RACEWAY SPAWNING
OF
LARGEMOUTH BASS

THESIS

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ABSTRACT

Largemouth bass (Micropterus salmoides) were spawned in outdoor raceways during three spawning seasons. During the first season, limited spawning success was achieved using year-class 4 Marion largemouth bass due to the late start of the experiments in the normal spawning season. During the second season, Florida largemouth bass (M. salmoides floridanus) were allowed to acclimate for 0-8 weeks. There was an inverse relationship between the length of the acclimation period and latency to first spawn, however, once spawning began the rate of spawning was similar across treatments. The second experiment was designed to test for differences in spawning success of acclimated bass treated with human chorionic gonadotropin (hCG; 4,000 IU/kg BW), [D-Ala^{6}Pro^9-N-ethylamide]-luteinizing hormone releasing-hormone (LHRH-A; 0.1 mg/ kg BW), salmon gonadotropin-releasing hormone (sGnRH; 0.1 mg/ kg BW), and Ovaprim (0.5 ml/ kg BW). The best hormone treatments in this study appeared to be hCG, Ovaprim, and sGnRH. The third experiment of the season involved treatment of unacclimated bass with hCG and LHRH-A. Due to the late start of this experiment, spawning success was low and no comparative information could be obtained. During the third season, unacclimated and 2-week acclimated Florida largemouth bass were treated with hCG or LHRH-A (0.5 mg/kg BW). Injections of hCG induced spawning quicker, produced more spawns and more fry than injections of LHRH-A or saline treated controls. Spawning success of acclimated bass was greater than that of unacclimated bass in all hormone treatments. Acclimation periods may have allowed stress associated with common fish culture practices to diminish and may have provided time for syncrony to develop between male and female largemouth bass. In the last experiment, injections of hCG in females resulted in spawns within 48 hours; however, spawns did not produce fry. This was attributed to overripeness of ova or a lack of syncrony with males. These results show that optimum spawning can be achieved when largemouth bass are allowed to

acclimate to raceway conditions for 2 weeks, and that hCG treatment of both sexes is the preferred hormone treatment for induction of spawning.

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CHAPTER I

INTRODUCTION

Largemouth bass (Micropterus salmoides) are one of the most highly prized, freshwater sportfishes in North America. To maintain stocks in heavily-fished reservoirs and lakes, hatcheries are under pressure to produce large quantities of bass fingerlings. Largemouth bass are traditionally spawned in ponds. Male and female broodfish are separated into different ponds before the spawning season. At the beginning of spawning season broodfish are stocked in spawning ponds which may be provided with artificial spawning substrates. Spawns are left in ponds to hatch. At the appropriate time, the pond may be drained or seined to remove fry. Fry are then distributed or allowed to grow in fertilized rearing ponds (Simco et al. 1986).

Production can be intensified through the controlled spawning of fish in raceways. Advantages of raceway spawning include: 1) the timing of spawning can be more easily controlled; 2) the spawning season can be compressed; 3) multiple spawns from the same broodfish can be collected; and 4) variables regulating fry production, such as aeration, temperature, feeding, predation, and disease and fungal infections, can be controlled. Spawning fish under controlled conditions also provides hatchery managers and researchers with data on spawning success of individual pairs. This allows better decisions to be made on environmental requirements such as photo-thermal regimes, hormone treatments, broodstock maintenance and selection, and egg and fry handling methods.

Research on the controlled spawning of fish has progressed along two main fronts: environmental manipulations and hormonal manipulations. Attempts at controlled spawning of largemouth bass have been aimed mainly at manipulation of photoperiod and thermal regimes (Snow 1970; Brauhn et al. 1972; Carlson and Hale 1972; Carlson 1973; Jackson 1979). Compressed photoperiod and thermal cycles have resulted in

gonadal advancement in Florida largemouth bass, M. salmoides floridanus (M. Stacell, Texas Parks and Wildlife Department, personal communication).

Two aspects of controlled spawning that have not been experimentally addressed are the effects of acclimation time and hormone injections on spawning success of largemouth bass in raceways. Following capture, handling, and transfer of broodfish from ponds to raceways, a period of acclimation may allow for more productive spawning. Based on their experience, A.E. Wood State Fish Hatchery personnel (San Marcos, Texas) allow broodfish 1-2 weeks of acclimation in raceways before pairing (J. Issac, Texas Parks and Wildlife Department, personal communication). A more detailed study of the effects of acclimation periods of differing lengths on spawning success should allow for a better determination of the optimum acclimation period.

One of the problems in fish culture is the lack of reproduction in some species held in captivity. Hormonal stimulation of reproductive function has been effective in a variety of fishes. Spawning can be induced either by direct effects at the gonads, or by inducing the secretion of gonadotropin (GtH) from the pituitary gland. Stevens (1970) found that mammalian luteinizing hormone (LH) was successful in inducing ovulation in largemouth bass. Human chorionic gonadotropin (hCG), which acts directly at the gonads, can induce ovulation or increase milt production in largemouth bass due to its LH-like activity (Wilbur and Langford 1975). It has also been successfully used in channel catfish, Ictalurus punctatus (Sneed and Clemens 1959), goldfish, Carassius auratus (Sneed and Clemens 1959), redtail black shark, Labeo bicolor (Naznov 1981), Sparrman's cichlid, Tilapia sparrmani (King et al. 1984), striped bass, Morone saxatilis (Stevens 1966), redear sunfish, Lepomis microlophus (Smitherman and Hester 1962), redbreast sunfish, Lepomis auritus (Smitherman and Hester 1962), and white crappie, Pomoxis annularis (Sneed and Clemens 1959).

