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Otolith shape in juvenile cod (*Gadus morhua*): Ontogenetic and environmental effects

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ABSTRACT

The objectives of this study were to describe the otolith shape development of cod from post-metamorphosis larvae to juveniles and to examine whether shape development is an ontogenetically determined process. Fish were reared under three different temperature regimes and two feeding levels. I examined shape by the length~width relationship of the otolith as well as the number and size of the lobes formed. Otolith size and crenulation is exclusively linked to fish size, indicating an ontogenetically determined development. Otolith shape is closely associated with the spatial development of the lobes. This process is linked with food consumption. Higher food consumption leads to a higher number of larger lobes, resulting in a more rectangular otolith. In conclusion: Otolith shape development consists of an ontogenetic component in the form of increasing size and crenulation and an environmental component impacting on the size and number of the lobes formed.

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1. Introduction

After Pannella's (1971) discovery of daily increment structures in fish otoliths, the interest of many fisheries scientists focused on internal structures of the otolith. In recent years, the shape of otoliths has become of increasing importance in relation to stock management. The major focus of these studies has been on spatial and temporal discrimination of stocks (Bolles and Begg, 2000; Campana and Casselman, 1993; Cardinale et al., 2004; Castonguay et al., 1991), populations (Mérigot et al., 2007; Pothin et al., 2006; Smith, 1992) and spawning aggregations (Jónsdóttir et al., 2006). These studies are based on measures of morphometrics (otolith length, width, weight, area and perimeter), shape indices (circularity and rectangularity) as well as shape in the form of Fourier descriptors. For These measures are coupled with discriminant function analysis, but also wavelet-transformation and curvature scale space analysis seem to be useful tools (Parisi-Baradad et al., 2005).

A shortcoming of these studies is that they only focus on the final goal – discrimination between groups - but, with a few exceptions (Bolles and Begg, 2000; Campana and Casselman, 1993; Cardinale et al., 2004), neglect to examine what factors cause the differences in shape. Knowledge on how genetic and environmental factors affect otolith shape would strengthen this shape based approach considerably. Common for many of these studies is that they do not describe shape other than in level of harmonics. Nevertheless, some interesting information on factors affecting shape development may be found. Significant effects of age (Bird et al., 1986), size (Campana and Casselman, 1993; Mérigot et al., 2007; Smith, 1992), year-class (Bolles and Begg, 2000; Campana and Casselman, 1993; Castonguay et al., 1991) and sex (Bolles and Begg, 2000; Campana and Casselman, 1993) are mentioned. Contrary to these, otolith shape of different fish species seems unaffected by one or several of these variables in other studies (Cardinale et al., 2004; Castonguay et al., 1991; Jónsdóttir et al., 2006; Tracey et al., 2006). To remove these size-related effects, the morphometric measures are standardised by subtracting the product of otolith length (or fish length) and the common within-group slope from the variable (Bolles and Begg, 2000; Cardinale et al., 2004; Jónsdóttir et al., 2006; Tracey et al., 2006) and the Fourier harmonics by division with the mean radius (Campana and Casselman, 1993; Jónsdóttir et al., 2006). But these standardization procedures are often not able to remove the size effect entirely (Campana and Casselman, 1993).

In the early life stages, an ontogenetic development from the circular, larval otolith to the oblongate otolith resembling the adults is known for a wide range of species (Brown et al., 2001; Jearld Jr. et al., 1993; Lagardère and Troadec, 1997; Modin et al., 1996). This change in shape is the result of the development of a progressing number of secondary growth centres, the origin of what will be called lobes in this study. The onset of lobe formation seems to be tightly coupled to processes of the ossification process during metamorphosis (Brown et al., 2001; Jearld Jr. et al., 1993; Lagardère and Troadec, 1997), where the number of lobes increases with fish age and size (Modin et al., 1996). The otoliths of larvae and juveniles are still small and the relative accretion rates large. Possible environmental and biotic effects are therefore easier and faster to detect than in adults. This makes these size classes an obvious model to examine whether it is genetic influence or conditions in the fish's environment that determines the shape of their otoliths.

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The objectives of this study are to examine whether otolith shape development in cod (Gadus morhua) is a genetically determined process and to what extent biotic and abiotic factors such as temperature and feeding level influence otolith shape. Under the assumption that shape development is a genetic process, the size of the otolith and the complexity of its shape are hypothesised to increase with fish size and age and modulated by biotic and abiotic factors.

