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ORIGINAL RESEARCH

Seasonal Differences in Plankton Community and Removal Efficiency of Nutrients and Organic Matter in a Subtropical Constructed Wetland

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Abstract The performance of free water surface flow constructed wetlands (CW) may be impaired by abiotic factors. The effects of seasons on the water quality improvement and on the community of plankton were evaluated in a CW system with the macrophytes *Cyperus giganteus* Vahl, *Typha domingensis* Pers., *Eichhornia crassipes* (Mart.) Solms and *Pontederia cordata* L. Water, plankton and macrophytes were sampled in the inflow and outflow during the dry and rainy seasons. Differences in temperature, precipitation, hydraulic loading rate (HLR), hydraulic retention time (HRT), inlet mass loadings and plant biomass between seasons affected the treatment efficiency. High precipitation and the consequent increase in HLR along with an increase in temperature and lower macrophyte biomass, were correlated to lower rates of removal efficiency during the rainy season. The season with higher macrophytes abundance coincided with high retention of zooplankton and solids. Higher nutrient levels in the dry season corresponded with a dominance and abundance of r-strategist planktonic species. To increase the removal efficiency of nutrients and organic matter by CW systems, care should be taken to decrease the HLR especially in periods of high precipitation.

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Introduction

Constructed wetlands (CW) are low cost easily maintained systems. As such, they may be the best alternative for the treatment of effluents in developing countries with serious water eutrophication problems (Baptista et al. 2008; Li et al. 2008). Several abiotic factors, such as water availability (Mann and Wetzel 2000), sediment quality (Kim et al. 2001), temperature, water level fluctuations (Ellery et al. 2003) and hydraulic loading rate (Travaini-Lima and Sipaúba-Tavares 2012) affect the operational efficiency of constructed wetland systems. Plants are highly important during the treatment since CW with macrophytes are more efficient in nitrogen, phosphorus, BOD_5, solids and thermotolerant coliforms removal than systems without any vegetation (Chung et al. 2008; Maltais-Landry et al. 2009).

Macrophytes in CW also have a strong influence on the structure and dynamics of the planktonic community, playing an important role in the complex interactions that occur in CW systems (Sinistro et al. 2006). The coverage of macrophytes is a major factor in the occurrence of phytoplankton species in wetlands, because they strongly modify the light conditions. Phytoplankton needs light and nutrients for photosynthetic production, and the availability of these factors directly influences the density and composition of the community (Izaguirre et al. 2001; O'Farrell et al. 2003; Izaguirre et al. 2004). The aquatic vegetation also affects the feeding habits of different species of the zooplankton community, as well as the size structure of zooplankton populations because macrophytes provide an important refuge area for these organisms

(Lansac-Tôha et al. 2003). Many studies (Jeppesen et al. 1998; Van Donk and Van de Bund 2002; Norlin et al. 2005) have evaluated the role of zooplankton trophic interactions in submerged plants, but the number of studies of these interactions in floating and emergent macrophytes is limited (Bobbink et al. 2008).

Hydrologic processes are closely tied to wetland ecology and are typically highly variable in space and time (Mitsch and Gosselink 2000), especially in sub-tropical regions where rainfall influence on the hydrologic processes changes with season. Wastewater composition in these areas varies throughout the year, according to the dry and rainy seasons (Trang et al. 2010). Management recommendations developed in temperate wetlands may not be directly applicable to humid tropical and subtropical areas because of extreme hydrological inputs e.g., high annual precipitation, and dissimilar processes and interactions among ecosystem components (Kaplan et al. 2011).

The objectives of this research were to: (1) estimate the potential of a subtropical free water surface flow CW system to remove incoming nutrients and organic matter during the rainy and dry seasons; and (2) study the spatial and temporal variations of planktonic organisms in the CW system and their relationships to possible limiting factors in the ecosystem.

Materials and Methods

Study Area and Sampling Sites

The CW system was built to treat the effluents of the Aquaculture Center of São Paulo State University (UNESP), southeast Brazil (21°15’ S and 48°18’ W) (Fig. 1a). Daily means of meteorological conditions during the summer rainy season (December 2009 to March 2010) were: 24.2 ± 1.4 °C air temperature, 26.9 ± 0.8 °C water temperature and 16.4 ± 19.5 mm rainfall, while during the winter dry season (June to August 2009) the daily means were 20.3 ± 2.4 °C, 21 ± 0.9 °C and 0.06 ± 0.2 mm respectively.

These effluents originated from six earthen ponds at the Aquaculture Center with areas varying between 1822 and 8067 m^2 (Fig. 1b). Some of these ponds received water from other smaller earthen ponds located further up the watershed. The CW system also treats effluents originated in a series of Upflow Anaerobic Sludge Blanket reactors (UASB) with volumes ranging between 50 and 908 L. During this study, the effluent used to feed the UASB reactors was swine manure.

The CW system has an area of 96.6 m^2 and is 71 m long, mean width of 1.36 m, shallow, and has a clayey bottom (Fig. 1c). At the beginning of both the rainy season and the dry season, the CW system was planted with four macrophyte species: *Cyperus giganteus* Vahl (20 m^2), *Typha domingensis* Pers. (13 m^2), *Eichhornia crassipes* (Mart.) Solms (10 m^2) and *Pontederia cordata* L. (3 m^2). Immediately after plant transplantation, the CW system was filled to a depth of approximately 0.30 m with a continuous water flow. The CW system receives the wastewaters of the aquaculture farm and UASB system through two concrete pipes in a spring box, allowing the entrance of water in a wide and uniform way. The wastewater, after passing through the CW system, is collected in another spring box and discharged into the Jaboticabal stream.

In each season the initial sampling of water and plankton started 1 month after the plants had been transplanted and wastewater loading began. At that time, the macrophyte biomass densely covered the designated area. Samples were collected at the inlet site (WI) of the CW system in the output of the influent spring box, and at the outlet site (WO) in the input of the effluent spring box (Fig. 1c). Collection was performed once a week in the early morning during the dry season (June to August 2009) and biweekly during the rainy season (December 2009 to March 2010). In the rainy season the sampling frequency was lower because the sample collection should happen after a rain event of at least 2 days. Water samples were collected in polyethylene bottles (500 ml) and stored at −20 °C for later nutrients quantification.

Biomass of aquatic macrophytes was monitored during the experimental period. All the macrophyte materials in a quadrat of 0.13 m^2 were harvested in triplicate and washed in the laboratory, at the end of the rainy (March) and the dry (August) seasons. The above and underground tissues were not separated, and the plants were dried to a constant weight in a fan forced oven at 60 °C until weight stabilization given by complete dehydration.