Recently, new hormones have become available, and show promise in these and other species. Superactive and degradation resistant mammalian luteinizing hormone

releasing-hormone analogs (LHRH-A), which act at the pituitary to induce GtH secretion, have been used with some success in Japanese medaka, Oryzias latipes (Chan 1977), gilthead seabream, Sparus aurata (Zohar et al. 1989), goldfish (Chang and Peter 1983), and a variety of salmonids (Billard et al. 1984; Crim and Glebe 1984; Fitzpatrick et al. 1984). Salmon gonadotropin releasing-hormone (sGnRH), [Trp^7 - Leu^8]- LHRH, which also acts via increased pituitary GtH secretion (Peter et al. 1985), elevates levels of the gonadal steroids, testosterone and estradiol, in Tilapia sparrmani (King et al. 1984) and testosterone and 11-ketotestosterone in male brown bullhead, Ameiurus nebulosus (Rosenblum and Callard 1987).

In some teleosts, dopamine acts as a GtH release-inhibitory factor (GRIF) by inhibiting GtH release from the pituitary (for review see Peter et al. 1986). Dopaminergic antagonists, such as domperidone and pimozide, potentiated the effects of LHRH-A injections in goldfish (Chang and Peter 1983; Sokolowska et al. 1985), Chinese loach, Paramisgurnus dabryanus (Lin et al. 1986), rainbow shark, Labeo erythrurus and redtail black shark (Shireman and Gildea 1989), African catfish, Clarias lazera (de Leeuw et al. 1985), coho salmon,Oncorhyncus kisutch, (Van Der Kraak et al. 1986), and orangemouth corvina, Cynoscion xanthulus (Prentice and Thomas 1987). Co-treatment with LHRH-A and a dopaminergic antagonist may prove to be a valuable tool for controlled spawning in many cultured fish species.

The goal of this study was to better define requirements for controlled spawning of largemouth bass in raceways. To accomplish this goal, several experiments were designed to investigate the effects of acclimation and/or hormone treatments on measures of spawning success such as spawning latency, total number of spawns, proportion of pairs spawning, and number of fry produced/spawn.

Experiment 1: Effects of Raceway Acclimation Time on Spawning.-On 15 December 1989, 30 male (mean weight = 320 g) and 30 female (mean weight = 400 g) bass were stocked into separate 0.1-acre earthen ponds. On 1 February 1990, three males and three females were removed from the holding ponds and stocked in separate ends of a 24 X 1.8 X 0.5 m deep divided concrete raceway. Flow rate (~25 gal/min) was limited to allow temperature in the raceway to approximate pond water temperature. This procedure was repeated on 15 February, 1 March, and 15 March 1990 and provided groups of bass acclimated to raceways for periods of differing lengths (2-8 weeks). On each occasion, males and females were placed in separate ends of additional divided raceways. Females were always placed at the headbox end. While in raceways, bass were fed live goldfish, Carassius auratus, to satiation every other day. On 1 April, partitions were removed from the raceways, allowing males and females to mix, and three 45 X 45 X 5 cm thick Spawn-Tex spawning mats (Blocksom and Company, Michigan City, Indiana) were equally spaced in each raceway. Feeding was terminated on this day. At this time, an additional three males and three females were placed in an undivided raceway, and 10 males and 10 females were placed in a freshly-filled 0.1-acre earthen pond to serve as control spawners. Pairs were kept together and allowed to spawn for 10 days. Mats were inspected twice daily for eggs.

Experiment 2: Effects of Raceway Acclimation on Hormonally-Induced Spawning.-On 2 April 1991, bass were seined from holding ponds and separated into two groups. One group (16 males and 17 females) was placed in a divided raceway and allowed to acclimate for 2 weeks; sexes were kept separate and fish were not fed during this time. Mean weights and total lengths were 243 g and 268 mm (males), and 281 g and 273 mm (females). Raceway flow (~25 gal/min) was limited to allow raceway water temperature to approximate ambient temperature. Mean weights and total lengths of bass in the unacclimated group (15 males and 15 females) were 278 g and 266 mm (males) and 301

g and 265 mm (females). Pairs of unacclimated bass (n=5 pairs/treatment) were injected with either 0.6% saline (control; 1 ml/kg body weight (BW)), human chorionic gonadotropin (hCG; Sigma Chemical Co., St. Louis, Missouri; 4,000 IU/kg BW), or [D-Ala^{6}Pro^9-N-ethylamide]-luteinizing hormone releasing-hormone (LHRH-A; Sigma Chemical Co.; 0.5 mg/kg BW). Each pair was randomly assigned to a 6.0 X 1.8 X 0.5 m deep raceway section. One Spawn-Tex mat was provided to each pair. Mats were inspected twice daily for spawns. Twenty-four hours after spawns were observed, mats were removed from the raceway and placed in 51 X 51 X 25 cm deep fiberglass hatching tanks supplied with 21° C well water at a flow rate of 1 gal/min. Aeration was provided by a 15 cm air stone placed underneath the mat. After swim-up, the number of fry/spawn was estimated. Bass were allowed to spawn for 2 weeks. On 16 April 1991, this experiment was duplicated using the bass previously acclimated to raceway conditions for 2 weeks.

Experiment 3: Female Only versus Female/Male Hormone Injections.-On 1 May 1991, bass were collected from holding ponds and allowed to acclimate to raceway conditions for 1 week. Males and females were held separately during the acclimation period. One week later, bass were weighed and measured as above and separated into four treatment groups (n = 4 pairs/treatment). The following treatments were used: 0.6% saline (1 ml/kg BW; control) in both sexes (BS); hCG (4,000 IU/kg BW) in both sexes (BH); 0.6% saline (1 ml/kg BW; control) in females (FS), only; hCG (4,000 IU/kg BW) in females (FH), only. Pairs were then assigned to raceway sections and allowed to spawn as previously described. Spawns were treated exactly as described in Experiment 2.

