https://doi.org/10.1590/S1413-415220210098

Artigo Técnico

Synthetic and natural hormones impact the zootechnical and morphological characteristics of Nile tilapia (*Oreochromis niloticus*)

Hormônios naturais e sintéticos impactam as características zootécnicas e morfológicas da tilápia do Nilo (Oreochromis niloticus)

Oscar Pacheco Passos Neto[1], André Bezerra dos Santos[1], Suetônio Mota[1]*

ABSTRACT

This work aimed to investigate the effects of the endocrine disruptors 17β-estradiol (E2) and 17α-ethinylestradiol (EE2) on Nile tilapia (*Oreochromis niloticus*) development, emphasizing the zootechnical and morphological aspects. The concentrations of E2 and EE2 tested were 250, 500, and 1,000 μg·L^{-1}. The evaluated compounds were capable of producing intersex individuals and causing zootechnical damage in Nile tilapia, with a significant decrease in the condition factor as the concentrations increased. Besides, these concentrations were also able to induce the development of morphological anomalies without any significant difference between them. E2 and EE2 exposure were shown to be lethal for Nile tilapia larvae, having no effect on the incubation time and the percentage of larvae hatching. Morphological anomalies such as head shape malformation, oral malformation, operculum malformation, belly retraction, distended abdomen with fluid accumulation (ascites), exophthalmos, signs of bleeding in the belly, and curved pectoral fin radii, were also observed, which impaired the fish development.

Keywords: endocrine disruptors; Nile tilapia (*Oreochromis niloticus*); zootechnical aspects; morphological aspects.

RESUMO

Este trabalho tem como objetivo investigar os efeitos dos desreguladores endócrinos 17β-estradiol (E2) e 17α-etinilestradiol (EE2) no desenvolvimento da tilápia do Nilo (*Oreochromis niloticus*) com ênfase no desempenho zootécnico e nos aspectos morfológicos. As concentrações de E2 e EE2 avaliadas foram de 250, 500 e 1.000 μg.L^{-1}. Os compostos estudados foram capazes de produzir indivíduos intersexo e causar danos morfológicos na tilápia do Nilo, com diminuição significativa no fator de condição, na medida em que as concentrações aumentaram em ambos os hormônios. Não foram observadas diferenças significativas no desenvolvimento de anomalias morfológicas entre as concentrações avaliadas. Também foi possível observar que as concentrações avaliadas de E2 e EE2 se mostraram letais para grande parte das larvas de tilápia do Nilo, não apresentando qualquer efeito no período de incubação dos ovos e na porcentagem de eclosão das larvas. As anomalias morfológicas mais observadas na presente pesquisa foram má formação da cabeça, má formação bucal, deformações no opérculo, retração ventral, abdômen distendido com acúmulo de líquido (ascite), exoftalmia, manchas hemorrágicas no ventre, curvatura nos raios das nadadeiras peitorais e atraso no desenvolvimento.

Palavras-chave: desreguladores endócrinos; tilápia do nilo (*Oreochromis niloticus*); aspectos zootécnicos; aspectos morfológicos.

INTRODUCTION

In the past few decades, many emerging pollutants have been detected and monitored in different water matrices. Among them, endocrine-disrupting chemicals (EDCs), have received global attention due to their estrogen effect, toxicity, persistence, and bioaccumulation in exposed individuals and populations (GAO *et al.*, 2020). EDCs reach aquatic environments from different sources such as wastewater, industrial contamination, hospital runoffs, solid waste, among others. In addition, the exposure of commercially and ecologically important marine and freshwater fish stocks to EDCs has seriously affected their production, besides leading to biotic toxicity and ecology dysfunction (REHMAN *et al.*, 2017; GOVARTHANAN *et al.*, 2022). The effects of many legacy chemicals with endocrine activity on wildlife are generally greater than those caused by current-use chemicals (MATTHIESSEN *et al.*, 2018).

[1] Universidade Federal do Ceará, Department of Hydraulic and Environmental Engineering – Fortaleza (CE), Brazil.
***Corresponding author:** suetonio@ufc.br
Conflicts of interest: the authors declare no conflicts of interest.
Funding: National Council for Scientific and Technological Development (CNPq), Process nº 481985/2012-3.
Received: 04/13/2021 – **Accepted:** 06/09/2021 – **Reg. ABES:** 20210098

EDCs cause disarray of endocrine axes and tissues by hampering different physiological parameters, including the brain, thyroid, immune, interrenal and gonadal systems in various fish species (KAR *et al.*, 2021). In addition, the main effects caused by EDCs in fish include abnormalities in the reproductive system of animals (47%), induction of vitellogenin (VTG) synthesis (20%), and species mortality (13%) (SILVA *et al.*, 2018). In addition to the development of female characteristics in male individuals, which generate abnormal production of VTG, low sperm count, and the appearance of intersex fish, EDCs can also act negatively on the nervous and immune systems, cause behavioral disorders, and affect the organisms' homeostasis in a general way (CZARNY *et al.*, 2017).

Among these endocrine disruptors, the 17β-estradiol (E2, natural estrogenic steroid) and 17α-ethinylestradiol (EE2, synthetic estrogenic steroid) are the most studied, as they are among the most widespread substances in the environment (CZARNY *et al.*, 2017). Also, according to these authors, some of these compounds (e.g., E2) are normally present in the urine of animals, and their concentration depends on sex, hormonal status, the existence of pregnancy, and the menstrual cycle phase. Synthetic forms, such as EE2, are present in contraceptive pills and hormone-based drugs.

The observed risks by the exposure to E2 in the health of aquatic organisms have raised concerns regarding what damage and/or health-related conditions are being expressed in terrestrial wildlife and the consequences for humans as a result of the lowest sensitive-concentration exposure to E2. In general, children and immature wildlife with lower body masses are at the greatest risk from elevated environmental E2 concentrations (NAZARI; SUJA, 2016).

Juárez *et al.* (2017) evaluated the effects of four concentrations (i.e., 100, 200, 300, and 400 $mg \cdot kg^{-1}$) of the estrogen EE2 on the sex ratio, growth, condition factor (F), and gonadosomatic index (GSI) of the progeny of XY-females. Significant differences were observed in wet weight, total length, and F during the experiment. However, final values in EE2-treated groups showed no significant differences compared with the control group. The group fed 200 $mg \cdot kg^{-1}$ of EE2 showed a significantly higher value of GSI than that observed in the control group. Increasing the concentration of EE2 makes it possible to increase the proportion of females without affecting growth or GSI.

