Bull Environ Contam Toxicol (2016) 97:632–638
DOI 10.1007/s00128-016-1925-1

Dynamics of Aromatase and Physiological Indexes in Male Fish as Potential Biomarkers of Anthropogenic Pollution

N. F. Guyón[1] · M. A. Roggio[2] · M. V. Amé[3] · D. A. Wunderlin[4] · M. A. Bistoni[1]

Received: 20 March 2016 / Accepted: 15 September 2016 / Published online: 26 September 2016

Abstract Endocrine disruption on aquatic wildlife is being increasingly reported, and the changes in gene aromatase expression are used as indicators. However, natural fluctuations in brain and gonadal aromatase expression and physiological indexes have not been previously measured in a fish species (*Jenynsia multidentata*) throughout a complete reproductive cycle, nor the biological effects of anthropogenic inputs on these responses. Accordingly, males were monthly collected over a year in both, a reference and a contaminated site. Physicochemical analyses of water samples were done and reflected a strong anthropogenic impact. Brain aromatase fluctuates along the reproductive cycle of this species and, noticeably, the increase of brain gene expression begins with a 1 month delay in the contaminated site. This mismatch is also evidenced for testes weight. Hepatosomatic index also revealed adverse effects in the polluted site. In turn, the alterations observed in biological responses could be affecting the reproduction of this fish species.

Keywords Water pollution · Sewage effluents · Biomarkers · Aromatase · Somatic indexes · Fish

✉ M. A. Bistoni
bistonimaria@gmail.com; mbistoni@unc.edu.ar

[1] IDEA-Instituto de Diversidad y Ecología Animal, CONICET and Facultad de Ciencias Exactas, Físicas y Naturales, Universidad Nacional de Córdoba, Av. Vélez Sarsfield 299, 5000 Córdoba, Argentina

[2] Facultad de Ciencias Exactas, Físicas y Naturales, Cátedra de Diversidad Animal II, Universidad Nacional de Córdoba, Avenida Vélez Sársfield 299, CP X5000JJC Córdoba, Argentina

[3] Facultad de Ciencias Químicas, Departamento de Bioquímica Clínica-CIBICI, Universidad Nacional de Córdoba-CONICET, Haya de la Torre esquina Medina Allende, Ciudad Universitaria, CP X5000HUA Córdoba, Argentina

[4] ICYTAC- Instituto de Ciencia y Tecnología de Alimentos Córdoba, CONICET and Facultad de Ciencias Químicas, Dpto. Química Orgánica, Universidad Nacional de Córdoba, Av. Juan Filloy s/n, CP 5016 Córdoba, Argentina

Endocrine disruption in wildlife is being increasingly reported worldwide, mainly on aquatic environments. Endocrine disrupting compounds (EDCs) includes many natural or manmade substances that are able to alter the functioning of the endocrine system, and therefore, represents a significant threat for aquatic organisms and human health. Wastewater treatment plants (WWTP) are commonly not designed to eliminate EDCs and therefore suppose unavoidable chemical pollution sources in freshwater ecosystems because of the incomplete elimination of pollutants during water treatment processes (Gomes et al. 2003). Among the environmental contaminants that have been identified in sewage effluents, xenoestrogens (e.g. phytoestrogens, mycoestrogens and 17-α-ethynylestradiol) are of special interest because of their ability to induce biological responses similar to those caused by the natural hormone 17-β-estradiol. Additionally, there are other chemicals frequently found in WWTP discharges that have a weak estrogenic activity (e.g. alkylphenols, bisphenol A and pesticides). In most South American rivers, due to insufficient infra-structure, untreated or poorly treated wastewaters are released into the environment (Bertin et al. 2011).

Biomarkers are early warning signals for detecting environmental stress and long-term negative effects on populations and communities. They also allow achieving cleaning actions before irreversible effects occur (Van der Oost et al. 2003). The general state of health individuals is used as marker to individual level since they are influenced by generic stressors ranging from physicochemical parameters to contaminants (Kime 1997). At lower level of biological organization, the study of changes in gene expression allows an early detection of toxic effects occurring due to pollution charges (Contardo-Jara and Wiegand 2008). In this regard, aromatase genes are increasingly used as indicators of endocrine disruption in ecotoxicological studies. Cytochrome P450 aromatase is the steroidogenic enzyme that catalyzes the conversion of androgens to estrogens (Gonzalez and Piferrer 2003). Euteleosts fish express two structurally and functionally different P450 aromatase isoforms, termed Cyp19a1a (preferentially expressed in gonads), and Cyp19a1b (preferentially expressed in brain). Interestingly, both aromatase genes are potential target for EDCs, mainly xenoestrogens. Many environmental contaminants can modulate its expression or activity altering the rate of estrogen production and disturbing local and systemic levels (Cheshenko et al. 2008). In fish species collected in rivers that receive WWTP discharges an increase in brain aromatase was observed (Geraudie et al. 2011), suggesting the presence of estrogenic compounds. However, the effects of this type of anthropogenic inputs on aromatase expression and physiological indexes throughout a complete reproductive cycle have not been previously analyzed in fish species.

