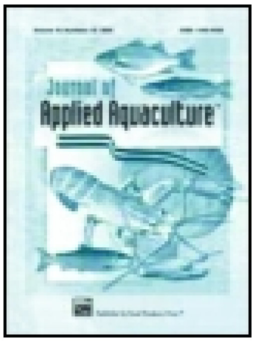

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Effect of feeding rate and hormonal treatments on the condition factor and the reproductive performance of the catfish, *Pangasianodon hypophthalmus*

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ABSTRACT

This paper aimed to evaluate the effects of feeding rate and the hormonal treatments for spawning induction on the condition factor, and the reproductive performance of the striped catfish, *Pangasianodon hypophthalmus*. A commercial diet with 35% of protein in three feeding rates – 1%, 2%, and 3% biomass, were supplied for 184 days – and two hormonal methods – hCG injections and GnRHa implants – were applied. A marked influence of the feeding rates on the monthly variations in body weight and condition factor was observed, with the highest values for fishes fed at 3% biomass. No spawning occurred in females in the 1% biomass group. The relative fecundity in both sexes, the fertilization, and the hatching rates were significantly higher in the 3% biomass group than observed in the 2% biomass group. Thus, the use of diets with 35% protein provided at 3% biomass to the breeders of *P. hypophthalmus*, is recommended.

KEYWORDS

Fertilization rate; hatching rate; relative fertility; spawning induction

Introduction

The striped catfish, *Pangasianodon hypophthalmus*, is a fast-growing fish, with a good flesh quality and an attractive appearance, and thus, it has been suitable for ornamental and human consumption purposes (Nguyen et al. 2013). Due to the abovementioned characteristics, the striped catfish has a known acceptance at international markets, reaching levels of production, distribution, and marketing like those achieved by tilapia, shrimp, and salmon (FAO 2020).

However, one of the main challenges for farming the striped catfish is achieving the final maturation of the gametes in both sexes. Despite the breeders reaching gonadic maturation in captive conditions, females are not able to spawn naturally, and the milt volume is low in males (Phan et al. 2009). Therefore, as observed in other farmed fishes, adequate feeding and diet

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composition management, combined with the use of hormonal treatments to promote the final maturation and release of the gametes have been valuable breeders management procedures for the controlled reproduction of the striped catfish (Bui et al. 2010; Nguyen et al. 2013).

Adequate feeding rates, both daily and seasonal, influences the condition factor and reproductive process of fishes. The condition factor is a good indicator of the general "well-being or fitness" of a fish population; such a parameter is based on the analysis of length-weight data and assumes that heavier fish of a given length are in better condition (Mozsár, *et al.*, 2015). The condition factor has been used in three types of analysis: (i) in comparing monospecific populations living under apparently similar, or different conditions of feeding and environmental conditions; (ii) in determining the gonad maturation; and (iii) as an indication of gross nutritional balance changes during chronic alterations in feeding activity or food supply (Bolger and Connolly 1989). In general, restricted rations have caused negative effects on the condition of fishes as stunted growth, inhibition of gonadal maturation, reduction in the fecundity, low fertilization and hatching rates as well as delay in maturation and spawning (Bobe and Labbé 2010), affecting the viability of eggs and larvae and reducing chances of survival in different species of fishes as African catfish, *Clarias gariepinus* (Adewumi 2006), Nile tilapia, *Oreochromis niloticus* (Bhujel, Little, and Hossain 2007) and Japanese amberjack, *Seriola quinqueradiata* (Higuchi et al. 2018).

It has been shown that the breeders of the striped catfish are able to consume different types of food, from those made on-farm with fish wastes, grains, and vegetables, as well as commercial diets. Indeed, these diets have been supplied to the breeders at different feeding rates, from 5.8% for diets made on–farm to 2.8% for commercial diets (Bui et al. 2010; Phuong 2013). Kabir et al. (2013) reported that breeders of *P. hypophthalmus* fed on diets with 35-40% of protein had higher growth rates, the largest diameter of oocytes, and higher percentages of mature oocytes when spawning was induced with hormonal treatments, suggesting that the required level of crude protein in diets for breeders of the striped catfish is 35%.

