EFFECTS OF STEROID AND PROSTAGLANDIN INJECTIONS ON

HYBRIDIZATION SUCCESS BETWEEN FEMALE CHANNEL

CATFISH AND MALE BLUE CATFISH

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EFFECTS OF STEROID AND PROSTAGLANDIN INJECTIONS ON HYBRIDIZATION SUCCESS BETWEEN FEMALE CHANNEL CATFISH AND MALE BLUE CATFISH

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THESIS ABSTRACT

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Hybrid catfish produced from female channel catfish, *Ictalurus punctatus*, and male blue catfish, *I. furcatus*, exhibit superior traits for commercial aquaculture when compared to those of the channel catfish. However, one of the primary obstacles in the production of these hybrids is the shortage of fingerlings available to producers which is due to low hybridization success of the parent species. Increasing hybridization success using traditional spawning methods such as pen spawning could result in a greater production of hybrid fingerlings. In the following experiments, hormonal pheromones were evaluated as a means of increasing the male blue's interest in the female channel during spawning so that the blue male might more readily mate with the female leading to increased spawning success.

In the first experiment, female channel catfish were injected with one of the following three hormonal pheromones: 17α, 20β-dihydroxy-4-pregnene-3-one (17,20β-P), (17,20β-P-20-glucosiduronate), and prostaglandin F-2α (PGF-2α). Females were then placed into traps in ponds containing either male channel or blue catfish. The male's responses to the pheromone treatments were recorded at various intervals over a ninety-six hour period. Males of both species were more likely to respond to a female injected with PGF-2α than a female injected with any of the other pheromones. Blue males were equally attracted to PGF-2α and 17,20β-P-20-glucosiduronate. Males were more susceptible to being attracted to females 48 to 96 hours after female injections.

In the second experiment, female channel catfish received PGF-2α injections during priming injections of LH-RHa used for induced spawning. Females receiving PGF-2α were paired with blue males in concrete tanks and spawning success was compared to channel females not receiving PGF-2α and paired with either blue or channel males. PGF-2α did not increase spawning success up to the level of channel catfish nor above spawning rates for hybrids where females did not receive PGF-2α injections. However, ovulation did appear to be positively influenced by additional PGF-2α injections.

These experiments prompt further investigation of the role of prostaglandins in attracting male blue and channel catfish to con-specific females during spawning which may be a reproductive isolating mechanism. Future research should also be directed towards the use of prostaglandins like PGF-2α as ovulation inducing agents for channel catfish.

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I. INTRODUCTION

The American catfish industry has grown to become one of the largest aquaculture industries in the U.S. Over US $410 million was generated by catfish growers in 2008, where nearly 595 million square meters of surface water was dedicated to catfish production (USDA 2009b). Total worth of the catfish industry is estimated to be around seven billion dollars after all associated industries such as feed mills, processors, and supply companies are included (USDA 2007). The industry has relied primarily on the channel catfish, *Ictalurus punctatus*, which accounts for approximately 70% of all aquaculture production in the U.S. (Goldburg et al. 2001), and yields up to 600 million pounds of processed channel catfish annually (USDA 2007).

The price of catfish in 1960 at the farm gate was $1.10/kg. Since then, the price has risen slightly to a price of $1.45-$1.82/kg in 2008 (USDA 2009a). Increased competition from imported products has kept the price relatively low (Haley 2007; Stickney 2008). Over 100 million pounds of catfish was imported during 2008, which was about a 20% increase over 2007 (USDA 2009a). Increased production cost resulting from the rising cost of grains has reduced profit margins for catfish growers and producers. Some catfish farmers have been forced out of business or have reduced production output as seen in annual decreases in the acreage of farm land dedicated to catfish production between 2002 and 2007 (Haley 2007). Around 147,000 thousand surface acres of water was used for catfish production in 2008, which was 10% less than

that used in 2007 (USDA 2009b). Producers need to find ways to reduce production cost and develop a higher quality product so they can demand a higher price.

Many improvements have been made in the channel catfish farming industry. Production factors such as growth, feed conversion, and survival, as well as processing traits involving meat yield and quality have been improved (Bosworth et al. 2004). Mass selection and crossbreeding have been used to identify rapid growing strains of channel catfish (Dunham et al. 1987; Rezk et al. 2003). Hatchery techniques have been improved which have led to better survival from the egg to juvenile stage in channel catfish (Phelps and Walser 1993; Walser and Phelps 1993; Small and Wolters 2003). Feed regimes, diet composition, temperature effects, and many other factors have been evaluated to identify strategies for obtaining better feed conversion (Andrews and Stickney 1972; Greenland and Gill 1979; Li, et al. 2008). Grading techniques (Lovshin and Phelps 1993; Green et al. 2004) and stocking densities (Engle and Pounds 1994; Losinger et al. 2000; Pomerleau and Engle 2003) have also been optimized, leading to better management strategies for catfish stocks.

A major breakthrough in the catfish industry has been hybrid catfish production. Hybrids produced from female channel catfish and male blue catfish, *Ictalurus furcatus*, have superior traits required for intensive aquaculture (Dunham et al. 2000). These Channel X Blue (CxB) hybrids show 30.0-60.0% greater survival in response to low dissolved oxygen (Dunham et al. 1983) and greater, microbial disease resistance (Smitherman et al. 1996; Wolters et al. 1996) than channel catfish. The hybrids are easier to seine for catfish farmers and are more susceptible to angler harvest (Tave et al. 1981; Dunham and Argue 1998). The hybrids also exhibit approximately 20.0% faster growth

to market size (Giudice 1966; Dunham and Brummett 1999), 11-14% better feed conversion (Smitherman et al. 1996), higher dress out percentages (Argue et al. 2003), and increased uniformity in size and shape (Dunham et al. 1982) than channel catfish. These traits could result in a 16.0% increase in net return on land, labor, and management for food fish production as compared to channel catfish (Ligeon et al. 2004), which has lead to increased interest in producing these CxB hybrids more efficiently (Bart 1994; Dunham et al. 1999; Dunham et al. 2000; Green et al. 2006).

CxB hybrid catfish production has been limited by traditional methods of channel catfish produciton. Typical open-pond spawning success rates of channel catfish average 30.0-50.0% (Silverstein et al. 1999). CxB hybrid open-pond spawning is rare although rate as high as 33.0% have been reported (Masser and Dunham 1998). Channel catfish spawning success can be improved through techniques like pen spawning, manual stripping of eggs, and hormone injection. Silverstein et al. (1999) obtained a 95.0% cage spawning rate for channel catfish injected LH-RHa along with pimozide. Tave and Smitherman (1982) obtained an 88.0% pen spawning success rate for channel catfish injected with hCG at 1,100 IU/kg. Hybrid pen spawning rates are variable (0-100%) and less than channel catfish rates (Smitherman et al. 1996). Masser and Dunham (1998) reported that Auburn University has only obtained an average pen spawning rate for hybrid production of 15.0% over a 14 year span. Tave and Smitherman (1982) obtained a hybrid pen spawning rate of 40.0% after female hCG injection; however, Tieman (1995) was unable to obtain any hybrid pen spawns with or without female CPE injection. The major factors causing this lack of hybrid spawning success are hard to identify due to the dynamic effects that the environment and the physiological systems of the two species

has on initiating reproductive activity. Higher open-pond or pen spawning success rates could lead to less labor, less facilities, and less brood fish required to produce the number of seed needed for the full-scale adoption of hybrids into the industry.

Studies show that low, hybridization success rates may be linked to a lack of male interest and performance (Bart 1994; Dunham et al. 1999; Green et al. 2006; Hutson 2006). Pen spawning of male channel and blue catfish paired with channel females injected with carp pituitary extract has resulted in females depositing high quality eggs (Dunham et al. 2000) that are not always fertilized by the males (R. A. Dunham and R. P. Phelps, Auburn Univeristy, Dept. Fisheries and Allied Aquaculture, personal communication). Giudice (1966) reported hybrid egg masses deposited in pens and aquaria were partially or fully eaten by the adults.

Artificial spawning, where eggs are hand-stripped from females and fertilized with sperm hand-stripped from males, is a guaranteed method for producing hybrids (Masser and Dunham 1998). However, artificial spawning also has male issues. Standard milt stripping procedures are not effective on many species of male catfish (Mansour et al. 2002; Viveiros et al. 2002). Male blue catfish are often sacrificed and the testes are extracted to obtain the sperm to fertilize channel catfish eggs. This can result in losing valuable brood fish (Bart 1994; Dunham et al. 1999; Hutson 2006). Dunham (1993) was able to strip blue male sperm after LH-RHa injections, but the sacrifice of blue males to extract the testes remains the primary method for obtaining sperm for artificial spawning. Green et al. (2006) made attempts to electrically stimulate testes of male blue catfish to increase spermatozoa concentrations released from extracted testes which was unsuccessful. Hutson (2006) investigated the effects of LH-RHa implants on male blue

catfish and found that sperm production could be increased almost 20.0% depending on the male strain. Bart (1994) found that male blue catfish sperm concentrations of at least $1.25x10^5$ spermatozoa per egg are required for acceptable fertilization rates of 50.0% or higher. Keeping up the production needed for CxB hybrid seed in the catfish industry needs further research on enhancing male blue catfish performance and their interest in channel females for both natural and artificial spawning methods.

Research on hormonal pheromones in fish has expanded throughout recent years. Hormonal pheromones are typically steroids or prostaglandins and have been identified in many fish species to play a role in manipulating male reproductive behavior. The best understood hormonal pheromones affecting male behavior are those of the goldfish, *Carassius auratus* (Stacey 2003). Female goldfish release two pre-ovulatory steroids, which act as primer and releasing pheromones on males causing them to increase milt production and increase swimming and searching behaviors respectfully, and two post-ovulatory prostaglandins which also increase milt production and further stimulates male courtship behavior (Stacey 2003; Stacey et al 2003). Similar reactions in which male physiology is manipulated, or attraction to females and courtship behavior is stimulated by hormonal pheromones have been seen in species like Eurasian ruffes, *Gymnocephalus cernuus* (Sorensen et al. 2004), brown trout, *Salmo trutta*, and lake whitefish, *Coregonus clupeaformis* (Laberge and Hara 2003) four-eyed sleepers, *Bosrticthys sinensis* (Hong et al. 2004) and Nile tilapia, *Oreochromis niloticus* (Pinheiro et al. 2003).

Hormonal pheromones are thought to be responsible in hybridization events for some fish species. Essington and Sorensen (1996) postulated that the hybridization of brown trout and Atlantic salmon, *Salmo salar*, observed in natural water bodies may be a

result of the similarities in male brown trout and Atlantic salmon to specific prostaglandins. Olfactory sensitivities between wild common carp, *Cyprinus carpio*, and goldfish to the common hormonal pheromones employed by the goldfish were observed by Irvine and Sorensen (1993). A similar sensitivity to two of the hormonal pheromones used by goldfish has also been seen in crucian carp, *Carassius carassius* (Burnard et al. 2008). In European natural water bodies where common carp, goldfish, and crucian carp inhabit, hybrids between all three species have been observed (Hanfling et al. 2005).

Sorensen et al. (1988) observed that injections of prostaglandin into female goldfish could cause the female to release some of the synthetic prostaglandin out into the water. One objective of this study was to evaluate the effectiveness of channel female injections with the hormonal pheromones 17α, 20β-dihydroxy-4-pregnene-3-one (17,20β-P), 17,20β-P-20-glucosiduronate, and prostaglandin F-2α (PGF-2α) to determine if channel and blue male attraction to channel females can be significantly increased. Another objective was to determine if injections of the same pheromones into blue males might attract female channel catfish. The last objective was to determine if the most effective blue male pheromone could be coupled with LH-RHa injected channel females to significantly increase vat spawning success, and determine if the most effective female pheromone injected into male blues might also significantly increase vat spawning success. The goal was to develop a possible injection method that might increase pen spawning success of female channel and male blue catfish.

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II. LITERATURE REVIEW

Natural spawning of channel and blue catfish

The reproductive biology of channel and blue catfish is thought to be similar (Rubec 1979; Graham 1999). Channel catfish typically reach sexually maturity in two to three years (Tucker and Robinson 1990) whereas blue catfish require around four to five years (Graham 1999; Wyatt et al. 2006). Channel catfish held in aquaculture settings begin spawning once water temperatures have begun to stabilize between 21 ºC and 30 ºC (Meyer et al. 1973; Tucker and Robinson 1990). Wyatt et al. (2006) reported optimal spawning temperatures at a hatchery in Texas of 21 ºC and 27 ºC for blue and channel catfish respectfully. A male will find some type of cavity in a secluded, semi-dark space to prepare a nest. He fans out as much debris from the area as possible before the spawning act. The female comes to the nest after being attracted to either the nest itself or the male via olfactory or social cues (Rubec 1979). The male is thought to be attracted to the female by pheromones (Timms and Kleerekoper 1972; Rubec 1979). The male and female embrace head-to-tail during the spawning event before eggs are laid and fertilized (Tucker and Robinson 1990). The channel female will lay around 6,600-8,800 eggs/kg of body weight in intervals over a 3 to 4 hour period (Meyer et al. 1973; Tucker and Robinson 1990). Blue females are reported to produce around 900-1,350 eggs/kg (Graham 1999). The spawning act of channel catfish may persist for 6 to 8 hours after the initiation of egg deposition (Dunham 1993). Once the act is complete, the male will drive

the female away. He will fan and pack the egg mass with his pelvic fins to provide aeration and remove waste products (Meyer et al. 1973; Tucker and Robinson 1990). He will guard the nest until the eggs hatch and fry are ready to leave the nest.

The exact type of communication cues causing female attraction to the nest and male, male attraction to the female, and the prolonged spawning event are not fully understood. Spawning in an environment where visual communication may be hindered by water turbidity and the darkness of the chosen nest sight suggest the involvement of other cues such as olfactory, gustatory, or auditory cues (Stacey et al. 2003). Rubec (1979) suggested brown bullheads, *Ameiurus nebulosus*, use pheromones to coordinate the reproductive act that is similar to that of channel and blue catfish. Observations by Rubec (1979) also suggested that pheromones in the urine of yellow (*A. natalis*), black (*A. melas*), and brown bullheads provide a prezygotic isolating mechanism that prevents hybridization between the species.

Hybridization in fish

Although channel and blue catfish can be found in overlapping habitats during the spawning season, reports of hybrids between the two species in nature could not be found. There are many factors that can result in reproductive isolation between closely related fish species. As noted by Hubbs (1955), hybridization in fish is first dependent on the successful meeting of eggs and sperm which requires two species to spawn during the same time of year and in similar habitats. Hybridization in the centrarchid genus *Lepomis* is a common occurrence in many natural waters. As much as 75% of a population of *Lepomis* has been identified as hybrids in some locales (Trautman 1957). Keenleyside (1967) commented on the similar seasonal and habitat spawning requirements of *Lepomis*

megalotis (longear sunfish), *gibbosus* (pumpkinseed), and *macrochirus* (bluegill) which was thought to be the primary factor influencing their hybridization. Keenleyside (1967) also demonstrated that males of the three species could readily distinguish between con-specific and hetero-specific females, which was hypothesized to provide a reproductive isolating mechanism between sympatric populations of the three species. However, Steele and Keenleyside (1971) found that male *L. megalotis* and *gibbosus* lack the ability to discriminate between con-specific or hetero-specific females either visually or non-visually, but female *L. gibbosus* preferred con-specific males based on non-visual cues and female *L. megalotis* preferred con-specific males based on visual cues. Childers (1965) reported that female *L. macrochirus* interbred with male *L. microlophus* (red ear sunfish) when the opercular flap of the male was removed.

Mate preference in closely related fish species that spawn during similar seasons and in similar habitats can be highly influenced by visual, olfactory, or auditory cues which can also provide a reproductive isolating mechanism between species. As previously mentioned, female *L. megalotis* are able to visually distinguish between con-specific and hetero-specific males, and prefer con-specific partners (Steele and Keenleyside 1971). Visual preference in con-specific mating partners has been observed in other fish species like the pupfishes *Cyprinodon maya* and *C. labiosus* (Kodric-Brown and Strecker 2001), cichlids *Haplochromis nyererei* and *H.* "zebra nyerere" (Seehausen and von Alphen 1998), hamlets in the genus *Hypoplectrus* (Fischer 1980), and the sailfin molly, *Poecilia latipinna* (Ptacek 1998). Olfactory preference in con-specific mating partners has been seen in species like the cichlids *Pseudotropheus emmiltos* and *P. fainzilberi* (Plenderleith et al. 2005), swordtails *Xiphophorus birchmanni* and *X. variatus*

(Wong et al. 2005), pupfishes *C. maya* and *C. labiosus* (Kodric-Brown and Strecker 2001), and three-spined stickleback, *Gasterosteus aculeatus* (Rafferty and Boughman 2006). Sound production has been identified in over 800 species of fish within 109 families (Kasumyan 2008). Difference in auditory signals during spawning has been observed in closely related species of at least six species of *Lepomis* (Gerald 1971) and the cichlids *Tramitichromis* cf. *intermedius* and *Copadichromis conophorus* (Lobel 1998) which were thought to aid in species recognition during spawning.

The fact that hybrids between female channel and male blue catfish have been obtained through artificial methods which fertilization and hatching rates are above 60.0% (Dunham et al. 2000) suggest that there is probably no gametic or developmental block inhibiting hybridization. Spawning of the two different species has occurred synchronously in aquaculture settings, suggesting no major environmental, inhibiting mechanism between the two species. Tave and Smitherman (1982) deducted that behavioral manipulation is a key to induce hybridization as hCG injection resulted in a 40.0% pen spawning success rate. Although hybrids can be obtained using the pen spawning method with hormone injection, the low rates of hybridization observed via open pond and pen spawning (0% observed by Tieman (1995), 15.0% observed by Dunham et al. (2000), and 40.0% observed by Tave and Smitherman (1982)) are probably a result of mate preference of the two species. The general similarity in appearance of the two species as evidenced by the misidentification of the species by some aquaculturist (Carmichael et al. 1992; Masser and Dunham 1998) may not be elaborate enough to provide a visual isolation mechanism. As mentioned previously, visual communication during spawning could even be hindered by water turbidity and the

darkness of the chosen nest. This leaves the possibility of olfactory or auditory cues playing a role in mate preference. Given the similarities in ictalurid spawning (Rubec 1979; Graham 1999), it may be possible that channel and blue catfish are employing pheromones like that of bullheads observed by Rubec (1979), resulting in their low hybridization rates.

Hormonal pheromones of fish

Pheromones have been described as 'substances which are secreted to the outside by an individual and received by a second individual of the same species, in which they release a specific reaction, for example, a definite behaviour or a developmental process' (Karlson and Luscher 1959). Many pheromones are recognized by olfaction or taste (Resink et al. 1987; Sorensen and Stacey 1999). Hormones are 'regulatory molecules synthesized in one cell or endocrine gland and transported to another cell or tissue' (Horton et al. 2006). During spawning, many fish use hormonal pheromones such as water-borne sex steroids, prostaglandins and their metabolites to induce physiological and behavioral effects among their species. Hormonal pheromones can have essential roles in mate attraction and gonadal maturation for some fish species (Stacey et al. 2003). Hormonal pheromones may be the part of the key components for successful spawning in channel and blue catfish.

