

Principal response curves technique for the analysis of multivariate biomonitoring time series

Paul J. van den Brink · Piet J. den Besten ·
Abraham bij de Vaate · Cajo J. F. ter Braak

Received: 5 October 2007 / Accepted: 9 April 2008
© Springer Science + Business Media B.V. 2008

Abstract Although chemical and biological monitoring is often used to evaluate the quality of surface waters for regulatory purposes and/or to evaluate environmental status and trends, the resulting biological and chemical data sets are large and difficult to evaluate. Multivariate techniques have long been used to analyse complex data sets. This paper discusses the methods currently in use and introduces the principal response curves method,

which overcomes the problem of cluttered graphical results representation that is a great drawback of most conventional methods. To illustrate this, two example data sets are analysed using two ordination techniques, principal component analysis and principal response curves. Whereas PCA results in a difficult-to-interpret diagram, principal response curves related methods are able to show changes in community composition in a diagram that is easy to read. The principal response curves method is used to show trends over time with an internal reference (overall mean or reference year) or external reference (e.g. preferred water quality or reference site). Advantages and disadvantages of both methods are discussed and illustrated.

P. J. van den Brink (✉)
Alterra, Wageningen University and Research Centre,
P.O. Box 47, 6700 AA Wageningen, The Netherlands
e-mail: Paul.vandenBrink@wur.nl

P. J. van den Brink
Department of Aquatic Ecology and Water Quality
Management, Wageningen University,
Wageningen University and Research Centre,
P.O. Box 8080, 6700 DD Wageningen, The Netherlands

P. J. den Besten · A. bij de Vaate
Centre for Water Management, Rijkswaterstaat,
Ministry of Transport,
Public Works and Water Management,
P.O. Box 17, 8200 AA Lelystad, The Netherlands

C. J. F. ter Braak
Biometris, Wageningen University and Research Centre,
P.O. Box 100, 6700 AC Wageningen, The Netherlands

Present address:
A. bij de Vaate
Waterfauna Hydrobiological Consultancy,
Oostrandpark 30,
8212 AP Lelystad, The Netherlands

Keywords Biological monitoring ·
Macroinvertebrates · Multivariate analysis ·
Principal component analysis · Principal response
curves · Water framework directive

Introduction

Biomonitoring of surface waters for the evaluation of their ecological quality is required by many authorities, including the European Union, as described in its water framework directive (Anonymous 2000). This type of monitoring typically involves several ‘water quality elements’ such as the composition of macroinvertebrate communities. Biomonitoring results consist of

lists of species with their densities, assessed at regular time intervals, to allow their dynamics to be analysed. Analysis of time series must therefore focus on the detection and interpretation of trends in time. Since the resulting time series are multivariate (i.e. involve multiple species), overall trends at the community level have to be detected, which may be a difficult task.

Multivariate techniques like principal component analysis (PCA) and nonmetric multidimensional scaling (NMS) are often used to reduce multivariate time series to fewer dimensions, e.g. two latent variables to produce a diagram indicating the changes in species composition of the samples over time (Ter Braak 1995; McCune and Grace 2002). The advantage of PCA and related methods over NMS is that PCA, because it uses the original species-by-sample matrix, allows not only the sample scores but also the species scores to be plotted in the ordination diagrams. This enables species to be identified that are responsible for the community changes observed. The advantage of NMS over PCA is that it is not restricted to Euclidian distances as a measure of similarity or dissimilarity (Van den Brink and Ter Braak 1998).

There are two drivers of dynamics in species composition, seasonality and the progression of years. Variation within individual years is typically (but not always) a result of seasonality, while between-year variation can, for instance, be the result of changes in weather conditions, ecological quality (water and habitat quality) or the introduction of exotic species. It is important for the interpretation of the results of an analysis that these two sources of variation in time are separated.

Since the time vector is often not a straight line in the multivariate diagrams, these diagrams can be highly cluttered, making them difficult to interpret (e.g. Kersting and Van den Brink 1997). Van den Brink and Ter Braak (1999) therefore developed the principal response curves (PRC) technique, a multivariate technique which overcomes this problem of cluttered diagrams in analysing time series resulting from microcosm and mesocosm experiments, in which several treatments are contrasted with a control. The method allows one to focus on the way the effect of a treatment (in their case a pesticide application) on a species assemblage changes over time, while excluding the variation between replicate microcosms and different sampling dates. The data used for the study described in the present paper were not obtained

experimentally but are the result of a biomonitoring programme. This paper demonstrates how PRC can be adapted to the analysis of time series obtained from biomonitoring programmes, as well as how trends can be detected at a single sampling site, and how different sampling sites can be compared, e.g. to estimate the degree of impairment at a particular site by contrasting it with a reference site.

Materials and methods

Example data sets

Artificial substrates have been used for decades in biomonitoring programmes for the evaluation of ecological quality (Cairns 1982). One of these programmes provided the data used for the present analysis. Between 1992 and 2001, macroinvertebrates in the Rhine and Meuse rivers were sampled on a regular basis at locations near the towns of Kampen and Grave, respectively, using a standardised artificial substrate consisting of glass marbles (De Pauw et al. 1994; Pashkevich et al. 1996). After the basket with the substrate had been emptied over a 1 cm mesh sieve, the animals were removed from the substrate by rinsing and additional brushing. The material that had passed through the sieve was collected on a 500 μm mesh sieve mounted under the coarser one, and preserved in ethanol. The animals were identified as much as possible to the species level. Most years were sampled six times (A through F), while 1998 was sampled only four times (A through D) and 1999 and 2000 seven times (A through G). All samples with the same code (e.g. A) were not sampled at the same time, so codes A through G can only be regarded as denoting the temporal sequence within years.

Since the data sets are only used here by way of example, they are not introduced in further detail. The monitoring activities at Kampen and Grave were part of a larger programme that was set up to evaluate long-term changes in the ecological quality of the surface water of the rivers Rhine and Meuse and to study the effects of invasive species (Marteijn et al. 1995).

