

Morphology and Ecological Characteristics of *Paracyclops Novenarius* Reid, 1987: A Cyclopoid Copepod Inhabiting a Highly Contaminated Aquifer in Central-North of Mexico

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Abstract

This work reports the freshwater copepod *Paracyclops novenarius*, in a water body with high arsenic concentrations. Morphologic analysis, abundances, body size of the copepod and physical and chemical variables of the water (including arsenic concentrations) were evaluated in two different climatic seasons (rainy and dry). Morphological analysis showed that the high arsenic concentrations do not affect the morphology of *P. novenarius*, including all its development and adult instars. The highest abundances of this species were found in the dry season for all development stages, with values of 1.51 and 1.50, 4.46, 0.21 ind/L⁻¹, for nauplii, copepodites, females, and males, respectively. However, these values are lower than other aquatic systems of the region and the world, without polluting agents. The highest arsenic concentration was found in the dry season (58 mg/L⁻¹) and the lowest during the rainy (54.5 mg/L⁻¹). The body size of the analyzed organisms was larger in rainy compared to dry, with an average of 637.2 ± 42 µm for females and 650 ± 37 µm for males. A Mann-Whitney U test showed significant differences in lengths by season and by arsenic concentration (U = 1284.5, U = 1284.5; p < 0.05). The results of this study could provide information for environmental impact assessments on aquatic systems.

1 Introduction

Arsenic (As) is a natural element present in the environment, including aquatic systems; however, in recent years, anthropogenic activities (e.g., mining, metallurgical processes, fossil fuel combustion, pesticide use) have caused an increase in average concentrations in epicontinental water bodies (Ravenscroft et al. 2009; Gutiérrez and Gagneten 2011). For example, in Matehuala, San Luis Potosi, Mexico, high concentrations of arsenic (up to 158 mg/L⁻¹) have been reported due to metallurgical wastes (Razo et al. 2004; Martínez-Villegas et al. 2013; Ruiz-Huerta et al. 2017; Mendoza-Chávez et al. 2021), which would lead to significant alterations in physical-chemistry parameters and generate multiple impacts in the aquatic biodiversity (Moreira et al. 2016).

One of the critical components in the aquatic biodiversity to assess these impacts on water systems is zooplankton, and within this is the class Copepoda H. Milne-Edwards, 1840, which contributes between 55–80% of the zooplankton biomass (Kjørboe 1998; Ordóñez-López and Ornelas-Roa 2003). In the world, 14,000 species are known, of which 3000 are freshwater species (Suárez-Morales et al. 2020). Moreover, their high sensitivity to physical and chemical changes in the environment make them suitable for use as bioindicators of metal and metalloids contamination (Gutiérrez et al. 2010; Gutiérrez and Gagneten 2011; Gutiérrez et al. 2012; Villagran et al. 2019).

Therefore, its study turns essential to understand better the toxic process in longer-term, such as changes in morphology and population dynamics which acquires great relevance from an ecological and environmental perspective.

In this work, we performed a detailed analysis of morphology, and we evaluated some ecological characteristics of the copepod *Paracyclops novenarius* Reid, 1987 (initially reported as *Paracyclops chiltoni* Thomson, 1882 by Mendoza-Chávez et al. 2021) inhabiting water polluted by arsenic as well as physical and chemical variables of the water (including arsenic concentrations) in two different climatic seasons (rainy and dry).

2 Material And Methods

The study area is in the city of Matehuala, San Luis Potosi, Mexico and corresponds to a shallow water body (< 2 m depth) known as “Club de Tiro”, which is part of an artificial complex of water contaminated with arsenic (Razo et al. 2004; Martínez-Villegas et al. 2013) (Fig. 1). The climate is arid; its annual average temperature is 19.3 °C with an average yearly rainfall of 450 mm. The predominant soil type is calcic to gypsic xerosol with a gradual increment of gypsum towards the center (Razo et al. 2004; CEFIM 2016) (for more details of the study site, see Mendoza-Chávez et al. 2021).

Biological samples were collected in two different seasons registered by INEGI (2002) (rainy = July 2017 and dry = December 2017). All samples were collected with a plankton net of 50 µm mesh by filtering a known volume and were fixed with 96% ethanol (Cervantes-Martínez and Gutiérrez-Aguirre, 2015).

To identify the species and some effect on the development of individuals due to the arsenic concentration, adult females and males collected (10 as a minimum of each collection) were analyzed with light microscopy Nikon Eclipse 50i and scanning microscopy JEOL-SM-6010. Some nauplii and copepodites were included in this analysis. Suárez-Morales et al. (2020) established the procedures for material preservation, preparation, and conservation; biological material is in the reference Collection of Zooplankton of ECOSUR at Chetumal (ECOCH-Z-10508).

Detailed morphology of prosomal and urosomal appendages along the development of the specimens was considered. The terminology for the armament of each appendage followed Huys and Boxshall (1991) and Karaytug and Boxshall (1999): antennule (= A1), antenna (= A2), mandible, maxillule, maxilla, maxilliped (= Md, Mxl, Mx, Mxp, respectively), first to sixth legs (= P1 to P6), Exp (= appendage, exopodal limb), Enp (= appendage, endopodal limb), setae on the first antennular male segment (setation elements A – H).

