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Metal Toxicity Can Affect Dragonfly Nymphs and Ostracods Predation Rates and Food Selectivity: Ecological Implications on Food Webs

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Abstract Predation is known to play a prominent role in maintaining ecosystem structure and functioning. Despite metals being known to potentially affect predation in aquatic ecosystems, few studies have been conducted, so far, with the aim of evaluating this interplay. In the present study, the effects of four metal salts (copper, cadmium, mercury, and manganese) on the feeding rates and food preference of the dragonfly *Tramea cophisa* and of the ostracod *Chlamydotheca* sp. were studied by performing laboratory ecotoxicity tests. Food preference was evaluated by offering four prey species to dragonfly nymphs and three to adult large ostracods. In general, the food preference of both predator species after being exposed to metal salts was not altered, compared with controls, but the feeding rate of *T. cophisa* decreased in comparison with controls, after exposure to each metal salt, except manganese. Contrastingly, predation rates of *Chlamydotheca* sp. increased after metal salt exposure. This difference in response can be explained by differences in life-history traits of these two organisms. Both species individuals preferred soft-bodied prey (Oligochaeta, Chironomidae) over water-dwelling crustaceans that are likely to be more difficult to prey upon. Tests evaluating the effects of metals and other chemicals on predation behavior may lead to a better understanding of biotic interactions that can be restricted by chemical stress, improving our understanding of possible food web disruptions underlying chemical stress.

Keywords Benthic invertebrates · Predation behavior · Metal toxicity · Food selection

1 Introduction

Prey capture is part of a predator foraging behavior and can influence the meta-community distribution,

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as well as prey behavior (Grainger et al., 2017). Predators conduct several determinant actions to assure prey capture success, such as finding, choosing, capturing, manipulating, and ingesting or rejecting prey (Kimbell & Morrell, 2015). Pollutants like metals may alter these actions, which may ultimately lead to reduced predation success rates and therefore diminished food acquisition (Walker et al., 2012).

Food intake reduction indeed appears to be a general response to contaminant exposure (McWilliam & Baird, 2002). For example, studies by Smith and Weis (1997) and Weis et al. (2003) have shown that metals like cadmium, mercury, and zinc may interfere with the ability of selection and the speed of prey capture by the fish *Fundulus heteroclitus*, resulting in a decrease in the amount of food ingested. Success in catching prey can be used as a sensitive behavioral biomarker to evaluate the sublethal effects of chemicals like metals on aquatic populations and indirectly on aquatic community structure (Weis et al., 2001). Crustaceans such as *Gammarus pulex* and *Hyallela azteca* have indeed been successfully used as bioindicators of metal and pesticide contamination in water and sediment through an evaluation of their feeding rates (Forrow & Maltby, 2000; Hatch & Burton, 1999).

Dragonfly and damselfly species (Odonata, Insecta) have recently been suggested as promising test species for toxicity testing (Miguel et al., 2017; Valente-Neto et al., 2016). Odonates play an important role in aquatic ecosystem dynamics (Costa et al., 2006) being relevant predators, among others, in freshwater food webs (Oliveira et al., 2013). Their diet may range from invertebrates (mainly other insects) to fish and amphibian larvae of suitable size for their ingestion (Corbet, 1999; Fulan & Dos Anjos, 2015). However, when prey-predator interaction occurs in contaminated environments, metals can be bioaccumulated in the food web due to their non-degradability (Yuan et al., 2017). In addition, because they live directly associated with the sediment in its nymph phase, they may accumulate more metals than other invertebrate taxa inhabiting the water column (Corbi et al., 2008, 2010) and may therefore be good bioindicators of metal contamination (Wayland & Crosley, 2006).

Like many Odonata, ostracods are invertebrates that are also in close contact with the sediment since most species are detritivores, feeding mainly on decomposing organic matter (Martens, 1995). However, filter-feeding and predaceous ostracod species also exist (Barnes, 1995). For example, Campbell (1995) observed that the ostracod *Australocypris insularis* is a predator on zooplankters, able to greatly reduce density of small copepods such as *Calamoecia clitellata* and *C. salina* besides other ostracods such as *Diacypris compacta* and *D. dietzi*. According to Duleba et al. (2005), several species of this Class are stenobiotic, i.e., very sensitive to environmental variations, and as such, good environmental indicators of changes in physicochemical water conditions. They are also known to be sensitive to pesticides, metals, and other pollutants related to oil spills (Khangarot & Das, 2009; Ruiz et al., 2013; Rocha et al. 2018).

