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Zooplankton as an Indicator: A Dramatic Shift in Its Composition Following a Sudden Temporal Brownification of a Tropical Oligotrophic Lake in Southern Mexico

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Abstract: Lake Bacalar, a fragile oligotrophic ecosystem located in the southeast of Yucatan Peninsula, Mexico, suffered from a sudden brownification after the tropical storm Cristobal in June 2020 in the Gulf of Mexico. The color change was the most visible effect of the storm, but all other water variables changed towards eutrophication. We used light traps and DNA barcoding of the zooplankton specimens based on previous baseline constructed for comparison with the species found after the change. A dramatic shift in the zooplankton community occurred: biomass was reduced to a minimum and 20 species of water mites, five copepods, three cladocerans, three chironomids and six species of fish larvae disappeared for a period of at least one year. They were replaced by three species of water mites, four cladocerans, one copepod, 23 chironomids and one ephemeropteran previously not registered, most of the species being characteristic of more eutrophic environments. The southernmost part of the lake, Laguna Xul-Ha, which conserved its oligotrophic characteristics, apparently became a refuge for the original fauna from the whole system. The ecosystem did not fully recover to its original condition until about two years later. While the system has returned to its original state after the storm described here, future changes in land use, including unsustainable tourism expansion, may compromise its resilience and induce hysteresis.

Keywords: eutrophication; tropical lake; freshwater; COI; DNA barcodes; conservation

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1. Introduction

The zooplankton is an essential part of the community in any freshwater ecosystem, being the intermediary between the primary producers (the phytoplankton) and the secondary consumers [1]. However, the use of zooplankton for biomonitoring is difficult due to the lack of taxonomic knowledge and which has led to employing molecular methods, particularly the COI gene, to overpass this problem [2]. Furthermore, in the first decade of the XXI century, the European Union Water Framework Directive omitted zooplankton as a biological quality element [3], which perdures today [4]. These latter authors demonstrated the value of zooplankton as an indicator of water quality in a reservoir in Portugal.

In a classic study, Brooks et al. [5] demonstrated the changes in the zooplankton community after introducing a planktivorous filter fish in a temperate lake. Recently, studies have shown how changes in some invaders and increased water clarity, linked to a filter feeding invader and temperature, caused three different shifts in the whole zooplankton community, modifying abundance and species composition, leading to a decline in the abundance of many cladoceran species and changes of copepod size over a 27-year period [6].

Recently, a new freshwater phenomenon was described, brownification. Tuvendal et al. [7] used this term for the first time to describe the fact of river waters becoming brown. Later, Graneli [8] defined it as "an increase in the yellow-brown color of lake and stream water caused mainly by a dissolved humic matter of terrestrial and wetland origin, which absorbs solar radiation strongly in the short wavelength of the visible spectrum". Since then, this phenomenon has been documented in temperate lakes and discussions of the causes are ongoing [9]. In general terms, the relationship between brownification and global warming effect in the temperate regions has been determined, including changes in land use [10]. Regarding biological communities, Hedström et al. [11] experimentally found an increase in fish mortality after brownification in a pond in Sweden. Concerning zooplankton, most studies on brownification have been devoted to demonstrating its effect under experimental conditions in mesocosm environments, indicating severe changes in picoplankton and some crustaceans [12,13]. The only research considering the entire zooplankton community is a long-term study [9]; however, most specimens have been defined to genus or family level. As a result, the authors did not find significant differences in the community composition, although the zooplankton biomass changed significantly after browning.

In tropical environments, there are no reports about brownification, except the sudden episode reported by Carrillo et al. [14] in the oligotrophic Lake Bacalar after the tropical storm Cristobal hit the western side of the Yucatan Peninsula from 3 to 9 June 2020. This sudden brownification happened less than a month after the storm, during the enclosure period, due to the COVID-19 pandemic, when all activities in the Lake and the nearby or shoreline towns like Bacalar were minimal or absent. The lake later shifted to a greenish, losing the original blue of its waters [14,15]. Total recovery of the original blue color did not appear until after two years (Figure 1). Lake Bacalar is a unique system hosting the biggest living microbialites in the world [16]. Due to the exceptional conditions of its blue waters, it has become an important tourist attraction in the southeastern Yucatan Peninsula. However, the development of this activity has been chaotic and has lacked consideration for the environment, particularly the microbialites [17].

Several years ago, in this lake, Elías-Gutiérrez et al. [18] elaborated a baseline of the zooplankton using the DNA barcodes establishing putative species based on Molecular Taxonomic Units (MOTUs), using the Barcode Index Numbers (BINs) [19]. The authors [18] used methods different from the traditional ones for collecting samples, utilizing light traps [20]. This method increased the known species number of the zooplankton in Bacalar from about 22 to more than 80, with a projection to nearly 120, considering the community in a broad sense, including many overlooked groups such as water mites, chironomids, other insect larvae, the first stages of fish, and decapod larvae among others as mysids. Traditionally, zooplankton has been considered only by rotifers, cladocerans, and copepods. Long ago, two important specialists proposed to include other groups, such as those that swim in short periods or have predominantly a benthic or littoral life cycle [21], and we agree with this idea. We observed that all the specimens attracted by the light traps swam to the traps suspended 2 m below the surface in Cocalitos sinkhole [18], with a depth of 38 m [14].

