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Testing the suitability of the abundance biomass comparison bioassessment method in a Mediterranean river

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Abstract: The Water Framework Directive (Directive 2000/60/EC) emphasises the need for simple tools and studies to characterise aquatic ecosystems. A wide range of methods has been developed, including different groups of biota and different taxonomic resolutions. Among these, the abundance biomass comparison (ABC) method is an important methodology widely used in marine benthic systems and well-founded from the ecological point of view. This method – with a slight modification using genera and families instead of species – was applied in a Mediterranean river (Eliche-Frío, northeast of Andalusia, Spain) using the macroinvertebrate community, together with the Margalef richness index and the Iberian BioMonitoring Working Party (*IBMWP*) to determine the quality of the water. The obtained results show the suitability of the ABC curves method to analyse the macroinvertebrate community and estimate the ecological status of river ecosystems. Although both, the genus and family aggregations, showed a similar trend, the values obtained with the family level indicate a worse state of contamination than those shown with the genus level. The comparison between genus and family levels with other biological indices shows that the evaluation obtained with family aggregation is more similar to those obtained with the Margalef and *IBMWP* indices than the evaluation based on genera; therefore, we could conclude that this level of taxonomic resolution is adequate for the use of the ABC method in assessing the ecological status of Mediterranean rivers.

Keywords: abundance biomass curves, Andalusia, *k*-dominance curves, macroinvertebrates, water quality

INTRODUCTION

The European Union Water Framework Directive [Directive 2000/60/EC] states that water resources must be subjected to ecological assessment, in order to achieve good ecological status for all European waters. This directive emphasises the need for simple tools and studies to characterise investigated ecosystems. However, it does not specify a standard methodology, proposing different methods suitable for the determination of ecological statuses. This idea is reflected in Spanish legislation in the Official State Newsletter (Es.: BOE – Agencia Estatal Boletín Oficial del Estado) [Orden ARM/2656/2008]. All of these documents recognise ecological assessment or "bioassessment" as fundamental to sustainable management, providing a more sensitive integrated assessment of river conditions over time compared to physical or chemical variables [Marchant *et al.* 2006].

An extensive range of methods has been developed for river bioassessment, including different groups of taxa [Alba-Tercedor *et al.* 2002; Kelly, Whitton 1995; Monaghan, Soares 2010; Munné *et al.* 1998; Philibert *et al.* 2006]. The choice of a particular group of organisms or method in river assessment is often based on the premise that the group is an indicator of the overall river condition.

In the Mediterranean area, many studies have implemented the use of macroinvertebrates in the biomonitoring and assessment of rivers and streams [Alba-Tercedor *et al.* 2002; Bonada *et al.* 2006; García-García *et al.* 2005]. Most of them are field-based methods that require the identification of macroinvertebrates at the family level, as well as a previous assignation of sensitivity weightings to each taxon, based on their tolerance to water-quality impairment. However, exploring methods well-founded from the ecological point of view, for example, using the changes in macroinvertebrate composition (abundance, dominance or biomass) as a response to disturbances in the system is, to date, less common [Brown 2001; Ismael, Dorgham 2003; Voicu *et al.* 2022].

In light of the need to use easy-to-apply tools to establish the ecological status of rivers, this study assessed a method that is widely used in benthic marine systems for detecting pollution, i.e., the abundance biomass comparison (ABC) method, which was developed by Warwick [1986]. The ABC method is adequate for quality evaluations applied with benthic or planktonic communities in marine systems [Estacio *et al.* 1997; Ismael, Dorgham 2003; Warwick *et al.* 1987], with benthic macroinvertebrate communities in wetlands [Dimuthu *et al.* 2018], and with bird populations in coastal wetland ecosystems [Meire, Dereu 1990], although its use in river ecosystems is less extensive. It is a well-founded ecological method that considers the structure of the community and the implications for the proper functioning of the aquatic ecosystem, through comparisons between the percentages of accumulated abundance and the percentage of accumulated biomass of macrobenthic communities ordered according to their dominance. The relative position of abundance and biomass on the plot can reveal pollution impacts. Relatively undisturbed sites have biomass curves above abundance curves and vice versa. This situation is a consequence of the larger size and lower abundance of organisms in unpolluted ecosystems.

In its original formulation, this method requires the identification of organisms at the species level; therefore, it does not comply with the premise of simplicity and speed in obtaining the results requested for assessing the quality of river ecosystems. Taking into account that a lower identification effort is an advantage when selecting evaluation tools, the present study had two objectives: (i) to evaluate the suitability of the method in a riverine ecosystem, and (ii) to evaluate the taxonomic resolution to be implemented. The results obtained with the ABC curves were compared with other biotic indices in different sections of a Mediterranean river, in order to detect its suitability as a method for assessing the quality of the freshwater ecosystem.