The feminizing effect of endocrine disruptors of estrogenic action has been observed in fish that live in rivers close to large urban centers in different locations worldwide (MEIJIDE *et al.*, 2016). Also, laboratory tests have confirmed that considerably low doses of hormones, on the order of microgram per liter ($\mu g \cdot L^{-1}$) and nanogram per liter ($ng \cdot L^{-1}$), are capable of causing losses in the development of several species of fish (NIEMUTH; KLAPER, 2015). Studies carried out in some states in Brazil demonstrate that such concentrations can reach the order of $\mu g \cdot L^{-1}$ in surface waters close to large urban centers (MACHADO *et al.*, 2014; CAMPANHA *et al.*, 2015), especially due to the low sanitation coverage and wastewater treatment plants that are not designed to remove EDCs and other micropollutants.

Nile tilapia (*Oreochromis niloticus*) is currently produced in more than 80 countries (FAO, 2018). Because it is a very robust and prolific species, it can be found easily in the environment, such as in Brazil. Studies developed with Nile tilapia (*O. niloticus*) indicated that biotransformation of diuron to active metabolites alters signaling pathways of the central nervous system, which may impact androgen and the stress response as well as behavior necessary for social dominance, growth, and reproduction (BOSCOLO *et al.*, 2018).

A study by Alcántar-Vázquez (2018) evaluated the effect of different combinations of the three most important estrogens (i.e., E2, diethylstilbestrol [DES], and EE2) on sex proportion, growth, and gonadal development of Nile tilapia (*Oreochromis niloticus*). Mixtures evaluated were E2-DES, E2-EE2, DES-EE2, and E2-DES-EE2. No significant differences in growth were observed at the end of the experiment between control fish and fish-fed estrogen mixtures.

Therefore, studies that evaluate the effects of endocrine disruptors in a controlled environment are necessary, as they make it possible to evaluate these compounds in isolation, especially when tested in concentrations higher than those found in the literature, but reported in some effluents of treatment plants. In addition, due to the low dilution capacity of many surface waters, the problem may be potentialized. This study aimed to investigate the effects of the endocrine disruptors E2 and EE2 on Nile tilapia (*O. niloticus*) development, emphasizing the zootechnical and morphological aspects.

MATERIALS AND METHODS

Research location

The research was developed through a joint work between the Aquatic Resources Laboratory of the Department of Fisheries Engineering (LARAq/DEP) and the Sanitation Laboratory of the Department of Hydraulic and Environmental Engineering, both located on the Campus of Pici, Universidade Federal do Ceará (UFC), Fortaleza, Ceará, Brazil. Nile tilapia eggs (*O. niloticus*) were obtained from the Aquiculture Station of the Department of Fisheries Engineering – UFC.

Experimental design

The present study lasted 92 days, during which the effects of E2 and EE2 on zootechnical performance and morphological development of Nile tilapia were evaluated at concentrations of 250, 500, and 1,000 $\mu g \cdot L^{-1}$. Table 1 shows the order and period (days) of the steps involved in developing the research.

A total of 21 experimental units (40 L aquariums) were used to evaluate 7 treatments with 3 replications (120 L per treatment), including the control treatment. Therefore, the effect of the hormones E2 and EE2 was assessed in three different concentrations, i.e., 250, 500, and 1,000 $\mu g \cdot L^{-1}$, resulting in six treatments, namely, E2-250, E2-500, E2-1,000, EE2-250, EE2-500, and EE2-1,000. In each aquarium, an incubator of benthic eggs of the cylinder-conical type was set up with an upward flow of water driven by an air pump (Figure 1). In each incubator, 20 Nile tilapia eggs were stored.

Two days after storage in the incubators, unfertilized (non-viable) eggs were removed. After hatching, individuals still holding a yolk sac were counted to calculate the hatching percentage as a function of the number of viable eggs that

Table 1 – Research stages and periods.

Stage	Period (days)
Experiment	92
Concentration test	7
Egg incubation period	7
Hormonal fish exposure	28
Growth period	50

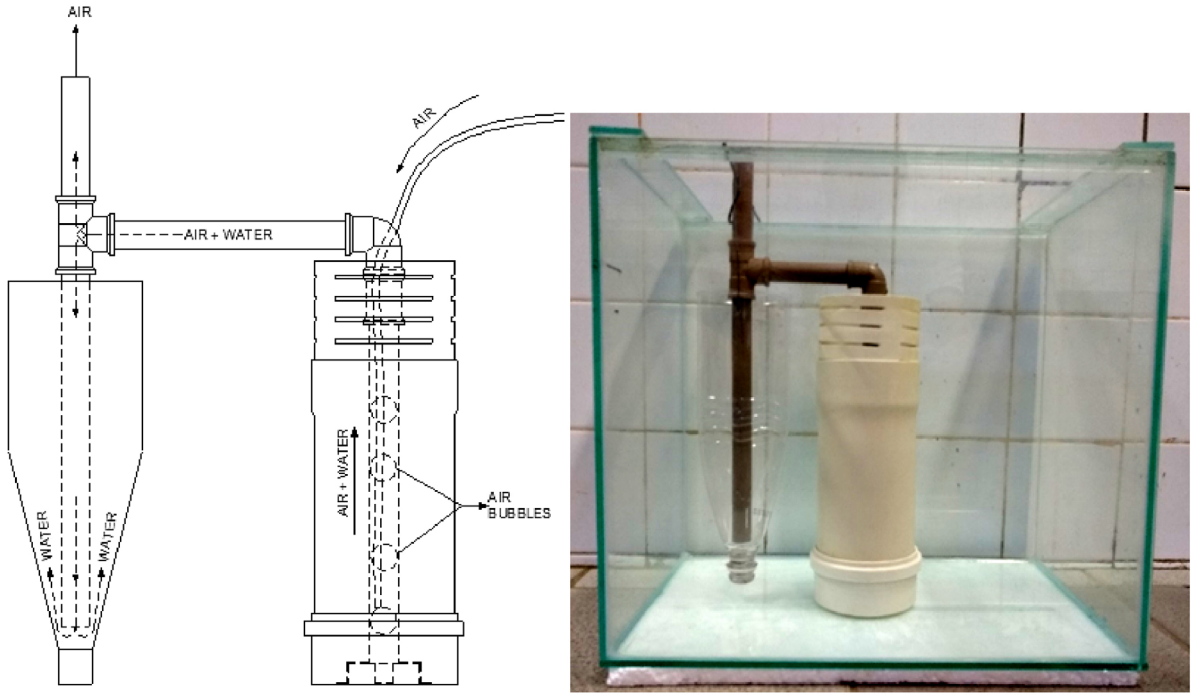


Figure 1 – Cylinder-conical incubator with ascending water flow driven by an air pump (airlift) used for incubating Nile tilapia eggs (*Oreochromis niloticus*) stored in different hormonal concentrations of E2 and EE2.

remained in the incubators. Four days after hatching, after the consumption of vitelline reserves, 10 individuals from each aquarium were randomly selected to remain in their respective aquarium under hormonal exposure for 28 days and for an additional period of 50 days to develop. Therefore, it was possible to observe and register their morphological characteristics.

