

13.4 Example: Gulf of Maine

invertebrates - functional resemblance

There are many situations where the standardisation of samples is required as a pre-treatment prior to analysis, but which needs to be done *separately within groups* of variables that may be identified by an indicator.[†] Here, we shall consider a dataset comprised of occurrences of $p = 91$ macroinvertebrate species recorded from intertidal areas at each of $N = 12$ exposed rocky headlands (surveyed in 2012) in the Gulf of Maine (Fig. 13.3, [Trott \(2022\)](#) [†]). For each species, we have additional information in the form of trait data. The trait data for each species consists of presence/absence information for a series of $q = 93$ individual trait variables. The trait variables are organised into 14 groups. These groups of traits are: Form, Position, Lifestyle, Trophic Relation, Body Shape, Body Support, Flexibility, Ecoengineer, Fertilization, Development, Size, Body Plan, Asexual Reproduction and Regeneration. The number of categories (trait variables) within each trait group varies, and any given species can be recorded as a '1' (indicating that they belong) to one or more of these categories within each trait group.



Fig. 13.3 Map of the Gulf of Maine showing 12 sites where macroinvertebrates were sampled by [Trott \(2022\)](#) . Satellite image: Google Earth.

Our initial agenda here may be to consider the relationships among the sites based on the species they contain in the usual way, e.g., using the Bray-Curtis (or Sørensen) resemblance measure directly on the species $\times$ site matrix. However, we may wish to nuance this calculation further by incorporating relationships among the species, based on their traits. In other words, if two sites do not share any species in common, they might nevertheless share two species that have similar traits. We can exploit the method of calculating taxonomic resemblance ('**Gamma+**', [Clarke et al. \(2006b\)](#)) in order to calculate, instead, a *functional resemblance*, using a *matrix of inter-species relationships built from the trait data* (e.g., [Myers et al. \(2021\)](#)).

Further to this aim, we shall consider our trait data matrix such that the classical role of 'samples' is given to the species (among which we shall calculate the resemblances), while traits are given the role of 'variables'. However, we want each trait *group* to be weighted equally in the calculation. To make sure the sum of values for each species within each trait group sums to 100 (so gets equal weight), we will want to apply a pre-treatment to standardise across the trait variables for each species ('sample'), but separately within each trait grouping. This standardisation would not be necessary if every species only had one trait within a group, but that is not the case here. For example, the mollusc species, *Adalaria proxima* (abbreviated ADP) is in two trophic categories: it grazes on algal fronds and blades (Tr-P-GF), and also grazes macro prey on the substratum (Tr-P-GSM).

Our analysis pathway looks like this:

- Open the trait data matrix in PRIMER 8.
- Standardise the 'samples' (species here) across the trait variables, separately within each trait grouping, using our new tool in P8, **Pre-treatment > Standardise....**
- Calculate functional resemblances among species using Bray-Curtis, based on the standardised trait data.
- Examine the inter-species functional relationships in an ordination (nMDS).
- Calculate functional resemblances among the 12 sites, by incorporating these species inter-relationships.
- Examine the functional relationships among the sites in an ordination (nMDS).

Open the trait data matrix

1. In PRIMER 8, click **File > Open...** and open up the file named '[Gulf_of_Maine_invert_traits.pri](#)' (found in the '[Gulf_of_Maine_inverts](#)' folder inside the '[Examples_P8](#)' folder), as shown below.

