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ORIGINAL ARTICLE

Evaluation of ecological quality in peri-urban rivers in Mexico City: a proposal for identifying and validating reference sites using benthic macroinvertebrates as indicators

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ABSTRACT

Conservation and management of aquatic ecosystems that are significantly influenced by urban activities requires the classification and establishment of potential reference sites. However, in Latin American countries, policies are not available that outlines the identification and evaluation of such sites. Therefore, this study represents a proposal for evaluating the ecological quality of peri-urban rivers in the conservation soil (CS) areas/zones of Mexico City. The proposal accounts for the zone's physicochemical, hydromorphological, and bacteriological characteristics along with its macroinvertebrate richness. Our evaluation was performed using a canonical correspondence analysis (CCA) and indicator values (IndVal) calculated for different taxa. River headwaters serve/work as a good physicochemical point for potential references sites. However, the hydromorphology of the CS has been gradually modified by numerous hydraulic alterations within the peri-urban zone. Using the CCA and IndVal, two types of sites were confirmed: sites in a good state of conservation and quality and sites modified by human activity, featuring lower discharge flow, poor quality hydromorphological values and Oligochaeta class organisms. At the sites featuring a good state of conservation and quality, higher hydromorphological values were positively correlated with discharge flow and certain macroinvertebrate taxa, including Nemouridae, Podonominae, Tanypodinae, Acarina, Baetis, Tipula, Antocha, Atopsyche, Glossosoma, Polycentropus, Hesperophylax *and* Limnephilus. *In the sites modified by human activity, the genus Simulium was classified as a disturbance-tolerant organism. The river reach within the urban zone is basically an open-air drainage ditch. Evaluations of the ecological quality of the riparian zone were used to identify the most important hydromorphological qualities and discharge flow parameters and to select the most appropriate factors that should be monitored in peri-urban rivers of the Mexico Basin.*

Key words: Peri-urban watersheds; ecological quality; reference sites; macroinvertebrates; Magdalena-Eslava River sub-basin.

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INTRODUCTION

The concept of *ecological quality* is defined as an expression of the structure and functioning of aquatic ecosystems and may be determined by the status of the biological elements that are supported by physicochemical and hydromorphological quality (Sánchez-Montayo *et al*., 2009). Ecological quality is influenced by geomorphology and climate in a hydrological basin as well as by local features such as land use, hydrodynamics, biological processes, and riparian vegetation (Munné and Prat, 2004). The concept of ecological quality is used as a reference point to achieve a better understanding of how ecosystem services (ES) are generated and to improve environmental standards and design monitoring strategies (Ruza-Rodríguez, 2005; Paetzold *et al*., 2010).

Ecological quality legislation, such as the Water Framework Directive (WFD) (European Commission, 2000), suggests that reference sites should be identified as a starting point for rehabilitating watersheds. A reference site is defined as a site with over 70% natural land use, no upstream water volume regulation, appropriate substrate diversity, stable banks with a proper riparian zone vegetation and no inputs from landfills (Hughes, 1995; Stoddard *et al*., 2006; Sánchez-Montayo *et al*., 2009). In addition, a reference site should not have been affected by large-scale disturbances and have hydromorphological and physicochemical characteristics that preclude significant negative effects on the ecosystem functions (Pardo *et al*., 2012).

However, the biggest challenge has been finding common approaches to defining ecological quality and anthropogenic intervention degree. In addition, the reference and rejection thresholds under which a site can be classified as a reference site should be identified (Pardo *et al*., 2012). Therefore, reference conditions are assembled from multiple sources depending on the spatial and temporal scale; thus, the term may refer to historical or current conditions (Bouleau and Pont, 2015).

Because of the variety of interpretations that can be applied to the term reference site, certain alternative definitions have been provided by Stoddard *et al.* (2006) that can help with practical applications. These definitions include the biotic integrity or natural state for which long-term variability is acceptable within relatively narrow limits; a state corresponding to conditions encountered in minimally disturbed areas (this definition coincides with other definitions cited above); and conditions that occurred prior to any human modification considered important by the authors. For example, Wallin *et al*. (2003) and Friberg *et al*. (2011) consider the period prior to the development of intensive agriculture and/or industrialization to be a reference condition regardless of previous human impact. Such a definition might imply the need for paleolimnological studies, which would be a difficult task when establishing reference sites in current studies. Thus, the diversity of reference conditions found in the literature reflects the need for geographical and academic intercalibration processes (Pardo *et al*., 2012).

The development of biotic indexes is an attempt to characterize the causal relationship between changes in biological composition and alterations in ecological quality by using organisms to indicate and track environmental changes (Friberg *et al*., 2011). The composition of benthic macroinvertebrates communities is frequently used as an indicator because i) the majority of these organisms are localized and representative of the area where they are collected; ii) their life cycles are relatively long and sensitive to alterations in the environment; iii) they are sensitive to stressors; and iv) they constitute a significant part of the trophic chain (Ferraro and Cole, 1990; Cortes *et al.,* 2013). These organisms may exhibit the influence of pressures on both terrestrial and aquatic environments, and they can be utilized to identify degradation levels in the system prior to employing physicochemical parameters (Sánchez-Montayo *et al*., 2009). These degradation characteristics include the food resource quantity and quality, habitat quality, riverbed structure, water flow regimens, water quality, biotic interactions and riparian zone condition (Sánchez-Montayo *et al.*, 2009; Pardo *et al.*, 2012). In general, the taxonomic level used to assign an indicator value for ecological quality in diverse biotic indexes is at the genus or family level because that resolution provides sufficient ecological information in statistical analyses and adequate data for sensitive and accurate bioassessments (Greffard *et al*., 2011). In addition, the use of functional groups of macroinvertebrates (*e.g.*, Merritt *et al*., 2008) can be directly correlated with ecological quality and provides additional taxonomic information (Cummins *et al*., 2005; Guilpart *et al*., 2012; Janushke *et al*., 2014). This approach is particularly sensitive to land-use impacts in the watershed, especially stream-side (riparian) vegetation that affects the stream/river system flowing through the landscape (Cummins *et al.,* 2005).

The ecological quality assessment of a water body is a relatively new and innovative strategy for water quality management (Bouleau and Pont, 2015). Earlier guidelines merely defined standards for water chemistry and only targeted the water used for specific purposes. This term is particularly difficult to apply in Latin America, and particularly in Mexico, where efforts at policy level are regional and the topic is new (Acosta *et al*., 2009; DOF, 2012). The majority of new knowledge is based on studies conducted in sub-moist temperate ecosystems. Tropical Latin America requires the development of specific regional and national guidelines, and baseline information that characterizes the typology of rivers must be generated. The development of methodological alternatives capable of evaluating the full range of ecological quality of Latin American rivers is crucial. An approach to determining potential reference sites includes the development of a protocol for evaluating the ecological quality of Andean rivers (CERA) and its application to two watersheds in Ecuador and Peru. This protocol was developed following the WFD, and it is an important reference for stream conservation in Latin America (Acosta *et al*., 2009).

Of particular interest are peri-urban rivers that often constitute a heterogeneous mosaic of agro-forestry and urban ecosystems that are subject to rapid and sudden anthropogenic effects (Allen *et al*., 2006). Anthropogenic impacts degrade these rivers, and conservation measures usually come second to the requirements of urban growth. When aiming to monitor ecosystem changes, it is important to understand the effect of urban impacts on benthic organisms (Pagliosa and Rodríguez, 2006; Whol, 2006).

The Mexico Basin, which supplies one of the most densely populated cities on the planet, contains several mountain streams (Dudgeon, 2008). However, these streams are impacted by changes in land use (urbanization occurring at 2500 m asl and below), recreational activities, and hydraulic projects. These impacts generally also affect other peri-urban rivers of the Mexico Basin (Legorreta, 2009).

The Magdalena-Eslava River sub-basin was selected as a study case because it is a relatively well-preserved forested area. This sub-basin provides ground and surface water that contribute up to 50% of Mexico City's surface water (Jujnovsky *et al*., 2010). As such, the identification of potential reference sites and determining the value of benthic macroinvertebrates as indicators of ecological quality would have regional applicability. A simple methodological strategy to evaluate the ecological quality of peri-urban mountain streams is required, and the necessary tools must be developed to make adjustments in public conservation policies in Mexico City. Thus, the goal of this study is to evaluate ecological quality in a representative peri-urban riparian watershed in the Mexico Basin using two methods: i) identify potential reference sites through an evaluation of the physicochemical and hydromorphological conditions of the river; and ii) estimate the ecological indicator value of benthic macroinvertebrates to characterize the ecological quality.