The abundance of individuals (individuals/L⁻¹) was estimated based on total counts performed with a stereoscopic microscope model Olympus SZ30. Body size (µm) of adult specimens (the number depending on the availability) was obtained by measuring the distance from the head to the furcal ramus, using an optical microscope model Olympus CX21 with a graduated eyepiece (Belmonte et al. 2006). Sizes (both males and females) were compared in function to the season and arsenic concentration with a Mann-Whitney U test. The sex ratio (F:M) was estimated by the relationship between the total number of females (F) and the total number of males (M) sexed (Dur et al. 2012).

In each sampling campaign, were measured *in situ* physical and chemical water variables: dissolved oxygen (mg/L⁻¹), temperature (°C), electrical conductivity (µS/cm³), pH, and salinity (PSU), using a multi-parameter model Hanna Instruments HI9829.

Finally, determination of arsenic in the water samples collected was made using the “Method 200.7 Rev. 4.4: Determination of Metals and Trace Elements in Water and Waste by inductively Coupled Plasma-Atomic Emission Spectrometry” (EPA 1994). The analysis was carried out in the National Laboratory of Agricultural, Medical and Environmental Biotechnology (LANBAMA), of the Potosino Institute of Scientific and Technological Research A.C. (IPICYT). The statistical analysis and the graphics generated were performed in the software OriginPro 2016 V.9.3.

3 Results

Normal and stable development was observed along the different instars, different arsenic concentrations in the media, and different sampled dates between the analyzed specimens (Figs. 2–5). All naupliar stages with the typical Labrum, A1, A2, Md, and one couple of spinulose caudal seta on each side of the body were present in Nauplius II to VI (Fig. 2a-c). Antennule armed with sabre-shaped masticatory process; the maxillule is differentiated as on setose, distal lobe, and first leg bud is differentiated in Nauplius VI (Fig. 2a-c).

As typically, the outer lateral furcal seta (seta III) is placed more proximally during Copepodite I and lateral furcal seta (II) is placed inwards; whereas dorsal seta (VII) is placed near its final place when the copepodite grows to instar V (Fig. 2d-g, 3a, b). Six antennular segments, as well as the first P1-P3, were developed during CV (Fig. 3c-f).

For adults (Figs. 4, 5) morphological features of the observed specimens corresponds to *P. novenarius*, such as the number of antennal segments, and antennal armature in females (8s, 12s, 6s, 5s, 2s + ae, 2s, 2s + ae, 7 s + ae), and males (8s + ae, 4s, 2s, 2s + ae, 2s, 2s, 2s, 2s, 2s + ae, 2s, 2s, 2s, 6s, 3s + ae, 11s + ae). All these features were stable in all the observed specimens of all collections.

Ornamentation of buccal and thoracic appendages corresponds to the mentioned species, including the presence of large setules on coxal, distal margin of P1-P3, and the absence of ornamentation in this distal margin on P4 (at least not identifiable with light microscopy). Furthermore, features related to sexual dimorphisms, such as the ornamentation of antennal basis, Enp3P1, and Enp3P3, also corresponds with *P. novenarius*.

Abundances of *P. novenarius* (including adult males, females and different instars) and arsenic concentrations are shown in Fig. 6. Abundances for copepodites (I-V) were slightly higher in dry (1.50 ind/L^{-1}) than rainy (1.02 ind/L^{-1}), whereas nauplii varied from 0.3 (rainy) to 1.51 ind/L^{-1} (dry). Abundances for adult females varied from 1.03 (rainy) to 4.46 ind/L^{-1} (dry), which were superior to adult males with 0.07 and 0.21 ind/L^{-1} for rainy and dry, respectively. Although the abundances were similar in both seasons, the dry season showed the highest abundances for nauplii, copepodites (I-V) and adults female and male, which corresponds to the highest arsenic concentration (58 mg/L^{-1}). On the other hand, the lowest abundances correspond to the lowest arsenic concentration (54.5 mg/L^{-1}) (Fig. 6).

Body size was large in rainy with $637.19 \pm 42 \mu\text{m}$ for females and $650 \pm 37 \mu\text{m}$ for males. In dry it was $607.3 \pm 28 \mu\text{m}$ and $616.5 \pm 40 \mu\text{m}$, for females and males respectively (Fig. 7). The Mann-Whitney U test demonstrated a significant difference in body size by season ($U = 1284.5, p < 0.05, n = 87$) as well as by arsenic concentration ($U = 1284.5, p < 0.05, n = 87$). Adult sex ratios were skewed towards the dominance of females in both seasons (rainy = 15:1, dry = 21:1) (Table 1).

Table 1
Seasonal sex ratios of *Paracyclops novenarius* in the study area

Season	Number of individuals sexed		Sex ratio F:M
	Females	Males	
Rainy	103	7	15:1
Dry	446	21	21:1

The values obtained for the physical and chemical variables and arsenic concentrations are presented in Table 2. The maximum value of dissolved oxygen was found in rainy (4.0 mg/L^{-1}), whereas the lowest value was found in dry (1.6 mg/L^{-1}). The maximum temperature was found in rainy ($22.8 \text{ }^\circ\text{C}$), and the minimum in dry ($20.0 \text{ }^\circ\text{C}$). The lowest value for electric conductivity was in dry ($3247 \mu\text{S/cm}^3$), whereas the maximum was in rainy ($3407 \mu\text{S/cm}^3$). The pH values were similar with 6.9 in rainy and 7.0 in dry. Salinity values ranged from 1.8 to 1.7 PSU. Arsenic concentration was 54.5 mg/L^{-1} in rainy and increased to 58 mg/L^{-1} in dry.

