 0.00^{ax}
C X C Non-spawned	75.0 ± 57.74^{ax}	25.0 ± 57.74^{ay}	25.0 ± 57.74^{ay}	75.0 ± 50.00^{ax}
C X B - Spawned	50.0 ± 70.71^{ax}	100.0 ± 0.00^{ax}	100.0 ± 0.00^{ax}	50.0 ± 70.71^{ax}
C X B Non-spawned	80.0 ± 42.16^{ax}	70.0 ± 48.30^{ax}	70.0 ± 42.16^{ax}	80.0 ± 42.16^{ax}
Frequency (per hour)				
C X C Spawned	2.9 ± 2.07^{ax}	0.8 ± 1.32^{bx}	3.6 ± 3.41^{ax}	3.3 ± 2.16^{ax}
C X C Non-spawned	2.5 ± 3.12^{ax}	0.8 ± 0.44^{axy}	0.5 ± 0.63^{ax}	2.3 ± 2.06^{ax}
C X B - Spawned	2.0 ± 2.83^{ax}	2.5 ± 2.12^{axy}	2.0 ± 1.41^{ax}	2.5 ± 3.54^{ax}
C X B Non-spawned	3.0 ± 3.37^{ax}	2.8 ± 2.86^{ay}	2.8 ± 2.86^{ax}	2.3 ± 2.06^{ax}
Duration (seconds)				
C X C Spawned	1.00 ± 0.00^{ax}	1.00 ± 0.00^{ax}	1.00 ± 0.00^{ax}	1.00 ± 0.00^{ax}
C X C Non-spawned	1.00 ± 0.00^{ax}	1.00 ± 0.00^{ax}	1.00 ± 0.00^{ax}	1.00 ± 0.00^{ax}
C X B - Spawned	1.00 ± 0.00^{ax}	1.00 ± 0.00^{ax}	1.00 ± 0.00^{ax}	1.00 ± 0.00^{ax}
C X B Non-spawned	1.00 ± 0.00^{ax}	1.00 ± 0.00^{ax}	1.00 ± 0.00^{ax}	1.00 ± 0.00^{ax}

* Non-spawners were observed at the time intervals based on the mean times for the spawners

DISCUSSION

Environmental Conditions in Experimental Runs

The overall environmental setting was appropriate for channel catfish spawning. Wellborn (1988) recommended a temperature of more than 24^0 and less than 30^0C for channel catfish reproduction. Chapman (1992) and Wyatt at al. (2006) stated that optimal temperature for spawning of this species should range 21 to 27^0C. The water temperature of this experimental system averaged 29.5 ± 0.35^0C and no significant differences between three combinations of channel x channel (C X C), channel x blue (C X B), and blue x blue (B X B) were found ($P > 0.05$) and could be considered near the upper acceptable temperature for spawning of channel catfish. Overall, the mean temperature in the experiment was higher than average temperature for spawning of blue catfish (21 – 24^0C, Goeckler et al. 2003) but may have been near optimum for channel catfish when held in aquaria and induced spawned. Phelps et al. 2011 found that 95.5 ± 21.3 % of the channel catfish females held at 28°C ovulated but only 52.9 ± 51.4% ovulated when held at 24°C. Although the mean temperatures showed the differences among the experimental runs (Table 6), no significant differences in spawning successes among the runs were found. Dissolved oxygen averaged 5.61 ± 0.44 mg L^{-1} with no significant distinctions in mean concentration among three fish crosses. Steeby (1987) found that spawning success of channel catfish was not affected by the DO level of more than 4.0 mg L^{-1}. Ammonia and pH of water in this experimental system averaged 0.29 ± 0.07mg L^{-1} and 6.74 ± 0.06, respectively. Generally, pH from 6.0 to 9.0 is considered as an optimal range for growth of channel catfish. The pH values below 4.0 to 5.0 or greater than 11.0 to 12.0 can affect survivorships of this species (Tucker and Robinson 1990). The pH and total ammonia nitrogen concentration for channel catfish growth and reproduction was around 6.5 to 9.0 and less than 1.0 mg L^{-1} (Chapman 1992). Thus, the pH and ammonia concentration in experiment were in the appropriate range for growth and spawning of this species.

Percent of Egg Releases and Fertilized Egg Masses between Different Parental Crosses

Several authors have hormone-induced channel catfish females to spawn in aquaria and fertilized eggs were obtained when paired with channel or blue males (Table 34). Similarly, blue females paired with blue males have given fertilized eggs when hormone-induced to spawn and held in aquaria (Dunham et al. 2000, Table 34).