Given this background, the aim of the present study was: First, to characterize the natural monthly fluctuations during a year in a reference site in aromatase expression and fish health indexes in the fish *Jenynsia multidentata*. Second, evaluate the effects of anthropogenic contamination on aromatase and condition indexes. *J. multidentata* is a fish widely distributed in the Neotropical Region (Malabarba et al. 1998) and it has been used as an excellent model in both laboratory and field studies mainly because of its ability to adapt to a wide variety of environments, including poor water quality conditions (Hued and Bistoni 2005). To the best of our knowledge; this is the first characterization of natural seasonal fluctuations in aromatase expression in *J. multidentata*. Furthermore, this work contributes to the understanding about the effects of anthropogenic pollution on this specific biomarker of endocrine disruption considering a complete reproductive cycle.

Materials and Methods

The Suquía River basin (Córdoba province, Argentina) originates at the San Roque dam, flows across Córdoba city, and drains into the depression of Mar Chiquita Lake. This lake and the mouth of its tributaries are considered as a Ramsar site (wetland of international concern included in the list of the Ramsar Convention) and it is located 150 km downstream from Córdoba City (Fig. 1). At the lower basin, the Suquía River receives sewage discharge from the only WWTP of Bajo Grande. The clearance capacity of this plant is overreached and, as a consequence, discharges wastewaters without previous treatment directly into the river (Hued and Bistoni 2005). The inputs of urban and industrial wastes as well as agrochemicals from many industries and crops established on the margins of this river have contributed to an increased amount of toxic effluents entering the river.

Sampling sites upstream and downstream of the WWTP discharge area were established (Fig. 1) considering previous reports on the water quality of the basin (Wunderlin et al. 2001; Valdés et al. 2014). Yuspe River is located 30 km upstream from the San Roque dam and is an already established reference sampling site. Primero River, the downstream site, is located at 70 km from the WWTP discharge and receives varying concentrations of contaminants from different sources.

Every 2 months, a water sample was collected at each sampling site to describe the water quality during 2010. Dissolved oxygen, conductivity, pH and water temperature were monitored *in situ* using a WTW multiparametric

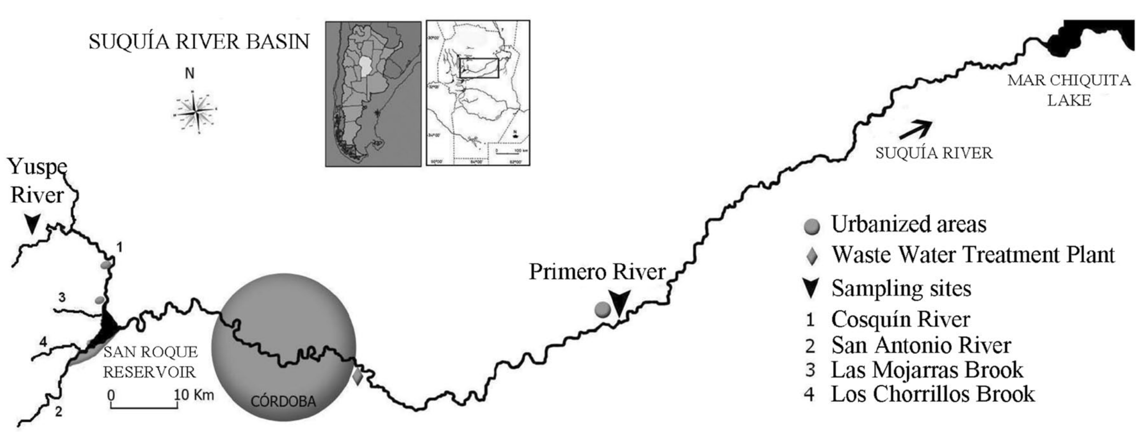

Fig. 1 Sampling stations selected along the Suquía River basin

equipment, while 5-day biological oxygen demand (BOD-5), ammonia–nitrogen, nitrite-nitrogen, nitrate-nitrogen, orthophosphate-phosphorous, chloride, sulfates, hardness, calcium, magnesium, total solids, and total coliforms were measured at the laboratory, according to APHA et al. (2005) methodologies. These physicochemical parameters were integrated into a Water Quality Index (WQI) (Pesce and Wunderlin 2000). Thirteen specimens of *J. multidentata* adult males were monthly caught at each site using a standard backpack electrofisher. The fish were transferred alive to the laboratory in oxygenated water tanks (20 L) and sacrificed with an overdose of tricaine methanolsulfonate (MS-222). Fish were weighted and total length recorded. After that, they were dissected and liver and gonad were weighed to determine of general state of health of individuals (van der Oost et al. 2003): Condition factor (CF = [(body weight/total length) × 100.000]); Gonadosomatic index as: GSI = [(gonad weight/(total body weight) × 100)] and Hepato-somatic index as: HSI = [(liver weight/(total body weight) × 100)]. Brains and gonads were quickly dissected and stored directly in RNAlater (QIAGEN) at −80°C for qPCR analysis. For *cyp19a1* mRNA quantification, total RNA was extracted from brain and gonadal tissue by the guanidine thiocyanate–phenol chloroform extraction method in accordance with Chomczynski and Sacchi (1987). Nonspecific reverse transcription was performed from individual tissue total RNA. Quantitative polymerase chain reaction was performed with a Bio-Rad iQ cycler and was used to amplify and measure the transcript abundance of *cyp19a1a* in testis and *cyp19a1b* in brain using specific *J. multidentata* primers for real-time polymerase chain reaction (Guyón et al. 2012a). Normalized expression levels for target genes were generated using the standard curve method with *J. multidentata* β-actin as reference gene (Larionov et al. 2005). Relative fold changes to reference sampling site were also calculated.

