

**Acute silver toxicity to *Cnesterodon decemmaculatus* (Pisces: Poeciliidae)  
in a river with extreme water-quality characteristics**  
**Toxicidad aguda de la plata sobre *Cnesterodon decemmaculatus* (Pisces: Poeciliidae)  
en un río con características extremas de calidad de agua**

Casares, María Victoria<sup>1\*</sup>; de Cabo, Laura I.<sup>1</sup>; Seoane, Rafael<sup>2,3</sup>; Natale, Oscar<sup>2</sup>

<sup>1</sup>Bernardino Rivadavia National Museum of Natural History, Avenida Angel Gallardo 470 (C1405DJR), Buenos Aires, Argentina. Tel. 54 011 4982 4494; fax: 54 011 4982 0306. <sup>2</sup>National Water Institute, Autopista Ezeiza-Cañuelas, Tramo Jorge Newbery km 1.62 (1802) Ezeiza, Buenos Aires, Argentina. <sup>3</sup>Faculty of Engineering, University of Buenos Aires, Avenida Las Heras 2214 (C1127AAR), Buenos Aires, Argentina.

\*[mvc251@hotmail.com](mailto:mvc251@hotmail.com)

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**Abstract.** A 96 h acute silver toxicity test was performed in order to determine silver toxicity ( $LC_{50}$ ) to a local fish species (*Cnesterodon decemmaculatus*) in a river with extreme water-quality characteristics (Pilcomayo River, South America) and evaluate a cross-fish-species extrapolation of the Biotic Ligand Model. The dissolved silver concentrations tested were 0.095, 0.148, 0.175 and 0.285 mg Ag L<sup>-1</sup>. The 96 h Ag  $LC_{50}$  calculated for *C. decemmaculatus* was 0.14 mg L<sup>-1</sup> (0.18 - 0.10) and the value predicted by BLM for *Pimephales promelas* was 0.051 mg Ag L<sup>-1</sup>. Test water elevated hardness may have exerted some protective effect. High mean water pH may have exerted a major protective effect by reducing silver free ion form and causing silver precipitation. The mortality pattern observed in this toxicity test may lend some support to a relationship between gill silver accumulation and mortality. A cross-fish-species extrapolation of Ag BLM for *P. promelas* was not valid in Pilcomayo River water and experimental conditions of this toxicity test.

**Keywords:** Silver; *Cnesterodon decemmaculatus*; Biotic Ligand Model; Extrapolation.

**Resumen.** Con el objeto de determinar la toxicidad de la plata en un pez nativo (*Cnesterodon decemmaculatus*), se llevó a cabo un ensayo estático de toxicidad aguda a 96 horas en un agua natural con características de calidad de agua, extremas (río Pilcomayo, Sudamérica). Asimismo, se evaluó una posible extrapolación inter-especie del Modelo del Ligando Biótico en el agua experimental. La concentración inicial de plata en solución en los distintos tratamientos fue de 0,095; 0,148; 0,175 y 0,285 mg Ag L<sup>-1</sup>. La  $CL_{50}$  a las 96 horas calculada para *C. decemmaculatus* fue de 0,14 (0.18 - 0.10) mg Ag L<sup>-1</sup> y el valor predicho por el BLM para *Pimephales promelas* fue de 0,051 mg Ag L<sup>-1</sup>. La elevada dureza del agua experimental pudo haber tenido algún efecto protector frente a la toxicidad de la plata. El valor medio de pH del ensayo fue elevado y posiblemente tuvo un gran efecto protector por reducción de la forma iónica libre y precipitación del metal. El patrón de mortalidad observado en este ensayo de toxicidad apoyaría la relación causa-efecto entre acumulación de plata en las branquias y mortalidad. La extrapolación inter-especie del BLM para *P. promelas* no resultó válida en el agua del río Pilcomayo y en las condiciones experimentales de este ensayo.

