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SALINDEX: A macroinvertebrate index for assessing the ecological status of saline "ramblas" from SE of the Iberian Peninsula

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ABSTRACT

SALINDEX: A macroinvertebrate index for the ecological status assessment of saline "ramblas" from SE of the Iberian Peninsula

A new index (SALINDEX) for assessing the ecological status of saline "ramblas" from the southeast of the Iberian Peninsula is presented. It was applied in the Protected Area of Ajauque Wetland and Rambla Salada (Murcia). The index is based on four metrics related with macroinvertebrate community: the richness of macroinvertebrate families (FR), the ratio between richness of Coleoptera and Hemiptera species (C/H) and the presence and abundance of species that act as indicators of a healthy (I+) and impaired (I-) status (including exotic species). Metric scores were calculated with regard to reference condition values for each of the 7 ecotypes established according to salinity and water flow velocity gradients. The four metrics that comprise the index have the same weight, although the three first (FR, C/H, I+) score positively and the last one (I-) scores negatively. An ecological status class was assigned to each resulting index value (–1, 0, 1, 2, 3) according with the criteria set out in the Water Framework Directive. SALINDEX is based in ecological patterns typical of inland saline waters ecosystems (e.g., lower taxonomic richness, predominace of Coleoptera and Hemiptera species, presence of halotolerant/halophilic species, etc.), and was able to detect anthropogenic perturbations, such as eutrophication and salinity drop processes, and any subsequent recovery. In contrast, the IBMWP index, commonly used for the ecological evaluation of Iberian streams, was unsuited to saline streams, and showed a negative correlation with the SALINDEX. The index presented here is the first tool specifically developed for assessing saline ramblas from the Iberian southeast and, although some weaknesses were observed, these can be corrected by improving ecotypes definition, increasing the number of reference stations and complementing with biological information from inland saline waters ecosystems for its application at peninsular level.

Key words: Ecological status, aquatic macroinvertebrates, saline "ramblas", Water Framework Directive, functional indicators.

RESUMEN

SALINDEX: Un índice de macroinvertebrados para la evaluación del estado ecológico de ramblas salinas en el sureste de la península Ibérica

Se presenta un nuevo índice (SALINDEX) para la evaluación del estado ecológico de las ramblas salinas en el sureste ibérico, el cual ha sido aplicado al Paisaje Protegido del Humedal de Ajauque y Rambla Salada (Murcia). El índice se basa en cuatro métricas de las comunidades de macroinvertebrados: riqueza de familias (RF), relación entre la riqueza de especies de coleópteros y hemípteros (C/H), presencia y abundancia de especies indicadoras de naturalidad (I+) y presencia de especies indicadoras de alteración (I-), incluidas las especies exóticas. La valoración de cada métrica se ha realizado atendiendo a las condiciones de referencia para los 7 ecotipos establecidos según el gradiente de salinidad y la velocidad del flujo de agua. Las cuatro métricas que componen el índice tienen la misma ponderación, aunque las tres primeras (RF, C/H, I+) puntúan de forma positiva si se cumplen las condiciones establecidas y la última (I-) puntúa de forma negativa. A cada posible valor resultante del índice (–1, 0, 1, 2 o 3) se le ha asignado una categoría de estado ecológico (malo, deficiente, moderado, bueno o muy bueno) en concordancia con las establecidas por la Directiva Marco de Agua. SALINDEX está diseñado sobre ciertos patrones ecológicos característicos de los ecosistemas salinos de interior (menor riqueza taxonómica, dominio de coleópteros y hemípteros, presencia de especies halotolerantes y halófilas etc.), siendo capaz de detectar perturbaciones de origen antrópico, como procesos de dilución y eutrofización, y la posterior recuperación del sistema. En cambio, el IBMWP, *uno de los índices más empleado para la evaluación del estado ecológico de los ríos ibéricos, resultó poco apropiado para ríos salinos, mostrando una correlación negativa con SALINDEX. El índice aquí presentado, constituye la primera herramienta específicamente diseñada para evaluar ramblas salinas en el sur-sureste ibérico y, aunque presenta algunas debilidades, éstas pueden ser subsanables en un futuro a través de una mejor definición de ecotipos, de un mayor número de estaciones de referencia y de completar la información biológica para su aplicación en los ecosistemas salinos a nivel peninsular.*

Palabras clave: *Estado ecológico, macroinvertebrados acuáticos, ramblas salinas, Directiva Marco de Agua, indicadores funcionales.*

INTRODUCTION

Based on the EU Water Framework Directive (WFD, Directive 2000/60/EC) the ecological status assessment of streams and wetlands is an important tool for the management, conservation and restoration of continental water bodies. Since the 1960's, many methods have been developed, including the bioassessment of streams using macroinvertebrates (Rosemberg & Resh, 1993; Metcalfe-Smith, 1994; Barbour *et al*., 1999, Bonada *et al*., 2006). One of the most widely-used indices for determining the water quality of Spanish streams is the IBWMP (Iberian Biological Monitoring Working Party) (Alba-Tercedor *et al*., 2002), an Iberian adaptation of the BMWP (Armitage *et al*., 1983) and is based on macroinvertebrate differential tolerance to pollution. However, as many others, this index was designed for freshwater streams and is not appropriate for saline streams, which are frequent in Mediterranean semiarid regions of the Iberian Peninsula: for example those that occur in the Segura, Guadalquivir, Júcar and Ebro basins.

In Iberian Southeast, saline "ramblas", that are watercourses with specific geomorphological features that make them different from all other temporary streams (Gómez *et al*., 2005), and associated wetlands are very common in marly sedimentary basins (Triassic and Miocene). They are characterised by hydrological extremes that result in drying and flooding, together with high salt concentrations that determine the composition of the community adapted to these extreme conditions (Gómez *et al*., 2005).

The GUADALMED project (Prat, 2002) developed a system of structural indicators of ecological status, called PRECE (Protocolo Rápido de Evaluación de la Calidad Ecológica) that comprises the IBMWP and another two indices (Jáimez-Cuellar *et al.,* 2002). This protocol includes saline streams in the "ramblas" ecotype without taking into account the heterogeneity of this group, which embraces a broad gradient of salinity (from very low to until hypersaline waters with values close to 300 g/L) and temporality.

