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Report of the Workshop on Age reading of Dab (*Limanda limanda*) (WKARDAB2)

17–20 November 2015.

Hamburg, Germany

ICES International Council for the Exploration of the Sea
CIEM Conseil International pour l'Exploration de la Mer

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Executive summary

Based on the results of a small-scale otolith exchange held in 2013 (Haslob, 2013) the ICES Planning Group on Commercial Catches, Discards and Biological Sampling (PGCCDBS) identified the need for an age reading workshop on dab otoliths (WKARDAB2). This workshop was hosted by the Thünen Institute of Sea Fisheries (Hamburg, Germany) 17–20 November 2015. Eight age readers from five countries (Belgium, The Netherlands, Iceland, Denmark and Germany) participated in the work shop. The workshop was chaired by Loes Bolle (The Netherlands) and Holger Haslob (Germany).

Two otoliths sets (exercise 1 and 2), both consisting of whole and sectioned otoliths, were aged before the workshop and on the first day of the workshop. The whole otoliths were aged before the workshop using WebGR, the sectioned otoliths were aged at the workshop using stereomicroscopes.

After exercise 1 and 2 were completed, differences in interpretations were discussed by projecting images on the screen. Images of whole and sectioned otoliths from exercises 1 and 2 were discussed and, in addition, images of neutral-red stained sections, supplied by Denmark and Belgium, were discussed. The main conclusions were:

- Stained sections appear to be a promising way to age dab otoliths.
- The biggest problem in the interpretation of dab otoliths is the edge of the otolith. Especially in the case of a translucent zone on the edge of the otolith in the early part of the 3rd quarter, there were clear discrepancies in the interpretation.
- Split rings often occur in dab otoliths, but this did not appear to be a major problem within the current group of experienced readers. In most cases, the whole group agreed on the identification of split rings.

Subsequently, exercise 3 was carried out to examine if the discussions had led to improvement in the consistency of age reading. For this exercise a new otolith set was used (consisting of whole and sectioned otoliths), that had been prepared prior to the workshop in WebGR. Unfortunately, WebGR failed during the workshop and the group had to switch to real material and stereomicroscopes. The results of exercise 3 did not show an overall improvement in the consistency of age reading.

The discussion on stained sections indicated the need to compare whole and stained sectioned otoliths in a calibration exercise. Images were made available at the workshop and it was attempted to initiate a 4th exercise. However, this failed again due to problems with WebGR. Therefore this exercise, in an elaborated form (include 3 methods: whole, sectioned and stained sectioned otoliths; include otoliths from 2 periods and several regions/countries), is now proposed as follow-up action.

No validation studies have been carried out for dab age reading yet. We propose a marginal increment study, to validate the timing of the deposition of opaque and translucent material on the edge of the otolith, as a second follow-up action. The results of such a study will help resolve the encountered problems with the interpretation of the edge of the otolith.

1 Introduction

1.1 Background

Dab is a widespread demersal species on the Northeast Atlantic shelf and distributed from the Bay of Biscay to Iceland and Norway, including the Barents Sea and the Baltic. In the North Sea it is one of the most abundant species distributed over the whole area in depths down to 100m, but it was also found occasionally down to depths of 150m. The main concentration of dab can be found in the southeastern North Sea. Dab abundance decreases towards the northern parts of the North Sea. Dab feeds on a variety of small invertebrates, mainly polychaete worms, shellfish and crustaceans. Dab is a by-catch species in fisheries for plaice, sole and demersal round fish, but up to 90% of the catches are discarded (ICES 2015).

Dab was assessed for the first time in the ICES WGNEW (ICES 2013). Since 2014, dab is assessed by the WGNSSK (ICES 2014a). Dab is currently defined as a data limited stock. No analytical assessment is carried out so far for this species and it is assessed applying the DLS 3.2. category rule (ICES 2012). The North Sea dab stock will be benchmarked in 2016. During a preparatory data collection work shop for this benchmark held in November 2015, several age based methods were identified which could be explored for North Sea dab as some countries do age readings for dab since several years on a regular basis. These methods (e.g. SURBAR, SAM) will have the potential to better inform the stock status of dab based on the age structure of the stock. Besides stock assessment purposes age readings are also important in a variety of other biological studies on dab. For instance, dab serves as an important indicator species in disease and toxicology studies and monitoring programmes due to its more sedentary life history. This underlines the need of age readings for this species which have to be calibrated from time to time between international laboratories by the means of calibration exercises and/or age reading workshops.

In 2008 the Planning Group on Commercial Catch, Discards and Biological Sampling (PGCCDBS, ICES 2008) identified dab as a species requiring an exchange and age reading workshop. An exchange of dab otoliths was performed for the first time in 2009 and based on this in 2010 the first work shop on age reading dab otoliths (WKARDAB, ICES 2010) was held in Hamburg. One otolith set was included in the exchange and the workshop. This set contained whole otoliths from 160 fish from the North Sea (area IVb) covering all seasons (40 fish per quarter). The main issues identified during this first workshop were:

- the interpretation of the first annual ring
- the identification of rings near to the edge of the otolith especially in material from the 3rd quarter
- confusion with the hyaline and opaque zone as growth rings
- difference of used light (transmitted vs. reflected)
- misinterpretation of split rings

A guideline for the preparation and reading of dab otoliths was prepared and a reference collection of otoliths was presented in the report of this workshop (ICES 2010).

The Planning Group on Commercial Catch, Discards and Biological Sampling (PGCCDBS, ICES 2008) identified the need for a follow up small-scale dab otolith exchange in 2013. Based on the results of this small-scale exchange (Haslob, 2013) the

PGCCDBS recommended a second age reading work shop on dab otoliths to be held in 2015 (ICES 2014b).

1.2 Terms of Reference

a) Analyse the results of an international otolith (image) exchange

b) Evaluate growth increment patterns

c) Compile international guidelines for the interpretation of growth structures in dab otoliths

d) Elaborate the reference collection of dab otoliths

e) Address the generic ToRs adopted for workshops on age calibration (see 'Guidelines for Workshops on Age Calibration').

1.3 Participants

The second Workshop on Age reading of Dab (WKARDAB2) was held in Hamburg from 17–20 November 2015. Eight age readers from 6 laboratories in 5 countries (Germany, The Netherlands, Belgium, Denmark and Iceland) participated in the workshop (Annex 1). The meeting was chaired by Loes Bolle (The Netherlands) and Holger Haslob (Germany).

1.4 Agenda

The meeting was opened at 10:00 am on Tuesday 17th November and closed at 12:00 pm on Friday 20th November. A detailed agenda was updated each day; the overall agenda is presented in Annex 2.

2 Age reading calibration (ToR a, generic ToR)

2.1 Introduction

Dab is generally considered to be a difficult species to age, as reflected by the results of the 2010 workshop and 2013 exchange (ICES 2010, Haslob 2013). The primary goal of this workshop and the pre-workshop exchange was to resolve interpretation differences between readers and laboratories. As different procedures are used to age dab otoliths (see Annex 3), different procedures were included in the pre-workshop exchange and in the workshop.

2.2 Methods

Two otoliths sets (exercise 1 and 2), both consisting of whole and sectioned otoliths, were planned for the pre-workshop exchange on WebGR. Unfortunately, several readers did not age the sectioned otoliths in exercise 2. Furthermore, the images of sectioned otoliths in exercise 1 were removed from WebGR because of poor image quality. Therefore, exercises 1 and 2 were completed during the first day of the workshop. All age readers participating in the workshop aged the sectioned otoliths using real material and stereomicroscopes.

For exercise 1, images of the whole otoliths were taken and then the same otolith was sectioned. Exercise 2 consisted of whole and sectioned otoliths from the same fish. All otoliths originated from the North Sea (IVbc). The otoliths in exercise 1 were collected in July; the otoliths in exercise 2 were stratified by month (Table 1). The (modal) age range was larger in exercise 2 than in exercise 1 (Table 2).

After exercise 1 and 2 were completed, otolith interpretations were discussed by projecting images on the screen. Images of whole and sectioned otoliths from exercises 1 and 2 were discussed. In addition, images of neutral-red stained sections, supplied by Denmark and Belgium, were discussed.

Subsequently, exercise 3 was carried out to examine if the discussions had led to improvement in the consistency of age reading. For this exercise a new otolith set was used which was comparable to the one used in exercise 2 (whole and sectioned otoliths from the same fish; stratified by month), but smaller in size. This exercise was prepared prior to the workshop in WebGR and it was intended to use WebGR during the workshop for carrying out exercise 3. However, WebGR did not work when all readers tried to annotate simultaneously and we had to switch to real material and stereomicroscopes. This caused a delay in the workshop programme and loss of otoliths in the analysis as some of the whole otoliths got mixed up.

The discussion on stained sections indicated the need to compare whole and stained sectioned otoliths in a calibration exercise. We had images available at the workshop and attempted to initiate a 4th exercise including these images. However, this failed due to problems with WebGR. Although the upload of the images to WebGR appeared to have succeeded, we were not able to include the images in a new WebGR exercise. This problem was not solved during the workshop. Therefore this exercise, in an elaborated form (include 3 methods: whole, sectioned and stained sectioned otoliths; include otoliths from more months and regions/countries), is now recommended as follow-up action (see section 6).

Eight age readers participated in both the workshop and the pre-workshop exchange. They all aged the whole otoliths of exercise 1 and 2 using WebGR, the sectioned otoliths of exercise 1 and 2 using microscopes (first day of the workshop), and the whole and

sectioned otoliths of exercise 3 using microscopes (third day of the workshop). Furthermore, these 8 readers are all considered to be experienced to highly experienced readers. Therefore, all calculations were done using the age readings of these 8 readers to determine modal age. Three more people participated in exercise 1 and 2 on WebGR prior to the workshop. Their age readings of whole otoliths were compared to modal ages based on the 8 'main' readers. This could not be done for the sectioned otoliths in exercise 2 because not everyone aged these using WebGR.

An ongoing discussion is whether loss of detail in images compared to real material reduces age reading quality. To address this question we compared WebGR and microscope results of the 5 experienced readers who had twice aged the sectioned otoliths in exercise 2; the first time using WebGR prior to the workshop and the second time using microscopes on the first day of the workshop.

Table 1 Otolith sets used in the pre-workshop exchange and workshop.

	Exercise 1		Exercise 2		Exercise 3	
Country	DE		NL		NL	
Area	IVbc		IVbc		IVbc	
Months	July		Jan-Dec		Jan-Dec	
Method	whole	sectioned	whole	sectioned	whole	sectioned
No. of otoliths	54	54	50	50	34	42
No. of readers	8 (+3)	8	8 (+3)	8	8	8

2.3 Results and discussion

A summary of the results of exercises 1–3 is presented in Table 2. The detailed results are presented in Annexes 4–10.

Overall agreement was low for the whole otoliths in exercise 1 (Table 2): 65% for the 8 main readers (i.e. experienced readers who participated in both the exchange and the workshop) and 63% for all 11 readers (when compared to modal age of the 8 main readers). All these otoliths were from July. The discussions of otolith images revealed that there was a strong disagreement on whether or not a translucent band on the edge of the otolith in July should be counted. This interpretation problem, which had already been identified during the workshop in 2010, was the main cause for low agreement in exercise 1. The readers who argued that the translucent edge should be counted (Readers 3, 4 and 7) were in the minority and consequently had low agreement and a positive bias compared to modal age (Table 2 and Annex 4).

Consistency between the readers was much higher for the sectioned otoliths of exercise 1: 78% overall agreement (Table 2), with no evident bias for any reader (Annex 5). It might appear that the interpretation differences in whole otoliths were resolved due to sectioning of the otoliths. This, however, was probably not the case. When the readers aged the sectioned otoliths (using microscopes, on the first day of the workshop) they were only informed that the otoliths were from the 3rd quarter, not specifically from July. All readers agree that a translucent band on the edge of the otolith in the 3rd quarter generally should not be counted. The problems arise for the early part of the 3rd quarter.

Exercise 1 revealed differences in the interpretation of a translucent band on the edge of the otolith in the early part of the 3rd quarter. Some argue that formation of the opaque zone in the remaining part of the year is unlikely and that therefore the opaque

zone has already been deposited (they do not count a translucent band on the edge of the otolith). Others argue that the opaque zone cannot have been deposited yet because of the amount of growth of the translucent zone after the last opaque zone (they count a translucent band on the edge of the otolith). To address this issue, a marginal increment study is proposed as follow-up action (see section 6). Examination of the edge of the otolith throughout the year should shed light on when the opaque and translucent bands are deposited. The study should include otoliths from different regions and age groups, as deposition of the opaque band is expected earlier in the season for fish from southern regions and for young (immature) fish.

Table 2. Summary of the results of exercises 1, 2 and 3. Readers 1–8 participated in the both the workshop and the pre-workshop exchange. Readers 9–11 only participated in the pre-workshop exchange.

	Exercise 1		Exercise 2		Exercise 3	
	whole	sectioned	whole	sectioned	whole	sectioned
Group results						
Modal age	1-6	1-7	1-9	1-10	1-9	1-10
Agreement	65% (63%)	78%	79% (72%)	63%	73%	68%
Bias	0.24 (0.20)	0.00	0.02 (-0.06)	-0.01	-0.03	-0.04
CV	17% (18%)	14%	9% (12%)	19%	12%	10%
APE	14% (15%)	10%	7% (9%)	14%	9%	8%
Agreement by reader						
Reader 1	80%	85%	78%	64%	79%	81%
Reader 2	74%	72%	80%	46%	62%	38%
Reader 3	37%	87%	98%	62%	94%	83%
Reader 4	35%	63%	72%	56%	88%	71%
Reader 5	93%	74%	70%	64%	68%	60%
Reader 6	89%	76%	72%	66%	62%	57%
Reader 7	19%	89%	88%	72%	62%	79%
Reader 8	81%	80%	73%	70%	68%	76%
Reader 9	54%		60%			
Reader 10	63%		52%			
Reader 11	56%		45%			

Exercise 2 consisted of otoliths from fish collected in all quarters of the year and only included 2 fish from July or August. The previously encountered disagreement on the interpretation of the edge in the early part of the 3rd quarter did not play an important role in this exercise. Consequently, overall agreement for the whole otoliths was higher than in exercise 1 (Table 2) and no structural bias over all ages was observed for any of the readers (Annex 6).

Overall agreement was lower for the sectioned otoliths than for the whole otoliths (Table 2). This was, at least partly, due to the fact that several of the age readers (Readers 1–5) were not accustomed to ageing sectioned otoliths. Those accustomed to sections (Readers 6–8) showed the highest percentages agreement. However, even for these readers, agreement dropped in sectioned otoliths compared to whole otoliths (Table 2 and Annex 7).

Precision (consistency between readers) alone is not the only basis on which a method should be evaluated. Accuracy (consistency with true age) is more important. At present, in the absence of validated material, a true evaluation of accuracy is not possible for dab. The outcome of the age readings clearly differed depending on the method, indicating that one of the methods is less accurate. Fish are generally assigned an older age when using sectioned otoliths compared to whole otoliths. This was evident for all readers combined (Figure 1) and for each reader separately (Annex 8). Question is whether age is underestimated when using whole otoliths or overestimated when using sections. In a validation study on two other flatfish species (plaice *Pleuronectes platessa* and sole *Solea solea*) it was concluded that age will be underestimated when using whole otoliths (Etherton, 2015).

Images of whole and (stained) sections were compared during the discussions. It became clear that more rings were counted near the edge of the otolith in (stained) sectioned otoliths compared to whole otoliths. This was not only the case for older fish in which the so-called cliff edge issue may occur (that is when the otolith gets thicker rather than larger in the horizontal plane), but also in young fish. The comparison also showed that the identification of the first annulus was more difficult in stained sections compared to whole otoliths. Although direct comparisons of non-stained sections and stained sections using the same otolith were not available during the workshop, overall the structures appeared clearer in stained sections.

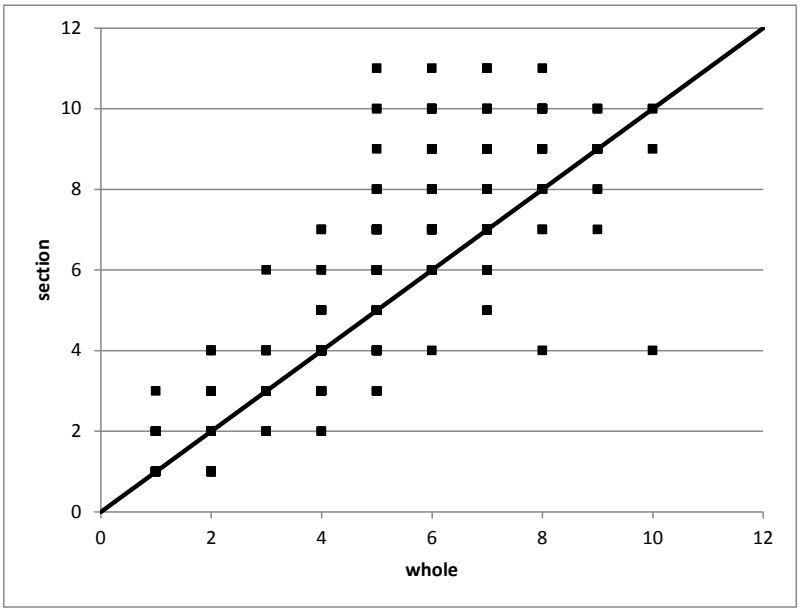

Figure 1. Comparison of age determinations based on whole and sectioned otoliths from the same fish. The y=x line indicates when both methods give the same result. 42% of the points are on the line (same age for whole and section), 41% are above the line (older age for section) and 17% are below the line (older age for whole).

Exercise 3 was carried out to examine if the discussions on how to interpret the structures had led to improvement in the consistency between age readers. The otolith set prepared for this exercise was comparable to that of exercise 2. Comparison of the overall results showed a small decrease in agreement for whole otoliths and a small increase for the sectioned otoliths (Table 2). This was highly variable at the level of the individual reader (Table 2 and Annexes 9–10). Apparently some readers got confused, whereas others became more consistent for one or both methods. However, sample

sizes were small, especially for the whole otoliths in exercise 3 (see section 2.2), and therefore small differences should not receive too much attention. The overall conclusion was that there was no clear improvement in the consistency between age readers.

We compared age determinations based on images and real material to examine if loss of detail in images compared to real material reduces age reading quality. This comparison could only be carried out for 5 readers and the sectioned otoliths of exercise 2 (see section 2.2). The expectation was a lower consistency when using images, but for 4 out of 5 readers, the opposite was the case (Table 3). Furthermore, there was no clear over or underestimation of age based on images compared to age based on real material (Figure 2).

Table 3. Comparison of age determinations based on images and real material.

