ICES WKAMDEEP2 REPORT 2018

ECOSYSTEM OBSERVATION STEERING GROUP

ICES CM 2018/EOSG:27

REF: ACOM, SCICOM, WGBIOP & WGDEEP

Workshop on Age Estimation Methods of Deep-Water Species (WKAMDEEP2)

17 -21 SEPTEMBER 2018

Cadiz, Spain

ICES
International Council for the Exploration of the Sea
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Recommended format for purposes of citation:

ICES. 2019. Workshop on Age Estimation Methods of Deep-Water Species (WKAMDEEP2), 17 -21 September 2018. Cadiz, Spain. ICES CM 2018/EOSG:27. 31pp.

Contents

Executive summary

Based on the previous work realized during the WKAMDEEP1 (2013), the Working Group on Biological Parameters (WGBIOP 2017) identified the need for an age reading Workshop on age estimation methods of deep-water species (WKAMDEEP2). This workshop was hosted by IEO (Cádiz, Spain; 1721 September 2018). The workshop was chaired by Ole Thomas Albert (Norway), Kélig Mahe (France), Juan Gil Herrera (Spain). During this workshop, the ageing of several deepwater species were reviewed: Blackspot seabream (*Pagellus bogaraveo*), Tusk (*Brosme brosme*), Greater silver smelt (*Argentina silus*), Blue ling (*Molva dypterygia*), Ling (*Molva molva*), Greater forkbeard (*Phycis blennoides*) and Black scabbardfish (*Aphanopus carbo*). The aims of the present workshop (WKAMDEEP2) were to assemble this group of experts in order to further develop the ageing protocol for all species and to estimate the precision of readings.

For each species, an easy to use ageing manual was agreed by all participants. These manuals are considered necessary and sufficient for a generic age reader of deepwater fish to provide reasonably accurate and precise age estimates of all the species. An exchange of 50 images by species was organized using the SmartDots tool. The results from all age readers showed relatively low precision, with a mean CV of 24%. However, this was mainly due to the inclusion of new age readers in early training phase. The expert readers showed precision that is considered adequate for stock assessments (mean CV 9%).

WKAMDEEP2 recommends that small exchanges of all species are scheduled in two and four years, followed by WKAMDEEP3 in 5 years in order to continue to improve both the quality and the capacity of age readings for deepwater species.

1 Administrative details

Expert Group name

Workshop on age estimation methods of deep-water species (WKAMDEEP2)

Chair(s)

Ole Thomas Albert (Norway), Kélig Mahe (France), Juan Gil Herrera (Spain)

Meeting venue

Cádiz, Spain

Meeting dates

17–21 September 2018

2 Terms of Reference

a) Collect and review the consistency of age data used in stock evaluations of deep-water fish, including, but not 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*), black-spotted sea bream (*Pagellus bogaraveo*), greater forkbeard (*Phycis blennoides*) and orange roughy (*Hoplostethus atlanticus*);

b) Review new information on precision and accuracy of age estimation of the seven first species listed above, for which WKAMDEEP1 agreed on individual ageing protocols, and revise those protocols as appropriate;

c) Review age estimation procedures, and propose new ageing protocols for deep-water species not considered by WKAMDEEP1;

d) Assemble age reading experts on deepwater species for training on age reading of several species, following the recommendation from WKAMDEEP1 to conduct age reading comparisons collectively for the whole group of slow-growing deepwater fish;

e) Conduct a small-scale comparison of otolith images from 100 individuals of each species and report on precision and between-reader biases.

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

WKAMDEEP2 will report by 1 December 2018 for the attention of ACOM, WGBIOP and WGDEEP.

3 List of participants

Workshop on Age Estimation Methods of Deep-Water Species (WKAMDEEP2)

1721 September 2018

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4 Introduction

4.1 Background

The workshop is a follow-up on a previous workshop, WKAMDEEP1 from 2013 (ICES, 2013), which provided a review of validation and corroboration work on each species, as well as a discussion of the procedures used by individual laboratories and recommendations for further development. Since there are very few age readers of most deepwater species, sometimes only one, the previous workshop decided that age readers of any of these species should preferably consider themselves as experts on the whole group of slow-growing deepwater fish. It was believed that this will facilitate age reading comparisons as a measure of quality assurance.

The aim of the present workshop (WKAMDEEP2) was to assemble this group of experts in order to further develop the competence and capacity for age reading of this group of fish. Since several laboratories have recruited new age readers lately, and since there has been a general increase of research effort on vulnerable species, it was felt that the most pressing issue was to develop easy to use species-specific age reading manuals. The purpose of these should be to make it easy for a generic deepwater age reading expert to take part in age reading on any specific species.

A secondary purpose of the meeting was to make everyone familiar with the present version of the SmartDots program, which is recommended by WGBIOP as a standard tool for exchanges (ICES, 2017), making it possible to contribute to further improvements of the program as a standard tool for age reading training and comparisons.

4.2 Implementation of the meeting

The species dealt with by the Group were: tusk (*Brosme brosme*), ling (*Molva molva*), blue ling (*Molva dypterygia*), greater silver smelt (*Argentina silus*), black scabbardfish (*Aphanopus carbo*), blackspot seabream (*Pagellus bogaraveo*) and greater forkbeard (*Phycis blennoides*).

The attendance and expertise in the Group was not adequate for addressing age reading methods for orange roughy (*Hoplostethus atlanticus*). For roundnose grenadier (*Coryphaenoides rupestris*) the WK notes that the French sampling program has been discontinued and available age data are not considered useful for the assessments. Therefore, although both species were listed in the terms of references, they had to be left untreated.

Among the deepwater species which are listed in the terms of references of this workshop, a preliminary review of WGDEEP 2018 (ICES, 2018) was realized to understand if stock assessment was built with or without ageing data:

Species	latin name	Ageing data used for stock assessment
Blackspot seabream	*Pagellus bogaraveo*	No
Tusk	*Brosme brosme*	Yes
Greater argentine	*Argentina silus*	Yes
Blueling	*Molva dypterygia*	Yes
Ling	*Molva molva*	Yes
Greater forkbeard	*Phycis blennoides*	No
Black scabbardfish	*Aphanopus carbo*	No
Orange roughy	*Hoplostethus atlanticus*	No
Roundnose grenadier	*Coryphaenoides rupestris*	No

The WK started on 17 September at Facultad de Filosofía y Letras of the University of Cadiz. After a short introduction, two guest lectures were given, one of an "approach to Cadiz´s history" by Rosa María Arniz, and another called "Inside Fish Otoliths: Matrix, mineral, growth" by Dr. Santiago Gómez from the Hard Tissue Laboratory (Faculty of Medicine) of the University of Cadiz.

A provisional agenda was presented and adopted (Annex 1). The outline of a generic short and easy-to-use age reading manual for deepwater species was discussed and a template was agreed. This template was used as basis for drafting species-specific manuals for the seven species considered by the Group. Each of these drafts were discussed in plenary and the agreed versions form the bulk of the report.

