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BIOLOGICAL DIVERSITY OF PHYTOPLANKTON AND PHYTOPERIPHYTON IN THE MADEIRA RIVER BASIN, AMAZON, BRAZIL

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Abstract

Floristic composition, abundance, richness and many other ecological patterns of phytoplankton and phytoperiphyton were studied in the Madeira River basin, between the Abunã River and Porto Velho City (Rondônia - Brazil), taking into account the hydrological period of the region. The results were correlated with abiotic factors, including the nutrients Na^+, K^+, Ca^{2+}, Mg^{2+}, $N\text{-}NO_3^-$, $P\text{-}PO_4^{3-}$ and chlorophyll-*a*. 283 taxa on the Madeira River and 327 taxa in the tributaries were identified belonging to six divisions and nine taxonomic classes. It was observed a considerable complexity in floristic composition, with a greater contribution from the division Chlorophyta, especially during the high water periods, when there was greater species richness recorded. Ecological indexes and statistical analysis confirmed strong seasonal pattern and differentiation between the species present in the white and clear waters.

Keywords: *floristic composition, diversity index, species richness, landscape ecology, Amazonia.*

1. Introduction

The Madeira River basin is located in the southwestern Amazonian region, and draining an enormous watershed frequently covered by Tropical Rain- Forest. The Madeira River stands out for help with a huge load of suspended sediment transported to the Amazon River basin, with low organic matter content and high clay and silt, both associated with metallic elements such as iron, manganese and mercury (Lechler *et al*., 2000; Alves *et al*., 2004; Dosseto *et al*., 2006; Guyot *et al*., 2007). Important anthropogenic activities as mining and gold mining, upstream from Porto Velho City, and the construction of the Santo Antônio and Jirau hydroelectrics, close to the Jaciparaná City (Rondônia State) can be highlighted. The hydroeletrics, as well as the formation of the respective reservoirs, will affect the speed of current flow and sediment suspended load, and therefore are also expected changes in the pattern of ecological diversity region (the authors note).

The Madeira River plays an important role in national integration through its Brazilian waterway, which has more than 920 km navigable between Porto Velho and its confluence with the Amazon River, about 150 km downstream of the Manaus City (Amazonas State). The waterway has allowed the colonization of the region for the flow of minerals and agricultural products, especially soybeans, beyond oil, timber and construction materials (Alves *et al*., 2004).

The first ecological studies in the Madeira River basin overlap with the research developed in the Amazon River basin should be noted in this regard the survey Gibbs (1967), which allowed the first estimates of the sediment load transported in the region. In the 80s studies were intensified in the Madeira River basin, especially in the headwaters and middle course. In this period began investigating the environmental impacts from mining and the effects of metal contaminants, especially mercury, on the aquatic fauna and flora, as well as the first research on the chemical and ecological aspects of the Madeira River. These studies have been widely disseminated and continue today.

The activities for the installation of hydroelectric plants on the Madeira River began in 2003. With the start of construction in 2008, several environmental studies intensified to better understand its biodiversity. However, floristic composition researches including composition of phytoplankton and phytoperiphyton in white waters are still relatively uncommon in the region, and studies of the scope that was given in this research had not been conducted in Madeira River basin. The main objective of this research was to study the floristic composition, abundance and species richness, and many other ecological factors of phytoplankton and phytoperiphyton organisms presents at the headwaters of the Madeira River basin, responding the questions: Could the seasonality be influencing significantly the nutrients concentration (N and P) in the water column? Which is the importance of seasonality (spatial and temporal variation) on phytoplankton and periphyton distribution?

2. Materials and Methods

2.1. Study area

The Madeira River is via the integration of the Western Amazon, being considered a binational river, by making the border between Brazil and Bolivia. It is formed from the confluence of the Beni and Mamoré rivers, both with springs in the Bolivian Andes (Fig 1), with a total length of 3600 km, and 1459 km located in Brazilian territory (SEDAM, 2002; Alves *et al*., 2004). The Madeira River basin has a total area of 1.4 x 10^6 km^2, representing between 20-23% of the Amazon basin, capturing 18% of the precipitation of rain and contributing with 15-17% of the water volume of the Amazon River (Molinier *et al*., 1995; Goulding *et al*., 2003; Bastos *et al*., 2006). The mean annual discharge of the Madeira River was estimated between 23,000 and 31,200 m^3 s^{-1} at the mouth near the Amazon River (SEDAM, 2002; Molinier *et al*., 1995; Goulding *et al*., 2003), with minimum values (low water) and maximum values (high water) ranging between 5000 and 48,000 m^3 s^{-1} (Pfeiffer *et al*., 1991; SEDAM, 2002).

The Andean rivers carry a huge load of suspended material containing clay-silty sediments rich in dissolved ions, which will sedimenting along the Amazonian lowlands bringing fertility to the floodplain. This load is estimated in 500 x 10^6 t $year^{-1}$, with 50% transported by the Beni River (Guyot, 1993). It is also estimated that the Madeira River basin may be responsible for the transportation of 37 up to 450 x 10^6 t $year^{-1}$ of sediment, depending on the hydrological period (Mortatti *et al*., 1989; Aalto *et al*., 2003; Latrubesse and Stevaux, 2003), reaching 14 x 10^8 t $year^{-1}$ during the rainy season (Guyot, 1993; Mortatti *et al*., 1989).

Located on the left bank of the Madeira River is the Abuna River (Fig 1), its main and largest tributary that is resulting from the confluence of Kharamanú and Chipamanú rivers, both from the Bolivian Andean Plateau. As Madeira River, the Abunã River is a binational, bordering between Brazil and Bolivia, through the Acre and Rondônia states (Alves *et al*., 2004). In the study area we also highlight the forest-river São Simão by the left margin, and the Mutumparaná, Cotia and Jaciparaná rivers by the right margin (Fig 1).

