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Erik H.W.G. Meesters

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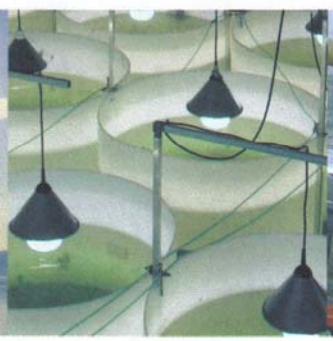


Wageningen IMARES is een
samenwerkingsverband tussen
Wageningen UR en TNO

Wageningen IMARES
Institute for Marine Resources & Ecosystem Studies

Location IJmuiden
P.O. Box 68
1970 AB IJmuiden
The Netherlands
Tel.: +31 255 564646
Fax: +31 255 564644

Location Yerseke
P.O. Box 77
4400 AB Yerseke
The Netherlands
Tel.: +31 113 672300
Fax: +31 113 573477

Location Texel
P.O. Box 167
1790 AD Den Burg Texel
The Netherlands
Tel.: +31 222 369700
Fax: +31 222 319235

Internet: www.wageningenimares.wur.nl
E-mail: imares@wur.nl

Report

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Ecotopes and species composition of the Westerscheldt: can ecotopes be distinguished by species composition?

Erik H.W.G. Meesters

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Nederlandse Samenvatting

In opdracht van het RIKZ is onderzocht of de soortensamenstelling van bodemmonsters van de Westerschelde die van te voren waren ingedeeld in bepaalde ecotopen ook significant van elkaar verschilden. Hiervoor is een multivariate statistische methode gebruikt die analyseerde of monsters uit hetzelfde ecotoop meer op elkaar leken dan monsters uit verschillende ecotopen. Ecotopen worden onderscheiden op basis van fysische verschillen (bv. verschillen in waterdiepte, zoutgehalte, gemiddelde troebelheid van het water, etc.). De monsters waren afkomstig uit 3 perioden: 1995-1997, 2000-2002 en 2003-2005. Deze perioden zijn afzonderlijk geanalyseerd.
Hoewel er 15 verschillende ecotopen in de database aanwezig waren, waren de hoeveelheid monsters per combinatie van ecotoop en jaar zo laag dat maar over 3 ecotopen een uitspraak gedaan kon worden (Z1, Z2 en B1). Uit de resultaten blijkt dat de ecotopen redelijk tot goed van elkaar te scheiden zijn indien we de monsters per periode middelen; dat wil zeggen voor elke periode zijn stations die elk jaar bemonsterd zijn gemiddeld en geanalyseerd. Toch zijn er zelfs dan altijd stations die niet passen in de vooraf gemaakte ecotopenindeling, maar die meer bij een ander ecotoop lijken te horen. Worden alle monsters apart bekeken, dan blijkt er een extreem grote variatie, met name door de tijd, in de samenstelling van de monsters te zijn waardoor het verschil tussen verschillende ecotopen veel minder duidelijk is. Gezien de grote fluctuaties in soortensamenstelling door de tijd lijkt het noodzakelijk om data van meerdere jaren bij elkaar te voegen om zo deze variabiliteit enigszins te verminderen. Verder dienen er meer monsters uit de andere ecotopen verzameld te worden om ook hier iets over te zeggen. Verder onderzoek zou ook kunnen kijken naar soorten die kenmerkend zijn voor de verschillende ecotopen en eventuele gevoelige soorten. Andere manieren om deze dit soort gegevens te analyseren worden ook kort aangegeven, maar vielen buiten de reikwijdte van dit onderzoek.

Summary

As a contract for the National Institute for Coastal and Marine Management (RIKZ) IMARES has analyzed a set of data from the Westerscheldt to investigate whether an *a priori* structure can be found in the data. A multivariate statistical analysis technique was used to determine whether the species composition of bottom samples from assumed different ecotopes was more similar in samples from the same ecotope than from samples from different ecotopes. Ecotopes are distinguished based on physical differences (e.g. differences in water depth, salinity, average turbidity, etc.). Samples came from 3 periods, 1995-1997, 2000-2002 en 2003-2005, which were separately analyzed. Although there were 15 different ecotopes in the database, there were too few samples of each ecotope-year combination to compare all ecotopes. Only three were compared (Z1, Z2, and B1). The results show that the three ecotopes were reasonably to well different from each other if samples for each period were averaged. In all cases, however, there remained stations that appeared to be closer to a different ecotype than the one they were assigned to. Variation of species composition of the samples is very large when samples were not averaged, especially over the years, resulting in very limited distinction power for the different ecotopes. Thus, averaging the samples over several years appears necessary to reduce the amount of variation in the samples. It will also be necessary to collect more samples from the other ecotopes since now only 3 ecotope types could be compared. Further research could focus on distinguishing characteristic species and key species for the different ecotopes. Other approaches for distinguishing the ecotopes are discussed, but were outside the scope of this research.

Introduction

Ecotopes are distinguished on the basis of physical parameters and the general assumption is that these differences in physical characteristics of the environment will be reflected in the composition of the biological communities within these ecotopes. There are now good descriptions of marine ecotopes in the Netherlands, but a formal test of the assumption that the biological communities of these ecotopes is significantly different has not yet been made. Since the proof is in the eating of the pudding, a set of data from RIKZ was given to IMARES to test this assumption. The data consists of biological samples from different ecotopes in the Westerscheldt. The hypothesis tested is that the ecotopes will have significantly different biological communities and consequently samples from within the same ecotope will be more similar than samples from different ecotopes. This hypothesis was tested within a multivariate framework.

Materials and methods

The technique used to visualize the similarity between samples and ecotopes is Non-metric MultiDimensional Scaling (MDS). The basis for an MDS analysis is a matrix with (dis)similarities between the different samples. Bray-Curtis similarity was chosen as an appropriate measure for the analysis of the data (after fourth root transforming the data to reduce the effect of quantitatively dominating species). Larger or smaller distances on a plot of the statistical output correspond to larger or smaller dissimilarities, respectively. The ordination of the points in space may indicate variables that are important for the discrimination of groups of points. MDS does not need any *a priori* knowledge of the grouping of the data. The data consisted of two sets of data, Samples of the MOVE programme were excluded because they are correlated through time being sampled at the exact same location. Samples with no species at all were excluded from the analyses because they do not give any valuable information and tend to distort the Multi-Dimensional Scaling (MDS) pictures.
Significance testing was done using the ANOSIM (analysis of similarities) test (Clarke & Green 1988). It is a simple non-parametric permutation procedure that uses the (rank) similarity matrix underlying the ordination or classification of samples. The main statistical package used for the analyses is Primer (Clarke & Gorley 2006) and the methods used in this report are extensively described in Clarke & Ainsworth (1993) and Clarke & Warwick (2003).

Periods

Samples are from three periods: 1995-1997, 2000-2002, and 2003-2005. And the middle year of each of the three periods is the year for which an ecotope map is available. So the samples from 1995-1997 can be compared with the ecotope map from 1996, the 2000-2002 can be compared to the ecotope map from 2001, etc.

Taxonomic determination levels

Taxa in the samples were analysed to different levels. The levels are:
A: identification to species level
B: identification not to species level, but no overlap with A.
G: identification down to genus level, but overlapping with level A.
X: Very course, comparable to the level of bivalves or worms
F: identification down to family level

Two taxonomic configurations were tested: only samples from A (determination down to species level) and samples from A, B, G, X (lumping all taxa together).

Results and Discussion

Available samples. Samples from the MOVE programme were excluded from the initial analyses for two reasons. Firstly, because of the temporal autocorrelation of the stations. The stations are sampled at exactly the same location each year. This creates a so called repeated measures design or hierarchical design with high autocorrelation between the samples. It is beyond the scope of the present investigation to address these complex matters within multivariate or univariate statistics. Secondly, the sampling in the MOVE programme appears to be not randomly (see table below).

Table 1. Total number of samples with and without the MOVE programme excluding samples with no species. Between brackets, the number of empty samples. In bold the number of samples used.

Period	Without MOVE samples	Including MOVE samples
95-97	**318** (37)	433 (37)
00-02	**296** (43)	437 (43)
03-05	**317** (35)	481 (35)

The number of samples with no species at all appear to be found only in the non-MOVE samples. This suggests that sampling has been different in the two programmes (possibly MOVE samples were not collected randomly). It seems highly unlikely that all samples taken in the MOVE programme contain species if there are so many samples in the other programme that do not.

Samples per Ecotope. How many samples are taken within each Ecotope? It is clear from Table 2 below and **Error! Reference source not found.** that little can be expected from the multivariate analyses with respect to ecotopes B3 to B8, Z3 and Z4, and Z7 and Z8. The most promising ecotopes to show any difference are B1, B2, Z1, Z2, Z5 and Z6. However, since the analyses method is reasonably capable of handling low sample numbers we will try initially to use as many ecotopes as possible.

