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Paracyclops chiltoni inhabiting water highly contaminated with arsenic: Water chemistry, population structure, and arsenic distribution within the organism☆

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ABSTRACT

We investigated population structure and arsenic bioaccumulation and distribution in zooplankton inhabiting highly contaminated freshwater with arsenic. We collected water and zooplankton samples over a 4 year period, determined environmental temperature as well as water temperature, pH, electrical conductivity (EC), total dissolved solids (TDS), oxidation-reduction potential (ORP), dissolved oxygen (DO), major cations and anions and total arsenic concentration. We identified zooplankton species and determined their abundance, length, sex ratios, and arsenic bioaccumulation and distribution in exposed organisms. At the study site, an extremophile, *Paracyclops chiltoni*, was found to survive in an environment with high concentration of arsenic, sulfate and fluoride in freshwater as a well-adapted organism. Results showed that the average arsenic concentration in freshwater was 53.64 ± 10.58 mg/L. Exposed organisms of *Paracyclops chiltoni* showed arsenic accumulation (up to 9.6 ± 5.4 mgAs/kg) in its body, likely in the digestive tract as well as typical abundance and length, which showed a relationship to environmental temperature and oxic conditions in freshwater. Metallotolerant copepods might help to better understand if arsenic methylation processes occur in freshwater aquatic organisms.

1. Introduction

Due to natural and anthropogenic processes and activities, arsenic (As) can be found in freshwater at concentrations that can cause stress to zooplankton, impacting their population ecology and morphology as well as their abundance, size, sex, and sex ratio (female:male), among other parameters (Conde-Porcuna et al., 2004; De la Lanza-Espino et al., 2011; Dinh et al., 2020; Karlsson and Winder, 2020; Zhao et al., 2018; Zhu et al., 2020). Concentrations of As as high as 3 mg/L have been reported to be lethal to zooplankton experimentally (Chen et al., 1999). However, it is fairly well known that all main groups of freshwater zooplankton, cladocerans, rotifers and copepods, bioaccumulate As (Alvarado-Flores et al., 2019; Byeon et al., 2020; Caldwell et al., 2011; Caumette et al., 2012; 2014 Rubio Franchini et al., 2015). This is likely due to the biotransformation of more toxic inorganic As species to less toxic arsenobetaine and arsenosugars species, as it has been demonstrated for marine and freshwater organisms, respectively (Caumette et al., 2012, 2014).

X-ray Fluorescence (XRF) studies on cladocerans, *Daphnia pulex* inhabiting in lakes with 0.25 mg/L of As and *Daphnia magna* exposed to As in laboratory cultures, indicate arsenic accumulation

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mainly in the gut of the organisms (Caumette et al., 2012; Wang et al., 2018), exhibiting concentrations that are approximately 10 times higher in the gut than in surrounding tissue (Caumette et al., 2012). Questions remain, however, on whether As biotransformation and accumulation would replicate in metallotolerant organisms inhabiting extreme concentrations of As in freshwater, as it is the case of this study. Extremophile calanoid copepods *Acartia* spp., *Temora longicornis*, and *Pseudocalanus* sp. have been reported to inhabit extreme conditions of salinity and temperature, but in marine environments (Barka et al., 2001; Hansen et al., 2006; Lee et al., 2003). However, to the extent of our knowledge, no metallotolerant species of zooplankton inhabiting highly contaminated freshwater has been reported yet. The study of As biotransformation and accumulation across a wide range of As concentrations, environments and groups of zooplankton might improve our understanding of As uptake by organisms, as zooplankton is the first link in the food chain and a water quality indicator.

In this study we investigated some indicators of population structure of *Paracyclops chiltoni* (a crustacean of the Copepoda Cyclopoid group) inhabiting highly contaminated As freshwater over time as well as As bioaccumulation and distribution in *P. chiltoni*. To better understand any relationships among the hydrogeochemistry of the environment and the organisms, major water parameters were determined and principal component analyses were carried out.

2. Materials and methods

Water and zooplankton samples were collected from an old excavation located in Matehuala, San Luis Potosi, Mexico. The excavation covers an area of 50 m^2, has blurred edges, serves as a private litter dump, and is nearly 2 m deep, while the height of the water level is 30 cm, approximately (Fig. 1). Previous studies at the sampling location showed extremely high concentrations of As in water (up to 158 mg/L) (Pelallo, 2006; Martínez-Villegas et al., 2013, Razo et al., 2004), due to the dissolution of metallurgical wastes from an inactive smelter located 200 m north the sampling point (Martínez-Villegas et al., 2013), suggesting that zooplankton inhabiting this water body might be a metallotolerant species that accumulate As. Local surface geology consists of a complex alluvial-gypsum geological transition that exhibits paleochannel and karst features (Gómez-Hernández et al., 2020).

Water samples were collected over time from 8 different sampling campaigns to determine As as well as major cations and anions. Water samples were taken in 60 mL polypropylene bottles previously washed and rinsed with deionized water. Bottles for As and cation determinations were washed with 2% Extran® and 10% HNO_3 acid, while bottles for anion determinations were washed with 2% Extran® only. All water samples were filtered through 0.45 μm filters, acidified to pH < 2 using concentrated HNO_3 (only for cations and As), and stored at 4 °C until analysis. For quality control and assurance, one laboratory blank, one field blank and one duplicate were collected at each sampling campaign.

Ambient temperature, water temperature, pH, electrical conductivity (EC), total dissolved solids (TDS), oxidation-reduction potential (ORP), and dissolved oxygen (DO) were measured on-site using a multiparameter probe (HANNA Instruments Model 9829 Handheld Multiparameter Water Quality Meter). Additionally, alkalinity was determined by titration using an Automatic Titration Kit of the HACH brand model AL-DT.