Selection criteria for blue males are not well defined and fish used in this study received less preliminary screening than did channel males. In this study blue catfish males were preliminarily screened and selected if they had a grayest body color and a prominent genital papilla. As part of the study, the spawning success of blue males x channel or blue females was compared based on the classification criteria used with the channel males. A total of nine criteria and subsets of each were used in describing male brooders. No criteria had subsets that differed in spawning success in the C X B or B X B pairings. Blue females responded similarly as did channel females when induced to ovulate with common carp pituitary with an ovulation rate of 69.2%. However, in the B X B cross, the percentage of spawns that were fertilized was 33.3% compared to the 91.3% rate obtained in the C x C cross. The percentage of spawns that were fertilized by a blue male did not differ whether paired with a blue or a channel female. This would suggest that the low rate of egg masses being fertilized is not a behavioral or pheromonal incompatibility but perhaps a question of male quality. Dunham et al. (2000) and Kristanto (2004) found that blue males' testicular development can be inconsistent. Adult male channel and blue catfish can be variable in their gonadal development with CVs of 40.7 and 42.5% in g testes/ kg male, respectively (Dunham et al. 2000). Mature channel males expressing classical indicators of maturity, such as body color, head shape and condition of the genital papilla, were selected for use in the present study but within the group there was variation among males. Eight sets of criteria were used and the fish classified into subsets within each criteria and the spawning success evaluated. Spawning success did vary by subset of a given criteria. For example for the criteria head width: body weight, channel males in the two highest categories represented 77% of the population and produced 87% of the total spawns while males in the lowest category (23% of the population) gave 13% of the total spawns. The current criteria used based on external appearance and morphology are not adequate to distinguish blue males with adequate gonadal development for successful reproduction when variation in gonadal development might vary as much as 42% (Dunham and Argue, 2000). Chatakondi and Davis (2013) gave subjective rating scale for evaluating maturity stages of blue male catfish base on the increase in characteristics of body coloration, and head and papilla conditions. They saw that blue males classified as darker colored skin, robust and muscular body with broad muscular head, and thicker genital papilla (stage 5) had significantly higher GSI and sperm production than those males raked from 2nd to 4th stage. However, in my research, blue males did not show similar traits as 5th-stage blue males in the study of Chatakondi and Davis (2013). This may have contributed to low spawning success seen in current study. According to Jerrod and Phelps' oral presentation in 2011, blue male catfish used in their research had better conditions of body coloration, head size, and papilla; so, the spawning success of hybridization from their study is also higher than the current study. These indicated that the low spawning success in channel x blue hybridization in the current study can be quality of blue males.

In this study, ovulation rates of C X C, C X B, and B X B from 1st to 5th experimental run were not significantly different. An average of 74.2% of the channel females induced to spawn with carp pituitary released eggs when paired with channel males and 70.0% did when paired with blue males. Hormone-inducement of blue females paired with blue males resulted 69.2% of the females in five experiments that released eggs. These ovulation rates are similar to those of several authors but the percent viable eggs have varied considerably (Table 34). In this study, 91.3% of the egg masses from the C x C pairs had viable eggs with an average viability of 77.4 ± 28.43% per egg mass. In the C x B, only 14.3% of the egg masses were viable with an average viability of 49.2 ± 37.59% per egg mass. Viability was also an issue with the B x B egg masses where 33.3 % of the egg masses were viable with an average viability of 55.9 ± 49.47% per egg mass.

These results suggest that although carp pituitary was effective in inducing females to ovulate in many cases the males may have not fertilized them. Lack of fertilized eggs has been reported by several authors with 40% of the egg masses obtained by Tave and Smitherman (1982) being fertilized in a channel female x blue male; 9% in the channel females x blue males, and 37 – 47% with channel females x channel males (Tieman 1995); and 15% in channel females x blue males (Dunham et al. 2000).

However, when the spawns were viable they often had high rates of egg viability. Tieman (1995) had 91% egg viability in channel females x blue males and 96% channel females x channel males. Dunham and Argue (2000) had egg viabilities of 73.6% for channel females x channel males, 64.4% for channel females x blue males, and 56.6% for blue females x blue males. Dunham et al. (2000) had 75% for channel females x channel males, and 79% for channel females x blue males.