	Sections of exercise 2	
	images	**real**
Group results		
Modal age	1-10	1-10
Agreement	75%	67%
Bias	0.08	0.01
CV	11%	16%
APE	8%	12%
Agreement by reader		
Reader 3	82%	60%
Reader 4	82%	58%
Reader 6	76%	74%
Reader 7	82%	70%
Reader 8	50%	74%

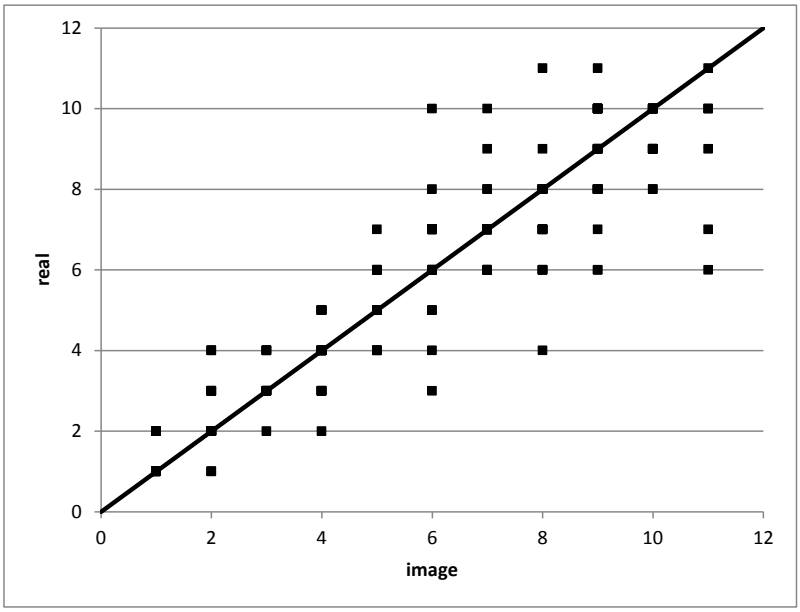

Figure 2. Comparison of age determinations based on images and real material. The y=x line indicates when both methods give the same result. 50% of the points are on the line (same age for image and real), 25% are above the line (older age for real) and 25% are below the line (older age for image).

2.4 Main conclusions

Stained sections appear to be a promising way to age dab otoliths. We propose to further explore this (see section 6).

The biggest problem in the interpretation of dab otoliths is the edge of the otolith. Especially in the case of a translucent zone on the edge of the otolith in the early part of the 3rd quarter, there were clear discrepancies in the interpretation. A marginal increment study is proposed to address this issue (see section 6).

Split rings often occur in dab otoliths, but this did not appear to be a major problem within the current group of experienced readers. In most cases, the whole group agreed on the identification of split rings.

To our knowledge no validation studies have been carried out for dab yet. Seizing any opportunity for direct or indirect validation is recommended (see section 6).

3 Growth increments (ToR b)

Measuring the increment of each annulus and comparing this between readers can be a valuable tool to examine interpretation differences between readers (Coad Davies *et al*. 2014). Differences in the interpretation of the first 2–4 annuli can be clearly identified in this manner. As growth decreases with age, it becomes more difficult in the later annuli.

Increment measurements can be extracted from WebGR if the age readers are specifically instructed how to make their annotations (Coad Davies *et al*. 2014). Basically, they need to annotate the nucleus and then annotate the annuli sequentially from the nucleus to the edge, at the end of the translucent zone, along a straight line and on a fixed axis.

We intended to use exercise 3 for this purpose. Unfortunately, WebGR failed during the workshop and we had to switch to microscopes and real material instead (see section 2.2). Data on growth increments were therefore not available.

4 Guidelines (ToR c, generic ToR)

International guidelines were compiled for the interpretation of dab otoliths (Annex 11). These guidelines were based on the expertise of the age readers and the discussions held during the workshop.

5 Reference collection (ToR d, generic ToR)

Three reference collections (images) were collated:

1) Agreed age for whole otoliths (Annex 12)

2) Agreed age for sectioned otoliths (Annex 13)

3) Age reading problems, including images of the same otolith whole and (stained)-sectioned (Annex 14)

For the agreed collections some examples with the highest agreements were compiled. For the collection with age reading problems examples with the lowest agreement were compiled. For the latter reference collection, images of both the (stained) sectioned otolith and the whole otolith were included, if available. The identified issues are described in the figure captions.

6 Follow-up actions (generic ToR)

We propose two follow-up actions. These follow-up actions require resources, therefore endorsement from WGBIOP is desirable.

1) International exchange including images of whole, sectioned and stained sectioned using the same otolith.

Proposed approach:

- WebGR exercise
- Include BE, DE, NL samples and from more countries if they so wish
- February & July samples
- Age range 1–10 years
- Reflected light images
- Water instead of oil must be used for the images of sections so the subsequent staining will not be hampered

2) Marginal increment studies to validate the timing of the deposition of opaque and translucent material on the edge of the otolith.

Proposed approach:

- IVc (BE, DE, NL, UK samples)
- IVb (BE, DE, NL, DK, UK samples)
- Va (IS samples)
- Cover as many months of the year as possible
- Two age groups: young fish (3–4 years) and older fish (6–7)
- Score the material on the edge (opaque or translucent)
- Optional: increment measurements including measurement of the marginal increment

7 Recommendation

WebGR is an excellent tool for otolith exchanges and workshops. However, there are several issues with the current version. The most critical problem at present is that it gets stuck, apparently related to if many people are working on WebGR simultaneously. We recommend that this problem is solved as soon as possible (Annex 15).

8 References

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ICES (2014b) Report of the Planning Group on Commercial Catches, Discards and Biological Sampling (PGCCDBS), 17–21 February, Horta (Azores), Portugal. ICES CM 2014/ACOM:34

ICES (2015) Report of the Working Group on the Assessment of Demersal Stocks in the North Sea and Skagerrak, 28 April–7 May, Copenhagen, Denmark. ICES CM 2015/ACOM:13

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Annex 1: List of participants

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Annex 2: Agenda

Workshop on age reading of dab (*Limanda limanda*) (WKARDAB2)

17–20 November 2015, Hamburg

Agenda

Tuesday 17 November

Welcome and housekeeping

Agenda, goals, otolith sets and ToRs

Present results exchange 2013 and workshop 2010

Reading exercise 1b

Reading exercise 2b

Discuss interpretations (images on screen)

Wednesday 18 November

Present results of exercises 1 & 2

Appoint subgroups for ToRs

Discuss interpretations (images on screen)

Guidelines (ToR c)

Reference collection (ToR d)

Overview laboratory procedures (generic ToR)

Social dinner

Thursday 19 November

Discuss interpretations (images on screen)

Guidelines (ToR c)

Reference collection (ToR d)

Overview laboratory procedures (generic ToR)

Instructions reading exercise 3 (w.r.t. growth increment analyses)

Reading exercise 3ab

Friday 20 November

Present results of exercise 3

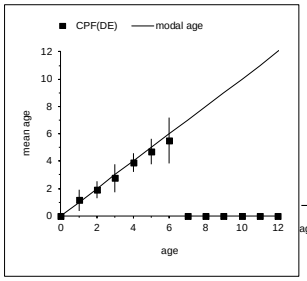
Formulate follow-up actions (generic ToR)

Reference collection (ToR d)

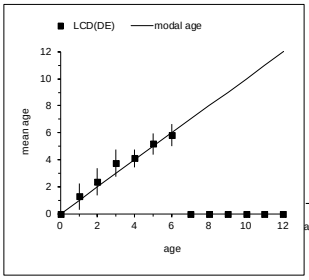
Annex 3: Laboratory procedures (generic ToR)

Country	Institute	Area	Age readings in stock assessments	Storage method (before preparation)	Preparation method	Count translucent or opaque	Lighting
Germany	Institute for Fisheries Ecology (TI) Cuxhaven	North Sea, western Baltic	no	in water (dried before embedding in resin)	whole in resin, between 2 glass plates	translucent	both
Germany	Institute for Sea Fisheries (TI) Hamburg	North Sea	yes	in water (dried before embedding in resin)	whole in resin, between 2 glass plates	translucent	both
Iceland	The Marine Research Institute (MRI)	North Atlantic Ocean (Va)	yes	frozen in water	whole in water	translucent	reflected
Denmark	DTU Aqua. National Institute of Aquatic Resources.	North Sea, Kattegat-Skaggerak, Baltic	yes	dry	whole in water (all samples)	translucent	both, mainly reflected
				dry	stained sections after soaking in water (test sample)	translucent	reflected
Belgium	Instituut voor Landbouw- en Visserijonderzoek (ILVO)	North Sea	no	dry	stained sections dry (all samples)	translucent	reflected
				dry	whole in water (all samples)	translucent	reflected
Netherlands	IMARES	North Sea	yes	dry	sections with oil (market sampes and offshore surveys)	opaque	both, mainly transmitted
				dry	whole in water (discards and inshore surveys)	opaque	both, mainly transmitted

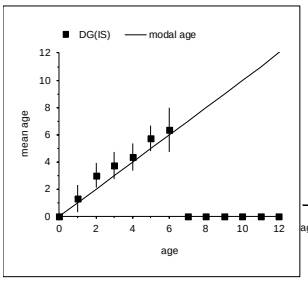
Annex 4: Exercise 1 – whole otoliths



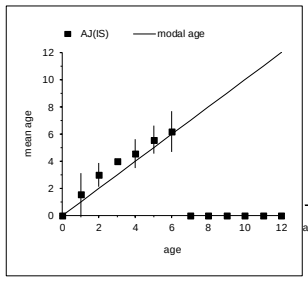
Age	modal	CPF(DE)
0	-	-
1	7	7
2	11	12
3	4	4
4	9	14
5	17	13
6	6	4
7	-	-
8	-	-
9	-	-
10	-	-
11	-	-
12	-	-
N	54	54
agreement	80%	
CV	15%	
bias	-0.19	
APE	15%	



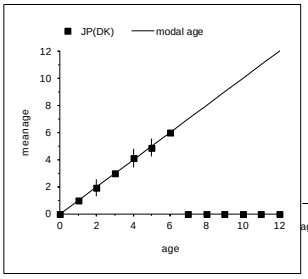
Age	modal	LCD(DE)
0	-	-
1	7	5
2	11	9
3	4	5
4	9	11
5	17	16
6	6	8
7	-	-
8	-	-
9	-	-
10	-	-
11	-	-
12	-	-
N	54	54
agreement	74%	
CV	15%	
bias	0.22	
APE	11%	



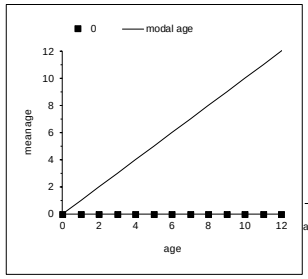
Age	modal	DG(IS)
0	-	-
1	7	5
2	11	3
3	4	10
4	9	10
5	17	9
6	6	14
7	-	3
8	-	-
9	-	-
10	-	-
11	-	-
12	-	-
N	54	54
agreement	37%	
CV	15%	
bias	0.61	
APE	15%	



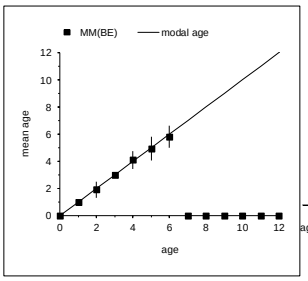
Age	modal	AJ(IS)
0	-	-
1	7	4
2	11	3
3	4	10
4	9	9
5	17	13
6	6	13
7	-	2
8	-	-
9	-	-
10	-	-
11	-	-
12	-	-
N	54	54
agreement	35%	
CV	16%	
bias	0.65	
APE	17%	



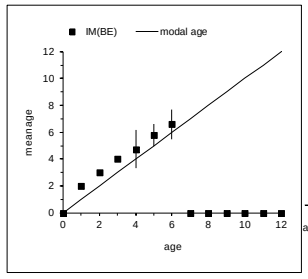
Age	modal	JP(DK)
0	-	-
1	7	8
2	11	10
3	4	4
4	9	10
5	17	16
6	6	6
7	-	-
8	-	-
9	-	-
10	-	-
11	-	-
12	-	-
N	54	54
agreement	93%	
CV	7%	
bias	-0.04	
APE	11%	



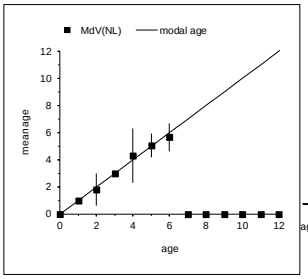
Age	modal	0
0	-	-
1	-	-
2	-	-
3	-	-
4	-	-
5	-	-
6	-	-
7	-	-
8	-	-
9	-	-
10	-	-
11	-	-
12	-	-
N	0	0
agreement		
CV		
bias		
APE		



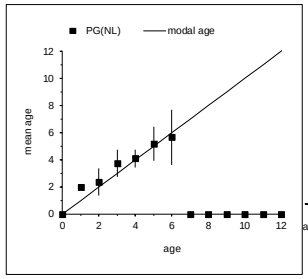
Age	modal	MM(BE)
0	-	-
1	7	8
2	11	10
3	4	4
4	9	10
5	17	16
6	6	6
7	-	-
8	-	-
9	-	-
10	-	-
11	-	-
12	-	-
N	54	54
agreement	89%	
CV	8%	
bias	-0.04	
APE	11%	



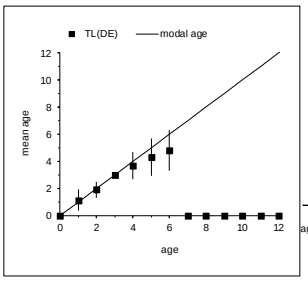
Age	modal	IM(BE)
0	-	-
1	6	-
2	5	6
3	4	5
4	8	7
5	15	7
6	5	15
7	-	3
8	-	-
9	-	-
10	-	-
11	-	-
12	-	-
N	43	43
agreement	19%	
CV	6%	
bias	0.84	
APE	20%	



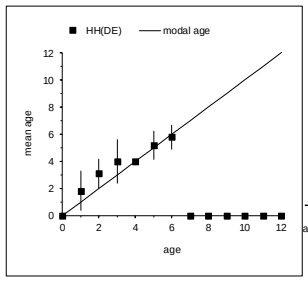
Age	modal	MdV(NL)
0	-	-
1	7	10
2	11	7
3	4	5
4	9	9
5	17	16
6	6	6
7	-	1
8	-	-
9	-	-
10	-	-
11	-	-
12	-	-
N	54	54
agreement	81%	
CV	14%	
bias	0.00	
APE	13%	



Age	modal	PG(NL)
0	-	-
1	7	-
2	11	14
3	4	5
4	9	14
5	17	12
6	6	8
7	-	1
8	-	-
9	-	-
10	-	-
11	-	-
12	-	-
N	54	54
agreement	54%	
CV	13%	
bias	0.30	
APE	18%	

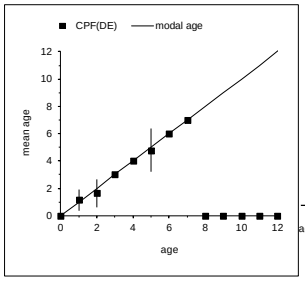


Age	modal	TL(DE)
0	-	-
1	7	7
2	11	11
3	4	9
4	9	16
5	17	10
6	6	1
7	-	-
8	-	-
9	-	-
10	-	-
11	-	-
12	-	-
N	54	54
agreement	63%	
CV	17%	
bias	-0.41	
APE	17%	

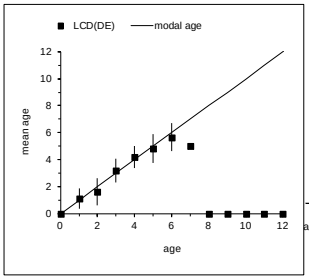


Age	modal	HH(DE)
0	-	-
1	6	2
2	11	4
3	4	10
4	9	14
5	17	14
6	5	8
7	-	-
8	-	-
9	-	-
10	-	-
11	-	-
12	-	-
N	52	52
agreement	56%	
CV	14%	
bias	0.44	
APE	21%	

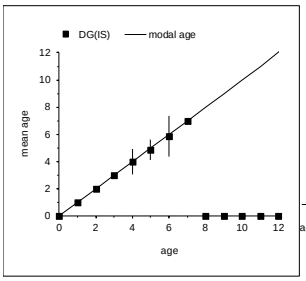
Annex 5: Exercise 1 – sectioned otoliths



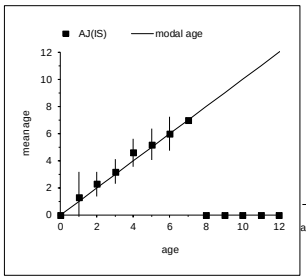
Age	modal	CPF(DE)
0	-	-
1	7	10
2	11	8
3	5	7
4	10	10
5	14	11
6	6	7
7	1	1
8	-	-
9	-	-
10	-	-
11	-	-
12	-	-
N	54	54
agreement	85%	
CV	15%	
bias	-0.11	
APE	10%	



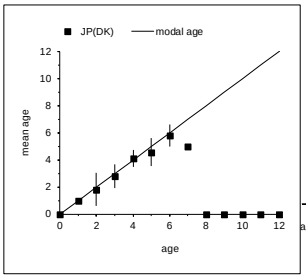
Age	modal	LCD(DE)
0	-	-
1	7	10
2	11	8
3	5	4
4	10	12
5	14	15
6	6	5
7	1	-
8	-	-
9	-	-
10	-	-
11	-	-
12	-	-
N	54	54
agreement	72%	
CV	18%	
bias	-0.11	
APE	11%	



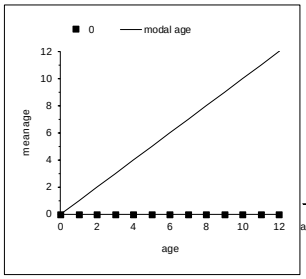
Age	modal	DG(IS)
0	-	-
1	7	7
2	11	11
3	5	6
4	10	10
5	14	15
6	6	3
7	1	2
8	-	-
9	-	-
10	-	-
11	-	-
12	-	-
N	54	54
agreement	87%	
CV	6%	
bias	-0.06	
APE	7%	



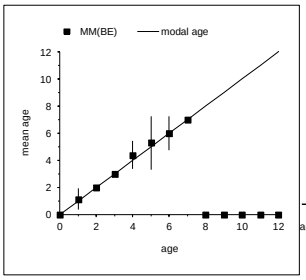
Age	modal	AJ(IS)
0	-	1
1	7	4
2	11	9
3	5	8
4	10	6
5	14	16
6	6	8
7	1	2
8	-	-
9	-	-
10	-	-
11	-	-
12	-	-
N	54	54
agreement	63%	
CV	21%	
bias	0.28	
APE	17%	



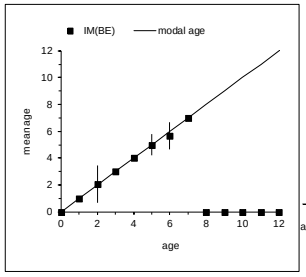
Age	modal	JP(DK)
0	-	-
1	7	10
2	11	8
3	5	5
4	10	15
5	14	11
6	6	5
7	1	-
8	-	-
9	-	-
10	-	-
11	-	-
12	-	-
N	54	54
agreement	74%	
CV	13%	
bias	-0.20	
APE	10%	



