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# Acute zinc toxicity to *Cnesterodon decemmaculatus* (Pisces: Poeciliidae) and application of the Biotic Ligand Model in Pilcomayo River water (South America)

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**Abstract:** The Biotic Ligand Model (BLM) is a chemical equilibrium-based model that incorporates the effect of physicochemical water characteristics on the bioavailability and toxicity of metals to aquatic biota. It was developed for four metals (Cu, Zn, Ag and Cd), two fish species and three daphnids. It is assumed that its predictions can be extrapolated between similar species. In this study, a cross-fish-species extrapolation of the BLM developed for zinc (Zn-BLM) was assessed in Pilcomayo River water. An acute zinc toxicity test was performed to assess zinc toxicity to the local fish Cnesterodon decemmaculatus. The dissolved zinc concentrations tested were: 3.74; 9.2; 21.6 and 26.4 mg Zn $L^{-1}$. The median letal zinc concentration (96-h Zn $LC_{50}$) calculated for *C. decemmaculatus* was 22.6 mg Zn $L^{-1}$ (17.5-27.6) and the predicted by Zn-BLM for *Pimephales promelas* in the test water was 1.71 mg $L^{-1}$. Zinc concentrations measured exceeded zinc solubility causing metal precipitation which derived in a 96-h $LC_{50}$ that most probably included both dissolved and precipitated zinc species. Nevertheless, speciation estimates showed that the more abundant zinc species in each treatment was the free ion. This higher proportion of zinc in its free ionic form would explain the low protective effect exerted by elevated water hardness. The difference between the observed zinc toxicity to *C. decemmaculatus* and the predicted by BLM for P. promelas may be due to the combination of inaccuracy in zinc dissolved measurements and a lower sensitivity of *C. decemmaculatus* to zinc exposure.

**Keywords:** $LC_{50}$, BLM, extrapolation, bioavalability.

**Resumen: Toxicidad aguda por cinc en *Cnesterodon decemmaculatus* (Pisces: Poeciliidae) y aplicación del Modelo de Ligando Biótico en agua del Río Pilcomayo (Sudamérica).** El Modelo del Ligando Biótico (BLM) es un modelo basado en el equilibrio químico. Incorpora el efecto de las características físico-químicas del agua en la biodisponibilidad y toxicidad de los metales sobre la biota acuática. Ha sido desarrollado para cuatro metales (Cu, Zn, Ag, Cd), dos especies de peces y tres de dáfnidos. Se asume que sus predicciones podrían ser extrapoladas a especies similares. En este estudio, se evaluó una posible extrapolación inter-especifica (peces) del BLM desarrollado para el zinc en agua del río Pilcomayo. Se llevó a cabo un ensayo de toxicidad aguda del zinc en el pez nativo *Cnesterodon decemmaculatus*. Las concentraciones de zinc disuelto aplicadas fueron: 0,13; 3,74; 9,2; 21,6 y 26,4 mg Zn $L^{-1}$. La concentración letal media del zinc ($CL_{50}$ 96-h) calculada para *C. decemmaculatus* fue 22,6 mg Zn $L^{-1}$ (17,5 - 27,6) y la predicha por el BLM para *Pimephales promelas* en el agua experimental fue 1,71 mg $L^{-1}$. Las concentraciones de zinc medidas excedían la solubilidad del metal lo que produjo la precipitación del mismo, derivando en una 96-h $CL_{50}$ que muy probablemente incluyó tanto especies de zinc disueltas como precipitadas. Sin embargo, las estimaciones de la especiación mostraron que la especie química del zinc más abundante en todos los tratamientos fue el ion libre. La mayor proporción de zinc en su forma iónica libre explicaría el bajo efecto protector de la elevada dureza del agua experimental. La diferencia entre la toxicidad del zinc observada y la predicha por el BLM podría deberse a la combinación de inexactitud en las mediciones de zinc disuelto y una menor sensibilidad de la especie experimental a la presencia de elevadas concentraciones de zinc.

