Seasonal comparison of the molar ratio of water and periphyton in a tropical dam (Lago das Niféias, São Paulo/SP)

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ABSTRACT
The molar ratio of water and periphytic material in a shallow mesotrophic dam was evaluated at four times of the year (spring, summer, autumn and winter) from isolated and combined enrichments by N and P. Four treatments were designed using nutrient diffuser substrate (polystyrene cups, filled with Agar solution and nutrients – control: no addition of nutrients; N+: 0.75 M; P+: 0.05 M; NP+: combined addition of the two salts, molar ratio N:P = 15). The opening of the cups was coated with a 20 µm mesh as a substrate for the periphyton. Collections were carried out on the 15th, 20th, 25th and 30th days of colonization. The greatest variability of abiotic limnological data was attributed to the type of treatment, followed by the annual scale. The PCA indicated a strong association between the addition of phosphorus and, mainly, combined addition with the higher levels of periphyton phosphorus content, as well as the treatments without phosphorus addition with the N:P ratio of periphyton. Phosphorus can be considered the driver of the molar ratio values found in both water and periphyton, followed by low temperature in the cold months.

Keywords: Periphyton, Molar ratio, Phosphorus.

INTRODUCTION

Periphytic algae play an important role in the functioning of shallow lakes and reservoirs, as they act as an important primary producer and source of food for the other trophic levels (Vadeboncoeur & Steinman, 2002). The structure and functioning of the periphyton in aquatic ecosystems are directly or indirectly influenced by numerous abiotic and biotic factors (Stevenson 1997). Abiotic factors, especially the concentration of nutrients in the water, are considered good predictors of periphytic algal abundance in tropical lakes and reservoirs (Vercellino & Bicudo, 2006). Considering the issue of enrichment in the aquatic environment, nitrogen and/or phosphorus is considered the main trigger of eutrophication, since such nutrients are commonly found to limit the growth of algae in general, including periphytic ones and, when available in larger quantities, are

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quickly assimilated, causing structural and functional changes in aquatic communities (Borchardt, 1996; Dodds, 20023).

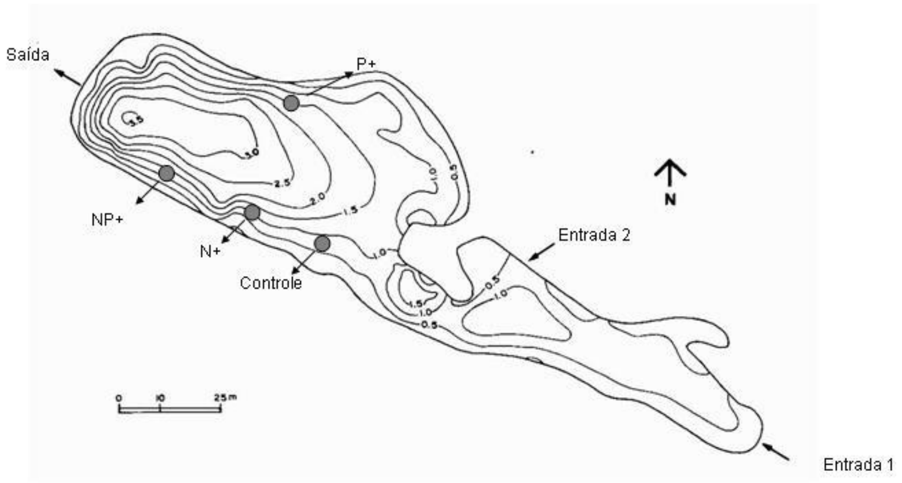
The use of periphyton to assess nutrient availability is still strongly centered in lotic systems, especially in temperate regions (Biggs, 1995; Borchardt, 1996; Francoeur *et al*., 1999). Studies in subtropic regions have been increasing and have given great impetus to this approach. They are mainly concentrated in some ecosystems, such as the Florida Everglades (Vymazal *et al*., 1994; McCormick & Stevenson, 1998; McCormick *et al*., 1998, 2001) and Lake Okeechobe, also in Florida (Havens *et al*., 1996; Zimba, 1998; Vadeboncoeur & Steinman, 2002). In tropical environments, and specifically in the Fontes do Ipiranga State Park (PEFI/São Paulo), there are studies with periphyton: in conditions of experimental oligotrophication in a eutrophic reservoir (Barcelos, 2003), addition of nutrients in an oligotrophic reservoir (Ferragut & Bicudo, 2009; Ferragut & Bicudo, 2010; Ferragut & Bicudo, 2012).

The objective of this study was to evaluate, in an experiment using a nutrient diffuser substrate, the influence of temperature and concentrations of nutrients, nitrogen and phosphorus, on the abundance of periphyton and on the water underlying the substrates, by means of molar ratio N/P. The objectives were: a) to compare the molar ratio of periphyton by nitrogen and phosphorus content with the molar ratio of water on a temporal scale in the four seasons of the year; b) to verify the limnological variables analyzed by statistically comparing them in their different enrichment treatments.

FIELD OF STUDY

The Lake of the Water Limphs (Figure 1) is located in the Fontes do Ipiranga State Park (PEFI), (parallels 23o38'08" S and 23o40'18" S and meridians 46o36'48" W and 46o38'00" W), a Conservation Unit circumscribed in the urban fabric of São Paulo. It is a polymythic mesotrophic system (Bicudo, D. *et al*. (2002).

Figure 1. Bathymetric map of the Lake of the Water Nympheas (PEFI, São Paulo) with the location of the entrances and exits and the experiments (control and treatments N+, P+ and NP+). Modified from Bicudo, C. *et al*. (2002).



MATERIAL AND METHODS

Experimental design - The experiment consisted of a control (without the addition of salts) and three enriched treatments: N+ (isolated addition of nitrogen, 0.75 M of NaNO3, P-limiting condition), P+ (isolated addition of phosphorus, 0.05 M of Na2HPO4, N-limiting condition) and NP+ (combined addition of the two salts, molar ratio N:P=15). The four treatments were positioned in the coastal region of the dam in the direction of the water flow and distanced from each other to avoid contamination between treatments (Figure 1). The nutrient diffusing substrates (SDN) were made of polystyrene cup (330 cm³, height 110 mm, opening diameter 80 mm) filled with 2% Agar solution and salts, according to the treatment. Its opening was coated with a 20 µm monofilament mesh (phytopankton mesh mesh), which served as a substrate for the development of the periphyton (area of 47.75 cm²). Details about the experimental unit, according to Fermino *et al*. (2004).