The treatment of each species by the Group started with an expert reader demonstrating how to interpret age on a few otoliths of that species, using the SmartDots program. Then all participants did age reading on a training set of 10 images, before the manual for that species was completed in plenary. A report was generated from SmartDots for each of the training sets and discussed in plenary. This included a revisit to some of the otoliths with less agreement between the readers. Afterwards the participants were prepared for the small-scale exchange that was finished directly following the workshop.

For each species considered by the Group, all the terms of reference were dealt with collectively by development of the age reading manuals and during the age reading discussions before and after age reading of the training sets. For species for which new information on age reading was available since WKAMDEEP1 (ICES, 2013), the corresponding references are included in the specific age reading manual. However, for most species there was no new information available and the previous WK report was therefore used as the only reference. The remainder of the report therefore focuses on the age reading manuals.

This small-scale exchange was initiated during the last days of the workshop and was finalized by each member separately and independently. A report of between-reader comparisons was generated for each of the seven small-scale exchanges and was added to the individual age reading manuals to indicate the level of precision that is attainable at present.

The generic ToRs adopted for workshops on age calibration by WGBIOP (ToR f in this WK) were addressed with the small exchanges that were conducted during and after the meeting. However, estimating age reading precision and between-reader bias were only minor elements in this WK, which focused on developing consistent and easy to use manuals for age reading of the individual species, including recommending a best practice. The purpose of the exchange was to make all participants familiar with the otoliths of the individual species, in order for all to be able to participate in the discussions leading to the agreed recommendations for ageing protocols. Some of the generic ToRs are therefore not particularly relevant for this WK.

5 Suggestions to improve SmartDots

The SmartDots program functioned reasonably well for viewing and annotation of otolith images. The Group considered that it is a bad practice to let the **fish length** be visible for the age reader during the first interpretation. Possible improvements may be to either not show the length at all or that it would require an active choice of the reader to show the length. In the last case, the reader's action may be recorded by the program and thus facilitate later investigations on the effect of knowing the fish length when interpreting age.

The possibility of fixing the reading axis for all participants of an exchange is good and makes it easier to compare and discuss the individual readings. For deepwater species it is usually necessary to use different axes for the first few zones and for the later zones. For some species, a broken (bended) axis is adequate, but for other species the two axes may not be connected. In later versions **disconnected axes** should preferably be supported. This may be done by introducing a special type of points to identify both the ending of one axis and the starting of the next from different locations within the same growth zone.

After performing a small-scale exchange with good results, it would be beneficial to be able to define the set of otoliths, with associated data, as a **reference collection**, which can further be used by individuals for training purposes. The interpretations made by the trainee may initially be saved as drafts and the program may provide comparisons of his/her interpretations with those of the trained age readers that participated in the exchange. Based on these statistics, the exchange coordinator may accept or reject the new interpretations, and if accepted they will be included in the reference collection, and the trainee will be accredited as official age reader of this species or stock.

It would be helpful to be able to go easily between individual otoliths during interpretation. It may give good support for the reader to be able to compare with his/her previous annotations. Similarly, it should be easier to go to the next otolith when working with a set of otoliths.

SmartDots generates a coloured list of filenames as the age reading proceeds. In the present version file names are shown in green when they have been read by any one reader. This makes it difficult for an age reader to keep track of one's own progress. We suggest using red, yellow and green to denote respectively that an image has not been annotated, not approved, or is annotated and approved.

When an age reader is done with an exchange set, a note should be sent automatically to the coordinator.

A growth line tool must be developed so that it can be used to compare different annotations of the same images and of all the images by one reader.

6 Results of the small-scale exchanges (ToR a-f)

6.1 Marginal otolith interpretation

As during WKAMDEEP1 (ICES, 2013), some discussion around the otolith edge observation was necessary to find an agreement between all readers. The age attribution may depend on the spawning period and the time of the opaque zone formation (Panfili *et al*. 2002). The age class estimation procedures are summarized at the following Table.

Table 1: The age class estimation procedures with N as number of complete annual zones (Panfili *et al.* 2002).

EDGE TYPE	DATE OF CAPTURE	
	1st semester	**2nd semester**
Translucent	N	N - 1
Opaque	N + 1	N

If the specimen is caught in the first semester, the age group corresponds to the number of complete annual zones (N) if the edge is translucent, but the fish is assigned to the following age group (N+1) if the edge is opaque. A specimen caught during the second semester is assigned to the age group that corresponds to the number of complete annual zones (N) if the edge is opaque and to the previous age group (N-1) if the edge is translucent (C.A.R.E., 2006).

6.2 Results of the exchange

During this meeting and after, all readers participated in the exchange for all species. All the age readings of each species were done after thorough discussions of the interpretation principles for that species, including completion of the short manuals in plenary. Although all age readers were experienced with one or several species, for each species there were only few age readers with previous experience in production ageing of that species. The results of the age reading comparisons across all age readers, experienced and unexperienced, are summarized in the following Table, which was generated by use of the standard EFAN spreadsheet by Guus Eltink (2000).

Table 2: Summary of the results of the exchange 2018 for deepsea species by experienced and unexperienced age readers combined.

Species	Scientific name	Sampling number	Range of modal age	Number of readers	Otolith preparation	Percentage of agreement	CV
Blackspot seabream	*Pagellus bogaraveo*	*50*	2-11	12	whole otolith	34.7	30.8
Tusk	*Brosme brosme*	*50*	6-13	11	whole otolith	48.4	11.5
Greater silver smelt	*Argentina silus*	*50*	3-14	12	whole otolith	68.7	8.7
Blue ling	*Molva dypterygia*	*50*	2-28	12	Transverse section	34.7	17.1
Ling	*Molva molva*	*79*	2-14	12	Transverse section	45.7	21.5
Greater forkbeard	*Phycis blennoides*	*50*	0-7	12	Transverse section	54.5	33.9
Black scabbardfish	*Aphanopus carbo*	*50*	4-10	11	Transverse section	36.7	25.6
					Mean :	*46.2*	*21.3*

For deepwater species, which are typically long-lived and difficult to age, a CV less than 10 is generally considered as good precision, but CV up to 15 may be more realistic and may as well be considered acceptable (ICES, 2013).

The above results showed that, for all these deepsea species, the reading is difficult with low percentage of agreement between readers and high Coefficient of Variation (CV) which is the consequence of low precision between readers (i.e. difference of several years among readers for the same otolith). A part of this imprecision is attributable to the inclusion of age readers with no or very limited experience with some or several of the species. Restricting the comparisons to the two highest ranked readers for each species (the “overall ranking” in the Eltink spreadsheet) resulted in a reduction in mean CV across species from 21.3 to 8.5 %. The table below summarise the precision between the two expert readers of each species.

