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Seasonal Diversity and Morphometric Variations of Rotifers in Relation to Selected Environmental Variables from a Tropical High-Altitude Lake in Mexico

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Abstract: We studied the species diversity and morphometric variations of rotifers from a high-altitude water body, the Llano reservoir, in central Mexico during September 2021 to August 2022. Samples were collected from four stations in the reservoir every month. During the study period we were able to identify 54 monogonont rotifer species of which *Polyarthra vulgaris*, *Lecane closterocerca*, *Trichocerca porcellus*, *Lepadella patella* and *Keratella cochlearis* were numerically the most abundant. Depending on the season, the total rotifer density varied from 250 to 2450 ind L^{-1}. Canonical correspondence analysis (CCA) showed that the species of *Trichocerca similis*, *Keratella cochlearis*, *Mytilina ventralis* and *Scaridium longicaudum* were directly related to temperature. Using rotifer species richness and abundance data, we derived the Shannon diversity index, saprobic index and rotifer trophic state index. Data on the geometric morphometrics showed that *Keratella cochlearis* was found in two of the three climatic periods of the year (dry and winter), while *Lecane closterocerca* and *Trichocerca porcellus* occurred during the three climatic seasons (dry, winter and rainy) and formed three distinct clusters in relation to body size.

Keywords: rotifera; morphometry; species diversity; saprobic index

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1. Introduction

Mexico is bestowed with a wide variety of epicontinental water bodies due to temporal and altitudinal variations, volcanic and seismic activities, salinity gradients, geographic location (connecting the American continent) and extreme ranges of hydrological, temperature and edaphic factors [1]. Studies on freshwater plankton are on the rise in Mexico, especially during the last three decades [2].

Among the freshwater plankton, zooplankton plays an important role due to its importance in the transfer of energy from primary producers such as phytoplankton to secondary consumers such as fish larvae. Rotifers, cladocerans and copepods form the bulk of freshwater zooplankton biomass and numerical abundances [3]. Rotifers occupy niches in different regions of freshwater bodies such as the limnetic, littoral and benthic zones. In addition, different physical (such as temperature), chemical (such as salinity) and biological factors (such as algal food, competition and predation) structure the rotifer communities in nature as they are sensitive to changes in these variables [4,5].

Rotifers are also widely used to indicate the trophic state of different inland water bodies. Different limnoecological approaches are available in which rotifer species serve as indicators of trophic state and other water quality related parameters [6]. Unlike laboratory-based simplified tests, responses of rotifers to both abiotic and biotic factors are not often direct, but their interactions are equally important [7].

To understand the ecology of rotifers and their role within aquatic systems, information relating to taxonomic composition, diversity indices, species-specific and total abundances are essential [8].

Monthly zooplankton sampling for periods of at least one year offers a greater possibility for understanding how the diversity and abundance of species vary in relation to the physical and chemical variables of the body of water [9,10]. Such data are also helpful to establish measures for the management and conservation of freshwater bodies [11]. Seasonal changes in rotifer community dynamics from temperate water bodies over a few decades provide data which are useful for ecological modeling. Such studies from tropical regions are not many. Regular field collections from a given station provide ample morphological variations [12]. Therefore, traditional morphometry, which quantifies the size and shape of lorica, forms the basis to indicate the occurrences of strong variations within a given species. With the help of multivariate statistical analysis, further inferences can be made [13,14]. Geometric morphometrics analyses the changes and differences among the structures of organisms by digital means, showing morphological changes resulting from the impact of biotic and abiotic factors [15]. Therefore, the use of geometric morphometrics has been increasing in the last decade and its use as a complementary tool in molecular analyses and phylogenetic comparisons is also on the rise. Geometric morphometrics analyses the variation in size and shape in biological structures through reference points placed in strategic positions on the organism called landmarks [16,17]. A currently well-known application of geometric morphometrics is in the description of new species that are similar even at the genetic level, hence an integrative approach combining geometric morphometrics with molecular tools is used to better interpret the processes that generate the variety of species [18].

In Mexico, studies on rotifer communities have been gaining research interest to apply limnological knowledge for the control of toxic cyanobacteria, establishing water quality indices and improving the survival rate of edible fish species. Still, long-term sample collection to study the diversity of rotifers is rare [19,20]. There are few high-altitude lakes and reservoirs in Mexico. Previous studies suggest that these water bodies have limnological characteristics similar to temperate lakes [21–23].

A previous study [22] in the Llano reservoir (State of Mexico) recorded 84 species of rotifers with a dominance of *Polyarthra vulgaris* and *Trichocerca bidens*. However, in this study, the geometric morphometrics of the common taxa was not considered.

The objective of this study was to document seasonal changes of rotifers and to record variations of geometric morphometrics of common species throughout an annual cycle and to relate them to selective physico-chemical variables of the Llano reservoir (State of Mexico).

2. Materials and Methods

2.1. Study Area

The study was carried out in the reservoir Llano, located at 2880 m.a.s.l. in the Municipality of Villa del Carbón, State of Mexico (19°54′24″ N and 99°39′07″ W) (Figure 1). It has a maximum depth of 35 m, but we collected samples from the littoral zone of <1 m depth. Water from the reservoir is mainly used for irrigation and recreational fishing of rainbow trout *Oncorhynchus mykiss* (Walbaum, 1792) and tourist activities that include boat rentals and hiking [22]. The four sampling points have macrophytes represented mainly by the genera *Elodea* and *Egeria* [24].

Figure 1. Sampling stations of the Llano reservoir from Central Mexico.

2.2. Sample Collection

Sampling was carried out every month starting in September 2021 and ending in August 2022. Four different sampling zones were selected based on the presence of aquatic vegetation (covering the largest possible area in the lake) as well as accessibility to the different stations.

For zooplankton samples, 80 L of reservoir water were filtered at each point at a distance of about 1 m from the shore and at a depth from the surface of 0.5 m using a 50 µm plankton net. The collected plankton was later concentrated to a volume of 250 mL and preserved in the field to a final concentration of 4% formalin. For species confirmation of sessile taxa, a few live zooplankton samples were also collected and transported to the laboratory for analysis.

2.3. Physical and Chemical Variables

The following water variables were determined at each sampling: temperature, dissolved oxygen, percentage oxygen saturation, specific conductivity and pH using a HANNA model HI 98194 multiparameter probe and YSI 55 probe. Water samples filtered through 0.22 µm Millipore (HA nitrocellulose) were collected in 30 mL polyethylene bottles placed in ice for the determination of nitrate and phosphate levels in the laboratory.

