BIOLOGICAL ASSESSMENT OF WATER QUALITY IN NORTHERN PORTUGAL USING A METHOD COMBINING SPECIES TOLERANCE AND DIVERSITY ALONG THE LONGITUDINAL AXIS

R.M.V. Cortes and A. Monzon

Dep. Florestal, Universidade de Tras-os-Montes e Alto Douro. 5000 Vila Real. Portugal.

Key words: macroinvertebrates, biotic index, typology. organic pollution, Portugal.

ABSTRACT

Based on the benthic macroinvertebrate community, a multiple biotic index was defined, determined by the diversity parameter H' from Shannon-Weaver and by the sensitivity of a list of organisms to eutrophication. This compiled list was derived using a multivariate technique —DECORANA— which allowed the characterization of a species according to its typological level and tolerance to organic loads. The method presented here takes into account the faunal composition and diversity patterns from the upper to the lower reaches and also the temporal variability. Thus, more reliable comparisons can be achieved in the evaluation of water quality for different order reaches of the river system, independent of seasonal fluctuations.

INTRODUCTION

As regards the indices for assessing the stages of deterioration and recovery of communities in flowing water in response to organic enrichment, two distinct procedures must be mentioned, the saprobien system devised by KOLKWITZ & MARSSON at the beginning of this century and later developed numerically by KNOPP (1954), PANTLE & BUCK (1955) and SLADECEK (1967), and on the other hand, the mathematical indices derived from diversity (H'-Shannon-Weaver, 1949; hierarchical diversity —e.g. KAESLER *et al.*, 1984; rarefaction curves —e.g. SIMBERLOFF, 1978) or community structure like the log-normal model of PRESTON (1948). Combining both aspects —tolerance of key species and a measure of diversity (n. of taxonomic groups)— are the biotic indices, exemplified by the work of WOODWISS (1964), adapted and improved later by numerous authors.

Pollution indices have been widely used to detect and quantify organic contamination in freshwaters and this has proved to be a consistent method in situations of heavy contamination. But lotic ecosystems are characterized by high resilience to disturbance and inversely low resistance (WEBSTER *et al.*, 1983), which makes these indices less accurate for moderate organic loads which do not disrupt the homeostatic equilibrium. On the other hand, there is an obvious longitudinal succession of species composition and, therefore, indicator taxa in some stretches are possibly absent in others or, if present, may reflect idfferent ecological factors. Diversity also has a strong spatial dynamism and can be related to stream order or environmental variability (VANNOTF *et al.*, 1980; STANFORD & WARD, 1983).

For these reasons we have established a biotic index which combines the tolerance of local benthic fauna to contamination (generally considering the identification to species level because it has a narrower spectrum), the natural spatial replacements of the organisms from source to mouth in the river system and also changes in diversity along the horizontal axis. The list of organisms was restricted to taxa which gave more stable temporal information, in order to reduce stochastic interference.

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MATERIAL AND METHODS

The biological survey was carried out in an area forming part of the Douro catchment and included several tributaries, along which 42 sampling stations were located (fig. 1). The purpose was to collect data from the different typological stream levels and also to assess the effects of organic input and its self-purification capacity.

The streams in this region are generally free from major sources of sewage and industrial pollution and from the effects of flow regulation. The water quality is related largely to soil structure and the underlying geology of the catchment. Nutrient concentrations and D.O.M. increase steadily downstream, but because the waters drain the mineral-poor granite and schist there is a low content of salts, not modified by the agricultural practices where the rough pasture and mixture of forest and (few) arable crops do not change the nutrient-poor water significantly. Nevertheless, some specific reaches are affected by sources of disturbance, mainly organic effluents. This is the case at sites C_1 and C_6 —receiving sewage from towns; B, influenced by fish-farming, and C„ C_{10}, To_1 and Tu_7— where irregular discharges from agroindustries occur. A different input occurs at T_3, which receives the run-off from spoil

