Article

Phytoplankton Biodiversity in Two Tropical, High Mountain Lakes in Central Mexico

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Abstract: Lakes El Sol and La Luna, inside the crater of the Nevado de Toluca volcano, Central Mexico, are the only two perennial high mountain lakes [HML] in the country. HML are considered among the most comparable ecosystems worldwide. These lakes are “extreme” environments with diluted, oligotrophic, and cold waters exposed to high UV radiation doses. In this paper, we document the phytoplankton species inhabiting these two extreme tropical ecosystems. The phytoplankton diversity of Lakes El Sol and La Luna is low compared to the global phytoplankton diversity from other Mexican inland waters. Nonetheless, the phytoplankton diversity turned out to be high compared to other HML worldwide, both temperate and tropical. The phytoplankton community in Lake El Sol was formed by 92 morphospecies and in Lake La Luna by 63; in both, the most diverse groups were Bacillariophyceae and Chlorophyceae. All species found in Lake La Luna were present in Lake El Sol, but 29 species present in Lake El Sol were absent in Lake La Luna. Nevertheless, 57% of the most frequent and abundant taxa in Lake El Sol were rare in La Luna, and 47% of the most frequent and abundant taxa in Lake La Luna were rare in Lake El Sol. Associated with their shallowness and polymictic thermal pattern, 87.5% of the Bacillariophyceae morphospecies are tychoplanktonic, while the remaining 12.5% are truly planktonic. Dominant taxa were Chlorophyceae in Lake El Sol and Cyanobacteria in Lake La Luna. The most extreme conditions of Lake La Luna (ultraoligotrophy, very low pH, high ultraviolet radiation) most likely explained the differences in the dominant species composition and lower species richness compared to Lake El Sol. Herein, we provide for the first time a taxonomic list of the phytoplankton community of Lakes El Sol and La Luna. This information constitutes a baseline needed to use these HML as sentinels of global and climate change.

Keywords: taxonomic richness; density; biomass; alpine lakes; Nevado de Toluca; El Sol and La Luna

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1. Introduction

Phytoplankton biodiversity responds to multiple stressors. The size of the lakes usually associated with habitat diversity correlates positively with phytoplankton species richness [1–3]. In contrast, phytoplankton species richness decreases as the trophic status increases [4–6], with higher doses of UV radiation [7,8], acidification [9,10], and fish introduction [11,12].

High mountain lakes [HML] have recently attracted scientific attention because they are extremely sensitive ecosystems to global changes [13,14]. The harsh conditions prevailing in HML [e.g., diluted, oligotrophic and cold waters, high UV radiation doses] classify these water bodies as extreme aquatic environments [15–17].

Catalan and Donato Rondón [18] consider HML among the most comparable ecosystems in the world, despite the climatic differences related to latitude and the uneven volcanic influence in some areas. Unfortunately, most of the limnological research on HML

has been done mainly in temperate areas, and comparatively few studies have so far been undertaken in HML of tropical latitudes [19], such as Mexico.

Mexico is considered a megadiverse country, one of the few that possess, all together, 70% of the vertebrate and vascular plant diversity worldwide [20]. However, little is known about the diversity of Mexican freshwaters, phytoplankton included, and it is unknown whether these ecosystems present the same trend of higher tropical biodiversity (latitudinal diversity gradient) that terrestrial communities show [21].

El Sol and La Luna are the only HML in the expanse between Panama and southern USA, located at the northern edge of the Neotropical region. These lakes are about 600 m away from each other, inside the crater of the Nevado de Toluca volcano, and they have the same origin, climate, and water regime [22]. Despite their closeness and similar geologic context, they differ in physical and chemical characteristics [23] and in their biological communities known so far (e.g., zooplankton and macrobenthos) [24–27]; nonetheless, their phytoplankton communities remained scarcely and inaccurately described.

Löffler [28] made the first study on the limnology and plankton composition of these two lakes, which were followed by further taxonomic, ecological, and paleolimnological investigations by Caballero [29], Banderas-Tarabay [30], Alcocer et al. [23], and Cuna et al. [31]. However, these works did not provide comprehensive data on their phytoplankton biodiversity, and many questions about their ecology remained open. Herein, we provide for the first time a taxonomic list of the phytoplankton communities of Lakes El Sol and La Luna. Moreover, the present study comparatively analyzes the phytoplankton biodiversity of these two unique tropical HML and provides insights into the differences found. This information constitutes a necessary baseline for future studies, which could evaluate possible changes in phytoplankton species in these lakes.

Considering that species diversity of terrestrial ecosystems increases in the tropics, and since recent studies on temperate HML, e.g., [16], have shown that they hold a rich eukaryotic planktonic community, we hypothesized that tropical HML El Sol and La Luna would display higher phytoplanktonic diversity compared to their temperate equivalents. Additionally, considering that phytoplankton responds to stressors, such as lake size, trophic status, acidification, and ultraviolet radiation, the biodiversity will be lower in Lake La Luna compared to Lake El Sol because of its smaller size, ultraoligotrophic status, more acidic pH, and higher doses of ultraviolet radiation.

2. Materials and Methods

2.1. Study Area

El Sol and La Luna crater lakes (19°06' N, 99°45' W, 4200 m a.s.l.) are around 80 km W-SW from Mexico City in the Central Mexican Volcanic Belt (Figure 1). Climate is cold alpine with monthly mean temperatures ranging between 2.8 °C in February and 5.8 °C in April [32]. Total annual precipitation is 1244 mm, concentrated between June and September and ranging from 17 mm in December to 270 mm in July [32]. The maximum depth of lake El Sol is 15 m, with a surface area of approximately 230,000 m², whereas that of lake La Luna is 10 m, with a surface area of 30,500 m². Both lakes are warm polymictic; La Luna is continuous, while El Sol is discontinuous, displaying frequent mixing events throughout the year [33]. Oligotrophic Lake El Sol displays lower transparency, and higher electric conductivity, pH, phosphorus, chlorophyll-a, and seston concentrations than ultraoligotrophic Lake La Luna (Table 1). In contrast, La Luna has higher nitrogen, particularly nitrate, concentrations than El Sol [22,23].

