

**WESTHER: A multidisciplinary approach to the identification of herring (*Clupea harengus* L.) stock components west of the British Isles using biological tags and genetic markers.**

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### Abstract

A considerable amount of research has been carried out on the complex of commercially important herring stocks to the west of the British Isles, from the south-west of Ireland and the Celtic Sea to the north-west of Scotland. Despite all this effort, the inter-stock mixing between components within this complex is still an unknown. The overall goal of WESTHER is to describe the population structure of herring stocks in this area through a large-scale analysis of the genetic, morphological, physiological and parasite faunal differences across spatial clines of herring stocks in these western European waters. All the different methods of stock discrimination employed have been applied to the same individual herring initially aiming to differentiate between spawning aggregations, thereby creating reference points to help describe juvenile and mixed adult aggregations. WESTHER's holistic approach allows apparent discrepancies implied by individual methods to be resolved and improves confidence in the results of stock identification. In this paper the data were analysed using various statistical techniques, for example discriminant analysis and classification trees, among others. These data analysis methods were used to predict a discrete outcome, as group membership, from the mix of variables (continuous, discrete and nominal) produced by the different techniques used to discriminate between stocks. The methods are being tested and refined using data from the first year's samples to give an assessment of the relative merits of the various phenotypic, chemical and genetic techniques for examining the stock structure of herring to the west of the British Isles.

Keywords: herring, classification and discrimination techniques, multi-disciplinary approach, stock components

## Introduction

Fish stocks (Ihssen et al., 1981) are identified on the basis of variation in characteristics between stocks, with the strongest influences on stock structure drawn from a suite of complementary techniques that cover multiple aspects of the biology and life history characteristics of a fish species (Begg and Waldman, 1999).

Genetic variation between stocks can provide a direct basis for stock structure but can prove inadequate where low and inconsistent levels of differentiation may still mask a high degree of demographic separation between stocks (Ward, 2000; Mariani et al., 2005). Phenotypic variation between stocks, on the other hand, can provide an indirect basis for stock structure, and although it does not provide direct evidence of genetic isolation between stocks, it can indicate prolonged separation of fish in different environmental regimes. It may therefore be applicable for studying short-term, environmentally induced variation and be highly applicable to fisheries management (Cadrin, 2000).

Stock identification is an evolving multidisciplinary field encompassing many techniques (Begg et al., 1999; Cadrin et al., 2005). A multi-disciplinary, integrated approach to any individual study maximises the likelihood of defining correctly the stocks, and different methods offer complementary perspectives on stock structure (Begg et al., 1999; Swain et al., 2005).

It is only really in the last fifteen years or so that any number of studies have been carried out using a broad suite of techniques for stock identification of exploited marine species, on cephalopods (Carvalho and Pitcher, 1989; Kassahn et al., 2003); bivalve molluscs (Wilding et al., 1998) and finfish (Haug and Fevolden, 1986; Waldman et al., 1988; Spanakis et al., 1989; Suneetha and Naevdal, 2001; Walsh et al., 2001; Stransky, 2003; Poulet et al., 2004). However these studies used either different samples of fish for the different methods compared, or different fish from the same samples. The only published study we have found to compare objectively among alternative approaches to stock discrimination by using the same individuals was performed on striped bass in the USA (Waldman et al., 1997) and there are two recently completed EU projects that have applied a suite of complementary techniques to the same individuals to detect stock structure: CODTRACE – QLRT-2000-01697 and HOMSIR – QLRT-1999-01438.

Atlantic herring (*Clupea harengus*) has played a pivotal role in the formulation of ideas relating to population structuring in marine fish, yet considerable uncertainty remains in the extent to which phenotypic and genetic differentiation coincide in such a highly mobile species. There is a long history of characterisation of herring to a particular 'race' or 'stock' using a wide variety of different techniques: morphometric and meristic characters (of whole fish and of otoliths), parasite tagging and genetic techniques, and examples on herring to the west of the British Isles include Wood (1936); Blaxter (1958); Parrish and Sharman (1958); Symonds (1964); Molloy (1975); Grainger (1976); King (1985); MacKenzie (1985); King et al. (1987) and Jörstad et al. (1991). These single technique studies have failed to address fully the interactions of life stage and fish population and none have been powerful enough to examine population identity over the scales that herring are distributed and, therefore, despite all this effort, the inter-stock mixing between components within this complex is still an unknown.

Herring in waters to the west of the British Isles are distributed over a wide area where several discrete fisheries exist (ICES, 2005). All the assessments carried out on these stocks show a great degree of uncertainty as to the current level of exploitation. This uncertainty is compounded by a lack of knowledge on the basis for the existence of the current stock units. Whilst the fisheries are managed in functional units, the herring

complex may not observe similar boundaries, or indeed current boundaries may contain more than a single discrete population. Hence it is vital that the interactions between stocks are understood, both in terms of the mixing of adults and the supply of juvenile recruits. In order to determine mixing the individual stocks of interest in the area need to be described and identified.

The overall goal of WESTHER is to describe the population structure of herring stocks in the area to the west of the British Isles through a large-scale analysis of the genetic, morphological, physiological and parasite faunal differences across spatial clines of herring stocks in these western European waters. All of the methods have been applied to the same individual herring, initially aiming to differentiate between spawning aggregations, thereby creating reference points to help describe juvenile and mixed adult aggregations.

Six techniques have been applied within the WESTHER project (body and otolith morphometry, parasites as biological tags, microsatellite DNA, otolith microstructure and otolith microchemistry (see reviews in Cadrin et al. (2005) for detail on the various methods) to form the basis of a study on stock structure in the area of interest. Full statistical analyses of the efficacy of each of these techniques to discriminate between the different spawning aggregations sampled will be carried out by the relevant project partners in due course.

In this preliminary analysis we present statistical analyses of several informative variables from four of the methods employed (body and otolith morphometry, parasites and otolith microchemistry), from samples collected from spawning aggregations within the first sampling year of the project (March 2003 to February 2004) to determine which mixture of variables gives the best discrimination between spawning populations.