Table 2. Number of samples from each Ecotope in the 3 periods. Most promising ecotopes are shaded.

	Period		
Ecotope	1995-1997	2000-2002	2003-2005
B1	31	23	44
B2	9	8	5
B3		2	1
B4	1	1	
B5	4	1	7
B6	3	7	3
B8	1		
Z1	161	142	147
Z2	40	35	42
Z3	3	8	1
Z4	2	5	1
Z5	9	4	20
Z6	15	14	9
Z7	1	1	1
Z8	1	2	1
Grand Total	281	253	282

Period 1: 1995-1997

For the combination of Year and Ecotope only few ecotopes have enough samples to allow for a meaningful comparison (see table below). The comparisons were restricted to ecotope Z1, Z2, and B1.

Table 3. Samples within year and ecotope for 1995-1997.

	year		
Ecotope	**1995**	**1996**	**1997**
B1	11	11	9
B2	3	2	4
B4			1
B5	1	3	
B6	2		1
B8	1		
Z1	51	51	59
Z2	14	15	11
Z3		1	2
Z4	1		1
Z5	2	4	3
Z6	5	3	7
Z7		1	
Z8		1	
Grand Total	91	92	98

Overall ANOSIM tests

Overall tests are not significant for Ecotope, but they are for Year. Apparently there is a strong temporal difference between all samples over the 3 years. Normally, no further interpretation should be permissible, but in this case there are many ecotope-year combinations that have only very few samples so we like to look at the pairwise comparisons even though the overall test indicates no significant effect between Ecotopes.

Identification level	ANOSIM Ecotope	ANOSIM Year
A	0.13	0.001
AFGB	0.10	0.001

Pairwise tests (A) Ecotopes

Groups (A)	R Statistic	Significance Level %	Possible Permutations	Actual Permutations	Number >= Observed
Z1, Z2	0.046	12.3	Very large	999	122
Z1, B1	0.037	18.3	Very large	999	182
Z2, B1	-0.006	48.5	Very large	999	484

The ecotopes can not be separated. There appears to be too much overlap in species composition between the different samples as indicated by the low R values. The R statistic is a measure of how well the two groups can be separated and varies between 0 and 1. Values close to 1 generally indicate almost perfect separation with no overlap between samples on an MDS plot.

Pairwise tests (A) Year

Groups (A)	R Statistic	Significance Level %	Possible Permutations	Actual Permutations	Number >= Observed
1995, 1996	0.114	0.1	Very large	999	0
1995, 1997	0.152	0.1	Very large	999	0
1996, 1997	0.030	2.5	Very large	999	24

There is evidence that the composition of the samples over the different years has changed or that at least one of the years, namely 1995, has a significantly different composition. The R statistic again indicates considerable overlap between the samples, so the amount of information that is contained in this significance appears limited. As noted by Clarke and Warwick (2003) it is not so much the significance that is important as this can be reached just by the high number of samples (and comparisons) that are possible, but much more if it is accompanied by a high R value.

MDS 1995-1997 (Taxonomic level: A)

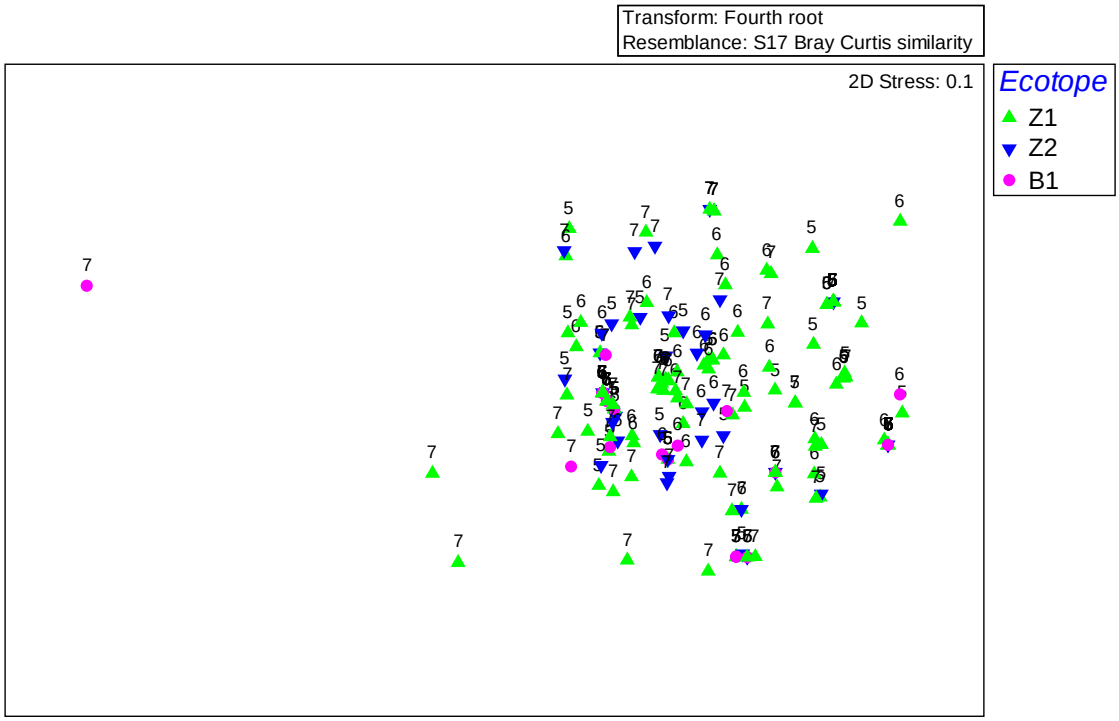

Figure 1. MDS plot of the samples from 1995-1997identified to level A with labels for years (last digit) and symbols for different ecotopes

The MDS plot also shows no clear separation of years or ecotopes. There appears to be some separation of samples from left to right that seems to be correlated with the year of sampling with samples from 1997 being more on the left side. However, there is a lot of overlap between the different samples.

Pairwise tests (AFGB) Ecotopes

When including the other taxonomic groups, the results appear very similar, though there are now slightly significant differences between Z1, Z2 and Z2,B1, however, still with a lot of overlap between the samples.

Groups (AFGB)	R Statistic	Significance Level %	Possible Permutations	Actual Permutations	Number >= Observed
Z1, Z2	0.038	11	Very large	999	109
Z1, B1	0.035	16.6	Very large	999	165
Z2, B1	0.073	6.6	Very large	999	65

Pairwise tests (AFGB) Year

Statistical differences between years remain the same as at the previous taxonomic level (A). Note that the R statistics are very low, indicating much overlap between the samples.

Groups (AFGB)	R Statistic	Significance Level %	Possible Permutations	Actual Permutations	Number >= Observed
1995, 1996	0.103	0.1	Very large	999	0
1995, 1997	0.116	0.1	Very large	999	0
1996, 1997	0.033	1.7	Very large	999	16

MDS (AFGB)

With some goodwill one can see the results of the ANOSIM analyses in the plot below. Samples of B1 are more consistently away from Z2 then from Z1. However, the overlap is clear, meaning that the samples are very similar or that samples within ecotopes vary so much that no distinction can be found. The effect of Year is not clear from the plot.

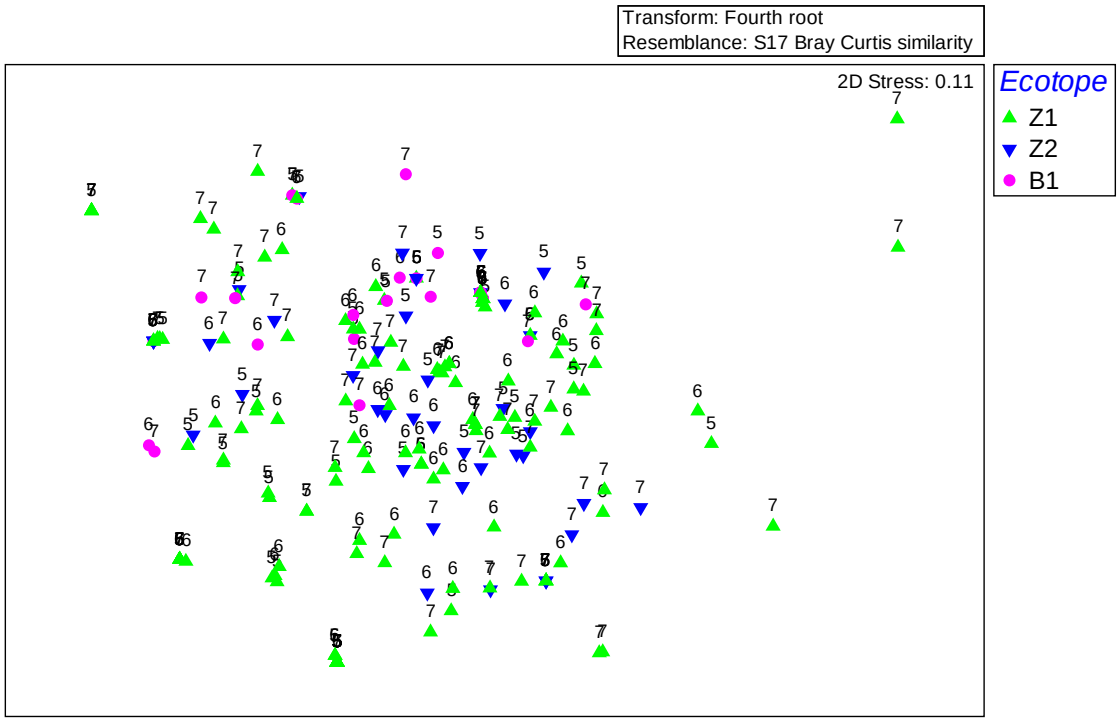

Figure 2. MDS plot of the samples identified to level AFGB with labels for years and symbols for different ecotopes

