Chapter 2

First Approach to Screening Endocrine Disruption Activity in Sediments from the Uruguay River (Uruguay Coast)

Noelia Rivas-Rivera and Gabriela Eguren

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.78736

Abstract

The Uruguay river basin supports intensive agricultural and forest production, and receives municipal sewage discharge and industrial effluent. Therefore, the river receives xenobiotic compounds which can be distributed in sediments, biota, water and particulate matter. There is evidence of the ability of several of these compounds to interfere with the endocrine system and the sediments are an important source. The aim of this study was to determine whether exposure of immature *Cyprinus carpio* to Uruguay river sediments undergo physiological and endocrine alterations. 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. The results showed that plasma vitellogenin levels increased along the river, and significant differences were found in exposed fish. Significant difference in hepatosomatic index was observed in fish exposed to sediment from an industrial site. In the histological analysis, only reproductive stage of males showed differences, where the number of primary spermatocyte accumulations was lower in exposed ones, and some exposed individuals from industrial sites presented with testis-ova. Our results suggest that the Uruguay river sediments are a source of endocrine-disrupting compounds available to the aquatic organisms.

Keywords: endocrine disruptors, sediments, bioassay, *Cyprinus carpio*, Uruguay river

1. Introduction

The endocrine system is the main regulator of important metabolic processes such as nutrition, development and reproduction. It is responsible for the maintenance of homeostatic mechanisms through regulatory actions positive and negative that allows keeping hormone levels in balance [1].

Several natural and synthetic chemical compounds released into the environment by human activities have the ability to interfere with the normal functioning of the endocrine system [2–4, 7, 8, 12–18]; these compounds are called endocrine disruptors (EDCs). The term endocrine disruptor defines, by both, to a compound or set of exogenous chemical compounds (natural or synthetics) that alter the normal functioning of the endocrine system through alterations in hormonal balance, causing effects on exposed organisms and including about his progeny [1, 5, 6].

A wide variety of EDC sources have been documented, including municipal sewage discharges and industrial effluents (e.g., pulp mills), as well as some pesticides and their metabolites [1, 7, 8]. Some of these compounds are persistent and lipophilic, and their concentrations are higher in sediments than in river water [9]. Growing scientific evidence shows that the EDCs can exert estrogenic, androgenic, antiandrogenic, and antithyroid actions on aquatic organisms and can induce alterations in the functional development and reproduction of fish [10–13]. Several 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 rereleasing 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 [9]. Several laboratories and field studies have reported that fish exposed to sediments experience significant alterations in endocrine functions [14–19].

Several approaches have been employed to show effects derived from the exposure to chemical compounds; however, those based on the use of biomarkers are the most widely used. These have been defined as "xenobiotically induced variations in cellular or biochemical components, processes, structures or functions that are quantifiable in a biological system" [20]. The first level of action of a compound or mixture of chemical compounds occurs in the biochemical-molecular component, triggering responses that tend to maintain the functioning of the organism within the homeostatic levels. If the exposure concentrations are high or are maintained during periods of prolonged time, the answers may not be enough to counteract the effect. In such a case, the agency triggers in the first instance mechanisms of compensation and then repair [21].

The Uruguay river is the natural border between Uruguay from Argentina and supports intensive agricultural and forest production and receives a variety of municipal sewage discharges and industrial effluents [22–24]. Since 1992, studies carried out by the Administration Commission of the Uruguay River (CARU) show the presence of some EDCs like chlorinated compounds (aldrin, dieldrin, HCH, HCB, DDT and its derivatives) and PCBs in fish [25]. Other works from the lower Uruguay river, detected in water and sediments several chemicals such as resin acids, phytosterols, polychlorinated dibenzo-p-dioxins, and dibenzofurans [26, 27]. At the time of the study was carried out, a large pulp mill was under construction at Fray Bentos along the Uruguay river and was associated with considerable controversy [28]. Pulp mill effluents have been associated with endocrine impacts in Canada as well as in other countries [29]. The main objective of this study was to determine whether exposure of immature common carp to Uruguay river sediments undergo physiological and endocrine alterations. *Cyprinus carpio* was exposed to sediments collected upstream and downstream of the construction site to provide a baseline prior to the initiation of effluent discharges from the new pulp mill facility. Indicators widely used for monitoring the effect of plasma vitellogenin levels, condition factor, liver and gonad somatic indexes and histology of gonads on the reproductive systems of fish were evaluated.

2. Methodological approach

The study was implemented applying a combination of field activities and laboratory, in order to evaluate the potential of the sediments of the Uruguay river of interfere with the normal functioning of the endocrine system and generate effects to reproductive level in fish.

Sediments have been selected as exposure matrix since that several compounds cataloged as endocrine disruptors have high affinity for them and that they have been identified in previous studies as one of the main sources of persistent estrogenic contaminants [6]. On the other hand, several crop protection compounds recognized as endocrine disruptors have been detected in water and fish in the Uruguay River [25, 30].

A battery of biomarkers was selected for the study, which included early warning signs (plasma vitellogenin levels) and late ones (condition factor, hepato and gonadosomatic indices and histological analysis of gonads). These were evaluated under controlled laboratory conditions by exposing juvenile individuals of *Cyprinus carpio* to sediments from different sectors of the Uruguay river.

The presence of vitellogenin, an estrogen-inducible protein, in plasma indicates a high internal concentration of estrogenic compounds, both of endogenous origin as exogenous (xenoestrogens) [31]. Therefore, the presence of detectable concentrations of vitellogenin in plasma of males or immature individuals has been proposed as a biomarker of exposure to xenoestrogenic compounds [1, 31–34]. Several studies have shown the interference of various compounds or mixture of these on the functioning of the endocrine system. Such is the case, where *Cyprinus carpio* males captured in the effluent channel of a plant of household waste treatment, presented levels of plasma vitellogenin significantly elevated [32]. These effects can lead to alterations in the structure of the trophic webs, causing changes in the transfer flows of matter and energy to and from the aquatic ecosystem. They can also cause a stock reduction or a loss in the quality of commercially exploitable species, by the accumulation of xenobiotics in tissues (bioaccumulation and/or biomagnification) [3, 35].