## Materials and Methods

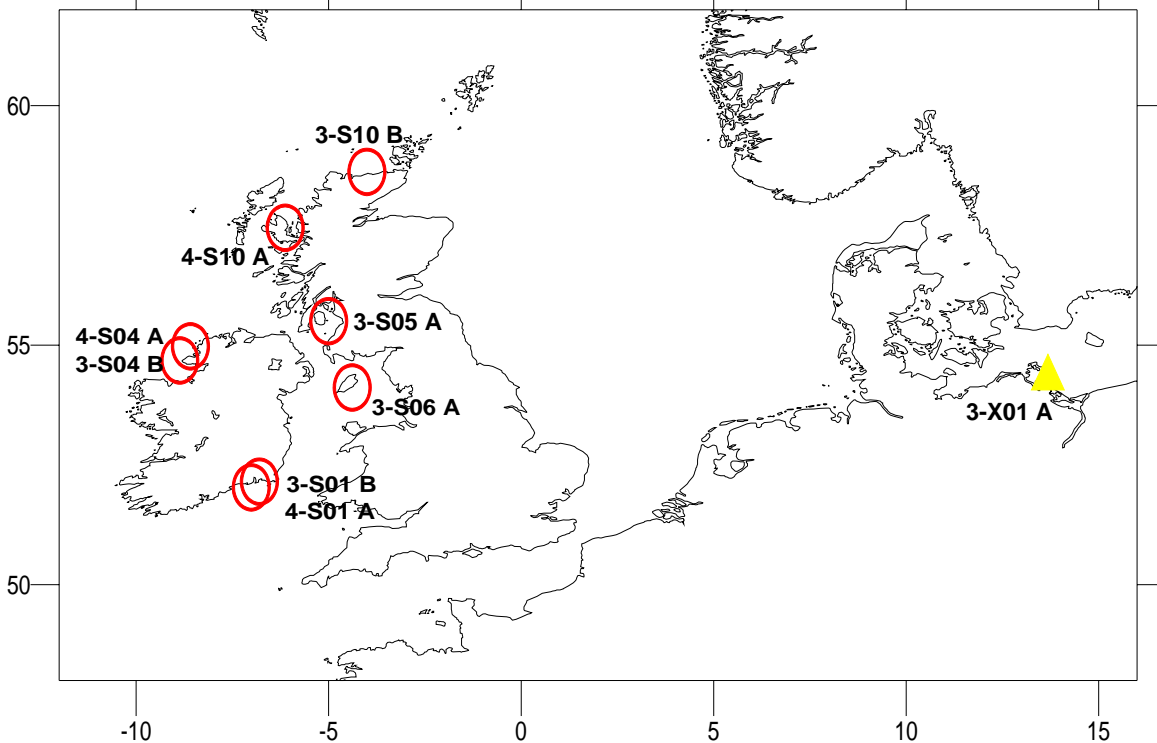
Samples were collected by various partners from both commercial vessels and research surveys between March 2003 and February 2004 (Figure 1 and Table 1). The sample from the western Baltic (Rügen – area 16) was collected to act as a biological outlier.

**Table 1.** Sampling data for herring analysed in the multidisciplinary multivariate analysis.

Sample ID	Geographical area	Collection date	Analysis code
3-S01B	Celtic Sea	December 2003	11
3-S04B	Donegal	October 2003	12
3-S05A	Clyde	March 2003	13
3-S06A	Irish Sea	October 2003	14
3-S10B	Cape Wrath	September 2003	15
3-X01A	Rügen	April 2003	16
4-S01A	Celtic Sea	February 2004	17
4-S04A	Donegal	January 2004	18
4-S10A	North Minch	February 2004	19

## Data exploration

Within each technique a full suite of variables was analysed giving a total number of combined variables of >100. Individual project partners provided their assessments of