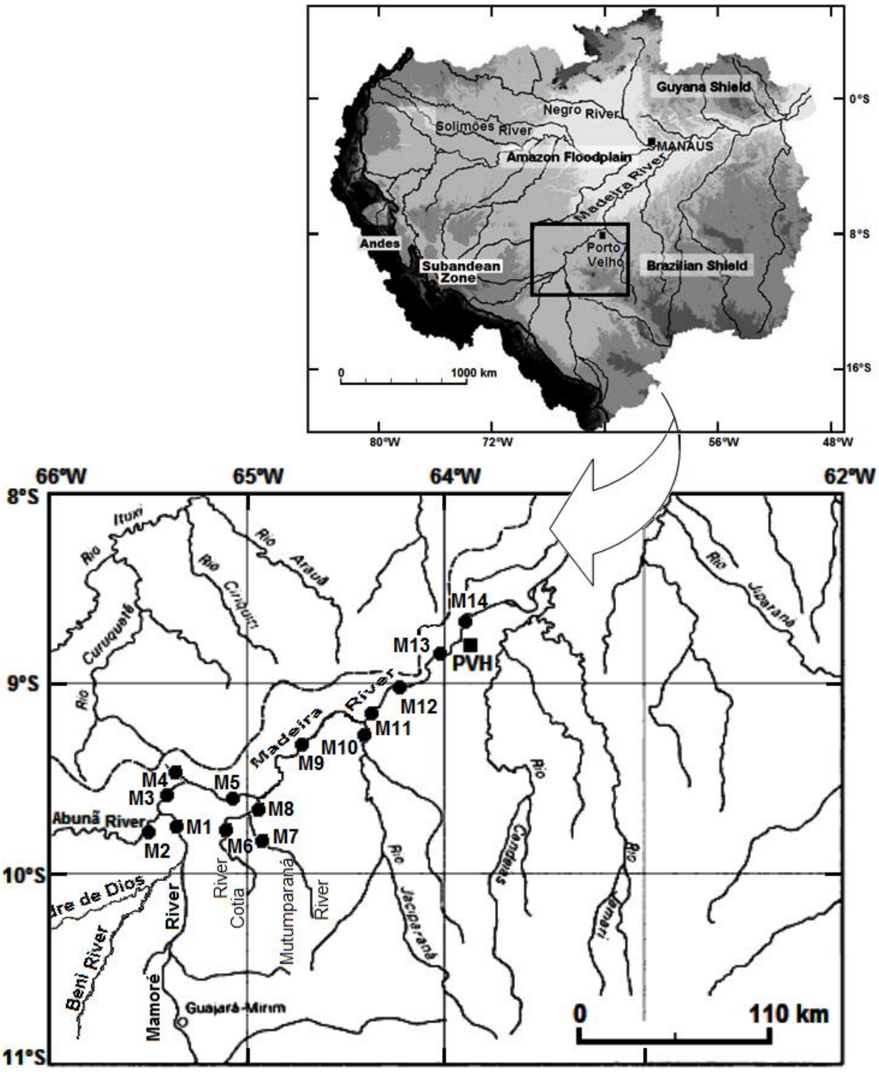

Fig 1: Location of sampling stations in the river Madeira (RO) and its tributaries (Source: Goulding *et al*., 2003 modified).

2.2. Analytical procedures

Samplings were undertaken at 14 sampling stations along the Madeira River basin from its confluence with Abunã River, in the border between Brazil and Bolivia, to Porto Velho City (Fig 1), during the high and low waters periods between the years 2003 and 2006. At each sampling station the limnological parameters water temperature (Temp °C), pH, conductivity (EC μS_{25} cm^{-1}), dissolved oxygen (DO mg L^{-1}) and total dissolved solids (TDS mg L^{-1}) were measured with a multi parameter WTW and YSI probe. Volatile (VSS mg L^{-1}) and fix (FSS mg L^{-1}) solids suspended were determined with Millipore's filters. Water transparency was measured with Secchi disc (∅= 0.30 m), and total turbidity (NTU) was determined with a portable turbidity meter. Na^+, K^+, Ca^{2+} and Mg^{2+} (mg L^{-1}) ions were determined by an atomic absorption spectrophotometer according to APHA (2012) procedures; nitrate (N-NO_3^- mg L^{-1}) was determined by cadmium column with NO_3^- to NO_2^- reduction in all filtered samples (Valderrama, 1981), and orthophosphate (P-PO_4^{3-} mg L^{-1}) was determined by ammonium molybdate method (Valderrama, 1981). Density of water (g cm^{-3}) was calculated from temperature table (Wetzel and Likens, 2000). Chlorophyll-*a* (Chl-*a* µg L^{-1}) was determined by colorimetric method (664 ηm) with extraction by acetone 90% in Whatman GF-F filters according to APHA (2012). All laboratorial analysis was developing in duplicate following the analytical procedures descript in Wetzel and Likens (2000) and APHA (2012).

The qualitative phytoplankton samples were collected with 20 µm plankton net and the quantitative phytoplankton samples with 10 liters bottle, both at the surface (0.0-0.3 m proof) of each sampling station. The phytoperiphyton was taken manually with soft brush, and all samples were fixed immediately with 1% acetic lugol´s solution. For identification of the phytoplankton the material was examined with a Zeiss phase contrast binocular microscope using lugol and/or methylene blue as contrast, and for the identification of the material the following bibliographies were consulted Bicudo and Bicudo (1970), Bourrelly (1970), Round (1971), Uherkovich, and Franken (1980), Van Den Hoek (1994) and Van Den Hoek *et al*. (1995). The nomenclature was checked according to the Guiry and Guiry´s Algae Base (2013). The score of material was done according to Utermöhl (1958) with an inverted microscope and Sedwick-Rafter's sedimentation chambers of 5 mL volume. Cells, colonies and filaments were quantified according to random fields´ method (Uhelinger, 1964). Based on the minimum area method and considering the samples with the greatest number of species, a constant number of fields were counted (n= 30). Chlorophyll-*a* was used as an indicator of algal biomass, as a measure to be effective in the evaluation of the biomass of photosynthetic phytoperiphyton communities (Castro *et al*., 2008). The score limit has been established according to the rarefaction curve of species, having been closed once reached the total of 100 individuals of the most common species (Bicudo, 1990).