MATERIALS AND METHODS

STUDY AREA AND SAMPLING

The study area, located in northeastern Andalusia (Jaén province), follows the valley of the Eliche-Frío River (Fig. 1), a tributary of the Guadalquivir River, the main river in southern Spain. This area has an average annual rainfall value of 579 mm, an average annual temperature between 12 and 15°C, and a runoff coefficient of 0.20 [MMA 2007]. The sampling sites are located close to the village of Los Villares (population close to 5,170 people). The urban sewage of this population and residential areas located along the riverbanks is, together with agriculture, one of the main sources of pollution. Moreover, the food industry emissions (mainly from olive oil extraction), along with the diffuse pollution generated by agriculture and livestock, also contribute to river pollution [Minea *et al.* 2022].

For this study, samples collected in the river during March 2004 were analysed. Three sampling sites were selected (see Fig. 1, Tab. 1). According to Spanish legislation [Orden ARM/2656/2008], the study area belongs to river typology 12 (calcareous Mediterranean mountain rivers). The river basin under study fulfils the requirements to be framed in this typology: (i) the altitude range for this typology is between 450 and 1280 m a.s.l.; (ii) Strahler river order ≤4; (iii) distance to the coast in a straight line of 50–255 km; (iv) catchment area of 15–1090 km^2; (v) conductivity >300 $\mu S \cdot cm^{-1}$.

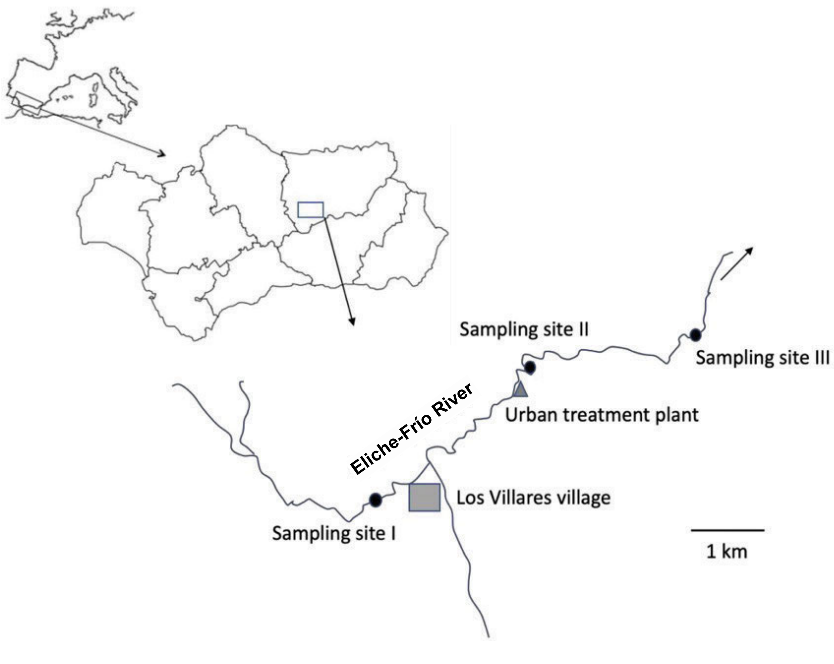

Fig. 1. Sampling sites (Andalusia, southern Spain); source: own elaboration

Table 1. Sampling sites information

Parameter	Site I	Site II	Site III
Coordinates (UTM)	30S0427075	30S0429535	30S0432186
Altitude (m a.s.l.)	582	526	430
Depth (min–max, cm)	18–72	14–45	20–39
Width (min–max, cm)	400–610	460–630	1040–1200
Flow velocity (min–max, $m\cdot s^{-1}$)	0.13–0.62	0.14–0.77	0.28–0.84
Temperature (°C)	17	15.4	14.4
Dissolved oxygen ($mg\cdot dm^{-3}$)	16	12.1	11.4
pH	8.7	8.7	8.5
Conductivity ($\mu S\cdot cm^{-1}$)	610	600	620

Explanations: UTM = Universal Transverse Mercator.
Source: own elaboration.

