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Ecotoxicological studies on the pejerrey (*Odontesthes bonariensis*, Pisces Atherinopsidae)

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The pejerrey (*Odontesthes bonariensis*, Cuvier and Valenciennes, 1835) is an Atherinid fish characteristic of the Southern sector of the 'del Plata' basin (Bonetto and Castello, 1985). This species not only possess ecological relevance (it could represent the largest fish biomass, especially in low diversity euryhaline lakes; Baigún and Delfino, 2001), but also has regional socioeconomic relevance due to commercial and sport fishing, the last representing an annual economic movement of $72 million for the Buenos Aires Province alone (Lopez *et al*., 2001). In addition, pejerrey is a promissory species for the aquaculture as a result of the high quality of its flesh, appreciated by local and foreign consumers; a fact that in recent years has driven important advances related to the larvae-culture (Miranda and Somoza, 2003), and caged culture (Colautti and Remes Lenicov, 2001) of the species.

On the other hand, pejerrey inhabit rivers and lakes located in the most densely populated and therefore most highly modified regions of Argentina due to human activities. These aquatic systems are usually natural receptors of pollutants released to the environment from point (industry, sewage) and non point (agriculture, urban runoff) sources. Previous studies testify to differing degrees of organic and inorganic contamination of these water bodies and their biota (Ringuelet, 1967; Catoggio, 1990; AGOSBA-OSN-SIHN, 1992; Ronco *et al*., 1995; Kreimer *et al*., 1996; Colombo *et al*., 2000; Menone *et al*., 2000; Villar *et al*., 2001).

Despite the ecological and socioeconomical relevance of the pejerrey, and the evidence of differing degrees of pollution in the water bodies that the species inhabit, no published studies on the potential effects of environmental pollutants on the wellbeing of the pejerrey were found at the beginning of our investigations in 1997, and little information on this subject is yet available. As a consequence, the aim of this publication is to review the advances in ecotoxicological aspects of *O. bonariensis* obtained up to the present in our laboratory (Carriquiriborde, 2004). The studies covered aspects such as lethal and sublethal responses from exposure to some organic and inorganic pollutants of environmental relevance, the influence of some physicochemical characteristics of the water on the toxicity of metals, the bioconcentration pattern of metals according to chemical speciation, and the vulnerability of the species in representative areas of the Southern sector of the del Plata basin.

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Methodological Approach

A summary of the ecotoxicological studies conducted during the last seven years by the working group of CIMA to assess adverse effects of pollutants of environmental relevance on *O. bonariensis* is shown in Table 1. The general conceptual approach for studies included the following aspects: 1) Evaluation of acute *lethal effects* of the selected toxicants on the species, in order to understand the relative toxicity of each contaminant, and the comparative sensitivity of *O. bonariensis* in relation to other fish species and aquatic organisms. Additionally, it provided information on the concentration/ response and time/response functions of the selected metals and pesticides. Acute lethal toxicity tests studies were also used to assess the dependence of the adverse effects of metals in relation to the physicochemical characteristics of the water and the source and age of the fish, 2) Evaluation of *sublethal effects* related to the toxicokinetics and toxicodynamics of the contaminants in order to quantify the bioconcentration of chemicals and their effects at the cellular levels in two main target organs after waterborne exposure to chemicals; the gills and the liver. Effects of chemicals at the cellular levels were investigated through the assessment of the response of the nucleolar activity and the DNA integrity. Selected biological responses were also evaluated as potential biomarkers of exposure and/or effect, 3) Evaluation of the *vulnerability* of *O. bonariensis* in the natural environment in order to identify the potential risk of lethal and sublethal exposure to the species in its main natural area of distribution. For this purpose, the toxic exposure ratio (TER) (Harrass, 1996) was calculated on the basis of the information reported in previous studies about the physicochemical characteristics and chemical concentrations in representative water bodies comprising the main distribution area of the species, and the sensitivity levels obtained in our studies.

Study of Lethal Effects

The study of the acute lethal effects of the metals (Cd^{+2}, Cu^{+2}, Cr^{+6} and Hg^{+2}) and the pesticides (the herbicide glyphosate and the insecticide cypermethrin)[1]

TABLE 1.

Ecotoxicological studies conducted with *O. bonariensis*

		METALS				ORGANICS			
		Cd	Cr	Cu	Hg	Cyp	Clp	End	Glf
LETHAL RESPONSES	Species sensitivity and chemicals relative toxicity	✔	✔	✔	✔	✔	✓	✓	✔
	Dose response and time effect	✔	✔	✔	✔	✔			
	Toxicity dependence with water chemistry	✔	✔	✔	✓	✔			
	Toxicity dependence with age of the organisms	✔	✔	✔	✓				
SUBLETHAL RESPONSES	Toxicokinetics	✔	✔						
	Citotoxicity	✔	✔						
	Genotoxicity	✔	✔	✓					

✔: concluded studies, ✓: ongoing studies, Cyp: cypermethrin, Clp: chlorpyriphos, End: endosulfan, Glf: glyphosate indicate that *O. bonariensis* sensitivity lies in the following order: cypermethrin > Hg^{+2} > Cd^{+2} > Cu^{+2} > Cr^{+6} > glyphosate (Table 2). Sensitivity to cypermethrin was two orders of magnitude greater than Hg(II) and six orders of magnitude greater than glyphosate, the least toxic of the tested chemicals.