Figure 1. Lakes El Sol and La Luna [lower panels], the Estado de Mexico and Mexico [upper panel].

Table 1. Environmental variables (average $\pm$ standard deviation, minimum, maximum) of Lakes El Sol and La Luna, Nevado de Toluca. (Temp = temperature, DO = dissolved oxygen, K_{25} = electrical conductivity, %SPAR = percentage of photosynthetic active radiation (PAR) at the bottom, $N-NO_2$ = nitrogen as nitrites, $N-NO_3$ = nitrogen as nitrates, $N-NH_4$ = nitrogen as ammonium, DIN = dissolved inorganic nitrogen, P-SRP = phosphorous as soluble reactive phosphorous, Si-SRSi = silica as soluble reactive silica, Chl-a = chlorophyll a). [* Statistically different ($\alpha < 0.05$)]. (Data from [22,23]).

Variable	Lake El Sol			Lake La Luna		
	X $\pm$ s.d.	Min	Max	X $\pm$ s.d.	Min	MAX
Temp ($^{\circ}C$)	9.2 $\pm$ 1.5	5.2	10.81	8.5 $\pm$ 1.7	5.7	10.9
DO ($mg\ L^{-1}$)	6.6 $\pm$ 0.6	5.76	7.74	6.8 $\pm$ 0.8	5.9	8.6
pH *	5.5 $\pm$ 0.3	4.9	6.1	4.7 $\pm$ 0.3	4.5	5.6
K_{25} ($\mu S\ cm^{-1}$) *	16 $\pm$ 1	15	18	14 $\pm$ 0.8	13	15
%SPAR *	10 $\pm$ 5	3	26	44 $\pm$ 12	17	53
$N-NO_2$ ($\mu g\ L^{-1}$) *	1.9 $\pm$ 0.4	0.56	4.3	0.5 $\pm$ 0.2	0.3	1.0
$N-NO_3$ ($\mu g\ L^{-1}$) *	66.0 $\pm$ 44.3	1.26	138	316.7 $\pm$ 28.0	278.7	362.6
$N-NH_4$ ($\mu g\ L^{-1}$)	38.1 $\pm$ 21.4	15.0	77.1	39.0 $\pm$ 20.7	15.0	77.1
DIN ($\mu g\ L^{-1}$) *	106.7 $\pm$ 57.7	16.9	185	356.4 $\pm$ 23.6	299.3	368.7
P-SRP ($\mu g\ L^{-1}$)	5.8 $\pm$ 3.6	1.1	11.1	4.0 $\pm$ 4.5	0.4	17.2
Si-SRSi ($\mu g\ L^{-1}$) *	68.9 $\pm$ 56.6	23.5	205.6	17.3 $\pm$ 28.4	3.3	108.4
DIN:P-SRP *	37 $\pm$ 44	2	143	244 $\pm$ 256	21	832
Chl-a ($\mu g\ L^{-1}$)	1.7 $\pm$ 1.6	0.2	5.1	0.6 $\pm$ 0.35	0.29	1.1

2.2. Field Sampling

Water samples for phytoplankton analyses were collected monthly over a full year (March 2000 to March 2001). Analyses were completed at the time, but the preparation of the material for publication was not possible until now. Hence, these valuable historical data represent an important baseline for further studies. Samples were retrieved from the central area of both lakes (average depth: El Sol 12.7 ± 0.4 m, La Luna 10.2 ± 0.4 m) at three water depths: surface (1 m below surface), mid water column, and bottom (1 m above sediment) using a Niskin or Van Dorn bottle. Samples for identification were kept refrigerated, and samples for counting were fixed with 1% Lugol's acetic iodine solution.

2.3. Laboratory Analysis

The identifications were made by first examining *in vivo* samples and further on material preserved at low temperature. These samples were analyzed using a Carl Zeiss phase-contrast microscope at $400\text{--}1000\times$. Bacillariophyceae were cleaned with hydrogen peroxide following standard procedures [34]. Selected diatom samples were prepared and observed with a scanning electron microscope (JEOL JSM-5300) to confirm identification. Identification was based on the following literature: Comas [35], Desikachary [36], Dillar [37–40], Ettl & Gärtner [41], Huber-Pestalozzi [42–44], Komárek and Anagnostidis [45], Komárek and Fott [46], Krammer and Langer-Bertalot [47–50], Philipose [51], Popovský and Pfiester [52], and Prescott et al. [53–55]. Phytoplankton taxonomy was according to AlgaeBase [56].

Cell counts were done by the Utermöhl method [57] with a Carl Zeiss D inverted microscope using 50- and 100-mL sedimentation chambers. The three water depths turned out to be similar and treated as replicates. The density values (cells mL^{-1}) were multiplied by the biovolume of each species using geometric formulas related to the shape of the cell [58]. A minimum of 15 measurements were made for each species, from which the average biovolume value was obtained. Biovolume values are reported as $\text{mm}^3 \text{ m}^{-3}$.

Identified taxa were appointed either as phytoplankton or tychoplankton. Tychoplankton is a common term applied to non-planktonic organisms, such as free-living or attached benthic organisms, that are carried into the plankton through a disturbance of their benthic habitat, or by winds and currents [59,60] (Diatoms of North America Glossary at <https://diatoms.org/glossary/tycoplanktonic>; accessed on 6 January 2022).