Laboratory Analyses

Water and plankton were sampled at 0.10 m under the water surface. Conductivity (Cond), pH and temperature (°C) were measured using a Horiba multi-probe (model U-10, Horiba Co), and dissolved oxygen (DO) was measured with a portable YSI oxygen meter (model 55, Yellow Spring Instr). Total suspended solids (TSS), total dissolved solids (TDS) and 5-day biochemical oxygen demand (BOD_5) were determined according to Boyd and Tucker (1992). Nitrate (NO_3), nitrite (NO_2), total phosphorus (TP) and soluble reactive phosphorus (SRP) were measured following Golterman et al. (1978) and ammonia (NH_4) was measured following Koroleff (1976). Chlorophyll-a (Chl-a) and thermotolerant coliforms (TC) were determined following Nusch (1980) and Greenberg et al. (1992), respectively. Analyses were performed immediately after sampling or samples were correctly stored under refrigeration until analyzed.

For zooplankton, 10 L of water were filtered through a net of 58 μm pore-size and concentrated to 50 ml. Formalin was added to reach 4 % final concentration. Cladocera and

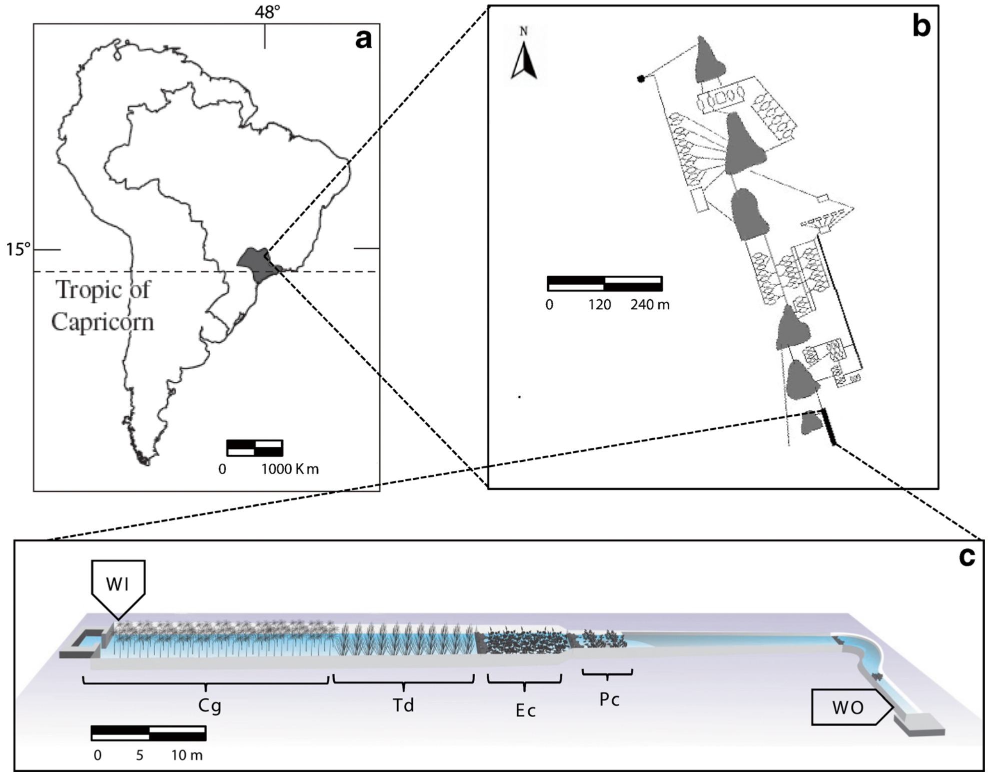

Fig. 1 Geographic location (**a**) and layout of the Aquaculture Center (**b**) and of the constructed wetland system (**c**), where WI = water inlet; WO = water outlet; Cg = *Cyperus giganteus*; Td = *Typha domingensis*; Ec = *Eichhornia crassipes*; Pc = *Pontederia cordata*

Copepoda were identified in a reticulated acrylic chamber under stereomicroscope (40 × augmentation). Rotifera were identified and counted in a Sedgewick-Rafter chamber under a Leitz microscope (100 × augmentation). Phytoplankton samples were collected with polyethylene bottles and preserved with Lugol 1 % solution. Phytoplankton abundance was estimated by counting the cells according to Utermöhl (1958) using sedimentation chambers following recommendations in Lund et al. (1958). Phytoplankton counting was carried out in an inverted microscope Axiovert 40 CFL (Carl Zeiss).

Constructed Wetland Hydraulic and Wastewater Loading

The hydraulic loading rate (HLR) of the CW system was calculated for the dry and rainy seasons as

$$q = \frac{Q}{A},$$

where q is the HLR (m h^{-1}); Q is the water inlet rate (m^3 h^{-1}) and A is the wetland land area (m^2) (Kadlec and Wallace 2009). Q was measured manually using a calibrated bucket and stopwatch. The hydraulic retention time (HRT) for the two seasons were measured as

$$HRT = \frac{V}{Q},$$

in hours (h); V is the maximum volume of the canal (m^3) and Q is the water inlet rate (m^3 h^{-1}). The mass loading rate of each physical and chemical parameter was calculated separately for the inlet and outlet in each season as

$$m = q \times C,$$

where m is the (specific) mass loading (g m^{-2} d^{-1}); q is the HLR (m h^{-1}) and C is the concentration (g m^{-3}). The removal efficiency (RE %) of mass loads by the CW system was measured by the formula

$$\text{RE}\ \% = \frac{m_i - m_o}{m_i} \times 100,$$

where m_i is the mass load in the inlet (g m^{-2} d^{-1}) and m_o is the mass load in the outlet (g m^{-2} d^{-1}).

Data Analysis

Two-way ANOVA was used to evaluate interactions between seasons and inlet/outlet CW effects of the hydrological and physico-chemical parameters and plankton community. Phytoplankton and zooplankton abundances were log10 transformed (log(x + 1)) to normalize the data and reduce the sample variance. For the macrophyte species, statistical comparisons between seasons and between species in each season were performed with one-way ANOVA. When significant differences were detected, the Fisher LSD test was used for post-hoc comparisons. The statistical analyses were carried out with Statistica 8.0 (StatSoft 2007) and were done at $P = 0.05$.

The relationships among the plankton, sampling sites and water quality were investigated using canonical correspondence analysis (CCA) (ter Braak 1988). Monte Carlo test was applied in order to verify the probability of the eigenvalues of the ordination axes having been randomly attributed (999 iterations; $P = 0.05$). The CCA was carried out with PC-ORD 5.15 (McCune and Mefford 2006).