Nutrient analysis was carried out using the standard techniques [25] with a Skalar San Plus model segmented flow auto-analyzer. The determination of nitrates and phosphates was made following standard procedure [26]. For chlorophyll a extraction, quantitative reservoir surface water was filtered through Millipore nitrocellulose filters with 0.45 µm pore size. These filters were used to carry out spectrophotometric (Model ELY 2000) analysis of chlorophyll a, extracting the material with 5 mL of 90% acetone and centrifuging for 15 min [27]. Chlorophyll a estimation was done following standard procedure [28]. To explain variations in the abundances of rotifers and to relate environmental variables to them, we performed the canonical correspondence analysis (CCA) using CANOCO 4.5. Environmental variables (except pH) and rotifer abundances were log (x + 1) transformed to approach normality conditions and homoscedasticity of the data. Monte Carlo test permutation (10,000 permutations) was used to evaluate the statistical significance of the relationships between rotifer species and environmental variables.

2.4. Zooplankton Identification and Quantification

Identification of rotifer species was carried out in two steps: first, using a Nikon model SMZ645 stereoscopic microscope for a tentative identification. Later, selected individuals were carefully separated using a finely drawn Pasteur pipette and transferred to a compound microscope (Nikon model E600) at different magnifications (40×–1000×) where they were identified to species level. When necessary, trophi were inspected using 11% sodium hypochlorate. For the correct determination of the species, specialized literature was used [29–32].

For quantification of the rotifers, we used an inverted optical microscope (Nikon model E100). From each fixed and concentrated zooplankton sample, a 1 mL aliquot was taken after thorough mixing and placed on a Sedgwick-Rafter chamber for counting each encountered individual of a rotifer species. From each sample, we selected 3 aliquots. The mean of 3 aliquots calculated as the density per species was later extrapolated to the whole sample. Later, the density of rotifers was expressed as number per liter.

2.5. Geometric Morphometrics

From the fixed zooplankton samples, the first 20 individuals encountered of each selected loricate rotifer species (based on availability during the different seasons) were separated. We measured the size (length and width) of the organism using a Nikon E600 microscope with a drawing tube and took photomicrographic images. The MorphoJ v 1.07a program was used to carry out the morphometric analysis using specialized techniques such as the use of "landmarks" or anatomical reference points to compare the shapes.

2.6. Indices

Based on species richness and abundances, Shannon-Wiener species diversity index was calculated for each size and season after standardization of abundances data using a rarefaction method [33]. The following formula was used to obtain the Shannon diversity index [34]:

$$H' = -\sum_{i=1}^{S} p_i \, ln p_i$$

H' = Shannon species diversity index (bits/individual).
P_i = Numerical proportion of each species within the total number of species per sample.
The saprobic index (S) [35,36] was calculated using the following formula:

$$S = \Sigma\ (\text{s.h})/\Sigma \text{h}$$

The saprobic index S is based on the following rating scale:

1.0–1.5: oligosaprobic; 1.6–2.5: β-mesosaprobic; 2.6–3.5: α-mesosaprobic and 3.6–4.4: polysaprobic [35].

The trophic state of the system was obtained using the following criteria: (a) TSI_{ROT} [37] and (b) total density of rotifers [38].

The rotifer trophic state index (TSI_{ROT}) proposed by Ejsmont-Karabin [6] includes 6 equations based on numbers, biomass and percentages of specific groups. In this present study, TSI_{ROT} was derived using only the equation for rotifers numbers:

$$TSI_{ROT} = 5.38 \ln (N) + 19.28,$$

where (N) is the total density of rotifers (ind L^{-1}).

A TSI_{ROT} number < 45 implies mesotrophic, between 45 and 55 implies mesoeutrophic, between 55 and 65 implies eutrophic and >65 implies hypereutrophic.

According to May and O´Hare [38], state

s can be determined as follows: when the total density of rotifers is ≤500 ind L^{-1}, oligotrophic; between 500 and 1000 ind L^{-1}, mesotrophic; between 1000 and 2500 ind L^{-1}, eutrophic; <3000 ind L^{-1}, hypereutrophic.

3. Results

The values of physical and chemical variables showed considerable variations during the three climatic seasons (dry−warm, from March to June; dry−cold, from November to February and rainy, from July to October). The temperature ranged from 10 to 23 °C, the lowest temperature during almost the whole year was at station 1, while station 2 was the highest. The conductivity data varied between 47 and 76 µS cm^{-1}, there was very little difference among the four stations. The dissolved oxygen variation remained between 6.0 and 11.0 mg L^{-1}, with station 2 having the highest value and station 1 having the lowest values over several months. Chlorophyll a levels ranged between 3 and 28 µg L^{-1} at station 3, with a high coverage of macrophytes the highest value was recorded, while the lowest values were at station 4, which did not have a large amount of aquatic vegetation (Figure 2).

The pH ranged from 7.0 to 9.5; at station 4 it had the highest value and at station 1 the lowest. Depending on season, the water depth ranged from 30 to 90 cm with the lowest transparency observed in the dry−warm season; during the rainy season we recorded the highest depth. Nitrates varied from 3 to 90 µg L^{-1}, the highest values corresponded to stations 1 and 3 while the lowest were from station 4. Phosphate levels ranged between 1 and 26 µg L^{-1}, station 2 had the highest levels, including for two months in which the levels were three times higher than the rest of the stations (Figure 2).

The common rotifer families were Trichoceridae with ten species, Lecanidae with eight species and Lepadellidae with seven species (Table 1). In all, 54 species of monogonont rotifers were found during the study period, of which *Polyarthra vulgaris*, *Lecane closterocerca*, *Trichocerca porcellus*, *Lepadella patella* and *Keratella cochlearis* were numerically the most abundant during the study period (Figure 3).

The total density of rotifers ranged between 250 and 2450 ind L^{-1}, depending on the sampling station and the season of the year. At station 1, minimum densities of >250 ind L^{-1} were recorded in September and maximum values of <1300 ind L^{-1} in April. At station 2, the highest value was <2450 ind L^{-1} in April, the highest of all values at all stations; on the other hand, the lowest value was >300 ind L^{-1} in October. At station 3, the highest abundance was <1400 ind L^{-1} in June, while the lowest value for this station was <400 ind L^{-1} in September. The highest rotifer densities were observed at station 4 during October (<1300 ind L^{-1}) and <350 ind L^{-1} in June (Figure 3). Densities of individual rotifer species also varied according to season and collection station. The highest densities reached for *P. vulgaris* were >850 ind L^{-1} in the month of June at station 2, while the highest for *K. cochlearis* were >1000 ind L^{-1} in April at station 2. *Trichocerca porcellus* contributed values of >450 ind L^{-1} in April at station 1, while *L. closterocerca* recorded > 200 ind L^{-1} in September at stations 2 and 4. *Lepadella patella* had its highest densities with >200 ind L^{-1} in January at station 1 (Figure 3).

