**Identifying blue whiting (*Micromesistius poutassou*) stock structure
in the Northeast Atlantic by otolith shape analysis**

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1 **Identifying blue whiting (*Micromesistius poutassou*) stock structure**
2 **in the Northeast Atlantic by otolith shape analysis**
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34 **Abstract**

Information on stock identification and spatial stock structure provide a basis for understanding fish population dynamics and improving fisheries management. In this study otolith shape analysis was used to study the stock structure of blue whiting (*Micromesistius poutassou*) in the northeast Atlantic using 1693 samples from mature fish collected between 37°N and 75°N and 20°W and 25°E. The results indicated two stocks located north and south of ICES Divisions VIa and VIb (54°5 to 60°5 N, 4° to 11°W). The central area corresponds to the spawning area west of Scotland. Sampling year effects and misclassification in the linear discriminant analysis suggested exchanges between the northern and southern stocks. The results corroborate previous studies indicating a structuring of the blue whiting stock into two stocks, with some degree of mixing in the central overlap area.

Keywords

Micromesistius poutassou, otolith shape, Fourier descriptors, stock discrimination, northeast Atlantic.

Résumé

60 L'identification et la connaissance de la structuration spatiale de stocks sont essentielles pour
61 étudier la dynamique des populations de poissons et ainsi gérer les pêcheries. Dans cette
62 étude, la forme des otolithes a été employée pour comprendre la structuration des stocks des
63 merlans bleus (*Micromesistius poutassou*) dans le nord-est de l'Océan Atlantique à partir de
64 1693 poissons matures échantillonnés entre 37°-75° N et 20°O-25°E. Les résultats ont indiqué
65 deux stocks séparés entre l'ouest de l'Irlande et de l'Ecosse correspondant à la zone de
66 reproduction. Les effets du facteur « année d'échantillonnage » et la mauvaise classification
67 dans l'analyse linéaire discriminante suggère des échanges entre les composants nordique et
68 méridionale. Ces résultats corroborent des études antérieures indiquant une structuration des
69 merlans bleus en deux stocks avec un certain niveau de mélange dans la zone de
70 chevauchement.

71 **Mots-clés**

72 *Micromesistius poutassou*, forme de l'otolithe, descripteurs de Fourier, discrimination de
73 stock, nord-est de l'Océan atlantique.

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Introduction

Stock identification and information on spatial structure provide a basis for understanding population dynamics and in turn to improve fisheries management. Several techniques can be used for stock identification, including tagging experiments, analysis of spatial variation of genetic or morphometric markers, differentiation of life-history variables, parasites and contaminant concentrations (Pawson and Jennings 1996; Cadrin et al. 2014). Otolith shape analysis is an efficient stock identification tool linked to genetic heterogeneity and the influence of environmental factors on otolith shape (Cadrin and Friedland 1999; Campana and Casselman 1993; Torres et al. 2000; Cardinale et al. 2004; Swan et al. 2006; Vignon and Morat 2010).

Otolith morphology is influenced by biotic and abiotic factors (Cardinale et al. 2004; Capoccioni et al. 2011). Analysis of otolith shape has previously been used for stock discrimination of various species (haddock: Begg and Brown 2000; cod: Galley et al. 2006; Petursdottir et al. 2006, Stransky et al. 2008; striped red mullet: Benzinou et al. 2013). Legua et al. (2013) applied otolith shape analysis to identify two distinct stocks of southern blue whiting (*Micromesistius australis*), an Atlantic and a Pacific stock.

Several basic descriptors exist to describe the external contour of otoliths (e.g., coefficient of form, roundness, circularity, rectangularity and ellipticity) in addition to more complex geometric morphometric analyses (Ponton 2006; Ramirez-Perez et al. 2010; Vergara-Solana et al. 2013), wavelet functions (Parisi et al. 2005; Sadighzadeh et al. 2014) and the geodesic method (Benzinou et al. 2013). Of these, the elliptical Fourier analysis currently remains the most widely used and powerful method to describe otolith shapes comprehensively (Aguera and Brophy 2011; Capoccioni et al. 2011; Fergusson et al. 2011; Legua et al. 2013; Paul et al. 2013). Otolith shape has the advantage of being unaffected by short-term changes in fish condition (Campana and Casselman 1993) or environmental variations (Campana 1999).