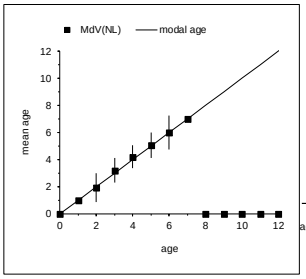
Age	modal	0
0	-	-
1	-	-
2	-	-
3	-	-
4	-	-
5	-	-
6	-	-
7	-	-
8	-	-
9	-	-
10	-	-
11	-	-
12	-	-
N	0	0
agreement		
CV		
bias		
APE		



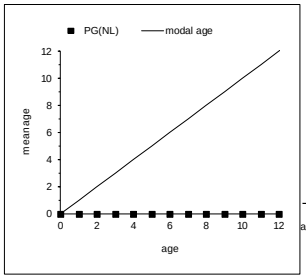
Age	modal	MM(BE)
0	-	-
1	7	6
2	11	12
3	5	5
4	10	8
5	14	13
6	6	7
7	1	2
8	-	1
9	-	-
10	-	-
11	-	-
12	-	-
N	54	54
agreement	76%	
CV	13%	
bias	0.17	
APE	9%	



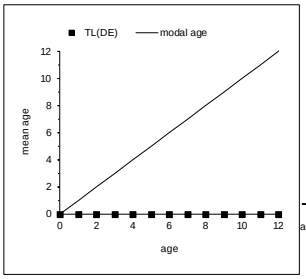
Age	modal	IM(BE)
0	-	-
1	7	8
2	11	9
3	5	5
4	10	12
5	14	14
6	6	5
7	1	1
8	-	-
9	-	-
10	-	-
11	-	-
12	-	-
N	54	54
agreement	89%	
CV	10%	
bias	-0.02	
APE	8%	



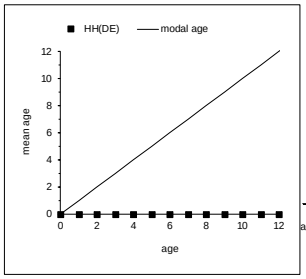
Age	modal	MdV(NL)
0	-	-
1	7	9
2	11	8
3	5	5
4	10	10
5	14	14
6	6	6
7	1	2
8	-	-
9	-	-
10	-	-
11	-	-
12	-	-
N	54	54
agreement	80%	
CV	13%	
bias	0.06	
APE	9%	



Age	modal	PG(NL)
0	-	-
1	-	-
2	-	-
3	-	-
4	-	-
5	-	-
6	-	-
7	-	-
8	-	-
9	-	-
10	-	-
11	-	-
12	-	-
N	0	0
agreement		
CV		
bias		
APE		

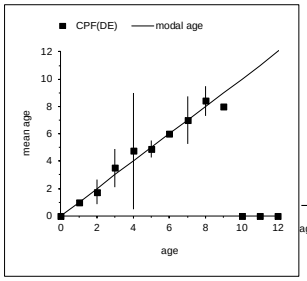


Age	modal	TL(DE)
0	-	-
1	-	-
2	-	-
3	-	-
4	-	-
5	-	-
6	-	-
7	-	-
8	-	-
9	-	-
10	-	-
11	-	-
12	-	-
N	0	0
agreement		
CV		
bias		
APE		

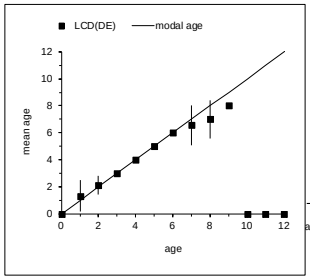


Age	modal	HH(DE)
0	-	-
1	-	-
2	-	-
3	-	-
4	-	-
5	-	-
6	-	-
7	-	-
8	-	-
9	-	-
10	-	-
11	-	-
12	-	-
N	0	0
agreement		
CV		
bias		
APE		

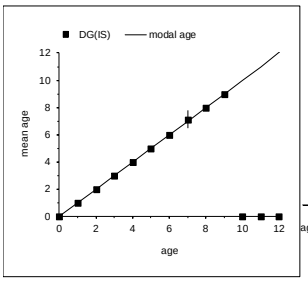
Annex 6: Exercise 2 – whole otoliths



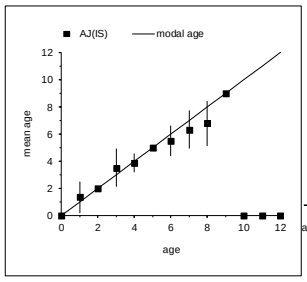
Age	modal	CPF(DE)
0	-	-
1	2	4
2	8	6
3	2	1
4	8	9
5	10	9
6	4	6
7	9	6
8	5	4
9	1	3
10	-	1
11	-	-
12	-	-
N	49	49
agreement	78%	
CV	17%	
bias	0.10	
APE	8%	



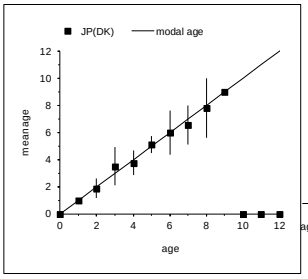
Age	modal	LCD(DE)
0	-	-
1	3	2
2	8	8
3	2	3
4	8	8
5	10	11
6	4	7
7	9	9
8	5	2
9	1	-
10	-	-
11	-	-
12	-	-
N	50	50
agreement	80%	
CV	8%	
bias	-0.16	
APE	7%	



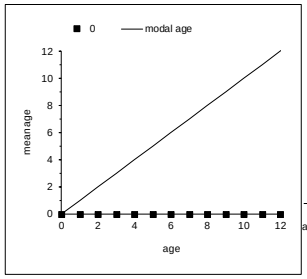
Age	modal	DG(IS)
0	-	-
1	3	3
2	8	8
3	2	2
4	8	8
5	10	10
6	4	4
7	9	8
8	5	6
9	1	1
10	-	-
11	-	-
12	-	-
N	50	50
agreement	98%	
CV	1%	
bias	0.02	
APE	5%	



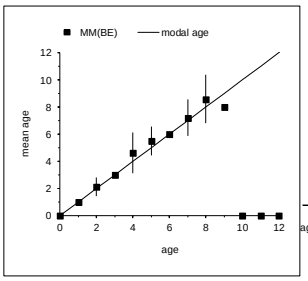
Age	modal	AJ(IS)
0	-	-
1	3	2
2	8	9
3	2	2
4	8	8
5	10	13
6	4	8
7	9	6
8	5	1
9	1	1
10	-	-
11	-	-
12	-	-
N	50	50
agreement	72%	
CV	9%	
bias	-0.26	
APE	8%	



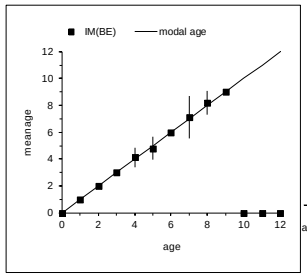
Age	modal	JP(DK)
0	-	-
1	3	4
2	8	7
3	2	3
4	8	7
5	10	11
6	4	5
7	9	10
8	5	-
9	1	3
10	-	-
11	-	-
12	-	-
N	50	50
agreement	70%	
CV	12%	
bias	-0.12	
APE	7%	



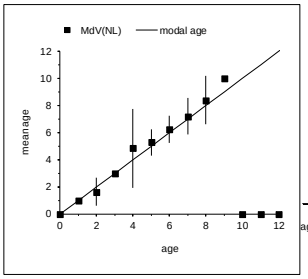
Age	modal	0
0	-	-
1	-	-
2	-	-
3	-	-
4	-	-
5	-	-
6	-	-
7	-	-
8	-	-
9	-	-
10	-	-
11	-	-
12	-	-
N	0	0
agreement		
CV		
bias		
APE		



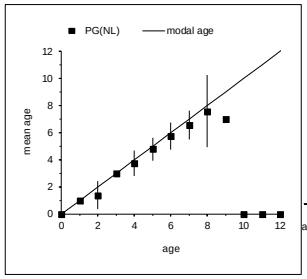
Age	modal	MM(BE)
0	-	-
1	3	3
2	8	7
3	2	3
4	8	4
5	10	8
6	4	10
7	9	8
8	5	4
9	1	2
10	-	1
11	-	-
12	-	-
N	50	50
agreement	72%	
CV	10%	
bias	0.30	
APE	8%	



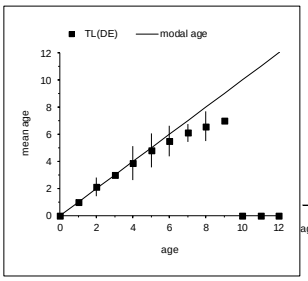
Age	modal	IM(BE)
0	-	-
1	3	3
2	8	8
3	2	2
4	8	9
5	10	9
6	4	5
7	9	7
8	5	4
9	1	3
10	-	-
11	-	-
12	-	-
N	50	50
agreement	88%	
CV	6%	
bias	0.02	
APE	6%	



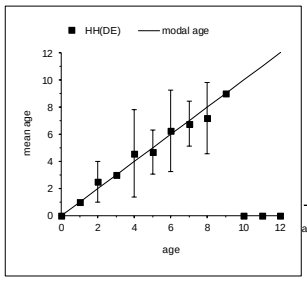
Age	modal	MdV(NL)
0	-	-
1	3	6
2	8	5
3	2	2
4	7	4
5	10	9
6	4	6
7	9	9
8	5	5
9	1	1
10	-	2
11	-	-
12	-	-
N	49	49
agreement	73%	
CV	15%	
bias	0.24	
APE	9%	



Age	modal	PG(NL)
0	-	-
1	3	8
2	8	3
3	2	4
4	8	8
5	10	9
6	4	8
7	9	8
8	5	-
9	1	2
10	-	-
11	-	-
12	-	-
N	50	50
agreement	60%	
CV	14%	
bias	-0.36	
APE	12%	

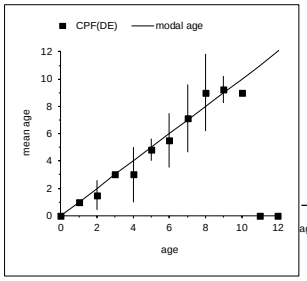


Age	modal	TL(DE)
0	-	-
1	3	3
2	8	7
3	2	5
4	8	8
5	10	9
6	4	13
7	9	5
8	5	-
9	1	-
10	-	-
11	-	-
12	-	-
N	50	50
agreement	52%	
CV	11%	
bias	-0.42	
APE	11%	

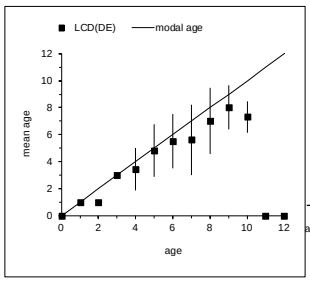


Age	modal	HH(DE)
0	-	-
1	3	3
2	8	5
3	2	5
4	7	10
5	10	6
6	4	8
7	9	5
8	5	5
9	1	2
10	-	-
11	-	-
12	-	-
N	49	49
agreement	45%	
CV	20%	
bias	0.00	
APE	16%	

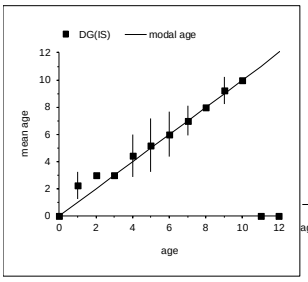
Annex 7: Exercise 2 – sectioned otoliths



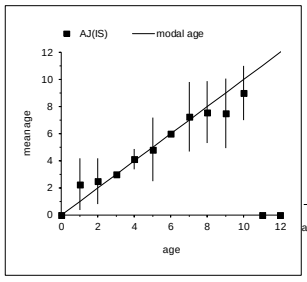
Age	modal	CPF(DE)
0	-	-
1	4	7
2	6	6
3	3	4
4	7	5
5	6	5
6	4	5
7	8	5
8	5	3
9	4	6
10	3	3
11	-	1
12	-	-
N	50	50
agreement	64%	
CV	16%	
bias	-0.18	
APE	14%	



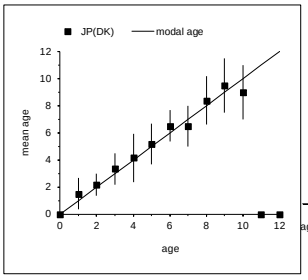
Age	modal	LCD(DE)
0	-	-
1	4	10
2	6	1
3	3	7
4	7	5
5	6	7
6	4	7
7	8	7
8	5	5
9	4	1
10	3	-
11	-	-
12	-	-
N	50	50
agreement	46%	
CV	14%	
bias	-0.82	
APE	20%	



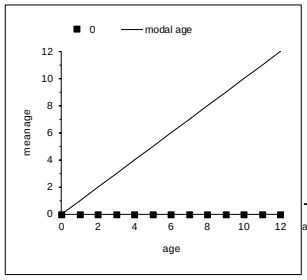
Age	modal	DG(IS)
0	-	-
1	4	-
2	6	3
3	3	10
4	7	6
5	6	6
6	4	4
7	8	8
8	5	6
9	4	3
10	3	4
11	-	-
12	-	-
N	50	50
agreement	62%	
CV	9%	
bias	0.32	
APE	19%	



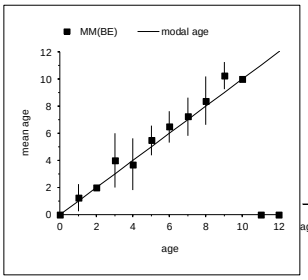
Age	modal	AJ(IS)
0	-	-
1	4	1
2	6	5
3	3	6
4	7	10
5	6	3
6	4	8
7	8	7
8	5	5
9	4	3
10	3	2
11	-	-
12	-	-
N	50	50
agreement	56%	
CV	18%	
bias	-0.02	
APE	16%	



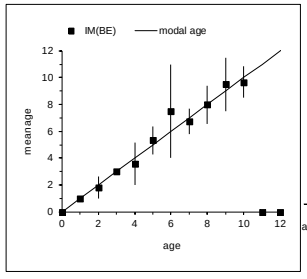
Age	modal	JP(DK)
0	-	-
1	4	2
2	6	7
3	3	4
4	7	7
5	6	4
6	4	7
7	8	7
8	5	5
9	4	4
10	3	2
11	-	1
12	-	-
N	50	50
agreement	64%	
CV	16%	
bias	0.10	
APE	10%	



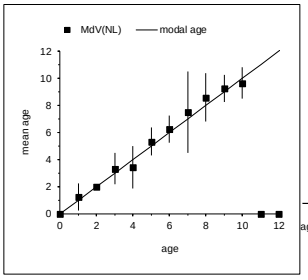
Age	modal	0
0	-	-
1	-	-
2	-	-
3	-	-
4	-	-
5	-	-
6	-	-
7	-	-
8	-	-
9	-	-
10	-	-
11	-	-
12	-	-
N	0	0
agreement		
CV		
bias		
APE		



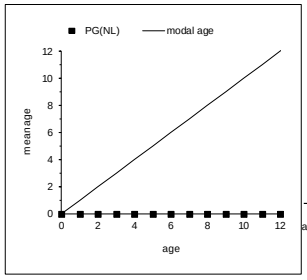
Age	modal	MM(BE)
0	-	-
1	4	3
2	6	8
3	3	2
4	7	5
5	6	5
6	4	5
7	8	9
8	5	4
9	4	1
10	3	7
11	-	1
12	-	-
N	50	50
agreement	66%	
CV	13%	
bias	0.32	
APE	11%	



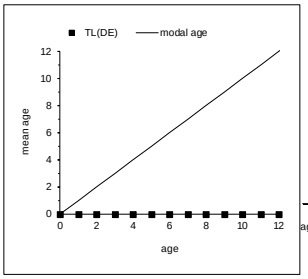
Age	modal	IM(BE)
0	-	-
1	4	5
2	6	6
3	3	4
4	7	5
5	6	4
6	4	5
7	8	9
8	5	3
9	4	5
10	3	3
11	-	1
12	-	-
N	50	50
agreement	72%	
CV	12%	
bias	0.06	
APE	12%	



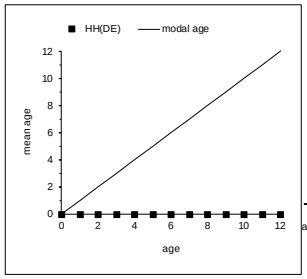
Age	modal	MdV(NL)
0	-	-
1	4	3
2	6	8
3	3	4
4	7	5
5	6	4
6	4	6
7	8	6
8	5	4
9	4	5
10	3	4
11	-	1
12	-	-
N	50	50
agreement	70%	
CV	14%	
bias	0.16	
APE	10%	



Age	modal	PG(NL)
0	-	-
1	-	-
2	-	-
3	-	-
4	-	-
5	-	-
6	-	-
7	-	-
8	-	-
9	-	-
10	-	-
11	-	-
12	-	-
N	0	0
agreement		
CV		
bias		
APE		

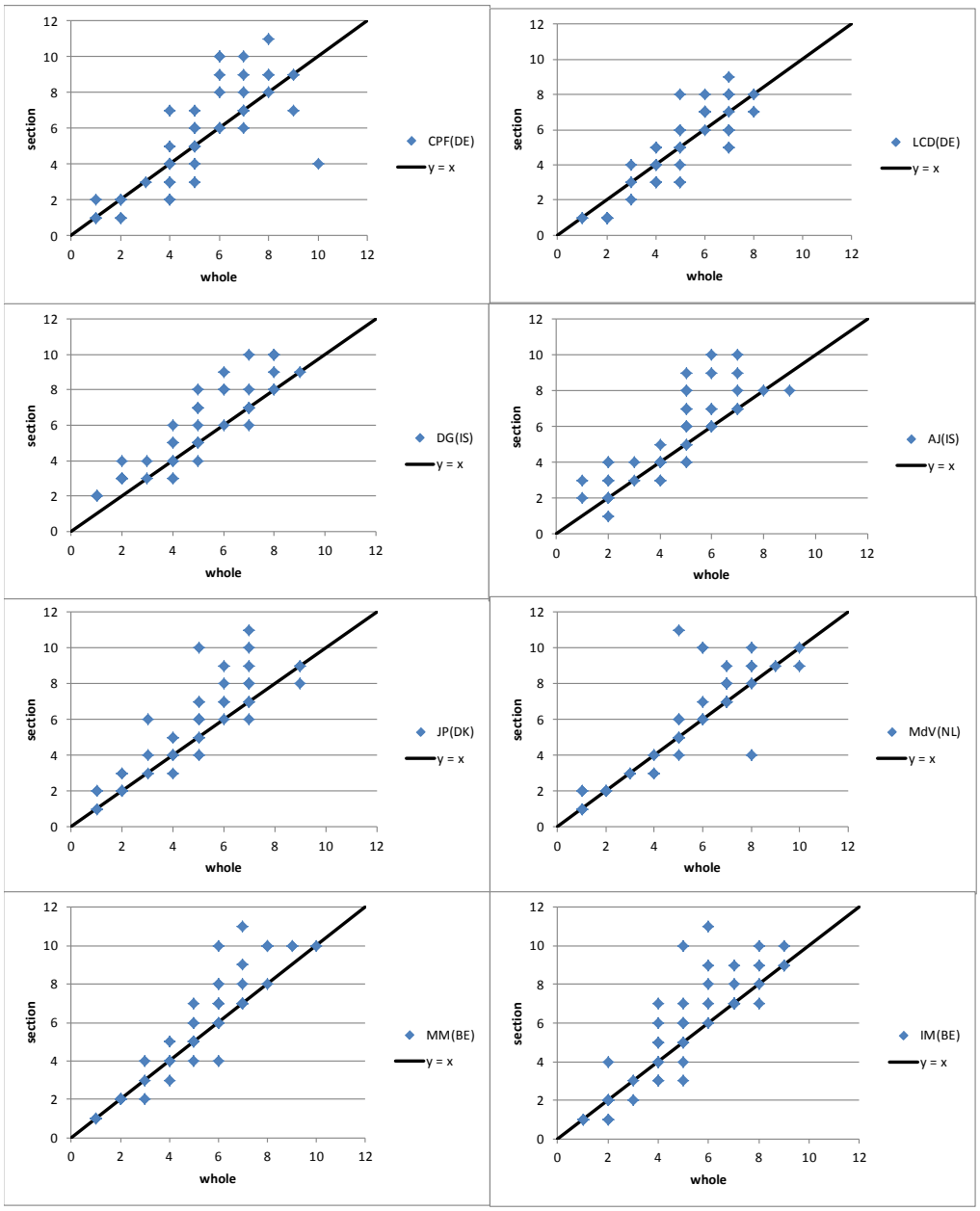


Age	modal	TL(DE)
0	-	-
1	-	-
2	-	-
3	-	-
4	-	-
5	-	-
6	-	-
7	-	-
8	-	-
9	-	-
10	-	-
11	-	-
12	-	-
N	0	0
agreement		
CV		
bias		
APE		

