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Trophic assessment of streams in Uruguay: A Trophic State Index for Benthic Invertebrates (TSI-BI)

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ABSTRACT

Human activities are radically changing natural land cover and increasing the delivery of soil, organic compounds, nutrients, toxic agrochemicals and other contaminants to aquatic ecosystems. The eutrophication of streams, rivers, lakes, reservoirs and costal zones is one of the most important consequences of human activities. In this study we assessed the trophic status of 28 wadeable stream reaches of the Santa Lucía basin, an important economic region of Uruguay. We developed a Trophic State Index of Benthic Invertebrates (TSI-BI), the first of its kind for South American lotic systems. The methodological approach consisted of determining the ambient trophic gradient via canonical correspondence analysis based on the benthic invertebrate abundance matrix and an environmental variable matrix. The rescaled site scores served as environmental variables in the weighted averaging model (WA), to weight the benthic abundances and then find the optimum and tolerance of each of the sampled genus. These data were used to estimate the TSI-BI scores. These scores, in conjunction with the total phosphorus concentrations (TP), were used to group the study reaches when running a cluster analysis. The basic statistical parameters of the defined groups serve as an input to identify the threshold values of TP and TSI-BI corresponding with the different trophic states. The boundaries of TSI-BI and TP demarcating mesotrophic and eutrophic states were 8 and 71 μg/l, respectively, and can be considered the limits between impaired and less altered reaches. The results also indicated that the trophic status of the reaches is related to land use intensity. A change in land use management seems to be critical for the preservation of one of the most important water supply systems in Uruguay.

1. Introduction

Human population growth and the ever-increasing demand for consumer goods have radically changed natural ecosystems by land clearing, agriculture, forestry, animal husbandry and urbanization. At the same time, the amount of available nutrients delivered to the biosphere has experienced an extraordinary increase. Considering the global production of agricultural fertilizers alone, the annual production of reactive nitrogen increased from ca. 15×10^6 teragrams (Tg) in 1860 to 187×10^6 Tg in 2005 (Galloway et al., 2008). Phosphorus fertilizer production shows a similar trend. According to Cordell et al. (2009), the addition of phosphorus to agricultural soils increased from ~2 Tg/year in 1900 to ~20 Tg/year in 2005. This has more than doubled P inputs to the environment over natural background P from weathering (Seitzinger et al., 2005; Bennett et al., 2001). Increased nutrient inputs to aquatic ecosystems cause a series of environmental, social and economic damage that may be covered by the concept of eutrophication.

The process of eutrophication has long been studied in lakes and reservoirs (Vollenweider, 1968; OECD, 1982). This has allowed the development of numerous eutrophication models and the categorization of these environments according with their trophic states. Traditionally, trophic categories are related with the values of primary productivity, biomass of primary producers and the nutrient load and/or the nutrient concentration in the water column (OECD, 1982). The theoretical background accumulated around the eutrophication process and trophic state classifications has an enormous importance for many practical purposes related with water use, watershed management and ecosystem restoration.

Although trophic state river classification based on autotrophic production can be traced far back in the past (Odum, 1956), the question of how nutrients are linked with the trophic state and how the trophic state may reveal system properties is much more recent, and essential to optimal management of fluvial ecosystems (Dodds, 2007).

The eutrophication of rivers is considered to be a dominant source of water quality impairment in the United States (USEPA,