Factors that contribute to the low rates of viable egg masses that have been observed often are not clear. As observed in this study, in many cases the females will release eggs but they were not fertilized by the male. Brood quality and the prominence of secondary sexual characteristics may be a factor. Neu (1995) found that channel catfish males with wider head widths had a greater spawning success. Fish that spawned had significantly larger head-to-total length ratio than those that did not spawn. The head-to-total length ratio of the spawners and non-spawners were 0.19 and 0.18, respectively. Body metrics and the ratios of body metrics are also criteria for assessing selecting female catfish brooders. Phelps et al. (2011) reported that the ratios of length-to-girth and length-to-width of channel female spawners were less than those of channel female non-spawners. They also found that channel females that have ratio of length-to-girth of less than 1.8 or length-to-width of larger than 5.5 spawned earlier than those in high value categories.

In this study, selection of brooders based on body coloration, papilla condition, and body metrics did not give differences between female spawners and non-spawners. In fact, spawning and non-spawning channel females paired either with channel or blue males showed the similar results of body metrics including body weight, body length, girth, and width. However, for channel females x channel males, ratios of length-to-width and length-to-girth differed between spawners and non-spawners. Categories based on ratios of body metrics had subsets with significantly higher rates of spawning than other subsets in the same category in the C X C cross. For the category length-to-width ratio, 100% of the channel females having the ratio of less than 5.5 spawned, almost doubling the spawning rate of females with the ratio of length-to-width larger than 5.5 (56.67%). Likewise, spawning success rates differed (P = 0.018) for channel females (x channel males) having length-to-girth ratios less than 1.9 relative to those with a ratio of larger than 2.0. Spawning rates were 94.2% and 37.5%, respectively. This had a trend that channel females having the similar total body length had larger measures of body girths and widths, they spawned more successfully. For body coloration and papilla conditions, channel females with dark abdomens had a significantly higher egg viability percent (91.1%) which was nearly two times higher than percentage of females with grey abdomen. The mean number of fry obtained from channel females that had poor papilla condition was less than that of the channel females with fair and good conditions.

Ratios of body metrics of channel males that spawned or did not spawn differed, but such ratios were similar between blue male spawners and non-spawners. Channel males paired channel females that spawned or did not spawn differed in ratio of length-to-head width. The ratio was smaller for spawners than non-spawners (5.39 ± 0.27 and 5.48 ± 0.43, respectively). The ratio of head width-to-body weight of channel males that is less than 5.5 also gave lower spawning success compared to channel males having a ratio larger than 5.5. More than 83.5% of channel males with ratio of width-to-weight ratios is larger than 5.5 spawned successfully (represented for more than 77% of total population) while only 42.9% of males having ratio of width-to-weight that is less than 5.5 spawned. Neu (1995) found a relationship between the head width of channel males and spawning success. According to his study, males that spawned had significantly larger head width-to-total length ratios than those that did not spawn. No other correlation was found between spawning success and any external characteristics or reproductive characteristics.

Generally, there was not much difference in external appearance of male blues that spawned and did not spawn. The blue male spawners and non-spawners had similar measurements of body weight, length, head width, and the ratios between those body metrics. Body coloration, head underside coloration, and papilla condition categories were also not distinct.

Several authors (Clemens and Sneed 1957, Tucker and Robinson 1990, Kelly 2004) recommend that male channel catfish brooders selected for spawning should have wide heads with muscular head pads, dark body coloration and a prominent genital papilla. In this study, there was no evidence that males with dark coloration or prominent papilla would give better spawning success except in regard to the coloration of the underside of the head. Channel catfish males where the underside of the head was dark had a 100% spawning success while only 54% of channel males with a white underside of the head spawned.

Behavior as a Predictor of Spawning Success

Successful reproduction is an expression of a complex set of factors such as physical condition or maturation (Brauhn and McCraren 1975, Babiker and Ibrahim 1979, Neu 1995), environmental conditions (Lenz 1947, Wolters 1993, Phelps et al.