Gulf_of_Maine_invert_traits

Gulf of Maine invertebrate traits
Other

Samples - Species

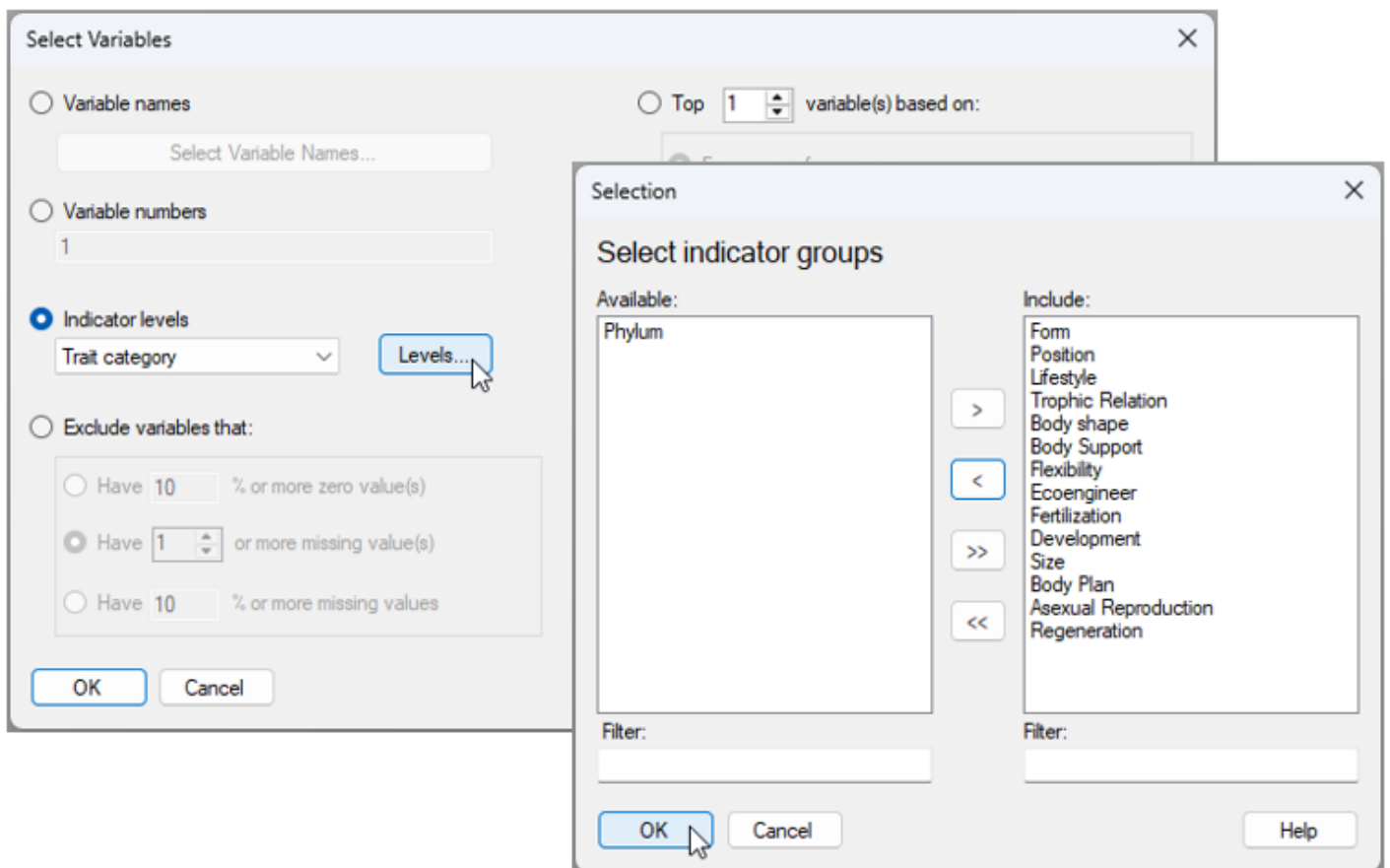
	ADP	AEP	AH	AS	AA	ANS	APP	AF
Ph-M	1	1	0	0	0	1	0	
Ph-B	0	0	1	0	0	0	0	
Ph-E	0	0	0	1	0	0	0	
Ph-EC	0	0	0	0	0	0	0	
Ph-N	0	0	0	0	1	0	0	
Ph-C	0	0	0	0	0	0	1	
Ph-H	0	0	0	0	0	0	0	
Ph-AR	0	0	0	0	0	0	0	
Ph-P	0	0	0	0	0	0	0	
Ph-A	0	0	0	0	0	0	0	
Ph-T	0	0	0	0	0	0	0	
F-F	1	1	0	1	1	0	0	
F-I	0	0	1	0	0	1	1	
F-R	0	0	0	0	0	0	0	
Po-E	1	1	1	1	1	1	1	
Po-I	0	0	0	0	0	0	0	
Po-Is	0	0	0	0	1	0	0	
Li-SW	0	0	0	0	0	0	0	
Li-C	1	1	0	1	1	0	0	
Li-B	0	0	0	0	1	0	0	
Li-T	0	0	0	0	0	0	0	

Variables - Traits

Note here that the 'Samples' are actually the species (the names are abbreviated). More information about the species can be seen by clicking **Edit > Factors....** Note also that the traits are variables. More information about the traits (also abbreviated) can be seen by clicking **Edit > Indicators....**

The first trait group is actually the name of the Phylum for each species. These are not actually traits, so let's start by selecting all of the traits that do not belong to the 'Phylum' group.

- From the 'Gulf_of_Maine_invert_traits' matrix, click **Select > Variables....** then choose (\$\bullet\$Indicator levels Trait category) and click the 'Levels...' button (Levels...). In the Selection dialog, first click the double right arrows button (>>) to move all of the groups to the 'Include' column on the right. Then click on 'Phylum' and the left arrow button (<), so that it appears back in the 'Available' column on the left (as shown below), then click **OK** (in both dialog windows).



