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Research Article

Functional feminization of the Mexican snook (*Centropomus poeyi*) using 17β-estradiol in the diet

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ABSTRACT. The Mexican snook, *Centropomus poeyi*, supports an important fishery in southeastern Mexico. Due to its protandrous hermaphroditism, females may be especially sensitive to overfishing because they tend to be larger and selective removal has the potential to negatively affect natural populations, thus making urgent the need to contribute on management strategies for the species. The objective of this study was to produce batches of reproductive females at an early age and a small size by using 17β-estradiol (E2) in the diet. In two separate experiments, we fed fish 0, 10, 20, 30, 40, 50, or 60 mg of 17β-estradiol per kg of food for 60 days during gonadal differentiation. Fish fed with 50 and 60 mg of E2 per kg of food resulted in 100% feminization, while the control group (0 mg of E2) was 100% male. Three years after feminization, sex-reversed fish remained female, producing oocytes and were capable of spawning, while fish in the control group were ripe males with running milt. E2-treated groups were significantly larger (weight and length) than the control groups for up to six months after treatment, but the difference was undetectable at 300 days post-treatment. Survival was high in all treatments (100%). We obtained high percentages of functional females using dosages of 50 and 60 mg kg^{-1} of E2 for 60 days, with 80% of the induced fish spawning and producing fertilized eggs and larvae. The results obtained in this study provide opportunities for broodstock management and sex manipulation.

Keywords: *Centropomus poeyi*; sex-inversion; functional females; snook culture; aquaculture

INTRODUCTION

Several centropomid species support important recreational and commercial fisheries in the Gulf of Mexico (Tucker, 2003; Alvarez-Lajonchère & Tsuzuki, 2008). Recent harvest rates in this region are lower than in the previous decade (FAO, 2011; Chávez-Caballero *et al.*, 2014). In Mexico, snooks command high prices. In 2013, the reported landing of snooks in Mexico was 8,085 t, with 75% of the total catch from the Gulf of Mexico (SAGARPA, 2017). Unfortunately, all snook species captured are reported under the generic category of "snooks" (FAO, 2011). This broad labeling of landed species impedes the assessment of fisheries at the species level.

Centropomus poeyi is an endemic species that occurs only in watersheds located between the Mexican states of Tamaulipas and Campeche that discharge into the Gulf of Mexico (Chávez, 1961, 1963). Contrary to *C. parallelus* and *C. undecimalis*, the Mexican snook spends most of its life cycle in rivers and lagoons, migrating to the ocean only to spawn (Chávez, 1981). The Mexican snook is a piscivorous species, reaching a total length of up to 110 cm and a weight of 14 kg, with females being significantly larger than males (Loran-Núñez *et al.*, 2012; Chávez-Caballero, 2014). Very little is known about the biology, ecology, or population status of the Mexican snook. While all snooks are considered protandric hermaphrodites (Alvarez-Lajonchère & Tsuzuki, 2008), only *C. undecimalis* is documented as such (Taylor *et al.*, 2000).

The potential for snook aquaculture has been highlighted, and specific studies have developed methods for culturing *C. undecimalis* and *C. parallelus* (Zarza *et al.,* 2006; Lajonchère & Tsuzuki, 2008; Cerqueira & Tsuzuki, 2009; Yanes-Roca *et al.,* 2009; Ibarra-Castro *et al.*, 2011; Contreras-García, 2015). However, there are no experimental studies on the culture of *C. poeyi* in captivity. In our facilities, we have observed that *C. poeyi* has advantages over other

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centropomids (*i.e.*, large size, high tolerance to handling, grow-out in a wide range of salinities, and fast growth). Broodstock management for *C. poeyi* is complicated because females range from five to ten kg. This makes operations expensive, not to mention the risks associated with maintaining large fish captive. Therefore, this study aimed to produce batches of reproductive females at an early age and a small size through induced sex inversion using 17β-estradiol in this hermaphroditic species. This natural estrogen has been frequently used in gonochoristic species; however, there are few studies on induced feminization in hermaphroditic fishes. In this study, we investigated the effect of different dosages of the steroid 17β-estradiol on the sex ratios, growth, reproductive viability, and survival of *C. poeyi*.

