Pergamon

0043-1354(94)E0125-P

Wat. Res. Vol. 29, No. 1, pp. 285–290, 1995
Elsevier Science Ltd. Printed in Great Britain

ARE BIOLOGICAL INDICES BMPW' AND ASPT' AND THEIR SIGNIFICANCE REGARDING WATER QUALITY SEASONALLY DEPENDENT? FACTORS EXPLAINING THEIR VARIATIONS

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(First Received December 1992; accepted in revised form April 1994)

Abstract—Biological indices based on macroinvertebrates offer advantages for testing water quality. Nevertheless, a common criticism is that these indices show seasonal dependence. To elucidate this matter, 60 sits were sampled over a two-year cycle, and the BMWP' and ASPT' biotic indices (adaptation of the Biological Monitoring Working Party Score System and Average Score Per Taxon for the Iberian Peninsula) were calculated. Annual variations in the indices were studied (considering, separately, only non-polluted sites, and grouping polluted and clean-water sites together) and compared for changes in temperature, the factor most characteristic of seasonality. Moreover, the correlations with physical factors (altitude, slope of stretch, flow, temperature, distance from the source) and chemical parameters (pH, conductivity, nitrates, nitrites, ammonium, phosphates, chlorides, sulphates, calcium, magnesium, sodium, potassium, COD, iron, copper, zinc, lead, nickel, oils and fats, detergents and pesticides) were analysed. Both indices were found to be negatively related with parameters indicative of pollution. From a multiple regression, a good score prediction using physical and chemical data (for BMWP' as for ASPT') was obtained. For non-polluted sites, the variability of the BMWP' (and its significance with respect to water quality) in relation to seasonality was quite reduced and not significant, but the ASPT' showed a significant dependence on temperature. The relationship of these indices to temperature in all sites (polluted and clean) was negative in both cases, indicating that the relationship is caused more by pollution than by seasonality.

Key words—biotic indices BMWP' and ASPT', river pollution, seasonality, physico-chemical factors

NOMENCLATURE

β = partial regression coefficient
B = regression coefficient of independent variables
COD = chemical oxygen demand
d.f. = degrees of freedom
F = Snedecor's statistic
p = probability
r = correlation coefficient
r_S = Spearman's rank correlation coefficient
R^2 = square of the multiple correlation coefficient
S.E. = standard error
t = t of Student

INTRODUCTION

Biological indices to assess water quality based on macroinvertebrates offer advantages over those using other organisms, since macroinvertebrates are easy to sample, at least with regard to qualitative measurements or relative abundance. In addition, good identification keys are available for most orders, and for many orders there is ample information concerning pollution tolerance (Hellawell, 1986; Jeffries and Mills, 1990; Mason, 1984). Nevertheless, the pronounced seasonal variability of some biotic indices (Murphy, 1978) and the regionality of others (Hellawell, 1978; Washington, 1984) constitute the greatest critcism against these methods.

In the last few years in Spain there has been broader use of the biotic index BMWP' (Alba-Tercedor and Sánchez-Ortega, 1988), an adaptation of the British BMWP system (Biological Monitoring Working Party Score System; Armitage *et al.*, 1983) for the Iberian Peninsula. Adaptations include the addition of new families, changes in some scores and correlations of particularly significant values representing degrees of pollution. Five levels of water quality were thereby established according to the Extended Biotic Index (after Ghetti *et al.*, 1983). In September 1991 this approach was adopted by the Spanish Society of Limnology (Alba-Tercedor and Prat, 1992). Because of the easy management of these indices and the high correlations with other European indices (Rico *et al.*, 1992) we have selected them for this study.

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Taking into account previous problems attributable to biological indices, we have studied whether temperature, and consequently seasonality (since temperature in our latitude is a good indicator of seasonality), significantly affects the BMWP' and ASPT' (Average Score Per Taxon) indices. Firstly, we discuss the variation in the BMWP' and ASPT' in relation to temperature in order to demonstrate the greater or lesser influence of seasonality. Secondly, we have related these biological indices to all the environmental variables measured, in order to determine which factors best explain their variation.

STUDY AREA

The study was carried out in the upper Genil River Basin, from the source of the river (on the northwestern slope of the Sierra Nevada Mountains, southern Spain) to the Iznájar reservoir, with a total surface area of 4500 km^2.

In this basin, aside from mainly urban waste water (most of the sewage waters are dumped without purification; S.A.S.-Univ. Granada, 1991) and agricultural pollution, which are more or less constant throughout the year, seasonal industrial pollution (December to March) takes the form of a by-product of olive-oil extraction, a toxic slurry known as *alpechin*. The seasonality of this pollution parallels temperature, since these dumpings occur in the colder periods of the year.

