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## **A reliable method for ageing of whiting (*Merlangius merlangus*) for use in stock assessment and management**

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### **ABSTRACT**

Accurate age estimation is important for stock assessment and management. The importance of reliable ageing is emphasized by the impending analytical assessment of whiting (*Merlangius merlangus*) in the Baltic Sea. Whiting is a top predator in the Western Baltic Sea, and is fished commercially although less extensively compared to the North Sea. Even though the species is considered one of the most difficult gadoids to age, few efforts have been made to shed light on the ageing problems. The aim of the present study was to identify and validate the 1<sup>st</sup> winter ring and to examine the visibility of the subsequent winter rings. Microstructure analysis was used to confirm the 1<sup>st</sup> winter ring. Additionally, otolith growth trajectories were obtained, confirming the allometric growth seen in many fish species. The method for ageing of whole otoliths presented in this study can be directly implemented in future ageing of whiting otoliths from the Baltic Sea – and potentially also adjacent areas where the conspecifics have similar growth rates.

*Keywords:* age estimation, otolith growth, validation, microstructure analysis, assessment

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## 23 **Introduction**

24 Whiting (*Merlangius merlangus*) is a commercially fished species throughout most of its  
25 distribution range. The increasing importance of the species in the North Sea and the Skagerrak  
26 is seen in the catches which have increased concomitantly with a decrease in catches of other  
27 gadoids such as cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*) (Anon.  
28 2012a/b). In the current EU directive regarding sampling of commercially important species, it is  
29 only obligatory to collect whiting in the North Sea, Kattegat and Skagerrak (EU 2010).

30 Analytical assessment is, currently, only conducted for whiting in the North Sea.

31 Acknowledging that whiting is a top predator in the western Baltic Sea, it is important to  
32 investigate its role, i.e. life-history traits, ecology and population dynamics. Such information  
33 can be used in multispecies modeling and for potential future analytical assessment. This  
34 emphasizes the importance of correct age estimation since under- or overestimation will  
35 influence ecological studies and bias the assessment (Beamish & McFarlane 1983; Campana  
36 2001; Reeves 2003; de Pontual *et al.* 2006). Whiting is considered to be a difficult gadoid to age  
37 (CEFAS 2005), and it is therefore essential to develop a reliable ageing method, which has  
38 potential application for other whiting stocks as well.

39 Vertebrae, scales, fin rays and otoliths are all used in ageing of fish, the latter being the primary  
40 method (Campana 2001; Campana & Thorrold, 2001). Routine ageing of otoliths is based on  
41 visual identification of growth zones (Campana & Thorrold 2001); an opaque zone is formed  
42 during the growth period (summer) and a translucent zone during periods of slow growth  
43 (winter). An annulus comprises both zones, but as 1<sup>st</sup> of January is set to be the birth date of all

44 fish, only the translucent zones are counted when ageing (Pannella 1974; Smedstad & Holm,  
45 1996).

46 Though commonly used, the traditional age estimation method has proven to be quite  
47 challenging in many gadoids such as Baltic cod (Hüssy 2010; Rehberg-Haas *et al.* 2012),  
48 European hake (Morales-Nin *et al.* 1998; de Pontual *et al.* 2006) and whiting (Polat & Gümüs  
49 1996; CEFAS 2005). Validation is required to ensure correct and reliable ageing (Beamish &  
50 McFarlane 1983). The most appropriate method to validate the age of a fish species is by  
51 mark/recapture studies marking both fish and otolith. This technique, however, is very time-  
52 consuming (Beamish & McFarlane 1983; Polat & Gümüs 1996; Campana 2001). Methods for  
53 identifying the 1<sup>st</sup> winter ring and investigating the seasonality in ring pattern have been applied  
54 such as breaking or grinding of otoliths (Polat & Gümüs 1996), microstructure analysis (Hüssy  
55 2010; Hüssy *et al.* 2010) or other methods (see review by Campana 2001).

56 The otoliths of whiting exhibit a similar annulus pattern as seen in many other fish species with a  
57 broad opaque zone forming during the growth season (spring-summer) and a narrow translucent  
58 zone during the period of reduced growth (winter) (Bowers 1954). As whiting grow larger,  
59 calcium carbonate is accumulated in the area around the nucleus, inhibiting the visibility of the  
60 1<sup>st</sup> and possibly also 2<sup>nd</sup> winter ring. This has been observed in whiting in the North Sea  
61 (Gambell & Messtorff 1964), the Irish Sea (Bowers 1954) and the Black Sea (Polat & Gümüs  
62 1996). In the latter study it was concluded that due to the thickness of the central area of the  
63 otolith, the risk of missing the 1<sup>st</sup> and 2<sup>nd</sup> annuli is high, hampering ageing based on whole  
64 otoliths. Problems relating to false winter rings, i.e. translucent zones formed during the year in  
65 response to changes in the environment, have additionally been reported for North Sea whiting  
66 (CEFAS 2005).

67 Different ageing methods such as grinding and breaking of the otoliths have been tested in  
68 whiting from other areas. Grinding of otoliths is a reliable but time-consuming method (Bowers  
69 1954; Gambell & Messtorff 1964; Polat & Gümüs 1996). Breaking of otoliths is a useful method  
70 for ageing of younger whiting (Polat & Gümüs 1996; CEFAS 2005), but as the fish grow older,  
71 the ring pattern becomes increasingly difficult to distinguish due to decreasing distances between  
72 the annuli (Gambell & Messtorff 1964). These studies have primarily focused on finding the best  
73 age estimation method, although investigating the seasonality in the edge formation is part of the  
74 age validation process. The first step in the validation process is to identify and validate the 1<sup>st</sup>  
75 winter ring (Campana 2001). The next step is to investigate the seasonality in the edge zone  
76 formation and to explore the consistency of the annulus pattern (Campana 2001). Both steps  
77 should theoretically be carried out for all age classes and for different years (Beamish &  
78 McFarlane 1983), though this is often difficult to achieve (Campana 2001).