2007), chemical stimulation (pheromones) (Pinheiro et al. 2003, Stacey 2003, Stacey et al. 2003) and behavior (Hioki and Suzuki 1995, Johnston 1995, Herb et al. 2003). This can be even more complex when the goal is natural hybridization between species (Chevassus, 1983, Masser & Dunham 1998, Allendorf et al. 2001). Spawning behavior of channel catfish was first described by Murphree (1940) and Lenz (1947) but little detail was given regarding courtship. Clemens and Sneed (1957) made periodic observations of channel catfish that were hormone-induced to spawn and were held in aquaria. They described various male reactions and responses in terms of aggressive or passive behavior. An aggressive male would bite and push the female for 20 or 30 minutes until female remained in a given location near the bottom of the aquarium. If the female swam off too far the male hazed her again until she returned to her original position. They did not specify at what point prior to spawning a given behavior was seen. The aggressive male behavior was not seen in the pairs of the present study monitored from 12 h prior to spawning up to spawning. Other behaviors as described by Clemens and Sneed (1957) for fish nearer the time of spawning were also seen in the present study (Table 35).

Common carp pituitary was equally effective in inducing female channel catfish to ovulate independent of whether the female was paired with a channel or blue male. Twenty-three of 31 channel females (74.2%) released eggs when paired with channel males and 21 of those spawns (91.3%) were successfully fertilized. Fourteen of the 20 channel females (70.0%) released eggs when paired with blue males but only two of the spawns (14.3%) were successfully fertilized. Generally, the ovulation percentage (egg release) of channel females injected with hormones and stocked in aquaria that have been reported of around 66.0 – 83.5% (Dunham et al. 1998, Dunham et al. 2000, Dunham and Argue 2000) are similar to ovulation rate of this study (70.0 – 74.2%). Other previous studies also showed that the successful spawning of channel females and blue males was limited, ranging 0.0 to 20.0% (Tave and Smitherman 1982; Tieman 1995) and averaging 15% (Dunham et al. 2000).

Exogenous hormones provided by the common carp pituitary were adequate to stimulate ovulation independent of which species of male a channel catfish female was paired. The broodfish were held in a common settling where the environmental conditions were similar whether the female was paired with a blue or channel male. Other factors were involved to give such a great difference in the rates of fertilized eggs. Pheromones are an important factor in stimulating successful reproduction in several species of fish (Kitamura et al. 1994, Volkoff and Peter 1999, Stacey et al. 2003). Pheromones have been shown to be an attractant in catfish, but their role in stimulating egg fertilization in catfish is less understood (Broach 2009).

Behavior is another component in successful reproduction by some species. A male angelfish (*Chaetodontopulus mesoleucus*) approaches a female and stimulates her spawning by showing his caudal fin, fully spreading all fins, and vibrating his caudal fin around the head of the female (Hioki and Suzuki 1995). Male paleback darters (*Etheostoma pallididorsum*) and butterflyfish (*Chaetodon trifascialis*) usually snuggle and push their snouts against abdomen or genital papilla of females to stimulate spawning (Johnston 1995; Yabuta and Kawashima 1997). Males and females of butterflyfish and angelfish often swim up together to water surface and release gametes after the pair moves forward to the water surface (Hioki and Suzuki 1995; Yabuta and Kaswashima 1997).

Spawning behaviors are considered as signals to attract attention of mating partners in bullhead catfish including yellow bullhead (*Ameriurus natalius*), brown head (*Ameiurus nebulosus*) and black bullhead (*Ameiurus melas*) so that spawning can occur (Wallace 1969). Clemens and Sneed (1957) did not observe any behavior the day before spawning that was a strong predictor that channel catfish spawning would occur. In the present study, no behavior from 12 h to just before spawning was a unique precursor to spawning; however, there were differences between spawners and non-spawners in the proportion of males or females that displayed a specific behavior, when the behavior occurred, and the duration of a behavior when displayed. Curling behavior, thought to be a precursor to dancing, was seen 6 to 1 h prior to spawning in 93.3% of the channel males that spawned and in the same time frame by 41.7% of the males that did not spawn. Spawners showed curling at a significant higher frequency than non-spawners at 6, 3, and 1 h prior to spawning, 15.2 ± 7.45 /h and 0.5 ± 0.25, respectively. Dancing was observed in 100% of channel male that spawned from 6 to 1 h prior to spawning but only in 25% of the non-spawners. Spawning males also differed in frequency of dancing relative to non-spawners, 6.77 ± 1.27 and 1.08 ± 0.31/h respectively.