MATERIALS AND METHODS

Broodstock and larvae production

Wild-caught broodstock was maintained in 63 m^3 circular plastic tanks (9 m diameter) with a daily 10% water exchange and natural photoperiod at the Marine Aquaculture Station, Biological Sciences Division, Juárez Autonomous University of Tabasco located in Jalapita, Centla, Tabasco (MAS-UJAT). In two separate spawning events, one mature female and two males (average weight 5.550 ± 223 and 3.000 ± 68 g, respectively) were induced using handmade cholesterol implants with 200 µg per fish of LHRHa (Syndel, Western Chemical, USA) and placed in a fiberglass spawning tank (4 m diameter, 1 m depth). Spawning began after 27 h; fertilized eggs were collected using a 400 µm soft-mesh net. Embryos were placed in 500 L fiberglass tanks, and hatching started 44 h later. The yolk-sac larvae were maintained in these tanks with a daily 80% water exchange and continuous aeration. Salinity (28.6 ± 1.9 ppm), dissolved oxygen (6.6 ± 1.1 mg L^{-1}), and temperature (27.1 ± 2.5°C) were monitored daily. At 15 days post-hatching (dph), salinity was gradually lowered (5 ppm per day) until the fish were in fresh water. Tanks were filled with salt water and stocked with a mix of the green microalgae *Tetraselmis chuii*, *T. suecica*, and *Nannochloropsis oculata* (2-4×10^5 cells mL^{-1}). Rotifer supplementation was initiated with *Brachionus rotundiformis* at 20 rotifers mL^{-1}; at 11 dph, the density was decreased to 15 rotifers mL^{-1} and *B. plicatilis* was added at a density of 15 rotifers mL^{-1}, making a total of 30 rotifers mL^{-1} until 22 dph. At 13 dph, *Artemia* nauplii were added to the mix at a rate of 30 ind mL^{-1}, until 22 dph. Larvae were weaned onto commercial food (Gemma Wean™, Skretting; proximate composition: 62% protein, 14% lipid, 8% ash, 0.2% fiber) beginning at 20 dph and continued for six months. After six months, fish were fed Europa™, (Skretting; proximate composition: 55% protein, 15% lipid, 12% ash, 1.0% fiber, 8.5% moisture).

Specimen procurement

Two experiments were carried out at the Tropical Aquaculture Laboratory, Biological Sciences Academic Division, Juárez Autonomous University of Tabasco. Juveniles were obtained from induced spawnings in October 2013 and October 2014. Experimental juvenile fish (120 and 240 individuals) were randomly selected for experimental groups I and II, respectively. Juveniles were 340 dph in experiment I and 227 dph in experiment II. Pre-treatment gonadal condition was determined with conventional hematoxylin-eosin staining.

Experimental design

Separate experiments were carried out using a completely randomized design with one factor (E2 dose) to evaluate the feminizing effects of E2 in food. Each experiment consisted of four treatments that were run in triplicate. In the first experiment, we evaluated 40, 50, and 60 mg E2 kg^{-1} of food. In the second experiment, we assessed the effects of 10, 20, and 30 mg E2 kg^{-1} of food. In both experiments, a control group (0 mg E2 kg^{-1} of food) was included. Ten juveniles were randomly placed in each experimental unit for the first experiment, with an initial average total length of 150.2 ± 1.6 mm, and a weight of 30.1 ± 9.8 g. For the second experiment, we used 20 juveniles per replicate, with an initial average total length of 73.7 ± 4.5 mm, and a weight of 2.8 ± 0.4 g. Both experiments were carried out in a closed recirculating system, consisting of twelve 2,000 L capacity circular tanks. All tanks were filled with non-chlorinated freshwater and kept under chemical and mechanical filtration during the experiment. The water was filtered over a homemade filter tank to remove residual E2 and sediments. This filter was built using a 200 L plastic tank (60 cm diameter, 90 cm height) containing three layers of filtering materials: a) 5 kg of granular activated charcoal (Silva *et al.,* 2012) contained in a plastic mosquito mesh bag (1 mm mesh size); b) 10 kg of crushed oyster shells; and c) 10 kg of fine gravel. Water passed by gravity to a 5,000 L reservoir tank and recirculated into the fish tanks by using a 1.5 HP suction pump equipped with a pressured sand filter (Jacuzzi L190, USA). Waste accumulated in the fish tanks was siphoned daily, and 30% of the water was replaced once a week. Replicates from the control group were maintained in similar tanks separated from the recirculating system.

Experimental diets

In both experiments, we fed the fish with commercial marine-fish food Europa® (55% protein) impregnated with the different dosages of E2. The protocol proposed by Popma & Green (1990) was used to impregnate the food with 17β-estradiol, varying the dosages for each treatment. Briefly, the desired dose of E2 (Sigma-Aldrich Co., USA) was dissolved in 100 mL 96% ethanol and sprayed onto 1 kg of the powdered food. Ethanol was left to evaporate at ambient temperature in a dark room, and afterward, the food was stored refrigerated at 4°C. Food for the control groups was impregnated with 96% ethyl alcohol only. Fish were fed until apparent satiation at 8:00, 11:00, 14:00, and 17:00 h, during 60 days for both experiments.

Water quality

Dissolved oxygen, temperature, and pH were measured in both experiments. Dissolved oxygen (6.02 ± 0.02 and 6.4 ± 0.03 mg L^{-1}) was measured with a YSI Model 55 oxygen meter (Yellow Springs, USA), and temperature (30.2 ± 0.14°C and 29.5 ± 0.16°C) with a digital thermometer (YSI 55, USA). The pH (6.5 ± 0.07 and 7.1 ± 0.09) was measured using a pH meter (Denver Instrument UB-10, USA). We measured ammonia (0.1 ± 0.01 and 0.14 ± 0.011 mg L^{-1}), nitrite (0.01 ± 0.01 and 0.012 ± 0.013 mg L^{-1}), and nitrate (0.5 ± 0.03 and 0.7 ± 0.04 mg L^{-1}) concentrations with a multiparameter meter (Hanna Instruments, HI 98311, USA) every week.