Arsenic and major cations (calcium, magnesium, sodium and potassium) were determined by Inductively Coupled Plasma

Fig. 1. Sampling point located in Matehuala, San Luis Potosi, Mexico. The sampling point is an excavation 2 m deep and 50 m^2 wide (14N: 332784, 2617530).

Optical Emission Spectroscopy (ICP-OES), using a Varian 730 ES spectrometer (EPA, 1994). Due to relatively high salinity because of gypsic and karstic geological settings, samples were diluted (1:5) for ICP-OES analysis. Calibration with reference samples and blanks as well as replicate analyses for quality control were carried out to ensure the reliability of the analytical data. The calibration curve was in the range of 0.05–20 mg/L, while the detection limit was 0.001 mg/L.

Anions (sulfate, chloride, nitrate and fluoride) were determined by High-Performance Liquid Chromatography/Ion Chromatography (HPLC-IC), using a Thermo-Dionex 1100 with ASRS-Ultra 300 4-mm suppressor, IonPac AS14A column and ED50 electrochemical detector (EPA, 1997). Calibration with reference samples and blanks and replicate analyses for quality control were carried out to ensure the reliability of the analytical data. Correlation coefficients in analytical curves were greater than 0.9993, while recovery percentages were greater than 90%.

Zooplankton samples were collected by grabbing and filtering different volumes of water (ranging from 12 to 100 L) in each sampling campaign. The sampling was carried out depending on the availability of water, ensuring the least possible impact on the ecosystem and also bearing in mind the small size and the shallow depth of the water body. After collection, all samples were immediately filtered through a 45 μm net for zooplankton. For quality control and assurance, a duplicate sample was meant to be collected in each sampling campaign. However, due to low water availability, only 5 out of 8 duplicate samples could be collected. After collection, the organisms were fixed in 99% ethyl alcohol in 15 mL Eppendorf tubes and kept at 4 °C until isolation, cleaning, counting and measurement using a Nikon SMZ 800N stereo microscope for further analyses. Then, a total of 180 organisms were picked for As bioaccumulation analysis. An additional zooplankton sample was collected to fix organisms in deionized water for As distribution analysis.

Zooplankton abundance was quantitatively determined by counting adult, copepodite, and nauplii individuals using an Olympus CX21 optical microscope. Countings were then normalized to the volume of water filtered in the field to report abundance in individuals per liter (ind/L). Zooplankton longitudinal length was measured for organisms using a Nikon SMZ 800N stereo microscope.

Zooplankton observations were carried out in 10 organisms dried at room temperature and mounted in an aluminum pin stub. For doing so, a Scanning Electron Microscopy (JEOL-SM-6010 and FEI Quanta 200) was used. SEM images were compared to specialized literature on morphological keys of the Subclass Copepoda, Order Cyclopoida (Karaytuğ and Boxshall, 1998; 1999; Mercado-Salas and Suárez-Morales, 2009; Suárez-Morales, 1996).

Arsenic bioaccumulation was determined in organisms fixed in alcohol 99% after rinsing the organisms 3 times with deionized water. For doing so, 5 replicates of 30 organisms each were digested at room temperature using 500 μL of HNO_3 in 3 mL Eppendorf tubes. Then, As was determined in the digests by Graphite Furnace Atomic Absorption Spectroscopy (GFAAS) using a Varian model AA2042, with a detection limit of 0.24 μg/L and practical limit of quantification of 2.80 μg/L, (Alvarado-Flores et al., 2019; DOF, 1994; Rubio Franchini et al., 2015). For this purpose, other 5 replicates of 30 organisms each were weighted and brought to constant weight in 5 Eppendorf microtubes at 60 °C in the oven to determine adult zooplankton dry weight, which was 7.77 ± 1.9 μg average per organism.

Additionally, micro-focused X-Ray Fluorescence (μ-XRF) data was collected at the XFM beamline 10.3.2 of the Advanced Light Source, Lawrence Berkeley National Laboratory, (Berkeley, CA, USA) to determine As distribution in *Paracyclops chiltoni*. Three organisms were placed onto a molybdenum foil using a stereomicroscope and were allowed to dry at room temperature. Samples were then frozen in liquid nitrogen and analyzed using a Peltier cooling stage (−22 °C) during the analysis to minimize beam radiation damage. All data were recorded with a solid state Canberra 7-element UltraLEGe detector (Canberra, ON). Elemental maps were collected at 11966.7 eV, using a 7 × 7 μm beam spot size, 4 × 4 μm pixels and 100 ms dwell time. Maps were then deadtime-corrected and decontaminated. Data were processed with custom LabVIEW software available at the beamline. https://sites.google.com/lbl.gov/lbnl-als-1032/software-download.

Relationships between abiotic and biotic parameters were determined using Principal Component Analysis (PCA) with physicochemical (ambient temperature, sample temperature, pH, EC, TDS, ORP, OD, cations, anions and As) and population structure (zooplankton abundance and length) data using software Origin(-Pro), Version 2016 (OriginLab Corporation, Northampton, MA, USA).

3. Results and discussion

In the study area, the average ambient temperature was 19.2 ± 2.9 °C during the sampling period. Average water temperature, pH, EC, ORP and DO were 20.9 ± 1.6 °C, 6.9 ± 0.2, 3.59 ± 1.24 mS/cm, 322 ± 15 mV, and 2.9 ± 1.5 mg/L, respectively (Table S1, Supplementary Material). These values were similar to previous values reported for the same study site (Gómez-Hernández et al., 2020; Martínez-Villegas et al., 2013; Pelallo-Martínez, 2006). Temperature, pH, ORP and DO were also in agreement with values of natural freshwater exhibiting suboxic-oxic conditions (Quiroz-Martínez et al., 2006; Essington, 2004; Ongley, 1997). In contrast, EC and TDS were slightly higher than typical values for freshwater (0–1 mS/cm and 1 g/L to 3 g/L, respectively) (Prajapati, 2018; USGS, 2016), highlighting the evaporitic nature of the geological settings.