2. Materials and methods

2.1. Sample preparation

500 juvenile cod of approximately 20 mm standard length (SL) and 60 days old were obtained from the Isle of Man Marine Laboratory, University of Liverpool and reared under different levels of controlled food and temperature conditions for 40 days. After acclimatisation to the facilities of the Danish Institute for Fisheries Research at a temperature of 12 °C for 7 days the fish were measured and weighed to the nearest 0.5 mm and 0.01 gram and distributed evenly over 12 experimental tanks (175 l). The fish were kept in 32 ppm seawater at full aeration and a light cycle of 16L:8D. The experimental setup consisted of three constant temperatures 5, 10 and 15 °C and two feeding levels, high and low with two replicate tanks for each temperature-food combination. The fish were fed pellets of dry food (Perla Marine, Skretting a/s Denmark, pellet size from 0.8 mm to 2 mm). High food corresponded to ad libitum feeding (approximately 4.0, 5.5 and 7.5% pellet dry weight/body dry weight per day for 5, 10 and 15 °C respectively) with food being provided continually by automatic feeders during daylight hours. Low food level corresponded to 65% of the high food level at each of the three temperatures. Every morning the amount of food provided was calculated, the leftovers from the previous day was collected and its dry weight estimated. No mortality occurred during the experimental period. The majority of fish were used in other experiments (Hüssy et al., 2004; Hüssy and Mosegaard, 2004). Every 10 days all fish were anaesthetised with MS 222, measured and weighed to the nearest 0.5 mm and 0.01 gram (fresh weight, FW) and 10 randomly selected fish from each temperature-food combination sacrificed. The present experiment was terminated after 80 days. Otoliths were extracted, rinsed in water and stored in labelled plastic bags. Fish were dried individually at 60 °C for 48 h (dry weight, DW). Sagittal otoliths are henceforward only called “otoliths”. Information on fish- and otolith sizes are summarised in Table 1.

2.2. Analysis of otolith shape

For the analysis of otolith data an image analysis system (IMAGE PRO, ver. 4) and frame grabber (Leica IM 50) coupled to a dissection microscope and digital camera (Leica DC300F) was used. Otoliths were oriented horizontally along the longest axis with the rostrum to the left and placed on a black background. Otolith area (OA) was measured using an edge detecting algorithm of IMAGE PRO from images of the whole otolith with the sulcus facing up at a magnification corresponding to 6.4 µm pixel^{-1}. With the same tool, the x and y coordinates of each pixel along the perimeter of the otolith was recorded. Otolith total length (OL) was measured as the longest distance between the rostrum and the post-rostrum (x_{max} - x_{min}) and otolith width (OW) as the longest distance between the dorsal and ventral edges (y_{max} - y_{min}).

This contour plot of x and y coordinates was subjected to the following routine in order to obtain an objective measure for the number and size of otolith lobes: First, a horizontal line, centred between the rostrum (x_{min}) and the post-rostrum (x_{max}) and the dorsal (y_{max}) and ventral (y_{min}) edge was drawn (Line length = 0.8 · (x_{max} - x_{min}), at y = (y_{max} - y_{min}) / 2 + y_{min}). Starting from the rostrum over the dorsal edge, the shortest distance (D_i) of each pixel i along the contour line to this line was calculated. Subsequently, a running average (R_i) spanning 15 pixel values was calculated from the distances. Using these two values, the base of a lobe was defined as the largest negative value of the difference between the “shortest distance” and the running average (D_i - R_i). Lobe bases (L_j) were numbered starting at the rostrum progressing over the dorsal edge, and the total number recorded called “number of lobes” (LN) in the following.

The area of the polygon enclosed by the lobe bases was calculated by first defining central x and y coordinates as $x_c = (x_{max} - x_{min}) / 2$ and $y_c = (y_{max} - y_{min}) / 2$. The areas of all triangles (A_n) defined by this central point and adjacent lobe bases was estimated using Heron's law:

$$A_n = \sqrt{\left[\left(s_n\left(s_n - a_n\right)\right)\left(s_n - b_n\right)\left(s_n - c_n\right)\right]},$$

where $s_n = 1/2\ (a_n + b_n + c_n)$ and the sides of the triangle $a_n = x_c y_c - L_j$, $b_n = L_j - L_{j+1}$ and $c_n = L_{j+1} - x_c y_c$. Polygon area (PA) was then calculated as

Table 1
Overview over fish and otolith size, lobe number and area in relation to treatment and sampling day (average ± standard deviation)

Variable	Day	High food			Low food		
		5 °C.	10 °C.	15 °C.	5 °C.	10 °C.	15 °C.
Fish size (mm)	10	35.4±2.9	38.0±6.3	35.8±6.3	35.9±2.2	36.8±4.5	37.0±4.0
	20	40.9±4.9	48.2±4.2	46.7±5.7	42.1±4.2	47.5±6.1	46.4±3.4
	30	45.3±6.1	55.8±5.0	59.0±2.8	43.7±6.1	52.7±3.8	53.9±3.8
	40	55.3±2.3	59.7±4.7	69.4±4.2	50.8±3.4	59.4±4.6	63.2±4.9
	50	54.1±2.7	70.9±4.4	69.6±7.3	48.9±6.0	61.9±4.2	72.2±5.5
Otolith size (mm^2)	10	0.771±0.112	0.847±0.188	0.847±0.208	0.740±0.090	0.833±0.143	0.887±0.139
	20	1.020±0.216	1.393±0.206	1.423±0.203	1.031±0.169	1.341±0.265	1.366±0.196
	30	1.114±0.197	1.802±0.289	2.139±0.311	1.132±0.227	1.641±0.215	1.897±0.161
	40	1.699±0.119	2.135±0.308	3.009±0.293	1.458±0.184	2.167±0.332	2.605±0.277
	50	1.635±0.127	2.943±0.336	3.178±0.525	1.476±0.310	2.371±0.230	3.158±0.357
Lobe number	10	8.2±1.7	9.2±1.5	10.7±2.2	8.0±0.9	9.0±2.4	8.7±1.5
	20	9.4±2.3	12.2±1.5	11.5±1.6	9.1±1.6	11.2±2.2	11.8±1.9
	30	9.4±2.5	14.0±1.4	15.6±1.8	9.5±1.5	12.5±1.4	13.9±1.7
	40	12.5±1.4	14.3±2.6	20.4±2.7	10.8±1.5	14.5±2.2	17.2±3.0
	50	10.0±1.8	19.8±2.2	20.3±3.0	9.8±3.8	15.2±3.8	17.0±3.0
Lobe area (mm^2)	10	0.186±0.038	0.214±0.076	0.169±0.039	0.185±0.029	0.210±0.056	0.207±0.037
	20	0.226±0.045	0.237±0.049	0.254±0.044	0.246±0.059	0.257±0.065	0.248±0.042
	30	0.237±0.061	0.292±0.077	0.301±0.062	0.231±0.054	0.267±0.047	0.314±0.042
	40	0.299±0.082	0.318±0.044	0.308±0.057	0.256±0.070	0.287±0.060	0.307±0.041
	50	0.292±0.070	0.296±0.046	0.321±0.039	0.295±0.087	0.339±0.093	0.399±0.045

