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Land Use and Basin Characteristics Determine the Composition and Abundance of the Microzooplankton

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Abstract The influence of watershed land use on microzooplankton was examined. Six rivers and a shallow lake located in rural (agriculture, livestock) and urban areas were sampled during 4 weeks at low water, low temperatures and 3 weeks at high water, high temperatures. The major aim of this study was to analyze the composition, richness and abundance of the microzooplankton in relation to land use, taking into account nutrient concentration, biological oxygen demand (BOD_5), conductivity, pH, transparency, dissolved oxygen, and chlorophyll-a. Redundancy analysis was used to assess microzooplankton response to environmental gradients. The composition and abundance can be considered good indicators of the land used and characteristic of the basin (broad range of conductivity water). The species composition show a gradient along the conductivity, pH and chlorophyll-a. *Brachionus* spp. were associated with saline waters on rural area and *Keratella* spp. (except *Keratella tropica)* were associated with urban water bodies. The microzooplankton abundance diminished by a factor of ten from the rivers in livestock–agriculture-dominated watersheds to those located in strictly urban areas. Urban rivers had low abundances of chlorophyll-a and microzooplankton despite the high concentration of nutrients. However, the effect of urbanization (mesotrophic/mesosaprobious state and lead presence) cannot be analyzed alone due to the potential effect of a filter-feeding invasive mollusk that colonizes the hard surfaces of harbor buildings and bridge pillars.

Keywords *Brachionus* · *Keratella* · Invaders · Urbanization · Agricultural practices

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1 Introduction

Zooplankton community composition has been widely related to environmental conditions such as resource availability, submerged macrophytes, predation by vertebrates and invertebrates as well as other biotic interactions. These factors have been largely analyzed in relation to the zooplankton abundance, composition, and structure in different aquatic environments, mainly lakes (McQueen et al. 1989, 1986; Schriver et al. 1995; Jeppesen et al. 2003).

The studies have been conducted mostly, in "natural" or non-human-dominated hydrosystems. However, it is known that human activities affect ecosystem functions through the disruption of pattern and rate of matter as well as energy flow (Ohl et al. 2007).

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The increasing human land use concerning mainly urbanization, agriculture activities, livestock rearing and deforestation, address a strong impact on the hydrology, geomorphology, and water quality of aquatic systems (Baer and Pringle 2000; Paul and Meyer 2001; Walsh et al. 2005). The effects range from runoff of fine sediments, nutrients, and pesticides (agricultural practices) to input of metals, oils, and road salts (urbanization) (Schulz and Liess 1999; Moore and Palmer 2005). These changes are reflected in the biodiversity, taxonomic composition and abundance of the aquatic communities (McKinney 2002; Walsh et al. 2005). Diversity loss, species invasion as well as chemical and organic pollution are also directly associated with human modifications (Allan and Flecker 1993; Faeth et al. 2005).

Studies that deal with algae, macroinvertebrates, and fish have reported a clear relationship between land use and population abundance or species composition (Gray 2004; Taylor et al. 2004; Morgan and Cushman 2005; Moore and Palmer 2005; Smith and Lamp 2008; Urrea and Sabater 2009; Utz et al. 2009).

Several investigations have reported that zooplankton, particularly the rotifers, respond rapidly to environmental changes due to their short life cycles and r-strategist attributes. Consequently, they are good indicators for assessing changes in limnological conditions and trophic status (Gannon and Stemberg 1978; Beaver and Crisman 1990; Attayde and Bozelli 1998; Duggan, Green and Shiel 2001, 2001; Whitman et al. 2004). However, zooplankton have rarely been included in biomonitoring schemes and investigations of land use effects. The link between watershed land use and zooplankton species richness has been recently explored, mainly in small streams, lakes and reservoirs (Ejsmont-Karabin and Kruk 1998; Lougheed and Chow-Fraser 2002; Dodson et al. 2005; Dodson 2008; Dodson and Lillie 2001; Hoffmann and Dodson 2005), but references to the relationship with abundance of zooplankton, and even more, the case of plain rivers, are scarce (e.g., Olguin et al. 2004; Claps et al. 2009).

Some areas of the alluvial plain of the Middle Paraná River are affected by livestock, farming, industrial and human settlements. Santa Fe (451,500 inhabitants) and Santo Tomé Cities (75,000 inhabitants) are included in this alluvial valley. Studies conducted in different rivers of the region, mainly secondary branches and tributaries, have described the zooplankton community over the last few decades (José de Paggi 1981; José de Paggi and Paggi 1998; Pecorari et al. 2006). However, the studies were not conducted simultaneously, which limits their comparability and the linkage between water quality and zooplankton.

Since the rivers of Paraná System are dominated by small zooplankton such as rotifer and nauplii (José de Paggi and Paggi 2007) the aim of this study was to test the hypothesis that land use and basin characteristics determine the abundance and composition of the microzooplankton, in six rivers and a shallow lake. Since in this complex ecological system the main environmental factors are regulated by the hydrological cycle, the study was performed throughout different hydroclimatic periods with the purpose to test if human influence persist during contrasting situations.

2 Study Area

Santa Fe City is surrounded by different water bodies that belong to the Paraná River Basin. It is bounded in the Southwest by Setúbal Lake and in the East by the Santa Fe, Salado and Coronda Rivers. Santo Tomé City is sited in front of Santa Fe City on the eastern side of the Salado River (Fig. 1).

The Setúbal Lake is approximately 35 km long and 5 m deep at the center. It is influenced by the Saladillos System in the Northwestern region, and the Leyes River at the Northeastern region. Setúbal Lake drains into the Santa Fe River and behaves as a semi-lotic water body with a low residence time. The Santa Fe River is a secondary channel of the Paraná River, with low salinity water and a water discharge that varies from 800 to 1,600 $m^3\ s^{-1}$.

The Saladillo Dulce and Saladillo Amargo Rivers make up the Saladillo System, that drains a large floodplain area (10,700 km^2). Its discharge ranges from 14 to 46 $m^3\ s^{-1}$ and is characterized by high conductivity and salinity due to phreatic input of chloride-sodium waters with sulfate enrichment. Its influence over Setúbal Lake has resulted in an increase in conductivity values of the right margin, especially at low-water periods.