Growth and survival assessment and histological analysis

Once the E2 exposure period concluded, we carried out three samplings (30, 180, and 300 days post-treatment) using a similar procedure in both experiments. At the end of each treatment, and in the subsequent samplings, weight was determined with an analytical scale (Ohaus®, USA), with an accuracy of 0.001 g, and we measured total length with digital vernier calipers (Carbon fiber Composites Digital®, USA), with an accuracy of 0.1 mm. Measurements were made on all specimens in each treatment. Survival was evaluated by counting the fish from each experimental unit at the end of the experiments. Subsamples were randomly taken from each replicate at each sampling time (n = 3, 2, and 1) totaling 18 per treatment in experiment I and 27 for experiment II (n = 3, 3, and 3) to determine sex ratio. Twelve (experiment I) and 33 (experiment II) fish per treatment remained after assessment of sex ratios for viability trials. Sampled fish were sacrificed with an overdose of tricaine methanesulphonate (MS-222; 1 g L^{-1}). Afterward, the fish were dissected, and the gonads were removed, fixed for 24 h in Bouin's solution, and then maintained in 50% ethyl alcohol. Samples were processed and stained with hematoxylin-eosin (H-E) (Aguilar *et al.*, 1996). Slides were examined with a compound microscope (Zeiss®, Germany) with 10x and 40x objectives. Sex was assigned histologically using the criteria for *C. undecimalis* adults as reported by Grier & Taylor (1998) and Grier (2000); for juveniles of the same species as reported by Huber *et al.* (2014); and as reported for other teleost fish species by multiple studies (Nakamura *et al.*, 1998; Devlin & Nagahama, 2002; Strüssmann & Nakamura, 2002; Meijide *et al.*, 2005).

Female maturation and viability

All remaining fish from treatments with 50 and 60 mg kg^{-1} (24) and the control groups (45) were implanted with a PIT tag (Avid Identification Systems, Inc., USA) in the body cavity and moved to the marine station (MAS-UJAT) for grow-out and spawning trials. At four years of age, during the spawning season (July-September), the fish kept for reproduction trials were anesthetized with clove oil (0.015 mL L^{-1}) and checked for maturity. Each remaining females and males were checked for maturity by taking oocytes or sperm from the genital pore with a polyethylene cannula. The fish were declared as mature when females presented vitellogenic oocytes and males if running milt was present. Oocyte diameter and maturity stage were determined for every fish. Five females (449.46 ± 161.33 g and 37.17 ± 4.47 cm) were selected for viability trials based on having oocytes larger than 150 µm in diameter. Ten males (317.36 ± 11.36 g and 34.50 ± 3.75 cm) with running milt were also selected. All selected fish were implanted with LHRH-a (150 µg kg^{-1}) handmade cholesterol-cellulose implants in the body cavity, near the pelvic fin. Fish were then placed in a 12.5 m^3 tank where salinity (30.67 ± 1.67), temperature (29.96 ± 0.86°C), dissolved oxygen (5.98 ± 0.43 mg L^{-1}), and pH (8.46 ± 0.22) were monitored three times a day. Five spawning trials were conducted using one female and two males per trial.

Statistical analysis

The effects of different E2 treatments on the sex ratio, growth (total length and weight), and survival rate, were analyzed using one-way ANOVA. Data expressed as percentages were arcsine-transformed before the ANOVA; however, sex ratios are reported as percentages. If the ANOVA revealed significant differences, Tukey's *post-hoc* test was performed. Normality (Shapiro-Wilk test) and variance homogeneity (Levene's test) were previously tested in all cases. All statistical analyses were performed using the Statgraphics Centurion® program, v XVI (level of significance $\alpha < 0.05$).

RESULTS

Feminization

A significantly higher percentage of females were obtained in the E2 treatments ($P < 0.001$). Induced females exhibited a normal pattern of ovarian development (Fig. 1) while the control groups consisted exclusively of males (Fig. 2). The fish fed with 50 and 60 mg of E2 kg^{-1} of food consistently produced 100% females, in the three consecutive samplings. Fish fed with 40 mg of E2 kg^{-1} of food produced significantly fewer (77.8% ± 5.6 SE, standard error) females ($P < 0.05$) compared to fish fed the highest dosages (Fig. 3). In the control group, 100% of males were consistently obtained at all sampling times, with different phases of spermatogenesis (Fig. 4).

In experiment II, a significant proportion of females were obtained in each treatment ($P < 0.05$). As in experiment I, the control group was all males, while fish fed the three E2 diets resulted in 37 to 63% females (Fig. 5). Fish fed 10 mg E2 kg^{-1} of food resulted in only 37.0 ± 6.4% females, a significantly lower percentage ($P < 0.05$) compared to those fed 20 and 30 mg E2 kg^{-1} of food (59.7 and 63.3% females ± 6.4 SE). Histological analysis confirmed that females developed ovaries with evidence of primary growth oocytes (Fig. 6).