Statistical analyses were carried out using Infostat Software Package (Di Rienzo et al. 2011). All results are expressed as mean ± standard deviation. Normal distribution of data was controlled using a Shapiro-Wilk's test ($p \leq 0.05$), and Levene test was used to check the homogeneity of variance. In order to find significant differences between sites, months and to see if the pattern of results is the same between sites over the months, a two factors variance analyses on ranks transformed variables was performed (Hollander and Wolfe 1973).

Results and Discussion

During the last decades the Suquía River basin has been intensively studied not only for the detection of detrimental changes in water quality due to anthropogenic activities, but also to determine the deleterious effects on aquatic biota (e.g. Pesce and Wunderlin 2000; Hued and Bistoni 2005; Guyón et al. 2012b; Hued et al. 2012). The historical reports of water quality reveal the progressive deterioration of this aquatic system and it was confirmed in this study. Water quality was severely affected downstream the WWTP showing significant differences for all the measured parameters between sites (Table 1). The drastic diminution of dissolved oxygen, the increase in total coliforms and the high concentration of nitrogen species (nitrate, nitrite and ammonia) were the main factors affecting water quality, and they are frequently associated with sewage inputs (Wunderlin et al. 2001). Other parameters such as conductivity, total solids, hardness, calcium, magnesium, chloride and sulfates were significantly increased in the polluted site. It has been proposed that these parameters are linked to urban, industrial and agricultural runoff (Wunderlin et al. 2001; Pasquini et al. 2012). The presence of pesticides, hormones, pharmaceuticals and heavy metals confirms those sources of pollution (Monferrán et al. 2011; Maggioni et al. 2012; Bonansea et al. 2013a; Valdés et al. 2014). WQI registered values ranged from 51 to 60 in Primero River. Hued and Bistoni (2005) pointed out a WQI value close to 50 seriously difficult aquatic life. The CF was not significantly different between sites when analyzing all the studied period ($F = 0.27$; $p = 0.607$; Fig. 2a). However, differences among months were observed when considering both sites together ($F = 5.73$; $p < 0.001$), indicating variations in the weight/length relationship along the year. The pattern of change was similar in both sites, as no interaction between months and sites was observed ($F = 0.50$; $p = 0.901$). The fact that there was no decrease in the CF suggests that the fish able to inhabit Primero River locality were not overtly affected by exposure to occurring contaminants with respect to somatic growth. This result is in line with studies showing not changes in this index in fish sampled in polluted places (Hinfray et al. 2010).

The contrast between the low HSI values recorded in males coming from the reference site (Yuspe River) and the high HSI values in samples from Primero River locality ($F = 76.31$; $p < 0.001$; Fig. 2b) may be linked to the presence of compounds capable of inducing metabolic activity in fish livers in the polluted site. Accordingly, elevated HSI have been detected in species exposed to WWTP effluents (Barber et al. 2007; Vajda et al. 2011). This increase in liver weight could be an unspecific response to contaminants, induced by an augmented demand of enzymatic activity. In this regard, Maggioni et al. (2012) reported increased levels of antioxidant enzymes in female livers of *J. multidentata* collected in the same polluted site (Primero River). Moreover, the same authors detected histopathological liver damages such as hypertrophia, hydropic degeneration, necrosis and fibrosis, all damages that have been associated with

Table 1 Water quality parameters of selected sampling sites in Suquía River basin

