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TECHNICAL NOTE

Screening of endocrine disruption activity in sediments from the Uruguay River

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Abstract Sediment constitutes an important sink of endocrine disruptor compounds; however, the potential of sediments to act as a source of endocrine disruptors should be more extensively investigated. The main objective of this study was to determine whether exposure of immature common carp to Uruguay River sediments undergo physiological and endocrine alterations. The lower Uruguay River watershed supports intensive agricultural and forest production, receives municipal sewage discharge and industrial effluent, and a new large pulp mill was constructed in 2006. A 30-day semi-static assay was performed using sediments from four sites along the Uruguay River and compared with an unexposed group in dechlorinated water as a negative control. We focused on two upstream and two downstream sites of a new elemental chlorine free pulp mill. The results showed that plasma vitellogenin levels increased in fish along the river and significant differences were found between the exposed and unexposed groups. Condition factor and gonadosomatic index were not different; however, a significant difference in hepatosomatic index was observed in fish exposed to sediment from an industrial site. A significant reduction in primary spermatocyte accumulation was observed in the exposed group compared with that in the control group, and some individuals exposed to sediments from industrial sites presented with testis–ova. Our results suggest that Uruguay River sediments act as an important source of estrogenic compounds that could be responsible for the alterations observed. Future studies are needed to identify the causal agents and determine exposure routes.

Keywords Endocrine disruptors · Common carp · Sediment · Vitellogenin · Laboratory-exposure

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Introduction

Growing scientific evidence indicates that some natural and synthetic chemical compounds interfere with normal endocrine functioning in humans and wildlife. Most of these studies have focused on detecting specific endocrine disruptor compounds (EDCs) in surface water and their potential effects on fish (Sellin et al. 2009, 2011a; Velisek et al. 2011). Recent studies have detected high concentrations of EDCs in sediments, suggesting that the sediments could be responsible for the observed alterations; however, bioavailability of EDCs is complex. Sediments could be acting as a sink and reducing EDC bioavailability or re-releasing the chemical compounds into the water and acting as a source. The possible exposure routes to aquatic organisms include direct uptake of free compounds across the gills or skin and ingestion of sediment particles (Peck et al. 2004). Several laboratories and field studies have reported that fish exposed to sediments experience

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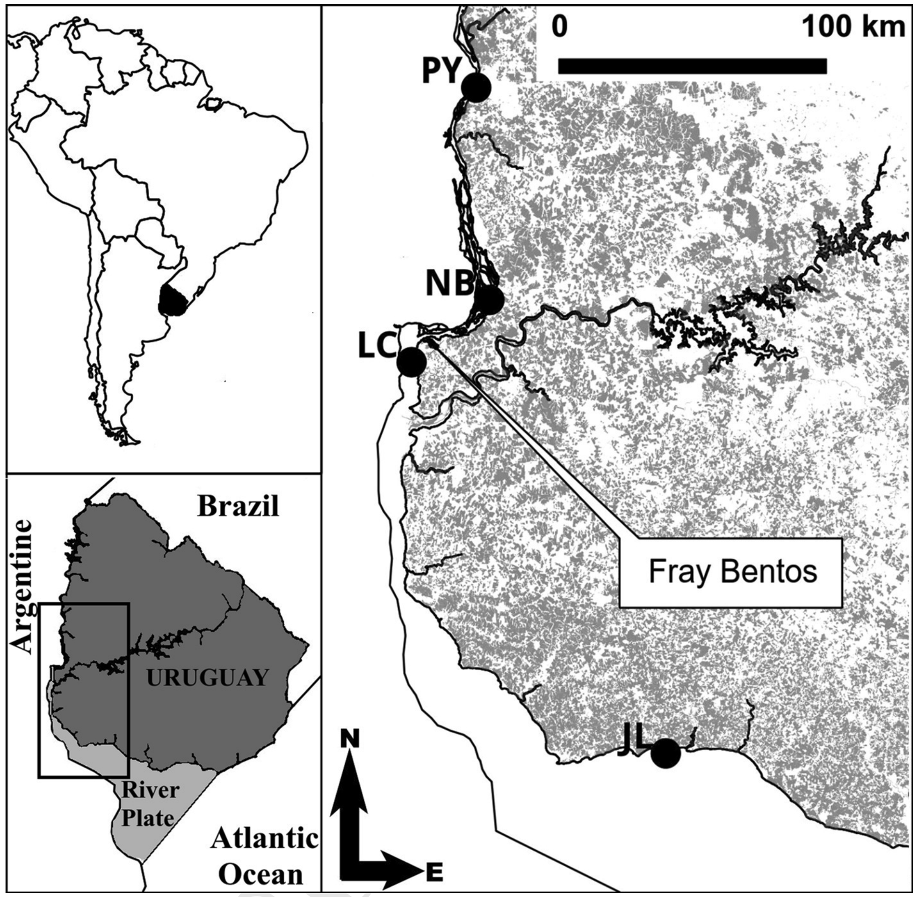

