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Aluminum as an endocrine disruptor in female Nile tilapia (*Oreochromis niloticus*)

T.G. Correia [a], A.M. Narcizo [a], A. Bianchini [b], R.G. Moreira [a,*]

[a] Instituto de Biociências, Universidade de São Paulo, R. do Matão, Trav.14, n. 321, 05508-090, São Paulo, SP, Brazil
[b] Instituto de Ciências Biológicas, Universidade Federal do Rio Grande, Av. Itália km 8, Campus Carreiros, 96201-900, Rio Grande, RS, Brazil

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ABSTRACT

The effects of aluminum on plasma ion, lipid, protein and steroid hormone concentration were evaluated in *Oreochromis niloticus* broodstock females. Lipid and protein concentrations from the gonads and liver were also measured. Experiments were performed at neutral and acidic water pH. Four groups of fish were tested for 96 h: 1) control conditions at neutral water pH; 2) control conditions at acidic water pH (CTR-Ac); 3) aluminum at neutral water pH (Al-N); and 4) aluminum at acidic water pH (Al-Ac). Aluminum and acidic water pH exposure caused no ionoregulatory disturbances. Total lipid concentration increased in the mature gonads and decreased in the liver, suggesting an acceleration of lipid mobilization to the ovaries in animals exposed to aluminum. However, a decreased protein concentration in ovaries was also observed. Exposure of control fish to acidic water pH caused an increased concentration of plasma 17α-hydroxyprogesterone. However, females exposed to aluminum at acidic water pH showed a decreased of plasma 17α-hydroxyprogesterone and cortisol. No differences in plasma 17β-estradiol were observed. The physiological mechanisms underlying the disturbances observed are discussed focusing on reproduction. We suggest that aluminum can be considered an endocrine disrupting compound in mature *O. niloticus* females.

1. Introduction

Aluminum (Al) is a harmful metal to the aquatic ecosystem, being responsible for events of toxicity with serious ecological consequences. To date, no normal physiological functions in biological systems are attributed to this metal (Nayak, 2002). Physiological alterations frequently observed in different fish species exposed to Al are mainly related to cardiovascular (Laitinen and Valtonen, 1995), hematologic (Barcarolli and Martinez, 2004), respiratory, ionoregulatory (Poléo, 1995), reproductive (Vuorinen et al., 2003), metabolic (Brodeur et al., 2001), and endocrine (Waring et al., 1996) disturbances, beyond structural gill damage (Peuranen et al., 1993).

In freshwater fish, Al causes acute ionoregulatory and respiratory disturbances due to the deposition of Al^{3+} on the gills (Poléo, 1995). Al also affects reproduction in the rainbow trout due to a concentration-dependent decrease in vitellogenesis. This effect is a consequence of a reduced expression of the mRNA encoding for vitellogenin synthesis (Hwang et al., 2000). In turn, acidic waters are known to impair fish reproduction, affecting fecundity, egg viability, spawning success (Mount et al., 1988), gonad development, and gamete production (Vuorinen and Vuorinen, 1991). These effects lead to serious consequences to fish populations such as a decreased number and size of fish (Mount et al., 1988).

In light of the above, it would be expected that fish exposure to Al at acidic water pH would lead to a more pronounced response than that observed after a single exposure to the metal. In fact, Al has shown to be more toxic to fish in acidic waters (Alstad et al., 2005). Therefore, the present study was undertaken to investigate the effects of Al on several physiological endpoints associated with ionic and osmotic regulation, reproductive hormones, and energy metabolism in mature females of the Nile tilapia *Oreochromis niloticus*.

2. Material and methods

2.1. Fish

Mature females of the Nile tilapia *O. niloticus* (Perciformes, Cichlidae) weighing 85.9 ± 9.8 g were obtained from a commercial fish farm. They were maintained in ponds at the Ponte Nova Fish Farm (23°35′33.8″S and 45°58′09.1″W) at Salesópolis (São Paulo State, Brazil).

2.2. Experimental design

Female fish ($n = 48$) were randomly divided into 4 groups (12 fish in each group), which were tested in duplicate. Fish from each replicate were held in a 145-L tank (6 fish per tank). The experimental groups tested were: 1) fish kept under control conditions in water at neutral pH (CTR-N); 2) fish kept under control conditions in acidic

* Corresponding author. Tel.: +55 11 3091 7531; fax: +55 11 3091 7568.
E-mail address: renatagm@ib.usp.br (R.G. Moreira).