Growth and survival

In the experiment I, the total length and final weight in all three samplings differed significantly among treatments ($P < 0.05$). At 30 days post-treatment, the longest (187.20 ± 12.0 mm) and the heaviest (55.46 ± 3.50 g) fish were obtained from the 60 mg E2 kg^{-1} treatment groups. Fish in the control group had the least growth (164.90 ± 11.7 mm and 35.93 ± 2.96 g). After 180 days post-treatment, the highest growth rates were found in the 60 mg E2 kg^{-1} of food treatment, and the lowest growth rates occurred in the control group. At 300 days, post-treatment, no significant statistical differences ($P > 0.05$) were found in total length and weight (Table 1). In experiment II, the total length and final weight for all three samplings differed significantly among the treatments ($P < 0.05$). At 30 days, the fastest growth in total length was observed in the treatment with 20 mg E2 kg^{-1} of food (187.20 ± 12.0 mm), and the greatest increase in weight was observed in the 30 mg E2 kg^{-1} group (55.46 ± 3.50 g). The least growth was observed in the control group. At 180 days, fish treated with 30 mg E2 kg^{-1} of food had the highest growth rates (215.57 ± 19.20 mm of total length and 58.80 ± 1.00 g of weight). Finally, at 300 days, significant differences persisted (Table 2). Survival was 100% in all treatments, for both experiments (Tables 1-2).

Sexual maturation and reproductive viability

All fish treated with 50 and 60 µg E2 kg^{-1} of food (experiment I) that were kept for sexual maturity assessments and viability tests were females producing oocytes (30 to 300 µm in diameter); while fish (45) from the control group were all males with running milt. Five of the females obtained through sex inversion had oocytes larger than 150 µm and were selected for induced spawning trials. Four of these females (80%) spawned, and produced fertilized eggs and larvae. An average of 386,425 ± 43,922 eggs was produced per female. The fertilization rate averaged 76.4 ± 14.71%. The average number of larvae obtained in each spawning trial was 243,621 ± 3,813.

DISCUSSION

Results indicate that strongly skewed populations with functional females can be obtained from immature males after feminization using 50 or 60 mg E2 kg^{-1} of food, for 60 days. Females obtained through sex inversion are fertile organisms of small size that can be induced to spawn and produce larvae. Induction of sex change in teleost fish is possible because germ cells are bipotential and their sensitivity to exogenous hormones allow disruption of the natural biochemical processes that govern sex differentiation, overriding normal developmental patterns even after gonadal differentiation has taken place (Devlin & Nagahama, 2002; Strüssmann & Nakamura, 2002). This plasticity in sexual development is the key to sexual manipulation in fish and can be effective even in the presence of genetic material for sex determination (Yamamoto, 1969; Goudie *et al.*, 1983; Pandian & Sheela, 1995; Piferrer, 2001; Devlin & Nahagama, 2002). Such manipulation of gonadal development by steroid induction has been used to obtain single-sex populations (Baroiller *et al.*, 1999; Devlin & Nahagama, 2002; Omoto *et al.*, 2002; Strüssmann & Nakamura, 2002), aiming to increase productivity by avoiding unwanted reproduction, favor the sex with fastest growth, and produce groups of females with enhanced production of eggs (Beardmore *et al.*, 2001; Piferrer, 2001; Devlin & Nahagama, 2002; Frisch, 2004).

In an extensive review, Piferrer (2001) cites over 50 species in which feminization has been carried out, with salmonids and cichlids been the most representative groups. These studies have examined the use of different steroids and dosages, lengths of steroid treatment and gonad development. With centropomids,