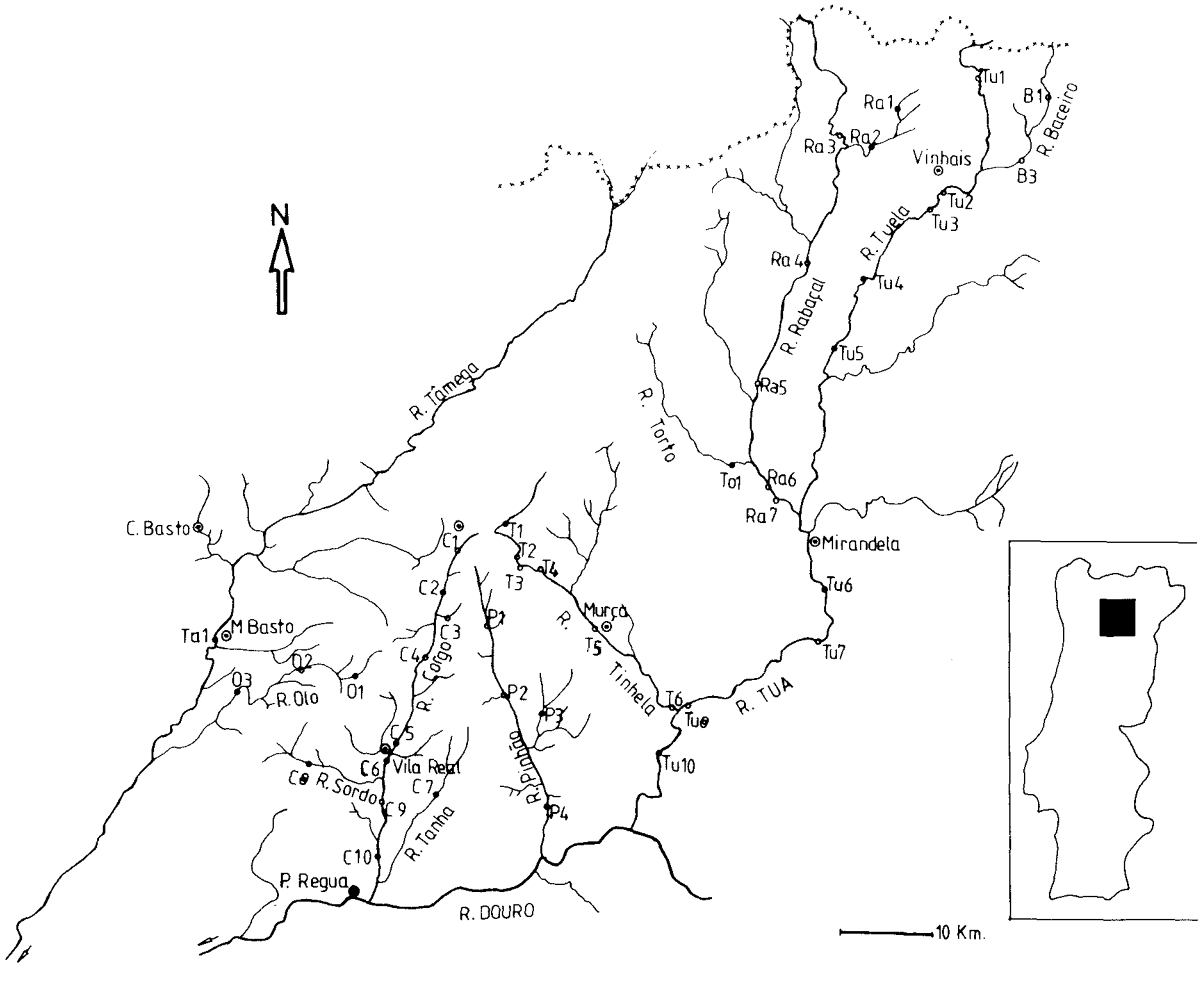

Figure 1.- Geographical localization of the studied area and selected sites.
Localización geográfica del area estudiada y puntos sclcccionados.

heaps resulting from mining, the major metals introduced being Arsenic and Zinc.

Table 1 shows low strong levels of contamination, rarely creating anaerobic conditions, because the low water hardness and residence time do not provide opportunity for algal or macrophytic blooms.

Each site was sampled over a period between 1 (4 samples) and 2.5 years (10 samples), covering each season. This procedure takes into account that many aquatic insects are seasonal and it was our purpose to obtain a representative species list for data analysis. Macroinvertebrates were sampled by a kicking method using a hand-net with a constant active removal effort of 4 minutes at each point. This technique covered all the major habitats within the sampling area. Measurements of 17 chemical parameters were made regularly and extensively in the area studied (Table 1 includes only the most representative ones).

To summarize and arrange the species data and to detect the relationship between taxa and sites, that is, the interaction between biotic and abiotic components, we used the DECORANA ordination method (HILL, 1979). This multivariate technique is based on a Reciprocal Averaging Analysis (R.A.) and uses a two-way weighted averaging algorithm, designed to correct the compression on the axis edges (by rescaling) and the systematic relation between the second and the first axes: arc-shoe effect (by detrending).

Plots were established for each sampling period (14 ordinations were created) with the objective of characterizing the taxa according to each typological level and to polluted and unpolluted reaches, paying particular attention to the species that explained most of the variance in each period for the first two eigenvectors. The respective diagrams appear in CORTES (1989) and MONZÓN & CORTES (1989).

