6.12 Two-way ordered ANOSIM designs

Under the non-parametric framework adopted in this manual (and in the PRIMER package) three forms of 2-way ANOSIM tests were presented on page 6.5: 2-factor nested, B within A (denoted by B(A)); 2-factor crossed (denoted A$\times$B); and a special case of A$\times$B in which there are no replicates, either because only one sample was taken for each combination of A and B, or replicates were taken but considered to be ‘pseudo-replicates’ (*sensu* Hurlbert (1984)) and averaged.¶

The principle of these tests, and their permutation procedures, remain largely unchanged when A or B (or both factors) are ordered. Previously, the test for B under the nested B(A) model (page 6.6) averaged the 1-way R statistic for each level of A, denoted $\overline{R}$, and the same form of averaged statistic was used for testing B under the crossed A$\times$B model with replicates (page 6.7); without replicates the crossed test used the special (and less powerful) construction of page 6.8, with test statistic the pairwise averaged matrix correlation, ρ_{av}. (There was no test for B in the nested model, in the absence of replicates for B). If B is now ordered, R is replaced by R^{Oc} where there are replicates (becoming $\overline{R}^{Oc}$ when averaged across the levels of A), or by R^{Os} where there are not (becoming $\overline{R}^{Os}$); there is no longer any necessity to invoke the special form of test based on ρ_{av} when the factor is ordered. The same substitutions then happen for the test of A, if it too is ordered: $\overline{R}$ and ρ_{av} are replaced by $\overline{R}^{Oc}$ and $\overline{R}^{Os}$. If A is not ordered, any ordering in B does not change the way the tests for A are carried out, e.g. for A$\times$B, the A test is still constructed by calculating the appropriate 1-way statistic for A, separately for each level of B, and then averaging those statistics.

*Table 6.3. **1-way and 2-way ANOSIM** (global) test statistics, for crossed and nested designs, with unordered or ordered factors, and with or without replication at the lowest level of the design. Also given are the existence (or not) of pairwise tests, details of the test constructions and examples of contexts in which they might be employed.*

No.	Type of design	Factors	Factor levels ordered?	Repli-cates?	Statistic used	Pairwise test?[1]	Construction of statistic	Examples
1a	1-way	A	Unordered	Yes	R	Yes	A: Standard 1-way ANOSIM statistic [2]	A: sites, with replicates in each
1b	1-way	A	Unordered	No	-	-	A: No basis for a test	-
1c	1-way	A	Ordered	Yes	R^{Oc}	Yes	A: ANOSIM form of seriation statistic for ordered categories [3]	A: impact levels, expecting monotonic response
1d	1-way	A	Ordered	No	R^{Os}	No	A; ANOSIM form of simple seriation statistic (no replicates) [3]	A: inter-annual trend or positions along a transect
2a	2-way crossed	AxB	A unordered B unordered	Yes	A: $\bar{R}$ B: $\bar{R}$	Yes Yes	A: Average of 1-way R for testing A across separate levels of B B: Average of 1-way R for testing B across separate levels of A	A: shores, B: treatment types (several applications) or A: locations, B: habitats (sites as replicates)
2b	2-way crossed	AxB	A unordered B unordered	No	A: ρ_{av} B: ρ_{av}	No No	A: Average of ρ among resemblance matrices (of A) across levels of B [4] B: Average of ρ among resemblance matrices (of B) across levels of A [4]	As 2a but each treatment only once on each shore, or A: sites, B: times, each site visited once at each time
2c	2-way crossed	AxB	A unordered B ordered	Yes	A: $\bar{R}$ B: $\bar{R}^{Oc}$	Yes Yes	A: As test 2a B: Average of 1-way R^{Oc} for testing B across separate levels of A	A: shores, B: increasing treatment impact levels or A: locations, B: water depths (sites as replicates)
2d	2-way crossed	AxB	A unordered B ordered	No	A: ρ_{av} B: $\bar{R}^{Os}$	No No	A: As test 2b B: Average of 1-way R^{Os} for testing B across separate levels of A	A: site, B: tidal height (transect down shore) or A: patch reefs, B: inter-annual trend
2e	2-way crossed	AxB	A ordered B ordered	Yes	A: $\bar{R}^{Oc}$ B: $\bar{R}^{Oc}$	Yes Yes	A: Average of R^{Oc} for testing A across B levels (i.e. 2c, switching A and B) B: As 2c	A: shores on latitudinal gradient, B: coarseness of sediment classes, replicate sites in each combination
2f	2-way crossed	AxB	A ordered B ordered	No	A: $\bar{R}^{Os}$ B: $\bar{R}^{Os}$	No No	A: Average of R^{Os} for testing A across B levels (i.e. 2d, switching A and B) B: As 2d	A: transect of sites along shore and B: depth transect at each site, sampling (once) the same set of depths
2g	2-way nested (B within A)	B(A)	A unordered B unordered	Yes	A: R B: $\bar{R}$	Yes No	A: As test 1a, but with levels of B as replicates (averaging within those) [5] B: As test 2a, but without pairwise tests [6]	A: protected/not protected areas, B: sites within each type (replicates are trawls within each site)
2h	2-way nested	B(A)	A unordered B unordered	No	A: R B: -	Yes -	A: As test 1a, but this time the sole levels of B are the only replicates B: No basis for a test	A: location, B: site (e.g. taken over a year but then time-averaged to give one sample for each site)
2i	2-way nested	B(A)	A ordered B unordered	Yes	A: R^{Oc} B: $\bar{R}$	Yes No	A: As test 1c, but with levels of B as replicates (averaging within those) [5] B: As test 2g	A: water depth groups, B: randomly chosen sites at each depth range, replicate grab samples at each site
2j	2-way nested	B(A)	A ordered B unordered	No	A: R^{Oc} B: -	Yes -	A: As test 1c, but this time the sole levels of B are the only replicates B: No basis for a test	A: distance from outfall, B: random sites at each distance, and 'pseudo-reps' (e.g. multicorer), pooled
2k	2-way nested	B(A)	A unordered B ordered	Yes	A: R B: $\bar{R}^{Oc}$	Yes No	A: As test 2g (ordered levels of B assumed representative as replicates) [7] B: As test 2c, but without pairwise tests [6]	A: dry/wet season, B: months (replicates as random days in month)
2l	2-way nested	B(A)	A unordered B ordered	No	A: R B: $\bar{R}^{Os}$	Yes No	A: As test 2h (ordered levels of B assumed representative as replicates) [7] B: As test 2d	A: site, B: points along transect (one transect at each site, randomly oriented and located)
2m	2-way nested	B(A)	A ordered B ordered	Yes	A: R^{Oc} B: $\bar{R}^{Oc}$	Yes No	A: As test 2i (ordered levels of B assumed representative as replicates) B: As test 2k	A: region, latitudinally arranged, B: transect of sites in each region (all at same depth), replicates within
2n	2-way nested	B(A)	A ordered B ordered	No	A: R^{Oc} B: $\bar{R}^{Os}$	Yes No	A: As test 2j (ordered levels of B assumed representative as replicates) B: As test 2l	A: seamounts in different depth classes, B: distance along single random transect on each seamount