The described indices do not work in saline systems due to the particularity of the saline biota, which includes halotolerant, halophilic and halobiont species adapted to osmotic stress (Williams, 1981; Williams y Feltmate, 1992). These communities show a lower taxonomic richness and, unlike in freshwater systems the orders Diptera, Coleoptera and Hemiptera show great richness and abundance (Ward, 1992; Millán *et al.,* 2001). For a wide salinity gradient (3-300 g/L), the relation between number of taxa and salinity is negative (Williams *et al.,* 1990). In Mediterrenean areas, freshening and eutrophication caused by an intensive agricultural hydric surplus are the most significant impacts on aquatic saline ecosystems. These impacts increase the number of macroinvertebrate species and families, reducing halophilic and halobiont taxa (Velasco *et al.,* 2006).

Most indices for wetland assessment were developed based on the macroinvertebrates found in coastal Almería, SE Spain (Ortega *et al.,* 2004) and based on crustaceans and insects from Catalonia (Boix *et al.,* 2005). However, these indices did not embrace the athalassohaline wetlands associated to drainage network systems, which are typical of the Iberian Southeast (Ramírez-Díaz, 1992; Vidal-Abarca *et al*., 2001; Gómez *et al*., 2005).

Table 1. Ecotypes according to saline habitat classes as function of current of velocity and salinity. *Ecotipos resultantes de la clasificación de los hábitats salinos en función de la velocidad del flujo y de la salinidad.*

Ecotype	*Velocity of flow*	*Salinity*
1	Lotic waters	Hiposaline (~3-~20)
2	Lentic waters	Hiposaline (~3-~20)
3	Lotic waters	Mesosaline (~20-~40)
4	Lentic waters	Mesosaline (~20-~40)
5	Lotic waters	α−hypersaline (~40-~100)
6	Lentic waters	α−hypersaline (~40-~100)
7	Lotic waters	β−hypersaline (~100-~140)

Bearing in mind the singularity of aquatic saline taxa and the lack of appropriate tools for their ecological status assessment, the objectives of this paper were: 1) To design an index based on the macroinvertebrate community for assessing "ramblas" and wetlands in the Iberian Southeast; 2) To apply the index in the Protected Area of Wetland of Ajauque and Rambla Salada; 3) To assess its response in the face of dilution stress and 4) To analyse the relationships between the index and environmental, biotic variables (structural and functional) and the IBMWP.

MATERIALS AND METHODS

Development of SALINDEX

To develop this index we have followed the criteria for multimetric index design provided by Paulsen *et al.* (1991) and Barbour *et al.* (1995, 1999) with some modifications. Firstly, the saline aquatic environments occurring in marly basins of the Iberian Southeast were classified. Subsequently, a group of metrics (parameters that respond to human stressors in a foreseeable way) were selected and scoring criteria for each were established. Finally, the metrics were integrated in the index (SALINDEX) and the overall scoring system was established.

Aquatic environments classification

Seven ecotypes were established based on the two parameters that determine the distribution and the abundance of the species: salt concentration (from hyposaline to hypersaline waters) and flow velocity (lentic or lotic waters) (Table 1). The characterization of the aquatic environments was made following the framework for thalassic waters (Montes & Martino, 1987). Temporality was not considered as an important variable since all the studied water bodies are permanent or semi-permanent.

Metric selection

The metrics were selected according to a bibliographic revision of ecological status indicators in saline aquatic ecosystems and their response to human stressors (Table 2). The richness of aquatic macroinvertebrate families (FR) and the coefficient of Coleoptera/Hemiptera species (C/H) have been used previously in another multimetric index (Ortega *et al.,* 2004). In addition, a further two metrics were included: presence and abundance of Coleoptera and Hemiptera species that indicate a good health (I+) and stressed (I-) status, including allocthonous species.

Table 2. Metrics selected for saline ecosystems and their predictable response to dilution (D) and eutrophication (E). ▲: increasing; ▼: decreasing; V: variable. *Métricas seleccionadas para ambientes salinos y su respuesta esperada ante las perturbaciones por dilución (D) y por eutrofización (E). ▲: incremento; ▼: disminución; V: variable.*

Indicator	*Label*	*Previous studies*	*Expectable response* D	*Expectable response* E
Family richness	FR	Williams *et al.,* 1990; Ortega *et al.,* 2004	▲	V
Coleoptera/Hemiptera coefficient	C/H	Greewood y Wood, 2003; Ortega *et al.,* 2004; Valladares *et al.,* 2004;	▼	▼
Indicator species of naturality	I+	Millán *et al.*, 1996; 2002; Sánchez-Fernández *et al.*, 2003	▼	▼
Indicator species of degradation	I-	Sánchez-Fernández *et al.*, 2003	▲	▲

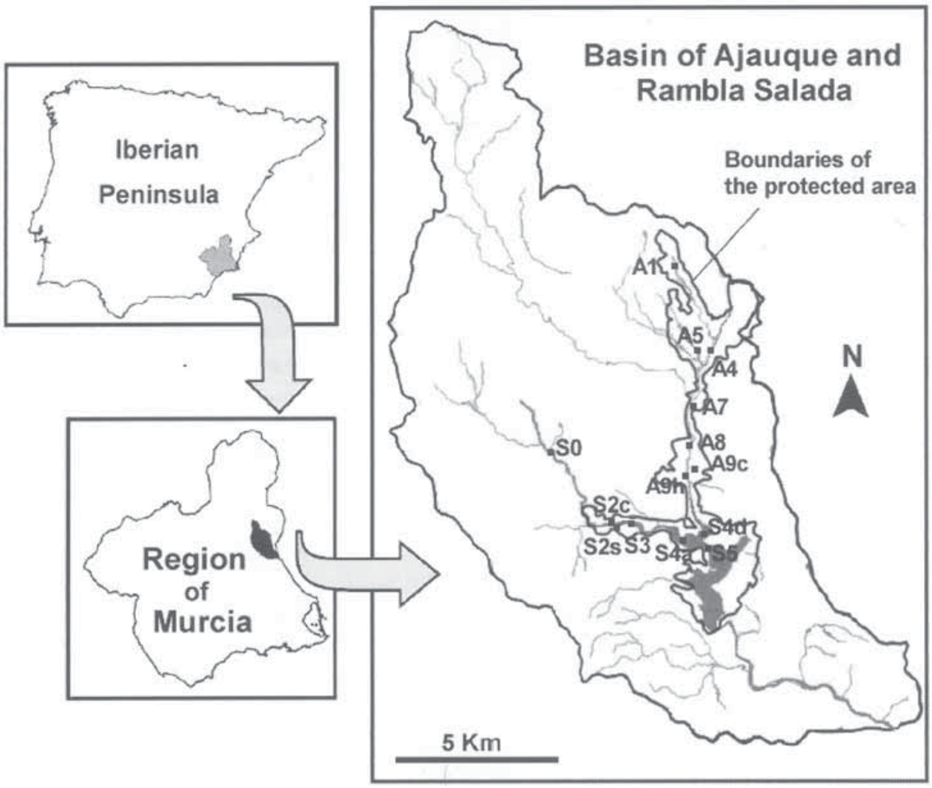

Figure 1. Location of the study area, delimitation of Protected Area of Ajauque Wetland and Rambla Salada and sampling stations. *Localización del área de estudio, Paisaje Protegido de Ajauque y Rambla Salada, y ubicación de las estaciones de muestreo.*

