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Fungi in eutrophic reservoirs in Paraíba State in semi-arid Northeast Brazil

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ABSTRACT
Fungi are important mediators of energy flow and nutrients because are excellent decomposers, active in biotic self-purification of aquatic ecosystems. Some species grow under adverse conditions of pH, conductivity and high concentrations of algal biomass. Studies have reported the isolation of cutaneous and systemic species of opportunistic human fungi from polluted freshwater. This work aimed to determine the fungal biodiversity in three eutrophic reservoirs in the Paraíba state, semi-arid northeastern region of Brazil. Over 12 months, physical, chemical and biological parameters of water quality were analyzed from three different sampling points in each reservoir. There were significant differences in water quality within and between reservoirs in times of drought and rainfall, indicating that seasonal climatic changes exert a strong influence over the dynamics and ecophysiology of these ecosystems. From a total of 132 water samples, 33 fungi taxa were isolated by the sample dilution and plating method, showing predominantly dematiaceous and hyaline *Hyphomycetes*, *Coelomycetes*, *Zygomycetes* and yeasts of which 13 were common in the three reservoirs. Taxonomic abundance and richness were higher during the rainy season in two of the three environments, all equally eutrophic. Canonical analysis showed the influence of macronutrients on fungal distribution.
KEY WORDS: fungal diversity; hyphomycetes; yeasts; eutrophic dams

RESUMO: Fungos em reservatórios eutróficos no Estado da Paraíba no semi-árido Brasileiro. Os fungos são importantes mediadores do fluxo de energia e nutrientes pois são excelentes decompositores, ativos nos processos de auto-purificação dos ecossistemas aquáticos. Algumas espécies crescem em condições adversas de pH , condutividade e altas concentrações de biomassa de algas. Estudos têm relatado o isolamento de espécies cutâneas e sistêmicas de fungos oportunistas humanos a partir de fontes de água-doce poluídas. Este trabalho teve como objetivo determinar a biodiversidade fúngica em três reservatórios eutróficos no estado da Paraíba, região nordeste semi-árida do Brasil. Em 12 meses, foram analisados parâmetros físico-químicos e biológicos da água de três amostras de diferentes pontos em cada reservatório. Houve diferenças significantes dentro e entre os reservatórios nas estações chuvosa e seca, indicando mudanças climáticas sazonais exercem uma forte influência sobre a dinâmica e ecofisiologia destes ecossistemas. De um total de 132 amostras de água, 33 táxons de fungos foram isolados pelo método de diluição e plaqueamento, mostrando predominantemente *Hyphomycetes* demáceos e hialinos,

Coelomycetes, *Zygomycetes* e leveduras, dos quais 13 eram comuns nos três reservatórios. Abundância e riqueza taxonômicas foram maiores durante o período chuvoso, em dois dos três ambientes, todos igualmente eutróficos. A análise canônica mostrou a influência dos macronutrientes na distribuição de fungos.
PALAVRAS-CHAVE: diversidade fúngica; hyphomycetes; leveduras; açudes eutróficos

Introduction

Fungi are major decomposers of organic matter and thus play a major role in the self-purification of aquatic and terrestrial ecosystems, acting efficiently through several exoenzymes that break down a great deal of chemical substances, especially humic substances (Ostroumov 2005, Grinhut et al. 2007). The ecological role of fungi in lake ecosystems has been reviewed by Wurzbacher et al. (2010). In general, yeast and various types of hyphomycetes (Ingoldian and aero-aquatic) which can be found spread in different areas of a lake, where the littoral zone is a "hotspot" for having an abundance of hyphomycetes, given the large amount of allochthonous organic debris (leaves, seeds, remnants of trunks) since it is a transitional zone from a terrestrial to aquatic environment. Some fungal species are quite tolerant to adverse conditions of water, such as high pH, low levels of dissolved oxygen, high salinity and high hardness levels (Rajashekar & Kaveriappa 2003, Steiman et al. 2004, Cantrell et al. 2006, Medeiros et al. 2008). These characteristics are usually seen in lentic aquatic environments in the semi-arid northeastern region of Brazil.

Fungi can be found in ultraoligotrophic environment up to eutrophic environment or even environments impacted by human activities (Wurzbacher 2010). Alishtayeh et al. (2002), Duarte et al. (2004) and Niyog et al. (2009) noticed that wastewater and heavy metals decrease the density and diversity of hyphomycetes, allowing pollution-resistant species to prevail. Reviewing the consequences of aquaculture on water quality, Sarà (2007) observed that even though they show different types of impacts, all kinds of aquaculture projects including fish and shrimp farms, have strong impacts on the microbial community, including fungi. In Guarapiranga Reservoir, São Paulo State, Brazil, Wellbaum et al. (2007) isolated twenty fungal taxa unique to the aquatic environment and thirty-four common to aquatic and terrestrial environments, those associated with submerged leaves. In Northeast Brazil, Saraiva (1998) studied samples from Lake Araçá in Pernambuco and isolated *Acremonium*, *Aspergillus*, *Penicillium* and *Cladosporium*. Cavalcanti & Milanez (2007) isolated *Aspergillus* and *Penicillium* at a higher frequency in soil and water samples from the Vale do Prata and Meio Reservoirs. In Paraíba, Ceballos et al. (1995) studied the spatial-temporal distribution of filamentous fungi and yeasts in three reservoirs - with different levels of pollution - in a semi-arid region, where they isolated *Aspergillus spp.*, *Penicillium spp.*, *Fusarium spp.*, *Cladosporium spp.*, *Curvularia spp.*, *Coelomycetes* (*Phoma spp.*), *Rhizopus spp.* and yeasts of the genera *Candida*, *Saccharomyces*, *Rhodotorula.* Recent taxonomic studies in the Brazilian semiarid region (Almeida et al. 2012; Barbosa et al. 2013) reported a wide diversity of fungi in aquatic environments. Almeida et al. (2012) reported 17 species of facultative aquatic conidial fungi, with five new records. While Barbosa et al. (2013) realized a study in streams and identified 151 species of fungi associated with submerged plant debris, being the most mitosporic fungi. However the approach of these works is about a non-clinical perspective. Although it is known the the clinical potential of a species also depends both on ecological and environmental conditions as the physiological conditions of the affected organism.