Means from 1995-1997 (A)

As an alternative approach I've selected only stations that were sampled in each of the 3 years and calculated the mean density over the 3 years (only including fully taxa determined down to species level). Only three ecotopes can be tested that have sufficient numbers of samples, namely B1, Z1 and Z2.
The ANOSIM gave an overall significance of 0.047. Pairwise comparisons are given below and indicate significant differences between Z1, Z2 and Z2, B1. This is also confirmed by the ordination in the MDS plot. Results for the all taxonomic levels together were similar. Note that the R statistics are now much better and that the separation of the samples in the MDS plot is much better. The results of an analysis determining the species differences between the different ecotopes are given in Appendix 1.

Table 4. Results of ANOSIM analysis on ecotope Z1, B1 and Z2 where only stations that were sampled in each of the three years have been used.

Groups	R Statistic	Significance Level%	Possible Permutations	Actual Permutations	Number>= Observed
Z1,Z2	0.203	3.6	38320568	999	35
Z1,B1	0.054	27.5	215553195	999	274
Z2,B1	0.663	0.1	6435	999	0

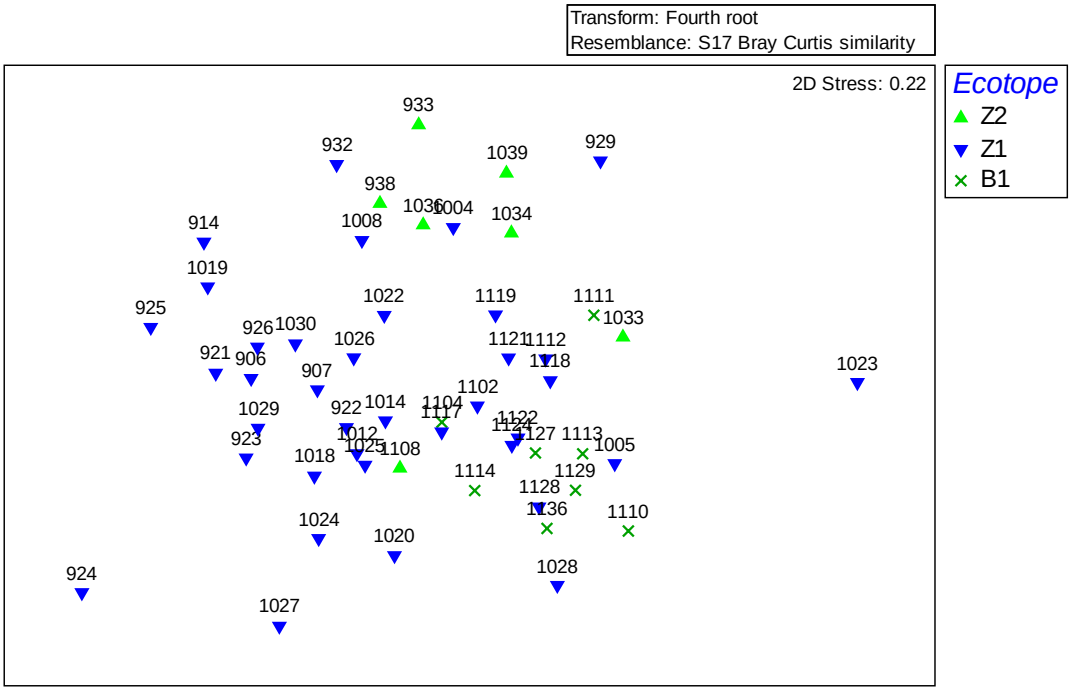

Figure 3. MDS plot of the samples from 1995-1997 identified to level A (everything determined to species level) with symbols for different ecotopes. Only stations that were sampled each of the three years were included.

The MDS plot shows an alignment of the samples in which Z1 samples are more or less present in the whole plot with some dominance in the left part, while the B1 samples are more confined to the lower right part and the Z2 stations to the upper right part. It may be well possible to relate this grouping to one or two environmental variables. There are a few questionable stations, e.g. 929, 1023, 1005, and 1028, these are more similar to Z2 or B1.

Period 2: 2000-2002

For the combination of Year and Ecotope only ecotopes Z1, Z2, and B1 have enough samples to allow for a meaningful comparison.

Table 5. Samples within year and ecotope for 1995-1997.

	year		
Ecotope	**2000**	**2001**	**2002**
B1	6	8	9
B2	3	3	2
B3	1	1	
B4		1	
B5	1		
B6	3	1	3
Z1	41	46	55
Z2	9	15	11
Z3	2	2	4
Z4	3	1	1
Z5	1	1	2
Z6	5	4	5
Z7			1
Z8	2		
Grand Total	77	83	93

Overall ANOSIM tests using all ecotopes

In the second period there appears a reasonable distinction between ecotopes and years possible. Again from the pairwise comparisons there appears to be considerable overlap, however, the R statistics for comparisons with B1 are clearly higher.

Identification level	ANOSIM Ecotope	ANOSIM Year
A	0.035	0.037
AFGB	0.04	0.017

Pairwise tests (A) Ecotopes

Groups (A)	R Statistic	Significance Level %	Possible Permutations	Actual Permutations	Number >= Observed
Z1, Z2	0.004	44.4	Very large	999	443
Z1, B1	0.126	0.2	Very large	999	1
Z2, B1	0.193	0.3	Very large	999	2

Z1 and Z2 differ significantly from B1, but not from each other.

Pairwise tests (A) Year

Groups (A)	R Statistic	Significance Level %	Possible Permutations	Actual Permutations	Number >= Observed
2000, 2001	0.017	13.4	Very large	999	133
2000, 2002	0.031	5.4	Very large	999	53
2001, 2002	0.028	5.3	Very large	999	52

MDS 2000-2002 (Taxonomic level: A)

The MDS shows considerable overlap between the samples from the different ecotopes, but B1 is more clearly separated from Z1 and Z2.

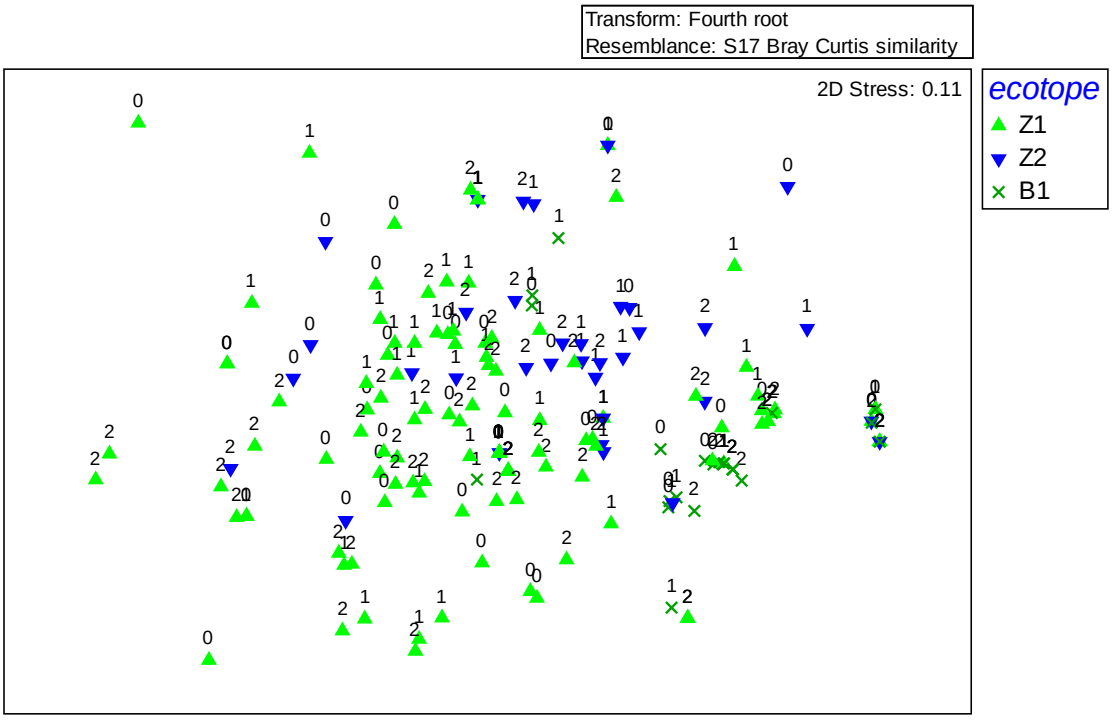

Figure 4. MDS plot of the samples from 2000-2002 identified to level A with labels for years (last digit) and symbols for different ecotopes

Pairwise tests (AFGB) Ecotopes

Groups (AFGB)	R Statistic	Significance Level %	Possible Permutations	Actual Permutations	Number >= Observed
Z1, Z2	-0.004	49.2	Very large	999	491
Z1, B1	0.121	0.3	Very large	999	2
Z2, B1	0.206	0.2	Very large	999	1

The conclusions are very similar as for the analysis which used only the species that were identified to species level. Ecotope Z1 and Z2 are significantly different from B1, although there is considerable overlap between de species composition of the individual samples.