The samplings included the annual scale of variation, being carried out in the spring (23/11-08/12/2001), summer (21/02-08/03/2002), autumn (03/05-18/05/2002) and winter (10/07-25/07/2002). For each period and treatment, four collections were carried out corresponding to the 15th, 20th, 25th and 30th days of periphyton colonization.

Abiotic variables - climatological variables (air temperature, rainfall, wind and solar radiation) were provided by the Meteorological Station of the Department of Atmospheric Sciences of the Institute of Astronomy, Geophysics and Atmospheric Sciences of the University of São Paulo, located about 800 m from the collection site.

The limnological variables analyzed were: underwater radiation (µmol.s-1.m-2, quantameter Li-COR, model LI-250); temperature (oC), pH and electrical conductivity (µS cm-1) by means of a YSI 600R multiparameter probe; dissolved oxygen (mg L-1, Winkler's method modified by azide, according to Golterman *et al*. (1978); alkalinity (mEq.L-1, according to Golterman & Clymo, 1971); and inorganic forms of carbon (mg. L-1), obtained from pH and alkalinity data, according to Mackeret *et al.* (1978). After filtering the samples in GF/F precalcined filters, under low pressure (< 0.5 atm), the following dissolved nutrients were determined and expressed by the concentration of the element considered: orthophosphate and total dissolved phosphorus (Strickland & Parsons, 1960); ammonium (Solorzano, 1969); nitrate and nitrite (Mackeret *et al.,* 1978) and orthosilicic acid (Golterman *et al*., 1978). Unfiltered samples were used for analysis of total phosphorus (TP) and total nitrogen (NT), according to Valderrama (1981). The collections were carried out with polyethylene bottles in the water overlying the treatments, in duplicates. The analyses were processed on the day of collection, except for total nutrients, which were determined in a maximum of 7 days.

Periphyton attributes - The samples (SDN) were collected randomly, through a random selection process, with three replications (n=3). In the laboratory, periphyton was immediately removed from surfaces by means of delicate brushing and jets of distilled or ultrapure water. The removed material was kept in a freezer at a known volume of ultrapure water for the chemical composition of the periphyton – nitrogen and phosphorus.

Statistical Analysis - To compare the means of the limnological data, it was performed using the Bonferroni multiple comparison method, with an overall confidence coefficient of 90% for each of the contrasts presented in the Generalized Linear Model (MLG), with a Gamma distribution of errors (Neter *et al*., 1996; Myers *et al*., 1999; Paula, 2004). Principal component analysis (PCA) was performed from a covariance matrix with data transformation by [log (x + 1)] to abiotic data. Data transformations were performed using the FITOPAC program (Shepherd, 1996) and multivariate analyses were performed using the PC-ORD version 4.0 program for Windows (McCune & Mefford, 1999).

RESULTS

Climatic variables - the daily climatic data presented refer to the period of 30 consecutive days of sampling in the four seasons of the year, starting on the day the SDN were placed in the Lake of the Water Nympheas. Higher average values of solar radiation, precipitation and wind speed were recorded in spring and summer. The winter period was characterized by lower average temperatures and, together with autumn, by low rainfall (Figure 2).

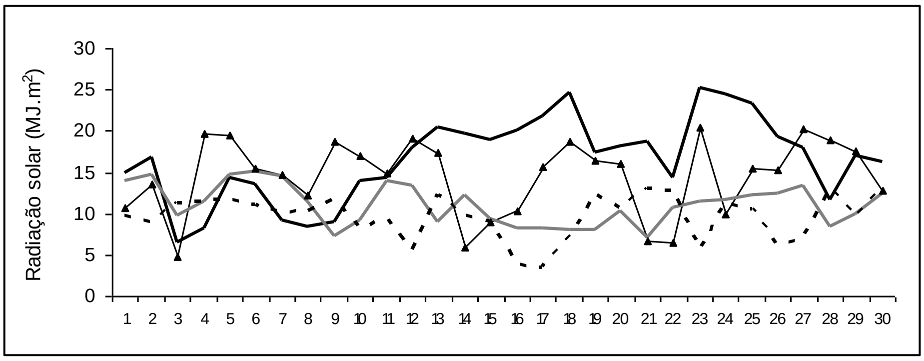
Limnological conditions - the mean values and standard error of the physical and chemical characteristics of the water of the treatments are presented by sampling period (Tables 1-4). Table 5 shows the average values of water temperature and underwater radiation measured in the treatments throughout the day (8:00 am, 10:00 am, 12:00 pm, 2:00 pm, 4:00 pm and 6:00 pm) at the four times of the year.

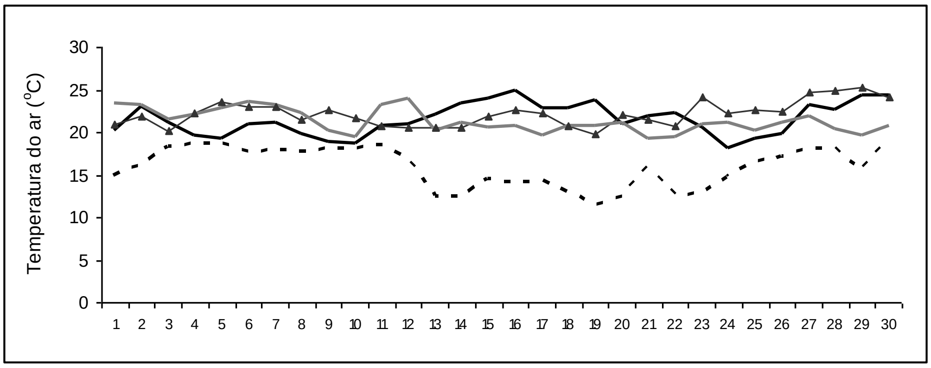
The variables water temperature, underwater radiation, pH, alkalinity, conductivity and orthosilicate did not show significant differences ($P > 0.10$) between treatments in the four seasons of the year (Tables 1-4). Between the seasons, these first two variables (water temperature and underwater radiation) varied throughout the year (Table 5), being clearly lower in winter. The highest recorded values of underwater radiation were in the spring, followed by summer and fall. The temperature showed significant differences in winter compared to the other seasons. In fact, in winter, the water temperature decreased by around 10 ^{o}C in relation to the other periods of the year (Table 5).