The present work studied the temporal and spatial variations of water quality and fungal population densities and diversity looking for associations between water quality and the presence of opportunistic fungi in three reservoirs located in the semi-arid northeastern region of Brazil, where there is intensive fish farming (*Oreochromis niloticus*).

Materials and methods

Study environments: This study was conducted in the following three reservoirs located in three different watersheds with high levels of anthropogenic disturbance in Paraíba State (AESA 2009). "Cacimba da Várzea" Reservoir (CV) (6º41'2.84"S; 35º46'37.3''W) is located in the Curimataú River basin, with a maximum capacity of 9,264,321 m³, total area of 1,027,900 m2 and maximum depth of 21.6 m. The annual rainfall average is around 601-800 mm (AESA 2006). "Várzea Grande" Reservoir (VG) (6°26'40.60"S, 36°20'1.85"W) is in the micro-region of Seridó, belongs to the sub-basin of the Seridó River (Piranhas River watershed), and has 21,532,659 m³ of maximum capacity. The annual rainfall average varies from 200 to 400 mm

(AESA 2006). "Acauã" Reservoir (Ac) (7°27.5 '3"S, 35°35'52.6"W) is located in Paraíba watershed, with an annual average rainfall of around 401 to 600 mm (AESA 2006). It has an area of 2300 ha and a maximum volume of 253,000,000 m³. All reservoirs have netcages with intensive fish farming(*Oreochromis niloticus* - Nile tilapia).

*Sample collection***:** Water samples were collected with Van Dorn bottles during twelve months (August 2008 to July 2009) at three sampling points in each reservoir - SP1: tributary river entrance; SP2: net cage proximity; and SP3: dam proximity – which were treated as replicates. Water samples were collected along the water column and a composite sample, prepared with the same volume of water from each level was used for analysis. The samples were placed in neutral plastic containers, identified and stored in thermal boxes at a temperature below 10°C until arrival at the laboratory.

Water quality parameters: The parameters evaluated were: temperature (T), pH, electrical conductivity (C), transparency (Trans), alkalinity (Alk), hardness (Har), dissolved oxygen (DO), biochemical oxygen demand (BOD), ammonia-N (am-N), nitrite-N (NO2-N) and nitrate-N (NO3-N), total phosphorus (TP) and soluble reactive phosphorus (SRP). All methods used were according to Clesceri et al. (1998). Chlorophyll-a (Chl-a) was assayed according to Wetzel & Likens (1991).

Trophic state index (TSI): The TSI average was calculated according to Carlson's principles (Carlson 1977) adapted by Toledo Jr. et al. (1983) for tropical environments.

Fungi: The dilution plating method (Clesceri et al. 1998) was used for the quantification of fungi. Aliquots of 0.1 ml of each dilution were spread on Petri dishes with potato dextrose agar (PDA).After incubation (for 5 to 7 days at 35°-36°C) the colonies were counted and then transferred to tubes containing PDA. Fungal concentrations were expressed in colony-forming units (CFU) per mL. Macro and micromorphological identification on permanent slides (Riddell 1950) was performed and biochemical tests were runwith the aid of the identification key from Larone (1995) and Hoog & Guarro (1995).

Statistics: Kruskal-Wallis analysis of variance (KW ANOVA) was applied for comparison of both physical and chemical parameters between samples collected, which was carried out within each reservoir (intra-reservoir) and between reservoirs (inter-reservoir). Canonical correspondence analysis (CCA; Canoco 4.5) was performed to visualize the distribution of fungi in relation to the distribution of physical and chemical parameters.

*Rainfall***:** The separation between dry and rainy seasons was determined following Sansigolo's principle (1989), which considers as a divider those months in which the monthly rainfall is 50% higher or lower than the previous month's rainfall. The historical rainfall reference for each region was calculated using data from the preceding eight years (August 1999 until July 2007) from PROCLIMA - Programa de Monitoramento Climático em Tempo Real da Região Nordeste.

Results

*Physical and chemical parameters***:** Some parameters showed higher values (TP, EC) and others, smaller values, compared with some reservoirs the belong to another basins into same state (Vieira et al. 2009) and located in other states (Leitão et al. 2006; Chellapa et al. 2008, Bezerra et al. 2014), although all dams belong to Braizilian semiarid region. The pH values ranges from natural to alkaline (Table I).