2.3. Data analyses

For the community structure analyses the following data were considered: species richness (S = taxa total number found); population density (D = ind mL^{-1}) calculated according to Weber (1973); equitability (J_s) according to Pielou (1966); relative abundance (R_a %) and total abundance (T_a %) calculated from direct score of the organisms in the Sedwick-Rafter's chambers. The frequency of occurrence of the taxa along the river (F_{river} %) and as a function of the hydrological period (F_{hydro} %) both were calculated following the equation $F=a.100/A$ (were a= number of samples that the taxa occurred, and A= total number of samples). The frequency results were classified as: a lot of frequent to F>70%, frequent to 40%<F≤70%, few frequent to 10%<F≤40%, and sporadic to F≤10%. The Shannon-Wiener specific diversity index (H_0) (Shannon, and Weaver, 1963), the species richness index (Margalef – S_g; Menhinick – S_n) and the Dajoz constancy index (Dajoz, 1978) were also calculated in accordance to the descriptor analysis (Magurran, 1988; Krebs, 1999). The modification rate of the community was estimated by the sucessional rate described by Lewis (1978). The relationship between the abiotic limnological parameters and the phytoplankton and phytoperiphyton were determined by the Principal Component Analysis (PCA) with a varimax rotation, and the spatial vs. temporal variability of phytoplankton and phytoperiphyton was studied applying two-way ANOVA hypothesis test.

3. Results and Discussion

3.1. Abundance and occurrence

Phytoplankton can contribute significantly with the global primary production of the aquatic ecosystems, forming the basis of their food chain. In this study 283 taxa were observed in the main channel of the Madeira River and 327 taxa in the tributaries. Both results were divided into six divisions and nine classes. In general, unique species of algae of white waters and clear waters were observed in the Madeira River basin, specially in the tributaries – Mutumparaná River (station M7) and Jaciparaná River (station M11), – as had been observed in previous studies (Aprile *et al*., 2013). The relative abundance analysis showed that of the six divisions found, the one with the highest abundance was the Chlorophyta. This division dominated the region with 56.2% of the total taxa identified on the Madeira River and 50.8% in the tributaries. Subsequently, we observed a predominance of Chromophyta division with 25.8% and 28.7% of the total, respectively (Figs 2A and 2B). The distribution of taxa per class showed greater abundance of *Zygnemaphyceae* with 129 taxa identified throughout the basin, followed by the *Bacillariophyceae* class with 70 taxa in the Madeira River and 85 taxa in the tributaries; *Euglenophyceae* with 31 and 35 taxa, respectively; *Chlorophyceae* with 27 and 34 taxa; *Cyanophyceae* with l8 and 24 taxa; *Chrysophyceae* with 3 and 9 taxa; *Oedogoniophyceae* with 3 taxa each; *Dinophyceae* with 1 and 5 taxa, and *Rhodophyceae* class with 1 taxa in the main channel of the Madeira River and 3 taxa identified in the tributaries (Figs 3A and 3B).

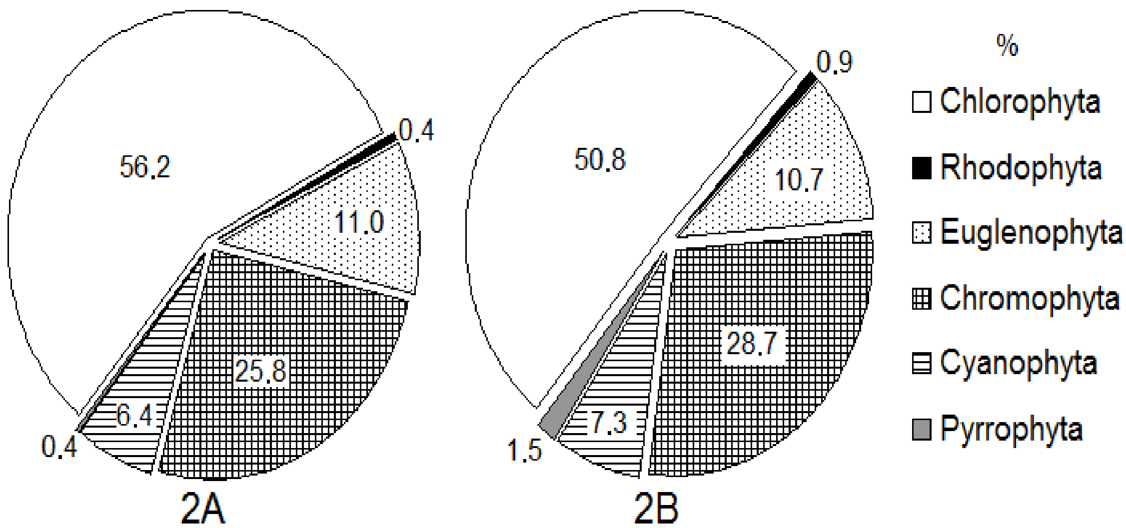

Fig 2: Taxonomic divisions in the (2A) main channel and (2B) tributaries of the Madeira River basin.