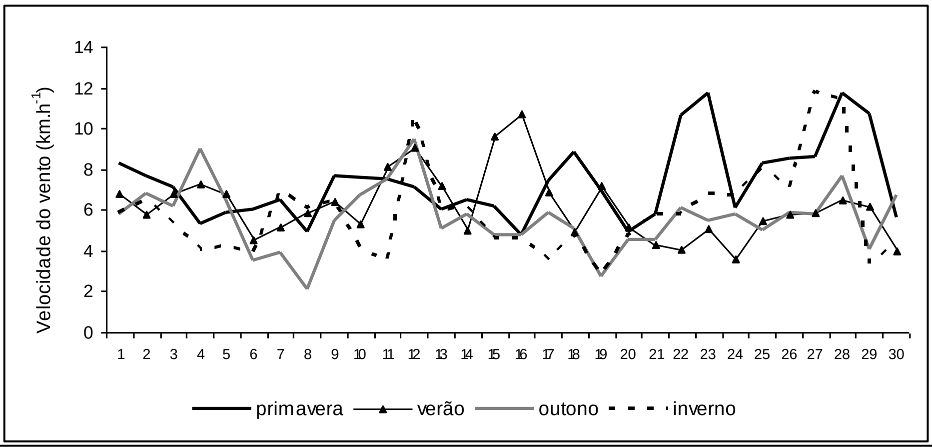
Regarding nutrients, only nitrite did not show significant differences, except in the autumn between the control and P+ treatments (Table 3). The other variables will be characterized by the seasons.

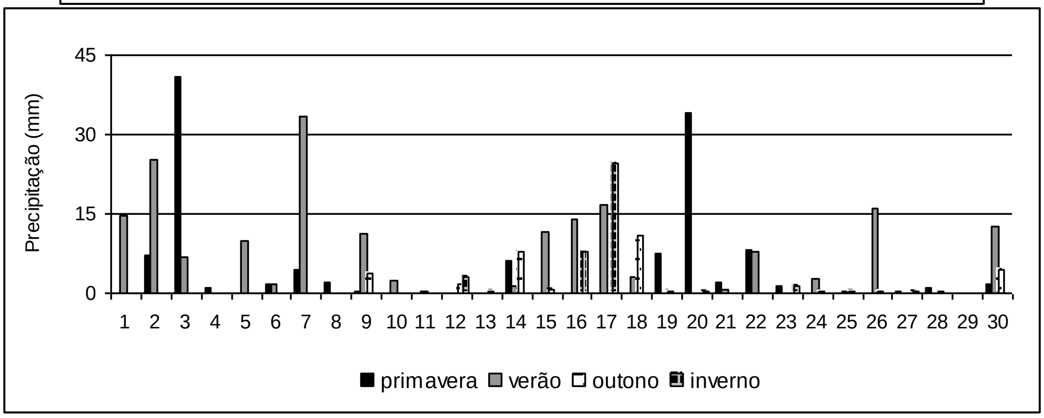
Primavera (Table 1) - Dissolved oxygen showed significant differences between treatments, with the lowest values recorded in the control. Only the N+ and NP+ treatments, with averages of 5.2 and 6.3 mg $^{L-1}$, did not show significant differences. Free CO2 and HCO3^{-} showed the lowest values in the treatments with phosphorus addition. Regarding the phosphorus series, the treatment with isolated addition of this nutrient presented the highest contents. The same trend, although less pronounced, was observed in relation to the treatment with combined addition of phosphorus and nitrogen.

Figure 2. Daily variation of solar radiation, average air temperature, wind speed and precipitation during 30 days at each time of the year in the PEFI area.









Regarding the nitrogen series, both nitrate and total nitrogen showed the highest levels in the treatments with isolated and combined addition of this nutrient. Ammonium presented values above the detection limit of the method only under non-enriched conditions (control).

Summer (Table 2) - Dissolved oxygen values were significantly higher in the NP+ treatment, while this same treatment presented the lowest values for free CO2. PT, PDT and orthophosphate, although in some cases below the detection limit of the method, were higher in treatments with phosphorus addition. The nitrate contents were higher in the treatments with nitrogen addition, with a significant difference only between the NP^+ and P+ treatments. Total nitrogen showed more constant mean values between treatments, with a slight increase in the N+ and NP+ treatments, but without significant differences. Ammonium, for most of the period, remained below the detection limit of the method.

Autumn (Table 3) – Oxygen levels were relatively low in all treatments, being higher in NP+. Carbon dioxide had the highest levels in the control and the lowest in the NP^{+} treatment ($p < 0.10$). The phosphorus values were the lowest found among all seasons of the year, and the PT, PDT and orthophosphate were almost always below the detection limit of the method. In addition, the highest average values were found in the treatments with the addition of this nutrient. Nitrate and NT showed higher levels in the N^{+} treatment, followed by the NP+ and control treatments. Ammonium had the highest levels in the control.

Winter (Table 4) – There was no significant difference between the dissolved gases in the treatments in this season of the year. The mean values of PT, PDT and orthophosphate were higher when compared to spring and autumn, and in the treatments with isolated or combined addition of P they were significantly higher. The nitrate contents, in general, were the lowest found throughout the year, while the ammonium levels were the highest. Particularly the N^{+} treatment stood out with the highest nitrate values.

Table 1. Minimum and maximum values and, in parentheses, mean and standard error (n = 8) of the limnological variables in the treatments performed in the Lake of the Water Limphies during the spring. Contrasts of treatments by the Bonferroni method applied in the Generalized Linear Model (GLM). Legend: NS = not significant; * significant difference ($P < 0.10$).