Variables	January		March		May		August		October	
	Yuspe River	Primero River	Yuspe River	Primero River	Yuspe River	Primero River	Yuspe River	Primero River	Yuspe River	Primero River
Ammonia–nitrogen	0.39 ± 0.54 a	5.00 ± 0.22 b	1.65 ± 0.67 a	1.52 ± 0.52 a	0.18 ± 0.03 a	1.31 ± 0.22 b	0.087 ± 0.003 a	11.69 ± 0.99 b	0.87 ± 0.64 a	0.86 ± 0.32 a
Biological oxygen demand after 5 days (BOD)	1.35 ± 0.07 a	4.50 ± 0.14 b	0.30 ± 0.14 a	4.11 ± 0.14 b	1.00 ± 0.14 a	1.58 ± 0.01 b	1.35 ± 0.01a	1.22 ± 0.01 b	1.71 ± 0.14 a	2.33 ± 0.01 b
Chloride	3.91 ± 0.02 a	89.33 ± 0.69 b	3.91 ± 0.03 a	77.61 ± 0.69 b	3.91 ± 0.01 a	80.54 ± 0.69 b	3.91 ± 0.04 a	112.76 ± 0.69 b	3.91 ± 0.02 a	79.08 ± 1.38 b
Conductivity [µS cm^{-1}][a]	72.00 ± 1.41 a	980.00 ± 7.07 b	49.00 ± 1.41 a	978.00 ± 2.83 b	82.00 ± 1.41 a	967.00 ± 1.56 b	148.00 ± 1.71 a	1351.00 ± 1.72 b	131.01 ± 2.83 a	1335.00 ± 1.78 b
Dissolved oxygen[a]	10.95 ± 0.07 a	7.56 ± 0.03 b	10.17 ± 0.03 a	7.36 ± 0.01 b	10.70 ± 0.14 a	6.88 ± 0.01 b	10.03 ± 0.01 a	4.84 ± 0.01 b	9.02 ± 0.03 a	5.57 ± 0.03 b
Nitrates-nitrogen	0.88 ± 0.09 a	31.07 ± 2.20 b	0.25 ± 0.03 a	7.24 ± 2.45 b	0.51 ± 0.12 a	9.11 ± 1.47 b	0.30 ± 0.01 a	2.65 ± 1.48 b	0.36 ± 0.08 a	7.50 ± 0.06 b
Nitrites-nitrogen	0.09 ± 0.04E-8 a	0.28 ± 0.01 b	0.09 ± 0.01 a	0.18 ± 0.09E-2 b	0.01 ± 0.01E-8 a	1.75 ± 0.02 b	0.011 ± 0.003 a	0.79 ± 0.04 b	0.076 ± 0.001 a	0.177 ± 0.002 b
Orthophosphates phosphorous	<DL	0.74 ± 0.16	<DL	0.28 ± 0.04	<DL	0.25 ± 0.03	<DL	1.37 ± 0.02	<DL	0.07 ± 0.04
pH[a]	8.75 ± 0.35 a	8.22 ± 0.03 a	7.92 ± 0.03 a	8.58 ± 0.02 b	8.15 ± 0.03 a	7.44 ± 0.01 b	8.94 ± 0.03 a	7.53 ± 0.01 b	8.80 ± 0.14 a	7.85 ± 0.14 b
Solids: total	76.40 ± 0.14 a	1008.40 ± 0.14 b	56.80 ± 0.28 a	701.60 ± 0.28 b	40.00 ± 1.41 a	400.00 ± 1.41 b	96.40 ± 0.14 a	891.60 ± 0.14 b	58.40 ± 0.28 a	676.00 ± 2.83 b
Sulfates	0.29 ± 0.33 a	132.90 ± 1.61 b	0.37 ± 0.03 a	132.27 ± 0.89 b	0.35 ± 0.06 a	67.14 ± 8.02 b	0.25 ± 0.04 a	121.99 ± 1.34 b	0.35 ± 0.06 a	67.14 ± 8.02 b
Temperature[a] [°C]	30.50 ± 0.28 a	24.40 ± 0.24 b	16.71 ± 0.49 a	22.60 ± 0.57 b	12.30 ± 0.28 a	11.70 ± 0.49 a	18.20 ± 0.57 a	15.00 ± 0.57 b	18.01 ± 0.35 a	17.30 ± 0.42 a
Total coliforms	1.50E + 04 ± 7.07 a	8.80E + 05 ± 7.07 b	2.00E + 02 ± 2.83 a	1.20E + 05 ± 2.83 b	5.00E + 02 ± 1.41 a	2.40E + 05 ± 1.41 b	2.20E + 03 ± 1.41 a	1.50E + 05 ± 1.41 b	5.00E + 02 ± 2.83 a	5.00E + 03 ± 2.83 b
Hardness	29.33 ± 7.32 a	337.53 ± 15.45 b	20.70 ± 0.07 a	317.41 ± 6.51 b	28.75 ± 1.63 a	281.75 ± 4.88 b	57.5 ± 3.3 a	292.68 ± 0.81 b	50.6 ± 0.1 a	289.23 ± 0.81 b
Calcium	7.14 ± 1.63 a	98.41 ± 6.84 b	6.45 ± 1.30 a	92.18 ± 3.42 b	8.76 ± 0.65 a	88.96 ± 0.65 b	14.75 ± 3.63 a	100.02 ± 1.96 b	8.30 ± 1.30 a	87.81 ± 0.33 b
Magnesium	2.79 ± 2.77 a	22.36 ± 7.90 b	1.12 ± 0.79 a	21.24 ± 1.58 b	1.68 ± 0.79 a	14.53 ± 1.58 b	5.03 ± 0.79 a	10.48 ± 0.99 b	7.27 ± 0.79 a	17.05 ± 0.88 b
WQI	74.00 ± 2.33	51.70 ± 1.89	82.70 ± 0.56	54.30 ± 1.00	88.70 ± 1.00	57.00 ± 1.80	88.70 ± 2.00	51.70 ± 1.13	78.70 ± 0.99	62.70 ± 0.71