Criteria establishment for metric scoring

Reference stations were selected from the DIVERSAL database, elaborated by the Ecología Acuática research group (Universidad de Murcia), containing information about aquatic macroinvertebrates from numerous saline environments of the Iberian Southeast (Granada, Córdoba, Jaén, Almería, Albacete, Murcia and Alicante) collected since early 80's and ongoing. This database includes information of collected individuals at family level with the exception of Coleoptera and Hemiptera, which are at species level. The reference stations were selected according to: a) dominance of natural vegetation and dryland farming; b) keeping historical salinity values.

The metric scoring criteria were obtained from the fauna collected in reference stations, following different procedures for each metric. The scoring range in the different habitats of the metrics richness of macroinvertebrate families (FR) and the Coleoptera/Hemiptera coefficient (C/H) was established, basically, from the 25th and 75th metric values, after adjustment to avoid overlapping between ecotypes. A Kruskal-Wallis non-parametric test was made to detect significant differences between ecotypes values.

To obtain the Coleoptera and Hemiptera species with the best health-indicator capacity an IndVal analysis (Dufrêne & Legendre, 1997) was performed using the software PC-ORD 4.20. This analysis estimates the indicator value (IV) with regard to the relative abundance and the occurring frequency of each specie in each previously defined ecotype. The final decision about which species are the best health-indicators was made taking into account our taxonomical experience and the available bibliography. Invasive allocthonous species were included as impairment-indicators.

Table 3. Sampling sites used for SALINDEX application. *Estaciones de muestreo empleadas en la aplicación de SALINDEX.*

A1	Pool on the headwater of the Rambla de Ajauque
A4	Rambla de Ajauque downstream of the confluence with the Sanel Wetland
A5	Derramadores wetland
A7	Rambla de Ajauque downstream of the Cabecicos Negros
A8	Rambla de Ajauque downstream of the diversion channel
A9c	Rambla de Ajauque at Ajauque wetland
A9h	Ajauque wetland
S0	Rambla Salada headwater
S2s	Hypersaline spring in Rambla Salada
S2c	Rambla Salada downstream of the diversion channel
S3	Rambla Salada at Finca de las Salinas
S4a	Rambla Salada upstream of the confluence with the Rambla de Ajauque
S4d	Rambla Salada downstream of the confluence with the Rambla de Ajauque
S5	Rambla Salada upstream of the Santomera Reservoir

Index application

Study area

The Protected Area of Ajauque Wetland and Rambla Salada is located in the north-east of the Province of Murcia (Fig. 1) and is a representative example of the natural ecosystems composed of saline "ramblas" and associated wetlands. This area is protected by environmental laws at regional level (declared Paisaje Protegido by Ley 4/92 de Ordenación del Territorio), at European level (Special Protection Area and Special Area of Conservation) due to the singularity and integrity of this saline zone and the presence of communities and species of community interest. The natural vegetation of the basin is halophilic, nitrophilic, gypsyc and thermophilic Mediterranean shrubs, although much is dedicated to irrigated crops.

The climate in the basin is semiarid with a mean annual temperature around 18° C and mean annual precipitation of below 300 mm, concentrated in autumn and spring. The study area is located in the Neogene Abanilla-Fortuna basin where gypsiferous marls from the late Miocene are abundant. Badlands are very common as a consequence of high erosion rates on marls which shaped a complex drainage network composed of numerous intermittent water courses that converge in two main streams, the Rambla of Ajauque and Rambla Salada, that flow to the Santomera reservoir. Associated to these courses are many wetlands and saline soils in the flood plain. On the other hand, the water bodies are highly mineralized as a result of lithology and climatic aridity, salinity ranging from 3 to 180 g/L. However, this feature has been modified by a freshening process due to the freshwater inputs from surrounding irrigation crops (Ballester *et al.*, 2003) and losses from the Tagus-Segura diversion channel. For instance the salinity of the Rambla Salada has fallen from 100 g/L in the early 1980's (Vidal-Abarca, 1985) to an average of 33 g/L in recent years (Velasco *et al.*, 2006). The minimum salinity value was recorded on 2 October 2003 when it fell to 3.5 g/L as a result of the diversion channel being emptied for repairs.

Assessment of the ecological status of the aquatic ecosystems in the protected area of Ajaque-Rambla Salada

The proposed index for monitoring and controlling the ecological status of the Protected Area of Ajauque-Rambla Salada was applied in 15 sampling stations (Table 3 and Fig. 1) which represent the diversity of the aquatic habitats in the area according to salinity, lotic or lentic waters and the degree of impairment.

We also included the station at the headwater of Rambla Salada (S0), even though it was located outside the protected area, since it is a good example of the reference status of ecotype 3 (lotic mesosaline) and belongs, of course, to the same basin. The sampling was made on late spring (30/05/2005).

Protocol for index application

Macroinvertebrate collection involved multihabitat samping in a 100 m reach following the protocol provided by Jáimez-Cuellar *et al.* (2002). The collected material was stored in 70 % ethanol and, then, a preliminary sorting was made, which was confirmed in the laboratory using taxonomic keys [see Nieser *et al.* (1994), for Hemiptera, Ribera *et al*. (1998) for a recompilation of the taxonomic keys used for Coleoptera and Tachet *et al.* (2000) for the rest]. Coleoptera and Hemiptera individuals were sorted at species level while the other orders were classified at family level. Finally, abundance classes were defined as the number of individuals collected in the reach: *rare* if occurs less than 3 individuals, *common* if between 3 and 15 and *abundant* if more than 15.