Variables	Control	N+	P+	NP+	L x N	L x S	C x NP	N x P	N x NP	P x NP
Temperature (°C)	21,0 - 23,6 (22.7 ± 0.8)	21,9 - 24,8 (23.3 ± 0.9)	23,4 - 27,4 (24.5 ± 1.4)	23,2 - 27,6 (24.8 ± 1.4)	NS	NS	NS	NS	NS	NS
Rad. subaq. ($\mu mol.s^{-1}.m^{-2}$)	1174,8	1183,3	1098,0	1224,7	NS	NS	NS	NS	NS	NS
Ph	6,1 – 6,3 (6.2 ± 0.03)	6,1 – 6,6 (6.3 ± 0.05)	6,3 – 7,0 (6.5 ± 0.10)	6,3 – 7,8 (6.8 ± 0.21)	NS	NS	NS	NS	NS	NS
Alkalinity (mEq.L-1)	0,290 – 0,317 (0,30 ± 0,001)	0,284 – 0,320 (0,30 ± 0,001)	0,273 – 0,319 (0,30 ± 0,001)	0,278 – 0,311 (0,30 ± 0,001)	NS	NS	NS	NS	NS	NS
Conductivity ($\mu S.cm^{-1}$)	35,0 – 70,9 (46,4 ± 4,45)	36,0 – 62,6 (45,3 ± 3,49)	35,0 – 58,3 (43.7 ± 3.2)	36,0 – 57,2 (44,9 ± 2,68)	NS	NS	NS	NS	NS	NS
Dissolved oxygen (mg. L-1)	0,51 – 2,60 (1,36 ± 0,25)	2,04 – 8,59 (5,23 ± 0,77)	1,34 – 8,98 (3,45 ± 1,08)	1,12 – 12,75 (6.26 ± 1.6)	*	*	*	*	NS	*
CO_2 free L^{-1})	11,64 – 19,32 (16,78 ± 1,09)	5,79 – 19,49 (15,29 ± 1,80)	2,15 – 18,97 (12,75 ± 2,53)	0,44 – 19,43 (13,04 ± 2,9)	NS	*	*	*	NS	NS
HCO_3 ($mg.L^{-1}$)	15,83 – 22,68 (18,61 ± 0,69)	12,88 – 21,28 (17.30 ± 1)	7,53 – 18,41 (13,19 ± 1,59)	8,66 – 18,45 (14,98 ± 1,41)	*	*	*	*	*	*
PT ($\mu g.L^{-1}$)	<4-33.3 (22,75 ± 4,04)	5,79 – 30,57 (21,27 ± 3,05)	41,11 – 174,85 (98,16± 16,14)	17,3 – 65,6 (43,22 ± 6,41)	NS	*	*	*	*	*
PDT ($\mu g.L^{-1}$)	<4 - 4.27 (4,03 ± 0,03)	<4 -5.19 (4.2 ± 0.15)	13,1 – 142,43 (50,87± 15,88)	<4 – 26,15 (11,99 ± 3,04)	NS	*	*	*	*	*

P-PO4 (µg.L^{-1})	<4	<4	<4 – 188,08 (51,36 ± 21,32)	<4 – 40,27 (12,97 ± 5,00)	---	---	---	---	---	---
NT (µg.L^{-1})	208,4 – 771,9 (465,9 ± 70,4)	262,6 – 1395,5 (705,5± 127,1)	91,0 – 834,3 (457,0± 115,1)	134,4–1607,2 (705,2±209,3)	*	NS	*	NS	NS	NS
N-NO$_2$ (µg.L^{-1})	<5	<5	<5	<5	NS	NS	NS	NS	NS	NS
N-NO$_3$ (µg.L^{-1})	<8	45,72 – 342,53 (131,18±32,5)	<8	42,46 – 240,67 (140,6 ± 0,91)	*	NS	*	*	NS	*
N-NH$_4$ (µg.L^{-1})	<4,2 – 7,34 (4,6 ± 0,99)	<4,2	<4,2	<4,2	---	---	---	---	---	---
Si-SiH$_4$O$_4$ (mg.L^{-1})	0,37 – 2,68 (1,18 ± 0,34)	0,40 – 2,58 (1.14 ±0.32)	0,35 – 2,38 (0.98 ± 0.3)	0,31 – 2,30 (0,96 ± 0,29)	NS	NS	NS	NS	NS	NS

< values below the method's detection limit

Table 2. Minimum and maximum values and, in parentheses, mean and standard error (n = 8) of the limnological variables in the treatments performed in the Lake of the Water Limphies during the summer. Contrasts of treatments by the Bonferroni method applied in the Generalized Linear Model (GLM). Legend: NS = not significant; * significant difference ($P < 0.10$).

Variables	**Control**	**N+**	**P+**	**NP+**	**C x N**	**C x P**	**C x NP**	**N x P**	**N x NP**	**P x NP**
Temperature (°C)	22,3 - 24,6 (22.9 ± 0.8)	22,3 - 25,0 (23.0 ± 0.9)	22,4 - 25,6 (23.4 ± 1.0)	22,5 - 25,3 (23.4 ± 0.9)	NS	NS	NS	NS	NS	NS
Rad. subaq. (µmol.s^{-1}.m^{-2})	1026,4	1058,9	1011,0	979,9	NS	NS	NS	NS	NS	NS
pH	5,9 – 6,3 (6.1 ± 0.03)	6,1 – 6,3 (6.2 ± 0.02)	6,1 – 6,4 (6.2 ± 0.03)	6,2 – 6,6 (6.3 ± 0.06)	NS	NS	NS	NS	NS	NS
Alkalinity (mEq.L-1)	0,233 – 0,253 (0,24 ± 0,001)	0,237 – 0,250 (0,24 ± 0,001)	0,246 – 0,263 (0,25 ± 0,001)	0,242 – 0,274 (0,25 ± 0,001)	NS	NS	NS	NS	NS	NS
Conductivity (µS.cm^{-1})	46,0 – 53,6 (49,2 ± 0,92)	42,6 – 52,2 (47,3 ± 1,22)	45,7 – 53 (49,3 ± 0,94)	43,0 – 56,3 (49.8 ± 1.7)	NS	NS	NS	NS	NS	NS
Dissolved oxygen (mg. L-1)	1,84 – 4,56 (3,12 ± 0,31)	2,48 – 4,56 (3,37 ± 0,26)	2,3 – 4,11 (2,89 ± 0,26)	3,09 – 8,63 (5,20 ± 0,65)	NS	NS	*	NS	*	*
CO$_2$ free (mg. L^{-1})	11,69 – 24,76 (15,75 ± 1,43)	10,35 – 16,33 (13,41 ± 0,75)	8,48 – 18,81 (13,80 ± 1,14)	4,95 – 16,08 (11,53 ± 1,46)	NS	NS	*	NS	*	NS
HCO3 (mg. L^{-1})	14,21 – 15,4 (14,67 ± 0,14)	14,37 – 15,32 (14,73 ± 0,13)	14,99 – 16,02 (15,42 ± 0,16)	14,76 – 16,69 (15,61 ± 0,23)	NS	NS	NS	NS	NS	NS
PT (µg.L^{-1})	12,27 – 25,07 (19,45 ± 1,67)	14,48 – 22,02 (18,67 ± 1,09)	20,2 – 32,99 (27,71 ± 1,66)	15,77 – 35,74 (23,69 ± 2,24)	NS	*	NS	*	NS	NS
PDT (µg.L^{-1})	<4 - 9.71 (6,25 ± 0,88)	<4 – 9,11 (6,24 ± 0,85)	4 – 12,96 (7,82 ± 1,46)	<4 – 10,91 (7,12 ± 1,08)	NS	NS	NS	NS	NS	NS
P-PO$_4$ (µg.L^{-1})	<4	<4 – 6,71 (4,33 ± 0,33)	<4 – 14,08 (6,39 ± 1,57)	<4 – 5,97 (4,34 ± 0,25)						
NT (µg.L^{-1})	208,5 – 529,7 (404,1 ± 44,2)	240,3 – 727,4 (513,7 ± 59,2)	286,2 – 758,0 (492,4 ± 57,3)	305,9 – 786,4 (516,1 ± 66,8)	NS	NS	NS	NS	NS	NS

