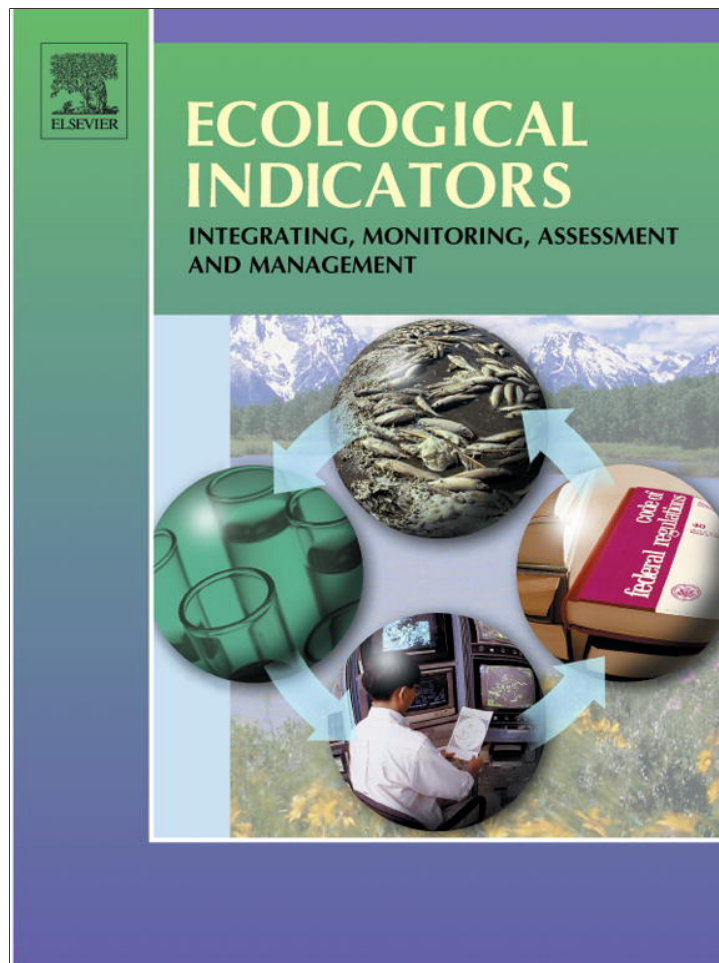


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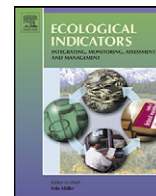
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Fish assemblage changes along a trophic gradient induced by agricultural activities (Santa Lucía, Uruguay)

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ABSTRACT

The input of nutrients into rivers and other aquatic ecosystems is one of the most common causes of damage to the integrity of the ecosystem. The use of biological communities for water quality assessment is a common and effective practice. Among these biological indicators, the fish community is one of the most used in monitoring programs and ecosystems studies and so there is much knowledge about their response to the process of eutrophication. In this study we analyzed the fish composition, diversity and evenness in fourteen reaches, of low stream orders within a basin with differing land use intensity.

The structure of the fish community was related with physiochemical water composition by canonical correspondence analysis, which enabled us to group the fish species according to their specific tolerance to eutrophication. Oligotrophic habitats were characterized by a higher evenness, larger individuals, a fish composition of at least 40% of sensitive species (*Crenicichla scotti*, *Gymnogeophagus gymnogenis*, *Gymnogeophagus* sp., *Heptapterus mustelinus*, *Hoplias malabaricus* and *Rhineloricaria* sp.) and less than 20% of very tolerant ones (*Astyanax fasciatus*, *Cnesterodon decenmaculatus*, *Corydoras paleatus*, *Cyphocharax voga*, *Hisonotus* sp., *Pimelodella australis*). Eutrophic reaches showed the opposite community features and a fish composition with more than 40% of very tolerant species and less than 20% of sensitive ones. Among the fourteen study reaches, four were classified as oligotrophic, six as mesotrophic and four as eutrophic.

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1. Introduction

Eutrophication is a severe and globally widespread problem that causes major environmental, economic and social damages. Land use changes (agriculture, forestry, urbanization, animal production, logging of native forests), result in major global changes that affect lotic systems (Allan and Castillo, 2007). More than a decade ago in 1998, the U.S. Environmental Protection Agency (EPA) reported that 40% of known water quality problems were caused by nutrient enrichment (Justus et al., 2010). Agriculture represents a diffuse source of nutrients and may be considered as the main cause of eutrophication of surface waters (Chambers et al., 2006; Freeman et al., 2007). Several studies have described how agricultural activities are strongly correlated with the increase of sediments and nutrients concentrations in streams (Ahearn et al., 2005; Allan et al., 1997; Chambers et al., 2006; Freeman et al., 2007; Karr and Schlosser, 1978; Strayer et al., 2003). In 2006, the U.S. Geological

Survey estimated that rivers in agricultural watersheds transport a high percentage of the nitrogen and phosphorus annually applied to crops (more than 50% and 20% respectively) (Justus et al., 2010). The continuing degradation of river ecosystems highlights the need and importance of monitoring these areas.

