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Systematic Review

Lipid Prospection Based on the Cellular Size of Phytoplankton Communities from Tropical Freshwater Ecosystems: A Systematic Literature Review

Jesús Bautista-Regil [1,*], Alberto J. Sánchez [1], Miguel Ángel Salcedo [1], Bertha Olivia Arredondo-Vega [2] and Violeta Ruiz-Carrera [1,*]

[1] Diagnóstico y Manejo de Humedales Tropicales, División Académica de Ciencias Biológicas, Universidad Juárez Autónoma de Tabasco, Villahermosa 86040, Tabasco, Mexico; alberthoj.sanchez@gmail.com (A.J.S.); mzalcedo@gmail.com (M.Á.S.)

[2] Laboratorio de Biotecnología de Microalgas, Centro de Investigaciones Biológicas del Noroeste S.C. (CIBNOR), La Paz 23205, Baja California Sur, Mexico; kitty04@cibnor.mx

* Correspondence: biol.jebar@gmail.com (J.B.-R.); violeta@ujat.mx (V.R.-C.)

Abstract: Eutrophication-resistant phytoplankton communities in freshwater ecosystems have a novel lipid potential to contribute to the development of tropical regions. The question that arises due to the unsustainability of their eutrophicated waters is how the recognition of the lipids of the resident phytoplankton progresses. Our aim was to provide an overview of the pico-, nano- and micro-cellular lipids of phytoplankton with a focus on eutrophic tropical freshwater ecosystems. Using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) framework, global and Latin American publications were retrieved based on search equations and specific questions. In total, 490 studies were reviewed. The inclusion criteria, in order, were (1) peer-reviewed articles, (2) articles investigating phytoplankton strains or communities from any aquatic environment, (3) articles on freshwater ecosystems, and (4) research in tropical climates. The contribution of freshwater phytoplankton was high and discontinuous, with a representation of 63% in the 21st century. Freshwater themes were resolved in the ecological context with phytoplankton or algae keywords, while microalgae were targeted using resource use keywords. On the tropical scale, technological themes on lipid microalgae were related to fatty acids, biofuels, biodiesel, antioxidants, and recombinant DNA. It is concluded that studies of the lipid composition of phytoplankton communities are delayed in the case of eutrophic tropical freshwater ecosystems.

Keywords: PRISMA; ecosystem services; tropical phytoplankton; eutrophication; cell size; lipid products

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1. Introduction

Freshwater phytoplankton include oxygenic photoautotrophs represented by microalgae and cyanobacteria of different cell sizes that can form extensive colonies and mixed communities with specialized and economically exploitable ecological functions in tropical regions [1]. Tropical aquatic ecosystems have shown a high diversity of phytoplankton species that provide ecosystem services [2] but are highly vulnerable [3]. One of the reasons for this is the uptake of organic matter into the ecosystem, which alters the phytoplankton community through changes in trophic cascades [4]. In the case of hypereutrophic ecosystems that have a trophic dynamic dominated by an exacerbated phytoplankton biomass due to the oversupply of phosphorus and nitrogen [3–6], the actions required to prevent organic photoautotrophic production or to restore environmental services are sometimes contradictory to basic human needs [2,6–8]. In this sense, the high biomass and persistence of phytoplankton in hypereutrophic ecosystems reflect the possibility of applying a technological approach to harness the metabolic raw materials generated from the surplus of phytoplankton biomass in stressed environmental conditions [9] for the well-being of local human populations, such as the extraction of valuable lipid biomolecules [10–12]. From this technological perspective, it is important to consider both lipid composition and biosynthetic metabolic capacity concerning cell sizes from picoplankton to macroplankton, for example, by studying biochemical adaptation through the production of lipids [13–19].

Within the biomolecular realm of phytoplankton, lipids constitute a heterogeneous and chemically diverse collection of components, mostly composed of fatty acid (FA)-derived metabolites, that influence the metabolic dynamics of organisms in eutrophic ecosystems [20,21]. In other words, the development of science requires diverse socio-ecological activities in multiple directions. Thus, it is important to conduct systematic studies on the intrinsic value of resident phytoplankton in ecosystems that are at the limit of environmental contingency and security.

Considering that the development of aquatic biotechnologies is one of the key areas of technological development [22], the lipid screening of eutrophicated freshwater phytoplankton opens up an opportunity to identify, based on cell size range, potential value-added bioproducts related to the community's suite of adaptations in the phycosphere. Among the adaptations important in the era of global change, the diversity of metabolic strategies in biogeochemical cycles and the biological carbon pump that assimilates carbon dioxide from the atmosphere should be mentioned [23]. Therefore, the revision of scientific information on phytoplankton lipids from tropical geographic regions is timely in regard to the exploration of patterns of information and bioeconomic sustainability transitions in naturally or anthropogenically perturbed ecosystems [24]. Based on the global theoretical knowledge across various subjects from tropical phytoplankton research, it is essential to establish a structured review framework. This systematic literature review, which adheres to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) framework [25], is focused on analyzing the current trends in research on the phytoplankton lipids of tropical freshwater ecosystems under eutrophic conditions, with a specific examination of the cell sizes of pico-, nano-, and micro-cells, aiming to gain insights into their potential roles as natural lipid bioreactors.