The saprobic index remained <1.6 (i.e., β-mesosaprobic) throughout the entire study period at the four stations where the samples were taken. Station 1 had an average value of 1.4 throughout the year, the lowest during September.

Station 2 had an average of 1.4 throughout the year. Station 3 had an annual average of 1.5. Station 4 had an average of 1.4, with August being the highest at 1.6 and February the lowest at 1.3 (Figure 4).

The Shannon-Wiener diversity index ranged from 0.6 to 2.7 bits ind^{-1}. Throughout the study period. At station 2, the lowest diversity (0.66 bits ind^{-1}.) was observed in June. The rotifer trophic state index (TSI_{ROT}) for all the stations during the different seasons remained within 49–61 (Figure 4).

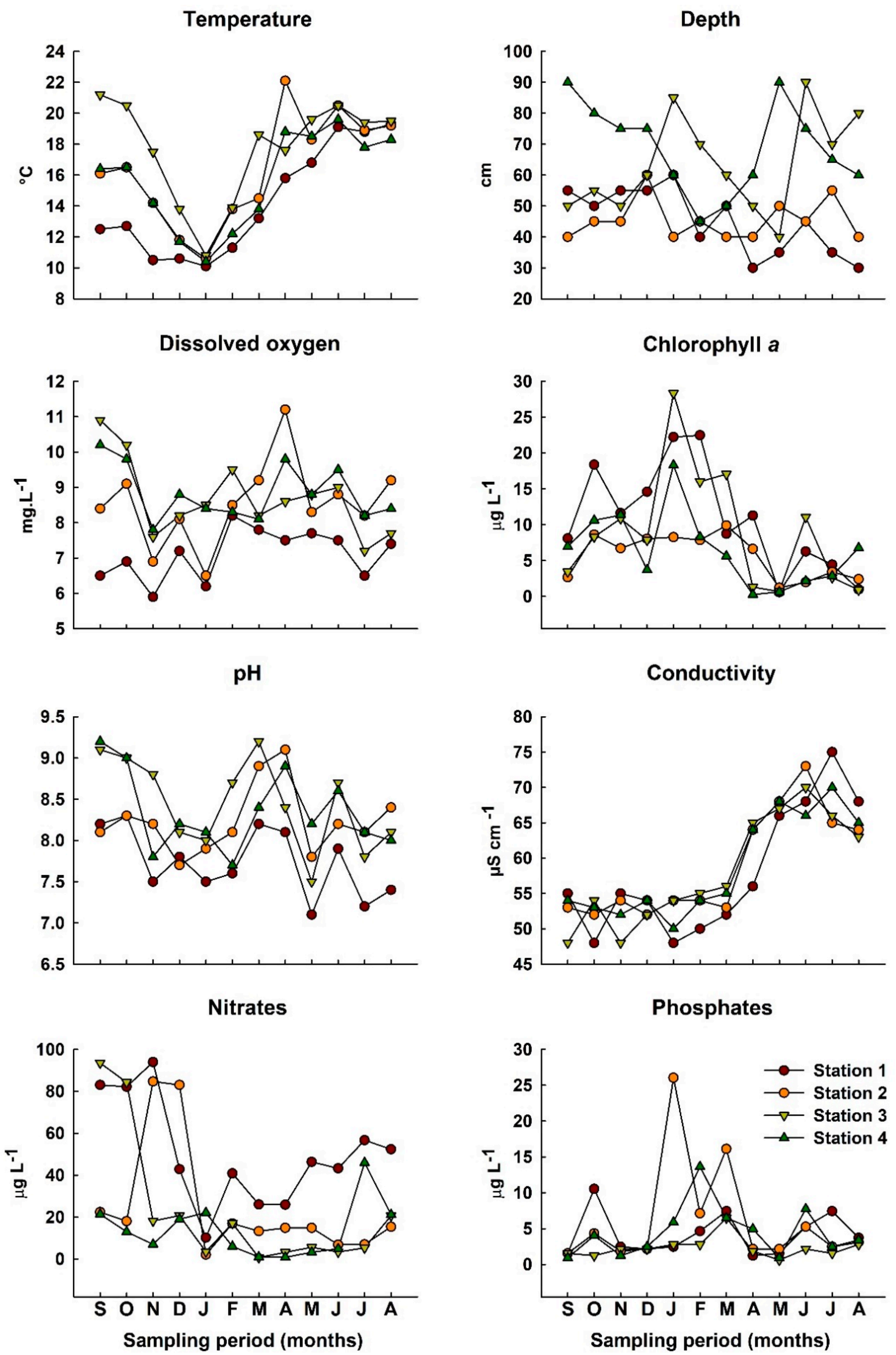


Figure 2. Physical and chemical variables (temperature, °C; depth, cm; dissolved oxygen, mg L^{-1}; Chlorophyll a, µg L^{-1}; pH, conductivity, µS cm^{-1}; nitrates, µg L^{-1} and phosphates, µg L^{-1}) of Llano reservoir.

Table 1. List of rotifer species observed in the Llano reservoir during September 2021–August 2022. Their presence (x) and absence (-) are shown during the sampling months (S = September, O = October. . . A = August).

