Technical review of the potential use of benthic macroinvertebrate biomonitoring, using the reference condition approach in the setting, monitoring and assessment of agri-environmental performance standards

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Technical review of the potential use of benthic macroinvertebrate biomonitoring, using the reference condition approach in the setting, monitoring and assessment of agri-environmental performance standards

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ABSTRACT

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The Reference Condition Approach is increasingly used to assess the ecological status of inland waters. In this report the advantages and disadvantages of this approach are discussed and a new methodology is presented. The advantage of this new approach is that it is flexible, dynamic, allows the inclusion of more data and can easily be combined with other approaches. The report concludes with a description of the multivariate Principal Response Curves technique and its use to detect trends in biological data. In the closing discussion a case is made for the adoption of species traits rather than species taxonomy in ecological quality assessment.

Keywords: biomonitoring, ecological waster quality assessment, reference condition approach, multivariate techniques

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Preface

Under Agriculture and Agri-Food Canada's (AAFC) Agriculture Policy Framework (APF), Environment Canada (EC) has committed to the development of environmental performance standards that will guide environmentally sustainable agricultural performance in support of common EC and AAFC goals for the environment. This standards development program (known as the National Agri-Environmental Standards Initiative (NAESI)) has four themes: Air, Water, Biodiversity and Pesticides.

The goal of the NAESI Water Theme is to establish agri-environmental performance standards that will protect surface and groundwater from the ecological effects of agricultural activities. The overall outcome will be to develop:

- environmental performance standards that would protect surface and ground waters from nutrients, sediments, microbial pathogens or excessive water withdrawal,
- levels of environmental performance that would be achieved by adoption of best management practices and new technologies
- monitoring and performance measurement tools specified with respect to proposed standards.

The NAESI project, is a five year project that will be conducted in four stages: 1) assessment, 2) development of standards, 3) testing of standards and 4) final product. The proposed work for the "Technical Review of the Utility of of Benthic Macroinvertebrate Biomonitoring, Using a Reference Condition Approach in the Assessment of Agri-Environmental Standards for Water" will partially fulfil the requirement for the assessment phase of the NAESI Water Theme.

The objective of this report was to produce a technical review of the potential use of of benthic macroinvertebrate biomonitoring, using a reference condition approach in the setting, monitoring and assessment of agri-environmental performance standards. This document will be used to help develop agri-environmental performance standards to protect the aquatic environment. The technical review should focus on agriculture land use practices; however, it will not be limited to this sector. The document includes a review and assessment of existing national and international approaches, analysis of existing published literature on in situ measures of exposure and effect, assessment of new and emerging indicators for evaluating environmental performance.

To be able to fulfil this objective we used example data sets from two sampling points in the Rhine and Meuse rivers in The Netherlands. We would like to thank Piet J. den Besten and Abraham bij de Vaate for making the Grave and Kampen data available to us.

Summary

The Reference Condition Approach is increasingly used to assess the ecological status of inland waters. In general, when using this approach, information on environmental conditions is compiled with inventories of plant and animal species that are found under these conditions. From these inventories, a site reference condition can be constructed which summarises the occurrence of species present under environmental conditions that are characteristic of undisturbed ecosystems. Once the reference condition has been established, any site suspected to be impacted can be assessed by comparing it with the reference sites, and its status determined.

This reference condition may be determined from existing sites, from models, from palaeolimnological reconstructions and from expert judgement. In this report the drawbacks of this approach are discussed. These drawbacks include the lack of agreement on how the degree of deviation from the reference condition, and the classification of ecosystems into quality classes should be measured, what the level of required information should be, the static nature of the approach, the lack of inclusion of functional endpoints and the exclusion of information on impacted sites in the assessment.

In this report a new methodology to define reference conditions is presented that might overcome the above drawbacks. This methodology is based on Cased Based Reasoning which is a problem-solving paradigm that is able to utilise the specific knowledge of previously experienced, concrete problem situations (cases) for solving new problems. The backbone of a predictive tool based on CBR is the construction of a case base containing all monitoring results of the region of interest and the judgement whether the sites are reference or not, which can be done using a sliding scale. A new test site can be compared with the case base in several ways, depending on the information available. The advantage of this approach is that it is flexible (not all information is needed to allow an assessment), dynamic (new information can be added on the spot), allows the inclusion of monitoring results of impacted sites and can easily be combined with other approaches such as simple ecological models and Bayesian statistical approaches.

