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Potential of the Biotic Ligand Model (BLM) to Predict Copper Toxicity in the White-Water of the Solimões-Amazon River

Giorgi Dal Pont[1,2] · Fabíola Xochilt Valdez Domingos[2] · Marisa Fernandes-de-Castilho[1] · Adalberto Luis Val[2]

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Abstract In this study, we evaluated the capacity of the Biotic Ligand Model (BLM) to predict copper toxicity in white-waters of the Solimões-Amazon River. LC_{50} tests using the species *Otocinclus vittatus* (Regan, 1904) were performed with Solimões-Amazon river water (100%) at 20%, 40%, 60%, and 80% dilutions. A sevenfold decrease in both dissolved and total Cu toxicity was observed in the experiment conducted with 100% when compared to 20% white-water, indicating that physicochemical characteristics of white-water attenuate Cu toxicity. There was agreement between the observed LC_{50} and the LC_{50} predicted by the BLM after the adjustment of critical accumulation concentration (LA_{50}) for *O. vittatus*. BLM modeling indicated that dissolved organic carbon (DOC) and pH were the most important water parameters influencing Cu toxicity, followed by Ca^{2+}. Our results highlight the first evidence that the BLM presents potential to predict Cu toxicity to aquatic organisms in the white-water of the Solimões-Amazon River.

Keywords Bioavailability · Metal toxicity · Toxicity prediction · Tropical aquatic environment · Water chemistry

The Amazon basin has been impacted by several sources of aquatic contamination including mining, industrial activity and urban effluents dumping. In the industrial zone in the city of Manaus (Amazonas State, Brazil), for example, the level of Cu in black-water from Igarapé do Quarenta can exceed 1000 µg/L (Sampaio 2000). High concentrations of Cu in freshwater can be toxic to fish, because it disrupts Na^+ and Cl^- active uptake mechanisms in the fish gills, which results in net ion loss (Laurén and McDonald 1985).

Metal toxicity in fish is modulated by water characteristics (U.S.EPA 2007). Thereby, geochemical factors such as competition and complexation can interfere with the amount of free metal available and, consequently, with the toxicity of metallic cations (Playle 1998). Matsuo et al. (2005) showed that an elevated concentration of either Ca^{+2} or dissolved organic carbon (DOC) was effective in protecting *Colossoma macropomum* against acute short-term Cu and Cd gill accumulation. Using these ligand properties in the water, Paquin et al. (2000) proposed a model—the Biotic Ligand Model (BLM), to predict metal toxicity to aquatic organisms. For the BLM, the complexing agents and competing cations present in water are determined and used to estimate the interaction of a given metal with a biological ligand, such as fish gills. Because the BLM provides a way to predict a toxic effect of a metal concentration to aquatic organisms based on the local water chemistry, this model is currently recommended by the U.S. Environmental Protect Agency (U.S.EPA) as the basis for setting criteria to predict Cu toxicity in fresh waters of the USA (U.S.EPA 2007).

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✉ Giorgi Dal Pont
giorgidalpont@gmail.com

[1] Laboratório de Estudos em Estresse Animal, Departamento de Fisiologia, Universidade Federal do Paraná, Curitiba, Parana ZIP 81531-970, Brazil

[2] Laboratório de Ecofisiologia e Evolução Molecular, Instituto Nacional de Pesquisas da Amazônia (INPA), Manaus, Amazonas ZIP 69060-001, Brazil

The BLM is parameterized to fit results of toxicity tests conducted with north-temperate species in water chemistries characteristic of the north-temperate climatic zone, which are different from those found in the Amazon. Besides that, the Amazon basin presents different types of water that reflect regional physicochemical, biological and geological characteristics—“clear”, “black” and “white” waters are known as the three main types (Sioli 1984). The white-water, as the Solimões-Amazon River, carries a high content of silt, that gives a “coffee-milk” color appearance, presents pH close to neutral and higher amounts of dissolved salts as Ca^{2+} and Mg^{2+} compared to the black and clear ones (Furch and Junk 1997). Despite these differences, all of the Amazon waters are considered as soft waters in comparison with the world average, due low concentrations of hardness cations (Sioli 1984). Such characteristics suggest that fish thriving in these environments are more susceptible to the toxic effects of metals, due to low alkalinity that thus decreases complexation of metals and due to low concentrations of major inorganic cations that compete with metals for binding to fish gills.

