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Macroinvertebrate Community in a Mediterranean Mountain River: Relationship with Environmental Factors Measured at Different Spatial and Temporal Scales

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Abstract: The macroinvertebrate community, physical–chemical water variables and hydromorphological indices were studied in the Turón River (Málaga, Southern Spain). Our study aims to improve the knowledge of the most influential environmental factors at different spatial and temporal scales in Mediterranean rivers, in order to establish better management of Mediterranean river ecosystems. To this end, in this work, seasonal sampling was carried out for one year to evaluate the effect of the characteristics of the drainage basin (i.e., geology, topography, land use) on the macroinvertebrate community. To this end, the environmental variables of the catchment basins were evaluated at three different scales: (i) watershed level, (ii) valley segment level and (iii) local level. The results showed that 13 environmental variables, 3 at the watershed scale, 5 at the valley segment scale and 5 at the local scale, influenced the macroinvertebrate community. Land use is the main explanatory variable at the watershed scale, while stream channel curvature is the most common variable at the valley segment scale, and the habitat assessment index is the variable with the strongest influence at the local scale. The influence of different spatial scales presented a seasonal variation. During spring, autumn and winter, the watershed scale exhibited the highest resolution (adjusted R^2 = 0.20–0.29), while in summer, the local scale became the most significant in explaining the presence of macroinvertebrate taxa (adjusted R^2 = 0.17). The obtained results emphasize the significance of temporal and spatial scales in Mediterranean rivers for adequate river ecosystem management.

Keywords: stream; spatial scales; macroinvertebrate; land use; hydromorphology; RDA–variation partitioning; biotic indices

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1. Introduction

The Water Framework Directive (WFD) 2000/60/EC establishes the basic principles of a sustainable water policy in the European Union, to maintain and improve aquatic communities and protect the ecological integrity of freshwater systems. An integrative analysis of ecosystem properties has become mandatory for river management; therefore, effective and easy-to-use tools that provide rapid information on ecological status are necessary for successful water management [1,2]. This integrative analysis requires a study perspective at different scales since river ecosystems constitute spatially nested hierarchies. Hierarchy theory postulates that physical and biological variables at a fine-scale spatial level are conditioned by variables at broader spatial scales [3–7]. In this sense, several studies have developed these concepts in theoretical frameworks in which the biotic community present at a point can be considered as the result of a series of filters ranging from the local habitat to regional and continental scales [8,9].

The use of biological indicators in river management presents several advantages, including low economic cost, rapid result generation and the capacity to offer a discriminating response due to their ability to show changes under environmental disturbances. The utilization of techniques involving live organisms, particularly macroinvertebrates, stands as the most comprehensive and practical approach available today for such purposes, constituting a fundamental tool in applied biomonitoring efforts for assessing water quality [2,10–14].

This study allows us to expand the current knowledge in the literature about the influence of environmental variables evaluated at different spatial and temporal scales in Mediterranean rivers. Thus, the inclusion of variables at different scales in studies on macroinvertebrate communities can improve our ability to predict the responses of Mediterranean rivers and generalize the predictions to other geographic ranges [15,16]. The obtained results are necessary to calibrate the appropriate scale for the analysis of the interactions among the environmental variables with human impact (i.e., land use) and the standardized ecological quality indices and physical–chemical variables [17].

Under this idea, the aim of this study was to explore the resulting macroinvertebrate community of a Mediterranean river as a consequence of its interaction with environmental variables measured at different spatial scales (watershed, valley segment and local) and temporal scales (spring, summer, autumn and winter), analyzing which variables have the greatest influence on macroinvertebrate communities, which is a key aspect in the management of river ecosystems.

2. Materials and Methods

2.1. Study Area and Sampling Sites

The Turón River is a Mediterranean river ecosystem with permanent waters located in the south of the Iberian Peninsula (Figure 1). The study area shows a Mediterranean climate, with a wet season in winter–spring and a dry season in summer–autumn. According to Spanish legislation [Orden ARM/2656/2008], the study area belongs to river typology TE09 (mineralized rivers of low Mediterranean mountains). The watershed is based on materials from the Cretaceous Miocene and mainly composed of marls, marl limestones, marly limestones and flysch clays [18]. The headwaters come from a large number of sources of karst origin. According to the Ardales gauge station, the average monthly flow of the Turón River is 0.29 $m^3\ s^{-1}$, with the winter months being those that provide the greatest amount of water and the summer and autumn months providing the lowest precipitation. The total length of its channel is 42.5 km, and its basin extends over an area of 252.9 km^2. The main tributaries are the Fuensanta Creek (permanent waters) and the Blanquillo Creek (temporary waters), both on its right bank. The main land use is natural, with areas of forests, bushes, rocks and pastures in areas of a high and medium slope; dryland agriculture in the open valleys; and irrigated areas in the plains, especially near the two towns: El Burgo and Ardales, where urban water discharges occur.