Pairwise tests (AFGB) Year

Groups (AFGB)	R Statistic	Significance Level %	Possible Permutations	Actual Permutations	Number >= Observed
2000, 2001	0.017	12.1	Very large	999	120
2000, 2002	0.039	2.9	Very large	999	28
2001, 2002	0.026	5.6	Very large	999	55

Again there is some evidence that there are year effects, however, note that the R statistics are very low, indicating a very large amount of overlap between the samples. This is also evident from the MDS plot below.

MDS (AFGB)

The MDS looks different, but it can be rotated any way, so if rotating clockwise the two plots from both identification levels may appear very similar.

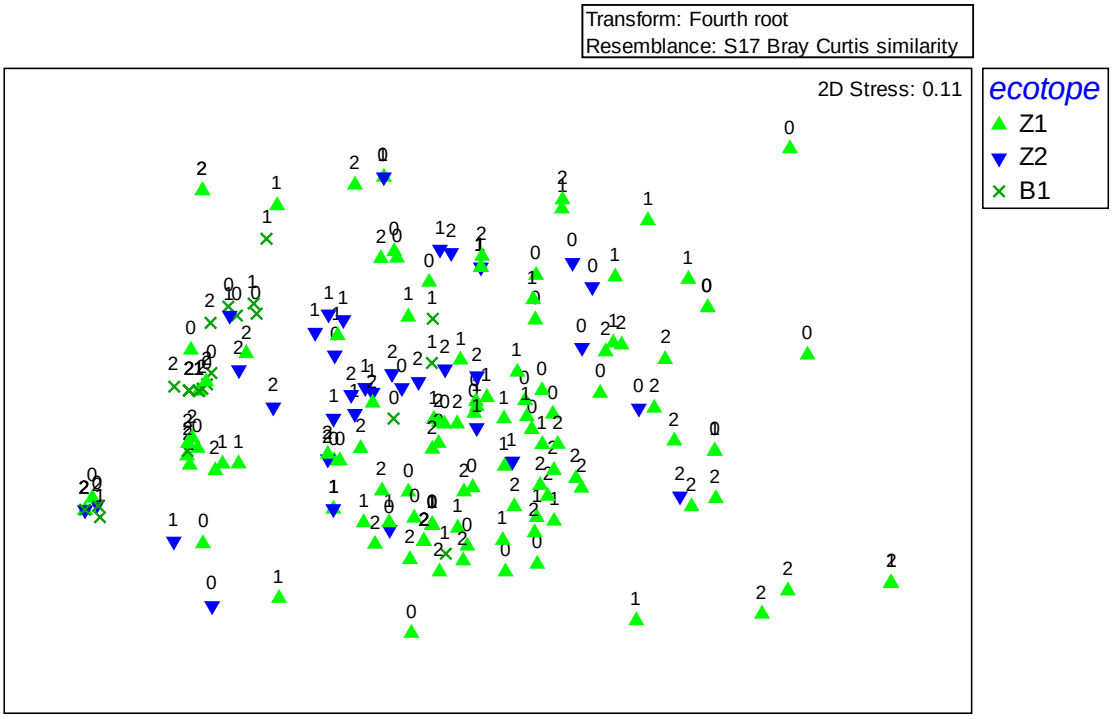

Figure 5. MDS plot of the samples identified to levels AFGB with labels for years and symbols for different ecotopes

Means from 2000-2002 (A)

For this period stations which were samples each year have been averaged as well and results are shown below. The overall ANOSIM test has a significance of 0.08 and pairwise comparisons show a significant difference between Z1,Z2 and Z2,B1.

Groups	R Statistic	Significance Level %	Possible Permutations	Actual Permutations	Number >= Observed
Z1, Z2	0.17	7.3	5985	999	72
Z1, B1	0.093	22.3	26334	999	222
Z2, B1	0.322	3.2	126	126	4

Especially the comparison between Z2 and B1 has a large R statistic indicating a high level of separation. Strangely, now Z1 and B1 are no longer significantly different, but Z1 and Z2 are. This may be caused by the fact that some stations are no longer part of the data set, because they were not sampled every year.

The MDS plot shown in Figure 6 indicates that station 1125 (B1) is very different from other stations in that Ecotope. Station 1036 (Z1) looks more like samples from Z2.

The results of an analysis determining the species differences between the different ecotopes are given in Appendix 1.

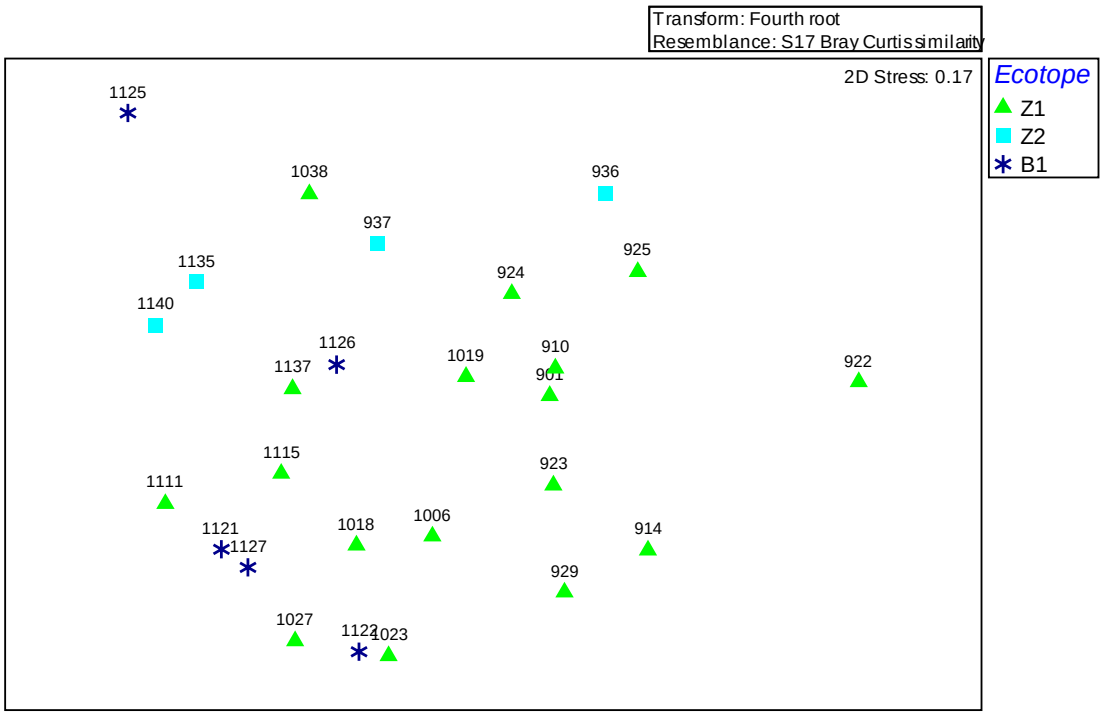

Figure 6. MDS plot of the samples from 2000-2005 identified to taxonomic level A (only samples identified to species level) with symbols for different ecotopes. Only stations that were sampled in each of the three years have been used.

Period 3: 2003-2005

Overall ANOSIM tests

For the combination of Year and Ecotope only few ecotopes have enough samples to allow for a meaningful comparison.

Table 6. Number of samples for each ecotope in each of the 3 years.

	year		
Ecotope	**2003**	**2004**	**2005**
B1	10	20	14
B2	1	2	2
B3	1		
B5		3	4
B6		2	1
Z1	50	45	52
Z2	12	16	14
Z3			1
Z4		1	
Z5	6	3	11
Z6	5	3	1
Z7	1		
Z8		1	
Grand Total	86	96	100

For the overall ANOSIM test only ecotopes Z1, Z2, and B1 could be used. The test indicates significant differences between ecotopes and years.