Fungi: A total of 33 taxa were found in all three environments: 24 were found in "Cacimba da Várzea" (Table II), 18 in "Várzea Grande" (Table III) and 24 in "Acauã" (Table IV). Most of them belong to the mitosporic fungi or hyphomycetes (Hoog & Guarro 1999). The remaining taxa were yeast, Coelomycetes, Zygomycetes and two taxa not identified (TNI).

Higher density was identified during rainy season in CV and VG. (Table V).

Table I. Mean values and standard deviation of physical, chemical and biological parameters of water quality during rainy and droughty seasons in three eutrophic dams: "Cacimba da Várzea", "Várzea Grande" and "Acauã" at northeast of Brazil (Paraiba state).

Canonical correspondence analysis (CCA): Alk and Har were excluded from the analysis for because they are parameters that are correlated with C. CCA was carried out with 499 permutations. In CV, CCA showed that axis 1 was represented by TP (0.755) and N-am (0.881), which explained 22.8% of the variance. T (0.648) and Chl-a explained 14.7% of the variance in axis 2. The Monte Carlo test showed that the axes were marginally significant for a non-random distribution of species in the graph (trace = 3.181, F = 1.210, P = 0.098). *Aspergillus niger*, *Aspergillus sp1* and *Aspergillus sp2* showed a positive correlation with T and negative correlation with Chl-a, OD and BOD. *Aspergillus flavus*, *Paecilomyces sp*. *Scopulariopsis sp*. showed a negative correlation with TP and am-N. TNI1, *Rhodotorula sp.*, *Trichophyton sp.* and black yeast were abundant when Chl-a concentrations were higher (Figure 1).

Table I. Mean values and standard deviation of physical, chemical and biological parameters of water quality during rainy and droughty seasons in three eutrophic dams: "Cacimba da Várzea", "Várzea Grande" and "Acauã" at northeast of Brazil (Paraiba state).

	T (°C)		pH		C (µS/cm)		Har ($mgCaCO_3/L$)		Alk ($mgCaCO_3/L$)	
Environments	**Rainfall***	**Drought****	**Rainfall**	**Drought**	**Rainfall**	**Drought**	**Rainfall**	**Drought**	**Rainfall**	**Drought**
CV†	27,6 ± 1,1	26,5 ± 0,7	8,0 ± 0,2	8,2 ± 0,1	2787,0 ± 426,7	3058,3 ± 82,8	447,7 ± 56,3	477,2±20,6	124,6 ± 42,4	152,3± 8,7
VG♦	27,0 ± 1,1	25,8 ± 1,3	8,6 ± 0,2	8,7 ± 0,3	1850,6 ± 117,0	1904,2 ± 145,4	232,5 ± 17,9	232,5±15,7	140,4 ± 30,4	155,5± 6,1
Ac¶	28,5 ± 1,7	28,3 ± 0,6	8,2 ± 0,7	8,6 ± 0,1	676,3 ± 92,0	736,3 ± 22,4	136,8 ± 16,7	142,1 ± 8,7	66,0 ± 14,4	74,3 ± 3,9

	DO (mg/L)		BOD (mg/L)		TP (µg/L)		SRP (µg/L)		N-am (µg/L)	
Environments	**Rainfall**	**Drought**	**Rainfall**	**Drought**	**Rainfall**	**Drought**	**Rainfall**	**Drought**	**Rainfall**	**Drought**
CV	8,4 ± 1,3	9,4 ±1,0	3,8 ± 0,4	4,5 ± 0,3	93,8 ± 60,1	135,3 ± 58,8	28,5 ± 11,8	28,6 ± 10,1	52,4 ± 48,8	56,8± 14,5
VG	8,0 ± 2,1	8,5 ± 2,4	4,5 ± 0,7	4,4 ± 0,9	111,7 ± 99,8	232,7 ± 28,8	32,2 ± 25,1	42,0 ± 22,1	54,1 ± 32,2	63,7± 35,6
Ac	10,0 ± 2,3	10,7 ± 2,0	4,6 ± 0,6	4,6 ± 0,7	141,4 ± 82,2	160,6 ± 32,4	43,0 ± 15,1	50,3 ± 25,6	58,2 ± 29,5	96,9± 42,7

	NO2-N (µg/L)		NO3-N (µg/L)		Chl-a (µg/L)		Trans (m)		Average annual rainfall
Environments	**Rainfall**	**Drought**	**Rainfall**	**Drought**	**Rainfall**	**Drought**	**Rainfall**	**Drought**	
CV	3,8 ± 2,2	16,6 ± 11,7	18,5 ± 6,5	23,1 ± 4,6	40,6 ± 24,8	44,6 ± 7,7	0,5 ± 0,1	0,6 ± 0,0	76,8 ± 56,9
VG	2,8 ± 2,0	11,3 ± 7,0	17,2 ± 12,4	21,6 ± 9,3	67,6 ± 24,9	42,3 ± 23,2	0,5 ± 0,2	0,6 ± 0,3	81,9 ± 76,4
Ac	7,9 ± 12,3	8,5 ± 5,3	20,9 ± 5,9	25,2 ± 10,5	51,1 ± 33,2	41,7 ± 22,0	0,8 ± 0,3	0,6 ± 0,0	69,5 ± 60,2

†CV – Cacimba da Várzea dam; ♦VG-Várzea Grande dam; and ¶Ac- Acauã dam

Table II –Isolated fungi in "Cacimba da Várzea" a northeast tropical Brazilian dam (Paraiba state).