Gonadosomatic (GSI) and hepatosomatic indices (HSI) reflect the dynamics of the use of energy by organisms. Changes in IGS are directly related to sex, age and reproductive stage [30]. Therefore, an acceleration of the maturation of the gonadal cells by exposure to xenoestrogens will be reflected in an increase in the IGS and vice versa. While the changes in the IHS are linked to alterations in the main functions of the liver as the synthesis and degradation of hormones and detoxification of xenobiotics. In this sense, exposure to xenobiotics can cause an increase in the size of the liver [37].

Although through changes in somatic indices, it is possible to demonstrate the existence of effects, it is not possible to elucidate the mechanism (s) by which changes were generated. In this sense, by analyzing histological sections of the gonads, it is possible to determine if the increase in size is due, for example, to acceleration in the maturation of the reproductive cells induced by exposure to xenoestrogens.

Finally, the condition factor reflects the degree of adaptability of the organism to environment, in terms of an adequate energy balance between physiological needs and the increase in body biomass. Therefore, exposure to natural or artificial stressors will cause changes in the storage and transfer of lipids and proteins tending to counteract the effect of the stressor in detriment of the increase in body weight [38].

Cyprinus carpio “common carp” is a teleost fish belonging to the Cyprinidae family. It is originally from Asia with a great ability to adapt to different media, has been widely introduced worldwide. In our country, the introduction was made for commercial purposes in the 1960s from Brazil [39]. It has wide ranges of temperature tolerances (12–32°C) and acidity (pH 5–10), resists low levels of dissolved oxygen (1–2 mg/l) and high turbidity. It is an omnivorous species, mainly benthophages [36]. About its reproductive characteristics, this species reaches its sexual maturity between 18 months and 2 years of life, depending mainly on the temperature of the water. The gonadal differentiation is within the category of differentiated gonochorist, starting at 50 days post-birth [40–42]. The Organisation for Economic Co-Operation and Development (OECD) and the United States Environmental Protection Agency (EPA) consider *Cyprinus carpio* as a good bioindicator specie for evaluation effects of endocrine disruption [43, 44] and has already been used in several studies [32, 40, 41, 45–48]. This allows the comparison of the results obtained with those generated in other studies.

3. Materials and methods

3.1. Sediment samples

The sediments samples were collected with an Eckman dredge in December 2006, transported at 4°C to the laboratory, the proportion of organic carbon in each sample was determined by calcination and then were stored at −20°C until bioassay. The zones were selected considering representative sectors of the diverse anthropic activities developed in the basin (**Figure 1**):

(a) Paysandu–PY (S. 32°19′ W58°06′): near to the mouth of the Sacra Stream. This stream receives the sewage and industrial effluents of the Paysandu city.

(b) Nuevo Berlin–NB (S 32°59′ W 58°04′): located at North of the Fray Bentos city. This site presents an important agricultural use, especially soybean and forestry. On the other hand, according to hydraulic studies carried out under the installation of a pulp mill in the city of Fray Bentos, this one zone would not be influenced by the plume of the effluent. Therefore, it could be considered as a preimpact point in future monitoring.

(c) Las Cañas–LC (S 33°15′ W 58°16′): located at South of the Fray Bentos city, is a tourist and agricultural area.

http://dx.doi.org/10.5772/intechopen.78736

(d) Juan Lacaze–JL (S 34°26′ W 57°27′): located in the Río de la Plata river (Colonia Department). It is an urban-industrial area and is directly influenced by discharge form an elemental chlorine bleached Kraft pulp mill (was considered a positive control).

3.2. Experimental design

The assay was performed in January 2007. Immature common carp were obtained from the National Direction of Aquatic Resources Fish Hatchery (DINARA – Villa Constitucion station). They were transported in plastic bags, which were injected with oxygen and placed in ice. Once received in the laboratory, total weight (g), total and standard length (cm) of the totality of the individuals were recorded (mean body length, 7.2 ± 0.9 cm; mean weight, 9.0 ± 3.2 g) The fish were acclimatized for 15 days in an aerated pool (800 L of dechlorinated water, renewed every 2 days). During the acclimation period as well as during the assay, they were fed *ad libitum* with commercial feed (Marplatense S.A., Montevideo, Uruguay) and were kept under controlled temperature conditions (22 +/− 1°C), light: dark cycle (12:12 h) and percentage of dissolved oxygen (89 +/− 1%).