The most understood, hormonal pheromone system is that used by the goldfish, *Carassius auratus* (Stacey 2003). The goldfish model involves a luteinizing hormone (LH) surge, steroids, and prostaglandins. During optimal environmental conditions for spawning, females undergo a gonadotropin or LH surge which starts the onset of final oocyte maturation. During this time, she releases two primary steroids, 17α, 20β-

dihydroxy-4-pregnene-3-one (17,20β-P) and 17,20β-P-20β-sulfate (17,20β-PS), which alters male behavior and physiology by increasing gonadotropin levels in males causing an increase in milt production, and swimming and searching behaviors. As the LH surge and steroid production decreases by females, and oocytes have matured, females undergo ovulation as a result of prostaglandin F-2α (PGF-2α) production, which also alters male behavior and physiology by increasing milt production and further stimulating male courtship behavior (Stacey 2003; Stacey et al 2003; Gerlach 2006).

Influences on male fish

Male behavior and physiology is primarily affected by pre-ovulatory steroids and prostaglandins (Gerlach 2006). Various studies with pheromonal steroids and prostaglandins have been done to determine how male behavior and physiology is affected. Hong et al. (2004) found that male, four-eyed sleepers, *Bosrticthys sinensis*, were attracted to artificial nests baited with 17,20β-P and a prostaglandin of the E series, and that these two compounds also increased spawning frequency. Eggs deposited in prostaglandin E (PGE) baited nests also showed high fertilization rates. Laberge and Hara (2003) found that PGF-2*α* induced an increased amount of male swimming activity for brown trout, *Salmo trutta*, and lake whitefish, *Coregonus clupeaformis*. Olsén et al. (2006) observed male gold fish with increased levels of luteinizing hormone and expressible milt shortly after being exposed to 17,20β-P injected females. Pinheiro et al. (2003) observed increased sperm volume and concentration in male Nile tilapia, *Oreochromis niloticus*, exposed to water-borne 17,20β-P. Stacey et al. (2003) found that 17,20β-P; 17,20β-PS; and PGF-2α induce swimming and searching behaviors, and increase milt volume in male goldfish.

Female attractiveness to male con-specifics has been induced through injections of specific hormonal pheromones. Volkoff and Peter (1999) observed increased male spawning behaviors (courting, chasing, and nudging) for several hours after injecting female goldfish with PGF-2α at approximately 0.67 mg/kg. Kitamura et al. (1994) observed increased courtship attempts by exposed males up to 90 minutes after injecting female oriental weatherfish, *Misgurnus anguillicaudatus*, with either PGF-2α, 15-keto-PGF-2α, or 13,14-dihydro-15-keto-PGF-2α at 2.5 mg/kg. Sorensen et al. (2004) injected female ruffes, *Gymnocephalus cernus*, with 4-pregnen-17,20β,21-triol-3-one (20β-S) which induced the release of a pheromone(s) that stimulated nudging and increased swimming activity in males. Dominant male Nile tilapia exhibited increased chasing frequencies as compared to the control when exposed to females injected with 17,20β-P at 0.2 mg/kg (Souza et al. 1998). Sorensen et al. (1988) injected non-ovulated female goldfish with PGF-2α at 0.4 mg/kg and observed that odorous cues produced by injected females were similar to those released by ovulated females which resulted in male reproductive behaviors (chasing and nudging). After injecting the females, Sorensen et al. (1988) found that high levels of PGF-2α were released by injected goldfish out into the water which was composed of 2.0% of the synthetic PGF-2α that was initially injected.

Effects on male catfish

These male behavioral and physiological traits in response to steroids have appeared in some species of siluriforms. Stacey et al. (2003) reported that two catfish, *Synodontis ocellifer* and *Clarias gariepinus*, respond to water-borne, un-conjugated and conjugated metabolites of androgenic and C-21 (17,20β-P-like) steroids respectfully including 3α,17-5βP-g; 3α,17-5β-5βP; and 5β-androstan-3a-ol-17-one.

Very little is known about the effects of steroid and prostaglandin exposure on male ictalurids. It has been documented that unidentified pheromones released by female channel catfish and brown bullheads do play a role in attracting male con-specifics (Rubec 1979). Mississippi fishermen have been known to use cages in rivers baited with ripe channel females to catch mature male channels (Timms and Kleerekoper 1972).

Steroid converting enzymes have been identified in channel catfish ovaries (Kumar et al. 2000). These enzymes include 3β-hydroxysteroid dehydrogenase (3β-HSD), cholesterol side chain cleavage (P450scc), 17α-hydroxylase/lyase (P450c17), and aromatase (P450arom). These enzymes begin with cholesterol and convert it to many different steroids which play a role in ovary development and maturation. One product produced during cholesterol conversion is 17,20β-P which has been shown as a male attractant in other species. The steroid 17,20β-P is suggested to be a maturation inducing steroid for channel catfish (Kumar et al 2000; Kazeto et al. 2005). However, Lambert et al. (1999) observed no significant effects on ovulation or fertilization when channel females were supplementally injected with 17,20β-P in conjunction with a priming dose of CPE. The enzymes also show a seasonal pattern in their presence, but are found predominately in the ovaries from February through June. The presence of these enzymes during the prime spawning season of channel catfish suggests that channel females may respond to injections of pheromonal steroids like 17,20β-P which could lead to final oocyte maturation or ovulation as seen in the Amur catfish, *Silurus osatus* (Miwa et el. 2001), *Heteropnustes fossilis* (Tripathi and Singh 1995), yellow catfish, *Pseudobagrus fulvidraco* (Lim et al. 1997), and Asian walking catfish, *C. batrachus* (Haider and Rao 1994). Injections of pheromonal steroids could also cause a production of male attracting

and milt increasing pheromones as seen in other species (Souza et al. 1998; Pinheiro et al. 2003; Stacey et al. 2003; Sorensen et al. 2004; Olsen et al. 2006).

There is very little research on prostaglandin production in channel catfish. Busby et al. (2002) found that the brown bullheads possessed the ability to breakdown PGE2 in hepatocytes, although the PGE-2 had no effect on glycogenolysis. Tikare et al. (1983) found that PGF-2α injections at 0.5 mg/kg induced ovulation and spawning activity in the female catfish, *C. batrachus*. It may be possible that PGF-2α injections into female channel catfish may induce ovulation and spawning activity as seen in *C. batrachus* and other species, as well as illicit male attraction responses (Kitamura et al. 1994; Laberge and Hara 2003; Stacey et al. 2003; Hong et al. 2004).

Influences on female fish

Pheromonal attractants released by male fishes have been observed and identified in many species. Male peacock blennies (*Blennius pavo*), black gobies (*Gobius jozo*), and sea lampreys (*Petromyzon marinus*) have all been reported to release a female sex attractant (Van Den Hurk and Resink 1992). The primary pheromone released by male *P. marinus* that attracts ovulating females has been identified as 3-keto petromyzonal sulfate, which may serve to control sea lamprey populations in the Great Lakes region (Siefkes et al. 2005). Steroid glucoronides released by male zebrafish, *Brachydanio rerio*, have been shown to induce ovulation in female con-specifics as well as attract them (Van Den Hurk and Resink 1992). African catfish, *C. gariepinus*, release glucorinated steroids which attract female con-specifics who have ovulated (Resink et al. 1987; Van Den Hurk et al 1987; Lambert and Resink 1991; Van Den Hurk and Resink 1992). Male round gobies, *Negobius melanostomus*, have been found to release pheromones that attract

female con-specifics, which are thought to be beneficial in guiding females to male nest which are often located in dark areas, possibly like the spawning areas of channel and blue catfish (Gammon et al. 2005).

Effects on female catfish

An issue that may contribute to low open-pond hybridization success is a lack of channel female attraction and gonadal response to males. As previously mentioned, observations of channel catfish mating have revealed that male channel catfish clean and prepare a nest site in a dark sheltered area. Males are then thought to release pheromones which attract the female and initiates courtship behavior (Lambert et al. 1999). This mating behavior is also thought to be similar in blue catfish and other ictalurids (Rubec 1979; Graham 1999). Fishermen and researchers have used traps baited with mature male channel catfish and black bullheads to catch female con-specifics (Rubec 1979). Research by Rubec (1979) suggests that urine of male black bullheads contains pheromones that attract female black bullheads injected with gonadotropin.

There is very little research on steroidal enzyme production in the testes of channel and blue catfish. Some steroidal enzymes identified in the zebra fish and *C. gariepinus* include uridine-diphosphoglucose dehydrogenase (UDPGD) and 3β-HSD (Van Den Hurk et al 1987; Van Den Hurk and Resink 1992). These enzymes are responsible for glucorinating different steroids and inactivating them, which then leads to their attraction effects on female con-specifics.

Schoonen et al. (1987) observed that culture conditions of male *C. gariepinus* affected the production of different steroids. When compared to feral *C. gariepinus*, testes from farm raised catfish showed a reduced production in important C-21 steroids

similar to 17,20β-P, which are thought to be important in sperm production and inducing spawning behavior. This lack of steroid production was thought to be due to a lack of gonadotropin secretion and an ensuing increased activity of cytochrome P-450 enzymes like 17α-hydoxylase and 11β-hydroxylase, which caused an increase in androgenic steroids. If blue catfish possess enzymes responsible for glucorinating steroids, it may be possible that steroid injections with a basic C-21 steroid like 17,20β-P into male blue catfish may lead to a cascading effect that will cause a release in glucorinated steroids. These glucorinated steroids could possibly induce ovulation and attract female channel catfish, overcoming a possible lack of gonadatropin secretion as seen in female *C. gariepinus,* which prevents ovulation and spawning behavior (Resink et al. 1989). Glucorinated steroids may attract ovulating female channel catfish into dark, sheltered spawning areas as seen in other species (Van Den Hurk and Resink 1992). Injections of 17,20β-P into male blue catfish may also overcome inhibition of gonadotropin release, which has been shown to be responsible for production of important C-21 steroids in male *C. gariepinus* (Schoonen et al. 1987). Steroid injections with 17,20β-P into male blue catfish could possibly even induce a gonadotropin surge as seen in male goldfish (Stacey 2003; Stacey et al. 2003).

Species specificity

Closely related species may share some similarity in the pheromones they use during spawning which could result in hybridization (Burnard et al. 2008). Common carp (*Cyprinus carpio*), goldfish, and crucian carp (*Carassius carassius*) have hybridized in European natural water bodies where at least one of the species has been introduced (Hanfling et al. 2005). Similar olfactory sensitivities of wild common carp and goldfish

to the common hormonal pheromones employed by the goldfish (17,20β-P; 17,20β-PS; PGF-2α; and 15-keto- PGF-2α) have been observed by Irvine and Sorensen (1993). Goldfish and crucian carp also show a similar sensitivity to 17,20β-P and PGF-2α (Burnard et al. 2008). Prostaglandins are thought to have a relatively universal action on many fish (Burnard et al. 2008). Hybrids between brown trout, *Salmo trutta*, and Atlantic salmon, *Salmo salar*, have been observed in nature. Essington and Sorensen (1996) observed similarities in male brown trout and Atlantic salmon in response to odorous PGF-2α and its derivatives which led them to postulate that the two species might employ the same pheromone system leading to their hybridization.

Many fish also show varying sensitivities and reactions to hormonal pheromones and their mixtures. Male goldfish can individually discriminate between the three primary pre-ovulatory pheromones (17,20β-P; 17,20β-PS; and androstenedione) used by females as the pheromones are released all at the same time but at varying concentrations throughout pre-ovulation (Poling et al. 2001). Brown trout exhibit increased locomotor activity in response to PGF-2α and 13, 14-dihydro-PGF-2α, while lake whitefish exhibit increased locomotor activity to PGF-2α and 15-keto-PGF-2α and rainbow trout, *Oncorhyncus mykiss*, exhibit no locomotor response or electro-olfactory sensitivity to any of the three prostaglandins (Laberge and Hara 2003). Male Atlantic salmon are highly sensitive to PGF-1α, PGF-2α, slightly sensitive to 15-keto-PGF-2α, and not sensitive to 13, 14-dihydro-PGF-2α (Moore and Waring 1996). Male Atlantic salmon sensitivities to PGF-1α and PGF-2α are also influenced by the sexual maturity of the male with more mature males being more sensitive. Males also exhibit increased levels of expressible milt in response to water borne PGF-1α and PGF-2α. Hong et al. (2004) found that male

B. sinensis were more likely to be attracted to artificial nest bated with 17α-P; 17α,20β-P; and PGE-2 but not PGF-2α, and that 17α,20β-P and PGE-2 baited nests were effective in inducing spawning while PGF-2α was ineffective.

Observations by Rubec (1979) suggested that pheromonal cues in the urine of yellow, black, and brown bullheads provide a prezygotic isolating mechanism that prevents hybridization between the species. It is possible that blue and channel catfish have innate responses to intra-specific pheromones and have learned to strongly discriminate them from pheromones of the other species. Blue and channel males may be employing highly species specific pheromones to attract females to nest; this, along with species specific pheromones used by females to attract males during the spawning act, may be the reason hybrids between the two are rarely obtained using the open-pond spawning method as the chance of the two species pairing together would be greatly reduced. Pairing the species in pens for induced spawning has resulted in better but still very low spawning success. By pairing the species in pens, aquaculturist may be overcoming the reproductive isolating mechanism in the form of female-attracting pheromones produced by males. The low success of pen spawning appears to be a result of an isolating mechanism in the form of species-specific male-attracting pheromones produced by females. However, it may be that blue males show at least a partial attractiveness and non-discrimination to pheromones released by channel females which results in low pen spawning success. Given the results of research on hormonal pheromones, it seems probable that injection of a hormonal pheromone(s) into channel females may be used to attract blue males and possibly increase pen spawning success.

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III. EFFECTS OF PHEROMONAL STEROIDS OR PROSTAGLANDIN ON ATTRACTING MALE BLUE CATFISH AND MALE CHANNEL CATFISH

Abstract

The effects of the hormonal pheromones 17α, 20β-dihydroxy-4-pregnene-3-one (17,20β-P), 17,20β-P-20-glucosiduronate, and prostaglandin F-2α (PGF-2α) injected into female channel catfish, *Ictalurus punctatus*, were investigated to determine if male channel and blue catfish, *I. furcatus*, could be attracted to the injected females. Females were injected IP at 0.5 mg/kg with one of the three pheromones or an ethanol control solution. Females were then evenly distributed into traps placed in ponds containing either channel or blue males. Traps were checked for captured males at various intervals up to 96 hours post-injection. Overall trapping efficiency based on the number of captures that could have been obtained was poor (4.8%). However, males were 17.86 (95% CI: 2.34, 142.86) times more likely to be captured with a PGF-2α injected female than with an ethanol-only injected female, or a 17,20β-P injected female, and 3.47 (95% CI: 1.23, 9.71) times more likely to be captured with a PGF-2α injected female than with a 17,20β-P-20-glucosiduronate injected female. Channel males responded to PGF-2α injected females on 36.1% of their opportunities as compared to only 2.8% for non-treated females ($P < 0.05$). Blue males responded to both PGF-2α and 17,20β-P-20-glucosiduronate injected females on 8.3% of their opportunities, and did not respond to

non-treated females throughout the study ($P > 0.05$). Males were 5.95 (95% CI: 1.57, 10.32) times more likely to be caught between 48 and 96 hours after female injection than between 12 and 24 hours post-injection. These results suggest that the overall best pheromone to attract blue and channel males was PGF-2α at 48 to 96 hours post-female injection.

Introduction

The hybrid between female channel catfish (*Ictalurus punctatus*) and male blue catfish (*I. furcatus*) exhibits better traits for commercial aquaculture than do channel catfish. These hybrids show 30.0-60.0% greater survival in response to low dissolved oxygen (Dunham et al. 1983b) and greater resistance to diseases such as *Flexibacter columnaris*, *Edwardsiella ictaluri*, and *Aeromonas hydrophila* than do channel catfish (Wolters et al. 1996; Masser and Dunham 1998). Seinability is greater for hybrids (Dunham and Argue 1998), and food conversion is around 10.0-15.0% better for hybrids than channel catfish (Wolters 1993; Smitherman et al. 1996). Hybrids also exhibit faster growth to market size (Giudice 1966; Dunham and Brummett 1999), higher dress out percentages (Argue et al. 2003), and increased uniformity in size and shape (Smitherman et al. 1996) than the parent species. These superior traits exhibited by hybrids can result in a 10.0% increase in a catfish farm's profit margin (Masser and Dunham 1998), and reduce fingerling production cost between 15-22.5% (Ligeon et al. 2004).

The promising traits exhibited by these hybrids and the possibility of increased profit have lead to increased interest in producing these hybrids. However, production of hybrids has been limited due to some type of reproductive isolating mechanism that exists between the two species (Tave and Smitherman 1982; Dunham et al. 1999).

Traditional spawning methods used for channel catfish production are not as successful for the production of hybrids. Open pond spawning rates of 30.0% were reported by Smitherman et al. (1996), and pen spawning has varied from 0-100.0% (Masser and Dunham 1998). Open pond spawning success for channel catfish averages between 30.0-50.0% (Wolters 1993; Silverstein et al. 1999). A production method for consistently obtaining hybrids is through manual stripping of eggs from the female channel and artificial fertilization with blue male sperm. Although various improvements have been made in the stripping of eggs and artificial fertilization (Dunham 1993; Bart 1994; Green et al. 2006; Hutson 2006), commercial production of hybrid fry is estimated to be around 325% higher than channel catfish fry production (Umali-Maceina 2007). This increased production price of fry is recovered by the increased profit associated with the superior culture traits exhibited by the hybrids.

The full scale adoption of hybrids to the catfish industry requires enhancement in spawning techniques. The spawning behavior of the two species is thought to be similar (Rubec 1979; Graham 1999). Communication factors that dictate the spawning behaviors of the species are not well studied. Spawning in an environment where visual communication can be hindered by water turbidity or darkness does suggest the involvement of other cues such as olfactory, gustatory, or auditory cues (Stacey et al. 2003). It is documented that unidentified chemicals released by mature female and male channel catfish do play a role in attracting the different sexes (Timms and Kleerekoper 1972; Rubec 1979). A 40.0% pen spawning success rate for hybrid production observed after injection of human chorionic gonadotropin (hCG) injection into female channel

catfish suggest behavioral manipulation with the use of hormones could be a key in increasing hybridization success (Tave and Smitherman 1982).

Hormonal pheromones affecting olfactory senses have been well studied in some species of fish. The hormonal pheromone model for the reproduction of goldfish, *Carassius auratus*, has been mapped out, which describes behavioral and physiological changes that occur in the two sexes during spawning (Stacey 2003). The goldfish model involves a luteinizing hormone (LH) surge, steroids, and prostaglandins. During optimal environmental conditions for spawning, females undergo a gonadotropin or LH surge which starts the onset of oocyte maturation. During this time, she releases two primary steroids, 17α, 20β-dihydroxy-4-pregnene-3-one (17,20β-P) and 17,20β-P-20β-sulfate (17,20β-PS), which alters male behavior and physiology by increasing gonadotropin levels in males. As the LH surge and steroid production decreases by females, and oocytes have matured, females undergo ovulation as a result of prostaglandin F-2α (PGF-2α) production, which also alters male behavior and physiology (Stacey 2003; Stacey et al 2003; Gerlach 2006).