79 Using whiting from the Western Baltic Sea, the objectives of this study are (1) to confirm the  
80 previous findings on whiting otoliths, i.e. the increasing difficulties in distinguishing the first  
81 annuli with increasing fish size and the seasonality in zone formation, (2) to identify and validate  
82 the first annulus and (3) to show individual otolith growth profiles, which will shed light on the  
83 changes in otolith growth rate from juvenile to adult. Additionally, a smaller sample of otoliths  
84 from the North Sea was examined to investigate whether similar problems regarding the  
85 decreasing visibility of the 1<sup>st</sup> winter ring exist.

86

## 87 **Materials and Methods**

### 88 **Sample selection**

89 Whiting were caught randomly during the extended BITS surveys in November 2011, January  
90 and May 2012. Stratified random sampling according to ICES square and depth stratification was  
91 conducted in the Fehmarn Belt (with a standard TV3-520 bottom otter trawl, OTB) in the  
92 southern part of the ICES subdivision 22 (fig. 1). The whiting were measured to the nearest cm  
93 below, weighed and the sagittal otoliths removed.

94 Fish used for identifying the 1<sup>st</sup> annulus by microstructure analysis were selected randomly from  
95 the peaks in the 2009, 2010 and 2011 cohort length distributions (i.e. 0-3 group), respectively  
96 (fig. 2). A total of 60 fish were selected (20 fish per survey), covering a length range of 8-30cm.  
97 Fish belonging to the 2009-2010 year-classes were subsequently used to test whether the  
98 increment pattern in older fish was consistent with the patterns observed in the first year of the  
99 same cohort. However, as year classes of whiting from other areas have been shown to overlap in  
100 length ranges (Gambell & Messtorff 1964; Flintegaard 1980; Armstrong *et al.* 2004), the tails of  
101 each cohort's length distribution were also sampled. These fish were also used in the edge  
102 formation analysis and for examining the visibility of the 1<sup>st</sup> annulus. Additional 11 fish in the  
103 size range 30-36cm were included in the latter analysis to confirm that only the 1<sup>st</sup> winter ring  
104 "disappeared" in the larger fish.

105 No samples were available for the 3<sup>rd</sup> quarter in the Fehmarn Belt surveys. To investigate the  
106 seasonality in the otolith edge formation, additional samples were taken with midwater otter  
107 trawl (OTM) in the acoustic survey performed by the German vessel, R/V Solea in September  
108 2011 in ICES subdivision 24 (fig. 1). 20 fish were randomly selected and otoliths from them only  
109 used for the edge formation analysis. Together with otoliths used for the identification of the 1<sup>st</sup>  
110 annulus, otoliths from a total of 80 fish were used in the edge formation analysis.

111 To test the applicability of this approach to other stocks, 15 otoliths from whiting in the North  
112 Sea were used in a separate analysis. Fish were randomly selected from a discard survey  
113 conducted with a multi-rig otter trawl (OTT, 90 mm mesh size) in June 2011 in the northeastern  
114 part of the North Sea (close to the Skagerrak). These fish covered a length range of 17-28 cm.

115

## 116 **Analyses**

117 Otoliths were investigated using three different methods: (1) ageing of whole otoliths, (2) ageing  
118 of ground otoliths and (3) examination of daily increment patterns, i.e. detection of zones with  
119 relatively smaller increments (low growth) assumed to correspond to the formation of a winter  
120 ring. The analyses were based on the following assumptions: (1) one year's growth corresponds  
121 to an opaque and a translucent zone; (2) this pattern is consistent throughout the life of the fish;  
122 and (3) periods of slow and fast growth (i.e. during winter and summer) can be observed as  
123 zones of decreasing and increasing daily increment widths. All image analyses were carried out  
124 in IMAGE PRO (vs. 5.0) and for the statistical analyses the Statistical Software *R* (R  
125 Development Core Team, 2009) was used.

126

## 127 **Ageing of whole otoliths**

128 The otoliths were placed in propylene glycol, sulcus facing upwards, and viewed under a  
129 stereomicroscope (Leica MZ12) at a 1.25x magnification corresponding to  $2.56 \mu\text{m pixel}^{-1}$  using  
130 reflected light in a standardized set-up. Images were digitized (Leica camera DFL290) using a

131 standard set-up. The distance from the nucleus to the 1<sup>st</sup> annulus ( $D_{\text{Traditional}}$ ) was measured on the  
132 anterior axis from the nucleus towards the tip of the rostrum (fig. 3).

133

#### 134 **Ageing of ground otoliths**

135 The otoliths were glued to a glass slide using thermoplastic resin (Buehler) and ground on both  
136 sides on a rotating disc with two different abrasive papers (grit 3  $\mu\text{m}$  and grit 1200  $\mu\text{m}$ ) to a  
137 thickness of approximately 500  $\mu\text{m}$ . The ground otoliths were viewed and treated according to  
138 the procedure above. The distance from the nucleus to the 1<sup>st</sup> annulus ( $D_{\text{Ground}}$ ) was measured  
139 (fig. 3).