Average As concentration in freshwater was 53.64 ± 10.58 mg/L, greatly exceeding those commonly found in natural waters (which range from $5x10^{-5}$ mg/L to 5 mg/L) (Smedley and Kinniburgh, 2002) as well as the concentration of As considered lethal for zooplankton (3 mg/L) (Chen et al., 1999) and guidelines for the protection of aquatic life and natural waters in Mexico and other countries (CCME, 2001; CONAGUA, 2020; EPA, 1995), supporting that our sampling point is consistently and highly contaminated with arsenic (Gómez-Hernández et al., 2020; Martínez-Villegas et al., 2013; Pelallo-Martínez, 2006).

Average cation concentrations were 437.45 ± 140.49 mg/L, 46.68 ± 10.15 mg/L, 181.68 ± 96.41 mg/L and 9.46 ± 3.16 mg/L for calcium, magnesium, sodium and potassium, respectively (Table S2, Supplementary Material). These values were within the ranges reported for cations in natural waters (Appelo and Postma, 2005). In the case of anions, average concentrations were 277.13 ± 63.93 mg/L, 2287.3 ± 890.38 mg/L, 189.13 ± 8.67 mg/L, 32.79 ± 6.32 mg/L, 2.00 ± 0.13 mg/L for bicarbonate, sulfate, chloride, nitrate, and fluoride, respectively (Table S2, Supplementary Material). While bicarbonate, chloride, and nitrate concentrations were within the ranges reported for these anions in natural waters (Appelo and Postma, 2005; Oram, 2020), sulfate and fluoride concentrations exceeded the values most commonly found in natural waters (900 mg/L and 1 mg/L, respectively) (Appelo and Postma, 2005; O'Riordan, 1990). Relatively high calcium and sulfate concentrations explain the high EC found in freshwater, which is consistent with the alluvial-gypsum geological transition reported for the study site (Gómez-Hernández et al., 2020).

Fig. 2 shows SEM images of the only species of micro-crustacean found to inhabit the arsenic-contaminated water. The observed

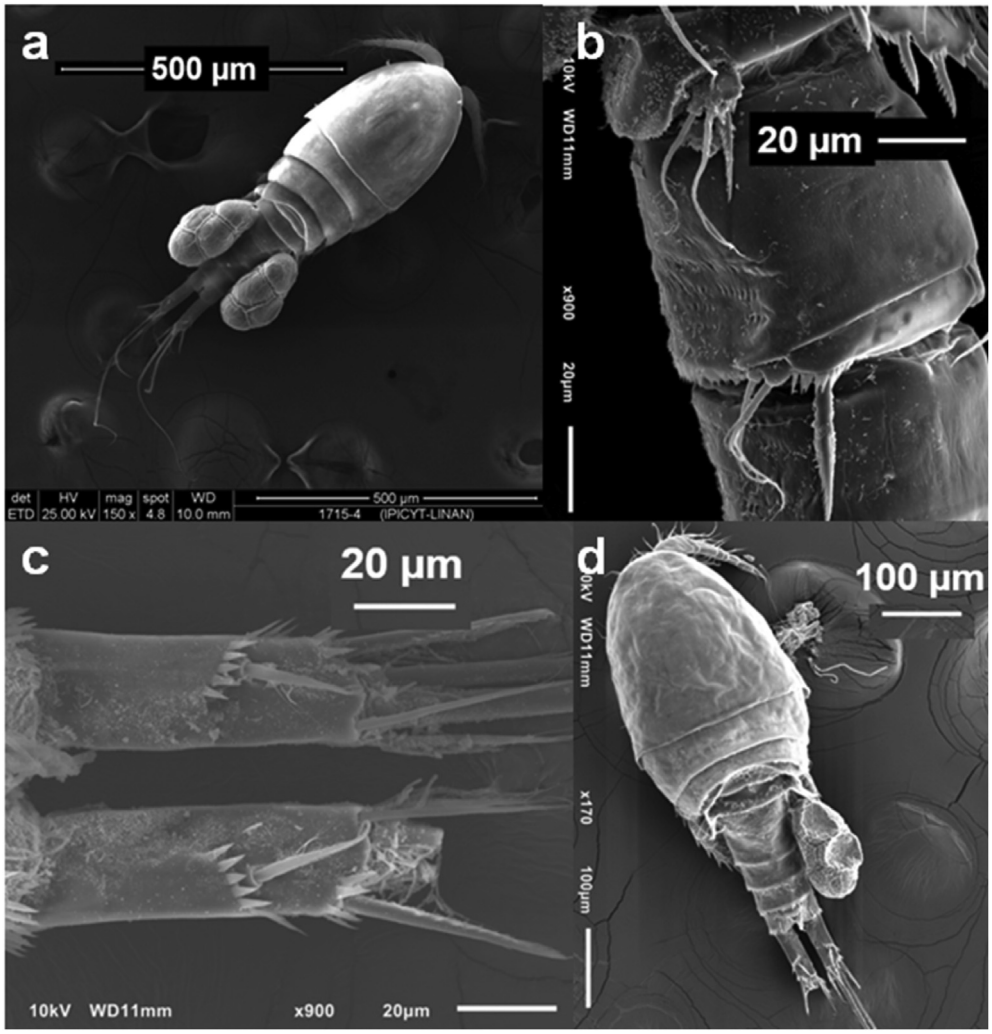

Fig. 2. SEM *Paracyclops chiltoni* images showing the a) habit (dorsal view), b) the fifth leg, c) the caudal rami, and d) the antenna of a female organism.