The LC50 values indicate that no general rule can be made relating to the *nature of the chemical* (organic or metal) and the *relative toxicity* on *O. bonariensis*. The observed relative order of toxicity for the tested chemicals was equivalent to that reported for *Oncorhynchus mykiss* and also for the mean available LC50 data for fish species. However, it was slightly different to that observed for *Cyprinus carpio*, which showed an inversion of the sensitivity to Cd and Cu. The lower sensitivity to Cd and higher to Cu was also a pattern reported for crustaceans, and distinctively different from that observed for *O. bonariensis* (Table 2).

The *relative sensitivity* of *O. bonariensis* in comparison with other fish species was, with the exception of glyphosate, always higher than that reported as the average value for all available fish species. In particular, when *O. bonariensis* was compared with the sensitivity of two well known species it was found to be closer to that reported for *O. mykiss* (a sensitive species) than for *C. carpio* (tolerant species). In relation to other Neotropical fish species, the sensitivity of *O. bonariensis* to all the studied metals ranged between 2.5 and 11 times higher than that reported for the 15 day old *Cichlasoma facetum* exposed in hard water (Bulus Rossini and Ronco, 2004). In addition, the sensitivity of *O. bonariensis* to cypermethrin was also higher than that reported for *Cichlasoma dimerus* (Domitrovic, 2000), for *Aequidens portalegrensis* to Hg (Hirt and Domitrovic, 1999) and Cd (Hirt and Domitrovic, 2002), and for C*nesterodon decemmaculatus* to Cu (Villar *et al*., 2000).

TABLE 2.

Relative sensitivity of *O. bonariensis* respect to a selected group of chemicals with environmental relevance, and in comparison with other well studied fish species and aquatic organisms.

	LC50(96h) (mg/L)					
Chemical	*O. bonariensis*	*O. mykiss*	*C. carpio*	Mean Fish	*D. magna*	Mean Crustaceans
Cypermethrin	0.0002	0.0005 [a]	0.001 [a]	0.002 [d]	0.003 [d]	0.006 [d]
Hg(II)	0.014					
Cd(II)	0.036 0.013 †	0.005 [c b] 0.003 †	7.45 [c b] 1.85 †	2.85 [c]	0.078 [c b] 0.013 †	2.50 [c]
Cu (II)	0.218 0.071 ‡	0.255 [c e] 0.035 ‡	0.65 [c]	3.62 [c]	0.016 [c e] 0.011 ‡	0.22 [c]
Cr(VI)	8.2	11.2 [c]	93.6 [c]	32.5 [c]	0.026 [c]	2.3 [c]
Glyphosate	147.5	74.9 [d]	315.5 [d]	71.5 [d]	57.4 [d]	42.3 [d]

Values of $LC50_{(96h)}$ for *O. bonariensis* are expressed as the mean and the 95% confidence interval. †LC50 value adjusted to a total hardness of 50 mg $CaCO_3$/L (USEPA, 2001). ‡BLM Normalized $LC50_{(96h)}$. Data from [a]Stephenson, 1982; [b]USEPA, 2001; [c]USEPA, 2004; [d]Orme and Kegley, 2004; [e]USEPA, 2003.

[1] **Lethal acute toxicity tests** were conducted following recommendations of the USEPA (1993). Static-renewal tests were employed to evaluate the 50% lethal concentration (LC50) for Cd^{+2}, Cr^{+6}, Cu^{+2}, Hg^{+2}, cypermethrin, and glyphosate. Fifteen or 30-day old organisms were used as test organisms. Assay were performed in hard water, (La Plata tap water; 255.3 mg $CaCO_3$/L) and reconstituted soft water (31.4 mg $CaCO_3$/L). Two hundred and fifty ml polyethylene or glass test chambers filled with 200ml of test solution were utilized for acute lethal toxicity assessment of metals and pesticide, respectively. Toxicity tests were conducted by triplicate using 5 or 10 organisms per replica in preliminary or final tests, respectively. Temperature was kept at 22 ± 1ºC and and pH 7.2. Larvae were fed with 24h *Artemia* sp. nauplii once at 48h exposure. Dead fish were removed and recorded every 24h during 96h. LC50 were estimated at each recording time by means of the Probit method (Finney, 1971) using a specific software (Probit; USEPA version 1.5).

In comparison with another relevant group in the aquatic environment such as the crustacean, the sensitivity observed for *O. bonariensis* was one and two orders of magnitude higher for cypermethrin and Cd, respectively and of the same order of magnitude for Cu, Cr, and glyphosate (Table 2). However, in comparison with *D. magna*, a micro-crustacean broadly used in toxicity bioassays, *O. bonariensis* presented higher sensitivity only to cypermethrin, a comparable sensitivity to Cd and glyphosate, and a markedly lower sensitivity to Cu and Cr.