This will create a dataset that is re-coloured with a blue background, showing only the selected subset of traits (excluding 'Phylum'), like this:

Gulf_of_Maine_invert_traits

Gulf of Maine invertebrate traits
Other

Samples - Species

	ADP	AEP	AH	AS	AA	ANS	APP	AF
F-F	1	1	0	1	1	0	0	0
F-I	0	0	1	0	0	1	1	1
F-R	0	0	0	0	0	0	0	0
Po-E	1	1	1	1	1	1	1	1
Po-I	0	0	0	0	0	0	0	0
Po-Is	0	0	0	0	0	1	0	0
Li-SW	0	0	0	0	0	0	0	0
Li-C	1	1	0	1	1	0	0	0
Li-B	0	0	0	0	1	0	0	0
Li-T	0	0	0	0	0	0	0	0
Li-Sh	0	0	0	0	0	0	0	0
Li-PA	0	0	1	0	0	1	1	1
Li-TA	0	0	0	0	0	0	0	0
Tr-Sa	0	0	1	0	0	1	1	1
Tr-Sp	0	0	0	1	0	0	0	0
Tr-P-SAW	0	0	0	0	0	0	0	0
Tr-P-S	0	1	0	0	1	0	0	0
Tr-P-P	0	0	0	0	0	0	0	0
T-P-GP	0	0	0	0	0	0	0	0
Tr-P-GF	1	0	0	0	0	0	0	0
Tr-P-GS	0	0	0	1	0	0	0	0

Variables - Traits

Any analyses from this matrix will now only be done on the sub-setted data. To keep things tidy, click **Tools > Duplicate** and the subsetted data will now be provided in the Explorer tree as a new matrix called 'Data1'. We can re-name this to 'Traits' by clicking **File > Rename Data** and typing in this desired new name, then clicking **OK**, as shown below.

PRIMER

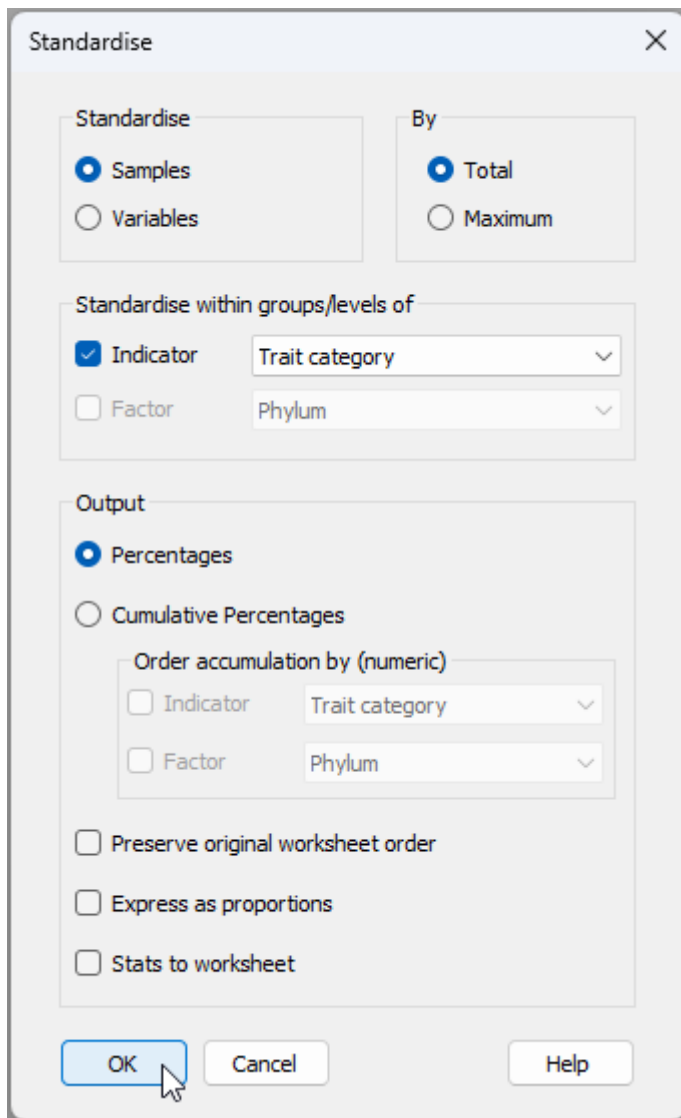
Rename

Traits

OK Cancel Help

Standardise the trait data

- From the 'Traits' data sheet, click **Pre-treatment > Standardise...** and choose (Standardise ☒ Samples) & (By ☒ Total) & (Standardise within groups/levels of ☒ Indicator Trait category) & (Output ☒ Percentages), then click **OK**, as shown below.