Similar hormonal pheromone interactions have been seen in other species. Hong et al. (2006) found that male black sleepers, *Bosrticthys sinensis*, were attracted to artificial nests baited with (17,20β-P) and a prostaglandin of the E series, and that these two compounds increased spawning frequency. Laberge and Hara (2003) found that PGF-2*α* induced increased amounts of male swimming activity for brown trout, *Salmo trutta*, and lake whitefish, *Coregonus clupeaformis*. Sorensen et al. (2004) observed similar results in which female Eurasian ruffes, *Gymnocephalus cernuus*, injected with trihydroxylated progesterone, 20β-S, stimulated increased amounts of male swimming

activity. Pinheiro et al. (2003) observed increased sperm volume and concentration in male Nile tilapia, *Oreochromis niloticus*, exposed to water-borne 17,20β-P. Stacey et al. (2003) reported that two siluriforms, *Synodontis ocellifer* and *Clarias gariepinus*, respond to un-conjugated and conjugated metabolites of androgenic and C-21 (17,20β-P-like) steroids respectfully.

Very little is known about the effects of pheromonal steroid exposure on ictalurids. Female channel catfish produce steroid converting enzymes such as 3β-hydroxysteroid dehydrogenase (3β-HSD), cholesterol side chain cleavage (P450scc), 17α-hydroxylase/lyase (P450c17), and aromatase (P450arom) during the prime spawning season for the species (Kumar et al. 2000). These enzymes are capable of converting cholesterol into important steroids like 17,20β-P, which is thought to be a maturation inducing steroid for channel catfish (Kumar et al. 2000; Kazeto et al. 2005). Channel female injection of steroids like 17,20β-P could induce final oocyte maturation and ovulation as seen in the other siluriforms, Amur catfish, *Silurus osatus* (Miwa et el. 2001), Asian walking catfish, *C. batrachus* (Haider and Rao 1994), *Heteropnustes fossilis* (Tripathi and Singh 1995), and yellow catfish, *Pseudobagrus fulvidraco* (Lim et al. 1997). Injections of pheromonal steroids could also cause a production of male attracting and milt increasing pheromones as seen in other species (Pinheiro et al. 2003; Stacey et al. 2003; Sorensen et al. 2004; Olsén et al. 2006).

There is little information on prostaglandins in channel or blue catfish reproduction. PGF-2*α* injections into female goldfish are used to initiate spawning behaviors in goldfish (Kobayashi and Stacey 1993; Kobayashi and Nakanishi 1999; Volkoff and Peter 1999). Brown bullheads, *Ameiurus nebulosus*, possess the ability to

breakdown PGE-2 in hepatocytes, although PGE-2 has no effect on glycogenolysis (Busby et al. 2002). Tikare et al. (1983) induced ovulation and spawning activity in the female *C. batrachus* by injecting females with PGF-2*α*. It may be possible that PGF-2*α* injections into female channel catfish may induce ovulation and spawning activity as seen in *C. batrachus* and other species, as well as illicit male attraction responses (Laberge and Hara 2003; Stacey et al. 2003; Hong et al. 2006).

One objective of this study was to determine the effectiveness of female injections with 17,20β-P, 17,20β-P-20-glucosiduronate, and PGF-2*α* on eliciting male blue and channel catfish's attraction to channel females. Another objective was to determine if injections of these same compounds into blue males might elicit female channel catfish attraction to blue males. The goal was to identify a hormonal pheromone that increases the attractiveness between blue males and channel females.

Methods and materials

Basic experimental protocol

Channel females and blue males were injected with one of three possible pheromones (PGF-2α, 17,20β-P, or 17,20β-P-20-glucosiduronate) or an ethanol solution for use as a control. Injected Kansas and Marion strain females were placed separately into traps and distributed equally by injected pheromone and genetic strain of female into two ponds stocked with either twenty Rio Grande blue or Kansas strain channel males. Injected Rio Grande blue males were placed separately into traps and distributed equally by injected pheromone into a pond containing twenty Marion strain channel females. Twelve traps were used in each pond (Fig. 1). Two, 96 hour long trials were carried out in which traps were checked for male and female captures at 6, 12, 24, 48, 72, and 96

hours post-fish injection during each trial. New channel females and blue males were injected and used for each trial but the same free swimming males and channel females were used throughout both trials. An outline of the number of fish injected by sex, species, and strain, and then placed into traps in the different ponds is depicted in Table 1. The male's responses to the injected pheromone of the female, the post-injection time, female strain, and individual female injected were recorded throughout both trials. The channel female's responses to the injected pheromone of the blue male, the post-injection time, and individual male injected were recorded throughout both trials.

Broodstock maintenance

All catfish used during the experiment were held at the E. W. Shell Fisheries Center and fed at approximately 1700 hours with a 32.0% crude protein extruded catfish diet. During colder months, fish were fed three times a week at 0.5% body weight per day. As water temperatures warmed to approximately 17 ºC, fish were fed five times a week at 1.0% body weight per day until the first experiment was to be conducted in early May, 2008.

Rio Grande blue males, approximately eight years in age, were held in a 0.1 ha pond at a density of 2,924.8 kg/ha (mean individual weight = 7.71 kg). Rio Grande blue males as well as Kansas channel males of unknown age classes were held in another 0.1 ha pond at a density of 928.7 kg/ha (mean individual weights = 4.80 and 3.58 kg respectfully). Marion channel females, approximately six years in age, were held in two, 0.1 ha ponds at densities of 1,196.6 and 1,335.9 kg/ha respectfully (mean individual weight = 3.72 kg). Kansas channel females, approximately five years in age, were held in a 0.1 ha pond at a density of 1,422.5 kg/ha (mean individual weight = 5.26 kg).

Trap preparation

Traps were constructed from cylindrical, white plastic barrels (80 cm in length and 51 cm in diameter; Fig. 2). A circular, 20.0 cm diameter hole was cut into the lower portion of one end of the barrel using a jigsaw. Plastic netting was folded to form an open cylinder 10.0 cm long with a 20.0 cm diameter (Fig. 3). The cylinder was attached to the opening of the container with zip ties so that 5.0 cm of the cylinder extended inside of the container. A 22.0 cm X 22.0 cm flap of plastic netting was connected to the side of the cylinder inside of the barrel. The flap was connected to the cylinder with two zip ties and a bungee strap allowing fish to enter, but closing once a fish had entered (Fig. 4). A 20.0 cm X 25.0 cm access door to the trap was cut on the upper horizontal side of the barrel so that the circular opening was positioned downwards (Fig. 2). Zip ties were used to hinge the doors, as well as keep them closed during the trials. Traps were held on the pond bottom by the use of rebar placed through the two ends of the barrel (Fig. 2). During the second trial, a second bungee strap was added to each trap to reduce the number of injected fish from escaping (seven fish in Trial-1 vs. two fish in Trial-2). A total of twelve traps were placed in each pond approximately 6.4 ± 0.65 m apart from each other and 1.0 m away from the pond bank.

Experimental pond stocking of free-swimming fish

Three ponds were drained for the removal of all other fish species. The ponds were given a day to dry before refilling. The ponds were filled with water supplied from a rain-fed reservoir. Each pond was approximately 644.0 m^2 and had an average depth of 1.4 m.

Fish were seined and transported to one of the three experimental ponds and stocked into their respective ponds and allowed to range freely. These fish were not injected with any pheromones during the experiment and were used during both trials. Females were selected based on overall healthy appearance and roundness of the abdomen. Males were selected based on overall healthy appearance, presence of fighting wounds, and their ability to fit through the 20.0 cm diameter trap door. The large, eight-year-old Rio Grande blue males (mean weight ± SEM = 8.17 ± 0.20 kg) were stocked into one of the ponds at a density of 311 fish/ha. Marion channel females and Kansas channel males, with average weights of 4.37 ± 0.14 and 3.92 ± 0.23 kg respectfully, were stocked separately at the same fish density as the Rio Grande blue males (1,359.1 and 1,219.1 kg/ha respectfully) into the other two ponds.

Hormone administration

Stock solutions of PGF-2α, and 17,20β-P (Sigma Chemical, St. Louis, MO), and 17, 20β-P-20-glucosiduronate (Steraloids Inc, Newport, RI) were prepared by diluting each compound in a 100% ethanol solution to achieve a concentration of 2.0 mg/ml of ethanol. Solutions were prepared one day prior to injection and stored in a refrigerator at approximately 5 ºC. A 100% ethanol solution was used as a control. Injections were given inter-peritoneal (IP) at the base of the pelvic fin with a 3.0 ml syringe and a 22.5 gauge needle. Female fish received injections of PGF-2α, 17,20β-P, or 17,20β-P-20-glucosiduronate at 0.5 mg/kg of body weight. Dose rates were based on results of Kobayashi and Stacey 1993, Zheng et al. 1997, Lambert et al. 1999, Volkoff and Peter 1999, Sorensen et al. 2004, and Olsén et al. 2006.

Before the first day of each trial, fish were seined from the holding pond and held overnight in a holding tank. The next day, fish were transported to one of the two experimental ponds. They were injected and placed into individual traps. Injections began at approximately 0700 hours and were finished by 0830 hours on the first day of each trial. During each trial, each of the two experimental ponds contained three randomly distributed replicates of control, 17,20β-P, 17,20β-P-glucosiduronate, and PGF-2α injected fish.

Stocking of injected fish

Immediately after injection, Marion and Kansas strain injected females were placed randomly into traps in the ponds containing the large, free-swimming Rio Grande blue males and Kansas channel males. In Trial-1, the pond containing the free-swimming Rio Grande blue males received eight Marion and four Kansas injected channel females (mean weights = 4.24 ± 0.17 and 5.67 ± 0.34 kg respectfully), and in Trial-2, four Marion and eight Kansas injected channel females (mean weights = 3.90 ± 0.38 and 5.80 ± 0.40kg). The same free-swimming Rio Grande blue males were used during both trials. Treatments were distributed equally among the strains for both trials in order to obtain three total replicates for each strain and treatment (Table 2). The pond containing the free-swimming, Kansas channel males received four Marion and eight Kansas injected channel females (mean weights = 3.82 ± 0.28 and 4.73 ± 0.14 kg respectfully), and eight Marion and four Kansas injected channel females (mean weights = 3.91 ± 0.28 and 4.83 ± 0.40 kg respectfully) during Trial-1 and Trial-2 respectfully. The same free-swimming Kansas channel males were used during both trials. Treatments were again distributed equally among the strains for both trials (Table 2). The pond containing the free-

swimming Marion channel females received twelve injected Rio Grande blue males (mean weight = 4.88 ± 0.28 kg; Table 2) during the first trial. Due to a lack of response in Trial-1, a second trial was not conducted with injected blue males.

Injected fish were collected at the end of each trial, stored in 20.0 m² concrete vats, and saved for later use in other spawning studies.

Data collection

There were two trials conducted on May 4th and 13th of 2008 respectfully with two days of separation between them, each following the same protocol. During each trial, traps were checked at 6, 12, 24, 48, 72, and 96 hours after fish injection. Trials were complete at 96 hours post-fish injection.

The same non-injected, free-swimming fish used during both trials were monitored for each time they entered a trap. The treatment, post-injection time, female strain, trial, and the injected fish they were attracted to were recorded. Each fish that entered a trap for the first time was pit tagged and returned to the pond. After the first trapping event was recorded, all fish that were trapped from there on were checked for pit tags and those without tags were given one. For each trapping, the tag number was recorded so that each fish's attraction response could be followed.

Dissolved oxygen (DO) and water temperature were recorded with an YSI, Y85 DO meter every morning (0700 hours) before checking the traps. Ammonia, nitrate, hardness, and alkalinity measurements were taken with a LaMotte, Fish Farm 8 fresh water test kit at approximately 1400 hours on the beginning and ending day of each trial. Also at approximately 1400 hours on the beginning and ending day of each trial, a Secchi

disk was used to measure the visibility, and a Denver Instruments, UP-5 meter was used to record the pH (Table 3).

Data analysis

Logistic regression analysis was performed on multiple data sets. All of the data sets analyzed contained the cumulative data from both trials. One data set was organized so that blue and channel male attraction responses to channel females could be modeled using pheromone treatment, post-injection time, female genetic strain, male species, trial, and the individual male entering the trap as predictor variables. Another data set was organized so that channel females eliciting a blue or channel male capture could be modeled based on the female's weight, genetic strain, and injected pheromone. Another data set was organized so that blue and channel male attraction responses to channel females could be modeled based on the individual females who elicited captures. No data set could be generated to analyze the Marion channel female's attraction response to the free-swimming blue males as no trappings of channel females were obtained. The response variable for each data set was coded as 1 for a capture event and 0 for a non-capture event. Multiple models were generated for each data set based on different variable selection procedures. The goodness of fit for these models were assessed based upon likelihood ratio p-values, Hosmer and Lemeshow p-values, the magnitude of McFadden's R^2 values, and the overall percent correct. Odds ratio estimates were estimated for each category within each predictor variable. Odds ratio estimates that did not contain a value of 1.0 within a 95% confidence interval (CI) were considered significant. For two of the data sets, stepwise selection provided models that fit more adequately for the data (Table 4 and Table 5).

Trapping efficiency for both male species was compared used Fisher's Exact test. Where data values were equal to zero for certain categories within variables, amended estimators were used by adding 0.5 to all of the observed frequencies within the variable being analyzed. Comparisons were made based on the number of observed captures out of the total number of opportunities for the different categories within the different variables. Likelihood ratio statistics less than 0.05 were considered significant.

All data analysis was performed using the Statistical Analysis System 9.1 (SAS Institute 2004).

Results

Channel female attraction to blue males

Capture distribution

No channel females were attracted to any of the blue males during Trial-1. Although no females were attracted during Trial-1, one injected blue male who escaped from his trap approximately 24 hours after injection reentered another trap containing a blue male injected with 17,20β-P-20-glucosiduronate at 48 hours post-injection. Due to the lack of response in Trial-1, only one trial was carried out in which blue males were injected with the hormonal pheromones.

Channel and blue male attraction to channel females

Male capture distribution

There were a total of twenty-three male capture events throughout both trials. The ethanol-only solution, (17,20β-P), (17,20β-P-20-glucosiduronate), and PGF-2α

treatments represented 4.4%, 4.4%, 21.7%, and 69.6% of the 23 total captures respectfully (Table 6). Channel males were captured more often than blue males (69.6% vs. 30.4%; Table 6). Marion and Kansas injected females elicited captures at a similar frequency (43.5% and 56.5% respectfully; Table 6). Approximately 4.3%, 4.3%, 34.8%, 30.4%, and 26.1% of the total captures occurred at 12, 24, 48, 72, and 96 hours post-injection respectfully (Table 6). There were no captures at six hours post-injection. Captures in Trial-1 and Trial-2 represented 73.9% and 26.1% of the total captures respectfully (Table 6).

There were nine different males (three blue males and six channel males) captured throughout the experiment for a total number of 23 captures. Two of the six channel males captured during the early part of the trials died before the trials were complete. The percent of males captured of the total population stocked for each species was calculated based on the average daily number of males still alive before the trials were complete. Thirty-one percent of the total channel males stocked were captured throughout both trials while only 15.0% of the total blue males stocked were captured throughout both trials.

Each male was coded with a single capital letter. Males labeled A, B, and C were blue males, and males D, E, F, G, H, and I were channel males. Males A, B, D, and H each represented 13.0% of the 23 total captures (Table 6). Males C, F, and I each represented 4.3% of the total captures (Table 6). Male E represented 8.7% of the total captures, and male G represented 26.1% of the total captures (Table 6).

From an opportunistic view, each male could have been caught a maximum of twelve times (six time periods at which traps were checked for each of the two trials).

However, channel male D was found dead during the second trial around 24 hours post injection, which would have reduced his total number of opportunities to be caught to only eight. Channel male E was found dead at the end of the first trial, which would have reduced his total number of opportunities to be caught to only six. Channel male G was captured six of his twelve (50.0%) total chances to be caught, followed by channel male D (37.5%), and channel male E (33.3%). Of the blue males, males A and B were each captured on 25.0% of their chances to be captured whereas blue male C was captured 8.3% of his given opportunities to be captured. The percentages of captures relative to the number of opportunities are given for each male in Fig. 5.

Distribution of elicited captures by females

There were twelve females throughout both trials who elicited at least one of the 23 total captures. Each female was coded with a single capital letter. Nine of the females (A, B, C, D, E, H, I, J, and K) each elicited one of the twenty-three total captures (Table 7). Females F and G (both injected with PGF-2α) elicited 8 (34.8%) and 4 (17.4%) of the 23 total captures respectfully and female L (injected with 17,20β-P-20-glucosiduronate) elicited two captures (Table 7). Of the twelve females who elicited at least one capture, six (50.0%) were injected with PGF-2α, four (33.3%) were injected with 17,20β-P-glucosiduronate, one (8.3%) was injected with the ethanol-only control solution, and one (8.3%) was injected with 17,20β-P (Table 8).

Three (25.0%) of the twelve females who elicited at least one capture were Kansas females, and nine (75.0%) were Marion females (Table 7). Over 37.0% of the 24

total Marion females injected elicited at least one capture and 12.5% of the 24 total Kansas females injected elicited at least one capture (Table 8).

Of the twelve females eliciting at least one capture, one (8.3%) was between 2.0 and 3.0 kg, three (25.0%) were between 3.0 and 4.0 kg, six (50.0%) were between 4.0 and 5.0 kg, one (8.3%) was between 5.0 and 6.0 kg, and one (8.3%) was between 6.0 and 7.0 kg (Table 8). There was one injected female in the 2-3 kg and 6-7 kg categories and each elicited at least one capture (Table 8). Thirty percent of the total pheromone-injected females between 3.0 and 4.0 kg elicited at least one capture, 24.0% the total injected females between 4.0 and 5.0 kg elicited at least one capture, and 11.1% of the total injected females between 5.0 and 6.0 kg elicited at least one capture (Table 8).

Odds ratio estimates

Treatment effect

The nine total males captured showed a strong preference when given a choice between ethanol-only, (17,20β-P), 17,20β-P-20-glucosiduronate, and PGF-2α injected females (P = 0.0006; Table 4). There was no significant difference in odds ratio estimates between the ethanol-only, 17,20β-P, and 17,20β-P-20-glucosiduronate treatments. The only significant differences in odds ratio estimates were between PGF-2α and the other treatments. Males were 17.86 (95% CI: 2.34, 142.86) times more likely to be captured with a PGF-2α injected female than with an ethanol-only injected female, or a 17,20β-P injected female. Males were 3.47 (95% CI: 1.23, 9.71) times more likely to be captured with a PGF-2α injected female than with a 17,20β-P-20-glucosiduronate injected female.

Males were 5.77 (95% CI: 2.52, 13.19) times more likely to be captured with a PGF-2α injected female than with a female injected with any of the other treatments.

Of the twelve total females who elicited at least one capture, the treatment that they were given had a significant influence on the numbers of capture they elicited (P = 0.0017; Table 5). There was no significant difference in odds ratio estimates between the ethanol-only, 17,20β-P, and 17,20β-P-20-glucosiduronate treatments. The only significant differences in odds ratio estimates were between PGF-2α and the other treatments. Females injected with PGF-2α were 29.41 (95% CI: 3.09, 250.00) times more likely to elicit a capture than an ethanol-only injected female, as well as a 17,20β-P injected female. There was very suggestive evidence that females injected with PGF-2α were 4.27 (95% CI: 0.99, 18.52) times more likely to elicit a capture than a 17,20β-P-20-glucosiduronate injected female. Females injected with PGF-2α were also 7.78 (95% CI: 2.73, 22.17) times more likely to elicit a capture than a female injected with any of the other treatments.