The use of biological communities for water quality assessment is a common and effective practice (Justus et al., 2010), that has been increasingly used since the middle of the last century (Hawkes, 1979; Giller and Malmqvist, 1998; Oberdorff et al., 2001; Rosenberg and Resh, 1993; Stainbrook et al., 2006). The presence of a “balanced” community in an aquatic ecosystem is an excellent indicator of low anthropogenic impacts (Araujo, 1998). While the use of benthic macroinvertebrates has been most widely used and studied, other studies have shown that the fish assemblage is particularly sensitive to different types of anthropogenic impacts (Adam and Bailey, 2011; Araujo, 1998; Bistoni et al., 1999; Brown, 2000; Harris and Silveira, 1999; Wolter et al., 2000) and may be used as bioindicators.

The use of fish communities as indicators of ecological integrity was first proposed by Karr (1981) and has since been frequently used worldwide (Adam and Bailey, 2011). Among the advantages of using the fish community as bioindicators are their ease of identification, their generally known life history, and the volume of

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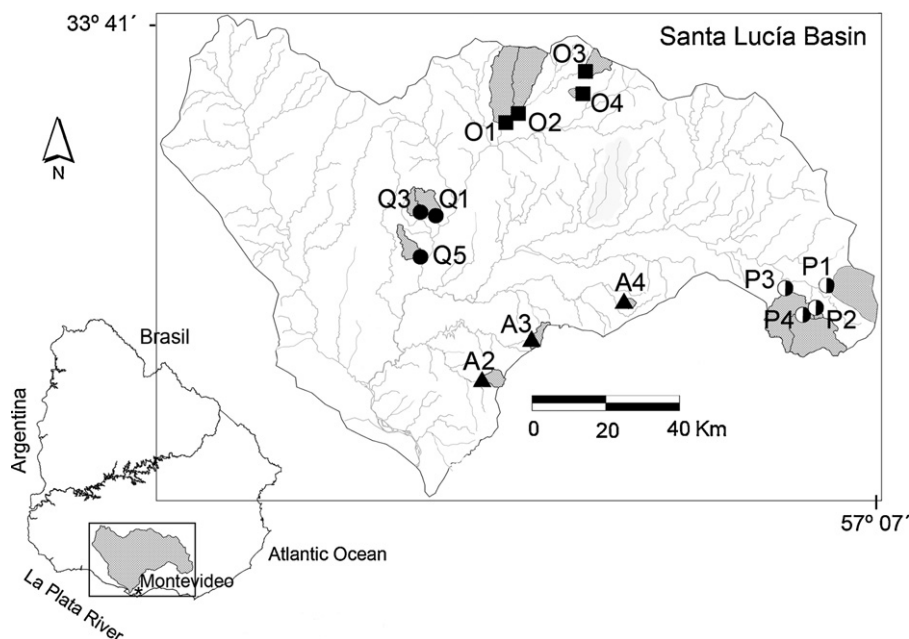


Fig. 1. Location of the Santa Lucía basin in Uruguay and detail of the study basins in each region indicated by letters.

accumulated knowledge about their trophic level and other ecological requirements (Araujo, 1998; Oberdorff et al., 2001). Moreover, since they may be located at the top of the food web, they allow us to have an integrated vision of the aquatic environment as a whole (Araujo, 1998; Oberdorff et al., 2001). This integration of information can also be considered in an ample spatial scale due to the high mobility of these organisms (Oberdorff et al., 2001).

Fish species differ in their tolerance to water quality changes which permits the creation of categories of species according to their ability to live in degraded environments (Araujo, 1998). In general, the first species to disappear are those with more specific diets, such as carnivores and insectivores (Harris and Silveira, 1999), and/or those without mechanisms to tolerate low oxygen levels. In highly degraded environments, omnivorous species are usually found along those with air-breathing mechanisms, such as Cyprinodontiformes, resulting in a more simple and hierarchical community (Bistoni et al., 1999).

Biological criteria are valuable for assessing human alterations to ecological integrity because they directly measure the conditions of the threatened resource, they detect the problems that other methods may miss or underestimate and provide a systematic process for measuring progress resulting from the implementation of water quality programs (Karr, 1991).

The aim of this study is to classify the most common fishes in the Santa Lucía basin according to their tolerance to water quality changes and to develop new biological indicators of environmental impairment (erosion, eutrophication). We also aimed to group the stream reaches according to their fish community and physiochemical composition and to add new biological criteria for the evaluation of the ecological integrity of aquatic ecosystems.

2. Materials and methods

2.1. Study area

This study was carried out in the Santa Lucía river basin, located in the south of Uruguay (33°41'–34°51'S; 54°59'–57°7'W, Fig. 1). It has an area of 13,310 km², a maximal altitude of 250 m above sea level and drains into the estuary of the Rio de la Plata. Although

only 9% of Uruguay's population live in the basin (ca. 300,000 inhabitants), it provides drinking water for the two million inhabitants of the metropolitan area of Montevideo.