However, for an adequate spawning and spermiation induction, some criteria for breeder selection should be observed: for females a modal diameter of oocytes greater than 1.0 mm, and for males, motile sperm after microscope examination (Legendre, Linhart, and Billard 1996; Legendre et al. 2000). Therefore to achieve spawning and increment sperm volume, two injectable hormonal products have been widely used for species belonging to the Pangasiidae family: salmon Gonadotropin-Releasing Hormone analog with some dopamine inhibitor (sGnRHa + Domperidone) and human chorionic gonadotropin (hCG) in variable doses and application times depending on the species, sex, and the farming site, with variable results in fertilization and hatching rates (Cacot et al. 2003, 2002; Legendre et al. 2000; Slembrouck et al.

2003). However, there is no information available on the use of GnRHa implants without a dopamine inhibitor, already evaluated in several fish species with remarkable results (Duncan et al. 2003; Rodríguez-montes De Oca et al. 2012).

Consequently, this paper aimed to evaluate the dietary regimes and the reproductive performance of the striped catfish breeders, *P. hypophthalmus* by 1) estimating the effects of three feeding rates, expressed in terms of % biomass on the monthly variations of the body weight, and the condition factor of breeders of striped catfish, 2) assessing the effects of the feeding rates on some growth parameters like absolute growth, relative growth and specific rate of growth and 3) estimating the potential additive effect of the feeding rates and two types of hormones applied to induce controlling breeding on some reproductive parameters such as relative fecundity in both sexes and fertilization and hatching rates (%).

Material and methods

Breeders management, feeding treatments, and estimated parameters

Two-year-old breeders of striped catfish (n = 144) were used and consisted of 72 females (1257.86 ± 265.81 g) and 72 males (1145.56 ± 232.21 g). These fish were randomly and equitably stocked into nine fiberglass tanks of 3 m3. Each tank had 16 individuals in a sex ratio about of 1:1. Tanks had constant aeration, and exchanges of water approximately 20% were made weekly. The fish were kept under a natural temperature and photoperiod (23°12′31.7” N and −106°25′28.18” W). Previously to start the experiment, all fishes were tagged with pit tags (HDX12, Biomark ®, USA) on the right dorsal side for identification and follow-up.

Breeders were fed on a commercial diet (Purina, Nutripec ®) (Table 1). The diets were supplied two times a day (9:00 and 17:00 hours), six days a week (from Monday to Saturday), according to three groups of breeders based on 1%, 2%, and 3% biomass with three replicates for each feeding rate. The

Table 1. Bromatological composition of the commercial diet, Purina, Nutripec ®, for stiped catfish, *P. hypophthalmus*, broodstock (data provided by the manufacturer).

Parameter	Content (%)
Moisture	12
Protein	35
Lipids	7
Raw fiber	5
Ash	13
Nitrogen free extract	28

feeding period lasted 184 days (from March to August 2017). Monthly, the total length (cm) and the body weight (g) of fishes from each feeding treatment were registered to estimate the biomass and adjust the amount of supplied food. Furthermore, those data were used to estimate the condition factor (*K*) throughout the feeding period. *K* was estimated as follow:

$$K = \frac{BW}{L^3} x100$$

Where BW is the body weight (g), and L is the total length (cm).

Furthermore, in order to demonstrate that all the fish had the same average body weight at the beginning of the experiment, and how it changed throughout the feeding period, the variation in body weight was plotted according to feeding rates.

Hormonal treatments

At the end of the feeding period, from each feeding rate treatment, 12 individuals with signs of sexual maturation were chosen randomly: six females and six males (two females and two males from each replicate). Female readiness was determined after observing soft abdomen and swollen genital papilla, followed by an intra-ovarian biopsy using a cannula of 2.66 mm diameter, with a modal distribution diameter of oocytes greater than 1.0 mm. Furthermore, the initial position of the germinal vesicle was also examined following the method described by Stoeckel (2000), whereby a sample of approximately 50 oocytes from each female was cleared in Serra's fluid (60% ethanol, 30% formalin, 10% acetic acid, by volume). Male readiness was determined by the production of milt at stripping by manually applying slight pressure on the abdomen. Spawning was induced in these fishes according to the following experimental treatments: a) brooders fed at 1% biomass + hCG; b) brooders fed at 1% biomass + GnRHa; c) brooders fed at 2% biomass + hCG; d) brooders fed at 2% biomass + GnRHa; e) brooders fed at 3% biomass + hCG; f) brooders fed at 3% biomass + GnRHa.

In the treatments with hCG (Intervet ®, Holland), two doses were applied to the females: the first dose was 500 U.I. Kg-1 of body weight, and the second one, 2000 U.I. Kg^{-1} of body weight, was applied eight hours later, as described by Legendre et al. (2000); only one dose of 2000 U.I. Kg^{-1} was applied to the males during the second application to the females. In the treatment with GnRHa (Ovaplant, Syndel ®, Canada), only one implant with 150 µg of sGnRHa was applied into the dorsal muscle of every fish for this treatment, using a Ralgun implanter.

