A Europe-wide system for assessing the quality of rivers using macroinvertebrates: the AQEM Project\(^1\) and its importance for southern Europe (with special emphasis on Italy)

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ABSTRACT

The AQEM Project aims to develop a Europe-wide system for monitoring the ecological quality of rivers using macroinvertebrates, to satisfy the requirements of the EU Water Framework Directive. Three main types of anthropogenic perturbation are being investigated: morphological degradation, water (organic) pollution and acidification (the last is not under investigation in Italy). The selection of reference and impaired study sites is discussed. Particular attention is paid to the problems encountered when defining reference conditions. The initial stages of the project highlighted the lack of a Europe-wide definition of river types. The future development of such a typology from the AQEM database is discussed. The standard AQEM data gathering methods are presented, from background information about sites to the microhabitat-based macroinvertebrate sampling method. The extended fieldwork methods used in Italy are described. These included the separate analysis of the invertebrate assemblages from each replicate, the recording of additional microhabitat variables for each replicate and the completion of large-scale survey techniques for each site (including RHS). The extended method was designed to enhance the important ecological information available from the dataset, particularly relevant in Italy where significant gaps exist in the taxonomic and ecological knowledge of many macroinvertebrate taxa. Preliminary and expected findings are presented, including examples of the range and habitat selection of two species of Ephemeroptera endemic to Italy, as well as data relating to the number of taxa found at a site with increasing numbers of microhabitat replicates taken. The importance of the AQEM Project not only for biomonitoring, but also for ecology, taxonomy and conservation, in Italy and for the south of Europe in general, is emphasised.

Key words: stream assessment, macroinvertebrates, stream typology, AQEM, reference conditions, multi-habitat sampling, mayfly

1. INTRODUCTION

The AQEM Project, otherwise known as "The Development and Testing of an Integrated Assessment System for the Ecological Quality of Streams and Rivers throughout Europe using Benthic Macroinvertebrates" is an ambitious Europe-wide project (involving 8 European countries) designed to put the EU Water Framework Directive (EU 2000) into practice. The primary aim of the project, as the title states, is to develop a method capable of functioning Europe-wide, which will describe the condition of our rivers as measured through their biology. The AQEM methodology uses the idea of the reference condition, that is (more or less) unimpaired sites, and compares these with sites suffering from the main perceived degradation types present in Europe: acidification, water (organic) pollution and morphological alteration. The final output will be in the form of a computer program, which will allow the user to obtain an overall site quality score as well as the degree to which a site is impacted by acidification and/or pollution and/or morphological degradation. The necessary input variables are expected to be: general site location data and stream type; a list of the macroinvertebrate taxa and number of individuals (from multi-habitat sampling) and selected parameters from the AQEM site protocol (depending on the kinds of impairments found).

Why is AQEM needed? The Water Framework Directive (WFD; EU 2000) legislates at a Europe-wide level but currently no Europe-wide assessment system exists. While many of the member countries individually have well established and informative monitoring programs, there is a need for an overall system. The AQEM method is not designed to replace or negate the work currently carried out by the individual states, but will instead be incorporated, for instance, as an addi-
ecology. Within AQEM, upgraded aquatic invertebrate and distribution of taxa, as well in terms of general essential knowledge, for example, regarding the identity of taxonomic knowledge. The project is therefore of biotic indication systems is strongly limited by the lack of comparable application in all EU member states. These taxa lists represent one of the intermediate tasks of the project and they will have a high intrinsic value in themselves. In many European countries (e.g., U.K., Germany, Austria, The Netherlands, Czech Republic, etc.), regional and country-wide macroinvertebrate species lists can be easily compiled for many of the most important taxonomic groups. Non-specialist researchers and trained technical staff - using appropriate identification keys - can identify species and define the overall taxa richness of streams and rivers for monitoring or conservation purposes. This is not the case in much of southern Europe, where new species remain to be found and the distributions of many described species are poorly understood.