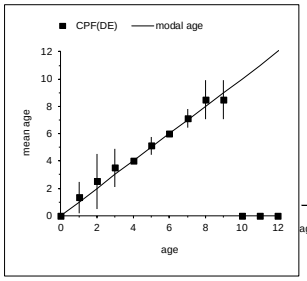


Age	modal	HH(DE)
0	-	-
1	-	-
2	-	-
3	-	-
4	-	-
5	-	-
6	-	-
7	-	-
8	-	-
9	-	-
10	-	-
11	-	-
12	-	-
N	0	0
agreement		
CV		
bias		
APE		

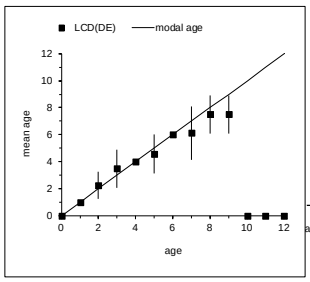
Annex 8: Exercise 2 – whole vs. sectioned otoltihs



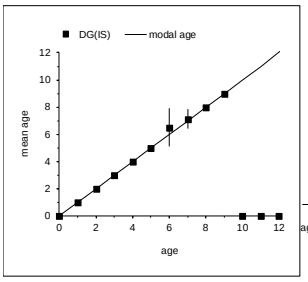
Annex 9: Exercise 3 – whole otoliths



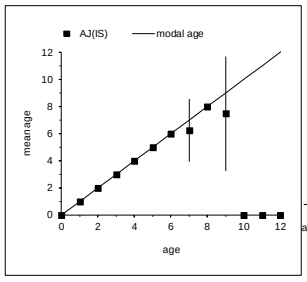
Age	modal	CPF(DE)
0	-	-
1	3	2
2	4	4
3	2	1
4	2	4
5	9	8
6	2	3
7	8	7
8	2	3
9	2	2
10	-	-
11	-	-
12	-	-
N	34	34
agreement	79%	
CV	14%	
bias	0.18	
APE	9%	



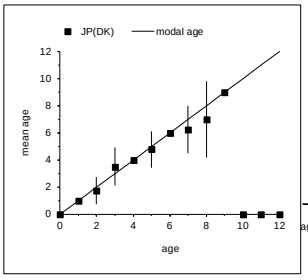
Age	modal	LCD(DE)
0	-	-
1	3	3
2	4	3
3	2	3
4	2	6
5	9	6
6	2	6
7	8	5
8	2	2
9	2	-
10	-	-
11	-	-
12	-	-
N	34	34
agreement	62%	
CV	13%	
bias	-0.38	
APE	10%	



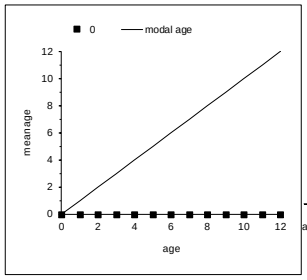
Age	modal	DG(IS)
0	-	-
1	3	3
2	4	4
3	2	2
4	2	2
5	9	9
6	2	1
7	8	8
8	2	3
9	2	2
10	-	-
11	-	-
12	-	-
N	34	34
agreement	94%	
CV	2%	
bias	0.06	
APE	7%	



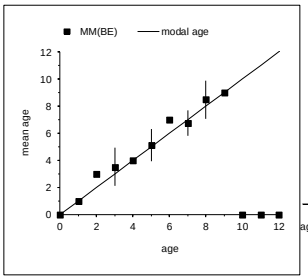
Age	modal	AJ(IS)
0	-	-
1	3	3
2	4	4
3	2	2
4	2	3
5	9	10
6	2	4
7	8	5
8	2	2
9	2	1
10	-	-
11	-	-
12	-	-
N	34	34
agreement	88%	
CV	6%	
bias	-0.26	
APE	8%	



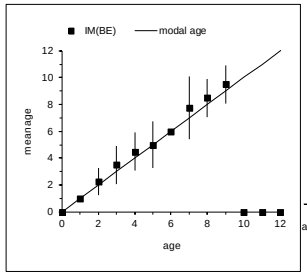
Age	modal	JP(DK)
0	-	-
1	3	4
2	4	3
3	2	1
4	2	6
5	9	7
6	2	6
7	8	4
8	2	1
9	2	2
10	-	-
11	-	-
12	-	-
N	34	34
agreement	68%	
CV	13%	
bias	-0.29	
APE	10%	



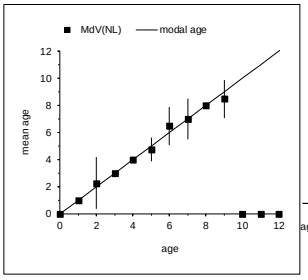
Age	modal	0
0	-	-
1	-	-
2	-	-
3	-	-
4	-	-
5	-	-
6	-	-
7	-	-
8	-	-
9	-	-
10	-	-
11	-	-
12	-	-
N	0	0
agreement		
CV		
bias		
APE		



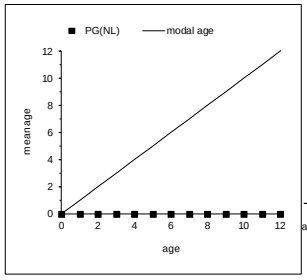
Age	modal	MM(BE)
0	-	-
1	3	3
2	4	-
3	2	5
4	2	4
5	9	6
6	2	4
7	8	8
8	2	1
9	2	3
10	-	-
11	-	-
12	-	-
N	34	34
agreement	62%	
CV	6%	
bias	0.21	
APE	10%	



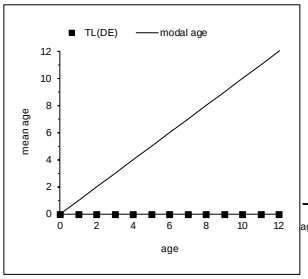
Age	modal	IM(BE)
0	-	-
1	3	3
2	4	3
3	2	2
4	2	4
5	9	7
6	2	3
7	8	4
8	2	2
9	2	5
10	-	1
11	-	-
12	-	-
N	34	34
agreement	62%	
CV	14%	
bias	0.32	
APE	10%	



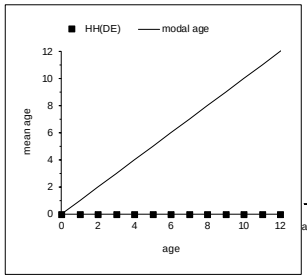
Age	modal	MdV(NL)
0	-	-
1	3	4
2	4	1
3	2	4
4	2	4
5	9	7
6	2	3
7	8	5
8	2	5
9	2	1
10	-	-
11	-	-
12	-	-
N	34	34
agreement	68%	
CV	11%	
bias	-0.03	
APE	8%	



Age	modal	PG(NL)
0	-	-
1	-	-
2	-	-
3	-	-
4	-	-
5	-	-
6	-	-
7	-	-
8	-	-
9	-	-
10	-	-
11	-	-
12	-	-
N	0	0
agreement		
CV		
bias		
APE		

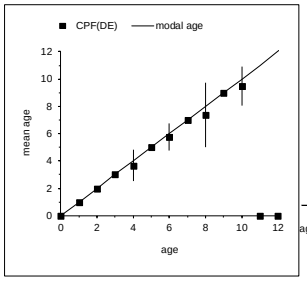


Age	modal	TL(DE)
0	-	-
1	-	-
2	-	-
3	-	-
4	-	-
5	-	-
6	-	-
7	-	-
8	-	-
9	-	-
10	-	-
11	-	-
12	-	-
N	0	0
agreement		
CV		
bias		
APE		

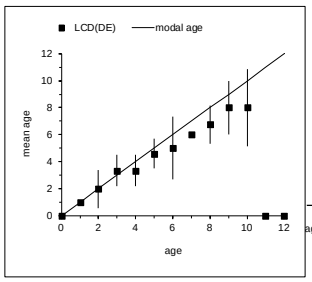


Age	modal	HH(DE)
0	-	-
1	-	-
2	-	-
3	-	-
4	-	-
5	-	-
6	-	-
7	-	-
8	-	-
9	-	-
10	-	-
11	-	-
12	-	-
N	0	0
agreement		
CV		
bias		
APE		

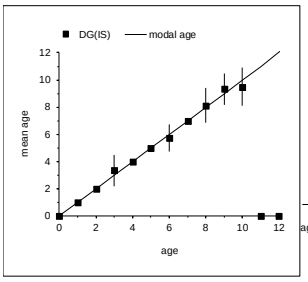
Annex 10: Exercise 3 – sectioned otoliths



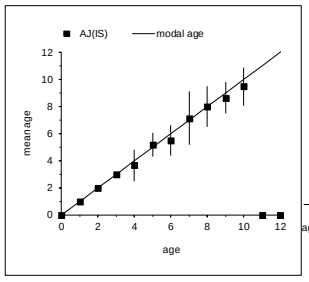
Age	modal	CPF(DE)
0	-	-
1	3	3
2	5	5
3	3	4
4	3	2
5	5	7
6	4	3
7	6	9
8	8	3
9	3	5
10	2	1
11	-	-
12	-	-
N	42	42
agreement	81%	
CV	5%	
bias	-0.19	
APE	6%	



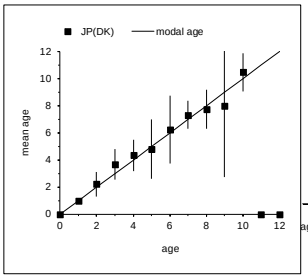
Age	modal	LCD(DE)
0	-	-
1	3	4
2	5	3
3	3	5
4	3	6
5	5	3
6	4	11
7	6	6
8	8	2
9	3	2
10	2	-
11	-	-
12	-	-
N	42	42
agreement	38%	
CV	14%	
bias	-0.71	
APE	13%	



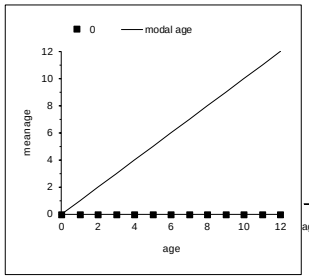
Age	modal	DG(IS)
0	-	-
1	3	3
2	5	5
3	3	2
4	3	4
5	5	6
6	4	3
7	6	7
8	8	5
9	3	5
10	2	2
11	-	-
12	-	-
N	42	42
agreement	83%	
CV	4%	
bias	0.02	
APE	5%	



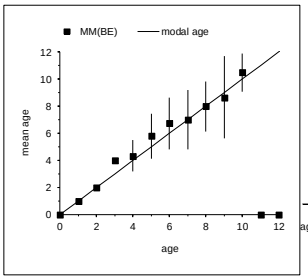
Age	modal	AJ(IS)
0	-	-
1	3	3
2	5	5
3	3	4
4	3	2
5	5	6
6	4	4
7	6	6
8	8	5
9	3	6
10	2	1
11	-	-
12	-	-
N	42	42
agreement	71%	
CV	8%	
bias	-0.07	
APE	6%	



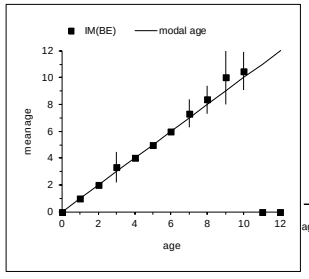
Age	modal	JP(DK)
0	-	-
1	3	3
2	5	4
3	3	3
4	3	4
5	5	6
6	4	3
7	6	7
8	8	7
9	3	2
10	2	2
11	-	1
12	-	-
N	42	42
agreement	60%	
CV	15%	
bias	0.05	
APE	9%	



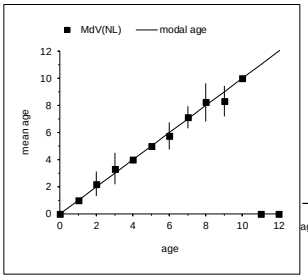
Age	modal	0
0	-	-
1	-	-
2	-	-
3	-	-
4	-	-
5	-	-
6	-	-
7	-	-
8	-	-
9	-	-
10	-	-
11	-	-
12	-	-
N	0	0
agreement		
CV		
bias		
APE		



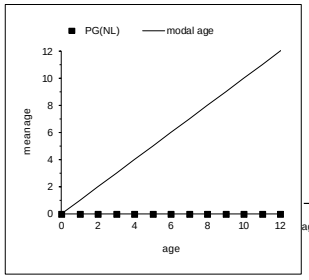
Age	modal	MM(BE)
0	-	-
1	3	3
2	5	5
3	3	-
4	3	5
5	5	4
6	4	5
7	6	6
8	8	8
9	3	3
10	2	2
11	-	1
12	-	-
N	42	42
agreement	57%	
CV	10%	
bias	0.26	
APE	9%	



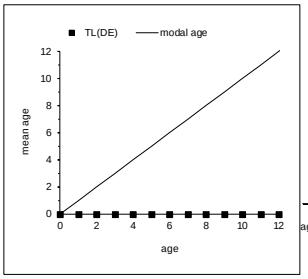
Age	modal	IM(BE)
0	-	-
1	3	3
2	5	5
3	3	2
4	3	4
5	5	5
6	4	4
7	6	4
8	8	7
9	3	4
10	2	2
11	-	2
12	-	-
N	42	42
agreement	79%	
CV	4%	
bias	0.24	
APE	6%	



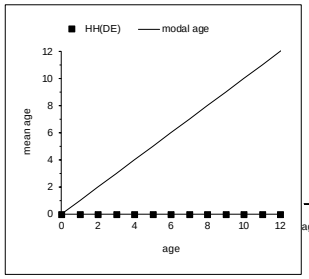
Age	modal	MdV(NL)
0	-	-
1	3	3
2	5	4
3	3	3
4	3	4
5	5	6
6	4	3
7	6	6
8	8	7
9	3	4
10	2	2
11	-	-
12	-	-
N	42	42
agreement	76%	
CV	7%	
bias	0.05	
APE	7%	



Age	modal	PG(NL)
0	-	-
1	-	-
2	-	-
3	-	-
4	-	-
5	-	-
6	-	-
7	-	-
8	-	-
9	-	-
10	-	-
11	-	-
12	-	-
N	0	0
agreement		
CV		
bias		
APE		



Age	modal	TL(DE)
0	-	-
1	-	-
2	-	-
3	-	-
4	-	-
5	-	-
6	-	-
7	-	-
8	-	-
9	-	-
10	-	-
11	-	-
12	-	-
N	0	0
agreement		
CV		
bias		
APE		



Age	modal	HH(DE)
0	-	-
1	-	-
2	-	-
3	-	-
4	-	-
5	-	-
6	-	-
7	-	-
8	-	-
9	-	-
10	-	-
11	-	-
12	-	-
N	0	0
agreement		
CV		
bias		
APE		

Annex 11: Ageing manual

Following guidelines can be used for reading both whole otoliths and (stained) sectioned otoliths.

- Be careful with cranking up the magnification, this might show too much detail. For older animals a higher magnification can be useful for looking at the edges of the otolith.
- Start in the middle of the otolith, the nucleus (birth of the fish)
- Use more angles/axes from the middle to the side
- The birthday of the fish is set on 1st January
- A translucent ring on the edge of the otolith at the beginning of the year should be counted. In the middle of the year, the new opaque ring shows up. At the end of the year, a translucent ring on the edge should not be counted until January.
    - Discussion on the deposition of the new opaque ring can be found in section 2.3 of this report.
- Most age-readers count the translucent rings (see the remark about the light)
- It is preferable to use the clearest one of the two otoliths (if possible)
- The first ring can be very small but should be counted anyway.
- Difference between a split ring and two rings. A split ring is a false ring and should not be counted. A split ring is mostly a short line, mostly thinner compared to other rings. The split ring usually merges into another ring if you follow it along the otolith.
- If on one side of the otolith more rings are visible compared to the other side, then always take the oldest age, although you can't see all the rings all around the otolith.
- The opaque ring is visible earlier in the season for young fish compared to older fish.
- Whole otoliths and sectioned otoliths can be read with transmitted or reflected light. With reflected light the translucent ring will show up as a dark ring, with transmitted light the opaque ring will show up as a dark ring. If in doubt about the interpretation of a structure, switching from reflected light to transmitted light (or the other way around) may help.

Points of attention when reading (stained) sectioned otoliths.

- Be careful that the otolith is cut through the nucleus, otherwise the first ring can be missed.
- If the nucleus is not visible turn over the slide and see if it appears on the other side. If the nucleus is still not visible maybe it is possible to have a look at the 'block' where the section is cut from. This can be aged or an extra slide can be cut.
- In stained sectioned otoliths a narrow red band shows up. This band is located at the transition from translucent to opaque. In stained sections the distinction between opaque and translucent is less clear
- When stained, look for rings that are clear (dark red) and that you can see on most or at least half of the otolith.

- Also when stained, the first 2 rings are often not as clearly visible as the other rings.
- When in doubt about the first 1 or 2 rings, it is recommended to use the whole otolith (if available) as a backup.
- It is necessary to use oil or water on the sectioned otoliths to obtain a clear view, unless the otoliths are covered with resin and an extra glass plate. On stained sections it is less necessary to use oil or water. Some readers use oil or water, some don't.

Points of attention when reading whole otoliths.

