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Research Article

Validation of an index of biological integrity based on aquatic macroinvertebrates assemblages in two subtropical basins of central Mexico

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ABSTRACT. The increasing degradation of freshwater ecosystems has demanded the development of methods that allow us to evaluate the ecosystem alterations. The Indexes of Biological Integrity (IBI) are a methodological approach to assess the condition of aquatic ecosystems. The objective of this study was to estimate the biological integrity and to validate the Index of Biological Integrity based on macroinvertebrate assemblages (IIBAMA) in 33 study sites rivers in the Lerma-Chapala and Pánuco river basins, in central Mexico. The Family-Level Biotic Index (FBI) was used to estimate the water quality and the Visual-Based Habitat Assessment (VBHA) was used to estimate the habitat quality. Spearman correlation analysis among IIBAMA, FBI, VBHA and water characteristics were made for validation of the IIBAMA. Besides, all variables were evaluated together by multivariate analyses. The rivers were classified in three of four biotic integrity categories, poor, regular and good, being poor the most common (88% of the study sites). We did not find study sites with excellent biotic integrity. The rivers of the Lerma-Chapala River Basin showed a worse ecological condition compare with the Pánuco Rivers Basin. We validated and recommended the using of the IIBAMA to assess the biological integrity of these two basins and rivers in central Mexico. This research represents the first efforts to validate an IBI based on aquatic invertebrate communities in a broad scale in Mexico and provide a general framework for their widespread use and to approach the validation and implementation of IBIs in other regions with similar conditions.

Keywords: biological integrity, biological monitoring, aquatic macroinvertebrates, freshwater ecosystem, environmental condition.

INTRODUCTION

The increasing degradation of freshwater ecosystems has recently demanded the development of methods that allow us to know the significance of the alteration due to human activities and to differentiate it from natural effects (Mercado-Silva *et al*., 2006b). The Index of Biological Integrity (IBI) is a methodological approach that combines structural and functional elements of aquatic ecosystems to assess the ecological condition (Moya *et al*., 2007). Biological integrity is defined as the environment´s capability to support and maintain a balanced and adapted community of organisms that have a specific composition, diversity and functional organization (Karr, 1981). The assessment of biological integrity in freshwater ecosystems allows a holistic estimation of the negative effects of the impact of human activities, and it is a methodology widely used to guide the management of aquatic resources in several parts of the world (Wente, 2000; Mercado-Silva *et al*., 2006a; Alexandrino *et al*., 2017). The quantification of biological integrity is obtained by adding the values of measurable ecological attributes, such as the structure, composition, and function of a biological community (Weigel *et al*., 2002).

The IBI is one strategy with right cost-benefit balance, and it is scientifically valid and oriented to i) facilitate the analysis of multiple study sites, ii) obtai-

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ning quick results, iii) providing scientific reports for easy access for the public, and iv) promoting environmentally healthy practices (Moya *et al*., 2007). Also, the IBI is used for biological monitoring for environmental risk assessments, because it measures the numerous biological conditions present and not only chemical ones; therefore, it becomes a significant source of information, describing expected environmental conditions in the absence of human impact (Alexandrino *et al.*, 2017). Also, the IBIs are designed to assess regional conditions (mostly the unit is one basin), representing regional processes (Moya *et al*., 2007).

However, it is not enough to generate and to apply the IBI, it must be validated before proposing it for extended use (Lyons *et al*., 2000; Ramírez-Herrejón *et al*., 2012). The validation consists of the analysis of IBI data and its correlation with the water physicochemistry and habitat variables. The validation is supported under the premise that water physical and chemical conditions and the physical condition of the habitat are the primary influence factors on the assemblages of biological communities of rivers (González-Zuarth *et al*., 2014). In this way, the aquatic macroinvertebrates assemblages and physical and chemical environmental conditions respond together to the natural and anthropic alterations of streams and rivers (Merritt *et al*., 2008).

In the development of these methodologies, the aquatic macroinvertebrates are used as a study model, due to the rich data that they provide (Bonada *et al*., 2006; Serrano-Balderas *et al*., 2016): a) aquatic macroinvertebrates are structured assemblages made up of taxa with broad ecological functions. Ranging from generalists to micro-specialists, they rapidly respond to anthropic and natural changes of freshwater systems, b) they are relatively sedentary and representative of the area where they are collected, c) they have relatively short life cycles, and they reflect the changes in their environment rapidly, and d) they live in or on the sediment allowing the accumulated organic matter to return to the trophic web.

Only two IBIs based on aquatic macroinvertebrates have been developed for freshwater ecosystems in Mexico. The first was developed by Weigel *et al*. (2002) in streams of the Sierra de Manantlán Biosphere Reserve. The second, the Index of Biological Integrity based on macroinvertebrates assemblages (IIBAMA) was developed by Pérez-Munguía & Pineda-López (2005) to estimate the environmental condition of rivers and streams in central Mexico, including the Mexican states of Guerrero, Jalisco, Hidalgo, State of Mexico, Querétaro, and Michoacán. The IBI of Weigel *et al*. (2002) in the west-central Mexico shows a methodological disadvantage because it is based on the taxonomic level of genera, increasing the difficulty of its application, contradicting the premise of the simple use of the index, *i.e.*, to facilitate the analysis of multiple study sites and obtaining quick results. On another hand, the IIBAMA is based on the taxonomic level of family, and this taxonomic resolution represents a confident indicator of the degradation level in river ecosystems (Serrano-Balderas *et al*., 2016; Wright & Ryan, 2016). The IIBAMA has been validated with independent data; however, the validation was done only for two rivers and two streams located in the Lerma-Chapala River Basin (LRB) and Balsas River Basin, in the Michoacán State (Pérez-Munguía *et al*., 2006; Piñón-Flores *et al*., 2014). IIBAMA represents a useful tool for the biological monitoring of the environmental quality in the Chiquito River in the Michoacán State (Piñón-Flores *et al*., 2014). However, it is not validated for its widespread use in other streams and rivers in wider regions of Mexico.

The Lerma River Basin is considered as the most degraded basin in Mexico (Cotler-Ávalos & Garrido-Pérez, 2010) and the Pánuco River Basin (PRB) has been considered a priority zone for conservation (Wikramanayake *et al*., 2002; Gutiérrez-Yurrita *et al*., 2013). For these reasons, both basins have streams and rivers located on an environmental condition gradient with different conservation status, which represent an appropriate model to validate the IIBAMA. Because of this, the present study focuses on estimating the biological integrity based on aquatic macroinvertebrate assemblages and validating the IIBAMA in the headwaters of 12 permanent rivers of Lerma-Chapala River Basin and Pánuco River Basin located in five Mexican states (Aguascalientes, Jalisco, Guanajuato, Querétaro, and San Luis Potosí) in Central Mexico.

