ICES WORKSHOP ON AGE VALIDATION STUDIES OF SMALL PELAGIC SPECIES (WKVALPEL)

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Contents

i Executive summary

The Workshop on age validation studies of small pelagic species (WKVALPEL) fo-cused on validating ageing criteria for small pelagic species (anchovy, horse macke-rel, chub mackerel, mackerel and sardine). The aim of the workshop was to collate information on existing ageing protocols and to use these to support development of a validated protocol to better standardize age estimates.

One of the main sources of error affecting ageing precision is the discrimination bet-ween the false ring and annulus. An ageing process follows a number of typical steps. First, an ageing methodology is established, based on scientific information, to obtain age data for a particular species. Once age results are available, some analysis is re-commended to improve precision among different readers and/or readings. The next step is to perform other studies that offer independent results used to support, or not, an accepted ageing methodology. Several matching and independent results help to corroborate certain ageing criteria. Each study determines how precision and/or trueness are enhanced. In general, these methods are included in indirect or semi-direct validation categories, as true ages are not actually known in any of them. Some other methodologies, usually more complex and costly, are considered strictly as va-lidation experiments, as results approach to real ages. Tagging-recapture experiments and rearing in captivity are included within this category.

The latest available information on ageing data (precision and/or validation studies) was presented for a number of different species of small pelagics. Methods highlighted included marginal increment analysis (MIA), marginal analysis (MA), length frequency distribution analysis (LFDA) and back calculation (BC). A synthesis table of the last annual growth workshops and exchanges by species is also presented. The goal, for each species (Engraulis encrasiculus, Sardina pilchardus, Clupea ha-rengus, Sprattus sprattus, Scomber scombrus, Scomber colias, Trachurus trachurus, Trachurus mediterraneus, Trachurus picturatus, Micromesistius potassou), was to add information on the exchange or workshop and to present the major difficulties that caused low Percentage of Agreement between the age readers as well as to recom-mend some guidelines to overcome those difficulties.

Given that several methods exist for validation of age readings of calcified structures, a summary table of age validation methods used for all small and medium pelagic species in European waters was developed with a focus on the feasibility for the small pelagic species and validation strength of the folowing methods: BC, LFDA, Weight frequency distribution (WFD), Progression of strong year-classes, MIA, MA, daily growth increments (DGI), Daily increments widths, Tag-recapture analysis and Cap-tive rearing.

ii Expert group information

Expert group name	WKVALPEL
Expert group cycle	Annual
Year cycle started	2019
Reporting year in cycle	1/1
Chair(s)	Kélig Mahe, France
	Pierluigi Carbonara, Italy
	Javier Rey, Spain
Meeting venue(s) and dates	22-24 October 2019, Boulogne sur mer, France (18 participants)

1 Precision, Trueness and Accuracy of Ageing data

Accuracy is the closeness of the estimate of a quantity (measured or computed value) to its true value (Fig. 1). **Precision** is the closeness of repeated measurements of the same quantity (Fig. 1). For a measurement technique that is free of bias, precision implies accuracy, but the two parameters are not identical (*In* Panfili et al., 2002). The third concept has **Trueness** as is a measure of how repeated measurements are located around the true value.

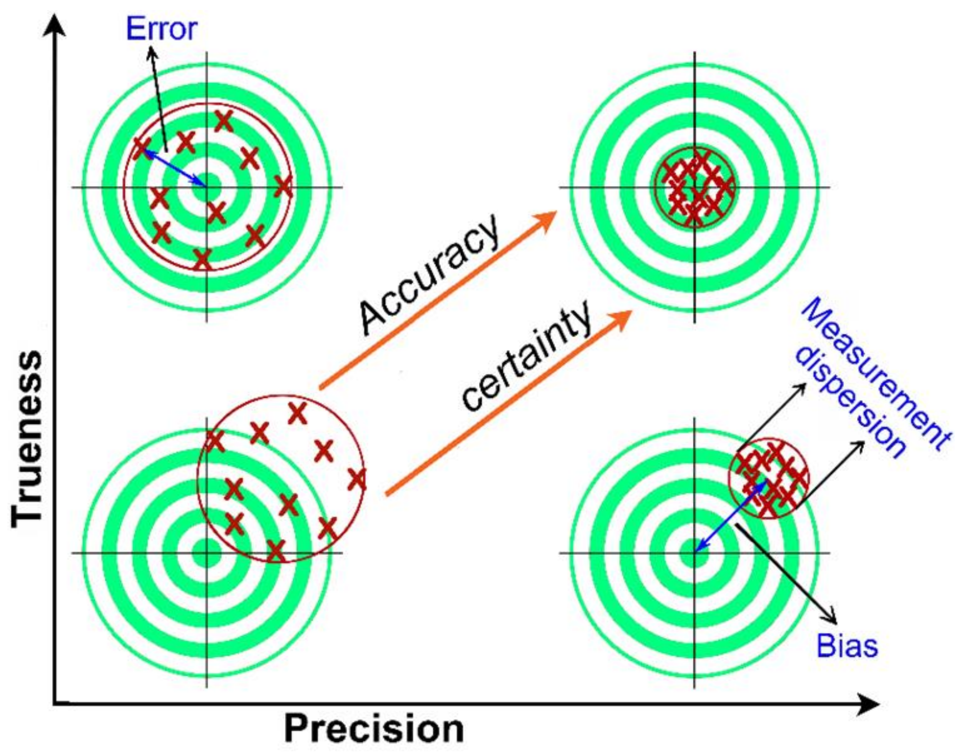

Figure 1: Relationship between TRUENESS, PRECISION and ACCURACY (*modified from* Villarraga-Gómez, 2016).

Precision is defined as the variability in the age readings. The precision's errors in age readings are described by the coefficient of variation (CV) and Percentage of Agreement (PA) by age group during the workshops and/or exchanges. This measure of precision is independent of the closeness to the true age (ICES, 2007). Conversely, the validation studies evaluate the accuracy of ageing data.

2 Review of ageing information

During this workshop, several presentations were made presenting the latest available information on the ageing data (precision and/or validation studies) of small pelagics. All presentations are presented in the form of a summary in this chapter.

2.1 Validation of the periodicity of growth increment formation in sprat (*Sprattus sprattus*) in the eastern North Sea

Francesca Vitale, Marianne Johansson, Birgitta Krischansson and Michele Casini

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Stock assessment procedures require an accurate and efficient determination of fish age for managing exploited fish populations. Routine techniques determine the age of an individual fish through the identification and count of periodic growth increments (*annuli*) on calcified structures such as otoliths. However, the interpretation of the annuli is often questioned and in need of validation. The present work attempts to validate the periodicity of formation of the growth increments on sprat otoliths (*Sprattus sprattus*) collected in the Skagerrak and Kattegat during 2003-2004, by the means of the widespread Marginal Increment Analysis. This is a novelty for this commercially important pelagic stock in the eastern North Sea. The results pointed out that the otolith hyaline and opaque zones were laid down once during the years analysed. The increment of the outermost translucent ring increased slowly from February to May conforming to the slow growth of sprat during the winter period while the deposition of the new translucent ring was completed during the summer period (June-July) (Fig. 2). This sinusoidal annual pattern was common for both Skagerrak and Kattegat and for all age groups. The results validate the periodicity of growth increment formation in this exploited sprat stock and revealed that summer is the most challenging period for age determination of this sub-stock.

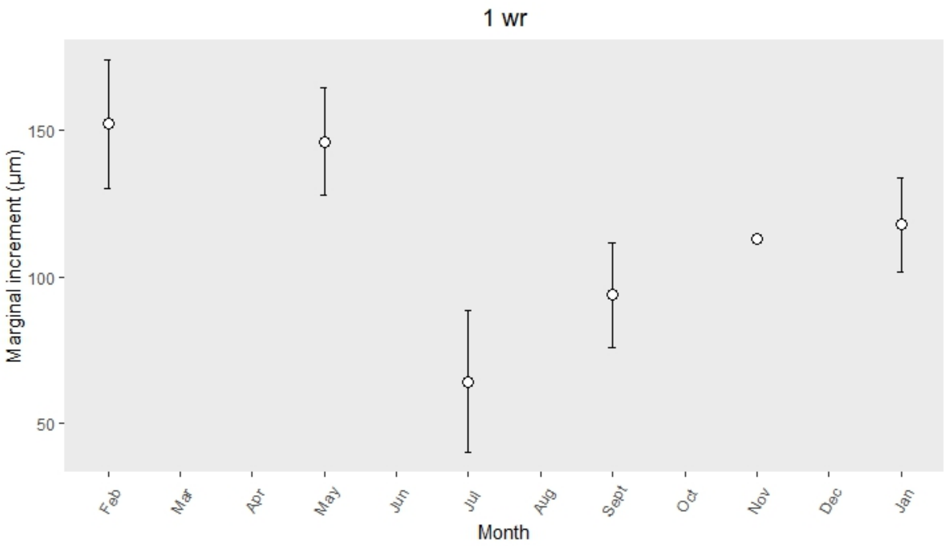

Figure 2: Marginal increment analysis for Srpatt in eastern North Sea

2.2 Age determination of Baltic sprat (*Sprattus sprattus balticus*) using otolith annual increments

Julita Gutkowska

Nmfri, Poland

Sprattus sprattus balticus (Schneider, 1908) is a subspecies of European sprat (*Sprattus sprattus*; Linnaeus, 1758) which has the ability to live in the waters of low salinity (Aps, 1991). Polish samples of sprat otoliths came from commercial catches and scientific surveys. Fish sampling is conducted in the three areas of the Baltic Sea – Gdańsk Basin (ICES Subdivision 26), Bornholm Basin (ICES Subdivision 25) and Arkona Basin (ICES Subdivision 24). During the last forty years the age determination of the Baltic sprat age was not discussed very often. The first informal ad hoc meeting took place, in Västervik (Sweden) in the 1980's (Grygiel, 1998), followed byworkshops in 1992 in Tallin (Estonia), in 1997 in Kaliningrad (Russia), in 2004 in Gdynia (Poland), in 2006 in Charlottenlund (Denmark) and in 2008 in Klaipeda (Lithuania) (Anon., 2006, 2008.). The latest otolith exchange started in spring 2004 and finished in fall 2005. Ten age readers from nine countries analyzed 754 otoliths. The results show that the range of disagreement in age reading was 28.9 - 88.9%, average 65.2% (Wilcoxon signed rank test) and the range of average convergence was from 36.2% (in the Estonian sample) to 72.3% (in the Polish sample), average 58.3%. During the latest workshop in Klajpeda, 95 otoliths were analyzed and the average percent agreement was 76.2%. One of the recommendations was to organize the workshops once in three years. Since this time, in 2016 the workshop on age estimation for sprat from Skagerrak and Kattegat (3.a), North Sea (4) and Celtic Seas Ecoregion (Divisions 6 and 7) was organized but not for the Baltic sprat (ICES. 2017). Any age validation methodologies were not used for the Baltic sprat but several methods were used for sprat from Skagerrak-Kattegat, like marginal increment analysis, weight frequency analysis and daily increment widths (Torstensen et al., 2004). At the workshop preliminary results of similar studies on Celtic Sea sprat were presented and discussed.

2.3 Age validation and growth pattern on Chub Mackerel and Atlantic Mackerel in ICES div. 9a.

Andreia V. Silva, Cristina Nunes, Georgina Correia, Pedro da Conceição, Delfina Morais, Diana Feijó and Patrícia Gonçalves.

Instituto Português do Mar e da Atmosfera, Portugal.

In Portugal, Chub Mackerel (*Scomber colias*) and Mackerel (*Scomber scombrus*) are middle-size pelagic species that inhabit shelf areas. The distribution of these two species overlap in the Iberian Peninsula coast. However, *S. scombrus* is more abundant in the north of Portugal and *S. colias* is predominant in the south (Martins et al., 2013). Both species show a fluctuating abundance off the Portuguese coast and are mainly captured by the purse-seine fleet which targets sardine (*Sardina pilchardus*). Recently, *S. colias* assumed an important role in the total Portuguese purse-seine landings (about 1/4 of the fish landed in Portuguese waters), in part likely because sardine abundance has decreased since 2006. On the other hand, *S. scombrus* landings show a decreasing trend over time which is associated with the Total Allowable Catch.

Both species show problems in age interpretation and very low levels of agreement were obtained in the last age readings workshops (ICES, 2016; ICES, 2019). In response to the workshop recommendations this study attempts to validate the age of *S. colias* and *S. scombrus* in Portuguese waters.

The age of both mackerels was determined from counts of trasparent annual growth zones in sagittal otoliths. The progression of diameter frequency was analyzed in 208 otoliths of *S. scombrus* from samples of 2018 obtained from Peniche harbour, to identify different age groups. Considering that the diameter of the first annual ring is directly proportional to the fish length and based on a specific sample collected by beach purse seine in Northeast Portuguese coast, the identification of the first annual ring for *S. colias* was investigated. Edge type analysis was performed, by examining the growing edge type of otoliths over time, in order to verify the existence of an annual growth pattern. Marginal increment analysis (MIA) was used for validating the periodicity of growth increment formation. Samples were collected monthly during 2006-2018 in Peniche and Matosinhos harbours. Growth parameters using a Von Bertalanffy (VB) growth curve were also estimated by year for both harbours.

The length range of the *S. colias* from the beach purse seiner samples was 9–25cm. The fish length/otolith radius relationship explained 78.4% of the variance observed (Fig. 3a). Only fish with 0 rings and translucent edge were used because it was assumed that this age class corresponds to the start of the deposition of the first annual ring. The mean of the first annual radius ring was estimated as 1.56 mm±0.16. The translucent ring that frequently appears laid down slightly closer to the nucleus should be considered as a check (false ring).

The distribution of each annulus of *S. scombrus* had a normal distribution with a decreasing otolith growth rate with age (Fig. 4b), except for the age group 4 and 5 which show a bimodal distribution indicating that problems exist in the age attribution.

The quartile proportion of the edge type for both species showed a growth seasonal pattern not fully clearly defined, though the opaque edge appears mainly during the 2nd and 3rd quarters

(Spring-Summer) (Fig. 5). For *S. colias*, the MIA did not show a clear pattern (Fig. 4a) but for *S. scombrus*, the MIA was higher in the first half of the year, between March and May, with highest values in April (Fig. 5b).

Regarding the estimated growth parameters using a VB curve, both species present significant statistical differences between years for both harbours (Likelihood Ratio Tests, *S. scombrus* - Peniche: $\chi 2$ = 74.4, df = 9, P < 0.0001; *S. scombrus* - Matosinhos: $\chi 2$ = 165.1, df = 9, P < 0.0001; *S. colias*- Peniche: χ^2 = 181.3, df = 12, *P* < 0.0001; *S. colias*- Matosinhos: $\chi 2$ = 325.2, df = 12, P < 0.0001).

From all the results obtained, *S. colias* shows some uncertainty in age determination from otoliths analyses, requiring further validation studies. The MIA and the frequency distributions of annuli radius (excluding age groups 4 and 5) of *S. scombrus* indicate a decreasing otolith growth rate with age and a formation of one annulus per year which gives some consistency to the present age estimations.

These are preliminary results of an ongoing work that should be considered of major relevance not only in terms of the improvement of the knowledge of the biology of these species but especially within the framework of the assessment of these fishery resources.

Acknowledgments

Authors would like to give special thanks to Rogélia Martins and Miguel Carneiro for collecting the samples of *S. colias* from the beach purse seiner.

a)

b)

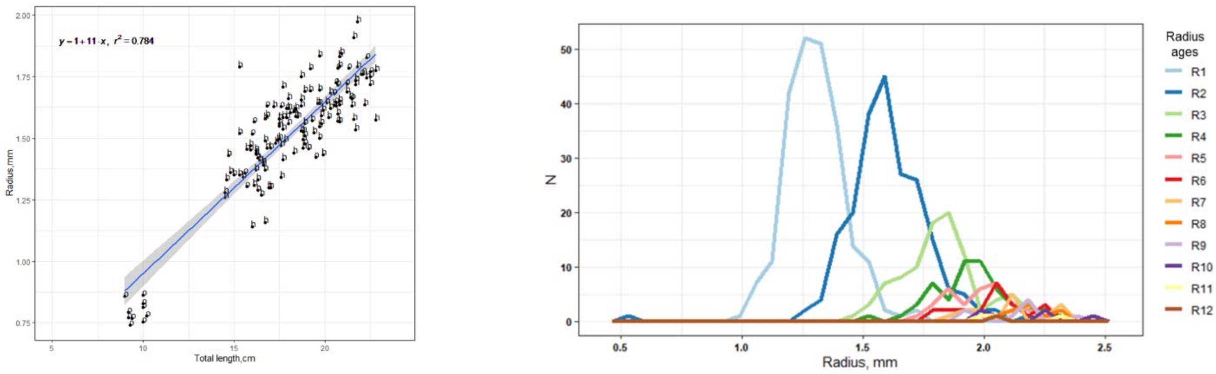

Figure 3 – a) *S. colias* otolith radius (mm) vs total length (cm) relationship from beach purse seine samples (2016-2017) and b) Annuli increment formation pattern (rings radius) in *S. scombrus* otoliths in Peniche harbour (2018).

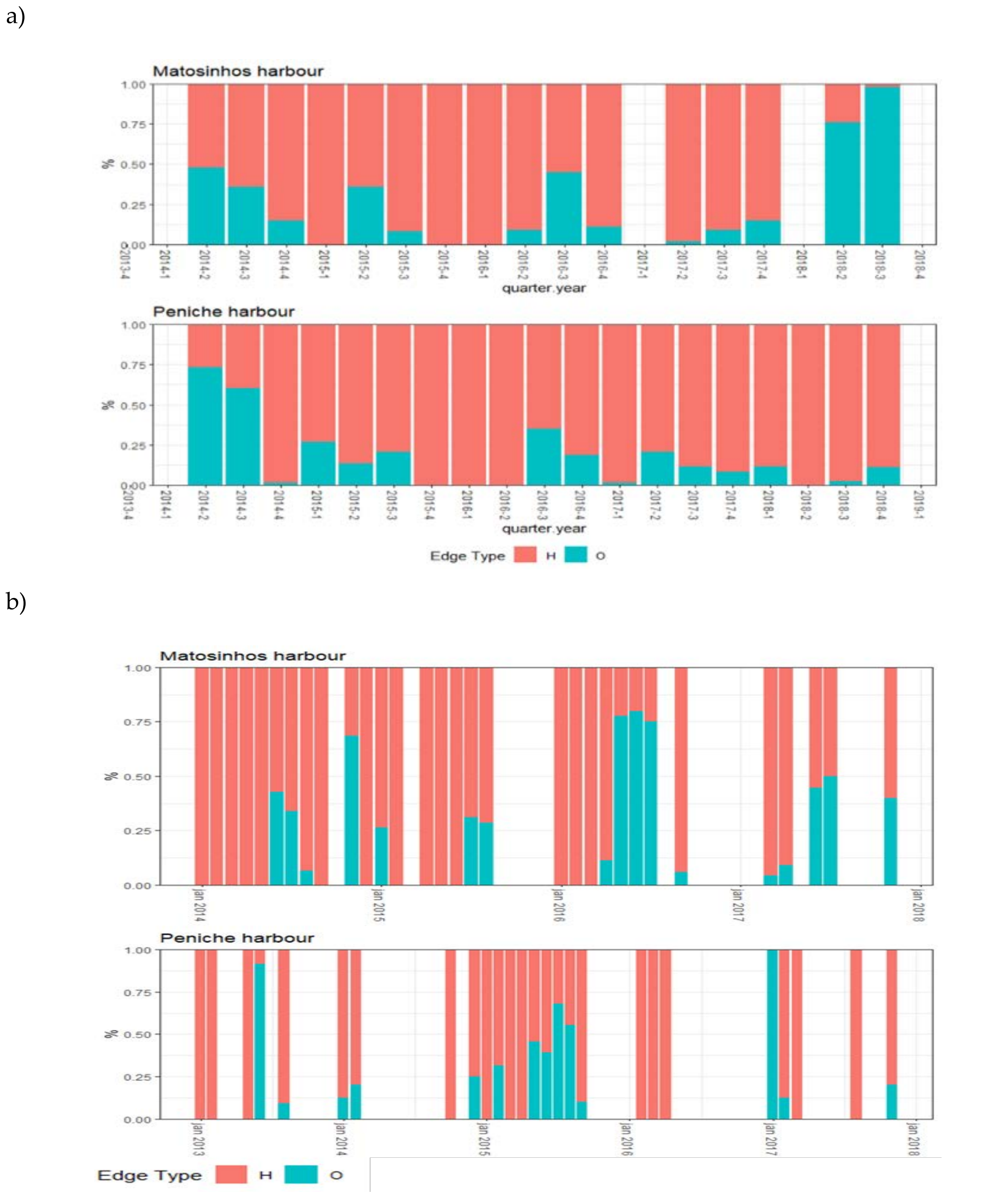

Figure 4 - Quartile proportion of edge type for a) *S. colias* and b) *S. scombrus* from Matosinhos and Peniche harbours.

2.4 Age validation and verification studies on blue whiting (*Micromesistius poutassou*)

Patrícia Gonçalves

Instituto Português do Mar e da Atmosfera, Portugal.

Blue whiting is a widely distributed species along the Northeast Atlantic. This species is considered as a single stock along their NEA distribution area for the assessment purposes. In recent years, several studies give indication of the existence of at least two distinct stock components, a northern and a southern, with the transition border at the Porcupine Bank. There is evidence of a slower growth pattern when moving north. The peak of spawning is also different between the southern (January-March) and the northern areas (March-April). The main age reading guidelines for this species, common for all the NEA are: to avoid the Baileys zone (larval ring zone) first ring should be considered around 50 - 56 e.p.u. (eye piece units) that it correspond to 8.33 – 9.33 mm. The main sources of age reading uncertaintyfor this species are: common age reading interpretation for the whole NEA distribution area; first ring interpretation, due to misinterpretation with the Bailey's zone; false rings and double rings. Due to these constraints on aging there is a need for age corroboration/validation studies. Taking this into account age corroboration studieswere prepared and presented during the last workshop on blue whiting age reading (WKARBLUE2) and helped with the clarification of the ring aging interpretation (ICES, 2017) (ICES, 2017). Those studies focused on:

(i) length based methods and back calculation:
  (ia) for the identification of the first ring for the Portuguese coast (Dores and Gonçalves, 2017);
  (ib) for the ICES divisions 2.b, 4.a, 6.a, 7.b, 7.c, 7.j, 8.c and 9.a; and Mediterranean and NAFO 1C to help on the identification of false and split rings based on multivariate modelling approach (Gonçalves and Dores, 2017);

(ii) marginal increment analysis (Elleboode and Chantre, 2017).