- With regard to split rings: On the whole otolith, for the first 3–4 years, the rings should be visible all the way round.
- A whole otolith should be read wet. For certain regions (e.g. Iceland) it is recommended to keep the otoliths in water or soak the otoliths overnight in water.
- If in doubt, move the otolith so you can look at it in different angles and "over the edge". (This is not possible for whole otoliths embedded in resin.)

Annex 12: Reference collection – agreed age whole otoliths

Fish 1 – area IVbc, September

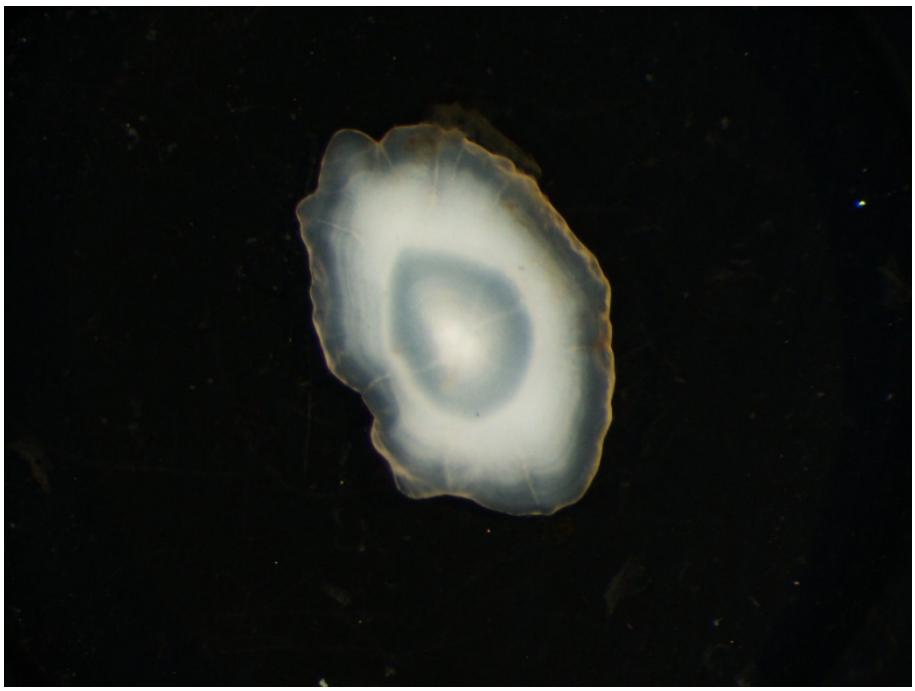


Figure A12_1: Whole otolith. Age 1 with 100% agreement. Dutch collection: DAB_NL_ex008.jpg. Male, 12.9cm, caught September 2013.

Fish 2 – area IVbc, September

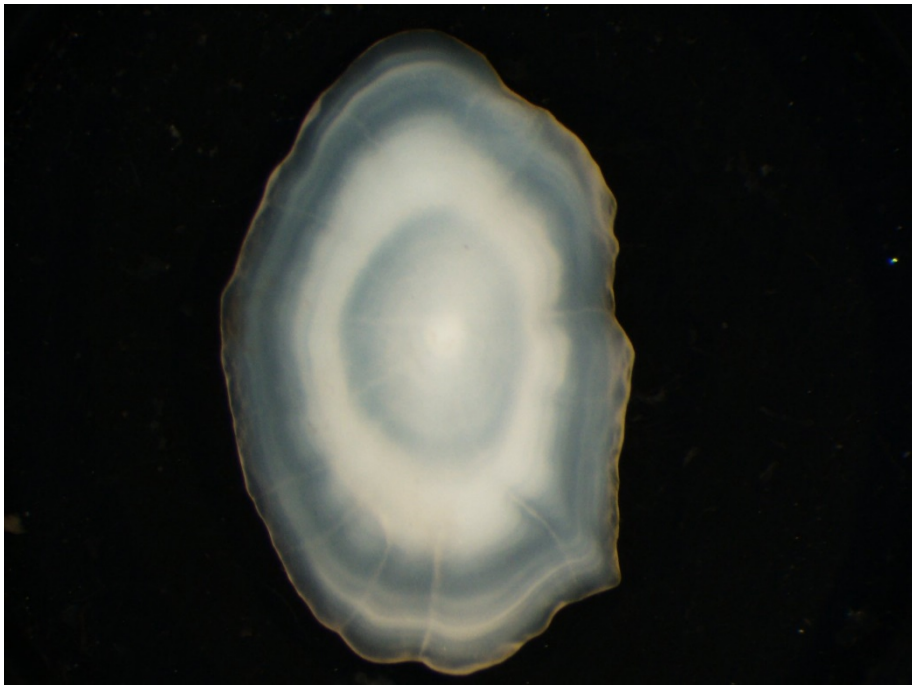


Figure A12_2: Whole otolith. Age 2 with 100% agreement. Dutch collection: DAB_NL_ex007.jpg. Female, 18.8cm, caught September 2013.

Fish 3 – area IVbc, January

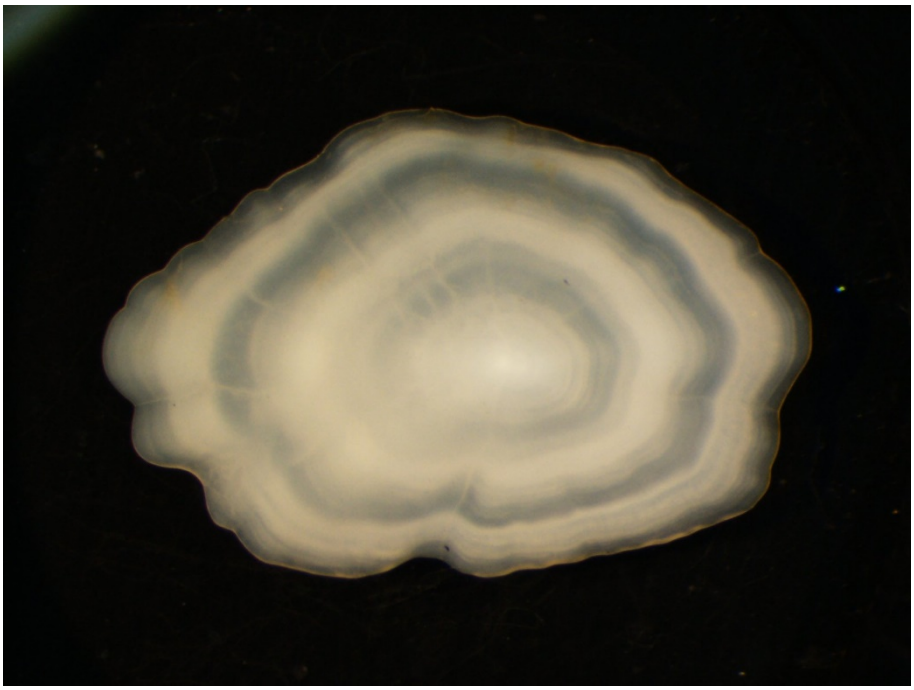


Figure A12_3: Whole otolith. Age 3 with 100% agreement. Dutch collection: DAB_NL_ex019.jpg. Female, 23.8cm, caught in January 2014.

Fish 4 – area IVbc, September

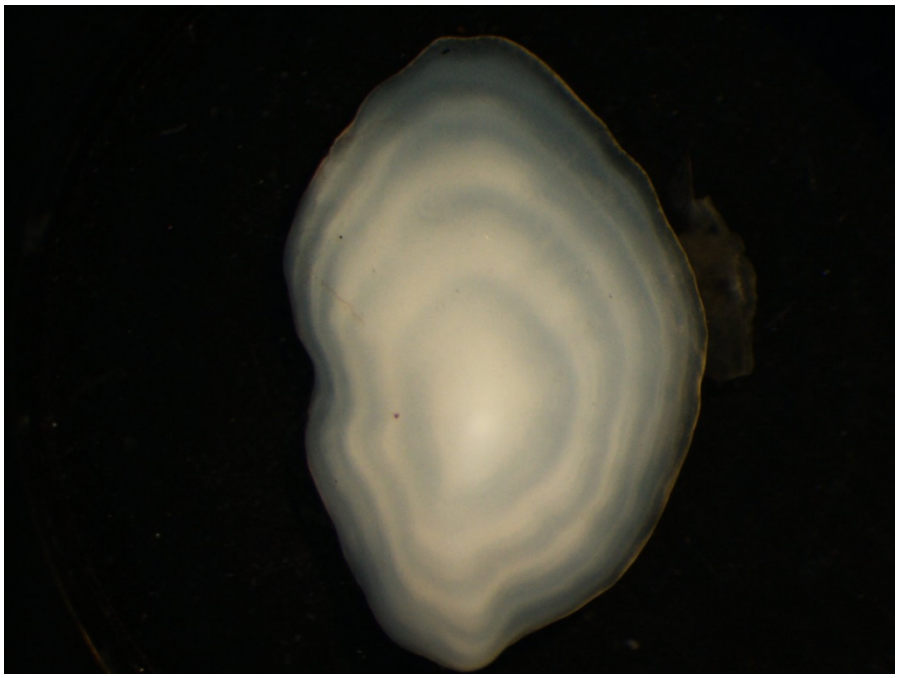


Figure A12_4: Whole otolith. Age 4 with 100% agreement. Dutch collection: DAB_NL_ex012.jpg. Male, 20.4cm, caught in September.

Fish 5 – area IVbc, January

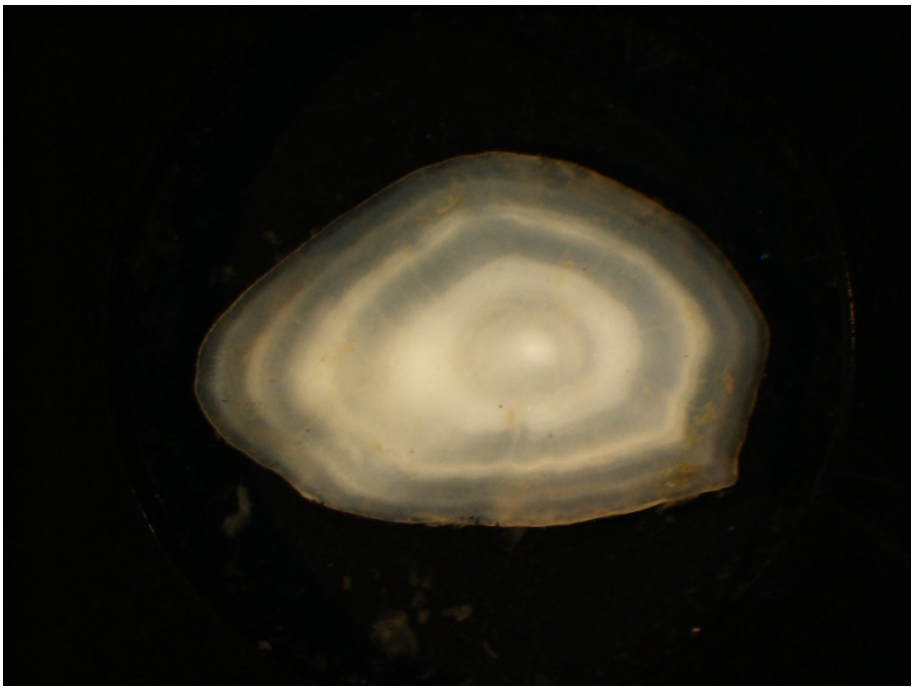


Figure A12_5: Whole otolith. Age 5 with 100% agreement. Dutch collection: DAB_NL_ex020.jpg. Female, 24.6cm, caught in January 2014.

Fish 6 – area IVbc, July

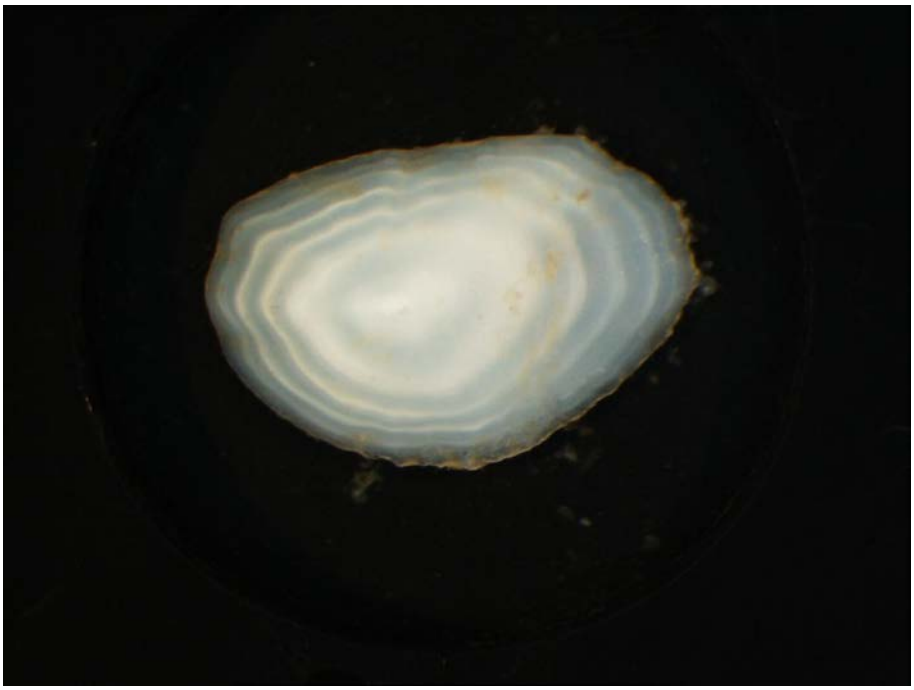


Figure A12_6: Whole otolith. Age 6 with 100% agreement. Dutch collection: DAB_NL_ex042.jpg. Female, 26.7cm, caught in July 2014.

Fish 7 – area IVbc, May

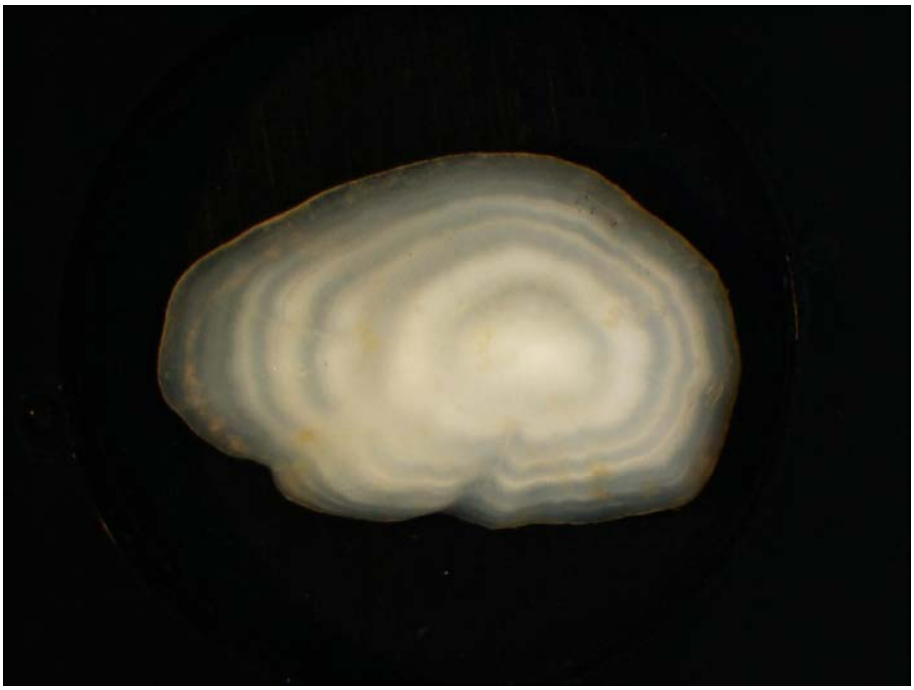


Figure A12_7: Whole otolith. Age 7 with 100% agreement. Dutch collection: DAB_NL_ex033.jpg. Female, 27.2cm, caught in May 2014.

Fish 8 – area IVb, July

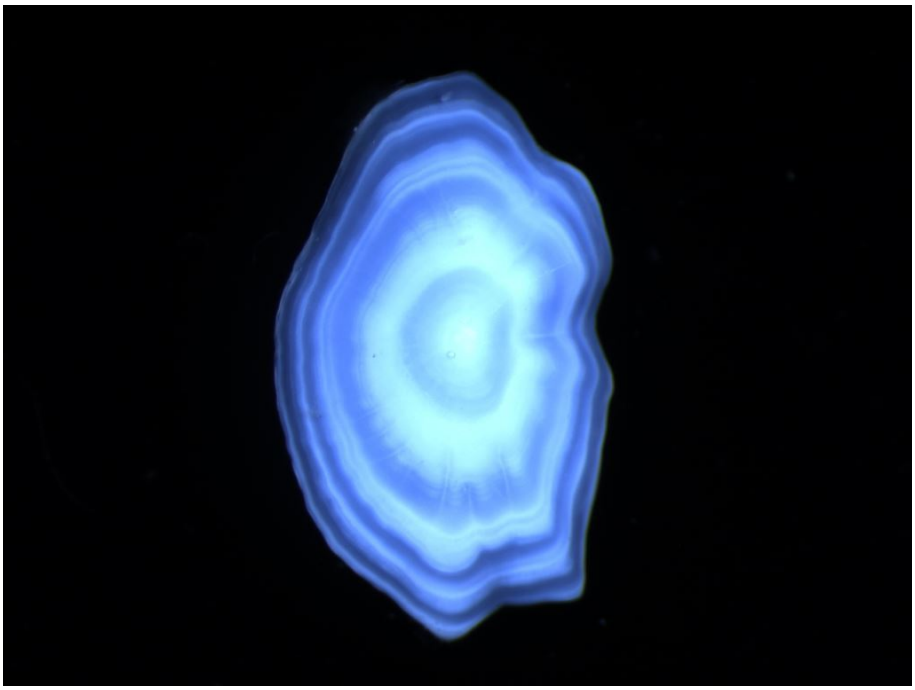


Figure A12_8: Whole otolith. Age 4 with 100% agreement. German collection: dab_EL_2014_Q3_M_002.jpg. Female, 25.5cm, caught in July.