METHODS

Study area and selection of sampling sites

The Magdalena-Eslava River sub-basin (Fig. 1) is located in the morphotectonic region of the Trans-Mexican Volcanic Belt at minimum extreme coordinates 463 915; 2126 293 and maximum extreme coordinates 475 774; 2134 715, and it has a total surface area of 50 km^2 (Ferrusquía-Villafranca, 1998). The Magdalena River originates at an elevation of 3650 m asl and spans 28.2 km to the edge of the Mexico City urban zone at 2300 m asl. The river then runs for 14.8 km through an area known as conservation soils (CS). There are two types of hydraulic river interventions: first, 90 gabion dams are concentrated along certain sections of the streams, and second, a water treatment plant is located in the transition zone between CS and urban soils (US). The gabion dams provide nutrient retention and changes in the self-cleaning dynamics in the sub-basin (Mazari *et al.*, 2014). The remaining 13.4 km of river runs through US, which is affected by urban discharge and channeled to deep drainage troughs. In addition, 4.5 km of the river within US has been piped and converted to roadway. The Eslava River is a tributary of the Magdalena River, and it begins at an elevation of 3557 m asl and spans 13.4 km until its confluence with the Magdalena, just as it enters the US area at 15 km. This river also contains 83 gabion dams in the CS area. These rivers provide 5% of Mexico's City surface water at a local level.

The climate of the region is sub-moist temperate (annual average temperature of 13.4°C and annual average precipitation between 1200 and 1500 mm), and it has abundant rains from June to October and a dry season

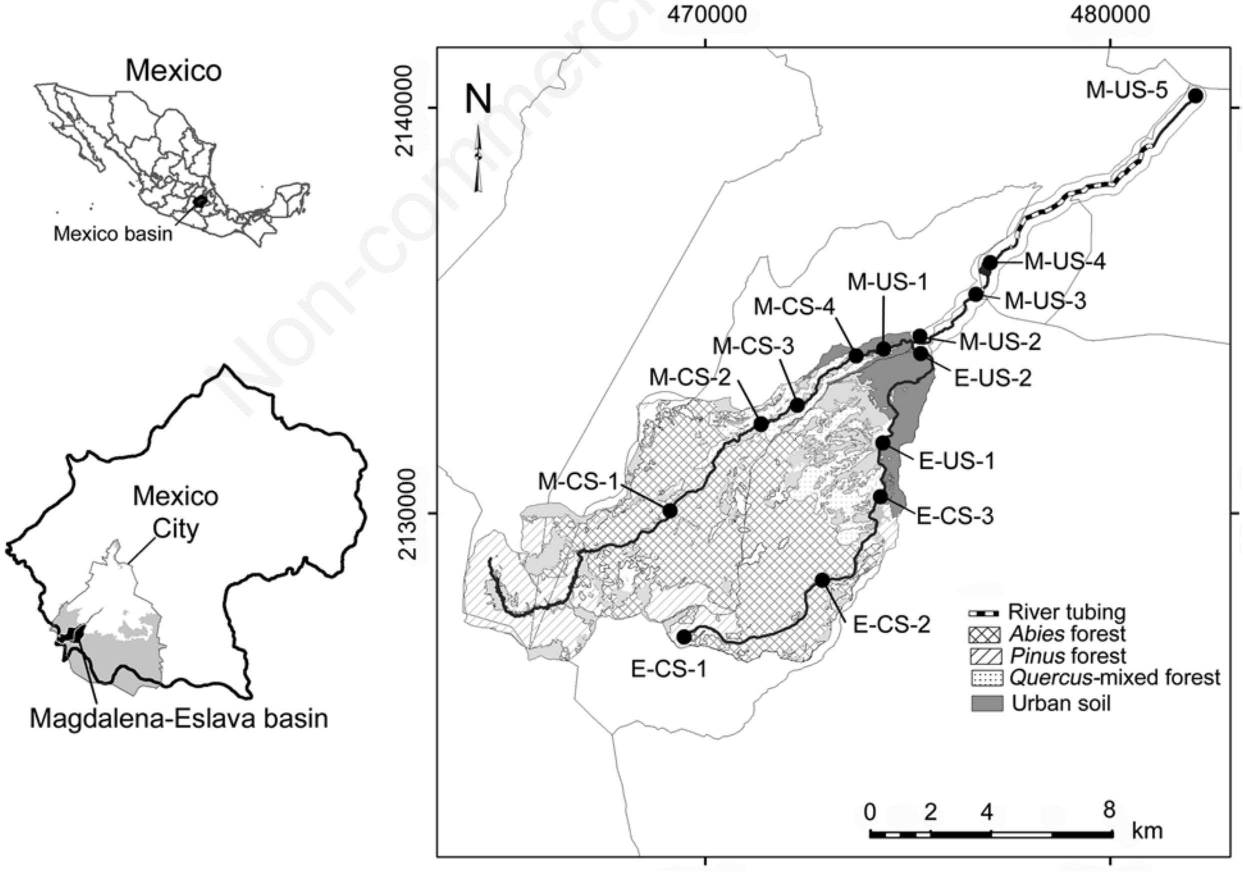

Fig. 1. Location of sampling sites in the basins of the Magdalena-Eslava rivers, Mexico. M-CS and M-US, Magdalena conservation soil and urban soil, respectively; E-CS and E-US, Eslava conservation soil and urban soil, respectively.

from November to May (García, 2004). Geological traits consist of rock packets alternating with andesitic to basaltic lavas (Ferrusquía-Villafranca, 1998). Forests of *Abies religiosa* (Kunth) Schltdl. and Cham., *Pinus hartwegii* Lindl. and *Quercus* spp. grow in the upper area of the sub-basin, with mixed forest occurring in the middle and lower areas (Ávila-Akerberg, 2010).

The sub-basin of the Magdalena-Eslava River was designated as a pilot area for the implementation of government conservation and restoration programs. One of the most important projects is the Master Plan for the Integral Rehabilitation of the Magdalena River (PUEC-UNAM-GDF, 2008; UAM-GDF, 2008). This project involves management and conservation actions that avoid jeopardizing its potential as a provider of ES. In 2009, in conjunction with the University Environment Program, this project conducted a study to present the *System of indicators for the rescue of the Magdalena and Eslava rivers* (PUMA-UNAM-GDF, 2009). The aim of this report was to provide Mexico City's government a tool that would allow it to monitor the performance progress of the goals outlined in the Master Plan. This sub-basin is of great importance for the future development of management plans and conservation in the Mexico Basin.

The selection of sampling sites was conducted following the set of rules proposed by the Freshwater Ecology and Management Research Group (FEM 2011), which were used to design an evaluation of ecological quality and create reference sites in high altitude Andean rivers (Acosta *et al*., 2009). The potential reference sites were preselected using a digital elevation model (Instituto Nacional de Geografía e Informática-INEGI, 2000), soil cover (Ávila-Akenberg, 2005), soil type (Registro Nacional Agrario-RAN, 2000), hydrologic network (Ávila-Akenberg, 2005) and weather station data from official climatological reports (ERIC III, version 3.2. Extractor Rápido de Información Climatológica, 2014). We also used information from previous research on the status of the hydrological ES and indicator system (PUEC-UNAM-GDF, 2008, 2009; Jujnovsky *et al*., 2010; Mazari *et al*., 2014).

Based on this information, three types of sites were established *a priori* within the sub-basin: potential reference sites (PRS), which were identified based on the definition of Stoddard *et al.* (2006), transition sites (TS) and degraded sites (DS).

Nine sampling sites were selected on the Magdalena River [four sites within the CS (M-CS) and five within the US (M-US)] and six sites on the Eslava River (four E-CS and two E-US sites). The goal of sampling site selection in the US was to characterize the local water quality. The potential reference sites were validated using estimates of physicochemical and bacteriological water parameters, hydromorphological quality (HQ), and benthic macroinvertebrate indicator values.