The Figure 1 shows an example of the dependence of the LC50 with the time of exposure[2]. The dependence of Cr^{+6} LC50 values with time show a good agreement with the model derived from the critical body residue concept (McCarty and Mackay, 1993), and was consistent with the fact observed for Cu and Ni, that binding of the metals to fish gills predicts acute toxicity better than free-ion activity (Meyer, 1999). In the figure, it is also possible to observe that the age of the organism and water hardness influence the values of the $LC50_{(t)}$ (a slight but significant increase of Cr^{+6} tox-

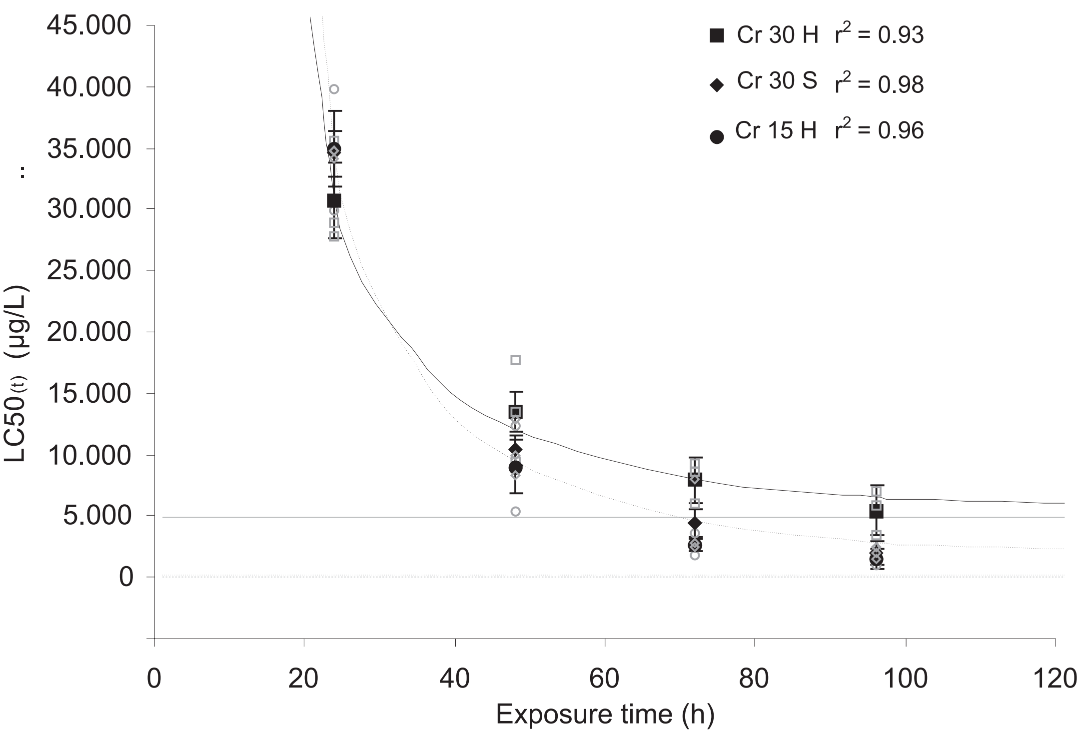

FIGURE 1. Time dependence of LC50 values for *O. bonariensis* exposed to Cr(VI).
Cr 30 H: 30d old organism exposed to Cr(VI) in hard water (255 mg $CaCO_3$/L); Cr 30 S: 30d old organism exposed to Cr(VI) in soft water (30 mg $CaCO_3$/L); Cr 15 H: 15d old organism exposed to Cr(VI) in hard water (255 mg $CaCO_3$/L); black geometric figures: indicate mean LC50 values; bars: 95% confidence limits; curves: adjusted model to the LC50 values; r^2: coefficient of determination; horizontal lines: incipient values ($LC50_{(\infty)}$).

[2] **Time dependency of the LC50 values** was explained by the following model: $LC50_{(t)} = LC50_{(\infty)} / [1- e^{(-ke \infty t)}]^2$, derived from the Critical Body Residue (CBR) concept and more specifically the Lethal Body Burden (LBB) approach (McCarty, 1986; McCarty and Mackay, 1993).
Effects of physicochemical characteristics of the water on Cd^{+2} and Cu^{+2} toxicity were assessed by means of empirical equations obtained from USEPA (2001) and Welsh *et al.* (1996), respectively. The equation used to explain the relationship between water hardness and Cd^{+2} LC50 was: $Ln(LC50_{Cd})= 1.205[Ln(hardness)]-3.949$. The equation used to explain the relationship between pH, DOC, and hardness and Cu^{+2} LC50 was: $Log(LC50_{Cu})= 0.192(pH) + 0.136(pH \infty \log DOC) + 0.166(Ca) -0.981$, replacing Ca by the hardness, expressed as mg $CaCO_3$/L, as the product of 0.4∞ [Ca], then the equation is $Log(LC50_{Cu})= 0.192(pH) + 0.136(pH \infty \log DOC) + 0.0664(Hardness) -0.981$. The parameters of these equations were recalculated for *O. bonariensis* using the LC50 values obtained in the laboratory at different hardness values.

icity was observed when organisms were younger and the water hardness lower). However, the effect induced by these two variables was accurately interpreted by the same time-response model. A similar behavior was also seen for Cd^{+2} exposed organisms (data not shown). In contrast with the model presented for Cu^{+2} by Villar *et al.* (2000), the developed model would provide a mechanistically based description of the relationship between exposure time and the LC50 for the studied metals.