Relative fecundity in both sexes and spermatocrit in males

To evaluate the fecundity in females, 3 g of eggs from each spawning were fixed in 4% formalin solution and subsequently counted. Additionally, the total spawning from each female was weighed, and the bodyweight of each spawned female was also recorded to estimate the relative fecundity expressed as the number of eggs kg-1 of body weight. In males, a gentle stripping was applied to collect the semen within a graduated syringe without a needle. The total volume of semen was measured, and the bodyweight of each striped male was recorded to estimate the relative fecundity expressed as the volume of semen mL kg-1 of body weight.

Additionally, two samples of semen of 1 mL from each male were drawn into microhaematocrit tubes 7.5 mm in length and 1.1 mm of internal diameter and were centrifuged at 12 000 r.p.m. for five minutes to estimate the spermatocrit (%), defined as the ratio of the packed volume of cells to the total volume of semen in the tube and multiplied by 100 (Rideout, Trippel, and Litvak 2004).

Fertilization and hatching rates (%)

For the oocyte fertilization, the dry method described by Slembrouck et al. (2003) was used. First, the milt of each male from each treatment was collected in new plastic graduated syringes (no needle) both for volume estimation and avoid direct air exposure, after a gently squeezing the abdomen, then, milt was diluted in saline solution (0.9% NaCl) to a ratio of 1:4 (milt: saline solution, vol/vol) and kept at 4°C until oocyte collection. Thereafter, the spawning of each female from each treatment was obtained by gentle stripping and collected into a dry plastic bowl.

The milt of one male was used to fertilize the eggs of one female into each treatment; therefore, six batches of fertilized eggs were obtained for each treatment. The diluted milt was poured over the eggs, then mixed delicately for one minute, until the sperm was homogeneously spread on the egg mass; the freshwater was then added to activate the gametes, and they were mixed once again for one minute. Finally, the fertilized eggs were rinsed to remove the excess milt before transferring them for incubation.

To estimate the fertilization and hatching rates, the method described by Unuma et al. (2004) was used. Samples of eggs from each spawning were taken in triplicate and drawn into microplates of ELISA with 96 wells, with one egg in each well, filled with fresh water at 28°C. The fertilization rate (%) was estimated five hours after fecundation by counting the total number of embryonated eggs in each microplate, and 24 hours after fecundation, when all the eggs had hatched, the total number of larvae was counted to estimate the total hatching rate (%).

Statistical analysis

K, the relative fecundity in both sexes, the spermatocrit in males, the fertilization, and hatching rates were expressed as percentages, thus they were statistically transformed before being analyzed by the arcsine of the square root method. Kolmogorov-Smirnov test was subsequently used to verify the normality of the results.

To assess the monthly variations in body weight and *K* regarding the feeding rates, two-way ANOVA was performed. Likewise, to evaluate the effect of the feeding rates and the hormonal treatments on the relative fecundity in females, on the volume of semen and spermatocrit in males, and on the fertilization and hatching rates, two-way ANOVA was performed too. In all cases, Holm–Sidak tests were performed to verify significant differences among treatments.

All statistical analyses were made with confidence levels of $P < .05$. Both statistical analysis and graphics were performed with Sigma Plot 11.0 software for Windows.

Results

Effect of the feeding rate on the variations in body weight and condition factor (K), and growth parameters

For changes in the body weight, there were no significant differences between fishes of the different feeding rates into the first two months (March and April). However, from May, a sustained increase was observed until the end of the feeding period (August), being the fishes fed at 3% of the biomass that reached a greater body weight (2451.15 ± 279.91 g), followed by fishes fed at 2% (1903.04 ± 310.67 g) (Figure 1A).

Regarding the condition factor (*K*), no significant differences were observed between fishes at the beginning of the experiment (March and April). However, from May, different trends were observed between feeding rates. For fishes fed at 1% biomass, a significant reduction was registered monthly, starting with 0.96 ± 0.13 in March and ending with 0.64 ± 0.07 in August. For fishes fed at 2%, were significant differences between the first month and the last one (0.97 ± 0.10 and 0.87 ± 0.12, respectively), although no significant differences were registered from April to July, with average values ranging from 0.93 to 0.90. For fishes fed at 3%, a monthly sustained increase was observed, starting with 0.95 ± 0.09 in March and ending with 1.21 ± 0.17 in August (Figure 1B).