140

#### 141 **Annulus pattern and individual growth trajectories**

142 The consistency of the sequential annuli was investigated and it was further tested whether there  
143 was a correlation between the 1<sup>st</sup> visible annulus in the whole otoliths and the 2<sup>nd</sup> annulus in the  
144 ground otoliths. This was done by comparing the distance from the nucleus to the 1<sup>st</sup> winter ring  
145 ( $D_{\text{Traditional}}$ ) in the whole otoliths with the distance from the nucleus to the 2<sup>nd</sup> winter ring  
146 ( $D_{2\text{Ground}}$ ) in the ground otoliths. Similarly, the 2<sup>nd</sup> visible annulus in the whole otoliths was  
147 compared with the 3<sup>rd</sup> annulus in the ground otoliths

148

#### 149 **Daily increment pattern and identification of the 1<sup>st</sup> annulus**



150 Microstructure analysis of the daily increments generates a similar pattern as the yearly banding  
151 with translucent zones corresponding to the period of slow growth (usually during the night) and  
152 opaque zones corresponding to the period of fast growth (day). One increment is comprised of a  
153 translucent and an opaque zone. Microstructure analysis or marginal increment analysis (MIA) is  
154 a good method to identify and validate the 1<sup>st</sup> winter ring (Campana 2001). Increment widths  
155 should display a sinusoidal cycle when plotted against time, i.e. during winter the widths  
156 decrease and during summer they increase (Campana 2001).

157 The ground otolith sections were viewed under a microscope (Leica DMLB) at a 10x  
158 magnification corresponding to  $0.46 \mu\text{m pixel}^{-1}$  using reflected light in a standardized set-up.  
159 Images were digitized (QImaging QIcam Fast 1394) using a standard set-up. The daily growth  
160 increments were investigated using the “caliper tool” in IMAGE PRO (vs. 5.0) which generates a  
161 profile of grey values ranging between 0, black, and 255, pure white. The beginning of an  
162 increment was defined as the rising point of inflection between the previous opaque zone and the  
163 subsequent translucent zone and was calculated from the divergence of individual pixel grey  
164 values from the running average. The distance from the nucleus on progressing days  $i$  was  
165 calculated as  $\text{Distance}_i = \text{Distance}_{i-1} + \text{Increment}_i$ . The  $\text{Distance}_{i-1}$  was standardized to the  
166 anterior axis by multiplying with the ratio between the length of the anterior axis and the length  
167 of the axis used for increment measurements. The  $\text{Increment}_i$  was standardized in a similar way,  
168 i.e. by multiplying with the ratio between the increment widths on the anterior axis and the  
169 increment widths on the axis used for increment measurements. Zones in which increments were  
170 difficult to distinguish were measured and added to the total distance, but leaving out the  
171 individual increments from the analysis. To reduce the inter-individual variation, the increment  
172 widths for each individual were standardized to the widest increment ( $\text{increment}_i / \text{increment}_{\text{max}}$ ).

173 This resulted in growth profiles (nucleus to edge) showing the increment widths in relation to the  
174 distance from the nucleus. The distance from the nucleus to the midpoint of the 1<sup>st</sup> zone with  
175 decreasing increment widths,  $D_{\text{Increment}}$ , was measured.

176

### 177 **Seasonal otolith edge formation**

178 The otolith edge was investigated to determine when the formation of the winter ring is initiated  
179 and ended. Four months were chosen (January, May, September and November) and 20-30  
180 otoliths were analyzed per month. The otoliths were ground in accordance with the procedure  
181 mentioned above and the edge of each otolith was inspected visually. Otoliths were categorized  
182 as having an opaque or translucent edge, respectively.

183

### 184 **North Sea otoliths**

185 Ageing of whole and ground otoliths from the North Sea was conducted in a similar way as with  
186 the Baltic Sea otoliths. Only otoliths of fish above 16 cm were included in this analysis as  
187 problems regarding the visibility of the 1<sup>st</sup> winter ring do not arise until the fish reach a certain  
188 size (Bowers 1954; Gambell & Messtorff 1964; Polat & Gümüs 1996).

189

## 190 **Results**

### 191 **Comparison of $D_{\text{Traditional}}$ and $D_{\text{Ground}}$**

192 The central area of the whiting otolith is thick and the zones less distinctive (fig. 4a).  
193 Comparison of whole and ground otoliths showed that the first annulus becomes increasingly  
194 difficult to detect by traditional ageing of whole otoliths as the fish grow larger (fig. 4a). There  
195 was a significant difference between the values of the ageing of the whole otoliths and the  
196 ground otoliths (paired t-test,  $df = 70$ ,  $p < 0.001$ ). This is also seen when comparing the actual  
197 distances from nucleus to 1<sup>st</sup> annulus,  $D_{\text{Traditional}}$  and  $D_{\text{Ground}}$  (paired t-test,  $df = 68$ ,  $p < 0.05$ ).  
198 The distance from nucleus to 1<sup>st</sup> annulus increases linearly with fish size up until a size of 16 cm  
199 in both whole and ground otoliths (fig. 5a). Thus,  $D_{\text{Traditional}}$  and  $D_{\text{Ground}}$  are not significantly  
200 different in fish  $< 17$  cm (ANOVA,  $df = 65$ ,  $p = 0.523$ ). At fish lengths  $\geq 17$  cm,  $D_{\text{Traditional}}$   
201 continues to increase linearly with fish length, whereas  $D_{\text{Ground}}$  stops at a threshold value of  
202 approximately 3600  $\mu\text{m}$ . This gives an interval of 1800 $\mu\text{m}$  ( $\sim 1800\text{-}3600\mu\text{m}$ ) from the nucleus in  
203 which the 1<sup>st</sup> winter ring can be assumed to lie within.