According to the criteria established by Rosgen [6] and the European Water Framework Directive [6], the studied channel has been divided into eight sections (T1 to T8; see Tables 1 and S1) based on its hydrogeological aspects, considering geology, geomorphology, land use, river valley and channel characteristics. Two sampling stations were added in the creeks Fuensanta (FS) and Blanquillo (AB). These creeks, tributaries of the main channel, are included due to their hydrological and ecological importance since they act as a pool of biodiversity [2] (Figure 1).

Table 1. Sampling site locations and annual mean and standard deviation data of environmental variables registered in this study.

Sampling Point	Latitude	Longitude	Altitude (m)	Temperature (°C)	pH	DO (mg L^{-1})	Alkalinity (mmol L^{-1})	TDS (mg L^{-1})
T1	−4.99133502	3.67737370	627	14.30 ± 0.96	8.3 ± 0.07	10.23 ± 0.66	3.5 ± 0.45	135.0 ± 23.80
T2	−4.97294618	3.67823537	571	14.43 ± 2.07	8.5 ± 0.12	9.60 ± 1.12	3.78 ± 0.59	132.5 ± 17.08
T3	−4.56590792	2.94793852	549	15.45 ± 3.81	8.5 ± 0.30	10.05 ± 0.42	3.93 ± 0.31	145.0 ± 31.09
T4	−4.93926584	3.67949044	525	16.78 ± 4.13	8.4 ± 0.36	7.93 ± 2.83	4.28 ± 0.56	175.0 ± 36.97

Table 1. *Cont.*

Sampling Point	Latitude	Longitude	Altitude (m)	Temperature (°C)	pH	DO (mg L^{-1})	Alkalinity (mmol L^{-1})	TDS (mg L^{-1})
T5	−4.91831657	3.68015175	502	16.10 ± 3.48	8.5 ± 0.12	10.15 ± 0.70	4.20 ± 0.48	182.5 ± 35.94
T6	−4.89629730	3.68298751	448	15.90 ± 3.81	8.4 ± 0.10	9.25 ± 1.04	4.23 ± 0.21	162.5 ± 25.00
T7	−4.87855210	3.68657774	380	15.85 ± 3.47	8.5 ± 0.20	9.08 ± 1.63	4.10 ± 0.08	167.5 ± 47.17
T8	−4.84686702	3.68883297	344	15.73 ± 2.99	8.6 ± 0.41	8.03 ± 4.42	4.63 ± 0.26	190.0 ± 21.60
FS	−4.94099278	3.67776248	571	15.10 ± 3.57	8.5 ± 0.08	9.25 ± 1.05	4.33 ± 0.36	242.5 ± 59.09
AB	−4.89911693	3.68201137	461	15.88 ± 3.44	8.2 ± 0.18	9.00 ± 0.93	5.13 ± 0.50	185.0 ± 92.56