Effect of the feeding rates and hormonal treatments on the fecundity in both sexes, and the spermatocrit

In the 1% biomass group, no spawning was obtained from any female at either hormonal treatment. Thus, the statistical analysis about the effect of the

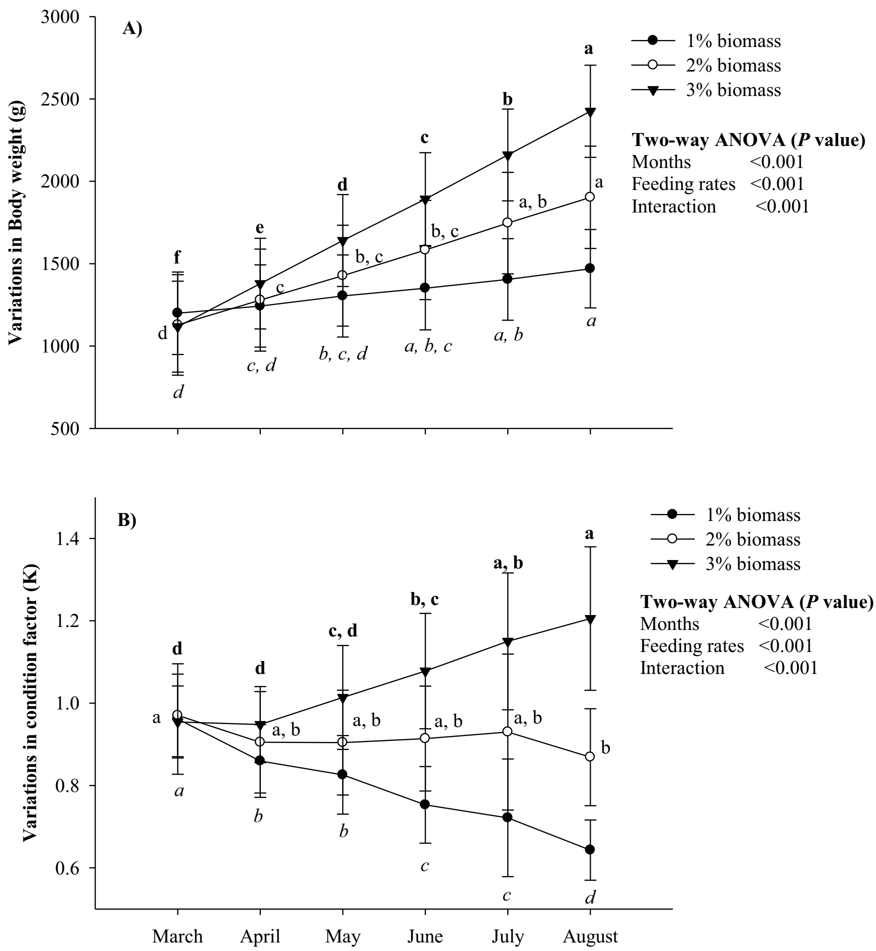

Figure 1. Monthly variations (mean ± standard deviation) in A) body weight and B) condition factor in broodstock of striped catfish, *P. hypophthalmus*, fed at different feeding rates. Different letters in the graphics, mean significant differences ($P < .05$). Italic letters for 1% feeding rate, Regular letters for 2% feeding rate, and Bold letter for 3% feeding rate.

feeding rate and the hormonal treatment on the relative fecundity in females was made only using data from the 2% and 3% biomass groups.

Significant differences ($P < .05$) were registered between feeding rates but not between hormonal treatments regarding the relative fecundity in females. The relative fecundity observed in females for the 3% biomass group was significantly higher (29.43 x 10^3 ± 6.06 x 10^3 eggs kg^{-1} for hCG and 30.43 × 10^3 ± 5.24 x 10^3 eggs kg^{-1} for GnRHa) than those in the 2% biomass group (18.81 x 10^3 ± 3.64 x 10^3 eggs kg^{-1} for hCG and 21.01 × 10^3 ± 5.57 x 10^3 eggs kg^{-1} for GnRHa) (Figure 2A).