N-NO$_2$ (µg.L^{-1})	5,55 – 10,87 (8,75 ± 0,61)	<5 – 14,03 (9,83 ± 1,14)	<5 – 11,86 (9,20 ± 1,07)	<5 – 14,87 (11,22 ± 1,74)	NS	NS	NS	NS	NS	NS
N-NO$_3$ (µg.L^{-1})	67,17–190,91 (132,52±12,94	76,35 – 422,39 (177,57±39,00	12,85 – 111,31 (58,59± 13,62)	40,75 – 741,78 (210,30±82,14	NS	NS	NS	NS	NS	*
N-NH$_4$ (µg.L^{-1})	<4,2	<4,2	<4,2 – 83,63 (14,36± 10,36)	<4,2	---	---	---	---	---	---
Si-SiH$_4$O$_4$ (mg.L^{-1})	0,24 – 2,96 (1.76 ± 0.4)	0,32 – 2,99 (1,63 ± 0,36)	0,31 – 3,17 (1,74 ± 0,37)	0,28 – 2,91 (1,69 ± 0,37)	NS	NS	NS	NS	NS	NS

< values below the method's detection limit

Table 3. Minimum and maximum values and, in parentheses, mean and standard error (n = 8) of the limnological variables in the treatments performed in the Lake of the Water Nympheas during the autumn. Contrasts of treatments by the Bonferroni method applied in the Generalized Linear Model (GLM). Legend: NS = not significant; * significant difference (*P* < 0.10).

Variables	**Control**	**N+**	**P+**	**NP+**	**C x N**	**C x P**	**C x NP**	**N x P**	**N x NP**	**P x NP**
Temperature (ºC)	20,0 - 21,5 (20.9 ± 0.5)	20,0 – 21,6 (20.9 ± 0.5)	21,2 – 21,8 (21.4 ± 0.2)	21,1 - 22,0 (21.5 ± 0.3)	NS	NS	NS	NS	NS	NS
Rad. subaq. (µmol.s^{-1}.m^{-2})	610,4	645,5	642,2	631,1	NS	NS	NS	NS	NS	NS
pH	5,8 – 6,0 (5.9 ± 0.03)	5,8 – 6,1 (6.0 ± 0.04)	5,9 – 6,2 (6.0 ± 0.03)	6,0 – 6,2 (6.1 ± 0.02)	NS	NS	NS	NS	NS	NS
Alkalinity (mEq.L^{-1})	0,203 – 0,241 (0,22 ± 0,001)	0,221 – 0,248 (0,23 ± 0,001)	0,238 – 0,248 (0,24 ± 0,001)	0,225 – 0,257 (0,24 ± 0,001)	NS	NS	NS	NS	NS	NS
Conductivity (µS.cm^{-1})	44,3 – 62,8 (50,2 ± 2,12)	44,9 – 53,1 (48,7 ± 1,24)	44,6 – 54,7 (49 ± 1.48)	45,6 – 54,1 (49,2 ± 1,35)	NS	NS	NS	NS	NS	NS
Dissolved oxygen (mg. L-1)	1,88 – 4,31 (2.60 ± 0.3)	1,72 – 3,76 (2,47 ± 0,22)	0,91 – 2,64 (1.88 ± 0.2)	2,13 – 4,21 (3,37 ± 0,27)	NS	*	*	*	*	*
CO$_2$ free (mg. L^{-1})	19,55 – 31,3 (24,1 ± 1,52)	16,03 – 28,03 (22,86 ± 2,04)	16,06 – 27,28 (20,67 ± 1,49)	14,22 – 22,7 (18,43 ± 1,17)	NS	NS	*	NS	*	NS
HCO$_3$ (mg.L^{-1})	12,37 – 14,69 (13,78 ± 0,27)	13,49 – 15,05 (14,37 ± 0,2)	13,72 – 15,66 (14,84 ± 0,2)	14,54 – 15,15 (14,79 ± 0,07)	NS	NS	NS	NS	NS	NS
PT (µg.L^{-1})	<4 – 11,77 (7,29 ± 1,21)	<4 – 8,86 (6,02 ± 0,83)	9,83 – 86,08 (45,32± 12,44)	<4 – 37,51 (15,51 ±3,85)	NS	*	*	*	*	*
PDT (µg.L^{-1})	<4 – 7,41 (4,96 ± 0,42)	<4 – 7,85 (4.97± 0.46)	<4 – 62,96 (17,76 ± 7,78)	<4 – 18,72 (9.4 ± 1.65)	NS	NS	NS	NS	NS	NS
P-PO$_4$ (µg.L^{-1})	<4	<4	<4 – 52,51 (14,74 ± 6,40)	<4	---	---	---	---	---	---
NT (µg.L^{-1})	687,1 – 933,1 (783,4 ± 31,4)	625,0 – 2929,2 (1077,3±270,4	229,5 – 817,2 (566,4 ± 75,7)	252,6 – 1499,3 (798,6± 147,7)	*	*	NS	*	NS	*
N-NO$_2$ (µg.L^{-1})	<5 – 8,91 (5,78 ± 0,68)	<5 – 13,23 (8,21 ± 1,18)	<5 – 16,24 (10,93 ± 2,41)	<5 – 15,68 (8,64 ± 2,06)	NS	*	NS	NS	NS	NS

N-NO$_3$ (µg.L^{-1})	73,53 – 192,85 (109,87±17,18	119,82– 477,06 (243,09±45,75	<8 – 69,25 (37,71 ± 8,5)	31,15 – 285,9 (144,22±32,34	NS	NS	NS	*	NS	*
N-NH$_4$ (µg.L^{-1})	<4,2 – 63,76 (23,74 ± 7,97)	<4,2 – 7,5 (2,68 ± 1,32)	<4,2 – 43,67 (11,68 ± 6,45)	<4,2 – 24,37 (4,11 ± 2,98)	---	---	---	---	---	---
Si-SiH$_4$O$_4$ (mg.L^{-1})	2,54 – 3,75 (3,22 ± 0,15)	2,59 – 3,79 (3,22 ± 0,16)	2,91 – 2,9 (3,41 ± 0,13)	2,84 – 3,84 (3,31 ± 0,15)	NS	NS	NS	NS	NS	NS

< values below the method's detection limit

Table 4. Minimum and maximum values and, in parentheses, mean and standard error (n = 8) of the limnological variables in the treatments performed in the Lake of the Water Limphies during the winter. Contrasts of treatments by the Bonferroni method applied in the Generalized Linear Model (GLM). Legend: NS = not significant; * significant difference ($P < 0.10$).

