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Effect of estradiol-17β on the sex ratio, growth and survival of juvenile common snook (*Centropomus undecimalis*)

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ABSTRACT. Sex control in fish is a promising technique for aquaculture, since it gives advantages associated with one sex. The aim of this study was to investigate the feminization of common snook (*Centropomus undecimalis*) by oral administration of two doses of estradiol-17β (50 and 100 mg E_2 kg^{-1} feed) and control treatment for 45 days and to evaluate their effects on the sex ratio, growth and survival of common snook juveniles. After this period, fish were fed only with commercial feed without hormone supplementation. During the period of E_2 administration, the control fish grew more than those in the other treatments. At the end of the experiment in the treatment with 50 mg E_2 kg^{-1}, 26.32% of the fish were male, 68.42% were female, and 5.26% were intersex. In the treatment with 100 mg E_2 kg^{-1}, 10% of the fish were male, and 90% were female. There was no difference in growth among the treatments after 11 months. This study showed that it is possible to obtain 90% common snook females using feeds with 100 mg E_2 kg^{-1} for 45 days without impairing the fish growth or survival.

Keywords: sex control, hormone treatment, marine fish, intersex, protandric.

Efeito do estradiol-17β na feminização, crescimento e sobrevivência de juvenis de robalo-flecha (*Centropomus undecimalis*)

RESUMO. O controle do sexo dos peixes é uma técnica promissora na aquicultura, pois permite obter vantagens associadas a um dos sexos. Sendo assim, o objetivo deste trabalho foi avaliar o efeito do hormônio 17β-estradiol (E2) na feminização, crescimento e sobrevivência de juvenis de robalo-flecha (*Centropomus undecimalis*). Durante 45 dias, os juvenis foram submetidos a três dietas (controle, 50, 100 mg E_2 kg^{-1} de ração) com três repetições cada. Após este período, os peixes passaram a ser alimentados somente com ração comercial sem adição de hormônio. Durante o período de administração do E_2, os peixes do controle cresceram mais que os dos outros tratamentos. No final do experimento, no tratamento com 50 mg E_2 kg^{-1}, 26% dos peixes eram machos, 68.42% eram fêmeas e 5.26% eram intersexo. No tratamento com 100 mg E_2 kg^{-1}, 10% dos peixes eram machos e 90% eram fêmeas. Após 11 meses não houve diferença de crescimento entre os tratamentos. Este trabalho mostrou que é possível obter 90% de fêmeas de robalo-flecha utilizando rações com 100 mg E_2 kg^{-1} durante 45 dias sem danos ao crescimento ou à sobrevivência.

Palavras-chave: controle do sexo, tratamento hormonal, peixe marinho, intersexo, protândrico.

Introduction

Sex control in fish is one of the most promising strategies in aquaculture. The main objective is to increase productivity based on advantages related to one of the sexes (BEARDMORE et al., 2001; FRISCH, 2004).

The production of monosex batches of the species *Lepomis macrochirus* avoids issues related to overpopulation in fish farms (WANG et al., 2008). In the case of *Hippoglossus hippoglossus*, the advantage of producing only females is related to the fact that the females grow faster and mature later than males (HENDRY et al., 2003). The same pattern occurs with European sea bass (*Dicentrarchus labrax*) females, which show 30 to 50% higher growth rates than males (GORSHKOV et al., 2004). In the case of the shortnose sturgeon *Acipenser brevirostrum*, the main advantage in the production of the species is to obtain caviar; therefore, monosex batches of females are much more economically interesting (FLYNN; BENFEY 2007).

One of the ways to manipulate the sex of fish is the use of exogenous steroid hormones. Several studies report the use of androgens or estrogens administered in immersion baths or in feeds at the beginning of fish gonad development (ROUGEOT

et al., 2002; FLYNN; BENFEY, 2007; ARSLAN et al., 2009).