The image shows a 'Standardise' dialog box with a close button (X) in the top right corner. It is divided into three main sections. The first section, 'Standardise', contains two radio buttons: 'Samples' (selected) and 'Variables'. The second section, 'By', contains two radio buttons: 'Total' (selected) and 'Maximum'. The third section, 'Standardise within groups/levels of', contains two options: 'Indicator' (selected) with a dropdown menu showing 'Trait category', and 'Factor' (unselected) with a dropdown menu showing 'Phylum'. Below this is an 'Output' section with two radio buttons: 'Percentages' (selected) and 'Cumulative Percentages'. Under 'Cumulative Percentages' is a sub-section 'Order accumulation by (numeric)' with 'Indicator' (unselected) and 'Factor' (unselected) options, each with a dropdown menu showing 'Trait category' and 'Phylum' respectively. At the bottom of the 'Output' section are three checkboxes: 'Preserve original worksheet order' (unselected), 'Express as proportions' (unselected), and 'Stats to worksheet' (unselected). At the very bottom are three buttons: 'OK' (highlighted with a mouse cursor), 'Cancel', and 'Help'.

The resulting data sheet (called 'Data1') can be renamed (using **File > Rename Data**, as we have done before) to (say) 'Standardised Data', for clarity. The standardised trait data sheet will look like this:

Standardised Data											
Gulf of Maine invertebrate traits											
Other											
	Samples - Species										
	ADP	AEP	AH	AS	AA	ANS	APP	AF	AR	BB	
F-F	100	100	0	100	100	0	0	0	0	0	
F-I	0	0	100	0	0	100	100	100	100	100	
F-R	0	0	0	0	0	0	0	0	0	0	
Po-E	100	100	100	100	50	100	100	100	100	100	
Po-I	0	0	0	0	0	0	0	0	0	0	
Po-Is	0	0	0	0	50	0	0	0	0	0	
Li-SW	0	0	0	0	0	0	0	0	0	0	
Li-C	100	100	0	100	50	0	0	100	100	100	
Li-B	0	0	0	0	50	0	0	0	0	0	
Li-T	0	0	0	0	0	0	0	0	0	0	
Li-Sh	0	0	0	0	0	0	0	0	0	0	
Li-PA	0	0	100	0	0	100	100	0	0	0	
Li-TA	0	0	0	0	0	0	0	0	0	0	
Tr-Sa	0	0	100	0	0	100	100	0	0	0	
Tr-Sp	0	0	0	14.286	0	0	0	0	0	0	
Tr-P-SAW	0	0	0	0	0	0	0	0	0	0	
Tr-P-S	0	50	0	0	100	0	0	0	0	0	
Tr-P-P	0	0	0	0	0	0	0	50	33.333	0	
T-P-GP	0	0	0	0	0	0	0	0	0	0	
Tr-P-GF	50	0	0	0	0	0	0	0	0	0	
Tr-P-GS	0	0	0	14.286	0	0	0	0	0	0	
Tr-P-GSM	50	50	0	14.286	0	0	0	0	0	0	
Tr-S-Fa	0	0	0	14.286	0	0	0	50	33.333	0	
Tr-S-Fl	0	0	0	0	0	0	0	0	0	0	
Tr-D-S	0	0	0	14.286	0	0	0	0	0	33.333	
Tr-D-SS	0	0	0	0	0	0	0	0	0	0	
Tr-H-F	0	0	0	0	0	0	0	0	0	0	
Tr-H-GP	0	0	0	0	0	0	0	0	0	0	
Tr-H-GF	0	0	0	0	0	0	0	0	0	0	
Tr-H-GS	0	0	0	14.286	0	0	0	0	0	0	

Note how the distribution of traits within a species for any particular trait group (in this example, the trait groups are identifiable by reference to the letters appearing before the dash '-' in the variable names, e.g., 'Po', 'Li', 'Tr', etc.) will sum to 100.

Calculate relationships among species based on traits

- From the 'Standardised Data' sheet, click **Analyse > Resemblance...** > (Measure $\bullet$ Bray-Curtis similarity) & (Analyse between $\bullet$ Samples), as shown below.

Resemblance

Measure

☒ Bray-Curtis similarity

☐ Euclidean distance

☐ Index of association

☐ Other

☒ Similarity ☒ Distance/dissimilarity

☐ Quantitative ☐ P/A

☐ Correlation ☐ Taxonomic P/A

(exc0-0 = excluding joint absences)

S1 Simple matching

Analyse between

☒ Samples

☐ Variables

☐ Add dummy variable

Value:

1

OK Cancel Help

The resulting resemblances among the species, based on their traits is shown below.