The main conclusions from those studies were: (ia) the size of first ring on otoliths off the Portuguese coast (southern component) is 8.5-11 mm (distance from the center to the anterior area), different from that described in literature based on blue whiting measurements from the northern areas (8.3-9.3 mm) (Fig. 5); (ib) this approach showed to be useful to help on annuli interpretation, mainly concerning the doubts due to false and double rings (Fig. 6); (ii) it is necessary to repeat the analysis for a larger sample size from each stock area (northern and southern).

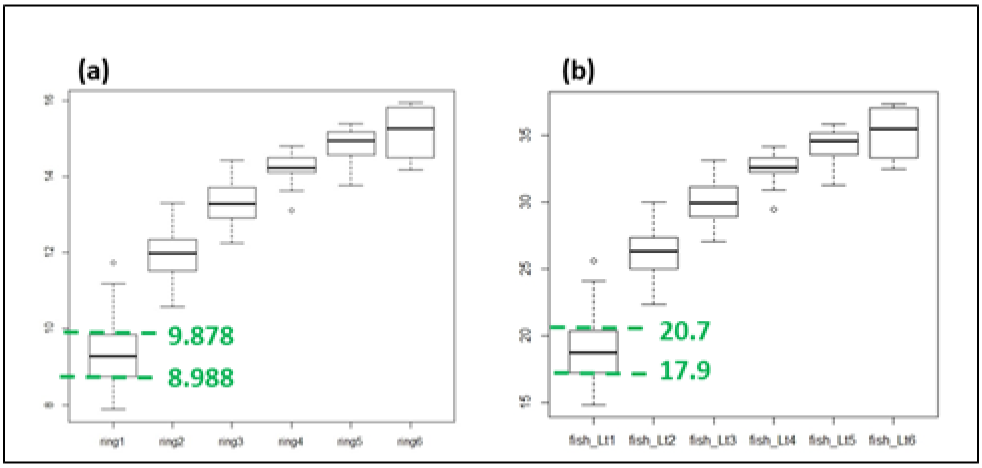

Figure 5 – (a) Otolith ring length (mm) and (b) fish length (cm) at each age group from 1 – 6.

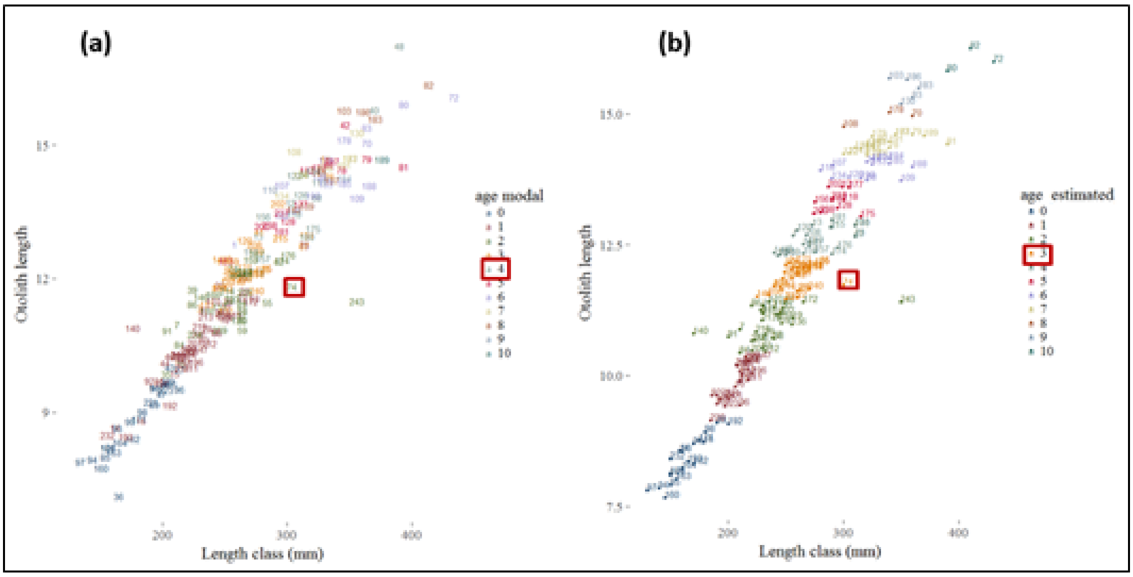

Figure 7 – Relation between the fish length (mm) and the otolith length (mm) by modal age (in colours) from (a) WKARBLUE2 and (b) estimated by an multivariate modelling approach. Fish ID identified in numbers.

2.5 Semi-direct and Indirect age validation for the horse mackerel in South Adriatic Sea (Central Mediterranean)

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COISPA Tecnologia & Ricerca, Stazione Sperimentale per lo Studio delle Risorse del Mare, Italy

During the workshop one example of a validation study on the T. trachurus in Mediterranean basin (Adriatic Sea), based on indirect and semi-direct methods was presented. The deposition of the one annulus was shown in samples from the Adriatic (Fig. 7).

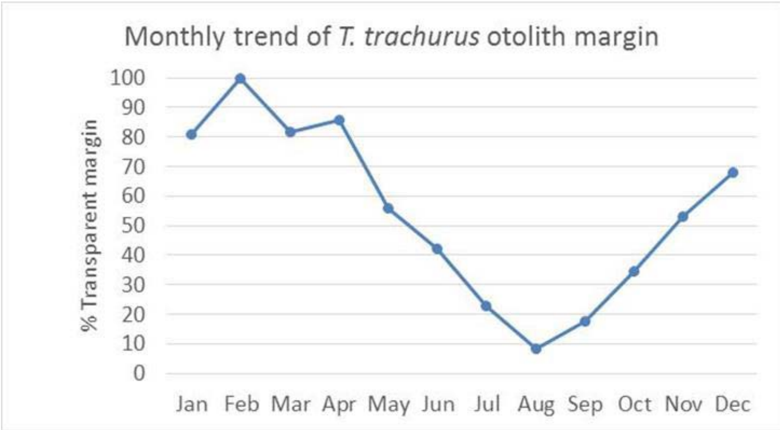

Figure 7. Monthly trend for the otolith edge of the T. trachurus in the Adriatic Sea.

Moreover, the back-calculation results (Table 1 & Fig. 8) were compared with the mean length of the mode (Bhattacharya method) from the winter Length Frequency Distribution (LFD) Survey (GRUND 2009).

Table 1. Back-calculation results for the T. trachurus in the Adriatic Sea.

		Rings											
N° Growth Increment	N° Specimens	1°	2°	3°	4°	5°	6°	7°	8°	9°	10°	11°	12°
1	143	72.64											
2	63	73.41	131.24										
3	250	73.18	131.25	188.15									
4	126	75.70	138.03	192.95	233.69								
5	68	80.76	140.24	190.90	229.97	258.02							
6	54	81.23	139.26	191.32	231.37	258.16	282.38						
7	28	77.06	142.38	194.46	231.03	259.05	280.65	301.79					
8	11	80.73	142.93	195.91	239.08	272.47	295.60	316.05	333.38				
9	7	76.43	144.43	194.61	234.33	266.09	287.86	306.40	320.32	334.22			
10	3	86.97	148.71	198.84	231.80	283.14	273.77	291.46	307.43	317.14	328.42		
11	1	79.49	147.63	191.43	225.50	249.84	269.31	313.11	317.98	327.71	347.18	371.51	
12	1	88.37	154.73	215.99	251.72	282.35	302.77	328.29	353.81	374.23	384.44	404.86	420.17
Tot. Number		755	612	549	299	173	105	23	12	5	3	1	1
Mean (mm)		75.09	136.36	191.24	232.40	260.00	283.49	305.63	326.24	332.74	343.38	388.19	420.17
Mean Increment (mm)			136.36	54.89	41.16	27.60	23.49	22.14	20.61	6.50	10.64	44.81	31.99
SD		11.35063	15.74223	17.824352	20.49372	23.1162	24.46939	20.83815	24.26462	23.7542	26.48223		
CV		15.12	11.54	9.32	8.82	8.89	8.63	6.82	7.44	7.14	7.71		

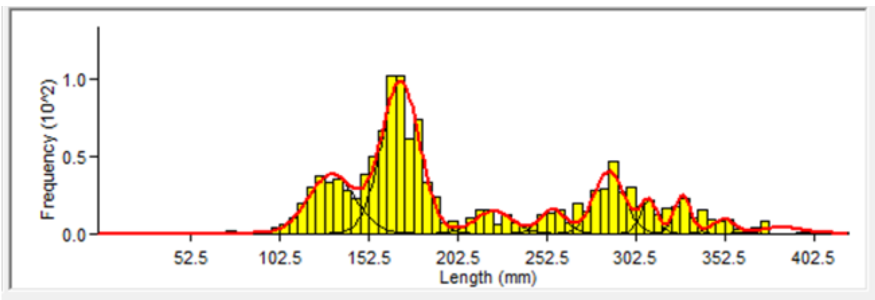

Figure 8. Length Frequency Distribution from the winter survey.

This analysis provided an indirect validation of the detected age group (Carbonara et al., 2018). The winter survey LFD was used in this analysis because the winter period seems to represent an age class (Table 2).

Table 2. Comparison (t-test) between the mean back-calculated length and the mean length of the mode (LFD).

Back-calculation				Bhattacharya method				t-Test
Mean Length	S.D.	N° specimen		Mean Length (mm)	S.D.	N° specimen		P-value
75.09	11.35	755	1° ring					
136.36	15.74	612	2° ring	133.87	19.59	252	1° mode	0.0502
191.24	17.82	549	3° ring	189.35	19.78	755	2° mode	0.0759
232.40	20.49	299	4° ring	230.62	7.32	164	3° mode	0.2826
260.00	23.12	173	5° ring	258.36	5.22	42	4° mode	0.6465
283.49	24.47	105	6° ring	287.51	8.99	174	5° mode	0.0511
305.63	20.84	23	7° ring	310.03	4.69	50	6° mode	0.1580
326.24	24.26	12	8° ring	328.87	4.67	57	7° mode	0.4414

This analysis show as the first ring back-calculated at a total length of 75.09 mm not have any correspondence in the LFD mode. While the other back calculated rings and mode did not present any significant difference (t-test p>0.05). In terms of accuracy, Campana (2001) indicated the analysis of discrete length modes as a robust approach to validating the interpretation of annuli.

The comparison of the growth curves from the otolith reading and LFD analysis from Medits survey (ELEFAN and Bhattacharys methods) did not show any statistical differences. This result could represent an indirect validation (Campana, 2001; Panfili et al., 2002) of the otolith age estimation criteria (Fig. 9).

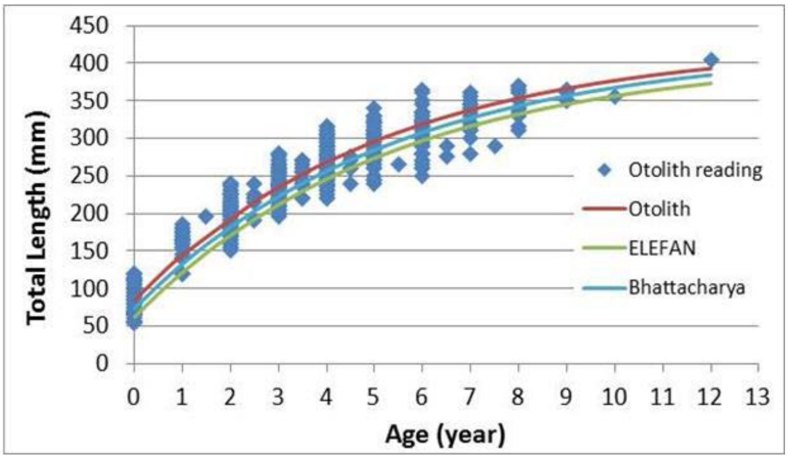

Figure 9. Growth curves comparison from otolith reading (L∞= 427.03 mm, k= 0.192 year-1, t0= -1.147 year) ELEFAN (L∞ = 409.5 mm, k= 0.188 year -1, t0= -0.875 year) and Bhattacharya L∞ = 421.47 mm, k= 0.186 year -1, t0= -1.032 year).

2.6 Age validation of the European anchovy (*Engraulis encrasiolus*) in the Western Mediterranean: 'a more accurate way to age anchovy'

Javier REY, Miriam DOMÍNGUEZ, Pedro TORRES & Alberto GARCÍA

INSTITUTO ESPAÑOL DE OCEANOGRAFÍA. Centro Oceanográfico de Málaga, Spain

Several studies on anchovy age and growth based on otolith microstructure have been published recently, although methodologies used were not analogous or easily reproducible. In order to set up a simple and reproducible reading process this study compares several otolith sections (frontal and sagittal) and magnifications (x400 and x200), to those used by other authors and a new alternative, frontal plane and lower magnification (x 200) (Fig.10). Greater difficulties were found to achieve legible samples of the sagittal sections. Moreover, when comparing the number of otoliths discarded, sagittal sections doubled in number compared to the rejected frontal sections. The results of this study point to otolith frontal plane at x200 magnification as the best option to age anchovy on a daily basis, both in reproducibility and accuracy perspective. From these daily growth increment (DGI) readings (Fig. 11 up), using OTOLab free software, a growth model and a growth strategy profile were obtained for this population (Fig. 11 down).

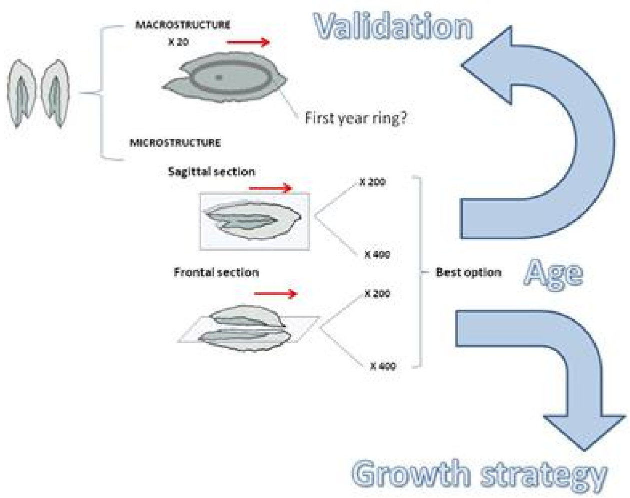

Figure 10. Study approach.

In addition, this study supports a fast growth hypothesis in Alboran anchovy,coupling biological and environmental traits where individuals attain 17.8 cm at the end of the first year (July). Moreover, microstructure outcomes **validate** the first annual ring, as recorded in macrostructure age readings, as a winter ring.

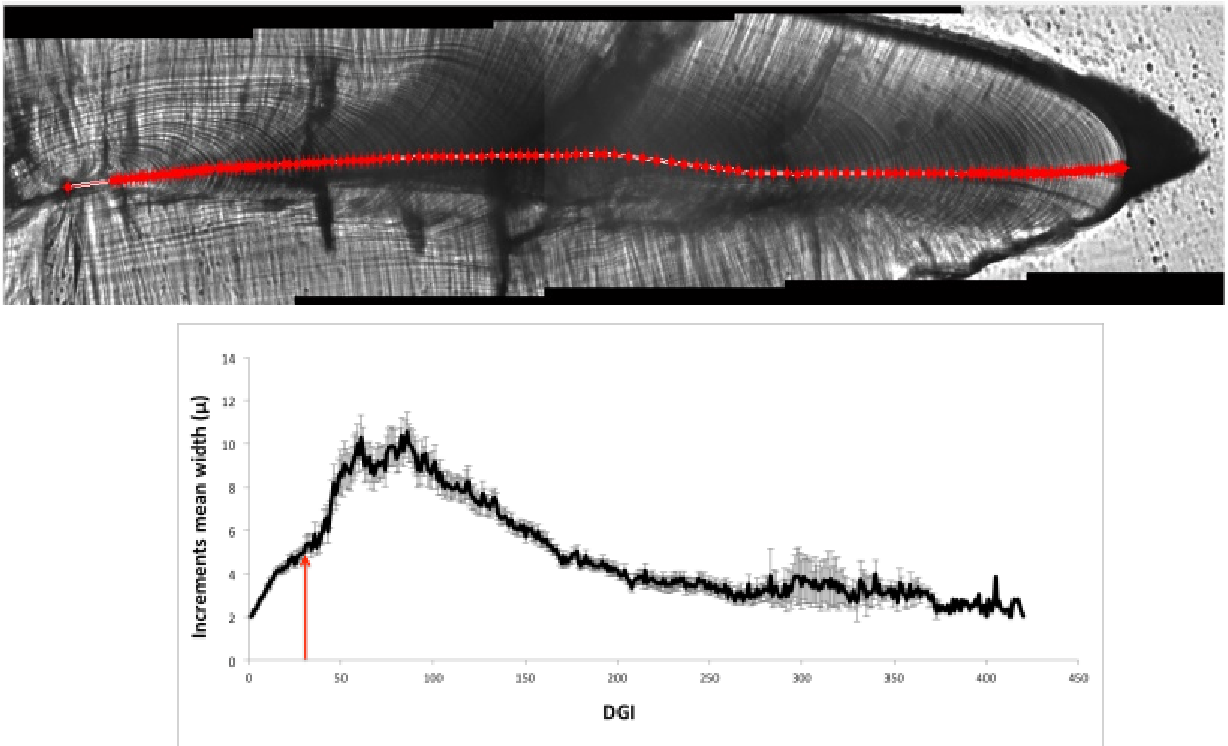

Figure 11. Left: panoramic view of an anchovy otolith throughout a frontal section (x400) from OTOLab free software. In red, daily DGI along the posterior growth axis. Right: anchovy growth history from x200 frontal DGI readings. Red arrow marks the end of larval phase.

2.7 Age validation of the Northeast Atlantic chub mackerel (*Scomber colias*) in Divisions 8.c and 9.a

This a summary of the poster By M.R. Navarro, J. Landa, B. Villamor, C. Hernández and R. Dominguez-Petit. 2019. Northeast Atlantic chub mackerel (*Scomber colias*): growth pattern and age validation in Northern Iberian waters. 5th International Sclerochronology Conference, 16-20 June 2019, Split, Croatia.

Atlantic chub mackerel is a middle-sized pelagic fish distributed in warm and temperate Northeast Atlantic waters. The bulk of the catches are taken in north western waters of Africa, but landings have increased significantly in the most recent years in the Iberian Peninsula (ICES, 2018; Villamor et al., 2017), resulting a new target species for both Portuguese and Spanish purse seiner fleets which partially replaces the important drop of sardine landings in both countries. Fishery advice has been recently recommended within ICES to be performed in the near future (ICES WKCOLIAS, 2020).

Based on samples from commercial catches and scientific surveys between 2011 and 2017, this study shows the growth pattern and parameters of chub mackerel and a holistic approach to age validation in Northern Iberian waters of interest for future assessment of this population. The age estimation criteria in *S. colias* applied in this study have been previously standardized among the European readers in a workshop (ICES 2016), and its consistency has been tested by periodical international calibration exercises. Absolute and relative otolith marginal increment analyses (AMD and RMD) and otolith edge nature analysis, based on specimens from two consecutive years (2011-2012), were performed, showing an annual periodicity in the formation of the hyaline and opaque annuli (opaque edge mainly from June to December). AMD and RMD show a similar trend (Fig. 12), which support the consistency in the age estimation Also, the consistency of the age interpretation is tested by the regularity of the otolith increments formation, that showed a unimodal distribution (Fig. 12), and with back-calculation analysis. The back-calculated mean length at age were estimated for two scenarios (Fraser-Lee; BPH), which showed almost identical values between them. Mean length at age from Direct Age Estimation (DAE) from commercial catches (LABs, in both semesters) and surveys (SURVs) and back-calculation were similar, especially for the most abundant ages (ages 2 to 5). These results also support the consistency of the age estimation.

In addition, length-frequency analyses were also performed for the period 2011-2017 with the purpose of corroborating the growth pattern (Bhattacharya's method and Length Frequency Distribution Analysis were used). Analyzing the growth performance index (Φ') and pattern of each method performed in this study (Fig. 13), three main different growth patterns were obtained:

a) a slow growth rate from direct age estimation/back-calculation (Φ': 2.74-2.81), showing 6-7 age groups between ~21 to ~40 cm of TL; b) an intermediate growth rate from Bhattacharya length-frequency analysis (Φ': 2.84), with 5 age groups between ~21 to ~40 cm of TL; c) a faster growth rate (Φ': 2.95-3.01) from LFDA length-frequency analysis, with 4 age groups between ~21 to ~40 cm of TL. Previous studies of *S. colias* in the Northeast Atlantic showed growth parameters estimated mainly from direct age estimation and/or back-calculation, all showing slow growth patterns (Φ':2.70-2.82). Only two studies showed growth parameters estimated from length frequency analysis, one using ELEFAN (Vasconcelos, 2006) from Madera Islands, showing an intermediate growth pattern (Φ': 2.86); and another using Bhattacharya (Nespereira, 1992) from Canary Islands, showing a slow growth pattern (Φ': 2.73).

This faster growth estimated in our length-frequency analysis, call into question the current otolith age estimation criteria, as this species could have a faster growth pattern than those estimated in otoliths. For this, the age and growth pattern has not been validated yet and further studies to corroborate/validate the age estimation are recommended. To extend the use of alternative methods to direct age estimation, such as length-frequency analysis, in other distribution areas is recommended and can help to confirm this faster growth rate obtained. Other direct validation studies, such as tag-recapture and daily increment analysis, can also confirm whether checks are being identified as true annuli in the age estimation process. In addition, other studies about the biology of this species (migration, feeding activity, etc.) would lead to a better understanding of the otolith growth pattern.

