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Improving the precision of ageing assessments for long rough dab by using digitised pictures and otolith measurements

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Abstract

Otoliths of long rough dab (*Hippoglossoides platessoides*) were analysed using both stereomicroscope and digitised pictures. Approximately 400 fish were aged twice by each technique. The use of digitised pictures gave consistently higher precision of age-determinations. This was shown both graphically and by use of the coefficient of variation. Age determined from digitised pictures was generally higher than that determined by using a stereomicroscope. This was particularly true for older specimens.

For some individuals a change in the zoning pattern in the otoliths was observed. The occurrence of these changes was not closely correlated with the visually determined maturation index.

For fish of similar size otolith radius was correlated with age. The growth rate also influenced otolith morphology. This additional information is incorporated in the outline of an objective age-determination suggested by this study. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Age-determination; Otolith morphology; Zoning pattern; Precision; Methodology; Hippoglossoides platessoides

1. Introduction

Age-determination of fish is of crucial importance in the assessment of fish stocks. Today a number of different techniques are used for age-determination (Boehlert, 1985; Lai et al., 1987; Kalish et al., 1996; Fletcher and Blight, 1996). The most frequently used method is still simple counting of annuli in the otoliths, as described by Jensen (1965) and Powles (1966).

Ring formation in otoliths is thought to be a result of the combined influence of biotic and abiotic factors, i.e. somatic growth, access to food, tempera-

ture, maturation, and light (Pannella, 1980; Campana and Neilson, 1985; Hopkins et al., 1986; Rijnsdorp, 1993). The resulting rings may therefore be difficult to interpret. For each species and area it is important to verify that the annuli reflect years (Beamish and McFarlane, 1983; Anon., 1994). Age validation using whole otoliths has been done for long rough dab in the Barents Sea (Isaksen, 1977), in seas around the British Isles (Bagenal, 1955) and in the NW Atlantic (Powles, 1965, 1966; Pitt, 1967). These validations were based on analysing frequencies of opaque and translucent marginal zones throughout the year.

A thorough validation of age-determination from wild fishes requires mark-recapture experiments or other situations where the age of the specimens is known (Beamish and McFarlane, 1983, 1987; Casselman, 1987). A comparison of several age

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readings from the same material will, however, give an indication of the consistency of the determinations, and an impression of how the variation in the determination may influence the age (Campana et al., 1995).

Several studies have shown a close relationship between individual age and the size of the otoliths (Templeman and Squires, 1956; Boehlert, 1985; Pawson, 1990; Fletcher and Blight, 1996; Cardinale et al., 2000). Compared to conventional age-determination through a stereomicroscope, methods that use otolith size are objective and have the potential to produce a high number of observations in a short time (Boehlert, 1985; Pawson, 1990; Cardinale et al., 2000).

This study describes how different techniques influence precision, as defined by Beamish and McFarlane (1983), in age-determination of long rough dab in the Barents Sea. Furthermore, it investigates the possibility of information about age being stored in otolith measurements and zoning patterns.

2. Materials and methods

Long rough dab were sampled with a modified shrimp trawl, Campelen 1800 with "rockhopper" gear (Engås and Godø, 1989), during a cruise with R/V "Jan Mayen" in May 1994. Aschan and Sunnanå (1997) gave details of trawl equipment and procedures. Trawl hauls were made between 200–487 m depth within the area 72°39′N and 74°42′N and 17°20′E and 24°35′E.

After the fish were caught, total length, sex and stage of maturity were recorded immediately for all long rough dabs in the trawl or a representative sample of 30 specimens thereof (Albert et al., 1994). The only maturation information required for this paper was whether or not the fish would spawn within the year of capture. Maturity stage was, therefore, visually recorded as, either "immature" (very small gonads relative to fish size), or "mature" (i.e. ripening, running or spent). In some cases it was difficult to distinguish between immature and early ripening or spent, and the maturity stage was recorded as "uncertain".

