Topics of limnological research in Mexico

Coordinator
Alfredo Pérez Morales

UNIVERSIDAD DE COLIMA

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En buen plan

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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.

Index

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.

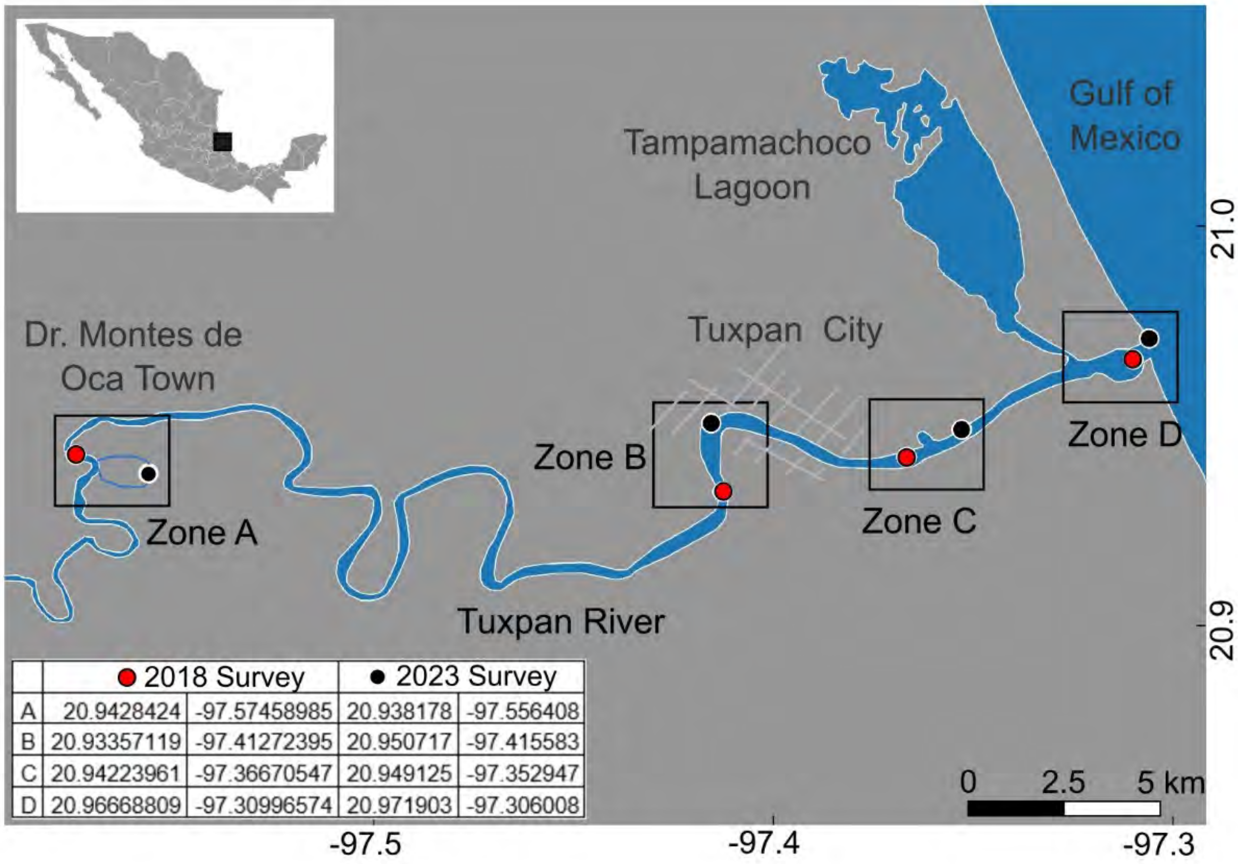
Material and Methods

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.



	🔴 2018 Survey		● 2023 Survey	
A	20.9428424	-97.57458985	20.938178	-97.556408
B	20.93357119	-97.41272395	20.950717	-97.415583
C	20.94223961	-97.36670547	20.949125	-97.352947
D	20.96668809	-97.30996574	20.971903	-97.306008

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 µm) and fixed with 4 % formalin final volume (Throndsen, 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 µm 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).