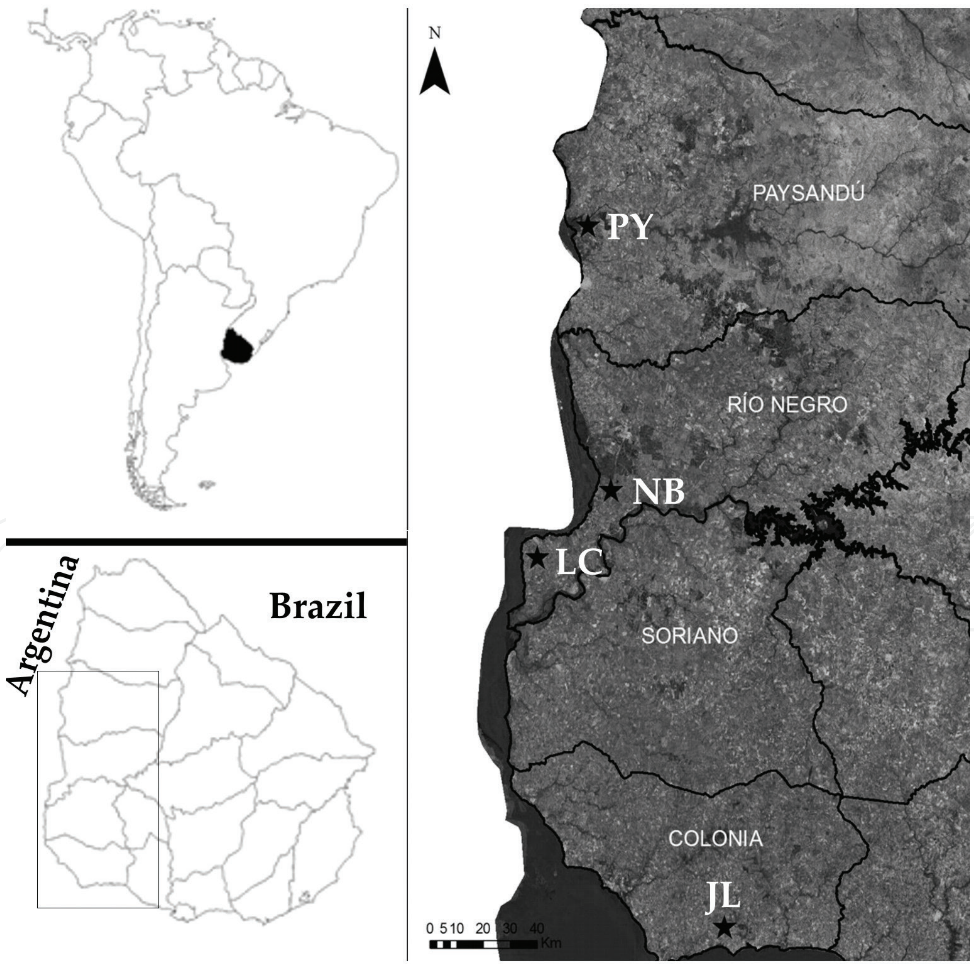

Figure 1. Study area and location of the sediment sampling zones. PY Paysandu, NB Nuevo Berlin, LC Las Cañas, JL Juan Lacaze.

Forty-eight hours prior to the start of the assay, the collected sediments were placed in 30-l tanks in a 1:10 ratio (sediment/water), in order to allow them to decant. Each of the treatments (sectors of the river) was analyzed in triplicate (total 12 fish tanks) and included three tanks with dechlorinated water and no sediment (negative control). Four fishes were placed in each tank randomly and were weighed and measured at the beginning of the assay. The spatial location at de laboratory of the treatments and controls was established randomly by using a table of random numbers. The assay was semi-static, with water replacement every 7 days and an exposure time of 30 days.

3.3. Plasma vitellogenin (VTG) levels

Once the bioassay was completed, blood samples were extracted from the vena caudalis using heparinized syringes for plasma VTG analysis (heparin solution 20 units/ml). Plasma was separated by centrifugation (Universal 32R, Hettich Zentrifugen) at 1500 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) [49]. 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 [50, 51].

3.4. Gonadal histology

The individuals were sacrificed by cervical dislocation, and the gonad was removed. Gonads were immediately weighed and fixed in 10% phosphate buffered formalin at pH 7.4. Then the tissue was dehydrated gradually through the passage through alcohol 70°, 96°, 100° and chloroform and embedded in paraffin. The cuts were made using a Reichert-Jung microtome at 5 μm. Finally, they were re-hydrated and stained with Harris's hematoxylin and eosin. The fish were sexed, and the reproductive maturity of the gonad cells was determined according to Smith and Walker in 2004 using an optical microscope with 10×, 20× and 40× eyepieces (Olympus Vanox; Tokyo, Japan) and photographed using a digital camera [36, 52–53].

3.5. Physiological indices

The somatic indices were calculated according to the following morphometric parameters: body weight (g), liver weight (g) and gonad weight (g).

For the condition factor, in first instance, was analyzed the length-weight relationship (log–log curve) from which the slope was obtained (p). This value was the allometric coefficient and was used in the equation Eq. 1.

$$K = \left(\frac{weight}{st.length^{p}}\right) \times 100 \tag{1}$$

The hepatosomatic index (HSI) and gonadosomatic index (GSI) of each fish were determined according to Eqs. (1) and (2)

http://dx.doi.org/10.5772/intechopen.78736

$$HSI = \left(\frac{liver\ mass}{body\ mass}\right) \times 100 \quad (2)$$

$$GSI = \left(\frac{gonad\ mass}{body\ mass}\right) \times 100 \quad (3)$$

3.6. 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; $p < 0.05$ was considered significant.

4. Results

4.1. Plasma VTG levels gonadal histology

Plasma VTG levels are presented in **Figure 2**. The values increased along a latitudinal gradient from Paysandu to Juan Lacaze (means: control = 0.360, Paysandú 6.128 and Juan Lacaze = 9989) accompanied of a reduction in the data dispersion. Only significant differences were observed among the sediment of exposed groups with the control (Tukey's HSD, $p < 0.05$); however, no differences were detected among the exposed groups.