Principal component analysis

We first analysed the Grave data set with principal component analysis to get an overview of the changes

in species composition over time. All analyses described in this paper were performed using the Canoco for Windows computer program, version 4.5 (Ter Braak and Šmilauer 2002). In all analyses, abundance data was $\text{Ln}(2x+1)$ transformed (see Van den Brink et al. 2000, for rationale) and the scaling used was adjusted to focus on inter-sample distances. Default values were chosen for all remaining options (Ter Braak and Šmilauer 2002).

Principal response curves

PRC using a reference time series

The PRC method enables researchers to contrast time series of impacted sites or treated sites with a time series of a reference site. A conventional PRC analysis achieves this by performing a redundancy analysis (RDA) and introducing the interaction between sampling date and treatment (being impacted or not) as a set of explanatory variables, and the sampling dates as a set of covariables (Van den Brink and Ter Braak 1999). This allows the variance to be partitioned (Borcard et al. 1992) into parts that can be attributed to differences in time (between sampling dates), to treatment differences in time, and to differences between replicates. In the PRC diagram, the first principal component of the variance explained by treatment differences in time is displayed on the y -axis, while time (sampling date) is shown on the x -axis. An axis showing the species scores is added to the diagram to show the affinity of the different species with the response displayed in the PRC diagram.

By way of example, we use the Grave data set as a reference (untreated control in the sense of the mesocosm experiments) and Kampen as the site of interest (treated system). Year is introduced as a set of indicator (0/1) covariables, and the products between the Year indicator variables and the indicator variable for Kampen as the set of explanatory variables (Van den Brink and Ter Braak 1999). In this way, the samples taken within a year play the role of replicates in mesocosm experiments. For obvious reasons, these samples are not true replicates and can therefore not be used for Monte Carlo permutation testing as used by Van den Brink and Ter Braak (1999).

After the first PRC component, more PRCs can be extracted from the remaining variation, by analogy to

what was described by Van den Brink and Ter Braak (1998). The second PRC shows the most important deviations from the first PRC that are present in the data set.

PRC using a reference point

As explained in the previous section, a conventional PRC uses a reference time series. In this section we present a novel method of time series analysis that uses a reference point only. The analysis method is similar to conventional PRC in the way that the first principal component is plotted against time, but differs from conventional PRC in terms of the precise definition of explanatory variables and covariables. The resulting plot displays approximate differences in species composition between the time series and the reference point.

The method of analysis is defined as follows. The first step is to add the reference point as a new sampling point to species data. The second step involves creating indicator (1/0) variables that indicate the sampling times of each sample. It is possible to use an indicator variable for each sampling date, but one can also choose to select a larger time scale, for example switching from date to years. The important aspect is that the reference point gets a zero value on each indicator variable. In the next step, an RDA of the modified species data is carried out, with the indicator variables as explanatory (environmental) data. Finally, a plot is created from the RDA results, in precisely the same way as in a conventional PRC, namely by plotting the (destandardised) canonical coefficients against time and adding the axis with the species scores (Van den Brink and Ter Braak 1999; Ter Braak and Šmilauer 2002). The definition of the indicator variables, using all zeroes for the reference sample, guarantees that the resulting plot displays the approximate differences between the samples and the reference.

The analysis presented here uses the Grave data set and a particular year (1992) as a reference. This analysis has the advantage that a particular preferred or undesirable starting position can be introduced as a reference. If, for instance, a site is degraded in the year 1992, changes in species composition away from this undesirable reference can be visualised using PRC. The reference can also be external, i.e. consist of a preferred reference image, e.g. defined by methodologies like RIVPACS (Bailey et al. 2004).

One may wonder whether some of these analyses could have been obtained by a more traditional method than the above technique. The answer is yes. The results of the analysis can be obtained by a simple RDA, without adding a reference sample, by using indicator variables for different years, but deleting the indicator for 1992. If an external reference is used, however, results cannot be obtained in an easier way. The attractive aspect of our technique is that it makes the reference explicit and that the reference can be freely chosen.

Results

Principal component analysis

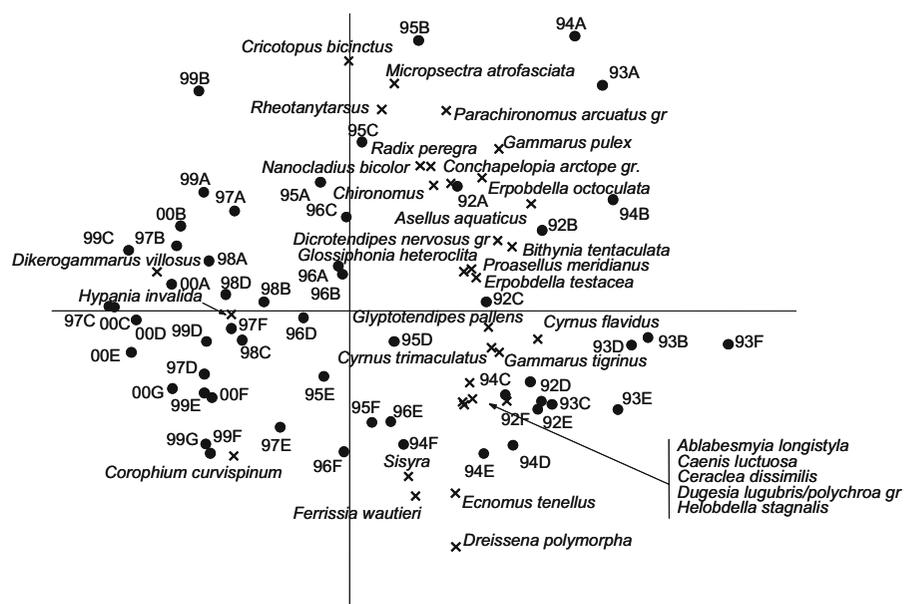
Figure 1 shows the PCA biplot of the Grave data set, displaying both species and samples, so that relative abundances of all species in all samples can be inferred. For instance, the gammarid *Dikerogammarus villosus* is shown to have a relatively high abundance value in the 97C sample because it is displayed far away from the origin on the same, left, side of the diagram. By contrast, this species is shown to have low abundance value in the 93F sample, since the species and site points are placed on opposite sides of the diagram. The species points should actually be interpreted as arrows, pointing from the origin in the direction of steepest increases in abundance. Thus, the

direction of the arrow indicates which samples have high abundance values and which ones have low ones, while the length of the arrow gives an indication of the magnitude of the differences. The exact interpretation method for this type of diagram can be found in Ter Braak (1995).