2. Materials and Methods

2.1. Search Strategy

This systematic review was conducted using the PRISMA framework according to Page et al. [25]. Scientific articles, abstracts, chapter books, and reviews were collected from the platforms Scopus, SpringerLink, ScienceDirect, Web of Science, and the Latin American platforms Scielo and Redalyc (List S1). In order to focus on key information, three questions were formulated based on the topic: 1. What is the status of scientific studies on tropical freshwater phytoplankton? 2. Is there sufficient scientific information focusing on the use of phytoplankton lipids based on cell size scale in tropical freshwater ecosystems? 3. What are the lipids of phytoplankton that are associated with metabolic stress due to eutrophication in the tropical environment? The search instruction was based on various equations from 15 terms linked by Boolean operators (AND, OR) and related to the research questions (Table 1). The cut-off date was February 2021. The equations were selected according to the highest contribution of publications from each platform, without restrictions (Table 2), although none of the search equations worked for Scielo.

Table 1. Search terms with Boolean operators in relation to the research questions.

	AND				
OR	Phytoplankton [1]	Lipids [1,2]	Cell size [2]	Freshwater [1,2]	Environmental stress [3]
		Lipidomic	Microplankton	Limnetic	Metabolic stress
		Fatty acids	Nanoplankton	Wetlands	
		Lipidic	Picoplankton	Tropical	

Note: Superscripts show the relationships of keyword columns to the research question order.

Table 2. Solved search equations in scientific information databases.

Platform	Search Equation
Scopus SpringerLink Web of science	(phytoplankton) AND (lipids OR lipidomic OR "fatty acids" OR lipidic) AND ("cell size" OR microplankton OR nanoplankton OR picoplankton) AND (freshwater OR limnetic OR wetlands OR tropical) AND ("environmental stress" OR "metabolic stress").
ScienceDirect	(phytoplankton) AND (lipids OR lipidic) AND ("cell size") AND (freshwater OR tropical) AND ("environmental stress" OR "metabolic stress").
Redalyc *	(fitoplancton) AND (lípidos OR lipidómica) AND ("tamaño celular" OR microplancton OR nanoplancton OR picoplancton) AND (agua dulce OR limnético OR humedales OR tropical) *.

Note: * Terms localized in Spanish.

2.2. Stages of the Systematic Review Process

The PRISMA method flowchart was used in this systematic review process step by step from the global scale (Figure 1). In the first step, four screenings were used to generate a number of environmental and experimental studies to be analyzed. The screening procedure was linked to the inclusion/exclusion criteria of the studies in the following order: (1) full original articles excluding abstracts, book chapters, and reviews; (2) phytoplankton species from all aquatic ecosystems, excluding articles focused on other biological groups; and (3) research on freshwater ecosystems, this screening including one sub-screening of articles related to or citing the eutrophication process, and the involucre species whose the cell size and dimensions were consulted in various studies recorded; and (4) tropical climate freshwater ecosystems only. Köppen–Geiger classification [26] was used to determine the climatic origin of the phytoplankton studied. For studies without climate zone records or laboratory studies, the geographical location of the institution responsible for the research was used to replace the climate reference.

For each screening, the publications on each scientific platform were retrieved to create a global database in Microsoft® Excel® V.230, without considering gray literature to avoid bias, and duplicate records were identified with Excel, highlighting duplicate value functions. Metadata such as the platform name, journal name, authors, year of publication, title, DOI, and abstract were recorded, as well as the fields of the type of environment, location of organisms studied or the address of the first author, and the type of climate (Table S1). In screens 3 and 4, the databases were transferred to the JabRef free program, including the keywords, and converted into an RIS file.

2.3. Data Analysis

The temporal trends of the phytoplankton articles were plotted according to aquatic environment (screening 2) with the support of the online software RAWGraphs 2.0R [27]. In the third screening, a spatial representation of the included studies was designed in Excel using the climate records and classification per country as layers. Additionally, articles related to or citing the eutrophication process were identified, as well as the cell size of the recorded species, whose cellular dimensions were consulted in various studies. The RIS files were used to perform the analysis of co-occurrences of the author keywords (KWs) in the VOSViewerR software v.1.6.18 [28].