Previous studies defined three main geological and landscape regions; the meta-morphic eastern hills, the crystalline north plain and the sedimentary south Plate plain and four main uses within the basin (Achkar et al., 2004; Arocena et al., 2008). The dairy farming is the main land use and economic activity, occupying 64% of the basin area. It is followed by intensive and extensive breeding of cattle for meat (7 and 17% respectively) and horticulture (7%), restricted to the metropolitan area of Montevideo city (Arocena et al., 2008). Many water quality problems in the basin have been addressed in the past, including increased sediment loads and the eutrophication of streams and reservoirs (Arocena et al., 2008). Chalar et al. (2011) carried out a study with the aim to assess the impairment of the water resources in the Santa Lucía basin, caused by the process of eutrophication. The study was based on the assemblage of the benthic macroinvertebrates and consisted in the diagnosis of 28 stream reaches covering the main regions of the landscape and land uses. One of the main results of the study was the positive relationship between the intensity of land use (% cultured areas), estimated by satellite images and the trophic state of stream reaches.

Considering these antecedents and due to logistic limitations we selected a sub sample of previous study reaches for the present survey. Fourteen stream reaches were selected in four regions of the Santa Lucía basin covering a wide range of land use intensity and all landscape regions. Region A corresponded to the sedimentary south plain dominated by dairy cattle production, region O was characterized by the crystalline south plain with extensive cattle breeding, region P was dominated by metamorphic eastern hills and extensive cattle breeding and region Q, in the north, had prevailing crystalline rocks and dairy cattle production (Fig. 1, Table 1). It is important to note that dairy is usually combined with crop production to meet the nutritional needs of cattle.

The choice of the study reaches in each sub basin was based on the availability of permissions and accessibility to the water courses and the absence of direct impacts or physical modifications of the channel (dams, channel rectifications). Selected reaches corresponded to permanent and wadeable stream sections of order

Table 1
Some important features of the study streams reaches at basin and reach scale (Ext. cattle = extensive cattle breeding). Percentage of crops and natural prairie from Bartesaghi and Achkar (2008).

Basin scale						Reach scale	
Study area	Area (km ²)	Geology	Main land use	Crops (%)	Natural prairie (%)	Depth (m) mean/max	Mean wet width (m)
A2	9	Sedimentary	Dairy cattle	60	36	0.5/0.9	2
A3	14	Sedimentary	Dairy cattle	61	19	0.6/1.2	3
A4	6	Sedimentary	Dairy cattle	67	29	0.4/1.0	2
O1	117	Crystalline	Ext. cattle	3	70	0.3/0.7	4
O2	100	Crystalline	Ext. cattle	5	80	0.4/0.7	5
O3	33	Crystalline	Ext. cattle	12	82	0.4/0.8	4
O4	6	Crystalline	Ext. cattle	1	89	0.3/1.0	2
P1	121	Metamorphic	Ext. cattle	1	69	0.4/0.9	6
P2	6	Metamorphic	Ext. cattle	16	67	0.2/0.5	2
P3	15	Metamorphic	Ext. cattle	11	65	0.3/1.0	3
P4	32	Metamorphic	Ext. cattle	16	69	0.3/0.7	3
Q1	35	Crystalline	Dairy cattle	66	30	0.3/0.5	3
Q3	18	Crystalline	Dairy cattle	52	42	0.6/1.0	8
Q5	30	Crystalline	Dairy cattle	74	18	0.6/1.0	9

2–4. Most of them exhibited less than 5 m of wet width and a less than 0.6 m of mean depth, riparian vegetation was scarce in most of the reaches (Table 1).

2.2. Fish sampling

One sampling survey was carried out in the 14 selected reaches between October 7th and 9th, 2007, under average to low water level and flow velocities conditions. Each stream reach was defined as 50 m long, fish sampling was performed by electro-fishing (Sachs team Elektrofishgeräte, 400 V backpack generator, GmbH (Type FEG 1000)). This technique is quite efficient for small streams (Dauwalter and Pert, 2003; Garner, 1996). In order to cover for habitat heterogeneity (pools, riffles, vegetated areas, substrate types), 10 transects regularly distributed along the reaches were sampled. In each of these 10 transects 3 current pulses (5 s each) were applied, one in the center and the other two at both margins of the stream. The samples collected in each pulse were integrated in a single sample by reach; resulting in a sampling effort of 30 electric pluses of 5 s each. It is important to note that the methodology used, did not aim to explain the intra-reach heterogeneity, but to obtain a representative sample of the fish assemblage of the reach. The collected fishes were euthanized with an overdose of anesthetic (3 Phenoxyethanol) and preserved with a solution of 10% formalin. Fish were then identified to the lowest taxonomic level possible, using scientific keys available for the different groups measured (0.1 cm) and weighed (0.1 g). Shannon evenness and diversity index were estimated on abundance basis (Shannon and Weaver, 1949). Rare species, captured in only one or two reaches were excluded from the study.