Physicochemical, bacteriological, and hydromorphological quality evaluation

Sampling was performed four times between September 2012 and September 2013 during the rainy season (R1) (September 2012), dry cool season (DC) (February 2013), dry warm season (DW) (April 2013), and subsequent rainy season (R2) (September 2013). The following physicochemical parameters were recorded *in situ* using an YSI 6600 multiparameter probe (Loveland, CO, USA): water temperature, specific conductivity (K_{25}), dissolved oxygen (DO) and pH. Discharge flow ($Q3\ m^3\ s^{-1}$) was calculated according to Gore (1996).

At each sampling station, 500 mL water samples were collected in sterile polypropylene bottles for the physicochemical analysis following the criteria established in the official Mexican guidelines and international standards NOM-001-SEMARNAT-1996 (DOF, 2003; APHA, 2005). The samples were stored at 4°C and analyzed three times in the lab within 24 h of collection. Nutrients [ammonium nitrogen, nitrate nitrogen, total nitrogen (TN), orthophosphate and total phosphorous (TP)] were analyzed using a portable spectrophotometer (Hach DR/2400) and digester (Hach DR/2000) (Hach, 2003; APHA, 2005). One liter samples were collected in sterile polypropylene flasks for bacteriological analysis, stored at 4°C and processed within 24 h of collection using the membrane filtration technique (APHA, 2005). Membrane filters (cellulose acetate, 0.45 µm, Millipore MF type HA) were placed in Petri dishes with 2.5 mL of membrane fecal coliform agar (m-FC) medium and incubated at 35°C for 24 h and with Kenner fecal (KF) *Streptococcus* agar for fecal enterococci (FE) and incubated at 44.5°C for 48 h (APHA, 2005).

The hydromorphological quality and anthropogenic activities were evaluated based on observations in the study area and adapted to the analysis established by CERA (Ecological Quality of Andean Rivers, Acosta *et al*., 2009). This method uses a scale of 24-120 points to classify the heterogeneity of the fluvial habitat in high-mountain rivers as determined by eight elements that could be altered by human activities, which include structure, continuity and natural condition of riparian vegetation and connectivity with the adjoining landscape, natural condition of the fluvial channel, depth regime, current velocity, channel heterogeneity, human trash and coarse sediments. The riparian vegetation (native and exotic species) was classified according to Ávila-Akerberg (2010). The sites with values higher than 100 were considered potential reference sites.

The similarity between sampling sites was examined as a function of their physicochemical, hydromorphological and bacteriological parameters using an ascendant hierarchical grouping analysis [Euclidean distance and Unweight Pair Group Method with Arithmetic Mean (UPGMA)], and a principal components analysis (PCA) to reduce the number of significant environmental variables. To measure the significance of variation in the physicochemical parameters, paired tests were performed using a Kruskal-Wallis analysis. Similarly, the Mann-Whitney test was used to identify seasonal differences between the parameters. All tests were performed using the STATISTICA 6.0 statistical software package (StatSoft, 2001). The significance value of the tests was set at P=0.05 to avoid type I errors. The environmental parameter data were transformed using the ln (X+1) function and then standardized. This process was necessary because of differences in the measuring units and the extreme variation of data between the US and CS areas.

Macroinvertebrate sampling

Collection points were selected at each sampling location according to a multihabitat criterion to obtain a representative sample and cover all possible habitats where the benthic macroinvertebrates might be found. An aquatic d-shaped net with a mesh size of 150 µm and a width of 30 cm was also used. Sampling was performed along a 50 m transect, sediments were removed over three minutes, and organisms were placed in a tray for sorting. Sampling was also conducted via manual examination and removal from the submerged faces of large rocks, branches, and leaves. A minimum of 100 individuals were collected from each location as a representative sample (by both techniques), deposited in plastic flasks and preserved in 70% ethyl alcohol. The individuals were sorted using an Olympus SZX7 stereoscopic microscope (Olympus Corporation, Tokyo, Japan) and were identified up to the genus level when possible using several sources (Merrit *et al*., 2008; Bueno-Soria, 2010; Dewalt *et al*., 2010). When genus-level determinations were not possible, the individuals were identified up to the subfamily, family, or class level.

The total absolute organism abundances were used for all statistical analyses, and only those taxa occurring in at least one site with an abundance of more than 1% during each of the sampling seasons were included in the analyses to minimize the influence of rare taxa and reduce the bulk and noise in the data set without losing much information (McCune and Grace, 2002). The data normality and variance homoscedasticity were analyzed using the Kolmogorov-Smirnov and Shapiro-Wilk tests. Benthic macroinvertebrate abundances were also transformed using the ln (X+1) function and later standardized using an unbiased standardization [standardize (n-1)], and the IndVal calculations used untransformed abundances.

Estimation of indicator species value

The indicator values of benthic macroinvertebrates were evaluated using two approximations and only performed at sites of CS, because the main goal was to identify potential reference sites and associated assemblages of benthic macroinvertebrates. First, a canonical correspondence analysis (CCA) (ter Braak, 1986) and Monte Carlo test (999 permutations, α=0.05) were performed to establish a relationship between the spatial and temporal distribution of benthic macroinvertebrates and the physicochemical and bacteriological characteristics of the water. The temporal analysis allows for the recognition of hydrological parameters related to the abundance of benthic macroinvertebrates that are not necessarily related to channel pollution. In addition, it allows for the selection and/or confirmation of potential reference sites and impacting factors (Dufrené and Legendre, 1997; Tornés *et al*., 2007). Statistical analyses were performed using the XLSTAT program (Addinsoft, 2013). The ecological quality categories were established according to taxa scores and axis characterizations obtained with the CCA analyses.

The second approximation determined the ecological indicator value of taxa (IndVal, Dufrené and Legendre, 1997). The IndVal method is based on the degree of habitat specificity (exclusivity to a habitat) and fidelity (frequency of occurrence within the same habitat) of the taxa in question, and both criteria were independently evaluated. Specificity, fidelity and indicator values were calculated for each family and genus using the following calculations:

$$Aij=N\ individualsij/N\ individualsi \qquad \text{(eq. 1)}$$

where:
Aij is the degree of specificity;
N individualsij is the average number of individuals of taxon i at all group j sites;
N individuals$i$ is the sum of the average number of individuals of taxon i in all groups.

$$B_{ij}=N\ sites_{ij}/N\ sites_j \qquad \text{(eq. 2)}$$

where:
Bij is the measure of fidelity;
N sitesij is the number of sites in group j where taxon i is present;
N sites$j$ is the total number of sites in this group.
Thus, the IndVal percentage for taxon i in group j is as follows:

$$IndVal=Aij*Bij*100 \qquad \text{(eq. 3)}$$

Higher specificity and fidelity of a taxon to a particular habitat, indicate a higher likelihood of its presence in samples from that habitat. Taxa with an IndVal equal to or greater than 50 are considered indicators for a given site, whereas those with an IndVal lower than 50 but greater than 25 are considered *detector Taxa*. Detector Taxa can provide information on environmental changes because they are found in more than one habitat (Tornés *et al*., 2007). The lowest selected weight of 30 was assigned to taxa that were only specific indicators for one ecological status because of the low diversity typical of mountain rivers and the theory of altitudinal zonation, as this value is influenced by abundance (Chang *et al*., 2014; Scheibler *et al*., 2014).

RESULTS

Typification of sites

The Magdalena and Eslava rivers within the CS can be described as tropical region mountain rivers because they exhibit characteristics of pronounced slopes, high oxygen content, lower water temperature, and low chemical element variation (Tab. 1). The river sections within the US were altered by the modification of hydromorphological elements that are typical of CS. The CERA HQ varied widely along the rivers (116-30 points), where the highest value was associated with CS headwaters and the lowest was associated with US reaches. In sites located in the middle of the CS, both rivers were subject to anthropogenic impacts, such as the gabion construction, uncontrolled grazing, unregulated tourist activities, and restaurant establishment, which occurred halfway down the basin to the end of the US. Regarding the hydrological regime, changes in Q3 were observed in the Magdalena River because of seasonal changes and influenced by the large number of dams. This result differs from that of the Eslava River, where changes in Q3 were drastic and indicated a shift from a perennial to a seasonal river over the past two years. Only the headwaters maintained surface water throughout the year. Human intervention in the US zone is also drastic. Drains have replaced the natural channel, the riverbed has been completely modified by the incorporation of lateral and central drainpipes or channeling, and the floodplain has been modified, especially by changes in riparian vegetation.