Resem1

Gulf of Maine invertebrate traits

Similarity (0 to 100)

Samples - Species

	ADP	AEP	AH	AS	AA	ANS	APP	AF
ADP								
AEP	86.905							
AH	24.691	24.691						
AS	68.878	67.092	22.222					
AA	64.286	63.095	35.802	46.071				
ANS	30.357	30.357	38.889	38.929	26.786			
APP	25	25	79.012	21.429	20.238	30.357		
AF	39.286	37.5	44.444	49.592	46.429	46.429	42.857	
AR	39.286	37.5	44.444	50.612	46.429	46.429	42.857	97.6
BB	38.095	38.095	43.21	47.092	20.238	51.786	48.81	23.2
BE	47.619	40.476	54.321	39.286	29.762	51.786	61.905	32.1
BV	27.381	28.571	79.012	21.429	23.81	37.5	89.286	35.7
BS	27.381	35.714	71.605	21.429	23.81	37.5	82.143	28.5
BU	46.429	57.143	24.691	45.663	27.381	37.5	32.143	33.9
BT	32.143	39.286	76.543	28.571	27.381	30.357	75	28.5
C	23.81	23.81	64.198	30.357	20.238	44.643	66.667	23.2
CA	38.095	39.286	59.259	47.092	35.714	21.429	57.143	

We mustn't forget that the role of 'samples' is being taken by the species here! (The traits were the variables that created this matrix). However, in subsequent analyses to follow, the species will be variables, and sites will be samples. So let's go ahead and change the properties of this resemblance matrix now.

From 'Resem1', click **Edit > Properties...** and change the following:

- Title 'Gulf of Maine invertebrates'; and
- Between $\bullet$ Variables then click '**OK**' (as shown below).

Resemblance Matrix Properties

Title:
Gulf of Maine invertebrates

Resemblance type

- ☒ Similarity
- ☐ Dissimilarity
- ☐ Distance
- ☐ Distance²
- ☐ Correlation
- ☐ R
- ☐ Rank

Between

- ☐ Samples
- ☒ Variables
- ☐ Other

History:
Resemblance: S17 Bray-Curtis similarity

Number of row/columns: 91

Description:

OK Cancel Help

We now have a resemblance matrix among the species which are clearly identified as variables, hence that is ready for ensuing analyses (see below).

Resem1

Gulf of Maine invertebrates
Similarity (0 to 100)

Variables - Species

	ADP	AEP	AH	AS	AA	ANS	APP	AF	AR	BE
ADP										
AEP	86.905									
AH	24.691	24.691								
AS	68.878	67.092	22.222							
AA	64.286	63.095	35.802	46.071						
ANS	30.357	30.357	38.889	38.929	26.786					
APP	25	25	79.012	21.429	20.238	30.357				
AF	39.286	37.5	44.444	49.592	46.429	46.429	42.857			
AR	39.286	37.5	44.444	50.612	46.429	46.429	42.857	97.619		
BB	38.095	38.095	43.21	47.092	20.238	51.786	48.81	23.214	23.214	
BE	47.619	40.476	54.321	39.286	29.762	51.786	61.905	32.143	32.143	
BV	27.381	28.571	79.012	21.429	23.81	37.5	89.286	35.714	35.714	
BS	27.381	35.714	71.605	21.429	23.81	37.5	82.143	28.571	28.571	
BU	46.429	57.143	24.691	45.663	27.381	37.5	32.143	33.929	32.738	
BT	32.143	39.286	76.543	28.571	27.381	30.357	75	28.571	28.571	
C	23.81	23.81	64.198	30.357	20.238	44.643	66.667	23.214	23.214	
CA	38.095	39.286	59.259	47.092	35.714	21.429	57.143	25	25	
CB	51.786	55.357	31.481	57.092	35.714	42.857	44.643	66.071	64.881	
CI	51.786	55.357	31.481	57.092	35.714	42.857	44.643	66.071	64.881	
CP	73.214	66.071	25.556	70.561	51.786	37.143	24.643	37.5	38.929	

As a next step, let's aim to visualise similarities among species, based on their traits, using a non-metric MDS ordination.

Visualise inter-species trait-based relationships

- From 'Resem1', click **Analyse > MDS > Non-metric MDS (nMDS)...**, and go with all of the default options in the dialog (just click 'OK'). The resulting 2D ordination ('Graph1' in the Explorer tree) is shown below. Different symbols/colours show the different phyla in which individual species (labeled by their abbreviations) belong.

Next, we shall use these trait-based relationships among the species to inform the analysis of functional turnover among the sites, based on the species they contain.

Calculate functional resemblances (Gamma+) among sites

- First, we need to open up the species $\times$ site matrix of presence/absence (occurrence) data in the same workspace (click **File > Open...**). This file is called 'Gulf_of_Maine_invert_occurrences.pri' (also found in the 'Gulf_of_Maine_inverts' folder inside the 'Examples_P8' folder).