In contrast to females, milt samples were obtained from males fed across all feeding rates groups. However, like females, significant differences ($P < .05$) were registered between feeding rate groups but not between hormonal

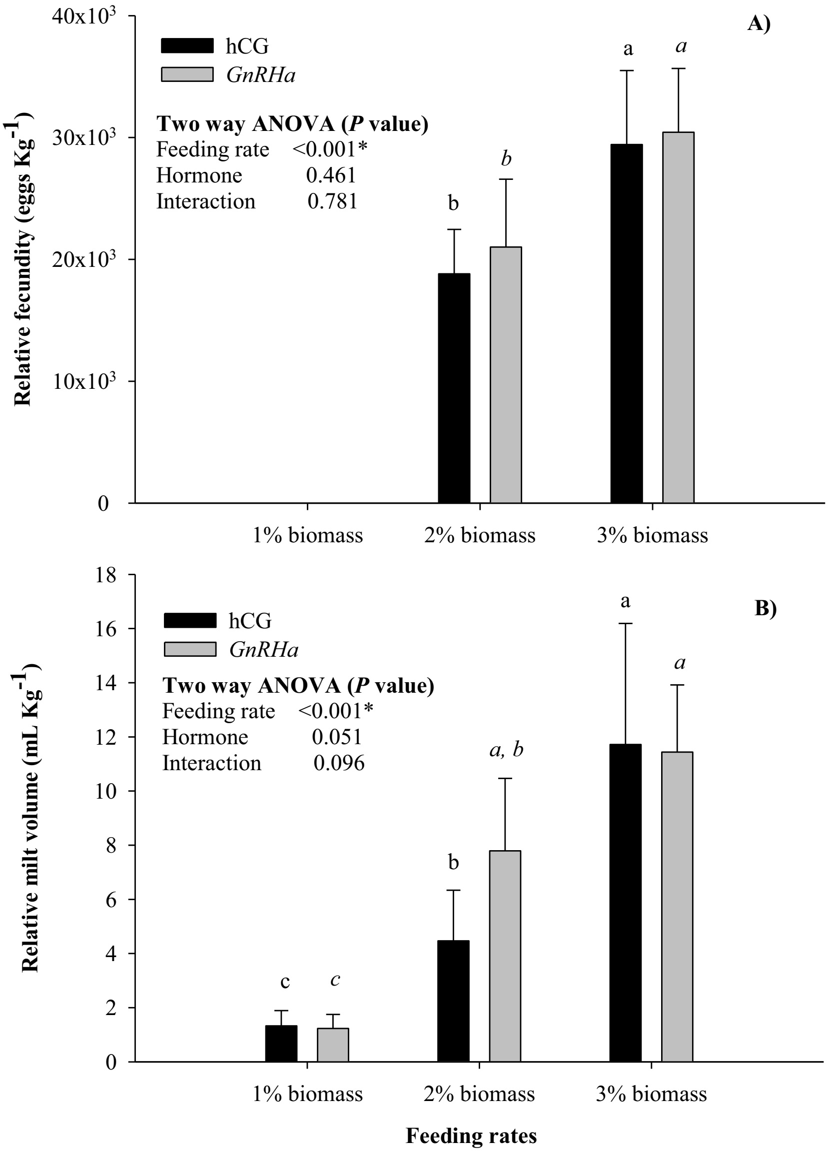

Figure 2. The mean ± standard deviation of A) the relative fecundity (eggs kg^{-1}) in females and B) relative milt volume (mL kg^{-1}) in males of striped catfish, *P. hypophthalmus*. Letters on top of the bars indicate significant differences between the treatments ($P < .05$). hCG = Human chorionic gonadotropic hormone; GnRHa = Gonadotropin releasing hormone analogue. * indicates significant differences between factors into the two-way ANOVA.

treatments regarding the relative milt volume (mL Kg^{-1}). Although significantly lower in males in the 1% biomass group (≤ 2 mL kg^{-1}) than those collected from males in 2% and 3% biomass groups, the highest relative milt volume was observed in the 3% feeding rate (11.72 ± 4.47 mL kg^{-1} for hCG and 11.44 ± 4.55 mL kg^{-1} for GnRHa) (Figure 2B).