The hormone estradiol-17β (E_2) is a natural estrogen that has been successfully used in different fish species, including *Pseudobagros fulvidraco* (PARK et al., 2004), *L. macrochirus* (WANG et al., 2008) and *Micropterus salmoides* (ARSLAN et al., 2009).

To ensure increased efficiency, the treatment should ideally be performed before sexual differentiation, during such period the gonad tissue is more sensitive to the action of exogenous steroids. Moreover, the hormone dose used and the duration of treatment are also essential for the treatment success (PIFERRER, 2001).

The common snook *Centropomus undecimalis* is a key marine fish found in tropical and subtropical regions of Florida, USA, down to the Southern Brazil (FIGUEIREDO; MENEZES, 1980). This fish is a protandrous hermaphrodite species (TAYLOR et al., 2000) that has been studied in the United States (YANES-ROCCA et al., 2009), Mexico (IBARRA-CASTRO et al., 2011) and Brazil (SOLIGO et al., 2011). Common snook has stood out as a species with potential for aquaculture because it is robust and euryhaline, adapts well to captivity, and has tasty white flesh and a high market value (CAVALLI; HAMILTON, 2007).

The first study of feminization of wild juvenile common snook in Mexico was recently published. The authors used 50 mg E_2 kg^{-1} in *Artemia* and commercial feed from 21 to 42 days and obtained a maximum of 90% females (VIDAL-LÓPEZ et al., 2012). Based on these results, this study aimed to investigate the effect of E_2 in the feed for 45 days in juveniles of common snook born in captivity and to evaluate its effects on the sex ratio, growth and survival following the hormonal treatment.

Material and methods

The experiment was conducted at the Laboratório de Piscicultura Marinha (LAPMAR) of the Universidade Federal de Santa Catarina (UFSC), in Florianópolis, Santa Catarina State, Brazil. The 235 juveniles of common snook *C. undecimalis* used in this experiment were obtained from the spawning of wild breeders caught by Danúbio Aquacultura company, Ltd. (CARVALHO FILHO, 2009). The fish were, on average, 14.17 ± 0.24 g and 12.15 ± 0.07 cm at the beginning of the experiment.

The experiment was performed in two stages. The first stage was feminization or the period of hormonal treatment. Three diets with increasing levels of estradiol-17β (E_2) hormone: 0 mg E_2 kg^{-1} feed (control treatment), 50 and 100 mg E_2 kg^{-1} feed were provided over 45 days (from February to March 2011). Each diet was considered as a treatment with three replicates. The second stage (post-feminization) was carried out from April 2011 to March 2012, when the fish were fed a commercial feed without hormone.

Feminization

The fish were distributed into nine 1-m^3 net cages fixed to a floating raft in the LAPMAR earthen ponds. Each net cage had 25 juveniles of common snook. Temperature and dissolved oxygen were 28.05 ± 1.05°C and 4.47 ± 0.85 mg L^{-1}, respectively, and the water salinity was 20 g L^{-1}.

A biometric (data on weight and length) assessment of all fish was performed before starting this stage, and ten fish were sacrificed for microscopic examination of gonadal tissue by histological analysis. All fish were measured and weighed at the end of the hormonal treatment, and six fish per treatment (2 fish per net cage) were randomly sacrificed for histological analysis.

Feed preparation

The feeds with different dosages of estradiol-17β (E_2) were prepared one week before starting the experiment. A stock solution of E_2 hormone dissolved in absolute alcohol was initially prepared for incorporation in the diet (commercial mash feed, 50% crude protein). An aliquot of that solution was diluted in 800 mL of commercial alcohol and homogeneously mixed in one kilogram of feed, calculating for each diet an aliquot to prepare the solution at the desired hormone dosage. The feed was then spread on a tray, protected from light, for 48h to complete alcohol evaporation. After drying, the feed was pelleted and then dried in an oven at 25°C for approximately 12 hours. Once ready, the feed was stored at 5°C. The control diet was prepared in the same manner using alcohol without hormone.

