

## Article

# Phenetic and Genetic Variability of Continental and Island Populations of the Freshwater Copepod *Mastigodiatomus ha* Cervantes, 2020 (Copepoda): A Case of Dispersal?

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**Abstract:** The diversity of freshwater zooplankton is still little known in Mexico, particularly in reference to insular zooplankton communities. Diaptomid copepods (Crustacea: Copepoda: Calanoida) are a widespread group worldwide, and Mexico harbours high diaptomid diversity. Based on a recent sampling of freshwater zooplankton on a Caribbean Island of Mexico, we present the first record of a diaptomid copepod from an island freshwater ecosystem. It shows the well-known tendency of Neotropical diaptomids to have restricted distributional patterns and high levels of endemism. The species recorded, *Mastigodiatomus ha* (Cervantes-Martínez, 2020) appears to have a restricted distribution in the Yucatan Peninsula (YP), and the island as well. In order to explore potential differences between the island and continental populations of this species, its phenetic and genetic diversity was analysed by performing morphological comparisons and also by exploring differences of the habitat conditions and genetic sequences (CO1 gene). Our analysis revealed a low (average = 0.33%) genetic divergence between both populations; likewise, both the morphology and habitat conditions closely resemble each other in these two populations. The low genetic divergence between the continental and island populations of *M. ha* suggests an early common origin of the species in the geological history of the YP.

**Keywords:** barcoding; Calanoida; diaptomids; freshwater; insular water bodies; new record



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## 1. Introduction

The diverse zooplankton community inhabiting the epicontinental and underground freshwater ecosystems of the Yucatan Peninsula (YP) can be largely constituted by calanoid copepods belonging to the most successful freshwater group; the family Diaptomidae. Diaptomids tend to have restricted distributional patterns, with many endemic species in the Neotropical region [1].

*Mastigodiatomus* is one of the most diverse genera in Mexico, currently including 13 species. The genus is widely distributed in the Neotropical region, including the Caribbean islands, Central America, and areas of the Southern United States [2,3].

Recently, Gutiérrez-Aguirre et al. [3] described three new species of the genus from Mexico; *Mastigodiatomus cihuatlan* (Gutiérrez-Aguirre, 2020), *M. alexei* (Elías Gutiérrez, 2020), and *M. ha* (Cervantes-Martínez, 2020). The latter was found in sinkholes (locally known as cenotes) in the northeastern continental zone region of the YP.

After 15 years of basic studies on the freshwater and anchialine zooplankton in Cozumel Island [4–6], this is the first report of a diaptomid copepod on a Mexican island. Previously, *M. ha* has been recorded in continental freshwater systems in the north-northeastern region of the YP [3]. In this study we analysed the phenetic and genetic distances between the island and continental populations, and specimens from the type

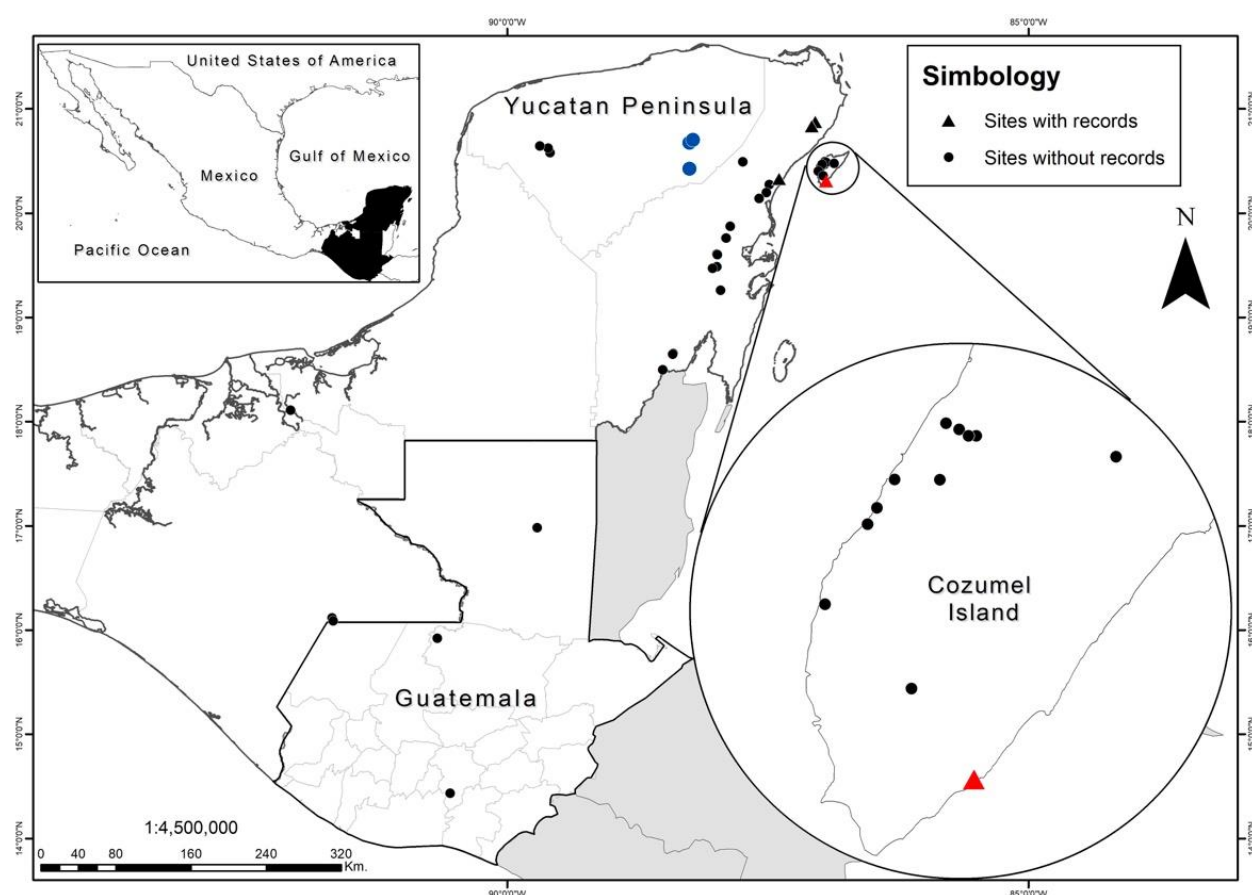
locality of *M. ha*, adding new molecular barcodes revealing that, despite the fact that these two populations were isolated for over 8000–6000 years, they are phenotypically and genetically similar.

This work confirms that YP copepod fauna provides the best-known Mexican region for harbouring the greatest diversity of the *Mastigodiatomus* species in Central America and the Caribbean region [2,3,7].

## 2. Materials and Methods

### 2.1. Study Sites and Sampling Methods

Cozumel Island is located off the northeastern coast of the YP, in the northwestern region of the Caribbean Sea (Figure 1) and, because of its area (~500 km<sup>2</sup>), it is the third largest island in Mexico and the most populated. An 18 km wide channel separates it from the continental YP.



**Figure 1.** Collection sites for zooplankton samples in the YP, Mexico (2005–2020), where diaptomids are present. Black circles are negative records of *Mastigodiatomus ha*; triangles are positive sites for records of *M. ha*. The red triangle is the place where the species was founded on Cozumel Island. Blue circles indicate probable presence of this species.

The YP is considered one of the largest karstic aquifers in Mexico and the world [8]; a system that includes Cozumel Island, which was once part of the continental YP geologic plate of the YP (~25 Mya). Like all karstic aquifers, underground aquatic systems are extended (freshwater or anchialine); sinkholes and some superficial lagoons are the surface features of karstic environments [9,10]. Cozumel island has some similar aquatic systems to those mentioned before, but they differ in area, size, and depth [11,12].

### 2.2. Zooplankton Analysis

During the last 15 years, a systematic sampling of freshwater and anchialine zooplankton has been performed on the YP and Cozumel Island (Figure 1), with high zooplankton

diversity and crustacean endemism [4–6,13]. Water samples for biotic and abiotic variables were collected on the southern part of the island, after the atypical influence of tropical storms in September–October 2020 on Cozumel Island [14].

The species classification of Copepoda was performed according to the methods outlined by Elías-Gutiérrez et al. [15] and Suárez-Morales et al. [16], several specimens were compared with the recently described *M. ha*, inventoried in the north-northeastern region of the YP continental plate [3].

Then, the phenetic variability between the continental vs. island populations of *M. ha* was determined, considering the morphological, environmental, and geological features of the locations inhabited by the copepods. Chemical environmental variables, related to the ionic content, and temperatures were measured in situ with a professional-plus YS Datasonde® (Xylem Inc., Yellow Springs, OH, USA). The trophic status of these systems was determined by the chlorophyll *a* concentration [9]. The sequence of the COI gene was used as evidence of the genetic variability between continental and insular populations of *M. ha*.

### 2.2.1. Continental Populations Analysed

1. Adult female holotype, dissected on semi-permanent slide (ECOCH-Z-10319), adult male allotype, dissected, mounted on semi-permanent slide (ECOCH-Z-10320), and 20 adult females and 20 males preserved in 96% ethanol and one drop of glycerine (ECOCH-Z-10321). Cenote 7 Bocas, Quintana Roo, Mexico, 20°52'36" N; 87°02'37" W, the type locality of *Mastigodiatomus ha*.