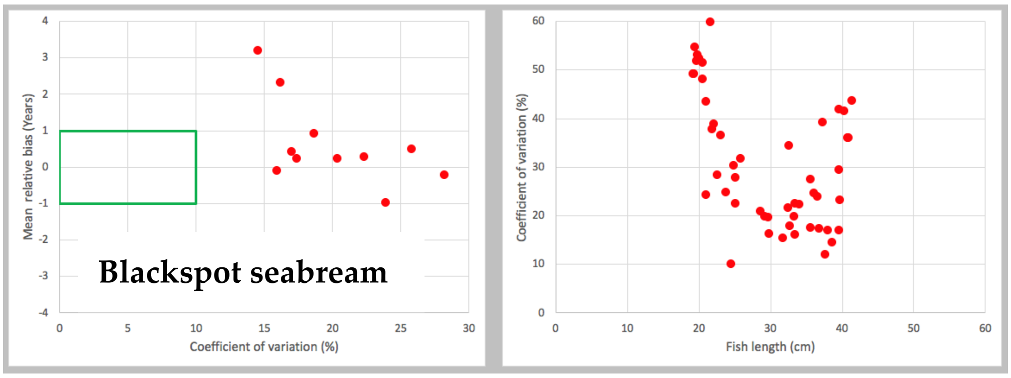
Table 3: Between-reader comparisons for the two highest ranked readers of each species.

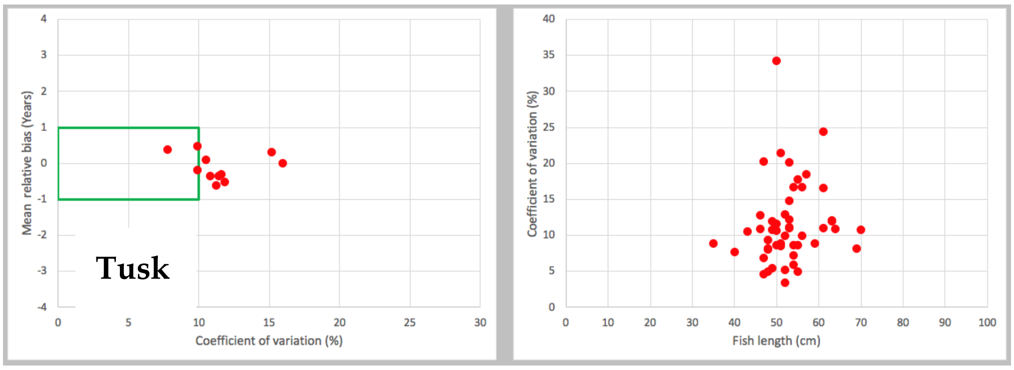
Species	Mean between-reader error	Percent agreement	CV
Blackspot seabream	0.1	30.0	15.7
Tusk	0.3	46.0	6.6
Greater silver smelt	0.2	72.0	2.6
Blue ling	0.4	48.0	3.9
Ling	0.2	39.2	9.1
Greater forkbeard	0.2	46.0	14.2
Black scabbardfish	0.1	44.0	7.3
Mean:	*0.2*	*46.5*	*8.5*

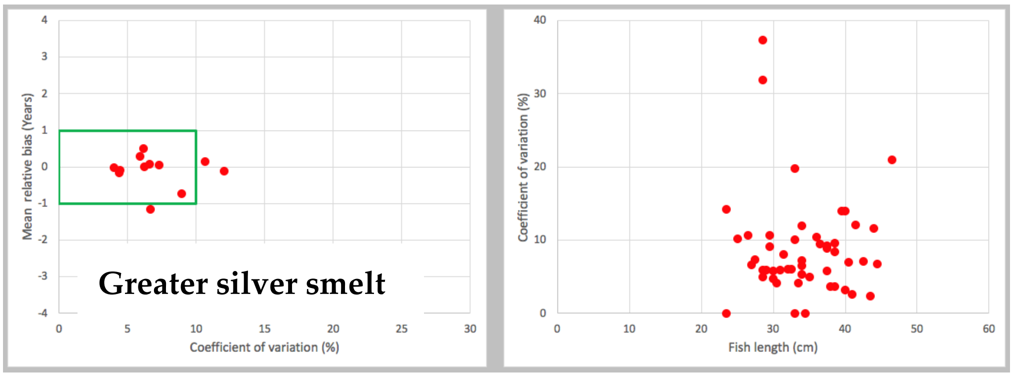
The table above shows that it is possible to achieve high precision (CV < 10%) and low between-reader bias (< 1 year) for most of these deepwater species. The generally low precision of the comparisons of all age readers (Table 2) was therefore mainly due to the inclusion of untrained age readers in the comparisons.

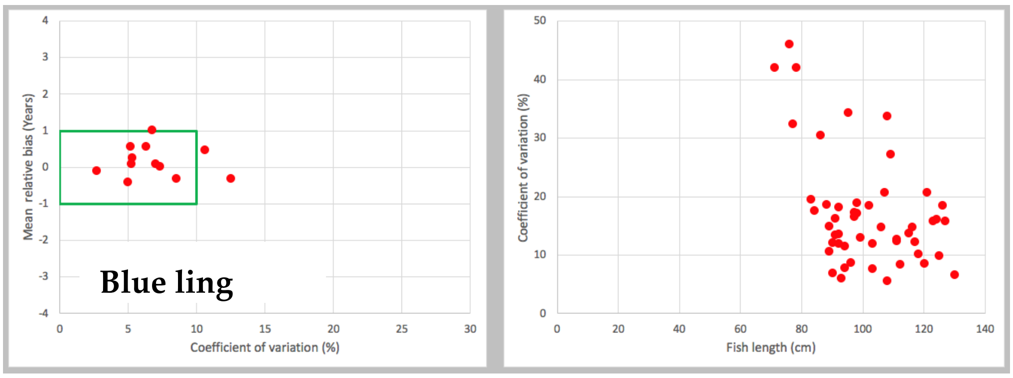
Table 2 and 3 also show that percent agreement is a poor measure of precision for these long-lived species. The mean percent agreement was similar between expert readers and unexperienced readers. As recommended by Campana (2001), the CV should therefore be the preferred measure of precision for these species.

Figure 1 shows that for all species except blackspot seabream, there were at least two age readers with CV < 15% and between-reader bias relative to the modal age less than one year. For four of the species there were at least one age reader with scores within the green box illustrating high precision and low bias. Greater silver smelt and blue ling were the two species for which most age readers scored within the green box, while for blackspot seabream, greater forkbeard and black scabbardfish there were no age reader within the green box. For ling, and possibly a few other species, the CV decreased with fish length, indicating that a source of discrepancy between readers is associated with the identification of the first annual zones.