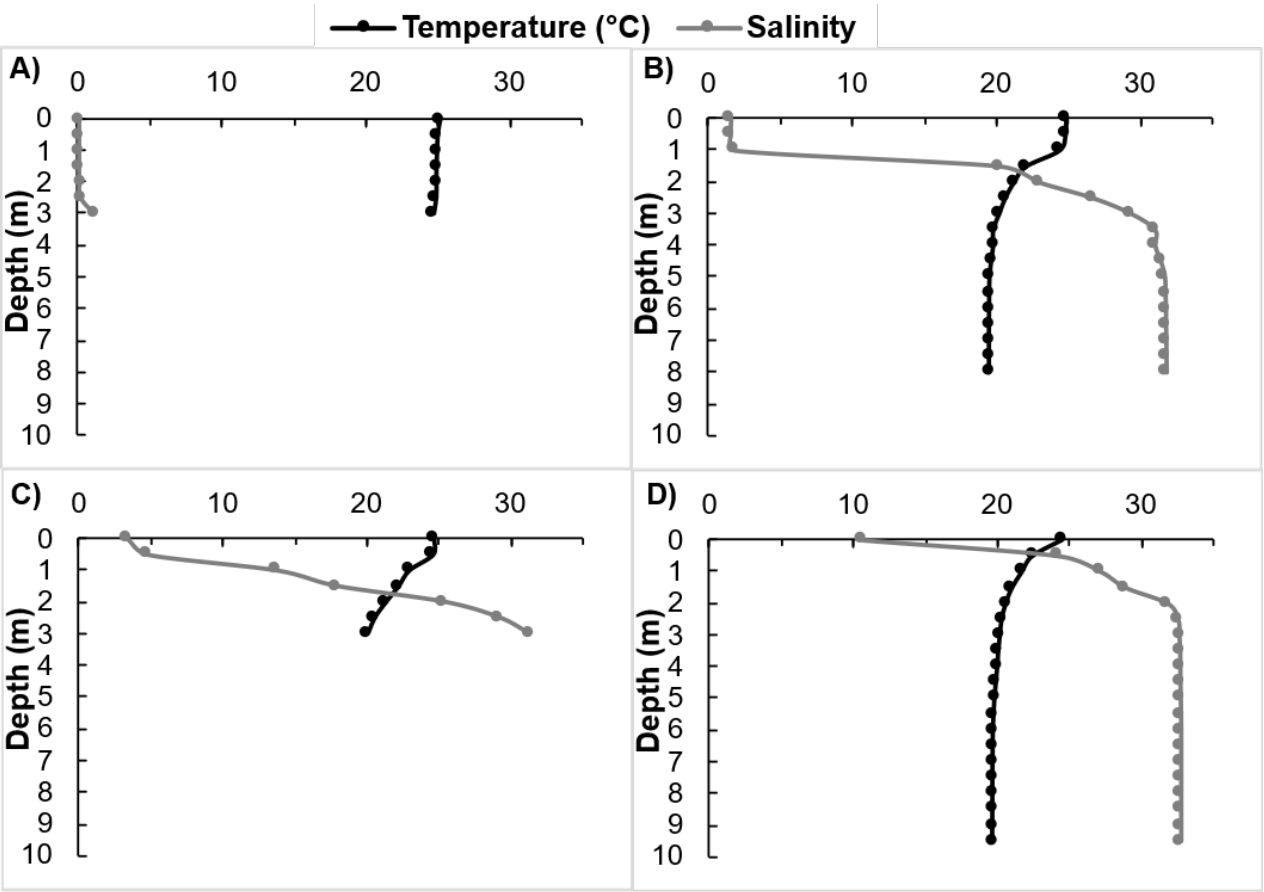


Figure 2. Vertical Profiles of Temperature and Salinity in the Four Zones Established in the Tuxpan River during the 2018 Survey: A) Zone A Far Away from the Mouth, B) Zone B Tuxpan City, C) No Urban Zone, D) Mouth of the River.

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 µm, while 2023 fluctuated between 153.1 and 660.9 µm (Fig. 3C) (Z=-2.30, p=0.02). Phosphate concentration in 2018 ranged from 0.7 to 3.5 µm and in 2023 from 36.8 to 1,068.8 µm, 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).