What we aim to do with this paper is to explore the wider possibilities of the AQEM Project and to emphasise its importance for the (relatively under-studied) south of Europe. We present our preliminary findings (including problems encountered), concerning two out of the four areas investigated in Italy. A further aim of the paper is to briefly illustrate the additional sampling and assessment options adopted in Italy, to reduce the gaps in ecological knowledge related to the use of aquatic invertebrates and to increase their suitability for ecological quality assessment in this European area.

2. METHODS

2.1. Selection of river type, reference conditions and investigated sites

It was not possible, even within this large project, to include all the typologies of rivers in Europe. The situation is further complicated by the fact that no comprehensive description of river types in Europe exists. The response to these problems was to use a simple “top-down” approach, employing for the most part the same variables as the EU Water Framework Directive (System A): ecoregions (according to Illies 1978); size classes (based on catchment area); geology of the catchment; altitude classes. The river types studied were chosen to be representative of the main river types and impact factors (acidification, organic pollution, hydraulic and morphological damage) in Europe and are shown in table 1.

Regarding reference sites, they should obviously be as little modified from pristine conditions as possible. The following is a summary of the guidelines given for the selection of reference sites for the AQEM Project (see Hering et al. in press, also Hughes 1994; Wiedenhöl & Johnson 1997).

- The reference conditions should genuinely reflect the unimpaired state of the rivers they represent (they should be of the same type).
There should be as little urbanisation, agriculture and silviculture as possible within the catchment area and, especially, floodplain. Natural climax vegetation or unmanaged forest should be present.

- Coarse woody debris should not be removed.
- No bed or bank fixation should be present, nor should overbank flow be prevented or controlled.
- The hydrograph and sediment transport system should not be altered (no or minor impoundments, reservoirs, weirs, water diversion, abstraction or pulse releases).
- There should be no point source or diffuse pollution (organic, nutrient, acidification, liming, thermal, other chemical or salinity).
- The biology of the site should not be impaired (alien species, changes in community composition of native species or impact of migration barriers).

Two out of the 4 river types in Italy were located in the northern and southern Apennines (mountainous areas). For the selection of study sites, local knowledge of the areas (presence of reference conditions and impact types) was used in consultation with the Po river authority (in the case of the northern Apennines, Piacenza and Parma) and with the Cilento park authority (for the southern Apennines, Salerno). In each case, the study rivers were selected first according to the likely presence of sites which would meet the strict requirements of the reference conditions (see Hering et al. 2001). This in itself was problematic (see results/discussion). The degraded sites, selected for morphological impact in the north and water quality in the south, were chosen using local information, e.g. presence of quarries, dams or significant sewage outfalls, backed up with field inspection of the chosen rivers.

### Tab. 1. Overview of stream types investigated in AQEM. Columns "Ecoregion": number according to Illies (1978). Column "geology class": cal = calcareous, sil = silicious, org = organic. Total number of sites investigated for each WFD river category (System A) are reported, together with the overall total.

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full list of the investigated sites with a general description is reported in Hering et al. (2001) and on the web site http://www.aqem.de.

2.2. Sampling procedure and site protocol

The data collected during the application of the AQEM methodology comes from both maps and fieldwork. There is an extensive set of background information about the site and catchment, gathered one time only, which is predominantly map based, but augmented with recording in the field. This includes data related to the catchment of the site, for example, stream order, catchment area, catchment density, geology, catchment land use, distance to source, presence of significant natural lakes. In addition, data are gathered upstream and downstream of each site (a total stretch of 20 km for a mid-sized river), for example, presence of dams, other structures, straightening and culverting. Data are also collected for the site itself (defined as a stretch of 1 km for a mid-sized river) including floodplain width, floodplain land use, altitude, slope, valley form, as well as details about trees (extent, shading, woody debris), physical modifications (bank/bed fixation, impoundments, channelling) and pollution.