**Palabras clave:** Plata; *Cnesterodon decemmaculatus*; Modelo de Ligando Biótico; Extrapolación.

## INTRODUCTION

Silver, as many other heavy metals enters the aquatic ecosystems as a by-product of many industrial and mining processes. Silver ion is one of the most toxic forms of a heavy metal, surpassed only by mercury and thus, it has been assigned to the highest toxicity class, together with cadmium, chromium (VI), copper and mercury (Ratte 1999). The primary effect of silver is on sodium and chloride uptake and efflux. When present as silver ni-

trate, silver is one of the most acutely toxic metals to freshwater rainbow trout (*Oncorhynchus mykiss*) with 96-h  $LC_{50}$  values in the range of 6.5–13  $\mu\text{g L}^{-1}$  in freshwaters that are generally low in organic matter content. Silver nitrate is highly toxic because it readily dissociates in water to yield ionic silver ( $\text{Ag}^+$ ), the most acutely toxic species of silver. Silver toxicity occurs in freshwater fishes as a result of  $\text{Ag}^+$  disabling first the carbonic anhydrase and later the enzyme  $\text{Na}^+/\text{K}^+$  adenosinetriphospha-

tase (ATPase) at the gill, leading to a net loss of sodium and chloride (Morgan et al. 2004). As a result, blood volume decreases and blood viscosity and pressure increase leading to fish mortality (Kennedy 2011).

The Biotic Ligand Model (BLM) (Di Toro et al. 2001) has been proposed as a tool to evaluate quantitatively the manner in which water chemistry affects the speciation and biological availability of metals in aquatic systems (Paquin et al. 2002). This model was developed using geochemical equilibrium principles, and provides site-specific toxicity predictions (Clifford and McGeer 2009). It is implicitly assumed that BLMs can be extrapolated within taxonomically similar groups, i.e., that BLMs developed for *P. promelas* can be applied to ecotoxicity data for other fish species, that BLMs for *D. magna* and *C. dubia* can be applied to ecotoxicity data for other invertebrates (Schlekat et al. 2010). The basis for a cross-species extrapolation is the assumption that the parameters, which describe interactions between cations (notably calcium, magnesium and protons), the toxic free metal ion ( $M^{n+}$ ) and the biotic ligand are similar across organisms, and that only intrinsic sensitivity varies among species (Schlekat et al. 2010).

A variety of water chemistry factors can protect against metal binding to the sites of action of toxicity either by cationic competition (sodium and protons in the case of silver), or by anionic complexation (e.g., hydroxide, (bi) carbonate, chloride, thiosulfate, sulfide, and most importantly dissolved organic matter), thereby preventing it binding to the toxicity sites (Wood 2008). One of the first and most well recognized of the modifying factors for metals is water hardness. However, it appears to be less important for silver toxicity (Kennedy 2011). Alkalinity, on the other hand, affects metal ionic species in water solution through their complexation with carbonates (Erickson et al. 1996; Lauren and McDonald 1986) and dissolved organic matter forms complexes with metals, which reduces the free form in the water, and therefore the amount of ionic metal available to bind to the gill sites (Matsuo et al. 2004).

Pilcomayo River water is characterized by high concentration of sulphates, chloride, calcium, magnesium, and total suspended solids. Moreover, most of the major ions mean concentration in Pilcomayo River water is at the 95<sup>th</sup> percentile compared to the 60 largest riv-

ers in the world (data in Gaillardet et al. 1999). Toxic waste spills containing high concentrations of arsenic and heavy metals are released daily from the mining district of Potosí, in Bolivia into Pilcomayo River waters. Mining of the Cerro Rico de Potosí ores began in 1545 however, since the introduction of the Crushing-Grinding-Flotation method of mineral processing (1985) the chronic contamination of the Pilcomayo River water and sediments has increased steadily (Smolders et al. 2003).

*Cnesterodon decemmaculatus* (Pisces: Poeciliidae; Jenyns 1842) is an endemic member of the fish family Poeciliidae with extensive distribution in neotropical America. The species attains high densities in a large variety of water bodies within the entire La Plata River and other South American basins. *Cnesterodon decemmaculatus* is a small, viviparous, micro-omnivorous, benthic-pelagic, non-migratory fish. This species, highly tolerant to extreme environmental conditions, is additionally easy to handle and breed under laboratory conditions (Menni 2004). Thereby, several authors have used *C. decemmaculatus* in bioassays (de la Torre et al. 1997, 2002, 2005; Ferrari et al. 1998; Gómez et al. 1998).

