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Efficacy of cGnRH IIa to Induce Spawning in Female Channel Catfish *Ictalurus punctatus*

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The use of hormones to induce spawning in fish is critical to the success of many fisheries and aquaculture programs. A wide variety of programs are dependent upon hormone treatment to complete final gamete maturation and ensure successful spawning. Spawning is a stressful period for all fish species. The handling required during the spawning of fish for artificial propagation complicates an already delicate situation. During normal spawning operations at a hatchery, it may be necessary to handle and examine individual fish weekly over a 6 – 8-week period. Such procedures can be extremely stressful to valuable broodstock which can compromise general fish health. Successful hormone treatment can reduce handling requirements thereby reducing the stress load related to overall fish handling.

Final gamete maturation in fish can be induced by the administration of a variety of hormones, which act on the hypothalamus-pituitary-gonadal (HPG) axis (Donaldson and Hunter 1983; Goetz 1983). Investigations have found synthetic analogues of gonadotropin releasing hormones (GnRHa) to be a very effective means of inducing final gamete maturation. Chicken gonadotropin releasing hormone analog 2 (cGnRH IIa) is a newer hormone option that has shown promise for spawning female Channel Catfish well as several other species (Quiniou et al. 2014; Sipos et al. 2019; Sipos et al. 2020; Broach & Ohs 2020; Quinou & Bosworth 2020). The U.S. Catfish industry has a growing need for induced spawning as the industry has continued to expand production of Hybrid Catfish (female Channel Catfish *Ictalurus punctatus* × male Blue Catfish *Ictalurus furcatus*). In female Channel Catfish (CCF), cGnRH IIa produced a greater percent ovulation than other common spawning hormones such as mammalian (mGnRH Ia) and salmon gonadotropin releasing hormone (sGnRH IIa) (Quiniou et al. 2014).

In this bulletin, we summarized the results of two pivotal efficacy studies conducted at active commercial catfish farms to demonstrate the effectiveness of cGnRH IIa to induce spawning in female Channel Catfish (CCF) *Ictalurus punctatus*.

Methods

Two studies (referred to as Study 1 and Study 2) were conducted during the hybrid catfish spawning season (May 25 - June 7, 2022) at two commercial fish farms located in Mississippi. In each study, test fish consisted of 40 mature female CCF broodstock, an additional 10 female CCF were collected from the same pond for fish health assessments, and an additional 5 male Blue Catfish (*Ictalurus furcatus*) were used to collect sperm for fertilization. Test fish were collected from a broodstock pond located at the test facility.

Each study consisted of a single-blinded, unbalanced design with 30 fish receiving the spawning hormone and 10 fish receiving a negative control treatment (sterile physiological saline). The following methods were the same for each study except where explicitly noted.

To establish the health of the overall broodstock population, a fish health assessment was conducted on 10 ready-to-spawn females (i.e., a soft, full abdomen (indicative of large, well-developed ovaries) and presence of a red, swollen, slit shaped vent) randomly sampled from the hauling truck to ensure there were no underlying health concerns. Of these 10 fish, 7 were subjected to non-lethal external assessments, including general external evaluations (checking for ulcerations, nodules, gill parasites, etc), and microscopic examination of wet mounts of gill tissue, skin scrapes, and scrapes of any lesions. The other 3 fish sampled were subjected to a full necropsy, which included visually examining internal organs, culturing posterior kidney on Bovine Serum Albumin (BSA) media, as well as the external examinations listed above.

Enrollment of female ready-to-spawn CCF into the study followed standard procedures used by commercial Catfish farms. Fish were collected from the broodstock pond, briefly checked for readiness, then loaded into a hauling truck for transfer to the hatchery where individual fish were systematically checked for readiness and allocated to individual mesh bags within a single holding tank. The initial screening of fish spawning readiness was conducted pond side by the farm manager. This initial sorting was based predominately on abdominal swelling indicative of ready-to-spawn females and the experienced judgment of a commercial Catfish farmer.

To evaluate the efficacy of cGnRH IIa as a spawning aid for use in female Channel Catfish, cGnRH IIa was administered via an intraperitoneal (IP) injection at a 20 µg cGnRH IIa/kg fish body weight (bw) Priming Dose followed approximately 16 h later by an 80 µg cGnRH IIa/kg fish bw Resolving Dose. The hormone was mixed so that each fish received an injection of 1ml/kg body weight. In each study, 40 fish were randomly assigned as either treated (30 fish) or control (10 fish), with control fish receiving sterile physiological saline. Mortalities were checked daily either during injection or when fish were checked to see if spawning had occurred, and general fish health observations were recorded. General fish behavior was monitored and recorded while the fish were in the spawning bags. Water temperature, dissolved oxygen concentration, hardness, alkalinity, and pH were collected daily throughout the study.