2.3. Environmental variables

Four samplings of environmental conditions were carried out the 14 reaches of the Santa Lucía basin during average to low water level and flow velocities conditions (December 2006, March, July and November 2007). Dissolved oxygen (DO) and conductivity were measured in situ at three points (upstream reach, middle reach and downstream reach), with Horiba probes (D-24 and D-25 models). In each sampling reach an integrated water sample of the three points was taken for analysis of nutrients (soluble reactive phosphorus (SRP), ammonium (NH₄), nitrate (NO₃), total nitrogen (TN), total phosphorus (TP), and total suspended solids (TSS)). For details on the analytical methods used see Chalar et al. (2011). The size of material on the bed of the study reaches was estimated during periods of low water, walking through the entire reach and

visually determining the proportion of silt (soft and fine <0.64 mm), sand (2–0.064 mm) and gravel (>2 mm), every few steps in at least 30 points per reach (Hauer and Lamberti, 2006).

Since fish species integrate the conditions of the habitat they live over the time and as they can move considerable distances, they are less affected by circumstantial environmental conditions. Therefore, we decided to use the mean values of the four seasonal samplings of all environmental variables, instead of the data obtained just at the moment of fish sampling.

2.4. Statistical analyses

Linear correlations between paired environmental variables were calculated by the non-parametric statistical methods of Spearman Rank Order Correlations. The data matrix was composed of 24 biological variables (55% of the species matrix was composed of zeros), 5 environmental variables (gravel content, DO, TSS, TP and NH₄) and 14 observations (reaches). First a DCA was run with CANOCO program version 4.5 (ter Braak and Smilauer, 2002), based on the logarithmic transformed abundance data in order to assess the length of the gradient. If the gradient length with the first axis is more than 2 standard deviations (as it was in this study = 2.034), an unimodal response model is considered suitable and then a CCA is recommended (Jongman et al., 1995). The Variance Inflation Factors (VIFs) of the selected variables were examined, with all of them being lower than 3. Peeters et al. (2004), recommended to include in the analyses those variables with VIFs < 20. A CCA was then run to explore the relationships between the composition of fish communities and the environmental variables. The different sampling reaches sorted along an axis may be associated with an environmental gradient defined by the variables best correlated with the axis (Jongman et al., 1995; Legendre, 2003). Also it was explored the variation explained by the first two eigenvalues of the environmentally constrained method of CCA (0.43), with that of the unconstrained DCA (0.47), concluding that no important environmental variable was missing (Jongman et al., 1995). A cluster analysis based on the species scores of the first and the second axis of the CCA, and using the Euclidian Distance and the Ward's method as amalgamation technique, was run in order to group the collected fishes according to their tolerance to eutrophication (Ward, 1963; StatSoft, Inc., 2007). Significant differences among the three trophic groups of streams were estimated by Kruskal–Wallis test (*H*). When no significant differences were found using this multiple comparison test, a paired group, Mann–Whitney *U* test, was run. These analyses were run with Statistica version 8.0 (StatSoft, Inc., 2007)

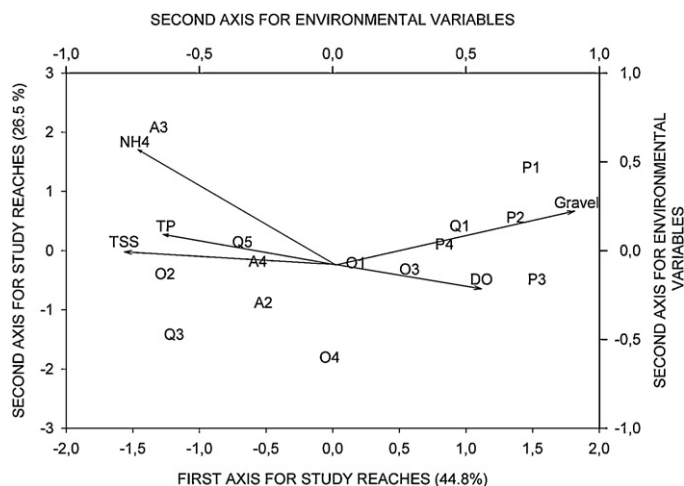


Fig. 2. The position of study reaches and environmental variables respect to the first two axes of CCA. Variables are represented by an arrow and their abbreviation: gravel, gravel content; DO, dissolved oxygen; TP, total phosphorus; TSS, total suspended solids and NH₄, ammonium. The length of the arrow is a measure of the importance of the variable and the arrowhead points at the direction of increasing influence.