Blackspot seabream
Mean relative bias (Years)
4
3
2
1
0
-1
-2
-3
-4
0
5
10
15
20
25
30
Coefficient of variation (%)
Coefficient of variation (%)
60
50
40
30
20
10
0
0
10
20
30
40
50
60
Fish length (cm)


Tusk
Mean relative bias (Years)
4
3
2
1
0
-1
-2
-3
-4
0
5
10
15
20
25
30
Coefficient of variation (%)
Coefficient of variation (%)
40
35
30
25
20
15
10
5
0
0
10
20
30
40
50
60
70
80
90
100
Fish length (cm)


Greater silver smelt
Mean relative bias (Years)
4
3
2
1
0
-1
-2
-3
-4
0
5
10
15
20
25
30
Coefficient of variation (%)
Coefficient of variation (%)
40
30
20
10
0
0
10
20
30
40
50
60
Fish length (cm)


Blue ling
Mean relative bias (Years)
4
3
2
1
0
-1
-2
-3
-4
0
5
10
15
20
25
30
Coefficient of variation (%)
Coefficient of variation (%)
50
40
30
20
10
0
0
20
40
60
80
100
120
140
Fish length (cm)

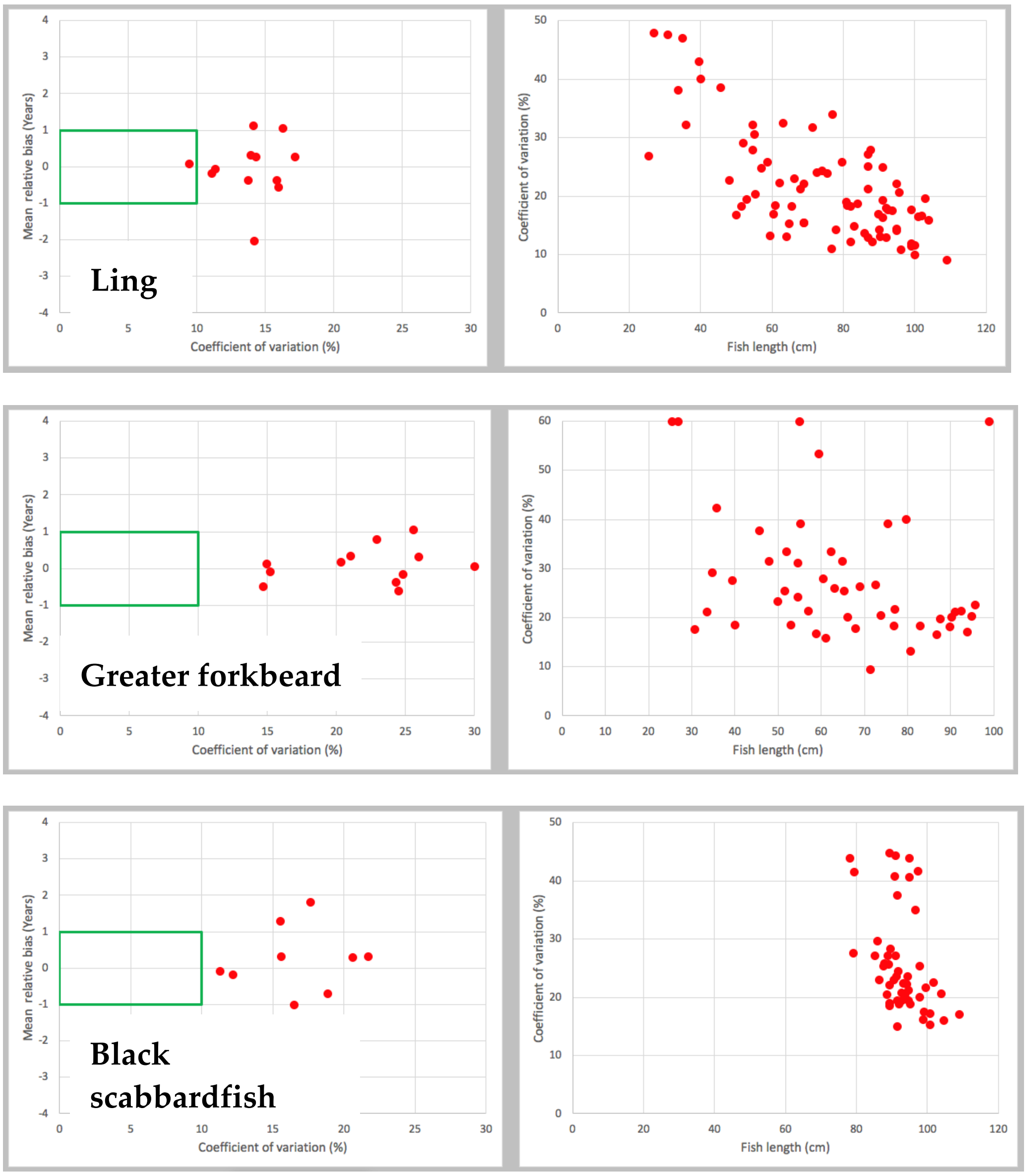


Figure 1: Left figures: Mean bias relative to modal age, versus CV, for each of the 11 age readers for each of the seven species. Green boxes indicate low bias and high precision. Right figures: Mean CV for all age readers combined versus fish length for each species.

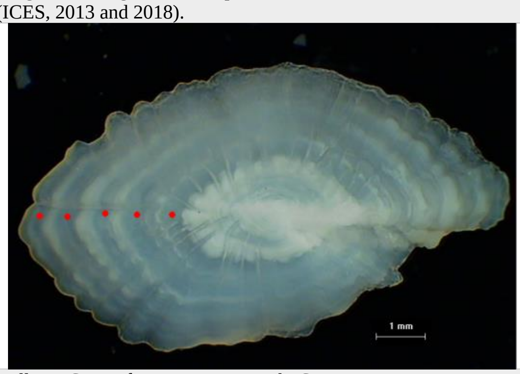
7 Age reading manuals (ToR a–f)

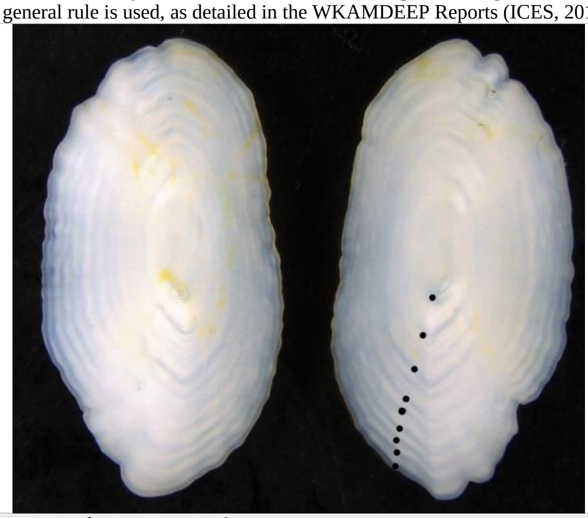
It seems clear that there is still potential for improved precision between age readers of deepwater fish, and further exchanges are therefore needed. A prerequisite is that all age readers base their interpretation on the same principles and rules. This was the rationale behind the Workshop's decision to establish consensual and easy to use manuals for each species.

A one-page template manual was first discussed and amended by the Group at the start of the meeting. Then this template was filled in for each species based on a demonstration of common practice by an expert reader of that species, followed by discussions in plenary. The finally agreed one-pagers are considered both necessary and sufficient as basis for a generic age reader of deepwater fish to be able to produce reasonably accurate and precise age estimates of each species.