Table 1.- Average values of chemical parameters for the different sampling stations. Values of hardness in mg $C_2CO_3l^{1}$. Valores medios de los parametros quimicos en las diferentes estaciones de muestreo. Valores de dureza en mg $C_2CO_3l^{-1}$.

	pH	O_2 $(mg.l^{-1})$	Hardness	Cond. $(\mu S.cm^{-1})$	Cl^-	$N\text{-}NO_3^-$	$P\text{-}PO_4^{3-}$
						$(mg.l^{-1})$	
O_1	6.4	10.1	7.0	10.1	2.6	<0.1	<0.1
O_2	6.3	9.5	6.5	25.6	2.8	<0.1	<0.1
O_3	6.5	10.0	15.0	29.5	3.6	0.1	<0.1
Ta_1	6.6	10.8	35.0	62.3	5.4	0.1	<0.1
C_1	5.9	8.8	27.5	88.1	8.3	0.3	0.1
C_2	6.3	9.9	11.0	27.0	3.6	0.1	<0.1
C_4	6.4	10.1	17.5	61.8	8.9	0.1	<0.1
C_5	6.5	10.1	17.5	59.5	5.3	0.1	<0.1
C_6	6.5	9.1	32.0	130.1	14.7	6.3	<0.1
C_7	6.9	10.9	45.5	108.5	9.4	0.3	<0.1
C_8	6.2	9.8	20.0	58.8	5.3	0.3	<0.1
C',	6.7	10.6	29.0	114.0	12.3	0.3	0.1
C_{10}	6.8	10.3	30.0	121.3	12.2	0.4	0.1
P_1	6.5	8.7	12.5	36.8	4.9	<0.1	<0.1
P_2	6.5	9.9	14.5	66.6	5.6	<0.1	<0.1
P_3	6.1	9.0	15.5	70.1	6.9	<0.1	0.1
P_4	6.6	9.9	29.0	106.7	10.1	0.1	<0.1
T_1	6.1	9.1	21.5	56.1	6.6	<0.1	<0.1
T_2	6.5	10.1	21.5	42.6	5.8	<0.1	<0.1
T_3	6.5	10.3	30.0	76.4	4.5	<0.1	<0.1
T_4	6.2	8.5	22.5	83.4	5.2	0.1	<0.1
T_5	6.7	9.3	31.0	74.0	6.1	0.1	<0.1
Ra_1	7.1	10.3	16.5	50.6	2.7	0.3	<0.1
Ra_2	7.3	10.1	43.9	105.7	4.9	0.2	<0.1
Ra_3	7.3	9.8	9.4	26.0	4.5	0.3	0.1
Ra_4	7.1	9.3	11.4	39.9	4.1	0.5	0.2
Ra_5	6.9	10.0	17.4	37.0	6.4	0.2	<0.1
Ra_6	7.0	9.5	11.6	44.1	5.5	0.5	<0.1
Ra_7	7.1	10.9	10.9	43.5	6.1	0.3	<0.1
To_1	7.0	9.8	19.9	70.8	9.2	0.8	0.1
B_1	7.0	10.0	11.5	28.3	3.6	0.8	<0.1
B_3	7.2	10.3	26.5	66.1	4.4	0.4	0.6
Tu_1	7.1	11.1	8.5	24.5	4.1	0.7	<0.1
Tu_2	7.4	10.3	25.7	64.4	4.3	0.5	0.6
Tu_2	7.3	11.6	21.1	54.4	1.8	0.2	<0.1
Tu_4	7.4	10.4	21.5	55.8	1.6	0.3	<0.1
Tu_5	7.1	9.9	20.2	55.2	4.3	0.2	<0.1
Tu_6	7.1	8.9	17.6	61.6	5.7	0.3	<0.1
Tu_7	7.1	8.8	19.0	81.3	8.3	0.4	<0.1
Tu_8	7.1	9.5	18.8	69.2	5.7	0.4	<0.1
Tu_{10}	7.1	11.0	37.5	95.8	6.0	0.1	<0.1

Biotic Index for North Portugal (B.I.N.P.)

The B.I.N.P. is based on the general consideration of two effects of pollution, reduction in community diversity (assessed here by H') and the progressive loss of taxa from clean water in response to organic enrichment and their replacement by tolerant fauna. In addition, B.I.N.P. defines a specific formula for each typological level in order to correct the longitudinal variation both in composition and diversity of benthic communities, which often leads to higher values in the upland streams as a result of the dominance of pollution-intolerant organisms. A type of differential index along the horizontal gradient was developed by GONZÁLEZ DEL TÁNAGO & GARCÍA DE JALÓN