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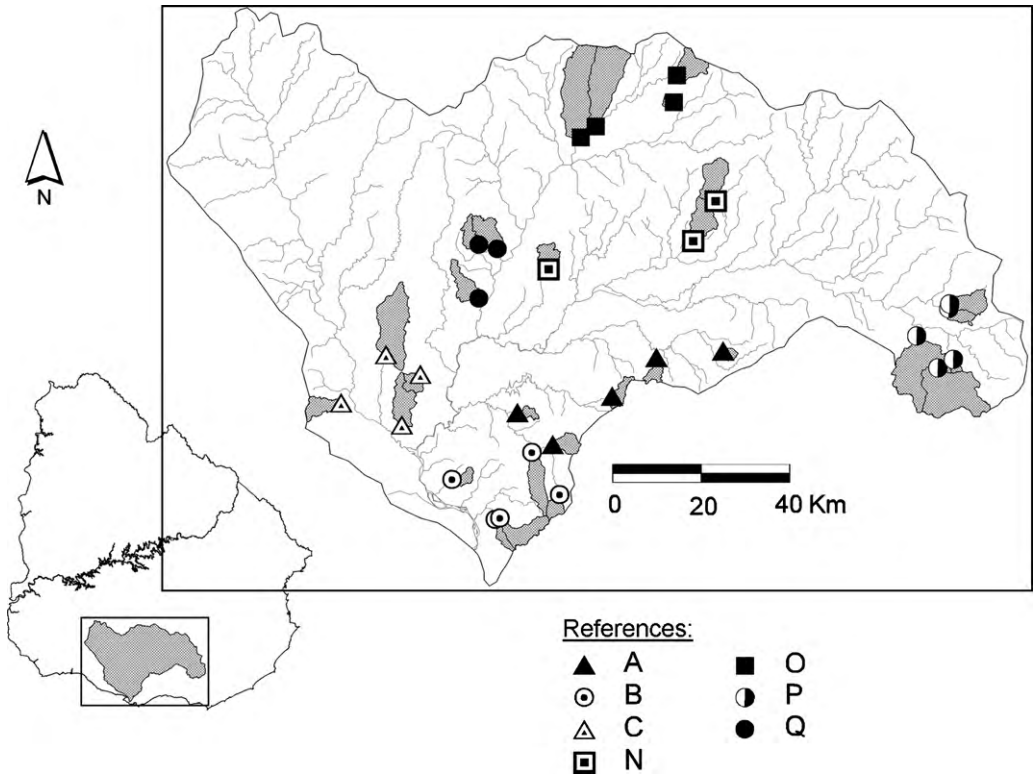

Fig. 1. Location of the Santa Lucia basin in Uruguay and detail of the study basins in each region indicated by letters (see Section 2.1).

2000; Reckhow et al., 2005), in the European Community (Drolc and Zagorc Koncan, 2002), and in general for most of the freshwater and coastal marine ecosystems in the world (Smith and Schindler, 2009). Nutrient enrichment produces excessive algal growth, which in turn adversely affects stream animal communities (Nordin, 1985). The most common ecological changes in stream invertebrates caused by eutrophication include the increased biomass (Bourassa and Cattaneo, 1998), the shift of macroinvertebrates from sensitive to more tolerant species (Allan, 2004; Chambers et al., 2006) and biodiversity losses (Nijboer and Verdonschot, 2004).

Benthic macroinvertebrate communities have been used extensively to assess overall water quality and stream health status. This is due to their high predictability response to different kinds of natural and anthropogenic stresses (Pearson and Rosenberg, 1978; Weisberg et al., 1997) and to many features that make them helpful as biological indicators (Rosenberg and Resh, 1993; Growns et al., 1997). In particular, it has been noted that different taxa show variation in their ranges of tolerance to nutrient levels (Beketov, 2004). This feature was explored to determine the pollution tolerance of each taxon by means of different methodological approaches to construct indices for the evaluation of diverse human impacts on water ecosystems (Word, 1978, 1980, 1990; Smith et al., 2001, 2007). Currently there is a growing trend towards the use of multimetric and multivariate indices developed from biotic and environmental properties of the ecosystems. These may be considered to evaluate the overall status of the ecosystems and as a measure of the ecological integrity of streams.

The aim of this study was to develop a Trophic State Index for Benthic Invertebrates (TSI-BI), to assess the trophic status of wadeable stream reaches and to present the associated optimal scores and tolerance for the main macroinvertebrate genera of the region. We also aimed to determine threshold values of TSI-BI and total phosphorus for the different trophic categories in order to develop regional nutrient and biological criteria for water management.

2. Materials and methods

2.1. Study area

Uruguay is covered by a dense and extensive network of rivers that flow either directly to the Atlantic Ocean or through the Rio de la Plata. Streams usually run slowly over softly undulating terrain and low meadows occupied by livestock and crops.

Point-source pollution from domestic sewage and industrial effluents represents an important localized impact on streams and rivers. Nevertheless, diffuse sources of nutrients and other contaminants, related with agricultural activities, cattle production and dairy farms, are a more widespread cause of disturbance to fluvial ecosystems and the main cause of eutrophication in Uruguay.

Eutrophication studies in Uruguay have been carried out mainly in reservoirs (Chalar, 2006, 2009; De León and Chalar, 2003; Conde et al., 2002) and lakes (Scasso et al., 2001). Past fluvial research was focused on point-source contaminant effects, and these studies may be considered the basis of Uruguayan biological monitoring in fluvial systems (Pintos et al., 1993; Arocena and Mazzeo, 1994; Chalar, 1994; Arocena, 1996, 1998; Arocena et al., 2000).