The phytoplankton species richness was determined as the total number of species recorded during the counts. The Shannon–Wiener diversity index was calculated using the Primer Program 6.1.6.3.

2.4. Statistical Analysis

Multiple variance analysis (MANOVA) was performed using STATISTICA V12.4 64 Bits, to determine if there were significant differences between both lakes. ANOVA was performed to identify significant differences in environmental (listed in Table 1) and biological variables (species richness, diversity, density, and biomass) between both lakes. The data were transformed to a base 10 logarithm to eliminate bias or external variability, except for pH. The confidence level was $\alpha < 0.05$.

Bivariate diagrams of abundance [$\log n + 1$] versus frequency of occurrence, also known as the Olmstead–Tukey diagram [61], were used. The diagram classifies the taxa into four categories: representative/dominant (high abundance and frequency), abundant/temporal (high abundance, low frequency), constant/frequent (low abundance, high frequency), and rare (low abundance, low frequency). The discrimination criterion is based on the mean of the relative frequency of appearance for the “X” axis and the respective mean of the natural logarithm of the sum of the relative abundance for the “Y” axis.

3. Results

As mentioned before, although both lakes are only 600 m apart and they share a common origin, climate, and water regime, Lakes El Sol and La Luna display differences in their physical and chemical characteristics. Lake El Sol is larger and deeper than Lake

La Luna; it has benthic vegetation (*Chara*) covering wide expanses of the littoral zone, and it currently holds a fish (rainbow trout) population. In addition, there are significant differences ($\alpha < 0.05$) between both lakes in terms of water transparency, nitrogen and silica concentrations, pH, and electrical conductivity (Table 1).

The phytoplankton species richness found in Lakes El Sol and La Luna included 92 taxa of which 47 were identified to species [51%] and 45 [49%] were identified as morphospecies [Table 2].

Table 2. Phytoplankton taxonomic list of HML El Sol and La Luna, Nevado de Toluca. According to AlgaeBase [42]. (X indicates the species presence. * Indicates tycho planktonic morphospecies). (Navicula NTA and N. NTB according to [15]).

Taxa	El Sol	La Luna
Phylum: Cyanobacteria		
Class: Cyanophyceae		
Order: Chroococcales		
<i>Limnococcus limneticus</i> [Lemmerman] Komárková, Jezbarová, Komárek & Zapomelová	X	X
Order: Nostocales		
<i>Anabaena</i> cf. <i>lapponica</i> Borge	X	
<i>Anabaena</i> sp.	X	
Order: Oscillatoriales		
<i>Limnoraphis birgei</i> [Smith] Komárek, Zapomelová, Smarda, Kopecký. Rejmánková, Woodhouse, Neilan & Komárková	X	
<i>Oscillatoria</i> sp.	X	X
Order: Synechococcales		
<i>Merismopedia</i> sp.	X	
<i>Synechocystis minuscula</i> Woronichin	X	
<i>Synechocystis</i> sp.	X	X
<i>Pseudoanabaena</i> sp.	X	
Phylum: Ochrophyta		
Class: Chrysophyceae		
Order: Chromulinales		
<i>Chromulina</i> sp.	X	X
<i>Chrysococcus minutus</i> [Fritsch] Nygaard	X	
<i>Dinobryon</i> cf. <i>soziale</i> [Ehrenberg] Ehrenberg	X	
<i>Ochromonas</i> sp.	X	X
Class: Synurophyceae		
Order: Synurales		
<i>Mallomonas</i> cf. <i>acaroides</i> Perty	X	
<i>Mallomonas</i> sp. 1	X	X
<i>Mallomonas</i> sp. 2	X	
Phylum: Bacillariophyta		
Class: Coscinodiscophyceae		
Order: Aulacoseirales		
<i>Aulacoseira nivaloides</i> [Cambrun] English & Potatova	X	X
<i>Aulacoseira</i> cf. <i>alpigena</i> [Grunow] Krammer	X	X
Class: Mediophyceae		
Order: Stephanodiscales		
<i>Cyclotella</i> aff. <i>quillensis</i> Bailey	X	X
<i>Cyclotella</i> sp.	X	X
Class: Bacillariophyceae		
Order: Bacillariales		
<i>Nitzschia</i> cf. <i>acidoclinata</i> Lange-Bertalot *	X	X
<i>Nitzschia</i> sp. *	X	X
Order: Cocconeidales		
<i>Achnantheidium minutissimum</i> [Kützinger] Czarnecki *	X	
<i>Psammothidium helveticum</i> [Hustedt] Bukhtiyarova & Round *	X	X
<i>Psammothidium levanderi</i> [Hustedt] Bukhtiyarova & Round *	X	X

Table 2. Cont.