	Fungi	Months of occurrence												Highest density month (UFC/mL)
		aug /08	sep/ 08	oct/ 08	nov/ 08	dec/ 08	jan/ 09	feb/ 09	mar/ 09	may/ 09	apr/ 09	jun/ 09	jul/ 09	
	Conidial Fungi													
1	*Acremonium sp.* (H)	x												aug/08 (R)
3	*Aspergillus flavus* (H)				x			x						nov/08 (D)
4	*Aspergillus niger* (H)							x	x	x				mar/09 (R)
5	*Aspergillus* sp.1 (H)					x		x	x	x	x			feb/09 (R)
6	*Aspergillus* sp.2 (H)								x	x				mar/09 (R)
7	*Aspergillus terreus* (H)						x							jan/09 (D)
8	*Cladophialophora bantiana* (H)	x												aug/08 (R)
9	*Cladosporium* sp. (H)				x		x	x					x	feb/09 (R)
10	*Exophiala* sp. (H)	x												aug/08 (R)
11	*F.N.I.* sp.1 (H)						x							jan/09 (D)
13	*Fusarium* sp. (H)				x									nov/08 (D)
15	*Mycelia sterillia* (A)									x		x		jun/09 (R)
16	*Ochrochonis* sp. (H)	x												aug/08 (R)
17	*Paecilomyces* sp. (H)			x	x		x		x	x			x	jul/09 (R)
19	*Penicillium* sp.2 (H)	x		x		x		x	x		x	x	x	mar/09 (R)
20	*Penicillium* sp.3 (H)						x	x	x			x		mar/09 (R)
21	*Penicillium* sp.4 (H)					x						x	x	jul/09 (R)
22	*Penicilliums* sp.5 (H)												x	jul/09 (R)
25	*Penicillium* sp.8. (H)										x	x		jun/09 (R)
26	*Scopulariopsis* sp. (H)				x			x						feb/09 (R)
27	*Tricophyton* sp. (H)											x		jun/09 (R)
	Zygomycetes													
29	*Rhizopus* sp.										x			may/09 (R)
	Yeasts													
31	*Black yeast*												x	jul/09 (R)
32	*Rhodotorula* sp.	x			x	x	x					x		jun/09 (R)
	Number of taxa	6	0	2	6	4	6	7	6	5	4	8	6	

SP1- tributary rivers entrance, SP2 –net-cages proximity, SP3 – proximity of the dam, R – rainy season (grey columns), D – drought season (white columns), (H) – Hyphomycetes, (A) – Agonomycetes, (C) – Coelomycetes.

In VG, CCA axes 1 and 2 both showed weak correlations with environmental variables (Figure 2). On axis 1, the variables with higher correlations were soluble SRP (0.333), BOD (0.268) and NO2-N (-0.228), which explained 19.6% of the variance. As for axis 2, Chl-a (-0.344), BOD (-0.283) and T (-0.284) explained 17.6% of the variance. The Monte Carlo test demonstrated that environmental variables did not explain the distribution of species (trace = 1.851, F = 0.901, p = 0.754). In Ac, CCA revealed that the first axis explained 30.2% of the variance, which was represented by Chl-a (0.804), while the second axis was represented by NO2-N (0.664) explaining, therefore, 21.2% of the variance. Monte Carlo test also shows that the axes were significant, that is, the distribution of fungi species was not random (trace = 3.099, F = 1.303, p = 0.048).

Table III. Isolated fungi in "Várzea Grande" northeast tropical Brazilian dam (Paraiba state)

	Fungi	Months of occurrence												Highest density month (UFC/mL)
		aug/ 08	sep/ 08	oct/ 08	nov/ 08	dec/ 08	jan/ 09	feb/ 09	mar/ 09	may/ 09	apr/ 09	jun/ 09	jul/ 09	
	Conidial Fungi													
1	*Acremonium* sp. (H)	x		x				x						oct/08 (D)
2	*Aspergillus candidus* (H)	x												aug/08 (R)
3	*Aspergillus flavus* (H)							x	x					feb/09 (R)
4	*Aspergillus niger* (H)		x	x	x				x	x	x	x		mar/09 (R)
5	*Aspergillus* sp.1 (H)						x	x	x		x			may/09 (R)
9	*Cladosporium* sp. (H)			x				x					x	oct/08 (D)
12	*F.N.I. sp.2* (H)									x				apr/09 (R)
14	*Geotrichum sp.* (H)												x	jul/09 (R)
17	*Paecilomyces sp.* (H)	x				x	x	x	x	x			x	jul/09 (R)
18	*Penicillium* sp1. (H)		x											sep/08 (D)
19	*Penicillium* sp2 (H)	x		x	x		x	x	x		x		x	may/09 (R)
20	*Penicillium* sp3. (H)			x					x					mar/09 (R)
21	*Penicillium* sp4. (H)								x	x	x	x	x	may/09 (R)
25	*Penicillium* sp8. (H)										x			may/09 (R)
26	*Scopulariopsis* sp. (H)								x			x		mar/09 (R)
	Zygomycetes													
29	*Rhizopus sp.*										x			may/09 (R)
	Yeasts													
30	*Candida sp.*	x								x				apr/09 (R)
32	*Rhodotorula sp.*	x	x	x		x	x	x	x			x		jul/09 (R)
	Number of taxa	6	3	6	2	2	4	7	9	5	6	4	5	

SP1- tributary rivers entrance, SP2 –net-cages proximity, SP3 – proximity of the dam, R – rainy season (grey columns), D – drought season (white column), (H) – Hyphomycetes, (A) – Agonomycetes, (C) – Coelomycetes.