Variables	**Control**	**N+**	**P+**	**NP+**	**C x N**	**C x P**	**C x NP**	**N x P**	**N x NP**	**P x NP**
Temperature (ºC)	14,4 - 16,0 (15.1 ± 0.5)	14,2 - 16,0 (14.9 ± 0.6)	14,4 - 16,2 (15.0 ± 0.6)	14,5 – 15,9 (15.0 ± 0.5)	NS	NS	NS	NS	NS	NS
Rad. subaq. (µmol.s^{-1}.m^{-2})	168,1	196,0	155,4	189,5	NS	NS	NS	NS	NS	NS
pH	6,2 – 6,5 (6.2 ± 0.03)	6,2 – 6,4 (6.3 ± 0.03)	6,2 – 6,5 (6.3 ± 0.04)	6,2 – 6,5 (6.3 ± 0.05)	NS	NS	NS	NS	NS	NS
Alkalinity (mEq.L^{-1})	0,231 – 0,254 (0,24 ± 0,001)	0,228 – 0,257 (0,24 ± 0,001)	0,230 – 0,258 (0,24 ± 0,001)	0,230 – 0,254 (0,24 ± 0,001)	NS	NS	NS	NS	NS	NS
Conductivity (µS.cm^{-1})	40,0 – 61,6 (48 ± 2.59)	39,0 – 49,1 (44,2 ± 1,32)	39,0 – 49,8 (43.2 ±1.34)	41,0 – 48,9 (44,6 ± 1,07)	NS	NS	NS	NS	NS	NS
Dissolved oxygen (mg. L-1)	3,64 – 6,58 (5,03 ± 0,45)	3,74 – 6,57 (5,09 ± 0,32)	3,95 – 7,49 (5,60 ± 0,45)	4,32 – 8,26 (5,85 ± 0,54)	NS	NS	NS	NS	NS	NS
CO$_2$ free (mg.L^{-1})	7,99 – 16,88 (14,2 ± 1,10)	9,5 – 17,29 (13,4 ± 1,02)	6,96 – 16,21 (12,72 ± 1,39)	6,47 – 16,71 (12,09 ± 1,48)	NS	NS	NS	NS	NS	NS
HCO$_3$ (mg.L^{-1})	14,07 – 15,48 (14,73 ± 0,18)	13,92 – 15,69 (14,68 ± 0,2)	14,05 – 15,72 (14,82 ± 0,19)	14,05 – 15,48 (14,75 ± 0,17)	NS	NS	NS	NS	NS	NS
PT (µg.L^{-1})	5,46 – 9,99 (7,84 ± 0,58)	5,46– 13,55 (8,21 ± 0,99)	13,07 – 86,72 (45,24 ± 9,73)	9,67 – 28,61 (15,92 ± 2,25)	NS	*	*	*	*	*
PDT (µg.L^{-1})	<4 – 5,35 (4,26 ± 0,18)	<44 – 4,62 (4,14 ± 0,08)	7,56 – 37,09 (16,66 ± 4,06)	4,18 – 7,12 (5,53.± 0,33)	NS	*	NS	*	NS	NS
P-PO$_4$ (µg.L^{-1})	4,36 – 11,53 (8,16 ± 8,09)	5,38 – 12,26 (8,72 ± 7,95)	8,57 – 26,27 (14,9 ± 8,37)	<4 – 9,23 (6.0 ± 8.45)	---	---	---	---	---	---
NT (µg.L^{-1})	507,6 – 651,1 (585,3 ± 15,3)	574,2 – 3040,4 (1032,9±288,8)	179,7 – 720,1 (438,3 ± 69,8)	344,2 – 875,2 (577,0 ± 71,6)	*	NS	NS	*	*	NS
N-NO$_2$ (µg.L^{-1})	<5	<5	<5	<5	NS	NS	NS	NS	NS	NS
N-NO$_3$ (µg.L^{-1})	<8 – 44,5 (21,96 ± 5,88)	90,08 – 524,13 (232,92± 52,5)	<8 – 70,32 (30,2 ± 9,07)	<8 – 351,33 (129,55±47,37)	*	NS	*	*	NS	*

N-NH_4 (µg.L^{-1})	<4,2 – 11,77 (5.7 ± 1.51)	<4,2 – 349,58 (70,51± 45,65)	<4,2 – 375,62 (54,44± 45,97)	<4,2 – 478,21 (71,07± 58,46)	---	---	---	---	---	---
Si-SiH_4O_4 (mg.L^{-1})	7,69 – 32,95 (22,24 ± 2,99)	9,89 – 45,04 (26,91 ± 3,8)	4,39 – 29,66 (17,71 ± 2,72)	12,08 – 25,26 (18,26 ± 1,68)	NS	NS	NS	NS	NS	NS

Table 5. Minimum and maximum values of water temperature (n = 32) and underwater radiation (n = 6) in the four seasons of the year (P: spring, V: summer, O: autumn and I: winter) in the Lake of the Water Nymphaeas. Contrasts of treatments by the Bonferroni method applied in the Generalized Linear Model (GLM). Legend: NS = not significant; * significant difference (*P* < 0.10).