During the experiment, the feed was provided daily to juveniles, four times a day until apparent satiation.

Post-feminization

In this stage, the fish were fed twice a day with commercial feed, without hormone supplementation until apparent satiation. During this time, the minimum temperature reached 16°C, and the maximum was 30°C. Dissolved oxygen and water temperature were 5.98 ± 1.25 mg L^{-1} and 21.73 ± 3.69°C, respectively. Two biometrics assessments were performed during this stage, one at the beginning of

December 2011 and another in early March 2012. In the last biometrics assessment, 75% of the fish from each treatment were sacrificed to sample the gonads and liver for histological analysis.

Biometrics and sampling procedures

During all biometrics assessments, the fish were anesthetized using 50 ppm benzocaine. The fish sampled for histology were euthanized with an overdose of benzocaine (100 ppm).

In the biometrics assessment performed at the end of the experiment, each specimen was dissected to remove the gonads and liver, which were weighed on a digital analytical balance to assess the gonadosomatic index (GSI) and hepatosomatic index (HSI), and later, the gonads were fixed for microscopic examination by histological analysis.

Histological preparation and analysis

The samples collected for histology at all stages were fixed in Davidson's solution for 24 hours and then preserved in 70% alcohol. Following dehydration and a clearing technique, the material was embedded in paraffin and cut using a microtome at a thickness between 5 and 7 μm. The slides were stained using hematoxylin and eosin.

The microscopic characterization of the gonads was performed using a LEICA DM-750 microscope, and images were recorded using a digital camera LEICA ICC50 HD. The sex of the fish was assessed according to Vazzoler (1996), Grier and Taylor (1998), Taylor et al. (1998) and Rhody et al. (2013) based on the evaluation of the gonad structures of all sampled fish.

Data analysis

Fulton's condition factor (K, %) was calculated according to the formula $k = ((W) \div (L^3)) \times 100$, where W = weight (mg) and L = length (mm).

Specific growth rate (SGR, % day^{-1}) was calculated by the formula SGR= 100× ((Ln Wf – LnWi) ÷ time), where W_f = final weight (g) and W_i = initial weight (g).

Weight gain (WG, g) was calculated by the formula $WG = (W_f - W_I)$.

Feed intake (FI, g fish^{-1}) was calculated by the formula FI = total intake ÷ number of fish per net cage.

Estradiol-17β consumption (E_2C, mg fish^{-1}) each dose was calculated by the formula $E_2C = C \times (E_2$ dose).

Survival (S, %) was calculated using the formula $S = 100 \times ((N_i - N_d) \div Ni)$, where N_i is the initial number of juveniles, and N_d is the number of juveniles dead.

Gonadosomatic index (GSI, %) and hepatosomatic index (HIS, %) were calculated according to the formula GSI = 100 × (gonad weight ÷ fish weight and HIS = 100 × (liver weight ÷ fish weight.

The results are shown as means ± standard deviation. The statistical treatment of data was performed using (one-way) analysis of variance, and Tukey HSD test was applied when significant differences were found. The percentage data were transformed using the arcsine function before being analyzed. In each case, the homoscedasticity of the data was tested using Levene´s test. Nonparametric data (the gonadosomatic index) were analyzed using the nonparametric Kruskal Wallis test followed by Dunn's test for comparison of means. The ratio between males and females was calculated and subsequently analyzed using the chi-squared (χ^2) test. All analyses were performed using the Statistic 7.0 software and at a 5% significance level.

Results

Histological examination of the gonads collected at the beginning of the experiment and at the end of the hormonal treatment (Figure 1), did not present evidence of complete sexual differentiation.