Taxa	S	O	N	D	J	F	M	A	M	J	J	A
Family: Asplanchnidae												
Asplanchna brightwellii Gosse, 1850	x	-	-	-	-	x	x	x	x	-	x	x
Asplanchna girodi Guerne, 1888	x	-	-	-	-	x	-	x	x	x	-	x
Asplanchna priodonta Gosse, 1850	x	-	-	-	-	x	-	-	x	-	-	x
Family: Brachionidae												
Keratella cochlearis (Gosse, 1851)	x	-	x	x	x	x	x	x	x	x	x	x
Platyias quadricornis (Ehrenberg, 1832)	-	-	-	-	-	-	-	-	x	x	-	-
Family: Collothecidae												
Collotheca campanulata (Dobie, 1849)	-	-	-	-	x	-	-	-	-	-	-	-
Family: Dicranophoridae												
Dicranophorus grandis (Ehrenberg, 1832)	x	x	-	-	x	-	-	-	-	-	-	x
Family: Euchlanidae												
Euchlanis dilatata Ehrenberg, 1830	-	-	-	-	-	-	-	-	-	x	-	-
Euchlanis mikropous Koch-Althaus, 1962	-	-	-	-	-	x	-	-	-	-	-	-
Euchlanis oropha Gosse, 1887	-	-	-	-	-	-	x	-	-	-	-	-
Family: Gastropodidae												
Ascomorpha ovalis (Bergendal, 1892)	x	-	-	-	-	-	-	-	-	-	-	x
Gastropus hyptopus (Ehrenberg, 1838)	-	-	-	-	-	x	-	-	-	-	-	-
Family: Hexarthridae												
Hexarthra intermedia (Wiszniewski, 1929)	-	-	-	-	-	-	-	-	-	x	x	x
Family: Lecanidae												
Lecane closterocerca (Schmarda, 1859)	x	x	x	x	x	x	x	x	x	x	x	x
Lecane decipiens (Murray, 1913)	-	x	-	-	-	-	-	x	-	-	x	-
Lecane flexilis (Gosse, 1886)	x	-	-	x	-	-	x	-	-	-	x	-
Lecane hamata (Stokes, 1896)	x	x	x	x	x	x	x	x	x	x	x	x
Lecane hastata (Murray, 1913)	-	-	x	x	-	-	-	-	-	-	-	-
Lecane inermis (Bryce, 1892)	-	x	-	-	-	-	-	-	-	-	x	-
Lecane luna (Müller, 1776)	-	-	x	-	-	-	-	x	-	-	-	-
Lecane lunaris (Ehrenberg, 1832)	x	-	x	-	-	-	-	-	-	-	-	-
Family: Lepadellidae												
Colurella obtusa (Goose, 1886)	x	x	x	x	x	-	-	x	-	-	-	x
Colurella uncinata (Müller, 1773)	-	x	x	-	-	-	x	-	-	-	-	-
Lepadella acuminata (Ehrenberg, 1834)	x	x	-	-	-	-	-	-	-	-	-	-
Lepadella ovalis (Müller, 1786)	x	x	x	x	x	-	x	x	x	x	x	x
Lepadella patella (Müller, 1773)	x	x	x	x	x	x	x	x	x	x	x	x
Lepadella triptera (Ehrenberg, 1830)	-	-	-	-	-	-	x	-	-	-	-	-
Squatinella lamellaris (Müller, 1786)	x	x	x	x	x	x	x	x	-	-	x	x

Table 1. *Cont.*

Taxa	S	O	N	D	J	F	M	A	M	J	J	A
Family: Mytilinidae												
Lophocharis oxysternon (Gosse, 1851)	x	x	x	-	x	-	-	-	x	-	-	-
Mytilina ventralis (Ehrenberg, 1830)	x	x	x	x	x	x	-	x	x	x	x	x
Family: Notommatidae												
Cephalodella physalis Myers, 1924	-	-	x	x	-	x	x	-	x	-	-	-
Cephalodella gibba (Ehrenberg, 1830)	x	x	x	x	x	x	x	x	x	x	x	x
Cephalodella ventripes (Dixon-Nuttall, 1901)	x	x	x	x	x	x	x	-	x	-	x	x
Notommata tripus Ehrenberg, 1838	x	x	x	x	-	-	x	x	-	x	x	-
Monommata arndti Remane, 1933	x	x	x	x	x	x	x	x	x	x	x	x
Family: Proalidae												
Proales decipiens (Ehrenberg, 1830)	-	x	x	-	-	x	-	x	-	-	-	-
Family: Scaridiidae												
Scaridium longicaudum (Müller, 1786)	x	x	x	x	x	x	x	x	x	x	-	x
Family: Synchaetidae												
Polyarthra vulgaris Carlin, 1943	x	x	x	x	x	x	x	x	x	x	x	x
Synchaeta pectinata Ehrenberg, 1832	-	-	x	-	-	-	-	-	-	x	-	-
Family: Testudinellidae												
Testudinella patina (Hermann, 1783)	x	x	x	x	x	x	x	x	-	-	-	x
Testudinella emarginula (Stenroos, 1898)	x	x	x	-	x	x	x	x	-	-	-	x
Family: Trichotriidae												
Macrochaetus subquadratus Perty, 1850	x	-	-	-	-	-	-	-	-	-	-	-
Trichotria pocillum (Müller, 1776)	x	x	x	x	x	x	x	x	x	x	x	x
Trichotria tetractis (Ehrenberg, 1830)	x	x	x	x	x	x	x	x	-	-	-	x
Family: Trichoceridae												
Trichocerca bicristata (Gosse, 1887)	-	-	-	-	-	-	-	-	-	x	-	-
Trichocerca bidens (Lucks, 1912)	x	-	-	-	-	-	x	-	-	-	-	x
Trichocerca cylindrica (Imhof, 1891)	-	x	x	x	x	x	x	x	x	x	-	-
Trichocerca elongata (Gosse, 1886)	x	x	x	x	x	x	x	x	-	x	x	x
Trichocerca longiseta (Schrank, 1802)	-	-	-	-	-	x	x	x	-	-	-	-
Trichocerca porcellus (Gosse, 1851)	x	x	x	x	x	x	x	x	x	x	x	x
Trichocerca ruttneri Donner, 1953	-	x	-	-	-	-	-	-	-	-	-	-
Trichocerca similis (Wierzejski, 1893)	x	x	x	x	-	-	x	x	x	x	x	x
Trichocerca tigris (Müller, 1786)	-	-	x	-	-	-	-	x	-	x	-	-
Trichocerca weberi (Jennings, 1903)	-	x	-	-	-	x	x	-	-	-	x	-

In this study, canonical correspondence analysis (CCA) was performed to determine the correlations between environmental variables and the rotifer abundances present at each station. At station 1, *Mytilina ventralis* and *Hexarthra intermedia* are directly related to temperature; on the other hand, *Trichocerca cylindrica* was related to dissolved oxygen. At station 2, *Trichotria pocillum* and *Lecane luna* were influenced by pH (Figure 5).