Table 2
Physical and chemical variables and arsenic concentration for each season

Season	DO (mg/L^{-1})	T ($^\circ\text{C}$)	EC ($\mu\text{S/cm}^3$)	pH	Salinity (PSU)	Arsenic (mg/L^{-1})
Rainy	4.0	22.8	3407	6.9	1.8	54.5
Dry	1.6	20.0	3247	7.0	1.7	58

*DO = dissolved oxygen, T = temperature and EC = electric conductivity

4 Discussion

In the world, around 30 species and subspecies of the genus *Paracyclops* Claus 1893 have been recorded in different types of freshwater habitats, distributed in temperate-cold latitudes and in tropical areas in which the genus tends to present more species (Karaytug and Boxshall 1998a, b; Karaytug et al. 1998; Mercado-Salas and Suárez-Morales 2009). In Mexico, four species of *Paracyclops* have been inventoried (Suárez-Morales et al. 2020). At the study site, previous work reported the presence of the species *P. chiltoni* (Mendoza-Chávez et al. 2021); however, in this work, the detailed morphological analysis by scanning and light microscopy confirmed that it is *P. novenarius*.

This species was reported for the first time in Colombia by Reid (1987), later by Gaviria (1994), and Gaviria and Aranguren (2007), inhabiting artificial asbestos containers, and it is well known that this material is carcinogenic (Barrera et al. 2010). Asbestos is composed of silicate fibers; the mineral is obtained in open quarries or shallow mines (Castellano-Alvarado et al. 1960), and according to its physical characteristics, it can be composed of SiO_4^- . In addition, in the region where *P. novenarius* was reported, the presence of heavy metals such as Cu, Cr, Ni, and Zn, have been reported, which exceed the contamination limits established by the EPA (Collazos-Santos, 2014). Probably, there is a relationship between the habitat of *P. novenarius*, living in environments with some pollutants, but further analysis is required to understand this.

In Mexico, it has not been reported in previous works (Mercado-Salas and Suárez-Morales 2009, 2012; Suárez-Morales 2020); thus, this is the first record of this species in Mexico. Additionally, *P. novenarius* is living in this site that significantly exceeds the concentration of arsenic considered lethal for zooplankton (3 mg/L^{-1}) (Chen et al. 1999), and could be recognized as an extremophile organism due to the ability to thrive in this habitat which for other organisms might be intolerably hostile or even lethal (Rampelotto 2013; Mendoza-Chávez et al. 2021).

The anamorphic development of *P. novenarius* during its naupliar, copepodid and adult instars observed in the freshwater analyzed system was typical of the cyclopoids, even with the extremely high and seasonally variable arsenic concentration in the analyzed population. Some differences were found in comparison with additional freshwater *Cyclopidae* species whose development is known (Dahms and Fernando 1992; Ferrari 2000); for instance, the number of added segments on each appendage or the number of setulae on each appendage segment, but this appears to be more related to the recognizable morphological differences between species, even at the earliest developmental stages (Suárez-Morales et al. 2007), than the effect of the contaminant (arsenic) on the *P. novenarius* morphology.

Morphological differences in other zooplankton groups (*Cladocera* and *Rotifera*) have been recorded because of metals such as Cd, Cu, or Pb (Gama-Flores et al. 2007; Pérez and Hoang 2017; Xue et al. 2017; Araujo et al. 2019; Pérez-Yañez et al. 2019). But to our knowledge, no morphological effect during the development of copepods has been recorded in the presence of metals or metalloids: the analyzed population appears not to be the exception.

Laboratory studies have shown that metals and metalloids affect copepods in a minor way in comparison with cladocerans and rotifers because these are relatively more tolerant to toxic action; this could be explained due to their ability to accumulation of heavy metals in the body (Gagneten and Paggi 2009; Caumette et al. 2011; Mendoza-Chávez et al. 2021).

On average, the abundances of adults were similar to the values reported by Gagneten and Paggi (2009) for the order *Cyclopoidea* inhabiting water polluted by heavy metals ($0.033\text{--}1.844 \text{ ind/L}^{-1}$). However, these values are low in comparison with other copepods inhabiting other aquatic systems without pollutant agents (up to $1,182 \text{ ind/L}^{-1}$) (Gerten and Adrian 2002; Mitsuka and Henry 2002; Cervantes-Martínez et al. 2005; Sarma et al. 2011; Gómez-Márquez et al. 2013; Cervantes-Martínez and Gutiérrez-Aguirre 2015), this suggests that arsenic concentration could play a key role in the abundances of *P. novenarius*; nevertheless, further studies are necessary to confirm this. On the other hand, individuals with egg sacs were observed along the two seasons studied, reflecting a constant development of all stages. Thus, even in these high concentrations of arsenic, *P. novenarius* reproduces; this could explain the presence of copepodites and nauplii in the periods surveyed.

Adult female and male lengths were within the ranges ($570\text{--}880 \mu\text{m}$ for females and $540\text{--}640 \mu\text{m}$ for males) reported by Reid (1987) for this species. On average, males were larger than females, which differs from the sexual dimorphism typically found in *Copepoda*, where males are smaller than females (Hirst and Kiørboe 2014). Statistical results showed a significant difference in body size by season and these variations could be related to factors such as temperature and food availability (Plath and Boersma 2001; Lin et al. 2013).