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water (CTR-Ac); 3) fish exposed to Al in water at neutral pH (Al-N) and; 4) fish exposed to Al in acidic water (Al-Ac).

2.3. Experimental conditions

The experiment was performed for 96 h in a semi-static system with daily water renewal, under a natural photoperiod. Fish were not fed during the test period to avoid the excess of organic matter in the water.

Al was added to the experimental media as $Al_2(SO_4)_3$ (Merck) from a stock solution prepared using Milli-Q water acidified (pH = 2.5) with 65% HNO_3 (Suprapur; Merck). Nominal Al concentration was 0.5 μg/mL for groups Al-Ac and Al-N. Water pH, at the beginning of the experiment, was approximately 7.0 and was adjusted with HCl and NaOH (Synth, Brazil) in order to reach the desirable values of 5.5 for the CTR-Ac and Al-Ac groups and 7.0 for the CTR-N and Al-N groups. Water pH was monitored with a digital pH meter (Gehaka, São Paulo, Brazil) before and after adjustments. The experimental media were continuously aerated, and water temperature (maintained around 21–22 °C) and dissolved oxygen content were daily monitored with an oximeter (model 55; YSI, Yellow Springs, OH, USA).

2.4. Water chemistry

The water used in the experiment was previously filtered through two cellulose filters (IMPAC® – CEP 10, São Paulo, SP, Brazil and Acqualimp® FICZ – 10, Valinhos, SP, Brazil) containing activated charcoal (5 μm) to remove the solid particles and reduce the organic matter content and any possible chemical contaminants. After these procedures, water was analyzed for the main physicochemical parameters (Na^+: 0.086 mM; K^+: 0.0027 mM; Ca^{2+}: 0.0057 mM; Mg^{2+}: 0.0016 mM; Fe: 0.0022 mM; Cl^-: 0.085 mM; and SO_4^{2-} 0.00002 mM; alkalinity: 16 mg $CaCO_3$/L; total carbon: 6.755 mg C/L; organic carbon: 3.934 mg C/L; and inorganic carbon: 2.822 mg C/L) and used in the 145-L test tanks. The Al stock solution was added to give a nominal final concentration of 0.5 μg/mL. The experimental media were left to stand for 12 h to allow decantation and to let the system to equilibrate. Al nominal concentration was then measured and readjusted to 0.5 μg/mL. This concentration was chosen in order to represent the aluminum concentration usually found in the main basins in São Paulo State (CETESB, 2008).

For each assay, an additional tank with the same water volume (145 L) was prepared following the same procedures described above. Water from this tank was used to renew the experimental medium every 24 h of test. Water samples were collected from the experimental tanks during the renewal period. These samples were filtered with 0.45 μm syringe filter (Minisart® – Sartorius, Goettingen, Germany) and used to quantify NH_3, NO_2, and dissolved Al concentrations. Total Al concentration was measured using an atomic absorption spectrophotometer (GBC, AAS 932 Avanta-Plus, IL, USA). Other analyses were performed according to the Standard Methods (2005).

2.5. Biological sampling and physiological analyses

After 96 h of exposure, fish were cryoanesthetized, and a blood sample (~2 mL) was collected by puncture of the caudal vein using a heparinized syringe. Fish were then killed by decapitation, following the animal care protocols approved by the Biosciences Institute (University of São Paulo) Ethics Committee (protocol 070/2008). Biometrical parameters were recorded, and the liver and gonads were immediately dissected and frozen in liquid nitrogen. The blood sample was centrifuged for 5 min at 655.1 × *g*, and the plasma obtained was collected and frozen in liquid nitrogen. All samples were transported to the laboratory and kept at −80 °C.

Concentrations of major plasma ions (Na^+, Ca^{2+}, K^+ and Cl^-) were measured using an atomic absorption spectrophotometer (GBC, AAS 932 Avanta-Plus, IL, USA). Plasma levels of 17β-estradiol (E_2), 17α-hydroxyprogesterone (17 α-OHP), and cortisol were quantified by enzyme-linked immunosorbent assay (ELISA) (E_2 and 17 α-OHP: Interteck, Virginia, USA; cortisol: Adaltis Inc., Montreal, Canada). Absorbance measurements were performed in a microplate reader (Molecular Devices, CA, USA).

