ANOSIM introduction

The series of ANOSIM (analysis of similarity) tests, accessed through **Analyse>ANOSIM**, operate on a resemblance matrix as the active sheet and carry out non-parametric tests for designs which broadly parallel univariate 1- and 2- way ANOVA (analysis of variance) crossed and nested cases, extended in PRIMER 7 to cover all combinations of crossed and nested 3-way designs. At the simplest level, a one-way layout of groups (e.g. of different times or sites or treatments), with replicates within each group, allows a test of the null hypothesis that there are no assemblage differences between the groups against an alternative which specifies that there are – but makes no assumption about the nature of those differences. However, another new feature in PRIMER 7 is the addition of a test of the same null hypothesis but against an alternative which specifies that the groups differ in a predetermined order, for example exhibiting a serial time trend or a continuous community change on approaching a point source impact. These ordered ANOSIM tests generalise, in a very natural way, the standard one-way ANOSIM R statistic to R$^\text{O}$ (superscript O for Ordered), defined in Chapter 6 of CiMC, and such ordering can be specified for all or any combination of factors in the 2- and 3-way designs. By narrowing the scope of the alternative hypothesis that the null is tested against, a greater degree of sensitivity (power) is obtained – though at the price of little or no sensitivity to detect group differences which do not conform to the hypothesised serial pattern. A crucial point to make is that the group designations (and specification of the group order under the alternative hypothesis) are made *prior* to seeing the data. ANOSIM is not a valid test of differences between groups generated by a cluster analysis, or other inspection of the data, other-wise the argument becomes entirely circular – use SIMPROF for these latter situations.

The simple 1-way R statistic is readily extended to a 2-way crossed layout, for example testing the null hypothesis that there are no differences between treatments (factor A), allowing for the fact that there may be site differences (factor B), in a case where all treatments are replicated at each site. Two-way crossed designs (A$\times$B) are symmetric, so the procedure can be reversed to give also a test for the hypothesis of no site differences given that there may be treatment differences – the routine gives both sets of tests automatically. The other 2-way option is a 2-way nested design, where the two factors are hierarchical, B(A), for example a top-level factor of treatment differences A (control *vs* impacted areas), with a second factor of different sites B within each treatment, and representative replicate samples from each site. A test can be carried out for significant differences between sites within a treatment, but at the next hierarchical level up, the primary interest would be in testing for assemblage differences due to treatment. This compares treatment differences against assemblage variation among sites within a treatment, rather than among the sample replicates at a site, and there is an example of this in Chapter 6, CiMC. A different style of test is required in the case of a 2- (or 3-)way crossed layout when there are no replicates (or no genuine replicates and it is wise to average the *pseudo-replicates* for each of the factor combinations). PRIMER 7 will automatically recognise situations in which replicates are not available and attempt to calculate a different test statistic ρ (in whatever combinations of 2- and 3-factor designs it is encountered).

Three-way crossed and nested designs, also now covered in PRIMER 7, are of the types: A$\times$B$\times$C, fully crossed; C(B(A)), fully nested, C within B within A; C(A$\times$B), C

nested in all combinations of A crossed with B; and B$\times$C(A), B crossed with C, where C is nested in A. These routines are all permutation tests, making a bare minimum of assumptions and consistent with the philosophy of the PRIMER routines that the primary information on relationships among samples is summarised in the ranks of the resemblance matrix – the basis for the preferred ordination technique of non-metric MDS. The tests apply to any resemblance matrix, so are equally effective at testing for assemblage change on biotic similarities, environmental change on Euclidean or other distances, change in biomarkers, particle sizes etc. None of the construction or concepts underpinning these tests is covered in this Section – that is all in the extensive Chapter 6 of CiMC, which includes the detailed Tables 6.3 and 6.4, listing the precise test statistics (and whether pairwise tests are possible when there are more than two levels of a factor) for every combination of: 1-, 2- and 3-way tests; unordered or ordered alternative hypotheses; and with or without replication. (That chapter finishes with some comments on the limits of construction of purely non-parametric tests, e.g. the non-existence of tests for interaction – a metric concept and the springboard for PERMANOVA). The below simply shows examples of the different tests and how the results windows are interpreted.

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