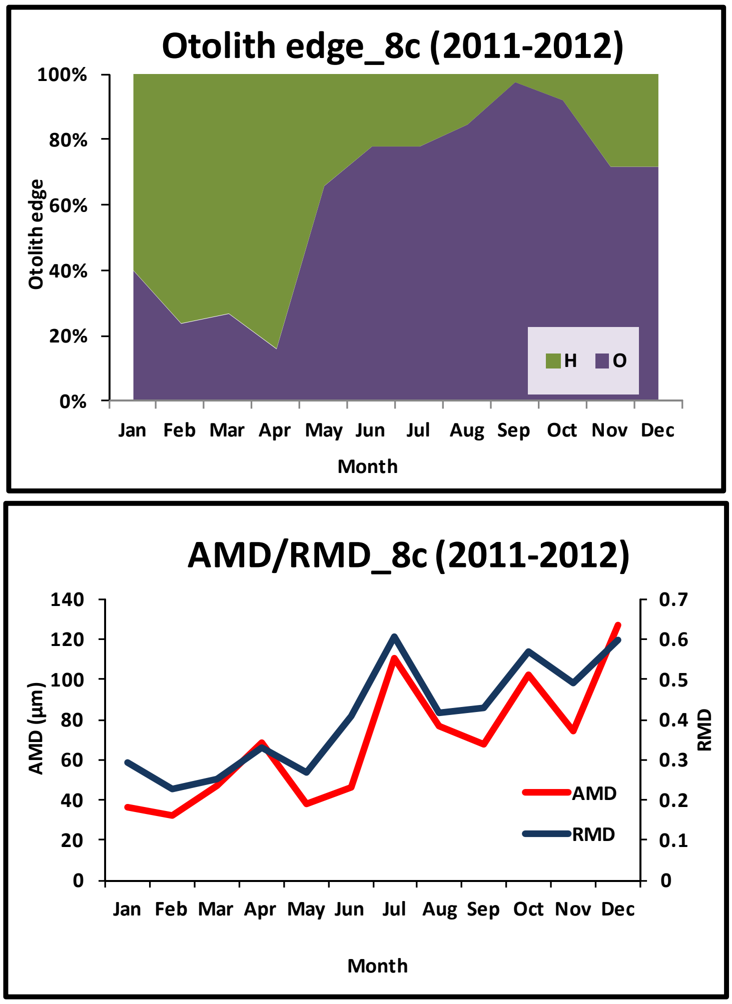

Figure 11: Monthly evolution (%) of hyaline and opaque otolith edges formation 2011-2012 (left panel) and Absolute (AMD) and Relative (RMD) otolith marginal increment analyses 2011-2012 (right panel).

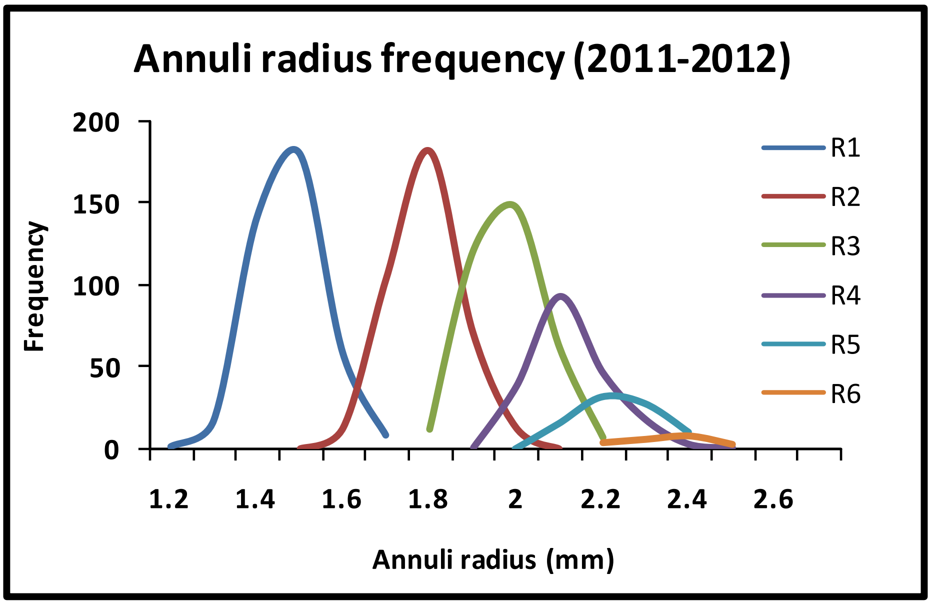

Figure 12: Anuli radius frequency 2011-2012.

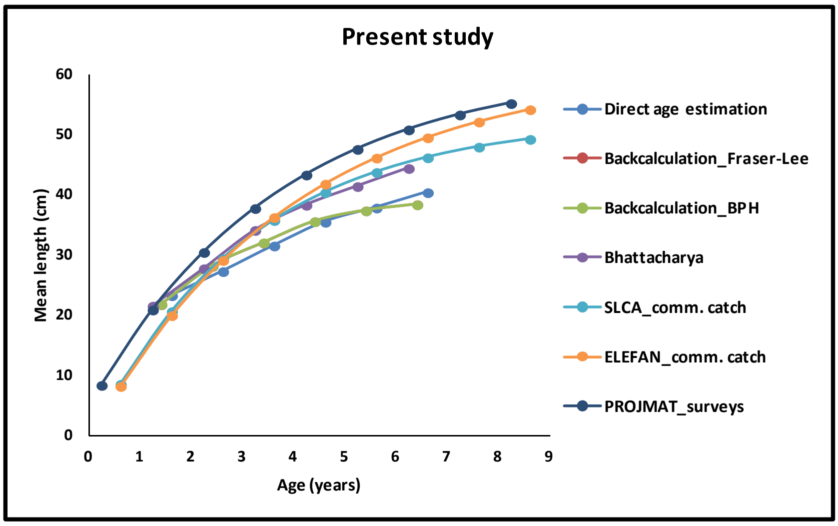

Figure 13: Growth curve of each method performed in this study

2.8 Seasonal formation of growth rings in the otoliths of the NEA Mackerel (*Scomber scombrus*) in ICES Divisions 8.c and 9.a North.

This a summary of the Working Document by Villamor, B., M.R. Navarro, C. Hernandez. C. Dueñas-Liaño, A. Antolínez. 2018. Study of seasonal formation of growth rings in the otoliths of the NEA Mackerel (*Scomber scombrus*) in ICES Divisions 8.c and 9.a North. Presentation to Workshop on Age Estimation of Atlantic mackerel, (*Scomber scombrus*) (WKARMAC2) 22–26 October 2018 San Sebastian, Spain. ICES CM 2018/EOSG:32

In this study, the marginal increment types (opaque or translucent) of the mackerel population of the Northeast Atlantic were assessed in the Southern area in relation to environmental and biological parameters. This study determines the seasonality in the formation of rings in the mackerel otoliths by monitoring hyaline/opaque edge in Divisions 8.c and 9.a North (Southern Component). Monthly samples were collected between January 2013 and December 2017 from commercial catches and spring and autumn research surveys.

The highest percentage of hyaline edge occurs between January and June, with a maximum in May every single year, except for the 2017 when the maximum is in June (Fig. 14). The minimum occurred between August and October. The variation in the proportion of hyaline edges was gradual over months and so was the delay in the formation of the opaque edge with age. In general, the minimum proportion of hyaline edges was observed around April at age 1, June at age 2 and August at ages 3 and older. The temporal delay in opaque-zone formation increase with age (Fig. 15), the growth of the younger mackerels of age-1 (all immature) resumes usually during March, mackerels of age-2 (mostly mature) start laying down the marginal opaque growth by May-June, and mackerels of 3 years and older (totally mature) start showing marginal opaque growth in June. Therefore, these results show a delay in the opaque edge formation with age for the Southern area (ICES Division 8.c and 9.a).

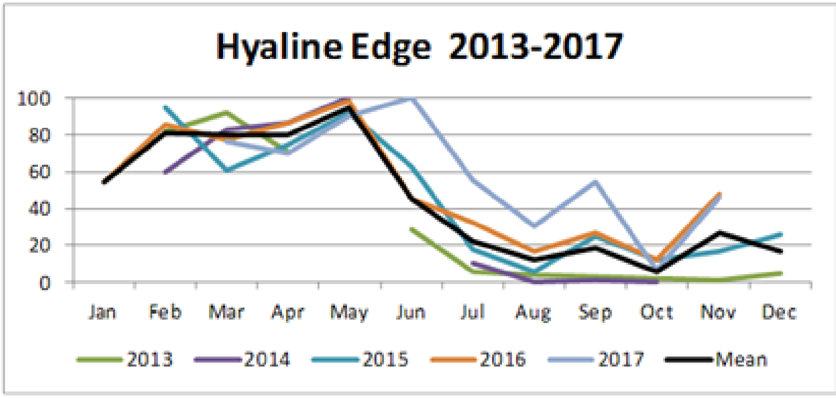

Figure 14: Evolution of hyaline edge formation (%) by year and for the whole study period, in Divisions 8.c-9.a North.

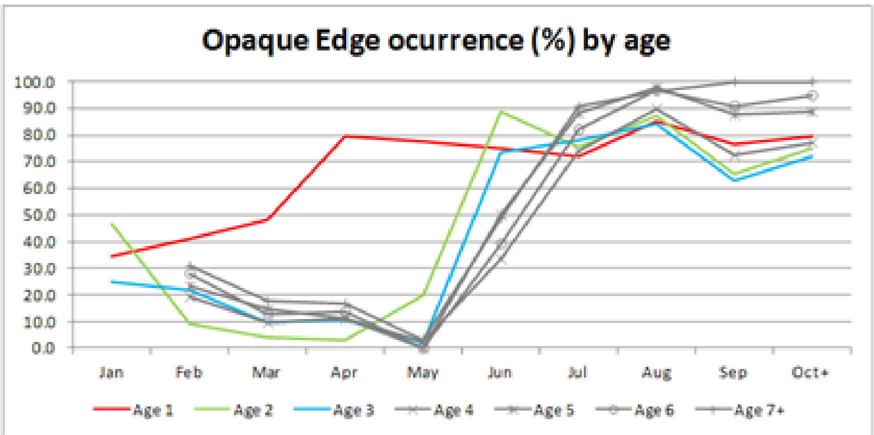

Figure 15. Opaque edge occurrence (%) by age for the total area (8.c-9.a North) and whole period.

In addition, the average temperature of the seawater, gonad somatic index (GSI) and Condition Factor (CF) were also calculated in order to get a yearly pattern for these parameters. The timing in the formation of rings in mackerel otoliths seems to link the temperature and food resources (Condition Factor) to the fast growth of the fish (Fig. 16 and 17).

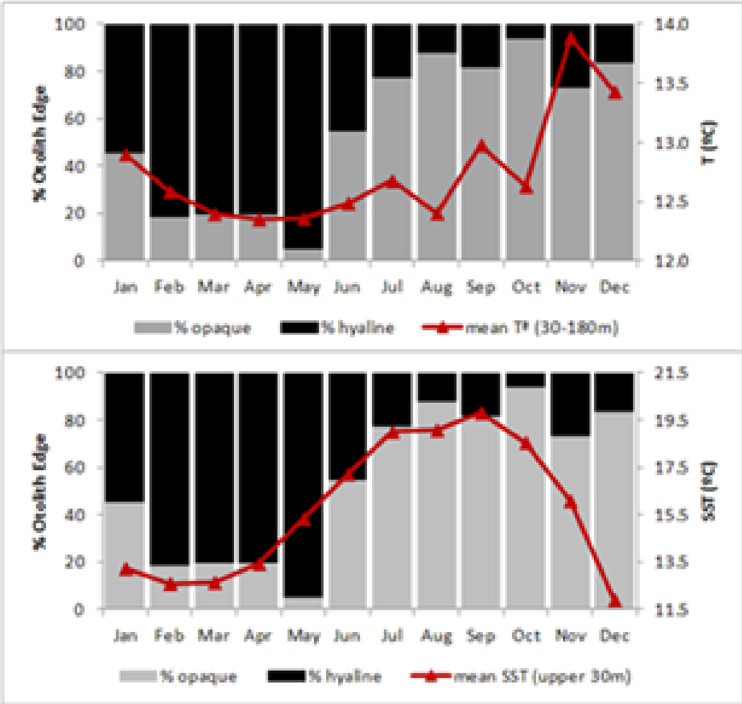

Figure 16. Monthly evolution of percentage of otolith edge formation and average monthly seawater temperature 30-180 m deep (top panel) and SST (bottom panel) for the whole study period.

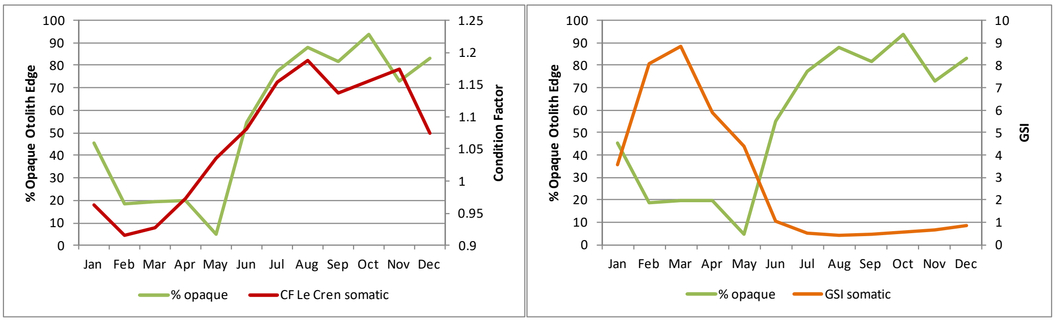

Figure 17. Evolution of percentage of opaque edge formation and Condition Factor (CF) (left panel) and Gonad somatic Index (GSI) (right panel) for the entire study period.

The season of formation of opaque and translucent zones may change during development and in relation to geographical distribution, as in the Atlantic cod (Høie et al., 2009) and Sebastes in the Pacific coast (Pearson, 1996). In this study, no geographical differences were found between areas 8.c and 9.a.N, nor with the one performed on the Portuguese coasts (ICES Subdivisions 9.a Central-North and South) by Gordo and Martins (1982). All these areas belong to the same Southern Component of the NEA mackerel. It is advisable to make this type of study for all distribution areas of mackerel in the Northeast Atlantic, from the south of the Iberian Peninsula to northern Europe (Norwegian and Icelandic coasts) to test whether or not there are seasonal differences in the formation of opaque-hyaline zones in otoliths and to study the factors influencing variation in otolith opacity.

2.9 Validation of age determination using otoliths of the European anchovy (*Engraulis encrasicolus L.*) in the Bay of Biscay

This a summary of the paper By A. Uriarte, I. Rico, B. Villamor, E. Duhamel, C. Dueñas, N. Aldanondo and U. Cotano 2016. Validation of age determination using otoliths of the European anchovy (*Engraulis encrasicolus* L.) in the Bay of Biscay Marine and Freshwater Research, 2016, 67, 951–966.

The paper presented the Validation of the age determination using otoliths of European anchovy along with a historical corroboration of the method and a summary of the annual growth in length. Validation of the age determination procedure using otoliths of European anchovy in the Bay of Biscay was achieved by monitoring very strong year- classes in successive spring catches and surveys. This was first achieved with the 1982 year class which showed a neat annual progression of modal lengths passing through the fishery until the age of 4 (Uriarte and Astudillo 1987). Validation of the proposed method was subsequently obtained through monitoring of the progression of the strong 1987, 1989 and 1991 year-classes, both by spring annual surveys and by continuous sampling of the commercial catches, coupled to the monitoring of the seasonal marginal edge formation of the otoliths. Since then, historical corroboration of the ageing method was obtained by the statistically significant cross-correlation between successive age groups by year-classes in catches and surveys (1987–2013).

Summary annual growth in length is also presented. Annuli consist of a hyaline zone (either single or composite) and a wide opaque zone, disrupted occasionally by some typical checks (mainly at age-0 and age-1 at peak spawning time). Age determination, given a date of capture, requires knowledge of the typical annual growth pattern of otoliths, their seasonal edge formation by ages and the most typical checks. Most opaque growth occurs in summer and is minimal (translucent) in winter. Opaque zone formation begins earlier in younger fish (in spring), and this helps distinguish age-1 from age-2+ (Fig. 18).

A Ttpical pattern of otolith growth is clearly shown by the oldest ages. Figure 1 of the paper presents typical pictures of otoliths from ages 1 to 5 in spring, both without or with false rings (or checks). In addition the first section of the electronic supplementary material of that paper contain another set of picture of otoliths of European anchovy in the Bay of Biscay throughout the year along with a seasonal characterization by age classes, as seen by incident light on whole mounted otoliths over black slides. In those Figures the typical growth pattern is clearly observed. Additional examples of typical otoliths at age 0 (autumn) and at ages 1 to 4 in spring for a recent cohort are shown here in Figure 19 (which were not included in the original published paper).

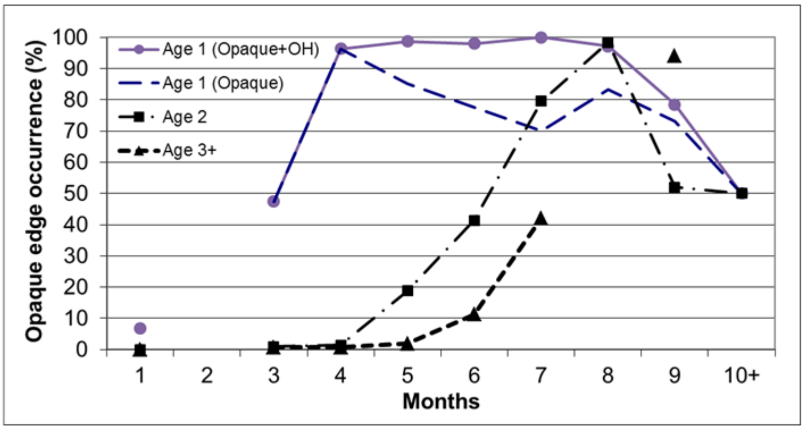

Figure 18: Occurrence of marginal opaque edges (adding up the opaque narrow and wide edges: ON + OW) by age class and month for the Bay of Biscay anchovy. Age-1 is shown either including the new semi-hyaline edges (opaque + OH) which occur during summer time or excluding them (Opaque = ON+OW). New semi-hyaline edges (OH) refer to the transition from opaque to hyaline not entirely visible all around the margin of the otolith which appear after having resumed (or completed) the annual marginal opaque growth.

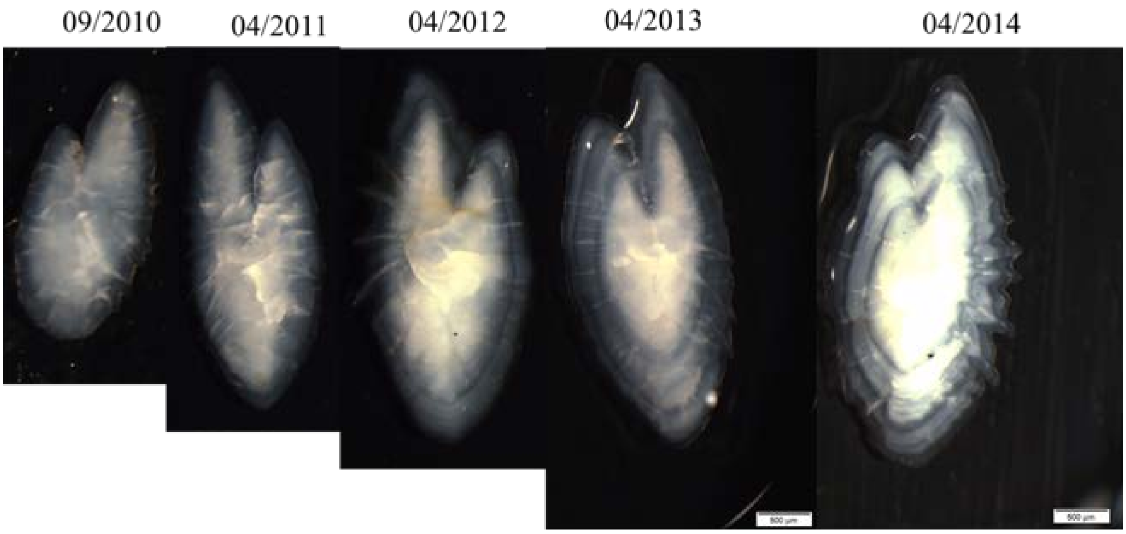

Figure 19: Typical Otoliths at age 0 (Autumn) and at ages 1 to 4 (Spring) corresponding to the 2010 year class (Source AZTI; in Uriarte et al. 2014 presentation).

2.10 Validation of the first annual increment deposition in the otoliths of European anchovy in the Bay of Biscay based on otolith microstructure analysis.

This is a summary of the paper by Naroa Aldanondo, Unai Cotano, Paula Alvarez and Andrés Uriarte (2016). Validation of the first annual increment deposition in the otoliths of European anchovy in the Bay of Biscay based on otolith microstructure analysis Ma-rine and Freshwater Research, 2016, 67, 943–950.

In order to validate the first annual increment deposition in European anchovy otoliths, early juveniles were captured in October 2012 in the southern Bay of Biscay. These individuals were maintained under a continuous feeding regime in a sea cage over a period of 6 months. From October 2012 to January 2013, lengths increased slightly or remained stable at around 9.8 cm. After this period, standard length increased significantly up to a mean value of 12.0 cm in April 2013. Likewise, the age of anchovies was estimated based on otolith microstructure analysis. The estimated age varied from 96 days (for individuals sampled in October 2012) to 293 days (for anchovies sampled in April 2013). A daily increment deposition rate was confirmed in otoliths of individuals maintained in the sea cage during the winter (Figure 3 of the paper proved such a relationship).

The general otolith daily growth pattern showed that increment widths increased rapidly and were broadest between 51 and 56 days, with a mean of 19.1 μm. Thereafter, the widths decreased steadily to 1.5 μm and remained almost constant until the end of the experiment. Figure 20 shows the typical daily growth increments according to the age of the anchovy juveniles throughout their first year of life and the general parallelism with the changes in temperature.