**Palabras clave:** $CL_{50}$, BLM, extrapolación, biodisponibilidad.

## Introduction

The chemical form of metals affects their bioaccessibility, bioavailability and effect and is influenced by physicochemical environmental conditions. The bioaccessible fraction of metal is the portion of environmentally available metal that actually interacts at the organism's contact surface and is potentially available for bio-absorption or bio-adsorption. Bioavailability is the extent to which bioaccessible metals cross biological membranes, expressed as a fraction of the total amount of metal the organism is proximately exposed, during a given time and under defined conditions [1].

The Biotic Ligand Model (BLM) [2] was proposed to evaluate how water chemistry affects the speciation and biological availability of metals in aquatic systems [3]. This model, and others which were developed to evaluate copper, zinc and nickel chronic toxicity to aquatic biota, are gaining acceptance within the regulatory community. They were developed using geochemical equilibrium principles, and provide site-specific toxicity predictions [4]. It is implicitly assumed that BLM models can be extrapolated within taxonomically similar groups, i.e., that BLM developed for *P. promelas* could be applied to ecotoxicity data for other fish species, that BLM models for *D. magna* and *C. dubia* could be applied to ecotoxicity data for other invertebrates. 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 (e.g., $Mn^{++}$) and the biotic ligand are similar across organisms, and that only intrinsic sensitivity varies among species [5].

Zinc, as many other metals, enters the aquatic ecosystems as a by-product of many industrial and mining processes. Zinc is an essential micronutrient with a well regulated physiology. It is present in over 300 enzymes [6]. In mammals and other higher vertebrates is taken up by the intestine, but in fish there is an additional important pathway for zinc absorption, namely the gills [7]. At intermediate exposure

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levels (e.g. 800 µg $L^{-1}$, approximately the 96-h $LC_{50}$ values in soft water) zinc can exert a toxic effect on fish by inhibiting the uptake of calcium [8]. The inhibition of calcium uptake creates an imbalance in the uptake and loss rates, leading to a net loss of calcium. Zinc and calcium appear to compete for the same apical voltage-independent calcium channel of the chloride cells [9, 10], such that while elevated levels of zinc inhibit calcium uptake, the converse is also true. To a lesser extent, zinc also disturbs sodium and chloride fluxes, resulting in a net branquial ion loss.

Several water chemistry variables can protect the organisms against metal binding to the toxicity sites by cationic competition (calcium, magnesium and protons), or by anionic complexation (e.g., hydroxide, (bi)carbonate, chloride, thiosulfate, sulfide, and dissolved organic matter), thereby preventing metals binding to the toxicity sites [11]. One of the first and most well recognized of the modifying factors for metals is water hardness. In particular, calcium and magnesium can modify the toxicity of metals by different degrees, with hardness-dependent toxicity thus varying as a function of calcium, magnesium, or the ratio between these two for some species [3]. Alkalinity, on the other hand, affects metal ionic species in water solution through their complexation with carbonates [12, 13]. Dissolved organic matter forms complexes with metals, which reduce the free form in water, and therefore the amount of ionic metal available to bind to the toxicity sites in fish gill [14].

Pilcomayo River, whose drainage basin includes parts of Bolivia, Argentina and Paraguay (South America), is characterized by its high concentrations of calcium, magnesium, chloride, sulphates and total suspended solids. Toxic waste spills containing high concentrations of arsenic and 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 [15]. In the lower basin, river conditions improve in relation to the concentration of heavy metals, with values below the freshwater quality criteria developed for the protection of aquatic life [15].

*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 [16]. Thereby, several authors have used *C. decemmaculatus* in bioassays [17,18,19,20]. *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*, *P. promelas* is quite tolerant to turbid, low-oxygenated water bodies, and can be found in muddy ponds and streams and in small rivers [21]. There is one previous work on the toxicity of metals to *C. decemmaculatus* in Pilcomayo River water. Casares et al. [22] found that a cross-fish-species extrapolation of the BLM developed for copper was valid for Pilcomayo River water characteristics and experimental conditions of the toxicity test.