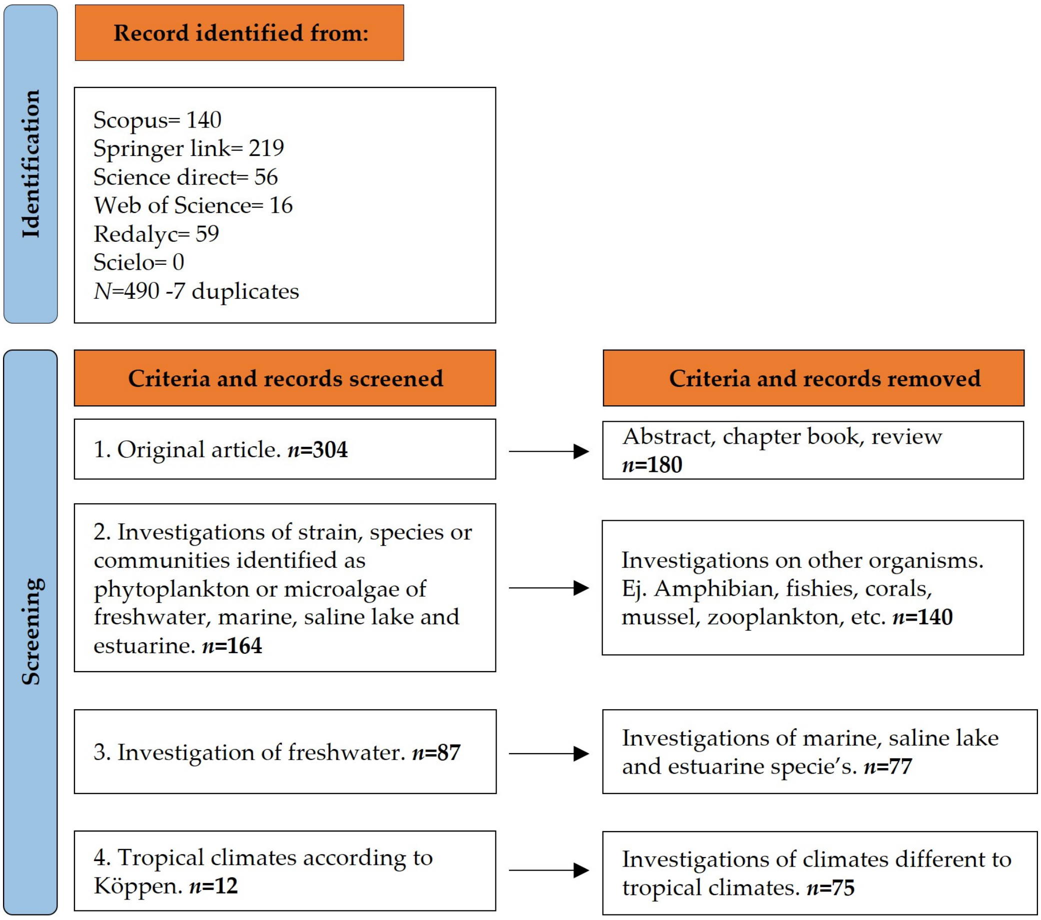

Figure 1. PRISMA flowchart showing the selection of scientific studies with the identification and screening steps in the systematic review. *n* = number of studies.

3. Results

3.1. Global Data Collection and Phytoplankton Studies over Time

Out of a total of 483 systematized global publications, 34% were scientific articles focusing on phytoplankton or microalgae studies on the species or community level (Figure 2a). Most phytoplankton articles were related to marine and freshwater environments, with similar proportions (45.12% y 44.51%, respectively), resulting in a total of 87 freshwater publications. A lower percentage addressed both marine and freshwater ecosystems (8.54%), with a few articles on saline lakes (1.22%) and estuarine ecosystems (0.61%). The timeline of freshwater phytoplankton research was intermittent from 1995 to 2022 and showed a greater increase over time compared to the proportion of studies on other aquatic environments (Figure 2b). In total, 96% of the articles on freshwater phytoplankton (87) collected in this literature review were published in this century (Figure 2).

3.2. Distribution of Freshwater Phytoplankton Articles by Climate and Country

Of the freshwater phytoplankton articles, 36% were based on locations in humid subtropical climates (Cfa), followed by cold and warm summer continental (Dfb and Dfa) and west coast marine climates (Cfb), which together contributed 35%. Rainforest (Af), monsoon (Am), and savanna (Aw) tropical climates accounted for 15%, mediterranean (Csa) climates accounted for 5%, while mediterranean cool summer (Csb), warm steppe (Bsh), cold steppe (Bsk), subtropical with dry winter (Cwa), warm desert (Bwh) and continental cool summer climates (Dwb) each represented between 2% and 1%. The total number of climates was registered for articles from 26 countries, with China having the highest number (24), followed by the USA (9), Brazil (7), Canada (5), and Colombia (5) (Figure 3). With the exception of studies from Brazil and Colombia, the contribution from Latin American countries was small.

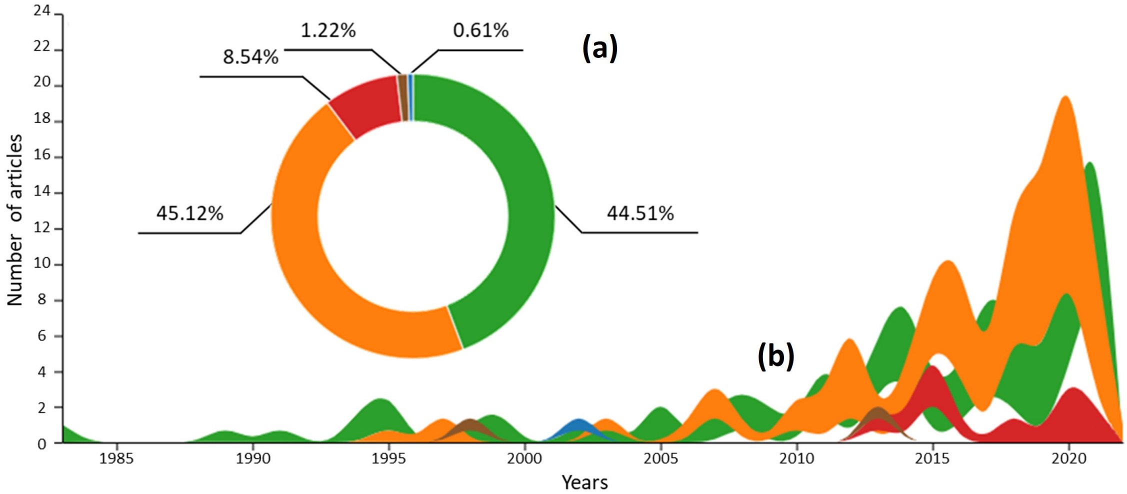

Figure 2. Global studies on phytoplankton in different aquatic environments. The total proportion of articles by type of environment (**a**). Timeline of the proportion of articles by type of environment (**b**). Symbology: ● freshwater, ● marine, ● marine and freshwater, ● saline lakes, ● estuarine.