Ecosystems inherently change in time and, together with irreversible processes like global climate change and invasive species, calls for a dynamic understanding of reference conditions. When reference sites are sampled in time, the question arises how a best visualisation of the dynamics in time is obtained. Therefore the report ends with a description of how the multivariate technique Principal Response Curves technique can be used to detect trends in community level time series. In the closing discussion a case is made for the adoption of species traits rather than species taxonomy in ecological quality assessment.

1 Introduction to the reference condition approach

Policy frameworks like the European Water Framework Directive (EU, 2000) and Agriculture and Agri-Foods Canada's Agricultural Policy Framework, set ambitious targets for maintaining the ecological integrity of agricultural landscapes, and the aquatic habitats they contain. In the European case, ecological standards are defined by using a typological framework and by describing good ecological status for each water type. Nowadays, such reference condition approaches are increasingly used to generate ecoregional typologies, and to define good status. For inland waters, reference conditions may be determined from existing sites, from models, from palaeolimnological reconstructions and from expert judgement (Moss et al., 2003). In general, information on environmental conditions is compiled with inventories of plant and animal species that are found under these conditions. From these inventories, a site reference condition can be constructed which summarises the occurrence of species present under environmental conditions that are characteristic of undisturbed ecosystems. Once the reference condition has been established, any site suspected to be impacted can be assessed by comparing it with the reference sites, and its status determined. The reference condition database, once formed, can be used repeatedly. When based on existing sites, the reference condition is established by standardized sampling of both the biota (in rivers, the focus is on benthic invertebrates) and the habitat conditions at a number of reference sites.

In previous studies, matching pairs of reference and test sites were compared for significant difference. This has a major disadvantage in that it has low sensitivity, caused by high variability in the single or limited number of reference sites (Norris & Georges, 1993). Increasing the number of reference sites to explain more of the variability leads to more complex asymmetric analysis (Glasby, 1997) and poses problems in finding reference sites that are both unimpaired and have a habitat similar to the test site (Linke et al., 2005). A recent development that overcomes this problem is the above mentioned reference condition approach, which uses whole sites, rather than multiple collections within sites, to serve as replicates (Reynoldson et al., 1997), and the variation within groups of unimpaired reference sites defines the acceptable range of communities (Linke et al., 1999).

2 Drawbacks of the reference condition approach

The reference condition approach is increasingly being adopted by regulatory scientists and water managers, as can be seen from the increase in published literature on the topic in recent years. Although the methodology may provide valuable information for environmental assessment, there are also some disadvantages and problems to overcome.

2.1 Typological framework

Each place on earth is unique, and river reaches are no exception. This is rather problematic since because each site is unique, there is no reference site that matches exactly. To overcome this problem some generalizations have to be made, and one of the main generalizations is the use of a typological framework. In such a framework, sites are classified in different water types and these types differ with respect to certain environmental factors. Such an approach has been used earlier in water quality assessment (Verdonschot 1990, Peeters & Gardeniers 1994). According to Moss et al. (2003), a typology should not be so complicated that conditions of high ecological quality cannot easily be defined and it should only use characteristics that are geographical and do not overlap with the variables used in measuring ecological status, otherwise a very confused system will result. The establishment of a sound typology seems therefore essential in using the reference condition approach. But, ecosystems are complex and their characteristics vary within large ranges, determined not only by external conditions such as weather and catchment characteristics but also by internal processes. The uptake of substances (nutrients, carbon) by primary producers, and their release by grazers and decomposers leads to an infinite number of normal combinations of values of thousands of measurable variables (Moss et al. 2003). Every ecosystem exists, even in the absence of human impacts, in many alternative states (May 1977, Scheffer et al. 1993) and this may hinder the establishment of sets of reference sites.

2.2 Assessment of deviation from reference condition

Descriptions of reference condition are developed to enable the assessment of ecological quality for a specific site which belongs to the same water type as those from the reference condition. A list of the physical and chemical conditions and the biological component of the reference condition alone are not enough to perform such an assessment. The situation encountered at a specific site should be compared with the reference condition and the distance from the reference situation should be determined. This requires an instrument with which the deviation from the reference conditions can be estimated in an objective way. Furthermore, once the distance is determined, the degree of deviation has to be evaluated in terms of quality classes, again in an objective way. Usually five quality classes are defined e.g. bad, poor, moderate, good and high. How to measure the degree of deviation from the reference condition, and the classification of ecosystems into quality classes is still an active areas of research.