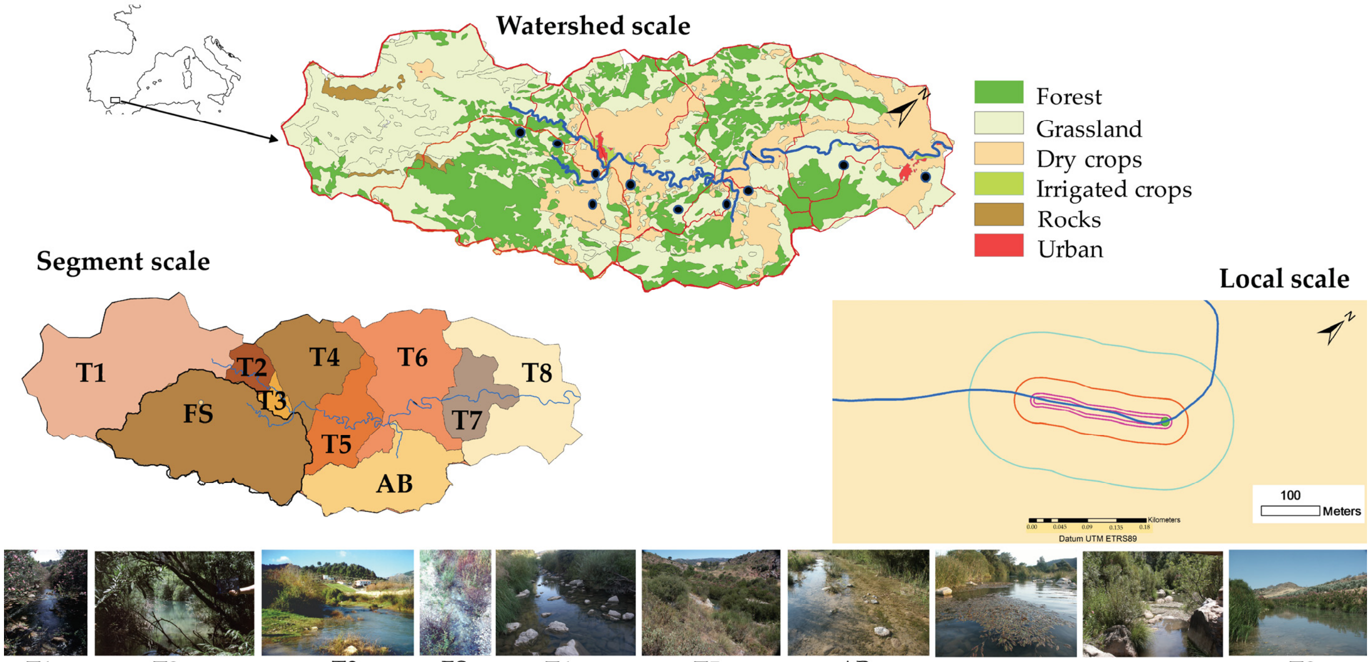

Figure 1. Location of the Turón River (Southern Spain), with indication of the three scales used in this study (watershed, segment and local) and photographs of the different sections of the river. At the watershed scale, the different land uses and sampling points (black dots) are represented. On the segment scale, the different catchments of the ten established river sections appear. Finally, on the local scale, the study area considered in each section is represented. The different color lines represent the different buffer used: (i) pink line = buffer of 5 m (LU5); (ii) brown line = buffer of 30 m (LU30) and (iii) green line = buffer of 100 m (LU100). Table 1 shows the geographical coordinates of the sampling points.

2.2. Environmental and Biological Data

Each section presents a water quality sampling point, both for physical–chemical variables and macroinvertebrate samples. The macroinvertebrate community was sampled seasonally from April 2000 to March 2001 at each sampling site using a hand net with a mesh size of 300 µm. A multi-habitat sampling was carried out according to the IBMWP protocol [10,11,19], which includes sampling all existing microhabitats at the sampling point. The collected organisms were stored in properly labeled bottles and fixed with alcohol (75% final concentration). In the laboratory, the samples were washed, with the organisms being carefully separated before the taxonomical identification was performed. The organisms were identified at the family level using a stereomicroscope and according to specific keys [20]. With these data, two biotic indices were calculated, the IBMWP [10,11,19] and the EPT [21]. The IBMPW index serves as a bioassessment tool for evaluating freshwater quality. This method takes advantage of the different sensitivities of macroinvertebrate families to different contaminants. By quantifying the presence of these families, the IBMPW index infers the degree of environmental stress and assigns a score ranging from

0 to 150. This score corresponds to five established classes of water quality: from "very poor" (<15), indicating highly polluted conditions, to "excellent" (>100), meaning nearly pristine environments. On the other hand, the EPT biotic index is based on the presence or absence of three orders of rheophilous insects: Ephemeroptera, Plecoptera and Trichoptera. These insects, with a preference for fast-flowing water, show a high sensitivity to pollution, which makes their presence or absence indicative of the quality of the water. The EPT index is obtained by adding the number of families of each order present in a sample of macroinvertebrates. The following environmental variables were also measured at the different sampling sites: temperature (°C), pH (Hanna piccolo 2), alkalinity (Aquamerck 1.11109.0001—mmol L^{-1}), dissolved oxygen (Aquamerck 1.11107.0001—mg L^{-1}) and total dissolved solids (Hanna DisST WP—mg L^{-1}).

The characteristics of the valley and river corridor were evaluated at different scales, i.e., watershed (10^5–10^6 m), valley segment (10^3–10^5 m) and local (10–10^3 m), using the information available in the REDIAM Environmental Information Network of the Ministry of Environment of the Government of Andalusia, the treatment of digital terrain models (DTMs), photointerpretation tools and a geographic information system (QGIS). At the watershed level, the following environmental variables were calculated: (i) geology (percentage of lithology class)—differentiating between calcareous, siliceous and detrital materials; (ii) mean basin slope (%); (iii) basin area (m^2); (iv) laminar erosion potential (t ha^{-1} $year^{-1}$) [22]; (v) land use percentage in the watershed (LUW)—differentiating between irrigated crop, dry crop, forest land, grassland, rock and urban; and (vi) C USLE factor (dimensionless) [23]. At the valley segment level, five environmental variables were obtained: (i) stream channel curvature (dimensionless) [24]; (ii) stream channel gradient (%) using the talweg line 1 km over the sampling point [25]; (iii) valley depth index (dimensionless) [24]; (iv) mean segment (upstream sampling point) QBR riparian index value (QBRmed) [26]; and (v) mean segment (upstream sampling point) habitat assessment score value (HASmed) [27]. Finally, at the local level, the following environmental variables were calculated: (i) land use over 200 m from the sampling point with a buffer of 5 m (LU5), (ii) with a buffer of 30 m (LU30) and (iii) with a buffer of 100 m (LU100) [28]; (iv) QBR index at the sampling point (QBRs); and (v) habitat assessment score at the sampling point (HASs). The land use categories employed on this scale are the same as those on the watershed scale.

2.3. Data Analysis

Figure 2 shows a flowchart with all the statistical analyses carried out. The discrimination of sampling sites and seasons based on the presence–absence of macroinvertebrates was carried out through two different but complementary statistical analyses. The first analysis involved a cluster analysis, grouping the sampling sites based on their macroinvertebrate assemblages. Jaccard's coefficient of similarity was utilized as the measure for agglomeration, employing the Unweighted Pair Group Method using Arithmetic Averages (UPGMA) as the clustering strategy [29]. The second analysis, Permutational Analysis of Variance (PERMANOVA) [30], was employed to infer significant differences in macroinvertebrate communities concerning the sampling site and season. PERMANOVA was performed using Jaccard's dissimilarity index. The analyses were carried out using the vegan package version 2.6-2 in R software (v.4.2.2.) [31].