The Kruskal-Wallis test revealed significant differences in the majority of the physicochemical and bacteriological parameters between the CS and US areas of both rivers, with a gradient of lower headwater concentrations to higher concentrations toward the US zone (H=11-24; P=0.002-0.005). Based on the Mann-Whitney test and ascendant hierarchical grouping analysis, three groups of sites were found to be equivalent for both rivers based on nutrient concentrations (TN and TP) and bacteriological concentrations (FE and FC) (U=0.000; P=0.029) (Tab. 1, Fig. 2).

First, the *potential reference* group covers the sites located at higher altitudes with the highest water quality (DO=6.74-8.1 mg L^{-1}; TN=0.79-2.12 mg L^{-1}; TP=0.36-0.56 mg L^{-1}; FC= -306 UFC 100 mL^{-1}; FE=0-738 UFC 100 mL^{-1}) and lowest anthropogenic disturbance. The soil is occupied by native vegetation, and human settlements are rare. The HQ, naturalness and heterogeneity of the channel were assigned above 100 points. The lowest score was associated with alterations of the channel by small gabions and human influence in certain locations caused by the construction of recreational structures (*e.g*., tourist cabins and restaurants).

Second, the *transition* group includes the first US site in the Magdalena River and last two CS sites in the Eslava River. Water quality is variable and includes natural annual variations in conjunction with the effects of human activity (DO=4.15-6.9 mg L^{-1}; TN=0.73-176.66 mg L^{-1}; TP=0.52-11.47 mg L^{-1}; FC=975000-27 x 10^6 UFC 100 mL^{-1}; FE=94000-11 x 10^6 UFC 100 mL^{-1}). Disturbances are in the form of human settlements. In this group, the HQ is low (≤ 99), which reveals significant alterations (90% modification) to the riparian vegetation in terms of both continuity and naturalness, altered channels related to the presence of gabions and degraded heterogeneous hydromorphological elements resulting from the establishment of human settlements in the riparian zones.

Third, the *degraded* group includes US sampling sites with high degradation and polluted water (DO=2.56-8.06 mg L^{-1}; TN=3.8-62.42 mg L^{-1}; TP=1.18-20.13 mg L^{-1}; FC=87000-44 x 10^6 UFC 100 mL^{-1}; FE=5000-15 x 10^6 UFC 100 mL^{-1}). Alterations at these sites are evident and include the total replacement of natural channels with drains for water from human settlements and total loss of HQ elements (≤30).

Macroinvertebrate classification

A total of 5360 benthic macroinvertebrate specimens belonging to 5 orders, 3 classes, 12 families and 3 subfamilies were identified (Tab. 2). The first two axes of the CCA (Fig. 3, Tab. 2) explained 79% of the total variation (P=0.0001, α=0.05) and indicated that the physicochemical and bacteriological variables, HQ, and benthic macroinvertebrate composition were interrelated. The first axis explained 53% of the variance and was correlated negatively with TN, TP, Q3, and HQ and positively with *Dytiscus*, *Hesperophylax*, Tanypodinae and Oligochaeta. The second axis explained 26% of the variance and was correlated negatively with TN, TP, and Q3 and positively with HQ and *Baetis*, *Tipula*, *Antocha*, *Atopsyche*, *Glossosoma*, *Simulium*, and Planariidae, Podonominae and Nemouridae. Both axes were driven by the better-preserved stretches of the Magdalena River during all seasons (primarily M-CS-1 and M-CS-2). The third axis explained 12% of the total variance and was correlated negatively with TN and TP and positively with HQ, Q3 and *Hydropsyche*, *Limnephilus*, *Epeorus* and Orthocladiinae. These conditions were documented in the middle portion of the Magdalena River in the rainy season at points M-

Tab. 1. Physicochemical and hydromorphological characteristics of the monitoring stations in the Magdalena-Eslava River sub-basin.

Site key/ altitude (m asl)	Distance points* (km) Coordinates	Land cover	Temperature (°C)	pH	K25 (μs cm–1)	DO (mg L^{-1})	Turbidity (NTU)	Q3 (m3 s^{-1})	TN (mg L^{-1})	TP (mg L^{-1})	CERA	Ecological status
Magdalena River Conservation Soils (M-CS-)												
M-CS-1 3099	0 X: 469065 Y: 2130102	*Abies* Forest	5.7-9-87 8.46 ±1.9	6.46-7.52 7.02±0.48	67-225 117±74.5	5.56-9.84 8 ±1.82	0.1-2.6 1.33±1.034	0.43	0.2-2.4 1.27±0.9	0.35-0.8 0.47±0.02	116	PRS
M-CS-2 2727	3.633 X: 471399 Y: 2132466	*Quercus* Forest	7.5-15.1 10.88±3.14	6.95-7.93 7.38±0.5	76-253 129.75±83.6	6.18-8.74 7.23±1.07	0.2-4.2 2.1±1.87	0.5	0.1-2.03 1.04±0.86	0.316-0.73 0.44±0.19	106	PRS
M-CS-3 2698	1.046 X: 472265 Y: 2132875	*Quercus* Forest	8.7-11.79 10.73±1.41	6.87-8.31 7.48±0.69	76-280 142.25±93.6	5.41-8.8 7.51±1.48	-14.6-6.5 1.1±9.5	0.5	0.3-1.26 0.79±0.48	0.343-0.58 0.46±0.09	86	PRS
M-CS-4 2591	1.926 X: 473584 Y: 2134007	Disturbed mixed forest	11.32-13.46 12.04±0.99	6.88-8.03 7.46±0.50	79-487 223±182.52	5.3-8.24 6.74±1.21	5.6-167.5 48±79.8	0.4	0.4-5.46 2.12±2.29	0.37-0.903 0.56±0.25	76	PRS
Eslava River Conservation Soils (E-CS-)												
E-CS-1 3557	0 X:469362 Y:2127247	*Pinus* Forest	6.69-10.46 9.05±1.75	5.55-7.7 6.28±0.1	54-162 91.8±48.41	4.78-10.18 8.1±2.5	0-1.05 0.66±0.47	0.005	0-1.7 1.01±0.8	0.09-0.8 0.36±0.3	116	PRS
E-CS-2 2965	4.033 X: 472925 Y: 2128548	*Abies* Forest	7.43-10.33 9.46±1.37	5.98-7.96 6.83±0.85	73-243 125±79.2	5.98-8.42 7.6±1.1	-0.8-3.4 1.45±1.8	0.03	0.06-1.13 0.8±0.5	0.186-0.963 0.5±0.34	110	PRS
E-CS-3 2769	2.632 X: 474143 Y: 2130330	*Quercus* Forest	11.99-13.17 12.43±0.52	6.12-7.4 6.9±0.62	79-100 89.25±11.9	6.41-7.37 6.9±0.5	12.2-77.8 47.5±30	0.01	0.8-3.93 2.01±1.5	0.14-0.96 0.52±0.34	69	TS
Magdalena River Urban Soils (M-US-)												
M-US-1 2515	0.687 X: 474215 Y: 2134142	Urban zone	12.15-12.48 12.48±0.17	7.11-7.44 7.44±0.16	87-418 93±189.4	5.16-9.06 5.16±1.95	14.5-37.9 14.5±13	0.13	0.166-2.8 0.73±1.39	0.603-0.723 0.72±0.07	69	TS
M-US-2 2475	1.124 X: 475259 Y: 2134397	Urban zone	10.34-13.15 11.68±1.41	7.17-7.2 7.19±0.015	109-2475 914.67±1351.52	1.73-8.7 4.15±3.95	13.1-159 67.47±79.73	0.4	6.66-176.66 63.83±97.7	0.36-32.5 11.47±18.22	35	TS
M-US-3 2493	1.899 X: 476742 Y: 2135610	Urban zone	11.53-17.6 14.2±2.55	7.24-7.86 7.53±0.32	237-2138 849.25±894.2	1.26-7.51 3.35±2.834	33-140.1 89.8±50.3	1.3	15.33-200 62.42±91.72	2.7-26.16 13.95±12.25	30	DS
M-US-4 2394	0.647 X: 476872 Y: 2136241	Urban zone	16.35-18.36 17.32±0.83	7.12-8.21 7.63±0.51	507-2004 934.5±716.35	1.61-5.71 3.13±1.96	19.4-102.3 69.43±39.4	ND	16.66-140 54.42±57.6	3.43-20.66 12.85±7.46	30	DS
M-US-5 2281	6.694 X: 482080 Y: 2140495	Urban zone	16.13-18-38 17.52±0-97	7.25-7.66 7.46±0.2	399-1692 824.75±593.02	1.59-3.45 2.56±1.03	21.5-78.3 55.63±26.32	ND	15.66-126.334.33-15.87 10.06±5.8330 52.083±50.87			DS
Eslava River Urban Soils (E-US-)												
E-US-1 2714	1.086 X: 474382 Y: 2130729	Disturbed mixed forest	10.53-13.21 12.07±1.2	6.48-7.43 7.04±0.4	98-335 175±110.8	6.83-8.74 8.06±0.88	6.4-44.3 23.05±15.9	0.01	0.9-9.9 3.8±4.12	0.36-3.1 1.18±1.3	30	DS
E-US-2 2580	2.692 X: 474564 Y: 2132191	Urban zone	11.48-17.07 14±2.32	5.95-8.06 7.27±0.91	13.14-1378 490.3±610.7	2.1-8.13 4.6±2.9	5.6-341.5 116.6±153.3	0.01	2.86-129 41.01±59.16	1.16-72.73 20.13±35.1	30	DS

