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The performance of three ordination methods applied to demersal fish data sets: stability and interpretability

L. M. MANJARRÉS-MARTÍNEZ

Laboratorio de Investigaciones Pesqueras Tropicales -LIPET, Universidad del Magdalena, Santa Marta, Colombia

J. C. GUTIÉRREZ-ESTRADA

Dpto. Ciencias Agroforestales, Campus de La Rabida, Universidad de Huelva, Huelva, Spain

J. A. HERNANDO & M. C. SORIGUER

Dpto. Biología, Facultad de Ciencias del Mar y Ambientales, Universidad de Cádiz, Puerto Real, Cádiz, Spain

Abstract The performance of robust principal component analysis (RPCA), detrended correspondence analysis (DCA) and non-metric multidimensional scaling (NMDS) with two demersal fish data sets were assessed in terms of their stability to bootstrap-generated sample variation and the method's ability to reflect a well-known depth gradient. Stability was assessed for both species and site orderings and configurations, using scaled rank variance (SRV) and Spearman (ρ) and Procrustes correlations (t_0). The NMDS site and species orderings showed the highest stability. DCA performed best in terms of site ordination stability, but NMDS performed best in terms of species ordination stability. In terms of matching the expected ecological variation, NMDS was the most sensitive method for the wider-depth gradient data and DCA was the most sensitive for the narrower-depth gradient data. According to the sensitivity and informative power criteria associated with the stability assessment, t_0 was the best methodological approach for site ordinations, and SRV for species ordinations.

KEYWORDS: assemblage, detrended correspondence analysis, non-metric multidimensional scaling, ordination, robust principal component analysis.

Introduction

Multivariate methods comprise a family of powerful tools for analysing large numbers of samples collected in surveys with different objectives: (1) to arrange samples along one or more environmental gradients (Bakus 2007; Ruokolainen & Salo 2006) to obtain a more interpretable view of the patterns of sampling units (e.g. sites) or assemblages (e.g. species) that would otherwise be too complex to understand (Pillar 1999; McGarigal *et al.* 2000; McCune *et al.* 2002); (2) to identify the main underlying environmental gradients that structure the data (Kenkel & Orlóci 1986; Kodama *et al.* 2002; Ruokolainen & Salo 2006) or reveal species–environment relationships (Cao *et al.* 2002); (3) to assess fisheries-related human impacts on assemblages (e.g. Cao *et al.* 2002); and (4) to reduce data dimensionality to obtain a parsimonious representation of individuals in a low dimensional space (Kenkel & Orlóci 1986; Gamito & Raffaelli 1992).

Multivariate methods for obtaining both direct and indirect ordinations have been used previously in community ecology. Direct gradient analysis has been used to determine how sample units or species are distributed in an n-dimensional space generated by environmental factors (McCune *et al.* 2002). By

Correspondence: Luis M. Manjarrés-Martínez, Centro Andaluz de Ciencia y Tecnología Marinas (CACYTMAR), Campus Universitario de Puerto Real, 11510 Puerto Real, Cádiz, Spain (e-mail: luis.manjarres@uca.es)

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contrast, indirect gradient analysis is based on patterns of covariation and association among species (Knox & Peet 1989; McCune *et al*. 2002). A group of these multivariate techniques for indirect ordination involves the Eigen analysis of a sum of squares and cross-products (SSCP) matrix (Kenkel & Orlóci 1986). The following metric methods are examples of this group: principal component analysis (PCA), principal coordinates analysis (PCoA), metric multidimensional scaling (MDS) and correspondence analysis (CA), which gave rise to detrended correspondence analysis (DCA). A recent version of PCA known as robust PCA (RPCA) was developed for coping with outliers (Croux *et al*. 2007). A different approach is known as non-metric multidimensional scaling (NMDS). Non-metric means that configurations are based on the rankings of distances. Therefore, NMDS derives a configuration in which the distances between all pairs of sample points are, as far as possible, in rank order agreement with their compositional dissimilarities (Minchin 1987), which makes this method well suited to non-normal data (McCune *et al*. 2002; Bakus 2007). Metric methods (e.g. PCA, CA and DCA) use distances that are proportional to the dissimilarities (Minchin 1987); therefore, DCA uses the Chi-Squared distance metric, whereas PCA uses the Euclidian one.

It is not possible to draw a clear conclusion from the studies that compare different metric Eigen value techniques. Under most conditions, DCA (Hill & Gauch 1980; McGarigal *et al*. 2000) has been found to be superior to PCA, although it has been suggested that this superiority could be attributed to differences in data standardisation that may lead to an undue emphasis on outliers (Hill & Gauch 1980). A similar conclusion arises from the studies that compare NMDS with metric Eigen value methods. Hill and Gauch (1980) reported that DCA ecological ordinations are more interpretable and successful than NMDS ordinations. These authors also found that NMDS does not ordinate species well, and that this method has only a marginal advantage over CA for sample ordinations. Bakus (2007) stated that DCA gives a slightly more realistic portrayal of intertidal community structure than NMDS, but recognised that this may not be the case in other communities. By contrast, Minchin (1987) considered NMDS applied with the Bray–Curtis dissimilarity coefficient to be the most robust and effective of the compared methods (PCA, PCoA, DCA and NMDS). Gauch *et al*. (1981), with vegetation data, also found NMDS to give 'better' results than CA and DCA, although this depended on the data set analysed (Gauch *et al*. 1981). In general, results from comparative studies depend strongly on the type of data set, gradient length, sampling pattern, data pre-treatment methods and distances or similarity measures.

Ordination methods have traditionally been used to detect spatial or temporal differences among demersal fish assemblages (e.g. Fariña *et al*. 1997; Kodama *et al*. 2002; Sousa *et al*. 2005; Bergstad *et al*. 2008), but the specific methods applied in each case are not based on previous assessments of the most appropriate technique for detecting these differences (Hurst 2005; Hurst *et al*. 2008). It is to be expected that different methods applied to species-station matrices of demersal fish assemblages will give different results. Consequently, the strengths and weaknesses of each method need to be considered before deciding which of the available methods best fits the collected data (Ruokolainen & Salo 2006; Bakus 2007). An assessment of the stability of ordination methods to sampling variation is important considering that interpretations may be misleading if they are based on dimensions that depict unstable variation trends that would not reappear in the analysis of other samples from the same sampling universe (Pillar 1999). This would influence the ability of multivariate analysis to detect and quantify ecological changes and patterns (Cao *et al*. 2002). Furthermore, the interpretability of the resulting ordinations in terms of underlying environmental gradients must also be a criterion for a comprehensive performance analysis, especially when working with field data and not simulated data.