the sum of all triangle areas (ΣA_j). Total lobe area was calculated as the difference between otolith and polygon area (OA – PA). Information on otolith biometrics are summarised in Table 1.

2.3. Etching of otolith surface

One otolith from each tank was chosen randomly to assess the effect of etching with hydrochloric acid (HCl). In particular, this procedure removes the protein matrix (IBACS, 2006). Otoliths were suspended in a net cage (mesh size 300 µm) within a chamber containing 0.3 l of 0.1 M HCl and a magnetic stirrer. After etching for 3 min. otoliths were rinsed in high alkalinity tap water and air dried. After 24 h, etched otolith area (OA_{etch}) was measured. Since lobe formation partly depends on otolith size (see results), this effect was removed ($LA_{rel} = LA / OA$). Accordingly, otolith area loss were standardised: $\Delta OA_{rel} = (OA - OA_{etch}) / OA$. Two otoliths were damaged during the procedure, leaving a total of n = 10. Due to etching the identification and comparison of lobe bases is not applicable. Therefore, the effect of etching was analysed only as total area loss, together with visual inspection.

Table 2
Variable used in the GLM analyses

Dependent	Variables	df	p	r^2
OA	*age+DW+age:DW*	266	***	0.97
	age	268	***	0.50
	DW	268	***	0.95
LN	*age+OA*	267	***	0.83
	age	268	***	0.27
	OA	268	***	0.80
LA	*OL+OW+LN*	266	***	0.64
	OL+OW	267	***	0.40
	OL	268	***	0.39
	OW	268	***	0.39
	LN	268	***	0.10

df = degrees of freedom: Significance level *** $p < 0.001$.

2.4. Statistical analyses

To establish whether otolith area and the formation of number of lobes are linked to the size or age of the fish, and to what degree