The Leyes River connects the main channel of the Paraná River with Setúbal Lake. Its mean discharge is

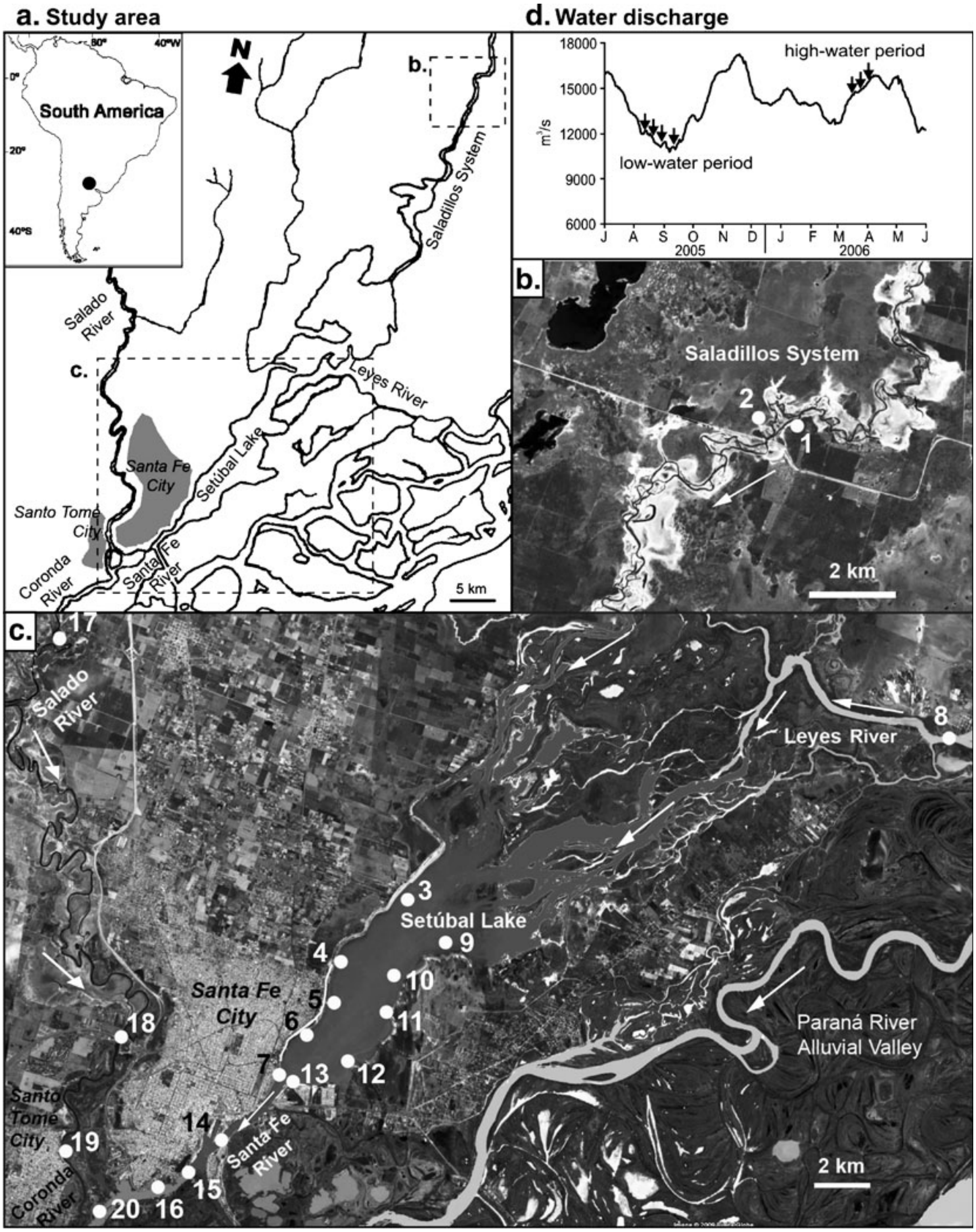

Fig. 1 **a** Map showing the location of the sampled rivers and lake. **b**–**c** Geographical position of the sampling sites in Setubal Lake (RM, *3* to *7*; LM, *9* to *13*) and the rivers Saladillo Dulce (*1*), Saladillo Amargo (*2*), Leyes (*8*), Santa Fe (*14* to *16*), Salado (*17* to *19*) and Coronda (*20*). The *white arrows* indicate the water flow direction. **d** Daily hydrometric level of the Paraná River from June 2005 to May 2006. The *arrows* indicate the sampling dates

1,550 $m^3 s^{-1}$ (Amsler et al. 2007), and it flows close to a small village.

The Salado River is a saline tributary of the Paraná River. It has a mean water discharge of 170 $m^3 s^{-1}$, and chloride-sodium waters with salt concentrations that range from 10 to 360 mg L^{-1} (Bonetto 1976). This river joins the Santa Fe River and forms the origin of the Coronda River.

The hyposaline waters from the Leyes-Setúbal left margin-Santa Fe-Coronda system has been classified as the carbonate-sodium type (Bonetto 1976).

At the Saladillos Basin intense agricultural and livestock rearing is practiced (Garcia de Emiliani and Anselmi de Manavella 1989), while the Setúbal Lake right margin and Santa Fe River receive pluvial drainage from urban runoff with evidence of connection sewage effluent to the storm drain system (Antoniolli 2007).

The Salado stretch studied also flows into a rural area (affected by agriculture and livestock), but 70 km upstream, it is influenced by industrial activities and metal pollution (Gagneten and Paggi 2008). Downstream, before it joins the Santa Fe River, it receives sewage effluent from Santo Tomé City. Coronda River, therefore, receives inputs from both rivers after it passes through the cities.

3 Materials and Methods

3.1 Sampling

Sampling was carried out weekly during two different hydroclimatic periods: August–September 2005 (four samplings) and March–April 2006 (three samplings). A total of 20 stations were sampled (Fig. 1); they were located at the Saladillos System (stations 1 and 2), Setúbal Lake right margin (RM) (3 to 7, in front Santa Fe City) and left margin (LM) (9 to 13), the Leyes River (8), the Santa Fe River (14 to 16, in front Santa Fe City), the Salado River (17 to 19, the last one in front of Santo Tomé City) and the Coronda River (20).

Microzooplankton samples were collected using a 20 l Schindler–Patalas plankton trap fitted with a 50 μm mesh net. Five traps were pooled at each station (100 l filtered in total), and the samples were preserved with formaldehyde (10%). The samples were collected from the center of the rivers, or at 30 m from the margin in an integrated water column at the lake. Conductivity, pH, dissolved oxygen concentration (DO) and water temperature were measured in the field in all stations using Hanna portable water checkers. Water transparency was measured with a Secchi disk of 20 cm in diameter.

Water samples were collected and transported to the laboratory to measure biological oxygen demand (BOD_5), nutrient content and chlorophyll-a (Chl-a) concentrations. In addition to these, concentrations of glyphosate and its major metabolite aminomethylphosphonic acid (AMPA) were measured because of local use of the herbicide, and lead because of probable input from cities.

3.2 Sample Analysis

Microzooplankton (rotifers+larval stages of copepods, nauplii+larval stages of *Limnoperna fortunei* (Dunker)) composition and abundance were analyzed in a total of 140 samples, using a Nikon Optiphot microscope at 100×. Microzooplankton were counted in a 1 ml Sedgwick-Rafter chamber, counting being stopped when 100 specimens of the most abundant species were reached; if necessary, the whole sample was counted. All organisms were identified to the lowest possible taxonomic level (except for bdelloid rotifers) following different authors (Koste 1978; Segers 1995; Ciros-Perez et al. 2001, among others).

Water samples for chemical analysis were filtered through Whatman GF/F glass-fiber filters, except for total phosphorus (TP) that was analyzed in non-filtered water samples. Nitrate (NO_3, cadmium reduction method) and TP (acid persulfate digestion method) were lectured with spectrophotometer using chemical sets of HACH Company. Ammonium (NH_4) concentration was determined by the indophenol-blue method using a Wiener chemical reactor.