This study aims to assess the performance of RPCA, DCA and NMDS with demersal fish survey data sets in terms of the stability to sample variation generated through bootstrap resampling and the extent to which the well-known depth gradient is reflected in the output of these multivariate methods. Thus, the work includes one non-metric method (NMDS) and two metric Eigenvalue methods with different approaches to the linearity assumption. Principal component analysis assumes a linear relationship between taxa and the ecological space determined by the underlying environmental gradients (McCune *et al*. 2002), whereas DCA does not make assumptions about the distribution of sample units and species in an environmental space (Bakus 2007).

Material and methods

Data source

Data on catch composition as well as fishing time and power by haul were collected in two bottom trawl research cruises conducted in the Colombian Carib-

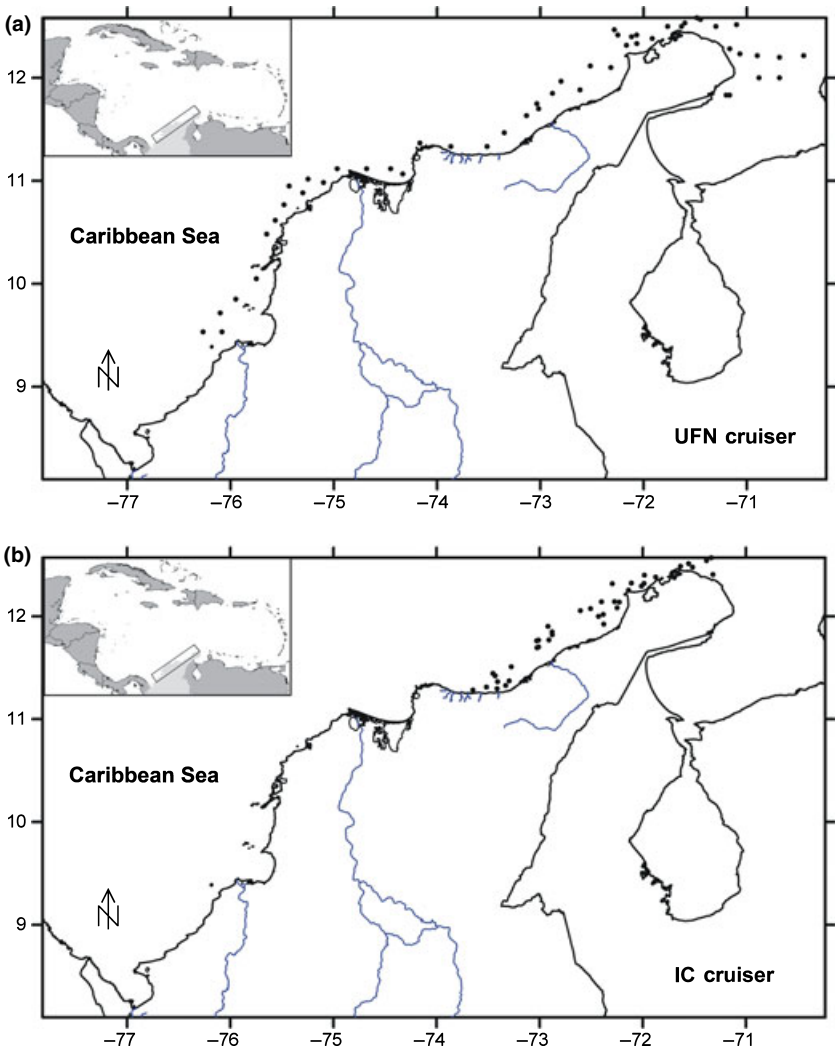

Figure 1. Spatial distribution of the fishing stations (sampling units) carried out on the (a) UFN and (b) IC cruises.

bean Sea (CCS) (Fig. 1): (1) The UNDP-FAO-NORAD 199806 (UFN), carried out from 15 to 21 June 1988 between Castilletes (11°51′0.1″ N, 71°19′60″ W) and Punta Arenas (8°37′24″ N, 76°52′55″ W), from 11 to 455 m depth, surveyed with 51 hauls (Strømme & Saetersdal 1989); and (2) the INPA-COLCIENCIAS 200112 (IC), carried out from 19 November to 7 December 2001, covering only the northern zone of the CCS, between Punta Gallinas (12°27′32″ N; 71°40′04″ W) and Palomino (11°15′12″ N, 73°44′15″ W) and from 10 to 88 m depth, with 39 hauls. Raw data from the two cruises were taken from the SIEEP database system (Duarte *et al*. 2005; Duarte & Cuello 2006). The UFN cruise catches were taken with a 31-m headrope bottom trawl (Strømme & Saetersdal 1989), and the IC cruise catches were made with a 20.5-m headrope bottom trawl (Garcia *et al*. 1998). As a result of differences in trawl size and towing velocity and duration, catches were standardised by calculating biomass densities (kg km^{-2}), i.e. by dividing the catch weight (kg) for each haul by the respective swept area a_i (km^2), which in turn was calculated by $a_\mathrm{i} = TV_\mathrm{i} \times t_\mathrm{i} \times W_\mathrm{i} \times 1852 \times 10^{-6}$ (King 2007), where, for each haul, TV_i is the towing velocity in knots, t_i is the duration of the tow in hours, W_i is the effective width of the trawl in metres and 1.852×10^{-6} is the conversion factor for expressing the swept area in squared kilometres.