The field-based data collected on every sampling occasion will be summarised briefly here. For a more extensive description see Hering et al. (2001). The method used for the macroinvertebrates is to sample the microhabitats found, in proportion to their % presence. This is a type of "multi habitat sampling" procedure (e.g., Barbour et al. 1999). The area sampled is chosen to include both a "riffle" and a "pool" area (loosely defined). The first step of this procedure is to estimate the cover of each habitat, by first considering two layers: a lower mineral layer (rocks, boulders, gravel etc.) the total of which must add up to 100%, and an upper organic layer (CPOM, macrophytes etc.) the total of which is likely to be less that 100%. To distribute the 20 sample replicates according to percentage presence, the two layers are considered as one and a sketch map drawn of the microhabitats and positions of replicates. Water depth and current velocity are measured for every replicate. Samples from the riffle area are pooled, as are samples from the pool area. The replicates are not necessarily divided equally (10/10) between riffle and pool, instead the numbers are weighted to match presence. The results section includes an actual example of a sketch map and position of replicates.

In addition, for each sampling period, water samples are taken for each site and subjected to extensive chemical and microbial analysis (for details see Hering et al. 2001).

2.3. Sampling strategy addenda adopted in Italy

In Italy, the method was extended, to enhance the "information potential" of the data and to allow greater comparability between the current study and related studies (past, in Italy and U.K., as well as future).

The standard AQEM method states that all the replicates taken from pools should be combined, as should all the replicates taken from riffles. In Italy, the replicates were kept as separate samples. This means that when the data are analysed, not only do we have information about which taxa were found in which sites, but also which taxa were found in which habitats. With this in mind, extra information was gathered to describe the habitat of each of the samples. The prevalent "functional habitat" (sensu Harper et al. 1992), according to those identified in a number of other Italian rivers (e.g., Crosa & Buffagni 1996; Buffagni et al. 2000; Buffagni 2001; Buffagni & Crosa, unpublished) was recorded. These were "margin with macrophytes", "margin without macrophytes", "macrophytes in current", "run-riffle" and "backwater" for lowland stretches and "riffle", "transition", "pool" and "bedrock" for upland segments.

In addition, for each sampling occasion, for each site, a River Habitat Survey (Raven et al. 1998a, 1998b; Buffagni et al., in preparation) was carried out. This survey assesses the physical habitat quality, including flow type information (which was also recorded for every invertebrate replicate taken), and physical modifications over a 500 m stretch, therefore gathering information at a scale larger than the AQEM invertebrate sampling methodology, which is based on one riffle-pool unit. The application of River Habitat Survey (together with information gathered from pages 1-3 of the AQEM site form) complies with the Water Framework Directive requirement for the hydromorphological assessment of rivers, to collect data that will yield a better understanding of biological and chemical data. One occasion for each site, two riparian indices were applied, the Buffer Strip Index and the Wild State Index (Braioni & Penna 1998) along with the Index of Fluvial Functioning (I.F.F.), designed to assess the overall quality of the riverine environment (Siligardi et al. 2000).

A count of Escherichia coli was included as part of the water analysis, to bring this analysis into line with Italian law. The general AQEM methodology assumes that two sampling dates are sufficient to characterise the benthic community and overall seasonality. For Italy, a third sampling date was adopted to better discriminate the overall seasonal trends (which presumably are of greater magnitude in southern than in northern Europe). In addition, the sampling of macroinvertebrate data three times per year is closer to the requirements of Italian legislation (D.L. 152/99), which prescribes four dates, but accepts three.

