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Toxicity of Reconquista River Water: Bioassays with Juvenile *Cnesterodon decemmaculatus*

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The Reconquista river is a body of water in the Province of Buenos Aires that harbours along its margins a great number of factories and some 3 million inhabitants, receiving residual sewage water and poorly treated or untreated industrial effluents. The water of the river presents a complex mixture of pollutants, mainly consisting of heavy metals (Ferrari et al 1994; Topalián et al 1990), which are found at concentrations higher than those allowed for the protection of aquatic life, as established by current legislation in Argentina. Permissible emission levels of pollutants entering the water are generally established by means of the results of laboratory toxicity trials with various aquatic organisms (USEPA 1985; Vitozzi and De Angelis 1991).

Our aim was to evaluate water quality of the Reconquista river through acute bioassays employing juvenile *Cnesterodon decemmaculatus* as test organism. In the same trials, tests were performed to determine the impact of pollution pulses simulated by adding Cd^{2+} to the samples. A preliminary report of our results has been published elsewhere (Demichelis et al. 1994).

MATERIALS AND METHODS

Surface water samples were collected from three points along the river, Cascallares (Cas), San Martin (Sin) and Bancalari (Ban), in February, March, April and May 1994. Fig. 1 shows the geographic location of the river and of the sampling sites. Samples were refrigerated and shipped to the laboratory within five hours after collection, then stored at 40°C. As it has been documented that the upstream Cas site presented lower pollution levels (Loez and Salibián 1990), samples from Cas were regarded as "controls" relative to those collected at the downstream sites.

Juveniles of *Cnesterodon decemmaculatus* (0.9 -1.3 cm fork length n=420) were gathered from a non-polluted natural pond located in the University campus and kept for 7-15 days in laboratory aquaria with aerated tap-water at room temperature (pH, 7.7; conductivity, 640 $\mu S.cm^{-1}$; hardness, $CaCO_3.L^{-1}$ 1,4 mM ; total alkalinity, $CaCO_3.L^{-1}$ 8,2 mM.)

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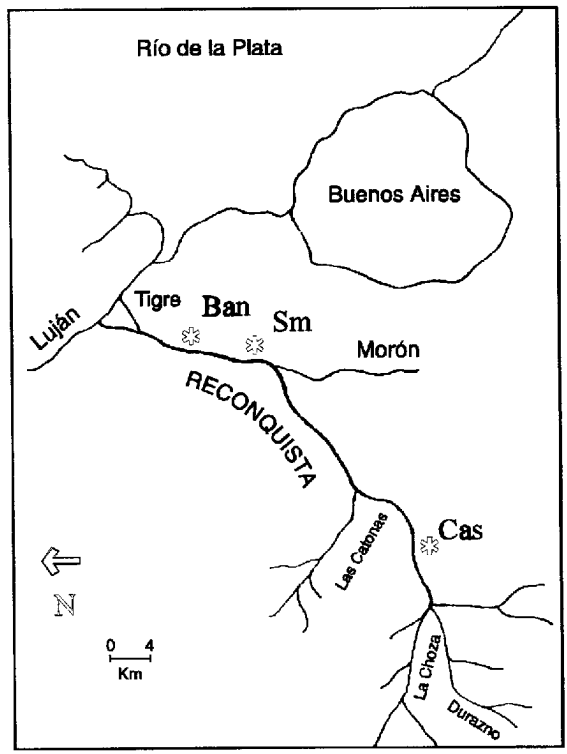

