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PART 3
Methods and Approaches

CHAPTER 11

Can Mediterranean River Plants Translate into Quality Assessment Systems? Venturing into Unexplored Territories

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Introduction

Attempts to develop macrophyte indices for assessing human impacts in Iberia that are suitable for use within the Water Framework Directive (WFD) (Council of the European Communities, 2000) have not been very successful. It became apparent that biological monitoring methods that had been applied in more northern temperate rivers of Europe were not performing well in Mediterranean rivers. The objective of this study was to review the development of macrophyte biological monitoring methods in Iberia and to examine which factors, both in the assessment methods and in the characteristics of the rivers, may be limiting the ability to produce macrophyte indices in Mediterranean rivers.

The precursor of most aquatic macrophyte monitoring methods in Europe is the Mean Trophic Rank (MTR) system (Holmes *et al.*, 1999). It was developed in the UK for the purposes of the Urban Wastewater Treatment Directive (Council of the European Communities, 1991). MTR scores each aquatic macrophyte based on an expert opinion of the species' preference along a perceived eutrophication gradient. Species strongly associated with eutrophication score highly (e.g. *Azolla filiculoides*), whereas species associated with oligtrophic conditions have low scores (e.g. *Nardia compressa*). To assess the eutrophic condition of a river section the mean of the species scores, weighted by a cover value, is calculated. By surveying macrophytes upstream and downstream of a waste water treatment works the extent of eutrophication caused by the treatment works can be established. In an assessment of the MTR, Dawson *et al.* (1999) stressed that care should be taken to ensure that the physical habitats of the two surveys are similar when making the comparison since physical differences in the sites can alter the MTR score. Macrophyte species-scoring indices appear to perform poorly in Mediterranean rivers, which are naturally eutrophic but have harsh ecological conditions, with high magnitude winter spates and extended summer droughts. Mediterranean rivers are often ephemeral (no flow in the summer) or intermittent (separate pools in a dry channel connected by subsurface flow) (Argyroudi *et al.*, 2008).

Within the Iberian Peninsula regions closer to the Atlantic coast (Lisbon, 702 mm yr^{-1}) receive more rain annually than eastern regions (Barcelona 596 mm yr^{-1}, Valencia 429 mm yr^{-1}), although

River Conservation and Management, First Edition. Edited by Philip J Boon and Paul J Raven.

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the driest regions are in the interior, such as Salamanca (385 mm yr^{-1}) (Reiser and Kutiel, 2010). However, more important than the annual rainfall in characterizing Mediterranean rivers is the distribution of rainfall throughout the year. Mediterranean areas tend to have high winter rainfall and long dry summers. The median Dry Days Since Last Rain (DDSLR) for northern regions is 21 in Salamanca and 22 in Barcelona, whereas in the more central Lisbon it is 65 and in the south of Portugal (Loulé) it is 83 (Reiser and Kutiel, 2010). Within Portugal this strong north-south division between northern temperate flow regime rivers and southern Mediterranean flow regime rivers is evident in the classifications of aquatic macrophyte communities (Dodkins *et al.*, unpublished information).

In the Guadiana river, which drains from Spain into the south of Portugal, there can be four months without flow, and up to 50% of the species found in the channel may be terrestrial plants (Ferreira *et al.*, 2001). The drying is a continuous and gradual process and therefore the yearly species succession represents the hydrological pattern within the river. The sediment within the channel is frequently of fine texture, with a strong association with the sediment deposited during drying and the nutrient concentration when the rain returns. Resuspension of these deposits also results in high natural turbidity.

Difficulties of developing indices for assessing Mediterranean Rivers

Low numbers of species

A previous macrophyte index (Aguiar *et al.*, 2009) was rejected for use in the WFD by the Portuguese Water Institute (INAG) since it contained hygrophytes and terrestrial species within the index which have a weak association with the water column. Thus, only helophytes (emergent) and hydrophytes (submerged or floating) could be considered for subsequent indices. Macrophyte surveys were conducted at 373 river sites by INAG (Aguiar *et al.*, 2008; INAG, 2008), recording macrophyte species in the river channel and along its banks. Over a thousand macrophyte species were found (Aguiar *et al.*, 2011). Birk *et al.* (2007) had developed a scoring system to represent the association of European macrophytes with the aquatic habitat. Using this assessment, helophytes and hydrophytes could be identified within the data. Helophytes and hydrophytes represented only 12% of the total number of taxa (Aguiar *et al.*, 2011) with 105 taxa being considered suitable aquatic macrophyte indicators. The north-south difference was evident, with sites in the south of Portugal only having a mean of 12.7 aquatic macrophytes per site (from the 105 species) compared with 13.9 in the north (significantly different at $P = 0.05$).