Despite their recognized functional relevance in aquatic ecosystems, the sensitivity of Odonata and Ostracoda to chemical stress has been poorly studied worldwide, especially regarding sublethal effects like predation behavior (Sloof 1983; Meyer et al., 1986; Khangarot & Ray, 1987; Rockwood et al., 1990; Havel and Talbot 1995; Khangarot & Das, 2009; Shuhaimi-Othman et al., 2011). Considering the above, the aim of the present study was to evaluate the effect of metal salts on the predatory behavior of nymphs of the dragonfly *Tramea cophisa* (Libellulidae, Odonata) and adults of the ostracod *Chlamydotheca* sp. (Ostracoda, Cyprididae). To this end, laboratory toxicity tests were conducted with four metals (copper, cadmium, manganese, and mercury) to evaluate their effects on predation intensity and food selectivity. We hypothesized that (1) with the increase in the concentration of the contaminant (metal salts), the organisms (predators) decrease the rate of predation; (2) at lower concentrations of the contaminant, the organisms show generalist feeding characteristics; (3) under exposure to the highest concentrations of metal salts, predators present greater food selectivity, opting for more sessile prey, with greater biomass.

Copper, manganese, cadmium, and mercury were selected as test compounds because they are common contaminants in aquatic environments (Campagna et al., 2008; Dornfeld et al., 2018; Gomes et al., 2020; Vogt et al., 2010; Watts & Pascoe, 2000). These compounds enter the aquatic environment both through natural sources and through human activity, e.g., mining activities agricultural runoff and industrial effluents (Hudspith et al., 2017; Lesch & Bouwman, 2018). Although copper and manganese are essential elements, in high concentrations, they are known to have adverse effects on aquatic organisms (Lima et al., 2019; Majumdar & Gupta, 2012). Cadmium is a highly toxic element with a high potential for bioaccumulation (Moiseenko & Gashkina, 2018), and mercury also has the potential to cause serious effects on growth, survival, and reproduction of invertebrates (Buch et al., 2017).

2 Material and Methods

2.1 Test Organisms and Culture Conditions

Tramea cophisa dragonfly nymphs were collected in the Mayaca reservoir, located in the municipality of São Carlos, SP, Brazil (21° 01′ S, 47° 53′ W). This small shallow reservoir is located in a permanent preservation area and has a surface area of 0.17 ha and a maximum depth of 1.20 m (Albuquerque 1990). The ostracod *Chlamydotheca* sp. was collected from 1,000-L plastic tanks at the Fish Farming Station of the Federal University of São Carlos (UFSCar). Care was taken in selecting nymphs of similar size to run the predation experiments, which required large sampling effort for obtaining both predator and prey. The need to not compromise *T. cophisa* population and the large amount of prey used, the number of replicas had to be limited.

In the laboratory, organisms were kept in plastic trays of 29 cm (width) × 34 cm (length) × 6.7 cm (height) and gradually acclimated to reconstituted water (ABNT, 2016) and test conditions (see below) for 7 days before starting tests. A preliminary study was made to determine best conditions to maintain healthy individuals in the laboratory (Lima et al., 2019). *T. cophisa* nymphs were fed with *Chironomus inquinatus* larvae (Insecta, Diptera) and small juveniles of *Artemia franciscana* (Crustacea, Anostraca), whereas *Chlamydotheca* sp. was fed with dense suspension of *Chlorella sorokiniana* (≈ 10^8 cells L^{-1}). Both species were fed daily ad libitum.

2.2 Test Concentrations and Chemical Analysis of Metal Salts

Compounds were evaluated and their respective stock solutions were copper sulfate (10 mg L^{-1}, $CuSO_4$, CAS no: 7758–98-7), manganese sulfate (100 mg L^{-1}, $MnSO_4$, CAS no: 7785–87-7), cadmium chloride (10 mg L^{-1}, $CdCl_2$, CAS no: 10108–64-2, Carlo Erba), and mercury chloride (10 mg L^{-1}, $HgCl_2$, CAS no: 7487–94-7, ACS Merck). The nominal test concentrations of each chemical were obtained by diluting their respective stock solutions in reconstituted water. The stock solutions and test concentrations were prepared immediately before testing. The concentrations of cadmium, copper, and manganese were determined in stock solutions using a Perkin Elmer PinAAcle 900 T flame and longitudinal Zeeman atomic absorption spectrometer which was calibrated with Cd, Cu, and Mn standards (Trace Metals Basis, Sigma-Aldrich) and for stock solutions of mercury chloride using an AAS, AAnalyst 400, PerkinElmer.

2.3 Experimental Design

To evaluate the effects of metal salts on predation and food selectivity of *T. cophisa* nymphs (mean length: 8.54 mm ± 2.88 mm) and *Chlamydotheca* sp. adult individuals (mean length: 4.32 mm ± 0.59 mm) were exposed for 24 h to four sublethal concentrations of copper sulfate ($CuSO_4$), cadmium chloride ($CdCl_2$), mercury chloride ($HgCl_2$), and manganese sulfate ($MnSO_4$) (Table 1), besides the control treatment (reconstituted water only). The selected test concentrations corresponded for LC_1, LC_5, LC_{10}, and LC_{20} values (LC_x = lethal concentration to x % of the test population) were derived from acute toxicity tests conducted in our laboratory with each metal salt, for both species (Lima et al., 2019).