2. Specimens obtained from Verde Lucero ( $n = 30$ , ECOCH-Z-10327; 20°52'09.57" N, 87°04'37.52" W) and Boca del Puma ( $n = 6$ , ECOCH-Z-10326; 20°52'179" N, 87°03'18" W) in Quintana Roo, Mexico were also analysed.

### 2.2.2. Cozumel Island Population

Twenty-two adult females and 36 adult males from 25 Horas Lagoon (20°18'39.7" N, 86°56'14.2" W) (ECO-CH-Z-10539). The acronym ECOCH-CH-Z refers to the Zooplankton Reference Collection held at El Colegio de la Frontera Sur, Chetumal, Mexico.

The morphological variability between the two groups of populations was analysed with dissected and whole specimens using a compound Nikon Eclipse 50i microscope. Light microscopic images of variable features were captured with a Lumenera Infinity1 Y-IDT camera (Teledyne Lumenera, Ottawa, Canada) and arranged in Adobe Photoshop V. 6.0 following the current taxonomic standards for the genus *Mastigodiatomus* [2,17,18].

The DNA extractions of the COI gene, PCR products, and sequence alignment between populations were conducted following the methods of Ivanova et al. [19], Hebert et al. [20], Prosser et al. [21], and Elías-Gutiérrez et al. [22], in accordance with the protocols of the Barcoding Laboratory of Life (ECOSUR, Chetumal, Mexico). Two specimens from the type area, three from the Verde Lucero, and two from the Boca del Puma were considered the continental populations in the genetic analysis, and 28 specimens from the Cozumel population were all included.

Cluster analyses of these sequences were performed to obtain a graphic representation of the divergences among the specimens by using Molecular Evolutionary Genetics Analysis (MEGA X) software (MEGA Freeware, University of Pennsylvania, Philadelphia, PA, USA).

Sequences were aligned to 600 base pairs (pb) with CLUSTAL W, and the Kimura 2 parameter (KP2) distance model was used to calculate the sequence divergences. Neighbour-joining (NJ) clusters were created with the gamma distribution model.

All sequences > 500 pb were added to the public data dataset named *Mastigodiatomus*, created in the Barcode of Life Data Systems portal (BOLD SYSTEMS, <http://boldsystems.org/index.php>, accessed on 15 April 2021). In the dataset DOI: 10.5883/DS-MMASTIGO (The Barcode of Life Data System, University of Guelph, Guelph, Canada, accessed on

15 April 2021), the individual sequences, trace files, collection data, and primer details are available.

### 3. Results

#### 3.1. Variability of Environmental, Genetic and Morphological Features

*Mastigodiaptomus ha* is a freshwater, free-living diaptomid copepod, that is apparently endemic to the north and northeastern zones of the YP. The aquatic ecosystems inhabited by this species showed the following environmental features: low elevations ( $13.2 \pm 6.0$  masl), oligo-mesotrophic conditions ( $\leq 0.70$  mg/m<sup>3</sup> of chlorophyll *a*), low oxygen concentrations ( $1.5 \pm 1.8$  mg/L), tropical climates ( $26.8 \pm 1.9$  °C), freshwater ( $0.7 \pm 0.2$  ppt), low conductivities ( $1396 \pm 559.2$  µS/cm<sup>3</sup>) (Table 1), and physically the sinkholes where *M. ha* was recorded are type 2, 3, and 4 sinkholes according to Hall [23] (Table 1).

**Table 1.** Limnological characteristics of the aquatic habitats inhabited by *Mastigodiaptomus ha* (Quintana Roo, Mexico). Elev = elevation (masl), T = water temperature, O<sub>2</sub> = dissolved oxygen (mg/L), EC= electrical conductivity (µS/cm<sup>3</sup>), Sal = salinity (ppt), TS = trophic state.

| Place Name    | Coordinates                    | Sinkhole Classification [23]                                     | Elev | T    | O <sub>2</sub> | EC   | Sal | TS           |
|---------------|--------------------------------|--|------|------|----------------|------|-----|--------------|
| Chemuyil      | 20°21'38.7" N<br>87°23'98.1" W | Vertical walls with wide aperture<br>(as a glass)                | 10   | 29.2 | 4.6            | 2274 | 1.1 | Oligotrophic |
| Verde Lucero  | 20°52'08.7" N<br>87°04'37.5" W | Vertical walls with wide aperture<br>(as a glass)                | 18   | 24.7 | 1.8            | 1414 | 0.8 | Mesotrophic  |
| 7 Bocas       | 20°52'35.8" N<br>87°02'37.5" W | Cavern, with lateral entrance<br>leading to a chamber with water | 16   | 25.6 | 0.3            | 1365 | 0.7 | Oligotrophic |
| Boca del Puma | 20°52'17.9" N<br>87°03'18" W   | Cavern, with lateral entrance<br>leading to a chamber with water | 18   | 26.3 | 0.7            | 1170 | 0.6 | Oligotrophic |
| 25 Horas      | 20°18'39.7" N<br>86°56'14.2" W | Superficial lagoon ("aguada")                                    | 4    | 28.6 | 0.2            | 742  | 0.4 | Oligotrophic |

This kind of aquatic ecosystem is common on the northern and the eastern fringe zones of the YP, where the most recent, highly permeable sediments (i.e., Miocene, Pliocene, and Quaternary) are widespread [8,24]. These regions are therefore dominated by underground currents or superficial lagoons that formed recently in geological time [8].

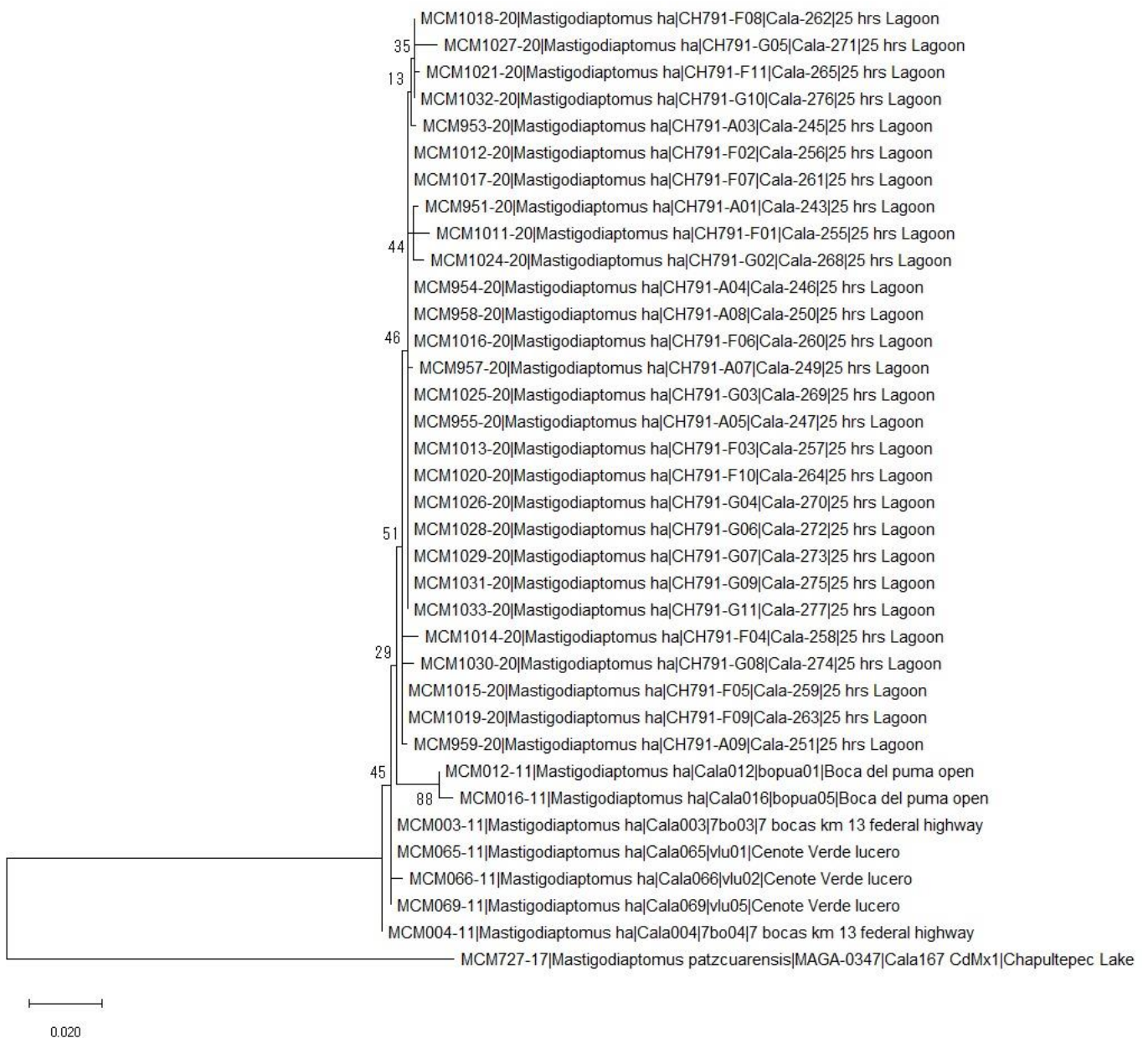
#### 3.2. Genetic Variability

The consensus tree with the highest log likelihood is shown in Figure 2. The bootstrap percentages of trees, in which the associated taxa were clustered together, are shown next to the branches. The extended dendrogram displays that the four continental populations and one island population analysed showed one group with low genetic divergence: the BOLDsystems generated the BIN AAU1038 (The Barcode of Live Data System, University of Guelph, Guelph, Canada, accessed on 15 April 2021). The genetic divergence between populations was 0.33% on average (Table 2).

