



# Topics of limnological research in Mexico

Coordinator  
Alfredo Pérez Morales

UNIVERSIDAD DE COLIMA



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*This book is dedicated to  
Dr. Singaraju Sri Subrahmanya Sarma,  
in gratitude for all his teachings in the world of limnology.*







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# Preface

This is a thoughtful and timely book, covering three key topics that will be of interest to many: research, limnology, and Mexico.

I shall begin with the latter. From the perspective of a nature-lover raised in the Mediterranean region, my experience with nature has always been shaped by landscapes where, over millenia, even the most remote and hidden corners have been influenced by human activities. While I have cherished Italy's natural beauty, the human imprint is ever-present. In contrast, Mexico, in my childhood fantasies, represented the opposite—an untouched and diverse natural paradise. It held a special place in my imagination, due to its vast biodiversity, pristine habitats, stunning landscapes, and rich cultural heritage.

My first trip to Mexico occurred after I had become a researcher, thanks to S.S.S. Sarma, to whom this book is dedicated. That visit, along with the warmth of Sarma's family and colleagues, solidified my idealized view of Mexico as a fascinating biodiversity hotspot. The country unfolded before me as an extraordinary place, rich in diverse landscapes, organisms, and cultures—all of which need preservation for future generations.

The task of preserving Mexico's ecosystems falls largely to scientists. Through the work of environmental researchers, we gain insights into the world around us: what habitats are, which organisms inhabit them, the roles different species play, and how they have adapted to their surroundings. This book serves as a great example of what scientific research can achieve with Mexico as a case study. The various chapters cover a broad range of topics: chemical and physical characteristics, environmental quality concerns, interactions between abiotic factors and living organisms, and the relationships among various species groups. The book also delves into different approaches to studying biodiversity and highlights the impact humans have on ecosystems and biodiversity. It does so by focusing on a range of organisms, including cyanobacteria, algae, phytoplankton, macrophytes, ciliates, zooplankton, crustaceans, hydrozoans, rotifers (my personal favourites), macroinvertebrates, and fish.

The importance of research in informing political decisions and actions, as well as Mexico's status as a biodiversity hotspot, are well-established facts and easy to agree on. However, the third topic, limnology, may be less familiar. Many people, including some

ecologists, might not be fully aware of what this term entails. Although considered technical jargon, limnology is simply the study of inland waters—lakes, rivers, streams, wetlands, and groundwater. Unlike its more popular counterpart, oceanography, limnology does not seem to capture the public's imagination. Could it be that the oceans, with their vast and mysterious depths, feel more captivating to most people than the inland waters?

This book takes a significant step in showcasing the relevance of limnology to our survival. Freshwater habitats, though they cover less than 1 % of the Earth's surface, are home to a substantial portion of the world's biodiversity—at least 10 % of all known species. Freshwater habitats and the biodiversity they support are under threat. Moreover, our survival depends on access to high-quality freshwater. This book not only highlights the beauty of limnology and the scientific methods used to study it, but it also draws attention to the major causes of biodiversity loss in freshwater ecosystems. It shows all readers what it means to deal with inland waters as a scientist interested in understanding ecosystems and protecting them.

I am confident that this book will influence future generations of scientists and help shape the study of limnology, both in Mexico, and far beyond its borders.

Diego Fontaneto  
*Verbania Pallanza, Italy*





# Introduction

Limnology studies continental aquatic ecosystems which include rivers, streams, lakes, reservoirs, cenotes, temporary bodies of water, flood plains, groundwater, polar waters, swamps, marshes, and flooded lands. Continental waters are generally made up of fresh water and are an essential natural resource for the survival of many species of animals and plants that inhabit the planet's biosphere. However, its scope includes knowledge of continental brackish and salt waters, such as coastal lagoons, marshes, estuaries, and inland seas.

The field of study of limnology is multidisciplinary, interdisciplinary, and in recent years, transdisciplinary. Its studies focus on understanding the interactions between aquatic organisms and their environment, based on different disciplines such as physics, chemistry, geology, biology, and mathematics. These relationships have helped to understand the dynamics and functioning of these complex ecosystems, the understanding of biogeochemical cycles, energy exchange processes in the food web, species ecology, functional links, ecological indicators, pollutants, and resource management, among others. Limnology also integrates evolutionary, ecological, and social factors that are determinants in the composition, distribution, and abundance of species in these ecosystems.

Mexico is a country that has a great variety of continental aquatic ecosystems which have been studied from different fields of knowledge, highlighting their enormous richness, biological diversity, and ecosystem services, which have been essential for the development of productive activities that generate national wealth and population well-being.

The book *Topics of Limnological Research in Mexico* is an academic and scientific dissemination work, made up with original investigations and reviews focused on studies of continental waters in Mexico. The works included in this book address diverse topics, from water quality with a focus on aquifers, dams, rivers, and environmental problems; microorganisms such as phytoplankton focused on composition by taxonomic groups, ecology, metabolism, temporality, and monitoring of potentially harmful cyanobacteria; zooplankton such as ciliates, rotifers, cladocerans, copepods, and hydrozoans, focused on their ecological role, population structure, taxonomy, distribution, diversity, richness, ecological indicators, and trophic status; macroinvertebrates determining their reproduction in captivity for cultivation purposes and on their diversity in natural ecosystems; as well as on the

socio-ecological challenges faced by regional fisheries. The aforementioned topics are highly professional research, which is at the forefront of advances in the development of limnology in the world and which generates knowledge of high scientific value. The works included in this book show the great effort that is carried out in Mexico to develop quality research and generate valuable knowledge in the field of limnology.

The University of Colima is located in the middle region of the Pacific Ocean and is continually generating knowledge derived from research on aquatic resources carried out by its faculties and research centers, which is why it always seeks to bring knowledge closer and contribute to the dissemination of scientific and technological research developed in Mexico. This book is an example of the collaboration between different university departments made up of researchers, professors, students, and various human resources that form a multidisciplinary and collaborative academic effort aimed at disseminating knowledge, not only for academic purposes, but also as a dissemination of science.

Alfredo Pérez Morales





# Analysis of the Ionic Quality of the Water in the North Aquifer and Cozumel Island, Quintana Roo, Mexico

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## Abstract

The Yucatan Peninsula comprises the states of Yucatan, Campeche, and Quintana Roo in the southeast of Mexico. Although it heavily relies on the aquifer as the primary source of water for socioeconomic and ecological needs, quality and quantity remain mostly unknown. Being a karstic region, the aquifer's recharge depends solely on precipitation with discharge occurring mostly through natural features like cenotes. The objective of this study was to characterize water quality from various sites from the northern part of the Yucatan Peninsula and to identify extraction region's potential on Cozumel Island. Ground and surface water quality from water bodies such as wells, cenotes, flooded caverns, and extraction wells within the central-northern Quintana Roo and Cozumel Island were analyzed to determine ion concentration ( $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{HCO}_3^-$ ,  $\text{Cl}^-$ ,  $\text{SO}_4^{2-}$  and  $\text{F}^-$ ), electrical conductivity, temperature, dissolved oxygen, pH, salinity, and chlorophyll-a. Results show heterogeneity in the environmental characteristics of all analyzed systems ( $n=231$ ), with 55 % Ca-Mg bicarbonate type, 24 % mixed type, and 21 % Na-K & Cl- $\text{SO}_4$  type. A high concentration of Cl- $\text{SO}_4$  in the coastal areas are a sign of seawater mixing with freshwater and can be used as indicators of possible intrusions at inland wells. Sites with mixed water types should be monitored to ensure their viability for socioeconomic water use. Water quality monitoring and potential extraction volumes from various sites must complement each other for sustainable water resource management. This analysis enhanced understanding of the hydrological behavior and heterogeneity of the karst aquifer.

## Keywords

Wells, sink holes, groundwater, karst, Yucatan Peninsula.

## Introduction

Groundwater is one of the most important sources of drinking water, supplying approximately 20-50 % of all demand worldwide (Steube et al., 2008; Brinkmann & Parise, 2012; Hartmann et al., 2015). In Mexico, groundwater covers 39.2 % of the total volume granted for all consumption uses, approximately 34,839 hm<sup>3</sup> per year as of 2018 (Conagua, 2019). The Yucatan Peninsula is located in the southeast of Mexico, and it is constituted by the states of Yucatan, Campeche, and Quintana Roo. The whole aquifer is the most viable source in the region to fulfill water needs of the population and for economic activities. According to the National Water Commission (Conagua), the region is divided into four main aquifers: Cerros y Valles, Xpujil, Yucatan Peninsula, and Cozumel (Conagua, 2020b). The four aquifers have an estimated annual water availability of 13,200 mm<sup>3</sup>/year and an extraction volume of 2,120 mm<sup>3</sup>/year; meanwhile only in the North of the state of Quintana Roo, the yearly water availability is estimated in 561.1 mm<sup>3</sup> with extraction volumes of 486.8 mm<sup>3</sup>/year (Conagua, 2021).

Cozumel is the third largest island in Mexico located in the state of Quintana Roo, approximately 17.5 km off the coast of the city of Playa del Carmen (Zack & Lara, 2003). It has a total surface area of 473 km<sup>2</sup>, with an average length of 30 km and an average width of 16 km (Conagua, 2020a), with an average elevation of 5 m and some hills up to 10 m in the south (Ward, 2004). On the island, population has increased from 1,500 inhabitants in 1936, to almost 90,000 inhabitants in 2015 with the urban area concentrated mainly in the western sector of the island (Hernández, 2001; Frausto-Martínez et al., 2016; INEGI, 2017). Tourism is the most important economic activity with a recorded arrival of 575,055 tourists and 3,391,241 visitors in 2015 (Segrado-Pavón et al., 2017).

Although the state of Quintana Roo is generally conceived as a region with high water reservoirs due to its large amounts of yearly rain and low hydric pressure (Conagua, 2019; 2020b), it is also very vulnerable. It has been reported that poor water quality reduces the possibilities of water use (as for drinking), resulting in health and environmental consequences, as well as a reduction in availability (Mishra et al., 2021). Chemical contaminants from human activities (estrogen, soap, fertilizers, pesticides, and petroleum products) in groundwater are one of the biggest threats to the available fresh water as recent studies suggest their presence in the Peninsula's aquifer (Medina-Moreno et al., 2014; Torres et al., 2014; Long et al., 2018); which along with a national 15.7 % of increase in national groundwater extraction from 2009 to 2018, compromise available water for future consumptive and non-consumptive use (Gondwe et al., 2011; Conagua, 2019).

As rainwater infiltrates into the aquifer and flows through the underground sediments, it accumulates elements (such as soluble ions) with which it comes into contact.

Piper Diagrams are known to be used to describe chemical water characteristics, which aid in the identification of 1) rainfall composition and infiltration, 2) sewage contamination, 3) dissolution of carbonate and minerals, and 4) mixing with saltwater (Back & Hanshaw, 1971). Ion analysis through piper diagram is important as it can provide a reference for water origins, status, flow, and recharge dynamics (Lagomasino et al., 2015). The aim of this study is to characterize water quality from the northern aquifer of the Yucatan Peninsula and to identify vulnerable water extraction regions in Cozumel through water concessions.

In this study, vulnerability was addressed based on the type of water (in relation to the ions present), its location, and in the case of Cozumel, the volumes of extraction granted within the Public Registry of Water Rights (REPDA).

## Materials and Methods

The analyzed area is the region called “*Acuífero Norte de Quintana Roo*” or Northern Aquifer of Quintana Roo, it is included in the polygon bounded from Cancún, Cozumel, Tulum, Cobá cities; and towards the West of Cobá, and Nuevo Xcán cities, into the geographic coordinates 20°15'N-86°44'W; 21°15'N-87°40'W, Quintana Roo, Mexico (Fig. 1).

Determinations of ionic content in natural waters that were made intermittently between 2008 and 2019 by local administrations, or by us (in the project QROO 2005-2008-01-19177 “Geohydrological Study and Evaluation of Polluting Sources of the Northern Aquifer of Q. Roo, Mex”), were also considered. These determinations were electrical conductivity, dissolved oxygen, and salinity concentration, done *in situ* with a calibrated multiparametric probe (Hydrolab-DS5x), by following standardized testing methods described in NMX-AA-007-SCFI-2013 (Secretaría de Economía, 2013) or by the American Public Health Association (APHA et al., 1996).

The considered source of data included coastal and lagoon areas, urban and rural areas, and catchment areas of rainwater for drinking water supply, which are under local administrations (Fig. 1). The physical and chemical characterization of water in the months of March and July to September of 2008-2019 was carried out through the analysis of water samples for cations and anions, such as  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{HCO}_3^-$ ,  $\text{Cl}^-$ ,  $\text{SO}_4^{2-}$  and  $\text{F}^-$ . A total of 231 sampling points were considered, 188 in the continent and 43 in Cozumel. For some of the sites, additional measurements of temperature, dissolved oxygen, pH, salinity, and chlorophyll-a (Chl-a) were taken.

To allow the comparison of the chemical results to the Mexican standard of drinking water and other official regulations, and because they are characteristic ions of karstic aquifers; the concentrations of  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{HCO}_3^-$ ,  $\text{Cl}^-$ ,  $\text{SO}_4^{2-}$  and  $\text{F}^-$  were considered.

The cation and anion concentrations were determined by certified laboratories where accuracy of the chemical analyses was controlled by the ion charge balance, proposed



by Fetter (2001). Ion concentrations were determined in the laboratory of the Technical University of Berlin, Department of Hydrogeology, or proceed from the records in the National Measurement Network on Water Quality (Red Nacional de Medición de Calidad del agua –RENAMECA-, and SINA). These certified laboratories use ion chromatograph, mass spectrometer, and flame atomic absorption spectrometer to calculate ionic concentration. Then, the reliability of these data was performed using the general criteria for the quality control of the analytical results (Secretaría de Economía, 2015) while  $\text{Chl-a}$  concentration was estimated using colorimetric methods (APHA et al., 1996).

The Cozumel water sample lacked  $\text{HCO}_3^-$ ,  $\text{Na}^+$  and  $\text{Mg}^{2+}$  ion content; an average of 314.4 (mg/L) alkalinity was estimated by considering reports from 183 sites within the Yucatan peninsula from 2012-2018 provided by *Sistema Nacional de Información del Agua (SINA)* from Conagua. According to an official water analyze norm, NMX-AA-036-SC-FI-2001 (Secretaría de Economía, 2001), alkalinity is to be estimated as  $\text{CaCO}_3$  mg/L by national institutions in Mexico. Bicarbonate ion ( $\text{HCO}_3^-$ ) content was estimated according to Lind (1985). Cozumel's sodium and magnesium ions were estimated by calculating an average value of water samples carried out by *Comisión de Agua Potable y Alcantarillado (CAPA)* from 90 wells in the catchment basin for every month from 2005-2019.

A Piper diagram was elaborated with the ion content information with the aid of an Excel (Microsoft 365) template published by Zenodo (Stosch, 2022). According to how water sites were positioned within the Piper diagram due to its ionic content, water samples were classified into one of the following three types and a specific dot color was assigned: Ca-Mg Bicarbonate (blue), Mixed (yellow) and Na-K & Cl- $\text{SO}_4$  (purple). Water sample sites were then positioned on maps of the state of Quintana Roo and Cozumel Island, using Open Source Software QGIS 3.34.1 'Prizren'.

Finally, Conagua was consulted about information from users registered in the REP-DA in Cozumel 2023. For each user, their geoposition, volume of water granted for annual extraction, and assigned use for extracted water were obtained. With this information, a map was created to locate each user within the island, depending on use.

With the granted extraction volumes and the location, a map was developed using Inverse Distance Weighting (IDW) to identify the areas with the greatest individual user potential for pressure on the aquifer due to the extraction volumes.

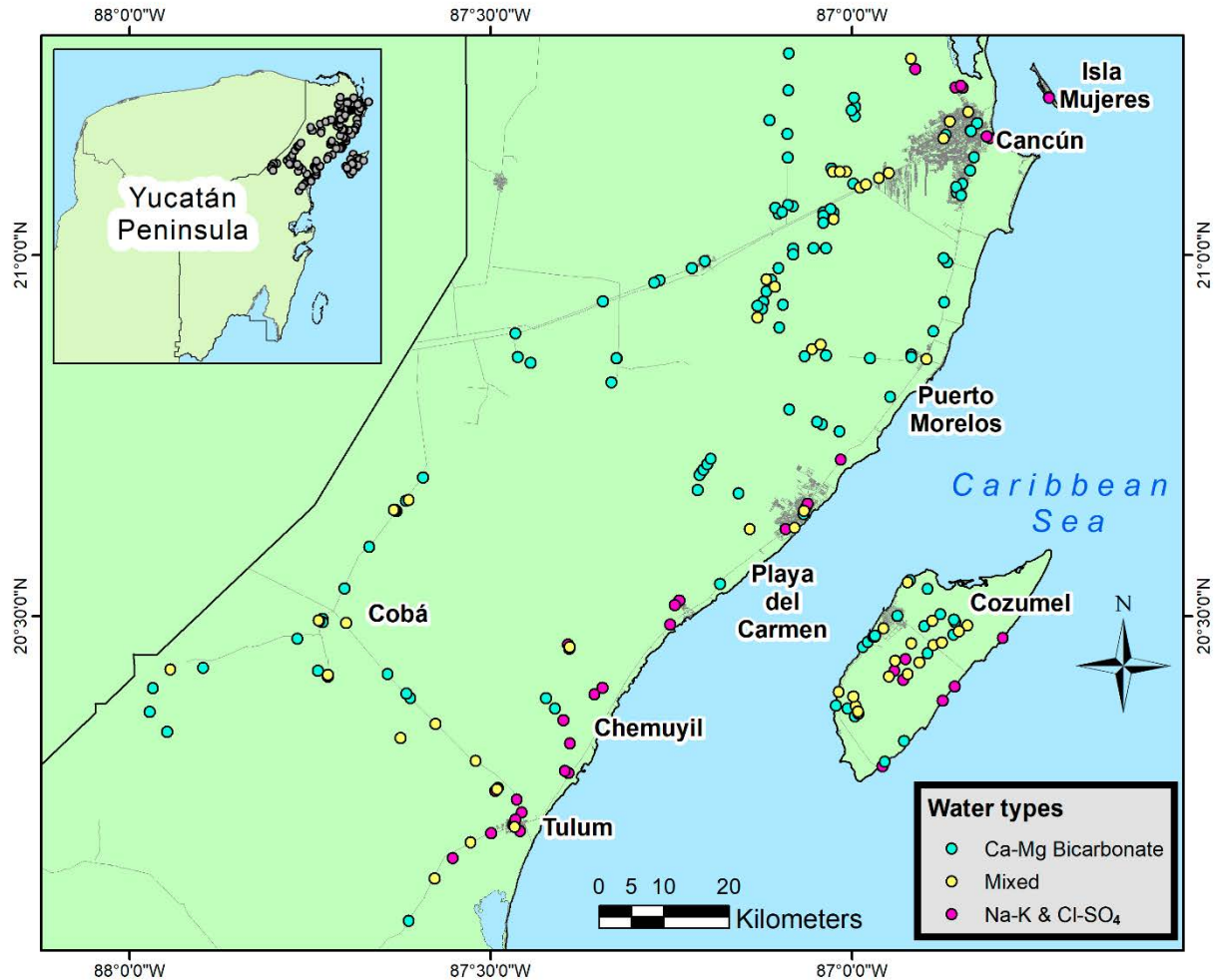


**Figure 1.** Water Types and Location of Sampling Sites.

## Results

A total of 231 water samples from within the state of Quintana Roo were analyzed and grouped according to their ion concentration within the Piper diagram. Water samples were sorted within one of three water type groups: 1) Ca-Mg bicarbonate being the one with most samples ( $n=127$ ), 2) Mixed type ( $n=56$ ) and Na-K & Cl-SO<sub>4</sub> with the least ( $n=48$ ).

As shown in Table 1, temperatures, dissolved oxygen concentrations, and pH showed very slight variations on their values among the different water types. As for Chl-*a* values, the lowest concentration was found in bicarbonated water type ( $\bar{x}=0.7\pm0.9$  mg/L) while it was almost 6 times higher in the other two water types described in this study.

**Fresh, bicarbonated water:** The North and West of the surveyed aquifer in the Yucatan Peninsula, with low Electrical Conductivity (EC) for the groundwater representing the best water quality. The EC ranges (Table 1) from 20 to 4,060  $\mu\text{S}/\text{cm}$  ( $\bar{x}=890\pm540$ ). Most of the sites correspond to deep wells ( $\sim 92\%$ ) that are in the areas of rainwater catchment basins (blue dots in Fig.

1) which subsequently go towards the urban areas of Cancún and Playa del Carmen. A smaller percentage is made up of epicontinental freshwater bodies like lakes and lagoons (~8 %).

According to the Piper diagram (Fig. 2), the water type that dominates in this region is the Ca-Mg Bicarbonate type. Such catchment basins are located within 5 to 20 km of the urban areas. In Cozumel, this type of water is found in the north-central region of the catchment area and in epicontinental lagoons to the east of the Island.

Mixed type waters: With EC values from 1,090 to 2,490  $\mu\text{S}/\text{cm}$  ( $\bar{x}=1,447\pm582$ ); mixed type, showed to be the second most classified systems as by their cation and anion content (yellow dots in Fig. 2) dominate in the Cozumel Island catchment area and some deep wells in the catchment area of Cancún and Playa del Carmen. Mixed waters are also part of superficial lakes and lagoons in this region.

Na-K & Cl- $\text{SO}_4$  type: This water type has EC values ranging from 1,020 to 53,321  $\mu\text{S}/\text{cm}$  ( $\bar{x}= 5,667\pm8,384$ ) and represent the most saline ( $\bar{x}=3.2\pm6.8$  ups) when compared to bicarbonate ( $\bar{x}=0.6\pm0.2$  ups) and mixed ( $\bar{x}=0.8\pm0.2$  ups) types. They are represented by purple dots, the least abundant of all analyzed water types in this study and are located near the coastline.

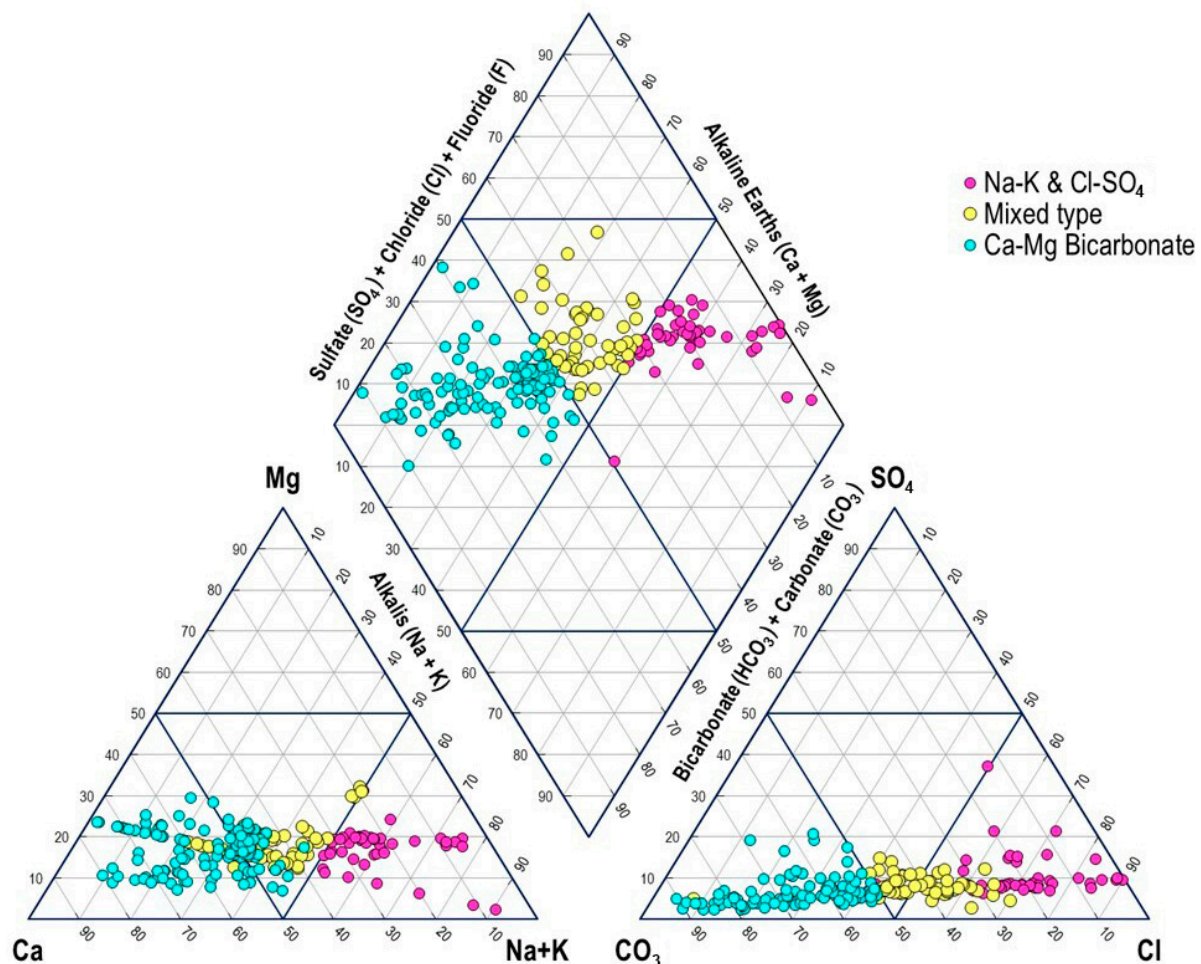


**Figure 2.** Piper Diagram with Samples Sorted by Water Type.



**Table 1.** Water Type Quality Parameters. (EC=Electrical Conductivity; Chl-a=Chlorophyll-a; SD=Standard Deviation)

| Water type                           | Ca-Mg Bicarbonate |     |     |       |     | Mixed   |      |     |       |    | Na-K & Cl-SO <sub>4</sub> |       |       |        |    |
|--------------------------------------|-------------------|-----|-----|-------|-----|---------|------|-----|-------|----|---------------------------|-------|-------|--------|----|
|                                      | Average           | SD  | Min | Max   | n   | Average | SD   | Min | Max   | n  | Average                   | SD    | Min   | Max    | n  |
| Ca <sup>2+</sup> (mg/L)              | 111               | 28  | 25  | 166   | 127 | 130     | 32   | 40  | 191   | 56 | 171                       | 84    | 40    | 585    | 48 |
| Mg <sup>2+</sup> (mg/L)              | 21                | 9   | 5   | 37    | 127 | 32      | 8    | 20  | 56    | 56 | 126                       | 230   | 23    | 1,416  | 48 |
| Na <sup>+</sup> (mg/L)               | 62                | 35  | 4   | 153   | 124 | 133     | 45   | 61  | 258   | 54 | 946                       | 1,704 | 122   | 10,090 | 47 |
| K <sup>+</sup> (mg/L)                | 5                 | 4   | 1   | 19    | 109 | 6       | 3    | 3   | 14    | 38 | 32                        | 46    | 4     | 199    | 41 |
| HCO <sub>3</sub> <sup>-</sup> (mg/L) | 361               | 90  | 117 | 579   | 127 | 348     | 88   | 200 | 566   | 56 | 377                       | 112   | 187   | 780    | 48 |
| Cl <sup>-</sup> (mg/L)               | 114               | 73  | 7   | 510   | 125 | 265     | 103  | 13  | 622   | 55 | 1,691                     | 2,973 | 195   | 17,603 | 48 |
| SO <sub>4</sub> <sup>2-</sup> (mg/L) | 31                | 18  | 6   | 100   | 124 | 56      | 24   | 12  | 113   | 56 | 300                       | 441   | 43    | 2,542  | 48 |
| F <sup>-</sup> (mg/L)                | 1                 | 1   | 0   | 2     | 45  | 1       | 1    | 0   | 2     | 17 | 1                         | 1     | 0     | 3      | 7  |
| EC (μS/cm)                           | 897               | 540 | 20  | 4,060 | 127 | 1,448   | 582  | 20  | 2,490 | 42 | 5,668                     | 8,384 | 1,020 | 53,321 | 48 |
| Temperature (°C)                     | 26                | 2   | 24  | 32    | 109 | 26      | 2    | 23  | 32    | 38 | 26                        | 2     | 25    | 31     | 41 |
| Dissolved Oxygen (mg/L)              | 3                 | 2   | 0   | 8     | 109 | 3       | 2    | 0   | 7     | 38 | 3                         | 2     | 0     | 6      | 41 |
| pH                                   | 7                 | 0.4 | 7   | 9     | 127 | 7.4     | 0.5  | 6.8 | 8.8   | 56 | 7.5                       | 0.4   | 7     | 9      | 48 |
| Salinity (ups)                       | 0.6               | 0.2 | 0.2 | 0.8   | 68  | 0.8     | 0.2  | 0.5 | 1.3   | 23 | 3.2                       | 6.8   | 0.9   | 35.7   | 25 |
| Chl-a (mg/L)                         | 0.7               | 0.9 | 0.1 | 4.1   | 68  | 4       | 15.8 | 0.1 | 76.5  | 23 | 4.3                       | 9.1   | 0.16  | 36.1   | 25 |

Among the sampling sites located on Cozumel Island, 18 were identified which belong to the Ca-Mg bicarbonate type, 18 to the mixed type, and 7 to the Na-K & Cl-SO<sub>4</sub> type (Fig. 3). It is important to highlight that in the center of the island, in the rain catchment area, there were 3 Na-K and Cl-SO<sub>4</sub> type samples.

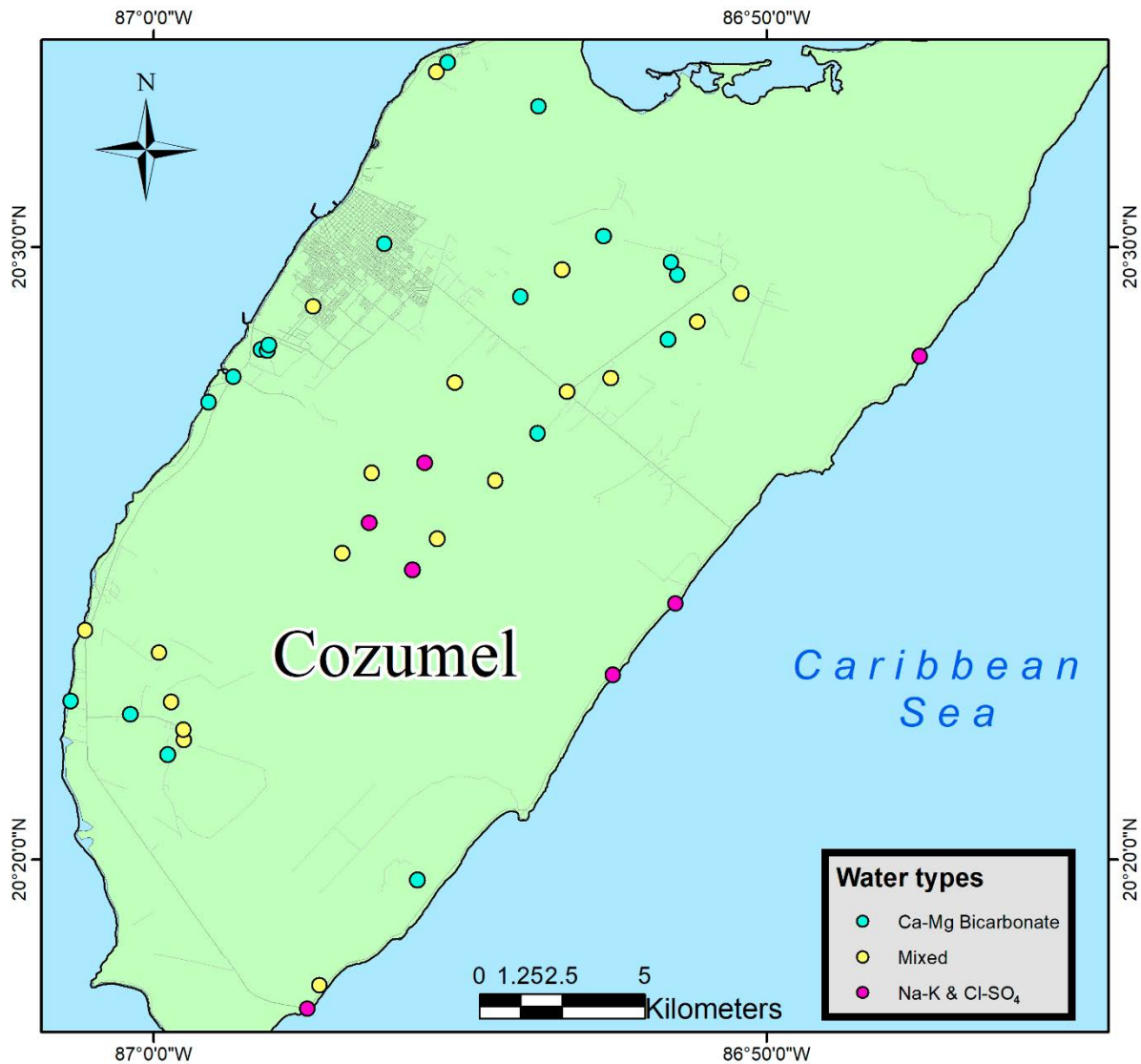


**Figure 3.** A Close Up of Water Types from Samples Taken in Cozumel.

Regarding the information of users registered at REPDA, Fig. 4 represents the 454 records that were identified within the Cozumel polygon, since a same user can be eligible for different extraction points with the same grant. A total of 56 sites were positioned outside the geographical limits of Cozumel, thus not considered in this study. The highest density of users was found in the center of the island and along the transversal highway.

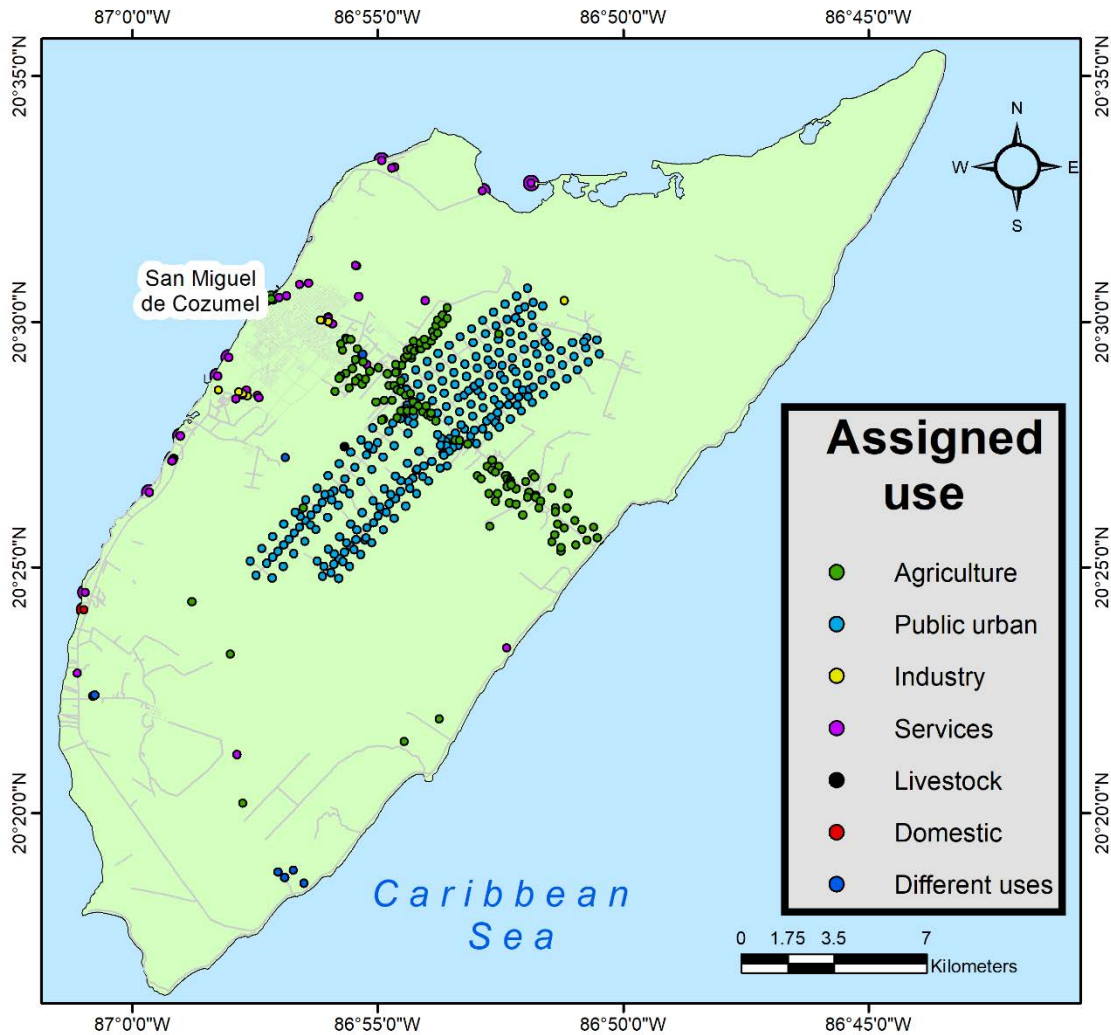


**Figure 4.** Location of Users Registered with REPDA and with the Concession for Water Extraction in Cozumel.

Figure 5 shows the potential water extraction granted for each user yearly. The greatest pressure can be identified in the North-West and South-West areas of the island.



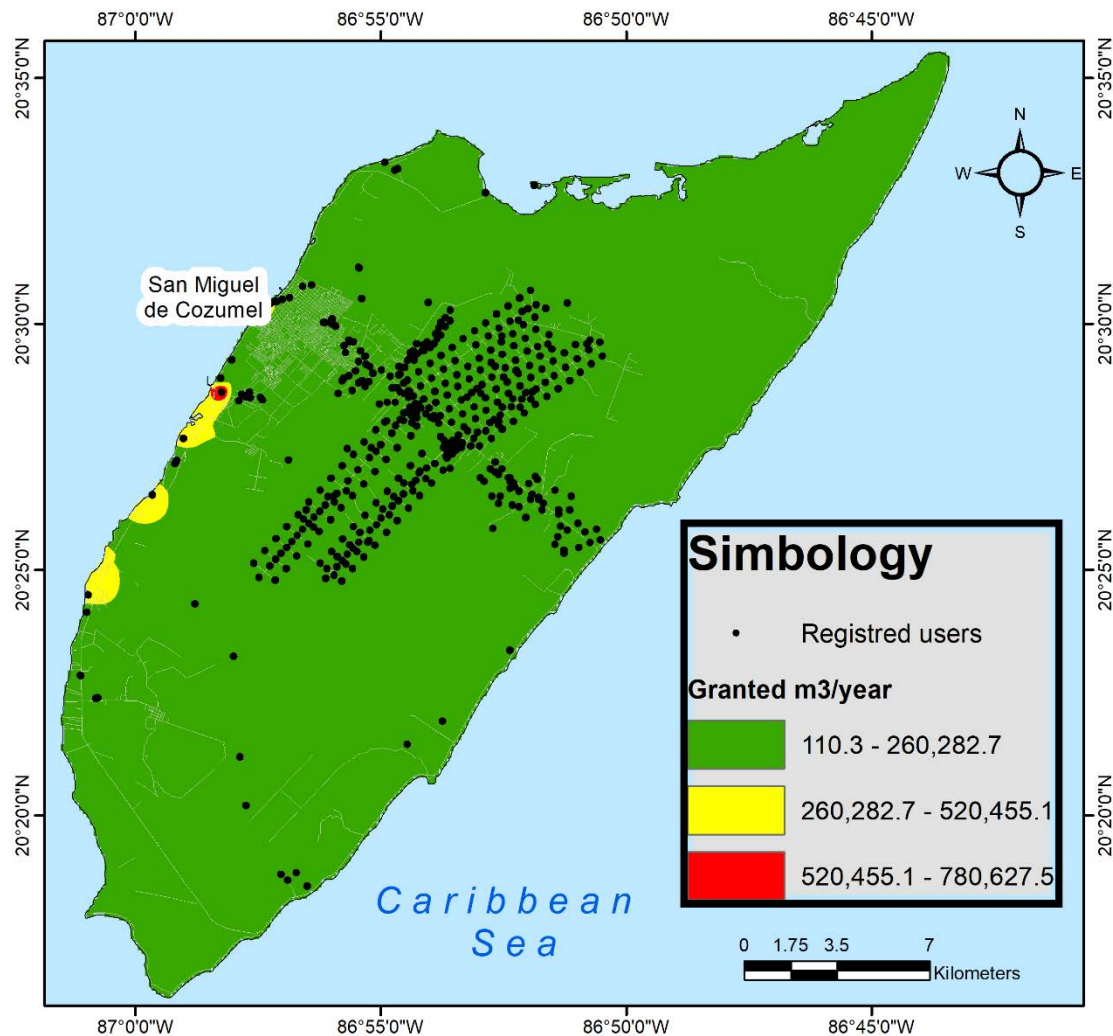


**Figure 5.** Individual Yearly Potential Water Extraction Granted to Users Subscribed to REPDA in Cozumel.

## Discussion

Although the Yucatan Peninsula hosts 4 main aquifers, according to Conagua's classification, the northern continental region of the state of Quintana Roo is part of aquifer 3105 "Yucatan Peninsula," while the aquifer on Cozumel Island is 2305 "Cozumel Island". Three main water types were identified on aquifers 3105 and 2305: Calcium-bicarbonate, Mixed, and Sulfated-Sodium–chlorinated. Conagua reports that the majority of the state of Quintana Roo contains calcium bicarbonate waters while on the coastal line they are sodium chloride type. Only south-central of the Yucatan Peninsula (around Xpujil) has calcium sulfate water been recorded (Conagua, 2021). The Conagua reports provide a good overall picture; but with the detailed analysis done here, it was possible to identify at least three different types

of water (only in the North of Quintana Roo), their location, and with an accurate ion charge balance, ranged between -4.99 to +5.0 % in the surveyed samples.

The westerly region of the investigation area showed low EC in the region where Ca-Mg bicarbonated waters were dominant. These types of waters are presumed to be originated by the dissolution of the carbonates that constitute calcareous rocks due to the dominant presence of evaporites and gypsum (Conagua, 2021). Further water quality analysis should be performed to determine if water within this type and location meets the criteria established within NOM 127 (Secretaría de Salud, 1996) which would make it suitable for potabilization processes. If the water does not meet the minimum standards established for potabilization, other uses could be suggested, such as agricultural or industrial, depending on the results of water quality analysis.

As previously mentioned, in the Yucatan Peninsula, sulfated natural waters are in the geohydrological zone of South Campeche and Quintana Roo and the chlorinated water type is described to be located usually along the coastal zone. In these coastal lines, the influence of seawater produces a mixing zone among them and the groundwater (Conagua, 2021). Fig. 1 shows that in the surveyed area, the Cl-SO<sub>4</sub> type waters are found along the coastal area: the wide range of EC of this water type (1,020-53,321 µS/cm) confirms that the presence of SO<sub>4</sub><sup>2-</sup> can be due to the dissolution of sulfate-rich minerals anhydrite (CaSO<sub>4</sub>) and gypsum (CaSO<sub>4</sub>·2H<sub>2</sub>O) as reported by Lagomasino et al. (2015), and to a greater extent, due to the influence of seawater causing a mixing among them and the groundwater. The presence of seawater is notable in the underground area of the east coast of the studied area. This influence of seawater towards the interior apparently presents a wedge shape: from the south of Tulum to the north of Playa del Carmen (in fact, these cities are above the area of seawater entry), as well as on the east margin of Cozumel Island. There is evidence of saltwater intrusion in the state of Quintana Roo and in Cozumel Island due to pumping rates and with a saline interface 22 m inland (Cardoso, 1993). According to Deng et al. (2017) considering the Quintana Roo aquifer with a 1 m rise in sea level, it is projected that inland saltwater intrusion could extend as far as 150 km under head-controlled conditions and 1 km under flux-controlled conditions. This entry of seawater into the underground region of the continental area occurs in recent Quaternary deposits (Sánchez & Pinto et al., 2015), both on the eastern margins of the Peninsula and the island; but it also occurs in the oldest deposits on the western edge of the island. Evidence about penetration of the seawater towards the continent in the coastal regions of Yucatan Peninsula, has been described by Steinich et al. (1996), and Steinich and Marin (1997), who have reported seawater penetration up to 110 km from the coast.

Black and Hanshaw (1971) have concluded that the primary factors influencing the chemical composition of water in the Yucatan Peninsula are the dissolution of carbon-

ate minerals and the blending of freshwater with underlying saline water, with seawater sites located at the right end of the triangle in the Piper diagram (Back & Hanshaw, 1971). Mixed-type waters should be evaluated according to parameters described in the NOM 127 (Secretaría de Salud, 1996) to confirm whether they are viable for use by institutions that provide domestic drinking water services. Although the chemical nature of mixed-type waters may not be suitable for potable use, perhaps (depending on their composition) they could be processed through techniques such as reverse osmosis which would allow their use as potable water. Additionally, they could also be used for other purposes such as agriculture or industry, as the quality requirements are not as stringent for these uses, although always considering that they do not contain compounds that could be harmful to human health. Unfortunately, water from Na-K & Cl-SO<sub>4</sub> type tend to be more of the saline-like type with average salinities of 3.2 ups, conductivities of 5,667  $\mu\text{S}/\text{cm}$ , and an elevated Chl-a content when compared to Ca-Mg Bicarbonate type, so their use is limited since they are not recommended for purification or agricultural use.

Cozumel shares characteristics with the Caribbean coast of the Yucatan Peninsula from the northern cape to Tulum such as upper Pleistocene eolianite, elevation, sedimentology, stratigraphy of subaerial and shallow-marine limestones (Ward, 2004). Miocene Pliocene are the sedimentary rocks present both, in the state of Quintana Roo from Chetumal to Cancun and on Cozumel Island (Ramos, 1975). For this reason, the island shows eolianites on the East which show classic terra rosa paleosol and vegemorph development, while from North to West there are eolianites without well-developed vegemorphs (Kelley et al., 2011). This might explain the prevalence of Ca-Mg bicarbonate type waters within the studied area.

Although a clear pattern of Na-K & Cl-SO<sub>4</sub> water types near the coastline of Quintana Roo was observed, the distribution is not so evident for the samples taken on the island, even though they occur around the east coast and in the center (Fig. 3). The center of the island is known to be reserved as an area for the recharge of the aquifer and the extraction of water to supply the needs of the inhabitants (Hernández-Flores et al., 2021). The presence of a water type associated with the influence of seawater mixing in this area could represent a thinning of the freshwater layer due to extraction practices, as it is the area where water is extracted to supply most of the island population's needs. This hypothesis is supported by a recent study that reports an increase in the number of extraction dejected wells (2015-2018) in that area due to the increase in the concentration of chlorides present in water samples (Hernández-Flores et al., 2021). For this reason, further studies to characterize water quality dynamics in this area are suggested.

Geographic information on the ionic content and quality of water provides an idea of possible sources that allow the extraction and use of water for different purposes. By over-

lapping the information of Figs. 3 and 5, it is possible to identify sites that with increase in exploitation, could harm the Cozumel aquifer. Highly vulnerable areas could be identified by the presence of Na-K & Cl-SO<sub>4</sub> water types with several water extraction sites nearby; in the case of Cozumel, it could be indicative of over-extraction and saline intrusion events in the aquifer, especially in the north-central part of the island. However, monitoring and updating the tools presented in this work are highly recommended, as the aquifer may be vulnerable to high extraction volumes and show indicators of degradation, such as the presence of brackish water. When Figs. 4 and 5 are combined, it is possible to see that the largest extraction of water volumes is found near the coastal area in the west of the island and those that belong to the “services use”. According to the Dupuit–Ghyben–Herzberg model, the thickness of freshwater lenses reduces in correlation to the nearness of shoreline, then the over-extraction of freshwater in this area (bicarbonate, or mixed) could increase the risk of marine intrusion such as has been observed in Tulum by Gondwe et al. (2011).

The use of maps providing information about ions in water, maximum volumes granted for extraction, and their location allows triangulation of information to obtain a more comprehensive overview of the water resource and its utilization. It's important to note that although agricultural use accounts for 130 extraction points in Cozumel, the volumes are not as high. However, it is the service sector that has shown high volumes of water concessioned for extraction, locating itself near the coastline. In Figure 5, the maximum annual extraction volumes are observed, with those of greater volume located near the coastline. This somehow prevents the deterioration of the thickest layer of freshwater, which is found in the center of the island (Lesser et al., 1978). This is because near the coastline, the thickness of freshwater thins out, although it also increases vulnerability to saltwater intrusion due to proximity to seawater. Beyond being a relief, it is an incentive for the development of research that describes the effect of “small” extractions but with a high density of users in the island recharge zone, so research addressing the cumulative extraction volumes in a geographical area is encouraged. All this, along with the idea of proposing strategies that encourage actions which allow us to sustainably manage water resources. It is suggested that this method of triangulating information between water types and REPDA data be used on mainland, as it provides a more integrative vision for resource management.

The method presented in this study allows to consider water types, water extraction potential, and their geographical location. Therefore, we believe it can be a useful tool for decision-making related to the proper management of the resource, such as granting new extraction concessions or identifying new areas of possible water supply with sufficient quality for potabilization.



## Conclusions

This study provides valuable insights into the water quality and extraction dynamics of the Yucatan Peninsula, particularly focusing on the northern Quintana Roo aquifer and on Cozumel Island. The analysis revealed three main water types: Ca-Mg bicarbonate, mixed, and Na-K & Cl-SO<sub>4</sub>, emphasizing the heterogeneity of the aquifer's environmental characteristics.

The prevalence of Ca-Mg bicarbonate type water, along with physicochemical analysis of the water, especially in the rain catchment basins, suggests that the water extraction provided complies with NOM-127-SSA1-2021 (Diario Oficial de la Federación, 2021). However, the presence of mixed and Na-K & Cl-SO<sub>4</sub> types, particularly in coastal areas, raises concerns about seawater intrusion and the need for continuous monitoring. The research highlights the importance of considering both water quality and extraction sites for sustainable water resource management. The study underscores the importance of incorporating this geospatial information into future management strategies, encouraging a balance between water use demands and the preservation of aquifer integrity.

Ultimately, this research contributes to a better understanding of the hydrological behavior of the karst aquifer in the region, thanks to the characterization of physicochemical variables and extraction potentials. The combination of the information exposed in the figures of this study offers a foundation for informed decision-making and emphasizes the need for continuous research and proactive measures to sustainably manage water resources in the Yucatan Peninsula and Cozumel.

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## Authors' Contributions

GHF conceived and designed the experiments, analysed the data, prepared figures and tables, and authored drafts of the paper. MAGA conceived and designed the experiments, prepared figures and/or tables, and authored drafts of the paper. ACM analysed the data and authored or reviewed drafts of the paper.

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# Limnological Variations of a Tropical Semi-arid River Dam System, Central México

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## Abstract

This study focused on the basic limnological variations of the Tula River-Zimapán dam under different climatic conditions (rains, post-rains, and dry cold). The sampling included three river sites and seven sampling sites in the reservoir. These included dam sites, water temperature, dissolved oxygen, total dissolved solids, water column profiles, nitrogen, and total phosphorus of composite samples from a 0.3-3 m layer. In the river, the same parameters are at 20 cm in depth. Physicochemical and nutrient spatial variations showed that the three riparian sites are a separate group from the reservoir sites as a response to different temperatures, oxygen, transparency, total dissolved solids, and nutrients; similarly, well-defined zones of the reservoir in June and February: riparian, transitional, and lacustrine. The temperature variation in each site water column was low; however, dissolved oxygen significantly varied in June and October. In surface water in June with initial rains, physical and chemical data peaked; in May and February, oxygen >4 mg/l, suitable for fish production, but not convenient in November because of frequent hypoxia conditions. Total nitrogen and phosphorous on the Tula River had values above 14 mg/l in the entrance of wastewater, decreasing in the remaining river sites with spring

waters nearby; in the reservoir, values were high for phosphorous and lower for nitrogen. The limnological conditions of the river dam system respond to the permanent entry of wastewater with organic matter, dissolved oxygen producers-consumers, and the potential nutrient loads generated by anthropogenic activities in the drainage basin and the reservoir surroundings.

## Keywords

Hypereutrophic, spring waters, fisheries, wastewaters, spatiotemporal variations.

## Introduction

In river-dam systems, one of the problems reflected by the water quality on dams is the accumulation of phosphorus and nitrogen whose primary source is particulate organic matter or dissolved organic matter carried by currents and runoff, as well as indigenous organic material, mainly vegetal, accumulated in the river basins. Depending on the climatic and land use conditions in the basin, the quantity and quality of the tributaries to the reservoir, there is an influence on the loading rates of sediments, minerals, and nutrients, accelerating the natural process of eutrophication with excessive growth of algae in natural or artificial systems, influencing water quality; each reservoir can respond differently regarding water quality to changing local and regional climatic conditions (Delazari-Barroso et al., 2009; Kazi et al., 2009). The sources of these compounds come from the urban population center as well as from agricultural and livestock areas with excessive nutrient loads from urban, farming, livestock, and industrial wastewaters have put at risk the different uses of water, aquatic biota, and human health; particularly in large human settlements where there is a deficient infrastructure for wastewater treatment (Kazi et al., 2009; Cunha et al., 2011, 2013; Fontana et al., 2014).

The Tula River feeds the Zimapán hydroelectric dam with wastewater. The high wastewater source originates from the megalopolis México City. This water is considered a high risk for human health due to a wide range of contaminants and emerging substances, as well as a high load of organic matter and nutrients, increased after intensive precipitation events.

The changes in water quality in dry and rain conditions can lead to several limnological spatiotemporal changes in physicochemical parameters in the water column, during the mixing and stratification patterns, water transparency, pH, and nutrients in the photic zone in the dam, and through the altitudinal gradient in the river. There are few studies at that level of detail in subsequent periods of drought and rainfall; water quality variations of tributaries may present spatial gradients along reservoirs in their riparian, transitional, and lacustrine zones (Thornton, 1990; Geraldés & Boavida, 2004; Olds et al., 2011; Azalina et al., 2012).

The study focused on determining under three different weather conditions (dry cold, rain, post-rain) the limnological spatiotemporal variations on the water temperature, dissolved oxygen, total dissolved solids, pH, water transparency, total nitrogen, and phosphorous in the river-dam system. This limnological spatiotemporal variations knowledge will be helpful in the prevention of diseases in fish and aquaculture activities caused by water quality and oxygen fluctuations, as well as water quality for farming activities using a pumping floating system.

## Study Site

The Zimapán reservoir, built for hydroelectric purposes in 1995, is located between the States of Queretaro and Hidalgo (Central Mexico); at a height of 1,870 m, it was considered the first project to take advantage of 95 % of México City wastewater for electric power generation. The main wastewater contributions to the reservoir come from the Tula and San Juan rivers. The reservoir has a storage volume estimated at 1,354 mm<sup>3</sup> and an approximate surface of 2,300 ha, with an average depth of 50-60 m and a maximum depth of 170 m in the curtain (20°40'N; 99°30'W).

The main affluent Tula River, in the state of Hidalgo, promotes high agricultural productivity in the Mezquital Valley, which 40 years earlier was an area of low farming yields due to the semi-arid climate and scarce surface water. The river also receives waste from cement, oil, thermoelectric industries, hospitals, and urban activities (Ramos et al., 2018). The second affluent San Juan River (state of Querétaro) drains the wastewater from the cities of San Juan del Río and Tequisquiapan, with chemical, metal, textile, and wine industries (López et al., 2021). This study works in the Tula River because it is a permanent affluent with an estimated flow of 12.3 m<sup>3</sup>/s compared with the estimated flow of 2 m<sup>3</sup>/s of the San Juan River.

The area has climate type BWh, very dry and warm, with average annual temperatures >22°C and the coldest month <18°C, with a summer rainfall regime and a prolonged dry season <https://sma.gob.mx>; May is the hottest month of the year with some rains; the annual temperature variation is 6.8°C. The average yearly rainfall is 432 mm, and the rainy season is from April to September. In June, the highest rainfall is an average of 79 mm; the lowest is in February and March, with an average of 8 mm, and the lowest temperature is 16°C. The outside vegetation of the dam is primarily thorny scrub and, to a lesser extent, grassland; the native plant communities are xerophilous scrub: submontane scrub, desert crash-cause scrub, and desert rosette scrub (González-Medrano, 2003).

Tula River wastewater contains a high load of organic material and organic pollutants, heavy metals, and pharmaceutical waste (Siemens et al., 2008; Sols et al., 2009; Rubio-Franchini et al., 2016; Lesser et al., 2018). Given the high organic load that the dam



has, the Riparian communities from the year 1997 have taken advantage of this reservoir for commercial fishing based mainly on tilapia (*Oreochromis* spp.). Later, sport fishing, besides productive activities, irrigated agriculture by pumping water inside the dam, incipient realty, and recently, ecotourism (López et al., 2007).

## Material and Methods

On a temperature-rain climate basis, wet (June), post wet (October), cold dry (Feb), physical and chemical data were sampled in seven sites considering the three reservoir zones (Thornton, 1990), one riparian San Juan River, one riparian Tula River, two transitional, and three lacustrine); on the river, three sites: México's wastewater entrance after the Requena dam, one after spring waters zone, and one before the entrance to the reservoir (Figure 1).

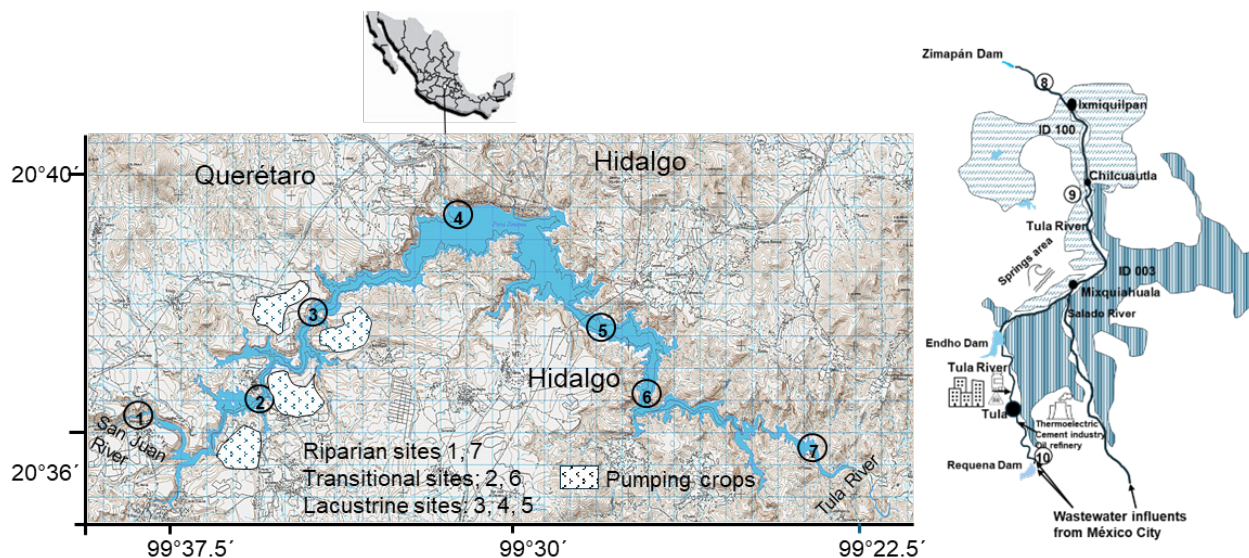


**Figure 1.** Location of Study Area River Tula with Sites 8, 9, and 10 (right) showing the Wastewater Influent, Irrigation Districts (ID) for Permanent Agriculture, Spring Waters Zones, and Important Cities; the Zimapán Dam with Sites 1-3 (Querétaro State) and Sites 4-7 (Hidalgo State), Pumping Crops Areas.

Composite samples from 0.3-3 m layer from water were collected using a PVC tube (3" Ø x 3 m). Historic temperature and rainfall data characterize the wet (June), post-wet (October), and cold drought (February) months in terms of the hydrological conditions of the reservoir—the following environmental variables at the site: water transparency on the reservoir was measured using a 21 cm diameter Secchi disk, on the river, with a 1 m long plastic transparent tube with a small 2 inches similar plastic Secchi at the bottom. In the water column, dissolved oxygen (mg/l), temperature (°C), pH, and total dissolved solids (g/l) using a multiprobe YSI (model YSI 556), water column registered 0.3-20 m depth.

Total nitrogen (mg/l) and total phosphorous (mg/l) were analyzed with a spectrophotometer Hach DR/2010, according to procedures in APHA (1995). The unfiltered water samples were on ice before being analyzed in the laboratory.

To evaluate the variability of each parameter in the water column of each site in different climatic conditions, average, standard deviation, and the coefficient of variation in % statistics were used. We considered that a %CV>20 would indicate considerable variations, allowing variations between sites in each study month, Spearman correlation, and the ordination diagram with Cluster analysis (Euclidean distance measure) using raw water column data. The Factorial F test gave us the significance of differences between seasons in the dam-river zones, with an average confidence of 95 %, and the Tukey confirmatory test (95 % confidence, 0.05 error). We get the graphing and analyses with Microsoft Excel and Statistica 10 software.

## Results

Descriptive statistics with average, standard deviation, and significant differences based on CV% of water column study variables among seven sampling sites for each sampled month are in Table 1.

**Table 1.** Variations of Water Temperature, Dissolved Oxygen, and TDS (Total Dissolved Solids) by Month Considering Water Column (0-20 m).  
Underlined= CV% <20, Significant Variation

| Temperature °C | 1     | 2     | 3     | 4     | 5     | 6     | 7     |
|----------------|-------|-------|-------|-------|-------|-------|-------|
| February       |       |       |       |       |       |       |       |
| Average        | 18.08 | 17.63 | 17.69 | 17.87 | 18.24 | 18.37 | 18.76 |
| Std. Dev       | 1.01  | 0.22  | 0.36  | 0.62  | 0.95  | 0.62  | 0.48  |
| n              | 13    | 13    | 13    | 13    | 13    | 13    | 13    |
| CV %           | 5.60  | 1.26  | 2.06  | 3.47  | 5.23  | 3.40  | 2.56  |
| June           |       |       |       |       |       |       |       |
| Average        | 27.05 | 23.11 | 23.49 | 23.07 | 23.31 | 23.09 | 21.11 |
| Std. Dev       | 2.41  | 1.20  | 0.26  | 0.93  | 1.39  | 1.20  | 0.67  |
| n              | 5     | 13    | 13    | 13    | 13    | 13    | 13    |
| CV %           | 8.92  | 5.20  | 1.09  | 4.01  | 5.95  | 5.19  | 3.18  |
| October        |       |       |       |       |       |       |       |
| Average        | 22.37 | 22.20 | 22.15 | 21.81 | 21.76 | 21.65 | 21.27 |
| Std. Dev       | 0.94  | 0.93  | 0.50  | 0.21  | 0.42  | 0.49  | 0.66  |
| n              | 11    | 12    | 12    | 13    | 13    | 13    | 13    |
| CV %           | 4.19  | 4.19  | 2.25  | 0.98  | 1.95  | 2.28  | 3.08  |

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| Dissolved oxygen   mg/L |       |       |       |       |       |       |       |
|-------------------------|-------|-------|-------|-------|-------|-------|-------|
| February                |       |       |       |       |       |       |       |
| Average                 | 6.74  | 6.88  | 6.88  | 6.84  | 6.68  | 6.64  | 6.52  |
| Std. Dev                | 0.36  | 0.12  | 0.13  | 0.21  | 0.31  | 0.21  | 0.14  |
| n                       | 13    | 13    | 13    | 13    | 13    | 13    | 13    |
| CV %                    | 5.36  | 1.72  | 1.86  | 3.05  | 4.58  | 3.20  | 2.20  |
| June                    |       |       |       |       |       |       |       |
| Average                 | 2.70  | 6.27  | 5.24  | 4.74  | 4.58  | 4.81  | 4.11  |
| Std. Dev                | 0.51  | 0.87  | 0.70  | 1.11  | 1.43  | 1.61  | 0.38  |
| n                       | 5     | 13    | 13    | 13    | 13    | 13    | 13    |
| CV %                    | 18.90 | 13.79 | 13.45 | 23.46 | 31.29 | 33.36 | 9.13  |
| October                 |       |       |       |       |       |       |       |
| Average                 | 4.56  | 5.52  | 5.18  | 5.05  | 2.74  | 3.25  | 2.36  |
| Std. Dev                | 1.34  | 1.43  | 1.60  | 1.02  | 0.36  | 0.58  | 0.90  |
| n                       | 11    | 12    | 12    | 13    | 13    | 13    | 13    |
| CV %                    | 29.26 | 25.87 | 30.95 | 20.20 | 13.26 | 17.96 | 38.11 |
| TDS   g/L               |       |       |       |       |       |       |       |
| February                |       |       |       |       |       |       |       |
| Average                 | 1.50  | 1.51  | 1.60  | 1.62  | 1.62  | 1.75  | 1.85  |
| Std. Dev                | 0.12  | 0.04  | 0.00  | 0.00  | 0.01  | 0.07  | 0.06  |
| n                       | 13    | 13    | 13    | 13    | 13    | 13    | 13    |
| CV %                    | 7.68  | 2.43  | 0.26  | 0.10  | 0.65  | 4.24  | 3.27  |
| June                    | 1.53  | 2.25  | 2.51  | 2.54  | 2.65  | 2.66  | 2.77  |
| Average                 | 0.39  | 0.03  | 0.01  | 0.03  | 0.06  | 0.05  | 0.05  |
| Std. Dev                | 25.65 | 1.17  | 0.46  | 1.06  | 2.26  | 1.88  | 1.69  |
| n                       | 5     | 13    | 13    | 13    | 13    | 13    | 13    |
| CV %                    | 65.59 | 44.50 | 39.82 | 39.31 | 37.73 | 37.54 | 36.12 |
| October                 |       |       |       |       |       |       |       |
| Average                 | 1.41  | 1.42  | 1.61  | 1.63  | 1.67  | 1.69  | 1.67  |
| Std. Dev                | 0.12  | 0.13  | 0.01  | 0.00  | 0.00  | 0.05  | 0.06  |
| n                       | 10    | 12    | 12    | 10    | 13    | 13    | 13    |
| CV %                    | 8.43  | 9.05  | 0.32  | 0.27  | 0.26  | 3.15  | 3.79  |

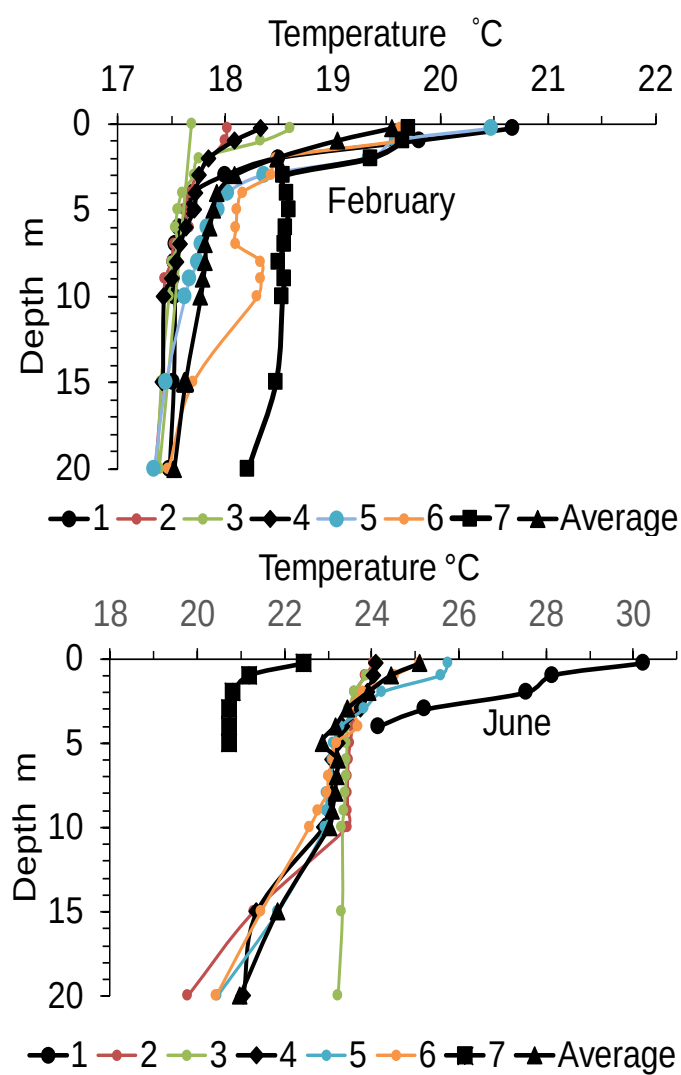
### *Limnological Variations in the Water Column Zimapán Dam*

Temperature in the water column (0.3 to 20 m), temperature values were 17.3 to 20.6°C in February and 20.3 to 26°C in June, with the influence of hot water springs (38°C) in site 1 with 30°C. The temperature variability between the seven sites occurred in the first 2 to 5 m depth, with a thermocline of 24°C in June from 5 to 10 m and in February with 22°C between 2 and 10 m. The variation is low, with CV less than 20 % at each site, with the most significant variation in June at site 1, with 8.9 % close to the hot water springs (Table 1 Fig. 2).

Dissolved oxygen had the lowest variability in February with a range of 6 to 7 mg/l, the most significant variability in space and time in June with values between 3 and 7 mg/l between

the surface and 10 m depth, with oxycline between 10 to 15 m and with 3 to 7 mg/l, with a subsequent decrease between 2 and 3 mg/l; October presented two zones, lower concentrations 1 to 4.5 mg/l between sites 5 to 7, and 3 to 7 mg/l between sites 1 to 4, site 7 stands out where the river enters. Then, hypoxia at 10 m, there was an increase to 3 mg/l at 15 m and up to 5 mg/l at 20 m. The most remarkable changes were in June in the influence of the Tula River in sites 4 to 6 with CV of 23.3 to 33.4 %; October presented significant variations in the area influenced by the San Juan River, from site 1 to site 4 (Table 1, Fig. 2).

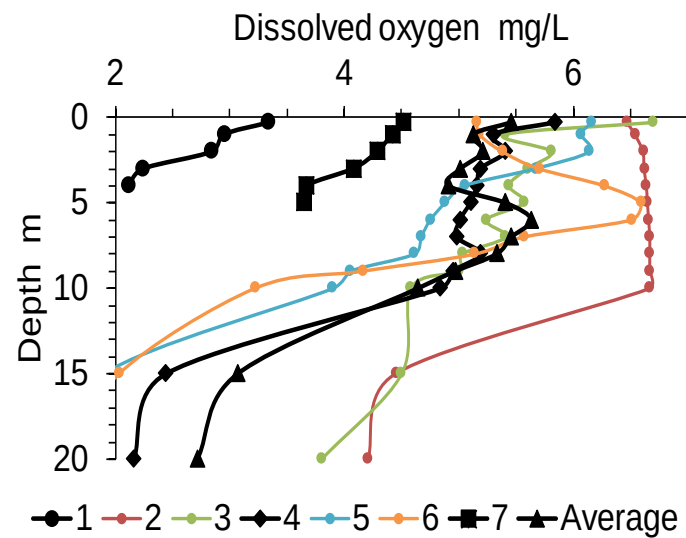
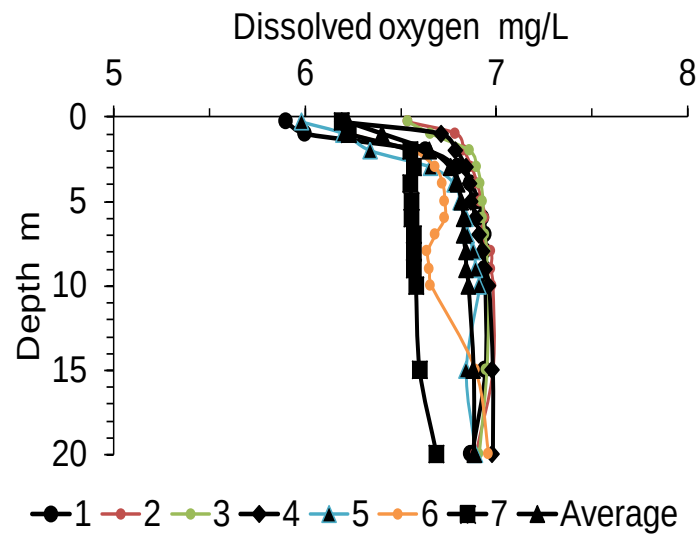
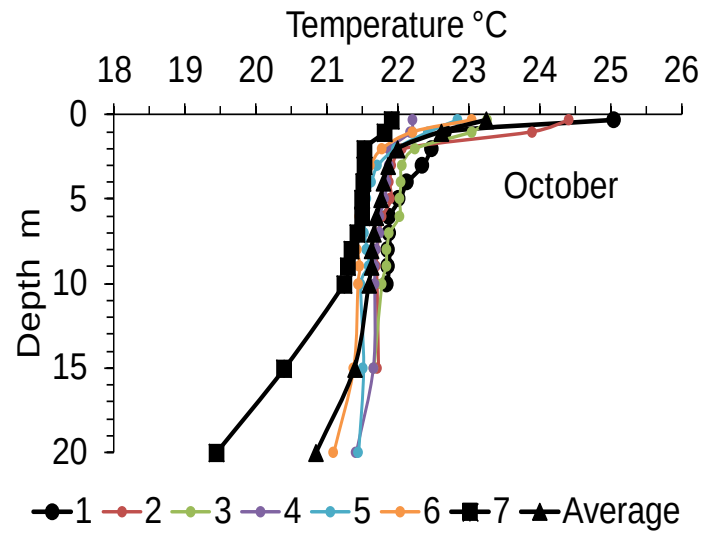
Total dissolved solids presented the lowest values in October and February, with the range 1.2 to 1.9 g/l, and the highest values in June, with 1.0 to 2.9 g/l: the variations with CV% were less than 20, except June in site 1, with CV% 25.6. The lowest values were between sites 1 and 4 in the three months and the highest in sites 5 and 7 in the Tula River influence area (Table 1, Fig. 2).



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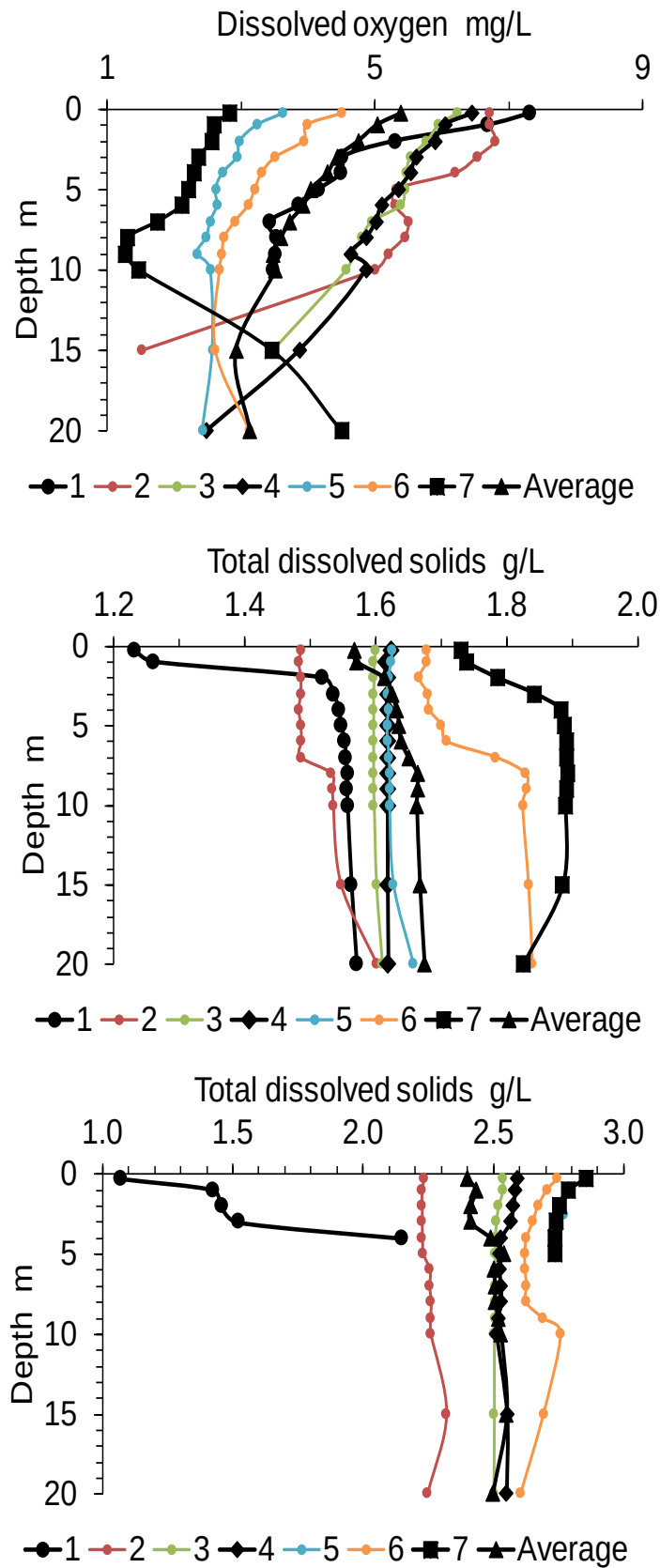


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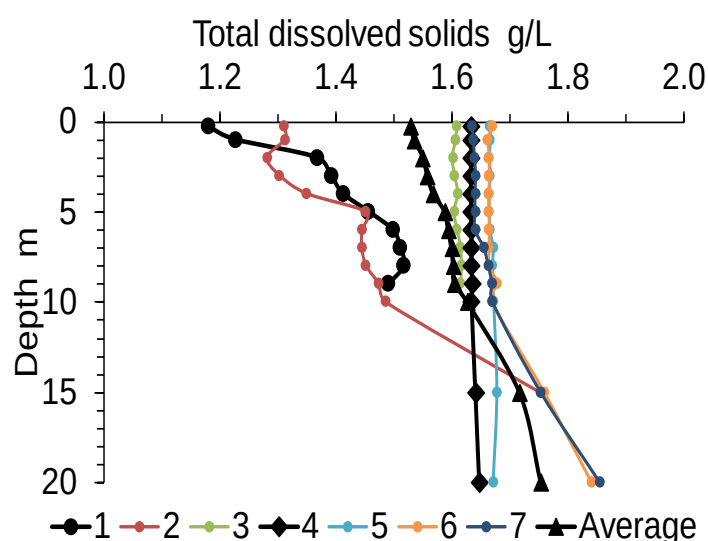


**Figure 2.** Water Column (Surface-20 m Depth) variations of temperature ( $^{\circ}\text{C}$ ), dissolved oxygen (mg/L), and total dissolved solids (g/L).

### *Zimapán Dam-Tula River system*

In the composite water sample (surface at 3 m) from sites 1 to 7 of the dam and in surface water (0.20 m) from sites 8 to 10 in the river, the water temperature of the river dam system presented variations between each month of study. However, the river dam did not show significant variations between the months studied,  $F(6,18)=1.0637$ ,  $p=0.4193$ . At the dam, the lowest values were in February, with averages of 18.6 to 20 $^{\circ}\text{C}$ , and in June, with high temperatures, 24.8 to 25.4 $^{\circ}\text{C}$ . The river had the lowest temperature values, ranging between 18 and 21 $^{\circ}\text{C}$ . In the dam, there were no significant differences between the three areas in June and October  $F(4, 12)=0.2263$ ,  $p=0.9184$ ; the lowest temperatures were in February, with significant differences  $F(2, 12)=15.723$ ,  $p=0.0004$  (Fig. 3A).

The changes in dissolved oxygen concentrations also did not present significant differences by study area and month  $F(6,18)=0.3958$ ,  $p=0.87204$ . The dam areas had the highest records in February but with a variation of 6.0-6.4 mg/l in June and October, with averages in the range of 3.7-6.4 mg/l; the river, with lower concentrations than the dam areas, with a range of 3.5-4.6 mg/l. The dam had no significant differences between the three zones  $F(4, 12)=0.5152$ ,  $p=0.7262$ ; the lowest oxygen concentrations were recorded in the riparian zone, while the highest were in the three zones in February (Fig. 3B).

Water transparency also had no significant differences between the months and study areas  $F(6,18)=1.6665$ ,  $p=0.1867$ . At the dam, the lake zone had the highest records, 6.0 m in February and 6.4 m in June, the lowest in the riparian zone, 1.1-2.8 m; the river,

its average values were almost the same, in the range of 0.5-0.8 m; the dam areas well differentiated from each other and the river with lower transparency. Between the areas and months of collection at the dam, there were no significant differences in the transparency values recorded  $F(4, 12)=1.5767$ ,  $p=0.2433$ . The lowest transparency values were recorded in the riparian zone, while the most significant transparency depth in the lake zone corresponded to February and June (Fig. 3C).

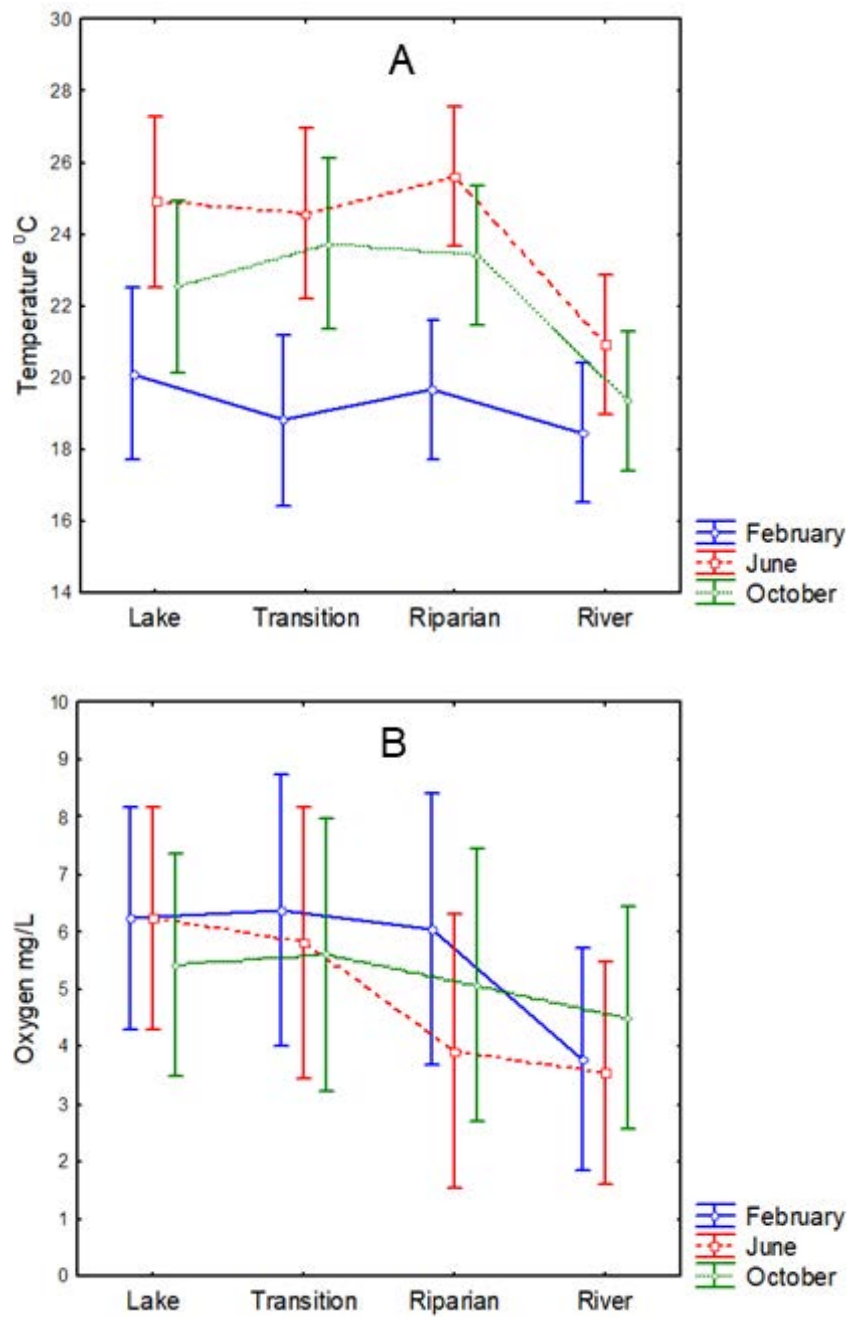
The average pH varied from basic conditions 7.7 to 8.7 in June and October, and February with values between 7.1 and 8.2 to neutral conditions 7.1; there were no significant variations  $F(6,18)=1.1180$ ,  $p=0.3907$  between the months and the areas of the river-dam system; The riparian zone shows the influence of river water, with values between 7.3 and 8.1, higher than those of the transition and lake zones. The river with the highest average values is 8.2 to 8.7. Between the areas and months of collection at the dam, there were no significant differences in pH values  $F(4, 12)=0.5389$ ,  $p=0.7102$ . The lowest pH values were in the three areas in February (Fig. 3D).

In TP, the highest concentrations were in the river, 7 to 22 mg/l. The dam areas had values close to 3 mg/l. There were significant differences  $F(6,18)=3.2314$ ,  $p=0.0247$  in the river dam system, with the river having the highest concentrations in the three months of the study. Between the areas and months of collection in the dam, there are no significant differences in total phosphorus values  $F(4, 12)=0.3633$ ,  $p=0.8301$ . The lowest total phosphorus values were in the transition and riparian zone in June and the highest in February in the riparian zone (Fig. 3E).

The TN, the highest values were in the river, 24 to 36 mg/l, and the dam areas, with values between 2 and 7 mg/l; there were significant differences  $F(6,18)=2.8097$ ,  $p=0.0414$  in the river-dam system. There were no significant differences in the dam  $F(4, 12)=0.8137$ ,  $p=0.54$ . The highest total nitrogen values were recorded in the riparian zone in February, while the lowest was in June and October in the three collection areas (Fig. 3F).

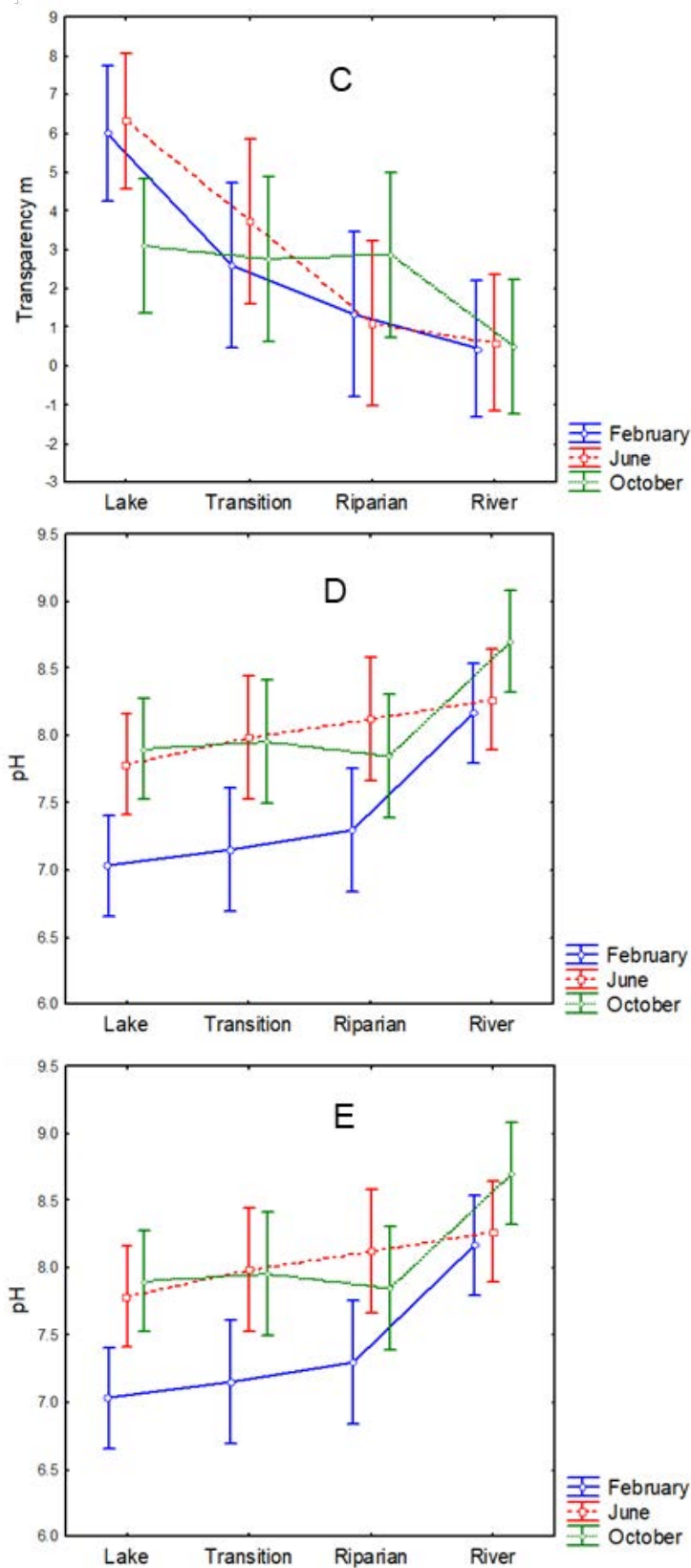
The limnological variables showed spatial changes in their values but were not significant or relevant compared with temporal changes between the three climatic conditions.





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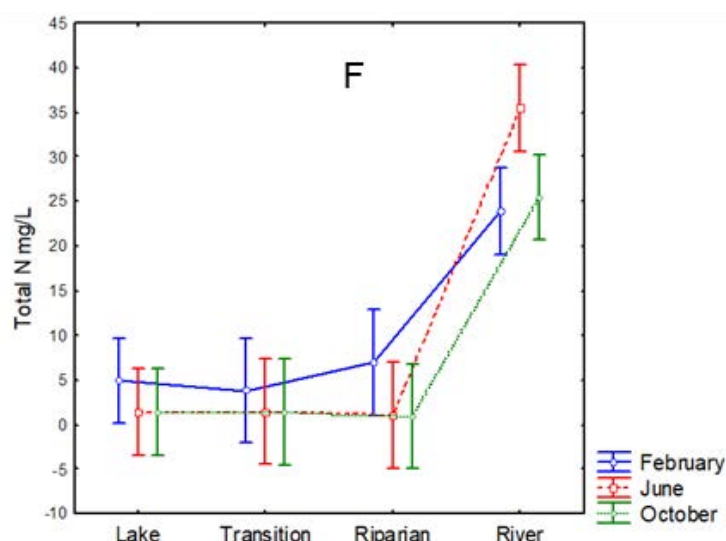


**Figure 3 (A, B, C, D, E, F).** Comparative Surface Water Variations Months vs Dam Zones and River of Physicochemical and Nutrient. F Factorial Test, Average, Confidence Bars 95%. There Are No Significant Differences in Months vs Zones. The River and the Dam Differ in Limnological Spatiotemporal Transparency, pH, Total P, and Total N Variations.

Tukey confirmatory test, with all the limnological parameters and for the tree climatic conditions studied, showed the river-dam differences in sites 8, 9, and 10 with temperature, oxygen, transparency, and nutrients; there were fewer differences in sites 1 to 7 of the reservoir (Table 2).

**Table 2.** Tukey Confirmatory Test (N =20, 95 % confidence, 0.05 error), to Detect Significant Differences (in bold) at Work Sites. River Sites 8,9 and 10 are Different in Most of the Parameters, but pH in Sites 5 and 6.

| Parameter      | 1    | 2    | 3    | 4    | 5     | 6     | 7    | 8      | 9      | 10     |
|----------------|------|------|------|------|-------|-------|------|--------|--------|--------|
| Temperature 0C | 0.90 | 0.89 | 0.86 | 0.87 | 0.98  | 0.95  | 0.74 | 0.33   | 0.26   | 0.21   |
| Oxygen mg/L    | 1.00 | 0.96 | 0.97 | 0.99 | 1.00  | 1.00  | 0.97 | 1.00   | 0.97   | 0.01   |
| Transparency m | 1.00 | 0.92 | 0.95 | 0.89 | 0.35  | 0.99  | 0.99 | 0.02   | 0.020  | 0.01   |
| pH             | 1.00 | 0.99 | 0.99 | 0.96 | 0.027 | 0.036 | 1.00 | 0.99   | 0.00   | 0.25   |
| Total P mg/L   | 1.00 | 1.00 | 0.99 | 0.99 | 1.00  | 0.99  | 0.98 | 0.35   | 0.13   | 0.004  |
| Total N mg/L   | 1.00 | 1.00 | 0.99 | 0.97 | 0.99  | 0.96  | 0.99 | 0.0002 | 0.0004 | 0.0001 |

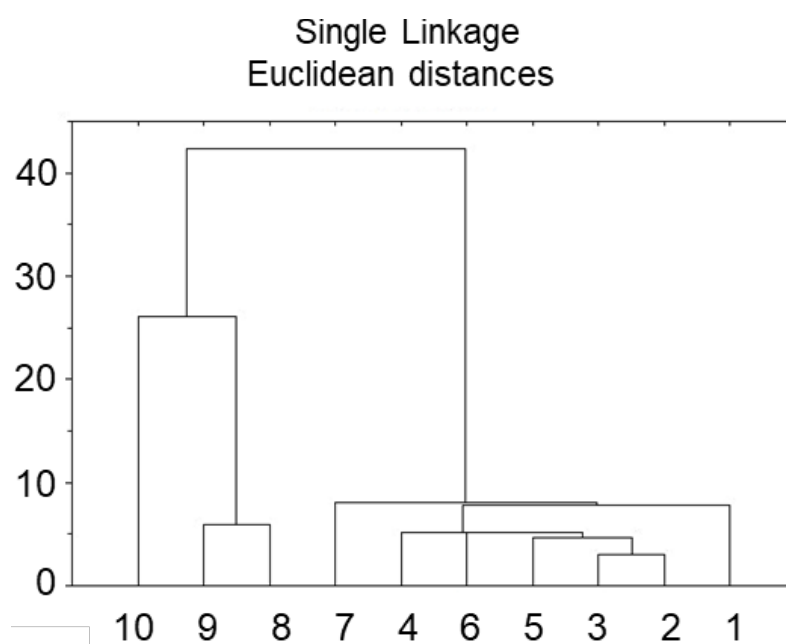
The Pearson correlation analysis between the variables in the ten stations in the three different climatic conditions showed that significant negative or inverse proportional

variations occurred between dissolved oxygen and total nitrogen nutrients, with  $r=-0.640$ , and with total phosphorus, with  $r=-0.548$ . Another important correlation was oxygen with pH, with  $r=-0.587$ . More TN, TP, and pH, less Oxygen (Table 3).