MATERIALS AND METHODS

Study area

The study area is in the LRB and PRB, found in the east-central region of Mexico (Fig. 1). Central Mexico has the most degraded basins in the country (Mercado-Silva *et al*., 2006b). Lerma-Chapala River Basin shows a distinct problem of physical and chemical anthropogenic transformation and is considered the most degraded in Mexico (Cotler-Avalos *et al*., 2004). This river basin also suffers from excessive water extraction to cover the needs of Mexico City inhabitants (Rascón *et al*., 2001). The Lerma-Chapala River Basin has been profoundly impacted by the loss of vegetation cover (>30%), expansion of cultivated pastures for livestock, increased agricultural activities combined with expanded industrialization and urbanization (Cuevas *et al*., 2010).

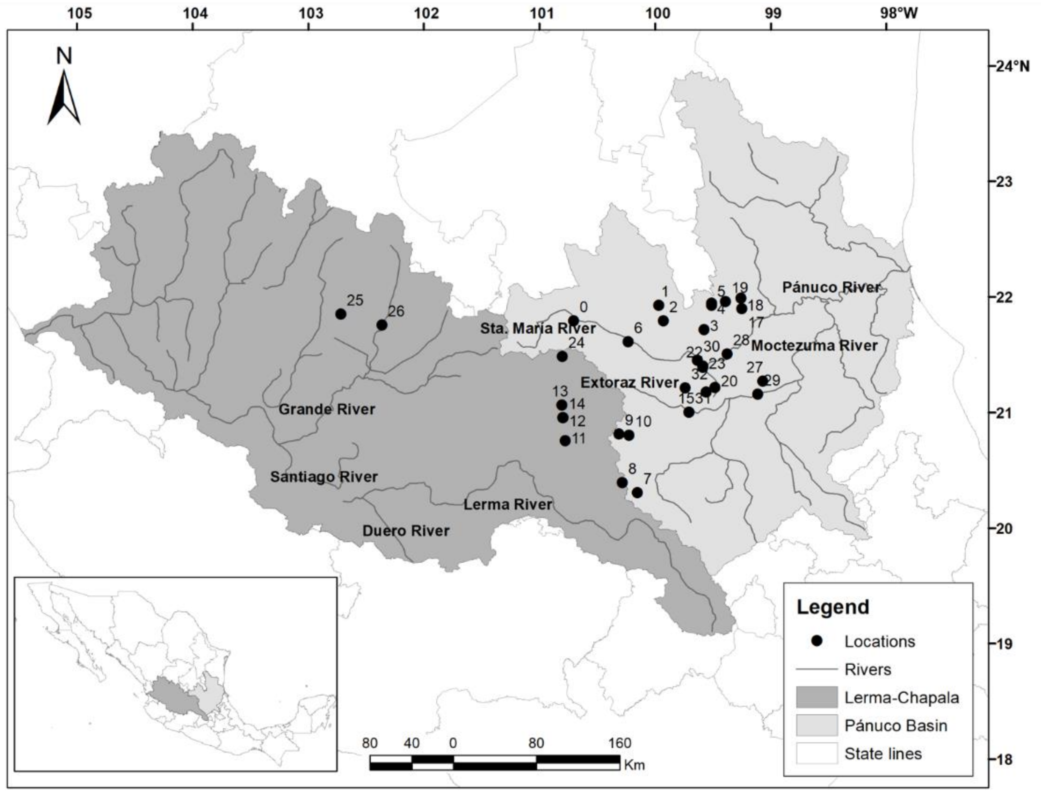

Figure 1. Sampling locations. 1) Fracción Sánchez, 2) La Planta-La Hacienda, 3) Puente La Plazuela, 4) Pinihuan, 5) Canoas, 6) Quinta Matilde, 7) El Realito, 8) Quiotillos, 9) El Salto, 10) Presa del Carmen, 11) Presa de Rayas, 12) Comonfort, 13) La Quemada, 14) Los Galvanes, 15) El Xote, 16) El Oasis, 17) Chuveje, 18) Carpintero, 19) Rascón, 20) Tamasopo, 21) Jalpan, 22) Ayutla, 23) Santa María, 24) El Carrizal, 25) Río Grande, 26) Calvillo, 27) El Salto de los salados, 28) Tancuilín, 29) Santa María (Tancoyol), 30) Río Moctezuma, 31) Concá, 32) Extoraz, 33) Río Blanco.

Meanwhile, the PRB shows a severe problem with water pollution and the water exploitation activities mainly for irrigation and drainage control. However, this basin harbors one natural protected area with high biodiversity, the Sierra Gorda Biosphere Reserve (Ruiz-Corzo & Pedraza-Ruiz, 2007). This Biosphere Reserve is characterized by their biological importance and the conservation status of their natural and ecosystem elements and process (Carabias-Lillo *et al*., 1999).

Both river basins suffer serious problems of environmental degradation, such as pollution and structural modification in the high parts of the basin, caused mainly by industry, livestock activity, and farming, as well as the increase in urban sprawl (Álvarez *et al*., 2008). In addition, at present, the headwaters of both drainages are being considered for special protection status as Water Reserves in Mexico by the National Commission of Water (Comisión Nacional del Agua, 2011).

The sampling sites are in permanent rivers from the headwater of San Pedro River and Calvillo River in Aguascalientes State (LRB), Grande River in Jalisco State (LRB), Laja and Apaseo rivers in Guanajuato State (LRB), rivers Extoraz, Huimilpan, Querétaro, San Juan, Jalpan, and Santa María in Querétaro State (PRB), and Verde River in San Luis Potosí State (PRB).

Data collection

A total of 33 study sites were selected from a habitat quality gradient (Fig. 1). The field work was done during the dry season (February-Abril 2014) for several reasons: i) dry season represents the more stable habitat conditions, ii) the low-flow phase of the river exposes aquatic macroinvertebrates for sampling, iii) human impacts are enhanced creating spatial variation along the length of the river system, and iv) for comparing to previous studies, because research on river ecology is commonly done during the dry season (Moncayo-Estrada *et al*., 2015).

Prior to the macroinvertebrates sampling, chemical and physical water characteristics were measured with a multimeter (HachHydromet Quanta, Loveland, Colorado, USA) including pH, dissolved oxygen (DO, mg L^{-1}), total dissolved solids (TDS, g L^{-1}), conductivity (C, mS cm^{-1}) and temperature (°C). The condition of the habitat was assessed by a Visual-Based Habitat Assessment (VBHA) proposed by Barbour *et al*. (1999), that includes variables as sinuosity, materials of the substrate and the banks, sediment retention points, condition of riparian vegetation and riparian zone, and the status of the floodplain (Table 1).

The macroinvertebrate samples were collected using a D-net (300 mm of diameter and 300 μm of mesh size) in all available habitats with a sample effort of 30 min per site, including all of the microhabitats in a section of the river (five times the width of the river, following the Official Mexican Standard NMX-AA-159-SCFI-2012). The macroinvertebrate individuals were separated from detritus in the field and were preserved in a solution of ethanol 80%, and the samples were transported to the Biotic Integrity Lab at UAQ-Campus Aeropuerto. The taxonomic identification of macroinvertebrates was made to the level of family based on specific keys (*e.g*., Merritt *et al*., 2008).