organisms key to *Paracyclops chiltoni* (Thomson, 1882) in the keys of, Karaytuğ and Boxshall (1999); Karaytuğ and Boxshall (1998), Mercado-Salas and Suárez-Morales (2009), Suárez-Morales (1996) (Fig. 2a). According to extensive observations and comparisons, all organisms showed a well-differentiated single segment of the fifth leg, with two apical setae of similar size, and an apical spine (Fig. 2b) as well as an antennule with 8 segments (Fig. 2d). Additionally, all the organisms showed a caudal rami that was 3.5–4 times longer than wide, ornamented with a short transverse row at the level of the caudal lateral seta and separated from each other from the base, by a length less than the width of one of the rami as well as an external seta of the fifth leg that was the same length as the middle one (Fig. 2c) as those reported by, Karaytuğ and Boxshall (1998), Karaytuğ and Boxshall (1999), Mercado-Salas and Suárez-Morales (2009), Suárez-Morales (1996). All morphological features matched those reported for the same species in the literature (Karaytuğ and Boxshall, 1998, 1999; Mercado-Salas and Suárez-Morales, 2009, Suárez-Morales, 1996). Additional genetic studies would help to better understand and increase knowledge on the biological characters of this metallotolerant zooplankton species inhabiting arsenic-contaminated water.

Table 1 shows total abundance, female and male abundance, copepodite and nauplii abundance, female and male length as well as sex ratio. Total average abundance (8.25 ± 10.77 ind/L) was generally within the abundance values reported for copepods inhabiting heavy metal contaminated water (0.2–20 ind/L) (Gagneten and Paggi, 2008) and epicontinental water (0.5 and 1182 ind/L) (Gómez-Márquez et al., 2013; Cervantes and Gutiérrez-Aguirre, 2014; Torres-Orozco and Zanatta, 1998; Villalba et al., 2017), except for the October 17 sample that showed a low abundance likely due to a relatively low water temperature (18.3 °C) compared with other samples. Haberman and Haldna (2017) reported that zooplankton abundance can decrease up to 3 times by one degree lowering of the temperature. On the other hand, *P. chiltoni* abundance was lower than the abundance values reported for cyclopoids inhabiting mesotrophic water levels (21.3 ind/L) (Cervantes and Gutiérrez-Aguirre, 2014).

Adult female and male lengths were within the lengths reported for cyclopoids, 556–857 μm in females and 531–751 μm in males (Karaytuğ and Boxshall, 1998), suggesting that *P. chiltoni* inhabiting highly contaminated water did not exhibit length changes. Sex ratio (female:male) was 8:1 ± 8.9:1 (Table 1), which was in agreement with adult sex ratios, which, in turn, was typically skewed towards the dominance of females in copepod field populations (Kiørboe, 2006), except for the May 18 sampling (Table 1), where sex ratio presented a higher population of males than females (0.5:1). During this sampling, the ambient temperature was the highest (22.66 °C). Sex ratio in copepods seems to show seasonal variability, the number of males increased with increasing temperature (Krupa, 2005). In this study, high variability in population structure

Table 1
Data of *Paracyclops chiltoni* structure population inhabiting contaminated water with arsenic values between 35.50 and 62.29 mg/L.

	April15	March16	August17	October17	December17	February18	May18	August18	Mean	SD	Min	Max
Total Ab (ind/L)	25.00	25.17	0.70	0.22	4.67	8.65	0.70	0.90	8.25	10.77	0.22	25.17
Female (ind/L)	10.00	24.00	0.62	0.17	4.46	4.80	0.40	0.50	5.62	8.17	0.17	24
Male (ind/L)	15.00	1.17	0.08	0.05	0.21	3.85	0.30	0.40	2.63	5.16	0.05	15
Copepodite	6.60	9.25	0.75	0.09	1.46	1.18	0.05	0.10	2.44	3.50	0.05	9.25
Nauplii	0.00	7.17	0.64	0.71	1.51	2.01	0.05	0.10	1.52	2.40	0.00	7.17
Length female (μm)	669.64	653.84	637.19	613.13	607.33	747.00	726.90	702.10	669.64	51.67	607.33	747.00
Length male (μm)	670.58	600.00	650.00	605.00	616.50	762.33	726.75	733.50	670.58	63.42	600.00	762.33
Sex Ratio (female:male)	4:1	20:1	14:1	4:1	21:1	1:1	0.5:1	1.25:1	8:1	8.9:1	0.5:1	21:1