The macroinvertebrate community was sampled at each sampling site using a rectangular hand net (20×10 cm) with a mesh size of 360 μm. All habitats, lotic and lentic facies, were sampled semi-quantitatively, with a sampling effort of 10 min in all microhabitats. The net was emptied to prevent the loss of organisms by clogging of the mesh, at least after each microhabitat sampling, or even earlier if it was saturated. The collected organisms were stored in properly labelled bottles and fixed with formaldehyde (4% final concentration). In the laboratory, the samples were washed and filtered through different mesh size sieves and deposited on white trays, in which the organisms were carefully separated. The organisms were identified at the genus and family level using a stereomicroscope (Leica MZ-12) and according to specific keys [Alba-Tercedor 1982; Bournaud *et al.* 1982; Brinkhurst 1971; Carchini 1983; Davies 1968; Dethier 1986; Faessel 1985; Franciscolo 1979; Gómez 1988; Mouthon 1982; Tachet *et al.* 1987; Verneaus, Faessel 1976]. Moreover, dry weights were determined using a microbalance (Mettler 0.01 mg), after drying to constant weight at 60°C. The following environmental variables were also measured in the water at the different sampling sites with a multi-parametric probe (YSI-556): conductivity ($\mu S\cdot cm^{-1}$), temperature (°C), pH, and dissolved oxygen ($mg\cdot dm^{-3}$). Depth, width, and surface flow velocity were also measured in a 100-m-long section of the river.

BIOLOGICAL DATA ANALYSIS

Different indexes were used in this research to evaluate the river quality: (i) the Margalef species richness index (*d*) [Margalef 1958]; (ii) the Iberian Biomonitoring Working Party (*IBMWP*) index [Alba Tercedor *et al.* 2002]; (iii) habitat quality index (*HQI*) [Pardo *et al.* 2002]; (iv) the ABC curves or *k*-dominance curves [Warwick 1986]. In the case of the *IBMWP* index, the Spanish legislation indicates five ecological quality ratios (*EQR* – very good, good, moderate, poor, bad) to describe the ecological water quality. The *EQR* takes values between 0 and 1, and for the typology in which the study area is located, the reference value is 150 (*EQR* = 1). The limits between classes are: 0.89 for the limit between very good and good status; 0.67 for the limit between good and moderate status; 0.45 for the limit between moderate and poor status; 0.22 for the limit between poor and bad status [Orden ARM/2656/2008].

The *W*-statistic was used to assess consistency between the evaluations obtained with the ABC method based on two taxonomical levels (genus and family) in the different sampling sites. This statistic uses the sum of the differences between biomass and abundance curves over each range of taxa [Clarke 1990]:

$$W = \sum_{i=1}^{s} \frac{B_i - A_i}{50(S-1)} \quad (1)$$

where: W = statistic, S = number of species, B = biomass, A = abundance.

This statistic can take values from +1, indicating a non-disturbed system, to –1, which defines a polluted situation. Values close to 0 indicate moderate pollution.

RESULTS AND DISCUSSION

Table 2 shows the values obtained for the Margalef, *HQI*, and *IBMWP* indices at the three sampling sites, together with the ecological quality ratios (*EQR*) values and ecological status. Site II showed the lowest values, followed by site III and site I, with the latter showing the best records for these indices. Thus, following the *EQR* criteria published by Spanish legislation for the *IBMWP* index in this river typology [Orden ARM/2656/2008], site I would have good quality, while sites II and III would have poor quality. The high values of the habitat quality index (*HQI* > 60) [Prat *et al.* 2012] in the three sampling points indicated that the habitat is well structured, with adequate development for the establishment of macroinvertebrate communities, thus biological indices can be applied without restrictions [Prat *et al.* 2012]. Accordingly, we can deduce that the differences among sampling stations are due to differences in water quality and not to the lack of adequate substrate (habitat) for the presence of a diverse community of macroinvertebrates.

Table 2. Ecological status and values of the different indices used to characterise the study sites

Index/status	Site I	Site II	Site III
Margalef richness index	5.98	3.24	4.38
IBMWP	109	58	66
HQI index	73	64	84
EQR values	0.72	0.39	0.44
Ecological status	good	poor	poor

Source: own study.

Figure 2 shows the abundance and biomass curves obtained at the three sampling sites with different taxonomic resolutions, using genus- (Fig. 2A) and family-level (Fig. 2B) identification. The evaluation obtained with both taxonomic levels indicates moderate or high pollution for the three sampling points. Sampling sites I and III show moderate pollution using the genus taxonomic resolution (abundance and biomass curves overlap), while site II shows poor quality (abundance curve over biomass curve). In contrast, the family-level analysis assesses poor environmental quality for all sites. To test the differences between