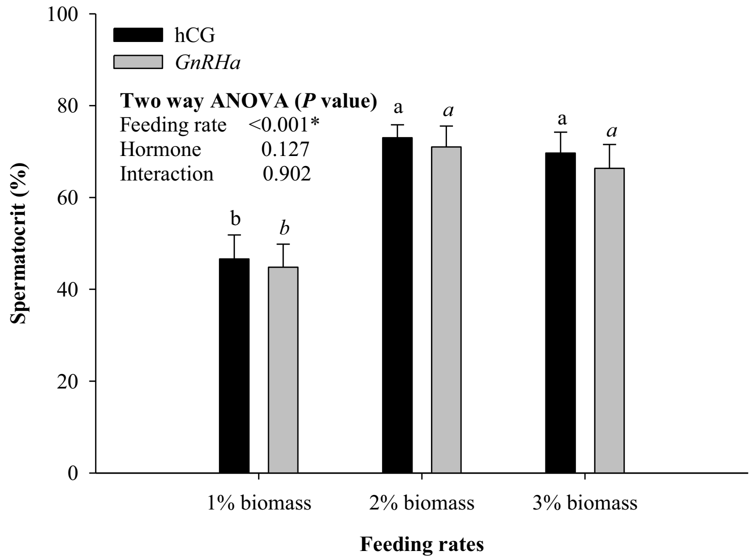

Figure 3. The mean ± standard deviation of the spermatocrit (%) in male broodstock of striped catfish, *P. hypophthalmus*. Letters on top of the bars indicate significant differences ($P < .05$). hCG = Human chorionic gonadotropic hormone; GnRHa = Gonadotropin releasing hormone analogue. * indicates significant differences between factors into the two-way ANOVA.

For the spermatocrit (%), significant differences ($P < .05$) were observed between feeding rates but not between hormonal treatments; the 1% biomass group had the lowest spermatocrit registered (approximately 50%) in both hormonal treatments. The spermatocrit were similar between the 2% and 3% biomass groups, with values from 66.3 ± 5.2% to 73.0 ± 2.8% in both hormonal treatments (Figure 3).

Effect of feeding rate and hormonal treatments on fertilization and hatching rates

Because no spawning was observed in females in the 1% biomass group, no fertilization and hatching rates were registered. Nevertheless, significant differences ($P < .05$) were observed between the 2% and 3% biomass groups but not between the hormonal treatments for these parameters. Fertilization rates of approximately 70% were registered for the 2% biomass group, while fertilization rates of approximately 85% were observed for the 3% biomass group (Figure 4A). In contrast, hatching rates of 63-65% were reported for the 2% biomass, while hatching rates greater than 80% were observed in the 3% biomass (Figure 4B).