As a consequence of the time between the two cruises, valid scientific names were standardised using the taxonomic database Fishbase (Froese & Pauly 2009) and FAO catalogues (Carpenter 2002). Only fish

species classified in Fishbase as demersal, bathydemersal or benthopelagic (Froese & Pauly 2009) were included in this study, as in the approach adopted in numerous previous works on demersal assemblages (e.g. Bianchi *et al.* 2000; Colloca *et al.* 2007; Massutí & Reñones 2005; García *et al.* 2007). Rare species were also removed from the matrix to avoid their strong distorting effect and obtain interpretable species ordinations (e.g. Hill & Gauch 1980; Clarke & Warwick 2001). Specifically, all species that never constituted more than 10% of the total biomass of any sample were removed according to the general approach suggested by Field *et al.* (1982), which has been used in several previous works (e.g. Manjarrés *et al.* 2001; Duffy-Anderson *et al.* 2006). A matrix of 50 species by 51 sites from the UFN data and a matrix of 38 species by 39 sites from the IC data was obtained. Downweighting of abundant species was used to obtain a more balanced picture of the sample ordinations, and the density biomass values were log-transformed, log $(X + 1)$ (Clarke & Warwick 2001; Kallianiotis *et al.* 2004; Bergstad *et al.* 2008). For the species ordination, the data matrix was standardised instead of log transformed (Clarke & Warwick 2001) to make the maximum use of the quantitative information on all species (Hill & Gauch 1980). This standardisation consisted in dividing each entry by its row (species) total and multiplying by 100, as recommended by Clarke and Warwick (2001). Taxon richness and Shannon diversity, as well as Bray–Curtis similarity were calculated for the two species-selected, log-transformed data sets using the program PRIMER version 6 (Clarke & Gorley 2006). The Shannon diversity was calculated based on biomass units (Wilhm 1968). The coefficient of variation in similarities was intuitively used as a measure of sample heterogeneity (Cao *et al.* 2002).

Statistical analysis

All the statistical analyses and graphical outputs were performed with programs written in the R software environment, version 2.10.1 (R Development Core Team 2009). Initially, a *t*-test was run to compare the mean richness of the two data sets, assuming homogeneous variances, as shown by an *F*-test. As a result of non-normal distribution, the median Shannon diversity indices of the two data sets were compared using the Mann–Whitney test ($\alpha = 0.05$). Robust principal component analysis was computed using the projection-pursuit-based GRID algorithm developed by Croux *et al.* (2007) and implemented in the function PACgrid of the R package pcaPP (Filzmoser *et al.* 2009). The DCA was run from the DECORANA command in the R package VEGAN (Oksanen *et al.* 2009). Non-metric multidimensional scaling ordinations were carried out using the isoMDS command in the R statistical software package MASS (Venables & Ripley 2002). Similarity matrices for NMDS were obtained by applying the Bray–Curtis coefficient (Clarke & Warwick 2001). The comparative analysis was based on two criteria. The first criterion was the robustness to the effect of bootstrap-generated sampling variation, which was tested by assessing the stability of both site and species orderings and configurations. The second criterion was the method's capacity to show specified types of expected ecological variation, based on the depth gradient that underlies the structure of the demersal fish data sets.

Bootstrap replicate solutions ($n = 1000$) were generated from the original $N \times P$ data (Knox & Peet 1989; Efron & Tibshirani 1993). Procrustes rotation was applied to make the bootstrap assessment of stability insensitive to reversals in direction and axis order, which are two well-known features of the axes of Eigen-analysis ordinations (Knox & Peet 1989; Pillar 1999). Target configurations were then determined with the scores on the first three axes produced by the different ordination methods applied to the original data set (X). As sampling has replacement, matrix X holds the scores of the sampling units that are in the bootstrap sample, but extracted from the reference scores (Knox & Peet 1989; Pillar 1999). The Procrustes rotation was implemented with the function PROCRUSTES from the R package VEGAN (Oksanen *et al.* 2009).

The overall stability of the first three original ordination axes (reference axes) was assessed with three approaches. The first used the function PROTEST of the package VEGAN to calculate a correlation-like statistic (t_0) derived from the symmetric Procrustes sum of squared differences (SS) between the original data set (X) and the Procrustes-rotated configuration of each bootstrap-generated data set (Y_{rot}) as $t_0 = \sqrt{(1 - \text{SS})}$ (Oksanen *et al.* 2009).

The second approach was to calculate Spearman's rank correlations (ρ) between Procrustes-rotated scores along bootstrap axes (X^*) and scores on reference axes (X), for both the stations (Knox & Peet 1989; Pillar 1999) and species, for which the respective P-values (p_ρ) were also obtained. The third approach was to calculate a coefficient termed scaled rank variance (SRV) to compare the stability in species orderings across axes (Knox & Peet 1989). The variance in rank was computed for each species from the Procrustes rotated scores for species occurring in

all the bootstrap samples, and then the variances were averaged across the n species for each axis. This mean variance was scaled to range from 0 to 1 using SRV = observed variance in rank/expected variance in rank, where expected variance in rank = $(n^2 - 1)/12$, and n is the number of items. SRV values near zero indicate very consistent species rankings, whereas values near 1.0 indicate that species ranks vary as much as random ranks (Knox & Peet 1989).

Distributions of t_0 and ρ generated from the 1000 bootstrap replicates (hereafter replicate axes) were depicted in box-and-whisker plots for each combination method-axis. As correlations were calculated in relation to each reference axis, these results could be interpreted in terms of accuracy. For the purposes of this study, accuracy (A) was referenced with the $Q1$–$Q3$ ranges, i.e. by the central 50% of the data (IQR). Thus, higher correlations indicate higher accuracy. The precision of the correlations was assessed with graphical information provided by the length of the whiskers and also the median coefficient of variation (VMe), which is a measure of the reproducibility, or closeness in value, of repeated measurements.

Several studies that use different methodological strategies show that demersal fish assemblages of the CCS are strongly associated with depth (Fariña *et al*. 1997; Garcia *et al*. 1998; Manjarrés *et al*. 2001; Labropoulou & Papaconstantinou 2004; Sousa *et al*. 2005; Massutí & Reñones 2005; Catalán *et al*. 2006). In this way, the three ordination methods are compared based on their ability to show the expected depth gradient for the following five depth strata: 10–30 m, 31–50 m, 51–100 m, 101–200 m, and >200 m. For most of the CCS, the first three strata correspond to the shelf proper (inner, middle and outer shelf, respectively), and the last two strata, to the slope (upper and intermediate slope).