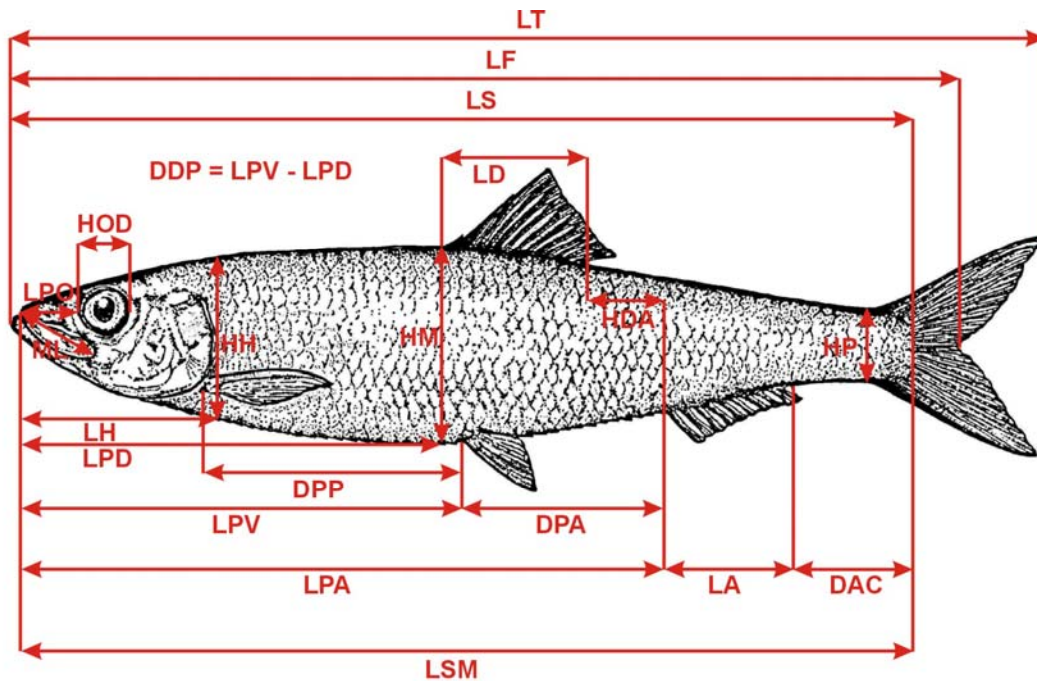


**Figure 1.** Geographical area from across which samples were taken. Sample labels refer to sample codes in Table 1.

**Table 2.** Variables used in the statistical analysis.

Technique	Workpackage	Abbreviation	Description
Body morphometry	02.1	DPA	See Figure 2
		DPP	See Figure 2
		HH	See Figure 2
		LA	See Figure 2
		OD	See Figure 2
Otolith morphometry	02.2	A04	Fourier descriptor of shape
		B03	Fourier descriptor of shape
		C03	Fourier descriptor of shape
		Area	Otolith aspect area
		Circ	Index of circularity of otolith outline
Parasites	02.3	Caeca	Numbers of pyloric caeca
	03	Anis	<i>Anisakis</i> spp.
		Dor	<i>Renicola cercaria doricha</i>
		Pyth	<i>Renicola cercaria pythionike</i>
Otolith microchemistry	06	Ba <sub>137</sub>	Barium concentrations in otolith core
		Na <sub>23</sub>	Sodium concentrations in otolith core
		Li <sub>7</sub>	Lithium concentrations in otolith core
		Sr <sub>88</sub>	Strontium concentrations in otolith core
		Mg <sub>25</sub>	Magnesium concentrations in otolith core

their best (most informative) variables for discrimination of the various spawning aggregations using either experience of the method, or statistical methods. The final suite of variables selected for the multi-disciplinary multivariate analysis presented here is given in Table 2 (and Figure 2 for a pictorial representation of the body morphometry measurements).



**Figure 2.** Morphometric distance and truss measurements used in WP 02.1.

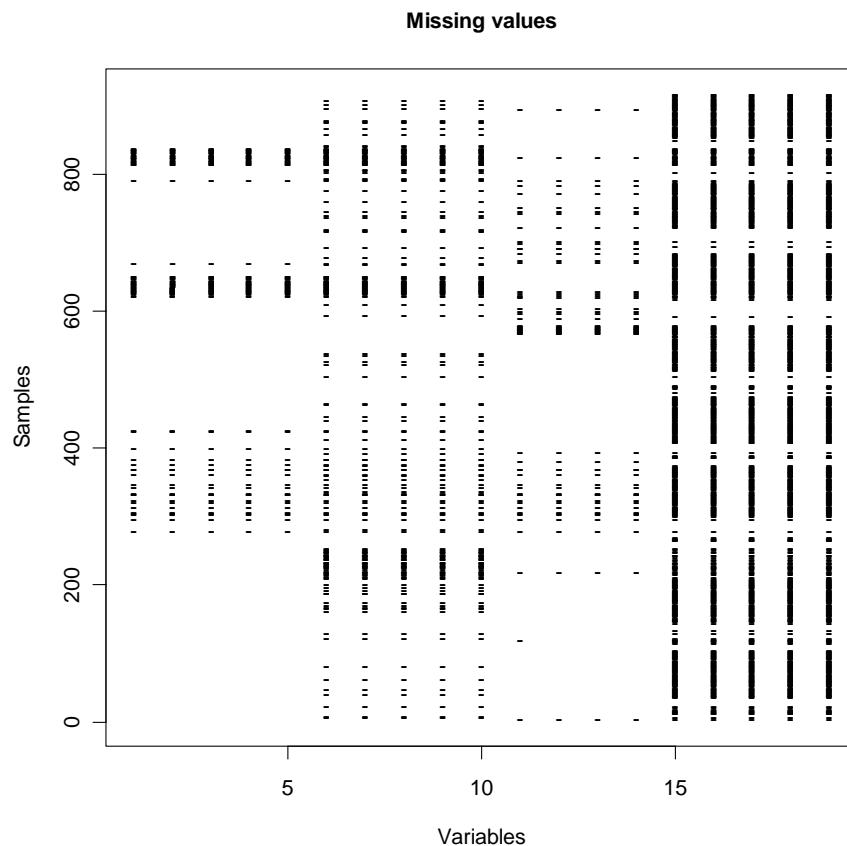
Since all variables in the morphometry workpackages (WPs) 02.1 and 02.2 were highly correlated ( $>0.9$ ) with fish length, the residuals of a cubic polynomial regression model applied on each variable, using LSM (tip of maxilla to end of caudal peduncle – see Figure 2) as the explanatory variable, were used in the analysis. LSM is the most stable measure of fish length. The correlations between variables of the other WPs and fish length were relatively low ( $<0.5$ ), except for Anis ( $=0.57$ ). Correlations between the selected variables in Table 2 and body mass were also low, except for Anis, which had a correlation of 0.54. Since this indicates that there are no strong linear effects of length or mass, it was decided to use these data without any pre-filtering (e.g., removing length and body mass effects).

When we combined the data from different WPs, we encountered a problem as not every fish was analysed for each technique. Since most statistical methods cannot deal with missing values, we only used those samples (fish) that were analysed by each technique. Note that there are still missing values in these combined data, but this is due to the fact that for some samples not every variable was measured in a work package. This process has the result that for some areas there are only ~15 - 30 fish analysed instead of the original 80 -100 (Table 3). This problem is visualised in Figure 3. The graph shows which fish were measured by each WP. A “-” indicates a missing value (or: not measured). Because there were only 13 fish in spawning area 13 (Clyde) that were measured by each WP (see Table 3), it was omitted from the statistical analysis.

**Table 3.** Numbers of fish measured for each workpackage (WP) / technique used in the multivariate statistical analysis (refer to Tables 1 & 2 for area and WP description).

Area	WP 02.1	WP 02.2	WP 02.3	WP 03	WP 06	Total no. in common
11	105	98	104	104	24	22
12	105	93	104	105	32	32
13	44	13	43	43	21	13
14	103	99	103	103	38	37
15	98	90	96	96	23	20
16	104	99	90	90	30	30
17	75	71	67	68	38	32
18	105	96	94	94	35	27
19	102	87	101	101	37	31

As well as the data from WPs 02, 03 and 06, there are also variables like length, mass, gutted mass, sex, maturity, age, year, month, latitude, longitude, vessel type, trawl type and catch depth available. We did not use these variables for classification purposes but some of them may be used to understand why we find particular groupings.



