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Nickel toxicity to cardinal tetra (*Paracheirodon axelrodi*) differs seasonally and among the black, white and clear river waters of the Amazon basin

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ABSTRACT

This study investigated the acute toxicity of nickel (Ni) to cardinal tetra (*Paracheirodon axelrodi*), within the three main water types of the Amazon basin: black (Rio Negro), white (Rio Solimões) and clear (Rio Tapajós) during the wet and dry season at pH 7 (representative of white and clear rivers) and pH 4 (representative of black waters). The influence of dissolved organic carbon (DOC) quality on Ni toxicity within the three waters was also explored via the use of DOC isolates. Differences in water chemistry, DOC quality and ion concentrations were shown between waters and between seasons. Toxicity of Ni was shown to vary between river waters, seasons, and pHs. Ni was significantly less toxic during the dry season at pH 4 in all three river waters; for example, black water during the wet season had an LC_{50} of 9.72 mg Ni/L compared to 41.5 mg Ni/L during the dry season. At pH 7, contrasting effects in toxicity between seasons were shown between black and clear waters (black: wet = 28.9 mg/L, dry = 17.3 mg/L; clear: wet = 13.8 mg/L, dry = 24.1 mg/L). There were no significant differences in Ni toxicity for white waters at pH 7 (white: wet = 22.2 mg/L, dry = 21.8). Overall, Ni was shown to be more toxic at pH 7 than at pH 4 except in black water during the wet season. Toxicity of Ni at pH 4 was negatively related to DOC concentration and amount of humic-like and fulvic-like DOC and positively related to fluorescence index. Therefore, at pH 4, Ni is more toxic in waters containing more allochthonous DOC, consisting of higher amounts of humic-like and fulvic-like components. LC_{50} values for the different DOC concentrates at the same DOC concentration of 4.5 mg/L (black: 26.8 mg/L; white: 73.3 mg/L; clear: 49.2) support the river water findings at pH 4 (Ni more toxic in presence of black DOC) indicating that DOC quality alone can influence Ni toxicity at this pH.

1. Introduction

Metal contamination of freshwaters worldwide is increasing as urbanization, mining and climate change impacts increase (Rockström et al., 2014). Nickel (Ni) is one such metal of concern with increased contamination of freshwaters occurring globally (Pyle and Couture, 2012). Ni constitutes approximately 2% of the earth's crust and reserves vary around the globe with Brazil containing the seventh highest reserves (Barrera, 2016). As anthropogenic processes such as mining encroach on many areas in Brazil, especially in the Brazilian Amazon, the relative risk that metals, such as Ni, pose to aquatic environments must be taken into consideration.

Metal bioavailability and toxicity to biota in aquatic systems are dependent on speciation of the metal in solution, uptake and binding to the target receptor, and subsequent depuration/detoxification processes. Speciation and bioavailability in turn depend on water quality characteristics, particularly pH, hardness, alkalinity,

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temperature and concentration and quality of dissolved organic carbon (DOC). In nature, the quality and quantity of DOC differs between waterways and within them both spatially and seasonally (Fellman et al., 2010; Johannsson et al., 2016). It has been widely shown that increased concentrations of DOC can significantly decrease toxicity of metals such as Ni (Hoang et al., 2004). Recent evidence also suggests that organic matter quality can lead to differences in toxicity of metals at fixed DOC concentrations (Al-Reasi et al. 2011, 2012; Wood et al., 2011), with most studies showing a protective effect of allochthonous aromatic optically dark DOC compared with autochthonous, optically light DOC.

The Amazon Basin in Brazil is characterised by three main water types: black, white and clear, which differ in physico-chemical properties such as pH, ionic composition, total suspended solids and DOC (Furch, 1984; Val and Almeida-Val, 1995). The black Rio Negro is acidic, low in ions and suspended solids, and contains high amounts of DOC leached from the surrounding vegetation, which stain the water a dark brown colour. White water rivers such as the Rio Solimões on the other hand are close to circumneutral pH and contain lower concentrations of DOC and higher amounts of ions and suspended solids which give the rivers a cloudy appearance. Clear water rivers are generally transparent, contain low amounts of ions and suspended solids, and vary in pH from acidic to circumneutral with DOC concentrations as low as ≤ 1 mg/L (Furch, 1984; Val and Almeida-Val, 1995). Marked seasonal differences in water quality between the wet and dry season have also been reported within each water type as a result of altered precipitation and flooding (Furch, 1984; Val and Almeida-Val, 1995).

Little information, however, is currently available on the toxicity of metals such as Ni within the different waters of the Amazon basin and whether such physico-chemical differences among rivers and between seasons (wet and dry) lead to significant differences in toxicity. It has been reported that Ni toxicity is influenced by pH, hardness, DOC concentrations and total suspended solids (TSS) (Hoang et al., 2004; Pyle and Couture, 2012; Pyle et al., 2002), all of which differ among the different waters and seasons of the Amazon basin (Sioli, 1984). It is therefore predicted that Ni toxicity will vary greatly among the three natural water types, as well as with season.

Cardinal Tetra (*Paracheirodon axelrodi*) are important ecologically and economically to the Amazon basin (de Oliveira et al., 2008; Crémazy et al., 2016). Cardinal are endemic to Brazil and the Amazon region and are the most common fish species in the Rio Negro (de Oliveira et al., 2008). Cardinal are also the most exported ornamental fish from the Amazon region, representing 80% of fish exported (de Oliveira et al., 2008; Crémazy et al., 2016). Its small size makes it easy to use in laboratory trials and it has been previously used in studies with metals such as copper (Duarte et al., 2009; Crémazy et al., 2016).

This study therefore aimed to investigate the acute toxicity of nickel to cardinal tetra (*P. axelrodi*), as assessed by classic 96 h LC_{50} tests, within the black (Rio Negro), white (Rio Solimões) and clear (Rio Tapajós) river waters of the Amazon Basin during the wet and dry season as well as a groundwater (Instituto Nacional de Pesquisas da Amazônia, INPA well water) low in ions and DOC which served as a reference water. Tests were performed at both pH 7.0 and pH 4.0 in all water types to capture the range of pHs seen in the natural waters (black water as low as pH 4, white water and clear water as high as pH 7). Investigations into the influence of DOC quality in determining toxicity were also conducted using the different DOC isolates from these rivers obtained using reverse osmosis (RO). Water chemistry in each of the test conditions was thoroughly characterized, with a particular emphasis on DOC quantity and quality, the latter assessed by a range of optical parameters. Our goal was to link any differences in toxicity with key water quality parameters and/or differences in DOC quality.

2. Materials and methods

2.1. Water chemistry

Water was collected from the black (S03 05.780, W60 21.352), white (S03 15.335, W60 14.861) and clear water rivers (S2 23 50.6869, W54 47 51.3569), both during the wet season (May–July 2015) and the dry season (October to November 2015) for use in the experimental trials. INPA well water was also collected and used in the dry season. Samples for determination of ionic composition and metal suites were collected from each river water before the start of experiments. Ionic compositions (Na^+, Ca^{2+}, Mg^{2+}, K^+) of waters ($n = 3$) were analysed using flame atomic absorption spectroscopy (Perkin-Elmer model 3100: Perkin-Elmer Inc, USA). Cl^- was measured using the colorimetric method described by Clarke (1950) ($n = 3$). Hardness was calculated from the Ca^{2+} and Mg^{2+} concentrations and alkalinity was measured using the method APHA Standard Methods for Examination of Water and Wastewater method 2320 (1992). Analyses for a suite of metals (Al, Cu, Pb, Fe and Ni) were conducted on the waters (black, white, and clear) collected during the wet season via inductively coupled plasma mass spectrometry (ICP-MS) (Agilent 7700x: Agilent Technologies, US), while graphite furnace atomic absorption spectroscopy (GFAAS) (Perkin-Elmer model 3100: Perkin-Elmer Inc. USA) was used for dry season samples ($n = 3$). To ensure results were comparable between instruments certified standards were used to develop calibration curves for both machines and the same quality control procedures conducted. This included metal analysis of method blanks, laboratory duplicates, and matrix spikes using certified standards. Percent recoveries ranged from 98 to 110%. Turbidity was determined using the EPA method 180.1: Determination of turbidity by Nephelometry and TSS was determined using Gravimetric analysis as outlined in the EPA Method 160.2.