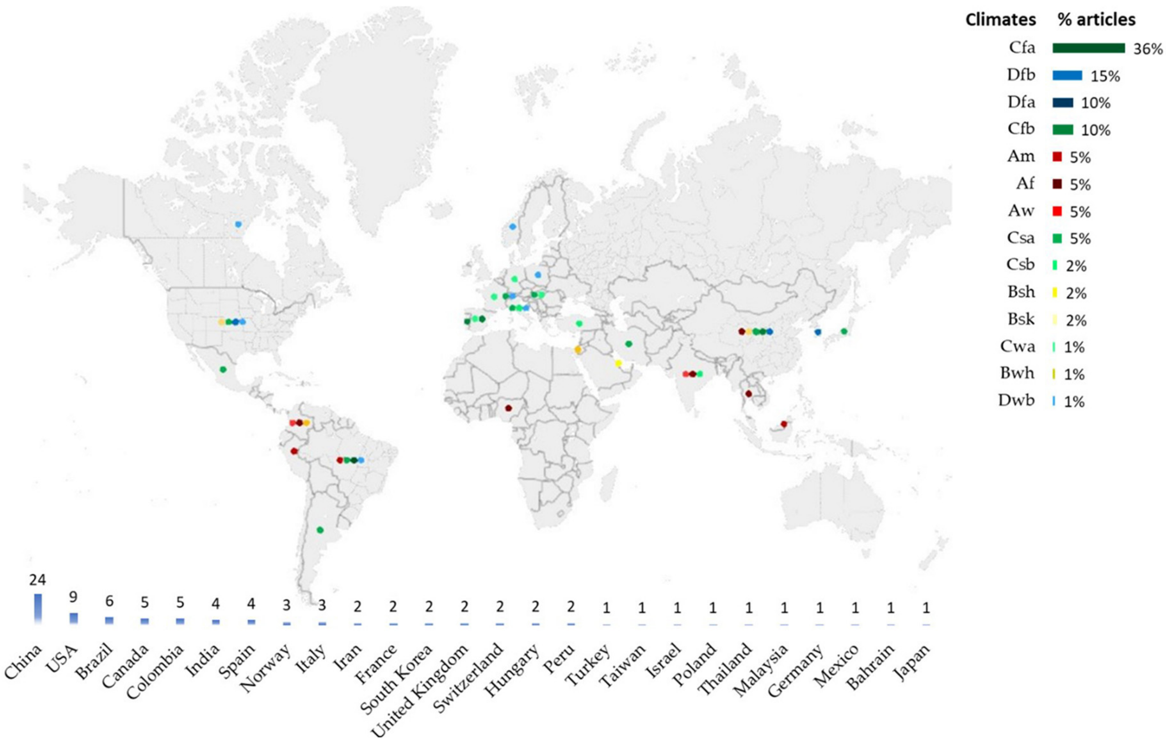

Figure 3. Location of freshwater phytoplankton screening studies by country and the number of articles for each type of climate recorded (n = 87). The locations were determined based on the coordinates provided in the respective studies or the institutional addresses of the authors for laboratory strains. Abbreviation of climate types: humid subtropical (Cfa), cold summer continental (Dfb), warm summer continental (Dfa), west coast marine(Cfb), Rainforest tropical (Af), monsoon tropical (Am) savanna tropical (Aw), mediterranean (Csa), mediterranean cool summer (Csb), warm steppe (Bsh), cold steppe (Bsk), subtropical with dry winter (Cwa), warm desert (Bwh), and continental cool summer (Dwb).

3.3. Co-Occurrence Analysis of Global Freshwater Phytoplankton

The co-occurrence analysis of freshwater screenings identified 361 KWs, of which 260 were linked to KWs in another paper (Table S2). In this co-occurrence network (Figure 4), the KW node of microalgae showed 12 repetitions with respect to phytoplankton, oxidative stress, and flow cytometry, which were repeated seven times, while *Microcystis aeruginosa* and algae were mentioned six and five times, respectively. Microalgae was the most recent term used to refer to algae as a function of the timeline. For these six nodes, we collected a total of 44 co-occurrences and 190 link streams. The node microalgae showed 49 links of which the nodes with lipids, fatty acids, biofuels, biodiesel, antioxidants, and recombinant DNA stood out, while the node phytoplankton showed 29 links with the nodes *Microcystis*, growth, biovolume, ultraviolet radiation, and photosynthesis.