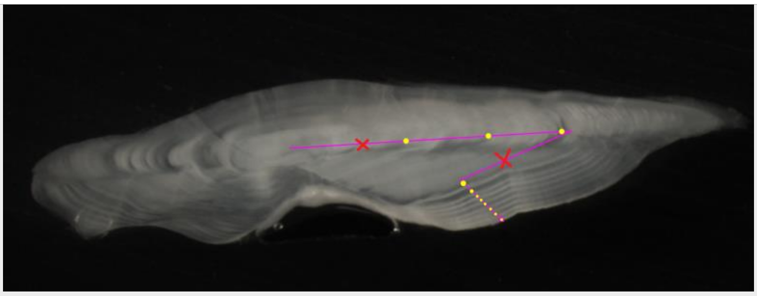
The Group recommends that new exchanges are arranged in two years and four years based on the agreed manuals. At the same time individual labs and age readers are encouraged to further develop the manuals with more specific and helpful information. After these two exchanges a new WKAMDEEP3 will be required to discuss the amendments and establish more robust (and presumably long-lived) manuals for each species.

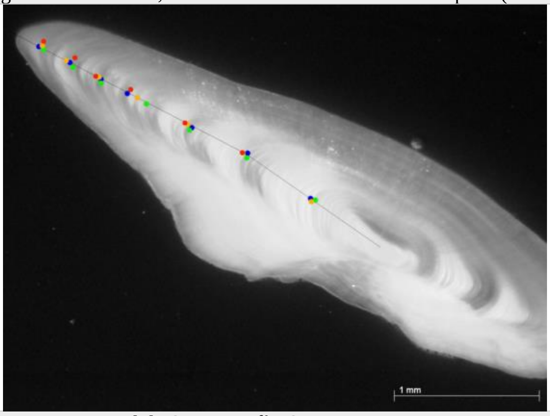
On the following pages are the manuals for each of the seven species considered by the Workshop. For practical use they may preferably be printed in A3 format.

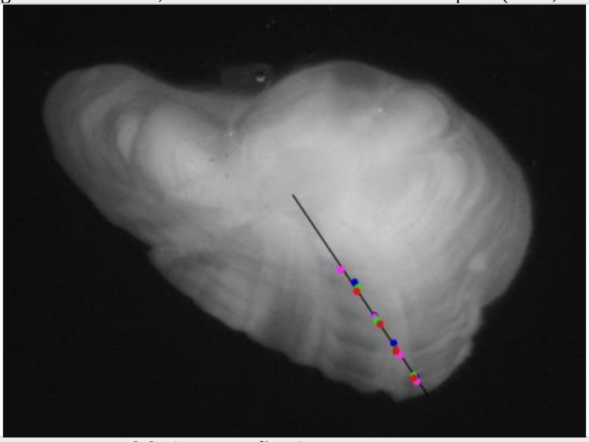
Age reading manual of Deep-water fish		Detailed description **– by labs or stocks if relevant** *Pagellus bogaraveo*
Manual updated by	WKAMDEEP2	Cadiz 17-21 September 2018 Reference: ICES CM 2019 / ACOM: 00
Species	*Pagellus bogaraveo*	Blackspotted seabream
ICES stocks	sbr.27.6-8 sbr.27.9 sbr.27.10	– Subareas 6–8 (Celtic Seas, the English Channel, and Bay of Biscay) – Subarea 9 (Atlantic Iberian waters) – Subarea 10 (Azores grounds)
Other areas	GFCM Mediterranean stocks	– Spain (GSA1), Marocco (GSA3) (assessments) – Age reading periodically in some other GSAs
Validation and corroboration	Partially corroborated	Tag-recapture experiments by IEO, daily increment analysis by HCMR (ICES, 2013).
Ageing structure	Otolith	
Storage before preparation	Small tubes or vials	Kept dry.
Preparation	Soaking	- The otolith is placed in a petri dish, sulcus-side up and immersed in water for some to 30 minutes (HCMR) -24 hours in water with thymol (1gram per liter) and glycrine (70:30) (IEO).
Storage after preparation	Small tubes or vials	Kept dry before long-time storing.
Method of visual inspection	Stereo microscope	– Reflected light against a black background, – Standardized magnification is required for reading directly in stereomicroscope or for images.
Imaging	Routinely	Calibrated images are routinely taken (HCMR)
Aging surface	Whole otolith	Measurements are taken from the left otolith
Reading axes	Usually the longest axis	Defined annuli are usually particularly found along the post-rostrum area through a straight line.
Available information during age reading	Sampling data	– Date of capture, – Sampling platform (survey, commercial catch, landings, etc) – Area – Sex – Fish lenght should not be taken in consideration during the first reading
Birthday	1st January	From nucleus until first translucent ring correspond to 0-group growth
Spawning period (quarter)	1st (Atl.) 1st-4th (Med.)	Its reproduction extends throughout the whole year with a peak period that varies according to Atlantic or Mediterranean region (ICES, 2013).
Identification of the first zone(s)	Measure size of first zone	Usually the radius from the core to the first translucent ring (2-3 mm) is used (HCMR). Longest diameter of first translucent ring could be used routinely. It is still an issue what constitutes the first zone.
The first several zones		As the otolith grows, the growth increments become narrower and the relative widths of each annulus decrease progressively and are less evident.
Transition zones	Yes	Transition zones are characterized by many checks. Zones are typically less defined along the short axis of the otoliths and age estimation should be interpreted along the post-rostrum axis.
Outer zones in older fish		Increments become narrower and more difficult to distinguish with increasing age. Such zones should be considered as annual zones and not as checks.
Edge		The annual deposition of increments has been tested by marginal increment analysis and the general marginal zone deposition rule is used, as detailed in the WKAMDEEP Reports (ICES, 2013 and 2018).
Reference images	Blackspotted seabream otolith with relatively clear growth pattern. TL: 24.4 cm Month caught: October Estimated age: 5	
*** Laboratories involved in this manual**	HCMR UNIBA IEO	Hellenic Centre for Marine Research, Greece University of Bari, Italy Instituto Español de Oceanografía, Spain
Discussion		Problem: Finding the 1st growth increment. Do not count 1st circular ring in nucleus.