Zooplankton and phytoplankton diversity were calculated at each site using the Shannon-Wiener (H') formula (Pielou 1975). Richness (S) was calculated as the total number of species present at each sampling site. Evenness or equitability (E) was calculated as H/H max, where H is the Shannon-Wiener index and H max = ln S.

The analyses of dominance and abundance of species were performed for phytoplankton and zooplankton. A species was considered dominant when its density was higher than 50 % of the total number of individuals present, and abundant when the number of individuals was higher than the mean density of all occurring species (Lobo and Leighton 1986).

Results

Water Quality

The CW system had significantly higher mean HLR in the rainy season (0.57 m h^{-1}) than in the dry one (0.32 m h^{-1}), whereas, the mean HRT was significantly higher in the dry season (3h23min) than in the rainy one (2h47min). For the calculations, the increase of HLR during the rainy season was attributed only to an increase in waste loading from the aquaculture system, because the measures of rainfall and runoff were not taken. It was observed that in the rainy season the depth of CW system increased, being approximately 0.60 m in the WI and 0.50 m in the WO, whereas in the dry season, the depth was approximately 0.30 m in the WI and 0.10 m in the WO.

The concentrations (Fig. 2) and mass loading means (Table 1) of the parameters studied were higher in the rainy season, when HLR was higher. The differences between the means of mass loadings in CW sampling sites depend on the season, as indicated by the significant ($p < 0.05$) season-site interaction effects of the two-way-ANOVAs. The nutrients (NO_3, NO_2, NH_4, SRP and TP) mass loadings were significantly lower in the WI during the rainy than during the dry season, due to the dilution effect from the higher HLR. The increase of water flow rate in the rainy season carried more solids (TSS) and organic matter (BOD_5) mass loadings to the effluent entering the CW. The removal of particles, chemical species and thermotolerant coliforms was more efficient in the dry season than in the rainy one. While the removal rates for 10 variables were 77–99 % in the dry season, they were only 47–68 % in the rainy season. With removal rates lower than 55 % (NO_2, NH_4, SRP, TP and TC during rainy season) the decrease of mass loadings from the WI to the WO was not significant ($p > 0.05$).

The DO was higher in the outlet sampling site than in the inlet one in the rainy season. Dissolved oxygen and pH were higher in the dry season than the rainy one, mainly in the WI sampling site ($p < 0.05$) (Table 1). The concentrations of TP, SRP, BOD_5, NO_2, NH_4, TSS, TDS and Chlorophyll-a were higher in the inlet sampling sites than in the outlet ones in both seasons (Fig. 2). In general, the CW system had higher organic loading and lower oxygen in the water entering the system than in the water leaving it.

Aquatic Macrophytes

Few specimens of *P. cordata* survived until the end of the dry season. Although the CW system was restarted before the rainy season, all *P. cordata* disappeared before the end of this period. All the other species had a significantly larger biomass during the dry than the rainy season. Emergent species had significantly larger biomass, mainly the *C. giganteus* (Table 2).

Plankton

The phytoplankton community comprised Cyanobacteria (10 species from 7 genera), Chlorophyceae (13 species from 11 genera), Zygnemaphyceae (3 species from 2 genera), Euglenophyceae (1 species), Bacillariophyceae (9 species from 7 genera) and Xanthophyceae (1 species) (Table 3). No phytoplankton group presented significant differences between seasons, sampling sites or interaction (Table 4). During the dry season over 70 % of the phytoplankton community were Chlorophyceae (mainly *Kirchneriella lunaris*) and the next abundant groups were Bacillariophyceae and Cyanobacteria; during the rainy season Bacillariophyceae and Chlorophyceae each formed about 40 % of the phytoplankton, the remaining 20 % being mostly Cyanobacteria (Fig. 3a). In both seasons phytoplankton species richness was high (above 27 species), while evenness values and species diversity index were higher in the rainy season ($p < 0.05$) (Table 4).

The zooplankton community comprised Rotifera (31 species from 17 genera), Cyclopoida Copepoda (1 species), Calanoida Copepoda (1 species) and Cladocera (4 species). Rotifera were the dominant group (above 97 %) in both seasons and sampling sites. The ANOVAs of zooplankton groups showed significant ($p < 0.01$) interactions between seasons and CW sampling sites, indicating that the differences in the abundances of zooplankton groups in the CW sampling sites depend on seasons. Copepoda in the WI were more abundant during the rainy than during the dry season (Fig. 3b, Table 4). Density of all zooplankton groups was only significantly

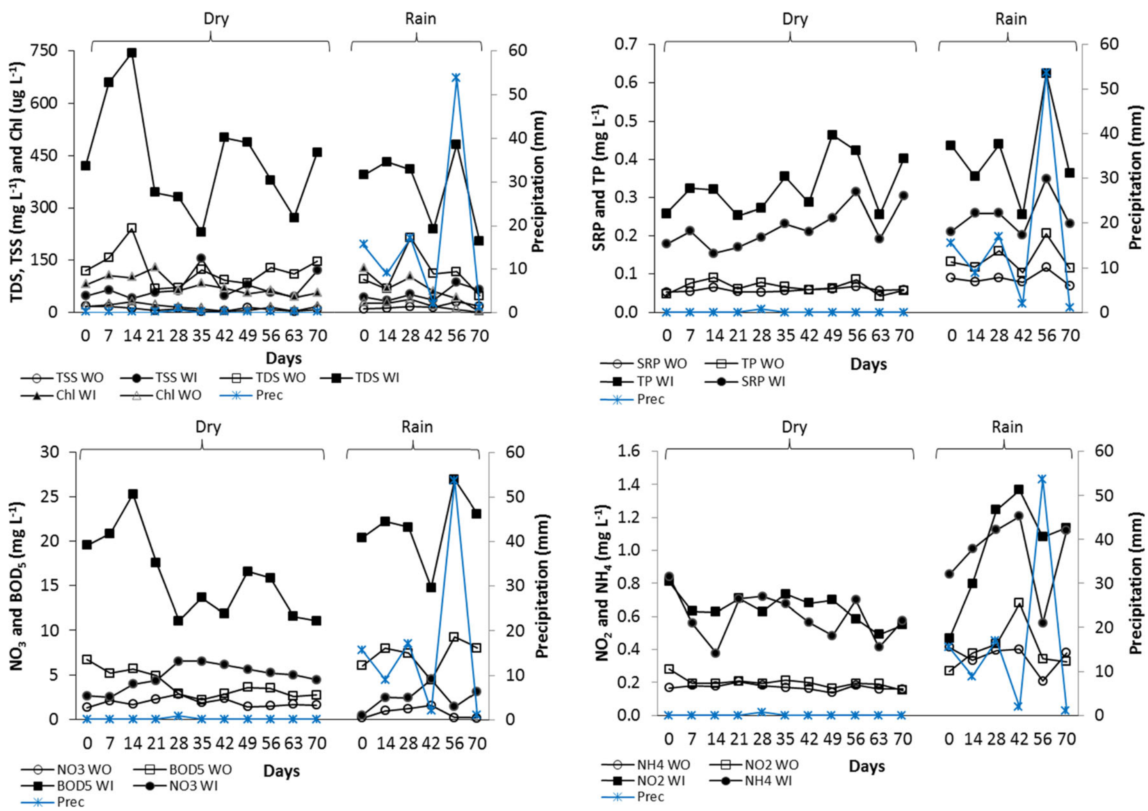

Fig. 2 Concentrations of Chlorophyll-a (μg L^{-1}), solids and nutrients (mg L^{-1}) in the CW system at the water inlet (WI) and water outlet (WO) in the dry and rainy seasons, against time (days), with a daily precipitation line (mm) included