**Table 3.** Pearson Correlations among All Sites of the River Dam System during Three Different Climatic Conditions. Important Correlation Above  $r = 0.5$

| Pearson Correlation, n=30   |         |
|-----------------------------|---------|
| Temperature vs Oxygen       | 0.1164  |
| Temperature vs Total N      | -0.2841 |
| Temperature vs Total P      | -0.5227 |
| Temperature vs Transparency | 0.2231  |
| Temperature vs pH           | 0.1101  |
| Oxygen vs Temperature       | 0.1164  |
| Oxygen vs Total N           | -0.6401 |
| Oxygen vs Total P           | -0.5476 |
| Oxygen vs Transparency      | 0.3086  |
| Oxygen vs pH                | -0.5875 |

In river-dam surface water, the physicochemical and nutrient variables allowed the definition of two zones: sites 10, 9, and 8 and sites 7 to 1 of the dam. The river group separates site 10, which had lower oxygen and higher TN and TP from the other sites, where oxygen increases and TN and TP decrease. Likewise, the horizontal zoning in the surface water of the dam presented sites 1 and 7 separated from the remaining sites; at the same time, the lake zone through sites 5, 6, and 4, and the area of influence of the San Juan River in sites 2 and 3 (Fig. 4).



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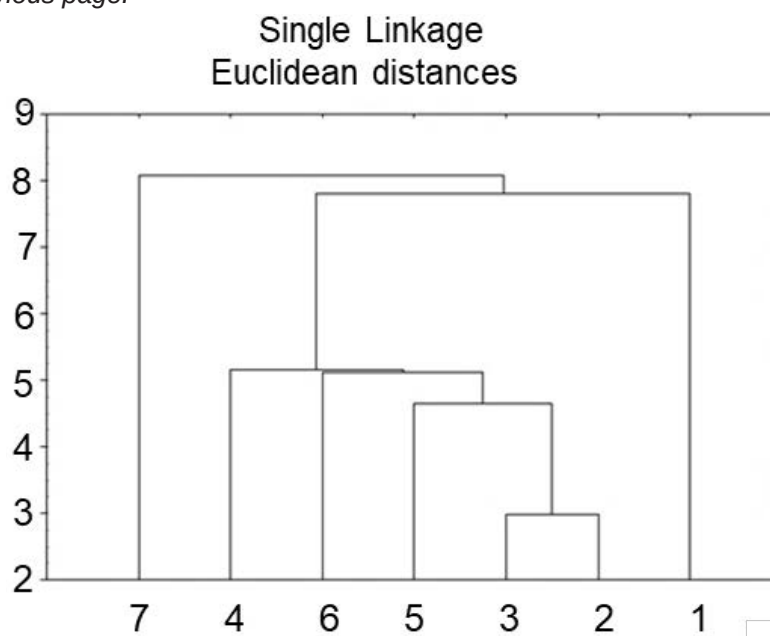


**Figure 4.** Ordination Diagram with All Study Variables of Sampling Sites, Left Together River and Dam Sites 1-10, Right Only Dam Sites 1-7.

## Discussion

Since 1972, the Tula River in the State of Hidalgo has received wastewater from the Megalopolis Mexico City. The water irrigates approximately 45,214 ha of crops in the Mezquital Valley, administered by two central irrigation districts (ID). It is one of the most polluting in the country, generating 40,942 million m<sup>3</sup> of wastewater annually (López et al., 2007).

Comparative drought-rain-pos rain conditions are essential to know the limnological variations in the river dam system. The limnological spatiotemporal variations will help to understand the increase and decrease in values and how to respond to the dam regarding water quality, eutrophication, and environmental quality for fisheries and sport fishing.

The region's semi-arid climate with ambient temperatures above 22°C and the lowest at 18°C had no significative water column variations (%CV <29) due to its slight annual variation (<6°C).

The environmental temperature, slope, current, bottom, tributaries, thermal springs, depth, and distance from the studied sites determined differences in the temperature, pH, dissolved oxygen, total dissolved solids, and nutrients in the river sites; likewise, in the dam zones, environmental temperature, winds, and the river's water incoming affect the mixture of temperature, dissolved oxygen, nutrients, and water transparency in the dam.

These vertical patterns varied in time, promoting the formation of thermocline and oxycline in June near the surface and up to 10 or 15 m at greater depths, as well as their breaking in the October mixing process; shallow places were more susceptible to increases in temperature and oxygen concentrations; as depth increased, temperature and dissolved oxygen decreased. When the dam is in the process of stratification and beginning of the mixing process, the oxygen presented significant variations (CV% greater than 20) as a product of the entry of the two rivers, particularly after the rains that transported oxygen-consuming compounds in the wastewater. The low variation of dissolved oxygen in conditions of cold drought in February, the lower entry of sewage, and the lower environmental temperature could be the drivers for the increase in oxygen solubility and the adequate concentrations of dissolved oxygen for aquatic life and aquacultural activities in the dam.

The total dissolved solids also showed differences between river entrances: Site 1 had less concentration than Site 7, and Site 1 had a dilution effect from wastewater from the thermal water spa area 8 km upstream. In contrast, Site 7 received water from the runoff in the area. The Tula River is the main contributor to nutrients, organic matter, and pollutants since it is an outlet conduit for sewage from Mexico City.

The decrease in the transparency at dam riparian sites illustrates the influence of the basin human activities on the water body's physicochemical characteristics; the increased transparency in the internal sites of the reservoir may be due to the suspended solids tending to settle in extended calm areas with high depth. Primary physical dynamics in the reservoir occur in the first 5-15 m of depth, the starting mixing process in October and even in February, causing the decrease of transparency throughout the reservoir. Storm runoff is the primary source of solid suspended material in the water bodies (Kemdirim, 2005). Similar results are for Lake Chapala, Mexico (de Anda et al., 2004) and the Aguamilpa reservoir (Rangel-Peraza et al., 2009).

The reservoir receives wastewater throughout the year. The oxidation of the organic matter that entered, the low water temperature with the lowest photosynthetic efficiency, the mixing conditions, decomposing organic material, and inorganic substances consuming oxygen promoted the decline of dissolved oxygen in riparian zones (Del Sontro et al., 2010). The condition of different turbulence and the mixture from surface to 5 m depth in which thermocline and oxycline occurred conditioned the different oxygen concentrations.

The average dissolved oxygen surface water remained over 4 mg/l in June and February. This finding suggests that the reservoir surface water is suitable for fish production (e.g., tilapia is the main fish caught in the reservoir). However, it is not convenient in November because of continuous conditions under hypoxia ( $DO < 2$  mg/l) with occasional sickness and massive fish kill (López et al., 2007).

The entrance of wastewater, with its high values of nitrogen, phosphorus, pH, and low transparency, showed the effect of dilution or self-purification after the entry point. The combined action of permanent turbulent flow along the slope and rocky substrates and the constant entry of high volumes of water from the springs between sites 10 and 9 helped reduce those variables' values. Still, high concentrations of nitrogen and phosphorus arrive at the dam. It is a fact that "partially treated" water enters site 1 of the dam, first through the Endho dam (State of Hidalgo), which functions as an oxidation pit, and then due to the permanently lotic character of the river.

The riparian zones in canyons, which have less depth and less exposure to the wind, presented a more significant occurrence of high DO due to primary productivity or low values due to the wastewater effect.

The Zimapán reservoir has the epilimnion in the first 5-10 m and adequate oxygen values for aquatic life ( $>4$  mg/l). In the hypolimnion, temperatures decreased and with values of hypoxia, in conditions of lower temperatures, the concentrations of DO usually lower by reaching hypoxia; with hypoxia and anoxia in the hypolimnion, there are conditions for the release of compounds such as methane, carbon dioxide, hydrogen sulfide, ammonia, iron, manganese, and phosphorus (Rangel-Peraza et al., 2012). Transparency, temperature, and DO are suitable for tilapia and black bass in this reservoir, but only at the first 15 m depth. Previous studies at Dam Zimapán present values of anoxia from 30 or 40 m depth in months from May to September and indicate that the reservoir presents a mixture of only 10-20 m of depth. It is considered a meromictic tropical reservoir with a permanent inlet of wastewater and dissolved oxygen that does not depend on physical and temperature dynamics and can also be important in the microbiological processes of production and consumption; previous studies reveal all physical, chemical, and biological processes occur from surface to 20 m depth, with anoxic waters from 40 to 70 m depth (López et al., 2007; Bravo-Inclán et al., 2008).

The reservoir showed an average pH of 7-8.4, considered a neutral or alkaline water body, consistent with other semi-desert soils with limestone rocks with carbonates and bicarbonate. Most Mexican waterbodies are rarely acidic, alkaline, or nearly neutral (Sarma & Elías-Gutiérrez, 1997; Torres-Orozco & Estrada-Hernández, 1997). Tula's river pH was considered highly alkaline (pH 7.7-9.6) with critically high values at site 10, where wastewater comes from México City; numerous spring waters before and after site 8 could explain pH values below 8.

NT and PT with concentrations, indicating nutrient excess because the source of nitrogen and phosphorus is the wastewater transported by the Tula and San Juan Rivers, as the contributions by livestock waste like those in some reservoirs in Brazil (Nogueira, 2001; Carvalho et al., 2002); the entry of high nutrient rates by river supply, affect water quality (Nogueira, 2001; Delazari-Barroso et al., 2009); the rates of denitrification by the action of anoxic sediments at the bottom and the permanent entry of organic matter are standard processes in dams with anoxic bottoms (Abe et al., 2003).

The TN concentrations in this dam can be part of the processes that promote the frequent algal blooms by cyanobacteria (*Microcystis* and *Anabaena*) in months of drought, high temperature, and little or no wind, resulting in high densities of cyanobacteria (López et al., 2007).

Based on the TP values (Lampárelli, 2004; Cunha et al., 2013), the reservoir always presented a hypereutrophic condition by the permanent inlets of wastewater. Dams like Zimapán, with high inflows of organic matter and accelerated eutrophication by nitrogen and phosphorus, present severe situations of degradation in water quality, with frequent cyanobacteria algal blooms and cyanotoxins, as well as precursors of trihalomethanes, and compounds with strong unpleasant odors generated from the decomposition of the biological material from animals, plants, and microorganisms (Mostofa et al., 2007; Conley et al., 2009).

This river-reservoir system is experiencing a frequently pronounced decline in water quality because of the excessive nutrient inputs delivered in untreated sewage effluents, pollution by metals and toxins from oil, cement, pharmacists, and other human impacts, physical disturbances included. There are similar conditions in other reservoirs (Daughton & Ruhoy, 2009; Fontana et al., 2014); because of this condition, the water from Dam Zimapán is beneficial for crop irrigation and is not considered healthy for human use or recreation.

The values of hypoxia DO in sites 1 and 7 are due to their consumption in the degradation process of the organic material that arrived by the trawls of both rivers and local runoffs in the reservoir surroundings after the rain occurred from June to September.

The self-depurating process in the river and reservoir mixing processes, bacterial activity in the water column, biological fishery actions, and minor rain events have promoted the decline of N and P; at the onset of research activities, the dam had data higher than 5 mg/l in the years 2002-2005 (López-Hernández et al., 2007).

The cluster diagram and Tukey analysis based on physicochemical and nutrients clearly show the separation between the river and the dam based on decreased values from the river and the differences between lotic and lentic systems. The reservoir has a typical pattern; the deeper sites (lake area) are always together regarding total nitrogen and phosphorous dilution. Site 7 receives the highest wastewater volume in the river, and site 1 has less pollutant loading.

This study confirms the water quality response regarding the permanent entry of organic matter, dissolved oxygen producers-consumers, and the potential nutrient loads generated by anthropogenic activities in the drainage basin and the reservoir surroundings. Similar dynamics exist in other Brazilian reservoirs (Geraldés & Boavida, 2003, 2004). In this research, wastewater with high pH, nitrogen, and phosphorous showed a significant negative relation with dissolved oxygen.



## Conclusions

Limnological spatiotemporal variations of water temperature, oxygen, dissolved solids, transparency, pH, total nitrogen, and phosphorous were evident between river and dam zones; river site variations had hydrological differences in current, influents, rocky/mud bottom, and anthropic activities in the surroundings. The dam sites, according to depth, wind influence in open and closed areas by mountains, ambient temperature, and the river entrance.

Primary physical dynamics based on temperature transparency and oxygen in the reservoir occur in the first 15 m of depth, the starting mixing process in October and even in February. The average dissolved oxygen surface water remained over 4 mg/l in June and February; surface water is suitable for aquaculture fish activity. However, it is not convenient in November because of continuous conditions under hypoxia ( $DO < 2$  mg/l).

The semi-arid climate of the region with ambient temperatures above 22°C, the lowest at 18°C and no significative water column variations, and its slight annual variation.

NT and PT with high concentrations indicate nutrient excess because the source of nitrogen and phosphorus is the wastewater transported by the Tula and San Juan Rivers and other contributions by livestock waste.

The reservoir always presented a hypereutrophic condition based on the total phosphorous values in the permanent inlets of wastewater; the TN concentrations can be part of the processes that promote the frequent algal blooms by cyanobacteria (*Microcystis* and *Anabaena*) in drought with little or no wind.

Zimapán Hydropower Dam, with high organic matter inflows and accelerated eutrophication caused by excessive nutrient inputs delivered in untreated sewage effluents, pollution by metals and toxins from oil, cement, pharmacists, and other human impacts, presents severe situations of degradation in water quality. Water can be used for irrigation purposes, not for human drinking or recreational activities.

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## Authors' Contributions

GFF worked with the physicochemical analysis and water column graphics; RAEG and GTP collected samples; LHM and GPA carried out statistical graphics procedures.

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# Temporal Characterization of Water Quality of Rivers in Contrasting Zones of Two Watersheds in Veracruz, Mexico

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## Abstract

Water quality is influenced by natural conditions and anthropogenic impacts along the zones of a watershed, being the human impact the most important factor due to pollution. In the state of Veracruz, Mexico, there are two important rivers that reach the Gulf of Mexico: La Antigua and Jamapa-Cotaxtla. In these rivers, there are people that have long term water quality monitoring activities, hence, the information generated can be used to understand the functioning of rivers in different zones of a watershed: Headwater at the highlands and floodplain at the lowlands. The objective of this work is to compare the water quality of both zones using information of physical, chemical, and microbiological parameters measured during the year of 2018 by community monitoring programs. We used a principal component analysis for detecting the most variable parameters and non-parametric analyses to detect significant differences for each measured parameter between the two zones. Also, we classified water quality according to different scales for different parameters. The results showed significant differences of water quality between the two zones of the watersheds, the highland river had the best quality. However, concentration of *Escheria coli* indicated pollution in both watersheds. We concluded that temperature, alkalinity, hardness, pH, and turbidity were traits of each region of the watersheds that respond to climatic seasons. The oxygen was severely depleted in the river at the lowland region of the Jamapa-Cotaxtla river most likely due to organic pollution, fecal contamination is constantly quite a problem affecting water quality in both zones of the watersheds.

## Keywords

*Escherichia*, floodplain, headwaters, monitoring.

## Introduction

Water quality is influenced by natural conditions and anthropogenic impacts along the regions of the watershed, being this last factor an emerging problem worldwide that deteriorates water quality for human use as well as ecosystems (Obiluno et al., 2013; Islam et al., 2021). Water flows from higher ground to rivers or creeks that finally reach lowlands and arrives to reservoirs, lakes, or the sea, changing the physical and chemical characteristics of the water through its way. Commonly, streams at the headwaters in a watershed are considered to have “good water quality” while streams at floodplains are negatively impacted by the pollutants that water has gathered while flowing downhill. For the understanding of the function of a watershed, this can be divided into three zones: headwater (source), transition (transfer or middle), and floodplain (depositional) (Nepal et al., 2014; Tang et al., 2020), where natural condition and anthropogenic activities interact and define the water quality.

Different traits of the water can be used as an indicator of water quality, they must be defined by physical, chemical, and microbiological parameters (Chidiac et al., 2023). These traits are natural properties of the water depending of the nature of the soil, altitude and vegetation, and they can be altered by the influence of anthropogenic activities that can modify oxygen concentration, transparency of water, temperature, pH, and microbiological biota by supplying, fertilizers, pesticides, and wastewater, being the latter the most usual negative impact worldwide (Tang et al., 2020). Evaluation of those mentioned parameters is necessary to preserve the natural properties of water. So, monitoring water quality is an issue that must be addressed by governments to face the worldwide water crisis.

The state of Veracruz has two important watersheds with rivers that flow to the Gulf of México, passing through the most important cities: The city of Xalapa in the upstream region in Antigua watershed; and the city of Boca del Rio in the downstream region of Jamapa-Cotaxtla watershed. In these two watersheds, two water quality monitoring initiatives have been established with the approach of Community Participatory Monitoring (Deutsch et al., 2009) using the protocols of the Global Water Watch Program from Auburn University, promoted by Global Water Watch Mexico (GWW, 2023). The data from these water quality monitoring initiatives can be used for understanding the differences and functioning of different zones within the watersheds (Ramos-Escobedo et al., 2019).

This work aims to determine and compare the temporal and spatial natural variation, as well as the anthropogenic impacts of the water quality traits of rivers at distinct zones: (headwater and floodplain) in two watersheds.

## Material and Methods

### *Study Sites*

**Headwater Zone:** The study site is the Pixquiac River, located at 1,460 m altitude close to the locality of Mariano Escobedo at the highlands of the La Antigua watershed, whose main drainage is the La Antigua river with a watershed area of 3,443.9 km<sup>2</sup>. Several tributaries come from the Cofre de Perote mountain (2,416 m) one of them is the Pixquiac river, that arises at the East of Cofre del Perote and supplies water to the Tillereo River that finally enters the La Antigua River (DOF, 2012). The Pixquiac River drains an area dominated by primary and secondary mountain cloud forest, with some agricultural lands and urban settlements. Mariano Escobedo is a locality with a small population (530 inhabitants) and close to the urban zones of Coatepec (55,720 inhabitants) and Xalapa (443,063 inhabitants) (INEGI, 2024).

**Floodplain Zone:** The study site was located close to the urban zone of Boca del Rio and Medellin de Bravo in the lowerland of the Jamapa-Cotaxtla watershed (<50 m altitude). This watershed of 3,995.4 km<sup>2</sup>, is defined by two rivers originated at the highlands of the highest mountain in Mexico: Pico de Orizaba (5,636 m) (DOF, 2008). The Cotaxtla River joins the Jampa River in the lower part of the watershed, this part of the river maintains the name of Jamapa while flowing through the floodplains of the state of Veracruz to the Gulf of Mexico, entering in the extended metropolitan area of Boca del Rio, Veracruz and receiving a strong urban influence. This watershed has a vegetation coverage highly fragmented by human activity, mainly agricultural and urbanization, with a negative impact in support services of the ecosystem (Ortíz-Lozano, 2013). Particularly, the floodplain zone is a deposition zone with an urbanization that displaced the primary vegetation. The study sites are in an urban zone with small remnants of vegetation close to localities of Puente Moreno (34,913 inhabitants), Playa de Vacas (791 inhabitants) and the urban zone of Boca del Río (144,550 inhabitants) (INEGI, 2024).

### *Sampling Design*

Sampling stations were selected following criteria established by Global Water Watch (GWW) protocols (Deutsch et al., 2009). Minimum 2 sites along the river, one located upstream a suspected pollution source (in this case human settlement) and one downstream from that.

**Headwater Zone:** Antigua watershed, three sampling stations: 1) Vado de las Monjas (VM) before a human settlement, 2) Seis de Enero (SE), and 3) Mariano Escobedo (ME), after the population of La Pitaya and Mariano Escobedo were established at river Pixquiac. The sites were sampled each month from January to December 2018 (except June) (Fig. 1).



Floodplain zone: Jamapa-Cotaxtla watershed, four sampling stations along the Jamapa River: 1) Arroyo Moreno (AM) an important stream that flows to Jamapa River, 2) Playa de Vacas (PV), before the joining of Arroyo Moreno and the population of Boca del Río, 3) Puente (P) in the joining of Arroyo Moreno and Jamapa, and 4) Jamapa (J), close to the mouth of the river in the Gulf of Mexico. The sites were sampled from January to December 2018 (Fig. 1).

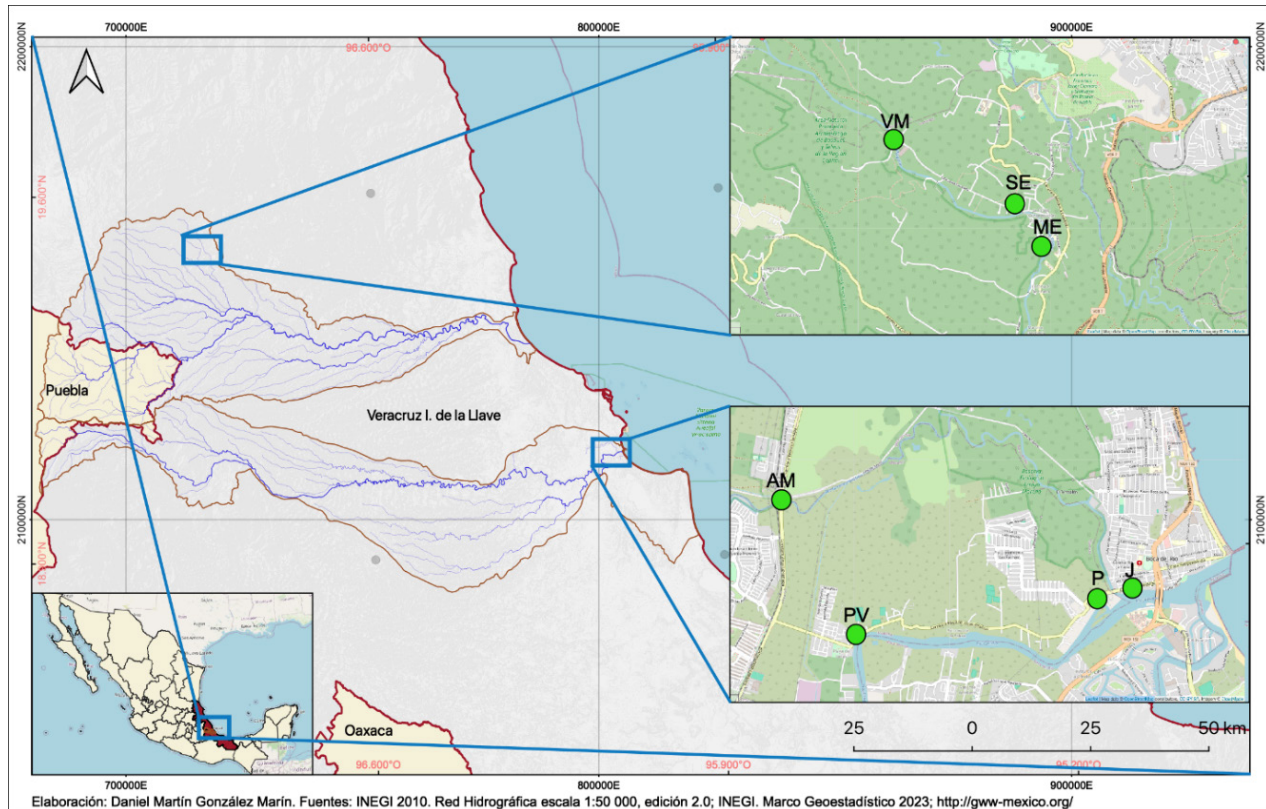


**Figure 1.** Sampling Site Locations (Green Circles). Headwaters Zone: Vado de Monjas (VM), Seis de Enero (SE) and Mariano Escobedo (ME); Floodplain Zone: Arroyo Moreno (AM), Playa de Vacas (PV), Puente (P) and Jamapa (J).

### Water Quality

Water quality was determined using the protocols and techniques considered in the data quality and quality assurance Plan of the Global Water Watch Program at Auburn University.

For the physicochemical analysis, we use the Alabama Water Watch Kit by LaMotte (Temperature, hardness, alkalinity, pH, dissolved oxygen, and turbidity; code 9844-02 LaMotte ©). All variables were measured *in situ*. Temperature was measured with an alcohol thermometer ( $\pm 0.5^\circ\text{C}$ ); pH measurements are based on comparing scale colors ( $\pm 0.5$ ) using indicator solution in a scale from 3 to 10.5; Hardness follows the titration method with EDTA (0-200 mg/l); Alkalinity follows the titration method with sulfuric acid (0-200 mg/l); Oxygen measured is based on the Winkler method, and turbidity is based on transparency

using a standard drop method (5-200 JTU). Determination of these physicochemical parameters were done once by sample, except for dissolved oxygen which were done using two replicates (Deutsch et al., 2009). For the Antigua watershed, June was missing.

The microbiological analyses were conducted using the Coliscam Easygel method by Micrology Labs, which consist of media with inhibitors that only allow the growth of coliforms and a sugar linked to a dye that make it possible to differentiate *Escherichia coli* from other coliforms (Colony Forming Units CFU/100 ml). The cultures were done using three replicates by sample indicated by GWW protocol (Deutsch et al., 2009). For the Antigua watershed, data for January, November and December were not available.

### *Statistical Analyses*

Principal Component Analysis was performed using all data to identify any temporal and spatial clusters in physical, chemical, and microbiological data (Jolliffe & Cadima, 2016). All data were transformed ( $\log_2(x+1)$ ) prior to the analyses (Zar, 2010). Components 1 and 2 (maximum variance explained) were selected according to eigenvalues and plotted to visualize clusters.

To detect significant differences between watershed zones in each parameter, Mann-Whitney U tests were used (data failed homogeneity of variances). Two approaches were used for explaining differences: annual mean values, and monthly mean values between regions. All analyses were performed using the software Statistica ver. 7 (Statsoft, Inc. ©).

## Results

Alkalinity and oxygen are the chemical traits that most heavily influenced the variation of water quality among data of the two zones of the watersheds. In addition, the temperature, the hardness, and microbiological traits also have a strong influence in the variation of water quality considering all data (explaining 64.6 % of total variation). Turbidity and pH do not contribute significantly to explain differences between the two zones studied (Fig. 2).

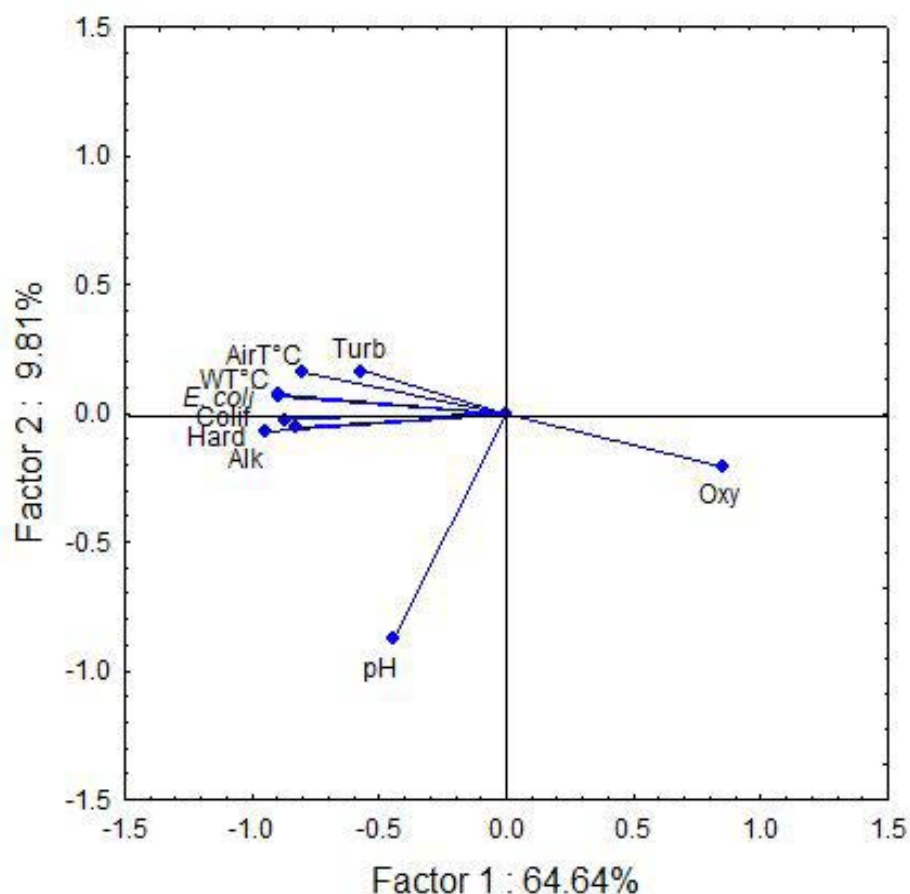


**Figure 2.** Contribution of Variables in Components 1 and 2 Considering Data Form the Headwaters Zone of the Antigua Watershed and Floodplain Zone of Jamapa-Cotaxtla Watershed.

The plot of data derived from PCA resulted in 3 groups, each with agglomerated data (Fig. 3). The headwater zone formed a compacted group (blue circle in the the plot) well separated from the floodplain zone (left side of the plot) indicating that water quality is different and, in this case, the headwater zone characterized by high concentrations of dissolved oxygen and low values of air and water temperatures, hardness, alkalinity and *E. coli* and coliforms. Only one datum split from the cluster, which had a pH value slightly lower than the rest of this watershed zone.

Floodplain zone was characterized by high values of air and water temperatures, hardness, alkalinity, and *E. coli* and coliforms but very low values of dissolved oxygen concentrations. In addition, pH values have an influence on this zone separating two groups (curved red lines in the plot) with neutral (group in the left-upside of the plot) and slightly basic pH (group in the left-downside of the plot) which corresponds to different stations and different months, so a local influence of seasons acts (salt wedge) directly in the pH.

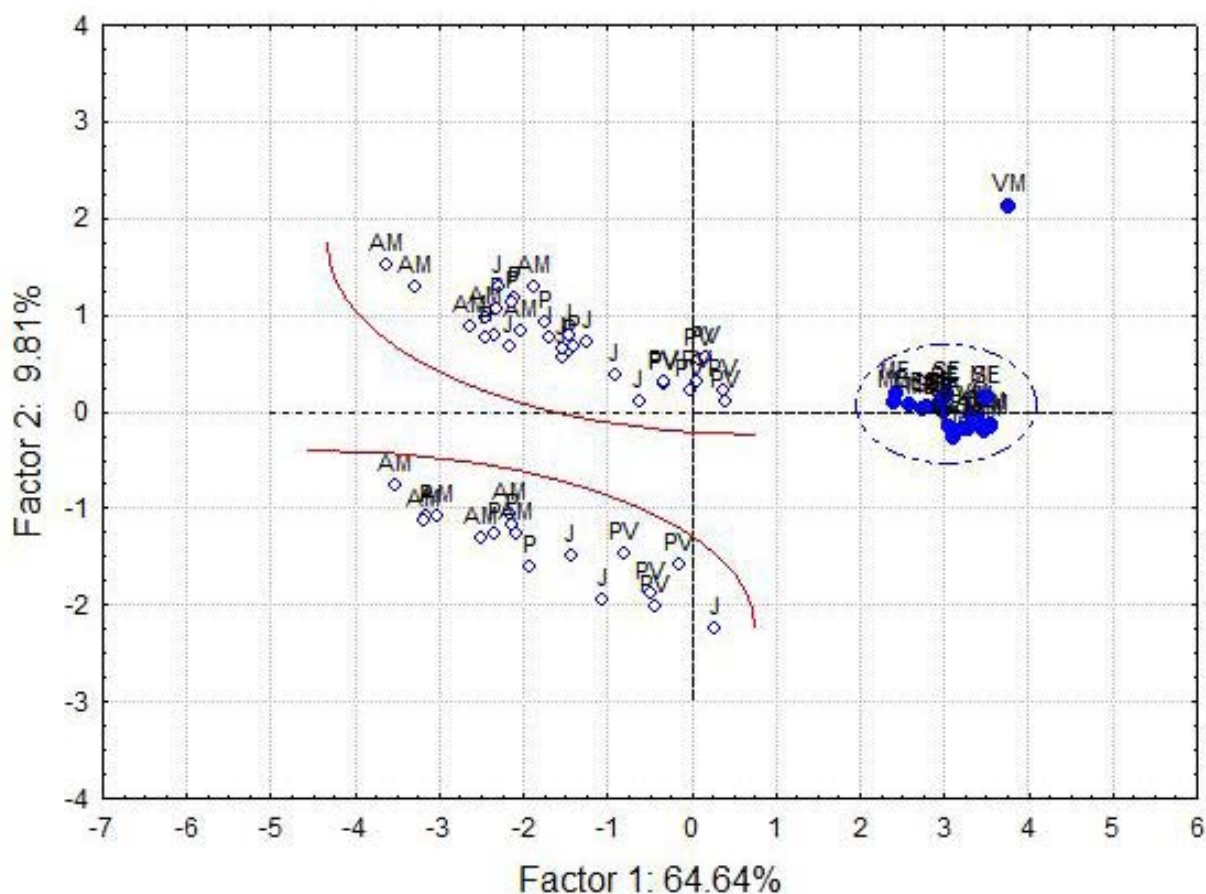


**Figure 3.** Plot of Data of the Headwaters Zone (Pixquiatic: Filled Circles) and Floodplain Zone (Jamapa-Cotaxtla: Empty Circles) Derived from PCA. Vado de las Monjas (VM), Seis de Enero (SE) and Mariano Escobedo (ME), Arroyo Moreno (AM), Playa de Vacas (PV), Puente (P) and Jamapa (J). Note Three Groups: Data in Blue Circle, and Two Groups Separated by Red Arcs.

The mean values of all variables were significantly different between the watershed zones (Table 1), so water quality is noteworthy different. However, significant differences between zones varied temporarily: water temperature, alkalinity and oxygen concentration were significant different between zones in all months; hardness was significantly different almost in all months except in October; pH was not significantly different in any month and turbidity did not show significant differences most of the time (except from January to March); *E. coli* concentrations have significant differences in February, March, May, August, September, and October as well as total coliforms except in February (Table 1).

In general, the water quality of both watersheds is affected negatively by fecal contamination (indicated by red color in Table 1). However, *E. coli* concentrations are acceptable for contact at headwater zone in some months of the year (indicated in yellow in table 1). Oxygen concentrations indicate good and very good water quality at the headwaters while at the floodplain region, the concentrations showed poor water quality in the river.



**Table 1.** Mean Values of Physicochemical and Microbiological Variables of the Headwaters Zone (H) of La Antigua Watershed and Floodplain Zone (F) of Jamapa-Cotaxtla Watershed. (\*) Significant Differences Mann-Whitney U Test ( $P < 0.05$ ) between Regions. (-) Missing Values. Scale Color Based in Deutsch et al. (2009); (a) Concentration Very Low for Aquatic Life; (b) >200-600/100 mL Limit for Human Contact (Recreational). Sample Number for Each Month H = 3, F = 4. Total Sample Number H = 33 (Except for Coliforms H = 24), F = 48). Number in Parenthesis is Standard Deviation.

| Month | Zone | Air °C           | Water°C          | pH             | Hardness<br>CaCO <sub>3</sub> mg/l | Alkalinity<br>mg/L | O <sub>2</sub> mg/l | Turbidity<br>JTU | E.coli<br>CFU/100 ml | Coliform<br>CFU/100 ml |
|-------|------|------------------|------------------|----------------|------------------------------------|--------------------|---------------------|------------------|----------------------|------------------------|
| J     | H    | 20.17*<br>(1.76) | 12.67*<br>(0.29) | 7.17<br>(0.29) | 20.00*<br>(0)                      | 30.00*<br>(5)      | 8.17*<br>(0.6)      | 2.00*<br>(0)     | —                    | —                      |
|       | F    | 24.38<br>(1.11)  | 24.38<br>(0.25)  | 7.25<br>(0.29) | 635.00<br>(421.78)                 | 285.00<br>(149.89) | 2.83                | 7.25<br>(8.62)   | 70766.67             | 59850.00<br>(38075.42) |
| F     | H    | 22.83<br>(2.75)  | 16.00*<br>(1)    | 7.00<br>(0)    | 16.67*<br>(5.77)                   | 25.00*<br>(0)      | 7.53*<br>(0.05)     | 2.00*<br>(0)     | 94.67*<br>(63.22)    | 3111.11<br>(346.94)    |
|       | F    | 26.13<br>(1.25)  | 26.88<br>(1.31)  | 7.38<br>(0.25) | 657.50<br>(396.67)                 | 237.50<br>(135.74) | 3.63                | 23.75<br>(17.97) | 49333.33             | 43950.00<br>(54980.81) |
| M     | H    | 22.0*<br>(1)     | 16.33*<br>(0.58) | 6.83<br>(0.29) | 20.00*<br>(0)                      | 26.67*<br>(2.89)   | 7.17*<br>(0.05)     | 2.00<br>(0)      | 527.78*              | 3700.00<br>(180.28)    |
|       | F    | 25.38<br>(0.48)  | 28.25<br>(1.19)  | 7.00<br>(0)    | 745.00<br>(296.25)                 | 240.00<br>(150.50) | 2.30                | 16.25<br>(22.5)  | 49433.33             | 37683.33<br>(34494.72) |
| A     | H    | 19.17*<br>(1.61) | 16.50*<br>(0.05) | 7.00<br>(0)    | 23.33*<br>(5.77)                   | 25.00*<br>(0)      | 7.00*<br>(0.26)     | 4.00<br>(1.73)   | 838.67               | 5572.33<br>(891.82)    |
|       | F    | 28.13<br>(1.18)  | 30.13<br>(2.02)  | 7.00<br>(0)    | 870.00<br>(260)                    | 242.50<br>(135.25) | 2.35                | 21.00<br>(32.88) | 42266.67             | 26050.00<br>(35301.43) |
| MAY   | H    | 21.67*<br>(1.53) | 17.67*<br>(1.15) | 7.00<br>(0)    | 13.33*<br>(5.77)                   | 28.33*<br>(2.89)   | 6.97*<br>(0.15)     | 2.00<br>(0)      | 444.44*<br>(452.55)  | 3505.56<br>(1683.78)   |
|       | F    | 29.13<br>(2.17)  | 32.00<br>(1.68)  | 7.13<br>(0.25) | 652.50<br>(401.78)                 | 171.25<br>(23.23)  | 2.13                | 3.50<br>(1.73)   | 53033.33             | 51433.33<br>(30506.08) |
| JUN   | H    | —                | —                | —              | —                                  | —                  | —                   | —                | —                    | —                      |
|       | F    | 28.63<br>(1.49)  | 28.38<br>(0.75)  | 7.00<br>(0)    | 102.50<br>(33.04)                  | 112.50<br>(14.43)  | 1.26<br>(2.53)      | 10.00<br>(4.08)  | 114133.33            | 47516.67<br>(16834.21) |
| JUL   | H    | 19.83*<br>(2.25) | 17.17*<br>(0.76) | 7.00<br>(0)    | 20.00*<br>(0)                      | 28.33*<br>(5.77)   | 6.97*<br>(0.16)     | 2.00<br>(0)      | 1105.78              | 2511.22<br>(925.76)    |
|       | F    | 30.38<br>(1.80)  | 31.13<br>(1.88)  | 7.25<br>(0.29) | 180.00<br>(74.83)                  | 130.00<br>(21.60)  | 1.38<br>(2.14)      | 2.00<br>(0)      | 100416.67            | 21383.33<br>(12837.95) |

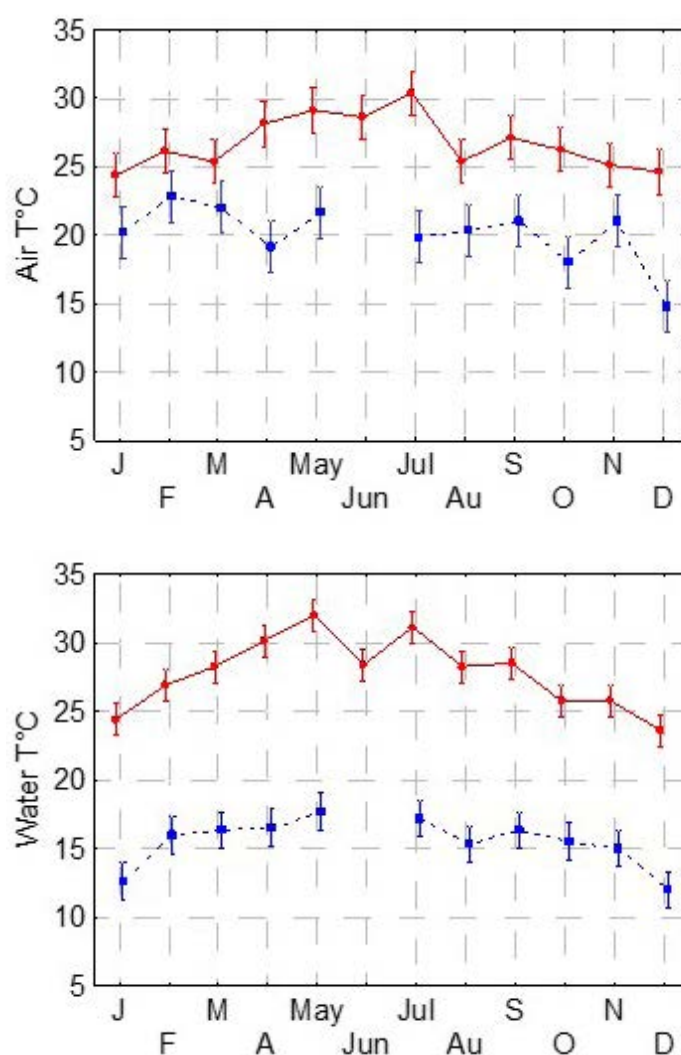
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|       |   |                  |                  |                |                    |                    |                 |                |                    |                          |
|-------|---|------------------|------------------|----------------|--------------------|--------------------|-----------------|----------------|--------------------|--------------------------|
| AUG   | H | 20.33*<br>(1.53) | 15.33*<br>(0.58) | 7.00<br>(0)    | 10.00*<br>(0)      | 36.67*<br>(2.89)   | 7.07*<br>(0.25) | 2.00<br>(0)    | 151.89*<br>(56.45) | 2879.67<br>(475.07)      |
|       | F | 25.38<br>(0.63)  | 28.25<br>(1.32)  | 7.13<br>(0.25) | 120.0<br>(24.49)   | 127.50<br>(31.75)  | 1.48            | 2.00<br>(0)    | 101566.67          | 65016.67<br>(28313.87)   |
| S     | H | 21.0*<br>(2.60)  | 16.33*<br>(0.29) | 7.00<br>(0)    | 10.00*<br>(0)      | 25.00*<br>(0)      | 7.00*<br>(0)    | 2.00<br>(0)    | 294.33*            | 1177.78<br>(1333.24)     |
|       | F | 27.13<br>(0.25)  | 28.5<br>(0.71)   | 7.13<br>(0.25) | 135.00<br>(47.96)  | 153.75<br>(72.27)  | 1.83            | 3.50<br>(1.73) | 94400.00           | 111950.00<br>(49124.92)  |
| O     | H | 18.0*<br>(1)     | 15.5*<br>(0.5)   | 7.00<br>(0)    | 40.00<br>(17.32)   | 30.00*<br>(0)      | 7.13*<br>(0.06) | 2.00<br>(0)    | 727.78*            | 3350.11<br>(852.06)      |
|       | F | 26.25<br>(0.87)  | 25.7<br>(1.04)   | 7.25<br>(0.29) | 105.00<br>(41.23)  | 162.50<br>(49.24)  |                 | 8.00<br>(4)    | 93733.33           | 145766.67<br>(95565.89)  |
| N     | H | 21.00<br>(3.70)  | 15.0*<br>(1)     | 7.00<br>(0)    | 16.67*<br>(5.77)   | 26.67*<br>(2.89)   | 7.40*<br>(0.1)  | 2.00<br>(0)    | —                  | —                        |
|       | F | 25.13<br>(1.93)  | 25.75<br>(0.96)  | 7.38<br>(0.25) | 252.50<br>(103.72) | 211.25<br>(94.90)  | 2.88            | 2.75<br>(1.5)  | 126300.00          | 151116.67<br>(97388.74)  |
| D     | H | 14.83*<br>(0.29) | 12.00*<br>(0.5)  | 7.00<br>(0)    | 20.00*<br>(0)      | 35.00*<br>(0)      | 8.17*<br>(0.15) | 2.00<br>(0)    | —                  | —                        |
|       | F | 24.63<br>(2.50)  | 23.63<br>(2.02)  | 7.25<br>(0.29) | 605.00<br>(457.27) | 250.00<br>(138.74) | 3.80            | 6.00<br>(4.62) | 125350.00          | 133500.00<br>(149433.12) |
| Total | H | 20.1*<br>(2.64)  | 15.5*<br>(1.79)  | 7.0*<br>(0.13) | 19.1*<br>(9.47)    | 28.8*<br>(4.51)    | 7.3*            | 2.2*<br>(0.73) | 523.2*<br>(648.75) | 3226.0*<br>(1425.35)     |
|       | F | 26.7<br>(2.28)   | 27.8<br>(2.78)   | 7.2<br>(0.24)  | 421.7<br>(371.05)  | 193.6<br>(103.69)  | 2.3             | 8.8<br>(13.56) | 85061.1            | 74601.4<br>(73913.29)    |



Figures 4 and 5 show variation through time of both watershed zones. All variables were always higher in the river at the floodplain than in the headwaters zone, mainly temperature, alkalinity, and hardness (Fig. 4), except the Oxygen concentration (Fig. 5). A temporal change is noticeable in June, the beginning of the rainy season, at the floodplain sites for most of the parameters. Also, in these sites, the hardness and alkalinity respond noteworthy to the dry period (November to May) and the rainy period (June to October) (Fig. 4). Microbiological parameters were higher (>100 times in mean) at the floodplain sites than in the headwaters zone (Fig. 5); no temporary tendency was detected at the floodplain sites. Even when there was an obvious variation at the sites in headwaters, no clear trend was identified in this zone.



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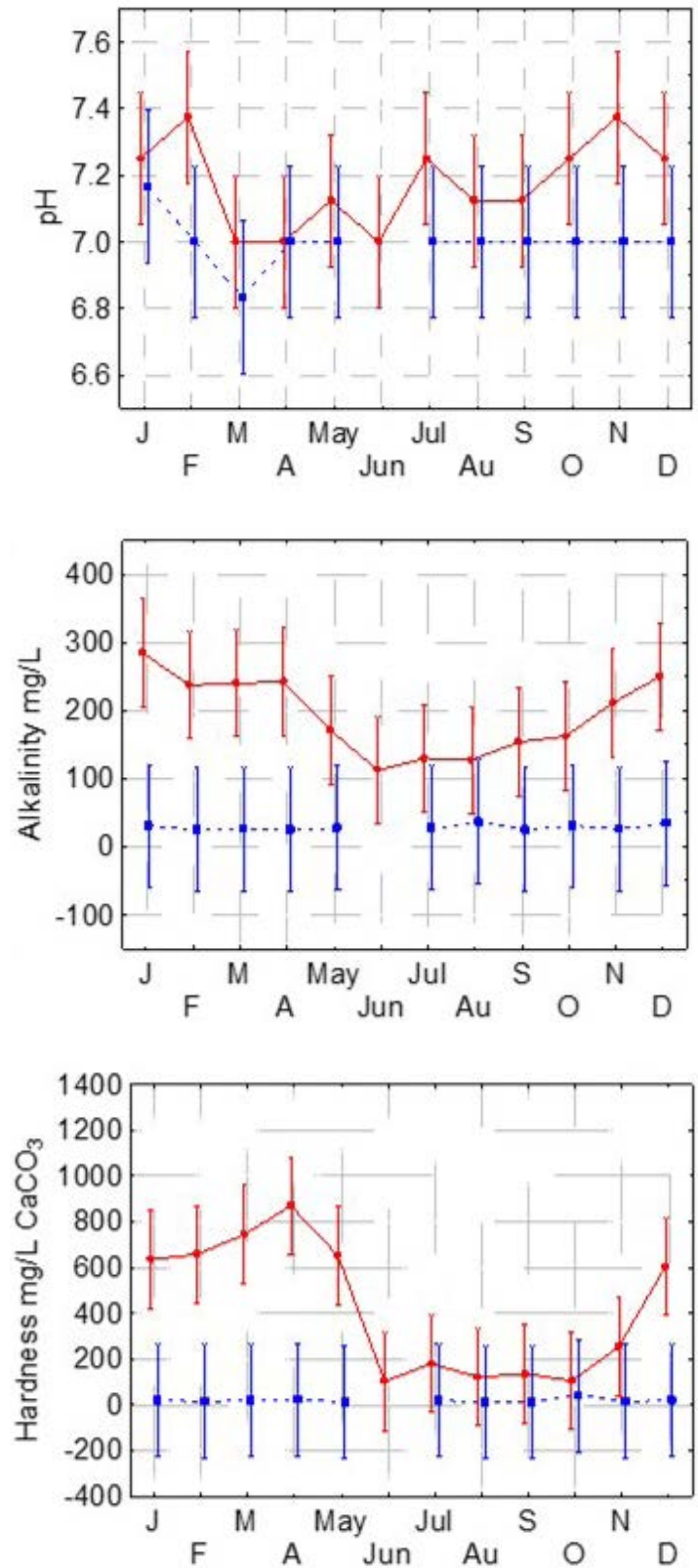
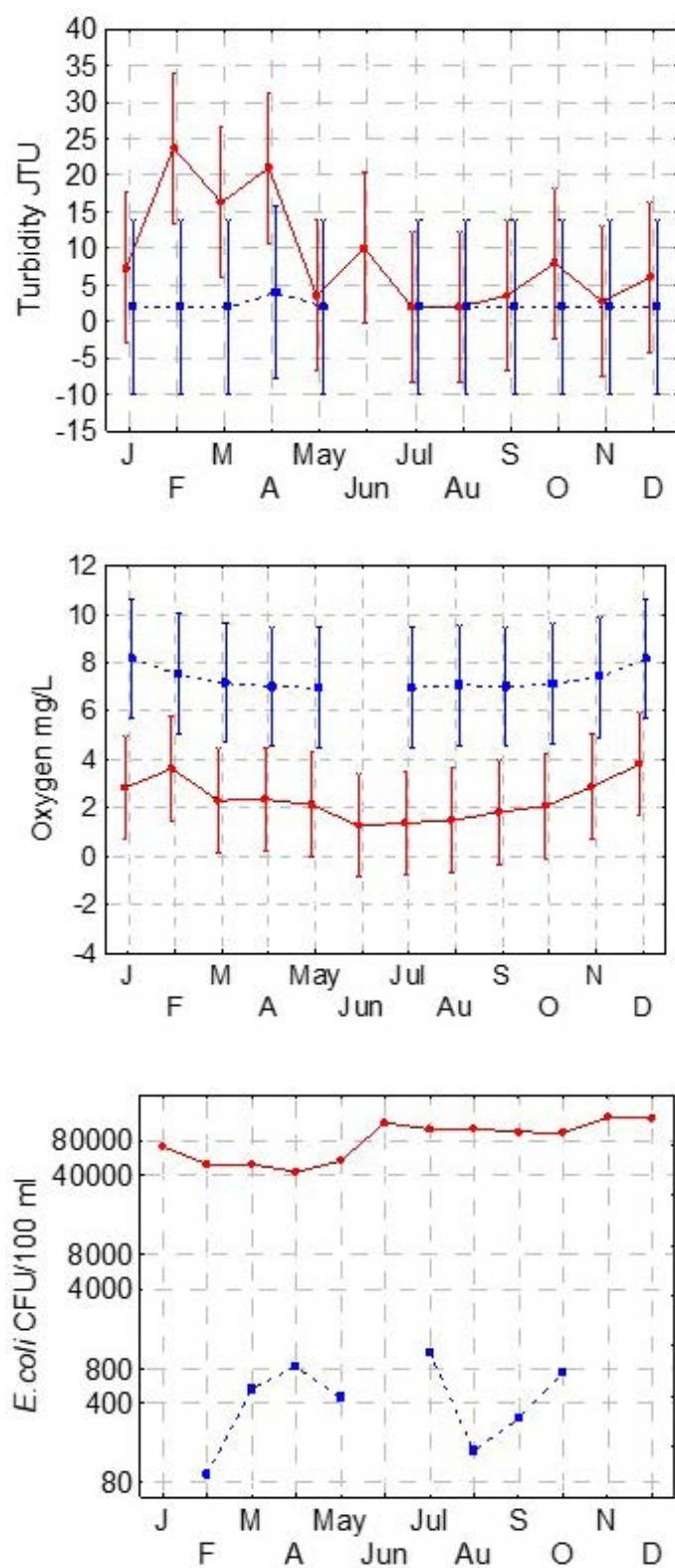


**Figure 4.** Monthly Mean Values of Air and Water Temperature, pH, Alkalinity, and Hardness. Whiskers Represent 95 % Confident Intervals. Headwaters in Blue Color, Floodplain in Red.





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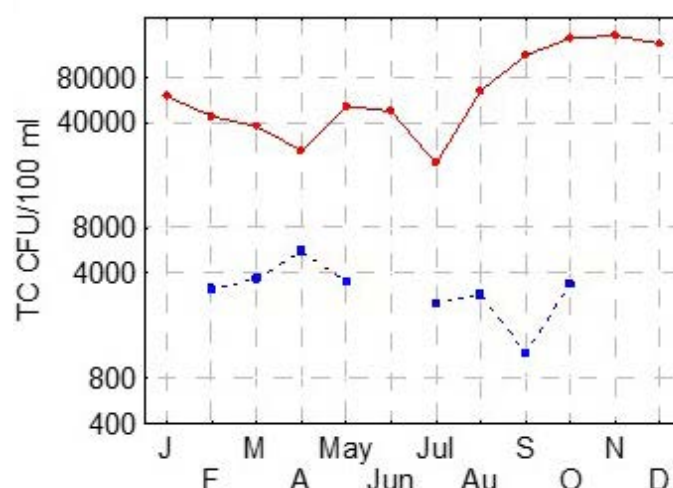


**Figure 5.** Monthly Mean Values of Turbidity, Oxygen, *Escherichia coli* and Total Coliforms. Whiskers Represent 95 % Confident Intervals. *E. coli* and Total Coliforms Lack Confident Intervals as They Are in Logarithmic Scale. Headwaters in Blue Color, Floodplain in Red.

## Discussion

As expected, floodplain sites had higher values in parameters related to weather and mineralization than headwaters zone. The high values of pH, hardness and alkalinity can be explained by three reasons. First, the geology in the watershed in these zones are extremely different, while in the headwaters igneous volcanic rocks are prevalent (andosol soil type), in the floodplains accumulated clayey sediments (vertisol soil type), mainly from the transference zone, prevail (INEGI, 2023). Second, load of diverse material that dissolves in water and naturally increases minerals concentrations from up to down of the watershed, rises hardness and alkalinity (Shajedul-Islam & Hoque-Majumder, 2020). Third, superficial water quality at floodplain zone is affected by marine influence specifically by the salt wedge and mixing processes that may homogenize water column. At Jamapa River, salt intrusion reaches 5 km along the river in the dry period (Perales-Valdivia et al., 2018), therefore, pH hardness and alkalinity increase in the affected zone as consequence of natural properties of marine water, such as carbonates and other minerals (Boyd et al., 2016). The pH in the different sampled sites of the floodplain is affected by the intrusion of the salt wedge and this is noticeable in the separation of the sites in the PCA analysis. Temporal variations were noticeable in hardness and alkalinity in the floodplain zone while in the headwater zone, it remains almost constant. The beginning of the rainy season in June affects alkalinity and hardness, lowering their values because of the higher supply of freshwater. The Jamapa region has a high capacity of buffering and hard water. In the case of the headwaters zone, the values were within the range considered typical for freshwater: soft water with a good

capacity of buffering, and only an atypical value of pH (6.5) was recorded in the Vado de las Monjas site. Turbidity values were higher for the floodplain zone only 3 months when strong winds from the north affect coastal zone (cold mass air coming from North America) and may affect the dynamics of the river by the influence of the sea and resuspending sediments and mixing water column (Perales-Valdivia et al., 2018).

Temperature is related to the altitude, so in the headwater zone, air and water temperature was lower than in the floodplain zone. Water temperature in the headwater zone was temperate (cloud forest) while in the floodplain zone, it was warm (coastal plain), and in both regions, a temporal variation was noticeable at the beginning of the dry season (May) with the increment of air and water temperature.

The parameters that showed critical water quality values were dissolved oxygen concentrations and fecal coliforms. While the headwaters zone had optimal concentrations of Oxygen, the floodplain zone was characterized all year by low concentrations that are not optimal for aquatic life. Depletion of oxygen is commonly caused by consumption by high bacterial activity decomposing organic matter (Harremoës, 1982), so this is an indicative of a high load of organic matter (Eriksen et al., 2022). The city of Boca del Río is located in the floodplain and other important localities which use Arroyo Moreno and the Jamapa River as destination of treated wastewater, so a high organic load may be entering the system. In the headwaters zone, dissolved oxygen concentrations were optimal all year long, so support of aquatic life is optimal in this region.

Concentration of *Escherichia coli* was the most important indicator of a negative impact in both zones of the watersheds. Although Pixquiac (headwaters zone) had 2 months with low values of this fecal bacterium, the high values of CFU of *E. coli* indicated an important negative impact most part of the year. Floodplain zone was heavily affected by fecal pollution all year in extremely high concentrations. In both cases, although the method used for estimation of fecal coliform differs from Most Probable Number (MPN), it is sure that these concentrations surpass the maximum permissible limit of concentration allowed for Mexican normativity for residual water to be released in rivers (250-600 MPN/100 ml; SEMARNAT, 2022). The temporal variation was not clear in the headwaters zone, while in the downstream region there is constant strong fecal contamination.

The fecal contamination of water of the rivers and the values observed in the two zone studies may be related to population and the capacity of the treatment systems of wastewater. In the headwater zone, the locality of Mariano Escobedo lacks a treatment plant wastewater operated by the municipality (Conagua, 2024). In the floodplain zone, in the Puente Moreno locality, there is a treatment plant with a capacity of 68 l/s while for Boca del Río city, only two treatment plants with a total capacity of 380 l/s (Conagua, 2024). According to the information generated between 2014 and 2016, SEMARNAT (2024) esti-

mated that 60 m<sup>3</sup> of residual water is generated by inhabitant in Mexico. This is equivalent to a production of 2 l/s for 1,000 inhabitants, which is a value that represents more than 277,000 l/s and superasses more than 220 times the capacity of the treatment plants established until 2023. So, a high load of organic matter derived from urban wastewater is received by the Jampa River, affecting optimal oxygen concentration for wildlife and incrementing fecal coliforms in the water and other pollutat found in municipal wastewaters.

In developing countries like México, the lack of information and resources to monitor has been acknowledged even in the National Hydric Program 2020-2024 (SEMARNAT, 2020) and this void is specially accentuated at the provinces of the country and rural areas (Hiriart et al., 2010; Jimenez-Cisneros et al., 2010). In general, the governmental monitoring of freshwater bodies in Mexico has not been systematic regarding techniques, criteria to determine water quality which makes assessments and comparisons extremely difficult (Jimenez-Cisneros et al., 2010); these disparities are even more complicated between the criteria used by water and health authorities. These discrepancies and the lack of budget to monitor within the government has resulted in a reduction of the parameters included in the water quality indexes and the frequency of monitoring with the consequent incomplete vision about the quality of the water of most Mexican water bodies (Hiriart et al., 2010). Data of this study show the relevance of community monitoring efforts in contributing to the knowledge of rivers at a local and regional scale. Community data obtained with standardized technique and methodologies contribute to build knowledge in countries like Mexico, where a void of information is the prevalent condition (Flores-Díaz et al., 2018).

## Conclusions

Temperature, alkalinity, hardness, pH, and turbidity were traits corresponding to altitude and soil type of each zone of watersheds that respond to climatic seasons. The oxygen concentration was quite different in the two watersheds, indicating a better condition of water quality in the headwaters zone (Antigua watershed) than in the floodplain zone (Jamapa-Cotaxtla watershed), being depleted by organic pollution derived from wastewater. For the two zones the fecal contamination (*E. coli*) was a constant problem throughout the year, and it is indicative of a failed treatment system of wastewater of population close to the rivers.

## Acknowledgements

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## Authors' Contributions

MGRE coordinated the monitoring program. JAAC and EAD collected and analyzed samples. JAAC generated the idea of the analyses of data and wrote the manuscript. MGRE revised the manuscript, interpreted results, and discussed them. JAAC, MGRE and EAD provided feedback to the final manuscript.

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# Environmental Problems on Water Resources: A Review at the Basin Level with Emphasis on Tuxpan River in Veracruz, Mexico

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## Abstract

**E**nvironmental conflicts arise from differing perceptions of situations and impacts where stakeholders have conflicting objectives or incompatible interests regarding water resources. The Tuxpan River basin, located in the northern part of Veracruz, has experienced an increase in population density, leading to escalating environmental challenges. To address these issues, a characterization of the water resource and an analysis of their underlying causes were conducted. A bibliographic review of academic articles and official documents was undertaken to assess the current state of the basin. The Tuxpan River basin features diverse morphological and topographic characteristics. The aim of this study is to identify the challenges related to water resource usage in Mexico's river basins with a particular focus on the Tuxpan River basin. This is achieved by compiling and analyzing available regional data, emphasizing the demand and distribution of water resources. Notably, the agricultural sector requires significant amounts of water for crops and livestock. These activities, combined with the climatic variability present in the Tuxpan River basin, impact water availability. Furthermore, increased precipitation in the upper regions—often degraded or sparsely vegetated—leads to sediment transport, which adversely affects the lower basin, making it more vulnerable to flooding.

## Keywords

Environmental issues, Gulf of Mexico, water management, basin, characterization.

## Introduction

Conflict and environmental issues are aspects to consider managing natural resources in Mexico. Environmental conflicts occur when human actions, whether intentional or unintentional, negatively impact natural resources, compromising human well-being. (Maya, 2019; Ortégón, 2019). In contrast, the environmental issue refers to any problem or challenge resulting from human activities or natural processes that negatively impact the environment, ecosystems, or natural resources.

Environmental issues and conflicts within river basins have escalated over the past few decades. These challenges have highlighted the need for sustainable water resource management (Rua de Cabo et al., 2006; Guerra, 2018; Maya, 2019; Ortégón, 2019).

Several basins in South America are experiencing disturbances linked to economic and productive activities, which have intensified with urban expansion and population growth. According to Aguayo et al., (2009), these activities have drastically altered land cover over the past 300 years. As a result, there has been a significant decline in vegetation, loss of natural habitats, disruption of hydrological and biogeochemical cycles, decline of native species, introduction of exotic species, and shifts in climate patterns associated with global change (Velázquez et al., 2002; Ureña-Retana, 2004; Ruiz et al., 2013).

A common issue observed in most river basins is the removal of forest cover, particularly in their upper regions. This poses a threat to the hydrological cycle by reducing both the availability and quality of water, leading to additional challenges in the provision of ecosystem services within the watershed (François et al., 2024). Deforestation is further driven by the overexploitation of water resources, the discharge of wastewater into water bodies, and unsustainable agricultural practices. If the relationship between ecosystems and productive activities within watersheds is not adequately studied, the provision of these services will continue to decline and further deteriorate (Manson, 2004).

Mexico is among the Latin American countries with the highest rates of deforestation. In 2019 alone, 321,000 ha were deforested, equivalent to 83.3 million tons of CO<sub>2</sub> emissions (WRI, 2020). Despite having extensive mangroves and seagrass ecosystems, Mexico has lost significant portions of these habitats due to coastal development associated with urban, industrial, and tourism expansion. Restoring and conserving mangrove and seagrass habitats could strengthen climate change mitigation and adaptation strategies by preserving blue carbon ecosystems. However, the loss of mangroves and seagrasses continues, making it essential to implement actions aimed at maintaining environmental services, increasing and preserving natural carbon sinks, reducing emissions from deforestation and forest degradation, and restoring and conserving marine-coastal ecosystems (Herrera-Silveira, 2020).

Deforestation is also driven by the overexploitation of water resources, the discharge of wastewater into water bodies, and unsustainable agricultural practices. If the relationship between ecosystems and productive activities within basins is not properly analyzed, the provision of ecosystem services will continue to diminish and deteriorate.

At the basin level, Matthews et al. (2000) highlight that vegetation provides crucial ecosystem services, including maintaining balance and supporting the proper exchange of energy and matter from the upper to the lower basin, soil stabilization, flow volume regulation, flow periodicity, and water filtration. These services are increasingly impacted by deforestation, agriculture, wildfires, and urban development, all of which disrupt the water supply network and its area of influence. This leads to reduced soil water availability and flooding in lower areas (Richard, 2016).

These issues are significant for informing decision-making processes related to development, public policies, and the management and administration of basins, not only in Mexico but also in other countries such as Colombia, Costa Rica, Uruguay, Peru, and Ecuador (Correa, 2005; Vargas, 2005; Maya, 2019). In certain cases, methodologies have been employed to identify socio-environmental conflicts, enabling the characterization of these challenges (Guerra, 2018; Maya, 2019; Bustamante, 2022)

The Tuxpan River basin, located primarily in the northern part of the state of Veracruz, has experienced a rise in population density, increasing from 6.9 million in 2000 to 8 million in 2020 (INEGI, 2017). Additionally, livestock and agricultural activities, along with oil and natural gas extraction, have intensified pressure on the river basin. However, environmental problems and conflicts related to water resources in the region have not been thoroughly characterized. This study aims to address this gap by identifying issues related to water resource use in Mexico's river basins, with a particular focus on the Tuxpan River basin. The analysis is based on a comprehensive review of the available data for the region.

## Materials and Methods

### *Bibliographic Analysis for Problem Identification*

This study was conducted through the compilation and analysis of documentary information, including scientific articles, ecological territorial planning documents, watershed management plans, and Action Plans for Integrated Watershed Management (PAMIC). A national reference framework for watershed management in Mexico was established.

A detailed analysis of these documents was carried out to examine environmental problems from the perspective of various authors. Environmental problems are defined as negative impacts or alterations to the environment that disrupt the balance of ecosystems and affect humans (Maya, 2019; Ortégón, 2019). Based on this definition, the environ-

mental problems of the Tuxpan River basin were identified using methodological tools proposed by Villareal (2015) and Ortégón (2019), which are considered highly applicable.

The methodology consisted of three steps:

1. Characterization of the Water Resource – Determining its coverage and area of influence.
2. Identification of Causal Factors – Finding direct and indirect causes that affect the water resource.
3. Environmental Problem Identification – Determining the specific environmental issues within the Tuxpan River basin.

This analysis helps reveal the current problems facing the basin and assess whether environmental issues exacerbate the impact on water resources (Fig. 1, Table 1).

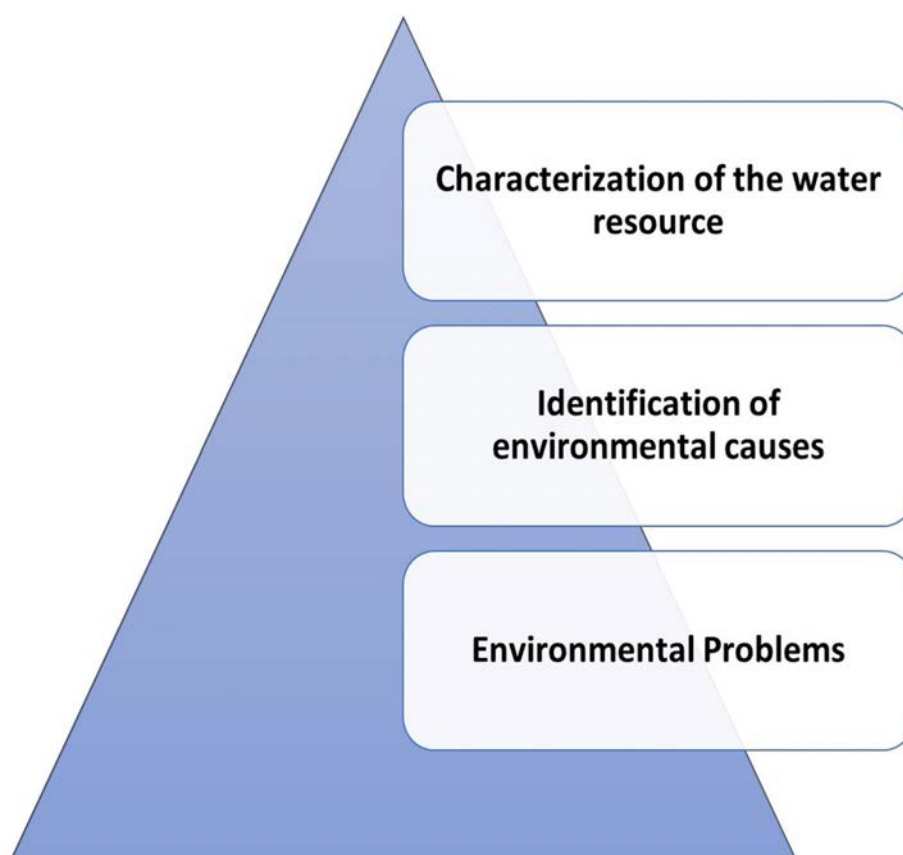


**Figure 1.** Description of the Process for the Identification of Environmental Issues.



**Table 1.** Bibliographic References Used to Identify the Different Aspects of Environmental Problems in the Tuxpan River Basin.

| Aspects to Identify Environmental Problems | References                                                                                                                                                                                                                                      |
|--------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Characterization of Water Resources        | INEGI-INE-Conagua (2007); INEGI (2013, 2016); Conagua (2016, 2017); INECC-FGM (2018); CONABIO (2019);                                                                                                                                           |
| Identification of Causes                   | Paruelo et al., (2005); Landa & Carabias (2008); García-Orozco et al., (2011); Ellis et al., (2012); FAO and ITPS (2015); Armenta-Montero & Ellis (2016); Melchor-Marroquín & Chagoya-Fuentes (2016); INECC-FGM (2018).                         |
| Environmental Issues                       | Gómez-Maya (2006); Hernández-Martínez (2008); Vergara et al., (2011); Ellis et al., (2012); Armenta-Montero & Ellis (2016); Melchor-Marroquín & Chagoya-Fuentes (2016); SEMARNAT (2016); INECC-FGM (2018); Medina-Núñez (2019); Cardona (2021). |

## Context of Basin Management in Mexico

In Mexico, effective basin-level management is uncommon. In 2007, three governmental agencies—the National Institute of Statistics and Geography (INEGI), the National Institute of Ecology and Climate Change (INECC), and the National Water Commission (Conagua)—established standardized topographic and hydrographic criteria for basin delimitation and nomenclature. This effort resulted in the identification of 1,471 basins, classified as exorheic, endorheic, and areal basins. These basins are organized into 37 hydrological regions, 25 Basin Councils, and 13 hydraulic-administrative regions (Cotler, 2008; Cotler et al., 2010; Torres et al., 2013).

Furthermore, studies on water availability have been conducted based on the proposed basin-level classification. It has been observed that Thirty-five percent of the country's population is found in the lower zones of the basins where the storage capacity predominates and enables productive activities. However, these areas face numerous threats, including the contamination of rivers, lakes, and aquifers, as well as the impacts of climate change. Changes in precipitation and temperature patterns are expected to disrupt the water balance within the basins (Cotler et al., 2010).

In Mexico, hydrological basin problems are particularly complex due to rapid population growth in recent years, which has significantly increased water consumption. On average, a person in Mexico uses 380 liters of water per day (INECC, 2020)—200 % more than the 100 liters per day recommended by the World Health Organization (WHO) for meeting basic consumption and hygiene needs (UNESCO, 2020). This excessive demand

has led to the overexploitation of 100 out of the 664 identified aquifers, while 26 of the 37 hydrological regions in the country are now in critical condition (Vargas, 2005).

Overexploitation is not the only challenge facing Mexico's hydrological basins; soil erosion is another severe issue, resulting from a combination of factors such as steep slopes, climate, improper land use, vegetation cover, and ecological disasters. Human activities, particularly agriculture, have greatly accelerated erosion rates (FAO & ITPS, 2015). Excessive cultivation and low humidity conditions deteriorate soil structure, further increasing the risk of erosion. For example, in southern Mexico, the periodic use of fire to eliminate agricultural residues is a major contributor to soil degradation. Conventional agricultural practices often involve burning stubble from previous harvests, leaving the soil exposed during the rainy season, which exacerbates erosion (Conagua, 2009).

Water pollution is another major issue affecting most of Mexico's basins, largely driven by human activities, particularly industrial, agricultural, and livestock operations. The central-western basins of Mexico are among the most severely impacted by this problem. Deforestation, often resulting from land-use changes for agricultural and livestock purposes, has significant consequences at the basin level, including the reduction of biodiversity, soil erosion, and landslides (Durán & Torres, 2003; Boehm, 2005; Conagua et al., 2009).

These environmental changes can be broadly summarized as disruptions to climatic elements, primarily caused by human activities in conjunction with deforestation, the burning of fossil fuels, and the contamination of water and soil. These factors contribute to long periods of drought and an increase in extreme weather events, such as torrential rains and high-category hurricanes (Granados-Sánchez et al., 2013).

## Tuxpan River Basin

### *The Characterization of the Water Resource*

The Tuxpan River Basin originates in the eastern part of the Sierra Madre Oriental. The upper section of the basin is marked by rugged terrain, with elevations reaching up to 3,000 meters above sea level, while the lower section consists of a vast coastal plain with elevations below 200 meters above sea level (Fig. 2) (INEGI, 2013). The basin is part of the Tuxpan-Nautla Hydrological Region (RH 27), and its primary tributaries include the Pan-tepec, Vinazco, Tecomate, Buenavista, and Tuxpan rivers. It spans across the states of Veracruz (75.6 %), Puebla (13.2 %), and Hidalgo (11.2 %), with Veracruz represented by 19 municipalities, Puebla by seven, and Hidalgo by five (INEGI, 2016). The Tuxpan River, the basin's main watercourse, stretches for 236 km, while the total length of the river network, including tributaries, extends to 11,283 km. Despite the vast extent of the basin's water network, the drainage system suggests that, in the event of heavy rainfall, the out-

flow response will be moderate. This means that the drainage outflow in response to climatic events will be slower, thus extending the concentration time for runoff. Most of the basin's elevations are below 500 meters above sea level, as shown in the hypsometric curve, which illustrates the distribution of contour lines (Fig. 3). The region's economy is primarily driven by livestock farming and agriculture, with oil and gas extraction also taking place (INEGI, 2013; INECC-FGM, 2018).

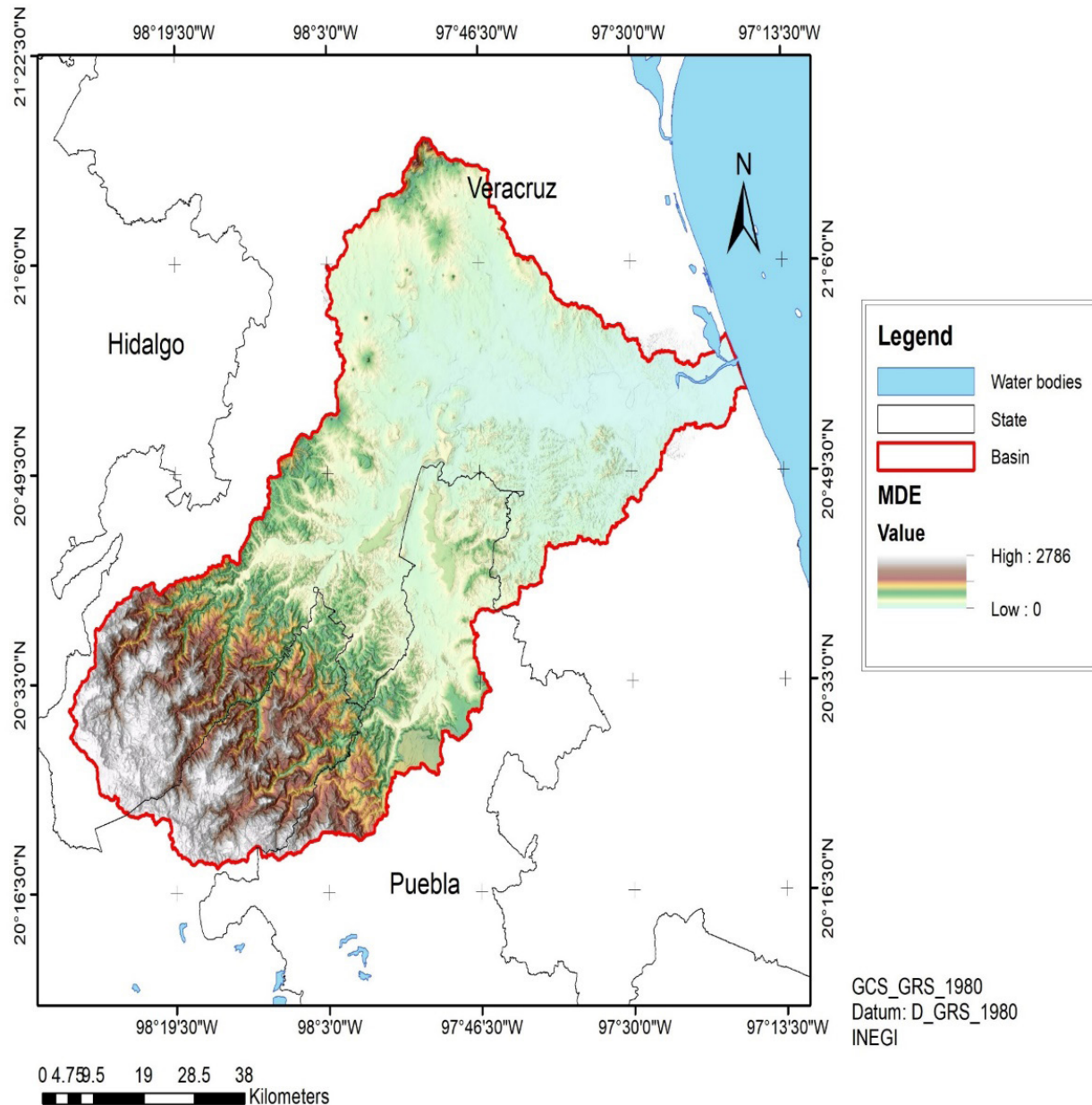


**Figure 2.** Location of the Tuxpan River Basin. INEGI (2016).  
(DEM, Digital Elevation Model).

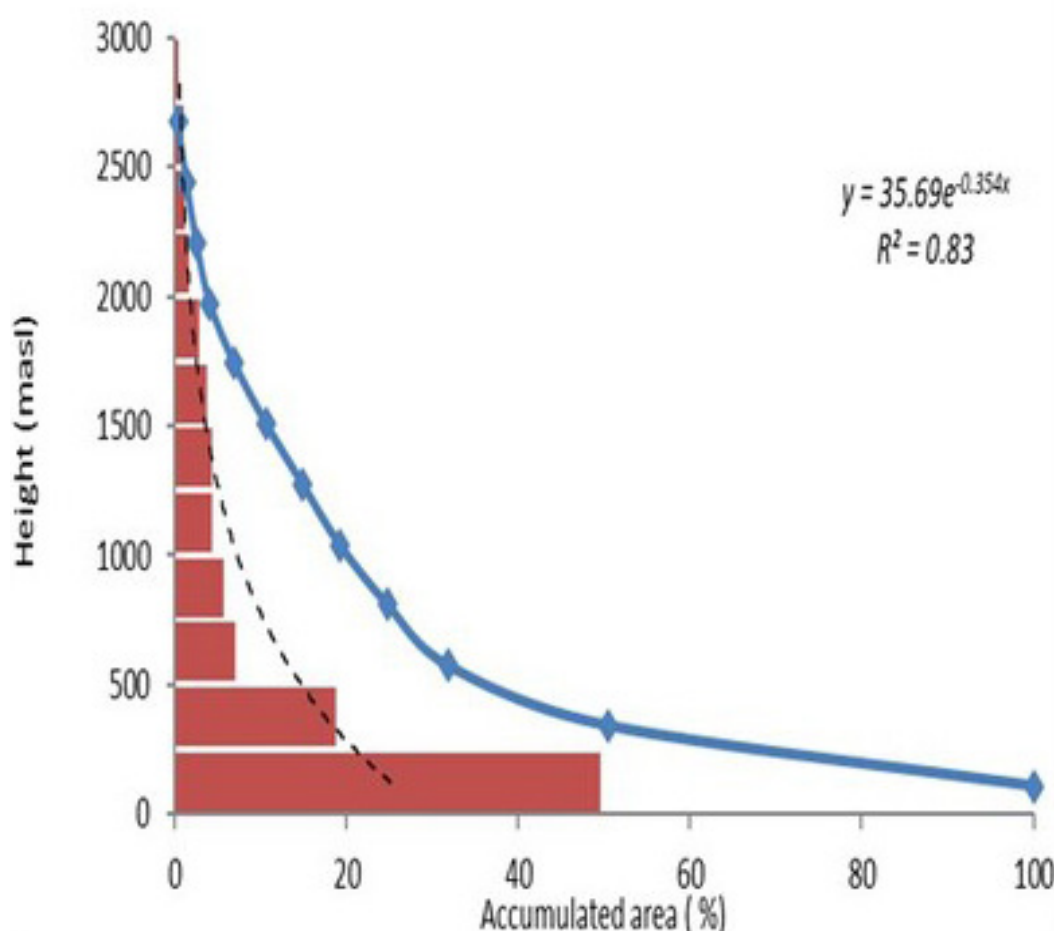


**Figure 3.** Hypsometric Curve and Frequency of Altitudes  
(Data Obtained from INEGI).

## Identification of Environmental Causes

The Tuxpan River Basin encompasses several rural areas. Although it does not have large urban centers, the existing urbanization places significant pressure on water resources, leading to political, economic, environmental, and social conflicts over water use. One of the outcomes of urbanization is the loss of vegetation, which undermines the stability of water resources, reducing their availability and coverage (Paruelo et al., 2005; Landa & Carabias, 2008; FAO & ITPS, 2015). The reduction in vegetation cover is driven by three main factors: demographic, social, and economic (García-Orozco et al., 2011). The Tuxpan River Basin, with a population density of 80 inhabitants per square kilometer, is particularly vulnerable to water erosion. The combination of population density, economic activities, topography, and precipitation contributes to widespread erosion, which affects the entire basin (Melchor-Marroquín & Chagoya-Fuentes, 2016).



In 2011, vegetation cover accounted for 27 % of the total area of the basin. The coniferous forests in the upper regions of the basin have been severely impacted, with a decline of up to 95 % (INECC-FGM, 2018). These changes in vegetation are largely a consequence of urbanization, where natural environments are increasingly being converted into agricultural and livestock areas.

These activities, compounded by the basin's climatic variability, significantly impact the availability of water resources. Furthermore, increased rainfall in the upper areas—often degraded or sparsely vegetated—results in sediment being transported downstream, which in turn exacerbates flooding in the lower basin (Ellis et al., 2012; Armenta-Montero & Ellis, 2016; Melchor-Marroquín & Chagoya-Fuentes, 2016).

According to the Public Registry of Water Rights (REPDA, 2014), the basin's water resources are allocated to seven different uses, including aquaculture, agriculture, industry, livestock, public-urban services, and domestic purposes, with surface water concessions granted for these uses. The Tuxpan River Basin's water demand is highest for agricultural, public-urban, services, and domestic purposes, with seven sub-basins categorized as having very high demand (INECC-FGM, 2018).

## Environmental Causes

The analysis of the Tuxpan River Basin highlights that the primary issue facing the region is the alteration of natural vegetation, which is being converted into agricultural and urban land, particularly from the upper to the lower basin. This has led to an annual loss of 16.86 km<sup>2</sup> of vegetation, with the greatest loss occurring in the middle-lower basin, where agricultural land use predominates. This land use has increased by 9.20 %, with the municipality of Álamo being the largest contributor to this trend (Ellis et al., 2012; Armenta-Montero & Ellis, 2016; Melchor-Marroquín & Chagoya-Fuentes, 2016; INECC-FGM, 2018).

The root causes of biodiversity loss stem from human activities, primarily the rapid population growth and unsustainable consumption of natural resources. At the current rate, natural systems are no longer able to meet human needs. The destruction of vegetation directly impacts water resources, reducing their availability and coverage (Paruelo et al., 2005; FAO & GTIS, 2015). Between 1999 and 2019, vegetation cover in the municipality of Tuxpan decreased by 0.17 % (1.55 km<sup>2</sup>) (Cardona, 2021), driven by the factors previously mentioned (García-Orozco et al., 2011).

The decline in vegetation, including oak forests, high evergreen forests, and sub-evergreen forests, exacerbates vulnerability in downstream areas. According to Ortiz-Lozano (2013), deforestation increases surface runoff, which impacts areas such as Álamo and Tuxpan. These regions have experienced extreme weather events, and the removal of vegetation leads to heightened water erosion and sediment transport, increasing the likelihood of flood-

ing. Ellis et al. (2012) reported return periods of 522 to 544 km<sup>2</sup> over periods ranging from 2 to 100 years, resulting in significant damage, economic losses, and human casualties, particularly in the agricultural and urban areas of Tuxpan and Álamo (Marroquín & Chagoya-Fuentes, 2016; Ibarra-Zabaleta et al., 2017; Rodríguez-Hernández et al., 2020).

## Environmental Problems

The influx of sediments from the upper regions of the Tuxpan River Basin, combined with water contamination from the oil and citrus industries, as well as power generation activities near the rivers, has led to the disruption and even disappearance of some water wells in the basin (Medina-Núñez, 2019). Additionally, the spillage of hydrocarbons into water bodies and soil in certain municipalities in the lower basin, such as Cerro Azul, Temapache, and Tuxpan, has caused significant contamination of both water and soil resources. This pollution has had detrimental effects on native vegetation in the region (Vergara et al., 2011).

Hydrocarbon contamination in water resources renders them unsuitable for human consumption and harmful to other living organisms, which cannot survive due to the high concentrations of carbon and other toxic substances. As a result, these organisms die, further degrading the quality of the water and making it unsafe for consumption (Gómez-Maya, 2006; Hernández-Martínez, 2008; Medina-Núñez, 2019). In the Álamo and Temapache regions, citrus juice companies also play a significant role in water contamination. These industries consume large amounts of water and produce toxic waste, which is discharged directly into drainage systems that are connected to local water sources, making the water unfit for use by the surrounding communities.

Demographically, the population in the lower basin has been steadily increasing (SEFIPLAN, 2022), resulting in higher demand for housing, education, healthcare, and commercial infrastructure. This growing demand is putting pressure on local vegetation types. The combined impact of population growth, infrastructure development, and industrial and port activities is reflected in the expansion of human settlements, which increased by 3.29 % (31.80 km<sup>2</sup>) between 1999 and 2019. Economically, the municipality of Tuxpan has been growing in tourism, port activities, industry, livestock, and agriculture. The first three sectors are primarily driven by the municipality's coastal location, which serves as a key transportation route for goods, machinery, and industrial equipment (Valdez-Lazalde et al., 2011).

The distribution of water for domestic use is a significant issue throughout the basin, as there is no centralized drinking water network to supply the municipalities. Water is only available in certain areas, and due to the poor condition of the pipes, it is not distributed daily. In some instances, water is not provided for several weeks because of the deteriorating infrastructure. This uneven distribution becomes remarkable during the dry season when the water levels in the sources from which the water is collected drop.

## Recommendations

Based on our review, we propose that the issues identified can be addressed through the strategies outlined in the PAMIC of the Tuxpan River Basin, along with ongoing management efforts. These proposals focus on the sustainable management of resources, ensuring their conservation and renewal. Additionally, we strongly recommend the establishment of a basin council to support the management of programs aimed at securing regular funding. To effectively tackle these challenges, it is also crucial to provide comprehensive training and guidance in environmental education.

Furthermore, we propose the implementation of mitigation mechanisms for environmental services. It is essential to seek basin-wide solutions rather than concentrating on isolated issues. As such, the state must reassess its policies and programs, adopting a strategy that acknowledges environmental problems and addresses market failures that have undermined equity, environmental sustainability, and the quality of life for the population.

Environmental concerns must be prioritized, as current environmental spending is insufficient to meet key challenges. Therefore, financial solutions are necessary, seeking both international and national support to secure resources for environmental projects.

Finally, the growing water shortage—both in terms of quality and quantity—facing the basin is increasingly recognized by the population as one of the most pressing environmental problems, both now and in the future. In many regions, this issue remains difficult to explain or substantiate, as the availability of water has decreased or deteriorated due to poor management. Consequently, this issue must be given higher priority across all sectors. Comprehensive management actions must be implemented throughout the basin, directing and regulating the use of land and natural resources in a way that enables society to meet its needs without causing environmental harm.

## Authors' Contributions

BERC and JLAM conceptualized this contribution. BERC compiled the literature, prepared the figures, and wrote the original manuscript. CFRG and KCGG wrote and edited the manuscript. All authors reviewed and approved the final document.

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# Prospective Analysis of Major Phytoplankton Groups in Some Freshwater Bodies in Campeche, Southeastern Gulf of Mexico

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## Abstract

A prospective analysis of major groups of phytoplankton and its abundance in some freshwater bodies of the state of Campeche is presented. Water-bottle quantitative phytoplankton samples were taken from seven freshwater bodies (waterholes, rivers, and a lagoon) in March and June 2019 and January 2020 (dry, rainy, and northern wind seasons, respectively). Twelve physicochemical parameters (water temperature, salinity, conductivity, pH, water hardness, dissolved oxygen, oxygen saturation, nitrates, nitrites, ammonium, phosphates, and silicates) were recorded. Phytoplankton was dominated by cyanobacteria (up to  $1.08 \times 10^6$  cell/l) in the windy and rainy seasons, followed by diatoms (up to  $3.40 \times 10^5$  cell/l) in the rainy season; chlorophytes and euglenophytes were rare. Nanoflagellate and dinoflagellate abundances were on the order of magnitude of  $10^3$  to  $10^5$  cell/l; both groups showed maximum averages in the rainy season. In general, wider ranges in values of physicochemical characteristics were recorded in the windy season. Water temperature (24.3 to 31.9°C;  $F=11.30$ ,  $p<0.005$ ) and pH (6.64 to 8.80;  $F=2.99$ ,  $p<0.005$ ) showed seasonality, as did nitrates, ammonium, and phosphates. Phosphate concentrations at five sites exceeded the maximum permissible limit (5 mg/l) of the water quality criteria for the protection of aquatic life in the rainy season (up to 69.96 mg/l) and the windy season (up to 71.99 mg/l). They also exceeded the maximum permissible limits (0.1 mg/l)

for waters for human use and consumption during the same seasons. Two sites, Ulumal (river) and Zoh Laguna (waterhole), showed strong ammonium contamination (up to 50.1 and 60.8 mg/l, respectively) in the rainy season.

## Keywords

Anthropogenic, eutrophication, cyanobacteria, microalgae, waterhole.

## Introduction

Freshwater phytoplankton are important because they are part of trophic webs as producers of oxygen and are indicators of environmental conditions of the water bodies in which they live (Naselli-Flores & Padisák, 2023). In this context, the study of phytoplankton communities through the analysis of fluctuations in their taxonomic composition is a key methodology for characterization of various types of freshwater ecosystems. Over time, it will facilitate obtaining metrics for the evaluation of the ecological health of aquatic habitats (Ebro Hydrographic Confederation, 2005).

In Mexico, studies of freshwater phytoplankton date back to 1843; however, some authors agree that the present knowledge of its ecology is limited (Montejano et al., 2000; Mora-Navarro et al., 2004; Novelo & Tavera, 2011). In particular, the study areas along the Yucatan Peninsula, restricted spatially, have provided little detailed information about the influence of hydrological parameters on phytoplankton composition and diversity (Sánchez-Molina & Zetina-Moguel, 1993; López-Adrián & Herrera-Silveira, 1994; López-Adrián & Barrientos-Medina, 2005, Álvarez-Góngora et al., 2012).

Phytoplankton studies in the state of Campeche have been focused on the coastal zone with an emphasis on harmful species (Poot-Delgado, 2016; Poot-Delgado et al., 2021). In the southern part of the state, studies on fluvial-lagoon water bodies predominated (Muciño-Márquez et al., 2014; Poot-Delgado et al., 2015, 2018). However, the state has favorable geohydrological conditions for the reception and storage of significant volumes of water from rainfall (Conagua, 2020). This allows the presence of temporary (waterhole) and permanent aquatic bodies, such as rivers, lakes, and lagoons (Rebolledo-Vieyra, 2010). Therefore, it is necessary to generate basic ecological information on the phytoplankton of the diverse freshwater bodies in the study area.

This pioneering study describes the abundance of predominant phytoplankton groups in response to limnological conditions during three climatic seasons (dry, rainy and windy) in some freshwater bodies in the state of Campeche.

## Materials and Methods

Seven freshwater bodies, located in the municipalities of Calkiní (1), San Francisco de Campeche (1), Champotón (2) and Calakmul (3) in the state of Campeche, southeastern Mexico, were sampled on March 8 (dry season) and June 17, 2020 (rainy season), and on 16 January 2020 (Fig. 1; Table 1). Sampling sites are distinct geographic locations where data are collected, often with unique environmental characteristics.