Feeding and maintaining water quality

During the period of exposure to hormones E2 and EE2 (28 days), the individuals were kept in their experimental units with artificial lighting controlled by a timer (photoperiod of 10 h of light and 14 h of dark) and constant aeration provided with a blower (nominal flow of 120 $L \cdot min^{-1}$) connected to several air stones (Figure 1). Tap water was used for supplying the aquariums, which remained reserved in a 1,000 L tank and kept under vigorous aeration for at least 24 h for chlorine volatilization.

The fishes were fed four times a day (i.e., 9 h, 12 h, 14 h, and 16 h 30 min) with specific powdered food for tilapia at the development stage, following the manufacturer's recommendation. However, during the development of individuals, this amount was adjusted according to the apparent fish satiety. The water quality parameters routinely assessed were dissolved oxygen (DO) and temperature, monitored daily with the aid of an oximeter; and pH, total ammoniacal nitrogen (NH_3 / NH_4^+; TAN) and nitrite (NO_2^-), measured every 5 days, by using colorimetric tests. Indirectly, starting from the TAN values and crossing the pH and temperature data, the concentration of non-ionized ammonia (NH_3) was obtained. The maintenance of water quality was carried out daily through the siphoning of feces and food remains, always before the first feeding. This management was divided into two stages:

- maintaining water quality during hormonal exposure;
- maintaining water quality after hormonal exposure.

During the hormonal exposure, the siphoned water, which contains feces and uneaten feed, was filtered with an acrylic blanket and returned to its respective aquarium. In this step, the siphoning time was sufficient only to remove debris, with no control over the drained volume. Once a week, all the water in the aquarium was renewed, and the hormonal dosage was readministered. The water drained from the aquariums during this renovation was deposited in a 1,000 L reservoir and treated with ultraviolet radiation for a week before final disposal, according to data presented by Birkett and Lester (2003) and Sornalingam *et al.* (2016).

After the hormonal exposure completion, the water from all aquariums was completely drained and deposited in a 1,000 L reservoir for UV treatment as already specified. The aquariums were then washed with soap and water and rinsed thoroughly.

In the second stage (50 days), to maintain water quality, the siphoned volume for removing particulate material started to be discarded, and the level of the experimental unit was recomposed with fresh tap water. Thus, a percentage of 10% of the total volume was standardized for daily replacement.

Preparation of the stock solution, administration of the hormonal dose, and concentration test

To reach the theoretical concentrations of 250, 500, and 1,000 $\mu g \cdot L^{-1}$, the amounts of 30, 60, and 120 mg, respectively, of the hormones E2 and EE2 were used for each treatment (120 L). The hormones were diluted in 10 mL

of ethyl alcohol (analytical grade) for the stock solution preparation, using a 250 mL conical flask, and processed in a 42 kHz ultrasonic processor for 120 s. Then, 200 mL of purified water (Milli-Q) was added to each flask, reaching a total of 210 mL. This mixture was processed in an ultrasonic processor for 240 s. A volume of 70 mL of this stock solution was carefully added to each experimental unit.

To ensure that the control was under the same conditions as the other treatments, except for the hormone presence, 10 mL of ethyl alcohol (analytical grade) was processed in the ultrasonic processor, for 120 s, in a 250 mL conical flask; 200 mL of purified water was added and processed for 240 s. Of this solution, 70 mL was added to each repetition (Table 2). For the concentration test and for the elaboration of the hormonal decay curve, seven aquariums were used, one referring to each treatment: C, E2-250, E2-500, E2-1,000, EE2-250, EE2-500, and EE2-1,000.

At pre-established intervals for 1 week (days 0, 2, 4, and 7), 500 mL water samples from each aquarium were taken to measure the hormonal concentration by high-performance liquid chromatography (HPLC).

Analytical methodology for measuring hormones

Initially, the samples (500 mL) were filtered through a glass fiber filter with a diameter of 47 mm and a porosity of 0.45 µm. For this purpose, a vacuum pump (MFS, VP-24) was used. The filtrate was then transferred to a flat-bottomed volumetric flask for subsequent concentration procedure. In this regard, solid-phase extraction (SPE) with Strata-X cartridges was used, which were coupled to a vacuum manifold manual processor (Applied Separations, Speed Mate 12) connected to a vacuum pump.

Initially, the cartridges were conditioned with 10 mL of methanol. The hormones were then extracted by the SPE cartridges keeping the water flow rate between 1.5 and 2.0 mL·min^{-1}. The identification and quantification of the compounds present in the eluate were performed using an HPLC (20A Prominence, Shimadzu), equipped with a UV-VIS detector (SPD-20A; 215 nm) and a C18 column (15 cm × 4.6 mm DI, 0.4 µm, Shimadzu). The run gradient elution applied was (acetonitrile/HCl 0.1%): increase from 15% to 80% acetonitrile in 10 min, returning to 15% in 4 min. The initial flow was 1.0 mL·min^{-1}, and after 5 min, the flow was increased to 2.0 mL·min^{-1}. The furnace temperature was maintained at 35 °C, and the injection volume at 20 µL.

Statistical analysis of the data

Water quality data analysis was performed for the physical and chemical parameters (i.e., DO, temperature, pH, nitrite, TAN, and non-ionized ammonia). For zootechnical variables (i.e., weight, length, and survival), descriptive statistics (i.e., mean, standard deviation, maximum, and minimum values) were applied. For hatching percentage, analysis of variance (ANOVA) at 5% significance was applied, considering the main effect of two groups: hormone and hormonal concentration. If so, Tukey's test was applied to compare the means using the systematic component (Equation 1).