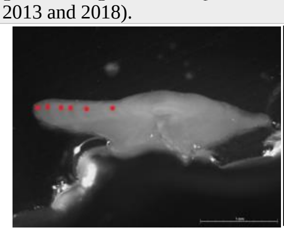
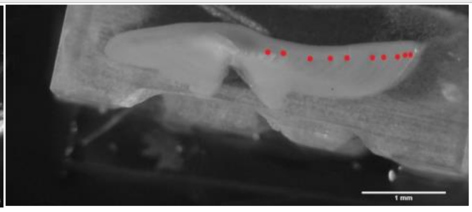
Age reading manual of Deep-water fish		Detailed description – by labs or stocks if relevant *Brosme brosme*
Manual updated by	WKAMDEEP2	Cadiz 17-21 September 2018 Reference: ICES CM 2019 / ACOM: 00
Species	*Brosme brosme*	Tusk
ICES stocks	usk.27.1-2 usk.27.12ac usk.27.6b usk.27.5a14 usk.27.3a45b6a7-912b	– Subareas 1, 2 (Northeast Arctic) – Southern Mid-Atlantic Ridge, excluding 12b – Rockall – Iceland and East Greenland – Subareas 4 and 7-9, divisions 3a, 5b,6a and 12b (North Atlantic)
Other areas		
Validation and corroboration	Partially corroborated	ICES. 2013. Workshop on Age Estimation Methods of Deep-water Species (WKAMDEEP), 21-25 October 2013, Mallorca, Spain. ICES CM 2013/ACOM: 83. 81pp.
Ageing structure	Otolith	
Storage before preparation	Paper envelopes	
Preparation	Soaking	Clearing in water or glycerol for 24 hours
Storage after preparation	Paper envelopes	
Method of visual inspection	Stereo microscope	– Reflected light, black background – Standardized magnification, – Reading otolith surface, convex side up
Imaging	Not routinely	Good quality images possible
Aging surface	Whole otolith	Reading on surface, convex side up
Reading axes	Usually the longest axis	Straight line, also for older individuals. Help from the short axes.
Available information during age reading	Sampling data	– Date of capture, – Sampling platform (survey, commercial catch, landings, etc) – Area – Fish length should not be taken in consideration during the first reading
Birthday	1st January	From nucleus until first translucent ring correspond to 0-group growth
Spawning period (quarter)	2nd-3rd	April-July
Identification of the first zone(s)	Measure size of first zone	Longest diameter of first translucent ring, could be used routinely
The first several zones		The 5-8 growth increments become narrower
Transition zones	Not a problem	Transition zones are not considered to be an issue for tusk otoliths.
Outer zones in older fish		Increments become narrower and more difficult to distinguish with increasing age. Such zones should be considered as annual zones and not as checks.
Edge		No distinct analysis has been carried out with respect to marginal zone deposition, so the general rule is used, as detailed in the WKAMDEEP Reports (ICES, 2013 and 2018).
Reference images	Tusk otoliths with relatively clear growth pattern. TL: 50 cm Month caught: March Estimated age: 9.	
*** Laboratories that regularly age this species**	IMR FAMRI MFRI	Institute of Marine Research, Norway Faroe Marine Research Institute, Faroe Islands Marine and Freshwater Research Institute, Iceland
Discussion		Problems: Overestimation of initial increments, many false zones between 1st and 2nd increment. Further recommendations: Polishing surface or transverse sections.

Age reading manual of Deep-water fish		Detailed description – by labs or stocks if relevant *Argentina silus*
Manual updated by	WKAMDEEP2	Cadiz 17-21 September 2018 Reference: ICES CM 2019 / ACOM: 00
Species	*Argentina silus*	Greater silver smelt (Greater Argentine)
ICES stocks	aru.27.123a4 aru.27.5b6a aru.27.5a14 aru.27.6b7-1012	– Subareas 1, 2, 4, and Division 3.a (Northeast Arctic, North Sea, Skagerrak and Kattegat) – Divisions 5.b and 6.a (Faroes grounds and west of Scotland) – Subarea 14 and Division 5.a (East Greenland and Iceland grounds) – Subareas 7–10 and 12, and in Division 6.b (other areas)
Other areas		–
Validation and corroboration	Partially corroborated	ICES. 2013. Workshop on Age Estimation Methods of Deep-water Species (WKAMDEEP), 21-25 October 2013, Mallorca, Spain. ICES CM 2013/ACOM: 83. 81pp.
Ageing structure	Otolith	
Storing before preparation	Paper envelopes	Kept in a dryer before long-time storing.
Preparation	Embedding	Embedding, sulcus down, in Epoxy on black plastic boards holding 12 otolith pairs
Storage after preparation	Paper envelopes	
Method of visual inspection	Stereo microscope	– Reflected light – Standardized magnification, increase magnification in outer zones for older fish.
Imaging	Not routinely	Good quality images possible
Aging surface	Whole otolith	
Reading axes	Longest axis on either otolith	Straight line, also for older individuals
Available information during age reading	Sampling data	– Date of capture, – Sampling platform (survey, commercial catch, landings, etc) – Area – Fish length should not be taken into consideration during the first reading
Birthday	1st January	From nucleus until first translucent ring correspond to 0-group growth
Spawning period (quarter)	2nd	Spawning occurs mainly in spring (March and April) but there is some evidence of an autumn spawning period for some individuals.
Identification of the first zone(s)	Measure size of first zone	Longest diameter of first translucent ring could be used routinely.
The first several zones		The first 10-12 growth increments become gradually narrower.
Transition zones	Not a problem	Transition zones are not considered to be an issue.
Outer zones in older fish		Increments become narrower and often more difficult to distinguish with increasing age. From age 10-12 years the zones will be very narrow and regularly spaced on the pointed edge of the otolith. Such zones should be considered as annual zones and not as checks.
Edge		No distinct analysis has been carried out with respect to marginal zone deposition, so the general rule is used, as detailed in the WKAMDEEP Reports (ICES, 2013 and 2018).
Reference images	Greater silver smelt otoliths with relatively clear growth pattern. TL: 42 cm Month caught: February Estimated age: 11	
*** Laboratories involved in this manual**	IMR FAMRI MFRI	Institute of Marine Research, Norway Faroe Marine Research Institute, Faroe Islands Marine and Freshwater Research Institute, Iceland
Discussion		

Age reading manual of Deep-water fish		Detailed description – by labs or stocks if relevant *Molva dipterygia*
Manual updated by	WKAMDEEP2	Cadiz 17-21 September 2018 Reference: ICES CM 2019 / ACOM: 00
Species	*Molva dipterygia*	Blue ling
ICES stocks	bli.27.5^{a}14 bli.27.5b57 bli.27.nea	-Subarea 14 and Division 5.a (East Greenland and Iceland grounds) -Subareas 6-7 and Division 5.b (Celtic Seas, English Channel, and Faroes grounds) -Subareas 1, 2, 8, 9, and 12, and Divisions 3.a and 4.a (other areas)
Other areas		
Validation and corroboration	Partially corroborated	ICES. 2013. Workshop on Age Estimation Methods of Deep-water Species (WKAMDEEP), 21-25 October 2013, Mallorca, Spain. ICES CM 2013/ACOM: 83. 81pp.
Ageing structure	Otolith	
Storage before preparation	Paper envelopes	Only 1 otolith is sampled. Kept in a dryer before long-time storing.
Preparation	Embedding Thin-sectioning	Embedding in polyester resin. Sulcus down Transversal thin-section 0.4 mm
Storage after preparation	Paper envelopes	
Method of visual inspection	Stereo microscope	– Reflected light with black background, – Clearing with oil – Standardized magnification
Imaging	Routinely	Calibrated images (tiff format) are routinely taken.
Aging surface	Transverse section	Unpolished section
Reading axes	Broken reading axis	1: Straight line along the longest axis until 3-4 years 2: Move closer to sulcus and read towards the proximal edge
Available information during age reading	Sampling data	– Date of capture – Sampling platform (commercial catch) – Area – Fish length should NOT be taken into consideration during the age readings
Birthday	1st January	From nucleus until first translucent ring correspond to 0-group growth
Spawning period (quarter)	2nd	March-July?
Identification of the first zone(s)		First false ring are identified from the studies on juveniles stages. The second visible ring is set as first zone.
The first several zones		The growth increments become narrower at 3-4 years.
Transition zones	Yes	Change in reading axis might be difficult to identify but is usually considered to be at 3-4 years.
Outer zones in older fish		Increments become narrower with increasing age and split zones are common.
Edge		No distinct analysis has been carried out with respect to marginal zone deposition, so the general rule is used, as detailed in the WKAMDEEP reports (ICES, 2013 and 2018).
Reference images	Blue ling otoliths with relatively clear growth pattern. TL: 108.0 cm Month caught: March Estimated age: 11	
*** Laboratories involved in aging this species**	IFREMER	IFREMER , France
Discussion		Problem: When switching reading axis, make sure not to count the same increment twice, also beware not to skip an increment. Recommendations: Define distance to first increment.