Identification level	ANOSIM Ecotope	ANOSIM Year
A	0.032	0.019
AFGB	0.035	0.012

Pairwise tests (A) Ecotopes

Groups (A)	R Statistic	Significance Level %	Possible Permutations	Actual Permutations	Number >= Observed
Z1, Z2	0.034	15.1	Very large	999	150
Z1, B1	0.067	1.7	Very large	999	16
Z2, B1	0.193	0.1	Very large	999	0

Z1 and Z2 are clearly different from B1, but are not different from each other. The R statistic is very low, indicating considerable overlap between the different ecotopes.

Pairwise tests (A) Year

Groups (A)	R Statistic	Significance Level %	Possible Permutations	Actual Permutations	Number >= Observed
2003, 2004	0.036	1.7	Very large	999	16
2003, 2005	0.008	21.5	Very large	999	214
2004, 2005	0.021	7.9	Very large	999	78

2004 is different from 2003 and 2005, but the R statistic indicates large overlap between the different years. Again the MDS shows these results as any general pattern is far from clear.

MDS 2003-2005 (Taxonomic level: A)

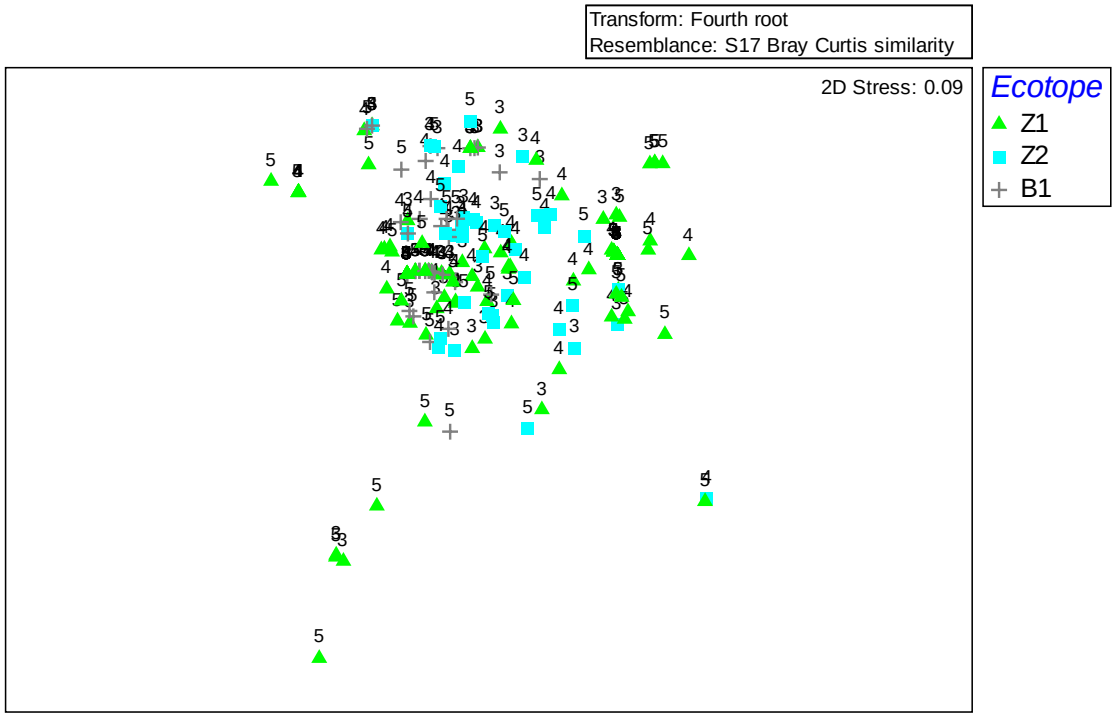

Figure 7. MDS plot of the samples identified to level A with labels for years (only last digit) and symbols for different ecotopes

The MDS is not very clear at discriminating the ecotopes or years. The lack of difference between Z1 and Z2 samples is clear from the fact that Z2 is positioned between samples from Z1.

Pairwise tests (AFGB) Ecotopes

Groups (AFGB)	*R Statistic*	*Significance Level %*	*Possible Permutations*	*Actual Permutations*	*Number >= Observed*
Z1, Z2	0.034	13.9	Very large	999	138
Z1, B1	0.063	1.3	Very large	999	12
Z2, B1	0.21	0.1	Very large	999	0

Pairwise tests (AFGB) Year

Groups (AFGB)	*R Statistic*	*Significance Level %*	*Possible Permutations*	*Actual Permutations*	*Number >= Observed*
2003, 2004	0.035	2.8	Very large	999	27
2003, 2005	0.02	6.2	Very large	999	61
2004, 2005	0.03	3.5	Very large	999	34

MDS (AFGB)

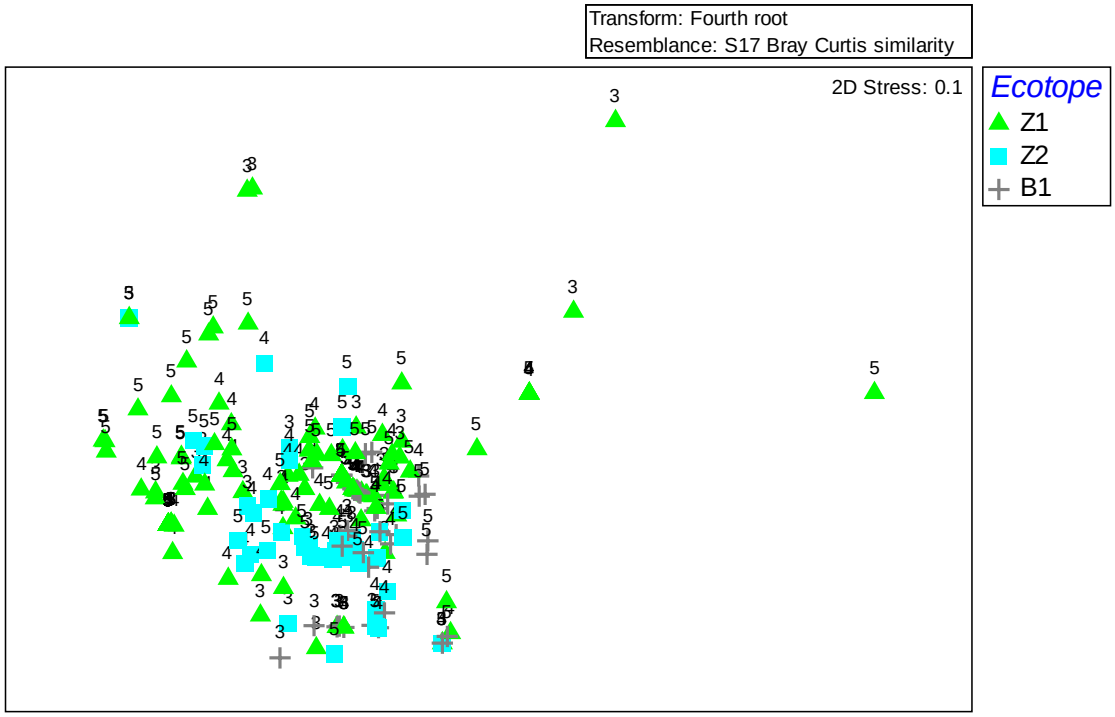

Figure 8. MDS plot of the samples identified to level AFGB with labels for years and symbols for different ecotopes

Means from 2003-2005 (A)

Stations that were sampled each year were averaged and the same analyses as for the other periods applied. The overall ANOSIM test 0.001 and pairwise comparisons indicated highly significant differences between all pairs of ecotopes..

