

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

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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 :

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

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

84 Introduction

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

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

100 Several basic descriptors exist to describe the external contour of otoliths (e.g., coefficient of
101 form, roundness, circularity, rectangularity and ellipticity) in addition to more complex
102 geometric morphometric analyses (Ponton 2006; Ramirez-Perez et al. 2010; Vergara-Solana
103 et al. 2013), wavelet functions (Parisi et al. 2005; Sadighzadeh et al. 2014) and the geodesic
104 method (Benzinou et al. 2013). Of these, the elliptical Fourier analysis currently remains the
105 most widely used and powerful method to describe otolith shapes comprehensively (Aguera
106 and Brophy 2011; Capoccioni et al. 2011; Fergusson et al. 2011; Legua et al. 2013; Paul et al.
107 2013). Otolith shape has the advantage of being unaffected by short-term changes in fish
108 condition (Campana and Casselman 1993) or environmental variations (Campana 1999).

109 Nevertheless, its biological interpretation is more complex than that of linear morphometric
110 descriptors (Stransky and MacLellan 2005). Some studies have combined elliptical Fourier
111 analysis and some basic descriptors of otolith contours to help the interpretation of results
112 (Campana and Casselman 1993; Begg and Brown 2000; Galley et al. 2006; Merigot et al.
113 2007; Fergusson et al. 2011; Legua et al. 2013).

114 Blue whiting (*Micromesistius poutassou*) is a pelagic gadoid whose distribution in the
115 northeast Atlantic extends from Spitsbergen in the north to Morocco in the south (Zilanov
116 1968; Bailey 1982; Heino and Godø 2002; Monstad 2004; ICES 2012). The blue whiting
117 fishery is one of the largest in the north Atlantic (Trenkel et al. 2014). Several European
118 nations have extensively fished this species, especially since the late 1990s, with annual
119 catches ranging from 100 000 to 2 400 000 tonnes (ICES 2013). All previous morphological,
120 physiological, genetic, parasite and otolith shape research suggested the existence of at least
121 two stocks in the north-east Atlantic, though geographic ranges differed between studies
122 (Karasev 1990; Isaev and Seliverstov 1991; Skogen et al. 1999; Ryan et al. 2005; Brophy and
123 King 2007; Was et al. 2008; Keating et al. 2014). Juveniles and adult blue whiting make
124 annual migrations in early summer to the main feeding areas, located primarily in the
125 Norwegian Sea in the north and in the Celtic Sea and Bay of Biscay in the south (Bailey 1982;
126 Carrera et al. 2001, Fig. 1). Previous stock structure studies focused primarily on the areas
127 located to the west of Scotland and Ireland, where spawning takes place in the water column
128 at the shelf break at depths of 250 to 450 metres (Bailey 1982; Isaev and Seliverstov 1991;
129 Standal 2006) and regions further north, with very few samples from the southern part of the
130 range, i.e. the Bay of Biscay and areas further to the South.

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

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

135

136 Materials and methods

137 Sample collection

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

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

151

152 Otolith shape analysis

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

158 *acusticus* facing up. The contour of each otolith was extracted using the automatic threshold
 159 in the TNPC software. To describe otolith contours, Elliptic Fourier Analysis (EFA; Lestrel
 160 1997) was carried out. For each otolith, the first 99 elliptical Fourier harmonics (H_i) were
 161 extracted and normalised with respect to the first harmonic using the TNPC software and
 162 were, thus, invariant to otolith size and rotation and starting point of the shape measurements
 163 (Kuhl and Giardina 1982). To determine the number of harmonics needed to reconstruct the
 164 otolith contour, the Fourier Power (PF) was calculated for each individual otolith k as a
 165 measure of the amount of contour rebuilt by each harmonic:

$$166 \quad PF(n_k) = \sum_{HI=1}^{n_k} \frac{A_{HI}^2 + B_{HI}^2 + C_{HI}^2 + D_{HI}^2}{2} \quad (1)$$

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

171

172 Statistical analyses

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

$$180 \quad P = \alpha + \alpha_s + \alpha_d + \alpha_y + \beta L + \beta_s L + \beta_d L + \beta_y L \quad (2)$$