Nevertheless, its biological interpretation is more complex than that of linear morphometric descriptors (Stransky and MacLellan 2005). Some studies have combined elliptical Fourier analysis and some basic descriptors of otolith contours to help the interpretation of results (Campana and Casselman 1993; Begg and Brown 2000; Galley et al. 2006; Merigot et al. 2007; Fergusson et al. 2011; Legua et al. 2013).

Blue whiting (*Micromesistius poutassou*) is a pelagic gadoid whose distribution in the northeast Atlantic extends from Spitsbergen in the north to Morocco in the south (Zilanov 1968; Bailey 1982; Heino and Godø 2002; Monstad 2004; ICES 2012). The blue whiting fishery is one of the largest in the north Atlantic (Trenkel et al. 2014). Several European nations have extensively fished this species, especially since the late 1990s, with annual catches ranging from 100 000 to 2 400 000 tonnes (ICES 2013). All previous morphological, physiological, genetic, parasite and otolith shape research suggested the existence of at least two stocks in the north-east Atlantic, though geographic ranges differed between studies (Karasev 1990; Isaev and Seliverstov 1991; Skogen et al. 1999; Ryan et al. 2005; Brophy and King 2007; Was et al. 2008; Keating et al. 2014). Juveniles and adult blue whiting make annual migrations in early summer to the main feeding areas, located primarily in the Norwegian Sea in the north and in the Celtic Sea and Bay of Biscay in the south (Bailey 1982; Carrera et al. 2001, Fig. 1). Previous stock structure studies focused primarily on the areas located to the west of Scotland and Ireland, where spawning takes place in the water column at the shelf break at depths of 250 to 450 metres (Bailey 1982; Isaev and Seliverstov 1991; Standal 2006) and regions further north, with very few samples from the southern part of the range, i.e. the Bay of Biscay and areas further to the South.

In the current study, analysis of the perimeter and elliptical Fourier descriptors of sagittal otoliths were employed to study stock structure of blue whiting in the north-east Atlantic

using individuals sampled over a wide geographic area, from Portuguese waters to Spitsbergen and Iceland.

Materials and methods

Sample collection

Blue whiting samples covering ten ICES Divisions between Spitsbergen and Portuguese waters (Fig. 1) were collected in 2006, 2008, 2010 and 2011 by seven institutes (IFREMER, France; CEFAS, UK (England and Wales); IMR, Norway; DTU, Denmark; MRI, Iceland, AFBI, UK (Ireland) and IPMA, Portugal) during international scientific surveys, on-board fishing vessels and from fish markets. This collection includes all samples used by Keating et al. (2014).

Sex and sexual maturity of the sampled individuals were determined by macroscopic examination of gonad and so only mature fish were selected in this study to minimize the effect of sexual maturity which can change otolith shape (Cardinale et al. 2004). This yielded a total of 1693 mature individuals ranging from 16 to 42 cm total length (mean 27.80, Table 1), corresponding to ages 1 to 13 years. The length for the 878 females ranged from 16 to 42 cm; while the 537 males had total lengths from 16 to 35 cm. Sagittal otoliths were extracted from all 1693 fishes.

Otolith shape analysis

Blue whiting otoliths are flat with rounded dorsal and ventral margins. The *rostrum* is large and straight and therefore, the *antirostrum* is indistinct (Fig. 2). Images of the whole left sagittal otoliths were scanned (Epson V750) under reflected light and stored with high resolution (3200 dpi). Image processing was performed using the image analysis system TNPC (Digital processing for calcified structures, version 7, www.tnpc.fr) with the *sulcus*

acusticus facing up. The contour of each otolith was extracted using the automatic threshold in the TNPC software. To describe otolith contours, Elliptic Fourier Analysis (EFA; Lestrel 1997) was carried out. For each otolith, the first 99 elliptical Fourier harmonics (H_i) were extracted and normalised with respect to the first harmonic using the TNPC software and were, thus, invariant to otolith size and rotation and starting point of the shape measurements (Kuhl and Giardina 1982). To determine the number of harmonics needed to reconstruct the otolith contour, the Fourier Power (PF) was calculated for each individual otolith k as a measure of the amount of contour rebuilt by each harmonic:

$$PF(n_k) = \sum_{HI=1}^{n_k} \frac{A_{HI}^2 + B_{HI}^2 + C_{HI}^2 + D_{HI}^2}{2} \quad (1)$$

where A_{HI} , B_{HI} , C_{HI} and D_{HI} are the parameters of the HI th harmonic, referred to as elliptical Fourier descriptors (EFD), and n_k is the total number of harmonics included. The value of n_k was chosen such that $PF(n_k)$ explained 99.99% of variance in contour coordinates or, in other words, such that otolith shape is reconstructed at 99.99% (Lestrel 2008).

Statistical analyses

Otolith perimeter (length of contour) was used as an integrative variable to study geographic and temporal differences in overall otolith shape. Keating et al. (2014) reported wider otoliths for a given otolith length for individuals sampled in the north-eastern part of the spawning area compared to the southwestern part; the perimeter should reflect such differences. Perimeter P was modelled as a function of sex (subscript s), ICES Division (subscript d), sampling year (subscript y) and total fish length L as well as all two-way interactions with total fish length. The full model is

$$P = \alpha + \alpha_s + \alpha_d + \alpha_y + \beta_L L + \beta_s L + \beta_d L + \beta_y L \quad (2)$$

where α 's are intercepts and β 's slopes of the linear model.

The model was fitted with a Gaussian error distribution using stepwise variable selection based on AIC to select the final model. Total fish length was centered by subtracting the mean length in the data (27.86 cm) to decouple the estimation of slopes and intercepts. An analysis of covariance (ANCOVA) was carried out for the final model and a Tukey HSD (honest significant difference) post-hoc test was used for pairwise comparison between intercepts α_d and slopes β_d of ICES Divisions (α -level=0.05).

To visualise geographic differences in the final model, the perimeter was predicted for all ICES Divisions for the year 2010 (corresponding to the year with data for the largest number of ICES Divisions) using $\hat{\beta}_{2010}$ and a standard total length of $L=28$ cm (corresponding to the mean length in data set). As the difference between ICES Division due to the interaction β_d with total length is better visible for larger individuals, predictions were also carried out for individuals of 38 cm total length.

To investigate potential explanations for otolith shape differences in more detail, multivariate analysis of elliptical Fourier descriptors of otolith contours was carried out. To reduce the number of dimensions and to avoid collinearity between descriptors, Principal Components Analysis (PCA) was applied to the EFDs (Rohlf and Archie 1984). Significant principal components (PC) were selected with the 'broken stick method' (Legendre and Legendre 2012). This method assumes that if the total variance (sum of the eigenvalues) is divided randomly among the various components, the expected distribution of the eigenvalues will follow a broken-stick distribution. The contribution of Fourier harmonics to the corresponding eigenvectors (PC loadings) was analyzed to understand which shape features had most influence on each PC. Next, two Redundancy analyses (RDA) were carried out. RDA is an extension of multiple regressions to multivariate response data and an extension of principal component analysis (Legendre and Legendre 2012). The first RDA aimed at removing the variance explained by total fish length. In the second RDA, the residuals of the first RDA,

referred to as RDA residual matrix, were related to the explanatory variables of interest, i.e. sex, ICES Division and sampling year. A permutation test was used to test the significance of each explanatory variable. The magnitude of multicollinearity between explanatory variables of interest, e.g. total length, sex, year of sampling and ICES Division was evaluated by calculating variance inflation factors (threshold value for VIF =10; Borcard et al. 2011).

To determine whether otoliths collected in different ICES Divisions could be distinguished based on their shapes, stepwise Linear Discriminant Analysis (LDA) was applied to select the discriminant Fourier harmonics (Rencher and Christensen 2012). To evaluate the resulting discriminant functions, the percentage correct classification of individuals to ICES Divisions was calculated using jack-knifed cross-validation and Wilk's lambda criterion (Klecka 1980).

Finally, a cluster analysis was performed on the normalised Fourier harmonics to group individuals with similar otolith shapes. For this, Ward's hierarchical algorithm based on squared Euclidean distances was used. To visualise differences in otolith shape between groups of individual fishes, the average otolith shape of each group was formed by the outline reverse Fourier transform using the first 34 normalised Fourier harmonics.