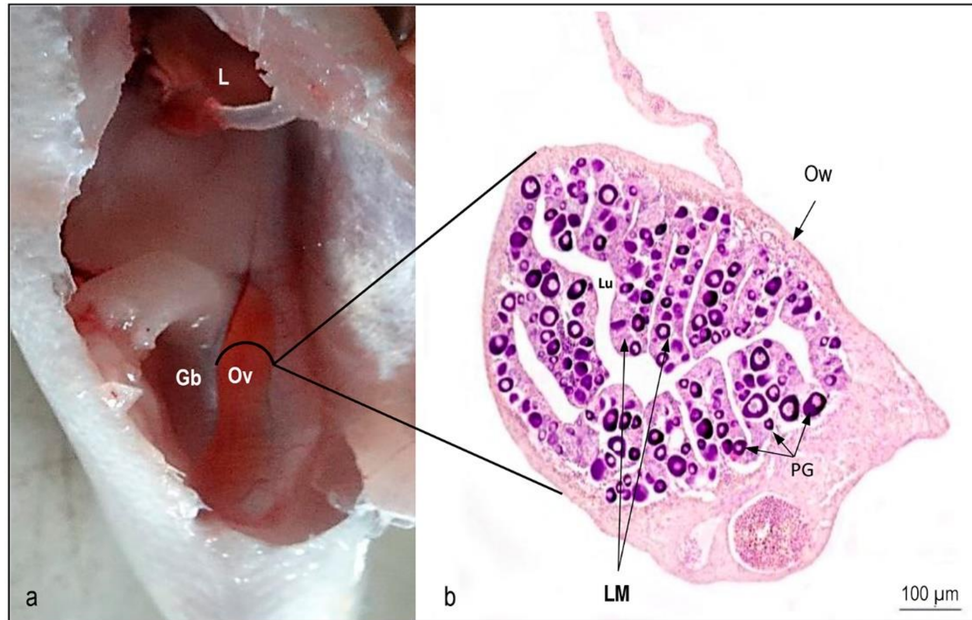

Figure 1. Characteristics of *Centropomus poeyi* ovaries. a) Anatomical position and macroscopic appearance of ovaries from females obtained after E2 treatment, b) microscopic view of a complete transverse section of an ovary. Liver (L); gas bladder (Gb); ovary (Ov); ovary wall (Ow); lamellae (LM); primary growth oocytes (PG); lumen (Lu). Hematoxylin and eosin.

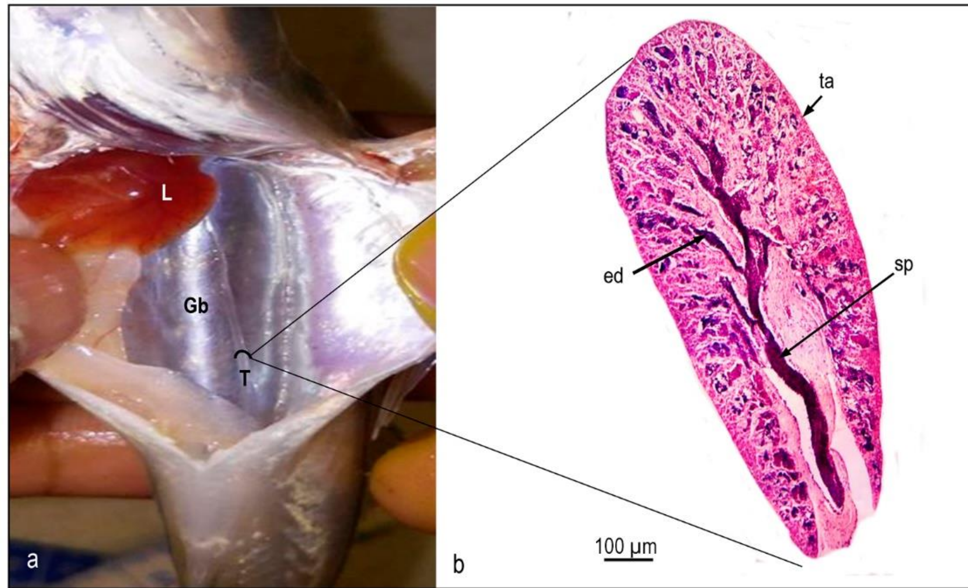

Figure 2. Characteristics of *Centropomus poeyi* testes. a) Anatomical position and macroscopic appearance of the testes from control group males, b) microscopic view of a complete transverse section of a testis. Liver (L); gas bladder (Gb); testis (T); efferent duct (ed); sperm (sp); tunica albuginea (ta). Hematoxylin and eosin.

specifically *C. undecimalis* and *C. parallelus*, positive results were obtained when the steroid was supplied using food or implants, obtaining high percentages of feminization in both species (Vidal-López *et al.*, 2012; Carvalho *et al.*, 2014; Passini *et al.*, 2016).

Histological evidence indicated that the fish used had begun to differentiate into males, supported with the appearance of the efferent duct, and an increase in the number and size of the primordial germinal cells. Therefore, we conclude that sex inversion was effective.

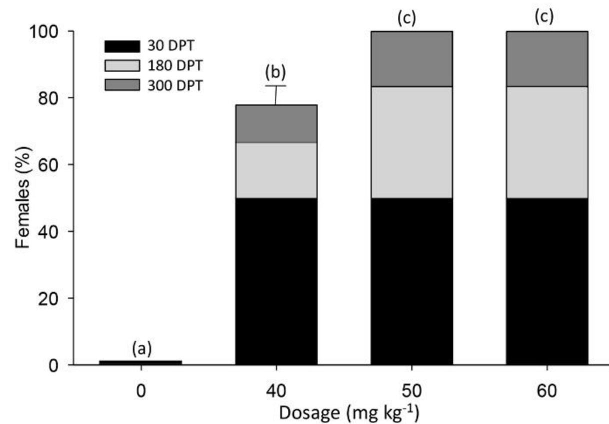

Figure 3. Average cumulative percent (± standard error) of *Centropomus poeyi* females obtained for the control and treatments fed high dosages of E2 in experiment I. Different color bars indicate percent females at each sampling time for a particular treatment. Different letters indicate statistically significant differences between treatments (ANOVA, $P < 0.05$). n = 18 for each treatment. DPT: Days post-treatment.