Fish were first checked for spawning approximately 24 hrs after receiving the resolving dose. Spawning checks were performed approximately every 3 hours. Fish which were ready to spawn were sedated with approximately 250 mg/L MS-222 prior to being strip spawned. For each fish stripped, the time, volume of eggs, egg color, bloodiness of the spawn, and the amount of clumping was recorded. After the fish were spawned, they were placed in a raceway to recover and were monitored for 24 hours. The fish that did not spawn during the study (both treated and control) were monitored for survival throughout the spawning period.

Collected eggs were fertilized and consolidated into large hatching jars. The hatching jars in Study 1 contained spawns from 7 to 9 fish. In Study 2 spawns from all 16 fish were consolidated into a single jar. The hatching jars were 93.5 L (24.7 gal) and were gravity fed water at a rate of 37.9 L/min (10 gal/min) from a large header tank. As the eggs grew closer to hatching, the flow rate was reduced to 19-23 L/min (5-6 gal/min). In Study 1, the inflow water was treated with a calcium reactor because the well water was too soft (less than 1 grain or 17 mg/L CaCO3) to allow for proper embryo development. Embryos were left to develop for 11 days (Study 1) or 7 days (Study 2) before the number of fry was enumerated by weight. The number of fry from each jar was then used to calculate hatching rate.

The cGnRH IIa treatment was determined to be successful to induce ovulation of female Channel Catfish if the treatment met two *a priori* conditions. The first was a significant difference in ovulation rate between the treatment and control groups. The second was the lower end of 95% confidence interval of the ovulation rate of the cGnRH IIa treated group was greater than 60%. In Study 1, this was determined using a logistic regression model (glm function, data family = binomial) to model the binomial of whether each fish produced a successful ovulation (produced > 100 mL of eggs when stripped). In Study 2, The typical glm function was not appropriate due to the data demonstrating complete separation of variables, which caused convergence issues and nonsensical outputs. Therefore, a Frith's bias-reduced logistic regression method was used (R function logistf, Frith 1993, Cornell Statistical Consulting Unit 2022, Heinze et al. 2023). For each study, the treatment effect was considered significant if it had a $P < 0.5$. The emmeans function (Lenth 2023) was used to determine the 95% confidence intervals of the probability of successful ovulation of each treatment group based on the model. The same analysis (logistic regression) was used to determine if mortality rates were significantly affected by the hormone treatment. All statistical analyses were conducted in Program R (RStudio, V 2021.09.1, RStudio: Integrated Development for R. RStudio, Inc., Boston, MA).

Results

Results of Study 1 - No mortalities were reported while the test fish (mean length, 60.3 cm; mean weight, 3.1 kg) were in the bags during the injecting and spawning procedures. All behavior was classified as normal despite the restriction of movement while fish were in the spawning bags. There were no abnormal findings during the general fish health observations and only three fish had ulcers on their tails. All spawned fish survived for 24 hours post spawn before being released back into a broodstock pond.

Water quality measurements throughout the study were within normal ranges for rearing healthy Catfish. Test fish were collected from a pond (12 ac, 48 ac-ft) located at the test facility with a mean temperature and DO of 25°C and 5.3 mg/L, respectively. Mean water temperature and DO was 26°C and 7.8 mg/L, respectively in the spawning tank. Water hardness and alkalinity in the spawning tank was 128 mg/L and 222 mg/L, respectively, and the pH was 8.2.

The hatching jars had predominately normal water quality conditions when measured. A calcium pump failure in the middle of the night during incubation caused an extreme drop in hardness and alkalinity to less than one grain (17 mg/L) for approximately 5 hours. Throughout the rest of embryo incubation, the mean temperature was 25.6 °C. The mean hardness and alkalinity when the calcium pump was working normally was 137 mg/L and 222 mg/L, respectively. Mean pH was 8.3. The DO was not measured in the incubation jars.

Overall, 96.7% (29/30) of females treated with cGnRH IIa successfully ovulated producing a mean of 473 mL eggs/spawn. Mean time to ovulation was 35 h after administration of the resolving dose. Of the control fish, 11% (1/9) fish successfully ovulated. The one control fish which did ovulate was accidently injected with the resolving dose and removed from the analysis. Based on the logistic regression model, there was a significant difference in ovulation success between the two treatment groups ($P< 0.001$, $Z=3.71$), and the lower end of the 95% confidence interval was greater than 60% (79.8%).