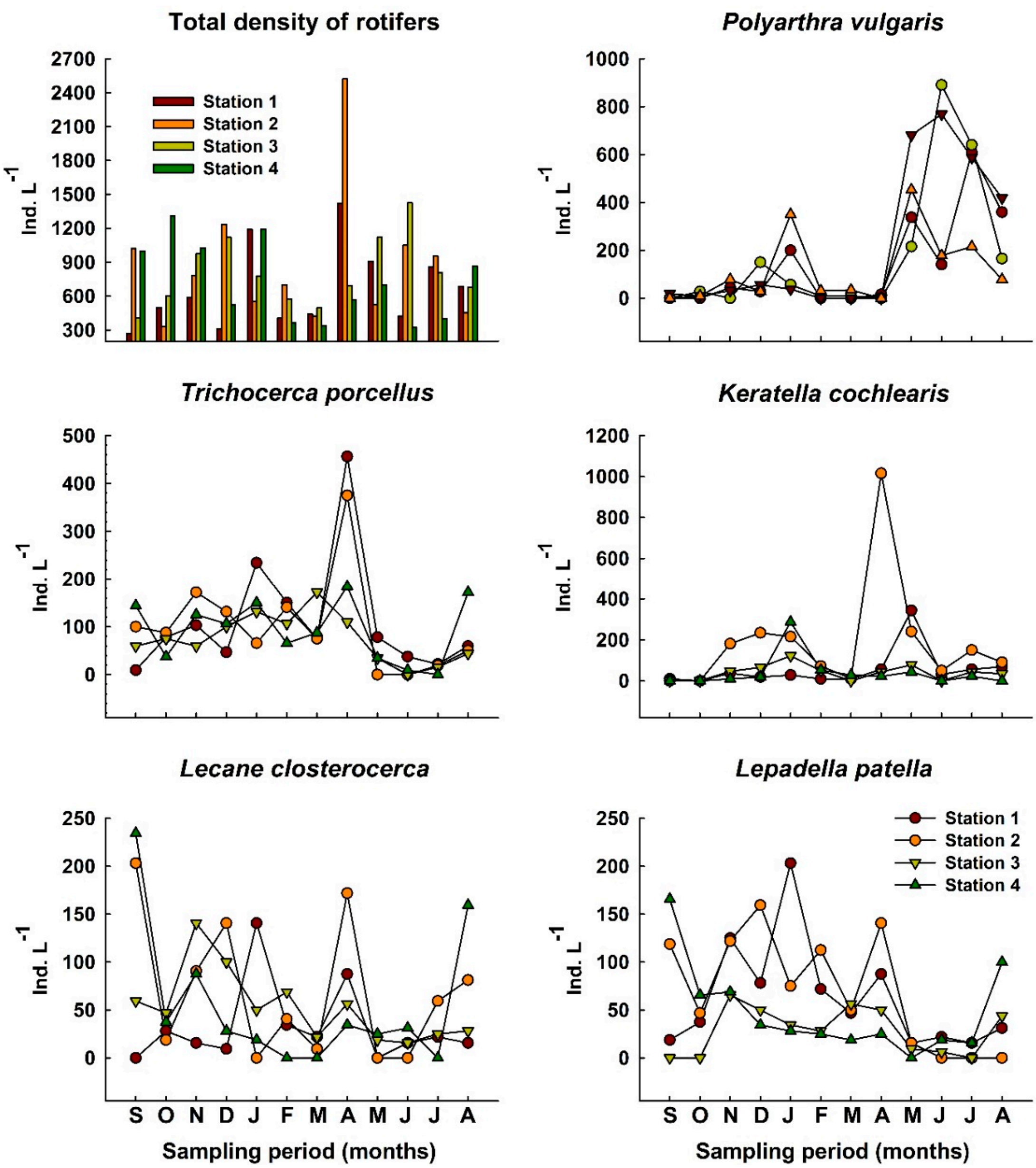


Figure 3. Seasonal changes in the total densities of rotifers (ind L^{-1}) and abundances (ind L^{-1}) of selected rotifer species from Llano reservoir during the sampling period.

At station 3, phosphate levels influenced *Lepadela patella*, *Trichotria tetractis*, *Trichocerca elongata* and *Testudinella emarginula*; while nitrates are related to *Cephalodella gibba*, *Colurella obtusa* and *Trichocerca porcellus*. At this station, additionally temperature influenced the abundances of *Lecane*. Finally, at station 4, oxygen levels had a relationship with *Trichotria pocillum* and *Lecane closterocerca*; while phosphate levels are associated with *Monommata arndti*, *Keratella cochlearis*, *Asplanchna priodonta* and *Trichocerca longiseta* (Figure 5). *Keratella cochlearis* was found in two of the three climatic periods of the year (dry and winter), the size range of body length varied from 152 µm to 170 µm, and body width from 54 µm to 60 µm. *Lepadella patella* only appeared in the dry and winter seasons as well, its body length range was from 108 µm to 121 µm and the width from 73 µm to 82 µm. On the other hand, *Lecane closterocerca* and *Trichocerca porcellus* occurred during the three climatic seasons (dry, winter and rainy). *Lecane closterocerca* had a body length which ranged from 92 µm to 105 µm while the width ranged from 65 µm to 79 µm. *Trichocerca porcellus* had a body length from 126 µm and 165 µm while the width ranged between 48 µm and 68 µm (Figure 6).

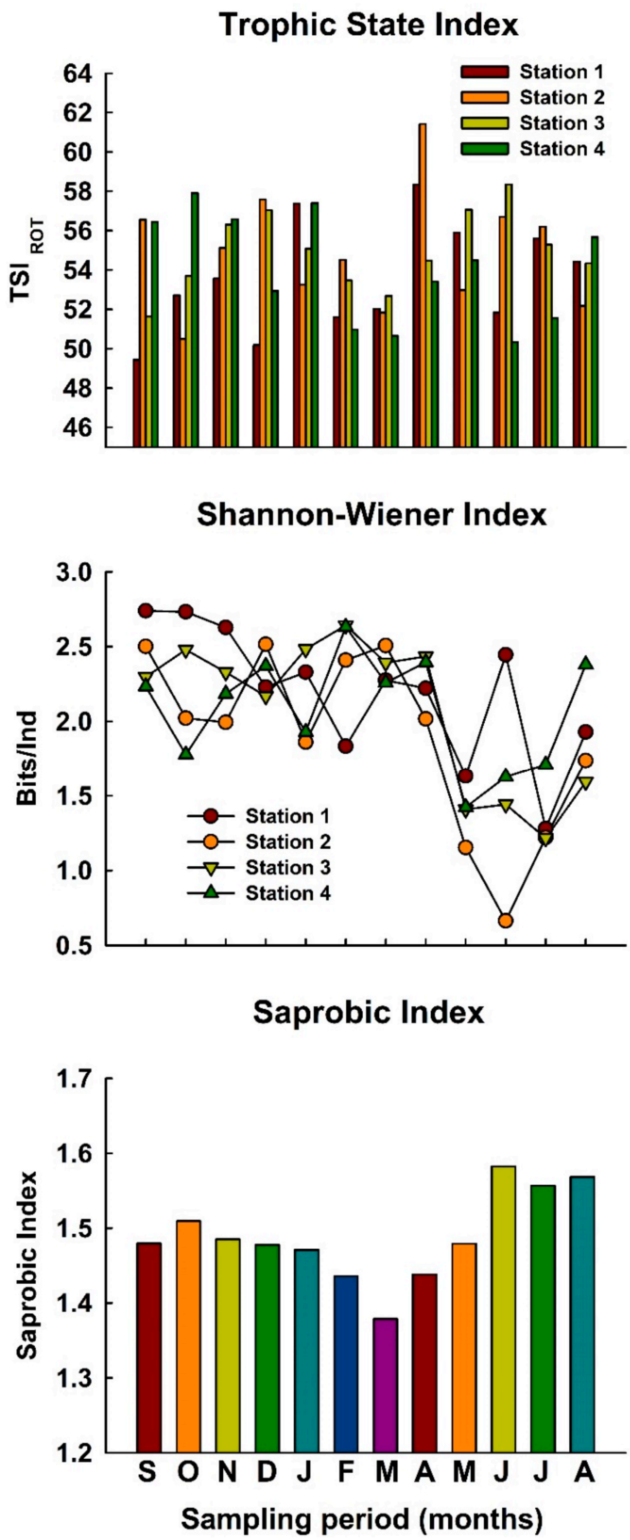


Figure 4. Water quality indices of Llano reservoir: rotifer trophic state index (TSI_{ROT}), Shannon-Wiener index and saprobic index.