A significant difference in body size by arsenic concentration was found. This differs from other zooplanktonic groups such as cladocerans, where body growth was not significantly altered by arsenic (Hoang et al. 2007). However, differences in mean length between individuals by season are minimal, compared with other studies where copepods showed a more significant difference in body size but without any pollutant agent (Cervantes-Martínez et al. 2005; Belmonte et al. 2006; Cervantes-Martínez 2021).

According to Fisher's principle, sex ratio (F:M) is expected to be 1:1 in a natural environment or skewed to the sex in which the female invested least in the embryo phase (Hirst et al. 2010). In this study sex ratio was skewed to females in both rainy and dry season with 15:1 and 21:1, respectively, which agreed with adult females outnumbering males in copepods populations (Hirst and Kiørboe 2002; Kiørboe 2006).

Water temperature and dissolved oxygen are variables inversely related (Lewis 1987; Khani and Rajaei 2017; Koralay et al. 2018); nevertheless, in this study, a direct relationship was observed, these variations might be explained more by biological effects (photosynthesis-respiration) than by physical aspects (Cervantes-Martínez and Gutiérrez-Aguirre 2015). pH values were closer to the neutrality, probably due to the limestone buffer, according to Razo et al. (2004) and Grochowska (2020). The electrical conductivity recorded in this work ($3247\text{--}3407 \mu\text{S/cm}^3$) is characteristic of freshwater systems in central-north Mexico due to the dominant processes of evaporation and salt precipitation (Alcocer and Escobar 1996). According to salinity, this system is classified as oligohaline (Strydom et al. 2002). In general, values of physical and chemical variables were lower than the reported in other studies (Copaja et al. 2016; Ali et al. 2016) in water bodies polluted by metals and metalloids.

Finally, arsenic concentrations were within the values (35–155 mg/L⁻¹) reported by Martínez-Villegas et al. (2013) and Mendoza-Chávez et al. (2021) at this site. These values exceeded the Mexican guidelines for the conservation of aquatic life (02. mg/L⁻¹) and for water quality (0.05 mg/L⁻¹) as well as international guidelines (EPA 1994; DOF 1994, 1998).

5 Conclusions

In this study, we reported for the first time in central-north Mexico the neotropical copepod *P. novenarius*. Additionally, this species was found inhabiting high arsenic concentrations. The morphological analysis allows us to conclude that arsenic does not affect the morphology in all development stages, and apparently, it affects some ecological aspects (body size, abundance, sex ratio). Further studies are required to know in detail more specific effects and mechanisms of action of arsenic on the life cycle of *P. novenarius*. Finally, knowing the probable impact of this metalloid on ecological characteristics such as abundance, sizes, distribution, and detailed morphology of plankton in a region recognized for high arsenic concentration in its aquifers, could lay the basis for using regional fauna for health analysis of continental aquatic systems in the region.

Declarations

Ethics approval and consent to participate. We collected from several freshwater ecosystems in Mexico. However, Mexican laws do not protect Zooplankton, thus, no specific permits for this type of field study are needed.

Consent for publication. Not applicable.

Availability of data and materials. The authors declare that the data supporting the findings of this study are available within the article.

Competing interests. The authors declare that they have no competing interests.

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Authors' contribution. All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by JLUC, ACM and MAGA. The first draft of the manuscript was written by JLUC and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Figures