A spatial autocorrelation analysis (Moran's index) was performed to determine the presence or absence of spatial autocorrelation [32]. Moreover, a Spearman's correlation analysis was conducted to assess the strength and direction of the relationships between the two different macroinvertebrate biotic indices used in this work, the IBMPW [10,11,19] and EPT [21], and the studied environmental variables.

Finally, to determine the influence of the variables included in each scale (watershed scale: 44 variables; segment scale: 28 variables; local scale: 52 variables) on the occurrence of individual taxa, a redundancy analysis (RDA) was conducted. This approach allows for selecting variables at different scale levels, explaining the variability in the taxon distribution. The selected variables were chosen through a forward selection process, following the guidelines set forth by Blanchet and colleagues [33]. After the variable selection in the RDA, a variation partitioning technique was employed to quantify the relative contribution of selected variables on the structure of the macroinvertebrate community (watershed scale: 11 variables; segment scale: 8 variables; local scale: 9 variables) [34]. This analysis deconstructed the variance (expressed as adjusted R^2), attributing it to a set of independent explanatory variables ($p < 0.05$), as well as the common variance collectively elucidated by these variables.

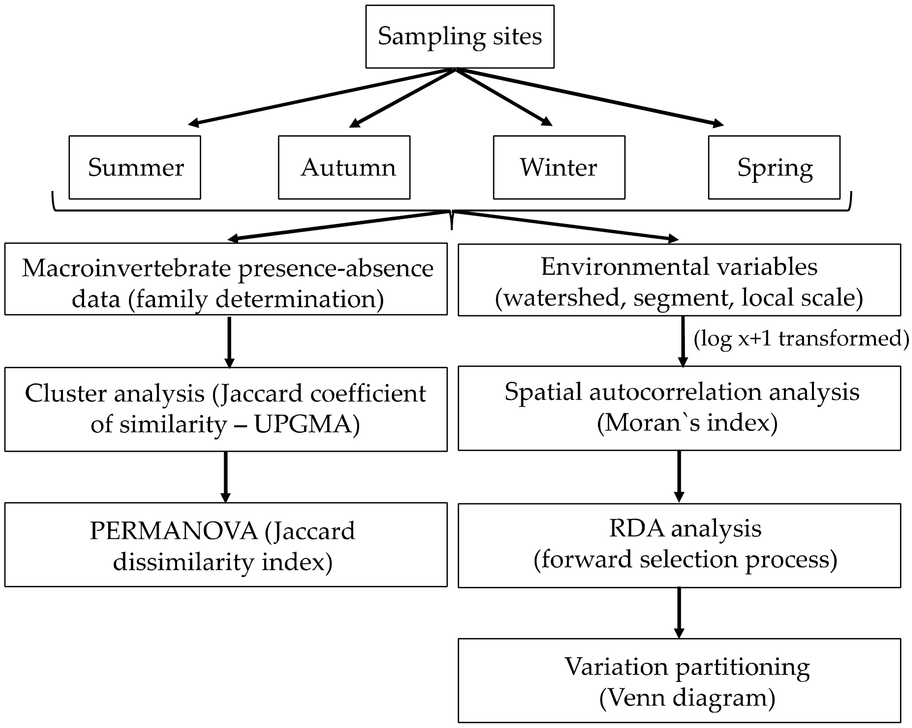

Figure 2. Flowchart with all the statistical analyses performed.

Before the analysis, the variables were transformed using the logarithm (x + 1), to reduce the influence of different scaled measurements [33]. The vegan package version 2.6-2 in R software (version 4.2.2) was used for the RDA and variation partitioning analyses [31].

3. Results

Table 1 shows the results obtained from the environmental variables at each of the sampling stations. A total of 78 taxa were collected in these sampling sites (Table S2), with a predominance (%) of the Trichoptera, Ephemeroptera, Gastropoda, Diptera, Coleoptera and Odonata families. Family richness ranged from 8 to 29 families per sampling point, with an average number and standard deviation of 20.29 ± 5.50.

The cluster analysis (Figure 3) and the PERMANOVA indicate different communities since there is no grouping of them according to the species recorded between sampling sites ($F = 2.1237$, $p < 0.001$) or seasons ($F = 3.3707$, $p < 0.01$).