The dependence of the estimated LC50 for *O. bonariensis* with water hardness was more evident for Cd^{+2} than for Cr^{+6}, and the relationship between the logarithms of these two variables was satisfactorily described by an empirical model proposed for cadmium by USEPA (2001), showing a positive linear correlation (Fig. 2). The figure also shows that *O. bonariensis* is within the group of sensitive species to Cd detaching from the more tolerant group (regression lines with higher *y*-intercept). Also the application of a model developed by Welsh *et al.* (1996) for *Pimephales promelas* to describe copper toxicity dependence with the water chemistry was used here to explain the behavior of this metal for the case of pejerrey. This model accurately predicted acute lethal effects of Cu on *O. bonariensis* exposed at different water hardness (data not shown, see Carriquiriborde and Ronco, 2002). Therefore, these models could be used to assess the risk of metal exposure for the species in the field as a function of physicochemical characteristics of the water bodies.

Sublethal effects [3]

Studied sublethal effects in laboratory exposure experiments covered the assessment of the bioconcentration and the response of nucleolar activity to chromium and cadmium; and the genotoxicity of cadmium, copper and chromium in gill and liver tissues.

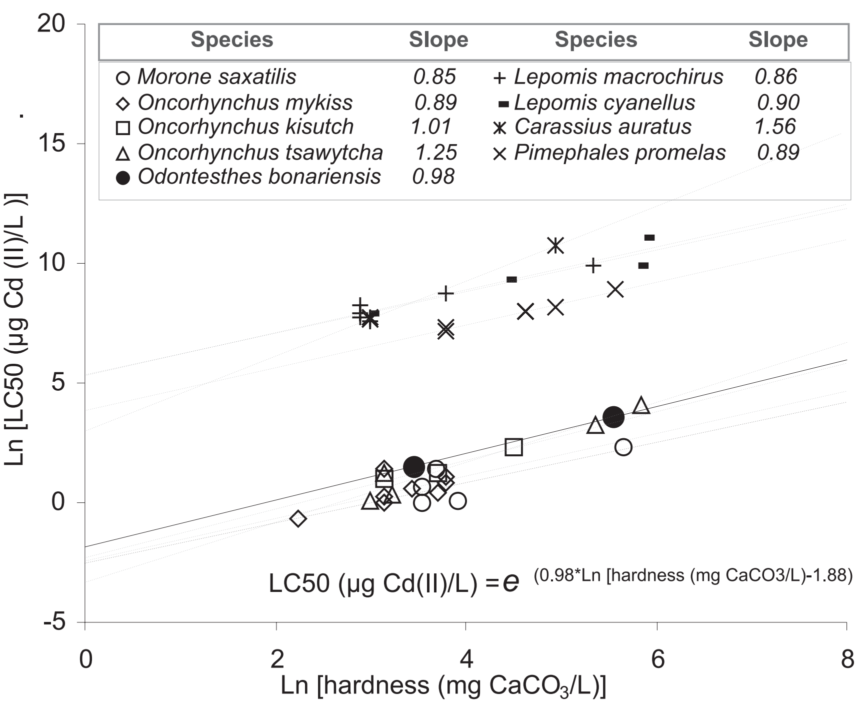

FIGURE 2. Dependence of Cd(II) LC50 for *O. bonariensis* with water hardness, and its comparison with other fish species (EPA, 2001).
Symbols: LC50 for different fish species (see insert); solid line and equation: linear regression function fitted for *O. bonariensis* at different hardness values; dotted lines: linear regression function fitted to Cd(II) LC50(96) data published for other species at different hardness values.

Bioconcentration depends on the relation between the uptake and elimination rate of chemicals. The study of the bioconcentration[4] of the Cd^{+2} and Cr^{+6} in the gills and liver of *O. bonariensis* showed that both metals bioconcentrate in the studied organs, each one following a characteristic pattern (Fig. 3). Small concentrations of waterborne Cd (≈ 0.1 μM) were highly accumulated in the gill (1000 fold), but only little Cd was accumulated in the liver (60 fold). On the other hand, relatively high concentrations of waterborne Cr (≈20 μM) were moderately bioconcentrated in the gill (only 2.5 fold), but presented low accumulation in the liver.

Under the assayed conditions, cadmium is mainly present as the free cation, Cd^{+2}, that quickly binds to gill surface to be later slowly up-taken (Pagenkopf, 1983; Playle 1998) passively through Ca^{+2} channels (Verbost *et al.*, 1989; Wicklund Glynn *et al.*, 1994). In a different way, chromium is mainly present as the anion $CrO_4^{-2}/Cr_2O_7^{-2}$ (Kotás and Stasicka, 2000) and it is believed that the metal is up-taken through transporters for SO_4^{-2} and PO_4^{-3} (Wetterhahn, 1979; Ottenwälder and Wiegand, 1988). As a consequence, these properties and mechanisms characteristic of each metal could be responsible for the differences observed in the pattern and the magnitude of the bioconcentration of Cd^{+2} and Cr^{+6} in the gills and liver of *O. bonariensis*. The higher affinity of the Cd^{+2} for the gill surface described by other authors agrees with the higher accumulation of the metal observed in this tissue. Furthermore, this could explain the observed correlation between the model derived from the CBR concept and the LC50(t), and may also explain the higher lethal acute toxicity of Cd^{+2} in comparison with Cr^{+6}. Moreover, according to Norey *et al.* (1990), the bioconcentration pattern of Cd observed in *O. bonariensis*, higher in the gill than in the liver, places the pejerrey within the group of sensitive species such as the salmonids to this metal.