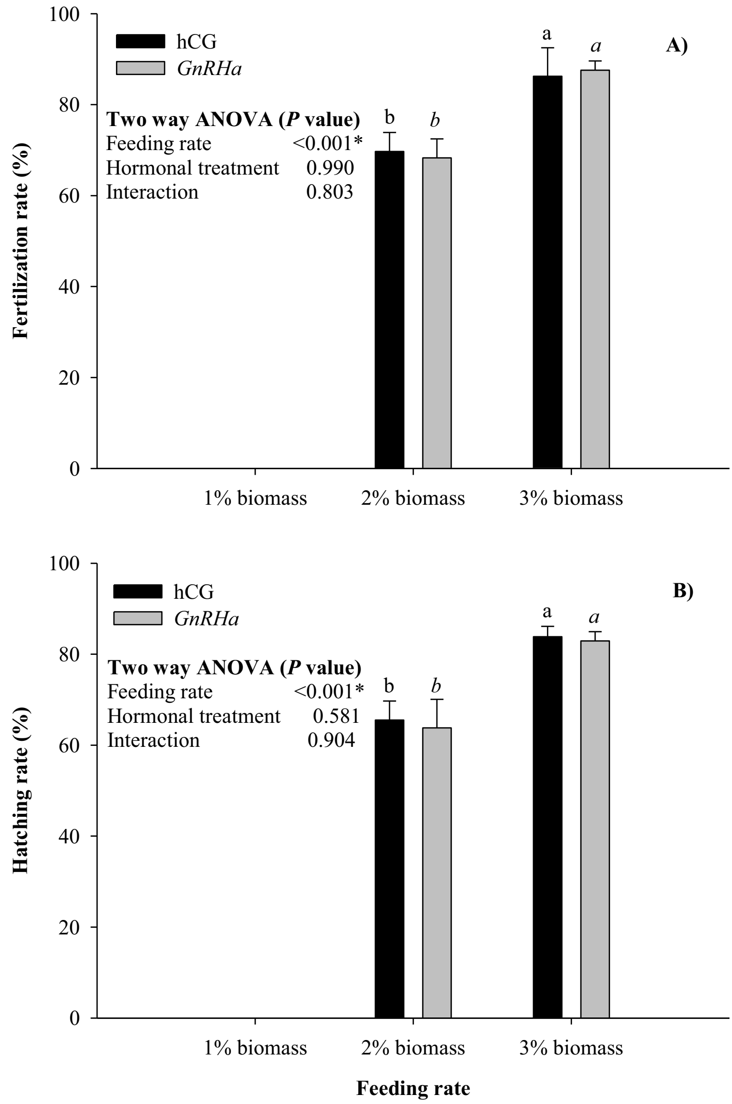

Figure 4. The mean ± standard deviation of A) the fertilization rate (%) and B) hatching rate (%) in spawning obtained from broodstock of striped catfish, *P. hypophthalmus*. Letters on top of the bars indicate significant differences ($P < .05$) between the treatments. hCG = Human chorionic gonadotropic hormone; GnRHa = Gonadotropin releasing hormone analogue. * indicates significant differences between factors into the two-way ANOVA.

Discussion

In this study, a commercial diet with 35% protein was supplied at three feeding rates for breeders of striped catfish: 1, 2, and 3% biomass. These feeding rates were chosen based on the data reported by Bui et al. (2010), who mentioned that overall feeding rates for breeders of striped catfish range from 0.2–10% body weight day^{-1} when they are fed pelleted feed, with a most commonly feeding rate of 2.8%. Additionally, in other freshwater species as African catfish, *Clarias gariepinus*, and Nile tilapia, *Oreochromis niloticus*, the feeding supplied at 3% biomass for breeders produced high oocyte diameter, high milt density, volume and sperm motility and a higher percentage of egg fertilization and hatching (Adewumi 2006; Bhujel, Little, and Hossain 2007).

The reproductive activities demand an expenditure of energy and that an increase in the reproductive effort would be expected an increasing amount of progeny produced per unit of food consumed, but this would be at the cost of reducing the rations and, thus, the energy available for maintenance and somatic growth (Lucas 2002). In some studies, Fulton´s condition factor has been considered as a simple proxy of energy reserves in the fish body. This index is based on the length-weight relationship and its applicability arises from the assumption that a heavier fish of a given length has greater energy reserves and consequently is in better condition (Bolger and Connolly 1989; Froese 2006). This fact explains the differences between values of the condition factor assessed for the breeders from each feeding rate group. Thus, those breeders fed at 1% did not have enough energy input to successfully achieve the final gonadic maturation, although was enough to maintain somatic growth; breeders fed at 2% had enough input of energy to support the production of gametes although fish fed at 3% biomass had higher fecundity and milt production. In rudd, *Scardinus erythrophtalmus*, a Cyprinid fish species, condition factor values ranging from 1.8 to 1.9 were observed during the pick of the reproductive season, (Mozsár, *et al*., 2015). Such values of condition factor were like those observed for fishes fed at 3% biomass (1.20 ± 0.27) in this work in August when breeders of striped catfish were induced to spawning.

Some natural and synthetic hormones have been used to induce the spawning and spermiation in silurid species. All these hormonal treatments have been solutions, either injected intramuscularly or intraperitoneally; among these products, solutions of carp pituitary extract, hCG, and GnRHa with some dopamine inhibitors have been the most used, with varied outcomes according to the type of hormone and application doses (Chad, Singh, and Mandal 2011; Chatakondi et al. 2011; Kutwal et al. 2017; Legendre, Linhart, and Billard 1996; Mosha 2018). Legendre et al. (2000) used hCG and sGnRHa + Domperidone injections to induce spawning in striped catfish, but both hormonal treatments led to similar results in terms of ovulation rate, hatching rate, and relative fecundity. In this work, in addition to hCG injections, implants with 150 μg of sGnRHa but without a dopamine inhibitor were applied to the fish, which also led to similar results in terms of relative fecundity in both sexes and fertilization and hatching rates. Thus, injectable solutions of hCG and GnRHa + domperidone and GnRHa implants without domperidone applied to breeders of *P. hypophthalmus* can be successfully used to induce spawning and spermiation with similar results.

However, despite the results obtained for the hormonal treatments, remarkable influences of the feeding rates on the relative fecundity and the fertilization and hatching rates were observed, and there was no effect of the hormones used to induce spawning and spermiation. For the 1% feeding rate group, no spawning was seen in females, and the volume of semen and spermatocrit were low in males (less than 5 mL and around 50%, respectively).

