

Summary of the PERMANOVA analysis

A summary of the essential commands associated with performing this PERMANOVA analysis of the holdfast data according to the 3-factor hierarchical experimental design is given in the table below:

Step	To implement in PRIMER:
1. Select variable subset	<i>From the original data sheet:</i> click Select > Variables... > (\$\bullet\$Indicator levels > Indicator name: Phylum), click Levels... > (Selection > Include: Mollusca), click OK .
2. Jaccard resemblance	<i>From the subset-selected data sheet:</i> click Analyse > Resemblance > (Measure \$\bullet\$Other > S7 Jaccard) & (Analyse between \$\bullet\$Samples), click OK .
3. Specify the design	<ul style="list-style-type: none">• <i>From the resemblance matrix:</i> click PERMANOVA+ > Create PERMANOVA design... > (Title: Three-way nested design) & (Number of factors: 3), click OK.• <i>Fill in the appropriate details of the design in the design file.</i>
4. Run PERMANOVA	<i>From the resemblance matrix:</i> click PERMANOVA+ > PERMANOVA... > (Design Worksheet: Design1) & (Num. permutations: 9999) & (default settings for the rest), click OK .
5. Ordination of centroids	<ul style="list-style-type: none">• <i>From the resemblance matrix:</i> click PERMANOVA+ > Distance Among Centroids... > Centroids > (Grouping factor: Area).• <i>From the resulting resemblance matrix among area centroids:</i> click Analyse > MDS > Non-metric MDS..., take all the defaults and click OK.• <i>To specify labels and symbols:</i> click Graph > Sample Labels & Symbols...

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