3. Results

3.1. Fish community structure

A total of 34 species were determined within the Santa Lucía basin but only 23 species were present in more than 2 sampling reaches and therefore included in this study. The mean specific richness was 10 with a minimum of 7 species at P1 and O4 (upper reaches) and a maximum of 16 at Q5. Mean fish abundance per sampling effort (SE-30 electric pulses of 5 s each), was 77 (variation coefficient = 80%), with a minimal value of 18 at O2 and a maximum of 234 ind SE⁻¹ at A3. Mean fish biomass was 249 g SE⁻¹ (variation coefficient = 57%), and was minimal at O4 (57 g SE⁻¹) and maximal at Q1 (556 g). Mean Shannon diversity was 2.6 bits ind⁻¹ (variation coefficient = 15%) and mean evenness was 0.79 (variation coefficient = 15%). Sampling reach P3 had the highest Shannon diversity and evenness (3.3 bits ind⁻¹ and 0.95, respectively) while A3 had the minimum values (2.0 bits ind⁻¹ and 0.65, respectively).

3.2. Canonical correspondence analysis (CCA)

The first eigenvalue represented an important gradient (0.274), while the second one was much weaker (0.162). The amount of the total variation explained by the environmental variation reached 61.1%. The first axis explained 21.6% of the total variation (total inertia) in the species data while the second explained 12.8%. Also, the first axis accounted for 44.8% of the total variation that could be explained (explainable inertia) by the species-environment relation while the second explained another 26.5%. The gravel content of the sediments showed the highest positive correlation with the first axis (0.91), followed by the DO (0.56), while the TSS showed the highest negative correlation with the first axis (-0.78), followed by the NH₄ concentration (-0.74), and TP concentration (-0.63) (Fig. 2). Ammonium was the environmental variable with the highest correlation with the second axis (0.61), followed distantly by gravel content (0.27), DO (-0.17), TP (0.14) and TSS (0.05). The TP concentration was also positively and significantly correlated with SRP, TN, TSS and conductivity. From these data we infer that the first axis represents a gradient of environmental degradation (eutrophication gradient). The species scores of the study reaches were arranged along this axis according to the specific fish tolerance to aquatic eutrophication. The more tolerant

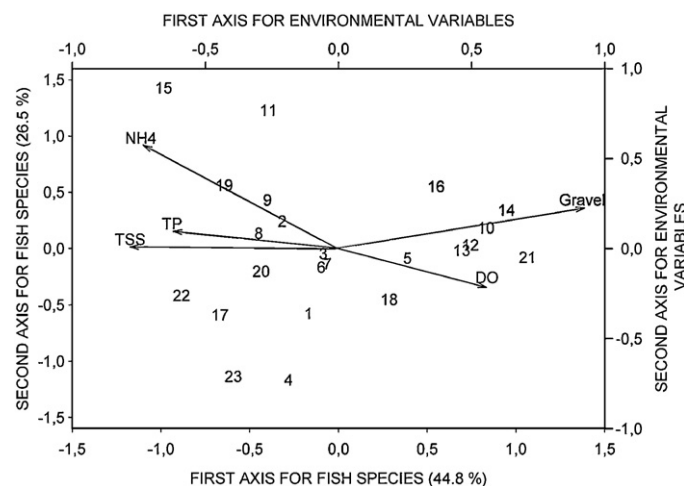


Fig. 3. The position of the fish species and environmental variables respect to the first two axes of CCA. Variables are represented by an arrow and their abbreviation in the case of: DO, dissolved oxygen; TP, total phosphorus; TSS, total suspended solids and NH₄, ammonium. The length of the arrow is a measure of the importance of the variable and the arrowhead points at the direction of increasing influence. Taxa are represented by numbers: 1 – *Astyanax eigenmaniorum*, 2 – *Astyanax fasciatus*, 3 – *Austaloheros scitulus*, 4 – *Australoheros fascetus*, 5 – *Bryconamericus iheringii*, 6 – *Characidium rachovii*, 7 – *Cheirodon interruptus*, 8 – *Cnesterodon decemmaculatus*, 9 – *Corydoras paleatus*, 10 – *Crenicichla scotti*, 11 – *Cyphocharax voga*, 12 – *Gymnogeophagus gymnogenis*, 13 – *Gymnogeophagus* sp., 14 – *Heptapterus mustelinus*, 15 – *Hisonotus* sp., 16 – *Hoplias malabaricus*, 17 – *Hyphessobrycon meridionalis*, 18 – *Hyphessobrycon uruguayensis*, 19 – *Pimelodella australis*, 20 – *Pseudocorynopoma doriae*, 21 – *Rhineloricaria* sp., 22 – *Steinacherina biornata*, 23 – *Synbranchus marmoratus*.

were located at the negative end of the first axis and the more sensitive species at its positive end (Fig. 3).

3.3. Fish tolerance and study stream classification

The cluster analysis exclusively based on the species scores along the first and the second axes of the CCA identified three main groups with more than 58% of similarity (Fig. 4). Group 1 was composed of the fish species more sensitive to habitat alterations and water quality, the third group corresponded to the most tolerant species, and the second group was integrated by ubiquitous species (Fig. 4).