Figure 1. Geographic location of river Reconquista and sampling sites

Organisms were fed daily with fish food (TetraMin®, TetraWerke, Germany) and water partially renewed every 72 hours. Seven test solutions were employed: a) hardwater, hardness 1.4 mM $CaCO_3.L^{-1}$ (USEPA 1987); b) hardwater + 4 ppm Cd^{2+} (as Cl^-); c) three river samples and d) two river samples+ 4 ppm Cd^{2+}. Solution a) was regarded as control solution b) was regarded as standard toxicity reference solution. The used concentration of Cd^{2+} corresponded to the 96 hr LC-50 for *Cnesterodon decemmaculatus* of the stage used in the assays. February assay was carried out omitting cadmium addition. Fishes were acclimated in hardwater (HW) for 48 hours, then split up into seven groups with 60 individuals (four replicates with 15 animals each) and lodged m glass containers with 40 ml of the test solution per animal (APHA 1992). Both acclimatization and assays were performed under constant temperature (20 ± 10°C) and photoperiod (12/12 D/L). Assays were semistatic; incubation media were renewed daily with freshly prepared solutions previously aerated over 40% of oxygen saturation at 20 °C. Fish were fasted during assays and cumulative mortality rate was recorded daily during 96 hours. Animals not responding to gentle prodding were considered dead and removed from assay containers.

Assayed river water samples were analyzed prior to tests (Table 1); dissolved oxygen, pH, conductivity, hardness and alkalinity were checked daily in all solutions.

Intergroup mortality rates (transformed as arc sine $\sqrt{P}$) were compared by means of a multiway factor ANOVA ($p<0.05$) and Bonferroni multiple comparison test was used to evaluate statistically significant group-time differences (Zar 1984). Stepwise regression analysis of assays with river water was carried out considering all physiochemical parameters (Table 1), mortality rates and time.

RESULTS AND DISCUSSION

Results are presented in Figs. 2 to 5. Mean cumulative mortality differed significantly between experimental bioassays, treatments and times.

Throughout, mortality recorded in control solutions failed to show statistically significant differences: in HW it was lower than 10% and in the reference solution containing 4 ppm Cd^{2+} (HW4) it was extremely high. This finding was taken to mean that animals were in comparable conditions of sensitivity. There were no differences between responses of animals exposed to Cas (the upstream sampling site) or HW in any bioassay.

In bioassays conducted in February (Fig. 2), mortality differed in Sm versus Ban batches and in both as compared to controls (HW and Cas), showing that sample toxicity increased downstream. In March (Fig. 3), mortality was low throughout, although toxicity tended to increase in downstream samples (Fig. 3, A), without reaching statistical significance. The addition of cadmium to the river water markedly increased mortality Sm4 (San Martin + 4 ppm Cd) and Ban4 (Bancalari + 4 ppm Cd) batches differed significantly from their reference (HW4), though not *inter se* (Fig. 3,B). In April (Fig. 4), Ban batches presented extremely high toxicity (Fig 4,A), which was further increased by the addition of Cd, with significant differences versus Sm4 and Ban4, as well as between the later two (Fig. 4, B). Samples taken in May (Fig.5) failed to exhibit toxicity (Fig. 5,A), presenting a mortality response profile similar to that of March. However, the addition of Cd raised fish mortality throughout (Fig. 5,B), an effect attributable to sample quality. In April bioassays (Fig. 4,B), the addition of 4 ppm Cd to Sm and Ban samples led to high mortality in both cases, though mean values were dissimilar. Almost without exception mortality as a fiction of time was evident from 48 hours exposure. The addition of Cd always increased mortality.

The analysis of the correlation between the physiochemical parameters of the water and the toxicity evaluated on biological systems is complicate because the chemical analysis is limited. Several authors have used the stepwise regression analysis in order to correlate the adverse impact of toxic media with physiochemical parameters (Meeter and Livingston 1977; Bomboi et al. 1990; Mulliss et al. 1996). In order to determine the relation between toxic effect on fishes and water quality, we performed

Table 1. Physiochemical parameters of water samples collected at three sites along the Reconquista river.

