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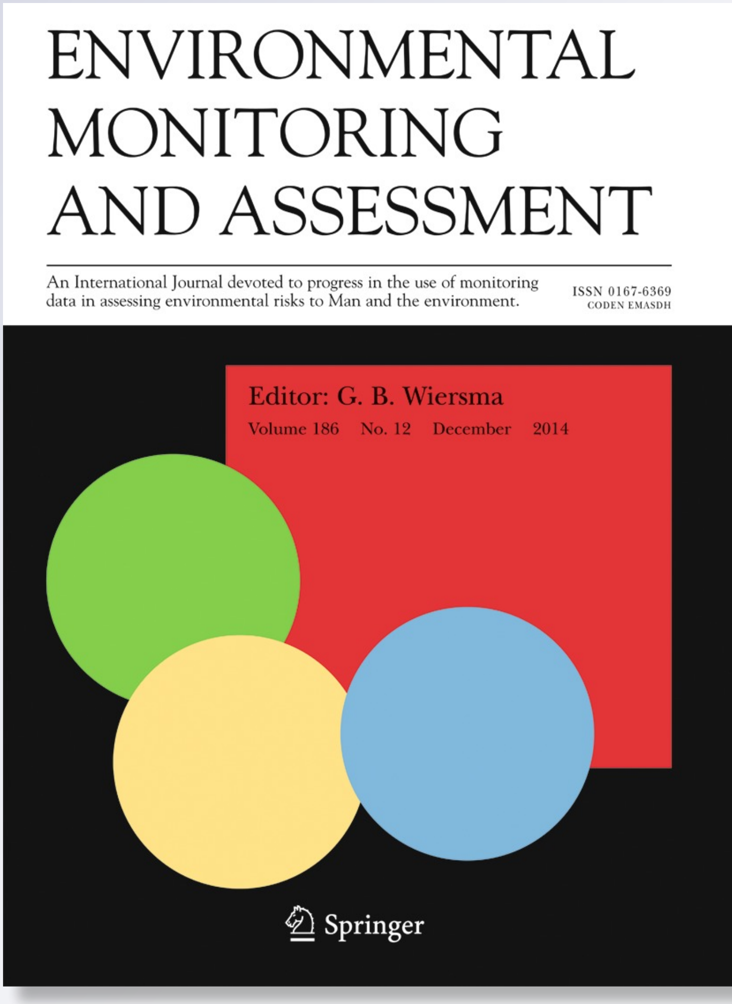

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Strategies to optimize monitoring schemes of recreational waters from Salta, Argentina: a multivariate approach

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Abstract Several recreational surface waters in Salta, Argentina, were selected to assess their quality. Seventy percent of the measurements exceeded at least one of the limits established by international legislation becoming unsuitable for their use. To interpret results of complex data, multivariate techniques were applied. Arenales River, due to the variability observed in the data, was divided in two: upstream and downstream representing low and high pollution sites, respectively, and cluster analysis supported that differentiation. Arenales River downstream and Campo Alegre Reservoir were the most different environments, and Vaqueros and La Caldera rivers were the most similar. Canonical correlation analysis allowed exploration of correlations between physicochemical and microbiological variables except in both parts of Arenales River, and principal component analysis allowed finding relationships among the nine measured variables in all aquatic environments. Variable's loadings showed that Arenales River downstream was impacted by industrial and domestic activities, Arenales River upstream was affected by agricultural activities, Campo Alegre Reservoir was disturbed by anthropogenic and ecological effects, and La Caldera and Vaqueros rivers were influenced by recreational activities. Discriminant analysis allowed identification of subgroup of variables responsible for seasonal and spatial variations. *Enterococcus*, dissolved oxygen, conductivity, *E. coli*, pH, and fecal coliforms are sufficient to spatially describe the quality of the aquatic environments. Regarding seasonal variations, dissolved oxygen, conductivity, fecal coliforms, and pH can be used to describe water quality during dry season, while dissolved oxygen, conductivity, total coliforms, *E. coli*, and *Enterococcus* during wet season. Thus, the use of multivariate techniques allowed optimizing monitoring tasks and minimizing costs involved.

Keywords Bacterial indicators · Recreational uses · Seasonal behavior · Statistical analysis · Surface water

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Introduction

Aquatic environments (AEs) such as lakes, ponds, reservoirs, rivers, and irrigation channels are known as surface waters. They differ in their origin, composition, uses, and quality. Humans use water in their daily lives for drinking, recreation, agriculture, and livestock and also for industrial processes. These anthropogenic activities and some natural events are the reasons by which surface waters are susceptible to changes in quality. The native flora and fauna of the area, seasonality, storm water runoff, illegal sewage discharges, industrialization, agriculture, and recreational human activities are some of the factors responsible for these changes, leading to a decrease in water quality (Cruz et al. 2012; David et al. 2013; Poma et al. 2012; Wunderlin et al. 2001).

On the other hand, natural environments are a good option for the population to carry out all sorts of recreational activities and the only possibility for those who cannot afford the costs of accessing to private pools or spas. In some cases, the users come into direct contact with water (i.e., bathing), and in others, they can establish secondary contact by various activities like canoeing or fishing (WHO 2006). When the water is microbiologically contaminated, with bacteria, viruses, and/or parasites, it becomes a potential source of diseases, mainly by the fecal–oral route. Other potential routes of infection due to recreational activities are as follows: ears, skin contact, eyes, nasal cavity, and respiratory tract, usually caused by *Pseudomonas aeruginosa* (WHO 2006). Epidemiological studies have shown that there is an increase in the incidence of illnesses strongly related with recreational activities (Craun et al. 2005).

The difficulty encountered in measuring pathogens present in the water column led to the use of bacterial indicators as established by different legislations (EC 1975; USEPA 1986; WHO 2006). Total and fecal coliforms are the most commonly used (Ashbolt et al. 2001; Cruz et al. 2012; EC 1975; Hong et al. 2010; Poma et al. 2012). Two other microorganisms that have consistently performed well as indicators of illness in epidemiological studies in freshwater are enterococci and *E. coli* (Marion et al. 2010; Wiedenmann et al. 2006). Although some studies failed to show good relationships between bacterial indicators and pathogens (Jiang et al. 2006; Lipp et al. 2001), they are still used worldwide to monitor surface water quality (Gao et al. 2011). Much of the discussions with regard to indicators and pathogen correlations are the result of studies with insufficient data (Wu et al. 2011).

Thus, a lot of effort has been done in research to find out a universal indicator, but it has not been identified yet, which could mean that individual characteristics will determine a particular indicator for each water body studied (Skraber et al. 2004). In order to find a good indicator, a large quantity of variables is often measured, and correlations are searched among them. When two or more of these variables are highly correlated, then just one or a group of them may be sufficient to characterize the variability between samples, which is important in order to reduce measurements. Therefore, environments might be characterized by the same set of variables, and comparisons could be made among them. In this sense, the use of multivariate statistical methods has become an essential tool in environmental science because they help to reveal and evaluate complex relationships in a wide variety of environmental applications (Samsudin et al. 2011).

Multivariate techniques are frequently used to evaluate water quality along rivers, reservoirs, groundwater, and other AEs (Agbaire and Obi 2009; Cruz et al. 2012; Farmaki et al. 2012; Hong et al. 2010; Noori et al. 2010, 2012; Ouyang et al. 2006; Poma et al. 2012; Shrestha and Kazama 2007; Shin et al. 2012; Samsudin et al. 2011; Wunderlin et al. 2001). They have been applied to large amounts of data, but in some cases, they were used to analyze the behavior of a unique AE and/or considering one or two microbiological parameters as indicators to evaluate microbial pollution. Cruz et al. (2012) studied water quality of shallow groundwater and surface water using 14 physicochemical and two microbiological parameters (total and fecal coliforms); Shrestha and Kazama (2007) analyzed the spatial and temporal variation of Fuji river basin, considering 12 parameters, with total coliform as microbial indicator; 19 parameters were measured by Shin et al. (2012) in a urban estuary in New Jersey; in this case, fecal coliform was the microbial indicator considered. Poma et al. (2012) evaluated water quality of 11 monitoring points located along Arias–Arenales River. In this case, 16 parameters were measured; ten of them were microorganisms including bacteria, viruses, and parasites.

In order to assess the quality of recreational waters and prevent user's diseases, systematic monitoring are usually carried out in most developed and developing countries (Craun et al. 2005; Chandran et al. 2011; Guida et al. 2009; He and He 2008; Mansilha et al.

2009; Palomino de Dios et al. 2011). Although monitoring is not so intensive in developing countries or it is only systematically done in the main recreational cities, people recreate themselves in almost any water body, without knowledge or regardless of their quality. Almost two thirds of the population in the province of Salta, located in the northwest of Argentina, live in absolute poverty, and diseases associated with this condition are widespread (Rajal et al. 2010). This was mainly due to the disrupted growth that Salta has suffered during the last decade, causing an expansion of the population to places where the basic services, such as water and sanitation, were not available. As a consequence, infant mortality (vulnerable group) during 2001 was 17 % higher in Salta compared to the rest of provinces of Argentina (Rajal et al. 2010).

The poor conditions, and in particular the pollution of water by pathogens, contribute to a major public health problem of the city (Rajal et al. 2010). In addition, the contamination may also produce a decrease on the quality of water bodies themselves, causing ecological disturbances (Fulazzaky 2012). Despite that, there is not sufficient background on water quality of AEs in the entire province that allows the quantification of the real condition of the source and its relationship with waterborne diseases. No new proposals for updating local legislations have been made in the country to control and/or mitigate these problems.

In the present study, a set of multivariate techniques have been applied for the first time on a group of parameters measured from various AEs that belong to the province of Salta, with different characteristics, to evaluate their quality. The AEs studied here were not regularly monitored by the national, provincial, or local environmental authority. Thus, there was neither information nor advice available to the users in terms of quality. The aims of this work were as follows: (a) to study the seasonal behavior of the measured variables in each AE; (b) to identify water quality problems related to recreational activities, considering a group of bacterial indicators; (c) to search associations among measured parameters; (d) to detect the potential sources of pollution that influence the quality of water bodies; and (e) to find a group of the parameters responsible for these seasonal/spatial variations, in order to reduce monitoring costs and achieve better water quality management.