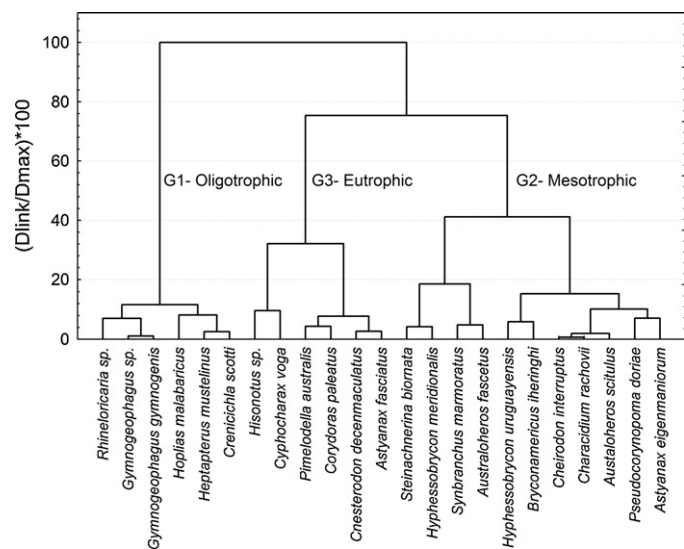


Fig. 4. Cluster analysis of fish species based on the first two axes of the CCA. G1 – sensitive species, G3 – tolerant species and G2 – ubiquitous species.

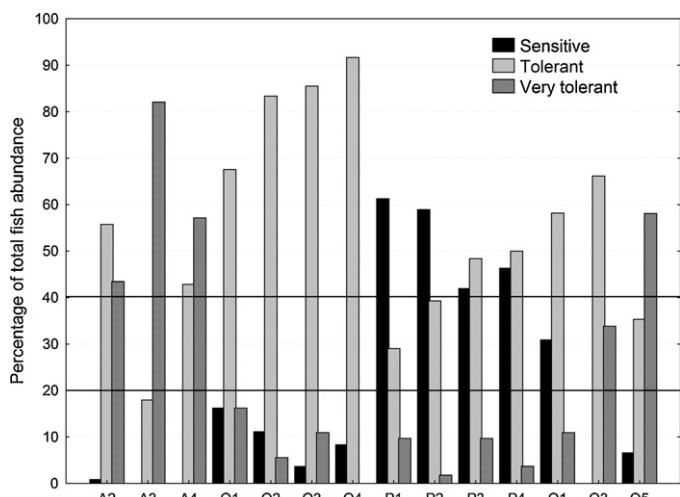


Fig. 5. Fish tolerance categories composition of study reaches. The eutrophic stream reaches show an abundance of tolerant species greater than 40% and less than 20% of sensible species, while the oligotrophic show the opposite composition.

Once we have classified the fish species according to their tolerance, we can explore the assemblages of each stream reach. According to Fig. 5, oligotrophic reaches (P1–P4), showed a community structure with more than 40% of sensitive species and less than 20% of very tolerant ones. In contrast, eutrophic reaches (A2–A4 and Q5) were dominated by very tolerant species (>40%) and sensitive species were poorly represented (less than 20%). In the middle of these extreme classes, mesotrophic conditions were prevalent.

Similar groups were formed when we ran a cluster analysis based on the relative composition of sensitive and very tolerant fish species. Oligotrophic reaches showed lower cultivated area, lower concentrations of nutrients (TN, SRP and TP), conductivity and TSS and higher DO concentration than eutrophic reaches (Table 2). The evenness of the fish communities, and the ratios of richness/abundance and biomass/abundance were higher in oligotrophic reaches than in eutrophic ones, while fish abundance was higher in eutrophic reaches (Table 2). All of the mentioned differences were significant according to Kruskal–Wallis test (*H*) or Mann–Whitney test *U*-test ($p < 0.05$).

Table 2
Emergent properties of fish assemblages, crop production within the basin and physicochemical features of the defined trophic groups of study reaches. Kruskal–Wallis (*H*) and Mann–Whitney (*U*) tests for significant differences between trophic groups: E, eutrophic; M, mesotrophic; O, oligotrophic. NS, not significant differences.