BOD_5 was determined by the dilution method after 5 days of incubation at 20°C (APHA, 2005). Chlorophyll-a was extracted in 25 ml of buffered acetone macerating into a glass grindex (90% acetone +10% distilled water) and stored at 4°C for 6–12 h in the dark. The extracts were filtered, acidified with 0.1 N HCl and measured with spectrophotometer at 664–750 nm, and 665–750 nm (APHA, 2005).

Glyphosate and AMPA determinations were performed by INTEC Laboratory (CONICET-UNL). Fifty milliliters of natural water sample was preconcentrated in a two-step procedure: firstly, the sample was percolated through a polymeric cartridge, LiChrolut EN, and secondly, through an anion-exchange column mechanism. Finally, concentrated samples were analyzed by ion-exchange chromatography followed by post-column reaction coupled to a fluorimetric detector. Linear calibration graphs were obtained between 5 and 200 $\mu g\ L^{-1}$. Limits of detection ranged from 2 μg L^{-1} for glyphosate and 4 μg L^{-1} for AMPA (Mallat and Barceló 1998). The determinations of lead (Pb) were performed by Atomic Absorption Spectrometry (Electrothermal atomization, STPF conditions). The samples were analyzed according to EPA standard 600/4–91/100, method 200.9. The method of calibration curve with aqueous standards certified was used.

3.3 Data Analysis

Diversity Index (Shannon and Weaver 1964) and Jaccard similarity coefficient (Jaccard 1942) were calculated. Normality and homogeneity of variances were checked with Kolmogorov–Smirnov and Bartlett tests, respectively, to select the application of either parametric or non-parametric methods. ANOVA with Tukey post test, or the non-parametric alternative Kruskal–Wallis with Dunn post test were used to test for significant differences between sites (Zar 1996). Comparison between periods was performed with Student's *t* test with Welch correction. Spearman's correlation coefficients were calculated to check for associations between environmental variables and microzooplankton abundance.

Cluster analysis based on Euclidean distance coefficient with unweighted pair group average linkage was used to identify similarities and differences among sites for each sampling period. Dendrograms were computed on the basis of normalized data ($\ln x{+}1$) for microzooplankton density, and for environmental parameters (pH, Secchi disk depth, conductivity, concentrations of dissolved oxygen, nitrate, ammonium and total phosphorus, BOD_5 and chlorophyll-a). Output from the distance matrix was used to perform a Mantel test in order to check linear correlation between environmental parameters and microzooplankton density in each period via 5,000 interactions ($p<0.01$). The Mantel test was also used for correlating microzooplankton density and chlorophyll-a concentration.

Multivariate constrained ordination method was used to analyze the relationship between abundance of microzooplankton species and environmental variables. Detrended Correspondence Analysis suggested that a lineal method was appropriate since the gradient length of species did not exceed three standard deviations. Accordingly, a redundancy analysis (RDA) was performed (ter Braak and Smilauer 2002). The data set of response variables was based on the abundance of species that appeared with a contribution of at least 20% of the total density. All the environmental variables measured were used as explanatory variables except hydrometric level. The species data were $\log_{10}(x{+}1)$ transformed. The forward selection option was used to identify the more significant subset of environmental variables. The significance of the first and of all axes was analyzed using Monte Carlo permutation test, under an unrestricted model of 999 permutations ($p<0.01$).

All statistical analyses were computed with PAST 1.76 (Hammer, Ø et al. 2001) and CANOCO 4.5 (ter Braak and Smilauer 2002) softwares.

4 Results

4.1 Environmental Variables

The period August to September 2005 was characterized by low-water discharge (range=10,570–11,603 $m^3\ s^{-1}$, Paraná Harbor) and temperatures (range=11.7–21.5°C), whereas from March to April 2006 high-water discharge (range=14,401–15,802 $m^3\ s^{-1}$) and temperatures (range=21.1–28.8°C) were registered (Fig. 1d). Pluvial precipitations occurred only during the second period.

Mean values and coefficients of variation of environmental parameters are shown in Table 1. At low-water discharge period, higher conductivity values were measured in all sampling sites with significant differences among them ($p=0.0001$). The Salado River followed by the Saladillos System had the highest concentrations. As regards water transparency, the lowest values were observed at the Saladillos in comparison with the other water bodies, except for the Setúbal right margin ($p=0.0001$). In addition, the left margin had significantly higher light penetration than the right margin ($p=0.01$).

Nitrate, ammonium and BOD_5 concentrations did not show significant differences between hydrological periods, but total phosphorous was significantly higher in high waters ($p=0.0001$) and the oxygen was significantly lower in high waters ($p=0.001$).

Euclidean distance analysis considering the measured water quality indicators (water transparency, conductivity, pH, DO, nutrients and BOD_5) showed a clear separation among water bodies. During low-

Table 1 Mean values and variation coefficient (%, in brackets) for environmental parameters in the study areas during low waters (August–September 2005) and high waters (March–April 2006)

	Secchi cm	Conductivity μS cm^{-1}	pH	DO mg L^{-1}	NO_3 mg L^{-1}	NH_4 μg L^{-1}	TP mg L^{-1}	BOD_5 mg L^{-1}
Low waters								
Saladillos	10 (31)	2,530 (32)	7.77 (3)	8.9 (14)	1.79 (26)	24 (60)	0.84 (121)	3.5 (40)
Salado	26 (37)	4,810 (41)	7.78 (3)	10 (26)	1.58 (47)	41 (69)	1.2 (67)	4.4 (50)
Setúbal RM	25 (16)	534 (37)	7.58 (2)	9 (13)	1.41 (51)	9.5 (41)	0.53 (128)	0.7 (60)
Setúbal LM	36 (40)	159 (24)	7.31 (1)	9.4 (14)	1.43 (34)	11 (118)	0.41 (144)	0.9 (54)
Leyes	28 (9)	160 (14)	6.92 (4)	8.8 (14)	1.5 (19)	25 (71)	1.57 (68)	3.1 (51)
Santa Fe	29 (15)	206 (18)	7.4 (2)	9 (19)	1.38 (54)	11 (27)	0.27 (58)	1.2 (71)
Coronda	32 (25)	261 (21)	7.3 (2)	9.6 (13)	2.25 (53)	22 (73)	0.59 (107)	4.5 (110)
High waters								
Saladillos	13 (33)	502 (74)	7.08 (4)	5.2 (12)	1.95 (41)	12 (121)	1.38 (47)	5.6 (13)
Salado	12 (37)	1,051 (30)	7.51 (3)	5.3 (37)	1.89 (29)	27 (76)	2.19 (30)	3.5 (15)
Setúbal RM	16 (20)	212 (41)	6.84 (1)	6.5 (13)	1.28 (46)	14 (73)	1.44 (68)	1.3 (67)
Setúbal LM	22 (5)	93 (17)	6.74 (0.2)	6.5 (13)	1.09 (49)	14 (100)	1.21 (81)	0.9 (75)
Leyes	17 (23)	94 (23)	6.74 (0.3)	6.4 (17)	1.81 (14)	8.5 (74)	1.15 (63)	1.8 (38)
Santa Fe	17 (11)	97 (20)	6.74 (0.3)	6.6 (4)	1.37 (26)	9.3 (69)	1.9 (42)	0.7 (30)
Coronda	13 (41)	128 (14)	6.77 (0.2)	7.9 (4)	2.83 (46)	12 (20)	2.55 (13)	4.2 (21)

RM right margin, *LM* left margin

water discharge (Fig. 2a), two main groups were determined. Saladillos System and Salado River were associated with high values of conductivity, total phosphorous and BOD_5. The other sites conformed a large group.

