Addressing the removal of rare species in multivariate bioassessments: The impact of methodological choices

Mark S. Poos*, Donald A. Jackson

Department of Ecology and Evolutionary Biology, University of Toronto, 25 Harbord Street, Toronto, Ontario, Canada M5S 3G5

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ABSTRACT

Multivariate analyses are important tools for the biological assessment of ecological communities. Despite the popularity of multivariate analyses in bioassessments, there is considerable controversy over how to treat rare species. As this debate remains unresolved, the objective of this study was to develop a methodology to quantify the impacts of removing rare species relative to other decisions inherent in multivariate analyses and to provide insight into their relative influence in our studies. Using fish species from a well-sampled system, we assessed the impact of several choices common to multivariate analyses, including the removal of rare species, ordination technique and measures of multivariate resemblance. Comparisons of multivariate analyses demonstrated the choice of ordination method explained 26% of the variation among the various results, followed by the choices regarding the removal of rare species (24.8%) and resemblance measure (11%). At the same time, the removal of rare species had important site-level impacts relative to full dataset, including a >9 fold change in sites impacted by the removal of single species, with an emphasis on removing species more correlated to anthropogenic stress. Our study demonstrates that the removal of rare species had similar or greater influence in multivariate analyses as other choices inherent in their calculation, such as the choice of ordination method. Better justifications for the removal of rare species, along with all decisions in multivariate analyses, are needed to move bioassessments forward.

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1. Introduction

Multivariate analyses have become important tools in biological assessments of aquatic communities (Norris, 1995; Wright et al., 2000). Multivariate analyses are widely used and have been shown to be accurate and sensitive ways to quantify biological impacts and recoveries (Linke et al., 2005). Several national bioassessment programs are based on multivariate measures, including those in the United Kingdom (e.g., RIPVACS; Wright et al., 2000) Australia (AUSRIVAS; Simpson and Norris, 2000), Canada (Reynoldson et al., 2001), as well as elsewhere (Bailey et al., 2004; Van Sickle et al., 2007). In total, approximately 30% of bioassessment articles use multivariate statistical methods (Dolédec and Stazner, 2010).

The application of multivariate analyses to bioassessments of aquatic communities has been a major point of controversy. Polarized debates remain regarding the use of multivariate metrics versus multivariate methods (Dolédec and Stazner, 2010), the taxonomic resolution needed for species identification (Arscott et al., 2006), and to some extent, the use of specific analytical methods (Marchant et al., 2006; Van Sickle et al., 2007). In particular, the treatment of rare species has received much attention in bioassessment studies (e.g., Faith and Norris, 1989; Norris, 1995; Cao et al., 1998; Marchant, 1999, 2002). On one hand, researchers often remove rare species with the perceived notion that they may add noise to multivariate analyses and provide little additional information beyond more common species (Gauch, 1982; McCune and Grace, 2002). On the other hand, researchers often retain rare species in multivariate analyses because they may be better indicators of ecosystem stress than common species (Faith and Norris, 1989; Cao et al., 1998, 2001), given the assumption that some or all of these rare species may be more sensitive to the stressor(s). In either case, the debate regarding the treatment of rare species has remained unresolved and researchers need to be aware of the impact of their decision of how to treat rare species (among others).

There are many difficulties in attempting to resolve the debate regarding the treatment of rare species in bioassessments and in other types of multivariate studies. For example, most multivariate approaches require several more decisions beyond whether or not to remove rare species, and these may reduce insight into the effect of rare species on resultant analyses. Researchers using multivariate methods typically must choose a type of resemblance measure (i.e., a distance or similarity coefficient) and ordination technique,
where such choices have been shown to significantly alter results (Podani, 2000; Hirst and Jackson, 2007; Poos et al., 2009). As such, the resolution to the debate regarding the impact of rare species cannot proceed until the effect of removing rare species is placed into a context comparable to other decisions inherent in multivariate bioassessments. Unfortunately relatively little effort has gone into comparing these methods of bioassessments (Norris, 1995; Marchant et al., 2006) and few studies have viewed decisions in analyses in a holistic manner (e.g., how do all decisions inherent in multivariate bioassessments impact results and which ones are of greater importance?).