*Pimephales promelas* (Pisces: Cyprinidae; Rafinesque 1820), one of the fish species for which BLM has been developed, is a temperate, holartic fresh water fish. As well as *C. decemmaculatus*, is quite tolerant to turbid, low-oxygenated water bodies, and can be found in muddy ponds and streams and in small rivers (<http://www.fishbase.org/summary/Pimephales-promelas.html>). There is one previous work on the toxicity of metals to *C. decemmaculatus* in Pilcomayo River water (Casares et al. 2012). The authors found that a cross-fish-species extrapolation of the Cu-BLM was valid within the Pilcomayo River water quality characteristics and experimental conditions of their toxicity test. With respect to Ag BLM, more work with a wider range of species will be required to improve its predictive capability (Bianchini et al. 2002).

The aim of this study was to evaluate a cross-fish-species extrapolation of the silver BLM in a river with extrem water-quality characteristics (Pilcomayo River water). In order to achieve this aim: (a) silver toxicity (96-h Ag LC<sub>50</sub>) to *C. decemmaculatus* was assessed in Pilcomayo River water, (b) BLM, version 2.2.3 was applied to predict acute silver toxicity to *P. promelas* (Ag LC<sub>50</sub>) under Pilcomayo River wa-

ter quality characteristics and (c) the predicted Ag LC<sub>50</sub> value for *P. promelas* was compared to the calculated for *C. decemmaculatus*.

## MATERIALS AND METHODS

### Study area

The Pilcomayo River in South America is a tributary to the large La Plata system. Its head-

waters are located in Bolivia along the eastern flank of the central Andes at an elevation of approximately 5,200 m (Figure 1). The river flows in a southeasterly direction until reaching the Chaco Plains along Bolivia's southern border with Argentina. Its total length is 2,426 km and its basin covers an area of approximately 288,360 km<sup>2</sup> (<http://www.pilcomayo.net/web/>).



Figure 1. Map of Pilcomayo River basin with the water sampling location (Misión La Paz, Argentina)

### Water sampling and chemical analysis

Discrete water samples for chemical analysis were taken 10 cm below the water surface and in triplicate from the navigation channel, left and right shore of Pilcomayo River in Misión La Paz International bridge (22° 22' 45" S – 62° 31' 08" W; 254 meters over sea level) in September 2009 (Figure 1). Water discharge (Q), pH and water temperature (T) were determined in situ. Dissolved concentrations of calcium (Ca), magnesium (Mg), chloride (Cl), potassium (K), sodium (Na), sulphate (SO<sub>4</sub>), hardness (Hard), alkalinity (Alk), dissolved organic carbon (DOC), total suspended solids (TSS),

total dissolved solids (TDS), total (T. Ag) and dissolved silver (D. Ag) concentrations were determined using Standard Methods test protocols (APHA, AWWA, WPCF 1992).

### Toxicity test

Water for the toxicity test was collected in pre-rinsed 10-L polypropylene containers. Samples were immediately placed into coolers and transported by plane to the laboratory. Later, water was centrifuged (2000 rpm during 15 minutes) and filtered through 47 mm, 0,45µm pore glass-fibre filters (Whatman GF/C). Dissolved silver background concentration in Pil-

comayo River water was below the method detection limit (ND).

Juvenile *C. decemmaculatus* were collected from a small pond, located in Reserva Natural Los Robles, Buenos Aires Province, Argentina (main chemical and physical parameters in Casares et al. 2012). Fish were kept at temperatures ranging from 20 to 24°C and pH ranging from pH 7.1 to 7.5 in an aquarium supplied with a continuous flow of aerated de-chlorinated tap water for 30 days. During this period and posterior laboratory and test water (centrifuged and filtered Pilcomayo River water) acclimation the fish were fed with a daily ration of commercial fish food. Acclimation to test water was performed by adding small quantities of test water to the aquarium until most of the water volume corresponded to test water. One day before and during the experiment, fish were not fed.