Spearman's correlations (Table 2) showed that both the IBMPW index (spring) and the EPT index (autumn) were strongly negatively correlated with soil type, urban and dryland agricultural use, and low stream channel confinement. There was also a positive correlation with basin slope, elevation and habitat quality. In summer and winter, the EPT index was negatively correlated with irrigated land use and positively correlated with valley confinement and habitat quality. The results of the spatial autocorrelation analysis (Moran's index—Table S3) show that most of the geographic and land use variables in the watershed do not have statistically evaluable values in the analysis, except for siliceous lithology, as it is highly localized in the lower section, and the land uses dry crop and forestland, as well as the degree of channel confinement, which show a clustered arrangement (agricultural valleys in the lower zone, natural areas in the headwaters, and embedded sections in the middle and upper sections). Of the physical–chemical factors, only alkalinity is significant, as it is related to the altitudinal gradient and the higher mineralization downstream. Likewise, elevation is a scaled factor. The biological indices IBMPW and EPT are strongly clustered, with the upper, middle and lower sections showing similar values among themselves, with a downward trend.

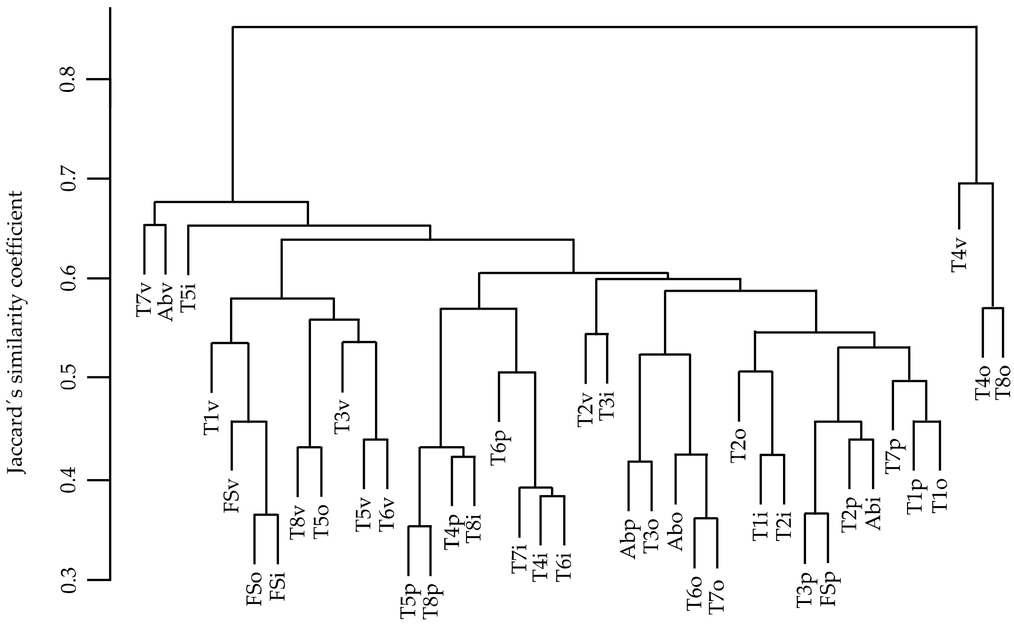

Figure 3. Cluster dendrogram grouping sites and seasons according to their macroinvertebrate species.

Table 2. Seasonal significant correlations among environmental studied variables and IBMPW and EPT biomonitoring indices.

	Spring		**Summer**		**Autumn**		**Winter**	
	IBMPW	**EPT**	**IBMPW**	**EPT**	**IBMPW**	**EPT**	**IBMPW**	**EPT**
Siliceous lithology	−0.69	-	-	-	−0.67	−0.78	-	-
LUw (urban)	−0.78	-	-	-	-	−0.72	−0.66	-
LUw (irrigated crop)	−0.76	-	-	-	-	−0.70	-	-
LUw (dry crop)	-	−0.66	-	-	-	-	-	-
Watershed gradient	0.67	-	-	-	-	0.65	-	-
Valley depth	0.70	0.65	-	0.68	-	0.74	-	0.77
Curvature	−0.82	−0.79	−0.66	-	−0.73	−0.68	-	-
Channel gradient	0.70	0.69	-	-	-	-	-	-
HASmed	-	-	0.75	0.72	-	0.73	0.73	0.75
QBRmed	-	-	-	0.70	-	-	0.64	0.66
Elevation	0.68	-	-	-	0.64	0.68	-	-
LU100 (irrigated crop)	-	-	-	−0.63	−0.81	−0.71	-	-
LU30 (irrigated crop)	-	-	-	−0.70	−0.80	−0.66	-	-
LU100 (forest land)	-	-	-	-	-	-	-	0.64
HASs	0.70	-	-	-	0.73	0.65	-	-
Temperature	-	-	-	-	0.71	-	-	-
pH	-	-	-	0.86	-	-	-	-
Alkalinity	-	-	−0.92	−0.72	-	-	-	-

The results of the variation partitioning and the proportion of the explained variance (adjusted R^2) at the three studied levels are summarized in Tables 3 and S4 and Figure 4.

This statistical analysis shows that 13 environmental variables (3 at the watershed scale, 5 at the valley segment scale and 5 at the local scale) influence the recorded variability, while other variables were not significant at any of the three scales under study. At the watershed scale, potential runoff, basin area and basin slope were not significant, which was also observed at the local scale, where the TDS, oxygen concentration and land uses in 100 m and 5 m buffers (LU100, LU5) were not significant.