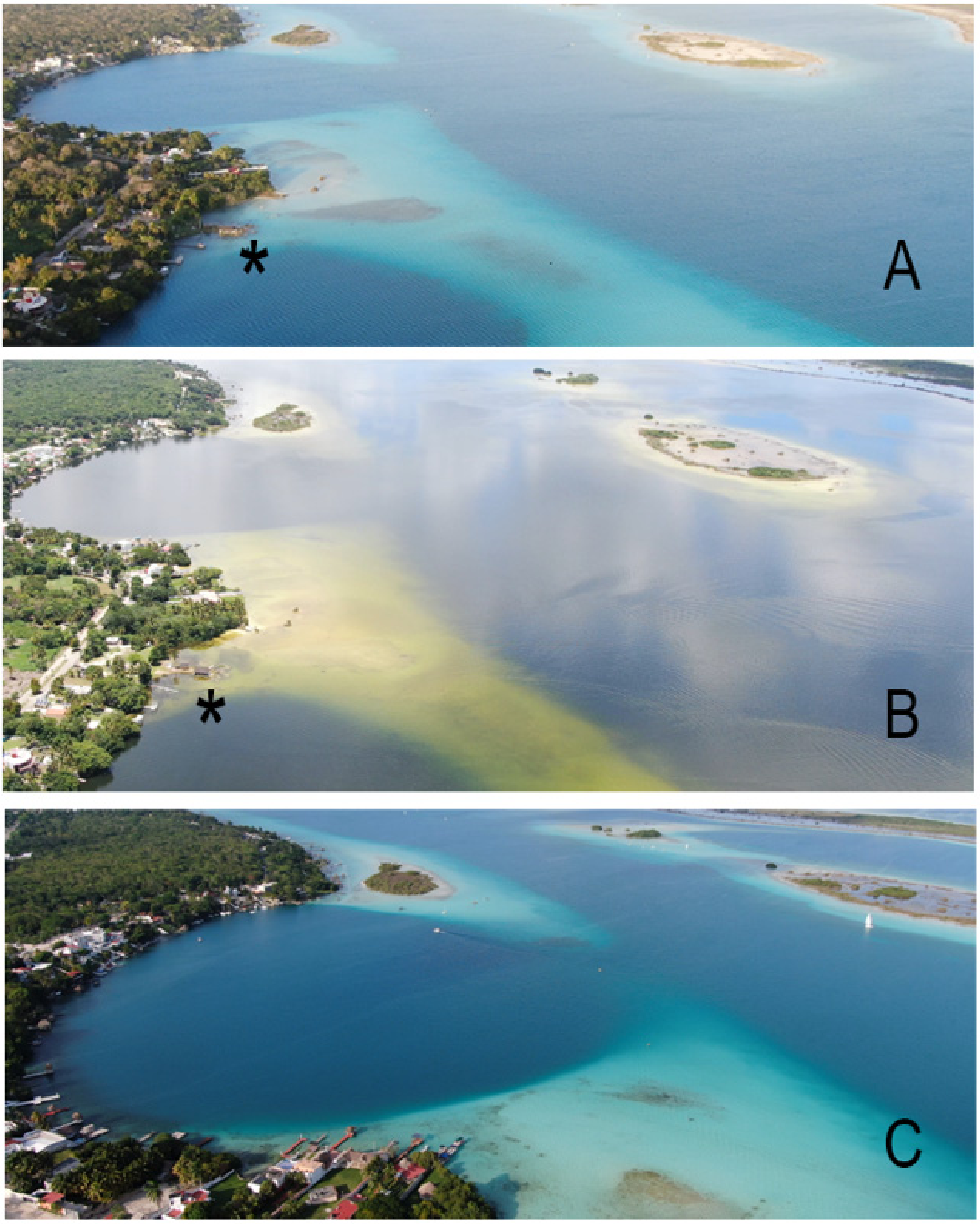

Figure 1. Study area in Lake Bacalar. Aerial photos taken in the Cocalitos sinkhole area; the sinkhole is shown in the foreground; the bottom shows La Laguna, another sinkhole. (**A**) Original colors on 9 March 2019. (**B**) After the brownification event 20 July 2020. (**C**) Recovery after two years, 6 June 2022 (Photos by ME-G). * The point where the light trap was deployed before and after the brownification.

The zooplankton community of Bacalar was stable with no shifts in main taxa, probably due to the minor environmental changes [14] owing to unique water chemistry [22,23] and the fact that its inflows almost exclusively derive from groundwater [23]. Unfortunately, the taxonomic impediment is high; about 80% of the MOTUs have not been identified to species level. Currently, we are in the process of describing this uncovered biodiversity [24].

However, based on the MOTUs, we have a good idea of the species diversity of Bacalar, and we constructed a baseline, available in a BOLD (boldsystems.org, accessed on 6 January 2025) dataset that can be consulted as a DOI (dx.doi.org/10.5883/DS-BACALAR). It includes a sequence of 806 specimens, each one with a photograph and all collecting data, representing 77 putative species. The idea behind this baseline was its use in future biomonitoring, using metabarcoding and eDNA technology. However, we could not continue with these developments because support for scientific research in Mexico has become extremely limited [25].

This work aimed to detect qualitative changes in the zooplankton community (considering it in a broad sense) in Lake Bacalar after the brownification event using the BINs after DNA barcoding [19] and Molecular Taxonomic Units (MOTUS) as indicators of the putative species, together with the identified species.

2. Materials and Methods

We used all previous zooplankton records of Lake Bacalar published by Elías-Gutiérrez et al. [18] as a baseline (dx.doi.org/10.5883/DS-BACALAR). Due to the urgency to establish the possible changes in the zooplankton community, we decided to repeat the original sampling with the light traps [18] and look at the effects of the brownification in the entire zooplankton community at least at two points of the lake, one in the north and another in the south. There is a good description of this lake in Carrillo et al. [14]. In Figures 1 and 2 we show the study area, and all points mentioned in this work.

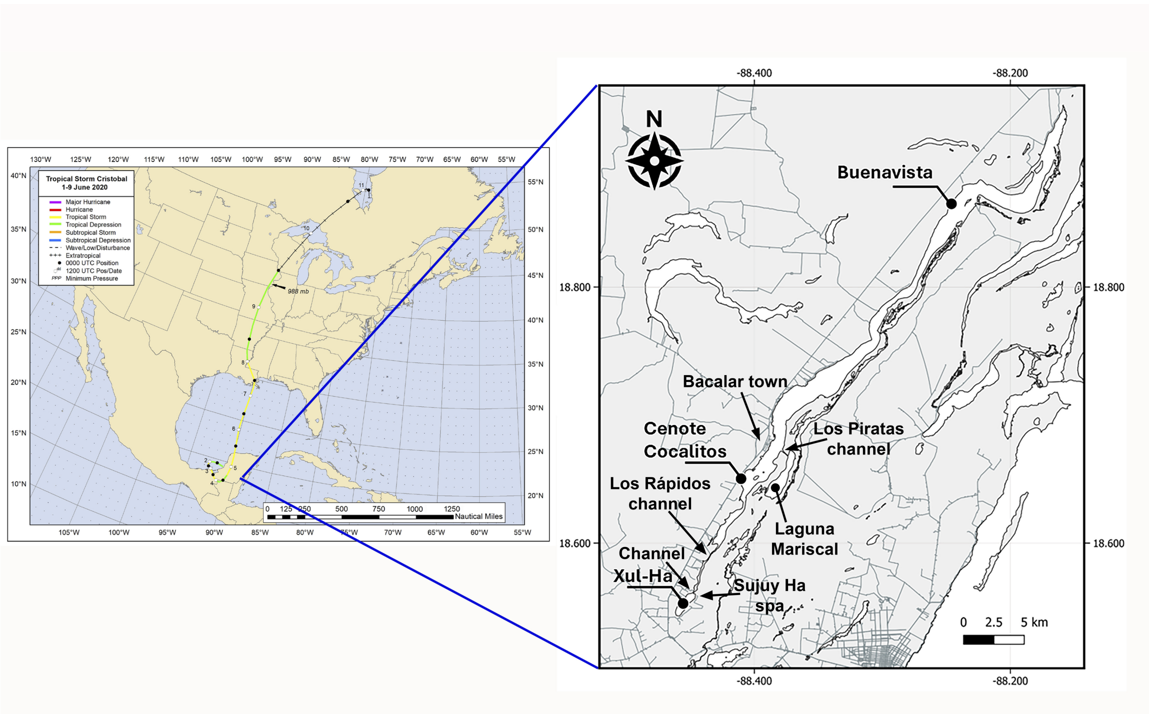

Figure 2. Study area. (**left**) Map showing the track positions for the tropical storm Cristobal, 1–9 June 2021 and the location of Lake Bacalar in the east of Yucatan Peninsula (Modified from Berg, 2021: https://www.nhc.noaa.gov/data/tcr/AL032020_Cristobal.pdf, accessed on 10 December 2024); (**right**) Lake Bacalar with the sampling points and localities mentioned in this work.

2.1. Sampling

A new sampling began on 18 July 2020, in the Cocalitos sinkhole (38 m maximum depth), located within Lake Bacalar (18.6510° N, −88.4097° W), where most of the previous baseline was established. Sampling continued from 20 September 2020, near Buenavista town shoreline (6 m maximum depth) (18.8657° N, −88.2460° W) to 20 June 2021. Only on 11 December 2020, the sampling was carried out in the southernmost lake connected to the Bacalar main system, Lake Xul-Ha (18.557° N, −88.446° W) (Figure 2). We undertook monthly sampling, using the light traps as documented for the nearby Cenote Azul [20].

Each trap was set at 2 m deep from the surface in all cases. After collection, the samples were sieved, washed, and fixed in 96° cold ethanol. All samples were stored at −18 °C for at least seven days. We measured the zooplankton volume with the displacement method [26]. At the same time, in the same points, we collected environmental data comprising temperature (°C), Secchi disk transparency (m), pH, conductivity (µs/cm), and total dissolved solids (TDS, ppm) with a Hanna HI9828 water quality multiparameter (Hanna Instruments, Woonsocket, RI, USA). We compared all this data with our historical archive.

We sorted the specimens from each sample under a stereomicroscope, and all representative morphospecies were photographed using a Zeiss stereomicroscope (Zeiss, Oberkochen, Germany) with an attached Eos Rebel T3i camera (Canon, Tokyo, Japan).