Taxa	El Sol	La Luna
Order: Cymbellales		
<i>Encyonema gracile</i> Rabenhorst *	X	
<i>Encyonema perpusillum</i> [Cleve-Euler] Mann *	X	X
<i>Gomphonema angustatum</i> [Kützing] Rabenhorst *	X	X
Order: Fragilariales		
<i>Staurosira</i> aff. <i>venter</i> [Ehrenberg] Cleve & Möller *	X	X
<i>Staurosira pseudoconstruens</i> [Marciniak] Lange-Bertalot *	X	X
<i>Staurosirella pinnata</i> [Ehrenberg] Williams & Round *	X	X
Order: Naviculales		
<i>Brachysira</i> sp. *	X	X
<i>Cavinula pseudocutiformis</i> [Hustedt] Man & Stickle *	X	X
<i>Frustulia rhomboides</i> [Ehrenberg] De Toni *	X	X
<i>Navicula</i> NTA *	X	X
<i>Navicula</i> NTB *	X	
<i>Pinnularia microstauron</i> [Ehrenberg] Cleve *	X	X
<i>Pinnularia</i> cf. <i>viridis</i> [Nitzsch] Ehrenberg *	X	X
<i>Pinnularia subcapitata</i> W. Gregory *	X	X
<i>Pinnularia</i> sp. 1 *	X	
<i>Pinnularia</i> sp. 2 *	X	X
<i>Sellaphora</i> sp. *	X	
<i>Stauroneis anceps</i> Ehrenberg *	X	X
Order: Surirellales		
<i>Stenopterobia</i> sp. 1 *	X	X
<i>Stenopterobia</i> sp. 2 *	X	X
<i>Surirella</i> cf. <i>linearis</i> Smith *	X	X
<i>Surirella</i> cf. <i>angusta</i> Kützing *	X	X
<i>Surirella</i> sp. *	X	X
Phylum: Haptophyta		
Class: Coccolithophyceae		
Order: Prymnesiales		
<i>Chrysochromulina</i> aff. <i>parva</i> Lackey	X	X
Phylum: Cryptophyta		
Class: Cryptophyceae		
Order: Cryptomonadales		
<i>Chroomonas nordstedt</i> Hansgirg	X	
<i>Chroomonas</i> sp.	X	X
<i>Cryptomonas</i> sp. 1	X	X
<i>Cryptomonas</i> sp. 2	X	X
Phylum: Miozoa		
Class: Dinophyceae		
Order: Gymnodiniales		
<i>Gymnodinium</i> sp.	X	X
Order: Peridinales		
<i>Parvodinium umbonatum</i> [Stein] Carty	X	X
<i>Peridinium</i> cf. <i>volzii</i> Lemmermann	X	
<i>Peridinium williei</i> Huitfeldt-Kaas	X	X
Phylum: Euglenozoa		
Class: Euglenophyceae		
Order: Euglenales		
<i>Euglena</i> sp.	X	
<i>Lepocinclis</i> sp.	X	
<i>Trachelomonas</i> sp. 1	X	
<i>Trachelomonas</i> sp. 2	X	
Phylum: Chlorophyta		
Class: Chlorophyceae		
Order: Chlamydomonadales		
<i>Chlamydomonas</i> sp. 1	X	X
<i>Chlamydomonas</i> sp. 2	X	X
<i>Carteria</i> sp.	X	X

Table 2. Cont.

Taxa	El Sol	La Luna
<i>Gemmellicystis planctonica</i> [Woronichin] Lund	X	X
<i>Microglena</i> sp.	X	
<i>Palmella</i> sp.	X	X
<i>Sphaerocystis schroeteri</i> Chodat	X	X
Order: Chlorellales		
<i>Oocystis lacustris</i> Chodat	X	X
<i>Oocystis</i> sp.	X	X
Order: Oedogoniales		
<i>Oedogonium</i> sp.	X	X
Order: Sphaeropleales		
<i>Ankistrodesmus</i> sp.	X	
<i>Coelastrum</i> sp.	X	
<i>Desmodesmus abundans</i> [Kirchner] Hegewald	X	X
<i>Desmodesmus spinosus</i> [Chodat] Hegewa	X	
<i>Gloeocystis</i> sp.	X	
<i>Monoraphidium minutum</i> [Nägeli] Komárková-Legnerová	X	
<i>Monoraphidium obtusum</i> [Korshikov] Komárková-Legnerová	X	X
<i>Tetradismus obliquus</i> [Turpin] Wynne	X	
Order: Trebouxiales		
<i>Botryococcus braunii</i> Kützinger	X	X
Phylum: Charophyta		
Class: Charophyceae		
Order: Charales		
<i>Nitella</i> sp.	X	
Class: Coleochaetophyceae		
Order: Chaetosphaeridiales		
<i>Chaetosphaeridium globosum</i> [Nordstedt] Klebahn	X	X
Class: Conjugatophyceae		
Order: Desmidiaceae		
<i>Cosmarium</i> cf. <i>exiguum</i> Archer	X	X
<i>Closterium lunula</i> Ehrenberg & Hemprich ex Ralfs	X	X
<i>Closterium</i> sp.	X	X
<i>Desmidium</i> sp.	X	X
<i>Euastrum</i> cf. <i>oblongum</i> Ralfs	X	X
<i>Micrasterias radiosa</i> Ralfs	X	X
<i>Staurastrum</i> sp.	X	X
Order: Zygnematales		
<i>Temnogametum iztacalense</i> Garduño & Martínez	X	X
<i>Spirogyra</i> sp.	X	
<i>Zygnema</i> sp.	X	X
Total	92	63

All 92 taxa were present in Lake El Sol, but only 63 ($\approx 68\%$) were in Lake La Luna (Table 2). The most diverse algal groups were Bacillariophyceae (El Sol 34.8%, La Luna 42.9%) and Chlorophyceae (El Sol 20.6%, La Luna 19%), accounting for > 50% of the species richness in both lakes. The species richness (S) in Lake El Sol averaged 32 ± 6.7 (15–41), while in Lake Luna, it averaged 19 ± 4.2 (10–25). S in Lake El Sol was significantly higher ($\alpha < 0.05$) than in Lake La Luna. The average value of the Shannon–Wiener index in Lake El Sol averaged 2.8 ± 0.6 (1.6–3.7), while in Lake La Luna, it averaged 2.4 ± 0.5 (1.1–2.9).

According to the Olmstead–Tukey diagrams, there were 21 (out of 92, $\approx 23\%$) species with high abundance and frequency in Lake El Sol and 17 species (out of 63, $\approx 27\%$) in Lake La Luna (Figure 2, Table 3). Both lakes shared 9 of these species (31%), while 12 were of high abundance and frequency [57.1%] exclusively in Lake El Sol, and 8 [47.1%] exclusively in Lake La Luna.