2.3 Level of required information

In many studies regarding reference conditions, extensive, large lists of animal and plant species are generated. Although it may be informative to collect as much detailed information as possible, it is questionable whether this is really necessary to assess ecological quality. For example, Marchant (2002) argues that limitations due to sampling implies that only common taxa will produce sufficiently strong signals for the interpretation of environmental gradients and thus for bioassessment.

2.4 Developments

Another drawback of the reference condition approach is that it can become a static tool, frozen at the time of establishment of the reference condition. Once reference conditions and associated species lists have been established, there is a strong probability that the methodology may not be able to deal with long-term ecological change within ecosystems. For example, many aquatic ecosystems on earth are threatened by invasions of alien species. Sala et al. (2000) predict that invasive species will be the major drivers of changes in freshwater biodiversity over the next 100 years. Examples of recently invaded aquatic ecosystems that are well documented are the River Rhine in Europe and the North American Great Lakes. The River Rhine was invaded by the Ponto-Caspian amphipod *Cheliocorophium curvispinum* in the 1990s resulting in remarkable changes in the benthic macroinvertebrate community composition (Peeters 2004). The latest successful invader in Western European hydrosystems is the amphipod *Dikerogammarus villosus*, present in the Rhine since 1995 (Figure 1, Bij de Vaate & Klink 1995) and since 1999 in the Moselle River (Devin et al. 2001). Together with the arrival of *D. villosus* in the Lower Rhine River a decline in macroinvertebrate species occurred (Van der Velde et al. 2000). It is expected that this species will invade the North American Great Lakes (Ricciardi & Rasmussen 2000). According to Ricciardi (2001) the rate of invasions increasing in the North American Great Lakes is supporting the invasional meltdown hypothesis of Simberloff and Von Holle (1999). Therefore, it is not the question whether invasions will take place in Canadian waters but what will be the long-term impact. Since invasions may result in very quick changes (within a few years) in species compositions (e.g. O'Dowd et al. 2003, Peeters 2004) it is questionable whether the reference condition approach can handle this. One possible solution to this drawback could be the monitoring of all reference sites each year. However, this would require an inordinate effort and would be expensive and inefficient.

2.5 Structure versus function

In most reference condition approaches, descriptions of environmental conditions and lists of plant and animal species that could be present are given. Although structural components (i.e. species and abundance) of the ecosystem may offer valuable information on the state of an ecosystem, they do not always provide sufficient insight into the processes operating within those ecosystems. Decomposition of organic matter, for example, is a major process in running waters in which macroinvertebrates play a key role. In general, the decomposition process changes from up- to downstream, but field studies have shown that differences also exist between different headwaters (Cummins 1974).

3 Possible methods to define reference conditions

In a reference condition approach, the structure of assemblages at disturbed sites is compared with that at reference sites to determine whether the former fall outside the range of expected conditions based on the latter. If environmental variables explain a substantial part of the variation in assemblage structure at reference sites, empirical models can be constructed to predict the structure of assemblages expected at undisturbed sites. Deviations from the predicted assemblages can then be used to assess effects of the disturbance (Tonn et al., 2003).

3.1 Empirical modelling

Many approaches have been used to model reference conditions (see Reynoldson et al., 1997 for an overview). Empirical modelling of the reference condition has generally followed the methods used to develop RIVPACS (Wright 1995) and AUSRIVAS models (Parson & Norris, 1996). In short, the reference sites are first classified into groups containing similar invertebrate communities using two-way indicator species analysis (TWINSPAN) (McCune & Mefford 1997). Second, from the large number of environmental variables measured at sites, a subset is chosen using stepwise discriminant function analysis (DFA) that best discriminates between the biological groups formed in TWINSPAN. Third, the selected variables are incorporated into a discriminant function to be used to assign sites into assemblage groups based on their environmental characteristics. This discriminant function can be used to predict the fauna expected at the test site in the absence of disturbance. In AUSRIVAS and RIVPACS, the severity of any environmental impact is assessed based on how much the number of taxa observed (O) deviates from the number expected (E), calculated as the O/E ratio. When the O/E ratio indicates impairment (i.e., less than the mean minus 2 SD for the reference site O/E), the types of organisms predicted to occur but not collected are used in interpretation (Reynoldson et a., 1997). An alternative approach to predict the occurrence of each species at a site from a common set of predictor variables is the use of Artificial Neural Networks (Joy & Death, 2004). A recent comparison of traditional (e.g. logistic regression, discriminant analysis) and these ANN techniques for predicting species presence/absence using both simulated and empirical data showed that the accuracy of ANN predictions outperformed the alternatives particularly with non-linear data (Olden & Jackson, 2002). ANNs also have advantages over traditional modelling methods, in that they are not dependent on particular functional relationships, need no assumptions regarding underlying data distributions and no a priori understanding of variable relationships (Olden & Jackson, 2001).