204

#### 205 **Comparison of $D_{\text{Traditional}}$ and $D_{2\text{Ground}}$**

206 The 2<sup>nd</sup> annulus was not difficult to distinguish in the whole otoliths investigated in this study,  
207 i.e. the 1<sup>st</sup> visible translucent zone observed in the whole otoliths in the 2+ groups corresponded  
208 well with the 2<sup>nd</sup> translucent zone seen in the otoliths after grinding (ANOVA,  $df = 41$ ,  $p =$   
209  $0.579$ ) (fig. 5b). Similarly when comparing the distance from the nucleus to the 3<sup>rd</sup> annulus in the  
210 ground otoliths with the distance from the nucleus to the 2<sup>nd</sup> annulus in the whole otoliths  
211 (ANOVA,  $df = 41$ ,  $p = 0.860$ ) and for the 4<sup>th</sup> annuli (ANOVA,  $df = 17$ ,  $p = 0.823$ ).

212

## 213 **Comparison of $D_{\text{Ground}}$ and $D_{\text{Increment}}$**

214 The daily increment widths generated a dome-shaped pattern with increasing widths during  
215 summer, where the fish grows, and decreasing widths during winter, when growth is stalled (fig.  
216 6a). The increment widths become very narrow, but they never disappear completely (fig. 6b).

217 By applying a fifth degree polynomial trend line to each growth profile, the midpoint of the area  
218 of consistently decreasing increment widths was identified visually and the distance from  
219 nucleus to the midpoint ( $D_{\text{Increment}}$ ) was recorded. This area corresponded well with the formation  
220 of a translucent zone (fig. 6c). Additionally, there appears to be a juvenile/settling zone  
221 approximately 1500  $\mu\text{m}$  from the nucleus, which should not be misinterpreted as the first winter  
222 ring (fig. 4b). This was confirmed by the microstructure analysis which showed continuously  
223 broad increment widths throughout the zone. A winter ring would have corresponded with a  
224 decrease in increment width. Based on the microstructure analysis, an interval for each winter  
225 ring could be obtained (table 1). The juvenile zone was only visible in the ground otoliths and it  
226 was narrower than the winter rings.

227 There was a high degree of consistency between  $D_{\text{Ground}}$  and  $D_{\text{Increment}}$  (ANOVA,  $df = 67$ ,  $p =$   
228  $0.905$ ) (fig. 6a/c). Combining the two methods provides an estimate of the maximum distance  
229 from the nucleus to the 1<sup>st</sup> annulus, which was found to be 3600  $\mu\text{m}$ .

230

## 231 **Growth trajectories**

232 The otoliths from the larger fish all showed a consistent winter ring pattern with decreasing  
233 distances between the annuli (fig. 7, table 1). The range of each winter ring was large, indicating

234 that significant variation in otolith growth exists (table 1). Additionally, the ranges overlapped,  
235 i.e. the upper limit for the 1<sup>st</sup> annulus was ~3600µm and the lower limit for the 2<sup>nd</sup> annulus was  
236 ~2900µm (table 1, fig. 5).

237

### 238 **Seasonal otolith edge formation**

239 The development in the opacity of the edge zone followed a seasonal pattern, although  
240 differences between otoliths existed. It was difficult to determine the degree of opacity of  
241 otoliths sampled in May and November as these months are part of a transition period in which  
242 the growth is either increased or reduced. Optical effects as well as the thermoplastic resin, in  
243 which the otoliths lay in, further complicated the interpretation. Most of the otoliths analyzed in  
244 January were observed to have a translucent edge, whereas in May approximately 30% had a  
245 translucent zone (fig. 8). In September, only a very small fraction had a translucent zone and this  
246 fraction increased in November to approximately 70% (fig. 8).

247

### 248 **North Sea otoliths**

249 Comparison of whole and ground otoliths did not show a similar consistent problem in relation  
250 to distinguishing the 1<sup>st</sup> winter ring (paired t-test, df = 14, p = 0.334). The distance between the  
251 nucleus and the 1<sup>st</sup> annulus was not significantly different (paired t-test, df = 14, p = 0.216).  
252 Nevertheless, the opaque zones were difficult to distinguish in two out of the fifteen otoliths, and  
253 grinding was necessary to ensure that the ageing was correct.

254

## 255 **Discussion**

256 This study confirmed earlier studies which have shown that traditional ageing of whiting otoliths  
257 is challenging and may result in underestimation of age (Gambell & Messtorff 1964; Polat &  
258 Gümüs 1996). In order to validate the winter ring formation, three issues were addressed: (1)  
259 seasonality of the otolith edge formation, (2) identification of the 1<sup>st</sup> winter ring and examination  
260 of the visibility of the 2<sup>nd</sup> and succeeding winter rings, and (3) individual otolith growth  
261 trajectories. Additionally, otoliths from North Sea whiting were examined to test the applicability  
262 of the present approach to other stocks.

263 Beamish & McFarlane (1983) were very strict about the validation of all ages and stated that  
264 extrapolation beyond the maximum validated age between populations can result in serious  
265 errors. They also pointed out that the only correct validation method is by mark/recapture.  
266 Considering the temporal scale of such a validation study as well as the fact that a large amount  
267 of the tagged fish would end up in the fishing nets during the first few years, the approach  
268 presented in this study is a valid substitute. The difficulties of obtaining whiting with known ages  
269 were also stressed by CEFAS (2005). With regard to validation of all ages, it was considered  
270 reliable to focus on identifying the 1<sup>st</sup> winter ring as the main issue in whiting appears to be the  
271 increasing thickness of the core area of the otolith with age, inhibiting the visibility of the 1<sup>st</sup>  
272 winter ring in whole otoliths (Bowers 1954; Gambell & Messtorff 1964; Polat & Gümüs 1996).  
273 This conclusion was supported in the present study which showed that from the 2<sup>nd</sup> winter ring  
274 and onwards, the annuli were always visible.

275 In accordance with earlier studies (Bowers 1954; Gambell & Messtorff 1964), the edge  
276 formation was found to vary over the seasons with most otoliths having a translucent edge in the

277 winter and early spring. Thus, one of the main requirements put forth in the beginning of the  
278 study is fulfilled, i.e. the synchronous appearance across all individuals of an opaque and a  
279 translucent zone corresponding to fast and slow growth, respectively.