During high-water period (Fig. 2b), the same environmental grouping was maintained. It could be also differenciated the Setúbal Lake RM with higher conductivity values than the left margin and lower Secchi disk readings, and Setúbal LM-Leyes River-Santa Fe River group with lower conductivity, and BOD_5 values and higher transparency. Even though there were some differences in the clustering distance, both matrix showed a Mantel correlation coefficient of r=0.859 (p=0.0001) indicating that differences in environmental water quality were maintained despite hydrological and temperature changes.

Highest values of total coliform bacteria were registered at Setúbal Lake RM and Santa Fe River, in the sampling stations with pluvial discharges (Antoniolli 2007). The presence of glyphosate herbicide in waters was only registered during low-water period at Setúbal Lake RM (stations 3 and 4) and Salado River (stations 18 and 19) with concentrations that ranged from 0.4 to 0.8 μg L^{-1}. Its metabolite AMPA was not detected. Regarding the heavy metal lead, it was found at Santa Fe River (station 14= 6 μg L^{-1}; station 15=8 μg L^{-1}) during low waters, and at Setúbal Lake RM (station 12=4 μg L^{-1}) during high waters.

4.2 Composition and Abundance of the Microzooplankton

Regarding rotifers, a total of 89 taxa were identified. *Brachionus* (13 spp.) and *Lecane* (21 spp.) were the most diversified genera (Table 2). The diversity of *Brachionus* was highest in Saladillos and Setúbal Lake.

Higher richness was observed during high-water period in comparison with low waters (77 vs. 40 rotifer species, respectively), mainly due to an increase in species of *Brachionus*, *Lecane*, and *Trichocerca*. The seasonality conditioned the presence of certain genera. For example, *Notholca* was present only at low temperature samplings while *Ploesoma* was found only at high temperature samplings, as expected from their thermal preferences (Koste 1978). The faunistic affinity between the two periods indicated by Jaccard's coefficient was 31%.

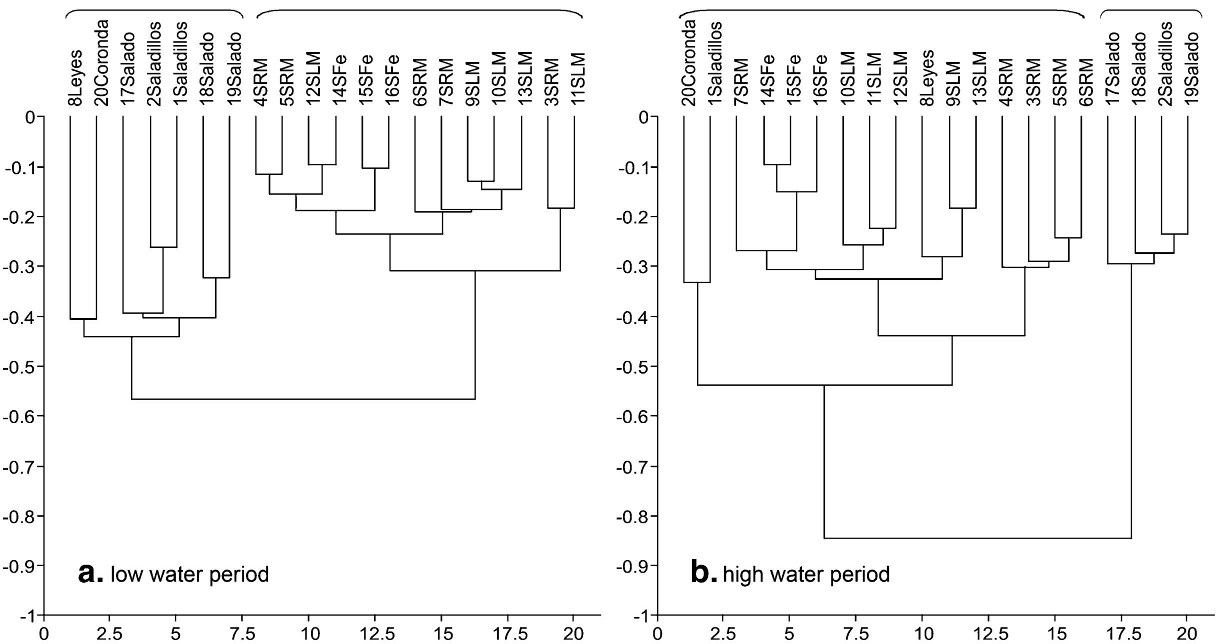

Fig. 2 Cluster analyses for 20 sampling sites on the basis of environmental variables during low-water (August–September 2005) and high-water (March–April 2006). Rivers Saladillo Dulce (*1*) Saladillo Amargo (*2*) Leyes (*8*) Santa Fe (*14–16*) Salado (*17–19*) Coronda (*20*). Setubal Lake *righ margin*: SRM (*3–7*) Setubal Lake *left margin*: SLM (*9–13*)

The cumulative richness was 64 (Setúbal Lake), 51 (Saladillos), 38, 31, 23 (Salado, Santa Fe, Coronda Rivers, respectively), and 27 (Leyes River).

A range of 1 to 22 species per sample were found with significant differences among habitat during low waters (KW=34.33, $p<0.0001$) and high waters (KW=34.28, $p<0.0001$). According to Dunn's post test, Saladillos differed from Setúbal Lake LM, Santa Fe, Coronda and Salado Rivers with higher values at the former during both periods. As regards diversity index, it was low in all the studied water bodies (Table 3).

In the rivers located in rural area, Salado and Saladillos, the most frequent assemblage was *Brachionus plicatilis*, *Brachionus angularis*, *Keratella tropica*, and *Synchaeta* spp., while in the rivers and lake located in urban area the typical assemblage was *Keratella cochlearis*, *Keratella americana*, *Keratella lenzi*, and *Brachionus ahlstromi*.

Microzooplankton abundance varied over a wide range with maximum and mean values higher during low waters (4 to 875 ind. L^{-1}) than high waters (5 to 406 ind. L^{-1}). Rotifers were the main contributors at low waters in all sampling sites. On the other hand, *L. fortunei* larvae dominated at high waters, with significantly higher density ($p=0.0001$), except at Saladillos and Salado Rivers where they were not registered. At Setúbal Lake, Leyes, Santa Fe, and Coronda Rivers they were present during the 3 weeks of the aforementioned period with contributions of 95% to total microzooplankton in many of the samples (Table 4).