The *sagittal* otoliths were then removed and stored dry in labelled envelopes. Both otoliths were cleaned and dried before they were weighed separately to

Table 1 Index of readability^a

- Believed to be reliable, good definition between translucent and opaque zones
- 2 Relatively clear zonation but not well defined, the error margin is expected to be ± 1 year
- 3 The zones are vaguely marked, and the error margin maybe ±2 years or more

 10^{-2} mg. Weights of otoliths that were broken or naturally deformed were excluded.

2.1. Age-determination

Age was determined as age-group based on the principle that a year-class starts as 0 group in the calendar year it has hatched and changes to 1, 2 groups, etc. every 1 January. For each age-determination an index of readability was also recorded (Table 1).

Age was determined from whole otoliths twice using stereomicroscope and twice from digitised pictures. The four age readings were labelled S_1 and S_2 (for the first and second stereomicroscope reading), and P_1 and P_2 (for the first and second picture reading). All readings were made by the same person and without any knowledge about the specimens the otoliths came from.

During the stereomicroscope readings reflected light, with otoliths immersed in glycerol on a black background were used. Fish younger than 8 years could usually be adequately determined using only the acentric otolith. For older fish the centric otolith was often used as confirmation and for determining the first or last annulus. Both the centric and acentric otoliths were honed on the outside using abrasive paper (granulation 500) in water. This resulted in better light permeability and solved the problem of an obscured inner annulus in older otoliths.

For age-determination using digitised pictures the medial face of the acentric otoliths was digitised and analysed using the computer software NIH Image 1.55. Only the acentric otoliths were used because

^a Rewritten after Jensen (1965).

NIH Image 1.55 is written by Wayne Rasband at US National Institutes of Health and is accessible through internet from anonymous ftp://zippy.nimh.nih.govor from floppies at NTIS, part number PB93-504868, 5258 Port Royal Road, Springfield, VA 22161, USA.

the zonation pattern seemed to appear clearer in the acentric than in the centric otoliths. The flat inside of the otoliths resulted in a level focal plane (Härkönen, 1986). Picture resolution was such that position of measuring points could be determined to 10^{-2} mm. The same procedure as during stereomicroscope reading was followed, i.e. reflected light, glycerol and black background.

Age and readability were determined from greyscale digitised pictures. During P_1 , both a picture of the otolith and the otolith itself under the stereomicroscope, were available. In this reading sequence, age was determined as part of a thorough analysis, including placements of different measuring points (Fig. 1). In the P_2 reading only digitised pictures were used.

The age-determination was carried out in the same manner during the first and second reading for both techniques. Due to the setup of the study the first reading for both techniques was, however, part of an extended analysis of the otoliths morphology and zonation pattern. Differences in the number of age-determinations between different readings are mainly due to the loss of some acentric otoliths during sampling.

Attempts to use sections of otoliths did not result in increased readability. Ages determined from sectioned otoliths (Bedford, 1975) were similar to the assessment made on whole otoliths. Based on the observed zonation pattern from the digitised images and the results of back-calculation of individual growth (Fossen et al., 1999), the authors do not believe that the use of whole otoliths may have resulted in a general underestimation of age among older specimens.

Generally, the width of yearly growth zones in otoliths decreases with increasing age, either gradually or more marked (Irie, 1960; Pannella, 1980; Gauldie and Nelson, 1990; Rijnsdorp et al., 1990). A sudden change from wide opaque and narrow translucent zones to narrow opaque and more distinct translucent zones, has been assumed to be a result of maturation (Rollefsen, 1933; Nordeng, 1961; Rijnsdorp and Storbeck, 1991; Godø and Haug, 1999). Since such patterns are often referred to in the literature, an investigation into the existence of spawning zones was included. This could give additional information about age. Two different zonation patterns were expected: (1) no change or a gradual decrease in the width of yearly zone formations; (2) a sudden change from wide opaque and narrow translucent

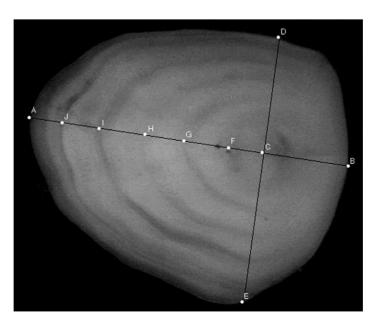


Fig. 1. Medial face of the acentric otolith from long rough dab determined to be 6 years old. Placement of measuring points (A–E) and zones expected to represent winter growth (F–J) are shown.

zones to narrow opaque and more distinct translucent zones. When, by visual inspection, the second pattern seemed appropriate, the age at which this change first occurred was recorded.