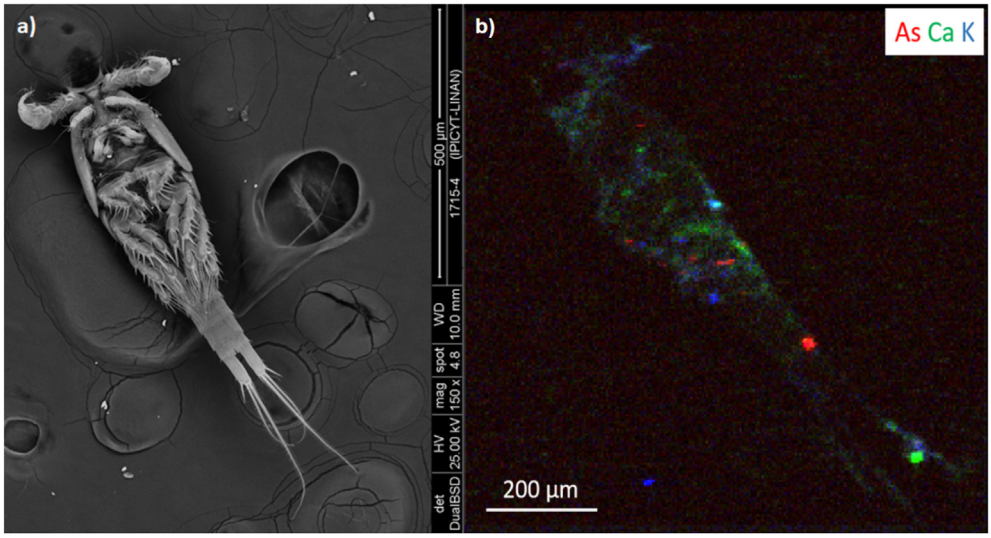

Fig. 3. a) SEM image of *Paracyclops chiltoni* living in highly contaminated freshwater and b) Tricolor-coded μ-XRF elemental map of arsenic in red, calcium in green and potassium in blue, revealing the presence of As in the prosome and the urosome of *P.chiltoni* The brightest red spot near the anus suggests the presence of As in the digestive tract of the organism. Acquisition done at −22 °C and 11966.7 eV with 4 × 4 μm pixels and 100 msec dwell time. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

was observed, perhaps due to environment settings surrounding the water body. As mentioned previously, it is next to a subsistence farm animal hatchery that may influence water hydrogeochemistry and zooplankton population structure.

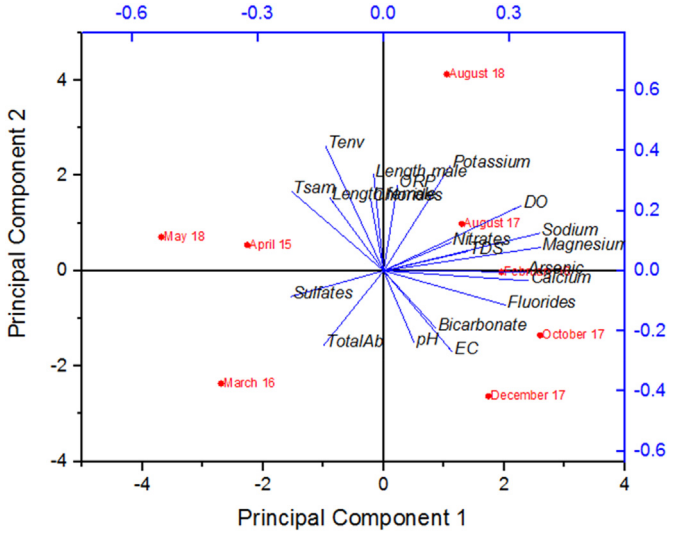

Fig. 4. PCA biplot, where two principal components (PC1 and PC2) are observed, as well as the contribution variables for each component.

Arsenic concentration in *Paracyclops chiltoni* was 9.6 ± 5.4 mg/kg, indicating that *P. chiltoni* uptakes As from freshwater and incorporates it in the body. These values were in agreement with those reported between 0.2 mg/kg and 11 mg/kg for copepoda and cladocera organisms inhabiting an environment down to 249 times less contaminated (Caumette et al., 2011). Questions remain on

Table 2
Eigenvectors.

	Coefficients of PC1	Coefficients of PC2	Coefficients of PC3
Tenv	−0.139	**0.417**	−0.052
Tsam	−0.220	0.265	−0.187
pH	0.073	−0.240	**0.355**
EC	0.164	−0.271	0.065
TDS	0.200	0.065	0.077
ORP	0.033	**0.286**	**0.312**
DO	**0.329**	0.218	0.104
Ca^{2+}	**0.346**	−0.033	0.025
Mg^{2+}	**0.374**	0.078	0.019
Na^{+}	**0.372**	0.125	0.122
K^{+}	0.159	**0.350**	−0.058
HCO_3^-	0.125	−0.191	**0.265**
SO_4^{2-}	−0.221	−0.085	0.072
Cl^-	−0.031	0.241	−0.355
NO_3^-	0.160	0.095	−0.409
F^-	0.291	−0.114	−0.323
As	**0.339**	−0.003	−0.084
TAb	−0.142	−0.246	−0.088
LF	−0.128	0.244	**0.321**
LM	−0.023	**0.321**	**0.329**

Table 3
Pearson's correlation matrix between biological and physicochemical parameters, showing values > 0.5 in orange and <-0.5 in blue.