Table 2.- Indicator taxa for H.I.N.P.
Taxones indicadorcs para cl B.I.N.P

Upper rhithron	*Lower rhithron*	*Potamon*	*Eutrophic conditions*
Plecoptera	Plecoptera	Trichoptera	Plecoptera
Siphonoperla torrentium	*Leuctra fusca*	*Oecetis testacea*	*Perla marginata*
Leuctra leptogaster	Ephemeroptera	*Hydropsyche exocellata*	Trichoptera
Protonemura pyrenaica	*Ecdyonurus venosus*	Heteroptera	*Hydropsyche pellucidula*
Nemoura minima ceciliae	*Baetis fuscatus*	*Micronecta meridionalis*	*Hydropsyche lobata*
Brachyptera risi	*Caenis luctuosa*	*Naucorus maculatus*	*Limnephilus guardarramicus*
Ephemeroptera	*Oligoneuriella duriensis*	Odonata	*Anomalopterygella chauviniana*
Baetis alpinus	Trichoptera	*Plactycnemis*	*Helycopsyche lusitanica*
Callyarcis humilis	*Cheumatopsyche lepida*	*Ophiogomphus serpentinus*	*Glossoma*
Habrophlebia fusca	*Calamoceras marsupus*	Crustacea	Heteroptera
Ecdyonurus forcipula/angelieri	*Mystacides longicornis*	*Atyaephira desmarestii*	*Sigara venusta*
Ecdyonurus aurantiacus	*Chimarra marginata*		*Parasigara infuscata*
Trichoptera	*Ceraclea*		Diptera
Plectrocnemia inflata	Heteroptera		*Prodiamesa olivacea*
Micrasema mininum	*Aphelocheirus montandoni*		*Chironomus* gr. thummi
Micrasema servatum	Odonata		*Chironomus* gr. plumosus
Micrasema moestum	*Boyeria irene*		*Tipula*
Micrasema togatum	*Pyrrhosoma nymphula*		Anthomyidac
Odontocerum albicorne	Bivalvia		Coleoptera
Larcasia partita	*Pisidum milium*		*Haliplus*
Ptilocolepus extensus	*Unio elongatulus*		*Agabus*
Philopotamus montanus			*Scarodytes*
Chaeopteryx lusitanica			Hirudinea
Allogamus ligonifer			*Glossiphonia complanata*
Rhyacophila adjuncta			*Batracobdella palludosa*
Rhyacophila intermedia			*Helobdella stagnalis*
Hydropsyche tibialis			*Erpobdella testacea*
Thremma tellae			*Erpobdellu monostriata*
Heteroptera			Bivalvia
Hespecorixa sahlbergi			*Sphurrium corneum*
Diptera			Gastropoda
Limoniidae			*Lymnaea peregra*
Dicranota			*Physa acuta*
Odonata			*Planorbarius corneus*
Cordulegaster bidentatus			Crustacca
Coleoptera			*Bragasellus cortesi*
Orectochilus villosus			Tricladida
Tricladida			*Dugesia gonocephala*
Polycelis felina			

(1984) for the upper Douro basin, which has a distinct geomorphological and rnorphometric character. The B.I.N.P. has already been described by CORTES (1989) for a smaller geographical area and lower typological range, for which this work provides some corrections and additional information.

The indicator species (table 2) were obtained by the above-mentioned partial ordinations for each sampling period. Because the ordination diagram mirrors the species data (although with some distortion —e.g. species points on the edge of the diagram are often rare species) we can make ecological inferences about the species represented in these diagrams. With Hill's scaling, site scores are weighted averages of the species scores and besides DECORANA is a good approximation to fitting bell-shaped response surfaces to the species data. That is, in this unimodal technique species that lie close to the point of a polluted site are therefore likely to be more resistant to that type of disturbance, and the expected probability of occurrence of an intolerant species increases with distance from the position of that site on the plot. In this way, we can obtain an indicator species, if it is possible to associate the axes scores with the underlying gradients (we used Pearson correlations between site scores and the environmental variables). On the basis of the B.I.N.P. it is clear (demonstrated by the ordination analysis) that eutrophication results in the upstream movement of representative taxa of lower reaches. As intolerant species we considered the organisms belonging to the upper rhitrhon, thus oligosaprobic ones, representative of zones with low nutrient content; ubiquitous taxa or particular taxa of specific biotopes were excluded.

The following formulae were established for the B.I.N.P. determination, depending upon the site position over the longitudinal profile:

Upper rhitrhon: $(C,.\ H') + (2\ s_i - s_d - 2\ s_t)$;
Lower rhitrhon: $(C_2.\ H') + (3\ s, - s_d - s,)$;
Potamon: $(C,.\ H') + (4\ s_i + s_u - s_t)$;

where: s_i – n. of intolerant species; s_t – n. of tolerant species; s_d – n. of species representative of the following reaches; s_u – n. of species representative of the preceding reaches.

For the coefficient C,—H' correction factor-we suggest— C_1: 3.0; C_2: 3.5; C_3: 4.0. The basis for this H' weighting is derived from the recognition that this index increases by an average of 0.5 units for each typological level (CORTES, 1989). Hydrobiological research carried out in Portuguese rivers (GRAÇA *et al.*, 1989) presented a common pattern of diversity variation, which was linked to the structurally more complex stony substrates in the upper areas, thus providing a higher number of species . Most macroinvertebrates could generally be identified to species level, except for Diptera, Oligochaeta and Hydracarina, where identification was taken to genus or family levels. Even if a consistency in the levels of identification was ensured we must point out that H' reflects the restrictions created by the absence of available keys for those groups.

For computing B.I.N.P. and in order to make it comparable and less subjective, it is essential to define the three typological levels, established by us on the base of the classification of ILLIES & BOTOSANEANU (1963) and on the morphology of the rhitrhonic streams, which appear in ELLIS (1989).

The *upper rhitrhon* —the main erosional region— comprises the crenon and the metarhitrhon, with mean monthly water temperatures of < 15 "C, oxygen levels near supersaturation, dominance of riffles with a substrate of gravel, cobbles and boulders.