A major failing of all these approaches is that they only employ information on reference sites, while often also much information on impaired sites is available which could potentially also be used to assess whether a test site is impaired or not. This is a particular problem when carrying out assessments of agricultural areas, where reference sites are often absent, or at best, geographically distant. There is also no systematic, scientifically-defensible method for determining the Discriminant Function or Neural Networks described above, which obscures the scientific interpretation and evaluation of the functions. The most important disadvantage of these techniques is, however, that they are static, i.e. when new information is gathered new discriminant function and/or neural networks have to be made. It also does not allow the incorporation of expert knowledge and/or other types of information available (e.g. in the form of simple known ecological relationships). In short, it is poorly suited for the assessment of the changing landscapes associated with intensive agriculture, and thus a new approach is urgently needed.

3.2 Cased Based Reasoning

A new methodology that potentially could overcome the above described problems is cased-based reasoning (CBR). CBR is a problem-solving paradigm that is able to utilise the specific knowledge of previously experienced, concrete problem situations (cases) for solving new problems. CBR is an approach that enables incremental, sustained learning since new experience is retained, making it immediately available for future problems (Aamodt & Plaza, 1994). A very important feature of case-based reasoning is its ability to learn. By adding present experience into the case base, improved predictions can be made in the future. Early applications of CBR are, among other, in diagnosis setting (clinical audiology, heart failure, building defects, aircraft fault diagnosis and repair), legal reasoning (criminal sentencing, patent law, injuries to workers, building regulations), and planning (warfare planning, manufacturing planning problems; Watson & Marir, 1994). Well known applications of CBR in medicine include aiding medical personnel to assess patient status, assistance in making a diagnosis, and facilitation of the selection of a course of therapy. In this example a case is defined as a set of variable values or features collected from a patient during a consultation. This case can be compared to earlier collected cases (patients) incorporated in a case base. From this case base the most similar cases can be extracted by applying for instance the nearest neighbour technique. From these similar cases some useful statistics like similarity in diagnosis and successful therapy between the cases can be calculated, and used for decision making. If the patient in this example is replaced by aquatic ecosystems and the diagnosis replaced by the assessment whether a site is a reference site or not its application within the reference condition approach becomes clear.

The backbone of a predictive tool based on CBR is the construction of a case base containing all monitoring results of the region of interest and the judgement whether the sites are reference or not, which can be done using a sliding scale. The monitoring results must consist of biological information and easy to measure environmental variables. A new test site can be compared with the case base in several ways. When only easily measured environmental variables are available for the test site, using the nearest neighbour method, the reference cases present in the case base that are most alike the test case can be extracted and used to predict the faunal composition of the test site under absence of anthropogenic stress. If the reference cases have very different faunal composition, the method can even check whether alternative stable states are possible at these environmental conditions. When biological data is also available for a test site, similar sites can be found in the case base based on both the biological and environmental data. From the reference cases having similar values for the environmental parameters, the expected biological community structure can be predicted and compared with the observed biological community structure in a manner similar to the O/E ratio approach described above. From the database, cases also having similar biological community structure to that observed at the test site can be extracted. In this way, we can evaluate whether these similar sites should be classed as reference or degraded sites. These judgments can be weighed using the similarity of the cases with the test site to make a prediction for the test site to be a reference or degraded site (e.g. 45% chance to be a reference site, 40% chance to be a moderately impacted site and 15% chance to be an impacted site). So two judgments on the ecological status are made, one based on the environmental data and one on the biological data, which can be evaluated on the basis of similarity. The advantage of this approach is that it is dynamic (new information can be added on the spot), allows the inclusion of monitoring results of impacted sites and can easily be combined with other approaches such as simple ecological models and Bayesian statistical approaches. The latter are needed because the reference condition approach heavily relies on their past experience with analogous cases. This also has some obvious drawbacks:

- often only a very few comparable cases will be available
- specific cases are easily over-generalised
- the uncertainty of the prediction is hard to assess

This led us to the idea that it would be good to seek the best of both worlds by using case-based reasoning as a mimic of the experts' approach and subsequently fine-tuning the results with the aid of simple ecological models and inclusion of other lines of evidence using Bayesian approaches. Branting et al (1997) named the integration of case-based reasoning and model-based reasoning, model-based adaptation and described an example dealing with a system for rangeland grasshopper management.