Materials and methods

Study area

The province of Salta is located in the northwest of Argentina. It has an area of 155,488 km^2 and a total of 1,214,441 habitants, with 44 % of the population (536,113 habitants) concentrated in the main city of Salta (INDEC 2010). The province belongs to a subtropical area with warm weather but showing variations in the different regions, due to the geographic differences (Baudino 1997). The orographic influences on precipitation result in two main seasons: wet season (WS) which spans from November to March and where the 90 % of annual rainfall is accumulated and dry season (DS) from April to October. The annual rainfall reported during monitoring years was 980 mm in 2008, 600 mm during 2009, and 640 mm in 2010, being 700 mm the mean for 1969–2009 (Fig. 1). Summer usually presents temperatures higher than 20 °C, and in winter, the average is lower than 14 °C, with January being the warmest month and July the coldest (PIDUA 2009).

Four water bodies located in two departments (the Province of Salta is politically divided into departments) that belong to the Province of Salta (Fig. 2) were selected for this study: the Arenales River that crosses the main city of Salta in Capital Department (Fig. 2a) and Campo Alegre Reservoir (Fig. 2b), Vaqueros River (Fig. 2c), and La Caldera River (Fig. 2c) located in La Caldera Department.

Arenales River

This river belongs to the Juramento–Salado watershed. It runs west–east, passes through the city of Salta

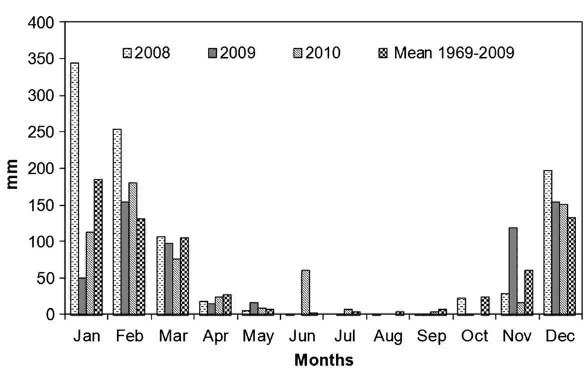

Fig. 1 Rainfall during monitoring period 2008–2010 (INTA 2011)

(Fig. 2a), and goes south where discharges into General Belgrano Reservoir. This reservoir provides hydroelectric energy and water for recreation, fishing, and irrigation for population located downstream (Aramayo et al. 2009). The regime of the river is torrential with high flow rate and presence of suspended particles (mainly clays) during the WS (Baudino 1997).

Before entering the city, the river suffers the impact of agricultural activities (Cruz et al. 2012), and while crossing the city, it is affected by different point and nonpoint discharges that decrease the ambient quality of the river water. The river also receives a high percentage of storm water, partly treated sewage from the wastewater treatment plant (WWTP) of the city of Salta, and effluents from small industries located on their margins (Poma et al. 2012). Industries produce effluents of different quality that increases the concentration of organic matter, phosphorus, total dissolved solids, sulfates, and chlorides reducing the percentage of dissolved oxygen in the water (Salusso 2005).

Campo Alegre Reservoir

La Caldera is a department located in the Province of Salta, 25 km north of the city of Salta. It covers an area of 1,000 km^2 with 7,763 habitants (INDEC 2010). It consists of two main villages: Vaqueros and La Caldera. The AE selected in this case is Campo Alegre Reservoir (Fig. 2b), an artificial lake located 4 km from La Caldera village and 30 km from Salta city. It was built to supply water for irrigation to the fields that belong to the "Mojotoro River Consortium" during the DS when the river's flow decreases. Its secondary function is as a water source for the drinking water plant of Salta city in case of failure of the primary reserves located in the area of Finca Las Costas. Finally, it is a recreational place where different activities, mainly of secondary contact (fishing and sailing, among others), are performed. Swimming (primary contact activity) is also done although it is forbidden in this AE.

Vaqueros and La Caldera rivers

These rivers belong to the high watershed of Mojotoro River. They are located in Vaqueros, a village in the department of La Caldera (12 km from the city of Salta). Mojotoro is the main river of La Caldera Department. This river is formed from the union of La Caldera and Vaqueros rivers (Fig. 2c). The first one comes from the north; drains Campo Alegre Reservoir; has meridional direction; and receives Santa Rufina, San Alejo, Yacones, and Wierna rivers as tributaries. The second starts at the confluence of Lesser and Castellanos rivers; it runs from west to east through the northern sector of the valley. The regimes of both rivers are linked to the seasonality, with increase in the flow rate during WS (Baudino 1997). During the DS (September to November), the flow rate decreases significantly (1.1 m^3/s). These rivers are used mainly for recreational purposes due to their proximity to the city.

Sampling location and collection

Samples were collected from specific monitoring sites selected on each AE, following the Standard Method for Examination of Water and Wastewater for surface waters (Eaton et al. 2005; Procedure 9060A). Four points along the Arenales River were selected (Fig. 2a) for this analysis: a low pollution area before entering the city (a_1), Los Sauces Park where the main activity is recreation (a_2), a third point before the WWTP (a_3), and a last one after it (a_4). Monthly monitoring was performed on this environment during February 2009 to March 2010. A total of 55 samples were analyzed.

Twenty one samples were monthly grabbed from Campo Alegre Reservoir during a whole year (May 2009 to April 2010) from two points (Fig. 2b): rocks–hill (b_1) and a rail (b_2). These points were selected because of their recreational use (bathers, fishermen, and sailors).

Finally, one point in La Caldera River and two points in Vaqueros River were selected for monitoring (Fig. 2c): one under the bridge (c_1) that connects Vaqueros and La Caldera towns and one before (c_2) and another after (c_3) the bridge that connects the main city (Salta, capital) with Vaqueros town, respectively. Due to the intensive recreational activities in both rivers, sampling frequency was different between seasons. It was conducted intensively during the WS, including weekends, when people density was higher. Additional samples were carried out during the DS (May and June representing autumn and winter respectively with no people attendance) to perform a comparison. Forty-two water samples from Vaqueros River were analyzed during December 2008 to May 2010, and 12 samples from La Caldera River during October 2009 to July 2010.

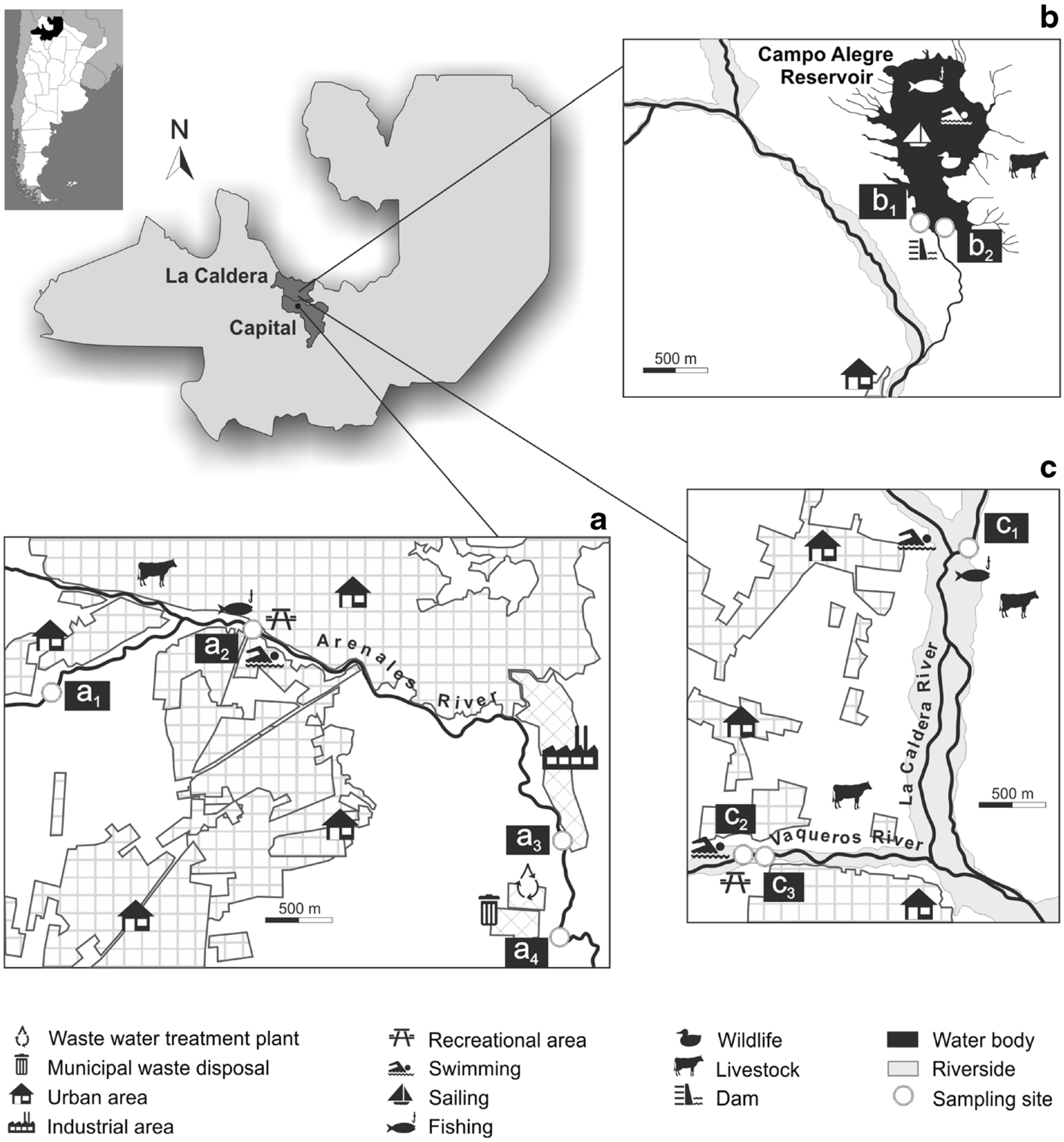

Fig. 2 Monitoring sites (*empty circles with the denomination in black squares*) selected in different aquatic environments studied that belong to Capital Department (Salta is the main city) and La Caldera Department in the Province of Salta, Argentina: **a** four points in Arenales River: a low pollution area (a_1) before entering the city, Los Sauces Park (a_2) and before (a_3) and after (a_4) the waste water treatment plant (WWTP); **b** two points in Campo Alegre Reservoir: rocks–hill (b_1) and a rail (b_2); **c** one site in La Caldera River: under the bridge (c_1) and two sites in Vaqueros River: before (c_2) and after (c_3) the bridge. The main activities developed in them are also marked with the indicated icons

Physicochemical and microbiological determinations

A multiparametric analyzer U10 (Horiba, Japan) was used for measuring in situ the following physicochemical variables: pH, conductivity (COND), turbidity (TURB), dissolved oxygen (DO), and temperature (*T*). Water samples were collected in 500-ml sterile containers and kept at 4 °C for microbiological analysis. Total (TC) and fecal coliforms (FC) were determined using the multiple tubes method on MacConkey broth (Britania, Argentina) incubating at 37 and 44 °C for 24 h, respectively, expressing result as the most probable number (MPN) per 100 ml (Eaton et al. 2005). Membrane filter technique was applied to determine: *E. coli* (ECL) in modified mTEC Agar (Fluka, USA) at 44.5 °C for 24 h (Method 1603, EPA 2002a), enterococci (EN) in mE Agar (Difco, USA) at 41 °C for 48 h and confirmation in esculin iron agar (EIA) at 41 °C for 20 min (Method 1106.1, EPA 2002b), and *Pseudomonas aeruginosa* (PA) using cetrimide agar (Britania, Argentina) and nalidixic acid at 37 °C for 24 h (Method W6-NHS-UK, HPA 2007).