MATERIAL AND METHODS

All along the principal course of the Genil River, as well as in the 26 tributaries within the basin, 60 sites were established and were sampled seasonally from March 1988 to February 1990.

In each site we made qualitative samplings of macroinvertebrates, using nets with 0.5 and 0.3 mm mesh to kick and sweep in all the different microhabitats. The content of each netting was deposited periodically in white trays to avoid losing organisms by overflow from the nets. Each sampling was considered finished when sweeps provided no new taxa. The specimens were conserved in alcohol for identification in the laboratory.

In situ measurements were taken of the pH, flow and temperature, and water samples were analysed in the laboratory for the following parameters: conductivity, nitrates, nitrites, ammonium, phosphates, chlorides, sulphates, calcium, magnesium, sodium, potassium, COD, iron copper, zinc, lead, nickel, oils and fats, detergents and pesticides.

The biological quality of the water was assessed by the BMWP'. At the same time, the ASPT' was calculated (dividing the BMWP' value at each sample by the number of families evaluated).

To determine which physical and chemical parameters best explain the BMWP' and ASPT' variations, we carried out simple correlations as well as stepwise multiple regressions (Dixon and Jennrich, 1983, Edwards, 1985; Sokal and Rohlf, 1979), a method used previously for similar purposes (Armitage *et al.*, 1983; Moss *et al.*, 1987; Rodriguez and Wright, 1988; Wright *et al.*, 1988; 1989; among others). For dependent variables we used the indices BMWP' and ASPT' × 100 in each case, and for independent variables the physical (altitude, slope of the stretch, flow, temperature, distance from the source) and chemical parameters. Previously, to avoid overdispersion of the data, following Digby and Kempton (1987), we applied a $\log_{10}(x+1)$ transformation to all the physico-chemical parameters necessary, except on the slope of the stretch of water, where an arctangential transformation was applied.

The analysis was considered finished when the reduction of the residual variance, after adding new variables, was no longer significant. We used different tolerance levels to allow a greater or smaller number of independent variables to enter the analysis. The tolerance is defined as protection against the loss of precision when the variables are highly correlated. Thus, no variable is entered into the classification function when the squared multiple correlation (R^2) with already entered variables exceeds $1 -$ tolerance, or when the entry would cause the tolerance of an already entered variable with the other variables to exceed $1 -$ tolerance (Dixon and Jennrich, 1983; Engelman, 1983).

RESULTS

The analysis of how biological indices are related to the environmental variables gave the following results.

Variation of the BMWP' index

To explain how the seasonal variations of temperature might influence the biological-quality index used, we have represented the average values of the BMWP' throughout the different samplings in the sites at the non-polluted upper reaches (Fig. 1A). In these sites the average values of the index varied, but variations remained within the limits of Alba-Tercedor and Sánchez-Ortega (1988) to be considered good water quality, regardless of the season of the year. These variations between the first four samplings were not statistically significant ($F = 1.21$; d.f. = 3, 24; $p = 0.33$) because the variation between samplings was similar to that between sites (MS [samplings] = 914.57 and MS [sites] = 756.15, respectively). Moreover the relationship between the BMWP' values in those non-polluted sites and temperature, although positive, was not significant ($r = 0.21$; $n = 56$; $p = 0.11$), nor was the relationship established between the classes of quality and temperature ($r_S = 0.04$; $n = 56$; $p = 0.74$).

On the other hand, the mean values of the BMWP' of all sampling sites (Fig. 1B) indicate that during the winter (March 1988 and January–February 1990, respectively) values were very low, corresponding to polluted waters considered "doubtful". As above, the differences between the BMWP' values obtained in all sampling sites during the first four samplings proved not to be significant ($F = 2.45$; d.f. = 3, 223; $p = 0.06$). Nevertheless, a correlation did exist with the temperature, although this was negative (Table 1). Thus, we found also a significant relationship between the quality classes established and the temperature ($r_S = 0.19$; $n = 447$; $p < 0.001$).