4 Multivariate analysis to show dynamics of reference sites and to contrast potential impacted sites with the reference condition.

When reference sites are sampled over time, it is important to get an overview of the dynamics of the species in time. Ecosystems inherently change in time and, together with irreversible processes like global climate change and invasive species, calls for a dynamic understanding of reference conditions. When reference sites are sampled in time, the question arises how a best visualisation of the dynamics in time is obtained.

Principal Response Curves is a multivariate ordination method especially designed for the analysis of data from microcosm and mesocosm experiments (e.g. Frampton et al., 2000; Van den Brink et al., 2000; Smit et al., 2002). The method is able to focus on the differences in species composition of different treatments with an untreated control in time (Van den Brink & Ter Braak, 1998; 1999). In this way the method is able to focus on that part of the variance that is explained by a treatment, by excluding the variance explained by differences between replicates and sampling data. The monitoring data collected when setting reference conditions are not obtained experimentally but are the result of a biomonitoring program. The nature of the data, however, is the same, ecosystems are sampled in time and a contrast between different systems (treatments against control or dynamics in time against an internal reference to view changes in time or reference sites against a possible impacted site) should be obtained. We will show, using an example data set, how changes in time can be visualised using an internal reference and how a possible impacted site can be compared to a reference site using the multivariate method of Principal Response Curves.

4.1 Example data sets

Macroinvertebrates from the Rhine and Meuse rivers were sampled at the locations Kampen and Grave, respectively, on a regular basis with a standardised artificial substrate, consisting of glass marbles (De Pauw et al. 1994, Paskevich et al. 1995). After emptying the basket with the substrate on a 1 cm mesh sieve, the animals were removed from the substrate by rinsing and additional brushing. The material was collected on a 500 μm mesh sieve mounted under the coarser one and preserved in ethanol. The monitoring activities at Kampen and Grave were part of a larger program that was set up to evaluate long-term changes in the ecological quality of the surface water of the rivers Rhine and Meuse.

4.2 Time series analysis with internal reference using PRC

For this analysis an internal reference was used that consisted mean abundance values for all species over all samples. When all samples are compared with this internal reference, this analysis can be regarded as a PRC analysis in the sense that a time series is compared to a control, with the difference being that normally an external control is used (untreated control) while in this example a reference sample (the mean of all others) was used as a contrast. All multivariate analyses described in this paper were performed after ln(2x+1) transformation of the species data (see Van den Brink et al., 2000 for rationale) using the computer program CANOCO for Windows 4.0 (Ter Braak & Smilauer, 1998). For this analysis the Kampen data set was used as an example and the resulting diagram is displayed in Figure 1. Because the average over time was used as the internal reference, the PRC diagram displays (by definition) a mean deviation of zero from this internal reference. After 1993 an increasing deviation from the years 1992 and 1993 is shown. Especially *Dikerogammarus villosus*, *Hypania invalida*, *Hydropsyche bulgaromanorum* and *Jaera istri* are indicated to have increased in abundance in time, *Dugesia lugubris/polychroa* gr, *Bithynia tentaculata*, *Asellus aquaticus* and *Hydropsyche contubernalis* to have decreased. The increase can also be evaluated in more quantitative terms: the indicated abundance of D. villosus in 2000 is exp(Δcdt*bk) = exp(0.6*4.5) = 15 times the abundance of 1992. Thirty-eight percent of the total variance could be attributed to between year variance, 62% by within-year variation. Of the variance explained by differences between the years, 56% is displayed in the diagram.