DOC was analysed at the beginning of each trial using a total carbon analyzer (Apollo 9000 combustion TOC analyzer: ©Teledyne Tekmar, Mason, USA) ($n = 3$). The TOC machine was calibrated as per manufacturer's instructions, using primary standard grade potassium hydrogen phthalate (KHP). QA/QC KHP standards were ran every 20–30 samples.

DOC quality was measured using a range of different absorbance and fluorescence measurements. River water samples and concentrates were adjusted to approximately pH 7 using 0.1 M NaOH. All absorbance and fluorescence scans were performed using a 1 cm quartz cuvette in a SpectraMax® M2e (VWR, Pennsylvania) spectrophotometer and replicated three times. SAC_{340} (2.303 × absorbance at 340 nm/DOC) was used to give an indication of aromaticity (Curtis and Schindler, 1997), fluorescence Index (FI) provided an indicator of DOC source (McKnight et al., 2001), while molecular weight was indicated by the absorbance ratio $Abs_{254/365}$ (Dahlén et al., 1996). $SUVA_{254}$ served as a measure of the UV-absorbing moieties (abs_{254}/DOC). Fluorescence excitation emission scans (FEEM) were conducted on all samples at emission wavelengths of 250–600 nm using steps of 10 nm excitation wavelengths between 250 and 450 nm. The excitation emission matrixes were then analyzed in MATLAB R2014b (MathWorks, Inc.© 1994–2016) to produce three-dimensional FEEMs and contour plots. Rayleigh scatter was removed and replaced with "not a number" values (NaN). The FEEMs were then modelled using parallel factor analysis (PARAFAC) based on the *a priori* assumption of 4 components (PLS-toolbox in MATLAB: Eigenvectors Research Inc, WA, USA)) (Al-Reasi et al., 2012; Johannsson et al., 2016). Tyrosine and tryptophan spectra were also included within the PARAFAC analysis to weigh the resolved components towards the *a priori* assumption that the DOC included only humic, fulvic and proteinaceous fluorophores (i.e, tyrosine and tryptophan-like fluorophores, Al-Reasi et al., 2011; De Palma et al., 2011).

2.2. Toxicity trials

Cardinal tetras (*Paracheirodon axelrodi*) that had been collected from black water in the wild were purchased from a supplier in Manaus, and ranged in size from 1.5 to 2.5 cm and weighed between 0.1 and 0.2 g. Fish were acclimated in 300-L tanks containing INPA well water for at least one month before use in experimental trials. Fish were fed TetraMin tropical fish flakes daily until satiation up until 48 h before the beginning of the experiment. Ni concentration within the food provided to the fish before exposure was not determined. All experimental work was approved by the Ethics Committee on Animal Experiments of INPA under registration number 026/2015, and conformed to national animal care regulations.

96 h static renewal acute toxicity tests were conducted in 2-L plastic containers, with each container, containing 1 L of test solution and ten fish. All containers and lids were previously acid washed using 10% nitric acid before the test. Eighty percent of the treatment water was renewed every 24 h. Each treatment in each trial consisted of two replicates. Fish were exposed to five Ni ($NiCl_2.6H_2O$: Sigma Aldrich) concentrations ranging from 7.3 mg/L-240 mg/L Ni plus the control (treatment water with no added Ni) at pH 4 and pH 7. Ni concentrations were chosen based on preliminary tests to ensure at least one concentration recorded minimum 90% survival and 100% mortality after 96 h pH was adjusted when needed using 1 M KOH or HNO_3. Measured concentrations of dissolved and total Ni recorded during each trial are provided in Supplementary Table 1. A natural light and temperature regime was used for the wet season experiments which represented a 12 h light: 12 h dark photoperiod and average temperature within test chambers of 27 °C, with conditions altered in the dry season experiments via the use of a chiller to maintain exposure temperature within test chambers to average of 27 °C to mimic the same conditions experienced during the wet season, with a natural light regime also employed.

Containers were continuously supplied with aeration. Lids were placed on top of the test chambers to prevent contamination and the escape of test subjects. Fish were monitored every 24 h for signs of morbidity or mortality. Fish displaying signs of persistent loss of equilibrium were shown in preliminary trials to die within 24 h and thus to reduce suffering of the fish, fish were determined to be morbid if displaying such signs, and were euthanized. All tests followed the recommendations in the OECD guidelines for the testing of chemicals: test no. 203 Fish Acute Toxicity Test and the recommendations listed in the draft revised version (Rufli, 2014). Survival of fish in controls for all treatments well water, black, white and clear waters with no added Ni was ≥90%. Fish were not fed during the trials. Water quality parameters: pH, temperature, conductivity, and oxygen (TPS WP-80, 81 & 82 m: TPS Pty Ltd. Australia), were measured every 24 h. Samples for determination of dissolved (filtered using Millipore PVDF 0.45 μm Sartorius filters: Sartorius, Germany) and total Ni concentration were collected before and after water change, every 24 h and analysed using GFAAS (Perkin-Elmer model 3100: Perkin-Elmer Inc. USA).

To try to determine mechanisms behind any differences in toxicity between the waters additional dilution and DOC concentrate experiments were conducted. To determine the effects of ions and/or DOC concentration on Ni toxicity at pH 7 and 4 in black and white river water, dilution experiments were conducted. Black and white river waters were diluted by 50% with INPA well water during the dry season. INPA well water contains a similar ionic composition to the black water with little to no DOC (Table 1), making it the perfect diluent to explore the role that DOC concentration and ions play in determining metal toxicity within the black and white waters. Fish were exposed to the same Ni concentrations, experimental set-up and conditions as outlined above. Trials were conducted at the same time as the river water dry season experiments. To determine the influence of DOC quality alone on the river water toxicity results, DOC concentrates were isolated from the black, white and clear river water using reverse osmosis (Vontron®ULP21-4021 polyamide membrane, Permution, model PEOS-0001, Curitiba, Brazil) following the recommendations of Serkiz and Perdue (1990). DOC concentrates were resinated to remove metals and cations using Amberlite IR-118 hydrogen form. DOC concentrates (black, white and clear) were added to INPA well water (which is virtually DOC-free, <0.04 mg/L DOC) to give a DOC concentration of 4.5 ± 0.1 mg/L. INPA well water was used as a control. Fish were exposed to the same Ni concentrations, experimental set-up and conditions as outlined for the river water trials. Trials were conducted during September 2015.