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

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

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

194 To investigate potential explanations for otolith shape differences in more detail, multivariate
195 analysis of elliptical Fourier descriptors of otolith contours was carried out. To reduce the
196 number of dimensions and to avoid collinearity between descriptors, Principal Components
197 Analysis (PCA) was applied to the EFDs (Rohlf and Archie 1984). Significant principal
198 components (PC) were selected with the ‘broken stick method’ (Legendre and Legendre
199 2012). This method assumes that if the total variance (sum of the eigenvalues) is divided
200 randomly among the various components, the expected distribution of the eigenvalues will
201 follow a broken-stick distribution. The contribution of Fourier harmonics to the corresponding
202 eigenvectors (PC loadings) was analyzed to understand which shape features had most
203 influence on each PC. Next, two Redundancy analyses (RDA) were carried out. RDA is an
204 extension of multiple regressions to multivariate response data and an extension of principal
205 component analysis (Legendre and Legendre 2012). The first RDA aimed at removing the
206 variance explained by total fish length. In the second RDA, the residuals of the first RDA,

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

212 To determine whether otoliths collected in different ICES Divisions could be distinguished
213 based on their shapes, stepwise Linear Discriminant Analysis (LDA) was applied to select the
214 discriminant Fourier harmonics (Rencher and Christensen 2012). To evaluate the resulting
215 discriminant functions, the percentage correct classification of individuals to ICES Divisions
216 was calculated using jack-knifed cross-validation and Wilk's lambda criterion (Klecka 1980).
217 Finally, a cluster analysis was performed on the normalised Fourier harmonics to group
218 individuals with similar otolith shapes. For this, Ward's hierarchical algorithm based on
219 squared Euclidean distances was used. To visualise differences in otolith shape between
220 groups of individual fishes, the average otolith shape of each group was formed by the outline
221 reverse Fourier transform using the first 34 normalised Fourier harmonics.

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

225 Results

226 Otolith perimeter increased linearly with total length as expected (Fig. 3a). Linear modeling
227 revealed that sex, ICES Division and year of sampling all influenced the intercept (i.e., $\alpha_s \neq 0$;
228 $\alpha_d \neq 0$; $\alpha_y \neq 0$) and the slope ($\beta_s \neq 0$; $\beta_d \neq 0$; $\beta_y \neq 0$) of the relationship between perimeter and total
229 length ($R^2=0.94$). The ANCOVA showed that after total length, sex was the most important
230 variable, i.e. variable with the largest mean sum of squares, for explaining variations in the

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

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

241 Among the 99 Fourier harmonics extracted to describe otolith shape, the first 34 harmonics
242 explained more than 99.99% of the between otolith variation and were thus used for the
243 multivariate analysis. Principal Components Analysis of these first 34 Fourier harmonics (i.e.
244 156 Coefficients of Fourier, see eq. 1) explained 84.01% of the total variance and the first and
245 the second PC accounted for 36.82% and 14.24% of the total variance respectively. Only the
246 first seven PCs were significant as determined by their eigenvalues exceeding the threshold
247 eigenvalue generated by the broken-stick model ($> 3.9\%$ of the total variance). In the RDA
248 when the first seven PCs were related to total length, the relationship was found to be
249 significant ($p < 0.001$). Therefore in the second RDA, the residuals from the first RDA were
250 used to test the explanatory variables of interest, i.e. sex, year of sampling and ICES Division,
251 as well as second order interactions. All VIF values were smaller than 1.7 providing no
252 evidence for multi-collinearity between explanatory factors. ICES Division ($p < 2 \cdot 10^{-16}$) and
253 the interaction between year and ICES Division ($p < 0.05$) both explained differences in otolith
254 shape (Table 3). Given no sexual dimorphism was found in this analysis (sex: $p = 0.538$; Table
255 3), males and females were combined in the subsequent LDA and cluster analysis.