Fig. 1 Study area and location of the sediment sampling zones. *PY* Paysandú, *NB* Nuevo Berlín, *LC* Las Cañas, *JL* Juan Lacaze. The *grey zones* on the Uruguayan territory show land under agricultural activity. The information was obtained from supervised classification of LANDSAT 5 satellite images

significant alterations in endocrine functions (Orrego et al. 2005; Kolok et al. 2007; Sellin et al. 2010, 2011b; Kolpin et al. 2013; Jessick et al. 2014). However, the potential of sediments to act as sources for endocrine disruptors should be more extensively investigated.

The lower Uruguay River watershed is characterized by intensive agricultural and forest production. This part of the river receives a variety of municipal sewage discharges and industrial effluents, and a new large pulp mill was constructed in 2006 (Paruelo et al. 2006; Céspedes-Payret et al. 2009; Vega et al. 2009). From 2005 to 2007 several chemicals such as resin acids, phytosterols, polychlorinated dibenzo-p-dioxins, and dibenzofurans have been detected in water and sediments from the lower Uruguay River and bioaccumulation and ecotoxicological effects have been observed in wild fish (Miguez et al. 2010; Saizar et al. 2010).

This study was designed to test the hypothesis that a variety of chemicals found in sediments can display endocrine disrupting activity. The main goal was to determine if immature common carp (*Cyprinus carpio*) exposed to sediments from the lower Uruguay River exhibit physiological and endocrine alterations as determined by condition factor, hepatosomatic and gonadosomatic indices, plasma vitellogenin levels, and a gonadal histological analysis.

Materials and methods

Sediment samples

Sediment from four sites along the lower Uruguay River (between S 30°11’, W 57°38’ and S 34°00’, W 58°21’) were collected with an Eckman dredge in December 2006, transported at 4 °C to the laboratory, and stored at −20 °C until bioassay. We focused on two upstream sites from a new elemental chlorine free (ECF) pulp mill: the Paysandú (PY) urban-industrial zone and the Nuevo Berlín (NB) agricultural and forested (*Eucalyptus* sp.) zone and two downstream sites: the Las Cañas (LC) agricultural and tourist zone and the Juan Lacaze (JL) urban-industrial zone, which are directly influenced by discharge from an elemental chlorine bleached kraft pulp mill (Fig. 1).

Experimental design

Immature common carp (mean body length, 7.2 ± 0.9 cm; mean weight, 9.0 ± 3.2 g) were obtained from the National Direction of Aquatic Resources Fish Hatchery. The fish were acclimatized for 15 days in an aerated pool (800 L of dechlorinated water, renewed every 2 days), maintained with a constant temperature (22 ± 1 °C), light:

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dark cycle (12:12 h), and dissolved oxygen (89 ± 1 %). Fish were fed commercial food (Marplatense S.A., Montevideo, Uruguay) ad libitum.