The diagram shows that most samples taken in the early 1990s are located on the right side of the diagram, while most of those taken in 1996 or later are situated on the left side. This means that the time vector, although not straight, runs approximately from right to left. The vertical axis seems to show seasonal variation, as most samples taken early in the year (A, B and C) are located above the horizontal axis, while those taken at the end of the year (D, E, F and G) are displayed in the lower part of the figure.

The figure indicates that the abundance of *D. villosus* and *Hypania invalida* increases with time, while other species (e.g. *Cyrrnus flavidus*) are shown to decrease in abundance over time. *Cricotopus bicinctus* is shown to be more abundant in spring, while *Ferrissia wautieri* is more abundant in the autumn. Figure 1 is relatively easy to interpret for specialists that are used to biplots, because variation within and between years is displayed on separate axes. Though the analysis shows the patterns in the data set in great detail, the biplot is not so easy to interpret or to communicate to laymen. When time is not a straight vector in the diagram and species are located all over the diagram, it is even more difficult

Fig. 1 PCA biplot showing the change in species composition at the Grave sampling site. The sampling points are denoted by dots, the species by crosses. Thirty-one percent of the total variation in species composition is displayed on the horizontal axis, another 13% on the vertical axis of the diagram. Only species with more than 30% of the variation captured by the first two components of the PCA are displayed in the diagram. This criterion was met by 32 out of 132 species. Note that these species were not deleted from the analysis



to extract the most important patterns from a data set, even for scientists familiar with biplots.

Principal response curves

PRC using a reference point

Figure 2 shows the results obtained for the Grave data set, using the year 1992 as a reference. The PRC diagram shows that community changes were greatest after 1995. The species composition at this site changes progressively in time, i.e. species either increase or decrease in abundance over time, or do not change at all. The species axis on the right shows the affinity of the species with the response shown in the diagram, i.e. how similar the real response of the species is to the indicated response. The PRC diagrams shows, like the PCA biplot, that *D. villosus* and *H. invalida* have increased in time, though other species are also shown to have increased in time (e.g. *Corophium curvispinum*, *Cricotopus intersectus* gr and *Corbicula fluminea*). Many species have negative weights, indicating that they have decreased in

abundance. Thus, the sign of the species scores indicates the direction of the changes in abundance, while the magnitude of the score reflects the size of the changes. The diagram indicates that the majority of the species have decreased in abundance, which means in this case that a few species have become dominant.

The increase in the abundance of species can also be evaluated in more quantitative terms, i.e. the increase in abundance between 1992 and 2000 for *D. villosus* is $\exp(c_{dt}[2000] \times b_k) = \exp(1.21 \times 5.6) = 877$ times the abundance in 1992. The real difference cannot be calculated because this species was absent in 1992 and increased to an abundance of 660 individuals in 2000. Fifty-five percent of all variation in species composition present in the data set was explained by within-year variation (including its interaction with year), whereas the other 45% was explained by between-year variation, 66% of which is displayed in the diagram.

PRC using a reference time series

The variance partitioning indicates that 22% of the variation in species composition for the total data set

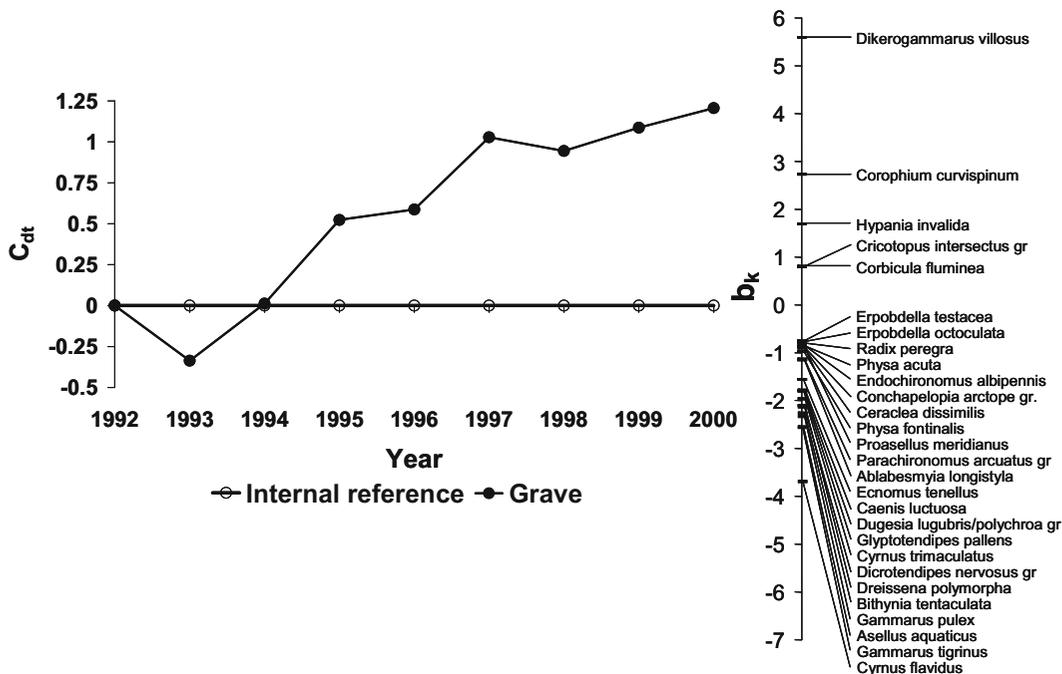


Fig. 2 Diagram showing the first component of the PRC of the Grave data set, using the year 1992 as an internal reference. Fifty-five percent of the total variation in species composition could be attributed to within-year variation, the other 45% to between-year variation; 66% of the latter is displayed in the

diagram. The *species weights shown in the right part of the diagram* represent the affinity of each species with the response shown in the diagram. For the sake of clarity, only species with a species weight larger than 0.75 or smaller than -0.75 are shown

could be attributed to between-year variation and 43% to within-year variation in species composition. The remaining 35% was explained by differences over time in species composition between the Grave and Kampen sites, 69% of which is displayed in the first PRC diagram.