Additionally, we estimated the Family-Level Biotic Index (FBI) proposed by Hilsenhoff (1987) as an auxiliary tool for the validation of the IIBAMA, because it is a rapid bioassessment procedure related to water quality which has been validated for the west central region of Mexico (Weigel *et al*., 2002). FBI is based mainly on the tolerance values for arthropods families and the number of individuals per family.

Index of Biological Integrity

The biological integrity was assessed using the IIBAMA proposed by Pérez-Munguía & Pineda-López (2005). The metrics and explanation of each variable of the index are described below:

1) Taxa Richness (TR).

This metric refers to the number of macroinvertebrates families founded in the sample. The taxa distribution is limited by the heterogeneity of ecological process (Hengeveld, 1996; Lambeck, 1997), for this reason, a high taxa richness can highlight a habitat heterogeneity (Williams, 1964; Currie, 1991; Tews *et al*., 2004). This habitat heterogeneity is related to the availability of fauna refuges, and it is associated with an increased speciation likelihood (Seto *et al*., 2004).

2) Ephemeroptera, Plecoptera and Trichoptera Richness (EPTR).

This metric must be calculated with the number of Families included in the Ephemeroptera (except the Baetidae family), Plecoptera and Trichoptera Orders (EPT) founded in the sample. These mentioned Orders are important biological groups because of their wide distribution, high abundance, and species richness, and are key elements for an ecological process such as the nutrients cycles in freshwater ecosystems (Righi-Cavallaro *et al*., 2010). These groups are associated with the transformation of organic matter into available nutrients for superior trophic levels (Graça *et al*., 2001; Boyero *et al*., 2012), and they represent the food of vertebrates and other macroinvertebrates (Ferro & Sites, 2007). The EPT Orders are sensitive indicator of right ecological conditions due to their low tolerance to environmental stress, which means the families composition and richness are negatively affected by degraded environmental conditions (Usseglio-Polatera *et al*., 2000; Callisto *et al*., 2001; Klemm *et al*., 2003; Ferreira *et al*., 2011). EPT is usually present in aquatic ecosystems with high water quality (Lemly, 1982; Buss *et al*., 2004; Bispo *et al*., 2006). For these reasons, EPT is considered a good indicator of water quality (Rosenberg & Resh, 1993).

3) The Richness of Sensitive Insects (RSI).

This metric refers to the number of Families of aquatic insects that are sensitive to environmental degradation. The insects are the most conspicuous group of macroinvertebrates of freshwater ecosystems (Macadam & Stockan, 2015). They can fly between freshwater bodies during adult stages as a survival strategy. However, the absence of sensitive insects is related with limiting conditions of temperature, dissolved oxygen, alkalinity, salinity, water flow rate, water level, aquatic vegetation cover and specific substrate (Ward, 1992). For these reasons, sensitive insects offer current and long-term information about environmental conditions.

4) The Richness of Sensitive Taxa (RST).

This metric combines the previous RSI with the rest of sensitive macroinvertebrate families. The sensitive taxa of aquatic macroinvertebrates (not insects), generally, spend no part of their lifecycle out of the water. For this reason, their presence can indicate an ecosystem where the habitat quality has been optimal for a long time.

5) Tolerance Value Average (TVA).

This metric refers to the average values of tolerance of the sample. The tolerance represents the capability of aquatic macroinvertebrate to survive under environmental degradation. The values of tolerance show a relationship among anthropic stress and the presence of aquatic organisms in a spatiotemporal way. For this reason, TVA indicates the condition of freshwater systems (Chutter, 1972; Winget & Mangum, 1979; Hilsenhoff, 1987; Lenat, 1993).

6) The number of Clingers Taxa (#CT).

This metric refers to the number of taxa that have life habits gripping to the substrate. These organisms are moderately sensitive to water pollution, and they depend

Table 1. Characterization of the study sites in Lerma-Chapala River basin and Pánuco River Basin based on the Visual-Based Habitat Assessment (VBHA). EB: Embeddedness, SD: Sediment deposition, CA: Channel alteration, FR: Frequency of riffles, BS(L&R): Bank stability (left and right bank), BVP(L&R): Bank vegetative protection (left and right bank), RVZW(L&R): Riparian vegetative zone width (left and right bank), C: Category, D: Description, O: Optimal, SO: Suboptimal, MG: Marginal, P: Poor. *Because of the large size of the table, we do not include the following variables: Epifaunal substrate/Available cover; Velocity/Depth combinations; Channel flows status, and Frequency of riffles. ES: El Salto, PC: Presa del Carmen, PR: Presa de Rayas, Com: Comonfort, LQ: La Quemada, LG: Los Galvanes, Xo: El Xote, RG: Río Grande, Cal: Calvillo, SS: El Salto de Los Salados, FS: Fracción Sánchez, PH: La Planta-La Hacienda, PP: Puente la Plazuela, Pin: Pinihuan, Can: Canoas, QM: Quinta Matilde, ER: El Realito, Qui: Quiotillos, EO: El Oasis, Chu: Chuveje, Car: Carpintero, Ras: Rascón, Tam: Tamasopo, Jal: Jalpan, Ayu: Ayutla, SM: Santa María, EC: El Carrizal, Tan: Tancuilín, SMT: Santa María (Tancoyol), RM: Río Moctezuma, Con: Concá, Ex: Extoraz, RB: Río Blanco.