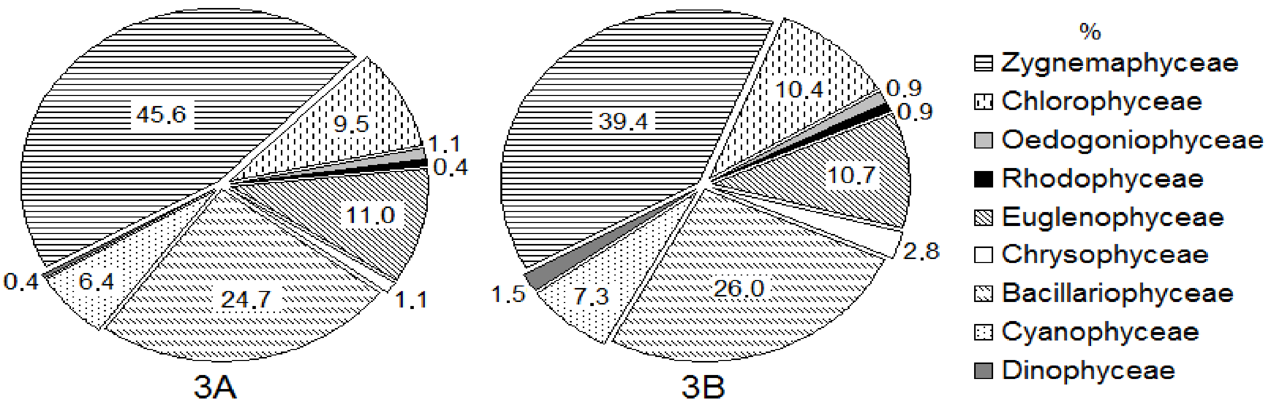

Fig 3: Taxonomic classes in the (3A) main channel and (3B) tributaries of the Madeira River basin.

The frequency of occurrence of taxa confirmed the trend seen in the relative abundance of the divisions, and the most frequent groups were Chlorophyta and Chromophyta with 45.7% and 38.3% in the Madeira River and 39.5% and 37.5% in the tributaries (Figs 4A and 4B). The frequency of occurrence of taxa per class, calculated for the Madeira River and tributaries for hydrological period (Figs 5A and 5B), indicated that the high water periods were the periods with highest occurrences of taxa, except for the *Rhodophyceae* class in the Madeira River, seen only during the low water periods. The high water periods have its maximum volume of water between January and February, and is marked by increased melting of the ice caps of the Andes, carrying large amount of suspended matter, mainly composed of fine particles of silt-sandy and sandy-clay, rich in mineral elements (Alves *et al*., 2004; Guyot *et al*., 2007). There is also increasing the volume of water in forest-rivers that feed the tributaries, and that carrying in their waters humic compounds from the decomposition processes of litter. It is a period marked by significant increase in the flow, with the transportation of large amount of macrophytes and residual of plants (branches, stems and leaves). It is believed that increasing the frequency of occurrence of phytoplankton and phytoperiphyton organisms during the flood is due to a greater transport of these organisms by the Madeira River basin, especially from the forest-rivers, which serve as a great outlet for the Amazon region. The various ecological relationships between the planktonic organisms, and among them its occurrence and abundance are directly related to fluctuations in environmental conditions (Paloheimo and Zimmerman, 1983; Paloheimo *et al*., 1983).

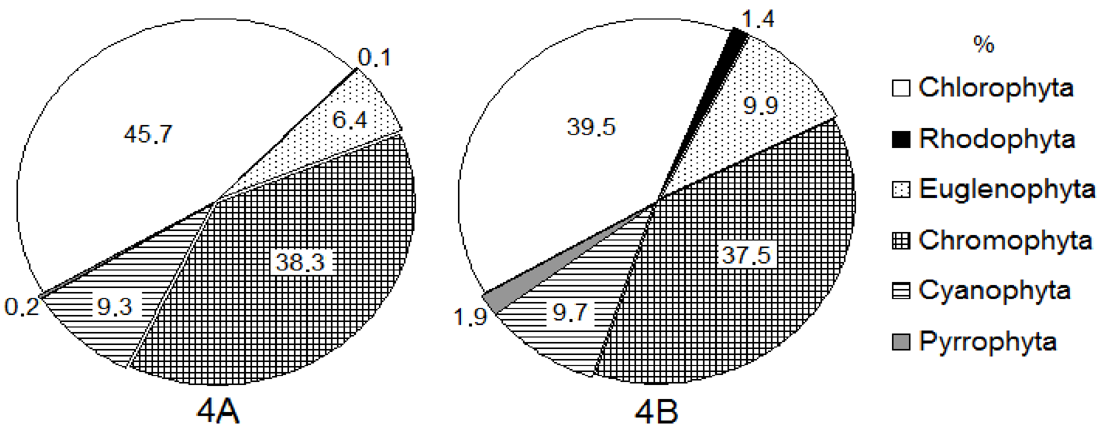

Fig 4: Frequency of occurrence of the taxonomic divisions in the (4A) main channel and (4B) tributaries of the Madeira River basin.

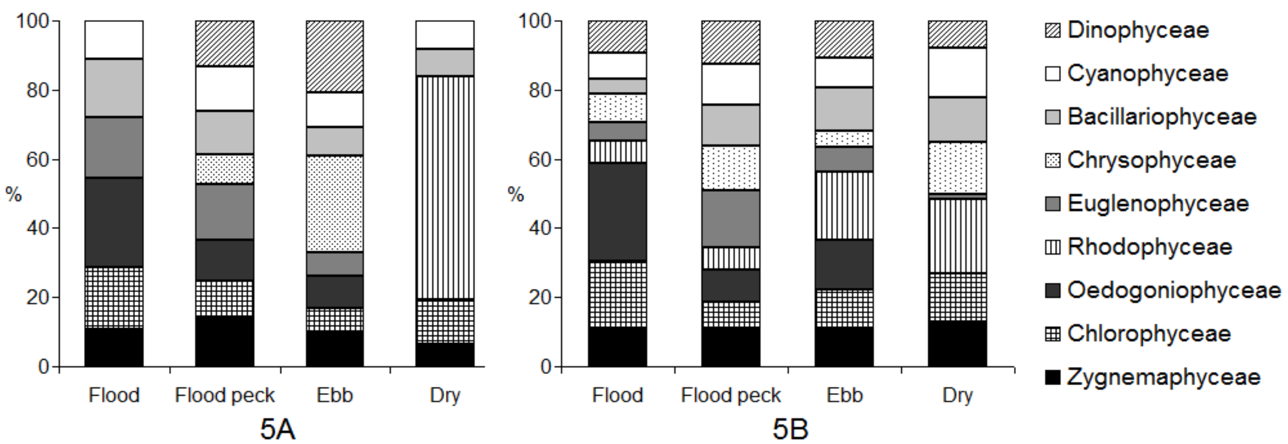

Fig 5: Frequency of occurrence of the taxonomic classes in the (5A) main channel (n= 1144) and (5B) tributaries (n= 935) according to hydrological period.