On the other hand, the AEs studied here were assessed regarding the water quality for recreational use, considering several regulations. The levels of five microbiological variables (TC, FC, ECL, EN, and PA) measured in each AE, were evaluated using recreational limit values (EC 1975; USEPA 1986; WHO 2006). In addition, the percentage of samples exceeding the established limits for recreational activities was calculated for all microbiological variables in each AE.

During monitoring, all variables were measured in each monitoring site of the different AEs, according to the sampling frequency described.

Statistical treatment of the data

The variability and correlations of water quality data were explored through statistical approaches. All microbiological variables and turbidity were natural log-transformed. A Mann–Whitney *U* test was performed first in order to evaluate the seasonal effects of the measured variables in each AE. For that, data obtained from physicochemical and microbiological variables measured in each AE were divided in two seasonal groups: WS and DS, according to the records of rainfall throughout the study period studied (Fig. 1). The Mann–Whitney *U* test is a nonparametric test applied to two independent samples (Lehmann 1975). It is used to test the null hypothesis that two samples come from the same population (Lehmann 1975). This nonparametric test was used, since the data were not normally distributed ($p < 0.001$), according to the Shapiro–Wilk *W* test. Also, box–whisker diagrams were built to show seasonal and spatial variations of five physicochemical (*T*, TURB, DO, COND, and pH) and five microbiological (TC, FC, ECL, EN, and PA) variables in each AE.

A group of multivariate techniques was applied to interpret results of complex data. Cluster analysis (CA) was performed to detect the differences and similarities between the AEs. This multivariate technique is used to classify objects into categories or clusters based on their similarity, and it provides relationships between any object and the entire data set (Farmaki et al. 2012). Clusters are typically illustrated by a dendogram which gives a visual summary of the clustering processes and presents a picture of the groups and their proximity, reducing the dimensionality of the original data (Shrestha and Kazama 2007). In this study, CA was performed by the Ward method using Euclidean distances (ED) calculated from nine variables (EN, ECL, TC, FC, *T*, TURB, DO, pH, and COND) for each AE. The most similar objects were those with the smaller distance between them (Farmaki et al. 2012). The cophenetic correlation coefficient (ccc) was also calculated in order to determine whether a given classification represented satisfactorily the relationships observed (Balzarini et al. 2008; Sokal 1986).

Many variables are generally measured in AEs to assess their quality. Sometimes, they can be correlated, allowing their reduction in order to save monitoring effort (money and time). In this case, two statistical methods: Canonical correlation analysis (CCA) and principal component analysis (PCA) were applied to the five AEs to search for correlations. The difference among these two multivariate methods is that CCA analyzes relationships between sets of data while PCA does it within one set (Laessing and Duckett 1979). CCA is used to determine linear relationships between two sets of variables, a predictor set and a response set (Laessing and Duckett 1979), and is applied to ascertain whether a group of measurements is related to another, determining particular attributes responsible for these relationships (Noori et al. 2010). In the present study, CCA was applied to evaluate the existing association among physicochemical and microbiological variables. These variables were divided in two groups: Five physicochemical variables (*T*, DO, TURB, pH, and COND) were included in the predictor data set and four microbiological variables (TC, FC, EN, and ECL) in the response set. Pearson's correlation coefficients (*r*) between the canonical axis and each component were obtained to facilitate the interpretation of the linear combinations. Coefficients <0.40 were considered as trivial, from 0.41 to 0.70 as moderate and >0.70 as heavy (Laessing and Duckett 1979). Biplots and correlation matrixes obtained from PCA were used to visualize relationships among nine variables (TC, FC, EN, ECL, *T*, TURB, pH, COND, and DO). PCA is designed to transform original variables into new ones called principal components (PCs), which are linear combinations of the original variables (Shrestha and Kazama 2007). CCA and PCA were applied for each AE separately.

Also, the loadings of the variables in the first PCs obtained from PCA were analyzed to identify the main pollution sources in the different AEs. Regarding this, a number of PCs which explains a relevant amount of variance were analyzed (more than 75 % in total). This can also be explained following Kaiser criterion (Kaiser 1959) which says that only PCs with eigenvalues exceeding the unity are considered as significant influence. The loadings of the variables that presented values equal or greater than 0.60 were considered as significant.

Finally, linear discriminant analysis (LDA) was applied in order to discriminate between AEs and to evaluate the parameters responsible for seasonal and spatial variations. LDA is another multivariate method widely used to distinguish between two or more groups of objects maximizing their differences. It allows the reduction of a large data set, selecting a few indicators responsible for water quality variations (Shrestha and Kazama 2007). The starting point of LDA is to find linear combinations of the original variables and construct the discriminant functions (Farmaki et al. 2012). For this purpose, LDA was implemented in standard and reduced mode, to separate between AEs depending on their characteristics and to allow the identification of subgroup of parameters responsible for spatial and seasonal water quality, without losing ability to distinguish between the AEs and leading to a lower measurement effort, shorter times, and minimal monitoring costs. All the possible combinations of 9, 8, 7, and 6 variables were investigated in the context of LDA. The linear combination of each set was evaluated through classification error rates (CERs) as a tool for assigning other water samples into one of the studied AEs.

Multivariate analyses were carried out on standardized dataset to eliminate the effect of data measurement scale. All statistical analyses were conducted using the statistical software package InfoStat (Di Rienzo et al. 2011).

For the water quality assessment through regulations and box plot diagrams, a larger dataset was used (130 cases) compared with the data used for statistical approach (80 cases), since the detection of ECL, EN and PA started in the middle of the monitoring. PA was only included in the water quality assessment because of the lack of monitoring data.

Results and discussion

Seasonal analysis

As it was mentioned before, data obtained from monitoring the different AEs were divided in two seasonal groups: WS and DS. Also, the concentration of the five microbiological indicators was assessed using recreational limit values established by legislations (EC 1975; USEPA 1986; WHO 2006), and the percentage of samples exceeding these thresholds was calculated.

Physicochemical variables

Water bodies exhibit temperature variations along with normal climatic fluctuations. These variations can occur seasonally and, in some AEs, over periods of 24 h (Chapman 1996). Due to the marked climate variability in the study region, temperature showed significant differences between seasons for all the analyzed environments (Table 1, Fig. 3a). The average temperature was 23.7±0.3 °C for the WS and 18.6±5 °C for DS (Table 1). Vaqueros River and Arenales River showed the lowest temperatures during winter (11.0 °C) and Campo Alegre Reservoir the highest during WS (30.0 °C).

No significant differences between seasons were observed for pH in any of the AEs ($p > 0.05$, Table 1, Fig. 3b). All the pH values ranged from 6 to 8 for all the AEs, except for Campo Alegre Reservoir, which showed the highest pH value during DS (reaching a maximum of 10.0), and Arenales River during the WS, when the pH reached its minimum value of 4.3. These changes in pH could endanger aquatic life (David et al. 2013) and are indicative of the discharge of certain effluents: domestic (detergent, sewage), from wastewater treatment plants, from industries like tanneries (chlorides and sulfates), and diffuse loadings (herbicides) from agriculture activities located upstream (Cruz et al. 2012; David et al. 2013; Salusso 2005).

The differences in conductivity between seasons were statistically significant in three of the four AEs (Table 1), excluding Vaqueros River ($p > 0.05$) where COND values remained constant during all monitoring year (Fig. 3e). The source of COND may be from dissolved salts due to poor irrigation management, minerals from storm water runoff, a failing sewage system (presence of chloride, phosphate, and nitrate), and an oil spill (David et al. 2013). In the case of DO, although it