Four replicates were used for each treatment, with each replicate consisting of a circular non-toxic

Table 1 Test concentrations for copper sulfate ($CuSO_4$), cadmium chloride ($CdCl_2$), mercury chloride ($HgCl_2$), and manganese sulfate ($MnSO_4$) evaluated in the predation rate and food selectivity tests with *Tramea cophisa* (Odonata, Libelullidae) nymphs and *Chlamydotheca* sp. (Ostracoda, Cyprididae) adults

Compound	Exposure concentration (μg L^{-1})	
	Tramea cophisa	*Chlamydotheca* sp.
$CuSO_4$ L^{-1}	0; 100; 170; 220; 300	0; 60; 105; 140; 190
$CdCl_2$ L^{-1}	0; 90; 170; 240; 360	0; 3; 6; 11; 22
$HgCl_2$ L^{-1}	0; 25; 60; 110; 220	0; 14; 44; 55; 170
$MnSO_4$ L^{-1}	0; 247,000; 265,000; 276,000; 290,000	0; 27,540; 32,726; 36,000; 40,388

plastic vessel (height 8 cm; diameter 11 cm) filled with 200 mL test solution and one individual of either *T. cophisa* or *Chlamydotheca* sp. Only one predator individual was tested per replica due to the observation, from preliminary tests, of cannibalism occurrence for both predator species, thus making impossible to use more than one predator individual per test recipient. During the 24 h exposure period, no food was offered to test organisms. After this period, organisms were rinsed with non-contaminated reconstituted water and transferred to test vessels containing 200 mL fresh prepared non-contaminated reconstituted water. Five specimens of each of the following prey species were offered to *T. cophisa* making prey density per replicate, n = 20: *Ilyocryptus spinifer* (Crustacea, Ilyocryptidae), *Artemia franciscana* (Crustacea, Artemiidae), *Chironomus inquinatus* (Diptera, Chironomidae), and *Dero furcatus* (Annelida, Randiellidae). For the ostracod predator *Chlamydotheca* sp., three specimens of each prey *C. inquinatus*, *A. fransciscana*, and *I. spinifer* were added (total prey per replicate = 12). Test organisms were held with their prey for 24 h, after which the number of each prey species consumed was verified.

2.4 Data Analysis

After exposure to metal salts, predation rate and food selectivity of predator species were obtained by Ivlev Index of Electivity (Ivlev, 1961) calculated by the equation: $E = (ri - Pi) / (ri + Pi)$, where E is the electivity index, *ri* is the relative abundance of each item in the stomach contents, and *Pi* is the relative abundance of each item in the environment after consumption. Stomach content was not analyzed. The relative abundance of stomach contents (*ri*) was calculated by subtracting the number of individuals of each prey remaining in each test vessel after 24 h of exposure, from the respective amount at the beginning of the test. Index values vary from − 1 to + 1 with the value zero indicating no selectivity; values lower than 0 indicate rejection of the food item and

Table 2 Mean specific predation rates and all prey consumptions by four dragonfly nymphs of *Tramea cophisa* after 24 h exposure to different concentrations of metal compounds: copper sulfate ($CuSO_4$), cadmium chloride ($CdCl_2$), mercury chloride ($HgCl_2$), and manganese sulfate ($MnSO_4$); *p* values (*) indicate significant differences as compared to controls

Metal	Concentration	Average of prey consumed per concentration				Average of total prey consumed per individual	Total predation and percentage	*p*
	μg L^{-1}	*I. spinifer*	*A. franciscana*	*C. inquinatus*	*D. furcatus*			
$CuSO_4$	Control	4.25 (±0.5)	4.25 (±0.5)	4.5 (±1)	4.75 (±0.5)	17.75 (±1.89)	71 (89%)	—
	100	2.5 (±0.58)	3.5 (±0.58)	3.25 (±0.5)	4 (±0.82)	13.25 (±0.96)	53 (66%)	0.0059*
	170	2 (±1.41)	4.25 (±0.96)	3.5 (±1)	4 (±0.82)	13.75 (±1.89)	55 (69%)	0.0239*
	220	2.5 (±0.58)	2.75 (±0.5)	3.5 (±0.58)	4 (±1.15)	12.75 (±1.5)	51 (64%)	0.0065*
	300	2 (±0.82)	1.75 (±0.96)	3 (±0.82)	3.5 (±1.29)	10.25 (±1.26)	41 (51%)	0.001*
$CdCl_2$	Control	2.75 (±0.5)	3.75 (±1.26)	5 (±0)	5 (±0)	16.5 (±1.73)	66 (82%)	—
	90	2.25 (±0.96)	3.25 (±0.96)	5 (±0)	5 (±0)	15.5 (±1.29)	62 (77%)	0.6068
	170	1.25 (±0.96)	3.25 (±1.26)	3.25 (±0.5)	4.5 (±0.58)	12.25 (±1.5)	49 (61%)	0.0102*
	240	2.5 (±0.58)	3.25 (±1.5)	4 (±0.82)	4.25 (±0.96)	14 (±2.45)	56 (70%)	0.1449
	360	1.75 (±1.26)	2.75 (±0.96)	3.5 (±1.29)	4.5 (±0.58)	12.5 (±1.91)	50 (62%)	0.0209*
$HgCl_2$	Control	4 (±0.82)	4 (±0.82)	3.5 (±0.33)	5 (±0)	16.5 (±0.58)	66 (82%)	—
	25	3.25 (±0.5)	3.25 (±0.92)	3.5 (±0.58)	4.75 (±0.5)	14.75 (±1.5)	59 (73%)	0.0708
	60	3 (±0.82)	3 (±0.82)	3 (±0.82)	4.5 (±0.58)	13.5 (±1.29)	54 (67%)	0.0059*
	110	3.25 (±0.5)	2.75 (±1.26)	3.25 (±0.5)	3.75 (±0.5)	13 (±1.63)	52 (65%)	0.0072*
	220	3 (±1.15)	2.25 (±0.5)	3.5 (±0.58)	4 (±0)	12.75 (±1.5)	51 (64%)	0.0040*
$MnSO_4$	Control	3.25 (±1.26)	3.5 (±0.58)	3.5 (±1)	4 (±1.41)	14.25 (±1.71)	57 (71%)	—
	247,000	3.25 (±2.06)	3 (±0.82)	3.75 (±1.89)	3.75 (±1.89)	13.75 (±2.99)	55 (69%)	0.7763
	265,000	4 (±0.82)	3.5 (±1)	4.5 (±1)	4.5 (±0.58)	16.5 (±1.91)	66 (82%)	0.1282
	276,000	2.5 (±0.58)	2 (±0.82)	4 (±1.15)	3.5 (±1.29)	12 (±2.16)	48 (60%)	0.1517
	290,000	3.25 (±1.26)	3 (±0.82)	2.5 (±0.58)	3 (±0.82)	11.75 (±1.5)	47 (59%)	0.0686