Basin	Sites	EB		SD		CA		BS(L&R)		BVP(L&R)		RVZW(L&R)	
		C	D	C	D	C	D	C	D	C	D	C	D
Lerma-Chapala	ES	O	Gravel, cobble, and boulder particles are 0-25% surrounded by fine sediment.^{1}	O	Less than 5% of the bottom affected by sediment deposition^{1}	O	Stream with normal pattern^{1}	O	<5% of bank affected^{1}	O	More than 90% of the riparian zones covered by native vegetation^{1}	P	Width of riparian zone <6 m^{4}
	PC	-	-	-	-	-	-	-	-	-	-	-	-
	PR	MG	Gravel, cobble, and boulder particles are 50-75% surrounded by fine sediment^{3}	P	More than 50% of the bottom changing frequently^{4}	P	Over 80% of the stream reach channelized and disrupted^{4}	P	60-100% of bank has erosional scars^{4}	P	Less than 50% of the streambank surfaces covered by vegetation^{4}	P	4
	Com	MG	3	P	4	MG	40 to 80% of stream reach channelized and disrupted^{3}	P	4	MG	50-70% of the streambank surfaces covered by vegetation^{3}	MG	The width of the riparian zone between 6-12 m^{3}
	LQ	SO	Gravel, cobble, and boulder particles are 25-50% surrounded by fine sediment^{2}	P	4	SO	Some channelization present, usually in areas of bridge abutments.^{2}	SO	5-30% of the bank in reach has areas of erosion^{2}	P	4	P	4
	LG	P	Gravel, cobble, and boulder particles are more than 75% surrounded by fine sediment^{4}	P	4	SO	2	MG	30-60% of the bank in reach has areas of erosion^{3}	MG	3	P	4
	Xo	P	4	P	4	SO	2	SO	2	SO	70-90% of the streambank surfaces covered by native vegetation.^{2}	P	4
	RG	MG	3	P	4	MG	3	SO	2	SO	2	SO	Width of riparian zone 12-18 m^{2}
	Cal	-	-	-	-	-	-	-	-	-	-	-	-
	SS	O	1	O	1	O	1	O	1	O	1	P	4
Pánuco	FS	P	4	P	4	MG	3	P	4	P	4	P	4
-	PH	SO	2	SO	5-30% of the bottom affected.^{2}	O	1	MG	3	MG	3	P	4
-	PP	SO	1	SO	2	O	1	O	1	O	1	SO	2
-	Pin	O	1	O	1	O	1	O	1	O	1	O	Width of riparian zone >18 m^{1}
-	Can	O	1	O	1	O	1	O	1	O	1	O	1
-	QM	O	1	O	1	O	1	O	1	O	1	O	1
-	ER	SO	2	P	4	O	1	O	1	O	1	O	1
-	Qui	O	1	O	1	O	1	O	1	O	1	MG	3
-	EO	O	1	SO	2	MG	3	SO	2	SO	2	SO	2
-	Chu	O	1	O	1	P	4	SO	2	MG	3	MG	3
-	Car	O	1	O	1	O	1	O	1	O	1	O	1
-	Ras	O	1	SO	2	O	1	O	1	O	1	O	1
-	Tam	O	1	O	1	O	1	O	1	O	1	O	1
-	Jal	SO	2	O	1	P	4	MG	3	MG	3	MG	3
-	Ayu	SO	2	O	1	P	4	SO	2	MG	3	MG	3
-	SM	SO	2	O	1	SO	2	SO	2	MG	3	P	4
-	EC	SO	2	SO	2	MG	3	SO	2	MG	3	MG	3
-	Tan	O	1	O	1	O	1	O	1	O	1	O	1
-	SMT	SO	2	MG	30-50% of the bottom affected.^{3}	O	1	SO	2	SO	2	SO	2
-	RM	O	1	O	1	SO	2	O	1	SO	2	SO	2
-	Con	O	1	O	1	P	4	O	1	P	4	P	4
-	Ex	MG	3	MG	3	SO	2	SO	2	P	4	MG	3
-	RB	O	1	O	1	MG	3	SO	2	SO	2	P	4

on biotope diversity and heterogeneity of flow patterns (Posada-García & Roldán-Pérez, 2013). Accordingly, the #CT depletion can indicate the loss of aquatic habitat heterogeneity and availability, caused by the riverbank's degradation (Pérez-Munguía & Pineda-López, 2004). Also, the land use change in the catch-

Table 2. Criteria to the assignation of the scores of each parameter of the Index of Biological Integrity based on aquatic macroinvertebrates assemblages (IIBAMA). TR: Taxa richness, EPTR: Ephemeroptera, Plecoptera, and Trichoptera Richness, RSI: Richness of sensitive insects, RST: Richness of sensitive taxa, TVA: Tolerance value average, #CT: Number of clinger taxa. "Y" represents the value obtained for each variable.

Variable	Category/Score 1	2	3	4	Response to degradation
TR	Y<23	23≤Y<27	27≤Y<30	Y≥30	Decrease
EPTR	Y<9	Y=9	Y=10	Y≥11	Decrease
RSI	Y<9	9≤Y<12	12≤Y<14	Y≥14	Decrease
RST	Y<10	10≤Y<12	12≤Y<14	Y≥14	Decrease
TVA	Y≥5.33	5.13≤Y<5.33	4.65≤Y<5.13	Y<4.65	Increase
#CT	Y<9	9≤Y<11	Y=11	Y≥12	Decrease

ment, that can increase in fine sediments depo-sition can reduce available habitat (Wood & Armitage, 1997) and food resource (*cf.* Yamada & Nakamura, 2002) for clinger organisms.

The index is calculated by the sum of the scores obtained from each variable (Table 2). The information about each variable of the index; tolerance value and life habit were obtained from Pineda-López *et al.* (2014).

Statistical analysis

The IIBAMA was validated through the comparison among the values of IIBAMA, and the values of FBI, VBHA, and the chemical and physical water characteristics. The correlations among IIBAMA with FBI, VBHA and water characteristics (pH, DO, temperature) were made by the Spearman correlation analysis (Zar, 1999) using the software SPSS ver. 20 (IBM Corp., 2011). All variables were evaluated together to analyze and to elucidate patterns of all measured parameters in both river basins, a principal component analysis (PCA) ordination was conducted using PAST ver. 3.07 (Hammer *et al.*, 2001). For this analysis, we normalize all variables using division by their standard deviations because the indices and variables were measured in different units. Additionally, to compare the differences between basins, we analyzed similarities (ANOSIM), which is a robust method to compare groups of multivariate sample units (Clarke, 1993; Anderson & Walsh, 2013).

RESULTS

We collected a total of 10,723 individuals, included in 86 families (Table 3), distributed in five classes: i) Insecta (eight families belong to Ephemeroptera, nine to Odonata, one to Plecoptera, 12 to Hemiptera, 10 to Trichoptera, one to Megaloptera, 14 to Coleoptera, 16 to Diptera, and one to Lepidoptera), ii) Maxillopoda (two families belong to order Decapoda, one to Amphipoda, and one to Isopoda); iii) Gastropoda (one family belong to Unionida order, one to Veneroida, two to Basommatophora, four to Neotaenioglossa; iv) Turbellaria (one family belonging to Tricladida order); and v) Acari (the order Hydrachnidia). From these groups, seven families were determined as very tolerant, 28 as tolerant, 29 such as intolerant, six as very intolerant, and 16 were not classified. Furthermore, we obtained 31 families with clinger's habits, 13 swimmers, ten climbers, five skaters, 11 burrowers, one hiker, and 15 were not determined (Table 3).

We obtained the following mean values, pH: 7.86 ± 0.41; TDS 363.54 ± 236.77 g L^{-1}; DO 4.13 ± 2.4 mg L^{-1}; and temperature: 21 ± 4.68°C, including both basins (Table 4). The habitat quality based on VBHA were estimated as Optimal for eight localities, Suboptimal in 16 localities, Marginal in four localities, Poor in four localities, and one site was not determinate. The FBI shows three localities with excellent conditions, two as very good, eight as good, 11 as fairly, five as fairly poor, three as poor, and one as very poor. Considering the IIBAMA, 87.88% of all sites shows a poor condition (IIBAMA<13), and 6.06 % moderate (13< IIBAMA<16) and 6.06% good (16<IIBAMA<21) (Table 4).

The rivers were classified in three of four biotic integrity categories, poor, regular and good (88%, 6%, 6% of the study sites respectively), we did not find study river locations with excellent biotic integrity. The associations of the IIBAMA scores showed significant correlations with measures of FBI, TDS, pH, DO (r = -0.38, P = 0.029; r = -0.39, P = 0.022; r = 0.559, P = 0.001 and r = 0.522, P = 0.002 respectively); however, there were no significant correlations with VBHA and temperature (r = 0.318, P = 0.076 and r = 0.208, P = 0.246 respectively) (Fig. 2).