In order to comply with the objectives of water quality surveillance, environmental authorities such as the national environment agency (DINAMA) and the water and sewage agency (DINASA) along with academic groups at the University of the Republic are developing a physicochemical and biological monitoring program of watercourses.

This study is a part of such efforts. It was carried out in the Santa Lucía river basin, located in the south of Uruguay (34°41′–34°51′S; 54°59′–57°7′W, Fig. 1). It has an area of 13,310 km^2 and drains to the estuary of the Rio de la Plata. Although only 9% of Uruguayans live in the basin (ca. 300,000 inhabitants), it provides drinking water for the two million inhabitants of the metropolitan area of Montevideo. Land use within the basin largely consists of dairy and meat cattle farming, agriculture and horticulture, while residential, forested,

Table 1
Regions and number of reaches sampled in the Santa Lucía basin and their main land uses (mean % and range) estimated using the land cover developed by Bartesaghi and Achkar (2008).

Region (number of reaches per region)	Culture mean (range)	Natural prairie mean (range)	Native vegetation mean (range)
A (5)	37 (22–60)	31 (18–46)	5 (0–12)
B (5)	54 (33–79)	26 (19–79)	7 (1–11)
C (5)	50 (40–70)	18 (11–25)	4 (5–16)
N (3)	14 (2–20)	49 (36–71)	7 (3–10)
O (4)	2 (0–7)	76 (75–82)	11 (6–18)
P (4)	0 (0–1)	60 (55–64)	8 (5–18)
Q (3)	36 (18–49)	30 (17–41)	7 (0–12)

and industrial areas occupy a smaller proportion of the catchment. Many water quality problems in the basin have been addressed in the past, including increased sediment loads and the eutrophication of streams and reservoirs.

Mean annual temperature in the region is around 17 °C, while mean annual precipitation is 1100 mm and evapotranspiration 1200 mm. Although evapotranspiration and temperature vary seasonally, precipitation does not, resulting in cold winters with high runoff and frequent floods, and warm summers with low flow. The basin covers three main geological and relief regions: (a) the metamorphic eastern hills, (b) the crystalline north plain and (c) the sedimentary south Plate plain. These regions also correspond to three main ecosystem units of the basin. These units were combined with the four main activities within de Santa Lucía basin: agriculture, horticulture, dairy cattle and extensive cattle breeding, resulting in seven regions, (A) the south plain with agriculture, (B) the south plain with horticulture, (C) the south plain with dairy cattle, (N) the north plain with agriculture, (O) the north plain with extensive cattle breeding, (P) the eastern hills with extensive cattle breeding, and (Q) the north plain with dairy cattle (Fig. 1 and Table 1).

2.2. Benthic macroinvertebrates

No significant differences in benthic assemblage were detected among the main ecological regions and no stratifying factor was further considered (*U* test). In each of these seven sampling regions we selected three to five permanent and wadeable stream reaches of orders 2–4. Four samplings were carried out these 28 reaches of the Santa Lucía basin during average to low water level and flow velocities conditions (December 2006, March, July and November 2007). Each stream reach was defined as 50 m long and divided into three portions, downstream, middle and upstream. A composite benthic macroinvertebrate sample was collected in each portion from downstream to upstream and then combined into the same container. The organisms were collected with a hand net (D-frame), equipped with 500 μm mesh, which was dragged for 1 min along all microhabitats present in the area. The collected samples were preserved with 70% ethanol and stored in plastic jars until the identification and counting of individuals in the laboratory. Taxonomic classification was made to the genus level, using regional keys when available (Lopretto and Tell, 1995; Merritt and Cummins, 1984). Macroinvertebrate abundances of the four samplings were averaged and the results expressed in number of individuals per sampling effort (Ind SE^{-1}).

2.3. Environmental variables

Dissolved oxygen (DO) and conductivity were measured in situ with Horiba probes (D-24 and D-25 models). In each sampling reach an integrated water sample of the three sections was taken for analysis of nutrients and suspended solids. The samples were kept refrigerated until filtration within 24 h and both fractions were stored frozen (−20 °C), until the determinations of dissolved and total nutrients within 30 days. Suspended solids were estimated by weight difference with a GF/F Glass Fiber Millipore filter (APHA, 1985). Dissolved nutrients were determined analytically in the filtered water. Soluble reactive phosphorus (SRP) was estimated using the technique described by Murphy and Riley (1962), ammonium following Koroleff (1970), and nitrate by the method of Müller and Widemann (1955). Total nitrogen (TN) and total phosphorus (TP) were determined in unfiltered water samples by the simultaneous analytical technique of Valderrama (1981). Substrate composition was visually estimated within the whole reach as the percentage of gravel (>2 mm), sand (2–0.064 mm), silt (soft and fine <0.64 mm) and clay (solid and slick <0.064 mm).