In this study, we evaluated the suitability of the BLM to predict Cu toxicity in the white-water of the Solimões-Amazon River using the ornamental fish species *O. vittatus*. We hypothesize that DOC and pH are the major parameters defining Cu toxicity, despite the higher levels of accompanying cations in the white-water from Solimões River.

Materials and Methods

The fish *O. vittatus* (Siluriformes; Loricariidae) is a native species that successfully thrives in white-water from the Solimões-Amazon River. Despite this biological adaptation, in ornamental trader establishments the species is frequently held in groundwater where they are able to adjust to the different chemical water characteristics. Thus, adult specimens (n = 500; Wt = 0.269 ± 0.041 g; Lt = 3.0 ± 0.4 cm) kept in groundwater for at least 2 weeks were obtained from a local facility. In the laboratory they were held for 2 weeks in aerated tanks (150 L) supplied with flow-through groundwater and fed once a day with commercial dry food. Feeding was stopped 48 h before starting and during experiments in order to reduce nonspecific Cu binding to food and waste products.

Laboratorial groundwater used in acclimation and experimental procedures is characterized by [Na^+], 2.01 mg/L, [K^+], 0.62 mg/L, [Ca^{2+}], 0.54 mg/L, [Mg^{2+}], 0.10 mg/L, [Cl^-], 1.31 mg/L; [SO_4^{2-}], 0.40 mg/L; [DOC], 0.9 mg/L; [Cu], 2.0 µg/L; alkalinity = 3.27 mg $CaCO_3$/L and pH = 6.92. Samples of the white-water were collected from the Solimões-Amazon River near Manaus city once a week, during 5 consecutive weeks, in the high-water season on July/August of 2009.

Experiment procedure consisted in acclimating all the 500 fishes to 20% white-water dilution (20% white-water + 80% groundwater), in which they were maintained for 1 week when a sample of 100 fishes was used for the 20% Cu LC_{50}-96 h determination. One extra acclimation week was added to each dilution step, to a maximum of 5 weeks in fish at 100% white-water experiment. No mortality was observed during this period. For each treatment (20%, 40%, 60%, 80% and 100% of white-water), 100 fishes were accommodated in 10 plastic aquaria (n = 10 at 0.8 g of fish/L) under flow-through system (0.2 L h) and monitored for 48 h as control of the Cu exposure condition. No mortality was registered. Then, 10 Cu concentrations (added as $CuCl_2 \cdot 2H_2O$) ranging from 3.85 to 1088.00 µg Cu/L were added and fish mortality was recorded for 96 h. In order to equilibrate Cu with the ligands in the white-water, this solution was prepared 48 h before use (Ma et al. 1999).

Water temperature (26.98 ± 0.78 °C) and oxygen concentrations (7.75 ± 0.25 mg/L) were monitored daily (YSI® model 85) and water samples were collected for determination of Ca^{2+}, K^+, Na^+, Mg^{2+}, Cl^-, SO_4^{2-}, DOC, pH, alkalinity, hardness, total and dissolved Cu. Due to methodological restrictions, only one sample of each treatment was assigned to determination of DOC and SO_4^{2-}.

Concentrations of K^+, Na^+, Ca^{2+}, Mg^{2+} and Cu were determined by atomic absorption spectrophotometry (AA). The detection limits and the operation specifications of the AA instrument are available at the supplementary electronic material. Chloride concentrations were colorimetrically determined at 480 nm (Zall et al. 1956). Alkalinity and hardness were determined by titration (APHA 2000–2320B and 2340C). DOC (Shimadzu® – TOC-VCPH) and SO_4^{2-} were determined according to the APHA 2000 (5310B) and (4500C) methodologies.

Data of Ca^{2+}, K^+, Na^+, Mg^{2+} and Cl^- were log-transformed prior to analysis to achieve normality. Water chemistry data were compared by one-way analysis of variance or Mann–Whitney analysis (pH) followed by Tukey and Dunn’s post-hoc tests. The significance level adopted was 95%.

Total and dissolved copper LC_{50}-96 h were calculated by using the Trimmed Spearman–Karber method (Hamilton et al. 1977). The BLM software 2.2.3 was used to predict Cu LC_{50}-96 h to *O. vittatus* using the fathead minnow model. Measured water physicochemical values were used as model input parameters for all experimental series. The content of humic acids was set at 20% (Ertel et al. 1986). Our data were evaluated in the BLM using the original set up for Fathead minnow and also an adjusted set up to *O. vittatus*. The adjustment was achieved using the speciation mode to calculate the mean critical accumulation concentration (LA_{50}) along the five treatments to *O. vittatus*. The obtained mean LA_{50} value (25.75 nmol/g dw) was inserted into the fathead minnow DAT file (Cu_Fathead_Minnow_06-10-07.DAT). Then we were able to evaluate the BLM predicted LC_{50} specific for *O. vittatus*, using the toxicity mode.