A 30-day semi-static assay (total water renewed every 7 days) was performed in January 2007 using the same environmental conditions as those used for acclimatization. Sixty fish were randomly allocated in 30 L aquaria (four per aquaria) into five groups with 12 fish in each group. One group was placed in dechlorinated water as a negative control, and the others were exposed to sediments from each zone (PY, NB, LC, and JL) a 1:10 w/v rate (Sellin et al. 2011b; Jessick et al. 2014). No mortalities were observed during the assay.

Plasma vitellogenin (VTG) levels

Once the bioassay was completed, blood samples were extracted from the vena caudalis using heparinized syringes for plasma VTG analysis. Plasma was separated by centrifugation (Universal 32R, Hettich Zentrifugen) at 1,500 rpm for 10 min, and plasma VTG was quantified using antibody pre-coated enzyme-linked immunosorbent assay kits (Biosense Laboratory, Bergen, Norway; product no. V01003402) (Nilsen et al. 2004). The microplates were measured at a wavelength of 492 nm in a Biorad 680 microplate reader spectrophotometer (Hercules, CA, USA). The VTG concentration was calculated based on a standard calibration curve and expressed in µg/mL.

Gonadal histology

Gonads were immediately fixed in 10 % phosphate buffered formalin at pH 7.4. They were dehydrated gradually (alcohol 70, 96, and 100 v/v% chloroform) and embedded in paraffin. Sections (5 µm, Reichert–Jung microtome) were rehydrated and stained with hematoxylin and eosin. The fish were sexed and the reproductive maturity of the gonad cells was determined according to Smith and Walker (2004) using an optical microscope (Olympus Vanox; Tokyo, Japan) and photographed using a digital camera.

Physiological indices

The hepatosomatic index (HSI) and gonadosomatic index (GSI) of each fish was determined by dividing the body mass of the fish by the mass of the tissues and multiplying by 100. Condition factor (K) was calculated according to the following equation: (K) = 100 (body mass without organs/standard length^{b}), where b is the allometric coefficient, estimated by linear regression after logarithmic transformation of body mass and standard length before starting the assay (n = 60).

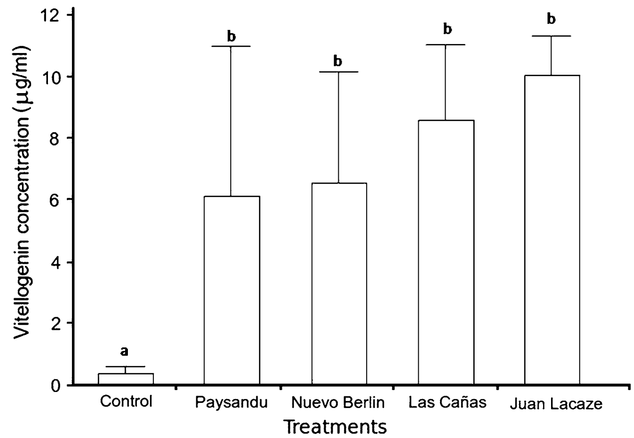

Fig. 2 Plasma vitellogenin levels in common carp exposed to sediments from the Uruguay River. *Vertical bars* indicate the 95 % confidence intervals. *Different letters* indicate significant differences

Statistical analysis

Normality and homogeneity of variance were verified and a single factor analysis of variance or Kruskal–Wallis test was used to determine differences between the physiological indices and plasma VTG levels. Statistical significance was confirmed by Tukey's post hoc test A $p < 0.05$ was considered significant.

Results

Plasma VTG levels and gonadal histology

Plasma VTG levels are presented in Fig. 2. The values increased along a latitudinal gradient from PY to JL. Significant differences were observed among the sediment-exposed groups and the control (Tukey's HSD, $p < 0.05$); however, no differences were detected among the exposed groups.

The gonadal histological analysis of females revealed that oocyte stages were not different between exposed and unexposed groups and that all oocytes were in the previtellogenic and perinucleolar stages (Fig. 3C, D). However, sediment-exposed males showed a significant reduction in the number of primary spermatocytes compared with those in the control group ($p = 0.01$) (Fig. 3A, B), and some individuals exposed to sediments from industrial sites (PY and JL) presented with testis–ova (Table 1).