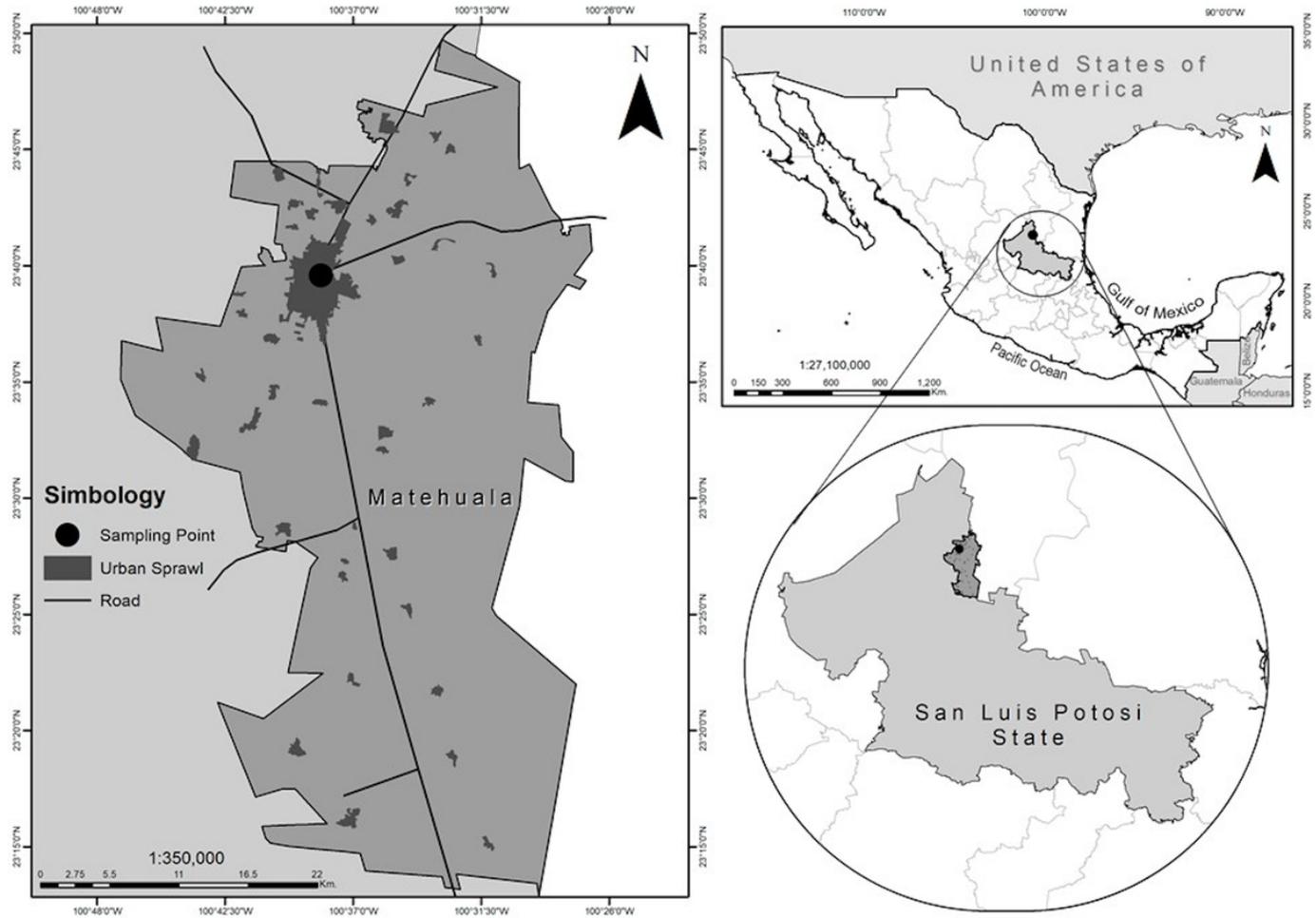


Figure 1

Location of the study area in Matehuala, San Luis Potosi

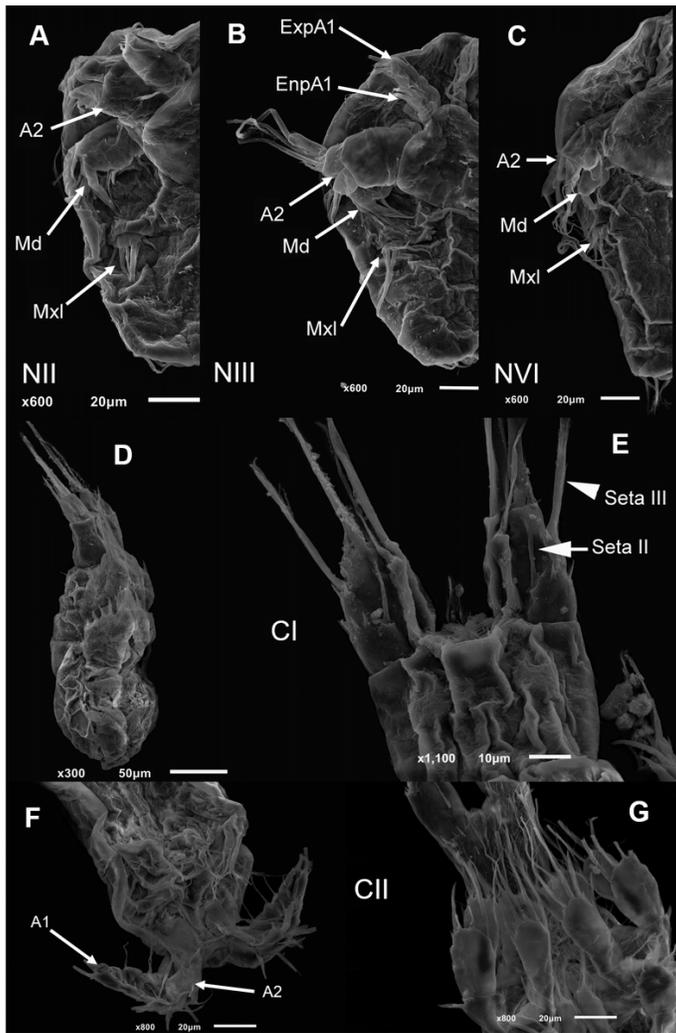


Figure 2
 Paracyclops novenarius, immature stages (collection 2017). a-c) Nauplii II-VI, d) Copepodite CI, lateral e) CI, anal somite and caudal rami, ventral f) Copepodite CII, prosome ventral g) CII, prosome and urosome, ventral