2.3. Statistical analysis

Dose-response curves (log-logistic) using the average dissolved Ni values for each Ni treatment were conducted on morbidity data to determine the concentration which is lethal to 50% (LC_{50}) of the fish using the 'drc' package in R (Ritz et al., 2015). 95% confidence limits were determined using the delta method. Statistical calculations to determine differences between LC_{50} values were conducted as outlined by Litchfield and Wilcoxon (1949). Linear regressions were used to determine and visualise significant relationships between water quality/chemistry and/or DOC quality

Table 1
Ionic composition, dissolved organic carbon (DOC) and total dissolved metal concentrations in the different river waters, as measured at the beginning of each trial. Values are the averages of three readings. Units are mg/L except turbidity, which is in NTU.

River waters	Season/experiment	DOC	Na	Ca	Mg	K	Cl	Al	Fe	Cu	Pb	Alkalinity ($CaCO_3$)	Hardness ($CaCO_3$)	Turbidity (NTU)	Total Suspended Solids
Black	Wet	8.75	1.33	0.16	0.11	0.34	0.74	0.167	0.167	0.002	<0.001	12.4	1	0.52	0.8
	Dry	5.84	0.78	0.17	0.08	0.61	0.5	0.158	0.062	<0.001	<0.001	7.7	1	0.13	0.5
	50%	3.16	0.93	0.12	0.04	0.71	0.52	0.078	0.031	<0.001	<0.001	5.5	0	0.26	<0.1
	Concentrate	4.43	1.49	0.08	0.004	0.58	2.18	<0.0025	<0.0025	<0.001	<0.001	12.1	0	0.26	<0.1
White	Wet	3.86	3.97	1.31	0.69	1.55	2.71	0.034	0.255	0.004	<0.001	40.7	6	3.38	5.9
	Dry	2.5	6.32	0.72	0.74	1.71	5.04	0.033	0.254	0.004	<0.001	33.0	5	3.12	3.4
	50%	0.87	3.43	0.38	0.38	1.1	3.62	0.016	0.127	0.001	<0.001	25.9	3	1.82	3.1
	Concentrate	4.61	1.7	0.07	0.004	0.72	4.19	<0.0025	<0.0025	<0.001	<0.001	17.6	0	0.26	<0.1
Clear	Wet	1.08	0.5	<0.01	0.24	1.49	0.34	0.061	0.151	0.002	<0.001	14.3	0	0.26	0.4
	Dry	0.41	0.67	0.13	0.26	0.98	1.51	0.06	0.149	0.016	<0.001	19.3	1	0.52	0.7
	Concentrate	4.42	0.79	0.08	0.006	1.54	1.15	<0.0025	<0.0025	<0.001	<0.001	15.1	0	0.26	<0.1
Well water	Dry	<0.4	0.55	0.04	0.007	0.73	1.85	<0.0025	<0.0025	<0.001	<0.001	8.3	0	0.26	<0.1
	Concentrate	<0.4	1.5	0.09	0.005	0.73	1.26	<0.0025	<0.0025	<0.001	<0.001	20.4	0	0.26	<0.1

Table 2
Indices which indicate characteristics of river water DOC. The results presented are the average of three measurements (n = 3).

River water	Season/experiment	SAC_{340} (cm^2 mg^{-1})[a]	FI[b]	$SUVA_{254}$[c]	$Abs_{254/365}$[d]	% Tyrosine-like[e]	% Tryptophan-like[e]	% Fulvic-like[e]	% Humic-like[e]
Black water	Dry	66.65	1.39	7.35	3.43	1.88	1.83	36.32	59.97
	Wet	43.69	1.41	4.98	3.63	1.07	0.52	34.16	64.25
	Concentrate	44.52	1.41	5.56	4.11	4.20	4.75	38.49	52.55
White water	Dry	64.95	1.54	7.32	3.45	2.48	3.18	48.45	45.89
	Wet	33.41	1.52	4.53	4.55	1.66	1.20	45.60	51.54
	Concentrate	34.29	1.55	4.90	4.69	4.33	6.53	47.08	42.07
Clear water	Dry	30.15	1.57	1.40	4.00	10.3	13.2	41.40	35.11
	Wet	57.58	1.51	7.31	3.95	6.36	7.98	45.70	39.97
	Concentrate	23.80	1.49	3.25	4.58	3.39	21.6	52.37	22.60
Well water	Dry	6.91	1.61	0.90	7.00	31.3	35.9	22.31	10.43

[a] $SAC_{340,}$ which is (cm^2 mg^{-1}) = (2.303 × absorbance at 340 nm)/DOC, gives an indication of aromaticity of DOC.
[b] Fluorescence Index (FI), which is emission intensity 450/emission intensity 500, at excitation 370 nm, gives an indication of DOC source (allochthonous or autochthonous).
[c] $SUVA_{254,}$ which is Abs_{254}/DOC, provides an estimate of the UV-absorbing moieties.
[d] $Abs_{254/365}$ gives an indication of molecular weight of DOC molecules.
[e] % relative abundance of each DOC component determined via PARAFAC analysis.

variables and LC_{50} values using SigmaPlot 13.

3. Results

3.1. Water quality and chemistry

The three river types were characterised by different water quality/chemistry (Table 1) and quality of DOC (Table 2). The white water contains higher concentrations of major ions and suspended solids (TSS) compared to the other two waters (black and clear) (Table 1). Similar ionic compositions (extremely low levels of all major ions) were observed between the black and clear river waters and INPA well water (Table 1). The black water contains the highest amounts of DOC and Al followed by the white water, than the clear and well water which both contained little to no DOC (≤1 mg/L) (Table 1). Differences in water chemistry between wet and dry seasons were also shown with slight changes in ion concentrations and decreases in DOC and Al between the wet and dry seasons (Table 1). Diluting the black water by 50% with well water led to nearly a 50% decrease in DOC concentrations (Table 1); however, since the well water has similar ionic composition to the black water, ion concentrations remained relatively the same (Table 1). Dilution of the white water also led to a decrease in DOC concentration, but also close to a 50% decrease in ion concentrations (Table 1).

3.2. DOC quality

Four fluorescent DOC components were determined by PARAFAC and accounted for 97.4% of the variability (Fig. 1). These include a humic-like (component 1), representative of high molecular weight, aromatic humic acid commonly associated with wetlands, flooded forests, and forested environments; fulvic-like (component 2), low molecular weight fulvic acid; and tryptophan-like and tyrosine-like (components 3 and 4 respectively), representative of protein compounds, free or bound amino acids (Fig. 1 and Table 2). The four components were determined based on descriptions provided in Fellman et al. (2010), Al-Reasi et al. (2012) and Johannsson et al. (2016).