**Figure 3.** Visualisation of missing values. The horizontal axis shows the 19 variables and the vertical axis the approximately 1000 samples. The first five variables (1 to 5) are from work package 02.1, the second five from work package 02.2, then four variables from work package 02.3 and 03, and finally the last five variables on the right side (15 - 19) are from work package 06. The symbol ‘-’ was used to identify a missing value.

### *Outliers*

Outliers or large observations might be influential in statistical techniques and demonstrate a requirement for data transformation. To identify these observations, a data exploration using dotplots, histograms, qq-plots and boxplots was performed. Several variables showed large/extreme observations, especially from WP 06. To reduce the effect of large observations in the statistical analysis, the parasite data (WP 03) were square root transformed and the otolith micro-chemistry data (WP 06) were log transformed. The other WP variables were used without a transformation. The application of a square root, log and no transformation is justified as we are dealing with different types of variables (Quinn and Keough, 2002). The choice for these particular transformations was based on the range and values of the data. The transformations also ensured homogeneity between the areas for most variables, which is important for discriminant analysis (see below).

The dotplots indicated that the Irish Sea (area 14) showed higher RES\_OD values. Rügen (area 16) had lower RES\_HH values, the Irish Sea had higher AREA values, many samples had zero abundance of Pyth, two samples had considerably lower values of Sr<sub>88</sub> and in the Irish Sea and off northwest Scotland (area 15) there were higher Li<sub>7</sub> values.

### **Statistical analyses**

To identify (i) which of the morphometric, parasite and otolith microchemistry variables were important for the discrimination and (ii) whether we could actually discriminate between spawning samples from different areas, various statistical methods were applied. We applied discriminant analysis (DA), classification trees, multivariate regression trees, multinomial logistic regression and neural networks for exploratory purposes. The results of DA and classification trees only are presented here but the other methods will be discussed later.

### *Discriminant analysis*

Discriminant analysis (DA) results in axes that give maximum separation of samples from different groups, and samples of the same group are as close to each other as possible. The most important underlying assumptions for DA (Klecka, 1980; Jolliffe, 2002; Krzanowski, 2000; Huberty, 1994; Hair et al., 1996) are homogeneity and normality, and the second assumption is required for the hypothesis tests. Another assumption of DA is that the size of the smallest group is larger than the number of variables, which indeed holds here.

Separation of groups can be measured in different ways. We used the sum between all groups means, also called the total Mahalanobis distance. DA cannot cope with missing values. The software used here, Brodgar ([www.brodgar.com](http://www.brodgar.com)), removes all samples with one or more missing values. Brodgar itself uses the DA routines from the statistical Fortran library IMSL (IMSL manual).

IMSL manual: <http://www.colostate.edu/Services/ACNS/swmanuals/imsl/STATVol2.pdf>

### *Classification trees*

In this method, the variable identifying the eight spawning areas is the response variable, and all variables in Table 2 were used as explanatory variables. The technique (Chambers and Hastie, 1992; De'Ath and Fabricus, 2000) tries to identify which of the variables in Table 2 are the best in discriminating between samples of different groups. Results are presented in a tree-style diagram. Just as in regression analysis, the optimal model has to be found. A tree pruning process was used for this (Chambers and Hastie, 1992; De'Ath and Fabricus, 2000).

## Results

### *Discriminant analysis*

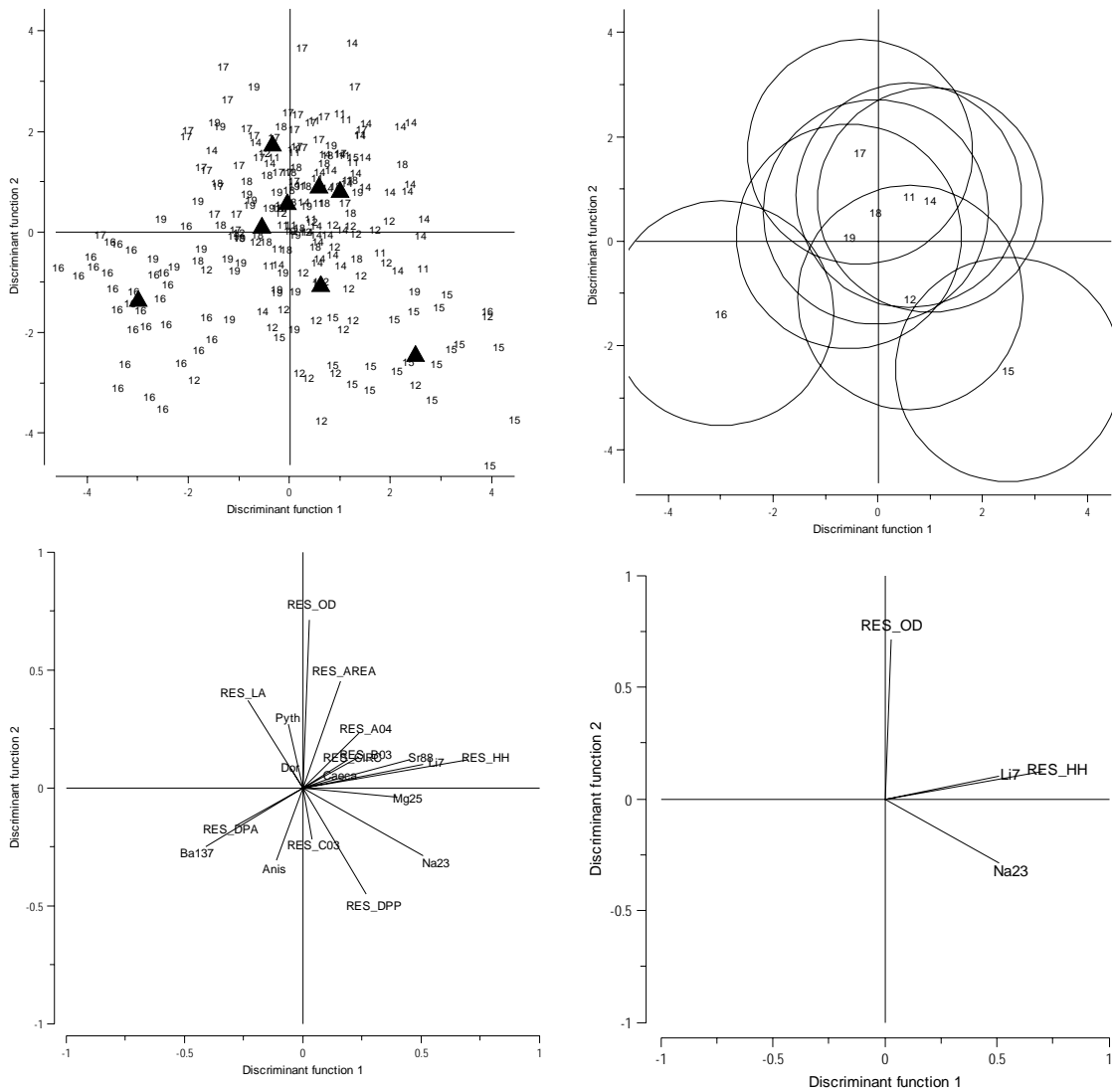
The data exploration indicated that for most variables, the assumption of homogeneity holds.