280 The microstructure analysis showed a consistent pattern with increasing increment widths in the  
281 period corresponding to summer, where the temperatures are high, and decreasing increment  
282 widths during winter (fig. 6a). This pattern persisted in the 2<sup>nd</sup> and 3<sup>rd</sup> year of life in the otoliths  
283 studied, and is thus considered to be representative for all year-classes. The observed increment  
284 pattern is seen in other gadoids like Baltic cod (Hüsey 2010; Hüsey *et al.* 2010), haddock and  
285 saithe (Quiñonez-Velázquez 1998), but also in other fish species, e.g. Atlantic herring (Clausen  
286 2006; Oeberst *et al.* 2006), boarfish (Hüsey *et al.* 2012), and sprat (Baumann *et al.* 2006).

287 The increment widths became successively narrower during winter, but never ceased completely  
288 as in eastern Baltic cod (Hüsey 2010; Hüsey *et al.* 2010), North Sea herring (Clausen 2006) and  
289 boarfish (Hüsey *et al.* 2012), where the increments disappear during winter concurrently with the  
290 formation of the translucent zone. The reason for the continuous increment formation in Baltic  
291 Sea whiting is not known and analyses of the increment pattern in older fish as well as in whiting  
292 from adjacent areas should be conducted to investigate this further.

293 The 1<sup>st</sup> annulus was identified in 0 to 3-group fish by applying microstructure analysis which  
294 confirmed the first translucent zone to be a winter ring associated with low water temperatures.

295 In some of the otoliths a translucent zone approximately 1500 µm from the nucleus was  
296 observed. Though the zone appeared translucent, no concurrent decrease in increment widths  
297 was observed, and the zone was thus not considered to be an annulus. This juvenile/settling zone  
298 may be similar to the one found in whiting from the Irish Sea and the North Sea, referred to as

299 the Bowers' zone, which is formed during late summer and likely relates to the change from  
300 pelagic to demersal habitat (Bowers 1954; Gambell & Messtorff 1964). It was generally easy to  
301 distinguish from the translucent zone formed during the first winter as the juvenile zone appeared  
302 close to the nucleus (~1500 µm). Bowers (1954) also noted that the translucent zone is much  
303 narrower than the actual annulus and this was also confirmed in the present study. More  
304 importantly, the microstructure analysis confirmed that this zone was not a winter ring since no  
305 decrease in increment widths was observed. The juvenile zone is only visible in ground otoliths,  
306 where microstructure analysis may reveal its nature.

307 The distance from the nucleus to the 1<sup>st</sup> winter ring showed large variation, the same applied to  
308 the succeeding winter rings (table 1). Whiting is a batch spawner, and in the North Sea and the  
309 Irish Sea, the species have been reported to spawn over an extended period (February to  
310 September) (Bowers 1954; Gambell & Messtorff 1964; Hislop 1975; Cohen *et al.* 1991), hence it  
311 does not seem unreasonable to have a large variation in otolith growth, i.e. larvae hatched late in  
312 the season will have a significantly reduced growth season. The 1-group fish (2011 cohort) used  
313 in this study ranged in size from 8-20 cm with the smallest fish being caught in May.

314 Whiting from the present study were capable of growing up to 20 cm within the first year of life.  
315 The rapid growth was also confirmed by the otolith growth trajectories which showed large  
316 otolith growth during the first year and then decreasing growth in the succeeding years (fig. 7).  
317 Bowers (1954) noted that from the second year and onwards, the growth is more moderate, i.e. 5-  
318 6 cm per year in Irish Sea whiting.

319 In most marine fish species, the initial growth is determining for growth later in life, hence a fish  
320 with a low growth rate in the first year will usually have slow growth throughout its life span



321 (Krohn & Kerr 1997; Armstrong *et al.* 2004; Rindorf 2008). This was also seen in the present  
322 study, where fish with the largest initial otolith growth generally achieved the overall largest  
323 otolith growth (fig. 7), corresponding to the highest length-at-age. The decrease in otolith growth  
324 with age is in agreement with the allometric growth seen in most species, especially after  
325 maturation where a proportion of the energy is allocated towards reproduction (Björnsson &  
326 Steinarsson 2002).

327 The fact that the ranges of the winter rings overlapped (table 1) was not surprising considering  
328 the large variation in hatching time and the resulting overlap in length distributions for the  
329 different year-classes. The large variation in length-at-age is also seen in whiting from other  
330 areas (Bowers 1954; Gambell & Messtorff 1964; Flintegaard 1980; Armstrong *et al.* 2004;  
331 CEFAS 2005). Similar overlap in the ranges of the winter rings are reported in Baltic cod (Hüssy  
332 2010).

333

#### 334 **Manual to ageing of whiting**

335 The otoliths showed a consistent winter ring pattern with decreasing distances between the  
336 annuli like in otoliths from Irish Sea and North Sea whiting (Bowers 1954; Gambell & Messtorff  
337 1964) as well as in other gadoids such as hake (Morales-Nin *et al.* 1998) and Baltic cod (Hüssy  
338 2010; Hüssy *et al.* 2010).

339 CEFAS (2005) provided guidelines for the ageing of North Sea whiting. It was generally  
340 recommended to break or section the otoliths, but if read whole the rostrum (i.e. the pointed part  
341 of the otolith) is considered the most reliable part of the otolith. In the present study, the post-  
342 rostrum or the anterior side of the otolith was found most suitable for ageing of both whole and

343 ground otoliths, as the winter rings were generally difficult to distinguish in the rostrum or the  
344 posterior side (fig. 3). Sectioning of the otoliths is not considered the most appropriate method  
345 for ageing of whiting as the annuli in older fish become very narrow and may be difficult to  
346 distinguish (Gambell & Messtorff 1964), especially in Baltic Sea whiting. Therefore grinding of  
347 otoliths is the preferred method. The present study enables reliable ageing of whole otoliths by  
348 following the guidelines below.