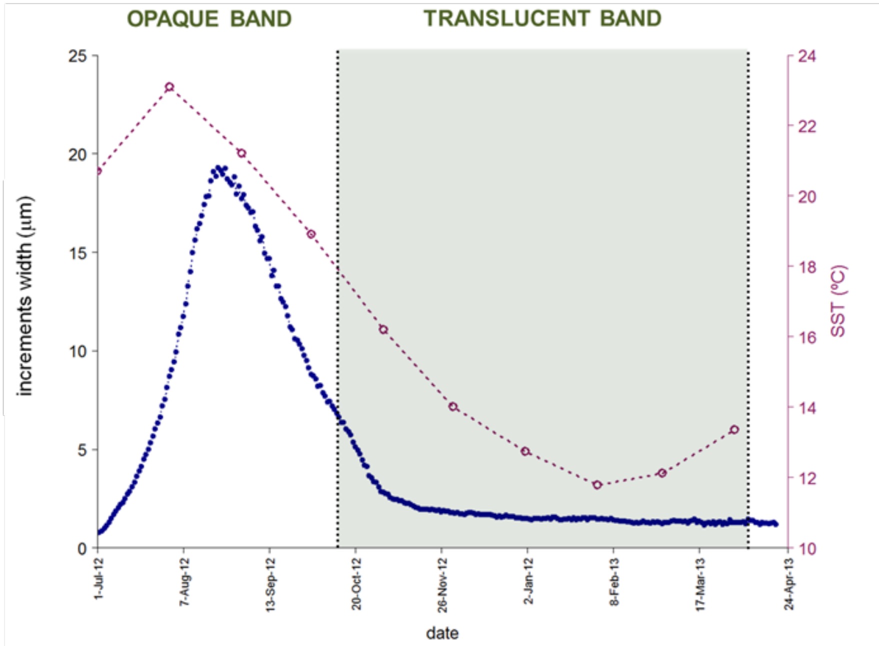

Figure 20: Average increment widths at age from otoliths of anchovy juveniles (blue dots) and the monthly mean sea surface temperature (SST) values obtained from data provided by the Aquarium of San Sebastian (438190N, 028W) (discontinuous line with circles). The shadow area, bounded by the vertical dashed lines, corresponds to the period of translucent band formation in the otoliths. (Source: Merging figures 6 and 7 of the Aldanondo et al. 2016 paper).

The present study also revealed that the first translucent band formation started in autumn and was completed by spring. This translucent band was characterised by a decline in increment widths, which were significantly narrower than those in the adjacent opaque band (Fig. 21). Examples of entire and polished otoliths showing the inner opaque area and the more translucent outer region appear in Figure 21.

Figure 21: Pictures of an entire otoliths (with incident light) at the end of the reared experiments, examined by Aldanondo et al. (2016) (left figure) and corresponding polished posterior part of the otoliths (with transmitted light) where the outer more translucent area can be seen (right figure) (Source: Aldanondo pers. comm.).

2.11 Corroboration of the position of the first false ring (check) for anchovy in the Bay of Biscay based on otolith microstructure analysis.

This is a summary of the Working Document presented in WKMIAS 2013 by C. Hernández, B. Villamor, J. Barrado, C. Dueñas and S. Fernández which corroborates the position of the first false ring (check) for anchovy in the Bay of Biscay.

Two methods were used, in the first, age was determined by identifying and measuring growth rings formed on sagitta otoliths. In order to support the identification of the first annual ring, the otolith radius of the first hyaline ring was measured and used as a gauge for exclude the first check in ageing older individuals. The results showed that increments widths have a normal distribution (Kolmogorov-Smirnov test, Presumed Check, R1, R2 and R3 values $p > 0, 05$) with a falling rate of otolith growth with age (Fig. 21; Fig. 22 a). This linearly decreasing interval between increments is a verification criterion that forms the basis of age estimation (May, 1965). In cases where the distance from the core to the first visible ring was < 852± 100 µm, this ring was assigned as a presumed check.

In the second, a method for age corroboration was used by means of the otolith microstructure and fish ages were determined by daily increment counts. Total number of daily increments in otoliths was counted to test whether identified macroscopically hyaline area is, in fact, a check or the first annulus. The growth of the entire sequence of opaque and translucent zones was analysed for a subset of selected otoliths in which the daily increment structure to the check and to the 1st annual ring (R1) was clear (Fig. 22 b). To ensure good-quality results, rigorous rejection procedures were applied to the otoliths, for this reason so far only six otoliths have been analyzed and read with confidence. For otoliths analysed the average distance fromthe core to the beginning of the check was 800 ± 69 µm at a mean age of 94±27 days.

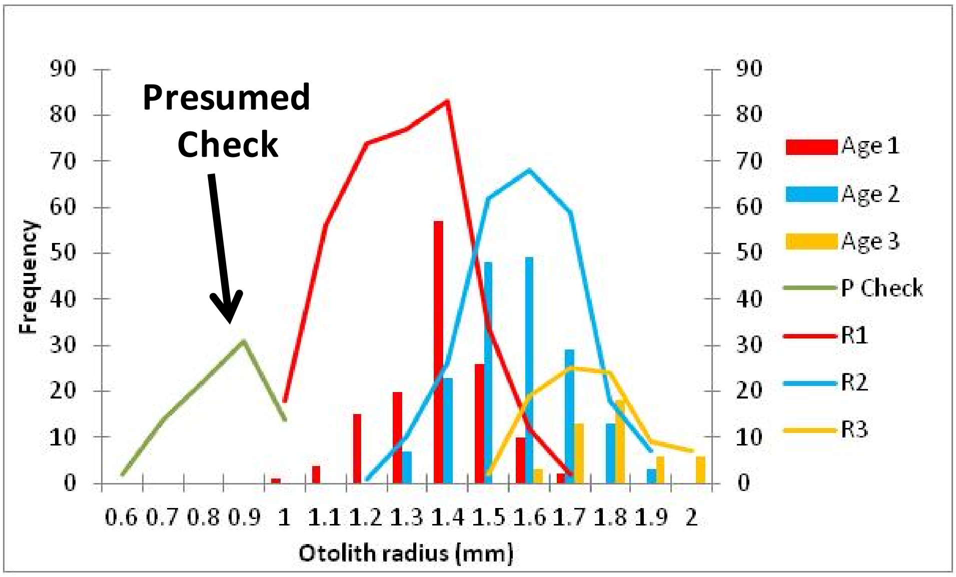

Figure 21: Frequency distribution of ring distances presumed check, R1, R2, R3 and tree annual age ring distances.

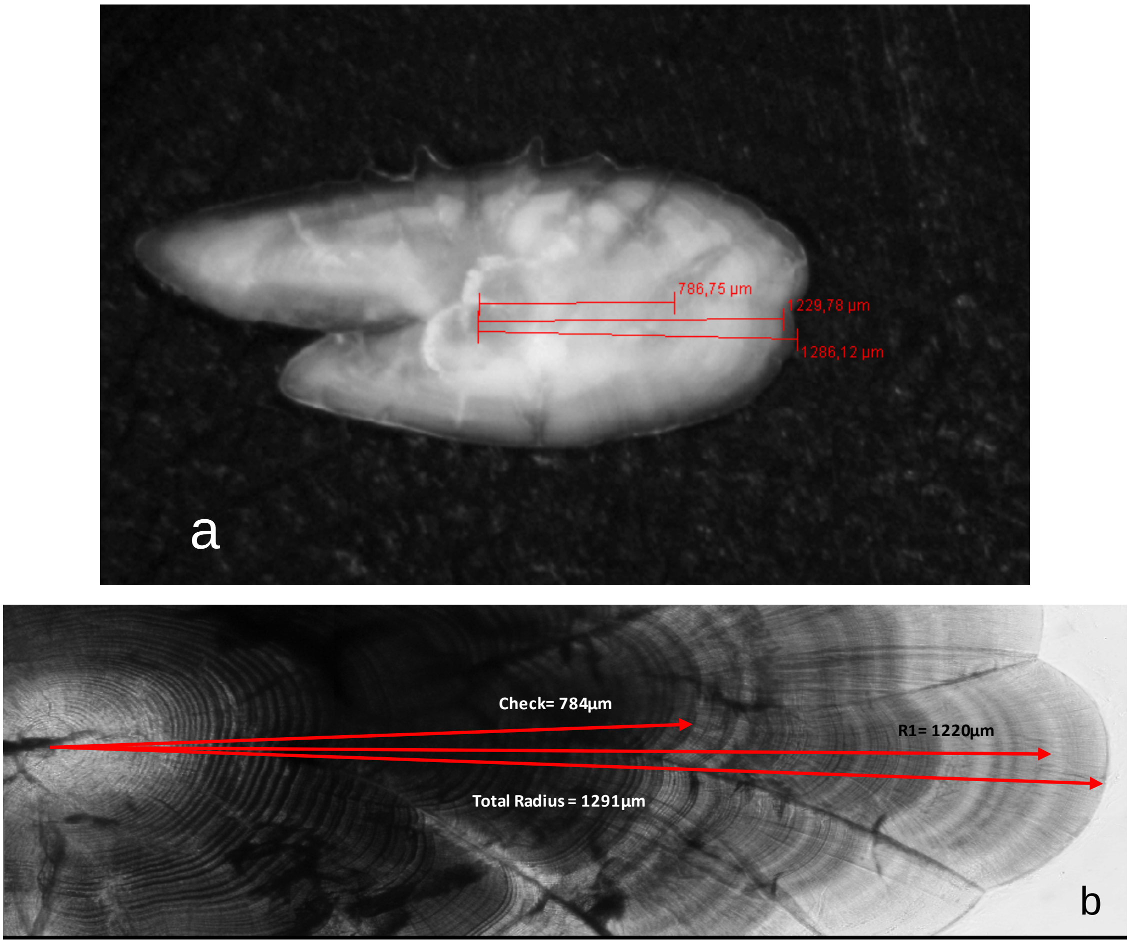

Figure 22: Measurements taken from the same anchovy otolith, (a) whole otolith and (b) otolith section. Check= false ring, R1= 1st annual hyaline ring and Total Radius. (Daily increments to Check = 73 days, Daily increments to R1= 185 days, Total Daily increments= 211 days).

We compare the results of age determination using whole otoliths with those determined through counts of daily increments in an attempt to corroborate the above method. The results obtained through the analysis of otolith microstructure indicated that the hyaline zone macroscopically identified as a check is not an annual growth zone because there were less than 365 daily increments seen in the otoliths before this ring was deposited. If we analyze the otolith macroscopically we found that the first check was at 852± 100 μm from the centre and if we look through the otolith microstructure it is at an average distance of 800±69 μm, the observed difference in both cases, apart from the difference in the number of specimens analyzed is due mainly to the difficulty in identifying macroscopically the exact position of the nucleus, it also would explain the high difference between maximum and minimum ring distances.

2.12 Validation of daily increment formation in otoliths of juvenile and adult European anchovy

This is a summary of the paper by P. Cermeño, A. Uriarte, A. M. De Murguia and B. Morales-Nin Validation of daily increment formation in otoliths of juvenile and adult European anchovy. Journal of Fish Biology (2003) 62, 679–691

The otoliths of juveniles and adults of European anchovy *Engraulis encrasicolus* held in aquaria were marked by immersion in oxytetracycline hydrochloride (OTC) at concentrations between 350 and 410mgl^{-1} for 12 h. Counts of microincrements between fluorescent bands validated the daily otolith increment formation. The otolith increments were easily readable at a magnification of 400 x with average increment widths of c. 1.1 mμ. Validation was successfully demonstrated in juveniles and adults maintained for short periods in the aquaria in the summer. For European anchovy captured as juvenile and reared to adults, however, increment formation appeared less than daily. The daily periodicity of the otoliths in juvenile European anchovy implies that counting of microincrements can be used to study their birth dates. The application of this technique to adults, however, may lead to the underestimation of actual age and further research needs to be done to clarify the reasons for the apparent loss of the daily rhythm over long periods.

2.13 Validation of daily increments deposition in the otoliths of European anchovy larvae (*Engraulis encrasicolus* L.) reared under different temperature conditions

This is a summary of the paper by N. Aldanondo, U. Cotano, E. Etxebeste, X. Irigoien, P. Alvarez, A. Martíınez de Murguıa, D.L. Herrero. Validation of daily increments deposition in the otoliths of European anchovy larvae (*Engraulis encrasicolus* L.) reared under different temperature conditions. Fisheries Research 93 (2008) 257–264.

European anchovy eggs (*Engraulis encrasicolus* L.) were hatched and larvae reared in the laboratory in order to validate the daily increment deposition in otoliths under different temperature condition. Additionally, the effect of temperature on otolith and larval growth and on the fish length and otolith radius relationship was also analysed. On sagittae, one to four increments can be depositioned during the embryonic stage and the first regular increment was formed the day after hatching. Temperature was found to have a significant effect on the increment deposition rate and otolith growth. A daily increment deposition rate was confirmed in larvae reared under conditions of higher temperatures (20.8 and 22.3 ◦C), while the apparent rate of increment formation of larvae reared at 17.6 ◦C was clearly lower. Standard length and otolith radius were closely related and this relationship was affected by both temperature and growth rates. The implications of the effect of these variables on otolith growth are further analysed in relation to non-daily pattern of increment deposition found at the lowest temperatures.

2.14 OTOLab free software for otolith analysis

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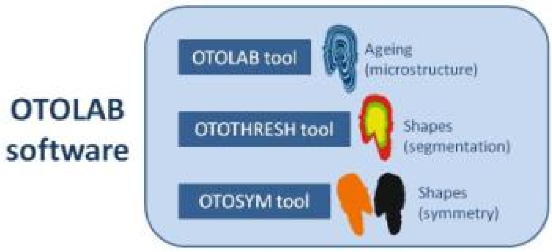

Both the Software and User's Guide will be available in 2020 at www.ieo.es

Responsible researcher: javier.rey@ieo.es

The purpose of **OTOLab software** is helping marine scientists in their research activities on otolith structure to estimate: 1) the age of an individual (**OTOLab tool,** Fig. 23) and 2) measurements of the otolith morphometry (**OTOTHRESH** and **OTOSYM tools,** Fig. 24 and 25). The software has been successfully used for hake, sardine and anchovy otoliths, both for ageing (designed for microstructure but also usable for macrostructure) and morphometry tasks. Doubtless, it will be also helpful with otoliths of other fish species, as well as other growth structures, namely clams shells and cephalopods beaks.

OTOLab is considered to be Open Source Software since the code is freely available so that every user can modify or improve its performances at his convenience. The Guide describes how to use the open source software OTOLab, which has been jointly developed by the engineers of UMA (University of Málaga) and the marine scientists of IEO.

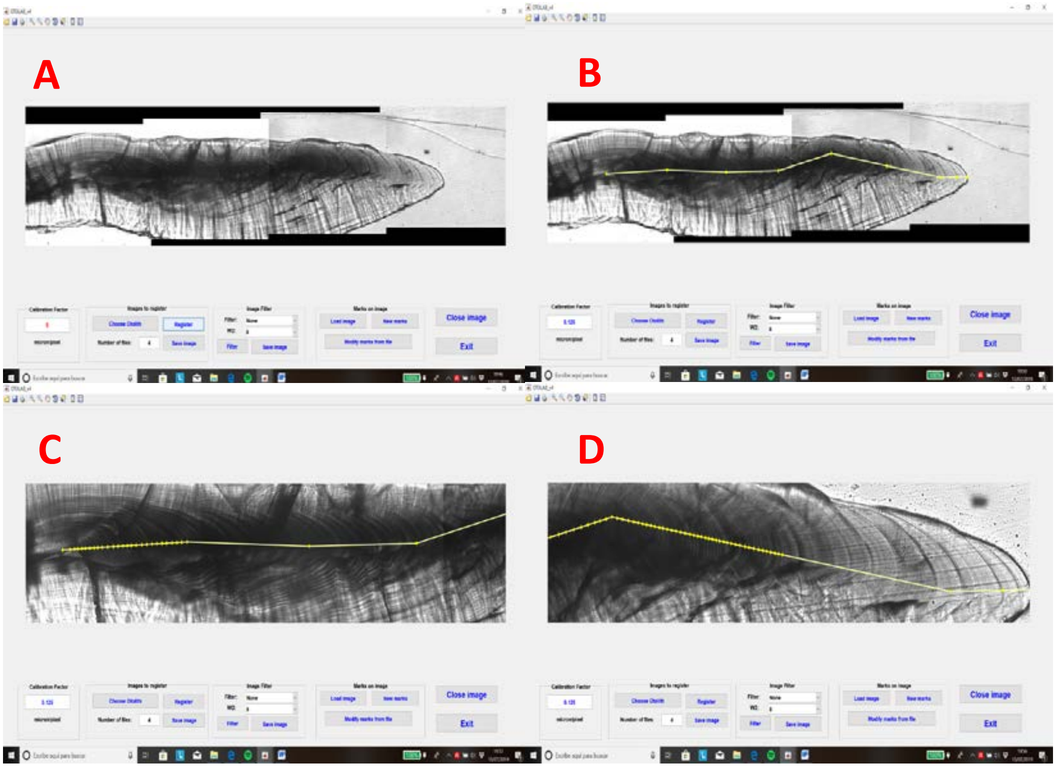

Figure 23. Adding marks process in an anchovy otolith (frontal section): A) tiling images (4 in this case), B) drawing a polyline, C-D) zooming and growth increments marking.

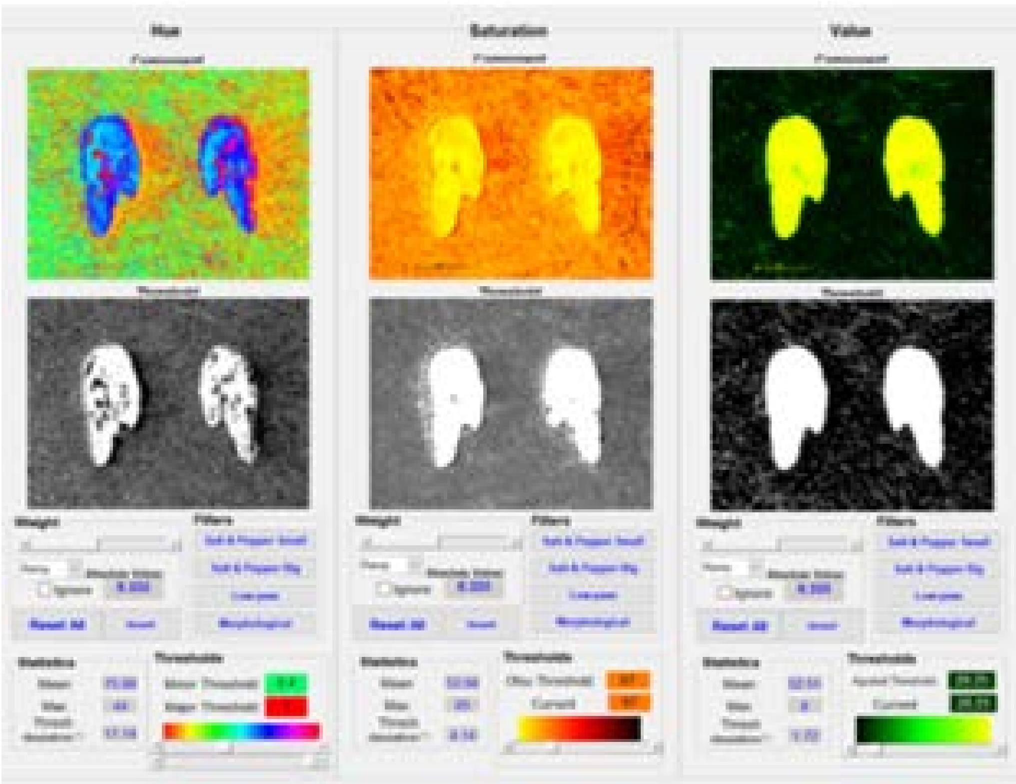

Figure 24. The OTOTHRESH tool components panel; from RBG image to a segmented black and white image.

Figure 25. The OTOSYM tool left and right otolith windows and information panels; from a segmented black and white image to morphological analysis.

3 Review of Ageing precision

A synthesis of the last annual growth workshops and exchanges by species was realized from the table which was originally created by Begona Villamor and Pierluigi Carbonara for the CRR 346 (Vitale et al., 2019). The goal, for each species, was to add any missing recent exchange or workshop and to present the major difficulties that caused low Percentage of Agreement (PA) between the age readers of those expert groups as well as to recommend some guidelines to overcome those difficulties.

During this meeting, the focus was on:

i. Updating the table with exercises (exchanges and/or workshops) that took place recently by species.
ii. Scrutinizing results from relevant reports to compile information on each species and to detect reasons that could lead to low PA between the age readers.
iii. Providing a complete review of the recommendations that were put forward during past workshops and exchanges in the terms of improving the low PA's.

The main changes from the CRR 346 Table were:

- ✓ Adding three additional columns in the table to include: Low PA Reasons, Recommendations and References. Those columns were completed with the most important information derived from previous exchanges and workshops.
- ✓ Adding Blue whiting (*Micromesistius poutassou*), another pelagic species, to the table.
- ✓ Reforming the table by splitting it into ten smaller tables, one by species, to make it easier to view and process and to ensure that the current format is more useful.

In this chapter e there is a table for each species.

3.1 Anchovy (*Engraulis encrasicolus*)

In the last otolith exchanges of Anchovy, which included areas/stocks of the Atlantic and Mediterranean, the Percentage of Agreement between all readers ranged from 58.5% to 74.3% by area (ICES, 2016; Villamor et al., 2019). The main reasons for that low PA were:

- Discrepancies in the otolith interpretation: different interpretations of the marks, growth bands and edges in different areas.
- The recommended age reading protocol in WKARAS 2 (ICES, 2016) has been inconsistently applied in some instances.
- Incorrect application of the age determination rule in the first half of the year for fish with birthdate in July.
- Anchovy otoliths are difficult to interpret. Given that there is no agreed collection of otoliths by areas, it is hard to assess the actual quality by areas.

There are somerecommendations below to tackle the above issues, such as:

- Follow the common protocol adopted in WKARA2 for all areas in order to standardize the anchovy age assignments and to improve the coherence of the age estimates.
- Production of a collection of age validated otoliths by areas (or at least of agreed age determination by experts)
- To carry out validation studies on age determination for the areas inhabited by the different anchovy stocks either via microincrement preparation (at least to validate the first annulus for each area) or by other methods as studies of progression of length frequency modes throughout time, for tracking cohorts, etc
- Intercalibration exercises by areas (for the different countries taking part in otolith age reading on the same stocks or adjacent stocks) are required. This be-comes compulsory for regions where several countries exploit the same stock. For the Mediterranean area, in particular, given the high sharing of anchovy fish stocks among several countries, these intercalibration exercises are required
- To review the convenience of setting the birthdate at the middle of the year for anchovies in some Mediterranean areas and to consider to move it to 1st January, because of the difficulties perceived during the exchange on the application of a changing rule for the first and second halves of the year (as associated to birthdate 1 July) for these stocks in the northern hemisphere (where winter marks are laid down around January-February).