Age reading manual of Deep-water fish		*Molva molva* Detailed description –by labs or stocks if relevant
Manual updated by	WKAMDEEP2	Cadiz 17-21 September 2018 Reference: ICES CM 2019 / ACOM: 00
Species	*Molva molva*	Ling
ICES stocks	lin.27.3a4a6-91214 (IEO and IMR) lin.27.5a lin.27.1-2 (IMR) lin.27.5b	-Subareas 6-9, 12, and 14, and in in divisions 3.a and 4.a (Northeast Atlantic and Arctic Ocean) - Division 5.a (Iceland grounds) - Subareas 1 and 2 (Northeast Arctic) - Division 5.b (Faroes grounds)
Other areas		
Validation and corroboration	No direct validation	ICES. 2013. Workshop on Age Estimation Methods of Deep-water Species (WKAMDEEP), 21-25 October 2013, Mallorca, Spain. ICES CM 2013/ACOM: 83. 81pp.
Ageing structure	Otolith	
Storing before preparation	IEO-5ml polypropylene tube IMR-Paper envelopes	Kept in a dryer before long-time storing.
Preparation	IEO-Sectioning IMR- Soaking	IEO-Embedding the left otolith in polyester resin, sectioning of 0.7 mm and mounting in metacrylate slides. IMR-Clearing in water at least 24h before ageing.
Storing after preparation	IEO-Box IMR-Paper envelope	IEO-Carton box (left otolith), 5ml polypropylene tube (right otolith). IMR-Paper envelope
Method of visual inspection	Stereo microscope	IEO-Reflected light, black background, 20x magnifications. IMR-Reflected light, black background, standardized magnification.
Imaging	IEO-Routinely IMR-Not routinely	IEO-Good quality images possible, composite image usually not necessary IMR- Good quality images possible, composite image usually not necessary.
Aging surface	IEO-Section IMR-Whole otolith	IMR-Reading on surface, and distal side up
Reading axes	Longest axis on either otolith	Straight line, also for older individuals
Available information during age reading	Sampling data	– Date of capture, – Sampling platform (survey, commercial catch, landings, etc) – Area – Fish length should not be taking in consideration during the first reading.
Birthday	1st January	From nucleus until first translucent ring correspond to 0-group growth
Spawning period (quarter)	2nd-3rd	May-September
Identification of the first zone(s)	Measure size of first zone	IEO-Longest radio of first translucent ring (Expected size: 0.8-1 mm) IMR- Longest diameter of first translucent ring could be used routinely.
The first several zones		Growth increments become narrower.
Transition zones	Not a problem	Transition zones are not considered to be an issue for ling otoliths.
Outer zones in older fish		Increments become narrower and more difficult to distinguish with increasing age, especially from age 5 years. Such zones should be considered as annual zones and not as checks.
Edge		No distinct analysis has been carried out with respect to marginal zone deposition, so the general rule is used, as detailed in the WKAMDEEP Reports (ICES, 2013 and 2018).
Reference images	IEO-Ling section otolith with relatively clear growth pattern. TL: 82 cm Month caught: March Estimated age: 7	
Laboratories involved in this manual	IEO IMR	Instituto Español de Oceanografía, Spain Institute of Marine Research, Norway
Discussion		

Age reading manual of Deep-water fish		Detailed description – by labs or stocks if relevant *Phycis blennoides*
Manual updated by	WKAMDEEP2	Cadiz 17-21 September 2018 Reference: ICES CM 2019 / ACOM: 00
Species	*Phycis blennoides*	Greater forkbeard
ICES stocks	gfb.27.nea	- Subareas 1-10, 12 and 14 (the Northeast Atlantic and adjacent waters)
Other areas		
Validation and corroboration	Not direct validation	
Ageing structure	Otolith	
Storage before preparation	Polypropylen tube (IEO) Paper envelope (IFREMER)	Dry with absorbent paper before storing
Preparation	Embedding Sectioning Mounting	Embedding the left otolith in polyester resin Cutting in 0.7 mm transverse sections Pasting into methacrylate slides (IEO)
Storage after preparation	Dry	Methacrylate slides are stored in cardboard boxes (IEO) Paper envelope (IFREMER)
Method of visual inspection	Stereo microscope	Reflected light, black background : 10x or 20x magnification (IEO) Transmitted light: standardized magnification (IFREMER)
Imaging	Routinely	Good quality images possible
Aging surface	Transverse section	
Reading axes	Straight axis	Transect from the nucleus towards the distal edge
Available information during age reading	Sampling data,	– Date of capture, – Sampling platform (survey, commercial catch, landings, etc) – Area – Sex – Fish length should not be taken into consideration during the age readings
Birthday	1st January	From nucleus until first translucent ring correspond to 0-group growth
Spawning period (quarter)	1st-3rd	March to July
Identification of the first zone(s)	Measure size of first zone	Longest radius of first translucent ring (expected size: 0.7 – 1 mm)
The first several zones		Growth increments become narrower.
Transition zones	Not a problem	Transition zones are not considered to be an issue
Outer zones in older fish		Increments become narrower and more difficult to distinguish with increasing age, especially from age 3 or 4 onwards. Such zones should be considered as annual zones and not as checks.
Edge		No distinct analysis has been carried out with respect to marginal zone deposition, so the general rule is used, as detailed in the WKAMDEEP Reports (ICES, 2013 and 2018)
Reference images	Otolith section with relatively clear growth pattern. TL: 40 cm Month caught: October Estimated age: 4	
*** Laboratories involved in this manual**	IEO IFREMER	Instituto Español de Oceanografía , Spain IFREMER, France
Discussion		Problems: Difficult to find 1st growth increment. False ring of a different shape in the nucleus. Recommendations: Define 1st growth increment (distance from core). Experiment with image quality.