All statistical analyses were performed using the 'lsmeans', 'multcomp', 'Vegan', 'MASS', 'CAR', 'FactoMinR', 'HH' and 'Ellipse' packages in the statistical environment R (R Core Team 2014).

Results

Otolith perimeter increased linearly with total length as expected (Fig. 3a). Linear modeling revealed that sex, ICES Division and year of sampling all influenced the intercept (i.e., $\alpha_s \neq 0$; $\alpha_d \neq 0$; $\alpha_y \neq 0$) and the slope ($\beta_s \neq 0$; $\beta_d \neq 0$; $\beta_y \neq 0$) of the relationship between perimeter and total length ($R^2=0.94$). The ANCOVA showed that after total length, sex was the most important variable, i.e. variable with the largest mean sum of squares, for explaining variations in the

intercept, with ICES Division and sampling year explaining less (Table 2). In contrast, year was the most important factor for explaining variations in the slope (Table 2).

The predicted perimeter at 28 cm total length decreased more or less continuously from North to South (Fig. 3b). Using a Tukey HSD pairwise comparison test, the partial intercept for ICES Division IXa ($\hat{\alpha}_{IXa}$) was found to be significantly smaller ($p < 0.05$) than the partial intercepts for all other Divisions. The Tukey HSD test did not identify any ICES Divisions for which the slope was different from all other Divisions. However, slopes also generally decreased from North to South with the exception of Iceland and Faroes Waters (Divisions Va and Vb), leading to a less marked North-South gradient for predicted perimeter for larger individuals of 38 cm (Fig. 3b).

Among the 99 Fourier harmonics extracted to describe otolith shape, the first 34 harmonics explained more than 99.99% of the between otolith variation and were thus used for the multivariate analysis. Principal Components Analysis of these first 34 Fourier harmonics (i.e. 156 Coefficients of Fourier, see eq. 1) explained 84.01% of the total variance and the first and the second PC accounted for 36.82% and 14.24% of the total variance respectively. Only the first seven PCs were significant as determined by their eigenvalues exceeding the threshold eigenvalue generated by the broken-stick model ($> 3.9\%$ of the total variance). In the RDA when the first seven PCs were related to total length, the relationship was found to be significant ($p < 0.001$). Therefore in the second RDA, the residuals from the first RDA were used to test the explanatory variables of interest, i.e. sex, year of sampling and ICES Division, as well as second order interactions. All VIF values were smaller than 1.7 providing no evidence for multi-collinearity between explanatory factors. ICES Division ($p < 2.10^{-16}$) and the interaction between year and ICES Division ($p < 0.05$) both explained differences in otolith shape (Table 3). Given no sexual dimorphism was found in this analysis (sex: $p = 0.538$; Table 3), males and females were combined in the subsequent LDA and cluster analysis.

The LDA provided discriminant functions for year-ICES Division combinations using the 34 Fourier harmonics and sampling year as explanatory variables. The overall jack-knifed classification success was 28.41% (Table 4). The analysis showed significant differences among groups of blue whiting sampled in different ICES Divisions and years (Wilks' $\lambda = 0.1769$; $F=24.843$; $p<0.001$). The misclassification percentage for each group was highest in the west of Scotland area (ICES Division VIa) and the Rockall area (ICES Division VIb), where individuals were misclassified to the Norwegian Sea (IIa) (Table 4). The misclassification percentages were particularly high from the Norwegian Sea to the West of Scotland in the North and from the Irish Sea and the Porcupine Bank to the Portuguese Waters in the South. When data from different years of sampling were grouped for a given ICES Division in the LDA, the overall jack-knifed classification success increased only by 6% to 34% (Wilks' $\lambda = 0.141$; $F=23.402$; $p<0.001$) (Table 5). With the aim of achieving a more reliable classification, certain ICES Divisions were combined. Combining ICES divisions into two areas, 71% of individuals were assigned correctly to the northern (from Norwegian Sea to West of Scotland: ICES Divisions IIa-VIa, $N=1009$) and the southern area (from Rockall to Portuguese Waters: ICES Divisions VIb-IXa, $N=684$) (Wilks' $\lambda = 0.893$; $F=28.834$; $p<0.001$). The hierarchical cluster analysis performed on the matrix of the 34 Fourier harmonics identified two clusters of fishes (Fig. 4). Cluster 1 ($N=573$) was exclusively composed of blue whiting sampled from the Norwegian Sea to the West of Scotland (ICES divisions: IIa, IIb, IVa, Va, Vb, VIa and VIb). All fish from Spitsbergen and Bear Island were found exclusively in cluster 1. Conversely, cluster 2 ($N=1120$) included individuals sampled in all ICES Divisions and years. For the southern ICES divisions (from Irish Sea to the Portuguese Waters) individuals were found in cluster 2.