As already mentioned, no spawning was obtained from females fed at 1% biomass and the average volume of semen obtained was low. McBride et al. (2013) have emphasized that poor feeding environments can lead to delayed maturation, skipped spawning, fewer spawning events per season, or fewer eggs produced per event. Indeed, it has been widely demonstrated that the fish endocrine system is sensitive to alterations in nutrient intake and that alterations in ration level and feeding time influence the fish metabolic hormones, even those involved in reproduction (Grone et al. 2012; MacKenzie, Van Putte, and Leiner 1998; Manor et al. 2014). Higuchi et al. (2018) observed that females of *Seriola quinqueradiata* subjected to restricted feeding had lower ovary weight, smaller ovarian follicles, lower levels of 17β–estradiol, and lower pituitary gene expression levels of gonadotropins than those females fed normally. In this work, female breeders of *P. hypophthalmus* were fed at 3% biomass on diets with 35% of protein and achieved relative fecundities among 29-30 × 10^3 eggs kg^{-1}, which is higher than recorded in other silurid species such as Asian redtail catfish, *Mystus nemerus*, with 17.69 ± 122 x 10^3 eggs kg^{-1} when breeders were fed on diets containing 35% protein to 3% biomass (Abidin *et al.*, 2006), and the silver catfish, *Rhamdia quelen*, with relative fecundities of 106.36 ± 40.08 and 128.05 ± 32.38 eggs kg^{-1} when females were fed with 28% and 40% protein, respectively at 2% biomass (Coldebella et al. 2011).

Regarding males, there is little information about the effects of restricted feeding on fish sperm production. Nevertheless, Asturiano et al. (2001) working with European sea bass, *Dicentrarchus labrax*, observed that although sperm quality is not affected by the diet, the survival of embryos and larvae was affected, which indicates a unique and striking long-term effect of diet–mediated sperm quality. In this work, a higher relative volume of semen was recorded in males in the 3% biomass group of around 11 mL of semen kg^{-1} of body weight; this is higher than recorded for 2% feeding rate, from 4 to 7 mL of semen kg^{-1}. Nevertheless, Tessaro et al. (2012) obtained volumes from 45 to 58 mL of semen kg^{-1} when male breeders of silver catfish, *R. quelen*, were fed with diets containing 30% of protein and 2% feeding rate but with different levels of digestible energy. Despite the relative semen volume being significantly different among the 2% and 3% biomass groups, the spermatocrit was similar for both feeding rates (≥ 70%). This result is higher than reported by Sanches et al. (2011) in other species of silurid fishes such as the silver catfish, *R. quelen*, and the acari, *Rhinelepis aspera*, with 35.52 ± 8.85% and

60.52 ± 11.18%, respectively, although they were fed a diet of 28% of protein, Nevertheless, males of Nile tilapia, *Oreochromis niloticus* fed 35% of protein, just reached a spermatocrit of 9.33 ± 4.31%. However, high spermatocrit values, like observed in this work, were registered for *R. quelen* in Brazil, but in natural conditions, reaching 65 ± 2.2% during the reproductive season (Borges et al. 2005).

Regarding the fertilization and hatching rates, the highest values were registered when breeders were fed at 3% biomass (>85% and >80%, respectively); however, no significant differences were observed between hormonal treatments, hCG and GnRHa. Nevertheless, Chad, Singh, and Mandal (2011) in *P. sutchi* reported significant differences when breeders were induced with carp pituitary extract (hCG), and two commercial products of sGnRHa with a dopamine blocker. They observed low fertilization rates with one GnRHa product (62–73%), but 82–91% with the other one and 87–93% with the carp pituitary extract, which is like that reported in this work. The hatching rates were slightly lower for carp pituitary extract and one GnRHa product (76–83% and 73-79%, respectively) than reported here.

Thus, the main conclusions, according to the results obtained, are: 1) the monthly variations in the body weight and the condition factor were influenced by the feeding rates, thus, fishes fed at 3% biomass reached the highest values; additionally such breeders reached higher relative fecundities in both sexes and fertilization and hatching rates; 2) to induce the spawning, the feeding rates had a marked influence on the relative fecundity in both sexes and on the fertilization and hatching rates, whereas there was no effect of the hormonal treatments; 3) the use of injected hormones such as carp pituitary extract, hCG and GnRHa with a dopamine inhibitor, as well as the use of GnRHa implants without a dopamine inhibitor, lead to advances, promoting spawning in females and increasing milt production in males of striped catfish, *P. hypophthalmus*, and 4) diets with 35% protein provided to breeders of *P. hypohthalmus* at 3% of biomass is recommended to generate high fecundities in both sexes and high fertilization and hatching rates.

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Disclosure statement

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