3. RESULTS AND DISCUSSION

3.1. Problems with the definition of reference conditions and the impact factors investigated

Regarding the two Italian Apennine areas, sites which simultaneously included all the characteristics required for reference conditions (see above and Hering et al. 2001) were not found. For example, for the northern
Apennines, it is difficult to meet the criteria for hydrologic conditions and regulation, relating to impoundments, pulse releases and retention of sediment. Of the three reference sites, only one has no significant impounding structure in the upstream continuum, but this site, on the Taro River, is not fully satisfactory either, as the site has a more mountainous character, compared to the river’s morphologically impacted downstream sites. In fact, for every river the reference sites are those located furthest upstream, meaning that they tend to be of steeper slope and smaller size. These variables in themselves shape the macroinvertebrate community, independent of other perturbations. The simultaneous study of reference sites present in three different subcatchments per area partly overcomes this problem (e.g. the reference site on the Trebbia River is at a much lower altitude than the impacted sites on the Taro). For the southern Apennines, even what appears initially to be the best reference site (along the Tanagro River, formally named Calore Lucano), with no upstream human habitation or cultivation, is not wholly natural. Large, fixed, stone weirs (to slow the dangerous run-off of flood-water) are present in the upstream continuum and water is abstracted, mainly for drinking, close to the source. Though this does not dry up the river, it can sometimes severely reduce its volume of water. “Lack of water” is a problem frequently encountered in this area of Southern Italy and many streams are naturally episodic. One potential reference site was abandoned as it was known to flow only underground during dry periods. Lack of water was also a problem in the northern Apennines: between the first and second sampling periods one reference site was abandoned for a site further downstream, as the original reference site had low-flow conditions (Ceno River).

In contrast, a third study area in Italy, involving small lowland streams (Novara Province, northern Italy), had problems deriving from too much water. These spring-fed streams (known as *fontanili*) belong to a peculiar, unique ecosystem where water chemistry, relatively constant water temperature and a characteristically stable hydrograph lead to a distinctive invertebrate community (e.g. Buffagni 1994; Buffagni & Gomba 1996). During exceptional flood events in the autumn of 2000 the nearby, large, River Ticino overflowed into the *fontanili*, washing away animals and leaving large sand deposits.

The choice of sites affected by the anthropogenic factors under investigation is as important as the choice of reference sites. It is obviously easier to detect the effect of an impact if it is the only variable which varies between sites; however, field ecology operates in the real world and such control over variables does not exist. Nevertheless, in the Northern Apennines, a single, main impact type, morphological degradation was found, while water quality remained high or fairly high at all sites (see Hering et al. 2001). The situation was more complicated for the Southern Apennines, where streams were affected by differing degrees of water pollution and/or morphological degradation. While this situation is not scientifically desirable, it reflects the reality of the rivers in this area and therefore offers the opportunity to describe more exhaustively the impact typology of the region.

3.2. A river typology for Europe

An area where AQEM has the potential to be very useful is in the development of a river typology for Europe. The choice of river types to be studied for AQEM has been made, through necessity, according to a simple top-down method (see methods). This work, however, has laid bare the fact that no comprehensive description of river types exists, neither for Europe as a whole, nor for many of the individual countries involved. Within Italy, a river typology has never been described (see Sandin et al. 2000).

The method employed for AQEM, however, was less than ideal. One factor is that it employed the ecoregions according to Illies (1978), which are developed at a scale which is likely not to be detailed enough for rivers. Italy is divided into two broad ecoregions, which, considering its complex biogeographical history, are unlikely to adequately represent the true situation. In contrast, Austria, a relatively small country, belongs to 6 European ecoregions (Moog et al. 2001; Schmidt-Kloiber et al. 2001). We envisage that the AQEM dataset, which gathers together a wide range of data types (biological, hydrological, geological) will contain the information necessary to make a more successful attempt at a river typology for Europe. In the U.K., with an extensive River Habitat Survey (Raven et al. 1998a) database, including thousands of sites, initial attempts to develop a nation-wide river typology involved dividing rivers into named categories. This was eventually rejected, as it inadequately represented the reality, which was a continuum of river types (Naura 1998). Multivariate methods (PCA) have been used to produce an ordination of all the sites, whose principal components are related to map-based variables, such as altitude, slope, distance to source and height of source (Jeffers 1998). This non-categorical approach is proving useful to compare habitat features and quality between sites (e.g. Raven et al. 1998b) and has also been used to link the River Habitat Survey with the distribution of the native white-clawed crayfish *Austropotamobius pallipes* (Naura & Robinson 1998). The same approach is being considered to predict the regional distribution of endemic, uncommon species of invertebrates (e.g. *Electrogera lunaris*, see below).