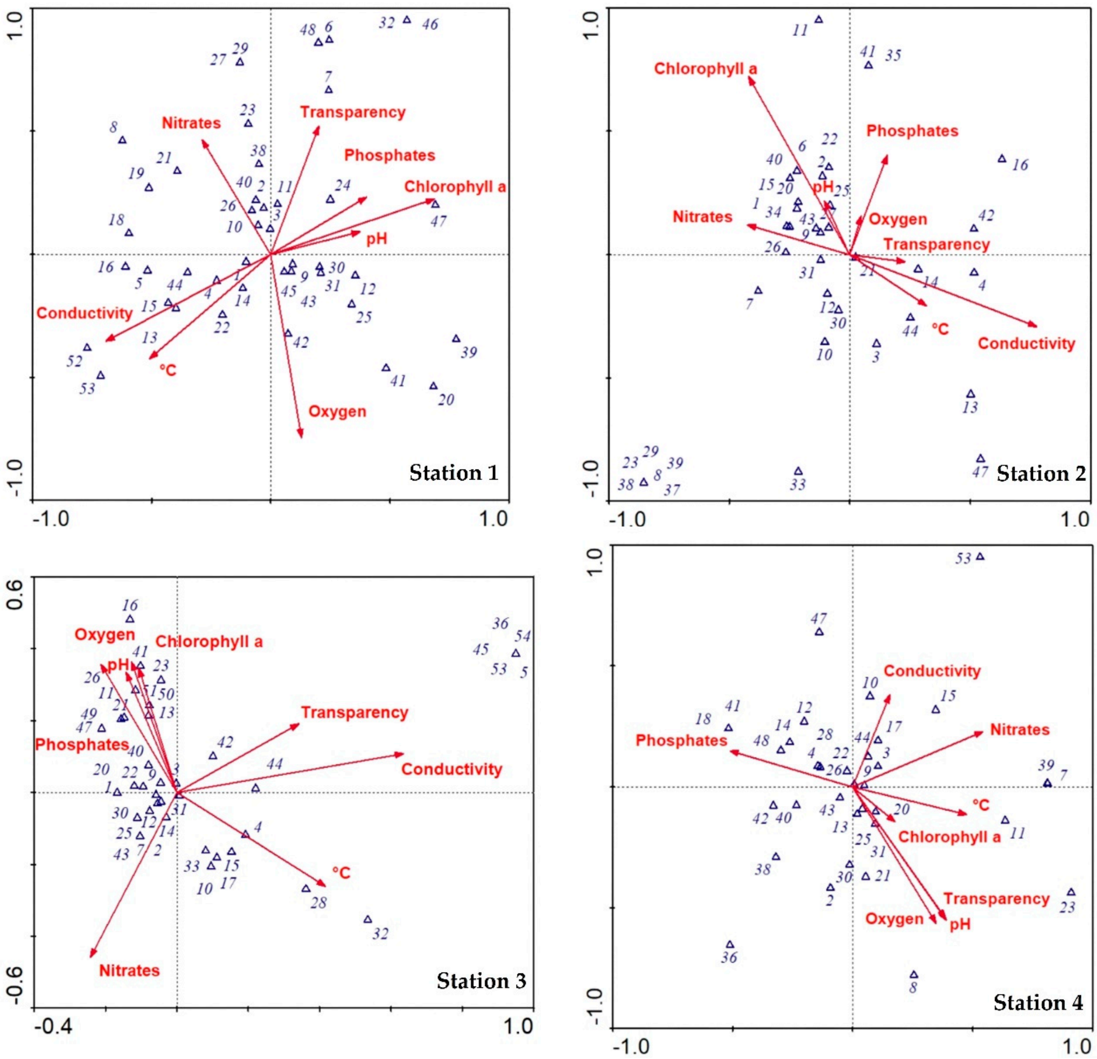
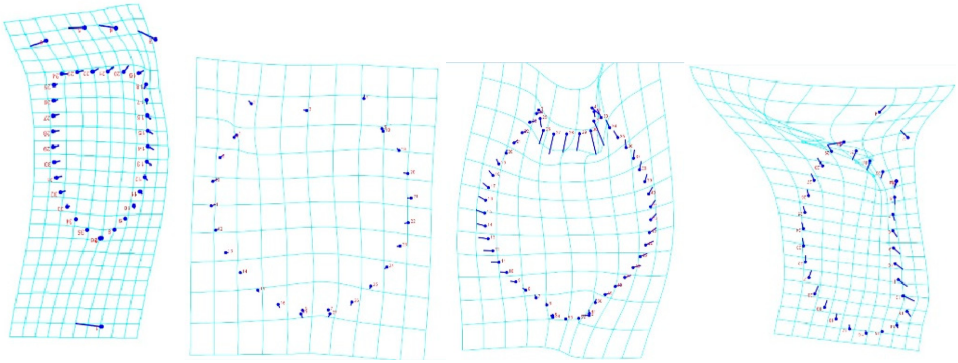
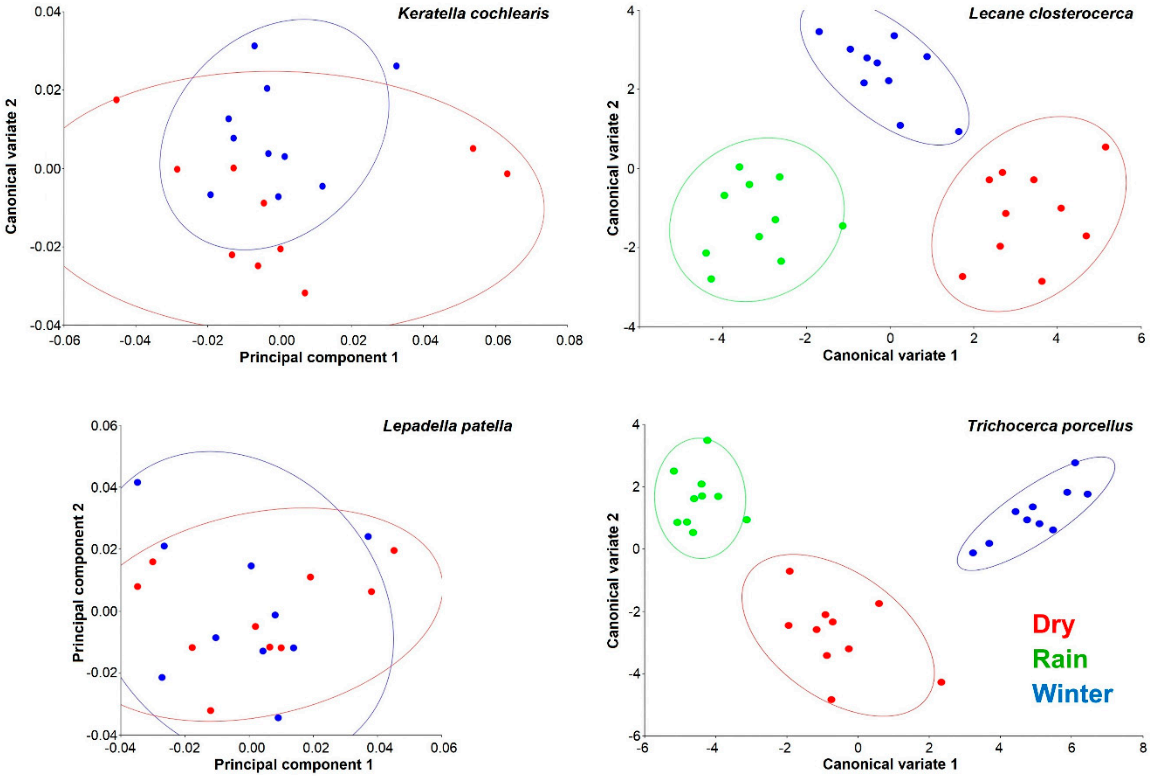