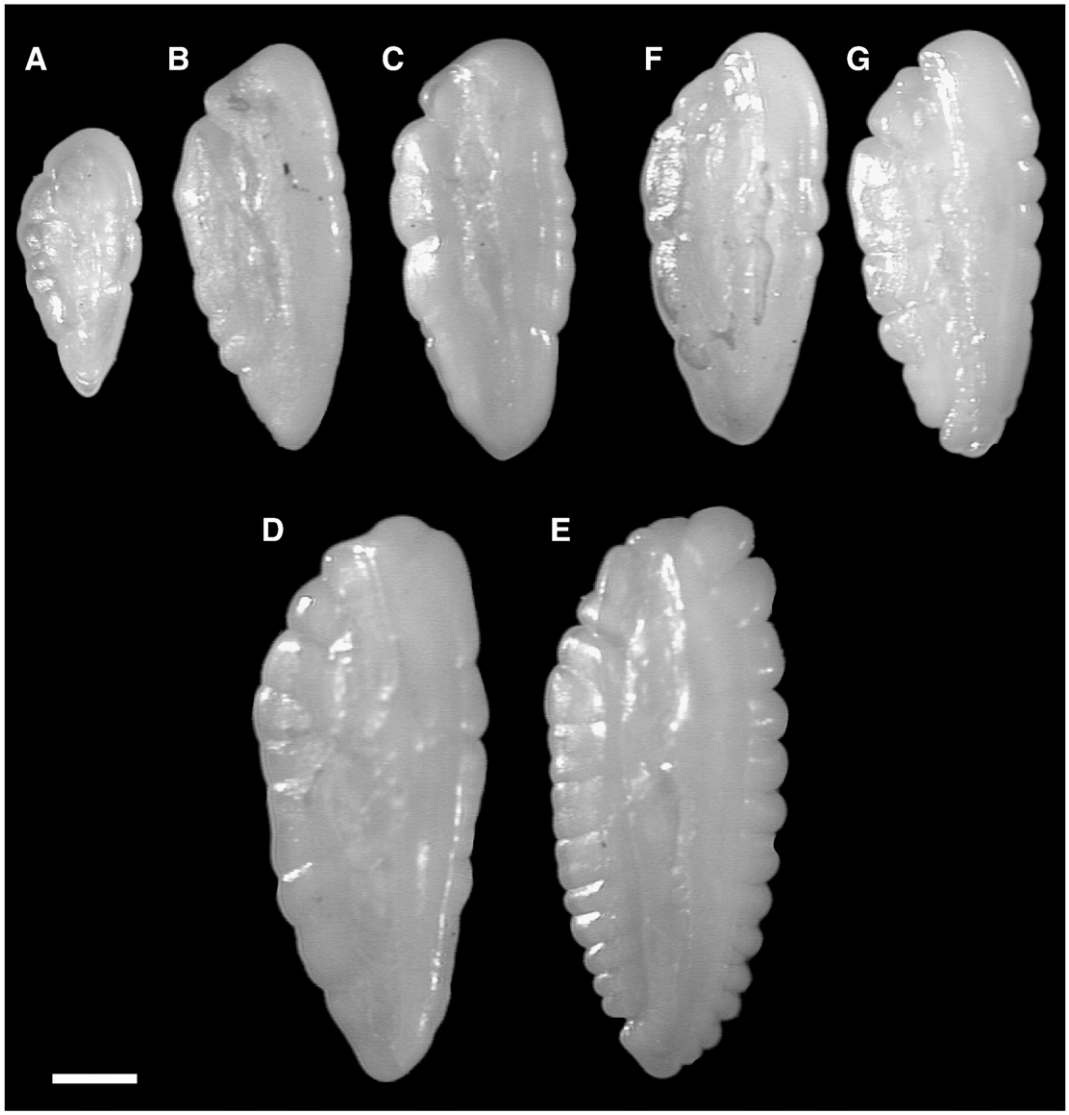

Fig. 1. Otoliths of juvenile cod, sulcus facing up, from A) the beginning of the experiment, B) the 5 °C. low food treatment, C) the 5 °C. high food treatment, D) the 15 °C. low food treatment, E) the 15 °C. high food treatment. Since size differences were considerable between temperature treatments, an example of otoliths from the 15 °C. at a fish size comparable to the 5 °C. treatment final sampling (10 days after experiment start) is shown for F) the 15 °C. low food treatment and G) the 15 °C. high food treatment Scale bar = 500 µm.

experimental temperature has an effect, the influence of these variables on otolith area (OA) and the number of lobes (LN) were examined with a generalized linear model (GLM) approach:

$$OA = age + DW + temp + \text{interaction terms} + \varepsilon$$

$$LN = age + DW + temp + OA + OL + OW + \text{interaction terms} + \varepsilon$$

These GLM models were reduced by stepwise backward elimination to include only significant terms. The effect of feeding was examined using covariance analysis (ANCOVA) with feeding level as covariate in the reduced GLM model.

A similar GLM-approach was used to examine whether the size of the lobes depends on total otolith area alone or/and on otolith shape in the form of number of lobes, otolith length and width:

$$LA = OA + OL + OW + LN + \text{interaction terms} + \varepsilon$$

Model reduction and test of feeding effect followed the procedure described above. All statistical analyses were carried out using "R" ver. 2.3.1. (http://wiki.r-project.org/rwiki/doku.php, last accessed 04/03/2008).

3. Results

3.1. Otolith area

The model that best fit the data was

$$OA = a + b \cdot age + c \cdot DW + d \cdot age \cdot DW + \varepsilon,$$

where $a = 4.04 \cdot 10^5$, $b = 1.44 \cdot 10^4$, $c = 3.36 \cdot 10^6$ and $d = -3.09 \cdot 10^4$. This model explained 96.9% of the variability in otolith area. Age, dry weight and their interaction terms were significant, while the effect of temperature was not significant. However, dry weight alone explained 94.5% of the variability (Table 2). The effect of food level on the otolith area – fish dry weight relationship was not significant (ANCOVA, df=268, p=0.79). This analysis showed that otolith total area depends exclusively on fish size in the form of dry weight.

3.2. Otolith length/width relationship

The otolith total length - width relationship differed significantly between treatments. Otoliths from the high food treatment were significantly wider than those from the low food treatment with respect to both intercept and slope of the relationship (ANCOVA, df=268, p=0.004; low food treatment: OW=216.7+0.35 OL, df=134, r^2=0.97, p<0.001; high food treatment: OW=188.9+0.39 OL, df=134, r^2=0.97, p<0.001), while temperature had no effect (ANCOVA, df=268, p=0.49). This result indicates that the proportions of the otolith were affected by food intake, even though otolith total area only depended on fish size.

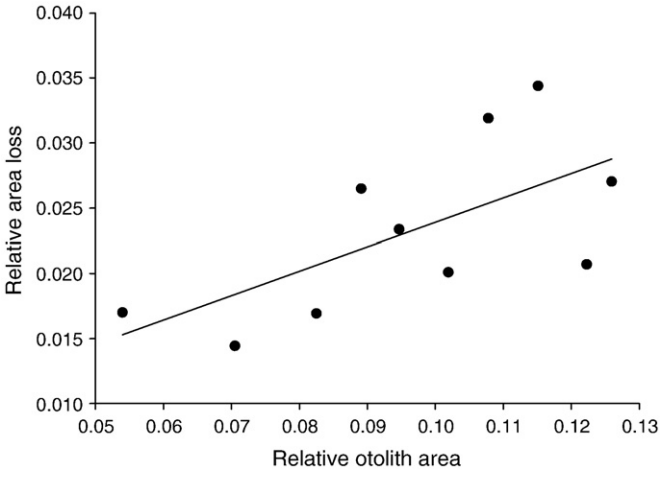

Fig. 2. Relative otolith area loss (ΔOA_{rel} = otolith area lost expressed as % of total area) in relation to relative lobe size (LA_{rel} = lobe area expressed as % of total area) of juvenile cod otoliths. ($\Delta OA_{rel} = 0.005 + 0.187\ LA_{rel}$; n=10, r^2=0.42, p<0.05).