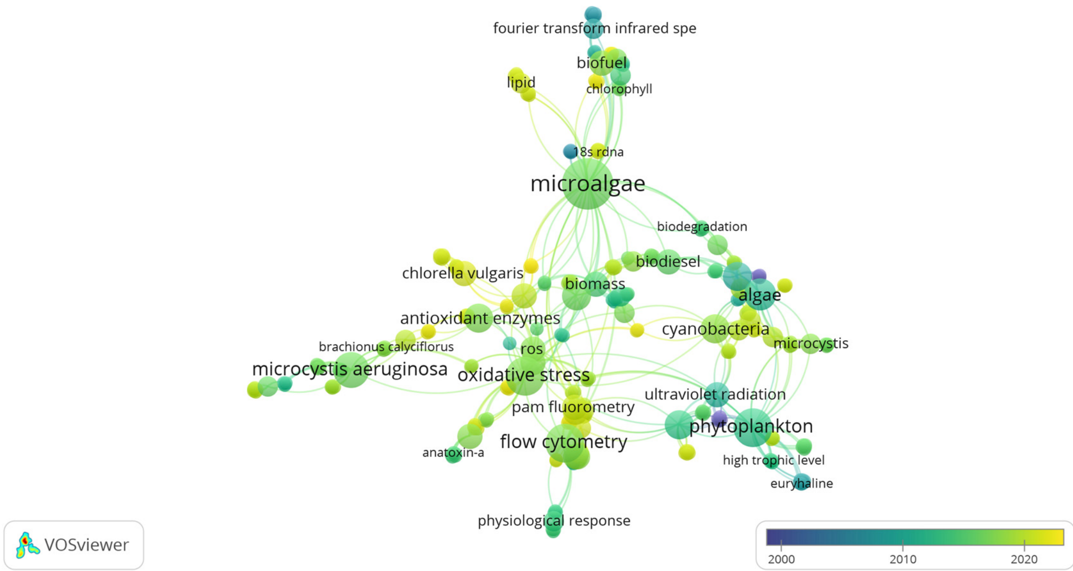

Figure 4. Co-occurrence network of keywords and link streams of freshwater ecosystems. The size of the node is proportional to the frequency of occurrence of the keyword it represents, the line length represents the degree of connection between two nodes, and the color indicates approximation to the time of publication.

Analysis of Freshwater Phytoplankton with Mention of the Eutrophication Process

From the freshwater screening, 18 articles, which represented 21% of the total, mentioned eutrophication in the text. In agreement with the co-occurrence analysis, 88 keywords were identified in this sub-screening. The node *Microcystis* again stood out with four repetitions, while allelochemicals, cyanobacteria, and microalgae were repeated twice each. The largest set of connected nodes was formed of 17 links (Table S3). On the timescale of the co-occurrence network (Figure 5), adaptation, cyanobacteria, and luteolin stood out as the most recent groups of keywords. It should be noted that in addition to the keyword allelochemicals, the keyword allelopathic effect was also registered.

The 18 articles registered 29 species belonging to 19 genera. Notably, the genus *Microcystis* had five identified species, while *Scenedesmus* had three (Figure 6). Figure 6 displays the phytoplankton size distribution by species [14,29–41], including 4 pico, 19 nano, and 10 micro.

Of the studies reviewed in this section, only five reported lipid accumulation in six phytoplankton species, all of which fell within the pico- and nanoplankton range (Table S4). These studies aimed to determine the impact of environmental stress factors, including the limited availability of phosphorus and high exposure to UV radiation, bisphenol A, and carbon nanotubes, on the variation in lipid content. The species *Mychonastes homosphaera* was the sole focus in explorations of its potential for biodiesel production [42–45].

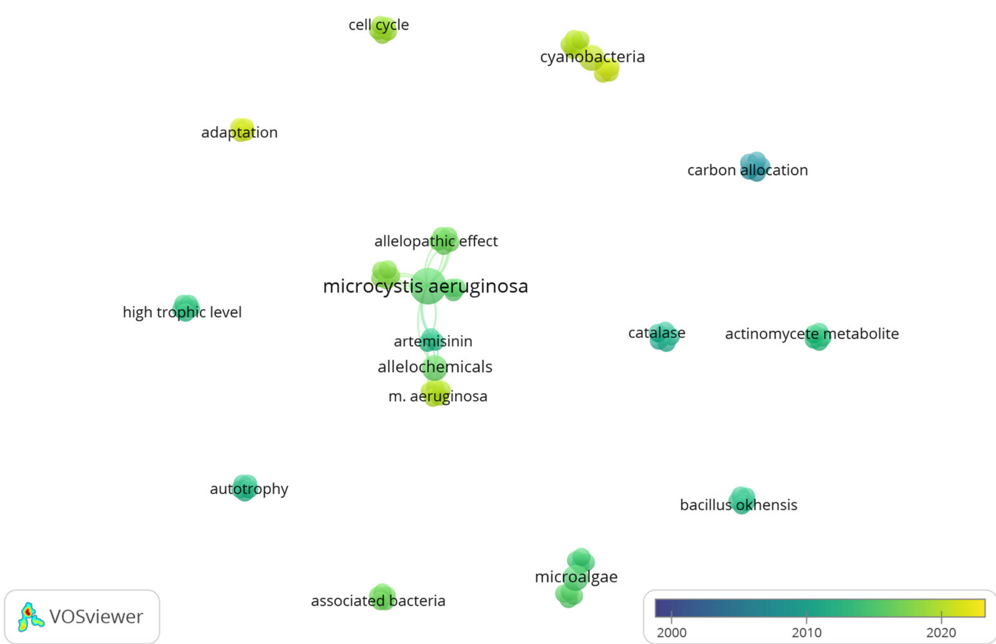

Figure 5. Co-occurrence network of keywords and link streams of articles citing the eutrophication process. The size of the node is proportional to the frequency of occurrence of the keyword it represents, the line length represents the degree of connection between two nodes, and the color indicates approximation to the time of publication.