Gulf_of_Maine_invert_occurrences

Gulf of Main invertebrate occurrences

Abundance

Samples - Sites

	Spt	BP	BIsI	BHd	PPt	MPt	ScPt	PMPt	RHd	R
AA	0	0	0	0	0	0	0	0	0	1
ADP	1	0	0	0	0	0	0	1	0	0
AEP	0	0	0	0	0	1	1	1	0	0
AF	1	1	0	1	1	1	1	1	0	0
AH	0	0	1	1	0	0	0	0	0	0
ANS	0	0	0	0	0	1	1	0	0	0
APP	1	1	1	1	1	1	1	1	1	0
AR	1	1	0	1	1	0	0	1	1	1
AS	1	0	0	0	0	0	1	1	1	0
BB	1	1	1	1	1	1	1	1	0	1
BE	0	0	0	0	0	0	0	0	0	0
BS	1	1	1	0	0	0	1	0	0	0
BT	0	0	1	0	0	0	0	0	1	0
BU	0	0	0	0	0	0	0	0	0	1
BV	1	1	1	1	1	1	1	1	0	0
C	1	1	1	1	0	0	0	1	1	0
CA	0	0	0	0	1	0	1	0	0	0
CB	0	1	1	1	1	1	1	1	1	1
CF	1	1	1	1	1	1	1	0	0	0
CFR	0	0	0	0	0	0	0	1	1	0
CI	1	1	0	1	1	1	1	1	1	0
CU	0	0	1	0	0	0	0	0	1	0

The sites have abbreviated names. Full names (as shown in the map in Fig. 13.3 above) can be seen by clicking on **Edit > Factors...** You will also see that the sites have been given a rank ordered value for their position along the Maine coastline ('West to East'), and have also been classified as occurring either north or south of Penobscot Bay, viz:

Factors

Edit Fill

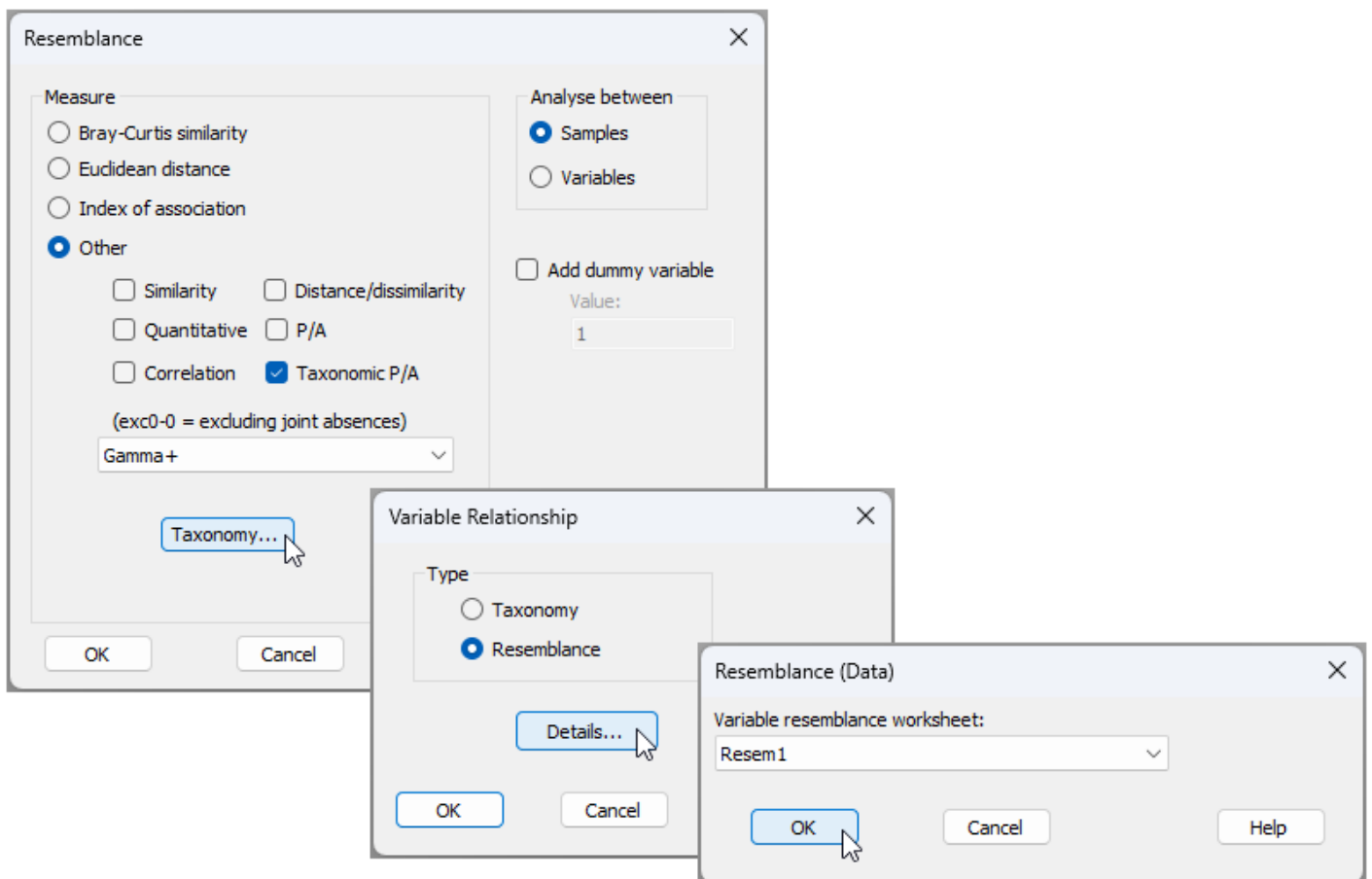
Add... Duplicate Combine... Rename... Rename Levels... Reorder... Delete... Key... Import... OK Cancel Help