DOC quality, differed among the river waters and INPA well water (Table 2). The DOC within the black water is allochthonous in nature (FI: 1.4). Aromatic (high SAC_{340} values: 44–67 cm^2 mg^{-1}), and of a higher molecular weight indicated by the lower $Abs_{254/365}$: 3.4–3.6, and is dominated by humic-like components (making up ≥ 60% of the DOC) (Table 2). The DOC within the white and clear waters on the other hand is mostly allochthonous in nature but with higher inputs of autochthonous DOC (FI: 1.5 and higher amounts of fulvic-like, tyrosine and tryptophan components). This DOC is less aromatic than the black water DOC (lower SAC_{340}: Rio Solimões: 33–65 cm^2 mg^{-1}; Rio Tapajós: 30–58 cm^2 mg^{-1}), and is likely to be of similar molecular weights ($Abs_{254/365}$ ratios: Rio

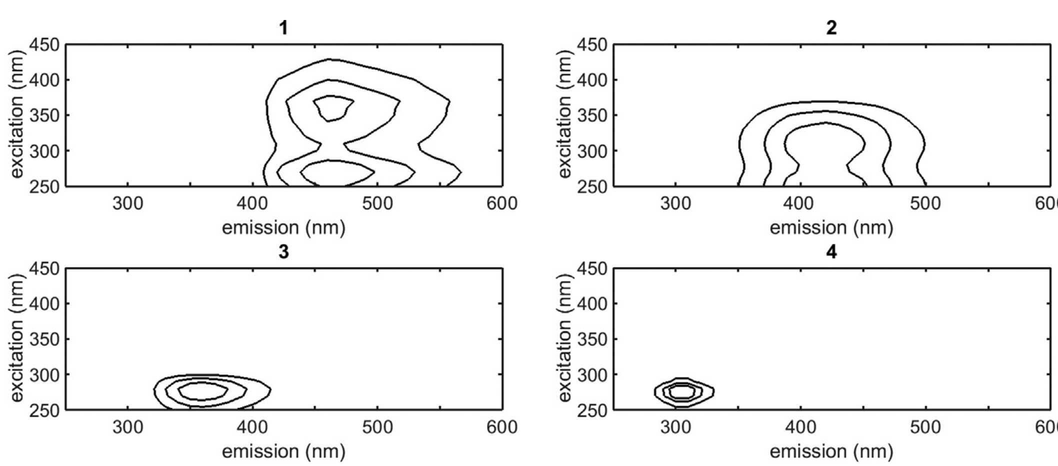

Fig. 1. The four components of dissolved organic carbon (DOC) determined by PARAFAC analysis. Component 1 = humic-like, component 2 = fulvic-like, component 3 = tryptophan-like and component 4 = tyrosine-like. These four components explained 97.4% of the variability in the excitation emission scans.

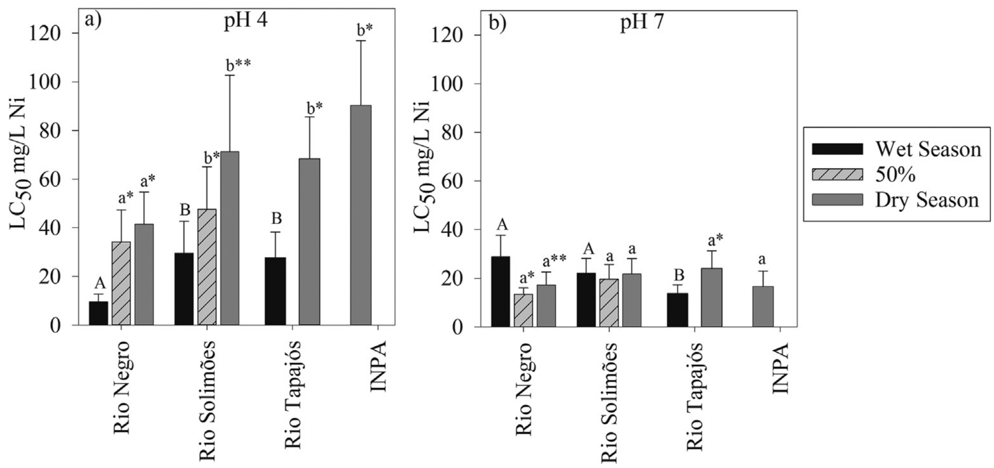

Fig. 2. 96 h LC_{50} values for nickel in mg/L within each of the three different river waters of the Amazon Basin: Rio Negro (black), Rio Solimões (white), Rio Tapajós (clear) at a) pH 4 and b) pH 7. Error bars represent their associated 95% confidence limits. Upper case (ABC) indicate significant differences between river water during wet season. Lower case (abc) indicate significant differences between waters in the dry season. *indicates significant difference in same water between seasons and ** indicates significant difference between the 50% and 100% dilutions.

Solimões: 3.5–4.6; Rio Tapajós: 4.0) (Table 2). INPA well water DOC is autochthonous in nature (FI: 1.6), consisting of mostly protein-like (tyrosine and tryptophan) DOC of low aromaticity (SAC_{340}: 6.91 cm^2 mg^{-1}), and low molecular weight (high $Abs_{254/365}$ ratio: 7.00) (Table 2). Difference in DOC quality was also shown among seasons. Specifically, higher SAC_{340}, $SUVA_{254}$, and $Abs_{254/365}$ values were recorded during the dry season for the black and white waters and lower values were recorded in the clear compared with those recorded in the wet season (Table 2).

Differences in DOC characteristics were also shown among concentrates, with SAC_{340} values highest in the black (45 cm^2 mg^{-1}) and lowest in the white (24 cm^2 mg^{-1}), FI and $Abs_{254/365}$ lowest in the black (FI: 1.41 and $Abs_{254/365}$: 4.11) and highest in the white (FI: 1.55 and $Abs_{254/365}$: 4.69) (Table 2). Dilution of the concentrates with well water led to increases in protein-like DOC (higher amounts of tryptophan and tyrosine-like DOC) and fulvic-like DOC and decreases in humic-like components compared with the river water from which the concentrate was isolated from (wet season black and white water and dry season clear) (Table 2). However, the ratio of the components remained similar with humic-like components still dominating within the black water DOC and fulvic-like components within the white and clear.

3.3. Toxicity of nickel

3.3.1. River waters

Negligible differences were shown between the total and dissolved Ni in treatment waters and among tests (Supplementary Table 1). Negligible differences were shown across trials in regards to water quality measured every 24 h (Supplementary Tables 2 and 3). pH in the pH 4 treatments varied from 3.9 to 4.4 within all trials and from 6.9 to 7.4 in the pH 7 treatment. Dissolved oxygen (% sat) varied from 50 to 80% and temperature ranged from 25 to 30 °C for all experiments. Conductivity varied between waters and between Nickel treatments. Toxicity of Ni within the waters of the Amazon basin varied depending on the pH, season, and river water. This is explored in the following sections.

3.3.1.1. Between pHs (4 and 7). Ni was significantly less toxic at pH 4 than at pH 7, as indicated by higher LC_{50} values, with the single exception of the black water during the wet season (9.72 mg/L Ni). LC_{50} values in waters from the other sites at pH 4.0 ranged between 27.83 (clear, wet) to 90.33 mg/L (well water) (Fig. 2a), while at pH 7 they ranged from 13.45 (50% black water) to 28.87 mg/L (black water, wet) (Fig. 2b).

3.3.1.2. Between wet and dry seasons. Significant differences were shown between the toxicity of Ni in black and clear water between the wet and the dry season at both pH's (Fig. 2a and b). However, no significant difference was shown between the seasons with regard to Ni toxicity within the white water at pH 7 (Fig. 2b). Ni at pH 4 was consistently more toxic in all river waters collected during the wet compared with the dry season (Fig. 2a). At pH 7 Ni was more toxic in black water collected during the dry and in clear water during the wet season (Fig. 2b).

3.3.1.3. Among waters. Significant differences in Ni toxicity among waters were also recorded at pH 4. Ni was significantly more toxic in black water (LC_{50} values: 9.71 mg/L Ni (wet season) and 41.46 (dry season)), compared with the other two waters during both the wet and dry seasons and well water (Fig. 2a). Toxicity of Ni was lowest in the well water (LC_{50}: 90.33 mg/L Ni) (Fig. 2a). Ni toxicity at pH 4 was significantly related to DOC concentration ($p = 0.02$; $r^2 = -0.31$) and DOC quality (FI ($p = 0.01$; $r^2 = 0.34$), amount of humic-like ($p = 0.01$; $r^2 = -0.30$) and fulvic-like DOC (mg/L C) ($p = 0.02$; $r = -0.31$)) (Fig. 3).