Salado and Saladillos Rivers achieved the highest density with *K. tropica, Brachionus plicatilis* and *Synchaeta* sp. as the main contributors. *K. tropica* was more important in Saladillos (mean=171 ind. L^{-1}, maximum=300 ind. L^{-1}) than Salado (mean=22 ind. L^{-1}, max=72 ind. L^{-1}). On the contrary, *B. plicatilis* was more important in Salado (mean=179 ind. L^{-1}, max=800 ind. L^{-1}) than Saladillos (mean=43 ind. L^{-1}, max=98 ind. L^{-1}). Other species present were *Brachionus caudatus*, *B. angularis* and *Brachionus austrogenitus*, but they only reached high densities at low waters. In the rural area, *Brachionus* were comparatively more abundant that in the urban area (Fig. 3).

At low and high water periods the cluster analysis, based upon the Euclidean distances of microzooplankton abundance separated the studied water bodies in two main groups: firstly Salado and Saladillos

Table 2 List of rotifer taxa founded

	Hw	Lw
Ascomorpha ecaudis (Perty)	1	
Asplanchna brightwelli Gosse	1	1
*Beauchampiella eudactylota (*Gosse)	1	
Brachionus ahlstromi Lindeman	1	
Brachionus angularis Gosse	1	1
Brachionus austrogenitus Ahlstrom	1	1
Brachionus bidentata Anderson	1	1
Brachionus budapestinensis (Daday)	1	
Brachionus calyciflorus Pallas	1	1
Brachionus caudatus Barrois and Daday	1	1
Brachionus havanaensis Rousselet	1	
Brachionus ibericus Ciros-Perez, Gómez and Serra		
Brachionus plicatilis (O.F. Müller)	1	1
Brachionus quadridentatus (Hermann)	1	1
Brachionus rubens Ehrenberg	1	
Brachionus urceolaris (O.F. Müller)		1
Cephalodella catellina (O.F. Müller)	1	1
C. sp.		1
Colurella colurus (Ehrenberg)	1	
Conochilus coenobasis Skorikov	1	
Conochilus unicornis Rousselet	1	1
Dicranophoroides caudatus (Ehrenberg)		1
Dicranophorus halbachi Koste		1
Dicranophorus robustus (Harring and Myers)		1
Epiphanes clavulata (Ehrenberg)	1	1
Euchlanis dilatata (Ehrenberg)	1	1
E. sp.	1	
Filinia sp.	1	1
Filinia opoliensis (Zacharias)	1	
Gastropus sp.		1
Hexarthra intermedia braziliensis (Hauer)	1	
Hexarthra mira (Hudson)	1	
Keratella americana Carlin	1	1
Keratella cochlearis (Gosse)	1	1
Keratella lenzi (Hauer)	1	1
Keratella tropica (Apstein)	1	1
Lecane bulla (Gosse)	1	1
Lecane aculeata (Jakubski)	1	
Lecane closterocerca (Schmarda)	1	
Lecane curvicornis (Murray)	1	
Lecane cornuta (O.F. Müller)	1	
Lecane decipiens (Daday)	1	
Lecane elsa Hauer	1	
Lecane furcata Murray	1	
Lecane flexilis (Gosse)	1	
Lecane hamata (Stokes)	1	
Lecane hastata (Murray)	1	
Lecane leontina (Turner)	1	
Lecane ludwigii (Eckstein)	1	
Lecane lunaris (Ehrenberg)	1	
Lecane papuana (Murray)	1	
Lecane proiecta Hauer		1
Lecane quadridentata (Ehrenberg)	1	
Lecane signifera (Jennings) f. ploenensis (Voigt)	1	
Lecane scutata (Harring and Myers)	1	
Lecane stenroosi (Meissner)	1	
Lecane tenuiseta Harring	1	
Lepadella acuminata (Ehrenberg)		1
Lepadella latusinus (Hilgendorf)	1	
Lepadella patella (O.F. Müller)	1	1
Lepadella quadricarinata (Stenroos)	1	
L. sp.	1	
Lophocharis salpina (Ehrenberg)	1	
Mytilina bisulcata (Lucks)	1	
Mytilina ventralis (Ehrenberg)	1	1
Monommata longiseta (O.F. Müller)	1	
Notholca acuminata (Ehrenberg)		1
Plationus patulus (O.F. Müller)	1	
Platyias quadricornis (Ehrenberg)	1	
Ploesoma truncatum Levander	1	
Pompholix sulcata (Hudson)	1	1
Polyarthra sp.	1	1
Ptygura sp.	1	
Sinantherina spinosa (Thorpe)	1	
Synchaeta sp.	1	1
Testudinella patina (Hermann)	1	
Trichocerca bicristata (Gosse)	1	
Trichocerca gracilis (Tessin)	1	1
Trichocerca pusilla (Jennings)		1
Trichocerca rattus (O.F. Müller)		1
Trichocerca similis (Wierzejski) f. grandis Hauer	1	1
Trichocerca tigris (O.F. Müller)	1	
Trichocerca stylata Gosse	1	
Trichocerca weberi (Jennings)	1	
T. sp	1	
Trichotria tetractis (Ehrenberg)	1	1
Wolga spinfera (Western)	1	1
Bdelloideos (sp. 1)	1	1
Bdelloideos (sp. 2)	1	

Hw high-water, *Lw* low-water

Table 3 Mean values and variation coefficients (%, in brackets) of species richness per sample and Shannon and Weaver Diversity index (H) in the study areas during low waters (August–September 2005) and high waters (March–April 2006)

	Richness	H
Low waters		
Saladillos	9.5 (36)	1.41 (11)
Salado	3.91 (58)	0.78 (60)
Setúbal RM	9.4 (46)	2.00 (43)
Setúbal LM	4.45 (77)	1.35 (44)
Leyes	6.8 (39)	1.45 (11)
Santa Fe	7.25 (60)	1.60 (50)
Coronda	7.75 (35)	2.00 (89)
High-waters		
Saladillos	20 (7)	2.19 (13)
Salado	10.78 (29)	1.69 (24)
Setúbal RM	5.67 (42)	1.59 (100)
Setúbal LM	4.66 (41)	1.32 (101)
Leyes	7.66 (53)	2.28 (49)
Santa Fe	4 (60)	1.00 (66)
Coronda	4.30 (81)	1.30 (81)

Rivers, secondly the remaining sampling sites. At low waters Salado and Saladilllos Rivers were clustered together jointly with the first station of Setúbal Lake RM (Fig. 4). Both dendrograms were significantly correlated (Mantel test, r=0.76, p=0.001).

4.3 Microzooplankton, Chlorophyll-a and Environmental Variables

Significant correlations between environmental variables and microzooplankton abundance are shown in Table 5. During both low and high water periods, conductivity, pH and chlorophyll-a concentration were positively correlated with abundance, while water transparency was negatively correlated. Correlations with species richness were higher at high water period, particularly those of conductivity, pH and chlorophyll-a concentration (Table 5).