Toxicity effect of silver on fish was tested in static systems (4L glass aquaria) with continuous artificial aeration, constant environmental temperature and natural laboratory photoperiod. Test water volume in each aquarium was 2L. Test silver concentrations were attained by spiking from stock solutions of 200 mg Ag L<sup>-1</sup>. The reagent-grade toxicants used to perform the stock solutions was AgNO<sub>3</sub> (Merck analytical grade).

To define the range of silver concentrations to be employed in the bioassay a nominal concentration of 1 mg Ag L<sup>-1</sup> was tested in an aquarium with 2L volume of Pilcomayo River water and 10 acclimated specimens of juvenile *C. decemmaculatus* for 96 h. Subsequently, the experimental design applied included different metal concentrations or treatments (T) with one control group (kept in test water and without metal addition). Fish (not sexed) taken from the acclimation tank were randomly distributed in the different experimental aquaria. Mean standard length of the specimens selected was 13.8 ± 1.2mm and each aquarium contained 10 specimens. Metal concentration in the experimental aquaria was adjusted prior to the fish transfer. Survival was registered four times a day during 96 h. Water pH, conductivity and dissolved oxygen were measured with portable probes from HANNA (HANNA instruments, Inc. Woonsocket, RI, USA) daily. At the beginning of the toxicity test, water samples were collected into polypropylene conical tubes and acidified to pH<2 with concentrated nitric acid (reagent-grade) for metal analysis.

Silver in samples was measured by graphite furnace atomic absorption spectrophotometry Perkin Elmer AAnalyst 800 (Perkin Elmer, Inc. Waltham, MA, USA). Method detection limit was 0.008 mg L<sup>-1</sup>.

### LC<sub>50</sub> calculations

The median lethal concentration (LC<sub>50</sub>) at 24, 48, 72 and 96 hours (24-h LC<sub>50</sub>, 48-h LC<sub>50</sub>, 72-h LC<sub>50</sub>, 96-h LC<sub>50</sub>) were calculated using PROBIT method (Finney 1978) and the statistical program StatPlus (Analyst Soft Inc.).

Version 2.2.3 of the BLM Windows Interface (available at [http://www.hydroqual.com/wr\\_blm.html](http://www.hydroqual.com/wr_blm.html)) was run in order to predict acute silver toxicity to *P. promelas* (toxicity mode). Pilcomayo River water quality parameters employed to run the BLM were temperature, pH, dissolved organic carbon, calcium, magnesium, sodium, potassium, sulphate, chloride and alkalinity.

### Metal speciation

BLM (speciation mode) was also used to assess chemical speciation of the measured silver concentrations.

## RESULTS

Water quality parameters of Pilcomayo River test water are shown in *table 1*.

**Table 1.** Test water main physicochemical parameters (data provided by Subsecretaría de Recursos Hídricos - Argentina). ND: not detected.

Parameter		
Q	m <sup>3</sup> s <sup>-1</sup>	19.3
pH	UpH	7.7
Ca	mg L <sup>-1</sup>	119
Mg	mg L <sup>-1</sup>	46.2
Cl	mg L <sup>-1</sup>	208
Na	mg L <sup>-1</sup>	143.9
K	mg L <sup>-1</sup>	12
SO <sub>4</sub>	mg L <sup>-1</sup>	364
DOC	mg L <sup>-1</sup>	3
Alk	mg L <sup>-1</sup> CaCO <sub>3</sub>	142
Hard	mg L <sup>-1</sup> CaCO <sub>3</sub>	485.16
T. Ag	mg L <sup>-1</sup>	ND
D. Ag	mg L <sup>-1</sup>	ND
TSS	mg L <sup>-1</sup>	546
TDS	mg L <sup>-1</sup>	945

Toxicity test mean water pH and temperature were  $8.3 \pm 0.2$  and  $25.1 \pm 1.2^\circ \text{C}$ , respectively. Within the first few hours from the beginning of the toxicity test, a precipitate was observed. No mortality was observed in the control group. Initial exposure silver concentrations were 0.095 (T1), 0.148 (T2), 0.175 (T3) and 0.285  $\text{mg Ag L}^{-1}$  (T4). Highest mortality was observed within the

first five hours in all treatments.