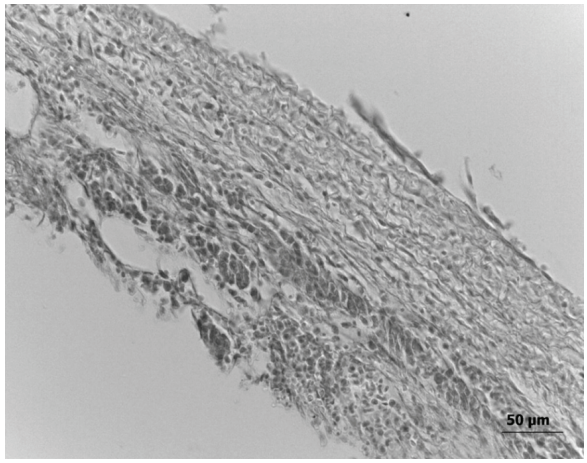

Figure 1. Photomicrographs of *C. undecimalis* gonads collected at the end of the hormonal treatment. No evidences of complete sexual differentiation were found. Bar indicates 50 μm.

The fish from different treatments showed no significant differences ($p > 0.05$) in weight (14.17 ± 0.24 g), length (12.14 ± 0.07 cm) and condition factor (0.761 ± 0.002%) before the onset of the experiment. The fish from the control treatment were significantly ($p < 0.05$) larger than the fish from the other treatments, after 45 days of hormone treatment. The weight gain and specific growth rate were also significantly higher ($p < 0.05$) in the control treatment (Table 1).

Feed intake was not significantly different ($p > 0.05$) in the treatments with 50 and 100 mg E_2 kg^{-1} and was significantly greater ($p < 0.05$) in the control treatment during the feminization experiment. The survival rates in the control and treated groups ranged from 78.67 to 89.33% (Table 1) after 45 days of rearing in feminization experiment.

Table 1. Effects of various dosages of estradiol-17β (E_2) on growth, survival, feed intake (F_1) and estradiol consumption in the feed (E_2 C) parameters of juveniles of *C. undecimalis* at the end of hormonal treatments.

E_2 dose (mg kg^{-1})	0	50	100
Final BW (g)	27.21 ± 11.20^a	20.18 ±6.26^b	20.01 ±6.96^b
Final BL (cm)	15.10 ±1.90^a	14.26 ±1.39^b	14.10 ± 1.61^b
K (%)	0.7518 ± 0.047^a	0.679 ± 0.054^b	0.691 ± 0.056^b
SGR (%)	1.39 ±0.10^a	0.90 ±0.06^b	0.78 ± 0.14^b
WG (g)	12.66 ± 1.36^a	7.01 ± 0.25^b	5.97 ±1.15^b
Survival (%)	84.00 ± 8.00	78.67 ± 6.11	89.330± 6.11^{ns}
FI (g fish)	20.02 ± 0.58^a	15.07 ± 0.31^b	15.84 ± 0.41^b
E_2C (mg $fish^{-1}$)	0.0 ± 0.0	31.16 ± 1.42	67.01 ± 0.75

Mean (± standart deviation) in the same row with different superscripts are significantly different ($p < 0.05$), ns = non significant ($p > 0.05$), BW = body weight, BL = body length, K = Fulton's condition factor, SGR = specific growth rate.

Two biometrics assessments were performed in the second stage of the experiments, with one performed in early December 2011 (Table 2) and another in early March 2012 (Table 3). No significant statistical differences ($p > 0.05$) were found among the treatments in both biometrics assessments.

Table 2. The body weigth (BW), body length (BL) and fulton's condition factor (K) of *C. undecimalis* eight months post-feminization.

E_2 dose (mg kg^{-1})	0	50	100
BW (g)	73.94 ± 23.66	76.78 ± 17.3	80.28 ± 24.35^{ns}
TL (cm)	21.36 ± 2.05	21.20 ± 1.77	21.86 ± 2.02^{ns}
K (%)	0.738 ± 0.076	0.800 ± 0.111	0.745 ± 0.59^{ns}

Mean (± standart deviation), ns = non significant ($p > 0.05$) BW = body weight, TL = total length, K = Fulton's factor.