For the index, we followed the macroinvertebrate sampling protocol described above. When the fauna composition was known, values obtained were compared with scoring criteria for each metric specified in Table 5. The metrics FR, C/H, I+ scored positively if all the criteria were fulfilled. The family richness or the Coleoptera/Hemiptera coefficient values, respectively, had to be inside the score range. The presence of the all health-indicator species (I+) added one point to the total score. While the occurrence of any impairment-indicator specie, reduced the total score by one point.

At last, to obtain the global score it is necessary to add all the individual metrics scored with no weighting. For each possible result (−1, 0, 1, 2 o 3) an ecological status category (bad, poor, moderate, good or high) exists according to the classes defined by WFD. The least perturbed status is represented by the classes good and high represents the objective for all the water bodies according to the WFD.

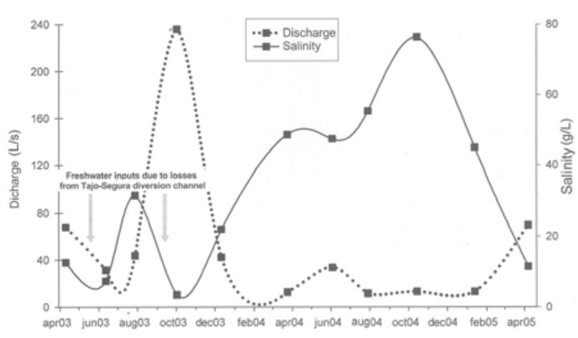

Figure 2. Variation of salinity and discharge during 2003-05 due to freshwater inputs to Rambla Salada. *Variación de la salinidad y el caudal durante el periodo 2003-05 debido a las entradas de agua dulce en Rambla Salada.*

Index assessment

Data obtained in the intensive survey of the Rambla Salada (station S3, Rambla Salada at Finca de las Salinas, lotic hypesaline, ecotype 5) during two years were used to analyse the response to large changes in salinity and the relationship with other environmental and biotic (structural and functional) variables, together with the index IBMWP. Sampling was performed with an almost nearly bimonthly frequency from 10/04/2003 to 07/04/2005. This study identified two different periods according to disturbance and recovery processes observed: the first was disturbed by large inputs of freshwater from the diversion channel (from April 2003 to October 2003) while during the second period (from December 2003 to May 2005) salinity recovered (Fig. 2). The highest discharges were found while diversion channel was emptied leading to the lowest salinity value (2 October 2003)

The aquatic macroinvertebrates were sampled following the previously mentioned protocol. Using the obtained biological data SALINDEX, the IBMWP score was calculated (Alba-Tercedor *et al.,* 2002) using original boundaries (labelled as IBMWP-o) (Alba-Tercedor & Sánchez-Ortega, 1988) and the ramblas ecotype boundaries (labelled as IBMWP-r) proposed by the Guadalmed proyect (Alba-Tercedor *et al.,* 2002). In some cases, when the index score did not exceed by more than 5 unities the quality class boundary (e.g., between *moderate* and *good*), it was classified as an intermediate class (e.g., moderate-good class), as recommended in Jáimez-Cuellar *et al.* (2002).

On each date, discharge was estimated from measurements of depth and current velocity along a cross-section of the run. Conductivity was measured in the morning with an ECmeter (TetraConR 325) that automatically calculates salinity. Stream metabolism rates were measured using an open-system, single-station approach (Odum, 1956). Water temperature and dissolved oxygen were measured *in situ* at 15 minute intervals over 24 hours on each date using a multi-parameter recorder (WTW, MultiLine P4). Reaeration coefficient (K_S) and ecosystem respiration (ER) were calculated following the night-time regression method (Thyssen & Kelly, 1985) using River Metabolism Estimator v. 1.2, an MS Excel spreadsheet available at `http://www.cawthron.org.nz` that also estimates gross primary production (GPP). In this method the reaeration coefficient is obtained from the slope of the linear regression between the night-time rate of change of stream dissolved oxygen versus the saturation deficit.

Moreover, chlorophyll *a*, total suspended solids and water dissolved nutrients (nitrite, nitrate, ammonium and orthophosphate), organic matter in sediment and macroinvertebrate density were calculated using the methods described in Velasco *et al.* (2006).

The mean density of each taxon was multiplied by the relative habitat area in order to obtain macroinvertebrate abundance in the reach. Benthic macroinvertebrate biomass was obtained from length-mass equations available for the same or nearest taxon from saline streams (Moreno, 2002; Barahona *et al.,* 2005) or from the general equations for macroinvertebrate families (Smock, 1980; Benke *et al.,* 1999).

Data analyses

All the variables were transformed to normalise distribution and equalise variance, with the exception of SALINDEX. The relationships between the SALINDEX score and C/H coefficient score, FR score, the IBMWP score and the environmental and biotic variables were studied through Spearman correlations. Furthermore, a one-way ANOVA analysis was carried out to detect meaningful differences between impaired dates (bad, poor or moderate ecological status) and healthy dates (good or high). All analyses were conducted by Statistica 6.0 (StatSoft, 2001) software.