Combining the ANCOVA on otolith perimeter which revealed a spatial gradient with the multivariate analyses (LDA and cluster analysis) two geographical areas of blue whiting stock structure were identified:

- Northern zone : from Norwegian Sea to West of Scotland (ICES Divisions IIa-VIa)
- Southern zone : from Rockall to the Portuguese Waters (ICES Divisions VIb-IXa)

To visualise differences in average shapes between clusters, the reconstructed outlines of the mean Fourier harmonics of the two clusters were plotted as overlay picture (Fig. 5). The main shape difference between clusters occurred in the direction of the small axis of the otolith, especially in the upper (dorsal) part.

Discussion

The use of linear morphometric descriptors has been found to be of limited use for *M. poutassou* (Keating et al. 2014). For this reason the perimeter was used in this study as a single additional morphometric descriptor which gave results in general agreement with the elliptical Fourier descriptors, except for the identification of a sexual difference. The number of selected Fourier harmonics (PF=34) was higher than previously identified for *M. poutassou* (PF=10, Keating et al. 2014) and *M. australis* (PF=14, Legua et al. 2013). A majority of studies using this technique compute the cumulated Fourier power PF (Fourier Power) using EFDs (Elliptical Fourier Descriptors) averaged across the full sample or a part of it, so that the selected harmonics describe the average otolith shape. In this study however, in order to ensure that each individual otolith in the sample was reconstructed with a precision of 99.99%, the PF value was calculated for each individual otolith such that individual shape was reconstructed at 99.99%. The maximum number of harmonics across individual otoliths was then used to statistically reconstruct each individual otolith in the sample. This value was higher than the cumulated PF using averaged EFDs.

ANCOVA and redundancy analysis were carried out to explore the effects of size (total fish length), sampling year and sex. Several authors have provided evidence for the importance of these factors for allometric otolith growth (Castanguay et al. 1991; Campana and Casselman 1993; Begg et al. 2001; Cardinale et al. 2004; Capoccioni et al. 2011). Moreover, Campana and Casselman (1993) found that sexual maturity can modify the final contour of otoliths. Therefore in our study, only otoliths from mature adults were used. Further, a significant correlation between total length of fish and morphological otolith parameters (perimeter and EDS) was found. To remove this effect the residuals of the first seven PC from the normalised elliptical Fourier descriptors were used.

The effect of sex on otolith perimeter was weak but significant. In contrast, somewhat surprisingly, no significant sex effect on overall otolith shape was found. A sex effect on otolith shape has been reported for other species, e.g. for herring (*Clupea harengus*, Bird et al. 1986), orange roughy (*Hoplostethus atlanticus*, Gauldie and Jones 2000), cod (*Gadus morhua*, Paul et al. 2013) and southern blue whiting (*Micromesistius australis*, Legua et al. 2013).