In the relationship among the values of IIBAMA, the values of water pH and water DO were positive and

Table 3. Families collected in rivers of Lerma-Chapala River Basin and Pánuco River Basin and attributes used for the Index of Biological Integrity based on aquatic macroinvertebrates assemblages (IIBAMA). UNK: Unknown, I: Intolerant, T; Tolerant, VT: Very tolerant, VI: Very intolerant, Clg: Clinger, Sw: Swimmer, Br: Burrower, Clb: Climber, Sk: Skater, Hk: Hiker.

Family	Tolerance value	Tolerance	Life habit	Class	Order
Baetidae	5	I	Clg	Insecta	Ephemeroptera
Ephemerellidae	3	I	Clg	-	-
Polymitarcyidae	UNK	UNK	-	-	-
Caenidae	6	T	Clg	-	-
Leptophlebiidae	3	I	Sw	-	-
Leptohyphidae	6	T	Clg	-	-
Heptageniidae	3	I	Clg	-	-
Ephemeridae	UNK	UNK	-	-	-
Gomphidae	3	I	Br	-	Odonata
Coenagrionidae	8	T	Clb	-	-
Lestidae	9	VT	Clb	-	-
Platystictidae	UNK	UNK	Sw	-	-
Macromiidae	UNK	UNK	-	-	-
Libellulidae	9	VT	Sw	-	-
Aeshnidae	3	I	Clg	-	-
Calopterygidae	6	T	Clb	-	-
Protoneuridae	UNK	UNK	-	-	-
Perlidae	1	VI	Clg	-	Plecoptera
Corixidae	9	VT	Sw	-	Hemiptera
Hebridae	UNK	UNK	Clg	-	-
Veliidae	6	T	Sk	-	-
Mesovellidae	UNK	UNK	Sk	-	-
Gerridae	5	I	Sk	-	-
Belostomatidae	10	VT	Clb	-	-
Naucoridae	5	I	Sw	-	-
Notonectidae	4	I	Sw	-	-
Saldidae	10	VT	Clb	-	-
Pleidae	UNK	UNK	Sw	-	-
Macroveliidae	UNK	UNK	Sk	-	-
Nepidae	UNK	UNK	-	-	-
Corydalidae	0	VI	Clg	-	Megaloptera
Hydroptilidae	4	I	Clg	-	Trichoptera
Polycentropodidae	5	I	Clg	-	-
Philopotamidae	3	I	Clg	-	-
Odontoceridae	0	VI	Clb	-	-
Hydrobiosidae	UNK	VI	Clg	-	-
Limnephilidae	3	I	Clg	-	-
Calamoceratidae	3	I	Clg	-	-
Lepidostomatidae	1	VI	Clg	-	-
Leptoceridae	4	I	Clg	-	-
Hydropsychidae	4	I	Clg	-	-
Gyrinidae	4	I	Sk	-	Coleoptera
Dytiscidae	6	T	Sw	-	-
Hydrophilidae	5	I	Clg	-	-
Helophoridae	5	I	Br	-	-
Staphylinidae	8	T	Clg	-	-
Hydraenidae	5	I	Clg	-	-
Psephenidae	4	I	Clg	-	-
Scirtidae	7	T	Clb	-	-
Dryopidae	5	I	Br	-	-
Elmidae	4	I	Clg	-	-

Continuation

Family	Tolerance value	Tolerance	Life habit	Class	Order
Limnichidae	3	I	Clg	-	-
Lutrochidae	3	I	Clg	-	-
Ptiliidae	UNK	UNK	-	-	-
Haliplidae	7	T	Clb	-	-
Tipulidae	3	I	-	-	Diptera
Ceratopogonidae	6	I	Br	-	-
Chironomidae	6	I	Br	-	-
Simuliidae	6	I	Clg	-	-
Syrphidae	10	VT	-	-	-
Dixidae	1	VI	Sw	-	-
Culicidae	8	T	Sw	-	-
Thaumaleidae	UNK	UNK	-	-	-
Tabanidae	6	T	-	-	-
Stratiomyidae	7	T	Br	-	-
Muscidae	6	T	-	-	-
Ephydridae	6	T	Br	-	-
Psychodidae	8	T	Br	-	-
Chaoboridae	7	T	Br	-	-
Athericidae	4	I	Br	-	-
Empididae	8	T	Br	-	-
Crambidae	5	I	Clb	-	Lepidoptera
Cambaridae	6	T	Sw	Maxillopoda	Decapoda
Palaemonidae	6	T	Hk	-	-
Hyalellidae	8	T	Sw	-	Amphipoda
Asellidae	8	T	Sw	-	Isopoda
Unionidae	UNK	UNK	-	Gastropoda	Unionoida
Corbiculidae	UNK	UNK	-	-	Veneroida
Planorbidae	7	T	Clg	-	Basommatophora
Pachychilidae	UNK	UNK	-	-	Neotaenioglossa
Hydrobiidae	7	T	Clg	-	Neotaenioglossa
Physidae	8	T	Clb	-	Basommatophora
Thiaridae	UNK	UNK	-	-	Neotaenioglossa
Pleuroceridae	6	T	Clg	-	Neotaenioglossa
Dugesiidae	1	VI	Clg	Turbellaria	Tricladida
Undetermined	5	I	Clg	Acari	Hydrachnidia

significant; pH showed basic values (7.27-8.65) and DO values were >2 mg L^{-1} in most of the study sites, which means an optimal condition for biological organisms. The total dissolved solids (TDS) and the FBI showed significant negative relationships as was expected. The water temperature showed a weak association with IIBAMA (r = 0.208, P = 0.246).

The PCA results showed that the majority of the variance was explained by VBHA, FBI, and IIBAMA, following by pH, TDS, DO, Temp (Table 5). In the ordination, a tendency gradient of segregation of data between basins (LRB and PRB) are showed (Fig. 3), and the ANOSIM demonstrates significate differences between basins considering all the measured variables (P = 0.03). The study sites of the LRB are located in the lower left quadrant of the ordination. They show a worse ecological condition compare with the PRB sites, including the water quality indicated by the FBI, the habitat quality indicated by the VBHA, the availability of dissolved oxygen, the acidification of the water (pH), the water temperature and the biotic integrity evidenced by the IIBAMA.