2.2. Morphometric measurements

Several morphometric variables were recorded during P_1 . The length of the otolith was measured as the continuation of the longest radius, whereas the width was measured as a straight line through the nucleus perpendicular to the length (Fig. 1). In addition, the software calculated several parameters from the picture: the circumference, the area, the minor and the major ellipse. The two latter measurements represent the biggest and smallest diameter in an ellipse fitted to the contour of the otolith. The shape of the acentric otolith was also estimated using the analogy to "condition factor" (C):

$$C = \frac{\mathrm{OW}}{\mathrm{OL}^b}$$

where b is calculated from the exponential relationship between otolith weight and otolith length, OW = $a ext{ OL}^b$ (where b = 2.405, a = 0.596), by minimising the sum of the squared differences between actual observations and the estimated exponential trajectory (Fig. 2). OL is the length of acentric otolith and OW is the weight of acentric otolith.

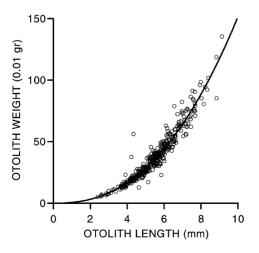


Fig. 2. Relationship between weight and length of acentric otoliths from long rough dab. Curve: estimated exponential relationship $(OW = 0.596 \times OL^{2.405}; N = 378; r^2 = 0.929)$.

3. Results and discussion

3.1. Precision versus reading technique

Incorrect age-determinations are often a result of absence of or failure to record the central translucent zone or incorrect determination in the edge region (Beamish and McFarlane, 1987). Such problems did not seem to occur often during this study. The centre of the otolith appeared as a small translucent spot, but became difficult to detect in large otoliths. An opaque zone surrounded the centre. Generally the zoning appeared most distinct along the line from the nucleus to the rostrum (the radius). In all cases where both the centric and acentric otoliths were clear so that the zoning could be studied in detail, the zonation pattern was similar.

Comparisons of the different age-determinations followed suggestions made by Richards et al. (1992) and Campana et al. (1995). To detect possible systematic errors between different age-determinations, ages from different determinations were plotted against each other (Fig. 3). The four age-determinations showed good agreement up to an age of about 10 years. The age of older specimens was often lower in stereomicroscope readings compared to readings from digitised pictures. Within the same technique, the second reading had a tendency to be lower compared to the first.

Age frequency tables for each of the comparisons in Fig. 3 were constructed to examine the precision in the age-determinations (Table 2). The agreement was best between the two P-readings, where 57% were given the same age; between the other comparisons the agreement fluctuated between 31 and 45%.

As shown in Fig. 3, discrepancy in mean age increased after an age of 10 years, while the confidence intervals increased after an age of approximately 14 years. Confidence intervals are, however, affected by number of observations. Fig. 4 shows how the coefficient of variation, which is less affected by the number of observations, varied with age for each of the comparisons in Fig. 3. The precision seemed to be relatively independent of age. However, comparisons of the two P-readings showed a tendency for higher coefficient of variation with increasing age. The coefficient of variation was generally lower

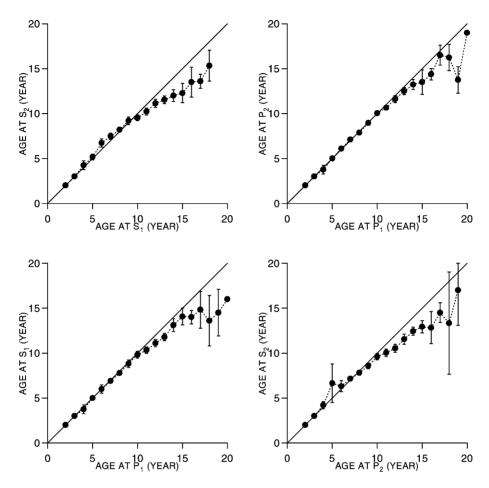


Fig. 3. For each age-group determined at one reading (x-axis), the figure shows mean age with 95% confidence limits determined at another age-reading of the same otoliths (y-axis). The line indicating identical age is also included.

between the two P-readings than between the two S-readings.