Fig. 3. Effect of etching with HCl on the otolith shape of juvenile cod otoliths with crenulated (A) and smooth (B) otolith edges. The white lines are the outline of the otoliths prior to etching. Scale bar = 500 µm.

3.3. Number of otolith lobes

The model that best explained the number of lobes was

$$LN = a + b \cdot age + c \cdot OA + \varepsilon,$$

where a=4.62, $b = -7.10 \cdot 10^{-2}$ and $c = 5.87 \cdot 10^{-6}$. This model explained 82.5% of variability in number of lobes, where otolith area alone explained 80% (Table 2). Food level had a significant effect on the intercept of the lobenr~otolith area relationship (ANCOVA, df=268, p=0.015), where otoliths from the high food treatment had a higher number of lobes (Fig. 1).

These results show that the development of otolith lobes is a process linked to the size of the otolith and therefore also to the size of the fish, but that ration level may affect the shape of the otolith with respect to the length~width dimensions as well as the number of lobes formed.

3.4. Otolith lobe area

Otolith lobe area was best described by the model

$$LA = a + b \cdot OL + c \cdot OW + d \cdot LN + \varepsilon,$$

where $a = -3.43 \cdot 10^4$, b=84.04, c=342.83 and $d = -1.82 \cdot 10^4$. This model explained 64.1% of the variability in lobe area, whereof otolith length and width alone only explained 39.5% (Table 2). The effect of food on this relationship was just outside the range of significance (ANCOVA, df=262, p=0.05). These results show that otolith shape is closely associated with the process of lobe formation, both with respect to lobe number and size (Fig. 1).

3.5. Etching of otolith surface

There was a significant positive correlation between relative otolith loss due to etching (ΔOA_{etch}) with relative lobe area (LA_{rel})

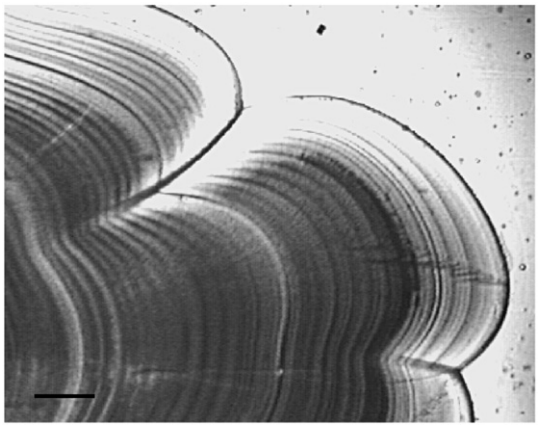

Fig. 4. Section of juvenile cod otolith in the dorso-ventral direction, ground to a thickness of ca. 50 µm. Scale bar = 30 µm.

(see Fig. 2). The effect of etching on otoliths with different crenulation of the edge is shown in (Fig. 3). Etching with HCl results in larger lobes being eroded faster than areas with a smoother edge and less pronounced lobes.

As a qualitative test, sections of the frontal lobe were ground from both the lateral and distal sides of the otolith to a thickness of approximately 50 µm (Fig. 4). These sections show that the area of the lobes is much more opaque than the between-lobe area.

4. Discussion

This study of lobe development in relation to fish size, age, temperature and feeding history provides new insight into otolith formation mechanisms. The objective was to examine whether shape development is an ontogenetically determined process and to determine the effect of environmental factors in cod from post-metamorphosis larvae to juveniles.

Cod otoliths are characterised by a blunt shape with crenulated edges along the dorsal and ventral edges. The onset of lobe formation starts at transition from larval to juvenile stage, where formation of secondary growth centres starts at the anterior end (Toole et al., 1993). The change in shape of the growing otolith is caused by the development of a progressing number of secondary growth centres (Brown et al., 2001; Hüssy et al., 2003; Jearld Jr. et al., 1993). This progression in formation of lobes continues well into the late juvenile stage, where average number of lobes have been observed to increase from 26 to 27 and 31 in < 15 cm, 15-20 cm and 20-30 cm long Baltic cod (own observation from samples of the spring and fall Baltic International Trawl Survey 1995/1996). This suggests that the development of number of otolith lobes found in this study is indeed an ontogenetic process reaching beyond the earliest life stages.