	Cascallares				San Martin				Bancalari			
	Feb	Mar	Apr	May	Feb	Mar	Apr	May	Feb	Mar	Apr	May
pH	9.3	8.6	8.0	7.8	8.9	8.2	7.9	7.7	7.9	8.3	7.6	7.7
Alkalinity ($mM\ CaCO_3.L^{-1}$)	10.4	7.9	8.5	4.0	11.5	9.5	9.2	5.6	10.8	9.5	10.8	5.6
Hardness ($mM\ CaCO_3.L^{-1}$)	1.3	1.5	1.3	0.8	2.0	1.6	1.4	1.2	1.9	1.4	2.0	1.2
Ammonia ($mg\ N\text{-}NH4^{+}.L^{-1}$)	0.8	0.4	1.7	0.4	16.1	6.8	10.6	6.5	14.7	5.9	16.7	6.3
Nitrites ($mg.\ N\text{-}NO_2^{2-}.L^{-1}$)	0.0	0.1	0.2	0.1	0.0	0.5	0.3	0.2	0.0	0.6	0.1	0.5
Phosphates ($mg\ PO4^{3-}.L^{-1}$)	2.6	2.4	1.8	1.5	4.1	2.8	4.4	2.2	5.1	2.4	6.9	2.4
Phenol ($mg.L^{-1}$)	0.3	0.5	0.4	0.9	0.9	0.7	0.9	0.7	0.9	0.3	1.7	1.1
BOD ($mg\ O_2.L^{-1}$)	6.5	2.8	4.5	2.6	22.0	19.8	23.3	10.9	23.8	11.7	53.5	8.4
Chlorides ($mg.L^{-1}$)	81	71	61	20	129	95	78	48	130	82	155	50

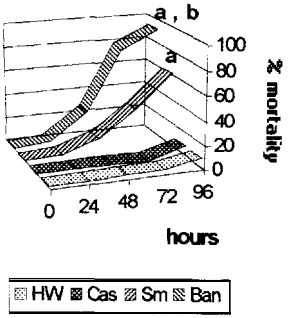

Figure 2. Cumulative mortality of juvenile *Cnesterodon decemmaculatus* exposed to samples of Reconquista river water (February bioassay). a: significantly different from HW and Cas at $p<0.05$; b: significantly different from Sm at $p<0.05$
HW (hardwater); Cas (Cascallares); Sm (San Martin); Ban (Bancalari).

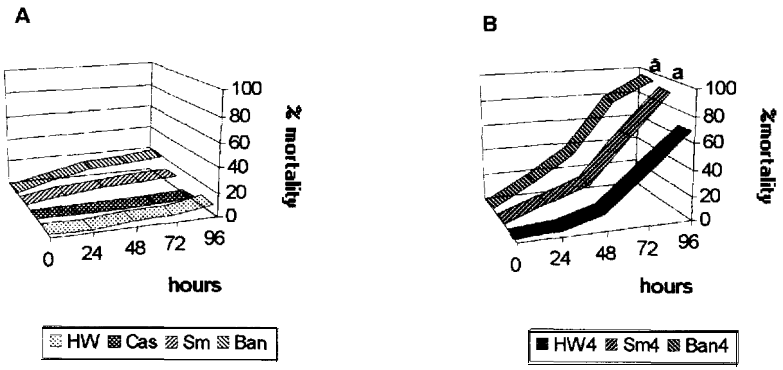

Figure 3. Cumulative mortality of juvenile *Cnesterodon decemmaculatus* (March bioassay)
A) Assay with Reconquista river water. B) Assay with river water containing 4 ppm Cd.
a : significantly different from HW4 at $p<0.05$.
HW (hardwater); Cas (Cascallares); Sm (San Martin); Ban (Bancalari); HW4 (hardwater + 4 ppm Cd); Sm4 (San Martin + 4 ppm Cd); Ban4 (Bancalari + 4 ppm Cd)

a stepwise regression analysis considering only the measured physiochemical parameters as independent variables (Table 1) and mortality percentages at 72 (P72) and 96 hours (P96) as dependent variables. Results of the four bioassays with river water were taken into account. Independent variables selected by stepwise analysis led to the following equations:

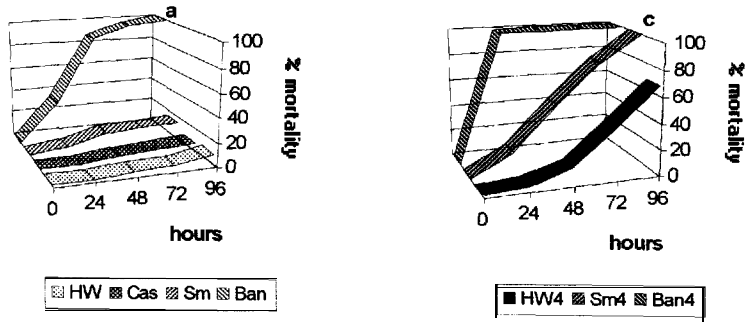

Figure 4. Cumulative mortality of juvenile *Cnesterodon decemmaculatus*, (April assay).
A) Assay with Reconquista river water. B) Assay with river water containing 4 ppm Cd.
a significantly different from HW, Cas and Sm at $p<0.05$; b: significantly different from Sm4 and HW4 at $p<0.05$; c: significantly different from HW4 and Ban4 at $p<0.05$.
HW (hardwater); Cas (Cascallares); Sm (San Martin); Ban (Bancalari); HW4 (hardwater + 4 ppm Cd); Sm4 (San Martin+ 4 ppm Cd); Ban4 (Bancalari + 4 ppm Cd)

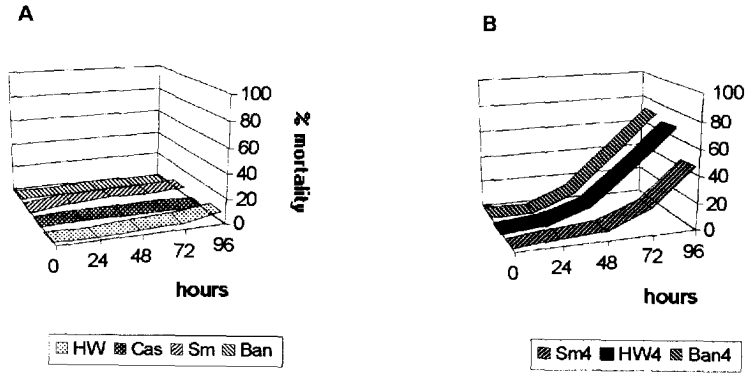

Figure 5. Cumulative mortality of juvenile *Cnesterodon decemmaculatus* (May assay).
A) Assay with Reconquista river water. B) Assay with river water containing 4 ppm Cd.
HW (hardwater) Cas (Cascallares); Sm (San Martin); Ban (Bancalari); HW4 (hardwater + 4 ppm Cd); Sm4 (San Martin + 4 ppm Cd); Ban4 (Bancalari + 4 ppm Cd)

$$P72 = 251.34 + 0.613\ Cl^{-} - 33.87\ pH - 33.26\ NO_2^{2(-)}$$
$$R^2 = 0.84\ ;\ R^2_{adj.} = 0.83;\ d.f. = 47;\ F = 79.28;\ p < 0.001$$

$$P96 = 225{,}81 + 0.702\ Cl^{-} - 30.74\ pH - 43.67\ NO_2^{2(-)}$$
$$R^2 = 0.89\ ;\ R^2_{adj.} = 0.88;\ d.f. = 47;\ F = 114.06;\ p < 0.001$$

On the basis of our results, it may be concluded that:

1. Water toxicity varies widely both spatially and temporally suggesting intermittent toxic spillage, as confirmed by analyzing variations in the measured sample physiochemical parameters (Table 1), particularly BOD, nitrite and chloride concentrations.
2. A predictive correlation may be established between river water toxicity and certain sample physiochemical parameters.
3. The addition of cadmium to river water invariably increases its toxicity; this effect is governed by sample composition.
4. The method described is suitable for test batteries recommended to evaluate the quality of contaminated freshwater bodies as it provides inexpensive, sensitive, relatively fast and reliable technique, grounded on the response of a sentinel species belonging to the native aquatic fauna of river basin.

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