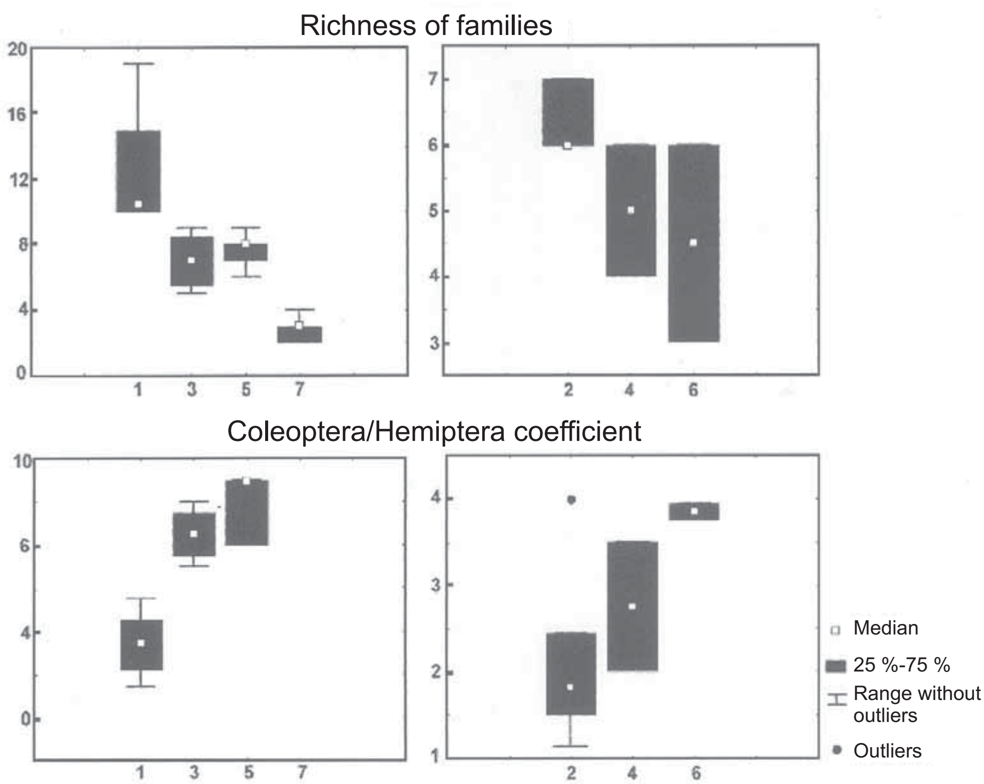

Figure 3. Boxplot of the metrics family richness and Coleoptera/Hemiptera coefficient for lotic and lentic ecotypes. *Boxplot de las métricas riqueza de familias y relación coleópteros/hemípteros para ecotipos lóticos y leníticos.*

Table 4. Results of IndVal analysis, showing the indicator value (IV) for each ecotype of species with p-value ≤ 0.1. *Resultado del análisis IndVal, mostrando el valor indicador (IV) para cada ecotipo de las especies con p-valor ≤ 0,1.*

Specie	*Ecotype*	*IV*	*p*
Helophorus fulgidicollis	1	16.7	0.082
Herophydrus musicus	2	18.6	0.055
Laccophilus minutus	2	20.1	0.052
Enochrus falcarius	5	70.7	0.001
Nebrioporus baeticus	5	26.7	0.063
Ochthebius cuprescens	5	29.9	0.032
Parcymus aeneus	5	31.3	0.013
Enochrus bicolor	6	22.1	0.053
Ochthebius notabilis	6	35.5	0.010
Ochthebius glaber	7	65.1	0.001

RESULTS

Development of the SALINDEX

The richness of macroinvertebrate families (FR) decreased when salinity increased, although ecotypes 3 and 5 (lotic mesosaline and hypersaline) showed no differences (Fig. 3). For close salinities, the lentic habitats exhibited a lower family richness than the lotic habitats. Meaningful differences were detected between ecotypes for this variable (*Kruskal-Wallis*, $H = 25$; $p = 0.0003$).

The Coleoptera/Hemiptera richness coefficient (C/H) increased with salinity, although for similar salinities this coefficient is usually lower in lentic habitats (Fig. 3). No Hemiptera species were observed in the ecotype 7 (lotic hypersaline) due to their lower tolerance to salinity. Significant differences were found in the C/H coefficient values (*Kruskal-Wallis*, $H = 17.2$; $p = 0.0042$) between ecotypes.

The species with the highest indicator values and $p \leq 0.05$ for the different ecotypes are shown in Table 4. Exceptionally, other species with *p*-value ranging between 0.05 and 0.10 were included as health-indicators depending on their ecological preferences (Millán *et al.*, 1996; 2002) and their distribution in the Murcia region (Sánchez-Fernández *et al.*, 2003). All the health-indicator species were Coleoptera *Enochrus falcarius* and *Ochthebius glaber* being the species with the highest degree of habitat specificity for hypersaline lotic waters ($p \leq 0.001$). However, the IndVal analysis did not identity any health-indicator species for ecotypes 3 and 4 (lotic and lentic mesosaline, respectively). Therefore, halophilic taxa were chosen to complete this metric because of to their large tolerance to osmotic changes (Millán *et al.*, 1996; 2002; Sánchez-Fernández *et al.*,

Table 5. Scoring criteria for each metric and ecotype. See Table 2 for metrics abbreviations. A: > 15 individuals, C: 4-15 individuals, R: < 4 individuals. *Condiciones de puntuación para cada métrica y para cada ecotipo. Ver Tabla 2 para las abreviaturas de las métricas. A: > 15 individuos, C: de 4-15 individuos, R: < 4 individuos.*

Ecotype	*Metrics*					
	FR	C/H	I+		I-	
			Ochthebius cuprescens	A		
1	10-15	4/1 - 7/1	*Helophorus fulgidicollis*	C	*Laccophilus hyalinus*	
			Herophydrus musicus	A		
2	4-7	2/1 - 3/1	*Laccophilus minutus*	A	*Sphaeroma serratum*	
3	4-10	7/1 - 8/1	*Ochthebius cuprescens*	A		
4	4-6	3/1 - 4/1	*Enochrus bicolor*	A	*Gammarus aequicauda*	
			Ochthebius cuprescens	A		> R
			Nebrioporus baeticus	A	*Micronecta scholtzi*	
5	4-9	> 8/1	*Enochrus falcarius*	A		
			Paracymus aeneus	C	*Hydroglyphus geminus*	
			Enochrus bicolor	A		
6	1-4	> 4/1	*Ochthebius notabilis*	A	*Yola bicarinata*	
7	1-4	NH	*Ochthebius glaber*	A		

Table 6. Results obtained from the application of SALINDEX for each sampling station in the Protected Area of Ajauque-Rambla Salada. Sampling sites abbreviations in Table 3. *Resultados obtenidos de la aplicación de SALINDEX a cada estación de muestreo del Paisaje Protegido de Ajauque-Rambla Salada. Abreviaturas de las estaciones en la Tabla 3.*

Station	*Ecotype*	*Salinity (g/L)*	*Metrics score*				*SALINDEX score*	*Ecological status*
			FR	C/H	I+	I-		
A1	2	9.5	0	0	0	0	0	Poor
A4	4	20.3	0	0	0	0	0	Poor
A5	4	36.3	1	0	0	0	1	Moderate
A7	1	5.5	1	0	0	0	1	Moderate
A8	1	4.5	0	0	0	0	0	Poor
A9h	4	48.5	1	0	1	0	2	Good
A9c	1	10.5	1	0	0	−1	0	Poor
S0	3	30.0	1	1	1	0	3	High
S2s	7	157.0	1	1	1	0	3	High
S2d	1	11.0	0	1	0	0	1	Moderate
S3	5	63.0	1	1	1	0	3	High
S4a	5	39.5	1	0	0	−1	0	Poor
S4d	3	25.5	1	0	0	−1	0	Poor
S5	2	26.0	1	0	0	−1	0	Poor

2003; Greewood & Wood, 2003), e.g. *Ochthebius cuprescens* and *Enochrus bicolor*.