Figure 5. Canonical correspondence analysis of environmental variables with rotifer species. The species are indicated in numbers: 1. *Cephalodella physalis*, 2. *Cephalodella gibba*, 3. *Cephalodella ventripes*, 4. *Polyarthra vulgaris*, 5. *Synchaeta pectinata*, 6. *Colurella uncinata*, 7. *Colurella obtusa*, 8. *Lepadella acuminata*, 9. *Lepadella patella*, 10. *Lepadella ovalis*, 11. *Squatinella lamellaris*, 12. *Monommata arndti*, 13. *Mytilina ventralis*, 14. *Keratella cochlearis*, 15. *Notommata tripus*, 16. *Asplanchna girodi*, 17. *Asplanchna brightwellii*, 18. *Asplanchna priodonta*, 19. *Ascomorpha ovalis*, 20. *Testudinella patina*, 21. *Testudinella emarginula*, 22. *Scaridium longicaudum*, 23. *Dicranophorus grandis*, 24. *Collotheca campanulata*, 25. *Trichotria pocillum*, 26. *Trichotria tetractis*, 27. *Lecane luna*, 28. *Lecane decipiens*, 29. *Lecane lunaris*, 30. *Lecane hamata*, 31. *Lecane closterocerca*, 32. *Lecane inermis*, 33. *Lecane flexilis*, 34. *Lecane hastata*, 35. *Euchlanis mikropous*, 36. *Euchlanis dilatata*, 37. *Macrochaetus subquadratus*, 38. *Lophocaris oxysternon*, 39. *Trichocerca bidens*, 40. *Trichocerca elongata*, 41. *Trichocerca longiseta*, 42. *Trichocerca cylindrica*, 43. *Trichocerca porcellus*, 44. *Trichocerca similis*, 45. *Trichocerca tigris*, 46. *Trichocerca ruttneri*, 47. *Trichocerca weberi*, 48. *Proales decipiens*, 49. *Gastropus hyptopus*, 50. *Euchlanis oropha*, 51. *Lepadella triptera*, 52. *Platyias quadricornis*, 53. *Hexarthra intermedia*, 54. *Trichocerca bicristata*.



(**a**)



(**b**)

Figure 6. (**a**). Transformation grids of *Keratella cochlearis*, *Lecane closterocerca*, *Lepadella patella* and *Trichocerca porcellus* (from left to right) using MorphoJ. (**b**). MorphoJ showing different lorica shapes of rotifers (*Keratella cochlearis*, *Lecane closterocerca*, *Lepadella patella* and *Trichocerca porcellus*) collected in three different seasons.

4. Discussion

Tropical high-altitude water bodies have characteristics similar to low-altitude temperate water bodies [39]. Central Mexico represents a high-altitude region of about 2000–4000 m.a.s.l., although most water bodies are located at about 2500 m.a.s.l. In Mexico, several limnological studies have been carried out on high-altitude water bodies, including those of Valle de Bravo [40], Lake Xochimilco [19,41,42], Cantera Oriente [9] and the Iturbide reservoir [43]. In the studies cited above, zooplankton samples were collected over a 12-month period and, therefore, seasonal changes in the physical and chemical variables and the plankton abundances can be comparable to other later studies such as ours.

The Llano reservoir is at a high altitude (2880 m.a.s.l.) and is of recreational importance to local communities. Previous studies on this water body recorded a minimum temperature of 10 °C and maximum of 22 °C throughout the year, as also observed in this work. This range is also comparable to that reported for other water bodies of central Mexico, such as Valle Bravo (1800 m.a.s.l.) [40], Madín Dam (2300 m.a.s.l.) [20], Iturbide reservoir (3300 m.a.s.l.) [43] and Benito Juárez reservoir (2540 m.a.s.l.) [44]. Recent studies indicate a considerable annual increase in water temperature during the past decades [45]. Here, no increase in the temperature during the different seasons was observed in this study as compared to that conducted five years ago [22].

Dissolved oxygen levels ranged between 6 and 11 mg L^{-1}, slightly higher than those reported for the Nevado de Toluca lakes, which present levels between 5 and 6 mg L^{-1} [23]. However, the DO range in the Llano reservoir was much lower (4–19 mg L^{-1}) as compared to the Cantera Oriente, another high-latitude water body of this region [10]. Regarding the pH levels, the present reservoir was nearly neutral to moderately alkaline, with levels from ca. 7 to 9. This is similar to the range reported from Lake Xochimilco [46] but slightly below the highest levels reported for Lake Huetzalin, situated at a similar altitude [41].

Nitrate levels generally remained between 3 and 45 µg L^{-1}, with some exceptions in which they reached up to 95 µg L^{-1}, slightly above the data reported previously [24] but lower than those reported for another high-altitude water body, the Iturbide reservoir [43].