**Table 2.** Sequence divergence distribution at each taxonomic level examined. To avoid confusion, in this analysis continental vs. island specimens were labelled as two different species, *Mastigodiaptomus ha* vs. *Mastigodiaptomus* sp. (= *M. ha*-island population), to perform a comparison between the populations.

| Label          | N  | Taxa | Comparisons | Min Dist. | Mean Dist. | Max Dist. | SE Dist. |
|----------------|----|------|-------------|-----------|------------|-----------|----------|
| Within species | 36 | 2    | 416         | 0.00      | 0.33       | 2.22      | 0.00     |
| Within genus   | 37 | 1    | 179         | 0.34      | 4.64       | 20.15     | 0.04     |

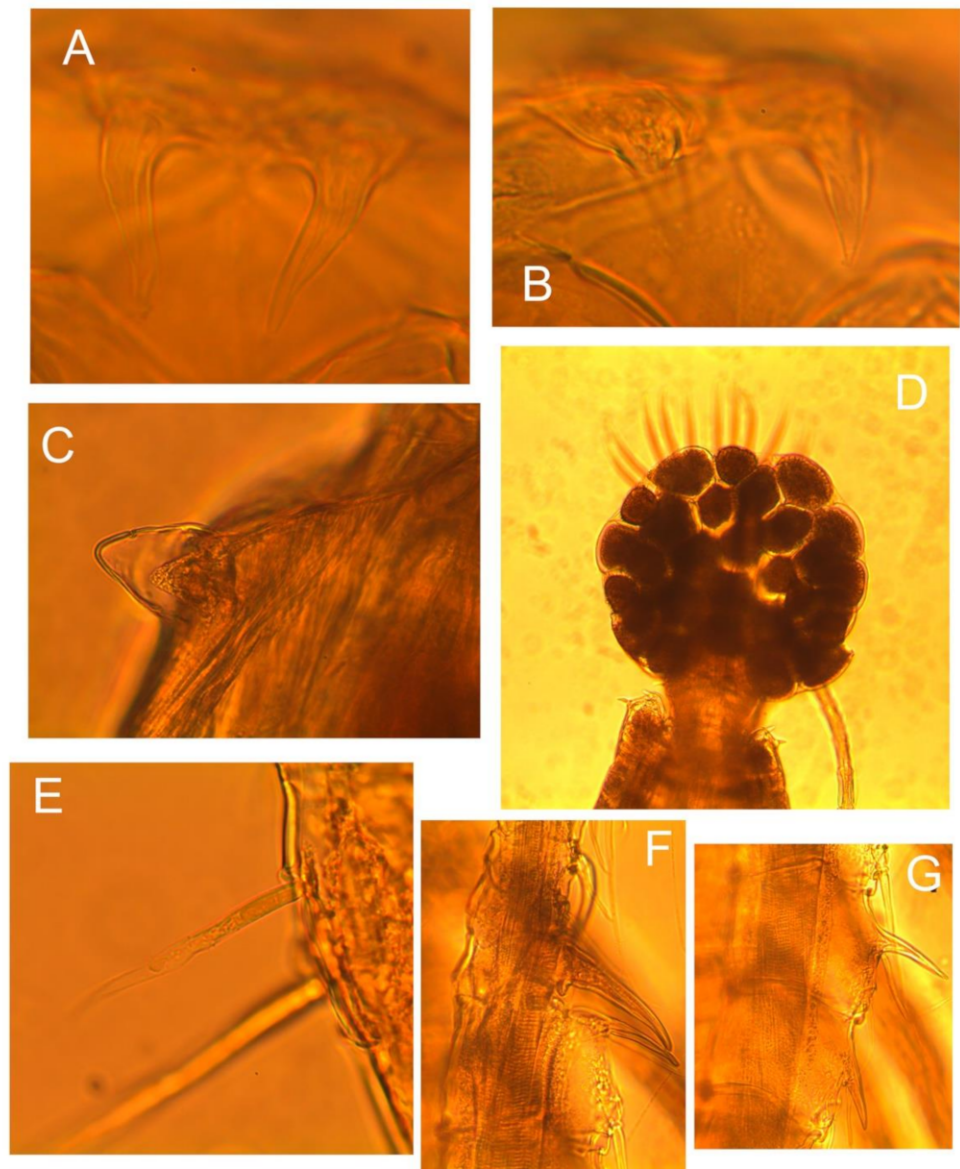




**Figure 2.** Analysis for *Mastigodiptomus ha* (Quintana Roo, Mexico) with the maximum likelihood method to observe genetic relationships between continental and insular populations (1000 replicates), based on the COI gene. The scale length represents the percentage of genetic distance between branches.

The body sizes of specimens of the island population of *M. ha* were larger than those of the continental population specimens; in the continental populations, the total body lengths were similar between females and males, ranging from 1.2 to 1.3 mm; whereas the females of Cozumel island are larger (i.e., 1.6 to 1.78 mm,  $n = 28$ ); males are also larger in the island population (1.45 to 1.5 mm,  $n = 35$ ).

The rostral points shape was also variable in all the analysed continental and island populations: short with rounded points, medium-sized with rounded points or even, large and acute in females (Figure 3A–B). Whereas only 20% of the continental females bear a dorsal keel-like process on the fifth prosomite, all the examined females of the island population (~60 specimens) possess one dorsal, triangle-shaped process (Figure 3C). Twenty percent of the surveyed island females bear large egg sacs with 23–30 eggs (Figure 3D), whereas continental females did not bear egg sacs.



**Figure 3.** *Mastigodiatomus ha*, adult female from 25 Horas Lagoon (island population): (A) large curved rostral spines; *M. ha*, adult female from Verde Lucero Lagoon (continental population): (B) short acute rostral spines; *M. ha*, adult female from 25 Horas Lagoon: (C) dorsal process, lateral view; (D) egg sac, dorsal view; (E) modified setae on antennular segment 11. *M. ha*, adult male from 25 Horas Lagoon: (F) right antennule segments 13, 14; (G) same, segments 15, 16.

In *M. ha*, the presence of modified setae on both female and male antennules was notable as a distinctive feature; these setae are large, flattened, and distally expanded (Figure 3E). In the island population, the spines of male right antennular segments 13–16 are proportionally larger than those of all the continental populations (Figure 3F–G).

#### 4. Discussion

After systematic surveys of freshwater and anchialine zooplankton were conducted for approximately 15 years in the YP, including on Cozumel Island, this is the first finding on record of a freshwater, free-living calanoid diaptomid on a Mexican island. In the southern region of Cozumel Island where *M. ha* was collected, the pools are flooded in the rainy period (July to October) and the 25 Horas Lagoon suffers a reduced catchment area during the dry season (March to May), with a proportional decrease in the copepod's population.

Therefore, it is hypothesised that *M. ha* is an endemic form of some freshwater systems located in the north-northeastern regions of the YP, and is probably present on other Caribbean islands with geological histories that are homologous to that of Cozumel Island.

The physiography of the YP has been influenced by successive marine regression and transgression processes: during the Cretaceous Period (~145 Mya) and the Pleistocene Yarmouth interglacial stage (220–170 Kya, during which the sea level rose 30 m above the current level), the YP was submerged [7,25]. The YP and Cozumel have been separate since the early Cenozoic Era (~65 MYa) [26]. Allopatric speciation has been advanced as a possible explanation for the divergence of two species of anchialine remipedes: *Xibalbanus cozumelensis* (island species) and *X. tulumensis* (continental). A weak gene flow between these two populations was deemed probable because the marine regressions likely gave place to intermittent communication between these populations, which is not the case for epigeal freshwater crustaceans.

The eastern coast of the YP remained submerged for about 33 MY, between the Paleocene and Oligocene periods. During these periods of marine regression, freshwater forms could have recolonised the emerged lands. According to Suárez-Morales [7], one of these forms was an ancestor of the genus *Mastigodiptomus*, probably related to *M. albuquerquensis*, currently deemed to represent a species complex. This ancestral form successfully radiated across the Yucatan Peninsula, including its central core and eastern coast [7]. The occurrence records of *M. ha* more or less seem to fit the area flooded by seas in the Pleistocene interglacial period, which suggests that this freshwater species could be relatively young (<220–170 Kya).

The most recent soils on the coastal fringe of the peninsula were formed during the Pleistocene Epoch (~1.8 Mya) and the Holocene Epoch (11 Kya) with predominant calcites and dolomites as the most common mineral (of organic origin) [27]. The current physiography of the YP (including its islands and its complex and extensive hypogean landscapes) was present approximately 8–6 Kya [7,27,28]. The specific natures (freshwater, anchialine, or strictly marine) of the cavernous ecosystems in the region were influenced by the intermittent drought/cold periods that took place between 8 and 7, and 6 and 5 Kya [7,28], called stadial and interstadial periods during the Holocene period (~9 Kya). These dry and cold conditions in the YP during the Holocene period [29], the high reproductive rates in copepods, and phoresis of micro-crustaceans by birds [30], probably represented adequate conditions promoting the dispersal of epigeal freshwater copepods through glacial refuges in emerged (probably cold lands), including the presence of *M. ha* in Cozumel Island and the eastern coast of the YP.