The median lethal concentrations ( $\text{LC}_{50}$ ,  $\text{mg L}^{-1}$ ) at 24, 48, 72 and 96 h (24-h  $\text{LC}_{50}$ , 48-h  $\text{LC}_{50}$ , 72-h  $\text{LC}_{50}$ , 96-h  $\text{LC}_{50}$ ) with their corresponding confidence intervals (quoted) calculated using PROBIT method and the 96-h  $\text{Ag LC}_{50}$  predicted by BLM for *P. promelas* in the toxicity test water are shown in table 2.

**Table 2.** The  $\text{LC}_{50}$  at 24, 48, 72 and 96 hours (24-h  $\text{LC}_{50}$ , 48-h  $\text{LC}_{50}$ , 72-h  $\text{LC}_{50}$ , 96-h  $\text{LC}_{50}$ ) with their respective confidence intervals calculated for *C. decemmaculatus* and the predicted 96-h  $\text{LC}_{50}$  by BLM for *P. promelas* in the test water.

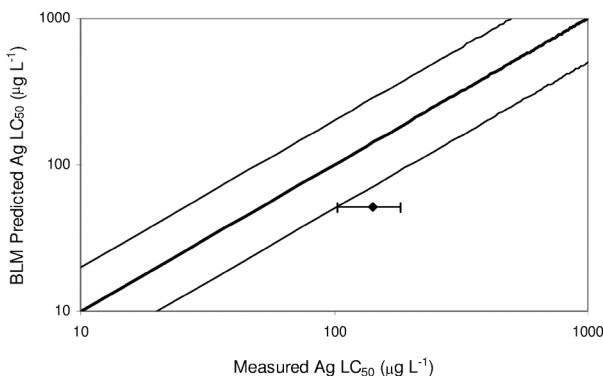
$\text{LC}_{50}$ ( $\text{mg Ag L}^{-1}$ )				
24-h	48-h	72-h	96-h	BLM
0.21 (0.15-0.33)	0.17 (0.12-0.22)	0.15 (0.11-0.19)	0.14 (0.10-0.18)	0.051

Figure 2 shows calculated silver toxicity to *C. decemmaculatus* compared with predicted silver toxicity ( $\text{LC}_{50}$ , in  $\mu\text{g Ag L}^{-1}$ ) using the Biotic Ligand Model. As it can be observed BLM prediction was not accurate within a factor of 2. BLM speciation output can be observed in figure 3. Silver speciation in the four treatments was practically the same with 60% of the silver as  $\text{AgCl}$  and 30% as  $\text{AgCl}_2$ . The free ion form represented the highest contribution (6%) amongst the remaining species group.

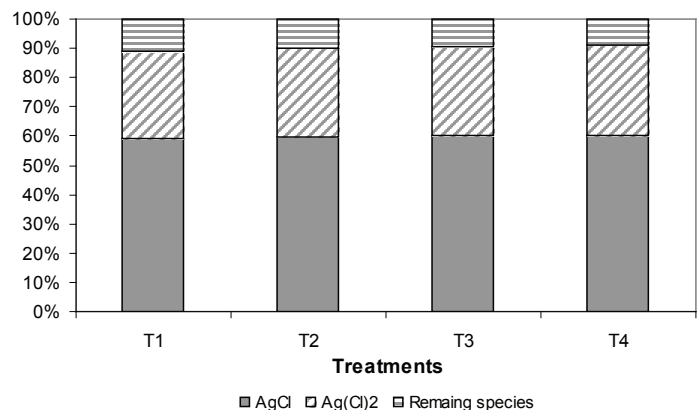
## DISCUSSION

Pilcomayo River water characterizes by its high water hardness. The ameliorative effects

of water hardness on copper toxicity have been well documented; whereas, its protective effect on silver toxicity seems to be not clear. For Paquin et al. (2002), when competing with silver at the biotic ligand, calcium tends to be of less importance than it appears to be for other metals. Bury et al. (1998) found that an increment on calcium concentration from 2 to 80  $\text{mg L}^{-1}$  had a small influence on the 96-h  $\text{LC}_{50}$  for rainbow trout and *P. promelas*. These authors reported a 96-h  $\text{Ag LC}_{50}$  for juvenile *P. promelas* of 0.008  $\text{mg L}^{-1}$ . Karen et al. (1999) reported that dissolved organic carbon was more important than hardness for predicting the toxicity of ionic silver in natural