[1] All pairwise tests are unordered, by definition. [2] $R = 2(\bar{r}_{Among} - \bar{r}_{Within})/M$, equivalently the slope of a linear regression of ranks of the biotic resemblances against ranks from a (0,1) model matrix for levels of A.
[3] R^{Oc} is the slope from a linear regression of ranks of biotic resemblances against ranks from a 'seriation with replication' model matrix and R^{Os} against a simple seriation model without replication; they are the (asymmetric) ANOSIM R forms of the (symmetric) RELATE Spearman ρ statistic. The distinction between ordered categories (R^{Oc}) and simple seriation (R^{Os}) is not crucial for calculation purposes (thus R^{O}).
[4] Matrix correlation (Spearman rank ρ) calculated between all pairs of biotic resemblance matrices (for levels of A) within levels of B, and then ρ averaged over the separate B levels to give ρ_{av} for A (vice-versa for B).
[5] Ranked resemblances are averaged within levels of B(A), and for all pairs across levels of B(A); the resulting averaged matrix is re-ranked and input to 1-way ANOSIM for levels of A, using B levels as replicates. The same is done for each of the pairwise tests, first selecting only resemblances for the requisite pair of A levels, then ranking, averaging and re-ranking before inputting the two levels to 1-way ANOSIM.
[6] The global test is the same as the crossed case but here the levels of B, even if similarly denoted (by 1, 2, .. say) have nothing in common across the levels of A, so a pairwise test of B1 v B2 (say) is meaningless.
[7] A nested factor might typically be a randomly located site (B) in a region (A). Ordered sites might come from transects of sites across each region (randomly directed so transect points are nested not crossed with region). If representative of the region's extent, transect sites could still be considered suitable replicates for a test of region, the 'randomness' coming from the stochastic nature of the environment being sampled.

Such a plethora of possibilities are best summarised in a table, and the later Table 6.3 lists all the possible combinations of 2-way design, factor ordering (or not) and presence (or absence) of replicates, giving the test statistic and its method of construction, listing whether or not pairwise tests make sense[†], and then giving some examples of marine studies in which the factors would have the right structure for such a test.

We have already seen unordered examples of 1-way tests (1a, Table 6.3) in Figs. 6.3 & 6.5, 2-way crossed (2a) in Fig. 6.7 and, without replication (2b), in Figs. 6.10 & 6.12; Fig. 6.6 is 2-way nested (2g). Examples of 2-way crossed without replicates, with one (2d) or both (2f) factors ordered, now follow.

[¶] *An example of the latter might be 'replicate' cores from a multi-corer deployed only once at each of a number of sites (A) for the same set of months (B); these multiple cores are neither spatially representative of the extent of a site (a return trip would result in multi-cores from a slightly different area within the site) nor, it might be argued, temporally representative of that month.*

[†] *If they do make sense, the PRIMER7 ANOSIM routine will give them. Performing such a 2-(or 3-) way analysis is much simpler than reading these tables! It is simply a matter of selecting the form of design (all likely combinations of 1-, 2- or 3-factor, crossed or nested) and then specifying which factors are to be considered ordered – the factor levels must be numeric in that case but only their rank order is used. Analyses that use specific numerical levels (unequally-spaced) can be catered for in many cases within the expanded RELATE routine, utilising a ρ statistic, see Chapter 15.*

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