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \varepsilon_{ijk} \quad (1)$$

where:

Y_{ijk} = hormone type i, hormonal concentration j and repeat k (i = 1, 2; j = 1, 2, 3; k = 1, 2, 3);

μ = populational average;

α_i = effect of type of hormone i;

β_j = effect of hormonal concentration j;

ε_{ijk} = residual error.

The values expressed as a percentage were transformed into arccosine to apply the statistical test. The data were submitted to the Lilliefors test to verify normality. Data analyses were performed using the Program of Statistical Applications in the Areas of Biomedical Sciences (BioEstat, version 5.0) at a significance level of 5%.

RESULTS AND DISCUSSION

Parameters of water quality

The water quality parameters showed slight variation between treatments, with no significant difference ($p > 0.05$), as shown in Table 3. DO concentrations achieved in the treatments were around 6.7 mg·L^{-1}, therefore higher than the value of 4.0 mg·L^{-1} considered as the minimum DO concentration in water for Nile tilapia to express its full zootechnical potential.

During the research, pH remained with an average value close to 7.5, which is in the optimal pH range between 6.0 and 9.0 for breeding Nile tilapia. Low average

Table 2 – Schematic representation of the experimental design, stock solution preparation, and hormonal dose administration.

Variable	Treatment (compound-concentration in µg·L^{-1})						
	Control-0	E2-250	E2-500	E2-1,000	EE2-250	EE2-500	EE2-1,000
Egg/Aq	20	20	20	20	20	20	20
Larvae/Aq	10	10	10	10	10	10	10
Horm. (mg)	0.0	30.0	60.0	120.0	30.0	60.0	120.0
AL. (mL)	10.0	10.0	10.0	10.0	10.0	10.0	10.0
US Cl. (s)	120.0	120.0	120.0	120.0	120.0	120.0	120.0
Water (mL)	200.0	200.0	200.0	200.0	200.0	200.0	200.0
US Cl. (s)	240	240	240	240	240	240	240
V/Aq (mL)	70.0	70.0	70.0	70.0	70.0	70.0	70.0

Egg/Aq: number of eggs per aquarium; Larvae/Aq: number of larvae per aquarium; Horm.: hormone; AL.: alcohol; US Cl.: Ultrasonic Cleaner; V/Aq: volume of stock solution per aquarium.

Table 3 – Dissolved oxygen (DO), temperature (Temp.), Hydrogen potential (pH), nitrite (NO_2^-), total ammoniacal nitrogen (TAN), and non-ionized ammonia (NH_3) from the water used in the cultivation of juvenile Nile tilapia (*Oreochromis niloticus*). Exposure to concentrations of 250, 500, and 1,000 µg·L^{-1} of the hormones E2 and EE2 during the hatching phase.

Treatment	DO (mg·L^{-1})	Temp. (°C)	pH	NO_2^- (mg·L^{-1})	TAN (mg·L^{-1})	NH_3 (mg·L^{-1})
C	6.8 ± 1.0	27.6 ± 1.2	7.6 ± 0.5	0.15 ± 0.09	0.25 ± 0.17	0.006 ± 0.002
E2-250	6.7 ± 1.1	27.4 ± 1.2	7.6 ± 0.5	0.28 ± 0.30	0.27 ± 0.15	0.007 ± 0.003
E2-500	6.8 ± 1.1	27.4 ± 1.2	7.6 ± 0.6	0.31 ± 0.25	0.32 ± 0.20	0.008 ± 0.003
E2-1,000	6.7 ± 1.1	27.4 ± 1.1	7.5 ± 0.6	0.22 ± 0.19	0.26 ± 0.18	0.006 ± 0.001
EE2-250	6.6 ± 1.2	27.2 ± 1.2	7.6 ± 0.5	0.38 ± 0.23	0.35 ± 0.24	0.009 ± 0.004
EE2-500	6.6 ± 1.2	27.2 ± 1.2	7.5 ± 0.6	0.19 ± 0.22	0.33 ± 0.19	0.008 ± 0.005
EE2-1,000	6.7 ± 1.2	27.2 ± 1.2	7.5 ± 0.5	0.37 ± 0.28	0.22 ± 0.21	0.005 ± 0.001

C: Control; Treatment: compound-concentration in µg·L^{-1}

concentrations were found for TAN and NH_3 (< 0.01 mg·L^{-1}) regardless of the treatment applied. Fish passively excrete ammonia through the gills, in which concentrations of non-ionized ammonia in water above 0.1 mg·L^{-1} reverse the excretion route, causing blood concentration to increase. Moreover, the accumulation of ammonia in the tissues leads to a generalized dysfunction of the cellular oxidative metabolism, mainly neurons.

Throughout the research, it was possible to observe that the water quality parameters were always within the comfort range of Nile tilapia cultivation. Thus, it can be inferred that any damage caused to individuals' development was due to the hormones E2 and EE2 exposure.

Nile tilapia zootechnical performance parameters

It was possible to observe a decrease in hormonal concentration over 1 week. However, the final concentration could still be measurable (data not shown), indicating that the individuals were subjected to hormone exposure during this time. As the hormones were replaced every 7 days, it is possible to state the exposure during the total experimental period of 28 days. During and after the individuals' hormonal exposure, high mortality was observed in treatments with E2 and total mortality of individuals exposed to treatments with EE2 (Table 4).

Due to this low survival, it was not possible to apply a statistical test to compare the zootechnical parameters such as weight, length, and survival. Thus, only descriptive statistics were applied. As shown in Table 4 for the hormonal concentration criterion, the tilapia survival in treatment E2-250 was four individuals (13.3%), and only one fish survived (3.3%) in treatment E2-500 until the hormonal exposure period completion. However, survival was 80.0% in the control (treatment C). When considering the type of hormone criterion, a survival of 5.6% for E2 and 0.0% for EE2 was achieved. However, the hormonal concentrations evaluated did not affect the hatching percentage, although lethal for the young forms of Nile tilapia. There was no significant difference in hatching percentage when considering hormonal concentration criteria ($p = 0.2870$) and the type of hormone ($p = 0.1135$).