Values are expressed in mg L^{-1} if not indicated directly, total coliforms correspond to E + 3 exponential values (i.e. 2.3 = 2300), and are expressed as MPN 100 m L^{-1} (most probable number per 100 mL). Different letters indicate significantly different values between sites ($p < 0.05$). Detection limit (DL) for orthophosphate phosphorous = 0.01 mg L^{-1}

[a]Field measurements during the monitoring

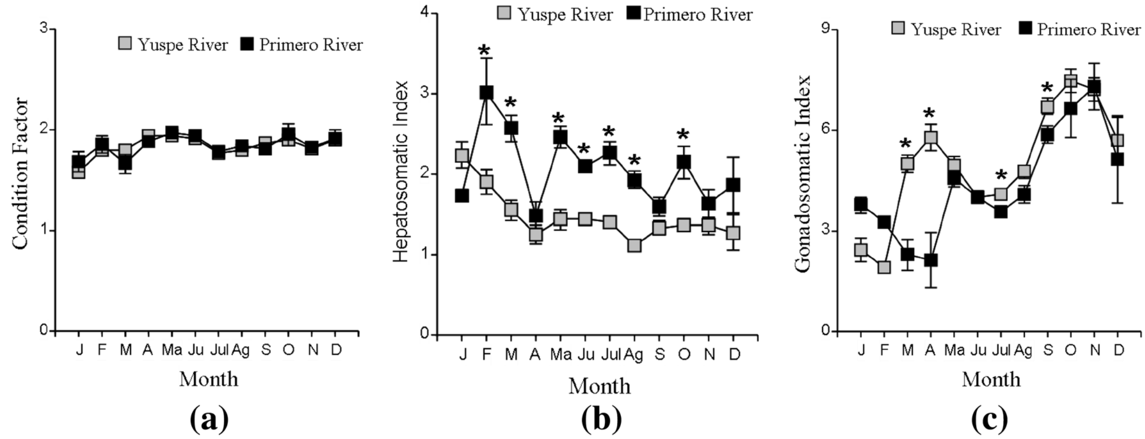

Fig. 2 Condition factor (**a**), Hepatosomatic (**b**) and Gonadosomatic (**c**) index measured in two sites with different water quality during an annual cycle. Significant differences between sites are indicated with *asterisk*

pesticide and wastewater exposure (Ballesteros et al. 2007; Hued et al. 2012). When considering both sites together, significant differences among months were observed ($F = 6.86$; $p < 0.001$), showing the highest values of HSI during summer (January, February, March). The presence of interaction between months and sites ($F = 3.40$; $p < 0.001$) indicated variations in the pattern of change at each site along the year.

When considering all the studied period, the gonad growth seems to be affected by exposure to the contaminants as is shown by the GSI reduction at the polluted site ($F = 19.31$; $p < 0.001$). This result is similar to the reported for other species after exposure to WWTP discharges (Hinfray et al. 2010; Vajda et al. 2011). When considering both sites together, significant differences were observed among months ($F = 31.28$; $p < 0.001$), showing the highest values of GSI during spring (September, October, November, December). Both sites showed two peaks in GSI during the breeding season that goes from August to April (Fig. 2c); however, the pattern of change was significantly different for each site, as indicated by an interaction between month and site ($F = 5.01$; $p < 0.001$). In the reference site, an increase in GSI was observed since July reaching a maximum in October, while the other increase started in March with maximum in April. At Primero River, a marked delay in the GSI increase was observed. The first peak started in August with maximum in November, while the second peak was in May. Consistent with this result, Bianco (2011) observed the same pattern when analyzing the reproductive cycle of *J. multidentata* females in the same year and sampling sites than this study. This author found a 1 month delay in the presence of embryos in the ovary in the site with elevated pollution. It might be possible that the amount of chemical compounds present in polluted environments can cause endocrine disruption and negative effect on fish reproduction (Jobling et al. 2002) and could also modify the beginning and length of the reproductive cycle.