3.4. Co-Occurrence Analysis of Freshwater Phytoplankton Studies in Tropical Environments

The co-occurrence network based on tropical screening (15%, screening 3) recorded 52 KWs, forming 23 nodes with at least one link stream with another (Figure 7). Similarly, microalgae were the most densely populated KW, showing 19 link streams with this node (Table S5). In this screening, most of the publications concerned approaches or topics on the use of microalgae (Table 3), of which four reported lipid accumulations evaluated using gravimetric methods, Nile red staining, high-performance liquid chromatography, no metabolite identity, or associations with eutrophication stress [46–49]. The topics examined over the past two decades included the isolation of microalgae, exopolysaccharides, total lipids, fatty acids, biomass for nutrients, pharmaceuticals, and biodiesel.

Table 3. Topics related to the use of freshwater microalgae as a resource in tropical ecosystems.

Topics	Lipidic Analysis Reported	Year	Reference
Isolation of microalgae with a high lipid content for potential use in aquaculture industry	Polar lipid content	2020	[46]
High production of *Chlorella sorokiniana* for its ability to synthesize fatty acids of industrial interest	Total lipid content	2012	[47]
Biodiesel production	Total lipid content Triglycerides	2014	[48]
Production of total lipids	Total lipid content Intracellular lipid presence	2016	[49]
Commercial use of microalgae	N/A	2020	[50]
Production of exopolysaccharides	N/A	2018	[51]
Biotechnological potential as fish feed, supplementary nutrients, pharmaceuticals, and the bioremediation of contaminated water	N/A	2016	[52]
Production of potentially useful biomass for aquaculture	N/A	2006	[53]

Note: N/A: did not report lipid analysis.

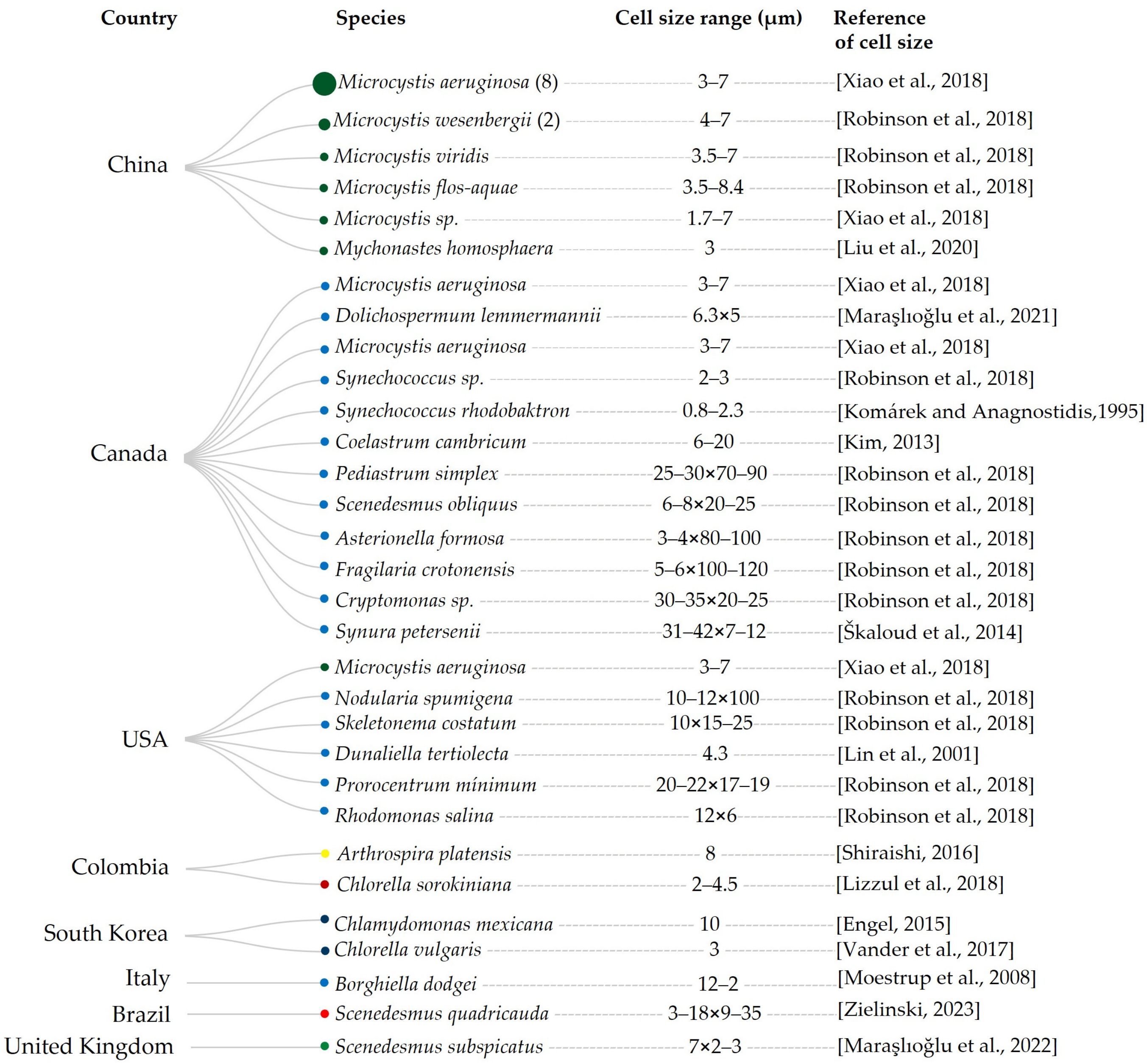

Figure 6. Freshwater phytoplankton species by country with size ranges reported in articles citing the eutrophication process. Climatic identity refers to the color of the image caption in Figure 3. Cell size range (peak, 2 µm; nano, 2–20 µm; micro, >20 µm) corresponds to the width or width × length dimension cited by the author [14,29–41].