The BLM predicted and experimentally measured LC_{50}-96 h values were graphically plotted to estimate the ability of the BLM to predict dissolved Cu toxicity. Pearson's correlation was applied to water chemistry parameters and LC_{50}-96 h data of experimental dissolved Cu. We also evaluated the influence of individual water chemistry parameters on dissolved Cu toxicity. Data from the 100% group was used as the main data source and then each parameter (e.g. DOC, Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}, Cl^{-}, SO_4^{2-}) was changed, one at a time, according to the range observed in the five experiments performed. For example, data from all parameters measured in the 100% experiment were combined with the range of Ca^{2+} from all the other groups (1.45–4.62 mg/L); then the LC_{50} was estimated and the coefficient of variation (CV) was calculated for all treatments.

All experimental procedures were conducted in accordance with Brazilian animal care regulations and approved by Instituto Nacional de Pesquisas da Amazônia (INPA) ethics committee.

Results and Discussion

Water was collected from the river over a 5-week period in order to complete the experimental series. As expected, it resulted in slight fluctuations in the water chemical properties among experiments (Table 1). However, a clear trend of decreasing concentrations of most parameters (Na^{+} was an exception) as well as decreasing pH was evident from 100% to 20% treatment, according to the relative proportions of white-water/groundwater.

Observed toxicity of dissolved and total Cu increased as the white-water physicochemical parameters concentrations decreased through the experimental dilutions. Dissolved Cu toxicity to *O. vittatus* was seven times lower ($LC_{50} = 572.11$ µg Cu/L) in the trial with 100% of white-water compared to the 20% group ($LC_{50} = 78.69$ µg Cu/L) (Fig. 1). Total Cu toxicity presented the same pattern as dissolved Cu resulting in a $LC_{50} = 697.60$ µg

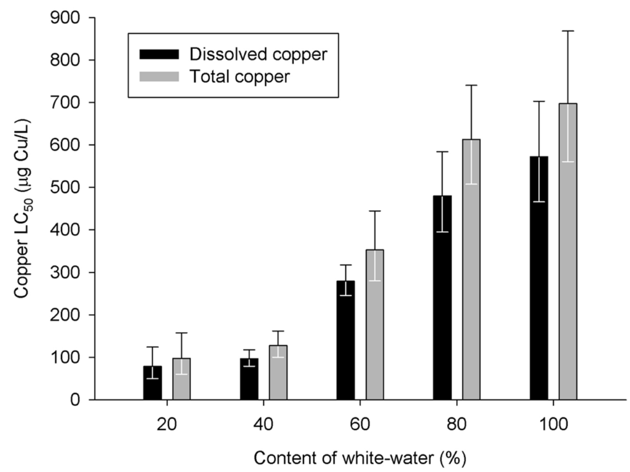

Fig. 1 Dissolved and total copper toxicity (LC_{50}-96 h) to *Otocinclus vittatus* in the white-water from Solimões-Amazon River (100%) and its dilutions (20, 40, 60 and 80%). *Bars* represent the mean observed LC_{50} values and *whiskers* represent LC_{50} *upper* and *lower* values

Table 1 Chemical parameters of the white-water from Solimões-Amazon River (100%) and experimental treatments (20, 40, 60 and 80%)