Table 1 Physicochemical and microbiological variables averaged over space for four aquatic environments in Salta, Argentina, for dry season (DS) and wet season (WS): temperature (*T*, in °C), pH, turbidity (TURB, in NTU), electrical conductivity (COND, in µS/cm), dissolved oxygen (DO, in mg/l), total coliforms (TC, in log MPN/100 ml), fecal coliforms (FC, in log MPN/100 ml), *E. coli* (ECL, in log CFU/100 ml), Enterococci (EN, in log CFU/100 ml), and *Pseudomonas aeruginosa* (PA, in log CFU/100 ml)

	n		Mean±SD		Range		*p* value*
	DS	WS	DS	WS	DS	WS	
Arenales River							
T	14	17	18.9±4.5	23.5±1.9	11.6–25.3	20.4–26.8	**0.0021**
pH	14	17	7.6±0.5	7.3±1.1	6.7–8.5	4.3–8.4	0.9683
DO	14	17	5.2±2.8	7.2±1.3	1.2–9.2	4.2–8.9	**0.0494**
COND	14	17	423.0±135.3	227.8±39.8	264.0–615.0	172.0–307.0	**<0.0001**
TURB	14	17	109.0±0.6	433.1±0.5	7.0–521.0	50.0–999.0	**0.0016**
TC	14	17	5.6±1.8	5.1±1.7	2.6–9.4	2.9–8.6	0.4383
FC	14	17	5.3±1.9	4.6±1.8	2.6–9.4	2.4–8.6	0.2333
ECL	14	17	4.1±1.6	4.3±1.4	1.7–6.6	1.3–6.2	0.5123
EN	14	17	3.5±1.4	3.6±0.9	1.9–5.7	2.3–5.5	0.9052
Vaqueros River							
T	3	14	13.8±3.7	23.4±3.3	11.5–18	17.7–29.7	**0.0117**
pH	3	14	7.8±0.2	7.7±0.7	7.7–8.0	5.9–8.5	0.8499
DO	3	14	9.1±0.6	8.2±1.4	8.4–9.6	5.3–9.3	0.1434
COND	3	14	92.7±3.8	83.5±23.4	90.0–97.0	53.0–130.0	0.4497
TURB	3	14	7.0±0.6	122.2±0.6	0.0–1.2	9.0–999.0	0.058
TC	3	14	1.9±0.4	3.6±0.7	1.5–1.9	2.4–4.6	**0.008**
FC	3	14	1.6±0.2	3.4±0.7	1.5–1.9	1.9–4.3	**0.0079**
ECL	3	14	1.1±0.3	2.7±0.5	0.9–1.4	1.6–3.8	**0.008**
EN	3	14	1.0±0.3	2.7±0.5	0.8–1.3	1.7–3.6	**0.0082**
La Caldera River							
T	2	9	15.6±2.6	23.9±2.9	13.7–17.4	20.2–29.7	**0.0339**
pH	2	9	8.5±0.2	8.4±0.2	8.3–8.6	8.1–8.8	0.6374
DO	2	9	8.4±1.1	8.2±1.5	7.6–9.1	5.2–9.0	0.6209
COND	2	9	243.5±19.1	196.1±17.3	230.0–257.0	169.0–230.0	**0.0446**
TURB	2	9	1.5±0.2	277.5±0.9	0.0–0.3	4.0–999.0	**0.0339**
TC	2	9	2.3±0.1	3.5±0.8	2.2–2.4	2.6–4.6	**0.0334**
FC	2	9	1.9±0.4	2.9±0.4	1.6–2.2	2.2–3.3	**0.0421**
ECL	2	9	1.5±1.2	2.8±0.6	0.7–2.4	2.2–3.9	0.1562
EN	2	9	1.6±0.5	2.5±0.3	1.3–1.9	2.0–2.9	**0.0339**
Campo Alegre Reservoir							
T	15	6	26.1±2.9	17.2±2.9	13.0–23.4	23.2–30.4	**0.0007**
pH	15	6	8.8±1.0	8.5±0.7	7.5–10.0	8.0–9.6	0.4361
DO	15	6	6.6±2.0	6.6±1.8	3.5–9.0	3.3–8.0	0.5812
COND	15	6	107±6.4	115.2±7.9	96.0–124.0	96.0–106.0	**0.0378**
TURB	15	6	12.4±0.5	13.8±0.2	1.0–40.0	8.0–25.0	0.3091
TC	15	6	2.8±0.8	2.4±0.7	1.5–3.9	1.9–3.6	0.225
FC	15	6	2.7±0.5	1.8±0.8	1.5–3.2	1.9–3.6	**0.0197**
ECL	15	6	2.5±1.1	1.2±0.7	0.0–2.8	1.9–3.7	0.1015
EN	15	6	1.5±0.8	0.6±0.6	0.0–2.5	0.6–2.1	**0.0138**

Bold numbers indicate significant values

*$p < 0.05$, statistical significance; Mann Whitney *U* test for the null hypothesis of no seasonal effect

SD standard deviation

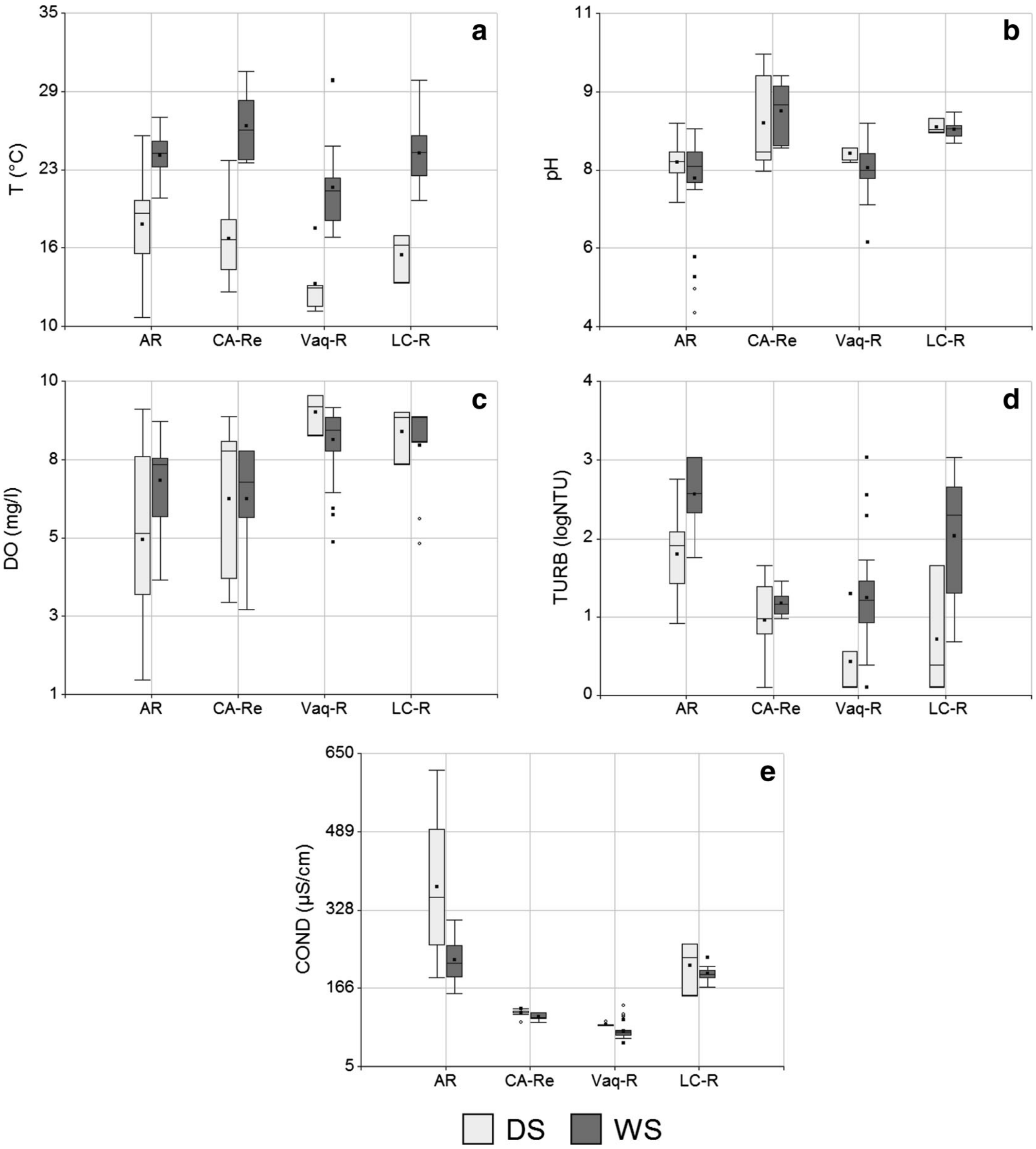

Fig. 3 Physicochemical variables monitored in the different aquatic environments during dry season (DS) and wet season (WS) and including all the monitoring points: Arenales River (AR): a_1 (a low pollution area), a_2 (Los Sauces Park), a_3 (before waste water treatment plant), and a_4 (after waste water treatment plant); Campo Alegre Reservoir (CA-Re): b_1 (rocks–hill) and b_2 (rail); La Caldera River (LC-R): c_1 (under the bridge); and Vaqueros River (Vaq-R): c_2 (before bridge) and c_3 (after bridge). The measured variables were as follows: **a** temperature (*T*), **b** pH, **c** dissolved oxygen (DO), **d** turbidity (TURB); and **e** conductivity (COND)

presented variability both between seasons and within environments (Fig. 3c), the only AE that showed significant differences was the Arenales River ($p < 0.05$). During WS, the increased turbulence contributes to the oxygenation of the river (Gatica et al. 2012). The concentration of DO is crucial for the survival of aquatic organisms; in fact, levels lower than 2 mg/l would kill fish (Berenzen et al. 2001; David et al. 2013). DO is also used to evaluate the degree of freshness of a river (Agbaire and Obi 2009). If water is contaminated with fertilizers, particulate matter, and/or industrial wastes, microorganisms will break down the contaminants, oxygen will be consumed, and anaerobic condition will be developed (Chapman 1996), and this will reduce the assimilative capacity of the AE.

The variable turbidity refers to cloudiness of water and has no health effects itself. Rainfall during WS increases the flow rate of rivers, causing resuspension of small particles like clays, which raise the turbidity of AEs. If these particles had microorganisms adsorbed to them, they would be resuspended too (Boutilier et al. 2009; Jamieson et al. 2005) and eventually desorbed from the solids, representing a potential risk of infection (Chandran et al. 2011). In this case, only Arenales River and La Caldera River showed significant differences on turbidity between seasons ($p < 0.05$, Table 1, Fig. 3d).

Microbiological variables

The concentrations of most microbiological indicators were different between seasons in three of the four AEs studied ($p < 0.05$, Table 1). In Campo Alegre reservoir, Vaqueros and La Caldera rivers microbiological values increased significantly during WS (Fig. 4) overcoming, most of the times, the limit values established for recreational waters (Table 2). Regarding this, Vaqueros River was the only AE that showed significant differences between seasons for all the bacterial indicators ($p < 0.05$, Table 1). Conversely, Arenales River was highly contaminated along the year presenting no significant differences between seasons for any bacterial indicators and overcoming, in most of the cases, the threshold values established (Fig. 4, Table 1). This situation may be due to the regular inflows of some illegal raw sewage discharges and to industrial effluents with high loads of microorganisms (Poma et al. 2012). In addition, this AE was the only one that showed that the concentration of some bacterial indicators (and some physicochemical concentration too, Fig. 3) was higher during the DS. This may be due to the decrease of the river flow rate during this season which produces the increment of pollutant concentrations, while dilution effect may exist during the WS (rainy season), attenuating these effects (Pesce and Wunderlin 2000).