Label	Site name	Site abbrev	West to East	Penobscot Bay
SPt	Sea Point	SPt	1	S
BP	Biddeford Pool	BP	2	S
BIsI	Bailey Island	BIsI	3	S
BHd	Bald Head	BHd	4	S
PPt	Pemaquid Point	PPt	5	S
MPt	Marshall Point	MPt	6	S
ScPt	Schoodic Point	ScPt	7	N
PMPt	Petit Mana Point	PMPt	8	N
RHd	Red Head	RHd	9	N
RoBl	Roque Bluffs	RoBl	10	N
BPt	Black Point	BPt	11	N
WQHd	West Quoddy Head	WQHd	12	N

7. Now we shall calculate functional (trait-based) resemblances (Gamma+) among the sites. From the 'Gulf_of_Maine_invert_occurrences' worksheet, click **Analyse** >

Resemblance..., and in the 'Resemblance' dialog window:

- Choose (Analyse between $\bullet$ Samples) & (Measure $\bullet$ Other > $\checkmark$ Taxonomic P/A > Gamma+), and click the 'Taxonomy...' button (Taxonomy...).
- In the 'Variable Relationship' dialog window, choose (Type $\bullet$ Resemblance), and click the 'Details...' button (Details...).
- In the 'Resemblance (Data)' dialog, choose (Variable resemblance worksheet: Resem1).
- Click 'OK' (3 times, once for each successive dialog window) to complete the operation. The dialog windows involved in this operation (i.e., to calculate Gamma+ on the basis of a resemblance matrix, which in our case is a trait-based resemblance matrix) are shown below:



The resulting resemblance matrix of Gamma+ values among the sites (called 'Resem2') is shown below.