2.2. DNA Extraction and Amplification

For samples after brownification in all possible cases, five individuals of each morphospecies were processed to obtain the DNA barcodes using the following method: DNA extraction was performed using a standard glass fiber method [27]. For the PCR procedures, refer to [18]. We used the zooplankton primers suggested by [28]. PCR products were visualized on 2% agarose gels (E-Gel 96 Invitrogen, Invitrogen, Waltham, MA, USA), and positive PCR products were selected for sequencing bidirectionally at Macrogen in Korea. All sequences were edited using Codon Code Aligner v. 3.0.1 and uploaded to the BOLD database (boldsystems.org) and are available in the public dataset DS-BACBEFAF; DOI: dx.doi.org/10.5883/DS-BACBEFAF, where we included the previous baseline.

2.3. Sequence Processing and Analyses

All sequences were processed using the tools from BOLD to construct an IdTree. We compared the BINs with the ASAP method to compare the putative species present in Bacalar. Although this study is not taxonomic, the idea was to compare the differences in taxa present before and after the brownification. To estimate if there were significant differences in the species assemblages before and after brownification, we performed in the dataset obtained a one-way ANOSIM, using the Jaccard index with 9999 permutations in Vegan software v. 2.4-3 for R [29].

For each major group, we created a simplified visual representation of the BOLD trees using the Mega software v. 7.0 [30]. We highlighted the clusters of MOTUs that emerged after the brownification of the lake, making them easily distinguishable by using different colors. We also included the number of specimens sequenced in total, with the purpose of giving support to the branches in each MOTU, but they do not represent abundance.

3. Results

Environmental variables are given in Table 1. Among the main changes after brownification, we detected a slight decrease in the temperature of around 1 °C, most evident in the northern part of the lake. Secchi transparency also decreased by about 1.5 m, and pH moved from alkaline to neutral. Conductivity also decreased slightly, and the total dissolved solids increased.

The zooplankton volume declined from values over 140 mL/L to 20 or 40 mL/L, except in May 2021, when 80 mL/mL were measured (Table 2). The single sample from Xul-Ha in December 2020 had a displaced zooplankton volume of 420 mL/L.

Table 1. Average values (±SD) of environmental variables in Bacalar.

	Before Brownification		**After Brownification**	
	Cocalitos	**Buenavista**	**Cocalitos**	**Buenavista**
Temperature °C	29.78 ± 1.54	29.1	29.05 ± 1.97	28.14 ± 1.92
Secchi transparency (m)	5.77 ± 1.02	ND	4.13 ± 1.72	2.42 ± 0.71
pH	8.49 ± 0.73	ND	7.42 ± 0.54	8.02 ± 0.56
Conductivity (µS/cm)	2497 ± 250	3370	2230 ± 67	2759 ± 377
TDS (ppm)	784	1004	1112 ± 38	1317 ± 83

Table 2. Volume of zooplankton collected by light traps before and after brownification.

Cocalitos (Before Brownification)		**Cocalitos (After Brownification)**	
Date	**Zooplankton Volume (mL/L)**	**Date**	**Zooplankton Volume (mL/L)**
19 July 2015	240	19 July 2020	20
17 August 2015	260	21 August 2020	20
24 April 2016	260	20 September 2020	20
17 August 2016	380	20 October 2020	20
30 November 2016	140	21 November 2020	20
		20 December 2020	>20
		21 January 2021	40
		20 February 2021	20
		23 March 2021	20
		22 April 2021	20
		18 May 2021	80
		20 June 2021	20
Xul-Ha, unique sample		11 December 2020	420

In total, including previous data and those after brownification, we have 1195 sequences with a length >500 bp for Lake Bacalar (Public dataset in BOLD: DS-BACBEFAF; DOI: dx.doi.org/10.5883/DS-BACBEFAF), representing 121 putative species after the BINs, with 26 being identified to species level (Supplementary Figure S1). ASAP gave 100 MOTUs (putative species) (Supplementary Figure S2), and the difference from the BINs is mainly due to the presence of singletons and the haplotype variations of *Arrenurus ecosur* and *Arctodiaptomus* cf. *dorsalis* that received more than one BIN assignation in BOLD and could be only haplotype variation (Supplementary Figure S1 and Figure 3). The latter are all included in a subset of the ASAP results (see Supplementary Figure S2). We considered all major clades for the analyses, and all were found in both analyses. Accordingly, with ASAP results before brownification, we found 77 species. Subsequently, we found 38 putative species not found before, and adding the species found in both, we found 61 putative species (Supplementary Table S1). This led to the disappearance of 54 (70%) of the putative species found before the brownification. The one-way ANOSIM suggested significant differences (R = 0.74; p-value = 0.007) in the species assemblages before and after brownification.

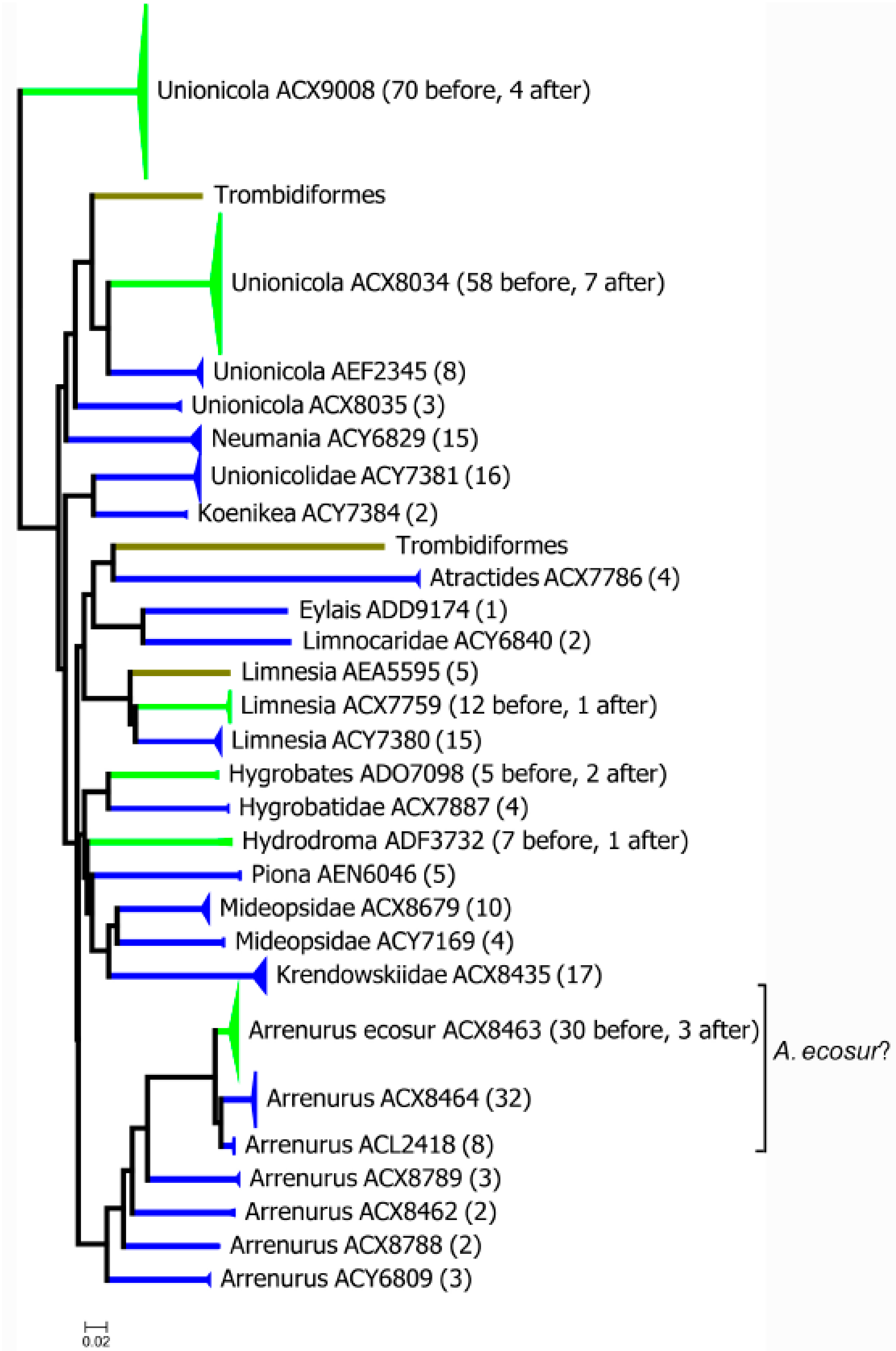

Figure 3. Water mites found in Lake Bacalar. After each name, the BIN appears. Between brackets, the number of sequenced specimens is given. Colors in branches are as follows: green when the MOTUs appeared both before and after brownification, and blue when they appeared only before. In brown are the ones that appeared only after brownification.