349 Based on the results from this study, guidelines for ageing routines of whiting were established:

- 350 • Preparation (propylene glycol for 15 min or distilled water for 24 hours to enhance the  
351 visibility of the opaque zones)
- 352 • Identify visible translucent zones
- 353 • Determine whether the edge is opaque or translucent
- 354 • Consult the catch date. If the fish was captured
  - 355 1) Before January 1<sup>st</sup>, the translucent edge is not counted
  - 356 2) After January 1<sup>st</sup>, the translucent edge is included in the count
- 357 • Measure the distance from the nucleus to the 1<sup>st</sup> visible translucent zone
  - 358 1) If 1500-3400  $\mu\text{m}$ , the translucent zone can be considered as the 1<sup>st</sup> winter ring
  - 359 2) If close to 3600  $\mu\text{m}$ , check the otolith growth trajectory, i.e. measure and compare  
360 the distances between the winter rings (c.f. fig. 7 and table 1)
  - 361 3) If > 3600  $\mu\text{m}$ , the 1<sup>st</sup> annulus is likely hidden

362 Note: If measuring the distance from the nucleus to the 1<sup>st</sup> visible translucent zone gives  
363 rise to either (2) or (3), grinding or sectioning of the otolith must be performed.

364

## 365 **Future perspectives**

366 The method developed in the present study likely applies to whiting from other areas as well,  
367 although the crosscheck, i.e. the maximum distance from the nucleus to the 1<sup>st</sup> winter ring, may  
368 differ somewhat between areas. In this study, a smaller amount of otoliths from whiting  
369 inhabiting the North Sea was examined and even though no apparent problem with  
370 distinguishing the 1<sup>st</sup> winter ring existed, some of the otoliths were thick which made it difficult  
371 to distinguish all of the winter rings. Nevertheless, decreasing visibility of the 1<sup>st</sup> annulus has  
372 been reported for otoliths from whiting in the North Sea (Gambell & Messtorff 1964), and  
373 whether this only applies to some subpopulations inhabiting the area, is yet to be investigated.  
374 This emphasizes the usage of the method developed in this study where grinding of the otolith is  
375 employed whenever doubt arises. More thorough analyses of whiting otoliths from other areas  
376 should be conducted to investigate whether similar or other problems regarding ageing of whole  
377 otoliths exist.

378 The results obtained in this study, together with results from previous ones, emphasize the need  
379 for a more holistic approach which incorporates length, catch date overall annulus pattern and  
380 application of a crosscheck (maximum distance from the nucleus to the 1<sup>st</sup> annulus). The  
381 stepwise method presented here can be directly implemented in ageing of whole otoliths and  
382 should provide correct age estimation, thereby ensuring the reliability and precision of analytical  
383 assessment of whiting.

384

## 385 **Acknowledgement**

386 The authors would like to thank the two reviewers for their suggested amendments which have  
387 improved the manuscript.

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462



463 **Tables**

464 Table 1 Whiting otolith growth.  $D_i$  is the distance from the nucleus to the respective winter ring.

465 Mean  $D_{i-1}$  is the distance between the 1<sup>st</sup> and 2<sup>nd</sup> winter ring (shown in the 2<sup>nd</sup> winter ring row),

466 2<sup>nd</sup> and 3<sup>rd</sup> winter ring and so forth. All measurements are in  $\mu\text{m}$ .

Winter ring number	Mean D	Range of $D_i$	Mean $D_{i-1}$	Range of $D_{i-1}$
Juvenile ring	1500	800-2100		
1	2600	1800-3600		
2	3900	2900-5200	1180	700-1600
3	5000	3800-6400	940	600-1400
4	6000	5200-6900	750	450-850
5	6400	5900-7000	650	570-680

467

468 **Figure captions**

469 Fig. 1 Sampling area. ICES subdivisions 22 and 24. The Femern Belt area is encircled.

470 Fig. 2 Length distribution for the 2009-2011 cohorts. The length distributions for the 2009-2011  
471 cohorts caught in November 2011, January 2012 and May 2012. Estimated numbers are based on  
472 the length proportions from a sample taken from each haul, i.e. the number in each length group  $i$   
473 for all hauls is calculated as  $N_i = \sum S_{i,h} \frac{W_h}{V_h}$ , where  $S_{i,h}$  denotes the numbers of length group  $i$  in  
474 the sample drawn from the haul  $h$ ,  $W_h$  is the total weight of whiting in the haul  $h$  and  $V_h$  is the  
475 weight of the sample drawn from haul  $h$ .

476 Fig. 3 Example of a whole, untreated Baltic Sea whiting otolith. The measurement axis is shown,  
477  $D$  = the distance from the nucleus to the 1<sup>st</sup> annulus.

478 Fig. 4 Ageing of whole and ground otoliths. Ageing of otolith from a fish, length of 22 cm,  
479 caught in May 2012. Image of (a) whole otolith and (b) ground otolith. Nucleus as well as the  
480 visual annuli are marked.

481 Fig. 5 Distance from nucleus to 1<sup>st</sup> and 2<sup>nd</sup> annulus as a function of fish length. (a) Distance from  
482 the nucleus to the 1<sup>st</sup> annulus ( $\mu\text{m}$ ) shown as a function of the fish length (cm) and (b) Distance  
483 from the nucleus to the 2<sup>nd</sup> annulus ( $\mu\text{m}$ ) shown as a function of the fish length (cm) (NB: the 1<sup>st</sup>  
484 visual annulus of the whole otoliths is plotted as this in reality corresponds to the 2<sup>nd</sup> annulus).  
485 Whole otoliths are shown with black dots and ground otoliths with red triangles.