In P_1 20% of the otoliths were classified to readability 1, while 58% had readability 2. The highest precision was found for readability 1. Eighty percent of these otoliths were given the same age in P_1 and P_2 (Fig. 5). Corresponding figures for readability 2 and 3 were 56 and 36%, respectively. The figures were 66, 25 and 20% for readability 1, 2 and 3, from the stereomicroscope readings. Differences between the age-determinations grew larger when the readability became poorer (Fig. 5). However, for P_1 and P_2 , 95, 88 and 67% of the otoliths with readability 1, 2 and 3, respectively, were estimated within ± 1 year. There

was no significant difference in readability between the sexes ($\chi^2 = 4.4$; d.f. = 1; p = 0.11), but it was substantially reduced with increasing age (Fig. 6). This is probably due to the covering of early formed zones by later formed material. Although the authors feel fairly confident in the method used, more work should be done to determine how sectioning of otoliths compares to the use of whole otoliths among older specimens (Whalen et al., 2000).

Use of digitised pictures gave higher age and better precision in the age-determinations than those obtained by stereomicroscope readings. The size and quality of the pictures made it easier to interpret the zoning pattern in the otoliths. Repeated interpreta-

Table 2 Age frequency tables of pairwise comparisons between different age-determinations^a

AGE (YEAR)	AG	E (YI		4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	SUM
AGE (TEAK)	2 3	1				.0					MINED		1.0	14	10	10	.,	10	12	20	1
AGE AT S ₁	3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18		4	3	1 10 1	8 4 1	1 4 34 13 4 1 3	5 12 32 9 12 4 1	3 13 18 15 8 7 2	4 9 14 14 6 8 2 1	1 1 8 9 12 8 3 4	1 1 2 3 10 19 13 5 1	1 7 4 8 2 3	1 1 2 4 3 3 1 1	1 2 3 1 1		1				4 4 11 18 55 65 45 52 46 58 39 23 14 6 5 3
	2	1	2						AGE	DETER	MINED	AT P ₂									1
AGE AT P ₁	2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20		3 1	3	1	10 1 1 1	1 22 9 1	2 32 5 1 1	1 5 29 5 3 2	5 26 12 4 2 2	7 25 14 1	1 1 5 26 10 6	8 12 5 4 1	2 14 4 4 1	1 3 4 2	2 1 1	3 1	1 1	1		1 3 4 8 11 26 47 42 40 46 56 28 34 14 8 6 4 4 4 1 1 3 83
	2 3 4	1	3						AGE	DETER	MINED	AT S ₁									1
AGE AT P ₁	5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20		1	3	8 2	8 4 3	24 11 2 1 1 1	1 1 28 18 4 4 4 1	6 17 8 5 2 1	1 19 11 12 2 2 1	2 5 19 8 5 2	1 2 6 21 13 5	1 7 7 8 4 3 1	1 9 3 3 2 1	1 3 5 2 2	2 1 1 1 1	2 1 1	1 1 1			3 4 8 11 29 48 42 40 46 56 28 35 14 8 6 5
	2	1	4						AGE	DETER	MINED	AT S ₂									1
AGE AT P ₂	2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18		4	3	1 8 3	5 3 2	4 22 11 6 1 1	8 18 18 8 7 4	1 6 17 17 7 10 2	1 4 12 16 8 6	5 5 10 8 6 2	2 6 9 11 4 8 5 3	1 5 6 6 2 1	2 4 4 3 1	1 1 1 1 1						4 4 4 10 13 33 38 47 49 46 49 29 25 14 5 5 2

^a Numbers in the tables indicate number of observations. The column to the right shows the number of age-determinations in the readings.