values higher than 0 denote positive selection (Harrison et al., 2005). Prior to statistical data analysis, all prey abundance values were logarithm transformed (log x + 1) to down-weight high abundance values aiming to approximate data normality. An array using the results of the Bray–Curtis similarity test was constructed and a permutational multivariate analysis of variance (PERMANOVA) was performed to compare results. Comparison between concentrations tested and controls was performed using the statistical program BioEstat 5.0 for analysis of variance (ANOVA).

3 Results and Discussion

3.1 Chemical Analyses

For all experiments, nominal concentrations of metal salts were measured for stock solutions. Concentrations of copper sulfate (10 mg L^{-1}), manganese sulfate (100 mg L^{-1}), cadmium chloride (10 mg L^{-1}), and mercury chloride (10 mg L^{-1}) were quantified. Accuracy (%) values obtained were 10.3 mg L^{-1} (100.3 ± 0.9%); 107 mg L^{-1} (107.3 ± 0.2%); 9.1 mg L^{-1} (91.1 ± 0.9%); and 9.3 mg L^{-1} (93 ± 1.8%), respectively. Nominal concentrations did not vary more than 10% from measured concentrations. Therefore, nominal initial concentrations were used to represent treatment concentrations.

3.2 Predation Rates

A voracious feeding behavior by unaffected *Tramea cophisa* nymphs was observed in the first hour after being transferred from treatment test solution to clean culture medium. This was very likely due to the food restriction that nymphs experienced during the 24 h exposure period. In line with this, Tomazelli et al. (2011) observed the same behavior for *Neuraeschna* sp. (Odonata, Aeshnidae) nymphs when offering the common carp *Cyprinus carpio* larvae (Cypriniformes, Cyprinidae) after a 27 h exposure period without food.

Table 3 Predation rate and prey consumption of ostracod *Chlamydotheca* sp. after 24 h exposure to copper sulfate ($CuSO_4$), cadmium chloride ($CdCl_2$), mercury chloride ($HgCl_2$), and manganese sulfate ($MnSO_4$). *p* values (*) indicate significant differences as compared to controls