Many fungi displayed a negative correlation with Chl-a, especially *Cladosporium sp*., *Paecilomyces sp*. and *Rhodotorula sp.* Despite axis 2, *Acremonium sp*. and *Penicillium sp3* showed positive correlations with NO2-N, while *Penicillium sp4* and fungi not identified FNI-1did not (Figure 3).

Discussion

The Hyphomycetes appeared in all three environments and were the dominant group. Most identified fungi were hyaline fungi (19 taxa) and to a lesser extent dematiaceous fungi (five taxa). In the hyaline hyphomycetes group, four species and two morphospecies of *Aspergillus* and eight morphospecies of *Penicillium* were identified, demonstrating the dominance of these genera. Other hyaline genera found were *Acremonium*, *Fusarium*, *Geotrichum*, *Paecilomyces*, *Scopulariopsis* and *Trichophyton.* The dematiaceous hyphomycetes *Cladophialophora bantiana*, *Cladosporium sp.,* *Exophiala sp.* and *Ochrochonis sp.* were present in all three reservoirs at low frequencies of occurrence, except *Cladosporium sp.* In Ac *Rhizopus sp.*, the only Zygomycetes recorded, was isolated from all three environments during the rainy season. In addition to the yeasts, *Rhodotorula sp.* was isolated from the three environments in both rainy and droughty periods, although appearing more often during the dry months; *Candida sp.* was isolated in VG and Ac and black yeast only in CV. Most taxa (13) such as *Acremonium sp.*, *Cladosporium sp.* and *Rhodotorula sp.* were common to all three environments. In the three reservoirs, there was higher species richness in the wet season (22) than dry season (9). In CV and the most frequent species were *Penicillium sp2* (75%), *Aspergillus sp1* (62,5%) and *Penicillium sp3* (50%) in the rainy season and *Paecilomyces sp.* (50%), *Penicillium sp2* (50%) and *Rhodotorula sp.* 50%) in the dry season. In VG 16 species were recorded in the rainy season

Table IV. Isolated fungi in "Acauã" northeast tropical Brazilian dam (Paraiba state).

	Fungi	Months of occurrence												Highest density month (UFC/mL)
		aug /08	sep/ 08	oct/ 08	nov/ 08	dec/ 08	jan/ 09	feb/ 09	mar/ 09	may/ 09	apr/ 09	jun/ 09	jul /09	
	Conidial Fungi													
1	*Acremonium* sp. (H)	x		x		x								jan/09 (D)
3	*Aspergillus flavus* (H)		x	x	x			x						nov/08 (D)
4	*Aspergillus Niger* (H)			x		x	x	x	x					feb/09 (R)
5	*Aspergillus* sp.1 (H)				x			x	x		x			feb/09 (R)
6	*Aspergillus* sp.2 (H)									x				apr/09 (R)
7	*Aspergillus terreus* (H)				x									nov/08 (D)
9	*Cladosporium sp.* (H)			x	x		x				x			oct/08(D)
10	*Exophiala sp.* (H)	x												aug/08(R)
11	*F.N.I.* sp.1 (H)							x			x			feb/may/09(R)
14	*Geotrichum sp.* (H)									x				apr/09(R)
15	*Mycelia sterillia* (A)										x			may/09(R)
17	*Paecilomyces sp.* (H)	x		x	x	x	x							sep/08(D)
19	*Penicillium sp2* (H)	x		x	x	x	x	x	x		x			feb/09(R)
20	*Penicillium sp3.* (H)			x										oct/08(D)
21	*Penicillium sp4.* (H)							x	x		x			mar/09(R)
22	*Penicillium sp5.* (H)												x	jul/09(R)
23	*Penicillium* sp6. (H)										x			may/09(R)
24	*Penicillium* sp7. (H)				x									nov/08(D)
25	*Penicillium* sp8. (H)						x					x		jun/09(R)
26	*Scopulariopsis* sp. (H)	x	x	x										aug/08 (R)
28	*Phoma sp.* (C)			x	x									nov/08(D)
	Zygomycetes													
29	*Rhizopus* sp.									x				apr/09 (R)
	Yeasts													
30	*Candida* sp.	x						x						feb/09 (R)
32	*Rhodotorula* sp.	x	x	x	x	x	x		x					nov/08 (D)
	Number of taxa	7	3	10	9	4	7	7	5	3	7	1	1	

SP1- tributary rivers entrance, SP2 – net-cages proximity, SP3 – proximity of the dam, R – rainy season (grey columns), D – drought season (white columns), (H) – Hyphomycetes, (A) – Agonomycetes, (C) – Coelomycetes.

and eight in the dry season, and the most frequent were *Paecilomyces sp.* (75%), *Penicillium sp2* (75%) and *Rhodotorula sp.* (62.5%) during the rainy period, and *Rhodotorula sp.* (75%) and *Penicillium sp2* (50%) during the dry season. In Ac 20 species were found in the rainy season and 14 in the dry season, and the most frequent fungi were *Penicillium sp2* (57.1%), *Penicillium sp4* (42.8%) and *Aspergillus sp1* (42.8%) in the rainy period, and *Rhodotorula sp.* (100%), *Aspergillus niger* (60%), *Asperillus flavus* (60%) and *Cladosporium sp.* (60%) in the dry period. Fungal density was higher during the rainy months, except in Ac which displayed higher densities in the droughty season. The range of mean fungal densities in Ac was always higher in comparison with the other reservoirs in both rainy and droughty seasons (Table V).