**Distance points is the distance in km between two sampling points. K_{25}, specific conductance standardized at 25°C; DO, dissolved oxygen; TN, total nitrogen; TP, total phosphorus; CERA, hydromorphological quality evaluation score (24-120) (Acosta* et al.*, 2009); PRS, potential reference site; TS, transition site; DS, degraded site; ND, no data (these sites were not accessible to the river). Minimum and maximum value, average, and standard deviation correspond to the four sampling stations.*

CS2-R and M-CS3-R. The fourth axis explained 9% of the variance and was correlated negatively with TP, HQ and Q3 and showed a positive relationship between TN contributions, *Polycentropus* and sample stations M-CS-3, M-CS-4, E-CS-1 and E-CS-3, where the concentrations of TN were higher (average 1.60-2.5 mg L^{-1}). Bacteriological groups and TP did not exhibit significant correlations with taxa or sample stations for the CS stations.

According to the correlation values derived from the CCA between benthic macroinvertebrate taxa, HQ and Q3, the following five related categories have been proposed for ecological quality (>0.5, P>0.05) (Tab. 3).

Ecological status 5: *potential reference sites*, which were indicated by organisms that had a clear signal for axis 2 and represent sites with the best conditions.

Ecological status 4: *good ecological quality without potential reference sites*, which were indicated by organisms that had a weight in the analysis below 0.5 in axis 2 and signals in axes 1 and 3.

Ecological status 3: *tolerant sites*, which were indicated by organisms that do not have a signal, have a strong weight within the analysis and could occur in either of the axes.

Ecological status 2: *greater nutrient concentration and reduced discharge flow sites*, which were indicated by organisms with signals in axes 1, 3 and 4 that lacked a strong (values above 0.5) preference for any axis.

Ecological status 1: *high nutrient concentration and lower discharge flow and HQ sites*, which were indicated by organisms that had signals exclusively in axis 3.

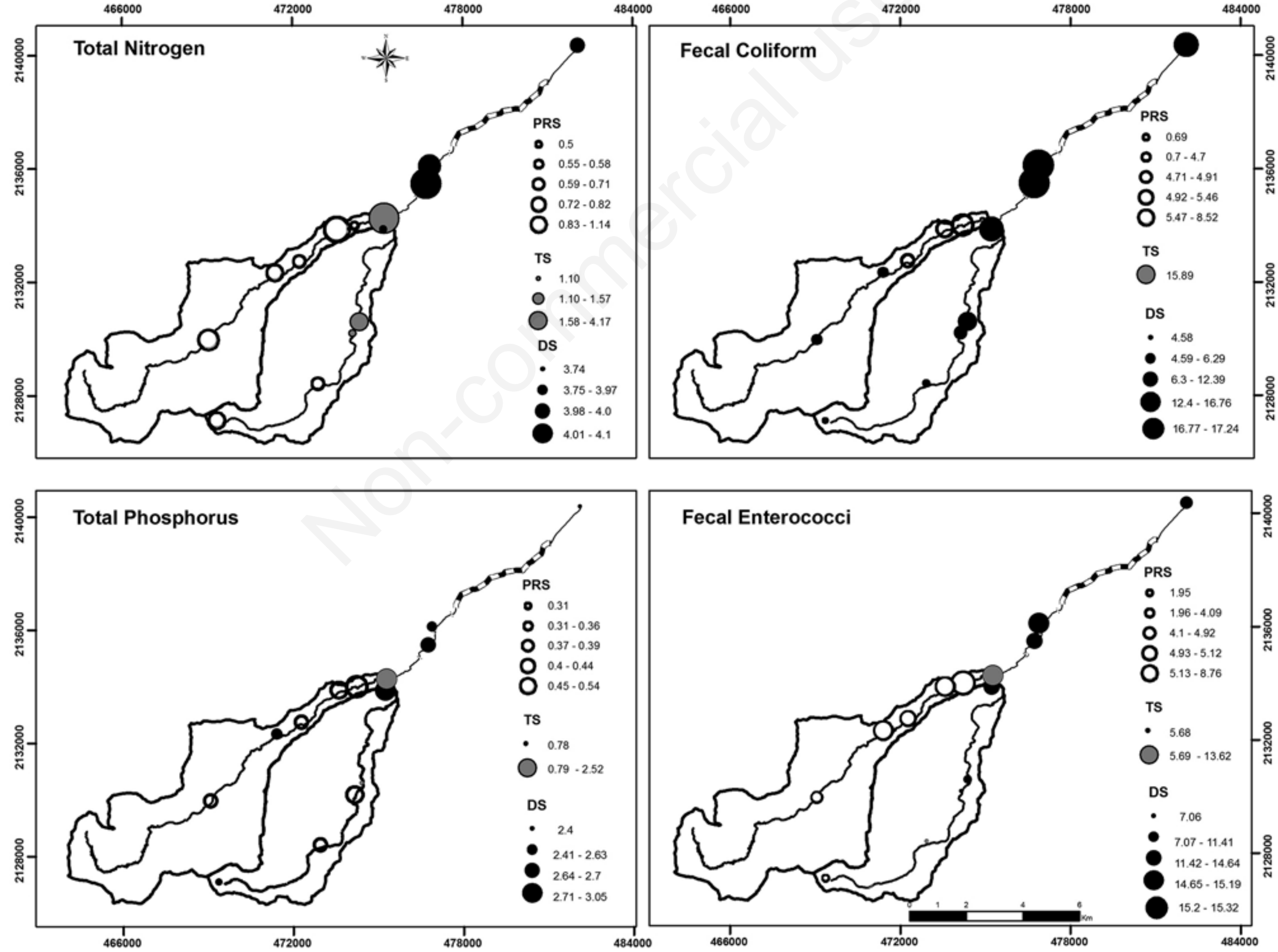

Fig. 2. Total nitrogen, total phosphorus, fecal coliform bacteria and fecal enterococci (n=16, mean values). The color of the circles indicate differences between sites according to the Mann-Whitney Test (α=0.05): white circles indicate potential reference sites PRS); grey circles indicate transition sites (TS); black circles indicate degradation sites (DS) in the Magdalena and Eslava Rivers. Site abbreviations correspond to those shown in Fig. 1.

Tab. 2. Value of the correlation (CCA) between environmental variables, sampling stations, and macroinvertebrate taxa in the Magdalena-Eslava River sub-basin. Site abbreviations correspond to descriptions in Fig. 1.