Based on these results, the study demonstrated the efficacy of the cGnRH IIa treatment based on the *a priori* criteria established in the study protocol. The secondary egg quality indices, egg color, blood presence, and egg clumping were assessed at the time of spawning and indicated normal to high quality spawns. All 31 spawns (29 treated, 1 control, and 1 control that accidentally received a resolving dose) were fertilized and pooled into four incubation jars as they spawned. Mean ± SD hatching rate was 23.8 ± 11.9% and ranged from 13.4% to 40.9% which was within the 20% target set in the protocol. All spawning data has been summarized in Table 1.

Results of Study 2 - There were multiple mortalities throughout the course of the study. In total, 15 of the 40 fish died during the study and another two fish were excluded (one male and one escaped). This decreased the sample size by 43%, resulting in seven control fish and 16 treated fish surviving through ovulation (23 total fish finished the study). During the pre-treatment fish health checks, six out of ten fish were noted to have digenean trematode metacercaria encysted on the body surface. These digeneans were presumed to be *Bolbophorus damnificus* due to it being relatively common in the area. Additionally, three of ten fish were noted to have signs of Proliferative Gill Disease. Post-spawning mortalities were high, 14 of 18 fish in the recovery tank did not survive 24 hours post spawning.

During the injection and spawning portion of the study, 30% of control fish died and 41.4% of treated fish died. There was no significant influence of treatment on the mortality rates ($z = 1.228$, $P = 0.478$). For this reason, it is likely that the high mortality rate was due to the overall poor health of the reference population and was not negatively influenced by the use of cGnRH IIa.

Water quality measurements throughout the study were within normal ranges for rearing healthy Catfish. Test fish were collected from a pond (10 ac) located at the test facility with a mean temperature and DO of 24 °C and 6.6 mg/L, respectively. Mean water temperature and DO was 26°C and 6.4 mg/L, respectively in the spawning tank. Water hardness and alkalinity in the spawning tank was less than 1 grain (<17.1 mg/L CaCO3) and 333.5 mg/L CaCO3, respectively, and the pH was 8.25. The hatching jar had normal water quality conditions when measured. The temperature was 26.1°C (78.9°F). The hardness and alkalinity were 51 mg/L CaCO3 and 308 mg/L CaCO3, respectively, and pH was 8.0.

Overall, 100% (16/16) of the surviving females treated with cGnRH IIa successfully ovulated producing a mean of 300 mL eggs/spawn. Mean time to ovulation was 31 h after administration of the resolving dose. Of the control fish, 2 of 7 fish (28.6%) released eggs. However, these spawns were deemed too poor of quality (eggs described as “hard”) to be fertilized and were not counted as successful ovulations. Based on the Frith’s logistic regression model, there was a significant difference in ovulation success between the two treatment groups ($P<0.001$, $X^2=15.1$), and the lower end of the 95% confidence interval was greater than 60% (62.7%).

Based on these results, the study demonstrated the efficacy of the cGnRH IIa treatment based on the *a priori* criteria established in the study protocol. The secondary egg quality indices (egg color, blood presence, and egg clumping) were assessed at the time of spawning. Overall, the indices indicated normal to high quality spawns in the cGnRH IIa treated fish. All 16 spawns were fertilized and pooled into one incubation jar. Mean hatching rate was estimated at 20% based on hatchery records for that season. This hatching rate was in range with the 20% target set in the protocol. All spawning data has been summarized in Table 1.

Discussion

Both studies conducted demonstrated the efficacy of cGnRH IIa, administered at 100 µg/kg body weight, consisting of priming (20 µg/kg) and resolving (80 µg/kg) doses, to induce ovulation of female CCF, even despite the mortality events which occurred. The high ovulation rates (> 95% in both studies) in the treated fish met both criteria for success that were laid out in the protocol. The ovulation rates observed in these studies were similar to the rate of 89-100% reported by Quiniou and Bosworth (2020) after using the same dose rate over three time points during the spawning season. A similar ovulation rate of 90.2% was also observed by Quiniou et al. (2014) using the same dose rate. For these reasons, we concluded that cGnRH IIa is effective at inducing ovulation in female Channel Catfish.

Final Study Reports were submitted to the FDA Center for Veterinary Medicine for review. Study 1 was accepted and will help fulfill the evidence of effectiveness requirements for the proposed indication in female Ictalurids. Study 2 was not accepted due to the pre-existing disease found in the reference population and some of the fish tested in the study.