The aim of this study was to evaluate a cross-fish-species extrapolation of the Zn-BLM under Pilcomayo River water quality characteristics. For this purpose, the acute zinc toxicity (96-h Zn $LC_{50}$) to *C. decemmaculatus* was assessed in Pilcomayo River water, and the calculated 96-h Zn $LC_{50}$ was compared to the predicted by BLM for the species *P. promelas* in the test water.

## Materials and Methods

*Study area*

The Pilcomayo River in South America is a tributary to the large La Plata River Basin, the largest water system in South America after the Amazon watershed. Its headwaters are located in Bolivia along the eastern flank of the central Andes at an elevation of approximately 5,200 m over the sea level. The river flows in a southeastern direction for about 670 km 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 $km^2$ [23].

*Water Sampling and chemical analysis*

Discrete water samples for chemical analyses were taken 10 cm below the water surface and in triplicate from the central channel, left and right shore of Pilcomayo River at Misión La Paz International bridge (22º 22′ 45” S – 62º 31′ 08” W; 254 meters over sea level) in May 2009. Samplings and *in situ* water quality determinations were performed by Subsecretaría de Recursos Hídricos de la Nación (SsRH-Argentina), Centro de Ecología Aplicada del Litoral (CECOAL) and Universidad Nacional de Salta (UNS). Laboratory analysis of chemical parameters was performed by the UNS and Comisión Nacional de Energía Atómica (CNEA-Argentina). Water discharge (Q), pH, electrical conductivity (EC) and water temperature (T) were determined *in situ*. Dissolved concentrations of calcium (Ca), magnesium (Mg), hardness (Hard), chloride (Cl), potassium (K), sodium (Na), sulphate ($SO_4$), alkalinity (Alk), dissolved organic carbon (DOC), total suspended solids (TSS) and total (T. Zn) and dissolved zinc (D. Zn) concentrations were determined using Standard Methods test protocols [24].

*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).

Juvenile *C. decemmaculatus* individuals were collected from a small pond, located in Reserva Natural Los Robles, Buenos Aires Province, Argentina (main chemical and physical parameters in [22]. Fish were kept at temperatures ranging from 20 to 24º 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 laboratory and test water acclimation period, 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 toxicity test, fish were not fed.

Toxicity effect of zinc on fish was tested in static systems (4-L glass aquaria) with continuous artificial aeration, constant environmental temperature and natural laboratory photoperiod. Test zinc concentrations were attained by spiking from a stock solution of 100 mg Zn $L^{-1}$. The reagent-grade toxicant used to perform the stock solution was $ZnCl_2$ (Merck analytical grade). To define the range of zinc concentrations to be employed in the bioassays nominal concentrations of 0.8, 33 and 60 mg Zn $L^{-1}$ were tested in an aquarium with 2L volume of Pilcomayo River test water and 10 acclimated specimens of juvenile *C. decemmacuatus* for 96 h.

The experimental design included four treatments with different metal concentrations and one control group (kept in test water and without metal addition). The dissolved zinc concentrations tested were 3.74 (T1), 9.2 (T2), 21.6 (T3) and 26.4 (T4) mg Zn $L^{-1}$. These concentrations were measured at the very beginning of the toxicity test; however, they are different to the nominal ones. This is owed to the fact that zinc precipitation increased as more $ZnCl_2$ was added to the aquaria. For this reason, zinc concentration in T4 was similar to T3. Dissolved zinc background concentration in Pilcomayo River water was 0.13 mg $L^{-1}$.

Fish (not sexed) taken from the acclimation tank were randomly distributed in the different experimental aquaria. Mean standard length of the specimens selected was 21.1mm and each aquarium contained 10 specimens. Metal concentration in the experimental aquaria was adjusted prior to 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 from each treatment were collected into polypropylene conical tubes and acidified to pH<2 with concentrated nitric acid (reagent-grade) for metal analysis. Zinc samples were measured by atomic absorption spectrophotometry (Perkin Elmer 1100B, Perkin Elmer, Inc. Waltham, MA, USA) after acid digestion ($HNO_3$:$HClO_4$:HF:HCl). The detection limit of the method for zinc was 0.018 mg $L^{-1}$.