In this study otoliths collected in several years were used. Combining data from different years has been done before in otolith shape analysis (DeVries et al. 2002; Tracey et al. 2006; Farias et al. 2009; Neves et al. 2011). Friedland and Reddin (1994) and Campana and Casselman (1993) found no year effect for the otolith shape of Atlantic salmon and cod. In contrast, both the ANCOVA for perimeter and the RDA for EFDs of *M. poutassou* in the present study indicated significant sampling year effects. The difference between sampling years could be explained by migrations for reproduction or feeding. Further, sampling was spread over different months. Consequently, the year effect could be due to true interannual differences, as identified in the previous small scale otolith shape study of *M. poutassou* (Keating et al. 2014), and/or due to seasonal differences such as found for pacific sardine

(*Sardinops sagax*) (Felix-Uraga et al. 2004; Vergara-Solana et al. 2013). However, a season effect was not tested in the present analysis due to a lack of sufficient sampling coverage across seasons and areas. In addition, a significant interaction between sampling year and geographical area was found in the RDA, which corroborates results obtained for other gadoids species (Castonguay et al. 1991; Begg and Brown 2000; Petursdottir et al. 2006). The results of this large scale study in which two broad blue whiting stocks were identified is in accordance with previous smaller scale studies which also suggested the existence of several stocks of the blue whiting in the northeast Atlantic (Karasev 1990; Isaev and Selivestof 1991; Skogen et al. 1999; Ryan et al. 2005; Brophy et al. 2007; Brophy and King 2007; Was et al. 2008; ICES 2012; Keating et al. 2014). Karasev (1990), comparing parasite types and infestation rates, concluded that there were three separate stocks from the north to the south: (1) Barents Sea, Spitzbergen and Iceland (ICES Divisions IIb, Va), (2) Norwegian Sea, Faeroes and Hebrides (ICES Divisions IIa, Vb, VIa), and (3) Porcupine Bank, Celtic Sea, Bay of Biscay and areas near Shetland (ICES Divisions VIIg-h & VIIIa-b). Other studies have investigated the existence of only two stock units. Although the genetic variability in blue whiting is low from Gibraltar to Norway (Mork and Gjæver 1995), differentiation has been found between the south, Bay of Biscay and Celtic Sea, and the north, Porcupine bank to Papa bank (Was et al. 2006; 2008). A recent study of blue whiting otolith shapes (Keating et al. 2014), which was focused on samples from ICES Divisions VIa and VIb, identified a geographical stock limit along a line from Porcupine Bank to Rockall Bank (Fig. 1). The location of spawning determines the probability of blue whiting eggs drifting northwards or southwards (Skogen et al. 1999) which in turn defines the division line between the two stock components. The division line, which varies between years, was determined to be approximately at 54.5°N corresponding to the northern edge of Porcupine bank. The Skogen et al. (1999) study covered the years 1976 to 1995, which was a period of strong subpolar

gyre activity hence probably a period with a constrained spawning area (Hátún et al. 2009), resembling the conditions for the data collection of the present study, thus the division line was expected to have been in a similar position.

Otolith shapes are influenced by environmental conditions and genetic traits (Farias et al. 2009; Vignon 2012). Vignon and Morat (2010) found that genetic variations only affected the otolith shape locally, mainly in the rostral and antirostrum parts. Conversely, Reichenbacher et al. (2007; 2009), concluded that the posterior and postero-ventral angles of sagittal otoliths differed as a consequence of environmental factors rather than different genetic information. In the present study, variations of otolith shape were observed locally in the dorsal and ventral parts (Fig. 5). Thus both environmental conditions and genetic components could explain the differences. However, genetic differentiation in blue whiting seems to be low from Gibraltar to Norway and there is a high probability of genetic mixing on the spawning grounds (Mork and Gjaever 1995). Therefore, the main factors influencing the observed otolith shape differences of blue whiting could be environmentally driven, such as temperature, salinity, food availability, water depth and oxygen. Temperature gradients have been found to influence the spatial distribution and growth of blue whiting (Kloppman et al. 2001; Hátún et al. 2009; Payne et al. 2012; Trenkel et al. 2015), and they might also explain, at least partly, the observed difference in otolith shapes, similar to what was found for *M. australis* (Legua et al. 2013).

In conclusion, otolith shape analysis has proven to be a useful tool to study the stock structure of blue whiting in the Northeast Atlantic.

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Fig. 1. Map of location of blue whiting samples collected between 2006 and 2011. Blue whiting feeding areas are located in the Norwegian Sea (IIa & IIb), the Celtic Sea (VIIg-j) and the Bay of Biscay (VIIIa & b).

Fig. 2. Photograph of a whole blue whiting otolith.