Since the terms "sex inversion" and "sex reversal" frequently appear interchangeably in the literature, we considered it necessary to reexamine this topic in the light of sex manipulation in hermaphroditic species: initially, Nakamura & Takahashi (1973) described sex reversal for gonochoristic species as the time when fish gonads are sexually undifferentiated but are susceptible to the action of exogenous steroids. Chan & Yeung (1983) further discussed this, defining the term as the transformation of an individual fish from one sex to another. Green *et al.* (1997) attempted to differentiate induction of sex change, in undifferentiated and differentiated fish, by arguing that the term "sex inversion" is defined as the process where the undifferentiated gonad is directed to a particular sex, and "sex reversal" as the induction of a differentiated gonad to become that of the opposite sex. In the case of hermaphroditic species, both terms stand, since sex change can be induced during both stages of gonadal development, supported by the successful sex inversion of juvenile sea bream, *Sparus aurata* (Condeça & Canario, 1999); common snook, *C. undecimalis* (Vidal-López *et al.*, 2012; Carvalho *et al.*, 2014) and fat snook, *C. parallelus* (Carvalho *et al.*, 2014). While sex reversal has been documented for adult blue-spotted grouper, *Epinephelus fario* (Kuo *et al.*, 1988), black porgy, *Acanthopagrus schlegeli* (Chang, *et al.*, 1994, 1995), black sea bass, *Centropristis striata* L. (Benton & Berlinsky, 2006), three-spot wrasse, *Halichoeres trimaculatus* (Nozu, *et al.*, 2009), and adult fat snook (Passini *et al.*, 2016). Contrary to this, Piferrer (2001) mentioned that for some gonochoristic species, once the sexual differentiation process is advanced, or the gonads are mature, it is more difficult to change the sex and may become impossible in some cases.

An important aspect of this type of study is the identification of an effective dose and an optimal time of steroid application, with the purpose of optimizing resources. We did not assess different times for steroid application, but the results of Vidal-López *et al.* (2012) in *C. undecimalis* were considered, suggesting that more than 21 days of exposure produced high rates of feminization. Nevertheless, in the present study, the assessment of different dosages was necessary, and we determined that dosages of 50 and 60 mg E2 kg^{-1} of food were highly efficient without compromising survival. The dose required for the feminization of fish species is highly variable. Piferrer (2001) detailed successful feminization using estradiol in food with dosages ranging from 1 mg kg^{-1} in *Mugil cephalus* to 400 mg kg^{-1} in *Poecilia reticulata*. The blackhead sea bream, *Acanthopagrus schlegelii*, can be feminized using as low as 4 mg kg^{-1} of estradiol in the diet;

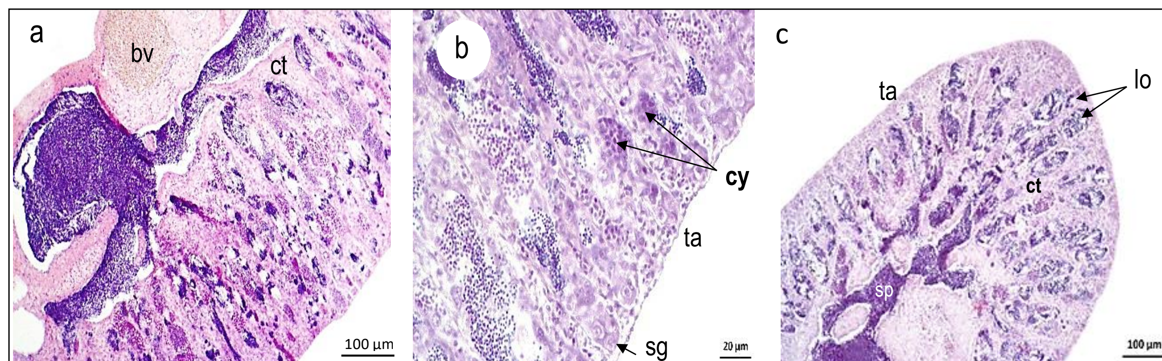

Figure 4. Unrestricted lobular testes from *Centropomus poeyi* obtained from males of the control group. a) Details from the transverse section of testes sampled at 60, b) 180 and c) 300 days post-treatment (100x; 40x and 10x, respectively). Efferent duct (ed); cysts (cy); spermatogonia (sg); sperm (sp); tunica albuginea (ta); blood vessels (bv); connective tissue (ct); lobules (lo). Hematoxylin and eosin.

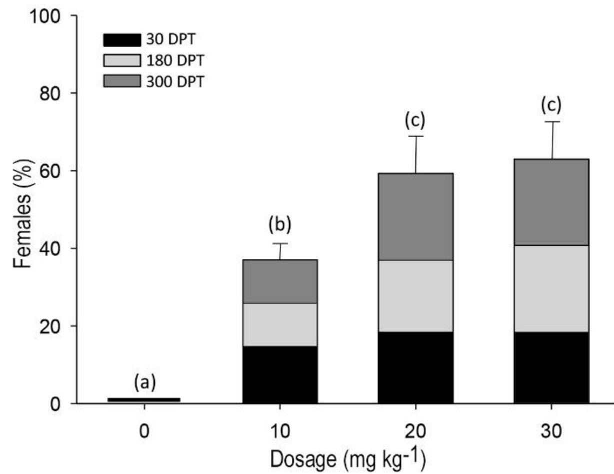

Figure 5. Average cumulative percent (± standard error) of *Centropomus. poeyi* females obtained in the experiment with 0, 10, 20 and 30 mg of E2 per kg of food. Different color bars indicate the percent of females at each sampling time for a particular treatment. Different letters indicate statistically significant differences between treatments (ANOVA, $P < 0.05$). n = 27 for each treatment. DPT: Days post-treatment.

