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Report of the Workshop on Age Estimation Methods of Deep-water Species (WKAMDEEP)

21–25 October 2013

Mallorca Island, Spain

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

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Executive Summary

The WKAMDEEP met at IMEDEA (UIB/CSIC) in Esporles, Spain from 21 to 25 October 2013 to review age determination methods and growth patterns of several deep-water fish species in order to pave the way for solid input data of future age-based assessments for these species.

The species dealt with by the Group were: tusk (Brosme brosme), ling (Molva molva), blue ling (Molva dypterygia), roundnose grenadier (Coryphaenoides rupestris), greater silver smelt (Argentina silus), black scabbardfish (Aphanopus carbo) and black-spotted sea bream (Pagellus bogaraveo).

All relevant information was collated in species-specific annexes, in order to have a protocol which was easy to consult and use for each species. The main report sums up the discussions in the group and presents results from the small WebGr-exchanges of each species made prior to and during the meeting. The body of the report is found in the species-specific annexes, which describe the general biological knowledge of growth and longevity for each species, review available ageing protocols, identify any unresolved problems with interpretation and recommend future actions.

1 Introduction

Based on input from WGDEEP and PGCCDBS, ACOM provided the following justification for establishing the present workshop.

Age determination is an essential feature in fish stock assessment to estimate the rates of mortalities and growth. Assessment of deep water fish stocks using age structured models has proved useful in establishing a diagnosis on stock status. However, the approach has several limitations and shortcomings such as stock structure, natural mortality and growth. Age data is provided by different countries and are estimated using international ageing criteria which have not been validated. Therefore, a WK should be carried out in order to make a general methodological review, evaluate available information on otolith growth patterns, age determination issues and ultimately pave the way for solid input data to age-based assessments (which has been a subject of concern to WGDEEP) thus, making progress towards a solution.

The necessity of age validation studies for all species assessed in WGDEEP is substantial. The stock-assessment is severely hampered by the lack of valid age-structured data and the fact that the agreement in the age-data supplied to the assessment is very low (as seen in previous exchanges).

For some of the shorter-lived species (e.g. tusk, greater silver smelt, greater forkbeard) techniques such as marginal increment analysis or length-modal analysis may be appropriate; while for longer lived species radiometric techniques (e.g. lead-radium) that have been refined in recent years for species such as orange roughy, could be applied.

The aim of the workshop was to identify cutting edge age estimation methods following validation studies conducted so far.

2 Implementation of the Meeting

The attendance and expertise in the Group was not adequate for addressing age reading methods of all the species listed in TOR a). Notably greater forkbeard (Phycis blennoides) and orange roughy (Hoplostethus atlanticus) were not discussed. The species dealt with by the Group were therefore restricted to: tusk (Brosme brosme), ling (Molva molva), blue ling (Molva dypterygia), roundnose grenadier (Coryphaenoides rupestris), greater silver smelt (Argentina silus), black scabbardfish (Aphanopus carbo) and black-spotted sea bream (Pagellus bogaraveo).

The WK started on October 21th afternoon at IMEDEA (UIB/CSIC). After a welcome from the IMEDEA Director, Dr.B.Morales-Nin, the general work procedure was agreed to and the Agenda was accepted (see Annex 1). Two short presentations on ageing problems and validating procedures for deep-water fish species opened the meeting, followed by a general discussion on the selected species.

It was considered important that all the information described in the ToRs was collated for each species, in order to have a species-specific protocol easy to consult and use. Therefore, it was agreed that the general results would be presented as the report and that all the items for each species will be reflected in individual Annexes. Thus, the experts presented advanced methodologies for the age reading of each species; followed by the joint discussion of the otolith image interchange and age interpretations performed prior to the meeting, using the WebGr tool. Finally, the draft report was compiled and reviewed before concluding the workshop. The final report revisions were performed by e- mail.

In annexes 2-8, the general biological knowledge of growth and longevity is described for each species and available ageing protocols are reviewed. Any unresolved problems with interpretation are identified and future actions are recommended.

3 Review of Background Information - Tor (a) and (b)

Addressing ToR (a) - Review information on age estimations, otolith exchanges, workshops and validation work done so far on the following species: tusk, ling, blue ling, roundnose grenadier, greater argentine, black scabbardfish, black-spotted sea bream, greater forkbeard and orange roughy

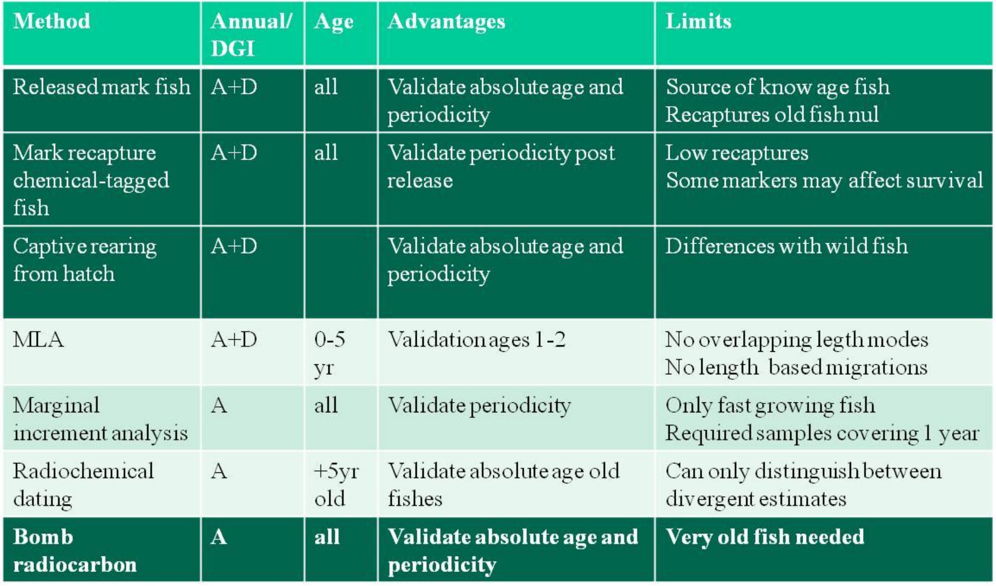
Addressing ToR (b) - Compile all available studies and results on validation of growth rates and longevity in deep water species, including but not limited to those listed above, and develop recommendations concerning the need and methods for validation studies in the ICES area

Status: Completed

The WK participants had different backgrounds ranging from technicians' performing routine age reading to researchers working on the life history of deep-water species. This mixed expertise provided the integration of different points of view and enriched the WK approach. However, the attendance and expertise in the Group was not adequate for addressing age reading methods of all the species listed in ToR a). Notably, greater forkbeard and orange roughy were not discussed. For each of the seven remaining species, the workshop reviewed efforts carried out in order to reach a common agreement for the interpretation of otolith growth zones. This includes an overview of exchange programs and workshops, summaries of identified remaining problems, as well as recommendations for future research and development of the ageing protocols. Particular areas of expertise from certain participants were also included in the protocols. The details for each species are found in the individual species annexes that compose ToR b).

The term age validation is used to denote the confirmation that the age estimation procedure provides unbiased age estimates, i.e. the average age estimates are precise and accurate. It can also be used in a more narrow sense to signify that identifiable zones in part of the otolith are formed annually. Validation can be direct or indirect (Campana 2001) (Table 1).

Table 1. Most frequent validation methods (from Campana 2001) with modifications. Dark green direct validation.A= annual, D=daily



Method	**Annual/ DGI**	**Age**	**Advantages**	**Limits**
Released mark fish	A+D	all	Validate absolute age and periodicity	Source of know age fish Recaptures old fish nul
Mark recapture chemical-tagged fish	A+D	all	Validate periodicity post release	Low recaptures Some markers may affect survival
Captive rearing from hatch	A+D		Validate absolute age and periodicity	Differences with wild fish
MLA	A+D	0-5 yr	Validation ages 1-2	No overlapping legth modes No length based migrations
Marginal increment analysis	A	all	Validate periodicity	Only fast growing fish Required samples covering 1 year
Radiochemical dating	A	+5yr old	Validate absolute age old fishes	Can only distinguish between divergent estimates
Bomb radiocarbon	**A**	**all**	**Validate absolute age and periodicity**	**Very old fish needed**

Several validation methods could not be applicable to deep-water fish due to the low survival rates after capture produced by barotraumas. If there are no results available from such rigorous validation studies, support for an ageing protocol may still be achieved by other approaches, generally referred to as age corroboration or verification. These include tag-recapture analyses without chemical markers, modal progression analyses and tracing of strong year classes over many years. The precision of age estimates is generally improved by means of otolith exchange schemes and age reading workshops which are scarce for deep water species in ICES waters, while the achieved precision is usually quite low. A summary of the previous results from these workshops is provided in Table 2. The reported precision varied considerably with CV from 8.3-22.6 %. There is no defined target for this measure, but based on simulations, Powers (1983) found that a CV of 10% or below would be acceptable for using the age estimates to calculate population rate parameters (i.e. growth and mortality) for use in stock assessments. Reference to a target CV of 10% for the most common age groups is made in Quinn and Deriso (1999). Only one of the previous exchanges listed in Table 2 has reported this level of precision.

Table 3 lists a few examples of available studies for some deep water species commonly occurring in ICES waters. It is recommended that the methods and experience developed in these studies be applied to other deep-water species as well.

Table 2. Summary of age reading workshops and exchanges for the target species. Details and references are given in the respective species annexes.

Species	ICES Areas	N	Ageing preparation	Number of readers	% agreement	CV	Workshop or exchange
Ling	Va	100	Whole in glycerol Whole in water	3		8.3 % 13.9%	WS 1997 EX 2012
Roundnose grenadier	VIa VIa IIIa	64 64 63	Transversal slide	11 6 7	30.2% 29.3% 30.7%	12.5% 14.9% 22.6%	EX 2006 EX 2011 WS 2007
Tusk	Va	300	Whole in glycerol Whole in glycerol	3 4	33.7%	20.8%	WS 1997 EX 2010
Black scabbard	Madeira Rockall Through	50 20	Whole left + right Sectioned	10 11		27% 22.3%	EX 1998-1999
Greater silver smelt							No workshops or exchanges
Blue ling							No workshops or exchanges
Black spotted sea bream							No workshops or exchanges

Table 3. List of selected validation and corroboration works on deep-water species.

Species	Type of study	Main methods	Reference
Greenland halibut	Formation of annual zones	OTC	Albert *et al*., 2009
Greenland halibut	Review of all validation and corroboration studies	C14, OTC, modal progression	ICES WKARGH 2011
Sebastes sp.	Longevity, average ages per length group	Lead-radium dating	Stransky *et al*., 2005
Sebastes sp.	Formation of annual zones	Tracing strong year class over years	Nedreaas, 1990
Sebastes sp.	Longevity	Lead-radium dating	Campana et al., 1990
Orange roughy	Longevity	Lead-Radium dating	Andrews et al, 2009

4 Results of the small-scale Exchanges - Tor (c)

Addressing ToR (c) - Evaluate the results of small exchanges of otolith images from the individual species before the meeting

Status: Completed

A small scale exchange of 50 otolith images for each species was initiated through WebGR (http://webgr.azti.es) a few months prior to the workshop. The calibrated images corresponded to the sagitta otoliths processed using the protocols employed in each laboratory. The purpose of the exchange was largely to make all participants familiar with the otoliths from each species, in order for everyone to be able to partake in the discussions leading to the agreed recommendations for ageing protocols. The results of the exchange will therefore not be presented in detail.

Due to server failure, all images and interpretations were lost, and a new exchange could only be established a couple of weeks before the workshop. However, 4-12 age readers interpreted all species before the end of the workshop. The spreadsheet program by Eltink (2000) was used to compare the age estimates between readers, and relative to the modal age for each sample.

Based on simulation, Powers (1983) found that a CV of 10% or below would be acceptable for using the age estimates to calculate population rate parameters (i.e. growth and mortality) for use in stock assessments. In the present small-scale exchange, the precision of the age estimates varied considerably between species (Table 4). Greater silver smelt was considered the easiest one by all age readers, and the mean CV for all the 12 age readers was only 7.5%. Also for ling and roundnose grenadier, the mean CV was relatively low compared to many other exchanges of long-lived species. For these three species, the precision is probably high enough to support age-structured analytical assessments.

The mean CV was much higher for tusk, black-spotted sea bream and particularly for black scabbardfish. If this exchange is representative of the present ageing results of these species, care should be taken when interpreting estimated year-class strength and population rates. However, for some of the age readers the CV was moderate for these species (9.7-12.9). Only a few of the age readers were trained in ageing these species thus, it may be that the CV will improve with more training. It is therefore recommended to undertake more exchanges and between-reader comparisons for these species.

For most deep-water species there are very few trained age readers and few labs that provide regular age data. Production ageing may be done by one or two people from a single lab. The WK recommends that training on age reading of deep-water species in the future be less focused on individual species and more on the group of species. With good ageing protocols and the general similarities of slow growing species, it should be achievable to educate age readers to become specialists on all the deep-water species simultaneously. This will make future exchanges and age-reading workshops more feasible for these species.

Table 4. Summary of the small exchanges made before and during the workshop. CV: Coefficient of variation; PA: Percent agreement; RB: Relative bias; "Length age 5" etc: Mean length at age of each species in 5 year increments (up to 20 years) expressed as the range across age readers.

	Greater silver smelt	Tusk	Ling	Black-spotted sea bream	Black scabbardfish	Roundnose grenadier
Mean CV	7.5	16.9	10.3	15.3	31.6	10.9
CV per reader	4.2–9.2	12.9–23.7	8.0–14.3	11.1–17.7	9.7–26.0	9.0–11.4
Mean PA	60	37	60	45	33	51
PA per reader	32–86	20–57	28–80	41–49	5–62	32–67
Mean RB	0.09	-0.18	-0.09	0.18	-0.3	-0.54
RB per reader	-0.3–0.6	-1.1–1.2	-0.9–0.4	-0.6–0.9	-4.0–3.6	-1.5–0.9
Length age 5	36–36	43–51	55–67	21–34	101–106	6–7
Length age 10	40–42	54–64	72–103	44–47	96–120	9–12
Length age 15	45–48	–		50–53	101–110	12–14
Length age 20						15–16
# age readers	12	10	9	4	6	4

5 Revised Age Estimation Procedures - Tor (d)

Addressing: ToR (d) - To revise the age estimation procedures and explore the possibilities of using supplementary information to verify estimated ages, including: otolith weight and/or morphometry, as well as length distribution in surveys and catches;

Status: Completed

The detailed ageing procedures are included for each species in Annexes 2 to 8 and a summary is found in Table 5. This chapter outlines the principles that the group consider particularly relevant for the ageing of deep-water species in general.

Table 5. Summary of ageing procedures by species.

Species	Structure	Preparation	Observation	Preferred reading axis	Comments
Tusk	Whole otolith	In water for 24 hours	Water	No preferred reading axis	
Ling	Whole otolith	In water for 24 hours	Water	Towards rostrum	Difficult for L>90cm
Blue ling	Transverse sectioned otolith (0.4 mm)	Unclear effects of polishing	Oil	Longest axis and close to the sulcus	Some use TNPC to guide the interpretation
Greater silver smelt	Whole otolith	In water for 24 hours or Mounted in Eukitt on black plastic plates	Water	Towards rostrum	
Roundnose grenadier	Transverse sectioned otolith (0.2 mm)		Oil	Start with longest axis and continue on sulcus side	Some use TNPC to guide the interpretation
Black spotted sea bream	Whole otolith		Water	Towards rostrum or post-rostrum	Some use Image Pro Plus System to guide the interpretation
Black scabbard fish	Transverse sectioned otolith (0.5 mm)	In 1:1 glycerin-alcohol for 24 hours	1:1 glycerin-alcohol	Start with ventral axis and bend towards sulcus side	Some use TNPC

For routine age determination of the majority of the species considered in this WK it is often considered sufficient to count annuli on the surface of the whole otoliths. The otoliths should be immersed in distilled water for 24 h prior to observation with a compound microscope. For some species transversal sections at core level are used for the whole length range, while for others only for the largest specimens. Albeit, the transversal sections do not improve the age interpretation for some species (e.g. ling).

In order to avoid age overestimation it is generally recommended to use the same magnification for otolith reading, irrespective of the otolith size. Not all laboratories use image analysis systems, but it was a general agreement that they are very useful for measuring annuli and to check the precision between readers. The WK also recommend that all labs should build up a register of calibrated and annotated otolith images, both for documentation purposes and for training of new age readers.

Regarding age interpretation of the species considered, it was clear that several issues were common for most, if not all of the species. Below is a summary of the discussions regarding issues associated with identification of the first zone, the occurrence of transition zones, and the characteristics of the slow growth zones of older specimens.

Identification of the first true annulus:

For most of the species the location of the first annulus is a question of concern. Most species have a complex zonation pattern in the central area, with rings that may or may not correspond to life history events (i.e. hatching, settlement marks) that obscure the annuli identification. Due to the lack of knowledge on the early lives of most deep-water fish, the interpretation of these initial rings is usually not clear. Moreover, some species have multiple or extended spawning periods (e.g. greater silver smelt) that may cause different ring patterns depending on the birth date and environmental conditions.

If feasible, it is recommended to measure otolith dimensions from juvenile fish, preferably down to 0 and 1-groups and construct a growth curve of an easily recognizable growth axis (Figure 1). By interpolation to the assumed time when the first annulus is formed, the expected size of this annulus is estimated. For species with multiple or extended spawning seasons it is important to capture the variability in otolith size at juvenile ages. Use of expected size to identify the first annulus may be justified if the frequency histogram of otolith sizes shows clear modal groups attributable to both 0 and 1 group.

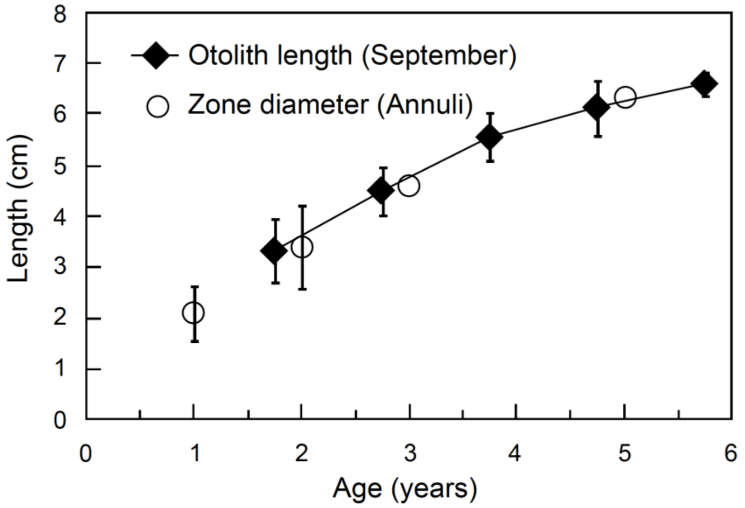


Figure 1. Mean otolith length of Greenland halibut at ages 1–5 in September (solid diamonds) and mean zone diameter at ages 1–5 for fish aged to more than 10 years (open circles). The bars indicate ± 2 SD. Age plotted as a fraction of a year and annuli assumed to be laid down on 1st January (from Albert et al., 2009).

Changing growth patterns along the fish life span:

In several species the otolith growth pattern (increment spacing, increment appearance) may change in the intermediate otolith zone, i.e. an area consisting of several annual zones between the juvenile fast-growth zones and the often regular, distinct and narrow zones of older ages. The pattern is well described for redfish (ICES, 2009) and is also recognized for Greenland halibut (Albert et al., 2009; ICES, 2011) and for several of the species in the present WK. These changes may be related to sex change (e.g. Pagellus), sexual maturity, migrations and/or diet changes.

In many cases it is recommended to count annuli along a curved or broken axis, with the bend or kink occurring within or at the end of the intermediate zones. The problematic ‘checks’ are usually more pronounced in the juvenile phase, while in the older years the problem of zones being vague or discontinuous becomes more serious. Consequently, there is often a danger of overestimating annuli along the first axis or reading direction, and a danger of underestimating annuli along the second axis or reading direction. The actual place where the shift in reading direction occurs is usually not well defined, and the present workshop was not able to add clarity to this question. It is advised that whenever possible validation studies with chemical marks should be used to clarify how zones should be interpreted around the inflection point of the reading axis.

The marginal otolith area:

The last annuli formed in old specimens tend to be very narrow and incomplete around the otolith perimeter. Generally they are first laid down in the area of the longest growth axis. Their narrowness may hinder their identification as annuli; also they can be confounded as checks within the growth zones. For some species (e.g. redfish) these zones may become so narrow for older individuals that they require microscopes of higher quatlity than what is usually available in ageing labs.

The otolith edge observation and the age class estimation procedures are summarized in Table 5. The age attribution may depend on the spawning period and the time of the opaque zone formation (Morales-Nin and Panfili 2002). The 1st January birth-date is established for all the studied species. For most species there has not been any edge analysis, and the following general rule is considered adequate and is illustrated in Figure 2.

Date of capture:

Quarter 1: Translucent zone formed at the edge and should be counted.

Quarter 2: On very young fish an opaque zone may be seen at the edge. All translucent zones should be counted.

Quarter 3: Opaque zone forms and should be visible. If only a thick translucent zone can be seen it is probably since last winter so it should be counted.

Quarter 4: Opaque zone is mostly formed. Translucent zone can be seen, especially in younger fish. Translucent zone on edge should not be counted until first of January.