Water quality issues

In terms of human health, the presence of pathogens in natural environments generally used for recreational purposes can be related to waterborne diseases (CDC 2008; Ashbolt et al. 2001; Marion et al. 2010). Lots of international legislations include and still use bacterial indicators to determine water quality (EC 1975; USEPA 1986; WHO 2006). Due to the lack of systematic monitoring and epidemiological studies, Argentina has established guidelines levels according to international standards (SRHN 2003).

The percentage of samples exceeding the established limits for recreational activities varied for the different AEs (Table 2). Campo Alegre Reservoir exhibited the best water quality in all cases while the Arenales River showed the worst (Table 2). In particular, the monitoring points from Arenales River were characterized by high microbial contamination (Fig. 4) which decreases the concentration of dissolved oxygen, especially during the DS (Fig. 3), to levels where the ecology of the river may be affected (MPCA 2009). In case of PA, more than 50 % of the samples from the three rivers studied exceeded the limits for these bacteria (Table 2). This microorganism was detected in higher concentrations during WS in Vaqueros River and Campo Alegre Reservoir (Fig. 4), but in case of Arenales River and La Caldera River, PA concentration was higher during DS. This may be due again to the high concentration of pollutants during the DS when water quantity in AEs decreases.

The presence of PA is common in recreational waters during WS (Guida et al. 2009; Papadopoulou et al. 2008) since it can be naturally present in the water and, in addition, it is usually shed by bathers. As PA requires minimal nutrients to grow, both bathers and natural nutrients can be responsible for this situation (Guida et al. 2009; Papadopoulou et al. 2008) that becomes a health risk. Coronel-Olivares et al. (2011) have suggested the inclusion of this microorganism in regulations for treated wastewater quality as a new indicator due to its importance as an opportunistic pathogen.

As it can be seen, water quality varied in the different AEs and within seasons evidencing that they were not always suitable for recreational uses (or even any use). Furthermore, the high microbial pollution found evidenced that there are sanitation issues to be solved and suggests that other microorganisms, like virus and parasites, may be also present. Thus, it is necessary to keep a systematic control to avoid waterborne-related diseases (WHO 2006).

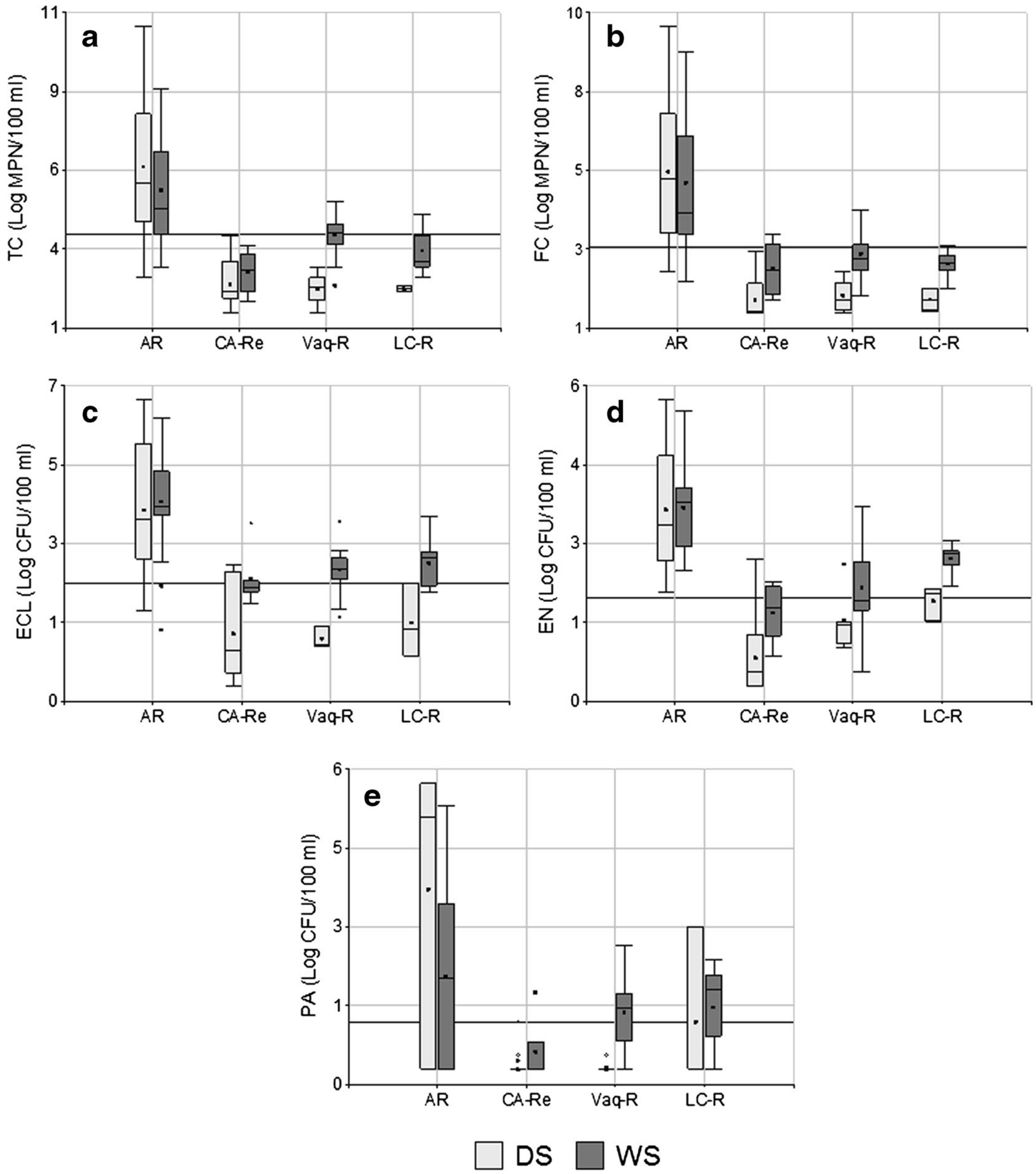

Fig. 4 Microbiological variables measured in the different aquatic environments during dry season (DS) and wet season (WS) and including all the monitoring points: Arenales River (AR): a_1 (a low pollution area), a_2 (Los Sauces Park), a_3 (before waste water treatment plant), and a_4 (after waste water treatment plant); Campo Alegre Reservoir (CA-Re): b_1 (rocks–hill) and b_2 (rail); La Caldera River (LC-R): c_1 (under the bridge); Vaqueros River (Vaq-R): c_2 (before bridge) and c_3 (after bridge). The measured variables were **a** total coliforms (TC); **b** fecal coliforms (FC), **c** *E. coli* (ECL), **d** Enterococci (EN), and **e** *Pseudomonas aeruginosa* (PA). *Black solid horizontal lines* represent recreational limit values for primary and secondary contacts (EC 1975; USEPA 1986; WHO 2006)

Similarity between the AEs

A great variability between upstream and downstream in seven of the ten variables measured at the four monitoring points in Arenales River was observed ($p<0.05$), except for temperature, turbidity, and conductivity ($p>0.05$). This may be probably due to the different impacts that the river receives from its catchments (Fig. 2a). Thus, the data obtained allowed the division of this AE in two parts: Arenales River Upstream (UpS) considered as a low-pollution site, including the monitoring sites a_1 and a_2, and Arenales River downstream

Table 2 Guideline values for recreational waters and percentage of samples that overcome these limits

Bacterial indicators	Concentration limits	Legislation	Arenales River	Vaqueros River	La Caldera River	Campo Alegre Reservoir
TC	10,000/100 ml LV,	EC 1975	88 % (51)	45 % (40)	18 % (11)	0 % (21)
	500/100 ml GV		100 % (51)	88 % (40)	73 % (11)	33 % (21)
FC	2,000/100 ml LV,		82 % (51)	28 % (40)	9 % (11)	10 % (21)
	100/100 ml GV		100 % (51)	95 % (40)	91 % (11)	43 % (21)
ECL	235[a] UFC/100 ml	USEPA 1986	97 % (31)	71 % (17)	58 % (12)	24 % (21)
	298[b] UFC/100 ml		97 % (31)	71 % (17)	50 % (12)	24 % (21)
EN	61[a] UFC/100 ml		100 % (29)	43 % (42)	92 % (12)	14 % (21)
	89[b] UFC/100 ml		97 % (29)	48 % (42)	75 % (12)	14 % (21)
PA	<10 UFC/100 ml	WHO 2006	56 % (16)	57 % (42)	58 % (12)	5 % (18)

The total number of samples for each variable is indicated in parenthesis

[a] Primary contact

[b] Secondary contact

LV limit value, *GV* guide value

(DoS) as a highly polluted site including a_3 and a_4. Gatica et al. (2012) also applied this division to Chocancharava River for statistical analysis due to the different degrees of contamination along it.

In order to check the validity of this differentiation, a cluster analysis (CA) was applied.

It is important to highlight that a high cophenetic correlation (>0.892) was found in CA, indicating that the classification obtained was a reasonably faithful representation.

As suspected, Arenales River DoS and UpS differed significantly (Fig. 5, ED=4.17). Arenales River DoS was strongly impacted by effluent from the WWTP and receives illegal discharges from industries, while Arenales River UpS was mostly impacted by recreational and upstream activities (Cruz et al. 2012; Poma et al. 2012) presenting more similarity with La Caldera and Vaqueros rivers (Fig. 5). The two latest, La Caldera and Vaqueros rivers, resulted the most similar environments (ED=1.76), which was expected considering the geographical closeness and geomorphology between them and their similar uses (Fig. 2c). Conversely, Campo Alegre Reservoir and Arenales River DoS were the most dissimilar environments (Fig. 5, ED=7.17). Campo Alegre Reservoir is used for recreation mainly of secondary contact and for water consumption (Fig. 2b); no effluent discharges were observed there.

Hence, five AEs will be considered from now on for the following analysis: Vaqueros River, La Caldera River, Campo Alegre Reservoir, Arenales River UpS, and Arenales River DoS.