There are two general arguments for the inclusion or exclusion of rare species in multivariate bioassessments. The first argument for removing rare species from bioassessments is that rare species provide limited interpretative value (Marchant, 1999). Proponents of this argument suggest that rare species may simply reflect stochastic sampling effects and therefore add noise rather than information, to the statistical solution (Gauch, 1982; Clarke and Green, 1988; Bailey et al., 2004). We refer to this argument as the statistical impact argument. Support for this argument has come from work in previous decades, which showed results from multivariate methods could be driven by the inclusion of rare species alone (see Cao et al., 2001; Dolédec and Stazner, 2010). To some degree this argument has been examined in the literature with analyses of certain aspects of developing a multivariate bioassessment, like data standardizations (Jackson, 1993a; Cao et al., 1999), resemblance coefficients (Jackson et al., 1989), ordination method (Marchant, 1990), or their combinations (e.g., data standardization and resemblance coefficients; Jackson, 1993a; Hirst and Jackson, 2007; taxonomic resolution and rarity; Arscott et al., 2006). Unfortunately, what has remained largely absent is a quantitative evaluation of the role of rare species in community assessments, including an evaluation which answers the practical question of how important rare species are relative to other decisions in multivariate analyses (but see Faith and Norris, 1989). In this context, the statistical argument can be tested as a hypothesis, with the prediction that differences among analyses should show considerable variation where rare species are removed relative to analyses where other decisions have been altered (e.g., resemblance coefficient, ordination method). Specifically, does the inclusion or exclusion of rare species lead to greater changes in community analyses than those arising due to other decisions (e.g., the well documented differences due to choice of ordination)?

The second argument for the inclusion or exclusion of rare species is that such species should be included in multivariate analyses because they are better indicators of ecosystem stress than are common species (Cao et al., 1999, 2001), i.e., common species tend to have broad ranges of tolerance to many conditions and therefore may not provide as good indicators. We refer to this argument as the biological impact argument. Support for this argument has come from empirical studies which note the importance of including rare species for conservation issues (Cao et al., 1999, 2001). Proponents of this argument suggest that exclusion of rare species may lead to an underestimation of differences between impacted and un-impacted sites (Cao et al., 1999, 2001). As one of the main goals of a bioassessment is to determine site-level impacts (Barbour et al., 1999; Wright et al., 2000), this argument assumes that the loss of rare species represent the loss of the strongest signals of impact, via decreases in species diversity or changes in community composition (Cao et al., 1998). Therefore the biological impact argument can also be tested as a hypothesis, with the prediction that sites where rare species were removed should be more affected across multivariate analyses than sites that had no species removed. If this result is not found, it would indicate that the exclusion of rare species is warranted as they do not provide meaningful information beyond that captured by more common species.

Given these definitions and hypotheses, the objective of our study was to determine the biological and statistical impact of removing rare species relative to other methodological decisions inherent in multivariate analyses (e.g., choice of ordination method and resemblance coefficient). For this evaluation, we use data of fish communities from of the Sydenham River as a model system because it has the highest diversity of aquatic fauna in Canada, as well as the highest number of species at risk in Canada (Poos et al., 2010). The Sydenham River is a 2725 km² watershed, which drains into Lake St. Clair, a connecting waterway of the Laurentian Great Lakes, between Lake Huron and Lake Erie (Poos et al., 2007). Although the human population is small (~100,000 within the watershed), anthropogenic impacts of turbidity remain an important concern, mostly due to land-use activities dominated by agriculture (Poos et al., 2010). The Sydenham River has undergone detailed sampling (Poos et al., 2007, 2008) which provides a high-quality dataset. As there are many types of rarity (Gaston, 1994), we define rare species as those which occur infrequently (i.e., at few locations or low prevalence). As well, we define species that have a conservation designation (e.g., endangered, threatened, special concern), as species at risk.

2. Materials and methods

Fishes were collected from the Sydenham River using the Ontario Stream Assessment Protocol (OMNR, 2007). The Ontario Stream Assessment Protocol represents a typical bioassessment protocol for monitoring impacts to aquatic systems (e.g., Barbour et al., 1999; Wright et al., 2000) and has been used effectively to monitor changes in riverine communities (Poos et al., 2008). We used fish rather than benthic macroinvertebrates as they are relatively easy to identify and enumerate and also have been used extensively in multivariate bioassessments (Bailey et al., 2004; Dolédec and Stazner, 2010); however the approach we propose is equally suited to macroinvertebrates or any other taxonomic group of species.