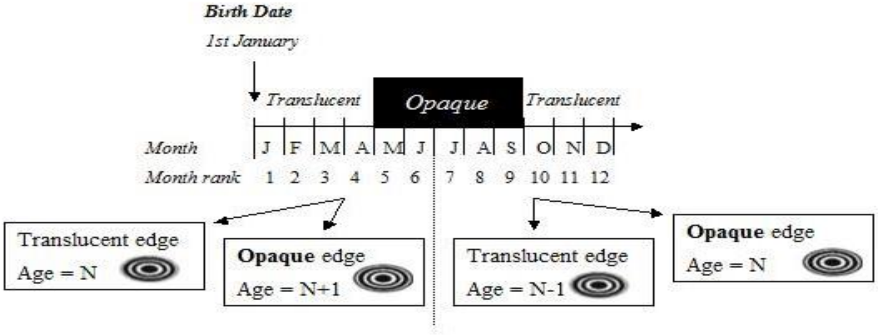


Figure 2. Figure describing the general rule for how to count the edge. N= number of complete annual zones (from Morales-Nin and Panfili 2002)

Table 6. Summary of otolith edge and age class atribution procedures by species.

Species	Spawning period	Opaque edge periodicity	Species	Spec
Tusk	April-July	No information		
Ling	March-August	No information		
Blue ling	February-May	No information		
Greater silver smelt	Extended or multiple periods throughout the year	No information		
Roundnose grenadier	May-November	August to March		
Black spotted sea bream	Throughout the year	May-September		
Black scabbard fish	September-December	July-December (Madeira) April-September (Mainland Portugal)		

5.1 Use of supplementary information - ToR (d)

The use of daily growth increments (albeit not validated) might help identify the temporal meaning of the first rings and also to locate the true annuli. A subsample of otoliths could be prepared and the enumeration of their daily growth increments may be used to determine at which age each check was laid down. Once the growth pattern is identified, measuring the distances from the otolith core to the true first annulus could be used for establishing an ageing protocol.

Length frequency analysis is feasible for fast growing species with short spawning seasons (Pauly 1983), therefore its use for deep-water species is limited to the fast growing juvenile phase. Moreover, in most cases the small sizes are not present in the landings or surveys, precluding the use of this method. The method may potentially be used to identify the first modal lengths and to clarify whether the first age is correctly determined.

However, analyses of modal groups of otolith weights could help in identifying more juvenile age classes. Since otoliths tend to grow even when the fish itself does not (Campana and Casselman, 1993; Cardinale et al., 2000; FAbOSA, 2002), the cubic root of otolith weight will be linearly related to age for a longer age span than the relation between fish length and age. Previous studies of otoliths from a wide range of long-lived deep-water species have shown that the relative weight of otoliths in relation to the somatic weight increase with age (Talman et al., 2003). It is therefore recommended to study the otolith weights for species where representative sampling of juveniles are feasible. Modal progression analyses may also be used on the otolith's annuli diameters or radius, and can then help in identifying the true annuli.

When possible, mark and recapture validation experiments are recommended. In order to solve the interpretation issues it is recommended to obtain the otolith, for instance in Pagellus marking and release experiments the length was collected, helping to solve the growth rate but not the otolith structure. Therefore, it is recommended to mark the fish externally and internally in order to have a check point on the otolith growth pattern and to collect the otoliths. This method, albeit requiring an extensive marking programme, has been applied to European hake and Greenland halibut as an aid to solve its age interpretation (Albert et al 2009; Mellon-Duval et al., 2010). For many deep-water species tagging programs will only be feasible with the application of new technological solutions. One example is the underwater tagging equipment (UTE) developed by Star-Oddi and applied as a tagging program on deep-water redfish (Sigurdsson et al., 2006).

It is strongly recommended to apply radiometric methods (i.e. bomb radiocarbon or lead-radium) to validate the ages interpreted for old fish. These methods have proved useful to validate Sebastes species (Andrews et al. 2002).

The use of image analyses software might help both with interpretations of seasonal zones and in comparisons of individual interpretations. During this workshop, some calibrated images from the small exchange with the best percentage of agreement among readers were interpreted with TNPC software (Figures 3 and 4). This image analysis allows for obtaining some help in the interpretation of calcified pieces and bettering the understanding of bias for one reader or between readers.

It was possible to compare the distances between growth rings and the nucleus from calibrated images to realise the growth curve by each otolith automatically. By comparing the otoliths interpretation approach of one species for one reader or several readers, the precision level of reading is estimated. Moreover, with these measurements, the position of the first growth rings could be calibrated and so to provide help with interpretation.

Different software allows analysing the gray-scale pattern along the reading axis, and this may help to identify the position of zones (Figure 3).

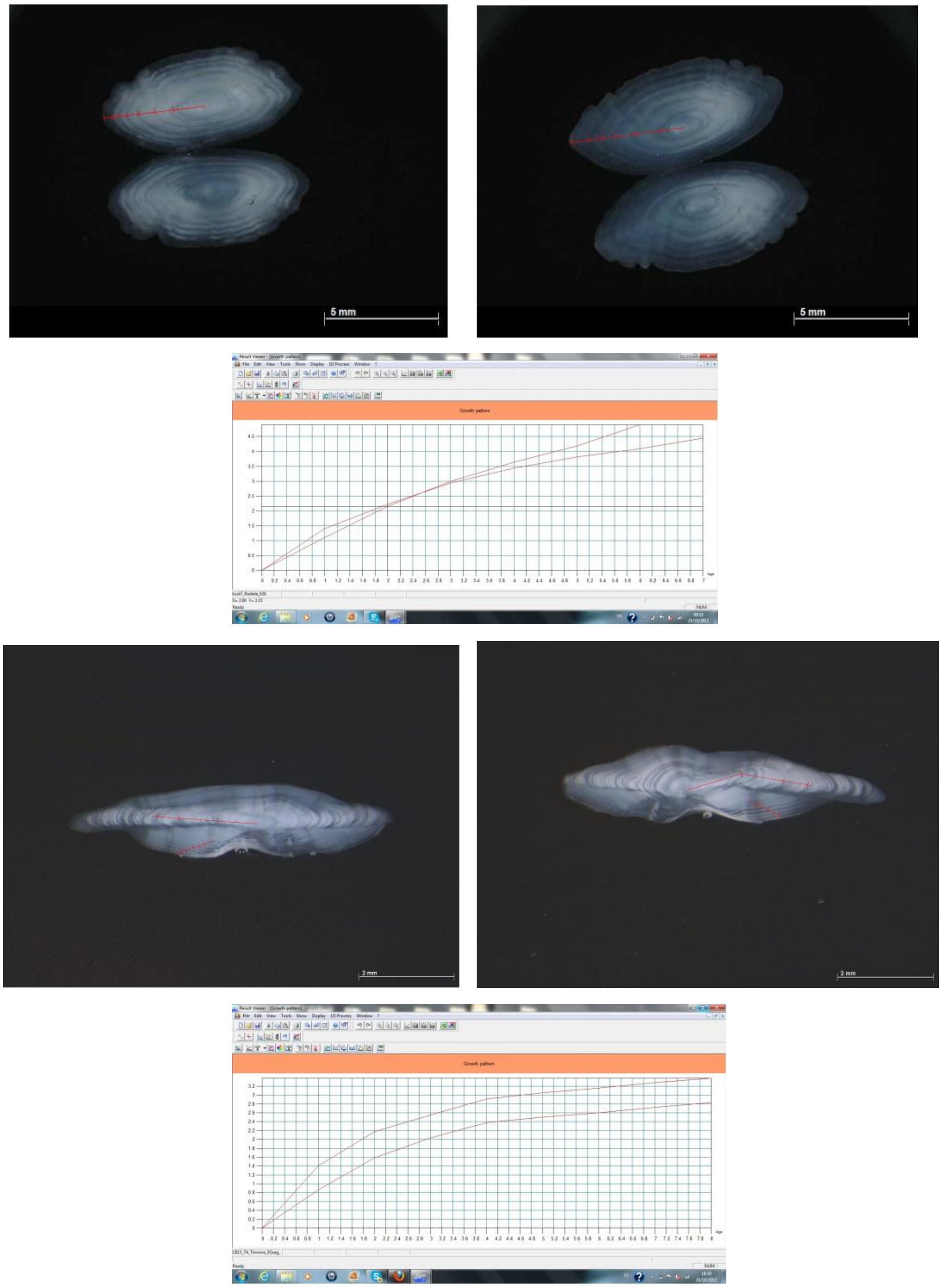


Figure 3. Whole otoliths of tusk (top) and blue ling (bottom) with annotated annuli and the resulting back-calculated growth curves.

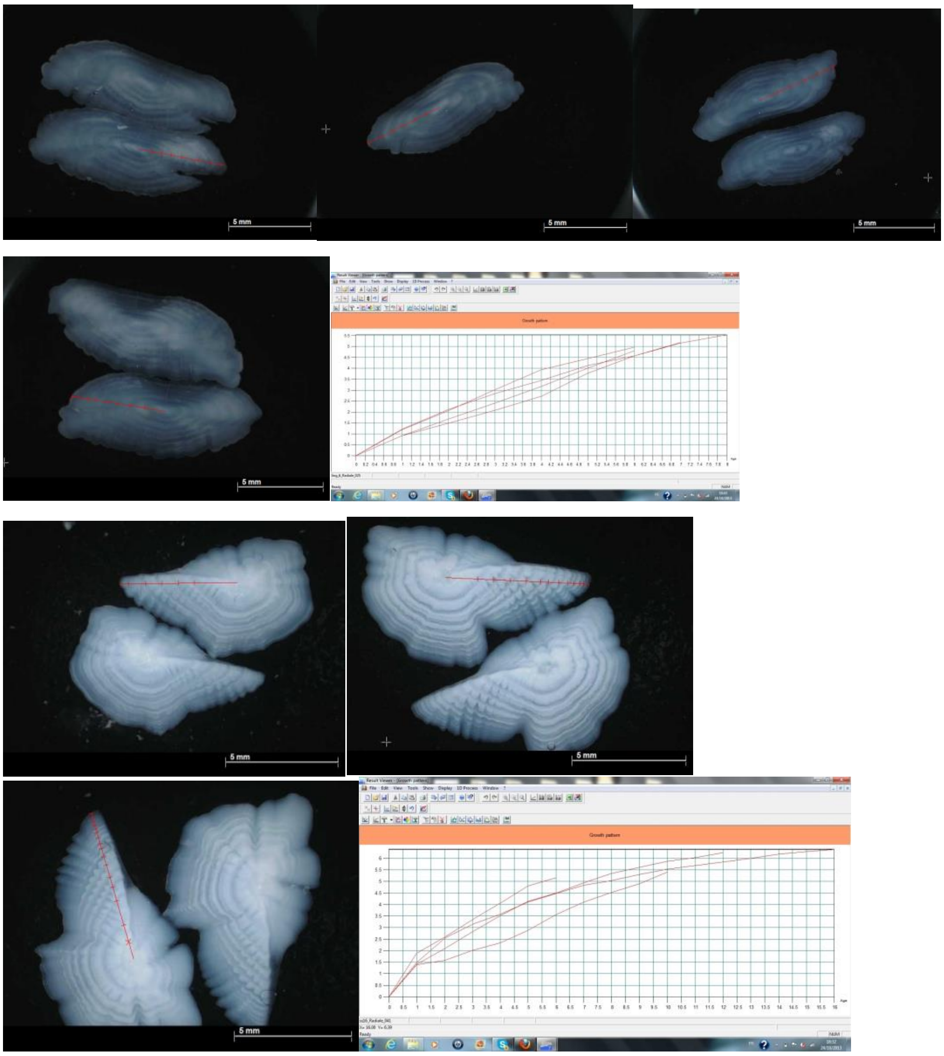


Figure 4. Whole otoliths of ling (top) and greater silver smelt (bottom) with annotated annuli and the resulting back-calculated growth curves.

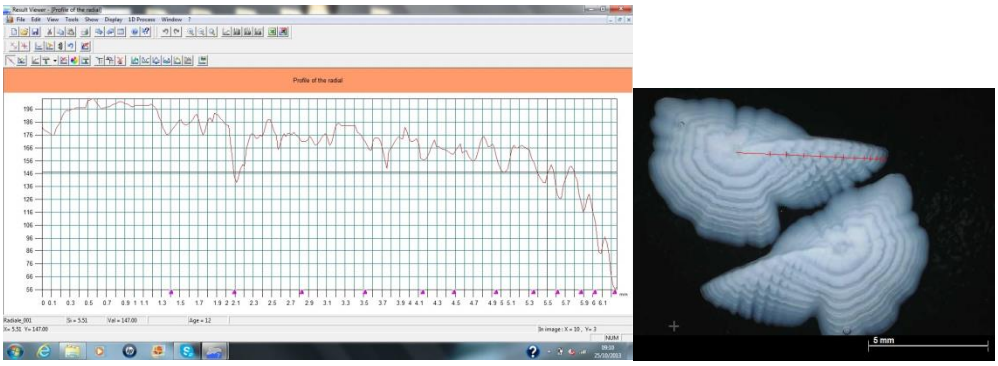


Figure 5. Annotated growth rings on a whole otolith of greater silver smelt. The left figure shows the grey scale profile of the image along the indicated reading axis, as well as the position of the annotated zones along the axis.

6 Publication on Ageing of DW Species - Tor (e)

Addressing ToR (e) - Develop a publication on ageing deep water fish based on analyses done prior to and during the meeting, and including descriptions of general patterns and advanced age estimation methods for deep water species individually and collectively;

Status: In development

The Planning Group for Commercial Catch and Discard Biological Sampling, PGCCDBS, has taken the initiative to publish an ICES Cooperative Research Report with a collation of age estimation protocols. One chapter will be devoted to the ageing of deep water species. The WK envisages a contribution based on the reports from WKAR2008, WKARGH2011 and WKAMDEEP2013. The PGCCDBS will submit the final draft of the publication by January 2015.

7 General - Tor (f)

Addressing ToR (f) - Address the generic ToRs adopted for workshops on age calibration (see 'PGCCDBS Guidelines for Workshops on Age Calibration').

Status: Finished

A small exchange was conducted before and during the meeting (see ToR c) and ToR f is partly addressed in the chapter for ToR c and also briefly in the individual species annexes. However, calibration of age readings was only a relatively minor element in this WK, which focused on describing existing protocols in a systematic and detailed manner, recommending a best practice based on available information on age validation and corroboration and also on practical knowledge of sclerochronology. The purpose of the exchange was largely to make all participants familiar with the otoliths of the individual species, in order for all to be able to partake in the discussions leading to the agreed recommendations for ageing protocols. Some of the generic ToRs are therefore not particularly relevant for this WK.

8 Recommendations

There are very few age validations made for deep water species, therefore the group recommend that validation methods be applied whenever possible.

The small exchange showed that age estimation of deep water species is still carried out with low precision for some species, notably tusk, black-spotted sea bream and black scabbardfish. The group therefore recommends performing more exchanges between laboratories for these species and that the WGDEEP evaluate the necessity to increase the quality of ageing data for each species assessed by the WG.

It is recommended to use image analysis systems for measuring annuli and that all labs should build up a register of calibrated and annotated otolith images both for documentation and for training of new age readers.

The workshop recommends that training on age reading of deep-water species in the future be less focused on individual species and more on the group of species. With good ageing protocols and the general similarities of slow growing species, it should be achievable to educate age readers to become specialists on all the species simultaneously. This will make future exchanges and age-reading workshops more feasible.

9 Recommendations for Future WKAMDEEP

This WK successfully agreed on ageing protocols for six deep water species. However, for several of the species uncertainty still remains on several critical issues. These must be resolved before accurate age estimation is attainable for these species. Recommendations were given to resolve some of these issues, and it is recommended that the WK meet again after four years (i.e. autumn 2017) to develop further with ageing protocols for a selected number of species.

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Annex 1: Ling

Background information

Ling, *Molva molva* L (Figure 1) can reach a very large size; it is a long thin fish up to 200 cm and 30 kg in weight. The largest on record was 212 cm long. It is found over a wide depth range, spanning from 15 to 1000 m. Usually, the younger fish are found in shallower waters. Ling is widely distributed in the warmer parts of the NE Atlantic. Ling is a demersal fish which lives and feeds on rocky bottoms at depths of 15 to 600 m or more, commonly from 100 to 400 m (Magnusson et al., 1997).

Distribution: In European waters, from northern Norway to the Mediterranean Sea. It occurs all around Iceland but is very rare in the colder waters north and east of the country. It occurs around the Faroe Islands, has been reported off southern Greenlandic waters and also in the Grand Banks off Canada (Magnusson et al., 1997).

The ling has a large mouth with sharp teeth, a classic predator on other fishes. It mostly eats herring, flatfishes, Norway pout and other codfishes. It can also eat benthic invertebrates. Spawning occurs offshore between March and August at a depth of 100-300 m (Magnusson et al., 1997). This takes place along the continental shelf break off south and west Iceland, in May and June (Jónsson and Pálsson 2013). Ling reaches sexual maturity at the age of 5 to 8 years when it has reached 60 to 80 cm in length.

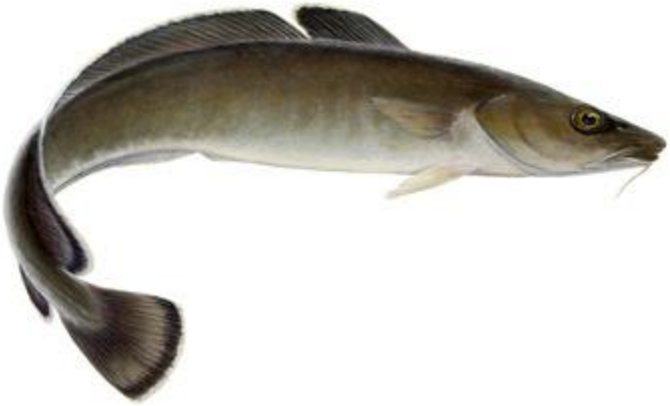

Figure 1. Ling *Molva molva* L. Drawing by Baldur Hliðberg.

Validation, Growth and longevity

There has been no direct validation of age estimation for ling.

Spawning occurs from March to July and eggs are pelagic. Major spawning grounds are located at 200 m depth from the Bay of Biscay to the Gulf of Norway at 100 to 300 m off southern Iceland, and at 50 to 300 m in the Mediterranean Sea (Magnusson et al., 1997). Growth is rapid (8-10 cm/year); at 3 years, a length of 37-62 cm is expected; at 5 years, 60 -75 cm and at 10 years, 99-102 cm (Table 1). It can reach at least 25 years of age (Jónsson and Pálsson, 2013).

Table 1: Growth and maturity of GSS from available studies. Estimated parameters of von Bertalanffy growth equation (L_∞, asymptotic length; K, coefficient of growth; t_0, theoretical time (year) at which the fish has zero length), maturity parameters (L_{50} and A_{50} are total length (L) and age (A) where 50% of the population have reached maturity) are given by area and by sex.

Area	Sex	VBF			Maturity		References
		L_∞ (cm)	K	t_0	L_{50} (cm)	A_{50} (years)	
Norway	Both	156.7	0.104	-1.40			WGDEEP, 2011
Norway	Both	141	0.43				Bergstad and Hareide, 1996
Iceland, Va	Both				75	7-8	WGDEEP, 2012
Faroes, Vb	Both	227	0.052	-0.93	70-80	7	WGDEEP, 2012
NE Atlantic	Both	160	0.09	1.179	70	5	Bergstad and Hareide, 1996
Nordic areas	Both				60-75	5-7	Magnusson et al., 1997

Table 2. Area, mean length of ling at age 3, 5 and 10 (N= number, SD= standard division) and source. Data compiled by the WK.

Area	Age 3	Age 5	Age 10	Source
Iceland (Va)	37.5 cm SD= 8.5 N= 31	60.2 cm SD= 12.9 N= 619	101.7 cm SD= 62.6 N= 284	MRI Iceland
Norway (IIa)	62.8 cm	74.5 cm	95.4 cm	IMR
Shetland (IVa)	51 cm N= 168	66 cm N= 140	102 cm N= 43	Angus, 2011
Faroe Islands (Vb)	53.9 cm SD= 9.0 N= 168	62.3 cm SD= 6.9 N= 140	99.4 cm SD= 7.9 N= 43	FAMRI Faroe Islands

Existing protocols and Results from previous workshops

There is an illustrated manual for age reading of ling (*Molva molva*) using otoliths, from a workshop in 1997 (Bergstad et al., 1997).

There was a ling otolith exchange in 1997 (Bergstad et al., 1998) and again in 2012 (Øverbø Hansen, 2012) with age readers from Norway, Faroe Islands and Iceland. The earlier study in 1997 stated that otoliths from ling greater than 90 cm of length, were considered robust and could rarely be read whole. Sectioning was attempted (transverse sections through the nucleus) but with limited success.

Conclusions from the 2012 ling otolith exchange was that there was some inconsistencies between the age readers but the differences were not very substantial and could easily be adjusted.

Before the WKAMDEEP 2013 in Esporles, there was a small exchange on ling otoliths using WebGR. The results from the annotations of this exchange highlighted that the problem (in most cases) is to do with edge growth. It is necessary to train an age reader and inform them when to count the first translucent zone (first year).

There are some initial guidelines/protocols in all the Institutes in regards to ageing ling.

Recommended ageing protocol

Since all labs have changed their methods for preparing otoliths for age reading ling;

<u>Suggestions made by the group:</u>

- Review of the old manual.

Choice of structure and preparations

Ling otoliths are viewed whole; submerged in water for at least 12 hours prior to ageing. They are read under a microscope with the sulcus-side down, against black background using reflected light. Today, images are not regularly used for ageing ling in any of the Nordic countries. For the small exchange carried out during this workshop, 50 digitised images of ling otoliths were analysed and annotated by age readers using WebGR software.

<u>Suggestions made by the group:</u>

- Otoliths should be kept dry after collection
- Soaking in distilled water for 24 hours is necessary to re-expose the annuli.
- Otoliths of ling should be read whole
- An effort should be made towards taking images of parts of the otolith samples for comparison and discussion within and between age readers.