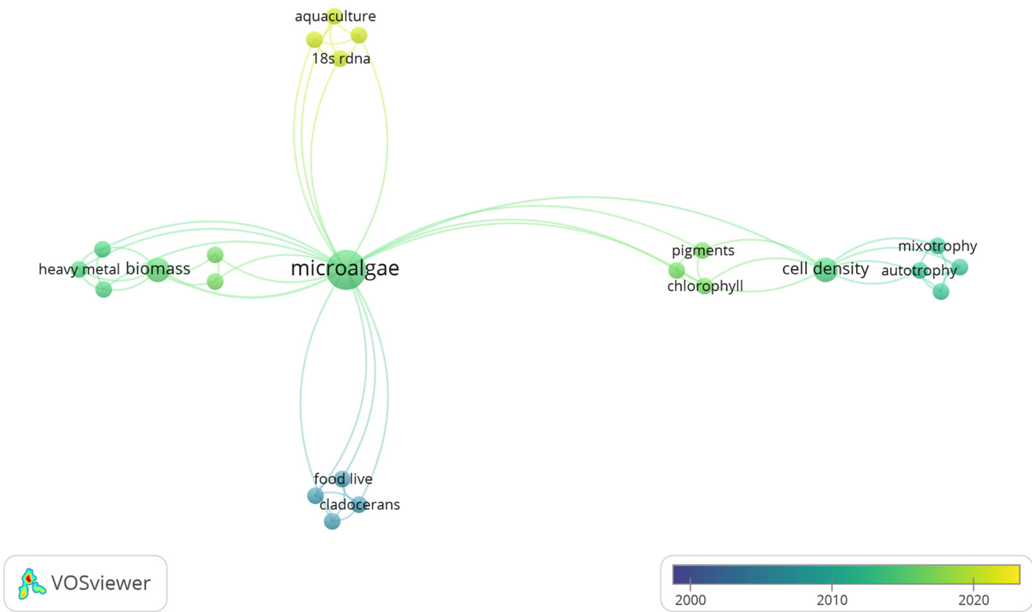

Figure 7. Co-occurrence network of keywords and link streams of tropical freshwater ecosystems. The size of the node is proportional to the frequency of occurrence of the keyword it represents, the line length represents the degree of connection between two nodes, and the color indicates approximation to the time of publication.

4. Discussion

Our investigation of eutrophic tropical freshwater phytoplankton focused on a systematic review aiming to identify the lipid content or lipid-based metabolites associated with phytoplanktonic communities by cell size in eutrophic ecosystems. In the case of the Scopus, SpringerLink, ScienceDirect, and Web of Science platforms, the collected database was satisfactory, but it was scarce for the regions of Latin and Central America, specifically Scielo. Adams et al. [54] emphasized the low number of publications of scientific articles from Latin American countries with highest impact factors, which led to a search for information from other relevant sources.

Nevertheless, the initial question about the progress of freshwater phytoplankton studies was answered by determining the timeline of phytoplankton studies for all aquatic ecosystems. This highlights the impetus to study of freshwater ecosystems due to the global crisis of water availability, demand, and quality [55] and the process of ecological transition towards sustainable technological innovations to achieve compliance with environmental sustainability criteria [56]. This information was important for distinguishing research efforts in countries in tropical zones with high phytoplankton biodiversity. The content of our survey of freshwater studies was numerically similar to that of Oliver et al. [57], which included 357 articles from the Web of Science database, refining the searches only for the terms eutrophic, urban, and cyanobacterial reservoirs, without terms related to lipids (or lipidomic or fatty acid or lipid) and cell scale. Similarly, the global review by Growns et al. [58] based on Scopus and the Web of Science, using the phrase "fatty acids and freshwater", identified 178 papers focusing on food webs, anthropogenic disturbance, seasonal or spatial variation, and the biomarkers of freshwater ecosystems, without mentioning lipid applications. These two systematic reviews excluded experimental studies, in contrast to the present review, so it is assumed that our survey comes closer to the recognition of phytoplanktonic lipids from eutrophicated ecosystems. It is noteworthy that most of the published papers from the 21st century coincidentally came from China, the USA, and Brazil [57,58], countries that are experiencing greater social and ecological stress due to the great loss of freshwater storage [7].

In addition, by mapping co-occurrences, it was possible to separate major phytoplankton research interests with selected articles based on freshwater and tropical ecosystems, even allowing us to distinguish current trends and connectivity with key information regarding focal species with known cell sizes. Throughout the freshwater co-occurrence network, studies focused on resource use and phytoplankton ecological knowledge were attested. In the ecological context, the lines of research centered on cladocerans as living food, microalgae, biomass, cell density, and, more recently, studies of pigments, chlorophyll, and genomic analysis.

The context of resource use, in turn, was used to decipher the technological orientations of the lipids of microalgae through their associations with fatty acids, biofuels, biodiesel, antioxidants, and recombinant DNA techniques. In addition, the third research question on lipids of phytoplankton that are associated with metabolic stress due to eutrophication in the tropical environment, only was associated to concepts of oxidative stress, without specifying the types of lipids or their composition. Here, the co-occurrence network indicated a low frequency for the keywords flow cytometry and, similarly, biovolume, being indicative of the scarcity of studies with optical counting of microalgal communities that may be composed of nano-, pico-, or micro-prokaryotes or planktonic eukaryotes in environmental samples [59].