The decrease of the richness of species in drought phase may be associated with reduced supply of dissolved nutrients, since there is a reduction in decomposition processes. However, the biggest change in the environment was the physical-chemical conditions. In periods of low water was observed a significant decrease in the levels of TDS in the Madeira River and $N\text{-}NO_3^-$ in the entire basin. According to some authors, the dynamics and taxonomic variety of the plankton depend on the geological and chemical characteristics of lake environments (Gates, 1983; Paloheimo *et al*., 1983). Seasonal changes of physical and chemical variables can and should be interfering with the distribution of planktonic organisms in the channel of the Madeira River. Studies have shown that changes in trophic levels within an aquatic environment can interfere with the whole supply of the trophic system (Sprules *et al*., 1983). The division Rhodophyta, of which the unique specie found in the main channel of the Madeira River was *Batrachospermum cayennense*, was the only one division that had the highest frequency of occurrence in the low water periods. However, due to the low sample size found, it is not possible to make any consideration of this behavior.

The most frequent divisions (Chlorophyta and Chromophyta) showed higher density in high water periods throughout the river basin (Fig 6), especially for *Zygnemaphyceae* class. From the results it was possible to confirm the trend of the division Chlorophyta species being highly cosmopolitan. In the Chlorophyta division the predominant genres were: *Closterium*, *Staurastrum*, *Cosmarium* and *Micrasterias*. In the Chromophyta division the predominant genres were: *Eunotia*, *Nitzschia*, *Surirella* and *Pinnularia*. In the division Phyrrophyta the taxa showed no occurrence equitable throughout the hydrological period, and in the main channel of the Madeira River the distribution remained concentrated between the flood and ebb periods. From these trends, observed throughout the study area, it is suggested that these behaviors may be associated with both hydrological and nutritional patterns.

3.2. Influence of nutrients

The general trend was to decrease the concentration of nutrients during the transition from the high water to low water periods (Fig 6). The exception was the concentration of chlorophyll-*a*, which increased during the dry season (Fig 6D). The chlorophyll-*a* values in the main channel of the Madeira River were significantly lower (mean 2.1±1.4 µg L^{-1}) than in the tributaries (mean 4.2±2.1 µg L^{-1}). Under the spatial perspective, the chlorophyll-*a* showed relatively homogeneous distribution on the Madeira River, contrary to what was observed in the rivers Cotia (station M6) and Mutumparaná (station M7). This trend is due to several limnological and hydrological factors acting simultaneously, particularly transparency, light intensity and flow velocity. According to some authors, the decrease in the concentration of chlorophyll-*a* in the ecosystem is expected with the increasing of the flow velocity (Taniguchi *et al*., 2005), which in this case always occurs during the high water periods, when the river flow can reach 50 x 10^3 m^3 s^{-1} (SEDAM, 2002). The flow velocity can influence direct or indirectly the productivity of lotic ecosystems, interfering in metabolic rates that control nutrient uptake by phytoplankton and phytoperiphyton. Analyzing the relationship between nutrient content, hydrological period and density of phytoplankton and phytoperiphyton organisms, it is observed that nitrate (Fig 6B) showed a direct relationship with the density of organisms in both the main channel of the river and tributaries. Several studies discuss the role of phosphorus, and in particular $P\text{-}PO_4^{3-}$, as a limiting factor to productivity, richness and abundance of phytoplankton, but in this case it seems $N\text{-}NO_3^-$ was the main driver of population growth pattern of algae. The highest concentrations of $N\text{-}NO_3^-$ were observed during of high water periods in the Madeira River (mean 1.31 mg L^{-1}), coinciding with the highest density of taxa of the most of identified divisions, while the lowest concentrations of $N\text{-}NO_3^-$ were determined in the tributaries, during the low water periods (mean 0.33 mg L^{-1}), when was observed the lowest densities of phytoplankton. Aquatic environments where the concentration of nutrients varies according to hydrological period they are subject to variation in the diversity and richness of phytoplankton, since the increase of the nutrients concentration give opportunity to the rare species to develop.

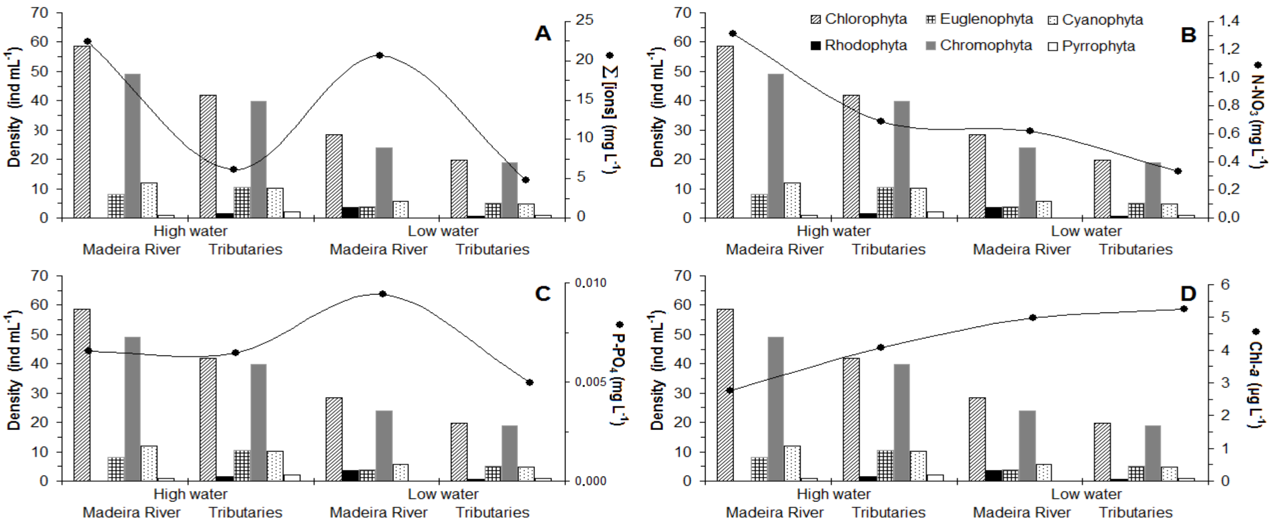

Fig 6: Relationship between the density of the taxonomic divisions and nutrient concentration (A) Na^+, K^+, Ca^{2+} and Mg^{2+} ions; (B) $N\text{-}NO_3^-$, (C) $P\text{-}PO_4^{3-}$ and (D) chlorophyll-*a* (bars with seasonal mean values).