Preferred reading axis

Both otoliths are used, if available. The one with the clearer annuli is selected and used for age interpretation. It is best to read ling otoliths along the axis from the nucleus to the rostrum attempting to follow the annulus around the whole perimeter of the otolith. As the fish grows and the otolith becomes thicker, the eye can lose the sight of the annuli, on one or both sides, in which case it is better to follow and count toward the rostrum. Otoliths from ling of a length greater than 90 cm can become very robust and are more difficult to age.

Determination of the first annulus

In all cases for ling, the 1st of January is the assumed birthdate. At present, the interpretation of what zone is expected to be the first annulus varies between readers. Due to the prolonged spawning season of ling, the first fast-growth zone (i.e. the nucleus and the opaque area deposited during the 0-group stage) may be of variable size and may sometimes seem very small compared with the subsequent opaque zones which may also be of variable width (Figure 2) (Magnusson et al., 1997).

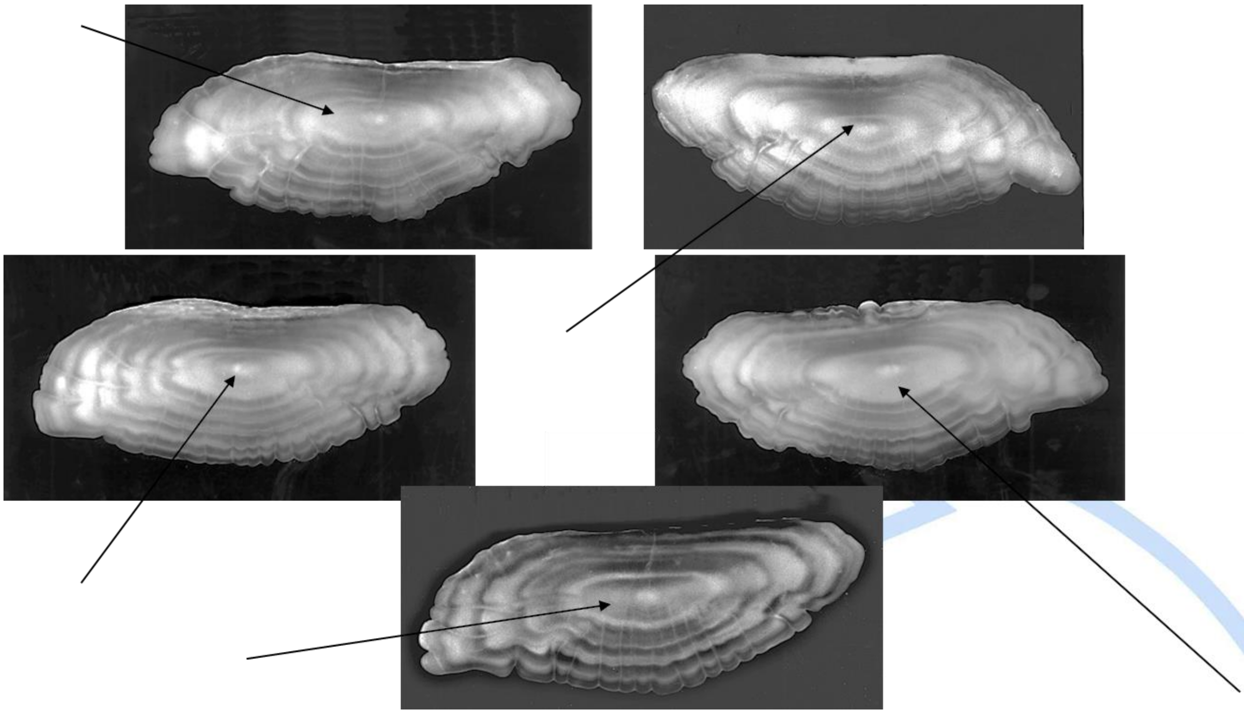

Figure 2. Arrows pointing at the first translucent zone (first year) in ling otoliths (Magnusson et al., 1997).

Identification of annual zones and checks in young fish

The appearance of checks in young fish is not considered to be a major issue for ling otoliths. Although there are some inconsistencies between readers, the interpretation of the first translucent zone as the first year should not be too problematic for readers (see Figure 3). The first translucent zone is the first winter growth. All age readers should count the translucent zones as annuli

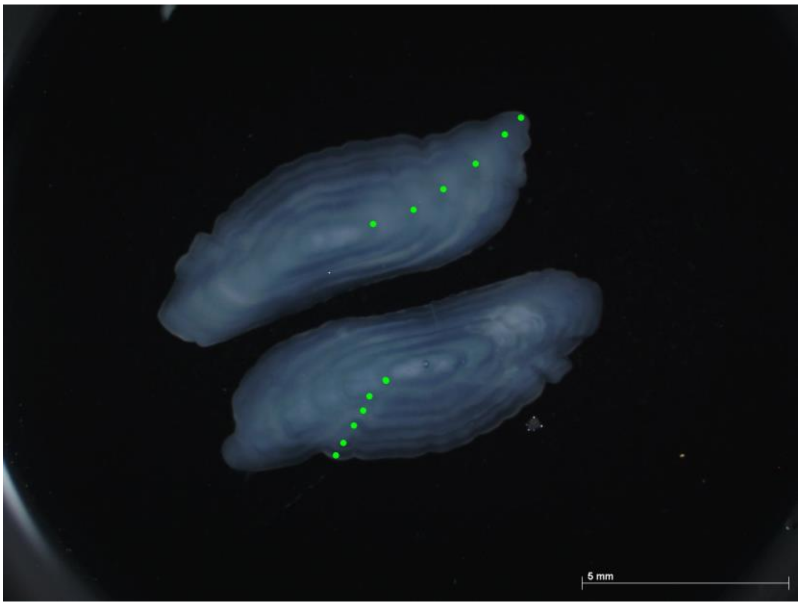

Figure 3. Date of capture is February. Interpretation: Almost all annuli very distinct. 6 years.

Transition zones

Transition zones are not considered to be an issue for ling otoliths.

Edge growth

Quarter 1 (January, February, March): Translucent zone formed at the edge and should be counted. (Figure 4)

Quarter 2 (April, May, June): On very young fish an opaque zone may be seen at the edge. All translucent zones should be counted.

Quarter 3 (July, August, September): Opaque zone forms and should be visible. If only a thick translucent zone can be seen it is probably since last winter so it should be counted (Figure 5).

Quarter 4 (October, November, December): Opaque zone is mostly formed. Translucent zone can be seen, especially in younger fish. Translucent zone on edge should not be counted until first of January.

Figure 6 contains the general rule of how to count the edge. There has not been an edge analysis of ling otoliths to date.

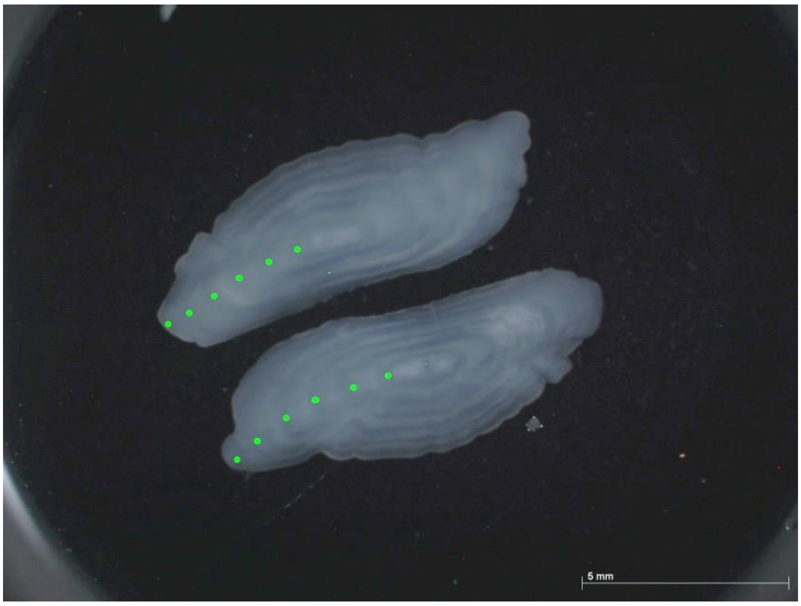

Figure 4. Date of capture is February. Interpretation: Almost all annuli very distinct. 6 years. Translucent zone on edge should be counted in February.

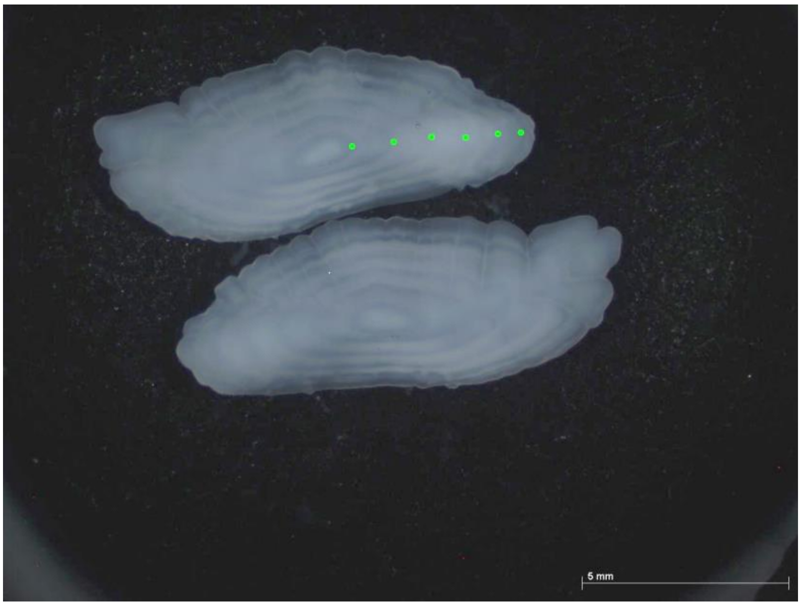

Figure 5. Date of capture is late August. Interpretation: Almost all annuli very distinct. 6 years old. Opaque zone on edge, therefore do not count the edge.

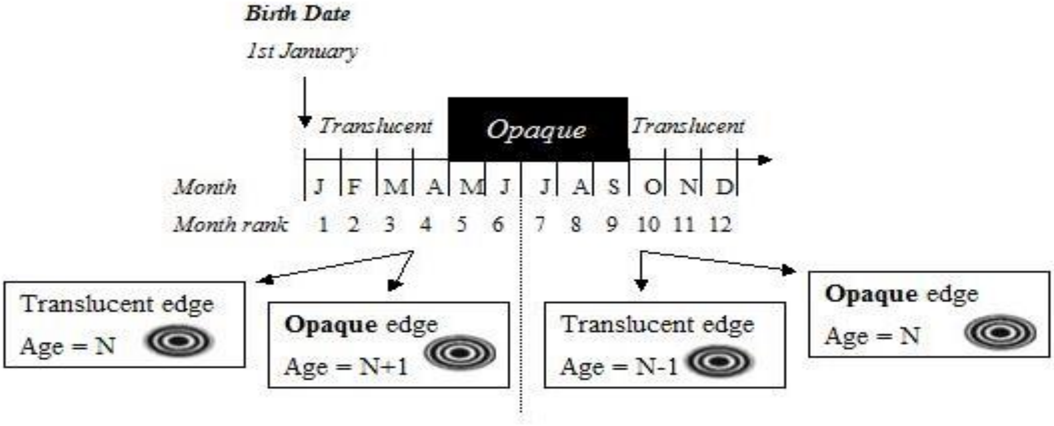

Figure 6. Figure describing how to count the edge from WKACM2 as WKARP 2010 (Kelig Mahé)

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Annex 2: Blue Ling

Background Information

Blue ling (Molva dypterygia, Fig.) is widely distributed in the North Atlantic from the west of the British Isles and further south to Morocco. This species congregates only for spawning. Spawning in the Icelandic area takes place from February to April, mainly in March at temperatures of 5-8°C. Spawning areas near the Faroes are reported west and south of the ridge from Scotland to Iceland including the Faroe Bank, the Bill Bailey Bank and the Lousy Bank; in 500 to 2000 m depth with most occurring at about 1000 m, in February to May in temperatures of 7.5 -8.7°C Spawning has also been observed at East Greenland in late summer (Magnusson et al, 1997).

Figure 1 : Blue ling (source : www.Fishbase.org; Salesjö, A.)

Validation, Growth and Longevity

Blue ling has been aged by counting the rings in otoliths from broken surfaces (Magnusson, 1982) or thin sections (Engas, 1983; Ehrich and Reinsch, 1985; Thomas, 1987; Bergstad, 1991, 1998, Magnusson et al., 1997, Magnusson, 2007). There is a growth rate difference between sexes after only 7 years of age (Magnusson, 1982). Ehrich & Reinsch (1985) showed that for females, the maximum length L∞ increases in distinct correlation from the north to the south.

Table 1 : Growth and Maturity of Molva dypterygia from available studies. Estimated parameters of von Bertalanffy growth equation (K, coefficient of growth; L∞, asymptotic length), maturity parameters (L50 and A50 are length (L) and age (A) where 50% of the population have reached maturity) are given by area and by sex.

Areas	Samples			Sex	Length (total length)		Maturity		References
	n	Age range (year)	Length range (cm)		L∞ (cm)	K	L50 (cm)	A50 (year)	
Faroe Bank	79	3-17		both	160	0,11	79	6,2	Magnussen, 2007
Faroe islands		5-20	72-107	male	104	0,197		6,4	Thomas, 1987
Faroe islands		5-20	77-125	female	116	0,17		8,1	Thomas, 1987
Shetland Islands		5-20	71-107	male	108	0,185			Thomas, 1987
Shetland Islands		5-20	71-133	female	137	0,13			Thomas, 1987
Faroe islands				female	116				Ehrich & Reinsch, 1985
Shetland Islands				female	137				Ehrich & Reinsch, 1985
British Islands				female	145				Ehrich & Reinsch, 1985
Iceland				female	138		75		Joenoes, 1961
Iceland				male	102				Joenoes, 1961

Table 2 : Length range (or mean length and SD) of different age groups. Data compiled by the WK.

AREAS	AGE 10	AGE 15	AGE 20	SOURCE
Faroese waters	93	109	127	Reinsch, 1987
ICES VIa	91	132	117	French data 2010
Iceland	87	112	137	Magnusson and Magnusson, 1995

Mean length-at-age estimated in Icelandic waters (Magnusson and Magnusson, 1995) are smaller than French estimates for ages of up to 15 or more years (Figure AB). In recent years, for older fish, Icelandic estimates are similar to French estimates but larger than the French calculated in 1994.

Mean length-at-age estimated from German catches in Faroese waters, (in the same area as the French catches) are similar to mean length-at-age from French otoliths readings for the age group of 8-12 years, i.e. the most abundant age group in current catches. For older ages, Reinsch (1985) estimated smaller lengths (Figure 2, C). Length-at-age was also estimated from fish from Faroese waters by Thomas (1987). The mean length-at-age from this study was similar to recent French findings of fish up to age 10 or 11 and much smaller for older age groups. However, the small mean length-at-age estimated at age 15 years and over by Thomas (1987) may be difficult to reconcile with large fish around 140 cm occurring in the stock, including in recent years, after a period of overexploitation in the 1990s and early 2000s.

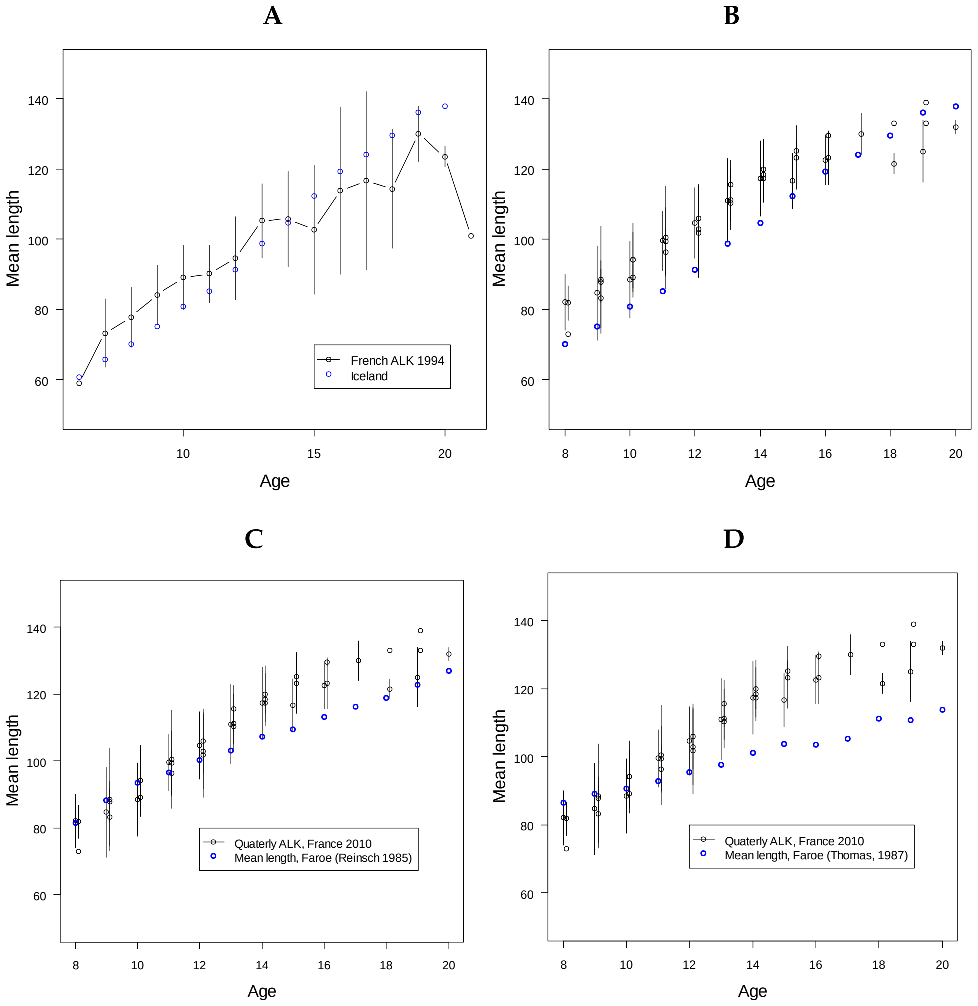

Figure 2: (A) Comparison between the mean length-at-age from France (age-length key from 1994) and Iceland (Magnusson and Magnusson, 1995); (B) quarterly mean length-at-age from France in 2010 and Iceland ; (C) quarterly mean length-at-age from France in 2010 and estimates from Faroese waters (Reinsch, 1985) ; quarterly mean length-at-age from France in 2010 and estimates from Faroese waters (Thomas, 1987).

Overall, all age estimates are in good agreement for ages of up to 10-12 years. For older fish, there are more discrepancies.

Tests were undertaken in 2013 in the sclerochronology center (France) to compare different preparation techniques of the blue ling otolith:

- Whole
- Polished whole
- Transversal slice through the nucleus
- Polished transversal slice
- Frontal slice
- Polished frontal slice

An image of a whole otolith from a blue ling at 76 cm total length has been attained. The calcified piece has then been embedded in resin. Polishing both surfaces of the otolith has been carried out in order to obtain a thin section of the entire otolith from the nucleus to the edges without losing any structure. However, the curved shape of the otolith makes this technique time-consuming and does not lead to an exploitable reading scheme (Fig. 3).

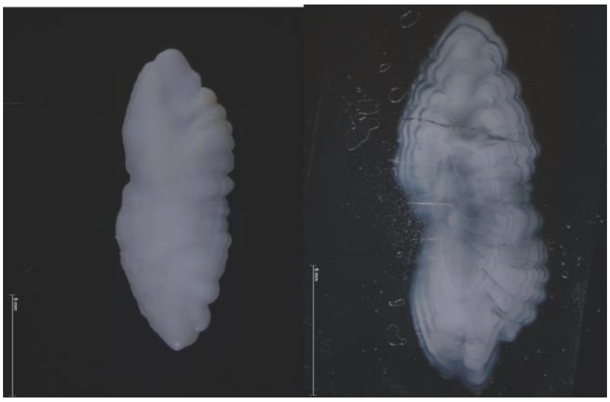

Figure 3: Otolith of blue ling (Lt :76 cm) : whole otolith versus polished whole otolith.