	Tenv	Tsam	pH	EC	TDS	ORP	DO	Ca^{2+}	Mg^{2+}	Na^{+}	K^{+}	HCO_3^{-}	SO_4^{2-}	Cl^{-}	NO_3^{-}	F	As	TAb	LF
Tsam	0.782																		
pH	-0.548	-0.636																	
EC	-0.566	-0.411	0.684																
TDS	-0.152	-0.166	0.250	0.098															
ORP	0.453	-0.052	0.091	-0.492	0.240														
DO	0.167	-0.205	0.114	0.242	0.468	0.372													
Ca^{2+}	-0.371	-0.331	0.059	0.399	0.366	-0.089	0.639												
Mg^{2+}	-0.117	-0.480	0.086	0.287	0.197	0.232	0.840	0.728											
Na^{+}	-0.081	-0.411	0.215	0.323	0.523	0.305	0.965	0.761	0.889										
K^{+}	0.587	0.161	-0.535	-0.402	-0.109	0.463	0.641	0.288	0.639	0.531									
HCO_3^{-}	-0.459	-0.301	0.526	0.590	-0.004	-0.058	0.136	0.596	0.247	0.253	-0.240								
SO_4^{2-}	0.142	0.108	0.189	0.229	-0.810	-0.217	-0.347	-0.514	-0.293	-0.440	-0.132	0.045							
Cl^{-}	0.649	0.708	-0.551	-0.081	-0.017	-0.229	0.175	-0.174	-0.018	-0.024	0.334	-0.522	0.045						
NO_3^{-}	0.190	0.369	-0.477	0.146	0.183	-0.376	0.289	0.352	0.329	0.231	0.277	-0.150	-0.352	0.759					
F^{-}	-0.433	-0.295	-0.222	0.256	0.285	-0.417	0.282	0.642	0.560	0.409	0.141	0.047	-0.568	0.160	0.716				
As	-0.244	-0.509	-0.078	0.202	-0.023	0.051	0.597	0.698	0.928	0.687	0.591	0.221	-0.251	-0.081	0.356	0.693			
TAb	-0.341	0.096	-0.187	0.042	-0.600	-0.521	-0.659	0.059	-0.350	-0.563	-0.343	0.433	0.282	-0.296	-0.078	0.063	-0.058		
LF	0.552	0.460	-0.018	-0.246	-0.093	0.493	0.140	-0.038	-0.190	0.014	0.234	0.283	0.210	-0.072	-0.395	-0.726	-0.367	0.000	
LM	0.603	0.368	0.008	-0.286	0.182	0.716	0.405	0.086	0.048	0.278	0.396	0.220	-0.060	-0.025	-0.270	-0.578	-0.199	-0.295	0.925

whether this might be a (maximum) range of As concentration in exposed organisms.

Fig. 3 shows an XRF elemental map of As distribution in *Paracyclops chiltoni* in the body of the organism. Arsenic was found to accumulate in the prosome and the urosome of *Paracyclops chiltoni*. The presence of As in *P. chiltoni* was confirmed by the characteristic Kα fluorescence emission peak at 10 543.4 eV in specific sample regions (Figure S1, Supplementary Material), mostly in the thoracic area and near the furcation or the anus, which suggest that As may be accumulating in the digestive tract of *P. chiltoni*. Our results were similar to those reported for organisms of cladoceran zooplankton (Caumette et al., 2012; Wang et al., 2018) and suggest that As may accumulate in the digestive tract of zooplankton across different zooplankton groups. For the very first time, we report a metallotolerant copepod inhabiting freshwater with extremely high concentrations of arsenic (up to 57.47 mg/L), which might help to better understand As biotransformation and toxicity in copepoda and zooplankton.

PCA was carried out to examine any relationships among the hydrogeochemistry of the environment and the organisms. PC1 (30.19%) combined dissolved oxygen, major cations of evaporite origin (Na, Mg, Ca) and As, while the PC2 (23.70%) combined environmental temperature, male length as well as ORP (Fig. 4). From left to right, PCA showed a clear separation between the samples collected in the warmest (March, April and May) and the coldest (August, October, December and February) months (Fig. 4). This, in turn, showed the lowest and highest DO concentrations, respectively, as well as the lowest and highest ambient temperatures, respectively. This information was in agreement with historical average monthly temperatures in Matehuala for the last 22 years for those months (SMN, 2020).

A third principal component (PC3; 18.32%) combined pH, bicarbonate, ORP and female and male lengths. These three main components explained 72.21% of the total variability of the data. Table 2 shows the coefficients of each parameter.

Fig. 4 and Table 2 suggest that PC1 is an abiotic factor related to the solubility of minerals and the oxic level of the system, which is supported by a positive correlation between the level of dissolved oxygen and the concentrations of sodium, magnesium, calcium and arsenic (Table 3). A negative correlation was found between dissolved oxygen and total abundance of zooplankton (Table 3). On the other hand, PC2 is a factor that combines abiotic and biotic aspects of the system, related to ambient temperature, ORP and female and male lengths (Fig. 4, Table 2). This is supported by a direct correlation between ORP and male and female lengths, as well as between ambient temperature and male and female lengths (Table 3). PC3 is a factor related to pH and bicarbonates (Table 2), which were directly correlated (Table 3).

4. Conclusions

In the study site, *Paracyclops chiltoni* was found to be an extremophile, able to survive in an environment with high concentrations of arsenic, sulfate and fluoride in freshwater as well as a well-adapted organism to this environment as determined by average length and abundance, which were, as expected, related to environmental temperature and oxic conditions in freshwater. *P. chiltoni* accumulates As and might provide insight on limited arsenic methylation in metallotolerant zooplankton, for which further studies are needed.

Author statement

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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References

Alvarado-Flores, J., Rubio-Franchini, I., Sofia Sanchez-Avila, A., de Jesos Ramirez-Tlalolin, G., Rico-Martinez, R., 2019. Arsenic toxicity, bioaccumulation and risk assessment: a case study in Tolimique Dam, Aguascalientes, Mexico. Cogent Environmental Science. https://doi.org/10.1080/23311843.2019.1650630.

Appelo, C.A.J., Postma, D., 2005. Geochemistry, Groundwater and Pollution, second ed. CRC Press, London.

Barka, S., Pavillon, J.-F., Amiard, J.-C., 2001. Influence of different essential and non-essential metals on MTLP levels in the Copepod Tigriopus brevicornis. Comp. Biochem. Physiol. C Toxicol. Pharmacol. 128, 479–493. https://doi.org/10.1016/S1532-0456(00)00198-8.