The *lower rhitrhon* corresponds mainly to the hyporhitrhon: mean temperatures may reach 20 °C, pools are common and sand with silty patches often cover larger materials.

Finally the *potamon* is the depositional zone where monthly temperatures rises in general to over 20 "C, oxygen defficiency may occur, flow is not turbulent and substrates are sand to fine silts.

So, the simplification of the 8 zones of Illies and BOTOSANEANU (1963) makes the B.I.N.P. less dependant on the personal criteria of the observer and avoids its variation in situations of slight change in physical conditions.

The typological classification of the stretch studied is therefore the first step for the B.I.N.P. determination in order to select one of the 3 formulae presented. After that, the site species list is compared with those on table 2 and the number of representative taxa of each ecological condition (higher and lower rhitrhon, potamon and contaminated streams) are counted. The B.I.N.P. can be computed after the calculation of H', corrected by the factor C,, which is dependent on the same typological level.

The B.I.N.P. computed for the streams studied ranged between −10 and +30. For reference purposes, we offer the following interpretation of the scale of index values, which are spread by 5 quality classes, to make it comparable with similar methods (e.g. B.M.W.P' of ALBA-TERCEDOR & SÁNCHEZ-ORTEGA, 1988):

> 10:	clean, unpolluted waters
0-10:	waters slightly enriched
< 0:	moderate eutrophication
< -5:	polluted waters
< -10:	highly polluted waters

RESULTS: SPATIAL AND TEMPORAL VARIATION OF THE B.I.N.P.

Figs. 2, 3, 4, 5 and 6 exhibit the index scores separately for each basin. Thus, it is possible to observe its spatio-temporal variation and also to assess the accuracy of B.I.N.P. in evaluating the disturbance created by organic inputs.

Fig. 2 refers to the absolute values in the Tua river (sites were sampled only during 1 year) and it appears that the index used is effective in measuring the organic enrichment in B_3 and To,. It also seems sensitive in detecting the slight and very temporary organic loads in Ra_5, Ra, and Ra_7 from small distilleries (and at the last 2 points the joint effect of self-purification after To,), which is higher in the autumn period.

The remaining figures represent the biotic index variation along the other streams obtained through the calculation of the average values for each season. They reveal the performance of the index in assessing the constant decrease of water quality in C_2 and C_6. Moreover —as an important feature— they show a general stability over the different seasons: this fact makes the B.I.N.P. a most appropriate method in biological surveillance, as it is more independent of seasonal fluctuations, which often arise because of the life cycles of many insects. However, this index is inappropriate for measuring the dramatic changes occurred in T_3, caused by heavy metals and suspended solids (fig. 4).

DISCUSSION

The composition and structure of aquatic communities depend on the overall physical and chemical characteristics, food availability and the biotic interactions (Bervoets *et* al., 1988). Thus, the degree of environmental stress cannot be evaluated by a reduced number of abiotic variables, not is it linked in linear form to these parameters (Tolkamp, 1985). This is in part because there is a stabilization in the chemical composition and the ac-