This study has shown that not only genetic effects influence otolith shape development. Feeding level is the major environmental variable that has a direct effect by affecting the number of lobes formed and probably also their area and thereby the otolith length~width relationship. But feeding level also acts indirectly on otolith size through somatic growth. Interestingly, temperature does not seem to have an effect on otolith shape. To understand this relationship between otolith shape and feeding level it is necessary to look at the basic mechanisms of otolith formation:

Otolith growth is an acellular process, where the bio-mineralisation depends on the endolymph chemistry (Borelli et al., 2003a,b) and the transport mechanisms of calcium carbonate and protein within the endolymphatic epithelium (Mayer-Gostan et al., 1997; Mugiya and Yoshida, 1995; Takagi and Takahashi, 1999; Tohse and Mugiya, 2004). It seems therefore likely that the spatial growth of otolith lobes is linked to the accretion of either calcium carbonate or protein. Thinly ground otolith sections show that the lobes are more opaque than the between-lobe area, indicating that lobes contain relatively more protein than the area between them (Hüssy and Mosegaard, 2004; Hüssy et al., 2004; Mosegaard and Titus, 1987; Mugiya and Muramatsu, 1982; Rice et al., 1985; Titus and Mosegaard, 1991; Volk et al., 1990; Watabe et al., 1982). The etching procedure used in the present experiment dissolves protein, leaving only the $CaCO_3$ rich parts of the otolith. During this treatment, the lobes of the otoliths were the first to disappear, while the interlobe areas remained virtually untouched. The larger the lobes, the faster was the loss of otolith material. The only study that previously examined protein accretion in relation to otolith biometrics found a correlation between optical density, etching effects and otolith shape (IBACS, 2006), where opaque areas of cod otolith lobes were also eroded faster than the area between them. However, the present results are only indicative. To assess the degree of protein-related lobe growth quantitatively, otoliths of similar size would be necessary to avoid any size-related effects. For this study, it was unfortunately not possible to rear fish for a longer period. Nevertheless, together with the qualitatively observed higher opacity within the lobes, this strongly supports the hypothesis that spatial growth of otolith lobes depends on the protein accretion process.

Protein synthesis is directly correlated with protein consumption (Houlihan et al., 1988), and the fractional rates of protein synthesis in a wide range of tissues are known to be proportional to whole body protein synthesis rates (Foster et al., 1992; Houlihan et al., 1988). The chain of processes seems therefore to range from protein consumption over synthesis and secretion of otolith-specific proteins into the endolymph to accretion thereof in the lobe area. The degree of crenulation eventually affects the length~width relationship of the otolith, in that high consumption leads to faster lobe growth in the dorsal and ventral axes. The processes involved in accretion of protein in the lobes rather than between them remain unresolved. But immunoreactive staining of otolith matrix producing cells has shown higher concentrations of these cells in the areas along the edge of the otolith, where lobe development is most prominent (Murayama et al., 2004, 2005; Takagi and Takahashi, 1999). This supports the hypothesis that lobe growth depends on synthesis and accretion processes of the organic matrix – and thus ultimately consumption. In addition to the genetically determined otolith shape development there seems to be a phenotypic response to feeding level. Indices from previous studies may easily be explained by this response:

Several authors point out possible reasons for differences in otolith shape such as temperature in silver hake (Merluccius bilinearis) inhabiting different geographical latitudes on Georges Bank (Bolles and Begg, 2000). Contrarily, Tracey et al. (Tracey et al., 2006) proposed genetic influence as key mechanism since temperature regimes in their area of study were very similar. Growth rate is also suggested, based on differences in the otolith size-fish size relationship (Bolles and Begg, 2000; Smith, 1992). But most of these suggestions are speculations and not based on statistical analyses.

Only a few studies have examined links between otolith shape and environmental/biotic variables. The most significant of these are feeding level and growth rate. Growth rates are highly correlated with the values of the first discriminant function analysis of otolith shape (Campana and Casselman, 1993). Growth rate seems to be a significant covariate of most shape variables and the effect persists even when individual otolith types are examined separately. Thus, growth rate contributes more variation to regional differences than stock origin (Campana and Casselman, 1993). Feeding levels, ranging from starvation to ad libitum, cause differences in both the general characteristics of otolith shape and finer details of outline crenulations in two species of tropical fish (Gagliano and McCormick, 2004). In Faroese known-age cod from two different populations both genetic but also environmental conditions in the form of temperature and food availability have significant effects on otolith shape (Cardinale et al., 2004). Environmental effects seem to be

acting through the effect of otolith growth rates and thus also feeding level (Cardinale et al., 2004). Together with the present results, these studies suggests that the finer details of otolith shape development are in fact a phenotypic response to feeding level and occur in a range of fish species.

The proposed phenotypic response of otolith shape to feeding level can also explain the patterns in shape observed in other studies (Bolles and Begg, 2000; Castonguay et al., 1991; Jónsdóttir et al., 2006; Mérigot et al., 2007; Pothin et al., 2006; Smith, 1992). Since fish somatic growth is also consumption-dependent, the proposed mechanism may also explain the effects of growth rate (Bolles and Begg, 2000; Campana and Casselman, 1993; Smith, 1992) and temperature (Bolles and Begg, 2000) referred to previously. Assuming differences in consumption and growth rates between groups, this response may to some degree also be the reason for differences between geographical areas (Castonguay et al., 1991; Pothin et al., 2006; Smith, 1992; Tracey et al., 2006) and year-classes (Bolles and Begg, 2000; Campana and Casselman, 1993; Castonguay et al., 1991).

Differences between age groups, particularly early in life (Bird et al., 1986), seem to be governed by the genetically determined nature of otolith shape development, while differences between size-classes (Campana and Casselman, 1993; Mérigot et al., 2007; Smith, 1992) and possibly sex (Bolles and Begg, 2000; Campana and Casselman, 1993) may be the result of a combination of genetic and environmental effects.