3.3. Advantages of applying the expanded sampling strategy along with large scale survey

The overall advantages and outputs deriving from the AQEM approach have been illustrated in Hering et al. (2001). In the present paper, emphasis is directed to
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the extra information gained by the addenda to the AQEM standard site protocol, such as the keeping of each replicate separate and the concurrent application of river habitat assessment methodologies.

Keeping the replicates separate for a site and recording microhabitat variables (see methods) dramatically expand the information content of the data, compared to taking one or two (riffle and pool) overall samples. One example is described below (3.4.); the simple but informative data given in figure 1 is obviously dependent on this method. The collection of this type of habitat-level data also makes it possible to answer questions about "best field practice".

Figure 2 shows, for a reference site, an intermediate site and a polluted site, the cumulative numbers of taxonomic units found, with increasing numbers of replicates. From these, decisions can be made about how many replicates need to be taken to adequately sample the fauna. The number of replicates can be based on the saturation curve of a rich site, such as in the Sammaro (Fig. 2, top). Also, the definition of such taxa saturation curves potentially gives information to support the reduction of the field effort required to apply the AQEM methodology for routine monitoring. In some southern European areas, invertebrate densities can be seasonally very high. In such cases, the standard method is relatively time-consuming and may result in a high degree of redundancy. A potential solution to this problem could be to further reduce the size of the river bed sampled for each replicate (at the moment between 1/20 and 1/16 m²). We feel, however, that this may increase the influence of chance effects on the invertebrate assemblages of each replicate, thereby making comparisons between replicates of the same and different habitat type less robust.

The AQEM methodology requires the use of a standardised list of microhabitats to allow objective sampling. This is the only credible approach for rivers where the invertebrate assemblages of "biologically relevant" microhabitats are not known in detail. However, we envisage that analysis of the separate AQEM microhabitats will identify a list of biologically-defined habitats for each river type studied. This is the "functional habitat" concept (habitats with a real ecological meaning, sensu Harper et al. 1992). Analysis of the species assemblages in each type of microhabitat can answer questions about the ecological uniqueness, importance or variability of the microhabitat. Microhabitat definitions could be merged or split further, depending on the findings, and this information can be used to de-
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Furthermore, the taxa assemblage data from the separate microhabitats can be further exploited, to ask questions about the effect of different impact types at a range of different scales. To give an example, not only can the question "What is the effect of organic pollution on the taxa present at the site?" be answered, but also, "What is the effect of organic pollution on the species assemblage of cobbles?" as well as "What is the effect of organic pollution on the number and equitability of microhabitats found at a site?" and the related "How do the microhabitats present at a site influence the taxa found at that site?". Such information is invaluable to develop impact-specific assessment tools. Habitat and impact specific metrics can be developed using biological indicators defined according to the level of taxonomic resolution necessary. For example, perhaps to detect organic pollution the identification of certain families of benthic invertebrates may be enough, but to detect morphological degradation indicator species can be found. In fact, it is known that invertebrate assem-

Fig. 2. Example of the cumulative number of taxa with increasing numbers of replicates at Southern Appennini sites (May 2000, Italy) with different organic pollution intensity (reference, slightly impacted, heavily impacted). River Sammaro: top; River Ripiti: middle; River Calore: bottom. bl: rocks > 40 cm (Megalithal); pg: cobbles >20 cm (Macrolithal); pf: cobbles >6 cm (Mesolithal); co: coarse gravel >2 cm (Microlithal); gh: gravel >0.2 cm (Akal); sa: sand >6 µm (Psammal); ph: Phytal; al: algae; tp: terrestrial plants.
blages from the same habitat at different sites can be more similar than those from different habitats within a site (e.g. Parsons & Norris 1996); furthermore, different impacts can have selective effects on benthic taxa and metrics (e.g. Metcalfe-Smith 1994; Rawer-Jost et al. 2000; Sandin & Johnson 2000).