To facilitate the understanding of the results, we will include the BIN number after the Linnean name of each taxon in the text and the figures.

After brownification, the total number of MOTUs recognized for Lake Bacalar increased to 100, as original species were replaced by others, which will be explained in the following paragraphs.

In the case of water mites, 20 taxa disappeared during the brownification period (Figures 3 and 4). Only *Piona* (BIN AEN6046) was found in Xul-Ha in December 2020. In the case of six MOTUs found in both periods, three common MOTUs declined: *Unionicola* (BINs ACX9008, ACX8034) and *Arrenurus* (BIN ACX8463). At least their appearance in the light traps became much rarer.

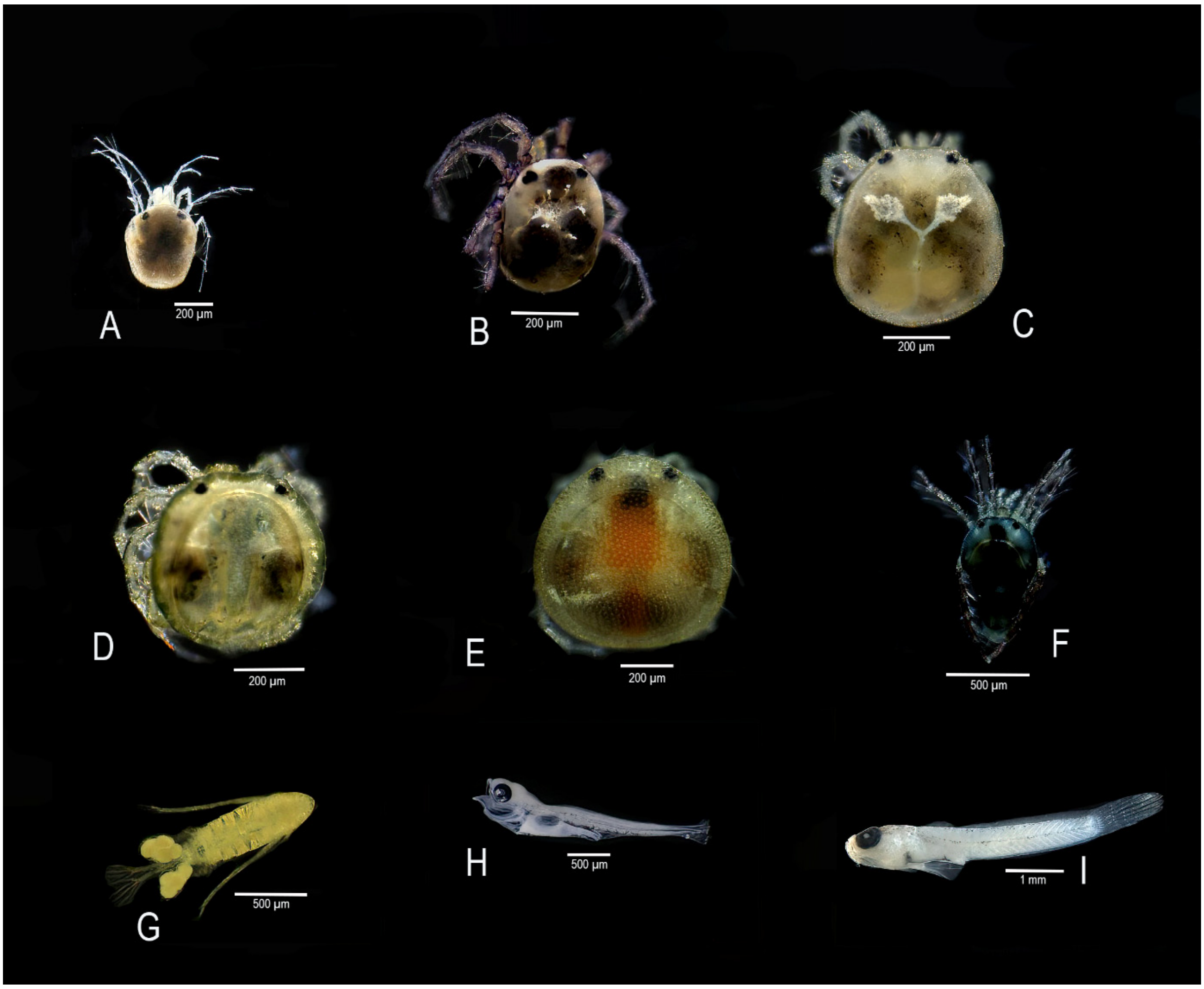

Figure 4. Examples of common species that were not found after brownification in Lake Bacalar. BINs are in brackets after the names. Water mites (**A**–**F**): (**A**) *Unionicola* (AEF2345); (**B**) *Neumania* (ACY6829); (**C**) *Koenikea* (ACY7381); (**D**) *Neoxystonotus* (ACX8679); (**E**) *Krendowskia* (ACX8435); (**F**) *Arrenurus* (ACX8464), Copepoda: (**G**) *Pseudodiaptomus marshi*. Actinopterygii: (**H**) *Bathygobius soporator* (AAA7195); (**I**) *Cyprinodon artifrons* (AAA8182).

The less dominant ostracods disappeared (BINs ADO1956, ABA7361); *Cypria* (BIN ACY1494) being the only species not affected (See Supplementary Figures S1 and S2).

Cladocerans were not common. *Ceriodaphnia* cf. *rigaudi* (BIN AAB5047) was dominant but became rare during brownification, and two Aloninae also disappeared (BINs AAB1004 and ACY0558). They were replaced by another Aloninae (BIN ACX5926), *Macrothrix elegans* (BIN AAD7091), *Grimaldina freyi* (BIN AES4225), and *Graptoleberis* cf. *testudinaria* (BIN ABW5248). All of them in small quantities (Figures 5 and 6).

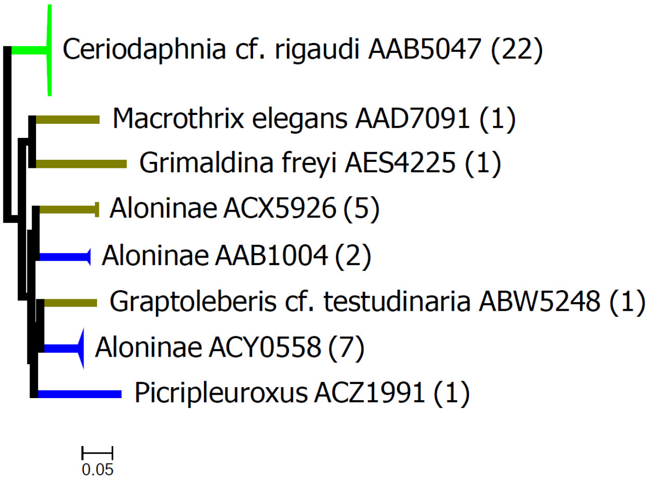

Figure 5. Cladocerans recorded in Lake Bacalar. After each name appears the BIN and number of specimens sequenced between brackets. The colors represent the same as Figure 3 in each branch.