Associations between physicochemical and microbiological variables

CCA results showed no significant correlation between physicochemical and microbiological variables in Arenales River, neither in UpS nor in DoS (data not shown). However, there was a significant correlation between those groups of variables in the other three AEs. In Campo Alegre Reservoir, the first two canonical axes were significant ($p < 0.05$), with correlation coefficients of 0.93 and 0.83, respectively (Table 3(A)). In the

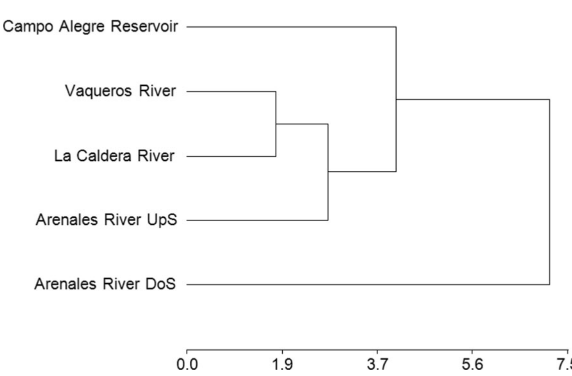

Fig. 5 Dendogram showing clustering for the different aquatic environments (ccc>0.892). *Vertical axis* represents the observations (aquatic environments) and *horizontal axis* the Euclidean distances (ED).

Table 3 Summary of canonical correlation analyses between microbiological and physicochemical variables for three aquatic environments: (A) canonical correlation coefficients and *p* values, (B) Pearson correlation coefficients between the first two canonical axes combining physicochemical variables (Axis 1_{PC} and Axis 2_{PC}) and each component, and (C) Pearson correlation coefficients between the first two canonical axes combining microbiological variables (Axis 1_{MB} and Axis 2_{MB}) and each component

	Campo Alegre Reservoir		La Caldera River		Vaqueros River	
(A)						
Canonical axes	*r*	*p* value	r	*p* value	r	*p* value
1	0.93	0.00001	1	0.002	0.92	0.03
2	0.83	0.01	0.97	0.09	0.65	0.4
(B)	Axis 1_{PC}	Axis 2_{PC}	Axis 1_{PC}	Axis 2_{PC}	Axis 1_{PC}	Axis 2_{PC}
T	0.61	0.08	0.46	0.47	−0.25	−0.38
pH	−0.46	0.07	0.32	0.33	0.17	0.26
COND	−0.68	0.49	−0.68	−0.70	−0.27	−0.42
DO	−0.55	−0.08	−0.36	−0.37	−0.25	−0.38
TURB	−0.23	−0.64	0.61	0.63	0.03	0.05
(C)	Axis 1_{MB}	Axis 2_{MB}	Axis 1_{MB}	Axis 2_{MB}	Axis 1_{MB}	Axis 2_{MB}
TC	0.55	−0.51	0.20	0.20	0.57	0.53
FC	0.81	−0.75	0.48	0.48	0.49	0.45
ECL	0.97	−0.90	0.91	0.91	0.89	0.82
EN	0.81	−0.75	0.73	0.73	0.99	0.91

case of La Caldera River and Vaqueros River, only the first canonical axis showed significant correlation ($p=0.002$; $p=0.03$) with coefficients of 1 and 0.92, respectively (Table 3(A)).

The first canonical axis of both groups of variables could sufficiently explain the relationship between them. In case of Campo Alegre Reservoir, the first linear combination in the predictor variables showed almost heavy and negative coefficient for COND (−0.68), moderate and positive coefficient for *T*, and moderate and negative for pH and DO (Table 3(B)). La Caldera River also presented moderate and negative coefficient for COND and positive and moderate coefficients for *T* and TURB (Table 3(B)). Vaqueros River did not show significant coefficients for this group.

For the microbiological variables (response data), Campo Alegre Reservoir showed positive and heavy coefficients for all indicators except for TC which loaded moderate (0.55). Vaqueros and La Caldera rivers showed heavy and positive coefficients for ECL and EN and moderate and positive coefficients for FC (Table 3(C)). Thus, it can be seen that high values of *T* were related with the presence of microorganisms. During WS, water temperature is higher, and these conditions are better for microorganisms' survival and growth causing the decrease of water quality and the increase of health risk on bathers who are exposed (Marion et al. 2010). Also, the presence of bathers is higher, and this also contributes to increase the microbiological load.

Although DO solubility naturally decreases with increasing temperature, it also decreases when microorganisms are present due to oxygen consumption (Gatica et al. 2012) resulting in the inverse relationship between this physicochemical variable and the concentration of microorganisms (Table 3). Regarding COND, it generally decreases during WS due to dilution effects (increase of flow rate), being the reason, in some cases, of the inverse relationships (Gatica et al. 2012)

On the other hand, in the second canonical axis, the physicochemical variable TURB presented negative and moderate coefficient for Campo Alegre Reservoir and positive and moderate coefficient for La Caldera River, showing a direct relationship with microbiological variables (Table 3(B, C)). This correlation was expected because it is well known that microorganisms can be adsorbed to particles (Brookes et al. 2004; Gao et al. 2011; Jamieson et al. 2004; Jamieson et al. 2005; Marshall 1980), which protect them in the AE and thus becoming a potential health risk (Chandran et al. 2011). Although this direct correlation between microorganisms and turbidity was also observed in Vaqueros River (Table 3(B, C)), it was not statistically significant.

This is probably due to a low solid concentration in water that may be not enough for microorganisms to attach to particles, leading to an eventual inactivation, death, or transport to other parts of the river. Only the variable COND showed a significant correlation with all microbial variables.

Summarizing, ECL was the microbiological variable that weighed the most in the canonical axes both in Campo Alegre Reservoir and in La Caldera River (Axes 1_{MB} and 2_{MB}, Table 3(C)), and COND was the physicochemical variable that most weighed in the first axis in both AEs (Table 3B). The microbiological variable EN also showed high correlation coefficients with the canonical variables, especially in Vaqueros River, and TURB weighted significantly in the second axis for Campo Alegre Reservoir and in both axes in La Caldera River.

These results confirmed an association between the microbiological and physicochemical characteristics of water samples in a multivariate sense.

Relationships between all variables

Biplots (Fig. 6) and correlation matrixes (Table S1) obtained from PCA showed relationships between the variables in each site. In case of biplots, right angles (90°) indicate poor correlation; almost straight angles (180°) indicate highly negative correlations, and near null angles (0°) show high positive correlations between variables. Correlation coefficients considered significant were the ones with p values of less than 0.05 (Table S1).

Considering all the AEs, the two first principal components (PC1 and PC2) explained between 63.6 and 75.2 % of the underlying total variability among water samples. In general, PC1 described water samples according to their microbiological characteristics whereas physicochemical variables were more represented in PC2 (Fig. 6). The correlation structure among variables varied between AEs (Fig. 6a–e).

As in CCA, temperature was also strongly correlated with microbiological variables (correlation coefficients >0.5; $p < 0.05$) in Vaqueros River, La Caldera River, and Campo Alegre Reservoir (Table S1). During WS, hot weather (T higher than 30 °C) increases water temperature allowing bacterial persistence and growth, which represents a potential health risk for bathers. The inverse situation occurs during DS when T values decrease (Table 1) causing, most of the time, bacterial inactivation. Also, microbiological variables were positively correlated among them in most AEs (Fig. 6). Regarding the physicochemical variables, significant and positive correlation could be found between DO and pH in Campo Alegre Reservoir (0.75) and in Arenales River UpS (0.76). When there is organic waste, microorganisms consume oxygen and produce CO_2 decreasing the pH (Frieder et al. 2012; Shrestha and Kazama 2007).

COND and DO were highly and negatively correlated in Arenales River DoS (−0.88) and slightly correlated in Arenales River UpS (−0.50), but they were not associated in other environments (Fig. 6). Organic matter load that comes from wastewater treatment plants and industrial effluents consume important amounts of oxygen, producing anaerobic conditions in the river (Shrestha and Kazama 2007). This was expected in both parts of Arenales River due to the high pollution caused by point and nonpoint sources (Cruz et al. 2012; Poma et al. 2012).

DO was negatively correlated with EN in Vaqueros River (−0.49), with ECL in Campo Alegre Reservoir (−0.58) and with FC in Arenales River DoS (−0.54), confirming the results obtained from CCA (Table 3).

In Vaqueros River, turbidity was negatively correlated with pH (−0.59), and in Campo Alegre Reservoir, this correlation was positive (0.68). Turbidity is a variable commonly used in monitoring because it may be related to the presence of microorganisms (Brookes et al. 2004; Chandran et al. 2011; Gao et al. 2011; Jamieson et al. 2004). Although positive correlation between TURB and most microorganisms within all AEs existed (Table S1), only La Caldera and Vaqueros River showed a statistically significant correlation between them (Fig. 6a, b). As it was said before, during WS, rainfall increases river flow rates causing a particle resuspension. This resuspension may also bring microorganisms adsorbed to particles from the bottom of the AE back to the surface (Boutilier et al. 2009; Jamieson et al. 2005). During DS, turbidity decreases, so free microorganisms in water are more exposed to environmental stressors and more prone to death. Also, some of them could sediment with particles and persist at the bottom of AEs for longer periods (Brookes et al. 2004; Jamieson et al. 2004).