DISCUSSION

This study demonstrates a successful validation of the IIBAMA using an independent dataset in the Pánuco and Lerma-Chapala river basins in central Mexico. It implies the availability of a new bioassessment tool for the ecological condition of streams and rivers on these two major basins. It was a first step to apply and perform the IIBAMA for the validation and application in a wide array of rivers in other basins in the country, even, in another region of the world. However, despite

Table 4. Values of the parameters measured and indices calculated in the sampling sites in Lerma-Chapala River Basin and Pánuco River Basin. FBI: Family biotic index, VBHA: Visual based habitat assessment, TDS: Total dissolved solids (ppm), DO: Dissolved oxygen (mg L^{-1}), Temp: Temperature (°C), P: Poor, G: Good, Mt: Moderate, F: Fair, FP: Fairly poor, E: Excellent, VG: Very good, SO: Suboptimal, O: Optimal, Mg: Marginal. ES: El Salto, PC: Presa del Carmen, PR: Presa de Rayas, Com: Comonfort, LQ: La Quemada, LG: Los Galvanes, Xo: El Xote, RG: Río Grande, Cal: Calvillo, SS: El Salto de los Salados, FS: Fracción Sánchez, PH: La Planta-La Hacienda, PP: Puente la Plazuela, Pin: Pinihuan, Can: Canoas, QM: Quinta Matilde, ER: El Realito, Qui: Quiotillos, EO: El Oasis, Chu: Chuveje, Car: Carpintero, Ras: Rascón, Tam: Tamasopo, Jal: Jalpan, Ayu: Ayutla, SM: Santa María, EC: El Carrizal, Tan: Tancuilín, SMT: Santa María (Tancoyol), RM: Río Moctezuma, Con: Concá, Ex: Extoraz, RB: Río Blanco.

Basin	Sites	Coordinates		IIBAMA		FBI		VBHA		pH	TDS	DO	Temp
		N	W										
Lerma-Chapala	ES	20˚23'21.1"	100˚16'48.9"	6	P	6.0	F	117	SO	7.56	443	5.3	13.86
-	PC	20°48'33.3"	100°18'33.9"	6	P	6.3	F	121	SO	7.46	190	2.85	18
-	PR	20°47'59.6"	100°13'22.1"	6	P	6.9	FP	31	P	7.64	112	1.24	16.76
-	Com	20˚45'03"	100˚46'25.6"	6	P	8.4	P	37	P	7.85	544	0.05	14.47
-	LQ	20˚57'06.0"	100˚47'40.8"	7	P	5.9	F	88	Mg	7.62	97	6.89	15.09
-	LG	21˚03'40.4"	100˚48'12.1"	6	P	6.9	FP	32	P	7.28	225	6.23	18.18
-	Xo	20˚57'08.5"	100˚47'42.7"	6	P	6.3	F	61	Mg	7.25	248	3.88	28.4
-	RG	21˚28'53.5"	100˚48'05.9"	6	P	5.8	F	82	Mg	7.48	162	0	15.03
-	Cal	21˚50'53.5"	102˚42'51.6"	6	P	5.0	G	-	-	8.26	294	0.61	24.37
-	SS	21˚45'18.2"	102˚21'31.2"	6	P	10.0	VP	54	Mg	7.27	672	0.93	13.05
Pánuco	FS	21˚47'20.3"	100˚42'04.1"	6	P	6.0	F	32	P	7.32	607	2.59	20.56
-	PH	21˚55'25.3"	99˚57'54.2"	6	P	6.7	FP	121	SO	7.55	874	1.86	22.63
-	PP	21˚47'27.3"	99˚55'29.5"	6	P	5.6	F	131	SO	7.63	754	4.15	19.11
-	Pin	21˚42'43"	99˚34'28.3"	6	P	3.4	E	200	O	7.68	838	1.98	19.35
-	Can	21˚56'36.7"	99˚30'35.4"	10	P	5.7	F	196	O	8.24	205	4.03	14.94
-	QM	21˚55'27.5"	99˚30'35.9"	8	P	7.8	P	189	O	7.96	229	3.72	15.88
-	ER	21˚36'24.9"	100˚13'46.1"	6	P	6.2	F	122	SO	8.61	130	1.52	29.31
-	Qui	20˚18'06.5"	100˚09'03.7"	8	P	8.1	P	112	SO	7.74	87	1.53	20.69
-	EO	20˚59'54.5"	99˚42'11.3"	7	P	5.8	F	133	SO	8.12	254	5.7	24.91
-	Chu	21˚10'17.9"	99˚33'26.1"	19	G	5.4	G	122	SO	7.97	202	4.8	20.83
-	Car	21˚53'45"	99˚14'44.8"	6	P	2.3	E	200	O	7.83	592	6.18	23.3
-	Ras	21˚59'12.8"	99˚15'16.8"	6	P	3.5	E	190	O	7.85	391	4.78	21.48
-	Tam	21˚57'18.5"	99˚23'15.4"	8	P	5.0	G	199	O	7.93	806	4.3	24.58
-	Jal	21˚12'44.8"	99˚28'5.4"	12	P	5.9	F	117	SO	7.83	175	4.6	21.99
-	Ayu	21˚23'18"	99˚35'11.7"	13	Mt	5.1	G	126	SO	8.49	198	8.06	21.58
-	SM	21˚23'50.9"	99˚35'04.7"	13	Mt	3.9	VG	126	SO	8.18	297	6.25	28.82
-	EC	21˚23'53.7"	99˚35'04.7"	11	P	4.8	G	140	SO	8.19	297	6.11	28.17
-	Tan	21˚16'04.3"	99˚03'59.9"	19	G	3.9	VG	176	O	8.34	152	6.44	21.83
-	SMT	21˚30'09.3"	99˚22'27.9"	7	P	5.3	G	150	SO	8.15	313	6.99	25.76
-	RM	21˚09'22.5"	99˚06'39"	6	P	6.7	FP	171	O	8.65	647	8.1	21.75
-	Con	21˚26'51.4"	99˚38'01.1"	6	P	6.8	FP	115	SO	7.12	501	1.54	28.16
-	Ex	20˚59'59.1"	99˚42'13"	11	P	5.5	G	121	SO	8.37	282	7.74	23.46
-	RB	21˚12'37.1"	99˚44'19.7"	11	P	5.1	G	149	SO	8.22	179	5.54	18.8

a successful validation of the index, the IIBAMA shows a moderate relationship among the environmental variables (r < 0.56), and our results differ from those of Piñón-Flores *et al.* (2014), because they demonstrated a strong positive relationship among the IIBAMA scores and the VBHA (r = 0.82) in rivers on the Chiquito River micro-watershed.