Channel female spawners and non-spawners displayed similar behaviors, but like the males, differed in the proportion of the fish displaying the behavior, the frequency/h and duration of the display. The greatest difference between channel female spawners and non-spawners was in rubbing behavior where the frequency/h at 3 and 1 h prior to spawning was 15 times more than that of non-spawners during the same time period. The rubbing behavior seen in the present study is similar to what Clemens and Sneed (1957) called long "runs", where the female moved over a 4 – 6 inch section of bottom in a wiggling motion, alternately beating the pelvic and pectoral fins against the bottom. These areas would be the areas where females that spawned later deposited their eggs. Dancing behavior was seen in 90% of the channel female spawners and 25% of the channel female non-spawners at all time intervals and the frequency/h was 3.2 times more for spawners than non-spawners. At the time period equal to 1 h before spawning, females that spawned released air bubbles more often/h than non-spawners, 21.60 ± 13.01 and 3.25 ± 3.81 times/h, respectively. However, average duration of curling behavior by channel females that spawned was less the duration of channel female non-spawners (7.7 ± 4.16 for the spawners and 26.8 ± 13.03 for the non-spawners).

Other behaviors monitored in this study, such as mouth widening, though commonly seen, does not appear to be a spawning related activity in channel catfish. Likewise, there were no differences in occurrence of swim-up behavior by channel females that spawned or did not spawn when paired with a channel male.

Spawning Behavior of C X C Compared to C X B

Hybridization occurs naturally in a number of fish species with more than a hundred hybrids identified (Hubbs 1955; Smith 1992). External fertilization, changes in living habitat quality and quantity, breakdown of behavioral isolating mechanisms, and competition for restricted breeding habitat are considered as the main factors leading to the hybridization of fish species in natural environment (Hubbs 1955; Herrington et al. 2008). In this study, broodfish were held under similar conditions but successful hybridization (C X B) occurred at a much lower rate than the natural spawning of C X C.

There were some behavioral differences between C X C and C X B pairs. In the majority of cases for a given behavior, a greater proportion of the male channel catfish would display the behavior than would male blue catfish, as well as display it more frequently/fish/h. Similarly, a greater portion of the females would display a behavior and do it more frequently/h when paired with a channel male versus a blue male. In some cases, the differences in behavior were more whether a pair spawned or not rather that what male was used.

The one behavior that was more common in blue males versus channel males was the swim-up behavior (*P* = 0.0001) where at 6 to 1 h before spawning, the frequency/h of this behavior was 2.52 times more in blue males than channel males.

Overall, channel females paired with channel or blue males gave similar ovulation rate to induced hormones; however, the percentage of egg masses that fertilized by channel males was much higher than that of blue males. The blue males paired with channel or blue females also gave low fertilized spawns. Thus, issue of spawning success was blue males. The blue males that spawned successfully or did not spawn did not give differences in terms of morphological characteristics. Additionally, blue and channel males paired with channel females generally showed similar spawning behaviors; so, environment could be a factor that affected spawning success. The temperature in the research averaged 29.5^{0}C that was higher than the mean temperature for spawning of blue catfish (21 – 24 ^{0}C, Goeckler et al. 2003). Jerrod and Phelps (2011) also found that blue catfish also spawned better as they were held in aquarium system at 28^{0}C. Aquarium system in hatchery might be not a good place for natural spawning of blue catfish. Lenz (1947) indicated that blue catfish were more sensitive to surrounding environment than channel catfish; so, conditions of experimental environments can affect negatively on spawning of blue catfish.

Behaviors, external body metrics, and egg and fry characteristics of C X C

Behaviors, external body metrics, and egg and fry characteristics were in some cases correlated. Channel males with larger head width-to-weight ratios curled more frequently (R^2=0.44, P = 0.04). The curling frequency of male spawners averaged 15.16 ± 7.45, whereas, the frequency of male non-spawners was 0.50 ± 0.25. The channel males with larger length-to-weight ratios had higher dancing frequency (R^2= 0.42, P = 0.04). The male spawners danced more frequently than the male non-spawners ($P < 0.0001$). There was a positive correlation between length-to-head width of channel males and hatching rate (R^2= 0.43, P=0.039).

Similar size females having greater abdominal widths or girths produced more eggs per female weights (R^2 = 0.48, P = 0.03). The percentages of hatched eggs also increased as the ratios of width-to-weight, girth-to-weight increased (R^2= 0.47, P = 0.03, and R^2= 0.48, P =0.03). The more frequently the females curled, the greater the number of fry obtained (R^2 = 0.57, P = 0.011).