Resem2

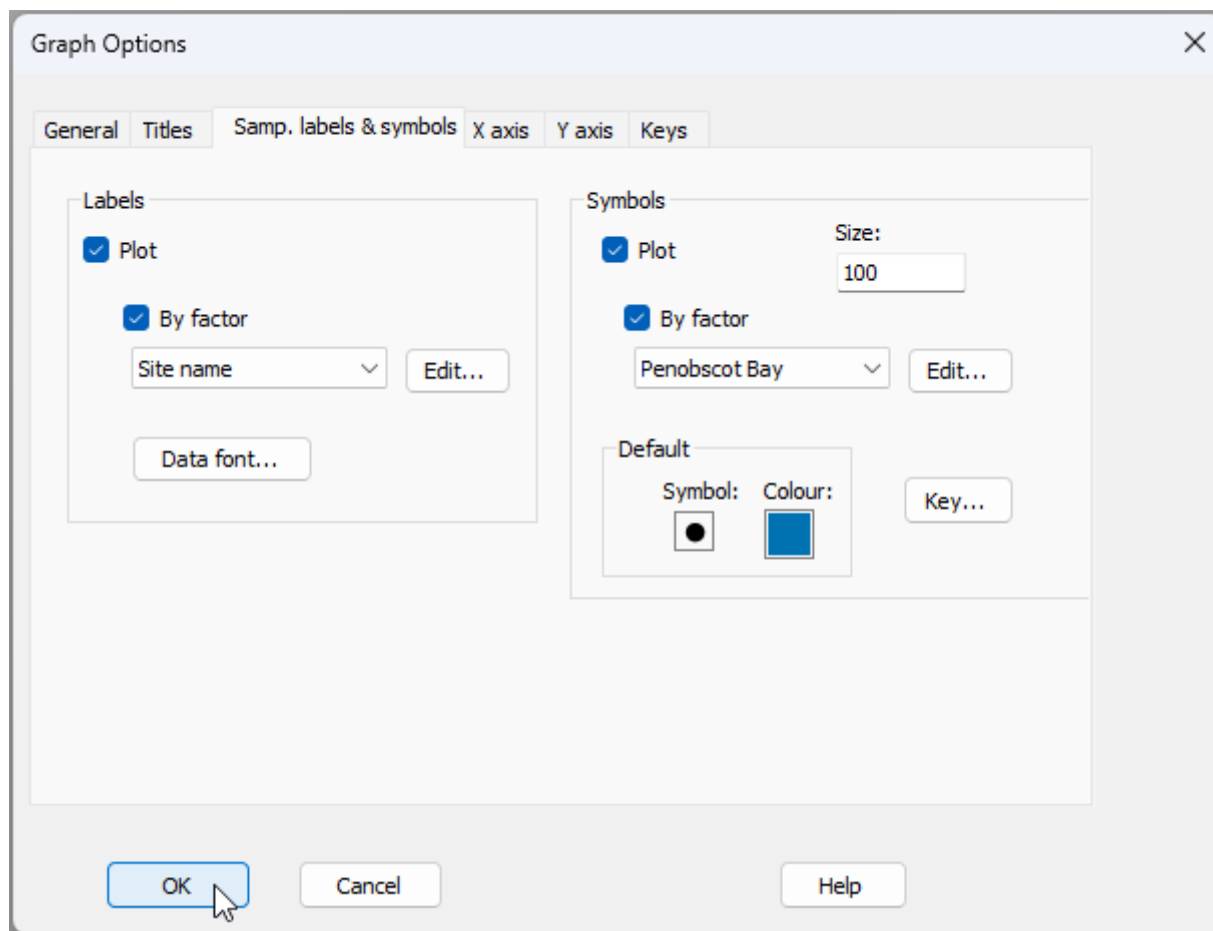
Gulf of Main invertebrate occurrences
Dissimilarity (0 to 100)

	Samples - Sites											
	SPT	BP	BIsI	BHd	PPt	MPt	ScPt	PMPt	RHd	RoBI	BPt	WC
SPT												
BP	5.3744											
BIsI	5.5122	5.1881										
BHd	4.2373	4.4618	3.9962									
PPt	6.21	2.8273	4.8778	2.9953								
MPt	4.9333	4.1816	5.8148	3.99	3.8353							
ScPt	4.1539	5.7213	7.7288	5.5054	5.6803	3.8588						
PMPt	5.7521	7.5095	6.1953	6.6509	8.0954	5.059	6.0106					
RHd	8.0944	8.4376	8.2215	6.7051	7.3283	8.6338	7.1213	7.7042				
RoBI	6.5	5.0083	7.8067	6.7359	6.3122	5.0294	5.0944	5.8051	6.6361			
BPt	8.2854	6.2704	7.8388	6.1272	7.0697	6.6552	4.8497	6.2225	5.8001	6.7433		
WQHd	6.1395	7.5173	7.0454	6.1653	7.1065	5.5993	5.1322	4.8605	6.7192	6.9964	5.1302	

Visualise functional turnover among sites

- We can now easily do an ordination (or indeed a cluster analysis or any other resemblance-based analysis) of the trait-based turnover among the sites. From 'Resem2', click **Analyse > MDS > Non-metric MDS (nMDS)**, just keep all of the defaults and click 'OK'.

9. From the resulting 2D nMDS ordination graphic (called 'Graph5' in the Explorer tree), click **Graph > Sample Labels & Symbols...** and choose to display labels by the factor of 'Site name', and symbols by the factor of 'Penobscot Bay', as shown in the dialog below, then click 'OK'.



The resulting nMDS graphic looks like this:



This plot shows there is a clear shift in functional traits of intertidal assemblages from sites located to the south (blue symbols) vs those located to the north (amber symbols) of Penobscot Bay. We can do statistical tests of relevant hypotheses about this using ANOSIM (from 'Resem2', click **Analyse > ANOSIM...**). Doing this shows that:

- This shift is statistically significant (one-way unordered ANOSIM of the factor 'Penobscot Bay', $R^2 = 0.546$, $P = 0.0022$); and
- There is a significant sequential change in trait-based relationships among these communities along the coastline (one-way ordered ANOSIM of the factor 'West to East', $R^2 = 0.422$, $P = 0.0026$).

[¶]Equally, and in a directly analogous fashion, there are situations where the standardisation of variables is required as a pre-treatment prior to analysis, but which needs to be done separately within groups of samples that may be identified by a factor.

[†]See also the following abstract by Thomas J. Trott, highlighted in the 4th World Conference on Marine Biodiversity: '[Traits matter: when rarity means more than abundance to functional diversity](#)'.

Revision #68

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