Euclidean distance analysis of chlorophyll-a showed the similar trends that microzooplankton. According to the Mantel test, both distance matrices were strongly correlated during low (r=0.837, p=0.001) and high-water stages (r=0.852, p=0.0001). In addition, the correlations between environmental variables and microzooplankton abundance were significant, being higher at low waters (r=0.830, p=0.0001) in comparison with high-water period (r=0.601, p=0.001). *Brachionus* spp. density was correlated with BOD_5 (r=0.38, p=0.02).

The two main axes of the RDA ordination diagram (Fig. 5) accounted for 93.5% of the cumulative variance that explained the species-environmental variation (first axis=74.8%; second axis=18.7%). The sum of the eigenvalues, which indicated the

Table 4 Mean values and variation coefficients (%, in brackets) of Chl-a, Mzoo, and percentage of rotifers, copepod nauplii, and mollusc larvae in the study areas during low-waters (August–September 2005) and high-waters (March–April 2006)

	Chl-a mg L^{-1}	Mzoo ind. L^{-1}	Rotifers%	Nauplii%	Mollusc larvae%
Low-waters					
Saladillos	70.55 (86)	321.43 (66)	96.66	3.34	0.00
Salado	93.55 (59)	237.80 (105)	92.85	7.15	0.00
Setúbal RM	6.8 (102)	29.08 (135)	94.05	4.99	0.96
Setúbal LM	1.85 (85)	10.03 (109)	89.53	9.47	1.00
Leyes	2.61 (38)	20.55 (115)	88.22	3.60	8.18
Santa Fe	3.24 (71)	17.98 (81)	92.55	7.12	0.33
Coronda	4.25 (35)	22.75 (66)	91.21	8.79	0.00
High-waters					
Saladillos	25.09 (47)	278.16 (26)	83.70	16.30	0.00
Salado	12.13 (42)	88.50 (68)	95.24	4.12	0.63
Setúbal RM	3.99 (53)	10.75 (51)	38.51	24.65	36.84
Setúbal LM	1.92 (57)	9.12 (44)	21.05	13.49	65.46
Leyes	3.74 (97)	16.99 (38)	29.72	23.19	47.09
Santa Fe	2.44 (57)	10.49 (70)	15.73	10.87	73.40
Coronda	2.30 (71)	9.87 (52)	22.39	15.10	62.51

Chl-a chlorophyll-a concentration, *Mzoo* microzooplankton abundance

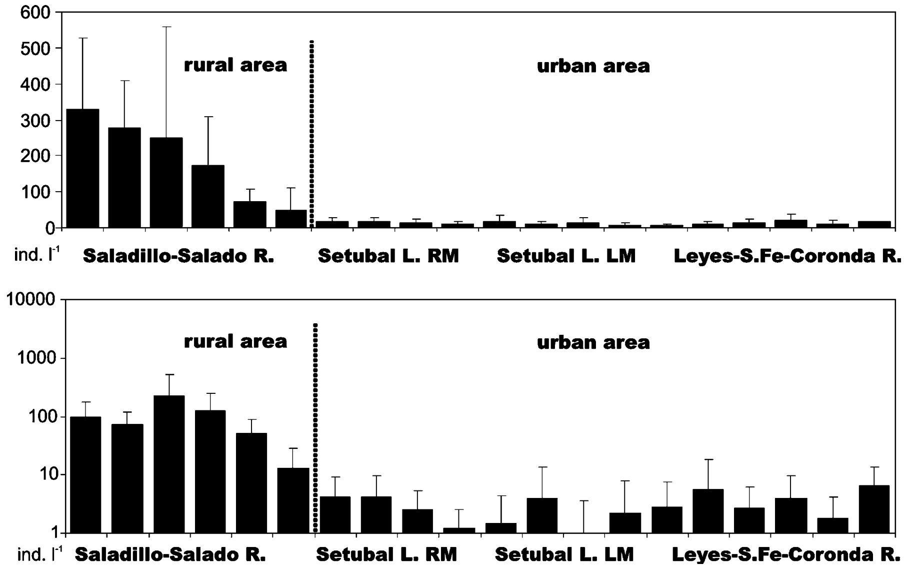

Fig. 3 Mean and standard desviation of abundance of microzooplankton (*upper*) and abundance of *Brachionus* (*lower*, logaritmic scale) in the studied waterbodies

proportion of the microzooplankton abundance variation, was 60% (first axis=0.48; second axis = 0.12). Forward selection reduced the environmental variables explaining the variance of the species to four (Monte Carlo permutation test for both axes: $p<0.01$). The first axis was defined by the combination of conductivity, pH and chlorophyll-a concentration. Samples positively loaded with increasing trends in these environmental conditions were plotted at the right of the graphic with Salado River low waters at the top (Group A) and Salado high waters and Saladillos System at the bottom (Group B). The species strongly associated were *Brachionus plicatilis, Synchaeta* sp., *K. tropica* and other species of

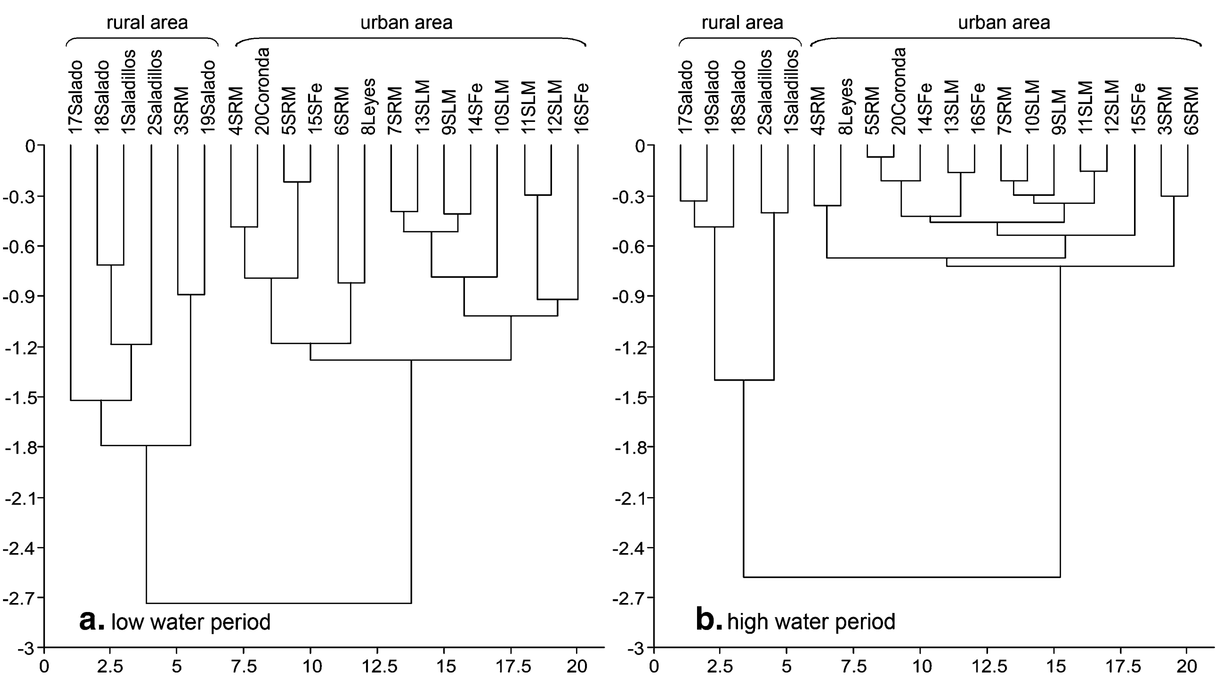

Fig. 4 Cluster analyses for 20 sampling sites on the basis of microzooplankton abundance during low-water (August–September 2005) and high-water (March–April 2006).The references are the same as in Fig. 2