Figure 2. Bivariate [Olmstead–Tukey] diagrams of abundance ($\log n + 1$) versus relative frequency (%) of the phytoplankton of Lakes (A) El Sol and (B) La Luna. (See species keys in Table 3).

In the Olmstead–Tukey diagrams of both lakes, tytoplanktonic and truly planktonic forms were included among the representative (large abundance and high frequency of occurrence) taxa. The tytoplanktonic forms were Bacillariophyceae (*Cavinula pseudocutiformis*, *Pinnularia microstauron*, *Encyonema gracile*, *Frustulia rhomboides*, *Encyonema perpusillum*, *Psamotidium helveticum*), Coleochaetophyceae (*Chaetosphaeridium globosum*), and Chrysophyceae (*Dinobryon* cf. *sociale*). The truly planktonic forms were Chlorophyceae with pods (e.g., *Oocystis lacustris*, *Sphaerocystis schroeteri*, *Gemmellicystis planctonica*, *Gloeocystis* sp., *Monoraphidium obtusum*), which gives buoyancy and grazing protection, and the podless *Synechocystis* sp. in Lake La Luna. Representative species of Dinophyceae, Chrysophyceae, Synurophyceae, Chlorophyceae, Cryptophyceae, and Euglenophyceae have flagellum (e.g., *Chrysochromulina* aff. *parva*, *Parvodinium umbonatum*, *Peridinium willei*, *Gimnodinium* sp., *Trachelomonas* sp. 1, *Carteria* sp., *Chlamydomonas* sp. 1), which can move freely to seek food and protection.

Phytoplankton density in Lake El Sol averaged $17,383 \pm 16,650$ cells mL^{-1} (2556 – $149,455$ cells mL^{-1}), while in Lake La Luna the phytoplankton density averaged 9361 ± 5355 cells mL^{-1} (188 – $25,499$ cells mL^{-1}). The density in Lake El Sol was significantly higher ($\alpha < 0.05$) than in Lake La Luna. According to their contribution to the global abundance, the chlorophyte *Oocystis lacustris* (58.2%) was the dominant species in Lake El Sol, followed by *Sphaerocystis schroeteri*, *Monoraphidium obtusum*, *Chrysochromulina* aff. *parva*, and *Gemmellicystis planctonica*. The other 87 species contributed less than 1%. In Lake

La Luna, the dominant species was the Cyanobacteria *Synechocystis* sp. (89%), followed by *Chrysochromulina* aff. *parva*, and *Chlamydomonas* sp. 1. The other 60 species contributed less than 1% (Table 4). The phytoplankton density data are provided in Supplementary Information Table S1.

Table 3. Representative species (large abundance and high frequency of occurrence) in Lakes El Sol and La Luna.

Lake El Sol	Lake La Luna
<i>Chaetosphaeridium globosum</i> [ChG]	
<i>Chryptomonas</i> sp. 1 [Crsp1]	
<i>Chrysochromulina</i> aff. <i>parva</i> [ChP]	
<i>Gemelliscystis planctonica</i> [GmP]	
<i>Limnococcus limneticus</i> [LiL]	
<i>Ochromonas</i> sp [Ocsp]	
<i>Oocystis lacustris</i> [OcL]	
<i>Parvodinium umbonatum</i> [PaU]	
<i>Sphaerocystis Schroeteri</i> [SpS]	
<i>Chryptomonas</i> sp. 2 [Crsp2]	<i>Chlamydomonas</i> sp. 1 [Chsp1]
<i>Cavinula pseudocutiformis</i> [CvP]	<i>Chroomonas</i> sp. [CrosP]
<i>Dynobryon</i> cf. <i>socialis</i> [DyS]	<i>Encyonema perpusillum</i> [EnP]
<i>Encyonema gracile</i> [EnG]	<i>Gymnodinium</i> sp. [Gysp]
<i>Frustulia rhomboides</i> [FrR]	<i>Mallomonas</i> sp. 1 [Masp1]
<i>Gloeocystis</i> sp. [Glsp]	<i>Peridinium willei</i> [PeW]
<i>Monoraphidium obtusum</i> [MoO]	<i>Psammothidium helveticum</i> [PaH]
<i>Palmella</i> sp. [Plsp]	<i>Synechocystis</i> sp. < 2 µm [Sycsp]
<i>Carteria</i> sp. [Ctsp]	
<i>Pinnularia microstauron</i> [PnM]	
<i>Temnogametum iztacalense</i> [TeI]	
<i>Trachelomonas</i> sp. 1 [Trsp1]	

Table 4. Dominant species in abundance (>1%) and biomass (>10%) in Lakes El Sol and La Luna.

Lake El Sol		La Luna	
Abundance	%	Abundance	%
<i>Oocystis lacustris</i>	58.2	<i>Synechocystis</i> sp.	89.1
<i>Sphaerocystis Schroeteri</i>	17.9	<i>Chrysochromulina</i> aff. <i>parva</i>	5.8
<i>Monoraphidium obtusum</i>	8.7	<i>Chlamydomonas</i> sp. 1	3.3
<i>Chrysochromulina</i> aff. <i>parva</i>	7.5		
<i>Gemelliscystis planctonica</i>	5.9		
Biomass	%	Biomass	%
<i>Monoraphidium obtusum</i>	19.0	<i>Gymnodinium</i> sp.	17.5
<i>Sphaerocystis Schroeteri</i>	14.5	<i>Parvodinium umbonatum</i>	16.2
<i>Oocystis lacustris</i>	10.6	<i>Synechocystis</i> sp.	14.8
		<i>Encyonema perpusillum</i>	14.7
		<i>Peridinium willei</i>	14.6