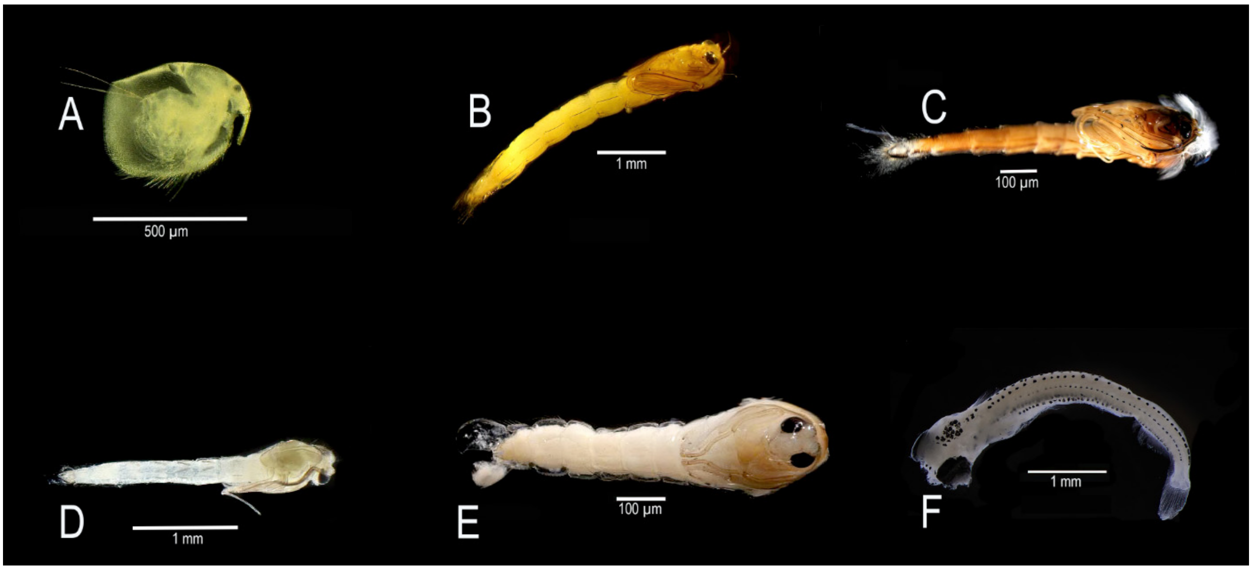

Figure 6. Examples of species that appeared after brownification in Lake Bacalar. BINs are in brackets after the names. Cladocerans: (**A**) *Grimaldina freyi* (AES4225). Chironomidae: (**B**–**D**) (ACR551, AEI8128, ADG7945); (**E**) *Coelotanypus* (ACX4768). (**F**) The rare Hemiramphidae, possibly belonging to the genus *Hyporamphus*, was found in Xul-Ha in December 2020.

The dominant zooplankton group was the copepods. In the case of cyclopoids, an important group of fish parasites disappeared during the brownification, the Ergasilidae (BINs ACX9579 and AEN6880), and was replaced by *Argulus* (BINs ADN4754 and ACX4312) in the samples (Supplementary Figures S1 and S2). *Thermocyclops inversus* (BIN AAB4353) became less common, and *Eucyclops* disappeared (BINs ABA1200 and AEA4964) (Figure 7), but it was not common before.

The most affected group was calanoids that in all previous samplings dominated the zooplankton. Notably, *Pseudodiaptomus marshi* (BIN AEO1594) disappeared entirely from the lake, except Xul-Ha in the southernmost part of Bacalar, highlighting the significant impact of brownification on this species and its distribution in the system.

Arctodiaptomus cf. *dorsalis* (BIN ACE4750 and others, see Supplementary Figures S1 and S2), almost disappeared but remained as a rare species (Figure 8). *P. marshi* was found in Lake Xul-Ha as a common zooplankter in December 2020 but was absent in the remaining part of Lake Bacalar (Figure 7).

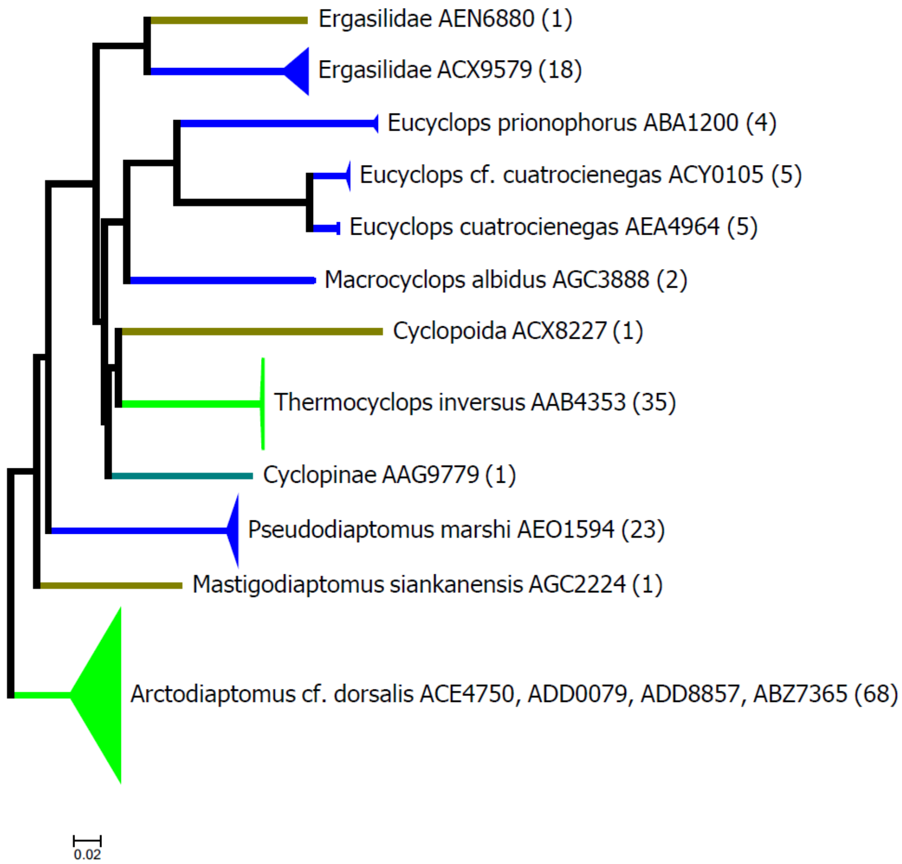

Figure 7. Copepoda from Lake Bacalar. After each name appears the BIN. The number of sequenced specimens is given between brackets. Colors are the same as in Figure 3.

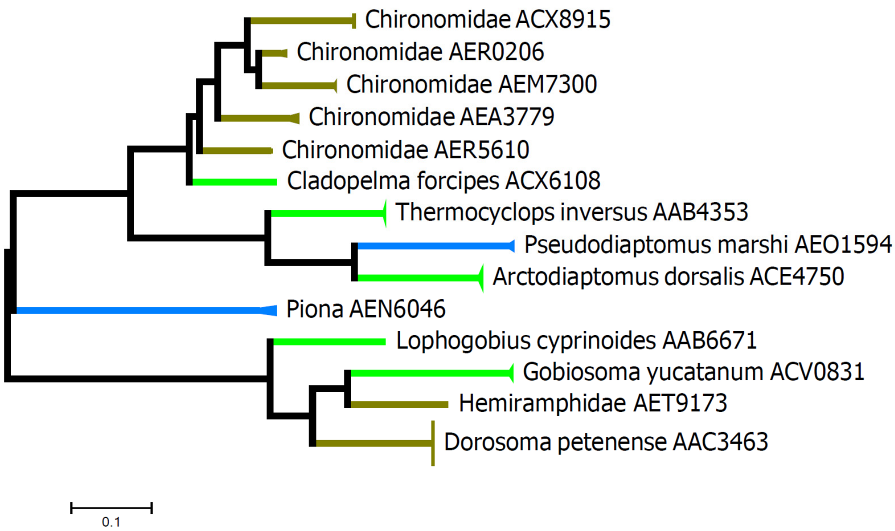

Figure 8. Species sequenced from Xul-Ha, collected on 11 December 2020. For illustrative purposes the colors in the branches are the same as in Figure 3. However, all of them were present after the brownification event. In this case, we sequenced from one to five representative specimens.