Age reading manual of Deep-water fish		Detailed description – by labs or stocks if relevant *Aphanopus carbo*
Manual updated by	WKAMDEEP2	Cadiz 17-21 September 2018 Reference: ICES CM 2019 / ACOM: 00
Species	*Aphanopus carbo*	Black scabbardfish
ICES stocks	bsf.27.nea	Subareas 1, 2, 4, 6–8, 10, and 14, and in divisions 3.a, 5.a–b, 9.a, and 12.b (Northeast Atlantic and Arctic Ocean)
Other areas	CECAF 34.1.2	– Madeira and Canaries
Validation and corroboration	No direct validation	Indirect validation using marginal increment analysis (ICES, 2013)
Ageing structure	Otolith	
Storage before preparation	Flip-top plastic vials	Dry well before storing.
Preparation	Soaking Embedding Sectioning	– Soaking in water for 24h – Embedding, sulcus down, in epoxy resin – High- or low-speed diamond saw; 0.5 mm sections. – Sections are glued on glass slides
Storage after preparation	Boxes	Glass slides are kept in paperboard boxes.
Method of visual inspection	Stereo microscope	– Transmitted or reflected light – Standardized magnification (approx. 48x) – Clearing with 1:1 glycerine:ethanol solution or an essential oil
Imaging	Routinely	Calibrated images are routinely taken. It is important to work with high quality images to facilitate growth increment interpretation.
Aging surface	Transverse sections	Across the nucleus.
Reading axes	Ventral axis	Straight line for measurements. However, the growth axis is curved, more noticeably for older individuals.
Available information during age reading	Sampling data	– Area – Date of capture – Sampling platform (commercial catch, survey) – Fish length should not be taken into consideration during the first reading.
Birthday	1st January	Usually there are translucent zones marking both the start and the end of the 0-group
Spawning period (quarter)	4th	Spawning is only reported for Madeira and the Canaries (ICES, 2013; Ribeiro-Santos et al., 2013).
Identification of the first zone(s)	Measure size of first zone (radius)	The radius from the core to the end of the 0 group measured along the ventral axis is estimated at 0.73±0.01 mm (ICES, 2013).
The first several zones		The growth increments become narrower after the first 5-6 increments, probably corresponding to the spawning event. Checks occur at younger ages.
Transition zones	Not a problem	Transition zones are not considered to be a problem in black scabbardfish otoliths.
Outer zones in older fish		Increments become narrower and more difficult to distinguish with increasing age. Such zones should be considered as annual zones and not as checks.
Edge		The annual deposition of increments has been tested by marginal increment analysis and the general marginal zone deposition rule is used, as detailed in the WKAMDEEP Reports (ICES, 2013 and 2018).
Reference images	Black scabbardfish otoliths with relatively clear growth pattern.	 TL: 92 cm. Month caught: September Estimated age: 6  TL: 106 cm. Month caught: November Estimated age: 10
*** Laboratories that regularly age this species**	IPMA DSI-DRP	Portuguese Institute for Sea and Atmosphere, Portugal Research Service of the Regional Fisheries Directorate of Madeira, Portugal
Discussion		Problem: 1st ring difficult, expected growth from core is 0.73 mm. The first increments are difficult. Recommendations: Consider staining or polishing. Experiment with image quality (contrast and gray scale). Use growth curve as a guide if necessary.

8 Recommendations

Recommendation	Addressed to
WGDEEP should indicate as soon as possible for which species and/or stocks age data are required or would be advantageous.	WGDEEP, WGBIOP
SmartDots should be further developed to allow the application to be used independently of ICES management, in order to facilitate further exchanges and training within and between national laboratories.	WGBIOP, ACOM
WKAMDEEP2 recommends organizing another exchange in 2020 with 50 otolith images of each species, and another exchange in 2022 with the same 50 images in random order and with additional 50 images of each species. After the last exchange, WKAMDEEP3 should be organised in 2023.	WGDEEP, WGBIOP, ACOM
Individual researchers and laboratories are encouraged to follow up on WKAMDEEP2 by continuing investigations to validate the age reading of each species, especially considering the validity of the first growth zones.	National laboratories

9 Recommendations for future WKAMDEEP

WKAMDEEP2 recommends that WKAMDEEP3 be scheduled in 5 years with the following preliminary terms of reference:

a) Collect and review the consistency of age data used in stock evaluations of deepwater fish;

b) Review new information on precision and accuracy of age estimation of each species and revise the individual protocols as appropriate;

c) Review individual protocols used by different laboratories and recommend a best practice for each species;

d) Assemble age reading experts on deepwater species for training on age reading of several species, following the recommendation from WKAMDEEP2 to conduct age reading comparisons collectively for the whole group of deepwater fish;

e) Analyse and evaluate the results of the previous exchanges of each species and report on precision and between-reader biases as well as measurements of growth zones.

10 References

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Annex 1

Workshop on Age Estimation Methods of Deep Water Species (WKAMDEEP2)

Facultad de Filosofía y Letras, Address: Av. Dr. Gómez Ulla, 1, 11003 Cádiz, Spain

17–21 September 2018

Provisional agenda

Working hours: 09:00-18:00, with lunch from 13:00-14:30 and coffee breaks at 11:00 and 16:00 (late start on Monday and early closing on Friday)

Monday 17

10:00-13:00

Juan: Welcome, practical information and adoption of the program

"Inside Fish Otolith- Matrix, Mineral, Growth" by Dr. Santiago Gómez from the Hard Tissue Laboratory (Faculty of Medicine) of the University of Cadiz

Kélig: Introduction to Smartdots and getting everyone up and going with the exchange system

14:30-18:00

Ole: Generic template for species specific age reading manuals

All age readers: Group according to species competence and adjust the generic template to a first draft for each species.

Tuesday 18

09:00-13:00

For each of the species tusk, ling and blue ling:

1) Expert demonstration of age reading using Smartdot (20 min)

2) Everyone practices individually (30 min)

3) Plenary discussions and adjustments of the draft manuals

14:30-18:00

All age readers individually: Ageing of sample sets of otolith images of tusk, ling and blue ling.

Report outline and distribution of responsibilities

Wednesday 19

09:00-13:00

For each of the species Black scabbardfish and Black-spotted sea bream:

1) Expert demonstration of age reading using Smartdot (20 min)

2) Everyone practices individually (30 min)

3) Plenary discussions and adjustments of the draft manuals

14:30-18:00

All age readers individually: Ageing of sample sets of otolith images of Black scabbardfish and Black-spotted sea bream.

Compiling of age reader comparisons on tusk, ling and blue ling from previous day,

Report writing

Thursday 20

09:00-13:00

For each species Greater forkbeard and Greater silver smelt:

1) Expert demonstration of age reading using Smartdot (20 min)

2) Everyone practices individually (30 min)

3) Plenary discussions and adjustments of the draft manuals

14:30-18:00

All age readers individually: Ageing of sample sets of otolith images of Greater forkbeard and Greater silver smelt.

Compiling of age reader comparisons of Black scabbardfish and Black-spotted sea bream from previous day,

Report writing

Friday 21

09:00-13:00

Compiling of age reader comparisons of Greater forkbeard and Greater silver smelt from the previous day.

Discuss all between-reader comparisons and output from Smartdot.

Agree on way forward, e.g. a small-scale exchange after the workshop.

Recommendations