however, this protocol requires five months of treatment (Chang & Lee, 1992). Strüssmann *et al.* (1996) obtained 100% females using 77 days of treatment with food impregnated with E2 at dosages of 20 mg kg^{-1} of food in the pejerrey, *Odontesthes bonariensis*. In centropomids, Vidal-López *et al.* (2012) presented histological evidence of significant feminization of the gonads in *C. undecimalis* by feeding 50 mg E2 kg^{-1} of food for 21 to 42 days. However, the reproductive viability of the females was not demonstrated. Likewise, complete sexual inversion was attained in adult males of this species, using implants at dosages ranging from 0.5 to 1.0 mg E2 kg^{-1} of fish (Passini *et al.*, 2016). In a closely related but smaller species (*C. parallelus*), Carvalho *et al.* (2014) produced up to 100% females when 25 mg E2 kg^{-1} of food was provided for 45 days. Unfortunately, in all cases, reproductive viability was not reported.

One important contribution of this investigation was the assessment of the treated fish at three different periods (30, 80, and 100 days post-treatment), to observe morphological and structural changes in the development of the gonad. Based on the histological analyses of 93 sex-inversed females, no abnormal morphological or structural changes were observed during gonad development, indicating that the ovaries followed a "normal pattern" of development. Furthermore, the high survival rate of the fish kept for viability tests and the successful production of larvae support the well-being of the females. However, it is important to mention that some studies have reported that exposure to estrogenic compounds can induce abnormalities in behavior and the structure and morphology of the gonads (intersex organisms and structural anomalies) and other organs. For example, Islinger *et al.* (2003) reported ultrastructural changes in the livers and testes of zebrafish, *Danio rerio*; Flynn & Benfey (2007) found mottled livers, swollen kidneys, and reduced gonad size in shortnose sturgeons, *Acipenser brevirostrum*; and Hendry *et al.* (2003) detailed irreversible injuries in Atlantic halibut, *Hippoglossus hippoglossus* L. including spinal deformities and high mortality rates, after E2 treatment. One possible explanation of these anomalies in growth and development may be related to altered interactions between sex steroids, growth hormone, and thyroid hormones (De Jesus *et al.*, 1992; Lin *et al.*, 1995) produced by the exogenous exposure to E2.

Growth was enhanced when juveniles were fed a diet containing 17β-estradiol. The highest dose of E2

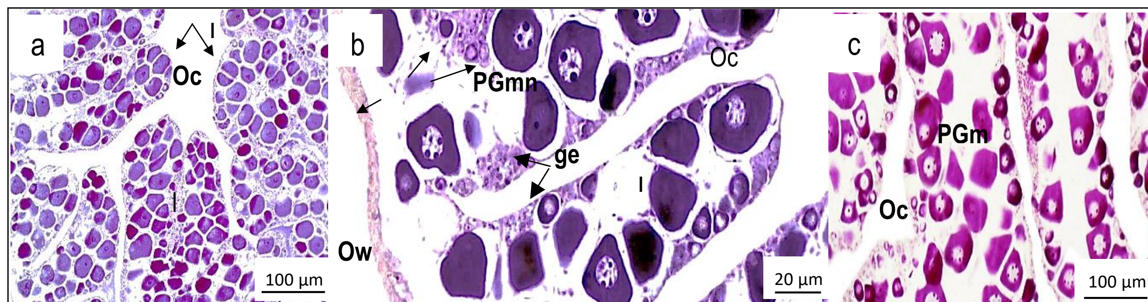

Figure 6. Cystovarian ovaries from *Centropomus. poeyi* obtained from E2-treated organisms. a) Details from transverse cuts of ovaries sampled at 60, b) 180 and c) 300 days post-treatment (100x; 40x and 10x, respectively). Ovarian cavity (Oc); lamellae (l); primary growth oocyte during multiple nucleoli stage (PGmn); germinal epithelium (ge); ovarian wall (Ow). Hematoxylin and eosin.

Table 1. Average values for weight and total length (± SE) of *Centropomus. poeyi* juveniles treated with 0, 40, 50 and 60 mg of E2 per kg of food (n = 30) at different days post-treatment (DPT). Survival was 100% in all treatments. Different letters indicate statistically significant differences ($P < 0.05$).