Additionally, the north and northeastern zones of the YP are considered to have low elevational contrasts, with plains lower than 20 masl, and these regions are apparently separated from the geologically older Meridional Peninsula by hills with elevation levels of 50 to 300 masl [31]. The groundwater ecosystems that span these plains are home to the greatest diversity of species and the highest number of endemic crustaceans in the YP [32]. In accordance with the distribution of other freshwater fauna, such as fish [33], the distribution of freshwater crustaceans that inhabit the plain region can apparently be explained by these geographic barriers.

The presence of *M. ha* individuals in this region supports the hypothesis that the YP is the area with the greatest known diversity and with the highest endemism of the *Mastigodiptomus* species in the Neotropical region, probably resulting from its habitat diversity and complex geologic history [2,7]. In the YP, seven diaptomid species have been recorded: *Arctodiptomus dorsalis* (Marsh, 1907); *Leptodiptomus novamexicanus* (Herrick 1895); *M. nesus* (Bowman, 1986); *M. reidae* (Suárez-Morales and Elías-Gutiérrez, 2000); *M. maya* (Suárez-Morales and Elías-Gutiérrez, 2000); *M. siankaanensis* (Mercado-Salas, Khodami, Kihara, Elías-Gutiérrez and Martínez-Arbizu, 2018), and *M. ha* (Cervantes-Martínez, 2020). The latter four are considered YP endemics [3,7,34]. The late Eocene marine regression exposed most of the YP [7]. This combination of geological, climatological, and



ecological conditions probably promoted the isolation conditions for local crustacean speciation and its current distributional patterns in the area [7].

Because low genetic divergence was revealed in and between the analysed continental and island populations of *M. ha*, it is hypothesised that the island population did not result from a very recent invasion process by humans, who have occupied the Cozumel island since 300 BC [35]. Instead, it is suggested that both the continental and island populations have an early common origin, and these populations were isolated after the Pleistocene Yarmouth interglacial stage; the slight morphological divergences featured, found within and between the examined populations of *M. ha*, support the previous statement, as well as a lack of evidence that the distribution of the *Mastigodiaptomus* species is promoted through resistance (or diapause) structures.

The genetic divergence found between populations was remarkably low, ranging between 0 and 2.22%: among the *Mastigodiaptomus* species, the greatest differences recorded within populations reach 2.76% on average [2]. The relatively low genetic, inter- and intra-population divergences found in *M. ha* might indicate the young age of the species. These low divergences have been found in freshwater fish whose current distributional ranges were glaciated during the Pleistocene period in America [36], and are probably related to the reduced population size in glacial refuges causing bottlenecks [37].

Morphological variabilities were described among and within the continental populations of *M. ha* in their original description [3]. In this study, when the continental populations were compared with the island population, a morphological variation was observed in body size (with striking differences between females and males), the rostrum shape, and the size of the spiniform process of the male right antennular segments 13–16.

In addition, the presence/absence of a dorsal process in females is a variable feature between and within populations of *Mastigodiaptomus texensis*, *M. amatitlanensis*, *M. albuquerquensis*, *M. montezumae*, and *M. ha* [38]. We speculate here that the variability of this character appears to be related to the presence of fish co-occurring with *Mastigodiaptomus*: the dorsal process is absent or very reduced in presence of predator fish, but mostly present and well-developed when fish predation is absent or negligible (unpublished data).

This is the third report of a *Mastigodiaptomus* species inhabiting Caribbean islands with an elevation below 100 masl: *M. nesus* was recorded on San Salvador Island, the Cayman Islands, the Bahamas, and Cuba; *M. purpureus* in Cuba [39], and *M. ha* in Cozumel (this report). Five species of this genus have been recorded at altitudes  $\geq 1000$  masl: *M. montezumae* [40], *M. patzcuarensis*, *M. albuquerquensis* [3,41], *M. suarezmoralesi* [38], and *M. amatitlanensis* [3]. It is likely that the ability of diaptomid genera to dwell in wide elevation and latitudinal ranges is a factor promoting the high regional diversity of *Mastigodiaptomus*.

Individuals of *Mastigodiaptomus* that inhabit island populations appear to be larger on average than their continental counterparts; for instance *M. purpureus*, exclusively recorded in Cuba, is one of the largest species (females = 2.5 mm long, males = 2.2 mm) [42,43]. The length of *M. nesus* in Cuban populations is 1.48 mm in females, and 1.34 in males [39] vs. 0.9–1.0 mm in both sexes in the continental populations [3,44]; the length of *M. ha* from Cozumel is 1.73 mm in females and 1.5 mm in males vs. 1.2–1.3 mm in both sexes in the YP continental populations [3]. As discussed above, it is likely that the presence of fish is related to decreasing copepod size, because in all mentioned cases in which larger island specimens occur, fish are absent.

## 5. Conclusions

After almost two decades of basic study on freshwater and anchialine zooplankton on Cozumel Island, this is the first report on *Mastigodiaptomus ha* (Cervantes-Martínez, 2020), a freshwater, free-living diaptomid copepod on a Mexican island.

This copepod was previously recorded in freshwater continental ecosystems on the north and northeastern region of the continental plate of the YP; this region is historically



and geologically similar to Cozumel Island, and slight morphologic and genetic differences were found between the continental and the island populations of *M. ha*.

Because low genetic divergences were observed between the analysed continental and island *M. ha* populations, both populations of the species probably resulted from the same founder effect.

The YP is the region in which the greatest number of copepod inventories has been documented and the greatest known diversity of the *Mastigodiatomus* species has been confirmed among the central and northern regions of Mexico, Central America and the Caribbean.

Finally, analyses combining morphological and genetic characteristics in the continental populations of freshwater copepods (and other zooplankton groups) are still scarce. These kinds of studies are even more rare for insular, freshwater organisms. Our study is the first work in Mexico and the Caribbean region, which combined the genetic and morphological tools in research on the freshwater copepod diversity in insular freshwater bodies.

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**Institutional Review Board Statement:** We collected from several freshwater ecosystems in Mexico. Zooplankton is not under any protection by Mexican laws; thus, no specific permits for this type of field studies are needed.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** All sequences the public dataset with the name *Mastigodiatomus* was created in BOLD database and is available in: DOI: 10.5883/DS-MMASTIGO.

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## Article

# Historical Zooplankton Composition Indicates Eutrophication Stages in a Neotropical Aquatic System: The Case of Lake Amatitlán, Central America

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**Abstract:** This paper presents a study of freshwater zooplankton biodiversity, deemed as a reliable indicator of water quality. The Guatemalan Lake Amatitlán, currently used as a water source, has shown signs of progressive eutrophication, with perceptible variations of the local zooplankton diversity. Biotic and abiotic parameters were determined at four sites of Lake Amatitlán (Este Centro, Oeste Centro, Bahía Playa de Oro, and Michatoya) in 2016 and 2017. The local composition, the species richness and abundance of zooplankton, and the system environmental parameters were analyzed during both years surveyed. Biological data suggesting eutrophication of this tropical system were obtained, including a high rotifer abundance (11 species: the rotifers *Brachionus havanaensis* (109 ind L<sup>-1</sup>) and *Keratella americana* (304 ind L<sup>-1</sup>) were the most abundant species in this lake). The presumably endemic diaptomid copepod species, *Mastigodiaptomus amatitlanensis*, was absent in our samples, but we report the unprecedented occurrence of two Asian cyclopoid copepods (i.e., *Thermocyclops crassus* and *Mesocyclops thermocyclopoides*) for Lake Amatitlán and Guatemala. The presence of larger zooplankters like adults and immature copepods (i.e., *Arctodiaptomus dorsalis*) and cladocerans (*Ceriodaphnia* sp.) at site “Este Centro” indicates a relatively healthy zooplankton community and represents a focal point for managing the conservation of this lake.

**Keywords:** conservation; eutrophication; exotic species; tropical lakes; zooplankton

## 1. Introduction

The knowledge of zooplankton in the Neotropical region is growing with fragmented studies. Therefore, it is likely that the species richness of zooplanktonic taxa is underestimated because of the presumably high diversity and scarcity of zooplankton taxonomists [1–3]. In addition, the progressive destruction of aquatic habitat and the progressive spread of exotic species threaten native biodiversity, ecosystem health, and environmental services.

The zooplankton community and abundance are closely linked to the trophic state of the water system; for this reason, its diversity has been deemed as an indicator of water quality [4]. In eutrophicated systems (at tropical and temperate latitudes), the dominance of microzooplankton is common, compared with larger organisms, owing to the increased availability of food and water conditions [5,6].