256 The LDA provided discriminant functions for year-ICES Division combinations using the 34
257 Fourier harmonics and sampling year as explanatory variables. The overall jack-knifed
258 classification success was 28.41% (Table 4). The analysis showed significant differences
259 among groups of blue whiting sampled in different ICES Divisions and years (Wilks' $\lambda =$
260 0.1769 ; $F=24.843$; $p<0.001$). The misclassification percentage for each group was highest in
261 the west of Scotland area (ICES Division VIa) and the Rockall area (ICES Division VIb),
262 where individuals were misclassified to the Norwegian Sea (IIa) (Table 4). The
263 misclassification percentages were particularly high from the Norwegian Sea to the West of
264 Scotland in the North and from the Irish Sea and the Porcupine Bank to the Portuguese
265 Waters in the South. When data from different years of sampling were grouped for a given
266 ICES Division in the LDA, the overall jack-knifed classification success increased only by
267 6% to 34% (Wilks' $\lambda = 0.141$; $F=23.402$; $p<0.001$) (Table 5). With the aim of achieving a
268 more reliable classification, certain ICES Divisions were combined. Combining ICES
269 divisions into two areas, 71% of individuals were assigned correctly to the northern (from
270 Norwegian Sea to West of Scotland: ICES Divisions IIa-VIa, $N=1009$) and the southern area
271 (from Rockall to Portuguese Waters: ICES Divisions VIb-IXa, $N=684$) (Wilks' $\lambda = 0.893$;
272 $F=28.834$; $p<0.001$). The hierarchical cluster analysis performed on the matrix of the 34
273 Fourier harmonics identified two clusters of fishes (Fig. 4). Cluster 1 ($N=573$) was
274 exclusively composed of blue whiting sampled from the Norwegian Sea to the West of
275 Scotland (ICES divisions: IIa, IIb, IVa, Va, Vb, VIa and VIb). All fish from Spitsbergen and
276 Bear Island were found exclusively in cluster 1. Conversely, cluster 2 ($N=1120$) included
277 individuals sampled in all ICES Divisions and years. For the southern ICES divisions (from
278 Irish Sea to the Portuguese Waters) individuals were found in cluster 2.

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

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

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

288 Discussion

289 The use of linear morphometric descriptors has been found to be of limited use for *M.*
290 *poutassou* (Keating et al. 2014). For this reason the perimeter was used in this study as a
291 single additional morphometric descriptor which gave results in general agreement with the
292 elliptical Fourier descriptors, except for the identification of a sexual difference. The number
293 of selected Fourier harmonics (PF=34) was higher than previously identified for *M. poutassou*
294 (PF=10, Keating et al. 2014) and *M. australis* (PF=14, Legua et al. 2013). A majority of
295 studies using this technique compute the cumulated Fourier power PF (Fourier Power) using
296 EFDs (Elliptical Fourier Descriptors) averaged across the full sample or a part of it, so that the
297 selected harmonics describe the average otolith shape. In this study however, in order to
298 ensure that each individual otolith in the sample was reconstructed with a precision of
299 99.99%, the PF value was calculated for each individual otolith such that individual shape
300 was reconstructed at 99.99%. The maximum number of harmonics across individual otoliths
301 was then used to statistically reconstruct each individual otolith in the sample. This value was
302 higher than the cumulated PF using averaged EFDs.

303 ANCOVA and redundancy analysis were carried out to explore the effects of size (total fish
304 length), sampling year and sex. Several authors have provided evidence for the importance of
305 these factors for allometric otolith growth (Castanguay et al. 1991; Campana and Casselman
306 1993; Begg et al. 2001; Cardinale et al. 2004; Capoccioni et al. 2011). Moreover, Campana
307 and Casselman (1993) found that sexual maturity can modify the final contour of otoliths.
308 Therefore in our study, only otoliths from mature adults were used. Further, a significant
309 correlation between total length of fish and morphological otolith parameters (perimeter and
310 EDS) was found. To remove this effect the residuals of the first seven PC from the normalised
311 elliptical Fourier descriptors were used.

312 The effect of sex on otolith perimeter was weak but significant. In contrast, somewhat
313 surprisingly, no significant sex effect on overall otolith shape was found. A sex effect on
314 otolith shape has been reported for other species, e.g. for herring (*Clupea harengus*, Bird et al.
315 1986), orange roughy (*Hoplostethus atlanticus*, Gauldie and Jones 2000), cod (*Gadus*
316 *morhua*, Paul et al. 2013) and southern blue whiting (*Micromesistius australis*, Legua et al.
317 2013).

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

328 (*Sardinops sagax*) (Felix-Uraga et al. 2004; Vergara-Solana et al. 2013). However, a season
329 effect was not tested in the present analysis due to a lack of sufficient sampling coverage
330 across seasons and areas. In addition, a significant interaction between sampling year and
331 geographical area was found in the RDA, which corroborates results obtained for other
332 gadoids species (Castonguay et al. 1991; Begg and Brown 2000; Petursdottir et al. 2006).