3.3. Ecological patterns

In the spatial-temporal aspect was observed a trend in reducing the number of taxa from the headwaters of the Madeira River (station M1) towards the station M14, near the Porto Velho City, in both hydrological periods. This trend was also observed in the relative abundance (A_r) showed in Table 1. This behavior is due to two factors acting together: 1) the biggest load of suspended sediments associated with a higher concentration of dissolved ions near the headwaters of the Madeira River, and 2) the presence of forest-rivers of clear water as the rivers Cotia and Mutumparaná in the upper basin, which contribute to the irradiation of taxa to the main channel of the Madeira River. As one moves from the headwaters to the Porto Velho City (station M14), we noted a reduction in the concentration of nutrients, especially nitrate, potassium, sodium and magnesium.

Diversity indices were applied aiming to describe the biological communities in terms of richness and uniformity of species. The Diversity Index (H_0) showed values close to 0.38 bits ind^{-1} in the basin during high water periods, and 0.53 bits ind^{-1} during the low water periods (Table 1). This trend was also observed in the calculation of evenness (J_s), confirming the seasonal pattern for the river basin. The evenness measure compared the Shannon-Wiener diversity with the distribution of species observed, maximizing the diversity. For this study, the values calculated for H_0 based on the Shannon-Wiener index were low, however, were quite consistent for Amazonian fluvial systems, particularly for lotic systems with a predominance of white waters with low transparency (Secchi <0.20 m) and limit of the euphotic zone (Z_{eu} <0.50 m). This fact restricts the photosynthetic activity in the main channel of the Madeira River only the top layer, unlike what occurs in the rivers of clear waters, where transparency and euphotic zone are much larger. The Menhinick (S_n) richness index of species for the Madeira River basin showed significant difference spatial-temporal, with higher values in the tributaries during the high water periods. The same trend was observed for the Margalef index (S_g) (Table 1). These calculations confirm the trend of the curves of abundance and density of the species, indicating that although there has been reduced richness during the low water periods (especially in drought phase) the reducing of the total number of individuals was more pronounced. The succession rate of added differences (SDI) of Lewis´s (Lewis, 1978) (Table 1) showed that there was a significant difference seasonal between the hydrological periods evaluated (p >0.05) and compared between the white waters of the Madeira River and clear waters of the tributaries.

Table 1: Ecological attributes applies to the analysis of the phytoplankton community structure.

	Madeira River		Tributaries	
Indexes	**H**	**L**	**H**	**L**
S (n)	192	91	204	123
D (ind ml^{-1})	128.3	62.3	105.8	50.0
J_S	0.051	0.081	0.049	0.076
A_r (%)	67.3	32.7	67.9	32.1
H_0 (bits ind^{-1})	0.38	0.53	0.38	0.52
S_g	27.1	12.8	29.7	17.8
S_n	5.7	2.7	6.7	4.0
SDI	0.67	0.33	0.68	0.32

H= high water level; L= low water level.

The highest values of specific richness (S) were determined for headwaters of the Madeira River, between the stations M1 and M4, during the high water periods (Fig 7A), probably influenced by discharge of clear waters from forest-rivers. The values of diversity (H_0) and evenness (J_S) increased significantly towards the station M14 (Figs 7B and 7C). The evenness was low (<0.08) indicating the presence of dominant taxa. In the main channel of the Madeira River were identified the following dominant taxa: Chlorophyta – *Closterium aciculare* T.West, *C. gracile* f. *gracillima* West, *C. kuetzingii* Brébisson, *C. praelongum* var. *brevius* (Nordstedt) Willi Krieger and *C. setaceum* Ehrenberg ex Ralfs; Chromophyta – *Aulacoseira granulata* var. *angustissima* (O.F.Müller) Simonsen. In tributaries: Chlorophyta – *C. aciculare* T.West, *C. lunula* Ehrenberg & F.G.Hemprich ex J.Ralfs, *C. pseudolunula* var. *concavum* K.Förster & F.Eckert, *Cosmarium decoratum* West & G.S.West and *Gonatozygon monotaenium* De Bary; Chromophyta – *A. granulata* var. *angustissima* (O.F.Müller) Simonsen, *A. granulata* (Ehrenberg) Simonsen and *Dinobryon cylindricum* O.E.Imhof; Cyanophyta – *Aphanothece clathrata* West & G.S.West, *A. saxicola* Nägeli and *Aphanizomenon flosaquae* Ralfs ex Bornet & Flahault; Pyrrophyta – *Glenodinium pernardiforme* (Lindemann) Schiller.