The nucleolar activity is mainly given by the product of ribosome biogenesis, which is directly linked with the protein synthesis of the cell. In the yeast, 75% of the most highly transcribed genes are ribosomal protein genes, representing an important energetic cost for the cell (Jelinsky and Samson, 1999). Consequently, it is tightly regulated (Leary and Huang, 2001; Jorgensen *et al.*, 2002) and is very sensitive to factors that induce cellular stress and DNA damage (Rubbi and Milner, 2003). Our experiments demonstrate that the nucleolar activity[5] in the gill and liver of *O. bonariensis* was affected by sublethal concentration of Cr^{+6} and Cd^{+2}, also showing a characteristic response depending on the metal and the organ. Whereas a general reduction of the nucleolar activity (assessed by the mean nucleolar area) was induced by Cr^{+6} both in the gills and the liver, a decrease of this parameter was induced by Cd^{+2} only in the gill. Conversely, a significant increase of the nucleolar activity was caused by this metal in the liver. The nucleolar activ-

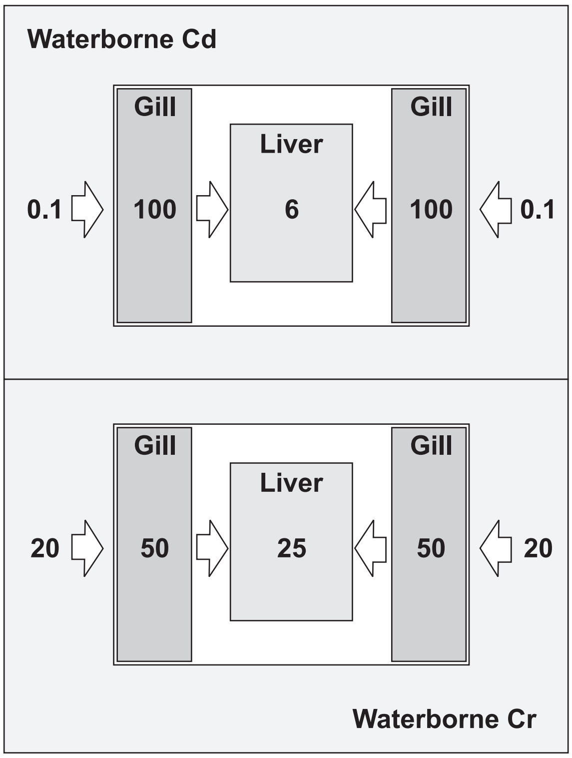

FIGURE 3. Chromium and cadmium bioconcentration patterns for *O. bonariensis* after 384h exposure.
Values: represent approximate micromolar concentrations; shades: symbolize concentration levels.

[3] **Sublethal exposure experiments** were performed using six-month old juveniles exposed in 20 L polyethylene test chambers to three sublethal concentrations of Cd^{+2}, Cr^{+6} or Cu^{+2} for a maximum period of 384 h. Assays were performed by triplicate using 2 organisms per replica per treatment (time/concentration). Fish were sampled at 6, 48, 192 and 384 h. Static renewal experiments were conducted using La Plata tap water, temperature was kept at 22 ± 1ºC, pH was 7.2, and juveniles were fed with adults of *Daphnia* sp. every 48h.

[4] **Bioconcentration** of Cd and Cr in gill and liver of *O. bonariensis* juveniles sampled at 48 and 384h were measured by means of electrothermic absorption spectroscopy using a GBC Scientific Equipment PTY. LTD. Model: AA902 equipped with a graphite furnace atomizer GF2000 and an autosampler, and following the operative manual guidelines. Tissues were previously digested following the method proposed by Handy and Depledge (1999).

ity assessment end points NOEC and LOEC found for each metal and tissue are shown in Table 3.

DNA integrity[6], assessed by means of the comet assay identifies double and simple strand breaks and labile alkaline sites induced in the DNA by chemicals. Results showed that all the tested metals (Cd^{+2}, Cr^{+6}, Cu^{+2}) were able to induce genotoxic effects on the studied tissues of *O. bonariensis*. It was evidenced as a significant increase in the frequency of DNA damaged nucleus presented in the gill and liver of metal exposed fish with respect to those in the control group. As was expected from a waterborne exposure, the three metals induced higher DNA damage in the gill than in the liver in a time dependent manner (Carriquiriborde *et al.*, 2002a, 2003). The NOEC and LOEC found for each metal and tissue is shown in Table 3.

The observed sublethal effects can also be linked to the bioconcentration pattern of the Cd and Cr, through the CBR concept. In the gill, organ with the highest concentration of both metals, the nucleolar activity was significantly reduced and was the only organ where DNA damage was observed. Reduction of the nucleolar activity can be related to the demonstrated capacity of Cd^{+2} at cytotoxic concentrations to induce "*in vitro*" and "*in vivo*" general inhibition of cellular proliferation, gene expression, and protein synthesis (Beyersmann and

TABLE 3.