Groups	R Statistic	Significance Level %	Possible Permutations	Actual Permutations	Number >= Observed
Z1, Z2	0.433	0.1	3365856	999	0
Z1, B1	0.154	4.1	600805296	999	40
Z2, B1	0.713	0.1	31824	999	0

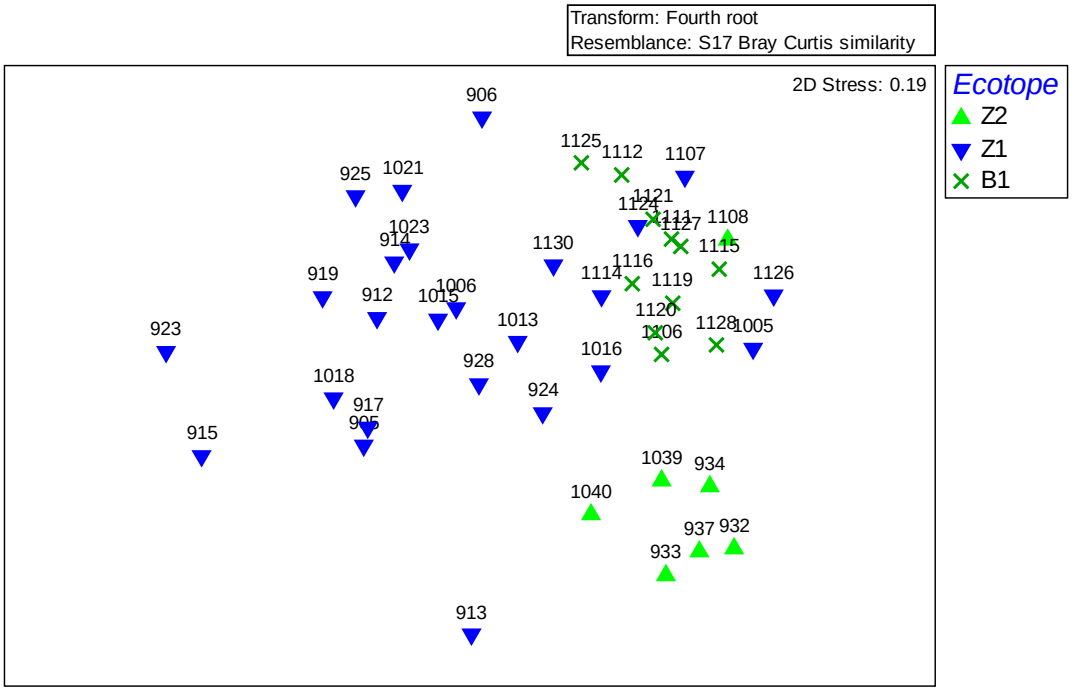

Figure 9. MDS plot of the samples identified to level A with labels for stations and symbols for different ecotopes

Ecotopes appear to be better distinguished in this period, especially Z2 and B1. The stress value indicates that the separation of the samples would be better in 3 dimensions. Even after averaging there are still samples that are very different from other samples. For example station 913 appears very different and also station 1108.

Discussion and conclusions

Samples from two monitoring programmes were analysed by a multivariate technique to assess whether an assumed grouping (ecotopes, distinguished on the basis of physical parameters) could be detected in the samples. Samples from the MOVE programme were found to be suspect for two reasons: they are from stations that are repeatedly measured without very little spatial distance between samples. However, this doesn't need to be a serious problem. One can take the average or adjust the multivariate analysis to accommodate the repeated design (e.g. Clarke et al. 2006). The other thing that is more disturbing is that samples from the MOVE programme do not include samples with no species at all, something which is very common with normal random sampling and is apparent in the samples from the other programme. At this point in time it was unclear whether sampling has been done non-randomly or if empty samples have been removed in this dataset. In either way analysing the two data sets as if they are one and the same seems not correct. Therefore the analyses were restricted to the first data set.

Data from the first period, 1995-1997, show no differences between ecotopes and only differences between the different years, however, there is very much overlap between the species composition of the different samples. A small difference between ecotope Z2 and B1 can be found when we look not only at the species level, but include all taxa. Even then, the amount of overlap between the different samples is very large. Only when we take stations that have been sampled every year in this period (not exactly at the same location, but nevertheless, close enough to be considered the same location), a difference between ecotopes emerges. Again Z2 and B1 are different, but now also a difference between Z1 and Z2 is found. Many Z1 stations appear to be still very similar to Z2 and though less, also to B1.

In the second period, 2000-2002, the ecotopes appear somewhat more different than in the first period. Ecotope B1 is significantly different from Z1 and Z2, but there is again much overlap between the samples. Also the year 2002 appears different from 2000 and 2001. If we average again the samples from stations that have been sampled each of the three years the differences become stronger, but now the difference between Z1 and B1 disappears.

In the third period, 2003-2005, differences are clear between ecotopes and years except for a comparison between Z1 and Z2 and between the years 2003 and 2005. When the means for the three years are taken, the differences become much more pronounced showing very little overlap between the different ecotopes (Z1, Z2, B1). There again some stations in each of the ecotopes appear to be more similar to stations in one of the other ecotopes.

From the analyses of the different data sets it appears that throughout the years the separation of the different ecotopes has increased. Remarkably this has been accompanied with an increasing number of different species within the samples: from the first to the third period the number of different species in the averaged samples increased from 70 (119 stations) to 87 (118 stations) and finally to 104 (119 stations). Two reasons are possible: either the number of species has increased or the species determination has improved. Fact is that in most cases there are significant differences between samples that have been assigned *a priori* to either one of the ecotopes Z1, Z2 or B1. However, often there are stations that appear to be better assigned to a different ecotope. It may be worthwhile to investigate these stations better and see why they do not conform to the average picture of the ecotope that they are thought to belong to.

Quickly, it became clear from data the that there are too few samples for most of the ecotopes. Future sampling should therefore give more attention to these missing ecotopes, mostly B3-B8, Z3-Z4, and Z7-Z8. As a rule of thumb, each of these should have at least 5 stations in each year. The analyses also indicated strong temporal fluctuations in the species composition of the samples and that better distinctions can be made if samples are averaged over several years. A consequence of the large temporal fluctuations is that changes in community structure as a consequence of environmental perturbations will be difficult to detect on a short notice. Possibly, one has to search for key species or very sensitive species in each ecotope.
Another possibility is to set up a repeated measures experiment in which stations are sampled repeatedly over time and the time trajectory is modeled through a multivariate seriation pattern (Clarke et al. 2006). Patterns can than be compared, but normally this would require more than 3 points through time.

Other techniques

In this report we considered the samples as already belonging to a certain ecotope. We can also start from another angle assuming that we do not know from which ecotope the samples come and see if we can detect structure in the samples. If we do that for the mean data from the last period we get the picture shown below.

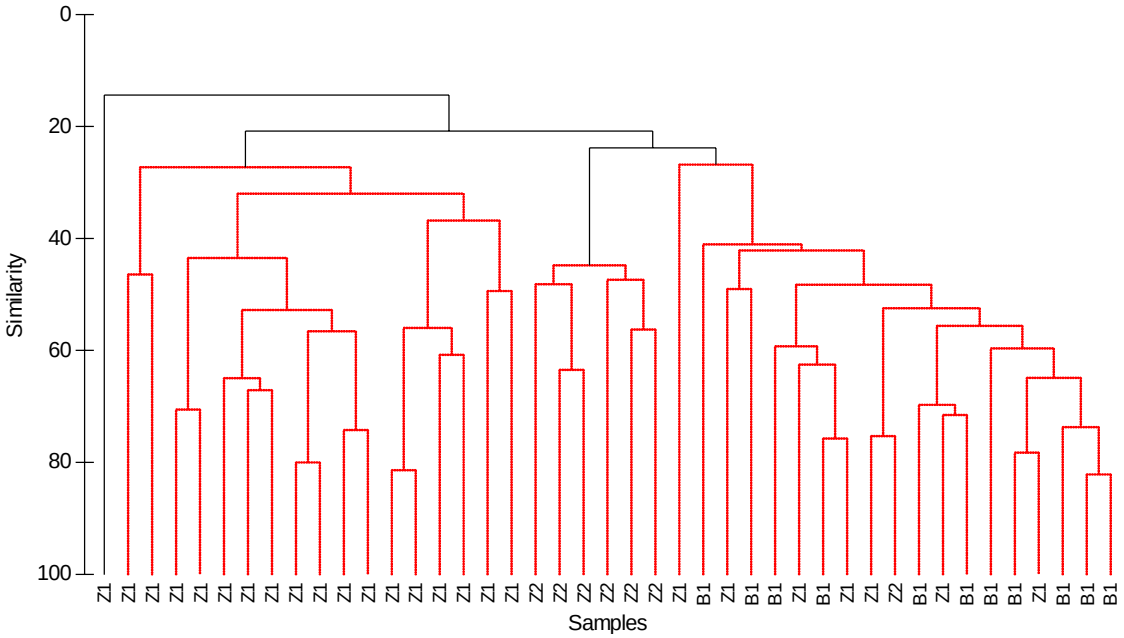

Figure 10. Cluster dendrogram of mean data from 2003-2005 using only station sampled each year. The connected red lines are not statistically different, assuming no initial grouping in the data.

From this analysis we can see 4 significant groups, one containing only samples from Z2, one containing Z1 samples and one containing mainly B1, some Z1 samples and one Z2 sample. Thus the ecotope structure is clearly present, but some stations clearly do not match the *a priori* grouping. In this case the biological samples are the start of the analysis, while before the ecotopes (thus the physical characteristics) are the start of the analysis. Since living organisms integrate many of the often complex interactions in nature, they may give a more accurate picture of the living conditions at the actual site. This approach seems also intuitively more in place, since it is directed at the living communities which are by definition the focus of many natural conservation programmes, not the physical environment. Biological communities can then be linked to physical variables through a multitude of techniques (e.g. multinomial regression, discriminant analysis, (multivariate) regression and classification trees etc.).