There was a negative relationship between the duration of rubbing by male channel catfish and the percentage of viable eggs (R^2= 0.70, P = 0.003). Duration of curling by male channel catfish was also negatively related to percentage of viable eggs (R^2= 0.44, P = 0.04) and percentage of swim-up fry (R^2= 0.44, P = 0.02). However, for female channel catfish, the duration of curling was positively related to percent hatch (R^2= 0.39, P = 0.05).

The above traits of morphological characteristics and behavior can be used to improve spawning efficiency under the proper settings. Brood selection based on morphological characteristics can be a practical method to improve fry production if brood management practices result in a high portion of brood stock with these traits. Phelps et al. (2011) found that females with a length-to-width ratio of < 5.0 had a 93% ovulation rate but only 13% of the females in the population met this standard. Behavior as seen in this study can be a predictor of spawning success and is associated with morphological characteristics, but only under a specific set of conditions can behavior be easily monitored. As discussed earlier, there was little difference in spawning behavior between male blue and channel catfish or how channel catfish females responded to males of either species, however there were significant differences in behavior by fish that spawned or did not spawn.

TABLE 34.*The percentages of ovulation (% Ovu), fertilized egg masses (% Fer) and %fertilization/spawn (% Fer/spawn) of this study and previous studies.*

	C X C				C X B				B X B		
Study	Spawning method	% Ovu.	% Fer.	%Fer. /spawn	Spawning method	% Ovu.	% Fer	%Fer/ spawn	% Ovu	% Fer	%Fer/ spawn
This study	Aquarium	74.2	91.3	77.4	Aquarium	70.0	14.3	49.2	69.2	33.3	85.3
Dunham et al. 2000	Aquarium	67.0		75.0	Pen	15		79			
	Stripped	91		49	Artificial	80		45			
Dunham & Argue 2000	Aquarium	83.5		73.6					58.4		56.6
Tieman 1995	Injected-pen			96.0	Aquarium	9		91			
Brahn 1971	Injected-tank	57.14									
Barrero et al. 2008	Injected pond	12.5		>80%							
Dunham et al. 1998	Aquarium	66.0		70.0							
Bates and Tiersch 1998	Aquaria	33.0		65.5							
Kelly and Kohler 1996	Recirculating	79.3									
Durland et al. 2000	Pond	72.3									
Jerrod and Phelps 2011	Aquaria	100	75			69.2	61.5		50	40	

TABLE 35. *Similar spawning behaviors as described by Clemens and Sneed (1957) and in the present study.*

Clemens and Sneed 1957	Present study
Female used pelvic and pectoral fins alternately beating against the bottom of aquaria (this behavior was called “runs” along the bottom).	Female also rubbed the aquarium bottom by using her pectoral fins and abdomen (rubbing behavior). This behavior sometimes was also exhibited by male.
Female sometimes gave off bubbles of gases, but no observations of the female going to the top were seen.	Both male and female released air bubbles from gills or mouth (air bubble releasing behavior). They also swim up to the water surface and gulf the air (swim-up behavior).
Male wrapped his tails around female’ eyes. If the female did not react to the behavior, the male was very brief (the behavior lasted about 5 seconds).	The curling activity was where the male or female used its tail to cover head of the other while the other partner shows little or no participation.
If male wrapped his tail around female’ eyes and she responded his behavior, she also covered his head. Both pelvic and pectoral fin were motionless during this behavior for 12 – 20 seconds.	Both females and males wrapped their caudal fin over the front portion of the head of its partner and quivered. The dance would take place over the aquaria bottom.