Wellbaum et al. (2007) found hyaline and dematiaceous hyphomycetes (*Aspergillus sp.*, *Penicillium sp.*, *Phoma sp.*, *Rhizopus sp.*, *Cladosporium sp.* and *Fusarium sp.*) associated with leaves collected from terrestrial and aquatic environments in Guarapiranga Reservoir, São Paulo. These authors showed that fungal diversity was

higher during the dry and cold season, even though the species were less abundant. However, the opposite was observed during the hot and humid period of the year. This phenomenon was related to the transport of organic matter by summer rains, which would decrease the concentrations of DO, and selected the most resistant species. In CV and VG density and richness were higher during the rainy season. In Ac, density and species richness were almost equal. Ceballos et al. (1995) monitored three northeastern Brazil reservoirs (Bodocongó, Açude Velho and Boqueirão Reservoirs) located in the semi-arid region of Paraíba, Brazil, and found a higher occurrence of *Aspergillus spp.* and *Penicillium spp.* in all three reservoirs, which had different levels of pollution. In that study, *Phoma sp.* was isolated only during the droughty season, even though according to Ceballos et al. (1995), it can be found during the rainy season as well but at low frequency. It is important to mention tha Coelomycetes (*Phoma sp.*) has been reported in very few studies and is considered as a group with rare frequency in the aquatic environment. But this rarity, according to Shearer et al. (2007) is due to lack of studies and specialized knowledge about this group. Ceballos et al. (1995) also reported that *Rhizopus*, along with the yeasts *Rhodotorula sp.* and *Candida spp.*, may also be present in dry and rainy seasons.

Table V. Density (CFU/mL) of taxa.

Reservoirs	RAINFALL*				DROUGHT**			
	Min.	Mean	Max.	Sum	Min.	Mean	Max.	Sum
CV†	633.3***	2,739.513	7,533.2	21,916.1	0	441.6	1,066.6	1,766.5
VG♦	466.6	1,867.025	4,900	14,936.2	33.3	683.3	1,233.3	2,733.2
AC¶	366.5	3,223.814	7,533.1	22,566.7	533.4	4,502.9	8,026.5	22,514.7

†CV – Cacimba da Várzea dam; ♦VG-Várzea Grande dam; and ¶Ac- Acauã dam.
*Rainfall for CV and VG: aug/08 and jan/09 to jul/09 (total:eight months). Rainfall for Ac: aug/08 and feb/09 to jul/09 (total: seven months).
**Drought for CV and VG:sep/08 to dec/08 (total:four months). Drought for Ac: sep/08 to jan/09 (total:five months).
***Sum of replicate densities (CFU/mL) by season.**

Raja et al. (2009) noted, in a study conducted in the Florida peninsula, that latitude difference (of 5º) is an important factor for fungal distribution patterns. In the present study, no significant variation in species diversity was found; the small difference in latitude (about 1º) between the three reservoirs was not enough to influence fungal distribution. The differences concerning species richness are probably related to the particular characteristics of the reservoirs and watersheds. The hyphomycetes fungi belong mostly to terrestrial environments but are adaptable to aquatic environments by introducing their propagules (spores or hyphae) into water bodies through the flow of rivers, rainwater and wind (Wurzbacher et al., 2010). According to these authors, the morphology of water bodies may facilitate the accumulation of organic matter in marginal dendritical zones with slow water flow and low aeration, which affects the metabolism and reproduction of hyphomycetes and, consequently, alters their biodiversity. The study of fungi in alkaline environments is rare. Steiman et al. (2004) studied an ecosystem with pH ranging from 9.4 to 9.8 (Mono Lake, California) and could not isolate fungi from the coastal region by the plating method, where he attributed this result to the high salinity environment. However, Cantrell et al. (2006) isolated hyphomycetes (nine species of *Aspergillus*, five species of *Penicillium* and *Cladosporium cladosporioides*) and black yeast (*Hortaewerneckii*) from hypersaline environments in Puerto Rico.

According to Wurzbacher et al. (2010), the pelagic area is predominantly inhabited by yeasts. However, for filamentous fungi, this area is considered a dispersal medium rather than a habitat itself. Yeasts inhabit freshwaters in higher proportions than marine waters (Kurtzman & Fell 2004). However, the three environments studied here showed lower species richness compared to the study by Ceballos et al. (1995), who found greater diversity of these microorganisms in eutrophic waters. Woollet & Hedrick (1970) isolated *Rhodotorula sp.* in three water bodies with different levels and types of pollution (low, domestic and industrial sewage discharges), exhibiting a significant increase in *Candida* density in the water with domestic pollutants. Ceballos et al. (1995) observed that the frequency of occurrence of yeasts increased with fecal contamination. Thus, species of