lower in the WO sampling site during the dry season. The densities of Cladocera and Copepoda in the WO were lower during the dry than during the rainy season. The CW system tended to have high zooplankton species richness (above 22 species), evenness (above 0.78) and diversity (Shannon-Wiener index between 2.47 and 2.76 nats ind^{-1}) (Table 4).

Table 1 Mean and standard deviation of pH, conductivity (μS cm^{-1}), DO (mg L^{-1}), mass loading of physical and chemical parameters (g m^{-2} d^{-1}), most probable number and standard deviation (MPN 10^3 100 ml^{-1}) of thermotolerant coliforms, and removal efficiency rates (RE %) in the water inlet (WI) and outlet (WO) of CW system for each season, and ANOVA results for all parameters

	Dry			Rainy		
	WI	WO	RE %	WI	WO	RE %
pH	6.8 ± 1.0^{a}	6.9 ± 1.0^{a}	–	5.9 ± 0.4^{b}	6.2 ± 0.4^{ab}	–
Conductivity	113.5 ± 64.7^{ns}	113.7 ± 64.3^{ns}	–	78.0 ± 1.2^{ns}	77.2 ± 2.3^{ns}	–
DO	6.6 ± 0.4^{a}	6.8 ± 0.4^{a}	–	4.2 ± 0.8^{c}	5.6 ± 0.6^{b}	–
BOD_5	51.7 ± 27.9^{b}	8.2 ± 4.7^{c}	77	102.6 ± 18.8^{a}	42.7 ± 15.9^{b}	58
Nitrate	16.0 ± 5.4^{a}	3.7 ± 1.2^{c}	80	9.5 ± 5.6^{b}	3.8 ± 3.5^{c}	60
Nitrite	2.0 ± 0.5^{a}	0.4 ± 0.2^{b}	79	0.2 ± 0.1^{bc}	0.1 ± .0.04^{c}	47
Ammonia	1.6 ± 0.4^{a}	0.3 ± 0.1^{b}	81	0.2 ± 0.1^{b}	0.09 ± 0.02^{b}	52
SRP	0.6 ± 0.1^{a}	0.1 ± 0.03^{b}	85	0.05 ± 0.01^{c}	0.02 ± 0.01^{c}	52
TP	0.9 ± 0.2^{a}	0.1 ± 0.04^{b}	84	0.08 ± 0.03^{b}	0.03 ± 0.02^{b}	55
TSS	163.3 ± 58.9^{b}	20.2 ± 15.3^{c}	88	252.7 ± 85.8^{a}	106 ± 66.2^{b}	58
TDS	1308.7 ± 831.5^{a}	243.0 ± 99.8^{b}	81	1504.3 ± 593.8^{a}	641.1 ± 328.1^{b}	57
Chlorophyll-a	0.23 ± 0.1^{a}	0.03 ± 0.02^{b}	87	0.33 ± 0.2^{a}	0.11 ± 0.1^{b}	68
Thermotolerant Coliforms	7.4 ± 1.8^{a}	0.051 ± 0.065^{b}	99	17.6 ± 2.9^{a}	9.4 ± 1.3^{a}	47

In each row, means followed by the same letter do not differ ($p < 0.05$); *ns* not significant difference, − not applicable

Table 2 Mean biomass and results of one-way ANOVA of the macrophytes during the dry and rainy seasons

Biomass (g DW m^{-2})	*T. domingensis*	*C. giganteus*	*E. crassipes*
Dry	2226.5[a]	2244.9[a]	743.4[b]
Rainy	1372.5[b]	2029.7[a]	352.1[c]
ANOVA Seasons	*	*	*

In each row, means followed by the same letter do not differ ($p < 0.05$); * = significant difference ($p < 0.05$) between seasons

Diaphanosoma birgei was the only Cladocera species frequently encountered throughout the study period (Table 5). The Cyclopoida and Calanoida copepods, *Thermocyclops decipiens* and *Argyrodiaptomus furcatus* respectively, were absent only at the WO in the dry season. There were more abundant Rotifera species at the WI in the dry and at the WO in the rainy seasons (Table 5).

The CCA extracted two axes which explain 38 % of the overall variance in plankton data (Fig. 4). The species-environment correlation was 0.89 for axis 1 and 0.85 for axis 2 indicating a strong relationship between the plankton distribution and the environmental variables used for the ordination. Monte Carlo's permutation test revealed that the ordination on axes 1 and 2 was statistically significant ($p < 0.05$). The first axis (24 % of the variance in plankton data) presents high positive association among TP, SRP, HLR, BOD5, NO2, NH4, TSS, TDS, Water temperature (°C), Chlorophyll-a, with Copepoda, Cladocera and most of the inlet sampling sites in the rainy season. All those variables were negatively related with DO, pH, Zygnemaphyceae, Chlorophyceae and most of the outlet sampling sites in the dry season. Thus, axis 1 represents a water quality gradient, with higher organic loading and lower oxygen in the water entering the system during the rainy season than in the water leaving it during the dry season. The axis 2 (14 % of the variance in plankton data) presents high association among TC, Euglenophyceae, Bacillariophyceae, Xanthophyceae, Cyanobacteria and most of the outlet sampling sites in the rainy season. All of them were negatively correlated with NO3, Rotifera and inlet sampling sites in the dry season (Fig. 4).