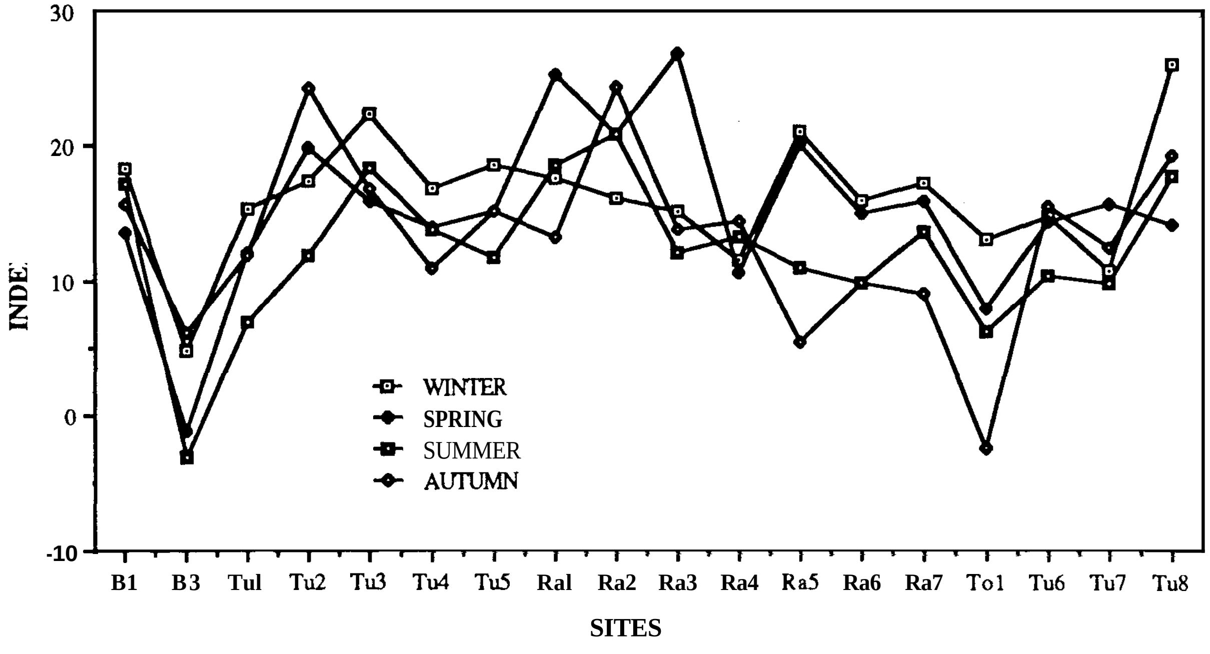

Figure 2.- Spatio-temporal variation of the biotic index B.I.N.P.in the Tua catchment.
Variación espacio-temporal del índice biótico B.I.N.P.en la cuenca del Tua.

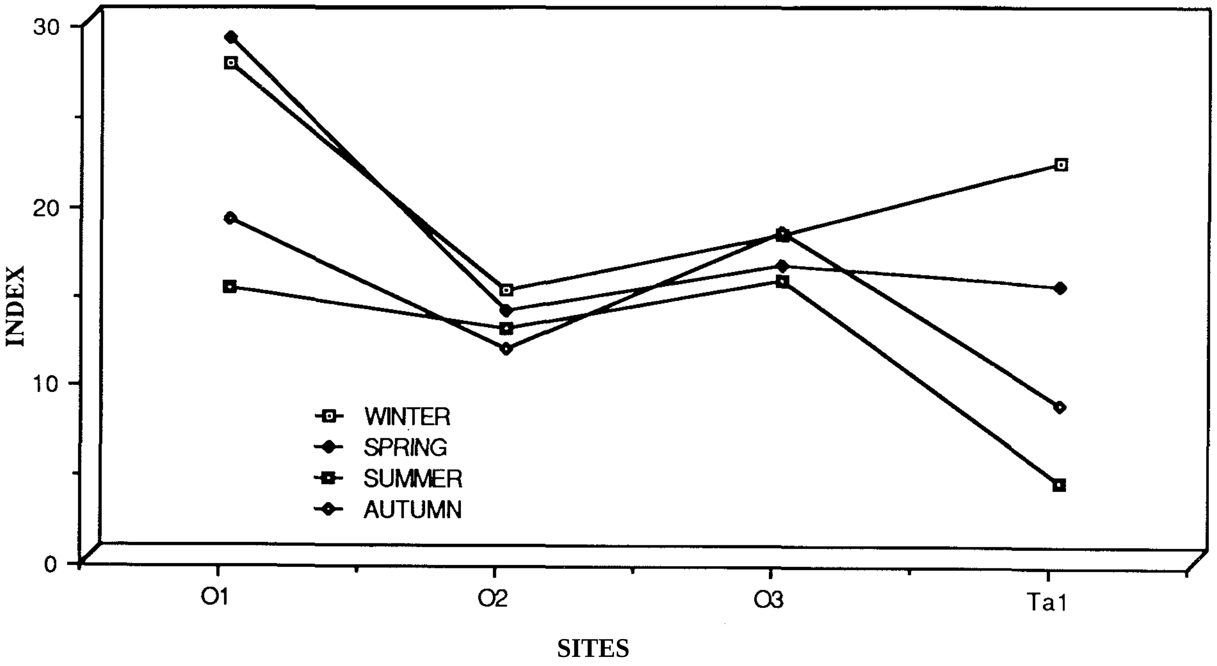

Figure 3.- Spatio-temporal variation of the biotic indexs B.I.N.P. in the Rivers Olo and Tamcga sampling stations
Variación espacio-temporal del índice biotico B.I.N.P. en las estaciones de muestreo del rio Olo y Tamega.