	Eutrophic Mean (range)	Mesotrophic Mean (range)	Oligotrophic Mean (range)	Significant differences ($p < 0.05$)
Abundance (ind SE ⁻¹)	157 (105–234)	40 (18–68)	51 (31–62)	H:E ≠ M; U:E ≠ O
Biomass (g)	280 (160–432)	232 (57–556)	245 (172–389)	NS
Richness (species number)	12 (11–16)	10 (7–11)	9 (7–11)	NS
Richness/abundance	0.1 (0.05–0.1)	0.3 (0.2–0.4)	0.2 (0.1–0.2)	H:E ≠ M; U:E ≠ O
Biomass/abundance	1.8 (1.4–2.4)	5.9 (1.6–11.1)	5.7 (2.9–12.5)	U:E ≠ M; E ≠ O
Shannon diversity (bits ind ⁻¹)	2.3 (2.0–2.5)	2.7 (2.3–3.1)	2.7 (2.3–3.3)	U:E ≠ M
Evenness	0.6 (0.6–0.7)	0.8 (0.8–0.9)	0.8 (0.8–0.9)	H:E ≠ M; E ≠ O
Crop area (%)	70 (63–76)	25 (1–67)	12 (1–17)	U:E ≠ O; U:M ≠ O
Gravel content (%)	13 (0–25)	30 (0–75)	94 (75–100)	H:E ≠ O; U:M ≠ O
Dissolved oxygen (mg/l)	7.3 (6.6–8.3)	8.3 (6.9–10.2)	9.6 (8.4–10.4)	H:E ≠ O
Conductivity (mS/cm)	0.9 (0.6–1.2)	0.4 (0.3–0.6)	0.2 (0.2–0.3)	H:E ≠ O; U:E ≠ M; M:E ≠ M
Total suspended solids (mg/l)	12 (8–16)	10 (4–23)	5 (3–6)	H:E ≠ O
NO ₃ (μg/l)	109 (74–158)	223 (58–798)	60 (30–111)	NS
NH ₄ (μg/l)	58 (15–165)	15 (6–21)	10 (3–16)	NS
Total nitrogen (mg/l)	3.8 (2.6–5.4)	3.4 (2.5–6.4)	2.1 (1.1–3.2)	U:E ≠ O
Soluble reactive phosphorus (μg/l)	172 (10–333)	93 (8–262)	11 (6–14)	H:E ≠ O
Total phosphorus (μg/l)	273 (159–502)	144 (19–361)	36 (17–77)	H:E ≠ O

4. Discussion

Fishes are considered to be good indicators of the ecological status of aquatic ecosystems. This is due to their ecological characteristics, such as living in the water all their life, occupying a wide range of ecological habitats and move in a variety of spatial scales (Ibáñez et al., 2010). Moreover, their life cycles are long, so that environmental conditions can be integrated in time and space. On the contrary, physical and chemical measurements are descriptors of the environmental conditions just at the moment of sampling. For these reasons we considered that the averaged values of the physical and chemical parameters registered seasonally are better related with fish assemblage. This relationship can be evaluated in the percentage of the total variation explained by the environmental variables in the canonical correspondence analysis. This value was in our study 61.1%, which can be considering as a very reasonable amount and equal to the value that could be obtained by unconstrained methods (e.g. correspondence analysis, data not shown).

The environmental gradient we determined was related to the eutrophication occurring in the Santa Lucía basin, which has profoundly altered the physiochemical and nutrient water composition of the more impaired basins (Chalar et al., 2011). This was supported by the significant differences found among the trophic categories of most of the environmental variables measured in this study. The oligotrophic reaches corresponded to basins dedicated to extensive cattle breeding, located in the metamorphic eastern hills. This practice based on natural prairies or with low fertilization rates, dominates in this kind of geology where superficial stony soils limits other uses and represents the activity with the lowest impact to aquatic ecosystems. The eutrophic reaches corresponded to basins dominated by intensive crop production and dairy, located mainly in the sedimentary south plain (A) and one reach in the crystalline rock geology (Q5). This is in agreement with the studies of soil erosion that indicates the region A, as one of the zones of the country with the highest rates of erosion induced by anthropogenic activities (DGRNR, 1998).

The fish species determined in the study reaches were arranged by canonical correspondence analysis along a strong environmental gradient, showing specific fish abilities and preferences for living in particular environmental conditions. The different performance of each fish species has being used to determine the specific tolerance to several water quality variables and asses ecosystem conditions, biotic integrity or ecosystem integrity (Ibáñez et al., 2010; Karr and Dudley, 1981; Meador and Carlisle, 2007; Meador et al., 2008).

The emergent properties of fish community like evenness and the ratios richness/abundance and biomass/abundance were also affected by eutrophication, showing lower values in eutrophic reaches. In eutrophic fish communities there was a more hierarchical distribution of total abundance, and total biomass was distributed in more and smaller individuals. The total number of fish species estimated in the present study (34), was high when compared with the mean ichthyologic richness (25 species) of the Pampa bonaerense province (Ringuelet, 1975) and to the total number of species (27), determined by Di Marzio et al. (2003) in Las Flores stream of the same province. The richness values observed in this work are similar to the observed for low order streams of Uruguay (between 14 and 32 species), using Multi Pass electrofishing sampling in 50 m reaches (Teixeira-de Mello et al., 2012).

The classification of fish species, according to their tolerance to environmental changes proposed in this study, agrees with previous observations made in the region. In this sense, the classification of *Rineloricaria* sp. as a species sensitive to anthropogenic impact agrees with the observations of Hued and Bistoni (2005) in Cordoba (Argentina) lowland streams, and the sensitive nature of *Heptapterus mustelinus* is consistent with that observed by Teixeira-de Mello, 2007, in small reaches of the lower Santa Lucia basin.