Total lipids from the liver and ovaries were extracted with a chloroform:methanol:water (2:1:0.5) solution, according to Parrish (1999) and adapted by Folch et al. (1957). Total lipid concentration was measured according to the spectrophotometer/colorimetric method described by Frings et al. (1972), using cod liver oil methyl-esters (Sigma Diagnostics, St. Louis, MO, USA) as a standard. Plasma samples were analyzed using the same method, but without previous lipid extraction.

Total protein from the liver and ovaries were extracted with perchloric acid, solubilized with KOH (Milligan and Girard, 1993), and measured according to the colorimetric method described by Lowry et al. (1951). Bovine serum albumin (Sigma Diagnostics INS, St. Louis, MO, USA) was used as a standard. Plasma samples were analyzed using the same method, but without previous protein extraction.

2.6. Statistical analyses

Data are expressed as mean ± SEM (standard error of the mean). Mean values for the different experimental conditions were compared using one-way analysis of variance (ANOVA) followed by the Tukey's test. The significance level adopted was 95% ($P<0.05$). Statistical analyses were performed using the software SIGMASTAT for Windows version 3.10 (Systat Software, San Jose, CA, USA).

3. Results

3.1. Water chemistry

The physicochemical characteristics of the water used in the experiments are presented in Table 1. Concentrations of nitrogen waste (ammonia and nitrite) were monitored over the experimental period (96 h), and values only slightly changed, without any significant variation. Dissolved Al concentrations also showed only a slight variation over the course of the experimental period, with a mean value of 0.6 and 0.5 μg/mL for Al-N and Al-Ac groups, respectively. Considering the pH corrections made with HCl or NaOH, water pH values were maintained within the desirable values.

3.2. Physiological biomarkers parameters

No significant isolated effects of the water pH adjustment or Al exposure on plasma ion (Na^+, K^+, Ca^{2+}, and Cl^-) concentrations were

Table 1
Physicochemical parameters of the experimental media used to perform tests with females of the Nile tilapia *Oreochromis niloticus*. Data are means ± SEM ($n = 3$).

Treatment	pH	T (°C)	OD (mg/L)	NO_2 (mg/L)	NH_3 (mg/L)	Dissolved Al (μg/mL)	Total Al (μg/mL)
Al-N	7.1 ± 0.05	21.0 ± 0.30	7.1 ± 0.10	–	0.04 ± 0	0.6 ± 0.1	1.6 ± 0.1
Al-Ac	5.7 ± 0.09	21.0 ± 0.24	7.2 ± 0.09	0.01 ± 0.01	0.03 ± 0	0.5 ± 0.1	1.7 ± 0.1
CTR-N	7.0 ± 0.10	22.2 ± 0.29	7.1 ± 0.08	0.01 ± 0	0.03 ± 0	–	–
CTR-Ac	5.8 ± 0.14	22.3 ± 0.22	7.0 ± 0.15	0.01 ± 0	0.02 ± 0	–	–

Table 2
Plasma ion concentration (mM) in female Nile tilapia *Oreochromis niloticus* kept under control conditions at neutral (CTR-N) and acidic (CTR-Ac) pH or exposed to aluminum at neutral (Al-N) or acidic (Al-Ac) pH for 96 h. Data are mean ± SEM (n = 7–9). Different letters indicate significant difference mean values among treatments (P<0.05).

Treatment	Na^+	Cl^-	K^+	Ca^{2+}
Al-N	221.0 ± 12.8^a	112.0 ± 9.4^a	8.45 ± 0.79^a	1.50 ± 0.34^a
Al-Ac	174.2 ± 9.7^{ab}	96.1 ± 11.6^a	11.18 ± 0.37^a	1.12 ± 0.18^a
CTR-N	175.4 ± 13.2^{ab}	80.6 ± 14.0^a	9.49 ± 1.18^a	2.16 ± 0.29^a
CTR-Ac	165.5 ± 18.4^b	91.6 ± 14.5^a	10.16 ± 1.06^a	1.41 ± 0.16^a

observed (Table 2). However, fish from the Al-N group showed a significantly higher Na^+ concentration than those from the CTR-Ac group (Table 2). Protein and lipid concentrations in the liver, gonads, and plasma showed different patterns of variations for the different experimental groups. Concentration of the gonad lipids sharply increased (~2-fold) in fish from the two experimental groups exposed to Al, when compared to their respective control groups ($P \leq 0.001$) (Fig. 1A). However, the concentration of hepatic lipids decreased in fish from Al groups at both neutral ($P \leq 0.001$) and acidic pH ($P = 0.008$) (Fig. 1B). No significant change in the concentration of plasma lipids was observed (Fig. 1C).