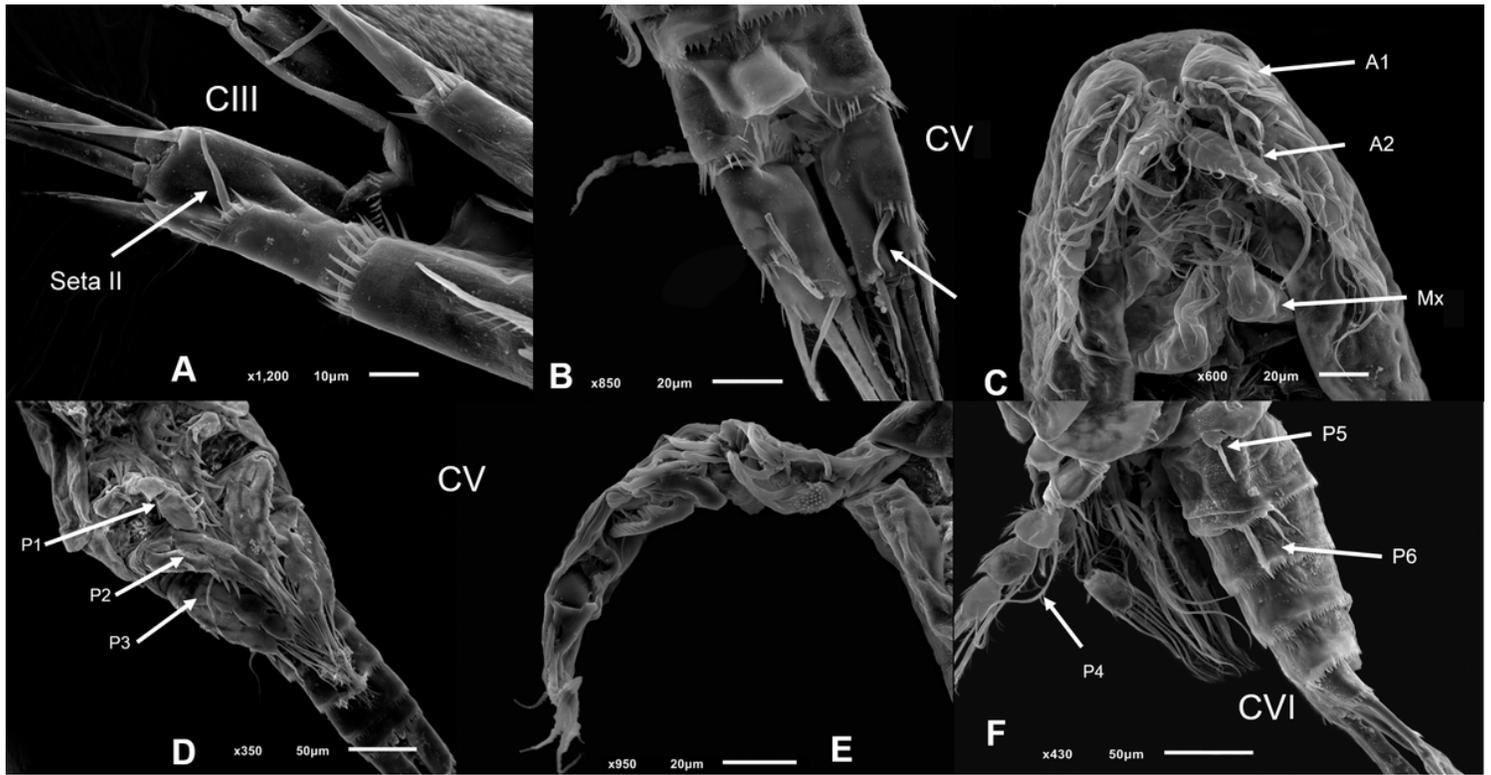


Figure 3
 Paracyclops novenarius, immature stages (collection 2017). A) Copepodite III, caudal rami, B) Copepodite V, anal somite and caudal rami, C) Copepodite V, prosome, ventral, D) Copepodite V, P1-P3, E) Copepodite V, A1, F) Copepodite VI, lateral