333 The results of this large scale study in which two broad blue whiting stocks were identified is
334 in accordance with previous smaller scale studies which also suggested the existence of
335 several stocks of the blue whiting in the northeast Atlantic (Karasev 1990; Isaev and
336 Selivestof 1991; Skogen et al. 1999; Ryan et al. 2005; Brophy et al. 2007; Brophy and King
337 2007; Was et al. 2008; ICES 2012; Keating et al. 2014). Karasev (1990), comparing parasite
338 types and infestation rates, concluded that there were three separate stocks from the north to
339 the south: (1) Barents Sea, Spitzbergen and Iceland (ICES Divisions I Ib, Va), (2) Norwegian
340 Sea, Faeroes and Hebrides (ICES Divisions IIa, Vb, VIa), and (3) Porcupine Bank, Celtic Sea,
341 Bay of Biscay and areas near Shetland (ICES Divisions VIIg-h & VIIIa-b). Other studies have
342 investigated the existence of only two stock units. Although the genetic variability in blue
343 whiting is low from Gibraltar to Norway (Mork and Gjaever 1995), differentiation has been
344 found between the south, Bay of Biscay and Celtic Sea, and the north, Porcupine bank to Papa
345 bank (Was et al. 2006; 2008). A recent study of blue whiting otolith shapes (Keating et al.
346 2014), which was focused on samples from ICES Divisions VIa and VIb, identified a
347 geographical stock limit along a line from Porcupine Bank to Rockall Bank (Fig. 1). The
348 location of spawning determines the probability of blue whiting eggs drifting northwards or
349 southwards (Skogen et al. 1999) which in turn defines the division line between the two stock
350 components. The division line, which varies between years, was determined to be
351 approximately at 54.5°N corresponding to the northern edge of Porcupine bank. The Skogen
352 et al. (1999) study covered the years 1976 to 1995, which was a period of strong subpolar

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

356 Otolith shapes are influenced by environmental conditions and genetic traits (Farias et al.
357 2009; Vignon 2012). Vignon and Morat (2010) found that genetic variations only affected the
358 otolith shape locally, mainly in the rostral and antirostrum parts. Conversely, Reichenbacher
359 et al. (2007; 2009), concluded that the posterior and postero-ventral angles of sagittal otoliths
360 differed as a consequence of environmental factors rather than different genetic information.
361 In the present study, variations of otolith shape were observed locally in the dorsal and ventral
362 parts (Fig. 5). Thus both environmental conditions and genetic components could explain the
363 differences. However, genetic differentiation in blue whiting seems to be low from Gibraltar
364 to Norway and there is a high probability of genetic mixing on the spawning grounds (Mork
365 and Gjaever 1995). Therefore, the main factors influencing the observed otolith shape
366 differences of blue whiting could be environmentally driven, such as temperature, salinity,
367 food availability, water depth and oxygen. Temperature gradients have been found to
368 influence the spatial distribution and growth of blue whiting (Kloppman et al. 2001; Hátún et
369 al. 2009; Payne et al. 2012; Trenkel et al. 2015), and they might also explain, at least partly,
370 the observed difference in otolith shapes, similar to what was found for *M. australis* (Legua et
371 al. 2013).

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

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

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

585 Fig. 3. a) Scatterplot of otolith perimeter as a function of body length and (b) predicted
 586 perimeter with 95% confidence intervals for best fitting linear model for male and female fish
 587 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).

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 (21.4±2.5)			118 (21.2±4.5)	113 (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 10 ⁻¹⁶
Sex	1	48.13	48.13	726.53	< 2 10 ⁻¹⁶
ICES Division	12	120.01	10.00	150.98	< 2 10 ⁻¹⁶
Year	3	24.24	8.08	121.97	< 2 10 ⁻¹⁶
Length:Year	3	7.46	2.49	37.52	< 2 10 ⁻¹⁶
Length:ICES Division	12	13.65	1.14	17.18	< 2 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 10 ⁻¹⁶
ICES Division	12	2.2 10 ⁻¹⁶
Sex	1	0.538
Year : ICES Division	36	2.2 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	Vla	Vlb	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
Vla	2011	47	6	2	8	2	19	7	2	180	6	4	0	3	13	60
Vlb	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	Vla	Vlb	VIIa	VIIc	VIIk	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
Vla	62	0	5	7	2	255	5	3	1	0	2	15	4	71
Vlb	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
VIIk	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