Fishes were sampled using a variety of approaches (see Poos et al., 2007); however, for this analysis only electrofishing data were used as it is the most commonly used method for the bio-monitoring protocols and regarded as the most effective gear type for sampling stream-fish assemblages and fish species at risk (Bohlin et al., 1989; Reynolds, 1996; Poos et al., 2007). Sampling sites were chosen at random across the entire Sydenham watershed. Species were collected at 50 sites in 2002 and 25 additional sites in 2003.

2.1. Evaluating decisions in multivariate bioassessments

Prior to analysis, four treatments of the removal of species were applied to the site-by-species matrix. Traditionally, researchers arbitrarily decide which characteristics define a rare species within a sample (Faith and Norris, 1989; McCune and Grace, 2002). Some researchers suggest eliminating species which occur at single sites because of the inflated correlations created by attempting to relate potentially random features at that site to its lone occurrence (Legendre and Legendre, 1998). Others suggest removing species that occur at less than five percent (McGarigal et al., 2000) or ten percent of sites (Marchant, 1990; McCune and Grace, 2002) or at even higher thresholds (Marchant et al., 1997). We used the treatments of: (1) the full dataset (All), (2) removing single-occurrence species (M1); (3) removing species found at less than five percent of sites (M5); and, (4) removing species found in less than 10% of sites (M10). These criteria represented 0, 4, 9, and 21 species, respectively of the 67 species dataset.

Resemblance coefficients were calculated from each of the four site-by-species matrices of which rarely sampled species were
removed. Several dozen resemblance coefficients have been developed for use with presence/absence data and their characteristics are well documented (Gower and Legendre, 1986; Legendre and Legendre, 1998; Podani, 2000). Choice of resemblance coefficients has been largely subjective and is often based on tradition or on a posteriori criteria without clear justification (Jackson et al., 1989). Different resemblance coefficients emphasize different aspects of the relation between observations so the exclusion of rare species may alter species relationships and subsequent analyses, such as ordinations (McGarigal et al., 2000). We used Jaccard's (J), phi (Φ), Russell and Rao (RR), and simple matching (SM) coefficients. These resemblance coefficients were chosen because they are commonly used in the literature and/or represent standard examples among the continuum of resemblance coefficients (Legendre and Legendre, 1998). For example, Jaccard's coefficient, which is similar to the Sorensen coefficient (Appendix A), does not consider joint absences; the phi coefficient is the correlation coefficient for binary data, whereas Russell and Rao and simple matching are variations which consider joint absences (Jackson et al., 1989; Legendre and Legendre, 1998; Podani, 2000). All the resemblance coefficients (R) were transformed in metric distances having Euclidean properties by D = √1−R (Gower and Legendre, 1986; Jackson et al., 1989) where R is the value estimated from the resemblance coefficient. This transformation to metric properties is effective in principal co-ordinates analysis (PCoA; Legendre and Legendre, 1998) as it preserves Euclidean space and eliminates potential negative eigenvalues, while at the same time it should have no effect on Non-Metric Multidimensional Scaling (NMDS) solutions due to its monotonicity in the NMDS estimation. We do note that the square-root transformation has the potential to affect large and small similarities to a different degree given it is a non-linear transformation.

Three types of ordination technique were compared for each combination of treatments excluding rare species and resemblance coefficients: PCoA, NMDS, and Correspondence Analysis (CA). These ordination techniques were chosen because they represent typical multivariate methods used by the majority of biologists (Legendre and Legendre, 1998; Podani, 2000; McCune and Grace, 2002). In addition, we chose these ordination methods as they are widely used in general ecological analyses and are popular multivariate methods used in bioassessments (Dolédec and Stazner, 2010). For example, PCoA using the simple matching coefficient is the same as a Principal Components Analysis (PCA) on binary data, another popular ordination technique. PCoA, PCA and CA represent eigenvector methods, which maximize either the linear (e.g., PCoA, PCA) or unimodal (e.g., CA) relationships of multivariate data (Legendre and Legendre, 1998). Alternatively NMDS differs fundamentally in that it can be used with either linear or non-linear data as relationships are fitted using an iterative search which minimizes the stress (i.e., maximizes the rank correlation) of the k-dimensional configuration relative to that based on a given resemblance measure (Legendre and Legendre, 1998). Whereas both NMDS and PCoA allow the user to choose a resemblance coefficient, CA (and PCA) does not provide the same diversity of options given its inherent resemblance coefficient (chi-square distance in CA, correlation/covariance matrix in PCA). Although chi-square distances can be used in other ordination methods (e.g., PCoA, NMDS), it is rarely done with ecological data, and so we include it only with CA (the most common approach).