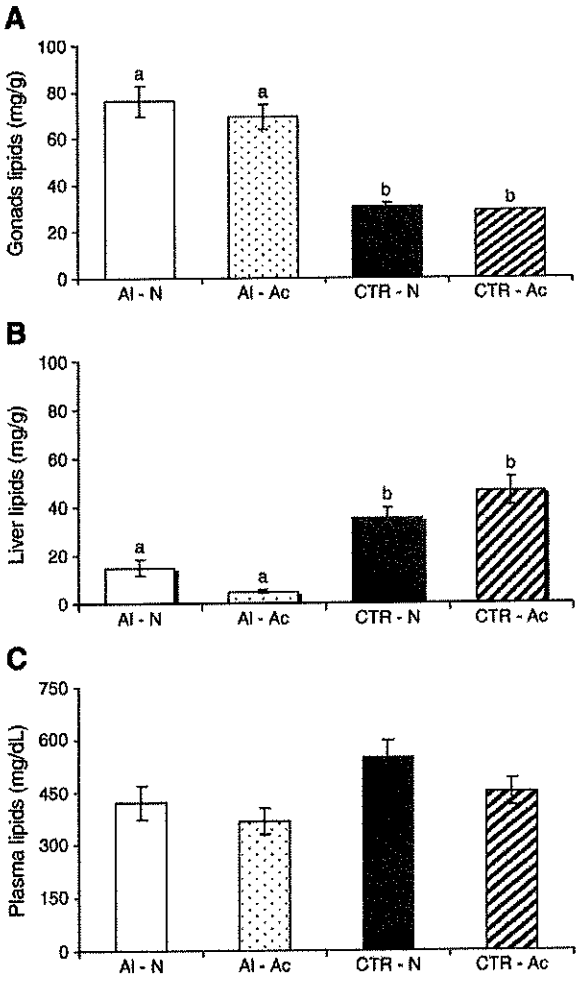

Fig. 1. Total lipid concentration in the (A) gonads, (B) liver, and (C) plasma in mature female *Oreochromis niloticus* exposed (96 h) to aluminum in neutral (Al-N) and acidic (Al-Ac) water pH. Fish were also kept in neutral (CTR-N) and acidic water pH (CTR-Ac) in the absence of aluminum. Data are mean ± SEM. Different letters indicate significant different mean values between treatments (P<0.05).