Variable	Premium.	See.	Out.	Inv.	P xV	P x O	P x I	V x O	V x I	O x I
Water Temp. (^{o}C)	21-27,6	22,3-25,6	20-21,8	14,2-16,2	NS	NS	*	NS	*	*
Rad. subaq. (µmol.s^{-1}.m^{-2})	1174,8-1224,7	979,9-1058,9	610,4-645,5	155,4-189,5	*	*	*	*	*	*

To evaluate the main trends of variation of abiotic limnological characteristics in the treatments, during the four seasons of the year, principal component analysis (PCA) was applied. The analysis summarized 62.8% of the variability of the data in its first two axes (Figure 3, Table 6). In main coordinate 1 (40.8%), the sampling units were positioned to the left of the axis in association with the highest phosphorus availability (PT and PDT) and to the right of the axis, in association with the highest nitrate (NO3) contents. These variables showed high correlations with this axis, respectively with values of -0.7; -0.6 and 0.9. Thus, at the ends of axis 1, the units related to the treatment with isolated addition of phosphorus (P+) were ordered on the left and, on the right, those related to the treatment with isolated addition of nitrogen (N+) and, in part, those with combined addition (NP+). Also, near the sampling units enriched with phosphorus, the conditions without enrichment (control) were ordered in the spring period, evidencing a greater availability of phosphorus in the Lake of the Water Limphies in this season of the year.

In coordinate 2, the sampling units were ordered on their positive side, fundamentally, due to the greater availability of ammonium (r = 0.9) and, on their negative side, by the highest values of water temperature (r = -0.4). Associated with ammonium were the observations of winter, regardless of the treatment and, on the negative side, the hottest seasons of the year (spring and summer).

In summary, axis 1 indicated that the greater variability of the abiotic data was mainly attributed to the type of enrichment, so that the highest availability of phosphorus was found in the treatment with isolated addition of phosphorus (in the majority) and greater availability of nitrate in the treatments with isolated but also combined addition of nitrogen. On the other hand, in axis 2, the ordering was more according to the time of year. It can be inferred, therefore, that the abiotic limnological variability was fundamentally conditioned by the type of treatment, although also by the annual scale of variation. It is also observed that phosphorus was temporally influenced, being more available under natural conditions (control) in the spring period.

Figure 3. Ordering by the ACP of the sampling units of the treatments (capital letters: C, N, P, NP) in the four seasons of the year (lowercase letter: p, v, o, i) throughout the experimental period (numbers: days of the experiment). Abbreviations - NH4: ammonium; NO3: nitrate; NT: total nitrogen; PDT: total dissolved phosphorus; total match; Temp: Water temperature. Vectors smaller than 0.200 were excluded.

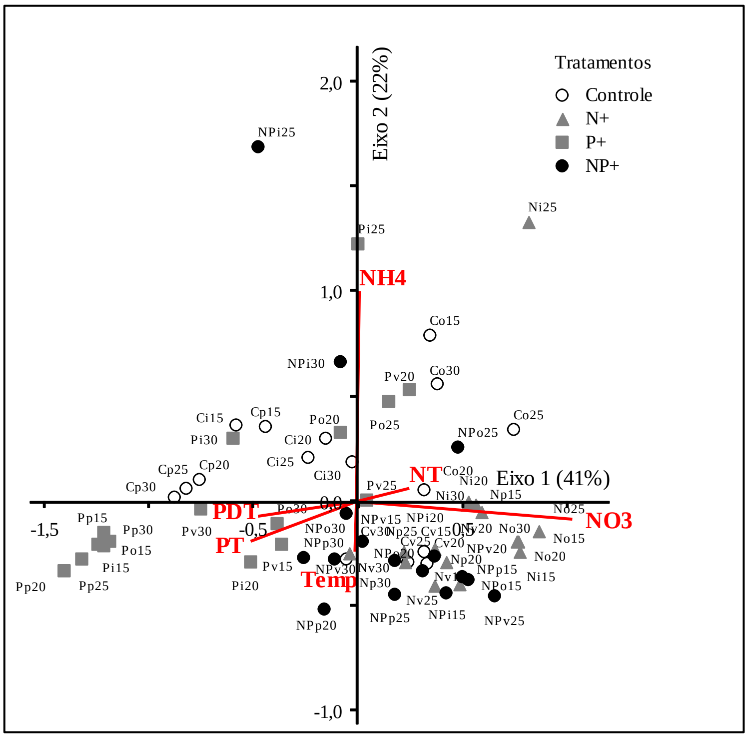


Table 6. Correlation of abiotic variables with axes 1 and 2. In bold, $r \geq 0.5$.

Variables	**Main Components**	
	1	**2**
Temp	-0,101	-0,451
Ph	-0,225	-0,074
Conductivity	0,113	-0,102
Dissolved oxygen	0,182	0,090
$CO_{2\ free}$	0,147	-0,008
HCO_3	0,040	-0,026
PT	**-0,654**	-0,399
PDT	**-0,627**	-0,249
NT	0,465	0,241
NO_3	**0,928**	-0,278
NH_4	0,122	**0,916**
Si	0,289	0,061
Variation Explained	**40,8%**	**22,0%**

N:P molar ratio - the **N:P molar ratio of water** (Figure 4) indicated clear phosphorus limitation (molar ratio N:P = 45-238) in natural conditions (control) in all seasons of the year. The isolated addition of N increased the limitation by P. On the other hand, the isolated addition of P changed the water to a condition of good resource availability ($10 \leq N:P \leq 20\text{-}32$), except for the summer that remained with limitation of P. Finally, the combined addition (NP+) made the medium P-limited, especially in the fall. Thus, under phosphorus addition conditions (P+ and NP+), the water ratio did not reflect the pre-established experimental conditions, i.e., nitrogen limitation (P+) and good resource availability (NP+).

The **N:P molar ratios of the periphyton** corroborated some trends observed for water, but, in general, the community responded much more sensitively to the experimental conditions (Figure 5). Thus, in the control and N addition condition, the N:P ratio of the periphyton was extremely high (N:P = 80-561), evidencing P-limitation (Figure 10). The isolated addition of P caused N-limitation (N:P < 10). Despite the ratios close to or equal to 10, in the summer and especially in the fall, the N content showed nitrogen limitation. With joint addition (NP^{+}), the ratio showed good availability (distribution) of resources.

Finally, in winter, the isolated or joint addition of P (P+, NP+) suppressed the limitation by P, however the community always remained N-limited, indicating the influence of another controlling factor, which we can assume exactly the low temperatures shown in the results of the sampled limnological and climatological data.

Figure 4. Mean values (n = 4) of the molar NT:PT ratio of water over the experimental period (30 days), per treatment (C = control; N+ = isolated nitrogen addition; P+ = isolated addition of phosphorus; NP+ = combined addition) and in the four seasons of the year in the Lake of the Water Lilies.