Summary of sublethal effects induced by Cd, Cr, and Cu in *O. bonariensis*

Metal	Tissue	Endpoint	Nucleolar Activity 48 h	Nucleolar Activity 384 h	DNA Damage 48 h	DNA Damage 384 h
Cd	Gill	NOEC	5	---	5	---
		LOEC	10 ↓	<1 ↓	10 ↑	<1 ↑
	Liver	NOEC	1	---	>10	---
		LOEC	5* ↑	<1 ↑	---	---
Cr	Gill	NOEC	100	---	---	---
		LOEC	500 ↓	<100 ↓	<100 ↑	<100 ↑
	Liver	NOEC	>1000	100	>1000	---
		LOEC	---	500 ↓	---	<100 ↑
Cu	Gill	NOEC	---	---	---	---
		LOEC	---	---	<10 ↑	--- ↑
	Liver	NOEC	---	---	>100	---
		LOEC	---	---	---	---

NOEC: no observed effect concentration, LOEC: lowest observed effect concentration; Values are expressed in μg/L; ↑Parameter significantly increased ($p<0.05$); ↓Parameter significantly decreased ($p<0.05$); *Significant differences ($p<0.05$) disappeared at higher concentrations; ---: no data.

[5] **Nucleolar activity** was evaluated in gill and liver cells sampled from juveniles exposed to Cd^{+2} and Cr^{+6} during 6, 48, 192, and 384h. Histological preparations and nucleolar area and number assessment were conducted following the method proposed by Arkhipchuk and Palamarchuk (1997) and adapted by Carriquiriborde *et al*. (2000).

[6] **DNA integrity** in the gill and the liver cells of *O. bonariensis* juveniles exposed to Cd^{+2}, Cr^{+6} and Cu^{+2} for 48 and 348h was assessed using the single cell gel electrophoresis assay (COMET assay) according to Singh *et al*. (1988). Tissue samples were pretreated following the methodology proposed by Deventer (1996) but using only trypsin to isolate cell from tissue. Qualitative analysis of DNA damage was performed scoring the observed nuclei in 5 levels, 0, I, II, III, IV according to the degree of DNA migration (tail).

Hechtenberg, 1997). In addition, it was observed that Cd induced DNA damage only in the gill, a reported effect only at cytotoxic concentrations of this metal (Beyersmann and Hechtenberg, 1997). The reduction caused by Cr^{+6} can be linked to the capability of this metal to induce cross links with proteins and DNA during its intracellular reduction from Cr^{+6} to Cr^{+3} (Stearns and Wettterhahn, 1994). In addition, both metals are able to generate general cellular injury by means of oxidative stress (Stohs and Bagchi, 1995). On the other hand, in the liver, while chromium (the higher concentrated of both metals, Fig. 3) still decreased the nucleolar activity and induced DNA damage, cadmium only increased nucleolar activity. The observed increase of the nucleolar activity induced by cadmium in the liver is consistent with the observation that small quantities of the metal induce protein synthesis, gene expression and cell proliferation (Beyersmann and Hechtenberg, 1997). The same authors state that the stimulation induced by cadmium on cellular signals at various stages of mitogenic cascades, proto-oncogene expression, DNA synthesis, and cell proliferation may be a clue for the interpretation of the carcinogenic action of this metal.

Vulnerability of *O. bonariensis* in the natural environment

Vulnerability of the species to be adversely affected by the Cd, Cu, Cr, and cypermethrin was assessed considering the information generated on the lethal and sublethal effects of these metals and information on water physical-chemistry and metal concentration reported for water bodies located within the main distribution area of the species.

It was shown in the previous sections that physicochemical characteristics of the water affect metal toxicity on *O. bonariensis*. The effect of the hardness, pH and DOC on Cd^{+2} and Cu^{+2} lethal acute toxicity can be easily assessed by means of empirical models, but no model is yet available for Cr. Consequently, in order to predict more accurately the vulnerability of *O. bonariensis* to these metals within the main area of distribution of the species, values of hardness, pH, DOC, and metal concentration were collected from the available literature and analyzed.

The reported pH range for the Lower Paraná River was 6.1-7.9 (Villar *et al*., 1999), for the Río de la Plata 5.8-9.6 (AA-AGOSBA-ILPLA-SHN, 1997), for Samborombón River 8.3-8.4 (Mercado, 2000), for Chascomús Lake 8.0-9.0 (Conzonno and Claverie, 1990), and for San Miguel del Monte pond 8.23-9.20 (Gabellone *et al*., 2001). In addition, mean hardness values, estimated as the sum of Ca^{+2} and Mg^{+2} concentrations reported in the literature, were expressed as calcium carbonate. Hardness interval values found for the Lower Paraná River were 22.5-32.0 mg $CaCO_3$/L (estimated from Villar *et al*., 1999), and for the Río de la Plata, 22.5-217.5 mg $CaCO_3$/L (estimated from AA-AGOSBA-ILPLA-SHN, 1997). Mean hardness values calculated for the lower, middle and upper sectors of the Samborombón River were 86, 87, 155 mg $CaCO_3$/L (estimated from Mercado, 2000). Hardness ranges obtained for Vitel and Chascomús ponds were 94.9-205.4 mg$CaCO_3$/L (estimated from Olivier, 1961), and 61.5-161.3 mg$CaCO_3$/L (estimated from Conzonno and Claverie, 1990), respectively. Hardness reported for Salado River (Guerrero's Bridge) was 262 mg $CaCO_3$/L. Finally, the mean and minimum DOC values reported were 2.8 - 4.2 for the Lower Paraná River and 4.0-7.9 mgC/L for the Río de la Plata (Villar *et al*., 1999). Minimum and maximum values found for the same parameter in some representative water bodies from the Salado River Basin were: Vitel pond 12.0-57.0 (Olivier, 1961) and Chascomús pond 7.7-18.7 (Conzonno and Claverie, 1990).