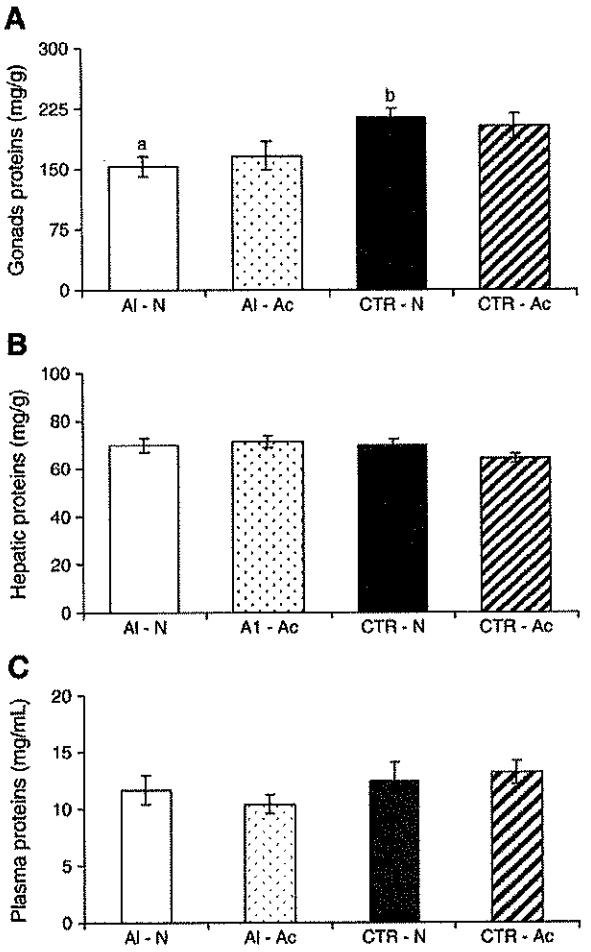

Fig. 2. Total protein concentration in the (A) gonads, (B) liver, and (C) plasma in mature female *Oreochromis niloticus* exposed (96 h) to aluminum in neutral (Al-N) and acidic (Al-Ac) water pH. Fish were also kept in neutral (CTR-N) and acidic (CTR-Ac) water pH in the absence of aluminum. Data are mean ± SEM. Different letters indicate significant different mean values between treatments (P<0.05).

Data from tissue protein concentrations revealed different patterns of response after Al exposure. Fish exposed to Al had a decreased concentration of protein in the gonads, but only at neutral water pH (Al-N) ($P = 0.004$). In acidic pH, Al exposure slightly decreased the concentration of the gonad proteins, but without showing a significant difference from the respective control (Fig. 2A). Concentrations of hepatic and plasma proteins were not altered by either Al or acidic exposure (Fig. 2B and C).

Short-term exposure to Al and/or acidic pH did not alter the plasma E_2 concentration (Fig. 3). However, the plasma concentration of the progestogen 17 α-OHP significantly decreased when fish were exposed to Al in acidic pH (Al-Ac) ($P = 0.001$). On the other hand, exposure to acidic pH in the absence of Al (CTR-Ac) significantly increased the 17 α-OHP concentration ($P = 0.001$) when compared with the neutral pH (CTR-N) (Fig. 4).

At neutral pH, Al exposure did not significantly change plasma cortisol concentrations. However, when fish were exposed to Al in acidic pH (Al-Ac), plasma cortisol concentrations significantly decreased compared to the levels observed in fish exposed to Al at neutral water pH (Al-N) ($P = 0.003$) (Fig. 5).

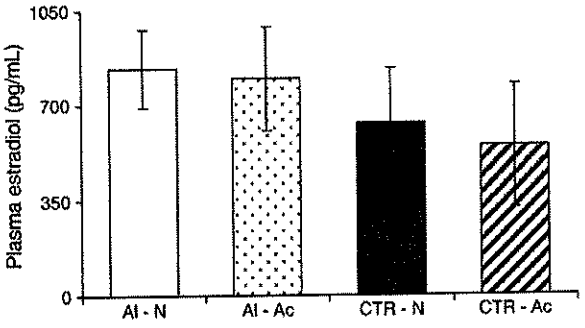

Fig. 3. Plasma estradiol concentration in mature female *Oreochromis niloticus* exposed (96 h) to aluminum in neutral (Al-N) and acidic (Al-Ac) water pH. Fish were also kept in neutral (CTR-N) and acidic (CTR-Ac) water pH in the absence of aluminum. Data are expressed as mean ± SEM.

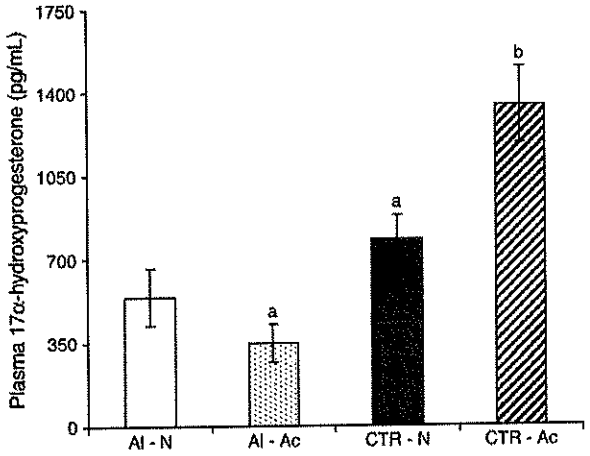

Fig. 4. Plasma 17α-hydroxyprogesterone concentration in mature female *Oreochromis niloticus* exposed (96 h) to aluminum in neutral (Al-N) and acidic (Al-Ac) water pH. Fish were also kept at neutral (CTR-N) and acidic (CTR-Ac) water pH in the absence of aluminum. Data are expressed as mean ± SEM. Different letters indicate significantly different mean values between treatments ($P<0.05$).