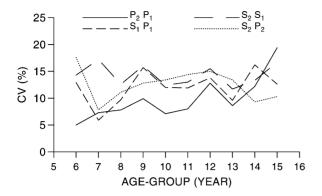


Fig. 4. The coefficient of variation (CV) for age-determinations from one reading of otoliths which formed an age-group at another reading. S_2S_1 : CV for age-determinations at S_2 for each of the age-groups at S_1 . P_2P_1 : CV for age-determinations at P_2 for each of the age-groups at P_1 . S_1P_1 : CV for age-determinations at S_1 for each of the age-groups at S_1 . S_2P_2 : CV for age-determinations at S_2 for each of the age-groups at S_2 for each of the age-groups at S_2 .

tions under the stereomicroscope could be affected by varying light conditions due to different placement of the otoliths under the stereomicroscope; this problem did not occur when using images. This might have contributed to the observed differences in precision between the two techniques.

To prevent counting of ring formations other than those that reflect years, the picture should indicate the real size of the otolith. The size of the otolith was unknown during P_2 , which may explain some of the larger differences between the two P-readings (Table 2). The results, however, show increased precision and higher age, which might suggest a more accurate age-determination from the P-readings than those obtained from the stereomicroscope readings.

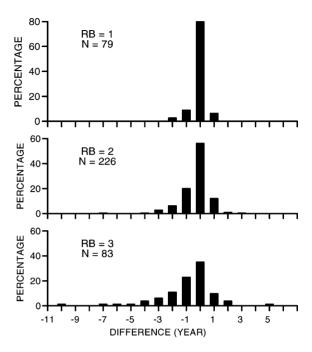


Fig. 5. Percentage of age-determinations at P_2 which differ from the age-determinations at P_1 with a certain number of years. Readability (RB) was determined during P_1 .

Age-determination of long rough dab in the Barents Sea is time consuming and relatively difficult. The precision in this study was, however, on the same level as that obtained from long rough dab ageing in the NW Atlantic (Brodie et al., 1990). Assuming that the zoning represents years, the precision observed here is sufficient for estimation of general stock parameters like age distribution, age of maturity, and length-at-age (Albert et al., 1994; Fossen et al., 1999).

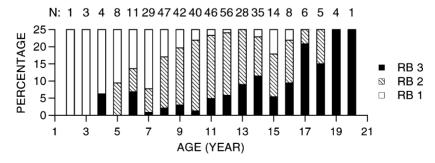


Fig. 6. Percentage of degrees of readability (1-3) within each age-group determined at P_1 . The number of observations is indicated above the bars.

For several species use of images may result in timesaving and higher precision and accuracy in the age-determination process (Cailliet et al., 1996). The time saving aspect has been difficult to show during this study due to repeated improvements in the way specific tasks were carried out. The authors feel that the quality of the digitised pictures used revealed important information about age and that this led to increased precision, and possibly accuracy, of the age-determination.

It is known that especially spring spawning fish species in temperate areas are exposed to high-energy losses in relation to spawning (Wootton, 1990; Jobling, 1995). Changes in energy costs might cause changes to the formation of zones in the otoliths (Pannella, 1980; Campana and Neilson, 1985; Hopkins et al., 1986; Rijnsdorp, 1993). The subjective observations of the type of zonation pattern revealed that in 84% of the otoliths a sudden change in growth had occurred during the last years before catch. These changes were seen in 88% of the 189 mature and in 56% of the 53 immature specimens. Thus, assuming correct maturity determinations, these ontogenetic changes in zonation were at least not directly associated with sexual maturation. However, approximately 60% of the changes occurred during the last year before the fish were caught, and the pattern was as such weakly defined.

In otoliths of long rough dab changes in zoning pattern can probably be classified along a continuum and it is therefore uncertain that this measurement has any biological meaning. Although such changes might be a result of maturation in several species, this investigation shows that such patterns might also occur in otoliths without being a result of maturation.