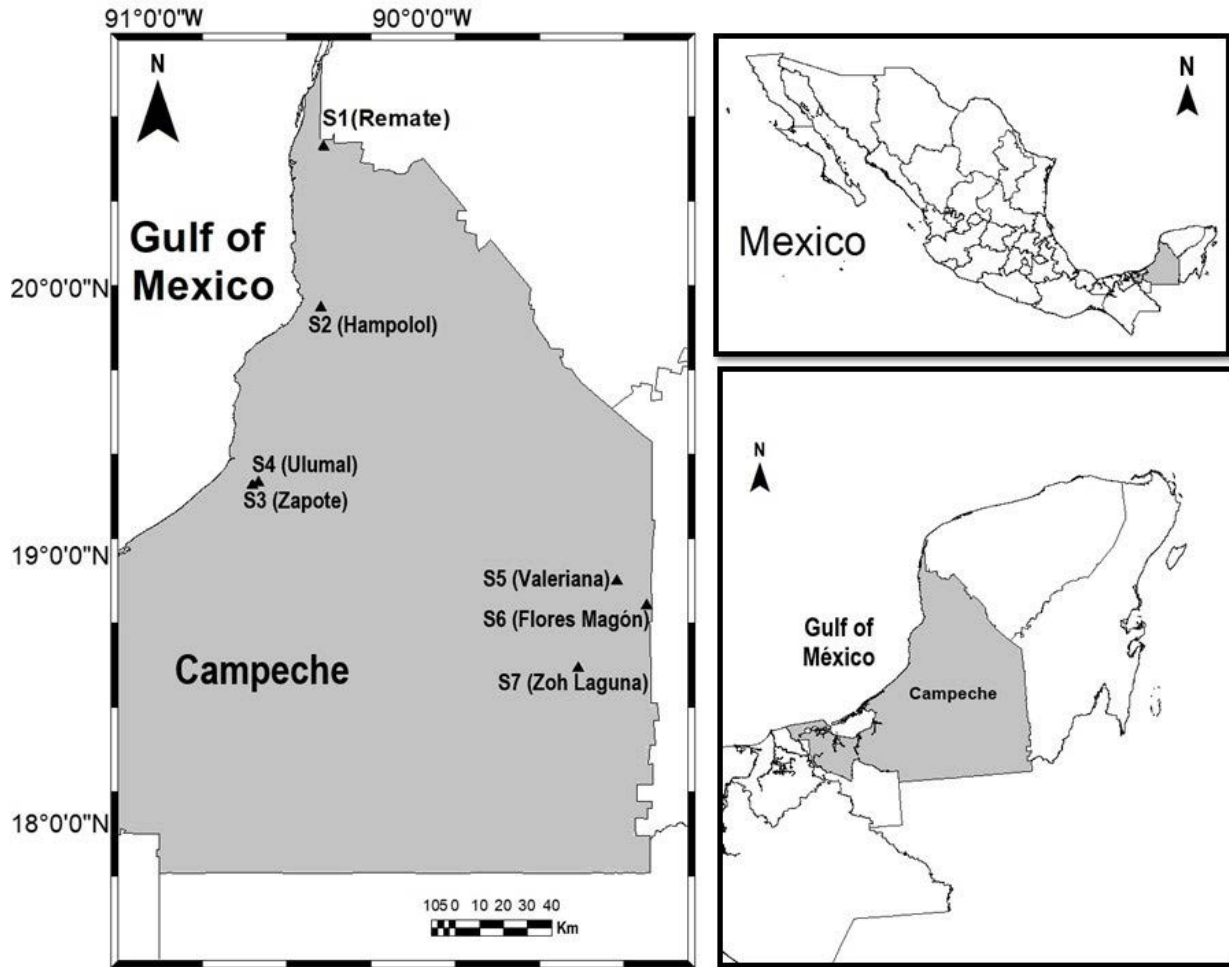


**Figure 1.** Study Area and Sampling Sites of Freshwater Bodies in the State of Campeche, Southeastern Gulf of Mexico.

### *Sampling Sites*

Remate: It is a natural spring or a waterhole, also called an “ojo de agua” (in Spanish), located 47 km from Calkiní, which runs through a channel 8 km long toward the sea. Low-impact ecotourism activities, such as swimming, are practiced at the site (Arriaga-Cabrera et al., 2002).



Hampolol: There are waterholes, and it is an ejido (a communal farmland) located in the southeast of the Yucatan Peninsula, 23 km from the municipality of San Francisco de Campeche. At this site, low-impact ecotourism activities are practiced, such as swimming and tourist walks (Arriaga-Cabrera et al., 2002).

Champotón River: It is a perennial river approximately 47 km long, with a general route from East to West that drains a basin of 650 km<sup>2</sup>. The National Commission for the Knowledge and Use of Biodiversity (Comisión Nacional para el Conocimiento y Uso de la Biodiversidad [CONABIO]) considers the Champotón River as a priority hydrological region, presenting a modification of the environment due to fires (in the headwaters), water pollution, domestic waste, and discharge from the sugar mill (Arriaga-Cabrera et al., 2002).

Calakmul Waterholes: They are situated in the municipality of Calakmul, in the southern part of the Yucatan Peninsula (Navarro-Olmedo et al., 2018). Calakmul is characterized by calcareous soil, which causes rainwater to filter quickly, and it has no permanent water bodies. However, there are areas where the characteristics of the terrain allow the accumulation of precipitation, forming water bodies known locally as “aguadas”; these are the only sources of water for wildlife and humans of the region during the dry season. The size of the water bodies varies from ca. 100 m<sup>2</sup> to several hectares. However, most are small, less than 1 ha, and dry quickly at the beginning of the dry season (Reyna-Hurtado et al., 2010).

**Table 1.** Characteristics of the Sampling Sites in Some Freshwater Bodies in the State of Campeche, Southern Gulf of Mexico.

| Municipalities | Geographic coordinates          | Stations/sites    | Type of water body* | Anthropogenic activity |
|----------------|---------------------------------|-------------------|---------------------|------------------------|
| Calkiní        | 20°32'3.88"N,<br>90°22'13.37"W  | Remate (S1)       | Waterhole           | Tourism and Recreation |
| Campeche       | 19°56'1.10"N,<br>90°22'49.25"W  | Hampolol (S2)     | Waterhole           | Tourism and Recreation |
| Champotón      | 19°17'9.44"N,<br>90°40'16.41"W  | Zapote (S3)       | River               | Tourism and Recreation |
|                | 19°16'51.59"N,<br>90°36'49.45"W | Ulumal (S4)       | River               | Tourism and Recreation |
| Calakmul       | 18°54'51.59"N,<br>89°16'41.44"W | Valeriana (S5)    | Lagoon              | Irrigation             |
|                | 18°49'18.27"N,<br>89° 9'46.57"W | Flores Magón (S6) | Waterhole           | Human Consumption      |
|                | 18°35'14.02"N,<br>89°25'3.48"W  | Zoh Laguna (S7)   | Waterhole           | Human Consumption      |

\*Conagua (2020).

### *Field Sampling and Data Collection*

Samples for quantitative analysis were collected from January 2019 to January 2020 at seven stations at an approximate depth of 1 m. The sampling dates for the analyses were grouped according to the meteorological conditions previously reported for the area (Ramos-Miranda et al., 2006): the dry season (February to May), the rainy season (June to September), and the windy (the northern winds) season (October to January).

Once the sampling sites were established, physicochemical parameters of the water were recorded (water temperature, salinity, conductivity, pH, dissolved oxygen (DO) and oxygen saturation (%DO) using a Hanna multiparameter water quality meter, model HI9828 (Woonsocket, RI, USA) and a Hach multiparametric probe, model HQ40d. Water hardness ( $\text{CaCO}_3$ ) was determined with a Hach DR890 multiparametric probe (Hach, Loveland, CO, USA). Similarly, water samples were collected for subsequent determination of inorganic nutrients (nitrates, nitrites, ammonium, phosphates, and silicates) in the laboratory. Water samples were taken using an amber glass bottle with a capacity of 2 l, poured into plastic bottles previously washed with neutral detergent (Extran), 0.1 %  $\text{H}_2\text{NO}_3$  and deionized water and subsequently were placed in a plastic refrigerator at 4°C for transportation, pending subsequent nutrient analysis.

Phytoplankton samples were taken using a plastic bottle (capacity 1 l). Samples were fixed in the field to a 1 % final neutral iodine solution and subsequently preserved by adding a stock (37 %) neutralized formalin solution to a final concentration of 4 % (Andersen & Throndsen, 2004).

### *Determination of Nutrients*

Laboratory analyses of macronutrients (nitrites, nitrates, ammonium, phosphates, and silicates) and water hardness were performed according to UNEP (1991).

### *Cell Counting*

Phytoplankton cells were counted according to Brierley et al. (2007), taking 10  $\text{cm}^3$  of the sample, which was placed in a sedimentation chamber (Utermöhl; Hydro-Bios Kiel, Germany) with a glass lid for 24 h. An Iroscope SI-PH inverted phase-contrast microscope, series 450 (Mexico City, Mexico) equipped with the 10x/0.25 Ph1 ADL and LD 25x/0.30 Ph1 objectives was used. Due to their size, among nanoflagellates (<20  $\mu\text{m}$ ), phototrophic and heterotrophic species were not differentiated. The abundance values are expressed in cells per liter (cell/l). To identify phytoplankton taxa, specialized literature was consulted: Schiller (1933, 1937), Cupp (1943), Dodge (1982), Komárek and Anagnostidis (1986a, b).

## Data Processing and Statistical Analyses

To determine statistically significant differences, a 95 % confidence level in physicochemical variables, including inorganic nutrients, and the phytoplankton abundance between seasons, the non-parametric one-way analysis of variance (Kruskal–Wallis test) was applied (Daniel, 1993; Boyer et al., 2000). Statistical tests were performed with the Statgraphics Centurion XV program, version 18.2.06; data were plotted using the STATISTICA 7 and MS Excel 365 programs.

## Results

### Physicochemical Variables

The measured environmental variables are given in Table 2. The surface water temperature reached its minimum in the windy season (24.3°C), while the maximum temperature (31.9°C) was recorded in the dry season. The seasonality of temperature was confirmed by significant differences between seasons. The concentrations of dissolved salts represented in the values of salinity, conductivity, and water hardness showed similar dynamics between seasons (see Table 2). However, no significant differences were found.

The pH values ranged from a minimum of 6.68 with a standard deviation (SD) of 0.38 during the dry season to a maximum of 8.8 in the rainy and windy seasons, with a greater SD (0.82) in the windy season. Significant differences in pH were found between seasons. Of note are the low values of oxygen saturation and DO content recorded during the dry and rainy seasons; however, no significant differences were observed between season (Table 2).

**Table 2.** Statistics for Meteorological/Climatic Seasons for Physicochemical Variables from Seven Sampling Sites (S1-S7), in 2021-2022 (Range and Mean  $\pm$  SD); the Average Minimum and Maximum Values of the Physicochemical Variables through the Study Period are Given in Bold.

| Season                      | t°C                | Salinity          | Conductivity (mV) | CaCO <sub>3</sub> (mg/l) | pH                   | DO (mg/l)         | %DO               |
|-----------------------------|--------------------|-------------------|-------------------|--------------------------|----------------------|-------------------|-------------------|
| Dry                         | 28.80-32.60        | 0.18-1.86         | 0.40-3.80         | 0.33-3.08                | 6.68-7.73            | 1.40-7.01         | 19.70-97.70       |
|                             | 30.60 $\pm$ 1.30   | 0.92 $\pm$ 0.71   | 2.10 $\pm$ 1.30   | 1.56 $\pm$ 0.89          | 7.14 $\pm$ 0.38      | 4.15 $\pm$ 1.94   | 57.07 $\pm$ 27.46 |
| Rainy                       | 28.70-30.9         | 0.23-2.42         | 0.35-3.68         | 0.16-7.35                | 7.10-8.80            | 1.72-6.20         | 21.70-80.30       |
|                             | 29.40 $\pm$ 0.70   | 0.96 $\pm$ 0.85   | 1.48 $\pm$ 1.30   | 2.31 $\pm$ 3.38          | 8.01 $\pm$ 0.67      | 3.86 $\pm$ 1.75   | 50.92 $\pm$ 23.41 |
| Windy                       | 24.30-31.90        | 0.08-1.83         | 0.18-3.59         | 1.56-13.18               | 6.64-8.80            | 1.61-8.84         | 19.40-112.80      |
|                             | 26.20 $\pm$ 2.60   | 0.99 $\pm$ 0.65   | 1.60 $\pm$ 1.30   | 4.58 $\pm$ 4.13          | 7.67 $\pm$ 0.82      | 5.52 $\pm$ 2.53   | 66.59 $\pm$ 35.37 |
| Differences between Seasons | F=11.30<br>p<0.005 | F=0.02<br>p>0.005 | F=0.43<br>p>0.005 | F=1.78<br>p>0.005        | F=2.99<br>p<0.005    | F=1.16<br>p>0.005 | F=0.47<br>p>0.005 |
|                             |                    |                   |                   | >500 <sup>1</sup>        | 6.5-8.5 <sup>1</sup> |                   |                   |

<sup>1</sup>Upper limits established for human consumption (DOF, 2022).

### *Inorganic Nutrients*

Data on inorganic nutrients are summarized in Table 3. Nitrites showed high values during the windy season; however, there were no significant differences between climatic seasons. Nitrate concentration was the highest during the dry season. Ammonium showed the maximum value during the rainy season. In general, the ammonium concentrations were low to moderate, except for Ulumal (river) and Zoh Laguna (waterhole) that presented strong ammonium contamination (up to 50.1 mg/l and 60.8 mg/l, respectively) in the rainy season. In both cases, significant differences occurred between seasons (Table 3).

**Table 3.** Statistics for Seasons for Inorganic Nutrients (mg/l) from Seven Sampling (S1-S7) Sites in 2021–2022 (Range and Mean  $\pm$  SD); the Average Minimum and Maximum Values of the Nutrient Concentrations through the Study Period are Given in Bold.

| Season                      | Nitrite           | Nitrates          | Ammonium          | Phosphates         | Silicates         |
|-----------------------------|-------------------|-------------------|-------------------|--------------------|-------------------|
| Dry                         | 0.35-5.38         | 6.63-45.25        | 0.14-7.94         | 1.61-14.86         | 18.40-55.80       |
|                             | 2.85 $\pm$ 1.50   | 28.43 $\pm$ 16.05 | 2.27 $\pm$ 2.67   | 4.38 $\pm$ 5.16    | 32.13 $\pm$ 12.66 |
| Rainy                       | 0.46-7.85         | 6.56-27.56        | 6.40-60.81        | 13.76-69.96        | 3.40-59.30        |
|                             | 1.79 $\pm$ 1.82   | 16.05 $\pm$ 8.11  | 23.64 $\pm$ 24.92 | 55.08 $\pm$ 21.14  | 28.35 $\pm$ 18.46 |
| Windy                       | 0.84-13.50        | 0.44-32.76        | 5.21-52.01        | 12.31-71.99        | 11.70-81.30       |
|                             | 3.25 $\pm$ 4.57   | 10.40 $\pm$ 10.87 | 12.37 $\pm$ 17.49 | 39.0 $\pm$ 23.64   | 31.49 $\pm$ 23.02 |
| Differences between Seasons | F=0.39<br>p>0.005 | F=3.9<br>p<0.005  | F=2.52<br>p<0.005 | F=11.32<br>p<0.005 | F=0.08<br>p>0.005 |
|                             | 0.06 <sup>1</sup> | 10.0 <sup>1</sup> | 0.50 <sup>1</sup> | 0.1 <sup>2</sup>   |                   |

<sup>1,2</sup>Upper limits established for human consumption (DOF, 2019<sup>1</sup>; 2022<sup>2</sup>).

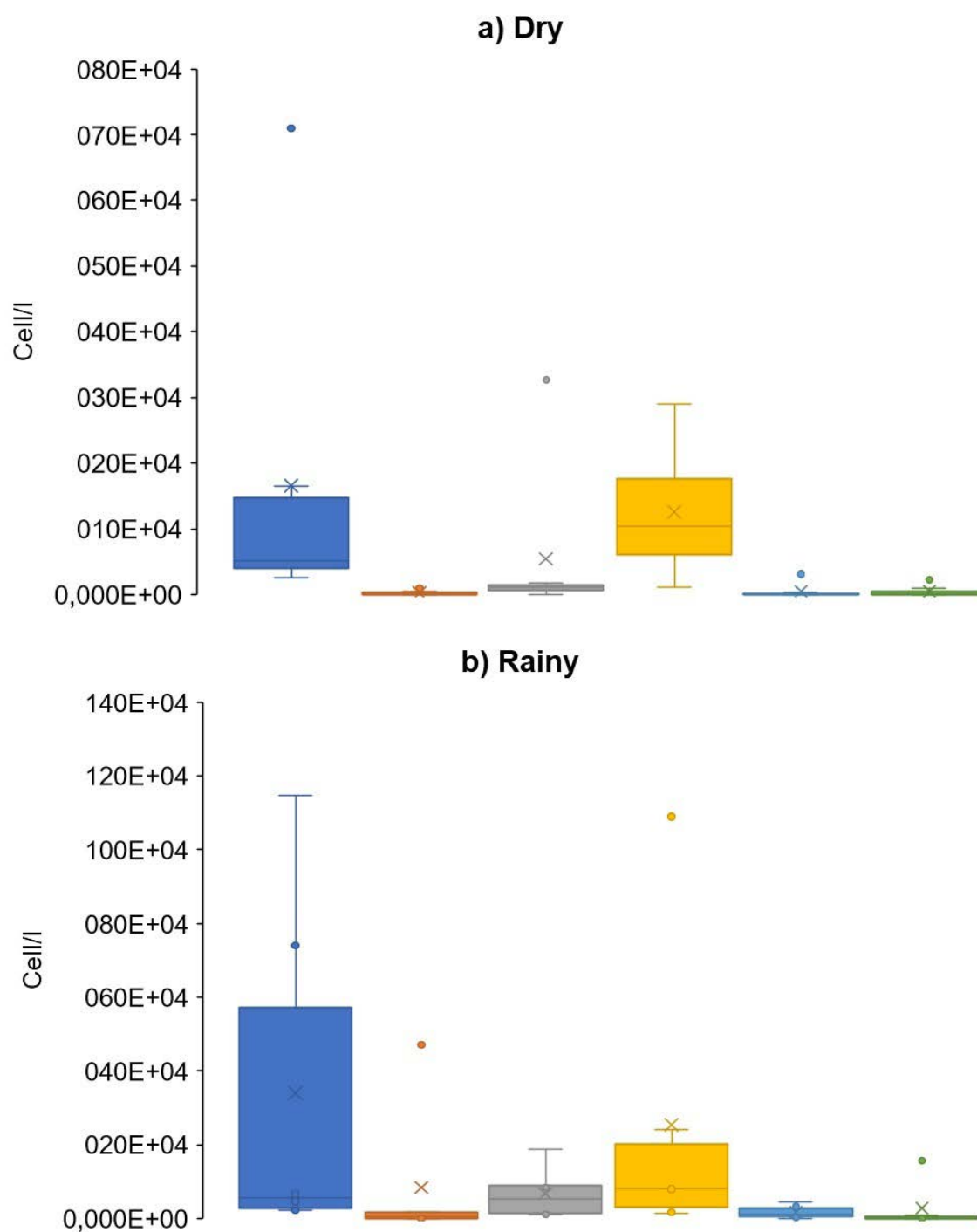
The average values of silicates during three climatic seasons were consistent across all three climatic seasons, ranging from 28.35 to 32.13 mg/l. The minimum values were recorded in the rainy season, and the highest values in the windy season. Silicate concentrations did not show significant differences between the climatic periods (F=0.08; p>0.005). The minimum phosphate values were recorded during the dry season, with the high values during the rainy and windy seasons. Significant differences were observed between seasons (F=11.32; p<0.005).

### *Major Phytoplankton Groups*

The variation in phytoplankton abundances during the three climatic seasons for the major phytoplankton groups is given in Table 4 and Figure 2. Cyanobacteria were the dominant taxonomic group, with the same maximum value of 1.08 $\times$ 10<sup>6</sup> cell/l during the rainy and windy seasons



(Fig. 2b, c) and minimum values on the order of magnitude of  $10^4$  cell/l in the dry season (Fig. 2a).



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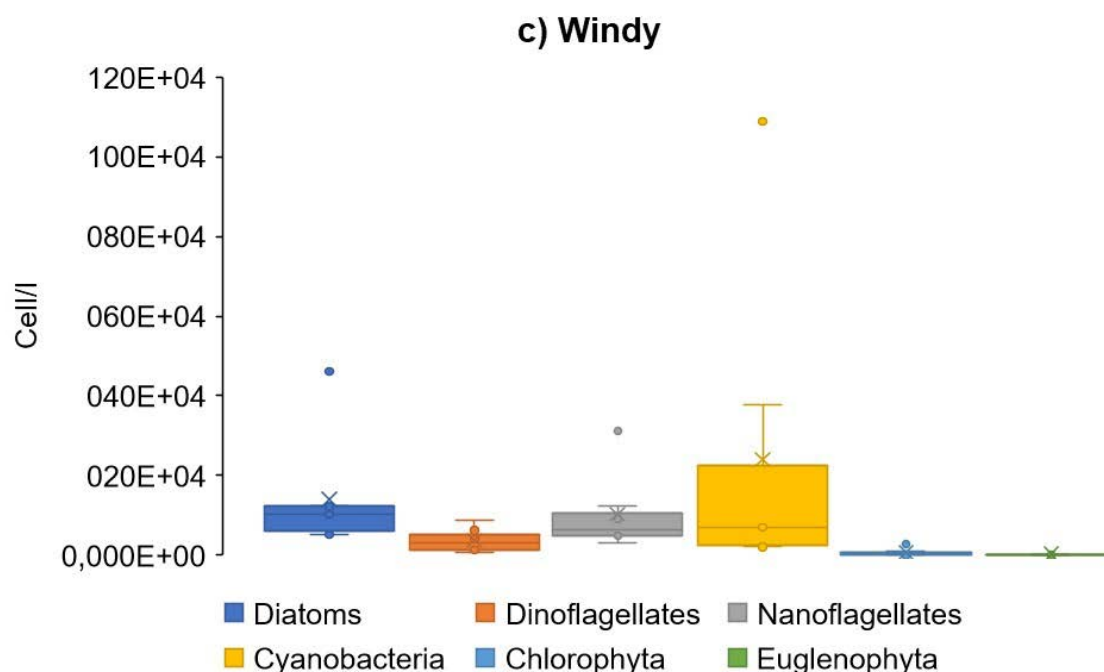


**Figure 2.** Abundances of Major Phytoplankton Groups in Three Seasons; a) Dry, b) Rainy and c) Windy in 2020-2021; Outliers are Indicated with a Circle.

Diatoms were the second dominant group, showing a maximum value of  $1.47 \times 10^6$  cell/l, with SD deviation of  $4.84 \times 10^5$  cell/l, during the rainy season (Fig. 2b). During the windy season, the minimum value was  $5.10 \times 10^4$  cells/l and SD  $1.44 \times 10^5$  cell/l (Fig. 2c).

Nanoflagellate and dinoflagellate abundances were on the order of  $10^3$  to  $10^5$  cell/l. Both groups showed their maximum averages during the rainy season (Fig. 2b). However, nanoflagellates showed their minimum abundance during the dry season (Fig. 2a). They were moderately abundant in the windy season at all sampling sites, except for Valeriana (in the dry season). Dinoflagellates were most abundant in the windy season with an order of magnitude of  $10^4$  cell/l (Fig. 2c).

Euglenophyta and Chlorophyta coincided in their minimum average values on the order of magnitude of  $10^3$  to  $10^4$  cell/l during the windy season (Fig. 2c) and their maximum average values on the order of  $10^4$  cell/l during the rainy season (Fig. 2b). For all major phytoplankton groups studied, no significant differences were observed between seasons (Table 4). Euglenophyta were scarce throughout all three climatic seasons studied.

**Table 4.** Statistics for Meteorological Seasons for Abundances of Major Phytoplankton Groups (Cell/l) from Seven Sampling Sites (S1-S7), in 2021–2022 (Range and Mean  $\pm$  SD).

| Season                      | Nanoflagellates                                    | Diatoms                                            | Dinoflagellates                                    | Cyanobacteria                                      | Chlorophyta                                        | Euglenophyta                                       |
|-----------------------------|----------------------------------------------------|----------------------------------------------------|----------------------------------------------------|----------------------------------------------------|----------------------------------------------------|----------------------------------------------------|
| Dry                         | 6.46x10 <sup>3</sup><br>-3.27x10 <sup>5</sup>      | 2.55x10 <sup>4</sup><br>-7.10x10 <sup>5</sup>      | 2.15x10 <sup>3</sup><br>-9.00x10 <sup>3</sup>      | 1.03x10 <sup>4</sup><br>-2.90x10 <sup>5</sup>      | 3.23X10 <sup>3</sup><br>-3.10X10 <sup>4</sup>      | 308<br>-2.20X10 <sup>4</sup>                       |
|                             | 6.34x10 <sup>4</sup><br>$\pm$ 1.29x10 <sup>5</sup> | 1.65x10 <sup>5</sup><br>$\pm$ 1.25x10 <sup>5</sup> | 5.01x10 <sup>4</sup><br>$\pm$ 3.54x10 <sup>4</sup> | 1.25x10 <sup>5</sup><br>$\pm$ 1.01x10 <sup>5</sup> | 1.71X10 <sup>4</sup><br>$\pm$ 1.96X10 <sup>4</sup> | 1.04X10 <sup>4</sup><br>$\pm$ 1.09X10 <sup>4</sup> |
| Rainy                       | 1.00x10 <sup>4</sup><br>-1.86x10 <sup>5</sup>      | 2.20 x10 <sup>4</sup><br>-1.47x10 <sup>6</sup>     | 3.00x10 <sup>3</sup><br>-4.70x10 <sup>5</sup>      | 1.35x10 <sup>4</sup><br>-1.08x10 <sup>6</sup>      | 3.00X10 <sup>3</sup><br>-4.40X10 <sup>4</sup>      | 8.50X10 <sup>3</sup><br>-1.57X10 <sup>5</sup>      |
|                             | 6.72x10 <sup>5</sup><br>$\pm$ 6.89x10 <sup>4</sup> | 3.40x10 <sup>5</sup><br>$\pm$ 4.84x10 <sup>5</sup> | 1.24x10 <sup>5</sup><br>$\pm$ 2.30x10 <sup>5</sup> | 2.53x10 <sup>5</sup><br>$\pm$ 4.17x10 <sup>5</sup> | 2.07X10 <sup>4</sup><br>$\pm$ 1.70X10 <sup>4</sup> | 8.31X10 <sup>4</sup><br>$\pm$ 1.05X10 <sup>5</sup> |
| Windy                       | 3.00x10 <sup>4</sup><br>-3.11x10 <sup>5</sup>      | 5.10x10 <sup>4</sup><br>-4.61x10 <sup>5</sup>      | 7.00x10 <sup>3</sup><br>-8.80x10 <sup>4</sup>      | 2.10x10 <sup>4</sup><br>-1.08x10 <sup>6</sup>      | 3.00X10 <sup>3</sup><br>-2.80X10 <sup>4</sup>      | 1.00X10 <sup>3</sup><br>-1.40X10 <sup>4</sup>      |
|                             | 1.02x10 <sup>5</sup><br>$\pm$ 9.74x10 <sup>4</sup> | 1.40x10 <sup>5</sup><br>$\pm$ 1.44x10 <sup>5</sup> | 3.66x10 <sup>4</sup><br>$\pm$ 2.94x10 <sup>4</sup> | 2.39x10 <sup>5</sup><br>$\pm$ 3.94x10 <sup>5</sup> | 1.15X10 <sup>4</sup><br>$\pm$ 1.15X10 <sup>4</sup> | 7.50X10 <sup>3</sup><br>$\pm$ 9.19X10 <sup>4</sup> |
| Differences between Seasons | F=0.43<br>p>0.005                                  | F=0.77<br>p>0.005                                  | F=0.98<br>p>0.005                                  | F=0.3<br>p>0.005                                   | F=1.64<br>p>0.005                                  | F=1.02<br>p>0.005                                  |

## Discussion

### *Environmental Characterization*

In general, the values of the measured physicochemical parameters showed variations related to other environmental changes, such as seasonality (dry, rainy, and windy), that had an impact on the volume of water in each water body, as well as on physical (leaching in the environment, concentration, dilution) and chemical (metamorphism, degradation of organic matter).

The values of salinity, hardness, conductivity, and pH were consistent at each point studied throughout the three climatic periods. This is because the state of Campeche is located on a platform of stratified carbonates, such as dolomites and limestones (Benítez et al., 2011). Generally, the pH of inland water bodies tends to be neutral (7), due to the high concentrations of calcium carbonates that are basic in character and is the main moderator of the carbonate-bicarbonate system (Stumm & Morgan, 1996). The values reported herein are within the maximum permissible limits for natural waters for human consumption (6.5-8.5).

In general, moderately to very oxygenated waters were observed, except for the sampling sites, Remate and Hampolol, that had slightly oxygenated waters. This could be

because these sites have abundant vegetation in their surroundings which prevents the aeration of water by winds and also affects the production of oxygen through photosynthesis (Arriaga-Cabrera et al., 2002). The afore-mentioned processes are reinforced by the fact that the DO highest concentrations were measured during the rainy and windy seasons, probably due to the movement of the water masses caused by the rains and wind, resulting in their oxygenation (De la Lanza-Espino & Gómez-Aguirre, 1999).

The inter-seasonal dynamics of nitrogenous compounds was similar to that reported for natural surface waters (Gama-Flores et al., 2010), except for Ulumal (river) and Zoh Laguna (waterhole) that showed strong ammonium contamination. Nitrite concentrations at the sampling sites exceeded the maximum permissible limit for waters for human use and consumption (DOF, 2022) of 1 mg/l during at least one climatic season. It should be noted that the settlements, such as Flores Magón, Valeriana and Zoh Laguna, have water used for human consumption by the surrounding population. In Remate, Hampolol, Zapote and Ulumal, the main use of water is recreational. Nitrites in natural waters are the result of the biodegradation of nitrates and ammoniacal nitrogen among other nitrogenous compounds and are indicative of fecal contamination in natural water bodies (Pacheco-Ávila & Cabrera-Sansores, 2003).

Nitrate high concentration is an important indicator of poor water quality. Its origin in natural waters is largely from the use of fertilizers in crop fields, which causes its significant increase in the receiving water bodies (Cabrera et al., 2003). For this reason, measurement of nitrate concentration is very important for the state of Campeche, which has had a notable increase in agricultural activity in recent years (Rendón-von Osten and Hinojosa, 2017). It could affect the sources of water supply for human consumption.

Phosphate concentrations during the rainy and windy seasons exceeded the maximum permissible limits (0.1 mg/l; DOF, 2022) for waters for human use and consumption. In addition, in Remate, Hampolol, Ulumal, Zoh Laguna and Flores Magón they exceeded the maximum permissible limit (5 mg/l) according to the water quality criteria for the protection of aquatic life (DOF, 2022) during the rainy and windy seasons. High phosphate concentrations could be due to possible leaching caused by rain and the significant discharge of anthropogenic (domestic) origin in nearby sites.

In particular, these bodies of water are ecosystems vulnerable to eutrophication because they act as receptors and destination points for pollutants generated and released into the environment from human activities (Pacheco et al., 2014).

Silicate concentrations of inland waters generally show a high variability that can range from 0.1 to 4,000 mg/l. However, in this study, a limited variability was observed, with a minimum of 3.40 mg/l and a maximum value of 81.30 mg/l (Table 3). These results are largely subject to the abiotic characteristics of the environment, such as temperature, salinity, and pH (Fuentes & Massol-Deyá, 2002). The above may be because silicates,



such as quartz, feldspar, mica, amphibole, pyroxene, olivine, and a great variety of clay minerals, are very abundant in the Earth's crust and come from the weathering and metamorphism of rocks (Rodríguez-Vega et al., 2015).

### *Biological Characterization*

Cyanobacteria were the most abundant group and were present at all sampling sites and during all climatic seasons studied (Fig. 1B). The highest cell abundances were observed in the Flores Magón and Zoh Laguna. These sites are considered a source of fresh water supply for human consumption. Cyanobacteria are considered the most common phytoplankton organisms associated with the eutrophication of freshwater systems (Vasconcelos, 2006). As a possible consequence, some volatile organic compounds, such as geosmin and methyl-isoborneol, are produced depending on the species of cyanobacteria; these have a direct impact on the organoleptic characteristics of the water (Pérez et al., 2008; Pérez-Morales et al., 2016).

This diversity pattern of diatoms is similar to that reported by López-Adrián & Herrera-Silveira (1994), Schmitter-Soto et al. (2001), and Sánchez et al. (2002), although the proportions differed. However, variability in diatom abundance may be due to nitrogen eutrophication, which impacts the amount of dissolved silicon (Krause et al., 2023), as can be observed in this study.

Our records of Euglenophyta are similar to those reported by Ayala-Castañares (1963), López-Ochoterena and Madrazo-Garibay (1990), and Muciño-Márquez et al. (2014): they contributed 10 % of the phytoplankton abundance in the Pom-Atasta Lagoon System (a part of a natural protected area of Laguna de Términos) and 8 % in Palizada, both in the state of Campeche.

## Conclusions

Based on quantitative water-bottle samples taken during three climatic seasons (dry, rainy, and windy) in 2019-2020 at seven freshwater bodies in the state of Campeche, changes in 12 physicochemical variables and proportions between major phytoplankton groups in terms of cell abundance were followed. In general, cyanobacteria and diatoms predominated; dinoflagellates and nanoflagellates, as well as chlorophytes and euglenophytes, were much less abundant. For all major phytoplankton groups studied, no significant differences were observed between climatic seasons. In general, wider ranges in the values of the physicochemical characteristics were recorded in the windy season (January). In addition, Ulumal (river) and Zoh Laguna (waterhole) showed strong ammonium contamination. The phosphate concentrations (related to anthropogenic eutrophication) in Remate, Hampolol, Ulumal, Zoh Laguna and Flores Magón exceeded the maximum permissible limit for both the protection of aquatic life and for human use and consumption in the rainy and windy seasons.

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## Authors' Contributions

Conceptualization, JAGF, CAPD, JRvO and YBO; data curation, JAGF, CAPD, JRvO and YBO; formal analysis, JAGF, CAPD, JRvO and YBO; funding acquisition JRvO and CAPD; investigation, JAGF, CAPD, JRvO and YBO; methodology, JAGF, CAPD, JRvO and YBO; resources, JAGF, CAPD, JRvO and YBO; software, JAGF, CAPD, JRvO and YBO; visualization, CAPD; writing—original draft, JAGF, CAPD, JRvO and YBO; writing—review and editing, JAGF, CAPD, JRvO and YBO. All authors have read and agreed to the published version of the manuscript.

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# On the Relevance of Monitoring the Thermal Structure, Community Metabolism and Phytoplankton Ecology of Inland Waters of Mexico in the Context of Global Change

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## Abstract

The relevance of aquatic bodies on global biogeochemical cycles has been recently highlighted. Thus, limnological research is increasingly needed to improve the models of ecosystems functioning. We also need to face Climate and Global Change. As the information on tropical systems is still scarce, tropical limnology is providing interesting elements to understand ecosystem's responses to environmental change. Particularly relevant are long-term monitoring efforts. Here we revise recent research on tropical limnology of aquatic ecosystems of Mexico that contribute to understand emergent and complex challenges in a panorama of water crisis, particularly, the rise of temperature and increased eutrophication. Our analyses showed long-term, interdisciplinary monitoring of ecosystems of the Mexican highlands are providing novel findings that contribute to global research questions in areas



like: community metabolism and carbon dioxide fluxes, physical forcing, water-level fluctuations, internal processes that affect vertical nutrient exchange, biodiversity, phytoplankton ecology, eutrophication progressions, plankton interactions, and phytoplankton blooms. This research contributes to the understanding of eutrophication and oligotrophication under warming scenarios, as well as aims to provide elements to mitigate and adapt to the increasing drought, and water crisis in Mexican territory.

## Keywords

Tropical limnology, global change, thermal stratification, eutrophication, net heterotrophy, long-term monitoring.

## Introduction

In recent decades, the linkage of aquatic systems functioning and Climate Change has been increasingly studied (IPCC, 2001). For example, it has been determined that changes in vertical stratification and the rise (upwelling) of water in different regions are related to warming of the surface layer. Baseline studies to understand these processes included research showing a strong positive relationship between the temperature of surface water and that of the surrounding air, at regional scales (e.g., Livingstone, 2003; O'Reilly et al., 2003). Aside from exploring the largest spatial scale possible, the access to time-series monitoring studies has been critical. For example, the study conducted by Boyce et al. (2010) shows a trend, in the last century, of decreasing chlorophyll coverage. Their results indicate this trend is related to the increase in surface layer temperature. They concluded that these trends may be linked to changes in vertical stratification and water upwelling, mainly because of elongation (or increase in depth) of surface layers, and limitation in nutrient supply from deep layers, as has been delineated by other studies, see Robertson et al. (1990), Falkowski et al. (1998), Behrenfeld et al. (2006), and Martínez et al. (2009).

Epicontinental aquatic systems have been useful models to observe the impact of global warming on the thermal characteristics of water bodies since epilimnetic temperature experiences a direct and rapid response to climatic conditions (Lee et al., 2012). Temperature also shapes the density gradient in the water column, therefore, if climatic conditions change, epilimnetic warming would induce changes in the intensity and duration of stratification, thermal stability, and the depth of the thermocline (McCormick and Fahnenstiel, 1999; Livingstone, 2003; Coats et al., 2006). In fact, lakes have recently been tagged as “sentinels” of Global Climate Change because they are sensitive to climate, respond quickly to change, and integrate information about changes in their watersheds (Pham et al., 2008; Williamson et al., 2008; Adrian et al., 2009). Examples of these observable changes are evapotranspiration, infiltration, water retention by soils, surface

and subsurface runoff, and underground recharge. The aquatic communities also experience short-term responses in this context, some examples include prokaryoplankton, phytoplankton, and zooplankton compositional changes related to turbulence, water-level fluctuations or nutrient availability, that frequently are related to some species blooms (e.g. Ramírez-García et al., 2002; Ramos-Higuera et al., 2008; Jiménez-Contreras et al., 2009; Ramos-Olvera et al., 2009; Valdespino-Castillo et al., 2014, 2017; Valeriano-Riveros et al., 2014; Nandini et al., 2019). Yet, much research is needed to understand the water bodies internal processes and their implications in ecosystem's functioning.

The increase in temperature is related to processes that have important ecological effects such as: the decrease in oxygen saturation due to the increase in temperature, or the alteration in the frequency of extreme wind/weather events (Mooij et al., 2005; Blenckner et al., 2007). Indirectly, temperature is also related to changes in trophic structure (Gyllström et al., 2005; Beklioglu et al., 2007; Jeppesen et al., 2007; Meerhoff et al., 2007), and complex interactions between temperature, nutrient recycling, and physical forces (Jeppesen et al., 2009; Jones et al., 2010; Merino-Ibarra et al., 2021) that are often determined by the conditions of each ecosystem. Moreover, temperature increase may determine the net metabolism of water bodies, for example by increasing respiration and carbon exportation to the atmosphere (Guimaraes et al., 2019).

There is convincing evidence that much of the variation in ecological and biogeochemical functioning of natural waters is related to changes in climate (Livingstone and Dokulil, 2001; Blenckner et al., 2007; Jones et al., 2010). As the studies are very limited in tropical inland waters, Lake Tanganyika, East Africa, is one of the most studied tropical systems. Its fisheries have crucial economic importance for the region. In a very paradigmatic study, O'Reilly et al. (2003) studied the sediments of the lake and concluded climate warming is diminishing productivity in Lake Tanganyika. By analysing C isotope records ( $\delta^{13}\text{C}$ ) in the sediments, their results show that primary productivity in Lake Tanganyika, may have decreased by around 20 % (from the 1950's to date), implying a decrease of approximately 30 % of the fishing load (O'Reilly et al., 2003).

The problem of scale is an additional challenge to study the effects of Climate Change on natural waters. In their study, Boyce et al. (2010) recognize that climate variability at a regional scale can induce variations on the large observed trends. Currently, different models are used to explore system responses to Climate Change in different regions (e.g., Peeters et al., 2002; Bell et al., 2006; Komatsu et al., 2006), and yet the variation associated with applying the models to different types of lakes receives little attention (Jones et al., 2010).

In this context of environmental change, we will briefly explore the global warming scenarios for Mexico. We will focus on the populated Central Highlands to frame the sce-

narios of change of the aquatic systems on this area in the context of Climate Change. This area concentrates recent limnological efforts in Mexico.

### *Mexico, Global Change and Inland Waters*

The Mexican territory is currently experiencing intense transformation related to human activities such as industry, commerce, minery, tourism, cattle raising, and agriculture. In this chapter, we do not intend to perform a general diagnosis on anthropogenic activities; instead, we shall briefly summarize the general scenarios of change in the panorama of Climate Change. Additionally, some authors have stated that in general, the regional impacts of global Climate Change on the functions and services of aquatic ecosystems may be even greater than the impacts of local anthropogenic activities (O'Reilly et al., 2003).

Climate Change models for the tropics foresee increases in temperature and evapotranspiration, conditions that generally translate into droughts of different magnitudes. Actually, the most likely general scenario for much of the Mexican territory is this (Hernández-Cerda & Valdez-Madero, 2004), as well as changes in the regime and magnitude of runoff, soil moisture, and evaporation (Mendoza et al., 2004). Drought could limit nutrient export to aquatic systems through altered weathering. And, although considerably smaller in magnitude, atmospheric transport of nutrients like P could increase (see Paytan & McLaughlin, 2007). The presence of extreme events could also alter the seasonality of nutrient export. Under a somewhat different approach, the model of Malmaeus et al. (2006) considers that mixing, mineralization, diffusion, and bio-absorption are temperature dependent. Their work highlights that lakes whose water has longer residence times will be more sensitive to increased temperatures.

Even under certain limitations, most atmospheric general circulation models based on Mexican territory, project different percentage decreases of precipitation in Mexico. For example, in the regionalized precipitation panorama for the period 2070-2099, a drier condition is expected, mainly in the Baja California Peninsula and in northwest and central Mexico (in terms of the percentage of average annual accumulated rainfall [see Martínez & Fernández, 2004]).

The 2023 drought monitor of INEGI (Mexican National Institute of Statistics, Geography, and Informatics [[www.inegi.org.mx](http://www.inegi.org.mx)]) integrates a time-series collection of climatic information of the Mexican territory. By comparing drought indicators through space and time, users of this platform may learn about the recent high-water vulnerability across the Central and Northern regions of the country. The drought indications depict heterogeneity in time, with a non-periodical distribution (Fig. 1; Conagua, 2023). Statistically, the States with the highest drought index are Durango, Chihuahua, and Coahuila. In the second order, Nuevo León, Zacatecas, San Luis Potosí, Aguascalientes, and Guanajuato, located in

the Central Highlands. The central region of Mexico depicts the highest population density and experiences relevant pressure over agriculture soils, rivers, reservoirs, and aquifers.

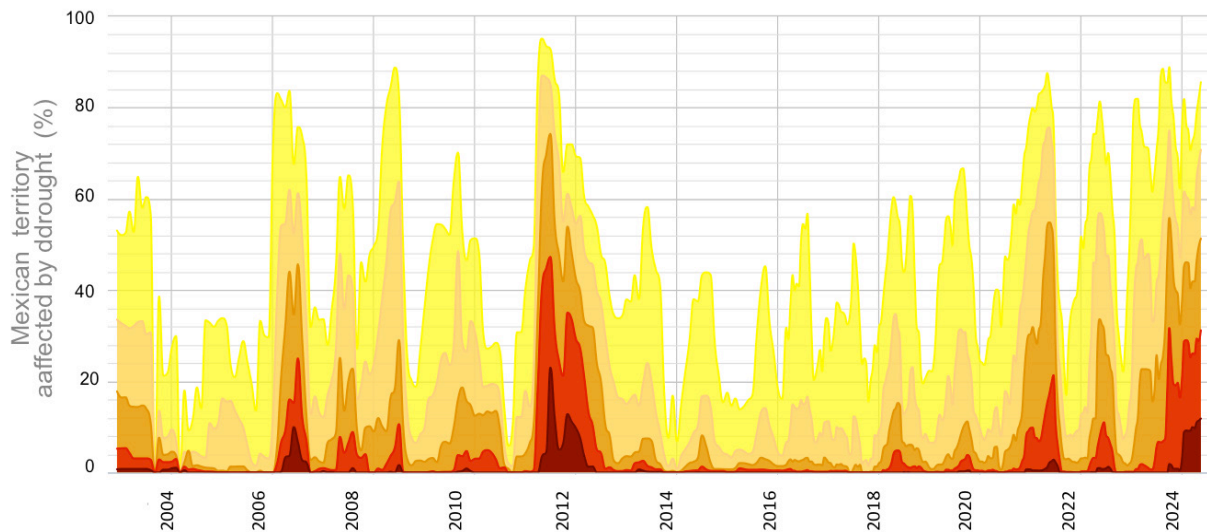


**Figure 1.** Percentage of Mexican Territory Affected by Drought through Time. Darker Colors (Towards Red and Brown) Depict the Highest Drought Intensity. Source: Modified from Monitor de Sequía en México, Conagua.

*Emerging Lessons Learned from Long-term Monitoring in Mexican Inland Waters. Case of study: Community Metabolism Monitoring in Valle de Bravo Reservoir*

Long-term monitoring of freshwater in Mexico includes only a few study cases, such as the more-than-two-decades interdisciplinary monitoring of Lake Alchichica (a soda, crater lake), and interdisciplinary monitoring of the Amanalco-Valle de Bravo Basin. The Valle de Bravo reservoir is also subject of remote sensing monitoring coupled with field work sampling (Arias-Rodriguez et al., 2020). As relevant research efforts comprehend the limnology of Lake Chapala (e.g., Moncayo-Estrada et al., 2012), and those of the Pátzcuaro lake (Ramírez-Herrejón et al., 2014), Zimapán reservoir (Bravo-Inclan et al., 2008), and Laja rivers (e.g., Mercado-Silva et al., 2006) in previous decades, long-term research efforts are still scarce for Mexican inland waters. Next, we will describe Valle de Bravo with more detail, as the first case of study.

Valle de Bravo (VB) is the last vase of the Cutzamala System, a system that interconnects seven reservoirs and a treatment plant and supplies close to 25 % of the freshwater supply to the superpopulated Central Mexican Highlands (including Mexico City and Toluca, see e.g., Ramírez-Zierold et al., 2010). A long-term monitoring of VB and its tributaries has taken place in the last two decades, by an academia-society partnership (see *Nuestro lago*, science divulgation series [Merino-Ibarra & Monroy-Ríos, 2004]). VB



behaves as a warm monomictic water body that has experienced drastic changes in water level in the last decade due to its management (fluctuations up to 10 m). Extraction and injection of water between the reservoirs of the Cutzamala system respond to urban water requirements. These water level fluctuations (WLF) affect tourism and all user's activities, as well as ecological structure and are related to drought conditions. For example, WLF have been related to changes in the plankton composition (Ramírez-García et al., 2002; Valdespino-Castillo et al., 2014; Valeriano-Riveros et al., 2014; Nandini et al., 2019), and to community metabolism (Valdespino-Castillo et al., 2014; Guimaraes et al., 2019), and biogeochemical N and P cycling (Barjau et al., 2022). In summary, in the warm season of the year (typically stratified during the warmest part of the year, from March to October), mixing events have occurred; these convey exchange of water (and nutrients) between the mixed surface layer and deeper layers, affecting reservoir's biota.

The long-term monitoring effort in the last two decades has contributed to the knowledge of the effects of Climate Change over tropical waters. Recent studies show that these effects are related to: a) community metabolism and carbon emission to the atmosphere (Guimaraes et al., 2019), and b) related to internal functioning, particularly to vertical heat and nutrient flux (Merino-Ibarra et al., 2021).

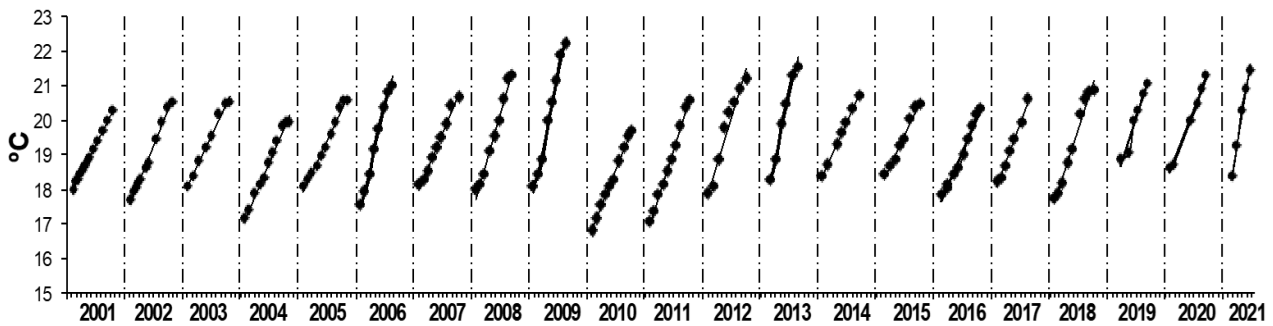
When VB water level is low, the frequency of the mixing increases due to boundary mixing, related to the geomorphology of the reservoir and the daily-wind regime of VB. Thus, whole-column or partial mixing may occur even in months when stratification typically occurs (e.g., Merino et al., 2003; Valdespino-Castillo et al., 2014). The mixing events promote the oxidation of organic matter in this eutrophic to hypertrophic system. By studying the photosynthesis:respiration ratio at ecosystem scale, Guimaraes et al. (2019) detected that, over a decade of observations, net heterotrophy prevailed. Their results show that increase in the reservoirs' temperature was directly, and significantly related to ecosystem respiration. The estimation of carbon released to the atmosphere corresponded, in average, to  $3.29 \text{ g C m}^{-2} \text{ d}^{-1}$ . This value lies within the range of carbon released to the atmosphere by aquatic systems, considering the global average. Other conclusions of this work highlight the need of long-term monitoring in tropical systems, in order to generate better models on the role of continental waters on global carbon cycling.

### *Vertical Nutrient Exchange in a Warming Scenario*

Nutrient concentration in surface waters is the result of allochthonous inputs, as well as of internal processes. While as nutrient runoff has been measured for decades in a large number of limnological studies, the processes governing nutrient inputs through vertical mixing remain obscure for the most part. This knowledge gap is a relevant handicap to fully understand the biogeochemistry and ecology of large-scale inland and ocean waters.

Recent research in VB has been useful to study these processes in a warming scenario (Merino-Ibarra et al., 2021). These authors studied heat budgets to assess vertical nutrient transport within the reservoir for more than two decades. They revealed that internal waves can cause vertical transport of heat and nutrients without breaking the stratification. Their results show that as a result of mixing, the hypolimnetic temperature increases (in the range of 0.009-0.028°C day<sup>-1</sup>). By estimating a water and nutrient balance, the authors built a physical-biogeochemical model to estimate vertical exchange between the epilimnion and the hypolimnion. The results of these balances during stratification show progressive hypolimnetic warming (Fig. 2a), and also that vertical exchange (and nutrient recycling) can increase up to fivefold when VB water level drops over 12 m in depth (Fig. 2b). Altogether, these efforts provide novel strategies to understand and to model eutrophication and oligotrophication in natural waters in a warming scenario.

a)



b)

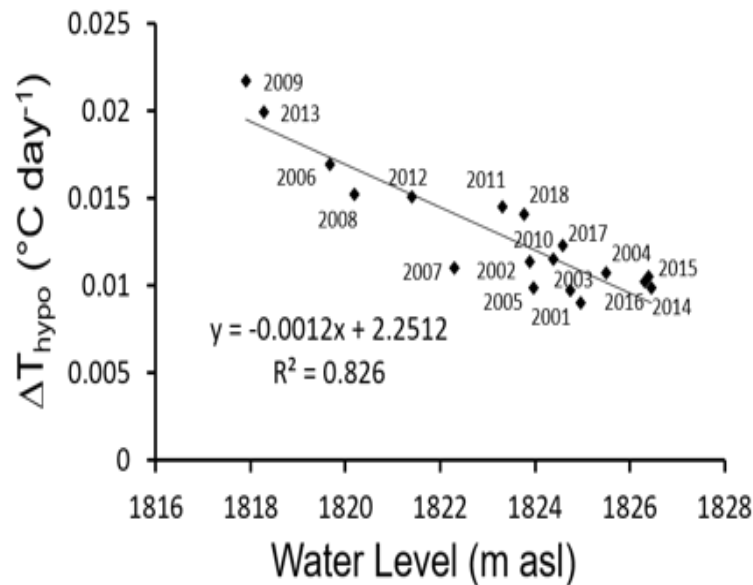


**Figure 2.** Hypolimnetic Water Warming and Water Level Relationship in VB: a) Hypolimnetic Temperature Variation during Each Stratification Period Sampled from 2001 to 2021 [The Slopes of the Regressions Were Used to Assess the Mean Hypolimnetic Warming Rate for Each of the Stratifications], and b) Mean Hypolimnetic Temperature Increase Rate During Each Annual Stratification Period Plotted Against the Mean Water Level Throughout 2001-2018. Modified from Merino-Ibarra et al., 2021.

*Case of study: Lakes of the Mexican Central Highlands, Perspectives in a Panorama of Climate Change*

As meteorological data has provided evidence of a recent regional warming trend (e.g., since 2000 *sensu* Caballero et al., 2016; or during 1966 to 2018 *sensu* Alcocer et al., 2022), paleolimnological data and phytoplankton studies have evidenced the ecological changes in different lakes of the Mexican highlands.

In the Alberca de Tacambaro lake, sediments and phytoplankton (2008 to 2011) were studied to investigate the effects of “El Niño” Southern Oscillation (ENSO). The results shown by Caballero et al. (2016), revealed changes in the hydrological cycle of the lake. A longer circulation period originated a relatively longer period of deoxygenation of the water column due to oxydation of a high organic matter load during warmer conditions. In consequence, this low-oxygen mixing period was significantly correlated to lower diatom diversity. Their results agree with the impacts of extreme climatic conditions associated to large-scale climatic oscillations like ENSO or the Pacific Decadal Oscillation (e.g., Wachnicka et al., 2013). Sediment changes were also correlated with the ENSO oscillation in the paleolimnological study reported by Ortega et al. (2021). Further evidence of changes in the phytoplankton composition related to Climate Change has also been reported in Lake Alchichica.

Lake Alchichica represents one of the few tropical lakes where long-term monitoring has occurred in Mexican territory. The results of this research have enlightened our understanding of protozooplankton, phytoplankton, zooplankton, hydrology, and biogeochemistry (e.g., Alcocer et al., 2000, 2007; Oliva et al., 2001, 2009; Ramos-Higuera et al., 2008; Ramos-Olvera et al., 2009). Alchichica is the largest of the Axalapascos lakes in the Cuenca Central, in the Mexican Highlands. This area is exposed to intense industrial and agricultural development. In a recent analysis of two decades of observations, Alcocer et al. (2020) showed the lake’s water temperature rose from 1993 to 2003 and remained similar until 2013. Phytoplankton time series analyses also showed that as the temperature increased, large-size phytoplankton (represented by the diatom *Cyclotella alchichicana*) abundance and biomass increased. In contrast, small-size phytoplankton as well as adult copepods decreased. The author concluded increase in temperature could have favoured the large-size over the small-size phytoplankton. And also affected zooplankton, negatively affecting calanoid copepods physiology and diminish the copepods’ food resource because large size phytoplankton was favored.

In the lakes of the Cuenca Oriental, Mexican highlands, planktonic dynamics and in particular, modifications in the annual pattern of blooms have been observed. Particularly critical are Lake Alchichica’s blooms, related to the cyanobacteria *Nodularia affine spumigena* (e.g., Macek et al., 2009). The occurrence of these blooms in Alchichica has been related to the increase in surface temperature and nutrient availability (Oliva et al., 2009) which

has effects on community structure and dynamics (Macek et al., 1994, 2009). The idea that Lake Alchichica may be sensitive to climatic fluctuations through changes in its mixing-stratification regime was explored in sedimentary cores by Caballero et al. (2003), however the favoring of diatom silica dissolution limits the potential for paleolimnological studies.

Being located in a region that may become hotter and drier, rapid responses to changes in climate are very likely to occur in lakes of the Mexican highlands. Under warming conditions of the Central Highlands of Mexico, conditions such as increase in epilimnion temperature, changes in thickness of the mixed layer, greater intensity and duration of stratification, and effects on vertical water exchange which may occur in a warming scenario, leading to oligotrophication should be studied. Moreover, diverse studies show that many aquatic systems have responded similarly to warming climate, regardless of their dimensions (see Adrian et al., 2009, and Williamson et al., 2009).

The wind regime appears to be an important source of supplementary energy (*sensu* Margalef, 1983) to aquatic systems. Therefore, if regional wind pattern is modified, the dynamics of biogeochemical processes, in particular nutrient recycling, could change significantly. It has been shown that the cold fronts (called “Nortes” in Mexico) may influence the dynamics of the mixed layer of the Gulf of Mexico (Villanueva et al., 2010) and can promote the vertical exchange of water to supply the nutrients that feed the production of the layer mixed. Its effect on the Mexican epicontinental bodies is still a subject of exploration, as are the possible changes in its frequency or intensity in Climate change scenarios (see CCA, 2011). If the frequency and intensity of these events increase, the stability of the stratification could be affected in water bodies, and the vertical exchange of water as well as the input of nutrients to the surface could be promoted. In this case, the effect of these meteorological phenomena could act against those that accentuate stratification (increased air temperature and greater drought).

### *Increasing Eutrophication in Mexican Inland Waters*

Limnological work has been reporting a global trend of increasing eutrophication and phytoplankton blooms (e.g., Pearl & Huisman 2008).

The information of Mexican ecosystems regarding eutrophication and phytoplankton blooms is heterogeneous and disperse. An useful effort to gather limnological experiences in the country took place in 2014, when more than 300 studies participated in the National Congress of Limnology, held in Mexico City. The presented studies focused on freshwater systems, such as lakes, rivers, reservoirs, wetlands, and groundwater. The abstract Conference book shows numerous studies that document signs of eutrophication in Mexican waters.

However, a comprehensive survey of nutrients concentration and eutrophication indicators in inland waters of Central Mexico is still needed. The map on Figure 3 summarizes



the concentration of total phosphorus (TP) in aquatic systems of Central Mexico. In this exploration (Neri-Guzmán, in press), 51 studies of water systems of Central Mexico were considered. When data were grouped into different categories, 72.55 % of the samples were in the range  $>3 \mu\text{M}$  of TP (Fig. 3), which corresponds to eutrophy and hypereutrophy (*sensu* Carlson and Simpson, 1996).

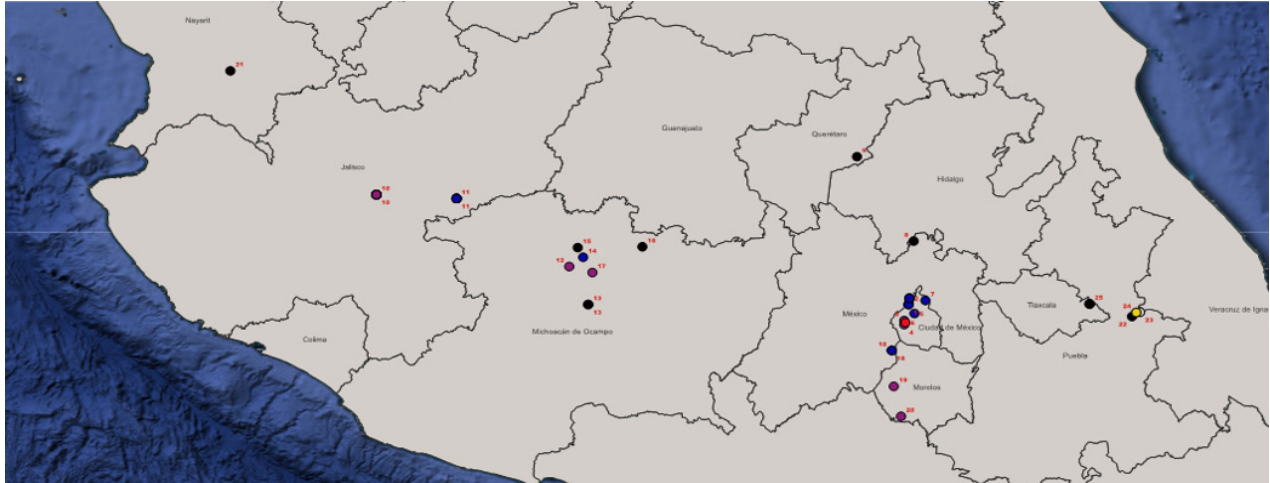


**Figure 3.** Total Phosphorus ( $\mu\text{M}$ ) in Water Systems of Central Mexico.  
Dark Blue Sample Color Indicates TP Concentration  $>3 \mu\text{M}$ .

**Table 1.** Limnological Parameters Summarized per State in Central Mexico  
(Modified from Neri-Guzmán, in Press).

| Mexican State                  | SD           | Chl-a                 | TP                    | TN           | BOD5        | CDO        | TSS           |
|--------------------------------|--------------|-----------------------|-----------------------|--------------|-------------|------------|---------------|
|                                | (m)          | (mg m <sup>-3</sup> ) | (mg L <sup>-1</sup> ) |              |             |            |               |
| Mexico City                    | 0.11 - 0.53  | 81 - 1320             | 0.37 - 10.65          | 1.27 - 46.69 | 9 - 102     | 169.4      | 8 - 140       |
| Mexico City - Estado de México | 0.32 - 0.63  | 54.9 - 6.1            | 8.03 - 28.61          | 1.11 - 4.53  | NA          | NA         | NA            |
| Estado de México               | 0.30 - 3.92  | 1.12 - 29.2           | 0.0002 - 0.32         | NA           | 2           | 14         | NA            |
| Hidalgo                        | 1.44 - 2     | 6.43 - 57.9           | 0.07 - 13.59          | 2.18 - 31.9  | 1.6 - 20.34 | 10.7 - 107 | 43.5 - 353.33 |
| Jalisco                        | NA           | NA                    | 0.4 - 2.31            | NA           | 1.9 - 25    | 2.18 - 76  | 59.5          |
| Jalisco - Michoacán            | NA           | NA                    | 0.62                  | 1.34         | 6.36        | 46.92      | NA            |
| Michoacán                      | 0.15 - 1.3   | 2.2 - 120             | 0.029 - 2             | 2.2          | 5.82 - 229  | 28.3 - 748 | 53.68         |
| Morelos                        | 0.09 - 5.20  | 0.04 - >100           | 0.003 - 5.5           | NA           | 30.02       | 88.94      | NA            |
| Puebla                         | NA           | 0.2 - 2.2             | 0.02 - 0.05           | NA           | NA          | NA         | NA            |
| Puebla -Tlaxcala               | Nula - Total | NA                    | NA                    | NA           | NA          | NA         | NA            |
| Tlaxcala                       | 0.08 - 0.2   | NA                    | NA                    | NA           | NA          | NA         | NA            |
| Overall                        | 0.08 - 5.20  | 0.04 - >1000          | 0.0002 - 28.61        | 1.11 - 46.69 | 1.6 - 229   | 2.18 - 748 | 8 - 353       |

SD (Secchi Disk Depth), Chl-a (chlorophyll a), TP (Total Phosphorus), TN (Total Nitrogen), BOD (Biologiccal Oxygen Demand), CDO (Chemical Oxygen Demand), NA (Not Available), and TSS (Total Suspended Solids).

In the exploration of water bodies of Central Mexico, Neri-Guzmán (in press) summarized the parameters considered to calculate the trophic state index (*sensu* Carlson and Simpson, 1996), per state. The integrated conditions indicate that most of the studied water bodies have reached conditions of eutrophy and hypertrophy (Table 1 and Table 2).

**Table 2.** Indication of Trophic State (*Sensu* Carlson and Simpson, 1996), Shown per State in Central Mexico (Modified from Neri-Guzmán, in Press).

| Mexican State                  | TSI SD        | TSI Chl-a     | TSI TP          | Trophic State               |
|--------------------------------|---------------|---------------|-----------------|-----------------------------|
| Mexico City                    | 69.15 - 91.80 | 73.70 - 101   | 89.48 - 137.96  | Eutrophic - Hypertrophic    |
| Mexico City - Estado de México | 66.65 - 76.42 | 69.90 - 70.10 | 133.89 - 152.22 | Eutrophic - Hypertrophic    |
| Estado de México               | 40.31 - 77.35 | 31.71 - 63.70 | 0 - 87.38       | Mesotrophic - Hypertrophic  |
| Hidalgo                        | 50 - 54.74    | 48.85 - 70.41 | 65.45 - 141.48  | Mesotrophic - Hypertrophic  |
| Jalisco                        | NA            | NA            | 90.60 - 115.91  | Hypertrophic                |
| Jalisco - Michoacán            | NA            | NA            | 96.93           | Hypertrophic                |
| Michoacán                      | 56.22 - 87.33 | 38.33 - 77.56 | 52.74 - 113.83  | Mesotrophic - Hypertrophic  |
| Morelos                        | 36.24 - 94.70 | 0 - >75.77    | 20 - 128.42     | Oligotrophic - Hypertrophic |
| Puebla                         | NA            | NA            | 47.37 - 60.60   | Mesotrophic - Hypertrophic  |
| Puebla - Tlaxcala              | NA            | NA            | NA              | NA                          |
| Tlaxcala                       | 83.20 - 96.40 | NA            | NA              | Hypertrophic                |
| Overall                        | 36.24 - 96.40 | 0 - 101       | 0 - 152.22      | Mesotrophic - Hypertrophic  |

TSI (Trophic State Index), SD (Secchi Disk Depth), Chl-a (Chlorophyll a), TP (Total Phosphorus), and NA (Not Available).

In the abstract collection of the 2014 National Congress of Limnology of Mexico, thesis and published studies, the study of cyanobacteria and other planktonic microbes that form massive blooms was frequent. Some examples of events of massive blooms listed on the book include: Valle de Bravo Reservoir, lakes of Central Mexico such as Zirahuén, Atexcac, Alchichica, Lake Texcoco, and Lake Chapultepec, as well as the coastal systems Pom-Atasta and Palizada del Este, in the Gulf of Mexico littoral.

One of the main challenges for Mexican freshwater ecosystems is related to the growth and toxicity of cyanobacteria. Among other phytoplankton species, cyanobacteria are the main biological agents related to blooms, and their development and ecological success is reported to benefit from warmer temperature (Pearl and Huisman, 2008). Recent studies on tropical limnology are contributing to the global understanding on the diversity, physiology,

and ecology of cyanobacteria and lake's microbiota. Some of these studies reveal a remarkable diversity in our territory, that includes in most cases, uncharacterized species (e.g., Becerra et al., 2020; Carmona et al., 2023). The toxic potential of some freshwater cyanobacteria and their impacts on different trophic levels has been also addressed, for example by Vasconcelos et al. (2010), Mendoza et al. (2012), Alillo-Sánchez et al. (2014), Pineda et al. (2019), and Ávila-Torres et al. (2023). Deep characterizations, such as genomic or metabolomic, of bloom forming plankters are still pending, in order to better understand the metabolic and ecological particularities of these organisms.

## Conclusions

In this mini review, we summarize some emerging findings of tropical limnology, focused on the studies of aquatic systems of Mexico that contribute to the global understanding of the effects of Climate Change on aquatic systems. Long-term, interdisciplinary monitoring of ecosystems of the Mexican highlands are providing integrated approaches of community metabolism and carbon dioxide fluxes, physical forcing, water-level fluctuations, internal processes that affect vertical nutrient exchange, biodiversity, phytoplankton ecology, eutrophication progressions, plankton interactions, phytoplankton blooms, and paleolimnological reconstruction, among others. Altogether, this research aims to contribute to the understanding of eutrophication and oligotrophication under warming scenarios. Increased drought risk, and water crisis in the Mexican territory highlight the relevance of interdisciplinary monitoring in inland waters of Mexico.

We also conclude that biogeochemical and chemical insights, as well as ecological studies based on long-time monitoring, are crucial to better understand ecosystems functions and responses. With this knowledge we will have more efficient toolboxes to mitigate and adapt to the environmental change we are facing.

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## Authors' Contributions

PMVC, MMI and JARZ conceptualization, first draft writing and editing. RJA, MBA and OAGN data integration and editing. JPCC and MANG compilation, data visualization and editing.



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# Middle-term Hydrological and Microalgal Study in the Lower Basin of the Tuxpan River, Veracruz, Mexico

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## Abstract

With the aim of analyzing the middle-term variation of hydrological factors and the composition, biomass, and richness of microalgae in the lower basin of the Tuxpan River during the “nortes” season in the region, two sampling surveys were performed in 2018 and 2023, obtaining *in situ* data (temperature and salinity) and surface samples for analysis of nutrients, Chl-a and microalgae species. In 2018, when high temperatures in a mostly freshwater environment and low nutrient concentrations were recorded, a greater variety of microalgae groups were present (5 clades). In 2023, lower temperature was recorded and a greater influence of seawater farther away from the river mouth was related to a higher diatom species richness, but just two clades of microalgae were recorded. Inter-seasonal variation was identified in the middle-term (cold front season), both in hydrological variables and in the composition, richness, and biomass of microalgae in the Tuxpan River. Saline intrusion, as an effect of the seawater wedge in the lower river basin, could promote changes in the composition of microalgae in the region.

## Keywords

Cold fronts season, diatoms, phytoplankton, river ecology, salt wedge.



## Introduction

Rivers are dynamic systems that transport freshwater from high elevations to lower regions, playing a crucial role in the water cycle by supplying this vital resource to different ecosystems and human activities (Angelier, 2019). The lower basin is especially relevant due to the accumulation of sediments and inorganic nutrients from upstream sources. Additionally, interactions with seawater induce alterations in river salinity (Perales-Valdivia et al., 2018; Angelier, 2019). Moreover, atmospheric fluctuations, including changes in air temperature, precipitation patterns, and wind intensity can impact temperature variability within river systems (Perales-Valdivia et al., 2018).

Microalgae, a polyphyletic group of microscopic primary producers, are particularly responsive to these hydrological changes and constitute the basis of the aquatic trophic networks (Reynolds, 2006). This community comprises species from diverse taxonomic groups, including diatoms, dinoflagellates, cyanobacteria, and chlorophytes. The occurrence of the different species under specific hydrological conditions has positioned microalgae as potential indicator organisms linked to environmental variation (Lemley et al., 2016; Wu et al., 2017). Hence, it is established that diatoms exhibit a preference for high-energy and well-oxygenated habitats, dinoflagellates, and cyanobacteria proliferate in response to increased inorganic nutrients levels, while some chlorophytes serve as indicators of high concentrations of reduced forms of nitrogen (Glibert, 2016). Moreover, among these groups, some species are specific to freshwater or marine environments, intermediate communities inhabiting brackish waters and all primary producers with chlorophyll-a (Chl-a) serving as an indicator of microalgae biomass.

On an annual scale, the variation of hydrological factors and microalgae in the rivers responds to the region's seasonality. In the Gulf of Mexico, this variability is influenced by the environmental conditions of the dry, rainy, and cold front seasons (cold mass air coming from the north of America) (de Velasco & Winant, 1996). However, it remains a notable scarcity of studies specifically examining seasonal variations across different years, which serve as proxies for middle- and long-term fluctuations. Specifically, the need for hydrological and microalgal information is evident in the northern part of Veracruz, compared to studies of the central coastal zone. Veracruz stands out among the Gulf of Mexico states, since about 30 % of the national runoff occurs within its boundaries (Pérez-Maqueo et al., 2011).

On the eastern coast of Mexico, the Tuxpan River discharges into the Gulf of Mexico, situated within the state of Veracruz. Its mouth is situated near the city of Tuxpan and the Tampamachoco Lagoon (INEGI, 2016). The presence of diatoms, dinoflagellates, and phytoflagellates has been recorded both within the Tuxpan River and at its mouth (Orduña-Medrano,

2012; Vázquez et al., 2021; Treviño-Butron, 2023). Influence from seawater has been recorded extending upstream as a product of the salt wedge (Contreras, 1983; Treviño-Butron, 2023). Studies have focused on species identification within annual cycles or climatic seasons, but to date, no study makes temporal comparisons in the middle or long term.

Due to the development of several projects carried out in 2018 and 2023 in the lower Tuxpan River basin, there are hydrological data and phytoplankton information that provide a suitable opportunity to explore the variation of these factors on a longer time scale. Therefore, the objective of this study was to analyze the middle-term variation of hydrological factors and the composition, biomass, and richness of microalgae in the lower basin of the Tuxpan River during the “*nortes*” season in the region.

## Material and Methods

### *Study Area*

The Tuxpan River is in the north of Veracruz. It's part of the hydrological region No. 27 Tuxpan-Nautla. The climate is hot and humid, with summer rains and winter rainfall of less than 5 %. There are rainy (June-September) cold fronts (known as “*nortes*”, October-March), and a dry season (April-May) with wide interannual variability (de Velasco and Winant, 1996). The lower Tuxpan River Basin consists of a coastal plain that varies from 100 m above sea level to the drop-in sea level at the mouth of the river. Economic activities are predominantly agriculture, livestock, and small-scale fishing, although natural gas and oil are also extracted (INEGI, 2016). The most important urban centers are the city of Tuxpan and Álamo Temapache; the community of Dr. Montes de Oca is in the latter municipality. Riparian vegetation has been replaced by cultivated pastures and crop fields, except for the Tuxpan mangroves belonging to RAMSAR site 1602 (Basañez-Muñoz, 2005).

### *Sampling and Analysis*

Two sampling surveys were conducted, the first from February 6 to 9, 2018, and the second on February 20, 2023, both during the “*nortes*” season of the region. In each of the surveys, four zones (A-D) were delimited along the lower Tuxpan River Basin considering the detection of the salt wedge, and in each zone a sampling site was established (Fig. 1). Zone A (depth 3.6 m) with high freshwater influence was in the community of Dr. Montes de Oca; zone B (depth 8.2 m) of brackish water with greater freshwater influence was in the urban area of the city of Tuxpan; zone C (depth 3.3 m) of brackish water with greater marine influence was in a less densely populated area; and finally, zone D (depth 15.2) with high marine influence was located at the mouth of the Tuxpan River.

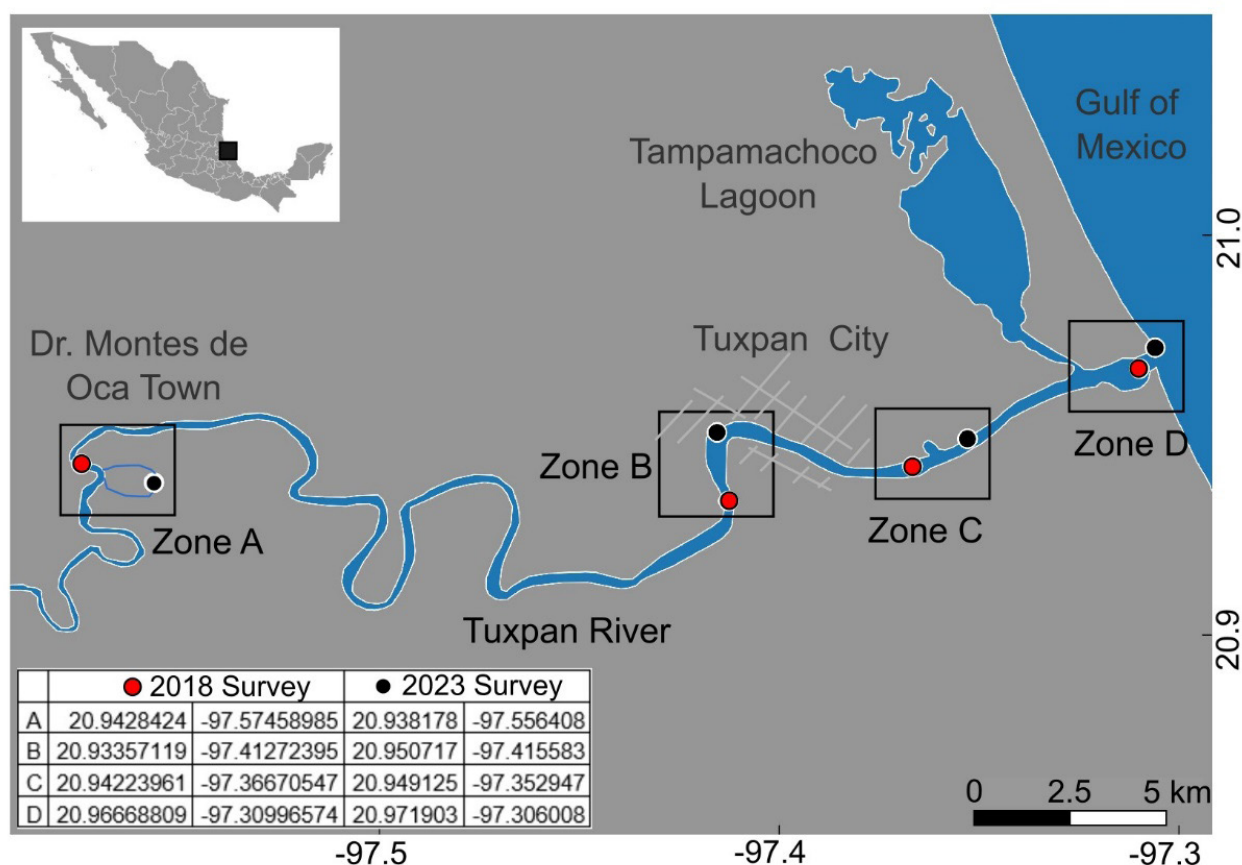


**Figure 1.** Map of the Lower Tuxpan River Basin Showing Study Zones (A-D) and Sampling Sites during the Surveys of 2018 and 2023.

At each sampling site, a single surface temperature and salinity measurement were determined *in situ*. In the 2018 survey, a single set of vertical temperature and salinity profiles was obtained at each site with a YSI 85 multiparameter to evaluate the influence of the salt wedge in the water column. To investigate the composition of phytoplankton species, a single phytoplankton sample was collected in each site using a plankton net (20  $\mu\text{m}$ ) and fixed with 4 % formalin final volume (Thronsdon, 1978). To analyze the concentration of Chl-a and inorganic nutrients, a single 500 ml surface water sample was taken at each site and filtered with 0.45  $\mu\text{m}$  membranes, following the indications of Aminot and Rey (2000), while the filtered water was used to determine the concentration of inorganic nutrients (nitrites+nitrates and phosphates) according to the colorimetric techniques described by Strickland and Parsons (1972) and A.P.H.A. (1998). For 2018, a spectrophotometer (Thermo Genesys UV) was used, while for 2023 a photometer (YSI Ecosense 9500) was employed.

## *Data Analysis*

The species were classified following the clades proposed by Adl et al. (2019) and according to their habitat (marine, freshwater, or brackish water) according to Guiry and Guiry (2023) and Vázquez et al. (2021). The taxonomic status was based on Algaebase (Guiry & Guiry, 2023). Two-way cluster analysis was employed to assess the similarity in species composition among the sampling sites for the two distinct sampling years using the Jaccard index; species with only one record were excluded. The analysis was executed using PCORD version 6.08 (McCune & Grace, 2002). To explore the relation between hydrological variables and species richness, Spearman correlation tests were performed; a U-Mann-Whitney test was performed to determine significant differences between the 2 surveys for each measured parameter including richness (Zar, 2010).

## Results

### *Hydrology*

Considering only the vertical profiles recorded in 2018, thermohaline variation was observed in the water column of the different sampling sites. At site A-2018, temperature (24.7°C) and salinity (0 ups) were recorded with very similar values along the 3 m of the water column, with a slight increase in salinity (1.1) at the bottom (Fig. 2A). At site B-2018, a surface temperature of 25°C was recorded, which at 2.5 m dropped to 20°C and remained so until the bottom, while salinity presented a pattern in reverse, with a surface salinity of 1.8 that at 2 m had already increased to 20 and at 4 m reached a salinity of 30 (Fig. 2B). At site C-2018, a similar pattern was identified, although the depth recorded was only 3 m (Fig. 2C). Site D-2018 presented a warm freshwater surface layer where in the first few centimeters of depth a decrease in temperature and salinity of 35.6 to 2.5 m was recorded (Fig. 2D).



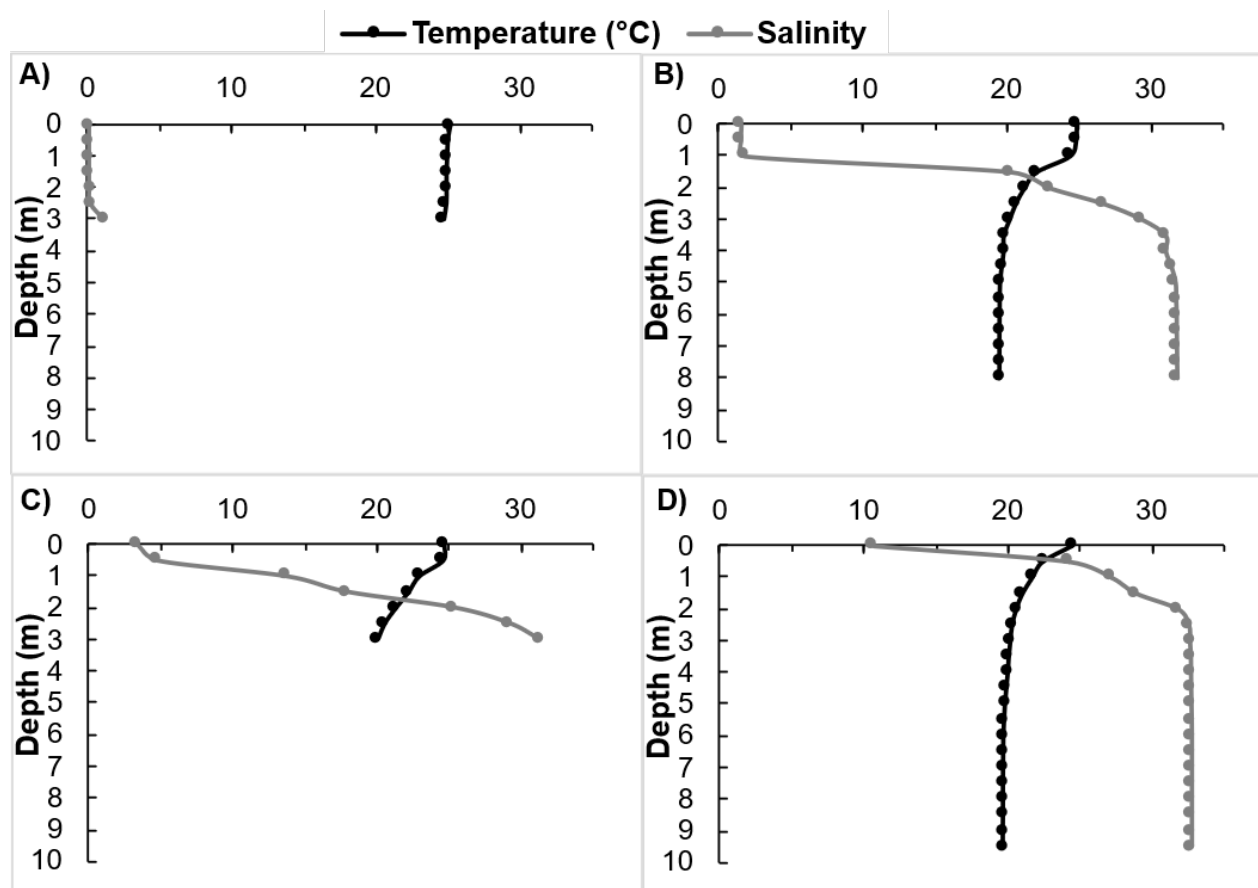


**Figure 2.** Vertical Profiles of Temperature and Salinity in the Four Zones Established in the Tuxpan River during the 2018 Survey: A) Zone A Far Away from the Mouth, B) Zone B Tuxpan City, C) No Urban Zone, D) Mouth of the River.

The water surface temperature rank recorded in 2018 (24.4-25.1°C) was higher than in 2023 (21-23°C;  $Z=2.31$ ,  $p=0.02$ ) (Fig. 3A). In contrast, higher salinity values were recorded in 2023 (7-38 ups) compared to 2018 (1-10.6 ups) ( $Z=-2.02$ ,  $p=0.04$ ) in both surveys the pattern of variation was similar, as a positive salinity gradient was identified from the river mouth to upstream (Fig. 3B). Inorganic nutrient concentration was recorded with higher values in 2023 than in 2018 for nitrates and phosphates ( $Z=-2.02$ ,  $p=0.04$ ;  $Z=-2.30$ ,  $p=0.02$ , respectively), and a positive gradient to the river mouth was identified in both samplings: nitrite+nitrate concentration in 2018 ranged from 8.39 to 82  $\mu\text{m}$ , while 2023 fluctuated between 153.1 and 660.9  $\mu\text{m}$  (Fig. 3C) ( $Z=-2.30$ ,  $p=0.02$ ). Phosphate concentration in 2018 ranged from 0.7 to 3.5  $\mu\text{m}$  and in 2023 from 36.8 to 1,068.8  $\mu\text{m}$ , the latter value was recorded at site A-2018; except for this record, phosphate concentration in both surveys presented a positive gradient to the river mouth (Fig. 3D).

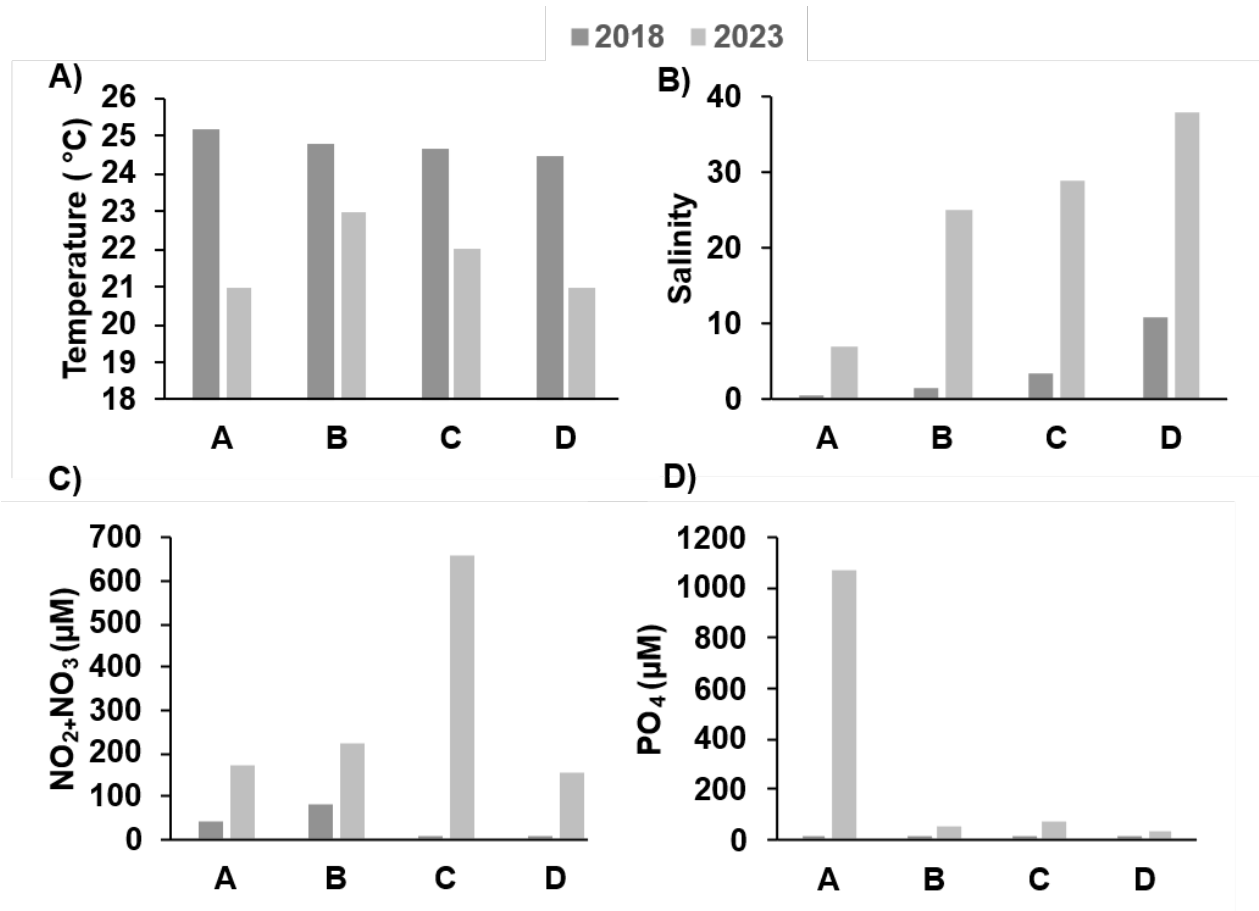


**Figure 3.** Surface Values of A) Temperature, B) Salinity and Concentration of C) Nitrites+Nitrates and D) Phosphates Recorded in the Four Zones Established in the Tuxpan River during the 2018 and 2023 Surveys.

### *Microalgae, Chl-a, and Relation with Hydrological Factors*

In both surveys, 188 microalgae species were recorded (Table 1): 151 were diatoms (Streptophyta, the most prevalent clade in the study), 17 were chlorophytes (Chlorophyta), 14 were dinoflagellates (Alveolata), 4 were euglenophytes (Excavata), and 2 were cyanobacteria (Eubacteria) (Table 2). In the 2018 survey, 73 taxa belonging to five clades were observed, three of which had their highest values in zone A (Chlorophyta, Eubacteria and Excavata), although in this zone, the Alveolata clade presented the lowest richness. The high species richness of the Streptophyta (17 to 23) was very similar in the sampling sites. In 2023, 129 species from only two clades (Alveolata and Streptophyta) were recorded. Diatoms and dinoflagellates showed a trend of lower species richness from zone A to zone D; in case of dinoflagellates, their presence was not recorded in zone A (Table 2). Mean richness by year was significantly different ( $Z=-2.02$ ,  $p=0.04$ ), but not richness in marine ( $Z=-1.15$ ,  $p=0.24$ ), freshwater ( $Z=1.87$ ,  $p=0.06$ ) or brackish water species ( $Z=-1.15$ ,  $p=0.24$ ).

