

Comparative study of *Candona neglecta* valves from the shallow and deep sites of Lake Mondsee

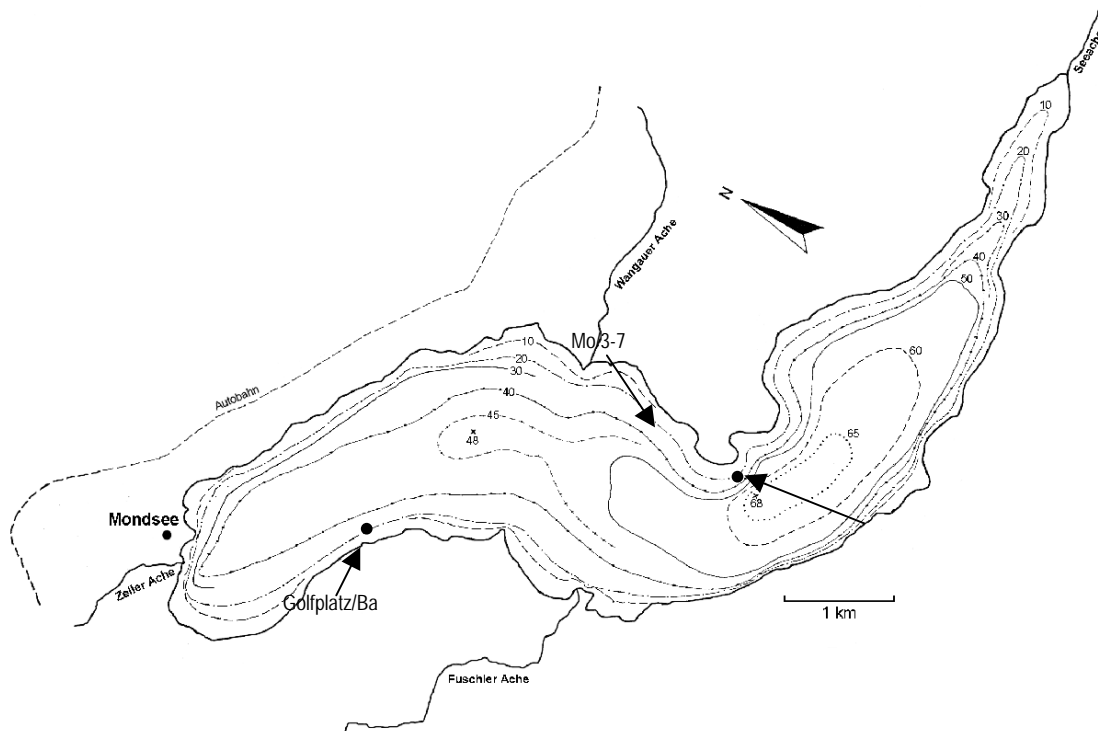
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60 valves of *Candona neglecta* female from different depths of lake Mondsee are compared to see if there is a difference between specimens from deep (65 m) and shallow (3 m-6 m) sites.



Lake Mondsee.

Open MORPHOMATICA to insert the digitalized specimen into the specimen folder. Activate the Cluster folder, go to *Cluster* in the menubar and choose *Select*, click the *Select All* button and *Apply*. Check under *Cluster – Properties* if *Normalize for Area* is selected. The valves are projected on top of each other. The difference between the valves is small, to see if there is a difference between the valves from the shallow parts and the deep parts, select valves from one depth only.



Valves from the deep/shallow sites, Normalized for Area.

More information concerning the differences between the different origins can be obtained, if the valves are compared without normalization. Activate *Cluster*, in *Properties* change to *Don't normalize* and select the valves from the different sites again. The valves from the deep sediment show a greater diversity in size than those from the shallow parts of the lake.



Valves from the deep/shallow sediment, No Normalization.

The data obtained with MORPHOMATICA can be used for further analysis in other programs. In both cases (Normalize for Area, Don't Normalize) all specimen are used for the Cluster analysis.

Select *Display Differences – Area Total* and copy the sheet with a click on the right mouse button. Paste the matrix into field A3 of an Excel worksheet, copy the list of specimen and insert it with *Paste special – Transpose* into field B2, into field A1 fill a title. Save the Excel book. Repeat the copy and paste with the other normalization and insert it into the next sheet of the workbook.

We use for further analysis the program Primer. Start Primer and select Excel files to be opened, in the dialog box choose sheet 1 and pick *Similarities – Ok* and *Dissimilarities – Ok*.