*$LC_{50}$ calculations*

The mean lethal concentrations ($LC_{50}$) at 24, 48, 72 and 96 hours (24-h $LC_{50}$, 48-h $LC_{50}$, 72-h $LC_{50}$, 96-h $LC_{50}$) were calculated using PROBIT method [25] 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) was run in order to predict acute zinc toxicity to *P. promelas* (toxicity mode). Pilcomayo River water quality input parameters to run BLM were: temperature, pH, dissolved organic carbon, calcium, magnesium, sodium, potassium, sulphates, chlorides and alkalinity.

*Metal speciation*

BLM was also run in the speciation mode to obtain an estimation of zinc speciation in each treatment.

## Results

Pilcomayo River test water main physicochemical parameters are shown in Table 1. All the parameters employed to run the BLM were within the range of values for which the BLM was calibrated.

No mortality was observed in the control group. Within the first few hours from the beginning of the toxicity test, water in aquaria turned cloudy, mostly in those aquaria where zinc concentration was the highest. A precipitated at the bottom of aquaria was also observed at the end of the test. Toxicity test mean water pH and temperature were 7.91 ± 0.21 UpH and 16.6 ± 1.51 º C, respectively.

**Table 1.** *Pilcomayo River test water main physicochemical parameters.*

| Parameter | | |
|---|---|---|
| | | |
| EC | µS $cm^{-1}$ | 945.3 |
| Ca | mg $L^{-1}$ | 73.33 |
| Mg | mg $L^{-1}$ | 30.5 |
| Cl | mg $L^{-1}$ | 101 |
| Na | mg $L^{-1}$ | 65.2 |
| K | mg $L^{-1}$ | 5.1 |
| $SO_4$ | mg $L^{-1}$ | 207.3 |
| DOC | mg $L^{-1}$ | 4.4 |
| Alk | mg $L^{-1}$ $CaCO_3$ | 110 |
| Hard | mg $L^{-1}$ $CaCO_3$ | 307.32 |
| T. Zn | mg $L^{-1}$ | 0.2 |
| D. Zn | mg $L^{-1}$ | 0.13 |
| TSS | mg $L^{-1}$ | 1637 |
| | | |

The parameters are expressed as follows: electrical conductivity (EC), calcium (Ca), magnesium (Mg), hardness (Hard), chloride (Cl), potassium (K), sodium (Na), sulphate (SO4), alkalinity (Alk), dissolved organic carbon (DOC), total suspended solids (TSS) and total zinc (T. Zn) and dissolved zinc (D. Zn) concentration.

The median lethal concentration (mg Zn $L^{-1}$) at 24, 48, 72 and 96 hours (24-h $LC_{50}$, 48-h $LC_{50}$, 72-h $LC_{50}$, 96-h $LC_{50}$) with their respective confidence intervals calculated for *C. decemmaculatus* using PROBIT method and the predicted 96-h $LC_{50}$ by BLM for *P. promelas* for the toxicity test water quality are shown in Table 2. The $LC_{50}$ value decreased from 24 to 72 hours and remained constant until the end of the toxicity test. The BLM predicted 96-h $LC_{50}$ for *P. promelas* was an order of magnitude lower than the calculated for *C. decemmaculatus*.