The maximum concentration of Cd, reported for the lower Paraná River, was 1 µg/L (Gómez *et al*., 1998). For the Río de la Plata, the range of maximum values reported for this metal was 1.0-3.7 µg Cd/L (AGOSBA-OSN-SIHN, 1992; Villar *et al*., 1998), with the highest value found in front of Berazategui. For the Salado River Basin, the observed range in Cd concentration was from 1.0-5.0 µg Cd/L, with higher concentrations found in the upper sector of the basin (Ronco and Argemí, 1998; Carriquiriborde *et al*., 2002b). In addition, the range of maximum values of Cr reported for the Lower Paraná River and Río de la Plata were 19.7 - 44.2 and 5 - 4,100 µg Cr/L, respectively (Villar *et al*., 1998; AGOSBA-OSN-SIHN, 1992; AA-AGOSBA-ILPLA-SHN, 1997). Extremely high values were observed in the Riachuelo River mouth with concentrations of 72,200 µg Cr/L (Kreimer *et al*., 1996). The values reported for the Salado River Basin ranged between 35 and 71 µg Cr/L, and again, the higher levels were found in the upper sector of the basin (Ronco and Argemí, 1998; Carriquiriborde *et al*., 2002b). Moreover, the range of copper concentrations reported within the distribution area of the species were 3-8 µg/L in the lower Paraná River, 4-12 µg/L in Río de la Plata (Villar *et al*., 1999), and between 12 to 16 in the Salado River Basin, with the highest value observed in del Monte pond (Carriquiriborde *et al*., 2002b). Finally, the interval of cypermethrin concentrations reported for streams and the mouths of affluent rivers of the lower Paraná River was 1 – 190 µg/L (Marino *et al.*, 2004).

A summary of the physicochemical parameter values corresponding to the worst case scenario (lowest values of hardness, pH, and DOC, and highest metal concentrations) was constructed from the published field data of water bodies and placed within the main distribution area of the species. The $LC50_{(\infty)}$ values corrected as a function of these physicochemical parameters and the toxic effect ratio (TER) values according to Harrass (1996), represented as a quotient between the adjusted lethal acute toxicity value and the reported field metal concentration, is displayed in Table 4.

According with the data presented above it is possible to assume that: a) the physicochemical characteristics reported for the water bodies of the southern sector of the del Plata basin showed a decrease of hardness and pH values from the South to the North and from the West to the East, showing the lower Paraná as the place of maximum vulnerability for the species to metal exposure, and b) the concentration of metal and cypermethrin near or greater than those that induced biological effects in *O. bonariensis* were reported for metals in the Río de la Plata and upper Salado River

TABLE 4.

Vulnerability of *O. bonariensis* to heavy metal acute exposure in the southern sector of the del Plata basin.

	Laboratory	Lower Paraná	Río de la Plata	Salado Basin
Maximum Reported Concentration (mg/L)				
Cd(II)		0.0010	0.0037	0.0050
Cu(II)		0.008	0.012	0.016
Cr(VI)		0.03	4.10	0.07
Cypermethrin		0.19 [c]	---	---
Water Physicochemical Parameters [a]				
Lower Hardness (mg $CaCO_3$/L)	255	19	23	80
Lower pH	7.8	6.1	5.8	8.2
Lower DOC (mg/L)	0.5 [b]	2.4	4.0	9.9
Lethal Acute Effects - Estimated $LC50_{(\infty)}$ (mg/L)				
Cd(II)	0.058	0.003 [d]	0.003 [d]	0.011 [d]
Cu(II)	0.097	0.054 [e]	0.07 [e]	1.18 [e]
Cr(VI)	6.36	6.36 [f]	6.36 [f]	6.36 [f]
Cypermethrin	0.0002	0.0019		
Acute Toxic Exposure Ratio				
Cd(II)		3	1	2
Cu(II)		7	6	74
Cr(VI)		236	2	90
Cypermethrin		0.01	---	---

$LC50_{(\infty)}$: value of incipiency; [a]Parameters that represent the worst scenario according to data in literature; [b]DOC was measured in laboratory water, but was not detected (detection limit = 1 mg/L). DOC value used was 0.5 mg/L, which is one-half the detection limit and is consistent with the recommended default DOC value for reconstituted waters (USEPA 2003). [c]Values reported for affuents of the lower Paraná River. [d]Value estimated using a regresion model by USEPA (2001); [e]Value estimated using a regresion model by Welsh *et al*. (1996). [f]no available model.