Discussion

Removal efficiencies in free water surface flow CW systems are influenced by several parameters related to annual cycles, such as water and air temperatures, solar radiation, humidity, rainfall, inlet pollutant concentrations, and vegetation (Kadlec 1999). Hydraulic characteristics such as the HLR and consequent HRT can also affect the treatment processes in these systems (Trang et al. 2010). The aforementioned parameters cause changes in the retention of nutrients, in the release of

Table 3 Composition and frequency of phytoplankton species in the water inlet (WI) and water outlet (WO), in the dry and rainy seasons

Taxa	Dry		Rainy	
	WI	WO	WI	WO
Cyanobacteria				
Aphanocapsa sp.	+	+	+	A
Chroococcus limneticus (Lemmermann)	+	+	A	A
Merismopedia sp.	+	+	+	+
Microcrocis pulchella (Werner)	+	+	−	−
Microcystis aeruginosa (Kützing) Kützing	+	+	+	+
Microcystis novacekii (Komárek) Compere	+	+	+	+
Microcystis panniformis Komárek et al.	+	+	+	+
Microcystis wesenbergi (Lemmermann)	+	+	+	+
Nostoc sp.	+	+	+	−
Sphaerocavum brasiliensis Azevedo et Sant'Anna	+	+	A	+
Chlorophyceae				
Ankistrodesmus gracilis (Ralfs)	+	+	+	+
Coelastrum microporum (Nägeli) Kützing	+	+	+	+
Coelastrum reticulatum (Dangeard) Senn	+	+	+	+
Crucigenia quadrata (Morren)	+	−	+	−
Desmodesmus armatus (Chord.) Hegerv	+	+	+	+
Dictyosphaerium pulchellum (Wood)	+	+	A	+
Kirchneriella lunaris (Kirchner) Möbius	D	D	A	A
Micractinium pusillum Fresenius	+	+	−	−
Pediastrum duplex (Meyen)	+	+	+	+
Pediastrum tetras (Ehrenberg) Ralfs	+	−	+	+
Scenedesmus acutus (Reed)	+	+	+	+
Sphaerocystis sp.	+	−	−	−
Tetraedron trigonum (Nägeli) Hansgirg	+	+	+	+
Zygnemaphyceae				
Closterium acutum Brébisson	+	+	+	−
Staurastrum lobatus (Börges.) Bourrely	−	−	+	−
Staurastrum sp.	+	+	+	+
Euglenophyceae				
Euglena sp.	+	+	+	+
Bacillariophyceae				
Aulacoseira sp.	A	+	A	A
Melosira granulata (Ehr.) Ralfs.	A	A	A	A
Melosira sp.	−	−	+	−
Melosira varians Agardh	+	+	A	A
Navicula sp.	+	−	+	−
Nitzschia amphibia (Grunow)	−	+	+	+
Pinnularia sp.	+	+	+	−
Synedra sp.	+	+	+	+
Tabellaria floculosa (Roth) Kütz.	+	+	+	A
Xanthophyceae				
Isthmochloron lobulatum (Nägeli) Skuja	+	+	+	+

+ = presence; − = absence; *A* abundant, *D* dominant

Table 4 Phytoplankton and zooplankton quantitative analyses: density, species richness, evenness, and Shannon–Wiener diversity (H') index in the WI and WO sampling sites in each season, and ANOVA results of planktonic groups density

	Dry		Rainy	
	WI	WO	WI	WO
Zooplankton total	454,606[a]	149,074[b]	460,807[a]	391,706[ab]
Cladocera (ind L^{-1})	1,333[a]	33[b]	2,800[a]	783[a]
Copepoda (ind L^{-1})	1,017[b]	33[c]	10,200[a]	6,783[ab]
Rotifera (ind L^{-1})	452,256[a]	149,008[b]	447,807[a]	384,140[ab]
Richness	32[ns]	22[ns]	28[ns]	27[ns]
Evenness	0.78[ns]	0.80[ns]	0.82[ns]	0.84[ns]
(H')	2.71[ns]	2.47[ns]	2.74[ns]	2.76[ns]
Phytoplankton total	337,105[ns]	465,085[ns]	196,380[ns]	184,360[ns]
Cyanobacteria (ind mL^{-1})	21,886[ns]	20,488[ns]	33,215[ns]	44,882[ns]
Chlorophyceae (ind mL^{-1})	247,475[ns]	408,603[ns]	74,360[ns]	75,219[ns]
Zygnemaphyceae (ind mL^{-1})	370[ns]	455[ns]	337[ns]	202[ns]
Euglenophyceae (ind mL^{-1})	303[ns]	135[ns]	286[ns]	354[ns]
Bacillariophyceae (ind mL^{-1})	67,054[ns]	35,387[ns]	88,165[ns]	63,636[ns]
Xanthophyceae (ind mL^{-1})	17[ns]	17[ns]	17[ns]	67[ns]
Richness	34[ns]	31[ns]	34[ns]	27[ns]
Evenness	0.36[b]	0.23[b]	0.63[a]	0.69[a]
(H')	1.26[b]	0.79[b]	2.21[a]	2.27[a]

In each row, means followed by the same letter do not differ ($p < 0.05$); *ns* not significant difference

chemical compounds and in biological activities of microorganisms and plants (Kadlec 1999; Kim et al. 2011). Based on the results given above, Fig. 5 presents a conceptual graphic model of the CW system performance in the dry and rainy seasons. The nutrients mass loading entering the CW system, the macrophyte biomass in it, and the HRT were high during the dry season. These factors facilitated sedimentation of particles and development of bacteria that were not washed away. As a result, the removal of nutrients and particles was efficient, leading to strong decreasing gradients between the input and output sites (mainly in nutrient mass loading). During the rainy season the rain increased the input of solids and organic matter mass loadings. This, together with the summer higher temperatures, enhanced decomposition processes in the CW system and account for the lower pH and DO concentration observed. Rain also increased HLR that reduced HRT, decreased nutrient mass loading through dilution, and decreased macrophyte biomass affecting fixation of the plants. The heterotrophic competition caused by higher organic matter and lesser oxygen concentration may affect the nitrification by restricting growth of nitrifying bacteria (Truu et al. 2005). Furthermore, the higher HLR may have created more anaerobic sediment conditions due to ponding. Therefore, probably the coupled nitrification-denitrification processes was inhibited, leading to lower removal efficiency rates of NH_4 and NO_2 than those measured in the dry season. As a result, decomposition and dilution were dominant processes in the CW system, and the removal of nutrients and particles was less efficient than in the dry season.