Byeon, E., Yoon, C., Lee, Jin-Sol, Lee, Y.H., Jeong, C.-B., Lee, Jae-Seong, Kang, H.-M., 2020. Interspecific biotransformation and detoxification of arsenic compounds in marine rotifer and copepod. J. Hazard Mater. 391, 122196. https://doi.org/10.1016/j.jhazmat.2020.122196.

Caldwell, G.S., Koch, I., Estrada, E., Reimer, K.J., 2011. Arsenic speciation in plankton organisms from contaminated lakes: transformations at the base of the freshwater food chain. Environmental Science & Technology. https://doi.org/10.1021/es2025092.

Caumette, G., Falnoga, I., Moriarty, M., Reimer, K.J., 2012. Arsenic distribution and speciation in Daphnia pulex. Science Of The Total Environment. https://doi.org/10.1016/j.scitotenv.2012.05.050.

Caumette, G., Koch, I., Estrada, E., Reimer, K.J., 2011. Arsenic speciation in plankton organisms from contaminated lakes: transformations at the base of the freshwater food chain. Environ. Sci. Technol. 45, 9917–9923. https://doi.org/10.1021/es2025092.

Caumette, G., Koch, I., House, K., Reimer, K.J., 2014. Arsenic cycling in freshwater phytoplankton and zooplankton cultures. Environ. Chem. https://doi.org/10.1071/EN14039.

CCME, 2001. Canadian Water Quality Guidelines for the Protection of Aquatic Life. Canadian Council of Ministers of the Environment, Canada.

Cervantes, A., Gutiérrez-Aguirre, M., 2014. Physicochemistry and zooplankton of two karstic sinkholes in the Yucatan Peninsula, Mexico. J. Limnol. 73 https://doi.org/10.4081/jlimnol.2014.976.

Chen, C.Y., Sillett, K.B., Folt, C.L., Whittemore, S.L., Barchowsky, A., 1999. Molecular and demographic measures of arsenic stress in Daphnia pulex. Hydrobiologia 401, 229–238. https://doi.org/10.1023/A:1003738427354.

CONAGUA, 2020. Ley Federal de Derechos Disposiciones Aplicables en Materia de Aguas Nacionales 2020. México.

Conde-Porcuna, J., Ramos-Rodriguez, E., Baquero, R., 2004. El zooplancton como integrante de la estructura trófica de los ecosistemas lénticos. Ecosistemas 2.

De la Lanza-Espino, G., Hernández-Pulido, S., Carbajal-Pérez, J.L., 2011. Organismos indicadores de la calidad del agua y de la contaminación (bioindicadores), 2da. Plaza y Valdés Editores, México.

Dinh, K.V., Dinh, H.T., Pham, H.T., Selck, H., Truong, K.N., 2020. Development of metal adaptation in a tropical marine zooplankton. Sci. Rep. https://doi.org/10.1038/s41598-020-67096-1.

DOF, 1994. NOM-117-SSA1-1994 (Norma Oficial Mexicana). Secretaria de Salud, Mexico.

EPA, 1997. EPA Method 300.1, Revision 1.0: Determination of Inorganic Anions in Drinking Water by Ion Chromatography (No. Revision 1.0). U. S. Environmental Protection Agency, Cincinnati, OH.

EPA, 1995. Office of Water Policy and Technical Guidance on Interpretation and Implementation of Aquatic Life Metals Criteria. United States of America.

EPA, 1994. Method 200.7, Revision 4.4: Determination of Metals and Trace Elements in Water and Wastes by Inductively Coupled Plasma-Atomic Emission Spectrometry (No. Revision 4.4). U. S. Environmental Protection Agency, Cincinnati, OH.

Essington, M., 2004. Soil and Water Chemistry: an Integrative Approach. CRC Press.

Gagneten, A.M., Paggi, J., 2008. Effects of heavy metal contamination (Cr, Cu, Pb, Cd) and eutrophication on zooplankton in the lower basin of the salado river (Argentina). Water, Air, Soil Pollut. 198, 317–334. https://doi.org/10.1007/s11270-008-9848-z.

Gómez-Hernández, A., Rodríguez, R., Lara del Río, A., Aurora Ruiz-Huerta, E., Aurora Armienta, M., Dávila-Harris, P., Sen-Gupta, B., Delgado-Rodriguez, O., Del Angel Rios, A., Martínez-Villegas, N., 2020. Alluvial and gypsum karst geological transition favors spreading arsenic contamination in Matehuala, Mexico. Science Of The Total Environment. https://doi.org/10.1016/j.scitotenv.2019.135340.

Gómez-Márquez, J.L., Peña-Mendoza, B., Guzán-Santiago, J.L., Gallardo-Pineda, V., 2013. Composición, abundancia del zooplancton y calidad de agua en un microreservorio en el estado de Morelos. Hidrobiológica 23, 227–240.

Haberman, J., Haldna, M., 2017. How are spring zooplankton and autumn zooplankton influenced by water temperature in a polymictic lake? Proc. Est. Acad. Sci. 66, 264. https://doi.org/10.3176/proc.2017.3.03.

Hansen, J., Sato, M., Ruedy, R., Lo, K., Lea, D.W., Medina-Elizade, M., 2006. Global temperature change. Proceedings Of The National Academy Of Sciences Of The United States Of America. https://doi.org/10.1073/pnas.0606291103.

Karaytuğ, S., Boxshall, G., 1999. Antennules of the male of Paracyclops (copepoda): functional significance and their importance in systematics. J. Crustac Biol. 19, 86–92. https://doi.org/10.2307/1549244.

Karaytuğ, S., Boxshall, G., 1998. The Paracyclops fimbriatus-complex (copepoda, Cyclopoida): a revision. Zoosystema 20, 563–602.