Metal	Concentration	Average of prey consumed per concentration			Average of total prey consumed per individual	Total predation and percentage	*p*
	μg L^{-1}	*I. spinifer*	*A. franciscana*	*C. inquinatus*			
$CuSO_4$	Control	0.5 (±0.58)	0.75 (±0.5)	1.5 (±0.58)	2.75 (±0.96)	11 (31%)	—
	60	0.5 (±0.58)	1 (±0.67)	0.75 (±0.5)	2.25 (±1.26)	9 (25%)	0.5549
	105	1.75 (±0.5)	0.75 (±0.5)	2.25 (±1.26)	4.75 (±0.92)	19 (52%)	0.026*
	140	2 (±0.82)	2 (±0.82)	2.25 (±1.5)	6.25 (±1.71)	25 (69%)	0.0118*
	190	2 (±0.82)	2 (±0.82)	2.75 (±1.26)	6.75 (±1.26)	27 (75%)	0.0028*
$CdCl_2$	Control	1.5 (±0.58)	1.25 (±0.5)	2.5 (±0.58)	5.25 (±0.96)	21 (58%)	—
	3	1.75 (±1.5)	1.5 (±0.58)	3.5 (±0.96)	6.5 (±1)	26 (72%)	0.1193
	6	1.5 (±1)	1.25 (±0.5)	3.5 (±0.58)	6.25 (±1.26)	25 (69%)	0.2521
	11	2.25 (±0.96)	1 (±0.82)	3.75 (±0.96)	7 (±0.82)	28 (77%)	0.0312*
	22	2.25 (±0.5)	1.75 (±0.5)	2.25 (±0.5)	6.25 (±0.96)	25 (69%)	0.1885
$HgCl_2$	Control	1.25 (±0.96)	0.25 (±0.5)	2.25 (±0.96)	3.75 (±0.96)	15 (41%)	—
	14	2 (±0)	1.25 (±0.96)	3.25 (±0.5)	6.5 (±1)	26 (72%)	0.0077*
	44	1.25 (±0.5)	0.5 (±0.58)	3.25 (±0.96)	5 (±0.82)	20 (56%)	0.0925
	55	2.5 (±0.58)	1 (±0.82)	3 (±0.82)	6.5 (±1.73)	26 (72%)	0.0313*
	170	2.25 (±0.96)	0.75 (±0.5)	3.25 (±0.96)	6.25 (±1.26)	25 (69%)	0.0193*
$MnSO_4$	Control	0.25 (±0.5)	1.25 (±0.5)	1.5 (±0.58)	3 (±1.16)	12 (33%)	—
	27,540	1.25 (±0.5)	1.5 (±0.58)	2.75 (±0.96)	5.5 (±0.58)	22 (61%)	0.0085*
	32,726	2.25 (±0.5)	1.25 (±0.5)	2.5 (±0.58)	6 (±0.82)	24 (66%)	0.0059*
	36,000	1.5 (±0.58)	1.25 (±0.5)	2.75 (±1.26)	5.5 (±1.29)	22 (61%)	0.0272*
	40,388	2.25 (±0.96)	1.5 (±0.58)	3.25 (±0.5)	7 (±0.82)	28 (77%)	0.0018*

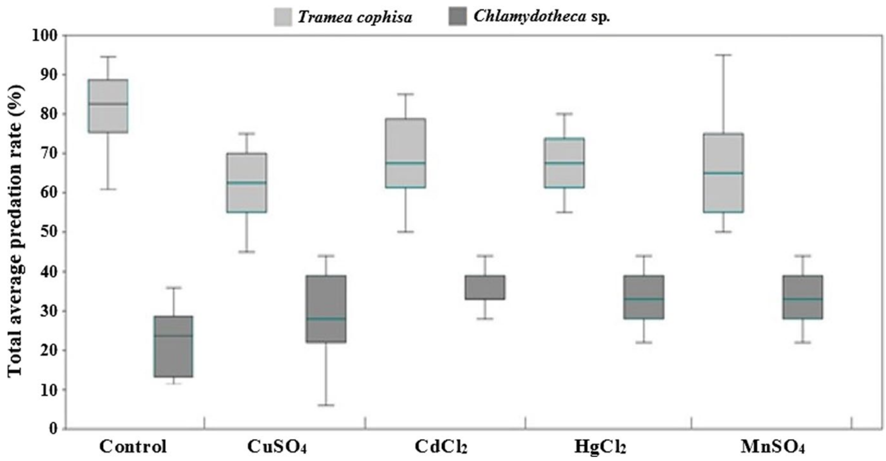

Fig. 1 Total average predation rates of the Odonata *Tramea cophisa* and Ostracoda *Chlamydotheca* sp. when exposed to metal salts: copper sulfate (CuSO4), cadmium chloride (CdCl2), mercury chloride (HgCl2), and manganese sulfate (MnSO4)

Prey consumption rates of *T. cophisa* indicate that most metal salt concentrations tested led to a significant decrease in predation rates as compared with controls, supporting our first hypothesis. However, for cadmium chloride, there was greater variability in prey consumption among replicates as observed at