Studying different concentrations of EE2 (i.e., 0, 10, 1,000, and 10,000 ng·L^{-1}), Boudreau *et al.* (2004) found that this hormone negatively affected several parameters of zootechnical performance of the fish *Fundulus heteroclitus*, including survival, having no effect on the incubation time and the hatching percentage of the larvae.

Table 4 – Zootechnical parameters of juvenile Nile tilapia (*Oreochromis niloticus*). Exposure to concentrations of 250, 500, and 1,000 µg·L^{-1} of the hormones E2 and EE2 during the egg incubation period and early stage of development. Hatching percentage (HP), weight (W), total length (TL), and survival (S). Mean ± standard deviation.

Treatment	HP (%)	W (g)	TL (cm)	S (%)
C[1,3]	94.7 ± 5.0	0.6 ± 0.5	3.2 ± 1.0	80.0
E2-250[2,3]	81.2 ± 6.5	0.4 ± 0.3	2.7 ± 0.9	13.3
E2-500[2,3*]	75.7 ± 15.3	0.76	3.7	3.3
E2-1,000[2,3]	86.7 ± 2.9	ns	ns	0.0
EE2-250[2,3]	88.3 ± 16.1	ns	ns	0.0
EE2-500[2,3]	75.7 ± 15.8	ns	ns	0.0
EE2-1,000[2,3]	81.0 ± 11.1	ns	ns	0.0
Concentration (F;p)	1.3839; 0.2870	-	-	-
C[1,4]	94.7 ± 5.0	0.58 ± 0.46	3.2 ± 1.0	80.0
E2[2,4]	81.2 ± 9.6	0.44 ± 0.32	2.9 ± 0.9	5.6
EE2[2,4]	86.7 ± 14.0	ns	ns	0.0
Hormone (F;p)	0.4456; 0.1135	-	-	-

ns: Total mortality of individuals; [1]C: Control; [2]Treatment: compound-concentration in µg·L^{-1}; [3]Hormonal concentration criterion; [4]Hormone criteria; *Only one individual survived.

Morphology

Other adverse effects caused by Nile tilapia exposure to E2 and EE2 were developmental delay and the presence of varied forms of deformities (Figure 2). No individual with a malformation was observed in the control treatment, while all surviving individuals from treatments E2-250 (four individuals) and E2-500 (one individual) presented abnormalities.

Figure 3 shows characteristic examples of malformations found in Nile tilapia in the present study after the 50-day growth period. Among the deformities present, it was possible to observe the retracted belly, a condition often associated with nutritional deficiency of physiological origin; mouth deformations; head shape deformations; exophthalmos (bulging eyes); distended abdomen with fluid accumulation (ascites); gills exposed due to operculum deformities; curved pectoral fins rays; and signs of bleeding in the belly, due to buoyancy problems and direct contact of this body region with the aquarium bottom.

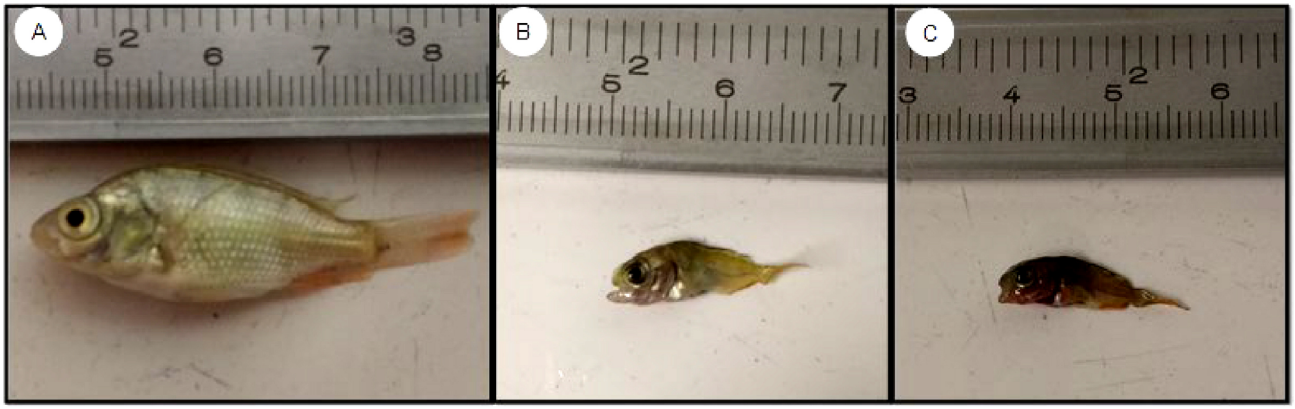
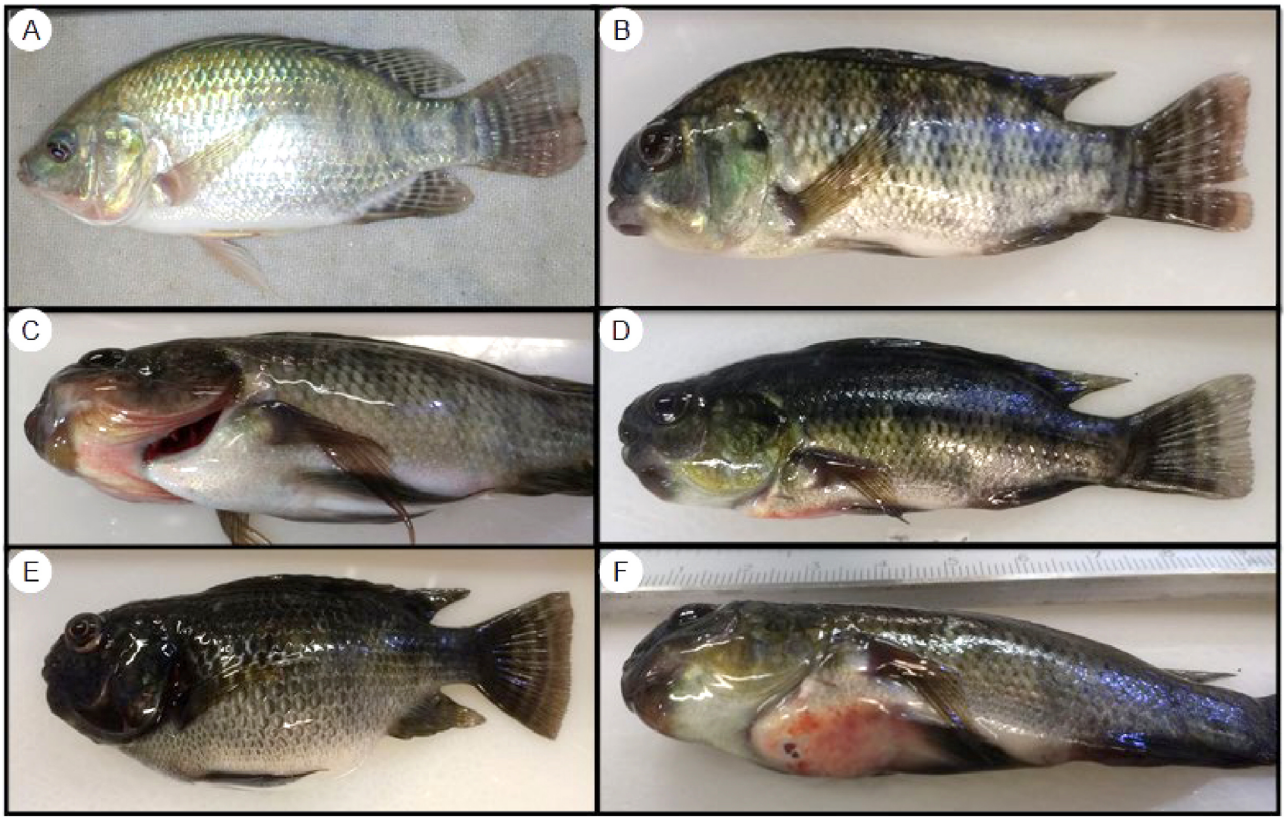