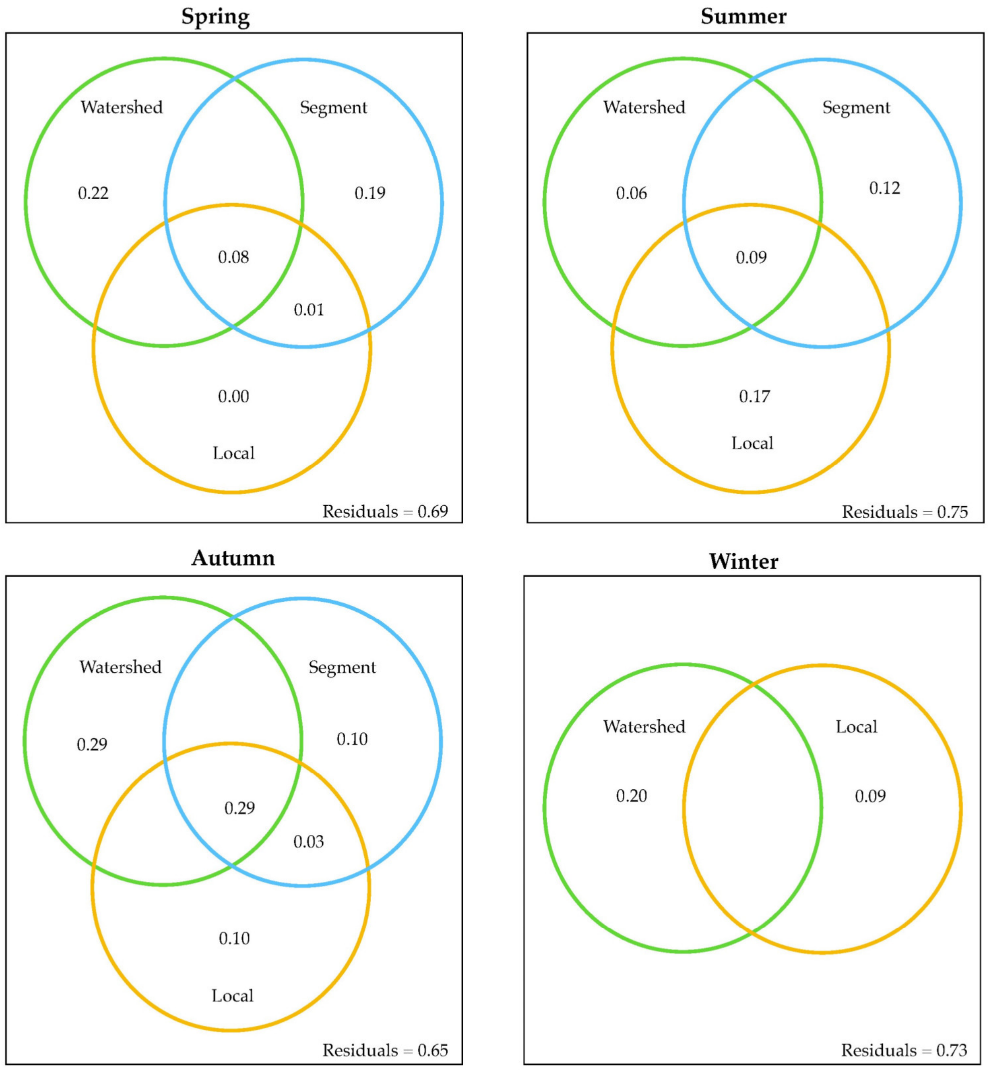

Figure 4. Venn diagram of the variation partitioning at the three scales and for the four seasons analyzed. In the winter season, the segment scale is not represented, since no significant variables were obtained in the previous RDA analysis.

In all seasons, land use is the main explanatory variable at the watershed scale. Stream channel curvature is the most common variable at the valley segment scale; while HAS is the variable that is most repeated at a local scale. The scales with the greatest explanation are different in each season; thus, in spring, autumn and winter, the watershed scale is the one with the highest resolution, whereas, in summer, the local scale is the most important for explaining the results of the presence of macroinvertebrate taxa (Figure 2). In the same way, the local scale is the one that contributes the least to the explanation of the results, except in summer, when it is the one that provides the greatest explanation. The three studied scales influence the presence of macroinvertebrate species in all seasons except in winter, in which the valley segment scale does not exert any influence on it. The total variance explained in the four seasons was always greater than 25%, with a maximum explanation in autumn and a minimum explanation in summer.

Table 3. Results of RDA and variation partitioning showing the contributions of environmental variables. W: watershed scale; S: valley segment scale; L: local scale.