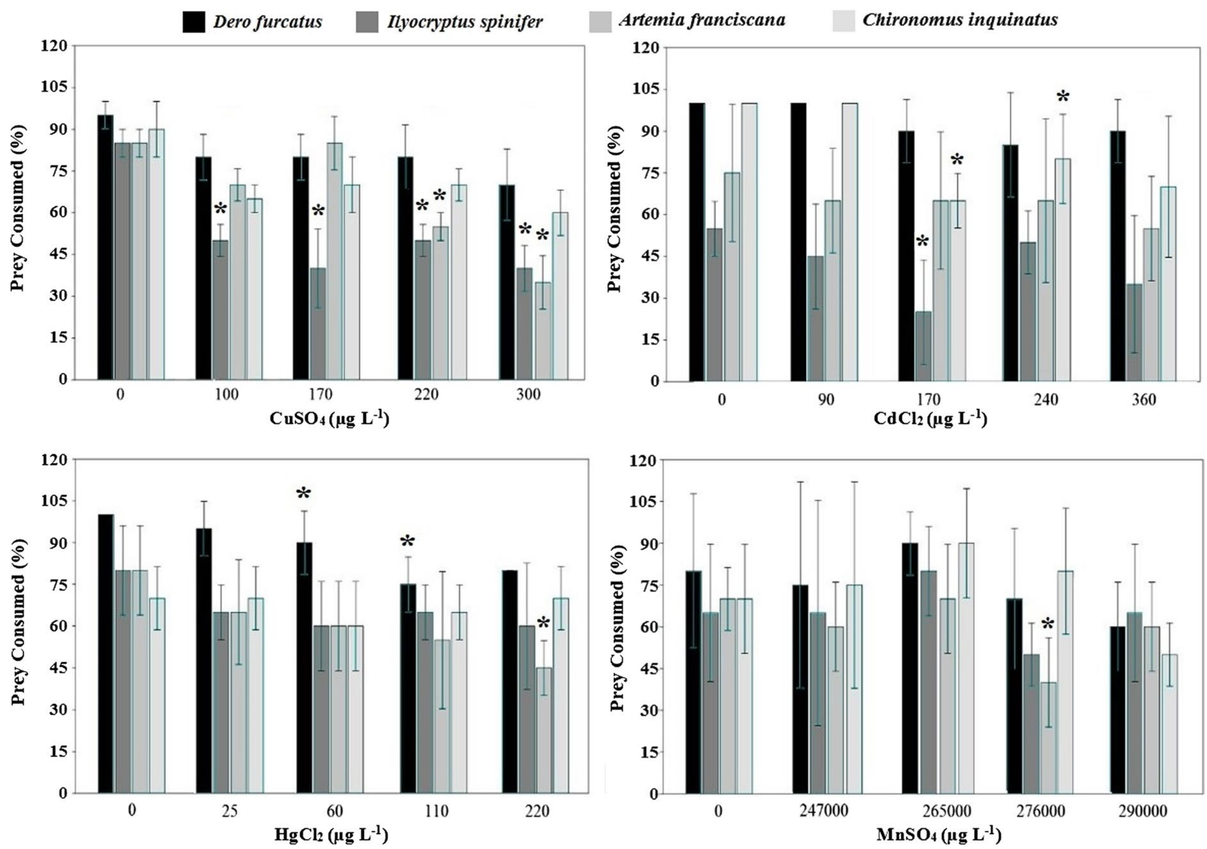

Fig. 2 Prey consumption rates of *Tramea cophisa* in relation to the number initially offered of each prey, in treatments without exposure (control) and in treatments with exposure to copper sulfate (CuSO4), cadmium chloride (CdCl2), mercury chloride (HgCl2), and manganese sulfate (MnSO4) for 24 h. Asterisks indicate significant differences

one of the highest concentrations tested (240 µg L^{-1}) resulting no significant difference from control. This might be explained by the low number of replicates used, in face of restrict availability of nymphs of similar size (Table 2). And only for nymphs exposed to manganese sulfate, there were no statistically significant differences in predation rates at any of the concentrations tested. In line with this, Lima et al. (2019) noted that this species is relatively non-sensitive to short-term exposure to manganese. However, this metal is an essential micronutrient being more or less toxic depending on the species, the stage of life and physical, and chemical characteristics of the water (Vieira et al., 2012).

So far, few studies have been conducted to evaluate metal effect on dragonfly feeding behavior, hampering comparisons between our results with studies also evaluating the metals tested in the present study. Nevertheless, previous studies have demonstrated a decrease in feeding rates of dragonflies following exposure to other chemicals. For example, a decrease in predation rate of *Pantala* sp. nymphs (Odonata, Libellulidae) when feeding on *Ictalurus punctatus* larvae (Siluriformes, Ictaluridae) after exposure in tanks chemically treated with larvicides (McGrinty, 1980). Tomazelli et al. (2011) also reported a reduction for predation rates of dragonfly nymphs (*Neuraeschna* sp.) on *C. carpio* fingerlings when nymphs were exposed to *Melia azedarach* (Meliaceae) extract. These authors also noted slowness in movements of dragonfly nymphs exposed to plant extract, which was concluded to be the cause of reduced predation rate.

The predation rate of the ostracod *Chlamydotheca* sp. increased compared to control in metal salts-affected treatments (Table 3). This increased predation rate could be caused by increased energy demand due to greater stress, which can be offset with greater predation. Alternatively, the increased predation rate could also be partly related with a greater energy

Table 4 Values of the Ivlev Electivity Index (IEI) obtained in the food selectivity tests for *Tramea cophisa* and *Chlamydotheca sp.* for the control and different metal treatments (24 h exposure) of copper sulfate ($CuSO_4$), cadmium chloride ($CdCl_2$), mercury chloride ($HgCl_2$), and manganese sulfate ($MnSO_4$)