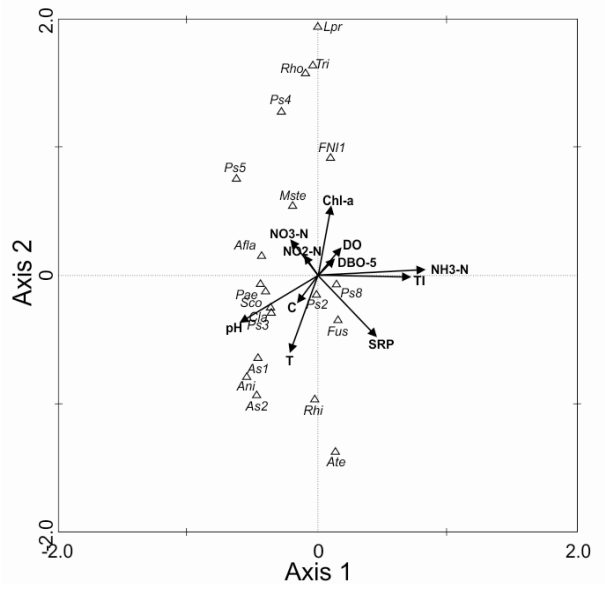

Figure 1. CCA of "Cacimba da Várzea" (p> 0.05). T – temperature; C – conductivity; Nm – ammonia nitrogen; Ni - nitrite Na - nitrate; DO – dissolved oxygen; BOD – biochemical oxygen demand; TP - total phosphorus; SRP - soluble reactive phosphorus and Chl – chlorophyll-a.

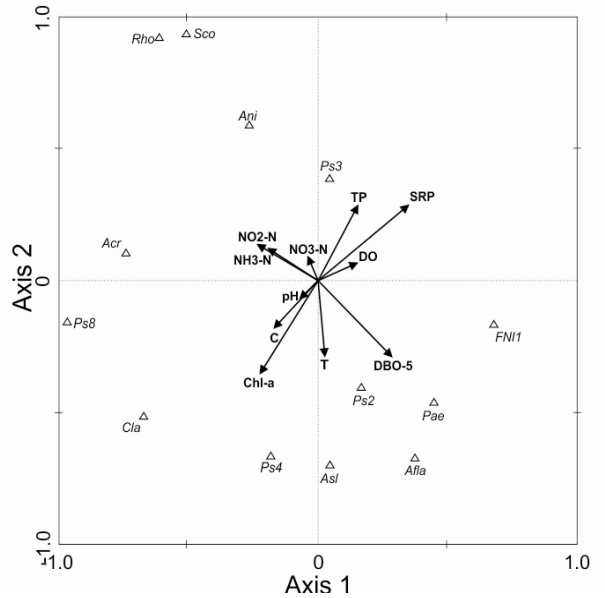

Figure 2. CCA of "Várzea Grande" (p>0,05). T – temperature; C – conductivity; Nm – ammonia nitrogen; Ni - nitrite; Na - nitrate; DO – dissolved oxygen; BOD – biochemical oxygen demand ;TP –total phsphorusm, SRP - soluble reactive phosphorus and Chl – chlorophyll-a .

Candida and Rhodotorula were more frequent in environments polluted with organic matter. Yeasts are associated with the daily life of humans and may represent bioindicators of water pollution (Woollet & Hedrick 1970).

Fischer et al. (2009) noticed that isolated fungi from submerged leaves in rivers in Switzerland

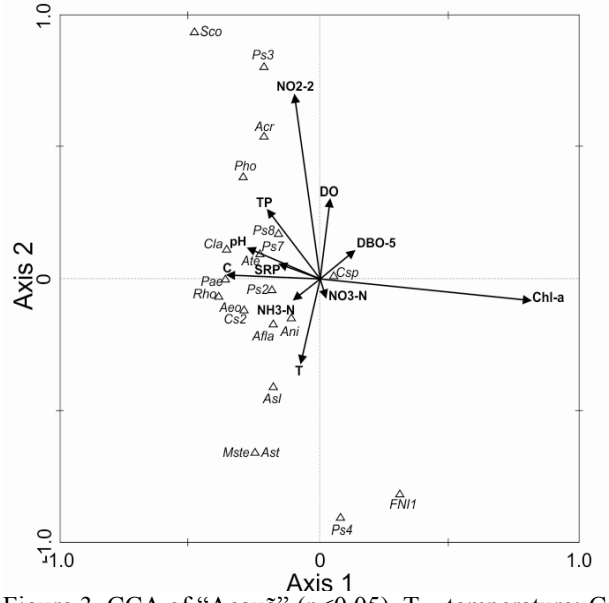

Figure 3. CCA of "Acauã" (p<0,05). T – temperature; C – conductivity; Nm – ammonia nitrogen; Ni - nitrite; Na - nitrate; DO – dissolved oxygen; BOD – biochemical oxygen demand; TP - total phosphorus; SRP – soluble reactive phosphorus and Chl – chlorophyll-a.

correlated significantly with electrical conductivity and nutrients (soluble reactive phosphorus, nitrite and nitrate), although the factor "type and abundance of vegetation "around the water body exerted a higher influence, because according to the authors, it is a source of carbon. In the present study, water quality seems to have exerted more influence than the vegetation around the reservoirs, in view of the fact that CV shared similar vegetation with VG but had higher fungal species richness. VG showed the highest values for electrical conductivity (2877 µS/cm) and hardness (457.5 mg CaCO3/L), which may have negatively influenced species richness. Ac differed from the other reservoirs due to the polluting discharges of the tributary rivers associated with untreated sewage water (Brito 2008). However, Ac had the same fungal diversity as CV.