Univariate Techniques. Individual species or species indices such as species richness can also be linked to environmental variables using state-of-the-art methods such as Generalized Linear Mixed Models and Generalized Mixed Additive Models that also provide ways to deal with temporal or spatial autocorrelation.

References

Clarke KR, Ainsworth M (1993) A method of linking multivariate community structure to environmental variables. Mar Ecol Prog Ser 92:205-219

Clarke KR, Gorley RN (2006) Primer v6: user manual/tutorial, Vol. Primer-e ltd, Plymouth

Clarke KR, Green RH (1988) Statistical desing and analysis for a 'biological effects' study. Mar Ecol Prog Ser 46:213-226

Clarke KR, Somerfield PJ, Airoldi L, Warwick RM (2006) Exploring interactions by second-stage community analyses. J Exp Mar Biol Ecol in press

Clarke KR, Warwick RM (2003) Change in marine communities: an approach to statistical analysis and interpretation.

Appendix 1: Ecotope composition

Differences between the ecotopes when the means of the stations are taken have been further analysed to look for the species that determine the amount of dissimilarity between the samples. the results of these analyses (so called SIMPER analysis) are given below. Av. Diss, the average dissimilarity between the samples; Diss/SD is the average dissimilarity divided by the standard deviation which is a measure of how constant the species is spread over the different samples (higher means that a species is mostly present in one ecotope and absent in the other), contrib%, the percentage a species contributes to the total dissimilarity; Cum.%, the cummulative percentage.

Period 1, 1995-1997

Groups Z2 & Z1

Average dissimilarity = 80.86

Species	Group Z2 Av.Abund	Group Z1 Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
hetefili	4.10	2.16	8.26	1.13	10.22	10.22
spiomart	2.12	1.08	6.26	0.72	7.74	17.95
hydrulva	3.34	0.28	5.75	0.85	7.11	25.07
bathpilo	2.33	0.92	5.34	0.90	6.60	31.67
macobalt	2.76	0.49	4.97	1.25	6.15	37.82
pygoeleg	2.28	0.23	3.89	0.93	4.81	42.62
nephcirr	0.36	1.04	3.87	0.55	4.79	47.41
aphemari	2.20	0.43	3.70	0.81	4.58	51.99
eurypulc	1.02	0.71	3.52	0.58	4.35	56.33
scolarmi	1.25	0.61	3.31	0.72	4.09	60.43
capicapi	1.00	0.83	3.08	0.64	3.81	64.24
nephhomb	1.01	0.52	2.99	0.67	3.70	67.94
neredive	1.62	0.00	2.59	0.57	3.20	71.14
hausaren	0.49	0.67	2.48	0.55	3.06	74.20
ceraedul	1.74	0.06	2.45	0.83	3.03	77.23
scroplan	1.42	0.03	2.00	0.70	2.47	79.70
corovolu	0.84	0.06	1.75	0.33	2.16	81.86
neresucc	0.86	0.24	1.72	0.54	2.12	83.99
polycorn	0.91	0.21	1.46	0.53	1.81	85.80
arenmari	0.69	0.14	1.26	0.55	1.56	87.36
crancran	0.50	0.10	1.12	0.51	1.39	88.75
coroaren	0.60	0.07	1.09	0.46	1.35	90.10

Groups Z2 & B1

Average dissimilarity = 81.93

Species	Group Z2 Av.Abund	Group B1 Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
bathpilo	2.33	3.64	9.25	0.95	11.29	11.29
hetefili	4.10	2.32	8.52	1.08	10.40	21.69
spiomart	2.12	0.22	7.40	0.84	9.03	30.72
hydrulva	3.34	0.94	6.53	1.03	7.97	38.69
hausaren	0.49	1.76	5.14	0.78	6.27	44.96
macobalt	2.76	0.22	4.82	1.41	5.88	50.84
pygoeleg	2.28	0.22	3.84	0.96	4.69	55.53
eurypulc	1.02	0.89	3.48	0.75	4.25	59.78
aphemari	2.20	0.00	3.27	0.77	4.00	63.77
neredive	1.62	0.22	2.93	0.64	3.58	67.36
scolarmi	1.25	0.00	2.62	0.68	3.20	70.56
capicapi	1.00	0.54	2.60	0.62	3.17	73.73
nephhomb	1.01	0.00	2.45	0.67	2.99	76.72
ceraedul	1.74	0.00	2.38	0.82	2.90	79.62
scroplan	1.42	0.00	1.97	0.69	2.40	82.02
nephcirr	0.36	0.22	1.93	0.38	2.35	84.37
neresucc	0.86	0.31	1.72	0.61	2.10	86.47
corovolu	0.84	0.00	1.60	0.31	1.95	88.43

polycorn	0.91	0.29	1.54	0.58	1.88	90.31

Groups Z1 & B1
Average dissimilarity = 73.65

Species	Group Z1 Av.Abund	Group B1 Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
bathpilo	0.92	3.64	13.81	1.40	18.75	18.75
hetefili	2.16	2.32	7.94	0.96	10.79	29.53
hausaren	0.67	1.76	7.49	1.02	10.17	39.70
eurypulc	0.71	0.89	5.16	0.80	7.01	46.71
nephcirr	1.04	0.22	5.02	0.72	6.82	53.53
spiomart	1.08	0.22	4.80	0.74	6.51	60.04
capicapi	0.83	0.54	4.58	0.73	6.22	66.26
hydrulva	0.28	0.94	4.26	0.81	5.79	72.05
macobalt	0.49	0.22	2.14	0.54	2.90	74.95
scolarmi	0.61	0.00	1.99	0.48	2.70	77.65
nephhomb	0.52	0.00	1.84	0.45	2.50	80.15
neresucc	0.24	0.31	1.59	0.42	2.16	82.31
polycorn	0.21	0.29	1.38	0.42	1.88	84.19
pygoeleg	0.23	0.22	1.16	0.42	1.58	85.77
crancran	0.10	0.22	1.14	0.37	1.54	87.31
aphemari	0.43	0.00	1.09	0.33	1.49	88.80
neredive	0.00	0.22	0.84	0.32	1.14	89.94
mytiedul	0.00	0.26	0.73	0.33	1.00	90.93

Period 2, 2000-2002
Groups Z1 & Z2
Average dissimilarity = 84.22

Species	Group Z1 Av.Abund	Group Z2 Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
hetefili	1.80	2.26	7.67	1.04	9.11	9.11
bathpilo	0.55	2.21	7.53	0.88	8.94	18.05
macobalt	0.79	1.35	5.18	0.87	6.15	24.20
pygoeleg	0.20	1.66	4.98	0.93	5.91	30.11
aphemari	0.60	1.08	4.69	0.72	5.57	35.68
hausaren	0.50	0.42	3.97	0.49	4.71	40.39
corovolu	0.25	1.33	3.85	0.53	4.57	44.96
scolarmi	0.50	0.88	3.78	0.73	4.49	49.45
spiomart	0.40	0.83	3.51	0.62	4.16	53.61
hydrulva	0.11	1.25	3.42	0.69	4.06	57.68
nephcirr	0.52	0.36	3.37	0.56	4.00	61.68
neresucc	0.19	0.95	2.90	0.55	3.44	65.12
eurypulc	0.32	0.62	2.51	0.49	2.98	68.10
magemira	0.25	0.21	1.97	0.37	2.33	70.43
neredive	0.07	0.76	1.76	0.48	2.09	72.52
polycorn	0.21	0.55	1.70	0.44	2.02	74.54
mya_aren	0.05	0.43	1.35	0.38	1.60	76.15
coroaren	0.10	0.40	1.28	0.37	1.52	77.67
nephcaec	0.15	0.16	1.27	0.30	1.50	79.17
scolsqua	0.00	0.34	1.24	0.33	1.48	80.65
mytiedul	0.21	0.32	0.98	0.37	1.17	81.81
crancran	0.23	0.00	0.94	0.24	1.12	82.94
ceraedul	0.10	0.30	0.93	0.41	1.10	84.04
strebene	0.15	0.11	0.92	0.33	1.09	85.13
cyatcari	0.04	0.42	0.90	0.39	1.07	86.20
nephhomb	0.25	0.00	0.89	0.32	1.06	87.26
parafulg	0.06	0.14	0.88	0.29	1.05	88.31
ophelima	0.12	0.17	0.88	0.29	1.04	89.35
bathsars	0.00	0.24	0.80	0.26	0.95	90.30