The results of DA are presented in Figure 4. The upper left panel in Figure 4 shows the scatterplot of discriminant axis 1 versus axis 2. Groups are represented by triangles. Instead of numbers and triangles, one can also draw circles that show the 90% confidence bands around a triangle (Krzanowski, 2000). These so-called 90% tolerance regions show the region in which 90% of the whole population of a spawning group is expected to be (see the upper right panel). Along the first two axes, four areas are separated from the rest. The eigenvalues (Table 4) indicate that these two axes represent 57% of the variation, and that the second axis is approximately as equally important as the first axis. This means that differences along either axis can be interpreted in the same way. There is a clear distinction between areas 15 (Cape Wrath) and 16 (Rügen). Areas 11 (Celtic Sea 2003), 14 (Irish Sea), 18 (Donegal 2004) and 19 (North Minch) are all in the middle and cannot be distinguished. Areas 12 (Donegal 2003) and 17 (Celtic Sea 2004) might be slightly different. However, the main difference is between areas Rügen and Cape Wrath (areas 16 and 15).

To obtain more insight into which of the variables are really important for the discrimination, three main options are available. The first option is to inspect the weighting factors, also called loadings. However, it is known (Huberty, 1994) that these factors can be unstable if the number of samples per group is small compared to the number of variables (as is the case here). A better option is to calculate correlations between the original variables and the discriminant axes. These correlations are plotted as lines from the origin to a point with the two correlation values in the lower left panel in Figure 4. However, rather than select qualitatively between 19 lines we chose an arbitrary cut-off level for these correlations of  $>0.5$ . From this pruning it is evident that areas 15 (Cape Wrath) and 12 (Donegal 2003) are associated with high values of  $\text{Na}_{23}$  (and area 17 – Celtic Sea 2004 - with low  $\text{Na}_{23}$ ). Samples from Rügen (area 16) have low values of  $\text{Li}_7$  and  $\text{HH}$ . However, a more detailed picture is obtained from the lower left panel showing that Rügen (area 16) might also be associated with high  $\text{Ba}_{137}$  values, and Cape Wrath (area 15) with large DPP values.

There is a third approach to identify the most important variables for discrimination. Distances between all the group averages (in the 7-dimensional DA space) can be measured using the total sum of Mahalanobis distance (Huberty, 1994). Leaving out an important variable (with respect to discriminating the areas) will result in a larger drop in the total sum of Mahalanobis distance compared to a less important variable. Stepwise removal of each variable indicates the least important variable, with a backward selection procedure then employed to remove repeatedly the least important variables. This process will give the order of importance of the variables (see Table 5).





**Figure 4.** DA results using all 19 variables; axes 1 versus 2. Upper left: Scores of axes 1 and 2. Upper right: confidence bands indicating the 90% probability of finding the real population averages. Lower left: correlations between axes and original explanatory variables. Lower right: same correlations as in lower left except that only those correlations larger than 0.5 are plotted.

**Table 4.** Eigenvalues (lambda) for the discriminant analysis using all 19 variables.

axis	Lambda	lambda as %	lambda cumulative %
1	2.076	32.344	32.344
2	1.605	25.007	57.351
3	1.310	20.401	77.752
4	0.788	12.268	90.020
5	0.415	6.468	96.488
6	0.149	2.328	98.816
7	0.076	1.184	100.000