The first PRC diagram resulting from the analysis is given in Fig. 3a. The diagram shows large differences in species composition between the two sites in the early 1990s, which become smaller at the end of the sampling period. The species *G. tigrinus*, *Caenis luctuosa* and *Dicrotendipes nervosus* gr. are more abundant at Grave, while *H. bulgaromanorum* and *Hydropsyche contubernalis* are more abundant at Kampen. In general, more species seem to show higher abundance values at Grave than at Kampen.

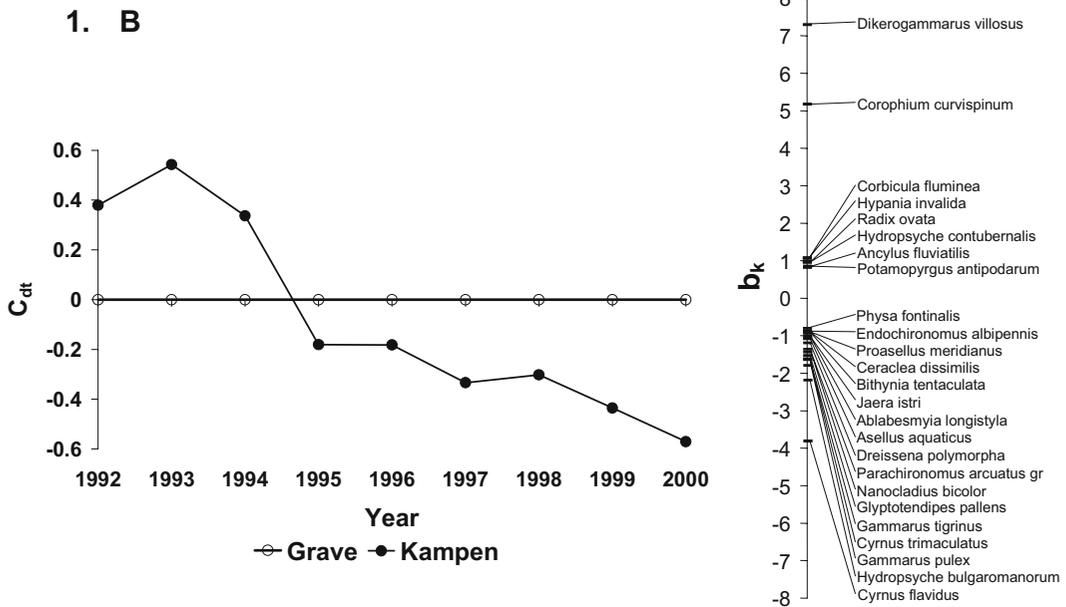
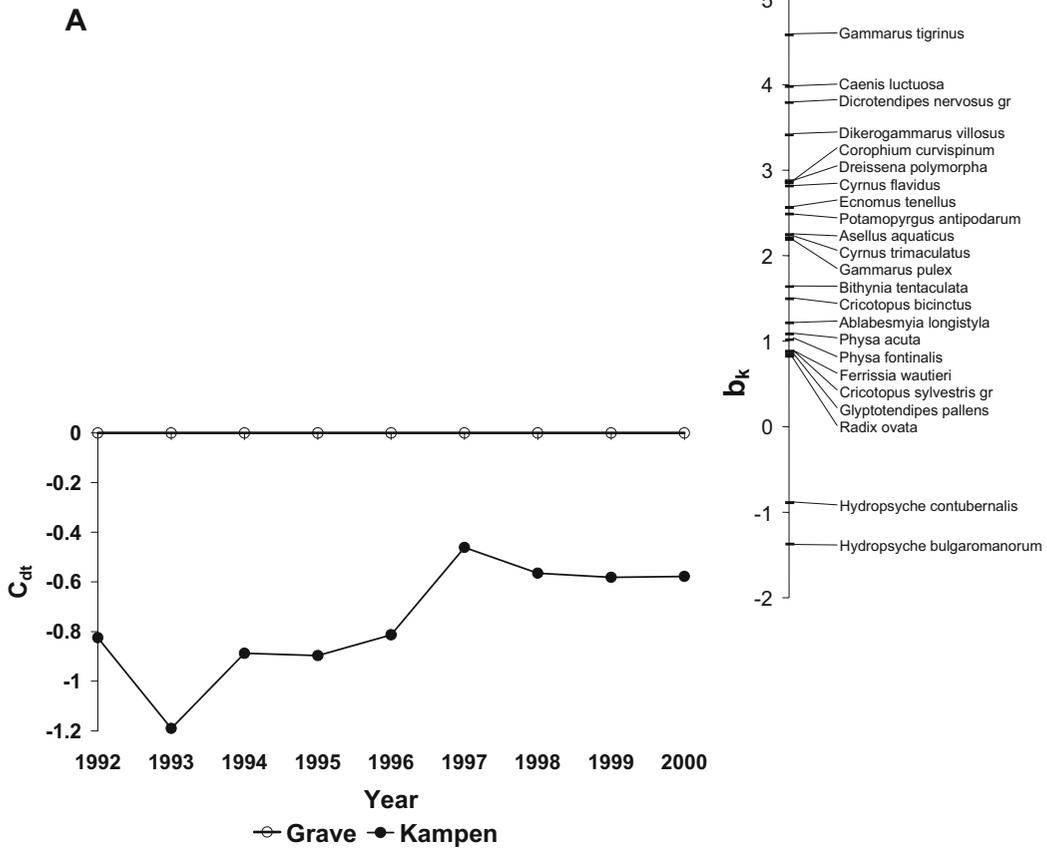
While the first PRC diagram shows 69% of the difference in species composition between the two sites, 31% remains to be displayed. Figure 3b shows the second PRC, which shows opposite differences in species composition between the early and late 1990s. For a good interpretation of the two PRC diagrams, they must be jointly interpreted (see Van den Brink and Ter Braak 1998 and Van den Brink et al. 2003 for examples). This is visualised in Fig. 4, where the *x*-axis shows the weights of the different species with the first PRC and the *y*-axis shows the weights with the second PRC diagram. When a species has an equal positive weight with both diagrams (as is almost the case for *C. curvispinum*), its response as actually indicated is the response resulting from the summation of the two diagrams. This derived response diagram is indicated in the upper right quadrant. The resulting response of a species that has a positive weight with the first and a negative weight with the second PRC is shown in the lower right quadrant. Figure 4 indicates that a few response patterns are present in the data set. Firstly, a lower abundance value at the Kampen site compared to the Grave site is shown during the whole sampling period. There are smaller differences at the end of the period for several species, with *C. luctuosa* and *D. nervosus* gr. having the highest weights. For these two species, the figure shows an increasing difference between the two sites, while the difference in abundance between the sites for *C. flavidus* is shown to decrease over time. The diagram also shows that although the first PRC diagram seems to show the same response for both *Hydropsyche* species, the second diagram qualifies this.