Variables/factors	Axis 1	Axis 2	Axis 3	Axis 4
Environmental variables	Negative: TN, TP, Q3 CERA	Negative: TN, TP, Q3 Positive: CERA	Negative: TN, TP Positive: CERA, Q3	Negative: TP, CERA, Q3, Positive: TN
Sampling sites	M-CS3-DW E-CS1-DC E-CS-1-DW E-CS2-R1 E-CS2-DC E-CS2-DW E-CS3-DC E-CS3-DW	M-CS1-R1 M-CS1-DC M-CS1-DW M-CS1-R2 M-CS2-DC M-CS2-DW E-CS2-R2	M-CS2-R1 M-CS2-R2 M-CS3-R1 M-CS3-R2	M-CS3-DC M-CS4-DC M-CS4-DW M.CS4-R2 E-CS1-R1 E-CS1-R2 E-CS3-R1 E-CS3-R2
Macroinvertebrates	Tanypodinae (F. Chironomidae) (0.24) Oligochaeta (0.211) *Dytiscus* (1.315) (F. Dytiscidae) *Hesperophylax* (1.114) (F. Limnephilidae)	Podonominae (0.3) (F. Chironomidae) *Baetis* (0.2) (F. Baetidae) *Tipula* (0.834) (F. Tipulidae) *Antocha* (0.843) (F. Tipulidae) *Atopsyche* (0.507) (F. Hydrobiosidae) *Glossosoma* (0.042) (F. Glossosomatidae) *Simulium* (0.4) (F. Simuliidae) Planariidae (0.7) Nemouridae (0.5)	Orthocladiinae (0.715) (F. Chironomidae) *Hydropsyche* (0.84) (F. Hydropsychidae) *Limnephilus* (0.412) (F. Limnephilidae) *Epeorus* (0.3) (F. Heptageniidae)	*Polycentropus* (0.064) (F. Polycentropodidae)

TN, total nitrogen; TP, total phosphorus; Q3, discharge flow; CERA, hydromorphological quality; DW, dry warm; DC, dry cool; R1, R2, rainy.

Tab. 3. Value and indicator status of macroinvertebrate taxa in the Magdalena-Eslava River sub-basin, which accounts for scores obtained in the classification and their correlation with environmental and hydromorphological variables.

Ecological quality	Very good hydromorphological conditions and permanent discharge flows	Good hydromorphological conditions and permanent discharge flows	No difference, which can occur in a wide range of conditions	Preference for greater nutrient concentrations (P and N)	Preference for greater nutrient concentrations (P and N) and flower discharge flows
Score	5	4	3	2	1
Indicator status	Potential reference sites	Good ecological quality	Tolerant	Sites with greater nutrient concentration, reduced flow	Sites with high nutrient concentration and lower discharge flows and hydromorphological quality
Families	Glossosomatidae Tipulidae Hydrobiosidae Nemouridae Heptageniidae	Baetidae Limnephilidae Hydropsychidae	Simuliidae Polycentropodidae Planariidae	Chironomidae Dytiscidae Limnephilidae	Chironomidae Oligochaeta
Subfamilies/Genera	Podonominae *Epeorus* *Tipula* *Glossosoma* *Antocha* *Atopsyche* Nemouridae	*Baetis* *Hydropsyche* *Limnephilus*	*Simulium* Planariidae *Polycentropus*	Tanypodinae Dytiscus *Hesperophylax*	Orthocladiinae

Macroinvertebrate IndVal

In the Magdalena River class I, only eight genera, one subfamily and one family were classified with an IndVal >30%, whereas in the Eslava River class I, one genus, one subfamily and one family were classified with an IndVal >30% and were related to the sample stations with high HQ and raised Q3 (Tab. 4). The lowest weight selected was 30, assigned to those taxa that were only specific indicators for one of the two classes. In class II, only one class with IndVal >30% in the Eslava River CS and no classes with IndVal >30% in the Magdalena River CS were observed. This class is characterized by low indicator values of the sampled organisms, which indicates low specificity and fidelity. *Simulium* was considered a *detector* taxon with a diffuse signal because it exhibited a different IndVal and lacked IndVal >30% in both classes for the same river (14 and 23 for the Magdalena River, and 25 and 7 for the Eslava River).

DISCUSSION

Typifying the sites

The physicochemical water composition and HQ helps to typify the Magdalena and Eslava rivers as high altitude mountain systems. However, they are altered in the middle portion of the basin by the presence of fish farms, restaurants, and recreational activities. These conditions promote highly polluted environments in most downstream sections of the rivers, which are surrounded by urban development that expanded by 29% between 1960 and 2000 (Chávez and García, 2011). Therefore, the degree of deterioration has increased over the 15 years that have passed since the last evaluation. Ecological quality drastically decreased within the CS zone, primarily because of the loss of vegetation cover and changes in channel structure (*i.e.*, gabion construction and channelling). These changes elicited a linear response of ecological degradation and changes in the composition and function of the benthic macroinvertebrate community (*e.g.*, Nijboer and Verdonschot, 2004; Pagliosa and Rodrigues, 2006; Clapcott *et al.*, 2012). CERA was used to catalog the HQ and heterogeneity of fluvial habitat directly related to the diversity of available niches for macroinvertebrates (Acosta *et al.*, 2009; Januschke *et al.*, 2014). The concentrations of nutrients and bacteriological variables in the water were used to identify sites that were subject to some type of human contamination. Usually, P and N are the limiting nutrients for biological activity because they are necessary for primary productivity, and benthic macroinvertebrates respond markedly to their enrichment (Fisher *et al.*, 2004; Nijboer and Verdonschot, 2004; Hering *et al.*, 2006; Pagliosa and Rodrigues, 2006). These characteristics suggest that the monitoring of peri-

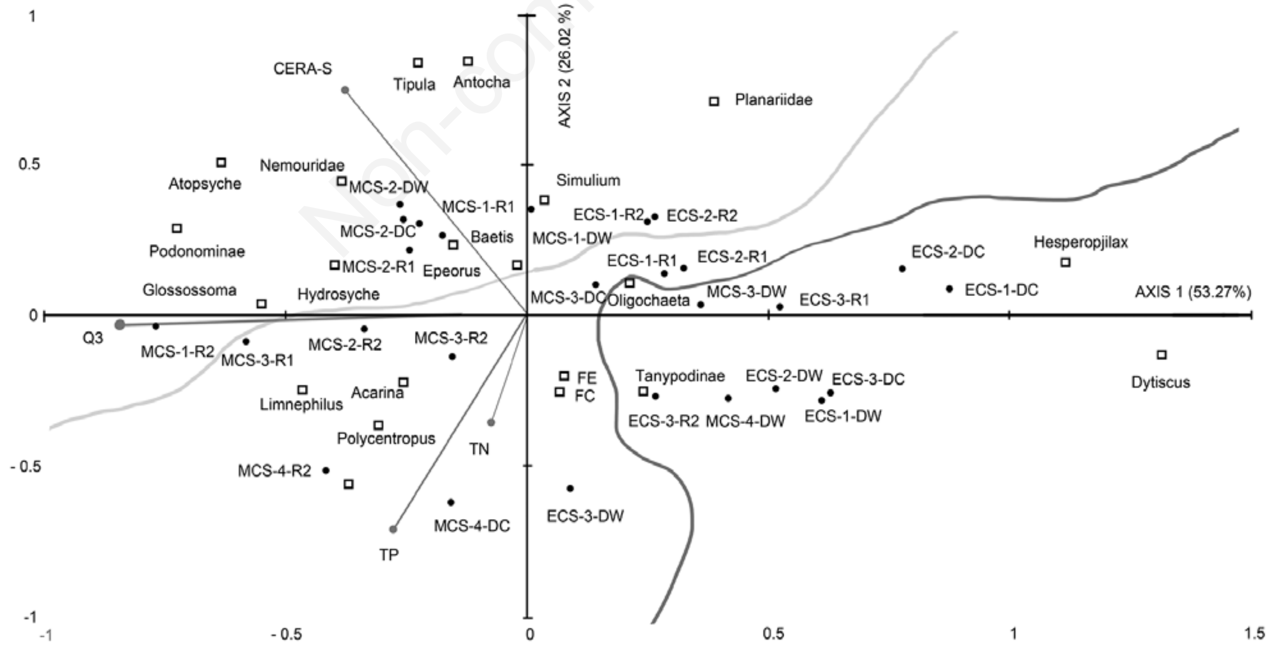

Fig. 3. CCA biplot explaining 79% of the total variance by the first two axes (P=0.0001, α=0.05). Axis 1 is on the left side in light gray, and axis 2 is on the right side in dark gray. The circles correspond to sampling stations, the rectangles correspond to biological data, and vectors correspond to environmental parameters. Site abbreviations correspond to those shown in Fig. 1. DW, dry warm; DC, dry cool; R1, R2, rainy.

urban areas should be based on those variables, which can be used to rapidly identify activities that are potential sources of pollution (Pagliosa and Rodrigues, 2006). The use of such variables presents time and space limitations related to nutrient monitoring and highlights the value of macroinvertebrates that monitor conditions over the aquatic portion of their life cycles and provide broad scale (at least reach scale) monitoring.