However, these associations between BMWP' index and quality classes with temperature may have been caused by pollution because the sites at lower altitude, with warmer temperatures, had greater

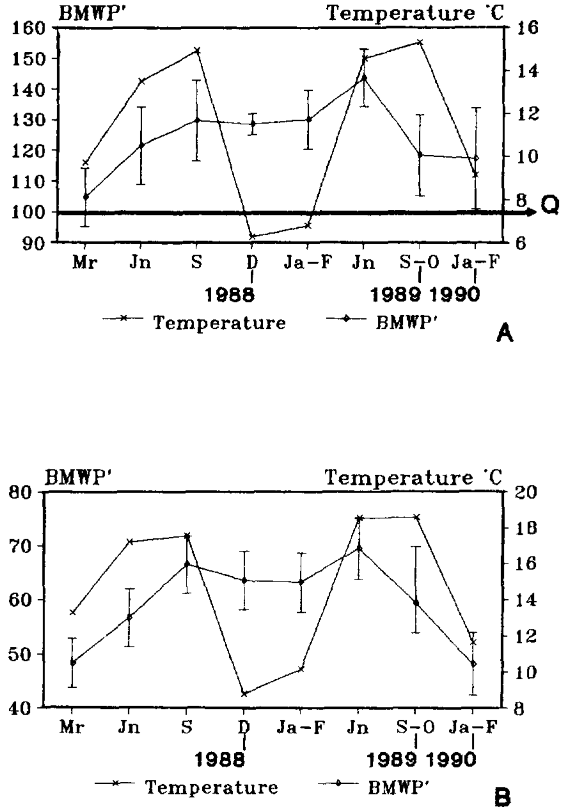

Fig. 1. Graphic representation of the average temperature values and of the BMWP' index for the different samplings. Graph A: in sites at the non-polluted upper reaches. Graph B: in all the sites of the Genil Basin. In the BMWP' index $x \pm$ S.E. is represented. Q indicates that above the line the values of BMWP' index correspond to non-polluted waters with a very good biological water quality.

Table 1. Correlation matrix between the different physico-chemical parameters and the biological indices BMWP' and ASPT'

	BMWP'	ASPT'
Temperature	−0.21***	−0.31***
pH	0.13*	0.16**
L Flow	−0.06	0.10*
L Conductivity	−0.50***	−0.49***
L Sulphates	−0.38***	−0.27***
L Nitrates	−0.47***	−0.45***
L Nitrites	−0.32***	−0.20***
L Ammonium	−0.42***	−0.57***
L Phosphates	−0.42***	−0.51***
L COD	−0.40***	−0.46***
L Calcium	−0.37***	−0.25***
L Magnesium	−0.30***	−0.27***
L Chlorides	−0.50***	−0.42***
L Sodium	−0.56***	−0.52***
L Potassium	−0.61***	−0.66***
L Iron	−0.29***	−0.27***
L Copper	−0.27***	−0.28***
L Zinc	−0.25***	−0.33***
L Lead	−0.07	−0.17**
L Nickel	−0.02	−0.08
L Pesticides	−0.30***	−0.33***
L Detergents	−0.27***	−0.36***
L Oils and fats	−0.13*	−0.21***
L Altitude	0.51***	0.44***
L Dist. origin	−0.37***	−0.29***
At slope	0.47***	0.50***

Note. The Letter L before the name of a variable signifies logarithmic transformation of that variable [$x' = \log_{10}(x + 1)$], while AT refers to arctangential transformation (* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$).

sewage input and, consequently, fewer taxa and lower scores. As we can see, the BMWP' index was positively related to altitude (Table 1). Also, a significant negative relationship can be found between this biological index and the parameters indicative of urban, industrial and agricultural pollution (nitrites, nitrates, ammonium, phosphates, COD, iron, copper, zinc, pesticides, detergents, oils and fats) as well as of water mineralization (some parameters of which also come from these types of pollution; e.g.: r(potassium/ammonium) = 0.44; $n = 449$; $p < 0.001$).

Thus, many factors might cause the variation of the BMWP' index. Therefore, to avoid cross-correlations and to determine which of those parameters best explain the variation of the biological index and whether temperature was one of those, a stepwise multiple-regression analysis was carried out, fixing the tolerance at 0.7 (see Methods).

We found that 50.4% of the variance of the BMWP' index (R^2) was explained in a highly significant way by only three parameters. These were, in order of entering them in the analysis: potassium, altitude and phosphates (Table 2). Even at a decreased tolerance limit ($t = 0.01$), temperature was not significant, indicating that other factors (all of them significant at $p < 0.005$): potassium, altitude, phosphates, copper and nitrites, explain the variation of the index ($R^2 = 0.54$; $F(5, 229) = 54.27$; $p < 0.0001$) better than temperature does.