There is a large variation in the number of taxa used in different indicator-based indices. For example, the Trophic Index of Macrophytes (Schneider and Melzer, 2003) uses only 49 species, whereas the Indice Biologique Macrophytique en Riviére (IMBR) (Haury *et al.*, 2006) uses 207 species. There has also been a tendency for an increase in the number of taxa used in indices, suggesting that indices with more taxa perform better. For example, the LEAFPACS method (Willby *et al.*, 2006) currently used for assessment of macrophytes in UK rivers uses 275 taxa, whereas the previous MTR system used only 120 taxa. The same has occurred with diatoms, with the original Trophic Diatom Index (Kelly and Whitton, 1995) having only 76 taxa whereas the new TDI has 667 taxa (Kelly *et al.*, 2008).

Small-scale physical habitat variation

With the advent of the Water Framework Directive, instead of assessment being focused on comparison of sites upstream and downstream of point sources, sites have to be compared with near-natural 'reference conditions'. The reference conditions are usually based on actual river sites, but these are rarely close to the monitoring station and are often on a different river system (and in some cases may even be located in a different country). Although rivers are classified into types that should have similar features, these are necessarily catchment- or reach-scale characteristics such as width, depth, alkalinity and slope. Physical variation at the habitat scale

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such as sediment type and the range of flow types may vary both within the reference sites, and between the reference sites and the monitoring site. This can result in pronounced differences in the habitat types within the reference sites, increasing perceived natural variation, and thus reducing the sensitivity of the index.

Non-causal relationships

Demars and Edwards (2009) criticized macrophyte monitoring methods that assign scores to species since the approach assumes that all the macrophytes at a specific site are limited by, or are responding to, the pressure gradient along which they are scored, which is unlikely to be true (Barendregt and Bio, 2003). Although the averaging of scores may produce good correlations across a Member State, on a site by site basis the score may be more associated with natural variation than with any pressure. For example, a small patch of light created by a fallen tree may permit vascular plants to grow in a natural river section that would normally have only bryophytes. These small-scale habitat changes are difficult to predict even when there is a sophisticated modelling process in place, such as Discriminant Function Analysis (Wright *et al*., 1998), to identify site-specific reference conditions.

The probable reason why so many macrophyte metrics for monitoring use species scores along a pressure gradient (e.g. nutrient enrichment) is that natural variation caused by other factors (such as small-scale physical habitat variation) can be reduced by the sampling or modelling approach employed, thus dampening, though not eliminating the influence of natural background variation.

Previous macrophyte indices in Iberia

Several different types of indices have been used within the Mediterranean region. The Índice de Conservação Macrofitico (Ferreira, 1994) was the earliest used in Portugal, although it was developed to assess the conservation potential of different reaches rather than for ecological quality assessment. The index had a scoring system based on the number of rare species found compared with the number of alien species found, and therefore was a measure of (native) species diversity.

The MTR system was applied in Portugal, though it functioned satisfactorily only in highly oligotrophic and highly eutrophic rivers. This was thought to result from the low number of scoring species found (80 out of a potential 120) (Szoszkiewicz *et al*., 2006). The Índice de Macrófitos (IM) (Suarez *et al*., 2005) is another plant scoring system developed for macrophytes in Spain. This index had a low correlation when regressed against log orthophosphate concentrations (r^2 = 0.165). Dodkins *et al*. (unpublished information) used the method of Lavoie *et al*. (2006) that had been applied to diatoms in Canada. Macrophyte species were scored based on their position along the main gradient in species change as represented by the first axis in a Detrended Correspondence Analysis (DCA). The advantage of this method is that the scores measure the main changes in the community between sites, and not only nutrient enrichment. The rank correlation with a nitrate gradient within the whole of Portugal was high (r^2 = 0.602); however, the index failed to show a significant separation between reference and impaired sites within southern river types.