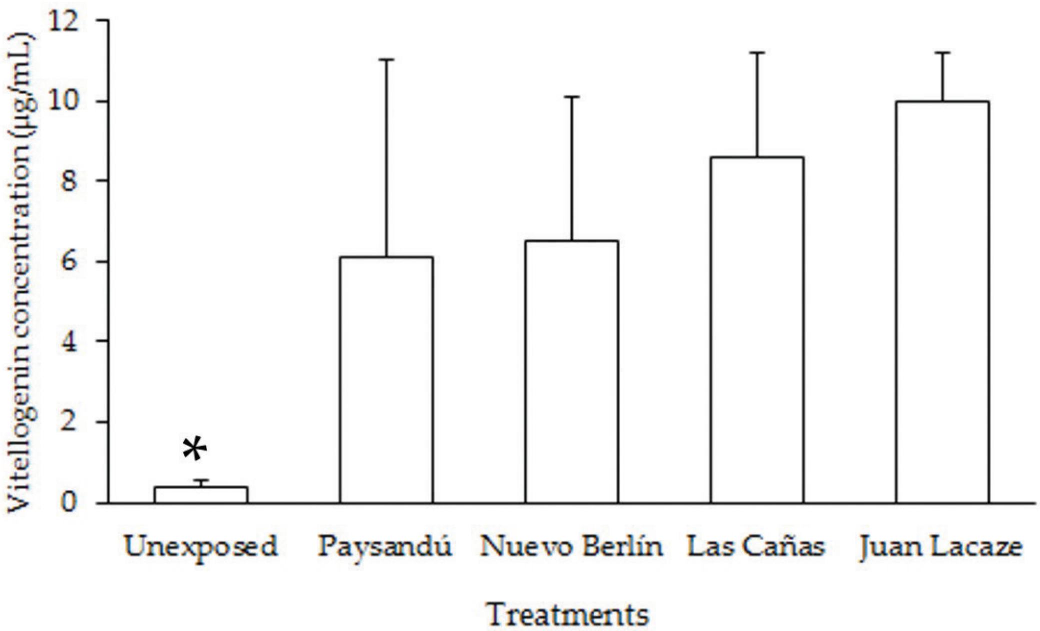

Figure 2. Plasma vitellogenin levels in common carp exposed to sediments from the Uruguay River. Vertical bars indicate the 95% confidence intervals. *Indicate significant differences ($p < 0.05$).

4.2. Gonadal histology

The gonadal histological analysis of females revealed that oocyte stages was not different between exposed and unexposed groups and that all oocytes were in the previtellogenic and perinucleolar stages according to the classification made by Smith and Walker [36] (**Figure 3C**, **D**). No significant differences were observed in relation to the size of the oocytes (control group range: 59–114 μm, Paysandu range: 79–113 μm, Nuevo Berlin range: 66–109 μm, Las Cañas range: 74–116 μm) nor in the amount of them within the displayed field.

Regarding testicular development, this in general does not have a clear differentiation, and it is only possible to observe a delay in maturity of the exposed individuals respect to

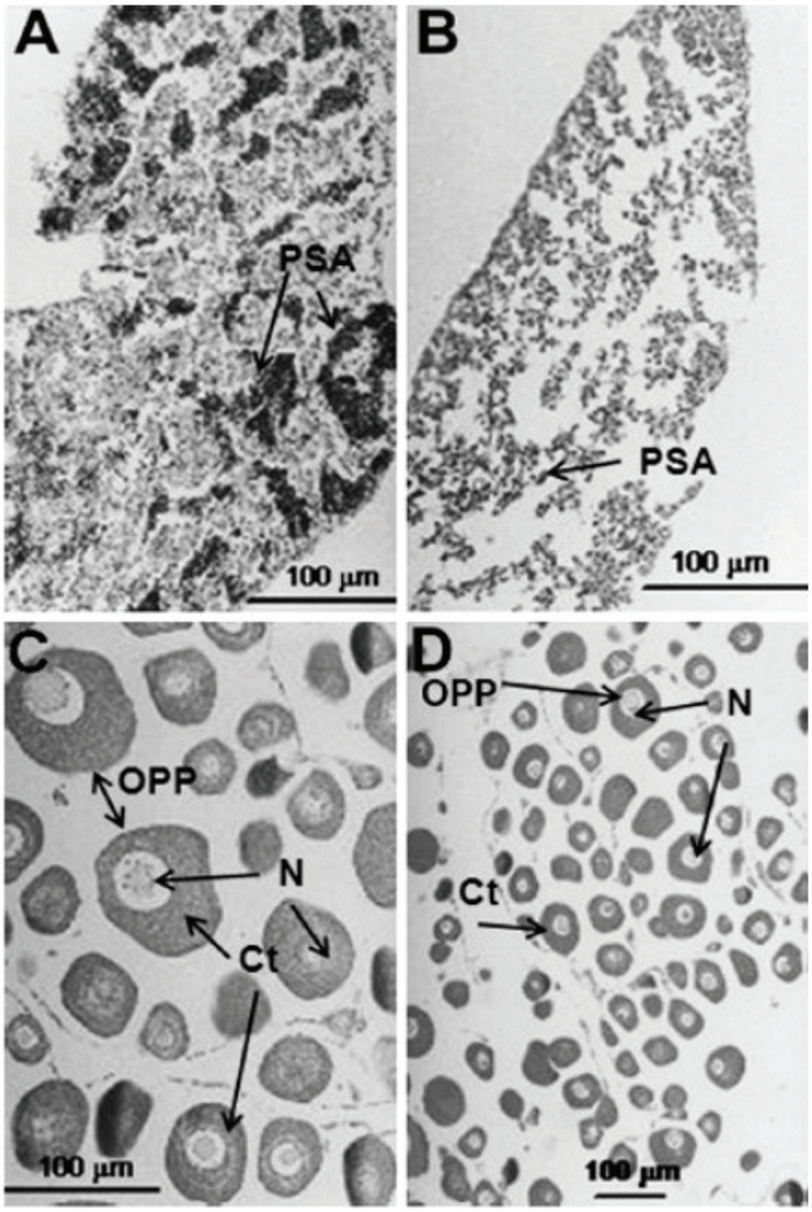

Figure 3. Photographs of gonadal histology. (A) male from control (20×); (B) male from Nuevo Berlin (20×); (C) female from the control (20×); (D) female from Nuevo Berlin (10×). PSA primary spermatocyte accumulation, OPP oocytes in previtellogenic and perinucleolar stage, Ct cytoplasm, N nucleus.