Additionally, *Cyphcharax voga*, classified as tolerant in this study, was observed in high abundances at impaired, eutrophic sites (Teixeira de Mello et al., 2011). In the same way, *Cnesterodon decenmaculatus* has already been reported as a pollution tolerant species by Hued and Bistoni (2005) and Teixeira-de Mello, 2007, and the tolerant natures of *Corydoras paleatus* and *Otocinclus* sp. are consistent with the findings of Hued and Bistoni (2005) and Teixeira-de Mello, 2007. However, *Rineloricaria* sp., *Gymnogeophagus* sp. and *Hoplias malabaricus* were grouped as sensitive species in the present study, different from the pattern observed by Teixeira-de Mello, 2007, in a sub-urban stream. The divergence could be related with the different kind of impairment in both studied systems, associated with the use of the basin (Teixeira-de Mello, 2007, urban – industrial; this study, agriculture), and shows the need of more biological and ecological studies of fishes.

The sensitive species group representing oligotrophic conditions was related with coarse sediments, low nutrient concentration and high oxygen concentrations. According to our own experience and that of some other authors in the region this group mainly contains fishes that have a large dependence on the substrate type. *Rineloricaria* sp. and *Gymnogeophagus gymnogenis* prefer sandy or fine gravel bottom streams and *H. mustelinus* largely prefers coarse gravel and rocky bottom sediments (Gonzalez-Bergonzoni et al., 2009; Teixeira de Mello et al., 2011). Additionally, most of the species classified as sensible are carnivorous, which agrees with the results of Harris and Silveira (1999).

In contrast to this group the tolerant species were found related to high TSS and nutrient concentrations as well as low oxygen conditions. This feature may be explained by the impact of soil erosion and the siltation process that changes the natural stream bottom composition and represents an important selection factor for fish species. In this group, the tolerant *C. voga*, a toothless detritivorous species, could be favored by the higher organic matter content sedimented in the streams of eutrophic sites. Previous studies have pointed out that the discrimination between good quality fish indicators and those tolerant to anthropogenic impacts in pasture agro-ecosystems results mainly from species' capacity for adaptation to siltation and hypoxia (Casatti et al., 2006; Meador and Carlisle, 2007). Our data are in agreement with these observations. The tolerant species *Otocinclus* sp. and *C. paleatus* have specialized mechanisms to breathe from the atmosphere (Nelson, 2006), while *C. decenmaculatus* is a water surface dweller adapted to breathe from the water/atmosphere interface and thus is resistant to hypoxia (Tagliani et al., 1992). Additionally it should be noted

that most of the eutrophication tolerant species in this study are omnivorous or detritivorous and thus could benefit from higher detritus, periphyton and macrophyte availability. This also agrees with the observation by Bistoni et al. (1999) about omnivorous fish being more commonly eutrophication tolerant.

The ecological integrity has been defined as a useful concept for addressing the global environmental crisis and as a guiding principle for sustainability. Ecological integrity is based on a holistic approach for the protection and conservation of watersheds that implies not only the biological, chemical, and physical bodies of water, but also the functional attributes of watersheds, as hydroecology, geomorphology, and natural disturbance patterns. Human activities within the watershed disrupt the natural balance among the ecosystem compartments and processes, leading to the loss of biodiversity, eutrophication and the deterioration of water quality. We showed how the fish community features (abundance, composition, species diversity, evenness), were related to the eutrophication process and the intensity of land use (% of crop area). Also, similar results were found in this region for the benthic community (Chalar et al., 2011). Now we are reinforcing the arguments for the use of biological criteria in the assessment of ecological integrity of streams, including the structure and assemblage of the fish community. Future studies on the physical structure of the channel, the status of the riparian zone and the condition of the hydrological regime, will be welcome to construct a holistic view of river ecological integrity.

5. Conclusions

The structure of the fish communities reflected the trophic status of the study reaches: oligotrophic habitats were characterized by a higher evenness, bigger individuals and a fish composition of at least 40% of sensitive species (*Crenicichla scotti*, *G. gymnogenis*, *Gymnogeophagus* sp., *H. mustelinus*, *H. malabaricus* and *Rineloricaria* sp.) and less than 20% of very tolerant ones (*Astyanax fasciatus*, *C. decenmaculatus*, *C. paleatus*, *C. voga*, *Hisonotus* sp., *Pimelodella australis*). Eutrophic reaches showed the opposite community features and a fish composition with more than 40% of very tolerant species and less than 20% of sensitive ones. Among the fourteen study reaches, four were classified as oligotrophic, six as mesotrophic and four were eutrophic.

Some disagreements between the tolerance assigned to a particular species of fish in previous studies and our own classification were found. This remarks the advantage to use the fish assemblage as a whole instead of indicator species to assess the degree of impairment of fluvial ecosystems.

Our data showed a strong relationship of the more impaired streams with a higher intensity of agricultural activity in the basins (crop production and dairy). These land use were associated with low oxygen concentration and higher conductivity, suspended solids, total nitrogen and phosphorus. We conclude that the use of fish assemblage composition is a valuable tool for stream ecosystem assessment and should be incorporated in national monitoring programs.

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