Mysidae, represented by an apparently undescribed species (BIN ACX6091), were common before brownification, after which they became rarer; however, they did not entirely disappear. The *Armases* larval stages (BIN ACX4604) and the amphipods (BIN ACX4754) were unaffected. Conversely, the Palaemonidae (BIN ACX4275) almost disappeared (Supplementary Figures S1 and S2).

The chironomid *Cladopelma forcipes* (BIN ACX6108) was common all the time. In contrast, other rarer species such as *Oxyethira pallida* (BIN AER5646) disappeared (Figure 9). The number of species of these dipterans increased dramatically after brownification. From eight putative species (BINs) to 18; of these, at least four became common (BINs ACR5551, AEI8128, ACX4768, and AEM7300) (Figures 6 and 9). The larvae of Ceratopogonidae flies became less rare during this period. Other larvae such as *Oxyethira pallida* (BIN AER5646) and Notonectidae (BIN ADN3958) disappeared.

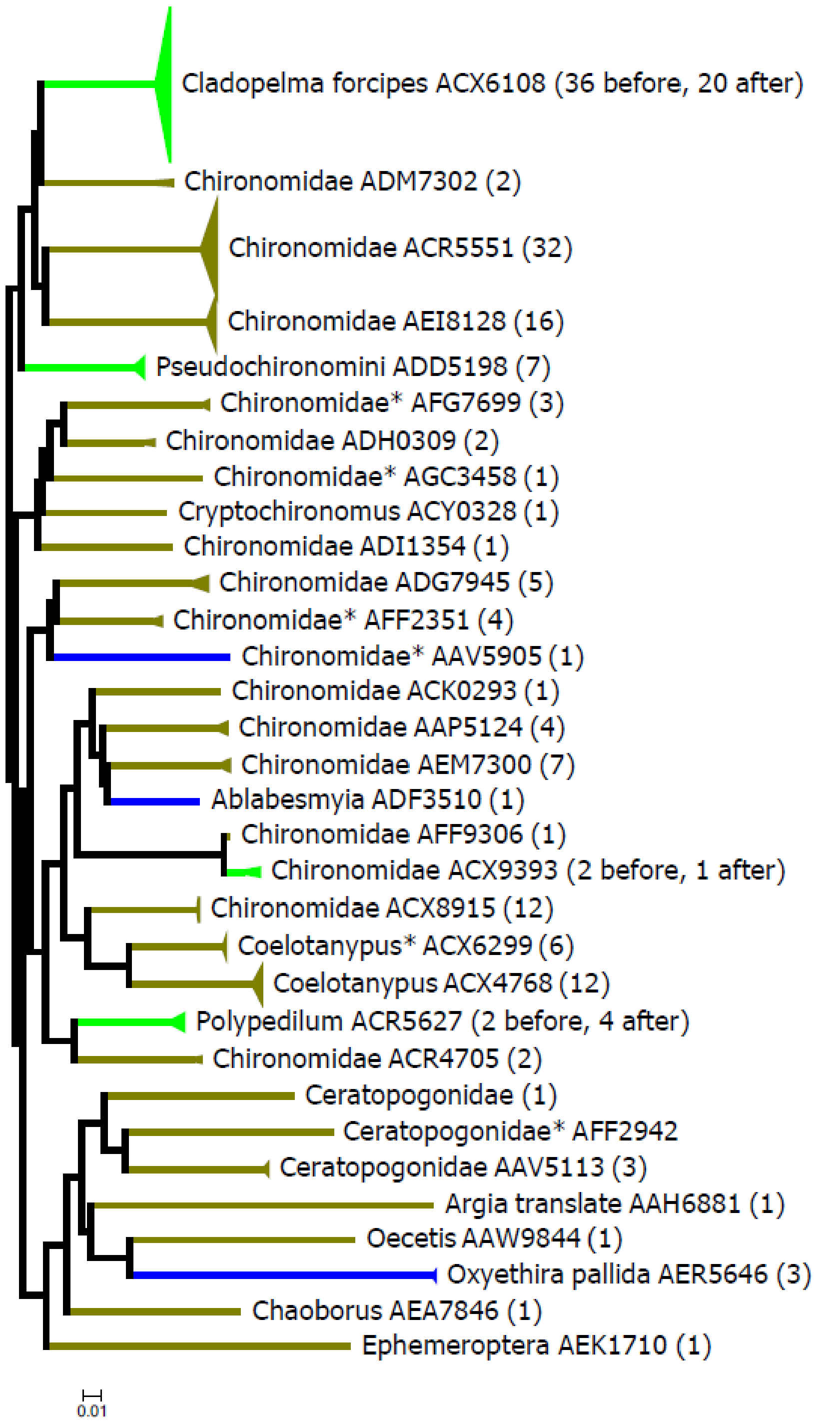

Figure 9. Meroplanktonic insects found in Lake Bacalar. After each name the BIN appears. The number of sequenced species is given in brackets. The colors are identical to Figure 3. * New records, being only found in Lake Bacalar.

Finally, fish larvae were severely affected. We did not find the six species previously recorded [31] during the brownification. Apparently, only *Lophogobius cyprinoides* (BIN AAB6671) did not become less common. Two other species, *Anchovia clupeoides* (BIN ACV0719) and *Gobiosoma yucatanum* (BIN ACV0831), persisted but became less common during the brownification (Figure 10).