For four decades, the Guatemalan Lake Amatitlán has shown signs of progressive eutrophication related to anthropic factors (i.e., peripheral population growth and urbanization, intensive use of water for agricultural irrigation), thus promoting the advancement towards eutrophication, related to the input of nearly 50% of the untreated residual urban and industrial waters from Guatemala City [7–10]. Because of this, some actions have been proposed to address this problem, either from the governmental level (i.e., Autoridad para el Manejo Sustentable de la cuenca del lago Amatitlán, AMSA 1996) or from descriptive studies of the lake involving the lake zooplankton biodiversity, like those by Basterrechea-Díaz (1997) [7] and Brandorff (2012) [11]; however, studies related with tropical epicontinental waterbodies have been more focused on environmental factors rather than biological community attributes or general limnology [12,13]; thus, the zooplankton biodiversity in Guatemala remains largely unknown [14], with only a few studies in Guatemalan lakes [15,16]. Most studies in Lake Amatitlán and Guatemala are more focused on current data instead of historical analysis.

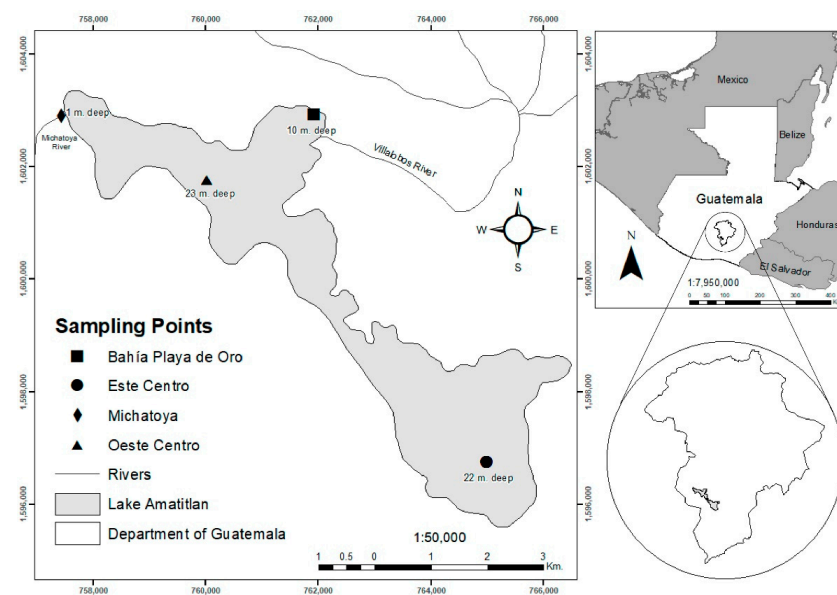
Based on the analysis of both, historical and current data of zooplankton biodiversity and environmental conditions of Lake Amatitlán, we present information on the zooplankton distribution, species richness, abundance, and its relation with successive changes of its trophic state.

## 2. Materials and Methods

### 2.1. Study Sites and Sampling Methods

Lake Amatitlán is the fourth largest lake in Guatemala, Central America, and one of the most emblematic waterbodies of this country. This lake is a warm monomictic waterbody in the highland of Guatemala, located at an altitude of 1186 m above sea level (m.a.s.l.), with an area of 15.2 km<sup>2</sup> and 11 km length and a maximum depth of 23 m. Its formation originated from volcanic activity of Pacaya, Fuego, and Agua in the late Quaternary [10,14,17].

Four sampled sites were considered: Este Centro (EC), Oeste Centro (OC), Bahía Playa de Oro (BPO), and Michatoya (MICH) to analyze the zooplankton species that inhabit the eastern and western regions of Lake Amatitlán (Figure 1). The latter two sites (BPO and MICH) are in the runoff of Villalobos and Michatoya rivers, respectively [14]. Water samples for biotic and abiotic variables were collected for 2016 and 2017 in the rainy (May–October) and dry seasons (November–April).



**Figure 1.** Location of Lake Amatitlán and sampling points for the biotic collection methodology. Water-filtered, vertical, and horizontal trawls as defined by Cervantes-Martínez & Gutiérrez-Aguirre (2015) [2].

### 2.1.1. Species Richness

Zooplankton samples ( $n = 8$ ) were collected by vertical and horizontal trawls with a 45  $\mu\text{m}$  plankton net between 1 and 22 m depth to ensure representative samples to evaluate the species richness in the lake, as it is well known that zooplankton tends to have vertical and horizontal migrations [2].

### 2.1.2. Species Abundance and Abiotic Variables

To estimate the zooplankton abundance, a known volume of water between 30 and 100 L was filtered through a 45  $\mu\text{m}$  zooplankton net. The water was determined with a 2.1 L<sup>-1</sup> capacity Van Dorn bottle [2,18]. Species abundance was determined by the account of two main groups: Rotifera and Copepoda, present in three aliquots of 1 mL each from the filtered samples, then the data were standardized as individuals per liter (ind L<sup>-1</sup>) in each sampled site [19].

Abiotic variables were measured in situ monthly for both years of study and in all the water columns, with the multiparametric probes WTW Cond 197i, WTW Oxi 1970i, and HACH HQ for water temperature ( $^{\circ}\text{C}$ ), pH, oxygen concentration O<sub>2</sub> (mg L<sup>-1</sup>), total dissolved solids (mg L<sup>-1</sup>), and conductivity ( $\mu\text{S cm}^{-1}$ ). With the actual environmental, richness, and zooplankton abundance data, a description of the trophic state of Lake Amatitlán was proposed.

## 2.2. Historical and Actual Records of Zooplankton and Environmental Parameters Analysis

Specific classification of Rotifera, Cladocera, and Copepoda of recently collected samples (collected in 2016 and 2017) was done according to Koste (1978) [20], Fontaneto & De Smet (2015) [21], Elías-Gutiérrez et al., (2008) [22], and Suárez-Morales et al., (2020) [23].

The presence/absence of the current zooplankton inventory was compared with previous surveys by Juday (1915) [24], Basterrechea-Díaz (1997) [7], and the record of copepods from the previous surveys of Wilson (1941) [25] and Brandorff (2012) [11], in order to analyze the historical composition of zooplankton of Amatitlán lake.

Historical environmental data recorded by Juday (1915) [24], Brezonik & Fox (1974) [26], Basterrechea-Díaz (1997) [7], and Ellenberg (2014) [27] were compared with the current data surveyed in this study.

## 3. Results

### 3.1. Species Richness

A total of 15 species of zooplankters including rotifers and crustaceans were found in the lake for 2016–2017 (Table 1); rotifers showed the highest species richness (80% of zooplankton species recorded), while copepods represented 20% of all zooplankton species in the lake.

We provide the first record of two cyclopoid exotic species (*Mesocyclops thermocyclopoides* and *Thermocyclops crassus*) for Lake Amatitlán and Guatemala. The endemic calanoid copepod, *Mastigodiptomus amatitlanensis*, was absent in our current survey and the record of *Arctodiptomus dorsalis* in Lake Amatitlán was confirmed here. Cladoceran crustaceans were very scarce in our samples; only a single specimen of *Ceriodaphnia* sp. was observed. The Brachionidae was the family with the highest species richness among rotifers in 2016 and 2017 (Table 1).

Nowadays, the east region (site EC) of Lake Amatitlán had the highest species richness in the lake (14 species), compared with the western region (9 species including the exotic *T. crassus* at OC). The largest zooplankters of the lake, including the cladoceran *Ceriodaphnia*, (~2 mm) [21], the calanoid copepod *A. dorsalis*, and the cyclopoid copepod *M. thermocyclopoides*, occurred in eastern region.

**Table 1.** Current and historical records of zooplankton species richness in Lake Amatitlán. Currently recorded species are shown in columns (1) EC, (2) OC, (3) BPO, and (4) MICH. Historical records are shown in columns 5–8, following data by Brandorff (2012) [11]; Basterrechea-Díaz (1997) [7]; Wilson (1941) [25]; and Juday (1915) [24], respectively. Presence (x), absence (-), new records (\*).