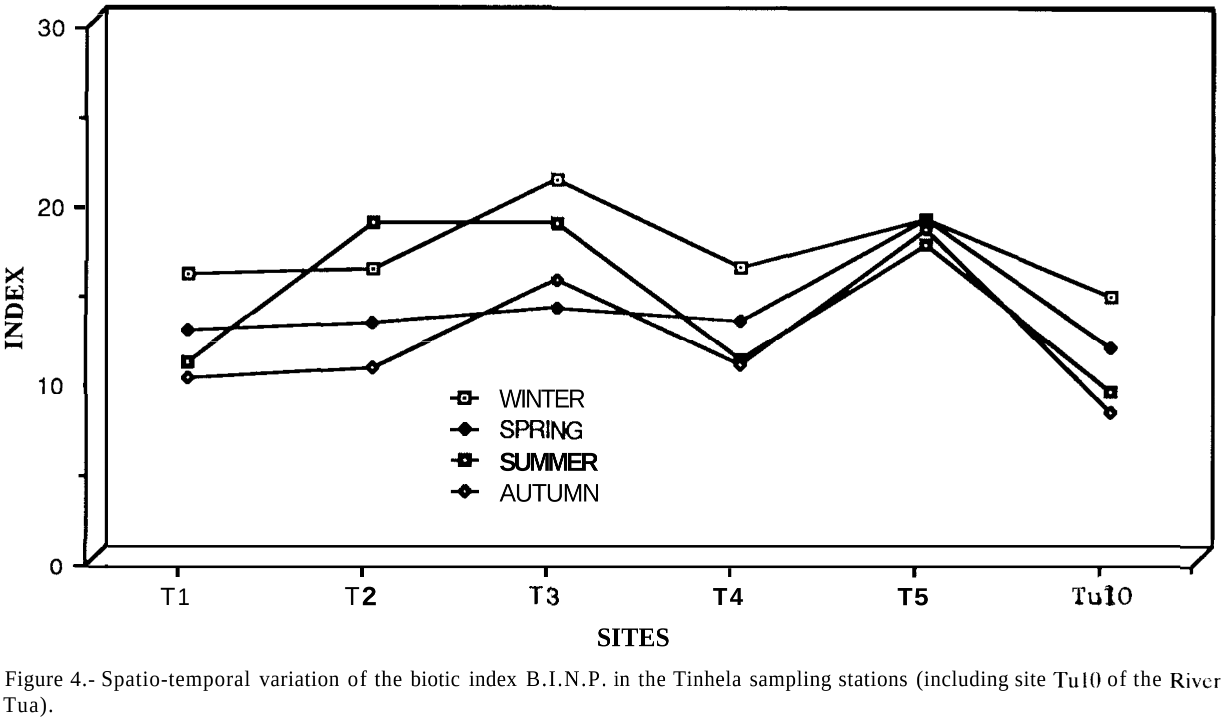

Figure 4.- Spatio-temporal variation of the biotic index B.I.N.P. in the Tinhela sampling stations (including site Tu10 of the River Tua).
Variación espacio-temporal del índice biótico B.I.N.P. en las estaciones de muestreo del rio Tinhela (incluyendo la estación Tu10 del rio Tua).

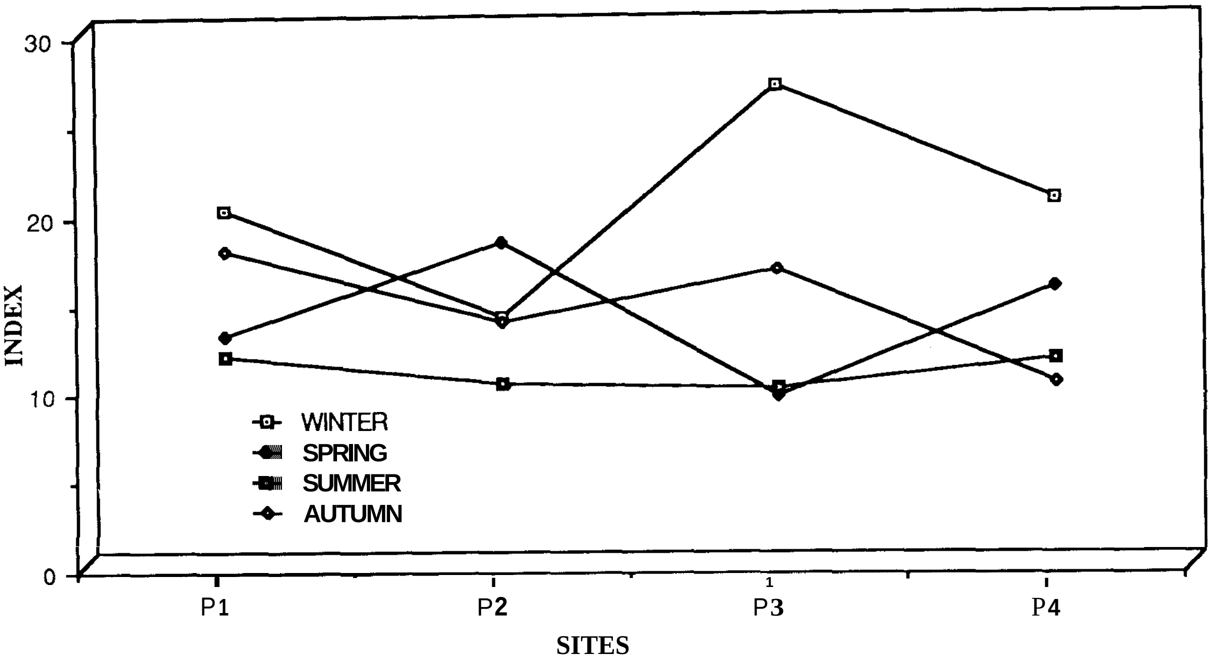

Figure 5.- Spatio-temporal variation of the biotic index B.I.N.P. in the River Pinhão sampling stations.
Variación espacio-temporal del índice biótico B.I.N.P. en las estaciones de muestreo del río Pinhão.