The BEnthic Assessment of SedimenT (BEAST) method was originally used to measure species similarity between reference conditions and an impaired site using invertebrates (Reynoldson *et al*., 1995). However, Aguiar *et al*. (2011) applied the same approach to macrophytes in Portugal. The Spearman's rank correlation of the index with nutrient gradients was less than 0.35 (equivalent to a rank r^2 of 0.12). The BEAST approach is not expected to correlate well with a single human pressure, since the index responds to multiple pressures, but this was a much lower correlation than found with the DCA metric of Dodkins *et al*. (unpublished information), and the BEAST approach also included terrestrial species. A RIVPACS-type approach (Wright *et al*., 1984) attempted by Aguiar *et al*. (2011) using macrophytes instead of invertebrates was also compared to the macrophyte BEAST method, but it also

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performed poorly (equivalent of an r^2 of <0.09 in a rank correlation with nutrient gradients).

Assessments of the vegetation structure and functional types provide an alternative to similarity measures or indicator species indices. The Riparian Vegetation Index (RVI) (Aguiar *et al*., 2009) developed for Portugal includes metrics such as riparian and aquatic species richness, exotic species richness, nitrophyllous species richness and riparian integrity. The RVI correlated well ($r^2 = 0.56$) with a combined pressure gradient in the south of Portugal, but in the north it performed less well ($r^2 = 0.31$) than other metrics. However, the RVI was rejected for the WFD because it used riparian plant species and riparian structure together with aquatic species.

Thus, all the methods previously attempted have proved either unsuitable for WFD application, or performed poorly in the Mediterranean rivers characteristic of the south of Portugal and Spain.

Possible solutions

There are several means that could be employed to resolve these problems. First, the definition of reference conditions at a site could be improved. Small-scale physical habitat variation is likely to be a large source of variation for macrophytes within the WFD, particularly since the comparison is not directly between two proximate sections of the same river. However, current modelling methods are not suitable for predicting the macrophytes at the habitat scale. Instead, all the available information could be used to produce a site-specific reference condition using expert judgement. The model for predicting the reference condition could be seen as a first phase, after which site-specific information, including sediment cores (Seddon *et al*., this volume) underlying geology (e.g. an acidic river running over alkaline rocks), and biogeography, could be used to improve the accuracy of the predicted reference condition and allow the uniqueness of the river in its natural state to be described. Since the same monitoring networks tend to be retained in subsequent monitoring periods, once an accurate reference condition is defined for a site it can be kept indefinitely. The current approach to developing reference conditions in the WFD (Wallin *et al*., 2003) implies that a general (or modelled) reference condition for a river type can be applied to a single unique site. This increases the apparent natural variation and may make small deviations from a near-natural state harder to detect.

Improving the performance of Mediterranean macrophyte indices could also be achieved by increasing the number of aquatic species in the index, either by expanding the non-vascular species, especially macroalgae, whose taxonomy and ecological requirements have yet to be resolved in Iberia, or by including hygrophyte species found in the dry river channel. These species indicate nutrient regime and hydrology in temporary Mediterranean rivers, but they are usually confined to the banks of temperate rivers. See Plates 15 and 16 show two rivers that have intermittent flow, with hygrophytes and terrestrial species being established in the channel.

Another way of increasing species numbers would be to combine the macrophytes and diatoms into a single metric. This combination is advocated in the WFD, because the biological quality element to be assessed is defined as 'macrophytes and phytobenthos' but it is not explicit as to how and at what stage within bioassessment this combination should take place. Nonetheless, diatoms are also part of the aquatic flora, and form a group with an important history as bioindicators (Kelly *et al*., 2009).