Species	Anchovy (*Engraulis encrasicolus*)											
WK/Exchange	**Exchange, 2014 / WKARA, 2016 (ICES, 2016c)**										**Exchange, 2018 (ICES, 2018)**	
Mode of Preparation	Whole otolith, in resin										Whole otolith, in resin	
Area	Bay of Biscay (ane.27.8)	English Channel (ane.27.7)	Gulf of Cadiz and Portuguese coast (ane.27.9a)	Alboran Sea (GSA 01)	Western Mediterranean Sea (GSA 06)	Gulf of Lion (GSA 07)	Southern Tyrrhenian Sea (GSA 10)	Strait of Sicily (GSA 16)	Western Ionian (GSA 19)	Aegean Sea (GSA 22)	Bay of Biscay (ane.27.8)	Strait of Sicily (GSA 16)
Agreement (%)*	74.3/80.8/90.9	66.7/80.4/-	68.5/76.4/75.7	58.9/63.5	60.9/59.6/-	73.4/75.1/-	62.9/62.0/67.3	58.5/59.9/85.6	61.9/60.2/73.5	70.0/78.3/97.1	71.1/82.9/90.7	56.1/59.2/96.3
CV*	45.1/22.4/11.4	127.6/73.9/-	49.1/34.7/33.0	58.7/71.1	49.9/59.2/-	31.3/30.3/-	67.2/86.7/58.1	78.7/73.8/11.2	60.9/73.3/55.3	55.7/42.8/6.7	41.1/25.6/9.3	58.5/56.5/8.8
APE	-	-	-	-	-	-	-	-	-	-	-	-
Low PA Reasons	1.Divergent otolith interpretation: different interpretations of the marks, growth bands and edges. 2.The age determination rules have in some instances been inconsistently applied. 3.Anchovy otoliths are difficult to interpret. Given that there is no agreed collection of otoliths by areas, it is hard to assess the actual quality by areas.										1.Incorrect application of the age determination rule in the first half of the year for fishes with birthdate in July. 2.Discrepancies in the interpretation of the growth pattern and marks in different areas.	
Recommendations	1.Adopt a common protocol for all areas in order to standardize the anchovy age assignments and to improve the coherence of the age estimates. 2.Produce an agreed collection of otoliths by areas.										1.Validation studies on age determination for the areas (1st ring). 2.Intercalibration exercises by areas. 3.Setting the birthdate at the middle of the year.	
References	*ICES Report of the Workshop on Age estimation of European anchovy (Engraulis encrasicolus) _ 2016*										*ICES Report of Otolith Exchange Analysis for Anchovy_2018*	

***(All readers/Expert readers/Stock readers)**

In the Table recommendations, in item 3 it is not completely correct what is written, what is recommended is that: To review the convenience of setting the birthdate at the middle of the year for anchovies in some Mediterranean areas and to consider to move it to 1st January.

3.2 Sardine (*Sardina pilchardus*)

At the same time, Sardine's otolith readers' agreement is also low, with a range between 64.9 and 77.4 %. Moreover, the samples coming from Mediterranean areas tend to have the lowest PA. Common reasons for disagreement among the readers are:

- Lower agreement due to identification of 1st ring.
- Lower agreement due to definition of edge nature.
- Lower agreement for old fish due to the interpretation of marginal growth rings.
- Difficulty in age reading for age group 1 and age group 2.
- Bigger disagreement due to the quality of otolith images.

Recommendations for improving the PA are:

- ➢ Provide more valuable tools to identify the first winter zone when is formed by a cluster of close hyaline marks.
- ➢ Validation studies of the first annual increment deposition through the study of the daily growth based on otolith microstructure analysis should be undertaken in some areas.
- ➢ Use of radius measurements to discriminate between checks and the first true winter ring.
- ➢ Inter-calibration exercises by areas.

Species	Sardine (*Sardina pilchardus*)						
WK/Exchange	**WKARAS, 2011 (ICES, 2011d)**			**Exchange, 2017 (ICES, 2018)**		**WKARAS2, 2019 (ICES, 2019)**	
Mode of Preparation	Whole otolith, in resin						
Area	Bay of Biscay North (Division 8.a)	Portuguese coast (Division 9.a)	Gulf of Cadiz (Division 9.a)	Northeast Atlantic (divisions 8.a, 8.b, 8.c, 9.a)	Mediterranean Sea (GSAs 01, 03, 06, 07, 09, 16, 22)	Northeast Atlantic (divisions 8.a, 8.b, 8.c, 9.a)	Mediterranean Sea (GSAs 01, 03, 06, 07, 09, 16, 22)
Agreement (%)*	73,1	76,5	77,4	64,9	66,7	72,8	65,6
CV*	17,3	18,1	10,9	57,9	104,8	50,5	72,4
APE	-	-	-	37,1	60,4	26,6	43,8
Low PA Reasons	1. Lower agreement due to identification of 1st ring. 2. Lower agreement due to definition of edge nature. 3. Lower agreement for old fish due to the interpretation of marginal growth rings. 4. Difficulty in age reading for age group 1 and age group 2. 5. Bigger disagreement due to the quality of otoliths images.						
Recommendations	1. Some first winter hyaline zones may be composed of several close hyaline marks forming a cluster. 2. Validation studies of the first annual increment deposition through the study of the daily growth based on otolith microstructure analysis should be undertaken in some areas. 3. Use of radius measurements to discriminate between checks and the first true winter ring. 4. Inter-calibration exercises by areas.						
References	*ICES Report of the Workshop on Age Reading of European Atlantic Sardine (WKARAS)_2011,* *ICES Report of the Workshop on Age Reading of European Atlantic Sardine (WKARAS)_2019 Annex 2: Report of WKARAS 2 small age reading calibration exercise*						

3.3 Herring (*Clupea harengus*)

In the last otolith exchange of Herring, the Percentage of Agreement between all readers ranged from 69.1% to 77.7% for North Sea, Irish Sea, Celtic Sea and West of Scotland sea areas. The last otolith exchange for the Baltic Sea produced Percentage of Agreement levels between 52 and 96%. The main reasons for that low PA were:

- Differentiation of first between false and true winter rings that is more often important in the first and second annual growth zones
- Determination and counting of winter zones on the edge (rostrum) for older fish
- Stock identification and stock mixing can cause disagreement on age 1 and 2 fish due to spring and autumn spawning fish
- Small sample size, lack of good image quality and issues with identifying 1st winter ring and edge from images

There are a few suggested solutions to tackle the above issues, such as:

- Recommend that there is a large-scale exchange for all sea regions which uses both images and otoliths (from the same fish) or standardized protocols used for image generation and annotation (Godiksen J.A., 2014 Report of the international. Norwegian spring-spawning herring Clupea harengus otolith and scale exchange).
- Creation of a standardized protocol for herring aging (WKVALPEL 2019).
- Compilation of reference collection of agreed age fish (Coad Davies et al., 2015).
- Any exchange which uses both otolith and scales should provide samples for both structures from the same fish in WKNSSAGE 2015 (ICES, 2015).

Species	Herring (*Clupea harengus*)														
WK/Exchange	**Exchange 2005-2007 (ICES, 2008)**	**WKARBH, 2008 (ICES, 2008b)**	**Exchange, 2008 (ICES 2008b)**	**Exchange, 2014 (Godiksen, 2014)**	**WKNSSAGE, 2015 (ICES, 2015)**		**Exchange, 2015 (Coad Davies et al., 2015)**				**Exchange, 2016**		**Exchange 2015-2017 (ICES, 2017)**		
Mode of Preparation	Whole loose otolith	Whole loose otolith	Stained otolith slices	Scales and whole otolith	Scales	Whole otolith	Whole loose otolith	Whole otoliths , in risen			Whole otholiths	Scales	Whole otolith, in resin		Stained otolith slices
Area	Baltic Sea	Baltic Sea		ICES Division IIa	ICES Division IIa		North Sea	Celtic Sea	Irish Sea	West of Scotland Sea	North Sea, Norwegian Sea		Baltic Sea (Subdivision 26)		Baltic Sea (Subdivision 30 and 32)
Agreement (%)*	80.4	86,9	76,3	67.6/-/69.1	81.0	75.0	73.6	75.2	77.7	69.1	69.0	87.0	88.0-94.0 (sample S1)	52.0-85.0 (samples S2 & S3)	87.0-96.0 (sample S4)
CV*	14.2	6,4	8,7	11.5/-/9.4	-	-	21.1	19.6	16.0	18.8	-	-	1.9-7.5 (sample S1)	3.9-20.0 (samples S2&S3)	4.0-8.1 (sample S4)
APE	-	-	-	-	-	-	14.8	14.2	11.6	13.6	-	-	-	-	-
Low PA Reasons	1. Distinguish between false and true winter rings that is more often important in the first and second annual growth zones. 2. Distinguish and counting of winter zones on the edge (rostrum) for older fish.			-	1. Differences between the scales and otoliths. 2. Several issues relating to identification of the first winter ring. 3. Age interpretation of the older fish confound by stock mixing issues.		-				1. Identification of the first winter ring and age interpretation of older fish cofounded by stock mixed issues. 2. Final conclusions cannot be reach based on the samples from neither of previous workshop nor exchange.		In the exchange took part some unexpirenced readers (with the area)		
Recommendations	The characteristics of the winter ring make it possible to detect false winter rings (*decreasing width of the daily rings prior to the winter ring, no daily ring formation during the winter ring formation and then progressively wider daily rings after the winter ring)*			A large scale exchange (images and the hard otoliths) and a larger collection from outside.	A large-scale exchange of good quality scales and otoliths from the same fish should be conducted follow by the workshop.		1. Compilation of a reference collection of agreed age fish. 2. Standardization of whether it is the count of "year" or "rings" which are used to define fish age for age reading exercises 3. Standardization of procedures for annotation of images used in exchanges.				Organize a future workshop		The otoliths which were used in the exchange should be from the same fish (slices and whole otoliths).		
References	*ICES. 2008. Report of the Workshop on Age Reading of Baltic Herring (WKARBH), 9–13 June 2008, Riga, Latvia. ICES CM 2008/ACOM:36. 37 pp.*			*Godiksen J.A., 2014. Report of the international Norwegian spring-spawnig herring (Clupea harengus) otolith and scale exchange 2014, 30 pp.*	*ICES. 2015. Report of the Workshop on ageestimation of Norwegian spring-spawnig herring (Clupea harengus) (WKNSSAGE)*		*Coad Davies J. et al., 2015, Report of the 2015 herring age reading exchange; 65 pp.*				*Summary in the Report of WGBIOP from 2018*		*Raitaniemi J. 2017. Baltic herring are reading intercalibration 2015-2017 (summary in WGBIOP report 2017)*		

3.4 Sprat (*Sprattus sprattus*)

In the last otolith exchange of Sprat, the Percentage of Agreement between all readers ranged from 68.6% to 94.9% for North Sea, Celtic Sea and 3a areas. The last otolith exchange for the Baltic Sea produced Percentage of Agreement levels of 76.1%. Although this PA is not discouraging, there are a few suggestions for improvement:

- Identification of the first winter zone - difficult to distinguish between opaque and translucent zones because there are many grey areas(potential opaque 'bands' within translucent zone) in the otoliths; can be difficult to determine if a true winter ring had been laid down
- The misinterpretation of the edge type and when to include a translucent zone at the edge in age count.
- Second annual growth zone – difficult to distinguish between false and true winter rings
- Difficulty in determining the winter rings in the otoliths of older fish (3+) which are located in the external part of the otolith.

There are a few suggested solutions to tackle the above issues, such as:

- All age readers should follow the established age reading protocol for sprat (WKARSPRAT, 2017).
- All exchange participants should take a part in the workshop after the exchange (WKARSPRAT, 2017).
- There should be a standardized protocol for image generation and annotation of the images (WKARSPRAT, 2017).
- Some readers had problems with the transition to work with microscopes and higher magnification. This could be solved by bilateral cooperation and help from in this respect more experienced readers (WKARBS, 2008).

Species	Sprat (*Sprattus sprattus*)									
WK/Exchange	Exchange 2004-2005 (ICES, 2006)	WKARBS, 2008 (ICES, 2008c)	Exchange, 2014 (Coad Davies et al., 2014)		Exchange, 2016 (ICES, 2017)		WKARSPRAT, 2016 (ICES, 2017)			Exchange, 2017
Mode of Preparation	Whole otoliths, with Canadian balsam or loose	Whole otolith, with nail polish	Whole otolith, in alcohol		Whole otolith, in alcohol		Whole otolith, in resin	Whole loose otolith		Whole otoliths
Area	Baltic Sea	Baltic Sea	North Sea	Cetic Sea	North Sea	Cetic Sea	Division 3.a	North Sea (divisions 4.b, 4.c)	Celtic Sea (divisions 6.a, 7.b, 7.g, 7.j)	Division 3.a
Agreement (%)*	53,7	76,1	62.0/78.0		88.1/91.4	94.3/96.6	68.6/67.8/-	81.5/81.0/-	94.9/94.4/-	80.0/-/91.0
CV*	-	17,1	44.0/45.0		16.3/10.0/-	12.7/5.5/-	22.8/22.3/-	20.4/21.7/-	12.1/12.5/-	22.0/-/8.0
APE	-	-	-	-	-	-	16.9	15.4	7.9	1.6/-/6.0
Low PA Reasons	1. Distinguish of the first winter zone 2. Distinguish between false and true winter rings that is more often important in the second annual growth zone. 3. Distinguish of the winter rings in the otoliths of older fish (3+) which are situated in the external part of the otolith.		1. Identification of the first annulus being probably due to the prolonged spawning period where a subset of a cohort may over winter as larvae and a winter-ring may not be discernible. 2. Bad quality photos. *(These are the Readers who have experience in reading Sprat otoliths from the North Sea area.)*		1. Interpretation of translucent band which has some opaque zones within it. 2. Misinterpretation of the edge type. 3. When to include a translucent zone at the edge in the count of age.		1. Distinguish between opaque and translucent zones because there are many grey areas in the otoliths. 2. Difficulties to determine if a true winter ring had been laid down.			-
Recommendations	1. Continue the exchange of otolith samples. 2. Meet regularly every third year. 3. All age readers should follow the report protocol regarding microscopes, sampling and methods.	1. Use of microscopes with high magnification for the age reading of Baltic sprat. 2. The exchange of sprat otolith samples must be continued.	1. Calibration workshop to be held on basis of the exchange, first a re-reading of the calibration set using set lines for annotation purposes. 2. Validation of the first annulus. 3. Application of microstructure data to provide guidelines for identification of subsequent annuli. 4. Expansion of the workshop to include samples from other eco-regions.		1. All age readers should take a part in the workshop after the exchange. 2. An agreed age reading protocol is described in the report and should be followed by age readers.		There has to be a protocol how to take the images and to mark the rings in the picture.			Following the age reading protocol was the main reason for improvement of the agreement between readers.
References	*Anon. 2006. Sprat age reading Workshop – Baltic Sea Regional Project. Charlottenlund, Denmark, 24-27 January, 2006; 16 pp.*	*Anon. 2008. Report of the Workshop on Age Reading on Baltic Sprat (WKARBS), 17-20 March 2008, Klaipeda, Lithuania. ICES CM 2008/ACOM:37. 28 pp.*	*Coad Davies J., Hüssy K., Worsøe Clausen L.2014. Report of the Sprat Exchange 2014 For the North Sea and Celtic Sea*		*ICES. 2017. Report of the Workshop on age estimation of sprat (Sprattus sprattus) (WKARSPRAT), 15-18 November 2016, Galway, Ireland. ICES CM 2016/SSGIEOM:19. 129 pp.*		*ICES. 2017. Report of the Workshop on age estimation of sprat (Sprattus sprattus) (WKARSPRAT), 15-18 November 2016, Galway, Ireland. ICES CM 2016/SSGIEOM:19. 129 pp.*			*Report of the 2017 3.a. Sprat age reading exchange*

3.5 Mackerel (*Scomber scombrus*)

For this species the last age reading exchanges and/or workshops show a PA low than the rest small pelagic species, with a range from 59.4 to 68.2%. The detected causes for this so far are:

- Lower agreement than previous exchanges due to new/different readers.
- Winter ring formation varies within and between areas.
- Seasonal differences in opaque-hyaline zone formation / Onset of maturity / timing of spawning / migration patterns / variation of the opaque edge.
- Difficulty to define the appearance of false or split rings.
- Bigger disagreement for ages above age 5 / subjective interpretation of growth patterns.

Also, in terms of practical issues, the age-reading was performed in a very new tool (SmartDots) so the Readers didn't have any previous experience with it. Furthermore, many Readers reported having problems with the position of the annotation line.

The recommendations for improving the agreement between readers are:

- Recalibration of all readers / calibration exercise / training new from experts.
- Reference collection / Agreed birthdate.
- Additional validation studies for otolith structures in all different districts (Atlantic done) / spatial and temporal coverage / length and age rate.
- Tagging and validation studies.

At the same time, there are a few suggested ideas to be used as guidelines, such as:

- ✓ Universal manual and protocol
- ✓ Calibration within laboratories and between readers
- ✓ Quality control (i.e. Validation studies), and
- ✓ Regular exchanges

Species	Mackerel (*Scomber scombrus*)		
WK/Exchange	**Exchange, 2014 (ICES, 2015c)**	**Exchange, 2018 pre-workshop (ICES, 2018)**	**WKARMAC2, 2018 (ICES, 2018)**
Mode of Preparation	Whole otoliths, fixed in resin/loose submerged in water (images only)		
Area	Northeast Atlantic (Subarea 2 and divisions 4-a-b, 6.a, 7.b)	Northeast Atlantic (divisions 2.a, 4.b-c, 5.a-b, 7.b, 7.j, 7.d, 8.b-c and 9.a)	Northeast Atlantic (divisions 2.a, 4.b-c, 5.a-b, 7.b, 7.j, 7.d, 8.b-c and 9.a)
Agreement (%)*	68.2/75.5	59.4/65.2	66.5/73.2
CV*	15.4/-	37.3/17.6	30.4/16.4
APE	-	-	-
Low PA Reasons	1. Lower agreement than previous exchanges due to new/different readers. 2. Winter ring formation varies within and between areas. 3. Seasonal differences in opaque-hyaline zone formation / Onset of maturity / timing of spawning / migration patterns / variation of the opaque edge. 4. Difficulty to define the appearance of false or split rings. 5. Bigger disagreement for ages above age 5 / subjective interpretation of growth patterns. *EXCHANGE PROBLEMS:* • *New tool (2nd time used)* • *Position of the annotation line*		
Recommendations	1. Recalibration of all readers / calibration exercise / training new from experts. 2. Reference collection / Agreed birthdate. 3. Additional validation studies for otolith structures in all different districts (Atlantic done) / spatial and temporal coverage / length and age rate. 4. Tagging and validation studies. *GENERAL SOLUTIONS* ◦ *Universal manual and protocol* ◦ *Calibration within laboratories and between readers* ◦ *Quality control (i.e. Validation studies)* ◦ *Regular exchanges*		
References	*ICES Report of the Workshop on Age Estimation of Atlantic Mackerel (Scomber scombrus) (WKARMAC2)_2018*		

3.6 Chub mackerel (*Scomber colias*)

Chub mackerel is yet another species with about five years of experience in age-reading exercises and calibrations, so a low percentage of agreement is justified (46.4-70.3%). The main reasons that led to this result were identified in the workshop's / exchange's reports:

- High growth rate, especially in small age groups.
- Difficulties in interpretation of young ages (identification of age 0 / 1st ring).
- Differences due to various spawning timing and conventional birthdates.

Implementation of the suggested protocol by all institutes and readers as well as regular exchanges and workshops could assist in the intercalibration of the readers. Moreover, validation studies for each area could detect any diversifications of different stocks, relating to the local environmental conditions.

Species	Chub mackerel (*Scomber colias*)														
WK/Exchange	**Exchange, 2015, pre-workshop (ICES, 2016a)**			**Exchange, 2015, WKARCM (ICES, 2016a)**						**Exchange, 2017 (ICES, 2018)**					
Mode of Preparation	Whole otoliths fixed in resin (images only)			Whole otoliths, fixed in resin / loose submerged in water (images only)											
Area	Division 8.c	Division 9.a	Western Mediterranean Sea (GSA 06)	Division 8.c	Division 9.a	CECAF - Mauritania	Western Mediterranean Sea (GSA 06)	Ligurain and North Thyrrenian Sea (GSA 09)	Southern Adriatic Sea (GSA 18)	Division 8.c	Division 9.a	CECAF - Canarias	Ligurain and North Thyrrenian Sea (GSA 09)	Aegean Sea (GSA 22)	NortWest Atlantic
Agreement (%)*	53,5	55,3	62,1	66,7	55,6	60,2	65,3	46,4	68,2	56.6/65.5/-	56.8/62.4/-	70.3/80.3/-	52.4/63.4/-	64.7/70.5/-	51.7/52.1/-
CV*	27,4	22,8	35,2	36,2	37,3	41,6	29,3	64,6	65,8	61.7/24.1/-	35.6/31.3/-	68.0/24.3/-	111-3/67.8/-	35.3/28.1/-	39.6/34.6/-
APE	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Low PA Reasons	1. High growth rate, especially in small age groups. 2. Difficulties in interpretation of young ages (identification of age 0 / 1st ring). 3. Differences due to various spawning timing and conventional birthdates.														
Recommendations	1. Regular exchanges. 2. Validation studies.														
References	*ICES Report of the Workshop on Age Reading of Chub mackerel (Scomber colias) (WKARCM)_2015*														

3.7 Horse Mackerel (*Trachurus trachurus*)

The last workshop carried out on horse mackerels (ICES, 2018 WKARHOM3) provided not very good result in term of PA (50.05% for whole and 49.45% for sectioned otolith) and consequently high CV percentages (19.00% for whole and 31.90% for sectioned otolith). The reported causes were:

- Identification of the first winter ring due to presence of several false rings.
- Overlapping of the transparent rings after 4th and 5th winter rings.
- Otolith preparation technique (whole/sliced; using different lights and clarification medium).