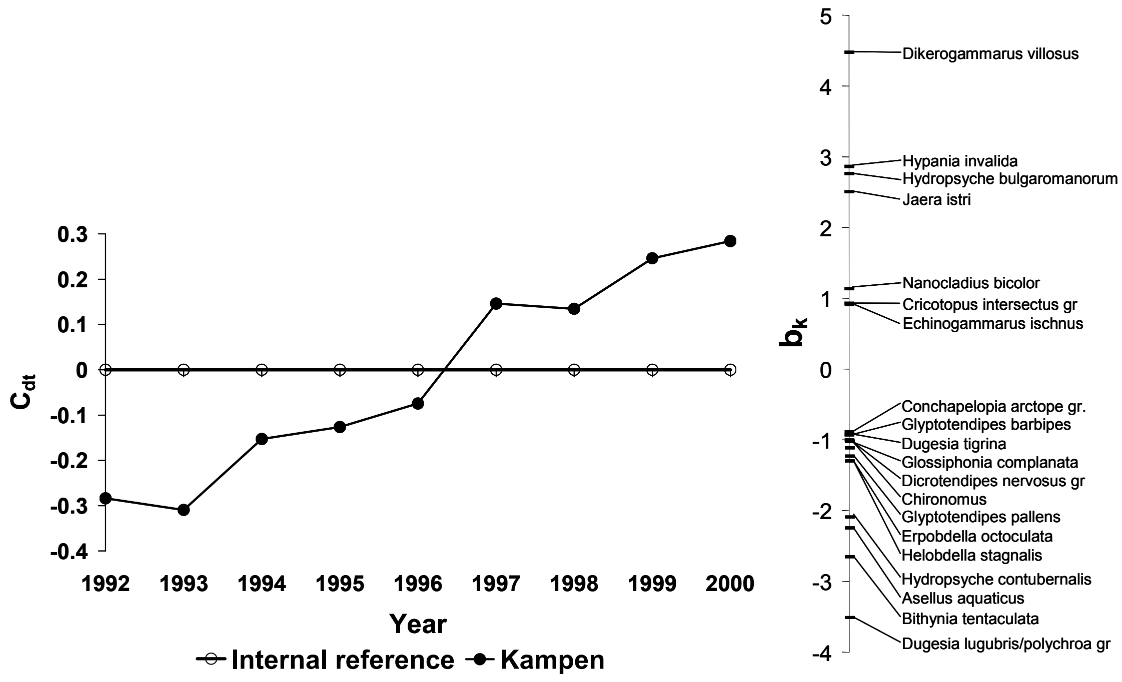

Figure 1. Diagram showing the first component of the Principal Response Curves analysis of the Kampen data set in which an artificial sample constructed of mean abundance values of all species was introduced as an extra sample. The years 1992 through 2000 were introduced as explanatory variables. Sixty-two percent of the total variation in species composition could be attributed to within year variance, the other 38% to between year variance of which 56% is displayed on the y-axis of the diagram.

4.3 Time series analysis with external reference using Principal Response Curves

In this section we discuss how to compare a time series with an external reference (i.e. reference condition), by comparing the Kampen with the Grave data set. This example is a PRC analogous to the examples described for ecotoxicological data sets (e.g. Van den Brink & Ter Braak, 1999; Smit et al., 2002 and Van den Brink et al., 2000), with small differences. In this example the Grave data set is used as the reference condition (untreated control in the sense of the ecotoxicological semi-field experiments) and 'Kampen' as the test site which ecological status has to be evaluated (treated system). 'Year' is introduced as covariable, the combination between 'Year' and 'Kampen' as an explanatory variable. In this way the samples taken within a year play the role of replicates analogous to the use of PRC in ecotoxicology. The PRC diagram resulting from the analysis is given in Figure 2.