Season	Scale Level	df	Adj. R^2	Variables
Spring	W	3	0.22	LUW (dry crop, irrigated crop, urban)
	S	3	0.19	Valley depth, curvature, channel gradient
	L	1	0.004	HASs
	S ∩ L		0.01	HASs, valley depth, curvature, channel gradient
	W ∩ S ∩ L		0.08	HASs, valley depth, curvature, channel gradient, LUW (dry crop, irrigated crop, urban)
Summer	W	2	0.06	LUW (dry crop, urban)
	S	2	0.12	HASmed, curvature
	L	3	0.17	HASs, pH, alkalinity
	W ∩ S ∩ L		0.09	LUW (dry crop, urban), HASmed, curvature, HASs, pH, alkalinity
Autumn	W	1	0.29	LUW (dry crop)
	S	3	0.10	QBRmed, HASmed, curvature
	L	4	0.10	HASs, pH, alkalinity, LU30 (irrigated crop)
	S ∩ L		0.03	QBRmed, HASmed, curvature, HASs, pH, alkalinity, LU30 (irrigated crop)
	W ∩ S ∩ L		0.29	QBRmed, HASmed, curvature, HASs, pH, alkalinity, LU30 (irrigated crop), LUW (dry crop)
Winter	W	5	0.20	LUW (dry crop, irrigated crop, urban), C USLE factor, siliceous lithology
	L	1	0.09	Temperature

4. Discussion

The results obtained in this study on water quality with the biotic indices (IBMWP and EPT) have allowed us to show some patterns. First, the IBMPW index presents important differences associated with pollution from urban, agricultural and livestock sources. In the upper reaches (T1 and T2), the biological quality is high, while in T3, a decrease is observed due to diffuse agricultural pollution. In the following sections, the index decreases, with the minimum values in T4 and T8, as a consequence of high urban pollution. The Fuensanta (FS) and Blanquillo (AB) creeks, which are not affected by human activity, have good biological quality with high values of the IBMWP index. In relation to the EPT index, a gradual decrease is observed downstream because the macroinvertebrate groups that make up this index generally prefer habitats with turbulent waters. However, a decrease in the EPT index is also observed in the sections affected by urban discharges (T4 and T8) and in AB due to the seasonality (summer drought). The results of the cluster and PERMANOVA analyses indicate differences in taxa between sampling sites and seasons, being in agreement with the results obtained with the biotic indices. In this way, a notable negative correlation is observed between the values of both indices, land uses at the basin level (urban and agricultural) and the low confinement of the channel. The low confinement of the canal indirectly favors these land uses due to greater accessibility and lower slopes of the riparian zone. This correlation also presents a seasonal variation, being highest in spring and autumn. Weaker correlations are observed in winter, due to higher flow and water quality, and in summer, with base water flow conditions and isolation from basin effects. Furthermore, it is also interesting to indicate the negative correlation with the use of local irrigation (LU100, LU30).

Our results also allow us to corroborate that the spatio-temporal distribution of the macroinvertebrate community is conditioned by different hierarchical biotic and abiotic variables at the studied scales [8]. A detailed analysis of the results at the scale level allows us to observe that, at the watershed scale, land use is one of the fundamental descriptors in all seasons of the year, whether due to dryland agricultural use, irrigation, urban use or the C USLE factor, which is also strongly influenced by land use. This result agrees with that reported by other authors [35], who confirm the importance of land use at the watershed level in the Mediterranean area, with numerous studies comparing its greater or lesser effect on the other two scales: local and valley segment [36–38]. Land use in a drainage basin has a greater influence on channel morphology, bank stability and river hydromorphology [39] than other natural characteristics such as geology and basin slope [40–42]. This explains the strong relationship between land use at the watershed scale and bioindicators in the Mediterranean climate [43], as well as in smaller watersheds [41].

At the valley segment level, the results are uneven. In winter, no significant variable appears (so, not included in the variation partitioning), surely as a consequence of the strong homogenizing effect of environmental variables due to the high circulating flow [44]. However, in the rest of the seasons, significant variables do appear, such as the average slope of the channel, the degree of channel enclosure (curvature) or the valley depth coefficient, which indicates that once more stable flow conditions occur, the valley typology and subsequent morphological characteristics of the channel are descriptors for the structure of macroinvertebrate communities. Furthermore, on this scale, in low water periods (summer and autumn), with greater stress due to flow reduction, the hydromorphology quality variables HASmed and QBRmed (mean value of the segment) are also significant, which indicates that the hydromorphological quality status of the banks and channels are important for determining the macroinvertebrate communities, thanks to the application of a more extensive spatial scale, which is more strongly correlated with the land use of the drainage basin [45,46].