Trial effect

There was a significant decrease in the number of captures between Trial-1 and Trial-2 (P = 0.0228; Table 4). Males were 3.04 (95% CI: 1.17, 7.92) more likely to be caught during Trial-1 than during Trial-2.

Effect of post-injection time

There was evidence that the post-injection time had an effect on the number of captures (P = 0.0844; Table 4). Because there were no captures at six hours post-injection, the six hour post-injection time category was excluded from the logistic regression analysis for the global model. The only observed significant differences in

odds ratio estimates for individual post-injections time were between 12 and 48 hours after hormone injection, and 24 and 48 hours post-injection. Males were 9.81 (95% CI: 1.16, 83.33) times more likely to be caught 48 hours after female injection than at 12 hours after female injection, as well as 24 hours after female injection. There was no significant difference in odds ratio estimates between 48, 72, and 96 hours post-injection. When compared collectively, males were 5.95 (95% CI: 1.57, 10.32) times more likely to be caught between 48 and 96 hours after female injection than between 12 and 24 hours post-injection.

Male effect

Although channel males accounted for 69.6% of the 23 total captures and blue males accounted for 30.4% (Table 6), the statistical difference in the odds ratio estimates for catching one species more than the other was not as distinct (P = 0.0563; Table 4). Despite some of the males being captured on multiple occasions, there was no significant difference in odds ratio estimates for capturing any one male more than another, assuming equal opportunities (P = 0.3755; Table 4) or actual opportunities (P = 0.4998) of capture for each male.

Female effect

Despite having multiple females who elicited more than one capture, the logistic regression model used to analyze the effects of individual females as predictor variables did not fit well as indicated by a non-significant likelihood ratio p-value of 0.193. Odds ratio estimates for comparing individual females to one another were therefore not valid to determine if any female attracted males at a greater frequency than any other female.

There was no significant difference in the odds ratio estimates for a male being attracted to one female strain more than the other (P = 0.4979; Table 4). Kansas females elicited 56.5% of the 23 total captures and Marion females elicited 43.5% (Table 6).

Although 37.5% of the 24 total Marion females and 12.5% of the 24 total Kansas females elicited at least one capture (Table 8), there was no significant difference in the odds ratio estimates for eliciting a male capture by using any one of the two strains (P = 0.7449; Table 5).

Female weight had no effect on the likelihood of capture (P = 0.2025; Table 5).

Interaction effects

Although the forward selected model for the data set for channel and blue male attraction to channel females found no statistically significant interaction effects between variables, there appeared to be some interaction between some of the variables. There appeared to be an equal distribution between trials for the control, 17,20β-P, and 17,20β-P-20-glucosiduronate treatments (Fig. 6) based on the 23 total trappings; but for PGF-2α, over 60.0% of the 23 total trappings occurred during Trial-1 as compared to less than 9.0% during Trial-2. Over 87.0% of the 16 total PGF-2α trappings occurred during Trial-1 whereas less than 13.0% of the total trappings occurred during Trial-2.

There appeared to be an equal distribution between male species for the control, 17,20β-P, and 17,20β-P-20-glucosiduronate treatments based on the 23 total trappings; but for PGF-2α, over 56.0% of the 23 total trappings were channel male captures whereas only 13.0% of the total trappings were blue male captures (Fig. 7). Approximately 81.0% of the 16 total PGF-2α trappings were channel male captures and 19.0% were blue male captures.

There appeared to be an equal distribution between female strain for the control and 17,20β-P treatments (Fig. 8) based on the 23 total trappings. But for 17,20β-P-20-glucosiduronate, all of the 5 total 17,20β-P-20-glucosiduronate trappings were elicited by Marion females and none were elicited by a Kansas female. For PGF-2α, over 52.0% of the 23 total trappings were elicited by a Kansas female whereas less than 18.0% of the total trappings were elicited by a Marion female. Thirty-three percent of the 16 total PGF-2α trappings were elicited by Marion females whereas 66.7% were elicited by Kansas females.

The percentage of total elicited trappings appeared equally distributed for Marion females between trials based on the 23 total trappings; but for Kansas females, all of the 13 total Kansas-elicited trappings occurred during Trial-1 and none occurred during Trial-2 (Fig. 9). There was a decrease in captures during Trial-2 for both species of males (Fig. 10).

There appeared to be female strain preference between the two male species (Fig. 11). Blue males were attracted to channel females on seven different occasions of which six (85.7%) were in response to Marion females and only one (14.3%) was in response to a Kansas female. Channel males were attracted to channel females on 16 different occasions of which 4 (33.3%) were in response to Marion females and 12 (66.7%) were in response to Kansas females.

Trapping efficiency based on total opportunities

There were a total of 480 captures that could have been obtained throughout both trials (each of the 40 males could have been captured at most 12 possible times). The

overall capture rate based on the total number of captures that could have been obtained was 4.8% (23 actual captures out of 480 possible). Overall capture rate based on the number of possible blue male captures that could have been obtained was 2.9% (7 captures out of 240 possible), and 6.7% (16 captures out of 240 possible males) for the channel male captures that could have been obtained.

There were 40 different males used throughout both trials. Overall capture rate based on the numbers of males present was 57.5% (23 actual captures out of 40 possible males). Overall capture rate based on the numbers of blue males present was 35.0% (7 actual captures out of 20 possible males), and 80.0% (16 actual captures out of 20 possible males) for the channel males present.

There were a total of 288 opportunities to choose from for each male throughout both trials for both species of male (12 traps per pond, 2 trials, 6 post-injection times, and 2 ponds). Overall capture rate based on the total number of opportunities presented was 8.0% (23 actual captures out of 288 opportunities). From the perspective of male species, there were a total of 144 opportunities presented for each male throughout both trials for each species (12 traps per pond, 2 trials, and 6 post-injection times). Overall capture rate based on the total number of opportunities presented was 4.9% and 11.1% for blue and channel males respectfully (Table 9). PGF-2α, (17,20β-P-20-glucosiduronate), Marion females, and the 48, 72, and 96 hours post injection times were the only factors for blue males that produced a higher percent capture than the overall 8.0% capture rate for both species combined, and 4.9% capture rate for blue males only (Table 9). There were a total of 36 opportunities for each treatment presented throughout both trials for blue males (3 replicates per treatment, 2 trials, and 6 post-injection times). Although not statistically

significant (P = 0.3007), the PGF-2α and 17,20β-P-20-glucosiduronate capture rates based on presented opportunities were 8.3% (3 captures out of 36 opportunities; Table 9) for each treatment whereas the control females did not elicit any blue male captures. There were a total of 72 opportunities per female strain for blue males (6 traps per pond, 6 post-injection times, and 2 trials). The capture rate for Marion females was significantly higher than those for Kansas females (P = 0.0418; Table 9). There were a total of 24 opportunities for each time period for blue males (12 traps per pond and 2 trials). The capture rates for the 48, 72, and 96 hour post-injection times were 8.3%, 12.5%, and 8.3% respectfully (P = 0.3046; Table 9).

PGF-2α, Kansas females, Trial-1, and the 48, 72, and 96 hours post injection times were the only factors for channel males that produced a higher percent capture than the overall 8.0% capture rate for both species combined, and the 11.1% capture rate for channel males only (Table 9). The PGF-2α capture rate for channel males based on presented opportunities (36.1%) was significantly higher than that produced by control females (P < 0.0001; Table 9). There were a total of 72 opportunities per female strain for channel males (6 traps per pond, 6 post-injection times, and 2 trials). The capture rate for Kansas females was significantly higher than those of Marion females (P = 0.0304; Table 9). There were a total of 24 opportunities for each time period for channel males (12 traps per pond and 2 trials). The capture rates for the 48, 72, and 96 hour post-injection times were 25.0%, 16.7%, and 16.7% respectfully (P = 0.0597; Table 9). There were a total of 72 opportunities per trial for channel males (12 traps per pond and 6 post-injection times). The capture rate for Trial-1 was significantly more than that of Trial-2 (P = 0.0304; Table 9).

Discussion

Overall trapping efficiency based on the number of total possible captures was low (4.8%); however, PGF-2α given to channel females significantly increased the likelihood of males being attracted to females as compared to the control. Channel males responded to PGF-2α injected females at significantly higher rate than to non-treated females (36.1% vs. 2.8%). Although not significant, blue males did respond to both PGF-2α and 17,20β-P-20-glucosiduronate injected females on 8.3% of their opportunities while never responding to non-treated females throughout the study.

Hormonal pheromones (affecting olfactory and gustatory senses) have evolved in fish as a mechanism to provide reproductive coordination for difference species (Stacey et al. 2003). The common forms of these pheromones are steroids and prostaglandins. As hormones, these steroids and prostaglandins can induce such physiological processes within a fish such as oocyte maturation, ovulation, milt production, etc. When these hormones are circulating in the fish they may be directly released or metabolized and then released out into the water. These hormones and metabolites can then serve as pheromones in which case they can serve many purposes such as indicating the reproductive status (ex. non-ovulated vs. ovulated) of a fish to a con-specific member of the same or opposite sex. Hormonal pheromones are generally highly conserved among species (Burnard 2008). However, it is believed that selective pressure for the evolution of species-specific pheromones might exist between species that spawn together in close proximity (Sorensen et al. 1988), are reproductively sympatric, and do not rely heavily on other sensory cues (Stacey et al. 2003).

Although the exact mechanism of the function of prostaglandins as hormones during fish reproduction is not completely understood, it is accepted that prostaglandins primarily influence ovulation. Water-borne prostaglandins are known to be effective as male attractants and spawning behavior stimulators in species such as Atlantic salmon, *S. salar* (Moore and Waring 1996), *S. trutta* and *C. clupeaformis* (Laberge and Hara 2003), and goldfish (Stacey 2003; Stacey et al 2003; Gerlach 2006). Injection of females with prostaglandins has resulted in attraction of males and induced spawning behaviors in oriental weatherfish, *Misgurnus anguillicaudatus*, (Kitamura et al. 1994) and goldfish (Volkoff and Peter 1999). Volkoff and Peter (1999) injected female goldfish at approximately 0.67 mg/kg and observed increased male spawning behaviors (courting, chasing, and nudging) for several hours after injecting the females. Kitamura et al. (1994) injected female oriental weatherfish with either PGF-2α, 15-keto- PGF-2α, and 13,14-dihydro-15-keto-PGF-2α at 2.5 mg/kg which all resulted in increased courtship attempts by exposed males up to 90 minutes after female injections. In the present study, PGF-2α injected IP at 0.5 mg/kg into channel females elicited the attraction of 31% and 15% of stocked channel and blue males respectfully primarily between 48 to 96 hours post-injection.

The nature of male attraction to PGF-2α injected females in this study was probably due to water-borne PGF-2α and/or metabolites released by the females. Sorensen et al. (1988) observed that odorous cues produced by non-ovulated female goldfish injected with PGF-2α at 0.4 mg/kg elicited the same male reproductive behaviors (chasing and nudging) as ovulated females did. High levels of PGF-2α were released by injected goldfish out into the water and it was estimated that 2.0% of the

synthetic PGF-2α that was actually injected into the goldfish was released out into the water. The results of Sorensen et al. (1988) also confirmed that PGF-2α-injected female goldfish release potent odorants that were detected by males using the same olfactory receptors that respond to prostaglandins of the F series.

Kumar's et al. (2000) observations of steroidogenic enzymes in channel catfish ovaries suggested that channel catfish should possess enzymes capable of metabolizing various steroids such as 17,20β-P and 17,20β-P-20-glucosiduronate during the month of May. The steroid 17,20β-P and similar steroids like 20β-S are known to be maturation-inducing steroids (MIS) in many species such as yellow perch, *Perca flavescens*, and brook trout, *Salvelinus fontinalis* (Goetz and Bergman 1978), striped bass, *Morone saxatilis* (King et al. 1997), *Pseudobagrus fulvidraco* (Lim et al. 1997), spotted seatrout, *Cynoscion nebulosus* (Pinter and Thomas 1999), *Silurus asotus* (Miwa et al. 2001), and *G. cernus* (Sorensen et al. 2004). The steroid 17,20β-P is suggested to be a MIS for channel catfish (Kumar et al 2000; Kazeto et al. 2005). However, Lambert et al. (1999) observed no significant effects on ovulation or fertilization when channel females were given 17,20β-P at 0.03 mg/kg along with carp pituitary extracts for induced spawning. Even if 17,20β-P is a MIS for channel catfish, it appears that IP injections of 17,20β-P and 17,20β-P-20-glucosiduronate at 0.5 mg/kg will not result in increased attraction of channel or blue males as seen with PGF-2α injections.

The dose rate for the steroid injections used in the present study may have not been in the appropriate dose rate to elicit male captures as well as PGF-2α injections did. Female Nile tilapia injected with 17,20β-P at 0.2 mg/kg produce cues that result in increased courtship behaviors among exposed dominant males (Souza et al. 1998).

Injections of 17,20β-PS into female *G. cernus* at around 0.2 mg/kg have also resulted in increased courtship behaviors among exposed males (Sorensen et al. 2004). However, Haider and Rao (1994) could not induce ovulation in *C. batrachus* with 17,20β-P even with dose rates as high as 2.0 mg/kg. The lower response to the steroids as compared to PGF-2α may be a result of the maturity stage of the males used.

During the final stages of gonadal maturation in female goldfish, steroid synthesis precedes prostaglandin synthesis. The steroids involved (17,20β-P and 17,20β-PS) are primarily involved in oocyte maturation whereas PGF-2α plays a role in inducing ovulation (Stacey et al. 2003). Thus, 17,20β-P and 17,20β-PS released by female goldfish signify to males that the female is undergoing oocyte maturation but is still not ready spawn. PGF-2α released by females signifies that females have ovulated and are ready to spawn. This might suggest that male goldfish may be more responsive post-ovulatory PGF-2α rather than pre-ovulatory steroids, as PGF-2α indicates that is female is ready to spawn. Bjerselius et al. (1995) observed sexually mature male goldfish avoiding water scented with 17,20β-P which was thought to be a reaction that might allow the male to avoid misdirected courting with non-ovulated females so that he could continue his search for an ovulated female. Dittman and Quinn (1994) observed precociously mature male Chinook salmon who avoided water borne 17,20β-P which was thought to be a result of the males' young age at maturity. Hong et al (2006) observed a unique pattern in which male *B. sinensis* were more likely to be attracted to artificial nest bated with 17α-P; 17α,20β-P; and PGE-2 but not PGF-2α, and that 17α,20β-P and PGE-2 baited nests were effective in inducing spawning while PGF-2α was ineffective. The significant response to PGF-2α in this study suggests that cues produced by PGF-2α injected females

were more attractive than those produced by females with the other ethanol-control solution and steroid treatments which may be a result of PGF-2α imitating females that have ovulated and are ready to spawn.

The lack of channel female captures with injected blue males could have been the result of a highly effective, reproductive isolating mechanism between the species. During the beginning of the natural spawning acts of these two species, males are known to find a nest and are then believed to use pheromones to attract con-specific females to the nest (Timms and Kleerekoper 1972; Rubec 1979). Time and energy in having to distinguish between a con-specific member of the opposite sex could be conserved the sooner a reproductive isolating mechanism appears during the spawning process. Female *C. gariepinus* are known to be attracted to con-specific males shortly after ovulation is complete (Resink et al. 1987). Ovulating female sea lampreys, *P. marinus*, are attracted to spermiating con-specifics (Johnson et al. 2005). Male Arctic char, *S. alpinus*, also release pheromones which attract females to spawning grounds and elicit female spawning behaviors (Sveinsson and Hara 1995). Egg development is of course a huge energy investment for females which has driven them to be very choosy when finding a mate (Moyle and Cech 2004). Ovulated channel catfish may only have specific receptors used for recognizing pheromonal cues produced by channel males to locate spawning nest. Pairing the blue males and channel females in pens for induced spawning has resulted in better spawning success than the open-pond spawning methods (Smitherman et al 1996). Aquaculturist using the pen spawning method may be overcoming the reproductive isolating mechanism in the form of female-attracting pheromones produced by males. In this study however, it cannot be confirmed that lack of channel female

captures with blue males was a result of a highly effective, reproductive isolating mechanism because the same blue male treatments were not used on blue females. The lack of channel female captures with blue males could have also been due to improper dose rates or injection location, lack of metabolizing the injected pheromones by the blue males, or the use of a non-attractive pheromone for female channels.

Trial effect

There was a drastic decline in captures from Trial-1 to Trial-2. The decline in captures may have been associated with the decline in morning temperature and DO levels between trials, or the addition of the second bungee strap to the trap before Trial-2 which may have made it more difficult for males to enter. The decrease in blue and channel male captures may have been due to a learned response of being trapped, which led to an ensuing avoidance of the traps for males after being captured. The decrease may have been the result of an increased threshold to detect pheromonal cues produced by injected females. The deceased could have been due to a seasonal change in sensitivity of pheromone receptors which has been reported in crucian carp, *C. carassius*, (Hamdani et al. 2008) and Atlantic salmon (Moore and Waring 1996). Furthermore, an accumulation of pheromones in the water after Trial-1 may have resulted in an over-exposure to pheromones for the males resulting in a decrease in captures. An overdose repellence to pheromones has been reported in insects such as the mountain pine beetle, *Dendroctonus ponderosae*, (Miller et al. 2005) and the casebearer, *Acrobasis nuxvorella* (Knutson et al. 1998). The decline in captures could have also been due to a buildup of natural repellent/suppressive pheromones for males. Goldfish (Bjerselius et al. 1995) and

Chinook salmon (Dittman and Quinn 1994) have exhibited an avoidance behavior to water borne 17,20β-P.

Effect of post-injection time

During spawning processes, male attraction to females is typically observed on up to point of ovulation for the females, and after ovulation during which the spawning act should take place (Stacey 2003). Female goldfish injected with PGF-2α become sexually active within minutes after being injected and produce pheromonal cues that result in increased male spawning behaviors (courting, chasing, and nudging) for several hours after injecting the females (Volkoff and Peter 1999). The best chance of obtaining a blue or channel male capture in the present study occurred 48 to 96 hours after injecting the females. With morning water temperatures averaging near 23 ºC, the degree hour capture response time for the treatments would have ranged from 1,104 hours to 2,208 hours, with an average response time of 1,656 degree hours. Phelps et al. (2007) observed a degree hour response time until ovulation of approximately 1,400 degree hours at water temperatures of 24 ºC for channel females given dual injections of LH-RHa at 120 µg/kg. In the present study, it could be that the hormonal pheromones injected were acting as ovulation inducing agents like LH-RHa, which might suggest that the cues released at ovulation and thereafter by channel females play a major role in attracting males during the spawning act. However, only two of the females used in this study had expressible eggs when observed at the end of the trials. Those two females were 17,20β-P and 17,20β-P-20-glucosiduronate injected females.

There was a steady decline in the number of male captures in this study from 48 to 96 hours post-female injection. Kitamura et al. (1994) observed a consistent decrease

in the number of courtship acts by male oriental weatherfish exposed to PG injected females at 30 minute intervals over the observed 150 minute trial.