4. Discussion

Some physicochemical water parameters, such as temperature and dissolved oxygen concentration, can cause significant alterations in fish physiology, thus confounding the interpretation of fish responses to water toxicants (Aragão and Araújo, 2006). Therefore, these parameters were monitored and kept constant throughout the entire experiment.

Regarding nitrogenous compounds, it is well established that ammonia is toxic to fish, even at low concentrations in the water. In addition, independent of the species sensitivity, fish size has a significant influence in determining the toxicity of this compound (Ruyet et al., 1997). Despite the difficulties to establish the threshold for toxicity in fish, Boyd (1990) and Lemarié et al. (2004) suggested that the toxic limit for freshwater fish is between 0.05 and 0.2 mg/L. In the present study, ammonia concentration throughout the experiment was always below 0.05 mg/L, suggesting that this nitrogen compound had no influence on fish responses to the experimental treatments tested.

As for ammonia, fish sensitivity to nitrite significantly varies among species. According to Willians and Eddy (1986), comparisons of the toxic effects of nitrite between species should be done carefully since water chemistry can modify the observed responses. According to Boyd (1990), safe nitrite concentrations are between 0.02 and 0.1 mg/L, and any value higher than that can be stressful to the fish. In the present study, nitrite concentration likely did not interfere with fish responses to the experimental treatments. This statement is based on the fact that the nitrite concentrations in water were always below this range. Certainly, the water renewal procedure was important to keep low levels of nitrogen compounds and also to maintain the dissolved Al concentration around 0.5 μg/mL. Therefore, physiological changes described in the present study are likely due to Al exposure and/or water pH.

Alteration in the gill ion regulatory function is considered the first toxic effect in fish exposed to acidic pH and Al (Buckler et al., 1995). Decreases in plasma Na^+ and Cl^- are the initial alterations observed in fish exposed to acidic pH, independent of the presence of Al in the water (Mount et al., 1988). This effect is mainly observed after acute exposure, and acclimation or physiological adjustments to these toxic effects are generally observed in fish after acute (Barcarolli and Martinez, 2004), subchronic (Brodeur et al., 2001), or chronic exposures (Vuorinen et al., 2003). In this context, the lack of effect of the acid pH or Al exposure on the ionic regulation of the female Nile tilapia suggests that this fish was not affected by the experimental conditions tested or had enough time to compensate for these presumed effects.

Acidic pH exposure can also interfere with fish growth and reproduction, affecting the liver's ability to metabolize carbohydrates and synthesize vitellogenin in brook trout (Tam et al., 1987). Sub-lethal Al concentration in acidic pH increases the swimming activity in Atlantic salmon (*Salmo salar*) with a significant loss of body mass, thus altering the energy budget in salmon living under this condition (Brodeur et al., 2001). Fish utilize glucose for their immediate energy source under stressing conditions and maintain hepatic glycogen by glyconeogenesis. In general, under a stressful condition, cortisol increases muscular catabolism, and the amino acids mobilized are used in the hepatic glyconeogenesis. In this case, changes in plasma protein levels are indicative of substrate mobilization from tissues, especially the liver and muscle, while changes in the gonad protein concentrations indicate substrate deposition, which in turn will be used for oocyte growth (Wendelaar Bonga, 1997). In the present study, mature Nile tilapia exposed to acidic water pH or Al separately, did not show changes in plasma cortisol. However, fish showed a decrease in plasma cortisol concentrations when simultaneously exposed to Al and acidic pH. In the South American fish *Rhamdia quelen* (Cericato et al., 2008) and *Brycon amazonicus* (Hori et al., 2008), deleterious effects on cortisol responses were also observed after acute exposure (96 h) to sub-lethal concentrations of agrichemicals and phenol, respectively.