3.2. Morphometric measurements

Based on the results above and due to the lack of the individuals actual age the age determined during P₁ was assumed to represent the true age. As during earlier investigations (Härkönen, 1986; Hunt, 1992), the different otolith measurements were strongly correlated with fish length but weakly with age (Table 3). The two exceptions were the otolith condition factor and the relationship between the otolith width and length which were weakly correlated both with fish length and age. An analysis of variance revealed a

significant difference in otolith condition factor between the lower and upper length quartile within each age-group ($F_{1159} = 9.415$; p = 0.003; for age-groups 7–14). The test showed that the longest fish within the age-groups had a higher otolith condition factor (mean C = 0.615; S.D. = 0.132; N = 79) than the smaller ones (mean C = 0.564; S.D. = 0.067; N = 82). The relationship between otolith width and length decreased slightly with increasing fish length and age (slope: -0.002; S.D. < 0.001; all data combined). An analysis of variance did not reveal any significant differences between age-groups (7–14 years, $F_{7259} = 1.324$; p = 0.24; only age estimates with readability 1 and 2 were used).

The observed differences in otolith condition factor between fast and slow growing fish maybe a result of the differences in growth rate and the space available for otolith growth (Pannella, 1980; Campana and Neilson, 1985; Gauldie, 1988; Gauldie and Nelson, 1990). If otolith growth is restricted due to lack of space, it seems likely to be a higher degree of restriction in slower growing fish since otolith growth also is a function of time. Otolith growth is fastest in the anterior—posterior direction of the otoliths. Therefore, if otolith growth is restricted, length growth. This will reduce the condition factor of otoliths among slower growing specimens and may explain the observed differences.

Among the different morphometric otolith variables in Table 3 the otolith radius appeared to be the one that was best correlated with age for both sexes. Otolith radius explained 63% of the observed variation in age (75 and 61% for females and males, respectively). A covariance analysis of otolith radius versus fish length (covariate) and age (factor) revealed a significant age effect ($F_{7263} = 7.45$; p < 0.001; age-groups 7–14, with readability 1 and 2 were used). The model explained 80% of the variation in the otolith radius and indicated that otolith growth was affected both by fish length and age. The effect of age showed that the otoliths among older specimens would have a tendency of being bigger than for the younger ones of same length. The effect of fish growth rate was, however, small compared to the observed variation in otolith size for a given fish length (Fig. 7). Similar relationships between otolith radius and length and age of the fish have been described for other species (Beamish

Table 3
Correlation (Spearman's coefficient of correlation) between fish length, age and otolith measurements^a

(1		/	0 , 0									
	Fish length	Age (female/male)	Weight centric otolith	Weight acen- tric otolith	Area	Circumference	Major ellipse	Minor ellipse	Otolith length	Otolith width	Otolith radius	Condition factor
Age (female/male)	0.715	1										
	(0.746/0.65	53)										
Weight centric otolith	0.950	0.744 (0.836/0.711)	1									
Weight acentric otolith	0.951	0.746 (0.782/0.701)	0.996	1								
Area	0.930	0.718 (0.780/0.671)	0.970	0.971	1							
Circumference	0.931	0.725 (0.776/0.677)	0.970	0.970	0.998	1						
Major ellipse	0.927	0.724 (0.780/0.660)	0.966	0.965	0.990	0.991	1					
Minor ellipse	0.915	0.696 (0.743/0.647)	0.955	0.965	0.988	0.982	0.958	1				
Otolith length	0.920	0.728 (0.773/0.671)	0.964	0.963	0.988	0.990	0.997	0.957	1			
Otolith width	0.863	0.610 (0.729/0.557)	0.908	0.910	0.954	0.949	0.924	0.966	0.921	1		
Otolith radius	0.896	0.787 (0.772/0.736)	0.929	0.926	0.939	0.942	0.950	0.908	0.954	0.816	1	
Condition factor	0.261	0.206 (0.127/0.166)	0.290	0.310	0.180	0.166	0.113	0.247	0.096	0.208	0.114	1
Otolith (width/length)	-0.279	$-0.378 \ (-0.389/-0.321)$	-0.279	-0.271	-0.224	-0.239	-0.313	-0.122	-0.323	0.022	-0.444	0.246

^a Number of observations varies around 325 depending on measurement in consideration. Correlation against age is splinted for each sex, only age-determination (P₁) with readability 1 and 2 are included. Number of observations varies within each sex, 80–85 for females and 190–216 for males.