It is likely that different ordination methods may emphasize rare species differently. For example, CA uses the chi-square metric that implicitly weighs species with the inverse of their commonness in the data set, which when dealing with abundance data will give higher weights to rare species (Legendre and Gallagher, 2001). Some studies have suggested that more currently favoured approaches (e.g., NMDS) may be more robust for dealing with rare species than alternative methods (e.g., PCoA, PCA), especially when non-linear relationships occur between variables (Cao et al., 2001); although other studies find NMDS less robust (Hirst and Jackson, 2007). For NMDS, a random set of 20 starting configurations was used as input configurations, and the solution having the lowest stress was retained. Random configurations were chosen in order to avoid biasing solutions towards those provided by PCoA results (i.e., one of the reasons argued for using NMDS is to avoid the incorrect solutions obtained from PCoA). All analyses were completed using the R programming language v2.7.0 using statistics libraries simba (Jurasiński, 2007), vegan (Oksanen et al., 2008), ecosplit (Goslee and Urban, 2007), MASS (Venables and Ripley, 2011) and labdsv (Roberts, 2010).

2.2. Assessing the statistical impact argument

The statistical impact of key methodological choices in multivariate bioassessments was assessed in several ways. First, all variants in ordination method, resemblance coefficients and exclusion of rare species were compared using Procrustes analysis (Jackson, 1995). Procrustes analysis is appropriate for comparing separate ordination results and was used because it is an orthogonal rotation that best matches two or more ordinations (Peres-Neto and Jackson, 2001; Paol et al., 2006). One advantage of using Procrustes analysis is that scaling/re-scaling or reflection of ordination axes is not required. In Procrustes analysis raw ordination solutions are scaled and rotated in order to find an optimal superimposition that maximizes their fit (Peres-Neto and Jackson, 2001). We did preliminary checks of variance explained by each ordination solution using broken-stick models (Jackson, 1993b; Legendre and Legendre, 1998) and found that ordinations generally were best represented by three-axes solutions, which were retained for further analyses. The sum-of-squared-deviations (i.e., Gower’s m2 statistic; Digby and Kempton, 1987) provide a metric dissimilarity measure evaluating the resemblance of two ordinations to one another, with higher m2 values indicating greater dissimilarity (Jackson, 1993a; Peres-Neto and Jackson, 2001). We calculated m2 distances between each pair of three-dimensional ordination solutions to produce a matrix of m2 distances between all thirty-six exclusion-distance-ordination combinations. The resultant 36-by-36 matrix of m2 distances was analyzed using PCoA to determine the relative effect of each methodological choice. This type of "ordination of ordinations", based on the matrix of m2 distances (see Digby and Kempton, 1987; Jackson, 1993a; Hirst and Jackson, 2007), provides a useful characterization of methodological decisions, where larger distances between objects represent more dissimilar ordination solutions. A minimum spanning tree was used to determine the most similar groups and superimposed onto the first two axes of the summary ordination diagram.

Variation partitioning of multivariate matrices can provide quantitative and objective determination of the influence of methodological choices, such as the removal of rare species. We quantified the variation of all treatments using a partitioning method of multivariate matrices (see Rundle and Jackson, 1996). As the squared deviations of points from the mean represents a measure of dispersion, where more deviant points have larger values, the sum of these distances (i.e., the various m2 statistics) represents a measure of variation. We totaled the sum-of-squared-deviations for within- and among-treatment groups (e.g., removal of rare species, ordination technique, and distance measure), similar to an Analysis of Variance (ANOVA) or variance components. As such, we compared the variation within a treatment (e.g., removal of rare species) to variation among treatments (e.g., distance measure and ordination method) to partition where the variation
is occurring. Although significance testing is possible (via simulation), we did not have strict hypotheses regarding our a priori expectations, therefore we did not develop a corresponding null model (Rundle and Jackson, 1996). Rather we use this approach as a means to quantify the relative effects of different decisions inherent in multivariate bioassessments.