Discussion

The trends in the PCA and PRC analyses of the macroinvertebrate communities described in the present paper are both in line with previously published ecological information on the rivers Rhine and Meuse (Van der Velde et al. 2002; Bij de Vaate et al. 2006), but PRC yielded a more condensed and easy-to-interpret overview. The communities in both rivers are strongly influenced by recent invasions of exotic species (Bij de Vaate et al. 2002). *Dikerogammarus villosus* is a Ponto-Caspian invader which was found for the first time in the lower branches of the river Rhine in 1994 (Bij de Vaate et al. 1995) and is currently developing relatively dense populations in that river (A. Bij de Vaate, Waterfauna Hydrobiological Consultancy, personal communication). The species is suspected to be more predatory than the other gammarids present in the Rhine and Meuse. Isotope analyses ($\delta^{15}\text{N}$) indicated that they are active at the same trophic level as fish species (Marguillier et al. 1998). Dick (1996) mentioned that differences in predatory/survival abilities of gammarid species are positively influenced by their body size. Therefore, *D. villosus* may have a considerable impact on populations of the North-American invader *Gammarus tigrinus*, the dominant gammarid in the river Rhine before the invasion of *D. villosus*, and on the amphipod *Corophium curvispinum*.

Soon after being recorded in the Rhine delta in 1987 (Van den Brink et al. 1989), the Ponto-Caspian amphipod *C. curvispinum* developed such dense populations that it became the most numerous macroinvertebrate on solid substrates. Monitoring data from artificial substrate samplings and other field observations have shown that the macroinvertebrate species richness was reduced by high densities of *C. curvispinum* (Van den Brink et al. 1991; Van der Velde et al. 1998). The animals collect suspended particles from the water column to construct tubes on

Fig. 3 Diagram showing the first (a) and second (b) principal response curves of the combined Kampen–Grave data set. The factors denoting the year were introduced as covariables, the combination of years and Kampen as explanatory variables. Forty-three percent of the total variation in species composition could be attributed to within-year variation, another 22% to between-year variation. The differences between the sites explained 35% of all variation, 69% of which is displayed on the *y*-axis of the first PRC (a) and another 18% on the *y*-axis of the second PRC (B). For the sake of clarity, only species with a species weight larger than 0.8 or smaller than -0.8 are shown



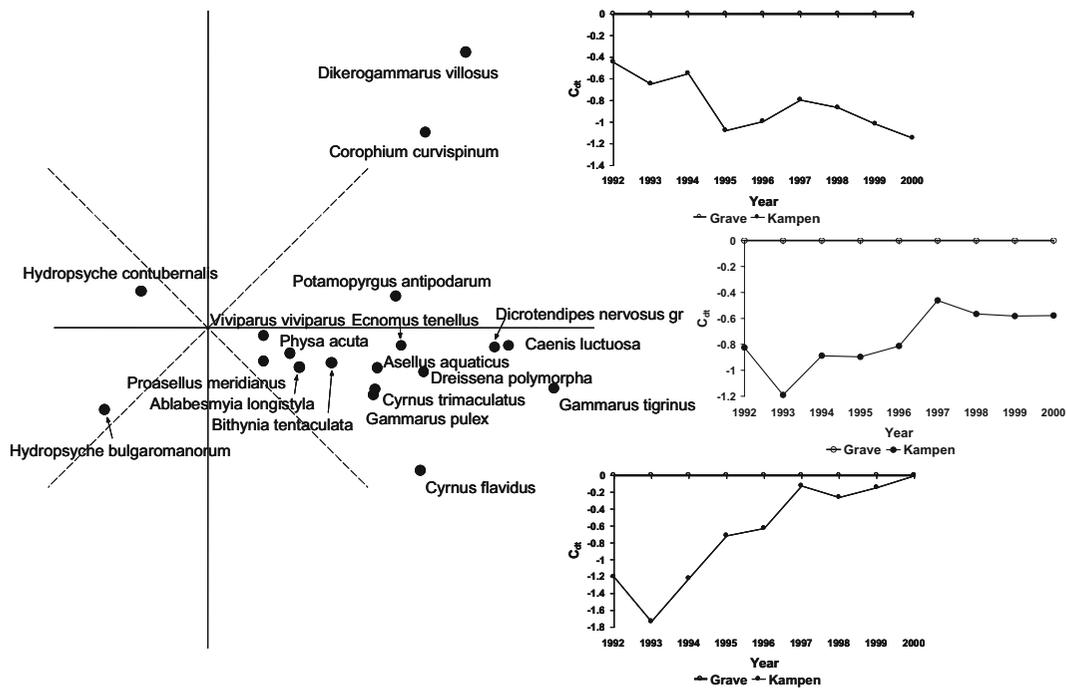


Fig. 4 Two-dimensional plot of the weights of the invertebrate species on the first and second PRCs, as given in Fig. 3a and b. The two diagrams in the corners apply to species that have equal weights on the two PRCs (except for the sign for the second PRC). The *one on the right* is the first PRC and applies to species with a high weight with the first PRC and a low one