2.4. Statistical analyses

Linear correlations and significant differences among groups of data were estimated by the non-parametric statistical methods Spearman Rank Order Correlations (r) and Mann–Whitney *U* test, respectively. The Trophic State Index of Benthic Invertebrates (TSI-BI) was developed using a combination of different multivariate statistical methods. Detrended correspondence analysis (DCA) and canonical correspondence analysis (CCA) were run with CANOCO program version 4.02 (ter Braak and Smilauer, 1999). The data matrix was composed of 96 biological variables (genera), 5 environmental variables (TP, DO, % gravel, % sand, % silt) and 28 observations (reaches). First a DCA was run based on the logarithmic transformed abundance data and using the downweigthing rare species option in order to assess the length of the gradient. The gradient length was more than 2 standard deviations; therefore the unimodal response model was considered suitable (ter Braak and Prentice, 1988). Prior to running the CCA the importance of each environmental variable was assessed by the forward selection procedure combined with a Monte Carlo Permutation test. Only those variables that had a significant contribution ($p \leq 0.05$) were included. Then, the Variance Inflation Factor (VIFs) was repeatedly examined and only those variables with VIFs < 20 were finally included in the CCA analysis (Peeters et al., 2004). To determine if other environmental variables have been missed we compare the first two eigenvalues of the environmentally constrained method of CCA (0.352 and 0.132), with those of the unconstrained DCA (0.38 and 0.164). As the CCA eigenvalues were only a bit lower than DCA ones it is not possible that important variables have been missed. This reasoning requires that the number of environmental variables is not large compared with the number of samples, as is the case (Jongman et al., 1995).

A CCA was run to explore the relationships between the composition of macroinvertebrate 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. The position of the reaches along the gradient is determined by the sample score on the axis. We rescaled the gradient by linear regression setting – in accordance with our CCA results – with the lowest score set to 10 (oligotrophic) and the highest to 1 (hypereutrophic). The rescaled positions of

the sampling reaches were used in a weighted averaging model (WA) as the environmental variable (Smith et al., 2001; Haase and Nolte, 2008). The WA model with classical deshrinking was selected and run on the C2 Data Analysis program, version 1.5.1 (build 1) (Juggins, 2007). Based on Haase and Nolte (2008), the TSI-BI for a given reach was estimated as:

$$\text{TSI-BI} = \frac{\sum \text{Op}_i \times \text{To}_i \times \text{Ab}_i}{\sum \text{To}_i \times \text{Ab}_i}$$

where Op is the optimum of each genus, To is the tolerance of each genus and Ab is the mean transformed genus abundance (Ind SE^{-1}) in each reach ($\text{Ab} = \log_{10}(\text{abundance} + 1)$).

Then a cluster analysis using the unweighted pair-group average as the amalgamation method and Euclidean Distances was run (StatSoft Inc., 2007). This cluster analysis, based on TP concentrations and TSI-BI scores as grouping variables was used to establish the limits between the trophic categories.

3. Results

A total of 96 genera were present in more than 3 sampling reaches and therefore included in this study. The mean number of genera in study reaches was 36, with a minimum value of 10 and a maximum of 56. Mean benthic abundance of the reaches varied between 49 and 450 individuals per sampling effort (Ind SE^{-1}), while total mean abundance was 173 Ind SE^{-1}.

3.1. Canonical correspondence analysis (CCA)

The first eigenvalue was fairly high (0.352), implying that the first axis represents a strong gradient, while the second one was much weaker (0.132). The amount of the total variation explained by the environmental variation reached 69.4%. The first axis explained 14.6% of the total variation (total inertia) in the species data and the second one explained 5.5%. Also, the first axis accounted for 50.7% of the total variation that could be explained (explainable inertia) by the species-environment relation while the second explained another 19.7%. Total phosphorus (TP), showed the highest positive correlation with the first axis (0.81); while dissolved oxygen concentration exhibited the highest negative one. TP was significantly correlated (r Spearman, $n = 28$, $p < 0.05$) with conductivity, suspended solids, nitrate, ammonium, total nitrogen and soluble reactive phosphorus and negatively with dissolved oxygen. The first axis therefore represents a gradient of nutrient enrichment associated with human activities. The scores of the study reaches are arranged along this axis according to their trophic states, with the more oligotrophic reaches at the negative end and the more eutrophic reaches having the opposite relationship. In addition, the gravel and lime proportion of the sediments were correlated with the first axis ($r = -0.67$ and 0.60, respectively), indicating an association of nutrient inputs with finer sediments. The most abundant fraction in the sediments of all the reaches was sand with varying contribution of gravel and silt. A greater proportion of fine sediments was observed in regions A, B and C where the intensity of land use was higher.