Species	Horse mackerel (*Trachurus trachurus*)														
WK/Exchange	Exchange/Workshop, 2012 (ICES, 2016b)					Exchange/Workshop, 2015 (ICES, 2015)					Exchange/Workshop, 2018 (ICES, 2018)				
Mode of Preparation	Sectioned otolith					Sectioned otolith		Whole otolith		Sectioned otolith	Sectioned otolith		Whole otolith		
Area	Ireland waters (Subarea 7)	Noth of Spain (divisions 8.c, 9.a)	Portugal waters (Division 9.a)	South of Spain (Division 9.a South)	Western Ireland	Division 7.d	Division 7.h	Northern Alboran Sea (GSA 01)	Corsica Island (GSA 08)	Corsica Island (GSA 08)	Division 8.c	Division 7.d	Division 8.c	Division 7.d	Ligurain and North Thyrrenian Sea (GSA 09)
Agreement (%)*	36,4	53,2	44,7	43,9	46,4	55,7	63,8	50,1	44,6	44	50,05		49,45		
CV*	26,9	42,3	54,7	43,8	21	16,8	25,9	69,7	32,1	28,9	19		31,9		
APE	-	-	-	-	-	-	-	-	-	-	-		-		
Low PA Reasons	1. Identification of the first winter ring due to presence of several false rings. 2. Presence of multiple rings that could le/ad to an age overestimation.					1. Identification of the first winter ring due to presence of several false rings. 2. Overlapping of the transparent rings after 4th and 5th winter rings. 3. Deformities in otolith preparation (whole/sliced; transmitted/reflected light). 4. False ring (reproductive) detected after the 2nd winter ring.					1. Identification of the first winter ring due to presence of several false rings. 2. Overlapping of the transparent rings after 4th and 5th winter rings. 3. Otolith preparation technique (whole/sliced; using different lights and clarification medium).				
Recommendations	1. The otolith reference collection (ICES, 2018 WKARHOM3) is an useful tool for training and intercalibration. It should be used prior the ageing process to minimize errors linked to true rings identification. 2. In the age estimation process, the position of the first annual ring should be the major point of the agreement procedure (FAO, 2002). 3. The latest WKARHOM3 stated that otoliths (whole/slices) must be analyzed under reflected light immersed in clarification medium (Sea water, thymol). 4. The observation of whole or sliced otolith have been proven equally reliable but the chosen method must be consistent in the process (ICES, 2018 WKARHOM3). 5. Sex, maturity, catch date and zone should be considered during readings (ICES, 2018 WKARHOM3). 6. Radii measurement analysis could be helpful in recognizing true winter rings (Jurado-Ruzafa & Santamaría, 2018) (ICES, 2018 WKARHOM3).														
References	*ICES Report of the Workshop on Age Reading of horse mackerel (Trachurus trachurus), Mediterranean horse mackerel (Trachurus mediterraneus) and blue jack mackerel (Trachurus picturatus) (WKARHOM)_2012*					*ICES Report of the Workshop on Age reading of Horse Mackerel, Mediterranean Horse Mackerel and Blue Jack Mackerel (Trachurus trachurus, T. mediterraneus and T. picturatus) (WKARHOM2)_2015*					*ICES Workshop on Age reading of Horse Mackerel, Mediterranean Horse Mackerel and Blue Jack Mackerel (Trachurus trachurus, T. mediterraneus and T. picturatus) (WKARHOM3)_2018*				

3.8 Mediterranean Horse Mackerel (*Trachurus mediterraneus*)

During the WKARHOM3 (ICES, 2018) the reported agreement between readers was 48.35% (PA) and the CV was 66.35%. In this workshop however only whole otoliths were analyzed, while in the prior workshop, WKARHOM2 (ICES, 2015), only sectioned otoliths were considered, obtaining similar results (PA 39.30-52.60%, CV 40.20-56.70%). The identified principal sources of error were:

- Identification of the first winter ring.
- False ring (reproductive) detected between the 1st and the 2nd winter rings.
- Overlapping of the transparent rings after the 3rd winter ring.
- Differences in the considered date of birth (1st Jan/1st July).
- Problems in assigning age of younger specimens due to intrinsic difficulties in interpreting age 1-2 (Karlou-Riga, 2000).

Species	Mediterranean horse mackerel (*Trachurus mediterraneus*)							
WK/Exchange	Exchange, 2012		Exchange/Workshop, 2015 (ICES, 2015)			Exchange/Workshop, 2018 (ICES, 2018)		
Mode of Preparation	Whole otolith		Sectioned otolith			Whole otolith		
Area	Mediterranean Sea (Italian waters)	North of Spain (divisions 8.c and 9.a)	Division 8.c	Division 9.a	Southern Adriatic Sea (GSA 18)	Division 8.c	Division 9.a	Ligurain and North Thyrrenian Sea (GSA 09)
Agreement (%)*	56,6	57,5	39,3	41,2	53,6	48,35		
CV*	28,7	30,5	40,2	41,7	46,7	66,35		
APE	-	-	-	-	-	-		
Low PA Reasons	1. Identification of the first winter ring due to presence of several false rings. 2. Presence of multiple rings that could led to an age overestimation. 3. Problems in assigning age of younger specimens due to intrinsic difficulties in interpreting age 1-2.		1. Identification of the first winter ring. 2. False ring (reproductive) detected between the 1st and the 2nd winter ring. 3. Overlapping of the transparent rings after the 3rd winter ring.			1. Identification of the first winter ring. 2. False ring (reproductive) detected between the 1st and the 2nd winter ring. 3. Overlapping of the transparent rings after the 3rd winter ring. 4. Differences in the considered date of birth (1st Jan/1st Jul). 5. Problems in assigning age of younger specimens due to intrinsic difficulties in interpreting ages 1-2.		
Recommendations	1. The otolith reference collection (ICES, 2018 WKARHOM3) is an useful tool for training and intercalibration. It should be used prior the ageing process to minimize errors linked to true rings identification. 2. In the age estimation process, the position of the first annual ring should be the major point of the agreement procedure (FAO, 2002). 3. The latest WKARHOM3 stated that otoliths (whole/slices) must be analyzed under reflected light immersed in clarification medium (Sea water, thymol). 4. The observation of whole or sliced otolith have been proven equally reliable but the chosen method must be consistent in the process (ICES, 2018 WKARHOM3). 5. Sex, maturity, catch date and zone should be considered during readings (ICES, 2018 WKARHOM3). 6. Radii measurement analysis could be helpful in recognizing true winter rings (Jurado-Ruzafa & Santamaría, 2018) (ICES, 2018 WKARHOM3).							
References	*ICES Report of the Workshop on Age Reading of horse mackerel (Trachurus trachurus), Mediterranean horse mackerel (Trachurus mediterraneus) and blue jack mackerel (Trachurus picturatus) (WKARHOM)_2012, Karlou-Riga, 2000*		*ICES Report of the Workshop on Age reading of Horse Mackerel, Mediterranean Horse Mackerel and Blue Jack Mackerel (Trachurus trachurus, T. mediterraneus and T. picturatus) (WKARHOM2)_2015*			*ICES Workshop on Age reading of Horse Mackerel, Mediterranean Horse Mackerel and Blue Jack Mackerel (Trachurus trachurus, T. mediterraneus and T. picturatus) (WKARHOM3)_2018, Karlou-Riga, 2000*		

3.9 Blue Jack Mackerel (*Trachurus picturatus*)

The accomplished workshops offered for the Blue Jack Mackerel a situation quite similar to what emerged for its congeneric. The PA ranged between 35.3 and 79.3% for sectioned otoliths (ICES, 2015 WKARHOM2) and has been reported at 56.3% for whole (ICES, 2018 WKARHOM3). The computed CV was 36.0-168.8% for sectioned otoliths (ICES, 2015 WKARHOM2) and 69.85% for whole ones (ICES, 2018 WKARHOM3). The main reasons of uncertainty were:

- Identification of the first winter ring.
- Winter rings not very well marked.
- Overlapping of the transparent rings after the 2nd winter ring.

Species	Blue jack mackerel (*Trachurus picturatus*)			
WK/Exchange	Exchange/Workshop, 2015 (ICES, 2015)			Exchange/Workshop, 2018 (ICES, 2018)
Mode of Preparation	Sectioned otolith			Whole otolith
Area	Azores	Tenerife	Southern Adriatic Sea (GSA 18)	Tenerife
Agreement (%)*	35,3	60,1	79,3	56,8
CV*	36	89,3	168,8	69,85
APE	-	-	-	-
Low PA Reasons	1. Identification of the first winter ring. 2. Winter rings not clearly marked. 3. Overlapping of the transparent rings after the 2nd winter ring.			
Recommendations	1. The otolith reference collection (ICES, 2018 WKARHOM3) is an useful tool for training and intercalibration. It should be used prior the ageing process to minimize errors linked to true rings identification. 2. In the age estimation process, the position of the first annual ring should be the major point of the agreement procedure (FAO, 2002). 3. The latest WKARHOM3 stated that otoliths (whole/slices) must be analyzed under reflected light immersed in clarification medium (Sea water, thymol). 4. The observation of whole or sliced otolith have been proven equally reliable but the chosen method must be consistent in the process (ICES, 2018 WKARHOM3). 5. Sex, maturity, catch date and zone should be considered during readings (ICES, 2018 WKARHOM3). 6. Radii measurement analysis could be helpful in recognizing true winter rings (Jurado-Ruzafa & Santamaría, 2018) (ICES, 2018 WKARHOM3).			
References	*ICES Report of the Workshop on Age reading of Horse Mackerel, Mediterranean Horse Mackerel and Blue Jack Mackerel (Trachurus trachurus, T. mediterraneus and T. picturatus) (WKARHOM2)_2015*			*ICES Workshop on Age reading of Horse Mackerel, Mediterranean Horse Mackerel and Blue Jack Mackerel (Trachurus trachurus, T. mediterraneus and T. picturatus) (WKARHOM3)_2018*

Suggestions to improve Horse Mackerels reading precision:

- The otolith reference collection (ICES, 2018 WKARHOM3) is a useful tool for training and intercalibration. It should be used prior the ageing process to minimize errors linked to true rings identification.
- In the age estimation process, the position of the first annual ring should be the major point of the agreement procedure (FAO, 2002).
- The latest WKARHOM3 stated that otoliths (whole/slices) must be analyzed under reflected light immersed in clarification medium (Sea water, thymol).
- The observation of whole or sliced otolith have been proven equally reliable but the chosen method must be consistent in the process (ICES, 2018 WKARHOM3).
- Sex, maturity, catch date and zone should be considered during readings (ICES, 2018 WKARHOM3).
- Radii measurement analysis could be helpful in recognizing true winter rings (Jurado-Ruzafa & Santamaría, 2018).

3.10 Blue whiting (*Micromesistius poutassou*)

During the exchange in preparation of the WKARBLUE (ICES, 2013) the percentage of agreement was 56.6% (PA) and the CV was 13.2, for the ICES Division 2.a, 5.a, 6.a and 6.b. In the workshop (ICES, 2013) for the same ICES divisions the PA increased to 57.1 with a CV of 13.4. The main reasons for such uncertainty between the readers were:

- Identification of the first ring;
- False rings (split rings);
- Edge interpretation.

In order to increase the accuracy and precision concerning the first ring interpretation it is suggested to conduct the following studies:

- Length-based methods.
- Marginal increment analysis.
- Daily increments.
- Regular exchanges and workshops.

Species	Blue whiting (*Micromesistius poutassou*)					
WK/Exchange	**Exchange, 2013 (ICES, 2013)**	**Workshop, 2013 (ICES, 2013)**			**Exchange, 2016 (ICES, 2017)**	**Workshop, 2017 (ICES, 2017)**
Mode of Preparation	Whole otolith					
Area	ICES Division 2.a, 5.a, 6.a and 6.b	ICES Division 2.a, 5.a, 6.a and 6.b	ICES Division 5.b	ICES Division 6.a	ICES Division 2.b, 4.a, 6.a, 7.b, 7.c, 7.j, 8.c and 9.a; and Mediterranean and NAFO 1C	ICES Division 2.b, 4.a, 6.a, 7.b, 7.c, 7.j, 8.c and 9.a; and Mediterranean and NAFO 1C
Agreement (%)*	56,6	57,1	74,1	54,6	63.7, 68.7, 68.7	64.1, 69.3, 69.3
CV*	13,2	13,4	40,1	17	55.7, 44.2, 44.2	53.4, 44.1, 44.1
APE	-	-	-	-	-	-
Low PA Reasons	1. Identification of the first ring. 2. False rings (split rings). 3. Edge interpretation.				1. Identification of the first ring; False rings (split rings). 2. Edge interpretation. *During the WKARBLUE2 results of corroboration studies concerning the otoliths collection from the whole area of distribution: length-based methods and marginal increment analysis were presented and helped on the clarification on the ring aging interpretation.*	
Recommendations	The use of existing methods of corroboration/verification were identified as recommended approaches to overcome the identified issues on blue whiting aging: 1. Length-based methods. 2. Marginal increment analysis. 3. Daily increments.				1. Daily increment analysis must be conducted prior to the next age reading workshop planned for 2021. 2. IPMA, will lead a study here corroboration studies (length-based methods, MIA, etc.) will be conducted by area of distribution in the Northeast Atlantic (southern and northern) (note: this study is already in preparation).	
References	*ICES. 2013. Report of the Workshop on the Age Reading of Blue Whiting, 10-14 June 2013, Bergen, Norway. ICES CM 2013/ACOM:53. 52 pp.*				*ICES.2017. Workshop on Age estimation of Blue Whiting (Micromesistius poutassou) WKARBLUE2, 6-9 June 2017, Lisbon, Portugal. ICES CM 2017/ SSGIEOM:22. 60pp.*	

3.11 Recommmendations to increase the ageing precision of small pelagic species

The age reading recommendations for accomplishing higher percentage of agreement between age readers were discussed in plenary and updated with the recommendations that were put forward during past workshops and exchanges. Those recommendations can be summarized for all small pelagic species as below:

- Validation / Verification / Corroboration studies on length-based methods, marginal increment analysis and daily increments in all different districts.
- Compilation of reference collection of agreed age samples.
- Adaptation of a common protocol for all areas to standardize the age assignments and to improve the coherence of the age estimates. Each area require specific guidelines in relation to differences in growth.
- Regular exchanges and workshops to assist the intercalibration within laboratories and between readers.
- Implementing quality control tools for the age the reading procedure.

4 Review of Precision/Corroboration/Validation methods used for each small and medium pelagic species

Several methods exist for validation of age readings of calcified structures (Campana, 2001). A summary of age validation methods used for all small and medium pelagic species in European waters was realized (Table 3). The validation studies carried out on small and medium pelagic species for each species/stock is presented at the end of each paragraph. The methods have been reported according to the terminology adopted in this Workshop (See section 5). Methods are categorized as; Precision, Corroboration or Validation. Under corroboration, the term "validation" is used to describe the type of study as either "indirect" or "semi-direct". The term "Corroboration" is used here as a measure of the consistency or repeatability of an age determination method. The term "Validation" is used here as the process of estimating the accuracy of an age estimation method.

Table 3 : Review of Precision/Corroboration/Validation methods used for each small and medium pelagic species.

Validation studies by species/stock

				Precision				Corroboration							Validation		
								Indirect Validation		Semi-direct validation		Indirect Validation					
Species	Area	Stock	Analitical Assessment	Several readings	Exchanges	Backcalculation	Verification	Modal Length/Frequency Analysis	Modal Weigth/Frequency Analysis	Nature edge	Marginal increment analysis	Daily increment widths	Daily increments between annuli	Progression of strong year-classes	Captive rearing from batch	Released marked fish	Bomb radiocarbon
Anchovy	English Channel	Ane 27.7	No	Y	Y												
	Bay of Biscay (Subarea 8)	Ane 27.8	Yes	Y	Y		Length distribution of anulli distance			Y	Y		Y	Y	Y		
	Galician waters (Division 9a North)	Ane 27.9a	No	Y	Y		Length distribution of anulli distance			Y							
	Gulf of Cadiz (Division 9a South)		Yes	Y	Y			Y		Y							
	Alboran Sea	GSA 01	Yes	Y	Y					Y			Y				
	Northwestern Mediterranean Sea	GSA 06	Yes	Y	Y			Y									
	Strait of Sicily	GSA 16	Yes	Y	Y	Y											
	Gulf of Lion	GSA 07	Yes	Y	Y												
	Southern Thyrrenian	GSA 10	No	Y	Y												
	Tunisia waters	GSA 12, GSA 13, GSA 14	No	Y	Y		Length distribution of anulli distance			Y	Y						
	Northern Adriatic Sea	GSA 17	Yes	Y	Y					Y							
	Western Ionian	GSA 19	Yes	Y	Y												
	Aegean Sea	GSA 22	Yes	Y	Y												
Mackerel	Portuguese coast (Division 9.a.Central-North) (Southern Component)	Mac 27.NEA	Yes	Y	Y					Y	Y						
	N and NW of Iberian Peninsula (Division 8.c and 9.a. North) (Southern Component)			Y	Y					Y							
	Bay of Biscay (Divsion 8bc) (Southern and Western Component)			Y	Y										Y		
	NE Atlantic (Subareas 6 and 4) (Western and Northern Component)			Y	Y											Y	
	NE Atlantic (Subarea 7 and Division 8abd) (Western Component)			Y	Y												
	NE Atlantic (Subarea 3 and 4) (Northern Component)			Y	Y												
	NE Atlantic (Subarea 2, 5 and 14) (Northern distribution)			Y	Y												
	Tunisia waters	GSA 12									Y						

				Precision				Corroboration							Validation		
								Indirect Validation		Semi-direct validation		Indirect Validation					
Species	**Area**	**Stock**	**Analitical Assessment**	**Several readings**	**Exchanges**	**Backcalculation**	**Verification**	**Modal Length/Frequency Analysis**	**Modal Weigth/Frequency Analysis**	**Nature edge**	**Marginal increment analysis**	**Daily increment widths**	**Daily increments between annuli**	**Progression of strong year-classes**	**Captive rearing from batch**	**Released marked fish**	**Bomb radiocarbon**
Horse mackerel	NE Atlantic	Hom.27.2.a. 4.a. 5.b. 6.a. 7.a–c.7.e–k	Yes	Y	Y						Y	Y	Y	Y			
	SE Atlantic (African waters)	NA	NA	NA	NA						Y	Y	Y				
	Adriatic Sea	GSA 18	No	Y	Y	Y		Y		Y							
Jack Mackerel	Canary Island	CECAF/34.1	Yes	Y	Y		Length distribution of anulli distance			Y	Y						
	Madeira Island	CECAF/34.1					Length distribution of anulli distance										
	Azores Island	27.10a2	Yes														
Mediterranean Horse Mackerel	Adriatic Sea	GSA 17	No					Y									
	South Adriatic Sea	GSA 18	No	Y	Y	Y		Y									
	Eastern Mediterranean (Hellenic seas)	GSA	No							Y							
Sprat	Skagerrak and Kattegat	spr.27.3a	Yes	Y	Y				Y		Y	Y					
	Skagerrak and Kattegat	spr.27.3a4	Yes	Y	Y					Y							
	Celtic Sea	spr.27.67a-cf-k	No	Y	Y					Y	Y		Y				
	Baltic Sea	spr.27.22-32	Yes	Y	Y							Y					
	North Sea	spr.27.4	Yes	Y	Y										Y		
Herring	Baltic Sea	Unclear	NA	Y	Y							Y					
	Norwegian Sea	her.27.1-24a514a	Yes	Y	Y										Y		
	North Sea, Skagerrak, Kattegat and Western Baltic	her.27.20-24 her.27.3a47d	Yes	Y	Y							Y					
	NE Atlantic			Y	Y												Y

				Precision				Corroboration							Validation		
								Indirect Validation		Semi-direct validation		Indirect Validation					
Species	**Area**	**Stock**	**Analitical Assessment**	**Several readings**	**Exchanges**	**Backcalculation**	**Verification**	**Modal Length/Frequency Analysis**	**Modal Weigth/Frequency Analysis**	**Nature edge**	**Marginal increment analysis**	**Daily increment widths**	**Daily increments between annuli**	**Progression of strong year-classes**	**Captive rearing from batch**	**Released marked fish**	**Bomb radiocarbon**
Chub mackerel	Bay of Biscay	27.8c	No	Y	Y	Y	Length distribution of anulli distance	Y		Y	Y						
	Galicia	27.9a	No	Y	Y					Y							
	North Portugal	27.9a	No	Y	Y		1st translucent ring id			Y	Y						
	Azores	27.10a2	No							Y							
	Gulf of Cadiz	27.9a	No			Y				Y							
	Madeira	CECAF/34.1	No			Y		Y		Y	Y						
	Canary	CECAF/34.1	No	Y	Y			Y		Y							
	Mauritania	CECAF/34.1	No	Y	Y	Y	Length distribution of anulli distance			-							
	Alboran Sea	GSA 01	No							Y							
	West Mediterranean (Murcia Coast)	GSA 06	No	Y	Y					-							
	West Mediterranean (Catalonian Coast)	GSA 06	No							Y							
	Tunisia waters	GSA 12								Y							
	Ligurian & North Thyrrenian Sea	GSA 09	No	Y	Y												
	Southern Adriatic Sea	GSA 18	No	Y	Y												
	Aegean Sea	GSA 22	No	Y	Y												
	Hellenic Sea	GSA22	No							Y	Y						
	Turkey waters	Unclear	No							Y							
Sardine	Bay of Biscay (Division 8c)	27.8c.9a	Yes	Y	Y					Y							
	Portugal coast (Division 9a CN		Yes	Y	Y		1st translucent ring id			Y		Y					
	Galician waters (Division 9a North)		Yes	Y	Y							Y					
	Adriatic Sea	GSA17	Yes	Y	Y							Y					
	Alboran Sea	GSA 01	Yes	Y	Y												
	W mediterranean	GSA 06	Yes	Y	Y												
	Tunisia waters	GSA 12, GSA 13, GSA 14								Y	Y						
Blue whiting	Portuguese waters (Southern component)	whb.27.1-91214	Yes	Y	Y	Y	1st translucent ring id	Y			Y						
	North East Atlantic (all areas)			Y	Y	Y	Rings length by modal age	Y			Y						
	Mediterranean Sea	-	No	Y	Y						Y						

4.1 Anchovy

Annual age

The majority of works attempting to validate annuli of anchovy apply the qualitative method of marginal increment analysis, edge nature (Giraldez and Torres, 2009; Donato and La Mesa, 2009; Millan and Tornero, 2009; Hernandez et al., 2016; Uriarte et al., 2016; Gaamur and Khemiri, 2019), one of the least rigorous methods (Panfili *et al*., 2002). So far, there are only three areas/stocks (Bay of Biscay, Gulf of Cadiz and Northern Western Mediterranean) where more accurate validation methods have been used and they have been published (Morales-Nin and Pertierra, 1990; Bellido et al., 2000; Aldanondo et al. 2016; Uriarte et al., 2016).