**Figure 2.** Calculated silver toxicity to *C. decemmaculatus* compared with predicted silver toxicity ( $\text{LC}_{50}$ , in  $\mu\text{g Ag L}^{-1}$ ) using the Biotic Ligand Model developed for *P. promelas*. The thicker line represents a 1:1 relationship. The thinner lines represent predictions within a factor of 2. The error bars represent 95% confidence intervals.



**Figure 3.** BLM speciation output for each treatment. Silver species are expressed as percentages of total dissolved silver concentration. Remaining species summarizes the contribution of the following species: free ion,  $\text{AgCl}_3$ ,  $\text{AgCl}_4$ ,  $\text{AgNO}_3$ ,  $\text{AgSH}$ ,  $\text{Ag}(\text{SH})_2$ , Torg Ag,  $\text{AgOH}$  and  $\text{Ag}(\text{OH})_2$ .

waters to *O. mykiss*, *P. promelas*, and *D. magna*. On the other hand, Erickson et al. (1998) reported a substantial decrease in silver toxicity to *P. promelas* (96-h  $LC_{50}$  increase from 0.005 to 0.012 mg L<sup>-1</sup>) when water hardness was increased from 48 to 249 mg L<sup>-1</sup> CaCO<sub>3</sub>. Bielmyer et al. (2007) found that *C. dubia* and *P. promelas* were less sensitive to silver in waters with a combination of higher hardness and dissolved organic carbon. In water with a hardness of 225 mg L<sup>-1</sup> CaCO<sub>3</sub>, these authors reported a 96-h  $LC_{50}$  for 7-days old *P. promelas* of 0.021 (0.019-0.023) mg L<sup>-1</sup>. Despite the opposite results reported, higher 96-h  $LC_{50}$  estimated for *C. decemmaculatus* may reflect some protective effect exerted by Pilcomayo River water elevated hardness.

High levels of sodium are thought to inhibit silver accumulation through competition; however, Bury and Wood (1999) found that one-third of the silver uptake continued in presence of a sodium channel and ATPase blockers or high levels of sodium in water. Erickson et al. (1998) reported no significant effects on silver toxicity to *P. promelas* when 2 meq L<sup>-1</sup> of sodium sulphate were added to test water. This means that alternatively or additionally, multiple pathways exist for apical silver uptake at the gill cells. If multiple silver pathways are also present in *C. decemmaculatus*, the high levels of sodium in Pilcomayo River water did not exert a protective effect against silver toxicity.

Pilcomayo River dissolved organic matter levels are relatively low. Increased levels of organic carbon are expected to affect silver bioavailability through complexation. Bury et al. (1998) observed that an increase of 0.3 to 5.8 mg L<sup>-1</sup> in dissolved organic carbon reduced acute silver toxicity to rainbow trout and *P. promelas*. Erickson et al. (1998) reported that increased levels of dissolved organic carbon (from 1 to 11 mg C L<sup>-1</sup>) significantly increased Ag  $LC_{50}$  for 30-day-old *P. promelas*. However, Rose-Janes and Playle (2000) highlighted the strong binding of Ag<sup>+</sup> to trout gills and the relatively weaker binding of silver to dissolved organic matter. In the present contribution, dissolved organic carbon did not exert an important effect on silver speciation, since the predicted percentage of silver bound to dissolve organic matter was very low (0.52 %).