| Species   | Current Data |   |   |   | Historical Data |   |   |   |
|---|--------------|---|---|---|-----------------|---|---|---|
|   | 1            | 2 | 3 | 4 | 5               | 6 | 7 | 8 |
| <b>Phylum: Rotifera Monogononta: Ploimida</b>                 |              |   |   |   |                 |   |   |   |
| <b>Family: Epiphanidae Haring, 1913</b>                       |              |   |   |   |                 |   |   |   |
| <i>Epiphanes macroura</i> Barrois & Daday, 1894 *             | x            | x | - | - | -               | - | - | - |
| <b>Family: Brachionidae Ehrenberg, 1838</b>                   |              |   |   |   |                 |   |   |   |
| <i>Anuraeopsis fissa</i> (Gosse, 1851) *                      | x            | - | - | - | -               | - | - | - |
| <i>Brachionus angularis</i> (Gosse, 1851) *                   | x            | x | x | x | -               | - | - | - |
| <i>B. calyciflorus</i> Pallas, 1766 *                         | x            | x | x | x | -               | - | - | - |
| <i>B. plicatilis</i> Müller, 1786 *                           | x            | - | - | x | -               | - | - | - |
| <i>B. havanaensis</i> Rousselet, 1911 *                       | x            | x | x | x | -               | - | - | - |
| <i>Keratella</i> sp.  | -            | - | - | - | -               | x | - | - |
| <i>K. americana</i> Carlin, 1943 *                            | x            | x | x | x | -               | - | - | - |
| <i>K. cochleraris</i> (Gosse, 1851)                           | -            | - | - | - | -               | - | - | x |
| <b>Family: Trichocercidae Haring, 1913</b>                    |              |   |   |   |                 |   |   |   |
| <i>Trichocerca</i> cf. <i>longiseta</i> (Schränk, 1802) *     | -            | x | x | x | -               | - | - | - |
| <i>T. pusilla</i> (Lauterborn, 1898) *                        | -            | x | x | x | -               | - | - | - |
| <b>Family: Asplanchnidae Eckstein, 1883</b>                   |              |   |   |   |                 |   |   |   |
| <i>Asplanchna sieboldi</i> (Leydig, 1854) *                   | x            | x | x | - | -               | - | - | - |
| <b>Flosculariaceae: Family: Trochosphaeridae Haring, 1913</b> |              |   |   |   |                 |   |   |   |
| <i>Filinia longiseta</i> (Ehrenberg, 1834)                    | x            | x | x | x | -               | - | - | x |
| <i>F. terminalis</i> (Plate, 1886) *                          | x            | x | x | - | -               | - | - | - |
| <b>Subclass: Bdelloidea *</b>                                 |              |   |   |   |                 |   |   |   |
|   | x            | - | - | - | -               | - | - | - |
| <b>Superclass: Crustacea Brachiopoda:</b>                     |              |   |   |   |                 |   |   |   |
| <b>Cladocera: Anomopoda</b>                                   |              |   |   |   |                 |   |   |   |
| <b>Family: Daphniidae Straus, 1820</b>                        |              |   |   |   |                 |   |   |   |
| <i>Daphnia</i> sp.  | -            | - | - | - | -               | x | - | - |
| <i>D. hyalina</i> Leydig, 1860                                | -            | - | - | - | -               | - | - | x |
| <i>Ceriodaphnia</i> sp.                                       | x            | - | - | - | -               | x | - | x |
| <i>C. lacustris</i> Birge, 1893                               | -            | - | - | - | -               | - | - | x |
| <i>C. pulchella</i> Sars, 1862                                | -            | - | - | - | -               | - | - | x |
| <b>Family: Bosminidae Sars, 1865</b>                          |              |   |   |   |                 |   |   |   |
| <i>Bosmina</i> sp.  | -            | - | - | - | -               | x | - | - |
| <i>Bosmina longirostris</i> O. F. Müller, 1776                | -            | - | - | - | -               | - | - | x |
| <b>Family: Chydoridae Stebbing, 1902</b>                      |              |   |   |   |                 |   |   |   |
| <i>Chydorus sphaericus</i> (O.F. Müller, 1785)                | -            | - | - | - | -               | - | - | x |
| <b>Copepoda: Calanoida Family:</b>                            |              |   |   |   |                 |   |   |   |
| <b>Diaptomidae G.O. Sars, 1932</b>                            |              |   |   |   |                 |   |   |   |
| <b>Subfamily: Diaptominae Kiefer, 1932</b>                    |              |   |   |   |                 |   |   |   |
| <i>Arctodiaptomus dorsalis</i> (Marsh, 1907)                  | x            | - | - | - | x               | - | - | - |
| <i>Mastigodiaptomus albuquerquensis</i> (Herrick, 1895)       | -            | - | - | - | -               | - | - | x |
| <i>M. amatitlanensis</i> (Wilson, 1941)                       | -            | - | - | - | -               | - | x | - |
| <b>Copepoda: Cyclopoida Family: Cyclopidae Kiefer, 1927</b>   |              |   |   |   |                 |   |   |   |
| <b>Subfamily: Cyclopinae Kiefer, 1927</b>                     |              |   |   |   |                 |   |   |   |
| <i>Thermocyclops crassus</i> (Fischer, 1853) *                | -            | x | - | - | -               | - | - | - |
| <i>Mesocyclops thermocyclopoideus</i> Harada, 1931 *          | x            | - | - | - | -               | - | - | - |
| <b>Subfamily: Eucyclopinae Kiefer, 1927</b>                   |              |   |   |   |                 |   |   |   |
| <i>Eucyclops serrulatus</i> (Fischer, 1851)                   | -            | - | - | - | -               | - | - | x |
| Nauplii   | x            | x | x | x | -               | x | - | x |
| Juvenile Cyclopoid  | x            | x | x | x | -               | - | - | - |
| Juvenile Calanoid   | x            | x | x | x | -               | - | - | - |

Our revision of the zooplankton community (Table 1) indicates that the historical data presented a great microcrustacean richness with the record of eight cladoceran species (*Daphnia* sp., *D. hyalina*, *Ceriodaphnia* sp. *C. lacustris*, *C. pulchella*, *Bosmina* sp., *B. longirostris*, and *Chydorus sphaericus*) and the three calanoid copepods: *A. dorsalis*, *Mastigodiatomus albuquerqueensis*, and the endemic *M. amatitlanensis*. The historical record of rotifers had the lowest species richness including three monogonont species. In our survey, the rotifer species richness increased significantly with 12 species not hitherto reported from the lake, including the record of organisms from the Subclass Bdelloidea.

### 3.2. Species Abundance

In this study, the total rotifer abundance was 522.7 ind L<sup>-1</sup>. Rotifers represent the most abundant group in the lake; their numerical abundance is considerably higher than that recorded for copepods, including immature stages (7.1 ind L<sup>-1</sup>). Cladocerans were almost absent from our samples.

Species with the highest abundance at all sites were the rotifers *B. havanaensis* (109 ind L<sup>-1</sup>) and *K. americana* (304 ind L<sup>-1</sup>), with a considerably lower abundance in the eastern area (9.3 and 121.8 ind L<sup>-1</sup>, respectively). Species of the family Brachionidae were the most abundant mainly in the western region (sites OC, BPO, and MICH), whereas the lowest abundance of rotifers occurred in the eastern region (site EC) (see Table 2).

**Table 2.** Abundance (ind L<sup>-1</sup>) calculated from zooplankton samples for all the studied points of Lake Amatitlán in 2017.

| Species                                 | Abundance (ind L <sup>-1</sup> ) |        |        |        |
|---|----------------------------------|--------|--------|--------|
|   | EC                               | OC     | BPO    | MICH   |
| <i>Brachionus angularis</i>             | 0.00                             | 0.00   | 71.56  | 1.87   |
| <i>Brachionus calyciflorus</i>          | 0.70                             | 0.70   | 85.56  | 4.67   |
| <i>Brachionus plicatilis</i>            | 0.00                             | 0.47   | 0.00   | 0.00   |
| <i>Trichocerca</i> cf. <i>longiseta</i> | 0.00                             | 0.00   | 14.00  | 12.60  |
| <i>Trichocerca pusilla</i>              | 0.00                             | 8.40   | 13.22  | 11.20  |
| <i>Asplanchna sieboldi</i>              | 1.40                             | 1.17   | 2.33   | 0.93   |
| <i>Filinia longiseta</i>                | 0.00                             | 1.40   | 28.00  | 13.07  |
| <i>Filinia terminalis</i>               | 8.17                             | 49.23  | 35.00  | 60.20  |
| <i>Brachionus havanaensis</i>           | 9.33                             | 153.53 | 108.89 | 165.20 |
| <i>Keratella americana</i>              | 121.80                           | 432.60 | 265.22 | 408.33 |
| Nauplii                                 | 3.50                             | 2.57   | 3.11   | 1.40   |
| Juvenile Cyclopoid                      | 5.83                             | 1.63   | 1.56   | 0.93   |
| Juvenile Calanoid                       | 2.80                             | 0.47   | 3.89   | 0.47   |
| <i>M. thermocycloides</i>               | 0.23                             | 0.00   | 0.00   | 0.00   |

The local copepod abundance was represented mainly by nauplii and juvenile stages of Calanoida and Cyclopoida (average = 2.6, 2.5 and 1.9 ind L<sup>-1</sup>, respectively), values resembling those recorded for the Rotifera like *B. plicatilis* (1.1 ind L<sup>-1</sup>) and *A. sieboldi* (2.3 ind L<sup>-1</sup>) in all the study sites, compared with adult copepods, where the abundance of the adult *M. thermocycloides* present only in EC was 0.23 ind L<sup>-1</sup>.

### 3.3. Environmental Variables

Environmental variables values in both analyzed years, in general, presented basic pH values (>8 ± 0.33), dissolved oxygen showed an average of 4.76 ± 5.21 and 4.65 ± 4.92 mg L<sup>-1</sup>, whereas temperature averaged 24 ± 1.31 °C, conductivity presented average values of 655.95 ± 59.52 and 678.23 ± 68.29 µS cm<sup>-1</sup>, and finally TDS showed average values of 339.99 ± 47.35 and 341.43 ± 30.30 mg L<sup>-1</sup>, respectively (Table 3).