3.2. Genus scores and TSI-BI reach classification

The TSI-BI was significantly correlated with the site scores defined by the CCA (r Spearman $= -0.95$, $p < 0.001$). Also, the WA model showed a good predictive performance with a high coefficient of determination (Table 2).

The rescaled site contributions (10-1), used in the WA model enabled us to estimate the optimal scores and the respective tolerance for each genus (Table 3

Table 2
Model performance. WA, weighted averaging; WATOL, weighted averaging tolerance; RMSE, root mean square error; R^2, coefficient of determination of the predicted and observed values.

	WA	WATOL
RMSE	1.11	1.01
R^2	0.86	0.88
Average bias	$1.3E^{-15}$	$-1.0E^{-15}$
Maximum bias	1.37	1.50

). The optimum (Op) is the numerical score of each genus indicating its "optimum" on the gradient from natural to sever impaired waters and the tolerance (To), describes the "power" of the genus score (i.e. whether the genus has a broad or narrow ecological amplitude) and it is used to weight the optimum.

Thirty-three genera composed of 8 Diptera, 7 Trichoptera, 7 Ephemeroptera, 7 Coleoptera, 2 Plecoptera, 2 Odonata and 1 Bivalvia exhibited optimal scores associated with oligotrophic stream conditions ($S_{10} > 8.5$, see also Trophic Reach Classification). Conversely, 32 genera including 9 Diptera, 9 Odonata, 4 Gastropoda, 3 Coleoptera, 3 Oligochaeta, 2 Bivalva, 1 Amphipoda and 1 Hirudinea, were strongly associated with hypereutrophic ambient conditions ($S_{10} < 5$).

Using the determined optimum genus scores (Table 3), the respective values of tolerance as weights and the abundance of each genus, the TSI-BI for the study reaches were estimated. The TSI-BI values were significantly correlated with CCA scores, indicating that the index summarized most of the information concerning the distribution of benthic organisms with respect to the trophic gradient (r Spearman $= -0.95$, $p < 0.001$).

3.3. Trophic reach classification

In order to classify the reaches according to their trophic states, a cluster analysis based exclusively on TSI-BI scores and TP concentration was run (Fig. 2). Three main groups with a similarity of 60% were defined. These groups were significantly different in terms of TSI-BI scores and TP concentration. Group 1 showed the minimal TP mean concentration and highest mean TSI-BI score (Table 4). Group 3 was composed of the highest values of TP and TSI-BI, while Group 2 showed intermediate TP concentrations and TSI-BI scores. These results indicated that the defined groups follow a gradient of trophic conditions of the study reaches from lower trophic levels (Group 1), to higher ones (Group 3). In order to define the lim-

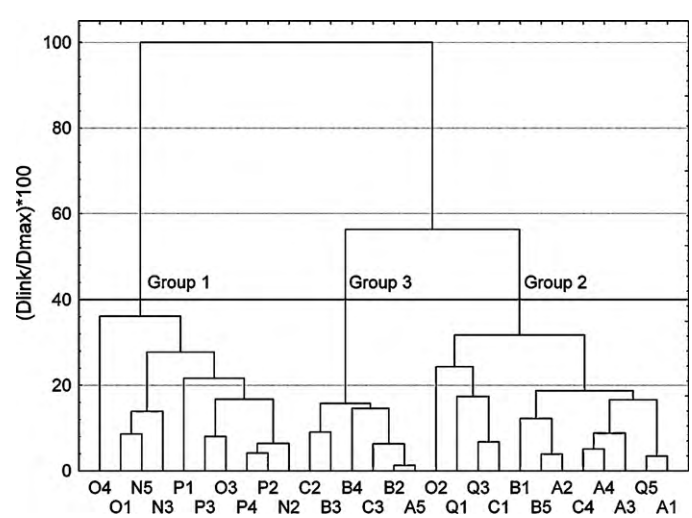

Fig. 2. Three main groups of reaches arranged according with their TSI-BI scores and TP concentrations and defined at 60% of similarity (solid line).