Therefore, these results (compared to CCA) also showed that relationships exist between variables and, sometimes, these associations can vary in each AE. In addition, PCA showed that it is possible to eliminate some microbiological variables that are strongly related when monitoring different AEs. Instead, the situation

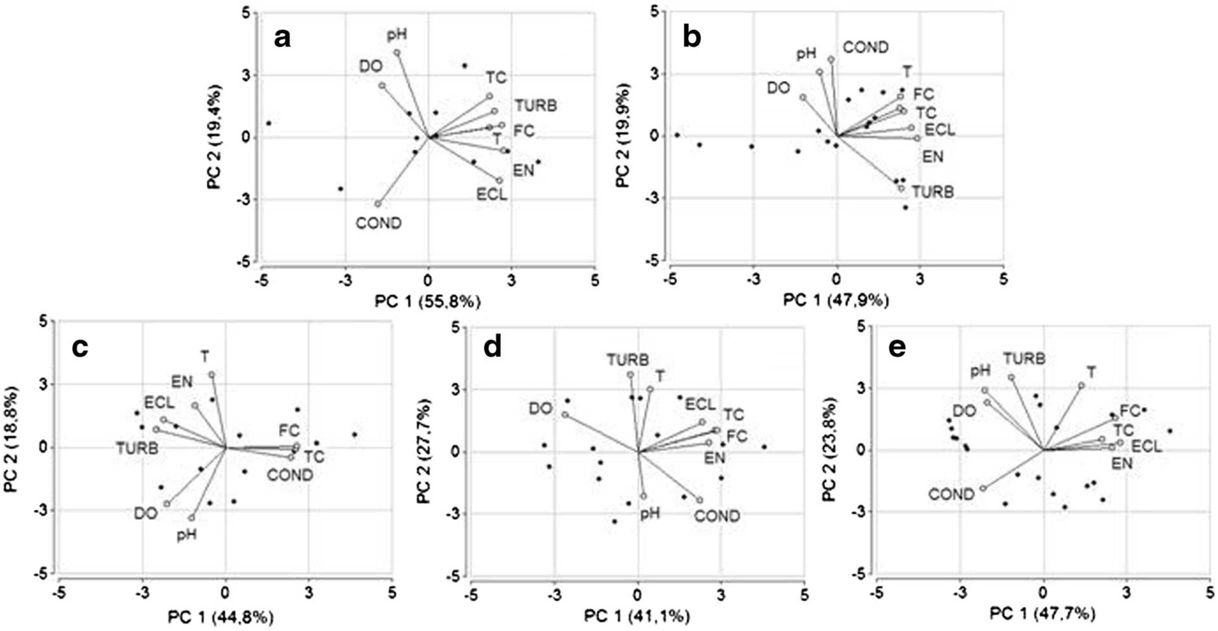

Fig. 6 Biplots of the first two axes of the principal component analysis performed on microbiological and physicochemical variables for each aquatic environment. *Filled circles* correspond to water samples, and *arrows with empty circles* correspond to the variables projections on the axes. **a** La Caldera River, **b** Vaqueros River, **c** Arenales River UpS, **d** Arenales River DoS, and **e** Campo Alegre Reservoir

with physicochemical variables is different because there are not always correlations among them, and if they exist, they are different in each AE, and other analyses are necessary to find out a reduced group of variables that affect each particular AE.

Identification of potential pollution sources

The loadings of the variables in the first PCs obtained from PCA were used to identify factors that affect water pollution in the different AEs.

In the case of Arenales River DoS, 81 % of the total variance was explained by three PCs. The first one (PC1) had positive loadings for TC, FC, ECL, EN, and COND and negative loading for DO (Table 4). The DO reduction may be due to an increase of the biochemical oxygen demand necessary to degrade the high organic matter load. Also, fecal bacteria are strongly related to municipal sewage and WWTP located along the river (Poma et al. 2012). Thus, PC1 represented microbiological contamination mostly due to anthropogenic activities. PC2 was mainly influenced by *T* (0.75) and TURB (0.92). These two variables can be associated with natural events such as seasonal variation and rainfall (the rainy season in Salta is during the summer). The latest produces an increment of eroded material and urban runoff (Wunderlin et al. 2001); therefore, this component may represent the seasonal effects. The dilution effect could also be observed through COND (−0.59). The third component (PC3) had strong positive loading for pH (0.76). Variation of pH can be indicative of the presence of certain industrial pollutant (Chapman 1996), concluding that this PC may represent industrial effects. This is justified because of the presence of industries that discharge effluents illegally in this particular part of the river.

In the case of Arenales River UpS, the 89 % of the variability was explained by four PCs. PC1 had high positive loadings for TC, FC, and COND (Table 4) and negative for DO, TURB, and ECL (−0.69, −0.82, and −0.73, respectively). Seasonal and dilution effects were also present here (TURB, COND, and DO). Therefore, PC1 represented microbiological and seasonal effects. For PC2, strong but opposite loadings appeared for *T* (0.73) and pH (−0.73). As the temperature increases, the decomposition rate of organic matter also increases, which causes the decrease in pH and higher DO consumption (−058). For that reason, PC2 could represent the effect of organic load from agriculture or industrial activities performed upstream (Cruz et al. 2012). PC3

Table 4 Variable loadings of standardized water quality data set for the five aquatic environments

Sites	Arenales River DoS			Arenales River UpS				Campo Alegre Reservoir			La Caldera River		Vaqueros		
Variables	PC1	PC2	PC3	PC1	PC2	PC3	PC4	PC1	PC2	PC3	PC1	PC2	PC1	PC2	PC3
T	0.14	**0.75**	0.1	−0.16	**0.73**	−0.06	**0.63**	0.45	**0.71**	−0.45	**0.75**	0.09	**0.77**	0.4	−0.33
pH	0.07	−0.54	**0.76**	−0.4	**−0.73**	0.24	0.4	**−0.68**	**0.65**	−0.27	−0.37	**0.81**	−0.2	**0.65**	**−0.61**
DO	**−0.79**	0.44	0.27	**−0.69**	−0.58	0.31	0.15	**−0.64**	0.51	0.04	−0.55	0.49	−0.39	0.39	**0.72**
COND	**0.68**	−0.59	−0.17	**0.78**	−0.1	0.26	−0.5	**−0.69**	−0.43	−0.34	**−0.61**	**−0.64**	−0.06	**0.78**	0.09
TURB	−0.08	**0.92**	0.07	**−0.82**	0.17	−0.01	−0.2	−0.37	**0.80**	0.3	**0.81**	0.25	**0.77**	−0.55	0.08
TC	**0.84**	0.25	0.43	**0.84**	−0.02	0.37	0.23	**0.69**	0.11	**0.61**	**0.75**	0.39	**0.80**	0.25	0.41
FC	**0.87**	0.25	0.29	**0.85**	0.01	0.41	0.24	**0.84**	0.34	0.15	**0.91**	0.12	**0.76**	0.28	0.44
ECL	**0.71**	0.35	−0.31	**−0.73**	0.27	0.39	−0.2	**0.89**	0.08	−0.26	**0.87**	−0.42	**0.89**	0.08	−0.13
EN	**0.78**	0.1	−0.16	−0.36	0.42	**0.76**	−0.1	**0.80**	0.02	−0.49	**0.92**	−0.13	**0.96**	−0.03	−0.19
Eigenvalue	3.7	2.5	1.08	4.03	1.7	1.25	1.01	4.29	2.14	1.18	5.02	1.75	4.31	1.79	1.43
%Total variance	41	28	12	45	19	14	11	48	24	13	56	19	48	20	16
Cumulative variance %	41	69	81	45	64	78	89	48	72	85	56	75	48	68	84

Bolded loadings are the most significant

was influenced just by EN (0.76), suggesting also microbiological contamination. In PC4, *T* had a positive and high loading (0.63) as a result of the seasonal variation.

For Campo Alegre Reservoir, the 85 % of the variability was described by three PCs. PC1 showed strong positive loadings for all microbiological variables TC, FC, ECL, and EN and strong negative loadings for the physicochemical ones pH, DO, and COND (Table 4). The fact that high pH is harmful to coliform bacteria (Hong et al. 2010) justifies that TC and FC decrease when pH increases. In addition, strong negative loadings for pH and DO could represent anthropogenic pollution sources and can be explained by saying that if dissolved organic matter is present in water, it will consume important amounts of oxygen creating an anoxic environment. Indeed, anaerobic fermentation processes will lead to an eventual formation of ammonia and organic acids. Hydrolysis of these acidic materials causes the decrease of water pH values (Vega et al. 1998). The low COND values indicate fewer amounts of dissolved solids as bicarbonates, producing instability in the buffer system which causes a decrease in pH indicating anthropogenic pollution. Therefore, this PC represented anthropogenic effects. PC2 had positive loadings for *T*, pH, and TURB (Table 4). Higher temperature and sunlight will increase algal photosynthesis and growth, raising the turbidity and pH (Hong et al. 2010). This component represented ecological (photosynthesis) and seasonal effects. PC3 had positive loading for TC (0.61) indicating also a microbial pollution.

For La Caldera River, 75 % of the total variance could be explained by two PCs. PC1 had positive loadings for all microorganisms, TURB, and *T* (Table 4). Torrential precipitations during WS result in soil erosion, and also, the resuspension of sediments increase the turbidity of the river. The presence of bathers during WS and the optimal water temperature lead to an increase of bacterial indicators. These microorganisms may be adsorbed to sediments which allowed their longer persistence in the environment. Thus, this factor represented recreational and seasonal effects and the adsorption of microorganisms to sediments. PC2 had positive loadings just for pH (0.81) and a moderate and negative loading for COND (−0.64). Lower conductivity indicates less dissolved salts; thus, the AE is more influenced by its own buffer system [CO_2/carbonate/bicarbonate] which explains its high pH (alkaline). Therefore, this factor represents mineralization effects.

For Vaqueros River, 84 % of the total variability was described by three PCs. PC1 had same effect as in La Caldera River: seasonal and recreational (Table 4). PC2 was positively influenced by pH (0.65) and COND

(0.78) suggesting mineral components of the river water. Finally, PC3 had positive loading for DO (0.72) and negative for pH (−0.61). pH and DO values are strongly influenced by environmental factors, and both variables are sensitive to changes in water quality (Wang et al. 2007); therefore, this component can be attributed to ecological effect.

Variables responsible for water quality changes

Knowing water quality issues of the studied AEs and seasonal variations, it is important to assess which are the variables responsible for these changes (Shrestha and Kazama 2007).