At pH 7 during the wet season the clear water recorded a significantly lower LC_{50} value in comparison to the black and white waters. The black water exhibited the highest LC_{50} value, followed by the white water and the clear (Fig. 2). In contrast the opposite occurred during the dry season with Ni at pH 7 in clear water less toxic than in the white water and most toxic in the black water; however, no significant difference in LC_{50} values was determined (Fig. 2).

3.3.1.4. Within the same water. Dilution of white water with well water significantly increased toxicity of Ni (LC_{50} 100%: 71.3 mg/L and 50%: 47.6 mg/L) at pH 4 (Fig. 2a), however had little effect at pH 7 (Fig. 2b). Within the black water on the other hand no significant difference was shown between the 100% and 50% dilutions at pH 4 (Fig. 2a). At pH 7, however, dilution by 50% led to a significant increase in toxicity from 17.29 mg/L Ni to 13.45 mg/L (Fig. 2b).

3.3.2. DOC concentrates

Significant differences in Ni toxicity were shown in the presence of different DOC concentrates at pH 4 (Fig. 4), with toxicity greatest

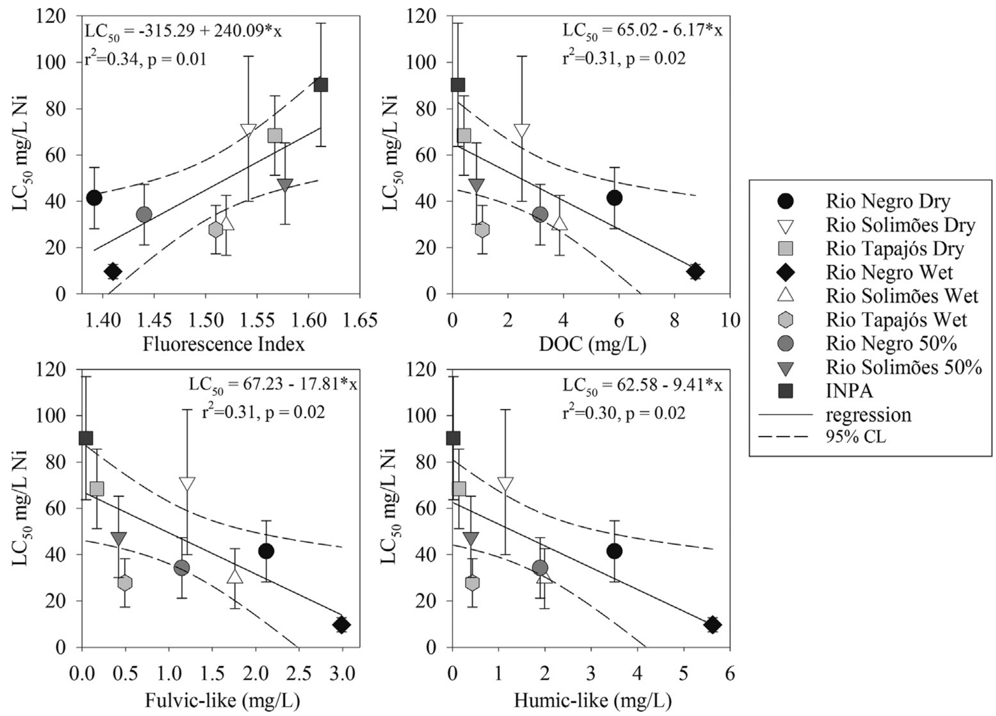

Fig. 3. Regression plots showing the significant relationships between LC_{50} values and Fluorescence Index, DOC concentration and the amount of humic and fulvic like DOC (mg/L C) as estimated using PARAFAC.

in presence of black water DOC. Ni was significantly less toxic in presence of well water (no DOC) than in the presence of black and clear water concentrates, indicating that the presence of these two DOC increases toxicity at pH 4. No significant differences among DOC concentrates were shown at pH 7; however, toxicity of Ni was significantly reduced in the presence of all three concentrates compared to well water which contained no added DOC (Fig. 3).

4. Discussion

4.1. Water quality and chemistry

Measured water chemistry within each of the three river waters was similar to previous published work (Furch, 1984; Val and Almeida-Val, 1995). The white water in comparison to the other two river waters (black and clear) contained approximately between 2 and 13 times greater ion concentrations and suspended solids. The black water on the other hand was characterised by the highest DOC concentrations during both the wet and dry season (approximately 2.3 (white) – 15 times (clear)). The black water also contained DOC of a more terrestrially derived nature (FI: 1.4) compared to the other waters (FI: 1.5–1.6). The black water DOC was also characterized predominately by humic-like components (60–64%) compared with the other two river waters which were dominated by fulvic-like components (white: 47–48% and clear: 41–46%), and well water which was dominated by protein-like DOC (66%).

SAC_{340} values for the black water (wet: 44 cm^2 mg^{-1} and dry season: 67 cm^2 mg^{-1}), white (dry season: 65 cm^2 mg^{-1}) and clear (wet season: 58 cm^2 mg^{-1}) were within readings previously reported for the black water(44–73 cm^2 mg^{-1}) (Duarte et al., 2016; Johannsson et al., 2016) and higher than values reported in the literature for waters from other sites such as Luther Marsh, a peat bog (39 cm^2 mg^{-1}). This indicates they were characterised by highly aromatic DOC (Richards et al., 2001; Al-Reasi et al., 2011; 2012). FI scores for the three rivers and well water (FI: 1.4–1.6) also fall among those reported from rivers in the US receiving terrestrial inputs of DOC and Antarctic lakes containing only autochthonous DOC (McKnight et al., 2001). $Abs_{254/365}$ values for the white (wet), clear and well waters (3.9–7), which serve as an indicator of molecular weight, were within ranges (3.7–15.7) reported previously for other freshwater DOC (Al-Reasi et al., 2012). Black and white waters during the dry season recorded slightly lower $Abs_{254/365}$ values (3.4–3.6) than previously reported indicating the DOC is likely to be of a higher molecular weight (Al-Reasi et al., 2012; Johannsson et al., 2016).

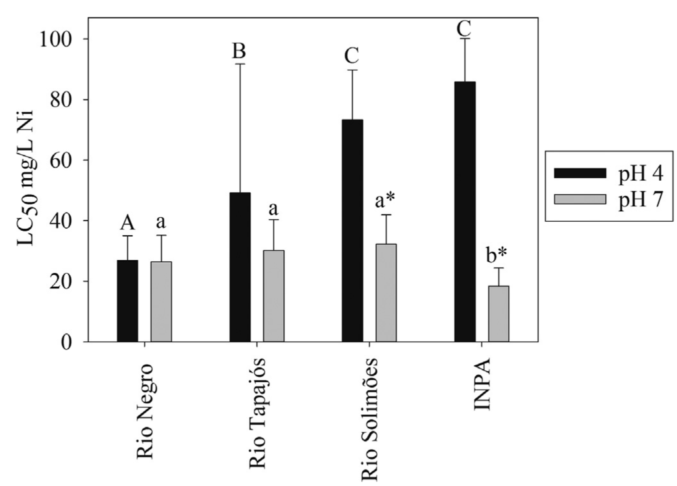

Fig. 4. 96 h LC_{50} values for nickel in mg/L in the presence of three DOC concentrates and INPA well water (control). Error bars represent their associated 95% confidence limits. Upper case (ABC) indicate significant differences between different treatments at pH 4. Lower case (abc) indicate significant differences between treatments at pH 7. *indicates significant difference in same treatment between pHs.