The results from this approach were analysed using two strategies: (1) visually examining the degree of correspondence between the relative positions of the sampling sites in the two-dimensional depth strata (McGarigal *et al*. 2000); and (2) using Welch's (1951), randomised version of the one-factor analysis of variance, to test the differences between depth strata in score means on axes 1 and 2 separately. When Welch's test was significant, *post-hoc* pairwise multiple comparisons between depth strata were performed using the Dunnet–Tukey–Kramer (DTK) test adjusted for unequal variances and unequal sample sizes, as implemented in the R package DTK (Lau 2009). The significance of the observed F-value from Levene's test, the P-value of Welch's test and mean differences from the DTK test were all tested by randomisation ($n = 5000$).

Results

Ecological-based comparison of data sets

The UFN data showed a slightly higher taxon richness than the IC data, but no significant differences ($P > 0.05$) were found between the two means. Shannon diversity was also higher for the UFN data, and the differences in relation to the IC data ($P < 0.05$) were significant. The respective coefficients of variation of these two indices were similar for the two data sets. Although the two data sets showed very similar mean Bray–Curtis similarities, the variability of the pairwise similarities was much higher for the UFN data set (Table 1).

Site score correlation

The correlation values for the site scores showed several differences in score accuracy between the three methods on each axis (Fig. 2). The two data sets resulted in different patterns on the first axis: for the UFN data (Fig. 2a), the accuracy tended to decrease from PCA to NMDS, while the opposite occurred for the IC data (Fig. 2b). By contrast, no differences between data sets were found for the next axes. The accuracy tended to increase from PCA to NMDS on the second axis. The accuracy of PCA and NMDS was higher than that of DCA on the third axis for both data sets. In short, on axis 1 RPCA obtained the highest score accuracy for the UFN data, and NMDS obtained the highest for the IC data. However, on axes 2 and 3, NMDS obtained the maximum accuracy for both data sets except on axis 3 with the IC data, where the score accuracy of RPCA was comparable to that of NMDS.

The comparison between methods on each axis showed that the score precision of the two data sets was not homogeneous (Fig. 3). On axis 1, RPCA

Table 1. Summary of ecological indices of the two input data sets used to investigate the performance of the three ordination methods (after elimination of rare species and log transformation)

	Taxon richness			Shannon diversity			Bray–Curtis similarity	
Data set	Total	Mean	CV (%)	Mean	Median	CV (%)	Mean	CV (%)
UFN	50	8.71	35.1	1.54	1.64	27.9	24.36	81.2
IC	38	7.85	35.9	1.37	1.43	31.4	25.10	68.4

CV, coefficient of variation; IC, INPA-COLCIENCIAS 200112; UFN, UNDP-FAO-NORAD 199806.

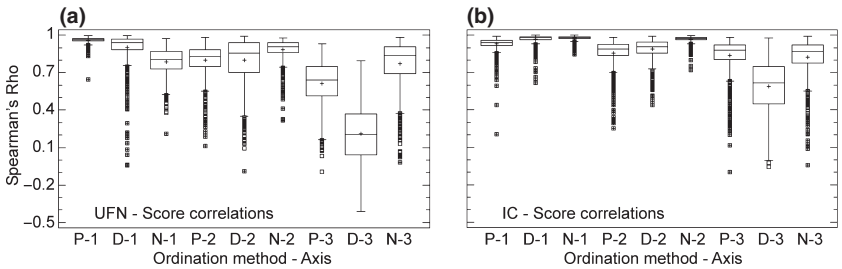

Figure 2. Comparison of distributions of Spearman's correlation coefficient (ρ) between site scores on the axes of the ordinations generated from original UFN (a) and IC (b) data sets and site scores on Procrustes-rotated axes of the ordinations generated from 1000 bootstrap replicates of these data sets, using the three first axes. Each box contains the central 50% of the data (interquartile range IQR). The centre-line indicates the median and the plus sign the location of the mean. Squares represent data below 1.5 IQR (outliers). Letters P, D and N on *x*-axis legend mean Robust PCA, detrended correspondence analysis and NMDS methods, respectively.

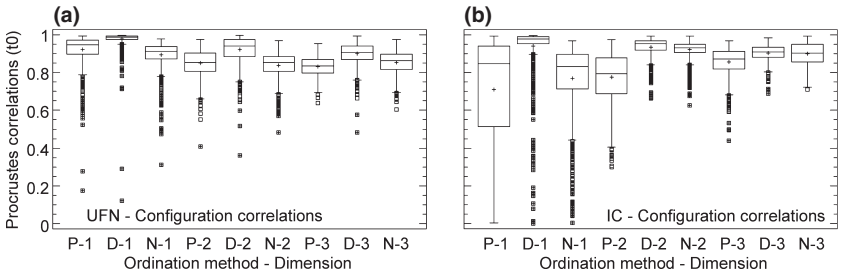

Figure 3. Comparison of distributions of Procrustes correlation (t_0) between original site ordination and each of the site ordinations generated for the 1000 bootstrap replicates of both UFN (a) and IC (b) data sets, using the three first dimensions. Symbols are the same as those used in Figure 2.

produced the highest precision with UFN data, and NMDS showed the highest precision with IC data. On axis 2, NMDS generated less scattered distribution of the correlation values than the other two methods with both data sets. On axis 3, NMDS had the highest score precision with the UFN data, whereas with the IC data, NMDS and RPCA had similar, higher precision levels than DCA (Table 1).

Procrustes correlation of site ordinations

Some differences in the site ordination accuracy patterns emerged when the two data sets were compared with base in t_0. IC data showed more t_0 accuracy heterogeneity than the UFN data (Fig. 3). The largest difference was the lower t_0 accuracy of the method-dimension combinations RPCA-1, NMDS-1 and RPCA-2 with the IC data (Fig. 3b) compared with the UFN data (Fig. 3a). A common feature of the two data sets was that DCA showed higher t_0 accuracy than NMDS and RPCA for the three dimensions.