Table 2. Results of the stepwise multiple-regression analysis between the BMWP' index and the physico-chemical factors measured

Dependent variable: BMWP'
Multiple R: 0.708
Multiple R^2: 0.504
Adjusted R^2: 0.498
$F(3, 231) = 78.247$; $p < 0.00001$
Intercept: −233.273

Variable	β	S.E.	B	S.E.	$t(229)$	$p =$
L Potassium	−0.389	0.055	−51.67	7.15	−7.082	0.00000
L Altitude	0.317	0.051	117.90	19.15	6.156	0.00000
L Phosphates	−0.248	0.048	−41.26	8.29	−4.977	0.00000

Note. Units: Potassium, Phosphates = mg/l; Altitude = m. Analysis for a tolerance of 0.7. Labelling as in Table 1.

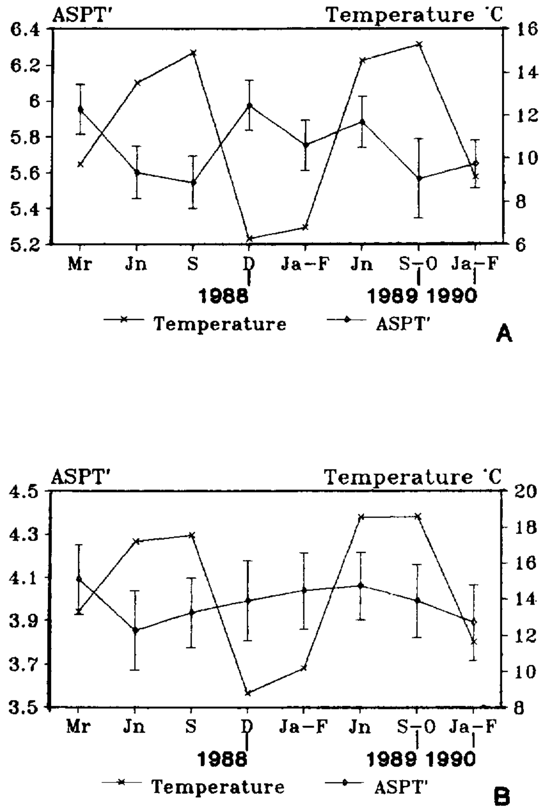

Fig. 2. Graphic representation of the average temperature values and of the ASPT' index for the different samplings. Graph A: in sites at the non-polluted upper reaches. Graph B: in all sites of the Genil Basin. In the ASPT' index $x \pm$ S.E. is represented.

Variation of the ASPT' index

In Fig. 2A the average values of the ASPT' and the temperatures of the different samplings in non-polluted upper reaches sites were represented. Although the differences between the first four samplings were not significant ($F = 2.52$; d.f. = 3, 34; $p = 0.08$), there was a negative relationship between the values of the index and temperature in the different samplings ($r = -0.38$; $n = 56$; $p < 0.01$). In colder periods, which have a lower average number of taxa, the ASPT', far from diminishing, increases (Fig. 2A) due to the presence of few taxa, but with a high value in the BMWP' score.

Moreover, the average values of the ASPT' reached at the river heads with clean waters were clearly higher than those obtained, on taking into account the overall data of the basin (Fig. 2B). In this case, again, no significant differences were found between the first four samplings ($F = 0.33$; d.f. = 3, 223; $p = 0.81$). Nevertheless, the correlation with temperature is significant and, even, superior to that obtained with the BMWP' (Table 1).

As with the BMWP', the ASPT' was negatively (and significantly) correlated with parameters indicative of pollution and water mineralization, and positively with factors such as altitude, slope and flow (Table 1).

A stepwise multiple-regression analysis between the ASPT' and the environmental variables indicated, with a high significance level, that 57% of the ASPT' variance was explained by three factors: potassium, ammonium and altitude (Table 3). Even after the degree of tolerance was reduced to 0.01 (as in the previous section) temperature did not appear among the variables that explain the variation of this index ($R^2 = 0.65$; $F(8, 226) = 52.88$; $p < 0.0001$). The variables were: potassium, ammonium, altitude, phosphates, lead, copper, slope and sulphates (all of these significant at $p < 0.05$).

Moreover, this again points out that, as with BMWP' index, the variation of potassium in the Genil Basin is the primary factor in ASPT' prediction. Potassium is related naturally with water mineralization, but in the study area part of its variability is also significantly explained by parameters indicative of pollution. The parameters were (in this order and significant at $p < 0.0001$): conductivity, ammonium and detergents ($R^2 = 0.70$; $F(3, 231) = 175.61$; $p < 0.0001$).