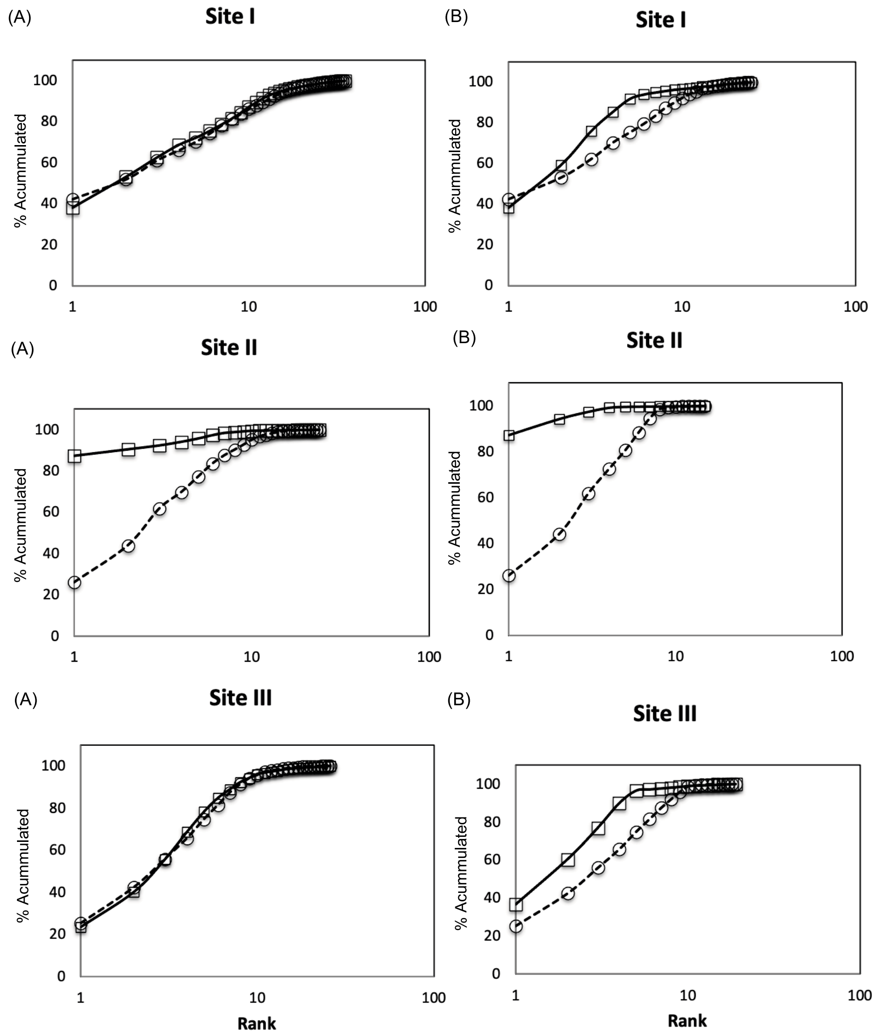

Fig. 2. Abundance biomass comparison (ABC) curves obtained in each sampling site using genus-level (A) and family-level (B) identification; ○ = biomass, ☐ = abundance; source: own study

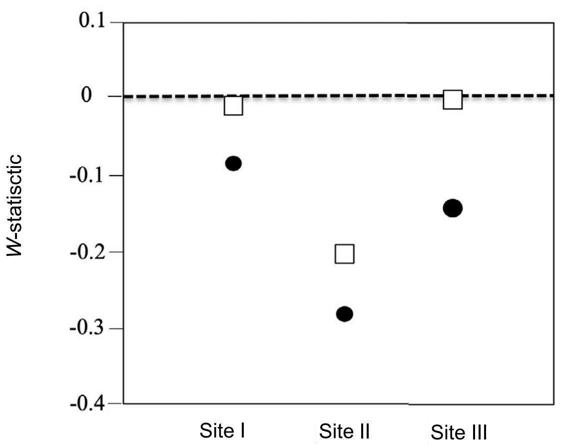

Fig. 3. *W*-statistic values in each sampling site using genus level and family level identification; ☐ = genus level, ● = family level; source: own study

both taxonomic levels, the *W*-statistic values were calculated (Fig. 3). Both, genus and family aggregations, show a similar tendency. However, the values obtained with the family level at all sites were below 0, indicating a worse pollution status than those shown when the genus level was used. In this last case, the *W*-statistic values were close to 0 on sites I and III, which indicates that the communities were moderately disturbed.