Phosphate values were between 2 and 15 µg L^{-1} throughout the year, except for one occasion in January, when they reached 25 µg L^{-1}, which was due to nutrient mixing dynamics, typical at that time of year for many water bodies of this region such as the Valle de Bravo reservoir [47] and the Benito Juárez reservoir [44].

Chlorophyll a levels were low, ranging between 2 and 28 µg L^{-1} representing an oligotrophic system with a tendency towards mesotrophy [22]. Most freshwater bodies in Central Mexico are eutrophic. For example, in Lake Xochimilco, Chl a levels are as high as 700 µg L^{-1} [21] or even higher at 900 µg L^{-1} in the Madín reservoir [20]. The high Chl a levels of Lake Xochimilco and the Madín reservoir are due to the fact that they receive partially treated waste waters, rich in organic matter from anthropogenic activities. However, in the Llano reservoir, the magnitude of wastewater entering the system is much lower; the reservoir is mostly filled with rainwater and underground sources. A previous study carried out on the same water body reports slightly lower values of Chl a [22]. This is because the sampling stations were different from those monitored in this work.

In this water body the rotifer species richness was 54, although this number was lower than that reported previously from this reservoir (84 species) [22] and a few other water bodies (75–80 species) such as Lake Xochimilco [42]. Yet, the number of rotifer species reported in this study can be considered high as compared to that of other water bodies in central Mexico, such as Valle de Bravo with 25 rotifer species [40] or the Nevado de Toluca with 34 species [48].

The total density of rotifers remained on average < 1000 ind L^{-1} throughout the year, except for the month of April, when there was a large increase in the population of *Keratella cochlearis*, a species that has been reported as dominant in water bodies from the center of the country such as the Valle de Bravo reservoir [40] and the Cantera Oriente lake [9]. Due to the values of total rotifer densities being <500 ind L^{-1}, for some months, the Llano reservoir could be considered an oligotrophic water body with a tendency to be mesotrophic for a few other months, but it never showed a eutrophic level [37,38]. When the trophic state index (TSI_{ROT}) was applied to this water body, the values oscillated between 49 and 61; therefore, this water body could be considered a meso-eutrophic system [6]. It is interesting to mention that the genus *Brachionus* was totally absent throughout the year, while it was reported previously [22] with a *Brachionus:Trichocerca* ($Q_{B/T}$) ratio [36] ranging from 1.0 to 1.3, suggesting a mesotrophic state of the reservoir. Now the Llano Dam can be considered as a mainly oligotrophic water body with a tendency to mesotrophy in some months of the year. The most abundant species throughout the study period were *Polyarthra vulgaris* and *Keratella cochlearis*. It has been suggested that, when the species of *Brachionus* are absent, the ecosystem will be dominated by *K. cochlearis* and *P. vulgaris* [49], as also observed in our study. The saprobic index values obtained in this study varied from 1.3 to 1.6 [35]. A range of 1.0–1.3 indicates an oligosaprobic nature of the water body.

From the saprobic values obtained in this study, it is reasonable to consider that this water body is practically oligosaprobic. For Lake Xochimilco the saprobic index values are >2.0, indicating a β-mesosaprobic condition of the lake [19]. The Shannon-Wiener diversity index had an interval between 0.6 and 2.7 bits/ind^{-1} depending on the sampling period and station. This range is lower than (3.7 bits ind^{-1}) than that reported for Lake Huetzalin in Xochimilco, a eutrophic system dominated by rotifers of the genus *Brachionus* [41].

Various environmental variables affect the abundances of different rotifer species. Among the most important are food availability, water temperature, dissolved oxygen levels and nutrient concentrations [50]. Canonical correspondence analyses (CCA) showed that the rotifer distribution and its relationship with environmental variables in the first two axes explained approximately 50% of the total variance. At station 1, *Mytilina ventralis* and *Hexarthra intermedia* were directly related to changes in water temperature; which is the main factor influencing the rotifers [51]; on the other hand, several species of the genus *Trichocerca* were positively related to the levels of dissolved oxygen (DO). Such is the case of *Trichocerca similis*, which had a positive relationship with water temperature at station 2 as reported previously [52]. At station 3, phosphate levels were positively related to the presence of *Trichotria tetractis* and *Squatinella lamellaris*; while nitrate levels were positively related to *Cephalodella gibba* and *Colurella obtusa*. Temperature had a high influence on the presence of the genus *Lecane*. The above has also been reported for several rotifer species in tropical water bodies [53].

The genera *Keratella* and *Polyarthra* were negatively affected by conductivity at this station, also shown earlier [52]. At station 4, DO levels had a positive relationship with *Trichotria pocillum* and *Lecane closterocerca*; while *Monommata arndti*, *Keratella cochlearis*, *Asplanchna priodonta* and *Trichocerca longiseta* were positively related to phosphates.

The results obtained from geometric morphometrics of 20 specimens for each species using MorphoJ software [16] showed that the transformation grids for the species *Keratella cochlearis* remained similar in shape throughout the sampling period; likewise, *Lepadella patella* had a similar lorica shape throughout the year. The species that showed considerable changes in shape during the study period were *Lecane closterocerca* and *Trichocerca porcellus*. Large variations in the morphology of the lorica of different monogononts have been documented [29]. These morphological differences for a given species have been attributed to cyclomorphosis, predation induced defenses and/or various infraspecific categories [54]. Despite indicating slight variations in the shape of the lorica of the species *Keratella cochlearis* and *Lepadella patella*, the MorphoJ-generated principal component plots showed no remarkable differences in the lorica shapes throughout the year. However, for *Lecane closterocerca* and *Trichocerca porcellus*, the different forms could be pooled into three distinct clusters representing the three climatic periods. Therefore, differences in the shape of the rotifers may be due to temperature differences [55] and nutrient availability; the option of cryptic speciation also exists. In fact, an excellent way to correctly determine a species is by combining molecular, morphological and morphometric tools [56].

5. Conclusions

Our study showed that the seasonal influence on rotifer species diversity was mainly due to temperature, dissolved oxygen and nutrients. Throughout the sampling period, we were able to observe 54 rotifer species. However, during any particular collection period, we observed only around 30 species. Therefore, seasonal studies yield higher species diversity than sampling sporadically. Geometric morphometrics of selected rotifers from monthly sampling also yielded morphometrically distinct groups of *Lecane closterocerca* and *Trichocerca porcellus*, while two other species, *Lepadella patella* and *Keratella cochlearis*, had a body size that overlapped. Although our identification is solely based on morphology, we believe such studies offer an insight into the effects of abiotic factors on the abundance dynamics of rotifers from a high-altitude water body. We also showed that geometric morphometrics is one of the tools for a future molecular approach. Therefore, future molecular analysis of rotifers will provide the final confirmation as to whether the morphometrically distinct groups observed in this study represent phenotypic plasticity or genetic differentiation.

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