

2-way crossed ordered test

The test for an ordered factor (A) in the 2-way crossed design parallels the construction seen earlier for the 2-way (unordered) crossed case, in that the 1-way R^{O} statistic is calculated separately for each level of the other factor (B) and those R^{O} values averaged to give the 2-way test statistic. This is compared with its null distribution calculated under the same constrained permutation procedure as for the previous 2-way crossed case – A labels are permuted only within the levels of B. The difference here, again, is that this is a perfectly viable test when there is no replication within the cells of the 2-way layout, provided there are enough ordered steps (a) in factor A or levels (b) of factor B to generate sufficient permutations, $(a!/2)^b$, for a sensible test. This number scales up very rapidly, so even a fairly minimal design will give some sort of test, e.g. $a=4$ transect sites sampled $b=2$ times gives 144 permutations and (at best) a $p<1\%$ level test for the presence of site ordering. The 1-way test ($b=1$) requires at least $a=5$ ordered steps, to give 60 permutations for a $p<2\%$ test.

Run **Analyse>ANOSIM>**(Model•Two-Way Crossed - AxB)>(Factors A: Position Ordered & B: Year Unordered) on same resemblance selection as above, of the two years 88 and 97, together. This will, of course, produce a massively significant *Position* effect, with average $R^{\text{O}} = 0.69$, and with (Max permutations: 9999) this is still off the top of the null distribution, $p<0.01\%$ (or, as a probability, $P<0.0001$). It is naturally a very powerful test, with 6×10^{16} possible permutations.

It did not matter in this case whether the *Year* factor was defined as *Unordered* or *Ordered*, since there were only two years. The test for *Year*, removing the effect of *Position* by comparing years only within each of the 12 levels for *Position*, is doomed to failure, unsurprisingly. There are no replicates on which to base such a test (applying the above formula for an ordered test, $a=2$ so $a!/2 = 1$ and, whilst $b=12$ is large, powering up 1 still gives 1, i.e. there is only one permutation which is the observed configuration of the labels!). ANOSIM simply says *No replication and <3 groups*.

However, if we were to take off the selection, so reintroduce the full set of 7 years, and specify that both factors are ordered then there are ample steps in both the spatial gradient of 12 points and a temporal time trend of 7 points for an ordered test of either factor, removing the effect of the other. The *Position* test now gives a very similar $R^{\text{O}} = 0.69$ as found for the two years alone but the *Year* test returns $R^{\text{O}} = 0.08$, with about 1100 of the 9999 permutations created under the null hypothesis giving larger R^{O} values than this ($p<11\%$), a non-significant result. (Incidentally, note it is always true that a test of factor A is completely unchanged by whether factor B is assumed ordered or not).

In fact, whilst the original study postulated serial change in coral communities along the onshore-offshore transect, so that an ordered test for the *Position* factor seems very appropriate, it is not so clear that it is relevant to test for a monotonic inter-annual trend – a drift of the community in time, ever further away from its original configuration. Local impacts in some years may be dominant, and the possibility that these have a differential effect on the transect gradient (an *interaction* of a type) suggests a very different approach, using the **Analyse>2STAGE** routine, which we shall

return to for these data in Section 14. Within the ANOSIM routines however, the 2-way crossed layout for an unordered factor with no replication leaves few options for a non-parametric test, though sometimes helpful is a fall-back test (available in PRIMER since the early versions), which is next described for the Exe estuary nematode data. Save and close workspace Phuket ws.

The screenshot displays the PRIMER software interface for an ANOSIM analysis. The main window shows the 'Analysed' data table for 'Phuket corals 88 & 97' with similarity values (0 to 100) for samples 88A1 through 88A5 across four transects (88A1, 88A2, 88A3, 88A4). The 'ANOSIM' menu is open, and the 'Two-Way Crossed - AxB' model is selected. The 'Factors' section shows 'A: Position' (Ordered) and 'B: Year' (Ordered). Two histograms are displayed: 'Graph19' (Position Test) and 'Graph21' (Year Test), both showing frequency distributions of the R statistic. Two ANOSIM result windows are also visible: 'ANOSIM3' and 'ANOSIM4'. 'ANOSIM3' shows results for the Position factor, including a Global Test with a sample statistic of 0.689 and a significance level of 0.01%. 'ANOSIM4' shows results for the Year factor, including a Global Test with a sample statistic of 0.683 and a significance level of 0.01%. Both tests used 9999 permutations.

ANOSIM3 Analysis of Similarities
 Two-Way Crossed - AxB
 Resemblance worksheet
 Name: Resem2
 Data type: Similarity
 Selection: 1-12,73-84

Place	Name	Type	Levels
A	Position	Ordered	12
B	Year	Unordered	2

Tests for differences between ordered Position groups (across all Year groups)
Global Test
 Sample statistic (Average R): 0.689
 Significance level of sample statistic: 0.01%
 Number of permutations: 9999 (Random sample from a large number)
 Number of permuted statistics greater than or equal to Average R: 0

Tests for differences between unordered Year groups (across all Position groups)
No replication and <3 groups

Outputs
 Plot: Graph19

ANOSIM4 Analysis of Similarities
 Two-Way Crossed - AxB
 Resemblance worksheet
 Name: Resem2
 Data type: Similarity
 Selection: 1-12,73-84

Place	Name	Type	Levels
A	Position	Ordered	12
B	Year	Ordered	7

Tests for differences between ordered Position groups (across all Year groups)
Global Test
 Sample statistic (Average R): 0.683
 Significance level of sample statistic: 0.01%
 Number of permutations: 9999 (Random sample from a large number)
 Number of permuted statistics greater than or equal to Average R: 0

Tests for differences between ordered Year groups (across all Position groups)
Global Test
 Sample statistic (Average R): 0.082
 Significance level of sample statistic: 11%
 Number of permutations: 9999 (Random sample from a large number)
 Number of permuted statistics greater than or equal to Average R: 1102

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