Fish 9 – area IVb, July

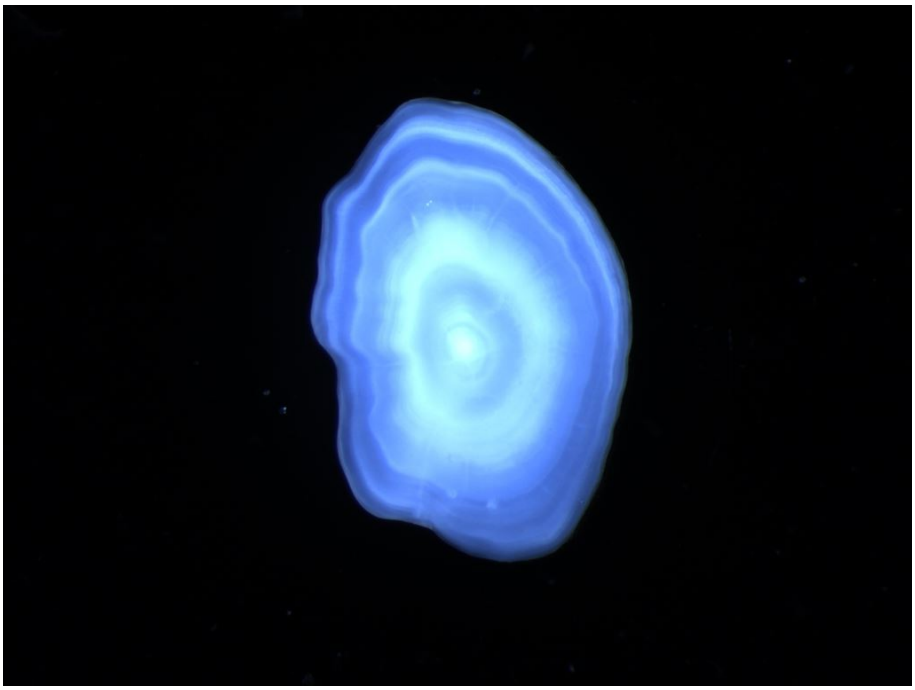


Figure A12_9: Whole otolith. Age 4 with 100% agreement. German collection: dab_EL_2104_Q3_M_010.jpg. Female, 24.5cm, caught in July.

Annex 13: Reference collection – agreed age sectioned otoliths

Fish 1 – area IVbc, September

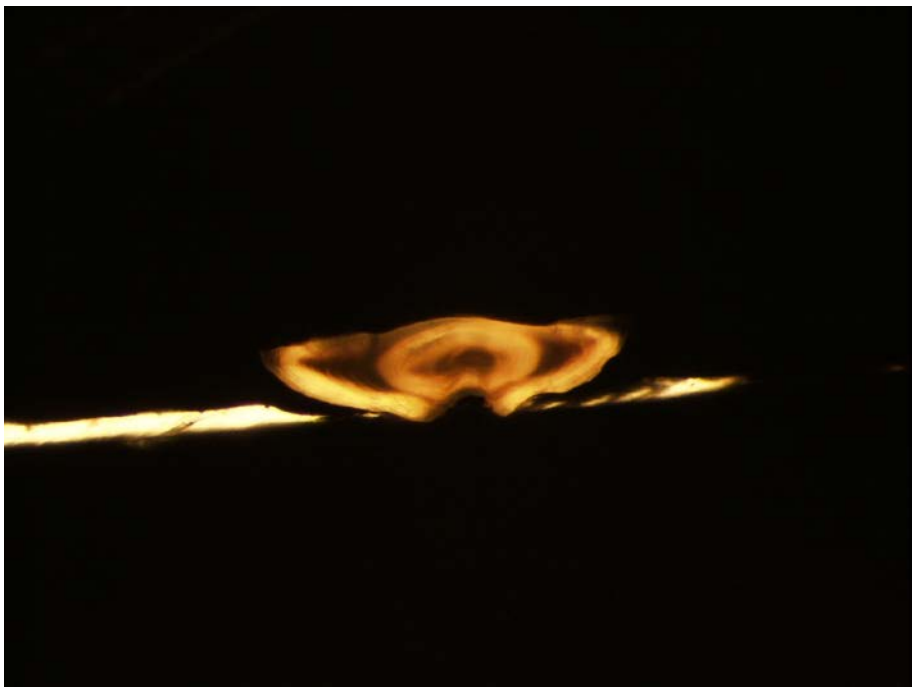


Figure A13_1: Section. Overall agreement of 88% with modal age 1. One reader assigned age 2. Dutch collection: DAB_NL_ex111. Female, 11.6cm, caught September 2013.

Fish 2 – area IVbc, September

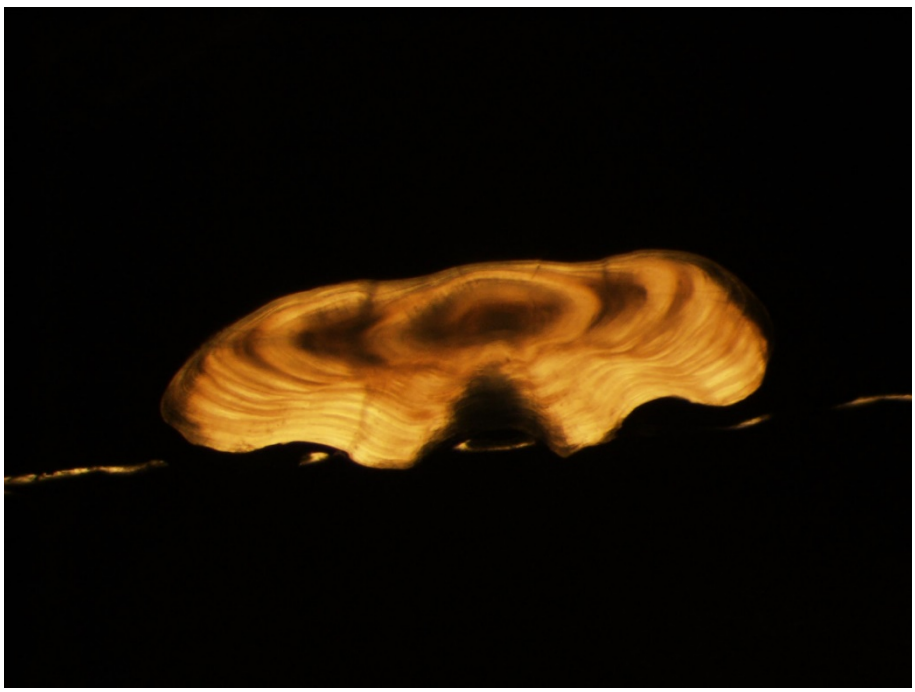


Figure A13_2: Section. Overall agreement 75% with modal age 8. Dutch collection: DAB_NL_ex116. Male, 22.8cm, caught in September 2013.

Fish 3 – area IVbc, January

Figure A13_3: Section. Overall agreement 100% on age 3. Dutch collection: DAB_NL_ex119. Female, 23.8cm, caught in January 2014.

Fish 4 – area IVbc, May

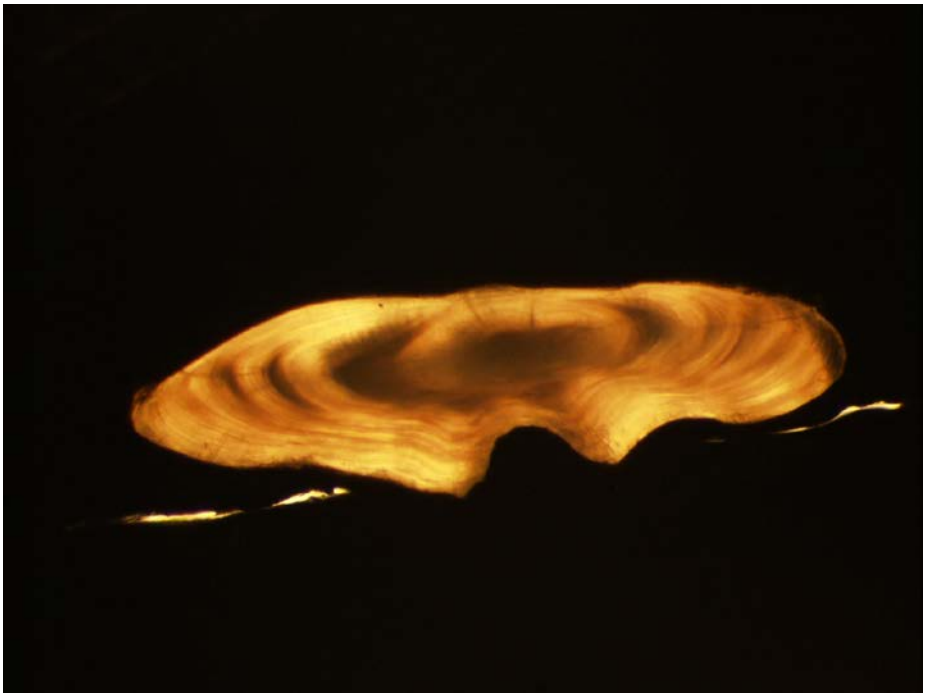


Figure A13_4: Section. Overall agreement of 75% with a modal age of 7. Two readers assigned age 6. Dutch collection: DAB_NL_ex133. Female, 27.2cm, caught in May 2014.

Fish 5 – area IVb, July

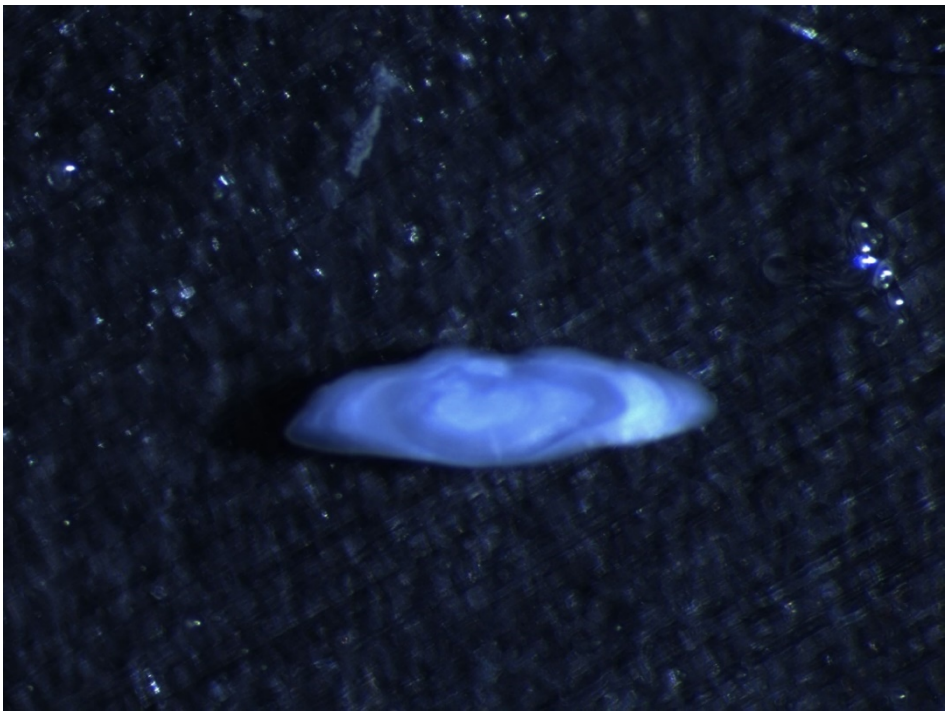


Figure A13_5: Sectioned otolith with 100% agreement on age 1. Male, 9.5cm, caught July 2014. File: dab_EL2014_Q3_s_116.jpg

Fish 6 – area IVb, July

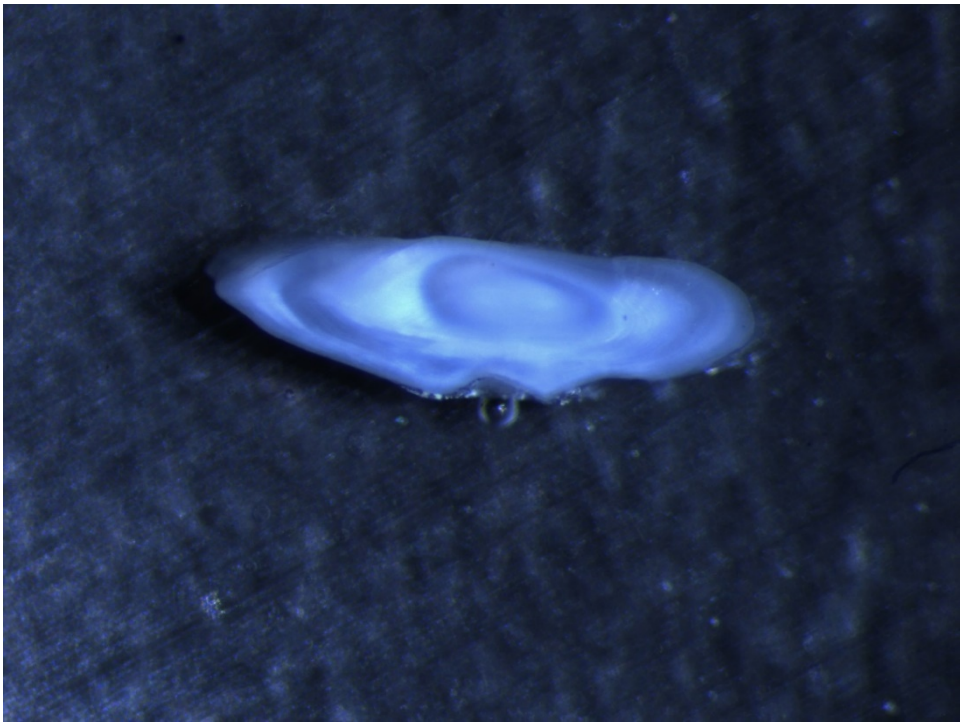


Figure A13_6: Sectioned otolith with 100% agreement on age 2. Female, 15.5cm, caught July 2014. File: dab_EL2014_Q3_s_109.jpg

Fish 7 – area IVb, July

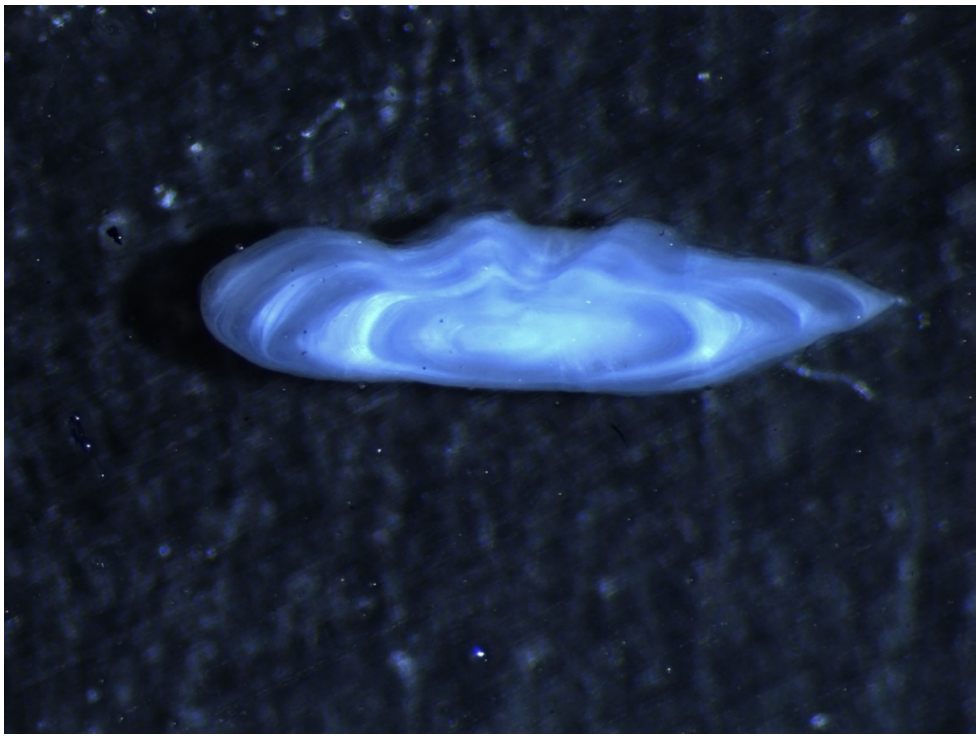


Figure A13_7: Sectioned otolith with 100% agreement on age 3. Female, 21.5cm, caught July 2014. File: dab_EL2014_Q3_s_122.jpg

Fish 8 – area IVb, July

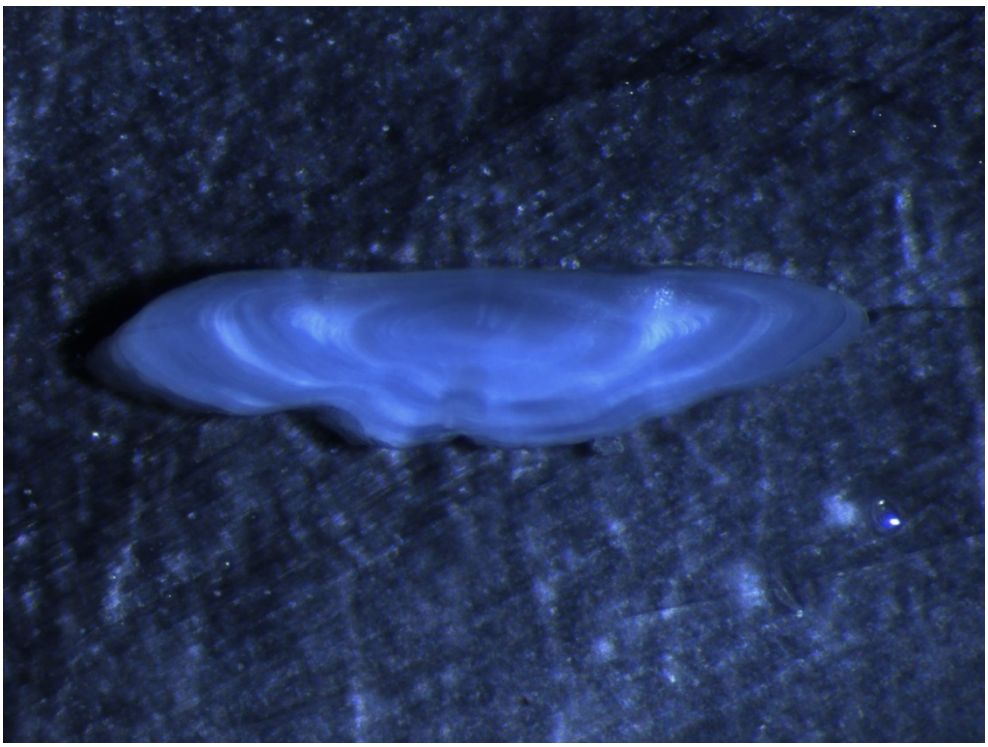


Figure A13_8: Sectioned otolith with 100% agreement on age 3. Female, 23.5cm, caught July 2014. File: dab_EL2014_Q3_s_023.jpg