Spatial variations

LDA performed in standard mode, using all the nine discriminant variables (Fig. 7a), gave a CER of 11.2 %, supporting the natural structure of the clusters generated by the CA (Fig. 5). The reduced mode of LDA was applied to search for a subgroup of variables that could describe water quality conditions without losing capacity to discriminate these AEs. First, LDA was built for physicochemical and microbiological variables separately. The CER was 21.2 % using just the five physicochemical variables and 32.5 % using only the four microbiological variables. Considering a combination of eight variables, the exclusion of TURB or *T* produced a decrease in the CER from 11.2 to 10 %. The variable that caused the highest increment in the CER was COND (27.5 %), which can be explained by the great difference of these measurements between the AEs studied. By using a combination of seven variables, removing now *T* and TC or *T* and FC, the CER decreased to 8.7 %. The combination of six variables (Fig. 7b) that showed the same CER than using nine variables was the one excluding TURB, *T*, and TC (CER=11.2 %). Thus, these six variables were the ones responsible of spatial water quality variations for the studied AEs. By measuring six variables (DO, COND, pH, EN, ECL, and FC), we are able to reach the same level of classification error for a water sample collected from one of these AEs. In Fig. 7, the symbols used for La Caldera could not be distinguished since they are mixed with the ones for Vaqueros and Arenales UpS rivers, which is in agreement with the high similarity found in CA between them (Fig. 5).

Seasonal variations

Seasonal analysis through Mann–Whitney *U* test showed that significant differences exist between seasons for some variables (Table 1), which suggested that some variables which weigh more in one season may not impact so much in the other. Therefore, LDA was also performed in order to evaluate the variables responsible for seasonal variability. Standard mode of LDA was applied considering all the nine variables for DS (Fig. 8a) and gave the lowest assignation error rate (5.88 %). Using the reduced mode, a combination of four variables (COND, DO, pH, and FC) resulted to be the one with less error rate (8.82 %), showing that they were the responsible for water quality variations during DS (Fig. 8b).

The same analysis was applied for the dataset during WS. The error using nine variables was 6.52 % for WS (Fig. 8c). A combination of five variables threw the lowest CER (8.70 %), being DO COND, TC, ECL, and EN the ones responsible for water quality changes during WS (Fig. 8d).

These results suggested that during DS, variables responsible for quality variations were different from the ones during WS. Besides that, COND and DO were present in the spatial and also in the seasonal analyses (dry and wet seasons). These variables seemed to be the ones that contribute to spatial and seasonal water quality variations.

During DS, physicochemical variables were the ones that mostly influenced the water quality while microbiological variables were more relevant during WS. This is in agreement with the fact that during WS, runoff contributed to microbial contamination and the people using the AEs for recreational purposes also reflected on bacterial indicators that exceed the limit values in most of the cases (Table 2). On the other side, during DS, physicochemical variables were the ones which most influenced on water quality. This may be because during this season, the lack of rainfall combined with sunny days leads to a decrease of the AEs' volume causing the concentration of some dissolved solids (David et al. 2013; Pesce and Wunderlin 2000) which are usually measured by the increment of conductivity values. On the other side, during DS, water temperature and turbidity decrease, so bacteria that are sensitive to these changes are more prone to inactivation;

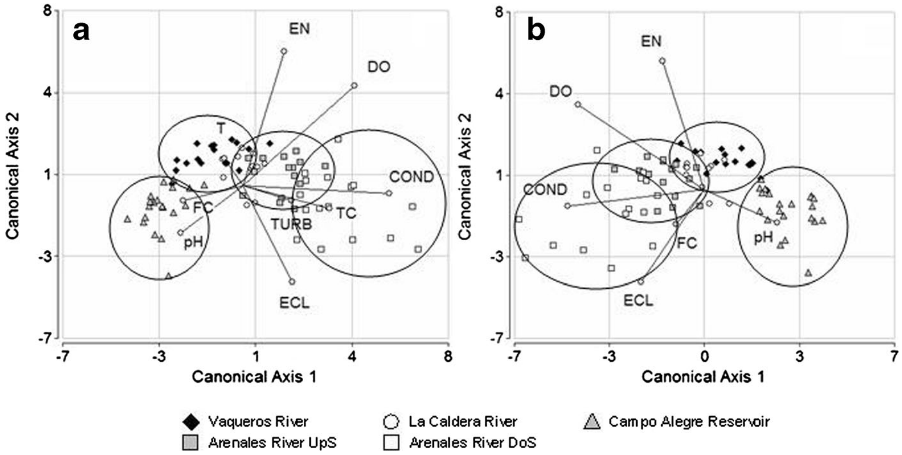

Fig. 7 Biplots of the first two axes obtained with a spatial linear discriminant analysis conducted (**a**) with all the nine variables (*T*, pH, DO, TURB, COND, TC, FC, EN, and ECL) and (**b**) with the combination of six variables (EN, DO, COND, ECL, pH, and FC) with the least classification error rate. La Caldera River values (*white circles*) could not be quite distinguished because they are mixed with Vaqueros and Arenales River UpS plots

therefore, they are not good indicators during this time of year.

As CCA and PCA, results from LDA also showed that both physicochemical and microbiological variables must be measured. Hence, the subgroup of variables obtained from seasonal and spatial analyses gave a combination of both groups, proving their relationship and showing that all these variables should be used to assess

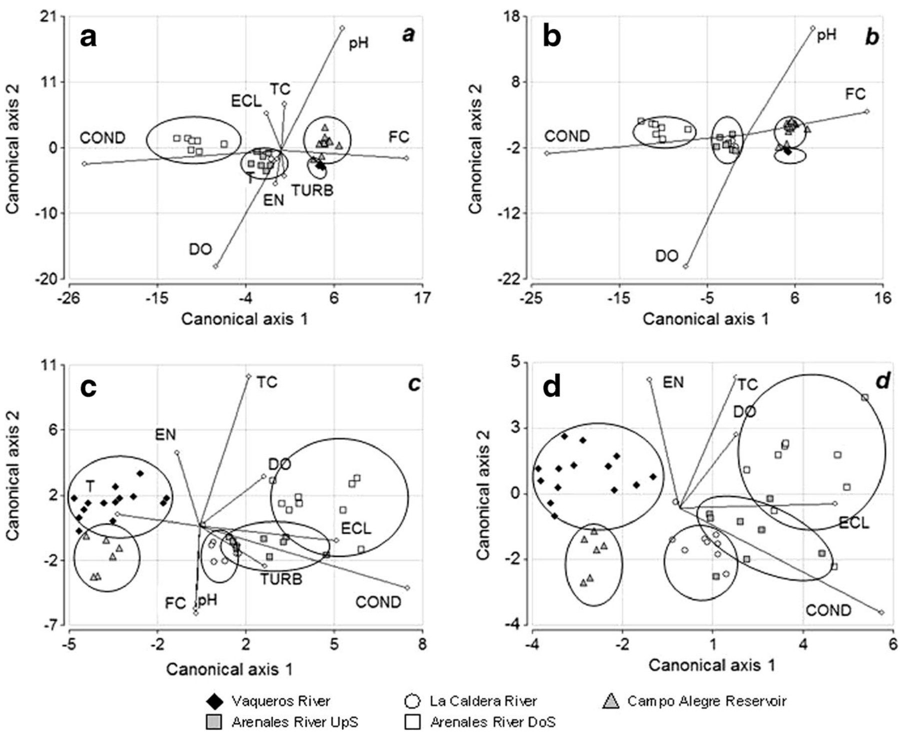

Fig. 8 Biplots of the two first axes obtained with a seasonal linear discriminant analysis during (**a**, **b**) dry season and (**c**, **d**) wet season conducted with **a** and **c**, all the nine variables (*T*, DO, TURB, COND, pH, TC, FC, ECL, and EN) and with the combination of **b** four variables (DO, COND, FC, and pH) and **d** five variables (DO, COND, TC, ECL, and EN) respectively, with the least classification error rate. La Caldera River values (*white circles*) during dry season (**a**, **b**) could not be quite distinguished because they were mixed with Arenales River UpS plots

the quality, regardless of the AE under study. Besides that, individual characteristics could be overlooked when using a reduced group of variables for monitoring purposes. In the case of water microbiology community, a new paradigm is emerging which proposes to utilize different indicator systems depending on the question asked (Yates 2007).

Conclusions

Seasonal analysis showed that significant differences exist between dry and wet seasons for most of the variables measured in each AE studied. This suggested that monitoring will still be important to be performed in both seasons, especially during intense recreational use to protect the health of users. Regarding recreational water quality, 70 % of the samples assessed exceeded at least one of the limit values established by legislation becoming unsuitable for recreational activities. In this sense, Campo Alegre Reservoir presented the best water quality while Arenales River showed the worst. The general poor water quality is of great concern because it can be the cause of waterborne disease outbreaks associated with recreational activities. The high microbial pollution found evidenced that there are sanitation issues to be solved and suggests that other microorganisms, like virus and parasites, may be also present, representing a risk for public health.

CA showed substantial difference in water quality between Arenales River UpS and DoS which were further divided in two AEs. Also, this analysis determined that Campo Alegre Reservoir and Arenales River DoS were the most different environments while La Caldera and Vaqueros River were the most similar ones.

CCA showed that an association between the microbiological and physicochemical variables existed in all AEs except for Arenales River DoS and UpS. In the case of Arenales River, the lack of correlation between both sets of variables was probably due to the fact that it receives waters from many different discharges, being those from the WWTP the most important in terms of flow rate and microbiological load. This may indicate that in a monitoring, both groups of variables will be important to be measured.

These results suggest that it cannot be discarded that differences in biotic compositions may be due to environmental conditions. To what extent abiotic factors control levels of pathogenic bacteria in each particular system is still an open question. Further research on these microbial communities and their relationships with environmental conditions is essential to understand their physiology as well as their ecological role under different conditions.

Results from PCA in each AE showed that it is possible to eliminate some microbiological variables that are strongly related when monitoring different AEs. This situation was different for physicochemical parameters, which correlations did not always exist or were different at each AE.

The loadings of the variables obtained from PCA allowed the determination of potential water pollution sources showing that in Arenales River DoS water quality was mainly affected by anthropogenic activities (industrial and domestic). In case of Arenales River UpS, water quality was mostly influenced by microbiological contamination and due to agricultural activities located upstream. Disturbances on Campo Alegre Reservoir could be due to anthropogenic and ecological effects. Water quality deterioration of La Caldera River and Vaqueros River may be a consequence of recreational activities, which happen constantly during WS.

Through discriminant analysis, we identified a reduced group of variables responsible for seasonal and spatial water quality variation. These results indicate that it is possible to monitor water quality of different AEs using fewer variables, thus optimizing task effort and reducing the costs involved.

Although the lack of background of water quality in the province and also in the country is still an issue to be solved, the application of these multivariate techniques may help in advancing one step in the development of new water monitoring strategies to optimally manage our water resources.

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