Thin transversal sections have been tested. Sectioned otoliths produced with a width of 0.6mm are very hard to interpret (Magnusson et al., 1997) because of unclear fale rings (checks). Thinner sections have been acquired using a high speed saw (Brillant 221, ATM society) which produces slices with a width of 0.4 mm. Images of such sections which have undergone a polishing treatment to reduce the width from approximately 0.1 to 0.15 mm have been used. (Fig. 4)

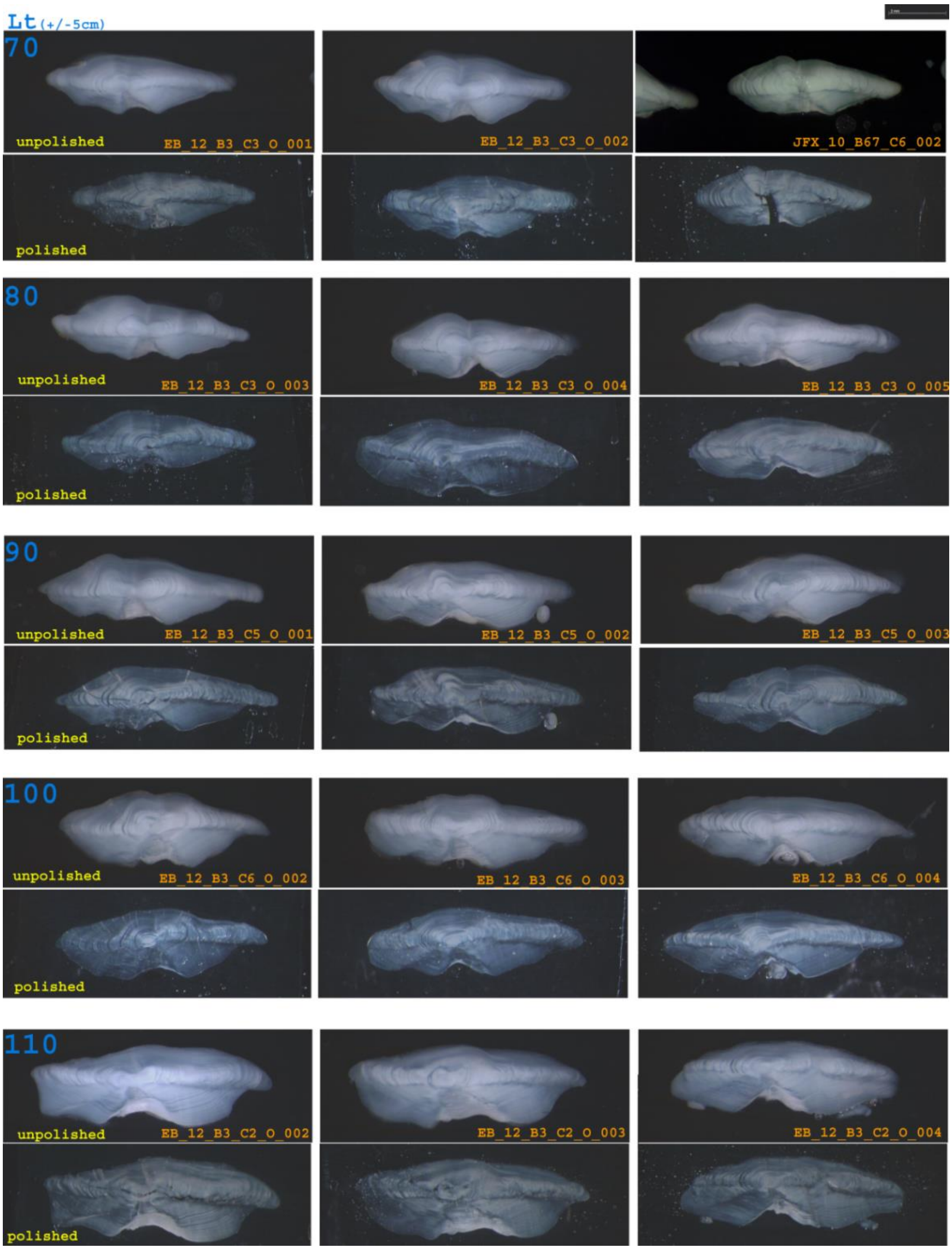

Figure 4: Otoliths from blue ling : Transversal slice of otolith versus polished transversal slice of otolith (source : Ifremer).

Thin sections along the frontal axis have also been tested in the hope to improve the identification of growth patterns. This uses the same procedure as for transverse sectioning of the otoliths. Nevertheless, the axis doesn't provide more than one or two sections through nucleus to the edges. Left and right otoliths from one fish of a specified total length have been removed; frontal and transversal sections have been obtained from both otoliths, pictured and thinly polished to compare structures revealed by these two techniques. The transversal slice appears to present the best resolution for ageing blue ling (Fig. 5).

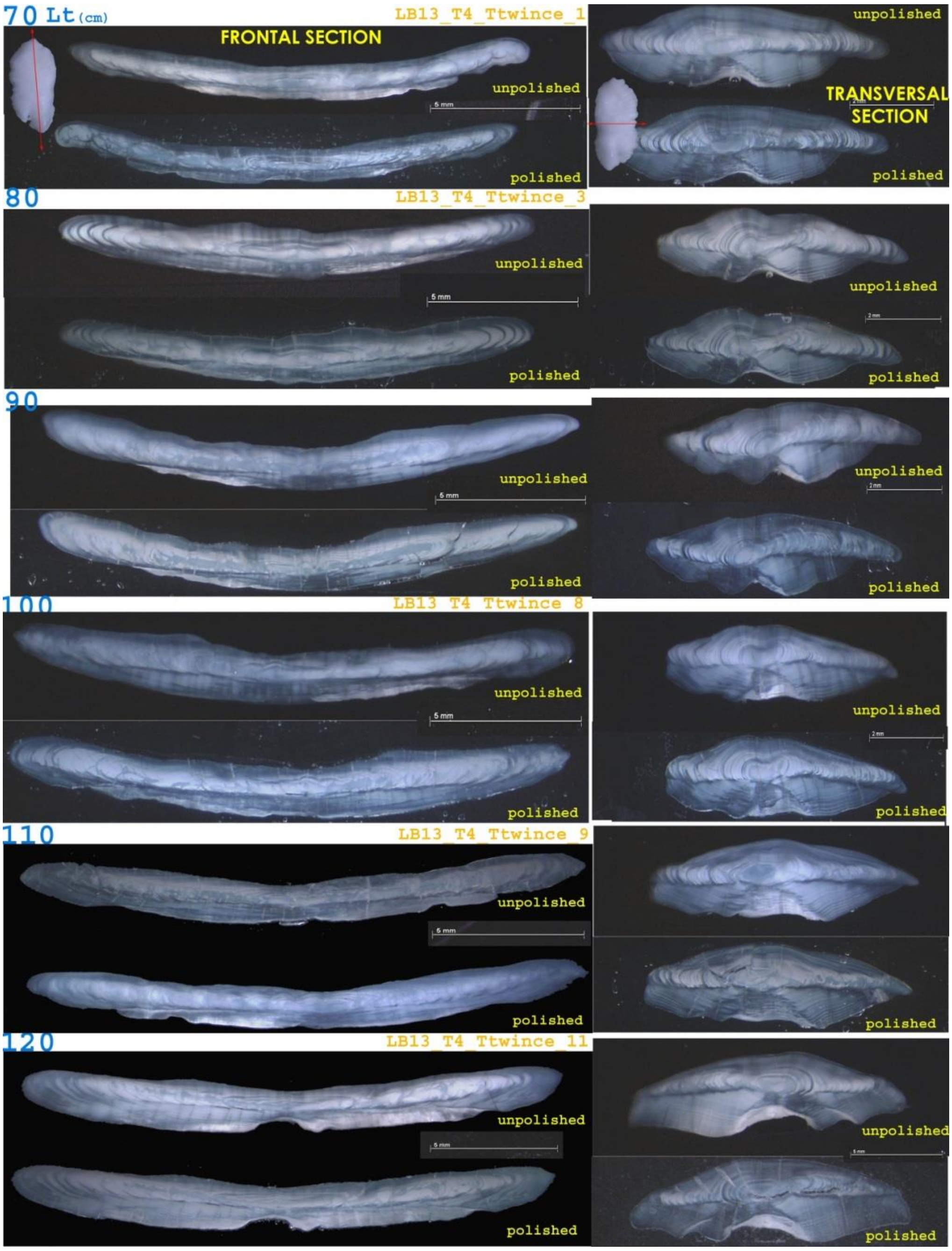

Figure 5: Left and rigth otoliths from blue ling : Transversal slice (with/without polishing) versus Frontal slice (with/without polishing) by length class (source : Ifremer).

There has been no direct validation of age estimation for blue ling.

Bergstad et al. (1998) carried out an indirect validation with length-frequency distribution analysis. The Icelandic data from groundfish surveys conducted every year in March showed modes among the smallest fish (from fish of TL<60 cm). The 1-group fish in March were mostly less than 20 cm with the 2-group varying between 20 and 40 cm. This indirect validation study showed that it was possible to achieve reasonable consistency among readers but only for otoliths from juveniles of up to 3-4 years old (Fig. 6; Bergstad et al., 1998).

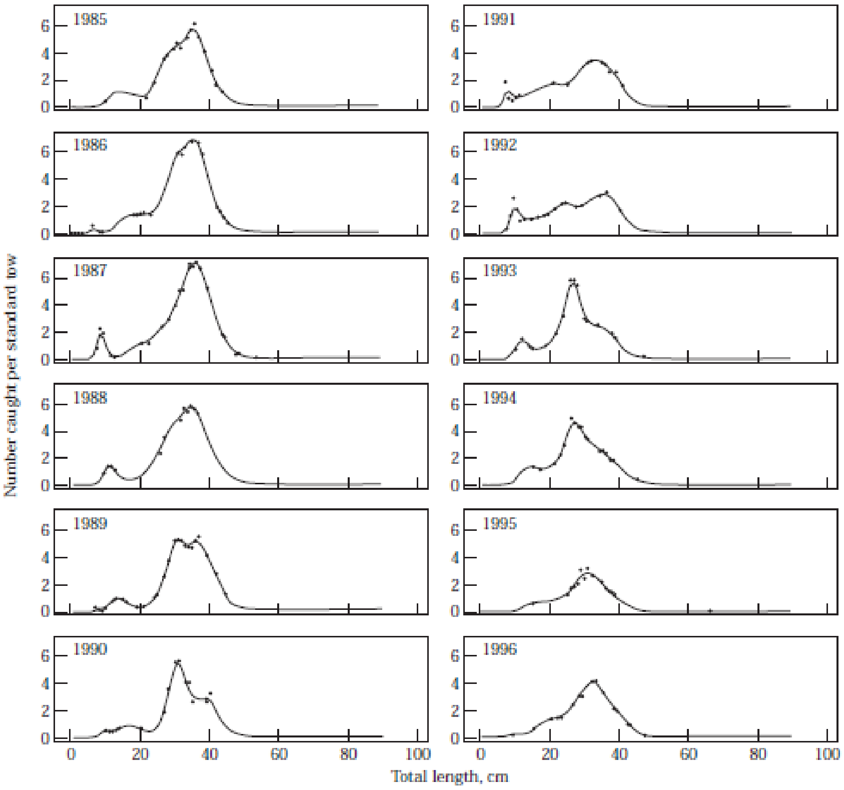

Figure 6: Length distributions of blue ling <60 cm caught by bottom trawl on the Icelandic groundfish surveys in March every year (In Bergstad et al., 1998).

Existing Protocols and Results from Previous Workshops

There have been no exchanges and/or workshops on ageing blue ling

Recommended Ageing Protocol

Choice of Structure and Preparations

The best structures for estimating the age of blue ling are sectioned sagittal otoliths. Sectioning is required because the internal structure of the otoliths from adult fish is thick, therefore cannot be interpreted whole.

During this workshop, all readers tested 4 techniques:

- Polished whole otolith
- Transversal slice
- Polished transversal slice
- Frontal slice

The technique of transversal sectioning presented the best results.

Preferred Reading Axis

Two axis of reading are used when ageing blue ling otoliths (Fig. 7).

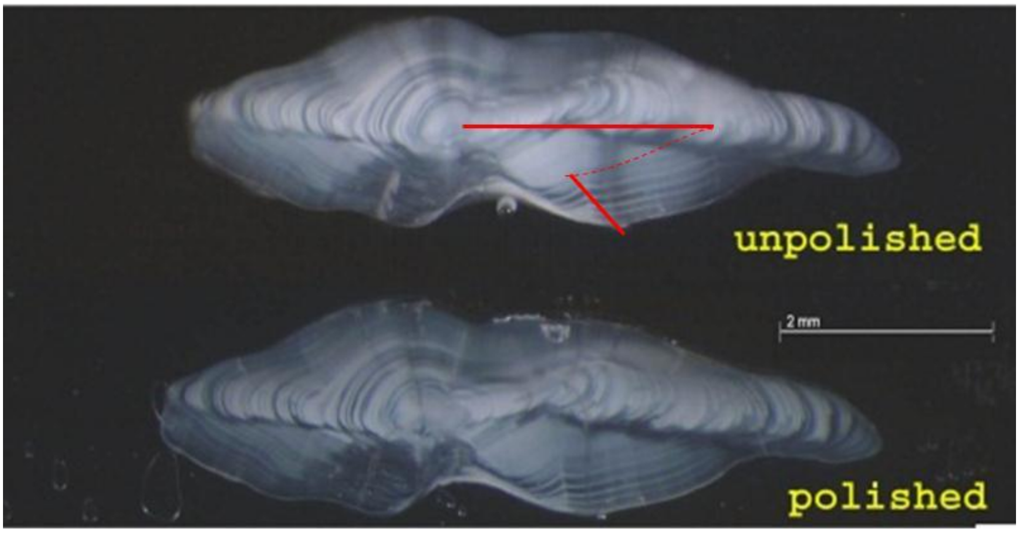

Figure 7: Interpretation axis for blue ling otolith.

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Annex 3 Greater Silver Smelt

Background Information

Greater silver smelt (GSS) (Argentina silus) (Figure 1) are distributed in European waters from the Bay of Biscay to the Barents Sea. They are found off the Faroe Islands, Iceland, southern Greenland and in North American waters from Newfoundland to Cape Cod. GSS are a bento-pelagic fish, i.e. they occur both close to the bottom and in the water column. They occur in temperatures of around 4 to 10°C and in depths from 150 to 1400 m. The younger fish are usually found in shallower waters than the older fish.

GSS can reach 60 cm in length and up to 30 years of age. Length at maturity is between 32 and 38 cm (5-8 years old). The spawning season is thought to be extended or it may occur at multiple periods with at least two peaks during the year. Minimum length at settling appears to be 9-10 cm total length (TL) (Bergstad, 1993). A distinct first mode at 12-15 cm TL in the winter distribution represents I-group juveniles which had recently recruited to near bottom zone (Bergstad, 1993).

In ICES, GSS is divided into two stock components, Division Va (Iceland) and other areas (WKDEEP, 2010). Common size in landings is from 40 to 50 cm.

Figure 1. Greater silver smelt Argentina silus

Validation, Growth and Longevity

There has been no direct validation of age estimation for GSS.

Maximum age can be up to 40 years old, but in the fishery they are usually between 6-14 years. Age range, sex, VBF parameters and maturity for GSS are listed in Table 1. Mean length at age for age 1, 5, 10 and 15 differ only with 4 cm between the Institutes from Iceland, Norway and Faroe Islands (Table 2).

Table 1: Growth and maturity of GSS from available studies. Estimated parameters of von Bertalanffy growth equation (L∞, asymptotic length; K, coefficient of growth; t0, theoretical time (year) at which the fish has zero length), maturity parameters (L50 and A50 are total length (L) and age (A) where 50% of the population have reached maturity) are given by area and by sex.

Area	Samples		Sex	VBF			Maturity		References
	n	Age range (year)		L_∞ (cm)	K	t_0	L_{50} (cm)	A_{50} (years)	
Iceland (Va)		0-19	both	47.9	0.17	-2.14	36	6	WKDEEP, 2010
			F	49.6	0.16	-2.30		6.5	
			M	45.8	0.19	-2.01		5.6	
Faroes (Vb)	1059	0-19	both	42.4	0.22	-1.12	34.8	6.8	WKDEEP, 2010
	556		F	43.9	0.22	-1.08	33.9	5.8	
	503		M	40.3	0.24	-1.11	35.5	7.6	
Norway		0-19	both	39.5	0.19	-2.13			WKDEEP, 2010
			F	41.7	0.19	-1.85		4.2	
			M	36.9	0.22	-2.02		5.1	
Norway			F	42.6	0.19	-1.95			Bergstad, 1993
			M	40.3	0.21	-1.95			

Table 2. Mean length (SD= Standard deviation, N= Number) of different age groups. Data compiled by the WK.

Area	Age 1	Age 5	Age 10	Age 15	Source
Iceland	19.1 cm (SD= 3.1, N=239)	33.9 cm (SD=2.7, N=578)	40.5 cm (SD=2.4, N=1472)	43.9 cm (SD=2.9, N=410)	MRI
Faroe Islands	15.4 cm (SD=2.1, N=49)	31.0 cm (SD=2.0, N=146)	38.5 cm (SD=2.3, N=95)	42.3 cm (SD=2.2, N=39)	FAMRI
Norway		29.9 cm (SD=1.9, N=132)	36.1 cm (SD=2.7, N=88)	39.4 cm (SD=3.6, N=43)	IMR

Existing Protocols and Results from Previous Workshops

Existing published protocols describing age reading methods of GSS, known to the group, are Magnusson (1990) and Bergstad (1995). The methods used were similar, reading the otolith whole on a black background, using a microscope with reflected light. The Institutes that are ageing GSS in the Northeast Atlantic (Iceland, Faroe Islands, Norway and Netherlands) have intern ageing protocols, reading whole otoliths which have been immersed in glycerol/distilled water or molded in epoxy resin.

There have been no workshops on ageing GSS. A comparison between age readers in Norway and Faroes of the same 270 otoliths gave very comparable results with high accuracy (Ofstad and Homrum, 2009).

Recommended Ageing Protocol

Choice of Structure and Preparations

Whole otoliths are usually mounted (e.g. with Eukitt) on black plastic plates or submerged in water (for at least 24 hours prior to reading), sulcus-side down, using reflected light against a black background. Today, images are not regularly used for ageing GSS in any of the Nordic countries.

For the small exchange exercise carried out during this workshop, 50 digitized images of GSS otoliths were analysed and annotated by age readers using WebGR software. The results from the annotations of this exchange highlighted that there were only minor problems ageing GSS compared to other deep water species. There was more or less overall agreement on the first translucent zone (first year) and the problem (in most cases) was the edge growth.

Suggestions made by the group:

- To count day rings on otoliths from small juvenile fish of different size in order to validate the first true annual zone as GSS have a prolonged or multiple spawning season.

- Perform an edge analysis on GSS otoliths to help support the use of the general rule on how to classify the edge (Figure 5).

- An effort should be made towards taking images of parts of the otolith samples for comparison and discussion within and between readers.

Preferred Reading Axes

It is best to read GSS otoliths along the axis from the nucleus to the rostrum (Bergstad, 1995). This was also agreed on by the group. Compared to other deep water species, most GSS otoliths have clear growth patterns (Figure 1). However, there can be otoliths which present not-so distinct growth zones (Figure 2).

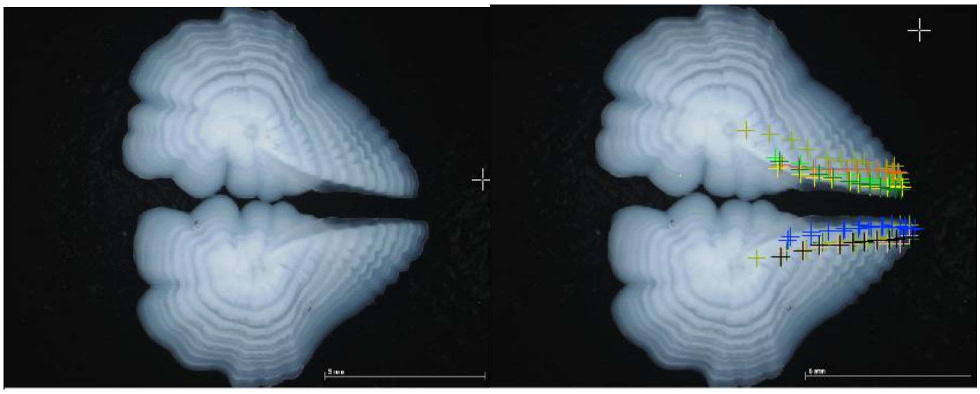

Figure 1. Image of GSS otoliths from a 42 cm long fish caught in February displaying clear patterns in the translucent and opaque zones. Left figure shows the otolith without annotations and the right figure with annotations. The age of this fish is estimated to be 11 years old.

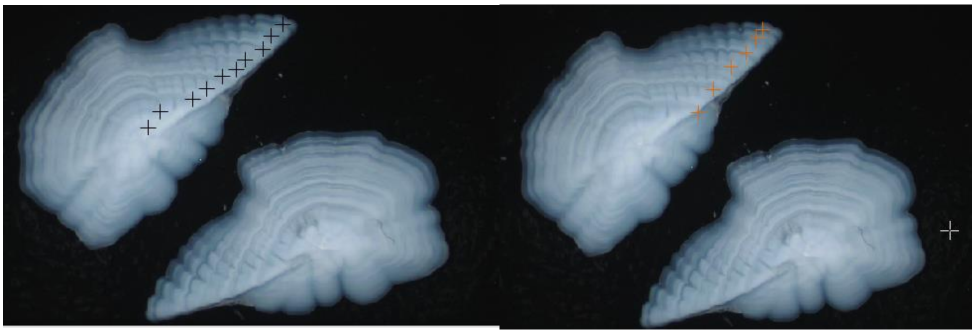

Figure 2. Image of GSS otoliths from a 40 cm long fish caught in October showing unclear patterns in the translucent and opaque zones. The left figure shows the otolith (with annotations) to be interpreted as 10 years old. However, the right figure displays the age of this fish to be estimated as 6 years old.

Determination of the First Annulus

In all cases for GSS, the 1st of January is the assumed birthdate. At present, the interpretation of what zone is expected to be the first annulus varies between readers. Due to the prolonged spawning season of GSS, the first fast-growth zone (i.e. the nucleus and the opaque area deposited during the 0-group stage) can often vary in size and may sometimes seem very small compared with the subsequent opaque zones which may also vary in width.

At present, the interpretation of what zone is expected to be the first annulus varied very little between readers in this workshop. Usually only one of the 13 readers counted an innermost, very narrow translucent zone as the first annulus with the rest counting the broader, more prominant translucent zone as the first annulus (Figure 3).

Images of GSS otoliths with a length range from 9 to 22 cm showed that the initial broader translucent zone appears to be made during the first winter (Figure 4). Counting day rings in otoliths from small fish can help on validate where to start counting the first true annulus.