Metal	(µg L^{-1})	Prey selectivity of *Tramea cophisa*				Prey selectivity of *Chlamydotheca* sp.		
		D. furcatus	*I. spinifer*	*A. franciscana*	*C. inquinatus*	*I. spinifer*	*A. franciscana*	*C. inquinatus*
$CuSO_4$	Control	0.9	0.7	0.7	0.8	−0.83	−0.75	−0.5
	60	0.6	0	0.4	0.3	−0.33	−0.58	0.08
	105	0.6	−0.2	0.7	0.4	−0.42	−0.75	−0.25
	140	0.6	0	0.1	0.4	−0.33	−0.33	−0.25
	190	0.4	−0.2	−0.3	0.2	−0.33	−0.33	−0.08
$CdCl_2$	Control	1	0.1	0.5	1	−0.5	−0.58	−0.17
	3	1	−0.1	0.3	1	−0.42	−0.5	0.08
	6	0.8	−0.5	0.3	0.3	−0.5	−0.58	0.17
	11	0.6	0	0.1	0.4	−0.25	−0.67	0.25
	22	0.4	−0.2	−0.3	0.2	−0.25	−0.42	−0.25
$HgCl_2$	Control	1	0.6	0.6	0.4	−0.58	−0.92	−0.25
	14	0.9	0.3	0.44	0.4	−0.33	−0.58	0.08
	44	0.8	0.2	0.2	0.2	−0.58	−0.83	0.08
	55	0.5	0.3	0.1	0.44	−0.17	−0.67	0
	170	0.6	0.2	−0.1	0.4	−0.25	−0.75	0.08
$MnSO_4$	Control	0.6	0.3	0.4	0.4	−0.92	−0.58	−0.5
	27,540	0.5	0.3	0.2	0.5	−0.58	−0.5	−0.08
	32,726	0.8	0.6	0.4	0.8	−0.25	−0.58	−0.17
	36,000	0.4	0	−0.2	0.6	−0.5	−0.58	−0.08
	40,388	0.2	0.3	0.2	0	−0.25	−0.5	0.08
Mean ± SD		0.66 ± 0.23	0.14 ± 0.30	0.24 ± 0.30	0.46 ± 0.26	−0.43 ± 0.20	−0.60 ± 0.15	−0.084 ± 0.21

demand of the predator for possible defense mechanisms against the toxic stress. In real aquatic ecosystems, indirect effects of chemical stress on predation rates may also play a prominent role. Pearson et al. (1981), for example, observed that the predator crab *Cancer magister* consumed a larger number of the snail *Protothaca staminea* in oil-contaminated sand when compared to uncontaminated sand. These authors associated this with a slower penetration of snails in the contaminated sand, making them easier to be preyed upon by the crabs.

Ostracoda *Chlamydotheca* sp. showed increased rates of predation in relation to the control (Fig. 1). Ostracods, for example, can ingest large quantities of food, in few minutes in contaminated systems and survive for several weeks without feeding, thus increasing their rate of predation at times (Vannier et al. 1998). On the other hand, *T. cophisa* showed lower rates of predation compared to control (Fig. 1).

3.3 Food Preferences: Ivlev Electivity Index

Figure 2 shows the consumption rates of *T. cophisa* dragonfly nymphs on each of four prey taxa. From this figure, it appears that *C. inquinatus* and *D. furcatus* were the most preferred prey species. The Ivlev Electivity Index (IEI) confirms this overall preference of *T. cophisa* for the *D. furcatus* (IEI = 0.66 ± 0.23; mean ± SD for all treatments) and *Chironomus inquinatus* (IEI = 0.46 ± 0.26; mean ± SD for all treatments), with not a single negative value, meaning no rejection for these preys at any tested metal salt concentrations (Table 4). These results confirm the food preference of *T. cophisa* for organisms with higher biomass