At the local level, the HASs habitat assessment index and the physical–chemical variables have the greatest influence on the presence of macroinvertebrate taxa, with a greater effect in summer and autumn, coinciding with lower values of the biological indicator IBMWP [2]. Similar data are shown in the literature for small Mediterranean rivers [47,48], as a consequence of the quick response of the macroinvertebrate community to local stressors. However, the physicochemical variables sampled (pH, temperature, alkalinity, TSD and dissolved O_2) are not very explanatory, only marginally the temperature in the winter season and more importantly pH and alkalinity in summer and autumn, although combined with the habitat assessment index (HASs) or land use (LU30). The absence of measurement of other physicochemical variables more strongly related to organic pollution, such as concentrations of nitrogen and phosphorus, total or in some of their dissolved forms, is surely part of the cause of the low contribution to explaining macroinvertebrate distribution or presence. This idea is also supported by the fact that the predictive power of water quality parameters for macroinvertebrate communities increases with the number of parameters measured [37]. Physical–chemical variables are often less important than hydromorphological variables, such as the water regime or sediments, which are variables controlled by other processes at the watershed and valley segment scales [49–51]. In rivers affected by discharges with high organic loads or toxic effluents or with a high area of intensive agriculture in their drainage basins, the opposite occurs, and water quality is the main factor in the structure of the macroinvertebrate community [43,49]. In the Turón River, the diffuse agricultural and direct urban discharges that occur in the dry season (summer and autumn) exert, as in other Mediterranean rivers [35,52,53], a greater weight on the water quality at the local scale. In the same way as at the local scale, once again, the hydromorphological indicator of the channel (HASs) exerts a strong influence on the macroinvertebrate community. However, the bank quality index (QBRs) at a local scale is not very relevant at any time of the year. This is explained by the fact that the QBR index appears to be correlated with the HAS in sites of high environmental quality, but with important differences with it in the case of river systems affected by erosive processes at the watershed scale, or by strong diffuse pollution and/or direct discharges [50,54–56]. It is notable that land use at the local level is only significant in the autumn sampling (LU30). The use of a sampling length of 200 m with buffers of 5, 30 and 100 m does not seem to be sufficient to reflect the effects of land use at this scale on macroinvertebrate biota. This result indicates the need, as has been previously demonstrated at the valley scale [28], to use greater sampling lengths and buffer widths, which will surely reflect the influence of land use on the macroinvertebrate species community [50].

It is necessary to highlight that the overlaps between the three spatial scales, with high explanatory power values in autumn and low or non-existent values in the rest of the seasons, are in accordance with those obtained in other European or Mediterranean areas [57,58]. Mediterranean river ecosystems are influenced by factors at a wide range of spatial scales, which makes it difficult to distinguish the relative roles of those spatial scales [59,60]. The differences between the broad and small scales suggest that some environmental (nutrients, water flow, velocity, substrate coarseness) or biotic variables (diversity at the genus or species scale, competition, predation, adaptations) that were not included in our study must have an important influence on the macroinvertebrate community [12,53,61,62]. The absence of measurements of other physicochemical variables more strongly related to organic pollution, such as concentrations of nitrogen and phosphorus, in either total or dissolved forms, may be a contributing factor to the low explanatory power of local-scale macroinvertebrate distribution or presence models. This shortcoming of our study is further supported by the observation that the predictive power of water quality parameters for macroinvertebrate communities increases with the number of parameters measured [37]. However, we can conclude that the richness of taxa and the biological quality indices are generally higher in river reaches with natural characteristics, which typically have a greater diversity of habitats, providing a wider range of conditions for macroinvertebrates to thrive. River reaches with altered characteristics, such as open valleys, poorly incised channels and low slopes, typically have lower richness of taxa and biological quality indices. These reaches commonly have a smaller range of habitats, which limits the diversity of macroinvertebrates that can survive.

It is important to indicate that scale-dependent environmental factors are responsible for the spatial and temporal heterogeneity of ecosystems [63]. The abundance, species composition and functional traits of aquatic macroinvertebrate communities in streams are influenced by these factors at multiple spatial and temporal levels [64], and they are related within a physical hierarchy, where larger-scale factors constrain the expression of smaller-scale factors [65]. Our findings corroborate that, in Mediterranean rivers, factors that operate at the watershed and valley segment scales act in synergy with local disturbance regimes to influence macroinvertebrate community taxon richness, including temperature, discharge concentration, and the frequency and intensity of floods and droughts [66–68], with the importance of each spatial scale varying throughout the year [12,66,69,70], as observed in other studies [66,69,70]

The results emphasize the importance of considering both temporal and spatial scales in effectively managing Mediterranean mountain rivers. They provide valuable insights for developing suitable watershed best management practices. An application of the study results highlights that adequate land use management at the watershed level might be more important than potential local improvements to the physical or riparian habitat for macroinvertebrate diversity, particularly in highly altered watersheds. Local improvements are not enough to counteract the negative impacts of inadequately managed watersheds, such as increased stress due to extreme events like droughts or floods and increased input of dissolved solids from direct/indirect pollution. Additionally, considering the medium- and short-term effects of climate change further reinforces this standpoint. Therefore, in the Mediterranean region, prioritizing watershed restoration and conservation should be more important than local restoration efforts.

In summary, this study sheds light on the intricate relationships between biological indices and environmental variables in Mediterranean rivers. While acknowledging the limitations in data availability and the study's scope, our findings emphasize the importance of considering diverse factors when assessing river health. Furthermore, our comparative analysis with other Mediterranean rivers underscores the potential applicability of these findings beyond this specific region. This study highlights the need for a holistic approach, encompassing spatial and temporal scales, to effectively manage and conserve mountain river ecosystems.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/su16051777/s1: Table S1: River segments' sub-watershed characteristics. Table S2: Macroinvertebrate taxa presence–absence at the studied sampling points. Table S3. Results of spatial autocorrelation analysis (Moran's index). Table S4. RDA statistically significant results.

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