The comparisons of the ordination precision did not reveal any consistent patterns between methods for each dimension (Table 2). The UFN data set showed higher t_0 precision than the IC data, except in the two-dimensional ordinations of DCA and NMDS. The DCA produced slightly higher t_0 precision levels than the other two methods for both data sets, with the only exception of two-dimensional ordinations with IC

Table 2. Median coefficient of variation (%) of Spearman's correlation coefficient (ρ) for site scores and Procrustes correlation (t_0) for site configurations, calculated between the ordinations generated from original UFN (UNDP-FAO-NORAD 198806) and IC (INPA-COLCIENCIAS 200112) data sets and the ordinations generated from 1000 bootstrap replicates of these data sets. Only the first three axes or dimensions are used

Data set	Ordination method	Score correlations (ρ)			Configuration correlations (t_0)		
		Axis 1	Axis 2	Axis 3	Dim. 1	Dim. 2	Dim. 3
UFN	RPCA	1.46	10.72	21.77	5.13	6.69	4.93
	DCA	6.60	15.99	91.64	1.47	5.43	4.84
	NMDS	10.59	5.93	15.78	4.91	6.00	5.37
IC	RPCA	3.14	7.75	9.80	26.49	12.96	6.49
	DCA	3.43	7.81	28.54	6.43	5.29	5.24
	NMDS	1.08	1.65	11.73	14.92	3.33	5.50

RPCA, robust principal component analysis; DCA, detrended correspondence analysis; NMDS, non-metric multidimensional scaling.

data, for which NMDS showed higher precision than DCA. Robust principal component analysis showed the worst t_0 precision levels with both data sets (Fig. 3, Table 2).

Species score correlation

The species score correlations for the two data sets showed similar accuracy patterns (Fig. 4). The higher score accuracy of NMDS was clear, particularly on axes 2 and 3. Robust principal component analysis showed the lowest score accuracy on all axes. Similar score precision levels were found for the two data sets, except in the NMDS-2 and DCA-3 combinations, which were far higher with the IC data (Table 3). Although precision ranking differences were found between data sets, a common general trend was that NMDS produced the most precise species score distributions and RPCA obtained the least precise species scores (Fig. 4, Table 3).

Table 3. Median coefficient of variation (%) of Spearman's correlation coefficient (ρ) for species scores and Procrustes correlation (t_0) for species configurations, calculated between the ordinations generated from original UFN (UNDP-FAO-NORAD 198806) and IC (INPA-COLCIENCIAS 200112) data sets and the ordinations generated from 1000 bootstrap replicates of these data sets. Only the first three axes or dimensions are used

Data set	Ordination method	Score correlations (ρ)			Configuration correlations (t_0)		
		Axis 1	Axis 2	Axis 3	Dim. 1	Dim. 2	Dim. 3
UFN	RPCA	36.23	35.92	34.16	68.80	28.54	19.34
	DCA	10.28	38.29	64.77	8.98	10.21	9.68
	NMDS	8.31	21.73	15.12	6.34	14.04	9.19
IC	RPCA	40.99	30.44	36.68	71.41	28.05	18.72
	DCA	6.31	34.94	37.09	7.94	9.08	9.21
	NMDS	6.63	10.67	13.47	13.27	11.91	9.36

RPCA, robust principal component analysis; DCA, detrended correspondence analysis; NMDS, non-metric multidimensional scaling.

Procrustes correlation of species ordinations

A similar t_0 pattern emerged for the two data sets based on both species accuracy and precision levels (Fig. 5). The main t_0 accuracy features of this common pattern were as follows: (1) NMDS and to a slightly lesser degree DCA showed higher Procrustes correlation values across most axes; and (2) RPCA showed very low t_0 accuracy levels. Furthermore, the two data sets showed very close t_0 precision values for each method-axis combination (Table 3).

SRV of species orderings

According to the bootstrap SRV criterion, the first three axes of NMDS species ordinations were less variable than those of DCA and RPCA, except for axis 1 with UFN data, for which DCA had a slightly lower SRV value than NMDS (Table 4). Robust principal component analysis had the worst performance on the three axes with both data sets. While RPCA showed similar, higher SRV values on the three axes, DCA and NMDS showed their minimum SRV values on axis 1 and intermediate levels on axis 2, except for NMDS applied to UFN data, for which the SRV value on axis 3 was lower than that on axis 2.

Matching to expected ecological variation

When the three multivariate techniques were performed on UFN data with RPCA (Fig. 6a) the samples from the different depth strata tended to be grouped. The plots for DCA (Fig. 6c) and NMDS (Fig. 6e) were both more sensitive in discriminating the intermediate-slope samples from the other depth strata. Other similarities between the DCA and NMDS

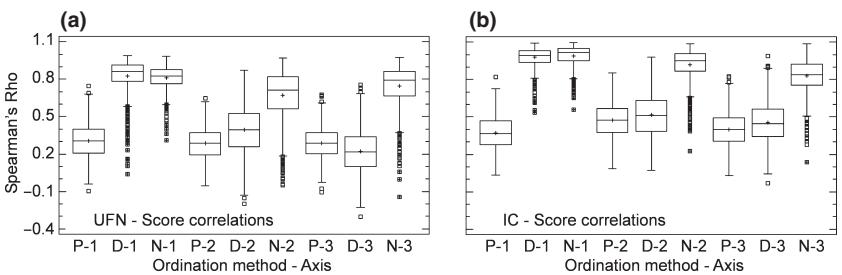

Figure 4. Comparison of distributions of Spearman's correlation coefficient (ρ) between species scores on the axes of the ordinations generated from original UFN (a) and IC (b) data sets and species scores on Procrustes-rotated axes of the ordinations generated from 1000 bootstrap replicates of these data sets, using the three first axes. Symbols are the same as those used in Figure 2.

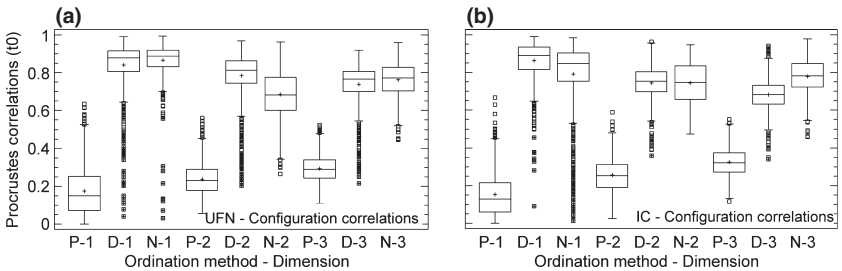

Figure 5. Comparison of distributions of Procrustes correlation (t_0) between original species ordination and each of the species ordinations generated for the 1000 bootstrap replicates of both UFN (a) and IC (b) data sets, using the three first dimensions (c and d). Symbols are the same as those used in Figure 2.