with the second. The figure only shows species for which more than 20% of the variation is captured by the first two PRC diagrams as displayed in Fig. 3. This criterion was met by 19 out of 138 species. Note that none of the species was deleted from the analysis

solid substrates, in which they live. In densities such as those found in the Rhine, the species was able to cover large parts of the substrates with these tubes, altering the environment for lithophilic communities. According to Kinzelbach (1997), *C. curvispinum* also outcompeted the freshwater isopod *Asellus aquaticus* and several species of chironomid larvae. The high densities of *C. curvispinum* observed in the river Rhine had a strong negative impact on another Ponto-Caspian invader, the zebra mussel (*D. polymorpha*, Van der Velde et al. 1998), which was a dominant species in the Rhine delta around 1990 (Bij de Vaate et al. 1992).

In addition to the freshwater isopod *Asellus aquaticus*, some gammarid species and several chironomid species, the caddis fly *Cyrcnus flavidus* is another species that decreased in abundance. *C. flavidus* is a common species in slow flowing and stagnant waters with well-developed aquatic vegetation. Predation on this species by the invaders is the most likely explanation for the negative trend. The euryoecious gastropod *Bithynia tentaculata*, which

showed a decreasing trend at Kampen, is one of the most common freshwater snails in The Netherlands. It owes its abundance to its feeding behaviour, in that the species can change from grazer to filter-feeder depending on the environmental conditions (Gittenberger et al. 1998). The same has been observed for the flatworm *Dugesia lugubris/polychroa* gr. *Dugesia* is a common species in the lower Meuse river, feeding on freshwater snails, especially *Bithynia tentaculata* (Reynoldson and Pearce 1979).

The PRC analysis comparing the Grave (Meuse) and Kampen (Rhine) data sets indicates that although all of the above observations are made at both sites, the intensity of the changes differs. Figure 4 shows the abundance values of the invaders *D. villosus* and *C. curvispinum* to be much higher at the Grave sampling site than at the Kampen sampling site, and this difference even increases in time. The opposite is true for a *Hydropsyche* species (*H. bulgaromanorum*), while for other species (*G. tigrinus*, *C. luctuosa* and *D. nervosus* gr.) differences remain constant in time (Figs. 3 and 4). Note that if Kampen had been used as

a reference, the line denoting Grave in the first PRC would be placed above the Kampen reference, with a difference exactly identical to that indicated in Fig. 3A.

Many different multivariate methods have been used to analyse biological time series of communities, with the ordination method PCA being most frequently used (e.g. Li and Kafatos 2000). Other methods, like nonmetric multidimensional scaling and clustering, have also been proposed but have some disadvantages compared to the use of ordination, as discussed by Van den Brink and Ter Braak (1998) and Pardal et al. (2004). In the present paper, however, PCA resulted in a difficult to interpret diagram, and much clearer visualisation was obtained when the same data set was analysed using PRC (compare Figs. 1 and 2). The PRC method presented in this paper, using an internal reference, can be regarded as a special case of PCA. PRC extracts the first principal component from the variation of interest, i.e. the differences between the samples and the reference. The method is flexible with regard to the type of reference used; in fact, any internal or external reference (e.g. preferred community composition) can be used. The analysis results in a diagram in which the time vector runs straight from left to right and differences are displayed on the y -axis. This mode of presentation is very powerful, especially for non-experts, since this is the same type of display we would use to disseminate univariate information. When multiple sites are being contrasted, a single PRC diagram can be used to show the differences between sites in time (Fig. 3), but if more detail is needed, a second PRC diagram can be added to the interpretation (Fig. 3b). The latter, however, complicates the interpretation (Fig. 4) and should only be done when the added detail is of key importance. Our example shows that the most dominant difference between the Grave and Kampen sites is constant over time (Fig. 3a), but if more detail is added by adding the second PRC, the differences increase in time for some species (*D. villosus* and *C. curvispinum*), while decreasing for other species (*C. flavidus*).

Often biomonitoring data sets do not only comprise of biological data but also of environmental data, like physico-chemical data, land-use data etc. Trends and relationships between the biological and environmental data set can be displayed together in a triplot, showing samples, species as well as the environmental variables. One can imagine that, when interested in relationships between biology and

environmental variables and their changes in time, these triplots get very complex and only showing a part of the variation of interest. A possibility to obtain a clearer overview is to perform separate PRC analyses on the biological and environmental data sets. In this way dynamics of the differences between the sites with respect to both the biological communities as the environmental factors are displayed in separate diagrams and their relationships can easily be inferred.

PCA and related methods, like PRC, are often criticised because of the linearity of the model underlying the methods (McCune and Grace 2002). PRC, however, shows that non-linear increases and decreases can both be shown in time, especially when the second PRC is also taken into consideration (Figs. 2, 3 and 4). This is a consequence of the nonparametric nature of the predictor variable set (all dummy variables coding for 'time \times impact'). The implied ANOVA model puts no constraint on the form of the principal response curves. The linearity is still there; the first axis is a linear combination of species log-abundance values. This is suitable for short-gradient situations (Ter Braak and Prentice 1988), where the short gradient is in the 'impact+ impact \times time' space. If the initial differences between sites are not very large, the gradients are by definition short, and PRC is likely to be a useful method to highlight site differences in time. We do not recommend PRC for "large-scale studies" with large initial differences among sites. The gradient in terms of the main effect of time is not at all restricted by linearity and can be of any complexity.