A combined diatom-macrophyte index

Within the 373 sites surveyed in Portugal, 105 aquatic macrophyte species and 281 diatom species were found, giving a total of 386 species. Weighted Average Partial Least Squared (WA-PLS) (ter Braak and Juggins, 1993), a more accurate form of Weighted Averaging (ter Braak and Looman, 1986), was applied to these species along a pressure gradient formed from the combination of nitrate and BOD. The species were thus scored to represent

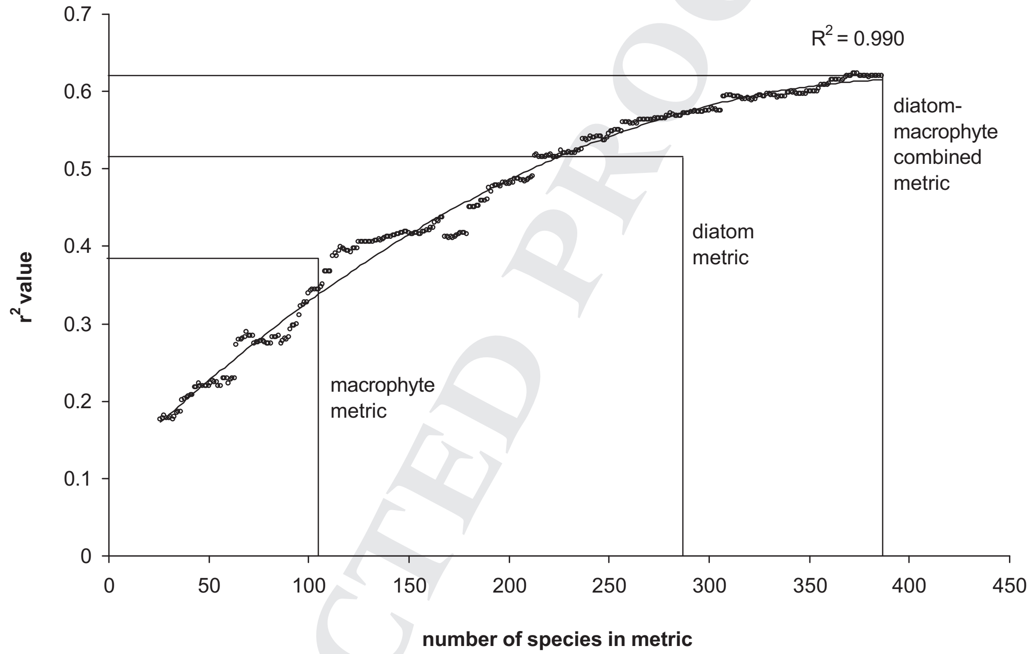

Figure 11.1 The number of species in the combined diatom-macrophyte metric plotted against the r^2-value when the metric (with this number of species) was regressed against the impact (combined nitrate and BOD). The fitted regression curve is a second order polynomial. The locations of the diatom only metric (281 species) and macrophyte only metric (105 species) are also shown. The line is not continued below 25 species since at this point many sites had no scoring species.

their optimum along the pressure gradient. The biological site scores, calculated as a weighted average of the species scores, had a high correlation ($r^2 = 0.620$) with the pressure gradient in the whole of Portugal. The index performed better in the northern region ($r^2 = 0.711$) than the southern region ($r^2 = 0.346$). When the index values were calculated separately for diatoms and macrophytes, diatoms had a higher correlation with the nutrient pressure gradient ($r^2 = 0.510$) than macrophytes (0.381).

The correlation of the combined diatom-macrophyte index against the pressure gradient was also assessed with consecutive and random removal of species from the index. Figure 11.1 shows that the performance of the metric had a clear relationship with the numbers of species in the index ($r^2 = 0.990$). Also, per species, the separate diatom index performed worse than the separate macrophyte index. Thus, increasing the numbers of species in an index that uses species scoring can increase the correlation with a pressure gradient, although indices should still be designed such that there is evidence of a causal relationship between the species and the pressures.

There are several benefits of combining macrophytes and diatoms. First, there are more macrophyte species in high discharge rivers than in low discharge rivers, whereas the converse is true for diatoms (Figure 11.2). The same pattern was also found in high and low nutrient rivers within this study, and in a study by Camargo and Jiménez (2007). Also, many macrophytes are not easily detected outside the late spring and summer growth period, whereas diatoms can be monitored throughout the year. Thus, the number of species that will be found at a particular site can be stabilized.