**Table 1.** List of Microalgae Taxa Recorded in the Sampling Zones (A, B, C, and D) of the 2018 and 2023 Surveys. 1=Present, 0=Absent. Code is in Reference to Fig. 5.

| Species                                    | Code    | A-2018 | B-2018 | C-2018 | D-2018 | A-2023 | B-2023 | C-2023 | D-2023 |
|--------------------------------------------|---------|--------|--------|--------|--------|--------|--------|--------|--------|
| <b>Clade Alveolata</b>                     |         |        |        |        |        |        |        |        |        |
| <i>Dinophysis caudata</i>                  |         | 1      | 0      | 0      | 0      | 0      | 0      | 0      | 0      |
| <i>Gonyaulax</i> sp. (cysts)               |         | 0      | 1      | 0      | 0      | 0      | 0      | 0      | 0      |
| <i>Gonyaulax</i> spp.                      | Gon spp | 0      | 1      | 1      | 1      | 0      | 1      | 0      | 0      |
| <i>Peridinium quadridentatum</i>           |         | 0      | 0      | 1      | 0      | 0      | 0      | 0      | 0      |
| <i>Peridinium</i> sp. 1                    |         | 0      | 0      | 0      | 0      | 0      | 1      | 0      | 0      |
| <i>Prorocentrum micans</i>                 | Pro mic | 0      | 1      | 1      | 1      | 0      | 0      | 0      | 0      |
| <i>Protoperdinium</i> cf. <i>thorianum</i> |         | 0      | 1      | 0      | 0      | 0      | 0      | 0      | 0      |
| <i>Protoperdinium</i> sp. 2                |         | 0      | 0      | 1      | 0      | 0      | 0      | 0      | 0      |
| <i>Protoperdinium</i> spp.                 | Pro spp | 1      | 1      | 1      | 1      | 0      | 1      | 0      | 1      |
| <i>Tripos furca</i>                        | Tri fur | 0      | 1      | 1      | 1      | 0      | 0      | 0      | 0      |
| <i>Tripos fusus</i>                        | Tri fus | 0      | 0      | 0      | 0      | 0      | 1      | 1      | 0      |
| <i>Tripos hircus</i>                       | Tri hir | 0      | 0      | 0      | 0      | 0      | 1      | 1      | 0      |
| <i>Tripos macroceros</i>                   |         | 0      | 0      | 0      | 1      | 0      | 0      | 0      | 0      |
| <i>Tripos muelleri</i>                     |         | 0      | 0      | 0      | 0      | 0      | 1      | 0      | 0      |
| <b>Clade Chloroplastida</b>                |         |        |        |        |        |        |        |        |        |
| <i>Closterium moniliferum</i>              |         | 1      | 0      | 0      | 0      | 0      | 0      | 0      | 0      |
| <i>Coenococcus</i> sp.                     |         | 0      | 1      | 0      | 0      | 0      | 0      | 0      | 0      |
| <i>Cosmarium obtusatum</i>                 | Cos obt | 1      | 1      | 0      | 0      | 0      | 0      | 0      | 0      |
| <i>Cosmarium</i> sp. 2                     |         | 1      | 0      | 0      | 0      | 0      | 0      | 0      | 0      |
| <i>Desmodesmus communis</i>                | Des com | 1      | 1      | 0      | 0      | 0      | 0      | 0      | 0      |
| <i>Desmodesmus subspicatus</i>             |         | 0      | 1      | 0      | 0      | 0      | 0      | 0      | 0      |
| <i>Eudorina</i> sp.                        | Eud sp  | 1      | 1      | 0      | 0      | 0      | 0      | 0      | 0      |
| <i>Pediastrum</i> sp. 1                    |         | 0      | 1      | 0      | 0      | 0      | 0      | 0      | 0      |
| <i>Pediastrum</i> sp. 3                    |         | 1      | 0      | 0      | 0      | 0      | 0      | 0      | 0      |
| <i>Pseudopediastrum boryanum</i>           | Pse bor | 1      | 1      | 1      | 0      | 0      | 0      | 0      | 0      |
| <i>Scenedesmus ecornis</i>                 |         | 0      | 1      | 0      | 0      | 0      | 0      | 0      | 0      |
| <i>Scenedesmus</i> sp. 1                   | Sce sp1 | 1      | 1      | 0      | 0      | 0      | 0      | 0      | 0      |
| <i>Scenedesmus</i> sp. 4                   |         | 1      | 0      | 0      | 0      | 0      | 0      | 0      | 0      |
| <i>Selenastrum</i> sp. 1                   | Sel sp1 | 1      | 1      | 0      | 0      | 0      | 0      | 0      | 0      |
| <i>Sphaerocystis</i> sp. 1                 |         | 1      | 0      | 0      | 0      | 0      | 0      | 0      | 0      |
| <i>Stauridium tetras</i>                   | Sta tet | 0      | 1      | 1      | 0      | 0      | 0      | 0      | 0      |
| <i>Tetradasmus dimorphus</i>               |         | 1      | 0      | 0      | 0      | 0      | 0      | 0      | 0      |
| <b>Clade Eubacteria</b>                    |         |        |        |        |        |        |        |        |        |
| <i>Merismopedia elegans</i>                |         | 1      | 0      | 0      | 0      | 0      | 0      | 0      | 0      |
| <i>Oscillatoria tenuis</i>                 |         | 1      | 0      | 0      | 0      | 0      | 0      | 0      | 0      |

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| <b>Clade Excavata</b>                               |         |   |   |   |   |   |   |   |   |
|-----------------------------------------------------|---------|---|---|---|---|---|---|---|---|
| <i>Euglena</i> sp. 1                                |         | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Euglena</i> sp. 2                                |         | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Phacus gigas</i>                                 |         | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Phacus pleuronectes</i>                          | Pha ple | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 |
| <b>Clade Stremenopile</b>                           |         |   |   |   |   |   |   |   |   |
| <i>Achnanthes brevipes</i>                          |         | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| <i>Achnanthes</i> cf. <i>armillaris</i>             |         | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| <i>Achnanthes</i> sp.                               |         | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Achnanthes</i> sp. 1                             |         | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| <i>Achnanthes</i> sp. 2                             | Ach sp2 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 |
| <i>Achnanthes</i> sp. 3                             |         | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| <i>Actinocyclus octonarius</i>                      | Act oct | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| <i>Actinocyclus octonarius</i> var. <i>tenellus</i> |         | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| <i>Actinoptychus aster</i>                          |         | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| <i>Actinoptychus senarius</i>                       |         | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| <i>Amphipleura lindheimerii</i>                     |         | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| <i>Amphiprora</i> cf. <i>kariana</i>                |         | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| <i>Amphora</i> cf. <i>proteus</i>                   | Amp pro | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 |
| <i>Amphora robusta</i>                              |         | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| <i>Asterionellopsis glacialis</i>                   |         | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| <i>Aulacodiscus</i> sp. 1                           |         | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| <i>Aulacoseira ambigua</i>                          |         | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Bacillaria</i> cf. <i>socialis</i>               | Bac soc | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| <i>Bacillaria paxillifera</i>                       | Bac pax | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 |
| <i>Campylodiscus simulans</i>                       |         | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| <i>Cerataulus smithii</i>                           |         | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| <i>Cocconeis</i> cf. <i>scutellum</i>               |         | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Cocconeis lineata</i>                            |         | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 0 |
| <i>Cocconeis placentula</i>                         |         | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| <i>Cocconeis scutellum</i>                          |         | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| <i>Cocconeis</i> sp. 1                              |         | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| <i>Coscinodiscus asteromphalus</i>                  | Cos ast | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 |
| <i>Coscinodiscus centralis</i>                      | Cos cen | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 |
| <i>Coscinodiscus concinnus</i>                      |         | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| <i>Coscinodiscus granii</i>                         | Cos gra | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 |
| <i>Coscinodiscus perforatus</i>                     | Cos per | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| <i>Coscinodiscus radiatus</i>                       |         | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| <i>Coscinodiscus</i> sp. 1                          | Cos sp1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| <i>Coscinodiscus wailesii</i>                       | Cos wai | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| <i>Cyclotella</i> cf. <i>operculata</i>             |         | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| <i>Cyclotella littoralis</i>                        | Cyc lit | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 |

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|                                                |          |   |   |   |   |   |   |   |   |
|------------------------------------------------|----------|---|---|---|---|---|---|---|---|
| <i>Cyclotella</i> sp. 1                        |          | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| <i>Cyclotella striata</i>                      | Cyc str  | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 |
| <i>Cyclotella stylorum</i>                     |          | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| <i>Cylindrotheca closterium</i>                | Cyl clo  | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 |
| <i>Cymbella tumidula</i>                       |          | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| <i>Cymbella turgidula</i>                      | Cym tur  | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| <i>Delicatophycus</i> cf. <i>delicatulus</i>   | Del del  | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 |
| <i>Denticula subtilis</i>                      |          | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| <i>Dinobryon</i> sp. 1                         |          | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Diploneis bombus</i>                        | Dip bom  | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| <i>Diploneis</i> cf. <i>fusca</i>              |          | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| <i>Diploneis</i> cf. <i>oblongella</i>         | Dip obl  | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 |
| <i>Diploneis crabro</i>                        |          | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| <i>Diploneis gruendleri</i>                    |          | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| <i>Diploneis incurvata</i> var. <i>dubia</i>   | Dip incu | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| <i>Diploneis litoralis</i>                     |          | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Diploneis pseudobombiformis</i>             |          | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| <i>Diploneis smithii</i>                       |          | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| <i>Diploneis</i> sp. 1                         |          | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| <i>Diploneis vacillans</i>                     |          | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| <i>Entomoneis alata</i>                        |          | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| <i>Entomoneis gigantea</i> var. <i>sulcata</i> | Ent giga | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 |
| <i>Eunotogramma</i> cf. <i>laevis</i>          |          | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| <i>Fragilaria capucina</i>                     | Fra cap  | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Gomphonema gracile</i>                      |          | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| <i>Grammatophora</i> sp. 1                     |          | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| <i>Gyrosigma balticum</i>                      | Gyr bal  | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 0 |
| <i>Gyrosigma</i> sp. 1                         | Gyr sp1  | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 |
| <i>Gyrosigma</i> sp. 2                         |          | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| <i>Halamphora</i> cf. <i>latecostata</i>       | Hal lat  | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 |
| <i>Halamphora</i> cf. <i>normanii</i>          |          | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| <i>Hantzschia amphioxys</i>                    |          | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| <i>Hobaniella longicruris</i>                  | Hob lon  | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 |
| <i>Leptocylindrus</i> sp.                      |          | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Licmophora paradoxa</i>                     |          | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| <i>Lyrella hennedyi</i>                        |          | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| <i>Mastogloia lanceolata</i>                   |          | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| <i>Mastogloia smithii</i>                      |          | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| <i>Mastogloia</i> sp. 1                        |          | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| <i>Melosira nummuloides</i>                    |          | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| <i>Melosira</i> sp.                            |          | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Navicula</i> cf. <i>cryptocephala</i>       | Nav cry  | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 |
| <i>Navicula</i> cf. <i>gregaria</i>            | Nav greg | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 |

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|                                             |          |   |   |   |   |   |   |   |   |
|---------------------------------------------|----------|---|---|---|---|---|---|---|---|
| <i>Navicula directa</i>                     | Nav dir  | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 |
| <i>Navicula platyventris</i>                | Nav pla  | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 |
| <i>Navicula recens</i>                      | Nav rec  | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 |
| <i>Navicula rhynchotella</i>                | Nav rhy  | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 |
| <i>Navicula</i> sp. 2                       |          | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| <i>Navicula</i> sp. 3                       | Nav sp3  | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 |
| <i>Navicula</i> spp.                        | Nav spp  | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 |
| <i>Nitzschia brevissima</i>                 | Nit bre  | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 |
| <i>Nitzschia</i> cf. <i>fluminensis</i>     | Nit flu  | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 |
| <i>Nitzschia</i> cf. <i>bicapitata</i>      |          | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| <i>Nitzschia</i> cf. <i>ovalis</i>          | Nit ova  | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 |
| <i>Nitzschia</i> cf. <i>tubicola</i>        |          | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| <i>Nitzschia</i> cf. <i>valdecostata</i>    |          | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| <i>Nitzschia kurzii</i>                     |          | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| <i>Nitzschia semirobusta</i>                |          | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| <i>Nitzschia sigma</i>                      | Nit sig  | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 0 |
| <i>Nitzschia sigmoidea</i>                  | Nit sgm  | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 |
| <i>Nitzschia</i> sp. 1                      |          | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| <i>Nitzschia</i> sp. 10                     | Nit sp10 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| <i>Nitzschia</i> sp. 2                      |          | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| <i>Nitzschia</i> sp. 3                      |          | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| <i>Nitzschia</i> sp. 4                      |          | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| <i>Nitzschia</i> sp. 5                      |          | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| <i>Nitzschia</i> sp. 6                      |          | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| <i>Nitzschia</i> sp. 7                      |          | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| <i>Nitzschia</i> sp. 8                      | Nit sp8  | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 |
| <i>Nitzschia</i> sp. 9                      |          | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| <i>Odontella aurita</i>                     | Odo aur  | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 |
| <i>Paralia sulcata</i>                      |          | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| <i>Petrodictyon gemma</i>                   | Pet gem  | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 0 |
| <i>Pinnularia acrosphaeria</i>              |          | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| <i>Pleurosigma angulatum</i>                |          | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| <i>Pleurosigma</i> cf. <i>delicatulum</i>   |          | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| <i>Pleurosigma latum</i>                    | Ple lat  | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| <i>Pleurosigma normanii</i>                 | Ple nor  | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 |
| <i>Pleurosira laevis</i>                    |          | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| <i>Psammodictyon</i> cf. <i>constrictum</i> |          | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| <i>Pseudauliscus oculatus</i>               |          | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 |
| <i>Pseudodictyocha dubia</i>                |          | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| <i>Rhabdonema</i> sp. 1                     |          | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 |
| <i>Rhaphoneis amphicerus</i>                |          | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| <i>Rhizosolenia setigera</i>                | Rhi set  | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 |
| <i>Rhoicosphenia abbreviata</i>             |          | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| <i>Rhopalodia</i> sp. 1                     | Rha sp1  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| <i>Shionodiscus oestrupii</i>               |          | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| <i>Sieminskia zeta</i>                      |          | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| <i>Skeletonema costatum</i>                 | Ske cos  | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 0 |

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|                                          |         |   |   |   |   |   |   |   |   |
|------------------------------------------|---------|---|---|---|---|---|---|---|---|
| <i>Stephanocyclus meneghinianus</i>      | Ste men | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 |
| <i>Surirella linearis</i>                | Sur lin | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Surirella recedens</i>                |         | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| <i>Surirella robusta</i>                 | Sur rob | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 |
| <i>Surirella</i> spp.                    |         | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| <i>Surirella striatula</i>               | Sur str | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 1 |
| <i>Synedrosphenia crystallina</i>        |         | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| <i>Thalassionema nitzschioides</i>       | Tha nit | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 1 |
| <i>Thalassiosira eccentrica</i>          | Tha ecc | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 |
| <i>Thalassiosira</i> sp.                 | Tha sp  | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| <i>Thalassiosira</i> sp. 1               | Tha sp1 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 |
| <i>Thalassiosira</i> sp. 2               |         | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| <i>Thalassiosira</i> sp. 3               |         | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| <i>Triceratium</i> sp. 1                 |         | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| <i>Trieres mobiliensis</i>               | Tri mob | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| <i>Tryblionella acuminata</i>            |         | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| <i>Tryblionella apiculata</i>            |         | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| <i>Tryblionella</i> cf. <i>victoriae</i> |         | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| <i>Tryblionella coarctata</i>            |         | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| <i>Tryblionella granulata</i>            | Try gra | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| <i>Ulnaria acus</i>                      | Uln acu | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 |
| <i>Ulnaria gouldardii</i>                |         | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| <i>Ulnaria sinensis</i>                  | Uln sin | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 |
| <i>Ulnaria</i> sp. 1                     |         | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| <i>Ulnaria ulna</i>                      | Uln uln | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |

**Table 2.** Species Richness per Taxonomic Clade in Both Surveys (2018 and 2023) and in the Zones of Each Survey (A-D).

| Clade          | 2018-2023 | 2018 Survey |    |    |    |    | 2023 Survey |    |    |    |    |
|----------------|-----------|-------------|----|----|----|----|-------------|----|----|----|----|
|                | Total     | Total       | A  | B  | C  | D  | Total       | A  | B  | C  | D  |
| Alveolata      | 14        | 10          | 2  | 6  | 6  | 5  | 6           | 0  | 6  | 2  | 1  |
| Chloroplastida | 17        | 17          | 12 | 11 | 2  | 0  | 0           | 0  | 0  | 0  | 0  |
| Eubacteria     | 2         | 2           | 2  | 0  | 0  | 0  | 0           | 0  | 0  | 0  | 0  |
| Excavata       | 4         | 4           | 4  | 1  | 1  | 1  | 0           | 0  | 0  | 0  | 0  |
| Stremenopile   | 151       | 40          | 19 | 17 | 23 | 22 | 123         | 58 | 43 | 46 | 37 |

Species richness in both surveys showed a negative gradient from zone A to D. In all zones, the richness recorded in 2023 was higher than in 2018 (Fig. 4A). In 2018, there was an increasing trend observed in Chl-a concentration ( $2.4\text{--}20.9\text{ mg m}^{-3}$ ) toward the river mouth. Additionally, Chl-a concentrations were higher in sites B, C, and D compared to the corresponding zones than in the 2023 survey; station A was the exception, because Chl-a was higher in 2023. In 2023, Chl-a showed a short variation among the four zones ( $4.7\text{--}5.2\text{ mg m}^{-3}$ ) (Fig.

4B). Not significant difference between mean survey years was detected ( $Z=1.15$ ,  $p=0.24$ ).

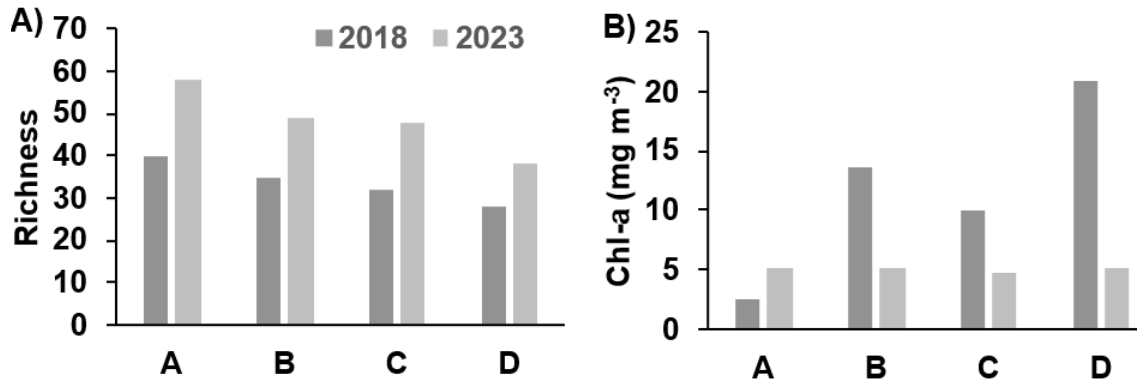


**Figure 4.** A) Microalgae Species Richness and B) Surface Chl-a Concentration Recorded in the Four Zones Established in the Tuxpan River during the 2018 and 2023 Surveys.

The two-way cluster analysis reveals substantial differences in species composition between the two study years and along the entire river (Fig. 5). In 2018, out of 38 species, 17 were freshwater, 16 were marine, and 5 were brackish. Notably, freshwater species like *Cosmarium obtusatum* (Schmidle) Schmidle, *Desmodesmus communis* (E.Hegewald) E.Hegewald, *Eudorina* sp., *Scenedesmus* sp. 1, *Selenastrum* sp. 1, and *Surirella linearis* W.Smith were exclusively observed in sites A and B. Additional species were recorded consistently across the entire river, including *Navicula* cf. *gregaria* Donkin, *Cylindrotheca closterium* (Ehrenberg) Reimann & J.C.Lewin, *Phacus pleuronectes* (O.F.Müller) Nitzsch ex Dujardin, *Ulnaria sinensis* B.Liu & D.M.Williams, *Surirella robusta* Ehrenberg, *Protoperdinium* spp., *Surirella striatula* Turpin, and *Ulnaria acus* (Kützinger) Aboal.

In contrast, the 2023 data involving 55 species indicates that 30 were marine, 15 were brackish, and 10 were freshwater. In the upper reaches (sites A and B), brackish species such as *Navicula* cf. *cryptocephala* Kützinger, *Navicula recens* (Lange-Bertalot) Lange-Bertalot, *Navicula rhynchotella* Lange-Bertalot, *Nitzschia* cf. *ovalis* H.J. Arnott, and *Nitzschia sigmaidea* (Nitzsch) W. Smith were predominant. Conversely, the lower reaches (sites C and D) were characterized by a prevalence of marine species, such as *Actinocyclus octonarius* var. *tenellus* (Brébisson) Hendey, *Coscinodiscus perforatus* Ehrenberg, *Diploneis bombus* (Ehrenberg) Ehrenberg, *Diploneis incurvata* var. *dubia* Hustedt, *Tryblionella granulata* (Grunow) D.G. Mann, *Pleurosigma latum* Cleve, and *Trieres mobiliensis* (Bailey) Ashworth & E.C. Theriot. *Ulnaria ulna* (Nitzsch) Compère was the only species present in all areas of both surveys (Fig. 5).

The Spearman correlation results indicated a significantly positive relation between phosphate concentration ( $\rho=0.90$ ,  $p>0.01$ ) and nitrite+nitrate concentration ( $\rho=0.83$ ,  $p>0.05$ ) with species richness. Conversely, there was a significantly negative relation between Chl-a concentration and species richness ( $\rho=0.73$ ,  $p<0.05$ ).



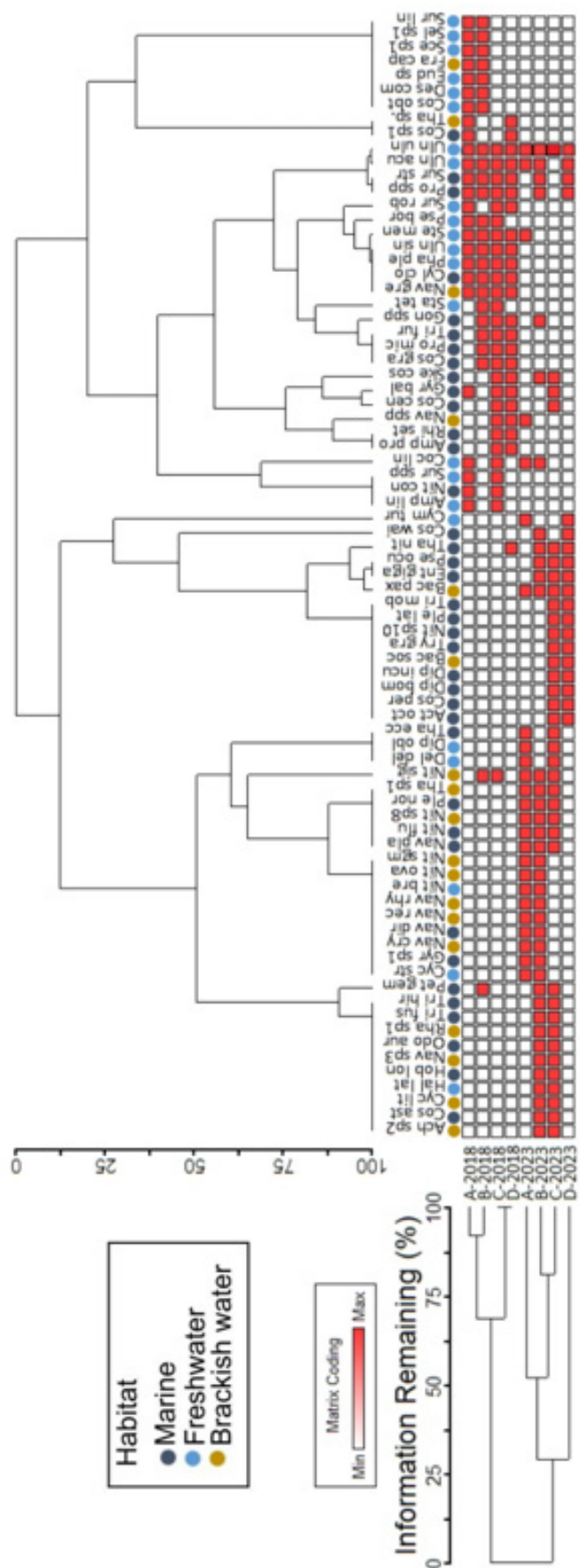


Figure 5. Two-way Cluster, Based on Jaccard Index.

## Discussion

The temperature, salinity, and Chl-a ranks recorded in both surveys (21-25.1°C, 1-38 ups, 2.4-20.9 mg m<sup>-3</sup> Chl-a) are within the ranges (21-29°C, 1-38 ups, 0.8-19.3 mg m<sup>-3</sup> Chl-a) reported by Treviño-Butron (2023) for the period from November 2022 to February 2023 in the Tuxpan River, corresponding to the cold front season. The Chl-a peak recorded in this study categorizes the Tuxpan River as a part of the group that integrates the 68 % of Mexican coastal water bodies that do not exceed a concentration of 50 mg m<sup>-3</sup>, as shown by De la Lanza and Gutiérrez Mendieta (2017). On the contrary, the concentrations of phosphates (0.7-1069 µm) and oxidized nitrogenous nutrients (NO<sub>2</sub>+NO<sub>3</sub> >100 µm) shown here, are higher than those reported by De la Lanza-Espino and Gutiérrez Mendieta (2017) for the Tuxpan River (0.4-8.6 µm NO<sub>2</sub>+NO<sub>3</sub>, 0.3-1.2 µm PO<sub>4</sub>) and even for the Tampamachoco Lagoon (0-55 µm NO<sub>2</sub>+NO<sub>3</sub>, 0-89 µm PO<sub>4</sub>). The results suggest an effect of waste products from livestock, agricultural, and fishing activities in the region, such as the tilapia farming that takes place locally in zone A, which could be related to the peak of phosphates in 2023. This nutrient increase, especially the recorded in the 2023 survey, suggests the development of the eutrophication process, although the effect on microalgae biomass was not detected.

In the 2018 survey, measurements of salinity in the water column detected marine intrusion as a wedge to ~50 km (zone A) along the basin of the river and extended the 30 km coverage that had been reported for the Tuxpan River by Contreras (1983). Salinity detected in 2023 indicates that the salt wedge influences superficial water as the values were elevated to 7 ups. This variation could be the result of the frequency and intensity of cold fronts that cause the mixing process and the sea level increase. The extension of the salt wedge plays an important role as this can separate superficial water from the bottom or could be mixed, creating a brackish water environment along the water column in some sites (Bilous et al., 2021).

Additionally, the increase in saline intrusion suggests an increase in salinization within the Tuxpan River. It has been estimated that salinization of freshwater bodies affects one out of three in the world (Gutiérrez-Cánovas et al., 2018), and according to Laghi et al. (2012), river salinization has implications for adjacent aquifers. Considering that the Tuxpan River has an important aquifer, widely used by the lower basin population, monitoring the influence of saline intrusion is suggested.

Variation in river salinity affects the composition of microalgae species, as has been reported in the La Antigua River on the central coast of Veracruz by Gaytán-Herrera et al. (2017). In 2018, the influence of the salt wedge produced two contrasting environments in the studied site: one characteristic freshwater in zone A and B and another brackish water/marine in zone C and D. The occurrence of two distinct groups indicated in the Jaccard

analysis showed typical freshwater species in the sites A and B as Chloroplastida as *Eudorina*, *Desmodesmus*, *Scenedesmus*, and diatoms as *Sellophora*, *Coscinodiscus*, and *Surirella linearis* W.Smith, while the zone C and D was characterized by the occurrence of marine species of dinoflagellates (*Prorocentrum micans* Ehrenberg, *Gonyaulax* spp., *Tripos* spp.) and diatoms (*Skeletonema costatum* (Greville) Cleve, *Rhizosolenia setigera* Brightwell). Few brackish water species were recorded this year, so this indicates a well salt wedge established without or minimal mixing through the water column.

On the contrary, in 2023, the marine intrusion was notable in zone A, with brackish water at the surface. As expected, zone B and C were brackish water, and zone D prevailed in marine conditions. So, most species in this survey period had a brackish water-marine affinity. From zone A to zone C, 12 species of 14 brackish water species occurred, while from zone C to zone D, 11 species of 24 marine species occurred with other registers in different stations. According to the literature, only six freshwater species were recorded.

The 73 and 129 species recorded in the study area in February 2018 and 2023, respectively, are well above the 12 species recorded for February 2011 and the 18 recorded for February 2012 at the mouth of the Tuxpan River by Orduña-Medrano (2012). The presence of five taxonomic groups in 2018 and only two in 2023 coincides with diversity recorded by other authors (Orduña-Medrano, 2012; Vázquez et al., 2021). The dominance of the diatoms in both surveys (55 and 95 % of species richness in 2018 and 2023, respectively, see Table 2) over microalgae richness is consistent with the information of Vázquez et al. (2021) showing that 17 of the 19 microalgae species they identified in the Tuxpan River (89 %) were diatoms. This taxonomic group has been of interest in the region. According to Treviño-Butron (2023), the abundance of the central diatoms of the Tuxpan River is greater in the brackish zone of the lower Tuxpan River basin. The month with the highest abundance is January, and there is a decrease in February.

Richness in the lower basin of the Tuxpan River is modulated by the load of nutrients more than temperature and salinity, in spite of the extensive salt wedge. However, salinity plays an important role in determining the occurrence of species with general affinities to freshwater, brackish water, or marine environments. In this study, we determined that for the *nortes* seasons, different conditions of mixing column water may vary among years but richness of phytoplankton species is determined by the loads of orthophosphate and nitrogen sources.

The only species that prevailed in both surveys were diatoms and dinoflagellates. While common exclusively by year, an euglenoid, *Phacus pleuronectes*, was present only in 2018. A diatom was common and exclusive of the survey in 2023, *Bacillaria paradoxa* J.F. Gmelin, a species considered a typical brackish water organism. The dinoflagellate *Tripos hircus* (Schröder) F.Gómez stands out, which can be considered a typical species

of brackish environments since it was not recorded in the extreme zones of both surveys (A and D); according to Guerra-Martínez and Lara-Villa (1996). This species has formed blooms in coastal lagoons of the Gulf of Mexico, so its presence indicates a potential risk of algal proliferation. The occurrence of *Ulnaria ulna* in all zones of both surveys indicates the prevalence for a long time of this diatom which although a typical freshwater species, can tolerate the influence of salt water (Vázquez et al., 2021).

Other species were representative of freshwater or marine habitats in the different years. *Stephanocyclus meneghinianus* (Kützinger) Kulikovskiy, Genkal & Kociolek was recorded in the survey of 2018 and only in zone A of 2023, so it was indicative of the freshwater environment concordant with our salinity values recorded. The marine species *Skeletonema costatum*, *Coscinodiscus centralis* Ehrenberg and *Gyrosigma balticum* (Ehrenberg) Rabenhorst in zones B and C of both surveys indicate the constant influence of the marine environment into the river. The restriction of marine species in zones C and D and the variability between the two surveys indicate the variation of richness related possibly to the final nutrient loads of the river, as our calculated correlation indicated.

It is suggested to continue with long-term evaluations to assess the possible salinization of the rivers due to a pattern of increasing influence of the salt wedge. In this study, only the cold front season was evaluated, so it is considered necessary to evaluate the intra-annual dynamics in this river. Additionally, it is suggested to include metrics associated with the abundance and diversity of microalgae due to the possible risk of algal blooms and their use as indicators of water quality.

## Conclusions

The results show that changes in the microalgal community of the lower Tuxpan River basin are associated with variations in environmental conditions in the middle-term. Largely, saline water intrusion is a factor that spatially modifies the composition, biomass, and species richness of microalgae, while nutrient concentration seems to be more related to middle-term variation. The presence of local factors, such as small-scale culture in riverine expanses, may be related to increased biomass and phosphate nutrients in the water. It is suggested to continue with the evaluation of annual variation, incorporating species density.

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## Authors' Contributions

CFRG, GV, JAAC, and ARCR collected the samples. GV, JAAC, and ARCR compiled the data. GV conducted the cluster analysis. CFRG wrote the original draft, and GV, JAAC, and ARCR contributed to writing and editing. All authors reviewed the final ms.

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# Phytoplankton from Two Dams in Central Mexico

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## Abstract

Reservoirs are environments where phytoplankton develop and sustain food webs. This study explores the structure of the phytoplankton community in two dams and its relationship with six limnological variables from El Llano and Taxhimay reservoirs during September and October 2022. Microalgae were collected in surface layer in three sampling sites with a 20 µm net mesh opening, which were preserved with formaldehyde. The specific richness, the Shannon-Wiener index, the canonical correspondence analysis (CCA), the association quotient, organic contamination, and diatom index were determined. The samples obtained during the two months were markedly different; both ecosystems recorded a warm temperature from 15 to 22°C, pH between 7-8, well oxygenation (5.8 to 9.6 mg/l), low mineralization, and conductivity. Fifty-five species were determined, and six are new records for Mexico. The abundance by taxonomic classes in all the sampling stations was markedly higher for Bacillariophyceae; in particular, observed in Taxhimay in T1 October, with 21,476 cell/ml. *Asterionella formosa* was dominant in both reservoirs, with 79.9 to 96.4 % of the abundance. The H' was recorded from 0.21 to 1.54 bits/ind., it represents variation between the sampling stations and the study months. The CCA positively related conductivity and temperature with *Aulacoseira granulata* var. *angustissima*, *A. granulata* f. *curvata*, *Monactinus simplex*, and *Microcystis panniformis*. The three indexes indicate

eutrophic environments. Furthermore, it is recommended to monitor phytoplankton, in particular, the abundance of *Microcystis* and other Cyanophyceae, as they are harmful algae.

## Keywords

Abundance, *Asterionella formosa*, *Aulacoseira granulate*, Bioindicators, Specific richness, State of Mexico.

## Introduction

In Mexico, there are more than 10,000 dams that are used for the generation of electrical energy, irrigation, flood control, food production, storage of drinking water, and ecotourism, among others (Arredondo-Figueroa & Flores-Nava, 1992; De la Lanza-Espino & García-Calderón, 2002); the study of these ecosystems is essential to know their relevance and impacts, as well as maintaining the availability of the ecosystem services they provide (Domínguez, 2019), which are essential for maintaining human society's well-being and sustaining life in general. However, despite their recognized importance, inland aquatic environments are among the most threatened ecosystems around the world (WWF, 2022). Climate change, domestic and industrial waste dumping, pollution from pesticides, invasions by exotic species, eutrophication and habitat destruction are the main threats to water bodies (Ghangrekar & Chatterjee, 2018). The diversity of species living in these ecosystems is dominated by organisms with poor swimming ability, such as plankton (Dodds & Whiles, 2010). Within this group, phytoplankton is a diverse community of microorganisms composed of different phyla, such as Cyanobacteria, Bacillariophyta, Chlorophyta, Charophyta, Chrysophyta, Dinoflagellata, Euglenophyta, among others (Wehr et al., 2015). They are important since they fix atmospheric carbon, produce oxygen, and generate biomass through photosynthesis, which is why they constitute the basis of the food webs of aquatic ecosystems (Carrasco-Vargas et al., 2014). Some phytoplankton species are used as biological indicators of water quality due to their short life cycle, and they are sensitive to changes in temperature, dissolved oxygen, pH, turbidity, conductivity, alkalinity, phosphorus, nitrogen, and silica (De Lanza-Espino & García-Calderón, 2002).

Phytoplankton as a bioindicator is a powerful tool that has been used to determine water quality where some change in conditions causes variation in the presence, absence, or abundance of some species, which provides us with information about the trophic state of the habitat where they are found (Bellinger & Sigeo, 2010). Bioindicators may have different degrees of tolerance to deterioration; some species are tolerant, while others are pretty sensitive, which helps determine conditions of contamination or eutrophication. In this way, there will be indicator species of good water quality with low levels of eutrophication and other abundant species in eutrophic environments (Nguyen, 2003). For example,

some studies have reported that *Microcystis*, *Aphanizomenon*, *Planktothrix*, *Dolichospermum*, *Cylindrospermopsis*, and *Oscillatoria* (Cyanobacteria); are indicators of eutrophic habitat. While, diatoms such as *Melosira* and *Fragilaria* have been found in waters contaminated with organic matter (Valeriano-Riveros et al., 2014), and Chlorophyta such as *Pandorina* and *Scenedesmus* are referred to contaminated with domestic sewage water (Chandel et al., 2023).

In the central area of Mexico, there are some limnological and phytoplankton analyses from the Zimapan Dam, Querétaro-Hidalgo where González-Fernández et al. (2023) indicated that phytoplankton richness suggests a eutrophic system with dominance of diatoms, Cyanophyta, and Chlorophyta with proliferations of *Microcystis aeruginosa*, *Botryococcus*, and *Stephanocyclus*. In another study, Ortega-Murillo et al. (2007) investigated the trophic state of the Mintzita Dam (Michoacán), based on the cellular concentration and distribution of phytoplankton, where they determined a mesotrophic to eutrophic condition with a dominance of *Ulnaria ulna* and *Cocconeis placentula* var. *euglypta*. In Puebla, González-Fernández et al. (2013) analyzed the composition of phytoplankton in three eutrophic reservoirs: the La Laguna, Los Reyes, and Necaxa dams, where the dominant species were *Woronichinia naegeliana*, *Microcystis wesenbergii*, *M. aeruginosa*, *Aulacoseira granulata*, *Fragilaria crotonensis*, and *Asterionella formosa*. For Morelos, Carrasco-Vargas et al. (2014) studied the phytoplankton of the eutrophic-hypereutrophic El Abrevadero reservoir with representatives of Euglenophyceae, Chlorophyceae, Cyanophyceae and Bacillariophyceae.

The State of Mexico is one of the entities where a high number of phytoplankton species has been recorded, with 668 species distributed in 14 classes, 40 orders, 81 families, and 209 genera, with greater dominance of green algae (307 species), followed by diatoms, cyanoprokaryotes, euglenids, with 262, 65 and 13 species, respectively (Garduño-Solórzano et al., 2009). For the tropical reservoir Valle de Bravo, the composition is dominated by *Cyclotella ocellata*, *Fragilaria crotonensis*, *Woronichinia naegeliana*, and *M. wesenbergii* that generate high biomass as a result of the trophic conditions of the system, in particular when the temperature and nutrient levels are high. In contrast, when the water level drops to a difference of up to 12 m, an increase in the biomass of planktic diatoms and a decrease in harmful algae have been evidenced (Valeriano-Riveros et al., 2014). Also, we know that this reservoir supplies drinking water to Mexico City through the Cutzamala system and that the cyanobacteria that develop as *Microcystis smithii*, *M. aeruginosa*, *M. viridis*, *M. flos-aquae*, *Aphanocapsa formosa*, and *Dolichospermum crassum* are species that produce microcystins with maximum values of 71 µg/l from July to September. Based on this information, the ecosystem requires constant monitoring to evaluate water quality (Martínez-Jerónimo et al., 2022). In particular, El Llano and Taxhimay dams in the State

of Mexico are sources for social development and economic activities. Razo-Paredes et al. (2016) analyzed the environmental and microbiological variables in El Llano Dam from July 2015 to May 2016, where the physical and chemical conditions favored the presence of diatoms and cyanobacteria. Additionally, Toledo-Trejo and Razo-Paredes (2018) indicated that the nutrients  $\text{SO}_4^{2-}$ ,  $\text{NO}_3^-$ , and  $\text{PO}_4^{3-}$  contributed to the growth of *M. aeruginosa* from the Taxhimay Dam. This reveals that knowledge of microalgae in these reservoirs is scanty, so this work explores the structure of the phytoplankton community in two dams (El Llano and Taxhimay) and its relationship with some limnological variables during September and October 2022.

## Materials and Methods

### *Study Area and Sampling*

El Llano Dam belongs to the physiographic province of the Trans-Mexican Volcanic Belt, and is located at the geographical coordinates 19°39'24"N 99°30'28"W (Fig. 1A), in the town of San Jerónimo Zacapexco in the municipality of Villa del Carbón, State of Mexico. It is an ecotourism park with an area of 40 thousand m<sup>2</sup>, at 2,800 m a.s.l. It is one of the primary sources of water supply for the nearby community. According to the Köppen classification, the climate is temperate-cold (Cw), located in a forested area surrounded by rivers (Toledo-Trejo & Razo-Paredes, 2018). The vegetation is mixed forest, pine forest, oak forest, and induced grassland, including cropped and reforested areas, and 73 species of vertebrates are known from the area (Moreno-Díaz, 2014).

The Taxhimay Dam is between 19°49'51"N and 99°23'46"W (Fig. 1B) in Villa del Carbón, at 2,200 m a.s.l. The water body covers an area of 365 ha, with 42 billion m<sup>3</sup>. It is characterized by being an area of important outcrops of springs that spring up in the wooded region due to the high forest cover that the Cerro de la Bufa and its foothills have; it has a high permeability caused by the predominance of igneous materials, which favor high rainwater retention (Toledo-Trejo & Razo-Paredes, 2018).



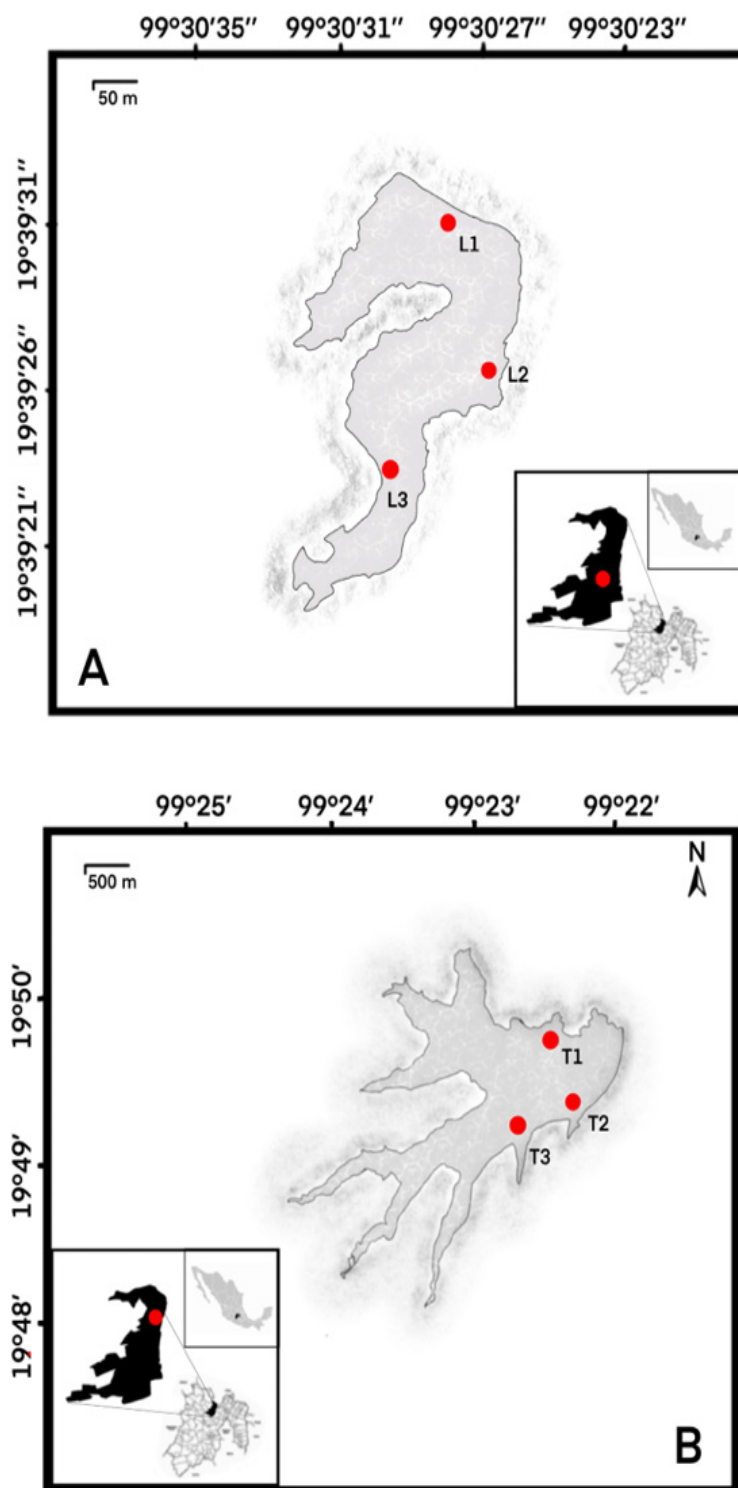


**Figure 1.** Study Area A) El Llano Dam L1, L2, L3; B) Taxhimay Dam T1, T2, T3, Red Dots Indicate the Sampling Stations.

Sampling sites were selected randomly in both dams (Table 1) in months with markedly different precipitation records; for September 2022 it was 150.5 mm and for October 2022 with 69 mm of precipitation (Conagua, 2023). 12 samples were obtained from the surface layer, where 10 l of water was filtered for each one with a net mesh size of 20  $\mu\text{m}$ . From the above, in a final volume of 50 ml, the biological material was preserved with formaldehyde at a final concentration of 4 %.

### *Limnological Variables*

A multiparametric model HANNA HI-9146 was used to record temperature, dissolved oxygen, and the percentage of dissolved oxygen saturation. The determination of total alkalinity was carried out by titration (APHA et al., 2005). In addition, the pH was measured using a Cole Parmer potentiometer, model Digi-sense, and the electrical conductivity with a Hanna equipment, model HI98312, in the surface layer of the water column.

### *Phytoplankton*

The phytoplankton was quantified by recording the volume of one ml of sample, and transferred to a preparation where it was placed on the stage under 200X and 400X in a Leica optical microscope (Semina, 1978). In addition, images were obtained with a 10 MP Rising Cam model C3CMOS 10000KPA camera. The works of Bourrelly (1981), Comas (1996), Komárek and Anagnostidis (2002), John and Tsarenko (2002), Figueroa-Torres et al. (2008), Bellinger and Sigge (2010), Wehr et al. (2015), Bicudo et al. (2016), Bicudo and Menezes (2017), Ponce-Márquez et al. (2019), and Bojorge-García and Cantoral-Uriza (2021) were used for taxonomic determination based on the classification indicated in the algae database (Guiry and Guiry, 2023).

### *Statistical Analysis*

To determine phytoplankton diversity, specific richness, and the Shannon-Wiener index, we used Magurran (2004). To categorize the phytoplankton species, an Olmstead-Tukey analysis was carried out according to Sokal and Rohlf (1981). The following criterion was applied: 1) Species with abundance and frequency values above the median were considered dominant; 2) Species with abundance values below the median, but above the median frequency were considered constant; 3) Species that showed values above the median abundance, but below the median frequency were occasional; 4) Species that recorded values below the median of both abundance and frequency were categorized as rare.

For Canonical Correspondence Analysis (CCA) we used the CANOCO 4.5 program (Ter-Braak, 1986). To normalize the data and ensure homoscedasticity, environmental variables (except pH) and phytoplankton abundances were transformed using the log (x+1) function. Forward selection, coupled with a Monte Carlo permutation test, was employed

to assess the statistical significance of the relationships between environmental variables and species abundance.

To analyze the water quality of the dams from phytoplankton, different water quality indices were applied: *i)* The “association quotient” (CA) based on the criteria of Thunmarck (1945), Nygaard (1947) and Gayral (1954) which is calculated with the following formula  $CA = Cy + Ch + Ce + Eu / De$ , where the number of species is represented by  $Cy$ =Cyanophyceae,  $Ch$ =Chlorophyceae (Chlorococcales),  $Ce$ =Central diatoms,  $Eu$ =Euglenophyceae and  $De$ =Chlorophyceae (Desmidiaceae), where the CA is interpreted as  $<1$ =oligotrophic, 1.1 to 2.5=mesotrophic, 2.6 to 2.9=eutrophic and  $>3.0$  saprotrophic (Ortega et al., 1994). *ii)* The organic pollution index (IP) of Palmer (1969), which is based on the reference of 20 phytoplankton genera that have been considered in 165 publications as tolerant to organic pollution in continental water bodies. The classification considers genera with a value of one when it is shallow organic contamination. At the same time, two indicates low organic contamination, three indicates intermediate conditions, four indicates moderate contamination, and five indicates high organic contamination. Based on the above, the following criterion was used: IP values  $\geq 20$  indicate environments with high organic contamination, IP values between 15 and 19 probable organic contamination, and IP  $< 15$  indicates low organic contamination. Finally, *iii)* Diatom Index ( $ID = \text{Central diatoms} / \text{Pennal diatoms}$ ), which is based on the richness of Central and Pennal diatoms, where values above 0.2 are considered characteristic of eutrophic waters (Nguyen, 2003).

## Results

### *Environmental Variables*

Table 1 shows the values of the six physical and chemical variables examined at each station in both dams during September and October. Both reservoirs are well-oxygenated environments between 4.4 and 9.6 mg/l, poorly mineralized with 20-40 mg/l  $\text{CaCO}_3$ , with a general pH of 7.0 to 8.0, temperature between 15 and 22°C, and conductivity of 62 and 171  $\mu\text{S/cm}$ .

**Table 1.** Values of the Physical and Chemical Variables in each Sampling Station of the El Llano (L) and Taxhimay (T) Dams, during September and October 2022.

| Limnological parameter                  | September/2022 |      |      | October/2022 |      |      |
|-----------------------------------------|----------------|------|------|--------------|------|------|
|                                         | L1             | L2   | L3   | L1           | L2   | L3   |
| 1. Temperature (°C)                     | 15.4           | 17   | 17.2 | 16.5         | 15.8 | 21.2 |
| 2. pH                                   | 7              | 9.1  | 8.8  | 7            | 7    | 7    |
| 3. Dissolved Oxygen (mg/l)              | 6.9            | 6.5  | 6.6  | 4.4          | 6.8  | 5.6  |
| 4. % Oxygen Saturation                  | 67.5           | 107  | 95   | 41           | 64   | 52   |
| 5. Alkalinity (mg/l CaCO <sub>3</sub> ) | 24             | 24   | 24   | 30           | 42   | 34   |
| 6. Conductivity (µS/cm)                 | 62             | 68   | 67   | 70           | 70   | 70   |
|                                         | T1             | T2   | T3   | T1           | T2   | T3   |
| 1. Temperature (°C)                     | 21.2           | 21.8 | 20.5 | 19.9         | 19.3 | 19.2 |
| 2. pH                                   | 7.9            | 8    | 7.8  | 8            | 8    | 7    |
| 3. Dissolved Oxygen (mg/l)              | 5.8            | 6.1  | 5.3  | 7.7          | 8    | 9.6  |
| 4. % Oxygen Saturation                  | 93.1           | 107  | 88.8 | 118          | 79.1 | 94.9 |
| 5. Alkalinity (mg/l CaCO <sub>3</sub> ) | 30             | 20   | 48   | 80           | 50   | 60   |
| 6. Conductivity (µS/cm)                 | 171            | 146  | 146  | 141          | 147  | 140  |

1, 2, 3 correspond to the sampling stations (station 1, station 2 and station 3).

### *Composition of Phytoplankton*

Table 2 shows the specific richness for both dams, which totals 55 *taxa*, 45 for El Llano, and 36 for Taxhimay (Fig. 2), where 26 species were recorded in both dams.



**Table 2.** Phytoplankton Determined in each of the Selected Stations: L1, L2, L3 (El Llano Dam) and T1, T2, T3 (Taxhimay Dam) for September and October 2022, Where "X" Indicates the Presence and "0" Absence of the Taxon. The Olmstead-Tukey O-T Value for El Llano (L) /Taxhimay (T); Where C=Constant, D=Dominant, O=Occasional, R=Rare; \* Corresponds to New Records for the Mexico, and \*\* New Records for the State of Mexico

| El Llano (L)                                                                                            |            |                                            |    |    |    |    |    |                |    |    |    |    |    |              | Taxhimay (T) |  |  |  |  |
|---------------------------------------------------------------------------------------------------------|------------|--------------------------------------------|----|----|----|----|----|----------------|----|----|----|----|----|--------------|--------------|--|--|--|--|
| Taxa                                                                                                    | O-T<br>L/T | L1                                         | L2 | L3 | L1 | L2 | L3 | T1             | T2 | T3 | T1 | T2 | T3 | October/2022 |              |  |  |  |  |
|                                                                                                         |            | September/2022                             |    |    |    |    |    | September/2022 |    |    |    |    |    |              |              |  |  |  |  |
|                                                                                                         |            | Phylum Cyanobacteria<br>Clase Cyanophyceae |    |    |    |    |    |                |    |    |    |    |    |              |              |  |  |  |  |
| <i>Dolichospermum planctonicum</i> (Brunnthal) Wacklin, L.<br>Hoffmann & Komárek 2009                   | C/D        | X                                          | 0  | 0  | X  | X  | 0  | 0              | 0  | X  | X  | X  | X  | X            |              |  |  |  |  |
| ** <i>Coelomorion microcystoides</i> Komárek 1989                                                       | C/-        | X                                          | 0  | 0  | X  | 0  | 0  | 0              | 0  | 0  | 0  | 0  | 0  | 0            |              |  |  |  |  |
| <i>Microcystis aeruginosa</i> (Kützing) Kützing 1846                                                    | -/D        | 0                                          | 0  | 0  | 0  | 0  | 0  | X              | 0  | X  | X  | X  | X  | X            |              |  |  |  |  |
| <i>Microcystis botrys</i> Teiling 1942                                                                  | -/D        | 0                                          | 0  | 0  | 0  | 0  | 0  | X              | X  | 0  | X  | X  | X  | X            |              |  |  |  |  |
| <i>Microcystis panniformis</i> Komárek, Komárkova, Komárkova-Legnerová, Sant'Anna, Azevedo & Senna 2002 | -/D        | 0                                          | 0  | 0  | 0  | 0  | 0  | X              | X  | X  | X  | X  | X  | X            |              |  |  |  |  |
| <i>Oscillatoria</i> sp. Vaucher ex Gomont                                                               | -/R        | 0                                          | 0  | 0  | 0  | 0  | 0  | X              | 0  | 0  | X  | 0  | 0  | 0            |              |  |  |  |  |
| <i>Planktothrix agardhii</i> (Gomont) Anagnostidis & Komárek 1988                                       | -/D        | 0                                          | 0  | 0  | 0  | 0  | 0  | 0              | X  | 0  | X  | X  | 0  | 0            |              |  |  |  |  |
| <i>Pseudanabaena</i> sp. Lauterborn, 1915                                                               | R/O        | X                                          | 0  | 0  | 0  | 0  | 0  | X              | 0  | X  | 0  | 0  | 0  | 0            |              |  |  |  |  |
| * <i>Radiocystis fernandoi</i> Komárek & Komárková-Legnerová 1993                                       | R/R        | X                                          | 0  | 0  | 0  | 0  | 0  | X              | 0  | 0  | 0  | 0  | 0  | 0            |              |  |  |  |  |
| <i>Sphaerocavum</i> sp. Azevedo & Sant'Anna, 2003                                                       | R/O        | X                                          | 0  | 0  | 0  | 0  | 0  | 0              | 0  | X  | 0  | X  | 0  | 0            |              |  |  |  |  |
| <i>Synechocystis</i> sp. Sauvageau, 1892                                                                | R/R        | X                                          | 0  | 0  | 0  | 0  | 0  | 0              | X  | 0  | X  | 0  | 0  | 0            |              |  |  |  |  |
| <i>Woronichinia naegeliana</i> (Unger) Elenkin 1933                                                     | -/D        | 0                                          | 0  | 0  | 0  | 0  | 0  | 0              | 0  | 0  | X  | X  | X  | X            |              |  |  |  |  |

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| Phylum Chlorophyta<br>Clase Chlorophyceae                                 |     |   |   |   |   |   |   |   |   |   |   |   |   |   |
|---------------------------------------------------------------------------|-----|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Coenocystis sp. Korshikov, 1953                                          | C/R | 0 | 0 | X | X | 0 | 0 | 0 | 0 | 0 | 0 | 0 | X | 0 |
| Crucigenia sp. Morren, 1830                                               | C/- | 0 | 0 | 0 | X | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Eudorina sp. Ehrenberg 1832                                               | D/- | 0 | 0 | X | X | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Haematococcus lacustris (Girod-Chantrons) Rostafinski 1875                | D/R | X | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | X | 0 | 0 | 0 |
| Hariotina reticulata P.A. Dangeard 1889                                   | D/- | X | X | 0 | X | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Monactinus simplex (Meyen) Corda                                          | -/D | 0 | 0 | 0 | 0 | 0 | 0 | X | 0 | 0 | X | 0 | X | X |
| Mucidosphaerium pulchellum (H.C. Wood) C. Bock, Proschold & Krienitz 2011 | D/D | X | X | X | X | 0 | 0 | 0 | X | 0 | 0 | 0 | X | X |
| **Neosporangiococcum sp. Deason, 1971                                     | R/- | 0 | 0 | 0 | 0 | 0 | 0 | X | 0 | 0 | 0 | 0 | 0 | 0 |
| Nephrocytium sp. Nägeli, 1849                                             | D/- | X | 0 | X | X | 0 | 0 | X | 0 | 0 | 0 | 0 | 0 | 0 |
| Oedogonium sp. Link ex Hirn, 1900                                         | C/- | 0 | 0 | 0 | 0 | 0 | 0 | X | 0 | 0 | 0 | 0 | 0 | 0 |
| Oocystis sp. Nägeli ex A. Braun, 1855                                     | D/R | X | X | X | X | X | X | X | 0 | 0 | 0 | 0 | X | 0 |
| Pandorina morum (O.F. Müller) Bory 1826                                   | D/- | X | 0 | X | 0 | 0 | 0 | X | 0 | 0 | 0 | 0 | 0 | 0 |
| *Paulschulzia pseudovolvox (P. Schulz) Skuja 1948                         | D/- | X | X | 0 | X | 0 | 0 | X | 0 | 0 | 0 | 0 | 0 | 0 |
| Sphaerocystis Schroeteri Chodat 1897                                      | D/- | X | X | X | X | 0 | 0 | X | 0 | 0 | 0 | 0 | 0 | 0 |
| *Yamagishiella sp. H. Nozaki, 1992                                        | D/- | X | X | X | 0 | 0 | X | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Phylum Charophyta<br>Clase Zygnematophyceae                               |     |   |   |   |   |   |   |   |   |   |   |   |   |   |
| Cosmarium sp.1 Corda ex Ralfs, 1848                                       | D/- | X | X | X | 0 | X | 0 | X | 0 | 0 | 0 | 0 | 0 | 0 |
| Cosmarium sp.2 Corda ex Ralfs, 1848                                       | D/- | X | 0 | 0 | X | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Mougeotia sp. C. Agardh, 1824                                             | D/- | X | X | X | X | X | 0 | X | 0 | 0 | 0 | 0 | 0 | 0 |
| Staurastrum cingulum (West & G.S. West) G.M. Smith 1922                   | D/R | X | X | X | X | X | 0 | X | 0 | X | 0 | 0 | X | 0 |
| Staurastrum sp. Meyen ex Ralfs, 1848                                      | R/R | 0 | 0 | 0 | X | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | X |
| Phylum Charophyta<br>Clase Klebsormidiophyceae                            |     |   |   |   |   |   |   |   |   |   |   |   |   |   |
| Elakatothrix sp. Wille, 1898                                              | R/- | 0 | X | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

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| <i>Phylum Euglenophyta</i><br><i>Clase Euglenophyceae</i>                       |     |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
|---------------------------------------------------------------------------------|-----|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| <i>Phacus</i> sp 1 Dujardin, 1841                                               | D/- | 0 | X | 0 | 0 | 0 | X | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Phacus</i> sp 2 Dujardin, 1841                                               | C/- | 0 | X | 0 | X | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Trachelomonas hispida</i> (Perty) F. Stein 1878                              | D/R | 0 | 0 | X | 0 | 0 | 0 | X | 0 | 0 | 0 | X | 0 | 0 | 0 | 0 |
| <i>Trachelomonas volvocina</i> (Ehrenberg) Ehrenberg 1834                       | D/O | X | X | 0 | 0 | 0 | 0 | 0 | 0 | X | 0 | 0 | 0 | X | X | 0 |
| <i>Trachelomonas rugulosa</i> var. <i>rugulosa</i> (F. Stein) G.A. Klebs        | C/R | X | X | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | X | 0 | 0 |
| <i>Phylum Dinoflagellata</i><br><i>Clase Dinophyceae</i>                        |     |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| <i>Ceratium hirundinella</i> (O.F. Müller) Dujardin 1841                        | R/D | 0 | 0 | X | 0 | 0 | 0 | 0 | 0 | 0 | 0 | X | 0 | X | X | 0 |
| <i>Peridinium willeyi</i> Huitfeldt-Kaas 1900                                   | D/D | 0 | X | X | 0 | 0 | X | 0 | X | 0 | X | 0 | X | X | X | X |
| <i>Peridinium</i> sp.                                                           | -/R | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | X | 0 | 0 |
| * <i>Peridiniopsis</i> sp. Lemmermann, 1904                                     | C/R | 0 | X | X | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | X | 0 | 0 |
| <i>Phylum Heterokontophyta</i><br><i>Clase Chrysophyceae</i>                    |     |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| <i>Dinobryon cylindricum</i> O.E. Imhof 1887                                    | D/D | X | X | X | X | X | 0 | X | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Dinobryon</i> sp.1 Ehrenberg, 1834                                           | D/D | 0 | 0 | X | X | X | X | 0 | X | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Mallomonas</i> sp. Perty, 1852                                               | D/D | X | X | X | X | 0 | 0 | 0 | 0 | X | X | 0 | X | X | 0 | X |
| <i>Clase Mediphyceae</i>                                                        |     |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| * <i>Acanthoceras</i> cf. <i>zachariassii</i> (Brun) Simonsen 1979              | -/D | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | X | X | 0 | X | 0 | X |
| <i>Clase Bacillariophyceae</i>                                                  |     |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| <i>Achnanthes</i> sp.                                                           | O/- | 0 | 0 | X | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Asterionella formosa</i> Hassall 1850                                        | D/D | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| <i>Cymbella mexicana</i>                                                        | -/R | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | X | 0 | 0 | 0 | 0 | 0 |
| <i>Fragilaria crotonensis</i> Kitton 1869                                       | C/C | X | X | 0 | 0 | 0 | 0 | 0 | 0 | X | X | X | 0 | 0 | 0 | 0 |
| <i>Epithemia</i> sp. Kützing, 1844                                              | C/- | 0 | X | 0 | 0 | 0 | X | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Frustulia</i> sp. Rabenhorst, 1853                                           | C/R | X | 0 | X | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Navicula</i> sp. Bory, 1822                                                  | R/R | 0 | 0 | 0 | 0 | X | 0 | 0 | 0 | X | 0 | X | 0 | 0 | 0 | 0 |
| <i>Clase Coscinodiscophyceae</i>                                                |     |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| <i>Aulacoseira granulata</i> f. <i>curvata</i> Simonsen, 1979                   | C/D | X | X | 0 | 0 | 0 | 0 | 0 | 0 | 0 | X | X | X | X | X | X |
| <i>Aulacoseira granulata</i> var. <i>angustissima</i> (O. Müller) Simonsen 1979 | R/D | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | X | X | X | X | X | X | X |

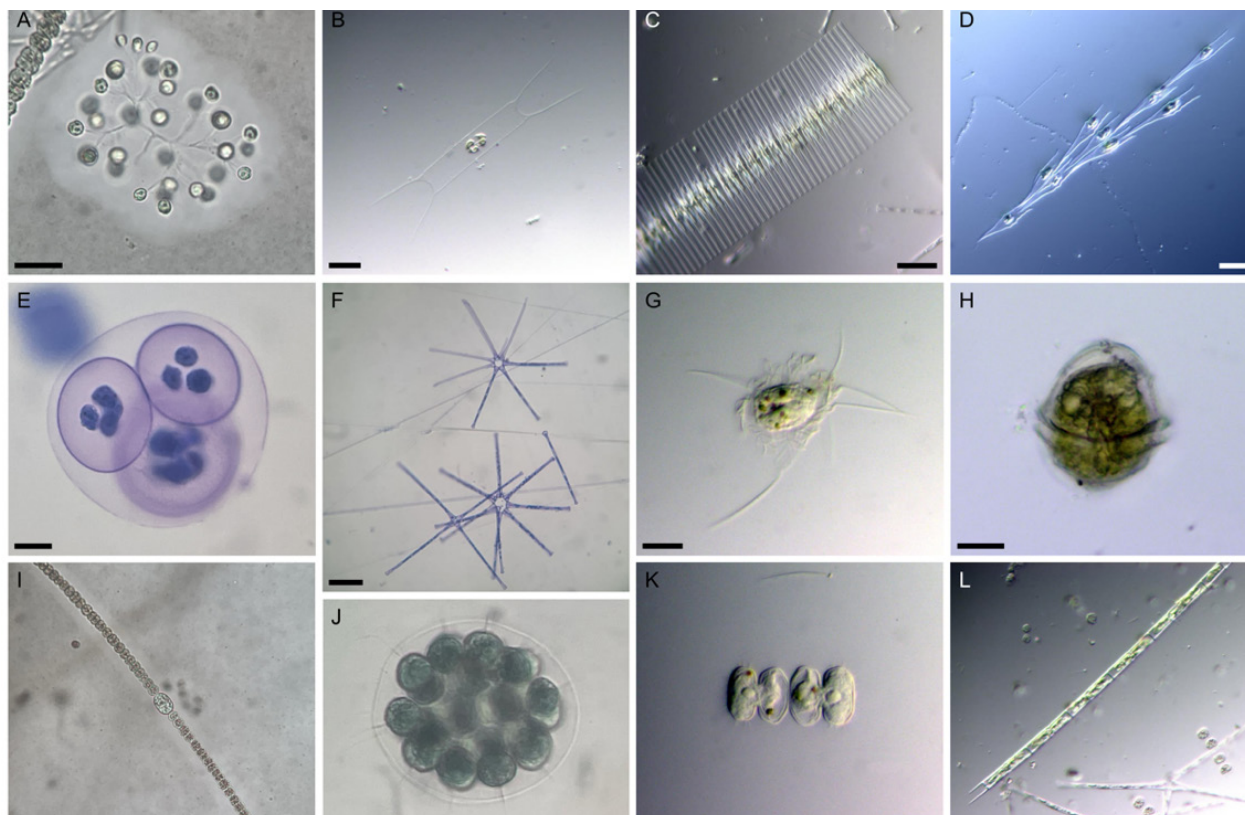


**Figure 2.** A) *Mucidosphaerium pulchellum*, B) *Acanthoceras cf. zachariassii*, C) *Fragilaria crotonensis*, D) *Dinobryon cylindricum*, E) *Paulschulzia pseudovolvox*, F) *Asterionella formosa*, G) *Mallomonas* sp. H) *Peridinium* sp. I) *Dolichospermum* Ç *planctonicum*, J) *Pandorina morum*, K) *Cosmarium* sp., L) *Aulacoseira granulata* var. *angustissima*. Scale Bars E, G, J, K=10 µm, A, B, D, H=20 µm, C, L=25 µm, F, I = 30 µm.

Taxonomic records for El Llano and Taxhimay dams (Fig. 3) indicate ten classes. The percentages concerning specific richness were the following: Chlorophyceae (27.3 %), Cyanophyceae (21.8 %), Bacillariophyceae (12.7 %), Euglenophyceae and Zygnematophyceae (9.0 %), respectively, Dinophyceae (7.2 %), Chrysophyceae (5.4 %), Coscinodiscophyceae (3.6 %), Mediophyceae, and Klebsormidiophyceae (1.8 %), each.



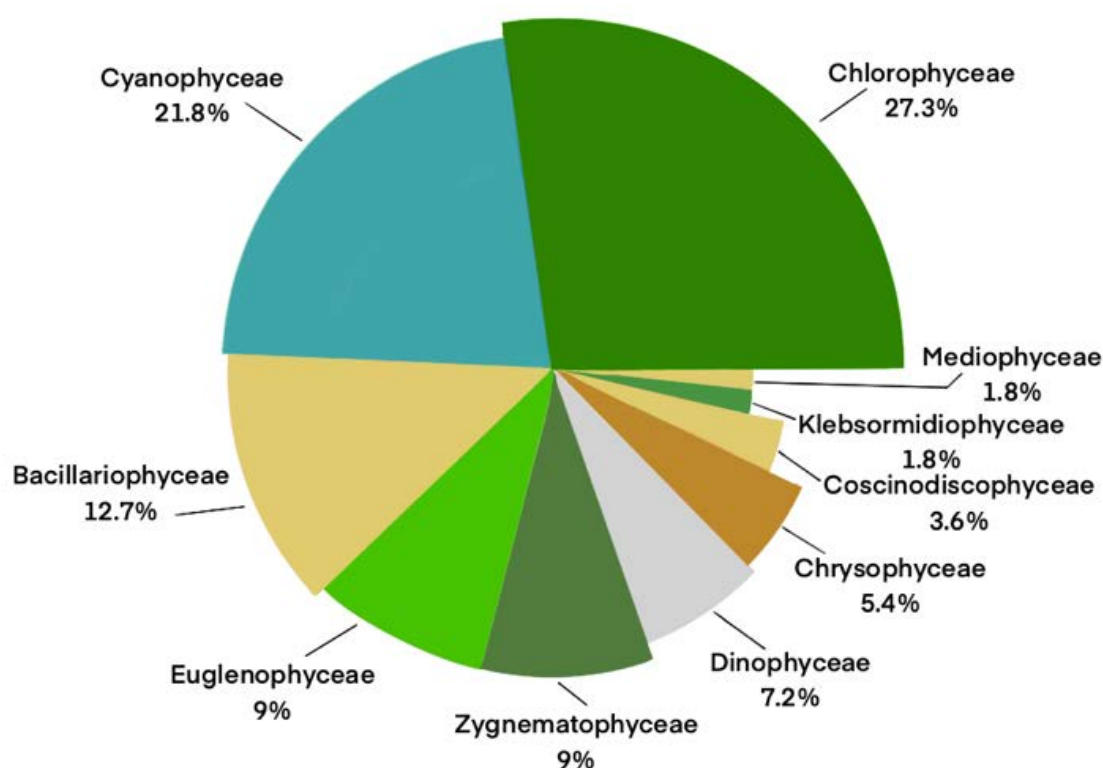


**Figure 3.** Percentage distribution by Taxonomic Classes of the Phycoflora Richness Recorded in the El Llano and Taxhimay Dams.

Based on the Olmstead-Tukey analysis, the following were recorded in El Llano: 22 dominant species, 12 constant, 10 rare, and 1 occasional. Of them, the most abundant was *Asterionella formosa*, with a percentage that ranged between 79.9 and 96.4. Meanwhile, *Aulacoseira granulata* f. *curvata*, and *Phacus* sp. 2 were constant. For its part, in Taxhimay, there were 17 dominant species, for example, *Asterionella formosa*, *Microcystis panniformis*, *Woronichinia naegeliana*, *Acanthoceras* cf. *zachariassii*, 14 rare, 3 occasional, and 1 constant.

In Fig. 4A, the specific richness (S) is indicated in each of the sites where it is observed that in El Llano, the record with the highest richness was 27 *taxa* (L1 in September), followed by 25 *taxa* (T1 in October). In contrast, those with the lowest richness were stations T2 in September and L2 in October, with 12 and 14 *taxa*, respectively. In general, the  $H'$  index was highest in the Taxhimay Dam, with 1.54 bits/ind. in T2 in October, and the lowest was in El Llano, with 0.21 bits/ind. in the L2 in October station.

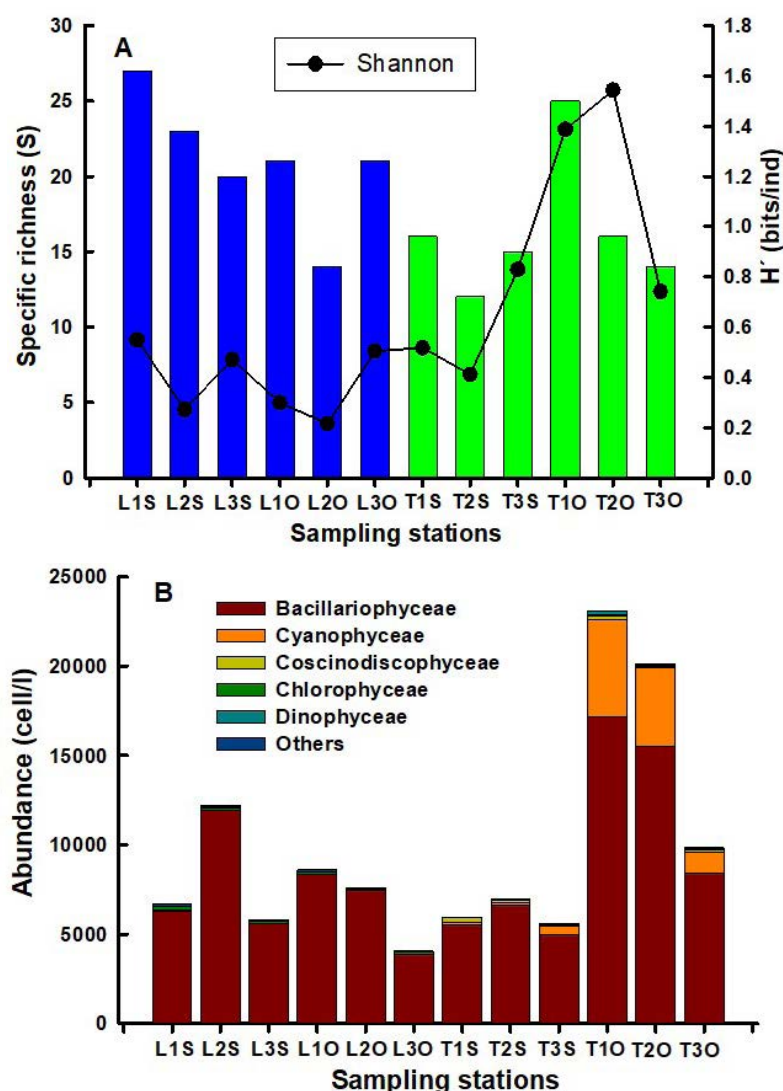


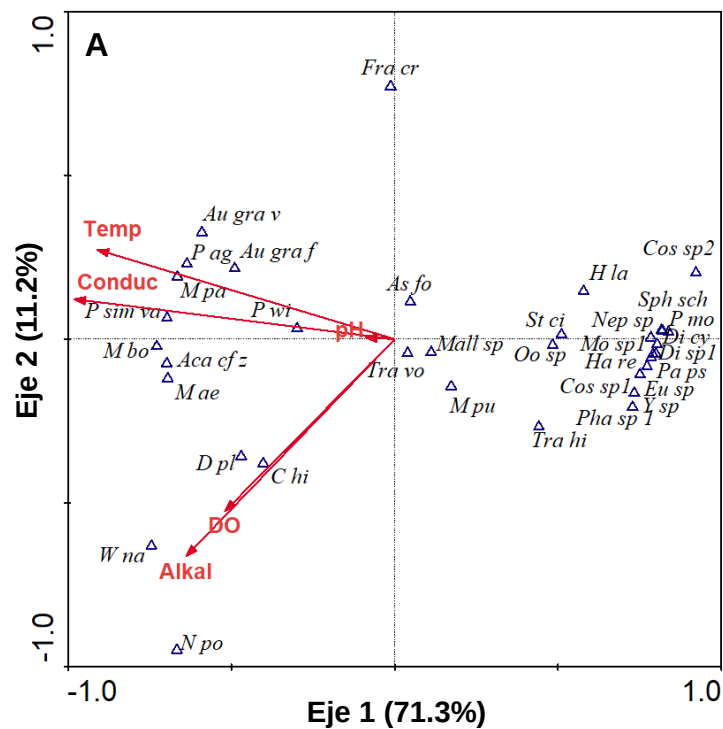
**Figure 4.** A) Specific Richness and Shannon Index in each of the Study Stations, 4 B) Abundance of Taxonomic Classes Recorded in each of the Dams, where L, Represents El Llano and T, Taxhimay; 1, 2, 3 each of the Stations; Finally, S and O Indicates the Month of Sampling (September and October 2022).

Regarding abundance, the highest record was at station T1 in October, with 21,476 cell/ml. In contrast, the lowest abundance was at station L3 in the same month, with 4,056 cell/ml (Fig. 4B).

The abundance by taxonomic classes in all the sampling stations studied was markedly higher for Bacillariophyceae, represented by the biomass of *Asterionella formosa* with 79.9 to 96.4 % (96,856 cell/ml). Also, during October in Taxhimay, the proliferation of the Cyanophyceae: *Dolichospermum planctonicum*, *Microcystis aeruginosa*, *M. botrys*, *M. panniformis*, and *Woronichinia naegeliana* were notable. Finally, the Chlorophyceae were represented

mainly by *Eudorina* sp., *Haematococcus lacustris*, *Mucidosphaerium pulchellum*, and *Yamagishiella* sp.

The CCA explained 82.5 % of the variation in the data where conductivity was significant ( $p < 0.01$ ,  $F = 8.23$ ,  $\lambda = 0.54$ ) and was positively related to *Aulacoseira granulata* var. *angustissima*, *Monactinus simplex*, *Aulacoseira granulata* f. *curvata*, *Planktothrix agardhii*, *Peridinium willei*, and *Microcystis panniformis* from samples collected in September at the Taxhimay Dam. At the same time, this variable was negatively related to *Cosmarium* sp.1, *Dinobryon* sp., *Dynobryon cylindricum*, *Eudorina* sp., *Hariotina reticulata*, *Mallomonas* sp., *Mucidosphaerium pulchellum*, *Nephrocitium* sp., *Oocystis* sp., *Pandorina* sp., *Phacus* sp.1, *Trachelomonas hispida*, and *Yamagishiella* sp. associated with samples from El Llanito (Fig. 5A and 5B). It is also observed that the species positively related to conductivity were positively related to temperature. For its part, *W. naegeliana*, *D. planctonicum* and *C. hirundinella* are related to alkalinity.



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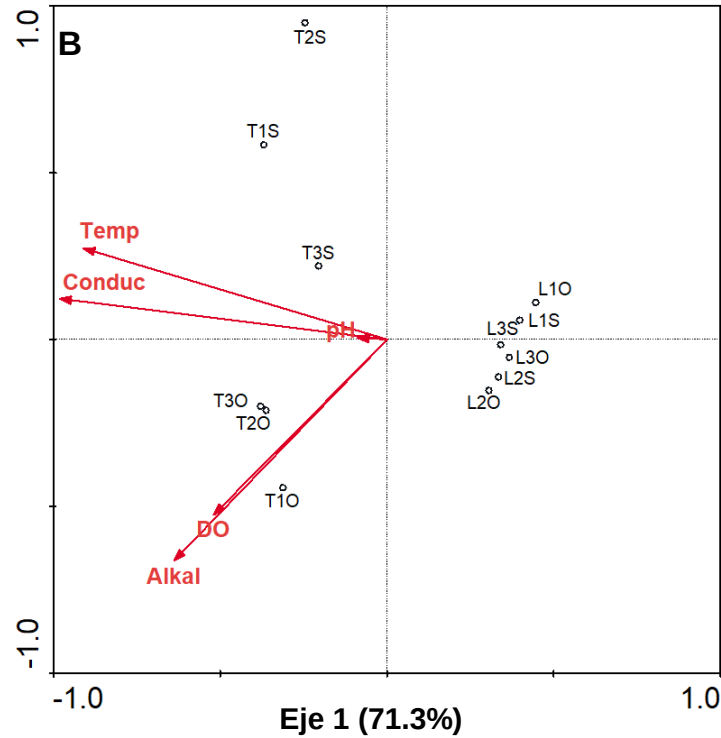


**Figure 5.** Canonical Correspondence Analysis (CCA) showing A) the Relationship of the Limnological Variables with the Species and B) the Relationship of the Limnological Variables with the Samples Collected in the El Llano and Taxhimay during September and October 2022. *Aulacoseira granulata* f. *curvata* (Au gra f. cur), *Aulacoseira granulata* var. *angustissima* (Au gra var. ang), *Monactinus simplex* (P. sim. var. chl.), *Microcystis panniformis* (M. pan.), *Planktothrix agardhii* (P. ag), *Navicula* sp. (Na sp.), *Oscillatoria* sp. (Os. sp.), *Acanthoceras* cf. *zachariassii* (Aca cf. zach), *Peridinium willei* (P wi), *Microcystis aeruginosa* (M ae), *Microcystis botrys* (M bo), *Sphaerocavum* sp. (Sph sp), *Dolichospermum planctonicum* (D. pl), *Woronichinia naegeliana* (W. na), *Ceratium hirundinella* (C hi) and *Cymbella mexicana* (C. mex).

### Water Quality Indexes

The association quotient (CA) registered values of 6.75 for El Llano ( $CA = 6 + 14 + 2 + 5/4 = 6.75$ ), in contrast to 10.5 in Taxhimay ( $CA = 11 + 5 + 2 + 3/2 = 10.5$ ); which indicates that they are saprotrophic environments. Considering the genera registered in the reservoirs, the IP, based on the genus contamination index obtained values of 6 for El Llano due to the presence of *Navicula* 3, *Pandorina* 1, and *Phacus* 2, which adds up to a total IP of 6; while for Taxhimay, it presented *Oscillatoria* with a value of 5 and *Navicula* 3, which indicated a value of 8. Therefore, based on these biological indicators, during this time of months of high and low rainfall, they were systems with low organic contamination. While, regarding the ID, it was 0.33 ( $2/6 = 0.33$ ) for El Llano and 0.50



for Taxhimay ( $2/4=0.50$ ). Both reservoirs recorded values  $>0.2$ , so the environmental condition of the systems indicates eutrophic conditions.

## Discussion

### *Environmental Variables*

El Llano and Taxhimay dams are reservoirs of temperate waters, with temperatures typically ranging between 15 and 22°C. Furthermore, mineralization levels remain low, usually between 30 and 80 mg/l  $\text{CaCO}_3$ . This is in contrast to levels observed five years ago in the study area, which ranged from 4 to 162 mg/l  $\text{CaCO}_3$  (Razo-Paredes et al., 2016). This low mineralization has also been recorded for Lake Zirahuén, Michoacán (Bernal-Brooks, 1998).

The pH levels ranged from neutral to basic (7-9), which were slightly similar to the 6-8.1 range previously documented in the study area by Razo-Paredes et al. (2016). This trend was also observed in the records from Taxhimay during 2018-2019, with values ranging from 7.0 to 9.0 (Saavedra-Martínez, 2020).

Regarding dissolved oxygen, values were from 5.8 to 9.6 mg/l, which indicates that in October 2022, the water surface was well-oxygenated. This suggests an essential photosynthetic activity during the study period and the values of this variable have increased when compared to the records of July 2017, where the values were from 1.0 to 4.6 mg/l (Toledo-Trejo & Razo-Paredes, 2018) and those quantified in Taxhimay between August 2018 and July 2019 with average values between 6.0 to 6.2 mg/l (Saavedra-Martínez, 2020).

The conductivity in both dams, ranging between 62-140  $\mu\text{S}/\text{cm}$  was recorded, indicating low conductivity values and confirming the records by Toledo-Trejo and Razo-Paredes (2018) and Saavedra-Martínez (2020). Concerning CCA, temperature and conductivity were positively related to diatoms: *A. granulata* var. *angustissima*, *A. granulata* f. *curvata*; green algae: *Monactinus simplex* and the cyanobacteria: *M. panniformis*. Alkalinity explains the secondary gradient of phytoplankton composition. Conditions that do not coincide with those analyzed for the phytoplankton of Chapala, Jalisco, where sulfate and alkalinity concentrations were the most important variables that determined the composition and richness of the lake's phytoplankton (Mora-Navarro et al., 2004).

The abundance of *Asterionella formosa*, *Aulacoseira granulata*, and *Microcystis aeruginosa* corresponds to species that grow better in eutrophic systems with high amounts of nutrients such as phosphates and nitrates. Information that has been quantified in Taxhimay is between 1.0 to 1.4 mg/l and 0.5 to 0.7 mg/l, respectively (Toledo-Trejo & Razo-Paredes, 2018). In addition, Chl a concentration was between 25.4 and 282.8  $\mu\text{g}/\text{l}$  when there were blooms; therefore, the limnological and phycological conditions indicate that they correspond to eutrophic systems (Saavedra-Martínez, 2020).

In particular, the development of populations of three *Microcystis* species suggests the establishment of monitoring that includes different climatic periods and sampling sites to learn more about the ecological dynamics of phytoplankton in the reservoirs of Villa del Carbón, State of Mexico, to support the management of alternative drinking water supply systems.

On the other hand, samples obtained during the two studied months have different levels of precipitation, September with 150 mm and October with 69 mm; this correspond according to the stage of ecological succession for reservoirs in the central zone of Mexico indicated by Arredondo-Figueroa and Flores Nava (2012) to the dilution phase in both dams during September, since the precipitation was greater during this period and therefore the phytoplankton community showed greater stability. Meanwhile, the biological material from October, when precipitation decreased (69 mm), increased primary productivity with the abundance of Bacillariophyceae of 36,404 cell/ml and Cyanophyceae with 11,016 cell/ml, so they represent a transition phase, when it is assumed greater amount of nutrients in the water column.

### *Phycoflora*

The specific richness of phytoplankton in both dams was 55 species; for El Llano, there were 45 taxa, while Taxhimay, 32 taxa, and 26 taxa were present in the two reservoirs. The most diverse groups in both were Chlorophyceae, with 27.3 %, Cyanophyceae 21.8 %, and Bacillariophyceae 12.7 %. For the El Llano, 41 taxa cited by Razo-Paredes et al. (2016) coincide; while, in Taxhimay Toledo-Trejo and Razo-Paredes (2018), indicated six taxa. Therefore, 26 species correspond to new records for this reservoir.

These environments contain only the 8.4 % phytoplankton diversity recorded from the State of Mexico (Garduño-Solórzano et al., 2009), where the taxa that develop in the two dams were: *D. planctonicum* (Cyanophyceae); *H. lacustris*, *M. pulchellum*, *Oocystis* sp., *S. cingulum* (Chlorophyceae), *A. formosa* (Bacillariophyceae), *A. granulata* f. *curvata*, and *A. granulata* var. *angustissima* (Coscinodiscophyceae); *Trachelomonas hispida*, *T. volvocina*, and *T. rugulosa* (Euglenophyceae); *C. hirundinella*, *P. willeii*, *Peridiniopsis* sp. (Dinophyceae), and *Mallomonas* sp. (Crysophyceae).

The following six taxa: *A. cf. zachariassii*, *Coenocystis* sp., *P. pseudovolvox*, *Peridiniopsis* sp., *R. fernandoi*, and *Yamagishiella* sp., correspond to new records from México, based in Novelo and Tavera (2023). Also, *Coelomoron microcystoides* and *Neospongio-coccum* sp. were new records for the State of Mexico.

In El Llano, the following diatoms were dominant: *Asterionella formosa* and *Aulocoseira granulata* f. *curvata*, which coincides with what was described by Toledo-Trejo and Razo-Paredes (2018), who indicated that *A. formosa* was also dominant in phytoplankton in the study area. Likewise, the dominant species in both dams was *A. formosa*, with

abundances of 43 and  $53 \times 10^3$  cell/ml for El Llano and Taxhimay, respectively; according to Bellinger and Sigeo (2010), values that indicate eutrophic lentic water bodies.

Taxhimay Dam, the development of *A. granulata* var. *angustissima* was dominant during October 2022; conditions that are explained by corresponding to a taxon whose ecological distribution in lentic environments of 13 lakes in central Mexico has been recorded under limnological conditions of average annual temperature of 16.6°C, pH 7.7 and trophic state from meso to hypertrophic (Ramírez-Nava et al., 2022). As well as in eutrophic reservoirs in Brazil (Bicudo et al., 2016). Therefore, this is a bioindicator of eutrophic environments, which allows us to add arguments to indicate the Taxhimay dam under ecological conditions of neutral to slightly basic, with pH between 7-8 and temperature 19.2 to 19.9°C where they grow in high percentages of the taxon, as has been observed in Lake Santa Gertrudis, Jalisco and San Juanico, State of Mexico, with abundances more significant than 31 % (Ramírez-Nava et al., 2022).

### *Water Quality Indexes*

The phycoflora of El Llano is distributed in six species of Cyanophyceae, in contrast to 11 species in Taxhimay. At the same time, the Chlorophyceae in El Llano were 14 *versus* five species in Taxhimay. A composition that allowed different water quality indexes to be considered: CA, IP, and ID, all with results that indicate that they are eutrophic systems. Besides, El Llano has a lower level of eutrophication than Taxhimay. In the latter, the dominant species were *A. formosa* and *M. aeruginosa*; their presence suggests a significant amount of wastewater and, therefore, a high concentration of nutriment. The presence of the same species has been recorded in the following seven dams: Zimapán (Gutiérrez-Hernández, 2003), Mintzita (Ortega-Murillo et al., 2007), La Laguna, Los Reyes, Necaxa (González-Fernández et al., 2013), El Abrevadero (Carrasco-Vargas et al., 2014), and Taxhimay (Toledo-Trejo and Razo-Paredes, 2018).

## Conclusions

The environmental conditions were in both reservoirs poorly mineralized (30-80 mg/l  $\text{CaCO}_3$ ), low conductivity (62-140  $\mu\text{S/cm}$ ), and well oxygenated; during the study of the phytoplankton structure of two months, where there were marked differences of precipitation records from September (150 mm) and October (69 mm).

Considering both dams, a specific richness of 55 species was obtained, of which the percentages concerning the specific richness were from the highest to lowest of Chlorophyceae (27.3 %), Cyanophyceae (21.8 %), and Bacillariophyceae (12.7 %), among other classes of lower record; new records were determined for the study area in Mexico. Also, the  $H'$  index was higher in the Taxhimay Dam with 1.54 than in El Llano with 0.21 bits/ind.

The Olmstead-Tukey analysis showed 22 dominant species for El Llano and 17 for Taxhimay. In particular, the abundance in both dams was represented by the Class Bacillariophyceae, of which *Asterionella formosa* was the most dominant species. In Taxhimay, it also indicated an abundance of the Cyanophyceae (*Microcystis aeruginosa*, *M. botrys*, *M. panniformis*, and *Woronichinia naegeliana*); taxa associated with eutrophic water body conditions. The CCA showed positive relations with the conductivity and warm temperature (15 and 22°C), and alkalinity with some species.

Based on the three water quality indexes used, the two dams indicate that they are eutrophic, in addition, the abundance of Cyanophyceae from Taxhimay in October suggests that they should be monitored in different climatic periods, since they can develop harmful proliferations.

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## Authors' Contributions

GGs formal analysis and investigation, data curation, writing—original draft preparation and review and editing. GFJM conceptualization, collected the samples, identification, statistical analysis and writing. BVV collected the samples, identification, writing. DLVK collected the samples, identification, writing. CAER conceptualization, collected the samples, original draft preparation, and review and editing. All authors have read and agreed to the published version of the manuscript.

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# Towards Molecular, Genetic, and Optical Monitoring of Potentially Harmful Cyanobacteria Blooms in Mexican Freshwater Bodies

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## Abstract

Programs for safe cyanotoxins-free water use in drinking and irrigation water are fundamental to avoid potential health risks and should be implemented. Data on limnological waterbodies, either natural or artificial, related to cyanobacterial bloom occurrence are essential to estimate the frequency of possible dangerous events and to establish strategies for monitoring and prevention. In Mexico, predominant cyanobacteria in freshwater algal blooms are *Microcystis* sp., *Planktothrix* sp., and *Raphidiopsis* spp. Cyanotoxins bioaccumulate in soil and plants through irrigation water and transfer to edible vegetables affecting plant physiology and food crop productivity. The harmful cyanobacterial blooms and cyano-

toxins production affect microbial diversity in waterbodies and soil. This work emphasizes standardized protocols using chemical, genomic, and optical techniques for potential toxic blooms in water bodies in the early stages.

## Keywords

Cyanotoxins, microcystin, cyanobacterial bloom, cyanotoxin bioaccumulation, molecular detection, abundance monitoring.

## Introduction

Bloom formation is a natural phenomenon where phytoplankton and cyanobacteria grow excessively, favored mainly by increased nutrients and the change of water flow conditions, and ultimately, by global warming. However, when toxins are produced, generally by cyanobacteria, they are known as cyanobacterial harmful blooms. These blooms have adverse environmental effects, including damage to flora and fauna, economic losses, and jeopardy to human health (Huisman et al., 2018; Anderson et al., 2021; Karlson et al., 2021). The control of harmful blooms in Latin America has gained greater importance in recent decades due to: a) the increasing scarcity of water, b) the deterioration of its quality, c) the lack of common regulations, monitoring efforts, and public access to the data generated, and d) protocols to evaluate harmful cyanobacteria (Aguilera et al., 2023).

Through the detection, monitoring, and prediction of cyanobacteria blooms, the quality of the water bodies can be determined to guarantee their safe consumption (Cannizzaro et al., 2019). The purpose of this chapter is to review the current knowledge on harmful cyanobacterial blooms, primarily in Mexico. Additionally, it aims to highlight the advancements in detection methods of cyanotoxins, to design a practical monitoring scheme for these increasingly prevalent and impactful phenomena. Among the techniques addressed in this chapter are mass spectrometry-based metabolomics, 16S or 23S rRNA gene sequencing, cyanotoxins biosynthesis gene amplification by polymerase chain reaction (PCR), quantitative-PCR (qPCR)-based probes, reverse transcription PCR (RT-PCR) and DNA arrays, high-throughput sequencing metagenomics, third generation sequencing technologies, as well as remote sensing, radiative transfer, as well as bio-optical sensing strategies are explored.

### *Diversity of Cyanobacterial Blooms in Mexico and Latin America: Status*

The microbial population dynamics on freshwater bodies are correlated to seasons leading to different nutrient loads, and temperatures. However, gradual increases in temperature, mainly due to global warming, and anthropogenic perturbations, such as discharges of wastewater, and agriculture runoffs, among other factors, trigger the eutrophication phenomena in which taxonomic and functional diversity is reduced, as well as other abiotic

variables. These changes ultimately harm the stability of the ecosystem (Tomasin-Ortiz, 2012; Zhu et al., 2019).

In a recent review (Aguilera et al., 2023) Latin American countries such as Argentina, Chile, Brazil, and Uruguay presented the highest number of harmful cyanobacterial taxa. The most frequently found genera were *Aphanizomenon*, *Dolichospermum*, *Microcystis*, *Planktothrix*, *Pseudanabaena*, and *Raphidiopsis*. Among them, *Microcystis* was continuously reported with values more than 25 % of the cases per country. In Mexico, blooms of harmful cyanobacteria in freshwater bodies have been studied, some recent examples are presented in Table 1.

**Table 1.** Microbial Diversity Reported Studies of Cyanobacterial Blooms in Natural and Artificial Water Bodies Located in Mexico.

| Water and Location (State)                                                                                     | Dominant Species Detected                                                                                                                                                                                                              | Year of Study | Predominant Toxin Detected                             | Reference                     |
|----------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------|--------------------------------------------------------|-------------------------------|
| Natural lake Zumpango, Reservoir, Chapultepec and Cuernavaca (CDMX)                                            | <i>M. aeruginosa</i> , <i>Planktothrix agardhii</i> , <i>M. flos-aquae</i> , <i>M. panniformis</i> , and <i>M. novacekii</i> , <i>M. wesenbergii</i> , <i>M. aeruginosa</i> , <i>Woronichinia naegeliana</i> and <i>Lyngbya birgei</i> | 2010          | MC-LR                                                  | Vasconcelos et al., 2010      |
| Santa María del Oro Crater Lake (Nayarit)                                                                      | <i>Limnoraphis robusta</i> ; <i>Microcystis aeruginosa</i>                                                                                                                                                                             | 2015          | MC-WR, MC-LR, MC-LA, MC-HiLR, MC-LF, MC-YR, and MC-LY. | Bustillos-Guzmán et al., 2020 |
| Lagunas de Montebello, National Park (Chiapas)                                                                 | <i>Planktothrix agardhii</i>                                                                                                                                                                                                           | 2017          | Not Measured                                           | Yanez-Montalvo et al., 2022   |
| Lake Alberca de Tacámbaro. (Michoacán)                                                                         | <i>M. aeruginosa</i>                                                                                                                                                                                                                   | 2018-2019     | Not Measured                                           | Montero et al., 2021          |
| The Apatlaco River (Morelos)                                                                                   | <i>Acinetobacter</i> , <i>Arcobacter</i> , and <i>Myroides</i> phyla                                                                                                                                                                   | 2019          | Not Measured                                           | Breton-Deval et al., 2019     |
| The Valle de Bravo Reservoir (CDMX)                                                                            | <i>M. wesenbergii</i> , <i>M. aeruginosa</i> , <i>Woronichinia naegeliana</i> and <i>Lyngbya birgei</i>                                                                                                                                | 2019          | Not Measured                                           | Nandini et al., 2019          |
| Zumpango Lake, Chapultepec Lake, Alameda Oriente Lake, and Virgilio Uribe Olympic Rowing-Canoeing Track (CDMX) | <i>Microcystis</i> , <i>Planktothrix</i>                                                                                                                                                                                               | 2020          | MC-LR                                                  | Pineda-Mendoza et al., 2020   |

| Water and Location (State)    | Dominant Species Detected                                                               | Year of Study | Predominant Toxin Detected | Reference                       |
|-------------------------------|-----------------------------------------------------------------------------------------|---------------|----------------------------|---------------------------------|
| El Palote Dam<br>(Guanajuato) | <i>Planktothrix</i> spp.,<br><i>Raphidiopsis</i> spp.,<br>and <i>Limnothrix redekei</i> | 2020          | Not measured               | Valdés-Santiago<br>et al., 2021 |

A common feature of harmful cyanobacteria blooms is the changes in microbial community composition over time, such as in the monomictic Alberca de Tacámbaro Lake. Although this lake was oligotrophic in 2006, with high microbial diversity with *Chroococcus minutus* and *Gloeocapsiopsis crepidinum* as dominant species, in 2018 and 2019, when a eutrophication phenomenon was evident, only the cyanobacterium *M. aeruginosa* was detected as the dominant species with a greatly reduced microbial diversity mainly due to anthropogenic activities (Hernández-Morales et al., 2011; Montero et al., 2021). A similar phenomenon was recorded in the “Lagunas de Montebello” National Park, where in recent years some of its lakes have shown eutrophication with *Planktothrix agardhii* as the dominant species with almost 97 % relative abundance, in contrast to the oligotrophic lakes, where the cyanobacteria *Cyanobium* was relatively more abundant and a greater relative diversity was seen (Yanez-Montalvo et al., 2022).