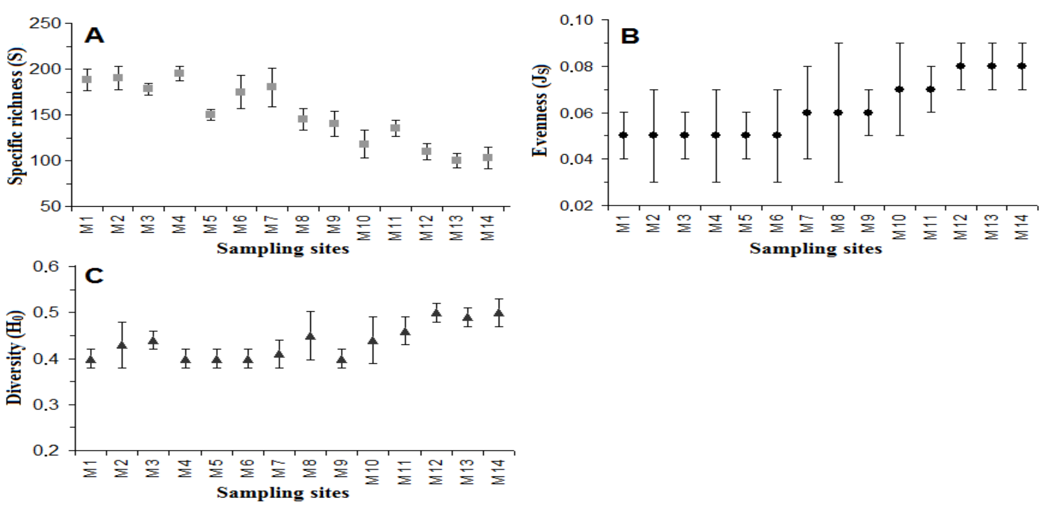

Fig 7: A) Specific richness (S), B) evenness (J_S) and C) diversity (H_0) of the phytoplankton and phytoperiphyton at the Madeira River Basin, Amazon.

The constancy index analysis (Fig 8), which illustrates the similarities in community composition in the Madeira River basin, took into consideration the absent taxa and present taxa, this accidental, accessory and constant. In general, the total of 283 taxa identified in the Madeira River and 327 taxa identified in the tributaries, the vast majority has ranked between accidental and accessory. The highlights were the taxonomic classes *Zygnemaphyceae*, *Oedogoniophyceae* and *Euglenophyceae* in Madeira River, with rates above 60% accidental (Fig 8A) and the classes *Rhodophyceae* and *Dinophyceae* in tributaries, with rates above 80% accessory (Fig 8B). The difference between the constants and accidentals number of taxa in the Madeira River is probably due to variation of some limnological patterns in the drainage basin, especially the load of suspended solids (Aprile *et al*., 2013). The constant species are those that best characterize the assemblies and generally are the species best adapted to the environment. The difference between the number of constant taxa in Madeira River and tributaries is probably due to the difference in the size of drainage basins. Normally, on equal terms, the larger area has the greater diversity. Of the total constant taxa 26 taxa were exclusive of the Madeira River, 12 taxa were exclusive of the tributaries, and 14 taxa were found in both Madeira River and tributaries. These two groups are the center of the phytoplankton assemblies with characteristics of the basin as a whole.

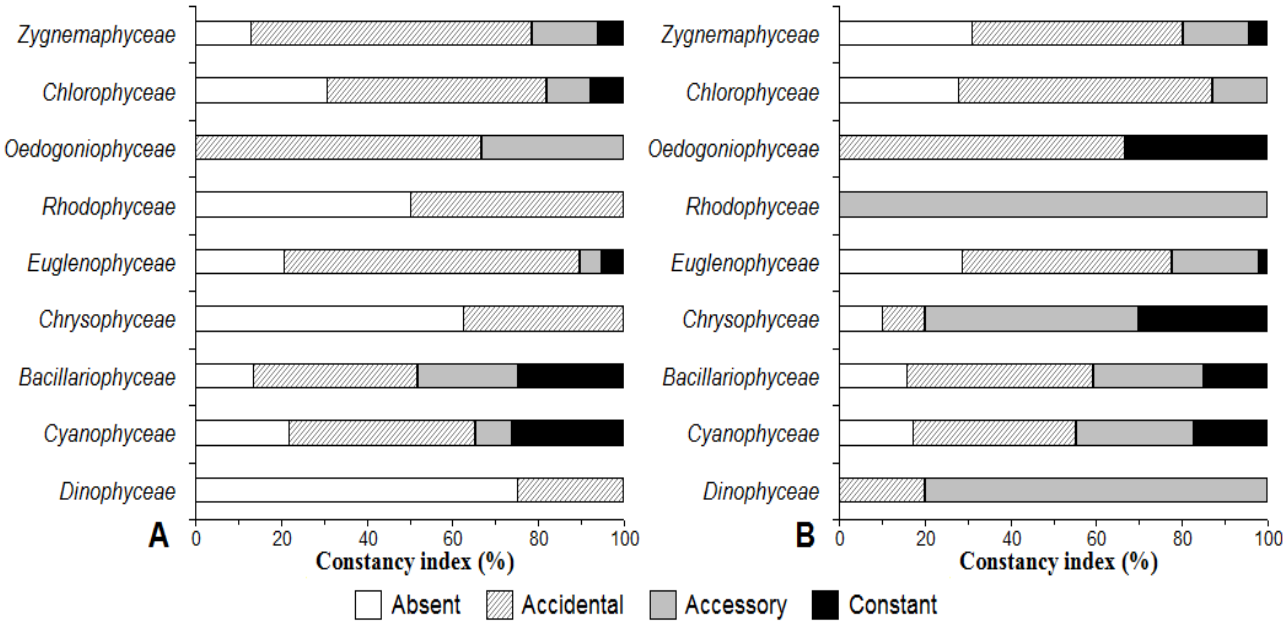

Fig 8: Dajoz constancy index (Dajoz, 1978) for the taxons identified in the Madeira River basin.

3.4. Statistical analysis

Based on the results of the physical, chemical, physical-chemical and biological variables measured, a principal component analysis (PCA) was developed, taking into account the seasonality (high and low waters periods), spatial distribution and climatic influence. The analysis allowed to indicate the variables associated with each factor, and to what degree of association, in order to identify which of the variables were primarily responsible for biological diversity and abundance of phytoplankton and phytoperiphyton. In the analysis presented in Table 2, it was decided to select three components to explain the behavior of the variables applied, with a total explanation percentage of 89.6%. Below that, with two components, the explanation percentage was 82.3%, and above that, with four components, the explanation percentage was 94.4%, or an increase of only 4.8% (Table 3). It was noted that the biological variables, represented by divisions with higher incidence (Chlorophyta, Chromophyta, Euglenophyta and Cyanophyta) in both the main channel of the Madeira River and tributaries, had a better connection between the factors (components) 1 and 2, projected that relationship in Fig 9.