The response of diatoms over much shorter time periods (Dixit *et al.*, 1992) than macrophytes

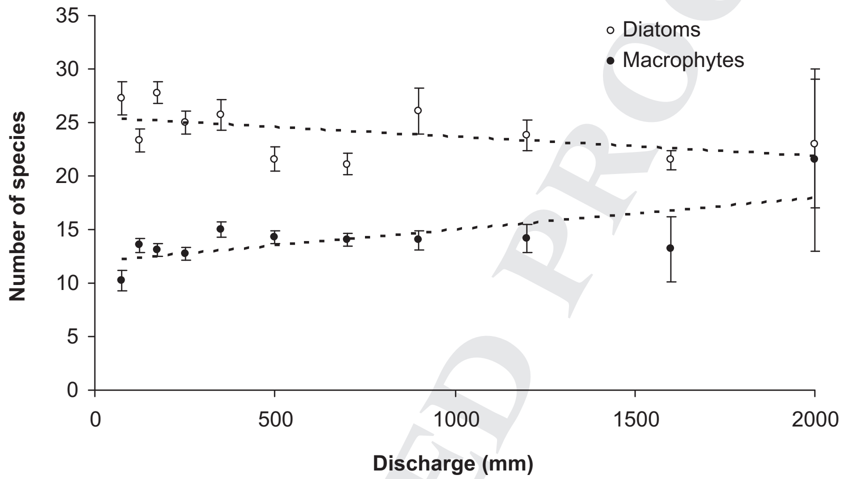

Figure 11.2 Mean number of diatoms and macrophyte species at different discharge categories (based on catchment rainfall runoff to that site). Vertical lines indicate standard error for each mean value. A linear regression for both the diatoms and macrophytes is shown as a dotted line. The diatom regression line is significant at P = 0.036, and the macrophyte regression line is significant at P = 0.017. The lines have significantly different slopes at P = 0.01.

(Seele *et al.*, 2000) helps to produce a more general measure of ecological quality when they are used in conjunction, making it useful for use in the WFD; the highly fluctuating diatom metric is effectively stabilized by the longer macrophyte response time.

The present method of combining indices from biological elements in the WFD is a 'one-out, all-out' method in which the status of the worst biological element determines the overall ecological quality. This approach has been criticized repeatedly for being over-sensitive (Moss *et al.*, 2003; Søndergaard *et al.*, 2005; Johnson *et al.*, 2006; Dodkins and Rippey, 2008; Moss, 2008; Noges *et al.*, 2009; Borja and Rodríguez, 2010). The alternative of averaging metrics is also problematic due to eclipsing, i.e. a failing metric being ignored owing to a dominance of other metrics reporting high status values (Suter, 1993; Dodkins and Rippey, 2008). By combining macrophytes and diatoms, which compete for similar resources of light and nutrients, a more balanced measure of the ecological status is produced without the same problems of eclipsing, since low scoring species, whether diatoms or macrophytes, will still have an influence on the final ecological status.

The future of biological indices?

Regulatory agencies may hope that biological indices developed for the WFD provide an integrated way to assess all the ecological problems a river may be subjected to, their causes, and management solutions. However, biological monitoring in the WFD gives a subjective estimate of ecological degradation based on a few parameters that are often subject to high natural variation. Ecological systems are not composed of separate physical, chemical, macrophyte, invertebrate, fish and phytobenthos components, but are complex highly interconnected and dynamic systems. The development of new indices, as well as the specification of monitoring methods, should reflect the information requirements, and thus there is plenty of scope for developing more specialized indices outside the scope of the WFD, for example to diagnose specific impacts. In such cases, combining information from all the different biological elements (as well as hydromorphological, riparian and chemical components) in a more structured way that reflects their ecological interactions may produce a more robust and sensible approach to diagnosis than separating indices based on the specializations of the monitoring scientists. Indeed, integrating biological elements together within indices may bridge the gap between subjective scoring systems and inhibitively complex ecological modelling, as well as producing indices that have a better causal relationship with impacts, within both Mediterranean and temperate rivers.

Acknowledgements

Thanks go to the Portuguese National Water Institute (Instituto da Agua, INAG) for funding this work and supplying the databases used.

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