	Content of white-water from Solimões-Amazon River				
	100%	80%	60%	40%	20%
DOC (mgC/L)	3.00	2.43	1.82	1.40	1.24
Sulfate (mg/L)	2.09	1.67	1.25	0.83	0.42
pH	7.13 (7.12/7.21)^{a}	7.11 (7.09/7.18)^{ab}	6.97 (6.70/6.99) ^{abc}	6.75 (6.62/6.93)^{bc}	6.48 (6.43/6.60)^{c}
Chloride (mg/L)	4.87 ± 0.66^{a}	4.81 ± 0.51^{a}	4.36 ± 0.91^{ab}	2.77 ± 0.50^{ab}	2.56 ± 0.50^{b}
Sodium (mg/L)	2.64 ± 0.04	2.50 ± 0.01	2.58 ± 0.01	2.56 ± 0.04	2.49 ± 0.03
Calcium (mg/L)	4.62 ± 0.06^{a}	4.1 ± 0.08^{ab}	3.15 ± 0.03^{bc}	2.48 ± 0.03^{cd}	1.45 ± 0.02^{d}
Potassium (mg/L)	1.15 ± 0.04^{a}	0.93 ± 0.04^{b}	0.79 ± 0.02^{c}	0.66 ± 0.02^{d}	0.64 ± 0.03^{d}
Magnesium (mg/L)	0.66 ± 0.002^{a}	0.58 ± 0.004^{ab}	0.45 ± 0.004^{bc}	0.32 ± 0.003^{cd}	0.19 ± 0.0^{d}
Hardness ($mgCaCO_3$/L)	39.90 ± 0.53^{a}	26.48 ± 0.64^{b}	21.58 ± 0.12^{c}	14.09 ± 0.29^{d}	8.15 ± 0.39^{e}
Alkalinity ($mgCaCO_3$/L)	23.43 ± 0.50^{a}	20.00 ± 0.51^{b}	15.42 ± 0.3^{c}	10.31 ± 0.39^{d}	4.68 ± 0.59^{e}

The five treatments are not exact dilutions of the 100% because batches of river water were collected in 5 distinct weeks. Chloride, sodium, calcium, potassium, magnesium, hardness and alkalinity are expressed as mean ± standard error of the mean (SEM) and pH data are expressed as median (min/max). Due to methodological restrictions only one sample of each treatment was assigned to determination of dissolved organic carbon (DOC) and sulfate. Lowercase letters represent differences among treatments ($p < 0.05$)

Cu/L at 100% and a $LC_{50} = 97.91$ µg Cu/L at 20%, but with higher values corresponding to 120% of the dissolved copper LC_{50}. Ten ornamental fish species of the Rio Negro previously analyzed had lower Cu LC_{50}-96 h values in experiments performed with the same source of groundwater (Duarte et al. 2009). One of the analyzed species (*O. hasemani*), from the same genus as *O. vittatus*, presented a dissolved Cu toxicity value thirty-one times lower (LC_{50}-96 h = 18.01 µg Cu/L) than the values observed here for 100% white-water. The results obtained here for *O. vittatus* suggest that the chemical composition of white-water also induced a protective effect towards Cu toxicity when compared to groundwater. This protective effect could be related to different Cu speciation patterns under the experimental conditions.

Most water chemical parameters, such as pH, Cl^-, Mg^{2+}, K^+, SO_4^{2-}, alkalinity, hardness, Ca^{2+} and DOC presented positive and significant correlation with dissolved Cu LC_{50}-96h (Table 2). Sodium was the only exception. De Schamphelaere and Janssen (2001) analyzing the individual effect of Ca^{2+}, Mg^{2+}, Na^+, K^+ and pH on Cu toxicity to *Daphnia magna*, observed a linear correlation between calculated EC_{50}-48 h and Na^+ but not between EC_{50}-48 h and K^+, wherein K^+ was shown to be a non-interfering parameter on acute Cu toxicity. However, our results showed a high significant correlation between K^+ and dissolved Cu LC_{50}-96 h. These results suggest that correlation alone does not prove causation, nor provide any indications as to which are the more important factors affecting Cu toxicity in white-water to *O. vittatus*. For this reason, we performed a modeling exercise with the BLM, where each factor was varied independently against a background of the 100% water for all other measured parameters.

DOC contributed to 37% of the observed variation (Fig. 2) representing by far the most important parameter influencing Cu toxicity to *O. vittatus* among the parameters evaluated in this study. We observed that even low DOC values (1.24–3.00 mg C/L) can modulate Cu toxicity in the soft white-water from the Amazon-Solimões River. DOC is known to bind to metals, decrease their bioavailability and consequently attenuate their toxic effects (Di Toro et al. 2001), but is important to consider that DOC sources are heterogeneous and the degree of protection towards metals relies on the type of DOC. Darker, aromatic-rich compounds of allochthonous origin, which present higher humic acid content, are more effective towards binding free metal ions, and reducing the toxicities of Cu, Ag, and Pb in freshwater environments (Wood et al. 2011). The DOC of Solimões-Amazon River represents 65% of the total organic carbon content (Aucour et al. 2003) and it is comprised by a mixture of allochthonous and autochthonous material (Mortillaro et al. 2012). Then it probably presents hybrid protective characteristics against metal toxicity. DOC can also interact with the gills of aquatic organisms, affecting osmoregulation processes such as Na^+ transport, diffusive permeability and electrical properties of fish gills, which can result in enhanced Na^+ homeostasis and attenuation of metal toxicity (Wood et al. 2011). Therefore, the influence of DOC towards Cu toxicity observed here is probably a combination of its positive effects on fish gills plus the complexation of free metal ions in the water column.