Although PRC has been used to analyse biomonitoring programmes aimed to study the field effects of pesticides (Schäfers et al. 2006; Leonard et al. 2000), we have not yet seen examples outside of this research field. We, therefore, end by discussing a few papers found in the open literature that deal with data sets for which PRC analysis could have been beneficial. Batzer et al. (2005) evaluated the effects of timber harvest on aquatic macroinvertebrates and used NMS for their multivariate analysis. Since their monitoring programme comprised multiple treatments (complete clear-cut, past retention and undisturbed reference) which were sampled in time (4 years), this data set would have been perfectly suited to PRC evaluation. In the paper, the authors present 6 NMS diagrams to show the effects of the timber harvest on

different aquatic macroinvertebrate families. If PRC had been used, this would not only have resulted in an easier-to-interpret diagram (time is a single direction), but the species responding to the treatments would also have been identified much more easily (one evaluation of the whole data set would suffice). A similar data set was gathered by Voelz et al. (2005) to evaluate the effects of urban land use on aquatic macroinvertebrates. These authors evaluated the data using all kinds of indices and quotients. Two rivers were sampled in multiple years at one site upstream and three sites within and downstream of urban areas. Since their aim was to compare these time series with regional references and single reference sites in the same rivers, this data set is ideal for an analysis using PRC. A much clearer overview of the effects of urban land use would have been obtained in comparison to the information provided by all the indices. It is also questionable whether these indices are suitable and able to detect biological impairment (Cao and Hawkins 2005). Stainbrook et al. (2006) evaluated the long-term ecological change in two rivers using fish data spanning 65 years (1936–2001) and macroinvertebrate data spanning 13 years (1988–2001). The data were evaluated using indices of biological integrity, but could also have been analysed by PRC, analogous to the example shown in Fig. 2, although some problems associated with missing data and inconsistency in sampling and identification procedures would need to be overcome. The same type of analysis could be applied to the macroinvertebrate and physico-chemical data set presented by Chambers et al. (2006), who evaluated the adoption of policies to reduce pollution in the former German Democratic Republic, following the re-unification of Germany in 1990, in terms of nutrient-related physico-chemical parameters and macroinvertebrate communities in a small rural watershed. The above list of examples is by no means exhaustive; these are just some examples which would have been suitable for PRC, based on a very limited review of the recent literature.

The present study demonstrates that PRC and related methods are powerful tools to analyse complex macroinvertebrate data sets and to detect major trends. For ecologists, PRC analysis offers a technique that supports an objective evaluation of their observations. The PRC diagrams also provide meaningful information on the species which contribute to these trends. Combining this with knowledge about the ecological

requirements of these species will provide decision-makers with a diagnostic tool for the ecological functioning of their water systems, e.g. as required by the EU Water Framework Directive.

References

- Anonymous (2000). Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy. *Official Journal of EU L*, 327, 1–73.
- Bailey, R. C., Norris, R. H., & Reynoldson, T. B. (2004). *Bioassessment of freshwater ecosystems using the reference condition approach*. Boston, USA: Kluwer.
- Batzer, D. P., George, B. M., & Braccia, A. (2005). Aquatic invertebrate responses to timber harvest in a bottomland hardwood wetland in South Carolina. *Forest Science*, 51, 284–291.
- Bij de Vaate, A., Breukel, R., & Van der Velde, G. (2006). Long-term developments in ecological rehabilitation of the main distributaries in the Rhine delta: fish and macroinvertebrates. *Hydrobiologia*, 565, 229–242.
- Bij de Vaate, A., Grejdanvus, K. M., & Smit, H. (1992). Densities and biomass of zebra mussels in the Dutch part of the Lower Rhine. In D. Neumann & H. A. Jenner (Eds.), *The zebra mussel, Dreissena polymorpha. Ecology, biological monitoring and first application in water quality management* (pp. 67–77). Stuttgart, Germany: Limnologie aktuell 4. Gustav Fischer Verlag.
- Bij de Vaate, A., Jazdzewski, K., Ketelaars, H., Gollasch, S., & Van der Velde, G. (2002). Geographical patterns in range extension of macroinvertebrate Ponto-Caspian species in Europe. *Canadian Journal of Fisheries and Aquatic Sciences*, 59, 1159–1174.
- Bij de Vaate, A., & Klink, A. G. (1995). *Dikergammarus villosus* Sowinsky (Crustacea: Gammaridae) a new immigrant in the Dutch part of the Lower Rhine. *Lauterbornia*, 20, 51–54.
- Borcard, D., Legendre, P., & Drapeau, P. (1992). Partialling out the spatial component of ecological variation. *Ecology*, 73, 1045–1055.
- Cairns, J. Jr. (1982). *Artificial substrates*. Ann Arbor, USA: Ann Arbor Science Publishers.
- Cao, Y., & Hawkins, C. P. (2005). Simulating biological impairment to evaluate the accuracy of ecological indicators. *Journal of Applied Ecology*, 42, 954–965.
- Chambers, P. A., Meissner, R., Wrona, F. J., Rupp, H., Guhr, H., Seeger, J., et al. (2006). Changes in nutrient loading in an agricultural watershed and its effects on water quality and stream biota. *Hydrobiologia*, 556, 399–415.
- De Pauw, N., Lambert, V., Van Kenhove, A., & Bij de Vaate, A. (1994). Comparison of two artificial substrate samplers for macroinvertebrates in biological monitoring of large and deep rivers and canals in Belgium and The Netherlands. *J. Env. Mon. Ass.*, 30, 25–47.
- Dick, J. T. A. (1996). Post-invasion amphipod communities of Lough Neagh, Northern Ireland: influences of habitat