**Table 3.** Historical and current environmental mean data of the water column recorded by previous surveys and this study. Juday (1915) [24], Brezonik & Fox (1974) [26], Basterrechea-Díaz (1997) [7], Ellenberg (2014) [27]. ND: no data available.

| Environmental Variables                                | 1910 [24] | 1969 [26] | 1985–1995 [7] | 2008–2013 [27] | 2016 *** | 2017 *** |
|--|-----------|-----------|---------------|----------------|----------|----------|
| pH   | ND        | 7.70      | 7.75          | 8.69           | 8.26     | 8.33     |
| Water temperature (°C)                                 | 19.86     | ND        | 22.75         | 25.00          | 24.46    | 24.23    |
| Conductivity ( $\mu\text{S cm}^{-1}$ )                 | ND        | 830       | 802           | 682            | 655.95   | 678.23   |
| TDS ( $\text{mg L}^{-1}$ )                             | ND        | ND        | 610           | ND             | 339.99   | 341.43   |
| Dissolved oxygen O <sub>2</sub> ( $\text{mg L}^{-1}$ ) | 4.74 *    | 8.40 **   | 4.20          | 8.90           | 4.76     | 4.65     |

\* Data originally recorded in cubic centimeters per liter of water. \*\* Original data recorded at surface. \*\*\* Data recorded in this study.

The historical data presented in Table 3 show pH with slightly neutral values in 1969 to 1985–1995, whereas in the first two decades of the XXI century, the pH increased to reach clearly basic values, over 8. The water temperature changed along the time, 19.86 °C in 1910 to 24.23 °C in 2017. Conductivity and total dissolved solids decreased on average by 18.29 and 44.10%, respectively.

#### 4. Discussion

The environmental parameters surveyed in this study can show the progressive eutrophication on Lake Amatitlán, according to the historical data recorded by authors like Juday (1915) [24], Brezonik & Fox (1974) [26] Basterrechea-Díaz (1997) [7], and Ellenberg (2014) [27]. The historical change in environmental and biological variables could reveal strong evidence of the current eutrophication of this lake. For instance, the observed changes of pH values, that is, an average of 8.26 and 8.33 in 2016–2017, differ in contrast from the values recorded in 1969 (7.70) [26], 1985–1995 (7.75) [7], and 2008 (9.3) [17].

The basic pH and the high concentration of dissolved oxygen at the surface promoted an increase of microzooplankters, like rotifers (especially *B. havanaensis* and *K. americana*), and a decrease of larger species like cladocerans and adult copepods, indicators of the system trophic state per se. Similar conditions have been recorded in American eutrophicated subtropical and tropical water bodies [4,28,29] as well as in other water bodies (i.e., temperate coastal water bodies) in which the replacement of larger copepod with smaller ones has been reported to the result from the eutrophication process [6].

Recently, phytoplankton blooming has been described as a consequence of this eutrophication progress in Lake Amatitlán, presenting a high concentration mainly in *Microcystis* sp. and *Dolichospermum* sp. cyanobacteria preceded by the diatom algae *Nitzschia* sp. at the surface of the lake [9], which in turn allows herbivorous zooplankters like brachionid rotifers to become dominant organisms in eutrophicated epicontinental waterbodies [20].

In earlier studies on Lake Amatitlán, the zooplankton community was largely dominated by cladocerans and copepods. In 1915 [24], zooplankton had a widely different composition compared with our results: rotifers were then the less abundant zooplankton group in the lake ( $0.3 \text{ ind L}^{-1}$ ), preceded by copepods ( $11.6 \text{ ind L}^{-1}$ ) and cladocerans, the most abundant zooplankton group at that time ( $14.4 \text{ ind L}^{-1}$ ). The system trophic state is also related to the zooplankters body size; that is, a stronger level of eutrophication is frequently expressed by a greater abundance and species richness of microzooplankters like small rotifers [4,6,28,29]. A possible explanation of the local absence or scariness of larger zooplankters (i.e., *Ceriodaphnia* sp., adult cyclopoid and calanoid copepods, including *M. amatitlanensis*) could result from the competition for available food [5], eventually explaining the strong dominance of small brachionid herbivorous rotifers like *B. havanaensis* and *K. americana*.

The presence and high abundance of these latter species, together with another species of *Brachionus* and *Keratella* at the east region of Lake Amatitlán, suggest that eutrophic conditions that make food available for these microphagous species [30].

In the case of *A. dorsalis*, this species is widespread in America [31] and has been recorded as an invasive exotic copepod in Asiatic waterbodies [32,33]. The environmental conditions of Lake Amatitlán seem to be adequate for the development of this species be-

cause it shows a selective feeding on phytoplankton; thus, it frequently inhabits moderately to strongly eutrophic environments [31,32], like Amatitlán lake.

It is well known that many diaptomid copepods tend to have restricted distributional patterns and endemic distributions in neotropical lakes [34]. Then, the local absence of the endemic copepod *M. amatitlanensis* in this study could be another indicator of the progressive eutrophication of Lake Amatitlán, because, since its description by Wilson (1941) [25], this species has not been recorded in other regional studies (i.e., Elías-Gutiérrez et al., 2008 [35]; Brandorff, 2012 [11]; and Gutiérrez-Aguirre, et al., 2020 [36]). It is probable that *M. amatitlanensis* occurs in other lakes of Guatemala (or Central America) and it is expected to be collected from adjacent systems. It is also probable that this species dwells at higher depths not easily reached by standard nets.

Our results showed a clear zonation; the eastern region (site EC) diverges from the other sites because of the absence of adjacent rivers (see Figure 1), its distance from the other sampling points (the closest site is OC, 7.04 km away), and its separation from other sites owing to a train riel that divides the lake in two [14]. Therefore, the EC area has the best conservation status of the lake, precisely where we found the greatest species richness and the larger zooplankters, with the copepods *T. crassus* (average body length of 0.56–0.93 mm) [37], *M. thermocycloides* (0.78–0.89 mm) [38], and *A. dorsalis* (0.77–1.13 mm) [31] among them. Thus, it is convenient to consider EC as a potential conservation site as it has better environmental conditions for the conservation and preservation of zooplankton biodiversity.

On the other hand, we report the presence of two exotic cyclopoid copepod species for the Central American Lake Amatitlán and Guatemala country, *M. thermocycloides* and *T. crassus*. *M. thermocycloides* is a native species from Taiwan and is well spread in Asia and Africa, and commonly widespread at tropical latitudes. This species has been recorded in lakes from South Mexico in epicontinental waterbodies from Chiapas state, Mexico, considering that their introduction may be related to anthropic factors (i.e., agriculture and aquaculture) [37,38]. This is the second record of the invasion of this species in Central American countries, as it has been recorded before in Costa Rican water bodies by Collado et al. (1894) [39], and the ecological potential of *Mesocyclops* use as biocontrol of vector mosquitoes like *Aedes aegypti* is well known [40–42]. Therefore, its finding in Guatemalan lakes represents a source for mass culture of this copepod to be used as biocontrol.

*Thermocyclops crassus* is commonly spread at tropical latitudes in Africa, Australia, and Asia; it was also recorded in Laurentian great lakes in the United States of America [43]; recorded for the first time in tropical lakes from Tabasco state, Mexico [37]; as well as in small ponds of San José Province in Costa Rica [39]. Being a thermophilic species, *T. crassus* has a narrow temperature tolerance [44], so it may be a local indicator of the temperature changes in the lake along time.

Finally, the physical, chemical, and biological conditions of the lake have clearly changed over time, from being a lake with oligotrophic characteristics to one with hypertrophic conditions in a relatively short period of time (100 years, approximately), allowing us to follow and describe the stages and speed of the eutrophication process of a large neotropical lake.

## 5. Conclusions

The historical analysis of zooplankton composition in the lake presented in this study reinforces the knowledge of its eutrophic state, suggesting a useful role of the zooplankton as a bioindicator and making possible the visualization of the changes in its composition over time, showing the progressive trophic state towards eutrophic or hypereutrophic conditions.

It is likely that the absence of the endemic species *M. amatitlanensis* is a warning sign regarding the accelerated loss of biodiversity and reinforces the idea that zooplankton is a great tool as a bioindicator of the health status for continental aquatic ecosystems, in both tropical and temperate latitudes.

Further studies analyzing bottom sediments to search resting eggs of zooplankton in Lake Amatitlán and around it can answer the question of the absence of *M. amatitlanensis*, where this type of knowledge is also scarce in inland aquatic systems of the region.

Finally, is convenient to consider the isolated site EC as a focal point for conservation as it presents better environmental conditions for the conservation and preservation of zooplankton biodiversity, owing to the record of the largest zooplankters found in this site.

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



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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  $53.64 \pm 10.58$  mg/L. Exposed organisms of *Paracyclops chiltoni* showed arsenic accumulation (up to  $9.6 \pm 5.4$  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.