Table 3. The growth, fulton's condition factor (K), the hepatosomatic index (HSI), the gonodosomatic index (GSI) of *C. undecimalis* eleven months post-feminization.

E_2 dose (mg kg^{-1})	0	50	100
BW (g)	271.04 ± 70.87	278.64 ± 84.14	258.83 ± 76.67^{ns}
TL (cm)	31.91 ± 2.77	31.66 ± 3.19	31.17 ± 3.03^{ns}
K (%)	0.878 ± 0.0656	0.8574 ± 0.1037	0.8335 ± 0.0871^{ns}
HIS (%)	1.73 ± 0.30^b	2.10 ± 0.46^a	1.82 ± 0.22^{ab}
GSI (%)	0.04 ± 0.01^b	0.14 ± 0.07^a	0.16 ± 0.04^a

Mean (± standart deviation) in the same row with different superscripts are significantly different ($p < 0.05$), ns = non significant ($p > 0.05$), BW = body weight, TL = total length.

However, HSI and GSI were also evaluated in the final biometrics assessment performed in March 2010 (final biometrics assessment of the study), in addition to weight, length and condition factor data (Table 3). Although the HSI of the fish treated with 50 mg E_2 kg^{-1} was significantly higher ($p < 0.05$) than the HSI of the control fish, the values of these two treatments were not significantly different ($p > 0.05$) from the HSI of fish treated with 100 mg E_2 kg^{-1}.

At the end of the post-feminization experiment, all control-treatment fish were male, and 80% of the fish released some semen following abdominal massage. Histological sections showed that the testicles had spermatogonia, spermatocytes, spermatids and spermatozoa (Figure 2a). No gonadal malformation was found, although 5.26% of the fish in the treatment with 50 mg E_2 kg^{-1} were noticeably intersex, which means, had intratesticular oocytes (Figure 2b). The females found in the treatments with 50 and 100 mg E_2 kg^{-1} (Figure 3a and b) had primary growth perinucleolar oocytes and approximately 45% of them had some primary growth oocytes with oil droplets.

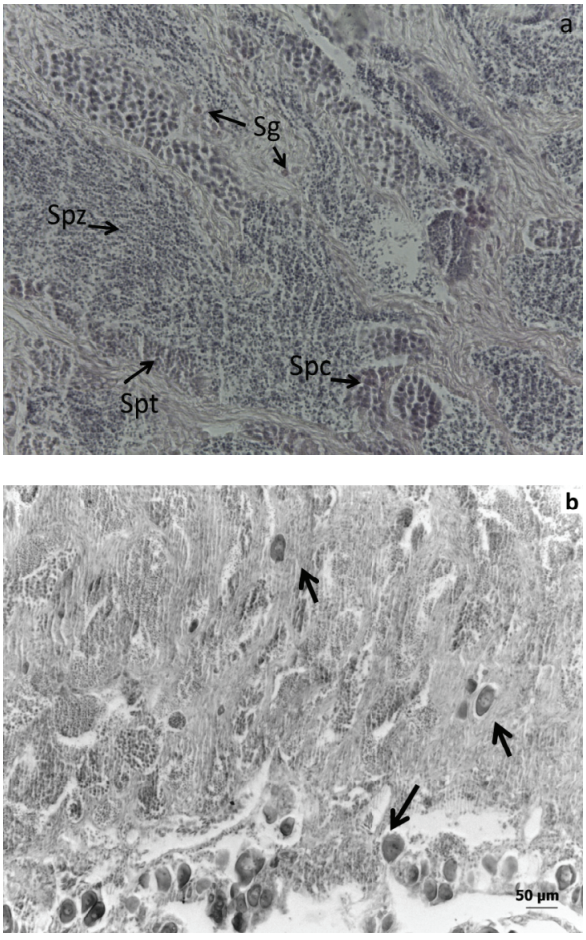

Figure 2. Photomicrographs of transverse sections of the gonads of male (a) and intersex (b) *C. undecimalis* at the end of the experiment. (a) Testis containing spermatogonia (Sg), spermatocytes (Spc), spermatids (Spt) and spermatozoa (Spz) from control fish, (b) intersex gonad from fish treated with 50 mg E_2 kg^{-1} showing intratesticular oocytes (arrow). Bar indicates 50 μm.