Validating the periodicity of annual growth increment formation (applied to the ageing method) is achieved by following the marginal otolith structure development throughout the year in several areas using qualitative method (edge nature): Bay of Biscay (Time series 1984-1989; ages 0-3+), Galician waters (Time series 2015-2016 ; ages 0-2+) Gulf of Cadiz (time series 2005-2008; ages 1-4), Alboran Sea (time series 1989-1992; all ages together), and North Adriatic Sea (time series Jan-Dec 2007; all ages together) (**Table 3**). Quantitave method (MIA) was only used in the Bay of Biscay [Time series 2004-2009; ages 1-4 (Uriarte et al., 2016)] and in Tunisian waters [ages 1-4 (Gaamur and Khemiri, 2019)]. The major pattern shows that the true translucent ring is formed in winter each year (hyaline edge) with a maximum growth in summer (opaque edge), but starting of the opaque edge during spring time changes with ages, being remarkably sooner at age 1 compared to older ages. Other rings may be present throughout the year mainly in summer (checks). However, this general pattern shows great inter-annual variability and is evidence for the complexity of age determination for this species as well as the difficulties in the recognition of the expected general growth pattern of the otolith throughout the year. It has been recognized in previous studies that a very high proportion of the variability observed in the anchovy growth between areas (and within the same area as well) is explained by changes in the habitat conditions, namely chlorophyll concentration and temperature (see, e.g., Basilone et al., 2004; Giraldez and Torres, 2009).

The age estimation criteria for Bay of Biscay anchovy were also indirectly validated by tracking year-classs abundance indices in successive spring catches and surveys and the historical correlation between the abundance of successive age groups of the same year classes in catches or in population estimates from surveys [Time series 1982-1992 and 1987-2013; ages 1-4 (Uriarte et al., 2016)]. In the Gulf of Cadiz (Time series 1989-1993; 4-18.5 cm) and in the NW Mediterranean Sea (time series 1984-1985 and 1987-1989) length frequency analysis methods were applied to corroborate the otolith interpretation and growth model parameters of anchovy (Pertierra, 1987; Morales-Nin and Pertierra, 1990; Bellido et al., 2000). In The Strait of Sicily, the back-calculation method was applied to anchovy to compare results from the growth model estimation [time series 2000-2001; ages 0-3 (Basilone et al., 2004)].

Based on different daily growth studies, the formation of the first annulus was validated and the position of the first false ring or check was corroborated in anchovy in the Bay of Biscay (Aldanondo et al., 2016; Hernandez et al., 2013) and in the North of Alboran Sea (Rey et al., 2019). Annual increment deposition in the otoliths of young-of-the-year European anchovy was validated in the Bay of Biscay (Aldanondo et al., 2016). Early anchovy juveniles were maintained in captivity from October 2012 until April 2013 and the first annulus was validated using daily increments counts. According to that, the first opaque band is completed in October-November, whereas the translucent band is completed by March-April in the Bay of Biscay. The position of the first check for anchovy in the Bay of Biscay and in Alboran Sea was also corroborated (Hernández, et al., 2013; Rey et al., 2019)). In the two methods, age was estimated by counting daily growth increments on sagittal otoliths.

In summary, there are several areas/stocks in which validations for the anchovy annual age determination have not been done yet. Age validation studies should be carried out for all anchovy stocks, and especially those that are assessed analytically. Precision in age readings may be improved by workshops and otolith exchange, but the validation of the annual deposition of seasonal zones and the checks in the otoliths represent the focal point to improve the precision in Anchovy age determination.

Daily age

The daily periodicity of micro-increment deposition was validated in early life stages of European anchovy (**Table 3**). These validations were done in rearing experiments and thus applicable to the species (*Engraulis encrasicolus)*, and not only the respective stock.

As far as anchovy is concerned, validation studies were carried out in individuals from Bay of Biscay. Daily increment deposition was validated in hatched eggs and larvae reared in the laboratory under different temperature conditions (Aldanondo et al., 2008). Additionally analysis of otoliths from wild juveniles marked by immersion in oxytetracycline hydrochloride (OTC) and reared until reaching adulthood over a period of 2 years (Cermeño et al., 2003) has been carried out for validation purposes. Furthermore, Aldanondo et al. (2008) demonstrated that increment deposition starts at hatching in anchovy.

Regarding daily growth, differences in reading criteria are maintained in some areas of the Mediterranean (Strait of Sicily and Adriatic Sea) (ICES WKMIAS 2013). It is necessary to carry out validation studies in these areas to advance the knowledge of growth rates and age , through the investigation of growth in the different phases of the life cycle of this species and to study what factors govern the formation of daily rings (experiments in controlled conditions: meso-cosmos).

4.2 Mackerel

Annual Age

The existing material on mackerel age validation studies is rather limited, particularly related to the actual annual age structures of mackerel otoliths.

Validating the periodicity of annual growth increment formation (applied to the the ageing method) is achieved by following the marginal otolith structure development throughout the year and has been studies in the Southern component of the NEA Mackerel (Divisions 8c and 9a) using qualitative method (edge nature): Division 9a Central and Central North [Time series 1981 and 2014-2017; all ages (Gordo and Martins, 1982; Silva and GonÇalves, 2019)] and in Division 8c and 9a North [Time series 2013-2017; ages 0-7+ (Villamor et al., 2018)]. Quantitave method (MIA) was also used in Portuguese waters (Division 9a) [Time series 2014-2017; all ages together (Silva and GonÇalves, 2019)] and in Tunisian waters (Gaamur and Khemiri, 2019).

The major pattern shows that the true translucentring is formed in winter each year (hyaline edge) with a maximum growth in summer (opaque edge), but laying down of the opaque edge during spring time changes with ages, being remarkably sooner at age 1 than at older ages. Seasonality of otolith zone formation can vary between fish species, and between populations within the same species and mainly in migratory species as NEA Mackerel. Although information on otolith zone formation is important for fish age estimation, there is incomplete knowledge of factors influencing variation in otolith opacity (Panfili et al., 2002). In Villamor et al. (2018), the marginal increment types (opaque or translucent) of the mackerel population of the Northeast Atlantic were assessed in the Southern area in relation to environmental and biological parameters. The main results show that timing in the formation of rings in mackerel otoliths seems to link the temperature and food resources (condition factor) to the fast growth of the fish.

The season of formation of opaque and translucent zones may change during development and in relation to geographical distribution (Høie et al., 2009; Pearson, 1996). In Villamor et al., 2018, no geographical differences are found between areas 8c and 9a N, nor with the one performed on the Portuguese coasts (ICES Subdivisions 9a Central-North and South) by Gordo and Martins (1982) and by Silva and GonÇalves (2019). All these areas belong to the same Southern Component of the NEA mackerel. It is advisable to make this type of study for all distribution areas of mackerel stock in the Northeast Atlantic, from the south of the Iberian Peninsula to northern Europe (Norwegian and Icelandic coasts) to test whether or not there are seasonal differences in the formation of opaque-hyaline zones in otoliths and to study what factors influence variations in otolith opacity. It could also be done for mackerel in all areas of Mediterranean and compare it with those of the Atlantic.

The validation of North East Atlantic mackerel annual age criteria was carried out in 1995 from ages 0 to age 8, using fish of known age, which were determined by mark-recapture experiments (Anon., 1995). Older ages could not be validated by this method because insufficient otoliths were available for validating above age 8. The collection was complied of otoliths obtained from tagged fish returns mainly in ICES Division IVa (Norwegian tagging programme). A new calibration exercise was made in the last workshop on mackerel age determination (ICES WKARMAC 2 2018) with otoliths of known age from tag and recapture experiments. These otoliths are from mackerel which were tagged between 20 and 28cm and recaptured after a known number of years. It has to be noted that the number of otoliths used in this exercise were quite low (only 28) and only otoliths from the northern area of the mackerel distribution were used. Therefore, the results should not be generalized. It would be useful to carry out similar experiments with more otoliths and otoliths from other areas. Continuity of the Norwegian experiments of tag-recapture of mackerel, especially in order to validate older ages (> 5 years old) is advisable.

Daily age

The daily periodicity of micro-increment deposition was validated in early life stages of Mackerel. These validations were done in rearing experiments and thus applicable to the species (*Scomber scombrus*) and not only the respective stock. The deposition of daily growth rings in larvae, post-larvae and juveniles of mackerel (*Scomber scombrus*) was validated by Migoya (1989) and D'Amours et al. (1990) in several areas in Northwest Atlantic, and by Mendiola and Alvarez (2008) in Northeast Atlantic. Migoya (1989) and Mendiola and Alvarez (2008) incubated mackerel eggs in the laboratory and showed that the deposit of the first increment in the otolith occurred on the hatching day and that the increments were formed daily. In addition, D'Amours et al. (1990) performed a validation experiment on mackerel juveniles in captivity, marking their otoliths with a fluorescent substance and showing that the increments were deposited on a daily basis.

4.3 Sprat

Table 3 gives an overview of the validation studies done for sprat (*Sprattus sprattus*) in the years from 1988 to present. The type of studies are categorised based on the method applied and fall under the overarching types; Precision, Corroboration and Validation (or True Age). A validation study was carried out on larval sprat in the North Sea area where the deposition of daily increments in sagittae was validated under laboratory conditions from 6 to 29 days (Alshuth, 1988a). No further validation studies have been conducted for sprat in this area and all studies carried out to date are considered "Corroboration" studies under which there are "Indirect Validation" and "Semidirect Validation" methods.

For sprat in the Skagerrak and Kattegat there have been both semi-direct and indirect validation methods applied. Relative marginal increment analysis (semi-direct method) was applied to sprat in the Skagerrak and Kattegat (Torstensen *et al.*, 2004), where the age range of the samples was 0-2 years and the samples used collected between February 2003 and January 2004. The same study included an otolith weight frequency distribution (OWFD), with OW from 0.22–2 mg. This method is a variant of the length frequency distribution analysis (Campana, 2001) and assumes that the otolith weight frequency modes correspond to the age classes in the population structure. At the WKVALPEL a recent study by Vitale et al. (in prep) looked at the edge zone formation in otoliths from age groups 1-4 captured in 2003 and 2004. This semi-direct method attempted to validate the periodicity of formation of the growth increments seen in sprat otoliths throughout the year in the Skagerrak and Kattegat. Using marginal increment analysis the seasonal growth pattern was confirmed across all ages. The outermost translucent zone increased slowly in width from February to May, coinciding with the period of slow growth of sprat during this period. The deposition of this translucent zone was completed between June and July, coinciding with the beginning of the summer growth period.

For Baltic Sea sprat, otolith (sagittae) microstructure has been examined in order to demonstrate (indirect validation) the structural differences between what are defined as true and false translucent (winter) rings (Mosegaard and Baron, 1999; ICES, 2008c). A decrease in the width of the daily increments is observed when a true translucent ring is deposited, no daily increments are formed during the winter ring formation and after the winter ring there is a gradual increase seen in the daily increment widths. When the winter ring is false it will not be possible to see this reduction in the daily increments widths before or directly after the translucent zone.

The most recent semi-direct and indirect validation studies on sprat to date were carried out on samples from the north Atlantic sea (Moore et al., 2019). A total of 533 otoliths, from ages 0 to 3 captured in ICES Divisions 6.a, 7.b, 7.j and 7 during 2011, 2013 and 2014 were used. Firstly, the deposition of the first winter ring was verified on a subset of otoliths by semi-direct methods. Daily increments were counted from the hatch mark (approx. 15 µm from the nucleus) to the first translucent zone, following the method described by Alshuth, 1988a. Following that, the semi-direct, marginal increment analysis method was used to validate the periodicity of formation of the growth increments throughout the year. Results showed that for both age 1 and age 2 year old fish the marginal increment ratio increased steadily from June to September, coinciding with the growth period and then stabilized, coinciding with a period of reduced growth between September and December when all otoliths examined had a translucent edge.

4.4 Herring

Table 3 gives an overview of the validation studies done for herring (*Clupea harengus*) in the years from 1999 to present. The type of studies are categorised based on the method applied and fall under the overarching types; Precision, Corroboration and Validation (or True Age). For herring the studies carried out to date are considered under the overarching titles of "Corroboration" and "True Age". For Baltic Sea herring, the same procedure described above for Baltic Sea sprat was applied to a herring sample (considered an indirect study). The sagittae otolith microstructure was examined in order to demonstrate the structural differences between what are defined as true and false translucent (winter) rings (Mosegaard and Baron, 1999; ICES, 2008c). A decrease in the width of the daily increments is observed when a true translucent ring is deposited, no daily increments are formed during the winter ring formation and after the winter ring there is a gradual increase seen in the daily increment widths. When the winter ring is false it will not be possible to see this reduction in the daily increments widths before or directly after the translucent zone.

For herring in the Norwegian Sea, the most direct forms of validation were applied. The studies examined the daily increment formation in otoliths from larvae and 0 group fish reared in captivity under laboratory and mesocosm conditions (Moksness, 1992 and Johannessen *et al.*, 2000). Fox *et al.*, 2004 examined otolith daily micro increment formation in herring larvae raised in captivity. The otoliths were marked by immersion of the larvae in an alizarin solution and later examined by means of light and scanning electron microscopy, with growth rates compared to results from previous studies.

A true validation study on Northwest Atlantic herring applied bomb radiocarbon analysis to validate the ages and improve age estimations of a series of archived otoliths from the 1960's and 1970's (Melvin and Campana, 2010). This is the most direct form of validation applicable to either very old fish or archived fish.

4.5 Horse Mackerel

In validation studies, two aspects shall be determined: (1) the increments are laid down according to a periodicity that can be related to a regular time-scale; (2) the age estimation structure has a consistent interpretable pattern of increments (Campana, 2001; Panfili et al., 2002). Both aspects have been poorly addressed in studies on *Trachurus* species.

To age horse mackerel (*Trachurus trachurus*) in the Northeast Atlantic, a variety of methods for processing different calcified structures as well as different age reading methods have been used. Abaunza *et al*. (2003) review an historical development of age interpretation criteria and validation methods in the Northeast Atlantic.

Annual age validation for northeast Atlantic horse mackerel (Abaunza et al., 2003) confirmed that one opaque and one translucent zone constitute one annual growth zone. Kerstan and Waldrom, (1995) validated the annual growth zones in otoliths of 0-2 years-old horse mackerel by counting the number of daily increments. Indirect age validation can be obtained from the comparison between ageing and the length-frequency distributions. This method confirmed the ageing of the first years of life (up to age 4) (Letaconnoux, 1951; Ramalho and Pinto, 1956; Barraca, 1964; Macer, 1977). Other method is based on the occurrence of annual year-marks, and has been tested by following identifiable year classes through successive year's age compositions (Eltink and Kuiter, 1989). Indications that a correct age determination method has been applied can be obtained by such an indirect validation technique. For example, in the catch in number of the western horse mackerel fishery, the extremely strong 1982 year class can be followed from 1984 to 1996 (ICES, 1999; Abaunza et al., 2003)

Waldron and Kerstan (2001) validated the age determination of horse mackerel otoliths from whole otoliths up to age four in the South-East and North-East Atlantic. They compared marginal increment (MI) widths (Kerstan, 1985), measured from annuli under a light microscope, with daily increment counts obtained with a scanning electron microscope (direct validation method). The estimated ages (0.6–4.3 years) agreed well for horse mackerel up to four years old. Examination of subsequent growth zones indicated that false rings and annuli are often of a similar appearance and that true annuli can only be identified if concurrent measurements of growth zone widths are available.

In the Eastern Mediterranean area (Greek Seas), the time of hyaline annual zone completion was estimated by the study of monthly marginal increments (quantitave), the 1st annulus formation was detected by comparing the progression by month of the smaller modal fish length with the respective otolith appearance during the year and the back-calculation method was applied to compare results from the growth model estimation (Karlou-Riga and Sinis, 1997). In the Adriatic Sea, length frequency analysis was applied to corroborate the otolith interpretation and growth model parameters of horse mackerel (Alegria Hernandez, 1984). In general, the annuli were validated until the 5th.

For the *T. picturatus* Garcia et al. (2015) reported in Azores Island a semi-direct validation study (Marginal Increment Analysis) on the deposition periodicity of the annulus. Moreover, for the Madeira area, Vasconcelos et al. (2006) report a validation of the coherency in the ageing criteria by partial radii measurement. Jurado-Ruzafa&Santamaría (2019) for the Canary Island report the validation of deposition periodicity of the annuli through qualitative (Marginal analysis in term of monthly opaque trend) and quantitative (Marginal Increment Analysis) approach. Moreover, Jurado-Ruzafa&Santamaría (2018) reported the frequency distribution of partial radii measurements in order to validate the coherency of the ageing criteria adopted. During the workshop some examples of the validation study on the *T. trachurus* in Mediterranean basin based on the indirect and semi-direct methods was presented. The deposition of the one annulus was demonstrated in the Adriatic by marginal analysis. Moreover the back-calculation results were compared with the mean length of the mode (Bhattacharya method) from the winter Length Frequency Distribution (LFD) Survey (GRUND 2009).This analysis provided an indirect validation of the detected age group (Carbonara et al., 2018). The winter survey LFD was used in this analysis because the winter period seems to represent an age class. This analysis showedthat the first ring, back-calculated at a total length of 75.09 mm did not have any correspondence to the LFD mode. While the other back calculated rings and mode did not present any significant difference (t-test p>0.05). In terms of accuracy, Campana (2001) indicated the analysis of discrete length modes as a robust approach to validating the interpretation of annuli.

The comparison of the growth curves from the otolith reading and LFD analysis from Medits survey (ELEFAN and Bhattacharys methods) did not show any statistical differences. This result could represent an indirect validation (Campana, 2001; Panfili et al., 2002) of the otolith age estimation criteria.This analysis showed the first ring back-calculated at a total length of 75.09 mm did not have any correspondence to the LFD mode. While the other back calculated rings and mode did not present a significant difference (t-test p>0.05). In term of accuracy, Campana (2001) indicated the analysis of discrete length modes as a robust approach to validating the interpretation of annuli.

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Validation techniques for annual growth structures (otoliths) of Mediterranean horse mackerel (*Trachurus mediterraneus*) carried out in European waters are summarized in Table 3. Accuracy cannot be evaluated in the Atlantic area since validation data are not available at the moment and the true age determination in this species is not possible. In the Hellenic Sea, the time of annulus completion was estimated by the study of monthly marginal increments (Karlou-Riga, 2000). Arneri and Tangerini (1984) studied the growth of the same species by otoliths and length frequency in young individuals (0-4 age groups) in the Adriatic Sea. . Recently Carbonara and Casciaro (2018) also studied this species in the southern Adriatic Sea for older ages (0-6) applying the same method.

4.6 Chub mackerel

There has been no direct validation study of age estimation of chub mackerel in the NE Atlantic Ocean and the Mediterranean Sea so far. Some semi-direct and indirect validation studies in these areas, like Marginal Increment Analysis (Quantitative), Nature of the otolith Edge Analysis (Qualitative) and Length frequency distribution analysis have been performed in some areas (Table 3).

The studies of the nature of the otolith edge performed show different time periods of opaque edge growth in each area. In the Bay of Biscay (ICES Division 8c) the opaque edge is mainly formed from June to December (Navarro *et al*., 2018, 2019), while in the Portuguese waters (ICES Division 9a), the opaque edge is laid down between May and August (Martins *et al.*, 1983) and in the Gulf of Cadiz (ICES Division 9a-S) the opaque edge occurs from March to October (Velasco *et al*., 2011). In the Canary, Madeira and Azores Islands the opaque edge occurs during March-September, May-July and May-October, respectively (Lorenzo *et al*., 1996; Vasconcelos, 2006; Carvalho *et al*., 2002). Regarding the Mediterranean waters, the opaque edge seen in the otoliths of chub mackerel in the Alboran Sea occurs between March-October (Velasco *et al*., 2011), while in the Catalonian waters, the opaque edge occurs between April-July (Perrota *et al*., 2005), in the Hellenic Sea between March-September (Kipparissis *et al*., 2000) and in the Turkey waters during the summer (Tuggac, 1997).

Only in a few areas an analysis of the marginal increment of the otoliths was performed. In the Bay of Biscay, AMD (Absolute Marginal Distance) and RMD (Relative Marginal Distance) were performed, showing a similar trend to that obtained in the qualitative study, with higher values (wider opaque edge) in the second semester (Navarro *et al*., 2018, 2019). In Portugal coast, a MI (Marginal Increment Analysis) was performed, showing a minimum in June and November and presenting a pattern not fully defined (Silva and Gonçalves, 2019). In the Madeira Island, an RMI (Relative Marginal Increment) was performed, with values of > 50% in May, September, October, December and January and a minimum in April, which did not coincide with that obtained in the qualitative study (Vasconcelos, 2006). In the Hellenic Sea, a MIR (Marginal Increment Ratio) was performed, showing a width decrease of the otolith edge in April for the age classes 1 and 2 and inJuly for the age class 3 (Kiparissis *et al*., 2000).

There are also some studies using back-calculation models in some areas: in the Bay of Biscay (Fraser-Lee and BPH equations) (Navarro et al., 2019), Portuguese Coast (Martins et al., 1983); Gulf of Cadiz (Rodriguez-Rhoda, 1982), Madeira Islands (Francis and Dahl-Lea equations) (Vasconcelos, 2006) Canary Islands (Francis equation) (Lorenzo *et al.,* 1995) and Mauritania (Monastyrsky equation) (Jurado *et al*., 2017).

Only three Length Frequency Analysis havebeen performed, one in the North and Northwest Iberian Peninsula (SLCA, PROJMAT, ELEFAN and Bhattacharya analyses) that showed a faster growth rate than that observed in direct age estimation in otoliths (Navarro et al., 2019), another one in the Madeira Islands (ELEFAN I analysis), that showed an intermediate growth rate, higher than that observed in direct age estimation (Vasconcelos, 2006) and a last one in the Canary Islands (Bhattacharya analysis) showing a slow growth rate, similar to that observed with direct age estimation (Nespereira, 1992).

No studies regarding daily increment validation have been performed for *Scomber colias,* although some daily increment studies have been performed for the congener specie (*Scomber japonicus*) in the Pacific Ocean (Higuchi *et al*., 2019; Takashi *et al*., 2014 and others).