Phytoplankton biomass (biovolume) in Lake El Sol averaged $1,074,505 \pm 688,770 \text{ mm}^3 \text{ m}^{-3}$ ($174,952\text{--}2,814,852 \text{ mm}^3 \text{ m}^{-3}$), while in Lake La Luna, the phytoplankton biomass averaged $155,607 \pm 144,026 \text{ mm}^3 \text{ m}^{-3}$ ($6640\text{--}630,982 \text{ mm}^3 \text{ m}^{-3}$). Biomass in Lake El Sol was significantly higher ($\alpha < 0.05$) than in Lake La Luna. Regarding the global biomass, the Chlorophytes *Monoraphidium obtusum*, *Sphaerocystis Schroeteri*, and *Oocystis lacustris* had a higher contribution in Lake El Sol. The other 89 species contributed less than 10%. In Lake La Luna the higher biomass contribution was by the dinoflagellates *Gymnodinium* sp., *Parvodinium umbonatum*, and *Peridinium willei*, followed by the Cyanobacteria *Synechocystis* sp., and the tytoplanktonic diatom *Encyonema perpusillum*. The other 58 species contributed less than 10% (Table 4). The phytoplankton biomass (biovolume) data are provided in Supplementary Information Table S1.

4. Discussion

Phytoplankton diversity from Mexican inland waters is estimated to be close to 1025 to 1100 species [62,63]. Taking this value as a reference, the total phytoplankton diversity found in the Nevado de Toluca HML lakes represents around 10% of the global phytoplankton richness reported for Mexican inland waters. Nonetheless, it must be noted that there is evidence of hidden phytoplankton diversity in these lakes, as indicated by the presence of a diverse Chrysophyte stomatocyst assemblage in the lake sediments [64], which is not reflected in the Chrysophyte [Synurales] diversity in this study, where only 3 morphotypes were identified. Relatively high values of the Shannon–Wiener diversity index, such as those recorded in Lakes El Sol and La Luna, are characteristic of unstable/astatic lakes; lower diversities are expected in stable lakes where the species are adapted and favor competitive exclusion, leading to the dominance of a few species [65].

The 45 morphospecies were morphologically clearly identified as different species; however, it was not possible to find a correct match for them in the available literature. This was the case, for example, for two diatom species previously reported for these lakes by Caballero [29] as *Navicula* NTA and *Navicula* NTB, which are new and possibly endemic taxa. Therefore, in this study, it is also very likely that at least some of the morphospecies are new to science, yet to be described, as exemplified with *Temnogametum iztacalense* Garduño & Martínez, a new species of Zygnemataceae recently described [66], and previously reported as *Mougeotia*. A comparable situation is repeated in other biological groups studied in this lake, as zooplankton investigations on Lakes El Sol and Luna revealed the presence of numerous new and very likely endemic species, e.g., [24,25,67,68]; so, it is not surprising to find new phytoplankton species in this study. Finally, it must be remembered that, in tropical inland waters, phytoplankton species composition is by far less known than their temperate counterparts, and therefore they are sub-represented in taxonomic keys.

However, for a better understanding of phytoplankton diversity in HML, it is important to consider that several tychoplanktonic taxa can be included in phytoplankton samples given that most HML are shallow and wind turbulence can mix the whole water column. In this regard, other variables have been similar, and a larger contribution of tychoplankton to tropical HML should be expected, since wind driven turbulence is present along the annual cycle given that tropical HML have no winter ice cover, which is a common feature in temperate HML [69] that limits resuspension for several months. This was clearly the case among the Bacillariophyceae in the Nevado de Toluca lakes, as tychoplanktonic taxa were the majority (28 taxa, 87.5%), with only 4 truly planktonic taxa (12.5%).

On the other hand, the phytoplankton species richness reported for HML outside Mexico was lower than in the Nevado de Toluca lakes, particularly those from the temperate region (Table 5). The highest species richness reported is 50 species found in 16 HML from the Adamello-Brenta Regional Park (46° N, 1936–2663 m a.s.l.), Italy [9]. However, this number must certainly correspond to a lower species richness per site, considering that it corresponds to an analysis of 16 lakes, while in the Nevado de Toluca the 92 taxa were present in a single lake (El Sol). It must be noted that the phytoplankton species lists reported from other HML worldwide also include tychoplankton, so they are comparable with Lakes El Sol and La Luna.

Table 5. Phytoplankton taxonomic richness (S), and percent (%) contribution per phytoplankton group in tropical (*) and temperate HML. (a: diatoms not included, b: includes epilithic diatoms). (Number of water bodies included in the study are below name). (Mex1: Mexico [63]; Mex2: Mexico [70]; Sol: Lake El Sol; Luna: Lake La Luna; ZL: Zempoala lakes, México [71]; SP: Lake San Pablo, Ecuador [19]; CR: Colombian reservoirs [72]; KL: Lake Kolsay, Kazakhstan [73]; LI: Laghetto Inferiore, Switzerland [74]; AL: Adamello-Brenta lakes, Italy [9]; TML: Tatra Mountains lakes, Slovakia [75]).