The selected impairment-indicator species (Table 5) were brackish Crustacean, such as *Gammnarus aequicauda* and *Shaeroma serratum*, and Coleoptera (*Laccophilus hyalinus*, *Hydroglyphus geminus*, *Yola bicarinata* and the Hemiptera *Micronecta scholtzi*) all of which commonly found in freshwater and/or eutrophic waters.

The scoring criteria for each metric and each ecotype are shown in Table 5.

Application of the SALINDEX

Ecological status of the aquatic ecosystems in the protected area of Ajauque-Rambla Salada

In general, stations with poor or moderate ecological status dominated in the protected area. We only found four stations without clear signs of impairment: one with a good ecological status and three with a high ecological status (Table 6). Rambla Salada showed the best preserved aquatic habitats, with the exception of the reach influenced by irrigated crops or by waters from the Rambla of Ajauque. In contrast, the aquatic habitats of the Ajauque sub-basin were more polluted and only one station (Humedal de Ajauque) showed good ecological status. On the other hand, it is important to highlight the good conservation status (high ecological status) of the Rambla Salada headwater (station Cabecera de Rambla Salada, S0) which is just outside the protected area. Moreover, no stations with a bad ecological status were found.

The family richness (FR) was the metric that scored most frequently. C/H and I+ appear to be more selective and, generally, they only scored when the ecological status was good or high. The metric I- scored in just four stations because of the presence of the brackish species *Gammarus aequicauda* and *Sphaeroma serratum.*

Assessment of the SALINDEX

The results of the application of the two indices is presented in Table 7: the index proposed in this paper (SALINDEX) and the IBMWP, with the original (IBMWP-o) and ramblas ecotype (IBMWP-r) scoring boundaries. SALINDEX classified the ecological status on four occasions as poor, three times as moderate and four times as good. It was able to detect the most intensive freshening process in October 2003, classifying it as poor. In addition, a good ecological status was assigned by SALINDEX to most of the sampling dates following repair of the channel (from December 2003 to April 2005). In contrast, the

Table 7. Results obtained from the application of SALINDEX and IBMWP (IBMWP-o: original classes; IBMWP-r: ecotype ramblas classes) in Finca de las Salinas (Rambla Salada). *Resultados de la aplicación de SALINDEX e IBMWP (IBMWP-o: acotaciones originales; IBMWP-r: acotaciones ecotipo ramblas) en la Finca de las Salinas (Rambla Salada).*

Date	*Metrics score*				*SALINDEX*	*IBMWP score*	*IBMWP-t*	*IBMWP-r*
	FR	C/H	I+	I-				
10/04/2003	0	0	0	0	Poor	33	Poor	Good
12/06/2003	0	1	0	0	Moderate	38	Poor/Mode	Good
28/07/2003	0	1	0	0	Moderate	42	Moderate	High
02/10/2003	0	0	0	0	Poor	61	Poor/Good	High
11/12/2003	1	1	0	0	Good	26	Poor	Good
24/03/2004	0	1	0	0	Moderate	28	Poor	Good
03/06/2004	0	0	0	0	Poor	30	Poor	Good
29/07/2004	0	0	0	0	Poor	38	Poor/Mode	Good
14/10/2004	1	1	0	0	Good	29	Poor	Good
13/01/2005	1	1	0	0	Good	27	Poor	Good
07/04/2005	1	1	0	0	Good	24	Poor	Good

isolated dilution events were not detected as in the case of April 2005. At last, a good ecological status was always found when metric FR scored.

Comparing the results obtained with SALINDEX with those obtained with IBMWP-o, six dates were classified with the same ecological status, two of them being intermediate classes. The results of the IBMWP-o yielded seven dates with poor ecological status (twice as poor-moderate intermediate class), one date with a moderate and one date with moderate-good class, the last coinciding with the freshening process (October 2003). After first time the channel was repaired, salinity recovered pre-dilution levels, although

Table 8. Spearman correlations between SALINDEX, metrics FR, C/H and IBMWP and the environmental and biotic variables (* at $p < 0.05$ and ** at $p < 0.01$). *Correlaciones de Spearman entre SALINDEX, RF, C/H e IBMWP y las variables ambientales y bióticas estudiadas (* a* p *< 0.05 y ** a* p *< 0.01).*

Variables	*SALINDEX*	*FR*	*C/H*	*IBMWP*
Water temperature	−0.61*	−0.66*	−0.12	0.79*
Dissolved oxygen	0.64*	0.72*	0.17	−0.56
Oxygen saturation	0.07	0.06	0.29	0.12
Conductivity	0.10	0.12	0.12	−0.20
Discharge	−0.10	0.00	−0.23	0.13
Benthonic organic matter	0.37	0.24	0.17	−0.65*
Chlorophyll *a* in water	−0.57	−0.72*	−0.17	0.37
Particulated organic matter	0.37	0.48	−0.12	−0.35
Total suspended solids	0.30	0.36	−0.12	−0.29
NO_3-N	0.03	−0.06	−0.12	0.04
NO_2-N	0.10	0.18	0.00	−0.26
NH_4-N	0.13	0.30	0.23	−0.20
PO_4-P	0.49	0.48	0.09	−0.12
GPP	−0.81*	−0.84**	−0.29	0.75*
ER	−0.74*	−0.84**	−0.17	0.78*
Biomass of *Cladophora glomerata*	−0.43	−0.36	−0.45	0.30
Biomass of *Enteromorpha instestinalis*	−0.20	−0.14	−0.07	−0.05
Biomass of epipelon	0.24	0.24	0.40	−0.38
Biomass of *Ruppia maritima*	−0.44	−0.48	−0.41	0.51
Richness of families	−0.75*	−0.85**	−0.32	0.86**
Biomass of macroinvertebrates	−0.57	−0.66*	−0.23	0.90**
SALINDEX	—	0.89**	0.64*	−0.73*

IBMWP-o did not detect the recovery in the ecological status, all the dates being classified as poor, with the exception of one date. On the other hand, IBMWP-r, assessed the ecological status as good on nine dates and as high on two dates, one of those being October 2003 which contrast with the physic-chemical changes observed.