Seasonal changes in microbial diversity are also well documented. In 2019, in the Valle de Bravo Reservoir, it was observed that *M. aeruginosa* was the dominant cyanobacterium in January, while *Woronichinia naegeliana* was dominant in September (Vasconcelos et al., 2010; Nandini et al., 2019). In the same way, the immediate effect of human activities on eutrophication is also clearly established, since in the Apatlaco River the phyla Proteobacteria and Bacteroidetes were abundant in almost all the sites sampled. In the contaminated ones, Acinetobacter, Arcobacter, and Myroides were the dominant phyla (Bretón-Deval et al., 2019).

### *The Effects of Cyanotoxins and the Tools for their Identification*

The presence of cyanotoxins in irrigation water, agricultural soil, and contaminated vegetables is concerning because it represents an indirect route of human exposure. The genera most recurrently reported producing cyanotoxin are *Microcystis*, *Anabaena*, *Oscillatoria*, and *Planktothrix*. In freshwater ecosystems from North and Central America, the most frequently reported cyanotoxins were microcystins (MC's), followed by anatoxins, cylindrospermopsins, and saxitoxins, and their producing genera identified were *Microcystis* spp., *Anabaena* spp., *Aphanizomenon* spp., *Lyngbya* spp., and *Raphidiopsis* spp. (Aguilera et al., 2018; Svirčev et al., 2019).

Soil accumulates cyanotoxins through irrigation with contaminated water which affects microbial diversity, microfauna, and plant development (Bouaïcha & Corbel, 2016).



The presence of MCs also leads to changes in bacterial diversity in soil (El Khalloufi et al., 2016). Plants can take up and bioaccumulate microcystin-LR (MC-LR) and cylindrospermopsin, in tissues, roots, seedlings, leaves, stems, and edible parts, affecting the physiology and morphology of them, hence, causing the loss of production (Machado et al., 2017; Levizou et al., 2020).

Studies carried out between 2000 and 2015 show that the cyanotoxins most frequently detected in water bodies in central Mexico are: microcystins, nodularins, cylindrospermopsins, and saxitoxins (Pérez & Sánchez, 2017). Cyanobacteria and cyanotoxin analysis techniques are divided into qualitative and quantitative methods to identify and measure their presence. Qualitative methods such as fluorescence microscopy which allows the identification of cyanobacteria by using specific fluorochromes (Jin et al., 2018), or flow cytometry which allows rapid and accurate quantification of cyanobacterial cells (Peniuk et al., 2016). In contrast, quantitative methods such as liquid chromatography coupled to mass spectrometry (LC-MS) are used to identify and quantify cyanotoxins in water samples (Oehrle et al., 2010).

### *Molecular Aspects of Cyanotoxins and Analytical Tools for their Identification*

Microcystins are cyclic heptapeptides. The great diversity in the structures of cyanotoxins determines their molecular targets leading to a range of toxic effects in exposed organisms (Baliu-Rodriguez et al., 2022). MC-LR is one of several structural variants. It is the most studied since it is widely distributed and because of its toxicity, and it is considered a human carcinogen under chronic exposure (Huisman et al., 2018; Hernández et al., 2023). In contrast, cylindrospermopsin and anatoxin are heterocyclic alkaloids that possess a multi-organ toxicity with severe lesions of the liver, kidneys, and gastrointestinal tract among others (Yang et al., 2021). Metabolomic analysis for cyanotoxins involves a comprehensive approach that seeks to identify and quantify the complete set of metabolites present in soil and water at a specific time, to understand their distribution pathway, their toxicity, and their effects on human health and the environment (Rinehart, 1998). The precise identification can be performed following a global analysis of metabolites under certain conditions and/or focusing on a specific analysis of a group of known and related metabolites in which their mass-to-charge ( $m/z$ ) ratios and retention times are often identified using reference standards. As a powerful analytical tool, liquid chromatography (LC) is common in metabolomic analysis. High-performance LC (HPLC) separates compounds based on their interactions in stationary and mobile phases. Gas chromatography (GC) can also be used, especially for volatile compounds. In the case of cyanotoxins, once separated, these compounds are introduced into the mass spectrometer, where they are ionized and fragmented; ionization can be performed by different techniques such as electrospray (ESI) or

chemical ionization (CI) in LC-MS, and electron impact ionization (EI) in GC-MS. Detection is based on the mass-to-charge ratio ( $m/z$ ) of the ions generated, which allows the identification of cyanotoxins and related metabolites. High-resolution mass spectrometry (HRMS) provides higher accuracy in ion mass determination, improving selectivity and reliability in identifying cyanotoxins. For compound identification, databases and spectral libraries are used to identify peaks obtained by mass spectrometry, and subsequently mass spectra are compared and correlated with known profiles of cyanotoxins and other metabolites (Lee et al., 2010). Finally, the data obtained is subjected to multivariate statistical analysis to identify patterns, correlations, and significant differences between samples. It is important to note that these techniques are currently the most widely used because certified standards offer some advantages in terms of sensitivity, selectivity, and accuracy in the quantification of cyanotoxins in complex matrixes, such as environmental samples.

### *Molecular Techniques for Monitoring Potentially Harmful Cyanobacteria Blooms*

In recent years, genetic and genomic strategies have been used to identify the presence of toxigenic species of cyanobacteria, as well as cyanotoxin biosynthesis operons. 16S rRNA V3-V4 region is commonly used to identify bacteria using amplicon sequencing. Identification of cyanobacteria relies on other genetic markers, including the *rpoC1* gene, intergenic regions, and conserved sequences of the 16S rRNA ribosomal gene (Rudi et al., 2000, Fergusson & Saint, 2003). Furthermore, other PCR-based methods, combined with indirect quantification using quantitative PCR (qPCR), provide high sensitivity (Asai et al., 2002; Rueckert et al., 2007). Specific cyanobacteria 16S rRNA gene amplification has been used before, as well as fluorescence probes targeting unique sequences in the 16S rRNA gene (Al-Tebrineh et al., 2010). DNA arrays targeting the 16S rRNA of various cyanobacteria groups have shown good results, particularly in detecting genera like *Anabaena* and *Microcystis* (Castiglioni et al., 2004).

Moreover, a qPCR assay capable of detecting and quantifying toxin biosynthesis genes from the microcystins, nodularins, cylindrospermopsins, and saxitoxins biosynthesis pathways have been developed (Al-Tebrineh et al., 2010). The *mcy* genes responsible for microcystins synthesis have been used to identify the toxigenic potential for the extensively studied microcystins. The *mcy* operon shares significant sequence homology with the nodularin synthesis operon (*nda* genes) and is commonly used to evaluate toxigenic potential using the same primer pairs (Pearson et al., 2016). In contrast, the cylindrospermopsin gene cluster (*cyrA-O* genes), spanning 43 Kb, presents gene arrangements in several cyanobacteria genera. Due to the limited conservation of *cyrN* and *cyrO* genes in many strains, *aoaA*, *aoaB*, and *aoaC* genes have been proposed as reliable markers (Mihali et al., 2008). Saxitoxin, synthesized by the *sxt* genes cluster, is well-characterized in speci-

fic genera. The characterization of this cluster has facilitated the investigation of molecular mechanisms, such as the regulatory effects of the genes *sxtY*, *sxtZ*, and *ompR* on the gene cluster (Kellmann et al., 2008). Anatoxin-a synthesis involves the *ana* gene cluster, displaying variations in the position and direction of certain genes. Understanding these gene clusters aids in unraveling molecular mechanisms and regulatory effects on toxin production (Mihali et al., 2011).

### *High-throughput Sequencing for Toxigenic Cyanobacteria Identification and Abundance Monitoring in Freshwater Ecosystems*

The process to determine toxic and non-toxic cyanobacteria poses challenges due to the high similarity in the 16S rRNA region. Exploring operon clusters becomes essential for accurate differentiation. Studies on *Microcystis* reveal effective differentiation through combined amplification of 16S rRNA and 23S rRNA (Otsuka et al., 1999). For microcystin operon detection, *mcyA*, *mcyB*, and *mcyE* are commonly employed (Ouahid et al., 2009). Techniques combining hybridization and PCR of the genes *mcyE* and *ndaF* have been developed for the simultaneous detection of microcystin and nodularin clusters in various cyanobacteria genera (Castiglioni et al., 2004; Rantala et al., 2008; Al-Tebrineh et al., 2010). Analyses using qPCR establish relationships between toxigenic genotypes and cyanobacterial populations and provide insights into spatiotemporal dynamics (Briand et al., 2009). However, identifying active biosynthesis clusters remains challenging (Al-Tebrineh et al., 2010). To overcome challenges in identifying active biosynthesis clusters, the application of reverse transcription PCR (RT-PCR) methods is promising. These techniques establish a significant correlation between gene expression levels and cyanotoxin production, offering valuable insights into environmental influences. Their exceptional sensitivity and specificity make them suitable for monitoring potentially toxigenic cyanobacteria, aiding the application of predictive methods for estimating cyanotoxin concentrations under specific conditions (Duan et al., 2022).

The previous strategies are based on the application of the amplification and sequencing of 16S rRNA genes or genes involved in cyanotoxin biosynthesis. Previous studies have shown that although these strategies are useful for identifying potential cyanotoxin-producing genera and species, some strains may not necessarily possess the complete biosynthesis pathway or regulation to produce cyanotoxins, *e.g.*, *Microcystis* *mcyE* gene (Linz et al., 2023). Hence, other strategies such as metagenomics using high-throughput sequencing are necessary to confirm the presence of cyanotoxin-producing species or to monitor the abundance of species with the critical genes for cyanotoxin production (Caserio et al., 2019). Furthermore, deep metagenomics data can be useful to evaluate the abundance and dynamics of cyanotoxin-producing species.

*Nanopore Sequencing Rapid On-site Cyanobacteria Identification.*

Third-generation sequencing technologies, such as Oxford Nanopore Technologies (ONT) platforms, have several advantages that can be exploited to monitor toxic cyanobacteria presence in freshwaters. This technology can obtain sequences of a wide range of fragment lengths from short (20 bases) to ultra-long (>4 million bases), for each of the individual DNA or RNA fragments in a sample. Furthermore, the library preparation protocols and computational requirements are minimal with an accessible cost (Potvin et al., 2023). The MinION flow cell can generate an average of 20-25 Gigabases (Gb) of sequencing data after 72h, this is enough data to obtain complete cyanobacterial genomes. PCR-free library preparation protocols reduce the cost of equipment and laboratory processing time and can generate highly accurate data. This can be used to evaluate cyanobacterial abundance in freshwater through direct sequencing. ONT sequencing data can be processed in real-time. The number of sequenced reads written in each file can be controlled, e.g., 1,000 reads to generate a file; thus, each generated file can be analyzed in a bioinformatic workflow while sequencing is running. Data is available to take actions according to results, for example, if potential toxigenic cyanobacteria are identified, amplification steps can be used to evaluate the presence of cyanotoxin biosynthesis clusters by specific PCR amplification according to identified species (Fig. 1). A recent study shows a strategy for rapid on-site detection of harmful algal blooms (RosHAB). RosHAB's study consists of freshwater sampling, genomic DNA preparation, metagenome sequencing, and data analyses using a MinION device. When positive or inconclusive results are obtained, PCR or amplicon sequencing can be useful to identify the toxigenic potential of the cyanobacteria present in samples by comparison with genes previously used to confirm toxigenic potential (Duan et al., 2022; Potvin et al., 2023). Examples of biosynthesis operons of the main cyanotoxins: nodularin, cylindrospermopsin, microcystin, saxitoxin, and anatoxin are shown in Figure 1 (Kellmann et al., 2008; Pearson et al., 2010; Mihali et al., 2011). Even so, if sequencing leads to concerning or inconclusive results, the sample can be reanalyzed using high-throughput metagenomics. Then, data is deposited in the International Cyanobacterial Toxin Database (ICYATOX; [www.icyatox.ca](http://www.icyatox.ca)).



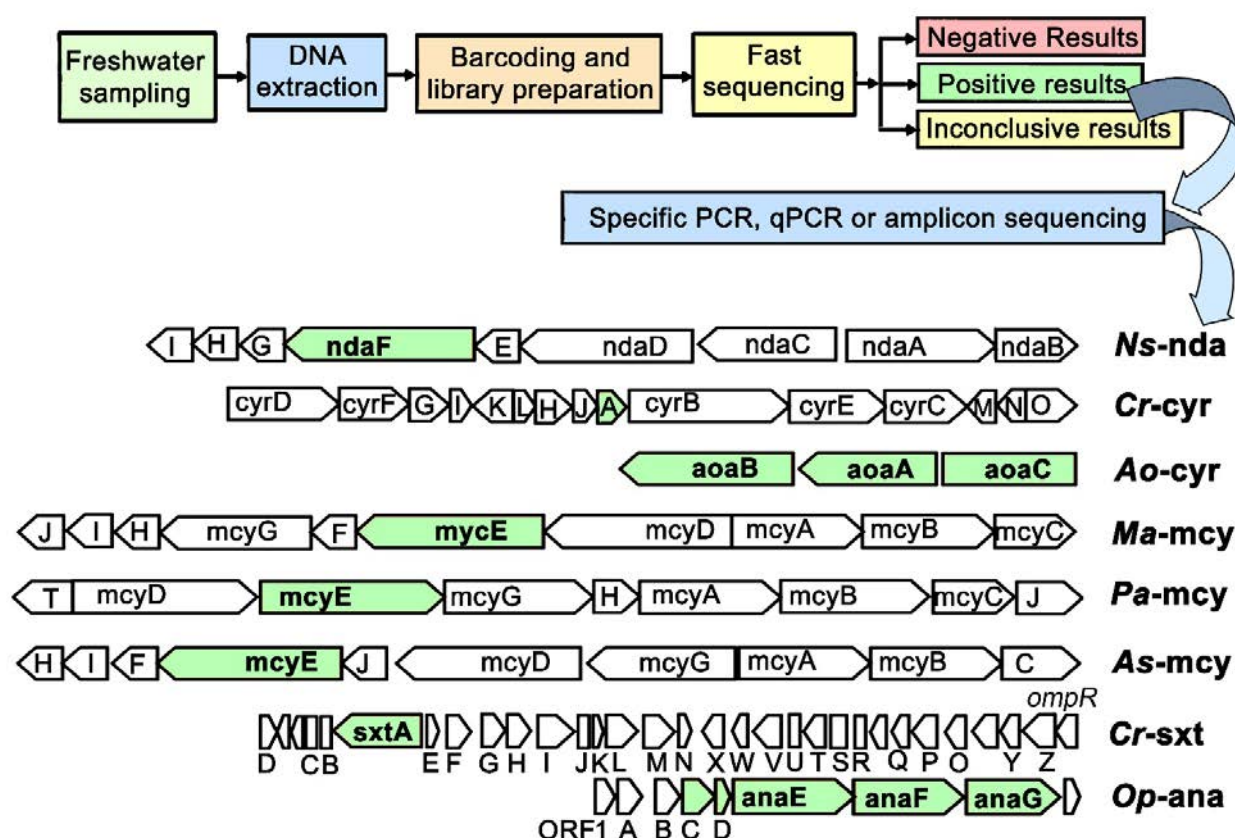


**Figure 1.** Rapid On-site Detection of Harmful Algal Blooms Strategy and Cyanotoxins Biosynthesis Clusters. The Biosynthesis Operons of *Nodularia spumigena* nodularin (Ns-nda); *Cylindrospermopsis raciborskii* cylindrospermopsin (Cr-cyr); *A. ovalisporum* cylindrospermopsin (Ao-cyr); *Microcystis aeruginosa* microcystin (Ma-mcy); *Planktothrix agardhii* microcystin (Pa-mcy); *Anabaena* sp. microcystin (As-mcy); *Cylindrospermopsis raciborskii* saxitoxin (Cr-sxt); *Oscillatoria* pcc. anatoxin (Op-ana) are shown.

Each new cyanobacterial genome and its metadata contributes to developing a more accurate reference database which will increase in length over time. Potvin et al. (2023) results show that a 30-minute Flongle cell sequencing run was sufficient to obtain reliable cyanobacterial relative abundance profiles, obtaining real-time results at the species level. Using this method, they identified *Anabaena*, *Calothrix*, *Chamaesiphon* spp, *Dolichospermum*, *Planktothrix*, *Pseudanabaena*, *Oscillatoria*, and *Nostoc*. Therefore, similar strategies can be used in Ibero-America to monitor locations where cyanobacterial harmful algal blooms have been reported monitoring their population abundance. If this strategy is made through seasons, and obtained data is correlated with other features, it can be useful to predict dangerous blooms in multiple locations.

## Optical Techniques for Monitoring Potentially Harmful Cyanobacteria Blooms

The need to monitor cyanobacteria in water bodies to prevent the release of toxins, odors, and water discoloration has led researchers to develop different sensing techniques (Moraire et al., 2014). Unique radiative and bio-optical properties must be exploited in optical methods for detecting cyanobacteria. For detection and quantification *in situ*, *in vivo*, in real-time, and in an automated manner, spectrofluorometric or spectroradiometric techniques must be used. In this way, it is important to determine the absorbance and/or reflectance spectra by non-destructive optical technique.

Through detection, monitoring, and prediction of cyanobacteria blooms, water quality in aquatic environments can be determined to guarantee its safe consumption and use (Cannizzaro et al., 2019). Cyanobacteria can be detected primarily using optical biosensors, spectrophotometry, and remote sensing (radiometer). Remote sensing is preferred for studying large water bodies where unmanned vehicles such as drones or satellites are used to perform indirect contact monitoring using optical devices. Using an appropriately structured algorithm will increase the relevant quality and resolution of data. The reflectance and spectral absorption characteristics of the optical sensors used should allow the determination of the presence, growth, and effects of cyanobacteria without having direct contact with them. Recent advances are driving the use of biosensors due to their excellent reproducibility, robust platform, high sensitivity, low cost, ease of use, and real-time identification. The main disadvantage is the lack of approval for it to be used as a verified method for determining quantitatively toxins. Biosensors use platforms based on immunoassays, optical principles, or based on highly sensitive nanomaterials to perform qualitative and/or quantitative determination of toxins. Biosensors can be exploited using four different strategies to quantify toxins: limit of detection (LOD) and/or dynamic range (DR), variation between measurements, assay time, and feasibility of the point-of-care (POC). For example, biosensors can use optical methods such as chemiluminescence, fluorescence, and optical immunoassay. Ultraviolet-visible (UV-Vis) spectrophotometry can be used to reach the detection limit of toxins produced by cyanobacteria (Malhotra et al., 2021). Among the water quality parameters that can be reviewed are total suspended solids (TSS), ultraviolet transmittance (UVT), dissolved organic matter (DOM), biological oxygen demand (BOD), and chemical oxygen demand (COD). The presence of cyanobacteria is verified in the range of 540 to 685 nm.

Recently, machine learning (ML) models from neural networks in optical radiometers have been introduced to quantify the presence of cyanobacteria in reservoirs, lakes, as well as inland and coastal waters using the wide spectral resolution of the phycocya-

nin (PC) photosynthetic pigments contained in them (Zolfaghari et al., 2022). Through the radiometer, it is possible to obtain hyperspectral images using remote optical reflectance sensing to detect cyanobacterial toxins in water bodies. The difficulty in detecting these toxins is that they tend to accumulate on the surface of water consumed by humans and animals since these are present in different species of cyanobacteria with very different toxicity, morphology, and cell sizes. Finally, pigments in water allow remote sensing of cyanobacteria through changing reflectance to obtain hyperspectral images using drones over wide areas (Kwon et al., 2020).

## Conclusions

The global increase in cyanobacterial blooms is an undeniable reality that continues to become more complicated by pollution. Chemical and molecular cyanotoxin analysis should be standardized, validated, and must be made accessible to communities using cyanotoxin-contaminated water. In addition, practical guidelines should be provided to prevent risks to human health and the ecosystem. It is necessary to investigate the effect of cyanotoxins on freshwater ecosystems and food crops, as well as alternatives for their degradation and control. Similarly, the constant monitoring of microbial populations is crucial to generate concrete actions and new strategies to avoid the causes that lead to this type of bloom and establish the original natural state of water bodies. This is an open field of research in Mexico that requires immediate attention.

## Authors' Contributions

JNGC, CPRC, RAR, ASRS and JGCM Writing. LVS, JLCG and RVB writing, review and editing.

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# Free Living Continental Aquatic Ciliates (Alveolata: Ciliophora) from Mexico: An Overview of their Species Richness and Distribution

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## Abstract

**W**e present the data of the ciliate species including free-swimming and sessile that have been recorded in Mexico inhabiting different types of continental water bodies. We found that 294 ciliate species were documented in 16 Mexican states through the period from 1922 to 2023. This species richness represents around 28.7 % of the more than 1,000 ciliate species recorded for Mexico and indicates that its study is still incomplete and that there exist possibilities for new records and new species descriptions.

## Keywords

Protists, historical approach, biogeographic areas.

## Introduction

Ciliates are a group of protists distinguished by the presence of cilia derived from kinetosomes with three fibrillar associates, two types of nuclei (nuclear dimorphism), and conjugation as a sexual process, and with more than 8,000 described species (Lynn, 2008, 2017). They are mainly heterotrophs and very common in aquatic ecosystems which have been studied around the world.

Their functional role as primary consumers has been documented, playing a key role in aquatic planktonic and benthic food webs, and the study of their diversity and ecology is essential to understanding limnological processes, providing valuable perspectives into ecosystem functioning. Ciliates participate in the microbial loop which is an important process of the trophic food web affecting carbon, nutrient, and energy flows being an important link in lake ecosystems to higher trophic levels such as copepods and fish (Pomeroy, 1974; Azam et al., 1983; Sanders et al., 1989; Sherr et al., 1991; Pomeroy et al., 2007). In sediments of freshwater ecosystems, benthic ciliate communities are usually the dominant protists (Finlay et al., 1988; Finlay & Esteban, 1998).

The community structure of ciliates is highly dynamic and responds rapidly to changes in the environment and ecosystem; for example, their abundance and biomass tend to increase along a trophic gradient from oligotrophy to eutrophy (Zingel, 2001). A progressive replacement of large cell ciliates (40-50  $\mu\text{m}$ ) by small cell ciliates (20-30  $\mu\text{m}$ ) has been observed as a trophic state increased in freshwater lakes as a response to changes in food availability (Beaver & Crisman, 1982). Aleya et al. (1992) suggested that some facultative anaerobic species such as *Loxodes striatus* may be significant contributors to denitrification in eutrophic lakes. Barouillet et al. (2022) documented that in recent times, some lowland lakes exposed to local human pressures have shown a decreasing  $\beta$ -diversity of ciliates, by changing assemblages of species with mixotrophs; benthic ciliates communities have also changed in response to increasing in deep water anoxia.

In Mexico, due to its geographic position where two biogeographical regions (Neotropical and Nearctic) are present (Morrone, 2019), the habitats that provide suitable conditions for their presence are distributed along the entire area, even so, the knowledge of the ciliate species is still incomplete despite its ecological importance. The purpose of this review is to compile the ciliate species that have been recorded in aquatic continental ecosystems to show their distribution across the country and the historical development of its taxonomic knowledge.

## Methodology

We search for the recorded binomial species from Mexico in continental aquatic environments including rivers, lakes, dams, temporal reservoirs, channels, swamps, wetlands, waterfalls, caves, fish farms, and plant reservoirs such as phytotelmata. We consulted López-Ochoterena and Roure-Cane (1970), Madrazo-Garibay and López-Ochoterena (1982), Aladro-Lubel et al. (2006), the search engine Google Scholar, and the <https://tesiuam.dgb.unam.mx/>, <http://ri.uaemex.mx/>, <http://redi.uady.mx/>, <https://www.riudg.udg.mx/>, <https://repositorioinstitucional.buap.mx/>, <https://ri.ujat.mx/>, <https://cdigital.uv.mx/>, <https://ri-ng.uaq.mx/>, <http://bdigital.dgse.uaa.mx:8080/>, <https://www.repositoriodigital.ipn.mx/>, <http://tesiuami.izt.uam.mx/>, <https://bibliotecavirtual.dgb.umich.mx/>, and <https://repositorio.unicach.mx/> databases.

The species recorded in published articles, books, book chapters, and thesis, were included encompassing a period from 1922 to 2023. Each ciliate genera and species names were checked by consulting Aescht (2001), Lynn (2008), and Mayén-Estrada et al. (2020), to eliminate the synonyms and to update their taxonomy. The ciliate species were organized according to the Mexican states and localities, and the number of species was counted. We grouped the genera according to their species number. We also performed a distributional map by states and one for biogeographical areas.

## Results

We obtained that, 153 genera including 294 ciliate species, have been reported for Mexico distributed along 16 states (Tables 1 and 2). Most of the genera contained only one species each, and only for the genus *Paramecium*, we found 14 species (Table 1). The total species richness obtained represents around 28.6 % of the more than 1,000 ciliate species reported for Mexico (Mayén-Estrada et al., 2020). Some examples of species are shown in Fig. 1.

**Table 1.** Genera of Ciliates and their Species Number Recorded in Mexican Continental Habitats.

| Number of species | Genera                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
|-------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 1                 | <i>Anigsteinia</i> , <i>Apoamphisiella</i> , <i>Apogastrostyla</i> , <i>Askenasia</i> , <i>Balantidion</i> , <i>Belonophrya</i> , <i>Bromeliophrya</i> , <i>Bromeliothrix</i> , <i>Bursaria</i> , <i>Bursellopsis</i> , <i>Calypotricha</i> , <i>Carchesium</i> , <i>Chlamydonellopsis</i> , <i>Cinetochilum</i> , <i>Cladotricha</i> , <i>Clathrostoma</i> , <i>Climacostomum</i> , <i>Codonella</i> , <i>Coriplites</i> , <i>Cotterillia</i> , <i>Cryptochilum</i> , <i>Ctedoctema</i> , <i>Cyrtohymena</i> , <i>Cyrtolophosis</i> , <i>Dexiostoma</i> , <i>Dexiotricha</i> , <i>Didinium</i> , <i>Discophrya</i> , <i>Enchelydium</i> , <i>Enchelyomorpha</i> , <i>Enchelys</i> , <i>Fragmocirrus</i> , <i>Gastrostyla</i> , <i>Glaucoides</i> , <i>Halteria</i> , <i>Heliophrya</i> , <i>Holophrya</i> , <i>Holosticha</i> , <i>Ileonema</i> , <i>Isocyclidium</i> , <i>Lambornella</i> , <i>Lembadion</i> , <i>Limnostrombidium</i> , <i>Linostomella</i> , <i>Loxocephalus</i> , <i>Loxophyllum</i> , <i>Luxophrya</i> , <i>Malacophrys</i> , <i>Microthorax</i> , <i>Monochilum</i> , <i>Monodinium</i> , <i>Multifasciculatum</i> , <i>Mykophagophrys</i> , <i>Neobryophyllum</i> , <i>Obertrumia</i> , <i>Odontochlamys</i> , <i>Onychodromus</i> , <i>Ophrydium</i> , <i>Opisthonecta</i> , <i>Parabryophrya</i> , <i>Paracolpidium</i> , <i>Paracolpoda</i> , <i>Paradileptus</i> , <i>Parapodophrya</i> , <i>Paroxytricha</i> , <i>Pelagohalteria</i> , <i>Phascolodon</i> , <i>Philasterides</i> , <i>Plagiopyla</i> , <i>Platynematum</i> , <i>Plesiocaryon</i> , <i>Pleuronema</i> , <i>Podophrya</i> , <i>Propygidium</i> , <i>Pseudoblepharisma</i> , <i>Pseudocyrtolophosis</i> , <i>Pseudoglaucoma</i> , <i>Pseudokeronopsis</i> , <i>Pseudomonilicaryon</i> , <i>Pseudourostyla</i> , <i>Rimaleptus</i> , <i>Rimostrombidium</i> , <i>Sathrophilus</i> , <i>Siroloxophyllum</i> , <i>Sphaerophrya</i> , <i>Sterkiella</i> , <i>Stichotricha</i> , <i>Stokesia</i> , <i>Strombidium</i> , <i>Systylis</i> , <i>Tachysoma</i> , <i>Telotrochidium</i> , <i>Thuricola</i> , <i>Tintinnidium</i> , <i>Trachelius</i> , <i>Trachelophyllum</i> , <i>Trichophrya</i> , <i>Trichodina</i> , <i>Trimyema</i> , <i>Trithigmostoma</i> , <i>Trochilioides</i> , <i>Urocentrum</i> , <i>Urosoma</i> , <i>Vorticellides</i> , <i>Zosterodasys</i> |
| 2                 | <i>Acineta</i> , <i>Actinobolina</i> , <i>Anteholosticha</i> , <i>Brachonella</i> , <i>Caenomorpha</i> , <i>Frontonia</i> , <i>Metacineta</i> , <i>Metopus</i> , <i>Nassula</i> , <i>Pelagovorticella</i> , <i>Platyophrya</i> , <i>Protocyclidium</i> , <i>Pseudovorticella</i> , <i>Rhabdostyla</i> , <i>Strobilidium</i> , <i>Trochilia</i> , <i>Uronema</i> , <i>Zoothamnium</i>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
| 3                 | <i>Amphileptus</i> , <i>Aspidisca</i> , <i>Chilodonella</i> , <i>Colpidium</i> , <i>Cothurnia</i> , <i>Drepanomonas</i> , <i>Epistylis</i> , <i>Spirostomum</i> , <i>Urotricha</i>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |

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| 4  | <i>Cyclidium, Glaucoma, Gonostomum, Lacrymaria, Leptopharynx, Loxodes, Spathidium, Tetrahymena, Tokophrya</i> |
| 5  | <i>Blepharisma, Litonotus, Opercularia, Stylonychia, Uroleptus</i>                                            |
| 6  | <i>Coleps</i>                                                                                                 |
| 7  | <i>Colpoda, Oxytricha</i>                                                                                     |
| 8  | <i>Stentor</i>                                                                                                |
| 11 | <i>Euplotes</i>                                                                                               |
| 12 | <i>Vorticella</i>                                                                                             |
| 14 | <i>Paramecium</i>                                                                                             |

**Table 2.** Mexican States and Continental Localities with Ciliate Records.

| States/Localities                                         | References                                           |
|-----------------------------------------------------------|------------------------------------------------------|
| <b>Aguascalientes</b>                                     |                                                      |
| Abelardo L. Rodríguez Water Dam                           | Tiscareño-Silva, 2008; Silva-Briano et al., 2022     |
| Aguascalientes City, Phytotelmata of Epiphytic Bromeliads | Romero-García, 2007                                  |
| Bordo El Salitre                                          | Tiscareño-Silva, 2008                                |
| Bordo La Cotorra                                          | Tiscareño-Silva, 2008                                |
| El Bordo                                                  | Tiscareño-Silva, 2008                                |
| El Huarache water dam                                     | Tiscareño-Silva, 2008                                |
| El Refugio Puddle                                         | Silva-Briano et al., 2022                            |
| El Tecuancillo Dam                                        | Silva-Briano et al., 2022                            |
| La Carreta Water Dam                                      | Tiscareño-Silva, 2008                                |
| La Providencia Water Dam                                  | Tiscareño-Silva, 2008                                |
| La Tomatina Pond                                          | Silva-Briano et al., 2022                            |
| Los Jiménez Tank                                          | Tiscareño-Silva, 2008                                |
| Lumbreras Water Dam                                       | Tiscareño-Silva, 2008                                |
| Malpaso Wáter Dam                                         | Silva-Briano et al., 2022                            |
| Rincón de Romos                                           | Flores and Martínez, 1993; Silva-Briano et al., 2022 |
| San Pedro River                                           | Tiscareño-Silva, 2008                                |
| San Pedro Swamp                                           | Tiscareño-Silva, 2008                                |

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| <b>Chiapas</b>                                                       |                                                                                                                                                                                                                                                                                                                         |
|----------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Montebello Lakes                                                     | Méndez-Sánchez, 2017;<br>Ramírez-Ballesteros et al., 2021                                                                                                                                                                                                                                                               |
| Montebello Lakes National Park, Phytotelmata of Epiphytic Bromeliads | Durán-Ramírez et al., 2020;<br>Ramírez-Ballesteros et al., 2021;<br>Durán-Ramírez and Mayén-Estrada, 2022                                                                                                                                                                                                               |
| Tziscão Lake                                                         | Méndez-Sánchez, 2017; Méndez-Sánchez et al., 2018b, 2022                                                                                                                                                                                                                                                                |
| <b>Guerrero</b>                                                      |                                                                                                                                                                                                                                                                                                                         |
| Cacalotenango Waterfall                                              | Teyer-Santiago et al., 2021                                                                                                                                                                                                                                                                                             |
| La Joya Cave                                                         | Sigala-Regalado, 2011                                                                                                                                                                                                                                                                                                   |
| <b>Hidalgo</b>                                                       |                                                                                                                                                                                                                                                                                                                         |
| Endhó Water Dam                                                      | Potekhin and Mayén-Estrada, 2020;<br>Melekhin et al., 2022                                                                                                                                                                                                                                                              |
| La Mora de Actopan Spring                                            | Sokoloff, 1936                                                                                                                                                                                                                                                                                                          |
| La Noria de Tula                                                     | Sokoloff and Ancona, 1937                                                                                                                                                                                                                                                                                               |
| Requena Water Dam                                                    | Melekhin et al., 2022                                                                                                                                                                                                                                                                                                   |
| Tlanchinol El Tegalome Phytotelmata of Epiphytic Bromeliads          | Durán-Ramírez et al., 2020; Durán-Ramírez and Mayén-Estrada, 2022                                                                                                                                                                                                                                                       |
| Tula River                                                           | Sokoloff and Ancona, 1937                                                                                                                                                                                                                                                                                               |
| Tula spring                                                          | Sokoloff and Ancona, 1937                                                                                                                                                                                                                                                                                               |
| <b>Mexico City</b>                                                   |                                                                                                                                                                                                                                                                                                                         |
| Atlapulco Channel                                                    | Moreno-Barón, 1976; Moreno-Barón and López-Ochoterena, 1976                                                                                                                                                                                                                                                             |
| Chapultepec Lakes                                                    | Bravo-Hollis, 1922; Sokoloff, 1931; Sámano and Sokoloff, 1931; Elliot and Hayes, 1955; Pérez-Reyes and Salas-Gómez, 1961; López-Ochoterena, 1962, 1965, 1992; López-Ochoterena and Barajas de López, 1963; González-Labastida, 1995; Lugo-Vázquez et al., 1998; Potekhin and Mayén-Estrada, 2020; Melekhin et al., 2022 |
| Consulado River                                                      | Elliot and Hayes, 1955                                                                                                                                                                                                                                                                                                  |
| Cuemanco Channel                                                     | López-Ochoterena, 1999                                                                                                                                                                                                                                                                                                  |
| Eastern Quarry (Cantera Oriente)                                     | Aladro-Lubel et al., 2007, 2009; Lugo-Vázquez et al., 2017; Saadi-Gómez, 2018; Sánchez-Sánchez, 2018; Potekhin and Mayén-Estrada, 2020; Melekhin et al., 2022                                                                                                                                                           |
| Fuentes Brotantes Lake                                               | Elliot and Hayes, 1955                                                                                                                                                                                                                                                                                                  |

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|--------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Instituto de Salubridad y Enfermedades Tropicales Pond | Pérez-Reyes and Salas-Gómez, 1961                                                                                                                                                                                                                                                                                                                                                |
| La Magdalena River                                     | Elliot and Hayes, 1955; Nomdedeu and López-Ochoterena, 1988; Mayén-Estrada et al., 2022                                                                                                                                                                                                                                                                                          |
| Los Dinamos Water Dam                                  | Potekhin and Mayén-Estrada, 2020                                                                                                                                                                                                                                                                                                                                                 |
| San Luis Tlaxiátemalco                                 | Pérez-Reyes and Salas-Gómez, 1961                                                                                                                                                                                                                                                                                                                                                |
| Tezozómoc Lake                                         | Cabral-Dorado, 2006; Ortíz-Haro, 2011; Sánchez-Rodríguez et al., 2011                                                                                                                                                                                                                                                                                                            |
| Tláhuac Lake                                           | Potekhin and Mayén-Estrada, 2020; Melekhin et al., 2022                                                                                                                                                                                                                                                                                                                          |
| Xochimilco Lake                                        | Bravo-Hollis, 1922; Beltrán, 1929; Sokoloff, 1930a-c; Sámano and Sokoloff, 1931; Elliot and Hayes, 1955; Pérez-Reyes and Salas-Gómez, 1961; Roure-Cané, 1969; Madrazo-Garibay and López-Ochoterena, 1973a, 1985; Moreno-Barón, 1976; Moreno-Barón and López-Ochoterena, 1976; Serrano-Limón and López-Ochoterena, 1992; López-Ochoterena, 1999; Potekhin and Mayén-Estrada, 2020 |
| <b>Mexico State</b>                                    |                                                                                                                                                                                                                                                                                                                                                                                  |
| Atarasquillo Wetland (=Chignahuapan Lake)              | Méndez-Sánchez, 2014, Méndez-Sánchez et al., 2018a                                                                                                                                                                                                                                                                                                                               |
| Chalco Lake                                            | Bravo-Hollis, 1924                                                                                                                                                                                                                                                                                                                                                               |
| Dolores Water Dam                                      | Pérez-Reyes and Salas-Gómez, 1961                                                                                                                                                                                                                                                                                                                                                |
| El Velo de la Novia Waterfall                          | Flores-Flores, 2012                                                                                                                                                                                                                                                                                                                                                              |
| Guadalupe Water Dam                                    | Pérez-Reyes and Salas-Gómez, 1961; Gallegos-Neyra et al., 2009                                                                                                                                                                                                                                                                                                                   |
| La Concepción San Pedro Spring                         | Flores-Flores, 2012                                                                                                                                                                                                                                                                                                                                                              |
| La Concha Water Dam                                    | Pérez-Reyes and Salas-Gómez, 1961                                                                                                                                                                                                                                                                                                                                                |
| Lerma Swamps                                           | Madrazo-Garibay and López-Ochoterena, 1985; Gallegos-Neyra et al., 2009; Potekhin and Mayén-Estrada, 2020; Melekhin et al., 2022                                                                                                                                                                                                                                                 |
| Los Remedios River                                     | Chilpa-Navarrete, 1998                                                                                                                                                                                                                                                                                                                                                           |
| Ocotál Lake                                            | Méndez-Sánchez, 2017                                                                                                                                                                                                                                                                                                                                                             |
| San Juan Teotihuacán                                   | Pérez-Reyes and Salas-Gómez, 1961                                                                                                                                                                                                                                                                                                                                                |
| San Miguel Almaya                                      | Melekhin et al., 2022                                                                                                                                                                                                                                                                                                                                                            |
| Sierra de Tepotzotlán Streams                          | González-Palma, 2016                                                                                                                                                                                                                                                                                                                                                             |
| Tiacaque Spring                                        | Méndez-Sánchez, 2017                                                                                                                                                                                                                                                                                                                                                             |
| Valle de Bravo Water Dam (Miguel Alemán Water Dam)     | Peštová, 2007; Gallegos-Neyra et al., 2009                                                                                                                                                                                                                                                                                                                                       |

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| <b>Michoacán</b>                                             |                                                                                                                                    |
|--------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------|
| Huarimio Caves                                               | Sigala-Regalado, 2011                                                                                                              |
| Los Espinos Crater Lake                                      | Rendón-López, 2008                                                                                                                 |
| Pátzcuaro Lake                                               | Ancona et al., 1940; Osorio-Tafall, 1944; López-Ochoterena and Roure-Cané, 1970                                                    |
| Tziranda Caves                                               | Sigala-Regalado, 2011                                                                                                              |
| Zacapu Fish Farm                                             | Herróz-Zamorano, 1998                                                                                                              |
| <b>Morelos</b>                                               |                                                                                                                                    |
| Cuernavaca Ricefields                                        | Santiago-Fragoso, 1969                                                                                                             |
| Salto de San Antón Waterfall                                 | Madrazo-Garibay and López-Ochoterena, 1973b                                                                                        |
| Zempoala Lake                                                | Pérez-Reyes and Salas-Gómez, 1961                                                                                                  |
| <b>Oaxaca</b>                                                |                                                                                                                                    |
| Analco Lake                                                  | Méndez-Sánchez, 2017; Méndez-Sánchez et al., 2020                                                                                  |
| El Estudiante Water Dam                                      | Méndez-Sánchez et al., 2020                                                                                                        |
| Ixtlán de Juárez Phytotelmata of Epiphytic Bromeliads        | Durán-Ramírez et al., 2015, 2020                                                                                                   |
| La Azucena Water Dam                                         | Méndez-Sánchez, 2017                                                                                                               |
| Santa Catarina Ixtepeji Phytotelmata of Epiphytic Bromeliads | Durán-Ramírez and Mayén-Estrada, 2022                                                                                              |
| Zimatlán de Alvarez Fish Farm                                | Rivas-Beltrán et al., 2023                                                                                                         |
| <b>Puebla</b>                                                |                                                                                                                                    |
| Alchichica Lake                                              | Lugo-Vázquez, 1993, 2000; Lugo-Vázquez et al., 1998; Peštová, 2007; Macek et al., 2008; Peštová et al., 2008; Sánchez-Medina, 2013 |
| Aljojuca Lake                                                | Lugo-Vázquez, 1993                                                                                                                 |
| Atexcac Lake                                                 | Lugo-Vázquez, 1993; Macek et al., 1994; Lugo-Vázquez et al., 1998; Peštová, 2007                                                   |
| Cuetzalan Phytotelmata of Epiphytic Bromeliads               | Durán-Ramírez et al., 2020, Durán-Ramírez and Mayén-Estrada, 2022                                                                  |
| Karmidas Caves                                               | Sigala-Regalado, 2011                                                                                                              |
| La Preciosa Lake                                             | Lugo-Vázquez, 1993; Sánchez-Medina, 2013                                                                                           |
| Quechulac Lake                                               | Lugo-Vázquez, 1993                                                                                                                 |
| Tecuitlapa Lake                                              | Lugo-Vázquez, 1993                                                                                                                 |
| <b>Querétaro</b>                                             |                                                                                                                                    |
| Amealco Wetland                                              | Potekhin and Mayén-Estrada, 2020                                                                                                   |
| El Cajón Water Dam                                           | Velázquez-Medina et al., 2020                                                                                                      |
| Los Riscos Cave                                              | Sigala-Regalado, 2008; Sigala-Regalado et al., 2011                                                                                |
| San Miguel Almaya Wetland                                    | Potekhin and Mayén-Estrada, 2020; Melekhin et al., 2022                                                                            |

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| <b>Quintana Roo</b>                                                |                                                                             |
|--------------------------------------------------------------------|-----------------------------------------------------------------------------|
| Bacalar Lagoon                                                     | Potekhin and Mayén-Estrada, 2020; Cruz-Jiménez, 2022; Melekhin et al., 2022 |
| Cenote Azul                                                        | Potekhin and Mayén-Estrada, 2020                                            |
| Chunyaxché-Sian Ka'an Lagoon                                       | Potekhin and Mayén-Estrada, 2020                                            |
| Sian Ka'an Biosphere Reserve                                       | Melekhin et al., 2022                                                       |
| <b>San Luis Potosí</b>                                             |                                                                             |
| Moctezuma River                                                    | Elliot and Hayes, 1955                                                      |
| <b>Tamaulipas</b>                                                  |                                                                             |
| Tamesí River                                                       | Elliot and Hayes, 1955                                                      |
| <b>Veracruz</b>                                                    |                                                                             |
| Acajete Phytotelmata of Epiphytic Bromeliads                       | Durán-Ramírez et al., 2019, 2020                                            |
| Bosque de Niebla Sanctuary                                         | Potekhin and Mayén-Estrada, 2020                                            |
| Bosque de Niebla Sanctuary Phytotelmata of Epiphytic Bromeliads    | Durán-Ramírez et al., 2019, 2020; Durán-Ramírez and Mayén-Estrada, 2022     |
| Coatepec Phytotelmata of Epiphytic Bromeliads                      | Durán-Ramírez, 2010, Durán-Ramírez et al., 2015, 2019, 2020                 |
| Galicia Grottoes                                                   | Sigala-Regalado, 2011                                                       |
| Jalcomulco Phytotelmata of Epiphytic Bromeliads                    | Durán-Ramírez et al., 2019, 2020                                            |
| Tuzamapan phytotelmata of Epiphytic Bromeliads                     | Durán-Ramírez et al., 2019, 2020                                            |
| Veracruz temporal pond near the harbor                             | Hernández-Anaya, 1981                                                       |
| Xalapa Phytotelmata of Epiphytic Bromeliads                        | Durán-Ramírez et al. 2015, 2020; Foissner, 2016; Foissner and Stoeck, 2011  |
| <b>Yucatan</b>                                                     |                                                                             |
| Oxkutzcab Biocultural Reserve Phytotelmata of Epiphytic Bromeliads | Durán-Ramírez et al., 2020, Durán-Ramírez and Mayén-Estrada, 2022           |
| Temozon Reservoir                                                  | Potekhin and Mayén-Estrada, 2020                                            |

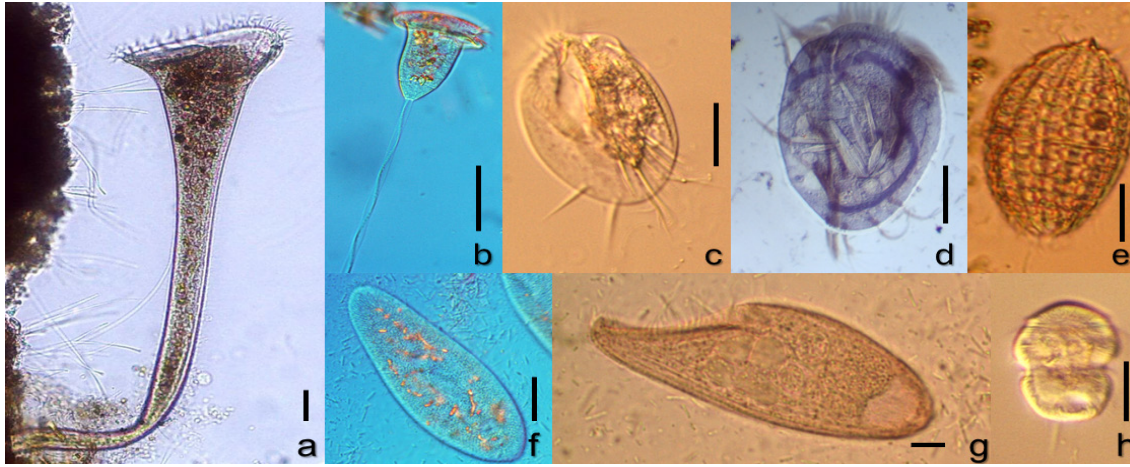


**Figure 1.** Some Ciliate Species from Continental Aquatic Ecosystems in Mexico. a. *Stentor coeruleus* from El Cajón Dam (Querétaro); b. *Vorticella* sp. from Cantera Oriente (Mexico City); c. *Euplotes* sp. from Cantera Oriente (Mexico City); d. *Euplotes eurystomus* from Tziscão Lake (Chiapas); e. *Coleps hirtus*, f. *Paramecium* sp. and g. *Blepharisma* sp. from Cantera Oriente (Mexico City), h. *Urocentrum turbo* from Montebello Lakes (Chiapas). Images a-c and e-h: Cells *In Vivo*; d: Stained with Harris' Hematoxylin Technique. Scale Bar: 10  $\mu$ m. Credits: M. Velázquez (a); V. M. Romero-Niembro (b, c, e, f, g); D. Méndez (d, h).

The largest number of species has been reported for central Mexico, being Mexico City, State of Mexico, Puebla, and Veracruz, the states that concentrate about 55 % of species richness (Fig. 2). For Mexico City, 201 species have been reported, and for Tamaulipas state, only one species was reported inhabiting a river (Table 1).

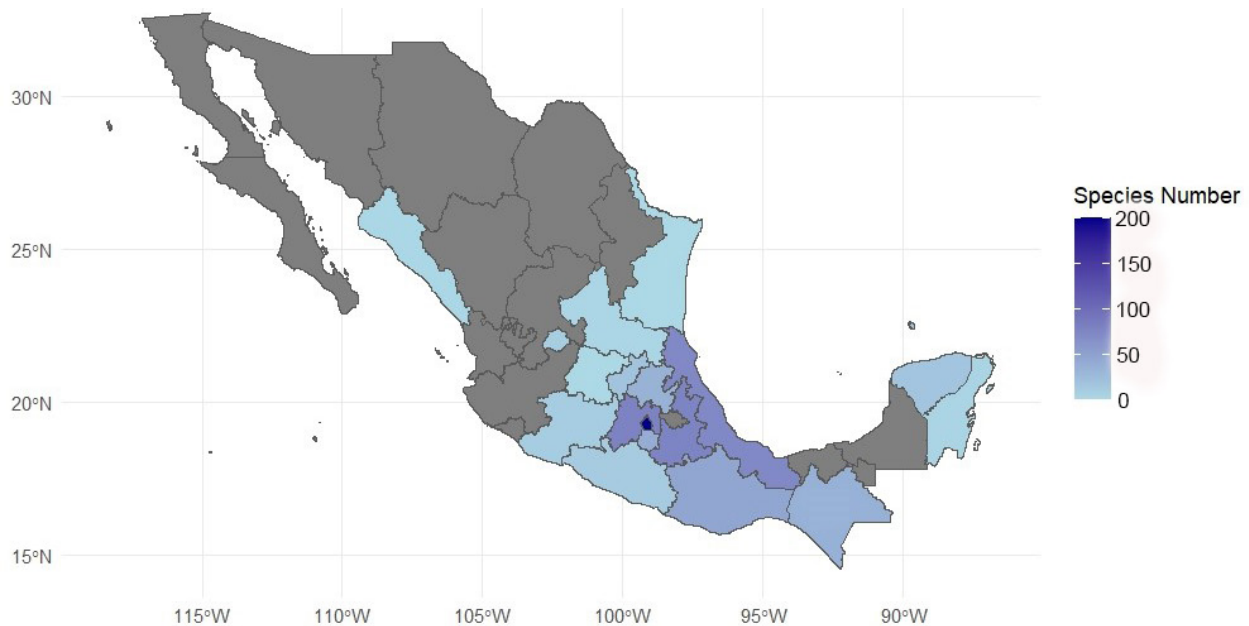


**Figure 2.** Number of Species in Continental Systems by Mexican States. The Gray Colour Indicates the Absence of Ciliate Species Record.

The biogeographical distribution of the continental ciliate species corresponds to two regions, Nearctic and Neotropical, and eight Mexican provinces (Fig. 3 and 4). The biogeographic regionalization is according to Morrone (2019).

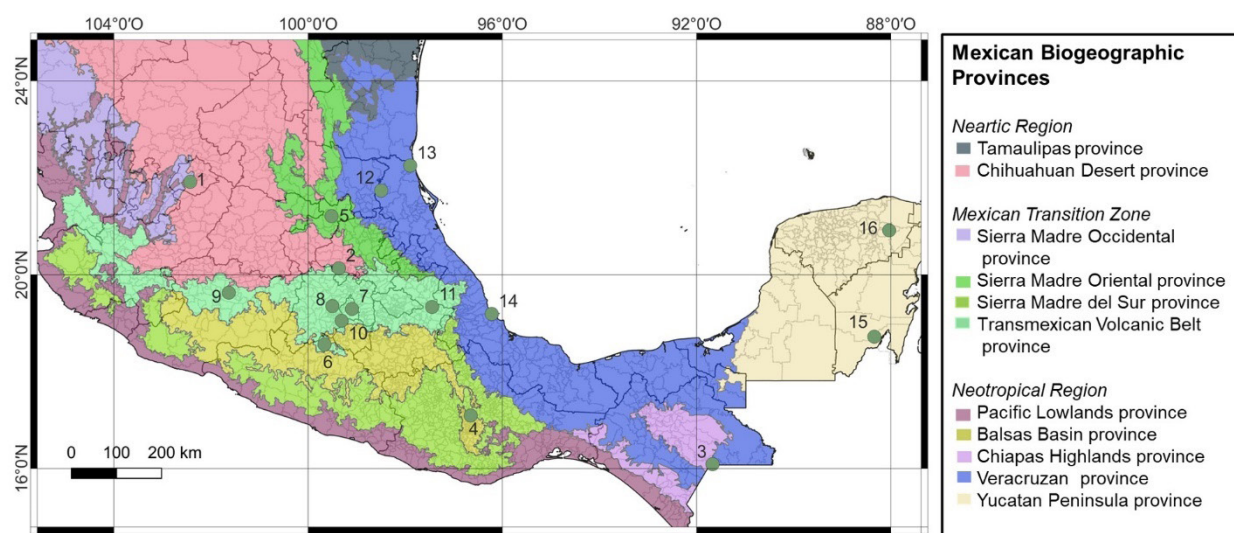


**Figure 3.** Biogeographic Mexican Provinces sensu Morrone (2019) of Mexican Republic States Where Species of Ciliated Protists Have Been Recorded in Continental Aquatic Environments. Chihuahuan Desert Province (<sup>1</sup>Aguascalientes State, <sup>2</sup>Hidalgo State); Chiapas Highlands Province (<sup>3</sup>Chiapas State); Sierra Madre del Sur Province (<sup>4</sup>Oaxaca State); Sierra Madre Oriental Province (<sup>5</sup>Querétaro State); Transmexican Volcanic Belt Province (<sup>6</sup>State of Guerrero, <sup>7</sup>México City, <sup>8</sup>State of México, <sup>9</sup>State of Michoacán, <sup>10</sup>State of Morelos, <sup>11</sup>State of Puebla) Province of Veracruz (<sup>12</sup>State of San Luis Potosí, <sup>13</sup>State of Tamaulipas, <sup>14</sup>State of Veracruz); Province of the Yucatan Península (<sup>15</sup>State of Quintana Roo, <sup>16</sup>State of Yucatan).

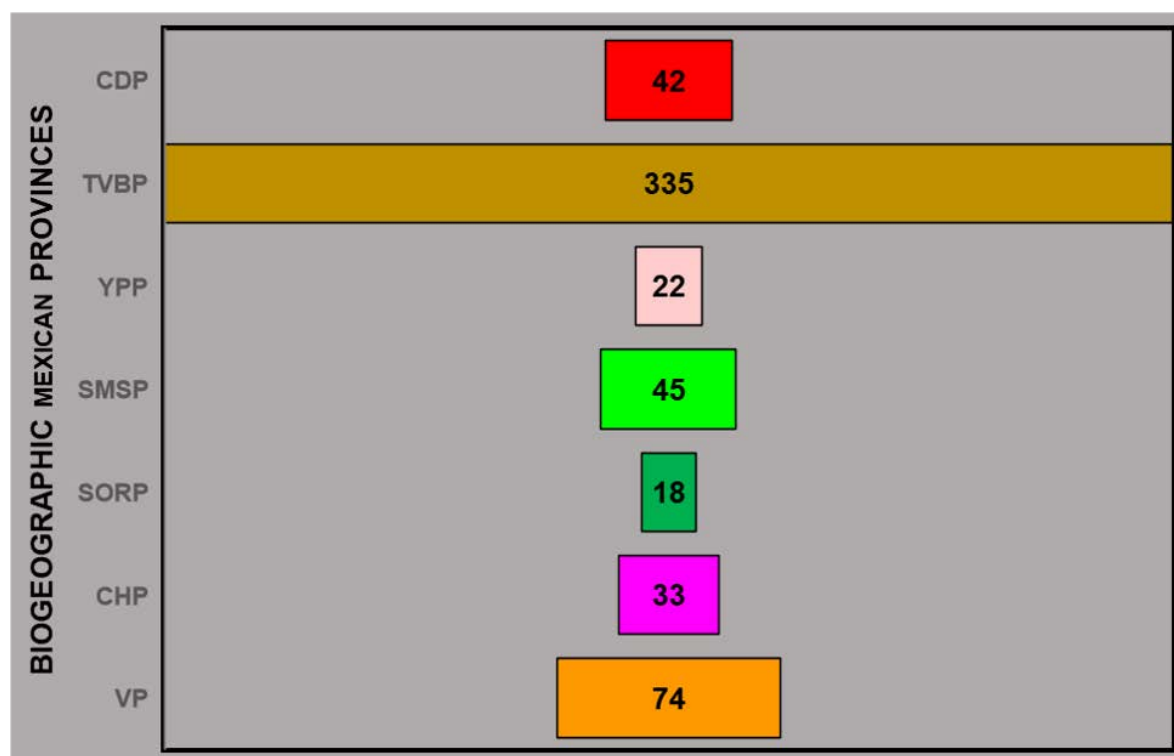


**Figure 4.** Number of Ciliated Protist Species by Biogeographic Provinces. Abbreviations: CDP, Province of the Chihuahuan Desert ; TVBP, Province of the Transmexican Volcanic Belt ; YPP, Province of the Yucatan Península ; SMSP, Province of the Sierra Madre del Sur; SORP, Province of the Sierra Madre Oriental; CHP, Province of the Chiapas Highlands; VP, Province of Veracruz.

Figure 5 shows the data of the historical analysis of the record of species over time between the period of 1922-2023. The first records were carried out by Bravo-Hollis (1922) for two lakes, Xochimilco and Chapultepec located in Mexico City; later, Sokoloff (1936) in Hidalgo state contributed with ciliate records. For the following decades, the works of Elliot and Hayes (1955) for San Luis Potosí, and López-Ochoterena (1965) for Mexico City could be mentioned. The diversity of ciliates since the year 2000 was focused on the study of very different continental systems as well as the coverage of a broader number of states (Durán-Ramírez, 2010; Sigala-Regalado, 2011; Méndez-Sánchez, 2017; Potekhin & Mayén-Estrada, 2020).



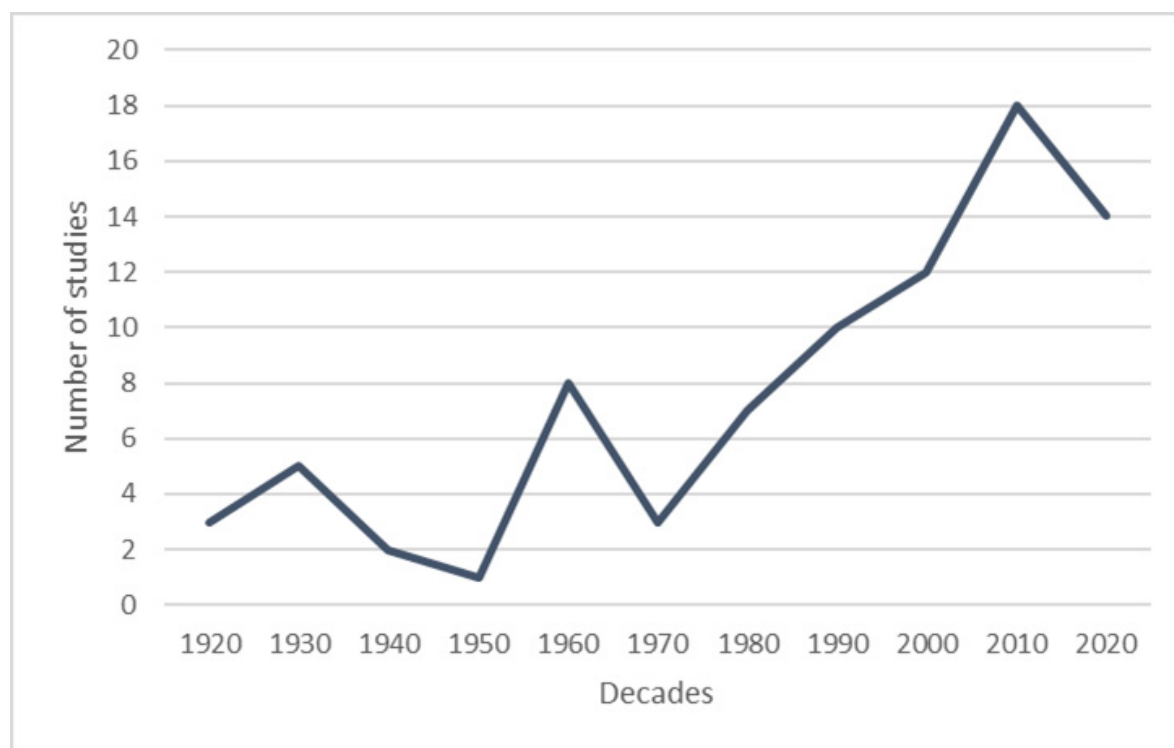


**Figure 5.** Number of Ciliate Species According to the Time Recorded in Mexican Continental Ecosystems.

However, there is a lack of studies in the north and west of the country where there are important bodies of water with a high risk of disappearance, such as Cuatro Ciéne-gas, Lake Chapala, Lake Cuitzeo, Pátzcuaro, and Yuriria, and important rivers such as the Balsas River, Lerma River, and even the border rivers such as the Bravo, Colorado on the northern border or the Usumacinta, Grijalva, and Suchiate, on the border with Guatemala and Belize. Despite the diversity of epicontinental water bodies in Mexico, the knowledge of the community of ciliates that inhabit them is scarce. Only records of ciliates account for 1.25 % of the water bodies inventoried by SINA (2020), and only a small fraction of natural environments is found within a Protected Natural Area or is categorized as part of a Ramsar site, as is the case of the Ciéne-gas de Lerma, the Lagos de Montebello National Park or the Bacalar Lagunar Complex.

Mexico has 28 main rivers (Maderey-Rascón & Carrillo-Rivera, 2005) and 6,332 inventoried wetlands (SINA, 2020) classified as marine, marsh, and lacustrine, occupying 6.5 % of the national territory. However, more than 60 % of these ecosystems have already been lost, except for the 142 protected Ramsar sites.

## Conclusions

In Mexico only 294 ciliate species have been recorded for 16 Mexican states, which indicates that the studies of the ciliate species across the Mexican Country's continental waters are still incomplete, being only about 28.7 % reported species. The environmental crisis that Mexico is facing due to the severe drought throughout its territory has caused some continental water bodies to have gradually dried out. This scenario makes all the previous biological information valuable and the current information can be a guide for making decisions in future strategies for ecological and conservation studies based on the ciliate's roles in ecosystems, considering that new ciliate records and new species descriptions are possible due to the geographic position of the country encompassing two biogeographical regions.

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## Authors' Contributions

Conceptualization RME; Data curation RME, CADR, VMRN, FOB; Formal analysis RME, CADR, VMRN, FOB; Investigation RME, CADR, VMRN, FOB; Methodology RME, CADR, VMRN, FOB; Software VMRN, FOB; Supervision RME; Validation RME; Visualization RME, CADR, VMRN, FOB; Writing RME, CADR, VMRN, FOB; Writing review, and editing RME.

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# Potential Use of Rotifer and Cladoceran Diapausing Eggs as a Tool for Taxonomical, Ecological, and Evolutionary Studies

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## Abstract

**R**otifers and cladocerans usually reproduce by parthenogenesis. However, under adverse conditions, females turn to sexual reproduction. The final output when females are fertilized is the production of diapausing eggs. They are a remarkable strategy of organisms to recolonize aquatic ecosystems when periods of dryness occur or when adverse conditions in the water prevail. Despite their prominent functionality, the knowledge of diapausing eggs in taxonomy, ecology, and evolution is still poor. Therefore, in this review, we discuss the gaps in the understanding of diapausing eggs and, moreover, their potential to develop further studies.



## Keywords

Dormancy, ephippia, resting egg, sexual reproduction, zooplankton.

## General Information about Diapausing Eggs

### *What is a Diapausing Egg, and Where Does it Come from?*

Rotifers and cladocerans are two of the most studied groups in zooplankton. These organisms usually reproduce by parthenogenesis. However, females change into sexual reproduction to generate diapausing eggs (see Figure 1) in response to several environmental stimuli that precede unfavorable conditions (Gilbert & Schröder, 2004; Schröder, 2005), environmental uncertainty (García-Roger et al., 2019), but also endogenous signals (Gilbert, 2007).

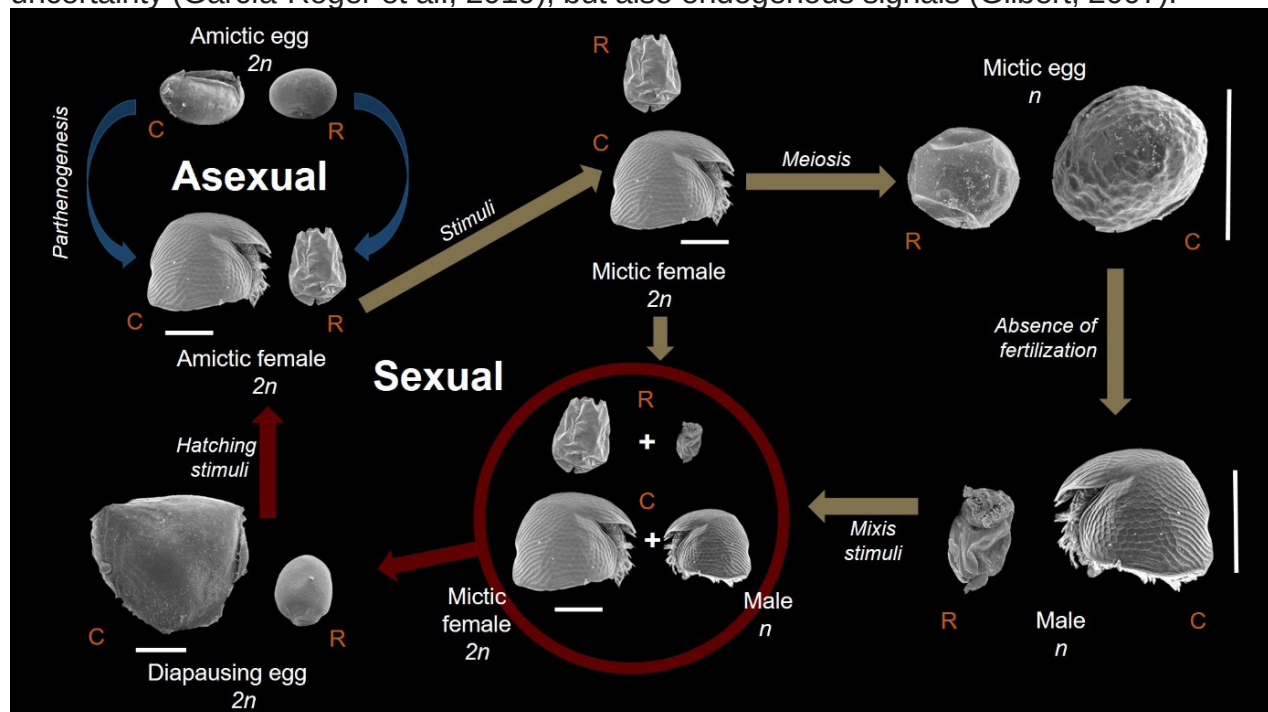


**Figure 1.** The Life Cycle of Monogonont Rotifers and Cladocerans. In the Life Cycle of Both Rotifers (R) and Cladocerans (C), the Asexual Phase Involves Amictic Females Producing Copies of Themselves in Amictic Eggs by Parthenogenesis. However, Particular Environmental Factors Will Eventually Trigger the Transition to Sexual Reproduction, Meaning that Meiosis Will Take Place in the Production of New Offspring. Females that Undergo this Shift Are Called Mictic Females, and They Produce Haploid Mictic Eggs. If a Mictic Egg Is Not Fertilized, a Haploid Male Will Hatch. If Fertilized, it Will Develop into a Diapausing Egg, and a New Diploid Female Will Hatch Under Stimuli. Scale bar = 100 μm. Figure Edited by Guerrero-Jiménez Gerardo.

Changes in photoperiod, crowding, or diet are some environmental factors that could induce the change to sexual reproduction (Gilbert & Williamson, 1983). Some studies also suggest

the role of endogenous signals that delay mixis to reproduce sexually despite environmental stimuli (Gilbert, 2003; 2007). Diapausing eggs (also called resting eggs in rotifers and ephippia - *in Greek "saddle"* - in cladocerans) are embryos that have arrested development after most nuclear divisions and are surrounded by thick-walled, resistant self-made envelopes or shells, which have the ability to persist for extended periods of dormancy (Gilbert & Wurdak, 1978). They are generally ovoid or spherical (Wurdak et al., 1978; Pourriot & Snell, 1983), but there can be some found with irregularities or even kidney-shaped (Guerrero-Jiménez et al., 2020). Diapausing eggs are characterized by a thick, dark, opaque shell that presents surfaces with a wide range of ornamentations (Wurdak et al., 1978) and accessory structures such as knobs, spines, grooves, lobes, wrinkles, and small holes; in cladocerans, peduncles can be found in some species attached to the posterior portion of the ephippia (Guerrero-Jiménez et al., 2020). The diapausing eggs of several rotifers have an operculum delimited by a suture, allowing the female to exit the egg during hatching (Pourriot & Snell, 1983), see Figure 2.

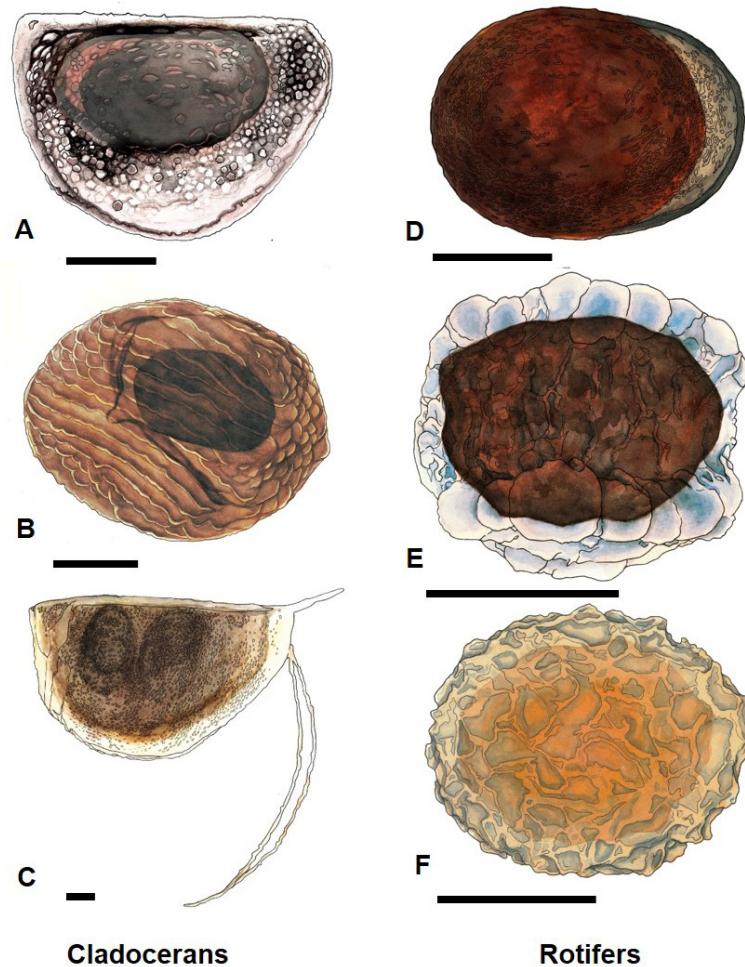


**Figure 2.** Different Ornamentation in Cladoceran and Rotifer Diapausing Eggs by Merging Optical and Scanning Electron Microscope Pictures. A) *Ceriodaphnia*, B) *Alona*, C) *Daphnia*, D) *Brachionus*, E) *Filinia*, F) *Lecane*. Scale Bar 50µm for Rotifers and 100µm for Cladocerans. Drawings Made by Armas-Chávez R. Natalia.

The mature diapausing egg appears to be composed of three layers in cladocerans: the first one being the ephippium with the ornamentation, the middle one containing the egg or eggs, and the internal layer compounded by a thin membrane (Guerrero-Jiménez et al., 2020). However, in rotifers two layers are generally observed: the external one showing the ornamentations and the internal layer enclosing the embryo, both usually separated by an extraembryonic space (Wurdak et al., 1978), see figure 3.

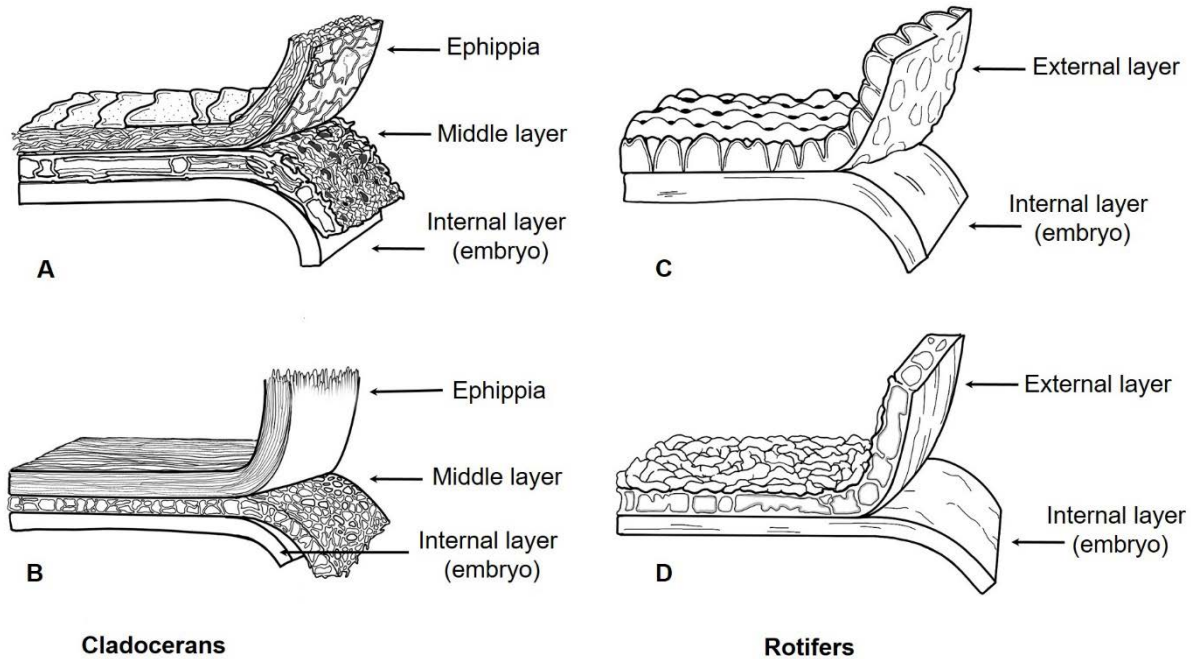


**Figure 3.** Transversal Cut of Diapausing Eggs of Cladoceran and Rotifer Species. The Figures Represent Layers Attached to Each Other in Some Portions. However, They May also Present a Space between Layers in Other Regions According to their Morphology.

Drawings Made by Armas-Chávez R. Natalia.

Piavaux and Magis (1970) confirmed the presence of chitin in the shell of mictic, amictic, and diapausing eggs of several species of rotifers and noted that chitin is regularly and specifically present in the internal layer. These dormancy structures hold one embryo in rotifers but can hold several in cladocerans.

#### *What is the Purpose of a Resting Egg?*

Diapausing eggs of cladoceran and monogonont rotifers allow a form of dormancy called diapause, a trait commonly found in organisms that inhabit harsh or unstable conditions and is controlled by an internal mechanism that initiates in response to environmental cues (Radzikowski, 2013; García-Roger et al., 2019). Diapausing eggs provide safety un-

der both biotic and abiotic unfavorable conditions over long periods of time by maintaining basal metabolism to ensure survival (Gremer & Sala, 2013), but diapause also serves the purposes of dispersal, habitat colonization (Schröder, 2005) and genetic variation (Pourriot & Snell, 1983). Despite of its functionality, the biological role of shell ornamentation and accessory structures is still unclear, although it is suggested that it may also have an important role as a defense in egg predation (Dumont et al., 2002). Pourriot and Snell (1983) have suggested that thickness of the shell in diapausing eggs and its reduced porosity allows them to resist the action of digestive enzymes when predated. The eggs accumulate in bottom sediments and create a resting egg bank, and once habitat quality improves or specific environmental cues are set (Radzikowski, 2013), resting eggs hatch, and hatchlings develop in the water column (Vargas et al., 2019). This happens after varying periods of dormancy, some remaining viable in lake sediments for many years (Gilbert & Wurdak, 1978). However, the viability of resting eggs and hatchlings might decrease as resting eggs age and are exposed to extreme conditions (Vargas et al., 2019).

### *Genetic Recombination*

As seen previously, rotifers and cladocerans combine asexual and sexual reproduction in their life cycle. This mechanism is designated as cyclical parthenogenesis (García-Roger et al., 2019), in which the asexual phase predominates, and organisms undergo several rounds of clonal reproduction followed by a sexual event (Rouger et al., 2016). The sexual phase produces genetic variation through recombination (King & Snell, 1977), which implies the exchange of DNA between male and female chromosomes during meiosis (Stapley et al., 2017). This starts the formation of the diapausing egg. Nevertheless, the production of diapausing eggs without fertilization has been reported for some species in both rotifers and cladocerans. Ruttner-Kolisko (1946) reported asexually produced diapausing eggs in *Keratella hiemalis*, and Schröder (1999) reported the same in the rotifer *Notholca squamula*. Gilbert (1995) reported the production of amictic diapausing eggs in the rotifer *Synchaeta pectinata*. In cladocerans, these early diapausing embryos from asexually produced diploid eggs are referred to as pseudosexual resting eggs. They are the result of a gene that suppresses meiosis, acting like the sexually derived resting eggs, but being genetically identical to their mother (Dodson & Frey, 2015). Some cladocerans have made the transition from cyclical parthenogenesis to obligate asexuality (Hebert, 1987), nevertheless, they produce pseudosexual resting eggs or pseudoephippia (Cerny & Hebert, 1993). Gilbert (1995) suggests that the production of diapausing amictic eggs in rotifers may be a strategy to increase the ability of clones to survive food limitations. On the other hand, pseudosexual eggs of *Daphnia* are probably a solution for the need to produce resting eggs in ponds that are too cold (Dodson & Frey, 2015).



### *Dispersion and Recolonization*

The resulting dormant stages settle at the bottom of the water body, creating an egg bank in the habitat that ensures the availability of eggs for future generations, although there are some reports where they were observed floating on the surface of lakes Guerrero-Jiménez et al. (2020). In addition, they may be dispersed in space passively by anemochory (Rivas Jr. et al., 2019), anthropochory (Perrigo et al., 2012), hydrochory (Ricci & Balsamo, 2000), and zoochory (Moreno et al., 2019). Therefore, when the diapausing eggs hatch, they can either replenish an existing population or establish a new one elsewhere (Vargas et al., 2019).

This bank is essential as it accumulates resting eggs produced at different periods, facilitating the preservation of genetic variants for active populations (De Stasio, 2007). The viable zooplankton egg bank is found in the top three centimeters of sediment, also known as active sediment (Pérez-Martínez et al., 2013). This stratum not only acts as a vital reservoir for the survival of these organisms but also plays a significant role in recruiting new genetic variants for active populations.

## Current Progress Using Diapausing Eggs

### *Taxonomy: Morphological Traits and their Purpose*

Records of diapausing egg taxonomy are historically scarce (Duggan et al., 2002). Furthermore, it has been suggested in latter years that further studies are required to understand diapausing eggs morphotype ultrastructure. In addition, Walsh et al. (2017) also mentioned that taxonomical descriptions were intricate due to a lack of feature standardization. On the other hand, similar conditions are met for cladocerans. Although some isolated studies have reported ultrastructure features of rotifers (Gilbert & Wurdak, 1978) and cladocerans (Kotov, 2013), Guerrero-Jiménez et al. (2020) first developed a useful key to identify rotifer diapausing eggs using six different taxonomical features easy to identify (see Table 1).

**Table 1.** Taxonomic Standardization with Six Categories to Identify Rotifer Diapausing Eggs of *Brachionus* Genus, According to Guerrero-Jiménez et al. (2020).

| Categories by Observation Order       |                                 |                                  |                                 |
|---------------------------------------|---------------------------------|----------------------------------|---------------------------------|
| 1. Egg Size                           | Small (<80µm)                   | Medium (80-120µm)                | Large (>120µm)                  |
| 2. Egg Shape                          | Regular                         | Regular                          | Regular                         |
| 3. Color                              | Brown                           | Brown                            | Brown                           |
| 4. Extraembryonic Space Size          | Small (1/8 of the Whole Embryo) | Medium (1/4 of the Whole Embryo) | Large (1/2 of the Whole Embryo) |
| 5. Ornamentation or Accessory         | Ornamentation                   | Ornamentation                    | Ornamentation                   |
| 6. Type of Ornamentation or Accessory | Holes                           | Wrinkles                         | Grooves                         |
| Species                               | <i>budapestinensis</i>          | <i>bidentatus</i>                | <i>calyciflorus</i>             |

Keys to identifying diapausing eggs using their taxonomy are still being developed in cladocerans. Thus, only 40 to 50 species on each taxon have been described in depth.

### *Ecology: Interaction with Environmental Factors*

Studies focused on the effects of different biotic and abiotic factors in diapausing eggs are surprisingly scarce, given the importance of these structures in rotifer and cladoceran life cycles. Among the available literature, effects of light, salinity, temperature, and pollutants have been studied on certain specific species. In fact, Stross (1966) and Vandekerckhove et al. (2005a) have suggested that two of the main factors implicated in activation and interruption patterns of dormancy are light and temperature. Several adverse environmental alterations lead to the production of resting eggs and different factors may be involved in their hatching, which makes it even more difficult to evaluate and establish a pattern for diapausing mechanisms. Among the factors that control diapause in Cladocera are photoperiod, temperature, population density, desiccation, and predation signals (Alekseev, 1990). Additionally, little is known about the endogenous mechanisms that restrict the activation of sexual reproduction regardless of the environmental stimuli that might trigger the sexual phase, as seen in the rotifer *Brachionus calyciflorus* (Gilbert, 2003) and *Hexarthra* populations (Gilbert, 2007).

### *Light*

Studies that consider the effects of light in diapausing eggs of rotifers and cladocerans mainly use white light, which encompasses all the wavelengths of visible light. However, experiments such as the work of Hagiwara et al. (1995) further study the effects of light by

testing six different intensities. In this study, it is concluded that hatching of *Brachionus plicatilis* caused by light depends on its intensity and irradiation period, but more hatchings were observed with more irradiation. On the other hand, in cladocerans, Vandekerkhove et al. (2005b) demonstrated different hatching patterns in 45 species from Denmark, Belgium/the Netherlands, and Spain using different photoperiods. They conclude that light is a key factor for the hatching of resting eggs when exposed to a long day photoperiod rather than under continuous illumination. Altogether, light is a determining factor in the hatching of both rotifer and cladoceran diapausing eggs. Indeed, preliminary analysis showed their natural reaction to different wavelengths of light.

### *Salinity*

Overall, studies have demonstrated that increment of salinity in water ponds decrease hatching rate, however, according to Santangelo et al. (2014) and Conde-Porcuna et al. (2018), not all species respond in the same way. In rotifers, a vast majority of studies are focused in marine rotifers belonging to *B. plicatilis* complex group. For example, Minkoff et al. (1983) observed that the hatching of rotifer *Brachionus plicatilis* s.s. diapausing eggs was affected by the increase of salinity. Other studies, such as the work by Gabaldón et al. (2015), also observed differences in the hatching patterns of *B. plicatilis* s.s. and *B. manjavacas* under different salinity conditions. Apparently, the optimal salinity for these species is under 15-18 g/L. However, it is not uncommon to observe hatching with higher salinities. In freshwater rotifers, Conde-Porcuna et al. (2018) demonstrated that species decrease hatchings dramatically when salinity exceeds 8 g/L. Cladocerans studies are more restricted, the most relevant such as the work of Santangelo et al. (2014) studied hatching patterns of 16 different taxa including cladocerans, using a salinity range of 30 g/L. They observed the lowest hatching values at high salinities, 16 and 32 g/L, respectively. On the other hand, Mabidi et al. (2018) in four South African wetlands proved that the hatching rate of cladocerans diminished with salinity levels of 2.5 g/L or higher.

### *Temperature*

This is one of the most direct stimuli for the activation of diapausing eggs, according to Vandekerkhove et al. (2005b). Indeed, experiments in laboratory conditions of May (1987) demonstrated that high temperatures are required for hatching. More specific data using rotifers demonstrated that at 25°C three species belonging to the *B. plicatilis* complex group drastically rushed their hatching rate, and also around 80 % of hatchings occurred in the first five days of exposure, compared to temperatures below 20°C (Walczyńska & Serra, 2014). In cladocerans, Vanderkerhove et al. (2004, 2005a) hatched several European species at 15°C. Komazawa and Endo (2002), using marine cladocerans, observed that at temperatures of 20°C or higher organisms did not hatch. Considering this information, ro-

tifer diapausing eggs seem to be more efficient at higher temperatures ( $>20^{\circ}\text{C}$ ) compared to those of cladocerans, who increase their hatchings at  $<20^{\circ}\text{C}$ .

### *Contaminants*

Heavy metals (Aránguiz-Acuña, 2016) and pesticides (Navis et al., 2013) have been proven to affect rotifer and cladoceran diapausing eggs by diminishing their hatching mechanism. Although the scarcely available studies are more focused towards cladocerans than rotifers. Oskina et al. (2019) showed that exposure time determines the negative effect of toxicants in hatching. Their experiments with the cladoceran *Moina macrocopa* demonstrated that exposure to concentrations of up to 80 g/L of Copper, Cadmium, Zinc, and Nickel for 30 days resulted in hatching rates superior to 80 %. However, when exposed for eight months, the hatching rate with concentrations of 30 g/L or higher was null. Thus, the structure of diapausing eggs is extremely efficient in short time periods. However, during long time periods, several external factors such as heavy metals may play an important role by corroding the external layer and/or permeating into the embryo, modifying the hatchlings and hatching patterns.

### *Evolution: Species-specific Hypothesis*

Specialists have discussed since several years ago whether ornamentation of diapausing eggs in rotifers and cladocerans are specific to their species. Although nowadays most authors (Gilbert & Wurdak, 1978; Kotov et al., 2018; Guerrero-Jiménez et al., 2020) support this species-specific hypothesis, a few have observed in the past that what seemed one species, produced two different types of diapausing eggs. However, these observations did not consider the possibility of encountering cryptic species which are individuals highly morphologically alike but with genetic divergence, and that therefore produce two different morphotypes of eggs. Thus, it is important to merge further taxonomical, ecological, and molecular analyses in order to expand the knowledge of evolutionary mechanisms in diapausing eggs.



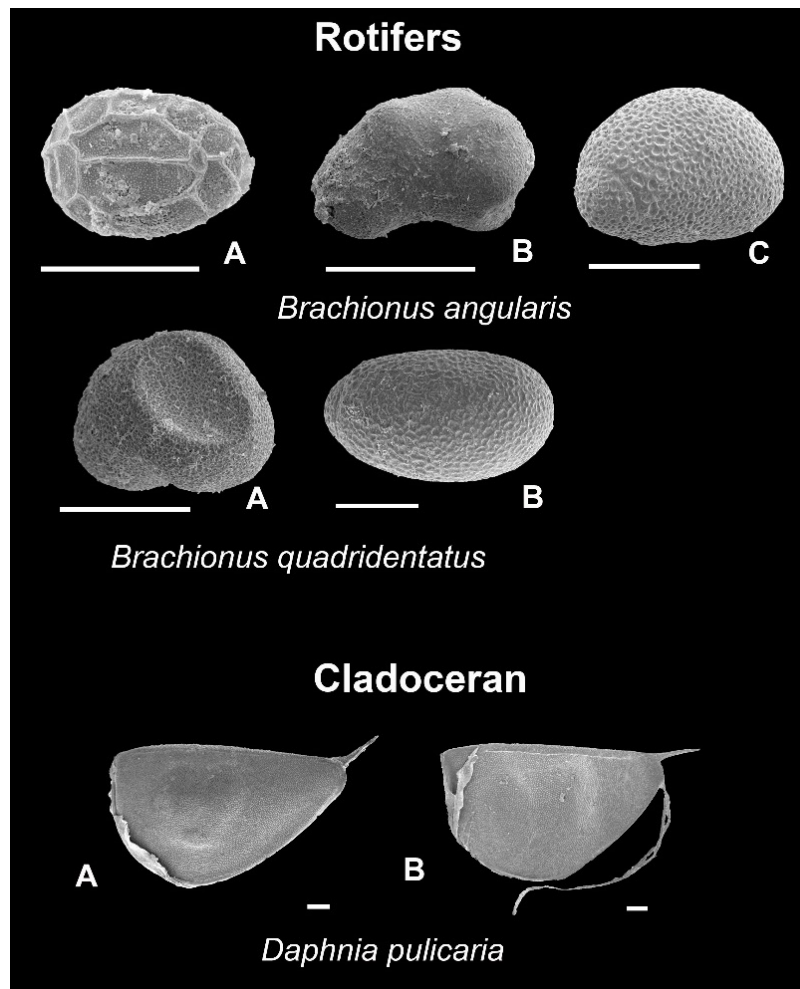


**Figure 4.** Cryptic Species with Clearly Different Morphotypes of Diapausing Eggs. Differences Are Based on Ornamentation, Size, and Shape. Scale Bar 50  $\mu\text{m}$  for Rotifers and 100  $\mu\text{m}$  for Cladocerans.

## Future Perspectives and Opportunities

There is still enough information to be uncovered about biodiversity and descriptions of rotifer and cladoceran diapausing eggs morphotypes. Ecological studies are limited to model species such as *B. plicatilis* complex group for rotifers and *Daphnia* species for cladocerans. In evolutionary studies, data about functionality of structures and the species-specific hypothesis are still speculative.

### *Biodiversity*

Molecular approaches using diapausing eggs are perhaps the most used tools to identify rotifer and cladoceran species nowadays. However, although DNA barcoding of diapausing eggs may yield higher numbers of taxa identified in comparison to taxonomic identification

(Moreno et al., 2017), these molecular studies often require complex methodologies and equipment that might not be accessible to most researchers. Furthermore, DNA barcoding without visual references does not provide solid evidence of biodiversity, especially in cryptic species. Considering this, rotifer and cladoceran diapausing eggs morphology already serves as a more cost-effective tool for identification of species, as their study requires relatively more simple methodologies and more accessible equipment. Furthermore, Vandekerckhove et al. (2005a) have suggested that diapausing egg banks may represent a complementary study approach of zooplankton species based on the wide assessment of community structure and diversity present in a single sample. However, despite its potential as a species identification tool, diapausing eggs are not widely studied, aside from a select set of keys available used with rotifers (Guerrero-Jiménez et al., 2020). In cladoceran species, there are available nomenclature keys in isolated studies (Kotov et al., 2018), although consistent tools to identify diapausing eggs are yet to be developed.

In summary, there are many opportunities to develop studies using taxonomical features in order to uniform the available and new characters that will be observed, which will increase the current list of morphotypes of diapausing eggs described.

### *Ecology*

Three phases within diapausing eggs can be studied: a) the factors that induce the females to produce them; b) the tolerance to adverse factors during dormancy; and c) factors that induce hatching patterns.

a) Most of the data available support that adverse conditions activate the production of diapausing eggs. However, delimiting what an adverse condition is between species is complicated due to the ecology and diversity of species, as well as within individuals. Furthermore, studies usually are focused on model species, such as *B. plicatilis* in rotifers and *Daphnia magna* in cladocerans. *B. plicatilis* is a marine species. Therefore, its studies mainly consider variability in salinity and temperature, which narrows the perspective on diverse factors that could be interacting with the species. In cladocerans, studies are mainly focused on temperature and food concentrations. Thus, there is a lack of information that considers the rest of the species and other factors such as different pollutants, light, and so on.

b) Two topics should be assessed regarding dormancy periods. Firstly, the ecology of the females to place diapausing eggs. Usually, it is assumed that they are placed only in sediments or floating on the surface of the water pond. However, water ponds present different dynamic and therefore species also present miscellaneous strategies to live in aquatic ecosystems which results in diapausing eggs to be found in places different from sediments. Secondly, the capability to tolerate adversity during dormancy is to be addressed. Indeed, studies are mostly focused on this line of investigation, although they are

still insufficient, perhaps by the fact that diapausing eggs are too resistant and no effect is assumed. Some studies using salinity and pollutants have demonstrated levels of deterioration within eggshells. For instance, García-Roger et al. (2006) showed how salinity affects the viability of diapausing eggs in *B. plicatilis*. Furthermore, Piscia et al. (2012) in Lake Orta, Italy, observed almost null hatching when they were exposed to copper as well as alterations in the morphology of the eggshells.

c) Finally, hatching patterns are the most studied phase. According to Pourriot and Snell (1996), in rotifers, light, temperature, and salinity are the main factors that determine their hatching patterns, while in cladocerans, data suggests light and temperature to be the key variables (Vandekerkhove et al., 2005). In both taxa, light is mandatory to hatch, however, species have displayed different hatching patterns when exposed to other factors such as temperature or salinity. In the case of contaminants, Oskina et al. (2019) demonstrated that cladoceran ephippia are quite resistant, and hatching rate is not affected in short exposure periods, but after long exposition, ephippia are drastically affected.