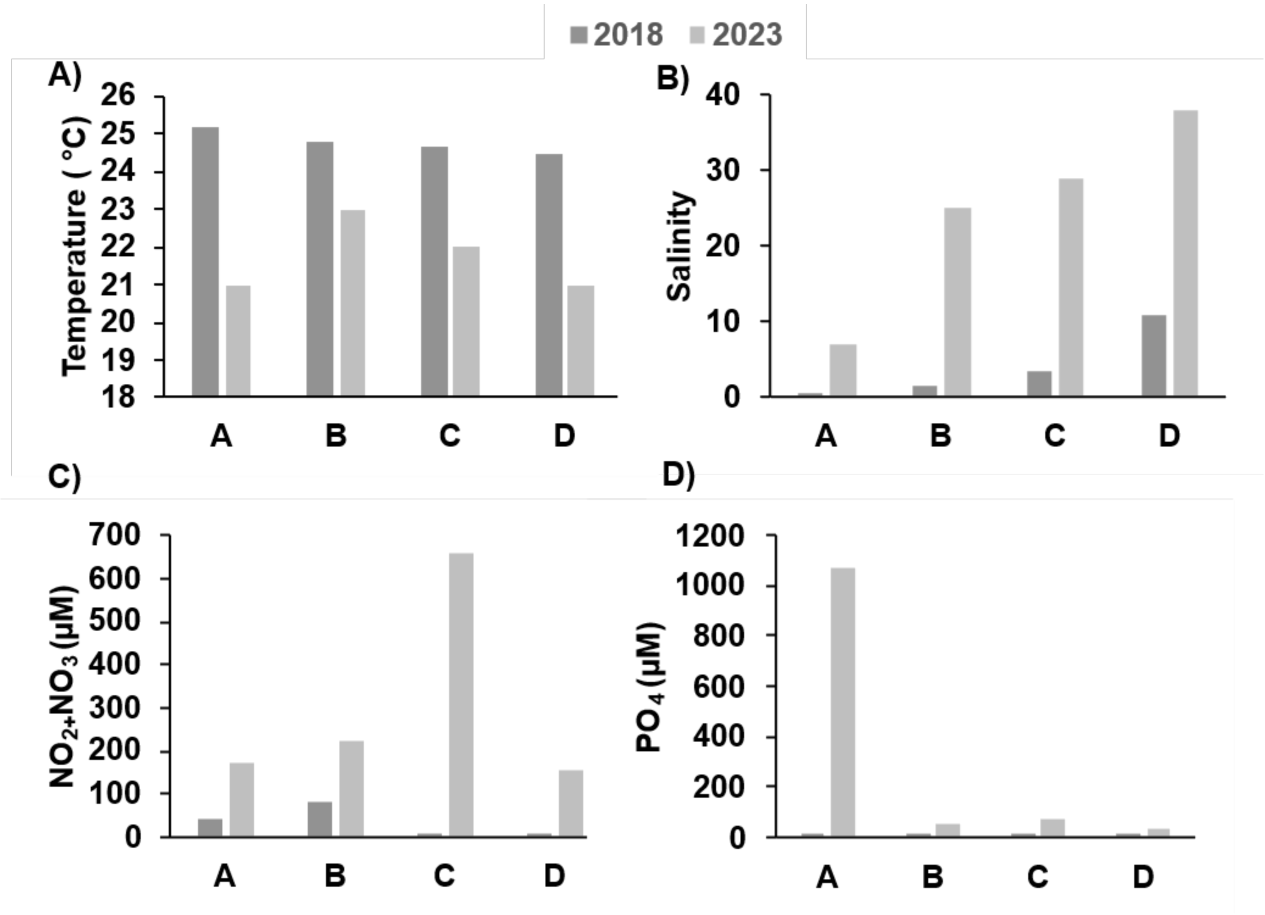


Figure 3. Surface Values of A) Temperature, B) Salinity and Concentration of C) Nitrites+Nitrates and D) Phosphates Recorded in the Four Zones Established in the Tuxpan River during the 2018 and 2023 Surveys.