Table 5 Spearman correlation coefficient among Mzoo, Chl-a, and environmental variables in the study areas during low-waters (August–September 2005) and high-waters (March–April 2006)

	Mzoo	Secchi	C	pH	DO	TP	BOD_5	Chl-a
Low-waters								
Mzoo		0.0000	0.0000	0.0016	0.8008	0.0323	0.0005	0.0000
Secchi	*−0.4698*		0.6938	0.4209	0.0000	0.6304	0.3337	0.0006
C	*0.6283*	−0.0447		0.0055	0.1173	0.0015	0.4949	0.0000
pH	*0.3470*	0.0912	0.3079		0.0004	0.0141	0.9482	0.0333
DO	0.0287	0.6511	0.4685	0.3881		0.5572	0.0342	0.2239
TP	0.2396	−0.0546	0.3486	0.2734	0.0666		0.0007	0.0002
BOD_5	*0.3816*	−0.4463	0.4375	−0.0074	−0.2371	0.3711		0.0002
Chl-a	*0.7734*	−0.5607	0.6824	0.2383	−0.1375	0.4095	0.5769	
High-waters								
Mzoo		0.0000	0.0001	0.0003	0.0015	0.3089	0.0316	0.0000
Secchi	*−0.5198*		0.0370	0.0011	0.0003	0.0126	0.1175	0.1225
C	*0.6691*	−0.5061		0.0000	0.0010	0.2220	0.0074	0.0000
pH	*0.6412*	−0.4120	0.9488		0.0024	0.1763	0.0114	0.0000
DO	*−0.4005*	0.4556	−0.4135	−0.3851		0.4280	0.1482	0.0074
TP	0.1336	−0.3204	0.1600	0.1769	−0.1043		0.6145	0.4896
BOD_5	0.2778	−0.2043	0.3423	0.3246	−0.1889	0.0663		0.0095
Chl-a	*0.8053*	−0.5319	0.7603	0.6763	−0.3424	0.0909	0.3321	

Correlation values are given in the lower triangle of the matrix, and the probabilities that the columns are uncorrelated are given in the upper. Significant correlations between microzooplankton and parameters (italicized cell entries)

Mzoo microzooplankton abundance, *Chl-a* chlorophyll-a concentration, *C* conductivity

Brachionus such as *B. austrogenitus, B. angularis* and *B. caudatus*. The second axis was correlated with increasing values of water transparency, with *Brachionus bidentatus* positively related and *K. cochlearis* negatively related. Samples during high-water period were arranged at the left of the graphic (Group C), while low-water samples were clustered in Group D with a highly dispersed ordination.

5 Discussion and Final Remarks

Human activities strongly influenced water quality of the studied environments. Two markedly differenciated areas could be identified in relation to the predominant human land use: rural (Salado and Saladillos Rivers) and urban areas (Setúbal Lake, Santa Fe and Coronda Rivers). Fluxes of nutrients were observed to water bodies located in both areas; according to the concentrations founded, the rivers and the lake are mesoeutrophic and eutrophic (Vollenweider 1968). In agreement, Saladillos, Salado, and Coronda Rivers can be considered a mesosaprobious zone along a gradient of nutrient and organic enrichment, with a BOD_5 concentration higher than 3 mg L^{-1} (Margalef 1983).

The enrichment of aquatic ecosystems with nitrogen and phosphorous derived from human activities is well documented in watersheds dominated by agricultural or urban land use (Knoll et al. 2003; Camargo and Alonso 2006). The sources of nitrogen related to basin use are mainly runoff processes from cultivated fields and livestock (Saladillos System, Salado River), sewage effluents, overflows of combined storm and sanitary sewers, and urban runoff (Setúbal RM, Santa Fe, Coronda Rivers mainly). The higher phosphorus values found at the high-water and rainy period (August–September 2006), may be associated with overflow waters that are in contact with terrestrial lateral areas and pluvial runoffs.

The physical and chemical conditions allowed to discriminate the water bodies in the two main land use area. Those rivers located in rural areas had comparatively lower transparency and higher conductivity, BOD_5, and ammonium concentrations in comparison with urban area. Coronda River also had high values of BOD_5 but due to its condition of water collector of Setúbal Lake and Santa Fe River. In accordance, herbicides inputs derived from agricultural activities were observed in Salado River, whereas lead content swept from Santa Fe City were found in Setúbal Lake and Santa Fe River. This

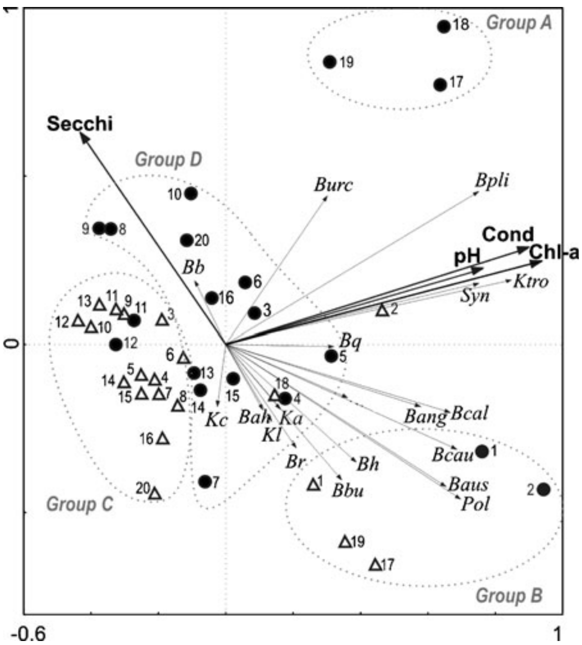

Fig. 5 Results of the RDA analysis performed with the environmental variables and rotifer taxa. *Bang B. angularis*; *Baus B. austrogenitus*; *Bbu B. budapestinensis*; *Bcal B. calyciflorus*; *Bh B. alhstromi*; *Bpli B. plicatilis*; *Br B. rubens*; *Burc Brachionus urceolaris*; *Bq B. quadridentatus*; *Ka Keratella americana*; *Kc K. cochlearis*; *Kl K. lenzi*; *Ktro K. tropica*; *Pol Polyarthra* sp.; *Syn Synchaeta* sp. The *numbers* indicate the sampling sites. *Black circles*, low-water period. *Triangles*, high-water period. Groups are indicated in the text

separation into two groups, in general, remained under the two contrasting hydroclimatic situations. Some parameters resulted in a decrease of conductivity during the high-water period due to water dilution, with not improvements in water transparency because it dependance on sedimentological regime (Amsler et al. 2007). The high nitrate, ammonium and BOD_5 values observed at both periods indicate that they are characteristic of the studied sites and did not depend on the hydrological conditions.