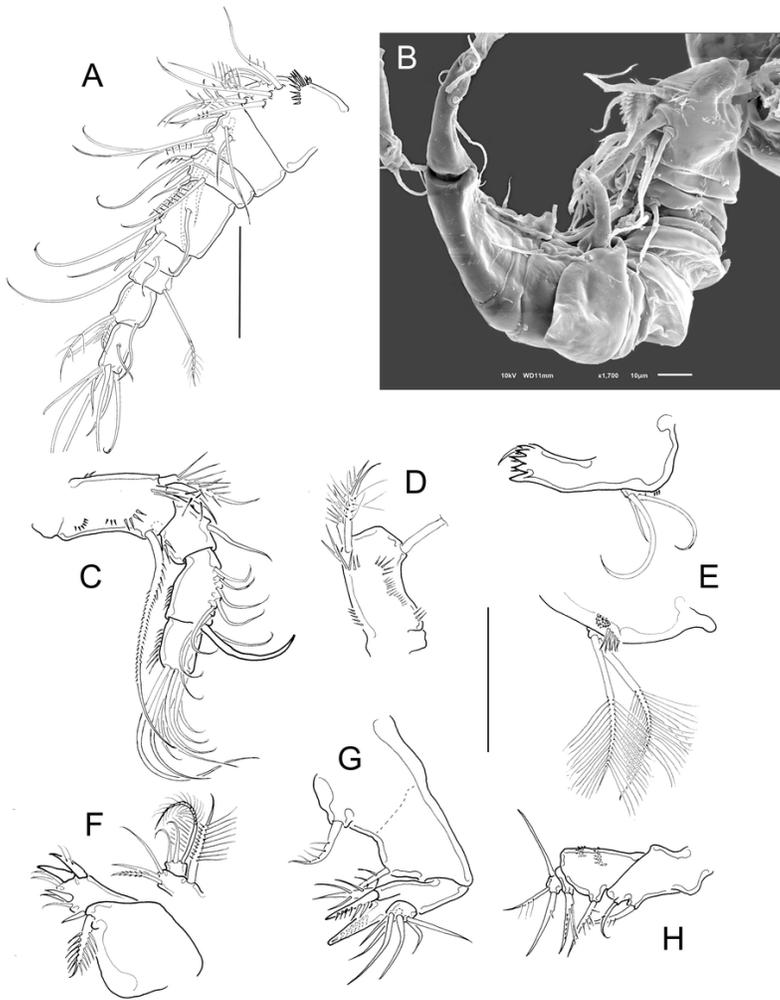


Figure 4

Paracyclops novenarius, adult A) Antennule, female, B) Antennule, male, C) Antenna, female, D) Antenna, basis, male, E) Mandible, posterior, anterior view separated, F) Maxillule, G) Maxilla, H) Maxilliped

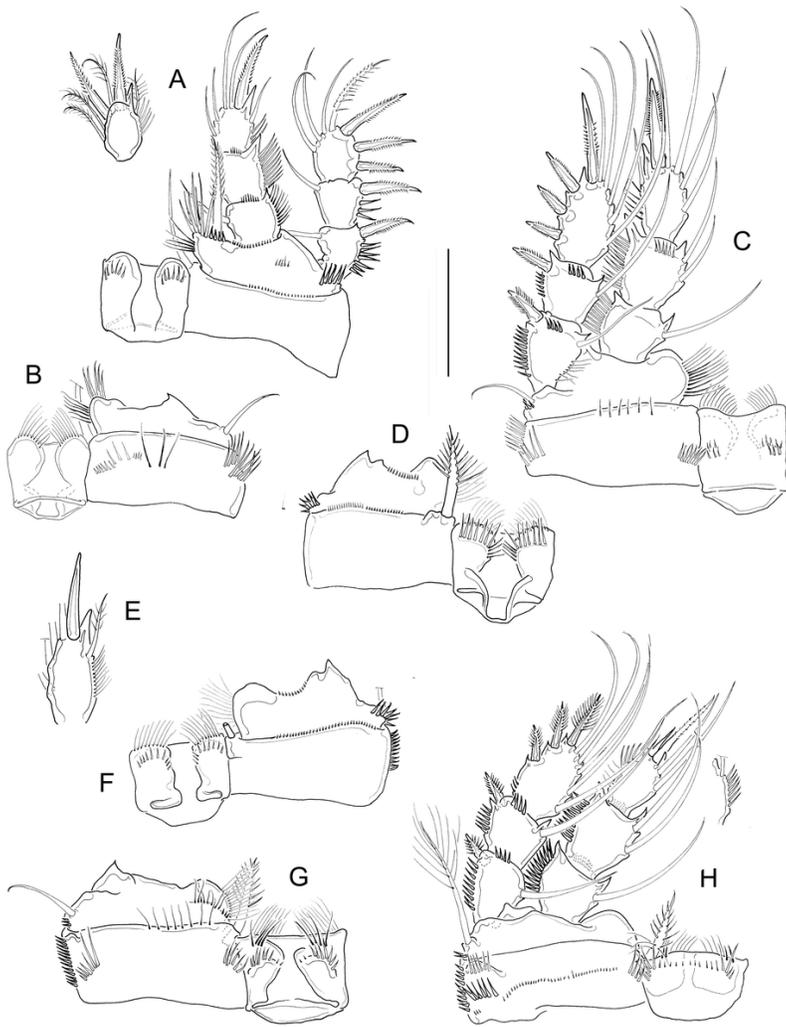


Figure 5
 Paracyclops novenarius, adult A) First leg, frontal, female, Enp3P1 separated, male, B) First leg, caudal, C) Second leg, caudal, D) second leg, frontal, E) Enp3P3, male: F) Third leg, frontal, G) Third leg, caudal, H) Fourth leg, caudal