Groups Z1 & B1
Average dissimilarity = 80.82

Species	Group Z1 Av.Abund	Group B1 Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
bathpilo	0.55	1.95	12.63	0.96	15.63	15.63
hetefili	1.80	2.09	10.55	1.07	13.06	28.69
hausaren	0.50	0.64	6.16	0.63	7.62	36.31
eurypulc	0.32	0.57	5.00	0.64	6.19	42.50
macobalt	0.79	0.46	4.75	0.68	5.88	48.38
polycorn	0.21	1.32	4.47	0.63	5.53	53.90
nephcirr	0.52	0.00	2.97	0.51	3.67	57.58
corolacu	0.06	1.19	2.90	0.46	3.59	61.17
scolarmi	0.50	0.00	2.55	0.47	3.16	64.33
aphemari	0.60	0.00	2.48	0.47	3.07	67.39
spiomart	0.40	0.00	2.23	0.39	2.76	70.15
corovolu	0.25	0.51	2.19	0.47	2.71	72.86
hydrulva	0.11	0.48	2.01	0.42	2.49	75.35
pygoeleg	0.20	0.45	1.84	0.50	2.27	77.62
crancran	0.23	0.20	1.63	0.33	2.02	79.64
cyatcari	0.04	0.51	1.46	0.47	1.80	81.44
neresucc	0.19	0.26	1.34	0.38	1.65	83.10
magemira	0.25	0.00	1.23	0.33	1.52	84.62
nephhomb	0.25	0.00	1.21	0.34	1.50	86.12
nephcaec	0.15	0.00	1.15	0.23	1.43	87.55
mytiedul	0.21	0.20	0.92	0.39	1.13	88.68
ophelima	0.12	0.00	0.81	0.23	1.00	89.68
micrsimi	0.12	0.00	0.68	0.22	0.84	90.52

Groups Z2 & B1
Average dissimilarity = 78.26

Species	Group Z2 Av.Abund	Group B1 Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
bathpilo	2.21	1.95	8.10	0.89	10.34	10.34
hetefili	2.26	2.09	7.68	1.04	9.82	20.16
pygoeleg	1.66	0.45	4.88	0.94	6.23	26.40
macobalt	1.35	0.46	4.78	0.83	6.10	32.50
hausaren	0.42	0.64	4.23	0.61	5.40	37.91
corovolu	1.33	0.51	4.14	0.61	5.28	43.19
polycorn	0.55	1.32	3.92	0.68	5.00	48.19
aphemari	1.08	0.00	3.69	0.61	4.72	52.91
hydrulva	1.25	0.48	3.47	0.82	4.43	57.34
eurypulc	0.62	0.57	3.43	0.64	4.39	61.73
neresucc	0.95	0.26	3.04	0.58	3.89	65.62
scolarmi	0.88	0.00	2.80	0.66	3.57	69.19
corolacu	0.33	1.19	2.75	0.51	3.51	72.71
spiomart	0.83	0.00	2.43	0.55	3.11	75.82
nephcirr	0.36	0.00	1.81	0.36	2.31	78.13
cyatcari	0.42	0.51	1.77	0.58	2.26	80.39
neredive	0.76	0.00	1.64	0.46	2.10	82.49
mya_aren	0.43	0.20	1.50	0.43	1.92	84.41
scolsqua	0.34	0.00	1.21	0.33	1.54	85.95
magemira	0.21	0.00	1.16	0.25	1.48	87.44
coroaren	0.40	0.00	1.10	0.34	1.41	88.85
mytiedul	0.32	0.20	0.85	0.38	1.08	89.93
bathsars	0.24	0.00	0.79	0.26	1.00	90.93

Period 3, 2003-2005
Groups Z2 & Z1
Average dissimilarity = 83.92

Species	Group Z2 Av.Abund	Group Z1 Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
bathpilo	3.25	0.34	9.45	0.98	11.26	11.26
hetefili	3.97	2.12	8.57	1.17	10.21	21.47
macobalt	2.87	0.59	5.81	1.21	6.92	28.39

nephcirr	1.03	1.45	5.02	0.75	5.98	34.38
aphemari	1.88	0.89	4.58	0.84	5.46	39.84
pygoeleg	2.32	0.15	4.26	0.89	5.08	44.92
ceraedul	1.66	0.00	3.37	0.98	4.01	48.93
eurypulc	0.73	0.20	3.06	0.57	3.65	52.58
scolarmi	0.64	0.31	3.00	0.49	3.58	56.16
neredive	1.63	0.00	2.92	0.93	3.48	59.64
hydrulva	0.99	0.11	2.35	0.70	2.80	62.45
arenmari	1.05	0.00	2.06	0.79	2.46	64.90
spiobomb	0.18	0.35	2.05	0.35	2.45	67.35
spiomart	0.31	0.11	1.85	0.33	2.20	69.55
crancran	0.73	0.04	1.84	0.51	2.19	71.74
neresucc	0.81	0.33	1.81	0.67	2.16	73.90
nephcaec	0.15	0.32	1.61	0.39	1.91	75.81
bathsars	0.85	0.00	1.59	0.51	1.90	77.71
coroaren	0.93	0.00	1.50	0.44	1.79	79.50
cyatcari	0.66	0.00	1.43	0.55	1.71	81.21
anaimuco	0.61	0.21	1.30	0.59	1.55	82.75
urotpose	0.29	0.18	1.29	0.39	1.54	84.30
corovolu	0.76	0.04	1.29	0.39	1.54	85.84
capicapi	0.15	0.25	0.99	0.36	1.18	87.02
carcmaen	0.38	0.17	0.99	0.43	1.18	88.19
nephhomb	0.27	0.18	0.91	0.34	1.09	89.28
polycorn	0.36	0.17	0.71	0.44	0.84	90.12

Groups Z2 & B1
Average dissimilarity = 74.78

Species	Group Z2 Av.Abund	Group B1 Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
hetefili	3.97	4.12	8.94	0.98	11.96	11.96
bathpilo	3.25	1.83	8.00	1.16	10.70	22.66
macobalt	2.87	0.99	5.93	1.35	7.93	30.59
eurypulc	0.73	1.15	4.19	0.84	5.61	36.20
pygoeleg	2.32	0.16	4.17	0.95	5.57	41.77
nephcirr	1.03	0.00	3.63	0.59	4.85	46.62
ceraedul	1.66	0.00	3.28	1.02	4.39	51.01
aphemari	1.88	0.16	3.17	0.78	4.24	55.25
neredive	1.63	0.00	2.88	0.94	3.85	59.10
neresucc	0.81	0.65	2.74	0.67	3.66	62.76
hydrulva	0.99	0.16	2.42	0.71	3.24	66.00
scolarmi	0.64	0.00	2.31	0.46	3.09	69.09
arenmari	1.05	0.16	2.20	0.80	2.94	72.03
cyatcari	0.66	0.37	2.04	0.63	2.73	74.76
crancran	0.73	0.00	1.71	0.51	2.29	77.05
bathsars	0.85	0.00	1.57	0.51	2.09	79.14
coroaren	0.93	0.00	1.48	0.45	1.98	81.13
spiomart	0.31	0.00	1.44	0.31	1.92	83.05
polycorn	0.36	0.36	1.33	0.48	1.78	84.83
corovolu	0.76	0.00	1.18	0.37	1.58	86.41
spiobomb	0.18	0.00	1.14	0.25	1.52	87.93
anaimuco	0.61	0.00	1.05	0.56	1.40	89.33
nephcaec	0.15	0.17	1.03	0.35	1.38	90.71

Groups Z1 & B1
Average dissimilarity = 76.64

Species	Group Z1 Av.Abund	Group B1 Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
hetefili	2.12	4.12	13.74	1.22	17.93	17.93
bathpilo	0.34	1.83	9.17	1.24	11.97	29.89
nephcirr	1.45	0.00	8.02	0.97	10.47	40.36
eurypulc	0.20	1.15	6.37	0.89	8.31	48.67
macobalt	0.59	0.99	5.79	0.87	7.56	56.23
aphemari	0.89	0.16	4.22	0.63	5.51	61.74

neresucc	0.33	0.65	4.00	0.61	5.21	66.95
nephcaec	0.32	0.17	2.44	0.45	3.18	70.13
polycorn	0.17	0.36	1.70	0.44	2.22	72.35
cyatcari	0.00	0.37	1.52	0.39	1.98	74.33
capicapi	0.25	0.18	1.51	0.40	1.97	76.30
spiobomb	0.35	0.00	1.42	0.35	1.85	78.16
scolarmi	0.31	0.00	1.37	0.31	1.79	79.94
hydrulva	0.11	0.16	1.30	0.33	1.70	81.64
pygoeleg	0.15	0.16	1.27	0.32	1.66	83.30
petrphol	0.35	0.00	0.94	0.28	1.22	84.52
nephhomb	0.18	0.00	0.90	0.26	1.18	85.70
urotpose	0.18	0.00	0.84	0.27	1.09	86.79
hausaren	0.00	0.18	0.81	0.27	1.06	87.85
laniconc	0.35	0.00	0.78	0.29	1.02	88.87
mytiedul	0.31	0.00	0.78	0.23	1.01	89.89
strebene	0.04	0.18	0.77	0.28	1.01	90.89

Signature: _____

Date: 14-12-2006