Fish 9 – area IVb, July

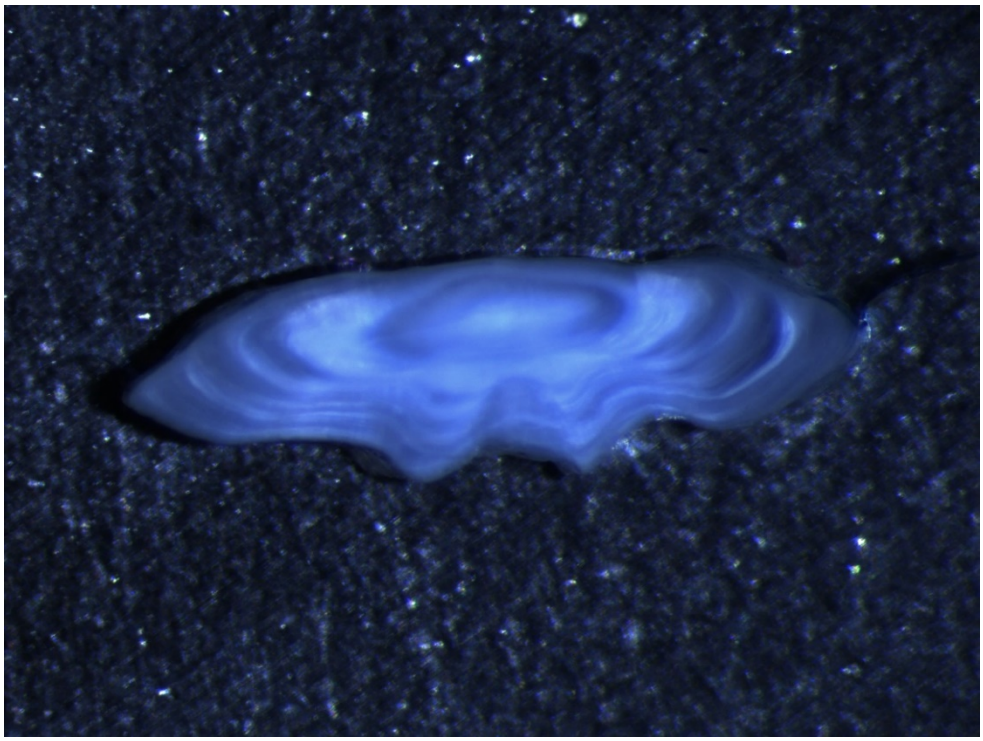


Figure A13_9: Sectioned otolith with 100% agreement on age 4. Female, 24.5cm, caught July 2014. File: dab_EL2014_Q3_M_s_014.jpg

Fish 10 – area IVb, July

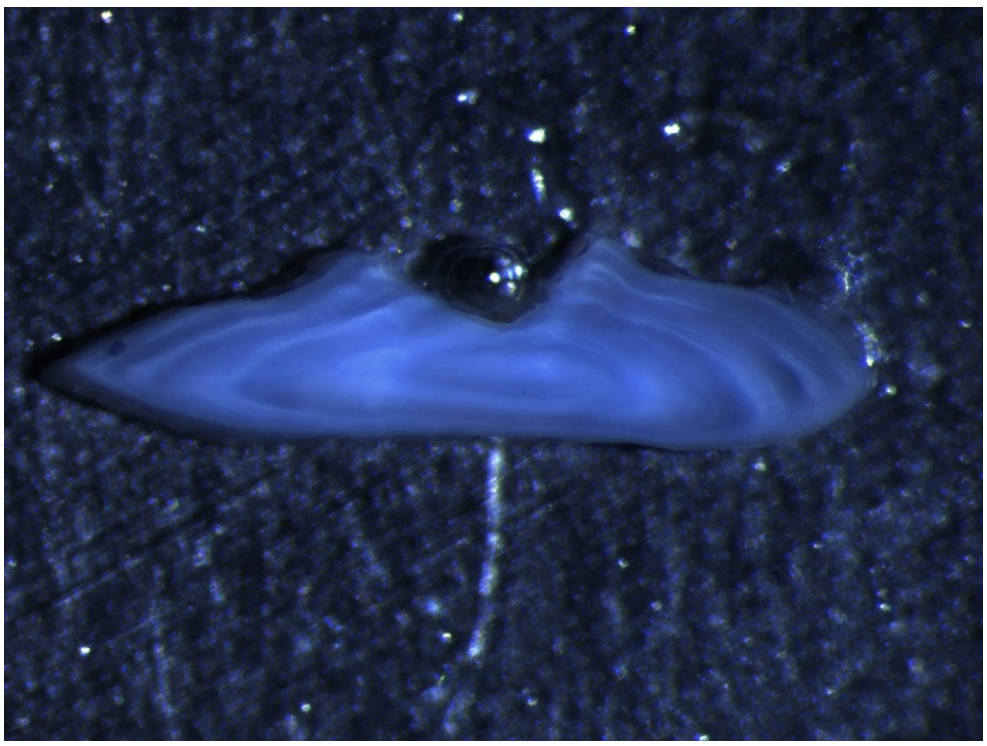


Figure A13_10: Sectioned otolith with 100% agreement on age 4. Female, 23.5cm, caught July 2014. File: dab_EL2014_Q3_M_s_017.jpg

Fish 11 – area IVb, July

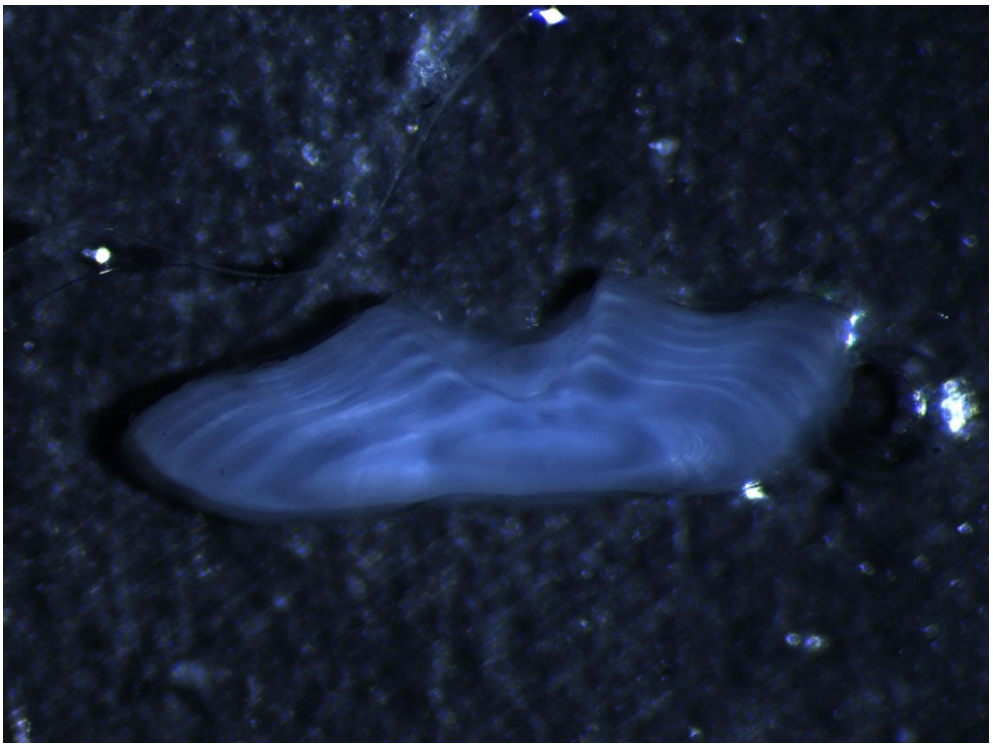


Figure A13_11: Sectioned otolith with 100% agreement on age 6. Female, 26.5cm, caught July 2014. File: dab_EL2014_Q3_M_s_013.jpg

Annex 14: Reference collection – age reading problems

Fish 1 – area IVb, month July

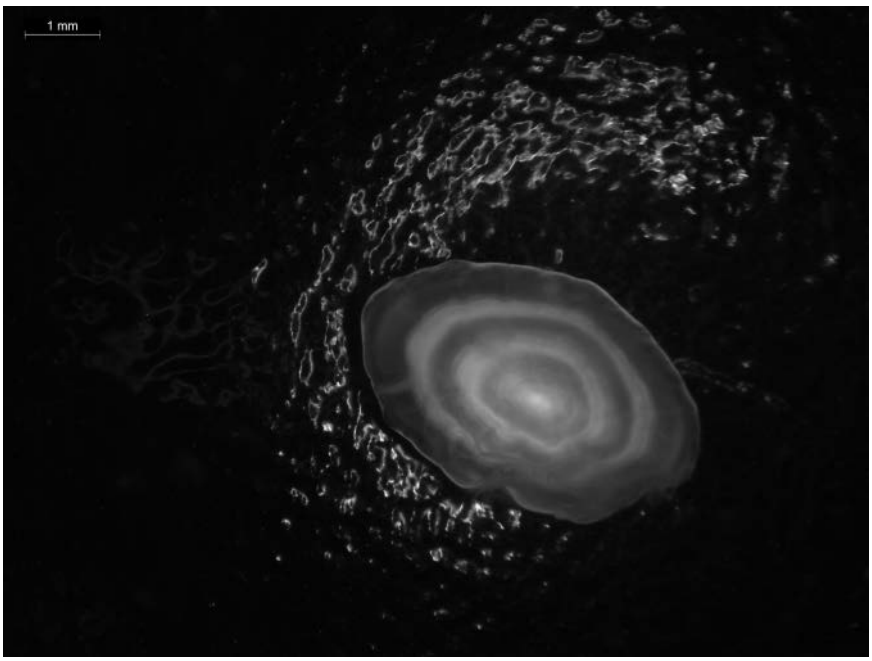


Figure A14_1a: Whole otolith. The edge is the problem in this whole otolith. It is like a big translucent ring with no obvious opaque ring(s). Most readers considered the vague opaque rings to be true rings. Most aged this otolith 4 years old, some aged it 5 years old. (Belgian collection: 05 CDDR05_DAB_4B_L_M_1.6_Wh_51850 05.tif).

Figure A14_1b: Stained section. In the section of the same otolith, the rings near the edge are clear. Most readers agreed that this otolith is 5 years old. (Belgian collection: 05 CDDR05_DAB_4B_L_M_3.2_S&S_51850 05.tif).

Fish 2 – area IVb, month July

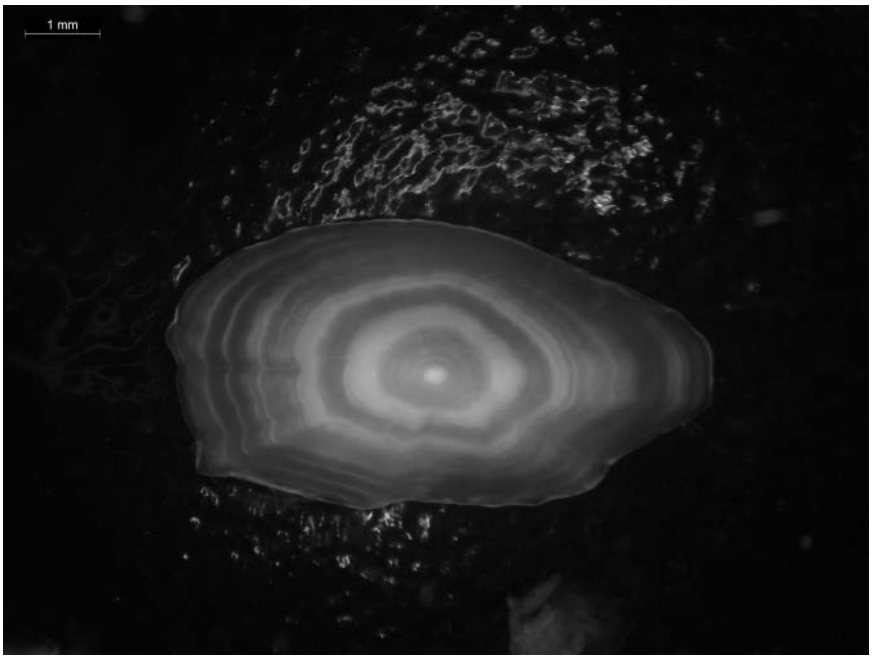


Figure A14_2a: Whole otolith. The whole otolith shows many split rings (several split rings close together are referred to as 'shadow' by some readers). Another problem is the edge, which appears to be translucent on the left and opaque on the right. The agreement was low for this otolith. (Belgian collection: 06 CDDR05_DAB_4B_L_M_1.6_Wh_51860 06.tif)

Figure A14_2b: Stained section. The stained section also shows split rings on the left side of the otolith, but not on the right side. Most age readers agreed on 7 years. (Belgian collection: 06 CDDR05_DAB_4B_L_M_3.2_S&S_51860 06.tif).

Fish 3 – area IVb, month July

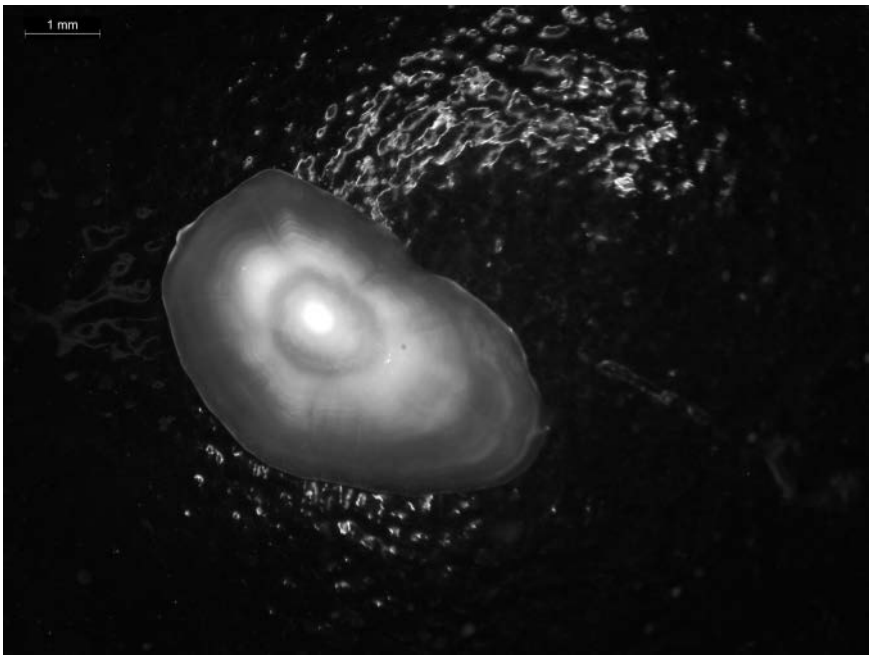


Figure A14_3a: Whole otolith. This whole otolith is a difficult one. The first translucent rings is obvious but the rings after that are hard to be sure of. Age readings ranged from 3–5. Some readers counted the translucent edge, others did not. Some counted a translucent ring within the broad opaque zone, others did not. (Belgian collection: 08 CDDR05_DAB_4B_L_M_1.6_Wh_51864 08.tif).

Figure A14_3b: Stained section. In the stained section, the rings from 2–6 are very clear, but it is hard to see the first ring. All age readers agreed that the first *clear* ring must be the second ring. They all agreed on age 6. (Belgian collection: 08 CDDR05_DAB_4B_L_M_3.2_S&S_51864 08.tif).

Fish 4 – area IVb, month July

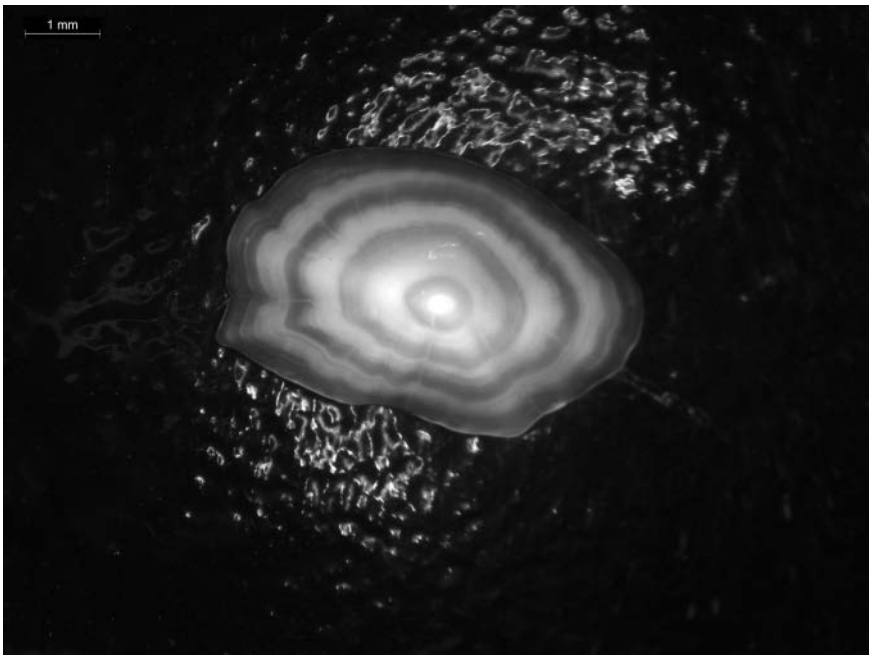


Figure A14_4a: Whole otolith. In the whole otolith there is uncertainty whether the rings near the edge are split rings, especially in the top left corner. Age determinations ranged from 5 to 7. (Belgian collection: 09 CDDR05_DAB_4B_L_M_1.6_Wh_51868 09.tif).

Figure A14_4b: Stained section. The sectioned otolith also shows split rings. The incomplete ring after the first true ring is a clear example of a split ring and should not be counted. Most age readers aged this otolith 5 years old. (Belgian collection: 09 CDDR05_DAB_4B_L_M_3.2_S&S_51868 09.tif).

Fish 5 – area IVb, month July

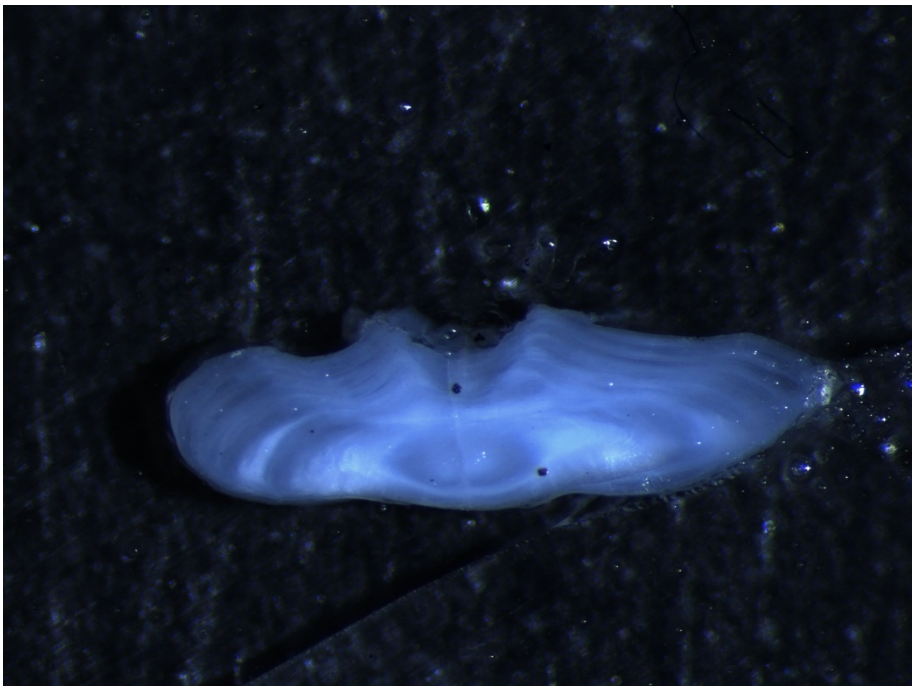


Figure A14_5a: Section. There was disagreement on whether or not to count a ring on the edge. Readers aged this otolith with age 5 or 6, one reader aged this otolith 7. The overall agreement was low with 50%. German collection: dab_EL_2014_Q3_s_100.jpg; Male, 21.5cm, caught in July.