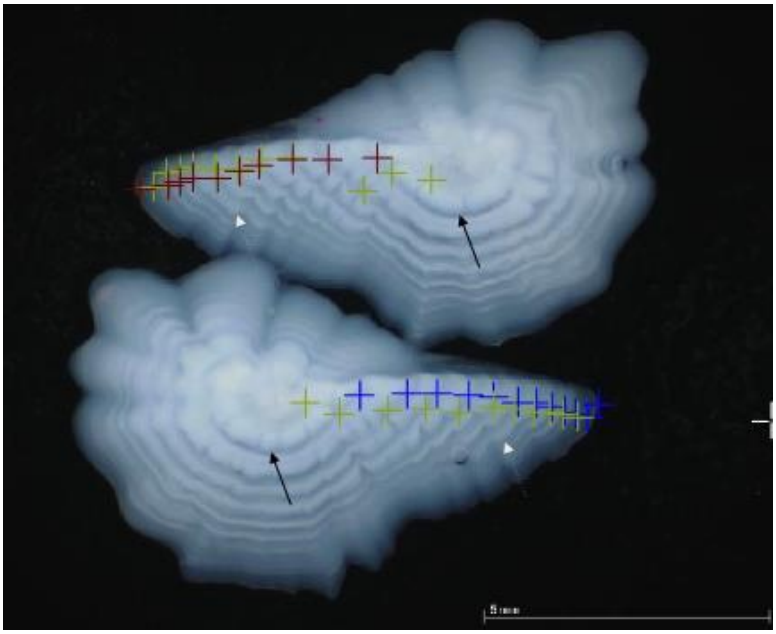

Figure 3. Image of GSS otoliths from a 40 cm long fish caught in February, showing that one of the readers count the innermost transulent zone which is believed to be a check (false ring). The broader, more translucent zone is marked with black arrows and the change in growth pattern is marked with light grey stipped arrows. The fish is interpreted to be 11 years old.

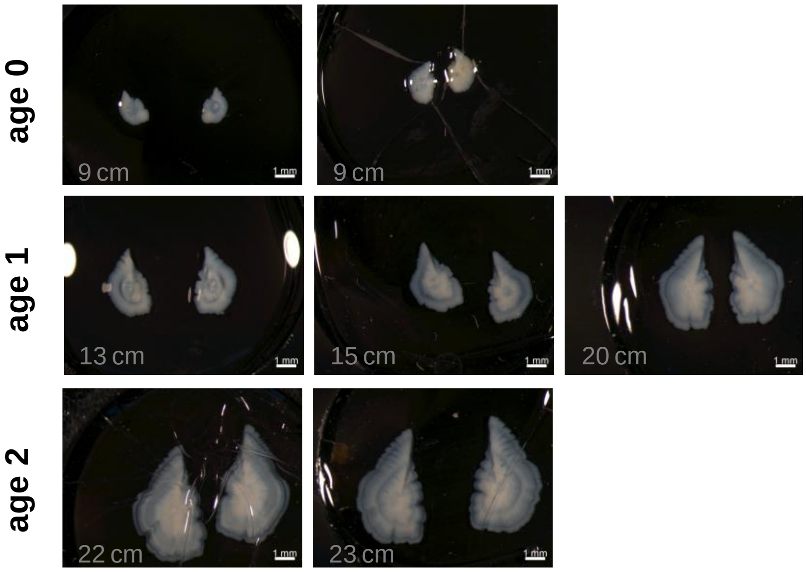

Figure 4. Images of small GSS otoliths sampled in August and their proposed age (photo courtesy Faroe Marine Research Institute).

Identification of Annual Zones and Checks in Young Fish

There is a change in appearance of annuli where the patterns of translucent zones become narrower (Figure 3). The first narrow zone, probably formed at first spawning, appeared in the age range of 4-9 years (Bergstad, 1993).

Transition Zones SLOW

Transition zones are not considered to be an issue for GSS otoliths.

Growth Zones

Increments closer to the edge become narrower, therefore should not be considered as checks but as annuli. However, these very narrow increments become more difficult to distinguish with increasing age. Such zones are thought to be a result of slower growth which may be related to maturation.

Edge Growth

No distinct analysis has been carried out with respect to marginal zone deposition, so the general rule showed in Figure 5 is used.

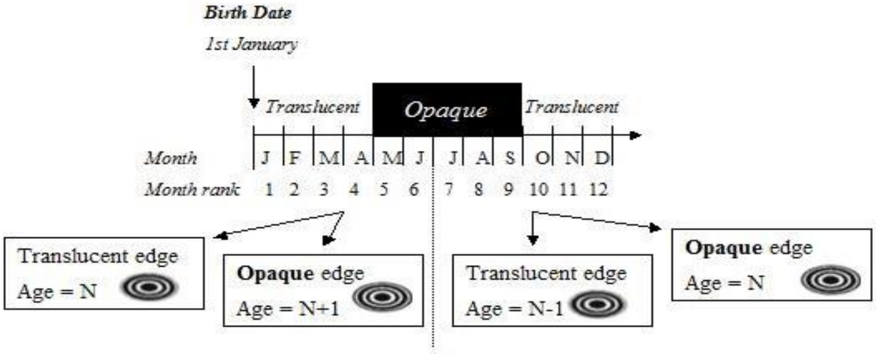

Figure 5. The general rule on how to count the edge (from WKACM2 as WKARP 2010).

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Annex 4: Roundnose Grenadier

Background Information

The roundnose grenadier (Coryphaenoides rupestris, Fig.) is widely distributed in the North Atlantic. Its area stretches from Norway to northwest Africa, in the east to the Canadian-Greenland coasts and the Gulf of Mexico in the west, and from Iceland in the north to the areas south of the Azores. It occurs at a depth of 180–2200 m (Haedrich & Merrett, 1988 ; Cohen et al., 1990 ; Swan & Gordon, 2001). To the west of the British Isles, females with maturing ovaries have been observed from February to December but they were more abundant from May to October. Spawning appears to extend from at least May to November (Kelly et al., 1996; Allain, 1999 and 2001).

Figure 1: Roundnose grenadier (source : Ifremer)

Validation, Growth and Longevity

The roundnose grenadier has been aged by counting the rings in otoliths and scales (Savvatimskiy, 1971 ; 1972 ; Koch, 1976 ; Borrmann, 1978 ; Bridger, 1978 ; Gordon, 1978 ; Savvatimskiy et al., 1978, Kosswig, 1981 ; 1983 ; 1984 ; 1986 ; 1989 ; Eliassen, 1986 ; Magnússon, 1986 ; 1987 ; Danke, 1987; Bergstad, 1990 ; Dupouy & Kergoat, 1992 ; Kelly et al., 1997 ; Draganik et al., 1998 ; Allain, 1999 ; Allain & Lorance, 2000). Age estimation with scales was higher for younger fish, but considerably lower than the results from otolith ageing in older fish. Bergstad (1990) indicated that scales may be unsuitable for determining age of the roundnose grenadier. Whole otoliths of roundnose grenadier can only be read from very small individuals (Kelly et al., 1997). As in many fish species, the females were heavier than males of the same length.

Table 1: Growth and Maturity of Coryphaenoides rupestris from available studies. Estimated parameters of von Bertalanffy growth equation (K, coefficient of growth; L∞, asymptotic length), maturity parameters (L50 and A50 are length (L) and age (A) where 50% of the population have reached maturity) are given by area and by sex.

Areas	Samples			Sex	Length (pre-anal length)		Maturity		References
	n	Age range (year)	Length range (cm)		Lpa ∞ (cm)	K	Lpa50 (cm)	A50 (year)	
West coasts of the British Isles	850	1-54		female	23,9	0,059			Allain & Lorance, 2000
West coasts of the British Isles	834	1-54		male	20,7	0,059			Allain & Lorance, 2000
Skagerrak	321	4-71		female	18,1	0,1	8	8	Bergstad, 1990
Skagerrak	365	4-64		male	14,7	0,105	12	10	Bergstad, 1990
Rockall trough		5-60		female	19,5	0,1	12		Kelly *et al*, 1997
Rockall trough		5-50		male	15,5	0,13	10	10	Kelly *et al*,, 1997

Table 2: Length range (pre-anal length, cm) of different age groups. Data compiled by the WK.

AREAS	AGE 20	AGE 25	AGE 30	SOURCE
VIa	12-17.5	13-17.5	15-18.5	Modal age from European exchange 2011
IIIa	14.5-17.5	14.5-19	17-21	Modal age from European exchange 2011

Length frequency distribution of roundnose grenadier (from MAR-ECO G.O. Sars Leg 2 2004 cruise; In Hunter 2009) was analysed by this WK group and it appears too difficult to distinguish some age classes. Conversely, the relations between the size of fish and the shape (height and length) of the otolith could help to estimate the age (Fig. 2).

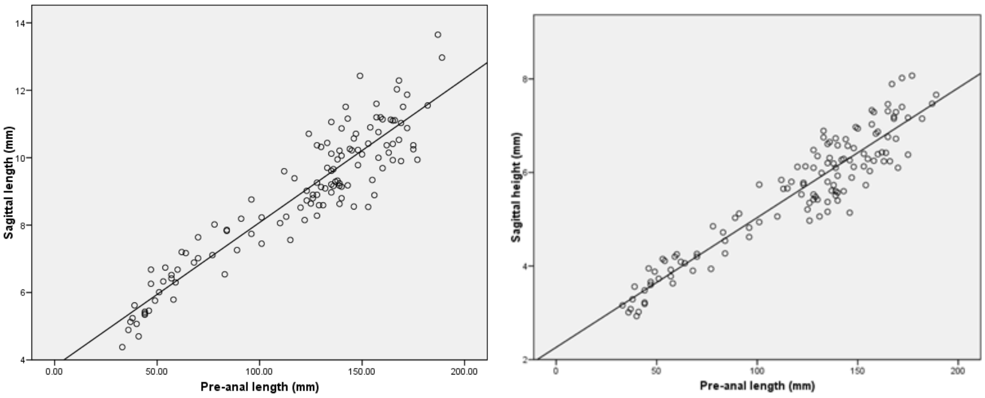

Figure 2: Comparison of pre-anal length (mm, PAL) of roundnose grenadier with sagittal length and sagittal height of otolith (mm). The equation of the line is sagittal length = (0.043 x PAL) + 3.808, R2 = 0.858, p = 0.000. The equation of the line is sagittal height = (0.028 x PAL) + 2.263, R-sq = 0.879, p = 0.000 (In Hunter 2009).

There has been no direct validation of age estimation for roundnose grenadier.

Nevertheless, Gordon and Swan (Gordon et al., 1996 ; Gordon & Swan, 1996 ; Gordon and Swan, 2001) carried out an indirect validation with marginal increment analysis. Based on the examination of the growing edge of otoliths from juvenile fish, they concluded that rings in the otoliths were formed annually. The broader, opaque zones which are generally considered to represent the growth phase were dominant from August to March. The thinner, translucent zones were more dominant from April to July. Compared to shelf species where it occurs in spring and summer, the deposition of opaque material in roundnose grenadier otoliths is delayed by 3-4 months (Gordon and Swan, 2001).

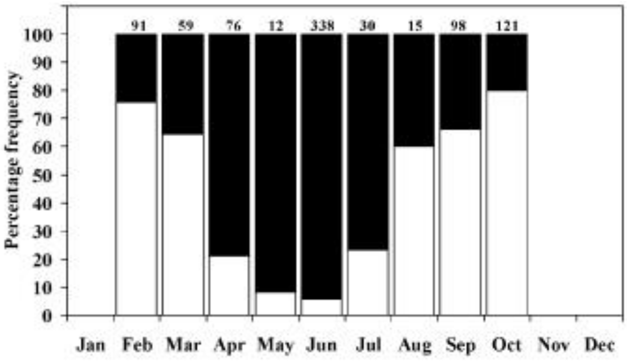

Figure 3: The percentage of otoliths with translucent (indicated by black shading) or opaque edges in different months (⚜ five fish per month excluded) (n= 840, In Swan & Gordon, 2001).

Existing Protocols and Results from Previous Workshops

The first roundnose grenadier age reading workshop was in 2007 (ICES, 2007) after exchange of otoliths was undertaken in 2006. The otolith exchange set in 2006 consisted of 66 otoliths (divisions CIEM Vb, VI and VII) attained during 2005. For each fish, there was a thin transverse section and two calibrated images with transmitted and the reflected light. The results from the otolith exchange showed the overall agreement as 30.2% with a precision of 7.0% CV. Only 2 otoliths were read with 80 % agreement. During the workshop in 2007, the image analysis system approach (software TNPC) showed that the lack of agreement could be due to two reasons: the first being a disagreement on the position of the first ring. The second reason is due to some readers not counting specific rings which were identified by other readers as true annual rings. For the position of the first ring, they analysed an otolith image estimated at 8 years old (Kelly et al., 1997). The age estimated was the same as the one published by these authors and it was consistent with validation of young fish age (Gordon & Swan, 1996). After discussions, a second reading showed the overall agreement of 38.1% with a precision of 6.5 % CV.

In 2011, a second otolith exchange was carried out with 6 readers. It was composed of 2 sets of otoliths from VIa (western Scotland ; n=64, the same as the exchange 2007 used one) and from IIIa (Skagerrak ; n=63) (Mahé et al., 2011). Only images of otolith sections were used during this exchange. The set of otoliths from VIa showed a CV of 14.9% and a percent agreement to modal age of 29.3%. The set of otoliths from IIIa showed a CV of 22.6% and a percent agreement to modal age of 30.7%. The results from both areas were very close to those from 2006. For the roundnose grenadier in the western Scotland (VIa) and in the Skagerrak (IIIa), there was an important bias between the readers and the modal age. In the western Scotland, with the same sampling between 2007 and 2011, the level of precision was very close in the two exchanges (2007 and 2011) but there was a bias between both modal ages and two readings by each reader. The sample from the Skagerrak was composed by younger fish than those of the sample from the western Scotland but the results showed the same bias. Sections of these otoliths remain very difficult to interpret.

Recommended Ageing Protocol (From ICES, 2007)

Choice of Structure And Preparations

The best structures for estimating the age of the roundnose grenadier are sectioned sagittal otoliths. Sectioning is required because the internal structure of otoliths from adult fish are thickand cannot be interpreted whole. All the otoliths are mounted in polyester translucide resin and transverse sections which pass through the nucleus are cut as described by Bedford (1983) with low-speed saw (Buehler Isomet) with a diamond blade. The result is approximately a 0.2 mm thick section. This technique starting from a fine cut (0.2 mm) requires 6 x longer than a cut usually used for the other species (0.35 mm).

Preferred Reading Axis

Two axis of reading are used when ageing roundnose grenadier otoliths (Fig.).

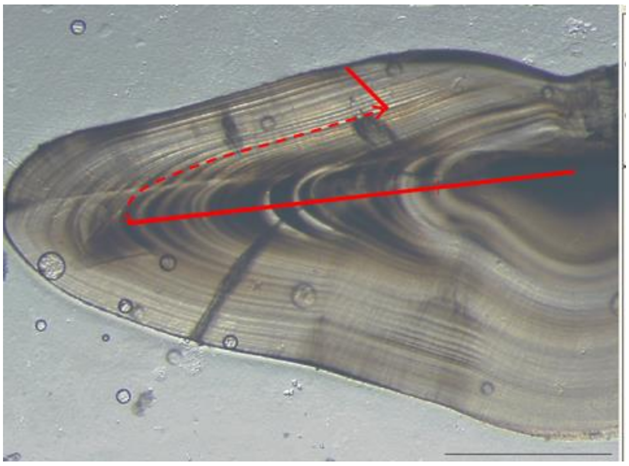

Figure 4: Interpretation axis for roundnose grenadier otolith.

Determination of the First Annulus

In all cases for roundnose grednadier, the 1st of January is the assumed birthdate. One annulus consists of one opaque and one translucent zone. For age estimation, the translucent zones are counted (Fig 5).The distance between the nucleus and the end of the first translucent ring was measured by all readers. The results showed very little differences from one reader to another. The distance between the first ring and the nucleus is on average 1.683±0.214 mm (min: 1.12 mm; max: 2.075 mm) with 40 otoliths.

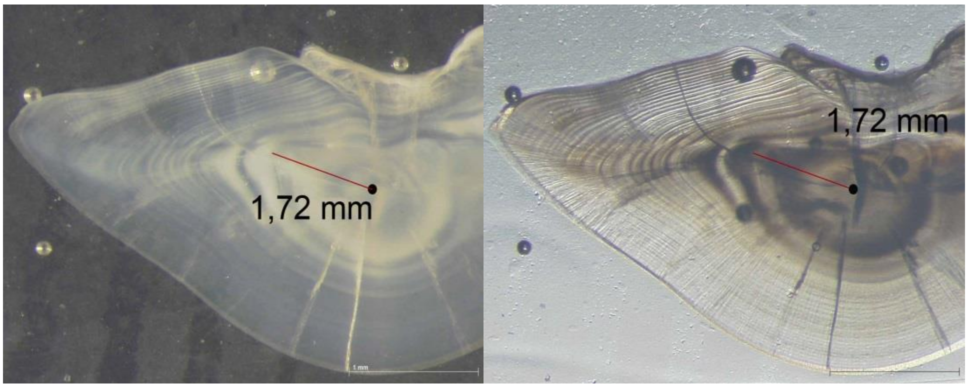

Figure 5: Image of sectioned otolith (fish with pre-anal fin length to 16.5 cm ; n°13). Digitised image with reflected light (A) and transmitted light (B). Black point represents the nucleus and the other tip of the red line shows the end of the first ring.

The first ring is detected with reflected or transmitted light. To help to place the first ring, it is possible to use the grey scale along the radial because the first important peak symbolizes the middle of the first ring. The difference in contrast is more marked as the transmitted light allows for better visualisation of the first ring.

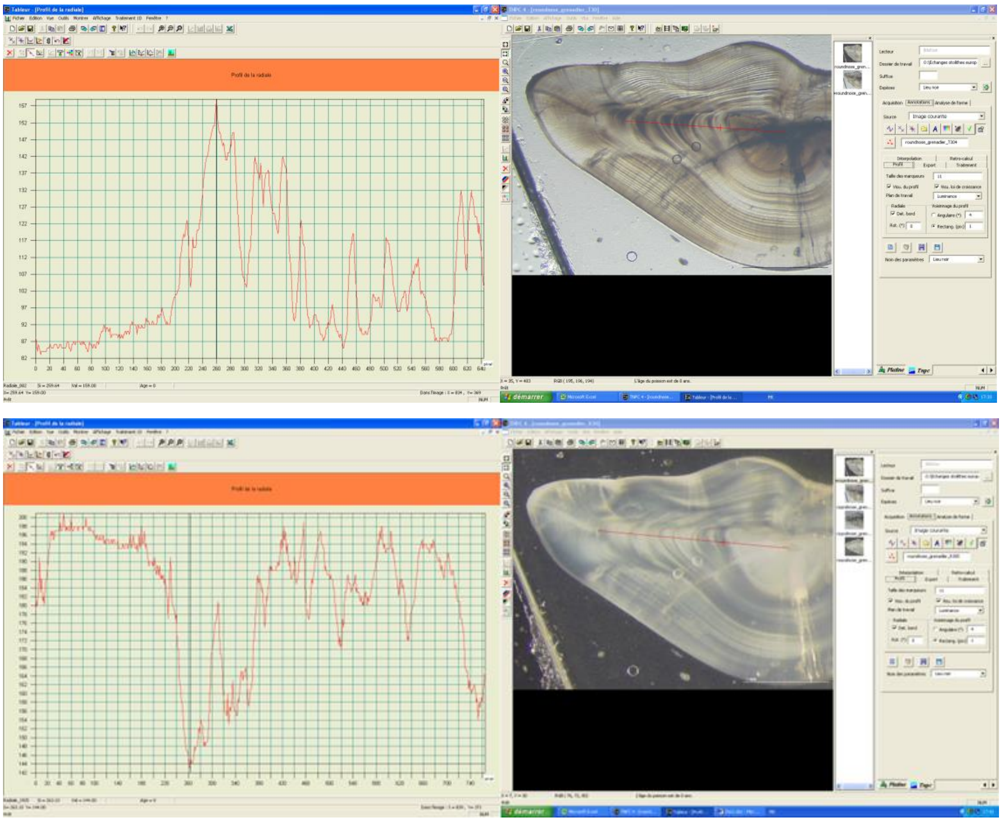

Figure 6: Image of sectioned otolith (fish n°30) with the grey scale along the radial (by software TNPC). Digitised image with transmitted light (A) and reflected light (B).

Edge Growth

Gordon and Swan (Gordon et al., 1996 ; Gordon & Swan, 1996 ; Gordon and Swan, 2001) carried out an indirect validation with marginal increment analysis. Based on the examination of the growing edge of otoliths from juvenile fish, they concluded that rings in the otoliths were formed annually. The broader, opaque zones which are generally considered to represent the growth phase were dominant from August to March (Gordon and Swan, 2001).

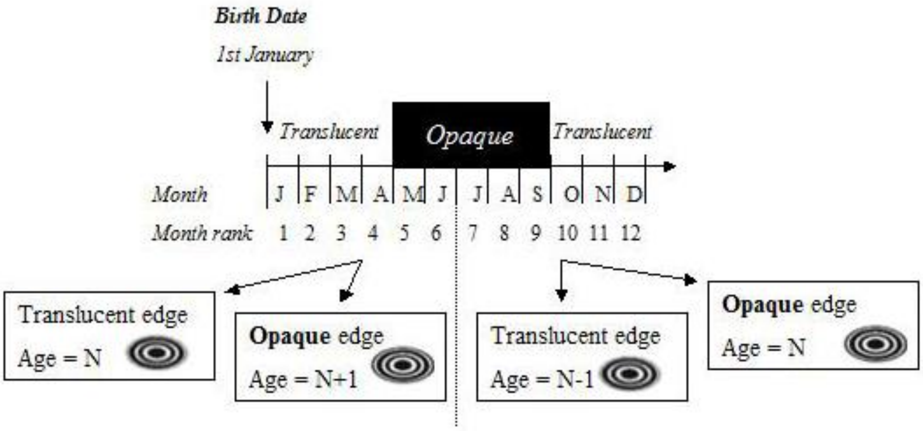

Figure 7: Approach to estimating Roundnose grenadier age (years) from otolith reading from the Atlantic. N is the number of translucent areas. Conventionally, the birth date is fixed at the 1st January as the birth date for all individuals (Williams and Bedford, 1974).