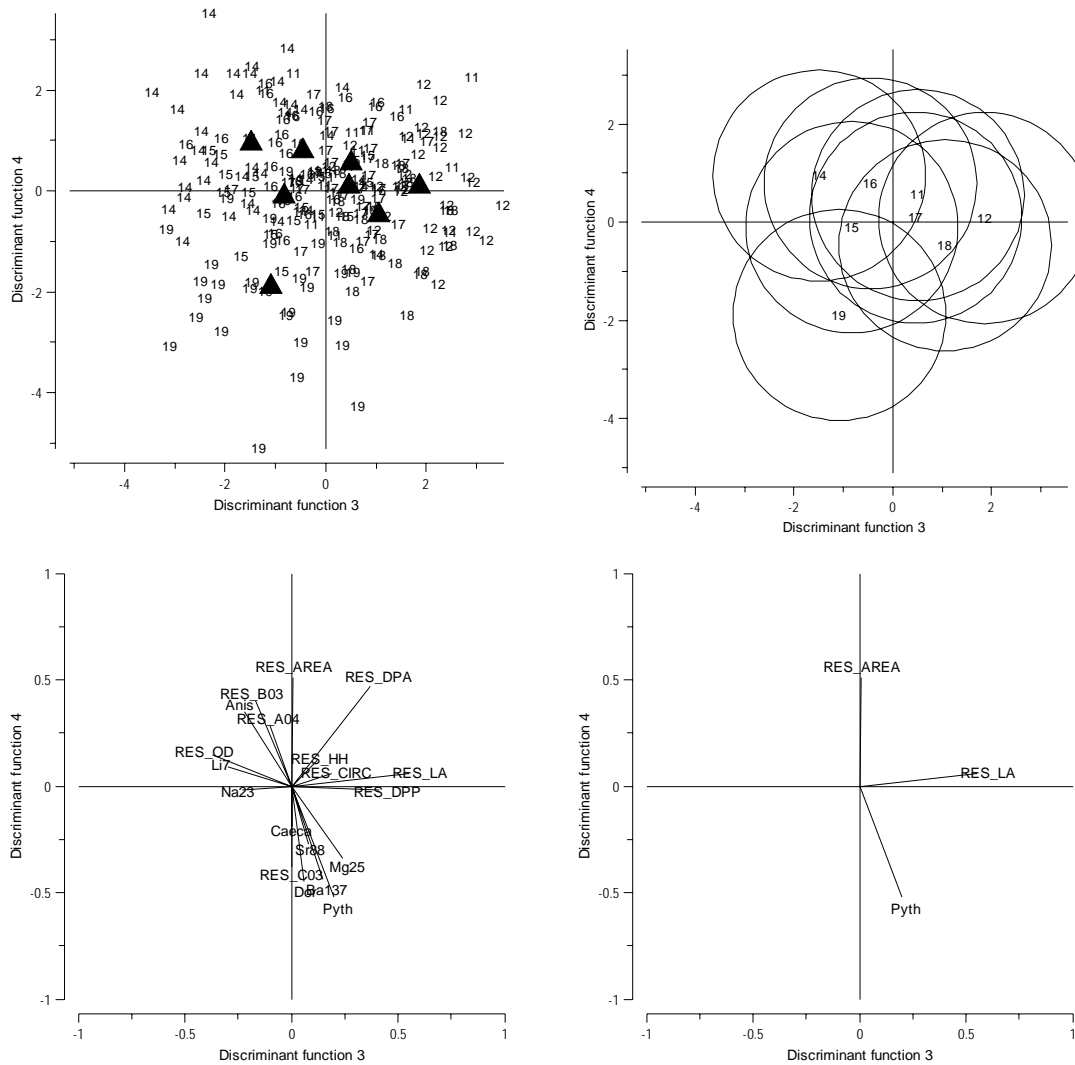
**Table 5.** Summary of the backwards selection.

Variables	Total Mah. distance	Dropped variable
19	408.9898	None
18	405.8074	Dor
17	401.6868	Caeca
16	390.1357	RES_A04
15	381.2488	RES_B03
14	374.8280	RES_C03
13	365.1359	RES_CIRC
12	345.7864	Pyth
11	324.9428	RES_AREA
10	302.3172	Li <sub>7</sub>
9	280.8975	Sr <sub>88</sub>
8	264.9802	Mg <sub>25</sub>
7	244.4703	Ba <sub>137</sub>
6	215.0878	Anis
5	182.8129	Na <sub>23</sub>
4	151.0967	RES_DPP
3	126.6851	RES_DPA

Since the algorithm for DA needs at least three variables, it cannot go any further. Variables that were not dropped are RES\_OD, RES\_HH, RES\_LA. Hence, the ten best variables are (from least to most important): Sr<sub>88</sub>, Mg<sub>25</sub>, Ba<sub>137</sub>, Anis, Na<sub>23</sub>, RES\_DPP, RES\_DPA and the following three variables: RES\_OD, RES\_HH and RES\_LA. Hence, all the body morphometry characters are among the five best discriminating variables.

The values and ratios between the first four eigenvalues in Table 4 indicate that it is also worthwhile to inspect axes 3 and 4 (Figure 5). The third axis, which represents about 20% of the variation, seems to show a difference between areas 12 (Donegal 2003) and 18 (Donegal 2004) on one side versus 14 (Irish Sea), 15 (Cape Wrath) and 19 (North Minch) on the other side. RES\_LA seems to be an important variable for this. The fourth axis (12% of the variation), seems to show a difference between area 19 (North Minch) and the other areas, and AREA and Pyth are also associated with this discrimination axis.

Various hypothesis tests indicated that the separation along the first, second and third axes were significant. These tests are based on normality. Among the other numerical output of DA is a classification table (Table 6 below). Rows of the table correspond to group memberships. Columns refer to the group to which the observation was classified by the algorithm.



**Figure 5.** DA results using all 19 variables; axes 3 versus 4. Upper left: scores of axes 3 and 4. Upper right: confidence bands indicating the 90% probability of finding the real population averages. Lower left: correlations between axes and original explanatory variables. Lower right: correlation between axes and original explanatory variables larger than 0.5.

**Table 6.** Classification table derived from the discriminant analysis on 19 variables.

	11	12	14	15	16	17	18	19
11	10.00	1.00	3.00	1.00	0.00	5.00	2.00	0.00
12	2.00	25.00	0.00	2.00	0.00	0.00	3.00	0.00
14	4.00	0.00	30.00	0.00	0.00	1.00	0.00	2.00
15	0.00	0.00	0.00	20.00	0.00	0.00	0.00	0.00
16	0.00	0.00	0.00	0.00	30.00	0.00	0.00	0.00
17	5.00	0.00	0.00	0.00	0.00	23.00	3.00	1.00
18	3.00	0.00	0.00	0.00	0.00	3.00	19.00	2.00
19	0.00	1.00	2.00	1.00	0.00	1.00	0.00	26.00

This output is interpreted as follows. Twenty-two samples were classified as from area 11 (Celtic Sea 2003). In the full 7-dimensional discriminant ordination diagram, ten of them were the closest to the group average of area 11. Therefore, ten out of the twenty-two samples were classified correctly. One sample was classified as from area 12 (Donegal 2003), three samples as from area 14 (Irish Sea), etc. Using this information, percentages of correctly classified samples can be calculated.