Table 4. Assessment of variability in species rankings through scaled rank variance (SRV) coefficient, after orthogonal Procrustes rotation. SRV are variances in ranks in species orderings from each axis, averaged across species present in all 1000 bootstrap samples, and scaled by the expected variance of a set of *n* random ranks

Data set	Ordination method	Axis 1	Axis 2	Axis 3
UFN	RPCA	0.875	0.875	0.887
	DCA	0.271	0.762	0.879
	NMDS	0.286	0.476	0.373
IC	RPCA	0.901	0.819	0.863
	DCA	0.199	0.783	0.832
	NMDS	0.165	0.290	0.410

UFN, UNDP-FAO-NORAD 199806; IC, INPA-COLCIENCIAS 200112; RPCA, robust principal component analysis; DCA, detrended correspondence analysis; NMDS, non-metric multidimensional scaling.

plots of UFN data were the grouping of the shelf samples, although NMDS produced a more scattered cluster of shelf samples than DCA, as well as the trend of the upper-slope samples to be located in an intermediate area of the plot, like a transition zone between the shelf and the upper slope. With IC data, which only include shelf depth strata samples, the RPCA ordination pattern (Fig. 6b) was also different from those of DCA (Fig. 6d) and NMDS (Fig. 6f). Likewise, DCA (Fig. 6d), and to a lesser extent NMDS (Fig. 6f), tended to display an assemblage composition gradation across the three shelf depth strata.

The Levene's test rejected the homoscedasticity hypothesis for several data set-method-axis combinations (Table 5). The subsequent application of Welch's test under randomisation showed that there were highly significant mean differences ($P < 0.01$) between depth strata for several data set-method-axis combinations (Table 6). With the UFN data set, significant differences were found for all tests involving RPCA and DCA, and for one of the tests involving NMDS: between depth strata on axis 1. With the IC data, the three methods showed identical results: significant mean differences between depth strata only on axis 2.

From the randomised *post-hoc* multiple comparisons implemented only for the significant Welch's tests carried out with UFN data (Table 7), highly significant differences ($P < 0.01$) were found for the following pairwise comparisons on axis 1: (1) for RPCA scores, inner shelf vs outer shelf, mid-shelf vs intermediate slope, and outer shelf vs intermediate slope; (2) for DCA and NMDS scores, intermediate slope vs all the other depth strata. On axis 2, RPCA detected significant mean differences for the comparisons inner shelf vs outer shelf ($P < 0.05$), outer shelf vs intermediate slope ($P < 0.01$), and upper slope vs intermediate slope ($P < 0.05$), whereas DCA only detected significant differences for inner shelf vs mid-shelf ($P < 0.05$). When this type of analysis was based on axis 2 scores generated from IC data (Table 8), a common result of all three ordination methods was the detection of significant differences for the three pairwise comparisons of shelf strata.

Discussion

In general, NMDS perform better than RPCA and much better than DCA in terms of site score stability, which suggests that with NMDS the relative position of the entities along the gradients underlying the main axes is more stable. However, based on the Procrustes correlation criterion, DCA perform slightly better than NMDS and far better than RPCA, which indicates that the site ordinations have a more stable internal structure with DCA. Therefore, for deciding which method is more recommendable for site ordinations, it should be taken into account that the score stability approach provides a measurement of the relative position of the entities (sites or species) on the ordination axes and not of the similarity in the

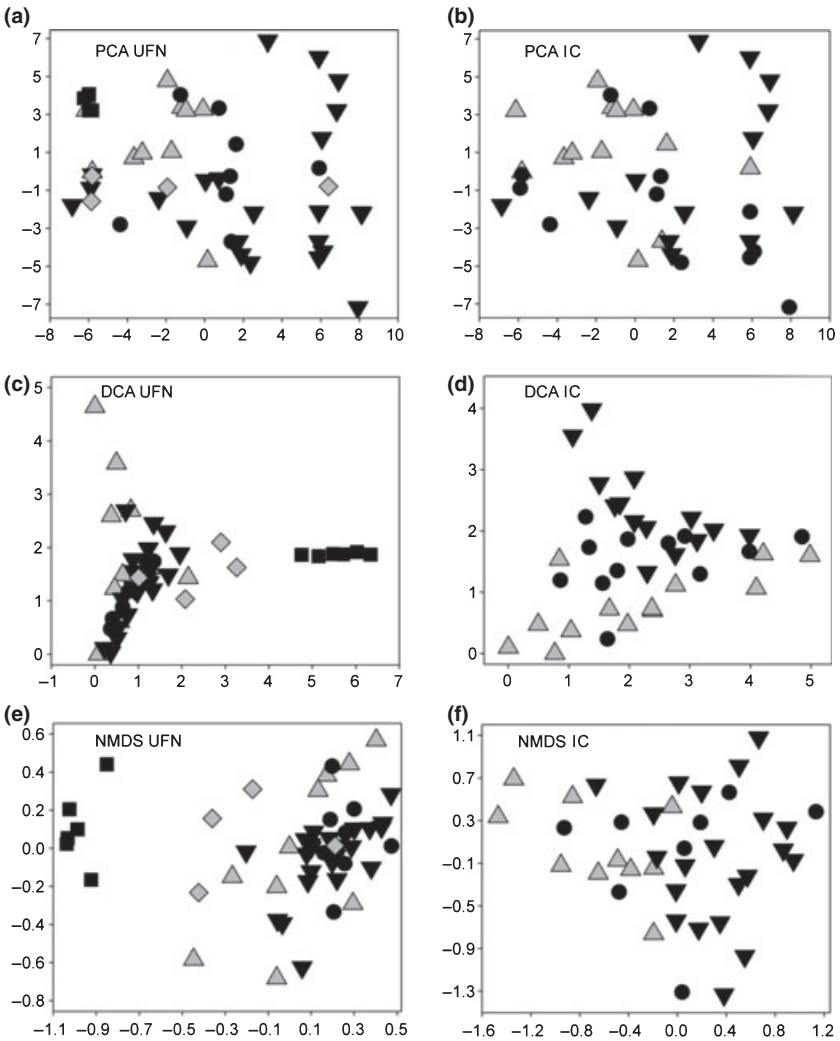

Figure 6. RPCA, DCA and NMDS two-dimensional ordinations by sites for the UFN (UNDP-FAO-NORAD 198806) and IC (INPA-COLCIENCIAS 200112) data sets. Symbol conventions represent the depth strata: gray triangle point up = 10–30 m (inner shelf), black circle = 31–50 m (mid shelf), black triangle point down = 51–100 m (outer shelf), gray diamond = 101–200 m (upper slope), black square = >200 m (intermediate slope).