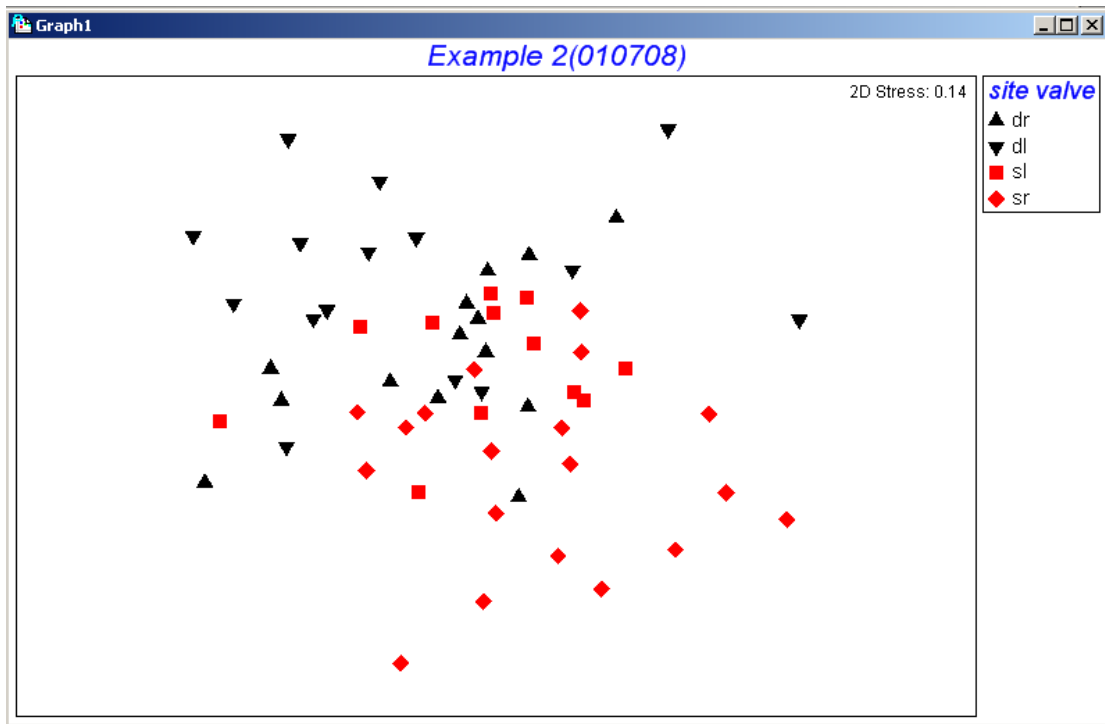
Make sure that the number of rows corresponds to the number of specimen that was used (if it does not, check in the Excel worksheet if something went wrong with copying the list of specimen, any blank field in column A and row 2 other than A2 will be the signal that it is the end of the matrix).

Go to *Edit* and select *Factors*, in the dialog box under *Factors* add a new factor and name it. Fill in the column with abbreviations, under *Factors* pick *Plot key* to give the abbreviations corresponding symbols that you like and sort the abbreviations with the up/down arrows to have them displayed on the side of the plot in the right order. When you are done confirm the abbreviations and plot key with OK. Select *Analyse* in the menubar and *MDS...*, ten restarts are usually sufficient.

The resulting plot needs to be modified, go to *Graph* and *Properties*, select *Display Symbol and Factor*, in the neighbouring field *Label and None*. If you made several columns with factors it is possible to select a factor, confirm with Ok. The plot can be rotated to display the results best (plot on the following page). The first plots are based on a matrix with normalization for area.

dl	Candona neglecta, deep sites, left valve
dr	Candona neglecta, deep sites, right valve
sl	Candona neglecta, shallow sites, left valve
sr	Candona neglecta, shallow sites, right valve

Abbreviations used in the plots below.

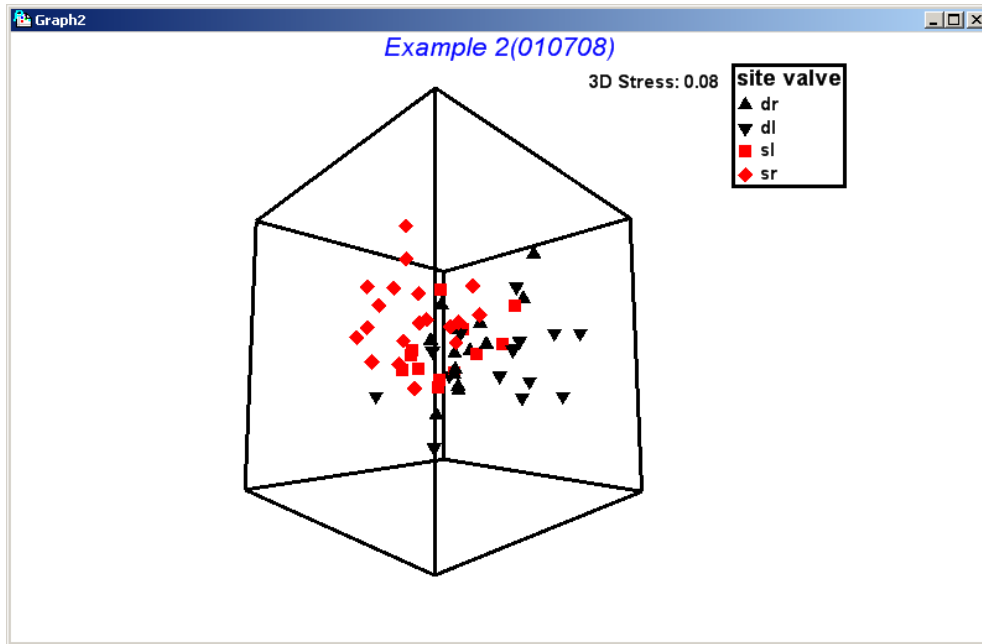


MDS plot using *Normalized for Area*.