486 Fig. 6 Increment width as a function of the distance from the nucleus. Ground otolith from 1-year  
487 old fish (length 18 cm) caught in November 2011. (a) Microstructure profile showing the  
488 increment widths as a function of the distance from the nucleus. (b) Increment widths of a

489 section of the otolith where the translucent area corresponds to the 1<sup>st</sup> annulus (7x magnification  
490 = 0.32  $\mu\text{m pixel}^{-1}$ ). (c)  $D_{\text{Ground}}$  shown with a straight line ( $\approx 2000\mu\text{m}$ ).

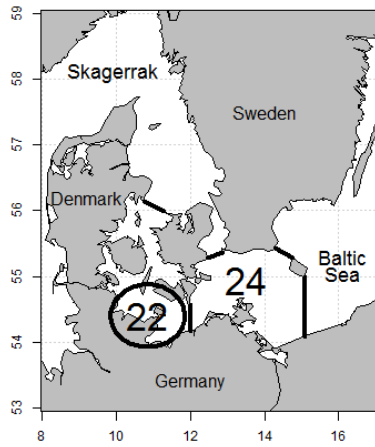
491 Fig. 7 Otolith growth trajectories. The distances from the nucleus to 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> annulus  
492 (based on ground otoliths) are shown as a function of the annulus number. The lines show the  
493 growth curves for 22 age 3 fish and 10 age 4 fish. Each line corresponds to an individual fish.

494 Fig. 8 Percentage of otoliths with an opaque edge zone. Otoliths with an opaque edge shown as a  
495 percentage of the total number of otoliths analyzed per month

496

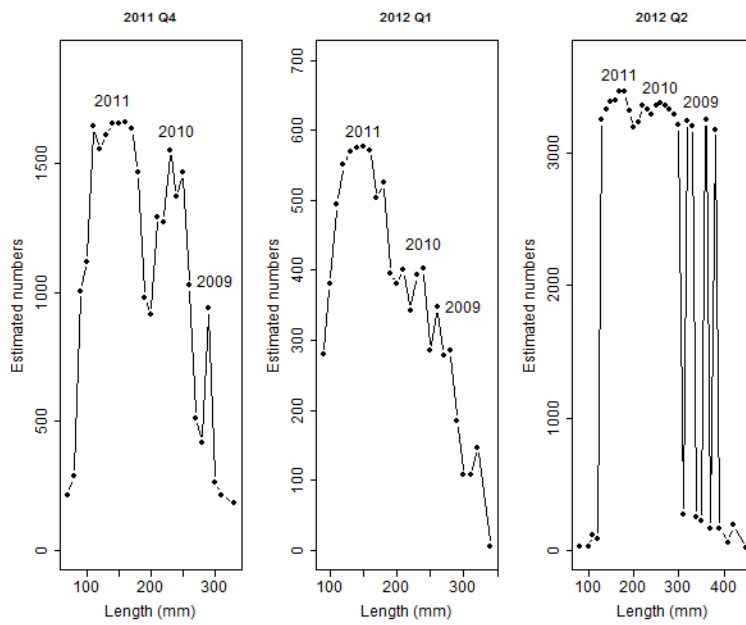
497 **Figures**

498 Fig. 1 Sampling area



499

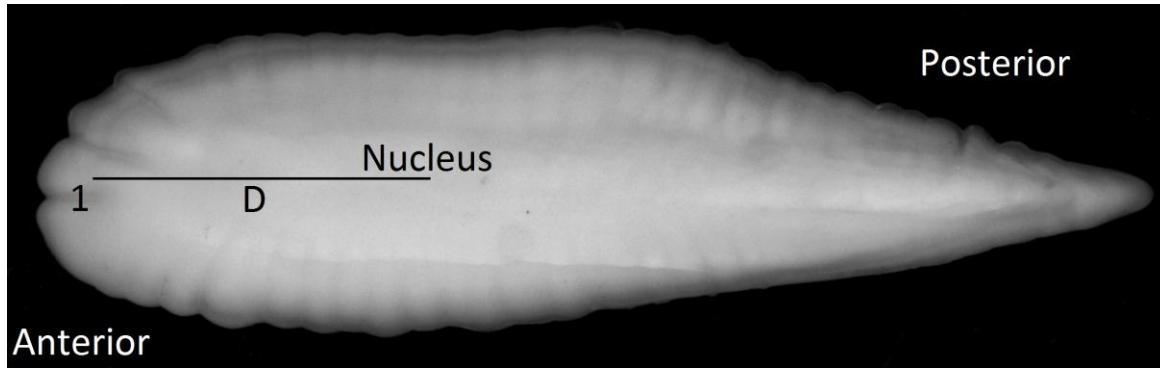
500 Fig. 2 Length distribution for the 2009-2011 cohorts