In some studies focusing on conservation physiology, animals are exposed to a mild stress to assess their capacity to react to environmental stressors (Wikelski and Cooke, 2006). However, not all environmental stressing conditions are able to induce a detectable

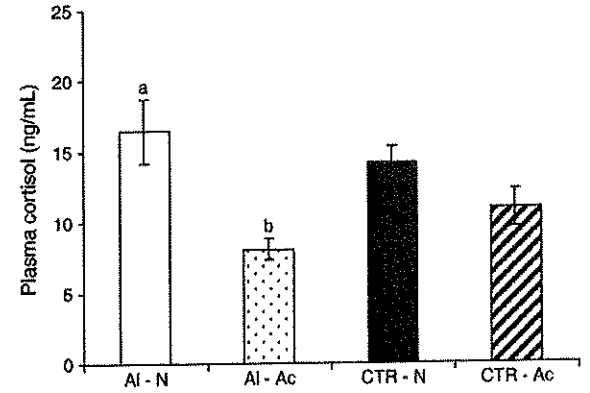

Fig. 5. Plasma cortisol concentration in mature female *Oreochromis niloticus* exposed (96 h) to aluminum in neutral (Al-N) and acidic (Al-Ac) water pH. Fish were also kept in neutral (CTR-N) and acidic (CTR-Ac) water pH in the absence of aluminum. Data are means ± SEM. Different letters indicate significant different mean values between treatments ($P<0.05$).

increase in plasma glucocorticoid concentrations (Rich and Romero, 2005; Wikelski and Cooke, 2006), as observed in the present study after fish acute exposure to acidic water pH or Al.

According to Rich and Romero (2005), changes in the responsiveness of the hypothalamic-pituitary-adrenal axis to adrenocorticotropin (ACTH) and arginine vasotocin (AVT) down-regulate corticosteroid responses during chronic stress in birds. Furthermore, these authors reported that basal corticosteroid concentrations and corticosteroid responses to acute stress were significantly reduced when birds were chronically stressed (e.g. captivity), as observed in the present study when fish were acutely exposed to Al in acidic water pH.

Therefore, it is clear that data on plasma glucocorticoid concentrations have to be interpreted carefully against a background baseline data. Increased glucocorticoid levels could indicate an adaptive response of healthy individuals to short-term environmental challenges (Wikelski and Cooke, 2006). On the other hand, when an expected stressor fails to activate the hypothalamus-pituitary-interrenal (HPI) axis, as observed in the present study after acute exposure to Al or acidic water pH in fish, it could be that the event is not actually perceived to be stressful or some mechanistic blockade in activation of the axis is occurring (Wingfield and Sapolsky, 2003).

Considering the gonad substrates, the decrease in protein concentration observed when females were exposed to Al in neutral pH can be related to the vitellogenin (VTG) absorption mechanism, which is mediated by the follicle-stimulating-hormone (FSH). It is known that Al can inhibit the activity of kinases (Katsuyama et al., 1989), but it can also activate cAMP-dependent kinases (Johnson and Jope, 1987). Many cell signaling pathways are regulated by the protein phosphorylation–dephosphorylation processes, which are also the basis for the control of many cell functions that are influenced by extracellular stimulus, such as hormones, mitogenics, carcinogenics, citocynes, neurotransmitters, toxic substances, or metabolites (Aoyama et al., 2003). Therefore, Al can interfere with the enzymes involved in VTG synthesis or even impair VTG incorporation by the oocyte or the cleavage process, since VTG is composed of highly phosphorylated molecules (e.g., phosvitin) (Mugiya and Tanahashi, 1998).

In general, female fish exhibit a decrease in the hepatic lipids concentration during the reproductive period, due to fatty acid mobilization and deposition in ovaries (Sheridan et al., 1983; Henderson and Tocher, 1987; Sheridan, 1988). Consequently, the results obtained for hepatic and gonad lipids in the present study after exposure to Al in neutral or acidic water pH can be interpreted as an energetic investment to accelerate spawn. We suggest that, mainly due to the mechanism of asynchronic oocyte development, *O. niloticus* has the physiological plasticity to accelerate the mobilization process of vitellogenesis, resulting in an increased lipid concentration in ovaries. Studies with yellow perch from lakes contaminated with other metals (Cd, Zn, and Cu) also did not respond by increasing their plasma cortisol and glucose levels and altered their capacity to build up and use glycogen and triglyceride reserves (Levesque et al., 2002), as observed in the present study with Nile tilapia. In the yellow perch, liver triglyceride was lower in fish from contaminated lakes due to an enhanced activity of triacylglycerol lipase, an enzyme that hydrolyses triglyceride reserves (Levesque et al., 2002). Therefore, the decreased lipid concentrations in the liver and the increased lipid concentrations in the gonads of *O. niloticus* exposed to Al could be explained by an increased triacylglycerol lipase activity after Al exposure.