Our results also showed that pH presents an important contribution to Cu toxicity (Fig. 2) and are consistent with other findings regarding its effects on chronic toxicity of Cu (De Schamphelaere and Janssen 2001, 2004). Shi et al. (1998) showed that Cu adsorption to total suspended solids (TSS, 200 mg/L) is influenced by pH. A high concentration of TSS is characteristic of the white-waters of the Amazon (30–230 mg/L) (Moreira-Turcq et al. 2003). Thus,

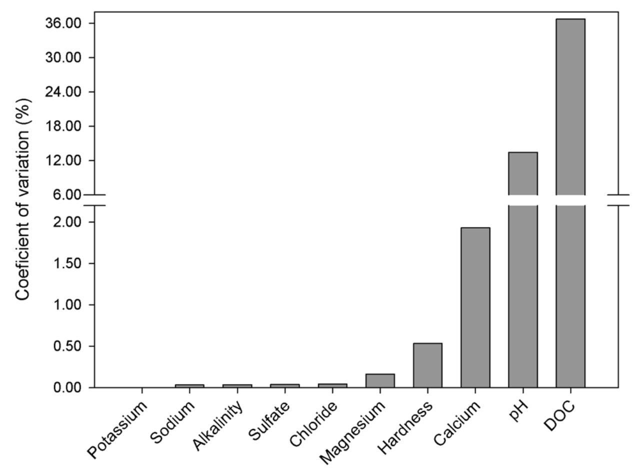

Fig. 2 Results of a BLM modeling exercise to determine the relative importance of various water quality parameters in contributing to the observed variation in dissolved copper LC_{50} as captured by the coefficient of variation (CV) across the range of each water quality parameter

Table 2 Pearson's correlation (R), p value (*p*) and coefficient of determination (R^2) between dissolved copper LC_{50}-96 h (to *Otocinclus vittatus*) and water chemical characteristics of Solimões-Amazon River

	Solimões-Amazon River water parameters									
	DOC	SO_4^-	Mg^{+2}	Ca^{+2}	K^+	Alkalinity	Hardness	Cl^-	pH	Na^+
R	0.988	0.975	0.968	0.964	0.956	0.965	0.956	0.946	0.934	0.369
p	0.001	0.004	0.006	0.007	0.010	0.007	0.010	0.014	0.020	0.540
R^2	0.976	0.951	0.938	0.931	0.914	0.932	0.914	0.896	0.872	0.136

considering the natural presence of TSS in white-water and the range of pH from these waters (5.0–7.0) (Santos and Ribeiro 1988), we suggest their interactions and possible effects on Cu toxicity should be studied. In fact, our results showed that the treatments that probably contained higher amounts of TSS (80% and 100%), based on qualitative visual observation, were also the most protective against Cu toxicity (Fig. 1).

Calcium contribution to Cu toxicity was 1.9%, while the remaining parameters contributed less than 1% (Fig. 2). Water hardness usually associated with Ca^{2+} or Mg^{2+} can modulate Cu toxicity in the aquatic environment by competing with metal ions to bind to the biotic ligand sites on fish gills, resulting in attenuation of metal effects (Playle 1998). However, the individual influence of Mg^{2+} remains unclear (Perschbacher and Wurts 1999). These authors demonstrated that only Ca^{2+} promoted effective protection against Cu toxicity when comparing the influence of individual hardness ions (Ca^{2+} and Mg^{2+}) on $CuSO_4$ toxicity in the catfish *Ictalurus punctatus*. Welsh et al. (2000) also demonstrated that Ca^{2+} concentration is more relevant than Mg^{2+} on Cu toxicity in waters with constant hardness in two salmonid species (*O. mykiss* and *O. tshawytscha*). In fact, calcium has been reported as the main ion capable of competing with Cu for binding to fish gills (Laurén and McDonald 1985; Paquin et al. 2000; Playle 1998).

Copper is known to act directly on fish osmoregulation processes by affecting gill permeability as well as sodium and potassium homeostasis (Matsuo et al. 2005; McGeer et al. 2000). Na^+ concentration is considered an important factor in the maintenance of homeostasis in situations of osmotic misbalance (Wood et al. 2011). However Na^+ concentration was quite similar in all experimental treatments (Table 1) and yielded a negligible CV (Fig. 2), indicating that here Na^+ concentration was a negligible factor regarding Cu toxicity on the different treatments performed with Solimões-Amazon River water.