CONCLUSION

Spawning success of catfish brooders may be affected by initial selection. Several differences between females that spawned or did not spawn were found in this study. For channel females x channel males, ratios of length-to-width and length-to-girth differed between spawners and non-spawners. Female spawners have smaller ratios of length-to-girth and length-to-width than those of female non-spawners. One hundred percent of the channel females having the ratio of length-to-width less than 5.5 spawned, almost doubling the spawning rate of females with the ratio of length-to-width larger than 5.5 (56.67%). Spawning success rates differed for channel females (x channel males) having length-to-girth ratios less than 1.9 relative to those with a ratio of larger than 2.0. Spawning rates were 94.12% and 37.50%, respectively. Additionally, channel females in experiments with the total length ranging 46.5 to 62.9 cm and having the larger girth (≥ 28 cm) and width (≥ 9 cm) spawned better than channel females with girth less than 28 cm and width less than 9 cm. Likewise, channel males that spawned and did not spawned also showed differences in several subsets of body coloration and the ratios of body metrics. Channel male spawners paired with channel females had smaller ratios of length-to-head width compared to the channel male non-spawners. The ratio of head width-to-body weight of channel males less than 5.5 also gave a lower spawning success compared to channel males having a ratio larger than 5.5. Males with dark coloration of the underside of heads spawned more successfully than those where the underside of the head was white in coloration. The spawners and non-spawners also exhibited differences in the proportion of the fish displaying the behavior, the frequency/h and duration of the display. Generally, curling and dancing behaviors were seen 6 to 1 h prior to spawning in higher percentages of the channel males that spawned than those of the non-spawners in the same time frame. Spawners showed curling and dancing at significantly higher frequencies than non-spawners. Channel female spawners and non-spawners displayed similar behaviors, but like the males, differed in the proportion of the fish displaying the behavior, the frequency/h and duration of the display. The greatest difference between channel female spawners and non-spawners was in rubbing behavior where the frequency/h at 3 and 1 h prior to spawning was 15 times more than that of non-spawners during the same time period.

In this study, the ovulation rate of channel females induced with carp pituitary (10 mg L^{-1}) was 74.2% when paired with channel males and 70.0% when paired with blue males. Hormone-inducement of blue females paired with blue males resulted in 69.2% of the females releasing eggs. However, the percent of viable egg masses from the C X C pairs was 91.3% and that was significantly higher than that from C X B (14.3%) and B X B (33.3%). The mean viability per egg mass of C X C, C X B, and B X B was 77.4, 49.2, and 55.9%, respectively. These results suggest that although carp pituitary was effective in inducing females to ovulate, in many cases the blue males may have not fertilized them and quality of the males may be this issue that causes the differences in percentages of fertilized egg masses.

Behavior is a factor that may affect spawning successes between combinations. There were some behavioral differences between C X C and C X B pairs. In the majority of cases for a given behavior, a greater proportion of the male channel catfish would display the behavior than would male blue catfish, as well as display it more frequently/fish/h. The percentage of channel males exhibiting curling behavior was nearly double the percent of blue males. The percentage of channel males showing dancing behavior was 57.4%, whereas only 8.33% of blue males exhibited this behavior. The frequency of curling and dancing behavior exhibited by channel males was significantly higher than those by blue males. Channel males also exhibited higher frequency of air bubble releasing behavior, whereas at 6 to 1 h before spawning the frequency/h of this behavior was 2.52 times more in blue males than channel males. Similarly, a greater portion of the females would display a behavior and do it more frequently/h when paired with a channel male versus a blue male. Channel females paired with channel (C X C) or blue males (C X B) had differences in rubbing, curling, and air releasing frequencies. Channel females paired with channel males often exhibited higher mean frequencies of rubbing, curling, and air releasing behaviors compared to the channel females paired with blue males.

In some cases, behaviors, external body metrics, and egg and fry characteristics were correlated. Channel males with larger head width-to-weight or length-to-weight ratios curled and danced more frequently. The male spawners curled and danced more frequently than the male non-spawners. There was a positive correlation between length-to-head width of channel males and hatching rate. Similar size females with having greater abdominal widths or girths produced more eggs per female weights. The percentages of hatched eggs also increased as the ratios of width-to-weight, girth-to-weight increased. The more frequently the females curled and danced, the greater the percentage of eggs hatched.

In short, spawning successes of C X C cross can be improved based upon selections of body ratios and external appearance of female and male channel catfish. Differences in spawning behaviors were more related to whether a paired spawned or did not spawn than the type of male a channel catfish female were paired with. In fact, there were not many differences in spawning behaviors between channel and blue males paired with channel females. In this study, blue males paired with channel or blue females gave low fertilized spawns compared to channel males paired with channel females. However, blue males gave fertilization rate of eggs/mass as similar as channel males Thus, the issue might be that the blue males did not release sperm when the females released eggs. This may have been due to such males not being mature enough to spawn or the experimental environment was not appropriate for spawning of blue catfish. The blue male selection criteria used was not adequate to distinguish those that spawned from those that did not. It is possible that the majority of blue males used in this study were not mature enough to spawn naturally under hatchery conditions. Additional work is needed in how to identify mature blue males capable of spawning naturally with channel catfish females.

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