Differences in ionic composition, DOC concentration and quality were shown among seasons. Increased levels of DOC during the wet season compared with those recorded during the dry season are likely to reflect increased flushing of the surrounding soils and forest inputs (Sousa et al., 2011) and have also been reported from other rivers within the Amazon basin and the Congo River basin (Sousa et al., 2011; Bouillon et al., 2014). Differences in DOC quality were also shown among the different seasons with higher SAC_{340}, $SUVA_{254}$, $Abs_{254/365}$ values recorded during the dry season for the black and white waters and lower values in the clear. This result is unexpected as it was assumed that higher SAC_{340}, $SUVA_{254}$, $Abs_{254/365}$ values associated with more aromatic and higher molecular weight DOC would be associated with the wet season due to flooding of the forests, however the opposite appears true for the black and white waters. Teixeira et al. (2011) also recorded higher $SUVA_{254}$ values for DOC from lakes along the Upper Paraná River floodplain during the dry season compared with the wet and suggested this may be due to increased primary production in the wet season as indicated by higher chlorophyll-a levels. Our results indicate that DOC quality differs between the wet and dry seasons within the Amazon basin and this must be considered when determining the quality of DOC in this region.

4.2. Nickel toxicity

LC_{50} values for Ni at pH 7 and pH 4 range between (13–29 mg/L) and (28–90 mg/L), respectively. Adult cardinal tetra used in this study appear to be tolerant to Ni at pH 7, when compared to other fish species exposed to Ni in soft waters and at a similar pH. 96 h LC_{50} value of 4 mg/L for adult fathead minnows (Birge and Black, 1980) and 0.5 mg/L for <24 h old larvae (Pyle et al., 2002), 10 mg/L for adult goldfish (Birge and Black, 1980) 8–9 mg/L for Arctic graylings, 17–18 mg/L for Coho salmon, 8–25 mg/L for alevin and juvenile rainbow trout, respectively (Buhl and Hamilton, 1991) have been previously reported in the literature. Currently no data exists for Ni toxicity to fish at a pH as low as 4, making it hard to determine whether or not cardinal are also a tolerant species at this pH. Ni is considerably less toxic (approximately 1000 times) to cardinal tetra compared with Cu (LC_{50} value of 46 and 56 μg/L) in well water used in this study, approximately 10–40 times less toxic in black water (LC_{50} value of 1090 μg/L) and approximately 100–400 times less toxic in white water (LC_{50} value of 194 μg/L) (Duarte et al., 2009; Crémazy et al., 2016).

Differences in water chemistry, DOC concentration and quality among rivers and seasons significantly influence toxicity of Ni within the Amazon basin, with toxicity of Ni shown to differ among the three river waters, between seasons and between pHs. Ni was shown to be consistently more toxic to cardinal tetras at pH 7 than at pH 4, except in Rio Negro water during the wet season (Fig. 2). This is not the first study to show that Ni toxicity decreases as pH decreases (Schubauer-Berigan et al., 1993; Pyle et al., 2002; Deleebeeck et al., 2007), nevertheless, it appears to be the first study to investigate its toxicity at a pH as low as 4, which is commonly associated with naturally acidic and anthropogenically acidified environments. Decreased toxicity of Ni at lower pH (in most waters) is likely to be due to competition between the Ni^{2+} ions and the H^+ for binding sites. It has also been suggested that changes in Ni speciation, notably, increases in $NiOH^+$ which is suggested to be more abundant at pH 7 than at pH 4.0, may also be bioavailable, thus leading to the increased toxicity shown at pH 7 (Pyle and Couture, 2012). However, metal speciation modelling using the Windermere Humic Aqueous Model (WHAM7) showed no significant difference in metal speciation in the four waters in this study at pH 4 and pH 7, indicating that this is unlikely. Therefore further research is needed to determine the direct mechanism of increased protection at lower pH and why black water collected during the wet season at pH 4 appears to be particularly toxic.

Differences in toxicity of Ni between seasons and among waters at pH 4 are likely to reflect differences in DOC concentrations and quality (FI, amount of fulvic-like and humic-like DOC) as indicated by the significant negative (DOC concentration and amount of fulvic and humic-like DOC) and positive relationships with FI (Fig. 3). LC_{50} values within the black, white and clear waters between the wet and dry seasons differed by a factor of 4.1, 2.4 and 2.4 respectively, and by a factor of approximately 3 between the three river waters during the wet season and approximately by factor of 2 during the dry season. All waters contained higher DOC concentrations and higher amounts of humic-like and fulvic-like DOC during the wet than the dry season (Table 2), with Ni toxicity shown to be highest during the wet season.

Ni was also shown to be most toxic within the black river water which contains higher amounts of DOC of a more allochthonous nature (lower FI), with high amounts of humic-like and fulvic-like components suggesting Ni is more toxic in river waters containing, allochthonous DOC, high in humic-like and fulvic-like components. This is also supported by the significant differences in Ni toxicity shown between the different DOC concentrates at pH 4, with Ni most toxic in presence of black water DOC which is allochthonous in nature, high in humic and fulvic-like components. Blewett et al. (2016) also showed that water collected from the mouth of a brown water stream containing DOC high in humic-like components was protective to sea urchin larvae at low concentrations of DOC but exacerbated Ni toxicity at high concentrations of DOC. The results at pH 4 dispute previous findings for other metals, such as Cu, which suggest a protective role of allochthonous humic-like DOC; however, studies showing a protective effect of allochthonous humic-like DOC tend to be conducted at pH 7 ± 1 (Al-Reasi et al. 2011, 2012; Richards et al., 2001; Ryan et al., 2004; Schwartz et al., 2004). Results for this study showed no effect of DOC quality at circumneutral pH, differing from results shown at pH 4 and from the previous studies involving Cu (Al-Reasi et al. 2011, 2012; Richards et al., 2001; Ryan et al., 2004; Schwartz et al., 2004). The decreased protection at pH 4 may reflect the effect of pH on the binding of such humic substances to cell membranes and their effects on cell permeability. Vigneault et al. (2000) showed that humic and fulvic acids (as indicated by humic and fulvic-like components in this study) bound more strongly to cell membranes at pH 5 compared to pH 7 and increased cell permeability. Alternatively, the binding of Ni to proteinaceous autochthonous carbon might be stronger than its binding to humic substances at pH 4. It is well established that Ni binds to proteins, this is in fact the basis of immobilized metal affinity chromatography in biochemical protein purification methods (Wong et al., 1991). In the Irving-Williams series of cation binding Ni binds more strongly to nitrogen containing moieties (i.e., amino groups) than carboxylic and phenolic groups typical of humic substances (Nieboer and Richardson, 1980). No matter the explanation, the observation is important as it appears contrary to expectations, i.e. Ni will be more toxic in black waters high in allochthonous, humic–like DOC at a realistic pH for these unique ecosystems. Season must also be taken into consideration as Ni toxicity may differ by greater than a factor of 4 within such waters during the wet compared with the dry season.