Figure 2 – Nile tilapia (*Oreochromis niloticus*) specimens at the end of the 28-day exposure period to the hormones E2 and EE2. (A) Individual with normal development; (B and C) individuals with developmental delay.



(A) Normal individual; (B, C, D, E and F) Head shape malformation; (B, C, D, E and F) Oral malformation; (C) Operculum malformation; (B, D and F) Belly retraction; (E) Distended abdomen with fluid accumulation (ascites); (E) Exophthalmos; (D and F) Signs of bleeding in the belly; (C, D and F) Curved pectoral fin radii.

Figure 3 – Nile tilapia (*Oreochromis niloticus*) specimens at the end of the 50-day growth period. Animals exposed to the hormones E2 and EE2 during the first 28 days of life.

Since only a few studies evaluate the effects of estrogenic substances on the morphological development of fish, especially during the early stages of life, it was not easy to compare the data obtained with those present in the literature. Besides, the methodological differences applied by each researcher, the different stages of fish development, the different concentrations tested, the different exposure times, the variety of compounds used, the different ways of exposing (in water and food), and the number of fish species assessed, make this task even more arduous.

Among the species most used for laboratory evaluation, the morphological effects caused by substances with potential for endocrine disruption found

in the literature can be cited: *Oryzias latipes* (internationally popular name, Japanese medaka) (PATYNA *et al*., 1999), *Danio rerio* (zebrafish) (ANDERSEN *et al*., 2001), *Cyprinodon variegatus* (sheepshead minnows) (ZILLIOUX *et al*., 2001), *F. heteroclitus* (mummichog) (BOUDREAU *et al*., 2004), *Pimephales promelas* (fathead minnows) (PARROTT; WOOD, 2002), *Poecilia reticulata* (guppy) (VOLKOVA *et al*., 2015) and *Melanotaenia fluviatilis* (murray rainbowfish) (WOODS; KUMAR, 2011). Among the species listed above, only zebrafish and guppy are easily found in Brazil. No study using Nile tilapia was found to compare the data obtained.

Boudreau *et al*. (2004), studying the effect of EE2 on the development of morphological changes in *F. heteroclitus*, in concentrations ranging from 0 to 10,000 ng·L^{-1}, found the same deformities observed in Nile tilapia in the present study. The researchers reported swelling of soft tissues near the anal region (ascites), craniofacial deformations, hemorrhage in different parts of the body, deformities in the mouth, abnormal coloration, developmental delay, deformations in the eyes, and curved fins rays, in addition to lordosis and scoliosis. Boudreau *et al*. (2004) also reported that some fish remained lying at the bottom of the experimental units, but they ate normally and survived until the experimental period completion.

Länge *et al*. (2001) found severe malformations in *P. promelas* exposed to low concentrations of EE2 (16 and 64 ng·L^{-1}) during their life cycle, such as atrophy, hemorrhages, distended abdomen, and curvature of the spine (lordosis and scoliosis). Lei *et al*. (2014) mainly observed scoliosis and enlarged abdomen in *O. latipes* exposed to estriol concentrations (E3) that varied from 5 to 5,000 ng·L^{-1}.

Porseryd (2018) found that zebrafish exposed to low EE2 concentrations during development showed both increased anxiety-like behavior and decreased fertility that were persistent in adulthood, even after a long remediation period in clean water. Alterations accompanied the altered behavior and lowered fertility in the testis and brain transcriptome of possible significance for the behavior and fertility effects.

Voisin *et al*. (2019) exposed mangrove rivulus (*Kryptolebias marmoratus*) for 28 days post-hatching (dph) to 4 and 120 ng·L^{-1} EE2, and raised for 140 days in clean water. The effects of EE2 were tissue and dose-dependent, and the results suggest that estrogen-responsive pathways, such as lipid metabolism, inflammation, and the innate immune system, were affected months after the exposure.

The bone deformations observed in the current study in the head region of Nile tilapia must be related to the effect of estrogens on bone calcification. According to the study by Oursler (1998), estrogens play a role in the development of different types of cells that are involved in the formation and modeling of bones. This information was corroborated by Armour *et al*. (1997), who observed an increase in bone calcification in rainbow trout (*Oncorhynchus mykiss*) exposed to E2. Boudreau *et al*. (2004) observed bone deformities in the fins, mouth, and head of *F. heteroclitus* exposed to EE2. These results seem to support the hypothesis that E2 was responsible for the deformities observed in this study. Bone changes in the spine were also observed in *O. latipes*, who received doses of E2 included in the diet (0.05 - 5.00 mg/kg of food).

The only deformity seen in Nile tilapia that seems to be unrelated to the bone structure was ascites. Several authors have reported this same deformity with different names, such as anal protrusion (LÄNGE *et al*., 2001) and anal swelling (BOUDREAU *et al*., 2004). Unlike what was observed in this study, Boudreau *et al*. (2004) reported that, after the end of EE2 exposure, ascites regressed in *F. heteroclitus*.