The information-potential of the data is further enhanced, when macroinvertebrate sampling is carried out concurrently with larger scale surveys, such as River Habitat Survey. Then questions can be asked across three different scales: the microhabitat, the riffle-pool unit and the section (500 m for RHS). Thus, the impact of morphological degradation or the frequency of flow types, assessed over hundreds of meters, can be compared with the taxa of a site, the taxa of a single microhabitat or the presence or absence of the microhabitats themselves.

3.4. Preliminary findings and expected gains in ecological and taxonomic information

Figure 1 shows an example of a sketch map, including microhabitat types and the position of replicates. Also shown are the microhabitat distributions of two Ephemeroptera species which are endemic to Italy. One, *Electrogena lunaris*, was found only in one habitat ("mesolithic", cobbles 6-20 cm). The other, *Habroleptoides pauliana* was more of a generalist, occurring in a wide range of habitats. This type of information for these species is completely new - *H. pauliana* has not been formally described in its larval stage yet and previously *E. lunaris* was only known from one river (in the Central Apennines) (see also the following paragraph), therefore no ecological information was available for the two species. In fact, in Italy part of the AQEM Project funding will be invested to investigate to the species level some of the invertebrate groups, to highlight taxonomic lacks as well as to define ecological preferences of a number of taxa, for which ecological traits are still to be defined (e.g., endemic species or taxa adapted to local conditions). In an intermediate step, AQEM will lead to the compilation of dedicated ecological databases concerning, for instance, data on feeding types and microhabitat preferences. Whilst this will not represent a totally new task for central and northern Europe, research is needed to do the same for the species of southern Europe. The AQEM Project has begun to address this knowledge deficit for this area of Europe. For some taxonomic groups (e.g., mayflies), use of the specific identification of taxa is being extended for bio-monitoring purposes, and a comparison of results with more traditional indices and assessment techniques (i.e. to the genus or family level) will be conducted.

The sampling, conducted with many replicates and taken from all the microhabitats present at a station, has already highlighted elements which are also important from the taxonomic point of view, above all for species whose rarity or sporadic occurrence has not until now allowed profound analysis. For example, *Electrogena lunaris*, whose previously known world distribution was restricted to a single site in the central Apennines (Belfiore et al. 1997), has now been found in many locations in the northern Apennines. This has helped to better define the diagnostic features for the species, as well as to describe the inter-population variability. Some species, up until now considered to have a wide distribution, showed noteworthy morphological differences between individuals collected in different areas (for example, *Baetis gr. alpinus*, the entire genus *Procloeon*). This calls into question their taxonomic status. Some other species, such as *Siphlonurus lacustris*, exhibited dramatic differences in habitat preference, suggesting that morphologically similar but distinct taxa may be present in Italy. Regarding the genus *Habroleptoides*, some of the species present in Italy are endemic and their nymphs have not been adequately described. A similar situation exists for much of Europe. The collection of large numbers of nymphs of cohabiting species potentially allows the definition of new taxonomic features useful for identification within this problematic genus. For other species, it has become evident that their descriptions are inadequate for Italy, having mainly been
based on finds from other European areas (e.g., *Baetis vernus*). The need for profound revision is now obvious. The gains in knowledge of species distributions resulting from such an in-depth sampling program are of great value. For example, significantly more is now known about the ranges of *Torleya major*, the meridional form of *Baetis vernus*, *Rhithrogena siciliana*, *Rhithrogena adrianae* and *Habroleptoides pauliana*.

The promotion of conservation, of species as well as habitats, is among the indirect outcomes of the AQEM Project in Italy. The identification of reference conditions and sites, as required by the Water Framework Directive, together with their conservation (e.g. HABITAT Directive, EU 1992), has the potential to alter management practices on the ground. Because, for AQEM, scientists are required search for, identify, describe and justify sites representing good or particularly interesting ecological conditions in their country, the value of these areas can be better appreciated by governments, local authorities and planning institutions. For example, for the Southern Apennines, where many of the study sites are within the Cilento National Park, the information gathered by the AQEM Project can be used directly by the Cilento Park Authority to identify high protection areas, as well as areas where ecological quality is not good, where remedial work could be targeted.

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