Phytoplankton plays a central role in the variability of ecosystems studied, and is significantly correlated (p= 0.01), especially with nitrate, temperature, TDS load and potassium levels. In hydrological periods of drought (low water), the concentrations of the divisions Euglenophyta, Rhodophyta and Pyrrophyta were greatly reduced, impeding to observe a clear relationship between these groups and the environmental factors studied. Particularly about the population of Euglenophyta, the group can be closely correlated with other groups of algae, which suggests the possibility that Euglenophyta using alternative nutritional sources such as organic matter with ethyl compound (Rosowski, 2003).

Table 2: Factor coordinates of the variables based on correlations active and supplementary* variables.

Variable	Factor 1	Factor 2	Factor 3
Secchi	0.7938	-0.4552	0.0831
Temp	-0.9018	-0.1464	-0.2798
pH	-0.5274	0.7740	0.0465
EC	-0.8426	0.5025	0.0544
TDS	-0.9034	-0.0798	0.0874
Na	-0.6522	0.7235	0.0545
K	-0.8631	-0.2448	-0.0450
Ca	-0.7761	0.5927	0.0474
Mg	-0.8376	0.5122	0.0555
N-NO_3	-0.7969	-0.5252	-0.0188
P-PO_4	-0.0677	0.0201	0.8957
Clh-*a*	0.1708	0.5754	-0.4466
Chlorophyta	-0.4619	-0.8783	-0.0178
Euglenophyta	-0.4619	-0.8783	-0.0178
Chromophyta	-0.4619	-0.8783	-0.0178
Cyanophyta	-0.4619	-0.8783	-0.0178
*DO	-0.0474	-0.7709	-0.0431
*FSS	0.8966	0.0477	-0.1229
*VSS	0.8313	0.2205	0.0884
*Density	0.6638	0.7323	-0.0108
*S	0.0219	0.8580	-0.0202
*S_g	-0.0947	0.8322	-0.0327
*S_n	-0.4075	0.6803	-0.0636
*N	0.6826	0.7262	0.0030
*Ln N	0.6756	0.7219	0.0017
Explain %	**45.3**	**37.4**	**6.9**
Accumulation %	**45.3**	**82.6**	**89.6**

Table 3: Eigenvalues of correlation matrix and related statistics.

Values	Eigenvalue	% Total variance	Cumulative eigenvalue	Cumulative %
1	7.2	45.3	7.2	45.3
2	6.0	37.4	13.2	82.6
3	1.1	6.9	14.3	89.6
4	0.8	4.8	15.1	94.4

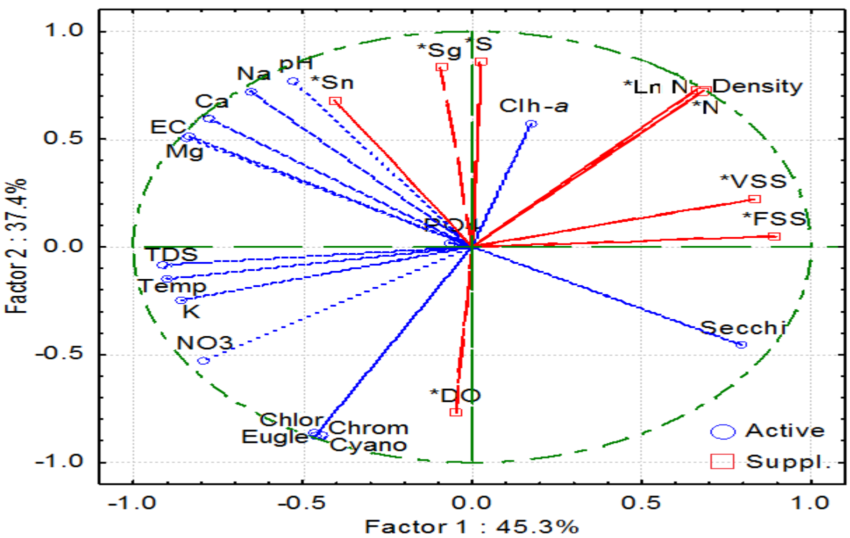

Fig 9: Projection of the variables on the factor-plane (1 x 2).

The two-way ANOVA (Table 4) was applied as hypothesis testing statistical tool to request if there was a significant interaction between the two selected sources of variability (i.e. sampling time and station), specially if temporal changes were significant or not. The results confirmed that the mean values were statistically meaningful and can be used to discuss the data. Besides, the hypothesis test confirmed presence of seasonality, indicating enough differences between high and low water levels on the phytoplankton and phytoperiphyton communities.

Table 4: 2-way ANOVA test to mean values (high and low water levels) in the Madeira River basin.

	Sum of Squares ($*10^3$)	**df**	**Mean Square** ($*10^3$)	**F**	***p***
Treatments	11613.94	24	483.91	77.6783	<0.0001
Blocks	856.03	27	31.71	5.0893	<0.0001
Residual	4036.86	648	6.23		
Total	16506.83	699*			

*df 699 = 25 variables X 14 sampling sites X 2 periods (high and low water levels) - 1

4. Conclusions

The results showed that there is a strong seasonality due to the hydrological period, with largest abundance and diversity during the high water periods (flood). In addition, the forest-rivers and small rivers that flow into the main channel of the Madeira River, contribute significantly to the increase of biodiversity in the basin. Ecological relationships analyzed and confirmed by statistical tests showed that the phytoplankton and phytoperiphyton communities reflecting both the physical-chemical and climate conditions of the Madeira River basin, responding mainly to nitrate concentrations, temperature, TDS load and potassium levels. The taxonomic groups cluster themselves strongly on the volume of water, and contrary to what is usually proposed in research of this nature, it was not due to phosphorous levels, but due to the nitrogen concentration ($N\text{-}NO_3^-$), considered the main drive-force of species abundance in the region.

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