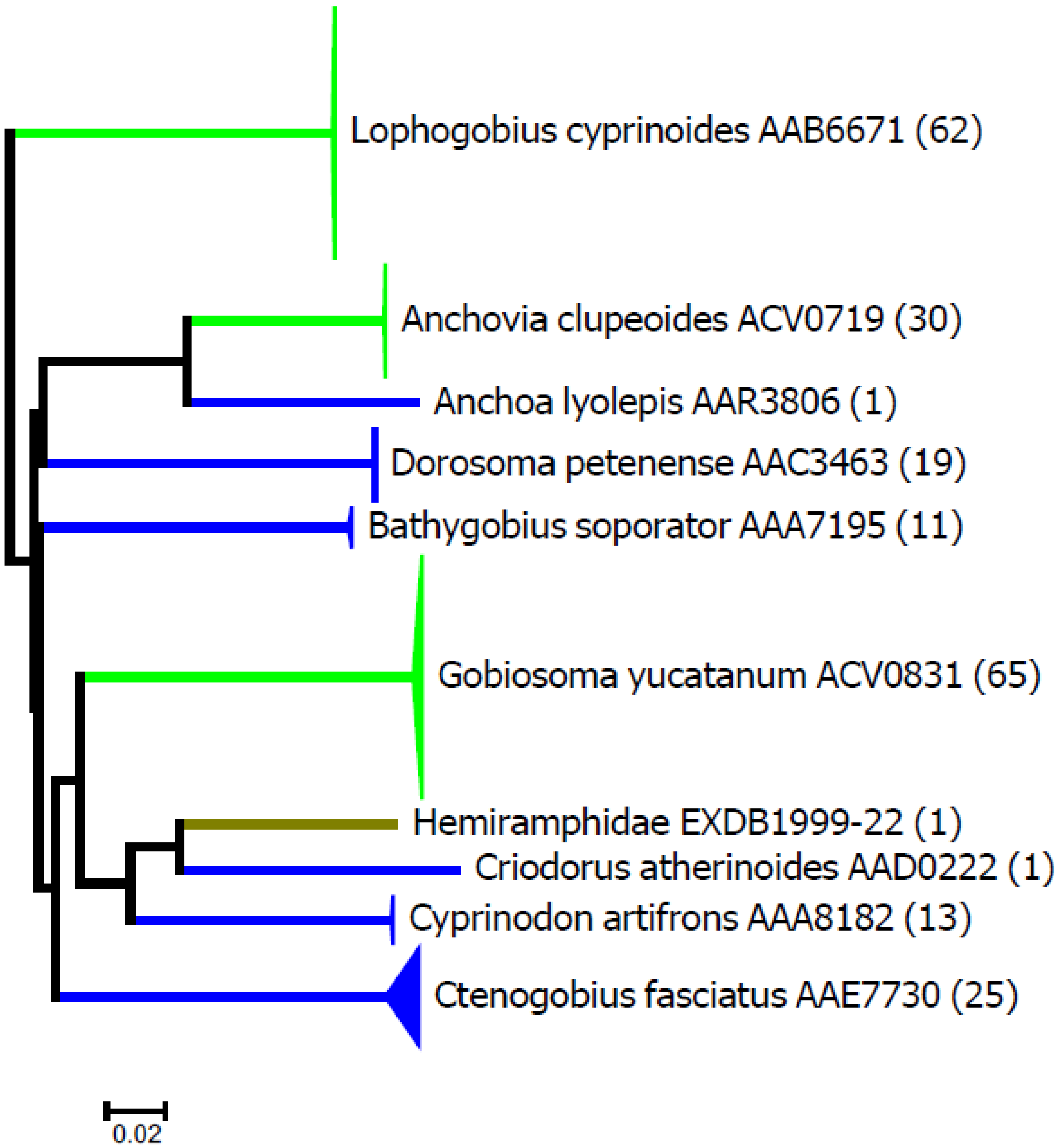

Figure 10. Fish larvae found in Lake Bacalar. After each name the BIN appears. The number of sequenced species is given between brackets. The colors represent the same as in Figure 3.

A unique larva of Hemiramphidae appeared in Xul-Ha (BIN AET9173), possibly belonging to the genus *Hyporhamphus*. It is a rare species in this system [32] (Figure 6). *Dorosoma petenense* (BIN AAC3463) was also rare before brownification in Bacalar; however, after this event, it was common in Xul-Ha (Figure 10).

4. Discussion

Brownification, a phenomenon previously observed in several temperate and boreal lakes [9–11,33,34], has now been documented for the first time in an oligotrophic tropical lake.

The environmental variables we measured clearly show changes after brownification, although their direct effect on the zooplankton community could not be measured. A combination of factors changed the trophic state of the system and affected the zooplankton and all the communities living in the lake. It is important to point out that this event occurred during the lockdown after the COVID-19 pandemic, so the activities in the lake and Bacalar town were minimal or absent, as previously mentioned.

The storm Cristobal hit the western shoreline of the Yucatan Peninsula, in the Gulf of Mexico (Campeche state) on 7 June 2020 [14] (Figure 2). The excess of water after its rains reached the eastern side, where Lake Bacalar is located, and less than one month later the color of the lake changed from blue to brown and then to greenish [14].

Reported values of nitrogen, phosphorous, and chlorophyll increased, and together with the physicochemical values that we measured, they confirmed the shift from an oligotrophic state to a mesotrophic one in the whole of Lake Bacalar [15], which did not change until 2022.

In the north of the lake, an intermittent creek was also flooded. However, we consider its amount of water insufficient in relation to the magnitude of the brownification event in the whole lake. Unfortunately, no data on the lake's water flows are available. We only roughly know the main surface currents through three narrow channels, one in front of Bacalar town (named Canal de los Piratas), with a main flow from Laguna Mariscal to Bacalar (Figure 2). The other two are Los Rápidos, where the biggest stromatolites are found, and the last channel, with no name, connecting the lake with the so-called Xul-Ha lagoon (see Figure 2). In these two, the flow is strong from south to north, and they are closely located in the southernmost part of the lake (Figure 11). There is no data about the volume of water passing through these channels, and Xul-Ha does not have any superficial inflow, although the surrounding landscape is elevated.

Most of the underground water flowing to the lake was enriched with nutrients when it percolated from the surface and passed through the south of the peninsula before arriving at the lake. The water arriving here is also richer in Ca^{2+}, HCO_3^- and Sr^{2+} as a result of dissolving the karst characteristic for this region [23]. Xul-Ha is connected to another more local groundwater system that comes from the western side, being one of the main inflows near the Sujuy-Ha spa (pers. obs. ME-G, MV-M) in the east of the lagoon (see Figure 1). As underground water is cooler than the lake water, this explains the declining average temperature after brownification. The excess of soluble tannins and nutrients [14,15] may explain the changes in coloration, the decrease in Secchi transparency and pH, and the increase of TDS values. Later, the growth of phytoplankton resulted in the green coloration of the waters. All the changes observed affected the zooplankton, which reacted almost immediately, as described.

When we started this sampling, we did not expect to see a dramatic shift in the zooplankton community. However, we knew the effectiveness of the light traps [20,35], and we knew that they are effective even in systems with low Secchi transparency [35]. The advantage of working with this previous methodology was, apart to ease the comparisons, the possibility of storing the samples and, after sequencing, keeping vouchers for the specimens instead of obtaining only different haplotypes. Additionally, we have a more complete picture of the biodiversity of this lake. Even though we do not know the species, mainly of mites and chironomids, we keep the sequenced specimens in the Zooplankton

Reference Collection at El Colegio de la Frontera Sur. With time, they will be described as we previously did with some of the water mites [24].