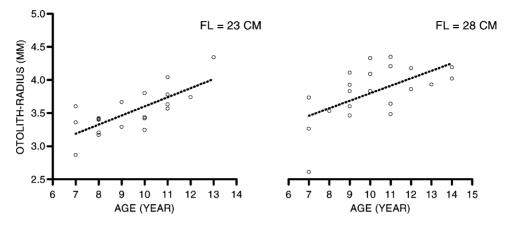


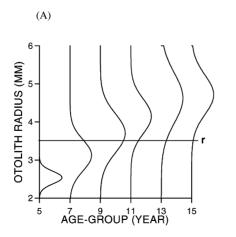
Fig. 7. The otolith radii plotted against age for long rough dabs of equal length. The two length groups with highest number of observations were used: (left) fish with a total length of 23 cm; (right) fish with a total length of 28 cm when caught. Only age-determinations with readability 1 and 2 were used. Smoothing (linear) based on single observations (Clevland, 1979).

and McFarlane, 1987; Pawson, 1990; Fletcher and Blight, 1996; Cardinale et al., 2000).

Wide variation in length-at-age, strong correlation between otolith measurements and between otolith measurements and fish length are likely to explain why combining several otolith measurements did not explain the variation in age much better than otolith radius did alone. These indications came through calculation of a multiple correlation coefficient.

The otoliths contain information both on fish age and rate of length growth. This information could be used to achieve a more objective and accurate age-determination. A first step might be to use this as a supplement to conventional age-determination, either to increase sample size or as an independent control of otolith readers. A possibility could be to invent a method that produces the most likely age based on otolith measurements together with fish length. This could be used as a reference for between reader comparisons, not only to see where the differences are but also related to a more objective base for comparison.

Based on the findings in this study we suggest that more work should be done to investigate the possibility of using otolith measurements to improve precision and accuracy in age-determination. Below is an



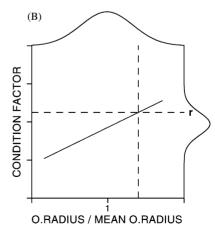


Fig. 8. (A) Probability distribution of otolith radius for six age-groups of long rough dab. (B) Schematic drawing of the relationship between otolith condition factor (y-axis) and otolith radius divided by the average otolith radius for the age-group (x-axis).

outline of possible ways to incorporate the additional information found in the otoliths.

For each age-group of long rough dab a probability distribution of otolith radius can be drawn (Fig. 8A). For any otolith radius this can be used to obtain the probability for a fish belonging to a certain age-group. One possibility would be to choose the age that maximises this probability. By considering several measurements simultaneously accuracy might be further improved.

The material shows that the otoliths condition factor contains information about individual growth rate. Due to the positive correlation between fish length and otolith measurements it will also give an indication of otolith size compared to age. For a given condition factor we can therefore get an indication of whether the fish has a large or small otolith radius compared to the average for its age-group (Fig. 8B). This can be incorporated to increase the possibility of finding the individual age.

To illustrate this an example is shown in Fig. 8. A fish with an otolith radius r (see line in Fig. 8A) has a similar probability of belonging to either the 7- or the 9-year-old age-group. Fig. 8B further indicates that the otolith condition factor of this fish is high (r). By using the intercept between the condition factor and the regression line,

condition factor

$$= a \left(\frac{\text{otolith radius}}{\text{average otolith radius for its age-group}} \right) + b$$

(Fig. 8B), we can see that the fish is expected to have a larger than average otolith radius for its age. This indicates that the fish grows faster than average. Therefore, when going back to Fig. 8A, the fish is more likely to be 7 than 9 years old.

To be able to bring this further, statistical work is needed to ensure that the estimates will be unbiased and to combine the different measurements in an appropriate way. Although such methods might work well with species like long rough dab they are likely to be more easily adapted to species with less within age-group variation in size, stage of maturity and otolith measurements. For such species it might be possible to obtain accurate and objective age-determinations with the use of a limited number of individual measurements.

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