Different water chemistry parameters appear to affect the toxicity of Ni within each water type differently. Within the black water at pH 7, dilution of this water by 50% led to a significant increase in Ni toxicity. This appears to be linked with the associated decrease in DOC concentration. This along with the significant difference between the well water (no DOC) and the three DOC concentrates at pH 7 supports previous studies which have shown that toxicity of Ni decreases with increases in DOC at circumneutral pH (Doig and Liber, 2006; Cloran et al., 2010; Custer et al., 2016). This is the first study to show contrasting effects between pH 4 and pH 7 in regards to the effect of DOC concentration on metal toxicity. This double edged sword of DOC, where at high pH's, DOC is protective and at low pH's DOC increases toxicity has also previously been shown for acidity alone, where increased DOC concentrations protected fish against respiratory stress at pH's above 4 and caused increased mortality at pH's of 4 and below (Holland et al., 2014).

In contrast to the black water, no significant difference was shown between the 100 and 50% dilutions within the white water at pH 7. However, at pH 4 dilution of the white water caused a significant increase in toxicity of Ni. The reduction in ions is likely to be responsible for the increased Ni toxicity within the 50% dilution water compared with the 100% white water. It is well established that increases in the amount of ions decreases toxicity of metals including Ni (Hall and Anderson, 1995; Hoang et al., 2004; Meyer et al., 1999). This is not surprising since ions such as Na^{+}, Ca^{2+}, and Mg^{2+} will compete with Ni for binding sites on the target animal (in this case tetras), leading to decreased binding of Ni and lower toxicity (Blewett and Wood, 2015; Hoang et al., 2004). Therefore, increases in this ion may also limit toxicity of Ni.

The Ni concentrations reflected in this study does not reflect environmentally relevant concentrations within the Amazon basin, however reflect concentrations previously reported in mine wastewaters from Brazil (Wildemand, 2004). Further research investigating the chronic toxicity of Ni to fish within the different waters of the Amazon Basin, at environmental relevant concentrations of Ni is needed to improve our understanding of Ni toxicity within this region. Although the direct physiological and/or chemical mechanisms behind why Ni toxicity differs between pHs, seasons and between waters of the Amazon Basin could not directly be determined from these experiments, results suggest that DOC concentrations and its quality along with ion concentrations may play a role.

5. Conclusion

- Toxicity and accumulation of Ni within the different waters of the Amazon basin was shown to vary depending on river water, season, pH, DOC and ion concentrations and DOC quality.
- At pH 4, contrasts in Ni toxicity among rivers and between seasons were linked with changes in DOC concentration and quality.
- These results have important implications for metal risk assessment and development of appropriate water quality guidelines, as results from bioassays in water collected during one season (dry or wet) may not be representative of that waterway during the other season. Results may also not be representative of other waterways within that region.
- Seasonal and spatial variability in DOC concentration and quality thus must be taken into consideration when analysing the toxicity and bioavailability of metals in freshwaters especially in regions were both circumneutral and naturally acidic waters such as the black waters within the Amazon basin coincide.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.watres.2017.06.044.

References

Al-Reasi, H.A., Wood, C.M., Smith, D.S., 2011. Physicochemical and spectroscopic properties of natural organic matter (NOM) from various sources and implications for ameliorative effects on metal toxicity to aquatic biota. Aquat. Toxicol. 103 (3–4), 179–190.

Al-Reasi, H.A., Smith, D.S., Wood, C.M., 2012. Evaluating the ameliorative effect of natural dissolved organic matter (DOM) quality on copper toxicity to *Daphnia magna*: improving the BLM. Ecotoxicology 21 (2), 524–537.

American Public Health Association, 1992. APHA Method 2320: Standard Methods of the Examination of Water and Wastewater. American Public Health Association, Washington, USA.

Barrera, P., 2016. 10 Top Nickel-producing Countries. Nickel. Investing News Network. http://investingnews.com/daily/resource-investing/base-metals-investing/nickel-investing/10-top-nickel-producing-countries.

Birge, W.J., Black, J.A., 1980. Aquatic toxicology of nickel. In: Nriagu, J.O. (Ed.), Nickel in the Environment. John Wiley & Sons, New York, pp. 349–366.

Blewett, T.A., Wood, C.M., 2015. Salinity-dependent nickel accumulation and oxidative stress responses in the euryhaline killifish (*Fundulus heteroclitus*). Arch. Environ. Contam. Toxicol. 68 (2), 382–394.

Blewett, T.A., Smith, D.S., Wood, C.M., Glover, C.N., 2016. Mechanisms of nickel toxicity in the highly sensitive embryos of the Sea Urchin *Evechinus chloroticus*, and the modifying effects of natural organic matter. Environ. Sci. Technol. 50 (3), 1595–1603.

Bouillon, S., Yambélé, A., Gillikin, D.P., Teodoru, C., Darchambeau, F., Lambert, T., Borges, A.V., 2014. Contrasting biogeochemical characteristics of the Oubangui River and tributaries (Congo River basin). Sci. Rep. 4, 5402.

Buhl, K.J., Hamilton, S.J., 1991. Relative sensitivity of early life stages of Arctic grayling, Coho salmon, and Rainbow trout to nine inorganics. Ecotoxicol. Environ. Saf. 22, 184–197.

Clarke, F.E., 1950. Determination of chloride in water improved colorimetric and titrimetric methods. Anal. Chem. 22 (4), 553–555.

Cloran, C.E., Burton, G.A., Hammerschmidt, C.R., Taulbee, W.K., Custer, K.W., Bowman, K.L., 2010. Effects of suspended solids and dissolved organic carbon on nickel toxicity. Environ. Toxicol. Chem. 29 (8), 1781–1787.

Crémazy, A., Wood, C.M., Smith, D.S., Ferreira, M.S., Johannsson, O.E., Giacomin, M., Val, A.L., 2016. Investigating copper toxicity in the tropical fish cardinal tetra (*Paracheirodon axelrodi*) in natural Amazonian waters: measurements, modeling, and reality. Aquat. Toxicol. 180, 353–363.

Curtis, P.J., Schindler, D.W., 1997. Hydrologic control of dissolved organic matter in low-order Precambrian Shield lakes. Biogeochemistry 36 (1), 125–138.

Custer, K.W., Hammerschmidt, C.R., Burton Jr., G.A., 2016. Nickel toxicity to benthic organisms: the role of dissolved organic carbon, suspended solids, and route of exposure. Environ. Pollut. 208 (Part B), 309–317.

Dahlén, J., Bertilsson, S., Pettersson, C., 1996. Effects of UV-A irradiation on dissolved organic matter in humic surface waters. Environ. Int. 22 (5), 501–506.

de Oliveira, S.R., Baptista de Souza, R., Nunes, E., de Carvalho, C., de Menezes, G., Marcon, J., Roubach, R., Ono, E., Affonso, E., 2008. Tolerance to temperature, pH, ammonia and nitrite in cardinal tetra, *Paracheirodon axelrodi*, an amazonian ornamental fish. Acta Amaz. 38 (4), 773–779.

Deleebeeck, N., De Schamphelaere, K., Janssen, C., 2007. A bioavailability model predicting the toxicity of nickel to rainbow trout (*Oncorhynchus mykiss*) and fathead minnow (*Pimephales promelas*) in synthetic and natural waters. Ecotoxicol. Environ. Saf. 67, 1–13.

DePalma, S.G.S., Arnold, W.R., McGeer, J.C., Dixon, D.G., Smith, D.S., 2011. Variability in dissolved organic matter fluorescence and reduced sulfur concentration in coastal marine and estuarine environments. Appl. Geochem. 26 (3), 394–404.