Aquatic Macrophytes

The similar-sized emergent macrophytes *C. giganteus* and *T. domingensis* had a higher biomass during the dry season. *Typha* species exhibit performance advantages when compared to smaller species (Hadad et al. 2006) such as *E. crassipes*. *E. crassipes* has a high production potential due to its vertical growth capacity, especially in high densities (Henry-Silva et al. 2008). However, *E. crassipes* had a lower biomass than the rooted species selected for this study, especially during the rainy season. Macrophytes act as a physical barrier that may retain particles, plankton and other microorganisms. Since the removal of suspended solids occurs mainly by physical mechanisms, such as sedimentation and filtration processes (Karathanasis et al. 2003), the larger plant biomass during the dry season worked as a physical barrier for TSS. Moreover the higher HRT favored the sedimentation process. Furthermore, the reduction of thermotolerant coliforms depends on sedimentation, solar radiation and inactivation by vegetation (Boutilier et al. 2009), processes that were favored during this season and caused higher TC removal rates. Submerged structures of macrophytes such as leaves, stems and roots also provide a substrate for the epiphyton development (composed of algae, debris, bacteria and other microorganisms and invertebrates), which represents an important food source for zooplanktonic Cladocera and Copepoda (Thomaz and Cunha 2010). Zooplankton may also benefit from the density of aquatic plants as refuge against possible predators mainly in more structurally complex plants that

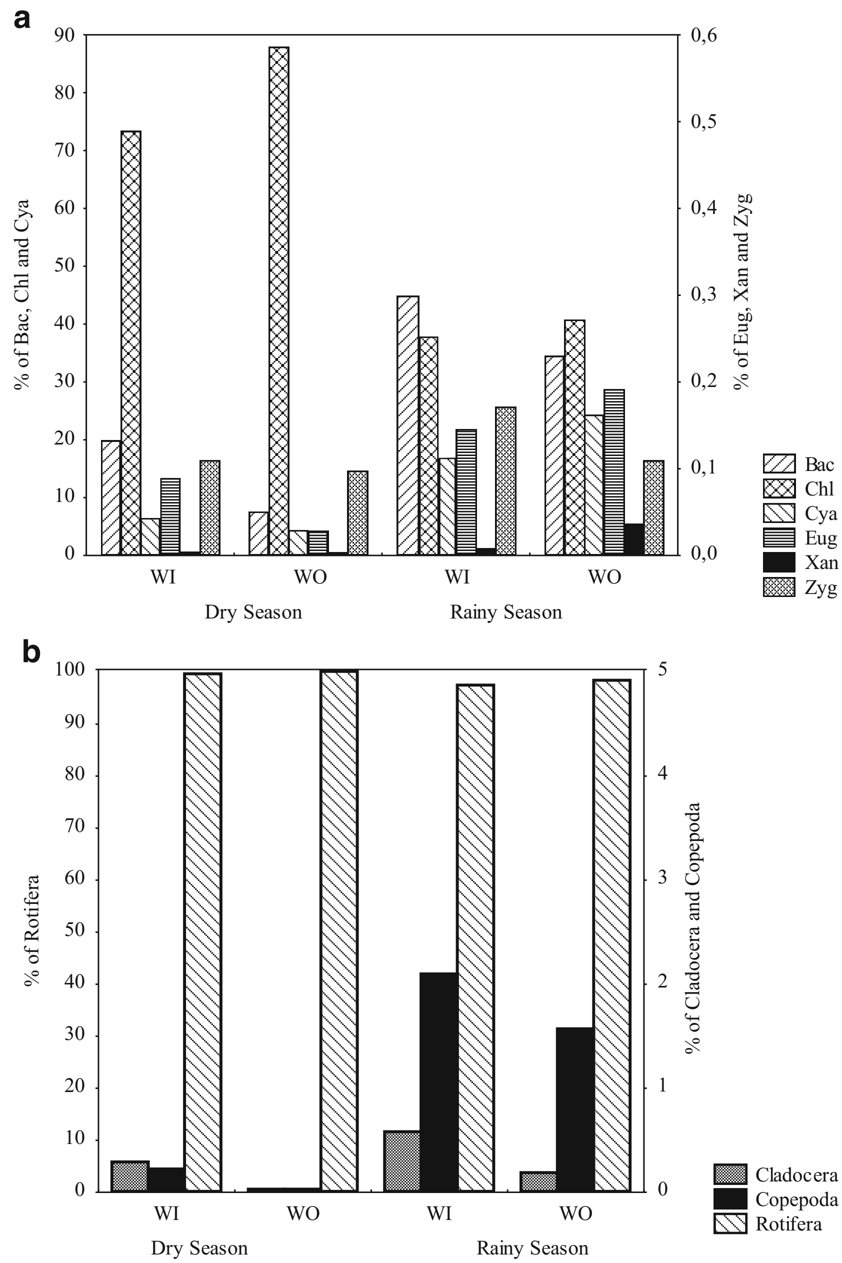

Fig. 3 Phytoplankton (**a**) and zooplankton (**b**) relative abundances (%) in the CW system at the water inlet (WI) and water outlet (WO) in the dry and rainy seasons, where Bac = Bacillariophyceae; Chl = Chlorophyceae; Cya = Cyanobacteria; Eug = Euglenophyceae; Xan = Xanthophyceae; Zyg = Zygnemaphyceae

provide advantageous conditions (Warfe and Barmuta 2004). Kuczynska-Kippen (2007) verified that macrophytes with more complicated spatial and morphological structure may offer better concealment conditions and also a more favorable nutritional source compared to morphologically simplified plants, like *Typha* sp. Among the macrophyte species in our study *E. crassipes* affords a denser habitat due to the high complexity of roots architecture hanging in the water column. Furthermore, in the dry season the high macrophyte biomass coincided with a decrease of zooplankton richness in the CW output and with significant differences in zooplankton density between the input and output sampling sites.

Higher HRT favored absorption processes of N and P fractions by macrophytes and microorganisms, because the contact time between water, biota and sediment was longer. Other authors also found that removal of N and P compounds increases with increasing retention time (Chung et al. 2008; Trang et al. 2010; Dong et al. 2011). Besides nitrogen assimilation into microbial and plant biomass, nitrogen removal in CW systems occurs through adsorption, ammonia volatilization and coupled nitrification-denitrification, which depend on the environmental conditions within the system. Ammonia volatilization occurs mainly at pH above 9 (Eighmy and Bishop 1989). The early morning pH measured in out CW system ranged between 5.9 and 6.9. However, algal and free-floating photosynthesis increases pH values during the day, promoting ammonia volatilization in wetlands (Vymazal and Kröpfelová 2008). The higher macrophyte density during

Table 5 Composition and frequency of zooplankton species in the water inlet (WI) and water outlet (WO), in the dry and rainy seasons