2.3. Assessing the biological impact argument

To assess impact of removing rare species from bioassessments, we compared the sites for which we removed rare species and evaluated any changes across the various multivariate analyses. Site-level differences were calculated for each pair-wise Procrustes analysis using vector residuals (Jackson, 1995). Vector residuals provide a means of investigating deviations in position of individual samples between two superimposed ordinations (Jackson, 1995; Paavola et al., 2006), i.e., the degree to which any given observation changes from one ordination to another, relative to the other observations. The length of the vector residual represents the deviation of the individual observation between two ordinations, with low values indicating close agreement between multivariate methods. We separated vector residuals between sites that had species removed from those sites that did not. For example in Fig. 1, we provide a typical example of site-level vector residuals across a comparison of multivariate analyses between two datasets – the complete (All) data set and the set with species occurring at 5% of sites removed. From this comparison, the biological impact of the removal of rare species can be assessed as the ratio of the mean of site-level vector residuals for sites where rare species were removed (i.e., mean of grey bars; Fig. 1) versus the mean of sites where species were not (i.e., mean of black bars; Fig. 1). Ratios over 1 indicate situations where the impacts of the removal of rare species were not randomly distributed, but related to site-specific differences.

3. Results

The multivariate analyses used in this study generally provided good representation of the data. Variance explained by the first three axes from all combinations of multivariate analyses ranged from 24.58% (PCoA with Jaccard’s distance and all species included) to 38.36% (PCoA using simple matching as a resemblance measure and species occurring at less than 10% sites removed; Appendix B). In all cases, the variance explained by each eigenvector multivariate method (PCoA, PCA, CA) increased with the exclusion of more species, as expected.

For fish species in the Sydenham River, the removal of rare species had similar impacts on resultant analyses to those arising from the choice of ordination type, and had a greater effect than choice of resemblance coefficient (Figs. 2 and 3). In considering the relative role of the various decisions to be made, the partitioning of variation provides a measure summarizing the relative impacts. Sources of variation in the various solutions were highest among ordination methods with 26% of the variation explained across all
comparisons, followed by the removal of rare species (25%) and resemblance coefficient (11%; Fig. 3). These results were also evident by the well-defined clustering of treatments of rarely sampled species (All, M1, M5) in close proximity to one another relative to the clustering of the differences in ordination technique (PCoA, NMDS, CA) or in resemblance coefficients (J, Φ, RR, SM) in the ordination of m² distances, i.e., the comparison of the various ordination results (Fig. 2). One clear exception to this result was the removal of 10% of the least occurring species, which showed deviations from the general multivariate groupings (Fig. 2A and B), and variation that exceeded most other choices (7%; Fig. 3A).

There was large variation among individual choices across multivariate bioassessments. Whereas CA demonstrated overall low amounts of variation among analyses (0.2%), there was large variation among analyses based on NMDS (22%; Fig. 3A) due to greatly differing solutions. Although some of these differences may be influenced by the smaller number of comparisons for CA (as a choice of resemblance measure is implicit and not selected), NMDS showed an almost seven-fold increase in variation over PCoA, which involved same number of comparisons and involved the same set of resemblance measure and treatment of rare species (Fig. 3A). These findings indicate that decisions such as which ordination method to choose may be as important as other choices such as the removal of rare species. In fact, the inclusion or exclusion of rare species did not impact the resulting multivariate analyses any more than the choice of ordination method (i.e., subtotals of 24.8% versus 26.2%, respectively). Further, levels of variation were similar between the removal of single occurrence species (6%) and species occurring at 5% of the sites (5.9%) as they were for using the entire dataset (6%; Fig. 3). Finally, the choice of resemblance coefficient showed lower levels of variation in general. Variation in chi-squared values was lowest (0.2%), followed by simple matching (1.5%), Jaccard (2.0%) and the phi (2.2%) coefficients (Fig. 3A). Resemblance measures showed generally low variation relative to other choices in multivariate methods (11%; Fig. 3B). These results do not appear to be driven by the choice or characteristics of the resemblance coefficient. Jaccard’s coefficient, which disregards joint absences, was shown to have similar site-level impacts than those that consider joint absences (e.g., simple matching; Appendices A and C).