Physiological indices

Mean (±standard error) values of the physiological indices in the exposed and unexposed groups are given in Table 2. No significant differences were observed among the groups

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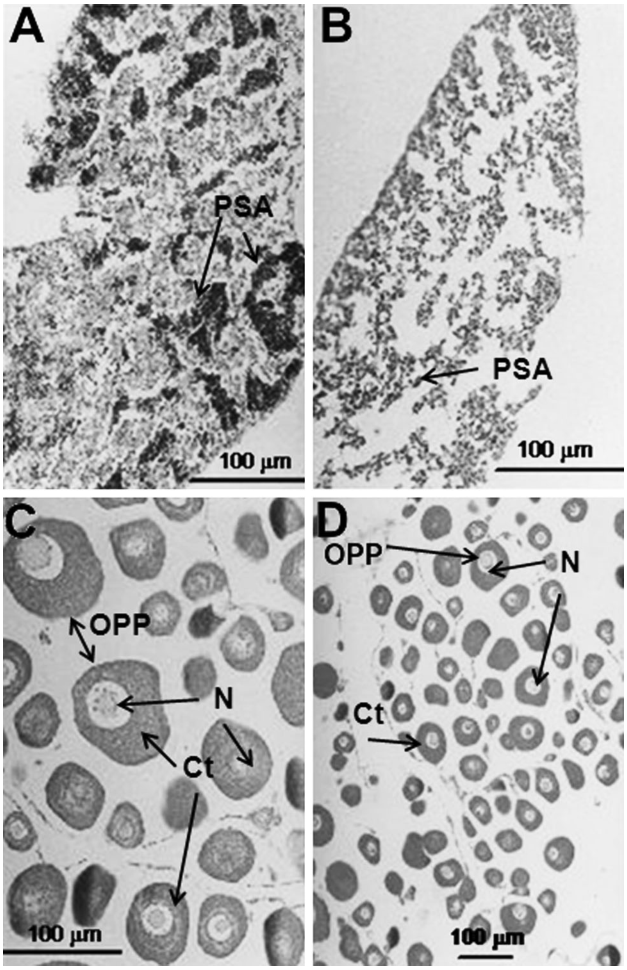

Fig. 3 Photographs of gonad histology in common carp exposed to sediments from different zones along the lower Uruguay River. **a** Gonads of a male from the control (magnification, 20×); **b** gonads of a male from the Nuevo Berlín (NB) fish group (magnification, 20×); **c** gonads of a female from the control (magnification, 20×); **d** gonads of a female from the NB group (magnification, 10×). *PSA* primary spermatocyte accumulation, *OPP* oocytes in previtellogenic and perinucleolar stage, *Ct* cytoplasm, *N* nucleus

Table 1 Sex distribution between treatments and the control and the occurrence of testis–ova

Treatment	Sex distribution (males/females per treatment)	Testis–ova frequency
Unexposed	9/3	0/9
Paysandú	9/3	1/9
Nuevo Berlín	4/8	0/4
Las Cañas	4/8	0/4
Juan Lacaze	10/2	1/10

Table 2 Physiological indices in the exposed and unexposed groups

Treatment	K^{α}	GSI^{β}	HSI^{γ}
Unexposed	2.6 ± 0.4 (12)	0.5 ± 0.6 (12)	1.3 ± 0.8 (12) a
Paysandú	2.8 ± 0.3 (12)	0.5 ± 0.6 (12)	1.7 ± 0.6 (12) ac
Nuevo Berlín	2.6 ± 0.5 (12)	1.3 ± 1.0 (12)	1.8 ± 0.6 (12) ac
Las Cañas	2.6 ± 0.4 (12)	0.9 ± 0.6 (12)	0.9 ± 0.6 (12) a
Juan Lacaze	2.8 ± 0.4 (12)	0.6 ± 0.8 (12)	2.5 ± 0.4 (12) bc

Values are given as mean ± standard deviations for each treatment, and respective n in parentheses

Treatments with statistical differences have different letters

α Condition factor

β Gonadosomatic index

γ Hepatosomatic index

for K or GSI; however, significant differences in HSI were detected. Post-hoc comparisons revealed that fish exposed to sediment from JL had significantly increased HSIs compared with those in the control ($p = 0.04$) and LC groups ($p = 0.02$).