Considering the NOM-001-SEMARNAT-1996 (DOF, 2003) criteria, the water in the locations considered reference sites in this study are appropriate sources of water for aquatic life conservation and human consumption. However, this appraisal is insufficient for evaluating ecosystem quality because it only considers water as a function of its potential use and not in terms of its own functional integrity. The ratio of FE to FC at a site could serve as an indicator of the origin (human or animal) of fecal waste material in an aquatic system (Toranzos *et al*., 2007). In both rivers, the presence of fecal contamination at the CS sites was primarily animal in origin (quotient lower than two), whereas at the US sites, it was of human origin (quotient greater than four). This result is consistent with the different land uses in both regions. In addition, the weighting of parameters and physicochemical and bacteriological parameters in the classification analysis confirms the importance of evaluating HQ and Q3 in the classification analysis.

A significant aspect when selecting potential reference sites is the prohibition on hydraulic alterations (*e.g*., gabion dams) (Munné and Prat, 2004; Sánchez-Montayo *et al*., 2009). This condition is not met in the CS zones of either of the studied rivers because the channels are regulated by a number of gabions constructed using local materials (boulders, rocks, etc.). These structures may significantly impact the magnitude, frequency and timing of discharge flows, which has been shown in other mountain rivers (Wohl, 2006; Brown and Pasternack, 2014). Moreover, the channels of both rivers may be modified by the local extraction of water for agricultural, grass, tourism and domestic activities.

Potential reference sites were located in the Magdalena-Eslava River sub-basin based on the physicochemical and bacteriological characteristics of the headwaters of both rivers. The evaluation of HQ revealed channel

Tab. 4. Indicator values of macroinvertebrate taxa (IndVal) in the Magdalena-Eslava River sub-basin. Site abbreviations correspond to descriptions in Fig. 1.

Organisms (	Class I: Magdalena River reference sites M-CS1-R1-2, DC, DC M-CS2-R1-2, DC, DW)			Class I: Eslava River reference sites (E-CS1-R1-2, DC-DW E-CS2-R2)			Class II: altered sites in Magdalena River conservation soils (M-CS3-R1-2, DC, DW M-CS4-R2, DC, DW)			Class II: altered sites in Eslava River conservation soils (E-CS2-R2, DC, DW E-CS3-R1-2, DC, DW)		
	S	F	IndVal (%)	S	F	IndVal (%)	S	F	IndVal (%)	S	F	IndVal (%)
Tanypodinae	0.21	0.88	18	0.65	1	65	0.09	0.71	6	0.05	0.71	4
Podonominae	0.96	1	96	0	0	0	0.04	0	0	0	0	0
Orthocladiinae	0.08	0.13	1	0.32	0.2	6	0.6	0	0	0	0	0
Acarina	0.79	0.88	69	0.08	0.6	5	0.12	0.29	4	0.01	0.29	0
Baetis	0.56	1	56	0.03	0.8	2	0.15	0.71	10	0.26	0.71	19
Polycentropus	0.26	1	26	0	0	0	0.73	0.14	10	0.008	0.14	0
Tipula	0.69	0.38	26	0.18	0.2	4	0	0.14	0	0.13	0.14	2
Antocha	0.81	0.5	41	0.19	0.2	4	0	0	0	0	0	0
Oligochaeta	0.23	0.5	12	0.07	0.2	1	0.13	0.71	10	0.56	0.71	40
Atopsyche	0.88	0.5	44	0	0	0	0.13	0	0	0	0	0
Simulium	0.16	0.88	14	0.41	0.6	25	0.33	0.71	23	0.1	0.71	7
Hydropsyche	0.37	0.25	9	0.2	0.2	4	0.43	0	0	0	0	0
Limnephilus	0.35	0.75	26	0	0	0	0.65	0	0	0	0	0
Glossosoma	0.62	0.75	46	0.02	0.2	0	0.36	0	0	0	0	0
Planariidae	0.24	0.63	15	0.67	1	68	0	0.29	0	0.09	0.29	3
Epeorus sp.	0.53	0.63	33	0	0	0	0.13	0.43	6	0.34	0.43	14
Nemouridae	0.83	0.5	42	0	0	0	0.17	0	0	0	0	0
Dytiscus	0	0	0	0.47	0.6	28	0.05	0.43	2	0.48	0.43	21
Hesperophylax	0	0	0	0.7	1	70	0	0	0	0.3	0.71	22

DW, dry warm; DC, dry cool; R1, R2, rainy; S, specificity; F, fidelity.

modifications that could be acceptable for a potential reference site provided that a monitoring plan is designed to evaluate ecosystem functioning. Monitoring is especially pertinent because it is difficult to locate completely conserved sites in peri-urban watersheds (Wallin *et al*., 2003; Munné and Prat, 2004; Stoddard *et al*., 2006; Sánchez-Montayo *et al*., 2009). Therefore, it is difficult to guarantee that ecosystem functions will be maintained over time because of on-going changes in settlements and hydraulic infrastructure (PUEC-UNAM, 2008).

Macroinvertebrate classification

The diversity of macroinvertebrates recorded in this study (12 families) was concentrated among the Chironomidae, Ephemeroptera, Trichoptera and Plecoptera groups, which is consistent with studies in other mountain rivers, such as that by Compin and Céréghino (2003), who found 283 species corresponding to these taxa and Coleoptera.

The CCA revealed that the indicator value of the taxa was consistent with the ecological characteristics of the sampling stations and suggested that higher HQ and Q3 contributed significantly to explaining the presence of sensitive organisms in the upper sub-basin areas within the CS. These results were also predicted by the River Continuum Concept model (Vannote *et al.* 1980), which states that in natural stream systems, biological communities of the headwaters form a temporal continuum of synchronized species replacements. Downstream communities are fashioned to capitalize on upstream processing inefficiencies, and both the upstream inefficiency (hydraulic intervention and organic pollution) and downstream adjustments are predictable.

Enterobacteria did not have a significant relationship with any environmental or biological variable, which means that contamination by organic material from animals and/or human waste was not a determining factor within the CS. The reduction of HQ elements in both rivers may be related to the presence of trout farms and bovine livestock. In addition, the large number of gabions, which modify the transportation of sediment, may affect the diversity of macroinvertebrates, a pattern observed in other rivers (*e.g*., Fisher *et al*., 2004; Nijboer and Verdonschot, 2004). Sites classified as having good HQ but no significant correspondence with Q3, which might have been caused by the permanence of the river throughout the year, were characterized by the presence of Podonominae, Planariidae, Nemouridae, *Baetis*, *Tipula*, *Antocha*, *Glossosoma* and *Simulium* and related to permanent flows. This finding corresponds with the ecological characteristics of these taxa as described in the literature. Podonominae are frequently found in water with high quantities of abrasive material (gravels and boulders), and their diet consists of periphyton (Ozcos *et al*., 2011). The *Simulium* genus grows in areas with a high current velocity is intolerant of organic contamination and requires a clean substrate upon which to anchor the silk strands that affix them to the substrate (Merritt *et al*., 2008). Two genera were found for the Tipulidae family, and they are intolerant of organic contamination and likely prefer slimy substrates and the presence of vascular hydrophytes and algae. The *Glossosoma*, *Atopsyche*, Nemouridae and Planariidae taxa are often found in upper mountain rivers in clean and cold water that is well-oxygenated (Merritt *et al*., 2008). This is a difference between the North American (NA) and Mexican fauna because most of the NA genera are considered gathering collectors and do not occur in fast waters (Merritt *et al*., 2008). The *Baetis* genus is generally associated with fast currents and can colonize different substrata, such as rocks, gravels, sands, branches, and leaves, where they feed on microalgae and particulate organic matter (Ozcos *et al*., 2011). In general, these taxa are representative of sites with good ecological quality and do not tolerate organic material contamination. However, they also do not tolerate low DO; therefore, the type of organic matter and flow conditions are determining factors. Tons of leaves are introduced to streams from the riparian zones of forested streams every year, and macroinvertebrate shredders tolerate these conditions and are dependent on them (Merritt *et al*., 2008). The presence of such organisms indicates that within the CS, such contamination is relatively insignificant.