Otolith shape apparently responds to altered food availability within a few days (Gagliano and McCormick, 2004), which suggests this variable to be a useful tool for the evaluation of body condition and recent growth, at least in juvenile fish. In order to evaluate the use of otolith shape, future studies should address the subjects of the histological relationship between saccular epithelium and the topography of the otolith as well as the physiological link between protein consumption and matrix synthesis. These studies should not just be limited to juveniles, but rather include all life stages.

5. Conclusions

We examined shape by the length~width relationship of the otolith as well as the number and size of the lobes formed. This study showed that otolith size and the overall number of lobes formed is exclusively linked to fish size, whereas otolith shape, in the form of length~width relationship as well as the number and size of lobes is also influenced by fish growth, mediated by feeding level.

With increasing fish size, otoliths become more crenulated due to the number of lobes formed. Higher food consumption causes more lobes to be formed, which become larger resulting in a relatively wider otolith. This shows that the development of otolith size and the general shape of the otolith is an ontogenetic process, but that the finer details of the otolith shape may be modulated by environmental factors, particularly food availability.

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References

Bird, J.L., Eppler, D.T., Checkley Jr., D.M., 1986. Comparison of herring otoliths using Fourier series shape analysis. Can. J. Fish. Aquat. Sci. 43, 1228–1234.

Bolles, K.L., Begg, G.A., 2000. Distinctions between silver hake (Merluccius bilinearis) stocks in U.S. waters of the northwest Atlantic based on whole otolith morphometric. Fish. Bull. 98, 451–462.

Borelli, G., Guibbolini, M.E., Mayer-Gostan, N., Priouzeau, F., de Pontual, H., Allemand, D., Puverel, S., Tambutte, E., Payan, P., 2003a. Daily variations of endolymph composition: relationship with the otolith calcification process in trout. J. Exp. Biol. 206 (15), 2685–2692.

Borelli, G., Mayer-Gostan, N., Merle, P.L., Pontual, H., Boeuf, G., Allemand, D., Payan, P., 2003b. Composition of Biomineral Organic Matrices with Special Emphasis on Turbot (Psetta maxima) Otolith and Endolymph. Calcif. Tissue Int. 72 (6), 717–725.

Brown, A.L., Busby, M.S., Mier, K.L., 2001. Walleye pollock Theragra chalcogramma during transformation from the larval to juvenile stage: otolith and osteological development. Mar. Biol. 139, 845–851.

Campana, S.E., Casselman, J.M., 1993. Stock discrimination using otolith shape analysis. Can. J. Fish. Aquat. Sci. 50, 1062–1083.

Cardinale, M., Doering-Arjes, P., Kastowsky, M., Mosegaard, H., 2004. Effects of sex, stock, and environment on the shape of known-age Atlantic cod (Gadus morhua) otoliths. Can. J. Fish. Aquat. Sci. 61, 158–167.

Castonguay, M., Simard, P., Gagnon, P., 1991. Usefulness of Fourier Analysis of otolith shape for Atlantic mackerel (Scomber scobrus) stock discrimination. Can. J. Fish. Aquat. Sci. 48, 296–302.

Foster, A.R., Houlihan, D.F., Hall, S.J., Burren, L.J., 1992. The effects of temperature acclimation on protein synthesis rates and nucleic acid content of juvenile cod (Gadus morhua). Can. J. Zool. 70, 2095–2102.

Gagliano, M., McCormick, M.I., 2004. Feeding history influences otolith shape in tropical fish. Mar. Ecol. Prog. Ser. 278, 291–296.

Houlihan, D.F., Hall, S.J., Gray, C., Noble, B.S., 1988. Growth rates and protein turnover in Atlantic cod, Gadus morhua. Can. J. Fish. Aquat. Sci. 45, 951–964.

Hüssy, K., Mosegaard, H., 2004. Atlantic cod (Gadus morhua) growth and otolith accretion characteristics modelled in a bioenergetics context. Can. J. Fish. Aquat. Sci. 61 (6), 1021–1031.

Hüssy, K., Mosegaard, H., Hinrichsen, H.-H., Böttcher, U., 2003. Using otolith microstructure to analyse growth of juvenile Baltic cod (Gadus morhua L.). Mar. Ecol. Prog. Ser. 258, 233–241.

Hüssy, K., Mosegaard, H., Jessen, F., 2004. Effect of age and temperature on amino acid composition and the content of different protein types of juvenile Atlantic cod (Gadus morhua) otoliths. Can. J. Fish. Aquat. Sci. 61 (6), 1012–1020.

IBACS, 2006. Integrated Approach to the Biological Basis of Age Estimation in Commercially Important Fish Species. Rep Final report (QQLRT-2001-01610).

Jearld Jr., A., Sass, S.L., Davis, M.F., 1993. Early growth, behaviour, and otolith development of the winter flounder Pleuronectes americanus. Fish. Bull. 91, 65–75.

Jónsdóttir, I.G., Campana, S.E., Marteinsdottir, G., 2006. Otolith shape and temporal stability of spawning groups of Icelandic cod (Gadus morhua L.). ICES J. Mar. Sci. 63 (8), 1501–1512.