**Table 2.** *The $LC_{50}$ at 24, 48, 72 and 96 hours with their respective confidence intervals calculated for C. decemmaculatus in Pilcomayo River water and the predicted 96-h $LC_{50}$ by BLM for P. promelas under Pilcomayo River test water physicochemical parameters.*

| $LC_{50}$ (mg Zn $L^{-1}$) | | | | |
|---|---|---|---|---|
| 24-h | 48-h | 72-h | 96-h | BLM |
| | | | | |
| 37.2 (26.6-47.8) | 23.6 (19.4-27.5) | 22.3 (16.8-43.8) | 22.6 (17.5-27.6) | 1.71 |
| | | | | |

Table 3 shows zinc speciation for each of the initial dissolved zinc concentrations measured. In the control group most of the zinc was complexed to dissolved organic matter (Org-Zn). In all treatments, zinc was mainly in its free ionized form followed by zinc bicarbonate ($ZnHCO_3$) and to a lesser extent by zinc carbonate ($ZnCO_3$). All zinc species increased with increasing concentration of total dissolved zinc; however, this increment was marked for the free ion especially in T3 and T4. The remaining species group summarizes the contribution of the following species: ZnOH, $Zn(OH)_2$, $ZnSO_4$, ZnCl and ZnS. Within this group, the species with higher contribution was zinc sulfate ($ZnSO_4$).

**Table 3.** *BLM speciation estimates for each of the zinc concentrations measured and the control group.*

| Treatment | Zinc Speciation (mg Zn $L^{-1}$) | | | | |
|---|---|---|---|---|---|
| | Free Zn | $ZnHCO_3$ | $ZnCO_3$ | Org-Zn | Remaining species |
| Control | 0.02 | 0.01 | 0.005 | 0.07 | 0.007 |
| T1 | 1.55 | 1.08 | 0.33 | 0.42 | 0.33 |
| T2 | 4.06 | 2.80 | 0.86 | 0.58 | 0.87 |
| T3 | 9.99 | 6.63 | 2.04 | 0.79 | 2.12 |
| T4 | 12.35 | 8.08 | 2.48 | 0.85 | 2.62 |

Zinc species are expressed in mg Zn $L^{-1}$. Zinc associated to dissolved organic matter is referred to as Org-Zn. The remaining species group summarizes the contribution of the following species: ZnOH, $Zn(OH)_2$, $ZnSO_4$, ZnCl and ZnS.

## Discussion

Development and calibration of the BLM model require comparison with suitable datasets that illustrate the effects of water chemistry on zinc toxicity [26]. Pilcomayo River water is characterized by high concentration of calcium and magnesium, which results in water with elevated hardness. Compared to sixty of the world largest rivers mentioned by Gaillardet et al. [27], Pilcomayo River is within the group of the ten rivers with highest major ions and suspended solids concentrations. It has been shown that water hardness, among other variables, exerts a major influence on the toxicity of some heavy metals to the aquatic biota [28]. Particularly, calcium and zinc share the same apical entry, therefore, a major protective effect of high calcium concentrations is expected. Many authors have reported a protective effect of water hardness against zinc toxicity. Bradley and Sprague [29] reported that water hardness exerted a major protective action than high alkalinity on zinc toxicity to rainbow trout (*O. mykiss*). Everall et al. [28] found a protective effect of hard water on zinc toxicity to brown trout (*Salmo trutta*) over a pH range of 4-6 and 8-9. In water with a total hardness of 204 mg $L^{-1}$ $CaCO_3$ these authors reported 96-h $LC_{50}$ values from 2.69 to 3.20 mg $L^{-1}$, and in soft water, the values reported were in the range of 1.07 to 2.31 mg $L^{-1}$. Judy and Davies [30] also found a significant decrease of zinc toxicity to *P. promelas* upon calcium addition to test water. Gómez et al. [19] reported for adult individuals of *C. decemmaculatus* in the Río de La Plata Estuary water (water hardness = 103 mg $L^{-1}$ $CaCO_3$) a 96-h Zn $LC_{50}$ value of 29.35 mg $L^{-1}$. Río de La Plata Estuary water hardness was much lower than Pilcomayo River (307.32 mg $L^{-1}$ $CaCO_3$) however, zinc toxicity to *C. decemmaculatus* in Pilcomayo River water was higher. Other authors have also encountered difficulties to predict not zinc but copper toxicity in natural waters by hardness alone [31,32] and their results may apply to other metals as well, in this case, to zinc.