WWTP discharges are linked with EDCs mainly xenoestrogens (Vajda et al. 2011). As a consequence, an increase in brain aromatase could be expected since in laboratory studies estrogenic compounds up-regulate its expression and activity (Vosges et al. 2011). Accordingly, Geraudie et al. (2011) found that aromatase activity was significantly upregulated in wild fish collected in river that receives WWTP effluents, suggesting the occurrence of estrogenic compounds and their involvement in aromatase activity modulation. In the study area, downstream the WWTP of Bajo Grande discharge, Valdés et al. (2014) detected estrogenic compounds like estrone. Given this background, we hypothesized to found elevated brain aromatase expression in males collected at this site. However, we found lower levels of mean seasonal values than the reference site (1.41 for Yuspe River and 1.21 for Primero River), without statistical differences between sites ($F = 3.11$; $p = 0.0801$). Laboratory studies showed that the pesticide chlorpyrifos and its commercial mixture with cypermethrin inhibit brain aromatase expression in *J. multidentata* females (Bonansea et al. 2013b). Both compounds have been detected in Primero River at concentrations that exceeded the international limits for aquatic life preservation (Bonansea et al. 2013a). Additionally, in a previous study conducted with wild females of this species collected in a pollution gradient, a *cyp19a1b* inhibition was observed downstream the WWTP of Bajo Grande (Guyón et al. 2012b). In this regard, fish are exposed to a complex mixture of pollutants some of which could act inhibiting aromatase expression. Fish brain aromatase is characterized by an elevated expression and activity during the spawning period in several species, suggesting a role in the control of the reproductive cycle (Gonzalez and Piferrer 2003; Cheshenko et al. 2008). Accordingly, when considering both sites together, significant differences among months were observed ($F = 20.29$; $p < 0.001$), with maximum values of aromatase expressed

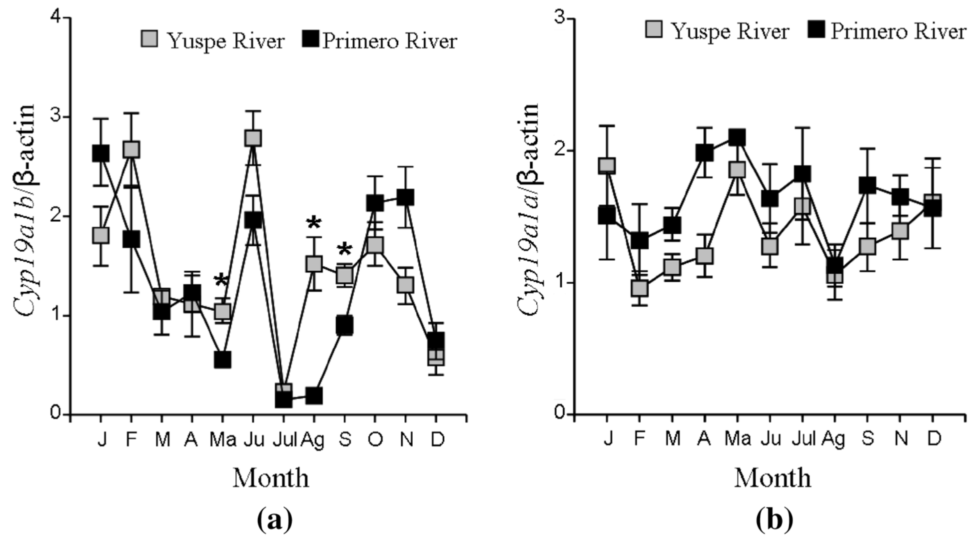

Fig. 3 Brain (**a**) and gonadal (**b**) *cyp19a1* expression normalized to β-actin in *J. multidentata* males, measured by qRT-PCR. *Asterisk* indicate significant statistical differences between sites ($p<0.05$)

during the reproductive season of this species according to the reproductive period description of Goyenola et al. (2011). In the reference site, the gene abundance showed an enhancement from August to October, and afterwards another increase in January to February. From March to July, brain aromatase expression was lower, however a peak was observed in June. In the polluted site an increase in aromatase expression was observed from September to November and another augment in January. This result shows a significant mismatch in the increase of the gene expression beginning with 1 month delay relative to reference site, indicating that the seasonal pattern of variation was different (Fig. 3a, $F=5.18$; $p<0.001$). Fishes from the reference sampling site had approximately 1.6, 6.4 and 1.5-fold higher *cyp19a1b* expression than fishes from the polluted sites in May, August and September, respectively.

This mismatch could be related to offsets in the beginning of the reproductive cycle due to pollution. Similarly to Yuspe River, a peak in June was observed in Primero River. In this regard, Gonzalez and Piferrer (2003) observed a peak of aromatase activity in *D. labrax* in a time when an overall reorganization of the gonadal tissues occurs after the spawning season and the authors suggested that these phenomena could somehow be connected.

Gonadal aromatase (Fig. 3b) showed significant differences between sites when considering the studied period ($F=5.89$; $p=0.017$), being 1.37 the value for Yuspe River and 1.65 for Primero River, but no differences were observed when considering month to month between sites. All along the studied period there were differences between months ($F=2.28$; $p=0.016$); and no interaction was registered between months and sites ($F=0.63$; $p<0.786$), indicating that the seasonal changing pattern was similar for both sites. The effects of WWTP effluents exposure on this parameter do not appear to follow a general rule. Lavado et al. (2004) informed lower gonadal aromatase activity in males of *C. carpio* collected downstream a WWTP discharge, while Douxfils et al. (2007) did not find differences in males of two species coming from sites upstream and downstream WWTP outfall. In *J. multidentata, cyp19a1a* expression was significantly higher in Primero River than Yuspe River. This result suggests the presence in the river of compounds capable of modulate this variable without causing an estrogenic response in *cyp19a1b* expression.