Male effect

There was very suggestive evidence (P = 0.0563) that channel males were captured more frequently than blue males. The difference in capture rates for blue and channel males may be linked to the difference in DO levels between the ponds or low temperatures close to the minimum threshold for spawning. The percent of the population of blue males captured (15.0%) is similar to natural hybridization pen spawning rates for channel females and male blue catfish obtained by Dunham et al. (2000). This may suggest that there is some factor (genetic, environmental, behavioral, etc) affecting what proportion of a given population of blue males will be attracted to, and spawn with, a channel female. Not obtaining a blue male population capture rate above 15.0% could mean that additional, hormonal pheromone injections into channel females along with standard hormone injections used for induced spawning may not increase pen spawning hybridization rates, or that another dose rate might be more appropriate. As mentioned previously, Lambert et al. (1999) observed no significant effects on ovulation or fertilization when channel females were given 17,20β-P along with carp pituitary extracts for induced spawning for the production of hybrids. However, Tripathi and Singh (1995) found that ovulation percentages increased significantly (from 59.0% up to 90.0%) when 17,20β-P and PGF-2α were used together to induce ovulation in vitro for *Heteropneustes fossilis*. Haider and Rao (1994) observed successful ovulation in all injected fish and high hatching rates when 17,20β-P injected at 1.0 mg/kg was given along with salmon gonadotropin injections at 10.0 mg/kg in *C. batrachus*. The additional injection of PGF-

2α, which was found to be effective in the present study, along with standard hormone injections used for induced spawning might produce an additive effect on pen spawning success rates and lead to an increase in natural hybridization pen spawning success of female channel and male blue catfish.

A similar situation was observed for channel males. Thirty-one percent of the channel males stocked for this study were attracted to channel females. This 31.0% channel male population capture rate is similar to and within the range (30.0-50.0%) of reported open pond spawning success rates for channel catfish obtained in other studies (Wolters 1993; Silverstein et al. 1999). This may also suggest that there is some factor (genetic, environmental, behavioral, etc) affecting what proportion of a given population of channel males will be attracted to, and spawn with, a channel female. This lack of obtaining a channel male population capture rate above 31.0% could once again mean that additional, hormonal pheromone injections into channel females along with standard hormone injections used for induced spawning may have no effect or could possibly produce an additive increase to the low, open pond spawning success rates for channel catfish.

Neu (1995) obtained pen spawning success rates of 31.0% and 15.0% for male channel catfish fed at either 0.5% or 3.0% body weight per day respectfully, suggesting feed rate could play a role in male spawning success. Waldbieser and Wolters (1999) performed a microsatellite study in which they observed approximately 36.0% of a channel male population spawning successfully with channel females in an open pond scenario. One key observation they noted was that some of the males who spawned successfully, spawned on multiple occasions. The average number of spawns per male

was 3.44, with the most spawns being six for one individual male. In the present study 31.0% of the channels male used in the study were trapped with multiple trappings of some individuals. The average number of traps per channel male was 2.66, and one channel male was trapped a total of six times. The low population capture rate as well as the average traps per male could be due to the shorter duration of the study period for present study (two weeks as compared to nine weeks in Waldbieser and Wolters (1999) study), as well as the smaller population of channel males (20 individuals as compared to 124 individuals). The multiple trappings of some males and the lack of trapping others observed in the present study suggest that there are differences in reproductive fitness in male channel catfish.

Three of the channel males that were captured in the study each responded to a common female more than once during Trial-1. Channel male D was trapped with a PGF-2α injected female (female F) at 48 and 96 hours after injection, channel male H was trapped with the same PGF-2α injected female at 72 and 96 hours after injection, and channel male G was captured by a different PGF-2α injected female (female G) at 72 and 96 hours after injection. In trial-2, channel male G re-entered the same trap that originally contained female G at 12 hours after injection. Once again, this trap contained a PGF-2α injected female (female J) although treatments had been previously, randomly selected for distribution into the traps. Male G entered the same trap three out of his six total times that he was captured; males D and H each entered their same traps containing female F, two out of their three total times captured. The response to a common female and the same trap on more than on one occasion in the same trial could have been the result of learned association or lingering effect that the PGF-2α treatment had as the actual traps

containing the females were not rotated within trials. A similar response was seen in a locomotor response study by Timms and Kleerekoper (1972) where male channel catfish were observed to stay in the same locale where holding water from a ripe female was introduced even after thirty minutes when the stimulus was removed. This reaction observed by Timms and Kleerekoper (1972) could have also been the result of a lingering effect that the female odor source had. The response to a common female in this study could have also been influenced by the monogamous nature of channels males as confirmed by Taterenkov et al. (2006). However, the application of PGF-2α is the most likely explanation for the three channel males being attracted to a common female and trap on more than one occasion.

Female effect

In this study, there was no overall difference in eliciting a male capture by using either a Kansas or Marion injected female. However, there appeared to be an interaction effect between male species and the strain of the female eliciting capture. The Kansas channel males used in the study responded more frequently to Kansas females than Marion females (16.7% vs. 5.6% of the male's given opportunities). This may be an example of same strain preference. Smitherman et al. (1984) observed significantly higher, spawning success rate for similar strain, paired channel broodstock than with mixed strain, paired broodstock. It could be possible that the higher spawning success for similar strain, paired broodstock observed by Smitherman et al. (1984) was a result of strain distinction among the brooders. The Rio Grande blue males used responded more frequently to Marion females than Kansas females (8.3% vs. 1.4% of the male's given opportunities). Dunham et al. (1983a) found that blue males hybridized more frequently

with channel females from crossbred strains than pure Kansas or Marion females, suggesting crossbred vigor in the form of mating readiness. Observations of Marion females being better capture elicitors for blue males suggest that this crossbred vigor observed by Dunham et al. (1983a) may be inherited more from Marion female parents of the crossbred offspring.

Conclusion

PGF-2α injected into channel females at 0.5 mg/kg significantly increased the frequency that male channels were captured. Male blues were not captured at the same rate as channel males, but were attracted to PGF-2α more than the control females. The effectiveness of PGF-2α in producing channel and blue male attraction responses suggest that PGF-2α could be coupled with standard hormone injections used for induced spawning in female channel catfish, and may produce higher spawning success rates between channel males and females, as well as blue males and channel females. The degree-hour capture response time for PGF-2α is similar to degree-hour response times for common ovulation inducing agents used for channel catfish. The difference in male species, population capture rates and low natural hybridization success could be the result in differences of attractive cues released by con-specific females during ovulation. Future research in methods to increase natural hybridization should therefore be directed towards determining attractive cues used by channel and blue females to attract their male con-specifics during ovulation. The effectiveness of PGF-2α also prompts further investigation of PGF-2α as a more effective ovulation inducing agent for female channel catfish. If capture rates for the male populations are directly correlated to male spawning success rates, then pre-screening male brooders with the use of females injected with

hormonal pheromones like PGF-2α could allow for the selection of male brooders who will spawn more readily. If a genetic factor is involved as a factor affecting capture rate and possibly spawning success rate, then pre-screening male brooders using females injected with hormonal pheromones could allow for selection of improved reproductive performance and development of male broodstock who spawn more successfully.

Table 1. The number, strain, sex, and species of fish that were injected with the four treatments during each trial and placed in traps and then into ponds containing either the free-swimming channel or blue males, or the channel females. Ponds 1, 2, and 3 contained the free swimming Marion channel females, Kansas channel males, and Rio Grande blue males respectfully which were used throughout both trials.

		Trial-1			Trial-2			Total			Grand total
Pond	Treatment	Marion channel females (*N*)	Kansas channel females (*N*)	Rio Grande blue males (*N*)	Marion channel females (*N*)	Kansas channel females (*N*)	Rio Grande blue males (*N*)	Marion channel females (*N*)	Kansas channel females (*N*)	Rio Grande blue males (*N*)	
1	Control			3						3	3
	17,20β-P			3						3	3
	17,20β-P-gluc.			3						3	3
	PGF-2α			3						3	3
	Total			12						12	12
2	Control	1	2		2	1		3	3		6
	17,20β-P	1	2		2	1		3	3		6
	17,20β-P-gluc.	1	2		2	1		3	3		6
	PGF-2α	1	2		2	1		3	3		6
	Total	4	8		8	4		12	12		24
3	Control	2	1		1	2		3	3		6
	17,20β-P	2	1		1	2		3	3		6
	17,20β-P-gluc.	2	1		1	2		3	3		6
	PGF-2α	2	1		1	2		3	3		6
	Total	8	4		4	8		12	12		24
Grand total		12	12	12	12	12		24	24		60

Table 2. Mean weights ((kg) mean ± SEM) of injected fish placed into traps in the three different ponds where three fish were stocked individually per treatment. Ponds 1, 2, and 3, contained the free swimming Marion channel females, Kansas channel males, and Rio Grand blue males respectfully which were used throughout both trials.

Pond	Treatment	Marion females (*N*)	Kansas females (*N*)	Blue males (*N*)
1	Control			4.49 ± 0.31 (3)
	17,20β-P			4.84 ± 0.70 (3)
	17,20β-P-gluc			4.92 ± 0.32 (3)
	PGF-2α			5.25 ± 0.91 (3)
	Grand mean			4.88 ± 0.28 (12)
2	Control	3.98 ± 0.19 (3)	4.76 ± 0.02 (3)	
	17,20β-P	3.98 ± 0.48 (3)	4.79 ± 0.59 (3)	
	17,20β-P-gluc	3.39 ± 0.64 (3)	4.61 ± 0.29 (3)	
	PGF-2α	4.17 ± 0.22 (3)	4.88 ± 0.26 (3)	
	Grand mean	3.88 ± 0.44 (12)	4.76 ± 0.34 (12)	
3	Control	4.16 ± 0.44 (3)	5.99 ± 0.68 (3)	
	17,20β-P	4.23 ± 0.50 (3)	5.93 ± 0.28 (3)	
	17,20β-P-gluc	4.05 ± 0.37 (3)	4.87 ± 0.53 (3)	
	PGF-2α	4.06 ± 0.13 (3)	6.25 ± 0.62 (3)	
	Grand mean	4.13 ± 0.34 (12)	5.76 ± 0.62 (12)	

Table 3. Water quality data (mean ± SEM) for all ponds used in the experiment. Ponds 1, 2, and 3, contained the free swimming Marion channel females, Rio Grande blue males, and Kansas channel males respectfully which were used throughout both trials.

	Pond 1	Pond 2		Pond 3	
	Trial-1	Trial-1	Trial-2	Trial-1	Trial-2
Temperature (ºC)*	23.7 ± 0.57	23.7 ± 0.58	22.8 ± 0.28	23.7 ± 0.50	22.9 ± 0.31
DO (mg/L)**	7.21 ± 0.30	5.13 ± 0.28	4.55 ± 0.11	7.74 ± 0.18	6.59 ± 0.12
pH	7.65 ± 0.30	6.95 ± 0.05	6.62 ± 0.25	8.12 ± 0.13	6.94 ± 0.50
Ammonia (ppm)	< 0.2	1.5	1.75 ± 0.25	1.15 ± 0.35	1.90 ± 0.10
Nitrate (ppm)	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
Hardness (ppm)	53.5 ± 1.5	47.5 ± 7.5	64.0 ± 16.0	57.5 ± 2.5	54.5 ± 9.5
Alkalinity (ppm)	57.5 ± 2.5	57.5 ± 2.5	62.0 ± 2.0	55.0 ± 5.0	57.5 ± 2.5
Sechhi disk visibility (cm)	85.0 ± 5.0	67.5 ± 7.5	71.75 ± 0.75	81.8 ± 0.8	95 ± 5.0

* Collectively for Ponds 2 and 3, morning temperature declined significantly (P-value $\leq$ 0.05) between trials.

** Collectively for Ponds 2 and 3, morning DO levels declined significantly (P-value $\leq$ 0.05) between trials. DO levels were also significantly less for Pond 2 compared to Pond 3.

Table 4. Model comparison between the global and stepwise-selected model for data set-1: channel and blue male attraction to channel females. Associated p-values are listed for each predictor variable. Associated p-values are listed for the likelihood ratio, and Hosmer and Lemeshow test statistics.

	Model	
	Global	Stepwise
Variable	P-value	P-value
Treatment	0.0004*	0.0006*
Male species	0.0563	***
Female strain	0.4979	***
Post-injection time	0.0844**	***
Trial	0.0184*	0.0228*
Individual male	0.3755	***
Model assessment values		
Likelihood ratio	<0.0001*	<0.0001*
Hosmer and Lemeshow statistic	0.0488	0.225
McFadden's R^2	0.26	0.15
Percent correct	86.0%	74.2%

* Associated p-value for variable is significant ($P \leq 0.05$)

** Associate p-value for variable is not significant ($P \geq 0.05$) for the entry level into stepwise model, but does contain significant differences within the categories for the variable

*** Variable was not significant ($P \geq 0.05$) at the level of entry chosen for the stepwise model

Table 5. Model comparison between the global and stepwise-selected model for data set-2: individual females injected. Associated p-values are listed for each predictor variable. Associated p-values are listed for the likelihood ratio, and Hosmer and Lemeshow test statistics.

	Model	
	Global	Stepwise
Variable	P-value	P-value
Treatment	0.0016*	0.0017*
Female weight	0.2025	***
Female strain	0.7449	***
Model assessment values		
Likelihood ratio	0.0001*	<0.0001*
Hosmer and Lemeshow statistic	0.0326	1.0
McFadden's R^2	0.33	0.28
Percent correct	86.20%	71.30%

* Associated p-value for variable is significant ($P \leq 0.05$)

** Associate p-value for variable is not significant ($P \geq 0.05$) for the entry level into stepwise model, but does contain significant differences within the categories for the variable

*** Variable was not significant ($P \geq 0.05$) at the level of entry chosen for the stepwise model

Table 6. Frequency data of the twenty-three total captures represented by each category within each variable. Percent values with different letters (a, b, and c) for different categories within the different variables are significantly different ($P < 0.05$).

Variable	Category	Number of captures	Percent representation within variable
Treatment			
	Control	1	4.3% [a]
	17,20β-P	1	4.3% [a]
	17,20β-P-gluc.	5	21.7% [a]
	PGF-2α	16	69.6% [b]
Male species			
	Blue	7	30.4% [a]
	Channel	16	69.6% [a]
Female strain			
	Marion	10	43.5% [a]
	Kansas	13	56.5% [a]
Post-injection time (hrs)			
	6	0	0.0%
	12	1	4.3% [a]
	24	1	4.3% [a]
	48	8	34.8% [b]
	72	7	30.4% [ab]
	96	6	26.1% [ab]
Trial			
	1	17	73.9% [a]
	2	6	26.1% [b]
Individual male (male species)			
	A (Blue)	3	13.0% [a]
	B (Blue)	3	13.0% [a]
	C (Blue)	1	4.3% [a]
	D (Channel)	3	13.0% [a]
	E (Channel)	2	8.7% [a]
	F (Channel)	1	4.3% [a]
	G (Channel)	6	26.1% [a]
	H (Channel)	3	13.0% [a]
	I (Channel)	1	4.3% [a]

Table 7. Number of trappings elicited by each of the twelve females for each injected treatment, who elicited at least one capture, and the percent of the twenty-three total trappings which they each elicited.

Female	Injected treatment	Female strain	Number of trappings	Percent representation of total trappings
A	17,20β-P-gluc.	Marion	1	4.35%
B	17,20β-P	Kansas	1	4.35%
C	17,20β-P-gluc.	Marion	1	4.35%
D	PGF-2α	Marion	1	4.35%
E	PGF-2α	Marion	1	4.35%
F	PGF-2α	Kansas	8	34.78%
G	PGF-2α	Kansas	4	17.39%
H	PGF-2α	Marion	1	4.35%
I	17,20β-P-gluc.	Marion	1	4.35%
J	PGF-2α	Marion	1	4.35%
K	Control	Marion	1	4.35%
L	17,20β-P-gluc.	Marion	2	8.70%

Table 8. Frequency data of the number of females who elicited a capture within in each category for each categorical variable. Percent values with different letters (a, b, and c) for different categories within the different variables are significantly different ($P < 0.05$).

Variable	Category	Number of female replicates	Number of females eliciting a capture	Percent representation within category
Treatment				
	Control	12	1	8.3% [a]
	17,20β-P	12	1	8.3% [a]
	17,20β-P-gluc.	12	4	33.3% [ab]
	PGF-2α	12	6	50.0% [b]
Female strain				
	Kansas	24	3	12.5% [a]
	Marion	24	9	37.5% [a]
Weight (kg)				
	2-3	1	1	100.0% [a]
	3-4	10	3	30.0% [a]
	4-5	25	6	24.0% [a]
	5-6	9	1	11.1% [a]
	6-7	1	1	100.0% [a]
	7+	2	0	0.0% [a]

Table 9. Capture rate based on the total number of opportunities presented throughout both trials for each pond containing their respectful male species. Blue male capture rates with different letters (a, b, c, and d) as well as channel male capture rates (w, x, y, and z) represent significant differences ($P < 0.05$) between different categories for the given variable.

Variable	Category	Maximum opportunities presented	Number of blue male captures	Number of channel male captures	Blue male capture rate	Channel male capture rate
Overall		144	7	16	4.9%	11.1%
Treatment						
	Control	36	0	1	0.0% [a]	2.8% [w]
	17,20β-P	36	1	0	2.8% [a]	0.0% [w]
	17,20β-P-gluc.	36	3	2	8.3% [a]	5.6% [w]
	PGF-2α	36	3	13	8.3% [a]	36.1% [x]
Female strain						
	Kansas	72	1	12	1.4% [a]	16.7% [w]
	Marion	72	6	4	8.3% [b]	5.6% [x]
Post-injection time (hrs)						
	6	24	0	0	0.0% [a]	0.0% [w]
	12	24	0	1	0.0% [a]	4.2% [w]
	24	24	0	1	0.0% [a]	4.2% [w]
	48	24	2	6	8.3% [a]	25.0% [w]
	72	24	3	4	12.5% [a]	16.7% [w]
	96	24	2	4	8.3% [a]	16.7% [w]
Trial						
	1	72	5	12	6.9% [a]	16.7% [w]
	2	72	2	4	2.8% [a]	5.6% [x]

Figure 1. Basic pond layout used for the experiment. Each large square represents a separate pond stocked with either channel or blue males, or channel females. Each fish symbol contained in the small rounded polygons represents a fish injected with one of the four possible treatments and placed in the trap. The twenty fish symbols not contained in the rounded polygons represents the free swimming channel or blue males, or channel females, stocked separately into the three ponds and used throughout both trials.