The SALINDEX scores showed a meaningful negative correlation with temperature, FR score and metabolic rates (GPP and ER), and a significant positive correlation with dissolved oxygen (Table 8). In contrast, the IBMWP score (IBMWP-o and IBMWP-r) presented an opposite pattern and was positively correlated with temperature, GPP, ER, FR score and macroinvertebrate biomass and negatively with organic matter in the sediments. Hence, the correlation between both index scores was negative. Only the C/H score was positively correlated with the SALINDEX score.

Meaningful differences were observed between healthy (good or high) and impaired (bad, poor or moderate) dates for some variables. Indeed, temperature ($F = 8.6$; $p \leq 0.05$), Chlorophyll *a* ($F = 8.6$; $p \leq 0.05$), GPP ($F = 16.6$; $p \leq 0.01$), ER ($F = 6.6$; $p \leq 0.05$), family richness ($F = 15.1$; $p \leq 0.01$) and macroinvertebrate biomass ($F = 6.5$; $p \leq 0.05$) were significantly greater on perturbed dates while mean dissolved oxygen values ($F = 6.8$; $p \leq 0.05$) were substantially lower on impaired dates.

DISCUSSION

Methodological questions

The development of an index for assessing ecological status is not exempt from questions inherent to a given methodology, such as the difficulty in defining ecotypes and establishing reference stations (Reynoldson *et al.,* 2000; Bonada *et al.,* 2002; Nijboer *et al.,* 2004) since aquatic ecosystems have long suffered stress in the Mediterranean Basin (Bonada *et al*., 2002; Sánchez-Montoya *et al*., 2005; 2007). In this paper, we have used the general classification system for thalassic waters to classify the saline environments with regard to the salinity gradient

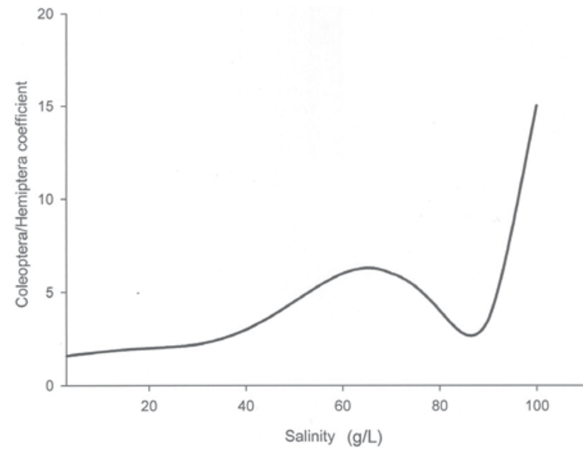

Figure 4. Hypothetical response of Coleoptera/Hemiptera coefficient to salinity changes. *Respuesta hipotética de la relación coleópteros/hemípteros frente a cambios en la salinidad.*

described by Montes & Martino (1987). This classification is based on shifts in algal communities (Hammer *et al.,* 1983) and other organisms in saline lakes (Hammer, 1986) as a result of salinity changes. However, the discontinuities along the salinity gradient that would determine quantifiable changes in the macroinvertebrate communities of saline streams is not well defined due to the high degree of tolerance of their biota to physical-chemical changes and the lack of scientific papers that study the response of community to salinity variations (Bunn & Davies, 1992, Nielsen *et al*., 2003, Pinder *et al*., 2005, Velasco *et al*., 2006). For instance, Velasco *et al.* (2006) in a study analysing the shifts in the composition and structure of the community in the face of salinity variations, carried out in Rambla Salada, found substantial differences when salinity surpassed 75 g/L. Below this level, the differences observed in community structure were greater between habitats than those between different levels of salinity. The results of the IndVal analysis seems to support this hypothesis since it did not detect any health-indicator taxa for mesosaline habitats (ecotypes 3 and 4), probably because the eurihaline response of many species that can live in a wide rage of salinity. This suggest that the upper boundary for mesosaline ecotypes could be higher than 40 g/L although further studies are required to improve the classification of saline aquatic ecosystems in the Iberian Peninsula, which would improve the robustness of the index and its area of application. The meaningful differences found in the coefficient values for the family richness and the Coleoptera/Hemiptera species between the proposed ecotypes appear to support the proposed classification, at least for the study area.

Response of the different metrics

Richness of families

The richness of families metric responded lineally in the fact of salinity changes, decreasing as salinity increased. In the Rambla Salada, when the FR metric scored (when richness of families was within the metric scoring range for reference sites for each ecotype), the ecological status class was, at least, good. Moreover, the SALINDEX score and the richness of families value were negatively correlated. The positive correlation found between the FR metric score and Chlorophyll *a* in water (impairment indicator) supports the usefulness of this metric. Boix *et al.* (2005) also found a negative correlation between the RIC index, based on the richness of Crustaceans and Insect taxa, and the Chlorophyll *a* and a positive correlation with dissolved oxygen. The FR metric is easy to use and does not require high taxonomic knowledge. To identify macroinvertebrate families, the graphic taxonomic keys of Tachet *et al*. (2000) are recommended.

Coleoptera/Hemiptera ratio

The C/H coefficient increased with salinity, since the richness of Coleoptera species decreased to a lesser extent than Hemiptera species. Nevertheless, for salinity levels above 70 g/L, the C/H tended to show a non-lineal response with regard to salinity (Fig. 4). In the 70-100 range this coefficient decreased because only one Hemiptera specie, *Sigara selecta*, tolerates this degree of salinity (Velasco *et al*., 2006) and only a small on Coleoptera species are able to support these conditions. Above 100 g/L the C/H coefficient trended to increase again because Hemiptera species disappeared. This metric appears to be inappropriate for assessing hypersaline habitats (ecotypes 5, 6 and 7) and we recommended it be excluded from in the index for these ecotypes.

Indicator species

Adding health indicator species to SALINDEX completes and improves the information given by the FR metric which is quite demanding since it only scores when all the health-indicator species for the ecotype occur in sufficient abundance. On the other hand, the inclusion of impairment-indicator species in the index, including allocthonous species, lowers the score in the case of the occurrence of any of such species, reflecting their impact in the native community. If these species are present, the index cannot classify the site as high. Thus, the occurrence of exotic species is a criterion that can be used to discard sites in the reference station selection process (Barbour *et al.*, 1999; Sánchez-Montoya *et al*., 2005; Kennard *et al*., 2006).