Microalgae, Chl-a, and Relation with Hydrological Factors

In both surveys, 188 microalgae species were recorded (Table 1): 151 were diatoms (Stremenopile, the most prevalent clade in the study), 17 were chlorophytes (Chloroplastida), 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 (Chloroplastida, Eubacteria and Excavata), although in this zone, the Alveolata clade presented the lowest richness. The high species richness of the Stremenopile (17 to 23) was very similar in the sampling sites. In 2023, 129 species from only two clades (Alveolata and Stremenopile) 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
Clade Alveolata									
Dinophysis caudata		1	0	0	0	0	0	0	0
Gonyaulax sp. (cysts)		0	1	0	0	0	0	0	0
Gonyaulax spp.	Gon spp	0	1	1	1	0	1	0	0
Peridinium quadridentatum		0	0	1	0	0	0	0	0
Peridinium sp. 1		0	0	0	0	0	1	0	0
Prorocentrum micans	Pro mic	0	1	1	1	0	0	0	0
Protoperidinium cf. *thorianum*		0	1	0	0	0	0	0	0
Protoperidinium sp. 2		0	0	1	0	0	0	0	0
Protoperidinium spp.	Pro spp	1	1	1	1	0	1	0	1
Tripos furca	Tri fur	0	1	1	1	0	0	0	0
Tripos fusus	Tri fus	0	0	0	0	0	1	1	0
Tripos hircus	Tri hir	0	0	0	0	0	1	1	0
Tripos macroceros		0	0	0	1	0	0	0	0
Tripos muelleri		0	0	0	0	0	1	0	0
Clade Chloroplastida									
Closterium moniliferum		1	0	0	0	0	0	0	0
Coenococcus sp.		0	1	0	0	0	0	0	0
Cosmarium obtusatum	Cos obt	1	1	0	0	0	0	0	0
Cosmarium sp. 2		1	0	0	0	0	0	0	0
Desmodesmus communis	Des com	1	1	0	0	0	0	0	0
Desmodesmus subspicatus		0	1	0	0	0	0	0	0
Eudorina sp.	Eud sp	1	1	0	0	0	0	0	0
Pediastrum sp. 1		0	1	0	0	0	0	0	0
Pediastrum sp. 3		1	0	0	0	0	0	0	0
Pseudopediastrum boryanum	Pse bor	1	1	1	0	0	0	0	0
Scenedesmus ecornis		0	1	0	0	0	0	0	0
Scenedesmus sp. 1	Sce sp1	1	1	0	0	0	0	0	0
Scenedesmus sp. 4		1	0	0	0	0	0	0	0
Selenastrum sp. 1	Sel sp1	1	1	0	0	0	0	0	0
Sphaerocystis sp. 1		1	0	0	0	0	0	0	0
Stauridium tetras	Sta tet	0	1	1	0	0	0	0	0
Tetradesmus dimorphus		1	0	0	0	0	0	0	0
Clade Eubacteria									
Merismopedia elegans		1	0	0	0	0	0	0	0
Oscillatoria tenuis		1	0	0	0	0	0	0	0

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

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

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

Continued on following page.

Continued from previous page.

Stephanocyclus meneghinianus	Ste men	1	1	1	1	1	0	0	0
Surirella linearis	Sur lin	1	1	0	0	0	0	0	0
Surirella recedens		0	0	0	0	1	0	0	0
Surirella robusta	Sur rob	1	0	1	1	0	0	0	0
Surirella spp.		1	0	1	0	0	0	0	0
Surirella striatula	Sur str	1	1	1	1	0	1	0	1
Synedrosphenia crystallina		0	0	0	0	0	0	0	1
Thalassionema nitzschioides	Tha nit	0	0	0	1	0	1	1	1
Thalassiosira eccentrica	Tha ecc	0	0	0	0	1	0	1	0
Thalassiosira sp.	Tha sp	1	0	0	1	0	0	0	0
Thalassiosira sp. 1	Tha sp1	0	0	0	0	1	1	1	0
Thalassiosira sp. 2		0	0	0	0	0	0	0	1
Thalassiosira sp. 3		0	0	0	0	0	0	0	1
Triceratium sp. 1		0	0	0	0	0	0	1	0
Trieres mobiliensis	Tri mob	0	0	0	0	0	0	1	1
Tryblionella acuminata		0	0	0	0	1	0	0	0
Tryblionella apiculata		0	0	0	0	1	0	0	0
Tryblionella cf. *victoriae*		0	0	0	0	1	0	0	0
Tryblionella coarctata		0	0	1	0	0	0	0	0
Tryblionella granulata	Try gra	0	0	0	0	0	0	1	1
Ulnaria acus	Uln acu	1	1	1	1	1	1	0	1
Ulnaria goulardii		0	0	1	0	0	0	0	0
Ulnaria sinensis	Uln sin	1	1	1	1	0	0	0	0
Ulnaria sp. 1		0	0	0	0	1	0	0	0
Ulnaria ulna	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 (2.4-20.9 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 (4.7-5.2 mg m^{-3}) (Fig.