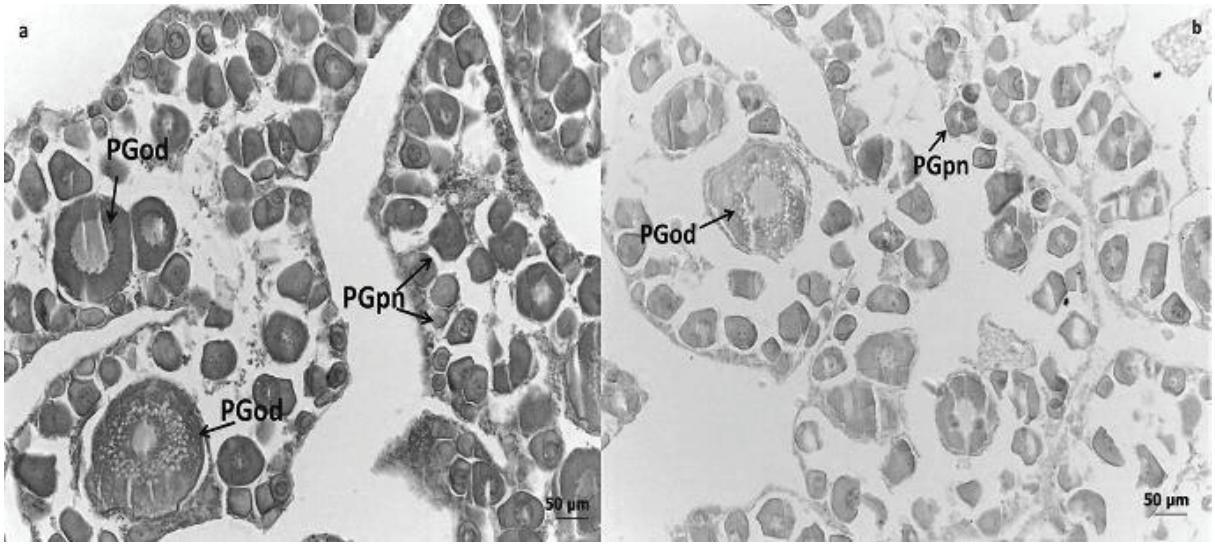

Figure 3. Photomicrographs of transverse sections of the gonads of female of *C. undecimalis* at the end of the experiment. (a) ovary of 50 mg E_2 kg^{-1} treated fish, (b) ovary of 100 mg E_2 kg^{-1} treated fish. Note that the oocytes present are in all cases at primary growth stage (PG) with oil droplet step (PGod) and perinucleolar step (PGpn). Bar indicates 50 μm.

Results of the sex ratio (Figure 4) assessed by microscopic analysis were significantly different among the treatments (χ^2, $p < 0.05$). The highest percentage of males was in the control, and the highest percentage of females was in the treatment with 100 mg E_2 kg^{-1}.

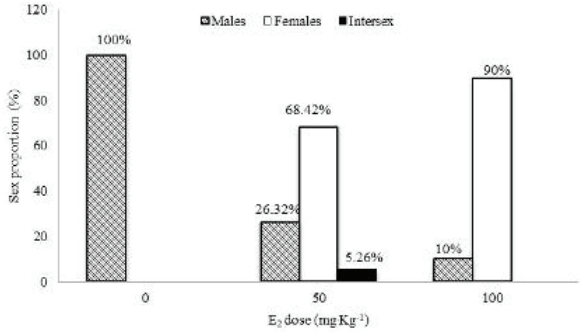

Figura 4. Effects of oral exposure to estradiol-17β (E_2) on sex proportions in *C. undecimalis* post-feminization. The results of the sex ratio assessed by microscopic analysis were significantly different among the treatments (χ^2, $p < 0.05$).