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Annex 5: Black-Spotted Sea Bream

Background Information

Black-spotted seabream (Pagellus bogaraveo) is found in the NE Atlantic, from the south of Norway to Cape Blanc, in the Mediterranean Sea and also in the Azores, Madeira, and Canary Archipelagos. It is a typical protandric hermaphrodite (Buxton and Garrat, 1990). Its reproduction extends throughout the whole year with a peak period that varies according to the region (Bauchot & Hureau, 1986). Generally, most individuals mature first as functional males and then transform into functional females. Sex inversion has been reported to take place between 23.9 and 39.0 cm TL (Krug, 1990; Gil, 2006; Chilari et al., 2006; Mytilineou et al., 2013). Length at first maturity in the Atlantic was estimated by Krug (1990) at an average length of 27.7 cm for males and 34.6 cm for females. This corresponds to the lengths of fish aged 5 and 8 respectively in W. Mediterranean by Gil (2006) at 30.1 cm for males and 35.7 cm for females while Jiménez (2010) recorded smaller lengths of 24.4 cm for males and 28 cm for females. In the Cantabrian Sea, Alcaraz et al. (1987) found similar values of 25 to 29 cm for males and 30-34 cm for females. In the E. Ionian Sea (E. Mediterranean), the mean size of spawning males was 29.7cm and for females, 29.9 cm has been reported (Mytilineou et al, 2013).The young individuals are found near the coast, while the adults on the slope of the shelf indicate an ontogenic migration towards deeper waters (Olivier, 1928; Desbrosses 1932; Morato et al., 2001; Spedicato et al., 2002; Mytilineou et al., 2013). The species grow to at least 80 cm TL (Bauchot and Hureau, 1986) and are considered a slow growing species. Gueguen (1969) reported a maximum age of 20 years. In the Azores Islands, a maximum age of 15 years was observed in a 56 cm length fish (Krug, 1994). More recently A. Garcia (pers. comm.) found a maximum age of 13 years in a specimen of the same length. No more than 10 years of age was reported from otolith readings in the Strait of Gibraltar area but two recaptures from the tag-recapture program have remained at sea for more than 10 years (J. Gil, pers. comm.). In the Eastern Ionian Sea (Greece), 15 years of age was estimated from otolith readings (A. Anastasopoulou, pers. comm.).

Several studies have focused on the age and growth of the black-spotted seabream. The following table compiles growth parameters and performance index for the species by sex and study area.

Reference	Study area	Method	Sex	t_0	k	L_∞ (cm)	Length (cm)	Age (years))
Anastasopoulou, A. (*pers. comm.*)	Ionian Sea	O	All	-1.37	0.16	47.1	14-43	2-15
J. Gil (*pers. comm.*)	Strait of Gibraltar	O	All	-0.57	0.157	62**	24-54	3-10
J. Gil (*pers. comm.*)	Strait of Gibraltar	T-R	All		0.062-0.147	62**	15-45	
Chilari et al., 2006	Ionian Sea	O	M	-1.81	0.11	49.2	15-40.2	2-9
			F	-2.23	0.10	49.5		
Chilari et al., 2006	Ionian Sea	S	M	-3.40	0.08	46.1	15-40.2	1-13
		S	F	-4.73	0.06	52.8		
Sobrino & Gil, 2001	Strait of Gibraltar	O	All	0.67	0.17	58.0**	11-54	0-8
Mytilineou & Papaconstantinou, 1995	N. Aegean Sea	O	All	-2.72	0.18	25.1 FL	7-17.58*	0-3
Krug, 1989	Azores	O	All	-1.55	0.12	58.5 FL	15.1-49*	1-14
Alcazar et al., 1987	Astrurian waters	O	All	-0.47	0.20	48.7	15-47	1-13
Sanchez, 1983	Cantabrian Sea	O	All	-0.53	0.21	51.6	13.7-49.6	1-12
Gueguen, 1969	Cantabrian Sea	O	All	-2.92	0.09	56.8 FL	17.2-49.9*	1-20
Ramos & Cendrero,1967	Cantabrian Sea	O	All	-1.02	0.13	53.9 FL	18.1-44.5*	2-12
Castro, 1990	Bay of Biscay	O	All	-0.66	0.17	54.2	19-50	1-11

* Fork Length (cm), O: otoliths, S: scales, T-R: tag and recapture

** Fixed (Largest individual)

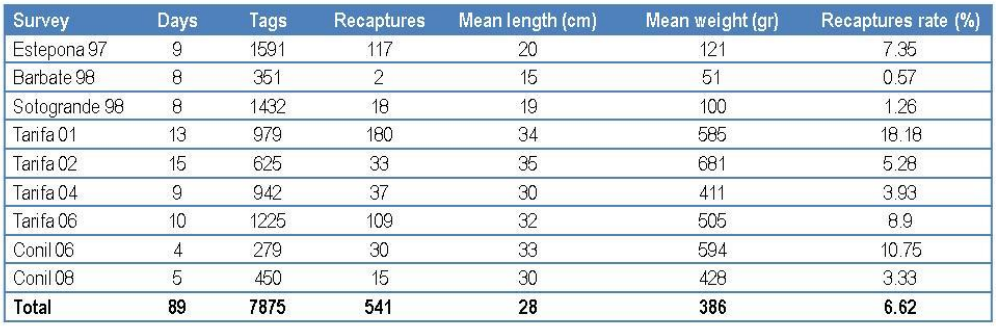
The length ranges (cm) found in the literature for ages 1, 5 and 8, based on otolith readings for the black-spotted seabream, can be found in the following table:

Area	Sex	Age 1	Age 5	Age 8	Reference
Ionian Sea	M	-	22-27	30-35	Chilari et al., 2006
	F	-	22-28	30-34	
Strait of Gibraltar	All	15-19	29-43	43-54	Sobrino & Gil, 2001
Strait of Gibraltar	All	-	29-43	43-51	J. Gil (*per. com.*)
Azores	All	12-18	23-36	34-44	Krug, 1989
Ionian Sea	All	19	29-35	37-39	A. Anastasopoulou (*pers. comm.*)
Astrurian waters	All	15-20	24-38	36-44	Alcazar et al., 1987
Cantabrian Sea	All	8-15	24-36	42-46	Gueguen, 1969

DOP (Azores, Portugal), HCMR (Athens, Greece) and IEO (Cadiz, Spain) are all involved in P. bogaraveo ageing.

Validation, Growth and Longevity

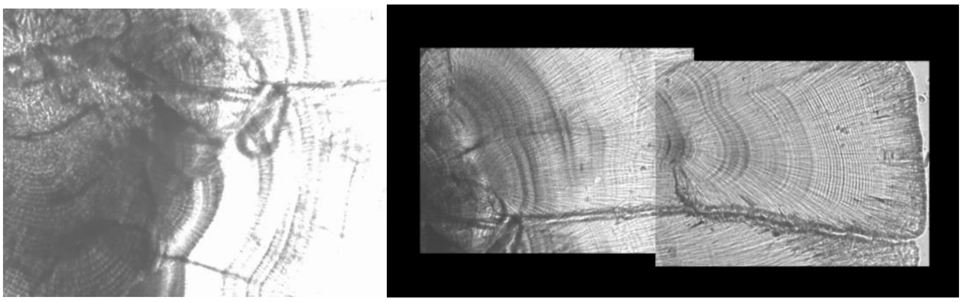
Tag-recapture experiments were conducted by IEO (Cadiz, Spain) between 1997 and 2008 in the south Mediterranean coast and Strait of Gibraltar waters. Summary information by survey can be seen in the following Table.



Survey	Days	Tags	Recaptures	Mean length (cm)	Mean weight (gr)	Recaptures rate (%)
Estepona 97	9	1591	117	20	121	7.35
Barbate 98	8	351	2	15	51	0.57
Sotogrande 98	8	1432	18	19	100	1.26
Tarifa 01	13	979	180	34	585	18.18
Tarifa 02	15	625	33	35	681	5.28
Tarifa 04	9	942	37	30	411	3.93
Tarifa 06	10	1225	109	32	505	8.9
Conil 06	4	279	30	33	594	10.75
Conil 08	5	450	15	30	428	3.33
Total	**89**	**7875**	**541**	**28**	**386**	**6.62**

Healthy fish were measured to the centimeter below and a tag was inserted in the dorsal muscle, close to the first rays of the dorsal fin. External tags were coloured and comprised of a spaghetti T-bar anchor of 4 cm long, containing a unique code and the contact address. Fish were always released in the same area as they were caught and all the information was recorded. Advertising in regards to the surveys, rewards and what to do in the case of catching a tagged sample (recapture) was provided internationally. Tagging surveys have made aware of the fact that countries involved in P. bogaraveo ageing are over estimating the age (J. Gil, pers. comm.).

A daily increment analysis on young individuals was carried out in the HCMR in order to detect and clarify the position of the first annual ring. The initial results indicated that all specimens in the range of 14 - 15 cm in length had completed the first annual ring. The following figure represents an individual with TL 90 mm with 167 daily increments.



Existing Protocols and Results from Previous Workshops

There have been no workshops on ageing black-spotted sea bream. However, all Institutes (DOP, HCMR and IEO) involved in P. bogaraveo otolith reading have internal protocols in order to improve agreement between readers and in return, provide more precise data. These protocols presented some guidelines for ageing P. bogaraveo to establish ageing criteria for the species.

Recommended Ageing Protocol

The criteria of the existing protocols adopted by all the Institutions are explained in the following sections.

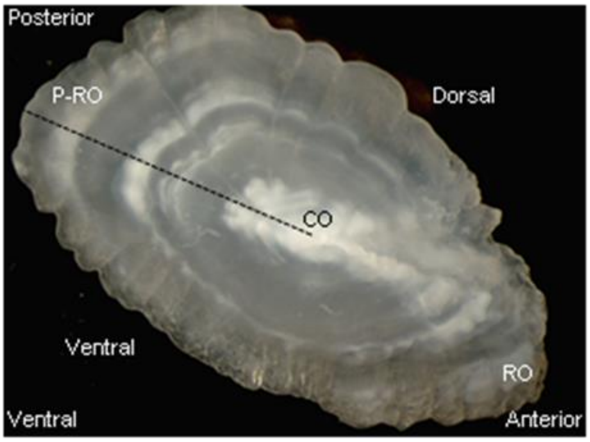
Choice of Structure and Preparations

Black-spotted sea bream has been aged by counting the annuli in otoliths (Ramos & Cendrero, 1967; Sánchez, 1983; Alcazar, 1987; Krug, 1989; Castro, 1990; Sobrino & Gil, 2001; Gil, 2006; Gil, 2010) and less often in scales (Chilari et al., 2006; Olivier, 1928; Gueguen, 1969).

The whole otoliths are used by all labs as it can produce satisfactory results without requiring expensive and laborious methods. The otolith is placed in a petri dish, sulcus side-up and immersed in water for 30 minutes (HCMR) or for 5 minutes in ethanol and glycerine (96%) (1:1) (IEO and DOP). This helps to reduce glare on the three dimensional surface of the otolith and/or improve the visibility of the growth bands on the otolith surface (clearing technique). Each otolith is observed under a binocular microscope with reflected light against a black background. The magnifications used tend to depend on the size of the structure. However, 8x magnification is often the preferred focus used to age P. bogaraveo. It is important to adjust the light and focus of the binocular microscope accordingly in order to identify the translucent and opaque rings. In DOP and HCMR, ages are usually estimated by reading digital images taken with a camera. Measurements are taken from the left otolith, but the right otolith can also be used if any problems are presented with the left. In this case, only the number of rings is taken into account without any measurement. In the case of IEO, the annuli are counted directly under the binocular microscope. It should be noted that the use of reflected light and a black background will cause translucent rings to appear darker and opaque rings lighter. For age determination only the translucent rings (annuli) are counted. For this we assume that one annulus represents one year of growth. The readings were performed twice and repeated approximately two to three months later. However, only two or more coincidental ageing estimates were accepted, i.e. if an agreement could not be reached, such otoliths are excluded from further analysis.

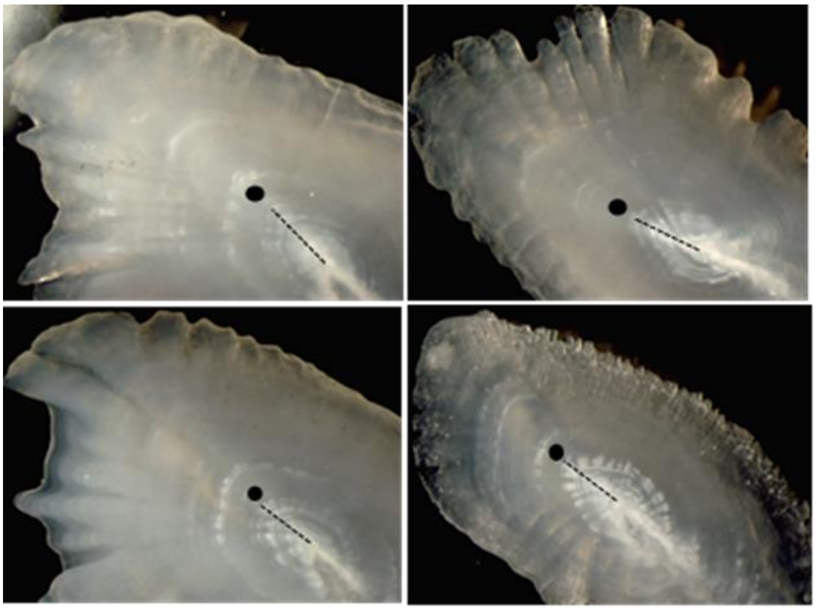
Preferred Reading Axis

Annuli in P. bogaraveo otoliths are not always prominent and distinct along the entire growth axis. Defined annuli are usually found along the fast growth axis of the otoliths, particularly along the post-rostrum (P-RO, dashed line), where a better contrast between rings can be found. However, when possible the reader should attempt to obtain identical counts over the rostrum (RO), before assigning an age. Consistent counts are most easily made from the core (CO) to the outer margin of the otoliths.



Determination of the First Annulus

In all cases for P. bogaraveo otoltihs, the 1st of January is the assumed birthdate. The core is surrounded by several narrow translucent rings. These checks (false rings) may occur when there is a change in diet, depth or habitat during the early life of the species. The first annulus is defined as the first prominent, translucent increment visualised after the nucleus. This increment presents a greater width than that of the checks (see black dots).

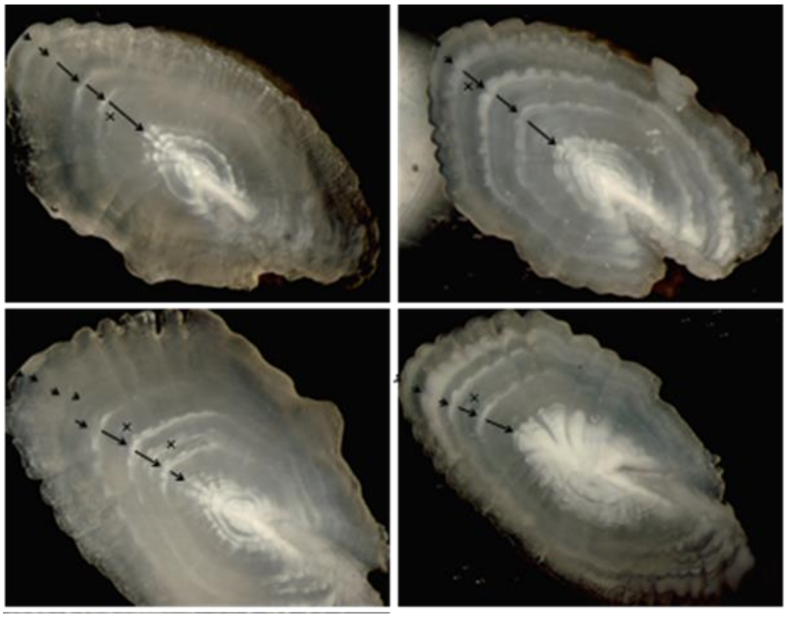


Identification of Annual Zones and Checks in Young Fish

Annuli are typically less defined along the short axis of the P. bogaraveo otoliths, thus as previously mentioned, age estimation should be interpreted along the long axis.

At times, translucent and opaque rings are well defined and clearly visible. However, most of the time the zones are split which makes annuli identification very difficult and subjective. deposition of checks may be a result of atypical temperatures, feeding or spawning conditions, stress or disease.

It is always difficult to define the appearance of checks on P. bogaraveo otoliths for all individuals. However, to overcome these difficulties it is important to be consistent in identifying such areas as true or false annuli, by always following the same criteria. Checks on P. bogaraveo otoliths are usually considered to be those rings that: i) are not as well defined as annual rings; ii) are usually not prominent along all axis, particularly along the short axis; and iii) are discontinuous or merge into annuli along the shorter axis.



Transition Zones

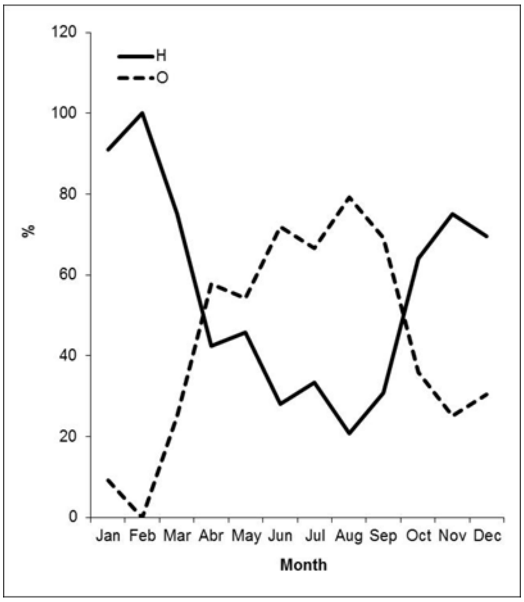
Transition zones in P. bogaraveo are characterized by many checks. To overcome the difficulties associated with the identification of true or false annuli along the transitional area, it is important to establish a common criterion as defined above.

Slow Growth Zones

As the otolith grows, the relative widths of each annulus decrease progressively and are less evident. Although, conditions affecting the life history of the fish can create unexpected relative width proportions between annuli. Thus, age reading in older fish can be a difficult task and great care must be taken in order to correctly identify the "older" opaque areas and annuli. In those specimens, the growth bands cannot be seen along all the otolith's axis. The suggestion is to always read along the long growth axis, particularly in the P-RO axis and compare the counts with the ones obtained along the RO axis.

Edge Growth

A translucent and opaque edge is deposited annually in all age-classes. Typically, translucent zone formation is usually seen in early fall through to winter while an opaque zone tends to form from late spring until the summer. The pattern deposition observed for P. bogaraveo is represented in the following graph.



The edge classification is not an easy task for age readers of P. bogaraveo, but it is easiest to interpret the edge along the fast growth axis of each otolith. In order to avoid the implications of misclassification bias on edge type could have on fish age, we adopted the January 1st as its birthdate, according with the international convention. To improve the fit of growth curves to the age data, the age of each fish should be converted into absolute decimal age. Absolute decimal age was calculated as the number of annual bands plus the percentage of the time of year (from January 1st) that had passed since the date of capture. For example, if the estimated age of an otolith from a fish caught in March was 7 the corrected age would be 7+ (3/12) = 7.25. This procedure will allow the minimization of the variability associated with a large sampling period.

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Annex 6: Black Scabbard Fish

Background Information

The black scabbardfish, Aphanopus carbo Lowe, 1839 (Actinopterygii: Trichiuridae) (Figure 1), occurs throughout the North Atlantic between 30oN and 70oN, from the strait of Denmark to the coast off the western Sahara, being most abundant to the south of the Faroe Islands, in the Rockall Trough, to the west of mainland Portugal, and around Madeira and the Canary archipelagos, but also occurring in Iceland, the Mid-Atlantic Ridge and Corner Rise, and the Azores (Nakamura and Parin 1993; Parin 1995; Pajuelo et al. 2008; Machete et al. 2011).

Figure 1. Black scabbardfish, Aphanopus carbo.

It is a benthopelagic species that is commercially exploited at depths between 200 m, in the northern section of the NE Atlantic (Nakamura and Parin 1993; Kelly et al. 1998), and 2300 m around the Canary Islands (Pajuelo et al. 2008).

Recently, it was found that A. carbo coexists spatially with the intermediate scabbardfish Aphanopus intermedius Parin, 1983 in the Azores, Madeira, the Canaries, and off the coasts of Morocco and the Western Sahara (Nakamura and Parin 1993; Stefanni and Knutsen 2007; Stefanni et al. 2009; Biscoito et al. 2011). The two species are morphologically very similar, yet both genetics (Stefanni and Knutsen 2007; Knutsen et al. 2009; Stefanni et al. 2009) and meristic characteristics (Tuset et al. 2010; Biscoito et al. 2011) have proven suitable for reliable identification.