The majority of headwaters of both river basins (29 of 33 study sites) have lost the ecological processes that kept the energy flux and river ecosystems functions. The rivers that presented normal and proper conditions are located in the PRB, while the LRB is represented only by sampling locations with poor condition. These results are evidence of the environmental problem that faces LRB and PRB. Some authors argue that the agriculture, livestock and timber forestry, as well as mining, organic pollution, channeling and damning of rivers, led to a continued deterioration due to the constant use of the soil, which promoted erosion, loss of vegetation cover and habitat disturbance for wildlife

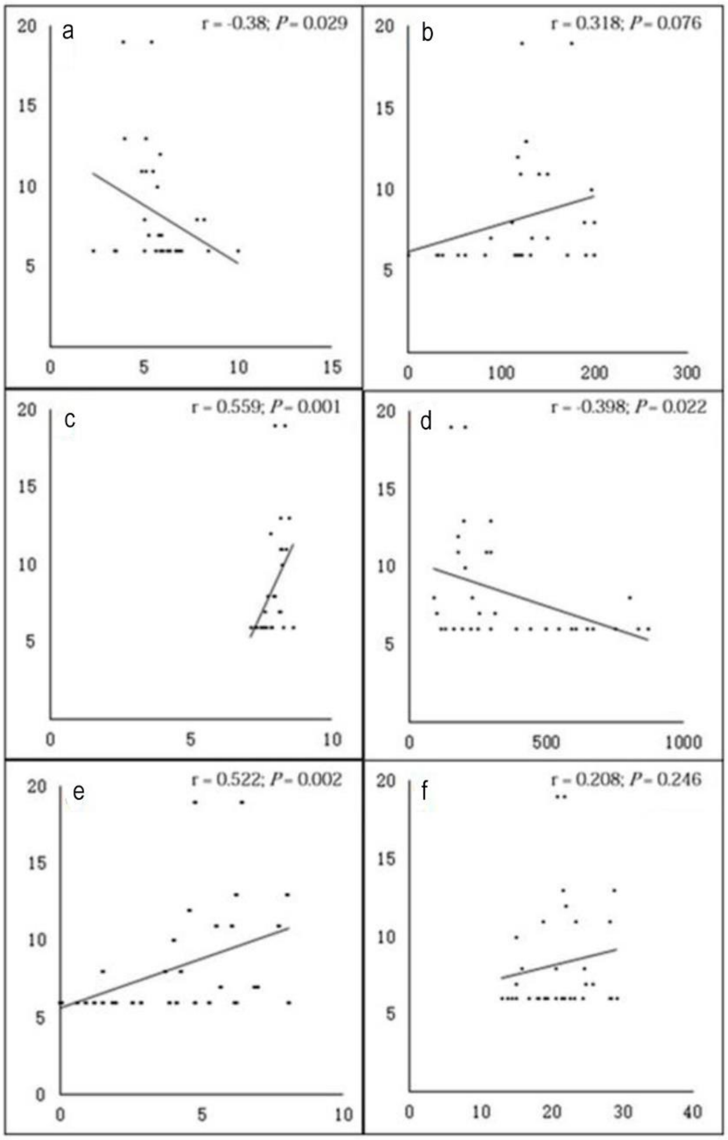

Figure 2. Correlations of the Index of biotic integrity based on macroinvertebrates assemblages (IIBAMA). All graphics shows the IIBAMA scores in the "Y" axes. The letters represent the environmental variable of correlation: a) Family biotic index (FBI), b) Visual based habitat assessment (VBHA), c) pH, d) Total dissolved solids (TDS), e) Dissolved oxygen (DO), f) Temperature.

species (Cotler-Ávalos & Garrido-Pérez, 2010). The cumulative effects of these practices can affect the physical hydrology, the riparian function, the water quality and channel morphology, which impinges on the aquatic invertebrates' communities (Reiter & Beschta, 1995).

The poor water quality of most of the study sites including both basins can be a consequence of agriculture, industry and drainage discharge, the main human activities (Cotler-Avalos *et al*., 2004; Alvarez *et al*., 2008). The agriculture practices significantly affect the water quality by contributing an excess of nutrients including sediments, through a process is known as leaching (Rai *et al*., 2012). It is evident by the dominance of highly tolerant taxa, and the loss of sensitive taxa; patter showed by the FBI analyses.

The optimal and suboptimal conditions of habitat mean that natural elements such as substrate at the bottom and habitat heterogeneity are stable and sustainable. However, some habitat elements such as riparian vegetation, vegetal bank (on the right riverbank), channel sinuosity, riffles frequency, and pool variability show a marginal category. The mechanisms of flux energy and dissipation remain, and

Table 5. Principal Component Analysis based on parameters measured and indices calculated in the sampling sites in Lerma-Chapala River Basin and Pánuco River Basin. IIBAMA: Index of biological integrity, FBI: Family biotic index, VBHA: Visual based habitat assessment, TDS: Total dissolved solids (g L^{-1}), DO: Dissolved oxygen (mg L^{-1}), Temp: Temperature (°C).

Physical and chemical variables			
	PC1	PC2	PC3
Eigenvalue	2.88	1.39	0.84
% variance	41.17	19.82	12.04
IIBAMA	0.38	-0.42	0.38
FBI	-0.44	-0.29	-0.44
VBHA	0.41	0.39	0.41
pH	0.45	-0.13	0.45
TDS	-0.12	0.73	-0.12
DO	0.43	-0.10	0.43
Temp	0.30	0.20	0.30

the present infrastructure is not common. The VBHA do not represent a short-term response to habitat degradation, it represents the long-term visual degradation process, such as was proposed by Allan (2004).

The significant correlation of the IIBAMA with environmental quality in most of the study sites means that poor biotic integrity was related to poor environmental quality. However, the found several sites in both basins that showed suboptimal habitat condition associated with poor biotic integrity could occur when the water properties were altered by local pollution. There are several ways and forms of water pollution, but this is one of the major causes of freshwater degradation worldwide and reflects the past, present, and future of human activities (Scholz & McIntyre, 2016). In this case, the wastewater discharge can attenuate the recuperation and maintenance of the composition and structure of macroinvertebrate communities and the dominance of tolerant taxa is reflected in this pattern. It has been found that wastewater treatment discharges are related with an increase in tolerance metrics (Poulton *et al.*, 2015).

The Ayutla and Santa María rivers located in PRB have a medium size large, where the macroinvertebrates communities have a diversity of functional feeding groups, such as collectors, grazers, predators and shredders. These functional feeding groups will be influenced by river width, the solar radiation, the allochthonous organic matter input, sediments size and substrate size (Vannote *et al.*, 1980). Moreover, in these kinds of sites with a low slope (<3%), allochthonous organic matter input from riparian vegetation (deciduous forest) and small sediment sizes; it is expected to find macroinvertebrates families with tolerance values from medium to high. Both study sites suffer from local anthropic negative effects of recreational activities at regional scales (people from other states) mainly in the dry season (March-April). These sites have regular biotic integrity and harbor degraded macroinvertebrates communities where the most sensitive taxa have lost. Trophic interactions have decreased, and the mechanisms of energy transfer from terrestrial systems to the aquatic system are negatively affected (Cotler-Ávalos & Garrido-Pérez, 2010). However, the excellent water quality (indicated by FBI) and the suboptimal habitat quality (VBHA), despite local organic contamination from tourism activities and mismanagement of wastewater, are evidence that the watershed area degradation is moderate, where the anthropic changes have not been enough to decrease their function and resilience, habitat structure and essential environmental services to people. Both sites showed regular biotic integrity, which means that the functional processes are present even with the loss of some sensitive taxa.