Estimate of Number of Fry/Spawn.-After swim up, fry were collected from hatching tanks and fixed in 10% formalin. Fry from one spawn were poured into a gridded tray with 49 cells. Each cell of the grid measured 3.65 X 3.65 cm. Fry were spread-out across

the grid system as evenly as possible. Fry within 10 randomly selected cells were counted for each sample. To validate this method, all fry from three samples were also counted. Estimates using the grid technique represented 98%, 95%, and 91% of the actual fry counts for these three samples. Fry number was expressed on a weight basis using maternal BW.

Statistical Analysis.-Multiple comparisons were performed using the Kruskal-Wallis test followed by non-parametric multiple comparisons test; pairwise comparisons were made using the Mann-Whitney *U*-test (Zar 1984). In all cases, $P<0.05$ was used for significance.

Results

Experiment 1: Effects of Acclimation Time on Spawning

Bass in the control pond spawned within 2 d after stocking. Although spawning was not actually observed, males were observed guarding nests and eggs were present on the substrate. In raceways, there was an inverse relationship between acclimation time and latency to first spawn (Table 1). Bass acclimated to raceway conditions for long periods (6 and 8 weeks) spawned within 2 d and produced a total of six spawns each. Bass acclimated for intermediate periods (2 and 4 weeks) spawned within 4 d; 2-week acclimated bass produced five spawns and 4-week acclimated bass produced four spawns. Unacclimated bass spawned after 7 d and produced three spawns.

Experiment 2: Effects of Raceway Acclimation on Hormonally-Induced Spawning

Both hCG and LHRH-A increased the numbers of pairs spawning and the total number of spawns (Table 2). Treatment with hCG was slightly more effective than LHRH-A injection. Although the number of pairs spawning in each hormone treatment was not affected by prior raceway acclimation, raceway acclimated fish produced more spawns and had a higher proportion of multiple spawning pairs, than did unacclimated fish. Three unacclimated (US) and three acclimated saline-treated pairs (AS) spawned, with a total of three and five spawns, respectively. Four unacclimated (UL) and four acclimated LHRH-A treated pairs (AL) spawned, producing a total of seven and nine spawns, respectively. Four unacclimated (UH) and four acclimated hCG treated pairs (AH) spawned, with a total of nine and 12 spawns, respectively.

Spawning latency was affected by both acclimation and hormone treatment (Table 3). AH pairs had the lowest spawning latency followed by UH, UL, AL, AS, and US pairs. Significant differences were detected between hormone treatments within each acclimation group ($P<0.05$). Mean spawning latency of AH pairs was significantly lower

than in AS and AL pairs, as was mean spawning latency of UH pairs compared to US and UL pairs. Mean spawning latency of AH pairs was significantly lower than in UH pairs.

Total fry production/spawn is presented in Figure 1. UH pairs had the highest total number of fry/spawn/kg BW followed by AH, UL , AS, US, and AL pairs, in descending order. No significant ($P>0.05$) differences were detected between treatments within each acclimation group, or between unacclimated and acclimated groups within each hormone treatment group. The lack of significant differences may be the result of the high variability in fry production within each group.

Figure 2 presents fry production as a function of multiple spawns in pairs of bass. In several groups (US, UL, UH, and AH), fry production declined with each successive spawn. However, in AS and AL pairs, the number of fry produced in second and third spawns was greater than in first spawns. This is probably due to the fact that several first spawns in these two groups produced no fry, while the second and third spawns were more successful.

Experiment 3: Female Only versus Female/Male Hormone Injections

Results of this experiment are reported in Table 4. Treatment of females only with saline (FS) resulted in a single spawn after 7 d. When females alone were injected with hCG (FH), all pairs spawned within 2 d and produced a total of seven spawns. Two saline treated pairs (male and female both injected; BS) spawned once each after 6 and 7 d, respectively, and three hCG treated pairs (BH) spawned a total of four times, with a mean spawning latency of 3.3 d. No significant differences were detected in mean spawning latencies between FS and FH groups, nor between BS and BH groups, nor between female only and both sex treatments.

Injecting females only with either saline or hCG resulted in very poor fry production, with the single FS spawn resulting in 21 fry/kg BW. Likewise, the seven FH spawns resulted in no or very little fry production. All spawns of BS and BH pairs produced

large numbers of fry. No differences were detected in the number of fry/spawn/kg BW between BS and BH groups, however, fry production was significantly greater when both sexes were injected compared to female only hCG treatment.

Table 1.-Effects of acclimation time on spawning latency (d) and total number of spawns produced by largemouth bass in raceways.

Acclimation time	Spawning latency (d)	Total number of spawns*
8 weeks	2 (2)	6
6 weeks	2 (2)	6
4 weeks	4 (1)	4
2 weeks	4 (1)	5
Unacclimated	7 (1)	3

Numbers in parentheses indicate number of pairs spawning on that day.
* total number of spawns produced by all pairs (n=3) in each treatment group over the 2-week test period.

Table 2.-Proportion of unacclimated and acclimated pairs of largemouth bass spawning one, two, and three times and total number of spawns produced after injection with saline, LHRH-A (0.5 mg/kg BW), or hCG (4,000 IU/kg BW).