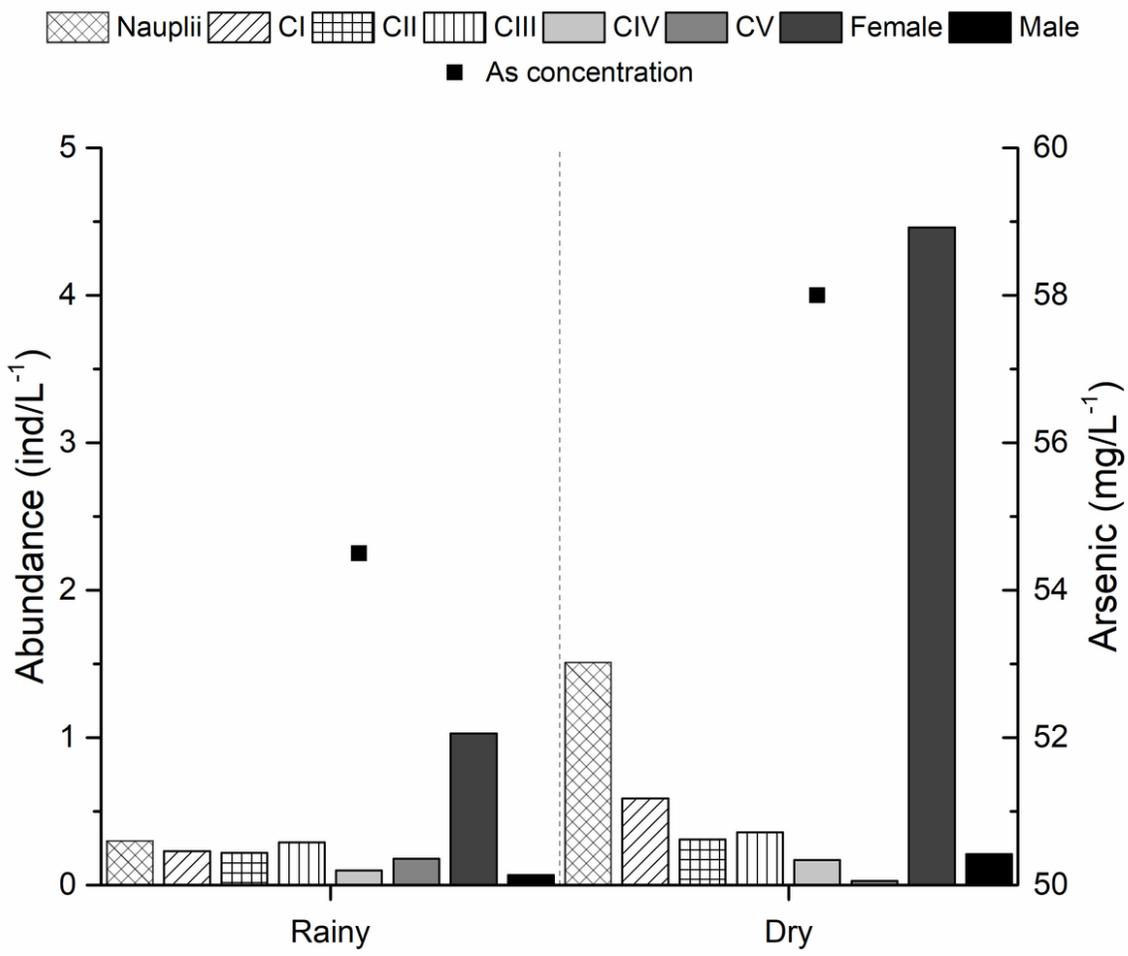


Figure 6

Abundances (ind/L-1) of *Paracyclops novenarius* (bars) and arsenic concentration (mg/L-1) (squares) in the seasons studied

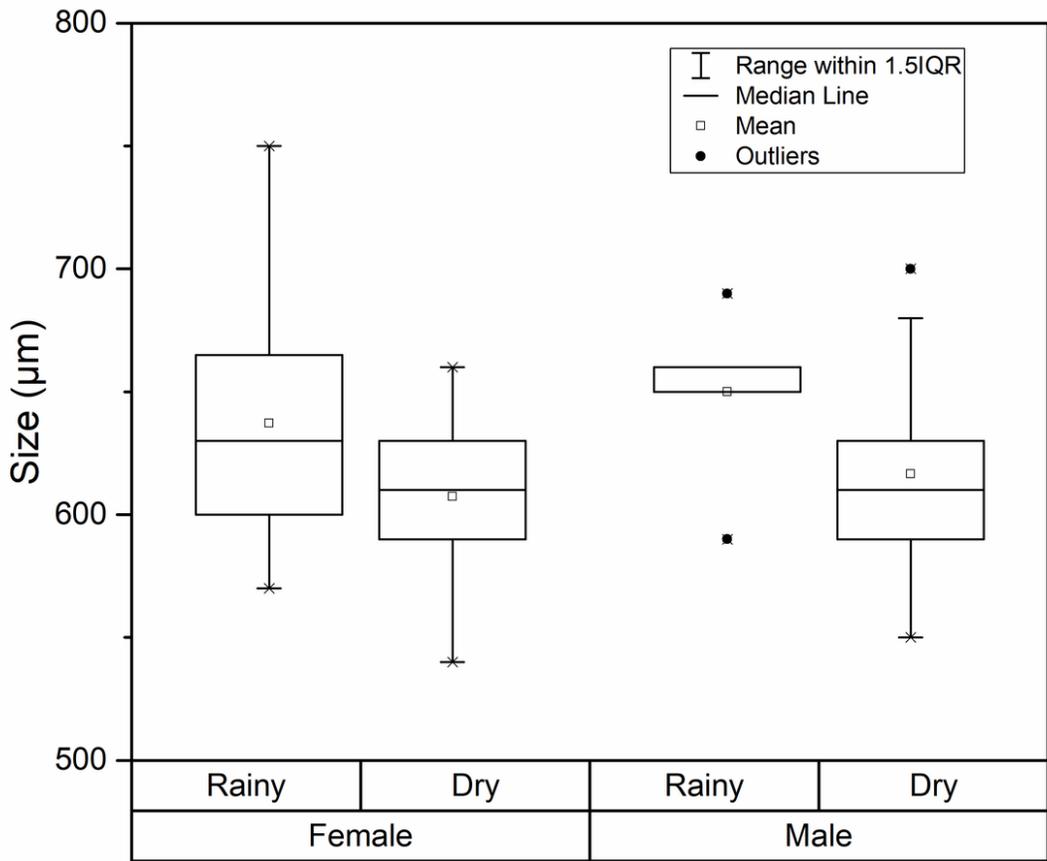


Figure 7

Body size of adult females and males of *Paracyclops novenarius* in rainy and dry seasons