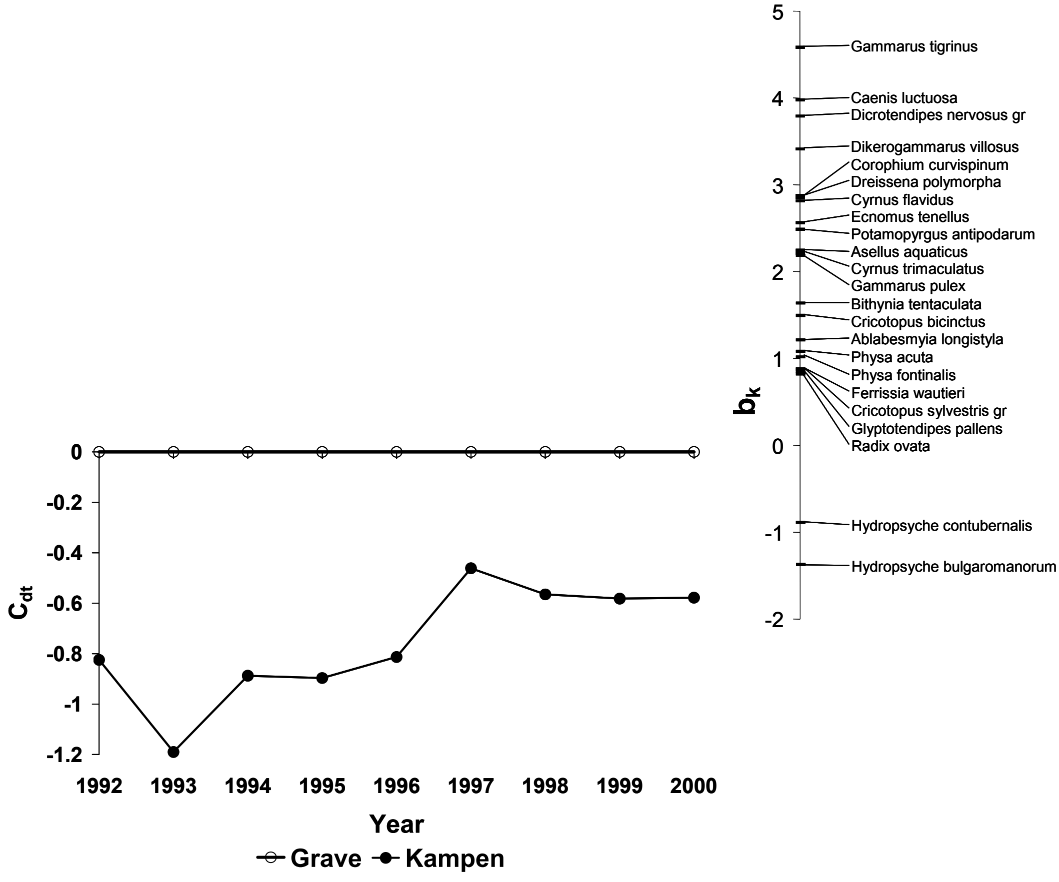

Figure 2. Diagram showing the Principal Response Curve of the combined Kampen-Grave data set. The factor year was introduced as covariables, the combination between year and Kampen as explanatory variables. Forty-three percent of the total variation in species composition could be attributed to within year variance, another 22% to between year variance. The differences between the sites explained 35% of all variation, of which 69% is displayed on the y-axis of the PRC diagram.

The analysis indicated that of the total data set 22% of the variance could be attributed to between year variance and 43% to within year variation in species composition. The remaining 35% could be explained by differences in species composition between the Grave and Kampen sites, of which 69% is displayed in the PRC diagram. The diagram shows large differences in species composition between the two sites in the early nineties, which become more similar towards the end of the sampling period. At Kampen, the species *Gammarus tigrinus*, *Caenis luctuosa* and *Dicrotendipes nervosus* gr. are more abundant, while *Hydropsyche contubernalis* and *Hydropsyche bulgaromanorum* are more abundant at Gave. In general more species seem to show higher abundance values at Grave compared to Kampen.

5 Discussion

Some of the previously-mentioned drawbacks of the Reference Condition Approach can be overcome by developing relevant definitions and appropriate tools. For establishing reference lists and for practical water management, the number of water types that will be distinguished should be limited. If there exists already a database with observations on macroinvertebrates, these data may be used to find out how many relevant water types are present. It is advisable to base the typology on geographical characteristics that do not overlap with the variables used in measuring ecological status (Moss et al. 2003).

Once the water types are defined, reference conditions should be developed. Different methods for creating reference lists have been proposed (e.g. contemporary data, historical data, expert judgements). The use of historical data might be problematic due to taxonomic difficulties. Taxonomy is not a static science, and names of species change throughout time. Furthermore, species that were formerly present and have been going extinct might never return, even when future conditions meet their requirements. Although expert judgement may provide valuable information it has the disadvantage of being biased by the expert because each expert has his/her own preferences. Therefore, in our opinion, the use of contemporary data is preferred because it reflects the current taxonomic knowledge as well as the contemporary state of the ecosystems. It is also recommended that we should begin to include functional aspects and processes in the description of the reference conditions in addition to structural characteristics.