	Saline	LHRH-A	hCG
One spawn			
Unacclimated	3/5	4/5	4/5
Acclimated	3/5	4/5	4/5
Two spawns			
Unacclimated	0/5	2/5	3/5
Acclimated	1/5	4/5	4/5
Three spawns			
Unacclimated	0/5	1/5	2/5
Acclimated	1/5	1/5	4/5
Total number of spawns			
Unacclimated	3	7	9
Acclimated	5	9	12

Table 3.-Number of pairs spawning and mean and range of spawning latencies (d) of unacclimated and acclimated largemouth bass injected with saline, LHRH-A (0.5 mg/kg BW), or hCG (4,000 IU/kg BW) (n = 5 pairs/treatment).

	Number of pairs spawning	Spawning latency	
		Mean	Range
Saline			
Unacclimated	3	9.3 [a]	7-12
Acclimated	3	7.3 [a]	4-10
LHRH-A			
Unacclimated	4	4.3 [a]	2-7
Acclimated	4	5.0 [a]	4-7
hCG			
Unacclimated	4	2.0 [b]	2
Acclimated	4	1.0 [b*]	1

Different superscript letters indicate significant differences ($P<0.05$) between hormone effects within acclimated and unacclimated groups.
*significant difference ($P<0.05$) between acclimated and unacclimated pairs within a hormone treatment

Table 4.-Proportion of largemouth bass pairs spawning, mean and range of spawning latencies (d), and mean number of fry/spawn/kg BW (range) after injections of saline and hCG (4,000 IU/kg BW) in females only or in both sexes.

	Both saline	Both hCG	Female saline*	Female hCG
Proportion of pairs spawning:				
One time	2/4	3/4	1/4	4/4
Two times	0/4	1/4	0/4	3/4
Total number of spawns	2	4	1	7
Mean spawning latency (d)	6.5[a]	3.3[a]	7	2.0[a]
(range)	(6-7)	(2-6)		(2)
Mean number of fry/spawn/kg BW	13734 [a]	12796 [a]	21	7.3 [b]
(range)	(12657-14811)	(5189-25956)		(0-42)

Different superscript letters indicate significant differences ($P<0.05$) within a row of means.
*females treated with saline were not included in the statistical analysis

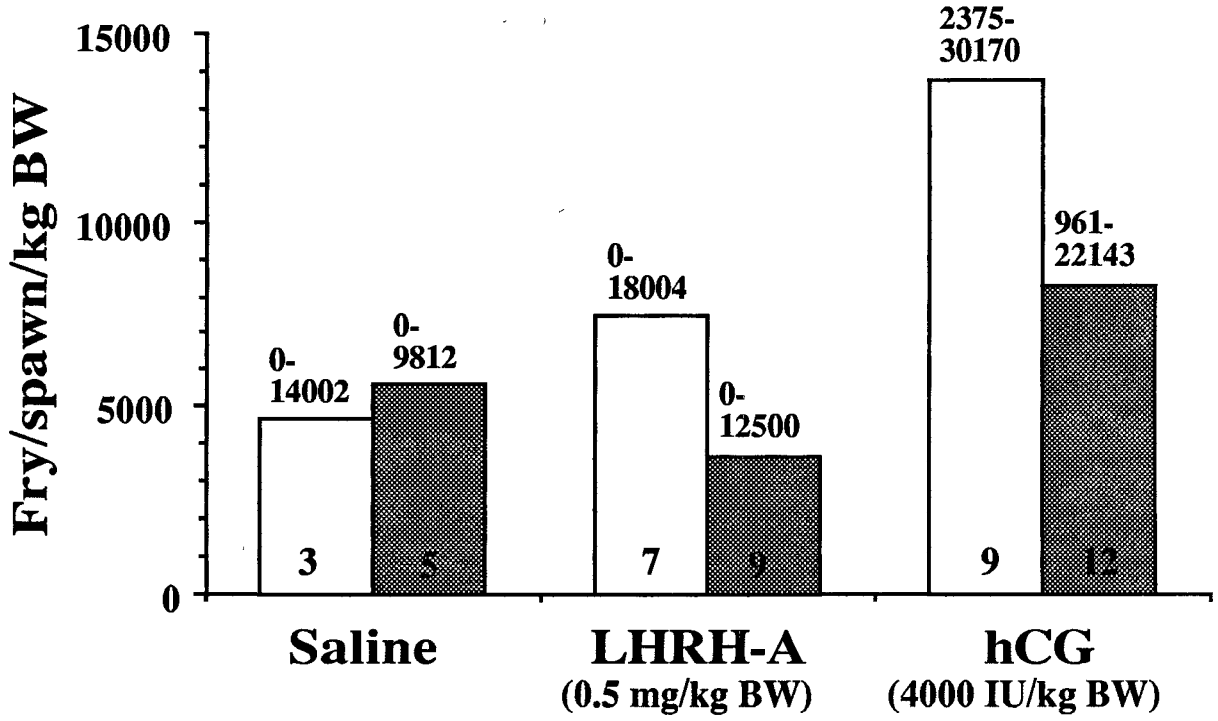

Figure 1.-Mean total number of fry/spawn/kg BW produced by unacclimated (open bars) and acclimated (solid bars) largemouth bass pairs receiving injections of saline, LHRH-A, or hCG. Number of spawns are reported at the bottom of each bar. Numbers at top of bars indicate range in fry production.