CONCLUSIONS

The concentrations of E2 and EE2 in the order of 250, 500, and 1,000 µg·L^{-1} proved to be lethal for Nile tilapia larvae, having no effect on the eggs' incubation time and the percentage of larvae hatching. Concentrations of E2 of 250 and 500 µg·L^{-1} were able to cause zootechnical losses in Nile tilapia, with a high mortality rate and significant delay in development.

The evaluated compounds were capable of producing intersex individuals and causing zootechnical damage in Nile tilapia, with a significant decrease in the condition factor as concentrations increased. Besides, these concentrations were also able to induce the development of morphological anomalies such as head shape malformation, oral malformation, operculum malformation, belly retraction, distended abdomen with fluid accumulation (ascites), exophthalmos, signs of bleeding in the belly, and curved pectoral fin radii that lasted throughout the experimental period, even after the contact with the hormone ceased.

AUTHORS' CONTRIBUTION

Passos Neto, O.P.: Conceitualização, Investigação, Escrita — Primeira Redação. Santos, A.B.: Conceitualização, Orientação, Escrita — Primeira Redação. Mota, S.: Conceitualização, Orientação, Escrita — Primeira Redação.

REFERENCES

ALCÁNTAR-VÁZQUEZ, J.P. Sex proportion in Nile tilapia *Oreochromis niloticus* fed estrogen mixtures: a case of paradoxical masculinization. *Latin American Journal of Aquatic Research*, v. 46, n. 2, p. 337-345, 2018. https://doi.org/10.3856/vol46-issue2-fulltext-9

ANDERSEN, L.; PETERSEN, G.I.; GESSBO, A.; ÖRN, S.; HOLBECH, H.; BJERREGAARD, P.; NORRGREN, L. Zebrafish Danio rerio and roach Rutilus: two species suitable for evaluating effects of endocrine disrupting chemicals? *Aquatic Ecosystem Health & Management*, v. 4, n. 3, p. 275-282, 2001. https://doi.org/10.1080/146349801753509177

ARMOUR, K.J.; LEHANE, D.B.; PAKDEL, F.; VALOTAIRE, Y.; RUSSELL, R.G.G.; HENDERSON, I.W. Estrogen receptor mRNA in mineralized tissues of rainbow trout: calcium mobilization by estrogen. *Febs Letters*, v. 411, n. 1, p. 145-148, 1997. https://doi.org/10.1016/s0014-5793(97)00680-7

BIRKETT, J.W.; LESTER, J.N. *Endocrine disrupters in wastewater and sludge treatment processes*. New York: Lewis Publishers, 2003. 310 p.

BOSCOLO, C.N.P.; PEREIRA, T.S.B.; BATALHÃO, I.G.; DOURADO, P.L.R.; SCHLENK, D.; ALMEIDA, E.A. Diuron metabolites act as endocrine disruptors and alter aggressive behavior in Nile tilapia (*Oreochromis niloticus*). *Chemosphere*, v. 191, p. 832-838, 2018. https://doi.org/10.1016/j.chemosphere.2017.10.009

BOUDREAU, M.; COURTENAY, S.C.; MACLATCHY, D.L.; BÉRUBÉ, C.H.; PARROTT, J.L.; VAN DER KRAAK, G.J. Utility of morphological abnormalities during early-life development of the estuarine mummichog, *Fundulus heteroclitus*, as an indicator of estrogenic and antiestrogenic endocrine disruption. *Environmental Toxicology and Chemistry*, v. 23, n. 2, p. 415-425, 2004. https://doi.org/10.1897/03-50

CAMPANHA, M.B.; AWAN, A.T.; SOUSA, D.N.R.; GROSSELI, G.M.; MOZETO, A.A.; FADINI, P.S. A 3-year study on occurrence of emerging contaminants in an urban stream of São Paulo State of Southeast Brazil. *Environmental Science and Pollution Research International*, v. 22, n. 10, p. 7936-7947, 2015. https://doi.org/10.1007/s11356-014-3929-x

CZARNY, K.; SZCZUKOCKI, D.; KRAWCZYK, B.; ZIELIŃSKI, M.; MIĘKOŚ, E.; GADZAŁA-KOPCIUCH, R. The impact of estrogens on aquatic organisms and methods for their determination. *Critical Reviews in Environmental Science and Technology*, v. 47, n. 11, p. 909-963, 2017. https://doi.org/10.1080/10643389.2017.1334458

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS (FAO). *The state of world fisheries and aquacultura 2018:* meeting the sustainable development goals. Rome: FAO, 2018. Available: http://www.fao.org/3/i9540en/i9540en.pdf. Acessed on: Nov 1, 2019.

GAO, X.; KANG, S.; XIONG, R.; CHEM, M. Environment-friendly removal methods for endocrine disrupting chemicals. *Sustainability*, v. 12, n. 18, p. 7615, 2020. https://doi.org/10.3390/su12187615

GOVARTHANAN, M.; LIANG, Y.; KAMALA-KANNAN, S.; KIM, W. Eco-friendly and sustainable green nano-technologies for the mitigation of emerging environmental pollutants. *Chemosphere*, v. 287, part 2, 132234, 2022.

JUÁREZ, V.J.; VÁZQUEZ, J.P.A.; ANTONIO-ESTRADA, C.; MARÍN-RAMIREZ, J.A.; DE LA TORRE, R.M. Feminization by 17α-ethinylestradiol of the progeny of XY-female Nile tilapia (Oreochromis niloticus). Effects on growth, condition factor and gonadosomatic index. *Turkish Journal of Fisheries and Aquatic Sciences*, v. 17, n. 3, p. 599-607, 2017. https://doi.org/10.4194/1303-2712-v17_3_16

KAR, S.; SANGEM, P.; AMUSHA, N.; SENTHILKUMARAN, B. Endocrine disruptors in teleosts: evaluating environmental risks and biomarkers. Aquaculture and Fisheries, v. 6, n. 1, p. 1-26, 2021. https://doi.org/10.1016/j.aaf.2020.07.013