Mature and spawning adults were only reported in Madeira (Figueiredo et al. 2003; Neves et al. 2009;Ribeiro Santos et al. 2013a), the Canaries (Pajuelo et al. 2008), and the northwest coast of Africa (Perera 2008) during the last quarter of the year. Estimated female length at first maturity (L50) was 103 cm around Madeira (Figueiredo et al. 2003) and 114 cm around the Canary Islands (Pajuelo et al. 2008). The possible mixture of black and intermediate scabbardfish specimens in the samples may have caused biased results (Farias et al. 2013). In more recent work, two different values were estimated: 111 cm for females from Madeira and 116 cm for females from Madeira and the west of the British Isles together (Ribeiro Santos et al. 2013a). These values are probably overestimated because the study did not use specimens from Madeira smaller than 92 cm in total length (Farias et al. 2013).

No eggs or larvae of black scabbardfish have ever been found; the smallest specimens recorded being 10 cm. The first one, together with a 15 cm long specimen, was found in the stomach of two long snouted lancetfish (Alepisaurus ferox Lowe, 1833) (Maul, 1950). More recently, another specimen of 10 cm in length (identified by DNA barcoding) was caught at the Senghor Seamount, off the northeast of Cape Verde (Hanel et al. 2010).

Several studies have focused on the age and growth of the black scabbardfish in NE Atlantic (Table 2). The estimated growth rate for the black scabbardfish was relatively rapid for all studies. Figure 2 represents the growth curves according to sex for all available studies, restricted to each sample's fish length range.

Differences found between studies on the estimates of L◎ and k appears to be related with the otolith preparation techniques. When using whole otoliths, it is difficult to identify the increments in the otolith terminal zone in older fish (Morales-Nin and Sena-Carvalho 1996; Vieira et al. 2009); hence the age of larger fish tends to be underestimated. In fact, in specimens over 110 cm TL the discrimination of growth increments becomes more difficult with age (Vieira et al. 2009). On the other hand, in the results from Kelly et al. (1998), the occurrence of split zones as a consequence of the otolith sectioning made the increments interpretation difficult, which resulted in the overestimation of the specimens' ages (Morales-Nin et al. 2002).

Based on Figure 2, growth estimates from Vieira et al. (2009) and Delgado et al. (2013) seem to be in agreement, without any meaningful area effect. The fact that age-at-length from Kelly et al. (1998) was consistently higher than in all the other studies implied a low k for fish from the west of the British Isles. Given the predominance of young immature specimens in this area, this result was not expected and was a consequence of age overestimation as explained above.

Table 1. Summary of black scabbardfish growth studies carried out in the NE Atlantic, namely the von Bertalanffy growth parameters. F, females; M, males; SD, standard deviation; NA, not available.

Area	Sex	N	TL	Age	L_∞ (±	k	t_0	Reference
W British Isles	both	230	75-120	4-32	*NA*	0.1	*NA*	Kelly et al. 1998
Mainland Portugal (ICES IXa)	F	248	64-131	5-13	135 ± 4	0.2	-2.0	Vieira et al. 2009
	M	206		4-10	124 ± 3	0.2	-1.7	
Madeira (CECAF)	F	334	58-151	0-8	142	0.3	-2.1	Morales-Nin and Sena-Carvalho 1996
	M	357	58-132		155	0.2	-3.3	
	both	649	58-151		139	0.3	-2.3	
	F	200	125-148	8-15	159 ± 4	0.1	-2.3	Vieira et al. 2009
	M	163		8-14	146 ± 1	0.1	-1.4	
	F	554	100-140	6-14	136 ± 5	0.2	-4.2	Delgado et al. 2013
	M				132 ± 5	0.2	-3.1	
Canary Islands (CECAF)	F	196	100-148	2-12	149 ± 2	0.2	-4.7	Pajuelo et al. 2008
	M	102	104-134	2-8	141 ± 4	0.3	-3.5	
	both	298	100-148	2-12	148 ± 2	0.2	-4.6	

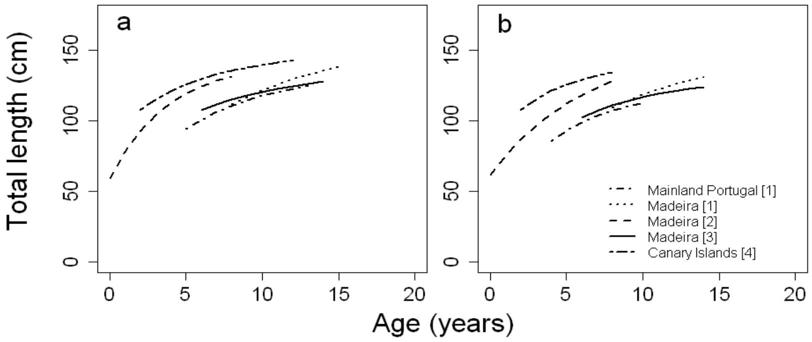

Figure 2. Growth curves for black scabbardfish from different studies, areas and methods. (a) females; (b) males. Growth parameters are from [1] Vieira et al. (2009); [2] Morales-Nin and Sena-Carvalho (1996); [3] Delgado et al. (2013); [4] Pajuelo et al. (2013). From Farias et al. (2013).

Validation, Growth and Longevity

Length frequency distributions for different ICES and CECAF management units in 2011 are presented in Figure 3. In general, the size distributions move towards higher values from north to south of the NE Atlantic. The smallest reported fish was caught off Iceland and was in the size class of 61 cm. The absence of small juveniles in the samples poses a major difficulty for this species' age estimation because age-0 specimens are not found.

In the Azores, a bimodal length distribution was found in samples from the south of Pico Island (Figure 3 g). This is a probable consequence of the mixing between specimens of black scabbardfish and intermediate scabbardfish, since the latter species has been described for this area (Stefanni and Knutsen 2007).

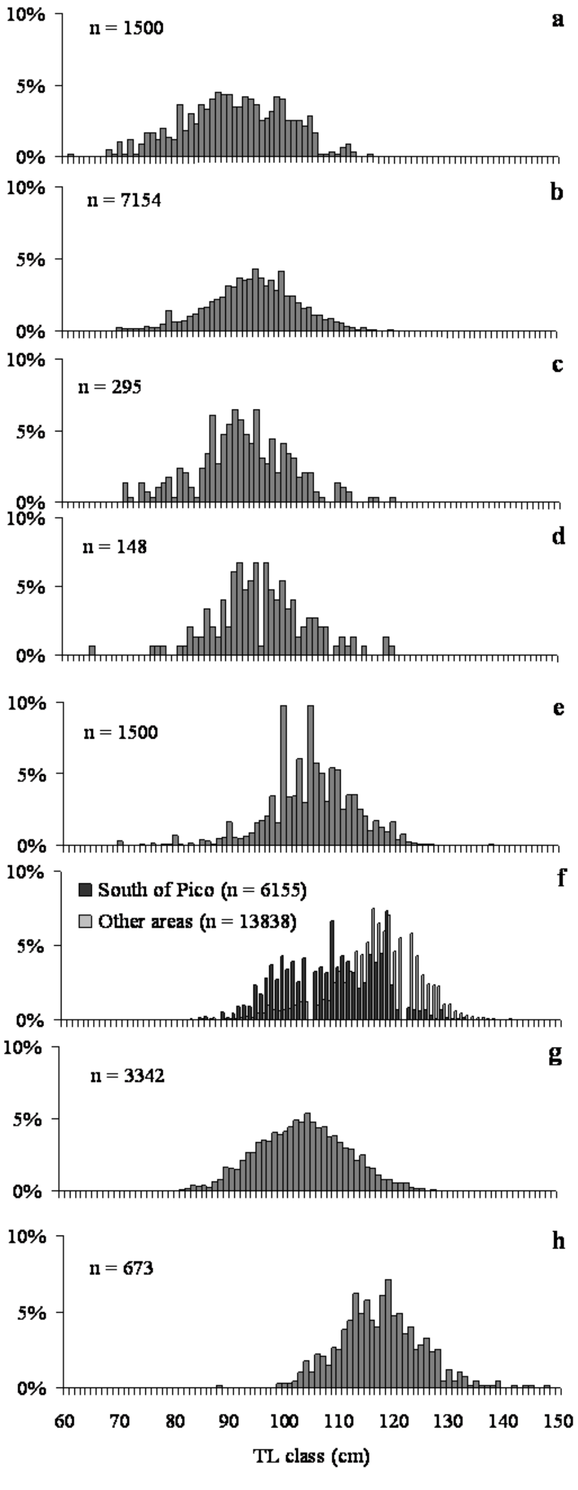

Figure 3. Length frequency distribution of black scabbardfish in 2011 from north to south: (a) Icelandic surveys (ICES Division Va); (b) on board observations of French trawlers (mostly in ICES Division VIa); (c) on board observations of Spanish trawlers off the west of the British Isles (VIb); (d) on board observations of Spanish trawlers (Subarea XII); (e) self-sampling Faroese exploratory surveys (Subarea X); (f) Portuguese longline fishery off mainland Portugal (IXa); (g) experimental fishery in the Azores (data are from 2005); (h) sampling of commercial landings in Madeira. Length frequency data are from [a-f] ICES (2012); [g] Machete et al. (2011); [h] Delgado et al. (2013). From Farias et al. (2013).

Based on published information, the length range for different age classes was analysed (Table 3). The differences found between the studies are mostly related with the otolith preparation techniques, as explained in the previous section.

Table 2. Length ranges found for ages 1, 5, 8, and 12 for the black scabbardfish.

Area	Sex	Age	Age 5	Age 8	Age 12	Reference
Mainland Portugal	F	*NA*	87-97	105-	120-	Vieira et al. 2009
	M	*NA*	85-95	100-	*NA*	
Azores	F	*NA*	*NA*	175-	140-	Vieira et al. 2009
	M	*NA*	*NA*	85-95	115-	
Madeira	F	*NA*	*NA*	107-	120-	Vieira et al. 2009
	M	*NA*	*NA*	107-	120-	
	F	58-70	104-	114-	*NA*	Morales-Nin and Sena-Carvalho 1996
	M	58-94	100-	130	*NA*	
Canaries	both	*NA*	*NA*	105-	125-	Delgado et al. 2013

There has been no direct validation of age estimation for black scabbardfish. The annual deposition of increments has been tested by marginal increment analysis (Morales-Nin and Sena-Carvalho 1996; Vieira et al. 2009). This subject is further explored below, under the section EDGE GROWTH.

Existing Protocols and Results from Previous Workshops

In the first studies on age and growth of the black scabbardfish, Morales-Nin and Sena-Carvalho (1996) used whole sagitta otolith, whereas Kelly et al. (1998) used thin sections embedded in epoxy resin. More recently, Pajuelo et al. (2008) used slightly burned whole otoliths immersed in 50% glycerol-alcohol for age assignment of specimens collected off the Canary Islands, while Delgado et al. (2013) used the left whole otolith immersed in a 1:1 glycerin-alcohol solution collected from fish caught off Madeira.

The protocol presently being followed in IPMA (Portuguese Sea and Atmosphere Institute, former IPIMAR) is described in Vieira et al. (2009). Right otoliths were embedded in epoxy resin blocks (7x6 otoliths per block) and transversely sectioned through the nucleus with a diamond-tipped saw blade (Labcut 230 Cutting Machine) rotating at 3700 rpm, following the technique proposed by Bedford (1983) and McCurdy (1985). Slides 0.5 mm thick were mounted in a glass slide with translucent glue, brushed with a 1:1 glycerin-alcohol solution, and observed in a stereomicroscope with a micrometric ocular under transmitted light and with a magnification of 18×. Otolith sections were photographed using the software TNPC 4.1 integrated in Visilog 6.3.0 (Fig. 4).

Figure 4. Transversal section of the right otolith of a black scabbardfish specimen caught off mainland Portugal. This specimen is a female with 103 cm TL. This section was photographed with the posterior side of the otolith facing up and the ventral side turned to the right. Magnification 37.5×.

More recently, the protocol developed in IMEDEA-CSIC/UIB (Mediterranean Institute for Advanced Studies) to prepare otoliths for laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) for trace elements analysis was adopted for preparing thin sections of black scabbardfish otoliths for age reading. Right otoliths were imbedded in a small block of resin (Buehler® Epo-Thin Low Viscosity Epoxy) with the sulcus acusticus facing down and the rostrum to the right. Thin sections with 0.5 mm were cut using a Buehler® Isomet low-speed cutting machine.

The sections made available in the present otolith exchange workshop were prepared following the previous protocol and were photographed with a Leica M165C stereomicroscope linked to an ALLIED Marlin F080-B digital camera, using the software AVT Smartview 1.7.2., under transmitted light and with a magnification of 33x (Figure 5).

Figure 5. Transversal section of the right otolith of a black scabbardfish specimen caught off Madeira. This specimen is a male with 106 cm TL. This section was photographed with the anterior side of the otolith facing up and the ventral side turned to the left. Magnification 33×.

The main advantage of this sectioning technique comparing with the previous one is the ability of manipulating each otolith individually and hence guaranteeing that the blade cuts exactly through the nucleus and that the cutting axis is perpendicular to the anterior-posterior axis.

The number of translucent increments is counted and the otolith edge is classified as opaque or translucent, considering that a valid increment is the one that can be followed all the way around the otolith. Afterwards, the fish age is assigned as the number of translucent increments, except if the fish was caught between the 1st January and the 30th June (as its birthdate is assumed to be the 1st of January. See section below) and the otolith border is opaque, in which case the age corresponds to the number of hyaline increments plus one.

For this species, there has been one single intercalibration exchange of otoliths, which occurred within the project Basblack (1998-2000) "Environment and biology of deep-water species Aphanopus carbo in the NE Atlantic: basis for its management". In this exchange four sets of otoliths were used: whole and burned right otoliths and thin transverse sections of the corresponding left otoliths from fish from Madeira, and whole left otoliths and thin transverse sections from the corresponding right otolith from fish from the Rockall Trough.

In the last few years, age readers from mainland Portugal, Madeira, and the Canaries have been working together in the age estimation of the black scabbardfish. In the near future, otoliths from fish from the Azores will start being analysed in DOP (Department of Oceanography and Fisheries of the University of the Azores).

Recommended Ageing Protocol

Choice of Structure and Preparations

The best structures for estimating the age of the black scabbardfish are sectioned sagitta otoliths. Clean and dry otoliths are embedded in epoxy resin and thin transversal sections with a thickness of 0.5 mm are cut through the otolith nucleus.

The sections are observed in a stereomicroscope (dissecting microscope) with transmitted light and a magnification of around 30x. Using a camera coupled to the microscope, images are saved for posterior analysis with TNPC 4.1. This software was developed by IFREMER and Noesis SA for the analysis and interpretation of calcified structures.

Preferred Reading Axis

The best axis for age reading in black scabbardfish sectioned otoliths is along the ventral side (Vieira et al. 2009).

For measuring the radius of each increment (distance from the nucleus to the end of the translucent increment) and comparing the measurements, a linear transect should be followed. Nonetheless, this rule is usually impossible to keep in larger fish because the growth axis in the last years is oblique to the axis in the first years (see Figure 6).

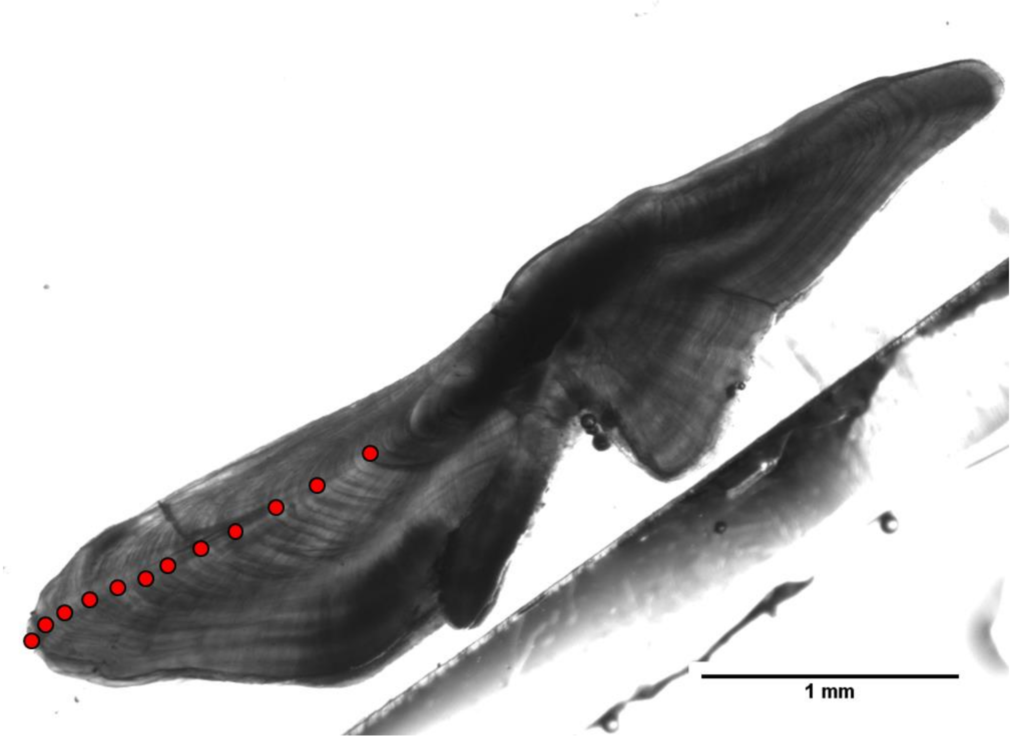

Figure 6. Transversal section of the right otolith of a black scabbardfish specimen caught off the Canary Islands in September. This specimen is a male with 118 cm TL. This section was photographed with the anterior side of the otolith facing up and the ventral side to the left. Magnification 33x. The estimated age for this specimen is 12 years old.

Determination of the First Annulus

In all cases for black scabbardfish, the 1st of January is the assumed birthdate since spawning occurs between September and December (Figueiredo et al 2003; Neves et al. 2009; Ribeiro Santos et al. 2013a), and that a pair of contiguous opaque and translucent increments are deposited annually.

The first annulus is defined as the first translucent increment visualised after the nucleus (see Figure 6). This increment is expected to appear at 0.73±0.01 mm and 0.56±0.02 mm from the nucleus on the ventral and on the dorsal side of the otolith respectively (Vieira et al. 2009).

Since no samples of juvenile black scabbardfish are available, these measurements were made on adult fish otoliths, assuming that the first well marked translucent increment after the nucleus was the first increment to be deposited.

Identification of Annual Zones and Checks in Young Fish

In black scabbardfish sectioned otoliths, many false increments (checks) are found along the growth axis, mostly in the area corresponding to the juvenile growth area. Hence, the agreed way to identify increments is considering bands (thick areas) instead of rings (lines). In the juvenile growth area, wide areas of rapid growth (opaque bands) are separated by a well-marked narrow area of slow growth.

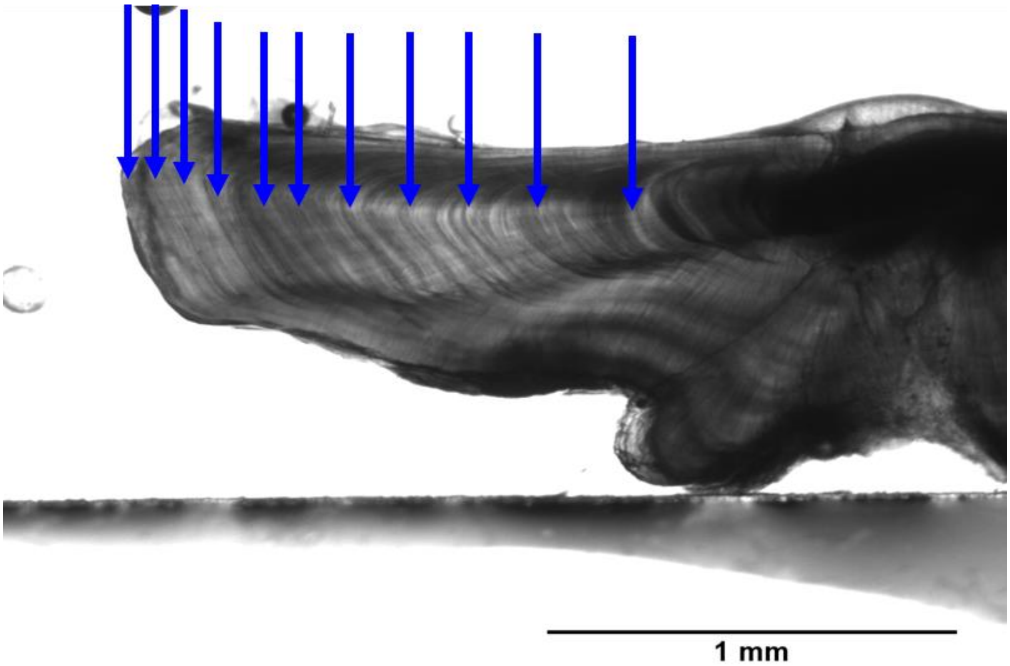

Figure 7. Transversal section of the right otolith of a black scabbardfish specimen caught off Madeira. This specimen is a male specimen with 106 cm TL. This section was photographed with the anterior side of the otolith facing up and the ventral side turned to the left. Magnification 52.8x. The blue arrows mark the translucent increments. The estimated age for this specimen is 11 years old.

In most otoliths, the first 5-6 increments are deposited relatively equidistant, at a distance of approximately 0.02 mm. The following increments are separated approximately 0.01 mm. From around the tenth increment on, the increments are very narrow and sometimes difficult to differentiate.

Transition Zones

In black scabbardfish sectioned otoliths, a change in the growth deposition pattern occurs around the first 5-6 increments. This change corresponds to the first maturity of this species.

Slow Growth Zones

In juvenile growth area, it may be difficult to distinguish the slow growth zones from the false increments (checks) that can be found throughout the fast growth zone. For clarification see the sections above.

Edge Growth

In specimens from Madeira, opaque increments were formed mostly between July and December and the highest occurrence of opaque margins was found in October (Morales-Nin and Sena-Carvalho 1996) (Figure 8).