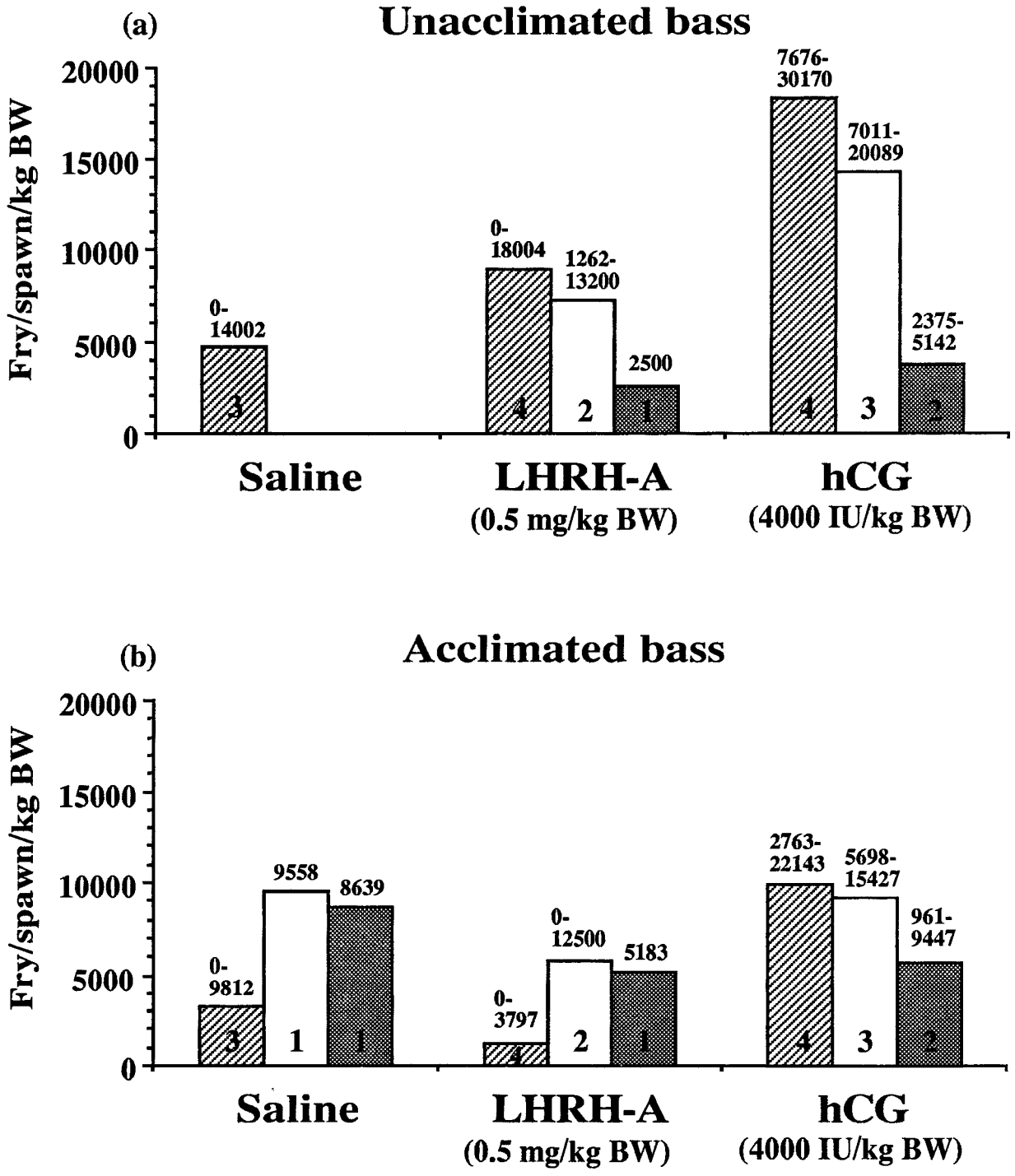

Figure 2.-Mean number of fry/spawn/kg BW as a function of spawns in unacclimated (a) and acclimated (b) largemouth bass receiving injections of saline, LHRH-A, or hCG. Number of spawns are reported at the bottom of each bar. Numbers at top of bars indicate range in fry production. Diagonal bars = first spawn; open bars = second spawn; solid bars = third spawn.

Discussion

The present experiments have shown that largemouth bass can successfully spawn under raceway conditions and that hormone treatments can be applied for the control of spawning in this species. Bass in ponds spawned within 2 d of pairing, whereas unacclimated bass in raceways required 7 d to initiate spawning. The longer spawning latency in the raceway is therefore probably not due to handling stress alone, but also involves stress due to confinement in the novel raceway environment. Carmichael et al. (1984) found that glucose levels were significantly higher in largemouth bass maintained in raceways than bass maintained in ponds, suggesting increased levels of stress in these fish. Stress due to confinement can induce negative effects on reproduction of some fishes. Oogenesis and ovulation are impaired when striped mullet, Mugil cephalus, are maintained in ponds (Abraham and Blanc 1966). Captivity of pike, Esox lucius, reduced the proportion of fish induced to ovulate with exogenous gonadotropin (GtH) and caused atresia of follicles (de Montalembert et al. 1978).

Raceway acclimated bass had shorter spawning latencies than unacclimated bass, suggesting that the acclimation period allows time for stress to diminish prior to pairing and leads to earlier spawning. This is further substantiated by the inverse relationship between acclimation time and spawning latency that was observed. The longer the bass were allowed to acclimate to raceway conditions, the shorter the latency to spawn after pairing. In addition, the total number of spawns was greater in acclimated than in unacclimated bass. This can be attributed to the longer period of time available for spawning due to shorter spawning latencies in acclimated bass. However, once spawning began in each treatment the number of spawns produced per day was not different between acclimated and unacclimated bass. The development of a higher degree of syncronization in males and females may also play a role in better spawning performance of acclimated bass. Behavioral and chemical stimuli may allow acclimated bass to spawn quicker. For example, before pairing, acclimated males had established territories and

begun sweeping behaviors. Additionally, some teleosts use pheromones as chemical stimuli to attract mates or to cue spawning activity (Liley and Stacey 1983). If bass produce pheromones, it is possible that acclimated bass performed better due to exposure to pheromones produced by potential mates.