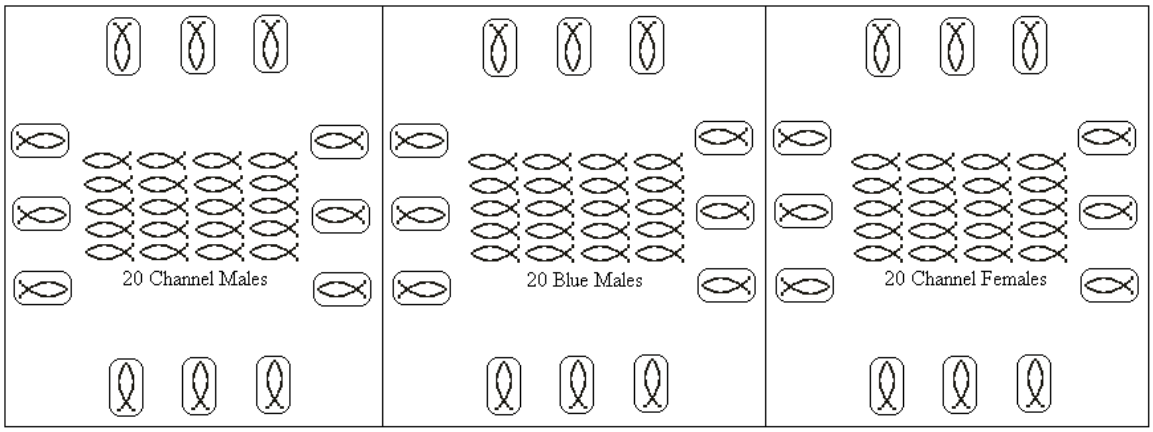
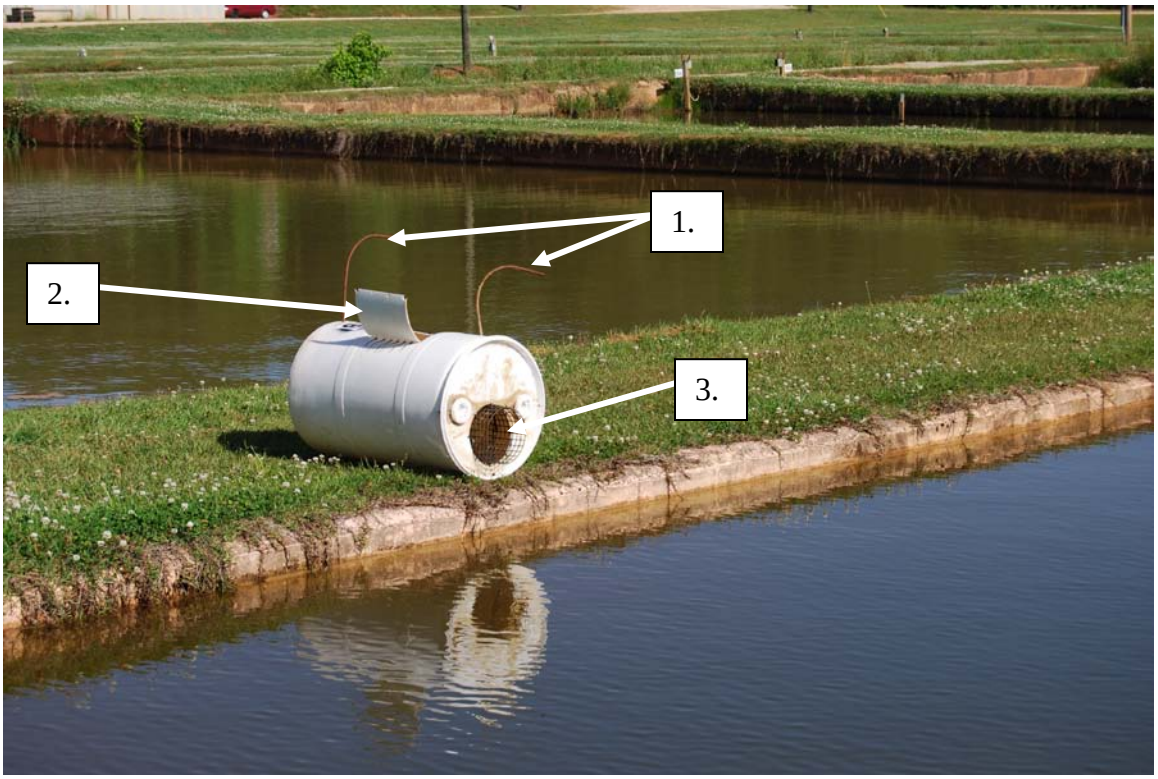
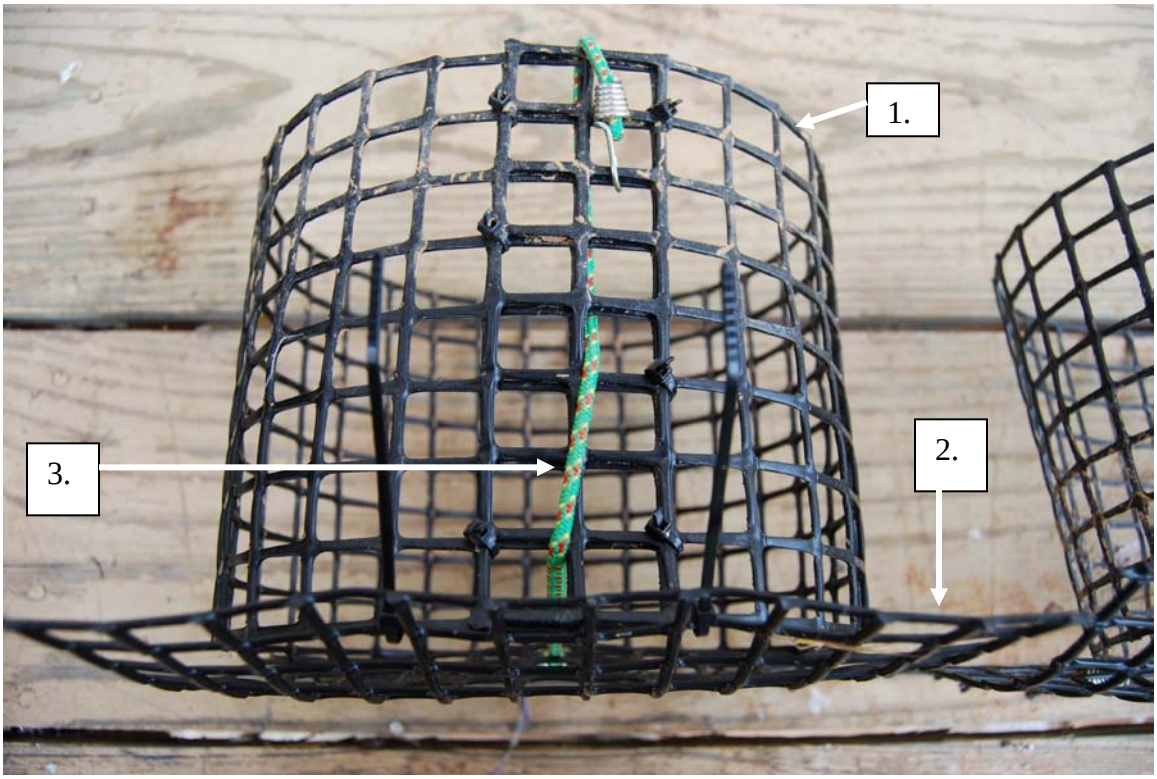


Figure 2. Picture of a trap with an open access door as well as the rebar used to hold the trap down on the pond bottom.



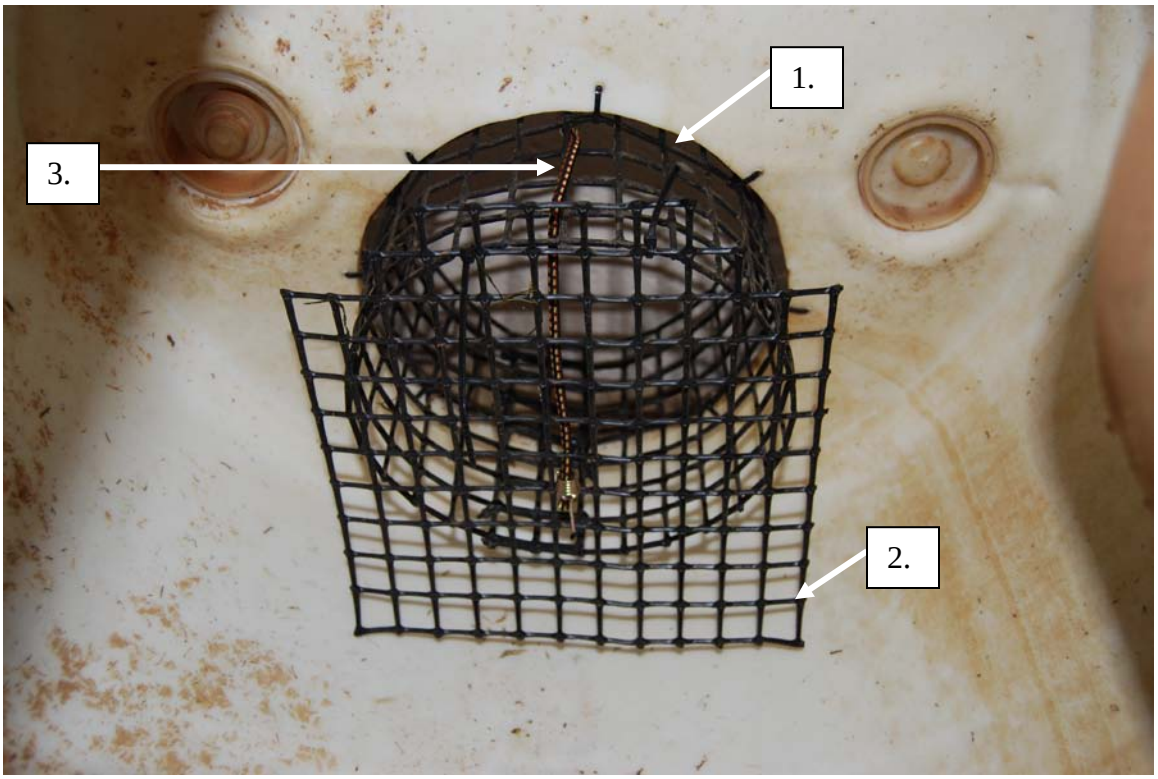
1. Rebar used to anchor trap.
2. Access door for brood stocking and removal.
3. Trap entrance.

Figure 3. Design of the plastic netting cylinder used for the trap door.



1. Outside entrance into trap.
2. Trap door.
3. Bungee cord used to pull door closed.

Figure 4. Inside of the barrel showing the placement of the cylinder extended into the inside of the barrel, as well as the design of the trap door.



1. Middle of the cylinder attached to barrel.
2. Trap door.
3. Bungee cord used to pull door closed.

Figure 5. Capture rate for each male based on each male's total number of chances to be captured. Each capital letter on the x-axis represents an individual male. The color represents the species of the male.

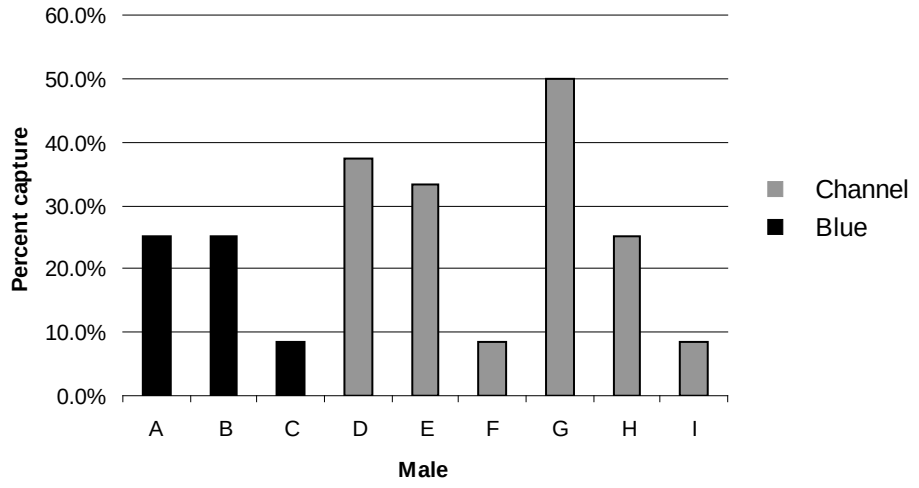


Figure 6. Percentage of the total trappings accounted for by each injected treatment during each trial where $N = 23$.

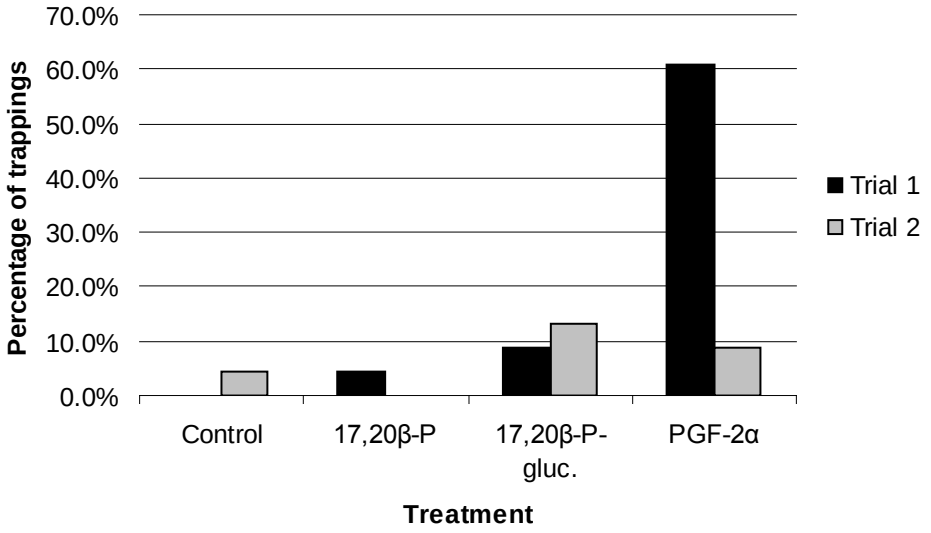


Figure 7. Percentage of the total trappings accounted for by each injected treatment and male species where $N = 23$.

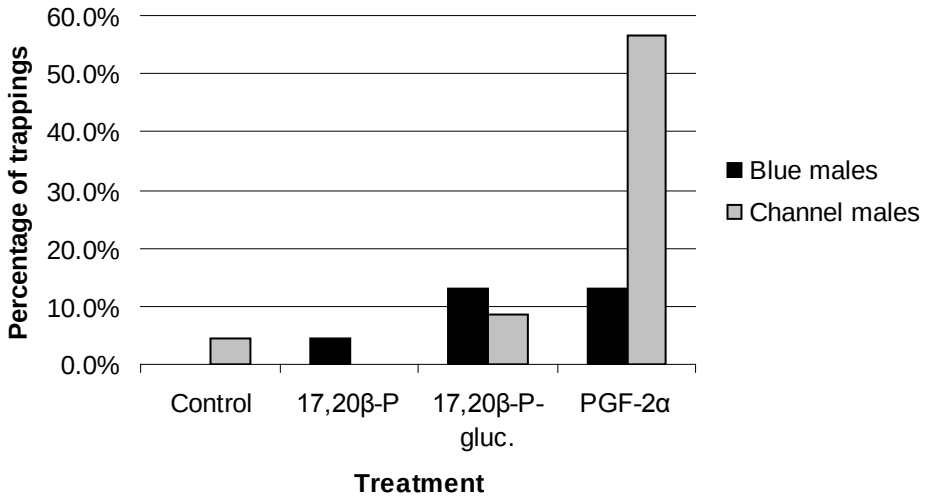


Figure 8. Percentage of the total trappings accounted for by each injected treatment and female strain where $N = 23$.

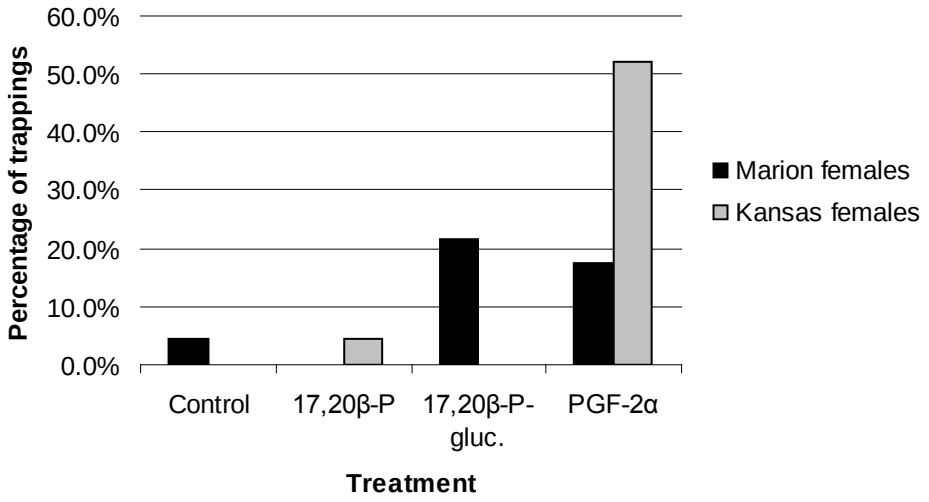


Figure 9. Percentage of the total trappings accounted for by female strain during each trial where $N = 23$.

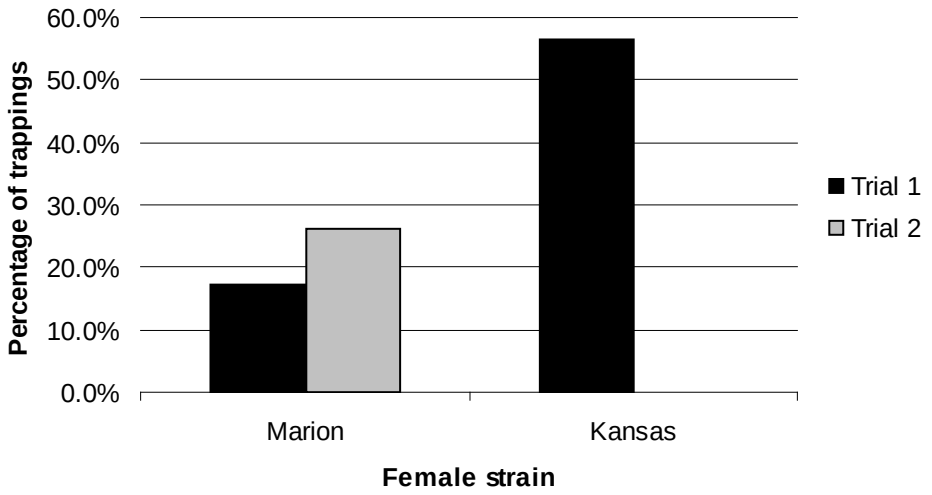


Figure 10. Percentage of total trappings accounted for by each male species during each trial where $N = 23$.