A net loss of chloride is also observed during silver exposure. The protective effect of high levels of chloride in water has been shown to be different among species. Some species

rely on branchial chloride uptake, which, in some species, is not inhibited by silver exposure, and others depend on multiple chloride uptake pathways. Apparently, chloride uptake pathways in *P. promelas* are not inhibited by silver exposure (Bielmyer et al. 2008). Given that chloride uptake pathways in *C. decemmaculatus* are unknown, we cannot suggest that high chloride concentration in Pilcomayo River water could have exerted a protective effect against silver toxicity. External chloride may also exert a protective effect through complexation. In this regard, BLM speciation output for each of the silver concentrations tested showed that 90% of the silver was as AgCl and AgCl<sub>2</sub>. High chloride levels have been shown to protect *O. mykiss* against silver toxicity presumably by complexation to form AgCl, thereby reducing the concentration of Ag<sup>+</sup>. However, the formation of AgCl does not appear to substantially influence silver sensitivity for any other fish species tested (Bielmyer et al. 2008). Therefore, it remains to determine experimentally whether changes in silver speciation related to changes in chloride concentration reduce silver toxicity to *C. decemmaculatus*.

Water pH is another water quality parameter that would affect silver toxicity through speciation. During the present toxicity test, mean water pH was 8.3. In this regard, Erickson et al. (1998) reported a reduction in silver toxicity with increasing pH. The 96-h  $LC_{50}$  at pH 8.6 was three times higher than at pH 7.2. In the present toxicity test, higher 96-h  $LC_{50}$  for *C. decemmaculatus* may reflect some protective effect against silver toxicity exerted by the elevated mean water pH.

Silver BLM  $LC_{50}$  prediction for *P. promelas* in Pilcomayo River water did not agree well with the  $LC_{50}$  calculated for *C. decemmaculatus*. Bielmyer et al. (2007) in their silver BLM validation study found that only 50% of the  $LC_{50}$  predicted values for *P. promelas* were accurate within a factor of two. Higher 96-h  $LC_{50}$  for *C. decemmaculatus* may show that this species is less sensitive to silver toxicity than other fish species. However, it must be taken into account that almost all of the studies previously cited on silver toxicity to *P. promelas* worked with younger life-stages of the species which are known to be more sensitive to pollutants. In this regard, Bielmyer et al. (2007) reported higher 96-h  $LC_{50}$  for *P. promelas* as fish aged and Grosell and co-authors (2002)

reported higher sodium turnover in smaller animals compared with larger animals in fresh water. Bianchini et al. (2002) studied  $LC_{50}$  values based on total silver as a function of the body mass. According to their results,  $\log LC_{50}$  progressively increased with  $\log$  body weight, independent of the species considered. For the mean body weight of the animals tested in the present contribution, the relationship found by these authors predicted a lower  $LC_{50}$  ( $0.45 \mu\text{g Ag/L}$ ) compared to the value calculated for *C. decemmaculatus* ( $140 \mu\text{g Ag/L}$ ). This difference may be another indicator of a lower sensitivity of *C. decemmaculatus* to silver toxicity.

In the present contribution, a clear pattern of mortality was observed. Most of the deaths occurred within the first five hours. Due to the fact that silver precipitation was observed, is possible that fish were in contact with higher silver concentrations during the first minutes to hours of the toxicity test. We did not measure silver accumulation on the gills but this pattern of mortality would correspond to a peak and decline in gill silver accumulation observed in static toxicity tests. Nebeker et al. (1983); Erickson et al. (1998) and Morgan et al. (2004) attributed this pattern of silver accumulation to changes in silver bioavailability through complexation with the increasing organic matter. In the present contribution, silver concentrations tested were higher compared to other toxicity tests performed, therefore, changes in silver bioavailability may have occurred mainly by silver precipitation.

## CONCLUSIONS

*C. decemmaculatus* higher Ag 96-h  $LC_{50}$  may reflect that this species is less sensitive to silver than other fish species. The mortality pattern observed suggests that toxicity can be attributed to the initial dissolved silver concentrations. The ameliorative effect of water hardness on silver toxicity is not clear; however, Pilcomayo River water elevated hardness may have exerted some protective effect. Dissolved organic carbon contribution in reducing silver bioavailability was negligible. Elevated mean water pH may have exerted a major protective effect. More research is needed to determine the effects of high sodium and chloride concentrations on silver toxicity to *C. decemmaculatus*. The mortality pattern observed in this toxicity test may lend some support to a relationship between gill silver accumulation

and mortality. A cross-fish-species extrapolation of the *P. promelas* Ag BLM was not valid in Pilcomayo River water and experimental conditions of this toxicity test.

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