In both unacclimated and acclimated fish, hCG was the most effective hormone treatment, inducing spawning faster and producing more spawns and fry than saline controls or LHRH-A treatment. Human chorionic gonadotropin acts directly at the gonads to induce ovulation and spermiation and apparently overrides the inhibitory effects of stress. These results are similar to those previously reported where hCG, at the same dosage used here, induced ovulation in female largemouth bass maintained in 100 or 110 L aquaria (Stevens 1970) , and induced ovulation and increased spermiation in largemouth bass maintained in 680 L water troughs (Wilbur and Langford 1975). Thus, it appears that hCG is an effective spawning inducer for largemouth bass under a variety of holding conditions. However, the dosage required to induce spawning in largemouth bass is considerably higher than in other species (Smitherman and Hester 1962; Stevens 1966).

The other hormone treatment used in this study, LHRH-A, acts through the pituitary gland to release endogenous GtH. It was not as effective as hCG in inducing spawning; this suggests that stress may be acting to inhibit LHRH-A-induced pituitary GtH secretion. Pituitary secretion of GtH is controlled by several hormones. Hypothalamic gonadotropin-releasing hormone (GnRH) stimulates and gonadotropin releasing-inhibitory factor (GRIF) inhibits secretion of GtH (Peter 1983). Dopamine (DA) inhibits basal and GnRH-stimulated GtH secretion in goldfish (Chang et al. 1984; Peter et al. 1986,1990) and has been implicated as a GRIF in a variety of other teleosts including, Chinese loach (Lin et al. 1986), coho salmon (Van Der Kraak et al. 1986) and rainbow and redtail sharks (Shireman and Gildea 1989). Norepinephrine has been shown to increase GtH secretion from perfused pituitary fragments in goldfish (Chang et al. 1984)

and, epinephrine and possibly norepinephrine have stimulatory effects on GnRH neurons (Peter et al. 1990). Intraperitoneal injections of epinephrine increased GtH levels in goldfish serum (Peter et al. 1986). In addition, research on the effects of corticosteroids on reproductive function in teleosts suggests cortisol is a normal component of the reproductive cycle and has been used to induce ovulation in several teleost species (for review see Donaldson and Hunter 1983). Responses to stress include changes in several of these factors that could interfere with pituitary GtH secretion. Immediate responses to stress include an increase in catecholamines, primarily epinephrine and norepinephrine (Mazeaud et al. 1977; Mazeaud and Mazeaud 1981). Carmichael et al. (1984) noted a peak in corticosteroids in largemouth bass 9 days after release from net confinement and Donaldson (1981) reported increased cortisol levels in various salmonids in response to stressors such as handling, anesthesia, net handling, and confinement. Hypothalamic concentrations of DA significantly decreased in response to five events of struggling in hypoxia for 5 minutes in rainbow trout, Oncorhyncus mykiss, suggesting an increased secretion of DA under these stressful conditions (Mazeaud and Mazeaud 1981). It is not clear how increased corticosteroid levels would affect spawning performance but, increases in epinephrine and norepinephrine due to stress could potentially elevate endogenous GnRH-induced pituitary GtH secretion. However, hypothalamic release of DA, as a stress response, could block both endogenous GnRH-induced and LHRH-A-induced GtH secretion. Thus, it appears that the inhibitory effects of DA could be magnified during stress and that DA is the logical factor acting as a GRIF in largemouth bass. This is further substantiated by the finding that acclimated bass treated with Ovaprim (0.5 ml/ kg BW; a commercially available treatment combining a sGnRH agonist and the dopaminergic antagonist domperidone) spawned quicker and produced more spawns than LHRH-A alone (Table 1; Chapter III). These results suggest that dopamine may be a GRIF in largemouth bass and may be responsible for poorer spawning success of LHRH-A treated bass in raceways.

Trends from multiple-spawning pairs suggest that most fry production occurs with the first spawn and declines with subsequent spawns. This is the same trend reported by Simco et al. (1986) for largemouth bass. Stevens (1970) and Wilbur and Langford (1975) both found lower hatching success of second or third ovulations induced by repeated hCG injections in largemouth bass. In those experiments, eggs were stripped and fertilized to test for viability. In the present experiment, pairs received only one injection and were allowed to spawn naturally. An attempt was made to determine the number of eggs/spawn but the three-dimensional structure and complexity of the Spawn-Tex mats prevented accurate estimates from being made, thus percent hatching values could not be derived. However, the decreased fry production in second and third spawns suggests that, as a practical matter, fish should be allowed to recover after their initial spawn, prior to inducing additional spawns.

Although large numbers of eggs were present, little or no fry production resulted from pairs in which only females received an hCG injection. This may be attributed to overripeness of ova in these females. Largemouth bass eggs have been reported to remain viable for up to 16 h after ovulation (Stevens 1970; Wilbur and Langford 1975). In Experiment 2, when both sexes were treated with hCG, spawns were observed 24 h after injection indicating that ovulation occurred within 24 h after treatment. When only females were treated with hCG, spawning did not occur until 48 h after injection. Assuming that ovulation again occurred within the first 24 h, the delay of spawning for an additional 24 h might have resulted in inviable ova. A second explanation involves a lack of milt production in untreated males. Pairs acclimated for 2 weeks spawned after 4 d and 1-week acclimated pairs receiving saline injections had a mean spawning latency of 6.5 d. Thus, the untreated males in the present experiment may not have reached the full spermiation stage, whereas the hCG treated females were induced to ovulate after 2 d. Production of relatively large numbers of fry in pairs receiving hCG and saline injections indicates that syncronization of reproductive cycles was achieved in these groups.