ecological information conveyed by the plots (Gamito & Raffaelli 1992). This last aspect is measured with the Procrustes correlation (t_0) (Pillar 1999; Oksanen *et al.* 2009). In addition, the site score correlation (ρ) was less sensitive to the method effects than the Procrustes correlation of site ordinations. Therefore, when this last criterion is prioritised, in general DCA yields the most stable site ordinations.

In terms of species stability, the ρ-based results indicate that NMDS is more robust than DCA and RPCA to sampling variation. In relation to t_0-based stability of the species ordination, NMDS and DCA would be appropriate for making spatial or temporal comparisons of species assemblages when sampling variation could be a determinant factor. However, according to the SRV, a third criterion used for comparing the stability of species ordination with different methods (Knox & Peet 1989), the relative species orderings along the axes are more consistently determined by NMDS. Besides showing higher sensitivity for detecting differences between ordination methods, the SRV criterion also provides evidence for comparing the three methods based on the interpretability of the axes. Knox and Peet (1989) suggested that interpretable axes must have SRV values lower than 0.5 (half the variance of random ranks) and, more stringently, first axes should have SRV values lower than 0.33 (a third of the variance of random ranks). According to this criterion, all three NMDS axes for the two data sets can be considered interpretable axes.

Table 5. *P*-values resulting from the randomization ($n = 5000$) of the Levene's test applied for assessing the homoscedasticity hypothesis for depth strata, taking as response variable the site scores yielded by the three ordination methods for the two data sets. Significant differences are indicated as (*) $P < 0.05$; (**) $P < 0.01$

Data set	Ordination method	Axis 1	Axis 2
UFN	RPCA	0.0052**	0.0856
	DCA	0.1210	0.0104*
	NMDS	0.0156*	0.0072**
IC	RPCA	0.4398	0.0010**
	DCA	0.1378	0.6700
	NMDS	0.1438	0.0228*

UFN, UNDP-FAO-NORAD 199806; IC, INPA-COLCIENCIAS 200112; RPCA, robust principal component analysis; DCA, detrended correspondence analysis; NMDS, non-metric multidimensional scaling.

Table 6. *P*-values resulting from the randomization ($n = 5000$) of the Welch test applied for assessing the hypothesis of no mean differences between depth strata, taking as response variable the site scores yielded by the three ordination methods for the two data sets. Significant differences are indicated as (***) $P < 0.001$

Data set	Ordination method	Axis 1	Axis 2
UFN	RPCA	0.0004***	0.0002***
	DCA	0.0002***	0.0006***
	NMDS	0.0002***	0.5278
IC	RPCA	0.5018	0.0002***
	DCA	0.9092	0.0002***
	NMDS	0.6392	0.0002***

UFN, UNDP-FAO-NORAD 199806; IC, INPA-COLCIENCIAS 200112; RPCA, robust principal component analysis; DCA, detrended correspondence analysis; NMDS, non-metric multidimensional scaling.

For DCA, only the first would be interpretable, and for RPCA, no axis is interpretable. Thus, both sensitivity and interpretability prioritise SRV as an appropriate approach for assessing and comparing the stability of species ordinations. Therefore, it can be stated that NMDS species ordinations are particularly robust to sampling variation. Based on criteria other than stability to sampling variation, Minchin (1987) found that NMDS performed well for species ordinations, whereas Hill and Gauch (1980) and Palmer (1993) reported that DCA performed well for sample ordinations. Furthermore, Palmer (1993) stated that DCA sample ordinations are generally more robust than DCA variable ordinations.

The methodological approaches used for assessing stability to sampling variation (ρ, t_0 and SRV) did not show the same sensitivity to data set effects. The two data sets differed in the number of samples n, but neither in the relative dimensionality, which is equal to $n - 1$ for both data sets, nor in the mean taxon richness. Given the shorter depth range and the lower diversity and variability in the similarity index of the IC data set, this seems to indicate that any different result from the two data sets would have to be attributed to differences in the underlying gradient length or the between-site heterogeneity in compositional similarity (Knox & Peet 1989; Gamito & Raffaelli 1992; Hurst *et al.* 2008). Considering that all the approaches tended to show similar stability results for the two data sets, it is reasonable to conclude that the application of any of the three approaches is not subject to an ecological-type restriction in terms of the length gradient or diversity, at least within the scope of the ecological differences between the two data sets used in this work.

In terms of matching to expected ecological variation, the 2-D site ordinations were consistent with evidence from the analysis of variance and post-hoc multiple comparisons test of site scores. In general, NMDS and DCA have a more consistent performance than RPCA, as they show a more informative picture of a strong change from the shelf and upper slope to the intermediate slope. Thus, these two methods are more successful in capturing a depth-structured pattern than previous studies have shown in the same area (Garcia *et al.* 1998; Manjarrés *et al.* 2001). However, a data set effect could be visually noted, which influenced the decision on the most sensitive method for showing the gradation in assemblage composition across shelf depth strata. Whereas NMDS tended to differentiate the shelf samples from the UFN data better, DCA was better for the IC data.