Doig, L.E., Liber, K., 2006. Influence of dissolved organic matter on nickel bioavailability and toxicity to *Hyalella azteca* in water-only exposures. Aquat. Toxicol. 76 (3–4), 203–216.

Duarte, R.M., Menzes, A., Rodrigues, L., Almeida-Val, V., Val, A., 2009. Copper sensitivity of wild ornamental fish of the Amazon. Ecotoxicol. Environ. Saf. 72, 693–698.

Duarte, R.M., Smith, D.S., Val, A.L., Wood, C.M., 2016. Dissolved organic carbon from the upper Rio Negro protects zebrafish (*Danio rerio*) against ionoregulatory disturbances caused by low pH exposure. Sci. Rep. 6, 20377.

Fellman, J.B., Hood, E., Spencer, R.G.M., 2010. Fluorescence spectroscopy opens new windows into dissolved organic matter dynamics in freshwater ecosystems: a review. Limnol. Oceanogr. 55 (6), 2452–2462.

Furch, K., 1984. In: Sioli, H. (Ed.), The Amazon: Limnology and Landscape Ecology of a Mighty Tropical River and its Basin. Springer, Netherlands, Dordrecht,

pp. 167–199.
Hall, L.W., Anderson, R.D., 1995. The influence of salinity on the toxicity of various classes of chemicals to aquatic biota. Crit. Rev. Toxicol. 25 (4), 281–346.
Hoang, T.C., Tomasso, J.R., Klaine, S.J., 2004. Influence of water quality and age on nickel toxicity to fathead minnows (*Pimephales promelas*). Environ. Toxicol. Chem. 23 (1), 86–92.
Holland, A., Kinnear, S., Duivenvoorden, L., 2014. The double-edged sword of humic substances: contrasting their effect on respiratory stress in eastern rainbowfish exposed to low pH. Environ. Sci. Pollut. Res. 21, 1701–1707.
Johannsson, O.E., Smith, D.S., Sadauskas-Henrique, H., Cimprich, G., Wood, C.M., Val, A.L., 2016. Photo-oxidation processes, properties of DOC, reactive oxygen species (ROS), and their potential impacts on native biota and carbon cycling in the Rio Negro (Amazonia, Brazil). Hydrobiologia 1–23.
Litchfield, J.T., Wilcoxon, F., 1949. A simplified method of evaluating dose-effect experiments. J. Pharmacol. Exp. Ther. 96 (2), 99–113.
McKnight, D.M., Boyer, E.W., Westerhoff, P.K., Doran, P.T., Kulbe, T., Andersen, D.T., 2001. Spectrofluorometric characterization of dissolved organic matter for indication of precursor organic material and aromaticity. Limnol. Oceanogr. 46 (1), 38–48.
Meyer, J.S., Santore, R.C., Bobbitt, J.P., DeBrey, L.D., Boese, C.J., Paquin, P.R., Allen, H.E., Bergman, H.L., DiToro, D.M., 1999. Binding of nickel and copper to fish gills predicts toxicity when water hardness varies, but free-ion activity does not. Environ. Sci. Technol. 33 (6), 913–916.
Nieboer, E., Richardson, D.H.S., 1980. The replacement of the nondescript term 'heavy metals' by a biologically and chemically significant classification of metal ions. Environ. Pollut. Series. B 3–26.
OECD, 1992. Test No. 203: Fish, Acute Toxicity Test. OECD Publishing, Paris. http://dx.doi.org/10.1787/9789264069961-en.
Pyle, G., Couture, P., 2012. In: Wood, C., Farrell, A., Brauner, C. (Eds.), Homeostasis and Toxicology of Essential Metals. Academic Press, Elsevier Inc, London, UK.
Pyle, G.G., Swanson, S.M., Lehmkuhl, D.M., 2002. The influence of water hardness, pH, and suspended solids on nickel toxicity to larval fathead minnows (*Pimephales promelas*). Water Air Soil Pollut. 133 (1), 215–226.
Richards, J.G., Curtis, P.J., Burnison, B.K., Playle, R.C., 2001. Effects of natural organic matter source on reducing metal toxicity to rainbow trout (*Oncorhynchus mykiss*) and on metal binding to their gills. Environ. Toxicol. Chem. 20 (6), 1159–1166.
Ritz, C., Baty, F., Streibig, J.C., Gerhard, D., 2015. Dose-response analysis using R. PLoS One 10 (12), e0146021.
Rockström, J., Falkenmark, M., Allan, T., Folke, C., Gordon, L., Jägerskog, A., Kummu, M., Lannerstad, M., Meybeck, M., Molden, D., Postel, S., Savenije, H.H.G., Svedin, U., Turton, A., Varis, O., 2014. The unfolding water drama in the Anthropocene: towards a resilience-based perspective on water for global sustainability. Ecohydrology 7, 1249–1261.
Rufli, H., 2014. Draft Revised Version of OECD Guideline for Testing of Chemicals: Test 203 Fish Acute Toxicity Test. https://www.oecd.org/chemicalsafety/testing/Draft-Update-TG-203-for-commenting.
Ryan, A.C., Van Genderen, E.J., Tomasso, J.R., Klaine, S.J., 2004. Influence of natural organic matter source on copper toxicity to larval fathead minnows (*Pimephales promelas*): implications for the biotic ligand model. Environ. Toxicol. Chem. 23 (6), 1567–1574.
Schubauer-Berigan, M.K., Dierkes, J.R., Monson, P.D., Ankley, G.T., 1993. pH-Dependent toxicity of Cd, Cu, Ni, Pb and Zn to *Ceriodaphnia dubia, Pimephales promelas, Hyalella azteca* and *Lumbriculus variegatus*. Environ. Toxicol. Chem. 12 (7), 1261–1266.
Schwartz, M.L., Curtis, P.J., Playle, R.C., 2004. Influence of natural organic matter source on acute copper, lead, and cadmium toxicity to rainbow trout (*Oncorhynchus mykiss*). Environ. Toxicol. Chem. 23 (12), 2889–2899.
Serkiz, S.M., Perdue, E.M., 1990. Isolation of dissolved organic matter from the suwannee river using reverse osmosis. Water Res. 24 (7), 911–916.
Sioli, H., 1984. The Amazon: Limnology and Landscape Ecology of a Mighty Tropical River and its Basin. Springer, Netherlands.
Sousa, E.d.S., Salimon, C.I., Figueiredo, R.d.O., Krusche, A.V., 2011. Dissolved carbon in an urban area of a river in the Brazilian Amazon. Biogeochemistry 105, 159–170.
Teixeira, M.C., Azevedo, J.C.R.d., Pagioro, T.A., 2011. Spatial and seasonal distribution of chromophoric dissolved organic matter in the Upper Paraná River floodplain environments (Brazil). Acta Limnol. Bras. 23, 333–343.
Val, A.L., Almeida-Val, V.M.F., 1995. Fishes of the Amazon and their Environment. Springer, Berlin.
Vigneault, B., Percot, A., Lafleur, M., Camplbell, P., 2000. Permeability changes in model and phytoplankton membranes in the presence of aquatic humic substances. Environ. Sci. Technol. 34, 3907–3913.
Wong, J.W., Albright, R.L., Wang, N., 1991. Immobilized metal ion affinity chromatography (IMAC) chemistry and bioseparation applications. Sep. Purif. Rev. 20, 49–106.
Wood, C.M., Al-Reasi, H.A., Smith, D.S., 2011. The two faces of DOC. Aquat. Toxicol. 105 (3–4 Suppl. L.), 3–8.