Treatment	Sex distribution (males/females)	Testis-ova (number/males)
Unexposed	9/3	0/9
Paysandu	9/3	1/9
Nuevo Berlin	4/8	0/4
Las Cañas	4/8	0/4
Juan Lacaze	10/2	1/10

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

Treatment	K	GSI	HSI
Unexposed	2.6 ± 0.4 (12)	0.5 ± 0.6 (12)	1.3 ± 0.8 (12)*
Paysandu	2.8 ± 0.3 (12)	0.5 ± 0.6 (12)	1.7 ± 0.6 (12)
Nuevo Berlin	2.6 ± 0.5 (12)	1.3 ± 1.0 (12)	1.8 ± 0.6 (12)
Las Cañas	2.6 ± 0.4 (12)	0.9 ± 0.6 (12)	0.9 ± 0.6 (12)*
Juan Lacaze	2.8 ± 0.4 (12)	0.6 ± 0.8 (12)	2.5 ± 0.4 (12)*

Values are given as mean ± standard deviations for each treatment, and respective n in parentheses. *indicates statistical differences between treatments ($p < 0.05$). K: condition factor, GSI: gonadosomatic index, HSI: hepatosomatic index.

Table 2. Physiological indices in the exposed and unexposed groups.

control. This delay is observed by a decrease in the number of accumulations of primary spermatocytes (**Figure 3A**, **B**). Some individuals exposed to sediments from industrial sites (Paysandu and Juan Lacaze) presented oocytes in testicular tissue (testis-ova) (**Table 1**).

4.3. Physiological indices

Values of the physiological indices in the exposed and unexposed groups are given in **Table 2**. No significant differences were observed among the groups for the condition factor (K) or gonadosomatic index (GSI); however, significant differences in HSI were detected. Post-hoc comparisons revealed that fish exposed to sediment from Juan Lacaze had significantly increased HSIs compared with those in the control ($p = 0.04$) and Las Cañas groups ($p = 0.02$)

5. Discussion

The results obtained in plasma vitellogenin levels indicate a marked effect of the treatments. Several known sources of endocrine disruptors are located in the Uruguay river basin, and previous studies have detected some EDCs in sediments [27] that could be responsible for the observed alterations. However, only significant differences were observed in Las Cañas and Juan Lacaze treatments. In the first one, the increase may be due to the contribution of known estrogenic pesticides such as chlorpyrifos, endosulfan, and cypermethrin from agricultural surrounding areas [54]. 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 [55]. Juan Lacaze receive untreated municipal sewage effluent containing a complex cocktail of natural (estrone or 17b-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 Juan Lacaze and where pulp mill effluent was discharged near the sampling site. In that sense, several works worldwide have documented the increase in vitellogenin plasma in juvenile individuals exposed to effluents from cellulose plants [14, 56, 57]. Elevated levels of VTG in males and immature females were clearly an estrogen-mediated response. It is important to note that VTG levels were not affected by the sex ratio, as shown by similar VTG concentrations in the Las Cañas and Juan Lacaze groups with opposite sex ratios, same when comparing the Paysandu and Nuevo Berlin treatments.

The gonad histology analyses indicated that female fish did not exhibit differences in maturation state. Unlike other works carried out under similar conditions, not differences in the stage of oocyte development were observed [33, 58, 59]. Whereby it could only be a trophoblastic but not protoplasmic growth, this could be checked by increasing the exposure time to see if there are changes in the stages in the individuals with the highest number of cells. However, sediment-exposed males presented delayed testicular maturation than that in the unexposed group. Jobling et al. reported that the induction of VTG in males is negatively correlated with testicular maturation, and Devlin and Nagahama observed retarded gonadal maturation in *C. carpio* males exposed to estrogenic compounds [60, 61]. Changes in sex ratios and intersex individuals have been reported in common carp exposed to EDCs [40, 41, 61]. However, the intersex condition occurs naturally in approximately 5% of the population in this species [7]. 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.

The condition factor showed higher values in all the treatments with respect to the control group; however, the differences are not statistically significant, and this agrees with the results obtained by Orrego et al. [14]. The significant increase in liver mass at Juan Lacaze 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 [62–64]. Increased protein synthesis generates proliferation of endoplasmic reticulum, which can be reflected in increased hepatocyte size [65–69].

6. 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). Finally, in relation to the adequacy of the bioassay developed to be applied as a monitoring tool, since Juan Lacaze sediments generated the greatest changes in the analyzed biomarkers, confirming their inclusion as a positive control. Likewise, the selection of negative controls (without sediment exposure) showed the lowest levels of changes as well as the lowest dispersion of values between replicates.

Acknowledgements

We express our thanks to the Departments of Virology and Cellular Biology (Science School) for cooperation with the processing of samples and to Mr. Angel Rosano and the Uruguayan Navy for their assistance in the fieldwork. This study was funded by the National Agricultural Research Institute (INIA) Project SA07 and the Environmental Science Master Program.

Conflict of interest

None.

Author details

Noelia Rivas-Rivera* and Gabriela Eguren

*Address all correspondence to: noeriv@gmail.com

Ecology and Environmental Sciences Institute, Sciences College, University of the Republic, Montevideo, Uruguay

References

[1] IPCS. Global assessment of the state of the science of endocrine disruptors. World Health Organization. International Programme on Chenical Safety. [Internet]. 2002. Available from: http://www.who.int/ipcs/publications/new_issues/endocrine_disruptors/en/ [Accessed: April 20, 2018]

[2] Ahmed RG. Perinatal TCDD exposure alters developmental neuroendocrine system. Food and Chemical Toxicology Journal. 2011;**49**:1276-1284. DOI: 10.1016/j.fct.2011.03.008

[3] Ahmed RG. Early weaning PCB 95 exposure alters the neonatal endocrine system: Thyroid adipokine dysfunction. Journal of Endocrinology. 2013;**219**(3):205-215. DOI: 10.1530/joe.13.0302

[4] Ahmed RG. Gestational 3,3′,4,4′,5-pentachlorobiphenyl (PCB 126) exposure disrupts fetoplacental unit: Fetal thyroid-cytokines dysfunction. Life Sciences. 2018;**192**:213-220. DOI: 10.1016/j.lfs.2017.11.033

[5] Argemi F, Cianni N, Porta A. Disrupción endócrina: perspectivas ambientales y salud pública. Acta bioquímica clínica latinoamericana. 2005;**39**(3):291-300