There was high site-level impact from the removal of rare species. In most cases, when rare species were removed from the analysis, the impacts were driven by differences in sites that contained species that were removed. For example, the site-level residuals were much higher in sites impacted by the removal of species than the complete set of data, i.e., All data (Fig. 4 and Appendix C). Recall that site-level vector residuals represent the degree to which any given observation changes in its relative position from one ordination to another (Olden et al., 2001; Paavola et al., 2006). Therefore, the ratio of the mean site-level residuals between sites impacted by species removal and those sites that did not have species removed provides an indication as to magnitude that rare species may alter site-level assessments. Overall, sites impacted by the removal of rare species had a 9-fold change in multivariate space when single-occurrence species were removed from the analysis, and 2-fold change when species having prevalence less than 5% were removed from the analysis. Interestingly, once species that occurred at less than ten percent of sites were removed from the analyses, there was virtually no difference between the two categories of sites (Fig. 4), and in some cases represented less of an impact (e.g., NMDS-J, NMDS-RR; Appendix C).

4. Discussion

One of the difficulties with assessing the importance of removing rare species in multivariate bioassessments is the lack of context from which to judge the impacts of the decision. For example, how can one evaluate whether the inclusion of rare species provides
redundant information with more common species or provides undue influence (i.e., the statistical impact argument; Gauch, 1982; Marchant, 1999, 2002; Bailey et al., 2004)? Alternatively how can one determine whether rare species are more sensitive to ecosystem stress than more common species (i.e., the biological impact argument; Faith and Norris, 1989; Cao et al., 1999, 2001)? Here we evaluated both the statistical and biological impact arguments as separate hypotheses using fish species data collected from a well-sampled aquatic system. Our study demonstrates that some methodological choices inherent in multivariate analyses may have greater influence relative to other choices, such as whether or not to remove rare species (Marchant et al., 2006). Although there are commonly held notions that rare species provide redundant information as compared to more common species, or unduly influence multivariate analyses (Marchant, 1999); in the case of fish species in the Sydenham River we show that rare species provided important site-specific information, in addition to the general comparison of sites and species. These results support the biological impact argument for the inclusion of rare species in bioassessments and not the statistical impact argument.

The importance of choices in multivariate analyses need to be better justified for bioassessment methodologies to progress (we expect parallel concerns for studies using multimetric approaches too). For example, this study demonstrates that the removal of rare species had similar or greater influence in multivariate analyses as other choices inherent in its calculation. Previous research has noted that differences in resemblance coefficients (Jackson et al., 1989; Cao et al., 1998; Poos et al., 2009) and ordination techniques (Jackson, 1993a; Podani, 2000) can lead to drastically divergent results. Yet despite this knowledge, researchers wishing to use multivariate analyses for bioassessments rarely justify these decisions. More often, researchers select methods based on past experience and assume that the resultant summary adequately models the underlying data, or they choose solutions which are most interpretable with regard to their a priori hypotheses (Jackson et al., 1989; Jackson, 1997; Podani, 2000). This approach may have severe consequences for the ultimate goal of inferring community responses for multivariate bioassessment. The comparison of choices inherent in multivariate analyses demonstrated the effect of rare species may not be equal across all ordination methods (Figs. 2 and 3). For example, there was great variation among the NMDS solutions relative to the CA and PCoA analyses, indicating that the NMDS results were more susceptible to differences arising from choice or resemblance measure and the inclusion or exclusion of rare species. As a result, the removal of rare species may be less of a concern than previously noted when used with some of these eigenanalysis-based ordination methods (e.g., Marchant, 1999; Marchant et al., 2006), whereas with other choices (e.g., NMDS used in ANNA; Linke et al., 2005) it may be equally or more important. Such results are contrary to the commonly held idea that NMDS is more robust to data characteristics than methods based on eigenanalysis and therefore a better standard alternative for the analysis of ecological communities (Cao et al., 2001, but see Hirst and Jackson, 2007) – rather we need to carefully evaluate our choices in each instance rather than simply assuming universal standards. Our results indicate that researchers must be mindful of the statistical decisions they make regarding ordination technique, resemblance coefficient and the exclusion of rarely sampled species (to name a few), as each may have potential to influence community responses and impact on meaningful conclusions. Other issues such as seasonal effects (Bailey et al., 2004), clustering method (Poos et al., 2009; Jackson et al., 2010), data standardization (Jackson, 1993a; Cao et al., 1999), taxonomic resolution (Arscott et al., 2006) and data quality (Bailey et al., 2004) also require adequate justification.