Despite the suggestion of Nielsen et al. (2003) that diapausing eggs might be more susceptible to the current environmental changes, studies are still focused in active stages. Thus, a huge opportunity to develop studies and contribute to the understanding of the whole process related with this type of eggs is open to exploration.

### *Evolution*

Studies that try to explain evolutionary processes are complex. Under the vast diversity of mechanisms that work in evolution, one of the most fascinating is how environmental pressure shapes morphological functionality within species. This environmental pressure might work in one or several stages of the life cycle of an organism, for example, in the life cycle of butterflies or beetles. All the stages in their life cycle present high morphological differentiations adapted to the functionality of the stated stage. In the same perspective, dormant stages of rotifers and cladocerans present an extraordinary array of morphological variabilities that integrate crucial mechanisms to recolonize aquatic environments. However, despite the importance of resting eggs as a stage in the life cycle of rotifers and cladocerans, studies focused on their relation with evolution and adaptation mechanisms are scarce. Indeed, diapausing eggs are often credited to be produced by females in unfavorable conditions. However, close observations have revealed that under regular conditions, the production of resting eggs is still present (personal observations, Gilbert & Schröder, 2004; Radzikowski, 2013; García-Roger et al., 2019). It is still unclear if, over time, the production of resting eggs has become a “default” action for rotifers and cladocerans, a doubt that can only be answered by performing more focused studies on this stage.

Another important topic in evolution is related to hatching patterns. Similar to morphology, hatching signals can be highly species-specific, as was observed in studies on egg banks in tropical aquatic systems by Araujo et al. (2013). This extensively studied phenomenon highlights the importance of understanding not only the function of the egg bank as a survival mechanism, but also the complexity of species-specific signals and responses to adverse conditions of hatching patterns. Moreover, a complete analysis of morphology-functionality will provide evidence to solve some species complex groups, as nowadays, cryptic species hypothesis in several taxa of rotifers and cladocerans is strongly accepted (Fontaneto et al., 2009; Garcia-Morales & Elias-Gutiérrez, 2013).

## Conclusions and Recommendations

Diapausing eggs comprise a key survival strategy for rotifers and cladocerans. The exploration of these dormant life stages, with their distinctive morphological features and remarkable resilience, opens avenues as we strive to comprehend the complexities and intricate dynamics of aquatic ecosystems.

So far, the significance of diapausing eggs has only been studied superficially. However, studies focused on these structures in taxonomy, ecology, and evolution, as well as other areas of biology, can provide a window into the past, allowing the reconstruction of historical environmental conditions and tracking changes in species composition over time.

This adaptive strategy for survival in adverse conditions also offers insights into the resilience of rotifers and cladocerans and their ability to withstand challenges caused by the impacts of environmental change. By delving into the intricate world of these resilient dormant stages, researchers can contribute not only to scientific knowledge, but also to the development of informed conservation and management strategies of aquatic environments.

## Authors' Contributions

GJG conceptualization, writing, supervision, and project administration. ANE, writing, and editing. ACRN writing, drawing, creative design, and editing. ASFS, RRJC funding acquisition and collecting samples for image references. AOA, SBM Scanning Electron Microscopic images.

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# Zooplankton Community and Trophic State in Lake Chapala

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## Abstract

Lake Chapala is the largest lake in Mexico, it supplies water for the city of Guadalajara and suffers a serious eutrophication problem. In the last 40 years, many indices related to trophic state, based on zooplankton abundance, have been developed for monitoring programs. In this study, we compared different trophic state indices (TSI) based on rotifers, cladocerans, and copepods at the end of the cold season during March 2023 at Lake Chapala, Mexico. We selected four sampling sites where limnological variables such as temperature, dissolved oxygen, pH, conductivity, depth, Secchi transparency, and zooplankton samples were obtained. The temperature was  $25.6 \pm 1.6^\circ\text{C}$ , dissolved oxygen  $7.3 \pm 0.4$  mg/l, oxygen saturation around 90 %, pH  $9.1 \pm 0.06$ , conductivity  $1012 \pm 5.3$   $\mu\text{S/cm}$  with a depth of  $4.23 \pm 0.13$  m and Secchi transparency of 0.46 m. The zooplankton community was composed of 19 species; 10 rotifers, 7 cladocerans, and 2 copepods. The dominant species were both copepods as well as *Ceriodaphnia* spp., *Diaphanosoma* cf. *birgei*, *Chydorus brevilabris*, *Filinia opoliensis*, *F. longiseta*, and *Horaella thomassoni*. Around 75 % of the total zooplankton abundance was represented by copepods, 13 % by rotifers, and 12 % by cladocerans. Carlson TSI based on Secchi transparency and  $\text{TSI}_{\text{ROT}}$  (BAC) indicate hypertrophic conditions, while  $\text{TSI}_{\text{ROT}}$  based on rotifer abundance results in mesotrophic conditions; for most TSI based on crustacean density, the eutrophic state was determined. The implications of calculations of TSI for zooplankton developed in temperate conditions may not completely match with observed conditions for Lake Chapala

where heterotrophic bacteria and cyanobacteria dominance play a key factor in the zooplankton community structuration. Formulation of indices in tropical conditions is important for monitoring water assessment.

## Keywords

Bioindicators, Cladocera, Copepoda, Rotifera,  $TSI_{CR}$ ,  $TSI_{ROT}$ .

## Introduction

Eutrophication is one of the main environmental challenges to solve in order to maintain water supply for human activities (Downing, 2014). The eutrophication process generates an increase in phytoplankton biomass with toxic potential and oxygen diurnal fluctuations, causing water quality and diversity reduction and the loss of ecosystem services (Janssen et al., 2020); moreover, climate change and cultural eutrophication enhance this problem (Moss et al., 2011). Hence, trophic state monitoring is critical to the adequate management and conservation of aquatic ecosystems (Dodds & Whiles, 2010).

For surveilling trophic levels on lakes, several trophic state indices have been developed. The Carlson trophic state index (TSI) based on total phosphorus, chlorophyll *a*, and Secchi transparency stands out as one of the earliest and most extensively employed classification schemes devised for assessing lake trophic conditions (Carlson, 1977). Additionally, the biological composition of a given lake is strongly influenced by its trophic state; for instance, a decrease in the average body size of zooplankton is correlated with an increase in trophic level; moreover, an increase in the overall abundance of rotifers and crustaceans exhibits a strong and positive correlation with the trophic state of lakes and some species have particular preferences for determined trophic state and water conditions (Ejsmont-Karabin, 2012; Ejsmont-Karabin & Karabin, 2013). Consequently, trophic state indices based on zooplankton have been proposed to estimate and classify trophic state conditions in freshwater ecosystems (*i.e.*, Sládeček, 1983; Ejsmont-Karabin, 2012; Ejsmont-Karabin & Karabin, 2013; Ochocka, 2021).

Freshwater zooplankton is a key component in the food webs of lentic ecosystems because it consumes phytoplankton, protozoans, and bacteria, and determines the abundance of secondary consumers such as macroinvertebrates and fish (Dodds & Whiles, 2010). Many species of zooplankton are abundant, some with wide geographical distribution, high reproductive rate, short generation time, and high sensitivity to environmental changes; therefore, they easily respond to environmental conditions (Beisner & Thackeray, 2023). Thus, these attributes make zooplankton an excellent tool for trophic state monitoring (Ejsmont-Karabin, 2012). Moreover, zooplankton is essential in enabling the provision of ecosystem services, including supporting fisheries, controlling phytoplankton

growth, and regulating carbon levels all of which enhance the quality of drinking water and irrigation. Additionally, they contribute to scientific advancements by providing model systems and ecological bioindicators (Declerck & de Senerpont Domis, 2023).

Sládeček (1983) proposed the *Brachionus/Trichocerca* quotient ( $B/T_Q$ ) and the saprobic index (SI) for rotifers to determine trophic conditions; nevertheless, genera *Brachionus* and *Trichocerca* are not always present and indicative weight of the species and individual saprobic index are not available for all rotifers. Additionally, indices for rotifers (Ejsmont-Karabin, 2012) and crustaceans (Ejsmont-Karabin & Karabin, 2013) were proposed in temperate lakes. Stamou et al. (2019) evaluated these indices in zooplankton communities of 16 lakes in Greece compared with the trophic state index based on Secchi transparency proposed by Carlson ( $TSI_{SD}$ ) (Carlson, 1977); they found that these indices exhibited a rise along the eutrophication gradient, but they inaccurately assess the trophic state. Ochocka (2021) developed a multimetric index ( $ZIPLA_S$ ) for Polish lakes considering the taxonomic composition and abundance, diversity, and stressor-sensitive species with the highest correlation with Secchi disk transparency.

In Mexico, there have been some attempts to calculate trophic state using zooplankton indicators in reservoirs and lakes (Muñoz-Colmenares et al., 2017; González-Gutiérrez et al., 2017, 2023; Moreno-Gutiérrez et al., 2018; Torres-Sánchez, 2020); nonetheless, all these studies only use rotifers whereas crustaceans have not been considered. Therefore, comparing these indices in a polymictic lake located at lower latitudes will provide us with information about how these indices respond in tropical conditions. Lake Chapala exhibits significant water quality decline related to an increase of nutrients (mainly nitrogen and phosphorus compounds) from domestic, livestock, industrial, and agriculture wastewater that enhanced primary production (Membrillo-Abad et al., 2016) with elevated presence of potentially toxic cyanobacteria (De Anda & Shear, 2001). Also, high concentrations of heavy metals such as cadmium, chromium, lead, mercury, copper, zinc, and, nickel have been recorded (Hansen et al., 1995). Earlier reports, discarded eutrophication as the main concern because light limitation, due to high inorganic clay turbidity, regulates phytoplankton productivity with a low annual average of  $5.4 \text{ mg/m}^3$  and lack of filamentous cyanobacteria without algal blooms (Limón & Lind, 1990).

Recently, degradation of water hyacinth (*Eichhornia crassipes*), inputs from the Lerma River, and excessive use of fertilizers in the agriculture catchment area were related to eutrophic conditions with cyanobacteria blooms of *Anabaena flos-aquae* (De Anda & Shear, 2001). Membrillo-Abad et al. (2016) summarized that Lake Chapala has been in eutrophic condition at least in the last 25 years with mean chlorophyll *a* concentration of  $11.7 \text{ mg/m}^3$  and mean Secchi transparency of 0.38 m. Therefore, monitoring and maintaining of water quality in Lake Chapala is a determinant task due to the increasing water



demand, because this lake supplies over 60 % of the freshwater requirements of the city of Guadalajara (Membrillo-Abad et al., 2016). Despite the physical and chemical characteristics of this lake being well known, biological data is not complete (Lind & Dávalos-Lind, 2001).

In Chapala 57 species of rotifers (mostly limnetic) were registered by Rico-Martínez et al. (2003); however, there is no data about abundance. For cladocerans and copepods, what we have in Lake Chapala is not clear with no records from recent zooplankton reviews in Mexico (Alcocer et al., 2023; Cervantes-Martínez et al., 2023). In the case of copepods, recent studies (Velázquez-Ornelas et al., 2021) have allowed us to know the diversity of species in the area. Some studies come from gut fish content analysis or gray literature, where taxonomical determination may not be accurate; in these studies, 14 cladocerans and 5 copepods have been registered (Rodríguez-Ruíz & Granado-Lorencio, 1988; Trotter, 1988). Thus, the objective of this study was to compare different trophic state indices (TSI) based on rotifers, cladocerans, and copepods at the end of the cold season during March 2023 at Lake Chapala, Mexico.

## Materials and Methods

### *Study Area*

Lake Chapala (Fig. 1) is a RAMSAR site located at the western edge of the Trans-Mexican Volcanic Belt in the eastern part of the state of Jalisco. It is a tectonic warm polymictic lake (20°06'08" - 20°18'08"N, and 102°42'00" - 103°25'20"W) with a maximum depth ( $Z_{MAX}$ ) of 10.5 m, and a mean depth ( $Z_{MEAN}$ ) of 7.2 m. Its maximum length ( $L_{MAX}$ ) is 77 km, and its maximum width ( $W_{MAX}$ ) 22.5 km, with a volume ( $V$ ) of 7.9 Km<sup>3</sup>. It is located at 1524 m a. s. l. in the Lerma-Santiago basin and has a catchment area of approximately 1,000 km<sup>2</sup>. By area and water volume, Chapala is the largest lake in Mexico, turbidic, with highly suspended clay and nutrient content (SRP=0.4-0.5 mg/l, TN=0.5-0.8 mg/l) where the dominance of the cyanobacteria *Microcystis aeruginosa*, *Aphanizomenon flos-aquae*, and *Anabaena* have been recorded (Dávalos-Lind & Lind, 2001; Alcocer & Bernal-Brooks, 2010).

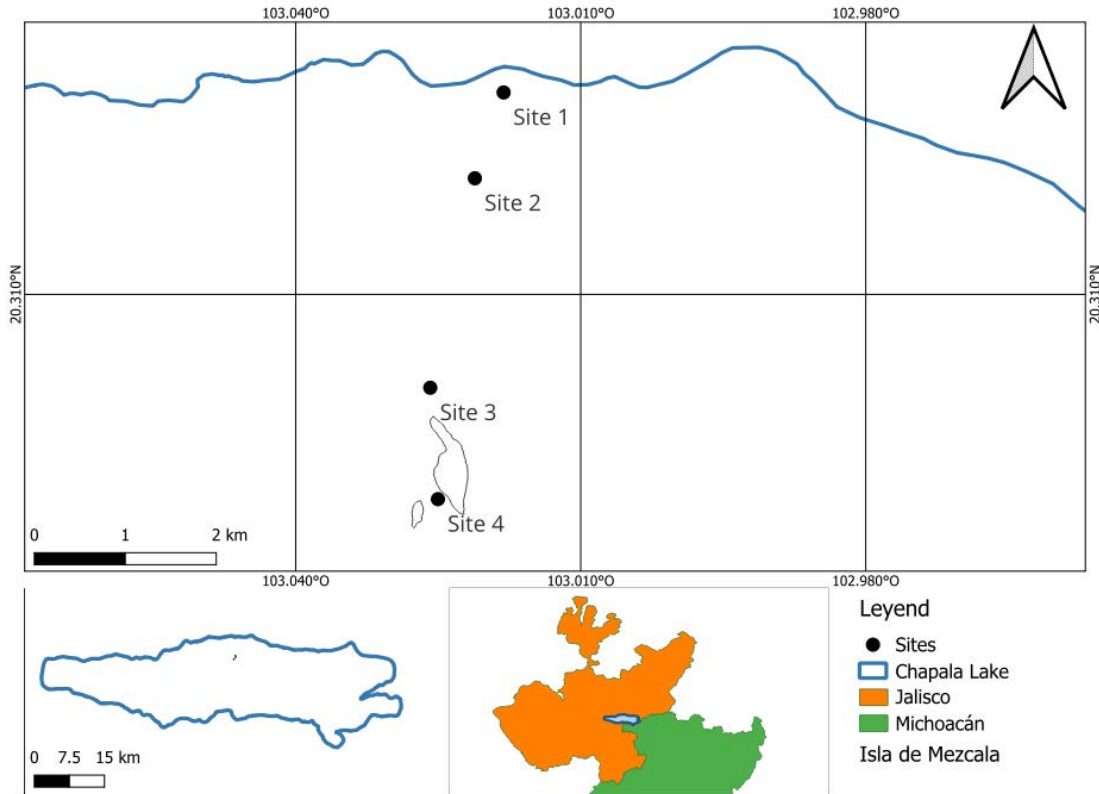


**Figure 1.** Lake Chapala Showing Sampling Sites.

### Sampling

We sampled four sites during March 2023 in a transect from the shore of the town of Mezcala to the island: site 1 inshore in the town of Mezcala (20°19'52.6"N, 103°01'05.3"W); site 2 between site 1 and the island of Mezcala (20°19'20.1"N, 103°01'16.2"W); site 3 close to the island of Mezcala (20°18'0.79"N, 103°01'33.1"W) and site 4 in vegetated area with *Schoenoplectus* sp. (20°17'18.6"N, 103°01'30.2"W). In each sampling point, temperature, dissolved oxygen, and conductivity were measured with a YSI85 probe, pH was registered with a potentiometer conductronic PC-20, depth with an ecosound Hondex digital, and transparency with Secchi disk. Zooplankton was sampled with vertical hauls from 1.5 m in the littoral zone up to 3 m depth in the pelagic zone using a Wisconsin planktonic net of 64  $\mu\text{m}$  mesh size. Later, samples were labeled with sampling data and fixed to a final formaldehyde concentration of 4 %.

### Samples Analysis

Each sample was analyzed under a Zeiss Axiostar plus microscope using a Sedgwick-Rafter counting chamber considering a minimum of five subsamples or 400 individuals of the most abundant species (Wetzel & Likens, 2001). Taxonomical determination was done with the taxonomical keys of Koste (1978), Elías-Gutiérrez et al. (2008a), Sarma and Nan-

dini (2017), and Sousa and Elmoor-Loureiro (2021). Zooplankton species photos were taken with an AxioScope A1 with camera AxioCam ICc 5. Names and status of the rotifers were verified in the Rotifer World Catalog database (<http://rotifera.hausdennatur.at/>); for cladocerans, we used Kotov et al. (2013) and for copepods Elías-Gutiérrez et al. (2008a).

## Data Analysis

### *Trophic State Indices*

We calculate the  $TSI_{SD}$  of three of four sampling sites according to the following formula (Carlson, 1977):

$$TSI_{SD} = 60 - 14.41 \ln(SD)$$

$TSI$  allows to determine trophic state based on a scale from 0 to 100, and the scale values correspond to different trophic conditions:  $0 < TSI \leq 40$  oligotrophic,  $40 < TSI \leq 50$  mesotrophic,  $50 < TSI \leq 70$  eutrophic, and  $70 < TSI \leq 100$  hypereutrophic.

We also consider trophic state indices based on rotifers (Ejsmont-Karabin, 2012) and crustaceans (Ejsmont-Karabin & Karabin, 2013) for polymictic lakes (POL) and indistinct mixis patterns according to the following formulas:

$$TSI_{ROT} \text{ POL (rotifer numbers)} = 4.64 \ln(N) + 25.36$$

$$TSI_{ROT} \text{ (rotifer numbers)} = 5.38 \ln(N) + 19.28$$

$$TSI_{ROT} \text{ POL (BAC, \%)} = 8.20 \ln(BAC) + 28.63$$

$$TSI_{CR1} \text{ POL (crustaceans numbers)} = 6.89 \ln(N) + 20.7$$

$$TSI_{CR1} \text{ (crustaceans numbers)} = 25.5 N^{0.142}$$

$$TSI_{CR6} \text{ POL (ratio Cladocera/Calanoida)} = 0.41 \ln(CL/CA) + 59.2$$

$$TSI_{CR7} \text{ POL (ratio Cyclopoida/Calanoida)} = 1.18 \ln(CY/CA) + 56.6$$

These indices calculate trophic state from abundance, bacterivorous percentage (BAC, %), and the ratio between crustacean groups and assumed that those lakes with a  $TSI_{ROT}$  and  $TSI_{CR}$  lower than 45 are mesotrophic, from 45 to 55 are meso-eutrophic, 55 to 65 eutrophic, and those over 65 are hypertrophic.

## Results

The mean values  $\pm$  standard deviation from the four sampling stations for temperature was  $25.6 \pm 1.6^\circ\text{C}$ , dissolved oxygen  $7.3 \pm 0.4$  mg/l, oxygen saturation around 90 %, pH  $9.1 \pm 0.06$ , conductivity  $1012 \pm 5.3$   $\mu\text{S/cm}$  with a depth of  $4.23 \pm 0.13$  m and Secchi transparency of  $0.46 \pm 0.005$  m (Table 1).

**Table 1.** Physical and Chemical Variables in Four Sampling Sites of Lake Chapala at the End of the Cold Season in 2023.

| Variables                         | S1    | S2   | S3   | S4   |
|-----------------------------------|-------|------|------|------|
| Temperature ( $^\circ\text{C}$ )  | 27    | 24.3 | 24.1 | 27   |
| Dissolved Oxygen (mg/l)           | 7.98  | 7.27 | 7.34 | 6.9  |
| Oxygen Saturation (%)             | 100.7 | 88.4 | 87.6 | 86.6 |
| pH                                | 9.06  | 9.19 | 9.21 | 9.14 |
| Conductivity ( $\mu\text{S/cm}$ ) | 1016  | 1009 | 1006 | 1017 |
| Depth (m)                         | 3.76  | 5.1  | 5.23 | 2.86 |
| Secchi Depth (m)                  | 0.46  | 0.47 | 0.47 | -    |

Table 2 presents 19 zooplankton species found in Lake Chapala; 10 rotifers, 7 cladocerans, and 2 copepods. We found 7 families of rotifers, the majority of species belonging to Trochosphaeridae and Brachionidae where *Filinia longiseta*, *F. opoliensis*, *Horaella thomassoni*, and *Keratella tropica* were the most abundant (Fig. 2A-F). We found five families of cladocerans where *Ceriodaphnia* spp., *Diaphanosoma* cf. *birgei*, and *Chydorus brevilabris* were more abundant (Fig. 3A-H). For copepods, we registered one calanoid and one cyclopoid (*Acanthocyclops* sp.) with a dominance of *Mastigodiatomus* cf. *albuquerqueensis* (Fig. 4A and 4F). We also found abundant *Trichodina* sp. (Fig. 2G) and a microturbellarian (Fig. 2H).



**Table 2.** Species Richness and Frequency of Metazoa Zooplankton Species Found in Four Sites of Lake Chapala at the End of the Cold Season of 2023.

| Species                                                            | S1 | S2 | S3 | S4 |
|--------------------------------------------------------------------|----|----|----|----|
| Crustacea                                                          |    |    |    |    |
| Branchiopoda                                                       |    |    |    |    |
| Anomopoda                                                          |    |    |    |    |
| Bosminidae                                                         |    |    |    |    |
| <i>Bosmina</i> cf. <i>longirostris</i> (O. F. Müller, 1785)        | -  | X  | -  | X  |
| Chydoridae                                                         | X  | X  | X  | -  |
| <i>Chydorus brevilabris</i> Frey, 1980                             |    |    |    |    |
| <i>Ovalona setulosa</i> (Megard, 1967)                             |    |    |    |    |
| Daphniidae                                                         | X  | X  | X  | X  |
| <i>Ceriodaphnia cornuta</i> Sars, 1885                             |    |    |    |    |
| <i>Ceriodaphnia</i> cf. <i>dubia</i> Richard, 1894                 |    |    |    |    |
| Moinidae                                                           | X  | X  | -  | -  |
| <i>Moina</i> cf. <i>micrura</i> Kurz, 1875                         |    |    |    |    |
| Ctenopoda                                                          | X  | X  | X  | X  |
| Sididae                                                            |    |    |    |    |
| <i>Diaphanosoma</i> cf. <i>birgei</i> Kořínek, 1981                |    |    |    |    |
| Maxillopoda                                                        |    |    |    |    |
| Copepoda                                                           |    |    |    |    |
| Calanoida                                                          |    |    |    |    |
| Diaptomidae                                                        |    |    |    |    |
| <i>Mastigodiatomus</i> cf. <i>albuquerqueensis</i> (Herrick, 1895) | X  | X  | X  | X  |
| Cyclopoida                                                         | X  | X  | X  | X  |
| Cyclopidae                                                         |    |    |    |    |
| <i>Acanthocyclops</i> sp.                                          |    |    |    |    |
| Rotifera                                                           |    |    |    |    |
| Eurotatoria                                                        |    |    |    |    |
| Monogononta                                                        |    |    |    |    |
| Flosculariaceae                                                    |    |    |    |    |
| Hexarthridae                                                       |    |    |    |    |
| <i>Hexarthra mira</i> (Hudson, 1871)                               |    |    |    |    |
| Trochosphaeridae                                                   |    |    |    |    |
| <i>Filinia longiseta</i> (Ehrenberg, 1834)                         | X  | X  | X  | X  |
| <i>Filinia opoliensis</i> (Zacharias, 1898)                        | X  | X  | X  | X  |
| <i>Filinia pejleri</i> Hutchinson, 1964                            | X  | X  | -  | -  |
| <i>Horaella thomassoni</i> Koste, 1973                             | X  | X  | X  | X  |
| Ploima                                                             |    |    |    |    |
| Brachionidae                                                       |    |    |    |    |
| <i>Keratella americana</i> Carlin, 1943                            | -  | -  | X  | -  |
| <i>Keratella tropica</i> (Apstein, 1907)                           | X  | X  | X  | X  |
| Euchlanidae                                                        | -  | -  | -  | X  |
| <i>Euchlanis dilatata</i> Ehrenberg, 1832                          |    |    |    |    |
| Lecanidae                                                          | -  | -  | -  | X  |
| <i>Lecane luna</i> (Müller, 1776)                                  |    |    |    |    |
| Notommatidae                                                       | -  | X  | -  | -  |
| <i>Cephalodella</i> sp.                                            |    |    |    |    |

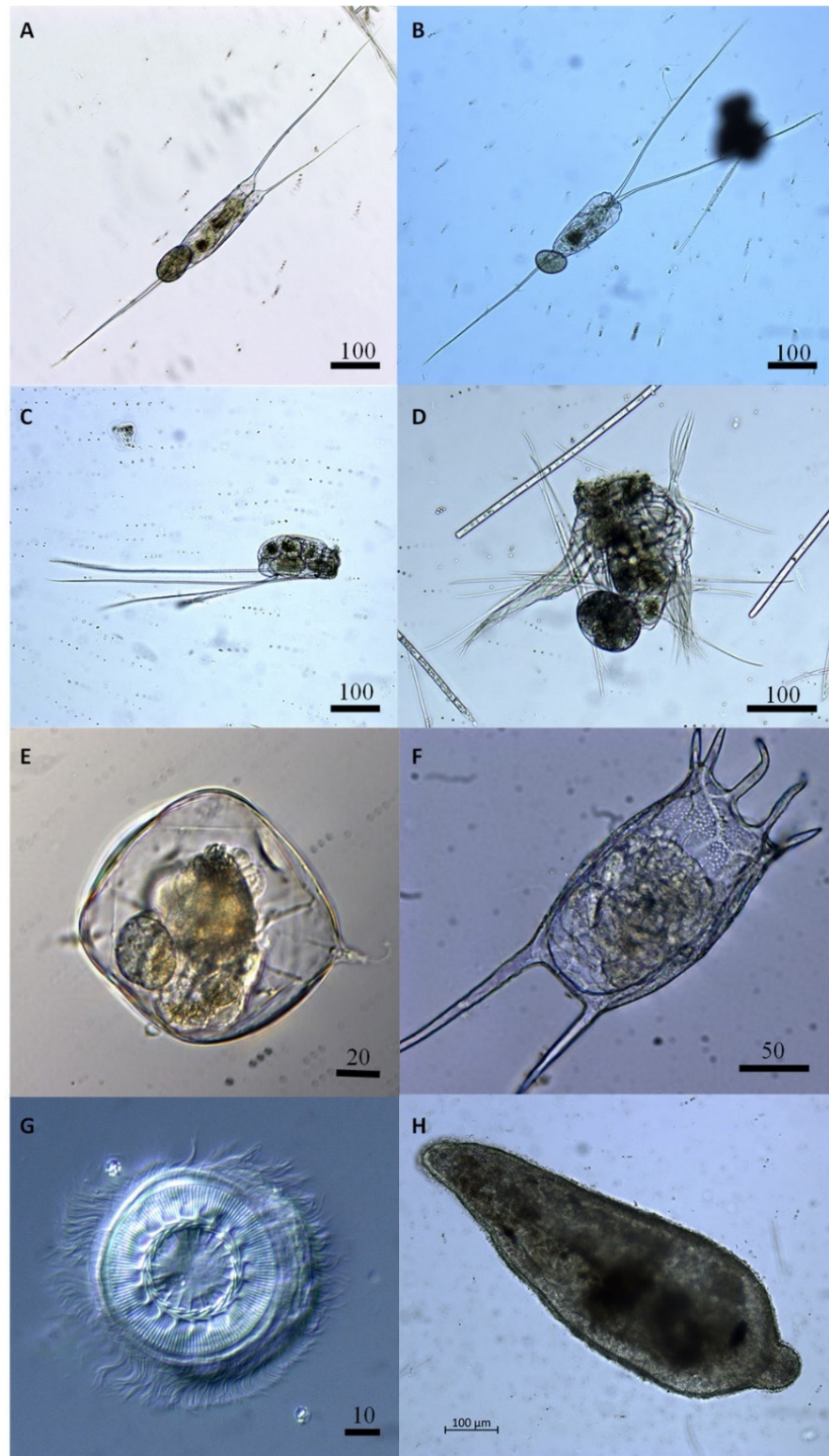


**Figure 2.** Rotifers, a Protist and Platyhelminth Found in Lake Chapala during the End of the Cold Season of 2023. A) *Filinia opoliensis*, B) *Filinia pejleri*, C) *Filinia longiseta*, D) *Hexarthra mira*, E) *Horaella thomassoni*, F) *Keratella tropica*, G) Protist *Trichodina* sp. and H) Platyhelminth microturbellarian. Scale Measurements are in Micrometers.



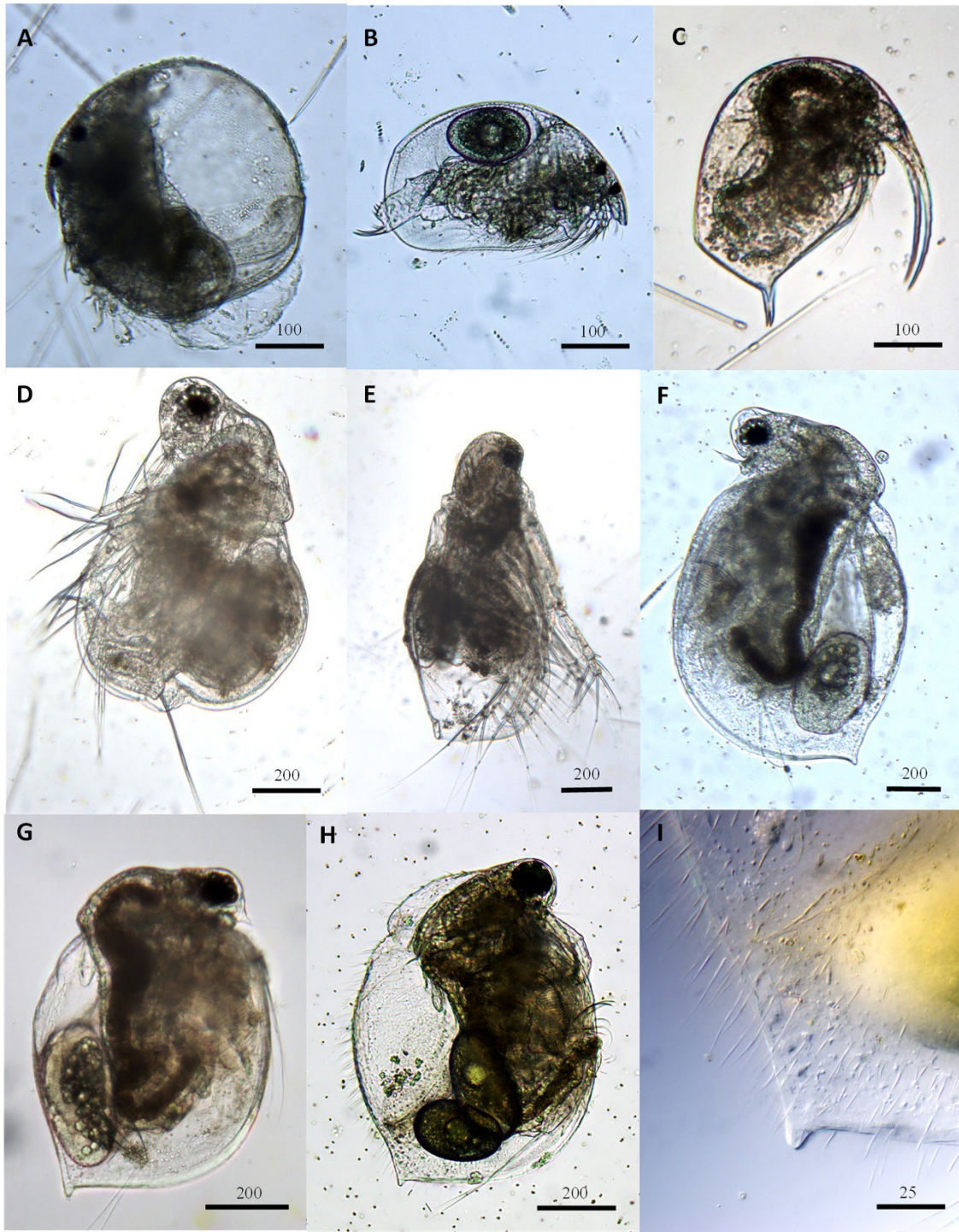


**Figure 3.** Cladocerans Found in Lake Chapala during the End of the Cold Season of 2023. A) *Chydorus brevilabris*, B) *Ovalona setulosa*, C) *Bosmina* sp., D) *Moina* cf. *micrura*, E) *Diaphanosoma* cf. *birgei*, F) *Ceriodaphnia* cf. *dubia*, G) *Ceriodaphnia* *cornuta*, H) *Ceriodaphnia* cf. *cornuta* and I) Postero-dorsal Margin of *Ceriodaphnia* cf. *cornuta*. Scale Measurements are in Micrometers.

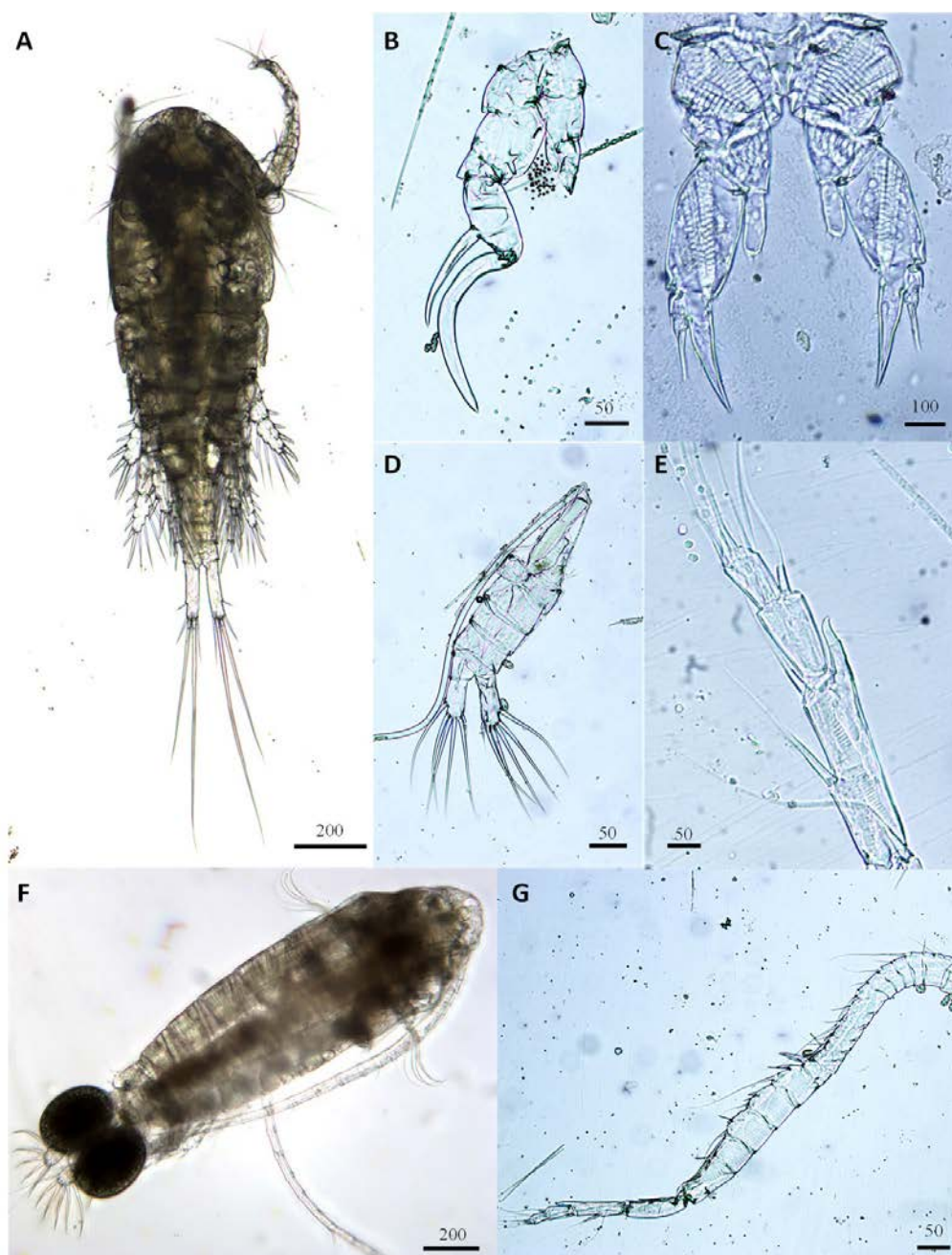


**Figure 4.** Copepods Found in Lake Chapala during the End of the Cold Season of 2023. A) Male of *Acanthocyclops* sp., B-G) *Mastigodiaptomus* cf. *albuquerquensis* B) Fifth Leg of Male and C) Female D) Urosomite of Male, E) Right Antennule, Segments 20, 21 of Male F) Female Dorsal View, and G) Right Antennule of Male. Scale Measurements are in Micrometers.

Zooplankton density was higher in site 4 with 330 ind/l, while site 1 had lower abundance with 194 ind/l. Abundance was mostly represented by copepods with close to 75 % ( $200 \pm 52$  ind/l), while rotifers and cladoceran had similar values with 13% ( $35 \pm 17$  ind/l) and 12 % ( $32 \pm 12$  ind/l), respectively (Fig. 5).



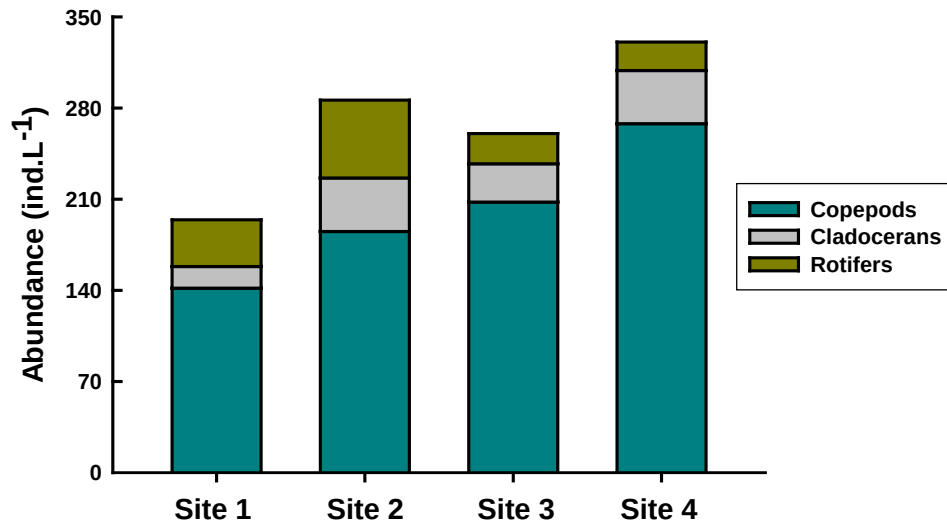


**Figure 5.** Abundance (ind./l) of Copepods, Cladocerans, and Rotifers in Four Sites of lake Chapala during the Cold Season in 2023.

Carlson TSI based on Secchi transparency determined hypertrophic conditions with values between 70.87 and 71.18. For  $TSI_{ROT}$  based on abundances for indistinct mixis pattern and for polymictic lakes, values ranged from 35.89 to 44.33, specifying a mesotrophic state; nevertheless,  $TSI_{ROT}$  for bacterivorous species was 66.39 for all sampling sites.  $TSI_{CR1}$  based on abundances for indistinct mixis pattern and polymictic lakes, determined in one sampling site meso-eutrophic conditions with a value of 52.33; however, for all the other sites, values ranging from 55.06 to 60.19 indicating the eutrophic state. For  $TSI_{CR6}$ , eutrophic conditions were calculated and for  $TSI_{CR7}$ , values were close to the eutrophic state (from 53.35 to 54.92) in three sites, while one site was calculated as eutrophic with 55.54 (Table 3).

**Table 3.** Trophic State Index Based on Rotifers ( $TSI_{ROT}$ ) and Crustacean ( $TSI_{CR}$ ) Densities in Four Sites of Lake Chapala during the Cold Season in 2023.

H=Hypertrophic, E=Eutrophic, M=Mesotrophic.

| Trophic State Index | S1          | S2        | S3          | S4          |
|---------------------|-------------|-----------|-------------|-------------|
| TSIZSD              | 71.18 (H)   | 71.18 (H) | 70.87 (H)   | -           |
| TSIROT Pol          | 41.97 (M)   | 44.33 (M) | 39.94 (M)   | 39.68 (M)   |
| TSIROT              | 38.54 (M)   | 41.28 (M) | 36.18 (M)   | 35.89 (M)   |
| TSIROT Pol BAC, %   | 66.39 (H)   | 66.39 (H) | 66.39 (H)   | 66.39 (H)   |
| TSICR1 Pol          | 55.59 (E)   | 58.05 (E) | 58.38 (E)   | 60.19 (E)   |
| TSI CR1             | 52.33 (M-E) | 55.06 (E) | 55.43 (E)   | 57.55 (E)   |
| TSICR6 Pol (CL/CA)  | 58.34 (E)   | 58.72 (E) | 58.48 (E)   | 58.49 (E)   |
| TSICR7 Pol (CY/CA)  | 53.35 (M-E) | 55.54 (E) | 54.92 (M-E) | 54.58 (M-E) |

## Discussion

Our results from environmental variables coincide with previous reports by Lind and Dávalos-Lind (2001), who mentioned that the annual Secchi depth is 0.5 m, with a mean annual temperature of 22°C. Reported pH values ranging from 8.54 to 8.9 and oxygen concentrations from 6.84 to 7.13 mg/l with oxygen saturation over 90 % due to wind mixing (Limón & Lind, 1990; de Anda & Shear, 2001).

From the 10 rotifers found, *Hexarthra mira*, *Filinia longiseta*, *F. opoliensis*, *Horae-lla thomassoni*, *Keratella americana*, *K. tropica*, *Euchlanis dilatata*, and *Lecane luna* were previously reported for Lake Chapala by Rico-Martínez and Silva-Briano (1993) and Rico-Martínez et al. (2003). *Euchlanis dilatata*, *Lecane luna*, and *Cephalodella* sp. were only found once in one sample; therefore,  $TSI_{ROT}$  was mainly calculated based on the other 7 species. According to Gilbert (2022), these 7 species are microphagous feeding on small particles where bacteria is an important component in their diet. Some studies have found that the trophic relations in Lake Chapala are not completely sustained by phytoplankton productivity, where bacteria play an important role with an annual production that represents 42 % of total edible small particles for zooplankton (Lind & Dávalos-Lind, 2001), which explains the dominance of bacterivorous rotifers.

Zooplankton community structure is strongly shaped by the interaction of different factors, such as vagility, physico-chemical variables, and biological attributes of the habitat, where selective predation is the main driving force (Beisner & Thackeray, 2023). In addition, cyanobacterial blooms also differentially affect zooplankton species and it is well known that the proportion of large-bodied cladocerans significantly decline into small-bodied species when microcystin concentration increased in lake sediments (Moss et al., 2011; Kâ et al., 2012; Pérez-Morales et al., 2015; Jiang et al., 2017).

Although there is currently no data on cyanotoxins in Lake Chapala, the samples revealed a high density of potentially toxic cyanobacteria, including *Aphanizomenon* sp., *Microcystis aeruginosa*, *M. wesenbergii*, and *Dolichospermum* sp. Moreover, large filamentous cyanobacteria such as those found in Lake Chapala mechanically interfere with the feeding process of large-bodied cladocerans (Gliwicz, 2003), while small cladocerans, rotifers, and copepods are more selective than large cladocerans (Thackeray & Beisner, 2023). Hence, selective predation and the dominance of cyanobacteria may shape the zooplankton community in Lake Chapala.

Despite food availability for rotifers not being limited in Lake Chapala, their abundances were low ( $35 \pm 17$  ind/L) in comparison with microcrustaceans ( $232 \pm 61$  ind/l), where cladocerans had similar abundances to rotifers ( $32 \pm 11.6$  ind/l), while copepods reached the highest abundances ( $200 \pm 53$  ind/l). Rotifer abundances are mainly affected by ex-

ploitative competition when food resources are scarce, interference competition with large-size cladocerans, and selective predation (Thackeray & Beisner, 2023). MacIsaac and Gilbert (1989) demonstrated in laboratory experiments that cladocerans smaller than 1.2 mm can easily coexist with rotifers such as we observed for Lake Chapala. Therefore, selective predation may explain low rotifer abundance; Nandini et al. (2011) showed how turbellarians reduce rotifer abundance through the production of toxins and direct predation and despite not registering turbellarian abundance, we observed many specimens in each counting. On the other hand, cyclopoid copepods also exert high predation pressure on rotifers, Sarma et al. (2019) found in field samples that *Acanthocyclops americanus* consume different rotifer species. Under laboratory conditions, it was observed that female *Acanthocyclops* consumed nearly twice as much as males, depending on food availability, ingesting between 40 to 400 rotifers per hour. Hence, the presence of *Acanthocyclops* in our samples may also lead to a decrease in rotifer abundance.

For cladocerans, *Diaphanosoma* cf. *birgei* is the ctenopod with more records in Mexico. Recently, Velázquez-Ornelas et al. (2021) registered this species for Laguna de Cajititlán and there is evidence of this species in the report of the international training course: “zooplankton a tool in lake management” (Rico-Martínez, 1992). Former studies (Rodríguez-Ruíz and Granado-Lorencio, 1988; Trotter, 1988) identified Chapala *Diaphanosoma brachyurum* (originally described for Europe); nevertheless, this species is not confirmed for México (Elías-Gutiérrez et al., 2008a); Dumont et al. (2021) mentioned that *D. brachyurum* is the ctenopod with many citations without figures or morphological evidence, while Alexiou et al. (2021) observed that insufficient knowledge of *Diaphanosoma* morphology resulted in *D. brachyurum* being the most commonly documented species globally. The specimens we found have the posteroventral margin with group denticles increasing in size and one setule between each group. Moreover, Elías-Gutiérrez et al. (2008b) mentioned that this is a complex species with at least 4 species in Mexico with divergence up to 14.5 % from its type locality.

*Ceriodaphnia* is a very confusing genera, records from Lake Chapala listed *Ceriodaphnia lacustris*, *C. pulchella* (Trotter, 1988), and *Ceriodaphnia* cf. *reticulata* (Rodríguez-Ruíz & Granado-Lorencio, 1988) and we also found three morphospecies but it does not coincide with previous records (Trotter, 1988). One of the species we found was determined as *Ceriodaphnia* cf. *dubia* (Fig. 3G) which is highly variable and widely distributed in Mexico. We also found two morphotypes of *C. cornuta* species complex (Fig. 3G and 3H), one of them is referred by Elías-Gutiérrez et al. (2008b) such as *Ceriodaphnia cornuta-rigaudi*; nevertheless, *C. rigaudi* is *species inquirenda* according to the Cladocera checklist (Kotov et al., 2013). Elías-Gutiérrez et al. (2008b) discussed that *Ceriodaphnia cornuta* belongs to the species complex *C. cornuta-rigaudi* with high morphological diversity, broad distri-

bution, and three lineages in Mexico. In addition, Berner (1985) worked with three typical forms of this species complex and mentioned that *C. rigaudi* is synonymous with *C. cornuta*. She also reported a *C. cornuta* form that lacks the rostral projection and it has a hairy surface carapace similar to the population found in this study, which was identified as *C. cf. cornuta*. *Moina micrura* s str. is a Palaearctic species but is considered a complex where at least three species are registered for Mexico. However, formal descriptions of these species have not been made (Elías-Gutiérrez et al., 2019). Chydorids also represent new records for Lake Chapala (i.e., *Chydorus brevilabris* and *Ovalona setulosa*).

On the other hand, *Acanthocyclops vernalis* and *Mastigodiaptomus albuquerquensis* were previously registered by Rodríguez-Ruíz and Granado-Lorencio (1988) and Trotter (1988), respectively. In addition, Velázquez-Ornelas et al. (2021) indicate the presence of *M. montezumae* in another lake close to Lake Chapala, but it was not recorded in our samples. However, due to the recent description of complexes of cryptic species in both genera, it is necessary to carry out genetic and detailed morphological studies to corroborate the identity of the collected specimens.

The trophic state condition of Lake Chapala is eutrophic. Lind and Dávalos-Lind (2001) mentioned that regarding nutrients, Lake Chapala indicates the eutrophic-hypertrophic condition. According to remote sensing analysis, Membrillo-Abad et al. (2016) showed that Mezcala is an area with high chlorophyll *a* concentration (up to 65 mg/m<sup>3</sup>) with low transparency and Carlson TSI from 40 and 70 cm in the inshore of the town of Mezcala. Cyanobacteria abundance is high with dominance of filamentous and colonial species such as *Anabaena limnetica* (Lind & Dávalos-Lind, 2001), *Aphanizomenon flos-aquae*, *Microcystis aeruginosa*, *M. flos-aquae* (Mora-Navarro et al., 2004). Despite the trophic state being clear, our idea was to compare some zooplankton trophic state indices to test how these come across Lake Chapala conditions.

Our data reveal mesotrophic conditions using rotifers abundances, hypertrophic state when calculations are based on the percentage of bacterivorous rotifers, and mostly eutrophic conditions using crustacean ratios and abundances. González-Gutiérrez et al. (2023) derived rotifers as indicators through the saprobic index, the *Brachionus:Trichocerca* quotient, and TSI<sub>ROT</sub> in a high-altitude the El Llano Reservoir, where they determined oligosaprobic, oligotrophic and meso-eutrophic conditions, respectively. Ejsmont-Karabin (2012) mentioned that discrepancies may be expected and the use of different methods and indices of different scales are not always in close agreement where TSI<sub>ROT</sub> BAC % does not seem to be a strong predictor of trophic state for polymictic lakes. Even though generally rotifers seem to be better indicators of trophic state than microcrustaceans because of their susceptibility to the negative influence of algal blooms and toxic effects of



cyanobacteria (Ejsmont-Karabin & Karabin, 2013), in Lake Chapala, microcrustaceans coincide with the eutrophic condition of Lake Chapala in a better way than rotifers.

It is also relevant to the role of copepods where calanoids were much more abundant ( $164 \pm 45$  ind/l) than cyclopoids ( $36 \pm 19$  ind/l). Ejsmont-Karabin and Karabin (2013) mentioned that usually, an increase in a lake trophy may cause an increase in the ratio Cladocera to Calanoida numbers and an increase in the ratio of Cyclopoida to Calanoida numbers; however, it does not occur in Lake Chapala during cold season. It is important to consider this study is based on a sampling taken during one moment; however, according to its water chemistry, Lake Chapala exhibits less than 20 % spatial and seasonal variation (Limón & Lind, 1990); hence, our observations may open a window to research on zooplankton dynamics and bioindicator in Lake Chapala.

## Conclusion

In summary, we registered, for the first time, for Lake Chapala *Filinia pejeri*, *Diaphanosoma* cf. *birgei*, *Ceriodaphnia* cf. *dubia*, *C.* cf. *cornuta*, *Ovalona setulosa*, and *Chydorus brevilabris*, where the zooplankton community may be mainly shaped by selective predation and dominance of bacteria (heterotrophic and cyanobacteria). TSI based on rotifers was not precise about Lake Chapala conditions, instead, TSI based on crustaceans was useful to determine trophic state.

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## Authors' Contributions

CAER: Conceptualization; sampling; identification of rotifers and cladocerans; photography; data analysis; original draft preparation; review, and editing manuscript. LCP: Description of the study area; photography; samples and data analysis; original draft preparation; review, and editing manuscript. OABM: Identification of copepods, writing, original draft preparation, review, and editing manuscript. All authors have read and agreed to the published version of the manuscript.

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# Seasonal and Diel Influence of Environmental Factors on the Parameters of a Zooplankton Community in a Tropical Coastal Lagoon

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## Abstract

Zooplankton communities show seasonal and diel changes mainly associated with the variability of abiotic and biotic factors. Community parameters are good descriptors for evaluating these changes. The study's objective was to analyze the seasonal and diel variation of the parameters of a zooplanktonic community in the La Mancha Lagoon (Veracruz state, Mexico), and the influence exerted by environmental factors. For a year, a 24-h cycle was completed each month, sampling zooplankton every 4 h during a new moon. Abiotic and biotic variables were recorded simultaneously. Univariate permutational analysis of variance, canonical correspondence analysis, and simple correlation analysis were used to analyze the data. Species richness, diversity, and dominance showed significant differences at the seasonal and diel levels. Seasonally, diversity and species richness showed their highest values in November-December and April, and low values in May, while dominance presented a completely inverse pattern. At the diel level, species richness and diversity showed high values at night, while dominance at dawn. Diversity was strongly associated with dominance inversely and directly correlated with species richness. Canonical correspondence ordination explained a high percentage of the constrained variance of the parameters-environment relationship (99.2 %), revealing salinity and diel effect as the most critical variables driving community structure. The influence of salinity was observed at a monthly and daily level. A negligible effect of biotic variables (chlorophyll *a* and densities of diatoms, dinoflagellates, and cyanobacteria) was also observed. Due to the high environmental variability in estuaries, abiotic factors may play a more important role in community structuring.



## Keywords

Abiotic factors, environmental variability, dominant species, predator-evasion, constrained ordination.

## Introduction

Zooplankton communities are of great importance in freshwater, brackish, and marine systems, playing an essential role in energy transfer in the aquatic food web between primary producers and higher consumers and contributing to nutrient recycling (Dvoretsky & Dvoretsky, 2021; Muñoz-Colmenares et al., 2021; Rosa et al., 2021). Furthermore, zooplanktonic organisms are susceptible to environmental changes. They are therefore considered good indicators of ecosystems, because a change in abiotic and biotic factors in aquatic systems results in a change in the relative composition and abundance of these organisms (Azevêdo et al., 2015; Arias et al., 2022; Guermazi et al., 2023).

An objective of aquatic ecological research is to know the response of planktonic communities to environmental conditions (Marques et al., 2009). Thus, many studies have demonstrated seasonal and diel changes in the species composition of zooplanktonic communities. Seasonally, these changes have been explained in terms of the influence of abiotic factors, such as salinity, temperature, dissolved oxygen, and light (Shi et al., 2015; Ge et al., 2021; Ursella et al., 2021; Arias et al., 2022), and/or by biotic factors, such as phytoplankton abundance, chlorophyll *a*, predation, and competition (Węgleńska et al., 1997; García-Herrera et al., 2022), which can act separately or in synergy (Liang et al., 2020; Hobbs et al., 2021; Muñoz-Colmenares et al., 2021; Guermazi et al., 2023).

Diel changes are associated with vertical migration, which is a ubiquitous characteristic in marine and freshwater planktonic communities (van Haren & Compton, 2013; Ursella et al., 2021), and these have been mainly related to food availability, predation avoidance and competition, and are generally modulated by diel and tidal cycles (Hobbs et al., 2021; García-Herrera et al., 2022). Indeed, several studies have observed a considerable increase in the number of species during the night (Marques et al., 2009; Primo et al., 2012) and for this reason, samplings throughout 24 h cycles can provide a better understanding of zooplankton richness.

Although the study of zooplankton communities is a multivariate process, because many species are captured in several samples, there are good univariate descriptors that allow condensing and summarizing information about the number, identity, and relative abundance of the species and, consequently, analyze changes in the community structure. These descriptors are community properties or community parameters, which are essentially species richness, diversity, evenness, and dominance (Morin, 2011), which ha-



ve been used in ecological studies of zooplankton communities (Liang et al., 2020; Arias et al., 2022; Romero et al., 2022; Taniguchi et al., 2023).

There are many studies on the structure of zooplankton communities in freshwater, estuarine, and marine systems. Still, in the particular case of Mexico, there are few studies that simultaneously address seasonal and diel changes in the structure of these communities. In this sense, the main objective of this study was to analyze the seasonal and diel variation of richness, diversity, evenness, and dominance of a zooplanktonic community in La Mancha Lagoon, and the influence exerted by different environmental factors, considering the hypothesis that community structure will be affected mainly by seasonal variability of abiotic factors and diel variation mainly by biotic factors.

## Materials and Methods

### *Study Area*

La Mancha Lagoon is a Ramsar site, a category defined as a site that provides for national action and international cooperation in the conservation of wetlands and the rational and sustainable use of their resources. This system is located in the state of Veracruz, Mexico ( $19^{\circ}33'55''$ – $19^{\circ}35'44''$  N and  $96^{\circ}22'41''$ – $96^{\circ}23'39''$  W). It is a small intermittent microtidal system with an approximate extension of 1,742 km<sup>2</sup> (Morgado-Dueñas & Castillo-Rivera, 2022). The region has a warm, sub-humid climate (Köppen climate classification: Aw2), with two climatic seasons: a dry season (mean monthly rainfall <60 mm) from November to May and a rainy season (mean monthly rainfall >100 mm) from June to October.

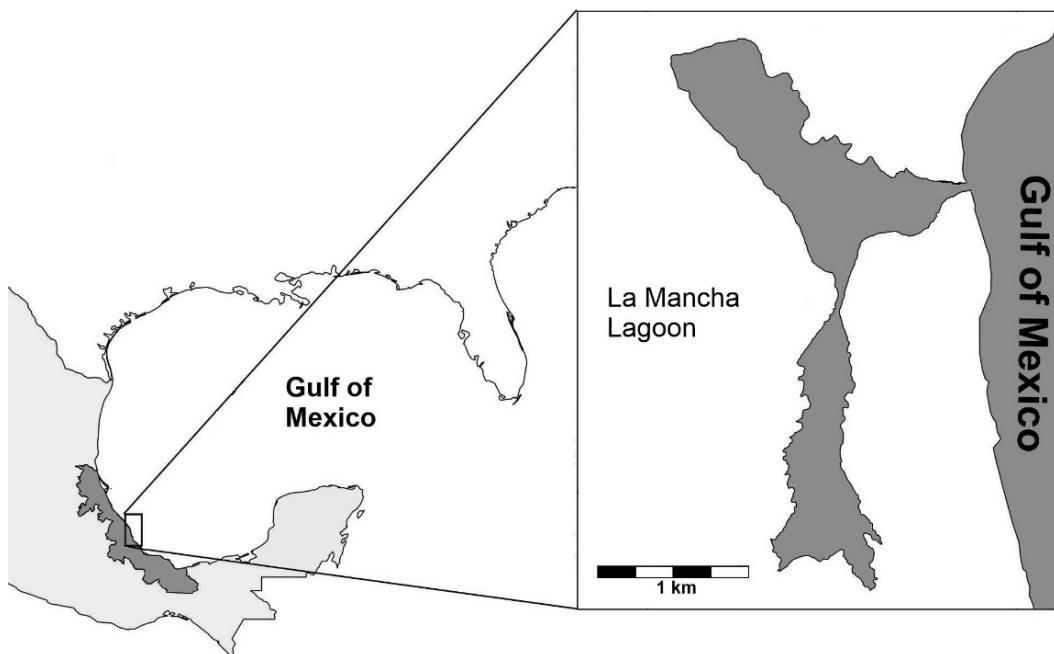


**Figure 1.** Geographical Location of the the La Mancha Lagoon.

### *Sampling Collections*

For a year, the zooplanktonic community at the mouth of La Mancha Lagoon was sampled through monthly diel cycles. Thus, 24-h cycle was completed each month, taking samples every four hours (6 samples per cycle) during the new moon period to maximize the light/dark effect (Castillo-Rivera et al., 2010). Zooplankton samples were obtained by circular surface hauls using a standard net (100 cm length, 30 cm diameter, and 150  $\mu\text{m}$  mesh size). Organisms were immediately preserved in 4 % borax-buffered formalin. Simultaneously, the biotic factors chlorophyll *a* (spectrophotometric method, SCOR, 1966), and abundance of diatoms, dinoflagellates, and cyanobacteria (Utermöhl method, using an inverted microscope Motic AE31), and the abiotic variables salinity (ATAGO S-10E Refractometer), temperature, dissolved oxygen (YSI 550A DO Instrument), water level (tidal phase), and time of day were recorded, according to Mecalco-Hernández et al. (2018). For quantitative analysis, the time of day was classified into diel periods, considering a light-dark gradient on an ordinal scale with values of 2 (day=10:00 and 14:00 h), 1 (twilight=06:00 and 18:00 h) and 0 (night=22:00 and 2:00 h).

### *Data Analysis*

Fifty-seven zooplankton components were recorded, corresponding to 55 species, in addition to two undetermined stages (zoea larvae of brachyura and fish eggs). Their abundances were expressed as individual numbers per cubic meter ( $\text{ind}/\text{m}^3$ ) (Mecalco-Hernández et al., 2018; Mecalco-Hernández & Castillo-Rivera, 2020). From these data, community parameters were estimated for each sample: species richness (number of species), diversity (Shannon-Winer index), evenness (Pielou index), and dominance (Simpson index).

Two-way univariate permutational analysis of variance (PERMANOVA) was applied to evaluate significant differences in the analyzed parameters among months and diel periods, as well as their interactions. PERMANOVA is a routine for testing the response of one or more variables to one or more factors based on a resemblance measure. This method is also appropriate because it uses multiple random permutations to obtain *p*-values; thus, the permutation procedure directly implies normality and homogeneity of variances (Anderson, 2001; Anderson et al., 2008). PERMANOVA was performed using Euclidean distance and permuted residuals under a reduced model, Type III (maximum permutations=999), according to the routine for univariate analysis (Anderson et al., 2008). This analysis was performed using the software PRIMER v7.

To evaluate the influence of environmental factors on community structure, canonical correspondence analysis (CCA) was applied to the parameter data matrix (dependent set: community parameters) and the factor data matrix (explanatory set: abiotic and biotic variables). Inter-set correlations from this analysis were used to determine the environmental

variables that were most important in determining the variability of community parameters (McGarigal et al., 2000). The significance of each of these factors was determined using 499 unrestricted Monte Carlo permutations. A biplot of parameters and explanatory factors was constructed to observe any pattern associated with these factors. All of these analyses were performed using the package CANOCO ver. 4.5 (ter Braak & Šmilauer, 2002). In addition, the degree of simple association between two variables was evaluated using non-parametric Spearman correlation analysis ( $r_s$ ).

## Results

The seasonal pattern of zooplankton diversity and species richness showed high values in November-December and April, and low values in May, July, and January (Fig. 2A). In contrast, dominance was high in May (defined by the copepods *Tortanus (Acutanus) setacaudatus* and *Pseudosiaptomus pelagicus*), October (by zoea larvae of brachyura), and March (by the copepod *Acartia (Acanthacartia) tonsa*), with low values in December and April, while evenness shows a completely inverse pattern to dominance (Fig. 2B).

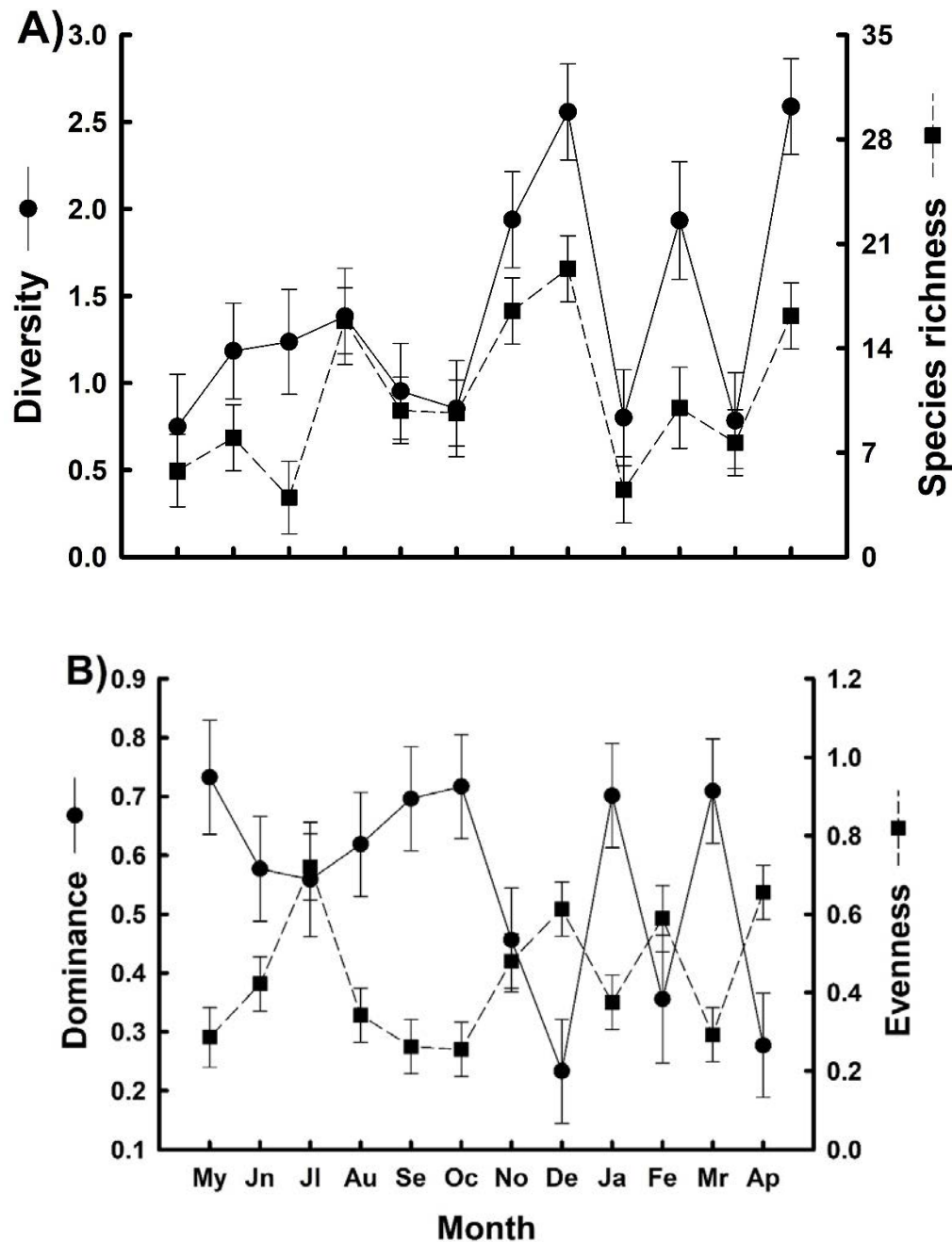


**Figure 2.** Monthly Variation of: A) Mean and Standard Error Values of diversity and species richness, and B) Mean and Standard Error Values of Dominance and Evenness (for each Monthly Value  $n=6$ ).

The diel variability of these parameters showed that diversity and richness presented high values at night (22:00 to 02:00 h) and low values at dawn (06:00 h) or dusk (18:00 h) and during the day (10:00 to 14:00 h) (Fig. 3A). On the contrary, the high value of dominance occurred at dawn (06:00 h) dominated by *A. tonsa* and zoea larvae, and low values during the night (22:00 to 02:00 h) while the evenness shows a reverse pattern (Fig. 3B). The community was dominated solely by the abundance of four zooplanktonic compo-



nents, *A. tonsa*, *P. pelagicus*, *T. setacuadatus*, and zoea larvae, which represented 89 % of the total abundance in number of the entire community.

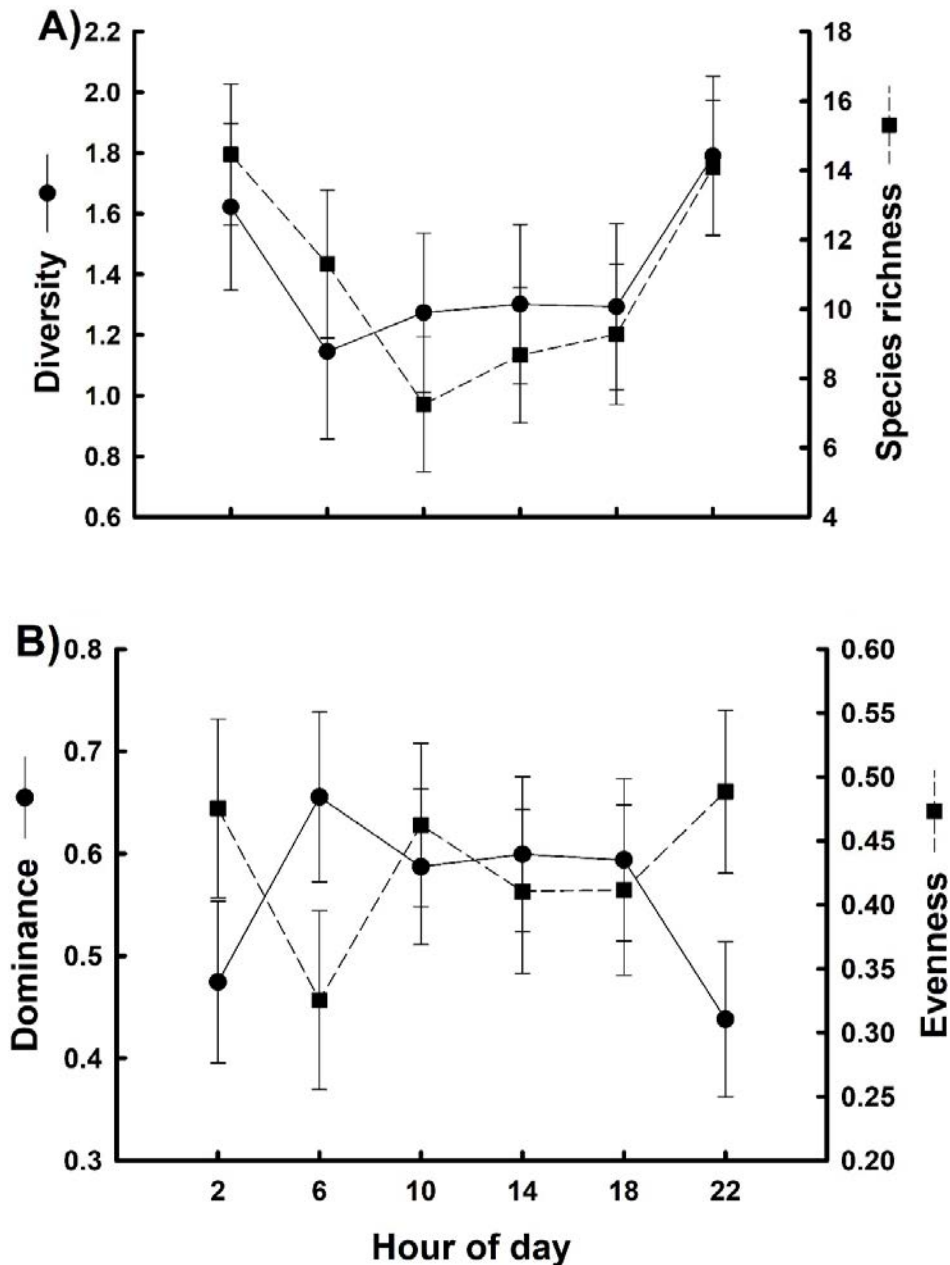


**Figure 3.** Mean and Standard Error Values by Hour: A) Diversity and Species Richness, and B) Dominance and Evenness (for each Hourly Value n=12).

According to the two-way univariate results (PERMANOVA), all parameters show significant differences among months. Similarly, at the diel level, there were significant differences except for evenness. All interactions between monthly and diel factors were not significant ( $P$ 's  $>0.3$ , Table 1).

**Table 1.** Results of Two-way Univariate Analysis of Variance (PERMANOVA) to Test the Effects of the Month and Diel Periods (Day, Twilight, and Night) on the Community Parameters.

| Source of Variance      | df | MS     | Pseudo-F | P (Permuted) |
|-------------------------|----|--------|----------|--------------|
| <b>Diversity</b>        |    |        |          |              |
| Months                  | 11 | 2.589  | 6.053    | 0.001        |
| Diel                    | 2  | 1.563  | 3.655    | 0.039        |
| Months x Diel           | 22 | 0.371  | 0.866    | 0.638        |
| Error                   | 32 | 0.428  |          |              |
| <b>Species Richness</b> |    |        |          |              |
| Months                  | 11 | 146.19 | 5.822    | 0.001        |
| Diel                    | 2  | 232.96 | 9.278    | 0.001        |
| Months x Diel           | 22 | 16.320 | 0.650    | 0.835        |
| Error                   | 32 | 25.109 |          |              |
| <b>Dominance</b>        |    |        |          |              |
| Months                  | 11 | 0.178  | 4.455    | 0.001        |
| Diel                    | 2  | 0.179  | 4.479    | 0.020        |
| Months x Diel           | 22 | 0.044  | 1.096    | 0.396        |
| Error                   | 32 | 0.040  |          |              |
| <b>Evenness</b>         |    |        |          |              |
| Months                  | 11 | 0.127  | 5.019    | 0.001        |
| Diel                    | 2  | 0.041  | 1.627    | 0.246        |
| Months x Diel           | 22 | 0.030  | 1.178    | 0.328        |
| Error                   | 32 | 0.025  |          |              |

In this sense, diversity was mainly inversely correlated with dominance ( $r_s = -0.981$ ,  $P < 0.0001$ ) and directly correlated with species richness ( $r_s = 0.747$ ,  $P < 0.0001$ ), while species richness and dominance were also negatively correlated ( $r_s = -0.693$ ,  $P < 0.0001$ ).

Concerning environmental factors' influence on community structure, the simultaneous ordination of parameters and environmental matrices in CCA showed that all canonical axes were significant ( $P = 0.010$ ). The first two axes explained 99.2 % (76.9 and 22.3 %, respectively) of the total constrained variance. Intra-set correlations indicated that salinity, the diel effect, and temperature were the most critical variables in structuring the zooplankton community. Likewise, according to Monte Carlo significance tests, only these factors significantly affected the community parameters. Both inter-set correlations and significance tests indica-

ted that biotic factors such as chlorophyll *a* and the abundance of diatoms, dinoflagellates, and cyanobacteria were unimportant in community structuring (Table 2).

**Table 2.** Inter-set Correlations Between Environmental Factors and Species Scores from the Canonical Correspondence Analysis. The Significance of these Factors (Unrestricted Monte Carlo Permutations) Is also Shown.

| Environmental Factors | Inter-set Correlations |        |             |
|-----------------------|------------------------|--------|-------------|
|                       | Axis 1                 | Axis 2 | P(Permuted) |
| Salinity              | -0.510                 | 0.104  | 0.002       |
| Diel Effect           | 0.363                  | 0.052  | 0.020       |
| Temperature           | -0.072                 | -0.284 | 0.038       |
| Tidal Stage           | -0.149                 | -0.156 | 0.192       |
| Dissolved Oxygen      | 0.133                  | -0.145 | 0.272       |
| Diatoms               | -0.129                 | -0.066 | 0.506       |
| Chlorophyll <i>a</i>  | 0.199                  | 0.068  | 0.608       |
| Cyanobacteria         | 0.181                  | -0.105 | 0.748       |
| Dinoflagellates       | 0.059                  | -0.023 | 0.934       |

In the CCA analysis biplot (Fig. 4), the length and direction of the arrows indicate the relative importance and direction of change that each factor has in the ordination. This diagram reveals a main ordination gradient related to salinity that is directly associated with species richness and inversely with dominance. Another major trend, perpendicular to the first, is related to temperature and is in the opposite direction of the diel effect, which shows that diversity is associated with low temperatures and evenness in the daytime.

Thus, salinity was the environmental variable that was most correlated individually with the community parameters, directly with diversity ( $r_s=0.575$ ,  $P<0.0001$ ), species richness ( $r_s=0.491$ ,  $P<0.0001$ ), and evenness ( $r_s=0.486$ ,  $P=0.0001$ ), and inversely with dominance ( $r_s=-0.536$ ,  $P<0.0001$ ). The direct relationship between salinity, diversity, and species richness can be observed at the seasonal and diel levels (Fig. 5A-B and 5C-D, respectively). Dominance has an inverse clear relationship with salinity only seasonally. Other abiotic and biotic variables did not show significant simple correlations with the parameters.

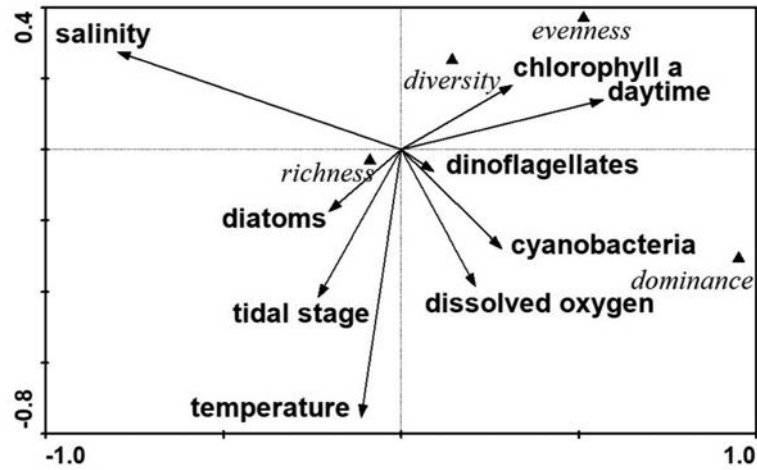
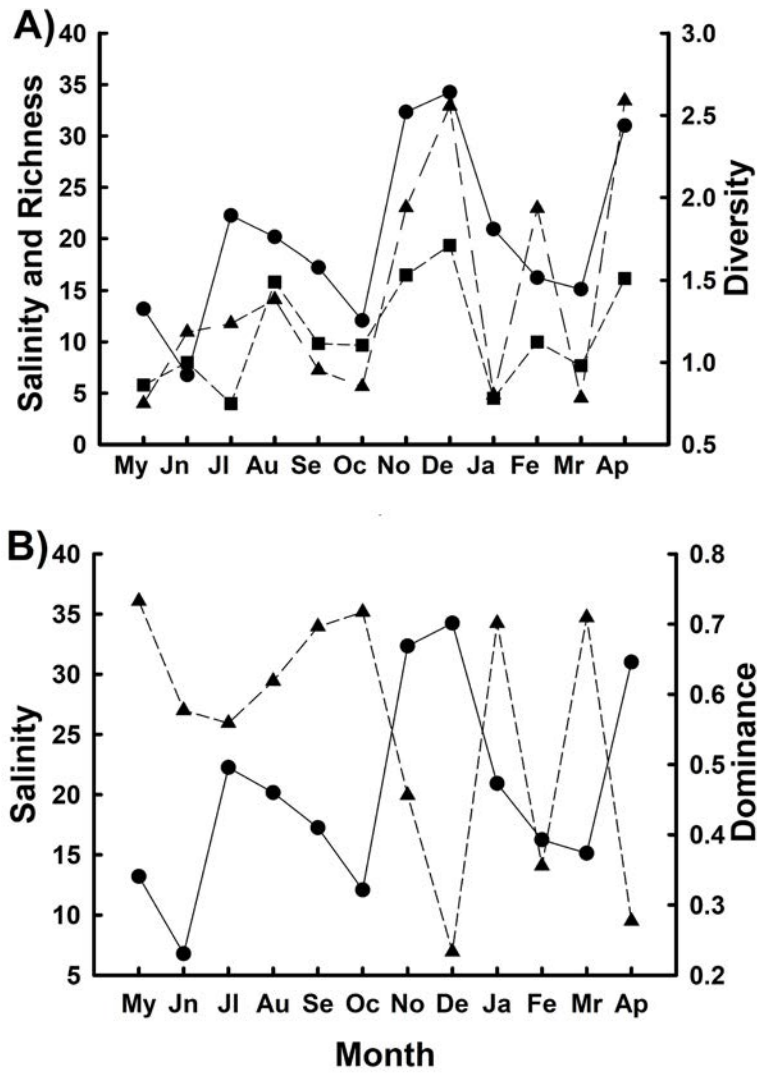


**Figure 4.** Bi-plot of Canonical Correspondence Analysis of the Overall Community Parameters and Environmental Variable Data Matrices. Data Points Represent Parameters (▲), and Arrows Represent the Direction and Relative Importance of Explanatory Variables.



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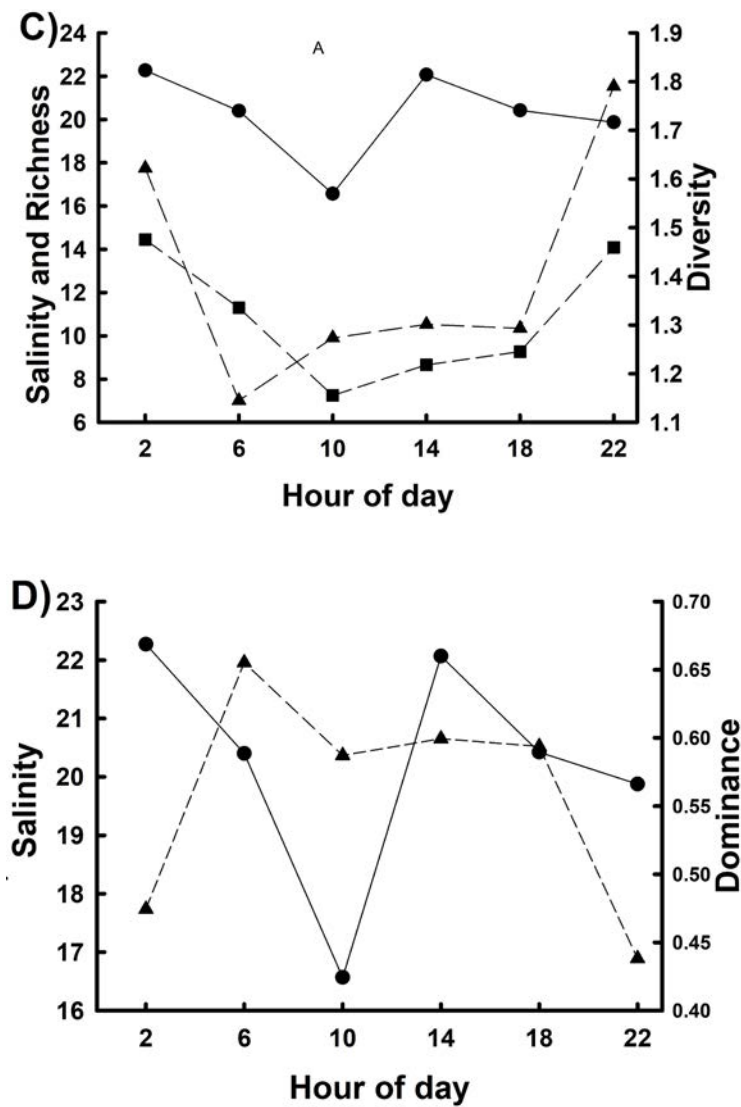


**Figure 5.** Monthly Mean Values of Salinity and Community Parameters: A) Direct Relationship between Salinity (●), Species Richness (■), and Diversity (▲), and B) Inverse Relationship between Salinity (●) and Dominance (▲) (for Mean Monthly Values  $n=6$ ). Mean Values by Hour of Salinity and Community Parameters: C) Direct Relationship between Salinity (●), Species Richness (■), and Diversity (▲), and D) Relationship between Salinity (●) and Dominance (▲) (for Mean Hourly Values  $n=12$ ).

## Discussion

Although the formal study of zooplankton in Mexican waters began more than a century ago, most of the studies carried out correspond mainly to research in freshwater and marine systems with central reference only to some groups of zooplankton (Mecalco-Her-

nández & Castillo-Rivera, 2020; Alcocer et al., 2022; Cervantes-Martínez et al., 2023), there are few studies in estuarine systems, especially in the state of Veracruz. Likewise, few studies have inferred diel changes in zooplankton communities from low-frequency temporal sampling which allows a more precise assessment of the timing of diel changes throughout the 24 h cycle and the influence of environmental factors on these changes over short periods.

In the research of zooplankton communities, many studies have used community parameters to describe changes in community structure adequately (Liang et al., 2020; Arias et al., 2022; Romero et al., 2022; Taniguchi et al., 2023). In the present study, all community parameters showed a significant monthly variation, which is related to the strong environmental variability that characterizes estuarine ecosystems and to the fact that zooplankton usually presents a relatively rapid response to environmental changes (Benfield, 2013; Guermazi et al., 2023).

In this way, diversity and species richness showed pulses in December and April and low values in May, while dominance showed a completely inverse pattern. Indeed, simple correlations showed that diversity and species richness had a strong inverse correlation with dominance. Concerning environmental stability, diversity can be determined primarily by evenness in habitats with low variability. However, in environments with strong variability, such as estuaries, few eurytopic species (tolerant to a wide range of ecological conditions) would be expected to be dominant, so diversity would mainly be inversely affected by dominance. Indeed, in La Mancha Lagoon, almost 90 % of the numerical abundance of the entire community was dominated by only four zooplankton components (*A. tonsa*, *P. pelagicus*, *T. setacuadatus* and zoea larvae).

At the diel level, all parameters showed significant differences among hours, except evenness. Species richness and diversity showed high values during the night and low values during the day. According to the PERMANOVA results, there was no significant interaction between the month and diel period factors, for all four parameters, indicating that the diel pattern was consistent across all sampled months. In this way, many studies have indicated that during the night there is a significant increase in the number of species (Marques et al., 2009; Primo et al., 2012). This behavioral pattern has been considered the most common characteristic in marine and freshwater planktonic communities, which is characterized by an evening ascent and a morning descent (van Haren & Compton, 2013; Ursella et al., 2021).

Considering the predator avoidance hypothesis, the vertical migration of zooplankton to deeper waters during daylight hours, where the probability of being detected by visually searching predators is lower than if they remained in shallow waters with more illuminated conditions, and during the night, under dark conditions, zooplankton migrate

upward to feed and shelter (Węgleńska et al., 1997; Ursella et al., 2021; Garcia-Herrera et al., 2022). These vertical patterns may also appear to be influenced by abiotic factors such as hydrological regime (inputs of freshwater flow), water currents, light variation, tidal phase, and salinity (Primo et al., 2012; Benfield, 2013; Hobbs et al., 2021; Ge et al., 2021).

The dominant zooplanktonic components, *A. tonsa* and zoea larvae, showed a typical crepuscular occurrence with pulses at dawn. In this sense, the increase in activity during twilight hours may be related to compensation strategies that attempt to resolve the trade-off between feeding and avoiding predators. Twilight conditions may mitigate visual detection by visual predators, but provide sufficient light to detect prey. Indeed, considering a predator-prey encounter model (Giske et al., 1994), for smaller zooplankters the risk of mortality will generally be reduced in accordance with a general decrease in ambient light.

Regarding the knowledge of biodiversity in zooplankton communities, studies over short periods throughout sampling in 24 h cycles can provide a better understanding of zooplankton richness. Indeed, in the present study, of the total 57 zooplanktonic components recorded, 13 (~23 %) were collected only at twilight and during the night.

About the multivariate influence of environmental factors on community parameters, the CCA ordination explained a high percentage of the constrained variance of the parameters-environment relationship (99.2 %), which implies that the environmental variables considered give an adequate description of the community structure. According to inter-set correlations and Monte Carlo significance tests, abiotic factors salinity and diel effect (related to light gradient) were the most critical variables driving community structure.

Indeed, salinity is one of the main factors driving the structuring of zooplankton communities in estuarine and marine systems (Benfield, 2013; Shi et al., 2015; Dvoretzky & Dvoretzky, 2017; Chen et al., 2023), affecting the richness and diversity species inversely (negative correlations) or directly (positive correlations), depending on whether the communities are dominated by freshwater or marine species (Paturej & Gutkowska, 2015; Helenius et al., 2017; Yuan et al., 2020).

In the present study, according to CCA results salinity has a positive effect on species richness, while it has a negative effect on dominance. These results were also confirmed by simple correlations, which indicated significant direct correlations of salinity with species richness and diversity and a significant inverse correlation between salinity and dominance. Higher salinities allowed the entry of a greater number of neritic species to the lagoon, thus increasing richness and diversity. In contrast, in low-salinity conditions only tolerant species remain dominant in the community. Thus, the effect of salinity was observed at monthly and diel levels. The light gradient showed that species richness was more significant during the night, while other abiotic factors such as dissolved oxygen and tidal stage showed little incidence on the community structure (low inter-set correlations and no

significant effect). This is because the dissolved oxygen values in the system are probably greater than the limits of lethal and sublethal hypoxic conditions. Likewise, although some studies have observed the importance of the tide in the structuring of the community (Marques et al., 2009; Primo et al., 2012), for the zooplanktonic community of La Mancha, the tide stage had little influence mainly because the southwest coast of the Gulf of Mexico is predominantly microtidal (Ellis & Dean, 2012), with <20 cm within the lagoon.

CCA results also showed the negligible effect of biotic factors on community structuring. Many studies have observed that zooplankton communities can be affected by biotic factors such as phytoplankton density (García-Herrera et al., 2022; Guermazi et al., 2023) and chlorophyll *a* (Liang et al., 2020; Muñoz-Colmenares et al., 2021; Chen et al., 2023), but in the present study, chlorophyll *a* and the densities of diatoms, dinoflagellates, and cyanobacteria were not significant. Thus, both abiotic and biotic conditions can have an important incidence in a structuring community, but their relative importance may vary among different environmental conditions and across temporal and spatial scales. While abiotic factors can affect community structure by setting the limits of abiotic tolerance of species, biotic factors may also affect diversity patterns by influencing species abundance and composition (Måsviken et al., 2023).

In this way, many studies have compared the relative effect of these factors on zooplankton communities, observing that both can act synergistically (Gray et al., 2012; Mecalco-Hernández et al., 2018; Yang et al., 2019; Chará-Serna and Casper, 2021) or at a different temporal scale (Gabaldón et al., 2019; Rollwagen-Bollens et al., 2020). In this way, the variability of abiotic factors can also modify the biological response of species (Begon & Townsend, 2021), as observed in La Mancha Lagoon, where the light/dark cycle affects predator-evasion patterns.

Notwithstanding the above, it would be expected that in environments with low environmental variability (stable), biotic factors such as food availability, predation, and competition would be the main regulators of the community. In contrast, in environments with high environmental variability, abiotic factors would play a more important role, as is also observed in the zooplanktonic community of La Mancha Lagoon and other similar systems (Sahuquillo & Miracle, 2019; Sgarzi et al., 2019).



## Conclusion

A better understanding of the zooplanktonic community dynamics of aquatic ecosystems can be achieved by sampling over 24 h. Thus, species richness was consistently greater during the night, which was related to predator-evasion strategies modulated by the light/dark cycle. The environmental variables considered in the present study explained a very high percentage of the community structure's temporal variability, allowing a more reliable interpretation of the results. The high environmental variability characteristic of estuaries caused the community structure to vary significantly among months and hours. As a consequence of this variability, diversity was mainly associated with dominance, and the abiotic factors salinity and diel effect played a more important role in determining the community structure, while the biotic variables chlorophyll *a*, and densities of diatoms, dinoflagellates, and cyanobacteria had negligible importance.

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# Utilization of Zooplankton in Environmental Risk Assessment in Mexico

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## Abstract

This brief review focuses on the use of zooplankton as a key indicator to evaluate environmental risk in the aquatic ecosystems of Mexico. It explores some ecotoxicological studies that assess the impacts of toxic agents on individual and population levels. The role of zooplankton in food webs, susceptibility to environmental changes, and significance as an indicator of both direct and indirect pollution effects are emphasized. The study underscores the crucial role of ecotoxicological tests in preventing environmental damage, guiding decision-making, and managing ecosystems. Special attention is given to the ecotoxicology of rotifers, cladocerans, and copepods, providing insights into cultivation protocols, their relevance in ecotoxicology, and key species used in Mexico. It further explores testing methodologies, advantages, and the range of pollutants assessed. The review also examines the geographical distribution of zooplankton research in Mexico, underlining the necessity to broaden investigations to diverse aquatic systems. Challenges, such as the importance of including native species in toxicity studies and the development of specific protocols for freshwater copepods, are addressed.

## Keywords

Ecotoxicology, Acute toxicity, Chronic toxicity, Rotifers, Cladocerans, Copepods.

## Introduction

### *Environmental Risk Assessment*

The environmental risk assessment (ERA) is a method that assesses the probability of adverse ecological effects arising from the exposure of organisms and communities to one or more chemical compounds, either currently happening or likely to occur (Di Lorenzo et al., 2023). In Mexico, researchers have incorporated the use of aquatic invertebrates into ERA for several years. The selection of these organisms in these evaluations is based mainly on ecological relevance (validity), reliability (reproducibility), representative test species, and sensitivity, as noted by Breitholtz et al. (2006). ERA is carried out through ecotoxicological studies conducted in both laboratory and field settings. Ecotoxicology is comprised of three disciplines: ecology, toxicology, and chemistry and examines the effects of toxic compounds at different levels of biological organization, including individuals, population, and community levels (Pastorino et al., 2024). Measurable outcomes encompass physiological homeostasis, reproductive behavior, morphological alterations, and mortality (Zimmermann and Sures, 2023). This approach employs sensitive organisms as reliable indicators, connecting compound exposure to organism response. These data enable ecotoxicologists to identify concentrations indicating adverse conditions (OECD, 2011).