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## 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<sup>2</sup>, 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<sub>3</sub> 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<sub>3</sub> (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<sup>2</sup> 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<sub>3</sub> 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  $7.77 \pm 1.9$  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  $7 \times 7$  µm beam spot size,  $4 \times 4$  µm pixels and 100 ms dwell time. Maps were then deattenuated 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

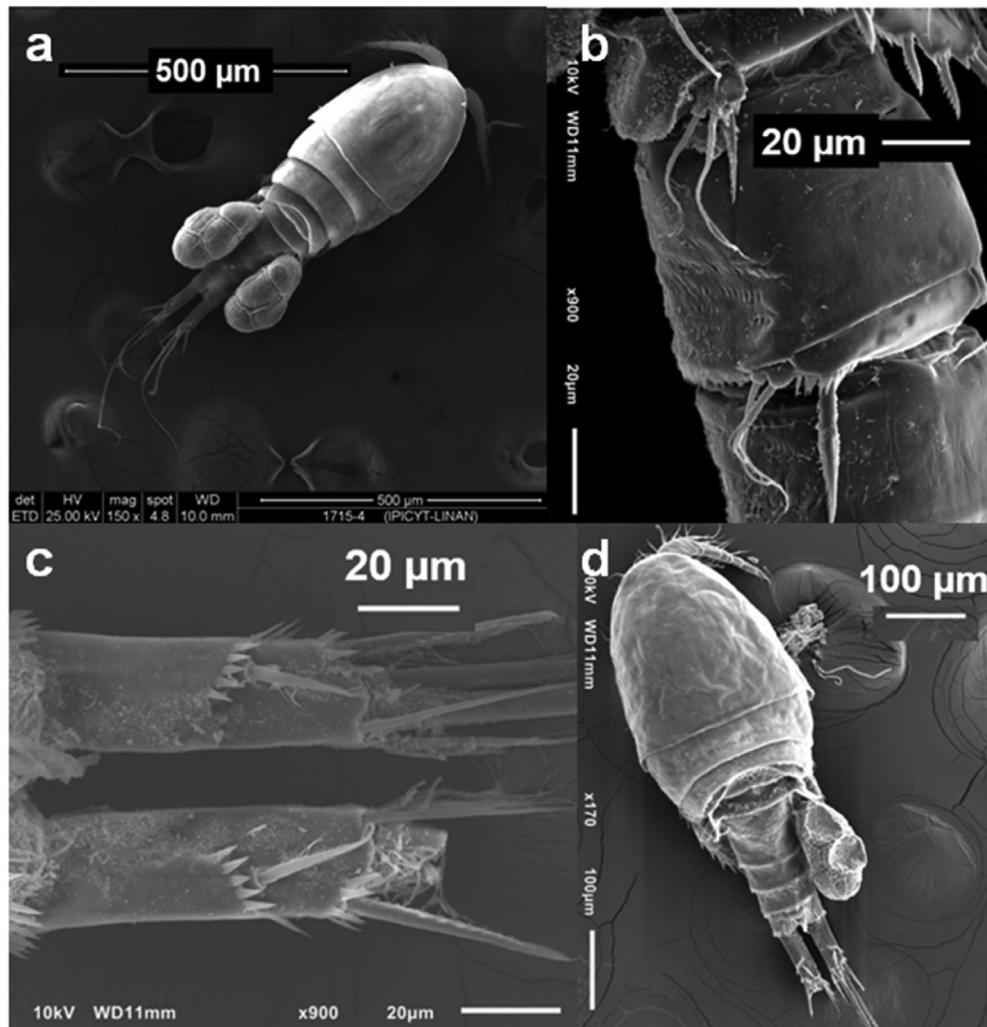
In the study area, the average ambient temperature was  $19.2 \pm 2.9$  °C during the sampling period. Average water temperature, pH, EC, ORP and DO were  $20.9 \pm 1.6$  °C,  $6.9 \pm 0.2$ ,  $3.59 \pm 1.24$  mS/cm,  $322 \pm 15$  mV, and  $2.9 \pm 1.5$  mg/L, respectively (Table S1, Supplementary Material). 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  $53.64 \pm 10.58$  mg/L, greatly exceeding those commonly found in natural waters (which range from  $5 \times 10^{-5}$  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  $437.45 \pm 140.49$  mg/L,  $46.68 \pm 10.15$  mg/L,  $181.68 \pm 96.41$  mg/L and  $9.46 \pm 3.16$  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  $277.13 \pm 63.93$  mg/L,  $2287.3 \pm 890.38$  mg/L,  $189.13 \pm 8.67$  mg/L,  $32.79 \pm 6.32$  mg/L,  $2.00 \pm 0.13$  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.

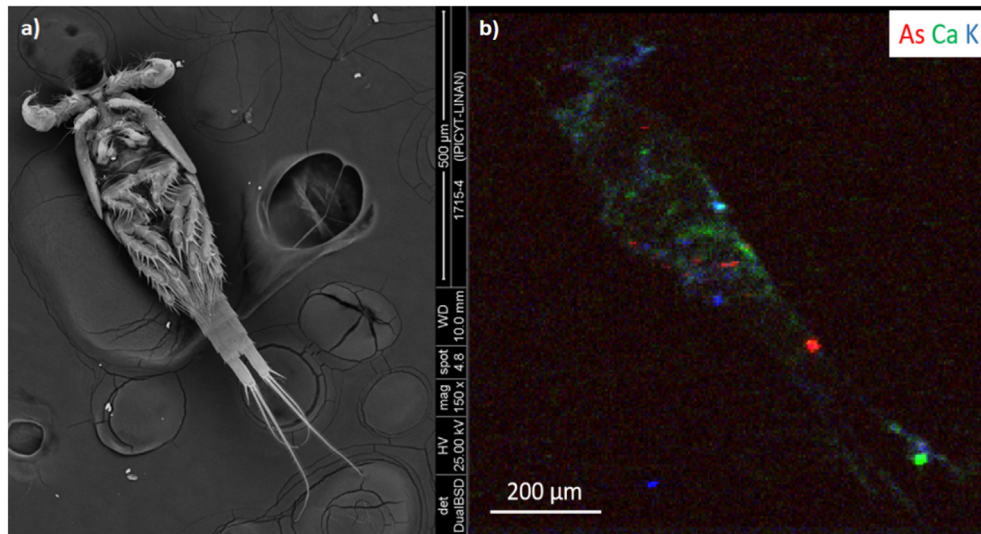
Table 1 shows total abundance, female and male abundance, copepodite and nauplii abundance, female and male length as well as sex ratio. Total average abundance ( $8.25 \pm 10.77$  ind/L) was generally within the abundance values reported for copepods

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  $8:1 \pm 8.9:1$  (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 (Kjørboe, 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.

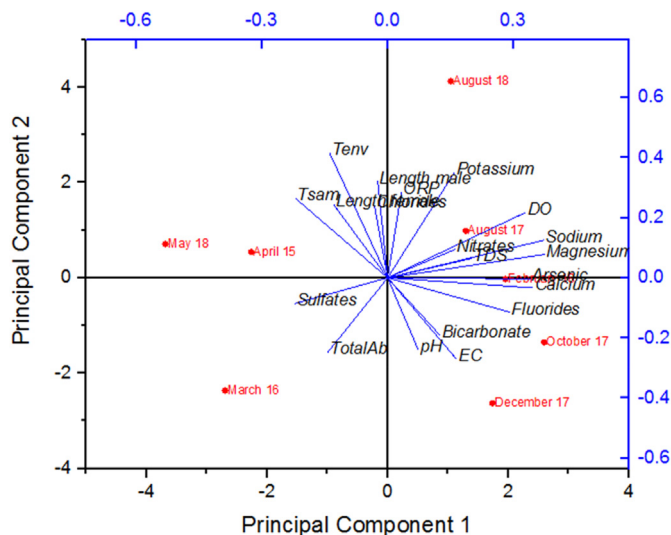
|                         | April15 | March16 | August17 | October17 | December17 | February18 | May18  | August18 | Mean   | SD    | Min    | Max    |
|-------------------------|---------|---------|----------|-----------|------------|------------|--------|----------|--------|-------|--------|--------|
| 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  $-22\text{ }^{\circ}\text{C}$  and 11966.7 eV with  $4 \times 4\text{ }\mu\text{m}$  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.

Arsenic concentration in *Paracyclops chiltoni* was  $9.6 \pm 5.4\text{ mg/kg}$ , 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



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

**Table 2**

Eigenvectors.

|                               | Coefficients of PC1 | Coefficients of PC2 | Coefficients of PC3 |
|-------------------------------|---------------------|---------------------|---------------------|
| Tenv                          | -0.139              | <b>0.417</b>        | -0.052              |
| Tsam                          | -0.220              | 0.265               | -0.187              |
| pH                            | 0.073               | -0.240              | <b>0.355</b>        |
| EC                            | 0.164               | -0.271              | 0.065               |
| TDS                           | 0.200               | 0.065               | 0.077               |
| ORP                           | 0.033               | <b>0.286</b>        | <b>0.312</b>        |
| DO                            | <b>0.329</b>        | 0.218               | 0.104               |
| Ca <sup>2+</sup>              | <b>0.346</b>        | -0.033              | 0.025               |
| Mg <sup>2+</sup>              | <b>0.374</b>        | 0.078               | 0.019               |
| Na <sup>+</sup>               | <b>0.372</b>        | 0.125               | 0.122               |
| K <sup>+</sup>                | 0.159               | <b>0.350</b>        | -0.058              |
| HCO <sub>3</sub> <sup>-</sup> | 0.125               | -0.191              | <b>0.265</b>        |
| SO <sub>4</sub> <sup>2-</sup> | -0.221              | -0.085              | 0.072               |
| Cl <sup>-</sup>               | -0.031              | 0.241               | -0.355              |
| NO <sub>3</sub> <sup>-</sup>  | 0.160               | 0.095               | -0.409              |
| F <sup>-</sup>                | 0.291               | -0.114              | -0.323              |
| As                            | <b>0.339</b>        | -0.003              | -0.084              |
| TAb                           | -0.142              | -0.246              | -0.088              |
| LF                            | -0.128              | 0.244               | <b>0.321</b>        |
| LM                            | -0.023              | <b>0.321</b>        | <b>0.329</b>        |





## 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

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envpol.2021.117155>.

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