Fig. 3. a) Scatterplot of otolith perimeter as a function of body length and (b) predicted perimeter with 95% confidence intervals for best fitting linear model for male and female fish with 28 and 38 cm total length. Northern ICES Divisions are in grey.

588 Fig. 4. Average individuals from the two clusters identified by hierarchical cluster analysis
589 using Ward's hierarchical algorithm based on the squared Euclidean distances for all blue
590 whiting (N=1693) on the first 2 dimensions. The dots with sample numbers represent
591 individuals.

592 Fig. 5. Mean otolith outline shapes formed with reverse Fourier transform of the outline using
593 the first 34 harmonics showing the overlap and variations between two clusters (arrows show
594 the divergence areas).

Draft

AXIS 2 (15.67%)

Table 1. Number of blue whiting otolith samples by year, sex (M: males, F: females) and ICES Division. Total length characteristics (mean±S.D.) are given in parentheses.

Area (ICES Division)	Sex	Year of sampling				Total
		2006	2008	2010	2011	
Norwegian Sea (IIa)	F			105 (32.1±2.8)		105 (32.1±2.8)
	M			96 (29.7±1.8)		96 (29.7±1.8)
Spitzbergen and Bear Island (IIb)	F			33 (33.4±2.2)		33 (33.4±2.2)
	M			9 (30.3±1.1)		9 (30.3±1.1)
Northern North Sea (IVa)	F		30 (28.0±1.5)	48 (28.1±5.6)	24 (22.1±3.7)	102 (26.7±4.9)
	M			67 (25.8±5.3)	20 (22.0±3.0)	87 (24.9±5.1)
Iceland Grounds (Va)	F	112 (27.4±2.1)			14 (31.5±1.3)	126 (27.8±2.4)
	M				52 (32.3±1.7)	52 (32.3±1.7)
Faroes Grounds (Vb)	F		26 (27.1±1.9)			26 (27.1±1.9)
	M		12 (26.3±1.0)			12 (26.3±1.0)
West of Scotland (VIa)	F			25 (30.9±1.2)	218 (33.0±2.8)	243 (32.8±2.7)
	M			35 (29.0±0.7)	81 (30.5±2.2)	118 (30±2.0)
Rockall (VIb)	F		30 (29.2±1.2)	15 (31.4±1.7)		45 (30.0±1.7)
	M		31 (26.7±0.7)	25 (29.3±1.3)		31 (27.9±1.6)
Irish Sea (VIIa)	F				16 (18.9±2.3)	16 (18.9±2.3)
	M				24 (17.5±1.4)	24 (17.5±1.4)
Porcupine Bank (VIIc)	F			28 (32.1±1.2)		28 (32.1±1.2)
	M			22 (29.5±0.9)		22 (29.5±0.9)
Celtic Sea (VIIg & h)	F	70 (25.1±4.1)				70 (25.1±4.1)
	M	32 (25.7±3.4)				32 (25.7±3.4)
Southwest of Ireland – West (VIIk)	F			23 (31.5±1.3)		23 (31.5±1.3)
	M			9 (29.7±0.7)		9 (29.7±0.7)
Bay of Biscay (VIIIa&b)	F	34 (22.2±3.5)			118 (24.2±4.4)	152 (23.3±4.3)
	M	12			118	113

		(21.4±2.5)			(21.2±4.5)	(21.1±4.5)
Portuguese Waters – East (IXa)	F		24 (25.2±1.8)		16 (20.8±2.7)	40 (23.9±2.9)
	M		33 (22.6±2.4)		16 (23.0±1.5)	49 (22.7±2.2)

Table 2. Analysis of covariance for otolith perimeter. Interactions between factors are noted by a colon (:) (df - degree of freedom; SS - sum of squares; MS - mean sum of squares).

Factor	df	SS	MS	F value	P value
Length	1	1597.84	1597.84	24121.02	$< 2 \cdot 10^{-16}$
Sex	1	48.13	48.13	726.53	$< 2 \cdot 10^{-16}$
ICES Division	12	120.01	10.00	150.98	$< 2 \cdot 10^{-16}$
Year	3	24.24	8.08	121.97	$< 2 \cdot 10^{-16}$
Length:Year	3	7.46	2.49	37.52	$< 2 \cdot 10^{-16}$
Length:ICES Division	12	13.65	1.14	17.18	$< 2 \cdot 10^{-16}$
Length:Sex	1	0.62	0.62	9.42	0.002176
Residuals	1647	109.1	0.07		

Table 3. Summary of Redundancy analysis (RDA) (n = 1693) of blue whiting otolith shapes (df - degree of freedom).