According to BLM speciation estimates, dissolved organic matter might exert an important protective effect at low zinc concentrations (control group). However, at higher concentrations, the estimations showed that most of the zinc was in its free ion form and the difference in concentration with other dissolved species (complexes) was superior in the highest concentrations tested. This may indicate a gradual saturation of the available anions to form complexes with zinc rendering in the increment of the free ionic zinc as the added metal increased. In this regard, the observed mortality pattern could be attributed to the higher concentration of the free zinc ion, the most toxic species. The high proportion of free zinc ion may also explain the low protective effect of elevated water hardness. Despite the presence of high concentration of calcium, zinc would have bound preferentially to the biotic ligand.

BLM prediction of 96-h $LC_{50}$ for *P. promelas* was an order of magnitude lower than the results obtained experimentally for *C. decemmaculatus*. In the cases in which water cloudiness was reported, as in the present contribution, BLM was not accurate in predicting zinc toxicity to *P. promelas*. Santore et al. [26] attributed this discrepancy to a combination of uncertainty in dissolved zinc concentrations measured and potential toxicity of the precipitated zinc that remained in suspension. According to the relationship between zinc solubility and pH showed by [26], zinc solubility reaches a minimum at approximately a pH of 8.5. This means that, in our test mean water pH, zinc solubility was near the minimum, which explains zinc precipitation during the toxicity test. Taking this into account, it is possible that the dissolved zinc concentration measured were uncertain and consequently derived in a 96-h $LC_{50}$ value that exceeded zinc solubility. Thus, in the cases in which a phenomenon of metal precipitation is observed, BLM does not appear to be a useful tool for predicting metal toxicity.

On the other hand, *C. decemmaculatus* might actually be a less sensitive species to zinc exposure than *P. promelas* considering that, and according to zinc speciation estimates, fish were exposed to very high concentrations of the free zinc ion during the toxicity test. In this regard, it has been established that there are marked differences in zinc sensitivity between various species. Non-salmonoid fish are known to be less sensitive than salmonoids [33]. Svecevičius [34] found for rainbow trout, three-spined stickleback (*Gasterosteus aculeatus*), roach (*Rutilus rutilus*), perch (*Perca fluviatilis*) and dace (*Leuciscus leuciscus*) in water with a mean pH value within the range of 7.9 - 8.1 and a hardness from 270 to 300 mg $L^{-1}$ as $CaCO_3$ values of 96-h $LC_{50}$ that ranged from 3.79 to 11.37 mg Zn $L^{-1}$. Likewise, Ebrahimpour and coworkers [35] reported for *Capoeta fusca* in water with a hardness of 40, 150 and 380 mg $L^{-1}$ $CaCO_3$, 96-h $LC_{50}$ values of 13.7; 74.4 and 102.9 mg Zn $L^{-1}$, respectively. Gül et al. [33] reported for adult guppies (*Poecilia reticulata*) 96-h $LC_{50}$ of 30.83 mg Zn $L^{-1}$ in water with a hardness of 230 mg $L^{-1}$ $CaCO_3$. *C. decemmaculatus* belongs as well as guppies to the *Poeciliidae* family and both species are known to be very opportunistic, colonizing poor quality habitats inadequate for most fish species. The highest tolerance of these species to unsuitable conditions could also be related to a lower sensitivity to zinc toxicity.

## Conclusions

Results in the present contribution suggest that a cross-fish-species extrapolation of the Zn-BLM for *P. promelas* was not possible for the water quality parameters and experimental conditions of the toxicity test. The initial dissolved zinc concentrations measured exceeded zinc solubility in the test water which derived in a 96-h $LC_{50}$ that most probably included both dissolved and precipitated zinc species. However, speciation estimates showed that fish were in contact with very high concentrations of the free zinc ion during the test. The results indicate that the difference between the observed zinc toxicity to *C. decemmaculatus* and the predicted by BLM may be due to the combination of inaccuracy in zinc dissolved measurements and a lower sensitivity of the experimental species to zinc exposure.