[6] Pombo M, Castro L. Disruptores endocrinos. [Internet]. 2005. Available from: http://www.seep.es/privado/documentos/congresos/C2005/Conferencias/Conferencia-Manuel%20Pombo.pdf [Accessed: April 20, 2018]

[7] Jobling S, Nolan M, Tyler CR, et al. Widespread sexual disruption in wild fish. Environmental Science & Technology. 1998;**32**:2498-2506. DOI: 10.1021/es9710870

[8] Pait AS, Nelson JO. Endocrine disruption in fish: An assessment of recent research and results. NOAA Technical. Memorandum. NOS NCCOS CCMA. Silver Spring. 2002;**149**:55

[9] Peck M, Gibson RW, Kortenkamp A, Hill EM. Sediments are major sinks of steroidal estrogens in two United Kingdom rivers. Environmental Toxicology and Chemistry. 2004;**23**:945-952. DOI: 10.1897/03-41

[10] Propper C. The study of endocrine-disrupting compounds: Past approaches and new directions. Integrative and Comparative Biology. 2002;**45**:194-200. DOI: 10.1093/icb/45.1.194

[11] Sellin MK, Snow DD, Schwarz M, et al. Agrochemicals in Nebraska, USA, watersheds: Occurrence and endocrine effects. Environmental Toxicology and Chemistry. 2009;**28**:2443-2448. DOI: 10.1897/09-135.1

[12] Sellin MK, Abbott K, Cowman T, et al. Occurrence and endocrine effects of agrochemicals in a small Nebraska, USA, watershed. Environmental Toxicology and Chemistry. 2011a;**30**(10):2253-2260. DOI: 10.1002/etc.615

[13] Velisek J, Stara A, Kolarova J, et al. Biochemical, physiological and morphological responses in common carp (*Cyprinus carpio* L.) after long-term exposure to terbutryn in real environmental concentration. Pesticide Biochemistry and Physiology. 2011;**100**:305-313. DOI: 10.1016/j.pestbp.2011.05.004

[14] Orrego R, Moraga-Cid G, González M, et al. Reproductive, physiological, and biochemical responses in juvenile female rainbow trout (*Oncorhynchus mykiss*) exposed to sediment from pulp and paper mill industrial discharge areas. Environmental Toxicology and Chemistry. 2005;**24**:1935-1943. DOI: 10.1897/04-251R1.1

[15] Kolok AS, Snowc DD, Kohnod S, et al. Occurrence and biological effect of exogenous steroids in the Elkhorn River, Nebraska, USA. Science of the Total Environment. 2007;**388**:104-115. DOI: 10.1016/j.scitotenv.2007.08.001

[16] Sellin MK, Snow DD, Kolok AS. Reductions in hepatic vitellogenin and estrogen receptor alpha expression by sediments from an agriculturally impacted waterway. Aquatic Toxicology. 2010;**96**:103-108. DOI: 10.1016/j.aquatox.2009.10.004

[17] Sellin MK, Conoan NH, Cox MB, et al. The anti-estrogenic activity of sediments from agriculturally intense watersheds: Assessment using in vivo and in vitro assays. Aquatic Toxicology. 2011;**105**:189-198. DOI: 10.1016/j.aquatox.2011.04.008

[18] Kolpin DW, Blazer VS, Gray JL, et al. Chemical contaminants in water and sediment near fish nesting sites in the Potomac River basin: Determining potential exposures to smallmouth bass (Micropterus dolomieu). Science of the Total Environment. 2013;**443**:700-716. DOI: 10.1016/j.scitotenv.201209063

[19] Jessick AM, Skolness S, Kolok AS. Sandy sediment and the bioavailability of 17b-trenbolone to adult female fathead minnows. Aquatic Toxicology. 2014;**148**:48-54. DOI: 10.1016/j.aquatox.2013.12.025

[20] Fossi MC. Nondestructive biomarkers in ecotoxicology. Environmental Health Perspectives. 1994;**102**(12):49-54

[21] Shugart L, McCarthy J, Halbrook R. Biological markers of environmental and ecological contamination: An overview. Risk Analysis. 1992;**12**(3):353-360. DOI: 10.1111/j.1539-6924.1992.tb00687.x

[22] Paruelo JM, Guerschman JP, Piñeiro G, et al. Cambios en el uso de la tierra en Argentina y Uruguay: marcos conceptuales para su análisis. Agrociencia. 2006;**X**:47-61

[23] Céspedes-Payret C, Pineiro G, Achkar M, et al. The irruption of new agro-industrial technologies in Uruguay and their environmental impacts on soil, water supply and biodiversity: A review. International Journal of Environment and Health. 2009;**3**:175-197. DOI: 10.1504/IJENVH.2009.024877

[24] Vega E, Baldi G, Jobbágy EG, Paruelo J. Land use change patterns in the Río de la Plata grasslands: The influence of phytogeographic and political boundaries. Agriculture, Ecosystems and Environment. 2009;**134**:287-292. DOI: 10.1016/j.agee.2009.07.011

[25] Comisión Administradora del Río Uruguay CARU. Segundo seminario sobre el río Uruguay y sus recursos pesqueros. Publicaciones de la Comisión Administradora del Río Uruguay. Serie Técnico-Científica. 1992;**1**(1):83

[26] Miguez D, Carrara MV, Carnikian A, et al. Evaluacion ecotoxicologica de sedimentos en una zona del Rıo Uruguay con puntos finales indicadores de toxicidad aguda, sub-letal, cronica, reproductiva y teratogenica. INNOTEC. 2010;**5**:3-10

[27] Saizar C, Boccardi L, Clemente J, et al. Lınea de base para evaluar el impacto de una planta de celulosa en el Rıo Uruguay. INNOTEC. 2010;**5**:11-22

[28] Korpivaara M. Botnia's pulp mill project in Uruguay moving ahead. Paperi Ja Puu. 2004;**86**:396-397

[29] McMaster ME, Hewitt LM, Parrott JL. A decade of research on the environmental impacts of pulp and paper mill effluent in Canada: Field studies and mechanistic research. Journal of Toxicology and Environmental Health, Part B:Critical Reviews. 2006;**9**(4):313-339. DOI: 10.1080/15287390500195869