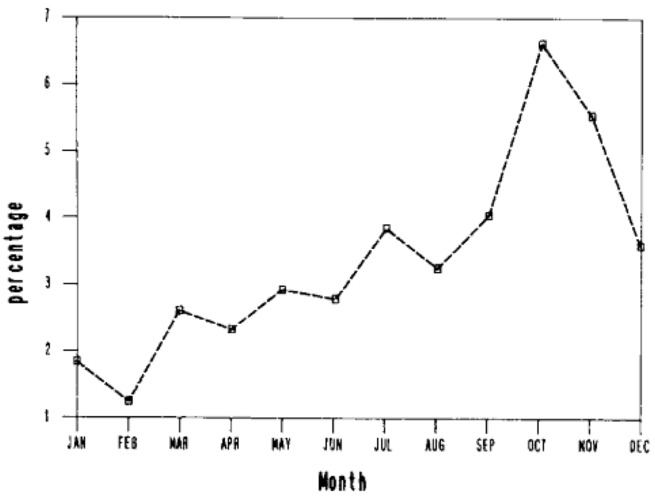

Figure 8. Monthly percentage of black scabbardfish caught off Madeira with opaque margins (Morales-Nin and Sena-Carvalho 1996).

In specimens from mainland Portugal, opaque increments were formed mostly between April and September (Figure 9).

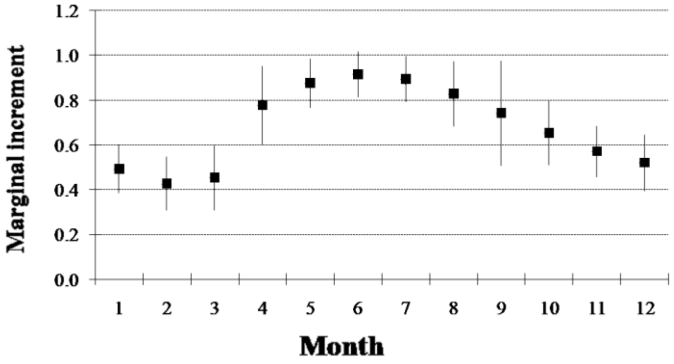

Figure 9. Monthly evolution of marginal increment analysis (MIA) in sectioned otoliths of black scabbardfish caught off mainland Portugal (Vieira et al. 2009).

Differences in otolith deposition were found between Madeira and mainland Portugal. These could result from environmental differences between the two areas (Madeira is typically tropical and mainland Portugal is temperate). Moreover, the otolith preparation technique was different between these studies. In the first study, whole otoliths were analysed whereas in the former study, thin sections were used. It has been shown that, when using whole otoliths for ageing large fish, the increments in the terminal zone of the otolith are difficult to distinguish and to classify as either opaque or translucent (Vieira et al. 2009).

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Annex 7: Tusk

Background Information

Biology and distribution

Tusk, Brosme brosme (Ascanius, 1772) (Figure 1), is a medium to large sized codfish, often caught between 40 to 70 cm long (Magnusson et al., 1997). The largest individual caught measured 120 cm (Frimodt, 1995). Sexual maturity is believed to be reached at the age of 8 to 10 years, at the total length of 45 to 60 cm (Choen et al., 1990; Magnusson et al., 1997). Longevity is expected to be at least 20 years of age (Choen et al., 1990; Table 1). Age range, VBF parameters and maturity for tusk are listed in Table 1. Expected mean length at age for age by area is shown in Table 2. Tusk mostly occur on hard bottoms at depths between 20 to 1000 m, with older fish usually found in deeper waters (Choen et al., 1990).

Distribution: Northwest Atlantic: New Jersey to the Strait of Belle Isle and on the Grand Banks of Newfoundland. Tusk is rare at the southern tip of Greenland. Northeast Atlantic: Off Iceland, in the northern North Sea, and along the coast of Scandinavia to the Murmansk Coast and at Spitsbergen, found in temperatures from 0 to 10 °C (Choen et al., 1990; Magnusson et al., 1997).

Tusk feed on a variety of crustaceans and fishes, such as: lobster, crabs, Norway pout and redfish. Spawning takes place at a depth of 200 to 400 m from April to July. Ripening adults, spawning and eggs have been observed in all parts of the distribution areas in the Northeast Atlantic (Magnusson et al., 1997 and references therein).

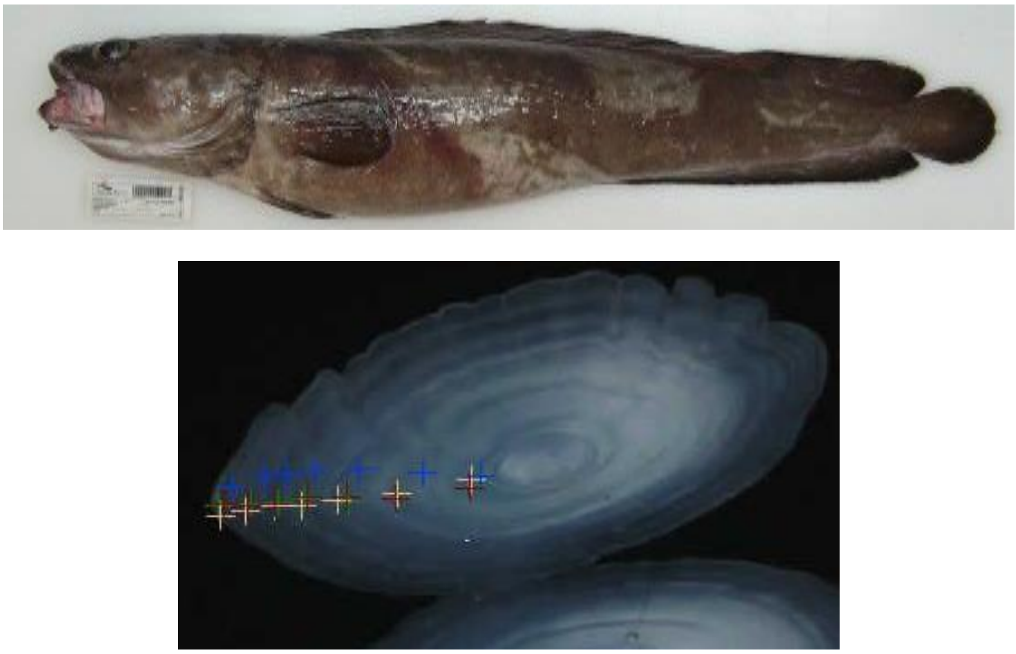

Figure 1. Tusk, Brosme brosme (Photo: Mar-Eco and a tusk otolith with annotations of annuli.

Table 1. Growth and maturity of Brosme brosme from available studies. Estimated parameters of von Bertalanffy growth equation (K, coefficient of growth; L∞, asymptotic length; t0, theoretical time (year) at which the fish has zero length), maturity parameters (L50 and A50 are length (L) and age (A) where 50% of the population have reached maturity) are given by area and by sex.

Area	Samples		Sex	VBF			Maturity		References
	n	Age range (year)		L_∞ (cm)	K	t_0	L_{50} (cm)	A_{50} (years)	
All		>20	both				45-60	8-10	Choen et al., 1990
Vb2	19	8-17		69	0.188				Magnussen, 2007
Nordic areas							40-45	8-10	Magnusson et al., 1997
I and II				79	0.099				Bergstad and Hareide (1996)
Div VIb				75	0.130				Bergstad and Hareide (1996)
Div IVa				73	0.15				Jennings et al., 1999
Iceland, Va							56-58	8-10	WGDEEP report, 2012
				84.3	0.13	0.19	44.8	4.8	Fishbase

Table 2. Mean length (SD=Standard Deviation, n=number of observations) of different age groups of tusk. Data compiled by the WK.

Area	Age 1	Age 5	Age 10	Source
Iceland	12.9 cm (SD= 2.3, n=22)	37.0 cm (SD=7.0, n=1015)	58.7 (SD=6.9, n=1040)	HAFRO
Norway		33.5 (SD=3.5, n=2)	57.0 (SD=4.4, n=57)	IMR
Faroe Islands		36.9 (SD=4.0, n=11)	49.0 (SD=4.6, n=781)	FAMRI

Validation, Growth and Longevity

There has been no direct validation of age estimation for tusk. Analysis of Icelandic length frequencies showed good correspondence between modes and the length of successive age groups obtained by ageing of otoliths for the age group of 2-4 years (Bergstad et al., 1998). A mode, found at 15 cm represented the II-group (Bergstad et al., 1998).

Existing Protocols and Results from Previous Workshops

An illustrated manual for age reading of tusk otoliths has been assembled by Bergstad and Hareide (1997).

The last exchange exercise was carried out in 2010, using 300 tusk otoliths collected from catches/landings in 2008 in Icelandic waters (Petursdottir and Finnbogadottir, 2011). During this exercise the tusk otoliths were soaked in glycerol for at least two weeks. The otoliths were viewed whole, immersed in glycerol sulcus-side up, using reflected light against a black background. Today all institutes (Iceland, Faroes and Norway) interpret whole tusk otoliths submerged in water, sulcus-side up, using reflected light against a black background. Other methods for ageing have been practiced e.g. sectioning/breaking; however there is no improvement on age agreement between readers.

Conclusions from the 2010 exercise:

There was only 1 otolith with complete agreement on age (9 years) by all 4 age-readers. The readers were all non-experts ageing tusk. The percentage agreement on age determination was 33.7% and the precision coefficient of variation was 20.8%.

The figures which caused most concern were the range of ages determined. The annotated images demonstrated the need to synchronize the interpretation, where to count the first annulus as well as to decide where the nucleus is located. The interpretation of annuli is highly inconsistent among the age-readers. This is largely due to the count of false rings (checks), therefore it is necessary to standardize the interpretation.

Recommended Ageing Protocol

Choice of Structure and Preparations

Tusk otoliths are viewed whole; submerged in water for at least 12 hours prior to ageing. They are read directly under a microscope, sulcus-side up, using reflected light against a black background. Today, images are not regularly used for ageing tusk in any of the Nordic countries.

For the exchange exercise carried out during this workshop, 50 digitized images of tusk otoliths were analysed and annotated by age readers using WebGR software.

Suggestions made by the group:

- Soaking in distilled water for 24 hours is necessary to re-expose the annuli
- An effort should be made towards taking images of parts of the otolith samples for comparison and discussion within and between age readers.

Preferred Reading Axis

Both otoliths are used, if available. The one with the clearer annuli is selected and used for age interpretation. The annuli are not counted along a fixed axis. The areas of the otolith where the annuli are most distinct are used for counting. A more detailed description of the preferred reading axes is not available at present.

Determination of the First Annulus

In all cases for tusk, the 1st of January is the assumed birthdate. At present, the interpretation of what zone is expected to be the first annulus varies between readers. Some readers believe that the first translucent ring is a result of larval settlement, while others suggest this zone represents the first winter growth (Figure 2).

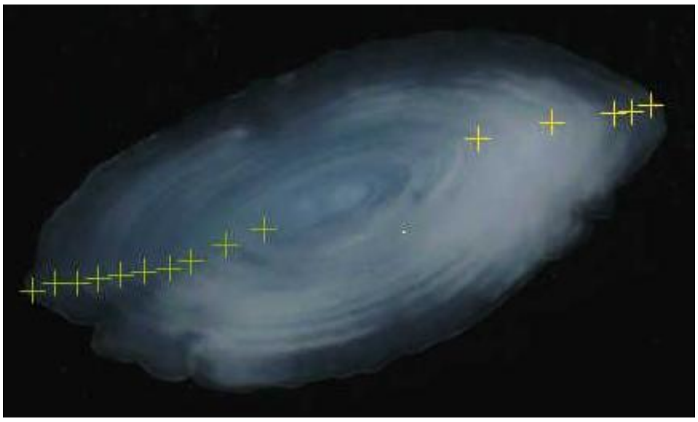

Figure 2 Example of different interpretation of zones found in the otolith from a 50 cm long tusk, 10 vs 5 years.

Suggestions made by the group:

- It has been proposed that the radius of the nucleus to the end of the first annulus should be measured from juvenile tusk otoliths. This measurement can be used to check the distance in other otoliths in order to have the same starting point for counting. Preliminary studies of 32 tusk otoliths during the meeting suggest that this might be a useful tool, but further work is needed.

- The group suggests that all age readers should consider the first distinct translucent zone (which is similar to the less distinct proceeding translucent zone) as the first annulus, as also suggested by Bergstad and Hareide (1997; see also Figure 3).

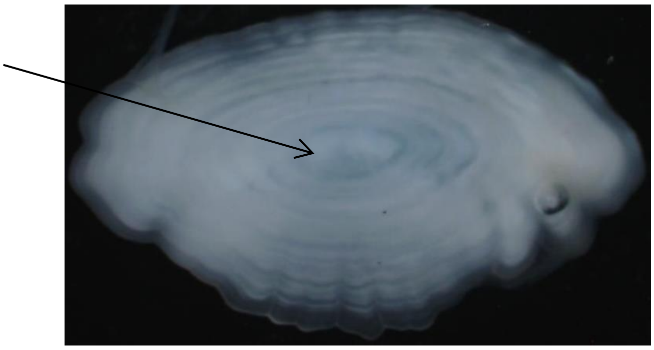

Figure 3. Image of an otolith originating from a tusk, 54 cm long, caught in September. Arrow point at the translucent zone which the group suggests should be interpreted as the first annulus.

- The group further suggests that an effort should be made to describe the youngest year classes; both with respect to length and otolith weight distribution (see Bergstad et al., 1998). A preliminary look at data provided from the Faroe Islands suggest that a number of modes can be seen in the material (see Figure 4). Average otolith weight for successive age groups obtained from otolith readings suggest that these modes correspond to these age groups. Since only a low number of observations and a possible sex dimorphism exist, it's suggested that this work should be continued when more data becomes available. The mentioned parameters should be compared to the reading of zonation pattern to reach a better understanding of otolith growth during, especially, the first year of life.

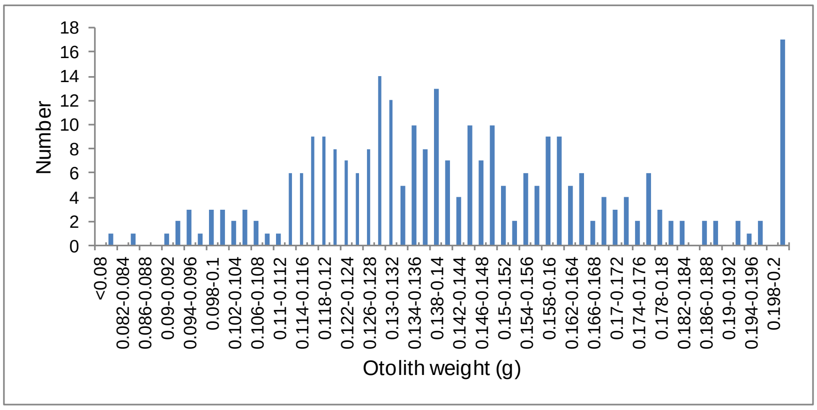

Figure 4. Frequency distribution otolith weight of tusk (N=284, length range 39-70 cm).

Identification of Annual Zones and Checks in Young Fish

Several readers interpretation of ageing is that the opaque fast-growth zones are seldom homogeneous i.e. checks seem to occur, and often in the middle part of the opaque zones, as also suggested by Bergstad and Hareide (1997). However, towards the edge of the otolith, annuli are often interpreted as being without checks (Figure 5 and 6).

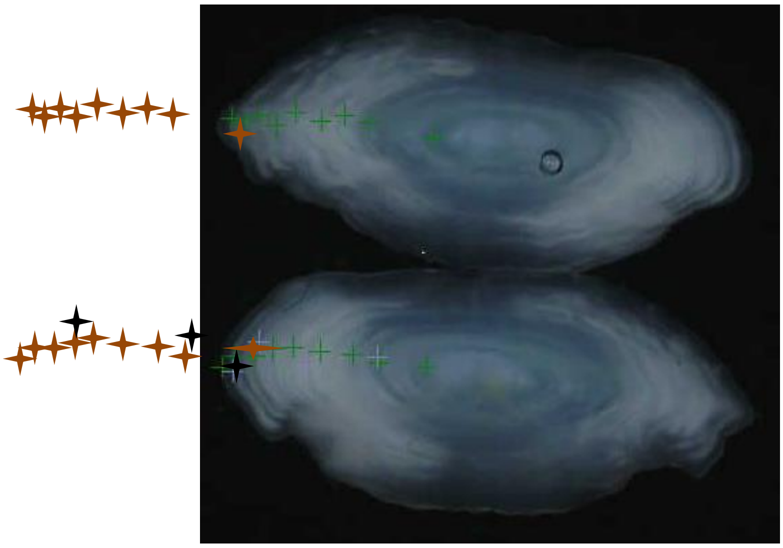

Figure 5. Image of an otolith originating from a tusk, 44 cm long.

Suggestions made by the group:

- Checks dividing the opaque zones close to the nucleus often occur similarly in subsequent opaque zones in several of the otoliths considered during the workshop. Age readers should be aware of such possibility as also noted by Bergstad and Hareide (1997).

Transition Zones

Transition zones are not considered to be an issue for tusk otoliths.

Slow Growth Zones

Increments closer to the edge become narrower, therefore should not be considered as checks but as annuli. However, these very narrow increments become more difficult to distinguish with increasing age. Such zones are thought to be a result of slower growth which may be related to maturation (Figure 6).

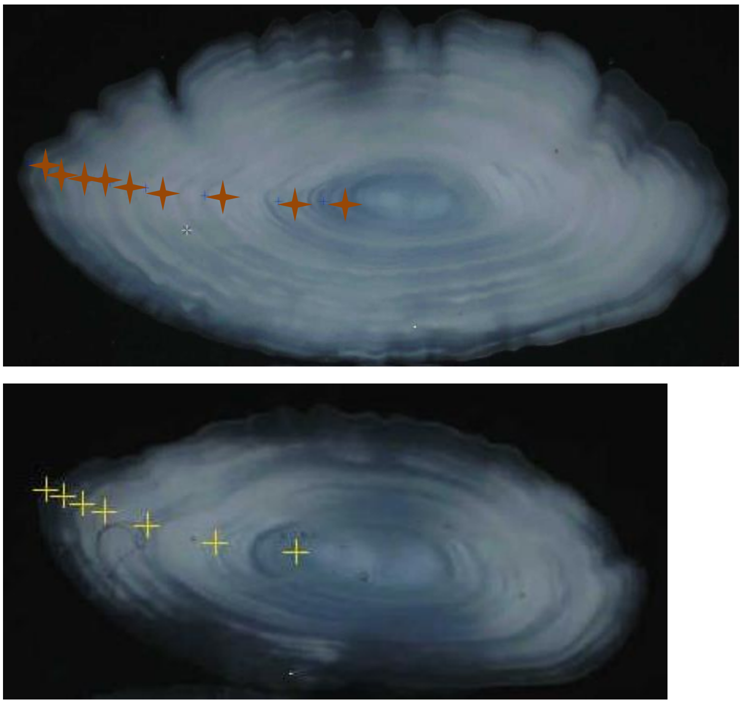

Figure 6. Images of otoliths with annotations originating from a tusk, 67cm long (above) and 55 cm long (below).

Edge Growth

No distinct analysis has been carried out with respect to marginal zone deposition.

Suggestions made by the group:

- Effort should be made to describe marginal zone deposition throughout the year.

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Annex 8: Agenda

Workshop on Age Estimation Methods of Deep Water Species (WKAMDEEP)

IMEDEA, Esporles, Spain, 21-25 October 2013

Working hours: 0900-1800, with lunch from 1400-1500.

Mon 21

1500 Welcome, Introduction, adoption of program, distribute responsibilities for each chapter, establish access to a common database with otolith images, …

- Beatriz: Age validation of deep water species
- Ole: Recent example of improved ageing protocols from validation studies of two deep-water species.

Tue 22

0900 Review of ageing protocols and literature for half of the species. (ToR a, b)

- Lise: Greater silver smelt
- Inês: Black scabbardfish
- Juan and Katerina: Black-spotted sea bream

1500-1800 Ageing of sample sets of otolith images

1500-1800 Report writing

Wed 23

0900 Review ageing protocols and literature for the other half of the species. (ToR a, b)

- Lise: Tusk
- Gróa: Ling
- Kélig: Roundnose grenadier
- Kélig: Blue ling

1500-1800 Ageing of sample sets of otolith images

1500-1800 Report writing

Thu 24

0900 Review of species annexes

Report writing

1500-1700 Report writing

1700-1800 Between-reader comparisons. (ToR c)

Fri 25

0900 General principles for ageing of DW fish (ToR e)

Finalising text and figures

1400 Closing of the meeting

Annex 9: Participant List

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Annex 10: Terms of Reference

a. Review information on age estimations, otolith exchanges, workshops and validation work done so far on the following species: tusk, ling, blue ling, roundnose grenadier, greater argentine, black scabbardfish, black-spotted sea bream, greater forkbeard and orange roughy;
b. Compile all available studies and results on validation of growth rates and longevity in deep water species, including but not limited to those listed above, and develop recommendations concerning the need and methods for validation studies in the ICES area;
c. Evaluate the results of small exchanges of otolith images from the individual species before the meeting;
d. To revise the age estimation procedures and explore the possibilities of using supplementary information to verify estimated ages, including: otolith weight and/or morphometry, as well as length distribution in surveys and catches;
e. Develop a publication on ageing of deep water fish based on analyses done prior to and during the meeting, including descriptions of general patterns and advanced age estimation methods for the deep water species individually and collectively;
f. Address the generic ToRs adopted for workshops on age calibration (see 'PGCCDBS Guidelines for Workshops on Age Calibration').