For most parameters (pH, hardness, alkalinity, DO, TP, SRP, N-am, NO2-N and NO3-N), the annual averages for the rainy season were slightly lower than for the droughty season, but species richness and density of fungi in the rainy season were higher, which may indicate rainfall as being the most important factor determining these characteristics, mainly because in semi-arid northeastern environments the rains are allochthonous factors that affect the physical, chemical and biological characteristics of water (Guimarães 2006).

Infections by opportunistic pathogens are often a result of human contact with these agents (usually saprophytes, inhabitants of water, soil and plants) through different transmission routes such as inhalation, ingestion and dermal contact with injured tissue. Individuals with low immunity are easily susceptible to these kinds of infections (Hoog & Guarro 1995, Porto et al. 2009, Nucci et al. 2010).

Several fungi found in aquatic animals from intensive farming systems have been isolated from sediment, water column and gills of mollusks (oysters and clams), particularly *Penicillium spp.* from the *Mucorales* species, *Aspergillus spp.* and *Cladosporium spp.* (Sallenave-Namont et al. 2000), and in fishes (Rand 1996). Fish diseases and cases of nutritional disorders have been related to the presence of mycotoxins in their diet, produced by *Aspergillus*, *Fusarium* and *Penicillium*, leading to mycotoxicosis. *Exophiala sp.*, *Ochrochonis sp.*, *Cladosporium sp.*, *Aspergillus sp.*, *Fusarium sp.*, *Penicillium sp.*, *Phoma sp.* and *Candida sp.* have been isolated from superficial and deep sites of the dermis of several aquatic animals, indicating them as potential opportunistic pathogens (Rand 1996). Parasitism in *Tilapia aurea* and *Tilapia mossambica* with *Paecilomyces lilacinus* was reported in two lakes in Puerto Rico (Rand et al. 2000). Moreover, an infection in the bladder and kidneys of red snappers (*Lutjanus campechanus*) by *Penicillium corylophilum* and *Cladosporium sphaerospermum*, causing injury and changes in behavior (Blaylock et al. 2001) has also been identified. Coelho et al. (2003) isolated *Rhizopus sp.* from the boga fish in the Mondego River in Portugal. These fungal genera were found in all three environments in this study, though the following appeared more often near tilapia netcages: *Aspergillus niger* (CV and VG), *Aspergillus sp1.* (CV and VG), *Paecilomyces sp.* (CV, VG and Ac), *Penicillium sp 2.* (CV, VG and Ac), *Penicillium sp3.* and *sp4.* (VG), *Scopulariopsis sp.* (VG), *Aspergillus flavus* (Ac), *Cladosporium sp.* (Ac) and *Phoma sp.* (Ac). However, much more investigation is required to determine possible infections in tilapia, including the possibility of contamination by mycotoxins, either directly through the presence of the fungus in the fish or the presence of such contaminants in the fish feed. And to determine to what degree mycotoxins can be harmful to humans when they feed on possible infected fishes. Pietsch et al (2013) reported the presence of mycotoxins in commercial fish feeds for aquaculture, harmful to the health of the animal, although risks still remain unknown and require further studies, including the risks to human health (Hinton 2000). These approaches have not been object of this study of this research. Although we can already question whether intensive farming with netcages are providing proper conditions for these fish or rather submitting them to stressful conditions, causing them to become vulnerable to microorganisms, such as fungi.

Some species of the genera *Aspergillus*, *Fusarium* and *Candida* have been shown to be the main pathogens in opportunistic fungal infections (such as aspergillosis, fusariosis and candidiasis) in immunocompromised humans. Furthermore, it has been observed that clinical cases of zygomycosis and phaeohyphomycosis (dematiaceous fungi - *Cladosporium* and *Exophiala*) in Latin America have increased (Nucci et al. 2010). They can also be agents causing superficial mycoses in nails, skin and scalp (*Candida* and *Acremonium*) and dermatophytosis (*Trycophyton spp.*) (Vermout et al. 2008, Porto et al. 2009). This is an important observation for many reservoirs supplying urban and rural communities. The water in these reservoirs need to be adequately treated before human use, otherwise there is a high risk of mycosis transmission to the public. Some studies have detected opportunistic fungi in bottled water (Yamaguchi et al. 2007, Hageskal et al. 2009) and even in water used for dialysis in hospitals (Varo et al. 2007), which passes through several treatment procedures that may have been insufficient.

Most Hyphomycetes genera and opportunistic yeasts have been found in association with contaminated water, indicating that these environments are vehicles for the transmission of these fungi. Further investigation is needed to identify the isolated species in addition to the epidemiological studies in communities that are supplied by these water bodies to determine if these are sources of opportunistic infections, since species isolated from the environment do not always match clinical case strains (Vicente et. al. 2008).

Water management is important and urgent in the semi-arid northeastern region of Brazil and, along with watershed management, can preserve water quality, and the biota as well, minimizing anthropogenic impacts. Fungi from natural biota are important to the balance of ecosystems, particularly in nutrient cycling, and can be essential in recovering environments through bioremediation (Gadd 2001, Gadd 2004). The management of eutrophic water reservoirs that supply water for human consumption and use should include special

techniques for treatment, to decrease the risks to rural and urban populations that depend on these waters.

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