[30] CARU. Comisión Administradora del Río Uruguay. Informe de avance Programa de Calidad de las Aguas y Control de la Contaminacion del Río Uruguay – Etapa I 1987 -1990. Publicaciones de la Comisión Administradora del Rió Uruguay. Serie Técnico-Científica. 1993;**2**(1):88

[31] Heppell SA, Denslow ND, Folmar LC, Sullivan CV. Universal assay of vitellogenin as a biomarker for environmental estrogens. Environmental Health Perspectives. 1995; **103**(7):9-15

[32] Folmar LC, Denslow ND, Rao V, et al. Vitellogenin induction and reduced serum testosterone concentrations in feral male carp (*Cyprinus carpio*) captured near a major metropolitan sewage treatment plant. Environmental Health Perspectives. 1996;**104**:1096-1101

[33] Bachmann Christiansen L. Feminisation of fish. The effect of estrogenic compounds and their fate in sewage treatment plants and nature. Environmental Project No. 729. Danish Environmental Protection Agency [Internet] 2002. Available from: https://www2.mst.dk/udgiv/publications/2002/87-7972-305-5/pdf/87-7972-306-3.pdf [Accessed: April 20, 2018]

[34] Vega-López A, Martínez-Tabche L, Domínguez-López ML, et al. Vitellogenin induction in the endangered goodied fish Girardinichthys viviparus: Vitellogenin characterization and estrogenic effects of polychlorinated biphenyls. Comparative Biochemistry and Physiology. 2006;**142**(3-4):356-364. DOI: 10.1016/j.cbpc.2005.11.009

[35] Rivas A, Granada A, Jiménez M, Olea F, Olea N. Exposición humana a disruptores endocrinos. Ecosistemas. Revista Científica y Técnica de Ecología y Medio Ambiente. 2005;**13**(3):7-12

[36] Smith BB, Walker KF. Spawning dynamics of common carp in the River Murray, South Australia, shown by macroscopic and histological staging of gonads. Journal of Fish Biology. 2004;**64**:336-354. DOI: 10.1111/j.0022-1112.2004.00293.x

[37] Eguren G. Evaluación del efecto derivado de la descarga de compuestos organoclorados al río Biobío usando biomarcadores en peces [thesis]. Universidad de Concepción (Chile). Centro EULA-Chile; 1997. 84 pp

[38] González P, Oyarzún C. Variabilidad de índices biológicos en Pinguipes chilensis Valenciennes 1833 (Perciformes, Pinguipedidae): ¿Están realmente correlacionados? Gayana (Concepc). 2002;**66**(2):249-253. DOI: 10.4067/S0717-65382002000200023

[39] Dirección Nacional de Recursos Acuáticos (DINARA). Carpa Común [Internet]. 2006. Available from: URL:http://dinara.gub.uy [Accessed: October 2006]

[40] Gimeno S, Komen H, Gerritsen AGM, Bowmer T. Feminisation of young males of the common carp, Cyprinus carpio, exposed to 4-tert-pentylphenol during sexual differentiation. Aquatic Toxicology. 1998;**43**:77-92. DOI: 10.1016/S0166-445X(98)00056-3

[41] Gimeno S, Komen H, Joblin S, et al. Demasculinisation of sexually mature male common carp, Cyprinus carpio, exposed to 4-tert-pentylphenol during spermatogenesis. Aquatic Toxicology. 1998;**43**:93-109. DOI: 10.1016/S0166-445X(98)00056-3

[42] Bongers ABJ, Zandieh Doulabi B, Richter CJJ, Komen J. Viable androgenetic YY genotypes of common carp (*Cyprinus carpio* L.). The Journal of Heredity. 1999;**90**:195-198. DOI: 10.1093/jhered/90.1.195

[43] Organisation for Economic Co-operation and Development. OECD Environment Health and Safety Publications. Series on Testing and Assessment No. 47. Detailed Review Paper on Fish Screening Assays for the Detection of Endocrine Active Substances. [Internet]. 2004 ENV/JM/MONO(2004)18. Available from: http://www.oecd.org [Accessed: October 2006]

[44] EPA/630/R-96/012. Special report on environmental endocrine disruption: An effects assessment and analysis. U.S. Environmental Protection Agency Washington, D.C. 1997;120 pp

[45] Schwaiger J, Spieser OH, Bauer C, et al. Chronic toxicity of nonylphenol and ethinylestradiol: Haematological and histopathological effects in juvenile Common carp (Cyprinus carpio). Aquatic Toxicology. 2000;**51**(1):69-78. DOI: 10.1016/S0166-445X(00)00098-9

[46] Sole M, Barcelo D, Porte C. Seasonal variation of plasmatic and hepatic vitellogenin and EROD activity in carp, *Cyprinus carpio*, in relation to sewage treatment plants. Aquatic Toxicology. 2002;**60**(3):233-248. DOI: 10.1016/S0166-445X(02)00009-7

[47] Lavado R, Thibaut R, Raldúa D, et al. First evidence of endocrine disruption in feral carp from the Ebro River. Toxicology and Applied Pharmacology. 2003;**196**(2):247-257. DOI: 10.1016/j.taap.2003.12.012

[48] Carballo M, Aguayo S, de la Torre A, Muñoz MJ. Plasma vitellogenin levels and gonadal morphology of wild carp (*Cyprinus carpio* L.) in a receiving rivers downstream of sewage treatment plants. Science of the Total Environment. 2004;**34**(1):71-79. DOI: 10.1016/j.scitotenv.2004.08.021

[49] Nilsen BM, Berg K, Eidem JK, et al. Development of quantitative vitellogenin-ELISAs for fish test species used in endocrine disruptor screening. Analytical and Bioanalytical Chemistry. 2004;**378**:621-633. DOI: 10.1007/s00216-003-2241-2