In the analysis of the co-occurrence of keywords from the sub-screening of freshwater articles that cited the eutrophication process, most were studies on the use of this process to restore or mitigate disturbances of the environmental health of these ecosystems with eutrophic conditions. Specifically, the articles mentioned the use of allelochemicals to mitigate the proliferation of *Microcystis aeruginosa* [60–62]. Likewise, other articles recorded the use of strains capable of capturing, accumulating, or detecting some types of contaminants. These included the potential of *Scenedesmus quadricauda* as a biomarker of the presence of lead [63] and *Chlamydomonas mexicana* for its ability to biodegrade Bisphenol A [43].

Microcystis is a genus undergoing extensive research in relation to eutrophic ecosystems, particularly regarding its proliferation and its connection to environmental factors. For instance, the swift bloom onset of *Microcystis aeruginosa* in a coastal lagoon has been linked to elevated total suspended solids, salinity, dissolved inorganic nitrogen, and diminished transparency levels [29]. Other factors that reduce the competitive advantage of toxic *Microcystis* are high light conditions or environments limited in nitrogen and light [64]. It is worth noting that the colonization of this cyanobacteria depends on physical conditions related to the natural forces that drive water turbulence, even with increasing buoyancy velocities, as some morphospecies form colonies of 100 µm to 1000 µm in diameter due to the excretion of extracellular polysaccharides [65]. Costa et al. [66] analyzed the incidence of cyanobacteria in freshwater environments using the search terms phytoplankton and eutrophication. Cyanobacteria such as *Microcystis* sp. and *Cylindrospermopsis raciborskii* (Woloszynska) were frequently cited in regard to lakes, followed by rivers, reservoirs, and the sea. Comparatively, the nanoplanktonic species of the genus *Microcystis* stood out among the estimates of different cell sizes of freshwater phytoplankton species recorded in this systematic review.

With respect to review present, the topics registered were microalgal isolation, exopolysaccharide production, total lipid accumulation, microalgal fatty acids and biomass for feed, supplemental nutrients, pharmaceuticals, and biodiesel, which occur with low frequency in the tropical freshwater phytoplankton literature according to cell size. Consequently, studies on the lipids of phytoplankton communities by cell size in eutrophic and hypereutrophic tropical freshwater ecosystems are lacking, especially in peri-urban and urban lakes with surface hydraulic disconnection [3].

In accordance with the global scientific community's aim to enhance decision making that aids in the preservation or restoration of ecosystems [67–70], this systematic literature review provides ample information for such purposes. However, we should emphasize the importance of conducting lipid studies to recognize the inherent value of the phytoplankton metabolic machinery as an emerging ecosystem property involved in the processes of resistance to anthropogenic stress resulting in the eutrophication that persists in many tropical ecosystems [71,72]. In turn, prospecting for lipids in phytoplankton communities in tropical regions offers the opportunity of one database to implement measures of exploitation of this aquatic resource in affected ecosystems depending on the intensity of eutrophication. The need for further research on oxygenic photoautotrophic communities is apparent in this review, as they have the potential to accumulate lipids with potential for facing multiple stressors in eutrophic environments. These communities could serve as natural bioreactors with social-technological benefits or as a biorefinery fed by eutrophic waters. Finally, this systematic literature review revealed scarce scientific production concerning lipids derived from pico-, nano-, and micro-plankton communities. Consequently, prospecting for lipids in phytoplankton communities in tropical regions represents an opportunity to utilize this aquatic resource in naturally or anthropogenically perturbated ecosystems by implementing measures that account for both the intensity and persistence of eutrophication or hypereutrophication. Under ex situ process conditions, physical and chemical parameters, the trophic mode, and nutrient levels could be adjusted to purposefully stress and alter the biochemical composition of the algal biomass. This may result in modifications to communities, as well as the amount and quality of lipid contents. Additionally, it is essential to consider post-harvest processing tactics [73]. Finally, it is important to use the PRISMA framework [25] for high-quality research in order to provide a suitable structure and selection criteria for methodically categorizing research studies. This results in a more comprehensive and thorough analysis than in the case of traditional reviews. The systematic use of PRISMA is essential for ensuring unbiased research related to the utilization of phytoplankton by cell size.

5. Conclusions

This study suggests that lipids have not been sufficiently documented in the communities of pico-, nano-, and micro-plankton inhabiting eutrophic freshwater tropical ecosystems. Additionally, scientific research on the lipids of microalgae species in tropical regions is less advanced compared to that focusing on non-tropical regions. It is imperative to recognize the potential of tropical phytoplankton and microalgae communities and to advance research on eutrophic ecosystems for the production of innovative lipid-based bioproducts using unconventional economic alternatives. In addition, research efforts species that have the ability to accumulate lipids under eutrophic conditions, potentially making them ideal candidates for the biotechnology industry.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/w15213774/s1: List S1: References collected from the platforms Scopus, SpringerLink, ScienceDirect, Web of Science, and Redalyc; Table S1: Global database; Table S2: Keyword list of global freshwater phytoplankton; Table S3: Keyword list of freshwater phytoplankton with mention of eutrophication process; Table S4: Freshwater phytoplankton studies that mention the eutrophication process and some approaches to lipid analysis; Table S5: Keyword list of tropical freshwater phytoplankton.

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