The good category of IIBAMA (two study sites, Tancuilin and Chuvejé) shows that macroinvertebrates communities are negatively affected which is evident by the loss of sensitive taxa. However, the communities still maintain the energy flux mechanisms, because the functional organization is preserved, evidenced by the presence of tolerant clingers taxa, taxa richness, EPTR richness. Good biotic integrity was associated with suboptimal habitat condition, and with very good water quality condition based on FBI. Good water quality conditions were present when the anthropic impacts had not embedded the substrates available for macroinvertebrates. The natural habitat structure and macroinvertebrates diversity are preserved, which proves the conservation of ecological integrity. This pattern of a suitable biological condition related to good habitat condition refers to ecological integrity, which can be associated with preserved ecosystem services and good condition of watershed area (Weigel & Dimick, 2011).

The site Chuvejé is in high altitude of the state of Querétaro (1,277 m over sea level, m.o.s.l.) and shows anthropic channel modification and high variations of the physical and chemical characteristics of water-related with its importance as a tourist destination. However, this site has an optimal habitat condition, which indicates that the dynamics of the river support alterations present in this place not directly influence the ecological processes and these impacts.

The positive relationships among the values of the IIBAMA with water quality (FBI), pH, DO, and the negative relationships with TDS and FBI (water quality depletion) demonstrate that the physical and chemical

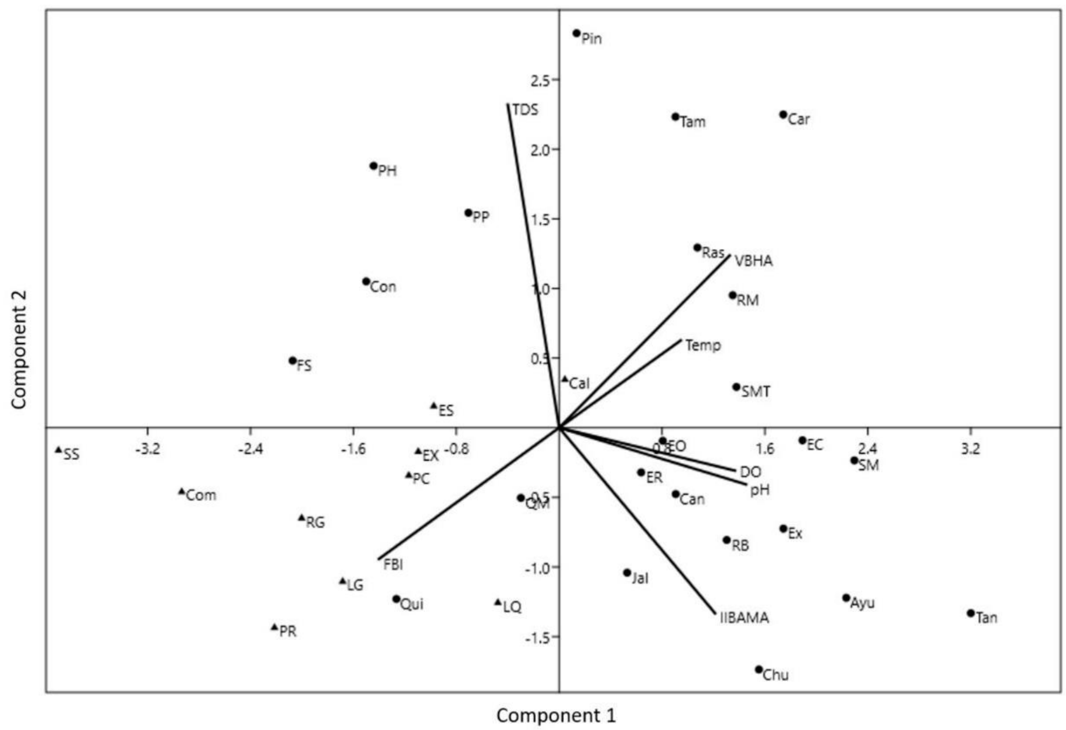

Figure 3. Principal Components Analysis based on parameters measured and indices calculated in the sampling sites in Lerma-Chapala River Basin and Pánuco River Basin. FBI: Family biotic index, VBHA: Visual based habitat assessment, TDS: Total dissolved solids (ppm), DO: Dissolved oxygen (mg L^{-1}); Temp: Temperature (°C), ES: El Salto, PC: Presa del Carmen, PR: Presa de Rayas, Com: Comonfort, LQ: La Quemada, LG: Los Galvanes, Xo: El Xote, RG: Río Grande, Cal: Calvillo, SS: El salto de los salados, FS: Fracción Sánchez, PH: La planta-La Hacienda, PP: Puente la Plazuela, Pin: Pinihuan, Can: Canoas, QM: Quinta Matilde, ER: El Realito, Qui: Quiotillos, EO: El Oasis, Chu: Chuveje, Car: Carpintero, Ras: Rascón, Tam: Tamasopo, Jal: Jalpan, Ayu: Ayutla, SM: Santa María, EC: El Carrizal, Tan: Tancuilín, SMT: Santa María (Tancoyol), RM: Río Moctezuma, Con: Concá, Ex: Extoraz, RB: Río Blanco. Triangles represent the sampling sites in Lerma-Chapala River Basin, circles represent the sampling sites in Pánuco River Basin.

processes in the river are determinant factors for ecological integrity evidenced by the aquatic macroinvertebrates assemblages. In another hand, the weak and no significant association between the IIBAMA, water temperature, and the VBHA indicates that in the headwaters of the two studied basins the water temperature, the habitat structure and stability by themselves, do not represent a determinant variable for biotic integrity. The water temperature is not a likely factor that determines the structure and composition in the aquatic macroinvertebrate's assemblages, especially in broad scale such as the basin (Friberg *et al.*, 2009; Buendia *et al.*, 2014). Moreover, the good habitat condition provides refuges for the fauna but, the current chemical condition affected by organic pollution can limit the aquatic macroinvertebrate assemblage's establishment. This process can occur when the watershed condition is stable, but the river has an additive point-source impact affecting the aquatic biota and ecosystems processes.

Our study demonstrates that the LRB and PRB are significantly degraded which coincides with Cuevas *et al*. (2010). However, the LRB is more degraded, and it has been affected by its physical, chemical and biological processes. While the PRB is mainly located into a Biosphere Reserve and its rivers harbor more stable and adapted biological communities and most of the sites the ecological process is close to the natural condition.

The IIBAMA, is a good estimator of the biological integrity in streams and rivers in the central basins Lerma-Chapala and Pánuco, it reflects patterns related with the physical and chemical processes. We validated and recommended the using of the IIBAMA with independent data to assess the biotic integrity in these two basins. However, we suggest using IIBAMA together with indexes to estimate the habitat and water quality, such as VBHA and FBI to assess the environmental quality of streams and rivers accurately, even in other regions with similar conditions. The

IIBAMA responded to a variety of stressors affecting the streams and rivers in the region, and it allows to differentiate in conditions status among the two basins assessed. With their implementation, the legislation efficacy or programs aimed at river ecosystem protection and restoration can be evaluated. This study is the first to validate an index of biological integrity based on aquatic macroinvertebrates in a broad scale in Mexico and provide a framework for their widespread use, and to approach the validation and implementation of other IBIs in other regions with similar ecosystems.

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