**Table 7.** The percentages of correctly classified samples per group.

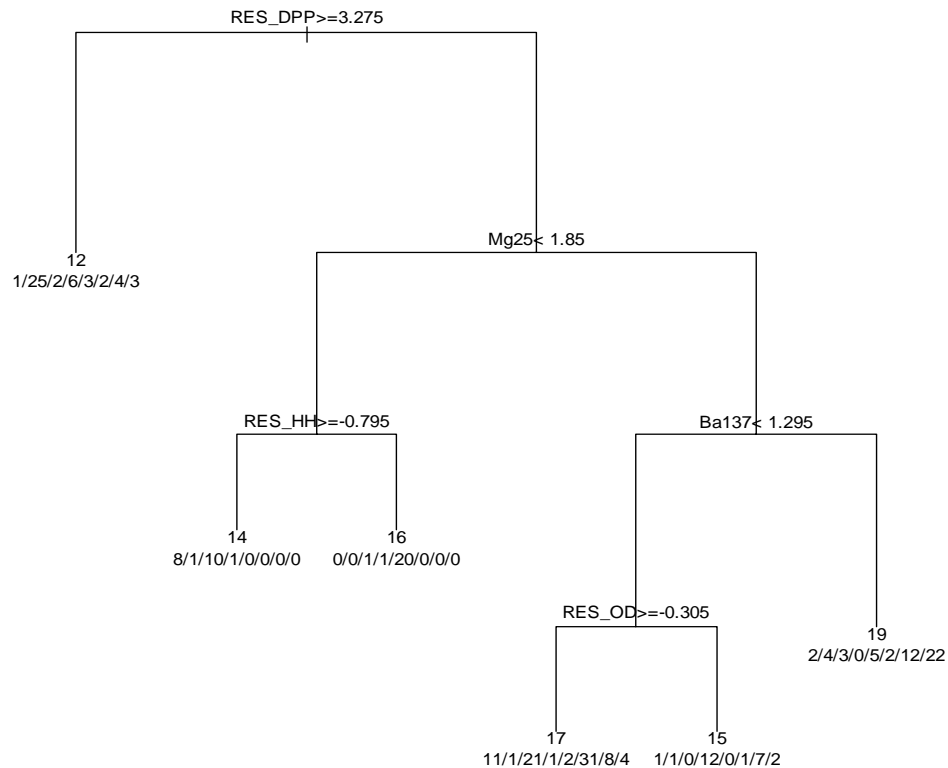
Area	% correctly classified
11	45.45
12	78.12
14	81.08
15	100.00
16	100.00
17	71.87
18	70.37
19	83.87

Thus all samples from areas 15 (Cape Wrath) and 16 (Rügen) were able to be classified correctly. Area 11 (Celtic Sea 2003) gave the lowest score, followed by areas 18 (Donegal 2004) and 17 (Celtic Sea 2004). The hit ratio (percentage of all correctly classified samples) and the maximum chance criterion (percentage of correctly classified samples relative to chance) are 79.22 and 16.02 respectively. Thus we could have classified 16.02% of the samples correctly by chance alone. In this analysis 79% of the samples were classified correctly. Another way to check whether the classification rate of 79% could have been obtained by chance is by using the Press Q statistic. Its value is 940.191, which is highly significant as the critical value ( $p < 0.01$ ) is 6.63. Therefore the classification rate of 79% is significant. The problem with the interpretation of classification results in DA is that it is recommended (Huberty, 1994) that the smallest group should have a sample size of at least 3 times the number of variables (19 in this case), which is 57 in this case ( $3 \times 19$ ). Hence, these classification results should be interpreted with care.

#### *Classification techniques*

The classification tree is presented in Figure 6. It reads as follows. If a sample has  $RES\_DPP > 3.275$  (or better: a residual DPP value of more than 3.275), then the sample is from area 12 (Donegal 2003). This is the left branch. The numbers below each area are the classification scores. The leaf was labelled as area 12 since that area had the highest score: 1 from area 11, 25 from area 12, 2 from area 14, 6 from area 15 etc. This part of the tree indicates that area 12 (Donegal 2003) can be singled out based on high (residual) DPP values, and there are only a few misclassified samples: 1 from area 11, etc.

If  $RES\_DPP < 3.275$ , then we follow the right branch of the tree and the samples are split up again based on  $Mg_{25}$ , but now the cut-off value is 1.85. Within this branch, the left sub-branch has HH as the next criterion, and the right sub-branch uses  $Ba_{137}$ .



**Figure 6.** Classification tree. The tree was pruned using the 1-SE rule.

Following all the branches in the tree, the classification tree shows that one can discriminate samples from:

- Area 12 (Donegal 2003) based on large (residual) DPP values. Samples in this area can be discriminated from the other areas because of large RES\_DPP values.
- Area 14 (Irish Sea) based on smaller RES\_DPP values ( $<3.275$ ), small  $Mg_{25}$  ( $<1.85$ ) and large HH ( $>-0.795$ ) values.
- Area 16 (Rügen) based on smaller DPP values ( $<3.275$ ), small  $Mg_{25}$  ( $<1.85$ ) and small HH ( $<-0.795$ ) values.
- The classification rules for samples from area 17 (Celtic Sea 2004) involve RES\_DPP,  $Mg_{25}$ ,  $Ba_{137}$  and RES\_OD.
- The same holds for area 15 (Cape Wrath).
- Area 19 (North Minch) involves RES\_DPP,  $Mg_{25}$  and  $Ba_{137}$ .

The classification scores at the bottom of each branch show that areas 12, 16, 15 and 19 are classified without too much error. Areas 14 (Irish Sea) and 15 (Cape Wrath)

have a large classification error (for example, 8 samples from area 14 (Irish Sea) were classified as area 11 – Celtic Sea 2003). The same holds for areas 15 (Cape Wrath) and 17 (Celtic Sea 2004). Areas 11 (Celtic Sea 2003) and 18 (Donegal 2004) were unable to be discriminated using this method.

We also applied a classification tree model that included the variables length, mass, sex, etc. Some of these variables could be equated to the use of life-history parameters as discriminatory variables, being broadly equivalent to length or mass at maturity. The analysis indicated that most of the latter variables could be more important than the morphometric, parasite and otolith micro-chemistry variables for classifying the samples (Figure 7). Area 17 (Celtic Sea 2004) can be discriminated based on smaller mass, and area 16 (Rügen) on larger length. For smaller fish with a smaller body mass at maturity, RES\_DPP is important (area 12 – Donegal 2003).