Karlsson, K., Winder, M., 2020. Adaptation potential of the copepod Eurytemora affinis to a future warmer Baltic Sea. Ecology and Evolution. https://doi.org/10.1002/ece3.6267.

Kiørboe, T., 2006. Sex, sex-ratios, and the dynamics of pelagic copepod populations. Oecologia 148, 40–50. https://doi.org/10.1007/s00442-005-0346-3.

Krupa, E., 2005. Population densities, sex ratios of adults, and occurrence of malformations in three species of cyclopoid copepods in waterbodies with different degrees of eutrophy and toxic pollution. J. Mar. Sci. Technol. 13, 1–12.

Lee, H., Ban, S., Ikeda, T., Matsuishi, T., 2003. Effect of temperature on development, growth and reproduction in the marine copepod Pseudocalanus newmani at satiating food condition. J. Plankton Res. https://doi.org/10.1093/plankt/25.3.261.

Martínez-Villegas, N., Briones-Gallardo, R., Ramos-Leal, J.A., Avalos-Borja, M., Castañon-Sandoval, A.D., Razo-Flores, E., Villalobos, M., 2013. Arsenic mobility controlled by solid calcium arsenates: a case study in Mexico showcasing a potentially widespread environmental problem. Environ. Pollut. https://doi.org/10.1016/j.envpol.2012.12.025.

Mercado-Salas, N., Suárez-Morales, E., 2009. A new species and illustrated records of Paracyclops claus, 1893 (copepoda: Cyclopoida: cyclopinae) from Mexico. J. Nat. Hist. 43, 2789–2808. https://doi.org/10.1080/00222930903108462.

Ongley, E.D., 1997. Lucha Contra la Contaminación Agrícola de los Recursos Hídricos. In: Estudio FAO Riego Y Drenaje - 55. FAO, Canadá.

Oram, B., 2020. Nitrates in the Environment. Water Research Center. URL. https://water-research.net/index.php/nitrates-in-the-environment.

O'Riordan, J., 1990. Water Quality: Ambient Water Quality Criteria for Fluoride. Ministry of Environment, British Columbia.

Pelallo-Martínez, N.A., 2006. Comportamiento químico de arsénico en sedimentos de sistemas acuáticos contaminados (Tesis Doctoral). UASLP, México.

Prajapati, S., 2018. Making sense of conductivity, TDS, and concentration in water treatment. Sensorex.

Quiroz-Martínez, H., Rodríguez-Castro, V.A., Universidad Autónoma de Nuevo León., Dirección de Publicaciones., 2006. Bioindicadores de contaminación en sistemas acuáticos : insectos acuáticos. Universidad Autónoma de Nuevo León, Dirección de Publicaciones, Monterrey, N.L.

Razo, I., Carrizales, L., Castro, J., Díaz-Barriga, F., Monroy, M., 2004. Arsenic and heavy metal pollution of soil, water and sediments in a semi-arid climate mining area in Mexico. Water Air Soil Pollut. 152, 129–152. https://doi.org/10.1023/B:WATE.0000015350.14520.c1.

Rubio Franchini, I., López-Hernández, M., Ramos-Espinosa, M., Rico-Martínez, R., 2015. Bioaccumulation of metals arsenic, cadmium, and lead in zooplankton and fishes from the tula river watershed, Mexico. Water, Air, & Soil Pollution 227. https://doi.org/10.1007/s11270-015-2702-1.

Smedley, P.L., Kinniburgh, D.G., 2002. A review of the source, behaviour and distribution of arsenic in natural waters. Appl. Geochem. 17, 517–568. https://doi.org/10.1016/S0883-2927(02)00018-5.

SMN, 2020. Estación 24021. CONAGUA, México.

Suárez-Morales, E., 1996. Clave de identificación ilustrada de los copépodos plánctico (Crustacea) de la Bahía de la Ascensión, Chetumal. Q. Roo. 17, 25–39.

Torres-Orozco, R.E., Zanatta, S.A., 1998. Species composition, abundance and distribution of zooplankton in a tropical eutrophic lake: lake Catemaco, México. Rev. Biol. Trop. 46, 285–296.

USGS, 2016. Saline Water. URL. The USGS water science school. accessed 8.20.18. https://water.usgs.gov/edu/saline.html.

Villalba, W., Márquez, B., Troccoli, L., Alzolar, M., López, J., 2017. Composición y abundancia del zooplancton en la laguna El Morro, Isla de Margarita, Venezuela. Rev. Peru. Biol. 24, 343. https://doi.org/10.15381/rpb.v24i4.14062.

Wang, N.-X., Liu, Y.-Y., Wei, Z.-B., Yang, L.-Y., Miao, A.-J., 2018. Waterborne and dietborne toxicity of inorganic arsenic to the freshwater zooplankton Daphnia magna. Environ. Sci. Technol. 52, 8912–8919. https://doi.org/10.1021/acs.est.8b02600.

Zhao, K., Wang, L., Riseng, C., Wehrly, K., Pan, Y., Song, K., Da, L., Pang, W., You, Q., Tian, H., Liu, S., Wang, Q., 2018. Factors determining zooplankton assemblage difference among a man-made lake, connecting canals, and the water-origin river. Ecol. Indicat. 84, 488–496. https://doi.org/10.1016/j.ecolind.2017.07.052.

Zhu, G., Noman, M.A., Narale, D.D., Feng, W., Pujari, L., Sun, J., 2020. Evaluation of ecosystem health and potential human health hazards in the Hangzhou Bay and Qiantang Estuary region through multiple assessment approaches. Environ. Pollut. 264, 114791. https://doi.org/10.1016/j.envpol.2020.114791.