Phytoplankton Phylum (Class)	Mex1	Mex2	Sol *	Luna *	ZL **	SP *	CR *	KL	LI	AL ^b	TML
	55	56	1	1	5	1	4	4	1	16	3
Cyanobacteria (Cyanophyceae)	187	65	9	3	12	1	6	3	2	1	1
Ochrophyta (Chrysophyceae and Synurophyceae)	37	10	7	3	2	0	2	0	4	16	11
Bacillariophyta (Coscinodiscophyceae, Mediophyceae, and Bacillariophyceae)	327	262	32	27	-	5	15	15	4	32	6
Haptophyta (Coccolithophyceae)	0	0	1	1	0	0	0	0	0	0	0
Cryptophyta (Cryptophyceae)	8	2	4	3	1	3	1	0	5	7	2
Miozoa (Dinophyceae)	1%	(<1%)	4%	5%	2%	11%	1%	0%	14%	9%	5%
Euglenozoa (Euglenophyceae)	28	6	4	3	2	2	3	0	5	7	6
Chlorophyta (Chlorophyceae)	95	13	4	0	7	2	9	2	0	0	1
Charophyta (Charophyceae, Coleochaetophyceae, and Conjugatophyceae)	9%	2%	4%	0%	13%	7%	9%	7%	0%	0%	2%
	233	307	19	12	21	13	37	7	8	6	10
	23%	46%	21%	19%	38%	48%	38%	25%	23%	7%	25%
	108	-	12	11	8	1	23	1	6	10	3
	10%	-	13%	17%	15%	4%	24%	4%	17%	12%	7%
Total (Richness per lake)	1025 (>200)	668	92	63	55 (1–40)	27	96 (6–49)	28 (6–15)	35	79	40

Increasing species diversity from the poles towards the tropics or the LDG (latitudinal diversity gradient) has been long postulated in terrestrial and marine ecosystems (e.g., [76]). However, the mechanisms behind this trend are still far from being understood, representing one of the significant contemporary biogeography challenges (e.g., [77]). The reduced climatic variation in areas close to the Equator favors tropical species to develop small thermal tolerance ranges and a restricted dispersion. These features, especially in mountainous environments (e.g., HML), generate rapid isolation between populations and develop new species [78]. Nevertheless, there is no evidence directly connecting ecological process gradients within communities at a local scale to the geographic gradient in biodiversity [79].

Regarding composition, Bacillariophyceae is the most diversified phytoplankton group in Mexico [80]; Oliva-Martínez et al. [63] report that Bacillariophyceae constitutes 31.9% of the total phytoplankton taxonomic richness, while Garduño Solórzano et al. [70] report they represent 40.6%, second only to Chlorophyta (46.3%); in accordance, it was also the most diverse group in the Nevado de Toluca lakes (34.8% in Lake El Sol and 42.9% in Lake La Luna). Cyanophyceae ranked second in Oliva-Martínez et al. [63], and third in Garduño Solórzano et al. [70] (18.2% and 9.8%, respectively); however, they graded fourth in both Lake El Sol (9.8%) and Lake La Luna (4.8%), after Bacillariophyceae, Chlorophyceae, and Conjugatophyceae (Zygnematophyceae). According to Rivera et al. [81], a rich Desmidiaceae (Conjugatophyceae) assemblage is a feature distinctive of many tropical HML; but in Lakes El Sol and La Luna, this is not the case, as there were only seven taxa ($\approx 8\%$ of the total community), all of which were present in both lakes.

Löffler [15], 1972 provides the first report of the phytoplankton composition of the Nevado de Toluca lakes with *Peridinium willei*, *P. volzii*, *Botryococcus braunii*, and *Dinobryon*. We found all these taxa in El Sol, but only *P. willei* and *B. braunii* in La Luna. The motility of Dinophyceae, in this case *P. willei*, along with a facultative mixotrophic behavior (feeding on, for example, *Synechocystis* sp.), allowed this species to be successful in the ultraoligotrophic waters of Lake La Luna [82].

In research on trophic relationships in HML, the importance of mixotrophy has been emphasized, a characteristic of nanoplankton flagellates ($<20\ \mu\text{m}$) (Dinophyceae, Chryso-

phyceae, Cryptophyceae, Chlorophyceae flagellates). These taxa represent the dominant fraction (>50%) of the phytoplankton of oligotrophic HML, particularly in lakes that lack fish [83]. Without the majority contribution of *Synechocystis* sp. in abundance in Lake La Luna, the dominant taxa are the mixotrophic flagellates, which feed on picophytoplankton (<2, *Synechocystis* sp.) or can absorb dissolved matter from the water column in nutrient-poor lakes, such as Lake La Luna [84].

As mentioned, solitary and rounded cyanobacteria *Synechocystis* sp. was the most representative (abundance) in Lake La Luna; its small cell size (<2 μm) results in a high surface to volume ratio that favors nutrient absorption by diffusion, an advantage under low nutrient concentrations, such as in Lake La Luna. Small cells facilitate the transfer of nutrients to higher trophic levels and therefore are a very important link in the trophic web of ultra-oligotrophic systems [84].

The predominance of Chlorophyceae, mainly Chlorococcal, in Lake El Sol agrees with its oligotrophic status [84], indicating photosynthesis is more important than mixotrophy. In contrast, in the ultraoligotrophic Lake La Luna, mixotrophy results in a more important process.

The differences in the phytoplankton species richness of Lakes El Sol and La Luna could be associated with the interplay of the following main drivers, which showed differences between both lakes and pose a challenge to phytoplankton development: [a] lake area, [b] presence/absence of macrophytes, [c] trophic status, [d] pH, [e] ultraviolet radiation (UVR), and [f] exotic fish introduction. However, not all variables have the same importance in controlling the phytoplankton community. First, the magnitude of the lake-to-lake difference of each variable differs, and second, the magnitude of the effect of each variable on phytoplankton also differs. Therefore, the result expressed in the phytoplankton community derives from the interaction of the variables that probably act synergistically in some cases, while antagonistically in others.