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## References

1. Fairbrother A, Wenstel R, Sappington K, Wood W (2007) Framework for metal risk assessment. Ecotoxicol Environ Saf 68: 145-227.
2. Di Toro DM, Allen HE, Bergman HL, Meyer JS, Paquin PR, Santore RC (2001) A biotic ligand model of the acute toxicity of metals I. Technical basis. Environ Toxicol Chem 20: 2383–2396.
3. Paquin PR, Gorsuch JW, Apte S, Batley GE et al (2002) The biotic ligand model: a historical overview. Comp Biochem Physiol Part C 133: 3–35.
4. Clifford M, McGeer JC (2009) Development of a biotic ligand model for the acute toxicity of zinc to *Daphnia pulex* in soft waters. Aquat Toxicol 91: 26–32.
5. Schlekat EC, Van Genderen E, De Schamphelaere KAC, Antunes PMC, Rogevich EC, Stubblefield WA (2010) Cross-species extrapolation of chronic nickel Biotic Ligand Model. Sci Total Environ 408: 6148-6157.
6. Sandstead HH (2000) Causes of iron and zinc deficiencies and their effects on brain. J Nutr 130: 347S-349S.
7. Hogstrand C, Verbost PM, Wendelaar Bonga SE, Wood C (1996) Mechanisms of zinc uptake in gills of fresh water rainbow trout: interplay with calcium transport. The Am Physiol Societ R1141-R1147.
8. Spry DJ, Wood CM (1985) Ion flux rates, acid–base status, and blood gases in rainbow trout, *Salmo gairdneri*, exposed to toxic zinc in natural soft water. Can J Fish Aquat Sci 42: 1332–1341.
9. Verbost PM, Flik G, Lock RAC, Wendelaar Bonga SE (1987) Cadmium inhibition of $Ca^{2+}$ uptake in rainbow trout gills. Am J Physiol 253: 216-221.
10. Verbost PM, Rooij JV, Flik G, Lock RAC, Wendelaar Bonga SE (1989) The movement of cadmium through freshwater trout branchial epithelium and its interference with calcium transport. J Exp Biol 145: 185–197.
11. Wood CM (2008) The biotic ligand model: Using physiology, geochemistry, and modeling to predict metal toxicity. Comp Biochem Physiol Part C: 148: 468-469.
12. Erickson RJ, Benoit DA, Mattson VR, Nelson HP, Leonard EN (1996) The effects of water chemistry on the toxicity of copper to fathead minnows. Environ Toxicol Chem 15: 181–193.
13. Lauren DJ, McDonald DG (1986) Influence of water hardness, pH, and alkalinity on the mechanisms of copper toxicity in juvenile rainbow trout, *Salmo gairdneri.* Can J Fish Aquat Sci 43: 1488–1496.
14. Matsuo AYO, Playle RC, Val AL, Wood CM (2004) Physiological action of dissolved organic matter in rainbow trout in the presence and absence of copper: sodium uptake kinetics and unidirectional flux rates in hard and soft water. Aquat Toxicol 70: 63–81.
15. Smolders AJP, Lock RAC, Van del Velde G, Medina Hoyos RI, Roelofs JGM (2003) Effects of mining activities on heavy metal concentration in water, sediment and macroinvertebrates in different reaches of the Pilcomayo River, South America. Arch Environ Contam Toxicol 44: 314-323.
16. Menni RC (2004) Peces y Ambientes en la Argentina Continental. Monografías del Museo Argentino de Ciencias Naturales 5: 50-53.
17. de la Torre FR, Demichelis SO, Ferrari L, Salibián A (1997) Toxicity of Reconquista river water: bioassays with juvenile *Cnesterodon decemmaculatus*. Bull Environ Contam Toxicol 58: 558–565.
18. de la Torre FR, Ferrari L, Salibián A (2005) Biomarkers of a native fish species (*Cnesterodon decemmaculatus*): application to the toxicity assessment of the water of a periurban polluted river of Argentina. Chemosphere 59: 577–583.
19. Goméz S, Villar C, Bonetto C (1998) Zinc toxicity in the fish *Cnesterodon decemmaculatus* in the Paraná River and Río de La Plata Estuary. Environ Pollut 99: 159-165.
20. Ferrari L, García ME, de la Torre FR, Demichelis SO (1998) Evaluación Ecotoxicológica del agua de un río urbano mediante bioensayos con especies nativas. Rev Museo Argentino de Ciencias Naturales. Vol Extra Nueva Serie 148: 1–16.
21. Page LM, Burr BM (1991) A field guide to freshwater fishes of North America and north of Mexico. Houghton Mifflin Company, Boston. 432 pp.
22. Casares MV, de Cabo LI, Seoane RS, Natale OE, Castro Ríos M, Weigandt C, F de Iorio A (2012) Measured copper toxicity to *Cnesterodon decemmaculatus* (Pisces: Poeciliidae) and predicted by Bitic Ligand Model in Pilcomayo River water: a step for a cross-fish-species extrapolation. J Toxicol doi:10.1155/2012/849315.
23. *Características de la cuenca del río Pilcomayo*. http://www.pilcomayo.net/web/
24. APHA, AWWA, WPCF (1992) American Public Health Association, American Water Works Association, Water Pollution Control Federation. Standard Methods for the examination of water and wastewater. 20th ed. Washington DC: APHA, AWWA, WPCF.
25. Finney DJ (1978) Statistical method in biological assay. London: Charles Griffin 508 pp.
26. Santore RC, Mathew R, Paquin PR, DiToro D (2002) Application of the biotic ligand model to predicting zinc toxicity to rainbow trout, fathead minnow, and *Daphnia magna*. Comp Biochem Physiol Part C 133: 271–285.
27. Gaillardet J, Dupré B, Louvat P, Allègre C J, (1999) Global silicate weathering and CO2 consumption rates deduced from the chemistry of large rivers. Chem Geol 159: 3-30.
28. Everall NC, MacFarlane NAA, Sedgwicks RW (1989) The interactions of water hardness and pH with the acute toxicity of zinc to the brown trout, *Salmo trutta* L J Fish Biol 35: 27-36.