In summary, urban wastes released from the WWTP of Bajo Grande, which has overreached its capacity, coupled with pesticides, industry and agricultural runoff from the margins of the Suquía River are the major causal factors and may have cumulatively negative effects on the environmental quality of the waters. Consequently, fish population studied is exposed to complex xenobiotic mixture, where the occurrence and activity of estrogenic and antiestrogenic compounds cannot be excluded. In *J. multidentata* coming from the reference site, brain aromatase fluctuates along the reproductive cycle with maximum values during the breeding season. The mismatch observed in the polluted site not only for gene expression but also for GSI values could interfere with the fish reproduction altering the beginning and length of the reproductive cycle. Moreover, the results make out the maintenance of the spatial deterioration in water quality that has been acting for more than 15 year, which not only provoke damage on organism's health but also could result in gradual loss of the reproductive function and important alterations at ecosystem levels upon long exposure.

Acknowledgments This work was supported by grants from the National Research Council (CONICET-PIP), the Science and Technology Secretary of the Province of Córdoba (SECyT), and the National Agency of Scientific and Technological Promotion (FONCyT).The present work is part of the PhD. thesis of N.F. Guyón and M.A. Roggio, who gratefully acknowledge fellowships from CONICET. We thank J.M. Kembro for language assistance.

References

American Public Health Association(APHA) (2005) In: Eaton AD, Clesceri LS, Rice EW, Greenberg AH (eds) Standard Methods for the examination of water and wastewater, 21th ed, American Water Works Association (AWWA), Water Environment Federation (WEF), Baltimore

Ballesteros ML, Bianchi GE, Carranza M, Bistoni MA (2007) Endosulfan acute toxicity and histomorphological alterations in *Jenynsia multidentata* (Anablepidae, Cyprinodontiformes). J Environ Sci Health 42:351–357

Barber LB, Lee KE, Swackhamer DL, Schoenfuss HL (2007) Reproductive responses of male fathead minnows exposed to wastewater treatment plant effluent, effluent treated with XAD8 resin, and an environmentally relevant mixture of alkylphenol compounds. Aquat Toxicol 82:36–46

Bertin A, Inostroza PA, Quinones RA (2011) Estrogen pollution in a highly productive ecosystem off central-south Chile. Mar Pollut Bull 62:1530–1537

Bianco R (2011) Impacto de la contaminación antrópica sobre la histología del ovario y el ciclo reproductivo de las hembras de *Jenynsia multidentat*a en la cuenca del Río Suquía, Córdoba, Argentina. Tesina de grado para optar al título de biólogo. Universidad Nacional de Córdoba, Córdoba, pp 31

Bonansea RI, Ame MV, Wunderlin DA (2013a) Determination of priority pesticides in water samples combining SPE and SPME coupled to GC-MS. A case study: Suquia River basin (Argentina). Chemosphere 90:1860–1869

Bonansea RI, Wunderlin DA, Amé MV (2013b) Optimization of pesticides analysis in water samples on the combination of solid phase extraction and solid phase microextraction coupled to gas chromatography mass detection. 6th SETAC World Congress/SETAC Europe 22nd Annual Meeting, Berlin 2013

Cheshenko K, Pakdel F, Segner H, Kah O, Eggen RI (2008) Interference of endocrine disrupting chemicals with aromatase CYP19 expression or activity, and consequences for reproduction of teleost fish. Gen Comp Endocrinol 155:31–62

Chomczynski P, Sacchi N (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 162:156–159

Contardo-Jara V, Wiegand C (2008) Molecular biomarkers of *Dreissena polymorpha* for evaluation of renaturation success of a formerly sewage polluted stream. Environ Pollut 155:182–189

Di Rienzo JA, Casanoves F, Balzarini MG, Gonzalez L, Tablada M, Robledo, CW. InfoStat version (2011) Grupo InfoStat, Facultad de Ciencias Agropecuarias. Universidad Nacional de Córdoba, Argentina. URL: http://www.infostat.com.ar

Douxfils J, Mandiki R, Silvestre F, Bertrand A, Leroy D, Thome JP, Kestemont P (2007) Do sewage treatment plant discharges substantially impair fish reproduction in polluted rivers? Sci Total Environ 372:497–514

Geraudie P, Hinfray N, Gerbron M, Porcher JM, Brion F, Minier C (2011) Brain cytochrome P450 aromatase activity in roach (*Rutilus rutilus*): seasonal variations and impact of environmental contaminants. Aquat Toxicol 105:378–384

Gomes RL, Scrimshaw MD, Lester JN (2003) Determination of endocrine disrupters in sewage treatment and receiving waters. Trends Anal Chem 22(10):697–707