- (a) Lake area. The area–species richness relationship is well-known [1,2]. Lake area is closely related to habitat diversity and then higher species richness [3]. The surface of Lake El Sol is almost eight times larger than Lake La Luna [23]. Additionally, Lake El Sol is deeper and holds a larger water volume than Lake La Luna.
- (b) Macrophytes. The presence and development of macrophytes are important in influencing phytoplankton biodiversity [85]. Lake El Sol displays an extended vegetated area (e.g., *Chara*) on the shallow littoral, while Lake La Luna has no macrophytes. While macrophytes compete with phytoplankton and reduce its biodiversity [86], macrophytes also provide microhabitats that harbor benthic diatoms that eventually incorporate into the water column, augmenting the taxonomic richness found in phytoplankton. To date, empirical evidence shows that the number of habitats in a region is almost always positively correlated with the number of species inhabiting it [3]. In fact, habitat diversity is often a better predictor of species richness than area [87,88].
- (c) Trophic status. An inverse relationship has been found between trophic status and phytoplankton biodiversity [4–6]. However, this relationship is expressed when comparing oligo- versus eutrophic lakes. The trophic (chlorophyll and nutrient concentrations) difference between Lakes El Sol and La Luna is marginal since the former is oligotrophic and the latter ultraoligotrophic. Phytoplankton in both lakes are restricted by the low nutrient concentration.
- (d) pH value. Acidification of HML diminishes phytoplankton biodiversity [9,10]. Both lakes have acid pH values. However, pH in Lake La Luna (4.7 ± 0.3) is lower than in Lake El Sol (5.4 ± 0.4), reaching limiting values for biota distribution [23,29].
- (e) UV radiation. UVR at HML is higher than at sea level, and often HML are transparent (oligotrophic) and with scarce UVR attenuation substances (e.g., chromophoric dissolved organic matter) negatively impacting phytoplankton biodiversity [7,8]. The light attenuation coefficient (K_d) is lower in Lake La Luna ($0.21 \pm 0.06 \text{ m}^{-1}$) than in Lake El Sol [$0.37 \pm 0.07 \text{ m}^{-1}$], mirroring the higher turbidity of Lake El Sol compared

to Lake La Luna. Consequently, UVR reaches all of the water column, down to the lake's bottom in Lake La Luna, while in Lake El Sol, UVR is attenuated in the top 25% of the water column. This translated into an inexistent depth refuge (UVR safe zone 0% of the water column) in Lake La Luna, while larger (UVR safe zone 75% of the water column) in Lake El Sol [89].

- (f) Exotic fish species introduction. During the 1950's there was a successful rainbow trout introduction in Lake El Sol that failed in Lake La Luna [31]. Fish introduction is associated with biodiversity loss in shallow lakes [11,12,90,91]. However, phytoplankton biodiversity in fish-stocked Lake El Sol was higher than in fishless Lake La Luna. In addition to promoting turbidity, rainbow trout also change the food webs, generate trophic cascade effects, and change the plankton functional groups with Cyanobacteria as a dominant group in fish-stocked lakes [92–94]. However, phytoplankton biodiversity in fish-stocked Lake El Sol was higher than in fishless Lake La Luna.

The reported effects of these drivers on biodiversity match our findings in some cases, while they did not in others (Table 6).

Table 6. Theoretical positive (+) and negative (−) effects of the main phytoplankton biodiversity drivers, and actual findings (✓ match, X no match) of the phytoplankton biodiversity of Lakes El Sol and La Luna.

	Surface Area	Macrophytes		Trophic Status	pH	UVR	Fish Introduction
		Coverage	Microhabitats				
Theoretical	+	−	+	−	+	−	−
Actual	✓	X	✓	X	✓	✓	X

Additionally, it seems Lake El Sol could be in a transitional stage between the clear-water (macrophyte dominated) and the turbid-water (phytoplankton dominated) states described as alternative stable states for shallow lakes. Regarding nutrient concentrations, there is a subtle difference between the trophic status of Lake La Luna (ultraoligotrophic) and Lake El Sol (oligotrophic). Concerning the effects of fish introduction on the ecosystem, the zooplankton of the fishless Lake La Luna is much larger than in the fish stocked Lake El Sol [89], suggesting rainbow trout is indeed affecting the plankton composition, as found by Lancelotti et al. [94] and others. Nonetheless, the rainbow trout abundance in Lake El Sol is unknown, so we do not know the actual effect on the trophic cascade.

Finally, the low pH combined with the high incident UVR could be the most important drivers in reducing phytoplankton diversity in Lake La Luna, as previously suggested for the zooplankton assemblage by Dimas-Flores et al. [26], while in Lake El Sol, the less extreme pH and higher turbidity that serve as protection against UVR, promote the phytoplankton diversity.

5. Conclusions

The phytoplankton species richness of Lakes El Sol and La Luna was higher than those reported for other tropical and temperate HMLs. The most diverse phytoplankton groups were Bacillariophyceae and Chlorophyceae. Most diatoms were tychoplanktonic, associated with the lakes' shallowness and warm polymictic regime. Regarding abundance, the Chlorophyte *Oocystis lacustri* was the dominant species in Lake El Sol, while the Cyanobacteria *Synechocystis* sp. in Lake La Luna. Regarding biomass, the Chlorophytes in Lake El Sol, and the dinoflagellates and Cyanobacteria in Lake Luna were dominant. The Chlorococcales characterized the oligotrophic Lake El Sol and the Cyanobacteria the ultraoligotrophic Lake La Luna. The larger phytoplankton species richness, density, and biomass in Lake El Sol than in Lake La Luna seems to be explained by the most extreme conditions in Lake La Luna expressed by its lower pH and the higher doses of UVR

associated with its higher transparency. A larger habitat diversity in Lake El Sol resulted from its larger area, and the presence of submersed macrophytes promotes phytoplankton species richness.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/d14010042/s1>, Table S1: Global density (cells mL⁻¹) and biovolume mm³ m⁻³) of the phytoplankton taxa recorded in Lakes El Sol and La Luna, Nevado de Toluca. (*Navicula* NTA and *N. NTB* according [12]). (NF = not found).

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