Discussion

The results from this study clearly show the hormone estradiol-17β (E_2) effect on the feminization of the gonads of common snook juveniles.

Vidal-López et al. (2012) achieved 90% common snook females using 50 mg E_2 kg^{-1} in *Artemia* and commercial feed for 21 and 42 days in fish with initial length of 4.6 cm, while the percentage of females in the present study, using 50 mg E_2 kg^{-1} in the feed for 45 days for fish with an initial length of 12 cm, was only 68.42%, and some intersex specimens were also found. However, the treatment with 100 mg E_2 kg^{-1} reached 90% females.

According to Piferrer (2001), three factors are critical for success in controlling the sex of juveniles: hormone dosage, hormone treatment and gonad development stage. Most likely, the different findings from Vidal-López et al. (2012) study may have resulted from the gonadal development stage given the difference in size of juveniles used in both of the studies. Wang et al. (2008) obtained 100% *Lepomis macrochirus* females and no intersex fish following hormone treatment for 60 days with 150 and 200 mg E_2 kg^{-1}. Gorshkov et al. (2004) also achieved 100% feminization of *Dicentrarchus labrax* using 12.5 mg E_2 kg^{-1} for 60 days.

The percentage of males found in the control treatment was already expected as the common snook is a protandrous hermaphrodite species according to Taylor et al. (2000).

The E_2 hormone may change the morphology of gonads, negatively affects survival and impairs growth in addition to changing the sex ratio (PIFERRER, 2001). In the present study, the use of E_2 did not affect survival but decreased the feed intake during the feminization experiment. Growth was also lower in the treated fish than in the control fish, most likely resulting from the decreased feed intake of the fish treated with E_2. The same estradiol effect on growth was also found in other species, including *Hippoglossus hippoglossus* (HENDRY et al., 2003) and *Micropterus salmoides* (ARSLAN et al., 2009).

After ceasing the supply of the feed with hormone, the juveniles of this study recovered their

growth and, at the end of the experiment, showed no differences from the control treatment. Wang et al. (2008) also evaluated this parameter in *L. macrochirus* and found the same result during and after the hormone treatment. According to these authors, this growth recovery following the hormone treatment may be regarded as compensatory growth. This growth is described as an accelerated growth phase after a period of adverse conditions for fish (ALI et al., 2003).

The HSI is used as a biomarker of exogenous estrogen to indirectly assess the vitellogenin production in an organism. Exogenous estradiol stimulates the hepatic production of vitellogenin in immature fish, increasing liver metabolism and thereby increasing the organ size, consequently increasing the HSI (VERSLYCKE et al., 2002; DEVLIN; NAGAHAMA, 2002). However, in this study, only the HSI of fish treated with 50 mg E_2 kg^{-1} was higher than that from the control. In the present study, the GSI was lower than 0.5 in all treatments. According to the classification conducted by Taylor et al. (1998) for wild *C. undecimalis*, GSI lower than 0.5 indicates an immature or regressed male and a female with only non-vitellogenic oocytes. In the present study, although the GSI were lower than 0.5, some females with vitellogenic oocytes were found, most likely resulting from the effect of E_2 on gonads.

Based on the results found in this study, parameters such as the dosage and length of treatments and the size of juveniles at the beginning of the experiment should be adjusted to improve the hormone-treatment effectiveness for the feminization of common snook juveniles.

Conclusion

This study showed that it is possible to obtain 90% common snook females of 12 cm by administering feed with 100 mg E_2 kg^{-1} for 45 days without impairing growth or survival. However, further studies are needed to design a protocol for 100% feminization.

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