Lagardère, F., Troadec, H., 1997. Age estimation in common sole Solea solea larvae: validation of daily increments and evaluation of a pattern recognition technique. Mar. Ecol. Prog. Ser. 155, 223–237.

Mayer-Gostan, N., Kossmann, H., Watrin, A., Payan, P., Boeuf, G., 1997. Distribution of ionocytes in the saccular epithelium of the inner ear of two teleosts (Oncorhynchus mykiss and Scophthalmus maximus). Cell Tissue Res. 289, 53–61.

Mérigot, B., Letourneur, Y., Lecomte-Finiger, R., 2007. Characterization of local populations of the common sole Solea solea (Pisces, Soleidae) in the NW Mediterranean through otolith morphometrics and shape analysis. Mar. Biol. 151 (3), 997–1008.

Modin, J., Fagerholm, B., Gunnarsson, B., Pihl, L., 1996. Changes in otlith microstructure at metamorphosis of plaice, Pleuronectes platessa L. ICES J. Mar. Sci. 53, 745–748.

Mosegaard, H., Titus, R., 1987. Daily growth rates of otoliths in yolk sac fry of two salmonid species at five different temperatures. In: Kallander, S.O., Farnholm, B. (Eds.), Proc. V Congr. europ. Ichthyol. Stockholm. Swedish Museum of Natural History, Stockholm, Sweden, pp. 221–227.

Mugiya, Y., Muramatsu, J., 1982. Time-marking methods for scanning electron microscopy in goldfish otoliths. Bull. Jpn. Soc. Sci. Fish. 48 (9), 1225–1232.

Mugiya, Y., Yoshida, M., 1995. Effects of calcium antagonists and other metabolic modulators on in vitro calcium deposition on otoliths in the rainbow trout Oncorhynchus mykiss. Fish. Sci. 61 (6), 1026–1030.

Murayama, E., Takagi, Y., Nagasawa, H., 2004. Immunohistochemical localization of two otolith matrix proteins in the otolith and inner ear of the rainbow trout, Oncorhynchus mykiss: comparative aspects between the adult inner ear and embryonic otocysts. Histochem. Cell Biol. 121 (2), 155–166.

Murayama, E., Herbomel, P., Kawakami, A., Takeda, H., Nagasawa, H., 2005. Otolith matrix proteins OMP-1 and Otolin-1 are necessary for normal otolith growth and their correct anchoring onto the sensory maculae. Mech. Dev. 122, 791–803.

Pannella, G., 1971. Fish otoliths: Daily growth layers and periodical patterns. Science 173, 1124–1127.

Parisi-Baradad, V., Lombarte, A., Garcia-Ladona, E., Cabestany, J., Piera, J., Chic, O., 2005. Otolith shape contour analysis using affine transformation invariant wavelet transforms and curvature scale space representation. Mar. Freshw. Res. 56 (5), 795–804.

Pothin, K., Gonzalez-Salas, C., Chabanet, P., Lecomte-Finiger, R., 2006. Distinction between Mulloidichthys flavolineatus juveniles from Reunion Island and Mauritius Island (south-west Indian Ocean) based on otolith morphometrics. J. Fish Biol. 69 (1), 38–53.

Rice, J.A., Crowder, L.B., Binkowski, F.P., 1985. Evaluating otolith analysis for bloater Coregonus hoyi: Do otoliths ring true? Trans. Am. Fish. Soc. 114, 532–539.

Smith, M.K., 1992. Regional differences in otolith morphology of the deep slope red snapper Etelis carbunculus. Can. J. Fish. Aquat. Sci. 49, 795–804.

Takagi, Y., Takahashi, A., 1999. Characterisation of ootolith soluble-matrix producing cells in the saccular epithelium of Rainbow trout (Oncorhynchus mykiss) inner ear. Anat. Rec. 254, 322–329.

Titus, R., Mosegaard, H., 1991. Selection for growth potential among migratory brown trout (Salmo trutta) fry competing for territories: Evidence from otoliths. Can. J. Fish. Aquat. Sci. 48 (1), 19–27.

Tohse, H., Mugiya, Y., 2004. Effects of acidity and a metabolic inhibitor on incorporation of calcium and inorganic carbon into endolymph and otoliths in salmon Oncorhynchus masou. Fish. Sci. 70 (4), 595–600.

Toole, C.L., Markle, D.F., Harris, P.M., 1993. Relationships between otolith microstructure, microchemistry, and early life history events in Dover sole, Microstomus pacificus. Fish. Bull. 91, 732–753.

Tracey, S.R., Lyle, J.M., Duhamel, G., 2006. Application of elliptical Fourier analysis of otolith form as a tool for stock identification. Fish. Res. 77 (2), 138–147.

Volk, E.C., Schroder, S.L., Fresh, K.L., 1990. Inducement of unique otolith banding patterns as a practical means to mass-mark juvenile Pacific salmon. Am. Fish. Soc. Symp. 7, 203–215.

Watabe, N., Tanaka, K., Yamada, J., Dean, J.M., 1982. Scanning electron microscope observations of the organtic matrix in the otolith of the teleost fish Fundulus heteroclitus (Linnaeus) and Tilapia nilotica (Linnaeus). J. Exp. Mar. Biol. Ecol. 58, 127–134.