Ecotoxicological studies play a crucial role in both preventing environmental damage and understanding the chemical characteristics of the environment. They are indispensable in decision-making processes related to resource protection and ecosystem management (Relyea & Hoverman, 2006). These assessments provide valuable insights into the effects of pollutants on aquatic organisms and ecosystems, thereby influencing the formulation guiding the development of environmental protection guidelines. Additionally, these studies help identify sensitive stress indicators, facilitating in the evaluation of mitigation measures (Montalvo & Luque, 2009).

Mexico has a wide variety of aquatic species that can be used in ecotoxicological tests. These tests are essential for conducting a comprehensive Environmental Risk Assessment (ERA), particularly studies focused on evaluating water quality over time and space in waterbodies impacted by pollutants (Santos-Medrano et al., 2007; Guzmán-Colis et al., 2011). In the present review, we emphasize the importance of ecotoxicological studies carried out in Mexico that use native zooplankton species as model organisms. We have compiled various relevant research that addresses the use of rotifers, cladocerans, and copepods in the assessment of environmental risk associated with wastewater treatment plant effluents, heavy metals, pesticides, cyanotoxins, pharmaceuticals, and microplastics, all of which are frequent pollutants in the aquatic environments of Mexico and other regions of the world.



### *Zooplankton Applications in Ecosystem Health*

Zooplankton is vital for aquatic ecosystems, contributing to food webs, nutrient cycling, biogeochemical processes, and algal bloom regulation (Declerck & de Senerpont, 2023). Marine zooplankton, spanning 12 phyla, is diverse, with copepods being the most abundant, constituting 80 % of biomass (Gasca, 2010; Peijnenburg & Goetze, 2013). In freshwater communities, rotifers, cladocerans, and copepods dominate, with rotifers significantly contributing to biomass (Sarma & Nandini, 2017; Elías-Gutiérrez & Ortiz, 2017).

Zooplankton is highly vulnerable to environmental changes, impacting other organisms through trophic interactions (Gutiérrez & Gagneten, 2011). Due to its ease of cultivation and sensitivity, with well-known nutritional requirements and short life cycles, it's an ideal model for ecotoxicological evaluations (Declerck & de Senerpont, 2023). Ecotoxicological tests use rotifers, cladocerans, and copepods as indicators to assess direct and indirect effects (Elías-Gutiérrez & Gagneten, 2011). For example, effluent quality is often assessed based on physical and chemical parameters alone; however, zooplankton have proven useful in assessing the effectiveness of wastewater treatment plants through acute toxicity tests (Torres-Guzmán et al., 2010).

### *Zooplankton Species Used Globally*

Researchers employ zooplankton models tested in controlled conditions or micro/mesocosms (Moreno et al., 2022). Microcosms maintain consistent but less complex conditions, while mesocosms, offering greater complexity, simulate controlled or natural environments indoors or outdoors (Lozano, 2020). Outdoor systems, while authentic, face weather unpredictability, complicating replication and causing data variation. Researchers must align conditions with their study focus and logistical factors, especially in ecotoxicology (Hjorth et al., 2006).

According to De Meester et al. (2023), the majority of zooplankton tests have centered around the *Daphnia* genus due to its well-researched ecology over the past 120 years. Copepod research has focused on risk assessment in both marine and freshwater environments, particularly emphasizing the naupliar stage (López, 2018). *Acartia tonsa* is common in bioassays for evaluating environmental hazards due to its ecological significance and adaptability (Rotolo et al., 2021). Studies indicate that *Tisbe battagliai*, *Tigriopus japonicus*, *Nitocra spinipes*, and *Mesocyclops leuckarti* are more sensitive to various contaminants than *Daphnia magna* (OECD, 2011).

Rotifers are cost-effective tools for environmental protection. The *Brachionus* genus (*B. plicatilis*, *B. calyciflorus*, and *B. havanaensis*) is recognized by the American Public Health Association for assessing xenobiotics, endocrine disruptors, and disinfectants in both freshwater and marine environments (Alayo & Iannacone, 2002; Sarma et al., 2014).

## Zooplankton Groups in Mexico for Environmental Risk Assessment

As of January 2024, in the Scopus database, there are roughly 450 research studies on zooplankton in Mexico, with 80 of them specifically focusing on environmental risk assessment. These studies have highlighted 50 different species of zooplankton with the primary attention given to three main groups: rotifers, cladocerans, and copepods. Other groups, including Anostraca (*Artemia franciscana*), Amphipoda (*Hyalella azteca*), and Ostracoda (*Diaphanocypris meridana*), have also been studied (Fig.1). The majority of research has been conducted in entities such as the State of Mexico, Mexico City, Aguascalientes, Veracruz, Baja California Sur, and Quintana Roo. However, there are still areas, like Oaxaca, Durango, Zacatecas, Colima, and Coahuila, where research is scarce. This emphasizes the need to promote research in these areas to gain a better understanding of the environmental risk in Mexico.

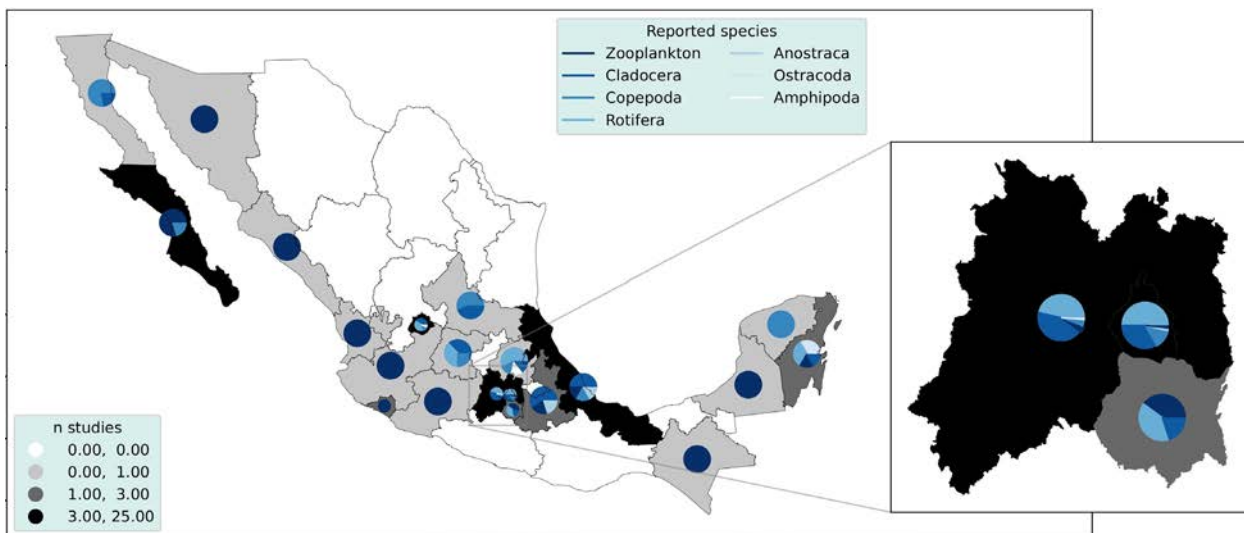


**Figure 1.** Distribution of Research on Zooplankton in Mexico with a Focus on Environmental Risk and the Main Groups Studied.

## Rotifers

### *Diversity, Behavior, and Reproductive Strategies*

Rotifers are microorganisms (approximately 50–2,000  $\mu\text{m}$  in length) ubiquitously found in diverse ecosystems, ranging from fresh and brackish waters to marine environments worldwide. Phylum Rotifera has two classes: Pararotatoria with subclass Seisonidea and Eurotatoria with subclasses Bdelloidea and Monogononta (Fontaneto & Plewka, 2021). Globally, approximately 2,300 species have been described, while in Mexico, around 400 species have been recorded (Sarma et al., 2021). In general terms, the life cycle of mo-

nogonont rotifers begins with the hatching of amictic females from diapause eggs. When environmental conditions are conducive, such as optimal temperature, salinity levels and food availability, reproduction occurs asexually through parthenogenesis.

Conversely, in unfavorable situations, the reproductive process takes a sexual phase. In the sexual phase, mictic females produce diapause eggs stored in the sediments of aquatic systems and can remain viable for many years (Fontaneto & Plewka, 2021). The distinctive ability of rotifers to produce diapause eggs makes them valuable resources for laboratory experiments, including ecotoxicological studies (Won et al., 2017).

### *Use of Rotifers in Ecotoxicology*

Early ecotoxicological studies involving rotifers commenced in the 1980s, as reported by Halbach et al. (1983). Since then, rotifers have been pivotal in evaluating environmental risks linked to a spectrum of emerging contaminants, including pharmaceuticals, pesticides, and microplastics. Additionally, they have contributed significantly to assessing heavy metals, emerging pollutants, and the impact of harmful algal blooms. Various reasons support the position of rotifers as model organisms in this ecotoxicology: a) their ease of culture and manipulation in the laboratory; b) a short life cycle (7-20 days), which facilitates short-term analysis of demographic parameters such as average lifespan, life expectancy, reproductive rates, generation time and increase rates; c) parthenogenetic reproduction, which ensures genetic homogeneity and rapid population growth; d) its high sensitivity to changes in water quality; and e) ecological relevance and reliability in reproducibility (Dahms et al., 2011; Rico-Martínez et al., 2017; Won et al., 2017). These characteristics meet the requirements to consider rotifers as representative test species in environmental risk assessments.

### *Main Rotifer Species in the Ecotoxicology of Mexico*

Recently, Sarma et al. (2021) carried out an exhaustive compilation of rotifer species present in freshwater bodies in Mexico. Among the rotifer families analyzed it was observed that the most diverse were Flosculariidae (50 species), Brachionidae (51 species), Lecanidae (68 species), Notommatidae (48 species), and Trichocercidae (31 species). It is relevant to highlight that the most significant diversity of species was found in the state of Mexico, where 323 species were recorded, followed by Michoacán with 164, Veracruz with 155, Aguascalientes with 150, and Yucatan with 129 species reported. In Mexico, various species of rotifers serve as model organisms in ecotoxicological tests. According to the data included in the Scopus database (November 2024), rotifer species used in ecotoxicological studies in Mexico include *Anuraeopsis fissa*, *Asplanchna sieboldii*, *Brachionus calyciflorus*, *B. angularis*, *B. rubens*, *B. havanaensis*, *Lecane hamata*, *L. luna*, *L. quadridentata*, *L. papuana*, *Euchlanis dilatata*, and *Plationus patulus*.

The basic rotifer bioassays include acute toxicity tests, like the 24 h LC<sub>50</sub> (lethal concentration for 50 % of the population) and the EC<sub>50</sub>, indicating the effective concentration inhibiting 50 % of biological responses to contaminants. Chronic toxicity tests assess life table parameters and population growth in organisms exposed to toxicants (Dahms et al., 2011).

In Mexican aquatic ecosystems, *B. calyciflorus* is commonly used in studies on environmental risks. For example, Zamora-Barrios et al. (2017) evaluated the effects of crude extracts of cyanobacteria detected in Lake Nabor Carrillo, which is part of what was once Lake Texcoco (Mexico City), on *B. calyciflorus* isolated from the same waterbody. Thus, demonstrating the impact that cyanobacteria blooms in tropical waters have on key species. The rotifer *B. angularis*, isolated from a pond in the Park of Tezozomoc (Mexico City), has been helpful in evaluating the effects of methyl parathion, a commonly used insecticide in Mexico, to eradicate insect pests (Gama-Flores et al., 2004). This study illustrates the potential risk associated with the presence of pesticides in aquatic systems and their impact on the population dynamics of invertebrates, which contend with constant changes in water quality and food availability. It also underscores the organisms' usefulness in short-term assessments of environmentally relevant pesticides in Mexico, particularly in agricultural areas. In Aguascalientes, Tovar-Aguilar et al. (2019) used the rotifer *L. papuana* to evaluate the effects of the pharmaceutical diclofenac, detected in surface and groundwater due to its extensive use in human and veterinary health. This study exhibits the vulnerability of zooplankton to relevant emerging pollutants and stresses the potential for bioaccumulation of xenobiotics, which can induce disturbances in the ecological structure of aquatic environments. Heavy metal pollution in aquatic environments is a growing concern due to high concentrations, persistence, and biomagnification. The first reports of lead biomagnification in predatory rotifers were published by Rubio-Franchini and Rico-Martínez (2008, 2011) at Niagara Dam in Aguascalientes. Their research focused on the species *A. brightwellii* and emphasized the importance of conducting *in situ* studies while confirming the findings through laboratory tests. Similarly, another study highlights the relevance of assessing the toxicity of metals (Al, Fe, and Zn) in the San Pedro River using acute toxicity tests on *L. quadridentata* (Torres-Guzmán et al., 2010). This approach allowed the estimation of each metal's contribution to overall toxicity, identifying zinc as the most toxic metal, underscoring the value of combining field and laboratory analyses for a comprehensive evaluation of metal pollution.

A recent study in Manatí Lagoon, a protected area in Cancún (Quintana Roo), evaluated the risk through zooplankton, including rotifers (Demidof et al., 2022). These key indicators of aquatic health underscore the urgency of addressing pollution in protected environments. This comprehensive approach, involving the evaluation of key organisms such as rotifers, highlights the urgent need for effective strategies to counteract the de-



trimental effects of heavy metal pollution in aquatic environments, especially in protected areas such as Laguna Manatí. The rotifers *P. patulus* and *A. sieboldii*, isolated from Lake Xochimilco (Mexico City), were used to analyze the effects of microplastics and their interaction with heavy metals on the predator-prey dynamics and demographic variables of the rotifers (Hernández-Lucero et al., 2023). This study revealed that mixtures of heavy metals and microplastics can accentuate the vulnerability of key species to pollution. Thus, it highlights the importance of rotifers in the ecological risk assessment of microplastics in Mexico, emphasizing the need to understand the interaction between these contaminants and aquatic fauna to preserve the health of ecosystems.

### *Challenges*

Most ecotoxicological studies with freshwater rotifers focus on the cities of Mexico and Aguascalientes. This is presumed due to the presence of groups of experts on the subject in these states. While there is evidence of ongoing efforts in the states of Veracruz and Yucatan. It's crucial to intensify these efforts and expand research to diverse aquatic systems in Mexico, leveraging the potential of rotifers. These organisms can be tools in developing mitigation strategies and deepening our understanding of the effects of pollution in different aquatic environments in the country. The study of the effects of heavy metals on rotifers is more common than research on other contaminants. It's essential to explore the environmental risks of emerging contaminants of global relevance, such as pharmaceutical waste and microplastics which still require more detailed investigations. Furthermore, it is crucial to consider more realistic scenarios in studies that reflect aquatic systems contaminated by a mixture of substances, an evaluation made possible thanks to the advantages that rotifers offer as model organisms. Surprisingly, in rotifer studies in Mexico, environmental genomics still needs to be fully integrated despite being a tool that could provide a deeper understanding of the toxicity mechanisms of various toxic substances.

## Cladocerans

### *Diversity, Behavior, and Reproductive Strategies*

Cladocerans, commonly referred to as “water fleas,” belong to the Phylum Arthropoda and the class Branchiopoda, encompassing four recognized Orders: Anomopoda, Ctenopoda, Haplopoda, and Onychopoda. With a size range of 0.25 to 18 mm, there are nearly 850 described species, including 150 recorded in Mexico (Cervantes-Martínez et al., 2023). They are the connection between the microbial loop, primary producers, and higher-level consumers (Kalinowska, 2015).

Cladocerans employ their thoracic appendages to filter particles, optimizing nutrient consumption for efficient growth and reproduction (Riisgård, 2015; Smirnov, 2017). Most

species exhibit facultative parthenogenesis, where males are associated with seasonality or environmental unpredictability. They produce resting eggs (ephippia), serving as a genetic reservoir and facilitating passive dispersal (Bernatowicz et al., 2018). However, certain species within the *Daphnia pulex* complex may display obligatory parthenogenesis (Huynh et al., 2023).

### *Use of Cladocerans in Ecotoxicology*

The utilization of cladocerans in bioassays is supported by their easy maintenance in laboratory settings and sensitivity (Terekhova et al., 2018). Since the 1970s, environmental agencies have standardized protocols for evaluating diverse toxicants using cladocerans (Versteeg et al., 1997). Toxicological studies on cladocerans involve acute tests (LC<sub>50</sub> determination within 24-48 h) (OECD, 2004; U.S. EPA, 2002) and chronic tests, assessing life history, physiology, and molecular responses over extended periods (>30 % of the life cycle) (Connors et al., 2022). Population growth and life table experiments offer vital insights into their fitness, stress responses, and reactions to chemical compounds (Sibly & Hone, 2002; Wilson et al., 2006; Sarma & Nandini, 2006).

### *Main Cladocerans Species in the Ecotoxicology of Mexico*

In Mexico, 17.6 % of the globally recognized cladoceran species have been identified, with ongoing efforts to expand this number. However, by 2008, only 1 % of watersheds had been thoroughly explored, indicating vast potential for further study (Elías-Gutiérrez et al., 2008a). Additionally, the use of molecular markers has brought to light a large number of cryptic species (Elías-Gutiérrez et al., 2008b).

The first study to use cladocerans as a model assay in Mexico was focused on evaluating the chronic toxicity of wastewater from the paper industry (Martínez-Jerónimo et al., 1993). Since then, there has been an exponential increase in publications exploring the effects of various chemical, physical, and biological compounds, such as heavy metals, pesticides, surfactants, personal care products, pharmaceuticals, hormones, phytotoxins, microplastics, and wastewater. These studies have been conducted across a limited range of species, including *Daphnia magna*, *D. pulex*, *D. laevis*, *D. exilis*, *D. schoedleri*, *D. ambigua*, *Ceriodaphnia dubia*, *Moina macrocopa*, *M. micrura*, *Alona glabra*, *Diaphanosoma birgei*, *Bosmina longirostris*, and *Macrothrix triserialis*. This surge in research highlights the growing recognition of the important role of cladocerans in aquatic ecotoxicology within the Mexican scientific community.

Cladocerans have proven to be a reliable model organism in Mexico's field of environmental assessment. A pioneering study conducted by López-López and Serna-Hernández (1991), linked seasonal zooplankton variation in reservoirs and identified species like *Bosmina longirostris*, *Diaphanosoma birgei*, and *Daphnia parvula* as excellent indicators of

water quality changes associated with eutrophication (Mendoza-Chávez et al., 2022). Recent research in San Luis Potosí revealed a correlation between arsenic levels and microcrustacean diversity in reservoirs, suggesting *Simocephalus punctatus* as a potential reliable bioindicator. Furthermore, saprobity indices have been devised for assessing water quality in the Xochimilco canals (Nandini et al., 2016).

Studies on urban lakes in Mexico City have focused on the impact of different feeding conditions, particularly in the presence of toxin-producing cyanobacteria. Cladocerans, including *Simocephalus mixtus*, *Daphnia mendotae*, *D. pulex*, *Moina micrura*, *M. macrocopa*, and *Ceriodaphnia dubia*, actively consume cyanobacterial cells, leading to alterations in filtration rates and fitness (Pineda-Mendoza et al., 2012; Pérez-Morales et al., 2014, 2020; Nandini et al., 2020) or inducing cyanotoxin production (Pérez-Morales et al., 2015). Additionally, cladocerans have been observed to accumulate heavy metals, cyanotoxins, and microplastics, subsequently transferring them within the food chain (Rubio-Franchini et al., 2016; Zamora-Barrios et al., 2019; Manríquez-Guzmán et al., 2023).

Research with Cladocera strains isolated from Mexican waterbodies examined the impact of Hexavalent Chromium on *Ceriodaphnia dubia*, emphasizing precise short-term assays, including volume, exposure duration, and temperature measurements (Martínez-Jerónimo & Martínez-Jerónimo, 2023). In Aguascalientes, a study revealed high susceptibility of the indigenous species *Alona guttata* to pesticides, even at concentrations similar to guava field applications, causing chronic exposure-related somatic growth alterations. Furthermore, the Holarctic species *Daphnia magna* has been utilized to evaluate the impact of toxic substances such as nonsteroidal anti-inflammatory drugs (NSAIDs) on oxidative stress and genetic material damage (González-González et al., 2014). Additionally, Mexican laboratories participated validating a two-generational reproduction test (Barata et al., 2017). Recent researches are focused on the effects of multiple stressors to assess the synergistic or antagonistic impacts of a wide range of emerging contaminants found in domestic, textile, and hospital wastewater, as well as pesticides. These contaminants include a mixture of detergents, metals such as Zinc, Cadmium, and Arsenic, anti-corrosion agents, cardioactive drugs, antibiotics, NSAIDs, antidepressants, etc. (Hernández-Zamora & Martínez-Jerónimo, 2019; Aguilar-Aguilar et al., 2023; Hernández-Zamora et al., 2023). All the works above highlight the relevance of cladocerans as valuable tools for understanding the response of aquatic ecosystems to factors such as pollutants, changes in feeding, and environmental conditions, providing crucial information for the management and conservation of aquatic resources in Mexico.

## Challenges

Mexican legislation designates *Daphnia magna* as a sentinel for toxicity tests (NMX-AA-087-SCFI-2010). However, smaller herbivores such as, *Diaphanosoma*, *Moina*, *Ceriodaphnia*, *Chydorus*, *Alona*, and *Macrothrix* genus, dominate Mexican freshwater ecosystems. These species have shorter lifespans, faster reproduction, lower fecundity, and potentially greater sensitivity than *D. magna*. Martínez-Jerónimo et al. (2008) have proposed to replace *D. magna* with the American cladoceran *Daphnia exilis* in ecotoxicological bioassays due to its taxonomic similarities. Following the same approach, Santos-Medrano and Rico-Martínez (2019) suggest determining the relative sensitivity of native species to *D. magna* using the formula proposed by Von der Ohe and Liess in 2004:

$$S = \log (LC_{50Daphnia\ magna} / LC_{50i})$$

Where:  $S$ =relative sensitivity;  $LC_{50Daphnia\ magna}$  = $LC_{50}$  value for *D. magna*, and  $LC_{50i}$  =experimental  $LC_{50}$  for a species  $i$ . A zero value indicates a sensitivity equal to that of *D. magna*, a positive value suggests that *D. magna* is less sensitive, and a negative value indicates that *D. magna* is more sensitive.

In a preliminary experiment designed to assess the impact of Potassium dichromate, *Daphnia magna* exhibited  $LC_{50}$  values of 0.93 at 24 h and 0.66 mg L<sup>-1</sup> at 48 h. The native species *C. dubia*, *D. laevis*, and *S. vetulus* showed greater sensitivity. *D. laevis* presented an  $LC_{50}$  of 0.34 mg L<sup>-1</sup> at 48 h, resulting in an  $S$  value of 0.28. *S. vetulus* was the most sensitive species, with a  $S=0.81$  (Fig.2).

Despite the absence of *D. magna* records in Mexican water bodies, recent studies identify it in Ciénegas del Lerma (Espinoza-Rodríguez, 2023). This underscores the importance of considering native species in ecotoxicological studies for a more realistic scenario and reducing the likelihood of introducing exotic species.



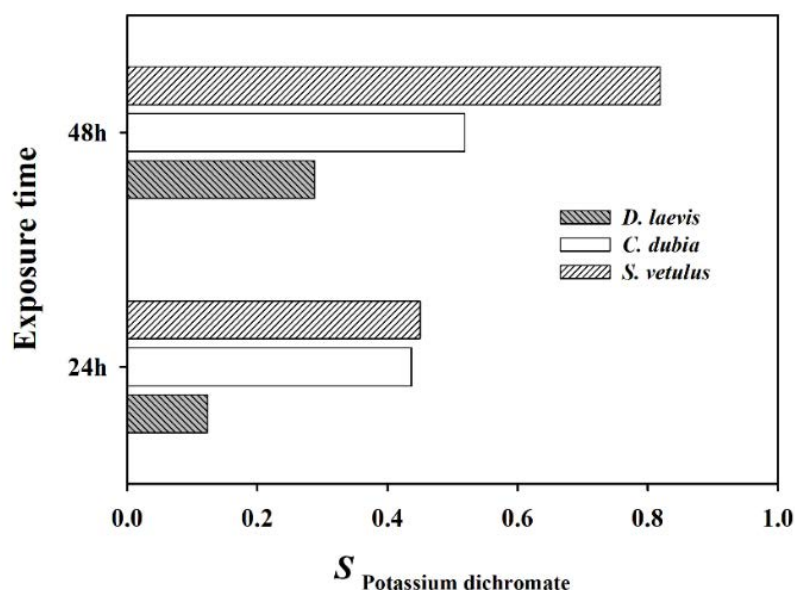


**Figure 2.** Relative Sensitivity Values of Native Species (*Ceriodaphnia dubia*, *Daphnia laevis*, and *Simocephalus vetulus*) Exposed to the Reference Toxicant (Potassium dichromate) during 24 and 48 h Experiments.

## Copepods

### *Diversity, Behavior, and Reproductive Strategies*

In general, most copepods are found in marine or brackish waters (~14,000 registered), with around 3,000 species in freshwater (Uc-Castillo et al., 2022). Copepods in continental waters are classified into three orders: Calanoida, Cyclopoida, and Harpacticoida (Dole-Olivier et al., 2000). Approximately 110 species have been documented in Mexican aquatic ecosystems (Gómez & Morales-Serna, 2014). Copepods typically measure between 1 and 5 mm in length, exhibiting a cylindrical body, segmented exoskeleton, and articulated appendages for swimming and feeding. Copepods display sexual dimorphism, and sexual reproduction is the most common form of reproduction; however, parthenogenesis has been observed in certain harpacticoid species (Poulin, 1996). Most copepods hatch from fertilized eggs, involving the union of a spermatophore, delivered by the male, to the copulatory pore of the female (Reid & Strayer, 1994). Copepod development involves eleven stages, including six naupliar and five copepodite stages with molting occurring between each stage and metamorphosis from the last naupliar to the first copepodite stage (Kwok et al., 2015).

### *Use of Copepods in Ecotoxicology*

Copepods are acknowledged as highly effective bioindicators of ecosystem pollution, with various toxicity testing protocols developed, including acute tests, multi-generation life cycle

tests, and short-term toxicity tests, assessing responses in individual actions, immune and endocrine processes, development, growth, and reproduction (Hussain et al., 2020). Since the 1940's, copepods have remained a popular model organism with most toxicity studies utilizing either static or static-renewal systems. Among copepod species, *Amphiascus tenuiremis*, *Nitocra spinipes*, and *Acartia tonsa* have seen the establishment of standardized full life-cycle testing protocols (Raisuddin et al., 2007). Unfortunately, freshwater copepods have not received sufficient attention, lacking protocols for toxicity tests. Nevertheless, *Mesocyclops* genera have been identified as a suitable option (Kulkarni et al., 2013).

### *Main Copepods Species in the Ecotoxicology of Mexico*

Research on copepods in Mexico has traditionally focused on their geographic distribution and taxonomic description. However, there is a growing interest and recognition of the important role copepods play in ecotoxicological studies. Several copepod species have become essential models for evaluating environmental impacts and conducting ecotoxicological tests. A simple search in the Scopus and Web of Science databases using the words “toxicology,” “copepods,” and “environmental assessment” shows that some of the marine species examined include *Acartia tonsa*, *A. clausi*, *A. spinata*, *A. lilljeborgii*, *Corycaeus amazonicus*, *Temora discaudata*, *Subeucalanus subcrassus*, *Acrocalanus longicornis*, *Calanus pacificus*, *Euterpina acutifrons*, and *Pseudodiaptomus euryhalinus*. Additionally, freshwater species such as *Acanthocyclops robustus*, *A. vernalis*, *A. americanus*, *Mastigodiatomus montezumae*, *Paracyclops novenarius*, and *Eucyclops chihuahuensis* have also been used.

The research on copepods in Mexico is limited but existing studies emphasize their importance. A specific study examined the reproductive response of the copepod *A. clausi* to the toxic dinoflagellate *Gymnodinium catenatum* suggesting that this species plays a crucial role in controlling red tides in Concepción Bay (Palomares-García et al., 2006). Another work identified the high tolerance of *P. novenarius* in a water body with high Arsenic concentrations ( $>50 \text{ mg L}^{-1}$ ), revealing that despite the high concentrations, there was no impact on its morphology or development (Uc-Castillo et al., 2022). Additionally, sensitivity and response were evaluated using oxidative stress biomarkers on *A. americanus* exposed to Cadmium, Chromium, Copper, Mercury, Manganese, Nickel, and Lead, emphasizing the need to understand how copepods, both marine and freshwater, respond to various environmental factors and contaminants (Sobrino-Figueroa et al., 2020). A recent investigation examines the impact of invasive species, such as *M. pehpeiensis*, not only from an ecotoxicological perspective but also considering their effects on biodiversity reduction and the potential impact on Mexican planktonic communities (Valencia-Vargas et al., 2023).

## *Challenges*

Ecotoxicological risk assessment commonly involves selecting species based on their sensitivity to various toxins and their suitability for laboratory cultivation. However, this approach often overlooks the diverse life history strategies copepods adopt in their natural environments. This oversight could have substantial implications for these species' vulnerability to contaminant exposure. Consequently, there is an urgent call for detailed and carefully orchestrated research within the realm of copepod ecotoxicology. Such research is pivotal in addressing the current disparity between ecological and ecotoxicological studies on copepods, with the goal of accurately identifying and incorporating representative species into assessments.

## Conclusions and Recommendations

The focus of this comprehensive brief review is on the significance of ecotoxicology in Mexican aquatic ecosystems, specifically directing attention towards zooplankton. The urgency to advance research in this field highlights the need to explore diverse ecosystems, particularly those in understudied regions. The inclusion of native species in toxicity studies emerges as a crucial aspect to ensure realistic outcomes and prevent the introduction of invasive species. Key approaches proposed for enhancing understanding include conducting detailed research on emerging contaminants (pesticides, animal pharmaceuticals, pharmaceuticals and personal care products, industrial compounds), heavy metals, detergents, as well as integrating environmental genomics into zooplankton studies. Furthermore, the establishment of standardized protocols, specifically with freshwater copepod species, is emphasized as an imperative need. Overall, these future perspectives collectively aim to strengthen the knowledge base in aquatic ecotoxicology in Mexico, providing valuable insights for the enduring preservation of the country's aquatic ecosystems.

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## Authors' Contributions

CAZB, UAR, MGRM, JMEH, and FJTM designed, analyzed, and wrote the study; JMEH and CAZB developed the graphic representation. FJTM acquired financial funds.

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# Exploring Zooplankton-Macrophytes Interaction Research in Mexico: Bibliometric Analysis

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## Abstract

This study conducted a bibliometric review of the Mexican scientific literature on the interactions between macrophytes and zooplankton. The findings suggest that comprehending the role and dynamics of each group is crucial for understanding the overall function of water bodies. Given the richness and distribution of both groups of organisms, Mexico presents a potential for further exploration of fundamental research questions and applications in ecology. The study highlights the need for more research on the impact of macrophyte removal on zooplankton communities, the role of macrophytes in mitigating anthropogenic stressors, the description of food webs, and different ecological interactions including allelopathy between primary and second producers. This work delves into discussing effective strategies for selecting editorial content and fostering collaborative efforts across different topics, all while considering the bibliometric results.

## Keywords

Aquatic plants, rotifer, cladocerans, bibliometric analysis, citation.



## Introduction

### *Importance of Zooplankton*

Epicontinental aquatic systems such as lakes, reservoirs, and rivers harbor a vast array of biological diversity (Moss, 2018). An essential biological component of freshwater ecosystems is zooplankton which is of utmost importance as it serves as a building block for the sustenance of various trophic levels (Pace & Orcutt, 1981; Sommer, 2012; Kuczyńska-Kippen & Joniak, 2015). Zooplankton is a group of microscopic aquatic animals composed of invertebrates and heterotrophic (consumers) organisms, among which the following stand out for their frequency, species diversity, and biomass: rotifers, cladocerans, and copepods, among other groups such as protozoa and ostracods (Canfield & Jones, 1996; Sommer, 2012; García-Chicote et al., 2018). A great diversity of zooplankton species is recognized, including 850 species of cladocerans, 2,000 rotifers, and 2,800 freshwater copepod species (Boxshall & Defaye, 2007; Wallace et al. 2019).

Rotifers, together with microcrustaceans such as cladocerans and copepods, with sizes from 200 to 6,000  $\mu\text{m}$ , have different feeding habits, most of them are consumers of algae, bacteria, and detritus; however, it is possible to find predators in these groups (Dumont & Negrea, 2002). These organisms have a central position in the aquatic trophic food web as primary consumers and, in addition, as a food resource for secondary consumers. Hence, they actively participate in various biotic interactions, which is why they have a great diversity of adaptations for optimal grazing (Lampert, 1997), predation avoidance, and intra and interspecific competition (DeMott, 1989; Diel et al., 2020); this has allowed the development of several areas of basic and applied research. Cladocerans, for example, have been used as an algal control tool to restore aquatic systems, mainly in temperate regions (Pereyatkina et al., 2009). On the other hand, cladocerans and copepods have been used for live feed production in aquaculture (Piasecki et al., 2004; Pearson & Duggan, 2018).

Zooplankton has generated significant interest in scientific research, mainly in their use as indicators of water health status (Jeppesen et al., 2011; Chandel et al., 2023), this is due to their high sensitivity to natural and anthropogenic environmental changes and perturbations reflected in changes in their specific richness, densities, life history traits, and even morphological effects (Sarma & Nandini, 2006; Alvarado-Flores et al., 2022). Ecological indicators provide information for biodiversity conservation as well as to ensure the sustainable use of resources and ecosystem services associated with these systems (Jeppesen et al., 2011; Berta et al., 2018). Likewise, the sensitivity and short life cycles of zooplankton have led some rotifers and cladocerans species to be the focus of both descriptive and experimental ecotoxicological studies in the field (Peither et al., 1996; Friberg-Jensen et al., 2003) and laboratory (Rico-Martínez et al., 2016; Pérez-Morales et al., 2020; Guo et al., 2023).

In aquatic systems, zooplankton are widely distributed in the littoral, pelagic, and benthic zones (Wallace et al., 2019). However, it is possible to find a more significant number of species in the littoral zone, where the presence of macrophytes considerably plays an important role in mediating food web dynamics (Perrow et al., 1999; Duggan et al., 2001; Geraldles, 2004).

### *Importance of Aquatic Plants*

The Neotropics are the regions with the most remarkable diversity of aquatic macrophytes (Murphy et al., 2019). Different types of macrophytes, such as submerged, floating, and emergent macrophytes, play an important ecological role associated with the physical, chemical, and biological characteristics of the water. Macrophyte-zooplankton interactions have been shown to be fundamental to the structure of aquatic communities as they influence higher trophic levels, which in turn are closely related to water quality (Perrow et al., 1999; Declerck et al., 2011).

Furthermore, they play an important role in cycling nutrients, providing habitats, purifying water, controlling diseases, and preserving cultural services (Thomaz, 2021). Applied approaches include controlling eutrophication caused by excessive loading of nutrients (Phosphorus and Nitrogen), the remotion of contaminants, and as food source for humans and livestock (Jeppesen et al., 2012; Singh et al., 2023). On the other hand, macrophytes could be of economic importance in cases such as species invasion, affecting navigation and excessive evapotranspiration, for example water hyacinth (*Eichhornia crassipes*) (Lugo et al., 1998).

Macrophyte diversity impacts invertebrate density and diversity, especially when considering plant structure. Morphologically complex macrophytes significantly affect animal populations and communities, for instance, in temperate zones, it has been observed that richer communities of larger-bodied organisms with higher densities of plant-associated cladocerans are present. In contrast, smaller-bodied zooplankton are typically found in subtropical water bodies (Merhoof et al., 2007; Thomaz & Cunha, 2010). The presence of macrophytes creates a rich substrate and abundant physical area through their leaves and roots, resulting in an increase of epiphytic algae and organic matter. This, in turn, attracts invertebrates for grazing and predators, leading to a higher diversity and abundance of animals in littoral zones (Dibble et al., 1996; Taniguchi et al., 2003).

In order to conserve biodiversity, it is essential to explore biological invasions. There is a global dispersion and introduction of macrophytes mainly for ornamental purposes (Lobato-de Magalhães et al., 2022; Bora & Padial, 2023). In certain ecosystems, invasive aquatic macrophytes have the ability to modify the complexity of habitats, which can lead to a reduction in water quality and availability (Thomaz & Cunha, 2010). This highlights the

importance of monitoring and managing invasive species to prevent them from negatively impacting aquatic ecosystems. Mexico is no exception and the problem is that, for example, the water hyacinth has been affecting a large number of aquatic ecosystems, as well as 10 other species throughout the country, although there are few studies that investigate, in depth, its effect on the ecological dynamics between species, the availability and use of water, as well as solutions and alternatives for the restoration, management, and conservation of these systems (Gutiérrez et al., 1996; Lugo et al., 1998; Martínez-Jiménez & Balandra, 2022).

### *Interaction Between Macrophytes and Zooplankton*

It is common practice to use macrophytes and invertebrates as bio-indicators of water quality due to the varying sensitivity of these organisms to pollution in their environments (Habib & Yousuf, 2014). The interaction between these two groups is crucial for several reasons. Macrophytes serve as a source of carbon and other nutrients that are taken up by heterotrophic bacteria, which in turn are consumed by the various zooplankton groups (Szabó-Tugyi & Tóth, 2020). This indirect source of food for zooplankton can be tapped during plant growth and even during their decomposition (Habib & Yousuf, 2014). In addition, the surface of macrophytes is exploited by periphyton for growth, which constitutes a food source for some members of zooplankton (Rautino & Warwick 2006).

The role of macrophytes in providing protection against predation for zooplankton has been questioned, as they seem to offer greater protection in temperate zones compared to tropical regions. In tropical areas, factors such as depth and behaviors like vertical or horizontal migration contribute to zooplankton survival. However, while it has been observed that zooplankton evade macrophytes, it has also been noted that macrophytes can serve as a habitat or refuge for cladocerans (Stansfield et al., 1997; Perrow et al., 1999; Montiel-Martínez et al., 2015; Arcifa et al., 2016). Moreover, macrophytes can influence the functional group richness and diversity of the invertebrate community, which in turn affects the abundance and distribution of zooplankton (Su et al., 2021; Wang et al., 2023). Some of the findings reveal an interesting chemical interaction in the food chain, macrophytes infochemicals trigger changes in demography growth of both phytoplankton and zooplankton; this can have a positive or negative effect depending on the species of macrophyte, finding species of rotifers, and cladocerans that grow particularly due to the presence of these (Duggan et al. 2001; Kuczyńska-Kippen & Nagengast, 2006; Espinosa-Rodríguez et al., 2016), however, is not clear and is missing empirical and experimental data for a better understanding.

The interaction between macrophytes and zooplankton is crucial for maintaining aquatic ecosystem balance and functioning, as it affects nutrient cycling, energy transfer,

and overall ecosystem health (Mulderij et al., 2007). By conducting a bibliometric analysis based on the search of Mexican literature, this study aimed to describe the publication trends on the scientific research in recent years, as recorded in indexed journal databases. This analysis allowed us to identify the journals with the highest publication frequency, the leading institutions, and the primary topic addressed. Additionally, it highlights the most cited works, thematic trends, and reference patterns in the publications, along with the collaboration networks among authors. These findings, along with a discussion on the concepts, their importance, and the understanding of the macrophyte-zooplankton interactions, will be elaborated upon of this work.

## Bibliometric Analysis

The study relied on bibliometric techniques to analyze the scientific output data obtained from the core collection of Web of Science® (WoS). The search method involved the utilization of the following keywords: “macrophyte\* OR aquatic plant\*”, which were subsequently refined by the term “zooplankton” and sorted by country (Mexico). This initial set of results was then subjected to a second logical operation, which was then applied using “zooplankton” filtered by “macrophyte\* OR aquatic plant\*”, followed by a final filter for the country. The results were from 1997 to 2023. The data from the documents obtained were exported as follows: the export selection was selected in the main WoS web page, then *Tab delimited file* was chosen, an emergent window asks for the records selection and it was marked *Full Record and Cited References* which includes the metadata of each document, finally, the text file was analyzed on R-language software using the Bibliometrix package (Aria & Cuccurullo, 2017). The package incorporates an interactive web interface (Biblioshiny) that permits an automatization of analysis, this involved the extraction and analysis of quantitative data related to the frequency and the impact of scientific publications. A three-field plot, including keywords, affiliation and source (journal) was obtained; a list of the most cited articles; a list of the topic tendencies during the last years; an occurrence network for dominant topics, and a network of collaboration within this two fields of study. A limitation of this package is the use of indexed databases, so those articles published under a different index database are omitted, and both the results and conclusions will be limited.

## Discussion

In the period from 1997 to 2023 (26 years), a set of 30 documents were compiled. According to the bibliometric analysis (Table 1), there was no annual growth rate (0 %) in the topics related to macrophytes and zooplankton interactions. However, an average of approximately 10 citations per document was found, which could benefit the academic community in the dissemination of knowledge, for example, articles such as van Donk and



van de Bund (2002), from 2002 to 2024 have a total of 435 citations, this article contributes to the impact of macrophytes on the plankton food web, showing a considerable gap in the scientific production and its impact worldwide. There has been a discussion (Leimu & Koricheva, 2005) on how different factors, such as the researcher's age, gender, the country where the research was conducted, the type and length of the article, the methodological strategies used, and the hypotheses proposed, can impact the citations of articles in the field of ecology.

**Table 1.** Main Information Data of Literature for Bibliometric Analysis.

| Description                        | Results   |
|------------------------------------|-----------|
| <b>MAIN INFORMATION ABOUT DATA</b> |           |
| Timespan                           | 1997:2023 |
| Documents                          | 30        |
| Annual Growth Rate %               | 0         |
| Document Average Age               | 8.17      |
| Average Citations per Doc          | 10.23     |
| References                         | 1453      |
| <b>DOCUMENT CONTENTS</b>           |           |
| Keywords Plus (ID)                 | 173       |
| Author's Keywords (DE)             | 125       |
| <b>AUTHORS</b>                     |           |
| Authors                            | 77        |
| Authors of Single-authored Docs    | 0         |
| <b>AUTHORS COLLABORATION</b>       |           |
| Co-Authors per Doc                 | 3.73      |
| International Co-authorships %     | 20        |
| <b>DOCUMENT TYPES</b>              |           |
| Article                            | 26        |
| Article; Proceedings Paper         | 3         |
| Review                             | 1         |

In the three-dimensional analysis of the interrelationships between the keywords of scientific articles, university affiliations, and publication journals (Fig. 1), the central role of the National Autonomous University of Mexico (UNAM) stands out. Currently, UNAM is recognized as a foremost actor in scientific research, covering a variety of research topics and consolidating its role as a leader in the generation of scientific knowledge, followed by the National Polytechnic Institute (IPN). These and other institutions contribute to knowledge on topics of interest in the present work. Although Mexico has about 20,825

km<sup>2</sup> of hydrophytic cover including coastal systems, the information generated is still limited (Palacio-Prieto et al., 2000). Similarly, the diversity of zooplankton is high, and there is still a tendency to increase the study of zooplankton in freshwater systems of Mexico (Elías-Gutiérrez et al., 1997; Gómez & Morales-Serna, 2014; Sarma et al., 2021). This is why academics, researchers, and their students could utilize these topics in their future research projects and, as a consequence, publish information on them. Aside from the above, the information generated would also provide insight into problems arising from anthropogenic activities, such as the increase in eutrophication, xenobiotic contamination, and plant invasion, topics of great interest nowadays.

Regarding the diffusion of research publications, it is noteworthy that the academic journal *Hydrobiologia* (Impact Factor=2.6, Q1) has indicated a significant preference, followed by *Allelopathy Journal* (Impact Factor=0.96, Q3), and *Ecohydrology & Hydrobiology* (Impact Factor=2.6, Q2) in terms of publishing (Fig. 1). These results indicate the relevance of the journals as platforms for the publication of studies related to the topics of zooplankton and macrophytes suitable for members of the National System of Researchers of CONAHCYT who have the challenge of publishing their research (Silva Payró et al., 2016; Díaz-Martínez, 2020). This finding highlights the significant relevance of carefully selecting journals for disseminating scientific knowledge in the Mexican context. Each of these journals addresses diverse themes, which allows for a detailed understanding of the different fields of study that receive particular attention. These results contribute substantially to the understanding of the editorial dynamics in scientific research in Mexico and highlight the strategic importance of editorial decisions in the process of knowledge dissemination on topics of interest.

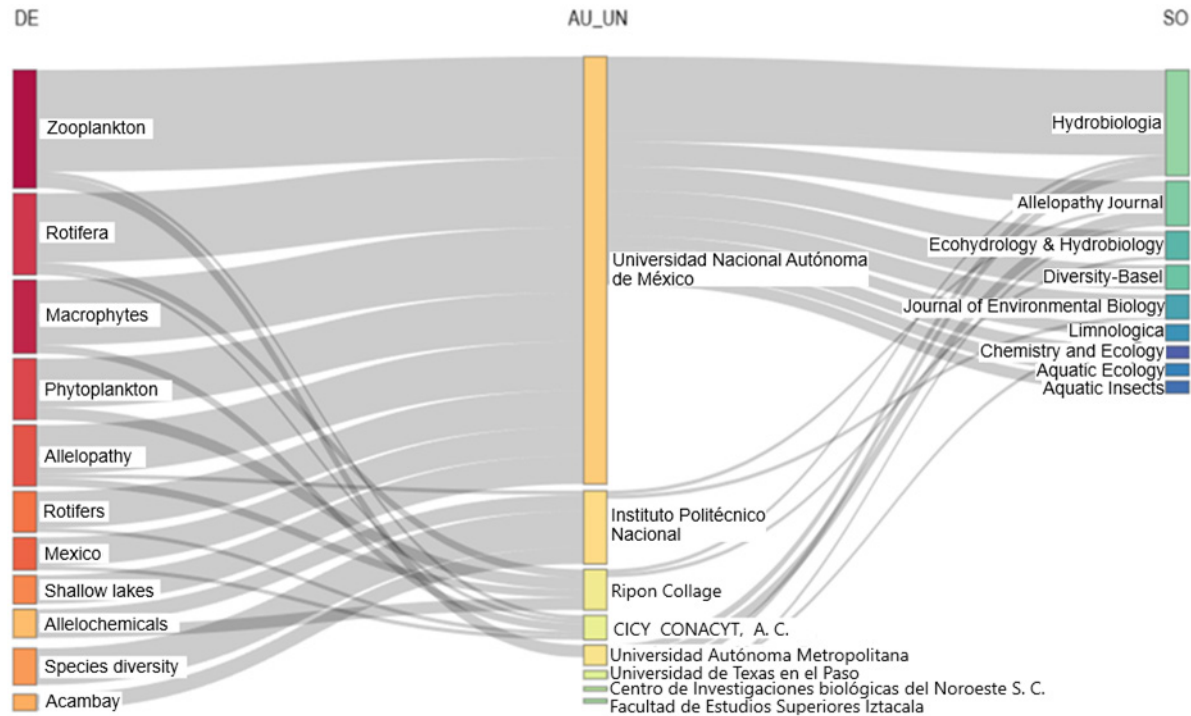


**Figure 1.** 3-field Graph, Showing the Interactions between DE: Keywords, AU\_UN: Affiliation, SO: Source (Journal), Data from the Literature Indexed in WoS Related to Macrophytes and Zooplankton in Mexico.

A classification of the most cited scientific articles in the bibliometric analysis (Fig. 2) indicates that in 10 articles, the number of citations varies from 12 to 43 in accordance with WoS, reflecting the breadth and relevance of the Mexican contribution to the global scientific community, for example in a study related to citation of marine and freshwater articles and average of citation of 19.4 in a range of 1 to 373 (Nash et al., 2017) was found. Mexican literature has made significant contributions in several areas, involving field and laboratory research. Studies have been carried out on the taxonomic description of species using traditional approaches (Elías-Gutiérrez et al., 1997). The use of isotopes has been added, for the study of biotic communities and their interaction with abiotic factors related to water quality (Zambrano et al., 2010; Muñoz-Colmenares et al., 2017). Research has been conducted on allochthonous fish and macrophyte species (Rocha-Ramírez et al., 2006). The potential impact of heavy metals on aquatic ecosystems can occur through several pathways, including the uptake of these metals by macrophytes. This may lead to their consumption by other aquatic organisms and aggravate the adverse effects of heavy metals on the ecosystem (De La Vega Salazar et al., 1997). Certain research gaps exist in the intriguing topic of fish-zooplankton predation mediated by macrophytes. Nonetheless, recent studies have addressed these gaps, revealing that

free-floating macrophytes, such as water hyacinth, can serve as habitats for *Chydorus brevilaris* or refuges for *Simocephalus vetulus* (Montiel-Martínez et al., 2015).

It is worth noting that recent research has demonstrated the significant contribution of macrophytes to the diversity of invertebrates in aquatic ecosystems. In comparison to open water areas, macrophytes have been found to foster a greater taxonomical and functional diversity of invertebrates (Enríquez-García et al., 2009; González-Gutiérrez et al., 2016; Jiménez-Santos et al., 2019; Espinosa-Rodríguez et al., 2021). These findings highlight the importance of macrophytes in promoting biodiversity and the need to consider their presence when assessing the health and ecological dynamics of aquatic environments.

Despite being a common practice in lake management in Europe and North America, the impact of the introduction and even removal of macrophytes on the taxonomic and functional groups of zooplankton has been little studied in Mexico. Recent research has shown that macrophyte removal has a significant influence on organisms over time, highlighting the need to better understand its effects (Espinosa-Rodríguez et al., 2021). The allelopathic effects of macrophytes in primary producers and their consumers is a topic that has been gaining attention currently. These effects can range from stimulating growth in *Simocephalus* spp. to interrupting growth in phytoplankton (*Scenedesmus acutus*) through interaction with *Egeria densa* (Espinosa-Rodríguez et al., 2016). The aforementioned contributions have provided valuable insights on diverse subjects, contributing significantly to the expansion of our knowledge at a regional as well as a global level.

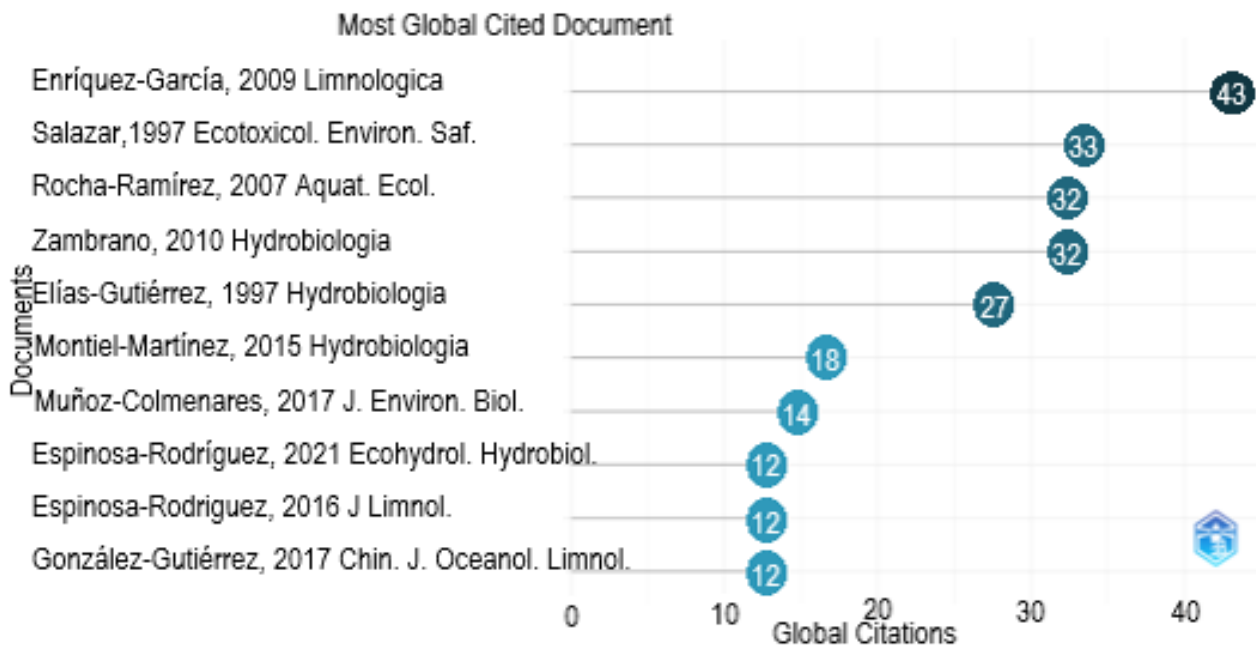


**Figure 2.** Documents with the Highest Number of Citations in the Area of Macrophytes and Zooplankton.



Figure 3 represents the Trend Topics through time and their temporal changes, 15 keywords with a high frequency of use and interest, mainly between 2010 and 2022. It is evident that certain topics were the subject of a more frequent study between 2010 and 2016 (“abundances” and “fish”), indicating a notable interest during that interval (articles from those years and the topics they address). Other topics, for their part, show an increase in their frequency of study between 2016 and 2018, suggesting a change in focus in research and an increase in the study and interest in the main topics of zooplankton and macrophytes (“*Daphnia*”, “submerged macrophytes”, “trophic state”). Additionally, a set of topics is identified that attracted notable attention between 2018 and 2022 (“diversity”, “population growth”, “zooplankton”, “communities”), showing the progress in research where the interest in understanding natural phenomena passes through autoecology to the ecology of communities, suggesting interest in a holistic approach, not just a descriptive one. This temporal representation offers insight into research trends and changing focus on different topics across these observed periods.

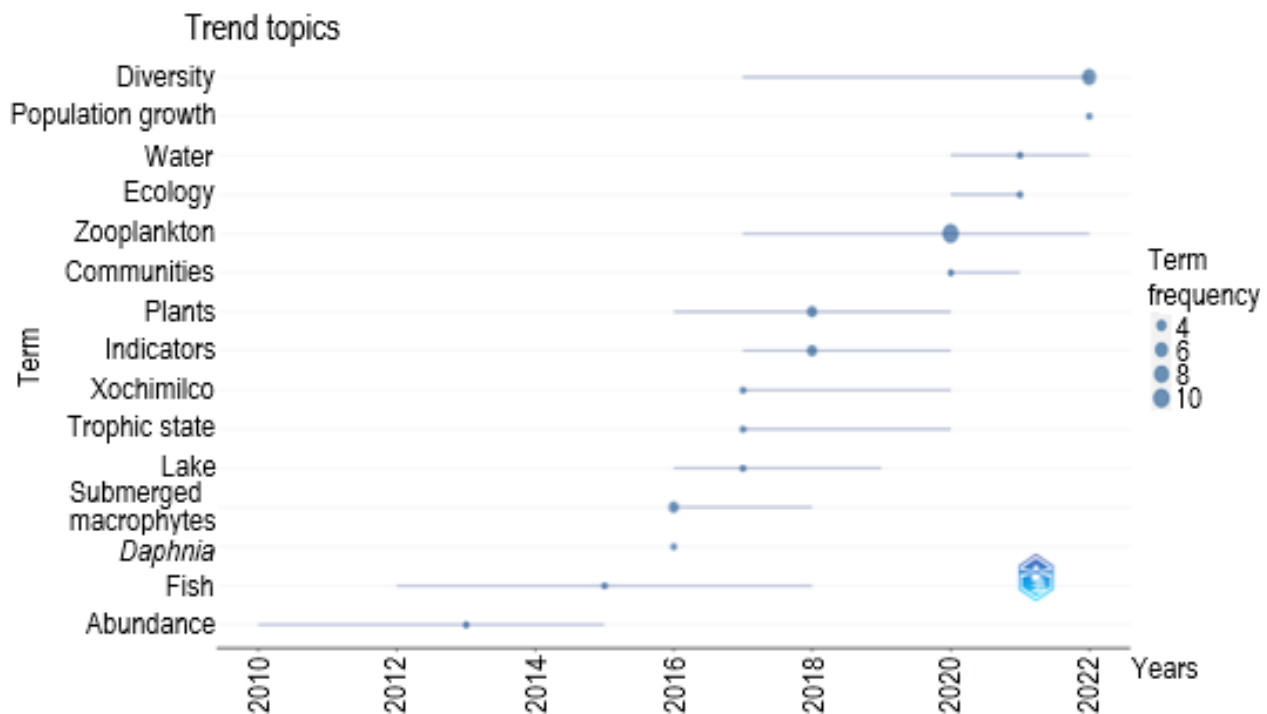


**Figure 3.** Topics and Their Trend in Relation to Publications Made in Mexico, Considering the Plus Keywords Provided in WoS Database.

The co-occurrence network analysis of the primary themes in Mexican literature related to zooplankton and macrophytes has revealed five distinct thematic groups (Fig. 4). The first group primarily focuses on zooplankton as a central theme. The second group highlights the interaction between fish and zooplankton evasion as relevant subtopics. In the third

group, researchers address water diversity and quality, along with bioindicators, highlighting the link between these research areas. The fourth group mainly represents the predator-prey relationship in rotifers. Finally, the fifth group emphasizes the study of macrophyte coverage and geographic information systems, which is inclined towards technological approaches and integrated ecology. These findings provide a comprehensive understanding of the thematic interconnection in scientific research in conjunction with the above results, offering specific insights into biological dynamics in the aquatic environment.

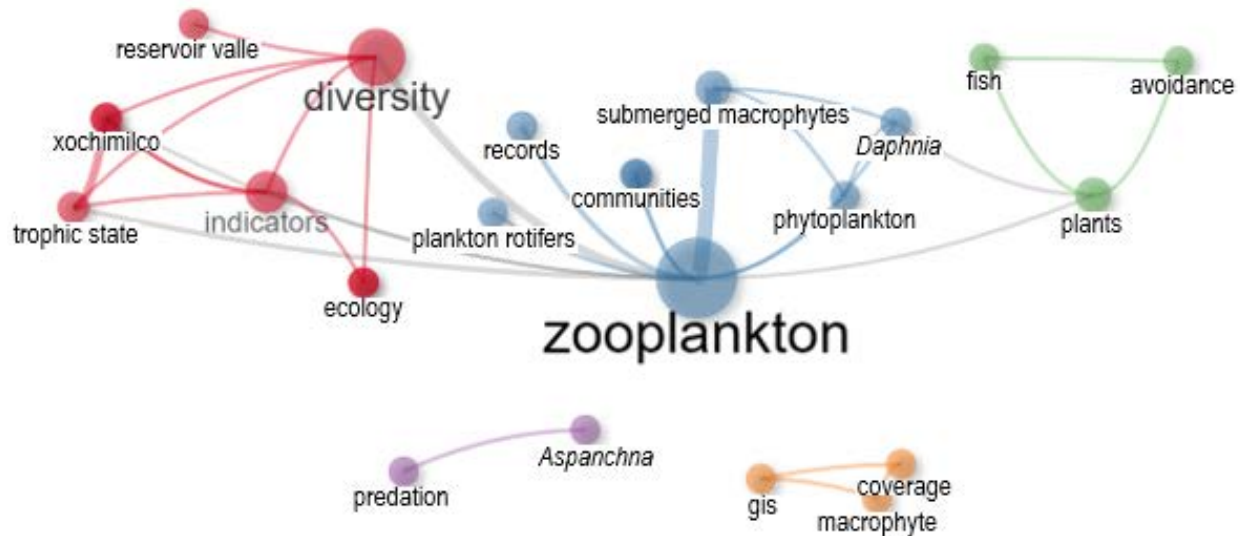


**Figure 4.** Network of Co-occurrence between the Dominant Themes of Focus in the Literature of Mexico.

The research on zooplankton and macrophytes in Mexican scientific literature shows a positive environment for collaboration, as evidenced by an average of 3.73 co-authors per document and 20 % of collaborations involving international co-authors (Table 1, Fig. 5). The collaboration network analysis reveals the presence of isolated clusters that reflect specialized scientific communities, varying in size from binary collaborations to groups with up to fifteen participants. Collaboration is crucial in scientific work, as well as in freshwater ecology, as it facilitates the convergence of diverse perspectives and skills, leading to the advancement of knowledge (Cullen et al., 1999). Identifying key actors and research producers in these groups enhances collaboration opportunities and promotes valuable synergy that contributes to the continuous progress of the scientific field. Collaboration among researchers establishes a breeding ground for exchange of ideas, access to funding, learning new skills, this promotes quality of results, which would be of great importance for this particular field of research conducted (Bansal et al. 2019).



**Figure 5.** Network Analysis of the Studies Distribution According to Authors in Mexican Literature in the Area of Macrophytes and Zooplankton.

## Conclusion

This bibliometric work focused on the description of the ecological importance and scientific productivity related to the interaction of zooplankton and aquatic plants in Mexico, showing slow progress with limited diffusion of publication through the last decades with a limited finding due to indexation of articles. We found a current interest in research going from the autoecology and description approach to understanding the ecological interaction mechanisms at a community level in remarkable topics of diversity, water quality, predation, food webs, seasonal variation, allelopathy, and ecotoxicology with some opportunities in remote sensing, genomics, biogeochemical, climate change, biomanipulation, and conservation as challenges for future studies. The interaction between macrophytes and zooplankton has been observed to yield interesting and noteworthy phenomena. However, the current state of research and publication in this area needs collaborative and participative initiatives that can provide funding support. Such efforts will facilitate knowledge development, as well as the restoration and conservation of natural habitats. By enhancing our understanding of this phenomenon, we can better protect and conserve the environment.

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## Authors' Contributions

JSMA conceptualization; JSMA drafting the initial manuscript; JSMA and FSMA methodology and data research; JSMA and FSMA data interpretation; JSMA and FSMA writing and editing the final manuscript.

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# The Freshwater and Brackish Hydrozoans of Mexico: An Overview of their Diversity

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## Abstract

Hydrozoans constitute a diverse cnidarian group with different morphologies and ecological strategies, occurring in all aquatic environments, including continental water bodies. The global diversity of freshwater hydrozoans has been scarcely addressed. The number of recognized species (~60) is substantially lower than other marine groups with continental representatives, such as sponges and crustaceans. The hydrozoan fauna of Mexico is relatively well known through inventories and recent studies on brackish and marine environments, although more effort is needed. Nevertheless, freshwater taxa are not included in the last checklists, leading to a gap in the understanding of the diversity of this group in Mexico. This chapter aims to provide an image of the diversity of freshwater and brackish-water hydrozoans in Mexico by reviewing 27 works that included hydrozoans recognized as inhabitants of these environments. Our search resulted in 15 taxa grouped into two subclasses, three orders, and seven families, of which Olindiidae was the richest. Seven taxa are recorded exclusively in freshwater environments, while eight are in lagoons, estuaries, or open oceans. Additionally, four hydrozoans are considered potentially invasive in other regions of the world, and few studies have monitored their populations in Mexico. Future perspectives in the study of freshwater hydrozoans include expanding research in freshwater environments and considering molecular tools for species identification.

## Keywords

Medusozoa, Cnidaria, polyp, estuarine environment, biodiversity, Hydrozoa.



## Introduction

The members of the class Hydrozoa constitute a diverse cnidarian group, including approximately 3,774 accepted species (Schuchert, 2024), which usually present a life cycle with a succession of different stages, including a larval, a benthic asexually reproducing polyp, and a sexually reproducing medusa (Santander et al., 2022). Nevertheless, several variants of this cycle exist in which the medusa may be absent or vice versa (Boero et al., 1992; Miglietta & Cunningham, 2012; Benthage et al., 2018). Hydrozoans occur in diverse aquatic ecosystems, going from freshwater to hypersaline environments, and from the surface to the deepest regions of the oceans (Jankowski, 2001; Benthage et al., 2018). Of the total hydrozoan species, few are representative of freshwater or brackish environments (Jankowski, 2001; Jankowski et al., 2008). For instance, only seven genera of Hydrozoa develop exclusively in freshwater environments, *i.e.*, *Hydra* Linnaeus, 1758, *Craspedacusta* Lankester, 1880, *Limnocyclus* Günther, 1893, *Astrohydra* Hashimoto, 1981, *Velkovrhia* Matjasic & Sket, 1971, *Halmomises* von Kennel, 1891, and *Calpasoma* Fuhrmann, 1939, comprising around 60 species (Deserti et al., 2023; Schuchert, 2024). On the other hand, there are more representatives of the brackish environments, such as some members of the orders Limnomedusae (*e.g.*, *Vallentinia* Browne, 1902, *Olindias* Müller, 1861, *Gonionemus* A. Agassiz, 1862), Anthoathecata (*e.g.*, *Moerisia* Boulenger, 1908, *Odessia* Paspalew, 1937, *Cordylophora* Allman, 1844) and Leptothecata (*e.g.*, *Blackfordia* Mayer, 1910) (Schuchert, 2024).

The success of freshwater and brackish hydrozoans involves diverse adaptations throughout their evolutionary history, such as the presence of solitary reduced polyps in Limnomedusae (an order of the subclass Trachylinae where most of its members lack this stage) (Benthage et al., 2018), and the development of adhesive structures to attach to macroalgae or seagrasses, commonly occurring in estuaries (Ahuatzin-Hernández et al., 2020). Unfortunately, their study has been scarce compared to those inhabiting marine ecosystems (Jankowski, 2001; Deserti et al., 2023), so taxonomy, evolutionary history, and ecological aspects of some of these taxa are poorly known (Jankowski, 2001). The knowledge regarding freshwater hydrozoans from Mexico is limited since most studies focus on new records or behavioral experiments (*e.g.*, Moreno-Leon & Ortega-Rubio, 2009; Rivera-de la Parra et al., 2016). Despite more effort needed, brackish species are better known in the country since they are included in taxonomical and ecological studies conducted in estuaries or coastal lagoons (*e.g.*, Álvarez-Silva et al., 2006; Ahuatzin-Hernández et al., 2020; Ocaña-Luna et al., 2021).

The number of studies concerning brackish-water environments in Mexico in the last years is comprehensive, likely due to some advantages of these environments, such as easy access and cheaper sampling costs (*e.g.*, Sarma et al., 2000; Hendrickx et al., 2007; Castellanos-Osorio, 2009; Benítez-Díaz Mirón et al., 2014; Ahuatzin-Hernández et al., 2020). A similar scenario happens with the freshwater biota of Mexico, which shows a progressive ad-



vance in the knowledge of the main taxonomic groups over the years (Calderón-Gutiérrez et al., 2017; Álvarez et al., 2023; Cervantes-Martínez et al., 2023). However, hydrozoans are not considered in these works, which could be associated with the benthic behavior of some tiny taxa (*Hydra*) and the seasonal fluctuation between polyp and medusae stages (e.g., *Craspedacusta*, *Moerisia*). Moreover, recent inventories about the medusozoans of Mexico do not include freshwater taxa, which represents a gap in the knowledge of this group (Segura-Puertas et al., 2003; Gasca & Loman-Ramos, 2014; Estrada-González et al., 2023).

Faunistic inventories are fundamental to improving the knowledge of the biodiversity of a region, which is necessary for applying good management of natural resources and conservation strategies (Cervantes-Martínez et al., 2023; Deserti et al., 2023). This task represents a challenge for a megadiverse country such as Mexico, so integrating dispersed literature about a taxonomic group allows us to improve the knowledge about that taxon, identifying gaps in the information, and recognizing future research fields. Herein, we gathered available published literature about freshwater and brackish-water hydrozoans from Mexico in order to provide a comprehensive picture of the state of the art and their biodiversity.

The information was integrated by reviewing available published literature, considering academic databases, such as Scholar Google, Scopus/Elsevier, ScieLO, and Web of Science/Clarivate, using keywords such as “Hydrozoa”, “freshwater”, “brackish-water”, “Mexico”, “medusa”, “polyp”, “anchialine fauna”, “Cnidaria”, both in Spanish and English. The information was assessed using the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-analysis) protocol (Page et al., 2021). The name of the taxa was cross-checked with WoRMS (marinespecies.org; April 2024) and GBIF (Global Biodiversity Information Facility) to retrieve additional occurrences and correct synonyms, invalid species, and typing errors. In addition, we identified non-native species based on the information provided in the literature, which could be useful for future monitoring of the impacts that these species could exert on native populations.

## Species Diversity

We found 27 works containing information about freshwater or brackish hydrozoans, resulting in 15 taxa grouped into two subclasses, three orders, and seven families (Table 1). The order Anthoathecata grouped nine taxa, distributed into five families, whereas Leptothecata was represented by one species. The order Limnomedusae was represented by five species grouped into one family. Seven of the 15 taxa were exclusively recorded in freshwater bodies, representing ~11.6 % and ~50 % of the worldwide and Neotropical freshwater known hydrozoans, respectively (Deserti et al., 2023; Schuchert, 2024). Five of the seven freshwater taxa were recorded in the polyp stage and two in the medusa stage. Family Olindiidae was the richest, matching previous studies (Jankowski, 2001) and supporting the hypothesis of independent origin in freshwater hydrozoan groups. This hypothesis states Olindiidae

as the most diverse freshwater cnidarian group (Jankowski et al., 2008), suggesting an older origin of this taxon as representative of freshwater medusozoans. *Pachycordyle* Weismann, 1883 was not considered in the literature of the Neotropical region (Deserti et al., 2023), so we add this genus to the knowledge of the diversity of this group. This taxon was recorded in a reef lagoon in the Mexican Caribbean (Ramos & Segura-Puertas, 2004).

**Table 1.** Freshwater and Brackish-water Hydrozoans Recorded in Mexico.

P=Polyp, M=Medusa, F=Freshwater, B=Brackish, O=Ocean.

| Taxa                                                                              | Morphology | Environment | Locality                                                     | Invasiveness | References                                                                                                |
|-----------------------------------------------------------------------------------|------------|-------------|--------------------------------------------------------------|--------------|-----------------------------------------------------------------------------------------------------------|
| Subclass<br>Hydroidolina<br>Collins, 2000                                         |            |             |                                                              |              |                                                                                                           |
| Order<br>Anthoathecata<br>Cornelius, 1992                                         |            |             |                                                              |              |                                                                                                           |
| Suborder<br>Aplanulata<br>Collins,<br>Winkelman,<br>Hadrys &<br>Schierwater, 2005 |            |             |                                                              |              |                                                                                                           |
| Family Hydridae<br>Dana, 1846                                                     |            |             |                                                              |              |                                                                                                           |
| <i>Hydra</i> sp.                                                                  | P          | F           | San Luis Potosi,<br>State of Mexico                          |              | Osorio-Tafall, 1943;<br>Zagmajster et al.,<br>2011; Rivera-de la<br>Parra et al., 2016                    |
| <i>Hydra vulgaris</i><br>Pallas, 1766                                             | P          | F           | State of Mexico,<br>Aguascalientes,<br>Chihuahua             |              | Marroquín-Cardona<br>et al., 2009;<br>Oseguera<br>et al., 2016; Armas-<br>Chávez<br>et al., 2022; GBIF    |
| <i>Hydra viridissima</i><br>Pallas, 1766                                          | P          | F           | Nuevo León,<br>State of Mexico,<br>México City,<br>Michoacán |              | Deserti et al.,<br>2023 (Retreived<br>from iNaturalist);<br>Retrieved from<br>iNaturalist through<br>GBIF |
| <i>Hydra oligactis</i><br>Pallas, 1766                                            | P          | F           | State of Mexico                                              |              | Deserti et al., 2023<br>(Retreived from<br>iNaturalist)                                                   |

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|                                                                              |   |     |                                                                                         |   |                                                                                               |
|------------------------------------------------------------------------------|---|-----|-----------------------------------------------------------------------------------------|---|-----------------------------------------------------------------------------------------------|
| Suborder Filifera<br>Kühn, 1913                                              |   |     |                                                                                         |   |                                                                                               |
| Family<br>Bougainvilliidae<br>Lütken, 1850                                   |   |     |                                                                                         |   |                                                                                               |
| <i>Nemopsis bachei</i><br>L. Agassiz, 1849                                   | M | B/O | Laguna Madre<br>and Open Ocean<br>(Tamaulipas),                                         |   | Mendoza-Becerril<br>et al., 2009;<br>Gutiérrez-Aguirre<br>et al., 2015                        |
| <i>Pachycordyle</i> sp.                                                      | M | B   | Puerto Morelos<br>(Quintana Roo)                                                        |   | Ramos and Segura-<br>Puertas, 2004                                                            |
| Family<br>Cordylophoridae<br>von Lendenfeld,<br>1885                         |   |     |                                                                                         |   |                                                                                               |
| <i>Cordylophora</i><br><i>caspia</i> (Pallas,<br>1771)                       | P | F   | Mandinga<br>Lagoon<br>(Veracruz),<br>Nuevo León                                         | X | Rioja, 1959;<br>Guajaro et al.,<br>1987; López-<br>Ochoterena and<br>Madrado-Garibay,<br>1989 |
| Family<br>Hydractiniidae L.<br>Agassiz, 1862                                 |   |     |                                                                                         |   |                                                                                               |
| <i>Cnidostoma fallax</i><br>Vanhöffen, 1911                                  | M | B   | Acapulco Bay<br>(Guerrero)                                                              |   | Guerrero-Ruíz et<br>al., 2015                                                                 |
| Suborder Capitata<br>Kühn, 1913<br>(sensu stricto)                           |   |     |                                                                                         |   |                                                                                               |
| Family<br>Moerisiidae<br>Poche, 1914                                         |   |     |                                                                                         |   |                                                                                               |
| <i>Moerisia</i><br><i>inkermanica</i><br>Paltchikowa-<br>Ostroumowa,<br>1925 | M | F   | In Ballast Water<br>of Tankers<br>Loaded at<br>Cayo Arcas<br>Oil Terminal<br>(Campeche) | X | Ahuatzin-<br>Hernández et al.,<br>2024                                                        |
| Order<br>Leptothecata<br>Cornelius, 1992                                     |   |     |                                                                                         |   |                                                                                               |

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|                                                     |   |   |                                                                                                       |   |                                                                                                                                                                    |
|-----------------------------------------------------|---|---|-------------------------------------------------------------------------------------------------------|---|--------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Family<br>Blackfordiidae<br>Bouillon, 1984          |   |   |                                                                                                       |   |                                                                                                                                                                    |
| <i>Blackfordia virginica</i> Mayer, 1910            | M | B | Tampamachoco and Pueblo Viejo Lagoons (Veracruz), Lagunar System Chantuto-Panzacola (Chiapas)         | X | Esquivel et al., 1980; Álvarez-Silva et al., 2003, 2006; Ocaña-Luna et al., 2021                                                                                   |
| Subclass<br>Trachylinae<br>Haeckel, 1879            |   |   |                                                                                                       |   |                                                                                                                                                                    |
| Order<br>Limnomedusae<br>Kramp, 1938                |   |   |                                                                                                       |   |                                                                                                                                                                    |
| Family Olindiidae<br>Haeckel, 1879                  |   |   |                                                                                                       |   |                                                                                                                                                                    |
| <i>Craspedacusta sowerbii</i> Lankester, 1880       | M | F | Sinaloa, Nuevo León                                                                                   | X | Guajardo et al., 1987; Moreno-Leon and Ortega-Rubio, 2009                                                                                                          |
| <i>Cubaia aphrodite</i> Mayer, 1894                 | M | B | Ascensión Bay, Puerto Morelos, Bojórquez lagoon, Mahahual reef, Chinchorro Bank (Quintana Roo)        |   | Canché-Canché and Castellanos-Osorio, 2005; Segura-Puertas and Damas-Romero, 1997; Suárez-Morales et al., 1999; Gasca et al., 2003; Ramos and Segura-Puertas, 2004 |
| <i>Octobulbacea brachymera</i> (Bigelow, 1909)      | M | O | Open Ocean (Tamaulipas), Acapulco harbor (Guerrero)                                                   |   | Bigelow 1909; Gutiérrez-Aguirre et al., 2015                                                                                                                       |
| <i>Olindias tenuis</i> (Fewkes, 1882)               | M | B | Ascensión Bay, Bojórquez Lagoon (Quintana Roo)                                                        |   | Canché-Canché and Castellanos-Osorio, 2005; Segura-Puertas and Damas-Romero, 1997                                                                                  |
| <i>Vallentinia gabriellae</i> Vannucci Mendes, 1948 | M | B | Costal Lagoon in Progreso, Bocas de Dzilam (Yucatan), Puerto Morelos, Bojórquez Lagoon (Quintana Roo) |   | Foster, 1973; Segura-Puertas and Damas-Romero, 1997; Ramos and Segura-Puertas, 1997; Ahuatzin-Hernández et al., 2020                                               |



Eight brackish hydrozoans were recorded either in lagoons, estuaries, or open ocean, mainly in the Gulf of México and the Mexican Caribbean. Coastal environments of Mexico are characterized by the strong influence of freshwater, mainly in the coastal lagoons of the Yucatan peninsula (Herrera-Silveira & Morales-Ojeda, 2010; Camacho-Ibar & Rivera-Monroy, 2014), causing mixing zones where marine and freshwater biotas are found. The eight brackish taxa were recorded in the medusa stage, likely due to most studies conducted in coastal environments focusing on zooplanktonic organisms, so benthic representatives fell out of the scope of the traditional zooplanktonic methods.

Most brackish medusae reported here belong to Limnomedusae and Anthoathecata, being characterized by a metagenetic life cycle, alternating a benthic polyp and a free-swimming medusa. In Limnomedusae, a mostly neritic group of the subclass Trachylinae, a reduced polyp is present, which represents an adaptation to inhabit coastal environments. This fact is evident since other trachylines inhabiting the open ocean develop from zygote to medusa via a free-swimming larva, lacking the polyp stage (Bentlage et al., 2018; Ahuatzin-Hernández et al., 2023). The polyp represents a derived character (novelty) in the evolution of Hydrozoa (Cartwright & Nawrocki, 2010), providing several advantages in the adaptation and colonization of new habitats, either by producing cysts resistant to environmental changes (longer life spans) or attaching to vagile substrates, driving long distances (dispersal).

Fifteen states of Mexico, either in continental or coastal environments, recorded hydrozoans. The states of Mexico and Quintana Roo showed the richest composition, while nine states only presented one taxon (Fig. 1). The diversity of freshwater hydrozoans of Mexico is poorly known. For instance, conventional inventories of medusozoans leave out the freshwater representatives (Segura-Puertas et al., 2003; Gasca & Loman-Ramos, 2014; Estrada-González et al., 2023), and those works addressing the freshwater diversity of Mexico don't consider hydrozoans in their scope (*e.g.*, Álvarez et al., 2023; Cervantes-Martínez et al., 2023; Gómez-Marín et al., 2024). This fact contrasts with investigations on coastal hydrozoans, which have been constant in recent years (*e.g.*, Canché-Canché & Castellanos-Osorio, 2005; Ahuatzin-Hernández et al., 2020; Mendoza-Becerril et al., 2020). The gap in the knowledge of freshwater hydrozoans biases the understanding of the diversity of both medusozoans and continental biota in Mexico, so more research in this sense is needed.

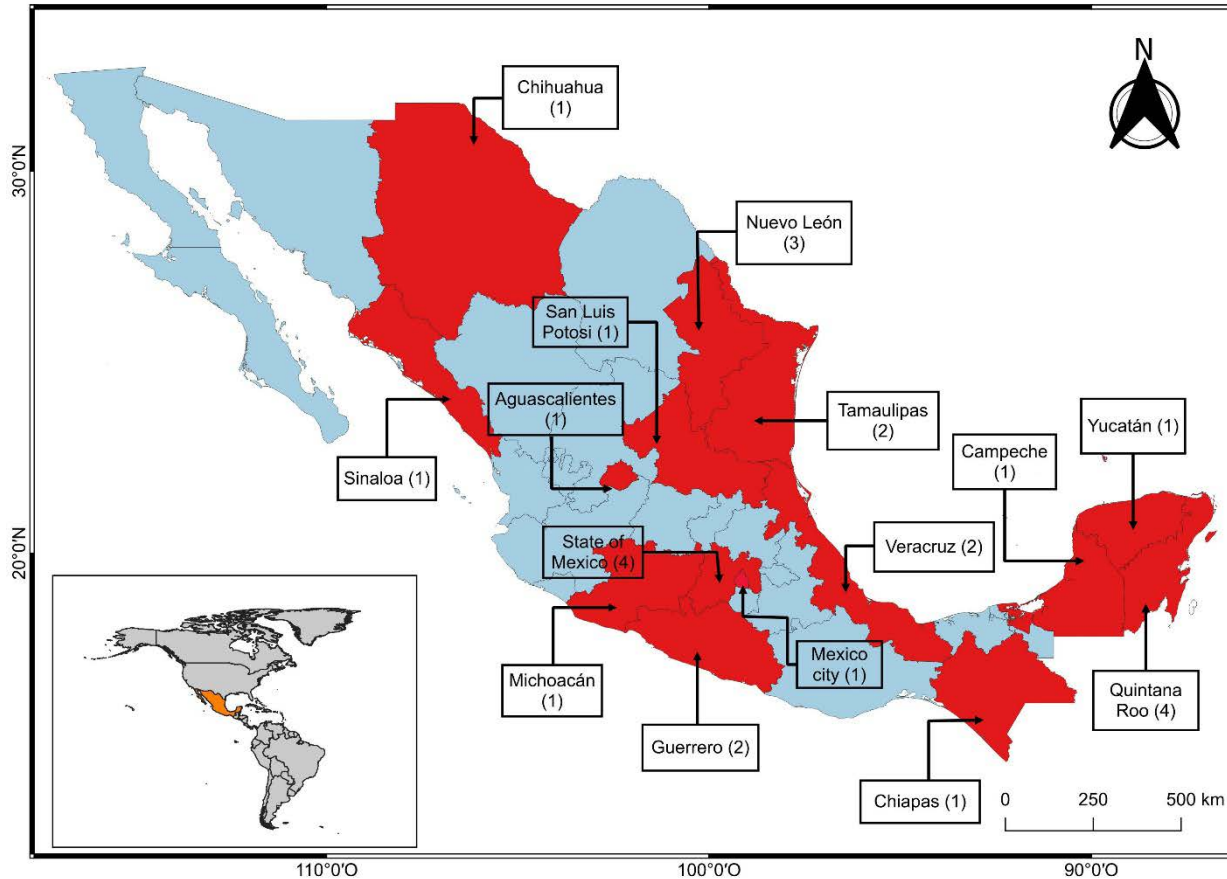


**Figure 1.** Mexican States with the Presence of Freshwater or Brackish-water Hydrozoans. The Number of Species per State Is Indicated in Parentheses.

## Invasive Species

Four of the 15 hydrozoans are considered non-native based on literature records, *i.e.*, *Cordylophora caspia* (Pallas, 1771), *Moerisia inkermanica* Paltschikowa-Ostroumowa, 1925, *Blackfordia virginica* Mayer, 1910, and *Craspedacusta sowerbii* Lankester, 1880. In the case of *M. inkermanica*, and *B. virginica*, recorded in brackish environments, the introduction in other regions of the world is attributed to maritime transport through the ballast water or bio-fouling (Nowaczyk et al., 2016; Ahuatzin-Hernández et al., 2024). The presence of these and other non-native species in the aquatic environments of Mexico encourages attention to the application of protocols that regulate the management of this type of water since it is one of the main vectors of invasion (Okolodkov et al., 2007; Okolodkov & García-Escobar, 2014).

On the other hand, the introduction pathways might be different regarding *C. caspia* and *C. sowerbii*, both recorded in continental water bodies. In this sense, a migratory route hypothesis by aerial dispersion due to bird migration is supported by observational evidence in *C. sowerbii* (Marchessaux et al., 2021). In this case, the role of the polyp stage,

which is drought-resistant, is crucial for the long-transport success. A similar scenario may happen with *C. caspia*, recorded in freshwater and brackish-water environments of Mexico on multiple occasions (Rioja, 1959; Guajaro et al., 1987; López-Ochoterena & Madrazo-Garibay, 1989), which suggests its establishment in the country, yet more research on the distribution of this species in Mexico is needed. Of the species treated here, only *C. caspia* is reported in the lists of non-native aquatic species of Mexico (Okolodkov et al., 2007). Therefore, this study adds three species to the knowledge of non-native hydrozoans in the country and encourages us to continue exploring continental and brackish-water ecosystems where other unrecorded species may occur.

## What do We Know about the Freshwater Hydrozoans of Mexico?

The knowledge about the freshwater hydrozoans of Mexico is scarce in many senses. The known number of species is likely underestimated due to the lack of research on this group. In addition, the taxonomy and evolution of the known taxa is also limited. In recent years, studies considering molecular data have shown that species previously considered cosmopolitan (*Hydra*, *Craspedacusta*, and *Cordylophora*) represent groups of species geographically delimited (e.g., Martínez et al., 2010; Schwentner & Bosch, 2015; Oualid et al., 2019; de Paula et al., 2024). However, studies conducted in Mexico regarding these taxa do not include molecular data or a suitable morphological analysis, so their taxonomic identity is uncertain.

The most studied genus of Mexico from freshwater environments is *Hydra*. Studies use it as a biological model to understand the effects of predation and the characterization of mycotoxins (Marroquín-Cardona et al., 2009; Rivera-de la Parra et al., 2016). Nevertheless, little is known about the relationship of this and other freshwater taxa with environmental variables. This fact leads to a gap in the knowledge of the ecology of these taxa. Thus, in the case of invasive species, their monitoring and prediction of further impacts on native populations is complicated.

More efforts are needed to improve the knowledge about the freshwater hydrozoans of Mexico. First, studies considering integrative taxonomy approaches are crucial to assigning an accurate identity to the Mexican taxa. Only then aspects related to the ecology of the species might shed light on their trophic relationships. Finally, these research lines could be integrated into an evolutionary frame to address issues such as biogeography, evolution, and distribution expansion by climate change through distribution models.

## Conclusions

The diversity of freshwater hydrozoans in Mexico has been overlooked over the years. In contrast, brackish hydrozoan diversity is better known due to constant research efforts in coastal environments. Four species are considered non-native; however, few works have monitored their distribution patterns and potential impact on native populations. Future perspectives on freshwater hydrozoans encompass global and local challenges. Hence, more research efforts are needed locally since other unrecorded species might occur in unexplored continental ecosystems. Due to a metagenetic life cycle involving benthic and planktonic stages, studies should include methodologies considering both types of environments.

Relevant topics with little information about Mexican freshwater hydrozoans include taxonomy, ecology, and potentially biotechnology. For example, *Hydra*, *Craspedacusta*, and *Cordylophora* have proved cryptic speciation, but Mexican records usually assign specimens to the type species, which are currently considered species complexes (Martínez et al., 2010; Schwentner & Bosch, 2015; Oualid et al., 2019; de Paula et al., 2024). The feeding and trophic ecology of *Hydra* has been studied in Mexico and other regions of the world (Rivera-de la Parra et al., 2016; Deserti et al., 2017; Morales et al., 2018). However, these aspects remain unexplored for the rest of the freshwater hydrozoans of Mexico, which are fundamental to knowing their ecology, mainly for those non-native ones. Finally, *Hydra* is one of the most widely implemented taxa as a biological model, being the basis of several studies concerning toxicology, regeneration, proteomics, and aging (e.g., Mocz, 2007; Galliot, 2013; Tomczyk et al., 2015; Murugadas et al., 2019), yet these approaches have not been implemented in the Mexican species. Addressing these lines would be crucial since it might change our view about the diversity of this group, recognizing new cryptic species distributed in the country, the potential impacts of those non-native ones, and their use as sources of natural compounds.

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## Authors' Contributions

JMAH and LVLD conceptualized the idea. JMAH reviewed the literature and conducted the analysis.

Both authors read and approved the final version of the manuscript.



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# Aquatic Macroinvertebrates Diversity in the Grijalva and Usumacinta Rivers, Mexico

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## Abstract

Aquatic macroinvertebrates are important and abundant components of the aquatic ecosystem, they transfer energy to higher trophic levels and can be used as bioindicator of aquatic ecosystem condition. The diversity of macroinvertebrates was determined through multihabitat diurnal sampling in three sections and five sites per section in the Grijalva and Usumacinta rivers, covering dry, rainy, and northern wind seasons. 75,482 individuals were recorded corresponding to 79 families, 128 genera, and 140 species, six species of mollusks, 11 of insects, and four of crustaceans for the Grijalva, and 17 of mollusks, 44 of insects, and 9 of crustaceans for Usumacinta were exclusive. The invasive mollusks *Tarebia granifera*, *Corbicula fluminea*, and *Melanoides tuberculata* accounted for 89.8 and 60.1 % of the abundance for the Grijalva and Usumacinta rivers. The maximum diversity values were in Lacantún section (Usumacinta) ( $H'=4.49$ ,  $J'=1.14$ ,  $D=1$  and Margalef 22.76), and seasonally in northern winds ( $H'=3.42$ ,  $J'=0.85$  and Margalef 15.19), meanwhile, dominance was in dry ( $D=0.93$ ). The dominant trophic groups were scrapers (74 %) and filter feeders (11 %), with maximum values in the Ostitán section (Grijalva) in rainy season. The collectors (10 %) were present in four sections, with the maximum value in the Tres Brazos section (Usumacinta) in the dry season. However, environmental variation reflects changes in the diversity of macroinvertebrates due to the function of these tropical rivers and their dependence on natural variation, hydraulic connectivity, and flow regulation. It is the first study that addresses the diversity of macroinvertebrates (three taxonomic groups) in two of the largest rivers in the country with contrasting ecohydrological conditions.



## Keywords

Macroinvertebrates, diversity, wetlands, Grijalva-Usumacinta basin

## Introduction

Aquatic macroinvertebrates (mollusks, crustaceans, and insects) are an important component in aquatic ecosystems for their abundance, in addition to being the intermediate link between primary producers and consumers, where their role as transformers and integrators of organic matter is relevant in the energy flows of the river systems (Barba-Macías et al., 2000). Mollusks, a group of marine origin, have a wide distribution and are associated with different types of habitats such as soft bottoms, logs and aquatic vegetation (Naranjo-García and Olivera-Carrasco, 2014). Furthermore, it is well known that, some bivalves are consumed in the lower basin of the Usumacinta River (Pantanos de Centla Biosphere Reserve), such as *Potamilus alatus* (Say, 1817), *Cyrtonaias tampicoensis* (Lea, I., 1839), *Pyganodon grandis* (Say, 1829), *Rangia cuneata* (Sowerby †, 1832), and *Pomacea flagellata* (Say, 1829). Other species such as *P. flagellata*, *Vitta usnea* (Röding, 1798), *Neritina virginea* (Linnaeus, 1758), *Biomphalaria obstructa (havanensis)* (Morelet, 1849), and *Rangia cuneata* are used as indicators of moderately or uncontaminated environments (Naranjo-García & Meza-Meneses, 2000). While, the invasive species *Melanooides tuberculata* (Müller, 1774), *Tarebia granifera* (Lamarck, 1816) and *Corbicula fluminea* (Müller, 1774) have been recorded with high abundances and probably generate an imbalance in the biodiversity of the aquatic environments that they colonize (Naranjo-García & Meza-Meneses, 2000; Cruz-Ascencio et al., 2003; Albarrán-Melzer et al., 2009; Sánchez et al., 2012; Barba-Macías & Trinidad-Ocaña, 2017).

On the other hand, 89 % of the recorded crustacean species are of marine origin, 10 % are freshwater, and 1 % are terrestrial (Álvarez et al., 1996). Crustaceans inhabit a variety of substrates such as muddy bottoms, logs, submerged, floating, and emerging aquatic vegetation. These organisms play an important role in energy transfer to higher trophic levels (Barba-Macías et al., 2000). Species such as: *Macrobrachium* spp. (Camacho-Sánchez, 2007), *Hyaella azteca* (Keithly et al., 2004), *Mysidopsis bahia* (Raz-Guzmán, 2000) are used as indicators of water quality (Álvarez et al., 1996; Cumberlidge et al., 2009; Schertzinger et al., 2018). Furthermore, within this group, penaeid shrimp, carideans, and crabs are important in commercial and coastal fishing, and contribute 1.4 % of national production (CONAPESCA, 2013).

Aquatic insects are distributed in a wide variety of ecosystems, such as streams, rivers, lakes, etc., and their distribution depends on the concentration of dissolved oxygen and water temperature (Bass, 1995; Castella et al., 2001; Li et al., 2001; Sullivan et al., 2004), and water chemistry, including pH, salinity, and concentrations of ions or elements,

as well as the presence of riparian vegetation (Subramanian et al., 2005). This group has been widely used as a bioindicator, where various authors with studies in temperate and tropical regions have found that the diversity and richness of species decreases in areas impacted by human activities (Stone & Wallace, 1998; Ometo et al., 2000; Benstead et al., 2003), in addition to presenting channels with a lack of boulders and the presence of terrigenous sediment, which drastically reduces the availability of niches and, as a consequence, a decrease in diversity (Allan et al., 1997). There are also other factors that, to a lesser extent, explain the structure and composition of the aquatic entomofauna, such as seasonal patterns (Miserendino & Pizzolon, 2003; Waite et al., 2004; Sporka et al., 2006; Joshi et al., 2007).

The Grijalva-Usumacinta basin (GUB) is characterized by its high-water wealth and biodiversity, in addition to contributing 30 % of the country's total surface runoff (García-García and Kauffer-Michel, 2011; Cruz-Paz et al., 2018). Previous studies have focused on the Usumacinta basin: mollusks and crustaceans (Trinidad-Ocaña et al., 2018) and insects for mountain areas of Tabasco (Benítez-Abud et al., 2016). However, the increase in anthropogenic activities causes greater pressure on the system due to the modification of land use (Kolb & Galicia, 2012), with the consequent decrease in riparian vegetation and an increase in the discharge of untreated wastewater, these affect water quality (Laino-Guanes et al., 2016). Therefore, records of invasive non-native species are increasingly frequent in the Grijalva-Usumacinta basin (Cruz-Ascencio et al., 2003; Rangel-Ruiz et al., 2011; Barba Macías et al., 2014).