These differences between different taxonomic groupings were also observed by AGARD *et al.* [1993] with the macroinvertebrate community in a tropical coastal environment. These authors compared species and family ABC curves and indicated that the aggregation of data to the family level produced an effect of increasing sensitivity to pollution and decreasing misclassification. This aggregation at the family level reduces the high dependence of the ABC curves on the single dominant species [CLARKE 1990]. In our case, the comparison between genus and family levels with other biological indices shows that the evaluation obtained with family aggregation is more similar to those obtained with the Margalef and *IBMWP* indices than the evaluation based on genera; therefore, we could conclude that this level of taxonomic (see Tab. 3) resolution is adequate for the use of the ABC method in assessing the ecological status in Mediterranean rivers. This result supports the suggestion by WARWICK [1988], who indicated that analyses of higher taxonomic levels might clearly reflect pollution gradients and considerably reduce the cost of an evaluation study.

Nevertheless, the information obtained from the ABC method showed some discrepancies with the other biotic indices. On the site I, the ABC method detected moderate water quality

Table 3. Families and subfamilies identified in the studied samples

Families and subfamilies	Site I	Site II	Site III
Dytiscidae	+	–	–
Dryopidae	+	–	–
Haliplidae	+	–	–
Ceratopogonidae	+	+	–
Sf. Ceratopogoninae	+	+	–
Sf. Dasyheleinae	–	+	–
Sf. Leptoconopinae	+	–	+
Chironomidae	+	–	–
Sf. Corynoneura	+	–	–
Sf. Diamesinae	+	+	+
Sf. Chironominae tr. Chironomini	+	+	+
Sf. Chironominae tr. Tanytarsini	+	+	+
Sf. Orthocladiinae	+	+	+
Sf. Tanypodinae	+	+	+
Limoniidae	+	–	+
Psychodidae	–	+	–
Simuliidae	+	+	+
Tabanidae	+	–	+
Tipulidae	–	+	–
Baetidae	+	+	+
Caenidae	+	+	+
Gerridae	+	–	+
Gomphidae	+	+	+
Nemouridae	+	–	–
Beraeidae	+	–	–
Ecnomidae	–	+	–
Hydropsychidae	+	+	+
Hydroptilidae	+	+	+
Leptoceridae	+	–	–
Odontoceridae	+	–	–
Rhyacophilidae	+	–	+
Valvatidae	+	–	–
Ancylidae	–	–	+
Physidae	–	+	–
Enchytraeidae	+	–	+
Lumbricidae	+	+	+
Lumbriculidae	+	+	+
Naididae	+	+	+
Hirudidae	–	–	+
Sf. Mermithoidea	–	–	+

Source: own study.

(genus level) or poor quality (family level). Conversely, this site has been classified as "good quality" by the *IBMWP* index. The information obtained in the ABC curves seems to be closer to reality than that offered by the *IBMWP*. The situation found on site I, i.e., a site impacted by human activity, with the presence of wastes and a low value of the riparian habitat – *QBR* < 30 (Ca.: *QBR* = qualitat bosc ribera) [Munné *et al.* 1998; Suarez *et al.* 2002], which does not correspond to a stream with good ecological quality. Moreover, the fish community is also poor, with only the presence of barbels (*Barbus barbus*), as a consequence of continuous discharges of organic matter from the olive oil industry [Morales-Mata *et al.* 2020]. On site II, the abundance curve was above the biomass curve, representing a site with a high pollution level. This sampling site is below the sewage water treatment plant, which was not working when the samplings were taken; thus, all the urban sewage was altering this site. In any case, at this sampling site, all indices detected the worst situation, with the lowest *W*-values and the longest distance between abundance and biomass curves. On the last sampling location (site III), there was a slight recovery of the *IBMWP* value, also detected by the Margalef diversity index, and by the ABC method. The increase in flow and water velocity below site II may cause the recovery of water quality, according to the natural self-purification process of rivers.

Focusing on the advantages of using the ABC method over other macroinvertebrate indices, the most evident advantage is that there is no need to know the species sensitivity weights, based on individual tolerance to water quality. However, there are also some disadvantages, such as the determination at the species level and the investment of time during the weighing of the individuals collected. These disadvantages are reduced by the aggregation process carried out in this work, since the level of taxonomic identification (i.e., family-level identification) required would be the same as that currently applied in most of the indices widely used in this type of evaluation (e.g., the *IBMWP* used in this work), and the weighing of these specimens grouped by family does not involve excessive time.

CONCLUSIONS

The objective of this study was to determine whether a method developed for the benthic marine community could be used for river assessment and monitoring using the macroinvertebrate community and to test the most appropriate level of taxonomic resolution to be implemented. Although the results shown are preliminary, as a consequence of a single sampling in a river ecosystem, and being aware that a larger study would be necessary to corroborate the results obtained here, they support the suitability of this methodology for quality evaluation in river ecosystems, with a family-level identification.

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