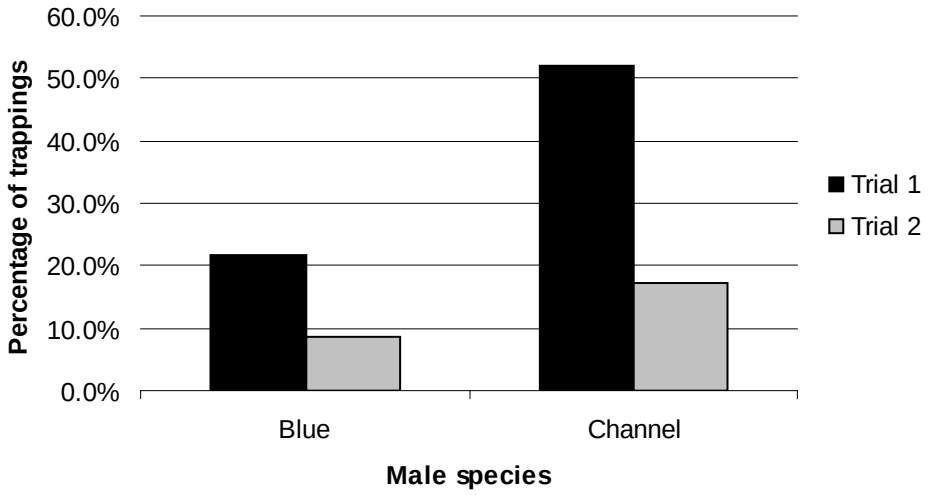
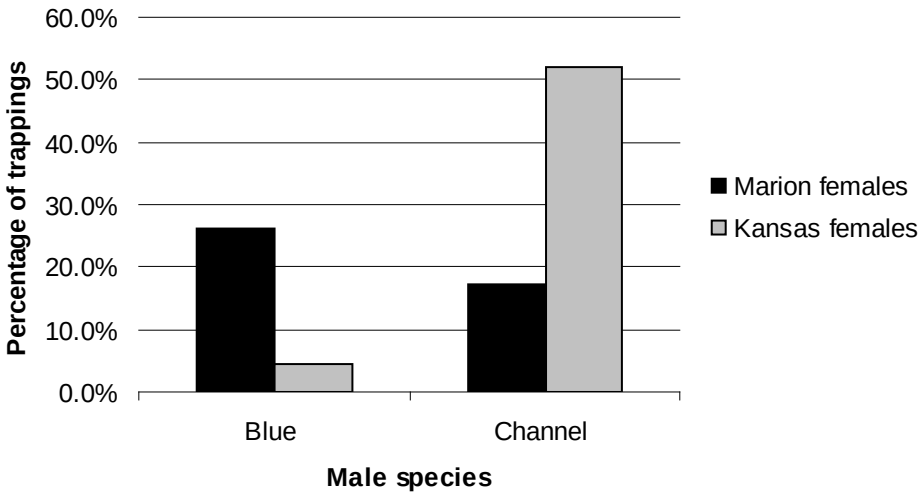


Figure 11. Percentage of total trappings accounted for by each male species and female strain where $N = 23$.



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IV. EFFECTS OF PGF-2α INJECTIONS ON NATURAL HYBRIDIZATION SUCCESS BETWEEN FEMALE CHANNEL CATFISH AND MALE BLUE CATFISH

Abstract

The effects of prostaglandin F-2α (PGF-2α) given along with LH-RHa used for induced spawning of channel catfish, *Ictalurus punctatus*, were investigated to determine if spawning success between female channel catfish and male blue catfish, *I. furcatus*, could be increased. PGF-2α was injected into channel females at 0.5 mg/kg during priming dose injections of LH-RHa. Females receiving the additional PGF-2α were paired with blue males in concrete tanks, and spawning success was compared to channel females (not receiving additional PGF-2α) paired with channel males or blue males. PGF-2α appeared to have no effect on successful fertilization events (success rates averaged 12.5% for females receiving and not receiving PGF-2α and paired with blue males); however, ovulation as well as hatch rates appeared to be positively and negatively influenced respectfully by the addition of PGF-2α. The results prompt further investigation to evaluate the effects of PGF-2α on ovulation in channel catfish.

Introduction

The hybrid between female channel catfish (*Ictalurus punctatus*) and male blue catfish (*I. furcatus*) has culture traits which are superior to that of channel catfish. These hybrids have a higher tolerance to low dissolved oxygen (Dunham et al. 1983), greater

resistance to microbial diseases (Masser and Dunham 1998; Wolters et al. 1996), better feed conversion ratios (Wolters 1993; Smitherman et al. 1996), and are easier to seine (Dunham and Argue 1998). Hybrids also exhibit faster growth to market size (Giudice 1966; Dunham and Brummett 1999), higher dress out percentages (Argue et al. 2003), and increased uniformity in size and shape (Dunham et al. 1982) than the parent species. These superior traits exhibited by hybrids make them a possible candidate to replace the channel catfish as the primary culture species for the catfish aquaculture industry in the U.S.

Unfortunately, production of these hybrids has been limited, and lack of fingerlings for culture has prevented hybrids from replacing the channel catfish as the primary cultured catfish in the U.S. Traditional spawning methods for channel catfish production (open-pond and pen spawning) are not very successful for the production of hybrids (Smitherman et al. 1996; Dunham et al. 2000). Hybridization success using traditional spawning techniques has yet to be increased up to the success rate of channel catfish. The lack of blue male's attraction to ovulating channel females has been one of the primary obstacles impeding natural hybridization success. However, in the previous chapter it was found that blue and channel males were strongly attracted to channel females injected with prostaglandin F-2α (PGF-2α) as compared to that of the control.

PGF-2α is a hormone that can stimulate ovulation in some fishes and can also serve as a pheromone after ovulation to induce female sexual behavior and/or attract a con-specific mate (Stacey and Goetz 1982; Stacey 2003; Stacey et al 2003). 'Hormonal pheromones,' which are typically steroids or prostaglandins, are known to produce significant behavioral and physiological responses during fish spawning (Burnard et al.

2008). Hormonal pheromones have been identified in many diverse fish species, but those of the goldfish, *Carassius auratus*, are the best understood (Stacey 2003; Stacey et al 2003). Male goldfish are attracted to female con-specifics during oocyte maturation (pre-ovulation) primarily by the steroids 17α, 20β-dihydroxy-4-pregnene-3-one (17,20β-P) and 17,20β-P-20β-sulfate (17,20β-PS). These steroids induce male courtship behaviors and also stimulate milt production. During post-ovulation when 17,20β-P and 17,20β-PS levels subside, males are primarily attracted to females by the two prostaglandins, PGF-2α, and 15-keto-PGF-2α, which further stimulate male courtship behaviors and milt production (Stacey 2003; Stacey et al 2003).

Injecting females with specific hormonal pheromones have caused them to become attractive to male con-specifics. Prostaglandin injections have been effective in attracting males and inducing spawning behaviors in oriental weatherfish, *Misgurnus anguillicaudatus*, (Kitamura et al. 1994) and goldfish (Volkoff and Peter 1999). Volkoff and Peter (1999) injected female goldfish with PGF-2α at approximately 0.67 mg/kg and observed increased male spawning behaviors (courting, chasing, and nudging) for several hours after injecting the females. Kitamura et al. (1994) injected female oriental weatherfish with either PGF-2α, 15-keto-PGF-2α, or 13,14-dihydro-15-keto-PGF-2α at 2.5 mg/kg and observed increased courtship attempts by exposed males up to 90 minutes after female injections. Sorensen et al. (2004) injected female ruffes, *Gymnocephalus cernus*, with 4-pregnen-17,20β,21-triol-3-one (20β-S) which induced the release of a pheromone(s) that stimulated nudging and increased swimming activity in males. Dominant male Nile tilapia, *Oreochromis niloticus*, exposed to females injected with

17,20β-P at 0.2 mg/kg exhibited increased chasing frequencies as compared to the control (Souza et al. 1998).

Attempts have been made to improve spawning success by coupling hormonal pheromones with standard hormones used for induced spawning such as luteinizing releasing hormone analogues (LH-RHa), human chorionic gonadotropin (hCG), and carp pituitary extract (CPE). Jieng-Chang et al. (1986) found that female silver carp, *Hypophthalmichthys molitrix*, receiving a priming dose of LH-RHa at 2.0 ug/kg 12 hrs prior to injection of either PGE-2 or 15-methyl-PGF-2α at 0.87 mg/kg spawned more successfully than those fish not receiving a priming dose of LH-RHa. Salmon gonadotropin injected at 10.0 mg/kg and supplemented with 17,20β-P at 1.0 mg/kg induced ovulation in all *Clarias batrachus* injected (N = 8) and resulted in higher hatch rates than those fish receiving salmon gonadotropin and supplemental injections of decorticosterone or progesterone at the same rate (Haider and Rao 1994). Lambert et al. (1999) injected female channel catfish with 17,20β-P at 0.03 mg/kg along with a priming dose of CPE at 2.0 mg/kg followed by a resolving dose of either 4 or 8 mg/kg but observed no improvement in ovulation rates (75% vs. 75%), egg production, fertilization rates, or hatching rates as compared to CPE injections given alone for manual stripping.

As PGF-2α proved to be the most potent pheromone to attract males in Chapter III, it was evaluated in this chapter to determine if it also impacted spawning success. In this study, PGF-2α injections were given along with LH-RHa injections to determine if natural hybridization success between female channel and male blue catfish could be enhanced with the additional attraction effect produced from PGF-2α injections.

Methods and Materials

Broodstock maintenance

All catfish used during the experiment were held at the E. W. Shell Fisheries Center and were under the care of the researchers beginning in March, 2008. Fish were fed at approximately 1700 hrs with a 32.0% crude protein extruded catfish diet. During colder periods, fish were fed three times a week at 0.5% body weight per day. As water temperatures warmed to approximately 17 ºC, fish were fed five times a week at 1.0% body weight per day until the first experiment was conducted in late May, 2008.

Rio Grande blue males, approximately eight years in age, were held in a 0.1 ha pond at a density of 1,942.8 kg/ha (mean weight = 3.68 kg). Kansas channel males of an unknown age class were held in a 0.1 ha pond at a density of 928.7 kg/ha (mean weight = 3.58 kg). Marion channel females, approximately six years in age, were held in two, 0.1 ha ponds at densities of 1,196.6 and 1,335.9 kg/ha respectfully (mean weights = 3.72 kg). Marion channel males of an unknown age class were held in a 0.02 ha pond at a density of 2,448 kg/ha (mean weight = 2.72 kg).

Spawning tank setup

Twenty-four concrete tanks, approximately 20.0 m² in size, were used as spawning enclosures. The tanks were drained and allowed to dry for one day prior to filling. The tanks were filled with water from a rain-fed reservoir. Once each tank was filled at a depth of approximately 66 cm, lime was added at 1,000 kg/ha and Aquashade® (Aquatic Eco-Systems, Apopka, FL) was added at 1.0 mg/L. Air diffusers were added to each tank to provide aeration. Tanks were covered with taut bird netting to prevent fish from escaping.

Spawning containers were constructed from cylindrical, white plastic barrels (80 cm in length and 51 cm in diameter). A circular, 20.0 cm diameter hole was cut into the lower portion of one end of the barrel using a jigsaw. A 20.0 cm X 25.0 cm access door to the fish and egg masses was cut on the upper horizontal side of the barrel so that the circular opening was positioned downwards. Zip ties were used to hinge the doors, as well as keep them closed during the trials. One barrel was added to each tank on the day prior to the first filling. Each container was held down by two, 10.2 cm X 20.3 cm X 40.6 cm cinder blocks placed on the sides of the container with rope looped over the top of the barrel.

Spawning induction

Marion strain channel females were seined from the holding pond and transported to a 20.0 m^2 holding tank one day before each trial. Fish were selected based on an overall healthy appearance, roundness of the abdomen, and redness of genital opening. To induce ovulation, females were injected with 120 ug/kg of a LH-RHa solution (Sigma Chemical, St. Louis, MO) in an initial 10.0% priming dose and a subsequent 90.0% resolving dose with a twelve hour interval between injections. Stock solutions of 50.0 ug/ml and 500.0 ug/ml concentrations were prepared by dissolving LH-RHa in a saline solution. Injections were given intramuscularly (IM) behind the adipose fin with a 1.0 ml syringe and 25 gauge needle.

Hormone treatment

One-third of the females induced to spawn were also given an additional pheromone treatment. Stock solutions of PGF-2α (Sigma Chemical, St. Louis, MO) were prepared by diluting the compound in 100.0% ethanol to achieve a concentration of 2

mg/ml. The additional pheromone injection was given inter-peritoneal (IP) at the same time as the LH-RH priming dose. Females receiving the additional pheromone were pit tagged behind the adipose fin for identification during the experimental stocking. The injections were given at a dose rate of 0.5 mg PGF-2α /kg body weight with a 3.0 ml syringe and a 22.5 gauge needle.

Experimental tank stocking

Each tank was stocked with a single pair of fish. Males were selected based on an overall healthy appearance and the presence of fighting wounds. The males were stocked shortly after the tanks were filled and at least eighteen hours prior to stocking the channel females. Females were stocked immediately after receiving the resolving dose of LH-RHa.

There were two spawning trials conducted. Eight Kansas strain males (mean weight = 3.90 ± 0.31 kg) and eight Marion (mean weight = 2.72 ± 0.25 kg) channel males were paired with non-PGF-2α injected females during Trial-1 and Trial-2, respectfully. Eight Rio Grande strain blue males (mean weight = 3.66 ± 0.25 kg) were paired with non-PGF-2α injected females during each trial. Eight Rio Grande blue males (mean weight = 3.98 ± 0.32 kg) were paired with PGF-2α injected females during each trial. The mean weights of the females paired with channel males, blue control males, and blue males with the PGF-2α treatment were 3.81 ± 0.20, 3.93 ± 0.17, and 3.34 ± 0.23 kg respectfully (Table 1).

Data collection

In each of the two, five day spawning trials, spawning containers were checked daily for the presence of egg masses during each trial at approximately 1300 hours. All

egg masses found were carefully taken from the containers and transported to the hatchery. Counting procedures were then performed on egg masses after being dissolved in a 1.5% sodium sulfite solution for approximately one minute until all eggs were separated from one another. Eggs were then rinsed in fresh water and added to a large graduated cylinder to determine volume and three sub-samples (1.0-3.0 ml) were taken to determine the number of eggs/ unit volume. Eggs were then added to MacDonald jars for incubation. Upon hatching, further counting procedures were then carried out on the larvae as well as swimup fry. Egg mass, eggs/kg female, egg number, fertilization rate, percent hatch, and percent survival were recorded.

Fertilization rates for dissolved egg masses were calculated one day after bringing the eggs into the hatchery. Three sub-samples approximately fifty eggs each were used to find the average percent of viable eggs in each spawn.

Newly hatched larvae were also counted volumetrically the same way as eggs. Upon swim up, fry were counted by weight using three, approximately 1.0 g sub-samples to obtain an average number of fry per gram. The large sample was pat-dried to remove as much water as possible for a more accurate measurement of the total weight of fry.

Dissolved oxygen (D.O.) and water temperature for at least four randomly selected tanks were recorded with a Y85, YSI DO meter every morning (0800 hours) during each trial. Morning pH levels for all tanks were also recorded with a Denver Instruments, UP-5 meter on the mornings of 24, 48, and 72 hours after the resolving dose injection. Ammonia, nitrate, hardness, and alkalinity measurements of three random tanks were taken with a LaMotte, Fish Farm 9 fresh water test kit at approximately 1400 hours on the beginning and ending day of each trial. D.O., water temperature, and ammonia

was also monitored in the hatchery daily from the beginning of egg incubation to swimup fry.

Data analysis

Logistic regression analysis was performed on one data set which contained the cumulative data from both trials. The data set was organized so that spawning success could be evaluated using spawning group (channel X channel, channel (without PGF-2α injection) X blue, and channel (with PGF-2α injection) X blue), male weight, female weight, the pH of the water on the day prior to and the day of spawning, average water temperature on the day of spawning, and trial as predictor variables. The response variable for each data set was coded as 1 for a successful spawning event and 0 for a non-successful spawning event. Multiple models were generated based on different, variable selection procedures (forward, stepwise, and backward). The goodness of fit for these models were assessed based upon likelihood ratio p-values, Hosmer and Lemeshow p-values, the magnitude of McFadden's R^2 values, and the overall percent correct. Odds ratio estimates were estimated for each category within each predictor variable. Odds ratio estimates that did not contain a value of 1.0 within a 95% confidence interval (CI) were considered significant. Spawning characteristics (eggs/kg female, fertilization rate, percent hatch, and percent survival), water temperature, D.O., and pH were analyzed using one-way analysis of variance followed by a Tukey-Kramer multiple comparisons test. P-values less than 0.05 were considered significant. Data analysis was performed using the Statistical Analysis System 9.1 (SAS Institute 2004).

Results

Spawning performance

All egg masses (fertile and non-fertile) that were collected for both trials were found approximately fifty-three hours after injection of the resolving dose of LH-RHa (day three at 1300 hours). Seven fertilized spawns were found during Trial-1 and two in Trial-2. Only two out of the sixteen (12.5%), channel (with PGF-2α injection) X blue gave fertilized spawns, and an additional two spawns were collected that were unfertilized. Two of sixteen pairs (12.5%) of channel (without PGF-2α injection) X blue gave fertilized spawns; no unfertilized spawns were collected. Five out of sixteen (31.3%), channel (without PGF-2α injection) X channel spawning pairs gave fertilized spawns. Logistic regression was unable to predict any significant influence of spawning group, male weight, female weight, the pH of the water on the day prior to and the day of spawning, water temperature on the day of spawning, and trial on spawning success using any selection procedure ($P = 0.3724$).

Spawning parameters for the fertile spawns that were collected are given in Table 2. The two females not receiving PGF-2α injections and paired with blue males produced around 4,754 and 5,630 eggs/kg. The egg masses produced were both above 90.0% fertile and had hatch rates above 90.0%. The two females receiving PGF-2α injections and paired with blue males produced slightly less eggs/kg (3,649 and 4,325 eggs/kg). Both egg masses produced by these females were at least 75.0% fertile but yielded poor hatch rates (39.0 and 47.7%). The five females paired with channel males produced an average of 5,905.8 ± 1,176.42 eggs/kg. These five egg masses had an average fertilization rate of 91.5 ± 0.05% and hatch rate of 78.6 ± 0.06%.

Water quality

The average pH throughout the experiment was 7.43 ± 0.04 (Table 3). There was a significant decrease ($P < 0.05$) in pH levels between trials (7.67 ± 0.06 vs. 7.19 ± 0.04 for Trial-1 and Trial-2 respectfully). The pH was also significantly lower ($P < 0.05$) on the day prior to spawn (7.24 ± 0.07) as compared to day of spawning (7.51 ± 0.05) and after spawning (7.55 ± 0.08). No significant difference ($P > 0.05$) in pH levels was detected between the fish spawning successfully and those not spawning.

The average D.O. level throughout the study was 6.68 ± 0.12 mg/L (Table 4). The D.O. decreased significantly between trials from 6.97 ± 0.14 mg/L in Trial-1 to 6.38 ± 0.15 mg/L in Trial-2 ($P < 0.05$). No significant difference in D.O. levels was detected for the days prior to spawning, of spawning, and after spawning.

The average morning water temperature throughout the study was 25.82 ± 0.27 ºC (Table 5). The temperature increased significantly from 24.68 ± 0.11 ºC in Trial-1 to 26.95 ± 0.26 ºC in Trial-2 ($P < 0.05$). The water temperature was also significantly higher on the day of spawning (26.39 ± 0.47 ºC) as compared to the day prior to spawning (25.06 ± 0.28 ºC) and the day after spawning (26.00 ± 0.55 ºC).

The average hardness and alkalinity in the spawning tanks was 61.4 ± 1.33 and 57.2 ± 7.71 mg/L respectfully. Ammonia and nitrates averaged 0.16 ± 0.02 and 0.061 ± 0.01 mg/L respectfully.

The average water temperature in the hatchery was 29.03 ± 0.47 ºC. The D.O. and ammonia averaged 5.13 ± 0.25 and 0.25 ± 0.06 mg/L respectfully. There were incidences after egg hatching when ammonia levels peaked above 0.5 mg/L.