In addition to the effects described above, Al also showed an endocrine disruptor effect, i.e., acting as an anti-steroidogenic stressor. In mature Nile tilapia, Al and/or acidic water pH exposure did not altered the plasma E_2 concentration. However, exposure to Al in acidic water pH decreased the plasma concentration of 17 α-OHP when compared with the acidic control group. Based on this alteration, a possible mechanism of action for Al can be discussed. Considering that progestogens are mainly produced under the control of the pituitary LH (luteinizing hormone) (Young et al., 2005), we suggest that suppression of the pituitary LH or even hypothalamic GnRH (gonadotropin releasing hormone) synthesis is occurring. However, due to the known effects of Al and other metals on the enzyme activities, the possibility of a stimulation of 20β-hydroxysteroid dehydrogenase (20β HSD), an enzyme that converts 17 α-OHP into 17α,20β-dihydroxy-4-pregnen-3-one (17,20 P), the main MIS (maturation-inducing steroid) in fish, cannot be ruled out (Young et al., 2005). In fact, this hypothesis corroborates our suggestion that Al is inducing the acceleration of spawn in *O. niloticus* females.

The 17 α-OHP decrease induced by the Al exposure in acidic water pH was paralleled by a decreased cortisol concentration, as discussed above. These changes in hormone profiles should be explained considering the pathways of steroid synthesis, since the fish species investigated has all the enzymes responsible for corticosteroid synthesis in the gonads, even if the 11-β hydroxylase (enzyme that generates cortisol from 11-deoxycortisol) gene expression was not abundant in the ovaries (Milla et al., 2009, for review). The low levels of cortisol and 17 α-OHP observed in the Nile tilapia exposed to Al in acidic water pH can also be explained considering the lowered lipid concentration in the liver, limiting steroid synthesis from cholesterol or by the fact that some xenobiotics (e.g., roundup) can inhibit the transport of cholesterol to the mitochondria (Walsh et al., 2000). Female Nile tilapia exposed only to acidic water pH in the absence of Al also showed disturbances in plasma 17 α-OHP, presenting higher 17 α-OHP values than females kept in neutral water pH. This finding suggests that, in fact, dysfunctions in the activity of the enzyme 20β HSD could be occurring.

Like in mammals, the FSH and LH control of the steroid synthesis in fish ovaries is a pathway that involves, at least partially, protein kinases (PKA) (Mendéz et al., 2003) and cAMP in the theca follicular layer (Planas et al., 1997). Substances that promote increases in intracellular cAMP also stimulate steroidogenesis (Kanamori and Nagahama, 1988), and cAMP antagonists partially block the steroidogenic action of gonadotropins (Planas et al., 1997). In fact, the LH stimulatory effect on the steroid synthesis in trout ovaries is related to cAMP/PKA activation (Mendéz et al., 2003). Therefore, the known effect of Al on abnormal phosphorylation by the activation of cAMP-dependent PKA can be the cause of the altered plasma level of 17 α-OHP in response to the LH action after Al exposure. In this context, it is important to note that Morrissey et al. (1983) observed that Al suppressed parathyroid hormone synthesis due to a decrease in cAMP levels. Therefore, we suggest that Al interferes with the final maturation phase and spawn in the Nile tilapia, inducing the production of eggs with lowered protein content.

In conclusion, Al exposure in acidic and neutral pH showed to be deleterious to the reproduction of the female mature Nile tilapia *O. niloticus* by accelerating lipid mobilization from the liver and deposition in the ovaries, decreasing protein deposition in eggs, and decreasing the plasma levels of 17 α-OHP. Based on these results, Al can be considered as an endocrine disrupting compound (EDC) for mature *O. niloticus* females. Therefore, we suggest that different reproductive biomarkers should be investigated in environmental monitoring programs in metal contaminated freshwaters, especially the acidic ones.

Acknowledgments

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