Factor	df	P value
Year	3	$2.2 \cdot 10^{-16}$
ICES Division	12	$2.2 \cdot 10^{-16}$
Sex	1	0.538
Year : ICES Division	36	$2.2 \cdot 10^{-16}$
Year : Sex	3	0.071
ICES division : Sex	12	0.059

Table 4. Jackknifed correct classification matrix of the linear function discriminant analysis for mature blue whiting (N=1693) between sampling ICES areas and year based on the first normalized 34 harmonics. The percentages in each row represent the classification into the ICES sampling Divisions given in columns (correct classification in grey square) sample sizes are given in parentheses. Overall classification success is 28.41%, Wilks' $\lambda = 0.354$.

		IIa	IIb	IVa			Va		Vb	VIa	VIb	VIIa	VIIg-h	VIIIa-b		%correct
		2010	2010	2008	2010	2011	2006	2011	2008	2011	2008	2011	2006	2006	2011	
IIa	2010	49	7	2	9	0	7	3	3	104	5	3	0	2	7	24
IIb	2010	12	6	1	1	0	4	1	0	15	0	0	0	0	2	14
IVa	2008	7	1	2	2	0	5	1	0	9	2	1	0	0	0	7
	2010	18	1	2	19	15	5	1	2	33	3	8	0	4	4	17
	2011	1	0	1	20	9	2	0	1	3	1	1	0	6	9	20
Va	2006	18	4	3	4	2	19	0	10	36	2	4	3	2	5	17

	2011	16	3	1	2	1	2	5	2	31	2	1	0	0	0	8
Vb	2008	9	0	3	0	1	6	1	4	10	3	1	0	0	0	11
VIa	2011	47	6	2	8	2	19	7	2	180	6	4	0	3	13	60
VIb	2008	11	1	2	3	0	4	2	7	17	8	2	1	0	3	13
VIIa	2011	0	1	0	3	2	0	0	1	0	0	2	22	7	2	55
VIIg-h	2006	7	0	0	7	2	3	0	0	12	2	14	3	6	46	14
VIIIa-b	2006	0	0	1	1	4	0	0	0	2	0	12	3	9	24	20
	2011	4	0	1	4	5	7	1	0	21	3	35	9	17	92	42

Table 5. Jackknifed correct classification matrix of the linear function discriminant analysis for mature blue whiting (N=1693) between sampling ICES areas based on the first normalized 34 harmonics. The percentages in each row represent the classification into the ICES sampling Divisions given in columns (correct classification in grey square), sample sizes are given in parentheses. Overall classification success is 34.44%, Wilks' $\lambda = 0.089$.

	IIa	IIb	IVa	Va	Vb	VIa	VIb	VIIa	VIIc	VIIIk	VIIgh	VIIIab	IXa	%correct
IIa	42	1	1	2	0	129	0	0	0	0	1	25	0	21
IIb	13	5	0	3	0	14	0	0	0	0	0	7	0	12
IVa	22	0	29	13	2	95	0	1	0	0	0	24	3	15
Va	12	5	22	33	5	71	3	7	1	0	3	15	1	19
Vb	3	1	8	4	2	19	0	0	0	0	0	1	0	5
VIa	62	0	5	7	2	255	5	3	1	0	2	15	4	71
VIb	2	1	5	6	2	38	31	2	6	0	1	4	3	31
VIIa	1	0	0	0	0	11	0	25	2	0	0	1	0	63
VIIc	0	0	0	3	0	28	7	0	6	0	0	0	6	12
VIIIk	0	0	0	1	0	22	4	0	5	0	0	0	0	0
VIIgh	4	0	0	1	1	67	0	4	3	1	1	19	1	1
VIIIab	8	1	4	6	1	124	3	11	8	8	7	76	7	29
IXa	0	0	0	3	0	11	2	0	0	0	0	1	78	82