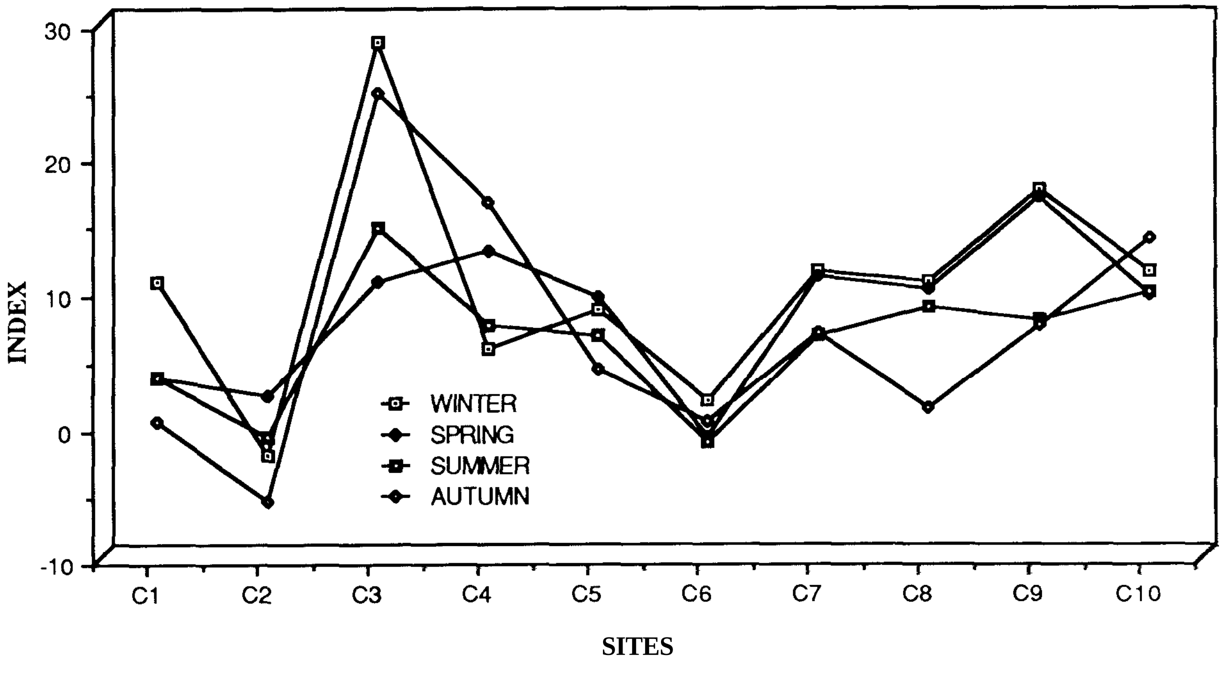

Figure 6.- Spatio-temporal variation of the biotic index B.I.N.P. in the River Corgo sampling stations
Variación espacio-temporal del índice biótico B.I.N.P. en las estaciones de muestreo del río Corgo.

quisition of a chemical inertia as the drained surface increases (FERNÁNDEZ ALÁEZ *et al.*, 1988). This means that upstream water is less mineralized and therefore more unbuffered — note that C_1 has a higher degree of contamination than C_2 but the ecological disturbance probably has a more important effect in this latter site (lower B.I.N.P.). This is the main difficulty for a direct interpretation of the ability of a biotic index like B.I.N.P. to detect faunal changes created by organic effluent discharges, simply by comparing the index scores with the chemical data.

The relative merit of the proposed index is the inclusion of reference organisms for each typological level and the determination of the diversity pattern along the spatial gradient. JENSEN & AAGAARD (1981) and VEKNEAUX (1983) agree on the necessity of comparing a polluted station with a reference one, to increase the efficiency of biological monitoring, and we believe B.I.N.P. provides a useful tool in this direction. A suitable method is fundamental when assessing moderate levels of enrichment which do not seriously affect the resilience of the ecosystem, like the cases discussed in this survey.

However, we should not deny that B.I.N.P. is more time consuming in identification and computing than traditional indices and has a regionally restricted usefulness. But, on the other hand, the method described here can be adapted to any other type of running water by choosing the local indicator species and coefficient values.

RESUMEN

VALORACIÓN BIOLÓGICA DE LA CALIDAD DEL AGUA EN EL NE DE PORTUGAL MEDIANTE UN MÉTODO QUE COMBINA LA TOLERANCIA DE LAS ESPECIES Y LA DIVERSIDAD A LO LARGO DEL EJE LONGITUDINAL

Partiendo de la comunidad bentica fue definido un índice biotico multiple, determinado por el parametro de diversidad H' de Shannon-Weaver y por la sensibilidad de una lista seleccionada de organismos a la eutrofización.

Esta lista fue elaborada a traves de la tecnica multivariante —DECORANA— que permite caracterizar las especies de acuerdo con su nivel tipologico y tolerancia a descargas orgánicas.

El método presentado aquí, toma en consideración la composición y los modelos de diversidad de los tramos superiores hacia los inferiores asi como la variabilidad temporal.

De esta forma, se pueden obtener comparaciones mas realistas en la evaluación de la calidad del agua en los diferentes tramos del sistema hidrico, independientemente de las fluctuaciones estacionales.

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