This contribution analyzes the diversity of aquatic macroinvertebrates in two basins with contrasting hydrological conditions, a dammed river (Grijalva), and one with free flow (Usumacinta) and if there are significant differences between sites and between basins, depending on the state of the ecological quality of the banks and the natural flow of the river expressed in better hydrological conditions, high values of riparian quality, high species richness, abundance, and biomass in relation with altered and unaltered river functionality.

## Materials and Methods

### *Study Area*

The study area includes hydrological region number 30 (RH30) formed by the Grijalva and Usumacinta rivers, both rivers make up the largest hydrological basin in México (Conagua, 2014). Its runoff area is 112,500 km<sup>2</sup> and represents 4.2 % of the continental national territory (Andrade-Velázquez & Medrano-Pérez, 2020), with a discharge of 4,500 m<sup>3</sup>/s, occupying second place in discharge to the Gulf of México after the Mississippi with an annual discharge of 18,000 m<sup>3</sup>/s (Yañez-Arancibia et al., 2009; Sánchez et al., 2015). Both rivers

are transboundary, they originate in Guatemala and cross the states of Chiapas and Tabasco in Mexico and converge on the coastal plain of Tabasco providing conditions for extensive wetland areas that mainly make up the Pantanos de Centla Biosphere Reserve (Barba-Macías et al., 2006; Conagua, 2014). The climate of the region is generally composed of three climatic groups: the warm-humid ones with approximately 93.5 % of the territory are temperate-humid with 6.3 % and a small portion of dry climate (Conagua, 2014).

The Usumacinta River annually transports around 59,397 hm<sup>3</sup> (including the contribution of the San Pedro River), its flow represents the largest contribution of water to the Laguna de Términos and the Pantanos de Centla Biosphere Reserve (PCBR) (Soria-Reinoso et al., 2022), this river does not have dams. While the Grijalva River is one of the largest rivers in the country, it annually transports a volume of 27,013 hm<sup>3</sup> and, in the rainy season, its flow increases. For this reason and to take advantage of the waters flow of this river, it was dammed with a system of four hydroelectric dams at the end of the 60's (Netzahualcóyotl, La Angostura, Chicoasén and Peñitas) (Conagua, 2014).

Three study areas were selected for each river, considering its main environmental, hydrological and ecological characteristics according to regulatory appendix F of the Ecological Flow Standard (MX-AA-159-SCFI-2012), where Flow Study Units were determined. Ecological, which consists of the scale of analysis in the territory of the basin. These evaluation units refer to surface water bodies (rivers, lakes, lagoons, or other wetlands) or part of them in the same hydrological basin and that have the same hydrological regime to which an environmental objective is assigned. These were validated by a panel of experts before the selection of reference areas. The areas chosen in the Usumacinta River were Lacantún, Jonuta, and Tres Brazos, while for the Grijalva River they were Amacohite, Ostitán, and Chilapa, in each study area five sampling sites were selected.

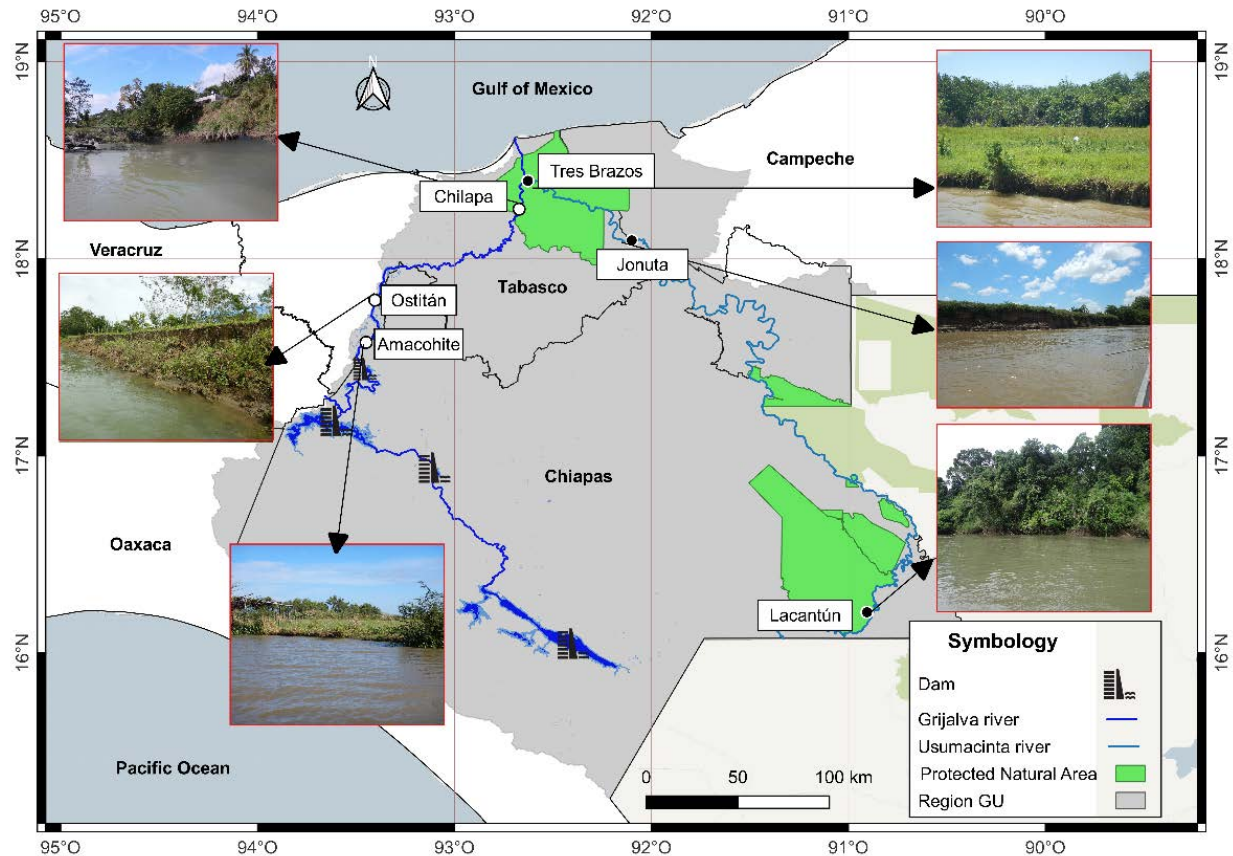


**Figure 1.** Sampling Sections of Grijalva and Usumacinta Rivers  
(Modified from Miranda-Vidal et al., 2023).

Ecological quality of the riverbanks at each selected site was evaluated using the Riverbank Quality Index (RQI) according to Miranda-Vidal et al. (in press), where seven attributes described in the protocol were evaluated; 1) riparian space (width) occupied by vegetation associated with the river, 2) longitudinal continuity of vegetation, 3) composition and structure of woody vegetation, 4) natural regeneration, 5) condition of banks, 6) transversal connectivity, and 7) vertical connectivity (González del Tánago et al., 2006; González del Tánago & García del Jalón, 2011) (Annex. 1).

### *Sampling*

Samples were collected in the rainy season (wettest period of the year; 950 mm of precipitation), “northern winds” (locally known as “nortes”, a condition where polar masses cross the Gulf of Mexico; 660 mm) and dry season (driest and hottest period of the year; 270 mm) (Ayala-Pérez et al., 2021; Cardoso-Mohedano et al., 2022) of an annual cycle of 2017-2018. At each site, the physicochemical parameters of the surface water were recorded using a Hanna HI9829 multiparameter for: temperature (°C), pH, dissolved oxygen (DO) (mg/L), conductivity (mS/cm<sup>-1</sup>), total dissolved solids (TDS) (mg/L) and salinity (ups).



Aquatic macroinvertebrates were collected following the Macroinvertebrate Sampling Protocol in continental waters for the application of the Ecological Flow Standard (NMX-AA-159-SCFI-2012). The biological collection material was carried out through a multihabitat approach with various collector equipment's. For infauna, a nucleator with an area of 0.0044 m<sup>2</sup> was used (in rivers and shallow wetlands) and a Petite Ponar dredge with an area of 0.025 m<sup>2</sup> in rivers more than 2.5 m deep. Epifauna was collected with different types of nets: dip net with a mesh size of 500 microns and an area of 0.082 m<sup>2</sup>, Renfro beam trawl net with a mesh size of 1 mm and an area of 2 m at the mouth with trawls of 25 m in length covering an area of 50 m<sup>2</sup>, seine net 15 m in length, with a mesh size of 1 cm on the sides covering four meters on each side and a 2 mm mesh size in the center in a length of 7 m and a drop of 2.20 m covering 62 m<sup>2</sup>. The use of each sampling equipment must be in accordance with the depth of the body of water and the conditions present in each location; at least three replicas must be taken per equipment used. All organisms were preserved in a plastic bag with 96 % alcohol.

### *Analysis*

The organisms were identified, counted and weighed; identification was carried out to the lowest possible taxonomic level with various taxonomic keys for mollusks using Burch and Cruz-Reyes (1987), Thorp and Covich, (1991) and Hershler and Thompson (1992). For crustaceans were used: Bousfield (1972), Chace (1972), Felder (1973), Williams (1984), Pennak (1978), Throp and Covich (1991), Raz Guzmán et al. (1992), and Raz-Guzmán and Sánchez (1996). The insects were identified using the keys from: Wiggins (2000), Merritt et al. (2008), and Bueno-Soria (2010).

These organisms were assigned to food groups according to their trophic specialization. Eight trophic groups were determined following categories proposed by Cummins and Klug (1979), Merritt and Cummins (1996), Liévano and Ospina (2007) and Tomanova et al. (2006). From these, Herbivores (H) that feed on aquatic vascular plants or filamentous algae, Omnivores (Om) that feed on diverse types of food, Collector-Shredders (C-Sh) that feed on coarse particulate organic matter, Scrapers (Ra) that are herbivores and feed on algae or microbes attached to rocks or other substrates, Gatherers (Ga) that consume fine particulate organic matter, Filter feeders (Ff) which is a special group of collectors that filter in the water column, Predators (P) that feed on other living organisms and Shredders (Tr) are organisms that cut or chew pieces of living or dead plant material, including all plant parts like leaves and wood.

Abundance and weight data were standardized to density (org/m<sup>2</sup>) and biomass (g/m<sup>2</sup>). Diversity indices H' Shannon (Shannon and Wiener, 1963), species richness (D) of Simpson (1949) and evenness (J') of Pielou (1966) were applied; the indices were calculated with the statistical package PAST version 2.17c (Hammer et al., 2001).

## Results

### *Environmental Description*

Riparian quality of the Usumacinta River showed a gradient of riverbank quality ranged from very good (scores of 139) at the Lacantún site to Tres Brazos (classified as poor score 41), while at Grijalva, the evaluation of riverbank quality ranged from moderate with scores of 70 and 73 (Chilapa and Amacohite sites, respectively), to poor in Ostitán site (score of 58) (Annexe 1). In Grijalva, sampling sites revealed that the attributes of the riverbank are degraded and altered in their functioning. Temperature was uniformly in both basins with values between 23 and 30°C, the minimum value being recorded in the Amacohite section. Sections corresponding to Grijalva basin (GB) recorded lower values of conductivity and total dissolved solids than those of Usumacinta basin (UB), but the minimum record was from the Amacohite section for conductivity (166.69 mScm<sup>-1</sup>) and 132.62 ppm to total dissolved solids (TDS) for the Chilapa section. Because they are freshwater environments, salinity is very low with records less than 1 ups. Dissolved oxygen registered values greater than 4 mg/L in the sections of both basins, the minimum value being recorded in the Jonuta section which registered 4.29 mg/L in the dry season (Table 1). However, data recorded corresponds to acceptable values for aquatic life.

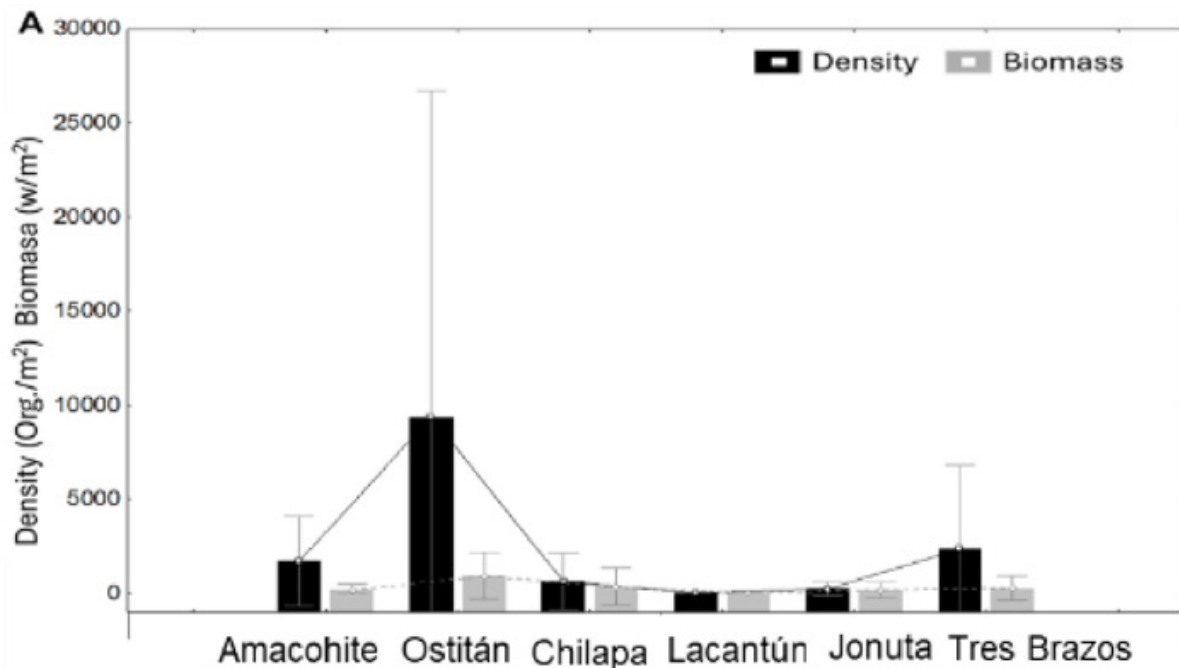
**Table 1.** Average Value of Physicochemical Parameters per Section and Season Sampling Areas of the Usumacinta and Grijalva Basin. R: Rainy, N: Northern Winds, D: Dry, Tr: Transparency, T: Temperature, OD: Dissolved Oxygen, TDS; Total Dissolved Solids.

| Basin      | Section     | Season | Depth (m) | Tr (m) | T (°C) | pH    | OD (mg/L) | Salinity (ppm) | Conductivity (mScm-1) | TDS (ppm) |
|------------|-------------|--------|-----------|--------|--------|-------|-----------|----------------|-----------------------|-----------|
| Grijalva   | Amacohite   | N      | 1.73      | 0.62   | 23.35  | 4.49  | 5.15      | 0.1            | 166.69                | 138.89    |
|            |             | R      | 1.73      | 0.62   | 30.02  | 7.24  | 4.81      | 0.17           | 368.20                | 184.80    |
|            |             | D      | 1.72      | 0.62   | 27.97  | 10.14 | 8.33      | 0.17           | 361.70                | 180.90    |
|            | Ostitán     | N      | 1.3       | 0.68   | 26.37  | 9.55  | 6.5       | 0.12           | 319.40                | 159.70    |
|            |             | R      | 1.23      | 0.68   | 29.87  | 7.39  | 4.93      | 0.2            | 360.20                | 219.20    |
|            |             | D      | 1.27      | 0.68   | 27.96  | 11.61 | 8.11      | 0.18           | 372.93                | 186.43    |
|            | Chilapa     | N      | 2.53      | 0.33   | 25.4   | 7.55  | 4.64      | 0.15           | 265.25                | 132.63    |
|            |             | R      | 2.53      | 0.33   | 31.49  | 7.58  | 5.34      | 0.17           | 471.10                | 235.60    |
|            |             | D      | 2.53      | 0.33   | 27.58  | 9.73  | 8.05      | 0.11           | 429.60                | 214.60    |
| Usumacinta | Lacantún    | R      | 1.08      | 0.36   | 24.99  | 8.24  | 7.02      | 0.2            | 417.37                | 208.75    |
|            |             | D      | 0.57      | 0.58   | 26.26  | 9.76  | 10.01     | 0.32           | 653.26                | 326.66    |
|            | Jonuta      | N      | 0.77      | 0.26   | 29.76  | 8.26  | 6.3       | 0.15           | 385.50                | 193.00    |
|            |             | R      | 1.8       | 0.54   | 28.68  | 7.99  | 5.66      | 0.16           | 384.50                | 192.25    |
|            |             | D      | 1.1       | 0.55   | 27.93  | 8.48  | 4.29      | 0.19           | 660.50                | 330.00    |
|            | Tres Brazos | N      | 1.9       | 0.3    | 30.31  | 7.99  | 6.73      | 0.24           | 377.13                | 188.75    |
|            |             | R      | 1.86      | 0.43   | 28.81  | 7.74  | 4.64      | 0.18           | 575.75                | 326.87    |
|            |             | D      | 1.83      | 0.58   | 28.65  | 8.64  | 4.3       | 0.94           | 1488.14               | 744.00    |

### *Aquatic Macroinvertebrates*

A total of 75,482 individuals corresponding to 79 families, 128 genera, and 140 species were collected. GB recorded 44 families, 60 genera, and 66 species, nine species of crustaceans were recorded (4,918 indiv.), 33 insect genera were recorded (317 indiv.), and for mollusks with 24 species (50,110 indiv.). While in UB 69 families, 111 genera and 117 species were recorded, 14 species of crustaceans were recorded (4,525 indiv.), 67 genera of insects (1243 indiv.) and 36 species of mollusks were recorded (14,369 indiv.). Species recorded exclusively in each basin were in GB 6 species of mollusks, 11 of insects, and 4 of crustaceans, while in UB there were 17 species of mollusks, 44 of insects, and 9 of crustaceans.

Regarding the density of macroinvertebrates, 70 % of the density was from the Grijalva basin and 30 % from the Usumacinta basin. Spatially, maximum values of density and biomass in Grijalva were in the Ostitán section (99.4 indiv./m<sup>2</sup> and 9.7 g/m<sup>2</sup>); while in Usumacinta, they were in the Jonuta section 24.8 indiv./m<sup>2</sup>, and 6.9 g/m<sup>2</sup> respectively (Fig. 2A). Temporally, maximum values of density and biomass in Grijalva were 112.5 indiv./m<sup>2</sup> in the rainy season, and 10.9 g/m<sup>2</sup> in northern winds, respectively; for Usumacinta, maximum temporal values were in the dry season, 31.9 indiv./m<sup>2</sup> and 5.3 g/m<sup>2</sup> respectively (Fig. 2B).



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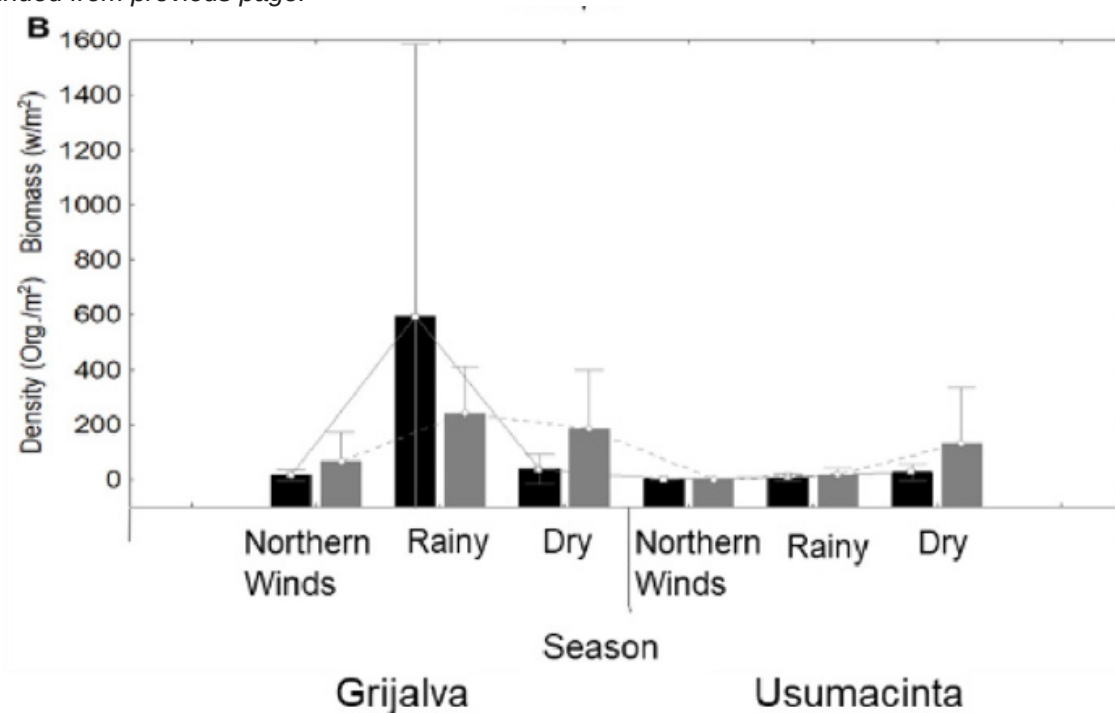


**Figure 2.** Mean and Standard Deviation of Density and Biomass of Aquatic Macroinvertebrates, Spatial (A) and Temporal (B) Distribution in Grijalva and Usumacinta Basins.

**Table 2.** Density and Biomass of AA<Aquatic Macroinvertebrates on Grijalva and Usumacinta Basins (\*Exotic Species, CG=Grijalva Basin, CU=Usumacinta Basin, D=Density, B=Biomass). Many Species Recorded Densities Less than One Individual per Square Meter

| Group/Species                 | GB |   | UB  |   | Group/Species              | GB |   | UB |   |
|-------------------------------|----|---|-----|---|----------------------------|----|---|----|---|
|                               | D  | B | D   | B |                            | D  | B | D  | B |
| <b>Mollusks</b>               |    |   |     |   | <i>Berosus</i> sp.         |    |   | 0  | 0 |
| <i>Anodonta</i> sp.           |    |   | 46  | 4 | <i>Brachycerus</i> sp.     | 1  | 0 | 1  | 0 |
| <i>Aroapyrgus clenchi</i>     |    |   | 141 | 1 | <i>Camelobaetidius</i> sp. | 1  | 0 |    |   |
| <i>Aroapyrgus</i> sp.         |    |   | 281 | 1 | <i>Campsurus</i> sp.       |    |   | 1  | 0 |
| <i>Biomphalaria helophila</i> | 1  | 0 | 57  | 1 | <i>Cheumatopsyche</i> sp.  | 0  | 0 | 0  | 0 |
| <i>Biomphalaria obstructa</i> | 1  | 0 | 69  | 1 | <i>Chimarra</i> sp.        |    |   | 0  | 0 |
| <i>Biomphalaria subprona</i>  |    |   | 1   | 0 | <i>Chironomus</i> sp.      | 28 | 0 |    |   |
| <i>Cochliopina francesae</i>  |    |   | 47  | 0 | <i>Chrysops</i> sp.        |    |   | 0  | 0 |

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|                                  |       |       |       |     |                          |    |    |     |
|----------------------------------|-------|-------|-------|-----|--------------------------|----|----|-----|
| <i>Cochliopina infundibulum</i>  |       |       | 5     | 0   | <i>Coryphaeschna</i> sp. |    | 1  | 0   |
| <i>Corbicula fluminea</i> *      | 2,520 | 3,400 | 75    | 141 | <i>Crambus</i> sp.       |    | 0  | 0   |
| <i>Cyrtonaias tampicoensis</i>   | 0     | 38    | 5     | 113 | <i>Cybister</i> sp.      |    | 0  | 0   |
| <i>Drepanotrema lucidum</i>      |       |       | 2     | 0   | <i>Desmopachria</i> sp.  | 0  | 0  |     |
| <i>Eupera cubensis</i>           | 101   | 1     | 169   | 3   | <i>Dythemis</i> sp.      |    | 0  | 0   |
| <i>Hebentancylus excentricus</i> |       |       | 426   | 2   | <i>Ellipes</i> sp.       |    | 0  | 0   |
| <i>Helisoma duryi</i>            |       |       |       |     | <i>Enallagma</i> sp.     | 18 | 0  | 6   |
| <i>Hydrobia</i> sp.              |       |       | 675   | 2   | <i>Enochrus</i> sp.      | 0  | 0  |     |
| <i>Lamellaxis micra</i>          | 0     | 0     |       |     | <i>Ephoron</i> sp.       | 28 | 0  | 233 |
| <i>Littoraria nebulosa</i>       |       |       | 0     | 0   | <i>Eretes</i> sp.        |    | 1  | 0   |
| <i>Lucidella lirata</i>          | 2     | 0     | 6     | 0   | <i>Erpetogomphus</i> sp. |    | 1  | 0   |
| <i>Margaritifera auricularia</i> | 2     | 103   |       |     | <i>Gelastocoris</i> sp.  |    | 0  | 0   |
| <i>Mayabina polita</i>           | 0     | 0     |       |     | <i>Glossosoma</i> sp.    | 0  | 0  |     |
| <i>Mayabina</i> sp.              |       |       | 111   | 2   | <i>Graptocorixa</i> sp.  |    | 36 | 0   |
| <i>Megalonaia</i> sp.            |       |       | 0     | 0   | <i>Gyretes</i> sp.       |    | 0  | 0   |
| <i>Megapitaria</i> sp.           |       |       | 252   | 6   | <i>Helichus</i> sp.      |    | 0  | 0   |
| <i>Melanoides tuberculata</i> *  | 39    | 1     | 355   | 49  | <i>Helobata</i> sp.      |    | 0  | 0   |
| <i>Mytilopsis leucophaeta</i>    | 14    | 2     | 835   | 20  | <i>Helochaeres</i> sp.   |    | 0  | 0   |
| <i>Neritina usnea</i>            | 478   | 102   | 2,359 | 440 | <i>Heptagenia</i> sp.    |    | 1  | 0   |
| <i>Neritina virginea</i>         | 0     | 0     | 14    | 0   | <i>Hetaerina</i> sp.     | 1  | 0  | 3   |
| <i>Physa</i> sp.                 | 35    | 0     | 209   | 1   | <i>Heterelmis</i> sp.    |    | 1  | 0   |
| <i>Planorbella duryi</i>         | 0     | 0     | 141   | 1   | <i>Hexatoma</i> sp.      |    | 1  | 0   |
| <i>Polymesoda caroliniana</i>    | 28    | 248   |       |     | <i>Hydrometra</i> sp.    | 0  | 0  |     |
| <i>Pomacea flagellata</i>        | 6     | 14    | 332   | 812 | <i>Hydrophilus</i> sp.   |    | 1  | 0   |
| <i>Potamilus alatus</i>          |       |       | 4     | 14  | <i>Lacophilus</i> sp.    |    | 1  | 0   |
| <i>Psoronaia crocodilarum</i>    | 15    | 630   | 30    | 429 | <i>Lachlania</i> sp.     |    | 0  | 0   |
| <i>Pyganodon grandis</i>         |       |       | 14    | 9   | <i>Leptohyphes</i> sp.   | 7  | 0  | 1   |
| <i>Pyrgophorus parvulus</i>      | 3     | 0     |       |     | <i>Leptonema</i> sp.     | 7  | 0  | 0   |

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|                                  |        |       |       |       |                          |    |    |    |
|----------------------------------|--------|-------|-------|-------|--------------------------|----|----|----|
| <i>Pyrgophorus coronatus</i>     | 20     | 0     | 2,799 | 15    | <i>Leptysma</i> sp.      |    | 0  | 0  |
| <i>Rangia cuneata</i>            |        |       | 1     | 0     | <i>Libellula</i> sp.     | 1  | 0  | 4  |
| <i>Stenophysa impluviata</i>     | 0      | 0     | 6     | 0     | <i>Limnogonus</i> sp.    | 0  | 0  |    |
| <i>Subulina octona</i>           | 1      | 0     | 10    | 0     | <i>Maccaffertium</i> sp. |    | 0  | 0  |
| <i>Succinea undulata</i>         | 0      | 0     | 15    | 0     | <i>Macrelmis</i> sp.     |    | 12 | 0  |
| <i>Tarebia granifera</i> *       | 54,199 | 2,782 | 9,293 | 2,090 | <i>Metrobates</i> sp.    | 0  | 0  |    |
| <i>Texadina sphinctostoma</i>    |        |       | 1464  | 5     | <i>Microcyloepus</i> sp. |    | 1  | 0  |
| <b>Crustaceans</b>               |        |       |       |       | <i>Neptopsyche</i> sp.   |    | 1  | 0  |
| <i>Armases cinereum</i>          |        |       | 4     | 1     | <i>Notonecta</i> sp.     | 0  | 0  | 3  |
| <i>Callinectes sapidus</i>       | 0      | 0     |       |       | <i>Omophron</i> sp.      |    | 0  | 0  |
| <i>Callinectes</i> sp.           |        |       | 0     | 0     | <i>Orchelimum</i> sp.    |    | 1  | 1  |
| <i>Cymadusa compta</i>           |        |       | 40    | 0     | <i>Palaemnema</i> sp.    |    | 0  | 0  |
| <i>Discapseudes mexicanus</i>    |        |       | 2,774 | 7     | <i>Paracleodes</i> sp.   |    | 0  | 0  |
| <i>Erichthonius brasiliensis</i> |        |       | 5     | 0     | <i>Paraponyx</i> sp.     | 0  | 0  | 0  |
| <i>Hyaella azteca</i>            | 0      | 0     | 4     | 0     | <i>Pelocoris</i> sp.     |    | 12 | 1  |
| <i>Macrobrachium acanthurus</i>  | 410    | 32    | 240   | 19    | <i>Petrophila</i> sp.    | 0  | 0  |    |
| <i>Macrobrachium carcinus</i>    | 25     | 4     | 22    | 3     | <i>Phyllocycla</i> sp.   | 0  | 0  | 20 |
| <i>Macrobrachium hubbsi</i>      | 27     | 2     |       |       | <i>Polypsectopus</i> sp. |    | 0  | 0  |
| <i>Macrobrachium olfersii</i>    | 113    | 11    | 642   | 286   | <i>Probezzia</i> sp.     |    | 0  | 0  |
| <i>Minuca</i> sp.                |        |       | 4     | 2     | <i>Progomphus</i> sp.    | 14 | 0  | 2  |
| <i>Uhlorchestia uhleri</i>       |        |       | 1     | 0     | <i>Ranatra</i> sp.       | 0  | 0  | 1  |
| <i>Pachygrapsus transversus</i>  |        |       | 1     | 1     | <i>Rhagovelia</i> sp.    | 2  | 0  | 37 |
| <i>Potimirim mexicana</i>        | 109    | 3     | 60    | 2     | <i>Rhantus</i> sp.       |    | 0  | 0  |
| <i>Pseudothelphusa hartmanni</i> | 0      | 7     |       |       | <i>Simulium</i> sp.      | 1  | 0  | 0  |

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|                                 |   |   |    |   |                           |    |   |   |   |
|---------------------------------|---|---|----|---|---------------------------|----|---|---|---|
| <i>Rhithropanopeus harrisii</i> |   |   | 1  | 0 | <i>Stenus</i> sp.         | 3  | 0 |   |   |
| <i>Uca pugilator</i>            | 0 | 0 |    |   | <i>Tabanus</i> sp.        |    |   | 0 | 0 |
| <b>Insects</b>                  |   |   |    |   | <i>Tanysphyrus</i> sp.    | 2  | 0 |   |   |
| <i>Aeshna</i> sp.               |   |   | 0  | 0 | <i>Thraulodes</i> sp.     |    |   | 4 | 0 |
| <i>Ambrysus</i> sp.             |   |   | 3  | 0 | <i>Tortopus</i> sp.       |    |   | 1 | 0 |
| <i>Anacroneuria</i> sp.         |   |   | 2  | 0 | <i>Traverella</i> sp.     |    |   | 1 | 0 |
| <i>Anaxipha</i> sp.             |   |   | 2  | 0 | <i>Trepobates</i> sp.     | 0  | 0 | 3 | 0 |
| <i>Aphylla</i> sp.              | 0 | 0 | 2  | 0 | <i>Triacanthagyna</i> sp. |    |   | 0 | 0 |
| <i>Argia</i> sp.                | 0 | 0 | 1  | 0 | <i>Tricorythodes</i> sp.  | 11 | 0 | 2 | 0 |
| <i>Baetis</i> sp.               | 9 | 0 | 58 | 0 | <i>Tropisternus</i> sp.   | 0  | 0 | 2 | 0 |
| <i>Belostoma</i> sp.            | 2 | 0 | 5  | 0 |                           |    |   |   |   |

Regarding the groups, mollusks represented 93 %, crustaceans 6 %, and insects 1 % of the total average density. Considering the density by basin, mollusks represented 98 % and crustaceans and insects 1 % respectively in the Grijalva basin (GB), while in the Usumacinta basin (UB) mollusks represented 83 %, crustaceans 15 %, and insects 2 %. Regarding biomass, in CG it registered 62 %, while CU 38 %. In both basins, mollusks represented more than 90 % of the total biomass.

Dominant species by density in the GB, were mollusks: *Tarebia granifera* with 77% while in biomass, they were *C. fluminea* (46 %) and *T. granifera* (38 %). For crustaceans, the dominant species in density were *Macrobrachium acanthurus* (28%), *M. olfersii* and *Potimirim mexicana* with 19 % respectively. *M. acanthurus* represented the largest biomass of crustaceans in the basin with 53%. In insects, the dominant species in density were *Chironomus* sp. and *Ephoron* sp. with (20 %) respectively, and *Enallagma* sp. (13 %), while the highest biomass was for *Progomphus* sp. and *Ephoron* sp. (18 %) respectively, followed by *Chironomus* sp. (16 %). In UB, the dominant species in density for mollusks were *T. granifera* (45 %), *Pyrgophorus coronatus* (13 %), and *Neritina usnea* (11 %). Regarding biomass, the dominant species were *T. granifera* (50 %) and *Pomacea flagellata* with 19 %. In crustaceans, the dominance in density was for *Discapseudes mexicanus* with 73 % of the density, while in biomass it was *M. olfersii* with 88 %. Regarding insects, the dominant species in density were *Ephoron* sp. (49 %), and *Baetis* sp. (12 %), while the species *Phyllocycla* sp. (45 %), and *Ephoron* sp. (13 %), were for biomass (Table 2).

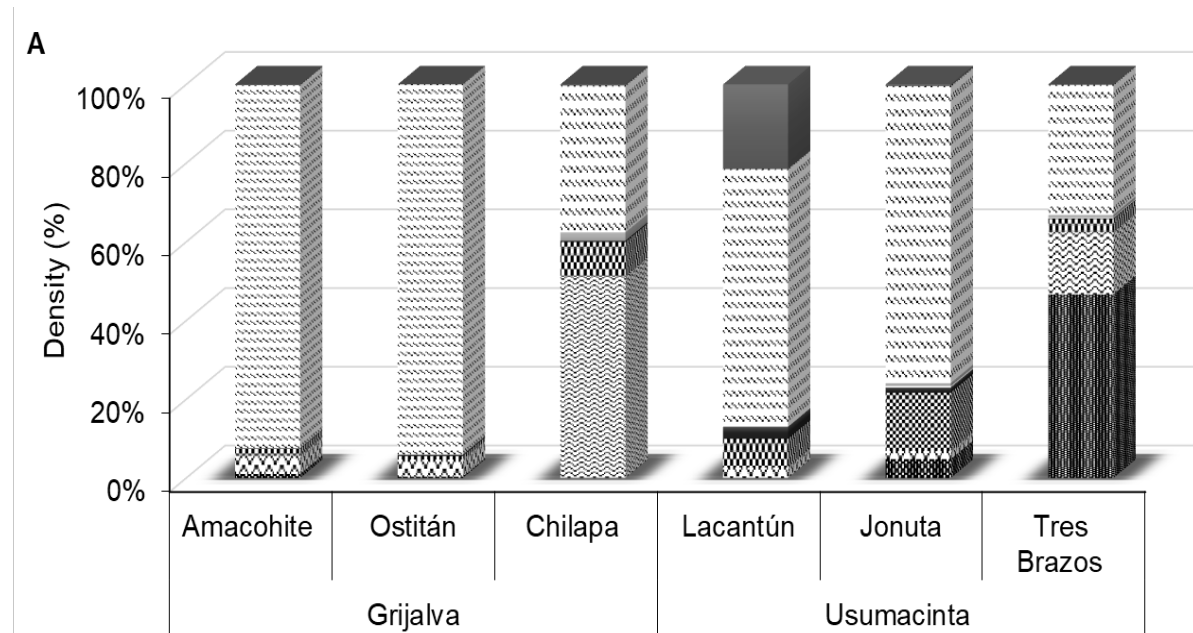
Diversity indices by basin, UB presented maximum values, spatially maximum diversity values were in the Lacantún section in upper basin ( $H'=4.49$ ,  $J'=1.14$ ,  $D=1$  and  $Margalef=22.76$ ). Temporally, maximum values were in the Northern winds ( $H'=3.42$ ,  $J'$

=0.85 and Margalef=15.19) and in the dry season ( $D=0.93$ ) (Table 3). Maximum values of diversity in GB were in lower basin section Chilapa ( $H'=1.78$ ,  $J'=0.48$  y  $D=0.71$ ), seasonally maximum values were in dry season ( $H'=2.28$ ,  $J'=0.79$ ,  $D=0.58$  y  $M=8.73$ ) (Table 3)

**Table 3.** Spatial and Temporal Diversity of Aquatic Macroinvertebrates in Grijalva and Usumacinta Rivers.

| Basin / Section | Grijalva       |         |         | Usumacinta     |        |          |
|-----------------|----------------|---------|---------|----------------|--------|----------|
|                 | Amacohite      | Ostitán | Chilapa | Tres Brazos    | Jonuta | Lacantún |
| Shannon_H       | 0.98           | 0.79    | 1.78    | 2.93           | 2.52   | 4.49     |
| Equitability_J  | 0.28           | 0.22    | 0.48    | 0.73           | 0.58   | 1.14     |
| Simpson_1-D     | 0.28           | 0.25    | 0.71    | 0.93           | 0.77   | 1.00     |
| Margalef        | 7.09           | 6.25    | 6.77    | 8.00           | 13.21  | 22.76    |
| Season          | Northern Winds | Rainy   | Dry     | Northern Winds | Rainy  | Dry      |
| Shannon_H       | 1.70           | 1.06    | 2.28    | 3.42           | 2.73   | 3.07     |
| Simpson_1-D     | 0.70           | 0.36    | 0.79    | 0.89           | 0.88   | 0.93     |
| Equitability_J  | 0.56           | 0.27    | 0.58    | 0.85           | 0.63   | 0.70     |
| Margalef        | 5.18           | 7.10    | 8.73    | 15.19          | 11.74  | 10.97    |

The dominant Trophic groups were the scrapers (74 %) and the filter feeders (11 %) present in all the sampled sections, presenting the maximum values in GB in the Ostitán section and the rainy season. Collectors (10 %) were present in four sections, having the maximum value in UB in the Tres Brazos section in the dry season (Fig. 3).



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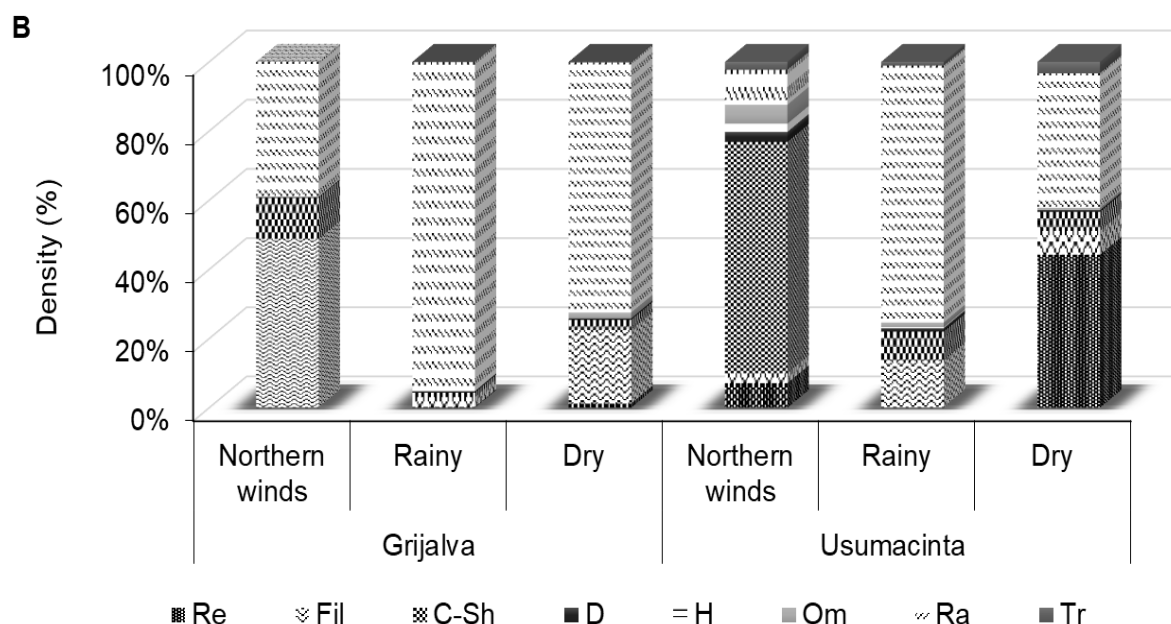


**Figure 3.** Spatial (A) and Temporal (B) Distribution of Trophic Groups in Grijalva and Usumacinta Rivers. Collector (Re), Filtering (Fil), Collector-shredders (C-Sh), Predator (D), Herbivores (H), Omnivores (Om), Scrapers (Ra) and Shredders (Tr)

Trophic groups per basin, presented significant differences with respect to density, mollusks ( $p \leq 0.001$ ), these differences occurred between Filter feeders BG over scrapers BU and filter feeder's BU over scrapers BU. Regarding crustaceans ( $p \leq 0.05$ ), these presented differences between gathering in BU over omnivores in BU and shredders in BU over omnivores in BU, and insects ( $p \leq 0.001$ ) the differences were presented between shredders in BG over predators BG, shredders BU over predators BG, and with a  $p \leq 0.05$  gathered BG over predators BG, shredders BU predators BU, predators\_BG: predators\_BU and shredders BU: shredders\_BG.

## Discussion

In the Grijalva River, it has been identified that main causes of modification of the river ecosystem are the construction of hydraulic infrastructure, urban growth, and pressure on aquatic resources related to primary activities (Sánchez et al., 2015; Sandoval-Rivera et al., 2022). This pattern of riparian quality from regular to poor, is a common condition that has also been observed in other basins (Escalona-Domenech et al., 2022). On the other hand, variation of parameters of temperature, salinity, and TDS in Grijalva, because of urbanization, changes in the use of land, modification of drainage networks, discharges of untreated water from the cities of Villahermosa and Tuxtla Gutiérrez, agricultural activities, oil industry and sugar mills (Ramos-Herrera et al., 2012; Marin, 2014; Musálem-Castillejos et al., 2018).

While in the Usumacinta River, the ecological condition of the river bank from very good to poor, could be explained by its location, since areas in Montes Azules Biosphere Reserve (MABR) in the state of Chiapas (Salinas-Rodríguez et al., 2021; Soria-Reinoso et al., 2022), so the riparian vegetation still presents natural conditions (Ochoa-Gaona et al., 2018), to Jonuta classified as good, this could be because there are unsustainable practices in the area (such as subsistence agriculture, use of agrochemicals, extensive plantations such as oil palm and in the lower areas of Tabasco, extensive livestock farming) (Salcedo-Garduño et al., 2019; Camacho-Valdez et al., 2022), and the Tres Brazos site, located in the Pantanos de Centla Biosphere Reserve in Tabasco, presented a poor riparian state. Due to economic activities such as agriculture, fishing, and livestock are developed in the area, coupled with presence of the oil and gas industry (Barba-Macías et al., 2006; Barba et al., 2018). These activities put the PCBR and its environmental services at risk and keep them under constant pressure (Barba Macías et al., 2014, 2015, 2018; Puc-Carrasco et al., 2016; Ochoa-Gaona et al., 2018).

Temporal variability of the physicochemical parameters of the water occurred due to the dynamics of tropical rivers, which depend on precipitation and flow rates (Soria-Reinoso et al., 2022), combined to this, human settlement and land use change in this river generate alterations in the physicochemical conditions of the water, mainly in dry season, when the volume of water decreases and, therefore, the concentration of discharges increases, increasing the concentration of contaminants and decreasing water quality (Salcedo-Garduño et al., 2022). It should be noted that in the Grijalva River, the depth did not show variation throughout the climatic seasons, which could be explained by the management that the dams carry out on the flow, since it is a characteristic of the reservoirs to compensate for low flows during the dry season and regulate high flows in the rainy season (Wang et al., 2016).

Tropical seasonality differs from the temperate zone whereas temperature differences in tropical streams are usually very subtle ( $\approx 10^{\circ}\text{C}$ ), where taxonomic composition, richness, and trophic guilds are driven by the seasonality through the contrasting (rainy and dry seasons) (Cortés-Guzmán et al., 2021).

Aquatic macroinvertebrate species richness was considerably lower in Grijalva (66 spp.) compared to those in the Usumacinta River (117 spp.). It is the first study that groups three phyla (mollusks, crustaceans, and insects) and therefore, the importance of the information generated. For group of mollusks, 24 species are recorded in the Grijalva River, compared to Mellano-Hernández et al. (2015) who reported 17 species of Gastropods and six species of Bivalvia, with *P. coronatus*, *M. impluviata*, *L. tenuipes*, *M. polita*, and *Pomacea flagellata* being dominant in abundance. For the Usumacinta River, in this study, 36 species of mollusks were recorded having a similarity with those reported by Trinidad-Ocaña et al. (2018) recording 35 species.

For the group of crustaceans in Grijalva, nine species were recorded, according to Álvarez et al. (2005), 72 species of crustaceans are registered for the State of Tabasco, of which

44 were counted for decapods and peracarids; It is considered that there is an underestimation of the records for invertebrates and especially crustaceans and insects since most of the studies have been carried out with species of commercial importance (Sánchez & Barba, 2005). For the Usumacinta River, 14 species were recorded, compared to what was reported by Trinidad-Ocaña et al. (2018) with 13 species. Insects in the Grijalva basin were recorded with 33 genera and for the Usumacinta River, 67 genera were recorded, which compared to Castillo et al. (2018) the value is lower than what they report (127 genera). This may be due in part to the fact that for some individuals of some orders, identification at the genus level was not possible.

73 % of total density was in the Grijalva River, due to a high number of exotic mollusk species, *Tarebia granifera*, with 65 % of total density (both rivers). This is the first study which groups three phyla (mollusk, crustaceans and insects), past reports are about a single zone *p.e.* Lacantún (Cortés-Guzmán et al., 2021), or just Insects (Castillo et al., 2018), or mollusk and crustaceans in Usumacinta (Barba-Macías et al., 2018). In Lacantún section 63, the genera of benthic macroinvertebrates were recorded from 39 families and ten orders of those 24 genera (40 %) have been previously reported from the Usumacinta River Basin (Castillo et al., 2018). In the Grijalva river, 17 species of Gastropod and six species of Bivalvia were recorded, being dominant in abundance *P. coronatus*, *M. impluviata*, *L. tenuipes*, *M. polita* and *Pomacea flagellata* (Mellano-Hernández et al., 2015),

Macroinvertebrate diversity was higher in the Usumacinta River, in the Lacantún section, and in northern winds, in Grijalva, the diversity was higher in the Chilapa section in the dry season. Spatial distribution of density by the taxonomic group in Grijalva, crustaceans and insects were higher in Chilapa, and mollusk in Ostitán, in Usumacinta mollusks and insects were higher in Jonuta, and crustaceans in Tres Brazos. Temporal distribution was in Grijalva, mollusks and insects higher in rainy, crustaceans in northern seasons, meanwhile, in Usumacinta highest density values were in dry season.

Scrapers group were dominant in all sections, with highest density values in Ostitan GB, in the rainy season, collectors were present in four sections with highest values in Tres Brazos in the dry season, differences between sections and rivers can be explained by differences in hydrological regime. The Usumacinta River is free-flowing with a maximum 220 discharge (averaged 3,632 m<sup>3</sup>/s) in the rainy season and a minimum (723 m<sup>3</sup>/s) in the dry season. This reflects the relationship between the rainfall regime in the rainy season with flow volume. The Grijalva River flow drastically modified, showed a decrease in volume to less than a half; being notorious in dry season with an increase in 156 (m<sup>3</sup>/s) of natural volume (Miranda-Vidal et al., 2023). Free-flow of the Usumacinta River has flow regimes well marked by seasonality (rainy and dry), with natural conditions of hydrological connectivity and flow regimes (Salinas-Rodríguez et al., 2021). These conditions facilitate the lateral and longitudinal migration of fish and their permanence in the aquatic systems of the floodplain (Castillo, 2011). In addition, hy-

hydrological connectivity is very important for the dynamics of aquatic and riparian vegetation (Mendoza, 2014), because it promotes the dispersal of seeds, fruits, or parts of the plant in the rainy season which enables individuals to develop for repopulation and, in addition, facilitates the flow and transfer of nutrients (Ochoa-Gaona et al., 2018).

Loss of seasonal flow variation in the Grijalva river compromised the seasonal variation of geomorphology creation and maintenance, reflected in the homogeneity of riparian and aquatic habitats (Wang et al., 2016). Furthermore, hydrological connectivity was altered for various aquatic species that require extreme flows to complete their life cycles (Metcalf et al., 2013). With such alteration to the flow, temporal pulses, and homogenization of levels (depth), the entry and permanence of invasive species are facilitated (Ormerod et al., 2011). Reduced flow favors soil and water salinization and decreases the inflow of freshwater to the sea, as in the mouth of the Grijalva River where the salt wedge has reached up to 46 km upstream (Alcérreca-Huerta et al., 2019). This threatens fluvial ecological processes and the permanence of freshwater species, especially in the RBPC (Mata-Zayas et al., 2017).

On the other hand, maximum values of richness and abundance recorded in the Usumacinta River could be so since it still has good natural conditions of flow and hydrological connectivity, so the heterogeneity of the landscape as a riparian condition represents an important role for aquatic macroinvertebrates. A similar situation occurs with fish diversity, Soria-Barreto et al. (2018) reported 172 species, through a search of bibliographic information and sampling carried out from the Lacantún River in Chiapas to the Centla Swamps in Tabasco and the Palizada River in Campeche. Contrary to the Grijalva River, only 92 species of fish have been listed (Gómez-González et al., 2015).

## Conclusions

Present work enriches information on the diversity and trophic groups of aquatic macroinvertebrates in the Grijalva River and Usumacinta River, one of the most important rivers in México, and due to their high water and natural wealth, they not only gather a large part of the biodiversity, but they also contribute 30 % of the country's total surface runoff.

Analysis of study and temporal zones for each basin allows us to demonstrate difference in macroinvertebrate diversity that exists between them. The Grijalva River had the highest density values represented by the exotic mollusk *Tarebia granifera* with 77 %. Other exotic species were *Melanoides tuberculata* and *Corbicula fluminea*. The Usumacinta River had the greatest richness and diversity of macroinvertebrates. Presence of trophic groups such as fragmenters and collectors are strongly related in the Usumacinta areas with better water quality. This relationship could be because the habitat where they are found has greater vegetation cover. However, trophic groups such as predators and scrapers are strongly associated with the Grijalva areas with lower water quality values and with evident human intervention.



Results confirm that rivers with natural flow regimes and high riparian quality values have greater diversity and trophic levels. The Usumacinta River has a natural seasonal flow corresponding to maximum and minimum flood seasons, providing various resources (habitat, food, shelter, connectivity) to aquatic populations. In addition, it provides various services such as supply and cultural services by obtaining water and food from the river and providing areas for sport fishing and recreation.

However, given the extension, complexity of the landscape, and the hydrological characteristics of the basin, it is necessary to carry out studies at a finer scale such as sub-basins and geomorphological units in such a way that the biogeographic and diversity patterns are explained, not only of macroinvertebrates, but of all the aquatic species that inhabit them.

Riparian habitats of the Usumacinta and Grijalva rivers are compromised by various anthropogenic activities and need urgent attention. Studies are needed to exhaustively evaluate the management of tropical forests to prolong and maintain the ecosystem services that the basins still have and focused on trophic groups, since this constitutes an approach to the ecology of the macroinvertebrate community where greater studies are necessary to understand the effects of changes in aquatic habitats on functional aspects of the community and its relationship with the quality of habitats and their environmental services.

## Authors' Contributions

EBM, JJF, sampling design, obtaining financing. EBM, JJF, CTO, FJMV sample collection, species identification, analysis. EBM, JJF, CTO, drafting of the document

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**Annexe 1.** Environmental Characteristics of the Selected Sites in the Usumacinta River; Season (D): Dry, (R): Rainy; Habitat: EMV (Emerged Vegetation, Trees and Shrubs *Phragmites australis*, *Typha dominguensis*), FFV (free floating vegetation, *Salvinia minima*, *Eichhornia crassipes*). SAV (Submerged Vegetation *Vallisneria americana*, *Cabomba palaeformis*) HA: Human Activities, MI: Marine Influence, RQI: Riparian Quality (Miranda-Vidal et al., 2023).

| Section                                          | Depth (m)            | Habitat                                                                            | HA                                                       | RQI          | MI  | Reference Image                                                                       |
|--------------------------------------------------|----------------------|------------------------------------------------------------------------------------|----------------------------------------------------------|--------------|-----|---------------------------------------------------------------------------------------|
| Lacantun<br>90°54'20.083"W<br>16°12'30.938"N     | 0.57 (D)<br>1.08 (R) | EMV<br>Substrate<br>Boulders<br>and Sand,<br>Moderate<br>Current.                  | Agriculture,<br>Fishing<br>Harvest,<br>Forest<br>Tourism | Very<br>Good | No  |    |
| Jonuta<br>92°05'59.676" W<br>18°05'42.492" N     | 0.90 (D)<br>1.80 (R) | EMV, FFV,<br>Substrate<br>Sand, Grav-<br>el, or Mud,<br>and Moder-<br>ate Current. | Agriculture,<br>Livestock<br>Fishing                     | Moderate     | No  |   |
| Tres Brazos<br>92°37'38.267"W<br>18°23'50.702" N | 1.83 (D)<br>1.96 (R) | EMV, SAV,<br>Substrate<br>Sand, Silt,<br>and Clay,<br>Strong Cur-<br>rent.         | Agriculture<br>Livestock<br>Fishing<br>Tourism           | Poor         | Yes |  |

**Annexe 2.** Environmental Characteristics of the Selected Sites in the Grijalva River; Season (D): Dry, (R): Rainy; Habitat: EMV (Emerged Vegetation, Trees and Shrubs *Phragmites australis*, *Typha dominguensis*), FFV (free floating vegetation, *Salvinia minima*, *Eichhornia crassipes*). SAV (Submerged Vegetation *Vallisneria americana*, *Cabomba palaeformis*) HA: Human Activities, MI: Marine Influence, RQI: Riparian Quality (Miranda-Vidal et al., 2023).

| Section                                         | Depth (m)            | Type of Habitat                                                               | HA                                                                      | RQI      | MI  | Reference Image                                                                       |
|-------------------------------------------------|----------------------|-------------------------------------------------------------------------------|-------------------------------------------------------------------------|----------|-----|---------------------------------------------------------------------------------------|
| Amacohite<br>93°26'56.016" W<br>17°34'40.449" N | 1.47 (D)<br>1.62 (R) | EMV,<br>Substrate<br>Boulders,<br>Gravel, and<br>Sand, Moder-<br>ate Current. | Agriculture,<br>Fishing<br>Aquaculture<br>Livestock,<br>Forest          | Moderate | No  |    |
| Ostitan<br>93°24'14.918" W<br>17°47'30.883" N   | 1.05 (D)<br>1.07 (R) | FFV,<br>Substrate<br>Sandy and<br>with Mod-<br>erate-slow<br>Current.         | Agriculture,<br>Fishing<br>Livestock,<br>Forest<br>River Trans-<br>port | Poor     | No  |   |
| Chilapa<br>92°40'19.311" W<br>18°15'14.303" N   | 2.16 (D)<br>2.53 (R) | EMV, FFV,<br>Substrate<br>Mud and Silt,<br>with Strong<br>Current.            | Agriculture,<br>Fishing<br>Livestock                                    | Moderate | Yes |  |





# Fishing Among Socioecological Challenges: The Case of the Zimapán Dam

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## Abstract

The Global Climate change crisis urgently demands a deeper and more comprehensive understanding of aquatic systems. In this study, we analyzed the Zimapán Dam, a relatively recently created ecosystem distinguished by its high complexity and distinct anthropization, where fishing is intricately connected with ecological, environmental, and social factors. We updated the list of fish species present in the dam and analyzed daily registers of the “13 de Junio” Fishing Cooperative, aiming to comprehend the dynamics of fishing activity. Additionally, semi-structured interviews were conducted with fishers to capture their perceptions of the situation, identify underlying causes, and explore potential solutions. The fish community is composed of eight species and two subspecies identified as exotics. On average, during the study period, the catch per unit effort of tilapia was 10 kg per fisher per day. The fishing activity has experienced notable variations over time, lacking evident consistency and was influenced by various factors. Several causes for the decrease in fishing and the gradual abandonment of the activity due to lack of profitability were pointed out by the interviewees, primarily the presence of black bass. This study underscores the need for integrated approaches and collaborative solutions addressing both the ecological problems and social dimensions of fishing. Ecosystems like the Zimapán Dam will become increasingly important in the context of global change; therefore, understanding them as key ecosystems on which we will depend in the near future is essential.

## Keywords

Anthropization; Black Bass; Exotic species; Hydroelectric Dam; Tilapia.

## Introduction

Throughout the Anthropocene, human activities have caused impacts on the environment, altering atmospheric, geological, hydrological, and ecosystem processes at multiple scales, ranging from local to planetary (Ruddiman, 2013). Nowadays, we are witnesses of the ecosystem disruptions resulting from the transformation of natural and social processes, such as land use change, transformation and degradation of the ecosystems, and alterations in biogeochemical cycles. In fact, ecosystems are no longer conceivable without human influences (Schmitz, 2016). There is a growing concern about the capacity of ecosystems to maintain their function in the context of global change. According to the Global Risks Report (World Economic Forum, 2019), water bodies are acknowledged as one of the systems experiencing the most significant social, political, and economic pressure globally, as nearly all human activities are intimately linked with water. For instance, estimates indicate that humanity expropriates more than half of the accessible drinking water (Gleick & Palaniappan, 2010).

Aquatic ecosystems are particularly affected, and it is estimated that aquatic populations have decreased by 76 % in epicontinental bodies of water since 1970 (WWF, 2014). Epicontinental water bodies are key to ensuring the water supply for human population and the sustainable development of nations (Bunn, 2016). The viability of these water bodies in the future is crucial and depends on the control that can be exerted over the sources impacting them, which, in turn, relies on an adequate understanding of their functioning (Sage, 2019). This situation is particularly concerning in Mexican aquatic ecosystems.

In our country, 77 % of the human population resides in areas where only 33 % of renewable water is found (Arreguín-Cortés et al., 2020). According to the Atlas of Water Vulnerability to Climate Change (Arreguín-Cortés et al., 2015), the most critical basins and aquifers in terms of population and economic activities are over-conceded and overexploited respectively. Besides the scarcity of availability, pollution represents another limiting factor; in Mexico, 70 % of bodies of water show some degree of contamination (Conagua, 2018). For example, 49 % of rivers and streams are categorized with a high or very high degree of ecohydrological alteration (Garrido et al., 2010). According to Sánchez (2017), in Mexico, more than 80 % of municipal wastewater generated by public-urban use is discharged into water bodies without any treatment. Likewise, 82 % of industrial wastewater is discharged untreated (Bunge, 2010). This, coupled with the expected impact of climate change on precipitation patterns, contributes to the challenges. The country's situation is projected to become critical by 2025 (Ávila, 2008).

Additionally, introduced species are among the main effects of human alteration of aquatic systems. Exotic species can reduce native fish populations (Rahel, 2000), modify community dynamics (Minns & Cooley, 1999), alter trophic structure stability (Vander Zanden & Rasmussen, 1999), and even lead to the extinction of native species (Lodge, 1993). The potential consequences on native fauna and ecosystems are severe, especially in areas of high diversity (Zambrano et al., 2006). In Mexico, exotic fish species are established in virtually all bodies of water in the central region of the country and are one of the most significant factors contributing to the loss of native species (Zambrano & Hinojosa, 1999; Contreras-MacBeath et al., 2014). Numerous effects generated by exotic species have been identified, such as changes in community structure, competition for food resources (Zambrano et al., 2010), modification of native species populations, trophic cascades, and algal blooms (Figueredo & Giani, 2005), increased turbidity (Miller & Cowl, 2006), and reduction of trophic pathways (Córdova-Tapia, 2011). Currently, some negative effects of these species are known, but many details about how they integrate into food webs and their ecological implications remain unclear. Both pollution and the introduction of exotic species act synergistically on epicontinental water bodies, generating direct and indirect changes in the quantity and quality of existing resources, which in turn can lead to changes in community structure and ecosystem functioning (McCormick et al., 2009).

Water-related issues in Mexico are becoming increasingly problematic. Climate change, water scarcity, exotic species, and poor water quality are persistent and worsening problems. Major cities will have less water, water quality will deteriorate, and conflicts over water will increase. Moreover, poverty and social inequality will rise, making the situation even more complex (Ávila, 2008). In summary, it is expected that in the coming years, Mexico will experience a loss of water security, affecting many people and exacerbating poverty and inequality, while also endangering the country's ecosystems. We are facing a water crisis, both nationally and globally, posing a significant challenge for limnology. Therefore, in recent years, research efforts have been directed towards understanding how ecosystems can continue to provide goods and services under different anthropization scenarios (Naeem et al., 2012). In summary, this crisis urgently demands a deeper and more comprehensive understanding of aquatic systems. In this regard, limnology plays a crucial role in identifying solutions for sustainable water management in a context of scarcity and global change.

### *Zimapán as an Example of a Highly Anthropized Complex System*

The Fernando Hiriart Balderrama “Zimapán” hydroelectric dam is situated in the “El Infiernillo” Canyon, marking the conclusion of a complex system comprising twelve dams along the Moctezuma River sub-basin in the Pánuco River region (Fig. 1). Its primary tributaries

are the Tula River, carrying wastewater from the metropolitan area and traversing the Mezquital Valley, known for its intense agricultural activity. This river also passes through the Miguel Hidalgo Refinery “Tula” and the Tula Thermal Power Plant. The other main tributary is the San Juan River, collecting wastewater from the industrial zone of San Juan del Río, as well as from the wine and cheese industry in Querétaro, and the community of Tequisquiapan, known for its goldsmithing. Thus, this dam is strongly influenced by human activities throughout the basin.

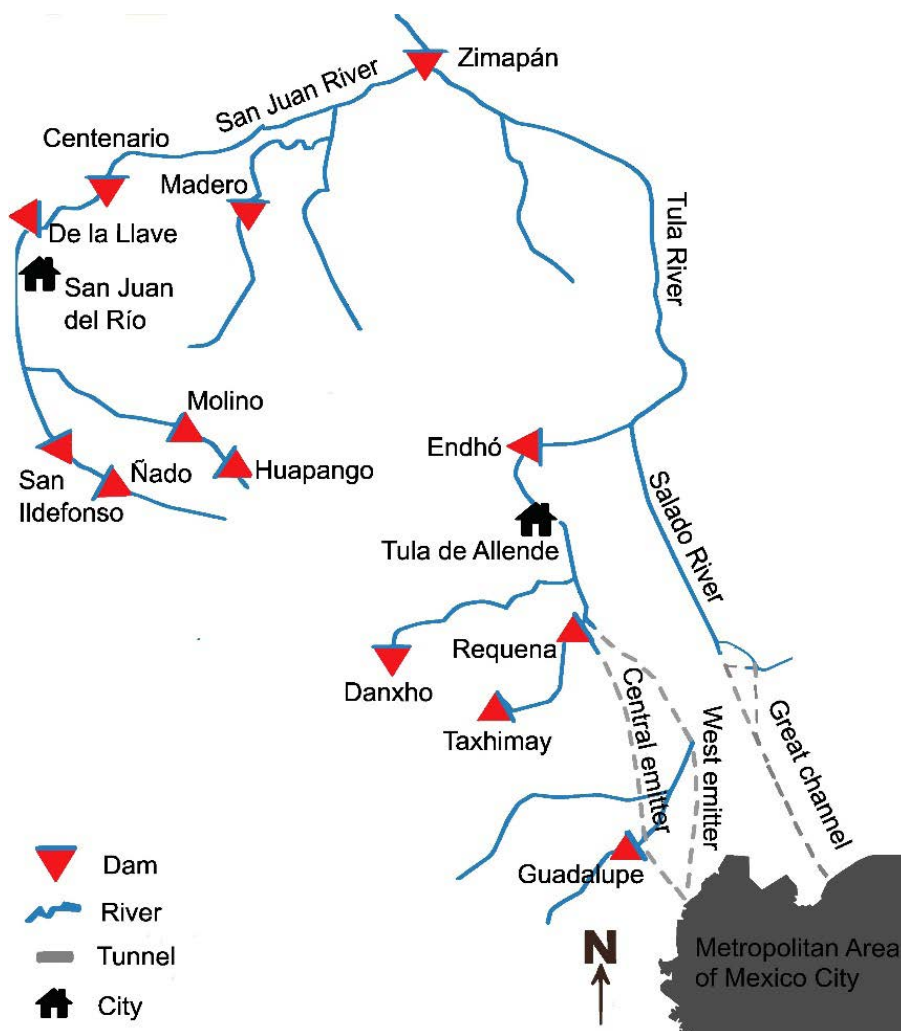


**Figure 1.** Hydrological System of Rivers and Dams that Converge in the Zimapán Dam. Based on Cortes-Silva et al., 2006.

Zimapán was built between 1990 and 1995 by the National Commission of Electricity (CFE) with the goal of generating electricity, featuring a 200 m high curtain dam forming a reservoir of 1,500 million m<sup>3</sup> of water in 2,300 ha. The dam's construction impacted the lives of twelve Otomi ejidos (community-owned lands) residing along the riverbanks, as well as 2,290 ha of cultivated and fruit-bearing lands (López-Hernández et al., 2021). The relo-



cation of families living on the dam's edges, such as Vista Hermosa and Rancho Viejo, led to a radical shift from traditional agriculture to fishing, a new activity posing a challenge for these communities (López-Hernández et al., 2007). Despite the challenges, fishing cooperatives were formed in Hidalgo and Querétaro, initiating fishing activities in 1998, which has since become the primary source of income in the area. To this end, two exotic species, tilapia (*Oreochromis* sp.) and carp (*Cyprinus carpio*), were introduced. The dam has been subjected to multiple introductions of exotic species over the years; for instance, according to personal communications the fishermen, in 2005, largemouth bass (*Micropterus salmoides*) were illegally introduced to promote sport fishing. Since 2013, fishing efforts were organized within 13 associative groups, comprising 192 fishermen from Querétaro and 455 from Hidalgo. Fishing is conducted using gill nets in accordance with established regulations, and an annual closed season is implemented from April 21 to June 20 (SAGARPA, 2011).

A recurring environmental problem affecting fishing activity is the entry of wastewater from the Tula and San Juan rivers due to improper hydrological management and extraordinary floodings of untreated wastewater. In 2002 and 2021, extraordinary floods of untreated water were recorded due to the retention incapacity of the Requena and Endhó dams, which resulted in massive fish deaths in 2002; and a few months after the entry of water in 2021, there was a massive proliferation of *Lemna gibba* directly affecting fishing activities. In recent years, there has been a growing concern among fishing cooperatives as the catch has been decreasing to a point where it is considered an unprofitable activity (López-Hernández et al., 2021).

The situation is concerning since in the riverside populations of the Zimapán Dam, fishing is the dominant economic activity, followed by agriculture in localities such as Tziquia and Tzibantzá (Querétaro), where water is extracted from the reservoir for drip irrigation of crops such as tomatoes, pumpkins, chili peppers, beans, and corn (López-Hernández et al., 2021). Livestock breeding is also practiced, mainly goats and sheep, and there is migration to nearby cities for jobs in the primary sector (SAGARPA, 2011). This situation led the Tecozautla Municipality, starting 2021, to promote the cultivation of tilapia through a scheme where the fish are placed in floating enclosures with the aim of increasing the size by three inches before their release on the dam.

Zimapán is a relatively recently created ecosystem, distinguished by its high complexity and distinct anthropization, where fishing is intertwined with ecological, environmental, and social factors. Given the growing anthropization trend in Mexico's water bodies, Zimapán represents a paradigmatic example of an ecosystem highly modified by human activities. Nonetheless, these complex systems are precisely the ones that pose new challenges for limnology and the approach to studying these water bodies. Within the framework of this study, an updated list of fish species present in the dam was compi-

led. Daily registers of the “13 de Junio” Fishing Cooperative, the largest in the dam, were analyzed, aiming to understand the dynamic of the fishing activity from the beginning of June 2021 through August 2023. Additionally, semi-structured interviews were conducted with fisherman from various communities aiming to encapsulate their perceptions regarding the situation, identify underlying causes, and explore potential solutions. With this information, the goal is to delve deeper into understanding how these various issues interplay and shape a complex socio-ecological situation.

## Materials and Methods

The Zimapán Dam is situated between coordinates 20°35'-20°40'N and 99°22'-99°37'W, at an elevation of 1,870 m above sea level, and has an extension of approximately 2,300 ha. The dam represents the territorial confluence of Hidalgo and Querétaro states, extending into the municipalities of Zimapán, Tasquillo, and Tecozautla in the western part of Hidalgo, as well as in the municipality of Cadereyta de Montes in Querétaro (Figure 2). The region's climate is classified as BSh, indicating a dry and semi-arid profile with an average annual temperature of 19.1°C. The prevalent vegetation in the area corresponds to xerophytic shrubland. The National Fisheries Institute identifies the reservoir as suitable for tilapia fishing, based on physicochemical conditions and the availability of food resources, both natural and artificially induced (López-Hernández et al., 2007).



**Figure 2.** Location of the Zimapán Dam and Neighboring Municipalities.

Six field trips were conducted to cover an annual cycle and consider different seasons of the year (October and November 2022, February, May, August, and November 2023). To assess species richness, various fishing techniques were employed during four

days during each visit. Five trap nets were set on the dam's shore, fixed to nearby rocks and left for six-hour periods. Casting nets with a diameter of three metres and mesh size of one inch were thrown from the quay and boats. Two sets of gill nets of 100 metres in length and five metres deep, with different mesh size openings (2, 3, 3.5, 4, and 4.5 inches) were installed and checked every 24 hours. Simultaneously, the species captured by commercial fishermen, who delivered their catches to the cooperative collection center, and those caught by sports fishermen at the dam were noted.

To analyze the fishing dynamics, a collaboration with the "13 de Junio" cooperative, was established. Access to the daily fishing logbook was obtained, going from June 2021 to August 2023, with a total of 4,923 records. This detailed record contains daily information on each fisher, including the daily kilograms captured of each species (tilapia, carp, and silver carp). It's important to highlight that the data in this logbook are considered of high quality as it's the same logbook used for fish commercialization. Using this information, the catch per unit effort (CPUE) was calculated, a metric allowing evaluation of fishery behavior (Díaz & Goenaga, 2003). The CPUE calculated represents the daily kilograms obtained by a fisherman over each week of the year. Additionally, the weekly number of fishermen delivering products to the collection center was examined to understand whether the quantity of active fishers' changes over time.

To comprehend the fisher's perception of the socio-environmental vulnerability of the fishing situation in the Zimapán Dam, semi-structured interviews were conducted. The main objective of these interviews was to clarify the economical relevance of fishing and acquire information about the evolution of the fishing situation over time. During these interviews, inquiries were made about the main issues identified by them, seeking information on potential solutions to improve the fishing activity. This approach provided a complete and detailed perspective of the locals' view, adding valuable insights complementing the technical and quantitative analysis of fishing at the Zimapán Dam.

## Results

The fish community in the dam consists of eight species distributed among three taxonomic orders: Cypriniformes, Perciformes and Siluriformes (Table 1). The order Cypriniformes is represented by a single family: Cyprinidae, with three species: *Hypophthalmichthys molitrix* (Silver carp), *Ctenopharyngodon Idella*, (Grass carp) and *Cyprinus carpio* (Common carp) with two subspecies: *Cyprinus carpio specularis* (Mirror carp) and *Cyprinus carpio koi* (Koi carp). The order Perciformes is represented by two families: Centrarchidae and Cichlidae. The family Centrarchidae includes *Lepomis macrochirus* (Bluegill) and *Micropterus salmoides* (Largemouth bass). The family Cichlidae contained *Oreochromis* spp. (Tilapia) and *Herichthys carpintis* (Pearlscale cichlid). In the order Siluriformes, the family Ictaluri-

dae was represented by a single species, *Ictalurus punctatus* (Channel catfish). The eight species and two subspecies identified are exotic species originating from various regions: America, Asia, and Africa. The Pearlscale cichlid is native to the Gallinas River basin and the upper Tamasopo River basin in Mexico.

**Table 1.** Fish Species and their Records in the Zimapán Dam.

| Order         | Family        | Species/Subspecies                                         | Local name          | Body shape                                                                            |
|---------------|---------------|------------------------------------------------------------|---------------------|---------------------------------------------------------------------------------------|
| Cypriniformes |               |                                                            |                     |                                                                                       |
|               | Cyprinidae    |                                                            |                     |                                                                                       |
|               |               | <i>Cyprinus carpio</i><br>(Linnaeus, 1758)                 | Carpa común         |    |
|               |               | <i>specularis</i>                                          | Carpa espejo        |    |
|               |               | <i>koi</i>                                                 | Carpa koi           |    |
|               |               | <i>Hypophthalmichthys molitrix</i><br>(Valenciennes, 1844) | Carpa plateada      |    |
|               |               | <i>Ctenopharyngodon idella</i><br>(Valenciennes, 1844)     | Carpa herbívora     |    |
| Perciformes   |               |                                                            |                     |                                                                                       |
|               | Centrarchidae |                                                            |                     |                                                                                       |
|               |               | <i>Lepomis macrochirus</i><br>(Rafinesque, 1819)           | Mojarra azul        |  |
|               |               | <i>Micropterus salmoides</i><br>(Lacepède, 1802)           | Lobina              |  |
|               | Cichlidae     |                                                            |                     |                                                                                       |
|               |               | <i>Oreochromis</i> spp.<br>(Günther, 1889)                 | Tilapia             |  |
|               |               | <i>Herichthys carpintis</i><br>(Jordan & Snyder, 1899)     | Mojarra tampiqueña* |  |
| Siluriformes  |               |                                                            |                     |                                                                                       |
|               | Ictaluridae   |                                                            |                     |                                                                                       |
|               |               | <i>Ictalurus punctatus</i><br>(Rafinesque, 1818)           | Bagre               |  |

The catch per unit effort (CPUE) analysis revealed that tilapia reached its peak during the month of November 2022, with an average of 23 kg/fisher/day (Fig. 3A). Similarly, carp recorded its highest average value in January 2023, achieving a CPUE of 9 kg/fisher/day (Fig. 3B). On average, during the study period, the CPUE was 10 kg/fisher/day for tilapia and 5 kg/fisher/day for carp. The fishing dynamics of both species were influenced



by various factors, including environmental conditions, seasonal changes, ecological interactions, and social dynamics. In October 2021, fishing was suspended preventively due to an extraordinary influx of wastewater into the reservoir. Throughout 2022, there was an uncontrolled proliferation of *Lemna gibba*, commonly known as duckweed. Duckweed is a fast-growing aquatic plant that quickly covers the water surface, blocking sunlight and hindering the growth of other photosynthetic organisms (Arroyave, 2004). This proliferation affected fishing activities during six months. After overcoming the contingency, the highest record of catch per unit effort was observed in November of the same year. However, this efficiency was followed by a rapid decrease in the following weeks.

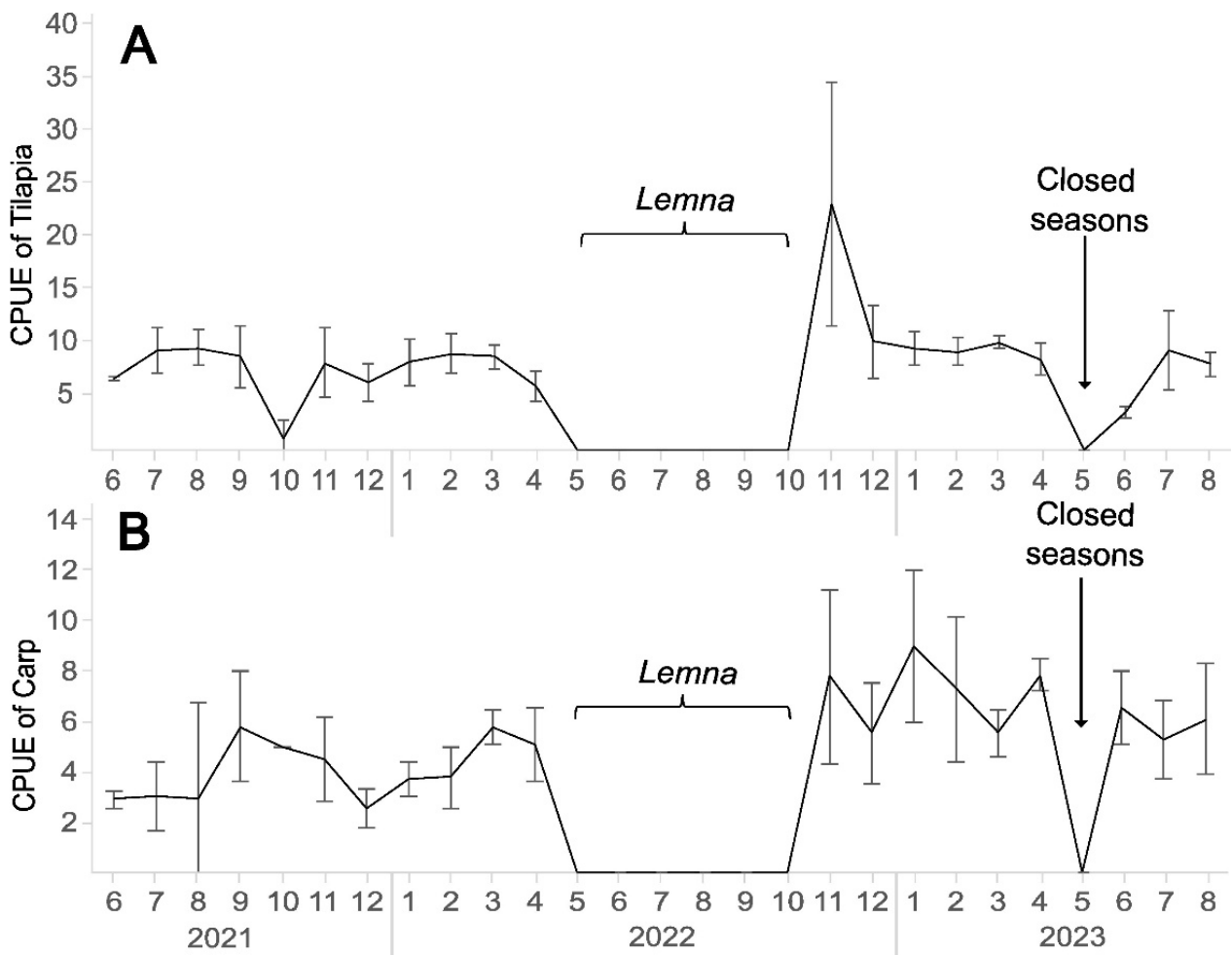


**Figure 3.** Mean and Standard Deviations of the Catch per Unit Effort (CPUE) for (A) Tilapia (*Oreochromis* sp.) and (B) Common Carp (*Cyprinus carpio*). The CPUE is Expressed as the Average Daily Catch in Kilograms per Fisher.

The fishing activity of the “13 de Junio” Fishing Cooperative has experienced notable variations over time, lacking evident consistency (Fig. 4). November 2022 stands out as the period of highest activity, with the participation of over 40 fishers. In contrast, during July 2023, the historical minimum activity was recorded, with only three fishers. On average, fishing activity remained at approximately 15 fishermen throughout the study period. Noteworthy is the dynamic of peaks where an increase in the number of fishermen is followed by a sharp decline in participation. This phenomenon is clearly observed in June 2021, at the conclusion of the annual closed season. Similarly, the pattern is also present after the contingency related to *Lemna gibba*, and after the closed season in June 2023.

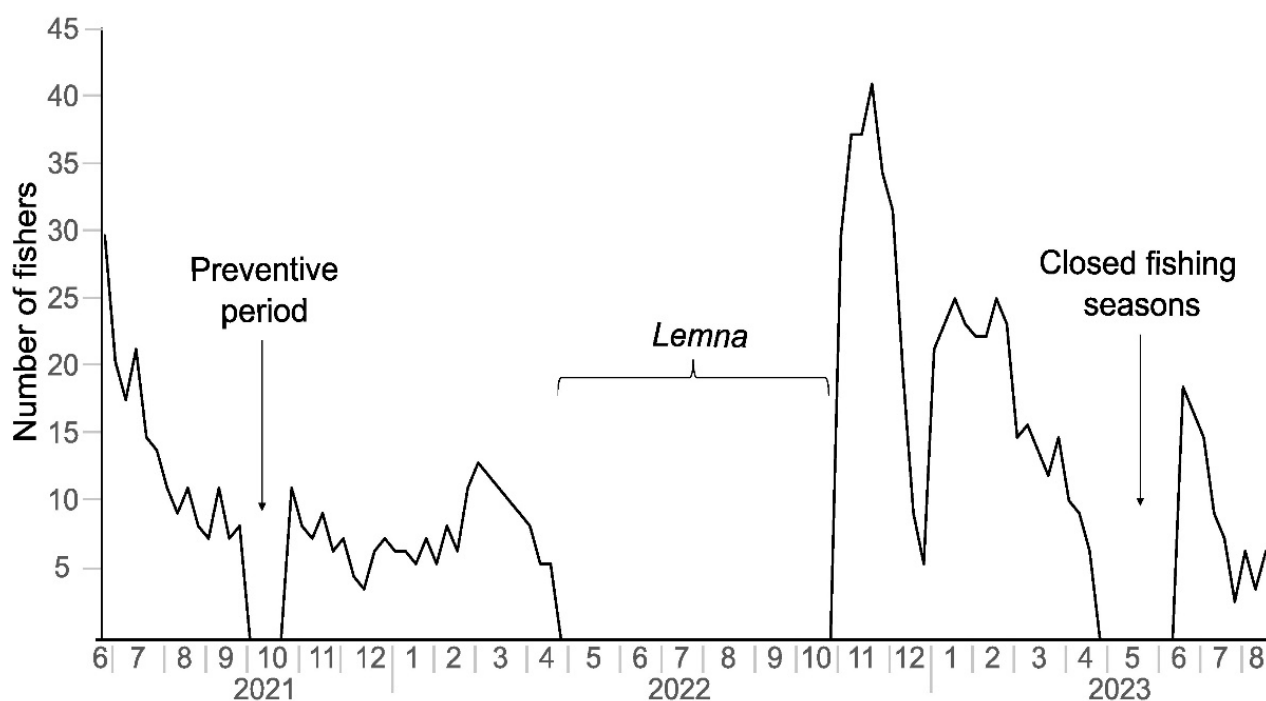


**Figure 4.** Behavior of Fishing Activities in the “13 de Junio” Cooperative.

In total, 43 interviews were conducted. Out of these interviews, 28 (65 %) corresponded to men, and 15 (35 %) to woman. Regarding the highest level of education among fishermen, 2 (5 %) have university education, another 2 (5 %) completed high school, 21 (49 %) attended middle school, 17 (39 %) have elementary education, and 1 (2 %) are in a situation of illiteracy. On the other hand, 31 (74 %) of the interviewees stated that fishing was their main economic activity, while 11 (26 %) indicated having another occupation besides fishing.

Several causes for the decrease in fishing and the gradual abandonment of the activity due to lack of profitability were pointed out by the interviewees. The introduction of carnivorous species, particularly the largemouth bass, a consequence of sport fishing, was

often referred to as a significant problem. Around 50 % of the interviewees mentioned that the largemouth bass feeds on tilapia, identifying that before its introduction, more tilapia was fished. “[...] initially, there wasn’t a fish that eats on tilapia... and when we opened it (the bass), there were fish in its stomach. I imagine they eat them because the fishing has been decreasing little by little, and yes, we attribute it to that, to the bass”. Despite efforts in tilapia fingerling restocking, fishers expressed concern about the lack of recovery in the captured quantities. It was mentioned that the largemouth bass, being carnivorous, could be negatively affecting the success of restocking. “[...] the bass is carnivorous, so, we believe that it also eats a lot of what is being stocked and also small fish. We have even caught bass weighing 4 kg with a tilapia weighing half a kilogram in its belly”. Furthermore, concern was highlighted regarding the increasing population of largemouth bass and the lack of economic benefits for commercial fishers. Restrictions on catching and commercializing largemouth bass were mentioned as a limitation. “We can only catch tilapia, not bass. They said we can’t catch bass”. “Yes; the stocking of tilapia. But this goes hand in hand with the utilization of bass or the reduction of its population. It’s useless to restock if you have an excess population of bass”.

Only 10 % of the fishermen acknowledge overexploitation as a cause of the decrease in catch. “The cause is that the reservoir was excessively exploited from the beginning”. “I think the causes might have been that, at the time when there was good fishing, maybe we didn’t know how to control it. I think we might have overexploited it then”. A lower percentage (8 %) of the interviewees considered water quality an issue, highlighting the decrease in oxygen in the reservoir. “Because yes, we have noticed, especially with the cages, we take measurements, and we realize that oxygen is already very low sometimes and other times it’s not”.

Around 15 % mentioned that fishing has become unsustainable, jeopardizing the economic security and causing the abandonment of fishing in Zimapán, some people are migrating in search of better opportunities. “Well, fishing is really low at the moment. That’s why we are leaving, because we must look for another job because if we don’t, if we just rely on this, we won’t survive”. “Very critical and very difficult. No more. That’s why there’s not many fishers, because, well, it’s not worth it anymore. We have to find elsewhere because fishing won’t do”. Regarding possible solutions, 23 % of the interviewees saw tilapia restocking as an alternative to increase daily catches. “Well, now we are restocking in higher quantities. Maybe it’s what affected us, at the beginning, when we started fishing, we didn’t repopulate, we just fished”.

## Discussion

The Zimapán hydroelectric dam stands out as a paradigmatic case of a complex system heavily influenced by human activities. Within this context, fishing, which initially emerged as an activity after the relocation of Otomi communities, has evolved into the primary source of income in the area. However, fishing in the Zimapán dam faces significant challenges, a product of the complex interaction between ecological, environmental, and social factors.

Exotic species represent a major concern for the conservation of aquatic ecosystems as they often displace native species, particularly in polluted habitats (Bourret et al., 2008). The fast loss of species and habitats in Mexico's central region, identified as a key ichthyic endemism center in the country (Miller, 2009), has raised concerns regarding the need to protect and conserve areas and species of ecological importance (Gutiérrez-Yurrita et al., 2013). In this regard, Mexico's National Strategy on Invasive Species is a comprehensive plan established by the Mexican government to address the management of invasive species. Its main objective is to prevent the introduction and spread of exotic species that may pose a threat to biodiversity and native ecosystems (CONABIO, 2010).

The species introduction phenomenon can trigger irreversible damages (Strayer, 2010). However, the continuous introduction of exotic species into artificial reservoirs is mainly attributed to socioeconomic factors (De la Lanza-Espino & García-Calderón, 2002). This circumstance presents a dilemma regarding the encouragement of the expansion of exotic species in these water bodies, posing a rising potential risk. In the Zimapán dam, 8 exotic species were recorded coexisting within the reservoir, raising interesting questions for future exploration about ecological interactions and the factors enabling their coexistence.

The introduction of largemouth bass as a measure to promote recreational fishing to diversify economic activities might be among the main causes of the decrease in tilapia population, thereby generating a socioecological conflict between both fishing practices. The presence of largemouth bass is identified by commercial fishermen as a key factor in the decline of catches. Furthermore, the coexistence of other exotic species suggests the possibility of competition for food resources and habitat, both with the native species (Pearlscale cichlid) and also tilapia (Córdova-Tapia et al., 2015). The presence of carnivorous species, such as largemouth bass and catfish, poses an additional challenge to the survival of tilapia fingerlings and juveniles.

The results reveal significant variability in fishing activity, characterized by peaks of participation followed by abrupt declines. These patterns highlight the susceptibility of fishing in Zimapán due to economic, ecological, environmental, and social factors. The interaction among species introduction, recreational fishing, and wastewater discharges could be generating complex and synergistic dynamics (Simberloff et al., 2013).



In 2021, precautionary measures after a flood caused by Hurricane Grace resulted in four weeks without records (from September 27th to October 24th) due to the severe flooding affecting Hidalgo state from the overflowing Tula River. In 2022, a few months after the floodings, *Lemna gibba*. In April of the same year, fishing was suspended due to the mandatory closed season. However, upon its conclusion in June, it was impossible to resume fishing activities as a great part of the reservoir was covered by *L. gibba*, making it impossible to set nets. Several months of that year were dedicated to removing it. Once the dam was cleared at the end of October, fishing activities resumed. November was the month with the highest presence of fishers, followed by a rapid abandonment. In 2023, the lowest number of active fishermen was recorded.

Interviews with the fishermen reveal a growing concern about the profitability of fishing. This concern is supported by the results of the analysis of catch per unit effort (CPUE), showing that, on average, fishermen obtain around 10 kg of tilapia/day and 5 kg of carp/day. The selling prices of tilapia is 40 Mexican pesos per kilogram (2.3 USD), while carp sells for one Mexican peso per kilogram (0.05 USD). Consequently, the average daily income of fishermen is 405 Mexican pesos (23.7 USD). However, this income is affected by associated expenses, such as transportation from their homes to the dam and the cost of gasoline for the boat used during checking of the nets (190 per day, 11.3 USD). Therefore, on average, a fisher earns 215 Mexican pesos (12.8 USD) per day of work. This situation generates uncertainty about the economic sustainability of fishing activities and poses a challenging outlook for the fishermen, raising legitimate concerns about the future of this activity at Zimapán Dam.

The restocking of tilapia emerges as a potential solution, according to the perception of some fishers, to increase daily catches and strengthen fishing activity at Zimapán dam. Even so, this approach faces significant challenges arising from the increased population of largemouth bass and the restrictions associated with their capture. The coexistence of these factors raises questions about the long-term viability of this strategy. It is imperative to reconsider fishing management strategies at Zimapán, considering not only tilapia stocking but also critically evaluating the continuous introduction of exotic species into bodies of water for socioeconomic purposes. This needs a relevant debate about the appropriateness of such practices given their long-term ecological impact (Simberloff et al., 2013).

The complex interaction between the introduction of several exotic species, the adaptation of local fishing to changing conditions, and the exploration of economic alternatives such as tourism, emphasizes the need to comprehensively understand the ecological and socio-economic dynamics of artificial reservoirs. This scenario underscores the importance of developing sustainable management strategies and biodiversity conservation. This could involve the implementation of sustainable land use practices, pollution reduction, population regulation of species like largemouth bass, effective fishing regulation, and the promotion of community participation in decision-making.

## Conclusions

Fishing at the Zimapán dam faces multifaceted challenges, from environmental issues to socioeconomic challenges. The water crisis, human impact, and a lack of sustainable management threaten the long-term viability of this activity, indicating complex problems occurring at different spatial and temporal scales. This study highlights the need for integrated approaches and collaborative solutions addressing both the ecological problems and the social dimensions of fishing in the ecosystem. Ecosystems like the Zimapán Dam will become increasingly important in a context of global change; therefore, understanding them as key ecosystems upon which we will depend in the near future is essential. We consider that such ecosystems will become more frequent, posing significant challenges for a field like limnology.

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## Authors' Contributions

RCB, MLH, and FCT conducted fieldwork. RCB and FCT performed data analysis. RCB, KERV, MLH, MAGP, and FCT wrote and reviewed the manuscript.

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# Conclusions

This book presents limnological research with various lines of progress, focused on continental environments in Mexico, showing certain trends in specific areas such as water quality, characterizing its ionic properties, contrasting between different aquifer systems, but above all, highlighting the environmental problems presented by aquatic resources; A relevant topic that stands out in recent research in Mexico is the study of plankton, which is not restricted only to its characterization or identification at a taxonomic level in the traditional way, but also the studies focus on highlighting its importance in the dynamics of ecosystems, energy transfer, and community structure, the tendency in these investigations is to carry out molecular and genetic studies with emphasis on ecological studies and species evolution, as well as adaptation to adverse scenarios such as Climate Change. On the other hand, the use of zooplankton species as indicators of environmental risk stands out, as well as a review of the studies focused on ecological interactions between zooplankton and macrophytes and one of the groups of great ecological importance and little studied in Mexico, the hydrozoans of continental waters.

Thus, this publication is an excellent reference for limnological research carried out in Mexico, not only for the topics and lines of research addressed, but also for the quality of the information presented and the effort made to achieve knowledge with solid arguments and scientific-academic support, which will undoubtedly support researchers and science students for current and future projects.

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This book takes a significant step in showcasing the relevance of limnology to our survival. Freshwater habitats, though they cover less than 1 % of the Earth's surface, are home to a substantial portion of the world's biodiversity—at least 10 % of all known species. Freshwater habitats and the biodiversity they support are under threat. Moreover, our survival depends on access to high-quality freshwater. This book not only highlights the beauty of limnology and the scientific methods used to study it, but it also draws attention to the major causes of biodiversity loss in freshwater ecosystems. It shows all readers what it means to deal with inland waters as a scientist interested in understanding ecosystems and protecting them.

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