4.7 Sardine

For sardine, most of the publications are related to environmental factors affecting growth and recruitment, and there is little work on validation. The main issues in the age attribution of sardine ages are the identification of the otolith edge and of the first annual ring (e.g. ICES, 1997; Soares et al., 2007, WKARAS 2005, 2011, 2016). At the age reading workshop 2011 the seasonality of the otolith edge was studied using a Binomial Generalized Linear Model (logit link function). The proportion of hyaline edges was fit using month, year and age as predictor factor variables. Results indicate that patterns with age differed in the Portuguese areas and in the Bay of Biscay. In the Portuguese areas, the variation in the proportion of hyaline edges was gradual over months and the delay in the formation of the opaque edge with age was also gradual; in general the minimum proportion of hyaline edges was observed around June at age 1, July at age 2 and August at ages 3 and 4. In the Bay of Biscay, the edge pattern changed substantially from age 1 to the remaining ages; at age 1, the hyaline proportion declines abruptly around May and remains close to zero until September while at ages 2-4 years it remains high most of the year and achieves minimum values in July-August. Also, the 2011 WKARAS workshop addressed the problem of first annual ring. Samples used at the Workshop showed the diameter of the first annual ring is directly proportional to fish length. Fish and otolith measurements for 0-group and 1-year old individuals with length between 11 and 16 cm had ring diameters between 2.0 and 2.9 mm (mean=2.4 mm, indicating that the first translucent ring forms at a distance around 1.1 mm from the otolith nucleus (corresponding to a diameter around 2 mm). This result were corroborated with a study regarding validation of ages 0 and 1 based on daily rings of sardine juveniles sampled in October 2008 and April 2009 off the northern Portuguese coast (6-12cm total length). Age in years and cohort determination was consistent with age in days for most juveniles, providing evidences that first annual ring is correlated with the length of fish at the time of its formation.

Regarding the daily validation, the periodicity of microincrement deposition was validated in early life stages of sardine (larvae), though in a few areas of distribution. In Portuguese waters and in the Bay of Biscay, the daily deposition was validated in sagittal otoliths of reared and wild sardine larvae from hatching to complete yolk-sac absorption (Re 1984; Alemany and Alvarez 1994; Alvarez and Alemany, 1997). Similarly, the validation of daily otolith increment formation was carried out by a mesocosm experiment on wild sardine late larvae growing under natural environmental conditions in the Adriatic Sea (Panfili, 2012).

Studies describing the larval and juvenile growth of wild sardines using daily increment widths have been performed for more areas. Sardines sampled in the eastern part of the Adriatic and in the Bay of Biscay were aged and larval growth analysis was characterized by increment widths (Dulèiæ, 1995). Also Almany et al. (2006) study the relation of survival and growth of sardine from Alboran Sea, with the wind and its relation with the date of birth by means of increments widths in larvae and juveniles. Alvarez (2005) and Gracía et al. (2006) used the same method in the Galician and Western Mediterranean waters. Finally, in Portuguese waters Meneses 2013, relate the temporal variability of daily sardine ages and growth with samples collected between 1989 and1993.

4.8 Blue whiting

A study using the blue whiting otoliths from the Portuguese coast was conducted to determinate the size of 1st ring (Dores and Gonçalves, 2017; ICES, 2017), the results showed a higher correlation of the modal age with otolith thickness and with otolith length. The results also showed, that the size of 1st ring on otoliths off the Portuguese coast (southern component) was between 8.5 – 11 mm (distance from the center to the anterior area) and different from the size described on literature based on blue whiting otoliths collected from the northern areas (8.3 – 9.3mm).

An age corroboration study applying a multivariate modelling approach (GLM) was conducted, using the otoliths from the 2017 WKARBLUE2 (Gonçalves and Dores, 2017), from the whole stock distribution area. The main analysis consisted on relating the rings length distribution by modal age, with other variables such as fish length, otolith total length, and otolith weight and otolith width. The results from this multivariate modelling approach showed to be very useful to validate ages, mainly the doubts due to false and double rings. It is planned that this study will be conducted by IPMA considering a larger sample size by area.

Marginal increment analysis was also performed using the otoliths from the 2017 WKARBLUE2 (Elleboode and Chantre, 2017) and by area (Mediterranean, Northern and Southern component). Although, an annual pattern was evident in the otoliths from the northern component, no clear results were obtained in the otoliths from the other areas. The analysis will be repeated for a larger sample size by area.

5 Propose the most appropriate ageing validation methods for small pelagic species

5.1 Ageing process

An ageing process follows several steps (Fig. 26). First, an ageing methodology has to be established for a particular species, based on scientific information. Once age results are available some analysis are recommended to improve precision among different readers and/or readings. These exercises are typically based on sharing a collection of otoliths (or images) which are used to perform blind readings in different laboratories and countries. These exchanges are useful to obtain comparable results and measures of precision (CV). Furthermore, international workshops are held periodically (every few years) to further analyse reading outputs and improve ageing methodologies.

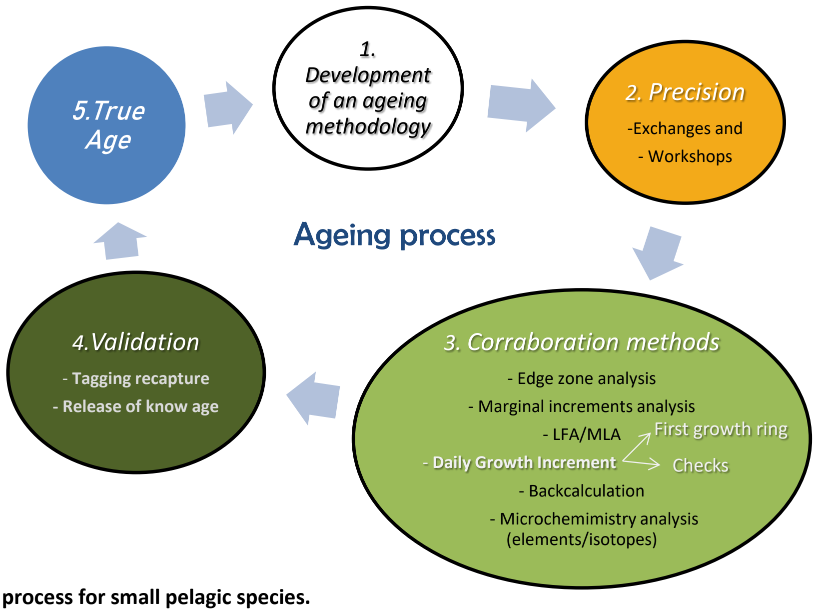

Figure 26: An ageing process for small pelagic species.

A next step is to perform other studies that offer independent results, related to diverse aspects of growth of the species. These studies are used to support, or not, our accepted ageing methodology. Several matching and independent results help to corroborate certain ageing criterion. Each type of study determines how precision and/or trueness are enhanced, usually increasing both at different levels. In general, these methods are included in indirect or semi-direct validation categories, as true ages are not actually known in any of them. When high difficulties exist to dermine the age of a particular species some other methodologies, usually more complex and costly, are considered such as strictly validation experiments, as approach to real ages. Tagging-recapture experiments and rearing in captivity are included within this category.

Although the final goal of obtaining true ages, considering all ages and natural environment, is impractical nowadays, using several methodologies in parallel help researchers to approach a closer knowledge of real ages and its uncertainty.

5.2 Validation studies for small pelagic species

Each validation study improves precision, trueness and accuracy at different degrees. Most common validation studies are outlinedin Table 4, based on Campana, 2001 and previous workshops (WSAVSG, 2013 and WKMIAS, 2013).

Table 4: Summary of age validation methodolgies, modified from Campane (2001) with methods used for small pelagic species according to the type of analysis (precision: yellow; corroboration: light green ; validation: dark green).

Method	Annual /Daily	Age	Advantages	Limits	Applicability to small pelagics	Type of analysis
Several readings	A+D	All	Reduce error	Subjectivity	YES	PRECISION
Exchanges	A+D	All	Reduce error	Subjectivity	YES	PRECISION
Backcalculation	A+D	All	Reduce error	Subjectivity	YES	PRECISION
Modal Length/Weight Frequency Analysis	A	0-5 yr	Validation of first ages	Modes as age groups assumption	YES	CORROBORATION
Edge Nature	A	All	Validate periodicity	At times edge nature is confusing	YES	CORROBORATION
Marginal increment analysis	A	All	Validate periodicity	Uncertain in slow growing/older individuals	YES	CORROBORATION
Life history events	A+D	All	Linked to biology	Troublesome in fitting rings and events	YES	CORROBORATION
Microstructure (DGI)	A+D	<3 yr	Validation of 1st years	Daily periodicity premise	YES	CORROBORATION
Strong year-class progression	A	All	Easy to follow this year-class	Unusual episode	YES	CORROBORATION
Microchemistry	A+D	All	Relationship with environment	Isolatedly does not give age information	YES	CORROBORATION
Aquaculture (rearing from hatching)	A+D		Validate absolute age and periodicity	Unnatural conditions	YES	VALIDATION
Tagging	A	All	Validate absolute age and periodicity	Low recaptures. May affect survival. Not for small individuals	YES	VALIDATION
Tagging + chemical marks	A+D	All	Validate periodicity post release	Low recaptures. May affect survival. Not for small individuals	YES	VALIDATION
Radiochemical dating	A	Plus 5yr old	Validate absolute age old fishes	Can only distinguish between divergent estimates	NO	VALIDATION
Bomb radiocarbon	A	old ages	Validate absolute age and periodicity	Restricted to very old fish or historical otolith collection	YES	VALIDATION
Known age fish	A+D	All	Real age information	Almost unavailable	NO	VALIDATION

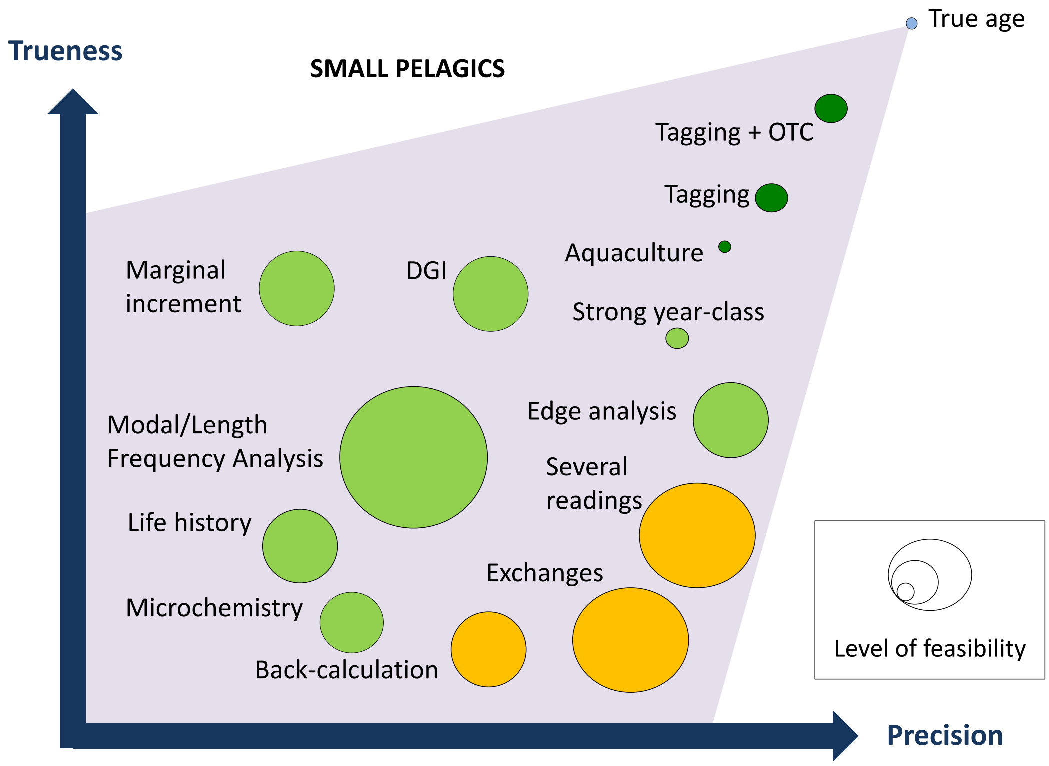

Figure 27: Different methodologies used in small pelagic ageing process related to trueness, precision and accuracy. Orange: precision methods; pale green: corroboration; dark green: validation and blue: objective.

Back-calculated lengths: This is an important method for obtaining estimates of an individual's size at age prior to capture date (Panfili et al., 2002). This method does not validate any age interpretation (Campana, 2001), simply show the consistency in the interpretation of the growth ring sequence regardless of whether it is correct or not. One method of using trends in patterns of growth as verification of how the ageing criteria were applied is to compare the length at age obtained from direct calcified structure readings and the back-calculated length at age. The results from the back-calculation (length at age) could be used in indirect age validation process if they are compared with mean length mode from length frequency distribution obtained by a survey carried out in the period of the hyaline ring formation. Exist a large amount of literature related to this topic (Campana, 2001; Panfili et al., 2002)

Requirements: The back-calculated length at age of previous size at age is an inexpensive tool but cannot stand alone. This method should preferably be coupled to a length frequency distribution from a survey carried out in the period of the hyaline ring formation or size distributions of individual age classes. To apply it correctly three assumption must be fulfilled (see Panfili et al., 2002)

Length Frequency analysis (LFD): Progression of length frequency modes though out time is one of the most basic of the length frequency analyses which is possible, and can be a reliable form of age corroboration in young, fast-growing fish. This method is difficult to apply in older age classes, where overlap between modes is broad.

Weight frequency distribution (WFD) is a variant of the length frequency distribution analysis, using individual weights instead of sizes.

Requirements and assumptions: Both methods are particularly suitable for short lived and fast growing species. For this reason this method has been applied to many of the pelagic species (Table 3).

Both of the above methods assume that the expected modes of the length/weight frequency would correspond to the population age-classes.

Random sampling procedure of fish is required to ensure that the samples collected are representative of the population. Low cost and takes advantage of biological data routinely obtained in fishery studies.

Progression of strong year-classes: This method can provide a strong, albeit qualitative, confirmation of growth increment periodicity. There are strong indications that the age reading method is accurate if the age composition of exceptionally good or weak year-classes can be followed over a long period of time (Abaunza et al., 2003).

Requirements: Catch at age available, strong (or weak) year-class well-defined and no marked age-structured migration or mortality. Low cost, if catch at age is available. However, these episodes could be unusual or difficult to identify.

Marginal increment analysis (MIA): These methods provide a validation of the deposition pattern of the annulus. Moreover, through these methods is also possible to detect the deposition of false rings during some period (reproduction, migration, etc.). MIA (quantitative) is sometimes differentiated from **edge analysis** (qualitative), but when used as a validation method, has similar properties.

In the small and medium pelagic species listed in Table 4, the use of the MIA (qualitative and quantitative) proved a method to corroborate annual increment formation, but there are some limitations on the use of such techniques. These are as follow: the difficulty in measuring small increments accurately, overall and particularly in older individual at the edge of the otolith; difficulties in interpreting opaque/translucent edge zones and the need for high contrast between growth zones.

Requirements: samples throughout the year. Low cost

Daily increments between annuli: This method can provide strong corroboration of the frequency of formation of the annuli. This method is valuable to identify the first winter ring but pre-supposes knowledge of dates of hatch and annulus formation.

Requirements: Validation of the formation of the daily increments growth for this approach to be valid. Low cost, but time consuming work.

Daily increments widths: This method can be used to identify the timing of growth zones, by linking the occurrence of translucent checks to the time of occurrence. It is based on an assumption that the growth increments used to validate a macroscopic structure are formed a daily basis. This validation method is only feasible for the 1st to the 3rd winter ring. Beyond these, the distance between the winter rings decreases making it impossible to detect the daily ring structure bordering these. For older ages, one must accept that the visible transparent zones are true winter rings, and as the occurrence and detection of false rings usually is highest within the first years of the individual of pelagic species, this assumption seems acceptable.

Requirements: validation of the formation of the daily increments growth for this approach to be valid. Low cost, but time consuming work.

Nowadays, Daily Growth Increments (DGI) analysis (from otolith microstructure) is being improved, by means of a new freely available digital tool, OTOLab, that allows the counting and measuring of each DGI throughout an otolith growth axis.

Tag-recapture analysis: The existence of otoliths from mark-recapture experiments are potentially the golden stones and could iron out many subjective assumptions related to age estimation. This provides a good growth comparison for age class.

Requirements: To carry out tagging-recapture experiments. Rather expensive, as the cost of tagging surveys is very high and recaptures are low (usually <5%) depending of fishermen collaboration.

Captive rearing: The only method to validate both absolute age and periodicity of growth structures. Mesocosms and outside cages provide improved and more natural rearing experiments for validation studies than indoor locations. Daily growth increments are less affected by environmental conditions than annulus formation.

Requirements: Aquaculture laboratory, mesocosms, ocean cage or outside enclosures. Expensive and may not mirror conditions in the wild.

General conclusions

• Age validation studies should be carried out for all pelagic species, and especially those that are assessed analytically.

• Precision in age readings may be improved by workshops and otolith exchanges and workshop, but validation of time deposition and periodicity of of seasonal zones (opaque and translucent) and checks (i.e. the spawning ring, migration ring) in the calcified structures (CS) are the key means to improve the precision and accuracy in the process of ageing by using CS.

• True age validation studies (i.e. tagging programs or captive rearing) cannot be easily applied to all species and stocks. Best practice for age determination would be to assess and compare other corroborative techniques (marginal increment analysis, marginal analysis, following strong year classes, length back-calculation, etc.) and combine the most appropriate to clarify the periodicity of CS growth and subsequently the correct interpretation of the annuli.

6 Method recommendations for small pelagic species

Using several corroboration methods is recommended for small pelagic species. Particularly, some highly feasible methods are preferred: length and modal frequency analysis, marginal increment analysis, edge nature and microstructure readings. If coincident results of these methods are obtained, a specific aging criterion could be supported. Also, life history and trait information (metamorphosis, recruitment, first maturity, migrations, etc.) can reinforce growth hypotheses.

When daily growth increment (DGI) readings are supported by other methods (edge and/or length analysis) they can be used to locate checks (false rings) in time and annual rings within the otoliths. Furthermore, checks could be linked to specific life events.

6.1 List of prioritised studies

Anchovy: There are several areas/stocks in which validations for the anchovy annual age determination have not been done yet. Age validation studies should be carried out for all anchovy stocks, and especially those that are assessed analytically:

1. Comparative first ring in different areas and historical DGI: Research by micro-increment counting on several selected otoliths by areas to validate first annual winter mark.

2. Other validations and corroboration methods by areas/stock, such as progression of length frequency modes throughout time to track cohorts, corroboration of inner consistency of age determination by following cohorts in catches and surveys and studies on the seasonal formation of hyaline and opaque edges3. Regarding daily growth, differences in reading criteria are maintained in some areas of the Mediterranean (ICES WKMIAS. 2013). It is necessary to carry out validation studies in these areas to advance in the knowledge of growth and age rates, through the investigation of growth in the different phases of the life cycle of this species and to study what factors govern the formation of daily rings (experiments in controlled conditions: meso-cosmos). Sardine (few validation studies in this species):

1. Complete studies to validate first annulus in order to standardize reading criteria in Atlantic and Mediterranean.
2. 2. Other validations and corroboration methods by areas/stock, such as progression of length frequency modes throughout time to track cohorts, corroboration of inner consistency of age determination by following cohorts in catches and surveys and studies on the seasonal formation of hyaline and opaque edges.

Mackerel (*Scomber scombrus*):

1. Corroboration of growth studies needed in all locations.
2. It is advisable to make studies of seasonal formation of growth rings in the otoliths for all distribution areas of mackerel in the Northeast Atlantic, from the south of the Iberian Peninsula to northern Europe (Norwegian and Icelandic coasts) to test whether or not there are seasonal differences in the formation of opaque-hyaline zones in otoliths and to study that factors are influencing variations in otolith opacity.

3. Continuity of the Norwegian experiments of tag-recapture of mackerel, especially in order to validate older ages (> 5 years old) is advisable.

Chub mackerel (*Scomber colias*):

1. Corroboration of growth studies needed in all locations.
2. 1.2. To extend the use of alternative methods to direct age estimation, such as length-frequency analysis in all distribution areas (especially with a wider length range in catches) is recommended.

Horse mackerel (*Trachurus* spp):

1. Corroboration of growth studies needed for other Trachurus spp and areas. (T. trachurus has been validated in N Atlantic (Abaunza, 2003).

Blue whiting:

1. Need to validate first annulus in Atlantic and Mediterranean.
2. Microstructure analysis

Sprat

1. Microstructure analysis to verify the position of first true winter ring which can be problematic in the North Sea
2. Contined work of otolith edge analysis to confirm the deposition of the growth zones across all areas.
3. Corroboration of growth studies, primarily in the Baltic

Herring:

1. Studies to identify the location of the first winter ring in both scales and otoliths

2. Corroboration of growth studies in all areas

6.2 Future perspectives in terms of validation of age for small and medium sized pelagic species

Tag-recapture and use of chemical agents for otolith marking: The existence of otoliths from the mark-recapture experiments (i.e Norwegian programme of mackerel) are potentially the golden stones and could clear up many subjective assumptions related to the age estimation of mackerel. It is of utmost importance that the dimensions and availability of such material is clarified and that efforts are made to reach agreement on potential availability for coordinated validation studies. Alternatively, chemical marker substances can be used in the tag-recapture experiments.

Validation of Life history events: Daily ring structures have been validated in otoliths of anchovy, sardine, herring, sprat and mackerel, (Alshuth 1988b; Moksness 1992; Alemany and Alvarez, 1994; Johannessen et al. 2000; Fox et al. 2004; Aldanondo et al., 2008; Mendiola and Álvarez, 2008). These studies give the potential for validating the first years of growth, making standards (L1, etc) and ruling out double structures in the first years of life.

Other validation methods: Indirect validation methods may be applied to check the accuracy of the age determination of a given species. For example; catch in numbers of mackerel in the fishery allows a tracing of the weak/strong year classes in successive years (ICES, 2013).

Corroborative methods for validation of annual rings, such as elemental or isotopic cycles (**microchemistry**), could potentially support the age determination (Campana, 2001). For this, however, it is necessary to require knowledge about the chemical environment in which the given species is found. Other age verification methods such as Bio-chronologiesstudies of growth-increment widths in the otoliths as supportive tool for age validation can be useful for the long lived pelagic species, such as horse mackerel and mackerel. And also for those pelagic species have large collections of otoliths, although they are not long-lived species (ICES, 2014).

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