In conclusion, controlled spawning of bass during the normal spawning season can be achieved through raceway acclimation of bass for 1-2 weeks, injecting males and females with 4,000 IU/kg BW hCG, and providing Spawn-Tex spawning mats. Longer acclimation periods are not warranted and may be prohibitive in most hatchery situations. LHRH-A injections alone were not as effective as hCG injections. The use of a dopamine inhibitor, such as pimozide or domperidone, in addition to LHRH-A might be an effective spawning inducer in largemouth bass.

CHAPTER III

ADDITIONAL EXPERIMENTS

Methods

1989 Experiments.-Experiments were conducted in indoor concrete raceways (24 X 2.4 X 0.75 m deep) at the A.E. Wood State Fish Hatchery (San Marcos, Texas) using year class-4 Marion largemouth bass (mean weight = 1300 g; mean total length = 400 mm). Marion bass are a domestic subspecies intergrade (M. s. salmoides X M. s. floridanus) stock originally produced at the Marion National Fish Hatchery, Marion, Alabama. This study used Marion bass produced at the NFHTC. Prior to the experiments, Marion bass were held in 0.1-acre earthen ponds at ambient temperature and photoperiod at the NFHTC until transferred to the A.E. Wood State Fish Hatchery on 16 May. At the state hatchery, Marion bass were maintained in one raceway and fed a commercial pelleted salmon feed (Biodiet) until needed for experiments. Raceways were supplied with San Marcos River water throughout the experiments.

On 16 May, 10 pairs of Marion bass were stocked into a raceway supplied with three wooden spawning boxes (0.75 m^2 X 150 mm deep) with rocks (25 to 100 mm diameter), three artificial turf mats (0.75 m^2), and three Spawn-Tex mats (0.75 m^2). Observations for spawning activity were made throughout the day for 14 d. Water temperature ranged from 23 to 27º C.

On 9 June, each of 24 pairs of Marion bass was placed in a 1 X 2 m spawning pen within a 0.75 m deep raceway; water temperature at this time was 25º C. Each pair (n=8 pairs/treatment) was injected with either 0.6% saline (control; 1.0 ml/kg BW), LHRH-A (0.5 mg/kg BW), or hCG (4,000 IU/kg BW). Spawn-Tex spawning mats were placed in each pen. Fish were reinjected 48 h later; water temperature was 24º C at this time. Observations for spawning activity were made for 48 h after each injection.

On 25 June, 2 male and three female Marion bass were placed in a raceway supplied with 2 Spawn-Tex spawning mats. Observations for spawning were made for 7 d; water temperatures were not recorded during this experiment.

On 8 July, two male and three female Marion bass were placed on each side of a divided raceway. Each side was supplied with three Spawn-Tex mats and one gravel pan (0.3 m diameter X 75 mm deep). Observations were made for 10 days.

1990 Experiments.-On 12 April, Florida largemouth bass that were used in the acclimation experiment (Chapter II) were stocked in a divided outdoor raceway at the NFHTC. Fifteen males (mean weight = 320 g) and 15 females (mean weight = 396 g) were separated and allowed to recuperate from the previous experiment for 12 d. Pairs were then randomly distributed to one-third sections of five trisected raceways (three pairs/raceway). Pairs in each raceway received one of the following treatments (n=3 pairs/treatment): 0.6% saline (1.0 ml/kg BW), hCG (4,000 IU/ kg BW), [D-Ala^{6}Pro^9-N-ethylamide]-LHRH (0.1 mg/ kg BW), sGnRH (Peninsula Labs, San Diego, California; 0.1 mg/ kg BW), or Ovaprim (Syndel Labs, Vancouver, British Columbia; 0.5 ml/ kg BW). Observations for spawning were conducted twice daily. Twenty-four hours after spawns were observed, mats were removed from the raceway in a metal tub and placed in 240 X 56 X 15 cm deep fiberglass trough supplied with 21º C well water at a flow rate of 1 gal/min. After 7 d of observations, all bass were returned to the divided raceway and separated by sex. Following a 10-day recovery period, animals were again assigned to random male/female pairs, and the experiment repeated.

On 25 May, 15 male (mean weight = 288 g) and 15 female (mean weight = 283 g) bass were seined from separate 0.1 acre ponds and randomly assigned to partitioned raceways (three pairs/raceway). Each pair (n = 5 pairs/treatment) was injected with either 0.6% saline (1.0 ml/kg BW), hCG (4,000 IU/ kg BW), or LHRH-A (0.1 mg/ kg BW). Bass were not allowed acclimate to raceway conditions before the experiment.

Observations for spawning activity were conducted for 7 days. Spawns were handled as in the preceding experiment.

Results

1989 Experiments

Although territorial and sweeping behaviors in males were observed, no spawns occurred during the first experiment. No spawns occurred after the first or second hormone injection during the course of the second experiment. One spawn occurred after 7 d in the third experiment resulting in about 1,000 swim up fry. No spawns occurred in the final experiment.