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Table 3
Benthic macroinvertebrate with their corresponding optimum and tolerance scores.

Taxon	Optimum	Tolerance
Oligochaeta		
Pristinilla	6.1	2.6
Dero	5.4	2.8
Allonais	5.6	3.0
Amphichaeta	5.5	2.3
Hirudinea		
Helobdella	5.2	2.8
Bivalvia		
Eupera	4.3	2.7
Corbicula	8.0	2.1
Pomacea	6.5	2.5
Pisidium	4.1	3.1
Diplodon	8.1	2.2
Gastropoda		
Gundlachia	5.6	2.9
Heleobia	6.2	2.6
Drepanotrema	4.0	2.2
Biomphalaria	4.6	2.8
Stenophysa	5.7	2.8
Amphipoda		
Hyalella	5.4	2.7
Decapoda		
Macrobranchium	6.8	2.2
Paleomonetes	7.6	1.7
Aegla	7.8	2.2
Isopoda		
Pseudosphaeroma	6.4	2.2
Ephemeroptera		
Caenis	6.2	2.7
Baetodes	6.6	3.0
Baetis	7.6	2.0
Dactylobaetis	9.4	1.2
Tricorythodes	8.9	1.3
Thraulodes	8.7	2.0
Ulmeritus	9.5	0.7
Haplohyphes	8.5	1.9
Leptohyphes	9.0	2.0
Campsurus	9.5	0.5
Odonata		
Ischnura	7.0	2.7
Nehalennia	6.3	1.3
Telebasis	4.7	2.9
Zoniagrion	3.3	3.8
Acanthagrion	5.5	1.3
Hyponeura	6.4	2.8
Hetaerina	8.0	1.7
Archeogomphus	5.5	4.2
Agriogomphus	9.3	1.0
Gomphus	8.2	1.4
Perithemis	3.6	2.8
Nannothemis	3.6	2.1
Erythemis	5.1	2.6
Aeshna	2.8	2.1
Anax	2.9	2.1
Plecoptera		
Anacroneuria	8.7	3.3
Paragripopteryx	8.6	1.4
Coleoptera		
Hydrocyphon	5.0	1.7
Hidrophilus	6.5	2.3
Hydrous	3.7	2.6
Laccobius	8.4	2.0
Berosus	4.3	2.7
Helichus	7.3	1.7
Dryops	8.1	1.8
Elmis	9.3	1.2
Riolus	7.9	2.0
Stenelmis	5.9	3.0
Esolus	7.7	2.4
Limnius	9.0	1.9
Oulimnius	6.4	1.0
Microcylloepus	9.1	0.9
Gyrinus	6.1	1.0
Donacia	7.0	0.9
Psephenus	8.8	2.0
Psephenops	9.4	0.8
Trichoptera		
Oxyethira	9.1	1.7
Ochrotrichia	9.0	1.4
Smicridea	9.5	0.7
Chimarra	8.9	2.7
Austrotinodes	8.4	1.8
Triplectides	8.3	1.2
Atanatolica	8.7	1.5
Diptera		
Chironomus	4.7	2.6
Dicrotendipes	6.0	2.9
Beardius	6.6	1.6
Pseudochironomus	9.4	1.3
Thienemannimyia	8.4	1.1
Nimbocera	6.7	2.5
Rheotanytarsus	8.1	2.1
Paramerina	8.0	2.0
Orthocladius	8.1	2.7
Cricotopus	8.4	1.0
Thienemaniella	8.7	1.3
Probezzia	7.0	3.9
Forcipomyia	7.5	2.8
Simulium	8.5	1.8
Odontomyia	4.1	2.0
Hemiptera		
Belostoma	5.7	2.6
Lethocerus	6.5	2.3
Ranatra	5.7	2.8
Tenegobia	5.2	3.1
Ectemnostegella	2.1	2.5
Centrocorisa	5.6	2.7
Pelocoris	9.7	0.3
Notonecta	6.1	2.5
Buenoa	4.9	3.8

its of each trophic state we consider the TP range of each group. As the maximum value of Group 1 (92 μg/l) overlapped with the minimum of Group 2 (71 μg/l) and the same occurred between Groups 2 and 3, we selected the minimal TP value as the threshold for trophic state classification (Table 5). This overlap did not occur with TSI-IB scores, where minimal and maximal values between trophic states categories coincided and so became the TSI-BI score thresholds (Table 5).