The removal of rare species may have unknowingly large effects for our interpretation of the biological conditions determining impacts or recovery in bioassessments. First, as rare species may not be as rare as perceived simply as a result of sampling protocols (Preston, 1948; Arscott et al., 2006), the removal of rare species may limit the number of species from which to assess the biological community. In our study, the removal of rare species not only limited the potential species undergoing assessment; it also coincided with the removal of species with conservation concern, including species at risk such as the Eastern Sand Darter (Ammodramys pellucida) and Blackstripe Topminnow (Fundulus notatus). Second, the removal of rare species may also remove species that are more sensitive to ecosystem stress. Large-scale agricultural activity and increases in turbidity has lead to severe declines in several species in the Sydenham River (Poos et al., 2007, 2010), and these were shown to be more related to changes in rare species (e.g., Eastern Sand Darter, Poos et al., 2008). A post hoc analysis corroborated that the species which occurred less frequently were also more associated with low anthropogenic stress (e.g., turbidity; Fig. 4). These

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results are in agreement with others who found that taxa which were more sensitive to stressors tend to be more restricted in their distribution and thus were found to have considerably lower probabilities of occurring at any given site (Clarke and Murphy, 2006; Van Sickle et al., 2007). Finally, and perhaps most importantly, the removal of rare species could fundamentally change conclusions of multivariate bioassessments. When rare species were removed from the analysis, sites impacted by this removal shifted in multivariate space to a greater degree than those not directly changed by this decision (e.g., >9-fold change for single occurring species; Fig. 4). Therefore, sites containing these more infrequently encountered species, i.e., those generally found at sites having less impacted environmental conditions are no longer highlighted to the same degree when these rare species are removed from the analysis. Likewise, as more species were removed there was less of an effect (i.e., M1 > M5 > M10; Fig. 3), likely due to the removal of more sensitive species at the initial stages (Fig. 4). Therefore the removal of rare species not only caused large site-specific differences in multivariate space, but that these site-specific differences were most likely related to the sensitivity of rare species to anthropogenic impacts. These results suggest that removing rare species has the potential to jeopardize the very rationale for developing a bioassessment (e.g., to determine impacted versus non-impacted sites) and must be considered carefully when developing the analytical methodology.

Of course, there are issues with the inclusion or exclusion of rare species in multivariate analyses which this study could not assess. For example, we utilize a holistic approach for determining the influence of removing rare species relative to choices inherent in multivariate bioassessments. This framework has the advantage of determining the influence of each choice while holding the other choices constant (i.e., we were able to partition out the variation of each choice relative to one another). Therefore, our results allow one to assess and appreciate the importance of each choice in a multivariate bioassessment. Such an approach is readily applicable to all multivariate approaches where choices in the removal of rare species, resemblance coefficient, and ordination (or clustering) are required. One cannot use our results to assess the robustness of individual methods (e.g., ANNA; Linke et al., 2005, BEAST; Readynoldson et al., 2000, RIPVACS; Wright et al. 2000, or AUSRIVAS; Simpson and Norris, 2000), nor should they be used to do so. Previous research into the impact of removing rare species on actual bioassessments can be found elsewhere (e.g., Faith and Norris, 1989; Clarke and Murphy, 2005; Van Sickle et al., 2007), but in many cases support our results that choices inherent in multivariate bioassessments can drastically alter results. Finally, there are many resemblance coefficients possible for multivariate bioassessments, which we did not evaluate and these measures also include many metrics based on species abundance. We have examined this issue in detail and have found no differences in our results using a variety of other resemblance coefficients, such as traditional coefficients (i.e., Sorensen, Kulczynski), unbound coefficients (i.e., chi-square) and one non-metric probabilistic coefficient (i.e., Raup–Crick; Poos and Jackson, unpublished data).