1990 Experiments

Data from the two sets of injections were pooled and are presented in Table 1. Hormone treatment appeared to affect spawning latency, total number of spawns produced, and the proportion of pairs spawning. Mean spawning latency was shortest in sGnRH treated pairs followed by hCG and Ovaprim treated pairs. LHRH-A had the longest mean spawning latency of any treatment. First spawns occurred within 2.5 d after injection with hCG, Ovaprim, and sGnRH, within 2 d with saline, and within 3.5 d with LHRH-A. Pairs treated with hCG produced the most spawns followed by saline and Ovaprim treated pairs. LHRH-A and sGnRH induced the fewest number of spawns. Additionally, hCG treatment induced spawning in five of the six pairs, while saline and Ovaprim injections resulted in four out of six pairs spawning. Three of the six pairs spawned in each of the LHRH-A and sGnRH treatments. Attempts were made to quantify the number of fry produced from each spawn, however, out of the 23 spawns produced, 10 produced no fry and four produced less than 200 fry. The remaining spawns produced between 1,000 and 2,000 fry. In the final experiment, two spawns were produced by unacclimated bass treated with hCG. Both spawns occurred 7 d after treatment. The first spawn produced 20 fry and the second spawn produced nine fry. No spawns occurred in the other treatment groups.

Table 1. Effects of hormone injections on mean spawning latency, total number of spawns after 7 days, and proportion of largemouth bass pairs spawning.

Treatment	Mean spawning latency	No. spawns after 7 days	Proportion of pairs spawning
saline	4.0	5	4/6
hCG	3.0	7	5/6
sGnRH	2.0	3	3/6
Ovaprim	3.5	5	4/6
LHRH-A	7.5	3	3/6

Discussion

The lack of spawning in the 1989 experiments may be due to a combination of possible factors. The peak spawning season for largemouth bass in central Texas is from late March to the end of April; water temperature during this period ranges from 17 to 22°C (Simco et al. 1984). While in holding ponds at the NFHTC, Marion bass were exposed to water temperatures greater than 22°C for 19 d and as high as 26°C before being transferred to the state hatchery raceways. Water temperature during the experiments ranged from 23 to 27°C. Exposure for an extended period of time to temperatures above the normal range for spawning may have led to gonadal regression. However, one spawn did occur, which suggests that gonadal regression may have not been complete and that gonadal recrudescence may have occurred during the time Marion bass spent in raceways. Gonadal condition was not examined until one female died near the end of the study; ovaries were ripe. Stress associated with capture, handling, transport, and captivity in a novel raceway environment may have also contributed to the poor spawning success.

In the 1989 hormone injection experiment, treatments did not induce spawning. Several factors may be responsible for these results. Confinement in very small pens may have been a stressor in addition to the aforementioned stressors. Treatment with hCG induced spawns 24 h after injection in acclimated Florida largemouth bass (Table 3).

The results from the 1990 hormone injection experiments indicate that hCG, sGnRH, and Ovaprim can be used to improve spawning success of bass. Injections of hCG proved to be the most successful, inducing more spawns in more pairs than other hormone treatments. Only sGnRH treated bass had a shorter spawning latency. Statistical comparisons of fry production between hormone treatments were not possible due to the limited number of spawns producing fry. Several factors may be responsible. Spawns were exposed to air during the transfer from the metal tub to the hatching trough. Some spawns may have been produced from bass that had spawned a number of times

during previous experiments. Second and third spawns are generally smaller than the first spawn produced by largemouth bass (Figure 2; Stevens 1970; Wilbur and Langford 1975).

Conclusions drawn from this experiment should be taken with caution due to the nature of the experimental design. The limited number of bass did not allow us to use unspawned bass in each experiment and due to the between experiment mixing it is not possible to determine how prior treatment affected subsequent spawning performance. However, this data suggests that hormonal treatments can be used to advance spawning in largemouth bass.

Unacclimated bass did not respond well to hormone injections. Stress induced by confinement in a novel raceway environment may have contributed to the limited spawning success of hCG and LHRH-A treated animals. An additional factor may have been the late starting date of this experiment; peak spawning occurs until the end of April in central Texas whereas this experiment began in late May. Bass may have undergone partial gonadal regression.

CHAPTER IV

SUMMARY AND RECOMMENDATIONS

Acclimation

Acclimation periods provide time for bass to adjust to raceway conditions and to alleviate stress associated with fish culture techniques, such as capture, handling, anesthesia, injections, and confinement. Acclimation periods longer than 2 weeks do not provide significant returns for the amount of time invested. Acclimation of largemouth bass to raceway conditions for 1-2 weeks offers adequate control of spawning and may allow for more productive spawning. Experiments investigating the effects of stress on the reproductive endocrinology of fish would provide valuable information on factors controlling reproduction in captive fishes.

Hormone Treatment

Human chorionic gonadotropin (4,000 IU/kg BW) was the most successful spawning inducing agent used in this study. Coupled with acclimation, it induced spawning consistently within 48 h in a high percentage (~80%) of treated pairs. Spawns were not adversely affected by the use of hCG, i.e., large numbers of healthy fry were produced. However, relatively large dosages are required to induce spawning in largemouth bass.

Other hormone treatments need to be more thoroughly tested before practical use can be recommended. The success of GnRH agonists (sGnRH and LHRH-A) to induce spawning may be limited by a gonadotropin releasing-inhibitory factor. However, GnRH agonists combined with dopaminergic antagonists (Ovaprim, LHRH-A + domperidone, and other formulations) may prove to be as effective as hCG at a lower cost. The cost of hCG in this study was $6.00/kg BW due to the high dosage required to induce spawning. The cost of a co-treatment of LHRH-A (0.1 mg/kg BW) and domperidone (1mg/kg BW) would be $2.02/kg BW, while the cost of Ovaprim (0.5ml/kg BW) was only 82¢/kg BW in this study. The spawning success of largemouth bass treated with different dosages of

each component and different combinations of GnRH agonists and dopaminergic antagonists should be investigated to provide an alternative to the use of hCG.

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