Taxa	Dry		Rainy	
	WI	WO	WI	WO
Cladocera				
Alona monacantha (Sars, 1901)	+	−	−	−
Bosmina hagmani Stingelin, 1904	+	−	+	−
Bosmina longirostris (O. F. Muller, 1785)	+	−	−	−
Diaphanosoma birgei (Korinek, 1981)	+	+	+	+
Copepoda				
Argyrodiaptomus furcatus (Sars, 1901)	+	−	+	+
nauplii	+	−	+	+
Thermocyclops decipiens (Kiefer, 1929)	+	−	+	+
nauplii	+	+	+	+
Rotifera				
Anuraeopsis fissa (Gosse, 1851)	+	−	−	−
Anuraeopsis navícula (Rousselet, 1910)	+	−	A	A
Ascomorpha sp.	+	+	+	+
Brachionus calyciflorus (Pallas, 1766)	A	+	+	A
Brachionus caudatus (Barrois & Daday, 1894)	A	+	A	A
Brachionus falcatus (Zacharias, 1898)	+	−	A	+
Brachionus havanaensis (Rousselet, 1911)	−	−	+	−
Brachionus quadridentatus (Hermann, 1783)	−	−	+	−
Cephalodella sp.	+	+	−	−
Colurella dicentra (Gosse, 1887)	+	+	−	+
Epiphanes macrourus (Barrois & Daday, 1894)	A	+	+	A
Epiphanes sp.	+	+	−	+
Filinia opoliensis (Zacharias, 1891)	−	−	+	+
Gastropus stylifer (Imhof, 1891)	A	A	A	A
Hexarthra intermedia (Hauer, 1953)	−	−	+	−
Keratella cochlearis (Gosse, 1851)	A	A	+	+
Keratella tropica (Apstein, 1907)	A	+	−	+
Lecane elsa (Hauer, 1931)	+	−	+	+
Lecane lunaris (Ehrb., 1832)	−	−	+	+
Lecane proiecta (Hauer, 1956)	+	+	+	+
Lecane signifera (Jennings, 1896)	+	−	−	−
Philodina sp.	+	+	+	+
Polyarthra sp.	A	A	A	A
Proales doliaris (Rousselet, 1895)	A	+	+	A
Proales globulifera (Hauer, 1921)	+	−	+	+
Proales sp.	A	+	+	+
Proalinopsis caudatus (Collins, 1872)	+	+	−	−
Synchaeta sp.	A	+	A	A
Synchaeta stylata (Wierzejski, 1893)	A	A	+	A
Trichocerca longiseta (Schrank, 1802)	A	A	A	A
Trichocerca rattus (Muller 1776)	A	A	A	A

+ = presence; − = absence; *A* abundant

the dry season, besides having contributed to efficient nutrient uptake, accounted for the higher DO concentration in that

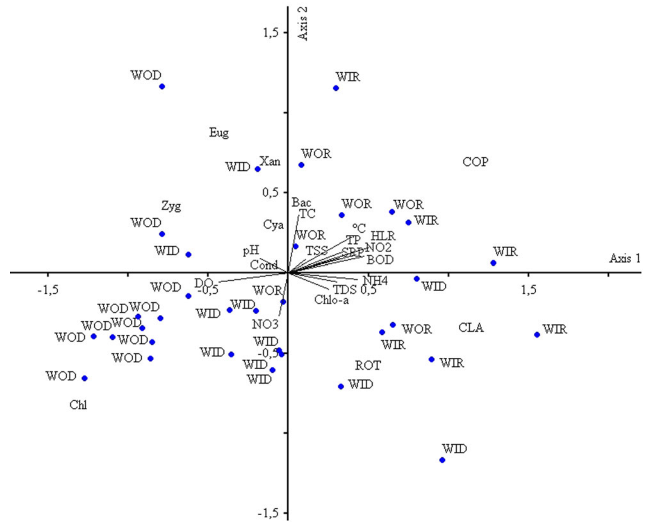

Fig. 4 Bi-plot of the canonical correspondence analysis (CCA) of plankton-environmental factors, where WID = water inlet in the dry season; WIR = water inlet in the rainy season; WOD = water outlet in the dry season; WOR = water outlet in the rainy season; COP = Copepoda; CLA = Cladocera; ROT = Rotifera; Bac = Bacillariophyceae; Chl = Chlorophyceae; Cya = Cyanobacteria; Eug = Euglenophyceae; Xan = Xanthophyceae; Zyg = Zygnemaphyceae

season since macrophytes release oxygen into the rhizosphere and parts of the root system, mainly around the root tips and on young laterals (Armstrong and Armstrong 1990; Wiebner et al. 2002). The increased oxygen availability in the upper layer of the sediment and at oxic microsites in the rhizosphere of macrophytes leads to an enhancement of the nitrification process (Maltais-Landry et al. 2009), which is a prerequisite for denitrification in anoxic zones at the bottom layer of the sediments.

During the rainy season the growth and development of *C. giganteus* and *T. domingensis* was impaired by the increasing water depth, due to high rainfall rates. This fact reduces the anchorage capacity of these species by decreasing biomass allocation to rhizomes and roots (Chen et al. 2010). *P. cordata* was not adequate for effluent treatment with high hydraulic loading since it was not capable of surviving until the end of the rainy season. Maine et al. (2006) also reported that this species was not able to survive in CW treatment systems. The large decrease in plant biomass, specifically of the sub-water parts, restricted the development of the microbial community and the filtering capacity of the substrate in the surface area. The stronger water current and the lower macrophyte biomass increased phytoplankton and zooplankton wash out, resulting in similar densities in the input and output sites. The higher HLR also carried larger amounts of organisms (mainly copepods) from the aquaculture ponds effluents into the CW system, and hampered the association of zooplankton with macrophyte stands. The high density of copepods in the rainy season (summer) also is related to water temperature (Bozkurt and Guven 2009).