For their application both metrics require high taxonomic resolution which must be considered a disadvantage. However, the low species richness in saline habitats and the use of a graphic guide with photographs and distinguishing features of the indicator species may facilitate their use. Besides, the use of Coleoptera and Hemiptera at family level has no indicator value in saline habitats due to different habitat preferences of the species to which belong these families, there being as many halophilic species as opportunistic species (e.g., Hydraenidae, Dytiscidade, Hydrophilidae, Corixidae). Indeed, the taxonomic resolution employed in the metrics I+ and I- seems to be sufficient.

Response of the SALINDEX to anthropic perturbation

The application of SALINDEX in the Rambla Salada at Finca de las Salinas during the period 2003-05, identified the dates when the system suffered a great dilution as a result of the massive input of freshwater from the Tagus-Segura diversion channel accident and confirmed the system's recovery after its repair. A clear response to perturbation was observed from the negative correlation of the SALINDEX score with GPP and ER. The freshwater inputs together with nutrient enrichment produced an increase in filamentous algae (Velasco *et al*., 2006) and, in turn, an increase in metabolic rates (GPP and ER) and the richness and biomass of macroinvertebrates. The metabolic rates, as other functional variables, seemed to respond clearly to anthropogenic perturbations (Gessner & Chauvert, 2002; Young *et al.,* 2004) reflecting shifts in ecosystem functioning, and could therefore be considered useful indicators for saline habitat assessment. However, the index appears to be less sensitive in detecting light perturbations, as occurred in April 2005 when freshwater input was short. Lastly, the index score was positively correlated with dissolved oxygen which indicates good quality water.

Comparison with the IBWMP

The inverse relation found between the SALINDEX and IBMWP was to be expected because of the differing composition and structure of the macroinvertebrate communities in freshwater and saline streams, together with the negative response of the family richness to salinity. The initial SALINDEX hypothesis is that, in a natural habitat, saline environments exhibit lower taxonomic richness than analogous freshwater habitats and a hypothetical perturbation would increase the taxonomic richness due to saline stress reduction. In contrast, the IBMWP is based on the inverse hypothesis and, in this case, the response of the community to pollution would be an increase in tolerant species (low score) and a decreasing in intolerant species (high score). The maximum IBMWP scores were obtained when there were a lot of intolerant families, such as those belonging to Plecoptera, Trichoptera and Ephemeroptera (Ward, 1992; Williams & Felmate 1992; Vivas *et al.,* 2002) which do not appear in saline streams, or do so intermittently. Other Odonata families, such as Aeshnidae, Coenagrionidae or Libellulidae, which commonly occur in lentic habitats of low mineralization (Vivas *et al.,* 2002), score quite high in the mentioned index (Alba-Tercedor *et al*., 2002) although their presence in saline "ramblas" could point to freshwater inputs. Nevertheless, in saline streams and wetlands the dominant taxa are Diptera, Coleoptera and Hemiptera (Ward, 1992) whose score in the IBMWP, in general, is low. Thus, if the IBMWP is applied to hypersaline "ramblas" with salinity above 100 g/L, where the reference community is composed of one or two Coleoptera species and some Diptera species, the score will always be low, indicating impairment. Indeed, in the present contribution, the IBMWP reached its maximum score in Rambla Salada during the long dilution period, classifying the ecological status as moderate-good using original boundaries and high using the "ramblas" ecotype boundaries. Thus, the IBMWP was not able to detect a clear dilution perturbation or recovery of the system after freshwater input cessation. Such results agree with the negative relation between the IBMWP score and conductivity found by Alba-Tercedor *et al.* (2002).

The SALINDEX and IBMWP indices were generally related with the same environmental variables but in an opposing way. For example a seasonal influence was observed in the scores of the two indices, temperature being correlated negatively with the SALINDEX score and positively with IBMWP score. This could have been due, partially, to their relationship with variables highly dependent on temperature o radiation such as the metabolic rates (Velasco *et al.*, 2003) or the macroinvertebrate biomass (Barahona *et al.,* 2005).

Given the results obtained, the IBMWP cannot be considered suitable for assessing the ecological status of saline streams, whether using original boundaries or the proposed "ramblas" ecotype. This ecotype has shown great variability in its macroinvertebrate communities and includes water bodies from a wide range of mineralization and water permanence, for which reason we recommend its division into subgroups for the correct application of WFD (Sánchez-Montoya *et al*., 2007). In the same way, other indices developed for freshwater streams, such as the index of canopy vegetation quality (QBR, *Qualitat del Bosc de Ribera*), have shown similar problems when applied to "ramblas" (Suárez *et al.,* 2002).

Application area and future perspectives for SALINDEX

The application of SALINDEX in the different aquatic ecosystems in the protected area of Ajauque-Rambla Salada identified the best preserved reaches and hydrological sectors, in addition to the most perturbed areas showing, generally, a better ecological status the aquatic habitats placed on Rambla Salada in comparison with those located on Ajauque. The index assigned a good or high ecological status to the stations surrounded by dryland farming (mainly cereals) and/or natural gypsic and halophilic vegetation, where freshwater and nutrient inputs were irrelevant. On the contrary, the stations where perturbation was detected were influenced by irrigation crops or organic pollution.

In the beginning, SALINDEX was designed as a component of an ecological indicator system for monitoring and controlling the ecological status of the Protected Area of Ajauque Wetland and Rambla Salada (Project Interreg III B Espacio MEDOC MedWet/Regiones). Such a system included, moreover, other indices or metrics based on hydrochemical features, vegetation, land uses, terrestrial invertebrates and aquatic and terrestrial vertebrates for the assessment of terrestrial and aquatic ecosystems of the protected area (Álvarez y Gómez, 2005). Nevertheless, the area to which SALINDEX could be applied includes the Iberian Southeast where "ramblas" and associated wetlands are the most frequent saline habitat.

In conclusion, SALINDEX is designed in accordance with ecological patterns typical of inland saline ecosystems (low taxonomic richness, predominance of Diptera, Coleoptera and Hemiptera, presence of halotolerant and halophilic species) and is the first tool specifically elaborated for these kinds of habitats. Finally, although some weaknesses were detected in the index and now are being studied by the authors of this paper, SALINDEX was able to detect the most important dilution processes and the system's recovery to date, then, it seems the most suitable tool for assessing the ecological status of saline "ramblas" and wetlands.

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