The species composition showed a gradient along with conductivity, pH and chlorophyll-a concentrations. This could be represented by the dominance of the genera *Brachionus* and *Keratella* (except *K. tropica*), in environments of rural and urban areas, respectively. *Brachionus* genus dominated the rotifer assemblage in the rivers with high salinity in rural area. The *Brachionus* species are sensitive to changes in the trophic status. In this sense, *B. angularis* and *B. caudatus* both euryhaline (Fontaneto et al. 2006), are good indicators of eutrophic conditions (Attayde and Bozelli 1998; Paturej 2006) and in this study they are also associated to the BOD_5 gradient. *B. plicatilis* and *Brachionus ibericus* are strictly haline species (Fontaneto op cit.) but scarcely associated to eutrophic waters, or to high BOD_5 values (Kameswara Rao and Chandra Mohan 1977) as it was recorded in this study. *Brachionus quadridentatus* sp. were correlated to salinity and alkaline waters as in Derry et al. (2003) study. In the rivers from rural area, the only abundant species of no-*Brachionus* genus was *K. tropica.* Duggan, Green and Shiel (2001, 2002) also observate its correlation with high trophic status. *K. tropica* was mainly abundant at Saladillos River, with a conductivity lower than Salado River, in agreement with Claps et al. (2009) who also found it preference for conductivity values lower than 2,000 $\mu S\ cm^{-1}$.

Dodson et al. (2007), in a large-scale study showed that the land use has a significant adverse effect on zooplankton species richness in lakes. Moreover in a previous also extensive study in 70 environments, Dodson et al. (2005), found that those located in agriculture area had higher richness than those located in urban areas. In acordance, the highest rotifer richness was found at Saladillos System (rural area). The right margin of Setúbal Lake, mainly at first stations, constitute a particular case, their higher richness in comparison with Saladillo is probably due the species introduction input from this river.

Langley et al. (1995) found a marked negative correlation between phosphate and rotifer richness in urban ponds. The environments located in urban areas seem to have the lowest number of species. However the response of the zooplankton richness, and rotifers in particular, could change under different seasonal and hydrological conditions. In the present study, at high waters, almost all environments increased in species richness, as a consequence of littoral species input from adjacent habitats by hydrological connections. Despite the changes in species richness, Diversity Index was low throughout the study indicating the dominance of tolerant species to the anthropogenic influences (Margalef 1974; Walsh et al. 2005).

The abundance of microzooplankton also responded differentially to the land use, without significant changes between the different hydrological situations as it was observed with the environmental variables trend. The organisms abundance diminished from the rivers emplaced in rural area to those located in strictly urban areas, near to Santa Fe and Santo Tomé Cities. The Leyes River also showed a low concentration of organisms in spite of receiving input from a small population (Fig. 4).

Chlorophyll-a values indicate high trophic level in rural areas and the microzooplankton abundance were more than ten times higher than in the aquatic bodies located in urban dominated land use. Ejsmont-Karabin and Kruk (1998) report a negative impact of agriculture area on rotifer density, which was not observe in the present study. The response of zooplankton to agriculture is probably also dependent on the type and concentration of pesticides in each region, as suggested by Dodson (2008).

In the Salado River, 70 km before the studied stations, Gagneten and Paggi (2008) recorded a dramatic impoverishment of the zooplankton (mean= 2.73 ind. L^{-1}) due to the negative effect of industrial activity. However, the passage of the river through a plain with intense rural activity, determined that the strongly eutrophic character of the studied meandric river enables an increase in zooplankton abundance. This positive relationship between eutrophy and zooplankton abundance has been found in lakes (Whitman et al. 2004) and rivers (Claps et al. 2009).

In urban area, despite having relatively high values of nutrients, the chlorophyll and microzooplankton abundances were comparatively low (Setúbal Lake, Santa Fe, Coronda and Leyes Rivers). Only the first station of Setúbal Lake showed high density of microzooplankton, probably because it receives the water inputs from Saladillos. Compared with previous years it was found a decrease of the microzooplankton densities of Santa Fe and Coronda Rivers. The maximum values were comparatively lower (33 and 54 ind. L^{-1}, respectively) than in 1974–1975 (130 and 145 ind. L^{-1}, respectively) reported by José de Paggi (1981). The Shannon and Weaver Index in both rivers were also higher in 1974–1975 (mean=3.1, SD=0.4). On the other hand, the Salado River increased its trophic level (maximum=430 ind L^{-1} in 1974 vs. 876 ind. L^{-1}).

Associated to the negative impact of urbanization, the effect of the invasive *L. fortunei* should be taken into account. This mollusk, which was introduced in the 1990s, has colonized the hard substrates as concrete bridge pillars, docks and harbor buildings at the western bank of Setúbal Lake, Santa Fe and Coronda Rivers. Its reproduction occurs during the warm season (Boltovskoy and Cataldo 1999), in coincidence with the highest larval abundance observed (March–April). Biological invasions are also a source of significant impacts on ecosystems (Morton 1997). Experimental and field data have shown that *L. fortunei* consume algae and zooplankton (Sylvester et al. 2004; Rojas Molina et al. 2009). Usually, the secondary branches of the Middle Paraná River such as the Santa Fe and Coronda Rivers, have more abundant and richer plankton than the main channel, because they are associated with less severe environmental conditions (José de Paggi and Paggi 2007). The lower zooplankton abundance than in previous decades found in these rivers suggests the impact of human activity, including the biological invasions.

Concluding, it can be stated that the results of this study support the hypothesis that land uses differentially influence the composition and abundance of the microzooplankton of the studied water bodies. The patterns of response of microzooplankton remain unchanged under contrasting hydroclimatic conditions. Higher abundance in Saladillos and Salado indicates a positive effect of rural area probably due to the enrichment of soils by livestock and fertilizer use, with the genus *Brachionus* and *K. tropica* as indicators

The lower abundance of microzooplankton in urban aquatic environments, indicates a negative effect on the community, especially if it is consider the density disminution in comparison with previous years despite the increasing eutrophication. *K. cochlearis*, *B. austrogenitus*, or larvae of *L. fortunei*, constitute the dominant assemblage at this environments, with subdominance of *Brachionus.*

The results suggest that river microzooplankton abundance and rotifer species assemblages can be consider a good indicator of the land use, even in contrasting hydrological situations. Human-dominated landscapes are increasing. The study of all communities living in water bodies can provide suitable tools for quality monitoring and management proposals. No single traditional criterion (e.g., macroinvertebrates, algae, or diversity-assemblage) can be safely used for monitoring the impact of land use on the aquatic bodies. The use of multiple indicators (at species or community attributes level) could provide more fine-grained information.

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