LÄNGE, R.; HUTCHINSON, T.H.; CROUDACE, C.P.; SIEGMUND, F.; SCHWEINFURTH, H.; HAMPE, P.; PANTER, G.H.; SUMPTER, J.P. Effects of the synthetic estrogen 17 α-ethinylestradiol on the life-cycle of the fathead minnow (*Pimephales promelas*). *Environmental Toxicology Chemistry*, v. 20, n. 6, p. 1216-1227, 2001. https://doi.org/10.1002/etc.5620200610

LEI, B.; KANG, J.; YU, Y.; ZHA, J.; LI, W.; WANG, Z.; WANG, Y.; WEN, Y. Long-term exposure investigating the estrogenic potency of estriol in Japanese medaka (*Orysias latipes*). *Comparative Biochemistry and Physiology, Part C*, v. 160, p. 86-92, 2014. https://doi.org/10.1016/j.cbpc.2013.11.001

MACHADO, K.S.; CARDOSO, F.D.; AZEVEDO, J.C.R.; BRAGA, C.B. Occurrence of female sexual hormones in the Iguazu river basin, Curitiba. *Acta Scientiarum. Technology*, v. 36, n. 3, p. 421-427, 2014. https://doi.org/10.4025/actascitechnol.v36i3.18477

MATTHIESSEN, P.; WHEELER, J.R.; WELTJE, L. A review of the evidence for endocrine disrupting effects of current-use chemicals on wildlife populations. *Critical Reviews in Toxicology*, v. 48, n. 3, p. 195-216, 2018. https://doi.org/10.1080/10408444.2017.1397099

MEIJIDE, F.J.; REY VÁZQUEZ, G.; PIAZZA, Y.G.; BABAY, P.A.; ITRIA, R.F.; LO NOSTRO, F.L. Effects of waterborne exposure to 17β-estradiol and 4-tert-octylphenol on early life stages of the South American cichlid fish *Cichlasoma dimerus*. *Ecotoxicology and Environmental Safety*, v. 124, p. 82-90, 2016. https://doi.org/10.1016/j.ecoenv.2015.10.004

NAZARI, E.; SUJA, F. Effects of 17β-estradiol (E2) on aqueous organisms and its treatment problem: a review. *Reviews on Environmental Health*, v. 31, n. 4, p. 465-491, 2016. https://doi.org/10.1515/reveh-2016-0040

NIEMUTH, N.J.; KLAPER, R.D. Emerging wastewater contaminant metformin causes intersex and reduced fecundity in fish. *Chemosphere*, v. 135, p. 38-45, 2015. https://doi.org/10.1016/j.chemosphere.2015.03.060

OURSLER, M.J. Estrogen regulation of gene expression in osteoblasts and osteoclasts. *Critical Reviews in Eukaryotic Gene Expression*, v. 8, n. 2, p. 125-140, 1998. https://doi.org/10.1615/critreveukargeneexpr.v8.i2.20

PARROTT, J.L.; WOOD, C.S. Fathead minnow lifecycle tests for detection of endocrine-disrupting substances in effluents. *Water Quality Research Journal of Canada*, v. 37, n. 3, p. 651-667, 2002. https://doi.org/10.2166/wqrj.2002.043

PATYNA, P.J.; DAVI, R.A.; PARKERTON, T.F.; BROWN, R.P.; COOPER, K.R. A proposed multigeneration protocol for Japanese medaka (*Oryzias latipes*) to evaluate effects of endocrine disruptors. *Science of the Total Environment*, v. 233, n. 1-3, p. 211-220, 1999. https://doi.org/10.1016/s0048-9697(99)00227-2

PORSERYD, T. *Endocrine disruption in fish: effects of 17α-ethinylestradiol exposure on non-reproductive behavior, fertility and brain and testis transcriptome*. PhD Thesis (Doctorate) – Södertörn University, Stockholm, 2018.

REHMAN, M.; ALI, R.; BILAL, S.; GULL, G.; HUSSAIN, I.; MUDASIR, S.; MIR, M. Endocrine disrupting chemicals (EDCs) and fish health: a brief review. *International Journal of Livestock Research*, v. 7, n. 11, p. 45-54, 2017. https://doi.org/10.5455/ijlr.20170812034344

SILVA, A.P.A.; OLIVEIRA, C.D.L.; QUIRINO, A.M.S.; SILVA, F.D.M.; SARAIVA, R.A.; CAVALCANTI, J.S.S. Endocrine disruptors in aquatic environment: effects and consequences on the biodiversity of fish and amphibian species. *Aquatic Science and Technology*, v. 6, n. 1, p. 35-51, 2018. https://doi.org/10.5296/ast.v6i1.12565

SORNALINGAM, K.; MCDONAG, A.; ZHOU, J.L. Photodegradation of estrogenic endocrine disrupting steroidal hormones in aqueous system: process and future challenges. *Science of the Total Environment*, v. 550, p. 209-224, 2016. https://doi.org/10.1016/j.scitotenv.2016.01.086

VOISIN, A.S.; KÜLTZ, D.; SILVESTRE, F. Early-life exposure to the endocrine disruptor 17-α-ethinylestradiol induces delayed effects in adult brain, liver and ovotestis proteomes of a self-fertilizing fish. *Journal of Proteomics*, v. 194, p. 112-124, 2019. https://doi.org/10.1016/j.jprot.2018.12.008

VOLKOVA, K.; REYHANIAN CASPILLO, N.; PORSERYD, T.; HALLGREN, S.; DINNETZ, P.; OLSÉN, H.; PORSCH HÄLLSTRÖM, I. Transgenerational effects of 17α-ethinyl estradiol on anxiety behavior in the guppy, *Poecilia reticulata*. *General and Comparative Endocrinology*, v. 223, p. 66-72, 2015. https://doi.org/10.1016/j.ygcen.2015.09.027

WOODS, M.; KUMAR, A. Vitellogenin induction by 17 β-estradiol and 17 α-ethynylestradiol in male Murray rainbowfish (*Melanotaenia fluviatilis*). *Environmental Toxicology and Chemistry*, v. 30, n. 11, p. 2620-2627, 2011. https://doi.org/10.1002/etc.660

ZILLIOUX, E.J.; JOHNSON, I.C.; KIPARISSIS, Y.; METCALFE, C.D.; WHEAT, J.V.; WARD, S.G.; LIU, H. The sheepshead minnow as an in vivo model for endocrine disruption in marine teleosts: a partial life-cycle test with 17α-ethynylestradiol. *Environmental Toxicology and Chemistry*, v. 20, n. 9, p. 1968-1978, 2001.