DISCUSSION

Although some authors, such as Murphy (1978), have demonstrated a temporal variability in biotic indices, we found in the present study that the variability of the BMWP' index, with respect to seasonality, is quite reduced. This agrees with Armitage *et al.* (1983) for the BMWP and ASPT

Table 3. Results of the stepwise multiple-regression analysis between the ASPT' index × 100 and the physico-chemical factors measured

Dependent variable: ASPT' × 100
Multiple R: 0.755
Multiple R^2: 0.570
Adjusted R^2: 0.565
$F(3, 231) = 102.189$; $p < 0.00001$
Intercept: −179.649

Variable	β	S.E.	B	S.E.	$t(229)$	$p =$
L Potassium	−0.406	0.053	−165.90	21.58	−7.686	0.00000
L Ammonium	0.340	0.048	−120.13	16.11	−7.457	0.00000
L Altitude	−0.218	0.048	254.68	55.80	4.564	0.00001

Note. Units: see Table 2 and Ammonium = mg/l. Analysis for a tolerance of 0.7. Labelling as in Table 1.

because, although they found a significant relation in the differences between maximum and minimum BMWP and ASPT values between seasons at each site, they considered that these differences were relatively small compared with differences between sites. As we have shown with the analyses of variance and regression analyses, the relationships between indices and temperature, more than being related to seasonal variations, are caused by pollution. There were other factors besides, and even more indicative than, temperature which influence the variation of the index.

It has been demonstrated that the ASPT is less sensitive than the BMWP index with regard to the degree of sampling effort and seasonal change (Armitage *et al.*, 1983; Pinder, 1989; Pinder *et al.*, 1987; Rodriguez and Wright, 1991). Thus, attempts to predict the ASPT index by means of environmental factors have produced better results than with the BMWP (Armitage *et al.*, 1983). In this study, using BMWP' index, we found no significant differences between seasons at either polluted or non-polluted sites. However, both in the case of river heads with clean waters and in the case of all sites (polluted and non-polluted) the ASPT' index was significantly related to temperature. For this reason, and despite the ASPT' offsetting the variations of the BMWP' index, as observed by Armitage *et al.* (1983) and Rodriguez and Wright (1991), the ASPT' is more dependent on temperature and therefore seasonality.

It is revealing that the first parameter included in the stepwise regression analysis was potassium. Potassium of the epicontinental waters originates naturally from the dissolution of evaporated salts and decomposition of silicates, and is therefore highly correlated with other cations and anions responsible for both water mineralization and conductivity. Nevertheless, the presence of potassium is occasionally associated with agricultural pollution such as the leaching of fertilizers, with urban pollution in the form of waste waters, and with the industrial pollution of *alpechín* (olive-pressing residue) dumping (Albi-Moreno and Fiestas Ros de Ursinos, 1960; Fiestas Ros de Ursinos, 1958, 1977), one of the principal industrial wastes in the study zone. Depending on the type of oil-pressing method (continual or traditional) the average potassium concentration in the *alpechín* is from 1.2 to 3 g/l (Instituto de la Grasa, 1980).

Because of part of the potassium variability was explained by parameters of pollution, we establish the significance of this parameter in water-quality studies.

It might appear that advantages ascribed to the ASPT in Armitage *et al.* (1983) and the ASPT' in Rodriguez and Wright (1991) would favour the use of ASPT' over the BMWP' in studies of the evolution of biological quality along a water course. In the present study, however, these two indices have proved to be highly correlated ($r = 0.82$; $n = 450$; $p < 0.0001$), and the good adjustment values of the variation of the BMWP' in terms of the environmental variables (R^2) disagree with those calculated by Armitage *et al.* (1983). These researchers found that the prediction of the values for this index were less successful than for the ASPT, since R^2 values obtained were scarcely 30% in equations with 10 independent variables. This seems to be due to the fact that, in non-polluted sites, the range of variation of the index was quite small. In the present study, by using data which range from clean to very polluted waters, we obtained better adjustment.

Furthermore, due to seasonal variation we found a greater dependence in the ASPT' than in the BMWP'. Because of this, and the difficulty of giving ASPT' values a specific meaning with regard to biological quality, we consider that using the BMWP' is valid. The different classes of quality obtained with the latter index correspond to wide intervals of the values; therefore, even when graphically tracing its evolution we find peaks and troughs, which do not affect the meanings with respect to biological water quality, so long as the values obtained do not go beyond these intervals.

Acknowledgements—We are grateful to the Dirección General de Obras Hidráulicas del MOPU for the financial support of the research project from which work has been realised, and the Ministerio de Educación y Ciencia for the predoctoral grant awarded to the first author. We also wish to thank David Nesbitt for the English version of the manuscript, Juan J. Soler for his valuable advice and assistance with statistical methods, and the research group of Comportamiento y Ecologia Animal of our Department for allowing us to use their computing facilities.

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