The sites positively correlated with higher Q3 and HQ values are represented by the following taxa: Orthocladiinae (most are gathering collectors), *Hydropsyche* (all are filtering collectors), *Limnephilus* (some are detrital shredders) and *Epeorus* (all are scrapers). These shredding and scraping organisms are sensitive to low concentrations of DO and associated with turbulence and high current velocities (Compin and Céréghino, 2003; Guilpart *et al*., 2012). However, most shredders and gathering collectors in NA occur in slower flow areas or in protected (low velocity) areas in faster water (Merritt *et al*., 2008). The difference from the previous axis is the positive relationship with Q3 represented by the rainy season, which reduces the discharge flow by more than half during the dry warm season.

All the Eslava River sites were negatively correlated with HQ and Q3, which might have been caused by the elevated number of channel alterations (gabions and local hydraulic derivations) and areas with lower HQ. The representative taxa are Tanypodinae, Oligochaeta and *Dytiscus*. Adults are able to tolerate low oxygen because they are air breathers, but the larvae of most are not tolerant of low DO because they require aquatic respiration, elevated organic material content and low current velocity. Coleoptera are reported in sites with low current velocity (Compin and Céréghino, 2003) because the larval and adult stages breathe atmospheric oxygen and do not depend on DO, However, certain species have cutaneous aquatic respiration and others have gills, such as Elmidae larvae (Merritt *et al*., 2008). Thus, these invertebrates are indicator species because they can tolerate a broad gradient of DO, and the species that do breathe air are good indicators of low oxygen conditions.

Macroinvertebrate IndVal

CS sites were separated into two classes (potential reference sites and altered sites) for each river based on the same physicochemical and bacteriological parameters established according to the ascendant hierarchical grouping and CCA. However, the IndVal method was more specific than the CCA for determining the ecological quality at the sampling sites and tolerance intervals for each taxon.

Twelve taxa that are indicators of good ecological quality were found in the CS, with most located in the Magdalena River and belonging to Nemouridae, Podonominae, Acarina, *Baetis*, *Tipula*, *Antocha*, *Atopsyche*, *Glossosoma*, *Hesperophylax* and *Limnephilus*; two taxa were found in the Eslava River: Tanypodinae and Planariidae. The taxa that can be considered better indicators of potential reference sites in the Magdalena River were the Acarina class, Nemouridae family, Podonominae subfamily, and *Epeorus, Atopsyche* and *Glossosoma* genera. These taxa have high values of fidelity and specificity; thus, they are representative of well-preserved sites, and a high frequency of occurrence would facilitate their use as bioindicators.

The results revealed that certain combinations of fidelity and specificity provided indicators of the sites. For example, *Antocha* has a high preference for well-preserved sites of the Magdalena River, but their frequency is low, which limits their fidelity. The opposite occurred with the Tanypodinae subfamily and *Baetis*, *Dytiscus*, and *Hesperophylax* genera, which showed a high occurrence in conserved sites of both rivers but varying specificity, which compromised the exclusivity conditions for a particular habitat. In these cases, the organism indicator value and weight of other variables should be considered as the elements of HQ. An example of a tolerant organism was *Simulium*, which maintained a frequency of occurrence in all types of sites and showed poor specificity values for each site. The importance of tolerant organisms is that they provide an early indication of changes in the conditions of ecological quality.

In general, these taxa are congruent with the CCA, although the low abundance and fidelity associated with Oligochaeta and Orthocladiinae explain the relatively insignificant IndVal values in relation to areas where the values were significant. This difference could be related to changing conditions during Q3, when it is more likely for organisms to decrease or increase in abundance and demonstrate changes in fidelity and specificity values. Macroinvertebrates associated with potential reference sites exhibited spatial preferences related to the characteristics of each river. Examples of such preferences are observed in the order Trichoptera, with *Hesperophylax* characteristic of the oligotrophic, low-flow habitat with little heterogeneity found in the Eslava River headwaters and *Limnephilus* characteristic of the higher flow, greater riparian heterogeneity and oligotrophic conditions of the upper portion of the Magdalena River. Both genera are detrital shredders found in headwaters where leaf litter is abundant, and they are key in the transfer of energy to other trophic levels (Bueno-Soria, 2010; Guilpart *et al*., 2012). Similarly, the three Chironomidae subfamilies exhibited different habitat preferences. Tanypodinae and Podonominae may be related to clean water. Tanypodinae can occur in a wide range of environments, and Podonominae is often typical of the rheophilic zone with low temperatures and high DO concentrations. The majority of Podonominae species are found in cold high velocity streams (Ogbeibu and Oribhabor, 2002). In this study, these taxa were related to sites with higher HQ and permanent flows. In contrast, Orthocladiinae are described as tolerant of organic and even heavy metal contamination and were found in the lower parts of the sub-basin, which have greater degrees of human influence and organic material pollution. Thus, it was possible to observe a difference in habitat preference that coincided with the longitudinal degradation gradient of the watershed and low values associated with specificity for a particular habitat.

The highest taxonomic resolution (subfamily and genus) was important for attaining a greater characterization of the associations between organisms and for determining spatial preferences for and ecological quality of the sampling stations. Because most families were represented by only one genus, the analyses performed using either families or genera were similar. Refinement of the monitoring methods presented in this study could be improved if adults were collected to allow for species identifications. In addition, more data on macroinvertebrate adaptations for specific habitat feeding preferences would provide valuable information.

CONCLUSIONS

The proposed evaluation for determining ecological quality in the peri-urban Magdalena-Eslava River sub-basin provided three important conclusions: i) monitoring within the CS zone is determined by Q3 and HQ because they are directly related to channel alterations; ii) these factors could be the most significant elements in evaluating disturbances of ecological quality and the structure and function of benthic macroinvertebrate communities in the remaining rivers in the Mexico Basin; and iii) analyses of nutrient concentrations and Enterobacteria abundance are necessary for evaluating transition sites that show greater evidence of human activity.

Ecological quality in the CS decreases as anthropogenic activities increase. In particular, an increase in the construction of gabions from the headwaters to the lower portions of the rivers induced changes in Q3 that masked seasonal effects (rainy and dry seasons) and affected the benthic macroinvertebrate communities. These hydraulic alterations and their impacts on hydrological dynamics have not been adequately evaluated as disturbances in the sub-basin. The full impact on macroinvertebrates benthic communities is currently underestimated and not fully understood; however, the construction of these structures continues to expand. The characterization of CS potential reference sites proved to be a difficult task because of differences in the description of conserved areas of geographic locations in the literature. However, it is important to assess the socioeconomic context and development of the entire study area to weigh the importance of potential reference status in locations that are in imminent danger of disappearing. Thus, this concept should be flexible and have adaptive capacity.

The suitability of CS potential reference sites was confirmed based on the calculated indicator values of 12 selected benthic macroinvertebrate taxa and was consistent with their known ecological traits. These 12 taxa are as follows: i) scrapers: *Antocha* and *Glossosoma* (fast water habitats); ii) detrital shredders: *Tipula*, *Hesperophylax,* and most *Limnephilus* (except for the last larval instar, which includes scrapers, and Tipulidae (other than *Tipula*), which are almost exclusively predators; the habitat is anywhere terrestrial riparian plant litter accumulates – slow water drop zones or accumulations in front of obstructions in fast water); iii) filtering collectors: none; iv) gathering collectors: certain Podonominae, *Baetis,* and Nemouridae (the habitat is mostly slow water); and v) predators: Planariidae, Acarina, Tanypodinae and *Atopsyche* (habitats include fast current locations, which are mostly out of the current, such as under rocks, and slow current locations).

A change in the structure of this taxonomic and functional macroinvertebrate community and the appearance of other taxa identified as tolerant to pollution could be related to land-use changes and their relationship to the physicochemical properties of water. Therefore, these taxa could be good indicators of potential reference sites in other rivers in the Mexico Basin.

The abundance of high mountain rivers in the Mexico Basin that have similar characteristics to the rivers sampled in this study demonstrates potential vulnerability to increased urban expansion. The sub-basin of the Magdalena-Eslava River functions as a pilot area for the implementation of conservation and management programs. Thus, this work is an important reference for evaluations and assessments of the effects of anthropogenic interventions in aquatic ecosystems near urban locations.

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