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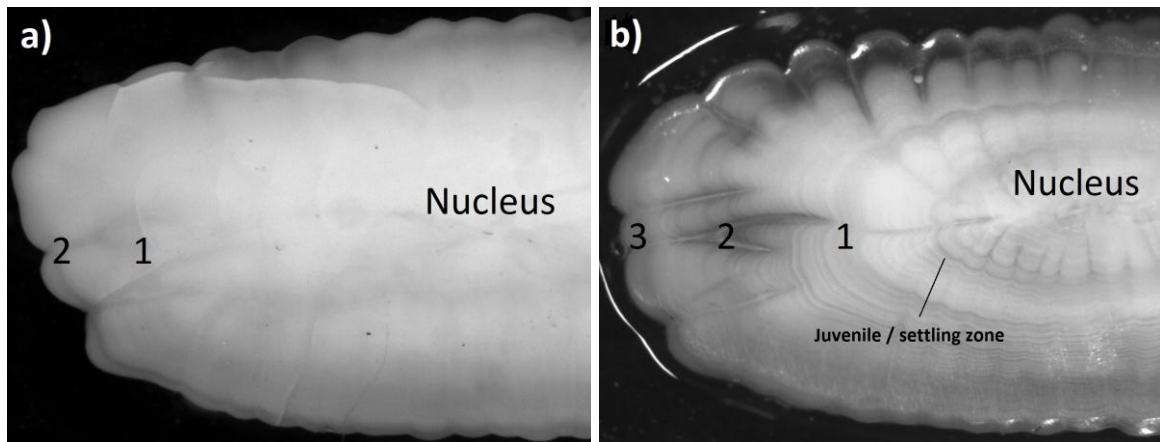
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503 Fig.3 Example of a whole, untreated Baltic Sea whiting otolith



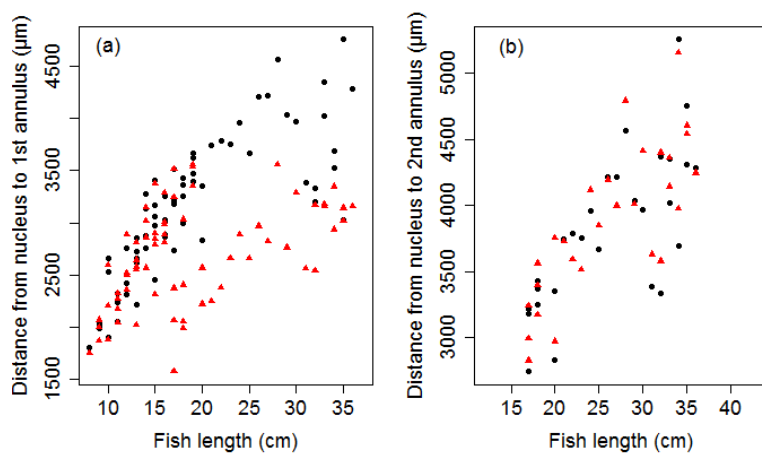
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505 Fig. 4 Ageing of whole and ground otoliths



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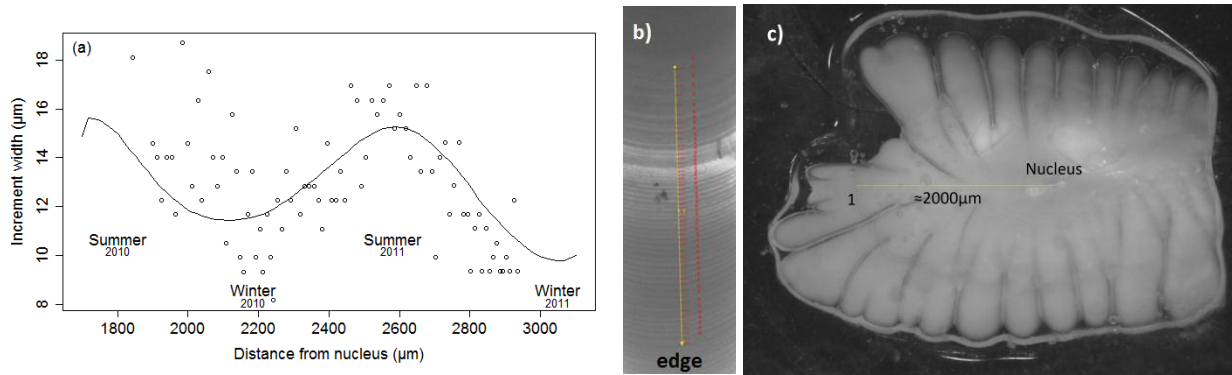
507 Fig. 5 Distance from nucleus to 1<sup>st</sup> and 2<sup>nd</sup> annulus as a function of fish length



508

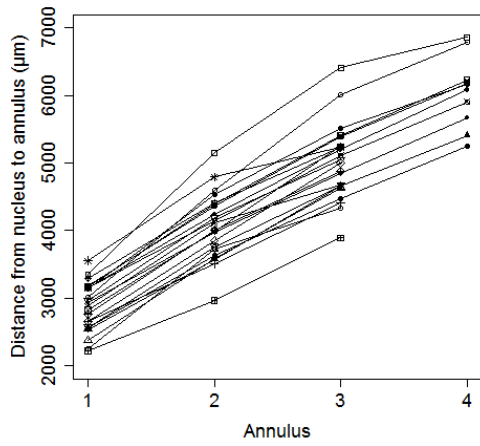
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510 Fig. 6 Increment width as a function of the distance from the nucleus



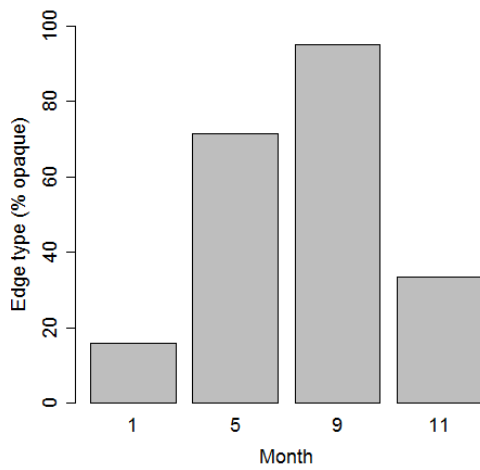
511

512 Fig. 7 Otolith growth trajectories



513

514 Fig. 8 Percentage of otoliths with an opaque edge zone



515