Despite the strong correlation observed between K^+ and dissolved Cu toxicity, this cation presented the lowest CV indicating that K^+'s contribution to attenuate Cu toxicity to *O. vittatus* was inconsiderable. Erickson et al. (1996) demonstrated that the increments of individual K^+ concentration in soft water from Lake Superior actually increased metal toxicity to larval fathead minnows. In fact, K^+ capacity to increase Cu toxicity in the white-water treatments may have been countered by DOC and pH protection role; which in fact, as hypothesized, was demonstrated to be more efficient to defining Cu toxicity in white-water from the Amazon-Solimões River.

The levels of natural trace metals as Cr, Mn, Co, Ni, Zn, Mo, Cd, Ba, U (Seyler and Boaventura 2003) and Hg (Maurice-Bourgoin et al. 2003) were reported in white-water of the Solimões-Amazon River. All reported concentrations are under the maximum allowed by the current Brazilian (CONAMA 2005) and USA (U.S.EPA 2016) legislation. We assume that the water used in the experiments could contain other metals as reported above, but there was no evidence of deleterious effects at least on the mortality level, as our control fish presented 100% survival during the control period (48 h).

After the adjustment of LA_{50} to *O. vittatus*, the BLM was able to predict Cu toxicity in the white-water, within a factor of plus or minus 2, suggesting that the model presents potential to be used as a tool to predict Cu toxicity for Solimões-Amazonas River fish (Fig. 3). On the other hand the prediction results obtained using the BLM's LA_{50} for fathead minnow were all outside the prediction limits (factor of ±2) (Fig. 3). Then we suggest recalibrating the BLM when using different species. The validation of models such as the BLM can be a valuable tool to predict Cu toxicity to fish in ion-poor waters. Field assays performed in different types of water including white, black and a mixture of white and black waters, have suggested that the BLM is suitable to predict Cu toxicity in Amazon waters (Bevilacqua 2009). Our results, obtained under laboratory conditions, indicate that the BLM has the potential to be used to predict Cu toxicity in white-waters from the Amazon.

The findings presented here with *O. vittatus* represent the first attempt to understand Cu toxicity in ion-poor white-waters from the Amazon under laboratory conditions. We identified that DOC and pH appeared to be major factors modulating Cu toxicity in *O. vittatus*

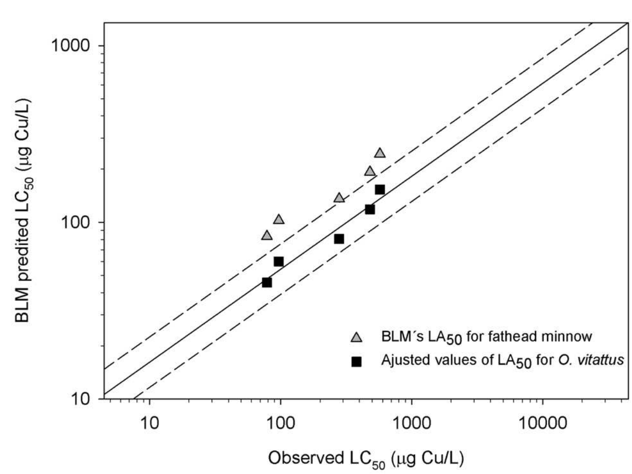

Fig. 3 Relationship between BLM predicted and experimentally measured dissolved copper LC_{50}-96 h to the ornamental fish *Otocinclus vittatus*. *Solid line* represents ideal fit between observed and predicted data (x = y). The *dashed lines* indicate an error within a factor of two between observed and predicted LC_{50} values. *Symbols* after adjusting the BLM LA_{50} for *O. vittatus* (*filled square*) and using BLM LA_{50} for fathead minnow (*open triangle*). Note that after the LA_{50} adjustment all data fitted the prediction interval

exposed to Cu in Solimões-Amazon River water. The influence of TSS on copper toxicity remains to be investigated. We also suggest the use of other fish species native from Solimões-Amazon River to evaluate the prediction capability of the BLM.

Our results highlight the first evidence that the BLM can be considered a promising tool for the prediction of Cu toxicity for regulatory purposes to Amazon aquatic environments. Further, these results suggest that the BLM, or similar tools to predict the bioavailability of Cu and other trace metals to aquatic organisms, may be applicable, or readily adaptable, to similar ion-poor waters that differ from the mid-latitude temperate waters originally used to develop and validate the models.

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