Researchers who wish to include rare species in multivariate bioassessments and minimize their impacts can choose from a variety of approaches whether they are using binary data or abundance data. Data transformations can downweight the influence of rare species (Jackson et al., 1989; Jackson, 1993a; Cao et al., 1999). For example, Legendre and Gallagher (2001) have suggested the use of Hellinger transformation for reducing the impacts of rare species. Researchers can also choose to reduce their data from abundance to presence–absence (as done here), which represents the strongest form of data standardization and has been suggested as less likely to bias estimates from resemblance coefficients (Cao and Williams, 1999). In some cases estimations can be used to remove the impacts of rare species, such as the use of Beal’s smoothing for presence/absence data (McCune, 1994; De Cáceres and Legendre, 2008). New quantitative approaches for identifying patterns in communities which do not require as many subjective decisions may provide robust alternatives (Jackson et al., 2010). One alternative is to use a global sensitivity analysis, where one compares several methods, including methods emphasizing different attributes (and choices within methods; Green, 1979; Jackson et al., 1989; Jackson, 1993a). If the methods produce similar results than one can have greater confidence that the results are more robust and representative rather than being dominated by the set of choices used in the analysis (Jackson, 1993a). Ultimately, the decision to include or remove rare species should be justified by the goals of the bioassessment. Naturally, researchers wish to limit their data to reflect the most appropriate number of species, the most practical resemblance coefficient and the most useful ordination technique; however, no such criteria exist. In the end, a more holistic view of multivariate bioassessments is needed move bioassessments forward, with better justification of all of decisions inherent in each analysis.

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Appendix A.

Algorithms for a selection of commonly used resemblance coefficients in multivariate analyses.

\[
\text{Jaccard} = \frac{a}{a + b + c}
\]

\[
\Phi_P(d) = \frac{ad - bc}{\sqrt{(a + b)(a + c)(d + b)(d + c)}}
\]

Simple matching = \[
\frac{a + d}{a + b + c + d}
\]

Russell and Rao = \[
\frac{a}{a + b + c + d}
\]

Chi-square = \[
\frac{(a + b + c + d)(ad - bc)^2}{(a + b)(a + c)(b + d)(c + d)}
\]

Sorensen = \[
\frac{2a}{2a + b + c}
\]

Kulczynsky = \[
\frac{a}{b + c}
\]

Raup–Crick = 1 − \text{prob} (j)

Note: all binary resemblance coefficients are expressed in terms of species/site associations using variables a, b, c, and sometimes d, where a is the number of species shared at a site, b is the number of species found only in one of the compared sites, c is the number of species found in the other of the compared sites, and d is the number of species absent from both sites (see Jurasinski, 2007). Raup–Crick is a probabilistic resemblance coefficient between sites based on presence/absence of species. It is defined as the probability
of finding shared species in common for each pair of sites. Legendre and Legendre (1998) recommend testing for the probability value for \( j \) through permutation.

**Appendix B.**

Summary of three-dimensional ordination results. Shown are eigenvalues for Principal Coordinates Analyses (PCA) and Correspondence Analyses (CA), with percent variance explained shown in parentheses. Stress values are shown for Non-Metric Multidimensional Scaling (NMDS). Note: we do not show variance explained for NMDS as it does not relate to the variation summarized by eigenanalysis ordination methods such as PCoA. Unlike all eigenanalysis ordination methods which increase the amount of variation explained with increasing numbers of axes, NMDS can actually have less variance explained with increasing numbers of axes (see McCune and Grace, 2002) because the variation considered does not represent the same type of information as in eigenanalysis methods.

<table>
<thead>
<tr>
<th>Ordination technique</th>
<th>Axis 1</th>
<th>Axis 2</th>
<th>Axis 3</th>
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<tr>
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<td>2.79 (19.37)</td>
<td>1.67 (24.58)</td>
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<td>0.21 (28.98)</td>
<td>0.12 (36.62)</td>
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</table>

**Note:** Abbreviations are: (1) ordination method – Principal Coordinates Analyses (PCA), Non-Metric Multidimensional Scaling (NMDS) and Correspondence Analyses (CA); (2) resemblance coefficient: Jaccard’s (j), phi (Φ), Russell and Rao (RR), and simple matching (SM).

**Appendix C.**

Site-level impacts of methodological choices in bioassessments. Shown are the ratios between mean site-level vector residuals from Procrustes analyses of sites having species removed and those sites having no species removed. Mean site-level vector residual values were separated by sites which had rare species removed (M1: \( n = 4 \); M5: \( n = 9 \); and M10: \( n = 21 \)); and compared with those sites that not.

**References**


Cao, Y., Williams, D., 1999. Rare species are important in bioassessment (Reply to the comment by Marchant). Limnology and Oceanography 44, 1841–1842.


McCune, B., 1994. Improving community analysis with the Beals smoothing function. Ecoscience 1, 82–86.