In this case, a three dimensional display helps to better understand the differences between the populations (see figure next page).

Go *Graph* and *Properties* again and select 3D instead of 2D in the dimensions field. The resulting graph shows the samples positioned in a three dimensional space. Most symbols have a thin line either going up or down that helps to position the symbols. There is an imaginary plane and the samples are either in, above or beneath that plane (imagine balloons filled with helium = above, or water = below) – the lines connect the symbols with this plane. The graph can be viewed from all around to find the best view of the results.

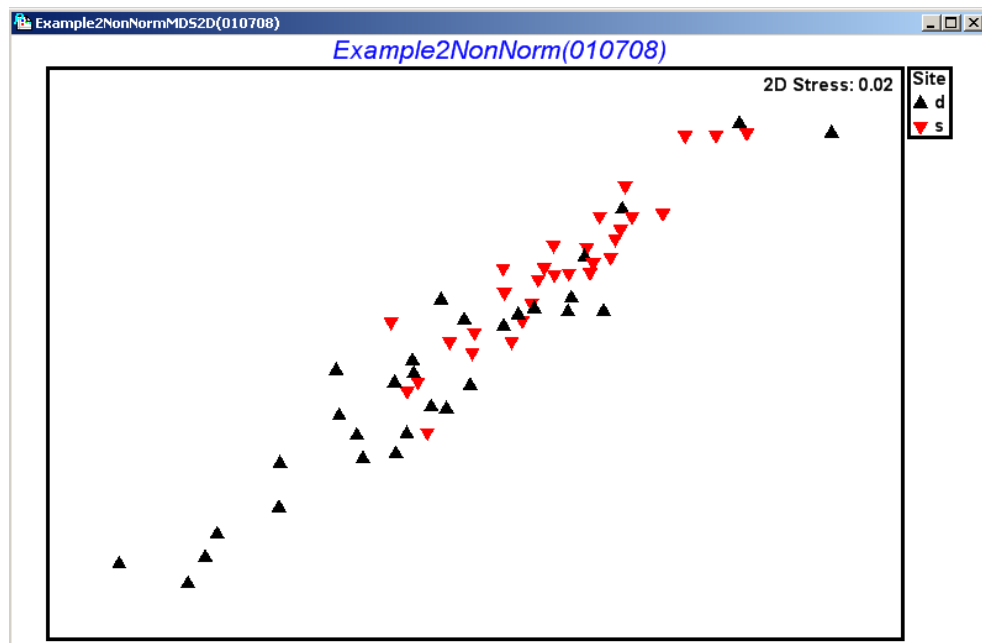
Most of the valves from the deep sediment of lake Mondsee are below the plane, right and left valves are grouped, whereas the valves from the shallow parts of the lake are mostly within or above the plane.



MDS-Plot in 3D.

To open the matrix containing the data from *Don't Normalize* it is necessary to save the first matrix as a Primer file or close it to enable the program to open the same Excel workbook again. In the first dialog box select sheet two as the source otherwise continue as before. Copy the factor list from the first matrix and use it for the new matrix.

The same effect as was shown in MORPHOMATICA above is visible in the plot, the variability is higher in the group of valves from the deep site.

MDS-plot with *No Normalization*.

Primer offers a randomisation test (Anosim) to evaluate the statistical difference between the populations. The example below was performed on the data produced with the setting *Normalize for Area*.

<i>Global Test</i>					
Sample statistic (Global R): 0.25					
Significance level of sample statistic: 0.1%					
Number of permutations: 999 (Random sample from a large number)					
Number of permuted statistics greater than or equal to Global R: 0					
<i>Pairwise Tests</i>					
Groups	R Statistic	Significance Level %	Possible Permutations	Actual Permutations	Number >= Observed
dr, dl	0.137	1.7	77558760	999	16
dr, sl	0.145	1.4	9657700	999	13
dr, sr	0.206	0.4	818809200	999	3
dl, sl	0.22	0.6	17383860	999	5
dl, sr	0.51	0.1	Very large	999	0
sl, sr	0.217	0.4	141120525	999	3

Result section of the Anosim analysis performed with Primer.

There is a rather high difference between left valves from the deep sediment compared to the right valves from the shallow sediment as compares to the other pair-combinations.