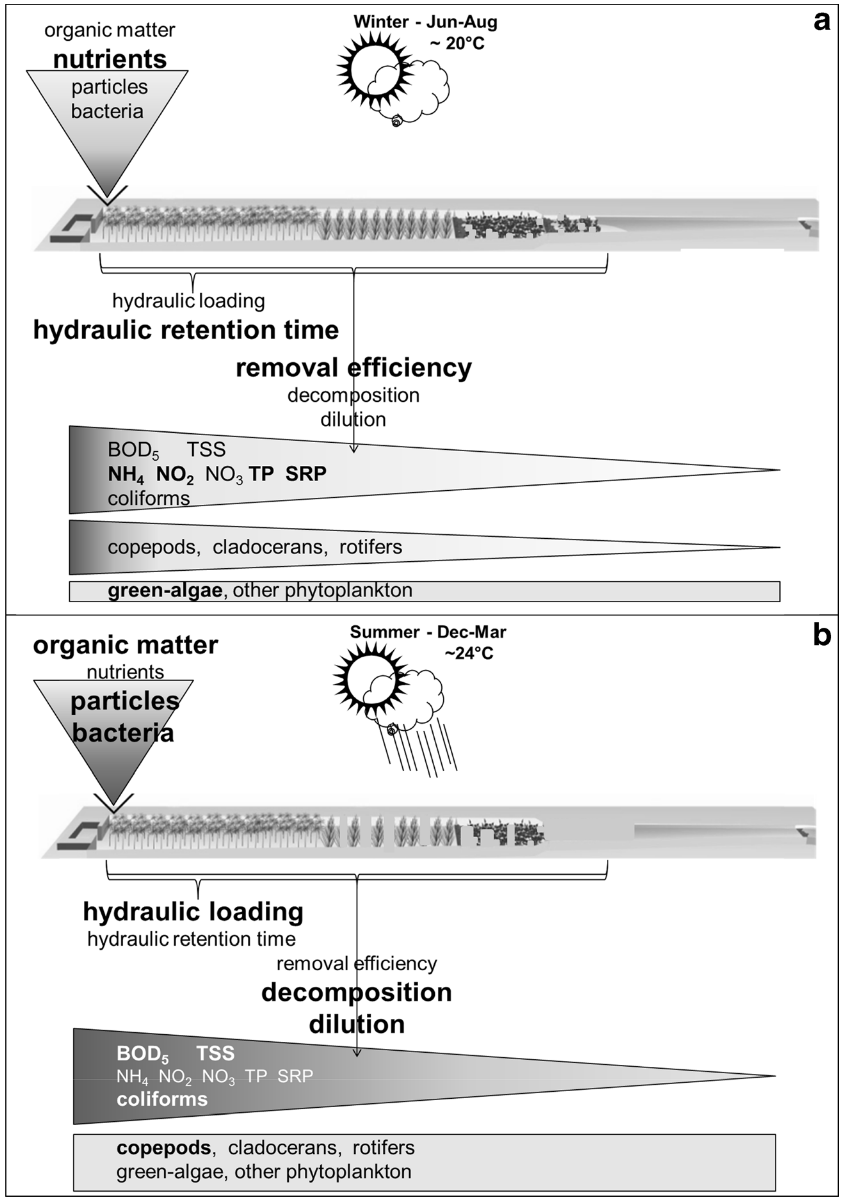

Fig. 5 Conceptual model of the functioning of the constructed wetland system during the dry (**a**) and rainy (**b**) seasons. Size and bold of words indicate relative importance. The *darker shading* means higher loadings or density of organisms

Phytoplankton

The higher nutrient mass loading in the dry season may account for the high Chlorophyceae density in that season, when the small and non-motile *Kirchneriella lunaris* was the single dominant species. The genus *Kirchneriella* can be called r-strategists (Cuvin-Aralar et al. 2004) which in the case of unicellular algae generally are small and present rapid growth when nutrients are abundant (Lampert and Sommer 1997). Because of this, the phytoplankton diversity and evenness were lower in the dry than in the rainy season. Also, the significantly lower Copepoda density entering the CW in the dry season may have contributed to limit phytoplankton diversity during that season; copepods are efficient grazers (as compared to rotifers, Morgan et al. 1980), so that with the lower amount of these grazers, phytoplankton species with competitive advantage like *K. lunaris* could become dominant. Moreover, in accordance with the resource-competitive theory (Tilman 1982), the lowest diversity occurs in places with few limiting resources, as occurred in the dry season.

Species of the genera *Melosira* generally are more abundant in moderate to high levels of water column turbulence due to their high capacity to adapt to low light intensity and their ability to perennate on sediments. As a consequence of the latter, resuspension of filaments provides a substantial inoculum of live cells to the water column causing growth and population increase (Reynolds et al. 1986). In this study *Melosira granulata* was abundant in both seasons, but in the rainy season the species *Melosira varians* also was abundant, due to resuspension of sediment caused by the high HLR. The highest abundance of *Aulacoseira* sp. in the WI site during the dry season and in both sampled sites during the rainy season can also be associated to high water flow (Sherman et al. 1998). Furthermore, the resuspension of the sediment may have increased survival of the thermotolerant coliforms (Katsenovich et al. 2009). Several of the phytoplankton species found in the CW system can potentially be harmful by producing cyanotoxins, such as the genera *Microcystis* and *Nostoc* (Prieto et al. 2007). However, neither of these genera were abundant and probably would not affect the other organisms. The Cyanobacteria species *Aphanocapsa* sp., *Chroococcus limneticus* and *Sphaerocavum brasiliense* showed higher densities only during the summer rainy season. The adaptive strategies of some species of Cyanobacteria, such as intense cell division at higher temperatures and the presence of gas vesicles, grant them competitive advantage over other groups of phytoplankton (Reynolds 1984) and eutrophic system conditions favor the establishment of blooms of species of this group (Huszar et al. 2000).

Zooplankton

In the zooplankton community Rotifera were more abundant than Cladocera and Copepoda in both seasons. The high loading mass of organic matter from the aquaculture ponds and UASB may have resulted in a detrital food chain in which rotifers had a competitive advantage. Bonecker and Aoyagui (2005) verified that generally Rotifera are a major consumer of bacteria mainly when these are at high density, as well as individuals in this group ingest debris particles in which the bacteria may be aggregated. Sipaúba-Tavares et al. (2002), studying the use of the floating macrophyte *E. crassipes* in constructed wetlands also observed Rotifera as the most abundant group, followed by Cladocera during the dry season and by Copepoda during the rainy one. In general, species with low tolerance of changing environmental conditions are eliminated, while those which can survive in turbulent habitats become dominant. Thus, Rotifera can establish more easily than Cladocera and Copepoda in systems with high hydraulic flow, such as the CW system studied.

Conclusions

The knowledge generated in this study contributed to the understanding the water regime limitations with regard to biogeochemical processes, removal efficiency of nutrients and organic matter, and performance of macrophyte species in constructed wetland systems in a subtropical climatic region, and thus to improve treatment efficiency. Higher macrophyte biomass, oxygenation, nutrient mass loading rates and hydraulic retention time in the winter dry season lead to better removal efficiency rates. In the summer rainy season the high rate of hydraulic load, temperature, higher inlet organic compounds mass loading and consequent decomposition process and decrease of oxygen concentrations impaired the CW treatment efficiency. Above all, the main factor for higher removal efficiency in the dry than in the rainy season was the highest biomass macrophytes, due to the direct and indirect effects on the improvement of water quality. The abundance of the zooplankton community changed due to the variation of climatic periods. The dominance and abundance of r-strategist planktonic species in the dry season was mainly related to higher nutrient levels. Thereby, the presence of plankton in association with macrophytes and other microorganisms adhered to the substrates should have influenced the removal of chemical compounds in the CW system. The authors suggest the need for interventions, such as engineering modifications to decrease the rate of hydraulic load especially in periods of high precipitation, in order to increase the removal efficiency of nutrients and organic matter by constructed wetlands in subtropical systems.

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