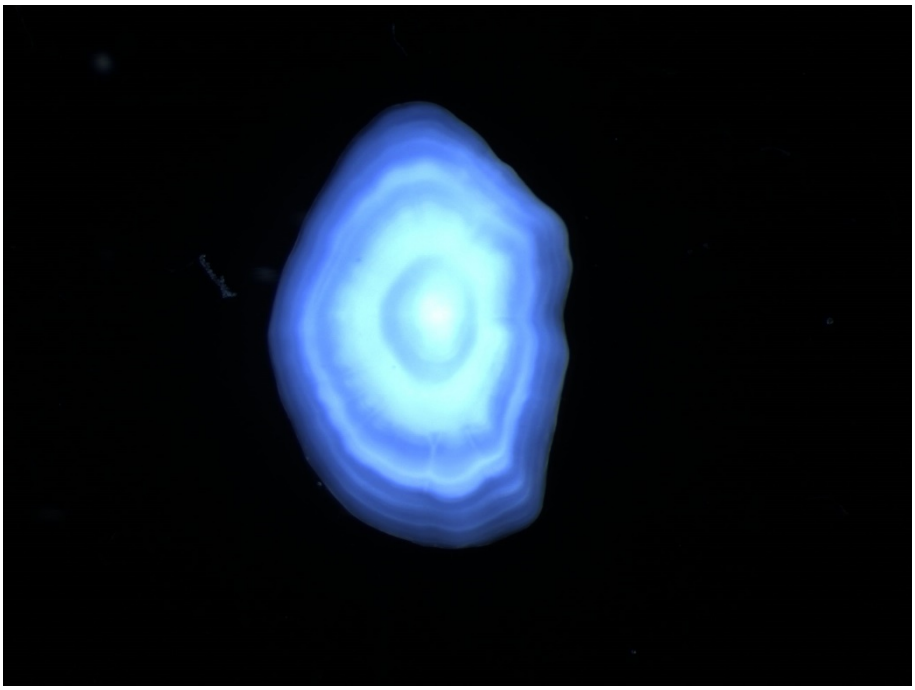


Figure A14_5b: Whole otolith. The edge is also the problem in the whole otolith. Readers aged this otolith with age 5 or 6. The overall agreement was low with 56%. German collection: dab_EL_2104_Q3_100.jpg; Male, 21.5cm, caught in July.

Fish 6 – area IV, July

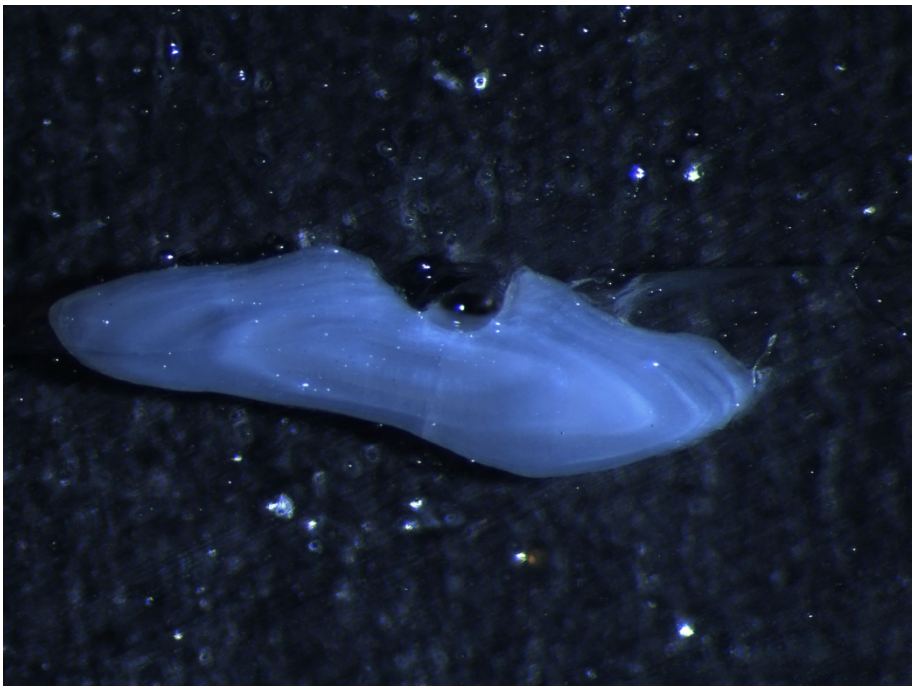


Figure A14_6a: Section. In this sectioned otolith the nucleus is not clear. The assigned age ranged from 3 to 5 with an overall agreement of 50%. Modal age was 5. German collection: dab_EL_2014_Q3_M_s_005.jpg. Female, 24.5cm, caught in July.

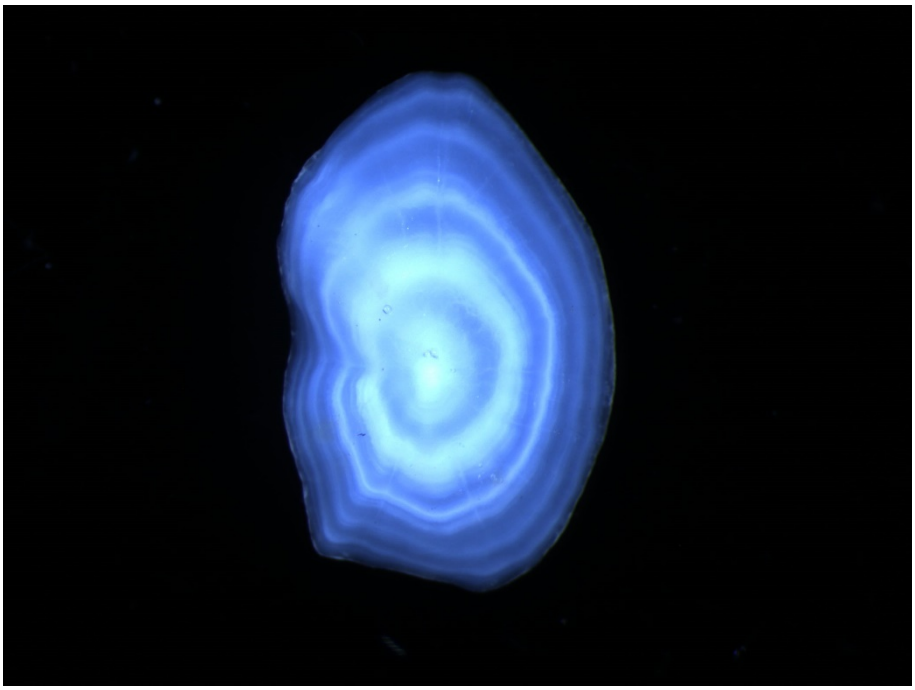


Figure A14_6b: Whole otolith. For the whole otolith the agreement was much higher with 78%. Readers assigned age 5 or age 6; modal age was also 5. German collection: dab_EL_2014_Q3_M_s_005.jpg. Female, 24.5cm, caught in July.

Fish 7 – area IV, July

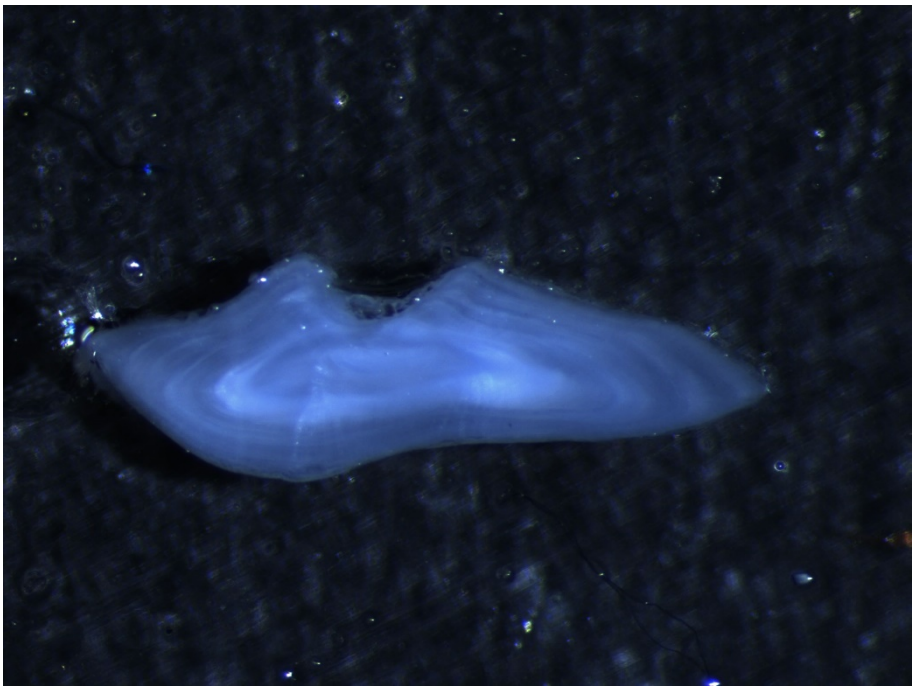


Figure A14_7a: Section. Another example where the edge of the otolith is the problem. Readers assigned age 5 or 6 to this otolith. The overall agreement was 50% and the modal age 5. German collection: dab_EL_2014_Q3_M_s_006.jpg. Female, 23.5cm, caught in July.

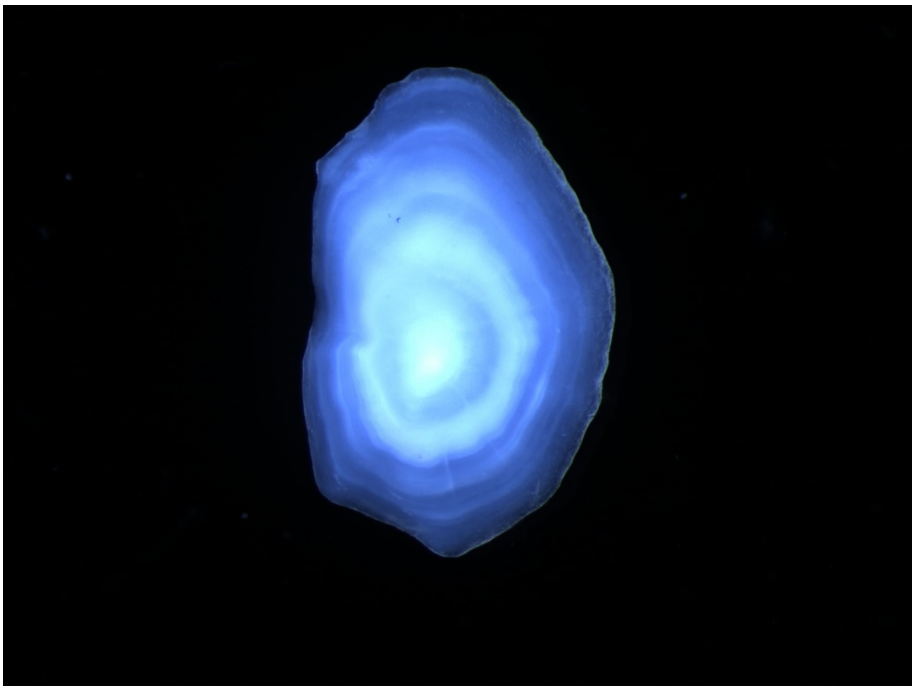


Figure A14_7b: Whole otolith. For the whole otolith the age ranged between 4–5 but with a higher agreement of 67% and a modal age of 4. German collection: dab_EL_2014_Q3_M_s_006.jpg. Female, 23.5cm, caught in July.

Fish 8 – area IVb, July

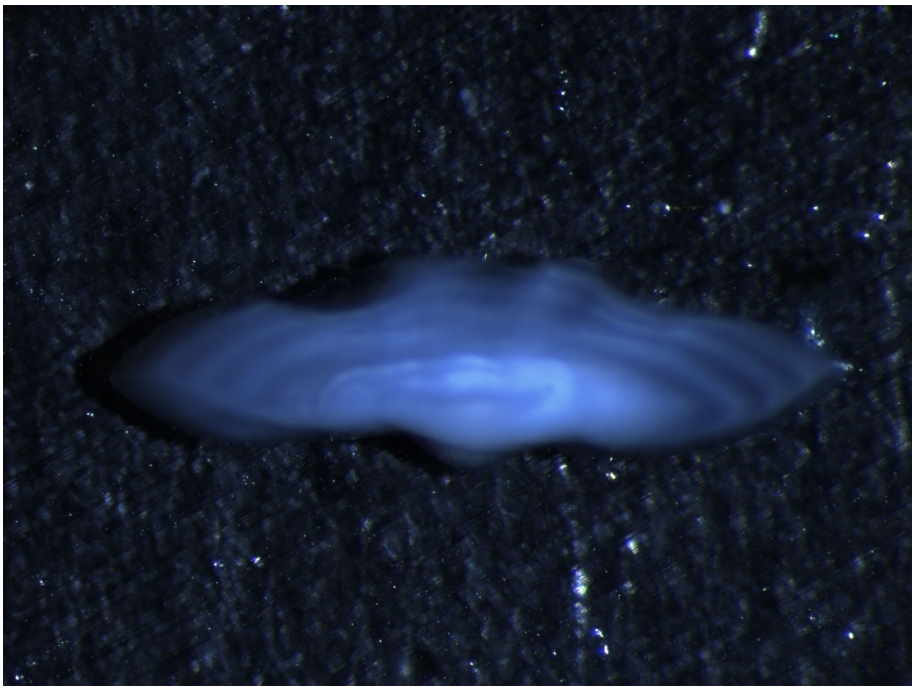


Figure A14_8a: Section. For this section the problem is the unclear nucleus and again the edge. It appears that this otolith was not sectioned exactly through the nucleus. The assigned age range was 3–5 years with a modal age of 5. The agreement was 50%. German collection: dab_EL_2014_Q3_M_s_0016.jpg. Female, 24.5cm, caughti in July.

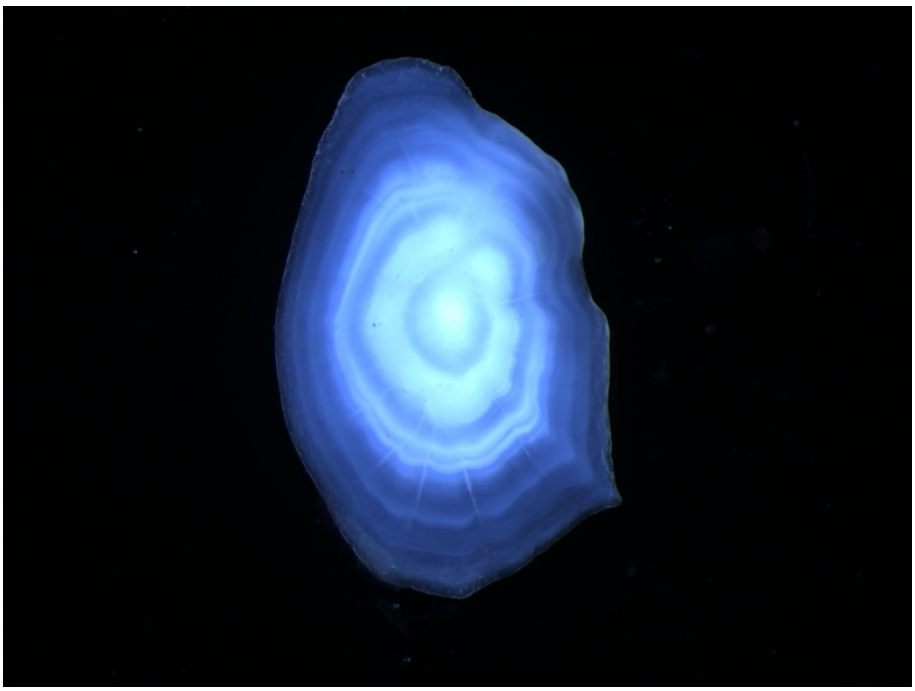


Figure A14_8b: Whole otolith. Here the edge is the problem. Readers assigned ages 5 or 6 with a modal age of 6 and an agreement of 56%. German collection: dab_EL_2014_Q3_M_s_0016.jpg. Female, 24.5cm, caughti in July.

Fish 9 – area IVb, July

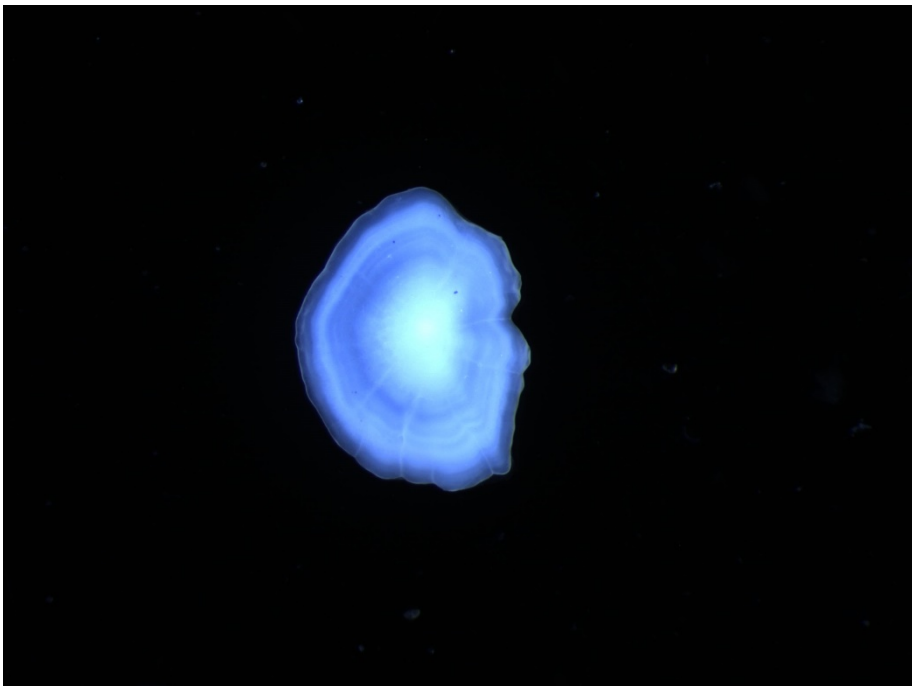


Figure A14_9a: Whole otolith. Here the edge is the problem. Readers assigned ages 1 or 2. The agreement was 56% with a modal age of 2. German collection: dab_EL_2014_Q3_119.jpg. Female, 12.5cm, caught in July.

Annex 15: Recommendations

RECOMMENDATION	ADRESSED TO
1. WebGR (the web based tool for age reading and maturity workshops) is an excellent tool. However, there are several issues with the current version. The most critical problem at present is that it gets stuck, apparently related to if many people are working on WebGR simultaneously. WKNARC2 recommends that this problem is solved as soon as possible.	ICES Secretariat