3.4. TSI-BI and the intensity of land use

We considered the percentage of cultivated area as a direct measure of the intensity of land use and the main human pressure to aquatic ecosystems, in accordance with the land uses described in the Study Area (Section 2.1, Table 1). We then explored the relationship between the intensity of land use and the proposed TSI-BI with the trophic reach classification. We found that the intensity of land use was negatively correlated with the TSI-BI scores (Fig. 3; $r = -0.71$, $p < 0.05$, $n = 28$) and positively with TP ($r = 0.70$, $p < 0.05$, $n = 28$). Considering our trophic classification based on TSI-BI scores, significant differences between mesotrophic and eutrophic reaches were determined (Mann–Whitney U test, $p < 0.05$), but not between eutrophic and hypereutrophic ones. The same feature was found for the trophic groups defined by total phosphorus thresholds values.

Table 4
Basic statistics of the three groups of reaches defined by cluster analysis. Threshold values between trophic classes for phosphorus concentration and TSI-BI scores in bold (see text for more details). SD, standard deviation, *n*, number of reaches per group.

	Group 1		Group 2		Group 3	
	TP (μg/l)	TSI-BI	TP (μg/l)	TSI-BI	TP (μg/l)	TSI-BI
Mean	47	9	253	7	744	5
Minimum	17	**8**	**71**	**6**	**383**	5
Maximum	92	9	502	8	1098	6
SD	30	0	125	0	309	0
n	10	10	12	12	6	6

4. Discussion

4.1. Estimation of TSI-BI

The methodology we used combined a direct gradient analysis (CCA) with a weighted averaging model (WA). CCA is a technique often used to detect spatial gradients as well as ordinate sites along them, while WA models have been widely used to determine species optima in ecological and paleolimnological studies (ter Braak, 1987; Hall and Smol, 1992; Rossaro et al., 2007). Moreover, the use of multivariate analysis (CCA, Multidimensional Scaling) in conjunction with weighted averaging models has been proposed more recently as an effective and objective method to develop benthic biotic indices for environmental quality assessments (Smith et al., 2001; Haase and Nolte, 2008). According to the highly significant correlation found between TSI-BI values and the ordination scores along the trophic gradient, the TSI-BI summarizes most of the ambient variability found among the study reaches. The established trophic gradient was mainly defined by TP at one side of the gradient and by DO at the opposite side. TP was also positively correlated with conductivity, suspended solids, nitrate, ammonium and total nitrogen, while DO was negatively correlated with them. These relationships indicate a common source of inputs of materials and a process related with these inputs that negatively affects the concentration of DO in the water. Natural cover from riparian vegetation is an exception in the Santa Lucía basin, and so light limitation of autotrophic production may be mainly attributable to suspended solids coming from erosion and wastewaters. At these impacted reaches, the heterotrophic activity is high and respiration processes cause DO depletion, explaining the TP side of our trophic gradient. On the other hand, in less perturbed reaches, suspended solids are low and DO concentration is higher. In these reaches autotrophic production may be higher than respiration, explaining the other side of the trophic gradient. These data justify the gradient found among the study reaches and are the basis of the TSI-BI proposed. They are also in agreement with the literature that defines the trophic state of aquatic ecosystems considering both autotrophic and heterotrophic productivity (Odum, 1956; Dodds and Cole, 2007).

4.2. Trophic state boundaries

Traditional models of eutrophication define aquatic ecosystems according to their nutrient supply in three trophic categories: oligotrophic, mesotrophic and eutrophic (Vollenweider, 1968; OECD, 1982). Ultraoligotrophic and hypereutrophic classes are also used in extreme trophic conditions. Although early studies of eutrophication were based on lake and reservoir data, the importance of this process in fluvial ecosystems is widely recognized. According to Dodds (2007), the trophic state is a fundamental property of the ecosystem structure directly linked to the biotic integrity and water quality of streams. In this context we propose a biotic index (TSI-BI) related with the TP concentration. The cluster analysis defined three main groups of reaches. According to the range and mean values of TP concentration of each group, they were, respectively, associated with mesotrophic, eutrophic and hypereutrophic conditions. The boundary value between mesotrophic and eutrophic classes (Table 5) was similar to those proposed by other authors for the same limit. Dodds et al. (1998) proposed a threshold value of 75 μg/l derived from frequency distributions of TP, TN, and chlorophyll, while Smith et al. (2007), suggested a boundary value of 65 μg/l TP, using invertebrates and nutrient data and a cluster analysis similar to our own. According to Smith et al. (2007), the boundary between mesotrophic and eutrophic categories represents the upper limit after which they began to observe the adverse effects of nutrient enrichment on aquatic invertebrate communities. Also, Dodds and Oakes (2004) suggested that in general the reference values should be suspect if they fall above 60 μg/l TP.