29. Bradley RM, Sprague JB (1985) The influence of pH, water hardness and alkalinity on the acute lethality of zinc to rainbow trout (*Salmo gairdneri*). Can J Fish Aquat Sci 42: 731-736.

30. Judy Jr RD, Davies PH (1979) Effects of calcium addition as $Ca(NO_3)_2$ on zinc toxicity to fathead minnows, *Pimephales promelas*, Rafinesque. Bull Environ Contam Toxicol 22: 088-094.

31. Van Genderen E, Gensemer R, Smith C, Santore R, Ryan A (2007) Evaluation of the Biotic Ligand Model relative to other site-specific criteria derivation methods for copper in surface waters with elevated hardness. Aquat Toxicol 84: 279–291.

32. Villavicencio G, Urrestarazu P, Carvajal C, De Schamphelaere KAC, Janssen, CR, Torres JC, Rodriguez PH (2005) Biotic ligand model prediction of copper toxicity to Daphnids in a range of natural waters in Chile. Environ Toxicol Chem 24: 1287–1299.

33. Gül A, Yilmaz M, Isilak Z (2009) Acute Toxicity of Zinc Sulphate ($ZnSO_4.H_2O$) to Guppies (*Poecilia reticulata* P., 1859) GU J Sci 22: 59-65.

34. Svecevičius G (1999) Acute toxicity of zinc to common freshwater fishes of Lithuania. Acta Zoologica Lituanica. Hydrobiologia 9: 114-118.

35. Ebrahimpour M Alipour H Rakhshah S (2010) Influence of water hardness on acute toxicity of copper and zinc on fish. Toxicol Ind Health doi: 10.1177/0748233710369123 .