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References

Broach, J. S., & Ohs, C. L. 2020. Efficacy of varying doses of cGnRH IIa on spawning performance of Pinfish and Pigfish. Aquaculture Research, 51(11), 4541-4550.

Cornell Statistical Consulting Unit. 2022. Separation and Convergence Issues in Logistic Regression. https://cscu.cornell.edu/wp-content/uploads/lgsbias.pdf

Donaldson, E.M. and G.A. Hunter. 1983. Induced final maturation, ovulation, and spermiation in cultured fish. Pages 351-403 in W.S. Hoar, D.J. Randall and E.M. Donaldson, editors. Fish physiology, volume 9, part B. Academic Press, New York.

Firth, D. 1993. Bias reduction of maximum likelihood estimates. Biometrika, 80(1), 27-38.

Goetz, F.W. 1983. Hormonal control of oocyte maturation and ovulation in fishes. Pages 117-169 in W.S. Hoar, D.J. Randall, and E.M. Donaldson, editors. Fish physiology, volume 9, part B. Academic Press, New York.

Heinze, G., Ploner, M., & Jiricka, L. 2023. Firth's Bias-Reduced Logistic Regression. R Package 'logistf' version 1.26.0. <https://CRAN.R project.org/package=logistf>

Lenth, R. 2023. emmeans: Estimated Marginal Means, aka Least-Squares Means. R package version 1.8.9, <https://CRAN.R project.org/package=emmeans>.

Quiniou, S. M., Bosworth, B., Chatakondi, N., & Oberle, D. 2014. Evaluation of a gonadotropin releasing hormone analog of cGnRH II as a spawning aid for Channel Catfish versus analogs of mGnRH I and sGnRH III. North American Journal of Aquaculture, 76(3), 281-288.

Quiniou, S. M., & Bosworth, B. G. 2020. Effects of a Gonadotropin-Releasing Hormone II a 5/20-µg/kg Dose versus a 20/80-µg/kg Dose on Channel Catfish Ovulation. North American Journal of Aquaculture, 82(3), 354-358.

R Core Team. 2023. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>.

Sipos, M. J., Lipscomb, T. N., Wood, A. L., Ramee, S. W., Watson, C. A., & DiMaggio, M. A. 2019. Evaluation of cGnRH IIa for induction spawning of two ornamental *Synodontis* species. Aquaculture, 511, 734226.

Sipos, M. J., Lipscomb, T. N., Wood, A. L., Ramee, S. W., Watson, C. A., & DiMaggio, M. A. 2020. Evaluation of cGnRH IIa for induction spawning of two ornamental *Epalzeorhynchos* species. Aquaculture Research, 51(1), 232-241.

	Study 1				Study 2			
	Treated			Control	Treated			Control
Spawn percentage	96.7% (n=29/30)			11% (n=1/9)	100% (n=16/16)			28.6% (n=2/7)
Mean time to spawn (h)	34.97 ± 7.06 (n=29)			48.73 (n=1)	31.0 ± 4.4 (n=16)			47.5 ± 0.01 (n=2)
Mean spawn volume (mL)	473.2 ± 141.7 (n=29)			500 (n=1)	300 ± 135 (n=16)			200 ± 0 (n=2)
Mean spawn volume/ kg body weight (mL/kg)	156.6 ± 38.0 (n=29)			172.4 (n=1)	135.4 ± 50.9 (n=16)			100 ± 47 (n=2)
Egg color	Bright 35.7% (n=10)	Normal 56.3% (n=5)	Pale 10.7% (n=3)	NA	Bright 31% (n=5)	Normal 56% (n=9)	Pale 13% (n=2)	Pale (n=2)
Blood in spawn	None 24.1% (n=7)	Little 69.0% (n=5)	Lots 6.9% (n=2)	NA	None 7% (n=1)	Little 93% (n=14)	Lots 0% (n=0)	Little (n=1) Lots (n=1)
Spawn clumping	Loose 68.0% (n=17)	Some 32.0% (n=8)	Clumped 0% (n=0)	NA	Loose 0% (n=0)	Some 100% (n=15)	Clumped 0% (n=0)	Clumped (n=0)
Hatch percentage	23.8 ± 11.9%			NA	Estimated at 20%			NA
Mean water temperature	26.2 ± 0.1°C (79.2 ± 0.2°F)				23.9 ± 1.5°C (75 ± 1.8°F)			

Table 1. Summary of cGnRH IIa pivotal efficacy studies conducted on female Ictalurids. In both studies the target dose tested was 100 µg/kg body weight (BW) (20 µg/kg BW followed by 80 µg/kg BW). Each Study was conducted at separate commercial catfish farms in the Mississippi Delta region.