Basin, and for cypermethrin in the lower Paraná River.

The TER, shows the Río de la Plata as the place where risk of lethal acute exposures to the three studied metals for *O. bonariensis* is the highest, not only as a consequence of the elevated metal level contents reported for its waters, but also resulting from the relatively low values of hardness and pH, and DOC that characterize this water body. In addition, risk of *O. bonariensis* to Cd and Cu lethal acute exposure should be considered in the lower Paraná River, as well as to Cd in the Salado River Basin. From another point of view, a high risk of acute lethal effects (TER<10) was found for Cd in the whole distribution area, for Cu in Río de la Plata and the Lower Paraná, and for Cr only in Río de la Plata. On the other hand, the lowest TER was obtained for cypermethrin in the mouth of affluent rivers of the lower Paraná River which drain from 'Pampa Ondulada', one of the most intensely cultivated areas of Argentina, indicating a high risk of exposure for *O. bonariensis* to this compound related to agricultural activities.

Concluding Remarks and Perspectives

In the present paper, ecotoxicological studies obtained during the last seven years in our laboratory on *O. bonariensis*, were reviewed. On the basis of the current knowledge, it is possible to state that:

- *O. bonariensis* is a very sensitive species to environmental pollution, and also presents a relative response pattern to chemicals comparable to most fish species.
- *Acute lethal effects* of waterborne Cd^{+2} and Cr^{+6} (and probably other metals) on *O. bonariensis* is mainly related to the ability of each metal to bind to the gill and accumulate there. The magnitude of the metal accumulation in the gills not only depends on its chemical nature but also on its bioavailability, which is conditioned by physicochemical characteristics of the water such as its hardness. The lethal acute toxicity of these metals also markedly depends on the age of the organism and the time of exposure up to 48h but, after this time variation of lethality is much lower.
- *Sublethal effects* of Cd(II) and Cr(VI) (and probably other metals) are also directly related to the distribution and concentration of the metal in the specific tissues. Reduction of the nucleolar activity induced by both metals is in good accordance with the general cellular damage proposed for these metals at cytotoxic concentrations, while the increase of the nucleolar activity induced by Cd may be related with its ability to stimulate protein synthesis, gene expression and cell proliferation at very low concentrations. As a consequence, nucleolar activity seems to be a good indicator of cell arrest or proliferation induced by metals in *O. bonariensis*. Genotoxic effects of Cd and Cr assessed by the COMET assay are in good correspondence with the general response observed for the nucleolar activity, and with the capacity to induce DNA damage as previously reported for these metals.
- According to the physicochemical characteristics and chemical concentrations reported for the water bodies included in the natural area of distribution of *O. bonariensis*, and the high sensitivity of the species to the studied environmental pollutants, it is possible to affirm that vulnerability to metal exposure is at a maximum in the soft and slightly acidic waters of the lower Paraná River, and that potential risk of exposure to this class of chemicals exist near urban areas, such as the occidental margin of the Río de la Plata. On the other hand, high potential risk of exposure to cypermethrin exists in rivers draining into agricultural areas.

Although unprecedented information on ecotoxicological aspects of *O. bonariensis* was summarized in the present paper, it only represents the beginning in understanding the effects of pollutants on this important natural resource and emblematic fish of the shallow ponds of the 'pampas' region. It is clear that a great amount and diversity of studies will still be necessary to have a clear insight on the impacts of toxicants on the species in its natural environment.

From the results obtained in the reviewed studies it is possible to establish a number of futures lines of research, however, many others would still be relevant. For example, the presented methodology could be used to complete the scheme presented in table 1, or to asses the adverse effects of other pesticides and chemicals of environmental concern. In addition, new experiments could be conducted to asses the more recently proposed Biotic Ligand Model as a tool to evaluate the effect of the physicochemical characteristics on the toxicity of metal. Furthermore, the observed response of the nucleolar activity to metals opens a new field to study possible mechanisms involved in such responses. On the other hand, it would be interesting to conduct field studies in selected regions to assess the potential risk areas and possible effects on *O. bonariensis* natural populations.

According to current challenges in ecotoxicology, studies with *O. bonariensis* could also be oriented to assess: i) chronic effects of pollutants, such as endocrine disruption, DNA damage/mutagenesis, deficiencies in the immune system, and neurological effects; ii) multiple effects by single pollutants, as for example multiple target sites and multiple modes of toxic action, and time- and organ-dependent effects; iii) effects of complex mixtures of pollutants, assessing the effects of wastewater treatment plant effluents, field runoff, pollutants and their degradation products, and complexes of chemical compounds; iv) multiple stressors, as for example assessing the join effect of UV, temperature and pathogens on pollutants toxicity; and v) ecosystem complexity, such as variations in species sensitivities, effects of propagation from organisms to populations and ecosystems, and identification of the stressor–effect relationship.

The challenges in ecotoxicology also call for novel bioanalytical methodologies such as modern molecular and genetic tools or sophisticated test systems for routine screening. However, it will be necessary to understand how subtle changes at the molecular level or laboratory scale affect *O. bonariensis* in its environment. It is evident that further studies will have to be undertaken to provide better tools in the species management and protection.

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