## Discussion

In this paper, two statistical techniques have been discussed in detail, namely discriminant analysis (DA) and classification tree models. All analyses were carried out using variables measured on the same individual fish, leading to a lower than planned sample size from each spawning aggregation sampled. For these analyses we have had to assume that these fish are representative of each of the sampled aggregations (and hence populations).

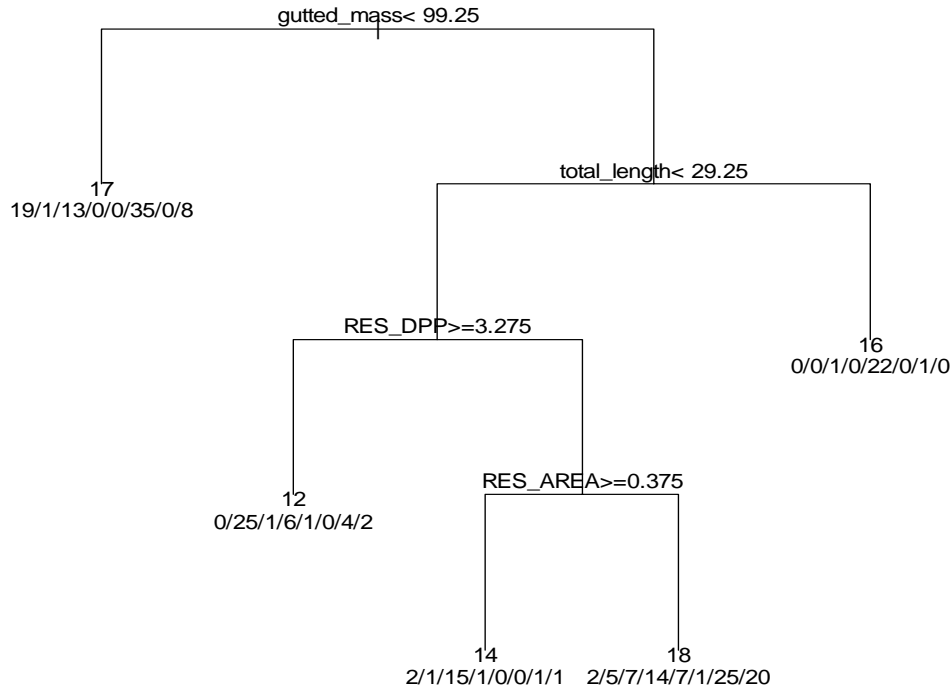
The DA showed that 57% of the variation could be associated with differences between areas 16 (Rügen – the biological outlier) and 15 (Cape Wrath), and to a lesser extent also areas 17 (Celtic Sea 2004) and 12 (Donegal 2003). Important variables seem to be Na<sub>23</sub>, RES\_HH and RES\_OD. Another 32% of variation could be related to differences between areas 14 (Irish Sea), 19 (North Minch) and 12 (Donegal 2003) with RES\_LA, AREA and Pyth as the most important variables. Another way of determining which variables are important is the backward selection method using the total sum of Mahalanobis distance. This method works on differences between groups in the 7-dimensional space (hence not only on the first 4 axes). Using this method, all five morphometric variables were deemed best for discrimination, followed by Na<sub>23</sub>, Anis and three of the four other chemical variables. Of the different methods used to assess the importance of the variables for discrimination, the Mahalanobis approach is probably the best, and most statistically informative, one.

Classification trees applied on the 19 selected variables singled out area 12 (Donegal 2003) based on RES\_DPP. Areas 12 (Donegal 2003), 16 (Rügen) and 19 (North Minch) were classified without too much error. Interestingly, including mass, gutted mass, length, sex, etc. into the tree model showed that area 17 (Celtic Sea 2004) could be singled out based on low gutted mass values and area 16 (Rügen) on larger lengths. For the remaining samples, RES\_DPP was the important variable; it classified samples from area 12 (Celtic Sea 2003).

Application of other statistical methods like neural networks and multivariate regression trees gave similar results. For example, the neural network analysis generated similar classification scores to those produced in the classification table from the DA. The multivariate regression tree analysis showed that we were able to make a similar classification of samples in that the same populations were able to be (or not) discriminated.

Currently our multinomial logistic regression model is over-parameterised and unable to be run. Decisions on further selection/de-selection of variables will need to be made (and these will be made as more samples become available).

### Tree models



**Figure 7.** Optimal classification tree using all variables.

It is not our intention in this paper to go into the detail of why particular areas may be more adequately separated than other, or why some variables might be more informative than others. To begin with we have not yet managed to derive a useful (or manageable) genetic data set from the initial nine spawner samples into a format that can be included in this level of analysis and it is critical to have a genetic technique included for a full comparison of techniques (Begg and Waldman, 1999). Furthermore, our ultimate aim is to use the best mix of variables that discriminate between the reference (spawner) collections in a mixed-stock approach to determine what possible mixtures of adults (and juveniles) may exist in the different ICES stock areas. To predict membership of putative spawning aggregations in potential admixtures of non-spawning adults collected in the four major areas in two different years it will be necessary to drop the Rügen sample (our biological outlier) from any future analysis of the sort presented here, because the prediction rules should be determined from the western stock complex samples only.

Nevertheless, the exercise presented here has been both a useful and an informative one. We have demonstrated that the multivariate statistical techniques employed are able to discriminate between the different areas and groups. The Rügen sample (area 16) was the first to be discriminated on axis 1 of the DA as it is the most distinct of the groups. However, it is not responsible for the majority of the variation

seen (accounting for just over a third of the 90% of variation seen in the first four axes). This gives some comfort that the western groups alone will be able to be distinguished in future analyses. Despite its strong differentiation from the rest of the groups/areas it was not, however, the only population to be perfectly discriminated in the DA classification table, as the Cape Wrath sample (area 15) was also perfectly classified.

It seems probable that life-history parameters will also be informative discriminators of the various stocks within the western stock complex and we can determine some parameters from the data we have and feed these into future analyses.

Our major drawback is that we have a good number of fish analysed for some techniques and very few analysed for others and this means that we are currently unable to carry out some of the statistical analyses we were hoping to perform. It is hoped that our experience of sampling within the first year of the project will lead to better comparison of samples in the second year and therefore in the final analysis.

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