Clear definitions should be available with respect to the water quality classes. There must be no lack of clarity on what is meant by 'good' or 'bad' ecological quality. A transparent, repeatable, and simple system should be developed to measure the distance, in ecological quality terms, between a test site and the reference condition.

The most important previously mentioned drawback in the traditional reference condition approach is the continuous change that takes place in ecosystems. A first step towards dealing with this challenge could be to evaluate species that are present at a site relative to those which exist at reference sites. What kind of species are they?: common, rare, indicator of certain conditions, invader, holder of a specific trait? This requires, however, considerable knowledge on autecology of species. This kind of information can be stored in a database to get easy access to available data. A second step to trace developments over time may be to establish regular monitoring of reference sites. The use of contemporary data for creating reference lists has the advantage that included sites can be monitored. To check whether ecological change is occurring in the reference sites, a subset of these sites can be monitored every 5 to 10 years. Analyses of these data with e.g. PRC (see chapter 3) may provide insight into any changes which have taken place.

Changes in the conditions of a water body (both environmental and biological) are reflected in altered composition of the macroinvertebrate community and, therefore, the composition of the community gives an indication of the intensity of the impact of different factors on that community (Peeters & Gardeniers, 1994). Within each community several groups of species can be distinguished, each determined by the impact of a specific environmental or biological factor. A species correlating with the intensity of a specific factor can be seen as an indicator. Indicator values may be derived from ecological and biological species traits (e.g. Usseglio-Polatera et al. 2000). Biological traits consist of characteristics on the life-history, like size of organism, life cycle duration, feeding habits, reproduction, whereas ecological traits consist of preferences of organisms regarding their habitat like type of substratum, current velocity, salinity, trophic status, saprobity. One of the first attempts to include species traits in an ecological assessment system was made by Peeters & Gardeniers (1994). They developed an assessment system for Dutch lowland streams in which they used macroinvertebrates to measure deviations from the reference condition by looking at current flow and substrate preferences of the benthic community as well as preferences for saprobity and trophic status and composition of functional feeding groups. Their approach is diagnostic in the sense that deviations from the reference situation are reflected in the quality classes obtained for the aspects mentioned above. The number of traits included in their system is limited and suggestions have been made to include larger numbers of traits in future biomonitoring (e.g. Dolédec et al. 1999).

Charvet et al. (2000) assessed the usefulness of species traits of benthic invertebrates from semi-natural reference sites as a potential benchmark for large-scale monitoring. They found that community structure based on ecological and biological traits was more stable along an environmental gradient compared to the one based on taxonomic composition. This approach could overcome the problem of functional redundancy associated with references based on taxonomy (i.e. is it bad if a species is replaced by another which is equally rare and performs the same function in an aquatic food web?).

CBR as described above could be used to build a flexible framework, capable of integrating the ideas described above into a single framework, which could generate reference conditions tailor-made for each site to be assessed. This framework could include biological data of impaired sites and recent data in its analysis and simple ecological relations and expert knowledge could be added. It would also be relatively easy to incorporate trait information into the CBR methodology, since there is no fundamental difference in looking for similar sites regarding species composition of regarding species traits present. In fact, both can be done together, illustrating the flexibility of the CBR methodology.

Applying the reference condition approach to a specific site provides the deviation of that particular water body from the reference conditions. As such, it provides information on ecological status, but it does not shed light on possible causes responsible for the deviation. For water managers, however, it is very relevant and interesting to diagnose which factor(s) cause(s) this deviation, because knowing the relevant factors, they can adjust their measures for restoration. For example, in agricultural streams, often a recognisable set of stress factors will occur (e.g. nutrient, pesticide and sediment addition, water withdrawal). It would be extremely useful to be able to attribute the measured deviation of a site from a reference state and/or good ecological quality to a specific cause or set of causes. If environmental pollution variables are also measured at the specific site possible causes for the impairment can be inferred. For explaining the absence of species also classification methodology like the Species At Risk (SPEAR, Liess and von der Ohe, 2005) concept can be included. The SPEAR concept groups species on the basis of their sensitivity to pesticides and their life-cycle traits that are known to influence recovery from toxicant effects. These and similar approaches describing the relation between species taxonomy, their traits, their sensitivity towards different stressors and their recovery potential can easily be included in the CBR methodology so not only an assessment of the ecological status of a site is obtained but also a possible cause for an impairment is provided.

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