Treatment E2 (mg kg^{-1})	0 DPT mean (±SE)	30 DPT mean (±SE)	180 DPT mean (±SE)	300 DPT mean (±SE)
0	33.12g ± 1.43 g^{a}	35.93 ± 0.96 g^{a}	49.18 ± 0.35 g^{a}	175.03 ± 1.93 g^{a}
	158.97 ± 2.53 mm^{a}	164.90 ± 1.22 mm^{a}	173.10 ± 1.66 mm^{a}	270.60 ± 1.43 mm^{a}
40	43.98 ± 2.25 g^{ab}	50.34 ± 1.93 g^{ab}	57.48 ± 0.30 g^{b}	179.97 ± 1.56 g^{a}
	174.27 ± 3.09 mm^{b}	183.60 ± 2.34 mm^{b}	198.30 ± 0.54 mm^{b}	272.00 ± 1.67mm^{a}
50	46.66 ± 1.75 g^{b}	54.89 ± 2.33 g^{b}	57.93 ± 0.33 g^{b}	178.10 ± 0.97 g^{a}
	175.63 ± 2.85 mm^{b}	187.20 ± 1.78 mm^{b}	195.37 ± 1.53 mm^{b}	282.6 ± 2.32 mm^{a}
60	47.47 ± 1.71 g^{b}	55.46 ± 1.17 g^{b}	58.80 ± 0.41 g^{b}	193.73 ± 1.02 g^{a}
	174.18 ± 2.95 mm^{b}	184.70 ± 184.67 mm^{b}	215.57 ± 1.84 mm^{c}	279.3 ± 1. 86 mm^{a}

Table 2. Average values for weight and total length (± SE) of *C. poeyi* juveniles treated with 0, 10, 20 and 30 mg of E2 per kg of food (n = 60) at different days post-treatment (DPT). Survival was 100% in all treatments. Different letters indicate statistically significant differences ($P < 0.05$).

Treatment E2 (mg kg^{-1})	0 DPT mean (±SE)	30 DPT mean (±SE)	180 DPT mean (±SE)	300 DPT mean (±SE)
0	13.06 ± 0.17 g^{a}	17.19 ± 0.24 g^{a}	35.13 ± 1.35 g^{a}	40.02 ± 1.36 g^{a}
	81.79 ± 1.12 mm^{a}	85.93 ± 0.96 mm^{a}	189.22 ± 2.93 mm^{a}	192.11 ± 5.39 mm^{a}
10	13.93 ± 0.13 g^{a}	18.36 ± 0.38 g^{a}	44.32 ± 2.49 g^{b}	48.32 ± 1.59 g^{b}
	91.13 ± 1.07 mm^{b}	98.12 ± 1.15 mm^{b}	197.87 ± 4.59 mm^{b}	209.53 ± 2.02 mm^{b}
20	13.98 ± 0.12 g^{a}	18.72 ± 0.40 g^{a}	61.88 ± 1.53 g^{c}	70.72 ± 0.89 g^{c}
	91.63 ± 0.95 mm^{b}	99.33 ± 2.47mm^{b}	207.00 ± 4.52 mm^{b}	237.00 ± 2.35 mm^{c}
30	13.86 ± 0.09 g^{a}	18.47 ± 0.25 g^{a}	63.37 ± 0.86 g^{c}	73.59 ± 1.31 g^{c}
	91.83 ± 0.62 mm^{b}	99.23 ± 2.45 mm^{b}	211.94 ± 1.84 mm^{c}	257.50 ± 2.75 mm^{c}

resulted in the fastest growth, remaining significant for up to six months; however, all fish reached the same size by the end of the study. Researchs supporting these findings indicate that orally administered E2 has significant growth-promoting effects in fish (Malison *et al.*, 1988; Satoh & Nimura, 1991; Hiroaki *et al.*, 1993; Blázquez *et al.*, 1998) perhaps because E2 can enhance growth through increased appetite, improved digestion, or increased absorption (Woo *et al.*, 1993). However, contrasting results have been published on the effects of E2 on fish growth; some researchers claim no effects (Matty & Cheema, 1978; Woo *et al.*, 1993), and others report detrimental effects (Funk *et al.*, 1973; Johnstone *et al.*, 1978; Goetz *et al.*, 1979; Wang *et al.*, 2008). As mentioned above, these effects might be related to the altered interactions between sex steroids and growth hormone, particularly in gonochoric species. Conley & Walters (1999) highlighted the importance of balance between androgens and estrogens, which is essential for normal sexual development, reproductive function, and normal growth of both sexes in vertebrates. Undoubtedly, the expression and activity of the aromatase enzyme play a fundamental role in maintaining this critical balance. In our study, the survival of *C. poeyi* was not affected by E2 administration, indicating that this protandric species can process up to 60 mg of E2 per kilogram of food with no deleterious effects. Reports indicate that the negative effects of steroid administration on survival depend on some factors, with the most important being treatment timing, treatment intensity, and species (George & Pandian, 1996).

In the present study, successful feminization and reproductive viability of the females was achieved for the first time in *C. poeyi*. These are major accomplishments for snook aquaculture since females currently used for larval production of large snooks are obtained from wild populations and require between three to four years after confinement to respond to hormonal induction (Ibarra-Castro *et al.*, 2011; Contreras-García *et al.*, 2015). On top of this, broodstock management is also expensive and risky due to the large size of the females. The production of small amounts of larvae from the sex-reversed females (approximately 240,000 per female) provides a practical and manageable solution to handling millions of larvae during every spawning event.

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