Table 5
Target values for trophic state classification.

	Group 1 Mesotrophic	Group 2 Eutrophic	Group 3 Hypereutrophic
TP (μg/l)	<71	71–383	>383
TSI-BI	>8	8–6	<6

Comparing the proposed thresholds values for TSI-BI is much more difficult. Nevertheless, as we used the same methodology in developing the TSI-BI as did Haase and Nolte (2008) when developing the Invertebrate Species Index (ISI) for streams in southeast Queensland, Australia, they are comparable. The methodology of these authors differed from ours in that they defined the thresholds values using a reference-site approach: "Sites meeting the minimal-disturbance criteria were those with remnant vegetation intact throughout the catchment and without cleared areas or other significant impacts upstream of the sampling site". They proposed an

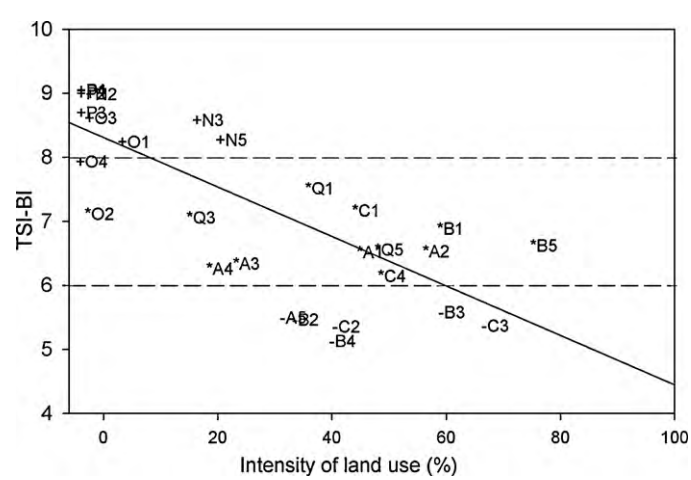

Fig. 3. Scatterplot of TSI-BI scores versus the intensity of land use indicating the trophic state classification of the sampling reaches according to Fig. 2 (+, mesotrophic; *, eutrophic; -, hypereutrofic). Dotted lines indicate threshold scores for TSI-BI between trophic states according to Table 5, and the solid line shows the linear regression adjustment.

ISI > 8.5 for reference sites and a range 8.5–7.3 for slightly impacted sites which were of an ecological quality class just 'below' reference condition. These values are similar to the boundary between mesotrophic and eutrophic classes (8) that we propose in this study. Moreover, we found a significant correlation between TSI-BI and the intensity of land use and significant differences in the intensity of land use between mesotrophic and eutrophic reaches. These results indicate that the index performs well when used to describe and predict the impact of anthropogenic activities on fluvial ecosystem integrity.

The selected reaches in this study covered an ample trophic gradient but oligotrophic sites were underrepresented and could not be identified as a distinct group. Among the 28 study reaches, 18 were classified as eutrophic or hypereutrophic. Among the mesotrophic ones only 3 showed a mean TP concentration < 25 μg/l and 7 reaches presented TSI-BI scores > 8.5, both limits associated with oligotrophic conditions (Dodds et al., 1998; Haase and Nolte, 2008). Further efforts should be made to sample a higher number of oligotrophic reference reaches, in order to understand the structure of undisturbed benthic communities and baseline nutrient concentrations.

5. Conclusions

In the present study, a trophic state classification is proposed for the first time for South American streams indicating the threshold values of TSI-BI and TP between mesotrophic and eutrophic categories. The defined optimum and tolerance scores enable us to assess (via the TSI-BI developed), the aquatic quality of the main ecoregions and land uses found at Santa Lucía basin. Once the optimum and tolerance scores are estimated, the use of the TSI-BI is very simple and rapid and should be incorporated into the national monitoring programs. Target values of >8 for TSI-BI and <71 μg/l for TP are proposed to distinguish between impaired and moderately to non-impaired streams.

Our results indicate a strong anthropogenic degradation of natural conditions in the study basin, which can be related with land use intensity. A change in land use management is clearly necessary in order to protect and preserve the main water supply system of half of the Uruguayan population and associated natural resources.

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