These results seem to be related to the differences between the two data sets in the depth gradient, which has been evident in several previous works that relate gradient length to the capacity of different methods to recover underlying gradients. According to Legendre and Legendre (1998), NMDS is an important alternative when data that represent complex ecological gradients are analysed. This is the case for the UFN data, which besides covering a larger area than the IC data, includes a much broader depth gradient. By contrast, in terms of matching to expected ecological variation, DCA performed worst for the data set with the strong underlying gradient (UFN). Most studies agree that DCA, despite the detrending and rescaling processes (Kenkel & Orlóci 1986), tends to evidence compression effects. This is especially true when it is applied to the data set with the widest underlying

Table 7. *P*-values resulting from the randomization ($n = 5000$) of the DTK test applied for the post-hoc pairwise comparison of means, taking as factor the depth stratum and as response variable the site scores yielded by the three ordination methods for the UFN (UNDP-FAO-NORAD 198806) data set. Only methods-axes combinations with significant Welch test *P*-values are included. Significant differences are indicated as (*) $P < 0.05$; (**) $P < 0.01$; (***) $P < 0.001$

Contrasted depth strata		Axis 1			Axis 2	
		RPCA	DCA	NMDS	RPCA	DCA
Inner shelf	Mid shelf	0.1514	0.9002	0.2944	0.3500	0.0210*
	Outer shelf	0.0024**	0.5980	0.4202	0.0236*	0.0524
	Upper slope	0.8316	0.0838	0.3580	0.2162	0.3640
	Intermediate slope	0.1594	0.0002***	0.0002***	0.2384	0.7918
Mid shelf	Outer shelf	0.3072	0.7380	0.6760	0.3428	0.4024
	Upper slope	0.3980	0.1164	0.0846	0.6238	0.3586
	Intermediate slope	0.0064**	0.0002***	0.0002***	0.0506	0.0810
Outer shelf	Upper slope	0.0790	0.1092	0.0948	0.8732	0.6648
	Intermediate slope	0.0002***	0.0002***	0.0002***	0.0010**	0.1774
Upper slope	Intermediate slope	0.1922	0.0024**	0.0036**	0.0380*	0.5564

RPCA, robust principal component analysis; DCA, detrended correspondence analysis; NMDS, non-metric multidimensional scaling.

Table 8. *P*-values resulting from the randomization ($n = 5000$) of the DTK (Dunnet-Tukey-Kramer) test applied for the post-hoc pairwise comparison of means, taking as factor the depth stratum and as response variable the site scores on axis 2 yielded by the three ordination methods for the IC (INPA-COLCIENCIAS 200112) data set. Significant differences are indicated as (*) $P < 0.05$; (**) $P < 0.01$; (***) $P < 0.001$

Contrasted depth strata		RPCA	DCA	NMDS
Inner shelf	Mid shelf	0.0208*	0.0400*	0.0442*
	Outer shelf	0.0002***	0.0002***	0.0002***
Mid shelf	Outer shelf	0.0246*	0.0150*	0.0048**

IC, INPA-COLCIENCIAS 200112; RPCA, robust principal component analysis; DCA, detrended correspondence analysis; NMDS, non-metric multidimensional scaling.

environmental gradient, because this limits the ability to use axis 1 scores as a linear proxy of position along the environmental gradient (McCune *et al.* 2002; Holland 2008). The tendency to compress the scores near the left end along axis 1 was evident in the output of DCA for the UFN data. Although several authors (Hill & Gauch 1980; Gamito & Raffaelli 1992; Bakus 2007) have reported that DCA ordinations are more interpretable than those of NMDS, this seems to depend on the kind of community analysed. DCA was developed to overcome the distortions inherent to CA; however, this method was mainly aimed at one-dimensional gradients (Holland 2008). Simulation studies (Minchin 1987) have shown that there may still be distortion in DCA plots applied to data sets with two underlying gradients.

Another relevant influence of the data set is that the clearest evidence of the effects of depth on the distribution of the CCS species is given by studies conducted at bathymetric scales such as that of the UFN cruise. The scores on the two major axes of both the RPCA and DCA ordinations performed with UFN data showed highly significant effects of the depth gradient, but in NMDS ordinations, this gradient was only depicted in the first axis. A different outcome was derived with the IC data, for which the effect of the depth gradient was only shown on the second axis of the three methods. The IC data ordinations only showing this relationship on their second axes seems to indicate that with a narrower depth gradient, factors other than depth would be more important in determining the assemblage distribution. Although there is ample evidence that demersal species distributions are mainly determined by the depth gradient at regional scales, several studies showed that other environmental factors determine which fish occur in a specific area (Longhurst & Pauly 1987; Kodama *et al.* 2002; Catalán *et al.* 2006; Bergstad *et al.* 2008). This indicates that depth needs to be mapped at a spatial resolution higher than that of the IC data, so that there is substantial spatial heterogeneity in the depth factor to be captured by the first axis.

The results indicate that in terms of matching to expected ecological variation, the performance of RPCA is only comparable to that of DCA and NMDS when there is a short gradient length like that underlying the IC data. The performance of RPCA is unsatisfactory with the UFN data, in which there is considerable environmental heterogeneity, as occurs in tropical habitats in which the beta diversity is high (Bakus 2007). The absence of a clear depth pattern in the RPCA plot seems to be related to the distortions that would be expected on theoretical grounds for

conventional PCA. Studies that compared ordination techniques (Kenkel & Orlóci 1986; Minchin 1987; Knox & Peet 1989; Clarke & Warwick 2001; Ruokolainen & Salo 2006) found that linear constraints of Eigen analysis may restrict the ability of many metric methods to summarise trends related to non-linear and non-monotonic species responses, which are biological traits of these assemblages (McGarigal *et al.* 2000).

In conclusion, the results show that the site ordinations yielded by DCA are the most stable to the effect of sampling variation in demersal fish surveys. NMDS yielded the most stable species ordinations, whatever the underlying gradient length of the data set. By contrast, there is a data set effect when the match to expected ecological variation is assessed: NMDS is a better choice for wider gradient length data sets and DCA for narrower gradient length data sets. Finally, a ranking of methodological approaches for evaluating stability in ordinations based on demersal fish assemblage data sets can be established by combining sensitivity and informative power criteria: Procrustes correlation for site ordinations, and SRV for species ordinations. These conclusions could be extrapolated to community data sets with similar richness, diversity, similarity and proportion of zero data levels, like those often yielded by benthic surveys.

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