[50] Biosense Laboratories. Preparation of plasma samples from fish – using syringes for blood sampling. [Internet]. 2004. Available from: http://www.biosense.com [Accessed: May 2006]

[51] Biosense Laboratories. Carp vitellogenin ELISA kit. Product Manual. Prod. N° V01003402. [Internet] 2005. Available from: http://www.biosense.com [Accessed: May 2006]

[52] Berois N, Bolatto C, Brauer MM, Barros C. Gametogenesis, histological gonadal cycle and in vivo fertilization in the whitemouth croaker (*Micropogonias furnieri*, Desmarest, 1823). Journal of Applied Ichthyology. 2004;**20**:169-175. DOI: 10.1111/j.1439-0426.2004.00523.x

[53] Aimale MA, Gatti J. Introducción a las técnicas histológicas. Cátedra de Anatomo-Histología. Universidad Nacional del Sur. Argentina. [Internet] 2006. Available from: http://anatomohistologia.uns.edu.ar [Accessed: May 2006]

[54] Mnif W, Hassine AIH, Bouaziz A, et al. Effect of endocrine disruptor pesticides: A review. International Journal of Environmental Research and Public Health. 2011;**8**:2265-2303. DOI: 10.3390/ijerph8062265

[55] Ng Y, Hanson S, Malison JA, et al. Genistein and other isoflavones found in soybeans inhibit estrogen metabolism in salmonid fish. Aquaculture. 2006;**254**:658-665. DOI: 10.1016/j.aquaculture.2005.10.039

[56] Karels A, Oikari A. Effects of pulp and paper mill effluents on the reproductive and physiological status of perch (*Perca fluviatilis*) and roach (*Rutilus rutilus*) during the spawning period. Annales Zoologici Fennici. 2000;**37**:65-77. DOI: 10.1016/S0273-1223(99)00707-6

[57] Karels A, Markkula E, Oikari A. Reproductive, biochemical, physiological, and population responses in perch (*Perca fluviatilis* L.) and roach (*Rutilus rutilus* L.) downstream of two elemental chlorine-free pulp and paper mills. Environmental Toxicology and Chemistry. 2001;**20**:1517-1527. DOI: 10.1002/etc.5620200715

[58] Kiparissi Y, Balch GC, Metcalfe TL, Metcalfe CD. Effects of the isoflavones genistein and equol on the gonadal development of Japanese Medaka (*Oryzias latipes*). Environmental Health Perspectives. 2003;**111**:1158-1163. DOI: 10.1289/ehp.5928

[59] Örn S. The Zebrafish as a model organism for evaluation of endocrine disrupters. [thesis]. Stockholm, Sweden: Swedish University of Agricultural Sciences; 2006

[60] Jobling S, Sumpter JP, Sheahan D, et al. Inhibition of testicular growth in rainbow trout (*Oncorhynchus mykiss*) exposed to estrogenic alkylphenolic chemicals. Environmental Toxicology and Chemistry. 1996;**15**:194-202. DOI: 10.1002/etc.5620150218

[61] Devlin RH, Nagahama Y. Sex determination and sex differentiation in fish: An overview of genetic, physiological, and environmental influences. Aquaculture. 2002;**208**:191-364. DOI: 10.1016/S0044-8486(02)00057-1

[62] Martel PH, Kovacs TG, Voss RH. Effluents from Canadian pulp and paper mills: A recent investigation of their potential to induce mixed function oxygenase activity in fish. In: Servos MR, Munkittrick KR, Carey JH, Van Der Kraak GJ, editors. Environmental Fate and Effects of Pulp and Paper Mill Effluents. Del Ray Beach, FL, USA: St. Lucie Press; 1996. pp. 401-412

[63] Williams TG, Carey JH, Burnison BK, et al. Rainbow trout (*Oncorhynchus mykiss*) mixed function oxygenase responses caused by unbleached and bleached pulp mill effluents: A laboratory-based study. In: Servos MR, Munkittrick KR, Carey JH, Van Der Kraak GJ, editors. Environmental Fate and Effects of Pulp and Paper Mill Effluents. Del Ray Beach, FL, USA: St. Lucie Press; 1996. pp. 379-389

[64] Coakley J, Hodson PV, van Heiningen A, Cross T. MFO induction in fish by filtrates from chlorine dioxide bleaching of wood pulp. Water Research. 2001;**35**:921-928. DOI: 10.1016/S0043-1354(00)00329-8

[65] Munkittrick KR, McMaster ME, Portt CB, et al. Changes in maturity, plasma sex steroids levels, hepatic mixed-function oxygenase activity, and the presence of external lesions in lake whitefish (*Coregonus clupeaformis*) exposed to bleached kraft mill effluent. Canadian Journal of Fisheries and Aquatic Sciences. 1992;**49**(8):1560-1569. DOI: 10.1139/f92-173

[66] Boer J, Brinkman U. The use of fish as biomonitors for the determination of contamination of the aquatic environment by persistent organochlorine compounds. Analytical Chemistry. 1994;**13**:397-404. DOI: 10.1016/0165-9936(94)85011-9

[67] De Matteis F. The role of cytochrome P-450 in drug metabolism and toxicity. In: Renzoni A, Mattei N, Lari L, Fossi MC, editors. Contaminants in the Environment a Multidisciplinary Assessment of Risk to Man and Other Organisms. CRC Press, Inc, Lewis Publishers USA. 1994;**81**-92. ISBN 0-87371-853-4

[68] Fossi M, Focardi S, Leonzio C, et al. Use of biomarkers to evaluate effects of xenobiotic componds in the Biobio basin (Central Chile). Bulletin of Environmental Contamination and Toxicology. 1995;**55**:36-42. DOI: 10.1007/BF00212386

[69] Munkittrick KR, McMaster ME, Van Der Kraak, et al. Development of methods for effects-driven cumulative effects assessment using fish populations: Moose River project. Society of Environmental Toxicology and Chemistry (SETAC). ISBN 1-880611; 2000