Observations

At the end of Trial-1 when all fish were removed from the tanks, some females were observed to have readily releasable over-ripe eggs when handled. However, the hormone treatment those females had received was not noted. At the end of Trial-2, seven out of eight PGF-2α injected females had releasable eggs while only five out of eight had releasable eggs for the channel (without PGF-2α injection) X blue and two out of eight for the channel (without PGF-2α injection) X channel spawning groups (Table 6). It should also be noted that one of the females receiving PGF-2α during Trial-2 did lay a non-fertile 190.0 g egg mass (and was also one of the seven females who released eggs when handled) and that 2 out 8 females in the channel female X channel male spawning group laid two fertile egg masses during Trial-2. No significant differences were detected between spawning groups for the total number of fertile and infertile egg masses laid during Trial-2, as well as the number of females releasing eggs at the end of Trial-2, using logistic regression analysis to model the females spawning group as a predictor for the presence of eggs ($P = 0.2406$; Table 6).

Discussion

The average pen spawning rate for channel catfish is reported to be between 30.0-50.0% (Wolters 1993; Silverstein et al. 1999); however, rates can be variable. Tieman (1995) obtained pen spawning rates of 47% and 37% for natural and hormone induced spawns respectfully for channel catfish. Busch and Steeby (1990) observed aquarium spawning rates of 74%, 68%, 50%, and 28% after injections of LH-RHa, HCG, CPE, and a control solution respectfully for channel catfish. Silverstein et al. (1999) observed cage spawning rates of 47%, 52%, 57%, and 95% for channel catfish injected with a control

solution, LH-RHa, pimozide, and LH-RHa with pimozide respectfully. Tave and Smitherman (1982) observed a pen spawning success rate of 88% for channel catfish injected with hCG. The spawning success rate (fertilized spawns) for channel catfish in the present study (31.3%) was below the normal range for pen spawning channel catfish when hormone induced.

Auburn University has obtained an average pen spawning rate for hybrid production of 15% over a 14 year span, but hybridization rates, like channel catfish spawning rates, are also variable (0-100%; Masser and Dunham 1998). Tave and Smitherman (1982) observed a 40% pen spawning success rate (N = 5) for hybrid production after female HCG injection. Tieman (1995) did not obtain any hybrid pen spawns with or without female CPE injection (N = 30). Dunham et al. (2000) obtained a pen spawning rate of 15% for hybrid production (N = 47). The spawning success rate for hybridization of channel (without PGF-2α injection) X blue catfish observed in this study (12.5%) is within the range of reported success rates.

PGF-2α did not appear to have any influence on the occurrence of fertilized egg masses produced from female channel and male blue catfish at the given dose rate and injection regime used. The actual spawning success rates for both blue male X channel female (with and without PGF-2α additional injections) spawning groups were 12.5% each which is within the range of reported rates (Tave and Smitherman 1982; Tieman 1995; Dunham et al. 2000). The lack of increased spawning success of female channel and male blue catfish may suggest that although PGF-2α injections alone are capable of attracting 15% of a blue male population as indicated in the previous chapter, their attraction effects are not strong enough to increase spawning success up to the rate of

channel catfish observed in this study (31.3%). The LH-RHa injected alone may have been enough to stimulate the release of prostaglandins, or other pheromones, in adequate concentrations that are naturally produced by the female which resulted in the similar 12.5% success rate for blue male X channel female (with and without PGF-2α additional injections). This would suggest that any resultant pheromone contribution following PGF-2α injection at 0.5 mg/kg as seen in the previous chapter is not effective in further stimulating fertilization success above the rate of LH-RHa injected alone.

The lack of increased spawning success could also be the result of the injected PGF-2α acting more as an ovulation inducing agent like LH-RHa rather than primarily acting as an attractant as discussed in the previous chapter, and as indicated by the increased ovulation rates in this study. The two additional non-fertilized egg masses found for blue male X channel female with PGF-2α additional injections, as well as observations of egg release made at the end of Trial-2, suggest that the additional PGF-2α injections given in conjunction with LH-RHa may have increased ovulation rates above those observed for channel females not receiving PGF-2α and paired with blue males. Prostaglandins are thought to play a role in modulating follicle rupture to stimulate ovulation (Sorensen et al. 1988; Goetz and Garczynski 1997). PGF-2α has been used to induce female spawning behavior and ovulation in many species. Goldfish injected with PGF-2α at various rates (0.2 – 1.0 mg/kg) become sexually active and release urinary cues that are attractive to males and stimulate milt production (Volkoff and Peter 1999; Kobayashi and Stacey 1993; Sorensen et al. 1988). Tikare et al (1983) induced profuse ovulation in all *C. batrachus* females ($N = 6$) by injecting them with either PGF-2α at 0.5 mg/kg or hCG at 4.0 IU/g. PGF-2α has been used to induce in vitro ovulation in a number

of species: rainbow trout, *Salmo gairdneri*, (Jalabert et al. 1978), yellow perch, *Perca flavescens*, and brook trout, *Salvelinus fontinalis*, (Stacey and Goetz 1982), *Heteropneustes fossilis* (Tripathi and Singh 1995), and Japanese eels, *Anguilla japonica*, (Kagawa et al. 2003). The ovulation response of female channel catfish in the present study suggests that PGF-2α given in conjunction with LH-RHa may be capable of inducing or enhancing ovulation in females as seen in many other fish species.

Prostaglandins tend to have a universal action which could result in the non-discrimination of con-specifics and hetero-specifics (Burnard et al. 2008). Male brown trout, *Salmo trutta*, and Atlantic salmon, *Salmo salar*, both respond equally well to odors of hetero-specific females as they do con-specifics which results in similar levels of strippable milt when exposed to the different females (Olsén et al. 2000). Essington and Sorensen (1996) had previously observed similarities in male brown trout and Atlantic salmon to PGF-2α and its derivatives, which led them to postulate that the two species might employ the same pheromone system which could result in the hybridization of the two species observed in natural water bodies. Irvine and Sorensen (1993) observed similar olfactory sensitivities between wild common carp, *Cyprinus carpio*, and goldfish to the common hormonal pheromones employed by the goldfish (17,20β-P; 17,20β-PS; PGF-2α; and 15-keto- PGF-2α). A similar sensitivity to 17,20β-P and PGF-2α has also been seen in goldfish and crucian carp, *Carassius carassius* (Burnard et al. 2008). Hybrids between common carp, goldfish, and crucian carp have been observed in European natural water bodies where at least one of the species has been introduced (Hanfling et al. 2005).

It is possible, however, that blue males might be more responsive to a different prostaglandin(s) as would normally be produced by blue females. Many fish show varying sensitivities and reactions to prostaglandins and their mixtures. Brown trout exhibit increased locomotor activity in response to PGF-2α and 13, 14-dihydro-PGF-2α, while lake whitefish exhibit increased locomotor activity to PGF-2α and 15-keto-PGF-2α and rainbow trout, *Oncorhyncus mykiss*, exhibit no locomotor response or electro-olfactory sensitivity to any of the three prostaglandins (Laberge and Hara 2003). Male Atlantic salmon are highly sensitive to PGF-1α and PGF-2α, slightly sensitive to 15-keto-PGF-2α, and not sensitive to 13, 14-dihydro-PGF-2α (Moore and Waring 1996). Males also exhibit increased levels of expressible milt in response to water borne PGF-1α and PGF-2α. Male Atlantic salmon sensitivities to PGF-1α and PGF-2α are also influenced by the sexual maturity of the male with more mature males being more sensitive; this could explain the lack of increased spawning success in this study where blue males were paired with one channel female, as results from the previous chapter indicated that dominant/ more responsive blue males to specific females injected with PGF-2α are present in a population. Hong et al. (2004) found that male *Bostricthys sinensis* were more likely to be attracted to artificial nest bated with (17α-P),(17α,20β-P) and PGE-2 but not PGF-2α, and that 17α,20β-P and PGE-2 baited nests were effective in inducing spawning while PGF-2α was ineffective. Observations by Rubec (1979) suggested that cues in the urine of yellow (*Ameiurus. natalis*), black (*A. melas*), and brown bullheads (*A. nebulosus*) provide a prezygotic isolating mechanism that prevents hybridization between the species. In the present study, it may have been beneficial to try different prostaglandins and perhaps mixtures of prostaglandins that more closely resemble what is

normally produced by blue females to attract the attention of blue males once the female has ovulated.

Hatching rates for hybrid production are variable depending on different factors and scenarios. Dunham et al. (2000) obtained a hatching rate of 66% for hybrid production using the pen spawning method as compared to 22% when using the artificial spawning method. Tieman (1995) obtained similar hatch rates of only 29% for hybrid production using the artificial spawning method. Tave and Smitherman (1982) observed hatch rates around 90.0% for naturally deposited hybrid egg masses after female injection with hCG using the pen spawning method. This is similar to the observed hatch rates found for channel (without PGF-2α) X blue spawning pairs seen in the present study (93.6-100%). The spawning data suggests that hybrid hatch rates may have been reduced due to the additional PGF-2α (93.6-100% without PGF-2α vs. 39.0-47.7% with PGF-2α). Haider and Rao (1994) observed an increase in hatch rates in *C. batrachus* injected with salmon gonadotropin and supplementary injected with 17,20β-P at 1.0 mg/kg (70.0%) compared to salmon gonadotropin alone (52%). Supplementation with decorticosterone or progesterone at the same rate had no effect (54.0% hatch rate).

The number of successful spawns declined drastically from seven spawns in Trial-1 to only two spawns in Trial-2. One factor that could have influenced this event was the significant change in water quality (pH, D.O., and water temperature) observed between trials (Table 3, 4, and 5). Mean water temperatures were 24.68 ± 0.11 ºC and 26.95 ± 0.26 ºC in Trial-1 and Trial-2 respectfully ($P < 0.05$). Water temperature is one of the major factors dictating reproductive seasonality and spawning success for many species including channel catfish (Zohar 1989; Tucker and Robinson 1990). Optimal

water temperature for spawning of channel catfish is reported to be around 25.5-26.5 ºC (Tucker and Robinson 1990). Midday (approximately 1400 hours) water temperatures in the present study were above 30.0 ºC on two out of four days during Trial-2. There were two out of four days during Trial-1 which water temperatures reached above 28.0 ºC and one out of four which temperatures reached slightly above 30.0 ºC. These high temperatures may have begun to exceed the threshold for spawning resulting in the observed reduced spawning success as spawning success can drop sharply at temperatures above 30.0 ºC (Lee 1979). Mean D.O. levels were 6.97 ± 0.14 mg/L and 6.38 ± 0.15 mg/L in Trial-1 and Trial-2 respectfully ($P < 0.05$). Mean pH levels were 7.67 ± 0.06 and 7.19 ± 0.04 in Trial-1 and Trial-2 respectfully ($P < 0.05$). Steeby (1987) observed no effect of DO levels on spawning success as long as DO levels were above 4.0 mg/L. In the present study, high water temperatures during Trial-2 was probably the major water quality factor leading to the decreased number of spawns as both DO and pH and even other factors such as un-ionized ammonia levels are/ can be correlated with temperature (Boyd 2000).

Conclusion

Additional PGF-2α injections given at 0.5 mg/kg given with the priming dose of LH-RHa do not appear to have any influence on the production of fertilized spawns by female channel and male blue catfish, but may increase ovulation success. Hatch rates appear to be negatively affected by the additional PGF-2α injections. Further experimentation where the sample sizes are increased during the optimal environmental conditions for spawning could provide a stronger and significant relationship between PGF-2α, spawning success, ovulation, and hatch rate.

Table 1. Average weights ((kg) mean ± SEM) of fish where females in all treatments were given LH-RHa at 120 μg/kg, and in treatment three were additionally given PGF-2α at 0.5 mg/kg.

Treatment	Males (kg)	Females (kg)
1. Channel ♀ X Channel ♂		
Kansas males (Trial-1) (*N* = 8)	3.90 ± 0.31	4.17 ± 0.24
Marion males (Trial-2) (*N* = 8)	2.72 ± 0.25	3.45 ± 0.24
Grand mean	3.31 ± 0.24	3.81 ± 0.20
2. Channel ♀(w/o PGF-2α) X Blue ♂		
Trial-1 (*N* = 8)	3.77 ± 0.35	3.81 ± 0.27
Trial-2 (*N* = 8)	3.56 ± 0.38	4.06 ± 0.21
Grand mean	3.66 ± 0.25	3.93 ± 0.17
3. Channel ♀ (w/ PGF-2α) X Blue ♂		
Trial-1 (*N* = 8)	4.59 ± 0.50	3.38 ± 0.33
Trial-2 (*N* = 8)	3.38 ± 0.29	3.29 ± 0.35
Grand mean	3.98 ± 0.32	3.34 ± 0.23

Table 2. Spawning parameters (mean ± SEM) for fertile collected tank spawns where females in all spawning pairs were given LH-RHa at 120 μg/kg and paired with blue or channel males, and some were additionally given PGF-2α at 0.5mg/kg and paired with blue males (*N* = 16 for each spawning group).

Spawning pair	Female weight (kg)	Eggs/ kg female	% fertile*	% hatch**
Channel♀ X Blue ♂ †	4.36	4,754.3	92.6%	100% [a]
Channel♀ X Blue ♂ †	3.88	5,630.3	96.2%	93.6%
Mean	4.12 ± 0.24	5,192.3 ± 438.00	94.4% ± 0.02	96.8% ± 0.03
Channel♀(with PGF-2α) X Blue ♂ †	3.36	3,649.2	93.6%	39.0%
Channel♀(with PGF-2α) X Blue ♂ †	3.30	4,325.3	75.6%	47.7%
Mean	3.33 ± 0.03	3,987.2 ± 338.03	84.6% ± 0.09	43.3% ± 0.04
Channel♀ X Channel ♂†	4.82	1,402.4	98.0%	70.9%
Channel♀ X Channel ♂†	4.72	6,331.8	70.5%	91.3%
Channel♀ X Channel ♂††	2.69	6,703.0	97.7%	88.9%
Channel♀ X Channel ♂††	2.54	6,770.7	97.8%	83.7%
Channel♀ X Channel ♂†	4.36	8,321.0	93.5%	58.0%
Mean	3.83 ± 0.50	5,905.8 ± 1,176.42	91.5 ± 0.05%	78.6 ± 0.06%
Grand mean	3.78 ± 0.28	5,320.9 ± 683.05	90.6 ± 0.03%	71.6 ± 0.07%

[a] Actual value was above 100% due to counting error
* % viable after egg mass collection
** % hatch of viable eggs
† Egg mass laid during Trial-1
†† Egg mass laid during Trial-2

Table 3. Morning pH levels (mean ± SEM) for tanks containing pairs that spawned and those that did not spawn throughout both trials. Variables with different letters are significantly different based on their grand averages; a, b, and c, and x, y, and z represent statistical significant differences ($P < 0.05$) for the grand averages for trials and days around the day of spawning respectfully. The letters e, f, and g also represent significant differences for the grand averages for spawners vs. non-spawners.

		Day prior to spawning	Day of spawning	Day after spawning	Average
Trial 1	Spawners (*N* = 7)	7.29 ± 0.26	7.56 ± 0.16	7.81 ± 0.18	7.55 ± 0.12
	Non-spawners (*N* = 17)	7.54 ± 0.09	7.56 ± 0.08	8.08 ± 0.07	7.73 ± 0.06
	Average	7.47 ± 0.10	7.56 ± 0.07	8.00 ± 0.08	7.67 ± 0.06 [a]
Trial 2	Spawners (*N* = 2)	7.14 ± 0.04	7.35 ± 0.11	6.91 ± 0.01	7.14 ± 0.09
	Non-spawners (*N* = 22)	7.00 ± 0.06	7.47 ± 0.08	7.11 ± 0.25	7.19 ± 0.04
	Average	7.01 ± 0.05	7.46 ± 0.07	7.09 ± 0.05	7.19 ± 0.04 [b]
Average	Spawners (*N* = 9)	7.25 ± 0.20	7.51 ± 0.13	7.61 ± 0.19	7.46 ± 0.10 [e]
	Non-spawners (*N* = 39)	7.23 ± 0.07	7.51 ± 0.06	7.53 ± 0.09	7.43 ± 0.04 [e]
	Average	7.24 ± 0.07 [x]	7.51 ± 0.05 [z]	7.55 ± 0.08 [z]	7.43 ± 0.04

Table 4. Morning D.O. levels (mg/L) (mean ± SEM) of randomly selected tanks (*N* = 4) on the days prior to, of, and after spawning during each trial. Variables with different letters are significantly different based on their grand averages; a, b, and c, and x, y, and z represent statistical significant differences (P < 0.05) for the grand averages for trials and days around the day of spawning respectfully.

	Day prior to spawning	Day of spawning	Day after spawning	Average
Trial 1	7.06 ± 0.14	6.69 ± 0.31	7.16 ± 0.26	6.97 ± 0.14 [a]
Trial 2	6.76 ± 0.26	6.03 ± 0.21	6.37 ± 0.19	6.38 ± 0.15 [b]
Average	6.91 ± 0.15 [x]	6.36 ± 0.21 [x]	6.76 ± 0.21 [x]	6.68 ± 0.12

Table 5. Morning water temperature (ºC) (mean ± SEM) of randomly selected tanks (*N* = 4) on the days prior to, of, and after spawning during each trial. Variables with different letters are significantly different based on their grand averages; a, b, and c, and x, y, and z represent statistical significant differences (P < 0.05) for the grand averages for trials and days around the day of spawning respectfully.

	Day prior to spawning	Day of spawning	Day after spawning	Average
Trial 1	24.35 ± 0.10	25.15 ± 0.06	24.55 ± 0.09	24.68 ± 0.11 [a]
Trial 2	25.78 ± 0.11	27.63 ± 0.15	27.45 ± 0.09	26.95 ± 0.26 [b]
Average	25.06 ± 0.28 [x]	26.39 ± 0.47 [z]	26.00 ± 0.55 [y]	25.82 ± 0.27

Table 6. Number of females per spawning group in Trial-2 (N = 8) who gave fertile egg masses, as well as the number of females who laid infertile egg masses and those who were running eggs at the end of Trial-2. Percentages with different letters (a, b, and c) are significantly different ($P < 0.05$). Groups 1, 2, and 3, correspond to the channel (without PGF-2α injection) X channel, channel (without PGF-2α injection) X blue, and channel (with PGF-2α injection) X blue spawning groups respectfully.

	Group		
	1	2	3
No. fertile egg masses	2	0	0
No. infertile egg masses	0	0	1
No. females running eggs	2	5	7*
Total No. of females releasing eggs	4	5	7
% of females releasing eggs	50.0% [a]	62.5% [a]	87.5% [a]

* One of the females running eggs also laid one of the infertile egg masses

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V. CONCLUSION

Hormonal pheromone injections into female channel catfish can manipulate male channel and blue catfish behavior, as well as possibly alter the physiology of injected females. Injections of PGF-2α caused female channel catfish to become significantly more attractive to channel males when compared to control females. PGF-2α as well as 17,20β-P-20-glucosiduronate injections made channel females become more attractive to blue males, but not significantly more than control females. Pheromone injections into channel females were most effective at eliciting male captures between 48 to 96 hours post-injection. PGF-2α injections at 0.5 mg/kg given in conjunction with a priming dose of LH-RHa does not appear to have any effect on increasing the number of fertile spawns produced by female channel and male blue catfish. The additional PGF-2α injections do appear to positively influence ovulation rates in female channels but also appear to negatively affect hatch rates. Results from both experiments prompt further investigation on the role of prostaglandins in attracting male blue and channel catfish during spawning, as well as the effects of prostaglandins on ovulation in channel catfish. An understanding of the roles of pheromones like PGF-2α used in channel and blue catfish spawning could provide a possible link to the low rates of hybridization observed via open pond and pen spawning.

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