Discussion

The main goal of this study was to determine whether immature common carp exposed to Uruguay River sediments exhibit physiological and endocrine alterations. Our results clearly indicate that natural and/or synthetic chemical compounds present in the sediments caused an abnormal and significant induction of the egg-yolk precursor protein VTG in all exposed groups. Elevated levels of VTG in males and immature females were clearly an estrogen-mediated response. The exposure to estrogenic compounds has a transitory effect on VTG production; however, exposure during early life stages could reduce survivability, and the effects on gonadal morphology and reproductive function may be permanent (Hutchinson et al. 2006).

Several known sources of endocrine disruptors are located in the Uruguay River watershed, and previous studies have detected some EDCs in sediments (Saizar et al. 2010) that could be responsible for the observed alterations. The urban-industrial sites (PY and JL) receive untreated municipal sewage effluent containing a complex cocktail of natural (estrone or 17β-estradiol) and synthetic estrogens used in oral contraceptives as well as surfactants used in soaps and detergents (alkylphenols and alkylphenolpolyethoxylates). Furthermore, the plasma VTG concentrations in fish were highest where deposition processes were predominant at JL and where pulp mill effluent was discharged near the sampling site.

The agricultural and forest river sectors (NB and LC) support intense soybean-wheat row crop production, and this agricultural system uses known estrogenic pesticides

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such as chlorpyrifos, endosulfan, and cypermethrin (Mnif et al. 2011). Additionally, phytoestrogens released by crops as a defense strategy may be reaching the river in overland runoff. In particular, soybeans contain high levels of genistein, daidzein, and glycitein, which can elicit alterations in endocrine function in wildlife and humans (Ng et al. 2006). It is important to note that VTG levels were not affected by the sex ratio, as shown by similar VTG concentrations in the LC and JL groups with opposite sex ratios. This was also true when comparing the PY and NB treatments.

The significant increase in liver mass at JL may have been caused by induction of the hepatic mixed function oxidase system in response to discharge of persistent organic compounds from the pulp mill effluent. Increased protein synthesis generates proliferation of endoplasmic reticulum, which can be reflected in increased hepatocyte size (Munkittrick et al. 1992).

The gonad histology analyses indicated that female fish did not exhibit differences in maturation state; however, sediment-exposed males presented delayed testicular maturation than that in the unexposed group. Jobling et al. (1996) reported that the induction of VTG in males is negatively correlated with testicular maturation, and Devlin and Nagahama (2002) observed retarded gonadal maturation in *C. carpio* males exposed to estrogenic compounds. Changes in sex ratios and intersex individuals have been reported in common carp exposed to EDCs (Gimeno et al. 1998; Devlin and Nagahama 2002). However, the intersex condition occurs naturally in approximately 5 % of the population in this species (Jobling et al. 1998). Thus, the presence of individuals with testis–ova observed in our study was possibly a natural phenomenon and may not have been caused by exposure to contaminated sediments.

Conclusions

This study is the first report about endocrine disruption in fish exposed to sediment from the lower Uruguay River. The results can be considered a reference condition for monitoring the impacts of the new ECF bleached kraft *Eucalyptus* pulp mill. Nonpoint (soybean–wheat crops) and point sources (municipal sewage and pulp mill effluent) can explain the VTG induction observed in immature fish, and suggest the presence and bioavailability of EDCs in the sediments. The specific agents responsible for the toxic effects were not identified because it was beyond the scope of this study. Future research is needed to identify the causal agents (natural or synthetic) and to determine exposure routes (e.g., grazing on sediments or bioconcentration from the water column).

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