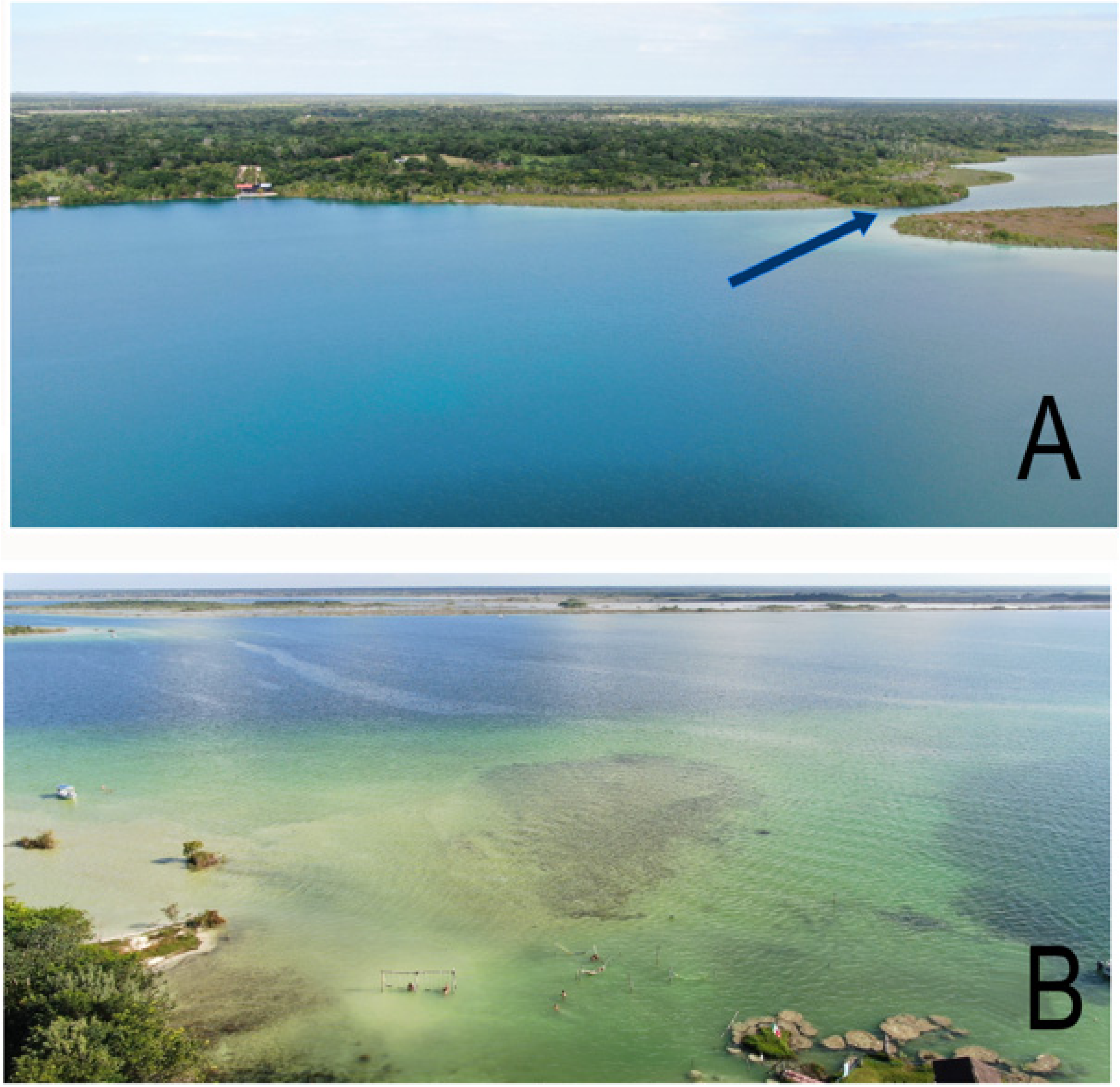

Figure 11. Aerial photos. (**A**) Xul-Ha Lagoon on 11 December 2020. The arrow shows the water flow to Lake Bacalar; (**B**) Cocalitos sinkhole area on 23 December 2020.

A limitation of the traps is that they have yet to be calibrated for quantitative analyses. However, they give an idea of the most common species, and the results can be compared with previous surveys. The changes in the catch of biomass were evident (Table 2; Supplementary Figure S3). It was surprising that the most common species disappeared from the system, and did not recover throughout the year, despite our sampling of both the northern and southern sites of the lake. We also did not expect that Xul-Ha would become a refuge for the species of the original fauna, where they coexisted with the new inhabitants of the lake (Figure 6). Possibly, a slight nutrient enrichment of the waters in this region led to the observed increase in biomass (Table 2; Supplementary Figure S3).

After examining sediments, Elías-Gutiérrez and Smirnov [36] concluded that the scarcity of cladocerans occurred also in other lentic systems from Yucatan. However, after brownification we found a few cladocerans typical of eutrophic environments, such as *Graptoleberis* cf. *testudinaria* (BIN ABW5248), *Macrothrix elegans* (BIN AAD7091), and *Grimaldina freyi* (BIN AES4225).

Among the most fragile species were the two dominant diaptomid copepods: *Arctodiaptomus* cf. *dorsalis*, which remained in scarce numbers, and *Pseudodiaptomus marshi*, which disappeared from the system during the brownification, together with most water mites. The few *A.* cf. *dorsalis* that remained became rarer whereas they previously appeared abundantly in the samples.

In the case of water mites, the disappearance of 20 of the 29 taxa registered previously and the decrease in appearance in the light traps prove their high sensitivity to changes. Previous studies in the Neotropics have documented a clear and predictable relationship between water quality and water mite fauna composition [37]. For example, at low water quality deterioration, sensitive species are replaced by more broadly tolerant species [38], and moderate water quality degradation decreases the species diversity and abundance of water mites, while severe pollution causes their disappearance [39]. Fish larvae were also negatively affected, as indicated by the disappearance of six species, but we do not know the effect of brownification on fish reproduction in tropical environments. The almost instantaneous vanishing of most taxa reflects brownification as a drastic event in Lake Bacalar, leading to community collapse.

Other groups, such as mysids and some larvae like the *Armases*, were less affected, while palaemonids were more susceptible to environmental deterioration. As we do not know their biology, we can only say that they are quite fragile to drastic environmental changes.

Among the insects, chironomids from eutrophic environments of southeastern Mexico appeared in the lake such as *Coelotanypus* (BIN ACX4768) together with other non-identified chironomids (BIN AEI8128). Two more species were distributed from Mexico to Costa Rica, known from eutrophic systems (BINs ACR5551 and AEM7300). All these species, together with rarer ones, took advantage of the brownification event. The change was sudden, especially for the insects, particularly Diptera. However, comparison with other studies is complicated because most of these have been long-term [40].

Finally, due to the urgency of the sudden brownification of Lake Bacalar following the tropical storm Cristobal [14] and the lack of funds for scientific studies already explained in the introduction, it was not possible to work similarly to what we did previously with the fish in the same lake using eDNA metabarcoding [41]. As a result, we decided to implement the light traps and follow the same approach as in the baseline study [18], storing the samples in cold conditions until we could sequence the specimens with the Sanger method.

Biomonitoring with metabarcoding has been proposed as a promising tool to replace the routine sampling and physical observations of organisms in the microscope. In general, metabarcoding cannot be used for quantitative analyses and is applicable only to track the species [42]. However, a crucial previous step to any metabarcoding study focused on a community is to have a baseline of the species for a given aquatic ecosystem [43]. Many limnologists recommend eDNA metabarcoding for biomonitoring [44–49]. However, the advantage of the method presented here against metabarcoding is registering the new species that appeared and not previously seen. With eDNA metabarcoding, we will only have haplotypes without the possibility of establishing if they are false positives.

Some limnologists consider the Sanger method obsolete, and nanopore technology seems a promising tool in the sequencing of individuals [50–52] which we consider a good alternative because demultiplexing allows us to link the sequence with the voucher in a collection, and the precision of this method has been improved with the new Flongle flow cells [53]. However, some limitations persist, such as the need for a higher number of specimens to lower the costs, so the Sanger method will continue being used in the short future.

5. Conclusions

The immediate shift in the zooplankton community following the brownification event in Lake Bacalar clearly indicates the event's impact on the entire ecosystem. The total number of species decreased from 77 to 61; however, there was a notable change in the composition of the Chironomidae and the water mites. In other cases, such as the copepods, six species disappeared, including one very common previously (*Pseudodiaptomus marshi*), while the most common became rare (*Arctodiaptomus* cf. *dorsalis*). Fish larvae were also sensitive to the change, with the disappearance of six species.

Although we did not evaluate all affected communities, the change in the phytoplankton community is evident, as indicated by the shift towards green coloration of the entire system. This alteration had implications for all other links in the trophic chain, a factor we unfortunately did not measure due to several limitations.

These changes were reflected in the environmental variables, such as the lowering of Secchi transparency and pH and an increase in conductivity and TDS.

When we finished the sampling, Lake Bacalar had not yet fully recovered. Almost two years passed before the lake returned to its initial color and Secchi transparency conditions.

Xul-Ha Lagoon is a system connected to Lake Bacalar, and its groundwater source differs from the main lake's. It is an important inflow in the southern part that retained its original blue color and became where the original fauna remained during the brownification.

Zooplankton is an excellent indicator of the lake's health and conservation status. Permanent periodical biomonitoring is urgently needed because tourist development in the region is increasing rapidly, often without consideration of the environment.

If this situation persists within the basin, including the region's groundwaters, we may reach a point of no return. Lake Bacalar could not recover from this accelerated eutrophication, leading to the loss of this unique ecosystem, which hosts the world's biggest microbialites (locally known as stromatolites).

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d17010058/s1, Supplementary Figure S1. BOLD tree of the 1216 sequences in the dataset DS-BACBEAF Bacalar before and after brownification. All specimens collected after brownification are preceded by the word "BROW". Supplementary Figure S2. Subsets (or putative species) proposed after ASAP analysis. Supplementary Figure S3. Changes observed in the biomass collected by the light traps in the month of December in Xul-Ha (2020) and Cocalitos sinkhole (2016 and 2020). All specimens collected after brownification are preceded by the word "BROW". Supplementary Table S1. List of all species found in Lake Bacalar before brownification, after it, and the ones found in both. All of them are found in the datataset DS-BACBEFAF; DOI: dx.doi.org/10.5883/DS-BACBEFAF.

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