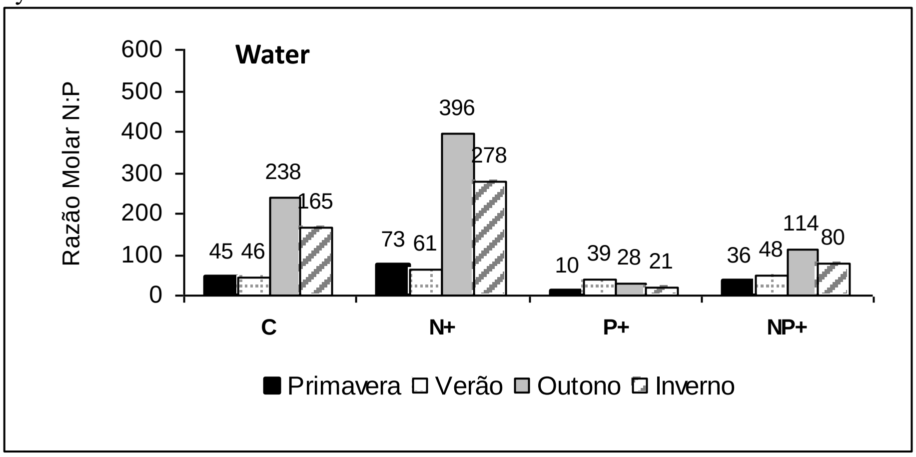
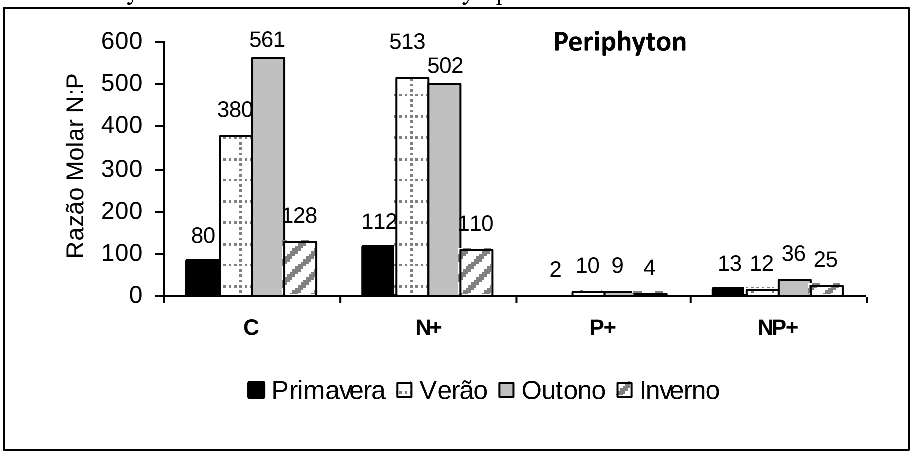


Figure 5. Mean values (n = 4) of the N:P molar ratio of the periphyton over the experimental period (30 days), per treatment (C = control; N+= isolated nitrogen addition; P+= isolated addition of phosphorus; NP+= combined addition) and in the four seasons of the year in the Lake of the Water Nymphaeas.



DISCUSSION

ABIOTIC CONDITIONS

The results obtained showed that the greatest source of abiotic variability was due to the type of treatment, or the isolated and/or combined addition of nitrogen and phosphorus, followed by the variability on an annual scale. In this case, with emphasis on precipitation, solar and underwater radiation, as well as temperature. Winter was characterized by the lowest values of these factors, with the temperature being lower by up to 10oC in relation to other times of the year. Contrasting is spring, with the highest values of the variables mentioned. Also, at this time, the highest phosphorus levels occurred under natural conditions (control), whose sampling units were associated with greater phosphorus availability (left side of ACP-1, Figure 3).

Considering the variations between treatments, in the conditions of combined enrichment, significantly higher levels of dissolved oxygen and lower levels of carbon dioxide were found, except for the winter, evidencing the greater photosynthetic activity in these nutritional conditions. The same trend was found in enrichment experiments carried out with mesocosms in an oligotrophic dam during the winter at PEFI (Ferragut, 1999). The manipulated nutrients responded coherently, with phosphorus (PT, PDT) being clearly higher in the treatment with isolated addition of phosphorus and nitrogen ($NO3^-$), in the treatments with isolated or joint addition of nitrogen. In general, however, the release of orthophosphate by the SDN was not sufficient to increase its contents in the water overlying the treatments, most likely due to the immediate assimilation by the periphytic community developed on the SDN, as well as by other components of the biota in the surrounding water. These trends were also found by Ferragut (2004) who also used SDN, but with different levels of phosphorus addition during the winter in an oligotrophic PEFI dam.

Razão Molar N/P

The N:P molar ratio of the periphyton has been widely used to indicate the nutritional status of the community, based on the optimal ratio (16) of Redfield (1958), proposed for marine plankton. In general, studies on periphyton of continental waters have used a molar ratio lower than 10 for N-limiting condition, greater than 20 for P-limiting, and between 10-20 for good resource distribution (Biggs, 1995; Borchardt, 1996; Stelzer & Lamberti, 2001). According to Borchardt (1996), enrichment studies have proven these values. Based on a literature review for the periphyton of continental waters, Kahlert (1998) considered the molar ratio above 32 as an indicator of P-limitation, less than 12, of N- limitation and optimal ratio of 18.

The present results on the chemical composition of periphyton were extremely sensitive to enrichments, providing answers consistent with the most commonly used ranges for nutrient limitation. Both the molar ratio and the nitrogen and phosphorus contents of the periphyton showed fundamental responses in the evaluation of nutrient limitation, corroborating studies carried out for other PEFI dams (Ferragut, 1999, 2004; Barcelos, 2003). Also, according to these studies, the N:P ratio of water was considered less predictive of nutritional limitation conditions, particularly under enrichment conditions.

FINAL CONSIDERATIONS

The results showed the role of periphyton in phosphorus retention, since the release of orthophosphate by the SDN did not cause changes in its contents in the water overlying the experiments, despite the marked increase of P in the periphyton. The results also allow us to infer that the Lake of the Water Ligrushes is much more sensitive to the supply of phosphorus and that, depending on the load of this nutrient, there may be profound changes in the structure and functioning of this system. They also showed the influence of low temperatures (winter) on the concentrations of N and P in the water and in the periphytic material. Finally, the importance of periphyton in nutrient retention in shallow ecosystems is highlighted.

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