Gonzalez A, Piferrer F (2003) Aromatase activity in the European sea bass (*Dicentrarchus labrax L.*) brain. Distribution and changes in relation to age, sex, and the annual reproductive cycle. Gen Comp Endocrinol 132:223–230

Goyenola G, Iglesias C, Mazzeo N, Jeppesen E (2011) Analysis of the reproductive strategy of *Jenynsia multidentata* (Cyprinodontiformes, Anablepidae) with focus on sexual differences in growth, size, and abundance. Hydrobiología 673:245–257

Guyón NF, Roggio MA, Ame MV, Hued AC, Valdes ME, Giojalas LC, Wunderlin DA, Bistoni MA (2012a) Impairments in aromatase expression, reproductive behavior, and sperm quality of male fish exposed to 17beta-estradiol. Environ Toxicol Chem 31:935–940

Guyón NF, Bistoni MA, Wunderlin DA, Amé MV (2012b) Inhibition of the brain cytochrome P450 aromatase isoform expression in *Jenynsia multidentata* reflects changes in water quality. J Braz Soc Ecotoxicol 7:97–104

Hinfray N, Palluel O, Piccini B, Sanchez W, Ait-Aissa S, Noury P, Gomez E, Geraudie P, Minier C, Brion F, Porcher JM (2010) Endocrine disruption in wild populations of chub (*Leuciscus cephalus*) in contaminated French streams. Sci Total Environ 408:2146–2154

Hollander M, Wolfe D (1973) Nonparametric statistical methods. Wiley, New York, p 503

Hued AC, Bistoni MA (2005) Development and validation of a biotic index for evaluation of environmental quality in the central region of Argentina. Hydrobiologia 543:279–298

Hued AC, Lo Nostro FL, Wunderlin DA, Bistoni MA (2012) Reproductive impairment of a viviparous fish species inhabiting a freshwater system with anthropogenic impact. Arch Environ Contam Toxicol 64:281–290

Jobling S, Beresford N, Nolan M, Rodgers-Gray T, Brighty GC, Sumpter JP, Tyler CR (2002) Altered sexual maturation and gamete production in wild roach (*Rutilus rutilus*) living in rivers that receive treated sewage effluents. Biol Reprod 66:272–281

Kime DE (1997) In: Endocrine disruption in fish. Kluwer Academic Publishers, Dordrecht, p 397

Larionov A, Krause A, Miller W (2005) A standard curve based method for relative real time PCR data processing. BMC Bioinform 6:62

Lavado R, Thibaut R, Raldua D, Martin R, Porte C (2004) First evidence of endocrine disruption in feral carp from the Ebro River. Toxicol Appl Pharmacol 196:247–257

Maggioni T, Hued AC, Monferran MV, Bonansea RI, Galanti LN, Ame MV (2012) Bioindicators and biomarkers of environmental pollution in the middle-lower basin of the Suquia River (Cordoba, Argentina). Arch Environ Contam Toxicol 63:337–353

Malabarba L, Reis R, Vari R, Lucena Z, Lucena C (1998) Phylogeny and classification of neotropical fishes. EDIPUCRS, Porto Alegre

Monferrán MV, Galanti LN, Bonansea RI, Amé MV, Wunderlin DA (2011) Integrated survey of water pollution in the Suquia River basin (Cordoba, Argentina). J Environ Monit 13:398–409

Pasquini AI, Formica SM, Sacchi GA (2012) Hydrochemistry and nutrients dynamic in the Suquía River urban catchment's, Córdoba, Argentina. Environ Earth Sci 65:453–467

Pesce SF, Wunderlin DA (2000) Use of water quality indices to verify the impact of Córdoba City (Argentina) on Suquía River. Water Res 34:2915–2926

Vajda AM, Barber LB, Gray JL, Lopez EM, Bolden AM, Schoenfuss HL, Norris DO (2011) Demasculinization of male fish by wastewater treatment plant effluent. Aquat Toxicol 103:213–221

Valdés ME, Amé MV, Bistoni MA, Wunderlin DA (2014) Occurrence and bioaccumulation of pharmaceuticals in a fish species inhabiting the Suquia River basin (Cordoba, Argentina). Sci Total Environ 472:389–396

van der Oost R, Beyer J, Vermeulen NP (2003) Fish bioaccumulation and biomarkers in environmental risk assessment: a review. Environ Toxicol Pharmacol 13:57–149

Vosges M, Kah O, Hinfray N, Chadili E, Le Page Y, Combarnous Y, Porcher JM, Brion F (2011) 17alpha-Ethinylestradiol and nonylphenol affect the development of forebrain GnRH neurons through an estrogen receptors-dependent pathway. Reprod Toxicol 33:198–204

Wunderlin DA, Diaz MP, Amé MV, Pesce SF, Hued AC, Bistoni MA (2001) Pattern recognition techniques for the evaluation of spatial and temporal variations in water quality. A case study: Suquia River basin (Córdoba-Argentina). Water Res 35:2881–2894