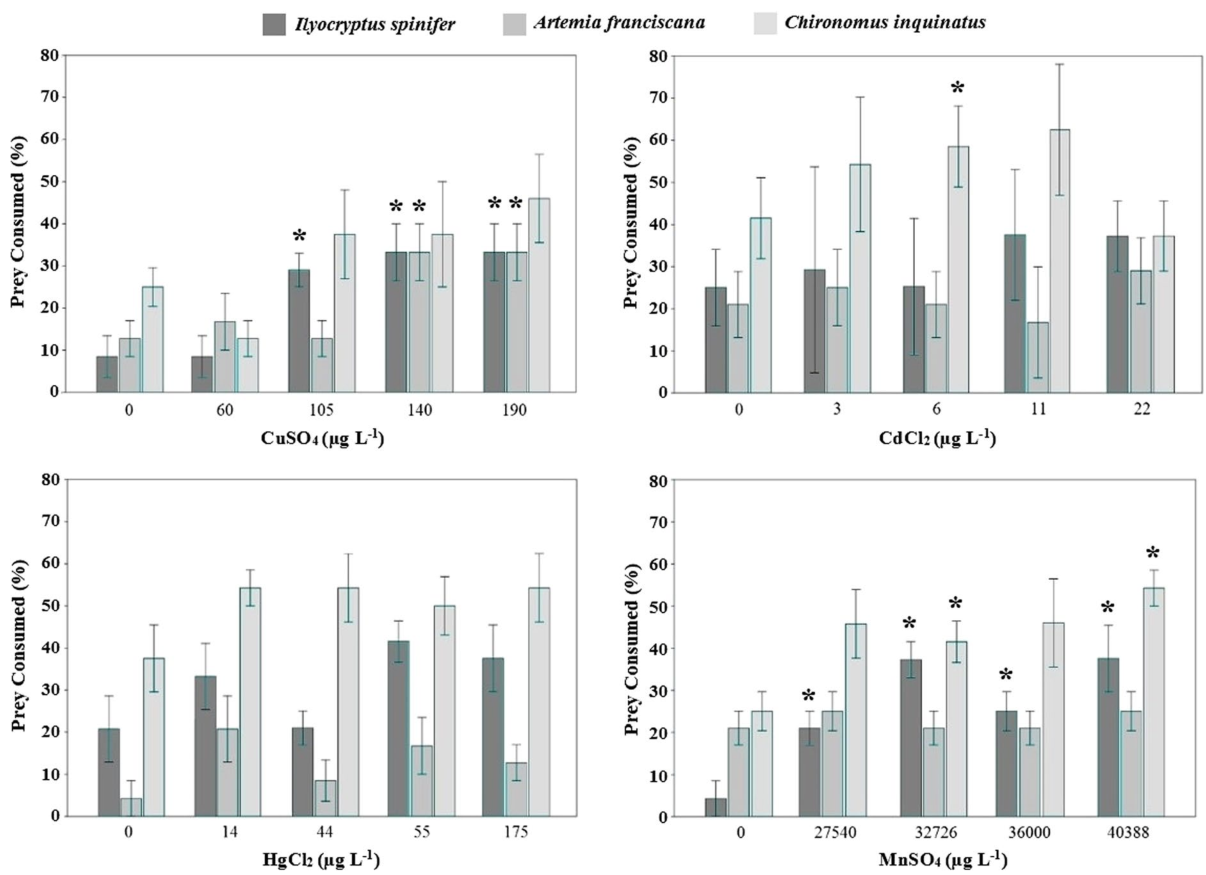

Fig. 3 Consumption rate of *Chlamydotheca* sp. (expressed as %) in relation to the number initially offered of each prey, for treatments without exposure (control) and in treatments with previous exposure to copper sulfate (CuSO4), cadmium chloride (CdCl2), mercury chloride (HgCl2), and manganese sulfate (MnSO4), during 24 h. Asterisks indicate significant differences

when exposed to higher concentrations of metal salts. Although these preys are not sessile, they have slower locomotion than *I. spinifer* and *A. franciscana*, corroborating our third hypothesis. These results also agree with those obtained by Alzmann et al. (1999) who found that the dragonfly *Gomphus pulchellus* preferred oligochaete and chironomid larvae over amphipods and ephemeropteran larvae. The relatively lower preference for *I. spinifer* (IEI = 0.14 ± 0.30) and, to a lesser extent, *Artemia franciscana* (IEI = 0.24 ± 0.30) may have several underlying reasons, including (i) their overall smaller size; (ii) their movement through the water column rather than being sessile; and, for *I. spinifer* specifically, (iii) the presence of thorns around the carapace (Kotov & Williams, 2000). However, in the lowest concentrations of the tested metal salts, *T. cophisa* showed a generalist feeding behavior, as indicated in our second hypothesis.

For *Chlamydotheca* sp., *C. inquinatus* chironomid larvae were the most consumed prey in the control in all tests performed, followed by *I. spinifer* and *A. franciscana* (Fig. 3; Table 4). *C. inquinatus* was also the most consumed prey species in the metal salt exposure treatments, for all tests performed, except in the treatment of 60 μg L^{-1} of $CuSO_4$, followed by *I. spinifer* that was less consumed only at concentrations of 60 μg. L^{-1} ($CuSO_4$) and in controls of $CuSO_4$ and $MnSO_4$. *A. franciscana* was most consumed only at the concentration of 60 μg. L^{-1} of $CuSO_4$. Thus, with these results, for *Chlamydotheca* sp., our second hypothesis that in low concentrations, the test organisms would be generalists, is not fully supported. Studies on the predation behavior of ostracods are scarce, but they are known to prey on a wide range of organisms including *Daphnia magna* (Ottonello and Romano 2011) and other cladocerans, as well as copepods, ostracods, oligochaetes, and insect larvae (Ganning, 1971; Meisch, 2000; Wilkinson et al., 2007). The preference for the chironomid prey over the two crustacean preys may have been due to the same reasons as discussed above for *T. cophisa*.

4 Conclusions

In this study, we observed that the tested metal salts did not influence food selectivity, but the predation behavior of nymphs of the dragonfly *T. cophisa* and adults of ostracod *Chlamydotheca* sp. was altered in relation to the control tests. In addition, this study evidenced that these effects are species specific. Such complex species-specific predator–prey interactions may have pronounced impacts on ecosystem structure and functioning following chemical stress. Future studies are needed to shed more light on predator species-specific prey preferences and how these are affected by metal and other chemical contamination.

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Data Availability The datasets used during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics Approval This article does not contain any studies with human participants performed by any of the authors. All applicable international, national, and institutional guidelines for the care and use of animals were followed.

Consent to Participate All authors inform consent to participate in this manuscript.

Consent for Publication Authors inform consent for the publication of any associated data and accompanying images.

Conflict of Interest The authors declare no competing interests.

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