- selection and mutual predation. *Journal of Animal Ecology*, 65, 756–767.
- Gittenberger, E., Janssen, A. W., Kuijper, W. J., Kuiper, J. G. J., Meijer, T., Van der Velde, G., et al. (1998). *De Nederlandse zoetwatermollusken. Recente en fossiele weekdieren uit zoet en brak water. Nederlandse Fauna Vol 2*. Utrecht, The Netherlands: KNNV Publishers.
- Kersting, K., & Van den Brink, P. J. (1997). Effects of the insecticide Dursban®4E (active ingredient chlorpyrifos) in outdoor experimental ditches: III. Responses of ecosystem metabolism. *Environmental Toxicology and Chemistry*, 16, 251–259.
- Kinzelbach, R. (1997). Aquatische Neozoen in Europa. *Newsletter der Arbeitsgruppe Neozoen*, 1, 7–8.
- Leonard, A. W., Hyne, R. V., Lim, R. P., Pablo, F., & Van den Brink, P. J. (2000). Riverine Endosulfan concentrations in the Namoi River, Australia: link to cotton field runoff and macroinvertebrate population densities. *Environ. Toxicol. Chem.*, 19, 1540–1551.
- Li, Z., & Kafatos, M. (2000). Interannual variability of vegetation in the United States and its relation to El Niño/Southern Oscillation. *Remote Sensing of Environment*, 71, 239–247.
- Marguillier, S., Dehairs, F., Van der Velde, G., Kelleher, B., & Rajagopal, S. (1998). Initial results on the trophic relationships based on *Corophium curvispinum* in the Rhine traced by stable isotopes. In P. H. Nienhuis, R. S. E. W. Leuven, & A. M. J. Ragas (Eds.), *New concepts for sustainable management of river basins* (pp. 171–177). Leiden, The Netherlands: Backhuys Publishers).
- Marteijn, E. C. L., Mulder, W. H., Noordhuis, R., Van Oirschot, M., & Prins, H. (1995). Biological monitoring of large freshwater bodies as integrated part of water management in The Netherlands. (Paper presented at the International workshop Monitoring Tailor-made, Beekbergen, The Netherlands).
- McCune, B., & Grace, J. B. (2002). *Analysis of ecological communities*. Glendon Beach, OR: MjM Software Design.
- Pardal, M. A., Gardosos, P. G., Sousa, J. P., Marques, J. C., & Raffaelli, D. (2004). Assessing environmental quality: a novel approach. *Marine Ecology Progress Series*, 267, 1–8.
- Pashkevich, A., Pavluk, T., & Bij de Vaate, A. (1996). Efficiency of a standardized artificial substrate for biological monitoring of river water quality. *Environmental Monitoring and Assessment*, 40, 143–156.
- Reynoldson, T. B., & Pearce, B. (1979). Predation on snails by three species of triclad and its bearing on the distribution of *Planaria torva* in Britain. *Journal of Zoology*, 189, 459–484.
- Schäfers, C., Hommen, U., Dembinski, M., & Gonzalez-Valero, J. F. (2006). Aquatic macroinvertebrates in the Altes land, an intensely used orchard region in Germany: correlation between community structure and potential for pesticide exposure. *Environmental Toxicology and Chemistry*, 25, 3275–3288.
- Stainbrook, K. M., Limburg, K. E., Daniels, R. A., & Schmidt, R. E. (2006). Long-term changes in ecosystem health of two Hudson Valley watersheds, New York, USA, 1936–2001. *Hydrobiologia*, 571, 313–327.
- Ter Braak, C. J. F. (1995). Ordination. In R. G. H. Jongman, C. J. F. Ter Braak, & O. F. R. Van Tongeren (Eds.), *Data analysis in community and landscape ecology* (pp. 91–173). Cambridge, UK: Cambridge University Press.
- Ter Braak, C. J. F., & Prentice, I. C. (1988). A theory of gradient analysis. *Advances in Ecological Research*, 18, 271–317.
- Ter Braak, C. J. F., & Šmilauer, P. (2002). *CANOCO reference manual and canodraw for windows user's guide: software for canonical community ordination (version 4.5)*. Ithaca, NY: Microcomputer Power.
- Van den Brink, P. J., Hattink, J., Bransen, F., Van Donk, E., & Brock, T. C. M. (2000). Impact of the fungicide carbendazim in freshwater microcosms. II. Zooplankton, primary producers and final conclusions. *Aquatic Toxicology*, 48, 251–264.
- Van den Brink, P. J., & Ter Braak, C. J. F. (1998). Multivariate analysis of stress in experimental ecosystems by Principal Response Curves and similarity analysis. *Aquatic Ecology*, 32, 161–178.
- Van den Brink, P. J., & Ter Braak, C. J. F. (1999). Principal response curves: Analysis of time dependent multivariate responses of a biological community to stress. *Environmental Toxicology and Chemistry*, 18, 138–148.
- Van den Brink, P. J., Van den Brink, N. W., & Ter Braak, C. J. F. (2003). Multivariate analysis of ecotoxicological data using ordination: Demonstrations of utility on the basis of various examples. *Australasian Journal of Ecotoxicology*, 9, 141–156.
- Van den Brink, F. W. B., Van der Velde, G., & Bij de Vaate, A. (1989). A note on the immigration of *Corophium curvispinum* Sars, 1895 (Crustacea: Amphipoda) into The Netherlands via the River Rhine. *Bulletin Zoologisch Museum Universiteit van Amsterdam*, 11, 211–213.
- Van den Brink, F. W. B., Van der Velde, G., & Bij de Vaate, A. (1991). Amphipod invasion on the Rhine. *Nature*, 352, 576.
- Van der Velde, G., Nagelkerken, I., Rajagopal, S., & Bij de Vaate, A. (2002). Invasions by alien species in inland freshwater bodies in Western Europe: the Rhine delta. In E. Leppäkoski, S. Gollasch, & S. Olenin (Eds.), *Aquatic invasive species of Europe. Distribution, impacts and management* (pp. 360–372). Dordrecht, The Netherlands: Kluwer.
- Van der Velde, G., Rajagopal, S., Van den Brink, F. W. B., Kelleher, B., Paffen, B. G. P., Kempers, A. J., et al. (1998). Ecological impact of an exotic amphipod invasion in the River Rhine. In P. H. Nienhuis, R. S. E. W. Leuven, & A. M. J. Ragas (Eds.), *New concepts for sustainable management of river basins* (pp. 159–169). Leiden, The Netherlands: Backhuys Publishers.
- Voelz, N. J., Zuellig, R. E., Shieh, S.-H., & Ward, J. V. (2005). The effects of urban areas on benthic macroinvertebrates in two Colorado plain rivers. *Environmental Monitoring and Assessment*, 101, 175–202.