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

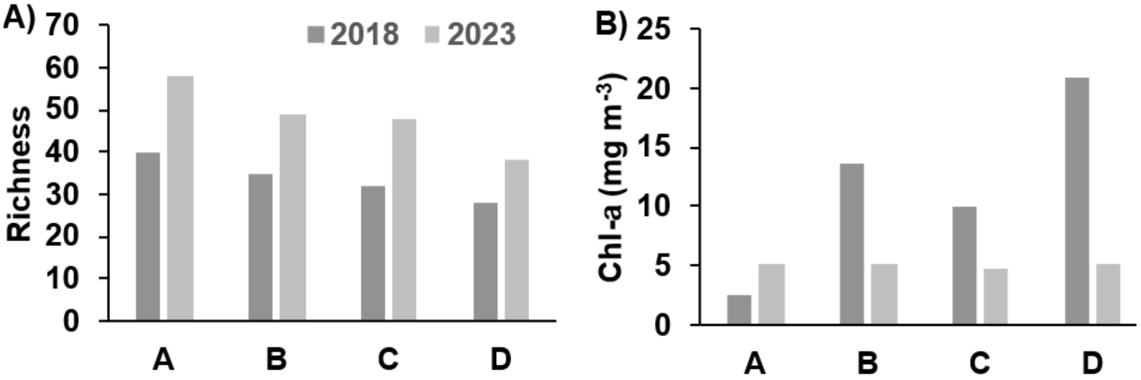

Figure 4. A) Microalgae Species Richness and B) Surface Chl-a Concentration Recorded in the Four Zones Established in the Tuxpan River during the 2018 and 2023 Surveys.

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, *Protoperidinium* spp., *Surirella striatula* Turpin, and *Ulnaria acus* (Kützing) 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* Kützing, *Navicula recens* (Lange-Bertalot) Lange-Bertalot, *Navicula rhynchotella* Lange-Bertalot, *Nitzschia* cf. *ovalis* H.J. Arnott, and *Nitzschia sigmoidea* (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 ($\rho=0.73$, $p<0.05$).

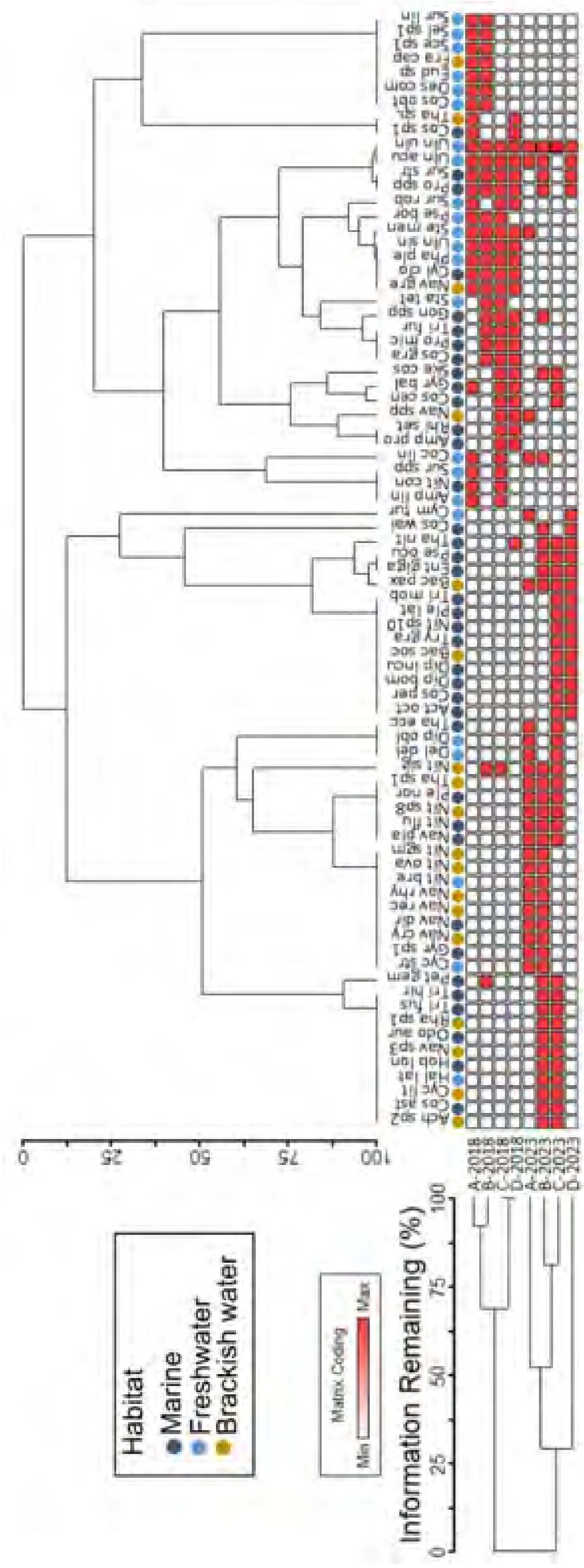

Figure 5. Two-way Cluster, Based on Jaccard Index.

]

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^{-3} Chl-*a*) are within the ranges (21-29°C, 1-38 ups, 0.8-19.3 mg m^{-3} 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^{-3}, 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_2+NO_3 >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_2+NO_3, 0.3-1.2 µm PO_4) and even for the Tampamachoco Lagoon (0-55 µm NO_2+NO_3, 0-89 µm PO_4). 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* (Kützing) 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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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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